Synthesis of ß-Keto- and ß-Hydroxy-α,α-difluorosulfonamides.

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

ß-Keto- and ß-hydroxy-α,α-difluorosulfonamides are both underexplored classes of molecules. There is only one report of each class of molecules in the literature, however very similar molecules are well known and are biologically active therefore we expect ß-Keto- and ß-hydroxy-α,α-difluorosulfonamides to be useful inhibitors of enzymes, especially proteases.

We have developed an efficient synthesis of ß-hydroxy-α,α-difluorosulfonamides via electrophilic fluorination of ß-ketosulfonamides which are derived from the reaction of *N*-protected α-amino acid methyl esters and methanesulfonamide carbanions. Both steps proceed in high yield without epimerization. Several protecting groups were investigated for the sulfonamide nitrogen and both dimethoxybenzyl and diphenylmethyl groups could be removed cleanly under mild conditions.

A new synthesis of difluoromethanesulfonamides has been developed which does not rely on expensive ozone depleting reagents. An investigation of difluoromethanesulfonamide carbanions revealed a dramatic dependence of stability on the cation with potassium and sodium salts being more stable than lithium. Difluoromethanesulfonamide carbanions reacted with aldehydes and ketones to give ß-hydroxy-α,α-difluorosulfonamides. Yields were high with nonenolizable substrates and lower with enolizable carbonyls. Excellent yields were obtained using 9-phenyl-9-fluorenyl protected α-amino aldehydes and, by using doubly protected *N*-benzyl-*N*-9-phenyl-9-fluorenyl-α-aminoaldehydes, excellent dr could be obtained. The reaction was extremely rapid and was complete within a few minutes, even at -128 °C.

The above synthesis required the use of the 9-phenyl-9-fluorenyl protecting group. However, we found that literature methods for the introduction of this group were unsatisfactory due to long reaction times, variable yield, and toxic reagents. We investigated new conditions

using a combination of phenylfluorenyl chloride, silver nitrate, and *N*-methylmorpholine which gave excellent yields of protected amines within 1 hour. By using TMS protecting groups the reaction could be extended to free amino acids. This approach also gave high yields rapidly with alcohols and carboxylic acids. Meanwhile thiols, sulfonamides, and amides reacted better with a mixture of 9-phenyl-9-fluorenyl alcohol and $BF_3 \cdot Et_2O$.

The IgA1 protease is an important virulence factor which contributes to diseases caused by a diverse group of bacteria. We investigated the synthesis of a series of chromogenic substrates that may be used to assay this enzyme and are derived from its natural substrate. We also investigated a series of peptidomimetics bearing C-terminal ß-keto- and ß-hydroxy-α,α-difluorosulfonamides, trifluoromethyl ketones and alcohols, and boronic acids, which we expect to be inhibitors of the enzyme. These inhibitors may lead to new to antivirulence therapies for the global fight against antibiotic resistance.

Acknowledgements

I would like to thank my supervisor professor Scott D. Taylor for his guidance, inspiration, and continuous support and tireless edits throughout my graduate research. I thank the other members of my advisory committee, Dr. Murphy, Dr. Tam, and Dr. Dmitrienko for the roles they played in the completion of this thesis.

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I thank Ryan Chung and Professor Jason E. Hein at the University of British Colombia for performing chiral HPLC work to determine the ee values of compounds in chapter 1; Edmond Chu for collaboration on the work described in chapter 1; and Yishu Zang for assistance preparing 4-nitroanilides described in Chapter 4.

I thank the members of the Taylor group, past and present for assistance and friendship: Matthew Diamandas, Ryan Moreira, Micheal Noden, Bradley Scott, Dr Robert Taylor and Bo Zheng and Dr Chuda Lohani. Special thanks to and Dr Braden Kralt for mentoring during the early years of my degree and proofreading portions of this work.

Dedication

This work is dedicated to my beloved wife Angeline.

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List of Abbreviations

°C	degrees Celsius
µL	microlitre
µmol	micromole
4-MP	4-methylpiperidine
AA	amino acid
Ac	acetyl
ACS	American Chemical Society
Ala	alanine
Alloc	allyloxycarbonyl
Ar	aryl
Asn	asparagine
Asp	aspartate
Bn	benzyl
Boc	butyloxycarbonyl
BOP	benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
br	broad signal
BSA	N,O-bis(trimethylsilyl) acetamide
Bu	butyl
CA	carbonic anhydrase
calcd	calculated
CAN	cerium ammonium nitrate
cat.	catalytic
Cbz	carboxybenzyl
CD	circular dichroism
CDC	Centers for Disease Control and Prevention
CDI	carbonyl diimidazole
CFC	chlorofluorocarbon
ChC	clostridium histolyticum collagenase
C_L	light chain
CMBP	cyanomethyltributylphosphorane
COMU	(1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate
conc	concentrated
COSY	homonuclear correlation spectroscopy
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCA	dichloroacetic acid
DCC	dicyclohexylcarbodiimide
DCE	dichloroethane
DCM	dichloromethane
DCU	dicyclohexyl urea
DEPBT	3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one
DFP	diisopropyl fluorophosphate

DFT	density functional theory
DIAD	diisopropyl azodicarboxylate
DIBAL	diisobutylaluminium hydride
DIC	*N,N'*-diisopropylcarbodiimide
DIPEA	*N,N*-diisopropylethylamine
DKP	diketopiperazine
DMAP	*N,N*-dimethyl-4-aminopyridine
DMB	dimethoxybenzyl
DMBA	*N,N'*-dimethylbarbituric acid
DMF	*N,N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPM	diphenylmethane
DPPA	diphenylphosphoryl azide
dr	diastereomeric ratio
e.g.	exempli gratia ("for example")
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDT	ethanedithiol
EDTA	Ethylenediaminetetraacetic acid
ee	enantiomeric excess
equiv	equivalents
er	enantiomeric ratio
ESI	electrospray ionization
Et	ethyl
et. al.	et alia (and others)
EtOAc	ethyl acetate
EXSY	chemical exchange spectroscopy
FAB	antigen binding fragment
Fc	crystallizable fragment
FDA	Food and Drug Administration
Fmoc	fluorenylmethoxycarbonyl
FRET	Förster resonance energy transfer
FT	Fourier transform
g	gram
Glu	glutamate
Gly	glycine
h	hour
HATU	Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium
HCTU	2-(6-Chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate
HFIP	hexafluoroisopropanol
HMDS	*hexamethyldisilazane*
HMPA	hexamethylphosphoramide
HMQC	heteronuclear multiple quantum coherence
HOAt	1-hydroxy-7-azabenzotriazole

HOBt	hydroxybenzotriazole
HPLC	high performance liquid chromatography
hPhe	homophenylalanine
HRMS	high resolution mass spectrometry
Hz	hertz
i	*iso*
IgA1	immunoglobulin A1
IgA1P	immunoglobulin A1 protease
Ile	isoleucine
J	coupling constant
KHMDS	potassium bis(trimethylsilyl)amide
L	litre
LD50	lethal dose killing 50% of the test sample
LDA	lithium diisopropylamide
LG	leaving group
LiHMDS	lithium bis(trimethylsilyl)amide
LRMS	low-resolution mass spectrometry
Lys	lysine
m	multiplet
m/z	mass to charge ratio
Me	methyl
Mesyl	methane sulfonyl
mg	milligram
MHz	megahertz
min	minute
mL	millilitre
mmol	millimole
MMP	matrix metaloproteases
mol	mole
mp	melting point
n	normal
NaHMDS	Sodium bis(trimethylsilyl)amide
ND	not determined
NHS	*N*-hydroxysuccinimide
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NOE	n*uclear Overhauser effect*
NOESY	nuclear Overhauser effect spectroscopy
OBO	4-methyl-2,6,7-trioxa-bicyclo[2.2.2]octan-1-yl
p	*para*
PAGE	polyacrylamide gelatinous electrophoresis
PBu3	tributylphosphine
PDB	Protein Data Bank
PEG	polyethylene glycol
Pfp	pentafluorophenol
PhF	9-phenyl-9-fluorneyl

PhSiH3	phenylsilane
PMB	paramethoxybenzyl
*p*NP	*para*-nitrophenol
ppm	parts per million
PyAOP	7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
q	quartet
R2	transverse relaxation rate
RNA	ribonucleic acid
RP	reverse-phase
rt	room temperature
RT	retention time
s	singlet
SAR	structure-activity relationship
sat.	saturated
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Selectfluor	1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)
Ser	serine
SIIS	solvent-induced isotopic shift
SN2	bimolecular nucleophilic substitution
SPPS	solid-phase peptide synthesis
STS	steroid sulfatase
t-	*tert*-
T2	transverse relaxation time
TAEA	tris(aminoethyl)amine
TBAF	tetrabutylammonium fluoride
TBDMS	tert-butyldimethylsilyl
TBS	*t*-butyldimethylsilyl
TEA	triethylamine
Temp	temperature
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl
TES	triethylsilane
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thr	threonine
TIPS	triisopropylsilane
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSE	trimethylsilylethyl
TMSOTf	trimethylsilyl triflate
Trp	tryptophan
Trt	trityl
UV	ultraviolet
v	volume
Val	valine

vide infra	see below
WHO	World Health Organization
wt	weight

List of Schemes

List of Publications.

Soley, **J**., Taylor, S. D. Synthesis of ß-Hydroxy-α,α-difluorosulfonamides from Carbanions of Difluoromethanesulfonamides. *J. Org. Chem*. **2021**, *86*, 6577-6591.

Soley, **J**., Taylor, S. D. A Mild, Rapid, Chemo- and Regioselective Procedure for the Introduction of the 9-Phenyl-9-fluorenyl Protecting Group into Amines, Acids, Alcohols, Sulfonamides and Amides. *J. Org. Chem.* **2020**, *85*, 4, 2068-2081.

Soley, **J.**, Chiu, E., Chung, R., Green, J., Hein, J. E., Taylor, S. D. Synthesis of β-Ketosulfonamides Derived from Amino Acids and Their Conversion to β-Keto-α,α-difluorosulfonamides via Electrophilic Fluorination. *J. Org. Chem*. **2017**, *82*, 11157–11165.

Lohani, C. R., **Soley**, **J**., Kralt, B., Palmer, M., Taylor, S. D. α-Azido esters in depsipeptide synthesis: C-O bond cleavage during azido group reduction. *J. Org. Chem*., **2016**, *81*, 11831-11840

Chapter 1 — Synthesis of ß-keto-α,α-difluorosulfonamides.

1.1 — Introduction

1.1.1 — ß-Keto-α,α-difluorosulfonamides: an underexplored class of molecules

Sulfa drugs, introduced in the 1930's, were the first major class of antibiotics used clinically and were also the first major class of drugs bearing a sulfonamide group.[1] Since then, many compounds containing the sulfonamide group have been developed into clinically useful drugs to treat a variety of medical conditions such as bacterial and viral infections, cancer, glaucoma, inflammation and dandruff. These have been reviewed by Supuran et al. in 2003 and 2013 and by Gulçin and Taslimi in 2018.[2-5] The sulfonamide group is one of the most important pharmacophores in medicinal chemistry. There were 72 FDA-approved drugs bearing the sulfonamide group as of 2018.[6] The majority of these molecules were aryl sulfonamides; however, a few have been alkyl sulfonamides. For example, sumatriptan and naratriptan are used to treat migraines while the methane sulfonamides: dofetilide, ibutilide, and sotalol are all used as anti-arrhythmia agents.[3]

Of the approved sulfonamides, 12 contain one or more fluorine atoms but none of these are α to the sulfonamide moiety.[6] Nevertheless, sulfonamides bearing α-fluorines are known in the literature and many are biologically active (Figure 1.1). Examples include inhibitors of *Clostridium histolyticum* collagenase (ChC),[7] matrix metaloproteases (MMP),[8] steroid sulfatase (STS),[9] and carbonic anhydrase (CA).[10-12] It has been known since the 1970's that α-

fluorosulfonamides have herbicidal properties and the commercially available herbicide pyrimisulfan contains a difluoromethane sulfonamide moiety.[13,14] The N-H of primary sulfonamides become approximately 1.5 pK_a units more acidic with for each fluorine atom α-to the sulfonamide and a coincident increase in lipophilicity is observed.[15] This can have a profound impact on bioavailability and interactions with enzymes. The CF_2 moiety can also be viewed as a non-hydrolysable replacement for an oxygen atom with similar electronic properties.[16]

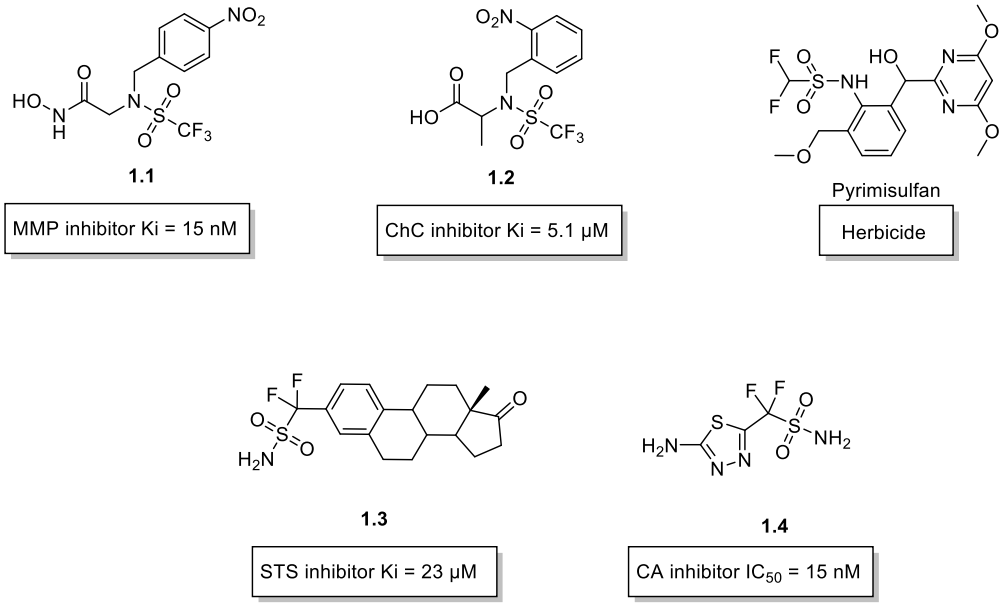
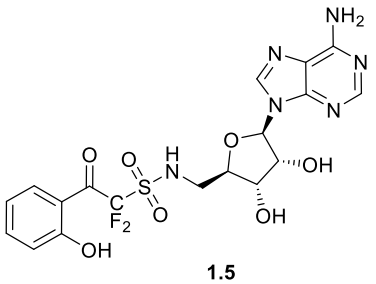


Figure 1.1. Structures of selected biologically active α-difluorosulfonamides.

There has been very little work done on ß-keto-α,α-difluorosulfonamides. Indeed, we are aware of only one report describing the synthesis of these molecules. This was the synthesis of sulfonamide **1.5** (Figure 1.2) by Vannada et al. in 2006 which is discussed below.[17] On the other hand, other closely related ß-keto-α-fluoro compounds are well represented in the literature, and in particular ß-keto-α,α-difluoroamides are common (*vide infra*).



1.5

Figure 1.2. Structure of ß-keto-α,α-difluorosulfonamide prepared by Vannada et al.

1.1.2 — α-Fluorocarbonyls as enzyme inhibitors

Carbonyl compounds bearing one, two, or three fluorine atoms adjacent to the carbonyl are known to be inhibitors of serine hydrolases. This is due in part to the tendency of these carbonyls to exist in a tetrahedral state. The tetrahedral state mimics the tetrahedral intermediate of amide hydrolysis this may be a hydrate or a hemiketal that is covalently bonded to the active site serine (Figure 1.3a). Some of the earliest examples of this are inhibitors of acetyl cholinesterase, which catalyzes the hydrolysis of the ester acetylcholine to acetate and choline (Figure 1.3b).[18,19] However, when the α-fluorocarbonyl **1.6** encounters the enzyme, hemiketal **1.7** is formed which is stabilized by the fluorine atoms and the reaction cannot proceed forward due to the lack of a suitable leaving group (Figure 1.3c).[18] Since this report, there have been many investigations into the use of fluorinated carbonyls as inhibitors of serine proteases and esterases, and while the specific structure will vary depending on the enzyme in question, the general mode of action is similar.

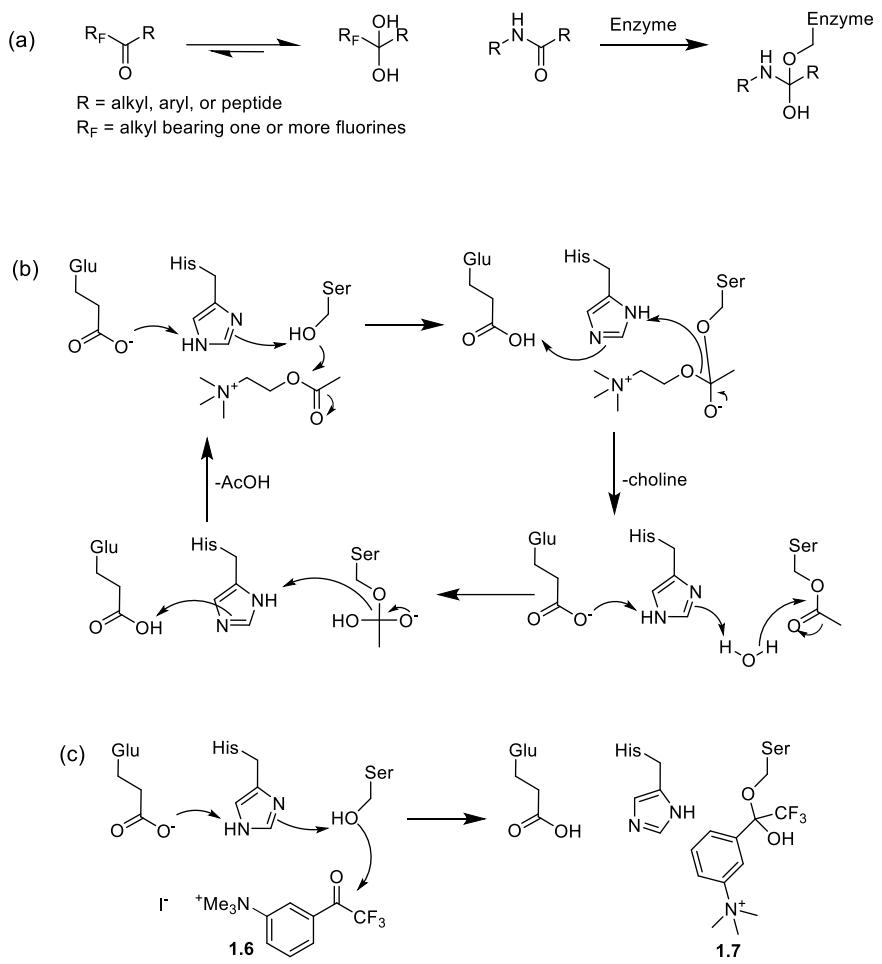


Figure 1.3. α-Fluorocarbonyls are transition state analog inhibitors of serine hydrolases. (a) Hydrated ketones resemble the tetrahedral intermediate of peptide hydrolysis; (b) Catalytic cycle of acetylcholinesterase; (c) The mechanism of an acetylcholinesterase inhibitor.

Many other α-fluorocarbonyls are also known enzyme inhibitors including ß-keto-α,α-difluoro esters,[20] and phosphonates.[21-25] ß-ester, and ß-amide α,α-difluorosulfonamides have also been reported,[11,26] as well as biologically active nonfluorinated ß-ketosulfonamides.[27] While there is only a single report on ß-keto-α,α-difluorosulfonamides, the most closely related class of molecules, the ß-keto-α,α-difluoroamides, are well studied. These include a number of peptidomimetics that are low or sub nanomolar inhibitors of important enzymes such as the aspartyl proteases pepsin[19] and renin,[28] as well as serine proteases such as chymase[29] and elastase (Figure 1.4).[30] The unfluorinated analogs are generally much less potent inhibitors. For example,

removal of the fluorines from amides **1.8** and **1.9** results in a 930- and 65-fold loss of potency, respectively.

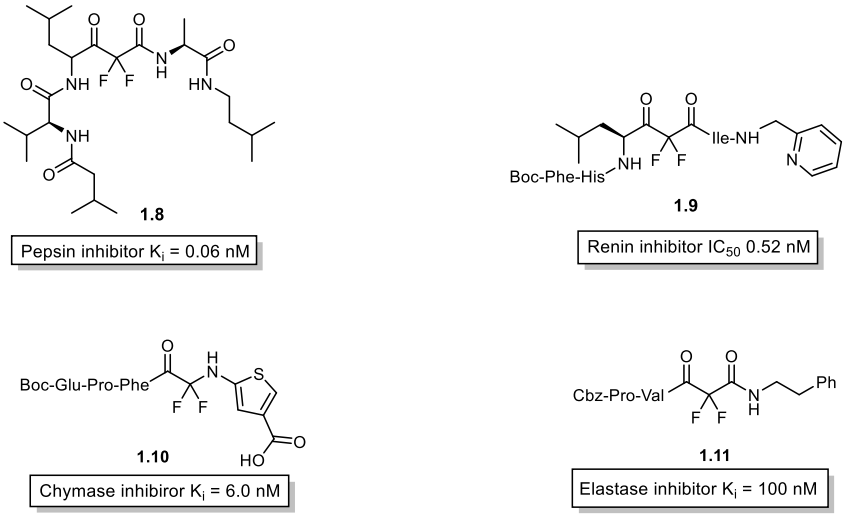
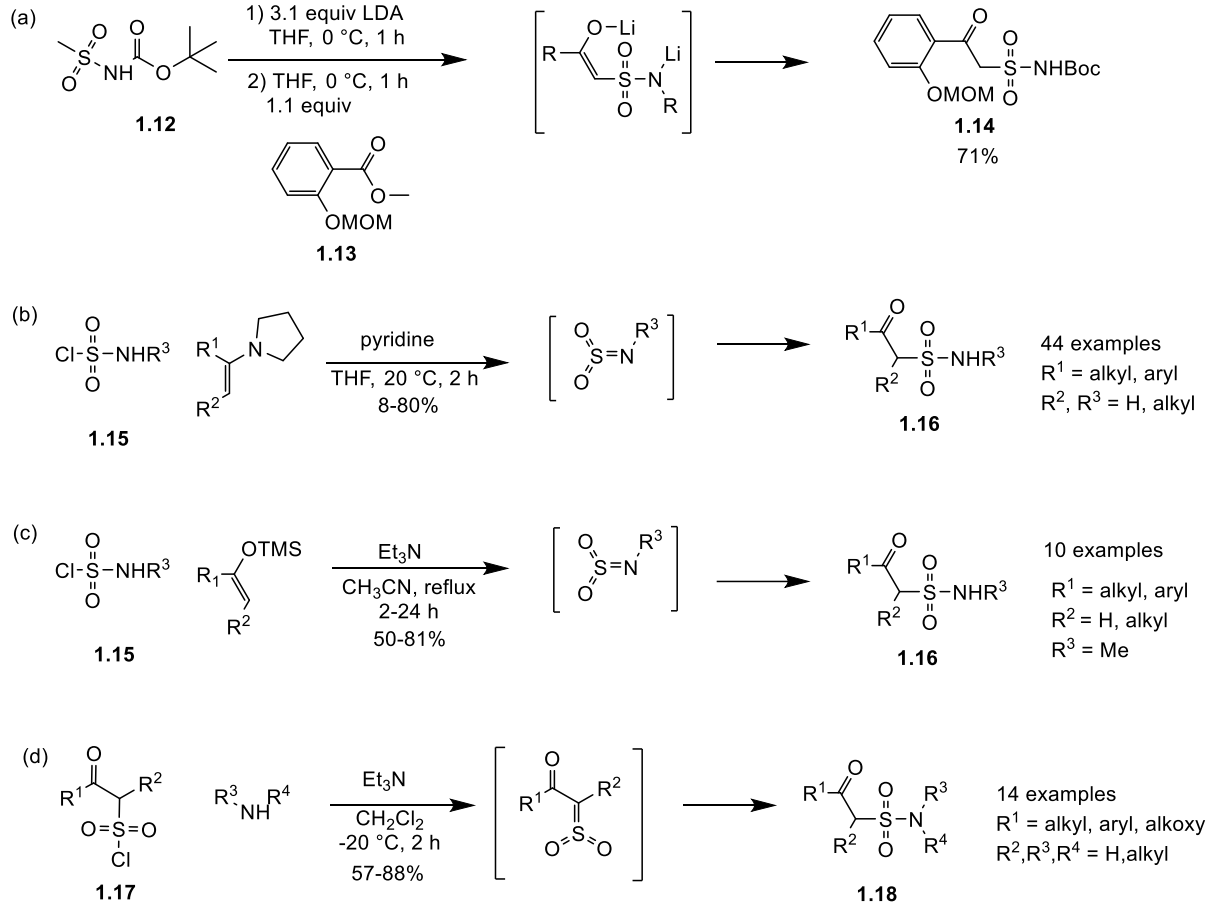


Figure 1.4. ß-Keto-α,α-difluoroamide containing enzyme inhibitors.

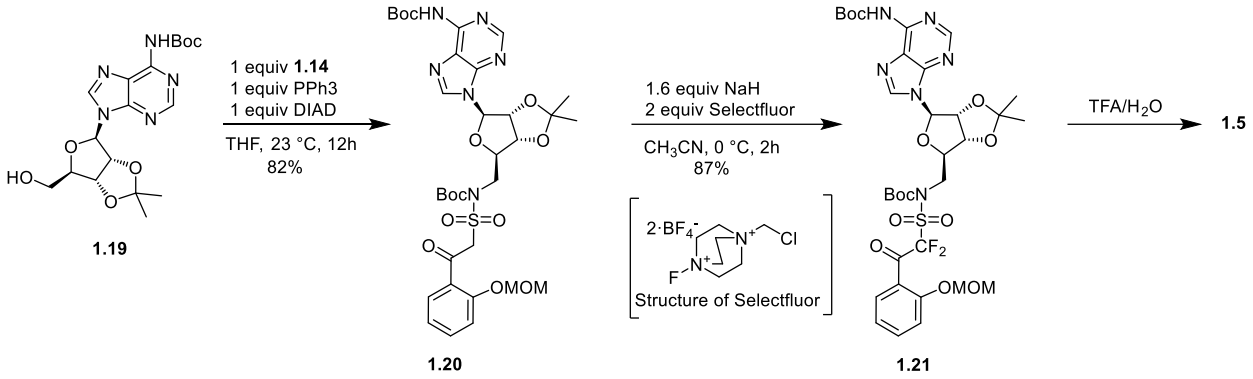
For the synthesis of ß-keto-α,α-difluorosulfonamide **1.5**, Vannada et al. first prepared the nonfluorinated ß-ketosulfonamide precursor **1.14** by reacting the dianion of *N*-Boc-protected methane sulfonamide **1.12** with methyl ester **1.13** (Scheme 1.1a).[17] The reaction required three equiv of base because both the methyl and sulfonamide *N*-H protons of **1.12** are acidic and the product, **1.14**, contains an additional acidic methylene proton which is lost to produce an enolate. The formation of this enolate prevents double addition. This dianion approach has also been used by others to prepare ß-ketosulfonamides.[27,31-33] Several other routes to ß-ketosulfonamides are also known (Scheme 1b-d). Sulfamyl chlorides, **1.15**, when in the presence of a base can react with enamines[34] or with TMS enol ethers[35] to give ß-ketones of type **1.16** after a hydrolytic workup (Scheme 1.1b and c). These approaches proceed through the in situ formation of a sulfonylimine (**1.16**) and are, therefore, limited to monoalkyl sulfonamides. ß-ketosulfonamides have also been prepared by reacting ß-ketosulfonyl chlorides **1.17** with primary or secondary

amines. This reaction presumably proceeds through a sulfene intermediate (Scheme 1.1d).[36,37] This approach allows access to tertiary sulfonamides (**1.18**).



Scheme 1.1. Literature methods for preparing ß-ketosulfonamides.

To obtain ß-keto-α,α-difluorosulfonamide **1.5**, Vannada et al. alkylated **1.14** under Mitsunobu conditions to give **1.20** which was subjected to electrophilic fluorination using Selectfluor and sodium hydride to give **1.21** (Scheme 1.2).[17] It is noteworthy that less than 2 equiv of base was used in the fluorination step because 1-(chloromethyl)-DABCO is produced as a reaction by product which can act as a base. Finally, deprotection with aqueous TFA yielded **1.5.**



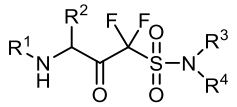
Scheme 1.2. Electrophilic fluorination of a ß-ketosulfonamide.

Selectfluor is one of many reagents that are available for electrophilic fluorination. The simplest reagent for this purpose is molecular fluorine but it is too hazardous and nonselective for most applications. All electrophilic fluorination reagents involve a weak fluorine bond, usually with nitrogen, but electrophilic fluorinating agents in which the fluorine is bonded to oxygen,[38] hypervalent iodine,[39] or xenon[40] are also known. Several groups have sought to quantify the fluorinating ability of N-F based electrophilic fluorinating agents.[41-43] Selectfluor is among the most powerful and practical of these and has been used extensively for the α-fluorination of carbonyl compounds including ß-ketophosphonates,[22-23] ß-ketosulfones,[44] ß-ketoesters,[45] ß-ketoamides,[45] and 1,3 dicarbonyls.[43] Selectfluor also has the advantage of being an easily handled non-hygroscopic solid which is relatively inexpensive, and the byproducts readily partition into the aqueous layer.

1.1.3 — Research objective

The primary objective of the research described in this chapter is to examine electrophilic fluorination as a means to prepare ß-keto-α,α-difluorosulfonamides of type **1.22** (Figure 1.5), which are derived from amino acids, with or without alkyl groups on the sulfonamide nitrogen. This includes developing an efficient synthesis of the unfluorinated ß-ketosulfonamides bearing

appropriate sulfonamide and N^{α} protecting groups and optimization of the electrophilic fluorination and deprotection conditions.



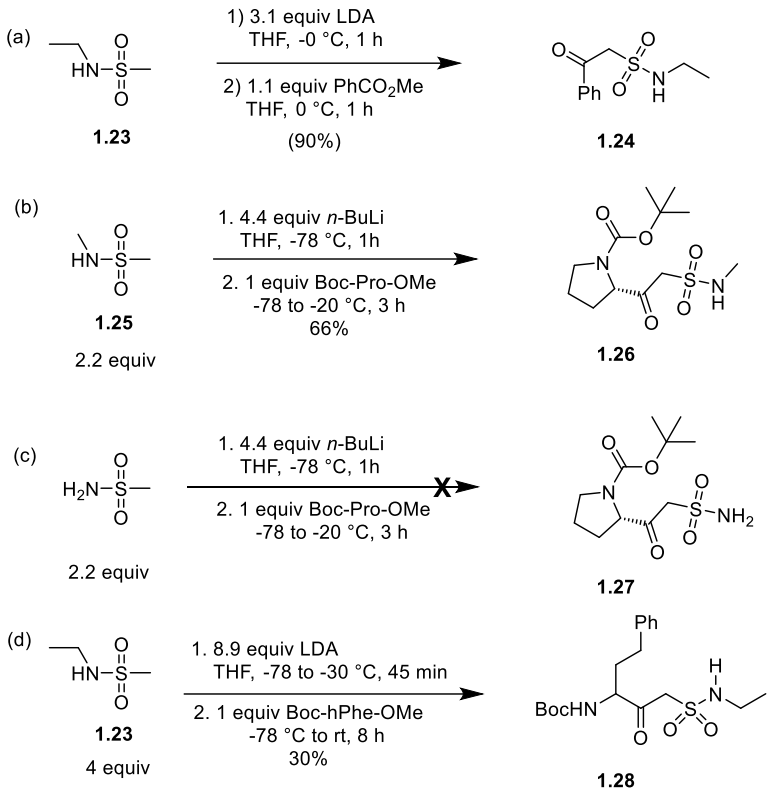
1.22

R^1 = H, Boc, Cbz or peptide
R^2 = amino acid side chain
R^3, R^4 = H, alkyl

Figure 1.5. General structure of ß-keto-α,α-difluorosulfonamides derived from amino acids.

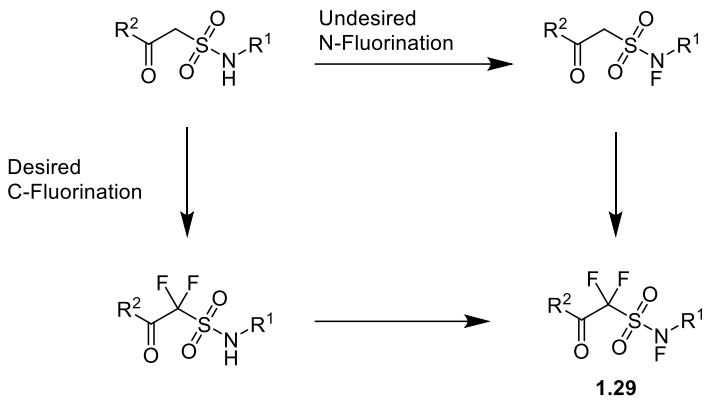
1.2 — Results and Discussion

Of the methods used to prepare ß-ketosulfonamides that were described in Scheme 1.1, the reaction with methyl esters (Scheme 1.1a) is the most appealing because methyl esters of *N*-protected amino acids are commercially available. Therefore, we began our studies using this approach and using methyl benzoate as a model ester. We were pleased to find that acylation of sulfonamide **1.23** with methyl benzoate using 3.1 equiv LDA occurred smoothly to give **1.24** and no over acylation at the sulfonamide nitrogen was observed (Scheme 1.3a). Under very similar conditions, the dianion derived from sulfonamide **1.25** reacted with Boc-Pro-OMe to give ß-ketone **1.26** in 66% yield (Scheme 1.3b). However, under the same conditions, methane sulfonamide, which contains no *N*-alkyl groups, did not produce the desired product **1.27** (Scheme 1.3c). Further difficulties were encountered if the amino acid included a carbamate proton. For example, using 3 equiv of the sulfonamide dianion, ß-ketone **1.28** was produced in very low yields from the reaction of Boc-homophenylalanine methyl ester and the dianion of **1.23**. Even when the amount of dianion was increased to 4 equiv only a 30% yield was obtained (Scheme 1.3d). These initial investigations were performed in part by Professor Scott Taylor.



Scheme 1.3. Initial investigations on the acylation of methane sulfonamide dianions.

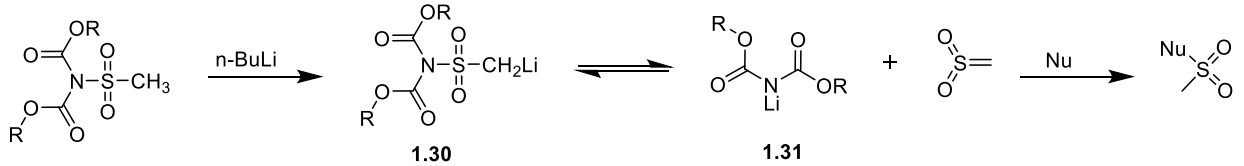
We anticipated that further difficulties might be encountered upon fluorination of these ketones because the sulfonamide nitrogen is prone to fluorination.[46] This might yield *N*-fluoro-ß-keto-α,α-difluorosulfonamides **1.29** (Scheme 1.4). Such compounds would be expected to have high reactivity, similar to the known *N*-fluoro-trifluoromethane sulfonamides.[47] We concluded that the sulfonamide nitrogen would need to be fully protected in order to obtain sulfonamides of type **1.22** in good yield.



Scheme 1.4. Potential undesired over-fluorination of an unprotected sulfonamide nitrogen.

1.2.1 — Selection of protecting groups

N-alkyl sulfonamides have previously been protected using common carbamate type protecting groups such as Boc, Cbz, Alloc,[48] and Fmoc.[49] Bis protection using common carbamate type protecting groups has been used occasionally;[50-53] however, sulfonamides protected in the way are highly labile. For example, both bis(Boc) sulfonamides and bis(Cbz) sulfonamides are labile to $Mg(ClO_4)_2$ or very low concentrations of TFA,[51] and the bis Boc sulfonamides could also be cleaved by amines.[52] Furthermore, we suspected that the anions derived from *N,N*-bis(alkoxycarbonyl)methanesulfonamides **1.30** might be prone to ß-elimination producing sulfene and anion **1.31.** For these reasons, we chose not to investigate carbamate protecting groups.



Scheme 1.5. E2 elimination of a biscarbamate protected sulfonamide.

Several alkyl groups have been used for the bis protection of the sulfonamide nitrogen. Among those are the allyl group which can be removed by a Tsuji Trost reaction,[9] the

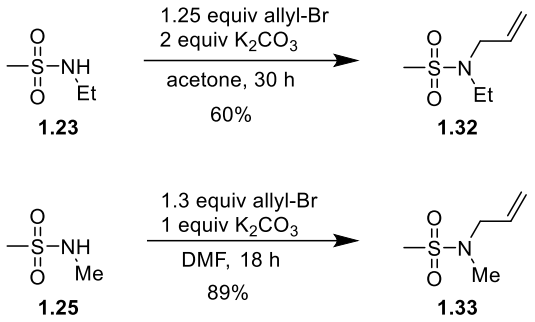
paramethoxybenzyl (PMB) group which is removed using high concentrations of TFA or by oxidation with CAN,[10] and the 2,4-dimethoxybenzyl (DMB) group which can be removed with lower concentrations of TFA.[9]

Additional alkyl groups used for bis sulfonamide protection include t-butyl,[10,54] prenyl,[55] and TMSE[56] groups, However, these have drawbacks. The TMSE group is removed by fluoride treatment which we expect to be incompatible with our fluorination conditions when TBAF or CsF is used as a base (*vide infra*). Prenyl groups are removed using very high temperatures and expensive silver salts, and *t*-butyl groups are removed under similar conditions to DMB groups, but bis-t-butyl is difficult to install. It is noteworthy that *N,N*-bis(benzyl)sulfonamides are readily prepared, but the benzyl group cannot be removed by the usual methods of hydrogenolysis or strong acids.[10]

The diphenylmethyl (DPM) group has also been used for sulfonamide protection and is removed by hydrogenolysis.[31] To the best of our knowledge this group has only been used for mono protection, possibly because *N,N*-bis(diphenylmethyl)sulfonamides would be too sterically demanding. However, we decided to pursue the DPM group because it is more acid stable than the DMB and PMB groups.

1.2.2 — Preparation of methane sulfonamides

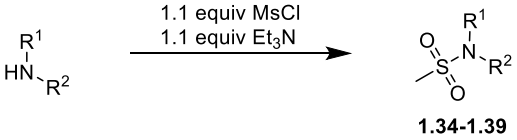
The first step was to prepare the *N,N*-dialkyl methanesulfonamides, which was accomplished in two ways. *N*-alkyl methane sulfonamides were prepared by allylation of the commercially available *N*-methyl or *N*-ethyl methanesulfonamides using allyl bromide to give **1.32** and **1.33** (Scheme 1.6). Sulfonamides **1.32** and **1.33** were prepared by Professor Scott Taylor and Edmond Chu, an undergraduate student in the Taylor group.



Scheme 1.6. Allyl protection of *N*-alkyl methane sulfonamides.

In the other approach, methanesulfonamides **1.34-1.39** were prepared by mesylation of the appropriate amine (Table 1.1). The yields were generally high and sulfonamides **1.33**, **1.34**, and **1.35** were purified by distillation, while **1.37**, **1.38**, and **1.39** were purified by crystallization, and sulfonamides **1.32** and **1.36** by chromatography. This provided us with methanesulfonamides which could be deprotected under reductive, oxidative, acidic, or palladium-catalyzed conditions.

Table 1.1. Preparation of methane sulfonamides by mesylation of amines.

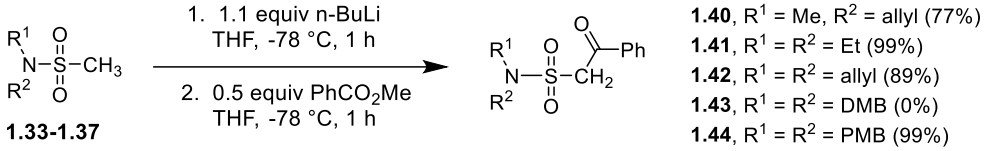


Entry	R^1	R^2	Solvent	Temperature	Time	Product	Yield (%)
1	Et	Et	THF	66 °C	2 h	**1.34**	89
2	Allyl	Allyl	THF	66 °C	2 h	**1.35**	90
3	DMB	DMB	THF	66 °C	2 h	**1.36**	81
4	PMB	PMB	DCM	0 °C	16 h	**1.37**	75
5	Ph_2CH	H	THF	-20 ° to rt	16 h	**1.38**	92
6	$-(CH_2)_4-$		DCM	0 °C	16 h	**1.39**	63

1.2.3 — Preparation of ß-ketosulfonamides derived from methyl benzoate

We selected methyl benzoate as a model ester since this molecule is inexpensive and non-enolizable. The reaction was performed by first generating the carbanion of sulfonamide **1.33-1.37** using *n*-BuLi at low temperatures followed by addition of methyl benzoate (Scheme 1.7). Two equiv of the carbanion relative to the ester were required because the methylene protons of

the product ß-ketone are acidic. With the exception of **1.43**, the reaction proceeded in high yield; however, the methanesulfonamide starting materials were often difficult to separate from the product which made the preparation of these ketones on a multigram scale labor intensive.



Scheme 1.7. Acylation of methane sulfonamides using methyl benzoate.

Theoretically, it should be possible to use equimolar quantities of the methanesulfonamides and methyl benzoate while using a two-fold excess of the base. Unfortunately, these attempts were not successful using *n*-BuLi or LDA.

We were surprised to find that the reaction was unsuccessful using the DMB-protected **1.36**. We initially suspected that the desired anion was not being formed on treatment with *n*-BuLi. We performed a control experiment in which the sulfonamide was treated with *n*-BuLi for 30 min at -78 °C and then quenched with D_2O (Figure 1.6). Complete deuterium exchange was observed as determined by examination of the ^1H NMR of the crude reaction mixture; the singlet corresponding to the methyl group at 2.7 ppm was slightly broadened and integrated to 2 protons.

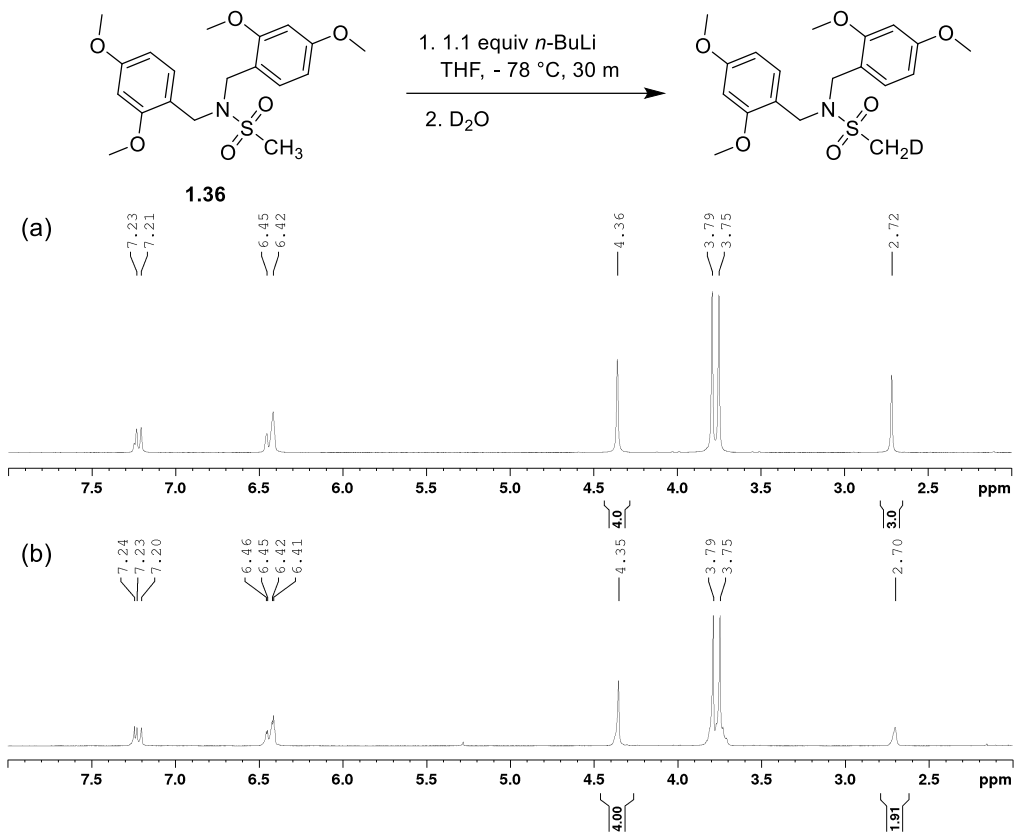
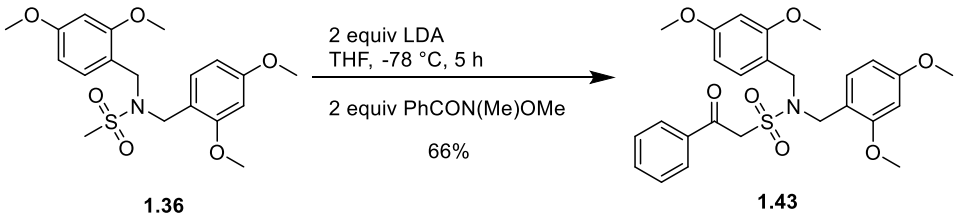


Figure 1.6. Deuterium exchange experiment with sulfonamide **1.36**. (a) ^{1}H NMR spectrum of **1.36**; (b) ^{1}H NMR spectrum of the crude product. The peak at 2.7 ppm corresponds to the SCH_3 or SCH_2D group which undergoes partial deuterium exchange. The peak at 4.35 ppm corresponds to the four benzylic protons.

The acylation of **1.36** was successful using the Weinreb amide of benzoic acid as shown in Scheme 1.8, where ketone **1.43** was obtained in 66% yield. It is unclear what role the amide plays in the reaction which makes it a more suitable substrate than an ester.

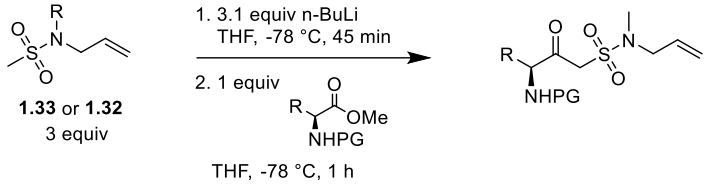


Scheme 1.8. Acylation of a methane sulfonamide **1.36** with methylmethoxy benzamide.

1.2.4 — Preparation of ß-ketosulfonamides derived from amino acids

Next, we turned our attention to ß-ketosulfonamides derived from amino acids. We selected the allyl-protected **1.33** as our model sulfonamide and chose a selection of amino acid methyl esters protected with either Boc or Cbz groups. We employed the same conditions as those used in Scheme 1.7 except an extra equivalent of the sulfonamide carbanion was required for substrates with an acidic carbamate proton (Table 1.2). The ketones in Table 1.2 were prepared in collaboration with Edmond Chiu and Professor Scott Taylor.

Table 1.2. ß-Ketosulfonamides from *N*-protected α-amino acids methyl esters.



Entry	Sulfonamide	R	Amino acid ester	Product	Yield (%)	er
1	**1.33**	Me	Boc-Val-OMe	**1.45**	97	>99:1
2	**1.33**	Me	Boc-Phe-OMe	**1.46**	93	ND
3	**1.33**	Me	Boc-Ala-OMe	**1.47**	84	>99:1
4	**1.33**	Me	Boc-Met-OMe	**1.48**	74	96:4
5	**1.33**	Me	Cbz-Val-OMe	**1.49**	65	>99:1
6	**1.33**	Me	Cbz-Ile-OMe	**1.50**	71	ND
7	**1.33**	Me	Cbz-Leu-OMe	**1.51**	83	ND
8	**1.33**	Me	Cbz-Tyr(tBu)-OMe	**1.52**	68	ND
9[a]	**1.33**	Me	Boc-Pro-OMe	**1.53**	95	ND
10	**1.32**	Et	Boc-hPhe-OMe	**1.54**	92	>99:1

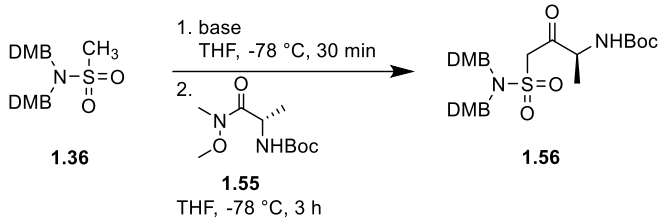
[a]2 equiv of BuLi and 2 equiv of **1.33** were used.

ß-Keto-sulfonamides **1.45-1.54** were obtained in good to excellent yields. For **1.45**, **1.47**, **1.48**, **1.49**, and **1.54** the enantiomeric ratio (er) was determined by preparing their enantiomers using the D-amino acid esters. The enantiomers were separable by chiral HPLC and in most cases the enantiomer was not detected with the exception of **1.48**, in which 4% of the enantiomer

was detected. The ee determinations were performed by Ryan Chung and Professor Jason E. Hein at the University of British Colombia.

Next, we examined DMB-protected methanesulfonamide **1.36** as a substrate for the reaction with amino acids. Unfortunately, **1.36** did not react with the methyl ester of Boc alanine but did react with the analogous Weinreb amide **1.55** (Table 1.3). It is well known that Weinreb amides react with carbon nucleophiles to give ketones because the tetrahedral intermediate does not break down at low temperatures.[57] In principle, the reaction should proceed to completion using 2 equiv of sulfonamide and *n*-BuLi (entry 2) or using 1 equiv of sulfonamide and 2 equiv of LDA (entry 6). To our surprise, three equiv of **1.36** and *n*-BuLi were required for full conversion to ketone **1.56** by TLC (entry 3) suggesting that, under these conditions, the tetrahedral intermediate is not well stabilized and decomposes to give **1.56**. Equimolar amounts of **1.36** and **1.55** with 3 equiv of LDA also gave **1.56** in moderate yield (entry 7). It is unclear what role the Weinreb amide functional group plays that makes it a more suitable substrate than a methyl ester.

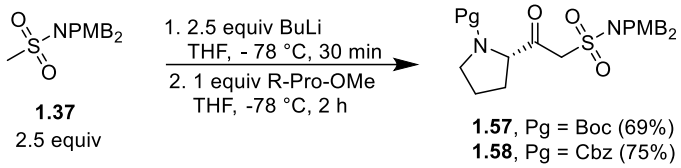
Table 1.3. Optimization of the synthesis of ketone **1.56**.



Entry	**1.36** (equiv)	Base	equiv	Yield
1	1.0	*n*-BuLi	1.0	4%[a]
2	2.0	*n*-BuLi	2.0	47%[a]
3	3.0	*n*-BuLi	3.0	92%[a](87%[b])
4	4.0	*n*-BuLi	4.0	97%[a]
5	1.0	LDA	1.0	6%[a]
6	1.0	LDA	2.0	18%[a]
7	1.0	LDA	3.0	67%[a] (64%[b])
8	1.0	LDA	4.0	59%[a]

[a] Yield was determined by HPLC. [b] Isolated Yield

Boc- and Cbz-protected ketones **1.57** and **1.58** were prepared in good yield from PMB-protected sulfonamide **1.37** and Boc- or Cbz-protected proline methyl ester (Scheme 1.9). As these proline substrates lack a carbamate proton, the reaction required less than three equiv of sulfonamide anion.

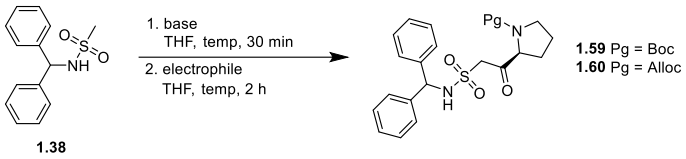


Scheme 1.9. Preparation of PMB-protected ß-ketosulfonamides derived from proline.

The DPM-protected sulfonamide **1.38** has an sulfonamide proton and so will react as a dianion; therefore, based upon the reactions of mono-alkyl sulfonamides **1.23** and **1.25** (Scheme 1.3), we expected that good yields would only be achieved with substrates lacking a carbamate proton such as those derived from proline. We optimized this reaction using Boc-Pro-OMe as the

electrophile (Table 1.4). When we applied our normal conditions (entry 1), none of the desired **1.59** was isolated. A modest yield was obtained by increasing the relative concentration of the electrophile (entry 2) and a slightly better yield was obtained when LDA was used instead of *n*-BuLi. In a literature example, sulfonamide **1.38** gave satisfactory results when reacted with a Weinreb amide if the anion was generated using *n*-BuLi-LiCl complex at -40 °C.[31] The influence of lithium chloride on the outcome of organometallic reactions is well documented, and may be related to the ability of LiCl to catalyze degradation of oligomers.[58] These conditions improved the yield of **1.59** (entry 5). Using LiBr, which is less hydroscopic and more soluble in THF than LiCl, resulted in a reduced yield (entry 6). The reaction also proceeded well when anhydrous lithium chloride was generated in situ by including an extra equivalent of *n*-BuLi and anhydrous HCl (entry 7). The increase in temperature, from -78 °C to -40 °C, was necessary to prevent precipitation of LiCl. It was not necessary to use a Weinreb amide, and proline methyl esters also gave **1.59** and **1.60** in satisfactory yields when the reaction was conducted at – 40 °C in the presence of LiCl (entries 8 and 9).

Table 1.4. Synthesis of ß-ketones from **1.38**.

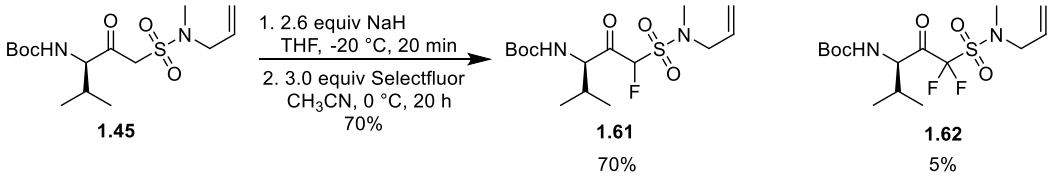


Entry	**1.38** equiv	Base (equiv)	Temp (°C)	Additive (equiv)	Electrophile (equiv)	Product	Yield
1	2.1	*n*-BuLi (4.2)	-78	-	Boc-Pro-OMe (1)	**1.59**	ND[a]
2	1	*n*-BuLi (2)	-78	-	Boc-Pro-OMe (1)	**1.59**	30%
3	2.1	LDA (4.2)	-78	-	Boc-Pro-OMe (1)	**1.59**	40%
4	1	*n*-BuLi (2)	-100	-	Boc-Pro-OMe (1)	**1.59**	15%
5	2.5	*n*-BuLi (5)	-40	LiCl (2.5)	Boc-Pro-N(Me)OMe (1)	**1.59**	83%
6	2.5	*n*-BuLi (5)	-40	LiBr (2.5)	Boc-Pro-N(Me)OMe (1)	**1.59**	36%
7	2.5	*n*-BuLi (7.5)	-40	HCl (2.5)	Boc-Pro-N(Me)OMe (1)	**1.59**	85%
8	2.5	*n*-BuLi (7.5)	-40	HCl (2.5)	Boc-Pro-OMe (1)	**1.59**	80%
9	2.5	*n*-BuLi (7.5)	-40	HCl (2.5)	Alloc-Pro-OMe (1)	**1.60**	66%

[a]A complex mixture was obtained.

1.2.5 — Electrophilic fluorination of ß-ketosulfonamides

We first attempted a fluorination reaction using the conditions of Vannada et al. (Scheme 1.2), which involved quantitative generation of an enolate using sodium hydride followed by cannulation into a solution of Selectfluor (Scheme 1.10). When we applied these conditions to ß-ketone **1.45**, very little reaction occurred. When the base was increased to 2.6 equiv and Selectfluor to 3 equiv, then a 70% yield of monofluorinated **1.61** was obtained but the desired **1.62** was observed in only trace amounts, even after stirring overnight.



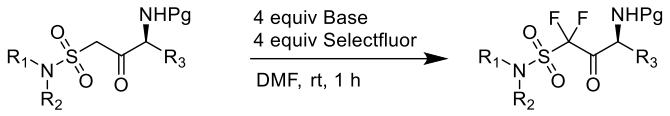
Scheme 1.10. Fluorination of ß-ketone **1.45** according to literature conditions.

We did not want to further increase the amount of sodium hydride for fear of epimerizing the α-carbon. We expected the acidity of ß-ketosulfonamides such as **1.45** to be similar to that of

ß-ketoesters (pK_a =10-12).[59] Therefore, we examined weaker bases CsF, Cs_2CO_3, DBU, and TBAF, using MeOH, CH_3CN, and DMF as solvents and the reactions were evaluated by TLC and ^{19}F NMR. Using DBU, the reactions were sluggish and incomplete after 24 h in all cases, Cs_2CO_3 in DMF or CH_3CN gave rapid difluorination within 15 min albeit with a significant amount of minor unidentified byproducts. Using MeOH as the solvent led to a cleaner reaction with Cs_2CO_3 within 1 h, while using TBAF or CsF in DMF even fewer side products; however, to drive the reaction to completion four equiv of Selectfluor and base were required. Isolated yields of **1.62** were 84% using CsF and 78% using TBAF (Table 1.5). These conditions were applied to several other ß-ketones and the difluorinated products were obtained in reasonable to excellent yield with the exception of methionine derivitive **1.65**. The sulfide moiety of **1.65** would be expected to react with Selectfluor giving α-fluorination through the intermediacy of a fluorosulfonium ion.[60,61] Similar yields were obtained using either CsF or TBAF (compare entries 1 and 2 and entries 15 and 16). Secondary sulfonamides were also tolerated, although with DPM the yields were lower (entries 15 to 18). None of the *N*-Fluorinated products of type **1.29** were observed from these reactions, either because it does not occur or because the nitrogen fluorine bond is hydrolyzed on workup.

The er values of **1.62** and **1.66** were determined by preparing the enantiomers of these amino acid derivitives, which were separable by chiral HPLC and the undesired isomer was not detected. The compounds in Table 1.5 were prepared in collaboration with Edmond Chiu and Professor Scott Taylor and the enantiomers were separated by chiral HPLC by Ryan Chung and Professor Jason E. Hein at the University of British Colombia.

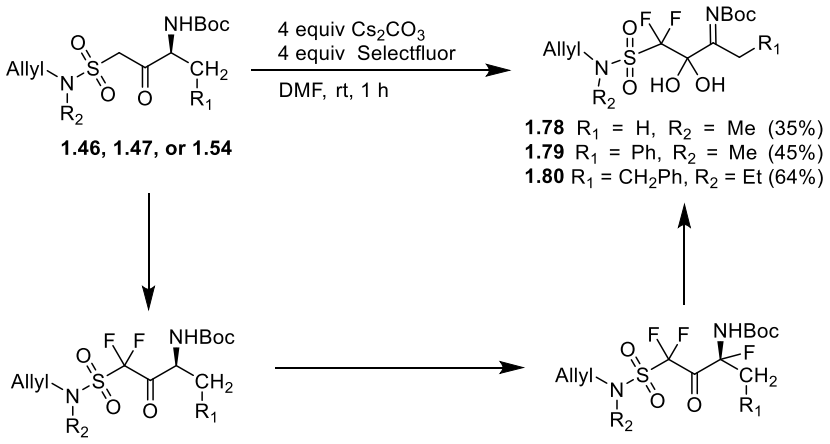
Table 1.5. Fluorination of ß-ketosulfonamides using Selectfluor and fluoride bases.



Entry	Substrate	Pg	R1	R2	Amino acid side chain	Base	Product	Yield	er
1	**1.45**	Boc	Me	Allyl	Val	CsF	**1.62**	84%	>99:1
2	**1.45**	Boc	Me	Allyl	Val	TBAF	**1.62**	78%	
3	**1.46**	Boc	Me	Allyl	Phe	CsF	**1.63**	84%	
4	**1.47**	Boc	Me	Allyl	Ala	CsF	**1.64**	76%	
5	**1.48**	Boc	Me	Allyl	Met	CsF	**1.65**	0	
6	**1.49**	Cbz	Me	Allyl	Val	CsF	**1.66**	93%	>99:1
7	**1.50**	Cbz	Me	Allyl	Ile	CsF	**1.67**	90%	
8	**1.51**	Cbz	Me	Allyl	Leu	CsF	**1.68**	82%	
9	**1.52**	Cbz	Me	Allyl	Tyr(OtBu)	CsF	**1.69**	74%	
10	**1.53**	Boc	Me	Allyl	Pro	CsF	**1.70**	69%	
11	**1.54**	Boc	Et	Allyl	hPhe	CsF	**1.71**	85%	
12	**1.56**	Boc	DMB	DMB	Ala	TBAF	**1.72**	64%	
13	**1.57**	Boc	PMB	PMB	Pro	CsF	**1.73**	87%	
14	**1.58**	Cbz	PMB	PMB	Pro	CsF	**1.74**	89%	
15	**1.28**	Boc	Et	H	hPhe	CsF	**1.75**	68%	
16	**1.28**	Boc	Et	H	hPhe	TBAF	**1.75**	74%	
17	**1.59**	Boc	DPM	H	Pro	TBAF	**1.76**	50%	
18	**1.60**	Alloc	DPM	H	Pro	CsF	**1.77**	47%	

In some cases side reactions occurred which ultimately led to oxidation of the α-amino ketones to fluorinated 1,2-iminoketones.[61] This occurred in some cases if Cs_2CO_3 was used as the base as shown in Scheme 1.11. The most likely mechanism involves difluorination of the methylene group which increases the acidity of the α-proton. Enolization at the α-carbon by Cs_2CO_3 leads to fluorination at the α-position. Finally, an E2 elimination by deprotonation of the amine yields the imine. It is also possible that fluorination occur at the amino acid nitrogen followed be loss of the α proton and ß-elimination. This occurred with **1.46**, **1.47**, and **1.54** where the side chain was derived from Phe, Ala, or hPhe but not with compounds **1.45**, **1.49**,

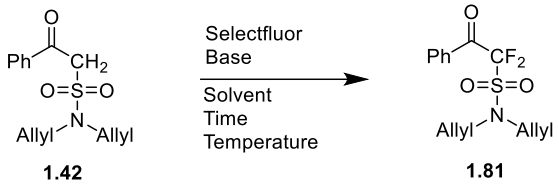
1.50, or **1.51** where the side chain side chain was derived from Val, Ile and Leu, presumably because the α-carbon of the latter amino acid derivitives is more hindered and does not quickly react with Selectfluor. Ketones **1.78-1.80** all existed in the hydrated state (as determined by $^{13}C\{^{1}H\}$ NMR) even in $CDCl_3$. In contrast to **1.62-1.77 which** all existed as ketones. The compounds in Scheme 1.11 were prepared by Professor Scott Taylor.



Scheme 1.11. Fluorination in the in the presence of Cs_2CO_3 leading to oxidation.

When we attempted the fluorination of **1.42** we found that the conditions used in Table 1.5 gave incomplete fluorination and a lower yield of **1.81** (Table 1.6, entry 1). We screened several bases and solvents, and the highest yield was achieved by using 4 equivalent each of TBAF and Selectfluor in DMF and by reducing the temperature to -10 °C (entry 15). This resulted in complete conversion to the difluorinated ketone with no side products detectable in the ^{19}F NMR of the crude reaction mixture. The reaction should require just 2 equiv of Selectfluor and base, unfortunately, lower yields were obtained with less than 4 equiv (entries 13 and 14).

Table 1.6. Optimization of the fluorination conditions for **1.42.**

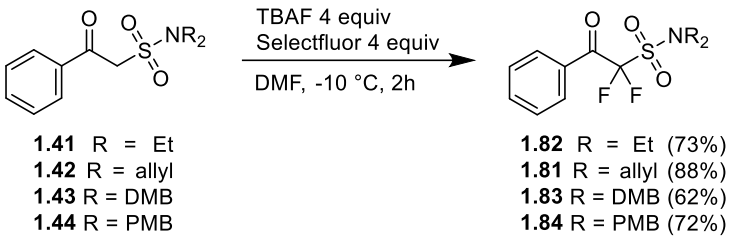


1.42 **1.81**

Entry	Base (equiv)	Selectfluor equiv	Solvent	Temp	Time	Yield(%)[a]
1	CsF (4)	4	DMF	rt	3 h	57[b,c]
2	TBAF (4)	4	DMF	rt	1 h	70
3	TBAF (4)	4	MeOH	rt	6 h	11
4	Cs_2CO_3 (4)	4	MeOH	rt	6 h	50
6	NaOMe (4)	4	MeOH	rt	6 h	10
7	TBAF (4)	4	CH_3CN	rt	8 h	46
8	TBAF(4)	4	Dioxane	rt	8 h	0
9	TBAF (4)	4	Water	rt	8 h	0
10	TBAF (4)	4	THF	rt	8 h	0[d]
11	TBAF (4)	4	DMSO	rt	8 h	0[d]
12	TBAF (4)	4	DMF	60 °C	1 h	40
13	TBAF (2)	2	DMF	-10 °C	2 h	50[c]
14	TBAF (3)	3	DMF	-10 °C	2 h	80[c]
15	TBAF (4)	4	DMF	-10 °C	2 h	100 (88[c])

[a]Yield determined by ^{1}H NMR using a 3-fluorotoluene internal standard.
[b]27% of monofluorinated material was also isolated.
[c]Isolated Yield.
[d]Some monofluorinated product was obtained.

The optimized conditions were also applicable to the other benzoyl methanesulfonamides (Scheme 1.12), although the yields were somewhat lower. Unlike most of the ß-keto-α,α-difluoromethanesulfonamides, DMB protected sulfonamide **1.83** appeared to form a hemiketal in methanol which was observed as a set of AB doublets in fluorine NMR. This persisted after being concentrated by rotary evaporation and redissolved in $CDCl_3$ twice, but was completely converted to the ketone after 16 h under vacuum.

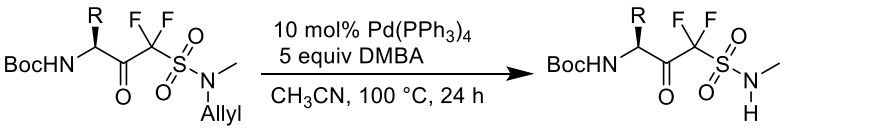


Scheme 1.12. Fluorination of ß-ketosulfonamides derived from methyl benzoate.

1.2.6 — Sulfonamide deprotection

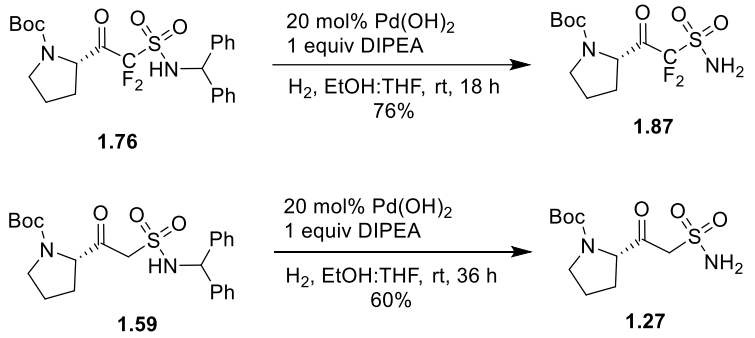
With the protected ß-ketosulfonamides in hand we set about investigating the conditions for deprotection. All the protecting groups that we have employed here have been used previously for sulfonamide protection, but none specifically for protection of ß-keto-α,α-difluorosulfonamides.

Deallylation of sulfonamides **1.62**, and **1.63** could be achieved using $Pd(PPh_3)_4$ and DMBA to give the secondary sulfonamides of **1.85** and **1.86**. Although the yields were good, the reaction required 24 h at 100 °C. This led to significant epimerization and the er of **1.85** was only 3:2 (Scheme 1.13). Once again this was determined by comparing the chiral HPLC trace of **1.85** and its enantiomer. Other deallylation methods for sulfonamides are known which proceed at room temperature. One approach uses DIBAL in the presence of a nickel catalyst, however this would also reduce the ketone group.[62,63] A metal-free method using ultraviolet light has been reported, but this can also cleave nitrogen sulfur bonds.[64] We did not pursue these methods. The separation of enantiomers was performed by Ryan Chung and Professor Jason E. Hein at the University of British Colombia. The deallylation reaction was performed by Professor Scott Taylor.



Scheme 1.13. Deprotection of allyl protected sulfonamides.

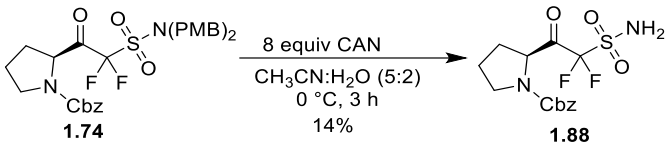
Removal of the diphenylmethyl group in **1.59** and **1.76** was accomplished via hydrogenation with Pearlman's catalyst and Hünig's base in ethanol-THF (Scheme 1.14).[31] The DPM group in **1.59** was removed more slowly than **1.76**, and after 36 h 60% of **1.27** was isolated and 14% of **1.59** was recovered.



Scheme 1.14. Hydrogenolysis of diphenylmethane protecting groups.

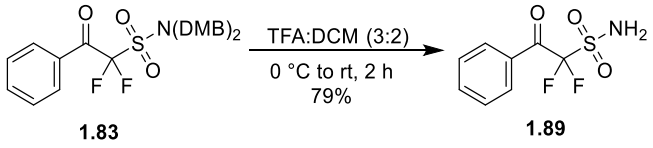
PMB groups are typically removed from sulfonamides using TFA; however, Blackburn observed that TFA-mediated PMB deprotection of sulfonamides was slower and lower yielding when one or two fluorines were added to the α-position.[10] This is due to the low basicity of the sulfonamide nitrogen, and we expect the situation to be even worse with ß-keto-α,α-difluoromethanesulfonamides due to the additional electron withdrawing ketone moiety. The same study also used CAN for PMB deprotection. When we employed this reagent for the deprotection of sulfonamide **1.74** a number of unidentified side products were observed and the desired sulfonamide **1.89** was obtained in a very poor yield (Scheme 1.15). This appears to be

due to the presence of the ketone since these deprotection conditions were effective on the ß-hydroxy-α,α-difluoromethanesulfonamides discussed in Chapter 2.



Scheme 1.15. Oxidative deprotection of sulfonamide **1.74**.

DMB protecting groups have previously been removed in good yield from α-fluorinated sulfonamides using TFA.[9] These conditions were also effective for sulfonamide **1.83** giving benzoyl difluoromethanesulfonamide **1.89** in 79% yield (Scheme 1.16).



Scheme 1.16. Acid-mediated deprotection of a DMB protected sulfonamide.

1.3 — Conclusions and future studies

In the project described in this chapter, a series of ß-keto-α,α-difluorosulfonamides were prepared by the reaction of the lithiated methane sulfonamide with the methyl ester of an amino acid or methyl benzoate. The previously reported literature protocol for this transformation employed the dianion of *N*-alkyl methane sulfonamides, however, this reaction gives poor results with amino acid methyl esters that contain a carbamate proton. We have shown that the monoanion of *N,N*-dialkyl methanesulfonamides is effective with these methyl esters giving our method a superior substrate scope over the literature method.

We prepared ß-ketosulfonamides containing a variety of *N*-alkyl groups including allyl, ethyl, PMB, DMB and DPM groups. The reaction with methyl esters proceeded smoothly with the first three derivatives, but challenges were encountered using the DMB protected **1.36** and

DPM protected **1.38.** These were overcome by reacting **1.36** with Weinreb amides rather than methyl esters (Table 1.3). It remains unclear what role the Weinreb amide plays in this reaction that makes it a more suitable substrate. To shed light on this reaction it may be useful to investigate the acylation of **1.36** with other ester derivatives such as anhydrides, acyl chlorides, or Pfp esters.

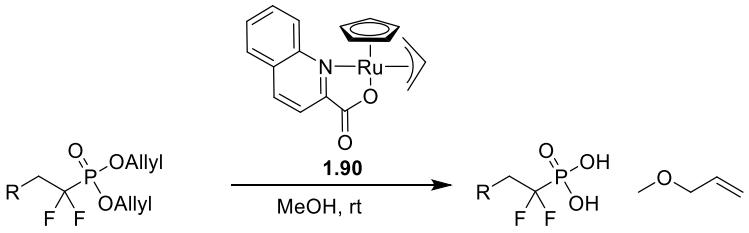
The DPM protected **1.38** reacts as a dianion; therefore, we only investigated the reaction of this compound with proline derivatives since these lack a carbamate proton. Still, difficulties were encountered (Table 1.4), and this reaction only gave satisfactory yields with methyl esters in the presence of lithium chloride. Again, it may be useful to employ more powerful acylating agents such as anhydrides, acyl chlorides, or Pfp esters to expand the substrate scope using this protecting group which may allow amino acids other than proline to be employed.

Conditions for electrophilic fluorination were optimized which avoid over fluorination or oxidation at the amino acid α-carbon. These conditions were effective using *N,N*-dialkyl sulfonamides, as well as the mono *N*-alkyl sulfonamides **1.28**, **1.59**, and **1.60** which reacted smoothly without noticeable nitrogen fluorination. It may be interesting to investigate these conditions using primary sulfonamides, such as **1.87**, which may or may not undergo nitrogen fluorination. The fluorination failed using a methionine derivative, presumably due to the reaction between the sulfide moiety and Selectfluor. It should be possible to carry out this transformation by first protecting the sulfide as a sulfoxide, however this was not attempted.

Deprotection of the ß-keto-α,α-difluorosulfonamides was investigated with all four protecting groups. Difficulties were encountered with PMB deprotection and unknown side reactions occurred. DMB and DPM groups were both removed cleanly by treatment with acid or hydrogenolysis respectively, the effectiveness of these transformations indicates that future

studies with these ß-ketones should focus on these protecting groups. This underscores the potential value in investigating more powerful acylating agents in order to broaden the substrate scope.

Allyl deprotection could be achieved by a Tsuji–Trost reaction using a palladium catalyst and DMBA as an allyl scavenger. However, the reaction required elevated temperatures and resulted in some degree of epimerization. Recently, Panigrahi et al., have published the deprotection of allyl groups from α-difluoro phosphonates under extremely mild conditions using ruthenium catalyst **1.90** (Scheme 1.17).[65] If these conditions are also effective for the deprotection of ß-keto-α,α-difluorosuolfonamides, then the allyl group would be superior to DMB and DPM in terms of atom economy and ease of removal.



Scheme 1.17. Mild ruthenium catalyzed Deallylation by the Berkowitz group.

1.4 — Experimental

1.4.1 — General experimental

All reagents and solvents were purchased from commercial suppliers and used without purification unless stated otherwise.

DMF was distilled from CaH_2 under reduced pressure and stored over activated 4 Å molecular sieves. THF was distilled from sodium metal in the presence of benzophenone under nitrogen. Acetonitrile was distilled from CaH_2 under nitrogen. Diisopropylamine was distilled from sodium metal. Methanol was distilled from CaH_2 and stored over activated 4 Å molecular

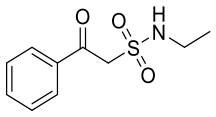
sieves. All $^{13}C\{^{1}H\}$ NMR spectra were proton decoupled. Chemical shifts (δ) for ^{1}H NMR spectra run in $CDCl_3$ are reported in ppm relative to the standard tetramethylsilane (TMS). Chemical shifts for $^{13}C\{^{1}H\}$ NMR spectra run in $CDCl_3$ are reported in ppm relative to the solvent residual carbon (δ 77.0 for central peak). Chemical shifts (δ) for ^{19}F NMR spectra run in $CDCl_3$ or DMSO-d_6 are reported in ppm relative to $CFCl_3$ (δ 0.0, external standard). Chemical shifts (δ) for ^{1}H NMR spectra run in or DMSO-d_6/D_2O mixtures are reported in ppm relative to DMSO residual solvent protons (δ 2.5). Chemical shifts for $^{13}C\{^{1}H\}$ NMR spectra run in DMSO-d_6 or DMSO-d_6/D_2O mixtures are reported in ppm relative to the solvent residual carbon (δ 39.5). The samples for high-resolution positive ion electrospray ionization mass spectrometry (HRMS-ESI^+) (ion trap) were prepared in 1:1 MeOH/ H_2O + 0.2% formic acid.

Chiral HPLC analyses were performed using a Chiralpak AS-RH column (250 × 4.6 mm) with CH_3CN/0.1% TFA in H_2O as eluent.

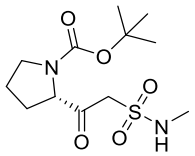
Methyl esters of the protected amino acids were either purchased or prepared via reaction of the corresponding Boc or Cbz protected amino acids with $SOCl_2$ in dry MeOH.[66] Weinreb amides were prepared by coupling the corresponding carboxylic acid with N,O-dimethoxyhydroxylamine according to standard procedures.[57] Bis(2,4-dimthoxybenzyl)amine was prepared according to the method of Reuillon.[67]

Unless otherwise specified all reactions were performed in flasks that were flame dried under a stream of argon and the argon atmosphere was maintained throughout.

1.4.2 — Experimental procedures for synthesized compounds

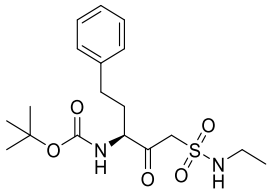


N-Ethyl-2-oxo-2-phenylethane-1-sulfonamide (**1.24**). Dry diisopropylamine (0.770 mL, 5.50 mmol, 3.3 equiv) was added to THF (6 mL) then cooled to 0 °C. *n*-BuLi (1.6 M in hexane, 3.25 mL, 5.20 mmol, 3.1 equiv) was added and the mixture stirred at 0 °C for 30 min. A solution of sulfonamide **1.23** (0.200 g, 1.67 mmol, 1.0 equiv) in THF (6 mL) was added and the mixture was stirred for 1 h at 0 °C. A solution of methyl benzoate (0.231 mL, 1.84 mmol, 1.1 equiv) in THF (2 mL) was added and the mixture stirred for 1 h at 0 °C. The reaction was quenched with sat. aqueous NH_4Cl and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo to give a pale yellow solid. Purification using flash chromatography, 75% EtOAc, 25% hexane, provided ketone **1.24** as an amorphous white solid (0.340 g, 90% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.96 (d, 2H, J = 8.3 Hz), 7.62 (t, 1H, J = 6.8 Hz), 7.48 (dd, 2H, J = 7.3 Hz), 4.94 (bt, 1H, J = 5.4 Hz), 4.42 (s, 2H), 3.27-3.14 (m, 2H), 1.21 (t, 3H, J = 7.8 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 190.2, 135.6, 134.5, 129.0 (2C), 57.3, 38.8, 15.3; *HRMS-ESI*$^+$ (*m/z*) calcd for $C_{10}H_{14}NO_3S$ $(M + H)^+$, 228.0689; found, 228.0688.



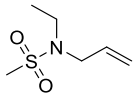
tert-Butyl (S)-2-(2-(N-methylsulfamoyl)acetyl)pyrrolidine-1-carboxylate (**1.26**). Sulfonamide **1.25** (240 mg, 2.20 mmol, 2.2 equiv) was dissolved in 10 mL of THF and cooled to –78 °C. *n*-BuLi (2.5 M in hexane, 1.76 mL, 4.40 mmol, 4.4 equiv) was added and the mixture was stirred

for 1 h before adding Boc-Pro-OMe (229 mg, 1.00 mmol, 1 equiv) dissolved in 3 mL of THF dropwise. The reaction was stirred for 3 h before quenching with 10 mL of sat. NH_4Cl. The mixture was extracted three times with 10 mL of DCM and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography with 60% EtOAc in hexanes to give **1.26** as a yellow oil, 202 mg (66%). ^1H NMR ($CDCl_3$, 300 MHz)(3:7 mixture of rotamers): δ 5.69 (m, 0.7H), 5.02 (m, 0.73), 4.58 (m, 0.7H), 4.26 (d, 1H, *J* = 14.3 Hz), 4.07 (m, 1.3H), 3.44 (m, 2H), 2.77 (d, 0.9H, *J* = 5.1 Hz), 2.73 (d, 2.1H, *J* = 5.1 Hz), 2.00 (m, 4H), 1.39 (s, 6.3H), 1.36 (s, 2.7H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz, 55 °C): δ 199.9, 154.8, 80.6, 64.8, 58.1, 53.3, 47.0, 29.4, 28.3, 24.2; HRMS-ESI$^+$ (*m/z*) calcd for $C_{12}H_{22}N_2NaO_5S^+$ $(M + Na)^+$ 329.1142, found 329.1154.

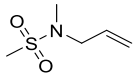


tert-Butyl (S)-(1-(N-ethylsulfamoyl)-2-oxo-5-phenylpentan-3-yl)carbamate (**1.28**). Diisopropyl amine (0.682 mL, 4.90 mmol, 9.8 equiv) was dissolved in 5 mL of THF. The mixture was cooled to -78 °C and then *n*-BuLi (1.6 M in hexane, 2.78 mL, 4.45 mmol, 8.9 equiv) was added. After being stirred for 10 min at 0 °C, this solution was cooled to -78 °C and sulfonamide **1.23** (0.198 mL, 2.10 mmol, 4.2 equiv) in dry THF (4 ml) was added over a period of 10 min. The reaction mixture was allowed to warm -30 °C over a period of 45 min and was then cooled to -78 °C and Boc-hPhe-OMe (0.146 g, 0.500 mmol) in dry THF (4 mL) was added. The solution was allowed to warm to rt and followed by TLC. After 8 h the mixture was cooled to 0 °C and quenched with sat. aqueous NH_4Cl. The mixture was extracted with ether (3 x 20 mL). The combined organic extracts were washed with brine and water, dried (Na_2SO_4), filtered and concentrated in vacuo to

give a yellow oil. Purification using flash chromatography (30% EtOAc, 70% hexane) provided ketone **1.28** as an amorphous white solid (0.057 g, 30% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29-7.08 (m, 5H), 5.22 (bt, 1H), 5.13 (d, 1H, *J* = 7.1 Hz), 4.43-4.31 (m, 1H), 4.28 (d, 1H, *J* = 14.4 Hz), 4.01 (d, 1H, *J* = 14.4 Hz), 3.11 (overlapping dq, 2H, *J* = 6.8 Hz), 2.65 (m, 2H), 2.25-2.10 (m, 1H), 1.88-1.71 (m, 1H), 1.44 (s, 9H), 1.18 (t, 3H, *J* = 7.1 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 200.1, 155.9, 140.2, 128.6, 128.4, 126.4, 80.8, 59.6, 58.2, 38.7, 31.8, 31.6, 28.3, 15.2; *HRMS-ESI*$^{+}$ (*m/z*) calcd for $C_{18}H_{32}N_3O_5S$ $(M + NH_4)^+$, 402.2057; found, 402.2051.



N-Allyl-N-ethylmethanesulfonamide (**1.32**). To a solution of sulfonamide **1.23** (1.00 g, 9.16 mmol, 1 equiv) in dry acetone (10 mL) allyl bromide (0.79 mL, 9.16 mmol, 1 equiv) and anhydrous K_2CO_3 (2.53 g, 18.3 mmol, 2 equiv) were added and the mixture stirred for 24 h then another 0.25 equiv of ally bromide was added and the mixture stirred for an additional 6 h. 50 mL of water was added and the solution extracted with 50 mL of DCM 3 times. The organic layer was dried over Na_2SO_4 and concentrated. Purification by flash chromatography (20% EtOAc, 80% hexane) gave sulfonamide **1.32** as a colorless liquid (0.89 g, 60% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.76 (m, 1H), 5.23 (dd, 1H, *J* = 1.2, 17.1 Hz), 5.18 (dd, 1H, *J* = 1.2, 11.2 Hz), 3.77 (d, 2H, *J* = 6.1 Hz), 3.21 (q, 2 H, *J* = 7.3 Hz), 2.79 (s, 3H), 1.12 (t, 3H, *J* = 7.3 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 133.0, 118.9, 49.3, 41.7, 39.3, 13.8; *HRMS-ESI*$^{+}$ (*m/z*) calcd for $C_6H_{14}NO_2S$ $(M + H)^+$, 164.0740; found, 164.0740.



N-Allyl-N-methylmethanesulfonamide (**1.33**). To a solution of sulfonamide **1.25** (10.9 g, 100 mmol, 1 equiv) in dry DMF (50 mL) allyl bromide (11.2 mL, 130 mmol, 1.3 equiv) and

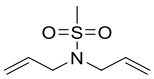
anhydrous K_2CO_3 (27.6 g, 200 mmol, 1 equiv) were added. The mixture was stirred for 18 h. Water was added (100 mL) and the mixture was extracted with Et_2O (3 x 150 ml). The organic layer was washed with water (3 x 100 mL), brine (1 x 100 ml) then concentrated by rotary evaporation. The resulting yellow liquid was purified by vacuum distillation to give sulfonamide **1.33** as a colorless liquid (13.3 g, 89% yield). Bp. = 67-70 °C (0.30 mm Hg). The ^{1}H NMR spectrum was identical to that reported in the literature.[68] ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.82 (m, 1H), 5.27 (dd, 1H, *J* = 1.6, 17.0 Hz), 5.24 (dd, 1H, *J* = 1.6, 10.1 Hz), 3.72 (d, 2H, *J* = 6.3 Hz), 2.79 (s, 3H), 2.67 (s, 3H).

General procedure 1.1 for the preparation of methane sulfonamides

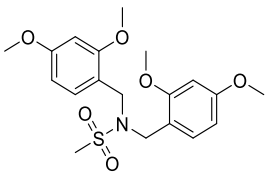
The amine (1 equiv) was dissolved in THF or DCM at a 1 M concentration and cooled to 0 °C. Triethyl amine (1.1 equiv) was added followed by dropwise addition of methane sulfonyl chloride (1.1 equiv) (**Caution**. Exotherm). When the addition was complete the reaction was stirred at room temperature or reflux until complete by TLC and then was diluted with 3 volumes of water and extracted three times with 1 volume of DCM. The combined organic layers were derived over $MgSO_4$, filtered and concentrated. The residue was purified by vacuum distillation, crystallization of flash chromatography as indicated.

N,N-Diethyl methanesulfonamide (**1.34**). According to general procedure 1.1 using THF as the solvent the reaction was complete after 1 hour at reflux. After vacuum distillation **1.34** was obtained as a pale yellow oil (bp = 81 °C at 1 mmHg). 26.7 g (89% yield) from 20.5 mL (198 mmol) of diethylamine. ^{1}H NMR ($CDCl_3$, 300 MHz) σ: 3.26 (q, 4H, *J* = 7.1 Hz), 2.81 (s, 3H),

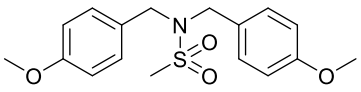
1.20 (t, 6H, J = 7.1 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) σ: 14.8, 39.3, 42.4; HRMS-ESI$^+$ (*m/z*) calcd for $C_5H_{14}NO_2S$ $(M + H)^+$ 152.0740, found 152.0740.



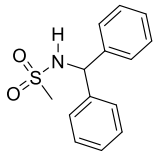
N,N-Diallyl methanesulfonamide (**1.35**). According to general procedure 1.1 using THF as the solvent the reaction was complete after 1 hour at reflux. After vacuum distillation **1.35** was obtained as a colourless oil (bp = 102 °C at 0.025 mmHg). 18.0 g (90% yield) from 14.1 mL (114 mmol) of diallylamine. ^{1}H NMR ($CDCl_3$, 300 MHz) σ: 5.67 (m, 2H), 5.22 (m, 4H), 3.79 (d, 4H, J = 6.3 Hz), 2.82 (s, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) σ: 40.0, 49.0, 119.3, 132.4; HRMS-ESI$^+$ (*m/z*) calcd for $C_7H_{14}NO_2S^+$ $(M + H)^+$ 176.0740, found 176.0740.



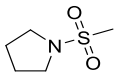
N,N-Bis(2,4-dimethoxybenzyl) methanesulfonamide (**1.36**). According to general procedure 1.1 using THF as the solvent the reaction was complete after 3 h at reflux. After flash chromatography, 20 to 60% EtOAc in hexane, **1.36** was obtained as a yellow oil which crystalized upon standing for several days. 11.3 g (91%) from 10 g (31.5 mmol) of Bis(2,4-dimthoxybenzyl)amine. ^{1}H NMR ($CDCl_3$, 300 MHz): σ 7.22 (2H, J = 8.16 Hz), 6.45 (m, 4H), 4.36 (s, 4H), 3.79 (s, 6H), 3.75(s, 6H), 2.72(s, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 7 MHz): σ 39.6, 44.9, 55.0, 55.3, 98.2, 103.9, 116.8, 130.8, 158.3, 160.4 HRMS-ESI$^+$ (*m/z*) calcd for $C_{19}H_{26}NO_6S^+$ $(M + H)^+$ 396.1475, found 396.1475.



N,N-Bis(4-methoxybenzyl) methanesulfonamide (**1.37**). According to general procedure 1.1 using DCM as the solvent the reaction was complete after stirring overnight at 0 °C. After recrystallization from 350 mL of hot (5:2) hexane:ethyl acetate **1.37** was obtained as white crystals, 23 g (75%), from 22 g (86 mmol) of *N,N*-bis(4-methoxybenzyl)amine. NMR data for this sulfonamide was identical to literature spectra.[69] ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.22 (d, 4H, J = 8.7 Hz), 6.87 (d, 4H, J = 8.6 Hz), 4.24 (s, 4H), 3.80 (s, 6H), 2.71 (s, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 159.3, 130.0, 127.4, 114.0, 55.2, 48.9, 40.1;



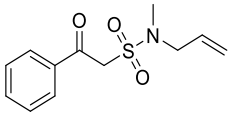
N-(Diphenylmethyl)methanesulfonamide (**1.38**). According to general procedure 1.1 using THF as the solvent the reaction was complete after stirring overnight at room temperature. After recrystallization from 200 mL of heptane, **1.38** was obtained as a white powder, 13.1 g (92%) from 10 g of amino diphenylmethane (54.6 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.3 (m, 10H), 5.75 (d, 1H, J = 7.2 Hz), 5.06 (d, 1H, J = 6.9 Hz), 2.65 (s, 3H); ^{13}C{^{1}H} NMR ($SO(CD_3)_2$, 75 MHz): δ 142.6, 128.8, 127.5, 127.5, 60.7, 41.6; HRMS-ESI$^-$ (*m/z*) calcd for $C_{14}H_{14}NO_2S^-$ (M - H)$^-$ 260.0740, found 260.0736.



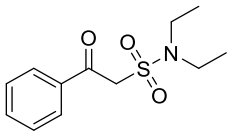
1-(methylsulfonyl)pyrrolidine (**1.39**) According to general procedure 1.1 using DCM as the solvent the reaction was complete after stirring overnight at 0 °C. After recrystallization by slow evaporation of ether **1.39** was obtained as colorless crystals, 9.6 g (63%) from 8.4 mL (105 mmol) of pyrrolidine. The Spectral data were identical to literature spectra.[70]

General procedure 1.2 For the preparation of benzoyl derivatives **1.40 – 1.44**

The sulfonamide **1.33** - **1.37** (1.0 equiv) was dissolved in one volume of THF at a concentration of 0.3 M and the solution was cooled to -78 °C. *n*-BuLi (1.1 equiv) was added slowly as a 2.5 M solution in hexanes and the reaction was stirred for 15 min before adding methyl benzoate (0.5 equiv) neat and dropwise. The reaction was stirred for another 1 hour at -78 °C before quenching with one volume of saturated aqueous ammonium chloride. The mixture was extracted three times with one volume DCM and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The ß-ketone was separated from the excess methane sulfonamide starting material by flash chromatography.



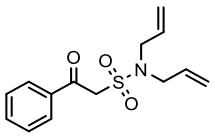
N-Methyl-N-allyl benzoylmethanesulfonamide (**1.40**). According to general procedure 1.2 after flash chromatography, 10 to 30% EtOAc in hexane, **1.40** Was obtained as a colorless oil, 1.43 g (77%) from methyl benzoate (1.00 g, 7.3 mmol, 0.5 equiv) and **1.33** (2.34 g, 15.7 mmol, 2.1 equiv). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.03 (d, 2H, *J* = 8.3 Hz), 7.63 (t, 1H, *J* = 7.5 Hz), 7.51 (t, 2H, *J* = 7.82 Hz), 5.80 (m, 1H), 5.25 (m, 2H), 4.59 (s, 2H), 3.78 (d, 2H, *J* = 6.4 Hz), 2.87 (s, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 189.5, 135.8, 134.4, 132.6, 129.4, 128.9, 119.1, 57.8, 53.3, 34.6; HRMS-ESI$^+$ (*m/z*) calcd for $C_{12}H_{16}NO_3S^+$ $(M + H)^+$ 254.0845 found: 254.0844.



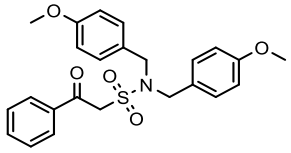
N,N-Diethyl benzoylmethanesulfonamide (**1.41**). According to general procedure 1.2 after flash chromatography, 5% EtOAc in benzene, **1.41** was obtained as a white solid 6.2 g (99% yield) from of methyl benzoate (3.65 mL, 23.8 mmol, 1 equiv) and **1.34** (7.19 g, 47.6 mmol, 2 equiv).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.03 (d, 2H, *J* = 8.0 Hz), 7.61 (t, 1H, *J* = 7.4 Hz), 7.49 (t, 2H, *J* = 7.7 Hz), 4.54 (s, 2H), 3.28 (q, 4H, *J* = 7.1 Hz), 1.19 (t, 6H, *J* = 7.1 z); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ, 189.2, 135.8, 134.1, 129.3, 128.8, 59.1, 42.9, 14.7; HRMS-ESI$^+$ (*m/z*) calcd for $C_{12}H_{18}NO_3S^+$ $(M + H)^+$ 256.1002, found 256.1001.

Alternatively **1.41** was obtained if potassium tert-butoxide (2.43 g, 21.7 mmol, 4 equiv) was dissolved in 20 mL of THF and cooled to 0 °C, **1.34** (816 mg, 5.4 mmol, 1 equiv) was added followed by methyl benzoate (1.36 mL, 10.8 mmol, 2 equiv) and the resulting mixture was stirred overnight at room temperature. The product was isolated as above to gave **1.41** in 86% yield.

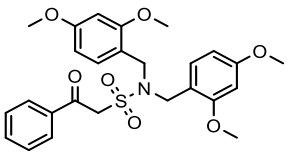


N,N-Diallyl benzoylmethanesulfonamide (**1.42**). According to general procedure 1.2 after flash chromatography, 1% ether in benzene, **1.42** was obtained as a white solid, 2.36 g (85%) from methyl benzoate (1.26 mL, 10 mmol, 1 equiv) and **1.35** (3.50 g, 20 mmol, 2 equiv). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.01 (m, 2H), 7.62 (tt, 1H, *J* = 7.5,1.3 Hz), 7.50 (t, 2H, *J* = 7.6 Hz), 5.81 (ddt, 2H, *J* = 16.8, 10.4, 6.5 Hz), 5.23 (m, 4H), 4.59 (s, 2H), 3.85 (d, 4H, *J* = 6.3 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 189.1, 135.7, 134.2, 132.7, 129.2, 128.8, 119.1, 59.8, 50.0; HRMS-ESI$^+$ (*m/z*) calcd for $C_{14}H_{18}N_1O_3S^+$ $(M + H)^+$ 280.1002, found 280.1002.



N,N-Bis(4-methoxybenzyl) benzoylmethanesulfonamide (**1.44**). According to general procedure 1.2 after flash chromatography, 0 to 10% EtOAc in benzene, **1.44** was obtained as a white solid,

335 mg (99%) from 95 μL (0.75 mmol) of methyl benzoate and **1.37** (502 mg, 1.5 mmol, 2 equiv). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.92 (d, 2H, *J* = 8.0 Hz), 7.60 (t, 1H, *J* = 7.4 Hz), 7.46 (d, 2H, *J* = 7.7 Hz), 7.20 (d, 4H, *J* = 8.6 Hz), 6.81 (d, 4H, *J* = 8.6 Hz), 4.42 (s, 2H), 4.32 (s, 4H), 3.76 (s, 6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 189.1, 159.2, 135.8, 134.1, 130.0, 129.1, 128.7, 127.4, 113.9, 60.0, 55.2, 50.6; HRMS-ESI^{+} (*m/z*) calcd for $C_{24}H_{25}NO_5SLi^{+}$ $(M + Li)^{+}$ 446.1608, found 446.1608.

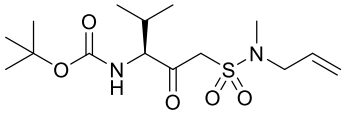


N,N-Bis(2,4-dimethoxybenzyl) benzoylmethanesulfonamide (**1.43**). Sulfonamide **1.36** (3.95 g, 10 mmol, 1 equiv) was dissolved in 100 mL of THF with gentle heating then cooled to -78 °C. Diisopropylamine (2.82 mL, 20 mmol, 2 equiv) was added followed by butyl lithium (1.6 M in hexane, 12.5 mL, 20 mmol, 2 equiv) and the solution was stirred for five min then *N*-methyl-*N*-methoxybenzamide (3.30 g, 20.0 mmol, 2.00 equiv) was added. The reaction was stirred at -78 °C for 5 h until TLC indicated that the methane sulfonamide had been completely consumed. The reaction was quenched with 10 mL of sat. NH_4Cl and warmed to room temperature. 150 mL of water was added, and the mixture was extracted three times with 100 mL of DCM. The combined organic layers were dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography, 0 to 60% EtOAc in hexane to give 3.3 g of a white solid (66%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.91 (d, 2H, *J* = 7.2 Hz), 7.58 (t, 1H, *J* = 7.1 Hz), 7.45 (t, 2H, *J* = 6.6 Hz), 7.25 (d, 2H, *J* = 7.9 Hz), 6.44 (d, 2H, *J* = 7.7 Hz), 6.38 (s, 2H), 4.45 (s, 4H), 4.41 (s, 2H), 3.78 (s, 6H), 3.76 (s, 6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 189.0, 160.5, 158.4, 136.0, 133.8, 131.1, 129.0, 128.6, 116.7, 104.0, 98.2, 59.7, 55.3, 55.1, 46.1; HRMS-ESI^{+} (*m/z*) calcd for $C_{26}H_{29}NO_7SLi^{+}$ $(M + Li)^{+}$ 506.1819, found 506.1819.

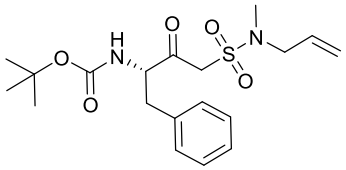
When the reaction was performed according to general procedure 1.2 **1.43** was not observed.

General procedure 1.3 for preparing ß-ketosulfonamides from amino acids.

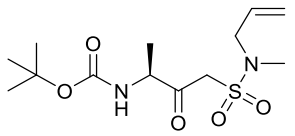
Sulfonamide (3.5 equiv) was dissolved in THF at a concentration of 0.1-0.2 M then cooled to -78 °C then *n*-BuLi (2.5 M in hexane, 3.5 equiv) was added dropwise and the reaction was stirred for 30 min. A solution of *N*-protected amino acid methyl ester was added as a solution in THF (aprox. 0.2 M) dropwise and the mixture was stirred at -78 ° for 1 hour before a sat. aqueous solution of NH_4Cl was added and the mixture extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography separated the ß-ketones from the excess methanesulfonamide.



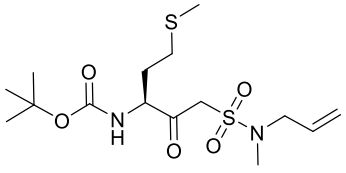
tert-Butyl (S)-(1-(N-allyl-N-methylsulfamoyl)-4-methyl-2-oxopentan-3-yl)carbamate (**1.45**). According to general procedure 1.3, **1.45** was obtained as an amorphous white solid (1.45 g, 97% yield) after flash chromatography (25% EtOAc, 75% hexane) from ester Boc-Val-OMe (1.00 g, 4.32 mmol) and sulfonamide **1.33** (2.25 g, 15.1 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.83-5.72 (m, 1H), 5.29-5.21 (m, 2H), 5.19 (1H, d, J = 8.6 Hz), 4.29 (dd, 1H, J = 4.6, 8.8 Hz), 4.23 (d, 1H, J = 14.3 Hz), 4.03 (d, 1H, J = 14.3 Hz), 3.78 (d, 2H, J = 6.1 Hz), 2.83 (s, 3H), 2.35-2.26 (m, 1H), 1.42 (s, 9H), 0.99 (d, 3H, J = 6.8 Hz), 0.81 (s, 3H, J = 6.8 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 199.0, 155.9, 132.5, 119.2, 80.3, 65.1, 58.1, 53.1, 34.5, 28.6, 28.2, 19.8, 16.8; *HRMS-ESI*$^+$ (*m/z*) calcd for $C_{11}H_{21}N_2O_5S$ $(M - C_4H_9 + 2H)^+$, 293.1166; found, 293.1164.



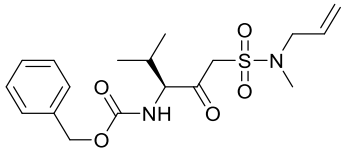
tert-Butyl (S)-(4-(N-allyl-N-methylsulfamoyl)-3-oxo-1-phenylbutan-2-yl)carbamate (**1.46**). According to general procedure 1.3, **1.46** was obtained as an amorphous white solid (0.370, 93% yield) after flash chromatography (25% EtOAc, 75% hexane) from ester Boc-Phe-OMe (0.279, 1.00 mmol) and sulfonamide **1.33** (0.521 g, 3.2 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.46-7.21 (m, 3H), 7.17 (d, 2H, *J* = 6.6 Hz), 5.82-5.69 (m, 1H), 5.26 (d, 1H, *J* = 18.5 Hz), 5.24 (d, 1H, *J* = 9.5 Hz), 5.10 (d, 1H, *J* = 6.9 Hz), 4.53-4.46 (m, 1H), 4.17 (d, 1H, *J* = 13.8 Hz), 4.03 (d, 1H, *J* = 13.8 Hz), 3.74 (d, 2H, *J* = 5.8 Hz), 3.20 (dd, 1H, *J* = 5.8, 14.4), 2.90 (d, 1H, *J* = 9.0, 14.3), 2.18 (s, 3H), 1.37 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 198.8, 155.5, 136.3, 132.4, 129.3, 128.8, 127.1, 119.3, 80.2, 61.3, 57.9, 53.1, 36.4, 34.4, 28.2; *HRMS-ESI*$^{+}$ (*m/z*) calcd for $C_{19}H_{29}N_2O_5S$ $(M + H)^{+}$, 397.1792; found, 397.1787.



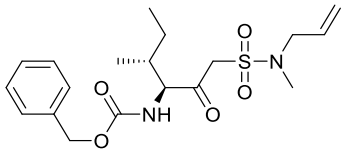
tert-Butyl (S)-(4-(N-allyl-N-methylsulfamoyl)-3-oxobutan-2-yl)carbamate (**1.47**). According to general procedure 1.3, **1.47** was obtained as an amorphous white solid (0.658, 84% yield) after flash chromatography (10% EtOAc, 90% benzene) from Boc-Ala-OMe (0.500, 2.46 mmol) and sulfonamide **1.33** (1.28 g, 8.61 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): 5.56-5.81 (m, 1H), 5.15-5.32 (m, 3H), 4.19-4.35 (m, 2H), 4.06 (one half of a doublet of doublets, 1H, *J* = 13.9 Hz), 3.74 (d, 2H, *J* = 5.6 Hz), 2.80 (s, 3H), 1.39 (s, 9H), 1.33 (d, 3H, *J* = 7.1 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): 199.7, 155.3, 132.3, 119.1, 80.2, 57.1, 55.9, 53.0, 34.3, 28.1, 16.3; *HRMS-ESI*$^{+}$ (*m/z*) calcd for $C_{13}H_{25}N_2O_5S$ $(M + H)^{+}$, 321.1475; found, 321.1479.



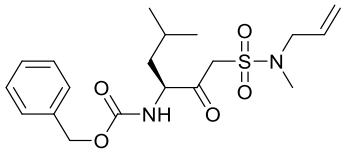
tert-Butyl (S)-(1-(N-allyl-N-methylsulfamoyl)-5-(methylthio)-2-oxopentan-3-yl)carbamate (**1.48**). According to general procedure 1.3, **1.48** was obtained as an amorphous white solid (1.12 g, 74% yield) after flash chromatography (33% EtOAc, 67% hexane) from Boc-Met-OMe (0.970, 3.68 mmol) and sulfonamide **1.33** (1.90 g, 12.8 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): 5.8-5.65 (m, 1H), 5.41 (d, 1H, *J* = 7.4 Hz), 5.24 (1H, d, *J* = 16.4 Hz), 5.19 (1H, d, *J* = 9.5 Hz), 4.32-4.44 (1H, m), 4.21 (1H, d, *J* = 13.8 Hz), 4.08 (1H, d, *J* = 13.8 Hz), 3.73 (d, 2H, *J* = 5.8 Hz), 2.79 (s, 3H), 2.45-2.55 (m, 2H), 2.10-2.23 (1H, m), 2.03 (3H, s), 1.77-1.90 (m, 1H), 1.39 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): 199.0, 155.6, 132.3, 119.3, 80.5, 59.4, 57.5, 53.0, 34.4, 30.1, 29.7, 28.2, 15.4; *HRMS-ESI*$^{+}$ (*m/z*) calcd for $C_{15}H_{32}N_3O_5S_2$ $(M + NH_4)^{+}$, 398.1778; found, 398.1773.



Benzyl (S)-(1-(N-allyl-N-methylsulfamoyl)-4-methyl-2-oxopentan-3-yl)carbamate (**1.49**). According to general procedure 1.3, **1.49** was obtained as an amorphous white solid (1.12 g, 65% yield) after flash chromatography (30% EtOAc, 70% hexane) from ester Cbz-Val-OMe (2.00 g, 8.02 mmol) and sulfonamide **1.33** (4.18 g, 28.0 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31 (s, 5H), 5.77-5.68 (m, 1H), 5.52 (d, 1H, *J* = 9.0 Hz), 5.27-5.19 (m, 2H), 5.09 (s, 2H), 4.40 (dd, 1H, *J* = 4.2, 9.0 H), 4.21 (d, 1H, *J* = 13.8 Hz), 4.02 (d, 1H, *J* = 13.8 Hz), 3.75 (d, 3H, *J* = 5.8 Hz), 2.79 (s, 3H), 2.34-2.27 (m, 1H), 0.99 (d, 3H, *J* = 6.9 Hz), 0.80 (d, 3H, *J* = 6.4 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 198.6, 156.5, 136.1, 132.4, 128.6, 128.3, 128.1, 119.2, 67.2, 65.5, 58.2, 53.0, 34.3, 28.8, 19.8, 16.7; *HRMS-ESI*$^{+}$ (*m/z*) calcd for $C_{18}H_{27}N_2O_5S$ $(M + H)^{+}$, 383.1635; found, 383.1627.

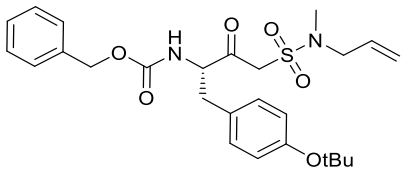


Benzyl ((3S,4R)-1-(N-allyl-N-methylsulfamoyl)-4-methyl-2-oxohexan-3-yl)carbamate (**1.50**). According to general procedure 1.3, **1.50** was obtained as an amorphous white solid (1.00 g, 71% yield) after flash chromatography (33% EtOAc, 67% hexane) from Cbz-Ile-OMe (1.00 g, 3.57 mmol) and sulfonamide **1.33** (1.87 g, 12.5 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.34 (s, 5H), 5.85-5.69 (m, 1H), 5.37 (bd, 1H, *J* = 9.0 Hz), 5.27 (d, 1H, *J* = 17.5 Hz), 5.25 (d, 1H, *J* = 9.5 Hz), 5.11 (s, 2H), 4.42 (dd, 1H, *J* = 4.2, 9.0 Hz), 4.22 (d, 1H, *J* = 14.3 Hz), 4.02 (d, 1H, *J* = 14.3 Hz), 3.78 (d, 2H, *J* = 5.8 Hz), 2.82 (s, 3H), 1.40-1.25 (m, 1H), 1.16-1.00 (m, 1H), 1.00 (d, 3H, *J* = 6.4 Hz), 0.89 (t, 3H, *J* = 7.4 Hz), ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 198.6, 156.4, 136.1, 132.4, 128.6, 128.3, 128.1, 119.3, 67.2, 65.4, 58.4, 35.6, 34.3, 24.1, 16.1, 11.5; *HRMS-ESI*$^{+}$ (*m/z*) calcd for $C_{19}H_{29}N_2O_5S$ $(M + H)^{+}$, 397.1792; found, 397.1785.

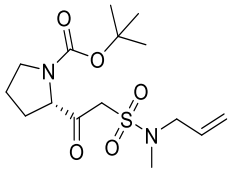


Benzyl (S)-(1-(N-allyl-N-methylsulfamoyl)-5-methyl-2-oxohexan-3-yl)carbamate (**1.51**). According to general procedure 1.3, **1.51** was obtained as an amorphous white solid (1.00 g, 83% yield) after flash chromatography (25% EtOAc, 75% hexane) from Cbz-Leu-OMe (0.848 g, 3.00 mmol) and sulfonamide **1.33** (1.56 g, 10.5 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32 (s, 5H), 5.79-5.65 (m, 1H), 5.25 (d, 1H, *J* = 17.8 Hz), 5.23 (d, 1H, *J* = 9.9 Hz), 5.10 (s, 2H), 4.42 (m, 1H), 4.25 (d, 1H, *J* = 13.7 Hz), 4.02 (d, 1H, *J* = 13.7 Hz), 3.75 (d, 2H, *J* = 5.6 Hz), 2.79 (s, 3H), 1.74-1.61 (m, 2H), 1.45 (m, 1H), 0.92 (d, 6H, *J* = 3.2 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ

199.7, 156.3, 136.1, 132.4, 128.6, 128.3, 128.1, 119.3, 67.2, 59.3, 57.5, 53.0, 39.3, 34.4, 24.8, 23.2, 21.3; *HRMS-ESI*$^+$ (*m/z*) calcd for $C_{19}H_{32}N_3O_5S$ $(M + NH_4)^+$,414.2057; found, 414.2048.

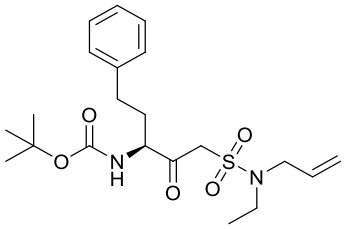


Benzyl (S)-(4-(N-allyl-N-methylsulfamoyl)-1-(4-(tert-butoxy)phenyl)-3-oxobutan-2-yl)carbamate (**1.52**). According to general procedure 1.3, **1.52** was obtained as an amorphous white solid (0.887 g, 68% yield) after flash chromatography (33% EtOAc, 67% hexane) from Cbz-Tyr(tBu)-OMe (1.0 g, 2.59 mmol) and sulfonamide **1.33** (1.23 g, 8.29 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38-7.24 (m, 5H), 7.02 (d, 2H, *J* = 8.3 Hz), 6.89 (d, 2H, *J* = 8.3 Hz), 5.82-5.67 (m, 1H), 5.53 (d, 1H, *J* = 7.3 Hz), 5.25 (d, 1H, *J* = 17.1 Hz), 5.23 (d, 1H, *J* = 9.5 Hz), 5.15 (s, 2H), 4.64-4.55 (m, 1H), 4.13 (d, 1H, *J* = 13.9 Hz), 4.00 (d, 1H, *J* = 13.9 Hz), 3.72 (d, 2H, *J* = 5.9 Hz), 3.15 (dd, 1H, *J* = 5.6, 14.2), 2.91 (dd, 1H, *J* = 8.3, 14.2), 2.27 (s, 3H), 1.30 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 198.4, 156.0, 154.5, 136.0, 132.2, 130.6, 129.7, 128.6, 128.3, 128.1, 124.4, 119.3, 78.5, 67.2, 61.7, 58.1, 53.0, 35.8, 34.3, 28.8; *HRMS-ESI*$^+$ (*m/z*) calcd for $C_{26}H_{38}N_3O_6S$ $(M + NH_4)^+$, 520.2476; found, 520.2467.

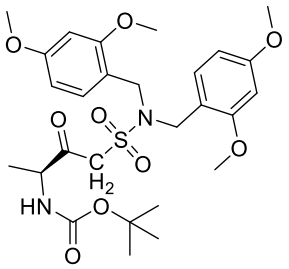


tert-Butyl (S)-2-(2-(N-allyl-N-methylsulfamoyl)acetyl)pyrrolidine-1-carboxylate (**1.53**). According to general procedure 1.3, except that only 2 equiv of the sulfonamide and *n*-BuLi were used, **1.53** was obtained as a pale-yellow oil (1.41 g, 95% yield) after flash chromatography (0 to 50% EtOAc in DCM) from Boc-Pro-OMe (1.0 g, 4.3 mmol, 0.5 equiv) and sulfonamide **1.33** (1.3 g, 8.74 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.78 (m, 1H), 5.26 (m, 2H), 4.39 (m,

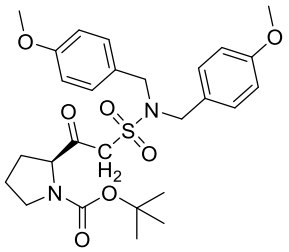
1H), 4.15 (m, 2H), 3.81 (m, 2H), 3.47 (m, 2H), 2.85 (s, 3H), 2.14 (m, 2H), 1.90 (m, 2H), 1.43 (2, 9H); 13C{1H} NMR ($CDCl_3$, 75 MHz): δ (major rotamer)199.8, 154.8, 132.6, 119.0, 80.3, 65.5, 57.8, 53.1, 47.0, 28.5, 28.3, 24.6; (minor rotamer) 199.3, 153.6, 132.4, 119.2, 80.7, 66.0, 56.9, 53.4, 46.8, 34.3, 29.4, 23.6; HRMS-ESI$^+$ (*m/z*) calcd for $C_{15}H_{26}N_2NaO_5S^+$ $(M + Na)^+$ 369.1455, found 369.1472.



tert-Butyl (S)-(1-(N-allyl-N-ethylsulfamoyl)-2-oxo-5-phenylpentan-3-yl)carbamate (**1.54**). According to general procedure 1.3, **1.54** was obtained as an amorphous white solid (0.189 g, 92% yield) after flash chromatography 20% EtOAc in hexane, from Boc-hPhe-OMe (0.146 g, 0.500 mmol) and sulfonamide **1.32** (0.261 g, 1.60 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32-7.14 (m, 5H), 5.38-5.25 (m, 1H), 5.30-5.20 (m, 3H), 4.35-4.27 (m, 1H), 4.25 (d, 1H, *J* =13.7 Hz), 3.95 (d, 1H, *J* = 13.7 Hz), 3.83 (d, 1H, *J* = 6.1 Hz), 3.27 (q, 2H, *J* = 7.1 Hz), 2.71-2.64 (m, 1H), 2.33-2.20 (m, 1H), 1.94-1.80 (m, 1H), 1.45 (s, 9H), 1.16 (t, 3H, *J* = 7.1 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 199.2, 155.6, 140.6, 133.1, 128.6, 128.5, 126.3, 119.0, 80.4, 60.0, 59.2, 50.1, 42.6, 32.2, 31.7, 28.3, 14.0; *HRMS-ESI*$^+$ (*m/z*) calcd for $C_{21}H_{36}N_3O_5S$ $(M + NH_4)^+$, 442.2370; found, 442.2365.

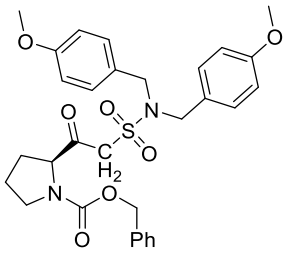


tert-Butyl (4-(N,N-bis(2,4-dimethoxybenzyl)sulfamoyl)-3-oxobutan-2-yl)carbamate (**1.56**). Sulfonamide **1.36** (1.35 g, 3.38 mmol, 3.1 equiv) was dissolved in 38 mL of THF and cooled to -78 °C. *n*-BuLi (2.5 M in hexanes, 1.35 mL, 3.38 mmol, 3.1 equiv) was added dropwise and the resulting solution was stirred for 30 min before adding Weinreb amide **1.55** (253 mg, 1.09 mmol, 1 equiv) as a solution in 7 mL of THF. After stirring for 4 h at –78 °C the reaction was complete by TLC and quenched with 5 mL of saturated ammonium chloride. The mixture was diluted with 200 mL of water and extracted three times with 200 mL of DCM. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was subjected to flash chromatography with 10% EtOAc in benzene which yielded 536 mg of **1.56** (87%) as a white solid. Additionally, 817 mg of **1.36** was recovered. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.35 (s, 2H), 7.19 (d, 2H, J = 8.0 Hz), 6.2 (m, 4H), 5.28 (d, 1H, J = 5.3 Hz), 4.4 (s, 4H), 4.30 (t, 1H, J = 6.7 Hz), 4.08 (d, 1H, J = 13.2 Hz), 3.85 (d, 1H, J = 13.4 Hz), 3.78 (s, 6H), 3.77 (s, 6H), 1.42 (s, 9H), 1.3 (d, 3H, J = 7.0 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 199.5, 160.6, 158.4, 155.2, 131.0, 116.5, 104.1, 98.3, 80.1, 59.8, 55.9, 55.4, 55.2, 45.9, 28.3, 16.9; HRMS-ESI$^+$ (*m/z*) calcd for $C_{27}H_{38}N_2O_9SLi^+$ $(M + Li)^+$ 573.2453, found 573.2450.

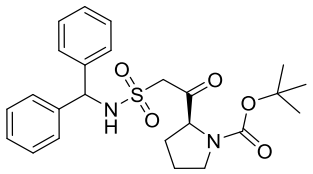


tert-Butyl (S)-2-(2-(N,N-bis(4-methoxybenzyl)sulfamoyl)acetyl)pyrrolidine-1-carboxylate (**1.57**)

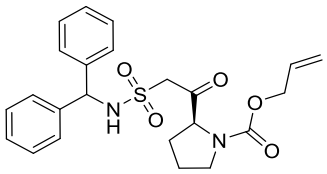
Sulfonamide **1.37** (3.66 g, 10.9 mmol, 2.5 equiv) was dissolved in 60 mL of THF and cooled to -78 °C before adding *n*-BuLi (2.5 M in hexanes, 4.36 mL, 10.9 mmol, 2.5 equiv). The solution was stirred for 30 min before adding Boc-Pro-OMe (1.00 g, 4.36 mmol, 1.00 equiv) dropwise as a solution in 5 mL of THF. The reaction was stirred for 2 h then quenched with 5 mL of saturated NH_4Cl. The mixture was diluted with 100 mL of water and extracted three times with 100 mL of DCM. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was subjected to column chromatography, 10% EtOAc in benzene, to give 1.60 g (69% yield) of **1.57** as a white amorphous solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (10:9 mixture of rotamers) 7.26 (d, 2H, *J* = 7.9 Hz), 7.20 (d, 2H, *J* = 8.0 Hz), 6.88 (d, 2H, *J* = 8.2 Hz), 6.84 (d, 2H, *J* = 8.1 Hz), 4.35 (m, 5H), 4.14 (d, 0.5H, *J* = 14.3 Hz), 4.11 (d, 0.5H, *J* = 14.3 Hz), 3.93 (d, 0.5H, *J* = 14.7 Hz), 3.81 (s, 6H), 3.76 (d, 0.5H, *J* = 14.3 Hz), 3.56-3.45 (m, 2H), 2.2-1.8 (m, 4H), 1.46 (s, 4.7H), 1.38 (s, 4.3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ (major rotamer) 199.7, 159.3, 154.8, 130.0, 127.4, 114.0, 80.3, 65.4, 60.9, 55.3, 50.2, 47.1, 28.7, 28.4, 24.7; (minor rotamer) 199.1, 159.4, 153.6, 130.1, 127.4, 114.1, 80.7, 66.1, 59.6, 55.3, 50.3, 46.8, 29.5, 28.2, 23.7; HRMS-ESI$^+$ (*m/z*) calcd for $C_{27}H_{37}N_2O_7S^+$ $(M + H)^+$ 533.2316, found 533.2316.



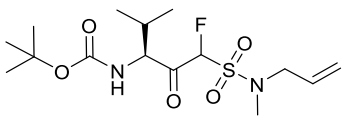
Benzyl (S)-2-(2-(N,N-bis(4-methoxybenzyl)sulfamoyl)acetyl)pyrrolidine-1-carboxylate (**1.58**) Sulfonamide **1.37** (2.70 g, 8.0 mmol, 2.1 equiv) was dissolved in 60 mL of THF and cooled to -78 °C and *n*-BuLi (2.5 M in hexane, 3.2 mL, 8.0 mmol, 2.1 equiv) was added dropwise. The red solution was stirred for 30 min before adding Cbz-Pro-OMe (1.00 g, 3.80 mmol, 1.00 equiv) as a neat oil. The orange solution was stirred for 2 h before quenching with 5 mL of saturated NH_4Cl. The mixture was diluted with 100 mL of water then extracted three times with 100 mL of DCM. The combined organic layers were dried over $MgSO_4$, filtered, concentrated, and then subjected to flash chromatography, 20 to 50% EtOAc in hexanes, to give **1.58** as 1.60 g (75%) of a white amorphous solid. In $CDCl_3$ at room temperature this sulfonamide existed as a mixture of conformational isomers, but in $(CD3)_2SO$ the rotamers resolved at 120 °C. ^1H NMR ($CDCl_3$, 300 MHz) (7:3 ratio of rotamers) δ: 7.35 (m, 5H), 7.20 (m, 4H), 6.88 (d, 4H, J = 8.5 Hz), 5.18 (d, 0.7H, J = 12.4 Hz), 5.12 (d, 0.7H, J = 12.4 Hz), 5.08 (m, 0.6H), 4.57 (dd, 0.7H, J = 7.9, 5.6 Hz), 4.3-4.0 (m, 5H), 3.82 (m, 7H), 3.60 (m, 2H), 2.7-1.8 (m, 4H); $^{13}C\{^1H\}$ NMR ($(CD3)_2SO$, 125 MHz, 120 °C): δ 198.6, 159.7, 154.7, 137.4, 130.2, 128.8, 128.7, 128.2, 127.9, 114.7, 66.9, 66.1, 60.3, 55.9, 51.2, 47.3, 28.8, 23.9; HRMS-ESI$^+$ (*m/z*) calcd for $C_{30}H_{38}N_3O_7S$ $(M + NH_4)^+$ 584.2425, found 584.2427.



tert-Butyl (S)-2-(2-(N-benzhydrylsulfamoyl)acetyl)pyrrolidine-1-carboxylate (**1.59**) *n*-BuLi (2.5 M in hexane, 12.9 mL, 33 mmol, 3 equiv) was dissolved in 20 mL of THF at -40 °C, hydrogen chloride (4 M in dioxane, 2.7 mL, 10.9 mmol, 1 equiv) was added. Sulfonamide **1.38** (2.81 g, 10.9 mmol, 1 equiv) was added as solution in 20 mL of THF to gave a red solution which was stirred at -40 °C for 30 min before adding Boc-Pro-OMe (1.0 g, 4.3 mmol, 0.40 equiv) as a solution in 10 mL of THF. The reaction was stirred for an additional 1 hour before quenching with 10 mL of saturated NH_4Cl solution. The mixture was diluted with 150 mL of water and extracted 3 times with 100 mL DCM. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, 0 to 50% EtOAc in DCM, to give 1.66 g (80%) of **1.59** as a white amorphous solid. ^1H NMR ($CDCl_3$, 300 MHz)(4:5 mixture of rotamers): δ 7.30 (m, 10H), 6.65 (d, 0.5H, J = 8.2 Hz), 6.16 (d, 0.4H, J = 8.9 Hz), 5.77 (d, 1H, J = 8.9 Hz), 4.44 (m, 0.5H), 4.06 (m, 1H), 3.77 (m, 1.5H), 3.35 (m, 2), 1.96 (m, 1H), 1.71 (m, 3H), 1.38 (s, 5H), 1.30 (s, 4H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 200.3, 199.9, 154.6, 153.5, 140.9, 140.5, 140.5, 128.8, 128.8, 128.8, 128.8, 128.7, 128.7, 128, 128, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 80.9, 80.5, 65.9, 64.2, 61.7, 61.5, 60.9, 58.5, 47, 46.7, 29.4, 28.4, 28.3, 28.2, 24.4, 23.6; HRMSESI- (*m/z*) calcd for $C_{24}H_{29}N_2O_5S^-$ $(M - H)^-$ 457.1792, found 457.1781.



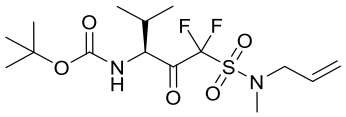
Allyl (S)-2-(2-(N-benzhydrylsulfamoyl)acetyl)pyrrolidine-1-carboxylate (**1.60**) *n*-BuLi (2.5 M, in hexane, 14 mL, 35.2 mmol, 7.5 equiv) was dissolved in 20 mL of THF at -78 °C, then hydrogen chloride (4 M in dioxane, 2.93 mL, 11.7 mmol, 2.5 equiv) was added. A white precipitate formed which dissolved when the temperature was raised to -40 °C. Sulfonamide **1.38** (3.06 g, 11.7 mmol, 2.5 equiv) was added as solution in 20 mL of THF to give a red solution which was stirred at -40 °C for 30 min before adding Alloc-Pro-OMe (1.0 g, 4.6 mmol, 1 equiv) as a solution in 10 mL of THF. The reaction was stirred for an additional 1 hour before quenching with to 10 mL of saturated NH_4Cl solution. The mixture was diluted with 150 mL of water and extracted 3 times with 100 mL DCM. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography, 20 to 50% EtOAc in hexane, to give 1.34 g (66% yield) of **1.60** as a white amorphous solid. ^{1}H NMR ($CDCl_3$, 500 MHz)(7:3 ratio of rotamers): δ 7.33 (m, 10H), 6.53 (br, 0.7H), 6.13 (d, 0.3H, J = 7.1 Hz), 5.90 (m, 0.6H), 5.81 (m, 1.4H), 5.30 (d, 0.6H, J = 17.2 Hz), 5.19 (m, 1.4H), 4.49 (m, 2.7H), 4.29 (m, 0.3H), 4.07 (d, 0.7H, J = 14.8 Hz), 3.94 (d, 0.7H, J = 14.8 Hz), 3.86 (d, 0.3H, J = 15.5 Hz), 3.80 (d, 0.3 Hz, J = 15.5 Hz), 3.46 (m, 2H), 2.04 (m, 1H), 1.80 (m, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ (major rotamer)199.4, 154.9, 140.8, 140.5, 132.6, 128.8, 128.7, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 118.2, 117.6, 66.3, 64.7, 61.7, 60.9, 46.7, 28.2, 24.3 (minor rotamer) 199.6, 153.9, 140.4, 140.3, 132.5, 128.8, 128.7, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 118.2, 66.3, 65.5, 61.6, 59.0, 47.2, 29.2, 23.4; HRMS-ESI$^+$ (*m/z*) calcd for $C_{23}H_{30}N_3O_5S^+$ $(M + NH_4)^+$ 460.1901, found 460.1901.



tert-Butyl ((3S)-1-(N-allyl-N-methylsulfamoyl)-1-fluoro-4-methyl-2-oxopentan-3-yl)carbamate (**1.61**). NaH (60% dispersion in mineral oil, 0.030 g, 0.746 mmols, 2.6 equiv), was rinsed three times with 5 mL of THF then suspended in 5 mL of THF. This mixture was cooled to 0 °C and ß-ketosulfonamide **1.45** (0.100 g, 0.287 mmol, 1 equiv) was added and stirred for 20 min. After 20 min the mixture was cannulated into a flask containing Selectfluor (0.305 g, 0.862 mmols, 3.0 equiv) in acetonitrile (5 mL) under argon atmosphere at -10 °C with stirring. This mixture was warmed to room temperature and stirred 20 h then quenched with water (50 mL) and extracted three times with diethyl ether (50 mL). The combined organic layers were dried with sodium sulfate, filtered, and concentrated. Purification using flash chromatography 15 to 35% EtOAc in hexanes provided monofluoro ketone **29** as a colorless oil (1:1 mixture of diastereomers, 0.074 g, 70% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.80 (d, 2H, *J* = 48 Hz), 5.79 (m, 2H), 5.31 (m, 4H), 5.20 (d, 1H, *J* = 8.5 Hz), 5.05 (d, 1H, 8.7Hz), 4.71 (d, 1H, *J* = 8.9 Hz), 4.63 (dd, 1H, *J* = 6.2 Hz, 6.5 Hz), 3.95 (dd, 2H, *J* = 5.7 Hz, 15 Hz), 3.83 (m, 2H), 2.94 (s, 3H), 2.93 (s, 3H), 2.43 (bs, 1H), 2.23 (m, 1H), 1.45 (s, 18H), 1.05 (d, 3H, *J* = 6.7 Hz), 1.02 (d, 3H, *J* = 6.7 Hz), 0.89 (d, 3H, *J* = 6.7Hz), 0.83 (d, 3H, *J* = 6.7 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 198.3 (d, *J* = 19.0 Hz), 197.4 (d, *J* = 19.9 Hz), 155.7, 155.6, 132.0, 131.9, 119.8, 119.7, 99.5 (d, *J* = 225.7 Hz), 98.9 (d, *J* = 224.9 Hz), 80.4, 80.4, 62.6, 61.1, 53.4, 53.3, 34.8, 34.8, 28.9, 28.7, 28.2, 19.9, 19.8, 16.8, 16.3; ^{19}F NMR ($CDCl_3$, 282 Hz): δ -184.4 (d, 1F, *J* = 47.9 Hz), -185.5 (d, 1F, *J* = 50.8 Hz); *HRMS-ESI*$^{+}$ (*m/z*) calculated for $C_{15}H_{28}FN_2O_5S^{+}$: 367.1698 found: 367.1697.

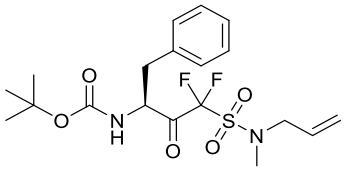
General procedure 1.4 for fluorination of ß-keto sulfonamides derived from amino acids.

To a solution of the ß-ketosulfonamide in dry DMF (0.025 M) CsF or TBAF 1 M in THF (4 equiv) was added followed by Selectfluor (4 equiv). The Reaction was stirred until complete by TLC (1 hour or less). Then the mixture was diluted with water then extracted with Et_2O (3x). The combined organic layers were washed with brine then dried over sodium or $MgSO_4$, filtered, and concentrated by rotary evaporation. flash chromatography of the residue provided ß-keto-difluorosulfonamides.

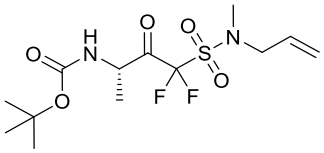


tert-Butyl (S)-(1-(N-allyl-N-methylsulfamoyl)-1,1-difluoro-4-methyl-2-oxopentan-3-yl)carbamate (**1.62**)**.** According to general procedure 1.4 using CsF, **1.62** was obtained as a colorless liquid (0.926 g, 84% yield) after flash chromatography (20% EtOAc, 80% hexane) from ketone **1.45** (1.00 g, 2.87 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.83-5.71 (m, 1H), 5.32 (d, 2H, *J* = 14.7 Hz), 5.06 (d, 1H, *J* = 9.3 Hz), 4.83 (d, 1H, *J* = 9.3 Hz), 3.95 (bs, 1H), 2.42-2.34 (m, 1H), 1.45 (s, 9H), 1.06 (d, 3H, *J* = 6.8 Hz), 0.84 (d, 3H, *J* = 6.8 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 195.4 (t, *J* = 26.3 Hz), 155.3, 131.8, 120.2, 115.6 (t, *J* = 297.6), 80.3, 61.0, 53.7, 35.0, 28.9, 28.1, 19.9, 16.0; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -106.5 (d, *J* = 246.6 Hz), -106.9 (d, *J* = 246.6 Hz), -108.0 (d, *J* = 246.6 Hz), -108.8 (d, *J* = 246.6 Hz); *HRMS-ESI*$^+$ (*m/z*) calcd for $C_{15}H_{27}F_2N_2O_5S$ $(M + H)^+$, 385.1603; found, 385.1603.

If TBAF was used instead of CsF then **1.62** was obtained in 78% yield.

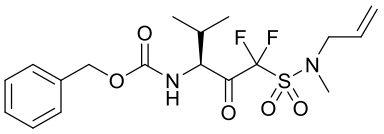


tert-Butyl (S)-(4-(N-allyl-N-methylsulfamoyl)-4,4-difluoro-3-oxo-1-phenylbutan-2-yl)carbamate (**1.63**)**.** According to general procedure 1.4 using CsF, **1.63** was obtained as a pale yellow amorphous solid (0.724 g, 84% yield) after flash chromatography (15% EtOAc, 85% hexane) from ketone **1.46** (0.800 g, 2.00 mmol).^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.36-7.24 (m, 3H), 7.16 (d, 2H, *J* = 6.8 Hz), 5.83-5.73 (m, 1H), 5.29 (d, 2H, *J* = 13.2 Hz), 5.12-4.85 (m, 2H), 3.92 (bs, 2H), 3.30 (bd, 1H, *J* = 13.9 Hz), 2.97 (s, 3H), 3.00-2.79 (m, 1H), 1.53 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 194.5 (t, *J*= 24.3 Hz), 154.7, 135.1, 131.7, 129.4, 128.7, 127.2, 120.2, 115.8 (t, *J* = 297.6 Hz), 80.5, 57.0, 53.7, 36.5, 35.0, 28.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -107.1 (d, *J* = 244.8 Hz), -107.2 (d, *J* = 244.8 Hz), -108.1 (d, *J* = 244.8 Hz), -108.3 (d, *J* = 244.8 Hz); *HRMS-ESI*$^{+}$ (*m/z*) calcd for $C_{15}H_{19}F_2N_2O_5S$ $(M - (CH_3)_3 + 2H)^+$, 377.0977; found, 377.0976.

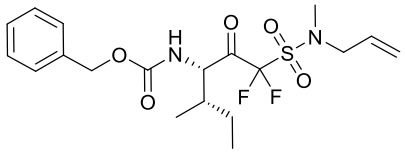


tert-Butyl (S)-(4-(N-allyl-N-methylsulfamoyl)-4,4-difluoro-3-oxobutan-2-yl)carbamate (**1.64**)**.** According to general procedure 1.4 using CsF, **1.64** was obtained as a white solid (0.131 g, 76% yield) after flash chromatography (20% EtOAc, 80% benzene) from ketone **1.47** (0.155 g, 0.484 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.80-5.68 (m, 1H), 5.29 (d, 1H, *J* = 15.7 Hz), 5.28 (d, 1H, *J* = 11.2 Hz), 5.09 (s, 1H), 4.81 (dq, 1H, *J* = 7.0, 7.0 Hz), 3.90 (bs, 2H), 2.95 (s, 3H), 1.41 (s, 1.5H, one half of the doublet corresponding to CH-C$\underline{H_3}$), 1.39 (s, 12H, 9H from (C$\underline{H_3}$)$_3$-C overlapping with one half of the doublet corresponding to CH-C$\underline{H_3}$); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 196.0 (t, *J* = 24.3 Hz), 154.7, 131.8, 120.2, 115.7 (t, *J* = 297.1 Hz), 80.4, 53.7, 52.3,

35.0, 28.2, 17.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -106.3, (d, *J* = 251.7 Hz), -106.7 (d, *J* = 246.6 Hz), -108.2 (d, *J* = 246.6 Hz), -108.8 (d, *J* = 253.5 Hz); *HRMS-ESI*$^+$ (*m/z*) calcd for $C_9H_{15}F_2N_2O_5S$ (M - $(CH_3)_3$ + 2H)$^+$, 301.0662; found, 301.0663.

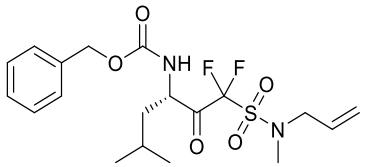


Benzyl (S)-(1-(N-allyl-N-methylsulfamoyl)-1,1-difluoro-4-methyl-2-oxopentan-3-yl)carbamate (**1.66**)**.** According to general procedure 1.4 using CsF, **1.66** was obtained as a colorless liquid (0.511 g, 93% yield) after flash chromatography (30% EtOAc, 70% hexane) from ketone **1.49** (0.500 g, 1.31 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.35 (s, 5H), 5.84-5.70 (m, 1H), 5.32-5.27 (m, 3H), 5.11 (s, 2H), 4.92 (dd, 1H, *J* = 3.7, 9.3 Hz), 3.92 (bs, 2H), 2.96 (s, 3H), 2.43-2.33 (m, 1H), 1.05 (d, 3H, *J* = 6.6 Hz), 0.82 (d, 3H, *J* = 6.8 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 195.0 (t, *J* = 24.7 Hz), 156.0, 136.0, 131.7, 128.6, 128.3, 128.2, 120.3, 119.6, (t, *J* = 297 Hz), 67.4, 61.5, 53.7, 35.0, 29.1, 19.9, 16.0; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -106.6 (d, *J* = 246.6 Hz), -107.3 (d, *J* = 244.8 Hz), -108.1 (d, *J* = 246.6 Hz), -108.5 (d, *J* = 244.8 Hz); *HRMS-ESI*$^+$ (*m/z*) calcd for $C_{18}H_{25}F_2N_2O_5S$ (M + H)$^+$, 419.1447; found, 419.1446.

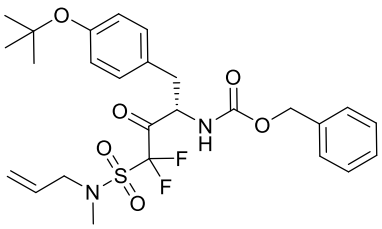


Benzyl ((3S,4S)-1-(N-allyl-N-methylsulfamoyl)-1,1-difluoro-4-methyl-2-oxohexan-3-yl)carbamate (**1.67**)**.** According to general procedure 1.4 using CsF **1.67** was obtained as a colorless oil (0.196 g, 90% yield) after flash chromatography (15% EtOAc, 85% hexane) from ketone 1.**50** (0.200 g, 0.504 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.33 (s, 5H), 5.80-5.71 (m, 1H), 5.40-5.25 (m, 3H), 5.10 (s, 2H), 4.90 (dd, 1H, *J* = 4.4, 9.3 Hz), 3.91 (bs, 1H), 2.95 (s, 3H),

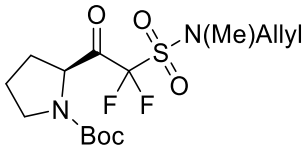
2.10 (bs, 1H), 1.38-1.21 (m, 1H), 1.06-0.95 (m, 4H, J = 6.6 Hz), 0.86 (t, 3H, J = 7.1 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 195.1 (t, J = 24.3 Hz), 156.0, 136.1, 131.8, 128.6, 128.3, 128.2, 120.2, 115.7 (t, J = 297.1 Hz), 67.3, 61.7, 53.7, 35.7, 35.0, 23.3, 16.2, 11.3; ^{19}F NMR ($CDCl_3$, 282 MHz): -106.2 (d, J = 246.6 Hz), -106.9 (d, J = 246.6 Hz), -108.1 (d, J = 246.6 Hz), -108.3 (d, J = 246.6 Hz); *HRMS-ESI*$^{+}$ (*m/z*) calcd for $C_{19}H_{27}F_2N_2O_5S$ $(M + H)^{+}$, 433.1603; found, 433.1604.



Benzyl (S)-(1-(N-allyl-N-methylsulfamoyl)-1,1-difluoro-5-methyl-2-oxohexan-3-yl)carbamate (**1.68***).* According to general procedure 1.4 using CsF **1.68** was obtained as a colorless oil (0.180 g, 82% yield) after flash chromatography (15% EtOAc, 85% hexane) from ketone **1.51** (0.200 g, 0.504 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32 (s, 5H), 5.87-5.69 (m, 1H), 5.38-5.25 (m, 3H), 5.10 (s, 2H), 5.03-4.18 (m, 1H), 3.91 (bs, 2H), 2.95 (s, 3H), 1.84-1.60 (m, 2H), 1.49-1.30 (m, 1H), 0.98 (d, 3H, J = 5.4 Hz), 0.93 (d, 3H, J = 5.6 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 195.6, (t, J = 23.7 Hz), 155.6, 135.9, 131.7, 128.4, 128.1, 128.0, 120.1, 115.7, (t, J = 297.1, Hz), 67.1, 55.3, 53.6, 39.6, 34.9, 24.8, 23.1, 20.9; ^{19}F NMR ($CDCl_3$, 282 MHz): -106.8 (d, J = 246.6 Hz), -107.58, -107.6, (d, J = 246.6 Hz), *HRMS-ESI*$^{+}$ (*m/z*) calcd for $C_{19}H_{27}F_2N_2O_5S$ $(M + H)^{+}$, 433.1603; found, 433.1603.

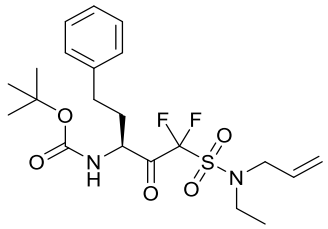


Benzyl (S)-(4-(N-allyl-N-methylsulfamoyl)-1-(4-(tert-butoxy)phenyl)-4,4-difluoro-3-oxobutan-2-yl)carbamate (**1.69**). According to general procedure 1.4 using CsF **1.69** was obtained as a colorless oil (0.180 g, 82% yield) after flash chromatography (15% EtOAc, 85% hexane) from ketone **1.52** (0.200 g, 0.504 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32 (s, 5H), 5.87-5.69 (m, 1H), 5.38-5.25 (m, 3H), 5.10 (s, 2H), 5.03-4.18 (m, 1H), 3.91 (bs, 2H), 2.95 (s, 3H), 1.84-1.60 (m, 2H), 1.49-1.30 (m, 1H), 0.98 (d, 3H, *J* = 5.4 Hz), 0.93 (d, 3H, *J* = 5.6 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 195.6, (t, *J* = 23.7 Hz), 155.6, 135.9, 131.7, 128.4, 128.1, 128.0, 120.1, 115.7, (t, *J* = 297.1, Hz), 67.1, 55.3, 53.6, 39.6, 34.9, 24.8, 23.1, 20.9; ^{19}F NMR ($CDCl_3$, 282 MHz): -106.8 (d, *J* = 246.6 Hz), -107.58, -107.6, (d, *J* = 246.6 Hz), HRMS-ESI^{+} (*m/z*) calcd for $C_{19}H_{27}F_2N_2O_5S$ $(M + H)^{+}$, 433.1603; found, 433.1603.

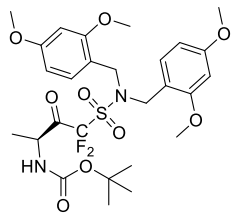


tert-Butyl (R)-2-(2-(N-allyl-N-methylsulfamoyl)-2,2-difluoroacetyl)pyrrolidine-1-carboxylate (**1.70**). According to general procedure 1.4 using CsF **1.70** was obtained as a colorless oil (756 mg, 69%) from 1.00 g (2.9 mmol) of **1.53.** ^{1}H NMR ($CDCl_3$, 300 MHz)(6:4 ratio of rotamers): 5.79 (m, 1H), 5.31 (m, 2H), 4.88 (m, 1H), 3.95 (br, 2H), 3.50 (m, 2H), 3.00 (s, 1.8H), 2.97 (1.2H), 2.31 (m, 1H), 2.12 (m, 1H), 1.90 (m, 2H), 1.43 (s, 5.4H), 1.39 (s, 3.6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ, (major rotamer)195.0 (m), 153.1, 131.7, 120.2, 115.8(ap t, *J* = 296.9 Hz), 80.8, 61.3, 53.7, 46.6, 35.0, 30.2, 28.1, 23.3(minor rotamer)194.9 (m), 154.0, 131.9, 119.9, 115.6

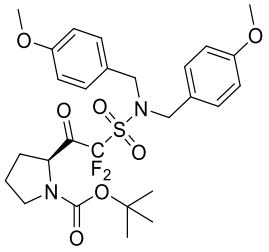
(ap t, J = 297.4 Hz), 80.2, 60.9, 53.7, 46.7, 35.0, 29.3, 28.3, 24.5; ^{19}F NMR ($CDCl_3$, 282 MHz): δ (major rotamer) -106.8 (d, J = 247.8 Hz), -108.8 (d, J = 247.7 Hz); (minor rotamer) -107.6 (d, J = 248.3 Hz), -108.6 (d, J = 247.2 Hz) HRMS-ESI$^+$ (*m/z*) calcd for $C_{15}H_{24}NO_5SF_2Na^+$ $(M + Na)^+$ 405.1266, found 405.1261.



tert-Butyl (S)-(1-(N-allyl-N-ethylsulfamoyl)-1,1-difluoro-2-oxo-5-phenylpentan-3-yl)carbamate (**1.71**)**.** According to general procedure 1.4 using CsF, **1.71** was obtained as a colorless oil (0.185 g, 85% yield) after flash chromatography (20% EtOAc, 80% hexane) from ketone **1.54** (0.200 g, 0.471 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.28-7.16 (m, 5H), 5.87-5.70 (m, 1H), 5.29 (d, 1H, J = 17.8 Hz), 5.27 (d, 1H, J = 8.3 Hz), 5.16 (d, 1H, J = 6.6 Hz), 4.88 (m, 1H), 3.96 (bd, 2H, J = 5.4 Hz), 3.42 (dd, 1H, J = 6.6, 7.1 Hz), 2.80-2.60 (m, 2H), 2.36-2.20 (m, 1H), 1.94-1.79 (m, 1H), 1.44 (s, 9H), 1.19 (t, 3H, J = 7.1 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 195.2 (t, J = 24.8 Hz), 155.0, 140.3, 132.5, 128.6, 128.5, 126.3, 120.0, 115.3 (t, J = 297.1 Hz), 80.5, 56.2, 50.4, 42.9, 32.6, 31.5, 28.2, 13.8; ^{19}F NMR ($CDCl_3$, 282 MHz): -107.3 (d, J = 244.8 Hz), -107.4, (d, J = 243.1 Hz), -108.6 (d, J = 243.1 Hz), -108.9 (d, J = 244.8 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{21}H_{31}F_2N_2O_5S$ $(M + H)^+$, 461.1916; found, 461.1917.

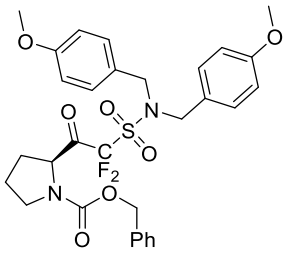


tert-Butyl (S)-(4-(N,N-bis(2,4-dimethoxybenzyl)sulfamoyl)-4,4-difluoro-3-oxobutan-2-yl)carbamate (**1.72**)**.** According to general procedure 1.4 using TBAF, **1.72** was obtained as a white solid (743 mg, 64%) after flash chromatography (25% EtOAc in hexane) from ketone **1.56** (1.1 g, 1.94 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.11 (d, 2H, *J* = 8.4 Hz), 6.36 (dd, 2H, *J* = 8.4, 2.3 Hz), 6.27 (d, 2H, 6.3 Hz), 5.08 (br, 1H), 4.87 (br, 1H), 4.50 (s, 4H), 3.76 (s, 6H), 3.65 (s, 6H), 1.43 (m, 12H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 196.0 (t, *J* = 23.2 Hz), 160.6, 158.2, 154.7, 130.5, 116.0, 115.8 (t, *J* = 297.9 Hz), 103.9, 97.8, 80.3, 55.4, 55.0, 52.3, 47.0, 28.2, 17.5; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -106.3 (d, *J* = 240.9 Hz), -108.0 (d, *J* = 240.8 Hz); HRMS-ESI^{+} (*m/z*) calcd for $C_{27}H_{36}F_2N_2NaO_9S^{+}$ $(M + Na)^{+}$ 625.2002, found 625.1995.

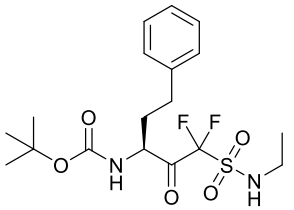


tert-Butyl (S)-2-(2-(N,N-bis(4-methoxybenzyl)sulfamoyl)-2,2-difluoroacetyl)pyrrolidine-1-carboxylate (**1.73**). According to general procedure 1.4 using CsF, **1.73** was obtained as an amorphous white solid (1.15 g, 87%) after flash chromatography (30% EtOAc in hexane) from ketone **1.57** (1.2 g, 2.25 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.11 (d, 4H, *J* = 8.7 Hz), 6.83 (d, 4H, *J* = 8.6 Hz), 4.93 (m, 1H), 4.36 (s, 4H), 3.80 (s, 6H), 3.52 (m, 1H), 2.35 (m, 1H), 2.18 (m,1H), 1.91 (m,2H), 1.42 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ (major rotamer) 194.8 (t, *J* = 23.6 Hz), 159.5, 153.0, 130.0, 126.0, 115.4 (t, *J* = 296.5 Hz), 113.9, 80.6, 61.4, 55.0, 50.6,

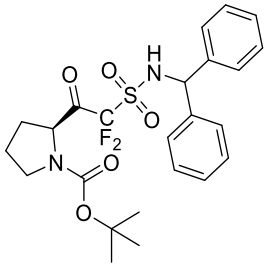
46.6, 30.3, 28.1, 23.2; (minor rotamer) 195.2 (t, J = 22.7), 159.4, 153.9, 130.1, 126.2, 115.4 (t, J = 297.8), 113.9, 80.0, 60.7, 55.0, 50.5, 46.8, 29.7, 28.2, 24.5 ^{19}F NMR ($CDCl_3$, 282 MHz)(5:4 mixture of rotamers): δ (major rotamer) -106.5 (d, J = 244.3 Hz), -108.3 (d, J = 244.4 Hz); (minor rotamer) -107.7 (d, J = 240.7 Hz), -108.8 (d, J = 240.5 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{27}H_{38}N_2O_7SF_2^+$ $(M + NH_4)^+$ 586.2393, found 586.2393.



Benzyl (S)-2-(2-(N,N-bis(4-methoxybenzyl)sulfamoyl)-2,2-difluoroacetyl)pyrrolidine-1-carboxylate (**1.74**)**.** According to general procedure 1.4 using CsF, **1.74** was obtained as an amorphous white solid (1.41 g, 89%) after flash chromatography (30% EtOAc in hexane) from ketone **1.58** (1.5 g, 2.65 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.33 (m, 5H), 7.10 (m, 4H), 6.84 (d, 4H, J = 7.6 Hz), 5.08 (m, 3H), 4.34 (d, 4H, J = 11 Hz), 3.80 (s, 6H), 3.59 (m, 2H), 2.36 (m, 1H), 2.18 (m, 1H), 1.96 (m, 2H); ^{13}C{^{1}H} NMR ($(CD_3)_2SO$, 125 MHz): δ (Major Rotamer) 194.8 (t, J = 22.9 Hz), 159.1, 153.9, 136.7, 129.9, 128.5, 128.0, 127.6, 126.5, 115.2 (t, J = 296.6 Hz), 114.0, 66.6, 61.3, 55.2, 50.8, 46.5, 29.3, 24.4; (minor rotamer) 194.8 (t, J = 22.9 Hz), 153.0 136.5 129.9 128.3 127.9 127.7 126.4, 115.3 (t, J = 296.6 Hz), 114.0 66.5 61.4 55.2 50.8 47.0 29.9 23.1; ^{19}F NMR ($CDCl_3$, 282 MHz)(10:9 mixture of rotamers): δ (minor rotamer) -107.8 (d, J = 241.4 Hz), -108.7 (d, J = 242.0 HZ); (major rotamer) -107.8 (d, J = 239.7 Hz), -109.0 (d, J = 239.6 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{30}H_{36}N_3O_7SF_2^+$ $(M + NH_4)^+$ 620.2237, found 620.2236.

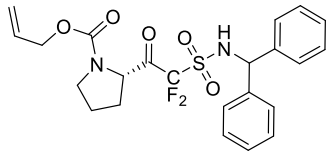


tert-Butyl (S)-(1-(N-ethylsulfamoyl)-1,1-difluoro-2-oxo-5-phenylpentan-3-yl)carbamate (**1.75**). According to general procedure 1.4 using TBAF, **1.75** was obtained as a white solid (0.078 g, 74% yield) after flash chromatography (15% EtOAc, 85% hexane) from ketone **1.28** (0.096 g, 0.250 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38-7.10 (m, 5H), 6.19 (s, 1H), 5.05 (d, 1H, *J* = 7.6 Hz), 4.78-4.73 (bt, 1H, *J* = 7.1 Hz), 3.35-3.10 (m, 2H), 2.84-2.61 (m, 2H), 2.32-2.18 (m, 1H), 1.89-1.71 (m, 1H), 1.43 (s, 9H), 1.43 (t, 3H, *J* = 7.1 Hz), 1.44 (s, 9H), 1.19 (t, 3H, *J* = 7.1 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 195.8 (t, *J* = 23.7 Hz), 155.9, 139.5, 128.7, 128.4, 126.6, 115.3 (t, *J* = 299.1 Hz), 81.5, 56.5, 39.3, 31.8, 28.1, 15.5; ^{19}F NMR ($CDCl_3$, 282 MHz): -108.3 (d, *J* = 244.8 Hz), -109.4, (d, *J* = 244.8 Hz); HRMS-ESI^{+} (*m/z*) calcd for $C_{18}H_{31}F_2N_3O_5S$ $(M + NH_4)^{+}$, 438.1869; found, 438.1862. If CsF was used instead of TBAF then **1.75** was obtained in a 68% yield.



tert-Butyl (S)-2-(2-(N-benzhydrylsulfamoyl)-2,2-difluoroacetyl)pyrrolidine-1-carboxylate (**1.76**). According to general procedure 1.4 using TBAF, **1.76** was obtained as a white amorphous solid (570 mg, 44%) after flash chromatography (15 to 20% EtOAc in hexane) from ketone **1.59** (1.2 g, 2.5 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz)(1:3 ratio of rotamers): δ 7.95 (s, 1H), 7.35 (m, 10H), 5.96 (m, 1H), 4.85 (m, 0.25H), 4.67 (m, 0.75H), 3.42 (m, 2H), 2.3-1.80 (m, 4H), 1.41 (m, 9H);

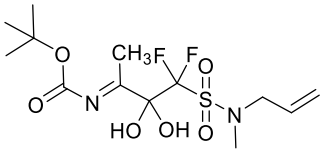
$^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ (major rotamer)194.4 (t, *J* = 22.5 Hz), 154.4, 141.0, 140.3, 128.7, 127.9, 127.4, 115.1 (t, *J* = 296.5 Hz), 81.1, 62.4, 61.2, 47.0, 29.4, 28.3, 24.3; (minor rotamer) 194.6 (t, *J* = 23.5 Hz), 153.4, 140.6, 140.5, 128.6, 127.8, 127.6, 114.2 (t, *J* = 294.3 Hz), 81.1, 62.5, 61.5, 46.6, 30.1, 28.2, 23.2 ^{19}F NMR ($CDCl_3$, 282 MHz)(1:3 ratio of rotamers): δ (minor rotamer) -106.7 (d, *J* = 252.7), -109.6 (d, *J* = 252.7 Hz); (major rotamer) -110.8 (d, *J* = 239.9 Hz) -111.7 (d, *J* = 238.6 Hz); HRMSESI- (*m/z*) calcd for $C_{24}H_{27}N_2O_5SF_2^-$ $(M - H)^-$ 493.1603, found 493.1610.



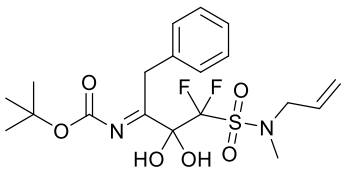
Allyl (S)-2-(2-(N-benzhydrylsulfamoyl)-2,2-difluoroacetyl)pyrrolidine-1-carboxylate (**1.77**). According to general procedure 1.4 using CsF, **1.77** was obtained as an amorphous white solid (665 mg, 41%) after flash chromatography (10 to 30% EtOAc in hexane) from ketone **1.60** (1.34 g, 3.00 mmol).^{1}H NMR ($CDCl_3$, 300 MHz)(1:5 ratio of rotamers): δ 7.56 (s, 1H), 7.31 (m, 10H), 5.79 (m, 2H), 5.19 (m, 2H), 4.88 (dd, 0.17H, *J* = 8.8, 4.4 Hz), 4.71 (m, 0.83H), 4.43 (m, 2H), 3.46 (m, 2H), 1.97 (m, 4H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ (major rotamer) 194.3 (t, *J* = 22.9 Hz), 154.7, 140.9, 140.1, 132.4, 128.7, 128.6, 127.9, 127.5, 117.9, 115.1 (t, *J* = 297.6 Hz), 66.6, 62.5, 61.7, 46.8, 29.4, 24.4; (minor rotamer) 194.3 (t, *J* = 297.6 Hz), 153.6, 140.9, 140.3, 132.6, 128.8, 128.7, 128.6, 128.1, 127.9, 127.5, 118.3, 114.5 (t, *J* = 297.2 Hz), 66.5, 62.5, 61.5, 47.0, 30.1, 23.3; ^{19}F NMR ($CDCl_3$, 282 MHz)(1:6 ratio of rotamers): δ (minor rotamer) -108.4 (d, *J* = 250.4 Hz), -109.2 (d, *J* = 250.0 Hz); (major rotamer) -110.6 (d, *J* = 239.5 Hz), -111.2 (d, *J* = 239.0 Hz); HRMS-ESI^+ (*m/z*) calcd for $C_{23}H_{28}N_3O_5SF_2^+$ $(M + NH_4)^+$ 496.1712, found 496.1713.

General procedure 1.5 for the preparation of iminoketones **1.78 – 1.80**.

To a solution of the ß-ketosulfonamide in dry DMF (0.025 M) Cs_2CO_3 (4 equiv) was added followed by Selectfluor (4 equiv). The reaction was stirred at room temperature for 1 hour. Then the mixture was diluted with water then extracted with Et_2O (3x). The combined organic layers were washed with saturated brine then dried (Na_2SO_4) and concentrated by rotary evaporation. flash chromatography of the residue provided imines **1.78 – 1.80.**

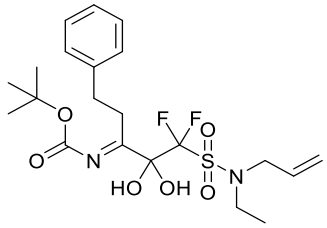


tert-Butyl (E)-(4-(N-allyl-N-methylsulfamoyl)-4,4-difluoro-3,3-dihydroxybutan-2-ylidene)-carbamate (**1.78**). According to general procedure 1.5, **1.78** was obtained as an amorphous white solid (0.041 g, 35% yield) after flash chromatography (30% EtOAc, 70% hexane) from ketone **1.47** (0.100 g, 0.312 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.70 (bs, 1H), 5.82-5.69 (m, 1H), 5.28 (bd, 2H, *J* = 13.4 Hz), 4.37 (s, 1H), 3.92 (bs, 1H), 2.95 (s, 3H), 1.73 (s, 3H), 1.48 (s, 9H), 1.43 (t, 3H, *J* = 7.1 Hz), 1.44 (s, 9H), 1.19 (t, 3H, *J* = 7.1 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 160.5, 148.7, 131.7, 121.7 (t, *J* = 295.1 Hz), 120.1, 83.1, 76.8 (t, *J* = 21.7 Hz), 53.6, 34.9, 27.9, 21.0; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -106.3 (bd, *J* = 243.3 Hz), -107.4 (d, *J* = 243.1 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{13}H_{22}F_2N_2O_6LiS$ $(M + Li)^+$, 379.1321; found, 379.1320.



tert-Butyl (E)-(4-(N-allyl-N-methylsulfamoyl)-4,4-difluoro-3,3-dihydroxy-1-phenylbutan-2-ylidene) carbamate (**1.79**). According to general procedure 1.5 **1.79** was obtained as an amorphous white solid (0.025 g, 45% yield) after flash chromatography (25% EtOAc, 75%

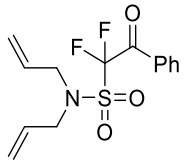
hexane) from ketone **1.46** (0.050 g, 0.126 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.35 (s, 1H), 7.40-7.21 (m, 5H), 5.89-5.73 (m, 1H), 5.34 (two overlapping doublets with J ~ 11.5 Hz, 2H), 4.2-3.7 (bs, 1H), 3.98 (s, 2H), 3.62 (d, 1H, J = 14.1 Hz), 3.36 (d, 1H, J = 14.1 Hz), 3.02 (s, 3H), 1.44 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 165.3, 148.2, 132.1, 131.7, 130.7, 128.7, 127.7, 121.7 (t, J = 297.1 Hz), 120.2, 82.80, 79.5 (t, J = 26.2 Hz), 53.6, 38.6, 35.0, 27.8; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -105.7; HRMS-ESI^{+} (*m/z*) calcd for $C_{15}H_{19}F_2N_2O_6S(M - C_4H_9 + 2H)^{+}$, 393.0926; found, 393.0933.



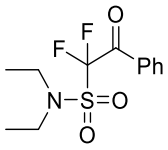
tert-Butyl (E)-(1-(N-allyl-N-ethylsulfamoyl)-1,1-difluoro-2,2-dihydroxy-5-phenylpentan-3-ylidene)carbamate (**1.80**). According to general procedure 1.5 **1.80** was obtained as an amorphous white solid (0.036 g, 64% yield) after flash chromatography (20% EtOAc, 80% hexane) from ketone **1.54** (0.050 g, 0.118 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.76 (s, 1H), 7.32-7.16 (m, 5H), 5.88-5.71 (m, 1H), 5.31 (d, 1H, J = 17.3 Hz), 5.30 (d, 1H, J = 7.7 Hz), 4.28 (s, 1H), 4.10-3.90 (m, 1H), 3.53-3.36 (m, 1H), 3.90-3.75 (m, 1H), 2.66-2.52 (m, 2H), 2.41-2.38 (m, 1H), 1.55 (s, 9H), 1.20 (t, 3H, J = 7.0 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 165.3, 148.5, 140.1, 132.2, 128.5, 128.4, 126.2, 120.7 (t, J = 296.5 Hz), 119.9, 83.1, 79.5 (t, J = 21.6Hz), 50.2, 42.8, 34.8, 28.8, 27.9, 13.6; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -107.2 (d, J = 243.1 Hz), -108.1 (d, J = 243.1 Hz); HRMS-ESI^{+} (*m/z*) calcd for $C_{21}H_{30}F_2N_2O_6NaS$ $(M + Na)^{+}$, 499.1685; found, 499.1686.

General procedure 1.6 Fluorination of benzoyl methane sulfonamides **1.41 - 1.44**.

The benzoyl methane sulfonamide was dissolved in dry DMF (0.25 M) and cooled to -15 °C before TBAF (1 M in THF, 4 equiv) followed by Selectfluor (4 equiv) were added. The reaction was stirred for 2-4 h until completed by TLC, then was diluted with four volumes of water. The mixture was extracted three times with four volumes of diethyl ether the then combined organic layers were washed three times with four volumes of water then dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, eluting with ethyl acetate and hexanes.

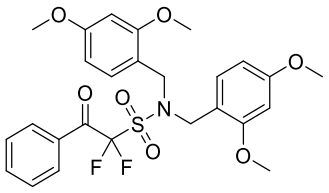


N,N-Diallyl-1,1-difluoro-2-oxo-2-phenylethane-1-sulfonamide (**1.81**) According to general procedure 1.6, **1.81** was obtained as white solid (1.31 g, 88%) after flash chromatography (10% ethyl acetate in hexanes) from **1.42** (1.32 g mg, 4.7 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.17 (d, 2H, *J* = 8.1 Hz), 7.67 (t, 1H, *J* = 7.5 Hz), 7.52 (t, 2H, *J* = 7.7 Hz), 5.82 (ddt, 2H, *J* = 16.9, 10.3, 6.6 Hz), 5.29 (m, 4H), 4.00 (d, 4H, *J* = 6.5 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ ν(F1) [ppm] 184.5 (t, *J* = 23.8 Hz), 135.1, 131.9, 131.7, 130.7, 130.6, 130.6, 128.7, 120.3, 116.8 (t, *J* = 295.5 Hz), 50.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -102.0; HRMS-ESI^{+} (*m/z*) calcd for $C_{14}H_{15}NO_3SF_2^{+}$ $(M + K)^{+}$ 354.0378, found 354.0372.



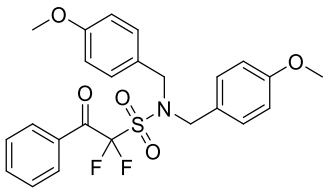
N,N-Diethyl-1,1-difluoro-2-oxo-2-phenylethane-1-sulfonamide (**1.82**) According to general procedure 1.6, **1.82** was obtained as white solid (600 mg, 73%) after flash chromatography (10%

ethyl acetate in hexanes) from **1.41** (714 mg, 2.8 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.17 (d, 2H, *J* = 7.6 Hz), 7.67 (t, 1H, *J* = 7.4 Hz), 7.51 (t, 2H, *J* = 7.7 Hz), 3.49 (br, 4H), 1.26 (t, 6H, *J* = 7.2 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 184.8 (t, *J* = 23.8 Hz), 135.2, 131.9, 130.7, 130.7, 130.7, 128.8, 117.1 (t, *J* = 295.3 Hz), 43.0, 14.3; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -102.0; HRMS-ESI^{+} (*m/z*) calcd for $C_{12}H_{16}NO_3SF_2^{+}$ $(M + H)^{+}$ 292.0814, found 292.0813.



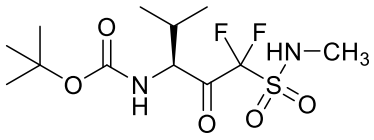
N,N-Bis(2,4-dimethoxybenzyl)-1,1-difluoro-2-oxo-2-phenylethane-1-sulfonamide (**1.83**)

According to general procedure 1.6, **1.83** was obtained as white solid (66 mg, 62%) after flash chromatography (10% ethyl acetate in hexanes) from **1.43** (100 mg, 0.2 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.17 (d, 2H, *J* = 7.9 Hz), 7.64 (t, 1H, *J* = 7.4 Hz), 7.49 (t, 2H, *J* = 7.8 Hz), 7.15 (d, 2H, *J* = 8.4 Hz), 6.36 (dd, 2H, *J* = 8.4, 1.8 Hz), 6.28 (d, 2H, *J* = 1.8 Hz), 4.55 (s, 4H), 3.75 (s, 6H), 3.66 (s, 6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 184.9 (t, *J* = 23.8 Hz), 160.6, 158.3, 134.9, 132.0, 130.8, 130.6, 128.7, 117.4 (t, *J* = 296.5 Hz), 116.1, 103.9, 97.8, 55.3, 54.9, 47.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -101.0; HRMS-ESI^{+} (*m/z*) calcd for $C_{26}H_{28}NO_7SF_2^{+}$ $(M + H)^{+}$ 536.1560, found 536.1551.



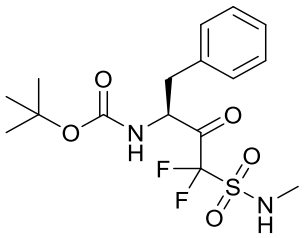
1,1-Difluoro-N,N-bis(4-methoxybenzyl)-2-oxo-2-phenylethane-1-sulfonamide (**1.84**) According to general procedure 1.6, **1.84** was obtained as white solid (67 mg, 72%) after flash chromatography (20% ethyl acetate in hexanes) from **1.44** (86 mg, 0.20 mmol). ^{1}H NMR

($CDCl_3$, 300 MHz) δ: 8.24 (d, 2H, *J* = 7.8 Hz), 7.68 (t, 1H, *J* = 7.33 Hz), 7.53 (t, 2H, *J* = 7.7 Hz), 7.14 (d, 4H, *J* = 8.4 Hz), 6.83 (d, 4H, *J* = 8.4 Hz), 4.39 (s, 4H), 3.80 (s, 6H);$^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz) δ: 184.6 (t, *J* = 23.8 Hz), 159.4, 135.1, 131.8, 130.7, 130.2, 128.7, 126.2, 117.1 (t, *J* = 296.1 Hz), 113.9, 77.4, 76.9, 76.5, 55.2, 50.6;^{19}F NMR ($CDCl_3$, 282 MHz) δ: -101.0; HRMS-ESI$^+$ (*m/z*) calcd for $C_{24}H_{23}NO_5SF_2^+$ $(M + Li)^+$ 482.1420, found 482.1420.



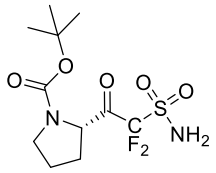
tert-Butyl (S)-(1,1-difluoro-4-methyl-1-(N-methylsulfamoyl)-2-oxopentan-3-yl)carbamate (**1.85**)

To a solution of **1.62** (50 mg, 0.130 mmol, 1 equiv) and DMBA (110 mg, 0.71 mmol, 5 equiv) in 2.5 mL dry acetonitrile in a pressure tube $Pd(PPh_3)_4$ (15 mg, 0.0130, 0.1 equiv) was added. The mixture was heated to 100 °C for 24 h. After cooling, the mixture was diluted with EtOAc and washed with sat. $NaHCO_3$ (3x), water (1x) and brine (1x), dried (Na_2SO_4) and concentrated by rotary evaporation. The residue was purified by flash chromatography (20% EtOAc, 80% hexane) to give **1.85** as a white amorphous solid (36 mg, 81%). ^1H NMR (DMSO-d_6, 300 MHz): δ 6.02 (bs, 1H), 4.98 (d, 1H, *J* = 8.6 Hz), 4.73 (dd, 1H, *J* = 2.9, 7.6 Hz), 2.92 (d, 3H, *J* = 3.9 Hz), 2.43-2.31 (m, 1H), 1.42 (s, 9H), 1.08 (d, 3H, *J* = 6.6 Hz), 0.85 (d, 3H, *J* = 6.8 Hz), $^{13}C\{^1H\}$ NMR (DMSO-d_6, 75 MHz): δ 195.5 (t, *J* = 22.7 Hz), 156.1, 115.2 (t, *J* = 297.0 Hz), 81.2, 61.4, 30.1, 28.6, 28.1, 19.9, 16.1; ^{19}F NMR (DMSO-d_6, 282 MHz): δ -105.8 (d, *J* = 249.6 Hz), -106.0 (d, *J* = 245.0 Hz), -110.2 (d, *J* = 246.1 Hz), -110.8 (d, *J* = 245.3 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{12}H_{23}F_2N_2O_5S$ $(M + H)^+$, 345.1290; found, 345.1290.



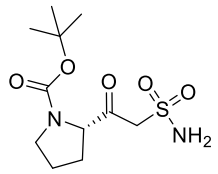
tert-Butyl (S)-(4,4-difluoro-4-(N-methylsulfamoyl)-3-oxo-1-phenylbutan-2-yl)carbamate (**1.86**)

To a solution of **1.63** (50 mg, 0.140 mmol, 1 equiv) and DMBA (110 mg, 0.71 mmol, 5 equiv) in 2.5 mL dry acetonitrile in a pressure tube $Pd(PPh_3)_4$ (15 mg, 0.0140, 0.1 equiv) was added. The mixture was heated to 100 °C for 24 h. After cooling, the mixture was diluted with EtOAc and washed with sat. $NaHCO_3$ (3x), water (1x) and brine (1x), dried (Na_2SO_4) and concentrated by rotary evaporation. The residue was purified by flash chromatography (30% EtOAc, 70% hexane) to give **1.86** as a white amorphous solid (41 mg, 76%). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.36-7.24 (m, 3H), 7.16 (d, 2H, *J* = 6.8 Hz), 6.10 (bs, 1H), 5.10-5.01 (m, 1H), 4.92 (d, 1H, *J* = 6.3 Hz), 3.30 (dd, 1H, *J* = 3.4, 17.1 Hz), 2.94-2.77 (m, 4H), 1.36 (s, 9H), $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 195.1 (t, *J* = 23.2 Hz), 155.6, 134.4, 129.2, 129.0, 127.6, 115.5 (t, *J* = 298.1 Hz), 83.4, 57.6, 36.0, 30.0, 28.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -106.8 (d, *J* = 246.6 Hz), -108.2 (d, *J* = 246.6 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{16}H_{23}F_2N_2O_5S$ $(M + H)^+$, 393.1290; found, 393.1290.



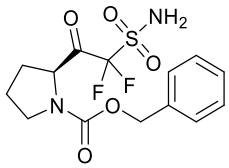
tert-Butyl (S)-2-(2,2-difluoro-2-sulfamoylacetyl)pyrrolidine-1-carboxylate (**1.87**). Sulfonamide **1.76** (200 mg, 0.4 mmol, 1 equiv) was dissolved in 5 mL of THF and 15 mL of EtOH. DIPEA (70 µL, 0.40 mmol, 1 equiv) followed by $Pd(OH)_2$ 20 wt% on carbon (56 mg, 0.08 mmol, 0.2 equiv). The mixture was stirred under a hydrogen atmosphere overnight then was filtered

through celite and rinsed with 100 mL of EtOAc. The eluent was washed once with 50 mL of 5% $NaHCO_3$ and once with 50 mL of 0.1 M HCl. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness then the residue was purified by flash chromatography using 20% EtOAc in hexanes to give **1.87** as (100 mg, 76%) of a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz)(4:1 mixture of rotamers): δ 6.28 (br, 1.6H), 5.64 (br, 0.4H), 5.07 (dd, 0.8H, *J* = 8.7, 4.1 Hz), 4.90 (dd, 0.2H, *J* = 8.5, 4.1 Hz), 3.57 (m, 1H), 3.46 (m, 1H), 2.37 (m, 1H), 1.98 (m, 3H), 1.45 (s, 7.2H), 1.41 (s, 1.8H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ (major rotamer) 194.9 (ap t, *J* = 23.0 Hz), 154.6, 114.7 (ap t, *J* = 295.9 Hz), 81.5, 61.6, 47.0, 29.3, 28.3, 24.4; (minor rotamer) 194.9 (ap t, *J* = 23.0 Hz), 154.6, 114.7 (ap t, *J* = 295.9 Hz), 81.2, 61.6, 46.5, 29.8, 28.2, 23.3; ^{19}F NMR ($CDCl_3$, 472 MHz): (minor rotamer) δ -106.7 (d, *J* = 257.6 HZ), -111.9 (d, *J* = 257.6 Hz); (major rotamer) -112.8 (apparent singlet); HRMS-ESI$^+$ (*m/z*) calcd for $C_{11}H_{17}N_2O_5SF_2^+$ $(M + H)^+$ 327.0821, found 327.0828.



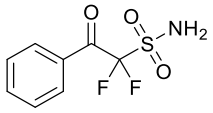
tert-Butyl (S)-2-(2-sulfamoylacetyl)pyrrolidine-1-carboxylate (**1.27**). Prepared by deprotection of **1.59**. Sulfonamide **1.59** (830 mg, 1.81 mmol, 1 equiv) was dissolved in 60 mL of EtOH and 20 mL of THF. DIPEA (320 µL, 1.81 mmol, 1 equiv) was added followed by $Pd(OH)_2$ 20wt% on carbon (254 mg, 0.36 mmol, 0.2 equiv) was added and the reaction was stirred under hydrogen atmosphere for 36 h. The mixture was filtered through celite concentrated. The residue was purified by flash chromatography, 20 to 50% EtOAc in hexanes to give **1.27** as 320 mg (60% yield) of a white solid. 113 mg (14%) of unreacted starting material was recovered. ^{1}H NMR ($CDCl_3$, 300 MHz)(7:3 ratio of rotamers): δ 5.64 (br, 2H), 4.60 (m, 0.7H), 4.40 (d, 0.7, *J* = 14.6

Hz), 4.26 (m, 0.9H), 4.11 (d, 0.7H, *J* = 14.7 Hz), 3.45 (m, 2H), 2.17 (m, 1H), 1.89 (m, 3H), 1.40 (s, 6.3H), 1.38 (s, 2.7H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ (major rotamer) 200.2, 154.7, 80.8, 64.6, 61.8, 47.0, 28.2, 28.1, 24.2; (minor rotamer) 200.8, 153.5, 81.1, 66.1, 59.4, 46.7, 29.3, 28.2, 23.6; HRMS-ESI$^+$ (*m/z*) calcd for $C_{11}H_{20}N_2O_5SNa^+$ $(M + Na)^+$ 315.0996, found 315.0984.



Benzyl (S)-2-(2,2-difluoro-2-sulfamoylacetyl)pyrrolidine-1-carboxylate (**1.88**). Protected sulfonamide **1.74** (250 mg, 0.415 mmol, 1 equiv) was dissolved in 20 mL of CH_3CN and cooled to 0 °C. CAN (1.82 g, 3.32 mmol, 8 equiv) was added dissolved in 8 mL of water. The mixture was stirred in an ice bath for 4 h until complete by TLC. 50 mL of water was added and the reaction was extracted 3 times with 30 mL of EtOAc, the combined organic layers were washed with 50 mL of water and 50 mL of brine then dried over $MgSO_4$, filtered, and concentrated. Residue was purified by flash chromatography, 5 to 50% EtOAc in hexanes. Fractions containing product were pooled and further purified by reverse phase chromatography using a Biotage Isolera One Flash purification system including a C-18 reversed-phase preparative Biotage column (30 g) using a gradient of 5 to 100% methanol over 20 min at 25 mL per minute (R_T = 14 min) giving **1.88** as 21 mg of a colorless oil (14% yield). This existed as a 1:8 ratio of rotamers as determined by ^{19}F NMR. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ; 6.74 (m, 5H), 5.59 (bs, 2H), 5.1 (m, 3H), 3.98 (m, 2H), 3.09 (m, 1H), 2.81 (m, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ ; 194.6 (ap t, *J* = 23.0 Hz), 155.1, 135.8, 128.6, 128.3, 127.9, 114.8 (ap t, *J* = 297.6 Hz), 67.9, 62.1, 47.0, 29.4, 24.5; ^{19}F NMR ($CDCl_3$, 282 MHz): δ (minor rotamer) -109.4 (d, 1F, *J* = 254.6 Hz), -110.8 (d, 1F, *J* = 254.0 Hz); (major rotamer) -112.3 (d, 1F, *J* = 239.5 Hz), -113.1 (d, 1F, *J*

= 240.0 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{14}H_{16}O_5N_2F_2NaS^+$ $(M + Na)^+$ 385.0640, found 385.0632.



1,1-Difluoro-2-oxo-2-phenylethane-1-sulfonamide (**1.89**). Protected sulfonamide **1.84** (86 mg, 0.16 mmol) was dissolved in 2 mL of TFA and cooled to 0 °C, 3 mL of TFA was then added dropwise and the mixture was stirred at room temperature for 2 h until complete. The reaction gradually developed an insense purple color. The reaction was diluted with 50 mL of toluene and 50 mL of *i*PrOH and concentrated in the presence of silica then purified by flash chromatography, 10 to 30% EtOAc in hexanes to give **1.89** as a white solid. 30 mg (79% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ ; 8.15 (dd, 2H, *J* = 8.5, 1.2 Hz), 7.73 (tt, 1H, *J* = 7.5, 1.4 Hz), 7.57 (t, 2H, *J* = 7.8 Hz), 5.09 (br, 2H); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ ; 185.2 (t, *J* = 24.7 Hz), 135.7, 131.4, 130.6 (t, *J* = 3.1 Hz), 129.0, 115.0 (t, *J* = 297.0 Hz); ^{19}F NMR ($CDCl_3$, 282 MHz): δ -102.8 (s, 2F); HRMS-ESI$^+$ (*m/z*) calcd for $C_8H_7F_2NNaO_3S^+$ $(M + Na)^+$ 258.0007, found 257.9997.

Chapter 2 — Synthesis of ß-hydroxy-α,α-difluorosulfonamides

2.1 — Introduction

Much like the analogous ketones discussed in Chapter 1, ß-hydroxy-α,α-difluorosulfonamides are a relatively unexplored class of molecules that have been reported only once. If these molecules could be easily accessed then we would expect them to be of interest because ß-hydroxysulfonamides and ß-hydroxy-α,α-difluoroamides are known to be biologically active. The synthesis of ß-hydroxy-α,α-difluorosulfonamides, with emphasis on those derived from amino acids in diastereomerically pure fashion, will be the subject of this chapter.

2.1.1 — ß-hydroxy-α,α-difluorosulfonamides

To the best of our knowledge, there is only a single report describing the synthesis of ß-hydroxy-α,α-difluorosulfonamides, and this report was not concerned with their biological activity.[71] On the other hand, closely related groups of molecules have been prepared and these are known to be enzyme inhibitors. The two most closely related groups are nonfluorinated ß-hydroxysulfonamides and ß-hydroxy-α,α-difluoroamides. Numerous ß-hydroxy-α,α-difluoroamides have been investigated as renin inhibitors, and examples are known with IC_{50} values in the low or sub-nanomolar region, such as peptidomimetic **2.1** (Figure 2.1).[28,72-75] Similarly, ß-hydroxysulfonamide-based renin inhibitors, such as sulfonamide **2.2**, have been investigated as treatments for hypertension (Figure 2.1).[76] It is therefore expected that ß-

hydroxy-α,α-difluorosulfonamides of appropriate structure might be potent inhibitors of renin, and perhaps other enzymes as well.

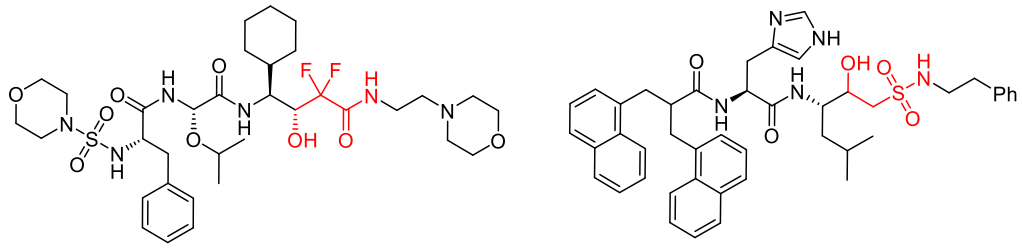
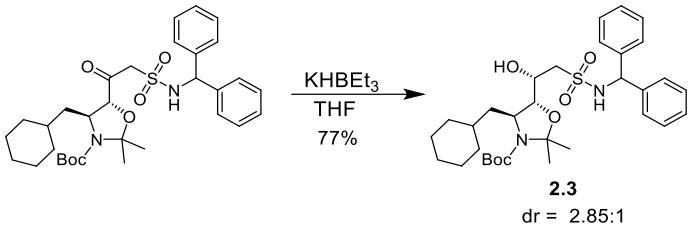


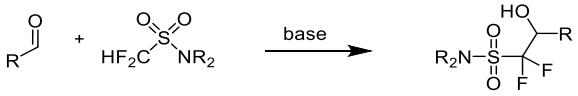
Figure 2.1. Structure ß-hydroxy-α,α-difluoroamide and ß-hydroxysulfonamide renin inhibitors.

There are two approaches to the synthesis of ß-hydroxy-α,α-difluorosulfonamides that are obvious to us. The first is by reduction of the analogous ketones. This method has been used previously for the synthesis of non-fluorinated ß-hydroxysulfonamide **2.3** (Scheme 2.1).[31] The synthesis of the ß-keto-α,α-difluorosulfonamides was discussed in the previous chapter and we expect that their reduction should be feasible and that, with sufficient experimentation, it might even be accomplished with a high dr. However, as part of our larger interest in peptidomimetics, a route that would be compatible with as many amino acid side chains as possible was desirable. The ß-keto-α,α-difluorosulfonamide derivatives of amino acids that can be accessed by the electrophilic fluorination route are limited to those which have side chains that can tolerate electrophilic fluorination and reaction with excess alkyl lithium, which does not include, for example, methionine and aspartate derivatives. Additionally, it is equally feasible that ß-hydroxy-α,α-difluorosulfonamides could be oxidized to ketones providing a synthesis that is complementary to that described in Chapter 1.



Scheme 2.1. Synthesis of ß-hydroxysulfonamides via carbonyl reduction.

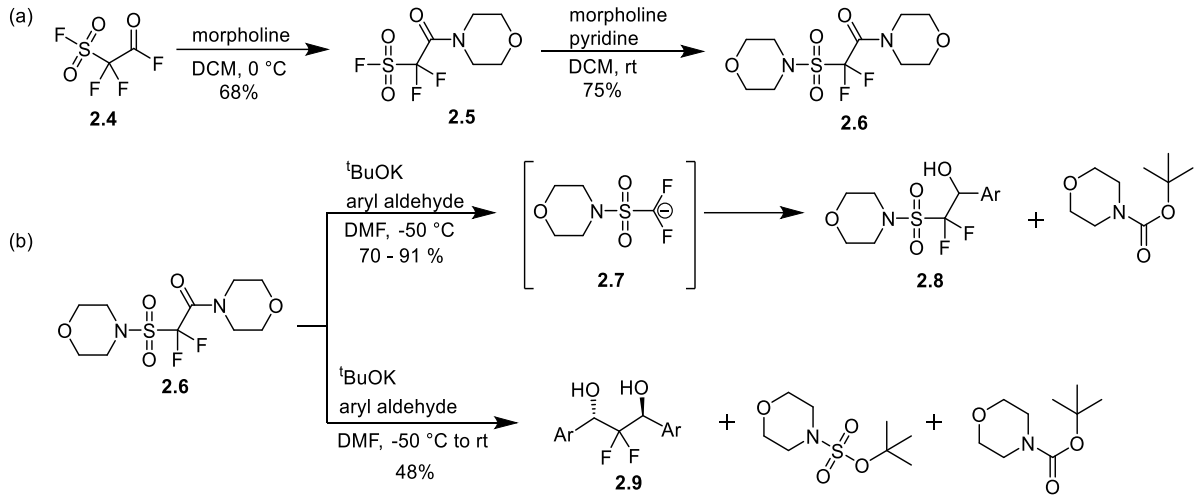
The second approach involves reacting carbanions of difluoromethane sulfonamides with aldehydes (Scheme 2.2). This approach would have the advantage of using the same fluorinated precursor for every derivative and would avoid late stage electrophilic fluorination which is problematic for certain substrates.



Scheme 2.2. General synthesis of ß-hydroxy-α,α-difluorosulfonamides.

An approach that is similar to that outlined in Scheme 2.2 was reported by Li and Liu in 2007, and this is the only report describing the synthesis of this class of compounds.[71] In their approach, the precursor **2.6** was prepared in a double nucleophilic displacement of fluorides from **2.4** in two steps (Scheme 2.3a). Amide **2.6** was reacted with an excess of potassium *tert*-butoxide in DMF in the presence of aryl aldehydes at low temperatures to give *tert*-butyl morpholine carbamate and difluoromethane sulfonamide carbanion **2.7**, which reacts with the aromatic aldehydes to give **2.8** in good yield (Scheme 2.3b). If the temperature was allowed to rise to rt then the sulfur atom was also attacked by *tert*-butoxide to give *tert*-butyl morpholine sulfonate with a second difluoromethane carbanion as a leaving group that can react with a second aldehyde to give anti-1,3-diols **2.9.** This method suffers from significant limitations. While **2.4** is commercially available, it is expensive, toxic, and volatile. The reaction requires anhydrous DMF and a 3-4 fold excess of aldehyde and base which is problematic if the aldehyde is

expensive or must be prepared. Furthermore, this approach failed entirely using enolizable aldehydes which would be required for our purposes. We reasoned that it should be possible to generate carbanion **2.7** from a difluoromethane sulfonamide and a suitable base because this approach was successful with the methane sulfonamides described in Section 1.2.3 and the difluoromethane sulfonamides should be more acidic due to the presence of electron withdrawing groups.



Scheme 2.3. Literature synthesis of ß-hydroxy-α,α-difluorosulfonamides.

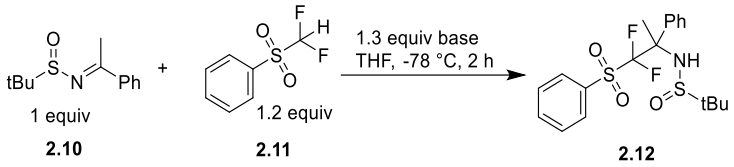
2.1.2 — Fluorinated carbanions as nucleophiles

Fluoroalkylation chemistry is markedly different from standard alkylation chemistry. A complete discussion of this topic is far beyond the scope of this work and has been reviewed.[77] There are, however, important features that must be summarized here regarding fluoroalkyl nucleophiles. Relative to alkyl carbanions, fluoroalkyl carbanions are harder nucleophiles and are kinetically unstable, and both of these factors make nucleophilic fluoroalkylations difficult.[78] One of the reasons for this instability is that fluorocarbanions can undergo α-elimination to expel fluoride and yield a carbene. However, fluoride is a very poor leaving group, especially if it is unsolvated. Indeed, 'naked' trifluoromethanide has been stored for several days at low

temperatures.[79] The α-elimination can only take place if a Lewis acid, such as a metal ion, is present to accept the fluoride. Lithium is a stronger Lewis acid than sodium or potassium, meaning that the lithium fluorocarbanions salts are less stable than sodium or potassium salts. The opposite is true for unfluorinated carbanions where the potassium salts are less stable due to the relative strength of the carbon-lithium and carbon-potassium bond.[80] Unfluorinated alkyl metal salts can generally be prepared and stored, many being commercially available. On the other hand, fluoroalkyl carbanions are generally prepared *in situ* and usually in the presence of the electrophile because they break down rapidly even at low temperatures.[81,82]

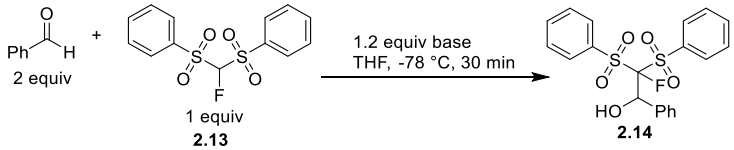
Alkyl lithium salts are generally less reactive than their sodium or potassium counterparts, due to relatively strong bonding between carbon and lithium and the greater tendency of lithium salts to form stable oligomers.[80] However, with fluorinated carbanions, reactivity can be difficult to predict. In some cases, the greater stability and reactivity of the potassium salts leads to a superior outcome, for example, when sulfone **2.11** reacts with sulfinyl imine **2.10** to give **2.12**, the yield is much higher using potassium than with sodium or lithium (Table 2.1).[83] In other cases the exact opposite trend is observed, for example, the lithium salt of **2.13** reacts with benzaldehyde to give much higher yields of **2.14** due to strong chelation between lithium and oxygen (Table 2.2).[84]

Table 2.1. The effect of counterions on nucleophilic fluoroalkylation of sulfinyl imine **2.10** with difluoromethyl sulfone **2.11**.



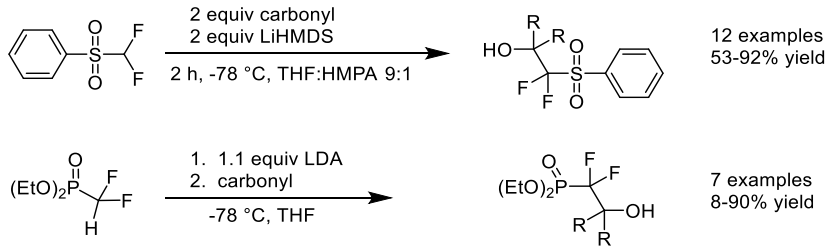
Base	Yield of **2.12** (%)
KHMDS	70
NaHMDS	29
LiHMDS	12

Table 2.2. The effect of counterions on nucleophilic fluoroalkylation of benzaldehyde with sulfone **2.13**.



Base	Yield of **2.14** (%)
KHMDS	27
NaHMDS	72
LiHMDS	92

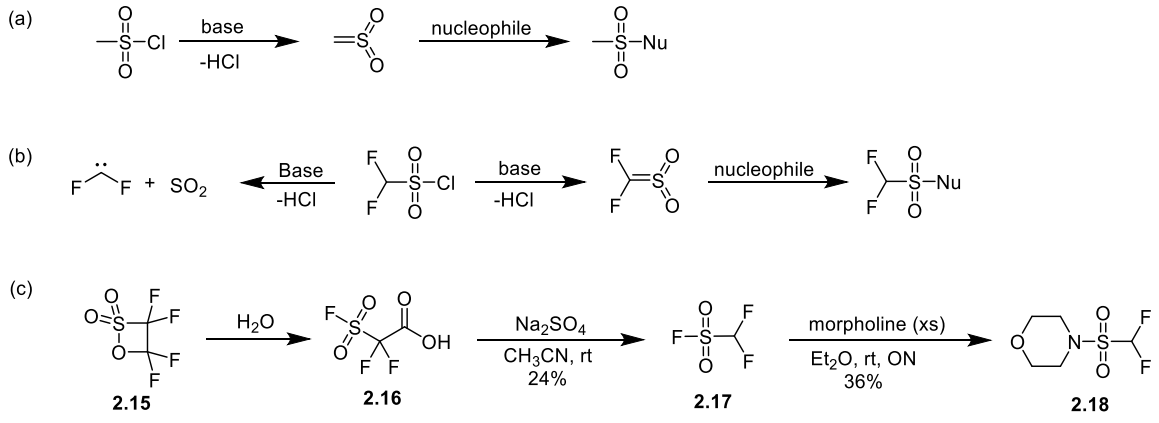
ß-hydroxy-α,α-difluoro phosphonates[85] and sulfones[86] have been prepared by reaction of carbonyl compounds with the lithium salts of difluoromethane phosphonates and sulfones (Scheme 2.4). Therefore, we were confident that a similar transformation would be possible using the lithium salts of difluoromethanesulfonamides, provided that we could easily access the difluoromethane sulfonamide starting materials.



Scheme 2.4. Synthesis of ß-hydroxy-α,α-difluoro compounds from lithiated fluorocarbanions and carbonyls.

2.1.3 — Difluoromethane sulfonamides

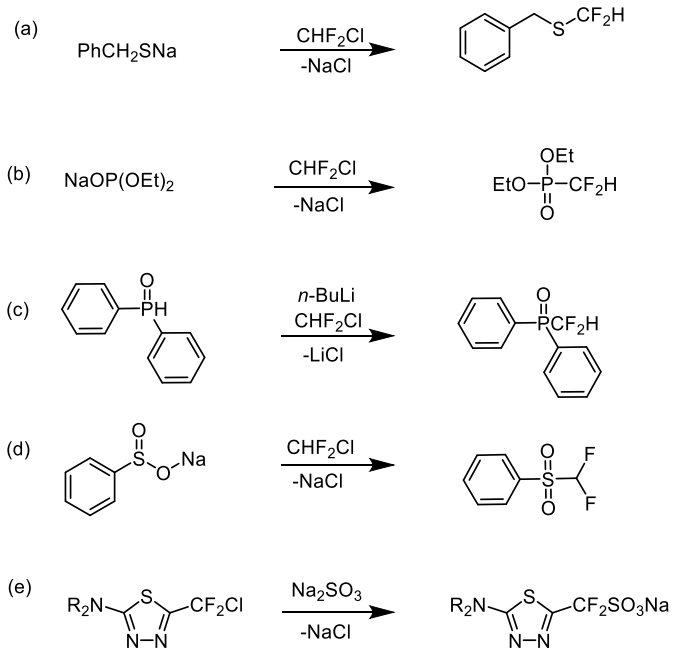
The most direct synthesis of sulfonamides is via sulfonylation of amines with the appropriate sulfonyl chloride (Scheme 2.5a). In most cases the transformation is straightforward and high yielding; however, difluoromethane sulfonyl chloride is an exception and sulfonylations with this reagent typically give low yields of difluoromethane sulfonamides.[14,87-90] This is because sulfonylations in the presence of an amine base proceed through a sulfene intermediate.[91] When difluoromethane sulfonyl chloride is used, it is possible that that reagent undergoes α-elimination rather than ß-elimination leading to difluorocarbene and sulfur dioxide (Scheme 2.5b). Furthermore, difluoromethane sulfonyl chloride is expensive, and is prepared from ozone depleting chemicals. Yields are slightly higher using difluoromethane sulfonyl fluoride **2.17** which reacts with morpholine to give **2.18** in 36 % yield (Scheme 2.5c).[92] Unfortunately **2.17** is prohibitively expensive although it can be prepared in a multistep low yielding procedure from **2.15**.



Scheme 2.5. Sulfonylation of nucleophiles with sulfonyl halides.

Another method would be alkylation of a precursor with a difluorohalomethane. Fluorine stabilizes carbocations, carbenes, and radicals more effectively than other halogens due to more effective orbital overlap. These species are even more electron deficient than unfluorinated

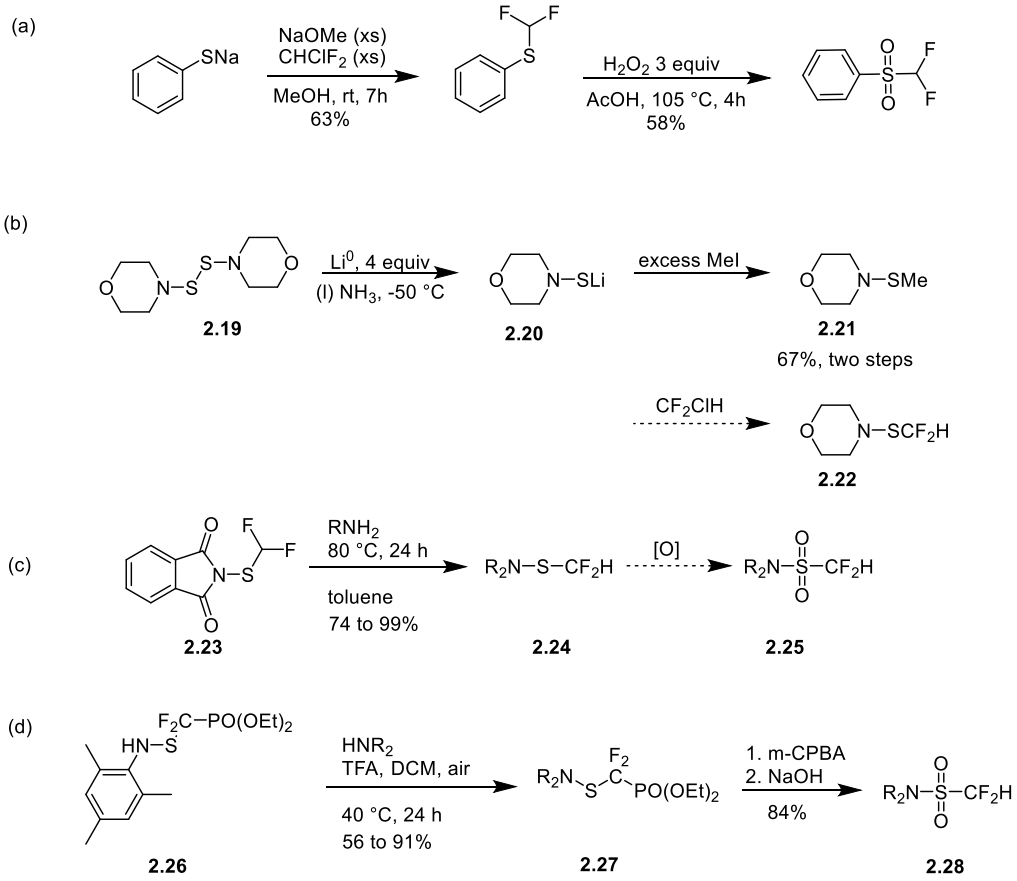
analogs due to fluorine's high electronegativity.[77] These properties allow mixed fluorohalomethanes to react with nucleophiles in ways that other mixed halomethanes cannot. This general approach has been used for the synthesis of a variety of α,α-difluoro compounds including sulfides (Scheme 2.6a),[93] phosphonates (Scheme 2.6b),[85] phosphine oxides (Scheme 2.6c)[94] sulfones (Scheme 2.6d),[95] and sulfonates (Scheme 2.6e).[11] This approach would be difficult for the direct synthesis of difluoromethane sulfonamides because it would require S-alkylation's of sulfuramidous acids which have occasionally been used as nucleophiles but not using halomethanes.[96,97]



Scheme 2.6. Synthesis α,α-difluoro compounds via alkylation with halomethanes.

Difluoromethyl phenylsulfone has also been made by difluoromethylation of thiophenol followed by oxidation (Scheme 2.7a).[98] Oxidation of sulfenamides to sulfonamides is well known, including examples with α-halogens.[99,100] Therefore, a route to difluoromethane sulfenamides should provide access to the desired sulfonamides. Sulfenamide **2.21** has been prepared by methylation of thiohydroxylamine **2.20** which in turn is prepared from its dimer **2.19**

(Scheme 2.7b).[101] The same reaction using chlorodifluoromethane should yield **2.22**. Alternatively, difluoromethylthiolation of amines has been accomplished in high yields using reagent **2.23** which yields sulfenamide **2.24** that could, theoretically, be oxidized to difluoromethane sulfonamide **2.25** (Scheme 2.7c).[102] A related reagent, **2.26**, for the transfer of a thiodifluoromethyl phosphonate group has recently been developed which reacts with a variety of nucleophiles including amines to give **2.27.** After oxidation of the sulfur atom, the phosphonate was hydrolyzed to yield the difluoromethane sulfonamide **2.28** (Scheme 2.7d).[103]



Scheme 2.7. Literature preparations and proposed routes to difluoromethane sulfones and sulfonamides by oxidation of sulfides and sulfenamides.

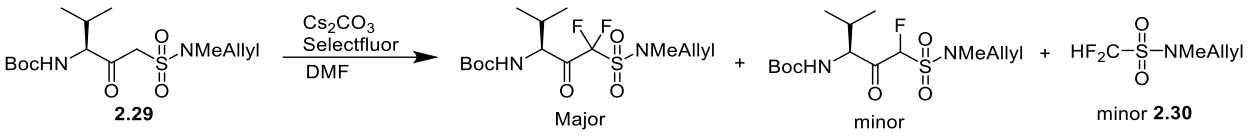
2.1.4 — Research objectives

The principal objective of this chapter is to develop an effective synthesis for ß-hydroxy-α,α-difluorosulfonamides derived from amino acids via the route outlined in Scheme 2.2. This requires an efficient synthesis of difluoromethane sulfonamide precursors, an exploration of the chemistry of the alkali metal salts of these compounds and their reaction with aldehydes, and the preparation of the target ß-hydroxy-α,α-difluorosulfonamides. Ideally, the approach should provide the desired alcohols diastereoselectively and without epimerization at the amino acid α-carbon. Finally, these sulfonamides should be deprotected under mild conditions to allow for their elaboration into peptidomimetics, which may act as inhibitors of renin and other aspartic proteases.

2.2 — Results and discussion.

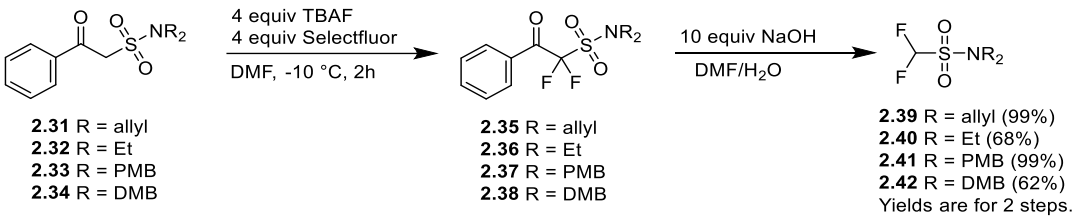
2.2.1 — Synthesis of difluoromethane sulfonamides.

In addition to the routes described in Section 2.1.3 for the preparation of difluoromethanesulfonamides, another possible approach is via decomposition of ß-ketosulfonamides as outlined in Scheme 2.8. We anticipated that this should be possible because we observed difluoromethane sulfonamide **2.30** as a side product during electrophilic fluorination of ß-ketosulfonamides, such as **2.29** in Chapter 1 (Scheme 2.8). If this approach could be achieved in high yield, then it would be superior to the above methods which are either low yielding or rely on expensive reagents that are themselves ozone-depleting or derived from ozone-depleting chemicals.



Scheme 2.8. Electrophilic fluorination of a ß-ketosulfonamide gives rise to a difluoromethane sulfonamide as a minor side product.

To investigate this approach, we subjected sulfonamides **2.31**-**2.34** (described in Chapter 1, section 1.2.3) to the fluorination conditions outlined in Section 1.2.5 to give ketones **2.35**-**2.38** (Scheme 2.9) which were not isolated. NaOH was added to the reaction mixture which gave **2.39**-**2.42** in good to outstanding overall yield.

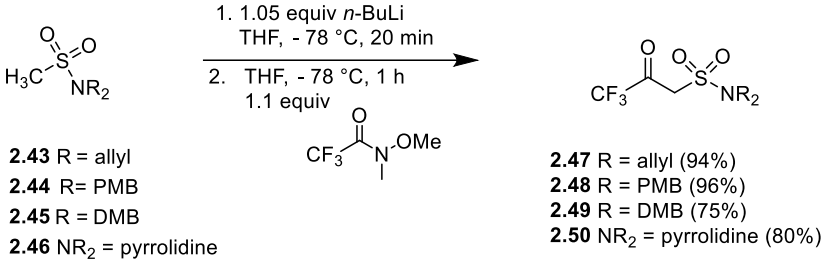


Scheme 2.9. Fluorination of ß-ketosulfonamides derived from methyl benzoate.

While this method does provide access to the desired difluoromethanesulfonamides in good yields, it suffers from two drawbacks. First, the reaction requires four equiv of Selectfluor which is inefficient. Secondly, the starting materials **2.31**-**2.34** are prepared using an excess of a methane sulfonamide which was difficult to remove from the sulfonamide product. Furthermore, this is wasteful in cases where the methane sulfonamide is derived from amines that are not commercially available and must be synthesized.

We reasoned that if we replaced methyl benzoate with a Weinreb amide then the synthesis could be performed without an excess of the methane sulfonamide which would simplify the isolation of the ß-ketone. When we performed this reaction using *N*-methyl-*N*-methoxy benzamide, we found that a significant amount of starting material remained which

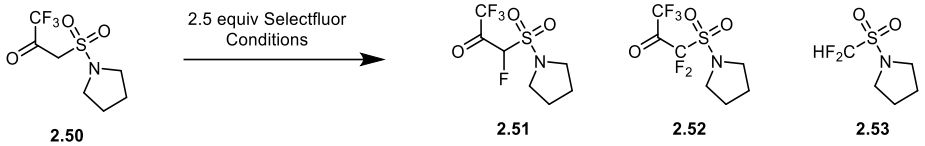
must be removed by tedious chromatography so this provides no benefit over a methyl ester. Next, we employed *N*-methyl-*N*-methoxy trifluoroacetamide, reasoning that this would be more electrophilic, leading to a higher yield. Also, the trifluoromethyl ketone product should increase the acidity of the methylene protons of the ß-ketone, facilitating fluorination. This approach gave ß-ketones **2.47-2.50** in good to excellent yields (Scheme 2.10). The purification of these molecules is facilitated by the absence of substantial quantities of methane sulfonamides **2.43-2.46** in the crude mixture.



Scheme 2.10. Trifluoroacylation of methane sulfonamides.

We selected ketone **2.50** as a model substrate for the fluorination reaction. We hoped to reduce the number of equiv of Selectfluor employed from 4 to at most 2.5. We attempted the reaction using a variety of solvents, bases, and temperatures (Table 2.3). The yields of **2.52** as well as its hydrolysis product **2.53** and the monofluorinated intermediate, **2.51** were estimated by ^{19}F NMR. The best result was obtained with lithium carbonate in DMF at room temperature (entry 21), although sodium and potassium bicarbonates also give quite high yields (entries 24 and 19). Good results could also be obtained with no base when the reaction was heated to 100 °C for 1 hour (entry 12). Only Cs_2CO_3 leads to monofluorination preferentially (entry 14).

Table 2.3. Optimization of fluorination conditions for **2.50**.



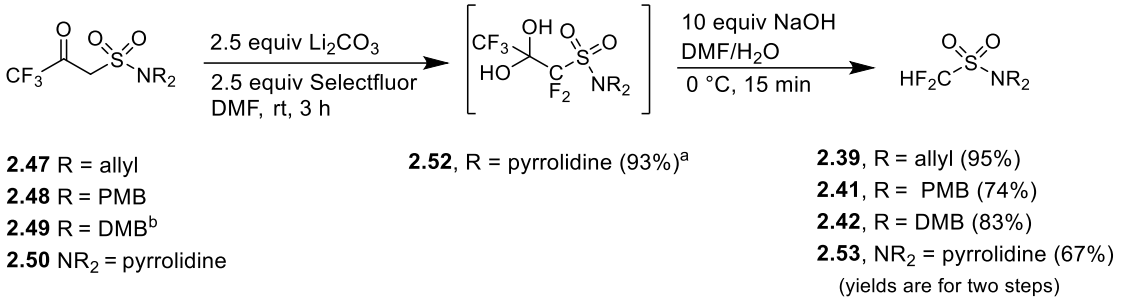
2.50 **2.51** **2.52** **2.53**

Entry[a]	Solvent	Temp	Base (2.5 equiv)	Time	**2.50** (%)	**2.51** (%)	**2.52** (%)	**2.53** (%)
1	Acetone	80	None	8 h	75	25	trace	0
2	AcOH	80	None	8 h		Complex Mixture		
3	CH_3CN	80	None	8 h		Complex Mixture		
4	Dioxane	80	None	8 h	22	15	0	0
5	Pyridine	80	None	8 h	25	0	50	0
6	Pyridine	RT	None	16 h	2	36	43	0
7	TEA	80	None	8 h		Complex Mixture		
8	Water	80	None	8 h		Complex Mixture		
9	DMF	45	None	16 h	trace	30	34	0
10	DMF	60	None	16 h	2	2.5	82	0
11	DMF	80	None	4 h	20	0	63	0
12	DMF	100	None	1 h	0	trace	78	0
13	DMF	120	None	30 m	0	0	72	0
14	DMF	RT	Cs_2CO_3	16 h	6	60	21	7
15	DMF	RT	CsF	16 h	0	38	44	3
16	DMF	RT	K_2CO_3	16 h	0	0	30	40
17	DMF	RT	K_3PO_4	16 h	0	8	47	8
18	DMF	RT	KF	16 h	0	20	65	trace
19	DMF	RT	$KHCO_3$	4 h	0	1	88	0
20	DMF	RT	KOAc	16 h	2	11	46	0
21	DMF	RT	Li_2CO_3	4 h	0	0	93	0
22	DMF	RT	Na_2CO_3	16 h	0	0	69	20
23	DMF	RT	NaF	16 h	1	36	60	0
24	DMF	RT	$NaHCO_3$	16 h	3	0	87	0
25	DMF	RT	NaOAc	16 h	0	12	79	0

[a]**2.50** (28 mg, 0.110 mmol, 1 equiv) and Selectfluor (100 mg, 0.282 mmol, 2.50 equiv) were dissolved in 1 mL of solvent then the base was added. After the indicated time, 3-fluorotoluene (0.11 mmol, 1 equiv) was added and the yield was estimated by ^{19}F NMR.

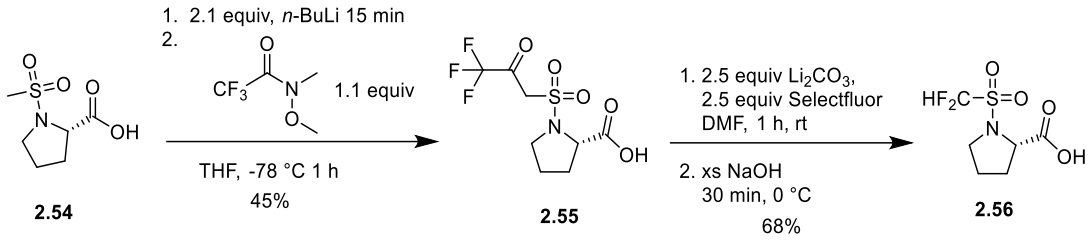
Pentafluoroketone **2.52** was prone to hydrolysis on silica and difficult to isolate; however, the monohydrate could be isolated by crystallization from hexanes in 20% yield. Fortunately, isolation was not necessary, and **2.52** could be hydrolyzed simply by adding aqueous sodium

hydroxide to the reaction mixture once fluorination was complete to give difluoromethanesulfonamide **2.53** in good yield (Scheme 2.11). This methodology was applied to sulfonamides **2.47**-**2.49** to give difluoromethane sulfonamides **2.39**, **2.41**, and **2.42** in good to excellent yields.



Scheme 2.11. Fluorination of trifluoroacetyl methane sulfonamides.
[a]Determined by ^{19}F NMR.
[b]Cs_2CO_3 was used instead of Li_2CO_3.

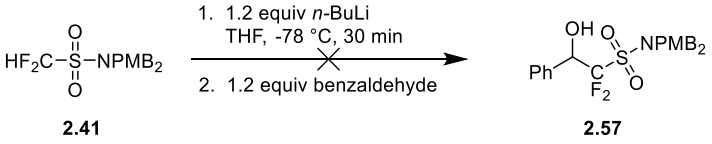
In addition to their use as precursors for making ß-hydroxy-α,α-difluorosulfonamides, difluoromethane sulfonamides are of interest in their own right, such as the herbicide pyrimsulfan; however, these are rare due to the difficulty of efficiently installing a difluoromethanesulfonamide moiety. Our approach provides straightforward access to this functional group without the need for ozone-depleting reagents which should pave the way for further exploration in new areas. For example, we have found that we could treat proline-derived sulfonamide **2.54** with 2.1 equiv *n*-BuLi and react the resulting dianion with *N*-methyl-*N*-methoxy trifluoroacetamide to give ketone **2.55** in 45% yield (Scheme 2.12). Treating **2.55** with Selectfluor in the presence of Li_2CO_3 for 1 h, followed by the addition of aq. NaOH, gave difluoromethane sulfonamide **2.56** in a 68% yield. Notably, both reactions proceed in the presence of an unprotected carboxylic acid. To the best of our knowledge, *N*-difluomethanesulfonyl amino acids are unknown in the literature (Scheme 2.12). The ee of **2.55** and **2.56** were not determined.



Scheme 2.12. Synthesis of an *N*-difluoromethanesulfonyl amino acid.

2.2.2 — Difluoromethane sulfonamide anions.

With the difluoromethane sulfonamides in hand we turned our attention to the reaction of their metalated derivatives with electrophiles using **2.41** as a model sulfonamide substrate. We had anticipated that the lithium salt of **2.41** would readily be prepared using *n*-BuLi and would react with electrophiles such as benzaldehyde and methyl benzoate to yield ß-hydroxy and ß-keto α,α-difluoro sulfonamides respectively. However, when sulfonamide **2.41** was treated with 1.1 equiv of *n*-BuLi for 15 min in THF at -78 °C followed by addition of benzaldehyde, none of the desired product **2.57** was obtained (Scheme 2.13). Indeed, only starting material was observed by ^{19}F NMR after quenching the reaction with NH_4Cl and extraction into ether. Similar results were obtained using methyl benzoate, benzoyl chloride and the Weinreb amide of benzoic acid.



Scheme 2.13. Attempted reaction of the lithium salt of **2.41** with benzaldehyde.

These results raised the question of whether or not the **2.41** was deprotonated by *n*-BuLi. To determine this, we performed a deuterium exchange experiment which involved treating, **2.41** with *n*-BuLi as above, followed by quenching the reaction with aq. deuterium chloride after 30 min and analyzing the resulting mixture by ^{19}F NMR. No $(PMB)_2NSO_2CF_2D$ was detected in the

^{19}F NMR spectrum and much of **2.41** remained unreacted; however, a small peak at -191 was observed corresponding to deuterium fluoride (Figure 2.2). Bis(4-methoxybenzyl)amine was the only new compound observed in the crude reaction by TLC.

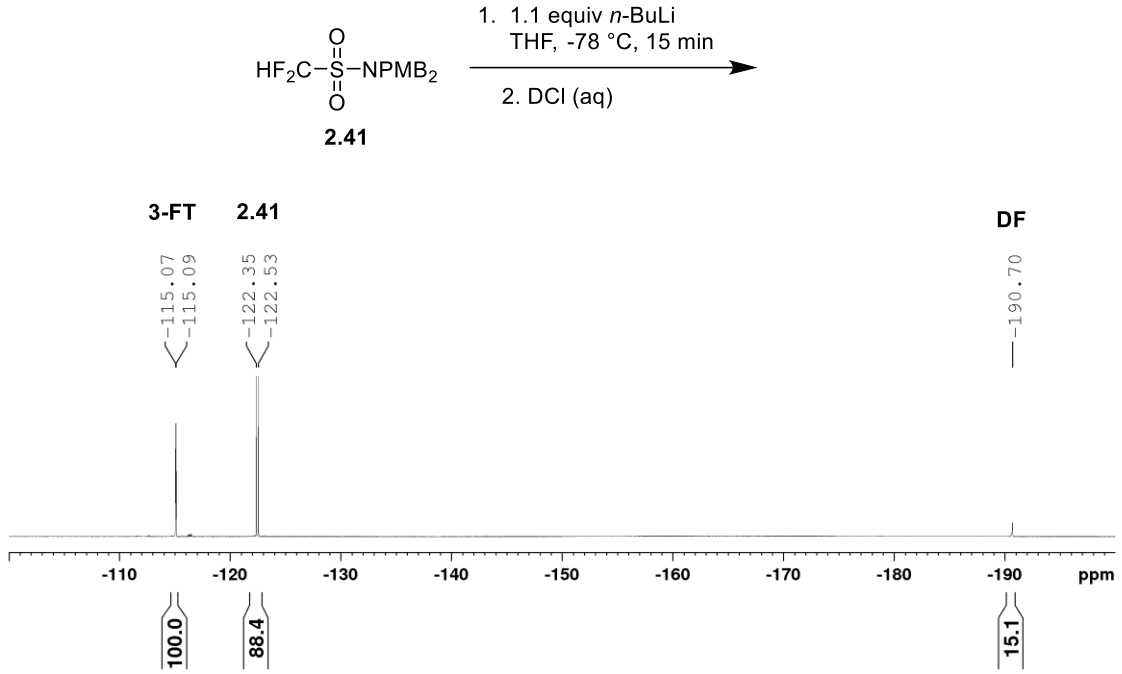


Figure 2.2. ^{19}F NMR spectrum of the reaction mixture of **2.41** after treatment with 1.1 equiv *n*-BuLi and quenching with DCl after 15 min. The doublet at -122 corresponds to **2.41** the broad peak at -190 corresponds to deuterium fluoride and the multiplet at -115 corresponds to the internal standard 3-fluorotoluene.

Under the same reaction conditions the analogous methane sulfonamide underwent complete deuterium incorporation as determined by ^{1}H NMR (Figure 2.3).

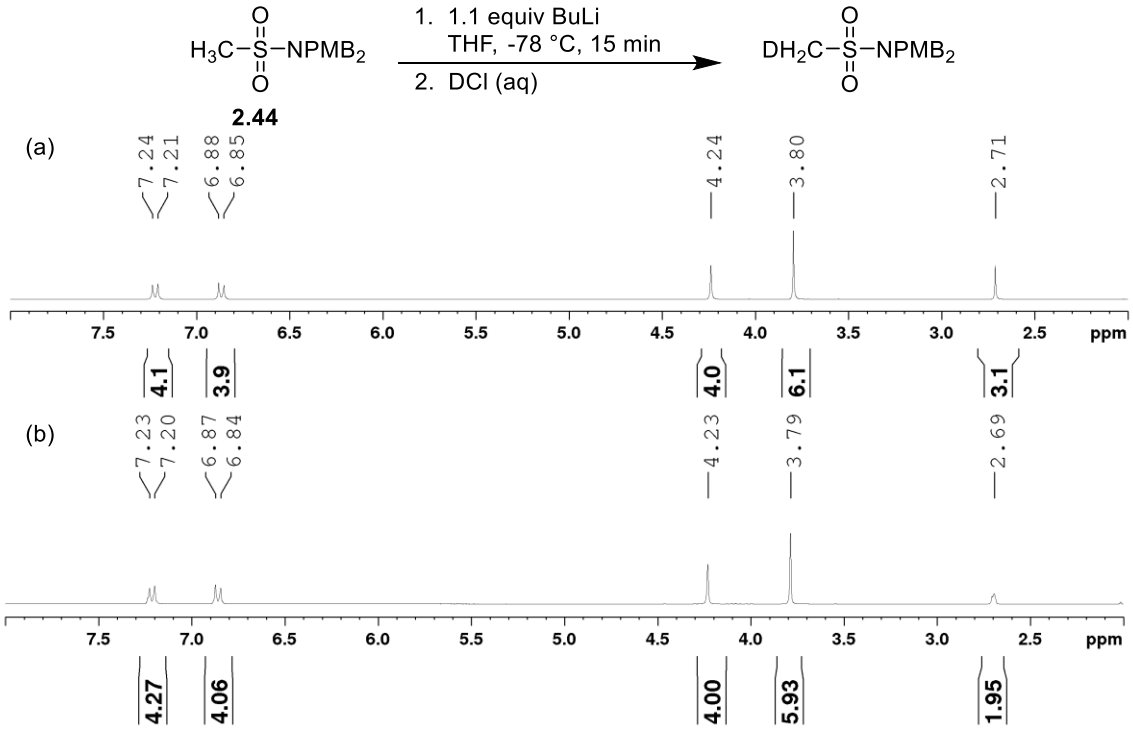


Figure 2.3. ^{1}H NMR spectrum of **2.44** after treatment with *n*-BuLi in THF at -78 °C then quenching with DCl (aq) and extraction into EtOAc.
(a) Starting material
(b) Crude product. The peak at 2.7 corresponds to the methyl group which undergoes partial deuterium exchange.

We thought it unlikely that the fluorinated derivative should be less acidic than methane sulfonamide; therefore, it seemed more likely that some side reaction was taking place involving more than 1 equiv of *n*-BuLi. To further investigate this possibility, we reacted **2.41** with different amounts of *n*-BuLi and determined the amount of **2.41** remaining by ^{19}F NMR using 3-fluorotoluene (3-FT) as an internal standard (Figure 2.4 and Table 2.4).

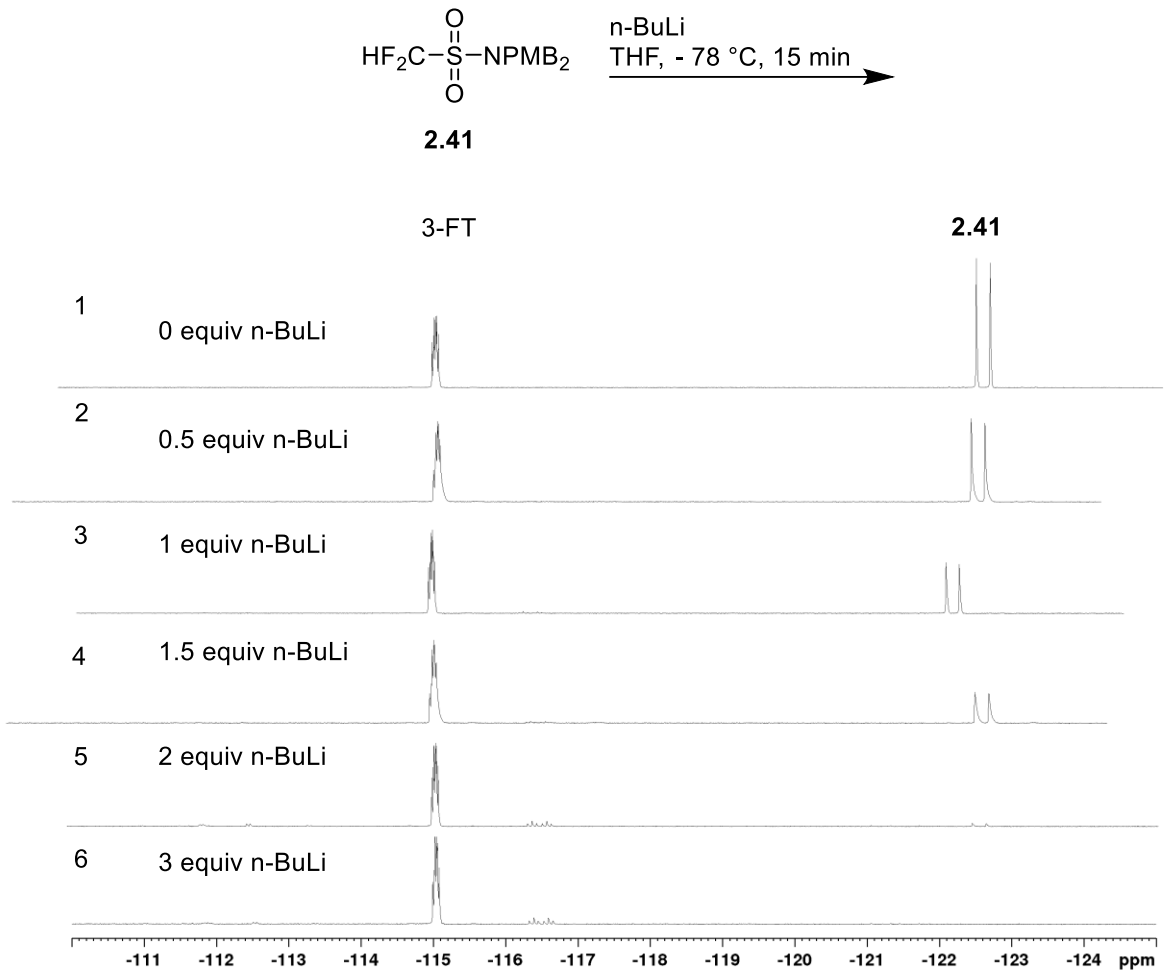


Figure 2.4. ^{19}F NMR spectra of the organic layer of the reaction mixtures of sulfonamide **2.41** after treatment with varying amounts of *n*-BuLi in THF at – 78 °C and quenching after 15 min with NH_4Cl and extracting into Et_2O. The doublet at 122.5 ppm corresponds to **2.41** and the multiplet at -115 ppm corresponds to the internal standard, 3-fluorotoluene (3-FT).

Table 2.4 Amount of **2.41** remaining in Figure 2.4.

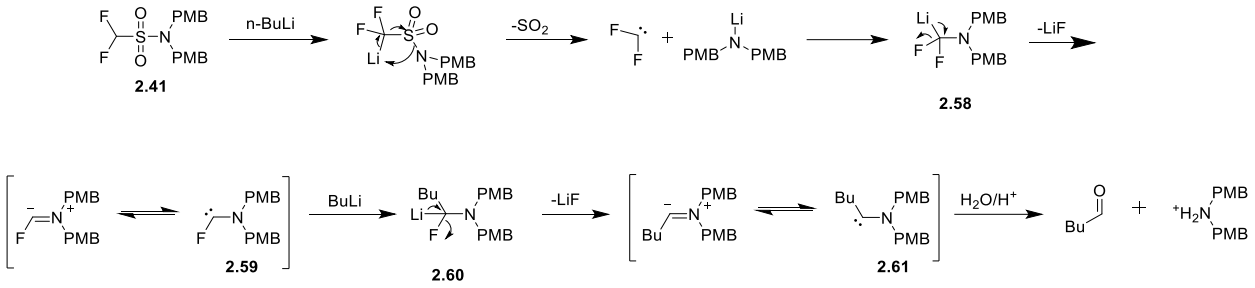
Entry	equiv *n*-BuLi	% **2.41** remaining
1	0	100
2	0.5	89
3	1.0	63
4	1.5	40
5	2	2.5
6	3	0

The reaction required 2 equiv of *n*-BuLi before almost all of **2.41** was consumed after 15 min at –78 °C. When 1.5 or more equiv of *n*-BuLi was used, a small doublet of triplets at approximately -116 ppm was observed which corresponds to 1,1-difluoropentane.[104] The remainder of the fluorine is expected to be converted to lithium fluoride which remained in the aqueous layer. These results are in contrast to the related difluoromethyl phenylsulfone, which is recovered unchanged after deprotonation with LiHMDS and normal workup.[105]

Considering the mechanism of decomposition, α-elimination could take place cleaving either a carbon-fluorine bond or the carbon-sulfur bond. α-Elimination to give lithium fluoride is known to be very favorable in the case of lithium trifluoromethide.[79,106] Fluoride α-elimination to produce fluorinated carbenes is also observed in the Grignard and organolithium derivatives of difluoromethane[107] as well by deprotonation of certain difluoromethyl alkanes.[108,109] Conversely difluoromethyl phenylsulfone decomposes to give difluorocarbene by α-elimination of a phenylsulfinic acid in the presence of sodium methoxide.[98]

A plausible mechanism for the major decomposition pathway is shown in Scheme 2.14. The lithium salt of **2.41** undergoes α-elimination to form sulfur dioxide, difluorocarbene and bis-PMB amide. The carbene reacts with the amide to give carbanion **2.58**. This would undergo a second α-elimination to lose lithium fluoride and yield carbene **2.59** which would be stabilized by resonance. Nucleophilic addition of a second molecule of *n*-BuLi would give carbanion **2.60** which can decompose yielding a second equivalent of lithium fluoride and producing stabilized carbene **2.61.** This molecule would persist until the reaction is quenched at which point it would be hydrolyzed to give bis-PMB amine and pentanal. Although it must be said that this mechanism is speculative and additional experiments are required to confirm the identity of the

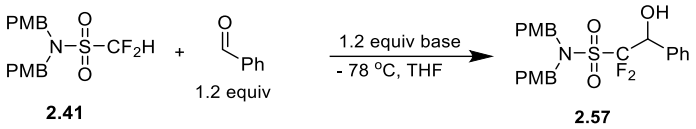
intermediates. 1,1-Difluoropentane most likely arises from the reaction of *n*-BuLi and difluorocarbene, although it could also form by a direct S_N2 attack of *n*-BuLi on **2.41**.



Scheme 2.14. Proposed mechanism for the decomposition of **2.41**.

We hoped that we would be able to intercept the lithium salt of **2.41** if it were generated in the presence of the electrophile. We selected benzaldehyde as a model substrate, anticipating that a nonenolizable aldehyde would be the best substrate (Table 2.5). When 1.2 equiv of *n*-BuLi was added to a mixture of benzaldehyde and **2.41**, no product **2.57** was formed. However, the amount of sulfonamide remaining was much greater than would be expected from the trend in Figure 2.4; therefore, we suspected *n*-BuLi reacted with benzaldehyde faster than it deprotonated **2.41** (entry 2). Not surprisingly, no product or decomposition was observed when the sulfonamide was added to a mixture of the *n*-BuLi and benzaldehyde (entry 3). Adding benzaldehyde to a mixture of *n*-BuLi and **2.41** gave none of the desired product but did give the lowest level of unreacted **2.41** suggesting that much of **2.41** had decomposed (entry 4). Next, we turned to more sterically hindered bases, namely, LDA, LiHMDS, and LiOtBu; however, these bases also yielded little to no product (entries 5-7). Warming the reaction to -20 °C (entry 8), adding TMSCl (entry 9) or LiCl (entry 10) had no effect on the outcome; however, adding HMPA did result in a small quantity of **2.57** being observed (entry 11).

Table 2.5. Reaction of sulfonamide **2.41** in the presence of benzaldehyde and lithium bases.



2.41 **2.57**

Entry	Base	Time (min)	Unreacted **2.41**[a] (%)	Yield (**2.57**)
1	-	15	100	0
2	*n*-BuLi	15	88	0
3[b]	*n*-BuLi	15	99	0
4[c]	*n*-BuLi	15	32	0
5	LiOtBu	15	65	Trace
6	LDA	15	87	0
7	LiHMDS	15	69	Trace
8[d]	LiHMDS	60	85	0
9[e]	LiHMDS	15	85	0
10[f]	LiHMDS	60	86	0
11[g]	LiHMDS	60	88	8

[a]^{19}F NMR yield determined after the reaction was quenched with NH_4Cl. 3-fluorotoluene as an internal standard.
[b]Base added to a solution of PhCHO at – 78 °C, stir 15 min, then add **2.41**. Stir 15 min at – 78 °C.
[c]Base added to a solution of **2.41** at – 78 °C, stir 15 min, then add PhCHO. Stir 15 min at – 78 °C.
[d]Reaction warmed to -20 °C after 5 min
[e]Reaction performed in the presence of 1.2 equiv TMSCl.
[f]Reaction performed in the presence of 4 equiv LiCl.
[g]Reaction performed in 5% HMPA/THF.

In all cases using LiHMDS, most of **2.41** remained unreacted, suggesting that this base is not strong enough to deprotonate **2.41** to an appreciable degree. KHMDS is a stronger base than LiHMDS,[110] therefore, we examined NaHMDS and KHMDS. These bases (1.2 equiv) were added to a solution of **2.41** in THF at -78 °C in the absence of an electrophile then quenched after 4 min with deuterium chloride in MeOD and analyzed by ^{19}F NMR (Figure 2.5). Most of **2.41** remained unreacted in the case of LiHMDS but was completely decomposed by NaHMDS or KHMDS, which are stronger bases. In all cases no deuterium incorporation was observed which rules out the possibility that the carbanion persists unreacted until workup.

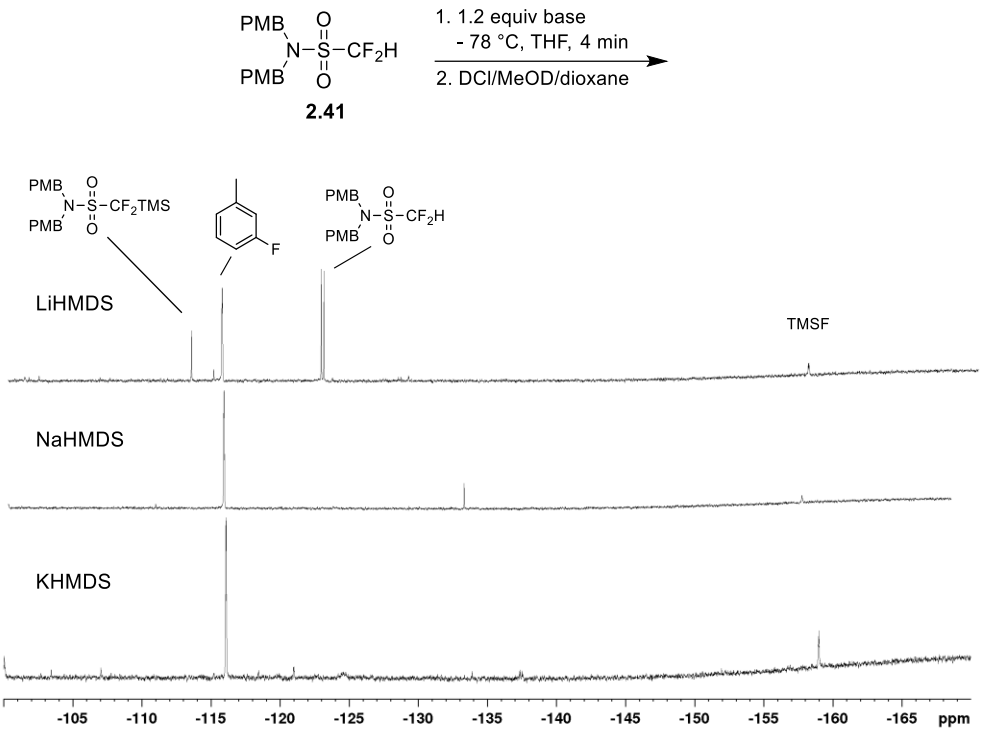
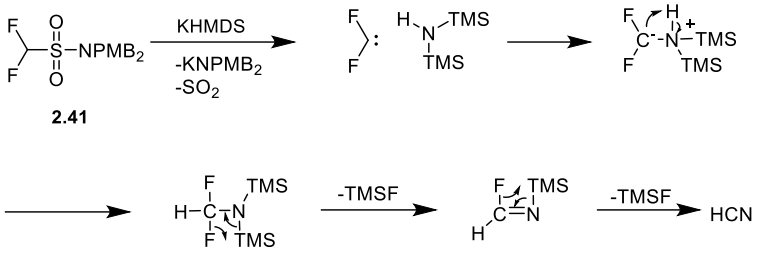


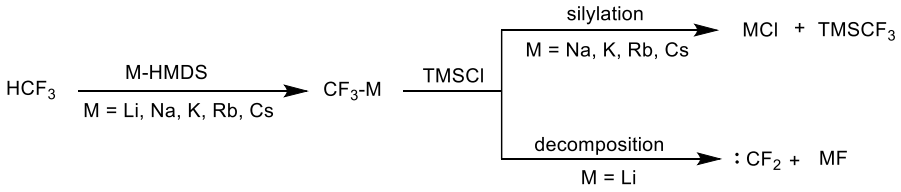
Figure 2.5. ^{19}F NMR spectra of the reaction mixtures of sulfonamide **2.41** after treatment with LiHMDS, NaHMDS or KHMDS in THF at – 78 °C and quenching after 4 min with DCl.

It is interesting to note that complete decomposition was observed with just 1.2 equiv of KHMDS or NaHMDS, while approximately 2 equiv of *n*-BuLi was required (Table 2.4). A plausible mechanism that would explain the difference is described in Scheme 2.15. Following deprotonation, the sulfonamide carbanion rapidly decomposes yielding difluorocarbene. The difluorocarbene then reacts with hexamethyldisilazane followed by a series of proton transfers and retro 2+2 reactions to give hydrogen cyanide and two equiv of fluorotrimethylsilane. This mechanism is very similar to that proposed by Zhang for the oxidation of ammonia to cyanide by difluorocarbene.[111]



Scheme 2.15. Proposed mechanism of decomposition of **2.41** with KHMDS.

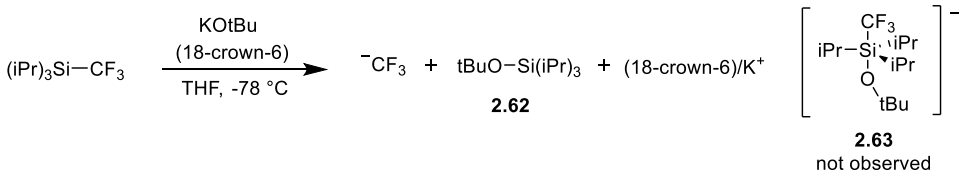
Another ion which decomposes rapidly to yield difluorocarbene is trifluoromethide. Computational studies on the CF_3 carbanion have shown that the counterion has a dramatic effect on the lifetime of this species, specifically decomposition to a metal fluoride becomes more favorable with smaller cations because of stronger bonds to fluoride.[106] DFT calculations predicted that trimethylsilylation of $^{-}CF_3$ should occur when the counterion is Na^+, K^+, Rb^+ or Cs^+, but α-elimination to difluorocarbene when the counterion is lithium (Scheme 2.16). This result is in agreement with experiments where KHMDS promotes the trimethylsilation of fluoroform but LiHMDS does not.[112]



Scheme 2.16. Decomposition of the $LiCF_3$ anion is favored over trifluoromethylation.

These results are also in good agreement with the synthesis of trifluoromethide anions by Prakash et al. In this study researchers prepared (18-crown-6)K^+ CF_3^- (Scheme 2.17).[79] Due to the chelating action of the crown ether, the potassium ion is not strongly associated with the CF_3 anion and a metal fluoride bond is unable to form. This means that a 'naked' fluoride would need to be eliminated in order for the molecule to decompose; this elimination is unfavorable enough that (18-crown-6)K^+ CF_3^- was stable for several days at -78 °C. Triisopropylsilyl

trifluoromethane is used as the starting material because this leads to a bulky by-product **2.62**, which prevented the formation of a pentacoordinate silicon side products such as **2.63**.



Scheme 2.17. Literature synthesis of a long lived CF_3 anion.

In the case of **2.41**, we suspect that decomposition initially proceeds by breaking the carbon-sulfur bond liberating difluorocarbene, sulfur dioxide and a lithium dialkylamide. We hoped that we might observe an increase in stability of the carbanion derived from **2.41** by switching to a potassium base. This is because we suspect **2.41** decomposes to give an amide (Scheme 2.14) and the lithium-nitrogen bond is stronger than the potassium-nitrogen bond, therefore, α-elimination from a salt of **2.41** should be slower when a potassium base is used.

We reasoned that if a potassium salt of **2.41** formed, then we should be able to trap it with deuterium cations and observe the deuterated sulfonamide by ^{19}F NMR. We knew that the anion completely decomposed after 4 min at -78 °C (Figure 2.5). To determine if deuterium exchange into **2.41** could be detected at lower temperature and shorter reaction times, 1.2 equiv of KHMDS was added to a solution of **2.41** in a 3:2 mixture of THF:Et_2O at -128 °C. The mixture was quenched with trifluoroacetic acid-d_1 after 10, 20 or 30 sec and the ^{19}F NMR spectra was recorded (Figures 2.6 and 2.7). Deuterium incorporation was observed by the appearance of a 1:1:1 triplet at -123 ppm, slightly upfield from **2.41**. A number of decomposition products are observed, two of these are unknown; a doublet of triplets at -136 and a set of AB doublets at -119 ppm. Fluorotrimethylsilane appears at -158 ppm and a singlet at 113 ppm appears which is

assigned to the trimethylsilyl difluoromethanesulfonamide, the same signal being observed on reaction of **2.41** with excess KHMDS and TMSCl.

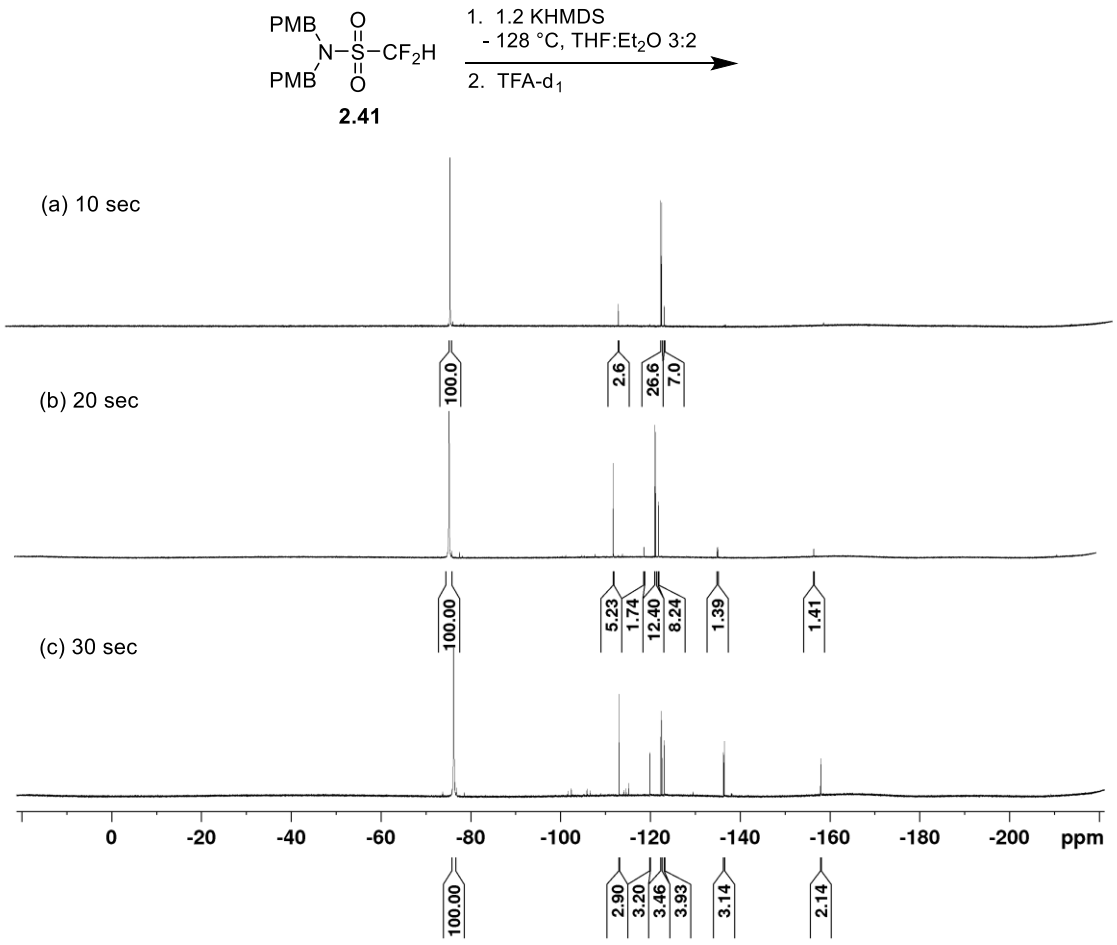


Figure 2.6. ^{19}F NMR spectra of the reaction of **2.41** with KHMDS at –128 °C in a 3:2 mixture of THF:Et_2O after quenching with TFA-d_1 after (a) 10, (b) 20, and (c) 30 sec. See Figure 2.7 for peak assignments.

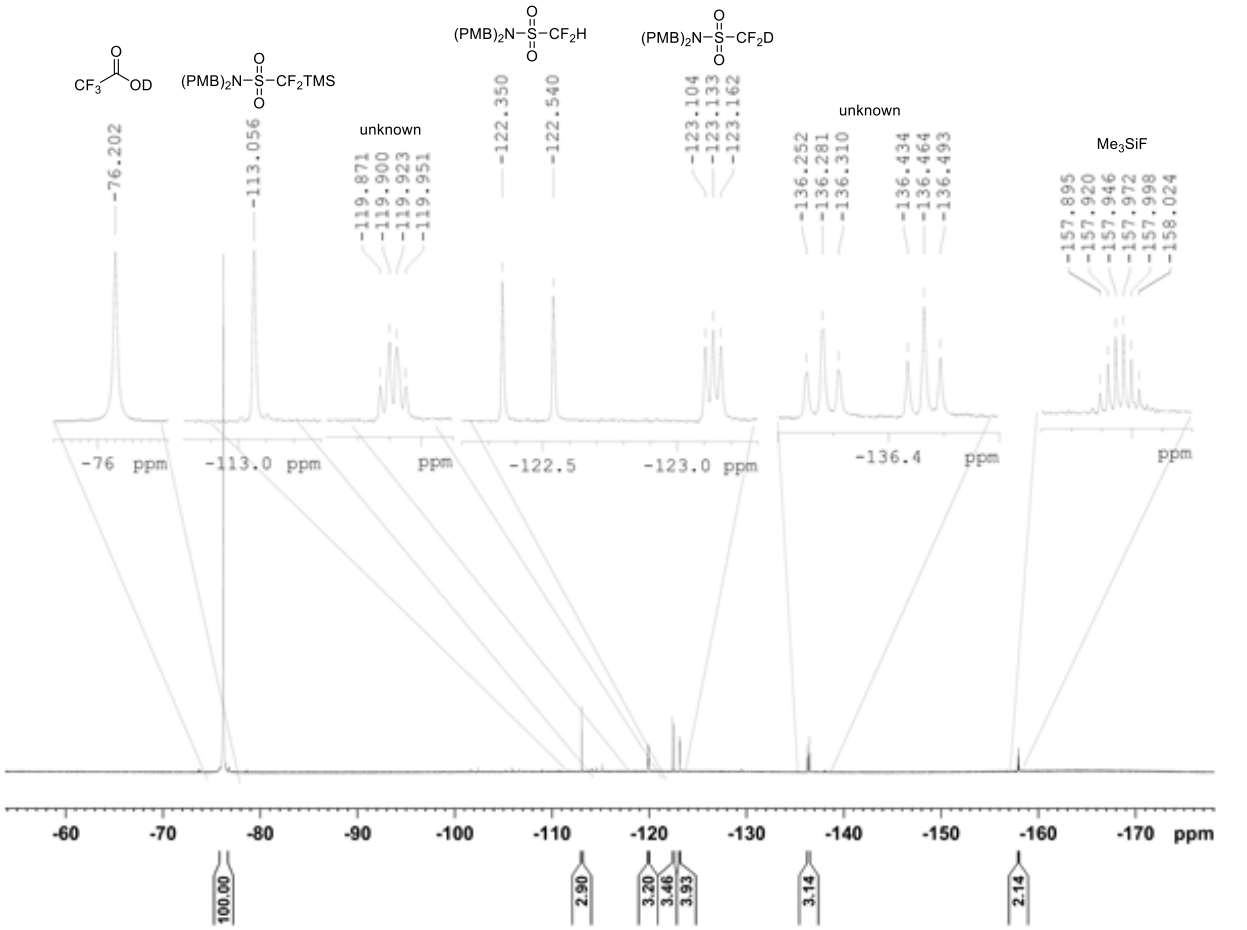


Figure 2.7. Expansion of Figure 2.6c.

Knowing that the carbanion could be generated briefly using KHMDS, we attempted the reaction in the presence of benzaldehyde at -95 °C in THF, and quenched the reaction after 5, 10 or 15 sec using TFA-d_1 (Figure 2.8). During this time period it is clear that the reaction is still actively occurring due to the gradual increase of product **2.57** observed at -105 and -118 ppm. The fluorines in **2.57** are diastereotopic, and give rise to different chemical shifts, the difference in chemical shift is large because one fluorine can hydrogen bond to the alcoholic proton. However, there is no sign of any deuterated sulfonamide at -123 ppm. This demonstrates that deprotonation is the rate limiting step of the reaction. None of the decomposition products that

were observed in Figure 2.7 were present, even though this reaction was run approximately 30 °C warmer. This indicates that 1,2 addition to the aldehyde is very much faster than α-elimination to the carbene, or that the aldehyde stabilizes the carbanion through coordination to potassium.

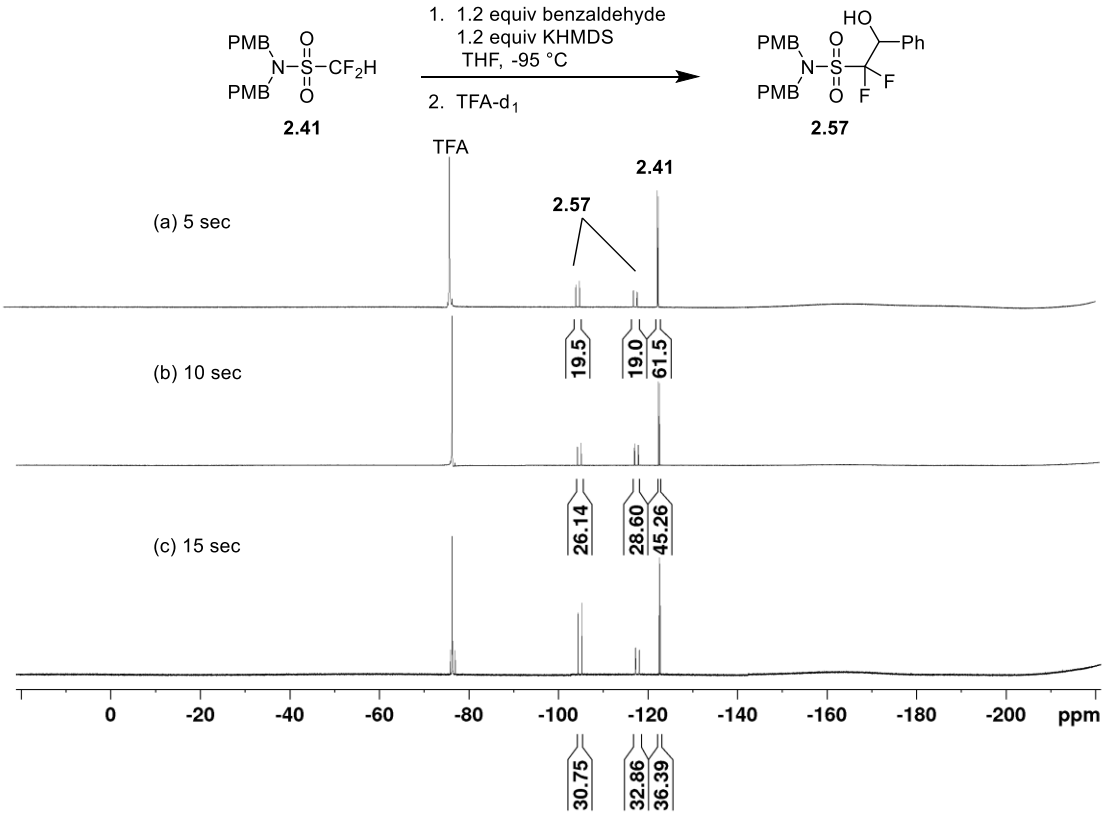
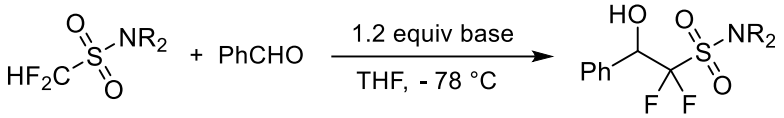


Figure 2.8. ^{19}F NMR spectra of the reaction of **2.41**, benzaldehyde, and KHMDS in THF at – 95 °C after quenching with TFA-d_1 after (a) 5, (b) 10, and (c) 15 sec.

The reaction of **2.41** with benzaldehyde using KHMDS provided **2.57** in almost quantitative yield in just 5 min at -78 °C (Table 2.6, entry 1). It occurred to us that the reverse reaction might lead to decomposition to difluorocarbene if the reaction time was too long; therefore, we repeated the reaction, this time stirring for one hour, but the yield remained excellent (entry 2). KOtBu and NaHMDS were also effective, but the product was obtained in

considerably lower yield (entries 3 and 4). NaOtBu did not result in any reaction after 1 hour (entry 5). Using KHMDS, difluoromethane sulfonamides **2.39**, **2.42** and **2.53** reacted with benzaldehyde to give alcohols **2.64 - 2.66** in good yield within 5 min at –78 °C (entries 6-8).

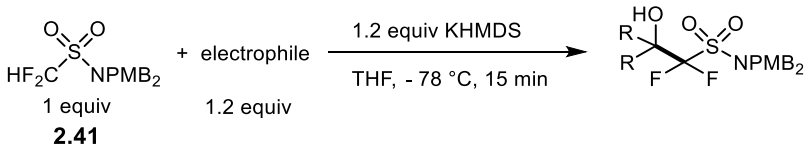
Table 2.6. Reaction of benzaldehyde with difluoromethane sulfonamides using Na^+ and K^+ bases.

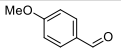
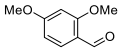
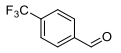
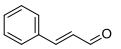
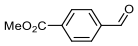


entry	R	base	time (min)	yield (%)[a]
1	PMB (**2.41**)	KHMDS	5	99 (**2.57**)
2	PMB	KHMDS	60	93
3	PMB	KOtBu	60	67
4	PMB	NaHMDS	15	42
5	PMB	NaOtBu	60	0
6	pyrrolidine (**2.53**)	KHMDS	5	71 (**2.64**)
7	allyl (**2.39**)	KHMDS	5	84 (**2.65**)
8	DMB (**2.42**)	KHMDS	5	88 (**2.66**)

We applied these conditions to a number of other carbonyl compounds (Table 2.7). The yields were high with non-enolizable aldehydes (entries 1-7) and ketones (entries 8-9). Reaction with cinnamaldehyde (entry 4) yielded only the 1,2-addition product which is consistent with the expected hardness of the fluorinated carbanion.[113] Meanwhile with methyl 4-carboxybenzaldehyde, addition was observed exclusively at the aldehyde position and not at the ester (entry 5). With enolizable aldehydes and ketones (entries 10 to 15) yields were generally lower. The worst-case example was phenyl acetaldehyde which yielded only 11% of **2.81** (entry 15). The yield could be dramatically improved by reducing the temperature to -128 °C and by increasing the equiv of aldehyde and KHMDS to 4 and 2 respectively (entries 16 and 17).

Table 2.7. Reaction of **2.41** with Aldehydes and Ketones.



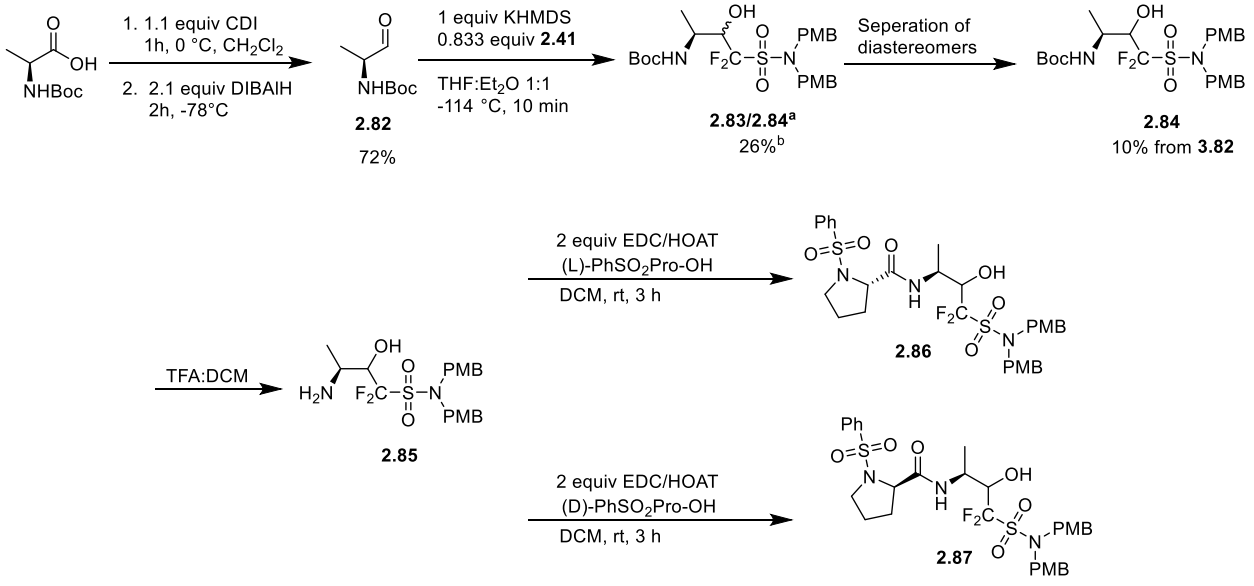
Entry	Electrophile	Yield (%)	Entry	Electrophile	Yield (%)
1		90 (**2.67**)	10		49 (**2.76**)
2		99 (**2.68**)	11		77 (**2.77**)
3		81 (**2.69**)	12		68 (**2.78**)
4		86 (**2.70**)	13		57 (**2.79**)
5		76 (**2.71**)	14		43 (**2.80**)
6		77 (**2.72**)	15		11 (**2.81**)
7		74 (**2.73**)	16		39 (**2.81**)[a]
8		86 (**2.74**)	17		61 (**2.81**)[a,b]
9		85 (**2.75**)			

[a]Reaction performed at -128 ˚C in 2:3 Et_2O:THF.
[b]4 equiv of aldehyde and 2 equiv of KHMDS

2.2.3 — Synthesis of ß-hydroxy-α,α-difluorosulfonamides derived from amino acids

We were particularly interested in ß-hydroxy-α,α-difluoromethanesulfonamides derived from amino acids. This required α-amino aldehydes in enantiomerically pure form. To prepare enantiomerically pure amino aldehydes such as **2.82** (Scheme 2.18), we used the procedure of Ivkovic which involves reacting an N^{α}-protected amino acid with CDI followed by reduction of the resulting activated amino acid with DIBAL-H.[114] When we subjected **2.41** to KHMDS in the presence of **2.82** at -78 °C, ß-hydroxy-α,α-difluoromethanesulfonamide, **2.83**/**2.84**, was obtained in only a 7% yield (Scheme 2.18). This was consistent with the low yield obtained with enolizable aldehydes (Table 2.7). The yield could be improved to 26% by reducing the temperature to -114 °C, unfortunately this material was obtained as a 1:1.2 mixture of

diastereomers, which were difficult to separate. A portion of one of the diastereomers was separated to give **2.84** in 10% yield from **2.82**. Given the tendency of **2.82** to epimerize under basic conditions, it seemed likely that epimerization would occur during the reaction with **2.41** and KHMDS, if this occurred then a mixture of 4 stereoisomers would be obtained and **2.84** would exist as a mixture of enantiomers. To examine this possibility the Boc group was removed by treatment with acid and the free amine was divided into two portions. The crude amine was coupled to either D or L *N*-benzenesulfonyl proline to give **2.86** or **2.87**. The diastereomers could be separated by HPLC which showed that epimerization was not significant (Figure 2.9). This can be rationalized if deprotonation of the sulfonamide, which is the rate limiting step, is faster than deprotonation of the aldehyde α-carbon. The absolute stereochemistry in **2.83** and **2.84** was not determined.



[a] 7% when the reaction was performed at -78 °C in THF, [b] combined yield as determined by ^{19}F-NMR

Scheme 2.18. Synthesis of a Boc alanine derivatives **2.86** and **2.87**.

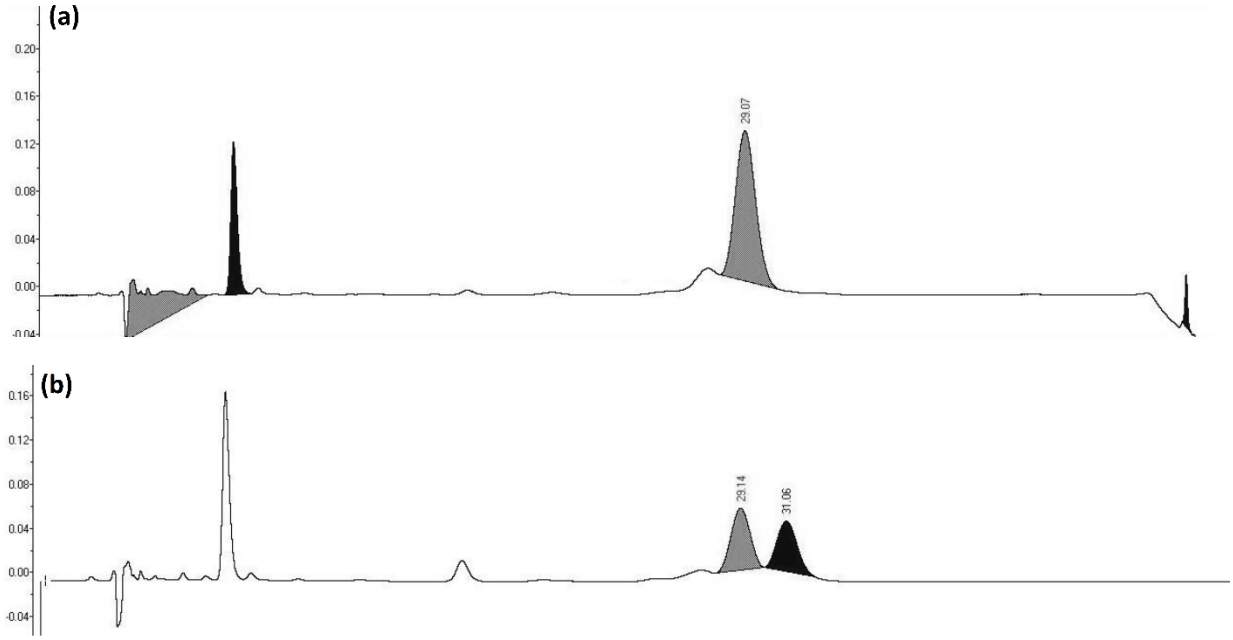
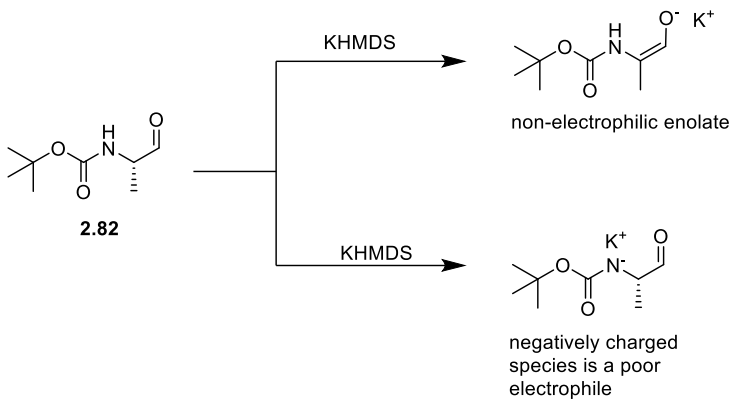


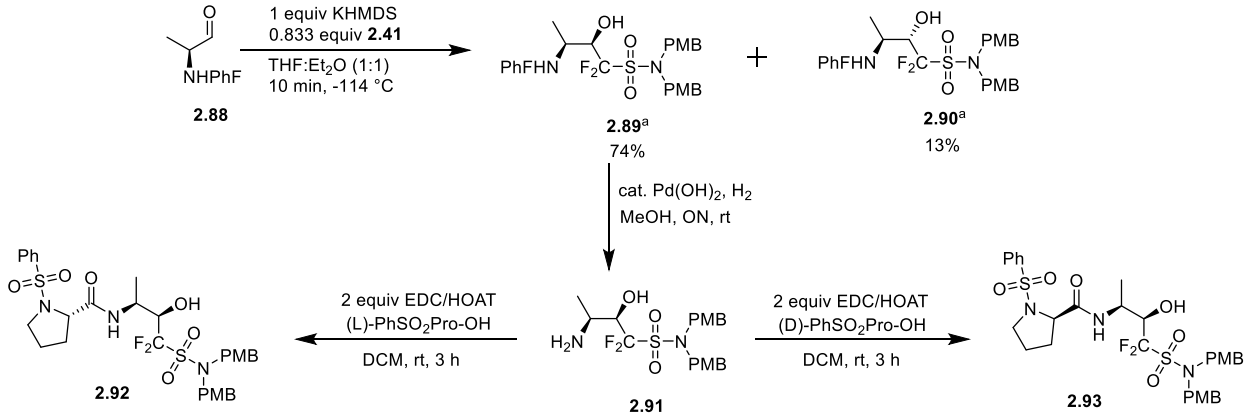
Figure 2.9. Analytical HPLC trace of Boc alanine derivatives **2.86** and **2.87.** (a) HPLC trace of compound **2.87** 0.1% TFA 47% CH_3CN:H_2O (b)HPLC trace of a mixture of compounds **2.86** and **2.87** 0.1% TFA 47% CH_3CN:H_2O.

It should be possible to further improve the yield of alcohol **2.83** by using a large excess of the aldehyde; however, the lack of diastereoselectivity in this reaction and the difficulty in separation remained problematic. The cause of the low yield is likely because **2.82** is deprotonated by KHMDS. The nitrogen may be deprotonated, resulting in a charged species which does not react due to electrostatic repulsion, or deprotonation may occur at the α-carbon resulting in an enolate which would not reaction with **2.41** (Scheme 2.19). We reasoned that deprotonation in both locations would be prevented by using the 9-phenyl-9-fluorenyl (PhF) protecting group. The PhF group is known to prevent enolization of α-amino aldehydes.[115] Additionally, the nitrogen is much less acidic since it is an amine rather than a carbamate. See section 3.1.1 for a detailed discussion of the PhF protecting group. The preparation of PhF protected amino aldehydes is described in Section 3.2.1



Scheme 2.19. Deprotonation of Boc-alanal leads to two possible unreactive side products.

When using PhF-alanal at -78 °C, a 60% yield of amino alcohol **2.89** was obtained and the dr was 5.1 to 1 (Scheme 2.20). By reducing the temperature to -114 °C, the yield was improved to 87% with a dr of 5.7:1. The diastereomers were separated and the major diastereomer was further derivatized to test for epimerization. The PhF group was removed by hydrogenolysis and the free amine **2.91** was divided and coupled to either D or L *N*-benzenesulfonylproline to give either **2.92** or **2.93**. HPLC of the crude material showed that no epimerization occurred (Figure 2.10a and b). The absolute stereochemistry of **2.89** and **2.90** is discussed below.



Scheme 2.20. Synthesis of a PhF alanine derivative **2.92.**

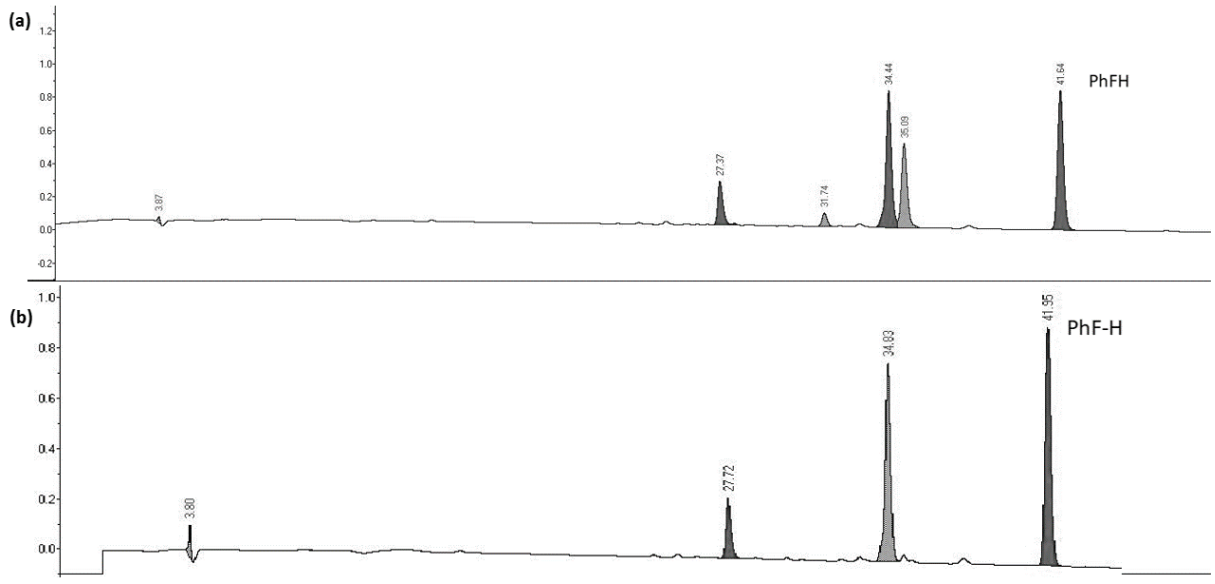


Figure 2.10. Analytical HPLC trace of Boc-alanine derivatives 2.92 and 2.93. (a) HPLC trace of a mixture of compounds **2.92** and **2.93** (b) HPLC trace of compound **2.92** 10 to 90% CH_3CN 0.1% TFA:H_2O over 45 min.

Using **2.41** and the PhF-protected amino aldehydes derived from methionine, t-butyl aspartate, and proline, we prepared several other ß-hydroxy-α,α-difluorosulfonamides (**2.97**-**2.102**, Table 2.8). Excellent yields were achieved with all PhF protected α-amino aldehydes. Methionine and aspartate were selected because the corresponding ß-hydroxy-α,α-difluorosulfonamides would be difficult to prepare using the electrophilic fluorination approach (after reduction of the ketone) described in Chapter 1: methionine is very sensitive to oxidation and incompatible with Selectfluor while aspartate is incompatible with the required 3-fold excess of alkyl lithium (see Chapter 1 for details). Proline was selected as we wished to incorporate the ß-hydroxy-α,α-difluorosulfonamide derived from proline into a peptidomimetic (discussed in Chaper 4). Unfortunately, the dr of these alcohols was modest, ranging from 2.3 to 5.7, and the diastereomers were difficult to separate except for proline derivatives **2.99** and **2.102**.

Table 2.8. Reaction of **2.41** with PhF-protected α-amino aldehydes and KHMDS

2.41 **2.88, 2.94-2.96** **2.89, 2.97-2.99** **2.90, 2.100-2.102**

Entry	Aldehyde	Products	Yield %	dr (RS:SS)
1	PhF-Ala (**2.88**)	**2.89** and **2.90**	87	5.7:1
2	PhF-Met (**2.94**)	**2.97** and **2.100**	93	5.5:1
3	PhF-Asp(tBu) (**2.95**)	**2.98** and **2.101**	94	2.3:1
4	PhF-Pro (**2.96**)	**2.99** and **2.102**	96	5.0:1

The absolute configuration in alcohol **2.99**, the major proline diastereomer, was determined to be 2R,3S (syn configuration) by X-ray crystallography (Figure 2.11). **2.89**, **2.97 and 2.98** was assigned as 2R,3S. For all of the compounds in Table 2.8 the ^{19}F NMR spectra showed the major isomer upfield of the minor isomer; therefore, the relative stereochemistry in each case is likely to be the same and **2.89**, **2.97 and 2.98** were also assigned as 2R,3S.

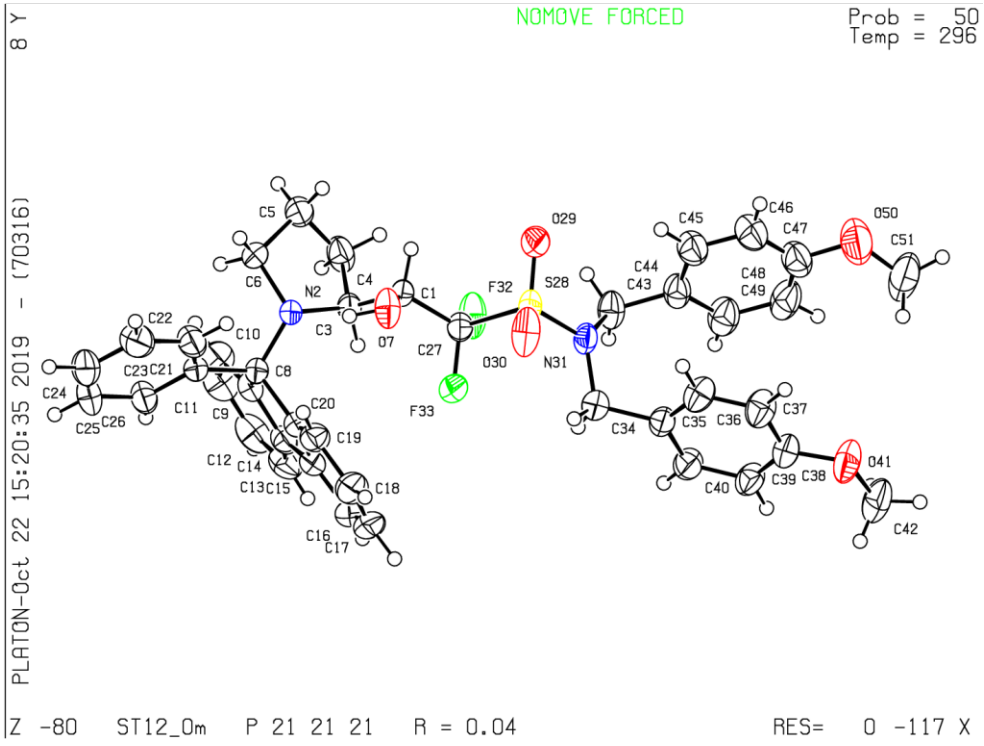


Figure 2.11. X-ray crystal structure of ß-hydroxy sulfonamide **2.89**.

This configuration can be explained by a mechanism involving chelation-controlled addition (Figure 2.12a). A Felkin-Anh model would predict the 2S,3S configuration (Figure. 2.12b). However, the standard Felkin-Anh model may not be an appropriate for nucleophilic addition to PhF-protected α-amino aldehydes. The PhF group forces the aldehyde into a conformation such that the α-proton is coplanar with the carbonyl which prevents enolization. Computational studies by Paz and Sardina have shown that the angle between the α-proton and the carbonyl may be either 0° or 180°.[116] A 0° angle would also explain the 2R,3S configuration (Figure 2.12c).

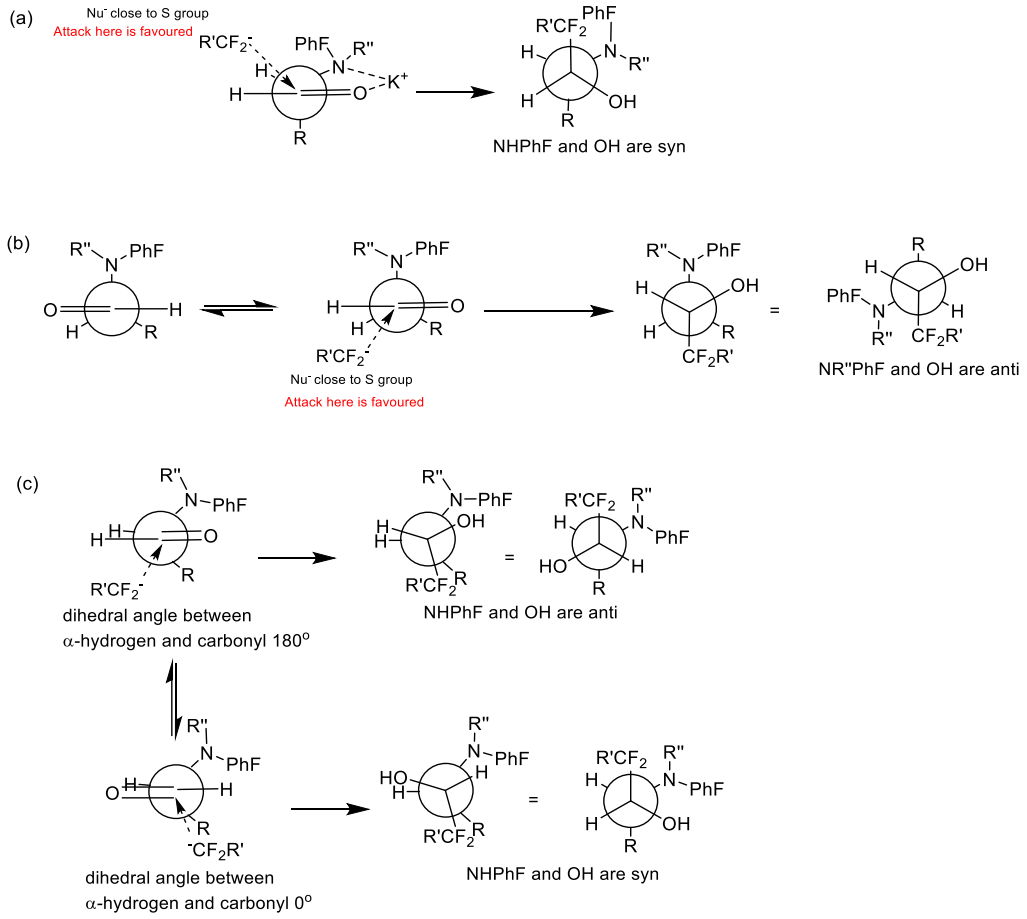
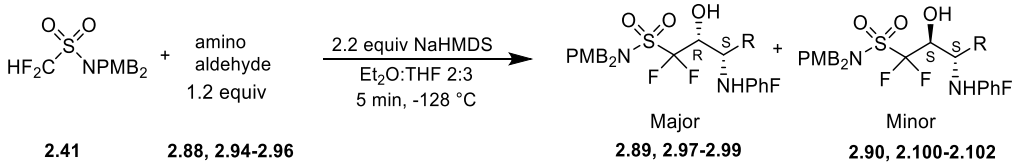


Figure 2.12. (a) Chelation mechanism predicts the syn isomer; (b) Felkin-Ahn model predicts anti isomer; (c) Paz/Sardina model predicts the syn isomer if the dihedral angle between the α-hydrogen and carbonyl is 0°.

In order to gain a better understanding of the factors that control diasteroselectivity, we repeated the between sulfonamide **2.41** and proline derivative **2.96** using NaHMDS because Na^+ chelates oxygen better than K^+. At -78 °C, the dr was significantly higher at 11:1 (Table 2.9 entry 1) and was further improved to 24:1 by lowering the temperature to -128 °C (entry 2). Curiously, the reaction required 2.2 equiv of NaHMDS and was incomplete when only 1.2 equiv was used (entry 3). This is seemingly at odds with the fact that 1.2 equiv of NaHMDS was sufficient to completely decompose sulfonamide **2.41** (Figure 2.5), although the lower temperature in this reaction may have an effect. These conditions were applied to **2.88**, **2.94** and **2.95**; however, these did not show any significant increase in dr and the yields were variable (entries 4-6). These results suggest that only aldehyde **2.96** reacts through a chelation-controlled mechanism while aldehydes **2.88**, **2.94**, and **2.95** react through the non-chelation controlled addition with the stereochemistry predicted by the Paz/Sardina model.

Table 2.9. Reaction of **2.41** with PhF protected α-aminoaldehydes and NaHMDS.

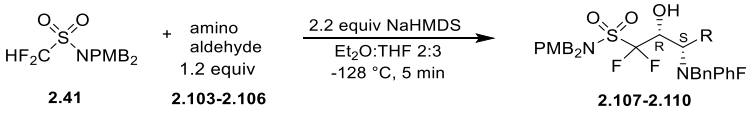


Entry	Aldehyde	Products	Yield (%)[a]	dr (RS:SS)[a]
1[b]	PhF-Pro (**2.96**)	**2.99** and **2.102**	99	11:1
2	PhF-Pro (**2.96**)	**2.99** and **2.102**	96[d]	24:1
3[c]	PhF-Pro (**2.96**)	**2.99** and **2.102**	65	19:1
4	PhF-Ala (**2.88**)	**2.89** and **2.90**	31	7.2:1
5	PhF-Met (**2.94**)	**2.97** and **2.100**	59	10.5:1
6	PhF-Asp(tBu) (**2.95**)	**2.98** and **2.101**	93	3.0:1

[a]Estimated from the ^{19}F NMR of the crude reaction mixture.
[b]Reaction run at -78 °C.
[c]1.2 equiv of NaHMDS used.
[d]Isolated yield.

The results in Table 2.9 led us to suspect that a tertiary amine would be necessary for high dr. Therefore, we prepared PhF protected aldehydes **2.103-2.106**, in which the nitrogen is protected by both benzyl and PhF groups (see experimental section for details). Using **2.41** and NaHMDS, all of these alcohols showed excellent dr (Table 2.10). For alcohols **2.107**, **2.108** and **2.110** only one isomer was observed in the ^{19}FNMR spectrum. In the case of aspartate derived alcohol **2.109**, a set of peaks was observed in the ^{19}F NMR spectrum which suggested 3% of a second diastereomer, but this was not separated and the identity of the second diastereomer is unconfirmed. Unlike the proline derivative, the diastereoselectivity of these molecules did not appear to be strongly dependent on the cation. Indeed, the aspartate and valine derivatives showed similar dr when 1.2 equiv of KHMDS was used instead of NaHMDS. In the case of valine, the yield was also much higher using KHMDS, this is probably because the valine aldehyde is quite hindered and reacts better with the longer-lived potassium carbanion.

Table 2.10 Reaction of **2.41** with *N*-benzyl-N-PhF protected α-aminoaldehydes

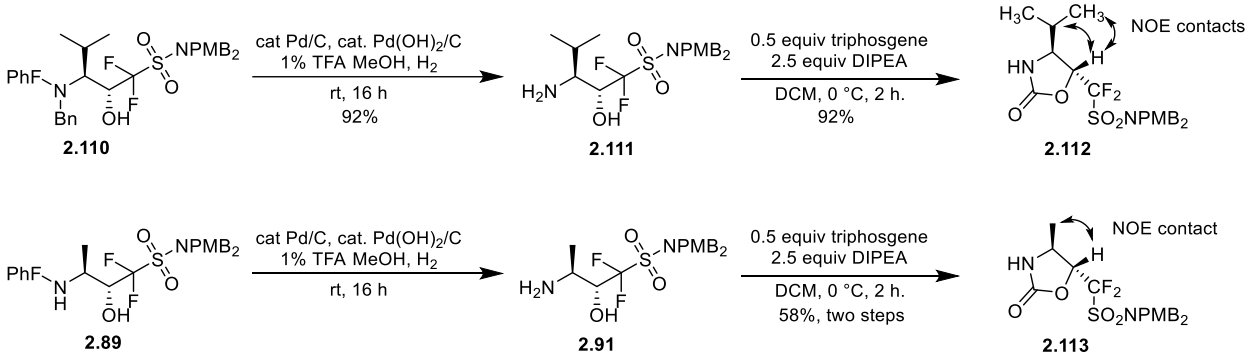


Entry	Aldehyde	Products	Yield	dr (RS:SS)[a]
1	PhF-BnAla (**2.103**)	**2.107**	81	>99:1
2	PhF-BnMet (**2.104**)	**2.108**	89	>99:1
3	PhF-BnAsp(OtBu) (**2.105**)	**2.109**	69	34:1
4	PhF-BnVal (**2.106**)	**2.110**	51	>99:1
5[c]	PhF-BnAsp(OtBu) (**2.105**)	**2.109**	70[a]	25:1
6[b,c]	PhF-BnVal (**2.106**)	**2.110**	96	>99:1

[a]Determined by ^{19}F NMR
[b]1.0 equiv of **2.41** was used.
[c]1.2 equiv of KHMDS was used instead of NaHMDS.

It would appear that proline is a special case and that the other derivatives are not produced under chelation control, therefore it no longer seemed appropriate to assign the

stereochemistry by analogy to the proline derivative. We selected ß-hydroxy sulfonamides **2.110** and **2.89** and deprotected these molecules to give ß-hydroxy amines **2.111** and **2.91** which were cyclized in the presence of triphosgene to give **2.112** and **2.113** (Scheme 2.21). In both cases, NOE contacts confirmed a trans relationship in the ring which confirmed that the absolute configuration was indeed 2R,3S which is the same configuration as the proline derivative. It would appear that the proline derivative progresses through chelation-controlled addition, while the other *N*-PhF and *N*-PhF-*N*-benzyl-protected α-amino aldehydes adopt a 0 °C conformation as predicted by the Paz-Sardina model. Both cases lead to a 2R,3S or syn configuration in the major product. The configuration in the remaining *N*-PhF and *N*-PhF-*N*-benzyl compounds can be assigned as 2R,3S for the major isomer by analogy to **2.89** or **2.110**.

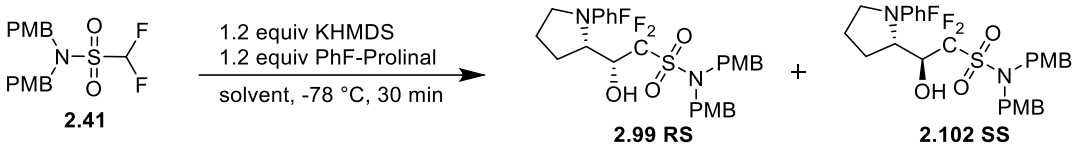


Scheme 2.21. Determination of stereochemistry in compounds 2.112 and 2.113.

We had originally planned to perform the synthesis of all ß-hydroxy-α,α-difluoromethanesulfonamides in 100% THF at -78 °C. However, during the course of these studies we found that it was useful to perform these reactions at lower temperatures. THF freezes at -108 °C; therefore, we employed a 1:1 mixture of THF and Et_2O for reactions at -114 °C and later discovered that a 3:2 mixture of THF:Et_2O remained liquid at -128 °C. To rule out the possibility of solvent effects, we performed the reaction between PhF-prolinal and **2.41** at -78 °C

in various mixtures of THF and Et_2O (Table 2.11). There was no significant change in dr up to 75% Et_2O, however at 96% Et_2O the dr fell almost to 1:1. Since all of our studies were performed between 0 and 50% Et_2O we can safely ignore any solvent effect on dr. A temperature of -128 °C is obtained by partially freezing a mixture of 86% methanol and 14% water.

Table 2.11. Solvent effects on the diastereoselectivity of the reaction of **2.41** and PhF-prolinal.



Entry	Solvent	dr (RS:RR)[a]
1	THF	2.5
2	THF:Et_2O 3:1	2.76
3	THF:Et_2O 1:1	2.82
4	THF:Et_2O 1:3	2.6
5	THF:Et_2O 1:25	1.14

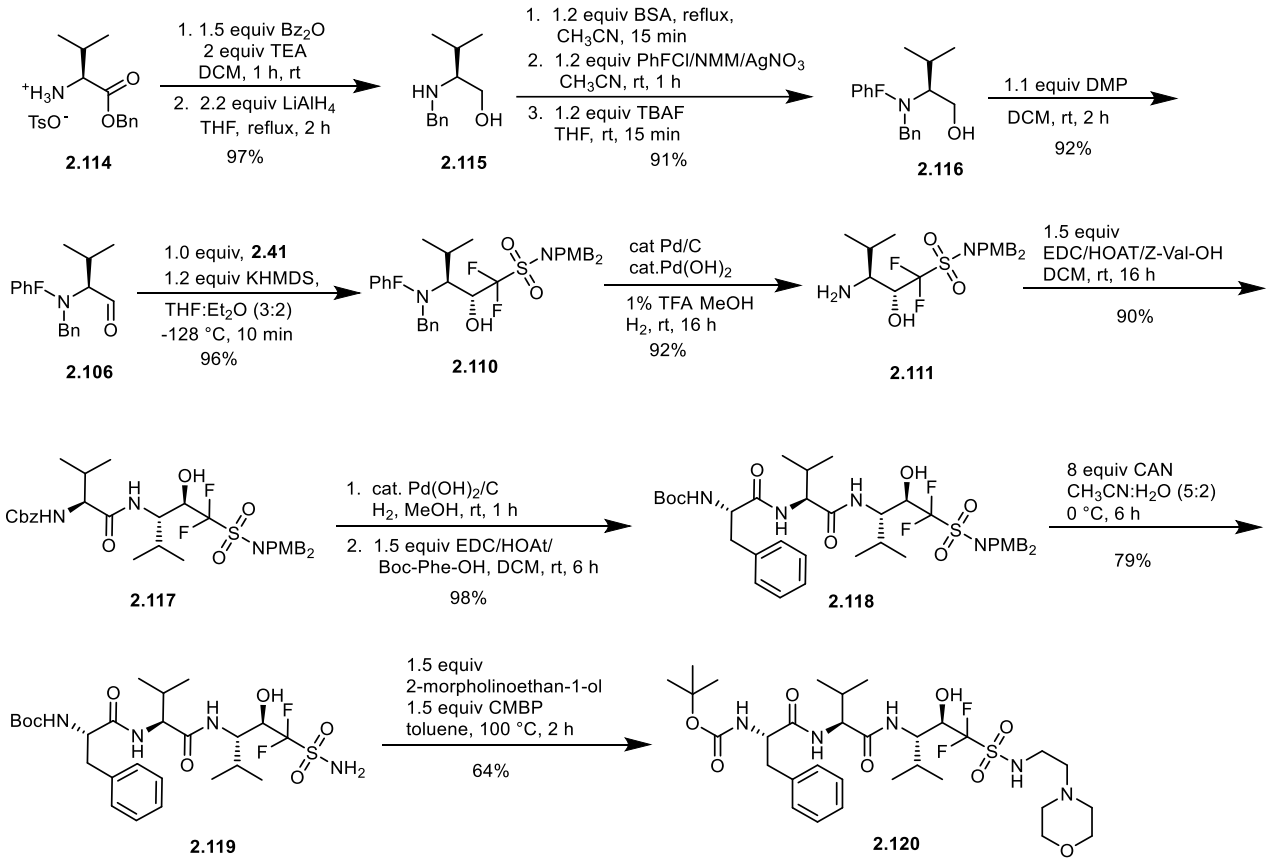
[a]Determined by ^{19}F NMR

2.2.4 — Synthesis of a peptidomimetic.

To demonstrate the applicability of our approach to peptidomimetics we sought to prepare **2.120** (Scheme 2.22). ß-hydroxy-α,α-difluorosulfonamide **2.120** is structurally similar to a reported ß-hydroxy-α,α-difluoroamide renin inhibitor **2.1** (Figure 2.1).[75] Beginning with commercially available amino acid ester **2.114**, the nitrogen was benzoylated, followed by reduction of both the amide and ester groups to give **2.115**. PhF protection of the amine with temporary protection of the alcohol group yields **2.116**. Attempts to oxidize this alcohol using Swern protocols gave inconsistent yields and side products. Fortunately, Dess Martin oxidation gave an excellent yield of **2.106** on a multigram scale. Reaction with sulfonamide **2.41** yielded ß-hydroxy-α,α-difluorosulfonamide **2.110**. The benzyl and PhF groups can both be removed by

hydrogenolysis with a small amount of acid present, but removal of the benzyl group was sluggish; therefore, we employed a combination of palladium and palladium hydroxide catalysts which is reported to give better results than either catalyst alone.[117] Under these conditions, the PhF group is completely removed in less than 1 hour, while the benzyl group requires overnight treatment to reveal **2.111**. Tripeptide **2.118** was constructed using standard peptide coupling methodology. PMB groups in sulfonamides are usually removed using TFA.[10] However, due to the α-fluorines, the sulfonamide nitrogen in **2.118** has reduced basicity, and an overnight reflux with TFA was required to remove PMB groups as demonstrated using sulfonamide **2.99** as a model system. PMB groups in sulfonamides have also been removed using CAN.[10] This reaction generates nitric acid which could result in removal of the Boc group; however, we found that this reaction on **2.118** proceeds at 0 °C using excess CAN, and provided primary sulfonamide **2.119** without significant loss of the terminal Boc group.

Finally, a Mitsunobu reaction furnished *N*-alkyl sulfonamide **2.120**. Using typical DIAD/PPh_3 conditions, only trace product could be observed by HPLC. This is probably due to the formation of a triphenylphosphine sulfonyl imine which is a known side reaction with primary sulfonamides.[118] To overcome this side reaction, we employed cyanomethyltributylphosphorane (CMBP) which cannot form similar side products and provided the desired monoalkylation in 64% yield.[119] Under these conditions no overalkylation was observed. The overall yield of **2.120** from commercially available **2.114** was 32%.

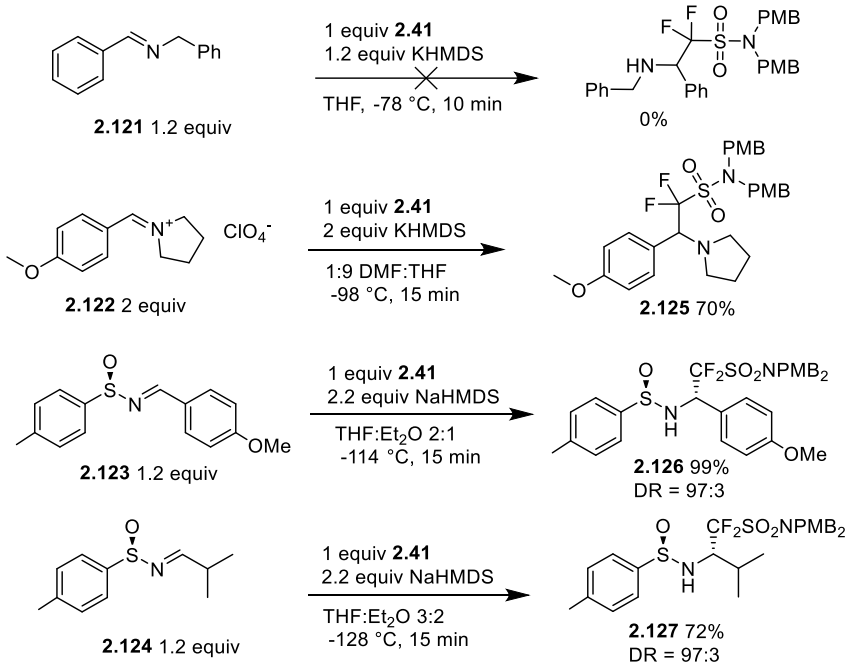


Scheme 2.22. Synthesis peptidomimetic 2.120.

2.2.6 — Reactions of α,α-difluoromethanesulfonamides with imines: preliminary results

Encouraged by our success with ß-hydroxy-α,α-difluoromethanesulfonamides we decided to further explore the chemistry of α,α-difluoromethanesulfonamide carbanions with non-carbonyl electrophiles, beginning with imines **2.121**-**2.124** (Scheme 2.23). None of the desired product was formed in the reaction with benzyl imine **2.121** and **2.41** was completely decomposed. Using the more electrophilic iminium salt, **2.122**, the ß-amino-α,α-difluorosulfonamide **2.125** was obtained in 70% yield. This reaction was complicated by the poor solubility of **2.122** in THF or THF:Et_2O mixtures, especially at low temperatures; however, **2.122** was soluble in 10% DMF in THF at -98 °C. Next, we attempted the reaction with sulfinylimine **2.123** which we expected would be more electrophilic than **2.121** and more soluble

than **2.122.** To our delight, the desired **2.126** was obtained with high dr and this was improved even further by changing the base to NaHMDS leading to a quantitative yield of **2.126** with 97:3 dr. The reaction was also successful with **2.124** leading to valine analog **2.127** with excellent dr, this reaction is particularly notable because **2.124** is enaminizable. The synthesis of ß-amino-α,α-difluorosulfones from sulfinimines is well known.[122-126] Therefore, the stereochemistry of **2.126** and **2.127** can tentatively be assigned by analogy to the sulfones which proceed through a boat like chelation controlled transition state.[126]

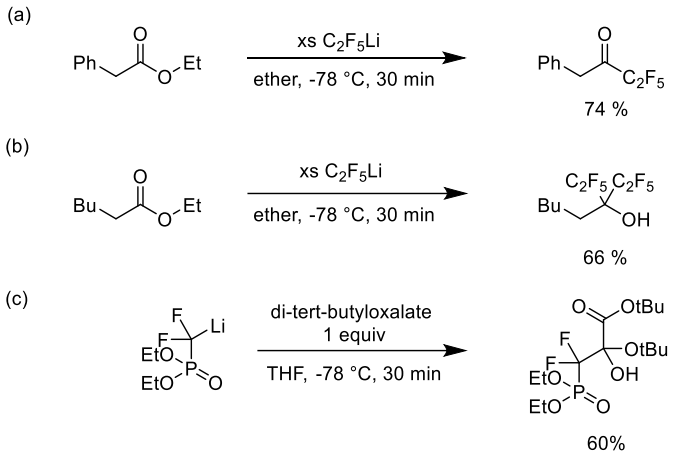


Scheme 2.23. Synthesis of ß-amino-α,α-difluorosulfonamides. Relative stereochemistry is shown, sulfinimines 2.123 and 2.124 were racemic.

2.2.5 — Reactions of α,α-difluoromethanesulfonamides with carboxylic acid derivatives: preliminary results

While nonfluorinated Grignard reagents and organolithiums generally add to simple esters twice to generate tertiary alcohols, fluorinated nucleophiles may add one or two times. The outcome of the reaction can be difficult to predict. For example, pentafluoroethyl lithium adds

just once to ethyl phenylacetate but twice to ethyl hexanoate (Scheme 2.24 a and b). The difference was shown to be due to the stability of the tetrahedral intermediate rather than to the formation of an enolate.[120] In some cases the tetrahedral intermediate is stable enough to be isolated (Scheme 2.23 c).[121]

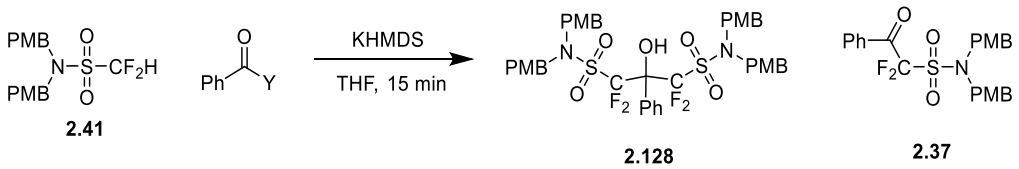


Scheme 2.24. Literature reactions for the addition of fluoroalkyl nucleophiles to esters.

We considered that the reaction of α,α-difluoromethanesulfonamides with esters would be a more efficient route to ß-keto-α,α-difluorosulfonamides than was described in Chapter 1. Unfortunately, reaction with methyl benzoate yielded none of the desired product (Table 2.12) probably because the methoxide leaving group is too poor and the reverse reaction dominates. When phenyl benzoate was used instead, double addition to the carbonyl was observed yielding a mixture of tertiary alcohol **2.128** and a number of unidentified side products. Using benzoic anhydride, a mixture of double and single addition was observed. By reducing the temperature to -128 °C and, using 2 equiv of KHMDS and 4 equiv of benzoic anhydride, single addition could be obtained as the main product giving **2.37**. We had hoped that this reaction would also be applicable to amino acid derivatives. Unfortunately, none of the desired ketone was obtained from the reaction using either $(Boc\text{-}Pro)_2O$ or the mixed anhydride obtained from Boc-proline

and isobutyl chloroformate. No product was obtained from the reaction of **2.41** with the Weinreb's amide of benzoic acid.

Table 2.12. Reaction of **2.41** with benzoic acid derivatives.

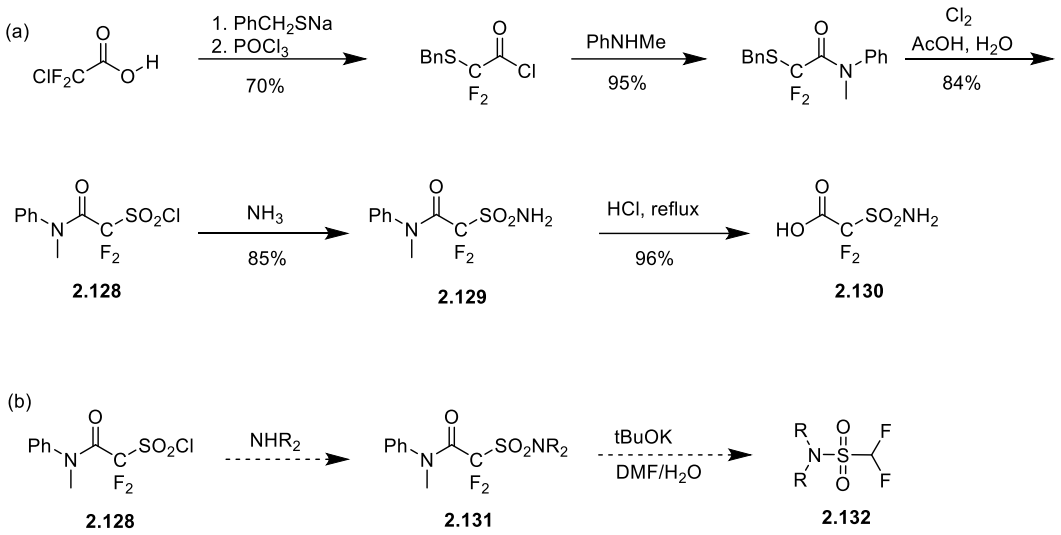


Entry	Electrophile	KHMDS	Temperature	Yield **2.128** (%)	Yield **2.37** (%)
1	methyl benzoate (1.2 equiv)	1.2 equiv	-78 °C	0	0
2	phenyl benzoate (1.2 equiv)	1.2 equiv	-78 °C	35	0
3	benzoic anhydride (1.2 equiv)	1.2 equiv	-78 °C	36	24
4	benzoic anhydride (4 equiv)	2 equiv	-128 °C	trace	65

2.3 — Conclusions and future studies.

In section 2.2.1 of this chapter, we have developed an efficient method for the synthesis of difluoromethane sulfonamides. This method is more efficient than previously reported methods and does not rely on ozone depleting chemicals. However, our route does involve several steps, namely mesylation of an amine, deprotonation using *n*-BuLi, acylation of the resulting carbanion, electrophilic fluorination, and basic hydrolysis. These steps limit the substrate scope. Boyle et al. have published the synthesis of ß-amido-α,α-difluorosulfonyl chloride **2.128** which is prepared in 4 steps from difluorochloroacetic acid. **2.128** reacts with ammonia to give the sulfonamide **2.129** which is hydrolyzed to give the carboxylic acid **2.130** (Scheme 2.25a).[11] We envision that sulfonyl chloride **2.128** could also react with other amines to give sulfonamides of type **2.131** (Scheme 2.25b). ß-Amido-α,α-difluorosulfonamides have already been shown to undergo alcoholysis with tBuOK in DMF to produce difluoromethane

sulfonamide carbanion which we hypothesize could be trapped with water to give the difluoromethane sulfonamides **2.132**.[71] This route would be superior in cases where the amine contains sensitive groups that would not tolerate *n*-BuLi or Selectfluor. This approach also delivers **2.132** in fewer linear steps from the amine; therefore, this would be attractive for the late stage difluoromethane sulfonylation of complex amines.



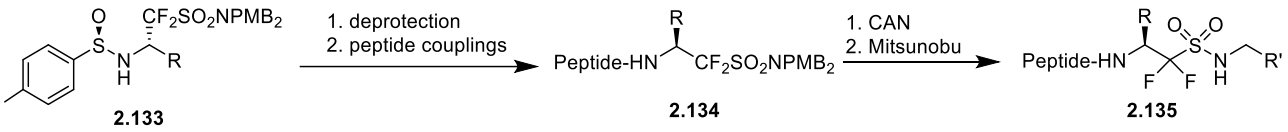
Scheme 2.25. (a) Literature synthesis of 2,2-difluoro-2-sulfamoylacetic acid; (b) Proposed synthesis of difluoromethane sulfonamides via a ß-amido-α,α-sulfonyl chloride.

We have shown that the lifetime of difluoromethanesulfonamide carbanions is strongly dependent on the counter ion, with potassium salts being longer lived than lithium salts; however, we have not yet thoroughly investigated the larger rubidium and cesium ions. Deuterium exchange in **2.41** could be affected by Cs_2CO_3 in a mixture of DMF and D_2O in 1 hour at room temperature without detectable decomposition. However, under these conditions **2.41** did not show any reaction with benzaldehyde, probably because carbonate is not a strong enough base to deprotonate a significant portion of the sulfonamide. However, if the cesium salt were generated quantitatively by a base such as CsHMDS, then we would expect the carbanion

to be longer lived for the reasons discussed in Section 2.2.2 and a this might allow for reactions with a larger range of electrophiles such as alkyl halides or epoxides.

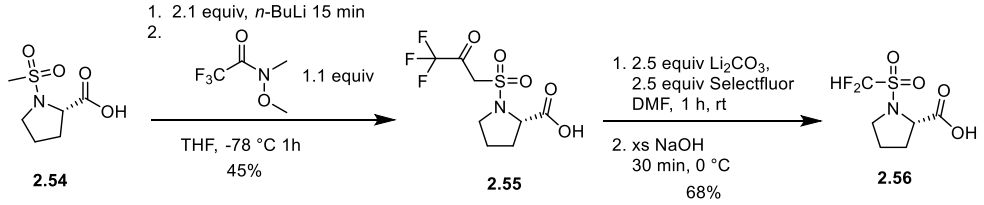
The chemistry of difluoromethanesulfonamide anions was explored, and in particular their reactions with aldehydes to give ß-hydroxy-α,α-difluoromethanesulfonamides. This reaction works well using non-enolizable aldehydes and ketones and modestly using enolizable substrates. Nevertheless, by employing PhF-protected α-amino aldehydes we were able to achieve excellent yields of ß-hydroxy-α,α-difluoromethanesulfonamides derived from amino acids, and by fully protecting the nitrogen with a PhF and a benzyl group, we were able to achieve good to excellent yields and excellent dr values. Furthermore, we have demonstrated that these can be deprotected and elaborated into peptidomimetics and we expect these to have applications as inhibitors of aspartyl proteases.

The sulfonamide carbanions also reacted effectively with sulfonyl imines to give ß-amino-α,α-difluorosulfonamides **2.133** in good yields with high diastereoselectivity. Future studies in the Taylor group will involve the incorporation of these molecules into peptidomimetics. This will involve deprotection of the amine followed by elaboration of the molecule with standard peptide couplings to give ß-amino-α,α-difluorosulfonamides **2.134**. Following deprotection, sulfonamides of type **2.135** will be obtained by Mitsunobu reaction with alcohols (Scheme 2.26). There is one other example of ß-amino-α,α-difluorosulfonamides which involves synthesis of the analogous sulfone followed by a laborious and low yielding conversion to the sulfonyl chloride which reacts with amines to give the sulfonamides of type **2.135**.[122] The proposed method would be superior in cases where the alcohol is readily available.

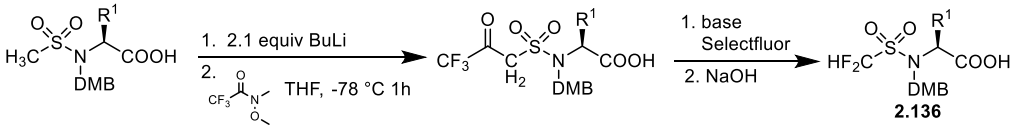


Scheme 2.26. Proposed synthesis of peptidomimetics incorporating ß-amino-α,α-difluorosulfonamides.

We have shown that proline derivative **2.56** can be prepared by the procedure outlined in Scheme 2.12 (shown again below). It would be of interest to determine if this procedure could be optimized and expanded to other amino acids to give compounds of type **2.136** as outlined in Scheme 2.27.

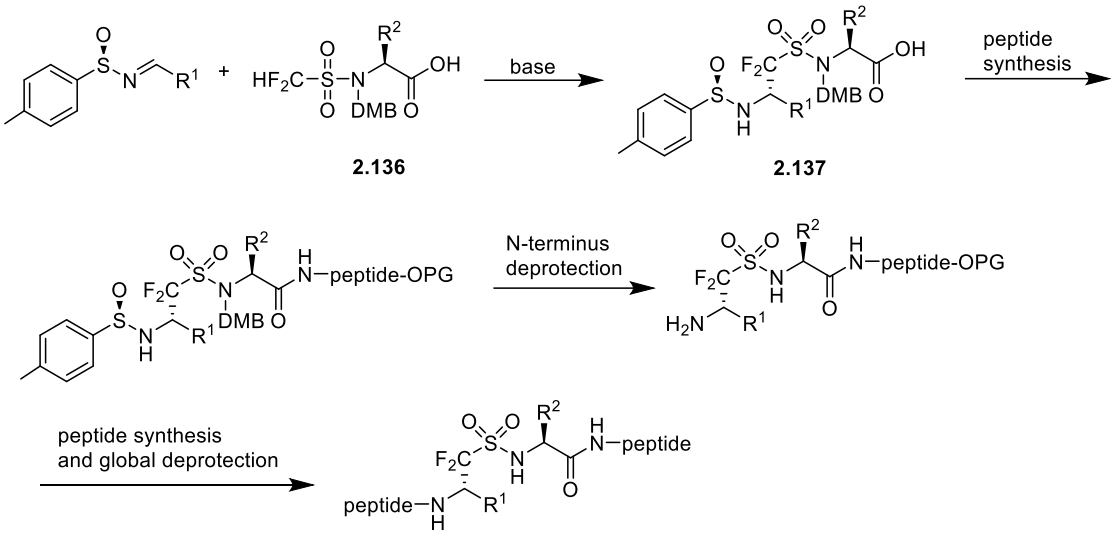


Scheme 2.12. Synthesis of a *N*-difluoromethanesulfonyl amino acid.

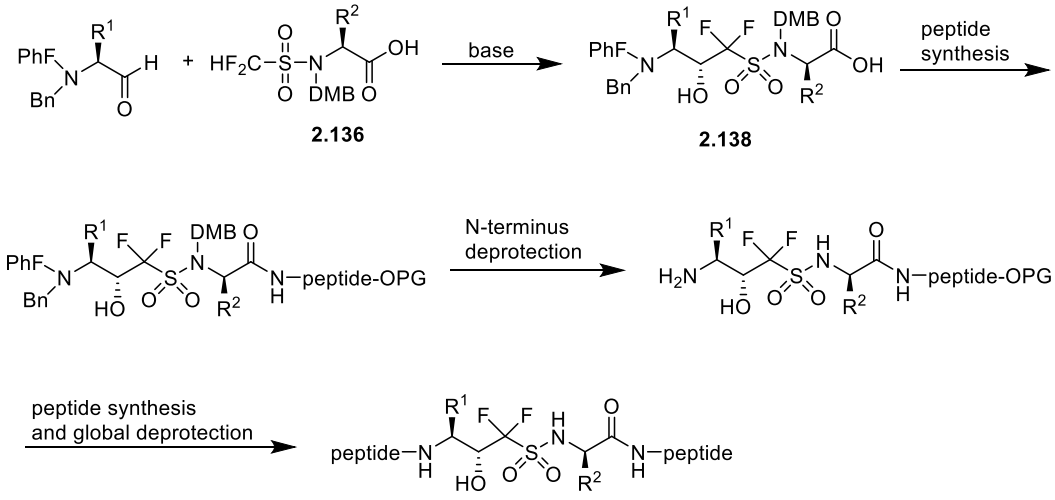


Scheme 2.27. Synthesis of a *N*-difluoromethanesulfonyl amino acids.

It would also be of interest if amino acid derivitives of type **2.136** could be used to prepare ß-hydroxyl and ß-amino sulfonamides of type **2.137** and **2.138** as outlined in Scheme 2.28 and 2.29. Although, the carboxylic acid of **2.136** may require protection in this transformation. Sulfonamides **2.137** and **2.138** could be added to a peptide chain followed by nitrogen deprotection and further peptide synthesis.



Scheme 2.28. Proposed synthesis of peptidomimetics incorporating α,α-difluorosulfonamides using compounds of type **2.136.**



Scheme 2.29. Proposed synthesis of peptidomimetics incorporating ß-hydroxy-α,α-difluorosulfonamides using compounds of type **2.136.**

2.4 — Experimental.

2.4.1 — General information.

All reagents and starting materials were obtained from commercial sources and used as received unless stated otherwise. Acetonitrile, DCM, methanol, and DMF were distilled from

calcium hydride. Diethyl ether, THF, toluene and hexane were distilled from sodium metal. All reactions were performed under an argon atmosphere using flame dried glassware unless stated otherwise. Reactions were monitored by TLC until deemed complete using aluminium backed silica plates. Plates were visualized under ultraviolet light (254 nm) and/or staining with $KMnO_4$ or cerium ammonium molybdate. Cooling of reaction mixtures to -78 °C was achieved using a dry ice-acetone bath, -98 °C with liquid N_2 /MeOH, -114 °C with liquid N_2 /EtOH, and -128 with liquid N_2 and 86:14 MeOH:H_2O.

All ^{13}C NMR spectra were proton decoupled. All ^{19}F NMR were not proton decoupled, in cases where yield is estimated by ^{19}F NMR the relaxation delay between scans is increased to 16 sec. Chemical shifts (δ) for ^{1}H NMR spectra run in $CDCl_3$ are reported in ppm relative to the standard TMS. Chemical shifts for ^{13}C NMR spectra run in $CDCl_3$ are reported in ppm relative to the solvent residual carbon (δ 77.16 for central peak). Chemical shifts for ^{19}F NMR spectra run in $CDCl_3$ are reported in ppm relative to the standard $CFCl_3$. High-resolution positive ion electrospray (ESI+) mass spectra were obtained using a quadrupole-orbitrap Mass spectrometer, dissolving samples in 1:1 MeOH/H_2O + 0.1% formic acid.

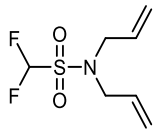
2.4.2 — Experimental procedures for synthesized compounds.

General procedure 2.1 Fluorination and cleavage of benzoyl methanesulfonamides.

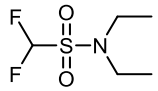
See Chapter, 1 Section 1.2.3 for the synthesis of benzoyl methanesulfonamides **2.31-2.34**.

The sulfonamide (1 equiv) was dissolved in DMF (10 mL per g of Selectfluor) and cooled to -20 °C then TBAF (1 M in THF, 4 equiv) and Selectfluor (4 equiv) were added. The mixture was stirred until complete by TLC (2-4 h) then 10 equiv of 6 M NaOH was added. The reaction was stirred for 1 hour, generally it is not possible to separate the difluoromethane sulfonamide from the ß-keto-α,α-difluorosulfonamide by TLC. The reaction was diluted with 4

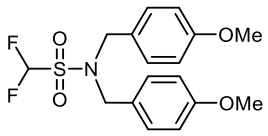
volumes of water and extracted three times with 2 volumes of diethyl ether. The combined organic layers are washed three times with an equal volume of water then dried over magnesium sulfate, filtered, and concentrated to dryness. The residue was purified by flash chromatography if necessary.



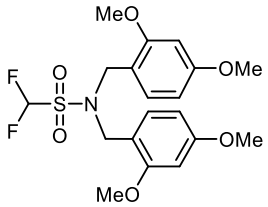
N,N-diallyl difluoromethanesulfonamide (**2.39**). Prepared according to general procedure 2.1 and obtained as a colorless oil without further purification. 1.50 g (99% yield) from 2.00 g (7.17 mmol) of **2.31**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.16 (t, 1H, *J* = 53.9 Hz), 5.78 (m, 2H), 5.28 (m, 4H), 3.93 (d, 4H, *J* = 6.1 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 132.0, 120.0, 114.0 (t, *J* = 282.7 Hz), 49.5; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -122.1 (d, *J* = 53.7 Hz); HRMS (ESI^+) m⁄z: $[M + Na]^+$ calcd for $C_7H_{11}F_2NNaO_2S$, 234.0371; found, 234.0361.



N,N-diethyl difluoromethanesulfonamide (**2.40**). Prepared according to general procedure 2.1 and obtained as a colorless oil after flash chromatography with 10% EtOAc in hexanes. 900 mg 68% yield) from 1.8 g of **2.32**. ^{1}H NMR ($CDCl_3$, 300 MHz):δ 6.12 (t, 1H, J = 54.0 Hz), 3.41 (q, 4H, J = 7.2 Hz), 1.21 (t, 6H, J = 7.1 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 114.2 (t, J = 280.3 Hz), 42.3, 14.3; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -122.3 (d, *J* = 53.6 Hz); HRMS (ESI^+) m⁄z: $[M + H]^+$ calcd for $C_5H_{12}O_2NF_2S$, 188.0551; found, 188.05440.



N,N-bis(2-methoxybenzyl) difluoromethanesulfonamide (**2.41**). Prepared according to general procedure 2.1 and was obtained as a white solid after flash chromatography with 15% EtOAc in hexane. 630 mg (99 % yield) from 755 mg of **2.33**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.16 (d, 4H, *J* = 8.2 Hz), 6.87 (d, 4H, *J* = 8.2 Hz), 6.04 (t, 1H, *J* = 53.8 Hz), 4.32 (s, 4H), 3.80 (s, 6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 159.6, 130.1, 126.7, 114.4 (t, *J* = 280.9 Hz), 114.2, 55.1, 50.0; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -121.08 (d, *J* = 53.8 Hz); HRMS (ESI^{+}) m⁄z: [M + Na]$^{+}$ calcd for $C_{17}H_{19}F_2NNaO_4S$, 394.0895; found, 394.0879.

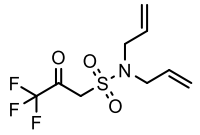


N,N-bis(2,4-dimethoxybenzyl) difluoromethanesulfonamide (**2.42**). Prepared according to general procedure 2.1 and was obtained as a white solid after flash chromatography 20 to 30% EtOAc hexane, 60 mg (62% yield) from 112 mg of **2.34**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.15 (d, 2H, *J* = 8.2 Hz), 6.43 (m, 4H), 5.84 (t, 1H *J* = 54.2 Hz), 4.43 (s, 4H), 3.75 (s, 6H), 3.72 (s, 6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 160.9, 158.5, 130.9, 115.9, 113.9 (t, *J* = 230.4 Hz), 104.3, 98.2, 55.2, 55.0, 46.1; ^{19}F NMR ($CDCl_3$, 283 MHz) : δ -121.94 (d, *J* = 54.5 Hz); HRMS (ESI^{+}) m⁄z: [M + H]$^{+}$ calcd for $C_{19}H_{24}F_2N_2O_6S$, 432.1287; found, 432.1286.

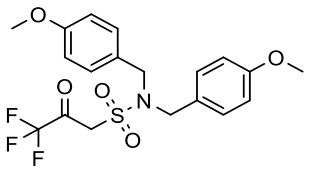
General procedure 2.2 for the preparation of trifluoracetyl methanesulfonamides 2.47 – 2.50

See chapter 1 for the synthesis of methane sulfonamides **2.43** – **2.46**.

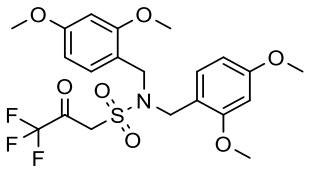
A solution of methane sulfonamide (1.00 equiv) in THF (0.1-0.33 M) was cooled to –78 °C and *n*-BuLi (2.5 M in hexane, 1.05 equiv) was added dropwise. The resulting solution was stirred for 15 min then *N*-methyl-*N*-methoxytrifluoroacetamide (1.10 equiv) was added as a neat liquid. Stirring was continued for 1 hour before the reaction was quenched with saturated NH_4Cl and extracted three times into DCM. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated to give crude products. Yields are calculated based on the molecular weight of the ketone form, however a ketone-hydrate mixture was typically observed by NMR.



1-trifluoroacetyl-N,N-diallyl methanesulfonamide (**2.47**). Prepared according to general procedure 2.The crude product was dissolved in 200 mL 1:1 Et_2O:hexane and extracted three times with 100 mL 5% Na_2CO_3, the combined aqueous layers were acidified to pH 1 and back extracted three times with 200 mL DCM. The combined DCM layers were dried over magnesium sulfate, filtered and concentrated to give **2.47** as a white solid (6.5 g, 94% yield) from 4.45 g (25.5 mmol) of **2.43**. ^{1}H NMR (acetone-d_6 : D_2O 1:1, 300 MHz): δ 6.19 (m, 2H), 5.63 (m, 4H), 4.25 (d, 4H, J = 6.3 Hz); $^{13}C\{^1H\}$ NMR (acetone-d_6 : D_2O 1:1, 75 MHz): δ 135.8, 125.3 (q, J = 288.0 Hz), 121.1, 94.1 (q, J = 32.6 Hz), 56.7 (quintet, J = 20.9 Hz), 52.3 (note that the methylene group of the hydrated ketone undergoes rapid deuterium exchange); ^{19}F NMR (acetone-d_6 : D_2O, 300 MHz): δ -83.6; HRMS(ESI$^+$) *m/z:* [M + H_2O + Na]$^+$ calcd for $C_9H_{14}F_3NNaO_4S$, 312.0488; found, 312.0497.

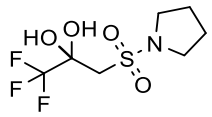


1-trifluoroacetyl-N,N-bis(4-methoxybenzyl)methanesulfonamide (**2.48**). Prepared according to general procedure 2.2. The crude material was dissolved in approximately 50 mL EtOAc and 250 mL hexane at reflux temperature, then slowly cooled to -78 °C overnight to yield 20.8 g white crystals. The mother liquor was evaporated and subjected to flash chromatography 0 to 10% EtOAc in DCM to yield a further 4.1 g. A total of 24.9 g (96% yield) of **2.48** was obtained from 20.1 g (60 mmol) of **2.44**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.19 (d, 4H, *J* = 8.0 Hz), 6.86 (d, 4H, *J* = 8.0 Hz), 4.68 (s, 2H), 4.31 (s, 4H), 3.80 (s, 6H), 3.22 (s, 2H); ^{13}C{^{1}H} NMR (9:1 acetone-d_6:D_2O, 125 MHz): δ 159.2, 130.0, 128.1, 122.7 (q, *J* = 288.1 Hz), 113.8, 91.7 (q, *J* = 32.1 Hz), 54.8, 50.6, 49.4 (Note that the methylene carbon undergoes exchange with D_2O); ^{19}F NMR ($CDCl_3$, 282 MHz): δ -78.6 (ketone), -87.0 (hydrate); HRMS (ESI$^+$) m⁄z: $[M + H_2O+Li]^+$ calcd for $C_{19}H_{22}F_3LiNO_6S$, 456.1275; found, 456.1268.

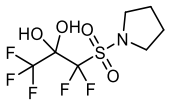


1-trifluoroacetyl-N,N-bis(2,4-dimethoxybenzyl)methanesulfonamide (**2.49**). The crude product was purified by flash chromatography, eluting with 0 to 10% EtOAc in DCM, to yield **2.49** as a white solid (368 mg, 75% yield) from 395 mg (1.00 mmol) of **2.45**. ^{1}H NMR (9:1 acetone-d_6:D_2O, 500 MHz): δ 7.11 (d, 2H, *J* = 8.7 Hz), 6.47 (s, 2H), 6.44 (d, 2H, *J* = 8.7 Hz), 4.35 (s, 4H), 4.05(s, 2H), 3.74 (s, 12H); ^{13}C{^{1}H} NMR (9:1 acetone-d_6:D_2O, 125 MHz): δ 160.7, 158.4, 130.7, 122.7 (q, *J* = 286.0 Hz*),* 116.2, 104.4, 98.0, 91.7 (q, *J* = 32.6 Hz*),* 54.9, 54.8, 54.2, 45.1 (note that the methylene group undergoes exchange with deuterium oxide); ^{19}F NMR (9:1

acetone-d_6:D_2O, 282 MHz): δ -86.5; HRMS (ESI^+) m/z: $[M + H_2O + Na]^+$ cald for $C_{21}H_{26}F_3NO_8SNa$, 532.1223; found, 532.1215.



1,1,1-trifluoro-3-(pyrrolidin-1-ylsulfonyl)propan-2-one (**2.50**). Prepared according to general procedure 2.2. The crude product was purified by recrystallization from hot Et_2O and hexane to give **2.50** (4.95 g, 80% yield) from 3.73 g (25.0 mmol) of **2.46** as a monohydrate. ^1H NMR (acetone-d_6, 300 MHz): δ 6.42 (s, 2H), 3.53 (s, 2H) 3.43 (m, 4H), 1.94 (m, 4H); $^{13}C\{^1H\}$ NMR (acetone-d_6, 75 MHz): δ 127.9 (q, J = 287.0 Hz), 97.0 (q, J = 32.0 Hz), 55.3, 52.7, 30.6; ^{19}F NMR (acetone-d_6, 282 MHz): δ -81.6; HRMS (ESI^+) *m/z:* $[M + H^+]$ calcd for $C_7H_{11}O_3NF_3S$, 246.0406; found, 246.0407.

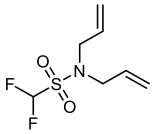


1,1,1,3,3-pentafluoro-3-(pyrrolidin-1-ylsulfonyl) propane-2,2-diol (**2.52**). Ketone **2.50** (280 mg, 1.14 mmol, 1.00 equiv) was dissolved in 10 mL of DMF and Selectfluor (1.00 g, 2.82 mmol, 2.50 equiv) was added. The reaction was placed in a preheated oil bath at 100 °C and stirred for 1 hour. The reaction was cooled, and an aliquot was removed and examined by ^{19}F NMR spectroscopy which indicated complete conversion. The mixture was diluted with 40 mL of water and extracted three times with 30 mL ether. The combined organic layers were washed three times 50 mL water and the aqueous washes were back extracted once with 100 mL ether. The combined ether layers were dried over magnesium sulfate, filtered, and concentrated to give 245 mg of a yellow solid. This was recrystallized from hot ether and hexane to yield **2.52** (68 mg, 20% yield) of the monohydrate as sharp colorless crystals. ^1H NMR ($CDCl_3$, 300 MHz) δ:

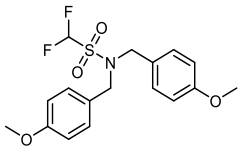
4.58 (br, 2H), 3.58 (m, 4H), 2.00 (m, 4H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 118.5 (tq, *J* = 298.0, 1.3 Hz), 116.9 (qt, *J* = 289.6, 2.3 Hz), 91.6 (qt, *J* = 55.6, 25.4 Hz), 49.3, 25.8; ^{19}F NMR($CDCl_3$, 282 MHz) δ: 81.4 (t, 3F, *J* = 11.2 Hz), -110.76 (d, 2F, *J* = 11.2 Hz); HRMS (ESI^+) *m/z:* calcd for $C_7H_{10}NO_4SF_5Na^+$ $(M+Na)^+$: 322.0143, found 322.0143.

General procedure 2.3 for the preparation of difluoromethanesulfonamides from trifluoracetyl methanesulfonamides

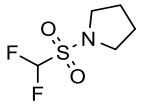
The sulfonamide (1.00 equiv) were dissolved in DMF (0.1 M). Selectfluor (2.50 equiv) was added followed by lithium carbonate (2.50 equiv) and the mixture was stirred for 3 h at room temperature until the reaction was complete as determined by ^{19}F NMR (The fluorinated and unfluorinated ketones were usually difficult to distinguish by TLC due to partial hydrolysis of the fluorinated ketone). The mixture was then cooled to 0 °C and 6 M aq. sodium hydroxide (10 equiv) was added. The mixture is stirred a further 15 min. The reaction was diluted with 4 volumes of water and extracted three times with 2 volumes of diethyl ether. The combined organic layers are washed three times with an equal volume of water then dried over magnesium sulfate, filtered, and concentrated to dryness. The residue was purified by flash chromatography if necessary.



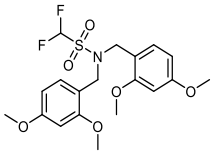
N,N-diallyl difluoromethanesulfonamide (**2.39**). Prepared according to general procedure 2.3. The crude material was purified by flash chromatography, eluting with 0 to 50% Et_2O in hexane, to give **2.39** (4.20 g, 95% yield) from 5.69 g (21.0 mmol) of **2.47**. This material was identical to that prepared according to general procedure 2.1.



N,N-bis(4-methoxybenzyl) difluoromethanesulfonamide (**2.41**). Prepared according to general procedure 2.3 and was obtained as a white solid after flash chromatography, 50 to 70% DCM in hexane, to give 2.55 g of **2.41** (74% yield) from 4.00 g (9.28 mmol) of **2.48**. This material was identical to that prepared according to general procedure 2.1.

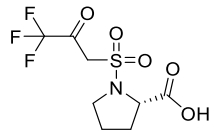


1-((difluoromethyl)sulfonyl)pyrrolidine (**2.53**). Prepared according to general procedure 2.3 and was obtained as a pure solid without further purification (250 mg, 67% yield) from 500 mg (2.04 mmol) of **2.50**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.18 (t, 1H, J = 53.9 Hz), 3.48 (m, 4H), 1.94 (m, 4H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 114.9 (t, J = 280.1 Hz), 48.6, 25.9; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -121.8 (d, J = 53.6 Hz); HRMS (ESI^+) m⁄z: $[M + Na]^+$ calcd for $C_5H_9F_2NNaO_2S^+$, 208.0214; found, 208.0215.

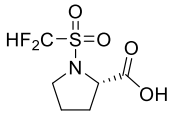


N,N-bis(2,4-dimethoxybenzyl) difluoromethanesulfonamide (**2.42**). Ketone **2.49** (970 mg, 1.98 mmol, 1.00 equiv) and Cs_2CO_3 (1.60 g, 5.00 mmol, 2.50 equiv) were dissolved in 20 mL DMF and cooled to -20 °C. Selectfluor (1.75 g, 4.93 mmol, 2.50 equiv) was added dropwise as a solution in 60 mL DMF over 3 h. Stirring was continued for two more h while the reaction was warmed slowly to 0 °C. 15 mL of 6 M NaOH was added and the mixture was stirred for 15 min. The mixture was diluted with 500 mL of water and extracted three times with 200 mL of Et_2O.

The combined organic layers were washed three times with 200 mL of water, dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 10 to 100% DCM in hexane, to yield **2.42** as a white solid (707 mg, 83% yield). This material was identical to that prepared according to general procedure 2.1.



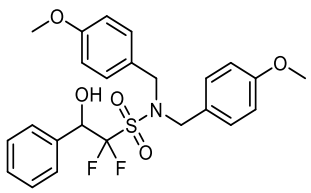
((3,3,3-trifluoro-2-oxopropyl)sulfonyl)-L-proline (**2.55**). Methane sulfonyl proline was prepared according to a literature procedure.[127] Methanesulfonyl proline (712 mg, 3.69 mmoles, 1 equiv) was dissolved in 20 mL THF and cooled to -78 °C under an argon atmosphere. *n*-Butyllithium (7.75 mmols, 3.87 mL in hexanes, 2.1 equiv) was added dropwise and allowed to stir for 15 min before *N*-methyl-*N*-methoxytrifluoroacetamide (636 mg, 4.05 mmols, 1.1 equiv) was added neat, dropwise. The reaction was quenched after 1 hour with 20 mL 10% citric acid and extracted three times with 20 mL methylene chloride. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography gradient elution 4:6 EtOAc:hexane to 98% EtOAc 2% AcOH to yield 475 mg colorless oil (45%). ^{1}H NMR (D_2O, 500 MHz): δ 4.53 (d of d, 1H, *J* = 9.0, 4.1 Hz), 3.55 (m, 2H), 2.36 (m, 1H), 2.11 (m, 1H), 2.01 (m, 2H); $^{13}C\{^{1}H\}$ NMR (D_2O, 125 MHz): 176.9, 122.2 (q, J = 288.1 Hz), 91.4 (q, J = 34.9 Hz), 60.8, 51.3 (broad signal due to deuterium exchange), 48.7, 30.9, 24.5; ^{19}F NMR (D_2O, 471 MHz): δ -86.29 (s, 3F); HRMS-ESI+ (*m/z*) $[M + H]^+$ calcd for $C_8H_{11}O_5NF_3S^+$: 290.03045 found:290.03015.



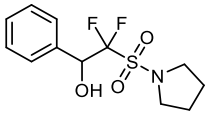
((difluoromethyl)sulfonyl)-L-proline (**2.56**). Ketone **2.55**(420 mg, 1.45 mmol 1 equiv) was dissolved in 30 mL of DMF and Li_2CO_3 (270 mg, 3.6 mmol, 2.50 equiv) followed by Selectfluor (1.28 g, 3.60 mmol, 2.5 equiv) were added as solids. The mixture was stirred at room temperature until complete by TLC then cooled to 0 °C. 10 mL of 6 M NaOH was added and the reaction was stirred for an additional 30 min and then quench with 100 mL of 1 M HCl and 100 mL of brine. The mixture was extracted 3 times with 100 mL EtOAc, the combined organic layers were washed with 100 mL 0.1 M HCl and brine. Then dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography 0 to 10% MeOH in DCM to give 225 mg (68% yield) as a white gum. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.93 (s, 1H), 6.35 (t, 1H, J = 53.8 Hz), 4.6 (dd, 1H, J = 8.5, 3.5 Hz), 3.66 (m, 2H), 2.41 (m, 1H), 2.2 (m, 1H), 2.07 (m, 2H); $^{13}C\{^1H\}$ NMR (D_2O, 75 MHz): δ 181.9, 116.9 (3, J = 279.7 Hz), 66.7, 52.3, 34.1, 27.5; ^{19}F NMR (D_2O, 282 MHz): δ-121.6 (dd, 1F, J = 277.2, 53.0 Hz), -122.8 (dd, F, J = Hz); HRMS-ESI+ (*m/z*) $[M + NH_4]^+$ calcd for $C_6H_{13}F_2N_2O_4S^+$: 247.0559 found:.247.0561

General procedure 2.4 preparation of ß-hydroxy sulfonamides 2.57, 2.64-2.66.

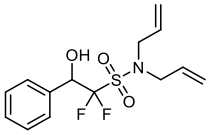
The sulfonamide was dissolved in THF (0.1 M) and benzaldehyde (1.20 equiv) was added. The mixture was cooled to -78 °C and then KHMDS (0.5 M in toluene, 1.2 equiv) was added dropwise. The reaction was stirred for 5 min then quenched with NH_4Cl. The mixture was diluted with water and extracted three times with DCM. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography using a gradient of 5 to 40% EtOAc in hexane to give the ß-hydroxy-α,α-difluorosulfonamide.



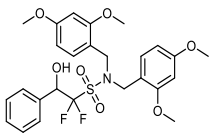
1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-2-phenylethane-1-sulfonamide (**2.57**). Prepared according to general procedure 2.4, colorless oil (128 mg, 99% yield) from 100 mg (0.269 mmol) of **2.41**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.53 (m, 2H), 7.42 (m, 3H), 7.10 (d, 4H, *J* = 8.5 Hz), 6.83 (d, 4H, *J* = 8.5 Hz), 5.43 (d, 1H, *J* = 21.6 Hz), 4.34 (s, 4H), 3.79 (s, 6H), 3.35 (s, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 159.4, 134.0, 130.2, 129.4, 128.4, 128.2, 126.4, 121.3 (dd, *J* = 293.4, 284.6 Hz), 114.0, 72.3 (dd, *J* = 26.9, 20.4 Hz), 55.3, 50.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -105.2 (d, 1F, *J* = 237.7 Hz), -119.4 (dd, 1F, *J* = 273.7, 21.2 Hz); HRMS (ESI$^+$) *m/z:* [M + Na]$^+$ calcd for $C_{24}H_{25}O_5NF_2NaS$, 500.1314; found, 500.1307.



2,2-difluoro-1-phenyl-2-(pyrrolidin-1-ylsulfonyl)ethan-1-ol (**2.64**). Prepared according to general procedure 2.4, colorless oil (47 mg, 71% yield) from 42 mg (0.227 mmol) of **2.53**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.51 (m, 2H), 7.42 (m, 3H), 5.35 (dd, 1H, *J* = 21.6, 8.0 Hz), 3.59 (m, 4H), 3.36 (bs, 1H), 1.98 (m, 4H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 133.8, 129.3, 128.3, 128.1, 122.3 (dd, *J* = 292.3, 284.7 Hz), 72.2 (dd, *J* = 26.2, 20.7 Hz), 48.9, 25.9; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -104.4 (dd, 1F, *J* = 240.5, 1.5 Hz), -118.6 (dd, 1F, *J* = 240.7, 21.6 Hz); HRMS (ESI$^+$) *m/z:* [M + H]$^+$ calcd for $C_{12}H_{16}F_2NO_3S$, 292.0813; found, 292.0814.



1,1-difluoro-2-hydroxy-N,N-diallyl-2-phenylethane-1-sulfonamide (**2.65**). Prepared according to general procedure 2.4, colorless oil (144 mg, 84% yield) from 114 mg (0.54 mmol) of **2.39**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.47 (m, 2H), 7.39 (m, 3H), 5.74 (m, 2H), 5.37-5.20 (m, 5H), 3.95 (d, 4H, *J* = 6.4 Hz), 3.20 (d, 1H, *J* = 3.4 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 133.9, 132.1,129.4, 128.4, 128.1, 121.0 (dd, *J* = 292.8, 284.1 Hz), 120.1, 72.1 (dd, *J* = 26.9, 20.5 Hz), 49.8; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -106.0 (d, 1F, *J* = 238.8 Hz), -120.3 (dd, 1F, *J* = 239.2, 21.7 Hz); HRMS (ESI$^+$) *m/z:* [M + Na]$^+$ calcd for $C_{14}H_{17}O_3NF_2NaS$, 340.0789; found, 340.0793.

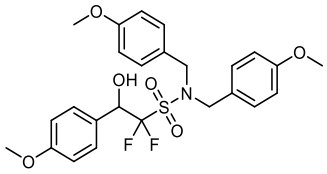


1,1-difluoro-2-hydroxy-N,N-bis(2,4-dimethoxybenzyl)-2-phenylethane-1-sulfonamide (**2.66**). Prepared according to general procedure 2.4, white solid (127 mg, 88% yield) from 116 mg (0.269 mmol) of **2.42**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.48 (m, 2H), 7.38 (m, 3H), 7.15 (d, 2H, *J* = 8.4 Hz), 6.36 (dd, 2H, *J* = 8.4, 2.4 Hz), 6.27 (d, 2H, *J* = 2.4 Hz), 5.30 (m, 1H), 4.50 (s, 4H), 3.75 (s, 6H), 3.62 (s, 6H), 3.40 (d, 1H, *J* = 3.2 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 160.5, 158.2, 134.0, 130.3, 129.2, 128.3, 128.2, 121.4 (dd, *J* = 294.3, 286.0 Hz), 116.2, 104.0, 97.9, 72.3 (dd, *J* = 26.4, 20.4 Hz, 55.3, 54.9, 46.5; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -105.1 (d, 1F, *J* = 238.2 Hz), -119.3 (dd, 1F, *J* = 238.2, 21.4 Hz); HRMS (ESI$^+$) *m/z:* [M + Na]$^+$ calcd for $C_{26}H_{29}O_7NF_2NaS$, 560.1525; found, 560.1526.

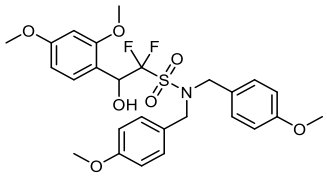
General procedure 2.5 for the synthesis of ß-hydroxy sulfonamides 2.67 - 2.81

Difluoromethane sulfonamide **2.41** (100 mg, 0.27 mmol, 1.00 equiv) and the carbonyl electrophile (0.32 mmol, 1.2 equiv) were dissolved in 5 mL THF and cooled to -78 °C. KHMDS (0.5 M in toluene, 640 µL, 0.32 mmol, 1.2 equiv) was added dropwise over approximately 1 minute and the reaction was stirred for an additional 15 min. The reaction was quenched with 10

mL of sat. NH_4Cl and extracted with 10 mL of DCM three times. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography to yield pure ß-hydroxy-α,α-difluorosulfonamides.

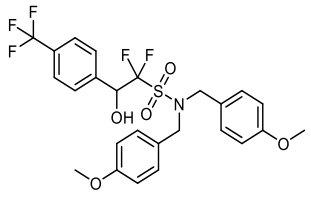


1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-2-(4-methoxyphenyl)ethane-1-sulfonamide (**2.67**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 43 mg 4-methoxybenzaldehyde (0.32 mmol) and was obtained as a white solid after chromatography eluting with 10 to 40% EtOAc in hexane (122 mg, 90% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.48 (d, 2H, *J* = 8.6 Hz), 7.14 (d, 4H, *J* = 8.7 Hz), 6.98 (d, 2H, *J* = 8.8 Hz), 6.86 (d, 4H, *J* = 8.7 Hz), 5.40 (dt, 1H, *J* = 21.2, 2.8 Hz), 4.37 (s, 4H), 3.86 (s, 3H), 3.83 (s, 6H), 3.33 (d, 1H, *J* = 3.2 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): d 160.4, 159.4, 130.2, 129.4, 126.4, 121.4 (dd, *J* = 293.1, 284.1 Hz), 114.0, 113.9, 71.8 (dd, *J* = 26.9, 20.4 Hz), 55.3, 50.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -105.0 (dd, 1F, *J* = 237.1, 1.9 Hz), -119.12 (dd, 1F, *J* = 237.1, 21.2 Hz); HRMS (ESI$^+$) *m/z:* [M + Li]$^+$ calcd for $C_{25}H_{27}O_6NF_2SLi$, 514.1682; found, 514.1687.

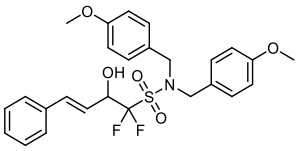


1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-2(2,4-dimethoxyphenyl)ethane-1-sulfonamide (**2.68**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 53 mg (0.32 mmol) 2,4-dimethoxybenzaldehyde and was obtained as a white solid after chromatography eluting with 10 to 40% EtOAc in hexane (144 mg, 99% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): d 7.47 (d, 1H, *J* = 8.3 Hz), 7.14 (d, 4H, *J* = 8.7 Hz), 6.85 (d, 4H, *J* = 8.7 Hz),

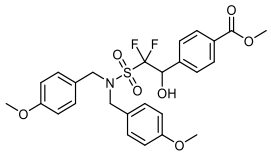
6.6 (dd, 1H, *J* = 8.5, 2.4 Hz), 6.53 (d, 1H, *J* = 2.3 Hz), 5.76 (ddd, 1H, *J* = 21.6, 6.0, 3.4 Hz), 4.37 (s, 4H), 3.88 (s, 3H), 3.86 (s, 3H), 3.82 (s, 6H), 3.71 (d, 1H, *J* = 6.1 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): d 161.6, 159.4, 158.8, 130.5, 130.2, 126.7, 122.0 (dd, *J* = 292.7, 285.7 Hz), 114.7, 113.9, 104.8, 98.7, 69.9 (dd, *J* = 27.3, 20.6 Hz), 55.8, 55.4, 55.3, 50.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -105 (dd, 1F, *J* = 236.1, 3.0 Hz), -117.3 (dd, 1F, *J* = 236.0, 21.7 Hz); HRMS (ESI^+) *m/z:* $[M + NH_4]^+$ calcd for $C_{26}H_{33}F_2N_2O_7S$, 555.1971; found, 555.1960.



N,N-bis(4-methoxybenzyl)-2-(4-trifluoromethylphenyl)-2-hydroxyl-1,1-difluoroethane-sulfonamide (**2.69**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 56 mg (0.32 mmol) 4-trifluoromethylbenzaldehyde and was obtained as a yellow oil after chromatography, eluting with 5 to 25% EtOAc in hexane (105 mg, 81% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.71 (m, 4H), 7.13 (d, 4H, *J* = 8.6 Hz), 6.87 (d, 4H, *J* = 8.7 Hz), 5.52 (dt, 1H, *J* = 21.0, 2.3 Hz), 4.38 (m, 4H), 3.83 (s, 6H), 3.55 (s, 1H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.5, 137.7, 131.5 (q, *J* = 32.5 Hz), 130.1, 128.6, 126.2, 125.3 (q, *J* = 3.7 Hz), 123.9 (q, *J* = 272.3 Hz), 120.9 (dd, *J* = 293.7, 285.5 Hz), 114.1, 77.2 (dd, *J* = 26.4, 20.7 Hz), 55.3, 50.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -62.7 (s, 3F), -105.2 (d, 1F, *J* = 238.0 Hz), -119.1 (dd, 1F, *J* = 238.2, 21.0 Hz); HRMS (ESI^+) *m/z:* $[M + NH_4]^+$ calcd for $C_{25}H_{28}F_5N_2O_5S$, 563.1634; found, 563.1625.

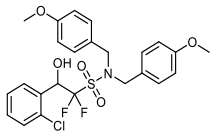


(E)-1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-4-phenylbut-3-ene-1-sulfonamide (**2.70**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 42 mg (0.32 mmol) cinnamaldehyde and was obtained as a yellow oil after chromatography eluting with 10 to 40% EtOAc in hexane (116 mg, 86% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.47 (m, 2H), 7.35 (m, 3H), 7.16 (d, 4H, *J* = 8.6 Hz), 6.93 (d, 1H, *J* = 16.5 Hz), 6.88 (d, 4H, *J* = 8.7 Hz), 6.35 (dd, 1H, *J* = 15.9, 6.4 Hz), 5.04 (m, 1H), 4.39 (s, 4H), 3.83 (s, 6H), 3.06 (s, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 159.4, 136.0, 135.8, 130.2, 128.7, 128.5, 127.0, 126.5, 121.7 (dd, *J* = 290.9, 286.2 Hz), 121.0 (dd, *J* = 2.82, 2.31 Hz), 114.1, 114.0, 71.8 (dd, *J* = 25.7, 22.3 Hz), 55.3, 50.2; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -107.1 (dd, 1F, *J* = 237.0, 5.2 Hz), -115.4 (dd, 1F, *J* = 236.8, 16.9 Hz); HRMS (ESI^{+}) *m/z:* [M + Na]$^{+}$ calcd for $C_{26}H_{27}O_5NF_2SNa$, 526.1470; found, 526.1449.

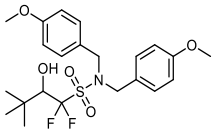


N,N-bis(4-methoxybenzyl)-2-(4-(methylcarboxy)phenyl)-2-hydroxyl-1,1-difluoroethane-sulfonamide (**2.71**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 52 mg (0.32 mmol) methyl 4-formylbenzoate and was obtained as a white solid after chromatography eluting with 5 to 25% EtOAc in hexane (110 mg, 76% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.1 (d, 2H, *J* = 8.3 Hz), 7.64 (d, 2H, *J* = 8.1 Hz), 7.12 (d, 4H, *J* = 8.6 Hz), 6.85 (d, 4H, *J* = 8.6 Hz), 5.52 (d, 1H, *J* = 20.8 Hz), 4.36 (s, 4H), 3.94 (s, 3H), 3.8 (s, 1H), 3.88 (s, 6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 166.7, 159.4, 138.9, 131.0, 130.1, 129.6, 128.2, 126.3, 121.1 (dd, 293.7, 285.2 Hz), 114.0, 72.0 (dd, *J* = 26.6, 20.7 Hz), 55.2, 52.2, 50.2; ^{19}F NMR ($CDCl_3$,

282 MHz): δ -105.0 (d, 1F, J = 238.4 Hz), -118.2 (dd,1F J = 238.3, 20.7 Hz), HRMS (ESI^+) *m/z:* $[M + NH_4]^+$ calcd for $C_{26}H_{31}F_2N_2O_7S$, 553.1815; found, 553.1808.

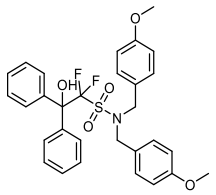


1,1-difluoro-2-hydroxyl-2-(o-chlorophenyl)-N,N-bis(4-methoxybenzyl)ethanesulfonamide (**2.72**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 45 mg (0.32 mmol) 2-chlorobenzaldehyde and was obtained as a colorless oil after chromatography, eluting with 10 to 40% EtOAc in hexane (106 mg, 77% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.81 (m, 1H), 7.41 (m, 3H), 7.15 (d, 4H, J = 8.7 Hz), 6.86 (d, 4H, J = 8.7 Hz), 6.09 (dd, 1H, J = 22.0, 3.3 Hz), 4.39 (s, 4H), 3.83 (s, 6H), 3.45 (d, 1H, J = 3.4 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.4, 134.1, 132.0, 130.4, 130.2, 129.8, 129.8, 129.5, 127.1, 126.3, 121.3 (dd, J = 294.1, 286.8 Hz), 114.0, 68.1 (dd, J = 27.4, 19.9 Hz), 55.3, 50.2; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -105.1 (d, 1F, J = 238.5 Hz), -119.0 (dd, 1F, J = 238.5, 22.0 Hz); HRMS (ESI^+) *m/z:* $[M + Na]^+$ calcd for $C_{24}H_{24}O_5NF_2SClNa$, 534.0924; found, 534.0949.

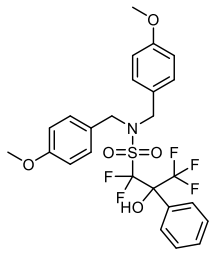


N,N-bis(4-methoxybenzyl)-1,1-difluoro-2-hydroxy-3,3-dimethylbutanesulfonamide (**2.73**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 28 mg (0.32 mmol) trimethyl acetaldehyde and was obtained as a colorless oil after chromatography, eluting with 10 to 40% EtOAc in hexane (91 mg, 74% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.14 (d, 4H, J = 8.6 Hz), 6.87 (d, 4H, J = 8.7 Hz), 4.10 (d, 2H, J = 15.2 Hz), 4.33 (d, 2H, J = 15.1 Hz), 4.08 (ddd, 1H, J = 25.7, 4.9, 1.0 Hz), 3.83 (s, 6H), 2.91 (d, 1H, J = 4.9 Hz), 1.16 (s, 9H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.4, 130.2, 124.5 (dd, J = 296.6, 290.8 Hz), 126.6, 114.0,

75.4 (dd, J = 24.0, 19.8 Hz), 55.3, 50.3, 35.8 (ap t, J = 2.0 Hz), 26.7 (dd, J = 3.2, 2.0 Hz); ^{19}F NMR ($CDCl_3$, 282 MHz): δ -102.1 (d, 1F, J = 233.0 Hz), -114.8 (dd, 1F, J = 233.0, 25.7 Hz); HRMS (ESI^+) *m/z:* $[M + NH_4]^+$ calcd for $C_{22}H_{33}F_2N_2O_5S$, 475.2073; found, 475.2065.

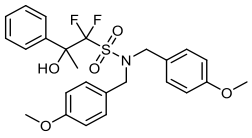


N,N-bis(4-methoxybenzyl)-1,1-difluoro-2-hydroxy-2,2-diphenylethanesulfonamide (**2.74**). Prepared according to general procedure 2.5 from 100 mg 100 mg (0.27 mmol) **2.41** and 58 mg (0.32 mmol) benzophenone and was obtained as a colorless oil after chromatography eluting with 5 to 25% EtOAc in hexane (128 mg, 86% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.73 (d, 4H, J = 6.7 Hz), 7.39 (m, 6H), 7.11 (d, 4H, J = 8.7 Hz), 6.82 (d, 4H, J = 8.8 Hz), 4.49 (s, 1H), 4.36 (s, 4H), 3.81 (s, 6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 159.5, 138.9, 130.2, 128.5, 128.1, 127.7, 126.5, 123.0 (t, J = 297.4 Hz), 114.0, 80.1 (t, J = 20.7 Hz), 55.3, 50.5; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -98.6 (s); HRMS (ESI^+) *m/z:* $[M + NH_4]^+$ calcd for $C_{30}H_{33}F_2N_2O_5S$, 571.2073; found, 571.2063.

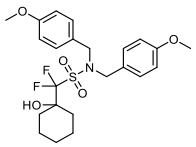


N,N-bis(4-methoxybenzyl)-1,1,3,3,3-pentafluoro-2-hydroxy-2-phenylpropanesulfonamide (**2.75**). Prepared according to general procedure 2.5 from **2.41** (100 mg, 0.27 mmol) and 2,2,2-trifluoroacetophenone (56 mg, 0.32 mmol) and was obtained as a white solid after chromatography eluting with 5 to 25% EtOAc in hexane (125 mg, 85% yield). ^{1}H NMR ($CDCl_3$,

300 MHz): δ 7.83 (m, 2H), 7.52 (m, 3H), 7.09 (d, 4H, *J* = 8.7 Hz), 6.85 (d, 4H, *J* = 8.7 Hz), 4.78 (s, 1H), 4.35 (bs, 4H), 3.82 (s, 6H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.6, 130.2, 128.4, 127.4, 126.0, 123.0 (q, *J* = 287.8 Hz), 120.0 (ap t, *J* = 297.4 Hz), 114.1, 78.4 (qt, *J* = 30.1, 21.8 Hz), 55.3, 50.6; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -72.8 (dd, 3F, *J* = 11.1, 8.6 Hz), -104.5 (dq, 1F, *J* = 244.9, 11.0 Hz), -105.6 (dq, 1F, *J* = 244.9, 8.7 Hz); HRMS (ESI^+) *m/z:* $[M + NH_4]^+$ calcd for $C_{25}H_{28}F_5N_2O_5S$, 563.1634; found, 563.1623.

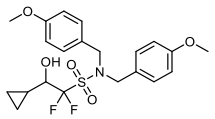


N,N-bis(4-methoxybenzyl)-1,1-difluoro-2-hydroxy-2-methyl-2-phenylethanesulfonamide (**2.76**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 38 mg (0.32 mmol) acetophenone and was obtained as a white solid after chromatography, eluting with 0 to 100% DCM in hexane (65 mg, 49% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.66 (d, 2H, *J* = 7.7 Hz), 7.43 (m, 3H), 7.1 (d, 4H, *J* = 8.5 Hz), 6.84 (d, 4H, *J* = 8.5 Hz), 4.37 (m, 4H), 3.9 (s, 1H), 3.82 (s, 6H), 1.97 (s, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.4, 139.0, 130.2, 128.4, 128.1, 126.7, 122.7 (ap t, *J* = 293.6 Hz), 126.4, 114.0, 76.0 (dd, *J* = 22.3, 20.9 Hz), 55.3, 50.3, 24.7 (d, *J* = 2.7 Hz); ^{19}F NMR ($CDCl_3$, 282 MHz): δ -105.5 (d, 1F, *J* = 236.3 Hz), -108.0 (d, 1F, *J* = 236.4 Hz); HRMS (ESI^+) *m/z:* $[M + NH_4]^+$ calcd for $C_{25}H_{31}F_2N_2O_5S$, 509.1916; found, 509.1925.

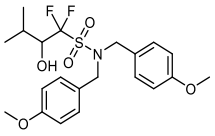


1,1-difluoro-1-(1-hydroxycyclohexyl)-N,N-bis(4-methoxybenzyl)methanesulfonamide (**2.77**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 32 mg (0.32 mmol) of freshly distilled cyclohexanone and was obtained as a colorless oil after chromatography eluting with 0 to 100% DCM in hexane (99 mg, 77% yield). ^{1}H NMR ($CDCl_3$,

300 MHz): δ 7.14 (d, 4H, *J* = 8.7 Hz), 6.86 (d, 4H, *J* = 8.7 Hz), 4.37 (s, 4H), 3.83 (s, 6H), 2.77 (s, 1H), 2.05 (m, 2H), 1.73 (m, 7H), 1.26 (m, 1H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.4, 130.2, 126.7, 123.8 (ap t, *J* = 291.9 Hz), 113.9, 74.5 (ap t, *J* = 20.8 Hz), 55.2, 50.3, 30.5, 25.2, 20.5; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -111.1 (s); HRMS (ESI^+) *m/z:* $[M + Na]^+$ calcd for $C_{23}H_{29}F_2NNaO_5S$, 492.1627; found, 492.1619.

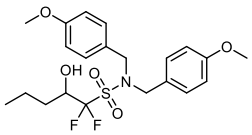


N,N-bis(4-methoxybenzyl)-1,1-difluoro-2-hydroxy-2-cyclopropylethanesulfonamide (**2.78**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 22 mg cyclopropanecarboxaldehyde (0.32 mmol) and was obtained as a colorless oil after chromatography, eluting with 10 to 40% EtOAc in hexane (81 mg, 68% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.16 (d, 4H, *J* = 8.6 Hz), 6.87 (d, 4H, *J* = 8.7 Hz), 4.38 (s, 4H), 3.83 (s, 6H), 3.75 (m, 1H), 2.9 (bs, 1H), 1.28 (m, 1H), 0.72 (m, 2H), 0.58 (m, 2H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.4, 130.2, 126.6, 122.5 (dd, *J* = 289.9, 287.1 Hz), 114.0, 74.1 (dd, *J* = 23.9, 21.5 Hz), 55.3, 50.2, 10.8 (ap t, *J* = 3.2 Hz), 3.0, 2.0; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -108.2 (dd, 1F, *J* = 237.4, 5.8 Hz), -114.6 (dd, 1F, *J* = 237.3, 16.0 Hz); HRMS (ESI^+) *m/z:* $[M + Na]^+$ calcd for $C_{21}H_{25}F_2NnaO_5S$, 464.1314; found, 464.1302.

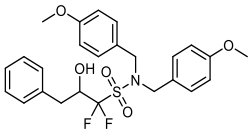


N,N-bis(4-methoxybenzyl)-1,1-difluoro-2-hydroxy-3-methylbutanesulfonamide (**2.79**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 23 mg (0.32 mmol) of freshly distilled isobutyraldehyde and was obtained as a colorless oil after chromatography, eluting with 10 to 30% EtOAc in hexane (68 mg, 57% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ

7.15 (d, 4H, J = 8.7 Hz), 6.87 (d, 4H, J = 8.7 Hz), 4.40 (d, 2H, J = 15.4 Hz), 4.34 (d, 2H, J = 15.3 Hz), 4.21 (ddd, 1H, J = 21.2, 9.0, 4.6 Hz), 3.83 (s, 6H), 2.78 (d, 1H, J = 5.4 Hz), 2.3 (s, 1H), 1.15 (d, 3H, J = 6.9 Hz), 1.11 (d, 3H, J = 6.8 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.4, 130.2, 126.6, 123.3 (dd, J = 292.3, 288.7 Hz), 114.0, 73.3 (dd, J = 24.0, 19.9 Hz), 55.3, 50.2, 28.7, 20.2, 16.2; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -107.1 (dd, 1F, J = 236.1, 2.5 Hz), -113.8 (dd, 1F, J = 236.1, 21.2 Hz); HRMS (ESI^+) *m/z:* $[M + Na]^+$ calcd for $C_{21}H_{27}F_2NnaO_5S$, 466.1470; found, 466.1456.



N,N-bis(4-methoxybenzyl)-1,1-difluoro-2-hydroxy pentanesulfonamide (**2.80**). Prepared according to general procedure 2.5 from 100 mg (0.27 mmol) **2.41** and 23 mg (0.32 mmol) of freshly distilled butyraldehyde and was obtained as a colorless oil after chromatography eluting with 10 to 30% EtOAc in hexane (52 mg, 43% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.13 (d, 4H, J = 8.6 Hz), 6.85 (d, 4H, J = 8.6 Hz), 4.35 (s, 4H), 4.28 (m, 1H), 3.81 (s, 6H), 2.67 (s, 1H), 1.73 (m, 3H), 1.49 (m, 1H), 1 (t, 3H, J = 7.2 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.4, 130.2, 126.5, 122.5 (dd, J = 289.6, 287.1 Hz), 114.0, 70.3 (dd, J = 24.5, 22.0 Hz), 55.3, 50.1, 31.3, 18.5, 13.7; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -108.8 (dd, 1F, J = 236.2, 5.4 Hz), -115.8 (dd, 1F, J = 236.2, 17.2 Hz); HRMS (ESI^+) *m/z:* $[M + Na]^+$ calcd for $C_{21}H_{27}F_2NnaO_5S$, 466.1470; found, 466.1459.

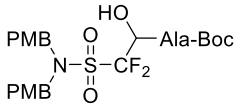


N,N-bis(4-methoxybenzyl)-1,1-difluoro-2-hydroxy-3-phenyl-propanesulfonamide (**2.81**). Prepared according to general procedure 2.5 using 100 mg of 100 mg (0.27 mmol) **2.41** and 38

mg (0.32 mmol) of freshly distilled phenylacetaldehyde. The product was isolated by RP-HPLC using a C18 column (10 μm, 150 mm × 20 mm, 10 mL/min flow rate) using a gradient of 20 to 100% acetonitrile in water over 60 min (RT = 45 min) yielding 9k as a colorless oil (15 mg, 11% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.36 (m, 5H), 7.17 (d, 4H, *J* = 8.6 Hz), 6.88 (d, 4H, *J* = 8.7 Hz), 4.55 (m, 1H), 4.39 (s, 4H), 3.84 (s, 6H), 3.25 (d, 1H, *J* = 14.1 Hz), 2.98 (dd, 1H, *J* = 14.1, 10.2 Hz), 2.7 (s, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 159.5, 136.4, 130.2, 129.5, 128.6, 127.0, 126.5, 122.3 (dd, *J* = 290.6, 287.3 Hz), 114.0, 71.6 (dd, *J* = 25.0, 21.5, Hz), 55.3, 50.2, 35.9; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -108.6 (dd, 1F, *J* = 236.3, 5.0 Hz), -115.9 (dd, 1F, *J* = 236.5, 17.2 Hz); HRMS (ESI$^+$) *m/z:* $[M + NH_4]^+$ calcd for $C_{25}H_{31}F_2N2O_5S$, 509.1916; found, 509.1898.

When the reaction was performed at -128 °C in a mixture of 3:2 THF:Et_2O the yield was increased to 52 mg (39% yield) and the product could be isolated by flash chromatography, 5% to 25% EtOAc in hexane.

When the reaction was performed at -128 °C in a mixture of 3:2 THF:Et_2O using 130 mg (4 equiv) of freshly distilled phenylacetaldehyde and 1.08 mL (2 equiv) of 0.5 M KHMDS in toluene then the yield was increased to 84 mg (61% yield) and the product was isolated by flash chromatography, 5 to 25% EtOAc in hexane.



tert-butyl (3S)-3-((2-(N,N-bis(4-methoxybenzyl)sulfamoyl)-2,2-difluoro-1-hydroxyethyl)amino)-2-oxobutanoate (**2.84**). Boc-alanal (**2.82**) was prepared according to a literature procedure.[114]

2.41 (371 mg, 1 mmol, 1 equiv) and **2.82** (208 mg, 1.2 mmol, 1.2 equiv) were dissolved in 10 mL of 1:1 Et_2O:THF and cooled to -114 °C. KHMDS (1.2 mmol, 1.2 equiv) was dissolved

in 5 mL of 1:1 Et_2O:THF, cooled to -114 °C, then transferred by cannula over a period of 4 min. The reaction was stirred for an additional 5 min then was quenched with 5 mL sat. NH_4Cl. The mixture was warmed, diluted with 20 mL of water, and extracted twice with 20 mL ether. The combined ether layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. ^{19}F NMR using 3-fluorotoluene as internal standard indicated that this mixture contained a 1:1.2 mixture of diastereomers in 26% yield. The mixture was subjected to several rounds of flash chromatography 0 to 12% EtOAc in 1:1 DCM: hexanes. 57 mg (10%) of the minor, more polar diastereomer was separated. None of the less polar, major diastereomer could be separated from unidentified impurities. ^1H NMR ($CDCl_3$, 300 MHz) σ: 7.10 (d, 4H, J = 8.4 Hz), 6.83 (d, 4H, J = 8.5 Hz), 4.94 (d, 1H, J = 8.0 Hz), 4.51 (d, 1H, J = 21.7 Hz), 4.37 (d, 2H, J = 15.1 Hz), 4.27 (d, 2H, J = 15.2 Hz), 4.20, (br, 1H), 3.80 (s, 6H), 3.64 (m, 1H), 1.45 (s, 9H), 1.30 (d, 3H, J = 6.7 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz) σ: 159.4, 155.6, 130.2, 126.5, 122.2 (dd, J = 293.4, 288.7 Hz), 114.0, 80.0, 71.6 (dd, J = 24.2, 19.8 Hz), 55.3, 50.3, 47.1, 28.4, 15.2 ^{19}F NMR ($CDCl_3$, 282 MHz): δ -108.9 (d, 1F, J = 238.5 Hz), -115.5 (dd, 1F, J = 238.7, 21.9 Hz) HRMS-ESI$^+$ (*m/z*) calcd for $C_{25}H_{34}N_2O_7SF_2Na^+$ $(M + Na)^+$ 567.19470, found 567.19584.

Determination of the extent of epimerization in 2.84

Compound **2.84** (47 mg, 0.087 mmol, 1 equiv) was dissolved in 2 mL of 25% TFA in DCM. The mixture was stirred at room temperature for 2 h. The mixture was diluted with 20 mL of EtOAc then washed twice with 20 mL of 10% Na_2CO_3. The organic layer was dried over $MgSO_4$, filtered, and concentrated, the residue was dissolved in 10 mL of DCM then divided into two flasks. To each was added HOAt (23 mg, 0.17 mmol, 4 equiv) EDC·HCl (33 mg, 0.17 mmol, 4 equiv) and either D or L *N*-benzenesulfonyl proline. The resulting mixture was stirred for 3 h then the unreacted HOAt ester was destroyed by the addition of TAEA (25 µL, 0.17

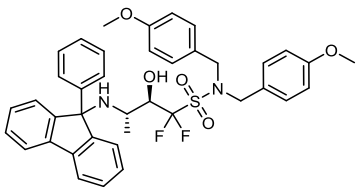
mmol, 4 equiv). After stirring an additional 10 min the reaction was diluted with 50 mL of EtOAc washed twice with 20 mL 0.1 M HCl, once with 5 % $NaHCO_3$ and once with brine. The organic layers was dried over magnesium sulfate, filtered and concentrated to give crude **2.86**, derived from *N*-benzenesulfonyl-L-proline, and **2.87**, derived from *N*-benzenesulfonyl-D-proline.

The crude material was subjected to HPLC. Isocratic gradient 0.1% TFA 47% CH_3CN:H_2O. The retention time was 29.1 min for **2.87** and 31.1 min for **2.86**. These compounds showed baseline separation on coinjection and in both cases there minor diastereomer was not observed (Figure 2.9).

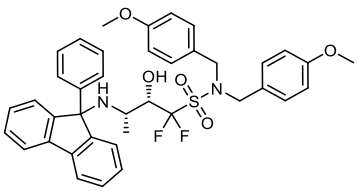
General procedure 2.6 for the reaction of 2.41 with *N*-PhF α-aminoaldehydes

See Chapter 3 for the preparation of amino aldehydes PhF-alanal (**2.88**), PhF-methioninal (**2.94**), *N*-PhF-O-(tBu)-Aspartal (**2.95**) and PhF-prolinal (**2.96**).

2.41 (0.4-1 mmol, 1.00 equiv) and PhF protected α-amino aldehyde (1.20 equiv) were dissolved in 10 mL of 1:1 THF:Et_2O per mmol of **2.41** then cooled to -114 °C. Separately KHMDS (0.5 M in toluene, 1.2 equiv) was diluted in 1:1 THF:Et_2O, 5 mL per mmol of **2.41**, cooled to -114 °C. The KHMDS was transferred to the reaction flask dropwise by cannula over 5-10 min. After the addition was complete the reaction was stirred for an additional 5 min before quenching with saturated NH_4Cl solution and extraction three times with DCM. The combined organic layers are dried over magnesium sulfate, filtered, and concentrated to yield a mixture of diastereomers and excess aldehyde.



2.89



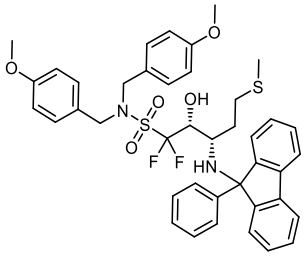
2.90

(2R,3S)-1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-3-((9-phenyl-9-fluorenyl)amino)-butane-1-sulfonamide (**2.89**) and *(2S,3S)-1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-3-((9-phenyl-9-fluorenyl)amino)butane-1-sulfonamide* (**2.90**). Prepared according to general procedure 2.6 from 208 mg (0.56 mmol) of **2.41** and 211 mg (0.67 mmol) of **2.88**. Flash chromatography, eluting with 5 to 25% EtOAc in hexane, yielded a mixture of **2.89** and **2.90**, which were separated on a second column with a gradient of 0 to 5% EtOAc in 1:1 DCM hexane. Combined yield 335 mg (87%). **2.89** is slightly more polar than **2.90**.

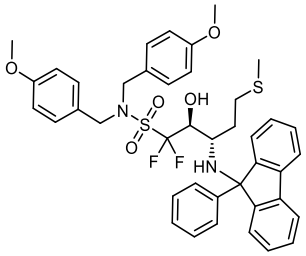
2.89 (syn isomer) was obtained as a white foam (285 mg, 74% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.78 (d, 1H, J = 7.5 Hz), 7.73 (d, 1H, J = 7.4 Hz), 7.45-7.2 (m, 11H), 7.14 (d, 4H, J = 8.5 Hz), 6.86 (d, 4H, J = 8.5 Hz), 4.33 (s, 4H), 3.90 (dt, 1H, J = 20.1, 4.7 Hz), 3.80 (s, 6H), 2.81 (m, 1H), 0.84 (d, 3H), J = 6.4 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 282 MHz): δ 159.3, 150.4, 147.6, 144.6, 140.8, 140.1, 130.3, 128.7, 128.6, 128.2, 128.0, 127.5, 127.0, 122.8 (dd, J = 293.5, 284.7 Hz), 125.9, 125.1, 120.3, 120.1, 113.9, 72.7 (dd, J = 26.1, 19.8 Hz), 72.5, 55.3, 50.3, 47.7, 21.9; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -109.0 (d, 1F, J = 238.5 Hz), -115.7 (dd, 1F, J = 238.4, 19.8 Hz); HRMS (ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{39}H_{39}O_5N_2F_2S$, 685.2542; found, 685.2568.

2.90 (anti isomer) was obtained as a white foam (50 mg, 13% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.66 (d, 1H, J = 7.6 Hz), 7.61 (d, 1H, J = 7.6 Hz), 7.33 (m, 3H), 7.24 (m, 4H), 7.17 (m, 5H), 6.98 (d, 4H, J = 8.5 Hz), 6.72 (d, 4H, J = 8.6 Hz), 4.16 (s, 4H), 3.71 (m, 7H), 2.6 (m, 1H), 0.85 (d, 3H, J = 6.7 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 159.3, 149.9, 148.6, 144.6, 140.9, 140.0, 130.1, 126.8, 126.0, 125.1, 124.8, 122.9 (dd, J = 293.5, 286.6 Hz), 120.3, 120.2,

113.9, 72.7, 71.1 (dd, J = 25.4, 19.3 Hz), 55.3, 50.2, 49.2 (d, J = 1.7 Hz), 17.5; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -106.2 (d, 1F, J = 239.6 Hz), -113.3 (dd, 1F, J = 239.6, 22.2 Hz); HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd for $C_{39}H_{39}O_5N_2F_2S$, 685.2542; found, 685.2568.



2.97



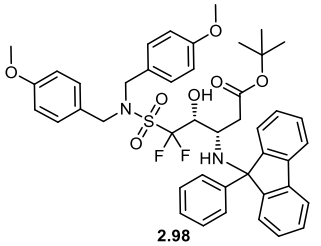
2.100

(2R,3S)-1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-5-(methylthio)-3-((9-phenyl-9-fluorenyl)amino)pentane-1-sulfonamide (**2.97**) and *(2S,3S)-1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-5-(methylthio)-3-((9-phenyl-9-fluorenyl)amino)pentane-1-sulfonamide*(**2.100**)**.** Prepared according to general procedure 2.6 from 448 mg (1.20 mmol) of **2.94** and 371 mg (1.00 mmol) of **2.41**. Flash chromatography, eluting with 5 to 30% EtOAc in hexane, gave a mixture of **2.97** and **2.100** in a 5.5:1 ratio (693 mg, 93% combined yield).

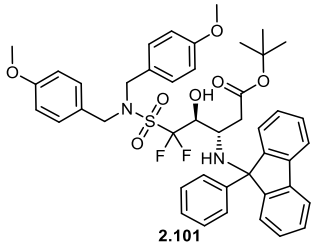
The mixture was subjected to additional chromatography, eluting with 0 to 5% EtOAc in DCM, and a portion of **2.97** was separated as a colorless oil (460 mg, 60% yield). **2.97** and **2.100** were not easily distinguished by TLC and fractions containing pure **2.100** were identified by ^{19}F NMR. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.74 (d, 1H, J = 7.5 Hz), 7.70 (d, 1H, J = 7.4 Hz), 7.4-7.2 (m, 11H), 7.10 (d, 4H, J = 8.6 Hz), 6.84 (d, 4H, J = 8.6 Hz), 4.29 (s, 4H), 4.05 (dt, 1H, J = 20.6, 3.6 Hz), 3.80 (s, 6H), 2.87 (m, 1H), 2.15 (m, 2H), 1.83 (s, 3H), 1.40 (m, 2H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.4, 150.4, 147.6, 144.7, 140.6, 140.2, 130.2, 128.72, 128.65, 128.5, 128.1, 128.0, 127.4, 126.8, 126.3, 125.9, 125.1, 120.3, 122.7 (dd, 293.1, 287.5 Hz), 120.0, 113.9, 72.4, 69.5 (dd J = 25.6, 19.1 Hz), 55.3, 51.3,50.2, 33.3, 29.6, 15.3; ^{19}F NMR ($CDCl_3$, 282

MHz): δ-107.9 (d, 1F, J = 237.5 Hz), -113.5 (dd, 1F, J = 237.5, 20.6); HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd for $C_{41}H_{43}F_2N_2O_5S_2$, 745.2576; found, 745.2593.

None of the minor diastereomer **2.97** was separated.



2.98



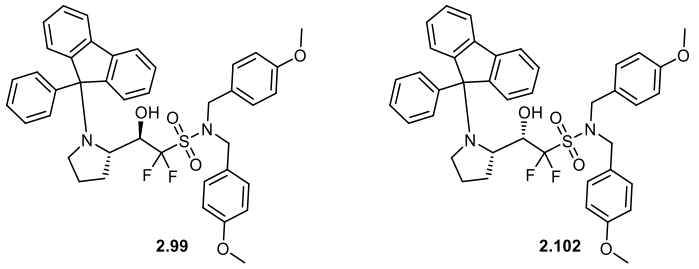
2.101

*tert-butyl(3S,4R)-5-(N,N-bis(4-methoxybenzyl)sulfamoyl)-5,5-difluoro-4-hydroxy-3-((9-phenyl-9-fluorenyl)amino)pentanoate (***2.98***)* and *tert-butyl (3S,4S)-5-(N,N-bis(4-methoxybenzyl)sulfamoyl)-5,5-difluoro-4-hydroxy-3-((9-phenyl-9-fluorenyl)amino)pentanoate(***2.101***).* Prepared according to general procedure 2.6 from 200 mg (0.480 mmol) of **2.95** and 149 mg (0.400 mmol) of **2.41**. Flash chromatography, eluting with 5 to 30% EtOAc in hexane, yielded awhite foam consisting of a mixture **2.98** and **2.101** in a 2.3:1 ratio (295 mg, combined 94% yield).

The mixture was subjected to additional chromatography 0 to 7% EtOAc in DCM and a portion of **2.98** (syn isomer) was separated as a white solid (192 mg, 61% yield). **2.98** and **2.101** were not easily distinguished by TLC and fractions containing pure **2.98** were identified by ^{19}F NMR. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.76 (d, 1H, J = 7.5 Hz), 7.71 (d, 1H, J = 7.4 Hz), 7.34 (m, 11H), 7.09 (d, 4H, J = 8.3 Hz), 6.82 (d, 4H, J = 8.4 Hz), 4.28 (s, 4H), 4.13 (m, 1H), 3.80 (s, 6H), 2.84 (s, 1H), 2.05 (m, 1H), 1.82 (dd, 1H, J = 17.5, 4.7 Hz), 1.42 (s, 9H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 171.6, 159.3, 150.7, 147.3, 144.2, 140.5, 140.2, 130.2, 128.84, 128.80, 128.5, 128.4, 128.1, 127.4, 127, 125.9, 125.8, 124.6, 122.6 (dd, J = 293.6, 282.9 Hz), 120.5, 120.2, 113.8, 81.6, 72.2, 70.0 (dd, J = 27.1, 20.4 Hz), 55.3, 50.3, 48.5, 37.3, 28.1; ^{19}F NMR

($CDCl_3$, 282 MHz): δ -107.9 (1F, d, J = 240.2 Hz), -116.5 (1F, dd, J = 240.1, 19.8 Hz); HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd for $C_{44}H_{47}N_2O_7SF_2$, 785.3067; found, 785.3029.

None of the minor diastereomer (anti isomer) **2.101** was isolated.



®-*1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-2-((S)-1-(9-phenyl-9-fluoren144yrrolidinedin-2-yl)ethane-1-sulfonamide (***2.99***) and (S)-1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-2-((S)-1-(9-phenyl-9-fluoren144yrrolidinedin-2-yl)ethane-1-sulfonamide(***2.102***).* Prepared according to general procedure 2.6 from 291 mg (0.780 mmol) of **2.41** and 320 mg (0.940 mmol) of **2.96**. The diastereomers were separated by flash chromatography eluting with 0 to 10% Et_2O in DCM. Combined yield 535 mg (96%) as a 5 to 1 mixture of **2.99**:**2.102**. Compound **2.102** is more polar than **2.99**.

2.99 (syn isomer) was obtained as a white foam (445 mg, 80% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.77 (d, 1H, J = 7.6 Hz), 7.61 (d, 1H, J = 7.3 Hz), 7.47 (m, 5H), 7.27 (m, 6H), 7.03 (d, 4H, J = 8.5 Hz), 6.78 (d, 4H, J = 8.5 Hz), 5.99 (s, 1H), 4.20 (s, 4H), 3.78 (s, 6H), 3.57 (td, 1H, J = 18.3, 6.2 Hz), 3.30 (t, 2H, J = 7.3 Hz), 3.04 (t, 1H, J = 6.8 Hz), 1.87 (m, 1H), 1.75 (m, 1H), 1.42 (m, 1H), 1.17 (m, 1H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.2, 148.3, 146.9, 142.6, 141.9, 139.0, 130.2, 129.2, 128.6, 128.5, 128.4, 127.7, 127.0, 126.0, 125.7, 122.4 (dd, J = 293.2, 282.9 Hz), 120.5, 119.7, 113.8, 77.6, 69.7 (dd, J = 26.7, 19.5 Hz), 57.8, 55.3, 50.5, 50.2, 31.6, 23.8; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -112.4 (dd, 1F, J = 238.4, 4.4 Hz), -117.1 (dd, 1F, J = 238.3, 18.2 Hz).

2.102 (anti isomer) was obtained as a white foam (90 mg, 16% yield)**.** ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.76 (d, 1H, J = 7.4 Hz), 7.64 (d, 1H, J = 7.4 Hz), 7.49 (m, 5H), 7.25 (m, 6H), 7.02 (d, 4H, J = 8.3 Hz), 6.78 (d, 4H, J = 8.4 Hz), 4.16 (s, 4H), 3.78 (s, 6H), 3.54 (m, 2H), 3.28 (m, 2H), 2.92 (m, 1H), 2.00 (m, 1H), 1.78 (m, 1H), 1.62 (m, 2H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 159.2, 148.2, 146.6, 143.6, 142.2, 138.9, 130.2, 128.9, 128.7, 128.5, 127.9, 127.8, 127.3, 127.2, 126.9, 126.3, 125.7, 122.6 (dd, J = 293.7, 283.3 Hz),120.2, 120.1, 113.8, 77.5, 69.8 (dd, J = 26.3, 17.3 Hz), 59.0, 52.4, 50.2, 27.2, 25.6; ^{19}F NMR ($CDCl_3$, 282 MHz): δ 110.0 (1F, dd, J = 239.3, 4.2 Hz), -114.1 (1F, dd, J = 239.2, 23.0 Hz); HRMS(ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{41}H_{41}F_2N_2O_5S$, 711.2699; found, 711.2718.

When Using NaHMDS. The reaction was performed by dissolving **2.41** (100 mg, 0.27 mmol, 1.00 equiv) and **2.96** (108 mg, 0.32 mmol, 1.2 equiv) in 5 mL of 3:2 THF:Et_2O. The solution was cooled to -128 °C and then NaHMDS (1 M in THF, 594 µL, 2.20 equiv) was added dropwise. The mixture was stirred until complete by TLC (5 min) then was quenched with 2 mL of saturated NH_4Cl. The mixture was diluted with water, extracted three times with DCM, the combined organic layers were dried over magnesium sulfate, filtered, and concentrated then the residue was purified by flash chromatography, 5 to 25% EtOAc in hexane, to yield a white solid consisting of **2.99** and **2.102** (96:4) (184 mg, 96% combined yield).

A crystal of **2.99** suitable for x-ray crystallography was obtained by dissolving pure **2.99** in a 1:1 mixture of DCM:Et_2O at room temperature. The solution was placed in a 7 mL vial and left open to air until completely evaporated.

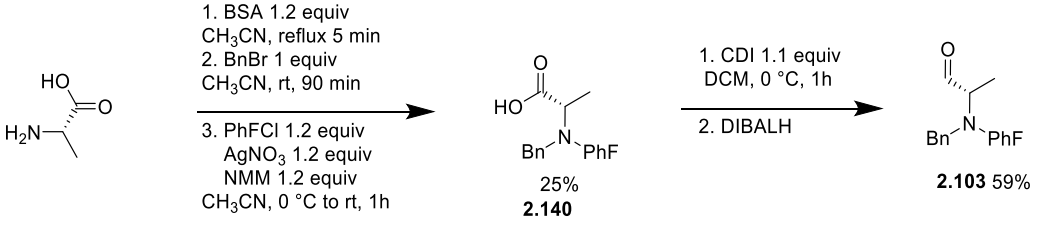
Determination of the extent of epimerization in 2.89

2.89 (68 mg, 0.1 mmol, 1 equiv) was dissolved in 10 mL of MeOH and 13 mg of 20 wt% $Pd(OH)_2$ on carbon was added. The mixture was stirred for 18 h under H_2 atmosphere until

complete by TLC. The reaction was filtered with a pad of celite and concentrated to give a crude mixture consisting of free amine **2.91** and 9-phenylfluorene. The crude material was dissolved in 10 mL of DCM and divided into two flasks. To each was added HOAT (27 mg, 0.2 mmol, 4 equiv), EDC·HCl (38 mg, 0.2 mmol, 4 equiv) and either D or L *N*-benzenesulfonyl proline. The reactions were stirred for 3 h then unreacted HOAt ester was destroyed by the addition of TAEA (30 µL, 0.2 mmol, 4 equiv) After stirring an additional 10 min the reaction was diluted with 50 mL of EtOAc washed twice with 20 mL 0.1 M HCl, once with 5 % $NaHCO_3$ and once with brine. The organic layers was dried over magnesium sulfate, filtered and concentrated to give crude **2.92**, derived from *N*-benzenesulfonyl-L-proline, and **2.93**, derived from *N*-benzenesulfonyl-D-proline.

The crude material was subjected to HPLC. 10 to 90% CH_3CN 0.1% TFA:H_2O over 45 min. The retention time was 34.5 min for **2.92** and 35.1 min for **2.93**. Both were diastereomerically pure and could be separated by coinjection. (Figure 2.10).

Synthesis of *N*-Benzyl-*N*-PhF-α-amino aldehydes 2.103-2.106



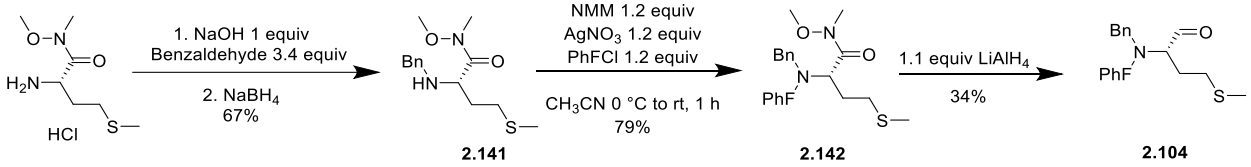
N-Benzyl-N-(9-phenyl-9-fluorenyl)-alanine (**2.140**). Freshly lyophilized alanine (178 mg, 2.00 mmol, 1.00 equiv) was placed in 5 mL of CH_3CN and BSA (586 µL, 2.40 mmol, 1.2 equiv) was added. The mixture was heated to reflux for 5 min until completely dissolved. After cooling to 0 °C, NMM (220 µL, 2.00 mmol, 1.00 equiv) and BnBr (237 µL, 2.00 mmol, 1.00 equiv) were added and the mixture was stirred for 90 min at room temperature. NMM (264 µL, 2.4 mmol, 1.2

equiv) and PhFCl (662 mg, 2.40 mmol, 1.20 equiv) and 10 mL CH_3CN were added and the mixture cooled to 0 °C. $AgNO_3$ (748 mg, 4.4 mmol, 2.2 equiv) was added as a solution in 7 mL of CH_3CN. A white precipitate formed immediately, and the reaction was stirred at room temperature for 1 hour then was quenched with 5 mL of MeOH. The precipitate was filtered off and the filtrate was concentrated. The residue was purified by flash chromatography eluting with 0 to 100% EtOAc in DCM to give (**2.128**) as a white foam (214 mg, 25% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.47 (br, 1H), 7.83 (d, 1H, *J* = 7.6 Hz), 7.75 (m, 1H), 7.68 (dd, 2H, *J* = 7.5, 2.0 Hz), 7.57 (m, 2H), 7.44 (m, 5H), 7.32 (m, 7H), 4.38 (d, 1H, *J* = 13.3 Hz), 4.01 (d, 1H, *J* = 13.3 Hz), 3.34 (q, 1H, *J* = 7.1 Hz), 0.93 (d, 3H, *J* = 7.2 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 177.2, 147.6, 145.7, 142.9, 141.1, 139.9, 138.2, 129.6, 129.1, 128.91, 128.87, 128.5, 128.3, 128.0, 127.8, 127.6, 127.2, 127.0, 126.0, 120.8, 120.3, 79.7, 55.3, 50.6, 12.5; HRMS (ESI$^+$) *m/z:* $[M + Na]^+$ calcd for $C_{29}H_{25}NO_2$, 419.1885; found, 442.1761.

329 mg of (9-phenyl-9-fluorenyl) alanine was also isolated (56% yield).

N-Benzyl-N-(9-phenyl-9-fluorenyl)-alanal(**2.103**). Compound **2.140** (210 mg, 0.50 mmol, 1.00 equiv) was dissolved in 5 mL of DCM and cooled to 0 °C then CDI (89 mg, 0.55 mmol, 1.10 equiv) was added and the mixture was stirred for 1 hour until complete by TLC. After cooling to -78 °C DIBALH (1 M in toluene, 1.1 mL, 2.2 equiv) was added dropwise over 20 min and stirring was continued for two h at –78 °C before another portion of DIBALH (1 M in toluene, 1.1 mL, 2.2 equiv) was added dropwise over 10 min. The mixture was stirred for an additional 2 h and then was quenched with 1 mL of EtOAc then 1 mL of MeOH and then was concentrated on silica. This was purified by flash chromatography eluting with DCM to give **2.103** as a colorless oil (124 mg, 59% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.27 (s, 1H), 7.76 (m, 3H), 7.64 (d, 2H, *J* = 7.4 Hz), 7.59 (d, 1H, *J* = 7.6 Hz), 7.46 (m, 3H), 7.3 (m, 9H), 4.25 (d, 1H, *J* =

13.2 Hz), 3.88 (d, 1H, *J* = 13.3 Hz), 3.17 (q, 1H, *J* = 6.7 Hz), 0.59 (d, 3H, *J* = 6.8 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 202.5, 148.5, 146.9, 143.3, 140.9, 139.9, 138.5, 129.6, 128.8, 128.7, 128.6, 128.4, 128.2, 128.0, 127.7, 127.5, 127.1, 126.8, 125.8, 120.6, 120.3, 78.9, 61.9, 50.1, 9.9; HRMS (ESI^+) *m/z:* $[M + H]^+$ calcd for $C_{29}H_{26}NO$, 404.2009; found, 404.1991.



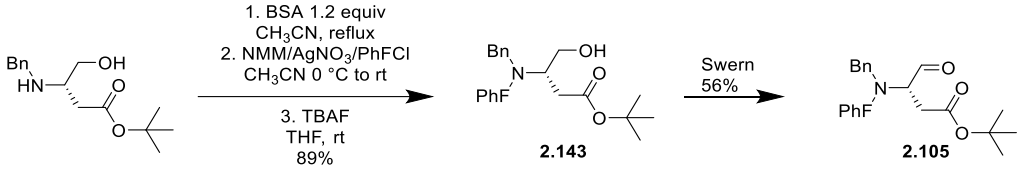
(S)-2-(benzylamino)-N-methoxy-N-methyl-4-(methylthio)butanamide (**2.141**). Methionine N,O-dimethylhydroxamide hydrochloride (670 mg, 2.93 mmol, 1 equiv) was dissolved in 6 mL of methanol and powdered NaOH (117 mg, 2.93 mmol, 1 equiv) was added followed by benzaldehyde (1.02 mL 10.0 mmol, 3.41 equiv). The reaction was sealed and heated to 60 °C overnight then was cooled to 0 °C and added to a suspension of $NaBH_4$ (975 mg, 25.0 mmol, 8.53 equiv) in 10 mL of THF and 10 mL of methanol. The reaction was stirred for 3 h while warming to room temperature then was quenched with 1 mL AcOH. The volume was reduced to approximately 10 mL and the mixture was diluted with 30 mL of 10% Na_2CO_3. The pH was adjusted to 12 with 6 M NaOH then the mixture was extracted with 20 mL of DCM 5 times. The combined organic layers were dried over magnesium sulfate, filtered, concentrated and the residue was purified by flash chromatography eluting with 5 to 50% EtOAc in hexane to give **2.141** as a viscous oil (552 mg, 67% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.35 (d, 2H, *J* = 7.3 Hz), 7.3 (t, 2H, *J* = 7.4 Hz), 7.23 (t, 1H, *J* = 7.2 Hz), 3.83 (d, 1H, *J* = 13.1 Hz), 3.74 (m, 1H), 3.58 (s, 3H), 3.56 (d, 1H, *J* = 13.2 Hz), 3.21 (s, 3H), 2.68 (m, 2H), 2.07 (s, 3H), 1.86 (m, 1H), 1.71 (m, 1H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 175.6, 140.1, 128.0, 128.0, 126.7, 55.6, 51.8,

32.5, 32.0, 30.7, 14.9; HRMS (ESI^+) *m/z:* $[M + H]^+$ calcd for $C_{14}H_{23}N_2O_2S$, 283.1475; found, 283.1503.

(S)-2-(benzyl(9-phenyl-9H-fluoren-9-yl)amino)-N-methoxy-N-methyl-4-(methylthio)-butanamide(**2.142**). Compound **2.141** (500 mg, 1.77 mmol, 1.00 equiv), PhFCl (587 mg, 2.13 mmol, 1.2 equiv) and NMM (235 µL, 2.13 mmol, 1.2 equiv) were dissolved in 10 mL of CH_3CN and cooled to 0 °C then $AgNO_3$ (362 mg, 2.13 mmol, 1.2 equiv) was added as a solution in 4 mL CH_3CN. A white precipitate formed immediately which darkened to brown/purple over time. The mixture was stirred for 1 hour then was filtered and concentrated onto silica. Flash chromatography, eluting with 0 to 25% EtOAc in hexane, yielded **2.142** as a white foam (727 mg, 79% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.7 (m, 8H), 7.27 (m, 10H), 5.06 (d, 1H, *J* = 14.0 Hz), 4.29 (d, 1H, *J* = 14.6 Hz), 4.00 (m, 1H), 3.1 (s, 3H), 2.62 (s, 3H), 2.36 (m, 2H), 1.78 (s, 3H), 1.51 (m, 2H);$^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 174.2, 148.8, 146.7, 144.5, 142.6, 141.0, 140.6, 128.7, 128.4, 128.2, 127.9, 127.7, 127.6, 127.5, 127.2, 126.5, 126.2, 120.1, 120.0, 80.1, 60.8, 54.8, 50.6, 31.8, 31.2, 30.8, 15.0; HRMS (ESI^+) *m/z:* $[M + H]^+$ calcd for $C_{33}H_{35}N_2O_2S$, 523.2414; found, 523.2428.

N-benzyl-N-(9-phenyl-9-fluorenyl)methioninal (**2.104**)**.** Compound **2.142** (261 mg, 0.5 mmol, 1 equiv) was dissolved in 5 mL of THF and cooled to -78 °C. $LiAlH_4$ (2 M in THF, 275 µL, 0.550 mmol 1.1 equiv) was added and the reaction was stirred overnight at 0 °C. The reaction was quenched by dropwise addition of 1 mL of EtOAc followed by 1 mL of MeOH and finally 5 mL of silica then was concentrated. Flash chromatography, eluting with 2.5% to 15% EtOAc in hexane, gave **2.104** as a white solid (78 mg, 34% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ 9.27 (s, 1H), 7.86 (m, 1H), 7.81 (d, 1H, *J* = 7.5 Hz), 7.69 (m, 3H), 7.51 (m, 3H), 7.44 (t, 1H, *J* = 7.4 Hz), 7.34 (m, 8H), 4.34 (d, 1H, *J* = 13.1 Hz), 3.97 (d, 1H, *J* = 13.2 Hz), 3.07 (d, 1H, *J* = 6.0

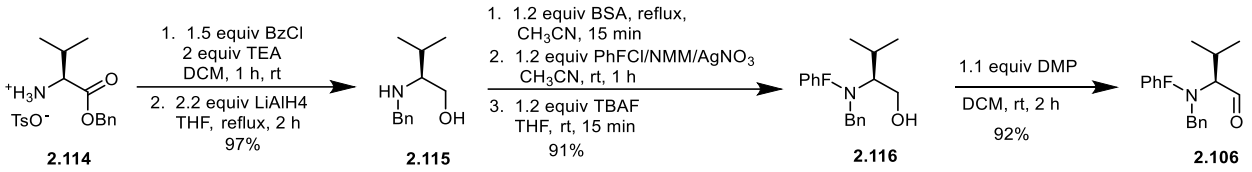
Hz), 2.1 (t, 1H, *J* = 9.1 Hz), 1.72 (s, 3H), 1.68 (m, 2H), 1.22 (t, 1H, *J* = 8.3 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 201.1, 148.2, 146.6, 143.2, 141.0, 139.9, 138.2, 129.7, 129.1, 128.8, 128.7, 128.4, 128.3, 128.1, 127.8, 127.6, 127.1, 126.9, 125.7, 120.8, 120.4, 78.9, 65.6, 51.1, 32.4, 26.2, 15.2; HRMS (ESI^+) *m/z:* $[M + Na]^+$ calcd for $C_{31}H_{29}NOSNa$, 486.1862, found, 486.1856.



N-(9-phenyl-9-fluorenyl)-N-benzyl-O4-tbutyl-aspartol (**2.143**). *N*-benzyl-O^4-tbutyl-aspartol was prepared according to a literature procedure.[128] *N*-benzyl-O4-tbutyl-aspartol (480 mg, 1.81 mmol, 1 equiv) was dissolved in 10 mL of CH_3CN and BSA (530 µL, 2.17 mmol, 1.20 equiv) was added. The solution was refluxed for 15 min then cooled to 0 °C and NMM (239 µL, 2.17 mmol, 1.2 equiv) and PhFCl (598 mg, 2.17 mmol, 1.20 equiv) were added followed by $AgNO_3$ (364 mg, 2.17 mmol, 1.20 equiv) in a solution of 4 mL CH_3CN. A white precipitate formed immediately. The mixture was stirred for two h then the reaction was quenched with 5 mL of MeOH. The precipitate was filtered off and the filtrate was diluted with 100 mL EtOAc and washed with 100 mL 5% Na_2CO_3. The organic layer was concentrated then redissolved in 10 mL of THF and TBAF was added (1 M in THF, 4.5 mL, 2.48 equiv). After 1 hour the TMS ether was completely consumed by TLC and the reaction was diluted with 100 mL EtOAc and then washed with 50 mL of 5% Na_2CO_3 then 50 mL of water. The organic layer was dried over magnesium sulfate, filtered, and concentrated. Then the residue was purified by flash chromatography, eluting with 15 to 30% EtOAc in hexane, to yield **2.131** as a white foam (814 mg, 89% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.68 (m, 6H), 7.33 (m, 12H), 4.17 (d, 1H, *J* = 14.2 Hz), 3.78 (d, 1H, *J* = 14.2 Hz), 3.32 (m, 1H), 2.9 (m, 2H), 2.22 (m, 1H), 2.08 (m, 1H), 1.93

(m, 1H), 1.23 (s, 9H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 171.6, 147.9, 146.6, 143.5, 140.94, 140.90, 140.1, 128.69, 128.66, 128.5, 128.4, 128.3, 128.0, 127.6, 127.35, 127.27, 127.1, 126.7, 126.2, 120.6, 120.0, 80.3, 79.6, 64.9, 56.1, 50.7, 36.7, 27.8; HRMS (ESI^+) *m/z:* $[M + H]^+$ calcd for $C_{34}H_{36}NO_3$, 506.2690; found, 506.2692.

N-(9-phenyl-9-fluorenyl)-N-benzyl-O4-tbutyl-aspartal (**2.105**). Oxallyl chloride (211 µL, 2.42 mmol, 3 equiv) was dissolved in 10 mL of DCM and cooled to -78 °C. DMSO (345 µL, 4.84 mmol, 6 equiv) was added dropwise and stirred for 10 min. **2.143** (408 mg, 0.81 mmol, 1.00 equiv) was added as a solution of 5 mL DCM, dropwise, then stirred for 15 min. Triethylamine (1.03 mL, 7.27 mmol, 9 equiv) was added dropwise and stirred for 5 min before warming to room temperature. After an additional 30 min the reaction was quenched wit 30 mL of water and extracted twice with 20 mL of DCM. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 0 to 30% EtOAc in hexane, to yield **2.105** as a white foam (227 mg, 56% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.28 (s, 1H), 7.92 (br, 2H), 7.76 (m, 4H), 7.50 (m, 3H), 7.4 (m, 9H), 4.30 (d, 1H, J = 13.2 Hz), 3.87 (d, 1H, J = 3.7 Hz), 3.74 (d, 1H, J = 13.2 Hz), 2.29 (dd, 1H, J = 16.2, 9.8 Hz), 1.83 (d, 1H, J = 15.3 Hz), 1.28 (s, 9H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 199.9, 170.1, 147.8, 146.3, 142.9, 140.9, 140.3, 137.8, 129.7, 129.0, 128.9, 128.8, 128.6, 128.3, 127.9, 127.6, 127.1, 126.5, 125.8, 120.8, 120.5, 80.2, 78.9, 63.1, 51.3, 31.8, 27.8, HRMS (ESI^+) *m/z:* $[M + H]^+$ calcd for $C_{34}H_{34}NO_3$, 504.2533; found, 504.2525.



N-benzyl valinol (**2.115**). Compound **2.114** (3.79 g, 10.0 mmol, 1.00 equiv) was dissolved in 50 mL of DCM then benzoic anhydride (4.00 g, 17.7 mmol, 1.77 equiv) and triethylamine (2.02 g, 20.0 mmol, 2.00 equiv) were added. The mixture was stirred until complete as determined by TLC (1 hour) then excess benzoic anhydride was destroyed by the addition of 1 mL of diamino propane and stirring for 15 min. The mixture was diluted with 200 mL of DCM then washed twice with 100 mL of 10% citric acid and once with 100 mL of 10% Na_2CO_3. The organic layer was dried over magnesium sulfate, filtered, and concentrated to give crude benzoyl valine benzyl ester which was used without further purification. The crude material was dissolved in 40 mL of THF then cooled to 0 °C. $LiAlH_4$ (2 M in THF, 11 mL, 2.2 equiv) was added dropwise and stirred until the ester was completely reduced (15 min) then the reaction was heated to reflux until the amide was completely reduced (2 h). The reaction was cooled to 0 °C, diluted with 50 mL of Et_2O and then quenched with 1 mL of water, then 1 mL of 3 M NaOH, then 3 mL of water. The suspension was stirred for 15 min at room temperature then dried with magnesium sulfate, filtered, and concentrated. The residue was purified by reversed phase chromatography using a Biotage Isolera One Flash purification system including a C-18 reversed-phase preparative Biotage column (30 g) using a gradient of 10 to 100% methanol over 30 min at 25 mL per minute (R_T = 19 min) to give **2.115** as a colorless oil (1.88 g, 97% yield). ^{1}H NMR of this material was identical to previously reported spectra.[129]

N-(9-phenyl-9-fluorenyl)N-benzyl valinol (**2.116**). Compound **2.115** (1.88 g, 9.74 mmol, 1 equiv) was dissolved in 50 mL of CH_3CN and bis(trimethylsilyl)acetamide (BSA, 2.85 mL,

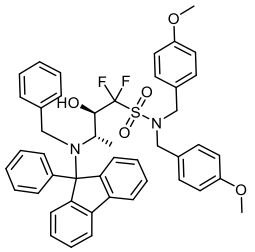
11.6 mmol, 1.20 equiv) was added and the mixture was heated to reflux for 20 min then cooled to 0 °C. PhF-Cl (3.23 g, 11.6 mmol, 1.20 equiv) and NMM (1.28 mL, 11.6 mmol, 1.20 equiv) were added followed by a solution of $AgNO_3$ (1.98 g, 11.6 mmol, 1.20 equiv) in 20 mL of CH_3CN. The mixture was warmed to room temperature and stirred for 1 hour and then 5 mL of methanol was added to quench the reaction. The mixture was filtered and concentrated to remove most of the CH_3CN then was diluted with 100 mL of water then extracted 3 times with 100 mL of 1:1 Et_2O:hexane. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved 20 mL of THF then TBAF (1 M in THF, 11.6 mL, 11.6 mmol, 1.2 equiv) was added and the colorless solution briefly turned a vibrant orange color which disappeared after 5 min. After 15 min the TMS ether was completely removed by TLC and the reaction was diluted with 100 mL of Et_2O and washed three times with 100 mL of water, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography, eluting with 1 to 15% EtOAc in hexane to give **2.116** as a white foam (3.82 g, 91% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.82 (d, 2H, *J* = 7.6 Hz), 7.73 (m, 4H), 7.63 (d, 2H, *J* = 7.1 Hz), 7.36 (m, 10H), 4.38 (d, 1H, *J* = 14.5 Hz), 4.27 (d, 1H, *J* = 14.6 Hz), 3.09 (m, 2H), 2.43 (m, 1H), 1.67 (bs, 1H), 1.35 (m, 1H), 0.51 (m, 6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 148.3, 148.2, 144.5, 141.7, 140.7, 140.4, 128.6, 128.4, 128.3, 128.0, 127.7, 127.5, 127.4, 127.3, 127.1, 126.8, 120.4, 120.3, 80.2, 65.0, 61.1, 51.8, 28.8, 23.1, 19.3; HRMS (ESI$^+$) *m/z:* $[M + H]^+$ calcd for $C_{31}H_{32}NO$, 434.2478; found, 434.2480.

N-(9-phenyl-9-fluorenyl)N-benzyl valinal (**2.106**). **2.116** (3.72 g, 8.59 mmol, 1.00 equiv) was dissolved in 100 mL of DCM and water (186 µL, 10.3 mmol, 1.20 equiv) was added. The reaction was immersed in an ice bath as Dess-Martin reagent (3.93 g, 9.27 mmol, 1.07 equiv) was added in small portions and then the reaction was stirred at room temperature. After 1 hour

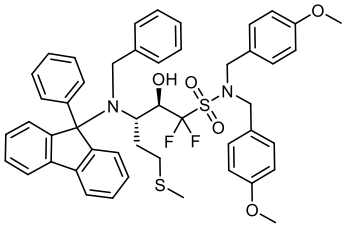
most of the starting material had been consumed and an additional 250 mg of Dess-Martin reagent was added. After an additional 1 hour the reaction was complete by TLC and the mixture was diluted with 300 mL of Et_2O and washed with 200 mL of 5% $Na_2S_2O_3$. The organic layer was dried over magnesium sulfate, filtered, and concentrated then the residue was purified by flash chromatography, eluting with 1 to 10% EtOAc in hexane, to give **2.106** as a white solid (3.42 g, 92% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.96 (d, 1H, *J* = 2.2 Hz), 7.85 (d, 2H, *J* = 7.3 Hz), 7.8 (d, 1H, *J* = 7.1 Hz), 7.72 (d, 1H, *J* = 6.9 Hz), 7.66 (d, 1H, *J* = 4.1 Hz), 7.63 (d, 1H, *J* = 4.4 Hz), 7.49 (m, 3H), 7.32 (m, 9H), 4.39 (d, 1H, *J* = 13.9 Hz), 4.12 (d, 1H, *J* = 13.9 Hz), 2.75 (dd, 1H, *J* = 10.0, 1.3 Hz), 1.38 (m, 1H), 0.81 (d, 3H, *J* = 6.6 Hz), 0.37 (d, 3H, *J* = 6.5 Hz);^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 204.9, 148.1, 147.6, 143.8, 140.7, 140.2, 139.9, 129.6, 129.1, 128.8, 128.7, 128.1, 127.9, 127.8, 127.5, 127.4, 127.1, 126.9, 120.8, 120.3, 79.8, 72.3, 52.6, 27.4, 21.0, 20.7; HRMS (ESI$^+$) *m/z:* [M +Na]$^+$ calcd for $C_{31}H_{29}NNaO$, 454.2141; found, 454.2124.

General procedure 2.7 for the reaction of 2.41 with *N*-Benzyl-*N*-PhF-α-amino aldehydes

A solution of **2.41** approximately (0.150 mmol, 1.00 equiv) and aldehyde **2.103** - **2.105** (1.20 equiv) were dissolved in 4 mL of 3:2 THF:Et_2O and cooled to -128 °C. NaHMDS (1 M in THF, 2.20 equiv) was added dropwise and the reaction was stirred until complete by TLC (5 min). Then the mixture was quenched with 10 mL NH_4Cl, extracted three times with DCM, dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography, 5 to 30% EtOAc in hexane, to give alcohols **2.107-2.119**.

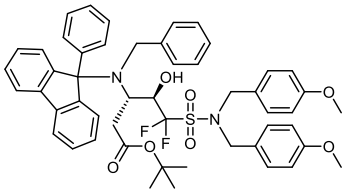


(2R,3S)-3-(benzyl(9-phenyl-9H-fluoren-9-yl)amino)-1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl) butane-1-sulfonamide (**2.107**). Prepared according to general procedure 2.7 from **2.41** (68 mg, 0.184 mmol, 1.00 equiv) and **2.103** (89 mg, 0.221 mmol, 1.20 equiv) as a white solid (115 mg, 81% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) : δ 7.69 (m, 8H), 7.32 (m, 10H), 7.05 (d, 4H, *J* = 8.2 Hz), 6.81 (d, 4H, *J* = 8.1 Hz), 4.55 (dd, 1H, *J* = 26.1, 4.4 Hz), 4.17 (m, 6H), 3.79 (s, 6H), 3.39 (q, 1H, *J* = 6.7 Hz), 1.31 (d, 1H, *J* = 4.7 Hz), 0.84 (d, 3H, *J* = 6.9 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 159.3, 147.7, 147.2, 143.6, 141.9, 140.8, 140.2, 130.2, 128.8, 128.5, 128.4, 127.8, 127.53, 127.45, 127.4, 127.2, 126.9, 126.6, 122.3 (dd, *J* = 295.9, 285.5 Hz), 120.4, 120.1, 113.9, 80.3, 74.8 (dd, *J* = 26.3, 19.4 Hz), 55.3, 52.4, 50.2, 50.1, 12.6; ^{19}F NMR ($CDCl_3$, 471 MHz): δ -103.9 (d, 1F, *J* = 235.2 Hz), -117.6 (dd, 1F, *J* = 235.2, 26.2 Hz); HRMS (ESI^{+}) *m/z:* [M + H]$^{+}$ calcd for $C_{46}H_{45}F_2N_2O_5S$, 775.3012; found, 775.2979.



(2R,3S)-3-(benzyl(9-phenyl-9H-fluoren-9-yl)amino)-1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-5-(methylthio)pentane-1-sulfonamide (**2.108**). Prepared according to general procedure 2.7 from **2.41** (52 mg, 0.140 mmol, 1.00 equiv) and **2.104** (78 mg, 0.168 mmol, 1.20 equiv) as a white solid (103 mg, 89% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.85 (m, 2H), 7.76 (t, 2H, *J* = 6.7 Hz), 7.69 (m, 4H), 7.48 (m, 2H), 7.35 (m, 5H), 7.29 (m, 3H), 7.07 (d, 4H, *J* = 7.8

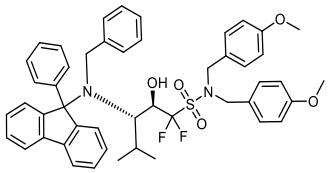
Hz), 6.86 (d, 4H, J = 7.8 Hz), 4.42 (d, 1H, J = 13.9 Hz), 4.35 (d, 1H, J = 14.1 Hz), 4.27 (dd, 1H, J = 26.5, 4.1 Hz), 4.18 (s, 4H), 3.84 (s, 6H), 3.26 (d, 1H, J = 8.0 Hz), 2.67 (bs, 1H), 2.4 (br, 1H), 2.33 (s, 1H), 1.8 (s, 3H), 1.6 (m, 1H), 1.35 (m, 1H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 159.4, 147.6, 145.9, 144.3, 141.3, 141.1, 140.2, 130.2, 129.0, 128.47, 128.45, 128.3, 127.8, 127.72, 127.70, 127.2, 127.0, 126.7, 126.6, 122.1 (dd, J = 289.2, 295.1 Hz), 120.6, 120.0, 113.9, 80.0, 71.1 (dd, J = 25.4, 20.2 Hz), 56.9, 55.3, 52.0, 50.2, 31.4, 28.8, 14.7; ^{19}F NMR ($CDCl_3$, 471 MHz): δ -102.6 (d, 1F, J = 232.9 Hz), -116.9 (dd, 1F, J = 232.8, 27.1 Hz); HRMS (ESI^+) *m/z:* [M + H]$^+$ calcd for $C_{48}H_{49}F_2N_2O_5S_2$, 835.3046; found, 835.3029.



tert-butyl (3S,4R)-3-(benzyl(9-phenyl-9H-fluoren-9-yl)amino)-5-(N,N-bis(4-methoxybenzyl)-sulfamoyl)-5,5-difluoro-4-hydroxypentanoate (**2.109**). Prepared according to general procedure 2.7 from **2.41** (61 mg, 0.167 mmol, 1.00 equiv) and **2.105** (100 mg, 0.20 mmol, 1.20 equiv) as a white solid (100 mg, 69% yield). A second species could be observed by ^{19}F NMR comprising ~3% of the mixture. This is presumed to be the minor, anti diastereomer and no attempt was made to isolate this material. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.78 (d, 2H, J = 7.5 Hz), 7.71 (m, 3H), 7.59 (d, 1H, J = 7.5 Hz), 7.47 (d, 2H, J = 7.1 Hz), 7.29 (m, 10H), 7.09 (d, 4H, J = 8.6 Hz), 6.84 (d, 4H, J = 8.6 Hz), 4.21 (m, 6H), 3.96 (m, 2H), 3.81 (s, 6H), 2.73 (d, 1H, J = 6.8 Hz), 2.45 (dd, 1H, J = 16.5, 8.5 Hz), 2.11 (dd, 1H, J = 16.6, 2.6 Hz), 1.34 (s, 9H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 171.9, 159.3, 146.5, 146.3, 143.6, 140.8, 140.6, 140.5, 130.2, 128.8, 128.6, 128.50, 128.46, 127.6, 127.5, 127.3, 127.19, 127.16, 127.1, 122.4 (dd, J = 294.8, 291.9 Hz), 120.3, 120.0, 113.9, 80.5, 80.2, 71.3 (dd, J = 24.1, 20.6 Hz), 55.3, 54.1, 51.7, 50.1, 34.4, 27.9; ^{19}F NMR

($CDCl_3$, 282 MHz): δ (Major diastereomer) -101.9 (dd, 1F, *J* = 234.3 Hz), -117.2 (dd, 1F, *J* = 234.3, 26.3 Hz); (minor diastereomer) -107.1 (d, 1F, *J* = 239.4 Hz), -119.8 (dd, 1F, *J* = 239.8, 23.0 Hz); HRMS (ESI^+) *m/z:* $[M + H]^+$ calcd for $C_{51}H_{53}F_2N_2O_7S$, 875.3536; found, 875.3514.

When the reaction was performed using 1.2 equiv of KHMDS instead of NaHMDS then a yield of 70 % was estimated by ^{19}F NMR and the presumed minor diastereomer comprised 4% of the crude.

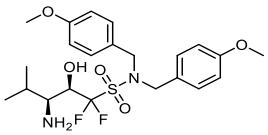


(2R,3S)-3-(benzyl(9-phenyl-9-fluorenyl)amino)-1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-4-methylpentane-1-sulfonamide (**2.110**). Compound **2.106** (2.90 g, 6.73 mmol, 1.00 equiv) and **2.41** (2.50 g, 6.73 mmol, 1.00 equiv) were dissolved in 60 mL of THF and 40 mL of Et_2O then cooled to -128 °C. KHMDS (1 M in THF, 8.07 mL, 1.20 equiv), was added dropwise and the mixture was stirred for 10 min until complete by TLC then was quenched with 10 mL of saturated NH_4Cl, diluted with 100 mL of water and extracted three times with 100 mL of DCM. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated then the residue was purified by flash chromatography, 5 to 20% EtOAc in hexane, to give 30 as a white solid (5.15 g, 96% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.77 (m, 4H), 7.63 (m, 4H), 7.42 (m, 2H), 7.27 (m, 8H), 7.06 (d, 4H, *J* = 8.6 Hz), 6.85 (d, 4H, *J* = 8.6 Hz), 4.53 (m, 2H), 4.18 (m, 5H), 3.84 (s, 6H), 2.96 (d, 1H, *J* = 9.5 Hz), 2.4 (bs, 1H), 1.55 (m, 1H), 1.11 (d, 3H, *J* = 6.6 Hz), 0.67 (ap t, 3H, *J* = 6.4 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.4, 147.7, 145.9, 141.6, 141.0, 140.2, 130.3, 129.7, 128.9, 128.3, 128.2, 127.9, 127.8, 127.6, 127.4, 127.0, 126.8, 122.2 (dd, *J* = 298.1, 288.8 Hz), 120.3, 119.8, 114.0, 80.4, 70.5 (dd, *J* = 26.7, 21.6 Hz),

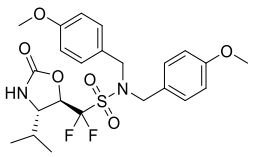
63.4, 55.4, 52.6, 50.2, 28.4, 23.4, 20.9, 20.7; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -99.8 (d, 1F, J = 229.3 Hz), -110.7 (dd, 1F, J = 227.5, 26.5 Hz); HRMS (ESI^+) *m/z:* $[M + H]^+$ calcd for $C_{48}H_{49}F_2N_2O_5S$, 803.33248; found, 803.3296.

When 2.2 equiv NaHMDS was used instead of KHMDS then the isolated yield was 51%.

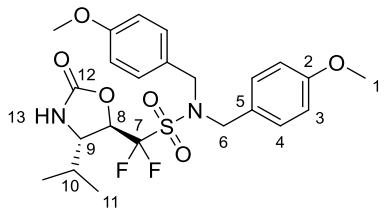
Determination of the absolute relative stereochemistry of 2.89 and 2.110.



(2R,3S)-3-amino-1,1-difluoro-2-hydroxy-N,N-bis(4-methoxybenzyl)-4-methylpentane-1-sulfonamide (**2.111**)**.** Compound **2.110** (4.95 g, 6.17 mmol) was dissolved in 100 mL of 1% TFA in MeOH. Pd/C 5 wt% (495 mg) and $Pd(OH)_2$/C 20 wt% (125 mg) were added and the mixture was stirred under an H_2 atmosphere overnight. The catalyst was removed by filtration with a pad of celite and the mixture was diluted with 300 mL of water and washed with 200 mL of hexane. Most of the methanol was evaporated from the aqueous layer and then Na_2CO_3 was added until a pH of 10 was achieved. The suspension was extracted 3 times with 100 mL of DCM. The combined DCM layers were dried over magnesium sulfate, filtered, and concentrated to give the free amine **2.111** as a white solid (2.69 g, 92% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.14 (d, 4H, J = 8.3 Hz), 6.86 (d, 4H, J = 8.3 Hz), 4.42 (d, 2H, J = 15.1 Hz), 4.32 (m, 3H), 3.83 (s, 6H), 2.89 (m, 1H), 2.1 (m, 1H), 1.05 (d, 3H, J = 6.6 Hz), 1.03 (d, 3H, J = 6.6 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 159.3, 130.2, 126.8, 124.2 (dd, J = 293.5, 288.4 Hz), 113.9, 71.0 (dd, J = 23.3, 20.2 Hz), 58.5, 55.2, 50.3, 29.4, 20.4, 17.5; ^{19}F NMR ($CDCl_3$, 471 MHz): δ -103.0 (d, 1F, J = 238.9 Hz), -112.1 (dd, 1F, J = 238.9, 20.6 Hz); HRMS (ESI^+) *m/z:* M + H]$^+$ calcd for $C_{22}H_{31}F_2N_2O_5S$, 473.1916; found, 473.1918.



1,1-difluoro-1-((4S,5R)-4-isopropyl-2-oxooxazolidin-5-yl)-N,N-bis(4-methoxybenzyl)methane sulfonamide (**2.112**). **2.111** (29 mg, 0.061 mmol, 1 equiv) was dissolved in 3 mL of DCM and cooled to 0 °C. DIPEA (27 µL, 0.153 mmol, 2.5 equiv) was added followed by triphosgene (9.1 mg, 0.0307 mmol, 0.5 equiv) in a solution of 1 mL DCM. The reaction was stirred for two h then was applied to a column of silica eluted using a gradient of 0 to 20% EtOAc in DCM. **2.112** was obtained as a colorless oil (28 mg, 92% yield). The relative stereochemistry of this compound was assigned based on NOESY contacts between the protons of the valine side chain and the proton α to the alcohol, which indicated that the geometry in the ring was trans. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.10 (ArH, d, 4H, *J* = 8.6 Hz), 6.82 (ArH, d, 4H, *J* = 8.7 Hz), 6.34 (NH, s, 1H), 5.21 (**H**CO, dt, 1H, *J* = 20.9, 6.6 Hz), 4.36 ($ArCH_2$, s, 4H), 3.94 ($C^{\alpha}H$, t, 1H, *J* = 7.2 Hz), 3.8 (OCH_3, s, 6H), 2.31 (C^{β}**H** m, 1H), 1.06 ($C^{\gamma}H_3$, d, 3H, *J* = 6.5 Hz), 1.03 ($C^{\gamma}H_3$, d, 3H, *J* = 6.5 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 159.4, 157.6, 130.2, 126.5, 120.9 (dd, *J* = 297.6, 288.3 Hz), 113.9, 75.2 (dd, *J* = 29.7, 20.3 Hz), 61.9, 55.3, 50.7, 27.6, 20.7, 18.8; ^{19}F NMR ($CDCl_3$, 471 MHz) δ: -106.5 (dd, 1F, *J* = 244.2, 4.0 Hz), -112.6 (dd, 1F, *J* = 243.6, 20.8 Hz); HRMS (ESI^+) *m/z:* $[M + Na]^+$ calcd for $C_{23}H_{32}F_2N_3O_6S$, 516.1974; found, 516.1962.



HMQC Peak Coordinates for **2.112** (300 MHz, $CDCl_3$)

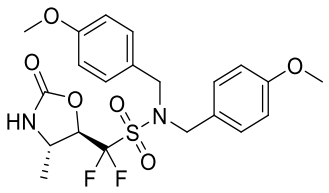
	^{1}H	^{13}C
$C^{1}H_3$	3.80	55.3
$C^{3}H$	6.82	113.9
$C^{4}H$	7.1	130.2
$C^{6}H_2$	4.36	50.7
$C^{8}H$	5.21	75.2
$C^{9}H$	3.94	61.9
$C^{10}H$	2.31	27.6
$C^{11}H_3$	1.06	18.8
$C^{11}H_3$	1.03	20.7

COSY Peak Coordinates for **2.112** (300 MHz, $CDCl_3$)

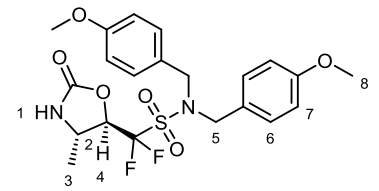
H^{3}-H^{4}	7.11 – 6.84
H^{8}-H^{9}	5.22 – 3.95
H^{9}-H^{10}	3.95 – 2.33
H^{10}-H^{11}	2.32 – 1.06

NOESY Peak Coordinates for **2.112** (300 MHz, $CDCl_3$)

H^{3}-H^{6}	7.–9 - 4.36
H^{4}-H^{6}	6.–2 - 4.36
H^{3}-H^{1}	6.–2 - 3.78
H^{13}-H^{9}	6.–4 - 3.93
H^{8}-H^{9}	5.–9 - 3.92
H^{8}-H^{10}	5.–9 - 2.29
H^{8}-H^{11}	5.–9 - 1.01
H^{9}-H^{10}	3.–3 - 2.29
H^{9}-H^{11}	3.–3 - 1.03
H^{10}-H^{11}	2.–0 - 1.02



1,1-difluoro-N,N-bis(4-methoxybenzyl)-1-((4S,5R)-4-methyl-2-oxooxazolidin-5-yl)-methanesulfonamide (**2.113**). **2.89** (50 mg, 0.073 mmol, 1.0 equiv) was dissolved in 5 mL of 1% TFA MeOH. 5 mg of 20 wt% $Pd(OH)_2$ on carbon and 10 mg of 10 wt% Pd on carbon were added and the mixture was stirred under hydrogen atmosphere for 16 h. The mixture was filtered then diluted with 20 mL of water and washed with 20 mL of hexane. The aqueous layer was basified with Na_2CO_3 until pH 10 and then extracted 3 times with 10 mL DCM. The combined DCM layers were dried over magnesium sulfate, filtered and concentrated then the residue was redissolved in 5 mL DCM and cooled to 0 °C. DIPEA (32 µL, 0.18 mmol, 2.5 equiv) was added followed by a solution of triphosgene (11 mg, 0.037 mmol, 0.5 equiv) in 1 mL DCM. The reaction was stirred for two h then was applied to a silica column and eluted with a gradient of 0 to 30% EtOAc in DCM to yield a colorless oil. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 7.04 (d, 4H, J = 8.4 Hz), 6.77 (d, 4H, J = 8.4 Hz), 5.63 (s, 1H), 4.69 (ddd, 1H, J = 15.1, 8.3, 5.1 Hz), 4.28 (m, 5H), 3.74 (s, 6H), 1.41 (d, 3H, *J* = 6.3 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$ 75 MHz) δ 159.5, 156.6, 130.2, 126.1, 120.0 (ap t, J = 288.2 Hz), 114.0, 79.4 (dd, J = 24.6, 20.5 Hz), 55.3, 50.4, 48.3, 21.8; ^{19}F NMR ($CDCl_3$ 283 MHz) δ -111.6 (dd, 1F, J = 239.6, 8.2 Hz), -117.0 (dd, 1F, J = 239.5, 15.5 Hz); HRMS (ESI^+) *m/z:* $[M + H]^+$ calcd for $C_{21}H_{25}F_2N_2O_6S$, 471.1396; found, 471.1374.



HMQC Peak Coordinates for (**2.113**) (300 MHz, $CDCl_3$)

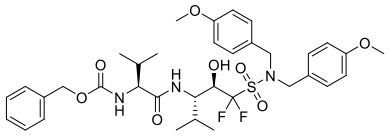
	^1H	^{13}C
C^2H^2	4.29	47.79
C^3H^3	1.41	21.97
C^4H^4	4.70	79.32
C^5H^5	4.28	50.65
C^6H^6	7.04	130.41
C^7H^7	6.78	113.98
C^8H^8	3.74	54.95

COSY Peak Coordinates for (**2.113**) (300 MHz, $CDCl_3$)

H^6-H^7	7.–4 - 6.78
H^4-H^2	4.–0 - 4.29
H^2-H^3	4.–8 - 1.41

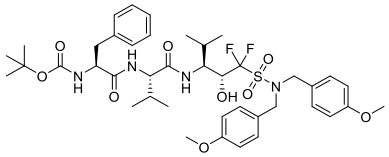
NOESY Peak Coordinates for (**2.113**) (300 MHz, $CDCl_3$)

H^8-H^7	6.–7 - 3.74
H^6-H^5	7.–4 - 4.29
	5.–4 - 4.28
	5.–4 - 1.40
H^2-H^3	4.–8 - 1.40
H^3-H^4	4.–0 - 1.42



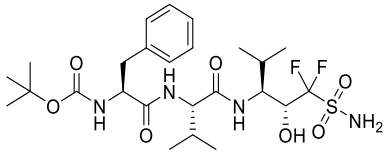
(1R)-1-(Cbz-Val-Valinolyl)-1,1-difluoro-N,N-bis(4-methoxybenzyl)methanesulfonamide (**2.117**). Cbz-Val-OH (2.13 g, 8.49 mmol, 1.49 equiv) was dissolved in 50 mL of DCM and HOAt (1.15 g, 8.49 mmol, 1.49 equiv) was added followed by EDC HCl (1.62 g, 8.49 mmol, 1.49 equiv) the resulting solution was stirred for 15 min then 31 (2.69 g, 5.70 mmol, 1 equiv) was added. The reaction was stirred overnight at room temperature then placed on a silica column and eluted with 0 to 25% EtOAc in DCM, which gave **2.117** as a white foam (3.63 g, 90% yield). ^1H NMR

($CDCl_3$, 300 MHz): δ 7.32 (s, 5H), 7.1 (d, 4H, *J* = 8.6 Hz), 6.83 (d, 4H, *J* = 8.7 Hz), 6.67 (d, 1H, *J* = 9.3 Hz), 5.65 (d, 1H, *J* = 8.8 Hz), 5.11 (s, 2H), 4.53 (d, 1H, *J* = 20.9 Hz), 4.33 (m, 6H), 4.09 (dd, 1H, *J* = 8.8, 7.1 Hz), 3.8 (s, 6H), 2.24 (m, 2H), 0.96 (m, 12H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 172.5, 159.4, 156.7, 136.1, 130.1, 128.5, 128.1, 127.9, 126.5, 123.0 (dd, *J* = 293.1, 289.0 Hz), 113.9, 70.4 (ap t, *J* = 22.0 Hz), 67.1, 60.9, 55.4, 55.2, 50.3, 30.2, 28.7, 20.4, 19.4, 17.84, 17.77; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -105.6 (d, 1F, *J* = 239.1 Hz), -114.2 (dd, 1F, *J* = 238.9, 21.1 Hz); HRMS (ESI^+) *m/z:* $[M + Na]^+$ calcd for $C_{35}H_{45}F_2N_3NaO_8S$, 728.2788; found, 728.2795.



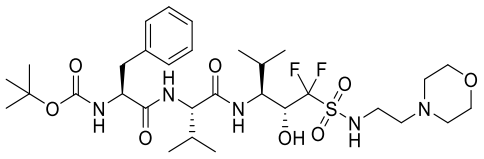
(1R)-1-(Boc-Phe-Val-Valinolyl)-1,1-difluoro-N,N-bis(4-methoxybenzyl)methanesulfonamide (**2.118**). **2.117** (3.63 g, 5.15 mmol, 1.00 equiv) was dissolved in 20 mL of MeOH and 182 mg of $Pd(OH)_2$ 20 wt% on carbon was added. The mixture was stirred under hydrogen for 1 hour until complete by TLC then filtered with a pad of celite and concentrated to dryness to give the crude amine. Separately, Boc-Phe-OH (2.05 g, 7.72 mmol, 1.50 equiv) was dissolved in 20 mL of DCM and HOAt (1.05 g, 7.72 mmol, 1.50 equiv) was added followed by EDC HCl (1.48 g, 7.72 mmol, 1.50 equiv). The mixture was stirred for 15 min then added to the crude amine. After stirring for 6 h at room temperature the reaction was filtered and the volume was reduced to approximately 20 mL then was purified by flash chromatography, eluting with 0 to 30% EtOAc in DCM, to give **2.118** as a white foam (4.14 g, 98% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.25 (m, 3H), 7.19 (m, 2H), 7.09 (d, 4H, *J* = 8.7 Hz), 6.82 (d, 4H, *J* = 8.7 Hz), 6.68 (d, 1H, *J* = 8.0 Hz), 6.48 (d, 1H, *J* = 8.3 Hz), 4.98 (d, 1H, *J* = 6.4 Hz), 4.52 (ddd, 1H, *J* = 20.9, 9.1, 4.8 Hz), 4.33 (m, 6H), 4.12 (d, 1H, *J* = 5.0 Hz), 3.79 (s, 6H), 3.08 (m, 2H), 2.22 (m, 2H), 1.4 (s, 9H), 0.96 (m,

9H), 0.87 (d, 3H, J = 6.8 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 172.8, 172.5, 159.2, 155.8, 137.2, 130.2, 129.5, 128.3, 126.8, 126.5, 123.3 (ap t, J = 291.4 Hz), 113.8, 79.4, 70.4 (ap t, J = 21.7 Hz), 59.7, 55.6, 55.2, 54.4, 50.3, 37.8, 30.4, 28.8, 28.4, 20.5, 19.3, 18.5, 17.4; ^{19}F NMR ($CDCl_3$, 282 MHz) : δ -106.9 (d, 1F, J = 239.5 Hz), -113.4 (dd, 1F, J = 238.4, 16.0 Hz); HRMS (ESI^+) *m/z:* $[M + H]^+$ calcd for $C_{41}H_{56}F_2N_4O_9S$, 818.37361; found, 819.3799.



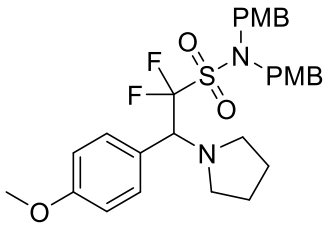
(1R)-1-(Boc-Phe-Val-Valinolyl)-1,1-difluoromethanesulfonamide (**2.119**). To a solution of **2.118** (2.00 g 2.44 mmol, 1.00 equiv) in 100 mL of CH_3CN at 0 °C, CAN (5.35 g, 9.75 mmol, 4 equiv) in 20 mL of water was added dropwise over 20 min. The resulting solution was stirred at 0 °C for 3 h then additional CAN (5.35 g, 9.75 mmol, 4 equiv) in 20 mL of water was added dropwise over 20 min. After 6 h total stirring the reaction was diluted with 250 mL brine and 250 mL 10% citric acid then was extracted three times with 250 mL of EtOAc. The combined organic layers were concentrated and then purified by reversed phase chromatography using a Biotage Isolera One Flash purification system employing a C-18 reversed-phase preparative Biotage column (30 g) on a gradient of 20 to 100% methanol over 30 min at 25 mL per minute (t_r = 22 min), giving **2.119** as a white powder (1.12 g, 79% yield). It was noted that an aqueous suspension of **2.119** was extremely prone to bumping when concentrating on a rotary evaporator, therefore we recommend that fractions containing product be diluted with a large volume of isopropanol/toluene or another suitable solvent to azeotropically remove the water. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.96 (d, 1H, J = 9.5 Hz), 7.26 (m, 5H), 4.3 (m, 4H), 3.14 (dd, 1H, J = 14.0, 4.7 Hz), 2.81 (dd, 1H, J = 13.9, 9.9 Hz), 2.27 (m, 1H), 2.14 (m, 1H), 1.37 (s, 9H), 0.94 (m, 12H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz): δ 174.2, 173.3, 157.5, 138.6, 130.3, 129.3, 127.6, 122.8 (dd, J

= 291.7, 286.9 Hz), 80.6, 70.4 (ap t, J = 22.6 Hz), 60.0, 57.2, 54.1, 38.9, 31.7, 29.5, 28.6, 20.7, 20.0, 18.1, 16.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -105.6 (d, 1F, J = 242.4 Hz), -119.8 (ddd, 1F, J = 242.0, 20.1, 5.4 Hz); HRMS (ESI^+) *m/z:* $[M + H]^+$ calcd for $C_{25}H_{41}F_2N_4O_7S$, 579.2659; found, 579.2665.

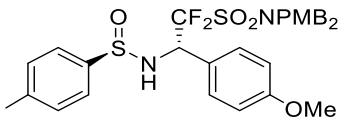


(1R)-1-(Boc-Phe-Val-Valinolyl)-1,1-difluoro-*N*-(2-morpholinoethyl)-methanesulfonamide (**2.120**). CMBP was prepared according to a literature procedure.[119] **2.119** (289 mg, 0.50 mmol, 1.0 equiv) was placed in 10 mL of dry toluene. 2-morpholinoethan-1-ol (98 mg, 0.75 mmol, 1.5 equiv) was added followed by CMBP (0.5 M in toluene, 1.50 mL, 0.75 mmol, 1.5 equiv). The reaction was heated to 100 °C for 2 h then the reaction was concentrated. The residue was subjected to flash chromatography eluting with 0 to 30% MeOH in DCM. Fractions containing product were pooled and further purified by reversed phase chromatography using a Biotage Isolera One Flash purification system including two C-18 reversed-phase preparative Biotage columns (30 g) connected in series on a gradient of 40 to 75% methanol over 60 min at 25 mL per minute (t_r product = 48 min, t_r starting material = 40 min). **2.119** was obtained as a white solid (220 mg, 64% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) : δ 7.3 (t, 2H, J = 7.2 Hz), 7.24 (m, 1H), 7.18 (d, 2H, J = 7.2 Hz), 6.67 (s, 2H), 5.16 (br, 2H), 4.98 (s, 1H), 4.49 (d, 1H, J = 22.0 Hz), 4.35 (q, 1H, J = 6.5 Hz), 4.22 (t, 1H, J = 5.8 Hz), 4.09 (m, 1H), 3.69 (t, 4H, J = 4.3 Hz), 3.36 (m, 2H), 3.08 (m, 2H), 2.53 (t, 2H, J = 5.7 Hz), 2.46 (m, 4H), 2.24 (m, 1H), 2.14 (m, 1H), 1.39 (s, 9H), 0.98 (d, 3H, J = 6.6 Hz), 0.94 (d, 3H, J = 6.6 Hz), 0.9 (d, 3H, J = 6.8 Hz), 0.84 (d, 3H, J = 6.9 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 172.4, 155.8, 136.7, 129.3, 128.5, 126.8, 122.5 (dd, J = 293.6, 288.5 Hz), 80.1, 70.1 (dd, J = 23.9, 20.9 Hz), 66.7, 59.3, 57.3, 55.7, 55.4, 53.1, 40.2,

37.6, 30.1, 28.6, 28.3, 20.4, 19.3, 18.0, 17.8; ^{19}F NMR ($CDCl_3$, 471 MHz) : δ -106.7 (d, 1F, *J* = 245.7 Hz), -115.7 (d, 1F, *J* = 245.0 Hz); HRMS (ESI^+) *m/z:* $[M+H]^+$ calcd for $C_{31}H_{52}F_2N_5O_8S$, 692.34992; found, 692.3559.

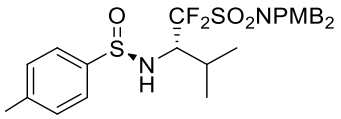


1,1-difluoro-N,N-bis(4-methoxybenzyl)-2-(4-methoxyphenyl)-166yrrolidinedin-1-yl)ethane-1-sulfonamide (**2.125**). 1-(4-methoxybenzylide166yrrolidinedin-1-ium perchlorate (**2.122**) was prepared according a literature procedure.[130] **2.41** (50 mg, 0.135 mmol, 1.0 equiv) and **2.122** (78 mg, 0.27 mmol, 2 equiv) were dissolved in 2.7 mL of THF and 300 µL of DMF. The mixture was cooled to –98 °C and KHMDS (1 M in THF, 270 µL, 0.27 mmol, 2.0 equiv). was added dropwise. The mixture was stirred for 15 min then was quenched with 10 mL brine. The reaction was extracted three times with 10 mL of DCM dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography, 0 to 20% EtOAc hexane, to give 53 mg colorless oil (70% yield). This material existed as a mixture of rotamers ~93:7 ^{1}H NMR ($CDCl_3$, 300 MHz) : δ 7.38 (d, 2H, J = 8.3 Hz), 7.13 (d, 4H, J = 8.3 Hz), 6.95 (d, 2H, J = 8.4 Hz), 6.83 (d, 4H, J = 8.3 Hz), 4.61 (dd, 1H, J = 19.6, 12.8 Hz), 4.34 (s, 4H), 3.85 (s, 3H), 3.82 (s, 6H), 2.69 (s, 4H), 1.72 (s, 4H); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 159.7, 159.2, 131.9, 130.1, 124.8 (dd, J = 292.9, 289.7 Hz), 127.2, 123.15, 123.13, 113.8, 113.5, 66.2 (dd, J = 23.3, 18.3 Hz), 55.2, 50.4, 50.2, 23.2; ^{19}F NMR ($CDCl_3$, 282 MHz): δ (Major Rotamer) 100.6 (dd, 1F, J = 232.6, 12.7 Hz), -103.1 (dd, 3F, J = 232.6, 19.7 Hz), (minor rotamer)-103.7 (dd, 5F, J = 261.5, 11.6 Hz), -106.1 (dd, 7F, J = 261.9, 18.9 Hz); HRMS (ESI^+) *m/z:* $[M+H]^+$ calcd for $C_{29}H_{35}F_2N_2O_5S$, 561.2229; found, 561.2226.



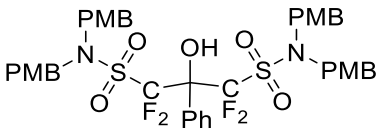
(S)-1,1-difluoro-N,N-bis(4-methoxybenzyl)-2-(4-methoxyphenyl)-2-(((R)-p-tolylsulfinyl)-amino)ethane-1-sulfonamide (**2.126**). (R,E)-*N*-(4-methoxybenzylidene)-4-methylbenzene-sulfinamide (**2.123**) was prepared according to a literature procedure.[132] **2.41** (50 mg, 0.135 mmol, 1 equiv) and **2.123** (44 mg, 0.162 mmol, 1.2 equiv) were dissolved in 2 mL of THF and 1 mL of Et_2O. The mixture was cooled to -114 °C and NaHMDS (1 M in THF, 300 µL, 2.2 equiv) was added dropwise. The reaction was stirred for 15 min then was quenched with 10 mL sat. NH_4Cl and extracted 3 times with 10 mL DCM. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography, 10 to 40% EtOAc in hexane, to yield 87 mg (99% yield) of **2.126** as a white solid. The DR was estimated as 97:3 by ^{19}F NMR. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.64 (d, 2H, J = 8.1 Hz), 7.28 (d, 2H, J = 8.4 Hz), 7.24 (d, 2H, J = 8.1 Hz), 7.12 (d, 4H, J = 8.5 Hz), 6.87 (d, 2H, J = 7.0 Hz), 6.84 (d, 4H, J = 7.0 Hz), 5.3 (m, 1H), 5.06 (d, 1H, J = 8.0 Hz), 4.43 (d, 2H, J = 15.1 Hz), 4.26 (d, 2H, J = 14.9 Hz), 3.82 (s, 9H), 2.39 (s, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 160.1, 159.4, 141.6, 141.2, 130.1, 129.9, 129.4, 126.5, 122.1 (dd, J = 290.0, 286.0 Hz), 125.9, 125.8, 114.0, 113.9, 58.8 (dd, J = 25.7, 21.0 Hz), 55.3, 55.2, 50.2, 21.3; ^{19}F NMR ($CDCl_3$, 282 MHz): δ (Major diastereomer) -103.2 (dd, J = 235.5, 8.5 Hz), -108.6 (dd, J = 235.6, 17.9 Hz), (Minor diastereomer) -104.6 (dd, J = 235.0, 7.7 Hz), -110.4 (dd, J = 234.9, 17.0 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{32}H_{35}F_2N_2O_6S_2^+$ $(M + H)^+$ 645.1899, found 645.1886.

When the reaction was performed using 1.2 equiv KHMDS the DR was 92:8, the reaction was complete by TLC, but the product was not isolated.

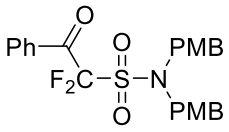


(S)-1,1-difluoro-N,N-bis(4-methoxybenzyl)-3-methyl-2-(((R)-p-tolylsulfinyl)amino)butane-1-sulfonamide (**2.127**). (R,E)-4-methyl-*N*-(2-methylpropylidene)benzenesulfinamide (**2.124**) was prepared according to a literature procedure.[131]

Compound **2.41** (50 mg, 0.135 mmol, 1.0 equiv) and **2.124** (34 mg, 0.16 mmol, 1.2 equiv) were dissolved in 1.5 mL of THF and 1 mL of Et_2O then cooled to -128 °C. NaHMDS (1 M in THF, 300 μL, 2.2 equiv) was added dropwise and the reaction was stirred for 15 min before quenching with NH_4Cl. The mixture was extracted three times with 10 mL of DCM then the organic layers were dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography using 5 to 30 % EtOAc in hexanes to yield 56 mg (72% yield) of **2.127** as a colorless oil. The DR was estimated at 97:3 by ^{19}F NMR. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.8 (3, 2H, J = 8.2 Hz), 7.35 (3, 2H, J = 8.0 Hz), 7.16 (3, 4H, J = 8.6 Hz), 6.87 (3, 4H, J = 8.6 Hz), 4.29 (m, 6H), 3.83 (s, 6H), 2.55 (m, 1H), 2.43 (s, 3H), 1.21 (d, 3H, J = 6.9 Hz), 1.03 (d, 3H, J = 6.8 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz): δ 159.4, 142.6, 141.7, 130.2, 129.6, 123.6 (ap. t, J = 288.6 Hz), 126.7, 125.8, 114.0, 62.8 (ap. t, J = 20.9 Hz), 55.3, 50.3, 27.7, 21.4, 20.7, 16.7; ^{19}F NMR ($CDCl_3$, 282 MHz): δ (Major diastereomer) -104.2 (dd, F, J = 235.7, 11.2 Hz), -105.2 (dd, F, J = 235.9, 14.4 Hz); (Major diastereomer) -96.3 (dd, 1F, J = 237.2, 8.6 Hz), -106.8 (dd, F, J = 237.2, 19.3 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{28}H_{35}F_2N_2O_5S_2^+$ $(M + H)^+$ 581.1950, found 581.1943.



1,1,3,3-tetrafluoro-2-hydroxy-N,N,N,N-tetrakis(4-methoxybenzyl)-2-phenylpropane-1,3-disulfonamide (**2.128**). Compound **2.41** (100 mg, 0.27 mmol, 1.00 equiv) and phenyl benzoate (64 mg, 0.324 mmol, 1.20 equiv) were dissolved in 5 mL of THF and cooled to – 78 °C. KHMDS (1 M in THF, 324 µL, 0.324 mmol, 1.2 equiv) was added dropwise and the reaction was stirred for 15 min. The reaction was quenched with 10 mL of sat. NH_4Cl and extracted with 10 mL of DCM three times. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography eluting with 10 to 60% EtOAc in hexane 41 mg white solid (35% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 7.86 (m, 2H), 7.49 (m, 3H), 7.04 (d, 8H, *J* = 8.6 Hz), 6.78 (d, 8H, *J* = 8.7 Hz), 4.97 (s, 1H), 4.26 (br, 8H), 3.77 (s, 12H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): δ 159.4, 130.2, 130.1, 129.9, 128.2, 128.0, 126.3, 121.3 (ap t, *J* = 302.1 Hz), 113.9, 79.4 (ap t, *J* = 20.9 Hz), 55.3, 50.6; ^{19}F NMR ($CDCl_3$, 283 MHz): δ -102.2 (d, 2F, *J* = 250.0 Hz); -103.3 (d, 2F, *J* = 251.7 Hz); HRMS (ESI$^+$) *m/z:* [M + NH_4]$^+$ calcd for $C_{41}H_{46}N_3O_9S_2F_4$, 864.2612; found, 864.2575.



N,N-bis(4-methoxybenzyl)-1,1-difluoro-1-benzoylmethanesulfonamide (**2.37**). Compound **2.41** (74 mg, 0.2 mmol, 1 equiv) and benzoic anhydride (180 mg, 0.8 equiv, 4 equiv) were dissolved in 3 mL THF and 2 mL of Et_2O. The mixture was cooled to -128 °C and then KHMDS (1 M in THF, 400 µL, 0.4 mmol, 2 equiv) was added dropwise. After stirring for 20 min the reaction was quenched with 10 mL of sat. NH_4Cl and extracted twice with 10 mL of DCM. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The

residue was purified by flash chromatography to yield 62 mg (65% yield) of **2.37**. ^{1}H NMR and ^{19}F NMR of this material was identical to an authentic sample prepared by electrophilic fluorination in Chapter 1.

Chapter 3 — Introduction of the 9-Phenyl-9-fluorenyl Protecting Group into Amines, Acids, Alcohols, Sulfonamides, Amides, and Thiols

3.1 — Introduction

3.1.1 — Background

During the course of our work on ß-hydroxy-α-difluoromethane sulfonamides (Chapter 2) we required α-amino aldehydes that were suitably protected for reaction with difluoromethane sulfonamide carbanions. Various *N*-protected α-aminoaldehydes have been reported.[132] Carbamate-protected α-aminoaldehydes are notoriously prone to racemization and are usually used immediately after they are prepared without purification.[114] *N,N*-dibenzylamino aldehydes are less prone to racemization,[133] but even these do racemize under basic conditions.[134] Garner's aldehyde provides excellent resistance to racemization, but it is limited to serine and threonine derivatives.[135] The 9-phenyl-9-fluorenyl (PhF) group (Figure 3.1) provides greater resistance to racemization than dibenzylamines and is more general than Garner's aldehyde so we decided to investigate this group for the protection of α-amino aldehydes.

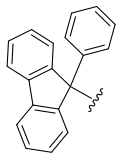
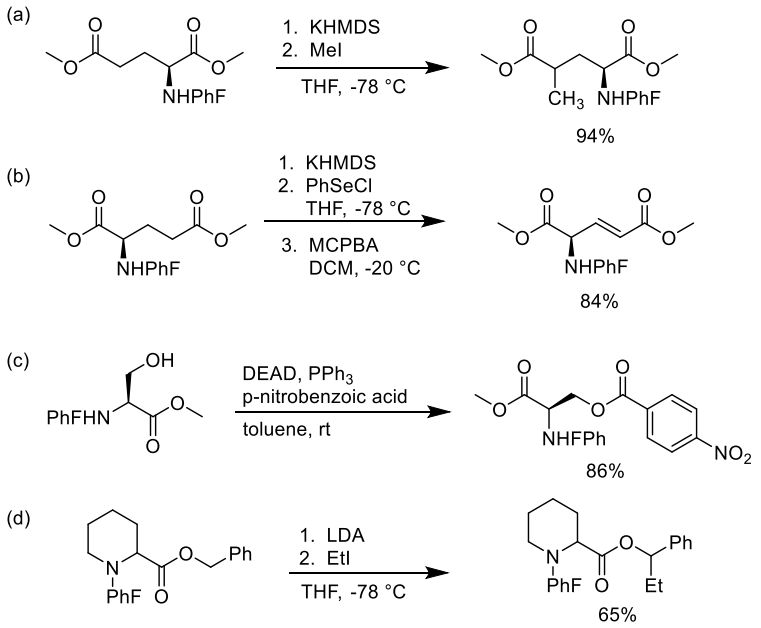


Figure 3.1. Structure of the PhF group.

The PhF group has been shown to preserve the configuration of α-amino aldehydes in the presence of silica, organic bases, and during nucleophilic additions.[115] The first example of a PhF amine was published in the 1930's,[136] but the benefits of using it as a protecting group for amines weren't realized until the 1980's when Henry Rapoport and coworkers began using it for the synthesis and protection of compounds such as amino aldehydes.[137,138] In addition to preventing racemization, the group is very useful in other situations where proton abstraction from the α-carbon must be avoided. PhF protection avoids alkylation at the α-position during ß- or γ-alkylations of aspartate or glutamate derivatives, (Scheme 3.1 a).[139] This ability to selectively functionalize at the less hindered ester is also useful for the synthesis of ß,γ-unsaturated glutamates while avoiding the α,ß-unsaturation (Scheme 3.1 b).[116] PhF protection also enables Mitsunobu reactions on serine derivatives while avoiding E2 eliminations which would lead to dehydroalanines (Scheme 3.1 c).[140] Even benzylic alkylation of a benzyl ester without alkylation at the α-carbon is possible (Scheme 3.1d).[137]



Scheme 3.1. Literature examples of reactions facilitated by PhF protected amines.

PhF deprotection can be accomplished using a variety of methods including using acidolysis,[115] hydrogenolysis,[141] TMSOTf and triethylsilane,[142] I_2 in methanol,[143] and Li or Na in ammonia.[144]

Both steric and electronic factors play a role in PhF protection. Sterically, the PhF group is similar to the trityl group which can also be used to shield the α-proton.[145] The bulk of the PhF group forces the molecule into a conformation where the C^{α}-H^{α} bond is co-planer with the carbonyl, which is unfavorable for enolization (Figure 3.2). This has been shown in the crystal structure of PhF protected α-amino aldehydes.[146]

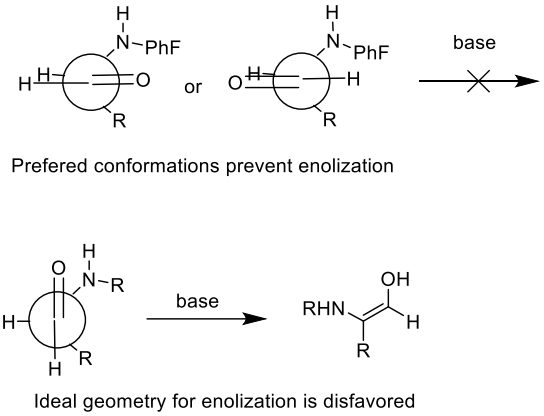
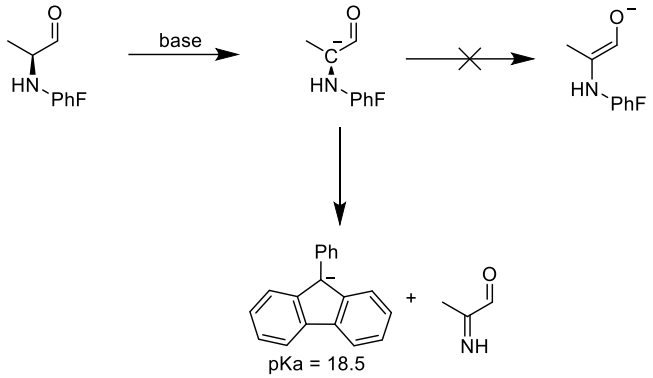


Figure 3.2. The PhF protected α-amino aldehydes adopt a conformation where the α-proton and carbonyl are coplanar.

Electronically the PhF group differs from the trityl group. Whether the fluorenyl cation is antiaromatic or simply nonaromatic has been a matter of some debate. Although the 12-electron π system would indicate the cation is antiaromatic, not all measurements and calculations support this classification.[147] Regardless of classification, the PhF cation is less stable than the trityl cation leading to increased reactivity and increased Lewis acidity. Therefore, PhF groups have increased hydrolytic and acid stability compared to trityl groups.[148]

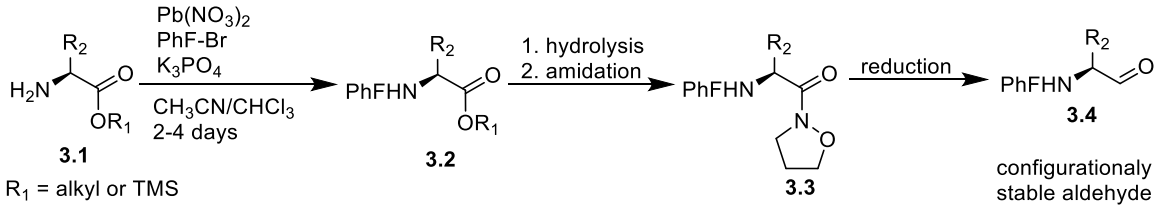
On the other hand, the PhF anion, with 14 electrons is aromatic and the negative charge is better stabilized than the trityl anion which is reflected in the pK_a values of triphenylmethane (33) and 9-phenylfluorene (18.5).[149] The highly stabilized anion is a reasonable leaving group and this gives PhF-protected α-amino aldehydes a tendency to eliminate rather than enolize (Scheme 3.2). Even this requires forcing conditions; (phenylfluorenyl)alanal has a half-life of 8 h when refluxing in THF with triethylamine, and under the same conditions no racemization is detected.[115]



Scheme 3.2. Deprotonation of α-amino aldehydes leads to decomposition not epimerization.

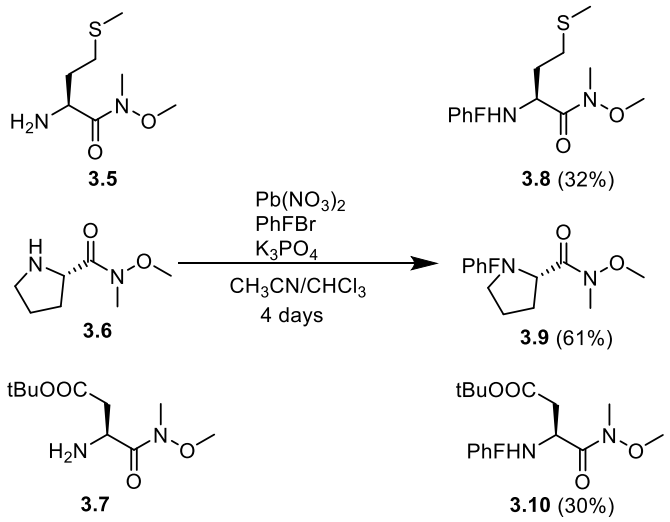
Despite its usefulness, the PhF protecting group is not widely used, possibly due to the issues that are sometimes encountered during its installation. The procedure originally developed by Rapoport is still generally used (Scheme 3.3).[150] In this procedure, an amino acid ester **3.1** is reacted with phenylfluorenyl bromide in the presence of potassium phosphate, which acts as a base, and $Pb(NO_3)_2$ which acts as a halide acceptor. The solvent is usually a mixture of chloroform and acetonitrile in which potassium phosphate and $Pb(NO_3)_2$ are insoluble; therefore, a Morton flask is used to facilitate mixing.[151] Rapoport later reported that the reaction also proceeds in the absence of $Pb(NO_3)_2$ if the reaction is conducted in nitromethane, though this procedure does not appear to have been widely adopted. The reaction is very slow, often

requiring several days and even then, yields of **3.2** can be variable.[137] Once **3.2** is obtained, it is readily converted to amide **3.3**, which is similar to a Weinreb's amide, and can be reduced to the configurationally stable aldehyde **3.4**. It is also possible to reduce **3.2** to the alcohol and then oxidize to **3.4** with standard methodologies.[152]



Scheme 3.3. N^{α}-PhF protection of α-amino acids and their conversion to N^{α}-PhF protected of α-aldehydes using the method of Rapoport.

We were interested in several aldehydes of type **3.4.** which were needed for work discussed in Chapter 2. We applied Rapoport's procedure to Weinreb's amides **3.5-3.7**. In our hands, these conditions were unsatisfactory as they provided protected amines **3.8-3.10** in poor to moderate yields after four days (Scheme 3.4). Therefore, we undertook a study to develop more efficient conditions for the installation of the PhF group.



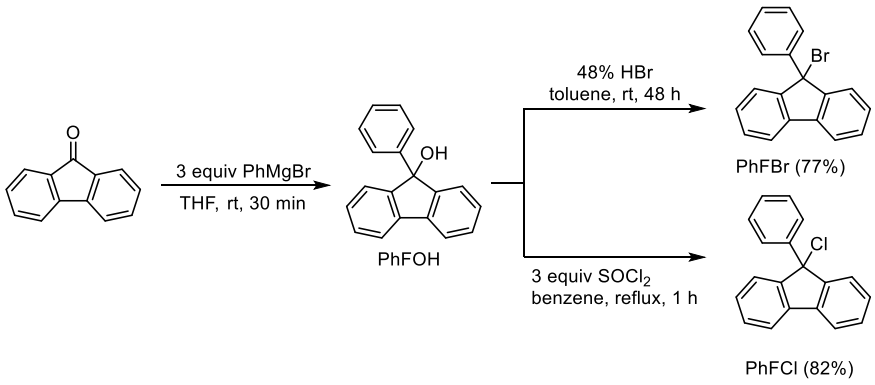
Scheme 3.4. Synthesis of PhF-protected Weinreb's amides using Rapoport's conditions.

3.1.2 — Research objective

The drawbacks of the literature method for introducing the PhF group are long reaction times, variable yields, limited substrate scope, toxic reagents, and specialized glassware. The primary objective of the work described in this chapter was to develop a method to install a 9-phenyl-9-fluorenyl protecting group which was superior to existing literature methods.

3.2 — Results and discussion.

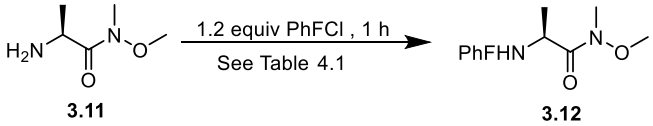
The reagent of choice for phenylfluorenation has been phenylfluorenyl bromide. This reagent is commercially available but is very expensive. It can be easily prepared from 9-fluorenone in two steps (Scheme 3.5), however, we decided to use the chloride instead for three reasons. First, we expected the chloride to be more stable to extended storage and less sensitive to hydrolysis. Second, the PhF group is similar to the trityl group is some respects, and the majority of tritylations use trityl chloride, so we hoped to have similar success with 176henylfluorenyl chloride (PhF-Cl). Finally, we found that the chloride was more easily prepared than the bromide. Both PhFBr and PhFCl are prepared from the corresponding alcohol. The bromide is prepared by treating the 176henylfluorenyl alcohol (PhFOH) with HBr for two days while the chlorination occurs after just one hour reflux with $SOCl_2$ (Scheme 3.5).



Scheme 3.5. Synthesis of PhFBr and PhFCl.

We began our studies using amine **3.11** as a model substrate, AgOTf to activate PhFCl and precipitate out the chloride ion, *N*-methylmorpholine (NMM) to sequester the acid produced in the reaction, and chloroform as solvent. Under these conditions, at – 20 °C, the corresponding PhF derivative **3.12** was obtained in 29% yield after 1 h (Table 1 entry 1). Performing the reaction at -78 °C in DCM resulted in a substantially increased yield (entry 2). As AgOTf is expensive we examined the much less expensive $AgNO_3$ as the activator. Using acetonitrile to help solubilize the silver nitrate and performing the reaction at 0 °C gave **3.12** in 92% yield (entry 3). Changing the base to triethylamine reduced the yield slightly and lead to increased unidentified side products (entry 4).

Table 3.1. Optimization of phenylfluorenation conditions

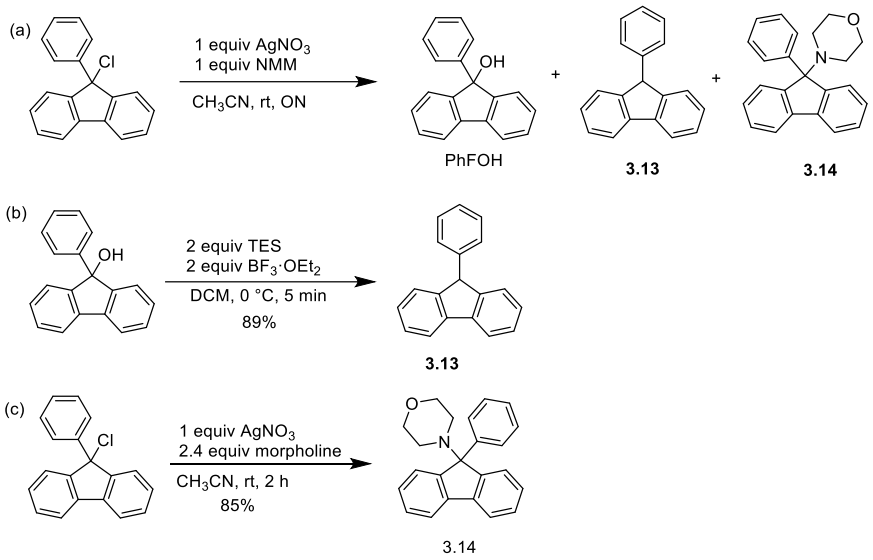


3.11 **3.12**

entry	Activator (1.2 equiv)	Base (1.2 equiv)	Solvent	Temp (°C)	Yield (%)
1	AgOTf	NMM	CH_3Cl	-20	29
2	AgOTf	NMM	DCM	-78	65
3	$AgNO_3$	NMM	CH_3CN	0	92
4	$AgNO_3$	Et_3N	CH_3CN	0	88

We noticed that small amounts of side products were formed in all of the above reactions including entry 3. In addition to the expected PhFOH, we isolated side products **3.13** and **3.14** which were identified by comparison to authentic samples (Scheme 3.6). These compounds were also produced in a blank reaction containing only PhFCl, $AgNO_3$ and NMM. Although the mechanism is unknown, **3.14** and **3.13** likely result from a radical process because if TEMPO is included in the reaction then **3.14** and **3.13** are not observed, although other unidentified side

products remain. A radical mechanism is not unexpected because radical processes are known to be initiated by silver nitrate.[153] During the synthesis of **3.13** (Scheme 3.6b) a red solution was briefly observed which we took to indicate formation of the PhF cation, and we recognized that this could be an alternative method for introducing the PhF group.

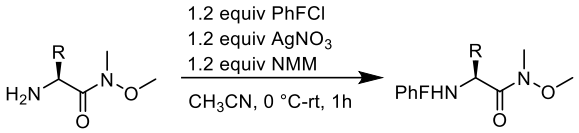


Scheme 3.6. Side products from the phenylfluorenation reaction.

3.2.1 — PhF protected α-amino aldehydes

With effective conditions in hand, we turned to our main target, protected amino aldehydes (Table 3.2). Good to excellent yields of PhF-protected Weinreb amides of amino acids were obtained and the PhF-protected products could easily be isolated by chromatography (Table 3.2). In most cases the amides could be easily reduced to the aldehydes using $LiAlH_4$ in excellent yields (Table 3.3). The aspartate derivative (entry 3) was obtained in a modest yield of 42% due to competing reduction of the *t*-Bu ester. The isoleucine derivative (entry 7) was obtained in 83% yield, but only after overnight treatment with 2 equivalents of $LiAlH_4$, presumably due to high steric hindrance of the bulky side chain.

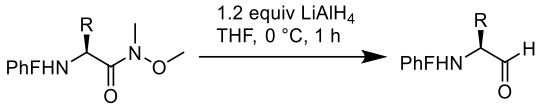
Table 3.2. Introduction of the PhF Group into Weinreb's Amides of Amino Acids.



Entry	Substrate	Product	Yield (%)
1[a]	H-Met-N(Me)OMe (**3.5**)	PhF-Met-N(Me)OMe (**3.8**)	87
2	H-Pro-N(Me)OMe (**3.6**)	PhF-Pro-N(Me)OMe (**3.9**)	70
3	H-Asp(OtBu)-N(Me)OMe (**3.7**)	PhF-Asp(OtBu)-N(Me)OMe (**3.10**)	82
4	H-Ala-N(Me)OMe (**3.11**)	PhF-Ala-N(Me)OMe (**3.12**)	92
5	H-Phe-N(Me)OMe (**3.15**)	PhF-Phe-N(Me)OMe (**3.18**)	84
6	H-Lys(Boc)-N(Me)OMe (**3.16**)	PhF-Lys(Boc)-N(Me)OMe (**3.19**)	76
7	H-Ile-N(Me)OMe (**3.17**)	PhF-Ile-N(Me)OMe (**3.20**)	86

[a]Tosylate salt and 2.4 equiv NMM were used.

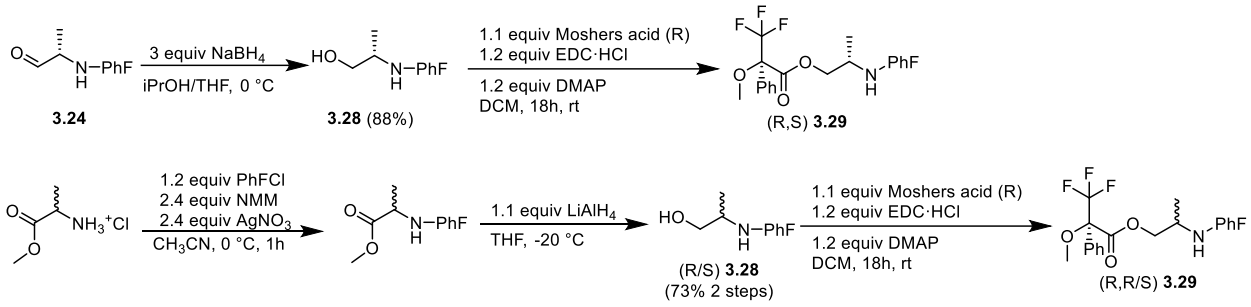
Table 3.3. Reduction of PhF-protected Weinreb's Amides of Amino Acids.



Entry	Substrate	Product	Yield (%)
1	PhF-Met-N(Me)OMe (**3.8**)	PhF-Met-H (**3.21**)	89
2	PhF-Pro-N(Me)OMe (**3.9**)	PhF-Pro-H (**3.22**)	87
3	PhF-Asp(OtBu)-N(Me)OMe (**3.10**)	PhF-Asp(OtBu)-H (**3.23**)	42
4	PhF-Ala-N(Me)OMe (**3.12**)	PhF-Ala-H (**3.24**)	89
5	PhF-Phe-N(Me)OMe (**3.18**)	PhF-Phe-H (**3.25**)	89
6	PhF-Lys(Boc)-N(Me)OMe (**3.19**)	PhF-Lys(Boc)-H (**3.26**)	92
7	PhF-Ile-N(Me)OMe (**3.20**)	PhF-Ile-H (**3.27**)	83[a,b]

[a]2 equiv of $LiAlH_4$ are used and the reaction is stirred overnight.
[b]Obtained as a single diastereomer.

To verify that the aldehydes were prepared without loss of stereochemical integrity we derivatized aldehyde **3.24** by reducing it to the alcohol **3.28** and forming Mosher's ester **3.29**. A mixture of **3.29** epimers was prepared from D/L alanine methyl ester hydrochloride (Scheme 3.7). ^{19}F NMR analysis of the Mosher's esters revealed that **3.29** was prepared as a single diastereomer from **3.24** which indicates that **3.24** was enantiomerically pure (Figure 3.3).



Scheme 3.7. Preparation of Mosher's esters.

(a) (b)

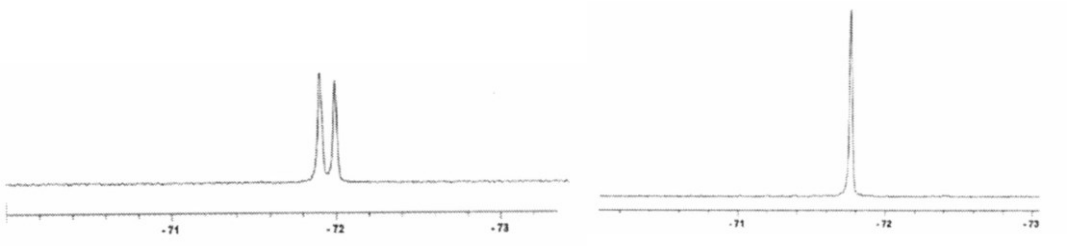
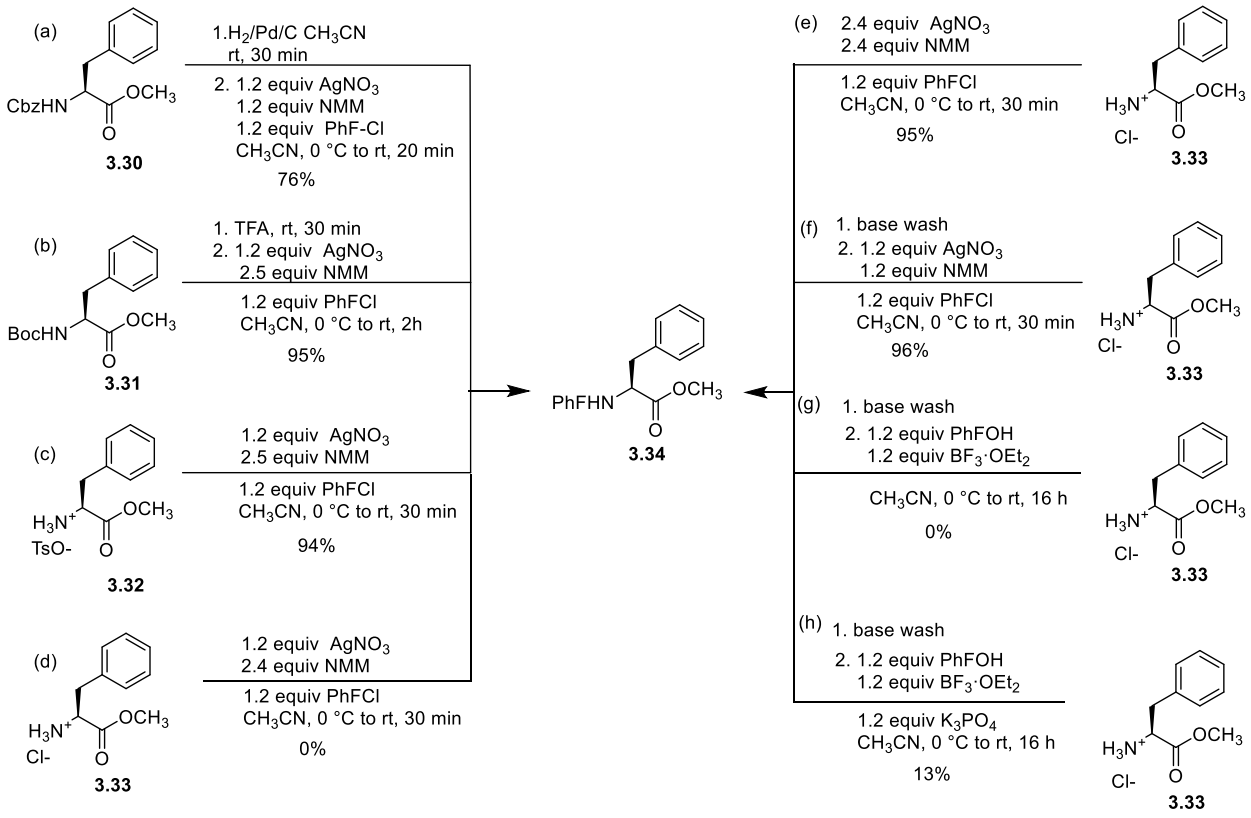


Figure 3.3. (a) ^{19}F NMR spectra of (R,R/S) **3.29** (b) ^{19}F NMR spectra of (R) **3.29**.

3.2.2 — Phenylfluorenation of amino acid methyl esters

Amino acid methyl esters are not available or stable as free amines but instead are stored as ammonium salts or N^{α}-protected compounds. Therefore, we decided to investigate protocols for the phenylfluorenation of phenylalanine methyl ester where the amine is obtained as a crude free amine or ammonium salt upon N^{α}-deprotection (Scheme 3.8) or from its commercially available hydrochloride or tosylate salts. PhF-Phe-OMe (**3.34**) was obtained in good yield in just

20 min from the crude free amine Phe-OMe obtained via hydrogenolysis of the Cbz group from Cbz-Phe-OMe (**3.30**) (Scheme 3.8a). The reaction with the TFA salt, generated after removal of the Boc group from Boc-Phe-OMe (**3.31**), was slightly slower than with the free amine and required 2.5 equiv NMM, but nevertheless proceeded in excellent yield (Scheme 3.8b). Using 2.5 equiv of NMM and our usual conditions, the tosylate salt **3.32** also proceeded in outstanding yield in just 30 min (Scheme 3.8c). Under our usual conditions, the chloride salt **3.33** did not react, even in the presence of 2.5 equiv NMM, possibly because the silver salt reacts with the hydrochloride salt faster than it reacts with PhFCl (Scheme 3.8d). However, if the equiv of $AgNO_3$ was increased to 2.4, then **3.34** was obtained in excellent yield after 30 min (Scheme 3.8e). Alternatively, converting the hydrochloride salt to the free amine via a base wash and then subjecting it to our usual conditions also gave **3.34** in excellent yield after 30 min (Scheme 3.8f). We also attempted the reaction by generating the PhF cation using PhFOH and $BF_3{\cdot}OEt_2$ (Scheme 3.8g). This was unsuccessful so the reaction was repeated in the presence of a base, NMM would form a complex with $BF_3{\cdot}OEt_2$ so we used K_3PO_4 instead (Scheme 3.8h). We reasoned that since K_3PO_4 is all but insoluble in acetonitrile it would not react with boron trifluoride to any significant extent; however, it should still react with the Brønsted acid byproduct and drive the reaction forward. This did not give the product in good yield and we believe the amine of Phe-OMe likely forms an unreactive complex with the boron trifluoride.

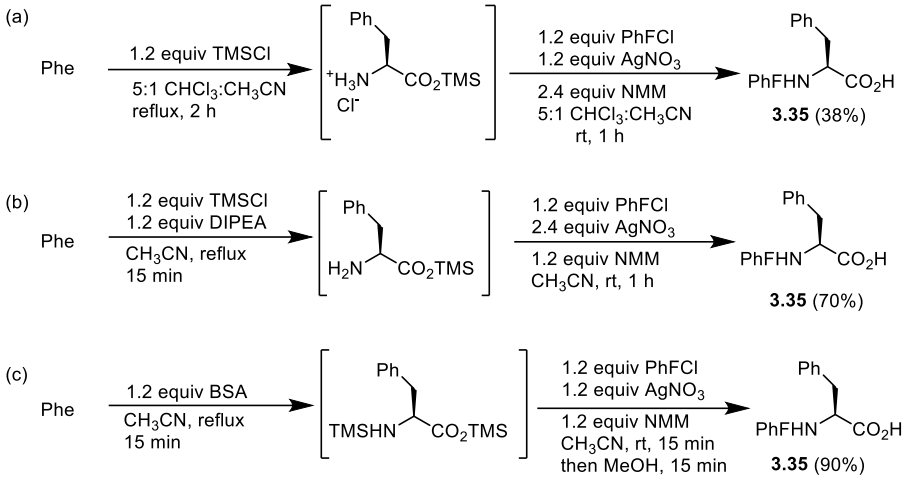


Scheme 3.8. Installation of the PhF Group into Phe-OMe.

3.2.3 — Phenylfluorenation of unprotected α-amino acids

Next, we turned to unprotected amino acids, and again phenylalanine served as a model substrate. The carboxylate requires temporary protection which can be accomplished by the *in situ* generation of a TMS ester which also serves to solubilize the substrate. First, we tried the conditions of Rapoport which involved refluxing a suspension of phenylalanine with TMS chloride in a mixture of chloroform and acetonitrile. This produces the hydrochloride salt of the TMS ester which, when subjected to our usual conditions, resulted in a low yield of the desired product **3.35** (Scheme 3.9 a). The yield was significantly improved by doubling the amount of silver nitrate and changing the solvent to acetonitrile, which improved the solubility of silver nitrate (Scheme 3.9 b). By changing the silylating agent to *N,O*-bis(TMS) acetamide (BSA) we

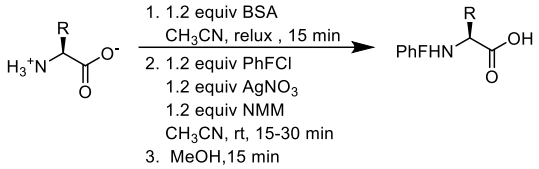
generated a neutral and halide free TMS ester which gave excellent yields of **3.35** under our normal conditions in just 15 minutes (Scheme 3.9 c).



Scheme 3.9. Phenylfluorenation of phenylalanine TMS ester.

We applied these conditions to several other amino acids (Table 3.4). The reaction proceeded rapidly to give high yields of the products in all cases except for tryptophan (entry 7) which gave a complex and strongly colored mixture. The products could be cleanly extracted into dilute base, acidified, and back extracted into DCM to give the desired products. Additional purification was not necessary except in the case of proline (**3.40**, entry 6) where minor impurities were carried into the DCM layer and were separated by flash chromatography.

Table 3.4. Phenylfluorenation of amino acids.



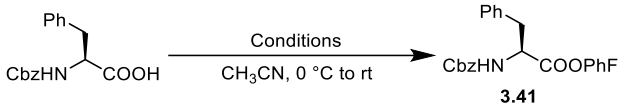
Entry	Amino acid	Yield (%)
1	Phe	90 (**3.35**)
2	Val	98 (**3.36**)
3	Met	99 (**3.37**)
4	Lys(Boc)OH	75 (**3.38**)
5	Asp(OtBu)OH·H_2O[a]	98 (**3.39**)
6	Pro	80 (**3.40**)
7	Trp[a]	0[b]

[a]2.4 equivalents of BSA were used.
[b]A complex mixture was formed.

3.2.4 — Phenylfluorenation of carboxylic acids

Encouraged by our success with amines we decided to try our conditions with other functional groups. The PhF group has usually been used for amine protection, but it has been used to protect carboxylic acids at least once in solution phase peptide synthesis[154] and occasionally as a linker in SPPS.[155] In these cases, PhF is useful due to its increased acid stability relative to the trityl group. We were pleased to find that our $AgNO_3$/PhFCl/NMM conditions gave ester **3.41** rapidly and in high yield from Cbz-Phe-OH (Table 3.5 entry 1). If the base was omitted, then the yield was reduced, and unidentified side products formed (entry 2). Perhaps because the product is not stable in the presence of one equivalent of nitric acid which is generated as a byproduct. No product was produced using $BF_3{\cdot}OEt_2$/PhFOH unless K_3PO_4 was added (entries 3 and 4).

Table 3.5. Phenylfluorenation of Cbz-Phe-OH.



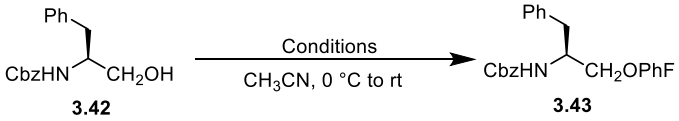
3.41

Entry	Activator (1.2 equiv)	Base (1.2 equiv)	PhF source (1.2 equiv)	Time (h)	Yield (%)
1	$AgNO_3$	NMM	PhFCl	1	84
2	$AgNO_3$	none	PhFCl	1	13
3	$BF_3{\cdot}OEt_2$	none	PhFOH	18	0
4	$BF_3{\cdot}OEt_2$	K_3PO_4	PhFOH	18	27

3.2.5 — Phenylfluorenation of alcohols

Next, we attempted the protection of primary alcohols using **3.42** as a model substrate. The primary alcohol was rapidly protected in excellent yield under our usual conditions to give **3.43** (Table 3.6)**.** Only a modest reduction in the yield occurred if the base was omitted (entry 2) suggesting that the PhF ether is more acid-stable than the related PhF ester **3.41**. Using $BF_3{\cdot}OEt_2$/PhFOH (entries 3 and 4), **3.43** was obtained in good yields, regardless of the presence or absence of base, although the reaction was slower without base.

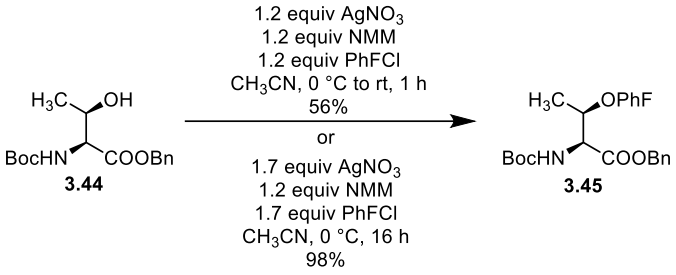
Table 3.6. Phenylfluorenation of a primary alcohol.



3.42 3.43

Entry	Activator (1.2 equiv)	Base (1.2 equiv)	PhF source (1.2 equiv)	Time (h)	Yield (%)
1	$AgNO_3$	NMM	PhFCl	1	93
2	$AgNO_3$	none	PhFCl	1	82
3	$BF_3{\cdot}OEt_2$	none	PhFOH	18	76
4	$BF_3{\cdot}OEt_2$	K_3PO_4	PhFOH	4	78

The secondary alcohol in **3.44** proved to be a more challenging substrate than the primary alcohol (**3.42**) and gave only 56% of the desired **3.45** under our standard conditions (Scheme 3.10). However, an excellent yield was obtained if the amount of $AgNO_3$ and PhFCl was increased slightly, and the reaction was stirred overnight.

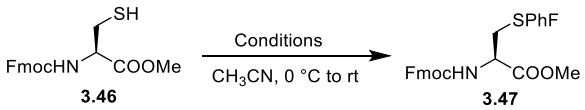


Scheme 3.10. Phenylfluorenation of a secondary alcohol.

3.2.6 — Phenylfluorenation of thiols

We expected that the reaction would be successful with thiols by analogy to alcohols; therefore, we were surprised that only 15% of **3.47** was obtained using our $AgNO_3$/PhFCl/NMM conditions and **3.46** (Table 3.7). We suspected that this was due to precipitation of a silver mercaptan. It is known that the combination of phenylfluorenyl chloride and silver nitrate results in the formation of 9-phenyl-9-fluorenyl nitrate, a reactive solid.[156] Therefore we prepared the PhF nitrate *ex situ*, filtered off the silver chloride byproduct, and repeated the phenylfluorenation of **3.46** with this solution. This led to a very good yield of **3.47** (entry 2). Using $BF_3{\cdot}OEt_2$/PhFOH, **3.47** was obtained rapidly and in excellent yield which was decreased slightly by the addition of base (entries 3 and 4).

Table 3.7. Phenylfluorenation of a thiol.

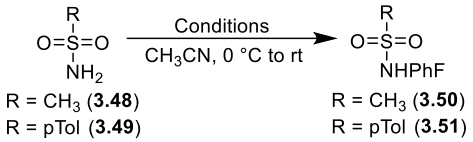


Entry	Activator (1.2 equiv)	Base (1.2 equiv)	PhF source (1.2 equiv)	Time (h)	Yield (%)
1	$AgNO_3$	NMM	PhFCl	1	15
2	none	none	$PhFNO_3$	1	86
3	$BF_3{\cdot}OEt_2$	none	PhFOH	1	95
4	$BF_3{\cdot}OEt_2$	K_3PO_4	PhFOH	1	80

3.2.7 — Phenylfluorenation of sulfonamides and amides

Trityl sulfonamides are rare and we are aware of only one example of a PhF sulfonamide.[157] When we applied our $AgNO_3$/PhFCl/NMM conditions to methane sulfonamide **3.48** and *p*-toluene sulfonamide **3.49** none of the desired products **3.50** and **3.51** were obtained (Table 3.8 entries 1 and 2). The crude mixtures contained several side products, chiefly compounds **3.13** and **3.14**. Under $BF_3{\cdot}OEt_2$/PhFOH conditions, sulfonamide **3.50** was obtained in a fair yield (entry 3) and both sulfonamides could be obtained in good yields if potassium phosphate was added (entries 4 and 5).

Table 3.8. Phenylfluorenation of sulfonamides.

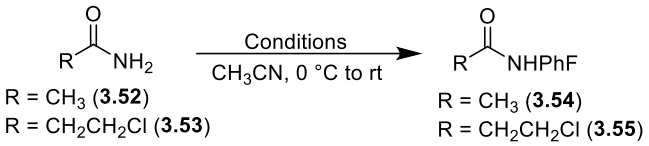


Entry	Substrate	Activator (1.2 equiv)	Base (1.2 equiv)	PhF source (1.2 equiv)	Time (h)	Yield (%)
1	**3.48**	$AgNO_3$	NMM	PhFCl	16	0[a]
2	**3.49**	$AgNO_3$	NMM	PhFCl	16	0[a]
3	**3.49**	$BF_3{\cdot}OEt_2$	none	PhFOH	16	55
4	**3.48**	$BF_3{\cdot}OEt_2$	K_3PO_4	PhFOH	16	84
5	**3.49**	$BF_3{\cdot}OEt_2$	K_3PO_4	PhFOH	16	78

[a]PhFOH and PhFH were observed as the major side products

While the trityl group is commonly used to protect amides, PhF amides are almost unheard of. PhF acetamide was first reported in 1937 but we are unaware of any more complex phenylfluorenyl amides.[136] We selected two simple aliphatic amides, acetamide (**3.52**) and 3-chloropropanamide (**3.53**), and these were unreactive under our $AgNO_3$/PhCl conditions (Table 3.9, entries 1 and 2). Using $BF_3{\cdot}OEt_2$ / PhFOH, the desired amides **3.54** and **3.55** were obtained in moderate yields (entries 3 and 4). When base was added only trace quantities of protected amides were observed (entries 5 and 6).

Table 3.9. Phenylfluorenation of amides.



R = CH_3 (**3.52**)
R = CH_2CH_2Cl (**3.53**)

R = CH_3 (**3.54**)
R = CH_2CH_2Cl (**3.55**)

Entry	substrate	Activator (1.2 equiv)	Base (1.2 equiv)	PhF source (1.2 equiv)	Time (h)	Yield (%)
1	**3.52**	$AgNO_3$	NMM	PhFCl	16	0
2	**3.53**	$AgNO_3$	NMM	PhFCl	16	0
3	**3.52**	$BF_3{\cdot}OEt_2$	none	PhFOH	16	45
4	**3.53**	$BF_3{\cdot}OEt_2$	none	PhFOH	16	44
5	**3.52**	$BF_3{\cdot}OEt_2$	K_3PO_4	PhFOH	16	trace
6	**3.53**	$BF_3{\cdot}OEt_2$	K_3PO_4	PhFOH	16	trace

The sulfonamides were protected in higher yields when a base was added while the amides showed the opposite trend. One explanation is that the acid byproduct formed catalyzes the reaction with amides, meanwhile sulfonamides are more acid labile and decompose under the acidic conditions (*vide infra*).

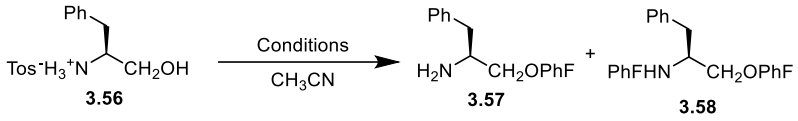
3.2.8 — Chemoselective phenylfluorenation

Having noticed differential reactivities in amines, alcohols, carboxylates, and thiols we next attempted to perform various phenylfluorenations chemoselectively. We chose a selection of substrates bearing two nucleophilic functional groups. In these experiments we used a small excess of the nucleophile, relative to the other reagents.

The primary alcohol in **3.56** could be protected in the presence of an ammonium salt to give **3.57** (Table 3.10, entry 1) using $AgNO_3$/PhFCl in the absence of a base; however, if a base was added than the doubly protected **3.58** was formed preferentially (entry 2). The alcohol could

also be selectively protected using $BF_3 \cdot OEt_2$/PhFOH conditions, although the reaction is slower, and the yield lower (entry 3).

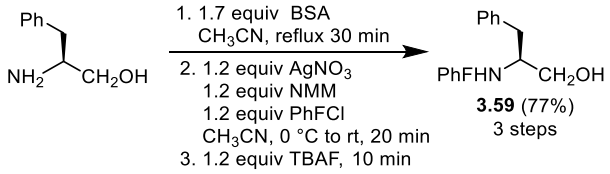
Table 3.10. Phenylfluorenation of an alcohol in the presence of an amine.



Entry	Activator (0.83 equiv)	Base	PhF source (0.83 equiv)	Time (h)	Temp (°C)	Yield **3.57** (%)	Yield **3.58** (%)
1	$AgNO_3$	None	PhFCl	1	0 - rt	80	0
2	$AgNO_3$	NMM[a]	PhFCl	4	-20	28	53
3	$BF_3 \cdot OEt_2$	none	PhFOH	16	0 - rt	69	0

[a]2.4 equiv

Amines could not be chemoselectively protected in the presence of alcohols and in order to perform this transformation the alcohol must be temporarily protected. By reacting phenylalanol with BSA to produce the TMS ether *in situ*, the amine could be protected followed by TMS deprotection with TBAF to give alcohol **3.59** in good yield over three steps in a one-pot process (Scheme 3.11). Alternatively, alcohol **3.59** could be readily obtained from **3.58** by selective removal of the PhF ether (*vide infra*).

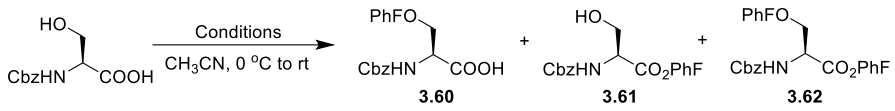


Scheme 3.11. PhF protection of an amine in the presence of an alcohol.

Carboxylic acids also require base for efficient protection so alcohols can be protected in their presence. For example, Cbz serine can be protected selectively at the alcohol to give ether **3.60** in a fair yield using $BF_3 \cdot OEt_2$/PhFOH or in good yields using $AgNO_3$/PhFCl without base

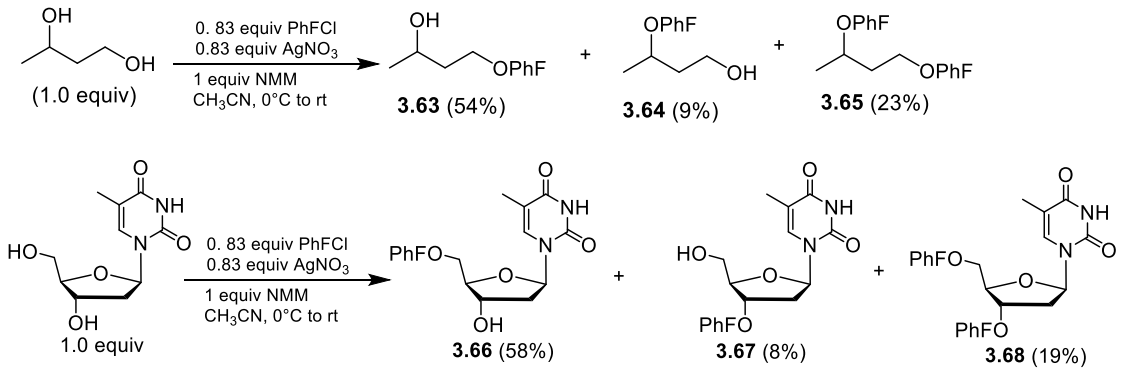
(entries 1 and 3, Table 3.11). On the other hand, if base is added then a moderate yield of the resulting ester **3.61** can be obtained, but this is contaminated with significant amounts of **3.60** and the doubly protected **3.62** (entry 2).

Table 3.11. Selective protection of Cbz Serine.



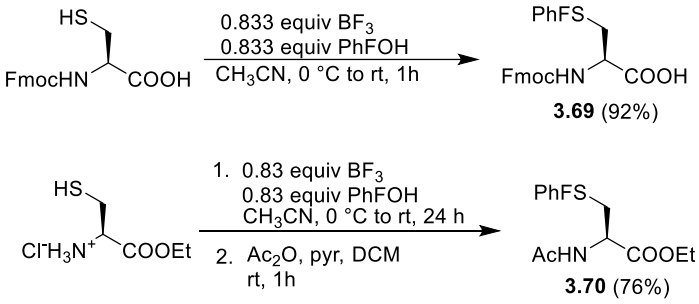
Entry	Activator (0.83 equiv)	Base (1 equiv)	PhF source (0.83 equiv)	Time (h)	Yield **3.60** (%)	Yield **3.61** (%)	Yield **3.62** (%)
1	$AgNO_3$	None	PhFCl	1	77	0	0
2	$AgNO_3$	NMM[a]	PhFCl	1	6	47	28
3	$BF_3{\cdot}OEt_2$	none	PhFOH	18	57	0	0

Next, we attempted to selectively protect primary alcohols in the presence of secondary alcohols (Scheme 4.12). We used 1,3-butanediol and thymidine as diols. Under our $AgNO_3$/PhFCl/NMM conditions, fair yields of the desired primary ethers **3.63** and **3.66** were obtained; however, the reactions also produced doubly-protected material (**3.65** and **3.68**) and secondary ethers (**3.64** and **3.67**). Almost no products could be obtained from the reactions using $BF_3{\cdot}OEt_2$/PhFOH, possibly because the diols form a bidentate complex with the boron trifluoride which prevents further reaction. Thymidine derivative **3.66** was previously reported by Hidehiko et al., however, their synthesis required harsher conditions (8 h reflux in pyridine) and provided **3.66** in only 37 % yield.[158]



Scheme 3.12. Chemoselective PhF protection of primary alcohols in the presence of secondary.

Thiols reacted much faster than the other protecting groups using $BF_3 \cdot OEt_2$/PhFOH; therefore, we attempted to selectively protect thiols under these conditions. We selected cysteine ethyl ester hydrochloride and Fmoc cysteine as model substrates (Scheme 3.13). The thiol of Fmoc-Cys-OH was readily protected in excellent yield in the presence of the carboxylic acid to give **3.69** in just 1 h. The thiol of the cysteine ethyl ester was also protected in good yield, but the reaction was slower, requiring 24 h, likely due to the poor solubility of the substrate. The resulting product was difficult to isolate so the free amine was acylated to give **3.70** which could be readily purified by flash chromatography.



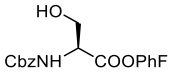
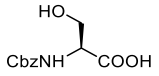
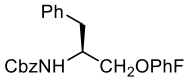
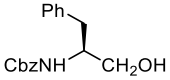
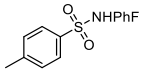
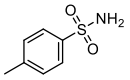
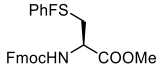
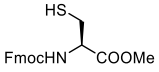
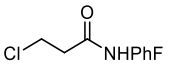
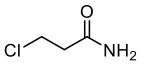
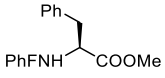
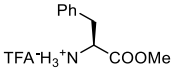
Scheme 3.13. Selective PhF protection of thiols.

3.2.9 — PhF deprotection.

There are a number of methods available for the deprotection of PhF amines (see introduction). Conditions for removing the PhF group from other functional groups have not

been established. We found that all of the functional groups examined could be deprotected under acidic conditions (Table 3.12). The ethers and esters were very acid labile and could be cleaved by 1% TFA/1% TIPS in DCM in a few minutes. The sulfonamides and thiols were more stable and were cleaved after 30 minutes in 10% TFA/1%TIPS, the thiol deprotection cocktail also included 1% ethanedithiol to prevent dimerization. Finally, the amines and amides were the most acid stable and were deprotected in 95% TFA/2.5% water/2.5% TIPS after 30 minutes. Therefore, the order of acid stability is amine ~ amide > sulfonamide ~ sulfide > ether ~ ester.

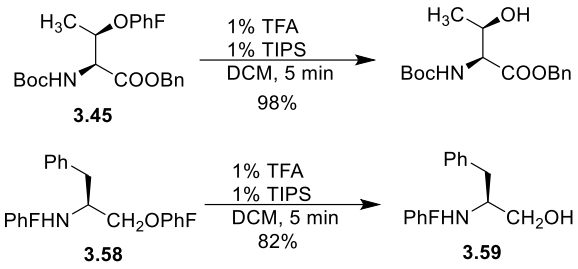
Table 3.12. Deprotection of PhF-protected functional groups.

Entry	Substrate	Deprotection cocktail	Time[a]	Product, Yield[b]
1	 **3.61**	1% TFA/1% TIPS, DCM	<10 min	 (86%)
2	 **3.43**	1% TFA/1% TIPS, DCM	<10 min	 **3.42** (93%)
3	 **3.51**	10% TFA/1% TIPS, DCM	30 min	 **3.49** (98%)
4	 **3.47**	10% TFA/1% TIPS, 1% EDT, DCM	30 min	 **3.46** (97%)
5[c]	 **3.55**	95% TFA,2.5% TIPS / 2.5% H_2O	30 min	 **3.53**
6[c]	 **3.34**	95% TFA, 2.5% TIPS / 2.5% H_2O	30 min	

[a]Time for complete dissaperance of the starting material as judged by TLC [b]Isolated yield. [c]Product was not isolated.

Due to their high acid lability, PhF ethers could be removed in the presence of other less sensitive protecting groups (Scheme 3.14). Treating ether **3.45** with 1% TFA/1% TIPS for 5

minutes in DCM cleanly removed the PhF ether without affecting the Boc group. Under the same conditions the PhF ether of compound **3.58** was removed while leaving the other PhF group (bound to the amine) intact.



Scheme 3.14. PhF ether deprotection in the presence of a PhF- or Boc-protected amine.

3.3 — Conclusions and future studies

In summary, we have introduced new conditions for the installation of a phenylfluorenyl group. We have shown that amines, alcohols, and acids are protected rapidly in high yields using $AgNO_3$/PhFCl/NMM. Furthermore, we have shown that thiols are protected rapidly in excellent yields using $BF_3 \cdot OEt_2$/PhFOH. These conditions also work for alcohols, sulfonamides and amides but the reaction is slower. These conditions are superior to the literature conditions which uses toxic $Pb(NO_3)_2$, requires very long reaction times, gives variable yields, and is limited in substrate scope. Our method allows for the chemoselective protection of alcohols or thiols in the presence of carboxylic acids or ammonium salts in excellent yields, and for protection of primary alcohols in the presence of secondary with fair selectivity. All of these functional groups can be deprotected under mild conditions with TFA and a cation scavenger, and ethers can be deprotected in the presence of moderately acid labile groups. We have demonstrated that protected α-amino aldehydes prepared with our method are configurationally stable and these served as building blocks for our work in Chapter 2. We believe that these protocols should provide ready access to PhF-protected functional groups which should encourage widespread

adoption of this very useful protecting group. In particular, new applications for this group include side chain protections of most amino acids, C-terminal protection in solution phase peptide synthesis, and use as a lipophilic tag for group assisted purification of very polar molecules.

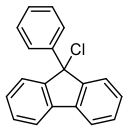
3.4 — Experimental

3.4.1. — General

All reagents and solvents were purchased from commercial suppliers and used without purification unless stated otherwise. Dimethylformamide (DMF) and acetonitrile were distilled from calcium hydride and stored over activated 4 Å molecular sieves. Tetrahydrofuran (THF) was distilled from sodium metal in the presence of benzophenone under nitrogen immediately before use. Dichloromethane was distilled from calcium hydride under nitrogen immediately before use. Benzene and toluene were distilled from sodium in the presence of benzophenone and stored over activated 4 Å molecular sieves. Potassium phosphate was dried by vigorous heating under vacuum with a Bunsen burner. Weinreb's amides were prepared from the coupling of methylmethoxy amine with Cbz protected α-amino acids using EDC HCl/HOBt in dichloromethane followed by hydrogenolysis using palladium on carbon in dry acetonitrile under a hydrogen atmosphere.

All $^{13}C\{^{1}H\}$ NMR spectra were proton decoupled. Chemical shifts (δ) for ^{1}H NMRspectra run in $CDCl_3$ are reported in ppm relative to the standard tetramethylsilane (TMS). Chemical shifts for $^{13}C\{^{1}H\}$ NMR spectra run in $CDCl_3$ are reported in ppm relative to the solvent residual carbon (δ 77.16 for central peak). The samples for high-resolution positive ion electrospray ionization mass spectrometry (HRMS-ESI^+) (ion trap) were prepared in 1:1 MeOH/H_2O + 0.1% formic acid.

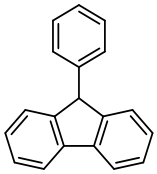
3.4.2. — Experimental procedures.



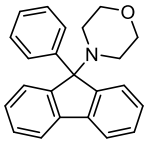
PhFCl

9-Chloro-9-phenylfluorene (PhFCl).[159] In a three necked flask equipped with an addition funnel, a condenser and a glass stopper was placed magnesium turnings (10.9 g, 450 mmol, 3 equiv) and iodine (381 mg, 1.50 mmol, 0.01 equiv). Bromobenzene (47 mL, 450 mmol, 3 equiv) was dissolved in THF (210 mL) and 25 mL of this solution was added and stirred 5 min until a gentle reflux was achieved, then the remaining solution was added dropwise over 1 h. When the addition was complete, the reaction was refluxed for an additional 1 h, until all of the magnesium turnings had reacted, and then cooled to 0 °C. 9-fluorenone (27.0 g, 150 mmol, 1 equiv) was dissolved in THF (120 mL) and added dropwise over 1 h. When the addition was complete the reaction was allowed to warm to rt and stirred until complete (30 min) as determined by TLC (5% ethyl acetate in hexanes). The mixture was poured into 1 L of ice/saturated ammonium chloride then extracted with dichloromethane (3 x 500 mL). The combined organic layers were washed with water (1 L), brine (1 L), then dried over magnesium sulfate, filtered, and concentrated to yield 9-phenyl-9-fluorenyl alcohol (PhFOH) as a white solid which was used without further purification. The alcohol was dissolved in benzene (450 mL) and thionyl chloride (32.6 mL, 450 mmol, 3 equiv) was added. The mixture was refluxed for 1 h then an aliquot was concentrated to dryness and dissolved in $CDCl_3$. $^{13}C\{^1H\}$ NMR determined that the reaction was complete based on the disappearance of the peak at 83 ppm and the appearance of a new peak at 75 ppm. The reaction was concentrated to a yellow solid which was suspended in hot pentane

and gravity filtered. The filtrate was allowed to cool and evaporate slowly to yield 34 g (82% yield) of PhFCl as yellow crystals over 4 crops. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.70 (d, 2H, *J* = 7.7 Hz), 7.49 -7.37 (m, 6H), 7.32-7.27 (m, 5H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 149.4, 141.3, 138.9, 129.3, 128.67, 128.4, 128.0, 126.6, 125.6, 120.4, 74.8 HRMS (ESI+) *m/z*: $[M - Cl]^+$ calcd for $C_{19}H_{13}$, 241.1012; found, 241.1013.



9-phenylfluorene (**3.13**). Prepared according to the general method of Vougioukalakis et al.[160] To a solution of PhFOH (516 mg, 2.00 mmol, 1 equiv) in dichloromethane (4 mL) cooled to 0 °C triethylsilane (637 µL, 4 mmol, 2 equiv) was added followed by $BF_3{\cdot}OEt_2$ (490 µL, 4 mmol, 2 equiv). The reaction was stirred until complete by TLC (5 min). The reaction was quenched with 5% sodium carbonate (10 mL) and extracted with dichloromethane (3 x10 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated to dryness. The residue was purified by flash chromatography using hexane which gave **3.13** as a white powder (484 mg, 89% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.89 (d, 2H, *J* = 7.5 Hz) 7.49-7.30 (m, 9H), 7.18 (d, 2H, *J* = 7.3 Hz), 5.14 (s, 1H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 148.0, 141.7, 141.1, 128.8, 128.4, 127.4, 126.9, 125.4, 119.9, 54.57; HRMS (ESI^+) *m/z*: $[M - H^-]^+$ calcd for $C_{19}H_{13}$, 241.1012; found, 241.1010.



N-(9-phenyl-9-fluorenyl)morpholine (**3.14**). PhFCl (276 mg, 1.00 mmol, 1 equiv) and morpholine (207 µL, 2.4 mmol, 2.4 equiv) were dissolved in acetonitrile (6 mL) and cooled to 0

°C. $AgNO_3$ (170 mg, 1.00 mmol, 1 equiv) was added as a solution in acetonitrile (4 mL) and the reaction was stirred for 2 h at ambient temperature. The mixture was filtered through a 3 cm silica plug and eluted with dichloromethane (50 mL) then concentrated to dryness. The residue was purified by flash chromatography using a gradient of 100% hexane to 10% ethyl acetate in hexane, which gave **3.14** as a white powder (279 mg, 85% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.73 (d, 2H, *J* = 7.4 Hz), 7.57 (m, 2H), 7.45-7.36 (m, 4H), 7.31-7.25 (m, 5H), 3.72 (t, 4H, *J* = 4.4 Hz), 2.50 (t, 4H, *J* = 4.0 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 146.6, 142.4, 140.7, 128.4, 128.2, 127.4, 127.3, 127.2, 126.2, 119.9, 78.1, 67.8, 48.1; HRMS (ESI+) *m/z*: calcd for $C_{23}H_{22}NO$, 328.1696; found, 328.1692.

General procedures for the phenylfluorenation of functional groups.

Unless otherwise specified, reactions were worked up by filtration through a short plug of silica (~5 cm for 1 mmol scale reactions) which was rinsed with ~50 mL of EtOAc. The eluent was concentrated under reduced pressure then purified by flash chromatography as specified.

General Procedure 3.1 for the phenylfluorenation of functional groups.

To a solution of acetonitrile at 0 °C containing substrate (0.166 M, 1 equiv), NMM (0.2 M, 1.2 equiv) and PhFCl (0.2 M, 1.2 equiv) a solution of silver nitrate (0.3 M, 1.2 equiv) in CH_3CN was added. The cooling bath was removed, and the white suspension was stirred until complete by TLC or a negative ninhydrin test (usually 1 h).

General procedure 3.2 for the phenylfluorenation of functional groups.

To a solution of acetonitrile at 0 °C containing substrate (0.166 M, 1 equiv), and PhFCl (0.2 M, 1.2 equiv) a solution of silver nitrate (0.3 M, 1.2 equiv) in CH_3CN was added. The cooling bath

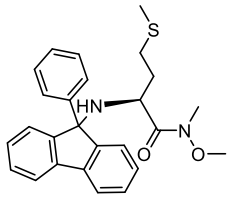
was removed, and the white suspension was stirred until complete by TLC or a negative ninhydrin test (usually 1 h).

General procedure 3.3 for the phenylfluorenation of functional groups.

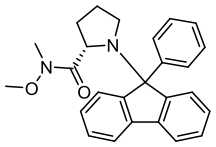
To a solution of acetonitrile at 0 °C containing substrate (0.1 M, 1 equiv) and PhFOH (0.12 M, 1.2 equiv) BF_3•OEt_2 (1.2 equiv) was added. The cooling bath was removed, and the reaction was stirred until complete or for 16 h.

General procedure 3.4 for the phenylfluorenation of functional groups.

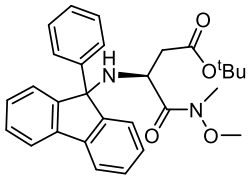
To a suspension of acetonitrile at 0 °C containing substrate (0.1 M, 1 equiv) PhFOH (0.12 M, 1 equiv), and K_3PO_4 (1.2 equiv) was added BF_3•OEt_2 (1.2 equiv). The cooling bath was removed, and the reaction was stirred until complete or for 16 h.



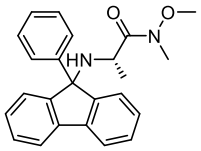
PhF-methionine methyl(methoxy)amide (**3.8**). According to General procedure 3.1 except that the tosylate salt of the methionine methyl(methoxy)amide (**3.5**) and 2.4 equiv of NMM were used. Obtained as a colorless oil 375 mg (87% yield) from (364 mg, 1.00 mmol) of **3.5**. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.68 (d, 2H, J = 7.2 Hz), 7.39 (m, 3H), 7.28 (m, 8H), 3.51 (br, 1H), 2.94 (d, 1H, J = 6.5 Hz), 2.85 (s, 3H), 2.83 (s, 3H), 2.67 (m, 1H), 2.45 (m, 1H), 1.95 (s, 3H), 1.53 (m, 2H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 176.1, 149.8, 148.9, 145.0, 141.4, 139.8, 128.3, 128.1, 128.1, 127.3, 127.1, 126.0, 125.8, 119.6, 119.5, 73.1, 60.3, 51.5, 34.2, 31.9, 30.9, 15.1; HRMS (ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{26}H_{29}N_2O_2S$, 433.1944; found, 433.1937.



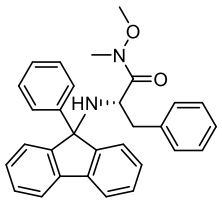
PhF-proline methyl(methoxy)amide (**3.9**). According to general procedure 3.1 **3.9** was obtained as a white amorphous solid (277 mg, 70% yield) from **3.6** (158 mg, 1.00 mmol). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.68 (d, 1H, *J* = 7.3 Hz), 7.60 (m, 4H), 7.44 (d, 1H, *J* = 7.4 Hz), 7.38 (t, 1H, *J* = 7.43 Hz), 7.31-7.13 (m, 8H), 3.67 (m, 1H), 3.28 (m, 1H), 2.90 (m, 7H), 1.93-1.59 (m, 4H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 177.3, 148.8, 148.0, 144.3, 141.1, 139.9, 128.3, 128.2, 128.1, 127.7, 127.6, 127.5, 127.1, 127.0, 126.6, 119.7, 119.5, 77.2, 60.3, 57.8, 50.3, 32.2, 31.9, 24.9; HRMS (ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{26}H_{27}O_2N_2$, 399.2067; found, 399.2078.



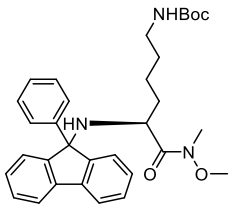
N^2-PhF-O^4-(t-butyl)aspartic acid methyl(methoxy)amide (**3.10**). According to General procedure 3.1 **3.10** was obtained as a white amorphous solid (389 mg, 82% yield) from **3.7** (232 mg, 1.00 mmol). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.64 (d, 1H, *J* = 6.9 Hz), 7.62 (d, 1H, *J* = 6.9 Hz), 7.41-7.13 (m, 11H), 3.33 (br, 1H), 2.96 (s, 3H), 2.75 (s, 3H), 2.33 (dd, 1H, *J* = 14.2, 8.2 Hz), 2.17 (dd, 1H, *J* = 3.3, 14.2 Hz), 1.45 (s, 9H), the NH proton was not observed; ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 169.9, 149.2, 148.6, 145.1, 141.4, 139.7, 128.3, 128.2, 128.0, 127.3, 127.1, 127.0, 126.2, 126, 119.5, 119.4, 80.3, 72.9, 60.4, 50.0, 41.7, 32.0, 28.2; HRMS (ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{29}H_{33}N_2O_4$, 473.2435; found, 473.2434.



PhF-alanine methyl(methoxy)amide (**3.12**). According to General procedure 3.1 **3.12** was obtained as a crystalline white solid (343 mg, 92% yield) from **3.11** (132 mg, 1.00 mmol). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.86 (d, 2H, *J* = 7.47 Hz), 7.48-7.42 (m, 3H), 7.35–7.17 (m, 8H), 3.59 (s, 1H), 2.86 (m, 7H), 1.08 (d, 3H, *J* = 7.05 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 177.1, 150.3, 149.4, 144.9, 141.2, 139.9, 128.23, 128.20, 128.10, 128.06, 127.5, 127.1, 126.8, 126.1, 125.4, 119.7, 119.6, 73.3, 60.3, 48.3, 31.9, 21.9; HRMS (ESI^{+}) *m/z*: [M + H]$^{+}$ calcd for $C_{24}H_{25}N_2O_2$, 373.1911; found, 373.1915.

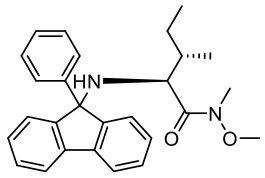


PhF-phenylalanine methyl(methoxy)amide (**3.18**). According to General procedure 3.1 **3.18** was obtained as an amorphous white solid (376 mg, 84% yield) from **3.15** (208 mg, 1.00 mmol). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.6 (d, 1H, *J* = 7.2 Hz), 7.54 (d, 1H, *J* = 7.5 Hz), 7.4-7.1 (m, 14H), 6.8 (t, 1H, *J* = 7.02 Hz), 6.5 (d, 1H, *J* = 7.2 Hz), 3.33 (br, 1H), 3.05 (d, 1H, *J* = 6.6 Hz), 2.87 (s, 3H), 2.84 (s, 3H), 2.6 (m, 2H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 176.1, 149.4, 149.3, 148.9, 145.4, 141.4, 139.4, 138.9, 128.2, 128.1, 128.0, 127.9, 127.7, 127.3, 127.0, 126.2, 125.5, 119.4, 119.0, 73.1, 60.3, 54.5, 41.4, 31.9; HRMS (ESI^{+}) *m/z*: [M + H]$^{+}$ calcd for $C_{30}H_{29}N_2O_2$, 449.2224; found, 449.2230.



N^{2}*-PhF-N*^{6}*-(boc)-lysine methyl(methoxy)amide* (**3.19**). According to General procedure 3.1 **3.19** was obtained as a white amorphous solid (407 mg, 76% yield) from **3.16** (289 mg, 1.00 mmol).

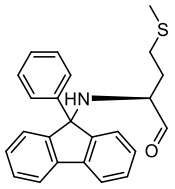
^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.61 (d, 1H, *J* = 6.9 Hz), 7.62 (d, 1H, *J* = 7.4 Hz), 7.41 (m, 3H), 7.31-7.17 (m, 8H), 4.41 (br, 1H), 3.44 (s, 1H), 3.01 (m, 2H), 2.81 (m, 7H), 1.43 to 1.13 (m, 15H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 176.6, 155.9, 149.9, 149.0, 145.01, 141.2, 139.8, 128.2, 128.0, 127.8, 126.0, 119.4, 78.8, 73.1, 60.1, 51.8, 40.4, 34.5, 31.8, 29.4, 28.4, 22.8; HRMS (ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{32}H_{40}N_3O_4$, 530.3013; found, 530.3039.



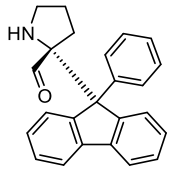
PhF-isoleucine methyl(methoxy)amide (**3.20**). According to General procedure 3.1 **3.20** was obtained as a colorless oil (356 mg, 86% yield) from **3.17** (174 mg, 1.00 mmol). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.55 (m, 2H), 7.35 (m, 3H), 7.3-7.1 (m, 8H), 3.25 (s, 1H), 2.77 (s, 3H), 2.71 (s, 1H), 1.6-1.15 (m, 4H), 0.70 (t, 3H, *J* = 7.36 Hz), 0.65 (d, 2H, *J* = 6.76 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz) δ:176.35, 149.6, 149.0, 145.7, 141.4, 139.8, 128.2, 128.04, 127.95, 127.7, 127.6, 127.0, 126.5, 126.1, 119.3, 73.1, 59.8, 56.4, 39.4, 31.7, 23.9, 16.3, 11.6; HRMS (ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{27}H_{31}O_2N_2$, 415.2380; found, 415.2381.

General procedure 3.5 for the reduction of PhF protected α-amino Weinreb amides.

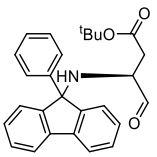
To a solution of Weinreb's amide in THF at a concentration of 0.1 M at -20 °C $LiAlH_4$ (2 M in THF, 1.1 equiv) was added dropwise. The reaction was stirred until complete by TLC (1 h) then was diluted with two volumes of cold diethyl ether and quenched with 50 µL of water followed by 150 µL of 1 M NaOH for ever 1 mmol of $LiAlH_4$ used. This was stirred for 15 minutes at 0 °C then dried over magnesium sulfate, filtered, and concentrated to dryness. The residue was purified by flash chromatography using a gradient of 0 to 20% ethyl acetate in hexane.



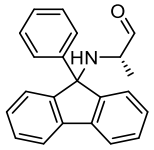
PhF-methioninal (**3.21**). 230 mg (89%) as a yellow oil was obtained from 300 mg (0.694 mmol) of **3.8** according to General procedure 3.5. ^{1}H NMR($CDCl_3$, 300 MHz) δ:9.31 (s, 1H), 7.70 (d, 2H, *J* = 7.5 Hz), 7.48 (m, 2H), 7.39-7.24 (m, 9H), 3.21 (br 1H), 2.76 (t, 1H, *J* = 5,6 Hz), 2.50 (m, 2H), 1.97 (s, 3H), 1.63 (m, 2H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 202.1, 149.2, 149.1, 144.4, 141.0, 140.2, 128.8, 128.6, 128.4, 128.1, 128.0, 127.4, 126.2, 126.1, 26.1, 125.5, 125.3, 120.1, 119.9, 72.9, 61.3, 30.9, 29.9, 15.4; HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd for $C_{24}H_{24}ONS$; 374.15731 found; 374.15753.



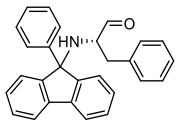
PhF-prolinal (**3.22**). 297 mg (87%) as a white solid as obtained from 398 mg (1 mmol) of **3.9** according to General procedure 3.5. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 9.14 (d, 1H, *J* = 4.3 Hz), 7.74 (d, 1H, *J* = 7.5 Hz), 7.63-7.39 (m, 6H), 7.35-7.2 (m, 5H), 7.13 (t, 1H, *J* = 7.5 Hz), 3.34 (m, 1H), 3.00 (m, 1H), 2.72 (m 1H), 1.82-1.6 (m, 4H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 201.7, 149.2, 145.7, 142.9, 142, 139.4, 129, 128.8, 128.5, 127.8, 127.7, 127.5, 127.4, 126.4, 125.9, 120.2, 120.1, 76.3, 66.3, 50.8, 28.4, 24.9 HRMS (ESI^+) *m/z*: $[M + MeOH + H]^+$ calcd for $C_{25}H_{26}NO_2$; 372.19581 found; 372.19510.



N^2-PhF-O^4-(t-butyl)aspartaldehyde (**3.23**). 112 mg (43%) as a white solid was obtained from 300 mg (0.63 mmol) of **3.10** according to General procedure 3.5. ^{1}H NMR($CDCl_3$, 500 MHz) δ: 946 (s, 1H), 7.72 (d, 2H, *J* = 7.4 Hz), 7.48-7.25 (m, 11H), 3.59 (s, 1H), 2.51 (dd, 1H, *J* = 15.9, 3.2 Hz), 2.09 (dd, 1H, *J* = 15.8, 5.8 Hz), 1.49 (3H, s); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz) δ:201.8, 170.4, 149.5, 149.1, 144.6, 141.1, 140.0, 128.8, 128.7, 128.4, 128.3, 128.2, 127.4, 126.2, 125.6, 125.3, 120.2, 120.0, 81.41, 72.9, 59.3, 38.2, 28.2; HRMS (ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{27}H_{28}O_3N$; 414.20637 found; 414.20665.

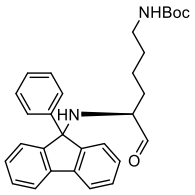


PhF-alaninal (**3.24**). 808 mg (89%) was obtained as a white solid from 1.08 g (2.9 mmol) of **3.12** according to General procedure 3.5. The NMR spectra were identical to literature data.[115]

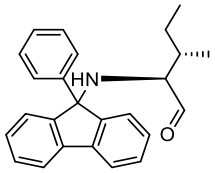


PhF-phenylalinal (**3.25**) 155 mg (89%) was obtained as a white solid from 200 mg (0.45 mmol) of **3.18** according to General procedure 3.5. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 9.26 (d, 1H, *J* = 2.2 Hz), 7.67 (m, 2H), 7.4-7.2 (m, 12H), 7.07 (t, 1H, *J* = 7.5 Hz), 6.96 (m, 2H), 6.63 (d, 1H, 7.6 Hz), 2.7-2.6 (m, 4H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 202.8, 148.6, 148.6, 144.2, 140.6, 140.6, 136.4, 129.5, 128.9, 128.7, 128.4, 128.3, 128.0, 127.8, 127.3, 126.9, 126.1, 125.7, 124.9, 120.0, 119.8, 72.8, 63.0, 37.7; HRMS (ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{28}H_{24}ON$; 390.18524 found;

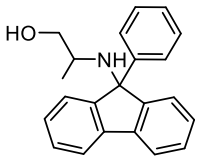
390.18591. This compound became discolored on standing and should be prepared immediately before use.



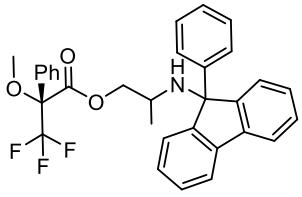
N^2-PhF-N^6-Boc-lysinal (**3.26**). 245 mg (92%) was obtained as a white solid from 300 mg (0.567 mmol) of **3.19** according to General procedure 3.5. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 9.20 (d, 1H, *J* = 1.8 Hz), 7.68 (d, 1H, *J* = 5.1 Hz), 7.65 (d, 1H, *J* = 5.2 Hz), 7.45 (m, 2H), 7.35-7.19 (m, 9H), 4.47 (br, 1H), 3.00 (m, 3H), 2.57 (m, 1H), 1.43 (s, 9H), 1.25 (m, 6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 203.3, 156.0, 149.3, 149.1, 144.5, 140.8, 140.5, 128.8, 128.5, 128.4, 128.0, 127.9, 127.4, 126.2, 125.6, 125.1, 120.1, 112.0, 79.1, 72.9, 61.6, 40.1, 30.8, 29.8, 22.1; HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd for $C_{30}H_{35}O_3N_2$; 471.2642 found; 471.2660.



PhF-isoleucinal (**3.27**). Prepared according to General procedure 3.5 except that 2 equiv of $LiAlH_4$ was used and the reaction was stirred overnight at 0 °C. 36 mg (83%) was obtained as a colorless oil from 50 mg of **3.20.** ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.31 (s, 1H), 7.65 (d, 2H, *J* = 7.3 Hz), 7.48 (m, 2H), 7.36-7.15 (m, 9H), 3.02 (br, 1H), 2.51 (m, 1H), 1.49 (m, 1H), 1.26 (m, 2H), 0.82 (d, 2H, *J* = 6.9 Hz), 0.65 (t, 3H, *J* = 7.4 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 204.6, 149.2, 149.0, 144.7, 140.8, 140.4, 128.6, 128.4, 128.3, 127.74, 127.70, 127.28, 126.2, 125.7, 125.6, 119.87, 119.77, 72.9, 65.3, 38.8, 25.3, 15.8, 11.6; HRMS-ESI+ (*m/z*) calcd for $C_{25}H_{26}ON^+$ $(M + H)^+$ 356.20170; found 356.20170.

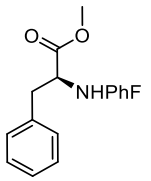


PhF-alanol (**3.28**). Was prepared as an enantiomerically pure sample from **3.24** according to the method of Lubell and Rapoport.[115] Additionally a racemic sample was prepared as follows. To a suspension of (DL)-alanine methyl ester hydrochloride (139 mg, 1.00 mmol, 1 equiv) in acetonitrile (6 mL) was added PhFCl (331 mg, 1.20 mmol, 1.2 equiv) and NMM (263 µL, 2.40 mmol, 2.4 equiv). The solution was cooled to 0 °C and $AgNO_3$ (408 mg, 2.40 mmol, 2.4 equiv) was added as a solution in acetonitrile (4 mL). The resulting suspension was stirred until complete as determined by a negative ninhydrin test (1 h), and then filtered through a short pad of silica and eluted with DCM. The mixture was concentrated to dryness and then dissolved in dry THF (10 mL) and cooled to -20 °C. $LiAlH_4$ (2 M in THF, 700 µL, 1.40 mmol, 1.4 equiv) was added dropwise and the reaction was stirred until complete by TLC (1 h). The reaction was diluted with diethyl ether (20 mL) and quenched by the addition of 150 µL of water then 450 µL of 1 M NaOH. The resulting suspension was stirred for 15 min then dried over magnesium sulfate, filtered, and concentrated to dryness. The residue was purified by flash chromatography using a gradient of 5% to 45% ethyl acetate in hexane, which gave (R/S) **3.28** (231 mg, 73 % yield). ^{1}H NMR of both samples was identical to literature data.[115]



Preparation of the Mosher's Esters of (L)- and (DL)-N-(9-phenyl-9-fluorenyl)phenylalanol (**3.29**). (S)-**3.28** (15 mg, 0.048 mmol, 1 equiv) 4-dimethylaminopyridine (9 mg, 0.08 mmol, 1.6 equiv) (R)-(+)-alpha-methoxy-alpha-(trifluoromethyl)phenylacetic acid (15 mg, 0.064 mmol, 1.4

equiv) and EDC HCl (14 mg, 0.075 mmol, 1.6 equiv) were dissolved in DCM (2 mL) and stirred at ambient temperature for 18 h. The mixture was then filtered through a short pad of silica and eluted with DCM. The filtrate with concentrated to dryness and used without further purification. ^{19}F NMR ($CDCl_3$, 282 MHz) δ : 71.77 (s, 3H). (R/S)-**3.29** was prepared in an identical manner from (R/S)-**3.28.** ^{19}F NMR ($CDCl_3$, 282 MHz) δ: 71.69 (s, 1.5H), 71.77 (s, 1.5H).



PhF-Phe-OMe (**3.34**).

Method i. Cbz-Phe-OMe (**3.30**, 313 mg, 1.00 mmol, 1 equiv) was dissolved in acetonitrile (6 mL) and Pd/C (10 wt.%, 30 mg) was added. The reaction was stirred under an atmosphere of hydrogen for 30 min until complete by TLC, then filtered through celite, the filtrate was subjected to General procedure 3.1.

Method ii. BocPheOMe (**3.31**, 279 mg, 1.00 mmol, 1 equiv) was dissolved in DCM (5 mL) and cooled to 0 °C. TFA (5 mL) was added and the reaction was stirred for 30 min until complete by TLC. The reaction was concentrated to dryness and the residue dissolved in water (10 mL) and then lyophilized to a white powder. The powder was dissolved in acetonitrile (6 mL) and PhFCl (331 mg, 1.20 mmol, 1.2 equiv) and NMM (262 µL, 2.4 mmol, 2.4 equiv) were added. The mixture was cooled to 0 °C and then a solution of $AgNO_3$ (204 mg, 1.20 mmol, 1.2 equiv) in acetonitrile (4 mL) was added. The cooling bath was removed, and the reaction was stirred until complete by ninhydrin test (2 h).

Method iii. Phenylalanine methyl ester tosylate (**3.32**, 351 mg, 1.00 mmol, 1 equiv) was subjected to General procedure 3.1 except that 2.5 equiv of NMM were used.

Method iv. Phenylalanine methyl ester hydrochloride (**3.33**, 215 mg, 1.00 mmol, 1 equiv) was subjected to General procedure 3.1 except that 2.4 equiv of NMM were used.

Method v. Phenylalanine methyl ester hydrochloride (**3.33**, 215 mg, 1.00 mmol, 1 equiv) was dissolved in acetonitrile (6 mL) and PhFCl (331 mg, 1.20 mmol, 1.2 equiv) and NMM (262 µL, 2.4 mmol, 2.4 equiv) were added. The solution was cooled to 0 °C and then a solution of $AgNO_3$ (408 mg, 2.4 mmol, 2.4 equiv) in acetonitrile (8 mL) was added. The cooling bath was removed, and the reaction was stirred 30 min until complete by ninhydrin test.

Method vi. Phenylalanine methyl ester hydrochloride (**3.33**, 215 mg, 1.00 mmol, 1 equiv) was dissolved in 5% aqueous sodium carbonate (20 mL) and extracted three times with dichloromethane (10 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. Residual water was removed by concentration from dry acetonitrile (10 mL). The residue was subjected to General procedure 3.1.

Method vii. Phenylalanine methyl ester hydrochloride (**3.33**, 215 mg, 1.00 mmol, 1 equiv) was washed as in **Method ii** then the residue was subjected to General procedure 3.3.

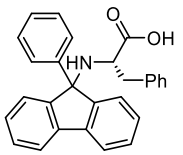
Method viii. Phenylalanine methyl ester hydrochloride (**3.33**, 215 mg, 1.00 mmol, 1 equiv) was washed as in **Method ii** then the residue was subjected to General procedure 3.4.

In all cases the residue was purified by flash chromatography using 2.5% ethyl acetate/97.5% hexane and silica gel which had been treated with hexane containing 1% triethylamine then rinsed with 2-3 column volumes of 2.5% ethyl acetate/97.5% hexane. **3.34** was obtained as a white amorphous solid. 76% (method i), 95% (method ii), 94% (method iii), 0% (method iv), 95% (method v), 96% (method vi), 0% (method vii), 13% (method viii). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.67 (d, 1H, J = 7.6 Hz), 7.63 (d, 1H, J = 7.6 Hz), 7.37-7.17 (m, 12H), 7.06 (m, 2H), 6.97 (t, 1H, J = 7.6 Hz), 6.65 (d, 1H, J = 7.5 Hz), 3.21 (s, 3H), 2.89-2.70 (m, 4H); ^{13}C{^{1}H}

NMR ($CDCl_3$, 75 MHz) δ: 176.2, 148.7, 148.5, 144.6, 141.0, 139.9, 137.6, 129.8, 128.3, 128.2, 128.1, 128.0, 127.8, 127.2, 127.1, 126.4, 126.3, 126.1, 125.1, 119.8, 119.7, 72.9, 57.6, 51.4, 41.4; HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd for $C_{29}H_{26}NO_2$, 420.1958; found, 420.1963.

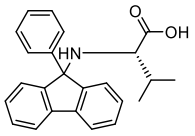
General procedure 3.6 for the synthesis of PhF protected amino acids.

To a mixture of the amino acid (1 mmol) in acetonitrile (2 mL) was added *N,O*-bis(trimethylsilyl)acetamide (BSA, 295 µL, 1.20 mmol, 1.2 equiv). The mixture was heated to reflux with stirring until a clear solution formed (generally 15 min). If necessary, an additional 1.2 equiv BSA was added and reflux continued until all the solids dissolved. The solution was diluted with acetonitrile (4 mL), cooled to 0 °C, then NMM (131 µL, 1.20 mmol, 1.2 equiv) and PhFCl (331 mg, 1.20 mmol, 1.2 equiv) were added followed by a solution of $AgNO_3$ (204 mg, 1.20 mmol, 1.2 equiv) in acetonitrile (4 mL). A white precipitate of AgCl formed immediately and the reaction was stirred at ambient temperature until complete as determined by a negative ninhydrin test, generally less than 30 min. Methanol (1 mL) was added and the reaction was stirred an additional 15 min. The precipitate was filtered off, the solution was diluted with diethyl ether (50 mL) then extracted with sodium hydroxide (0.1 N, 3 x 20 mL). The combined aqueous layers are acidified with citric acid (6 g). Sodium chloride (6 g) was added and then the mixture was extracted with dichloromethane (3 x 25 mL). The combined dichloromethane layers were dried over magnesium sulfate, filtered, and concentrated to yield pure product. Further purification was not necessary unless otherwise noted.

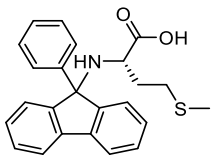


PhF-Phe-OH (**3.35**). Obtained as a white amorphous solid (365 mg, 90% yield) from phenylalanine (165 mg, 1.00 mmol) according to general procedure 3.6. ^1H NMR($CDCl_3$, 300

MHz) δ: 7.68 (d, 1H, *J* = 7.1 Hz), 7.66 (d, 1H, *J* = 7.1 Hz), 7.34 – 7.00 (m, 16H), 6.64 (d, 1H, *J* = 7.6 Hz), 2.88–2.78 (m, 2H), 2.67 (m, 1H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 177.4, 148.1, 147.0, 143.5, 140.6. 140.5, 136.2, 129.6, 128.9, 128.8, 128.5, 128.1, 127.9, 127.5, 127.1, 126.0, 125.9, 124.6, 120.2, 119.9, 72.7, 57.0, 39.6; HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd for $C_{28}H_{23}NO_2$, 406.1802; found, 406.1807.

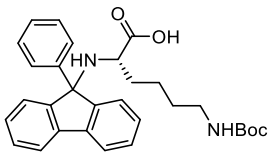


PhF-Val-OH (**3.36**). Obtained as a white powder (348 mg, 98% yield) from valine (117 mg, 1.00 mmol) according to general procedure 3.6. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.69 (d, 1H, *J* = 7.12 Hz), 7.68 (d, 1H, *J* = 7.28 Hz), 7.50 (m, 2H), 7.38-7.16 (m, 9H), 2.38 (d, 1H, *J* = 2.4 Hz), 1.80 (m, 1H), 1.00 (d, 3H, *J* = 6.7 Hz), 0.86 (d, 3H, *J* = 6.7 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 180.3, 148.5, 148.1, 144.5, 141.1, 140.4, 128.6, 128.5, 128.4, 127.8, 127.5, 127.4, 126.4, 126.2, 125.7, 120.0, 119.9, 72.8, 61.1, 32.6, 19.2, 18.7; HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd for $C_{24}H_{24}NO_2$, 358.1813; found, 358.1800.

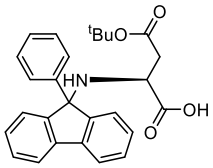


PhF-Met-OH (**3.37**). Obtained as a white amorphous solid (389 mg, 99% yield) from methionine (149 mg, 1.00 mmol) according to general procedure 3.6. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.90 (br, 2H), 7.70 (m, 1H), 7.45 (m, 3H), 7.38 (m, 2H), 7.30-7.18 (m, 6H), 2.81 (t, 1H, *J* = 6.0 Hz), 2.49 (m, 2H), 1.97 (s, 3H), 1.75 (m, 2H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 178.2, 147.6, 146.8, 143.1 140.9, 140.4, 129.0, 128.96, 128.5, 128.1, 127.8, 127.6, 126.05, 126.00, 125.4,

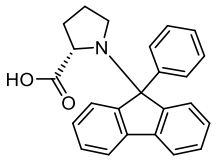
120.2, 120.1, 73.0, 55.4, 33.2, 30.3, 15.2; HRMS (ESI$^+$) *m/z*: [M + H]$^+$ calcd for $C_{24}H_{24}NO_2S$, 390.1533; found, 390.1521.



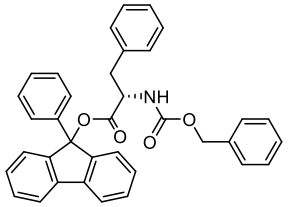
PhF-Lys(Boc)-OH (**3.38**). Obtained as a white amorphous solid (368 mg, 75% yield) from Boc-Lys-OH (246 mg, 1.00 mmol) according to general procedure 3.6. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.67 (m, 2H), 7.38-7.00 (m, 13 H), 5.55 and 4.54 (br, 1H), 2.98 (m, 2H), 2.58 (t, 1H, *J* = 5.3 Hz), 1.41–1.24 (m, 15H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 178.1, 156.2, 148.62, 147.5, 143.7, 140.8, 140.6, 128.8, 128.7, 128.5, 128.0, 127.8, 127.5, 126.1, 126.0, 125.2, 120.1, 120.0, 79.2, 73.0, 55.6, 40.0, 33.6, 29.6, 28.4, 22.2; HRMS (ESI) *m/z*: [M + H]$^+$ calcd for $C_{30}H_{35}N_2O_4$, 487.2602; found, 487.2607.



PhF-Asp(OtBu)-OH (**3.39**). Obtained as a white amorphous solid (420 mg, 98% yield) from Asp(tBu)OH (207 mg, 1.00 mmol) according to general procedure 3.6. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.73 (d, 1H, *J* = 7.59 Hz), 7.69 (d, 1H, *J* = 7.59 Hz), 7.45-7.20 (m, 11H), 2.80 (dd, 1H, *J* = 5.0, 3.3 Hz), 2.67 (dd, 1H, *J* = 3.3, 17.3 Hz), 1.89 (dd, 1H, *J* = 17.3, 5.0), 1.41 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 175.5, 170.8, 148.8, 147.2, 143.3, 140.6, 140.3, 129.1, 129.0, 128.6, 128.5, 128.2, 127.6, 125.8, 125.2, 124.8, 120.5, 120.3, 81.7, 72.5, 52.8, 37.4, 28.1; HRMS-ESI$^+$ *m/z*: calcd for $C_{27}H_{28}NO_4^+$ [M + H]$^+$ 430.2024., found 430.2017.

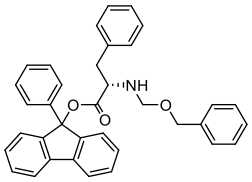


PhF-Pro-OH (**3.40**). Obtained as a white amorphous solid (285 mg, 80% yield) from proline (115 mg, 1.00 mmol) according to the General procedure 3.6 except that the reaction was stirred for 2 h and the crude product was purified by flash chromatography, using a gradient of 10% to 100% ethyl acetate in hexane. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 10.24 (br, 1H), 7.75 (d, 1H, *J* = 7.5 Hz), 7.64 (d, 1H, *J* = 7.64 Hz), 7.56 (d, 1H, *J* = 7.5 Hz), 7.49-7.15 (m, 11H), 3.41 (m, 1H), 3.24 (dd, 1H, *J* = 2.5, 9.1 Hz), 3.06 (dd, 1H, *J* = 8.3, 18.8 Hz), 1.95 (m, 1H), 1.7 (m, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 175.0, 146.1, 144.8, 141.6, 140.7, 139.4, 129.5, 129.2, 128.8, 128.2, 128.0, 127.0, 126.1, 125.9, 120.5, 120.2, 77.0, 62.7, 50.9, 31.1, 25.0; HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd for $C_{24}H_{22}NO_2$, 356.1656; found, 356.1646.

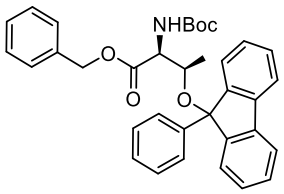


Cbz-Phe-OPhF (**3.41**). All reactions used 299 mg (1 mmol) of Cbz-Phe-OH as the substrate and were purified by flash chromotography using a gradient of 0 to 25% ethyl acetate in hexane. Methods i-iv correspond to entries 1 to 4 in Table 3.5. **Method i**: Using General procedure 3.1, 454 mg (84%) of **3.41** was obtained after 1 h. **Method ii**: Using General procedure 3.2, 74 mg (13%) of **3.41** was obtained after 1 h. **Method iii**: Using General procedure 3.3, no product was obtained after 18 h. **Method iv**: Using General procedure 3.4, 149 mg (27%) of **3.41** was obtained after 18h. The product was a white solid. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.70 (d, 1H, *J* = 7.6 Hz), 7.69 (d, 1H, *J* = 7.4 Hz), 7.4-7.05 (m, 21H), 5.06 (m, 3H), 4.76 (dd, 2H, *J* = 14.1, 6.3

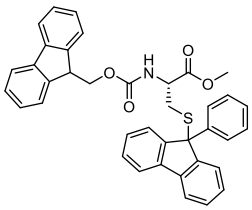
Hz), 3.1 (d, 2H, J =6.2 Hz); $^{13}C\{^{1}H\}$ NMR$\{^{1}H\}$($CDCl_3$, 75 MHz) δ: 169.2, 155.6, 146.1, 145.9, 140.6, 140.4, 136.2, 135.7, 129.4, 129.3, 129.3, 128.56, 128.54, 128.4, 128.2, 128.1, 128.0, 127.7, 127.0, 125.0, 124.6, 120.24, 120.21, 89.9, 66.8, 55.0, 38.2; HRMS (ESI^+) *m/z*: $[M + NH_4]^+$ calcd for $C_{36}H_{33}N_2O_4$, 557.2435; found, 557.2453.



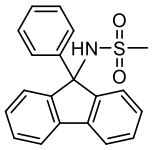
Cbz-O^1-PhF-phenylalanol (**3.43**). All reactions used 285 mg (1 mmol) of Cbz-phenylalanol (**3.42**) as the substrate and were purified by flash chromotography using a gradient of 10 to 25% ethyl acetate in hexane. Methods i-iv correspond to entries 1-4 in table 3.6 **Method i**: Using General procedure 3.1, 490 mg (93%) of **3.43**was obtained after 1 h. **Method ii:** Using General procedure 3.2, 428 mg (82%) of **3.43** was obtained after 1 h. **Method iii**: Using General procedure 3.3 400 mg (76%) of **3.43** was obtained after 18 h. **Method iv**: Using General procedure 3.4 412 mg (78%) of **3.43** was obtained after 4 h. The product was a white solid ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.70 (d, 1H, J = 7.6 Hz), 7.69 (d, 1H, J = 7.5 Hz), 7.43-7.22 (m, 21H), 5.21 (d, 1H, J = 8.8 Hz), 5.1 (s, 2H), 3.96 (br, 1H), 3.01 (m, 4H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 155.7, 146.9, 146.7, 143.3, 140.9, 140.6, 138.0, 136.7, 129.5, 129.3, 129.2, 128.57, 128.39, 128.28, 128.23, 128.11, 127.3, 126.4, 125.6, 125.3, 125.2, 120.1, 120.0, 88.6, 66.5, 63.3, 52.4, 38.2; HRMS (ESI^+) *m/z*: $[M + Na]^+$calcd for $C_{36}H_{31}NO_3Na^+$, 548.2196; found, 548.2211.



Boc-Thr(PhF)-OBn (**3.45**). **Method i**: Using General procedure 3.1, 306 mg (56%) of **3.45** was obtained after 1 h from 309 mg (1 mmol) of Boc-Thr-OBn (**3.44**). **Method ii**: To a solution of **3.44** (309 mg, 1.00 mmol, 1 equiv) in acetonitrile (6 mL) PhFCl (470 mg, 1.7 mmol, 1.7 equiv) and NMM (131 µL, 1.20 mmol, 1.2 equiv) were added. The solution was cooled to 0 °C and then a solution of $AgNO_3$ (289 mg, 1.7 mmol, 1.7 equiv) in acetonitrile (4 mL) was added. A white precipitate of AgCl formed immediately. The mixture was stirred for 16 h while slowly warming to ambient temperature. The reaction was filtered through a short plug of silica and eluted with ethyl acetate then concentrated to dryness. The residue was purified by flash chromatography using a gradient of 5% to 25% ethyl acetate in hexane to give **3.45** as a white solid (541 mg, 98% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.67 (m, 2H), 7.41-7.21 (m, 14H), 7.09 (t, 1H, *J* = 7.4 Hz), 6.97 (d, 1H, *J* = 7.4 Hz), 5.53 (d, 1H, *J* = 9.7 Hz), 5.22 (d, 1H, *J* = 12.3 Hz), 5.08 (d, 1H, *J* = 12.3 Hz), 4.20 (d, 1H, *J* = 9.7 Hz), 4.05 (m, 1H), 1.49 (s, 9H), 0.72 (d, 3H, *J* = 6.1 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 171.1, 156.2, 148.6, 145.8, 143.8, 140.5, 140.2, 135.3, 129.3, 129.2, 128.5, 128.4, 128.3, 128.15, 128.13, 128.12, 128.0, 127.1, 126.3, 126.2, 125.5, 120.1, 120.0, 88.0, 79.9, 71.0, 67.1, 59.7, 28.4, 19.5; HRMS (ESI$^+$) *m/z*: $[M + Na]^+$ calcd for $C_{35}H_{35}NNaO_5$, 572.2407; found, 572.2405.

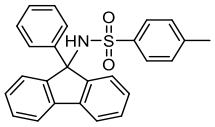


Fmoc-Cys(PhF)-OMe (**3.47**). All reactions were purified by flash chromotography using 0 to 40% ethyl acetate in hexane to give **3.47** as a white solid. Methods i to iv correspond to entries 1 to 4 in Table 3.7 **Method i**: Using General procedure 3.1, 53 mg (15%) of **3.47** was obtained from 165 mg (0.6 mmol) of Fmoc-Cys-OMe (**3.46**). **Method ii**: PhFCl (165 mg, 0.6 mmol, 1.2 equiv) was dissolved in acetonitrile (2.5 mL) and $AgNO_3$ (102 mg, 0.6 mmol, 1.2 equiv) in acetonitrile (2.5 mL) was added at 0 °C. The mixture was stirred for 10 minutes then the white precipitate was filtered off and the filtrate was added to a solution of **3.46** (178 mg, 0.5 mmol, 1 equiv) and NMM (66 µL, 0.6 mmol, 1.2 equiv) in acetonitrile (5 mL). The reaction was stirred at ambient temperature until complete by TLC (1 h). The mixture was filtered through a short silica plug and eluted with ethyl acetate then concentrated to dryness. flash chromotography yielded 255 mg (86%) of **3.47**. **Method iii**: Using General procedure 3.3, 286 mg (95%) of **3.47** was obtained after 1 h from 175 mg (0.5 mmol) of **3.46**. **Method iv**: Using General procedure 3.4, 236 mg, (80%) of **3.47** was obtained after 1 h from 175 mg (0.5 mmol) of **3.46**. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.77-7.67 (m, 4H), 7.59-7.21 (m, 17H), 5.17 (d, 1H, J = 8.1 Hz), 4.33-4.19 (m, 4H), 3.61 (s, 3H), 2.29 (d, 2H, J = 5.1 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 170.9, 155.6, 148.7, 144.0, 141.4, 141.2, 139.9, 139.7, 128.7, 128.49, 128.47, 128.44, 128.3, 127.8, 127.7, 127.2, 126.95, 125.62, 125.56, 125.3, 125.201, 120.20, 120.14, 120.07, 67.2, 63.5, 52.6, 47.2, 31.8; HRMS (ESI$^+$) *m/z*: $[M + Na]^+$ calcd for $C_{38}H_{31}NO_4NaS^+$, 620.1866; found, 620.1867.

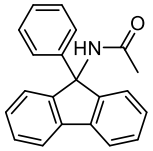


N-(9-phenyl-9-fluorenyl)methanesulfonamide (**3.50**). **Method i**: Using General procedure 3.4, 247 (74%) mg of **3.50** was obtained from 95 mg (1 mmol) of methanesulfonamide (**3.48**) as a crystalline white solid after 16 h reaction time and flash chromotography using 0 to 100% ethyl

acetate in hexane. **Method ii**: Using General procedure 3.1 no product was obtained. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.74 (d, 2H, *J* = 7.5 Hz), 7.49-7.26 (m, 11H), 5.38 (s, 1H), 2.46 (s, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 147.1, 142.3, 140.1, 129.5, 128.8, 128.4, 128.0, 125.9, 125.8, 120.6, 71.5, 42.9; HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd for $C_{20}H_{17}O_2NNaS$, 358.0872; found, 358.0882.

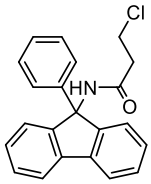


N-(9-phenyl-9-fluorenyl)toluenesulfonamide (**3.51**). All reactions used 171 mg (1.00 mmol) of p-toluene sulfonamide (**3.49**) as a substrate and were purified by flash chromotography using 5 to 25% ethyl acetate in hexane. **Method i**: no product was obtained using General procedure 3.1. **Method ii**: Using General procedure 3.3, 346 mg (84%) of **3.51** was obtained after 16 h. **Method iii**: Using General procedure 3.4, 190 mg (55%) of **3.51** was obtained after 16 h. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.40 (d, 2H, *J* = 7.6 Hz), 7.25-7.08 (m, 9H), 6.96 (m, 4H), 6.76 (d, 2H, 8.0 Hz), 5.9 (br, 1H), 2.19 (s, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 146.2, 142.8, 142.5, 140.1, 137.7, 128.8, 128.7, 128.6, 127.9, 127.7, 127.1, 126.0, 125.7, 119.8, 71.3, 21.5; HRMS (ESI^+) *m/z*: $[M + NH_4]^+$ calcd for $C_{26}H_{25}N_2O_2S$, 429.1631; found, 429.1636.

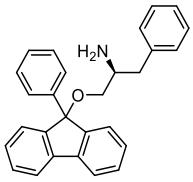


N-(9-phenyl-9-fluorenyl)acetamide (**3.54**). All reactions used 59 mg (1.00 mmol) of acetamide (**3.52**) as a substrate. **Method i**: No product was observed using general procedures 1 or 4. **Method ii**: Using General procedure 3.3, 139 mg (45%) of **3.54** was obtained as a white solid after 16 h reaction time and flash chromotography using 0 to 50% ethyl acetate in hexane. ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.71 (d, 2H, *J* = 7.46 Hz), 7.63 (d, 2H, *J* = 7.46 Hz), 7.39 (t, 2H, *J* =

7.31 Hz), 7.27 (m, 7H), 6.42 (s, 1H), 1.90 (s, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 169.8, 148.9, 141.4, 139.9, 128.8, 128.5, 128.3, 127.2, 125.8, 125.2, 120.0, 70.0, 23.9; HRMS-(ESI^+) *m/z*: $[M + Na]^+$ calcd for $C_{21}H_{17}ONNa$, 322.1202; found, 322.1209.

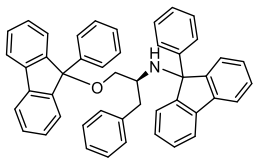


3-Chloro-N-(9-phenyl-9-fluorenyl)propanamide (**3.55**). All reactions used 107 mg (1.00 mmol) of 3-chloropropanamide (**3.53**) as a substrate. **Method i**: No product was observed using general procedures 1 or 4. **Method ii**: Using General procedure 3.3, 151 mg (44%) of **3.55** was obtained as a white powder after 16 h reaction time and flash chromotography using 0 to 50% ethyl acetate in hexane. ^{1}H NMR(DMSO-d_6, 300 MHz) δ: 8.87 (s, 1H), 7.84 (d, 2H, *J* = 7.5 Hz), 7.58 (d, 2H, *J* = 7.5 Hz), 7.39 (t, 2H, *J* = 7.5 Hz), 7.3-7.15 (m, 7H), 3.75 (t, 2H, *J* = 6.1 Hz), 2.71 (t, 2H, *J* = 6.2 Hz); $^{13}C\{^{1}H\}$ NMR (DMSO-d_6, 75 MHz) δ: 169.1, 148.9, 142.5, 139.3, 128.3, 128.1, 127.8, 126.7, 125.6, 125.0, 120.0, 69.3, 41.0, 38.2; HRMS (ESI^+) *m/z*: $[M + K]^+$ calcd for $C_{22}H_{18}ONClK$, 386.0709; found, 386.0723.



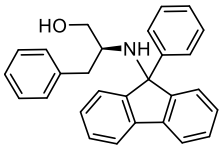
O^{1}*-(9-phenylfluoren-9-yl)phenylalanol* (**3.57**). **Method i**: Compound **3.56** (162 mg, 0.600 mmol, 1.2 equiv) was dissolved in minimal acetonitrile (8 mL) with gentle heating then cooled to 0 °C. PhFCl (138 mg, 0.500 mmol, 1 equiv) was added followed by a solution of $AgNO_3$ (85 mg, 0.50 mmol, 1 equiv) in acetonitrile (2 mL). The reaction warmed to room temperature and stirred for 1 h then concentrated in the presence of silica. The product was purified by flash

chromatography using ethyl acetate and silica gel which was previously equilibrated with 2% NH_4OH in ethyl acetate. Compound **3.57** was obtained as a white amorphous solid (156 mg, 80% yield). **Method ii**: Compound **3.56** (387 mg, 1.20 mmol, 1.2 equiv) was dissolved in acetonitrile (15 mL) with gentle heating. PhFOH (256 mg, 1.00 mmol, 1 equiv) was added followed by BF_3•OEt_2 (122 µL, 1.00 mmol, 1 equiv). The resulting solution was stirred for 18 h at room temperature. The mixture was diluted with ethyl acetate (30 mL) and washed with 5% Na_2CO_3 (30 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated to dryness. The residue was purified by flash chromatography which gave **3.57** as a white amorphous solid (269 mg, 69% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.67 (d, 2H, *J* = 6.9 Hz), 7.4-7.1 (m, 16H), 3.15 (br, 1H), 2.95 (m, 2H), 2.82 (m, 1H), 2.45 (m, 1H), 1.40 (s, 2H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 147.2, 147.1. 143.6, 140.8, 140.7, 139.2, 129.3, 129.1, 128.42, 128.31, 128.25, 128.19, 127.23, 126.2, 125.6, 125.43, 125.36, 120.04, 120.02, 88.5, 67.7, 52.9, 40.8 HRMS (ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{28}H_{26}NO$, 392.2009; found, 392.2013.



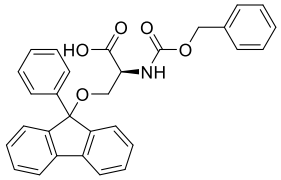
N,O^1-bis(9-phenyl-9-fluorenyl)phenylalanol (**3.58**). To a solution of **3.56** (387 mg, 1.20 mmol, 1.0 equiv) in acetonitrile (16 mL) PhFCl (276 mg, 1.00 mmol, 0.833 equiv) and NMM (316 µL, 2.88 mmol, 2.4 equiv) were added then cooled to -20 °C. $AgNO_3$ (170 mg, 1.00 mmol, 0.833 equiv) was added as a solution in acetonitrile (4 mL) and the reaction was stirred for 4 h. The mixture was filtered through a short plug of silica and eluted with ethyl acetate then concentrated to dryness. The residue was purified by flash chromatography, using a gradient of 0% to 30% ethyl acetate in hexane, to give **3.58** as a white solid (168 mg, 53% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.60 (m, 3H), 7.52 (d, 1H, *J* = 7.5 Hz), 7.38-7.01 (m, 23H), 6.86 (t, 1H, *J* = 7.4 Hz),

6.71 (m, 3H), 2.86 (dd, 1H, J = 13.0, 8.6 Hz), 2.49 (m, 4H), 2.10 (m, 1H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 150.4, 149.9, 147.6, 147.1, 145.5, 143.8, 140.9, 140.6, 140.4, 140.3, 139.9, 129.5, 129.0, 128.9, 128.3, 128.21, 128.18, 128.08, 128.06, 127.9, 127.7, 127.6, 127.2, 127.0, 126.2, 125.8, 125.7, 125.6, 125.5, 125.2, 125.1, 119.9, 119.7, 119.6, 88.3, 73.0, 65.1, 54.7, 40.9; HRMS (ESI^+) *m/z*: $[M + H]^+$ calcd for $C_{47}H_{38}NO$, 632.2959; found, 632.2940. 109 mg (28%) of compound **3.57** was also obtained from this reaction.



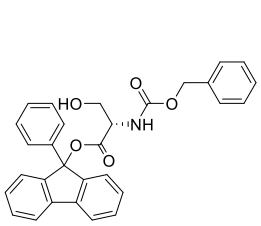
N-(9-phenyl-9-fluorenyl)phenylalanol (**3.59**). To a solution of Cbz-protected phenylalanol (285 mg, 1.00 mmol, 1 equiv) in MeOH (10 mL) 10 wt. % Pd/C (30 mg) was added. The mixture was stirred until complete by TLC (1 h) then filtered through a pad of Celite and concentrated to dryness. The residue was dissolved in acetonitrile (3 mL). BSA (414 µL, 1.70 mmol, 1.7 equiv) was added and the mixture was heated to reflux for 30 min. The solution was cooled to 0 °C and diluted with acetonitrile (4 mL). PhFCl (331 mg, 1.20 mmol, 1.2 equiv) and NMM (131 µL, 1.20 mmol, 1.2 equiv) were added followed by a solution of $AgNO_3$ (204 mg, 1.20 mmol, 1.2 equiv) in acetonitrile (4 mL). The reaction was stirred at room temperature until complete as determined by a negative ninhydrin test (20 min). The reaction was filtered through a short plug of silica and eluted with ethyl acetate and then concentrated to approximately 10 mL. Tetrabutylammonium fluoride (1.20 mL, 1 M in THF, 1.2 equiv) was added and the reaction stirred until the TMS group was completely removed by TLC (10 min). The reaction was diluted with ethyl acetate (10 mL) then washed with 5% sodium carbonate, (2 x 20 mL), and aq. hydrochloride acid (0.1 M, 20 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography using a gradient of 5% to 35% ethyl acetate in

hexane, to give **3.59** as a white foam (303 mg, 77% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.64 (d, 1H, *J* = 7.6 Hz), 7.58 (d, 1H, *J* = 7.5 Hz), 7.26 (m, 5H), 7.10 (m, 8H), 6.74 (m, 2H), 6.66 (d, 2H, *J* = 7.6 Hz), 2.99 (dd, 1H, *J* = 10.7, 2.9 Hz), 2.68 (dd, 1H, *J* = 3.7, 10.7 Hz), 2.56 (m, 2H), 2.48-2.18 (m, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 149.7, 149.5, 145.0, 141.0, 139.9, 138.8, 129.4, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.3, 126.3, 125.9, 125.2, 125.1, 120.1, 119.8, 72.8, 63.2, 55.7, 40.3; HRMS (ESI^{+}) *m/z*: $[M + H]^{+}$ calcd for $C_{28}H_{26}NO$, 392.2020; found, 392.2009.

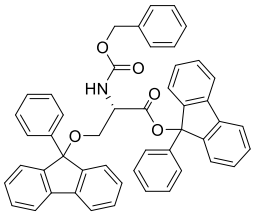


Cbz-Ser(OPhF)OH (**3.60**). **Method i**: Cbz-Ser-OH (239 mg, 1.00 mmol, 1 equiv) and PhFCl (230 mg, 0.833 mmol, 0.833 equiv) were dissolved in acetonitrile (6 mL) and cooled to 0 °C. A solution of $AgNO_3$ (141 mg, 0.833 mmol, 0.833 equiv) in acetonitrile (4 mL) was added. The reaction was stirred at room temperature for 1 h, and then filtered through a short plug of silica and eluted with ethyl acetate and concentrated. The residue was purified by flash chromatography, using dichloromethane then 1% acetic acid, 30% ethyl acetate, 69% dichloromethane, which gave **3.60** as a white foam (308 mg, 77% yield). **Method ii**: Cbz-Ser-OH (287 mg, 1.20 mmol, 1.2 equiv) was dissolved in acetonitrile (10 mL). PhFOH (256 mg, 1.00 mmol, 1 equiv) was added followed by BF_3•OEt_2 (122 μL, 1.00 mmol, 1 equiv). The resulting solution was stirred overnight at room temperature. The reaction was concentrated in the presence of silica and purified by flash chromatography which gave **3.60** as a white foam (271 mg, 57% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 9.89 (br, 1H), 7.71 (m, 2H), 7.40-7.18 (m, 16), 6.31 (minor rotamer, d, 0.2 H, *J* = 7.1 Hz), 5.80 (major rotamer, d, 0.8 H, *J* = 8.7 Hz), 5.15

(m, 2H), 4.48 (major rotamer, d, 0.8H, *J* = 8.6 Hz), 4.43 (minor rotamer, br, 0.2H), 3.53 (dd, 1H, *J* = 2.3, 8.9 Hz), 3.32 (dd, 1H, *J* =3.0, 9.4 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 175.8, 156.1, 146.1, 142.7, 141.0, 140.6, 136.2, 129.53, 129.47, 128.6, 128.52, 128.47, 128.3, 128.3, 128.2, 127.4, 125.5, 125.4, 125.3, 120.2, 120.1, 88.8, 67.3, 63.5, 54.4; HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd for $C_{30}H_{25}O_5NNa$, 502.1636; found, 502.1612.



3.61



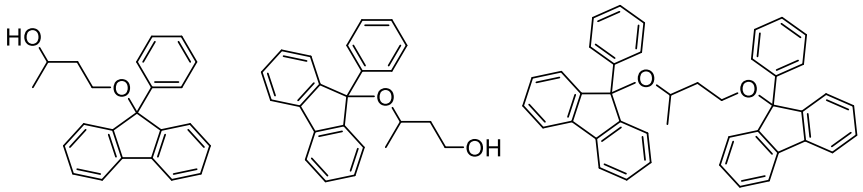
3.62

Cbz-Ser-OPhF (**3.61**) and *Cbz-Ser(PhF)-OPhF* (**3.62**). Cbz-Ser-OH (287 mg, 1.20 mmol, 1.0 equiv) NMM (131 µL,1.20 mmol, 1.0 equiv) and PhFCl (276 mg, 1.0 mmol, 0.833 equiv) were dissolved in acetonitrile (6 mL) and cooled to 0 °C. A solution of $AgNO_3$ (170 mg, 1.00 mmol, 0.833 equiv) in acetonitrile (4 mL) was added. The reaction was stirred for 2 h then filtered through a plug of silica and eluted with ethyl acetate then evaporated to dryness. The residue was subjected to flash chromatography, using a gradient of 0% to 100% ethyl acetate in hexane which separated **3.60**, **3.61**, and **3.62**.

3.60 was obtained as a white solid (29 mg, 6 % yield).

3.61 was obtained as a white amorphous solid (225 mg, 47% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.71 (d, 2H, *J* = 7.5 Hz), 7.32 (m, 16H), 5.68 (d, 1H, *J* =7.3 Hz), 5.09 (s, 2H), 4.52 (m, 1H), 4.04 (d, 1H, *J* = 9.2 Hz), 3.92 (d, 1H, *J* = 9.8 Hz), 2.42 (br, 1H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 168.2, 156.3, 146.1, 146.0, 140.8, 140.5, 140.4, 136.1, 129.4, 128.6, 128.5, 128.4, 128.2, 128.1, 127.9, 125.1, 124.7, 124.6, 120.2, 120.2, 90.1, 67.1, 63.4, 56.6; HRMS (ESI^+) *m/z*: $[M + NH_4]^+$ calcd for $C_{30}H_{29}O_5N_2Na$, 497.2082; found, 497.2064.

3.62 was obtained as a free flowing white solid (99 mg, 28% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.74 (d, 2H, *J* = 7.7 Hz), 7.68 (d, 2H, *J* = 7.5 Hz), 7.40-7.15 (m, 26H), 6.97 (d, 1H, *J* = 7.4 Hz), 5.64 (major rotamer, d, 0.9H, *J* = 8.6 Hz), 5.46 (minor rotamer, d, 0.1H, *J* = 7.0 Hz), 5.06 (m, 2H), 4.46 (major rotamer, d, 0.9H, *J* = 8.6 Hz), 4.26 (minor rotamer, d, 0.1H, *J* = 6.4 Hz), 3.70 (major rotamer, d, 0.9H, *J* = 8.1 Hz), 3.62 (minor rotamer, d, 0.1H, *J* = 9.5 Hz), 3.20 (d, 1H, *J* = 9.0 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 168.0, 155.7, 146.29, 146.26, 146.2, 146.1, 142.5, 140.9, 140.8, 140.7, 140.5, 140.3, 136.3, 129.5, 129.4, 129.3, 128.53, 128.49, 128.33, 128.27, 128.1, 128.1, 128.0, 127.7, 127.2, 125.6, 125.4, 125.1, 125.1, 120.1, 120.1, 120.0, 90.1, 88.6, 66.9, 63.8, 54.8; HRMS (ESI^{+}) *m/z*: [M + K]$^{+}$ calcd for $C_{49}H_{37}O_5NK^{+}$, 758.2314; found, 758.2290.



3.63 **3.64** **3.65**

Phenylfluorenation of 1,3-butanediol (**3.63**, **3.64** and **3.65**). **Method i**: PhFCl (276 mg, 1.00 mmol, 1 equiv) was dissolved in acetonitrile (6 mL) and cooled to 0 °C. NMM (110 µL, 1.00 mmol, 1 equiv) and 1,3-butane diol (107 µL, 1.20 mmol, 1.2 equiv) were added, followed by a solution of $AgNO_3$ (170 mg, 1.00 mmol, 1 equiv) in acetonitrile (4 mL). A white precipitate formed immediately. The cooling bath was removed and the reaction was stirred for 1 h at rt. The mixture was then filtered through a short plug of silica and eluted with dichloromethane. The mixture was subjected to flash chromatography using a gradient of 0 to 25% ethyl acetate in hexane.

3.63 (R_f = 0.15 in 10% ethyl acetate/90% hexane) was obtained as a colorless oil (179 mg, 54%). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.57 (d, 2H, *J* = 7.5 Hz), 7.29-7.07 (m, 11H), 3.87 (br,

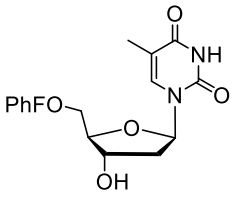
1H), 3.16–3.01 (m, 2H), 2.95 (s, 1H), 1.65-1.42 (m, 2H), 1.04 (d, 3H, J = 6.1 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 147.0, 146.9, 143.2, 140.9, 140.7, 129.23, 129.21, 128.39, 128.30, 128.28, 127.3, 125.5, 125.3, 125.2, 120.1, 120.0, 89.0, 67.5, 62.2, 38.4, 23.2 HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd for $C_{23}H_{22}O_2Na$, 353.1512; found, 353.1533.

3.64 (R_f = 0.1, 10% ethyl acetate/90% hexane) was obtained as a colorless oil (30 mg, 9%). ^1H NMR($CDCl_3$, 300 MHz) δ: 7.61 (m, 2H), 7.35-7.10 (m, 11H), 3.74 (m, 1H), 3.53 (m, 1H), 3.37 (sextet, 1H, J = 5.9 Hz), 2.22 (br, 1H), 1.52 (m, 2H), 0.89 (d, 3H, J = 6.3 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ: 148.3, 147.4, 143.9, 141.3, 140.4, 129.4, 129.1, 128.3, 128.2, 128.1, 127.3, 126.4, 126.12, 125.6, 120.2, 120.1, 88.7, 69.8, 60.1, 39.8, 21.9 HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd for $C_{23}H_{22}O_2Na$, 353.1512; found, 353.1515.

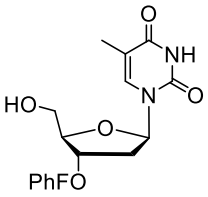
3.65 (R_f = 0.6, 10% ethyl acetate/90% hexane) was obtained as a white solid (66 mg, 23%). ^1H NMR($CDCl_3$, 300 MHz) δ: 7.57 (m, 4H), 7.3-6.9 (m, 22H), 3.22 (sextet, 1H, J = 6.2 Hz), 2.85 (dd, 2H, J = 5.8, 7.3 Hz), 1.67 (m, 1H), 1.37 (m, 1H), 0.60 (d, 3H, J = 6.2 Hz); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 75 MHz) δ:148.5, 147.8, 147.66, 147.6, 144.2, 143.6, 140.75, 140.73, 140.70, 128.91, 128.87, 128.78, 128.13 128.05, 128.02, 127.95, 127.85, 126.93, 126.90, 126.3, 126.0, 125.7, 125.6, 125.3, 125.2, 119.9, 119.8, 88.38, 88.36, 68.1, 60.6, 38.7, 21.7; HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd for $C_{42}H_{34}O_2Na$, 593.2451; found, 593.2458.

Method ii: 1,3-butane diol (129 μL, 1.44 mmol, 1.2 equiv) was dissolved in acetonitrile (10 mL) and cooled to 0 °C. PhFOH (309 mg, 1.20 mmol, 1 equiv) and K_3PO_4 (256 mg, 1.20 mmol, 1 equiv) were added followed by BF_3•OEt_2 (142 μL, 1.20 mmol, 1 equiv). The resulting suspension was stirred for 18 h at room temperature. The reaction was concentrated in the presence of silica and purified by flash chromatography. **3.64** was obtained as a colorless oil (9

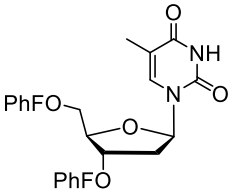
mg, 2% yield), **3.63** was obtained as a colorless oil (104 mg, 26% yield). **3.65** was obtained as a white solid (15 mg, 4% yield).



3.66



3.67



3.68

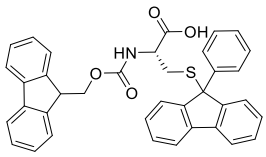
Phenylfluorenation of thymidine (**3.66**, **3.67**, **3.68**). Thymidine (290 mg, 1.20 mmol, 1.2 equiv) was dissolved in DMF (6 mL) and cooled to 0 °C. PhFCl (276 mg, 1.00 mmol, 1 equiv), NMM (110 μL, 1.00 mmol, 1 equiv) and acetonitrile (6 mL) were added. A solution of $AgNO_3$ in acetonitrile (4 mL) was added dropwise and the reaction stirred at ambient temperature. After 4 h a white precipitate of AgCl was filtered off and the reaction was concentrated to dryness. The residue was subjected to flash chromatography using a gradient of 10 to 80% ethyl acetate in hexane.

3.66 (R_f = 0.2, 50% ethyl acetate/50% hexane) was obtained as a white solid (281 mg, 58% yield). ^{1}H NMR($CDCl_3$, 500 MHz) δ: 7.85 (s, 1H), 7.75 (d, 1H, *J* = 7.4 Hz), 7.70 (d, 1H, *J* = 7.4 Hz), 7.45 (t, 1H, *J* = 7.2 Hz), 7.35–7.20 (m, 10H), 6.49 (t, 1H, *J* = 6.4 Hz), 4.61 (s, 1H), 4.00 (s, 1H), 3.36 (d, 1H, *J* = 8.7), 3.18 (d, 1H, *J* = 9.0 Hz), 2.47 (m, 2H), 1.48 (s, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz) δ: 164.4, 151.0, 146.4, 145.8, 143.0, 141.2, 140.3, 136.2, 129.7, 129.4, 128.6, 128.5, 128.4, 127.7, 125.5, 125.4, 124.8, 120.3, 111.3, 89.2, 86.5, 85.1, 72.9, 63.6, 41.2, 11.8; HRMS (ESI$^+$) *m/z*: $[M + Na]^+$ calcd for $C_{29}H_{26}N_2NaO_5$, 505.1734; found, 505.1728.

3.67 (R_f = 0.25, 50% ethyl acetate/50% hexane) was obtained as a white solid (38 mg, 8% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 8.58 (s, 1H), 7.68 (m, 2H), 7.4-7.24 (m, 12H), 7.08 (s, 1H), 6.20 (t, 1H, *J* = 6.9 Hz), 4.04 (d, 1H, *J* = 2.5 Hz), 3.70 (m, 1H), 3.51 (d, 1H, *J* = 11.8 Hz),

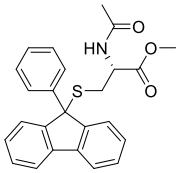
3.04 (d, 1H, J = 11.4 Hz), 2.16 (ddd, 1H, J = 13.6, 6.0, 2.9 Hz), 1.77 (s, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz) δ: 163.5, 150.2, 147.1, 147.0, 142.4, 141.0, 140.6, 136.4, 129.7, 129.6, 128.5, 128.3, 128.2, 127.0, 125.9, 125.7, 125.6, 120.3, 120.2, 111.0, 89.0, 86.3, 86.2, 73.0, 61.8, 39.3, 12.4; (HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd for $C_{29}H_{26}N_2NaO_5$, 505.1734; found, 505.1737.

3.68 (R_f = 0.6, 50% ethyl acetate/50% hexane) was obtained as a white solid (66 mg, 19%). ^1H NMR($CDCl_3$, 500 MHz) δ: 8.66 (s, 1H), 7.78 (d, 1H, J = 7.6 Hz), 7.70 (d, 1H, J = 7.6 Hz), 7.67 (d, 1H, J = 7.6 Hz), 7.59 (d, 1H, J = 7.5 Hz), 7.56 (s, 1H), 4.79 (m, 1H), 7.42 (m, 2H), 7.36-7.05 (m, 21H), 6.95 (d, 1H, J = 7.6 Hz), 6.40 (dd, 1H, J = 5.9, 8.3 Hz), 4.03 (d, 1H, J = 1.9 Hz), 3.89 (d, 1H, J = 6.2 Hz), 3.00 (dd, 1H, J = 10.3, 1.8 Hz), 2.39 (dd, 1H, J = 10.3, 2.0 Hz), 2.34 (dd, 1H, J = 13.6, 5.7 Hz), 1.87 (m, 1H), 1.38 (s, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz) δ: 163.7, 150.3, 147.1, 146.9, 146.1, 145.7, 143.0, 142.5, 141.1, 140.6, 140.5, 134.0, 135.8, 129.54, 129.51, 129.3, 129.0, 128.6, 128.5, 128.4, 128.3, 128.23, 128.18, 127.6, 127.0 126.0, 125.6, 125.3, 124.5, 120.2, 120.12, 120.10, 111.1, 89.1, 88.8, 85.8, 84.9, 74.4, 62.9, 40.4, 11.5; HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd for $C_{48}H_{38}N_2NaO_5$, 745.2673; found, 745.2642.



FmocCys(PhF)OH (**3.69**)**.** FmocCysOH (230 mg, 0.671 mmol, 1.2 equiv) was dissolved in acetonitrile (10 mL) and PhFOH (142 mg, 0.554 mmol, 1 equiv) was added. The solution was cooled to 0 °C and BF_3•OEt_2 (68 µL, 0.56 mmol, 1 equiv) was added dropwise. The resulting solution was stirred at 0 °C until complete by TLC (1 h). The mixture was diluted with ethyl acetate (30 mL) then washed twice with 10% citric acid (20 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated to dryness. The residue was purified by flash chromatography using 100% hexane followed by 70% ethyl acetate/29% hexane/1% AcOH.

Compound **3.69** was obtained as a white solid (358 mg, 92% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.76 (d, 2H, *J* = 7.2 Hz), 7.67 (m, 2H), 7.59 (d, 2H, *J* = 6.9 Hz), 7.5-7.1 (m, 15H), 5.08 (d, 1H, *J* = 7.6 Hz), 4.32 (m, 2H), 4.19 (m, 1H), 4.07 (m, 1H), 2.33 (m, 2H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz) δ: 175.1, 155.8, 148.6, 148.4, 143.8, 143.6, 141.2, 141.0, 139.7, 139.6, 128.6, 128.4, 128.3, 127.7, 127.6, 127.1, 126.8, 125.6, 125.4, 125.2, 125.1, 120.1, 112.0, 67.2, 63.5, 52.7, 47.0, 31.4; HRMS (ESI^{+}) *m/z*: $[M + NH_4]^+$ calcd for $C_{37}H_{33}N_2O_4$, 601.2156; found, 601.2166.



AcCys(PhF)OEt (**3.70**). HCl·H-Cys-OEt (222 mg, 1.20 mmol, 1.2 equiv) was suspended in acetonitrile (25 mL) and PhFOH (256 mg, 1.00 mmol, 1 equiv) was added followed by BF_3•OEt_2 (126 µL, 1.00 mmol, 1 equiv). The suspension immediately became clear and new precipitates formed after stirring for 24 h. After 24 h the reaction was quenched with methanol (1 mL), filtered, and concentrated. The residue was dissolved in 30 mL ethyl acetate (30 mL) and washed three times with 10% Na_2CO_3 (15 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated to dryness. The crude material was subjected to Ac_2O:pyridine:DCM (1:1:8, 10 mL) for 1 h. The mixture was diluted with dichloromethane (50 mL) and washed with 10% Na_2CO_3 (20 mL) and 10% citric acid (2 x 20 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated to dryness. The residue was purified by flash chromatography using a gradient of 0% to 40% ethyl acetate in hexane. Compound **3.70** was obtained as a white solid (329 mg, 76% yield). ^{1}H NMR($CDCl_3$, 300 MHz) δ: 7.70 (d, 2H, *J* = 7.5 Hz), 7.5-7.2 (m, 11H), 5.78 (d, 1H, *J* = 7.7 Hz), 4.44 (dt, 1H, *J* = 7.9, 4.9 Hz), 4.08 (m, 2H), 2.35 (dd, 1H, *J* = 12.7, 5.4 Hz), 2.22 (dd, 1H, *J* = 12.7, 4.4 Hz), 1.86 (s, 3H), 1.17 (t, 3H, *J* = 7.1

Hz);$^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz) δ:170.3, 169.5, 148.6, 148.5, 141.0, 139.6, 139.5, 128.43, 128.32, 128.25, 128.2. 128.1, 127.5, 126.7, 125.4, 125.3, 120.1, 112.0, 77.6, 77.1, 76.7, 63.2, 61.5, 51.0, 31.6, 22.8, 13.9; HRMS (ESI^+) *m/z*: $[M + Na]^+$ calcd for $C_{26}H_{25}N_2O_3Na^+$,454.1447; found, 454.1451.

Procedures for the deprotection of PhF groups

Deprotection of PhF ester **3.61.** To a solution of **3.61** (50 mg, 0.11 mmol) in dichloromethane (5 mL) triisopropylsilane (50 µL), and trifluoroacetic acid (50 µL) were added. The mixture was stirred until complete by TLC (5 min). The reaction was then diluted with hexane (15 mL) and extracted with aq. NaOH (0.1 N, 10 mL). The combined aqueous layers were acidified with hydrochloride acid (1 N, 5 mL), saturated with sodium chloride, then extracted with dichloromethane (3 x 10 mL). The combined dichloromethane layers were dried over magnesium sulfate, filtered, then concentrated which gave Cbz-Ser-OH as a white solid (20.6 mg, 86% yield) whose ^1H NMRspectrum was identical to an authentic commercial sample.

Deprotection of PhF ether **3.43**. To a solution of **3.43** (100 mg, 0.190 mmol) in dichloromethane, 10 mL. triisopropylsilane (100 µL) and trifluoroacetic acid (100 µL) were added. A red color appeared briefly and disappeared after a few seconds. The colorless solution was stirred for five min until complete by TLC then the mixture was applied directly to a silica gel column and eluted with a gradient of dichloromethane to ethyl acetate to yield Cbz-phenylalanol (**3.42**, 50 mg, 93% yield).

Deprotection of PhF sulfonamide **3.51**. Compound **3.51** (120 mg, 0.292 mmol) was dissolved in dichloromethane (9 mL) and triisopropylsilane (100 µL), and trifluoroacetic acid (1 mL) were added. The reaction was stirred until complete by TLC (15 min) and then concentrated to dryness in the presence of silica. The product was purified by flash chromatography, using a gradient of

100% hexane to 100% ethyl acetate, which gave p-toluenesulfonamide (**3.49**) as a white solid (49 mg, 98% yield).

Deprotection of PhF sulfide **3.47.** Compound **3.47** (100 mg, 0.167 mmol) was dissolved in DCM (5 mL) then 50 µL each of triisopropylsilane and ethanedithiol were added followed by 500 µL of TFA. The reaction was stirred for 30 minutes until complete at ambient temperature. The mixture was diluted with DCM (30 mL) and washed with monosodium citrate (0.5 M, 30 mL) then dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography to yield Fmoc-Cys-Ome (**3.46**) as a white solid (58 mg, 97%).

Deprotection of PhF amide **3.55**. Compound **3.55** (10 mg, 0.029 mmol) was dissolved in a mixture of trifluoroacetic acid, triisopropylsilane and water (95:2.5:2.5) (1 mL) and stirred at room temperature. The reaction was followed by TLC (UV, 20% ethyl acetate in hexane) until the starting material has been completely consumed (10 min). The product was not isolated.

Deprotection of PhF amine **3.34**. Compound **3.34** (10 mg, 0.042 mmol) was dissolved in a mixture of trifluoroacetic acid, triisopropylsilane and water (95:2.5:2.5, 1 mL) and stirred at room temperature. The reaction was followed by TLC (UV, 20% ethyl acetate in hexane) until the starting material has been completely consumed (30 min). The product was not isolated.

Deprotection of PhF ether **3.45.** Compound **3.45** (100 mg, 0.182 mmol) was dissolved in DCM (10 mL) then triisopropylsilane (100 µL) and trifluoroacetic acid (100 µL), were added. The reaction was stirred at ambient temperature until complete by TLC (15 min) then concentrated in the presence of silica. The product was isolated by flash chromatography using gradient elution 10% to 60% ethyl acetate in hexane to yield Boc-Thr-OBn (29 mg, 98%).

Partial deprotection of PhF ether **3.58**. Compound **3.58** (50 mg, 0.079 mmol) was dissolved in DCM (5 mL) then triisopropylsilane (50 µL) and trifluoroacetic acid (50 µL) were added. The

reaction was stirred at ambient temperature until complete by TLC (1 h) then concentrated in the presence of silica. The residue was subjected to flash chromatography, using a gradient of 0% to 50% ethyl acetate in hexane, to give **3.59** (26 mg, 82% yield).

Chapter 4 — Synthesis of peptidomimetic substrates and inhibitors of IgA1 protease.

4.1 — Introduction.

This chapter concerns the synthesis of a series of potential inhibitors and substrates of the IgA1 protease produced by *Haemophilus influenzae (H. influenzae)*. These studies could result in the development of antivirulence factors for the treatment of bacterial infections. Before these potential inhibitors and substrates can be introduced, a discussion of virulence factors and antivirulence therapy is warranted, beginning with the need for antivirulence therapy which is brought on by the growing threat of antibiotic resistance.

4.1.1 — Antibiotics and antibiotic resistance.

Among the first antibiotics deployed were the sulfa drugs during the 1930's, but the most well-known antibiotic is, without a doubt, penicillin, for which Alexander Fleming received one third of a Nobel prize in 1945. During his Nobel lecture, Fleming warned of the threat of widespread resistance to antibiotics that would result from the overuse, and the misuse of antibiotics.[161,162]

It would appear that history is slowly bearing out Fleming's fear: in 2010, the World Health Organization (WHO) declared antibiotic resistance one of the three greatest threats to human health and the Infectious Disease Society of America called for the development of 10 new antibiotics by the year 2020.[163] Although several new classes of antibiotics have been

approved in the 21st century, resistance quickly follows. Table 4.1 shows the year in which major antibiotics were first deployed on a large scale alongside the date at which resistance to said antibiotic was observed clinically.[1,164-170] Resistance has been observed to every major class of antibiotics deployed in the clinic so far.

Table 4.1. Emergence of antibiotic resistance over time.

Antibiotic	Year Deployed	Clinical resistance observed
Sulfonamides	1930s	1940s
Penicillins	1943	1946
Streptomycin	1943	1959
Chloramphenicol	1947	1959
Tetracycline	1948	1953
Erythromycin	1952	1988
Vancomycin	1956	1988
Methicillin	1960	1961
Ampicillin	1961	1973
Quinolones	1967	1969
Cephalosporins	1960s	1960s
Linezolid (oxazolidinones)	2000	2001
Daptomycin (lipodepsipeptides)	2003	2005
Retapamulin (pleuromutilins)	2007	2014
Fidaxomicin (Tiacumicins)	2011	2019
Bedaquiline (Diarylquinolines)	2012	2014

The selective pressure imposed by antibiotics is easily understood as the classical targets for antibiotics are inhibition of the synthesis of DNA, RNA, proteins, folate, or the cell wall, and depolarization of the membrane.[1] All of these are necessary for the viability of bacteria *in vitro*; therefore, any resistant mutants which neutralize or otherwise avoid these antibiotics will have an enormous fitness advantage whenever the antibiotic is present. Even if a significant tradeoff exists in which the resistant mutants have reduced fitness in some other area, non-resistant bacteria are generally killed off by the antibiotic leaving a largely empty niche to be filled by resistant clones.

Research into novel classes of antibiotics has been declining among large pharmaceutical companies.[171] The reason for this sluggish and limited development may be economic rather than scientific. The cost of bringing new drugs to market is extraordinary, and for antibiotics the potential return on investment is low. Antibiotics are generally administered over a short period of time meaning that only a small number of doses are needed. Furthermore, good stewardship of our antibiotic arsenal dictates that new drugs be held in reserve and used only when existing drugs have failed. Therefore, for any new class, particularly in the first few years, total sales will be low. This issue, combined with the short time-period between deployment and resistance, leads to a low return on investment for antibiotics.

Unfortunately, even with the most diligent antibiotic stewardship, resistance cannot be completely avoided because it exists at low levels in the general population of bacteria.[164] We should expect that antibiotic resistance would predate modern antibiotics in light of the fact that most antibiotics, particularly early examples, are either natural products or derivatives thereof. Antibiotics have been produced by organisms for their own defense for millions of years and antibiotic resistance should evolve concurrently.[173]

Toxicity is also a concern with most antibiotics. For example, high toxicity has prevented the A54145 family of lipodepsipeptides from seeing clinical use.[174] Unfortunately, toxicity may be unavoidable given the importance of the human microbiome, which is also vulnerable to antibiotics.

It is clear that greater efforts should be made toward the continued development of new drugs to combat the threat of antibiotic resistance. However, given that resistance appears to be an inevitable consequence of antibiotic use, and that antibiotics are, by their nature, prone to problems with toxicity, it seems that alternative forms of treatment should be investigated.

4.1.2 — Virulence factors and antivirulence therapy.

Due to the toxicity of certain antibiotics, the rapid appearance of resistance, and the need to constantly produce novel drugs, alternative approaches are attractive. One such approach is antivirulence therapy. Before this can be introduced, a brief discussion of virulence factors is warranted. Virulence is a measure of the severity or harmfulness of a disease. Virulence factors can be defined as bacterial products which are not necessary for viability *in vitro* and not necessarily beneficial to the bacterium *in vitro* or *in vivo*, but which cause disease either by preventing an immune response or damaging host tissues.[175,176] Virulence factors are usually not produced by bacteria unless they have infected the host, this is the result of tight gene regulation and reflects the high metabolic cost of virulence.[177]

Virulence factors include adhesins which allow bacteria to adhere to specific surfaces and form biofilms, toxins which may cause cell death in host cells or the microbiome, effectors which mimic host proteins, siderophores which sequester iron from the blood, any mechanism that allows for immune system evasion, specialized secretory systems which allow delivery of effector molecules directly to eukaryotic cells, and quorum sensing systems which lead to the activation of virulent genes.[1,177] Critically none of these are necessary for the viability of the bacterium *in vitro* which is in sharp contrast to the targets of antibiotics.

An ideal antivirulence therapy would be one which deactivates one of the aforementioned factors, but which has no effect on the viability or growth rate of bacterium *in vivo*. In other words, the therapy is neither bactericidal nor bacteriostatic. In specific cases, virulence therapy would not induce resistance (*vide infra*). Potential other benefits are reduced toxicity to the host and to the microbiome, the harmlessness of inappropriate or prophylactic use, low transfer of resistance between species, and the potential for combination therapy with traditional antibiotics.

The mechanism by which antivirulence therapies could avoid resistance is critical and warrants careful consideration because this is not equally true for all virulence factors. In a 2014 review, Allen et al. divide virulence factors into 3 categories: (1) those which are not beneficial; (2) those which are beneficial to the bacterial community as a whole and; (3) those which are beneficial to the individual bacterium.[178]

The first category, virulence factors which confer no benefit to pathogens, uselessly consume energy and should be selected against over time.[179]

An example of the second class is a toxin (Figure 4.1a). *Clostridium difficile* releases toxins which damage the host or microbiome cells. This leads to diarrhea which helps to spread the disease which benefits the bacterial community. The condition can be treated with Bezlotoxumab, which is a monoclonal antibody that binds to one of these toxins and prevents damage to the epithelial cells and colitis.[180] We shall assume that some small fraction of bacteria in the infection are resistant, which in this case means that they produce a mutant version of the toxin which is not neutralized by Bezlotoxumab. Since the fraction of resistant bacteria is assumed to be small, only a small quantity of toxin is produced which is not harmful to the host. Resistance should not increase for 2 reasons: first, increasing transmissibility gives no particular advantage to the bacterium which produce the mutant toxin, rather it affects the community as a whole. Second, the treatment is not bactericidal, therefore nonresistant bacteria will remain in the niche, preventing the exponential growth of resistant bacteria through competition. In this example, any mutants which produce no toxin at all will have a competitive advantage because the production of toxins is metabolically expensive. This treatment will select against virulence in the long term.

An example of the third category is an adhesin (Figure 4.1b). Urinary tract infections caused by *E. coli* can form biofilms through adherence to bladder cells using structures known as pili and the assembly of pili can be inhibited with pilicides.[181] Although the pili are not essential for the survival of the bacteria, if they are not present then the bacteria cannot adhere to the urinary tract and are flushed out with the normal stream of urine. Resistant bacteria are those which produce mutant pili whose construction is not inhibited by pilicides. Resistance will increase for three reasons. First, resistant bacteria will remain in the urinary tract and the susceptible bacteria will not, which provides a clear fitness advantage to resistant bacterium on an individual level. Second, the resistant bacteria can grow exponentially in the now empty niche. Thirdly, horizontal gene transfer of resistance is very likely because a large variety of bacteria display pili. This is not to say that the approach is useless, but simply that it does not offer any special advantage to avoid bacterial resistance over traditional antibiotics.

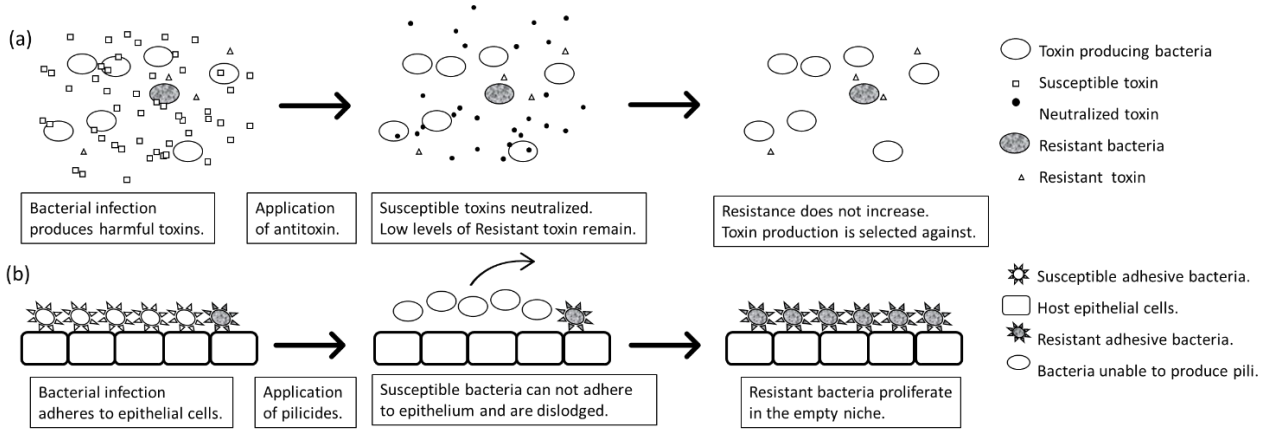


Figure 4.1. Generalized bacterial infections producing virulence factors. (a) Bacteria produce harmful toxins. Treatment with antitoxin does not cause resistance. (b) Bacteria adhering to the epithelium using pili. Treatment with pilicides causes resistance.

Because antivirulence therapy is neither bacteriostatic nor bactericidal, it would be expected to be less toxic than traditional antibiotics which can harm the host microbiome. However, this also means that there is no obvious mechanism by which antivirulence therapy can

clear an infection and the treatment may need to continue for a very long time to maintain low virulence.[178,182] Therefore, the main use of antivirulence is simply to avoid the damaging symptoms of bacterial infections until it can be cleared either by the immune system or using traditional therapies. This is not necessarily a limitation, since antivirulence therapy could be given prophylactically to high-risk individuals, such as those who have had surgery. The antibiotic is held in reserve until an infection is confirmed so that there is no risk of promoting resistance through inappropriate antibiotic use.[182]

4.1.3 — The IgA1 antibody

The IgA1 antibody is produced in humans and other great apes. It is one of the primary antibodies used for defense against invasive bacteria. IgA is the most abundant class of antibody expressed by humans and consists of two subclasses: IgA1 and IgA2. IgA1 is the primary antibody expressed in serum and on mucosal surfaces. The solution state structure of IgA1 was studied in 1999 by Boehm et al. and it is best understood in the context of related antibodies IgA2 and IgG.[183,184]

IgA1 is often produced as a dimer, however for simplicity only the structure of the monomer is shown (Figure 4.2). In their monomeric form, IgA1, IgA2, and IgG all consist of two antigen binding fragments or Fab fragments and one Fc fragment or crystallizable fragment (Figure 4.2). Each fragment consists of a total of four domains for a total of twelve domains per antibody.

Each antibody contains 4 polypeptide chains, two heavy and two light. The light chains contain one constant domain and one variable domain, each light chain makes up half of a Fab fragment. The heavy chains also contain a constant and variable domain making up the other half of a Fab fragment and are linked to two additional constant domains through a hinge region.

Together the additional constant domains of the two heavy chains make up the Fc fragment. The function of the Fab fragments is to carry the hypervariable antigen binding region which binds to foreign substances such as pathogenic bacteria. Meanwhile the function of the Fc fragment is to bind to effector cells and initiate a response.

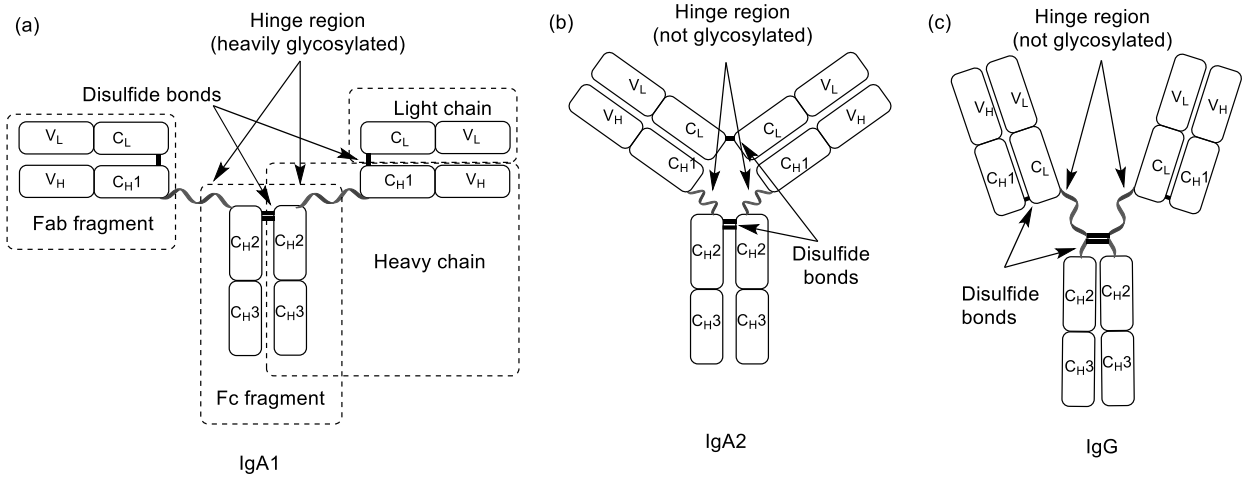


Figure 4.2. Structure of common antibodies. (a) Structure of the IgA1 antibody; (b) Structure of IgA2 antibody; (c) Structure of IgG antibody.

The antibodies differ from each other in how the chains are joined. In IgA1 the heavy chains are linked by disulfide bonds in the C_H2 domain, and each heavy chain is linked to a light chain by a disulfide bond between the C_H1 and C_L domains. The hinge region is 23 amino acids long and very mobile allowing for a 23 nm separation between the antigen binding regions and gives the molecule an overall 'T' shape. In IgA2 the heavy chains are also linked by disulfide bonds in the C_H2 domains, but the heavy chains are linked to the light chains by noncovalent interactions. The light chains are linked to each other through disulfide bonds in the C_L domains and this, combined with a hinge region that is only ten residues long gives the molecule an overall 'Y' shape. Finally, in IgG the heavy chains are linked through disulfide bonds in the hinge region and each heavy chain is linked to a light chain through disulfide bonds in the C_H1

and C_L domains. Although the hinge region is 22 residues long, the disulfide bonds restrict mobility and result in an overall 'Y' shape for the molecule.

It is evident from the overall shape of the antibodies that the hinge region of IgA1 is the most accessible to attack from proteases and therefore it is rich in prolines and contains five glycosylations that are not present in the IgA2 and IgG. [185] These features make IgA1 resistant to hydrolysis by most mammalian and bacterial proteases. Boehm et al. suggest that the greater mobility the Fab regions in IgA1 allow for a binding to a greater range of antigens.[184]

4.1.4 — The IgA1 protease

In 1973 Mehta and coworkers were interested in studying the Fc domain of IgA1, but common proteolytic enzymes either did not hydrolyze the antibody or did so nonspecifically, destroying the Fc domain. An enzyme was eventually isolated from human feces that specifically attacked the hinge region and yielded the intact Fc domain. This marked the first isolation of an IgA1 protease.[186]

IgA1 proteases are a polyphyletic group of proteinases which all cleave the hinge region of human IgA1 after a proline and generally have a very narrow substrate scope. IgA1 proteases have been isolated from a wide range of bacteria including Gram-positive and Gram-negative examples, and are either serine, cysteine, or metalloproteases. This striking example of convergent evolution suggests that the cleavage of this antibody must convey significant selective advantage to the bacteria that produce IgA1 proteases. Each protease cleaves the hinge region in one of several locations, those which cleave after a single proline are designated type 1 and those which cleave after two prolines are designated type 2, the cleavage sites are summarized in Figure 4.3.[186,187] Notably the hinge region contains an octameric repeat (-

STPPTPSP-) and enzymes that cleave at one position in the octameric repeat do not cleave at the analogous position in the other repeat.

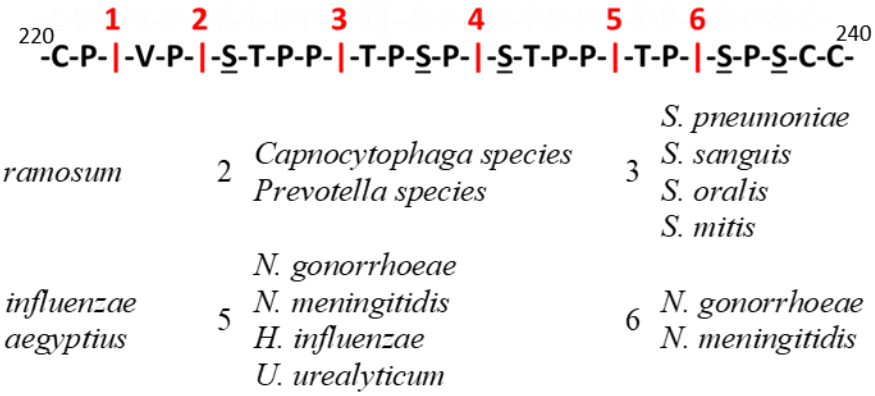


Figure 4.3. Cleavage sites of various IgA1 proteases produced by different bacteria. The sequence of the hinge region is shown with the glycosylated residues underlined.[185] The bacteria are numbered according to the position which they hydrolyze.

H. influenzae produces both type 1 and type 2 IgA1P; the crystal structure of the type 1 enzyme ($IgA1P_{Hinf1}$) was obtained by Holyoak and coworkers in 2009.[188] Using this structure (Figure 4.4) Holyoak was able to shed light on the mechanism of action of the enzyme and how it is able to cleave IgA1 with such remarkable specificity.

$IgA1P_{Hinf1}$ is a serine protease and consists of a large ß-helical domain which supports the other domains. The protease domain is chymotrypsin-like (see Section 1.1.2 for a general mechanism). A unique D-loop extends over the catalytic site. This D-loop is normally in a closed position which prevents substrates from entering the active site. Meanwhile, additional domains 2, 3, and 4 extend away from the ß-helical domain and provide a binding site for the antibody. The largest of these, domain 2, forms one side of a large binding pocket (Figure 4.4). Holyoak and coworkers performed docking simulations which predict that first the Fc domain interacts with both the protease domain and domain 2, this stabilizes loop D in an open conformation and allows the hinge region to fully extend without steric clash between the Fab domain and the

protease domain. The hinge region can then insert into the active site cleft. The correct positioning is aided by domains 3 and 4 and by the binding of the Fab fragment to the reverse side of the protease domain which ensures that the glycosylations of the hinge region are facing away from the active site. Hydrolysis can then occur, and one Fab fragment is separated. This complex method of substrate recognition reduces the degrees of freedom of the mobile antibody and allows for cleavage of a glycosylated hinge. Hydrolysis of the second hinge region occurs through the same mechanism independently of the first.[189]

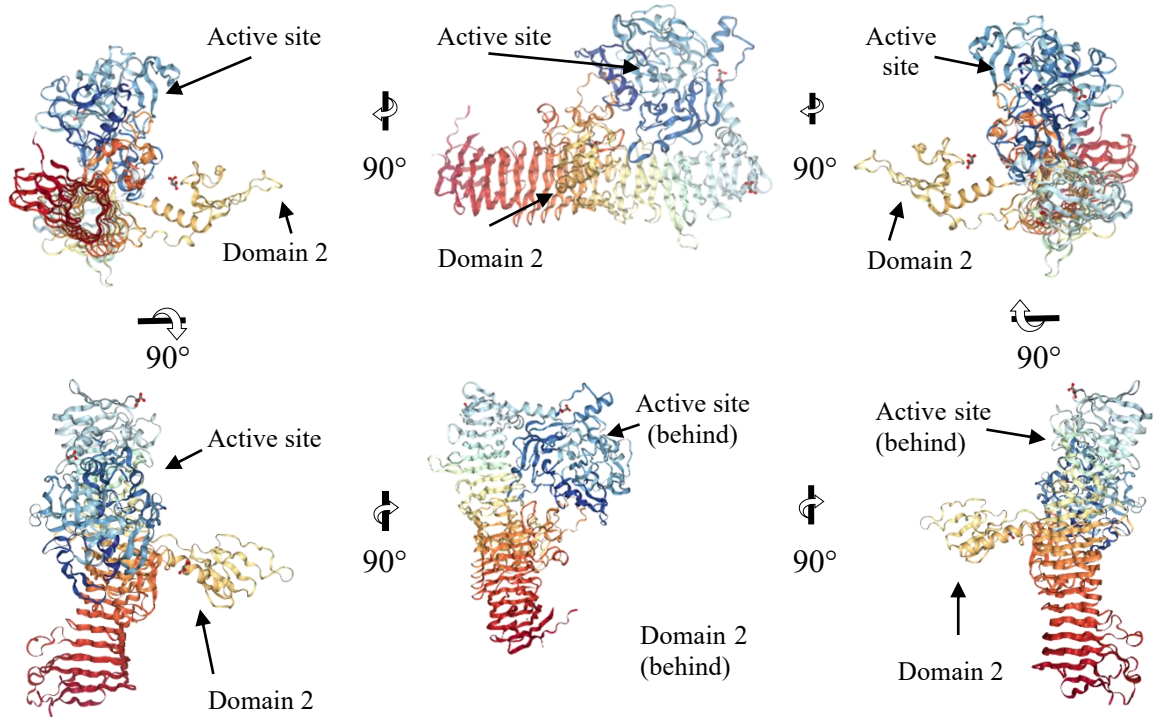


Figure 4.4. The Crystal structure of the type 1 IgA1 protease from *H. influenzae* as viewed from various angles showing the relative position of domain 2 and the active site. Images from the RCSB PDB (rcsb.org) of PDB ID H309.[188]

One additional important substrate exists. The enzyme produced by *H. influenzae* and some other species is produced as a proenzyme. The proenzyme contains a large C-terminal domain which inserts into the outer membrane and creates a pore which allows the remainder of

the enzyme to translocate. Once in the extra cellular space, the enzyme can cleave itself at an autoproteolytic site and be released from the cell.[190] The hydrolysis may also occur intermolecularly.[191] This site is cleaved in the absence of the long-range interactions with the IgA1 antibody which are normally necessary to stabilize the enzyme active site in an open position which has implications for the design of small molecule inhibitors.

4.1.5 — IgA1 Protease and Virulence

IgA1P is a virulence factor because it allows bacteria to colonize areas of the body they otherwise could not. The enzyme is not necessary for the survival of the bacteria *in vitro*, and closely related strains of bacteria that do not produce this enzyme are not pathogenic.[192,193]

IgA1P causes disease by allowing bacteria to evade the immune system. Following hydrolysis of IgA1, the Fab and Fc fragments remain intact, and the Fab fragment can still bind to antigens, including IgA1 producing bacteria, but this can no longer be communicated to effector cells through the Fc domain so the antibody is neutralized. This has two additional important effects. The first effect is that the bacterium can become coated in these Fab fragments which reduces the ability of other, intact antibodies to detect the bacterium.[194] The second effect stems from the Fab fragments having a net positive charge which can promote adherence of bacterium to host cells electrostatically.[195]

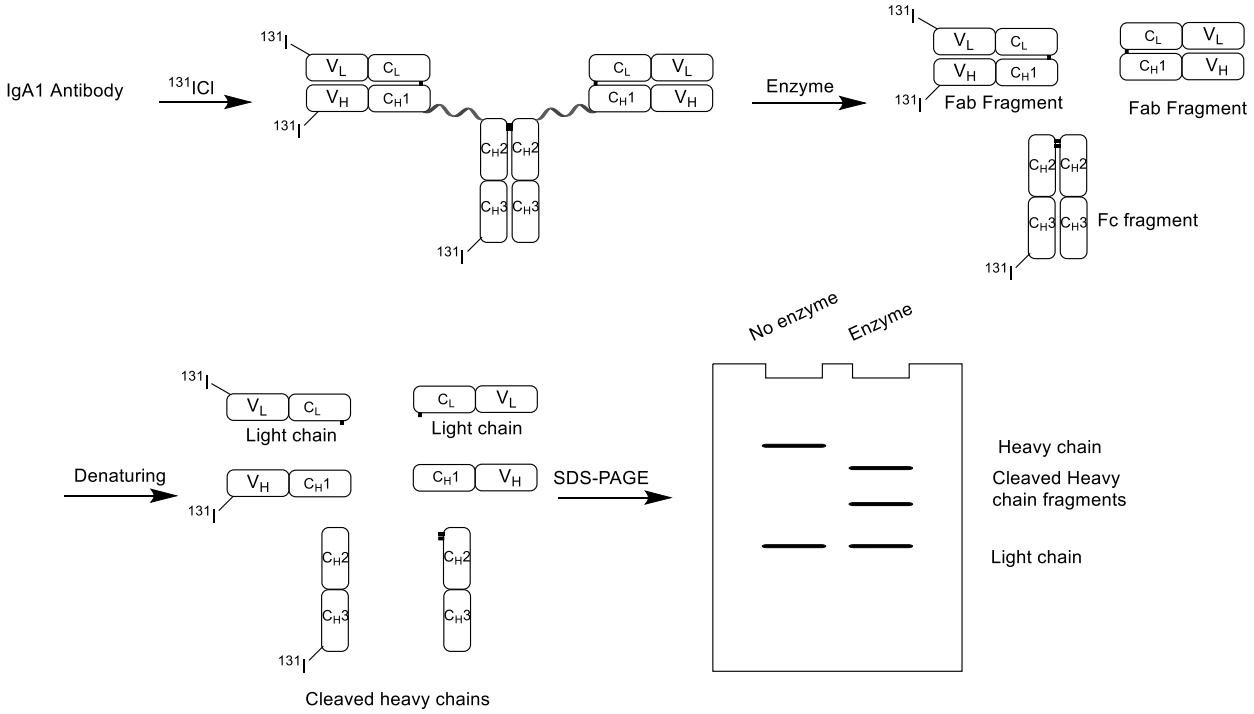
IgA1P should be most effective in environments where IgA1 is the most prevalent antibody, such as in serum.[183] The enzyme is secreted into the serum where it benefits the entire community of bacteria by targeting IgA1 antibodies preventing the detection of bacteria by the immune system. If a hypothetical antiviral therapeutic is applied which inhibits IgA1P then bacteria cells will once again be identified for destruction. As before, we should assume that a small portion of the population will be resistant to this therapy by producing an enzyme which is

not inhibited by the hypothetical antivirulence drug. In this case a small amount of resistant enzyme is produced which neutralizes only a small amount of IgA1, but since the serum is well mixed there is still enough intact IgA1 remaining to be effective. Critically, the resistant IgA1P provides no benefit to the resistant individuals over the nonresistant individual, assuming that the environment is well mixed.[178] Furthermore, mutants which produce no IgA1P will have a selective advantage since the secretion of the enzyme is metabolically expensive. In this example, once the hypothetical antivirulence therapy is applied, then the immune system can clear the infection meaning that there is no need for an additional bactericidal or bacteriostatic drug and resistance should not increase. This makes IgA1P an ideal target for antivirulence therapy.

4.1.6 — Assays of IgA1 protease activity

The study of IgA1P has been limited by the lack of a convenient assay. To the best of our knowledge three assays have been developed that have been used to screen IgA1P inhibitors. The earliest method of assaying the enzyme involves using the IgA1 antibody labeled with a radioactive isotope of iodine, usually ^{131}I or ^{125}I. The iodine is incorporated into the antibody using a general method developed by Hunter and Greenwood: radioactive iodide is oxidized with chloramine T to give iodine monochloride in the presence of the antibody, then any of the aromatic residues can react through electrophilic aromatic substitution to give the labeled enzyme (Scheme 4.1).[196] Several studies have used this method to assay IgA1P.[187,189,197] Once labeled the antibody is incubated with the enzyme where it is cleaved into the Fab and Fc domains which is analyzed by SDS-PAGE and quantified by autoradiograph. The intact light chain is separated from the cleaved heavy chain under the denaturing conditions of SDS-PAGE. This method suffers from several drawbacks. The first is the cost of materials. The natural

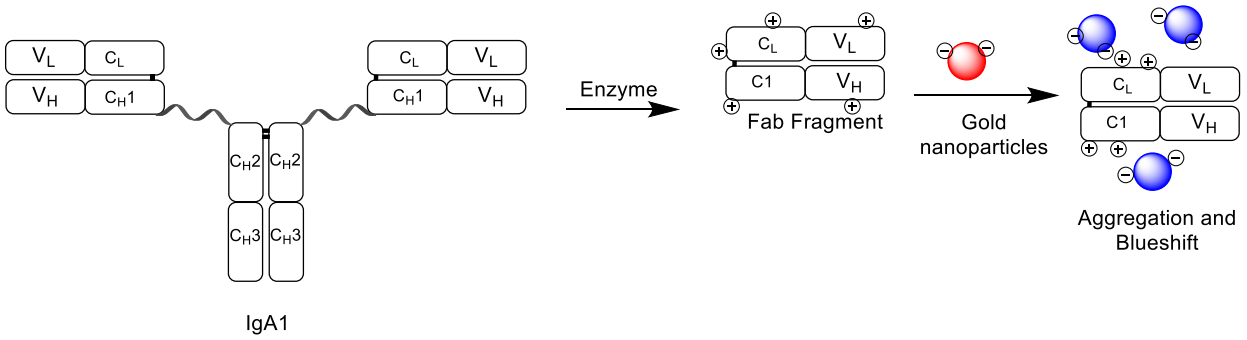
substrate is isolated from human myeloma plasma and is commercially available but very expensive. Secondly radioactive iodine requires specialized precautions and equipment and is also expensive. Finally, throughput is low, SDS-PAGE is slow and precludes monitoring the reaction in real time. This limits the search for inhibitors in the above studies to only a few, rationally designed compounds. For decades this was the only available assay for IgA1P which limited the study of the enzyme.



Scheme 4.1. Early Assay for IgA1 protease activity using ^{131}I labeled antibody.

The next assay was developed by Garner et al. in 2013.[198] Briefly, gold nanoparticles were modified with citrate so that they bore a negative charge. These are red when fully dispersed but undergo a blueshift when aggregated. The enzyme and antibody are incubated for 18 hours and then the nanoparticles are added. After IgA1 is cleaved enzymatically the Fab Domain of IgA1 is positively charged and interacts with the gold nanoparticles electrostatically,

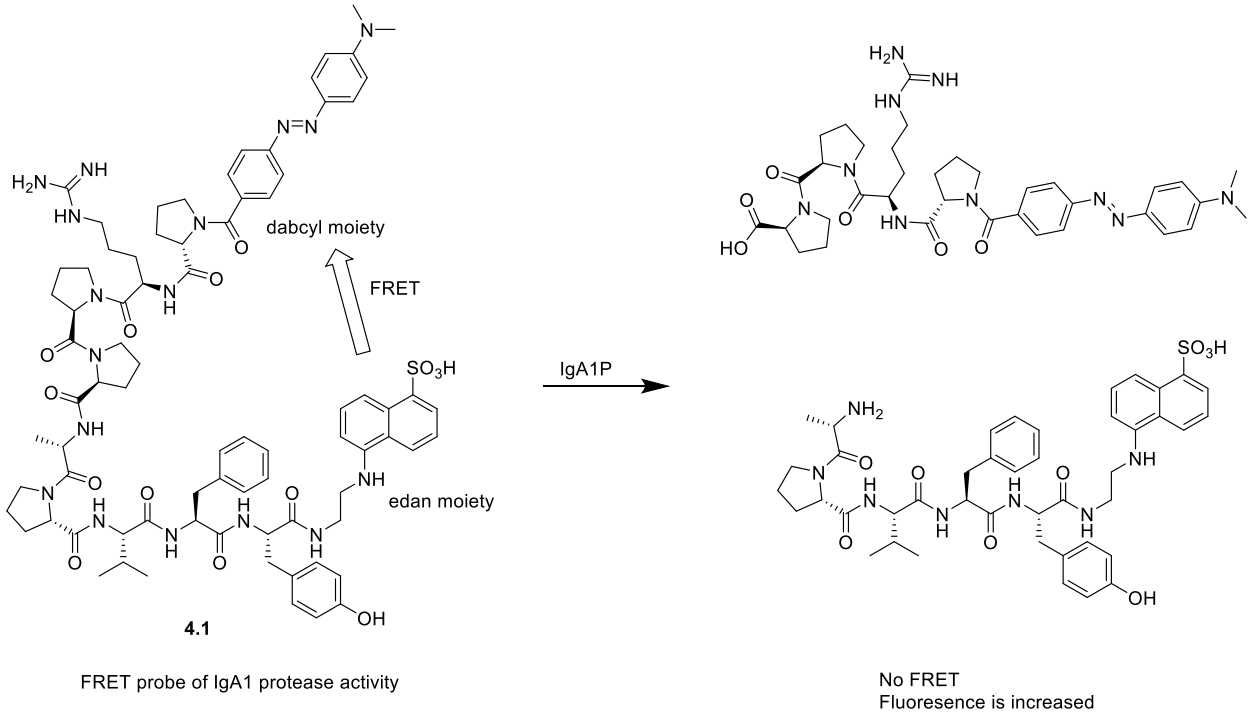
leading to aggregation and a blue shift in the spectrum (Scheme 4.2). Using this method Garner et al. were able to screen approximately 100 compounds for IgA1P inhibition. Several inhibitors were identified; however, these were only tested against enzyme produced by *S. pneumoniae*, which is a type 2 metalloprotease. This assay represents an improvement over the previously discussed method as it can be performed in a 96 well plate and the use of radioactive material is eliminated. However, the assay still requires overnight incubation and uses considerable quantities of the antibody.



Scheme 4.2. Assay of IgA1P activity based on aggregation of the FAB domain with charged gold nanoparticles.

The final assay is based on FRET and was independently developed by the Kritzer group concurrently with the nanoparticle assay.[199] This is the first assay to use an artificial substrate, **4.1**. The peptide is cleaved by IgA1P, separating the FRET donor accepter pair and the change in fluorescence can be measured (Scheme 4.3). Unlike the previous two assays, the measurement is non-destructive so multiple readings from the same experiment can be obtained more easily which allows for higher throughput. The artificial substrate was designed to mimic the autoproteolytic site of $IgA1P_{Ngon2}$. Surprisingly, the probe was effective with several different proteases produced by different species including both the type 1 and type 2 enzymes, and both serine and metalloproteases, which have different cleavage sites in the hinge region of IgA1. In

2019, this group was able to use this probe to perform a high throughput screening of 47 000 small molecules as inhibitors of $IgA1P_{Hinf1}$.[200]



Scheme 4.3. Assay of IgA1 protease activity based on cleavage of an artificial substrate resulting in loss of FRET.

Currently there is no assay available which can monitor the activity of the enzyme in real time using an easily accessible chromogenic substrate, such as a *p*-nitrophenyl ester or amide.

4.1.7 — Inhibitors of the IgA1 protease

The first small molecule inhibitors for any IgA1P were short peptides based on the hinge region of IgA1 that were reported in 1988. These included either one or two copies of the octameric repeat and several modifications thereof.[187] In particular, Burton et al. were interested in replacing the hydroxyl containing amino acids with cysteine and dimerizing these with disulfide bonds. In all cases the dimers were worse inhibitors than the monomers indicating that

the enzyme binds to only one hinge region at a time. This result agrees with later docking simulations.[188] The best inhibitor was an octapeptide with an IC_{50} of 50 μM using $IgA1P_{Ngon1}$ and 200 μM using $IgA1P_{Ngon2}$ (Figure 4.5).

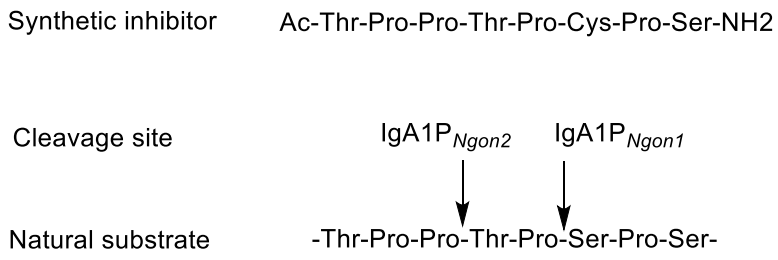


Figure 4.5. Early peptide inhibitor of IgA1P which mimics the IgA1 hinge region.

This octapeptide was not a substrate of the enzyme. Perhaps because the peptides bind in the binding cleft, but in the absence of the Fc fragment, the D-loop is not stabilized in an open conformation and the inhibitors cannot insert deeply in the active site, even if the length of the peptide was extended to 25 residues only a very poor substrate was obtained.[199] Substrates can be obtained if peptides are based on the autoproteolytic site. This was demonstrated in 1991 by Wood and Burton using decapeptides derived from the self-cleavage site of $IgA1P_{Ngon2}$ to produce the first synthetic substrate of any IgA1 protease.[201] Since the self-cleavage site is naturally hydrolyzed in the absence of the IgA1 antibody it is not surprising that these peptides were better substrates than those derived from the hinge region. However, this was only demonstrated using enzymes derived from *N. gonorrhoeae* and not *H. influenzae*.

The first small molecule inhibitors of $IgA1P_{Hinf1}$ were small peptidyl boronic acids that were prepared by Bachovchin et al. in 1990 (Table 2).[197] The C-terminal carboxylate is replaced by a boronic acid. Boronic acids are well known serine protease inhibitors which bind in the active site as transition state analogs.[202] These effectively inhibited types 1 and 2 $IgA1P_{Hinf}$ and $IgA1P_{Ngon,}$ all four of which are serine proteases, but not $IgA1P_{Ssan2}$ which is a metalloprotease.

All four of the susceptible proteases were inhibited at submicromolar levels and this is the first series of potent IgA1P inhibitors.

Table 4.2. Peptidyl boronic acid inhibitors of serine IgA1 proteases.

		N. gonorrhoeae		H. influenzae		S. sanguis
		Type 1	Type 2	Type 1	Type 2	Type 2
	Hinge-cleavage site	PTP	TPP	PSP	TPP	TPP
	Self-cleavage site	APP	RPP	DVP	DVP	
Entry	Inhibitors	Ki (μM)				
1	H-boroPro-OH	[a]	[a]	[b]	[b]	33
2	Ac-boroPro-OH	[a]	18	65	12	55
3	H-Ala-boroPro-OH	[a]	14	80	73	7.2
4	Boc-Ala-boroPro-OH	3.7	1.2	18	4.5	26
5	Ac-Ala-Pro-boroPro-OH	0.016	0.004	1.3	0.013	[b]
6	Boc-Ala-Pro-boroPro-OH	0.063	0.035	5.9	0.03	[b]
7	H-Ala-Pro-boroPro-OH	62	85	[b]	47	[b]
8	H-Ala-Pro-boroVal-OH	[c]	[a]	[c]	[c]	[c]
9	Boc-Pro-Thr-boroPro-OH	13	[a]	3.2	[a]	[b]
10	Boc-Pro-Thr(OBn)-boroPro-OH (**4.2**)	3.2	[a]	0.25	50	[b]
11	MeO-Suc-Ala-Ala-Pro-boroPro-OH	0.052	0.028	7	0.019	[b]
12	MeO-Suc-Ala-Ala-Pro-boroVal-OH	[c]	[a]	[a]	25	[b]

[a] Ki >100 μM. [b]no inhibition at 1000 μM. [c]not determined.

Much can be learned from the structure of the molecules in Table 4.2. First, α-amino boronic acids alone are not sufficient to inhibit as shown by the mono and dipeptides which are at best, marginal inhibitors (entries 1-4). Only those peptides with a C-terminal proline showed good inhibition and those with a C-terminal valine substitution were less active (entries 8 and 12). Furthermore, entries with a free N-terminus were poor inhibitors (entries 1,3,7, and 8), the cation evidently is not accommodated by the active site.

Both type 2 enzymes cleave the hinge in the second octameric repeat after the double proline and those inhibitors that bear a double proline are indeed potent inhibitors of both type 2

enzymes (Entries 5, 6 and 11). These were also potent inhibitors of the IgA1P$_{Ngon1}$, but not for IgA1P$_{Hinf1}$. This can be explained by examining the self-cleavage sites of these enzymes: both type 1 and 2 IgA1P$_{Ngon}$ enzymes self-cleave after a double proline while both IgA1P$_{Hinf}$ enzymes self-cleave after an aspartate-valine-proline site.[193,199] The aspartate-valine-proline boronic acid was not prepared which would presumably be a potent inhibitor for both IgA1P$_{Hinf1}$ and IgA1P$_{Hinf2}$.

The best inhibitor of IgA1P$_{Hinf1}$ was boronic acid **4.2** (entry 10) which closely resembles the hinge-cleavage site of this enzyme. If the threonine were exchanged for a serine it would be even more similar and might be a better inhibitor. In the hinge region of IgA1, this serine would be glycosylated, the glycosylation is mimicked by the benzyl ether in **4.2** and, removal of this ether resulted in a moderate loss of activity (entry 9).

The discovery of additional inhibitors has been slow due to the lack of a convenient assay however recently a high throughput screening of chemical libraries has identified 85 inhibitors of IgA1P$_{Hinf1}$ (Figure 4.6).[200] Ester **4.3** was identified as a lead compound which was a potent inhibitor, although still more than 20-fold less potent than boronic acid **4.2**. Analogs of **4.3** were prepared and begin to show structure activity relationships that make this a promising starting point for the rational design of inhibitors.

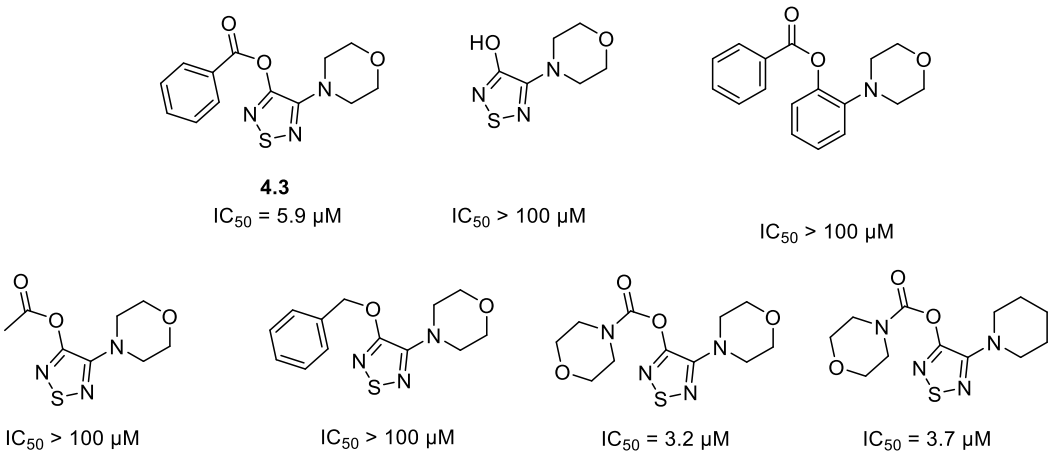


Figure 4.6. Analogs of an IgA1P inhibitor identified by high throughput screening.

4.1.8 — Research Objective

The principal objectives of the research described in this chapter are to prepare a series of peptides or peptidomimetics based on the hinge region or autoproteolysis site of IgA1P$_{Hinf1}$, which we anticipate will serve either as substrates or inhibitors of the IgA1P$_{Hinf1}$. Peptidomimetics corresponding to the cleavage sites in the hinge or autoproteolysis sites and bearing the ß-keto and ß-hydroxy α,α-difluorosulfonamide groups, were prepared. Since peptides containing boronic acid,[197] or trifluoromethyl ketone[19] groups are known to be good inhibitors of serine proteases, we also prepared potential peptide-based inhibitors bearing these functionalities. Additionally, since a simple colorimetric assay for the enzyme would be helpful for screening inhibitors and for kinetic studies, peptides containing C-terminal *p*-nitrophenyl esters and *p*-nitroanilides were also prepared. When we began this study, the Holyoak group was investigating the production of IgA1P$_{Hinf1}$ using a yeast expression system. We wished to prepare as many candidate inhibitors and substrates as possible in advance, which could be investigated for biological activity once the enzyme was available.

4.2 — Results and discussion

4.2.1 — *p*-Nitrophenyl ester based IgA1P substrates

Early on, we realized that our studies of the IgA1P enzyme would be facilitated by the development of a chromogenic assay based on the hydrolysis of a 4-nitrophenyl ester. To that end we sought to prepare two candidate molecules (Figure 4.7). We believed that these were strong candidates for enzyme substrates based on their similarity to known boronic acid inhibitors of $IgA1P_{Hinf1}$ (see Table 4.2). Ester **4.4** contains the DVP sequence which corresponds to the three residues that are immediately towards the N-terminus to the autoproteolytic cleavage site and Ester **4.5** contains a PSP sequence which corresponds to the three residues that are immediately towards the N-terminus to the site of hydrolysis in the hinge region.

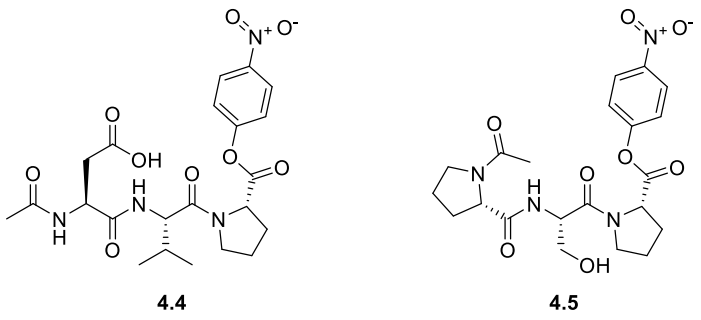
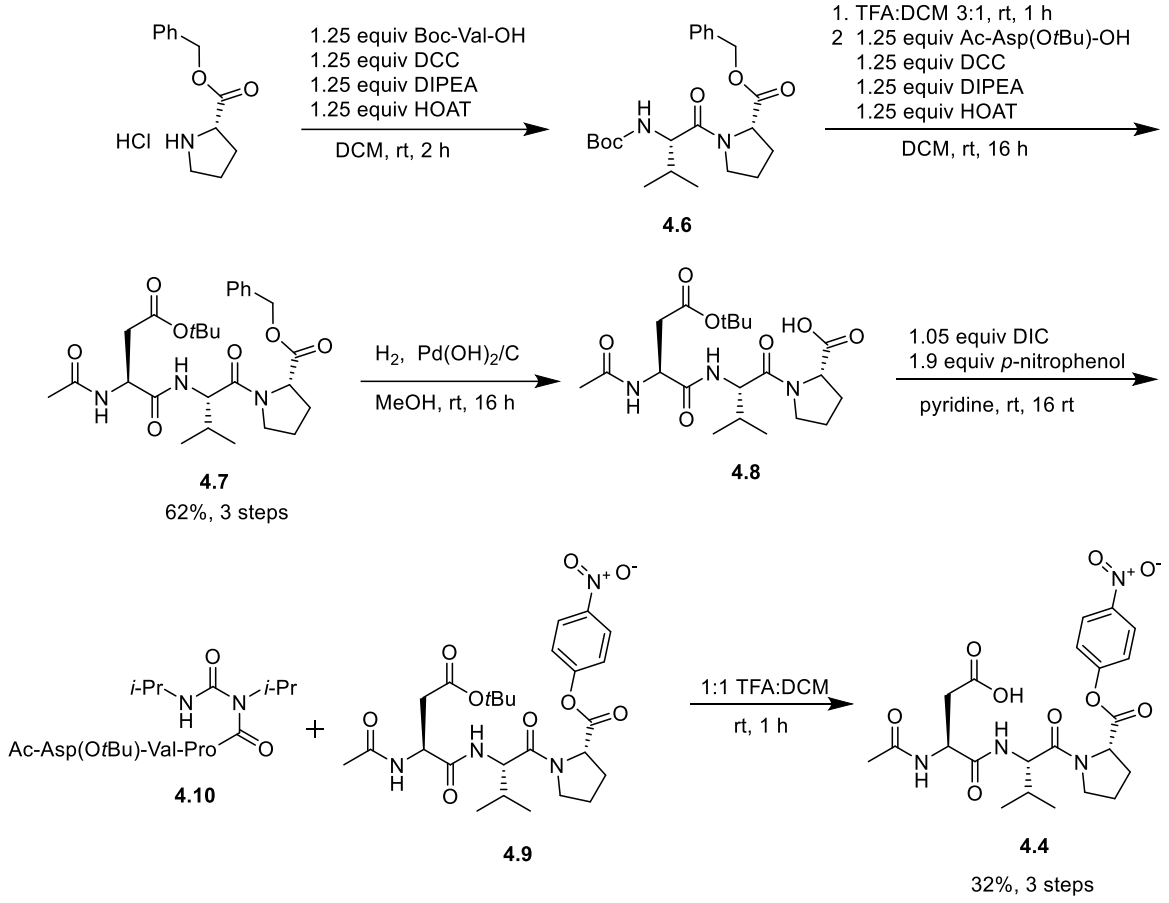


Figure 4.7. Structure of 4-nitrophenyl ester candidate substrates.

We began the synthesis of these compounds using standard solution phase peptide synthesis techniques (Scheme 4.4). Tripeptide **4.7** was prepared through two peptide couplings from proline benzyl ester hydrochloride. After removing the C-terminal benzyl ester by hydrogenolysis, we attempted to form the 4-nitrophenyl ester on the resulting acid, **4.8**, using DIC/4-nitrophenol in pyridine. Pyridine has previously been used as a solvent for the synthesis of 4-nitrophenyl esters.[203] Although some product was formed, **4.9** was obtained as an

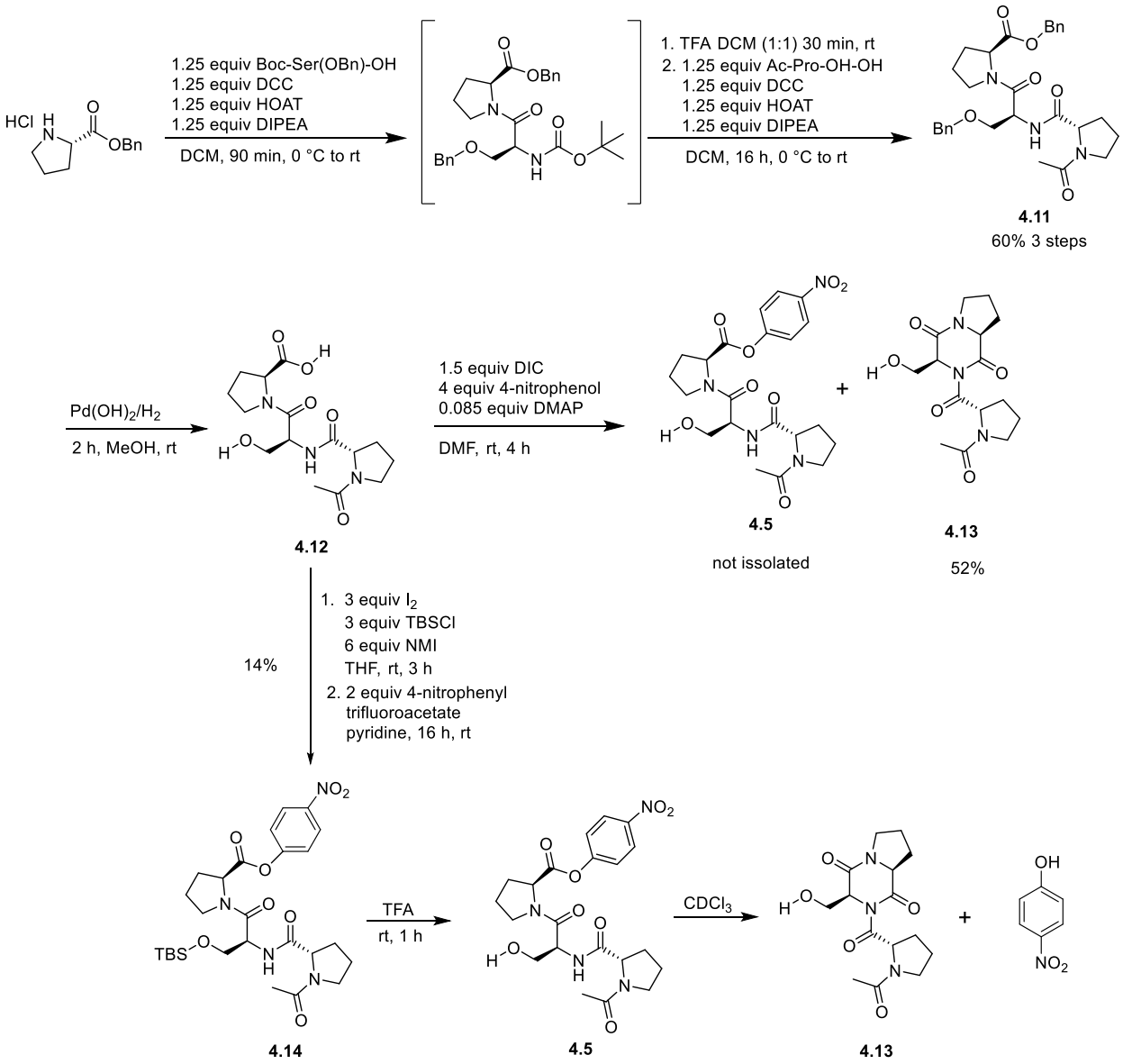
inseparable mixture with *N*-acyl urea **4.10**. The mixture was subjected to TFA to remove the side chain t-butyl ester to give **4.4** which was separated from the *N*-acyl side product by HPLC.



Scheme 4.4. Synthesis of 4-nitrophenyl ester **4.4.**

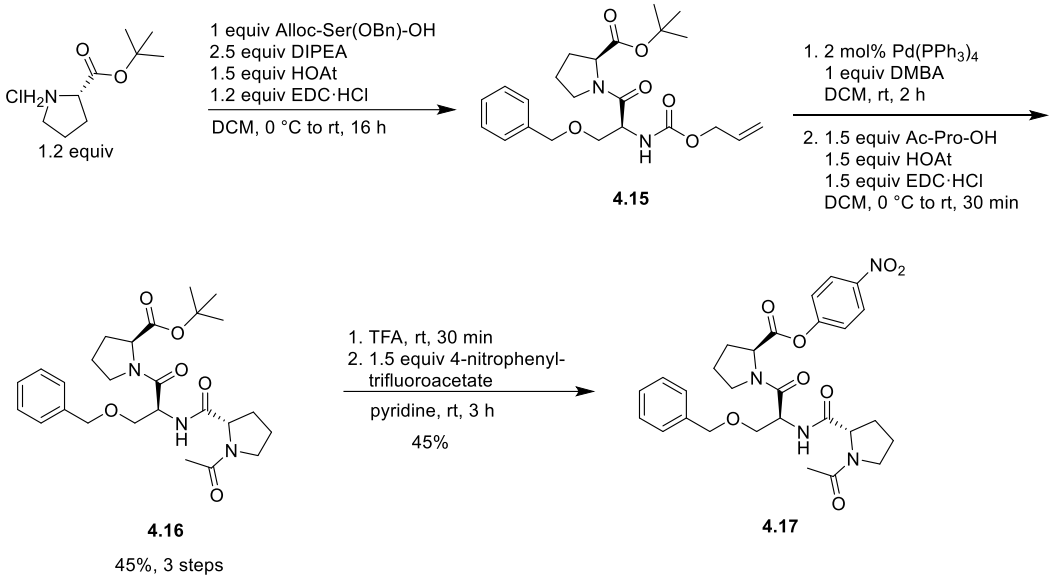
We attempted to prepare ester **4.5** using a similar approach (Scheme 4.5). Tripeptide **4.11** was prepared with standard peptide couplings then deprotected to give **4.12**. We attempted to form 4-nitrophenyl ester **4.5** with DIC and 4-nitrophenol in DMF using DMAP as a nucleophilic catalyst; however, none of the desired product was observed in the mass spectrum of the crude material. Instead diketopiperazine **4.13** was isolated. When the side chain of serine was protected with a TBS group, then the esterification could be performed using 4-nitrophenyl trifluoroacetate.[203] The silylation was performed on tripeptide **4.12** and then the crude material

was subjected to the esterification conditions which provided **4.14**, albeit in very poor yield. The TBS group could then be removed with TFA and 4-nitrophenyl ester **4.5** was isolated. Unfortunately, **4.5** was unstable and existed only long enough to characterize by ^{1}H NMR and HRMS. The NMR sample slowly turned yellow and TLC analysis showed that this was decomposing to yield **4.13** and 4-nitrophenol.



Scheme 4.5. Attempted synthesis of 4-nitrophenyl ester **4.5**.

Given that **4.14** was more stable than **4.5**, we suspected that a benzyl ether would also slow the rate of DKP formation (Scheme 4.6). A benzyl ether was thought to mimic a glycosylation in the boronic acid inhibitors discussed in Table 4.2; therefore, we suspected that this modification would also lead to a better substrate. Tripeptide **4.16** was prepared from proline *tert*-butyl ester. Following deprotection with TFA, the 4-nitrophenol group was installed using 4-nitrophenyl trifluoroacetate in pyridine.[203] This yielded **4.17** which was stable enough to be isolated.

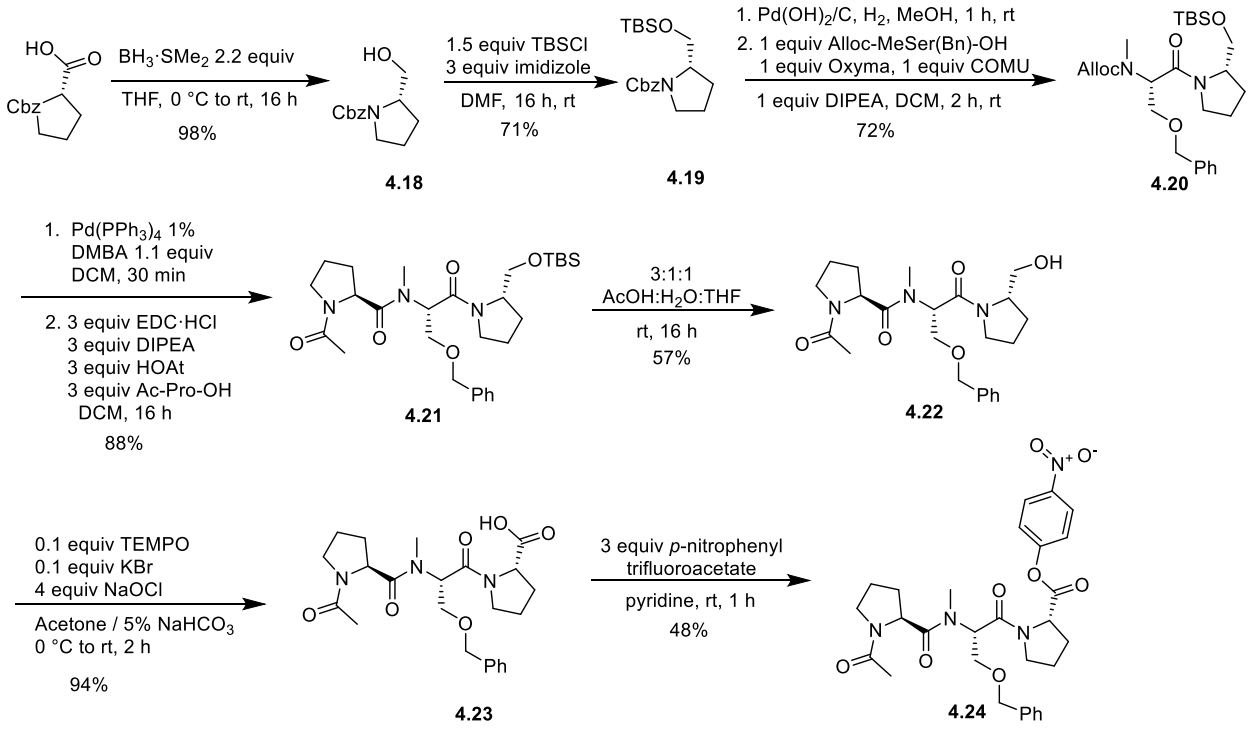


Scheme 4.6. Synthesis of 4-nitrophenyl ester **4.17.**

We next investigated the stability of **4.4** and **4.17** (Figure 4.8). When these esters were placed in buffer at pH 7.5, an increase in the absorbance at 405 nm was observed over a period of two hours which indicated the production of 4-nitrophenol. However, the rate of increase was far greater with PSP derivative **4.17** than with the DVP derived **4.4**, and we concluded that ester **4.17** was too unstable for use as an enzyme substrate. We suspected that 4-nitrophenol was

produced due to DKP formation and hypothesized that this side reaction could be minimized by *N*-methylation of the serine residue.

Initial attempts to prepare Ac-Pro-*N*-MeSer(Bn)-Pro-O*p*NP (**4.24** in Scheme 4.7) were hindered by the propensity of the *N*-methyl serine proline dipeptide esters to undergo DKP formation which is a common occurrence with *N*-methylated dipeptide esters.[204] In order to circumvent this problem we elected to begin the synthesis by reducing Cbz proline to alcohol **4.18** which was protected with a TBS group giving **4.19**. Tripeptide **4.21** was then constructed by standard peptide coupling techniques without the possibility of DKP formation. The TBS group was removed by treatment with mild acid and the carboxylic acid was restored by a TEMPO bleach oxidation to give **4.23**. Finally, the *p*NP ester was installed using 4-nitrophenyl trifluoroacetate in pyridine. The rate of *p*-nitrophenol production in buffer at pH 7.5 was much slower with *N*-methylated **4.24** compared to the unmethylated **4.17** (Figure 4.8). Therefore, we believe that esters **4.4** and **4.24** are good candidates to examine as potential IgA1P$_{Hinf1}$ substrates.



Scheme 4.7. Synthesis of Ac-Pro-MeSer(Bn)-Pro-O*p*NP **4.24.**

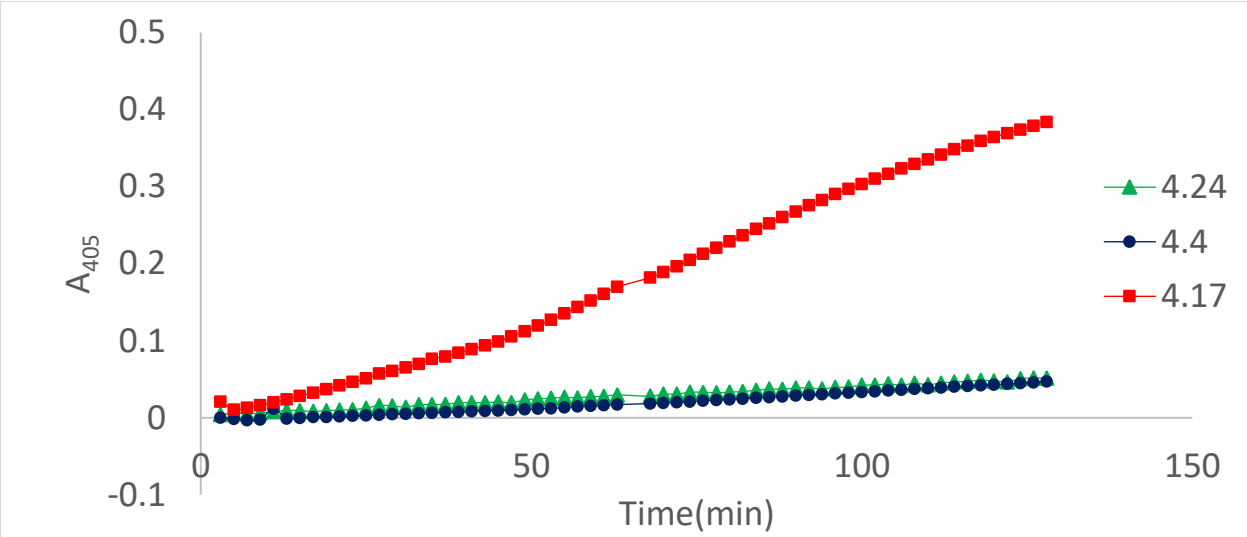


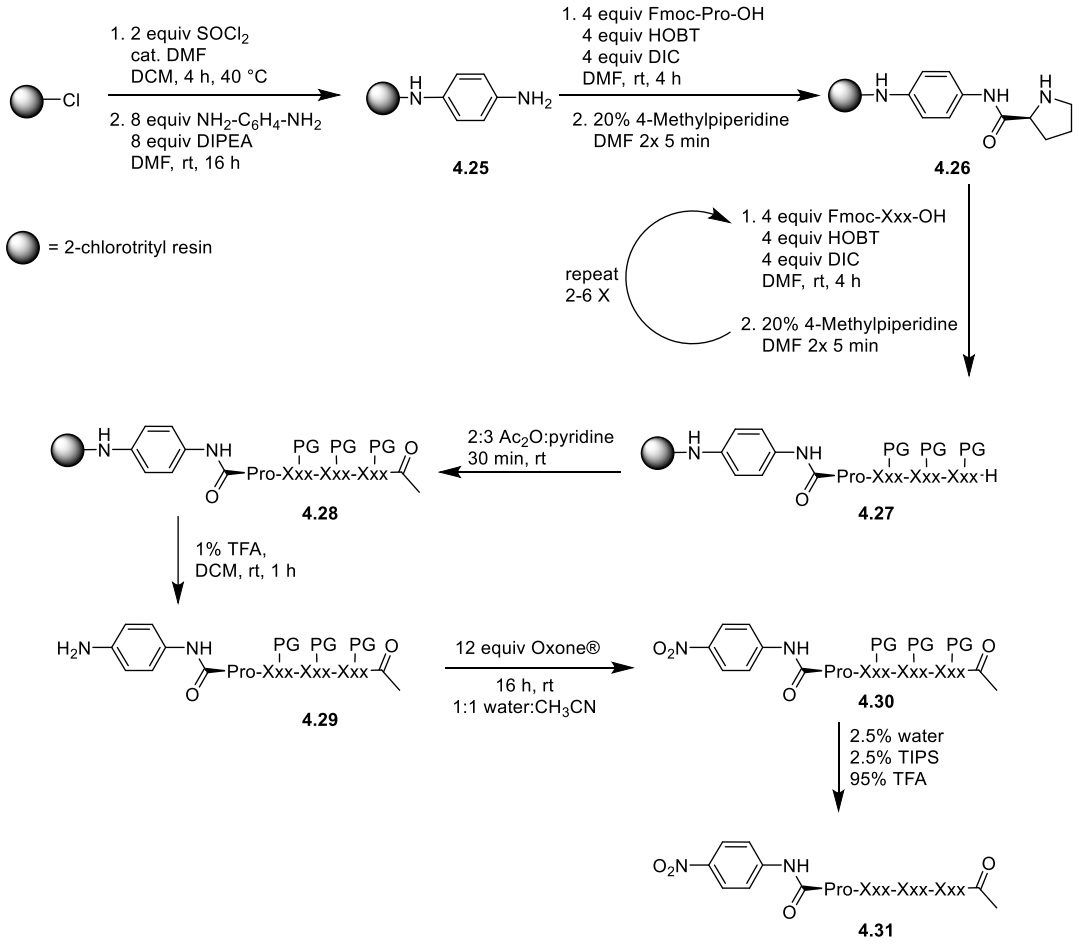
Figure 4.8. Hydrolysis of 4-nitrophenyl esters in buffer over time. A 200 µM solution of the ester in 20 mM TRIS 50 mM EDTA at pH 7.5 is incubated at 37 °C for 2 h in triplicate and absorbance at 405 nm is recorded every 2 minutes.

4.2.2 — *p*-Nitroanilide based IgA1P substrates

Although esters **4.4** and **4.24** were more stable than **4.17**, we were concerned that these might still be too labile for enzyme studies. The $IgA1P_{Hinf1}$ is produced with a 6 histidine C-terminal tag which aids in purification.[188] The imidazole side chains of this tag might catalyze the hydrolysis of the 4-nitrophenyl esters, or the ester might react with any other surface exposed nucleophilic amino acid.[205,206] To mitigate the possibility of hydrolysis or attack by nucleophilic residues (other than the catalytic serine) we decided to prepare the more stable 4-nitroanilides.

Two series of 4-nitroanilides were prepared according to the Fmoc solid phase method of Abbenante (Scheme 4.8).[207] The syntheses were performed on polystyrene-supported 2'-chlorotrityl resin which was loaded with 1,4-diaminobenzene to give **4.25**. The peptide chain was constructed using standard Fmoc-SPPS techniques, amino acid couplings were performed using

DIC/HOBt and Fmoc deprotection was performed using 20% 4-methylpiperidine in DMF. This was repeated as necessary to give peptides of type **4.27**. Some of the peptides contained amino acid side chains that required protection. *tert*-Butyl groups were used to protect Thr or Asp residues and trityl groups was used to protect Asn residues, Gln was used without protection. Serine residues had a permanent *O*-benzyl group on its side chain. The *N*-terminus was capped with acetic anhydride and the peptides were cleaved from resin using 1% TFA to give 4-aminoanilides **4.29**. Oxidation to nitro anilides **4.30** was accomplished with Oxone® and final deprotection was performed using TFA for those peptides bearing temporary protecting groups to give the final peptides **4.31.** These were subjected to normal-phase flash chromatography which gave the desired final peptides in all but one example (Table 4.3).



Scheme 4.8. Synthesis of 4-nitroanilides based substrates.

Similar to the 4-nitrophenyl esters, we prepared the Ac-Asp-Val-Pro and Ac-Pro-Ser(Bn)-Pro C-terminal 4-nitroanilide derivatives (entries 1 and 2 in Table 4.3). We also extended these peptides with the residues found in the hinge region and self-cleavage site to yield peptides with 4 to 7 residues for a total of 10 peptides (entries 3-10, Table 4.3). We anticipated that there would be a tradeoff between longer peptides which could mimic the natural substate to a greater extent and smaller peptides which could more easily enter bypass the active site lid, but we were unsure what the optimal length would be. The analytical HPLC chromatograms of peptides **4.32** and **4.37-4.40** showed single peaks. Peptides **4.33-4.36** showed a minor peak which eluted slightly ahead of the major peak and accounted for 5 to 10% of the

material. We were unable to achieve baseline separation by HPLC and this minor product was not separated. No additional peptides could be identified by HRMS. It is possible that these additional peaks arise from epimers; however, peptides that are proline-rich sequences are known to give rise to multiple cis-trans conformers, and such conformers have been reported to be separable by HPLC in some cases.[208] Therefore, it is possible that the extra peak in the HPLC chromatograms of compounds **4.33**-**4.36** is due to a conformational isomer. One method to distinguish between epimers and conformational isomers would be to analyze these peptides by HPLC at increased temperatures. Finally, the presence of peptide **4.41** was confirmed by HRMS; however, after subjecting the crude material to flash chromatography, several peaks were observed in the HPLC chromatograph and we were unable to obtain this compound in high enough purity for enzyme studies (see the Appendix for copies of HPLC and HRMS data). The compounds in Table 4.3 were prepared by Yishu Zang, an undergraduate student in the Taylor group, under the supervision of this author.

Table 4.3. Yield, retention times, and mass spectrometric analyses of *p*-nitroanilides **4.32-4.41**.

Entry	Peptide	Retention time(min)[a]	Yield(%)	*m/z* (calc.)	*m/z* (obs.)
1	Ac-Pro-Ser(Bn)-Pro-pNA (**4.32**)	26.0	46	552.2453(M+H)	552.2474
2	Ac-Thr-Pro-Ser(Bn)-Pro-pNA (**4.33**)	24.5	57	653.2930(M+H)	653.2925
3	Ac-Pro-Thr-Pro-Ser(Bn)-Pro-pNA (**4.34**)	24.5	31	750.3457(M+H)	772.3295
4	Ac-Pro-Pro-Thr-Pro-Ser(Bn)-Pro-pNA (**4.35**	25.0	30	853.4067(M+Li)	853.4104
5	Ac-Thr-Pro-Pro-Thr-Pro-Ser(Bn)-Pro-pNA (**4.36**)	24.5	27	948.4462(M+H)	948.4459
6	Ac-Asp-Val-Pro-pNA (**4.37**)	21.5	11	492.2089(M+H)	492.2089
7	Ac-Ala-Asp-Val-Pro-pNA (**4.38**)	25.5	12	585.2279(M+Na)	585.2289
8	Ac-Gln-Ala-Asp-Val-Pro-pNA (**4.39**)	22.5	28	691.3046(M+H)	691.3072
9	Ac-Ile-Gln-Ala-Asp-Val-Pro-pNA (**4.40**)	28.5	14	826.3706(M+Na)	826.3747
10	Ac-Asn-Ile-Gln-Ala-Asp-Val-Pro-pNA (**4.41**)	-	trace	918.4316(M+H)	918.44299

[a]Gradient: 5:95 MeCN:H_2O (+0.1% TFA) to 95:5 MeCN:H_2O (+ 0.1% TFA) over 40 min.

To determine the stability of the 4-nitrophenyl amides in aqueous solution, peptide **4.37** was dissolved in 20 mM Tris, 50 mM EDTA buffer at pH 7.5 and incubated at 37 °C for 24 hours (Figure 4.9). The absorbance was recorded at 380 nm (the λ_{max} of 4-nitroaniline) and no significant increase was observed over that time period.

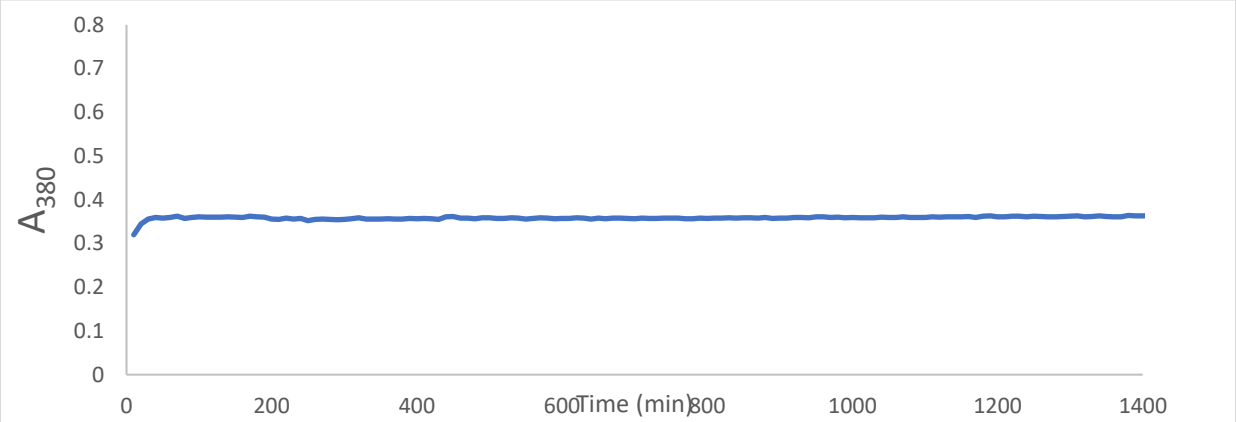


Figure 4.9. Hydrolysis of 4-nitrophenyl esters in buffer over time. A 250 µM solution of **4.37** in 20 mM TRIS, 50 mM EDTA at pH 7.5 was incubated at 37 °C for 24 h and the absorbance at 380 nm was recorded every 10 minutes.

4.2.3 — Boronic acid based IgA1P inhibitors

The first potential inhibitors that we prepared were peptidyl boronic acids. There were several reasons for this. First, it had already been demonstrated that small peptides with C-terminal boronic acids act as inhibitors of IgA1 proteases (Table 4.2).[197] Additionally, peptidyl boronic acids and esters are well-known inhibitors of other serine proteases and some are found in FDA approved medications.[209,210] Finally, boronic acids have been used as ligands for affinity chromatography.[211-213] It occurred to us that a potent boronic acid inhibitor of an IgA1P could be used to prepare an affinity column which would facilitate purification of the enzyme.

Bachovchin had already demonstrated that Boc-Pro-Thr(OBn)-boroPro-OH was a good inhibitor of $IgA1P_{Hinf1}$ (Table 4.2).[197] However, this molecule is more similar to the cleavage site of other IgA1 proteases. The cleavage site of $IgA1P_{Hinf1}$ has a -Pro-Ser-Pro- sequence, therefore; we anticipate that peptide **4.42**, which contains a serine in place of the threonine, would be a better inhibitor (Figure 4.10). The self-cleavage site of $IgA1P_{Hinf1}$ occurs after an Asp-Val-Pro sequence, therefore **4.43** would also be a candidate inhibitor.

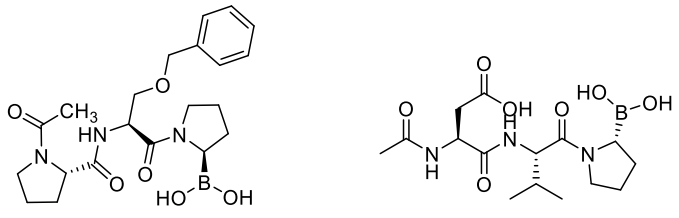
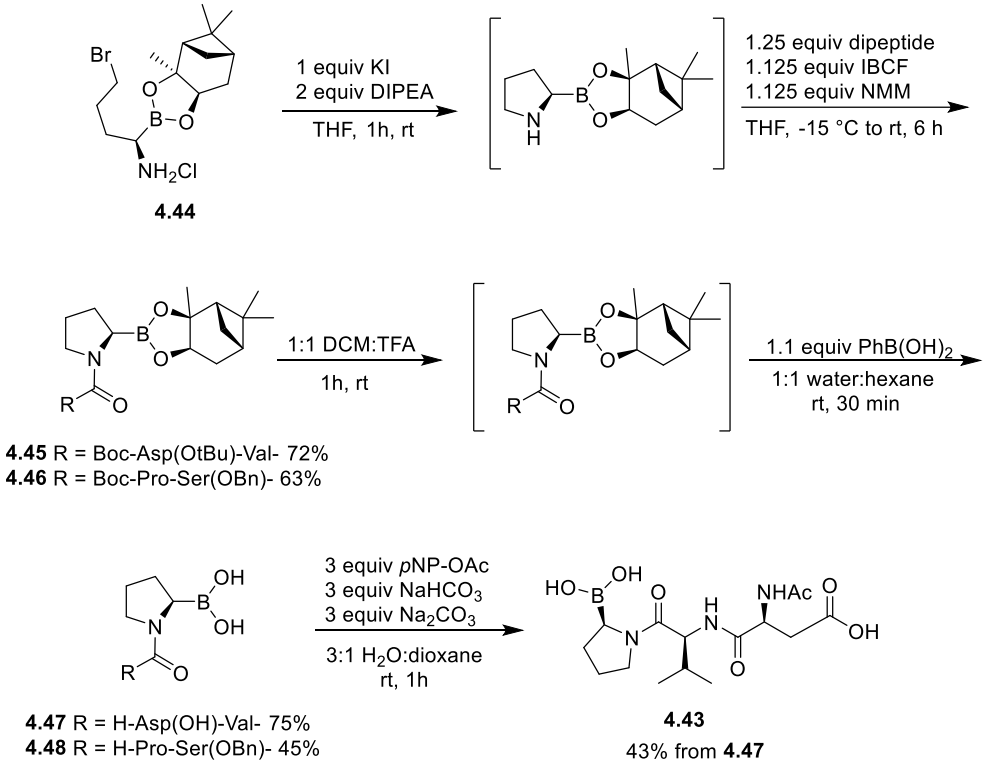


Figure 4.10. Tripeptides bearing C-terminal boronic acids as potential inhibitors of $IgA1P_{Hinf1}$.

It has been observed that the purification of boronate esters by chromatography can often be problematic due to over absorption on silica gel which leads to tailing, poor resolution, and low recovery.[214] Therefore, it was desirable that the synthesis of the tripeptides be done with as few chromatography steps as possible. We began by preparing precursor **4.44** according to the

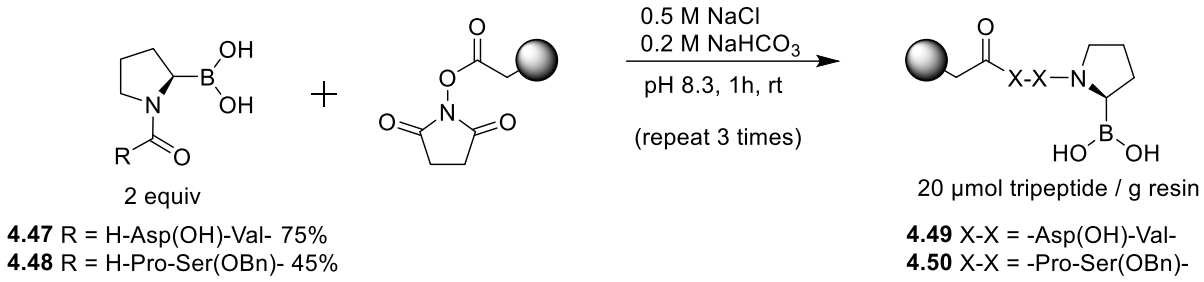
method of Wityak (Scheme 4.9).[215] Amine **4.44** was cyclized in the presence of DIPEA with potassium iodide added as a nucleophilic catalyst (Scheme 4.10). The reaction mixture was cooled to -15 °C, and then a dipeptide, activated as a mixed anhydride with isobutyl chloroformate and NMM, was added to the mixture. The resulting tripeptides were purified by flash chromatography. Deprotection was performed in two steps using TFA to remove the *tert*-butyl protecting groups, followed by transesterification with phenyl boric acid which gave **4.47** and **4.58** as well as phenylboronic acid pinanediol ester, which partitioned into hexane. The final tripeptides were purified by RP HPLC. Tripeptide **4.47** was acylated with 4-nitrophenyl acetate to give the candidate inhibitor **4.43**. Peptide **4.48** will be acylated in the near future.



Scheme 4.9. Synthesis of boronic acid tripeptides.

Tripeptides **4.47** and **4.48** were also loaded onto Sepharose solid support column using a standard procedure.[216] A solution of the tripeptide in was incubate with the resin for 1 hour, then

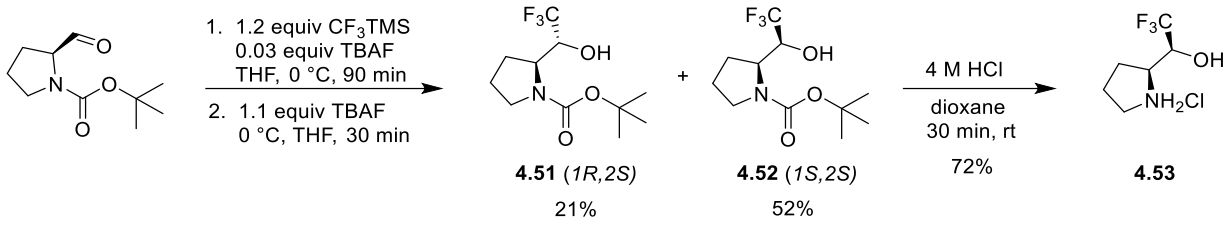
the amount of bound tripeptide was estimated removing an aliquot of the loading cocktail and determining the amount of tripeptide remaining by RP HPLC and comparing this to a standard of known concentration. The loading step was repeated three times until there was no change in the concentration of tripeptide, giving a total of approximately 20 µmols of tripeptide per gram of resin. This resulted in resins **4.49** and **4.50** which are potential affinity columns which may be useful in the isolation of active IgA1P, assuming that **4.42** and **4.43** are inhibitors of IgA1P.



Scheme 4.10. Loading of boronic acid tripeptides onto a solid support.

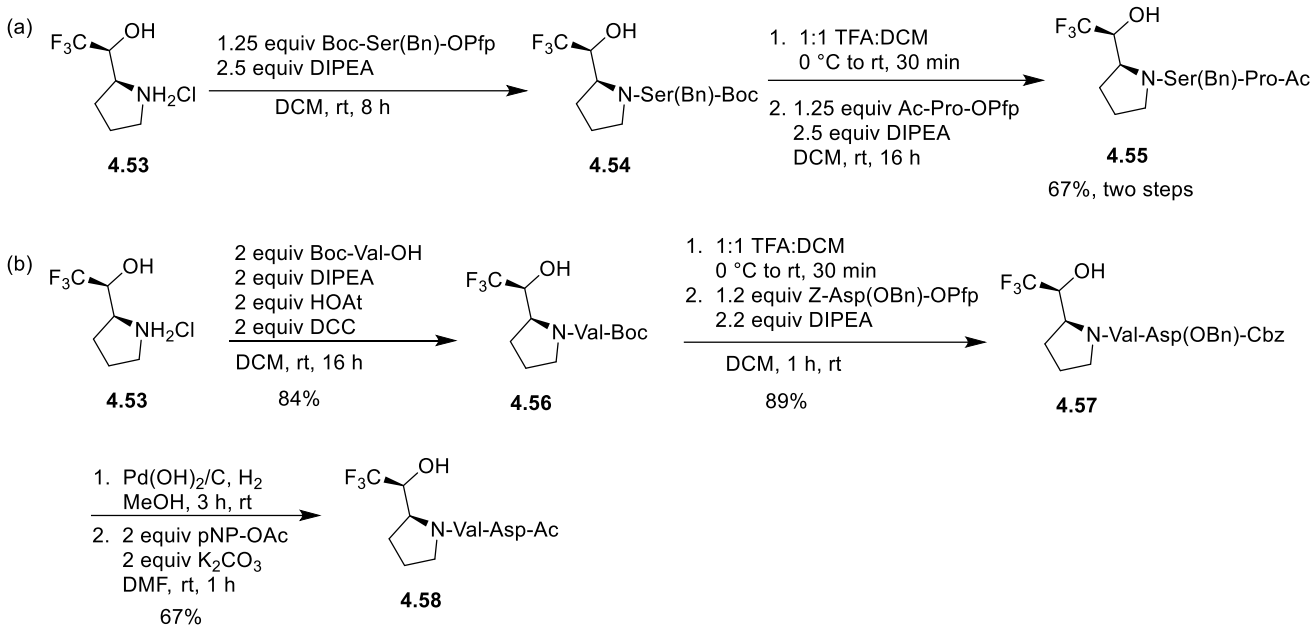
4.2.4 — Trifluoromethyl alcohol and ketone based IgA1P inhibitors

The next class of potential inhibitors that were preprared were peptidyl trifluoromethyl ketones as these types of compounds have been shown to be good inhibitors of serine proteases (See Section 1.1.2 for details). We also prepared the corresponding trifluoromethyl alcohols as peptidyl trifluoromethyl alcohols are also known to be inhibitors of serine proteases, although they tend to be less potent than the corresponding ketones.[217] All of the inhibitors were prepared from trifluoromethyl alcohol **4.53** (Scheme 4.11).[218] Trifluoromethyl alcohols **4.51** and **4.52** were prepared from Boc prolinal (Scheme 4.11). Diastereomers were separated by flash chromatography. Only **4.52**, the major isomer, was carried forward. The Boc group in **4.52** was removed using HCl in dioxane to give **4.53**.



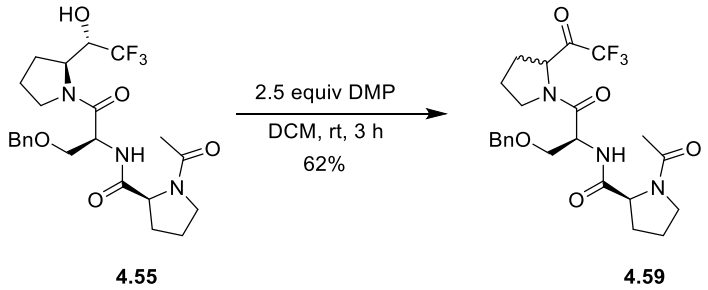
Scheme 4.11. Synthesis of trifluoromethyl prolinol **4.53.**

Tripeptides containing **4.53** were prepared using standard solution phase peptide chemistry (Scheme 4.12). Dipeptides **4.54** and **4.56** were found to be single diastereomers which indicated that no epimerization of the α-carbon occurred in the trifluoromethylation of Boc-prolinal. These were extended by acidic deprotection of the Boc groups followed by acylation with Pfp esters of the next amino acid. Multiple conformers are expected for peptides containing multiple prolines.[208] The NMR spectrum of tripeptide **4.55** indicated that it existed as a mixture of several rotamers. The peaks corresponding to the various rotamers coalesced to a single set of peaks corresponding to one of the rotamers upon heating to 100 °C. The N-terminal acylation of **4.57** was performed using 4-nitrophenyl acetate which avoided *O*-acylation at the trifluoromethyl alcohol of **4.58**.



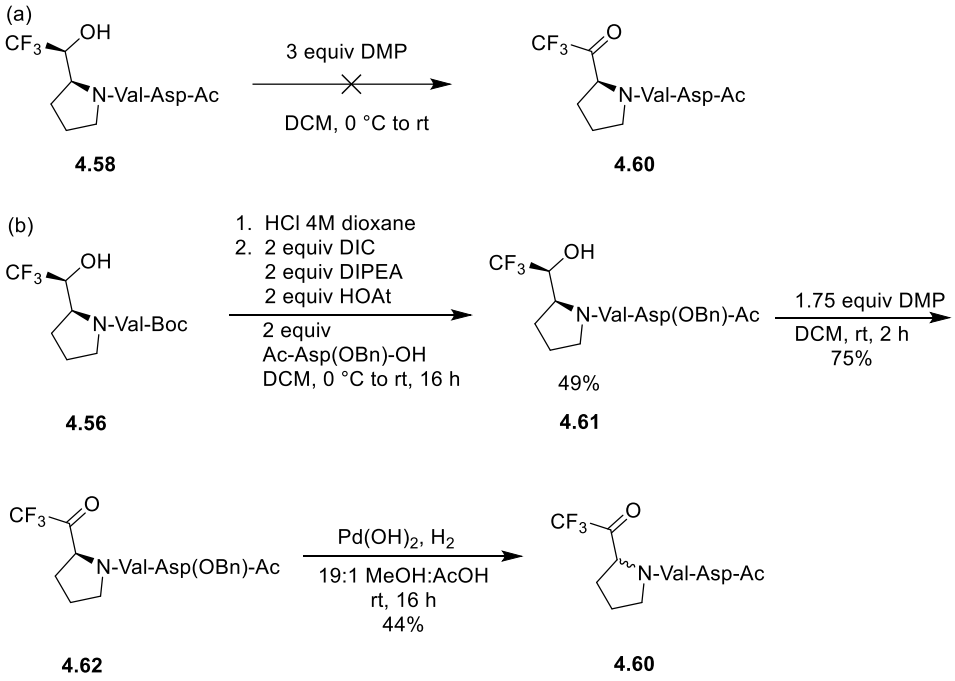
Scheme 4.12. Preparation of trifluoromethyl alcohol tripeptides.

Next, the tripeptides were oxidized to the trifluoromethyl ketones. Trifluoromethyl alcohols are reported to resist oxidation with DMSO or metal-based reagents, and Dess-Martin reagent is the method of choice, although even this typically requires an excess of the oxidant.[218] This approach successfully yielded the PSP derivative **4.59**, which was obtained as a mixture of isomers which may arise from epimerization of the proline α-carbon or from conformational isomers (Scheme 4.13). ^{19}F NMR spectroscopy indicated that the **4.59** existed primarily in the hydrated form in 4:1 D_2O:CD_3CN solvent, which is typical for trifluoromethyl ketones.[219]



Scheme 4.13. Dess Martin oxidation of trifluoromethyl alcohol 4.55.

Unfortunately, the same transformation failed on alcohol **4.58** (Scheme 4.14 a). However, the transformation was successful if a benzyl ester protecting group on the aspartate side chain was present (Scheme 4.14 b). Ketone **4.62** was obtained from **4.61** as a single isomer as judged by ^{19}F NMR; however, after hydrogenolysis of the benzyl group, the final ketone **4.60** was obtained as a mixture of isomers, presumably due to epimerization of the proline α-carbon



Scheme 4.14. Synthesis of Ac-Asp-Val-Pro trifluoromethyl ketone 4.60.

4.2.5 — Sulfonamide based IgA1P inhibitors

Unlike the trifluoromethyl compounds and boronic acids that were prepared above, ß-keto-α,α-difluorosulfonamides can be incorporated into peptidomimetics at an interior position rather than strictly at the C-terminus. Therefore, we began our studies of sulfonamide-based inhibitors by considering artificial substrate **4.1**. The termini of **4.1** are labeled with a FRET pair, however these were not necessary for hydrolysis by the enzyme and **4.63** is also a substrate.[199]

Although **4.63** is derived from the cleavage site of $IgA1P_{Ngon2}$, this was a substrate for proteases from several different species, including $IgA1P_{Hinf1}$, which all cleaved in the same location between proline and alanine (Figure 4.11).[200] We reasoned that we could create an analog by substituting a ß-keto-α,α-difluorosulfonamide into the structure to give **4.64** which we hypothesized would be able to enter the active site of the enzyme and form a covalent adduct with the active site serine. We further reasoned that since **4.1** was a general substrate of IgA1 proteases then **4.64** might be a general inhibitor.

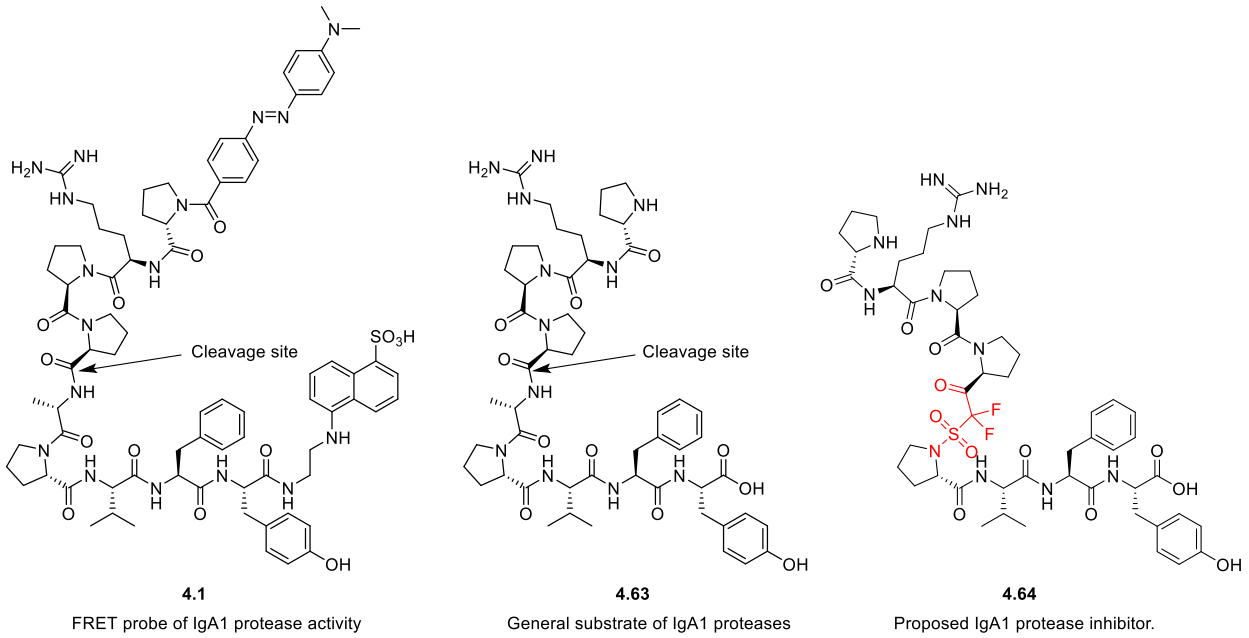
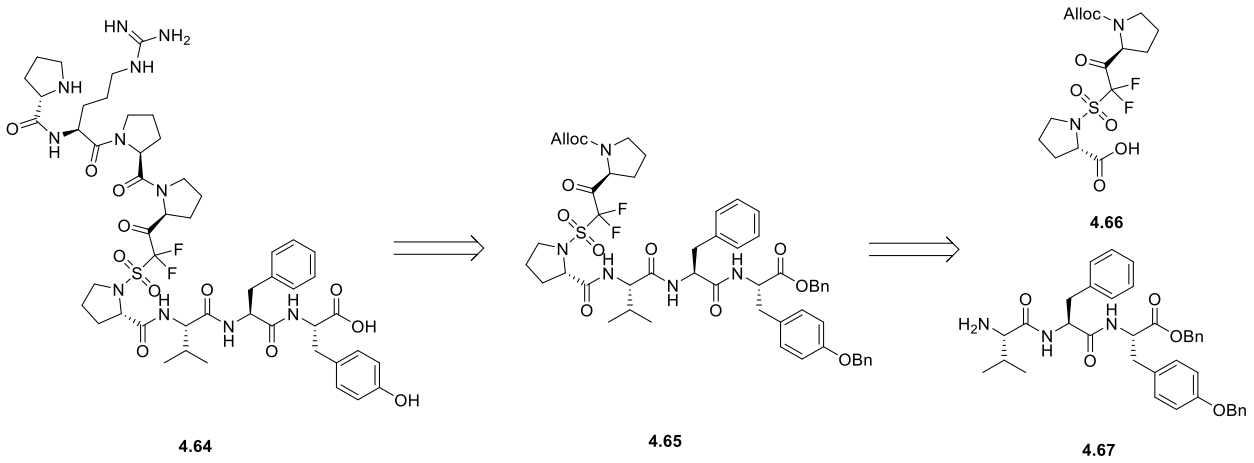


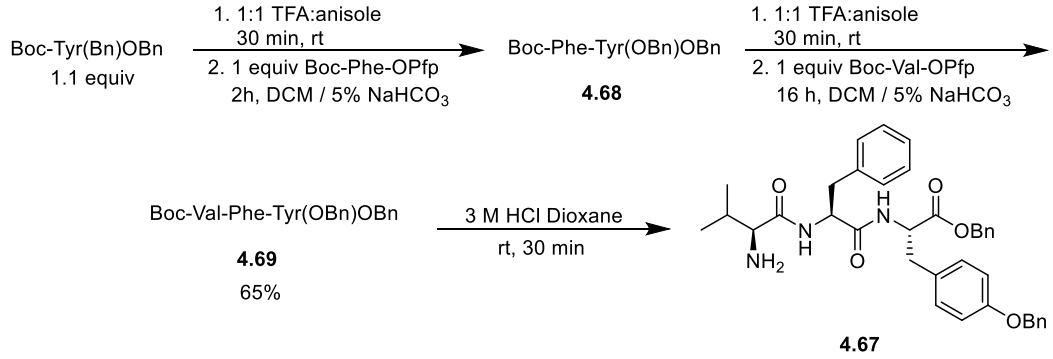
Figure 4.11. Proposed General IgA1P inhibitor derived from an artificial substrate.

We envisioned that **4.64** would be prepared by extension of peptide **4.65** using standard peptide couplings (Scheme 4.15). **4.65** would be prepared by condensation of fragments **4.66** and **4.67** meanwhile **4.67** could be assembled by solution phase peptide synthesis while fragment **4.66** would be prepared using the techniques described in Chapter 1.



Scheme 4.15. Retrosynthesis of **4.64.**

The first step was to prepare fragment **4.67** (Scheme 4.16). BocTyr(Bn)OBn was deprotected with TFA followed by acylation with the Pfp ester of Boc-Phe to give dipeptide **4.68**. Similar conditions with the Pfp ester of Boc-Val gave the C-terminal tripeptide **4.69**. Deprotection with HCl in dioxane gave amine **4.67** which was ready to be coupled to the central fragment **4.66.**



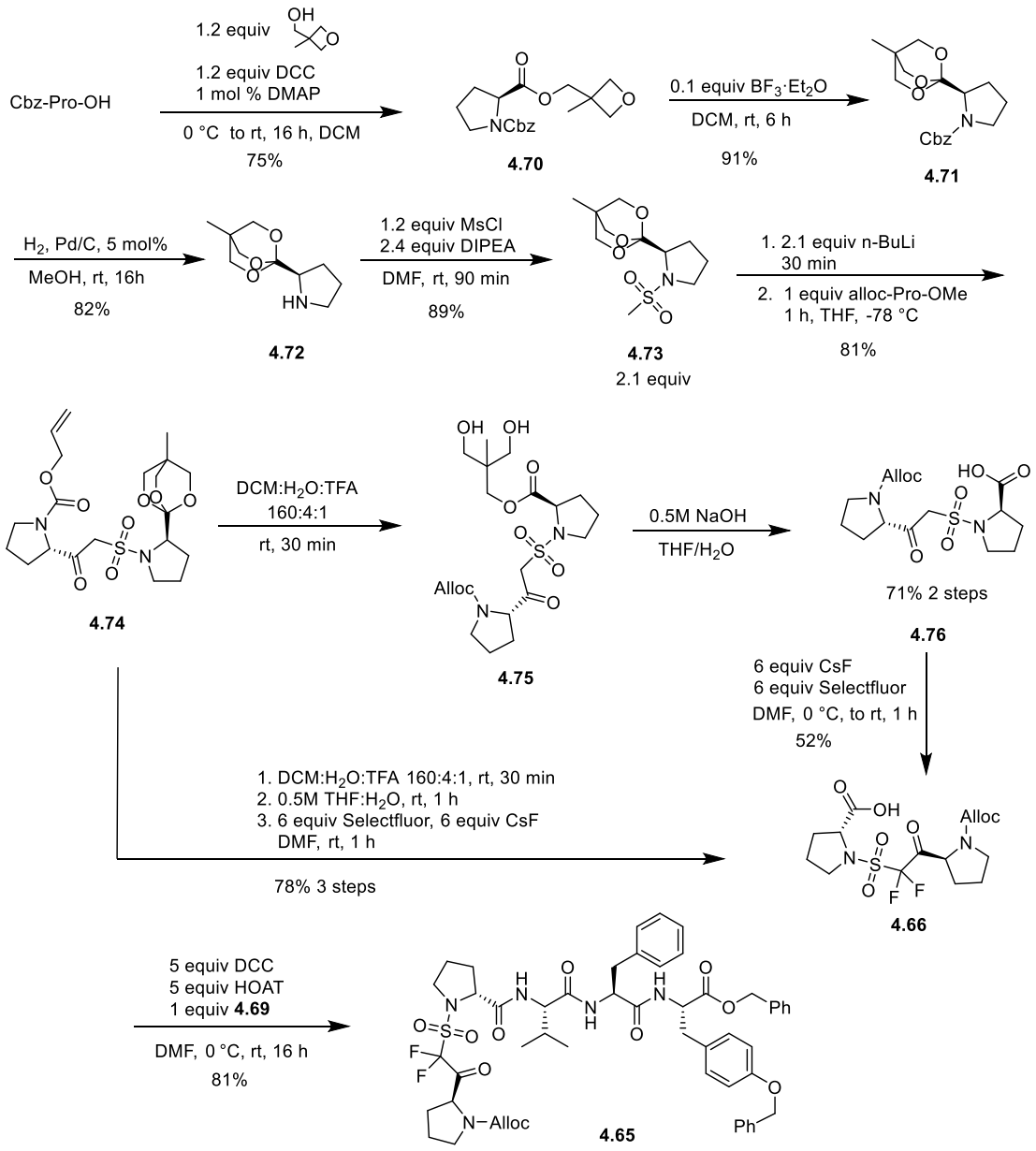
Scheme 4.16. Synthesis of peptide fragment **4.67.**

The synthesis of **4.66** began with the installation of an OBO protecting group on Cbz-proline. This was accomplished in two steps using the general method of Zhdanko and Nenajdenko (Scheme 4.16).[220] This gave **4.71** with the carboxylic acid protected as an ortho ester

which tolerates strong bases and nucleophiles without risk of epimerization. Next, the Cbz-protecting group was removed by hydrogenolysis to furnish **4.72**. The mesyl group was installed with MsCl giving **4.73**.

With the OBO group successfully installed, the most acidic site in **4.73** is the sulfonamide methyl group. We expected **4.73** to be slightly less acidic than a methyl sulfone which would mean a pK_a in the range of 25 to 30.[221,222] The lithium salt of **4.73** reacted with alloc-Pro-OMe to give **4.74** The alloc group is selected here because OBO group removal requires treatment with acid followed by base, and because it can be removed in the presence of the benzyl groups used to protect fragment **4.67.**

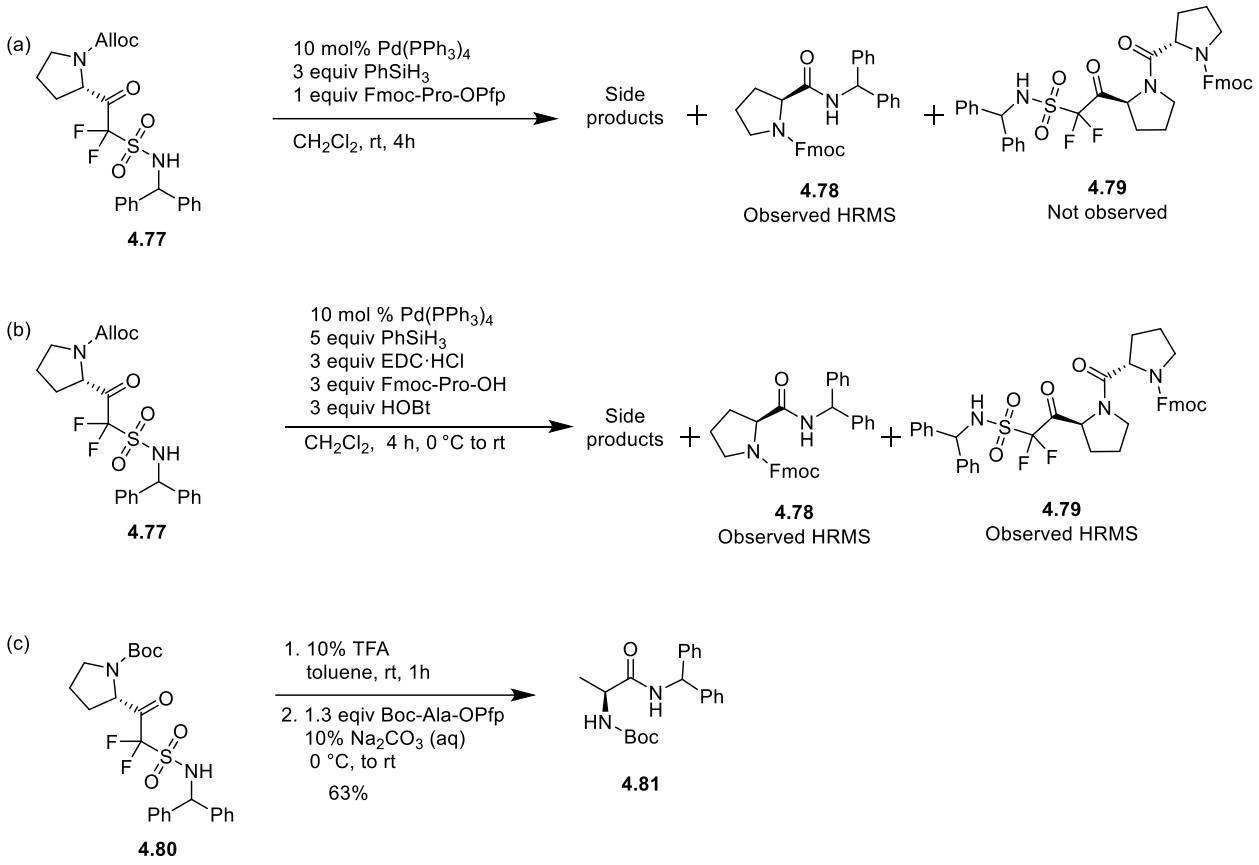
OBO deprotection was accomplished according to a literature procedure using mild aqueous acid to give ester **4.75**, which was not isolated and was hydrolyzed under basic conditions to give acid **4.76**.[220] Electrophilic fluorination of **4.76** using Selectfluor and CsF gave **4.66** in modest yield. The yield was significantly improved if **4.76** was not isolated in which case **4.66** was obtained in 78% yield from **4.74**. Sulfonamide **4.66** is similar to a dipeptide; however, it contains a central sulfonamide group rather than an amide. Therefore, there is little risk of epimerization at this step since sulfonamides do not form oxazolones, which is a problem frequently encountered with amides.[223] Coupling of fragments **4.66** and **4.67** using DCC/HOAt proceeded smoothly to give **4.65**.



Scheme 4.17. Synthesis of peptidiomimetic **4.65**.

The next step was to remove the N-terminal alloc protecting group. Coupling tripeptide Cbz-Pro-Arg(NO_2)-Pro-OH followed by hydrogenolysis would yield **4.64**. However, before we attempted this transformation, we wanted to test the conditions on a simpler substrate to determine if dipeptides could be prepared from ß-keto-α,α-difluorosulfonamides derived from α-amino acids. To this end, we prepared alloc-protected **4.77** (see Table 1.5 entry 18) anticipating

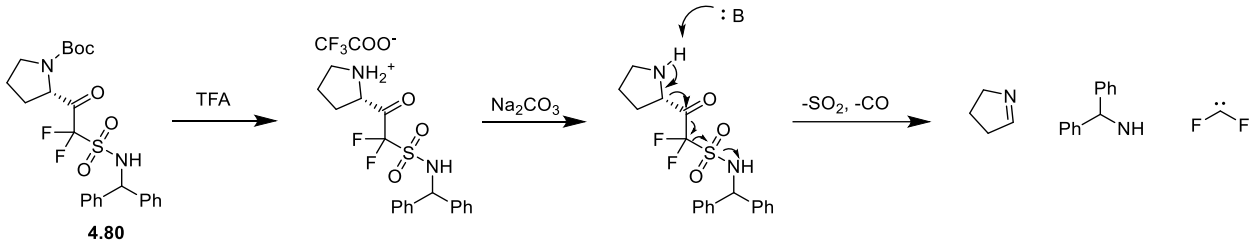
that we would be able to remove the alloc group with $Pd(PPh_3)_4$ and phenylsilane and then obtain dipeptide **4.79** using a Pfp ester (Scheme 4.18a). Unfortunately, we found that **4.79** was not observed in the crude reaction mixture which consisted of numerous side products, one of which was amide **4.78** as determined by HRMS. When we attempted the reaction using Fmoc-Pro-OH and EDC/HOBt, we observed a small amount of **4.79** by HRMS, but the crude reaction mixture was still dominated by unidentified side products including **4.78** (Scheme 4.18b). We also prepared Boc protected **4.80** (see Table 1.5, entry 17) and attempted the transformation (Scheme 4.18c). In this case the dominant product was **4.81**; however, the reaction contained fewer unidentified side reactions and **4.81** was readily isolated.



Scheme 4.18. Attempted synthesis of ß-keto-α,α-difluorosulfonamide dipeptides.

The side reaction to produce **4.81** obviously involves the production of amino diphenylmethane, which might arise by hydrolysis of the sulfonamide. However, similar products were also observed under anhydrous conditions (Scheme 4.18a and b), furthermore ß-keto-α,α-difluorosulfonamides have been shown to hydrolyze under basic aqueous conditions to give difluoromethanesulfonamides, not amines (Chapter 2 Section 2.2.1). A plausible mechanism that explains the side product is shown in Scheme 4.19. First TFA deprotection yields an ammonium salt, which does decompose in solution as determined by ^{19}F NMR. When base is added a series of elimination reactions occur liberating aminodiphenylmethane as well as sulfur

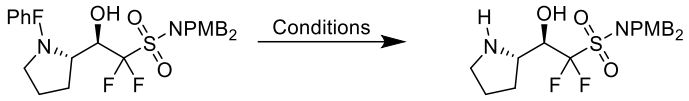
dioxide, difluorocarbene, carbon monoxide and an imine. Sheehan and Frankenfeld proposed a similar mechanism to explain the base promoted decarbonylation of an α-amino acid yielding an imine, carbon monoxide and toluene sulfonic acid.[224] Despite some experimentation, we were unable to perform the desired transformation cleanly and turned to the synthesis of sulfonamide-based inhibitors derived from the Pro-Ser-Pro and Asp-Val-Pro tripeptides.



Scheme 4.19. Proposed mechanism for the decomposition of 4.80.

We began with proline derivative **4.82** (See Table 2.8 entry 4). The diastereomers of **4.82** were readily separable by flash chromatography but only the major (R,S) isomer was used (see chapter 2 for details). Various methods were investigated to remove the PhF protecting group (Table 4.4). Hydrogenolysis under a hydrogen atmosphere was sluggish in EtOAc/EtOH even if acid was added (entries 1 and 2). 10% $Pd(OH)_2/C$, H_2, DCM/MeOH, 1% TFA gave a complex mixture (entry 3). In chapter 3, the use of 95:2.5:2.5 TFA:H_2O:TIPS was effective for deprotecting a PhF amine, but in this case led to partial loss of the PMB protecting groups before PhF deprotection was complete (entry 4). Employing 2 equiv of I_2 in refluxing MeOH did not produce any reaction even after 16 h (entry 5). Triethylsilane in DCM/MeOH in the presence or absence of TFA were also unsuccessful (entries 6 and 7). However, performing the reaction with triethylsilane and cat. Pd/C in DCM/MeOH was effective (entry 8) and the reaction rate was increased by the addition of a small amount of acid (entry9).

Table 4.4. Optimization of PhF deprotection from sulfonamide **4.82.**

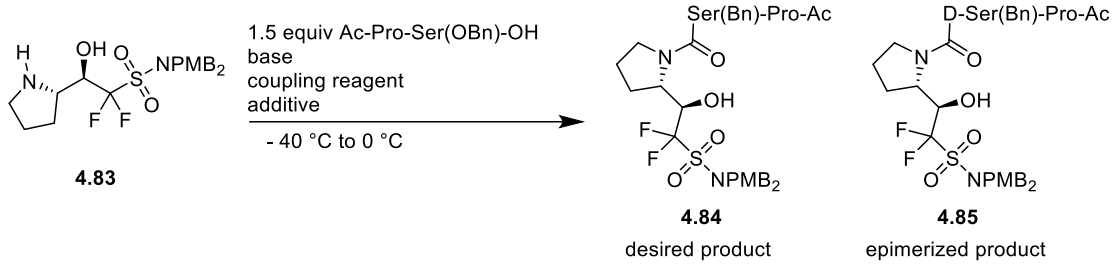


4.82 **4.83**

Entry	Conditions	Result
1	10 wt. % of 20% $Pd(OH)_2/C$, H_2, EtOAc/EtOH (2:3), rt, 16 h	Trace product
2	EtOAC/EtOH/AcOH (12:8:1), H_2, rt, 16 h	Trace product
3	10% $Pd(OH)_2/C$, H_2, DCM/MeOH, 1% TFA	Unknown side reactions
4	95% TFA, 2.5% TIPS, 2.5% water, rt, 16 h	Partial loss of PMB groups
5	2 equiv I_2, MeOH, reflux, 16 h	No reaction
6	DCM:MeOH:TES (1:1:1), 16 h	No reaction
7	DCM:MeOH:TES (1:1:1), 3% TFA, 16 h	No reaction
8	DCM:MeOH:TES (1:1:1), 10 wt. % of 10% Pd/C	Complete after 16 h
9	DCM:MeOH:TES (1:1:1), 10 wt. % of 10% Pd/C, 3% TFA	Complete after 30 min

Next a fragment coupling was performed between Ac-Pro-Ser(Bn)-OH and amine **4.83** using a variety of coupling reagents on a small scale (Table 4.5). Yields were estimated by HPLC, and all of the coupling reagents except for DPPA showed some degree of epimerization at the serine α-carbon. DPPA is reported to give particularly low levels of epimerization in segment couplings.[225]

Table 4.5. Optimization of the coupling conditions for tripeptide **4.84.**



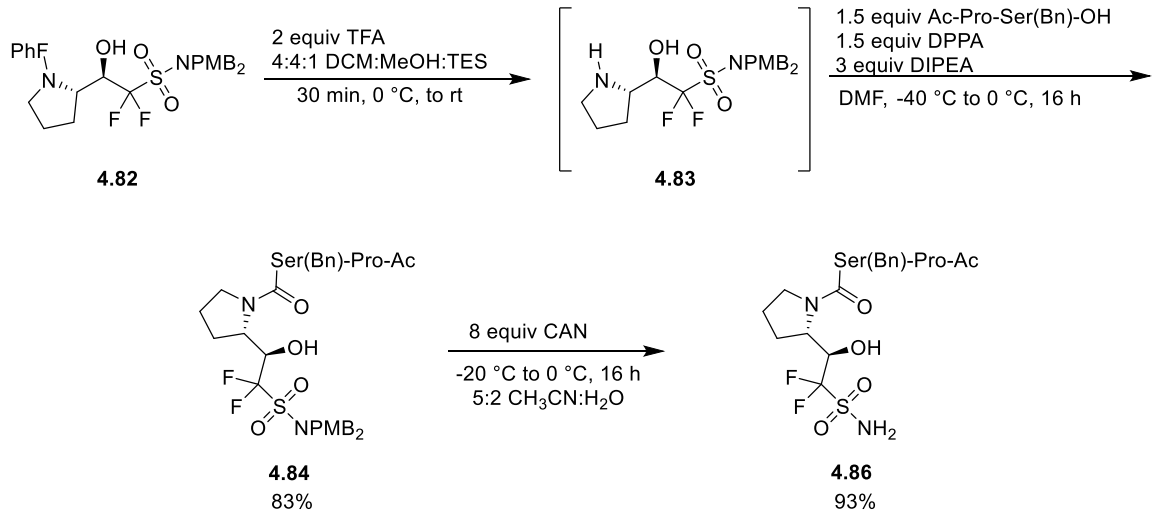
4.83 **4.84** desired product **4.85** epimerized product

Entry[a]	Coupling agent 1.5 equiv	Base 3 equiv	Solvent	Additive 1.5 equiv	**4.85** (yield 2 h)	**4.84** (yield 2 h)	**4.85** (yield 24 h)	**4.84** (yield 24 h)
1	DEPBT	DIPEA	THF	-	17%	52%		
2	DEPBT	K_2CO_3	THF	-	0%	8%	7%	75%
3	DPPA	DIPEA	DMF	-	0%	33%	0%	72%
4	DPPA	K_2CO_3	DMF	-	0%	28%	0%	33%
5	HATU	DIPEA	DMF	-	11%	16%		
6	HCTU	DIPEA	DMF	-	19%	17%		
7	PyAOP	DIPEA	DMF	-	8%	31%		
8	HATU	DIPEA	DMF	HOAt	25%	17%		
9	HCTU	DIPEA	DMF	HOAt	24%	17%		
10	PyAOP	DIPEA	DMF	HOAt	15%	17%		

[a]Reactions were performed with 10 mg of **4.83** and the indicated base, additives, and dipeptide in 1.4 mL of solvent. The mixture was cooled to -40 °C then the activating agent was added in 100 µL of solvent then the mixture was warmed to 0 °C.

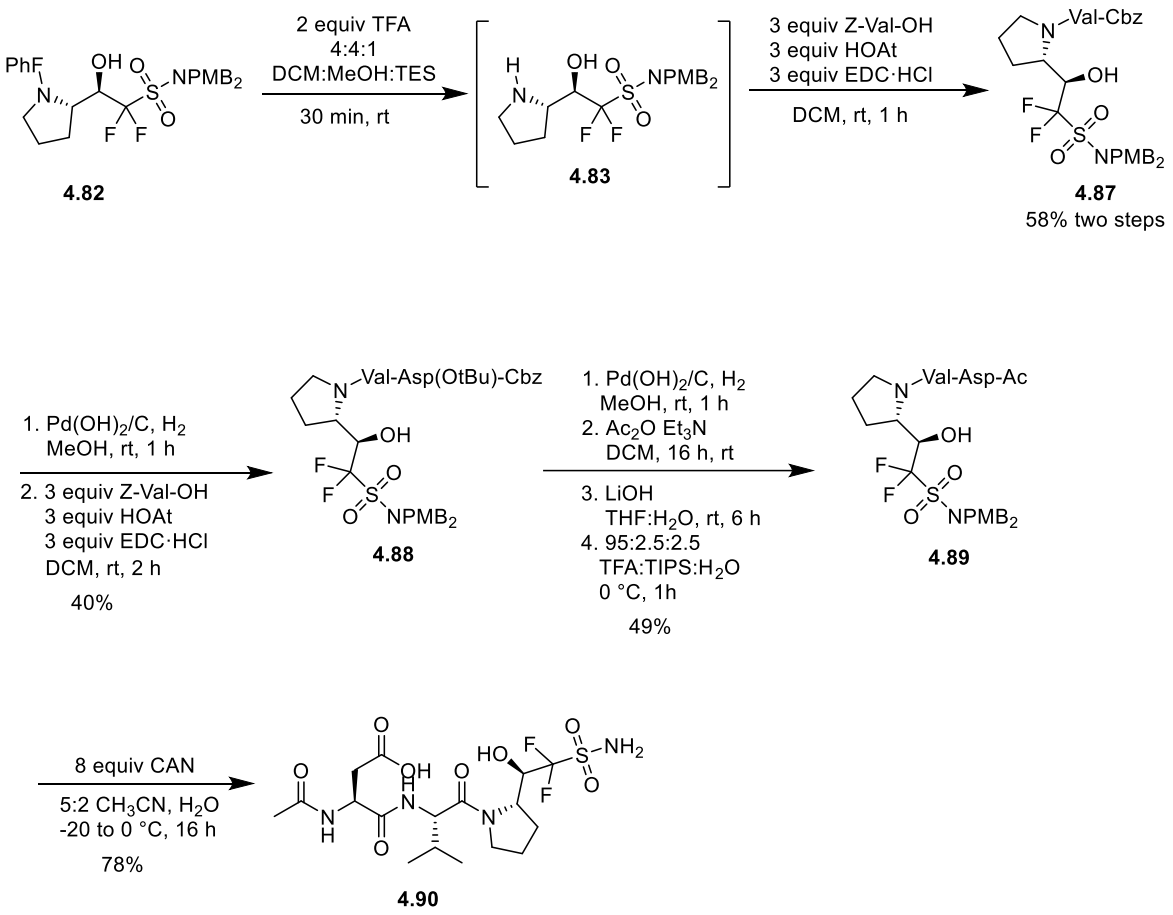
When the reaction was scaled up, **4.84** was obtained in good yield without detectable epimerization as determined by HPLC (Scheme 4.20). Various methods have been reported for the deprotection of PMB-protected sulfonamides including, $AgSbF_6$ and trimethoxybenzene,[226] $FeCl_3 \cdot 6H_2O$ in DCM or DCE at 60 °C,[227,228] KBr and Oxone in nitromethane,[229] or titanium chloride;[230] however, none of these were successful in our hands. CAN or TFA have also been used.[10] TFA required high temperatures and prolonged reaction times which resulted in competing loss of the benzyl ether. Cerium ammonium nitrate successfully removed the PMB groups without effecting the benzyl ether which provided **4.86** in excellent yield after

purification by flash chromatography. This material was judged to be homogenous by HPLC/HRMS (see Appendix Figures A.23 and A.24) but the presence of at least four conformers made characterization by NMR challenging.



Scheme 4.20. Synthesis of tripeptide 4.86 by fragment coupling with DPPA and oxidative debenzylation.

Unfortunately, the fragment coupling between amine **4.83** and Ac-Asp(OtBu)-Val-OH led to epimerization; therefore, this tripeptide was prepared by stepwise couplings using EDC·HCl and HOAt which provided tripeptide **4.88** albeit in low yields (Scheme 4.21). The N-terminal Cbz group was removed by catalytic hydrogenation however acylation using 4-nitrophenyl acetate failed to provide any product. Therefore, we employed Ac_2O and base, which resulted in acylation of the amine as well as partial acylation of the α-difluoro alcohol. The crude mixture was hydrolyzed with lithium hydroxide followed by TFA to remove the *O*-acylation as well as the *t*-butyl ester giving **4.89**. PMB deprotection proceeded smoothly giving **4.90** using the same oxidative debenzylation conditions as above.



Scheme 4.21. Synthesis of **4.90** by stepwise peptide synthesis followed by oxidative debenzylation.

The next step would be to oxidize the ß-hydroxylsulfonamides to ketones using the Dess-Martin reagent; however, during preliminary studies with this reaction we encountered difficulties in analyzing the reaction mixtures. Specifically, the ketones appeared to give extreme tailing when analyzed by RP-HPLC. This phenomenon, combined with the presence of multiple conformers, the possibility of keto/hydrate mixture, and the high likelihood of epimerization of the carbonyl α-carbon, made assessment of purity challenging. Additional work will be required to adequately analyze these mixtures. However, **4.86** and **4.90** are also candidates for IgA1P$_{Hinfl}$ inhibition.

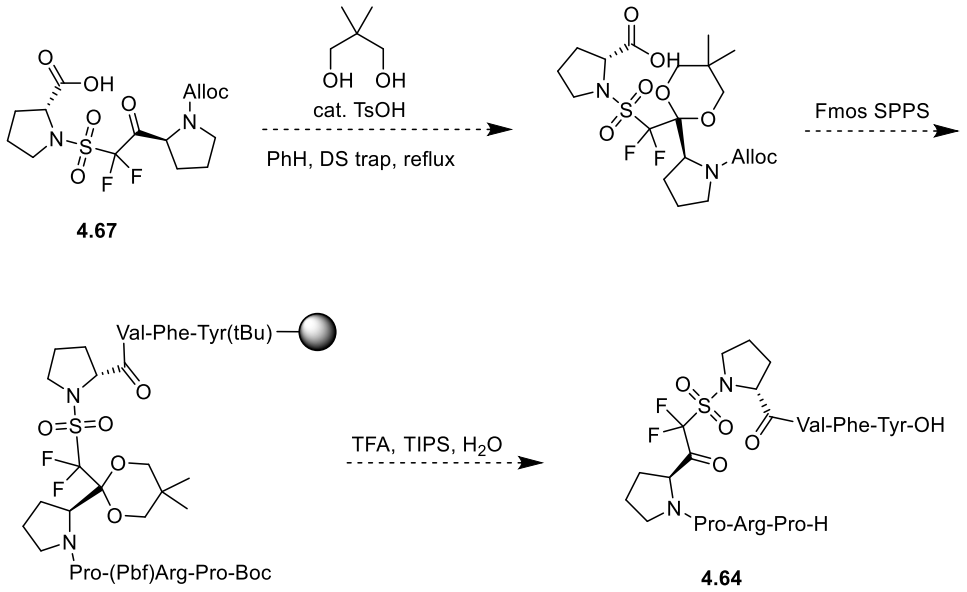
4.3 — Conclusions and Future Studies.

In this chapter we have described the synthesis of some potential substrates and inhibitors of the type 1 IgA1 protease derived from *H. influenzae*. These were designed to mimic the cleavage site in the hinge region of the IgA1 antibody, or the autoproteolytic site which is hydrolyzed by IgA1P during its secretion.

We successfully prepared three tripeptides as potential chromogenic substrates bearing C-terminal 4-nitrophenyl esters. However, these were hydrolytically unstable; therefore, we also prepared nine peptides of varying length bearing the more stable 4-nitroanilide group. Future studies with these compounds will determine which, if any, can be hydrolyzed by $IgA1P_{Hinf1}$. The studies with 4-nitroanilides will also determine the optimal length of the substrate peptides which will influence the rational design of future inhibitors.

A number of potential inhibitors were prepared that consist of tripeptides bearing various non-hydrolyzable groups on the C-terminus. In particular, we investigated C-terminal boronic acids, trifluoromethyl alcohols, trifluoromethyl ketones and ß-hydroxyl-α,α-difluorosulfonamides. The preparation of ß-keto-α,α-difluorosulfonamide tripeptides is underway. We have shown that the boronic acids are readily loaded on to a solid support providing a potential affinity column for IgA1P.

The synthesis of octapeptide **4.64** was attempted, but was unsuccessful due to difficulties extending the chain from the ß-keto-α,α-difluorosulfonamide residue. It should be possible to overcome this side reaction by protecting the ketone (Scheme 4.22). A suitable protecting group would be the 2,2-dimethyl-propane-1,3-diol acetal which as previously been used in peptide synthesis and is removed by the mild acidic deprotection conditions typically used in Fmoc SPPS.[231]



Scheme 4.22. Proposed synthesis of peptide **4.64** utilizing ketone protection.

Future investigations on the inhibition of $IgA1P_{Hinfl}$ will determine which of these candidate molecules are inhibitors of the enzyme. Should some of these compounds be inhibitors, then the x-ray crystal structure of these inhibitors bound to $IgA1P_{Hinfl}$ will be obtained in collaboration with the Holyoak group. Overall, these studies could lead to the development of new antivirulence therapies for treating bacterial infections.

4.4 — Experimental

4.4.1 — General experimental

All reagents and solvents were purchased from commercial suppliers and used without purification unless stated otherwise. Dimethylformamide (DMF) and acetonitrile were distilled from calcium hydride and stored over activated 4 Å molecular sieves. Tetrahydrofuran (THF) was distilled from sodium metal in the presence of benzophenone under nitrogen immediately before use. Dichloromethane was distilled from calcium hydride under nitrogen immediately before use. Benzene and toluene were distilled from sodium in the presence of benzophenone

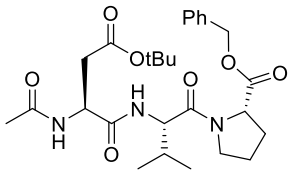
and stored over activated 4 Å molecular sieves. 1,4-diaminobenzene was recrystallized from hot ethanol and stored under argon.

All ^{13}C NMR spectra were proton decoupled. Chemical shifts (δ) for ^{1}H NMR spectra run in $CDCl_3$ are reported in ppm relative to the standard tetramethylsilane (TMS). Chemical shifts for ^{13}C NMR spectra run in $CDCl_3$ are reported in ppm relative to the solvent residual carbon (δ 77.16 for central peak). The samples for high-resolution positive ion electrospray ionization mass spectrometry (HRMS-ESI$^+$) (ion trap) were prepared in 1:1 MeOH/H_2O + 0.1% formic acid.

Analytical HPLC was accomplished with a reversed-phase C18 column (10 µm, 250 mm × 4.6 mm, 1 mL/min flow rate). Preparative HPLC was accomplished using a C18 column (10 µm, 150 mm × 20 mm, 10 mL/min flow rate).

4.4.2 — Experimental procedures for synthesized compounds

Cbz-prolinol was prepared according to a literature procedure.[232] Dipeptides Boc-Pro-Ser(Bn)-OH, Boc-Asp(OtBu)-Val-OH, and Ac-Pro-Ser(Bn)-OH were prepared by the reaction of the appropriate Pfp ester with H-Ser(Bn)-OH or valine. Alloc-*N*-methyl-*O*^{3}-benzyl-serine was prepared according to method of Zhang.[233]

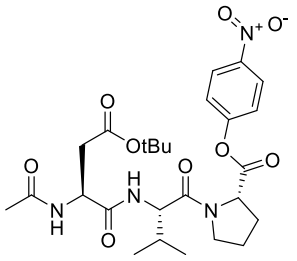


Ac-Asp(OtBu)-Val-Pro benzyl ester (**4.7**). Boc-Val-OH (1.08 g, 5 mmol, 1.25 equiv), HOAt (680 mg, 5 mmol, 1.25 equiv), and DCC (1.03 g, 5 mmol, 1.25 equiv) were dissolved in 30 mL of DCM at 0 °C and stirred for 10 min. Proline benzyl ester hydrochloride (966 mg, 4 mmol, 1 equiv) and DIPEA (870 µL, 5 equiv, 1.25 equiv) were added. The mixture was stirred at room temperature for 2 hours. TAEA (295 µL, 2 mmol, 0.5 equiv) was added and the mixture stirred

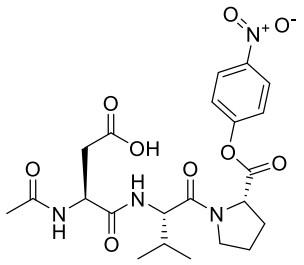
for 10 minutes until the unreacted HOAt ester was consumed then cooled to 0 °C and allowed to stand 30 minutes to encourage precipitation of DCU. The suspension was filtered through a pad of celite and rinsed with 70 mL of DCM. The filtrate was washed twice with 100 mL of 10% citric acid, twice with 100 mL of saturated $NaHCO_3$ and once with 100 mL of brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude dipeptide was dissolved in 5 mL of DCM, 15 mL of TFA was added and stirred for 1 hour at room temperature then the solvent was evaporated.

Separately Ac-Asp(OtBu)-OH (1.16 g, 5 mmol, 1.25 equiv) HOAt (680 mg, 5 mmol, 1.25 equiv), and DCC (1.03 g, 5 mmol, 1.25 equiv) were dissolved in 30 mL of DCM at 0 °C and stirred for 10 min. The dipeptide TFA salt from above was added along with DIPEA (870 µL, 5 mmol, 1.25 equiv) and the mixture stirred overnight at room temperature. TAEA (295 µL, 2 mmol, 0.5 equiv) was added and the mixture stirred for 10 minutes until the unreacted HOAt ester was consumed then cooled to 0 °C and allowed to stand 4 hours to encourage precipitation of DCU. The suspension was filtered through a pad of celite and rinsed with 70 mL of DCM. The filtrate was washed twice with 100 mL of 10% citric acid, twice with 100 mL of saturated $NaHCO_3$ and once with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography 50 to 100% EtOAc in hexane to give **4.7** as 1.27 g of white foam (62% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.32 (m, 5H), 7.05 (d, 1H, *J* 8.6 Hz), 6.87 (d, 1H, *J* = 8.27 Hz), 5.16 (d, 1H, *J* = 12.3 Hz), 5.09 (d, 1H, *J* = 12.3 Hz), 4.67 (m, 1H), 4.53 (m, 2H), 3.74 (m, 1H), 3.64 (m, 1H), 2.85 (dd, 1H, *J* = 16.8, 4.6 Hz), 2.54 (dd, 1H, *J* = 16.9, 5.9 Hz), 2.18 (m, 1H), 2.01 (m, 7H), 1.42 (s, 9H), 0.95 (d, 3H, *J* = 6.7 Hz), 0.88 (d, 3H, *J* = 6.8 hz); ^{13}C {^{1}H} NMR ($CDCl_3$, 125 MHz): δ, 171.84, 171.49, 170.43, 170.17, 170.13, 135.66, 128.62, 128.36, 128.26, 81.83, 66.94, 58.97, 55.89, 49.58, 47.27, 36.98,

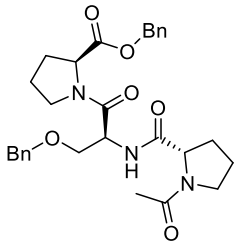
31.46, 29.09, 28.09, 25.00, 23.33, 19.35, 17.61; HRMS-ESI$^+$ (*m/z*) calcd for $C_{27}H_{40}N_3O_7^+$ (M + H)$^+$ 518.2872, found, 518.2861.



Ac-Asp(OtBu)-Val-Pro 4-nitrophenyl ester (**4.9**). Tripeptide **4.7** (1.27 g, 2.46 mmol, 1 equiv) was dissolved in 30 mL of methanol and $Pd(OH)_2$, 20 wt% on carbon (127 mg, 0.136 mmol, 10 mol%) was added was added. The reaction was stirred under a hydrogen atmosphere overnight then filtered through a pad of celite. The solution was concentrated to give 970 mg of crude **4.8** as a white solid which was dissolved in 60 mL of pyridine and cooled to 0 °C. p-Nitrophenol (640 mg, 4.60 mmol, 1.9 equiv) was added followed by DIC (400 µL, 2.56 mmol, 1.05 equiv) the solution was warmed to rt and stirred overnight then concentrated in the presence of silica and subjected to flash chromatography on a gradient of 0 to 100% EtOAc in DCM. This yielded 1.2 g of a mixture of the desired p-nitrophenyl ester and an *N*-acyl urea side product which was carried forward to the next reaction, however a small portion of analytically pure **4.9** was found in some fractions. ($CDCl_3$, 500 MHz): δ 8.28 (d, 2H, *J* = 8.9 Hz), 7.32 (d, 2H, *J* = 8.9 Hz), 7.11 (d, 1H, *J* = 8.4 Hz), 6.90 (d, 1H, *J* = 8.1 Hz), 4.81 (m, 1H), 4.69 (dd, 1H, *J* = 7.8, 5.7 Hz), 4.58 (t, 1H, *J* = 7.7 Hz), 3.90 (m, 1H), 3.74 (m, 1H), 2.91 (dd, 1H, *J* = 16.9, 4.6 Hz), 2.58 (dd, 1H, *J* = 16.9, 5.9 Hz), 2.45 (m, 1H), 2.13 (m, 7H), 1.47 (s, 9H), 1.01 (d, 3H, *J* = 6.7 Hz), 0.94 (d, 3H, *J* = 6.7 Hz); ^{13}C {^1H} NMR ($CDCl_3$, 125 MHz): δ 171.7, 170.62, 170.59, 170.3, 170.0, 155.5, 145.6, 125.4, 122.4, 82.0, 59.3, 56.0, 49.6, 47.4, 36.8, 31.4, 29.2, 28.1, 25.4, 23.4, 19.3, 17.7; HRMS-ESI$^+$ (*m/z*) calcd for $C_{26}H_{37}N_4O_9^+$ (M + H)$^+$ 549.2555, found 549.2554.



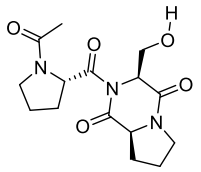
Ac-Asp(OtBu)-Val-Pro 4-nitrophenyl ester (**4.4**). A mixture of 1.2 g of crude **4.9** and side product **4.10** were dissolved in 12 mL of DCM and 12 mL of TFA was added. The reaction was stirred for one hour and then concentrated. The reside was subjected to flash chromatography, 0 to 10% MeOH in DCM with 1% AcOH. Fractions containing product were further purified by HPLC using a gradient of 25 to 40% CH_3CN in water (0.1% TFA) over 40 min to yield 392 mg of white solid (32% in three steps from 1.27 g of **4.7**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.66 (br, 1H), 8.24 (d, 2H, *J* = 7.5 Hz,), 7.83 (d, 1H, *J* = 6.0 Hz), 7.40 (br, 1H), 7.26 (d, 2H, *J* = 7.9 Hz), 4.91 (m, 1H), 4.69 (m, 1H), 4.55 (m, 1H), 3.96 (m, 1H), 3.73 (m, 1H), 2.91 (d, 1H, *J* = 15.2 Hz), 2.72 (d, 1H, *J* = 14.3 Hz), 2.41 (m, 1H), 2.07 (m, 7H), 0.95 (d, 3H, *J* = 5.7 Hz), 0.90 (d, 3H, *J* = 5.2 Hz); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 173.9, 171.9, 171.5, 171.0, 169.5, 155.2, 145.5, 125.3, 122.3, 59.4, 56.4, 49.6, 47.8, 36.1, 31.0, 29.1, 25.1, 22.6, 18.9, 18.0; HRMS-ESI$^+$ (*m/z*) calcd for $C_{22}H_{49}N_4O_9^+$ $(M + H)^+$ 493.1929, found 493.1930.



Ac-Pro-Ser(Bn)-Pro benzyl ester (**4.11**). Experimental procedure. Boc-Ser(Bn)-OH (1.48 g, 5.00 mmol, 1.25 equiv), HOAt (680 mg, 5.00 mmol, 1.25 equiv), DIPEA (870 µL, 5.00 mmol, 1.25 equiv), and DCC (1.03 g, 5.00 mmol, 1.25 equiv) were dissolved in 30 mL of DCM at 0 °C. The

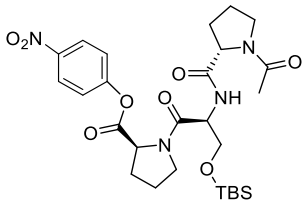
mixture was stirred for 15 minutes and then H-Pro-OBn·HCl (940 mg, 4 mmol, 1 equiv) was added as a solution in 10 mL of DCM and the reaction was stirred 90 minutes at room temperature. The mixture was cooled to 0 °C then filtered through celite, rinsed with 100 mL of DCM then the filtrate was washed 3 times with 50 mL of 0.1 M HCl, 3 times with 50 mL of 5% $NaHCO_3$, and once with 50 mL of brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give crude dipeptide. The residue was suspended in 5 mL of DCM at 0 °C and 15 mL of TFA was added. The reaction was stirred until complete removal of the Boc group was observed by TLC then concentrated.

Separately acyl proline (785 mg, 5.00 mmol, 1.25 equiv), HOAt (680 mg, 5.00 mmol, 1.25 equiv), DIPEA (870 µL, 5.00 mmol, 1.25 equiv), and DCC (1.03 g, 5.00 mmol, 1.25 equiv) were dissolved in 30 mL of DCM at 0 °C and stirred for 15 minutes before adding the deprotected dipeptide TFA salt as a solution in 10 mL of DCM. The reaction was stirred overnight at room temperature. The mixture was cooled to 0 °C then filtered through celite, rinsed with 100 mL of DCM then the filtrate was washed 3 times with 50 mL of 0.1 M HCl, 3 times with 50 mL of 5% $NaHCO_3$, and once with 50 mL of brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography 1 to 10% MeOH in DCM to yield **4.11** as a white foam, 1.25 g (60% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.3 (m, 11H), 5.16 (d, 1H, J = 12.6 Hz), 5.07 (d, 1H, J = 12.5 Hz), 4.86 (m, 1H), 4.4 (m, 4H), 3.53 (m, 6H), 2.3-1.8 (m, 11H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 171.6, 171.3, 170.5, 168.8, 138.0, 135.7, 128.5, 128.3, 128.2, 128.1, 127.7, 127.6, 73.2, 70.0, 66.8, 59.7, 59.1, 51.2, 48.2, 47.1, 29.0, 28.3, 24.9, 24.9, 22.5; HRMS-ESI$^+$ (*m/z*) calcd for $C_{29}H_{36}N_3O_6^+$ $(M + H)^+$ 522.2599, found 522.2599.



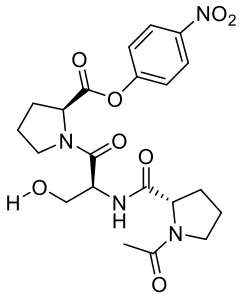
(3S,8aS)-2-(acetyl-L-prolyl)-3-(hydroxymethyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**4.13**). Benzyl ester **4.11** (1.23 g, 2.36 mmol, 1.00 equiv) was dissolved in 20 mL of MeOH and $Pd(OH)_2$ (20 wt% on carbon, 123 mg) was added. The suspension was stirred under a hydrogen atmosphere for 2 hours. The mixture was the filtered through celite and concentrated to give **4.12** which was used without further purification.

The residue was dissolved in 20 mL of DMF and 4-nitrophenol (1.3 g, 9.3 mmol, 4 equiv), DIC (550 µL, 443 mg, 3.52 mmol, 1.49 equiv) and DMAP (25 mg, 0.20 mmol, 0.086 equiv) were added. The mixture was stirred for four hours and TLC analysis showed that this consisted of a mixture of **4.12** and **4.13** but not **4.5.** The mixture was concentrated, and the residue was subjected to flash chromatography with a gradient of 0 to 15% MeOH in DCM to give 400 mg of **4.13** as a white solid (52% yield). $^{13}C\{^1H\}$ NMR showed that this compound existed as a mixture of rotamers in DMSO-d_6 which coalesced when heated to 100 °C. ^1H NMR (MeOD, 300 MHz): δ 3.17-2.90 (m, 4H), 2.77 (t, 1H, J = 7.4 Hz), 2.12-1.92 (m, 4H), 1.81 (m, 1H), 0.8-0.5 (m, 11H); ^{13}C $\{^1H\}$ NMR ($CDCl_3$, 125 MHz, DMSO-d_6 100 °C): δ 171.1, 168.2, 167.8, 162.5, 62.3, 57.9, 53.8, 53.7, 46.6, 44.2, 28.2, 27.4, 23.6, 21.3, 21.1; HRMS-ESI$^+$ (*m/z*) calcd for $C_{15}H_{22}N_3O_5^+$ $(M + H)^+$ 324.1554, found 324.1555.



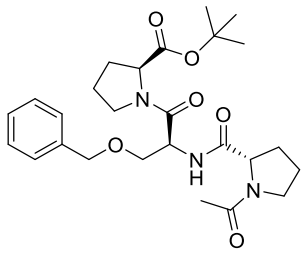
Ac-Pro-Ser(TBS)-Pro 4-nitrophenyl ester (**4.14**). Tripeptide **4.12** (906 mg, 2.66 mmol, 1.0 equiv) was prepared as above and dissolved in 60 mL of THF and iodine (2.0 g, 7.9 mmol, 3.0 equiv),

TBSCl (1.2 g, 7.9 mmol, 3.0 equiv), and *N*-methylimidazole (1.34 mL, 1.38 g, 16.8 mmol, 6.0 equiv) were added. The reaction was stirred for 3 hours at room temperature. The mixture was concentrated in the presence of silica and subjected to flash chromatography, 1% AcOH, 0 to 10% MeOH in DCM. This provided the TBS ether as a mixture with inseparable impurities which was dissolved in 20 mL of pyridine and 4-nitrophenyl trifluoroacetate (1.25 g, 5.3 mmol, 2.0 equiv) was added. The mixture was stirred overnight at room temperature and then was diluted with 100 mL of EtOAc and washed three times with 100 mL of 0.1 M HCl, 3 times with 100 mL of 5% Na_2CO_3, and once with 50 mL of brine The organic layer was dried over $MgSO_4$, filtered, and concentrated then the residue was purified by flash chromatography (0 to 6%) MeOH in DCM to give 200 mg of an amorphous white solid (14% yield for two steps). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.15 (d, 2H, *J* = 9.1 Hz), 7.31 (m, 1H), 7.21 (d, 2H, *J* = 9.1 Hz), 4.74 (m, 3H), 3.74 (m, 4H), 3.49 (m, 1H), 3.36 (m, 1H), 2.02 (m, 11H), 0.74 (s, 9H), -0.08 (s, 3H), -0.1 (s, 3H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 171.2, 170.6, 169.6, 169.6, 155.5, 145.3, 125.0, 122.4, 63.3, 59.6, 59.3, 52.8, 48.2, 47.2, 29.1, 28.0, 25.7, 25.1, 24.9, 22.4, 18.1, -5.7, -5.8; HRMS-ESI$^+$ (*m/z*) calcd for $C_{27}H_{41}N_4O_8Si^+$ $(M + H)^+$ 577.2688, found 577.2687.



Ac-Pro-Ser-Pro 4-nitrophenyl ester (**4.5**). Compound **4.14** (190 mg, 0.33 mmol) was dissolved in 4 mL of TFA and stirred for 1 hour. The reaction was quenched 30 mL of ice water saturated with $NaHCO_3$. 30 mL of brine was added and the mixture was extracted 5 times with a 3:1 mixture of $CHCl_3$:*i*PrOH. The combined organic layers were died over $MgSO_4$, filtered, and

concentrated. Working quickly, the residue was purified by flash chromatography 0 to 10% MeOH in DCM to give a colorless oil (R_f = 0.1 in 5% MeOH in DCM). The product was immediately examined by ^{1}H NMR and HRMS which confirmed the identity of the material. However the solution gradually turned yellow and after several hours TLC analysis showed that a significant amount of the product had decomposed by DKP formation releasing 4-nitrophenol and **4.13**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.2 (d, 2H, *J* = 8.8 Hz), 7.41 (d, 1H, *J* = 7.7 Hz), 7.28 (d, 2H, *J* = 8.8 Hz), 4.8 (m, 1H, *J* = 6.9 Hz), 4.67 (m, 1H, *J* = 4.2 Hz), 4.4 (dd, 1H, *J* = 7.3, 3.5 Hz), 3.79 (s, 4H), 3.6-3.4 (m, 2H, *J* = 9.1 Hz), 2.5-1.8 (m, 11H); This compound decomposed before a carbon NMR could be obtained. HRMS-ESI$^+$ (*m/z*) calcd for $C_{21}H_{27}N_4O_8^+$ $(M + H)^+$ 463.1823, found 463.1823.



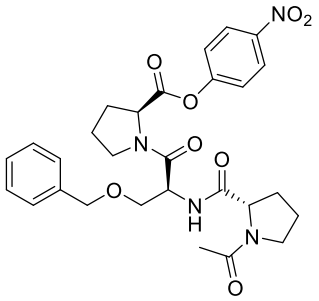
Ac-Pro-Ser(Bn)-Pro t-butyl ester (**4.16**). H-Ser(Bn)-OH (1.00 g, 5.10 mmol, 1.00 equiv) and NaOH (205 mg, 5.10 mmol, 1.00 equiv) were dissolved in 20 mL of water and 25 mL of THF and cooled to 0 °C. Allyl chloroformate (653 µL, 6.1 mmol, 1.2 equiv) was added dropwise and stirred for 5 hours until complete. The THF was removed by evaporation under reduced pressure and the mixture was adjusted to pH 12 with 1 M NaOH then washed with 30 mL of DCM. The aqueous layer was acidified to pH 1 with 1 M HCl and then extracted three times with 30 mL of DCM and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved in 50 mL of DCM and HOAt (1.04 g, 7.65 mmol, 1.50 equiv), DIPEA (2.2

mL, 12.8 mmol, 2.5 equiv), EDC·HCl (1.2 g, 6.1 mmol, 1.2 equiv) and H-Pro-O^{t}Bu·HCl (1.04 g, 6.1 mmol, 1.2 equiv) were added. The reaction was stirred overnight then diluted with 200 mL of EtOAc and washed twice with 50 mL of 10% Na_2CO_3 twice with 50 mL of 10% citric acid and once with 50 mL of brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give crude **4.15**.

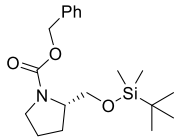
The crude dipeptide was dissolved in 35 mL of DCM. DMBA (780 mg, 5.00 mmol, 1.00 equiv) and $Pd(PPh_3)_4$ (92 mg, 0.08 mmol, 1.6 mol%) were added the mixture was stirred for 2 hours until complete allyl deprotection was observed by TLC. The reaction was extracted three times with 30 mL of 0.1 M HCl. Then the combined aqueous layers were adjusted to pH 12 and saturated with NaCl then back extracted five times with 30 mL of 3:1 CH_2Cl_2:*i*PrOH. The combined organic layers was dried over $MgSO_4$, filtered and concentrated.

Separately acyl proline (942 mg, 6.00 mmol, 1.50 equiv), HOAt, (816 mg, 6.00 mmol, 1.50 equiv) and EDC·HCl (1.15 g, 6.00 mmol, 1.50 equiv) were dissolved in 30 mL of DCM at 0 °C and stirred for 30 minutes before adding the deprotected dipeptide as a solution in 10 mL of DCM. The reaction was stirred for 30 minutes until complete then was diluted with 200 mL of EtOAc and washed with 50 mL of 10% Na_2CO_3, twice with 50 mL of 10% citric acid, and once with 50 mL of brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, 0 to 10% MeOH in EtOAc, to give **4.16** as 1.19 g of white foam (45% yield). **4.16** existed as a mixture of rotamers in $CDCl_3$. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.27 (m, 5H), 6.95 (m, 1H), 4.84 (m, 1H), 4.44 (m, 4H), 3.53 (m, 6H), 1.96 (m, 11H), 1.38 (s, 9H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ (major rotamer) 171.3, 170.8, 170.4, 168.4, 138.0, 128.2, 127.6, 127.5, 81.1, 73.2, 69.9, 59.8, 59.7, 51.2, 48.1, 47.0, 32.1, 30.6, 29.0, 28.4, 27.9, 24.9, 24.7, 22.4; (minor rotamer) δ 171.7 169.9 169.6 167.8 137.6 128.4 127.7 127.3

81.4 71.2 69.3 61.9 59.9 50.7 46.7 46.4 32.2 30.7 28.9 28.4 27.8 22.8 22.4 22.2; HRMS-ESI$^+$ (*m/z*) calcd for $C_{26}H_{38}N_3O_6^+$(M + H)$^+$ 488.2755, found 488.2745.

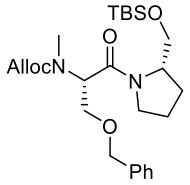


Ac-Pro-Ser(Bn)-Pro 4-nitrophenyl ester (**4.17**). *t*-Butyl ester **4.16** (487 mg, 1.00 mmol, 1.00 equiv) was dissolved in 10 mL of TFA and stirred for 30 minutes, until complete by TLC, then concentrated. The residue was dissolved in 3 mL of pyridine and 4-nitrophenyltrifluoroacetate (352 mg, 1.50 mmol, 1.50 equiv) was added and the reaction was stirred for 3 hours. The solution was diluted with 100 mL of EtOAc then washed twice with 10% citric acid and four times with 1% Na_2CO_3 the organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, 0 to 20% MeOH in EtOAc, to give 250 mg of **4.17** as a white foam (45% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.17 (d, 2H, *J* = 8.8 Hz), 7.42 (d, 1H, *J* = 7.1 Hz), 7.23 (m, 5H), 4.92 (m, 1H), 4.65 (m, 1H), 4.47 (m, 3H), 3.78 (m, 2H), 3.69 (m, 2H), 3.52 (m, 1H), 3.40 (m, 1H), 2.12 (m, 11H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 171.5, 170.7, 169.8, 169.3, 155.4, 145.4, 137.7, 128.3, 127.6, 127.5, 125.1, 122.4, 73.4, 69.9, 59.7, 59.4, 51.1, 48.2, 47.3, 29.1, 28.3, 25.2, 24.9, 22.5; HRMS-ESI$^+$ (*m/z*) calcd for $C_{28}H_{33}N_4O_8^+$ (M + H)$^+$ 553.2293, found 553.2290.



O-tert-butyldimethylsilyl Cbz-prolinol (**4.19**). Cbz-prolinol (884 mg, 4.00 mmol, 1.00 equiv) was dissolved in 1.8 mL of DMF then imidazole (816 mg, 12 mmol, 3 equiv) and TBSCl (900 mg, 6

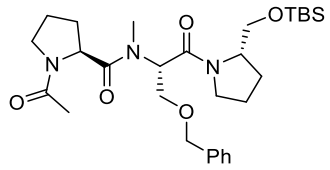
mmol, 1.5 equiv) were added and stirred overnight. The mixture was diluted with 100 mL of ether and washed three times with 100 mL of 0.1 M HCl and once with 100 mL of 10% Na_2CO_3. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, 20% EtOAc in hexane to yield 1.00 g of colorless oil (71%). **4.19** existed as a 1:1 mixture of rotamers in $CDCl_3$ as determined by 2D-EXSY NMR which showed chemical exchange between the peaks at 3.5 and 3.75 ppm. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.36 (m, 5H), 5.19 (m, 2H), 3.92 (m, 1H), 3.60 (m, 4H), 1.95 (m, 4H), 0.92 (m, 9H), 0.07 (m, 6H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 154.8, 137.1 and 136.8, 128.4, 128.1 and 127.9, 127.8 and 127.7, 66.8 and 66.5, 63.8 and 63.1, 59.0 and 58.4, 47.2 and 46.9, 28.3 and 27.5, 25.9 and 25.7, 23.9 and 22.9, 18.2, -3.5 -5.5 HRMS-ESI$^+$ (*m/z*) calcd for $C_{19}H_{32}NO_3Si^+$ $(M + H)^+$ 350.2146, found 350.2148.



Allyl ((S)-3-(benzyloxy)-1-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)pyrrolidin-1-yl)-1-oxopropan-2-yl)(methyl)carbamate (**4.20**). Compound **4.19** (350 mg, 1.00 mmol, 1.00 equiv) was dissolved in 5 mL of MeOH and $Pd(OH)_2$ 20 wt% on carbon (35 mg, 0.05 mmol, 5 mol%) was added. The mixture was stirred under a hydrogen atmosphere for 1 hour until complete then was filtered through a pad of celite and concentrated.

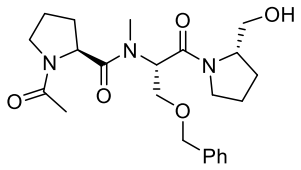
Separately alloc-*N*-methyl-O-benzyl serine (293 mg, 1.00 mmol, 1.00 equiv) was dissolved in 10 mL of DCM and Oxyma (142 mg, 1 mmol, 1 equiv) and DIPEA (176 µL, 1 mmol, 1 equiv) were added followed by COMU (428 mg, 1 mmol, 1 equiv) the mixture was stirred for 10 minutes before adding the deprotected amine as a solution in 5 mL of DCM**.** The

reaction was stirred for 2 hours then was diluted with 50 mL of EtOAc and washed with 50 mL of 10% citric acid then 50 mL of 10% Na_2CO_3. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography 5 to 30% EtOAc in hexane to yield 355 mg of a colorless oil (72% yield). **4.20** existed as a mixture of rotamers in $CDCl_3$ as determined by 2D-EXSY NMR which showed chemical exchange between the peaks at 4.97 and 5.15. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.28 (s, 5H), 5.89 (m, 1H), 5.21 (m, 3H), 4.55 (m, 4H), 4.09 (s, 1H), 3.65 (m, 6H), 2.91 (m, 3H), 1.93 (m, 4H), 0.84 (m, 9H), -0.02 (m, 6H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ (major rotamer) 167.8, 156.8, 137.9, 132.8, 128.3, 127.7, 127.6, 117.3, 72.9, 66.7, 66.3, 62.3, 58.8, 56.1, 47.3, 29.7, 26.9, 25.8, 24.2, 18.1, -5.7; (minor rotamer) 167.2, 156.0, 137.9, 132.7, 128.3, 127.7, 127.6, 117.8, 73.2, 67.0, 66.4, 62.3, 58.9, 57.0, 46.9, 30.1, 26.9, 25.8, 24.2, 18.1, -5.7; HRMS-ESI$^+$ (*m/z*) calcd for $C_{26}H_{43}N_2O_5Si^+$ $(M + H)^+$ 491.2936, found 491.2937.

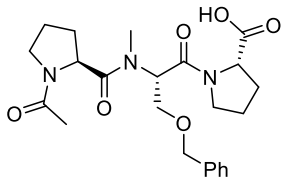


O-tert-butyldimethylsilyl-(Ac-Pro-N-methylSer(Bn)-prolinol) (**4.21**). Dipeptide **4.20** (355 mg, 0.724 mmol, 1 equiv), $Pd(PPh_3)_4$ (8 mg, 7 µmol, 1 mol%), and DMBA (126 mg, 0.81 mmol, 1.1 equiv) were dissolved in 5 mL of DCM and stirred for 30 minutes until complete by TLC. Separately Ac-Pro-OH (345 mg, 2.20 mmol, 3.00 equiv), HOAt (299 mg, 2.2 mmol, 3.00 equiv), DIPEA (390 µL, 2.2 mmol, 3.00 equiv) and EDC·HCl (422.4 mg, 2.2 mmol, 3 equiv) were dissolved in 5 mL of DCM at 0 °C and stirred for 15 minutes. Then the entire mixture was transferred to the alloc deprotection reaction and stirred overnight. TAEA (150 µL, 1 mmol) was added to the reaction and stirred 15 min to destroy unreacted HOAt ester and the mixture was dissolved in 100 mL of EtOAc then washed with 50 mL of 10% citric acid and 50 mL of 10%

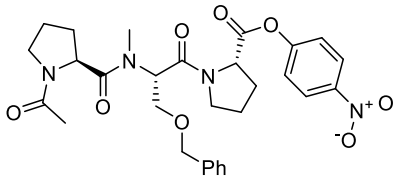
Na_2CO_3. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography, 0 to 10% MeOH in EtOAc, to yield 355 mg of a colorless oil (88% yield). **4.21** existed as a mixture of rotamers in $CDCl_3$ as determined by 2D-EXSY NMR which showed chemical exchange between the peaks at 5.61 and 5.41 and the peaks for the *N*-protons at 3.1 ppm. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29 (m, 5H), 5.61 (m) and 5.41 (t, J = 7.1 Hz)(1H), 4.84 (m, 1H), 4.53 (m, 2H), 4.10 (m, 1H), 3.65 (m, 8H), 3.12 (m, 3H), 1.97 (m, 11H), 0.85 (m, 9H), 0.00 (m, 6H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ (major rotamer) 172.5, 169.0 168.3, 137.9, 128.3, 127.8, 127.6, 72.9, 66.9, 62.3, 58.6, 56.7, 54.9, 48.0, 47.3, 31.6, 31.1, 28.8, 26.8, 25.8, 24.7, 24.2, 22.3, -5.48, -5.50; (minor rotamer) 172.4, 169.4, 167.4, 137.3, 128.5, 127.9, 127.6, 73.0, 66.0, 62.1, 58.7, 56.5, 54.3, 47.5, 46.7, 30.6, 30.4, 28.8, 26.9, 25.8, 24.5, 24.4, 22.1, -5.48, -5.50 HRMS-ESI$^+$ (*m/z*) calcd for $C_{29}H_{48}N_3O_5Si^+$ (M + H)$^+$ 546.3358, found 546.3354.



Ac-Pro-N-methylSer(Bn)-prolinol (**4.22**). Tripeptide **4.21** (355 mg, 0.651 mmol) was dissolved in 5 mL of 1:3:1 THF:AcOH:H_2O and stirred overnight at rt then concentrated. The residue was purified by flash chromatography, 0 to 10% MeOH in DCM, to give 160 mg of a white foam (57% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31 (m, 5H), 5.37 (t, 1H, J = 6.7 Hz), 4.80 (dd, 1H, J = 8.0, 3.9 Hz), 4.57 (d, 1H, J = 12.0 Hz), 4.49 (d, 1H, J = 11.9 Hz), 4.15 (m, 1H), 3.64 (m, 8H), 3.17 (m, 3H), 1.96 (m, 11H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 172.6, 170.8, 169.3, 137.6, 128.4, 127.8, 127.8, 73.3, 67.5, 66.4, 61.3, 56.7, 55.1, 48.1, 47.9, 32.0, 28.7, 28.1, 24.6, 22.2, 20.7; HRMS-ESI$^+$ (*m/z*) calcd for $C_{23}H_{34}N_3O_5^+$ (M + H)$^+$, 432.2493 found 432.2492.

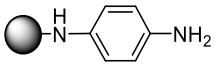


Ac-Pro-N-methylSer(Bn)-proline (**4.23**). Tripeptide **4.22** (80 mg, 0.18 mmol, 1.0 equiv) was dissolved in 1.5 mL of acetone then cooled to 0 °C. 1.5 mL of 5% $NaHCO_3$ was added followed by potassium bromide (28 mg, 0.18 mmol, 1.0 equiv), TEMPO (28 mg, 0.18 mmol, 1.0 equiv), and 6% sodium hypochlorite (825 μL, 0.74 mmol, 4.0 equiv). The mixture was stirred for 90 minutes at room temperature then was diluted with 20 mL of water and washed with 20 mL of ether. The aqueous layer was adjusted to pH 1 with 1 M HCl and the extracted 3 times with 20 mL of 10% MeOH in DCM. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, 1% AcOH, 10% MeOH, 89% DCM to give **4.23** as 78 mg of a white foam (94% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.88 (br, 1H), 7.24 (s, 5H), 4.97 (m, 2H), 4.47 (m, 3H), 3.63 (m, 6H), 3.08 (m, 3H), 1.99 (m, 11H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 172.4, 171.3, 170.1, 137.4, 128.5, 127.9, 73.2, 66.7, 57.4, 57.2, 48.4, 47.4, 28.6, 28.0, 24.9, 24.4, 21.5; HRMS-ESI$^+$ (*m/z*) calcd for $C_{23}H_{31}N_3O_6Na^+$ (M + Na)$^+$ 468.2111, found 468.2107.



Ac-Pro-N-methylSer(Bn)-proline 4-nitrophenyl ester (**4.24**). Tripeptide **4.23** (38 mg, 0.085 mmol, 1.0 equiv) was dissolved in 2 mL of pyridine and *p*-nitrophenyltrifluoroacetate (60 mg, 0.26 mmol, 3.0 equiv) was added and the mixture was stirred for 1 hour at rt. The reaction was diluted with 50 mL of EtOAc and washed 3 times with 20 mL of 10% Citric acid and 3 times

with 20 mL of 10% Na_2CO_3. The organic layer was died over $MgSO_4$, filtered and concentrated to dryness. The residue was purified by HPLC using 40% CH_3CN in water to yield **4.24** as a colorless oil, 23 mg (48% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ. 8.14 (m, 2H), 7.20 (m, 7H), 5.32 (m, 1H), 4.84 (m, 1H), 4.48 (m, 3H), 3.70 (m, 6H), 3.11 (m, 3H), 2.03 (m, 11H); ^{13}C {^1H} NMR ($CDCl_3$, 75 MHz): δ 172.5, 169.9, 169.4, 169.1, 155.4, 145.4, 137.7, 128.3, 127.67, 127.66, 125.1, 122.3, 73.2, 67.3, 59.3, 56.7, 55.7, 48.1, 47.0, 32.5, 28.9, 28.7, 25.2, 24.7, 22.2; HRMS-ESI$^+$ (*m/z*) calcd for $C_{29}H_{35}N_4O_8$ $(M + H)^+$ 567.2449, found 567.2461.



Resin-bound 1,4-diaminobenzene (**4.25**). Polystyrene supported 2-chlorotrityl resin (1.00 g, 1.50 mmol, 1.00 equiv) was swelled in dry DCM under reflux for 15 minutes. Thionyl chloride (392 µL, 5.4 mmol, 3.6 equiv) was added followed by DMF (22 µL, 0.30 mmol, 0.2 equiv). and the suspension was refluxed for 4 hours. The resin was transferred to a disposable polyethylene cartridge for peptide synthesis and rinsed with DMF (3 x 3 min) and DCM (3 x 3 min). A solution of 1,4-diamino benzene (1.3 g, 12 mmol, 1.5 equiv) and DIPEA (2.1 mL, 12 mmol, 1.5 equiv) in 8 mL of DMF was added. And the mixture was stirred overnight at room temperature. After rinsing with DMF (3 x 3 min) and DCM (3 x 3 min) a solution of 9:1 DCM methanol was added and stirred for 30 minutes to cap any unreacted sites. Then the resin was rinsed DCM (3 x 3 min) and dried under vacuum.

General procedure for amino acid coupling on a solid support.

The resin was swelled in DMF for 15 minutes then Fmoc-AA-OH (4 equiv), HOBt (4 equiv), and DIC (4 equiv) 0.5 M in DMF were added. The cartridge was mixed for 4 hours at room temperature and the cartridge was drained then the resin was rinsed with DMF (3 x 3 min) and DCM (3 x 3 min).

General procedure for Fmoc deprotection.

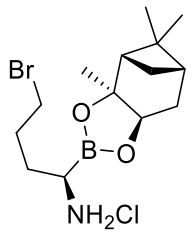
Fmoc groups were removed by two 5-minute treatments with 20% 4-methylpiperidine in DMF followed by rinsing with DMF (3 x 3 min) and DCM (3 x 3 min).

General procedure for the synthesis of peptides **4.32-4.41**

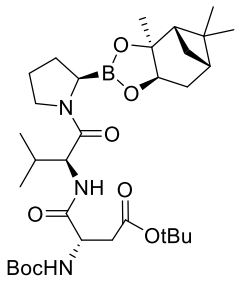
Peptides **4.32** to **4.41** were synthesized from 100 mg, 0.15 mmol, of resin **4.25** according to the general procedures for Fmoc SPPS. Once all amino acid residues were added the N-terminal amine was capped with a solution of 2:3 acetic anhydride: pyridine with mixing for 30 min. The acylated peptides were rinsed with DMF (3 x 3 min) and DCM (3 x 3 min) followed by cleavage with 1% TFA in DCM for 1 hour. The solvent was removed and the crude 4-aminoalilide peptides were dissolved in 6 mL of 1:1 CH_3CN:H_2O with 12 equiv of Oxone® and the mixture stirred overnight at room temperature. Brine was added and the mixture was extracted with 9:1 DCM:MeOH three times. The combined organic layers were dried over $MgSO_4$, filtered and concentrated then purified by flash chromatography 1 to 10% MeOH in DCM.

For peptides which bore side *t*-butyl side chain protecting groups, deprotection was accomplished with 95:2.5:2.5 TFA:H_2O:TIPS for 1 hour followed by concentration under reduced pressure. This was followed by a second round of flash chromatography 1 to 10% MeOH in DCM.

The purified 4-nitroanilides (Table 4.3) were analyzed by analytical HPLC and HRMS. See Appendix Figures A1-A20 for copies of data.



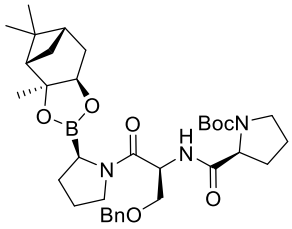
((R)-4-bromo-1-((3aS,4S,6S,7aR)-3a,5,5-trimethylhexahydro-4,6 methanobenzo[d][1,3,2]-dioxaborol-2-yl)butyl)chloro-l4-azane (**4.44**). Compound **4.44** was prepared according to a literature procedure.[215]



Ac-Asp(OtBu)-Val-boroPro pinanediol boronate ester (**4.45**). Compound **4.44** (100 mg, 0.273 mmol, 1.0 equiv) was dissolve in 2 mL of THF and potassium iodide (45 mg, 0.27 mmol, 1.0 equiv) and DIPEA (95 µL, 0.55 mmol, 2.0 equiv) were added. White precipitates formed and the mixture was stirred for 1 hour at room temperature before cooling to -15 °C. A negative ninhydrin test indicated that cyclization was complete.

Separately Ac-Asp(OtBu)-Val-OH (132 mg, 0.34 mmol, 1.25 equiv) and NMM (31 µL, 0.31 mmol, 1.125 equiv) were dissolved in 2 mL of THF and cooled to -15 °C. *iso*-Butyl chloroformate (40 µL, 0.31 mmol, 1.125 equiv) was added as a solution in 1 mL of THF and the mixture was stirred for 10 minutes before being transferred by cannula to the flask containing the crude amine. The mixture is stirred for 6 hours while warming slowly. Then the reaction was concentrated on silica and purified by flash chromatography, gradient elution 0 to 100% EtOAc in hexane to give pure 123 mg of **4.45** as a white solid (72% yield). ^{1}H NMR ($CDCl_3$, 300 MHz):

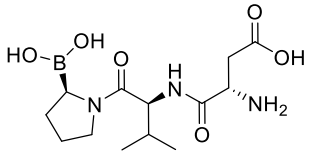
δ 6.96 (d, 1H, *J* = 9.1 Hz), 5.63 (d, 1H, *J* = 8.7 Hz), 4.47 (t, 1H, *J* = 8.3 Hz), 4.37 (br, 1H), 4.23 (m, 1H), 3.72 (t, 1H, *J* = 7.9 Hz), 3.43 (m, 1H), 3.10 (dd, 1H, *J* = 10.0, 6.9 Hz), 2.81 (dd, 1H, *J* = 16.7, 5.3 Hz), 2.54 (dd, 1H, *J* = 16.7, 5.1 Hz), 2.3-1.7 (m, 10H), 1.4-1.3 (m, 25H), 0.91 (d, 3H, *J* = 6.7 Hz), 0.87 (d, 3H, *J* = 6.7 Hz), 0.79 (s, 3H); ^{11}B NMR ($CDCl_3$, 96 MHz): δ 30.74 (br) and 21.81 (br); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 171.0, 170.5, 169.2, 155.4, 85.8, 81.3, 80.2, 77.8, 55.5, 51.2, 46.8, 39.5, 39.4, 38.1, 37.1, 35.4, 31.4, 28.6, 28.3, 28.0, 27.3, 27.2, 27.1, 26.2, 24.0, 19.0, 17.7; Note that a signal for the R_2HC-$B(OR)_2$ carbon was not observed due to quadrupolar coupling effects. However, the chemical shift of the unobserved peak can be determined by examination of the HMQC spectrum which shows a cross peak for C^{α}-H^{α} of 44.7-3.09 ppm. HRMS-ESI$^+$ (*m/z*) calcd for $C_{32}H_{55}N_3O_8B^+$ $(M + H)^+$ 620.4078, found 620.4089.



Boc-Pro-Ser(Bn)-boroPro pinanediol boronate ester (**4.46**). Compound **4.44** (400 mg, 0.8 mmol, 1.0 equiv) was dissolve in 10 mL of THF and potassium iodide (132 mg, 0.8 mmol, 1 equiv) and DIPEA (279 µL, 1.6 mmol, 2 equiv) were added. White precipitates formed and the mixture was stirred for 1 hour at room temperature before cooling to -15 °C. A negative ninhydrin test indicated that cyclization was complete.

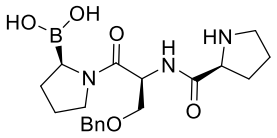
Separately Boc-Pro-Ser(Bn)-OH (392 mg, 1 mmol, 1.25 equiv) and NMM (99 µL, 0.9 mmol, 1.125 equiv) were dissolved in 8 mL of THF and cooled to -15 °C. *iso*-Butyl chloroformate (116 µL, 0.9 mmol, 1.125 equiv) was added as a solution 4 mL of THF and added to the dipeptide and the mixture was stirred for 10 minutes before being transferred by cannula to the flask containing the crude amine. The mixture was stirred for 6 hours while warming slowly

to room temperature then concentrated on silica and purified by flash chromatography, 0 to 100% EtOAc in hexane, 450 mg of **4.46** as a white foam (66% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.28 (m, 5H), 7.07-6.71 (m, 1H), 4.93 (br, 1H), 4.53 (d, 1H, *J* = 11.8 Hz), 4.43 (d, 1H, *J* = 11.8 Hz), 4.24 (d, 1H, *J* = 7.6 Hz), 3.6-3.4 (m, 6H), 3.12 (t, 1H, *J* = 8.21 Hz), 2.3-1.8 (m, 13H), 1.39 (s, 9H), 1.37 (s, 3H), 1.29 (d, 1H), 1.23 (s, 3H), 0.79 (s, 3H); ^{11}B NMR ($CDCl_3$, 96 MHz): δ 32.3 (br); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 172.2, 167.7, 154.2, 138.0, 128.4, 128.1, 127.5, 85.7, 80.3, 73.1, 70.3, 60.7, 51.2, 50.0, 46.8, 44.5, 39.5, 38.1, 35.4, 30.9, 28.5, 28.2, 27.2, 27.0, 26.1, 24.5, 24.0, 23.6; HRMS-ESI^{+} (*m/z*) calcd for $C_{34}H_{51}N_3O_7B^{+}$ $(M + H)^{+}$ 624.3815, found 624.3825.

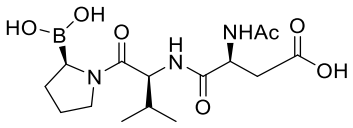


Asp-Val-boroPro (**4.47**). Boronate ester **4.45** (118 mg, 0.19 mmol, 1.0 equiv) was dissolved in 2 mL of DCM then 2 mL of TFA was added. The reaction was stirred for 1 hour and then the solvent was removed under a stream of N_2 gas. The residue was dissolved in 3 mL of water and phenyl boronic acid (24 mg, 0.2 mmol, 1.05 equiv) was added. Hexane, 3 mL was added and stirred 30 minutes before being discarded, this washing was repeated a total of three times and the aqueous layer was purified by HPLC using a gradient of 10:90 CH_3CN:H_2O (0.1% TFA) to 30:70 CH_3CN:H_2O (0.1% TFA) which yielded 49 mg of a white solid (75% yield). ^{1}H NMR (D_2O, 300 MHz): δ 4.29 (m, 2H), 3.66 (t, 1H, *J* = 8.7 Hz), 3.39 (m, 1H), 2.85 (m, 3H), 1.96-1.78 (m, 4H), 1.54 (m, 1H), 0.81 (m, 6H); ^{11}B NMR (D_2O, 96 MHz): δ 19.4 (s); ^{13}C {^{1}H} NMR (D_2O, 75 MHz): 172.5, 170.0, 168.2, 57.0, 49.3, 47.4, 34.9, 29.4, 26.9, 26.6, 18.2, 17.1; Note that a signal for the R_2HC-$B(OR)_2$ carbon was not observed due to quadrupolar coupling effects. However, the chemical shift of the unobserved peak can be determined by examination of the

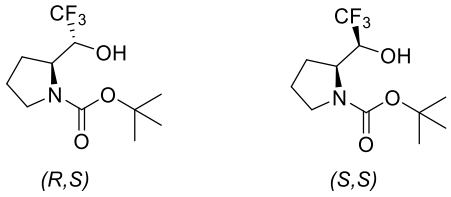
HMQC spectrum which shows a cross peak for C^{α}-H^{α} of 48.6-2.85 ppm. HRMS-ESI$^+$ (*m/z*) calcd for $C_{13}H_{23}N_3O_5B^+$ $(M - H_2O + H)^+$ 312.1725, found 312.1723.



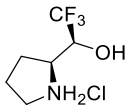
Pro-Ser(Bn)-boroPro (**4.48**)**.** Boronate ester **4.46** (88 mg, 0.14 mmol, 1.0 equiv) was dissolved in 2 mL of DCM then 2 mL of TFA was added. The reaction was stirred for 1 hour and then the solvent was removed under a stream of N_2 gas. The residue was dissolved in 3 mL of water and phenyl boronic acid (18.3 mg, 0.15 mmol, 1.07 equiv) was added. Hexane, 3 mL, was added and stirred 30 minutes before being discarded, this washing was repeated a total of three times and the aqueous layer was purified by HPLC using a gradient of 10:90 CH_3CN:H_2O (0.1% TFA) to 30:70 CH_3CN:H_2O (0.1% TFA) which yielded 25 mg of a white solid (45% yield). ^{1}H NMR (D_2O, 300 MHz): δ 7.42 (m, 5H), 4.64 (d, 1H, *J* = 11.8 Hz), 4.59 (d, 1H, *J* = 11.9 Hz), 4.39 (dd, 1H, *J* = 8.2, 6.2 Hz), 3.87 (dd, 1H, *J* = 10.8, 4.8 Hz), 3.75 (m, 2H), 3.41 (m, 3H), 3.01 (dd, 1H, *J* = 10.8, 6.8 Hz), 2.43 (m, 1H), 2.04 (m, 6H), 1.65 m, 1H); ^{11}B NMR (D_2O, 96 MHz): δ 30.2 (br) and 19.5 (s); ^{13}C {^{1}H} NMR (D_2O, 75 MHz): δ 169.3, 167.7, 136.7, 128.7, 128.5, 128.4, 73.1, 67.5, 59.4, 51.9, 47.3, 46.4, 29.7, 26.7, 26.6, 23.6; Note that a signal for the R_2HC-$B(OR)_2$ carbon was not observed due to quadrupolar coupling effects. However, the chemical shift of the unobserved peak can be determined by examination of the HMQC spectrum which shows a cross peak for C^{α}-H^{α} of 48.5-2.97 ppm. HRMS-ESI$^+$ (*m/z*) calcd for $C_{19}H_{27}N_3O_4B^+$ $(M - H_2O + H)^+$ 372.2089, found 372.2097.



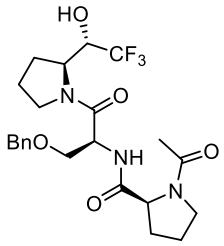
Ac-Asp-Val-boroPro (**4.43**). Compound **4.47** (50 mg, 0.112 mmol, 1.0 equiv) was dissolved in 3 mL of water. The solution was buffered with $NaHCO_3$ (29 mg, 0.336 mmol, 3.0 equiv) and Na_2CO_3 (36 mg, 0.336 mmol, 3.0 equiv) and then 4-nitrophenyl acetate (61 mg, 0.336 mmol, 3.0 equiv) was added. The reaction was stirred at room temperature for 1 hour until complete as determined by ninhydrin test and then 50 mL of 0.2 M HCl was added. The solution was washed 3 times with 50 mL of DCM then the aqueous layer was neutralized with minimum ammonium acetate and concentrated. The residue was purified by RP HPLC, 1 to 10% CH_3CN in H_2O over 40 minutes, RT = 40 min. This yielded 18 mg of a white solid (43% yield). ^1H NMR (D_2O, 300 MHz): δ 4.55 (dd, 1H, *J* = 7.6, 6.1 Hz), 4.27 (dd, 1H, *J* = 8.1 Hz), 3.65 (ap t, 1H, *J* = 8.9 Hz), 3.42-3.30 (m, 1H), 2.8 (dd, 1H, *J* = 11.2, 6.6 Hz), 2.73 (m, 1H), 2.64 (dd, 1H, *J* = 16.8, 7.7 Hz), 2.00-1.75 (m, 7H), 1.52 (m, 1H), 0.79 (dd, 3H, *J* = 5.2 Hz), 0.77 (dd, 3H, *J* = 5.3 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{15}H_{26}BN_3NaO_7^+$ (M + Na) 394.1756 found 394.1759. This compound was not soluble enough in D_2O or $DMSO_{d6}$ to obtain a ^{13}C NMR.



Boc-(S,R)-prolinolyl trifluoromethane (**4.51**) *and Boc-(S,S)-prolinolyl trifluoromethane* (**4.52**). Compounds **4.51** and **4.51** were prepared according to the method of Podichetty,[234] except that the mixture of diastereomers was separated by flash chromatography, 0 to 5% EtOAc in DCM (**4.52** is more polar than **4.51**). Both diastereomers gave NMR spectra that were identical to literature which revealed that the major diastereomer was the *S,S* isomer.[235]



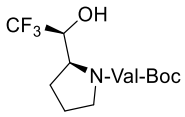
(S,S)-prolinolyl trifluoromethane hydrochloride (**4.53**). Compound **4.52** (836 mg, 3.11 mmol) was dissolved in 8.5 mL of 4 M HCl in dioxane and stirred at room temperature. After 30 minutes the solution was concentrated to dryness then dissolved in 30 mL of water and the pH was adjusted to 12 with NaOH. The solution was extracted 3 times with 30 mL of 3:1 $CHCl_3$:*i*PrOH and the organic layers were concentrated. The free amine was dissolved in 0.1 M HCl and concentrated to dryness to give **4.53** as a grey powder 459 mg (72% yield). ^{1}H NMR ($(CD_3)_2SO$, 300 MHz): δ 9.56 (br, 2H), 7.10 (br, 1H), 4.60 (m, 1H), 3.69 (m, 1H), 3.14 (m, 2H), 1.93 (m, 4H); ^{13}C {^{1}H} NMR ($(CD_3)_2SO$, 125 MHz): δ 124.5 (q, *J* = 283.1 Hz), 66.7 (q, *J* = 30.1 Hz), 57.67, 45.14, 24.29, 23.29; ^{19}F NMR ($(CD_3)_2SO$, 282 MHz): δ -75.9 (d, *J* = 7.17 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_6H_{11}NOF_3^+$ $(M + H)^+$ 170.0787, found 170.0787.



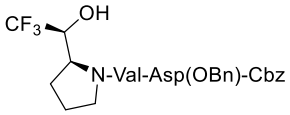
Boc-Pro-Ser(Bn)-Prolinolyl trifluoromethane (**4.55**). Compound **4.53** (822 mg, 4.00 mmol, 1.00 equiv) was placed in 30 mL of DCM and Boc-Ser(Bn)-OPfp (2.3 g, 5.00 mmol, 1.25 equiv) was added followed by DIPEA (1.29 g, 10 mmol, 2.5 equiv) the mixture was stirred until complete by TLC (8 h) and then the unreacted Pfp ester was destroyed by the addition of 225 µL of Tris(2-aminoethyl)amine. This mixture was stirred for 30 minutes then was diluted with 200 mL of EtOAc and washed twice with 100 mL of 10% citric acid, twice with 100 mL of $NaHCO_3$ and once with 100 mL of brine. The organic layer was dried over $MgSO_4$, filtered and concentrated

to give **4.54** which was used without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.32 (m, 5H), 5.59 (d, 1H, *J* = 7.9 Hz), 4.72 (m, 1H), 4.53 (m, 3H), 4.41 (m, 1H), 4.31 (m, 1H), 3.78 (m, 1H), 3.69 (m, 1H), 3.61 (m, 1H), 3.42 (m, 1H), 2.11 (m, 1H), 2.01 (m, 2H), 1.79 (m, 1H), 1.43 (s, 9H); ^{13}C {^{1}H} NMR ($CDCl_3$, 125 MHz): δ 170.2, 155.4, 137.3, 128.5, 128.0, 127.8, 124.4 (q, *J* = 256.8 Hz), 80.0, 73.5, 70.4, 68.8 (q, *J* = 29.1 Hz), 58.4, 52.4, 48.3, 28.3, 24.9; ^{19}F NMR ($CDCl_3$, 471 MHz): δ -75.3 (d, 3F, *J* = 6.9 Hz); HRMS-ESI^{+} (*m/z*) calcd for $C_{21}H_{30}O_5N_2F_3^{+}$ $(M + H)^{+}$ 447.2101, found 447.2100.

The dipeptide **4.54** was dissolved in 10 mL of TFA and stirred for 20 minutes until complete by TLC and then was concentrated. The residue was dissolved in 30 mL of DCM and Ac-Pro-OPfp (1.60 g, 5.00 mmol, 1.25 equiv) was added followed by DIPEA (1.29 g, 10 mmol, 2.5 equiv). The reaction was stirred overnight then was diluted with 200 mL of EtOAc and washed twice with 100 mL of 10% citric acid, twice with 100 mL of $NaHCO_3$ and once with 100 mL of brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography, 0 to 10% MeOH in EtOAc, to give 1.3 g of **4.55** as a white solid (67% yield). This existed as a mixture of rotamers which coalesced by heating the compound in DMSO to 100 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.52 (d, 1H, *J* = 7.0 Hz), 7.31 (m, 5H), 4.89 (dd, 1H, *J* = 12.5, 6.2 Hz), 4.5 (m, 5H), 4.31 (m, 1H), 3.71 (m, 3H), 3.55 (m, 1H), 3.41 (m, 2H), 2.23 (m, 1H), 2.03 (m, 9H), 1.78 (m, 1H); ^{13}C {^{1}H} NMR (DMSO-d_6, 100 °C, 125 MHz): δ 172.0, 169.3, 169.0, 138.8, 125.8 (q, *J* = 288.7 Hz), 128.5, 127.8, 127.8, 73.0, 70.4, 68.4, 59.8, 57.2, 51.8, 48.1, 47.4, 32.2, 29.2, 25.2, 24.7, 24.0, 22.3; ^{19}F{^{1}H} NMR (DMSO-d_6, 100 °C, 471 MHz): δ -75.04 (s, 3F); HRMS-ESI^{+} (*m/z*) calcd for $C_{23}H_{31}O_5N_3F_3^{+}$ $(M + H)^{+}$ 486.2210, found 486.2211.

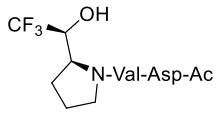


Boc-Val-Prolinolyl trifluoromethane (**4.56**). Boc-Val-OH (217 mg, 1.00 mmol, 2 equiv), HOAt (136 mg, 1.00 mmol, 2 equiv) and DIPEA (172 µL, 1 mmol, 2 equiv) were dissolved in 10 mL of DCM and cooled to 0 °C then DCC (206 mg, 1.00 mmol, 2 equiv) was added. The mixture was stirred for 15 minutes before adding **4.53** (103 mg, 0.50 mmol, 1 equiv) the reaction was stirred overnight at room temperature and then filtered through a pad of celite and rinsed with 100 mL of EtOAc. The filtrate was washed 50 mL of each of water, twice with 10% citric acid, twice with 10% Na_2CO_3 and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated and the residue was purified by flash chromatography, 20 to 30% EtOAc in hexane to give **4.56** as 154 mg of white foam (84% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.42 (d, 1H, *J* = 6.5 Hz), 5.25 (d, 1H, *J* = 9.2 Hz), 4.32 (m, 3H), 3.81 (m, 1H), 3.40 (m, 1H), 1.94 (m, 5H), 1.38 (s, 9H), 0.93 (d, 3H, *J* = 6.7 Hz), 0.85 (d, 3H, *J* = 6.7 Hz); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ, 172.9, 156.0, 124.8 (q, *J* = 283.2 Hz), 79.7, 70.3 (q, *J* = 28.8 Hz), 58.9, 57.1, 48.2, 31.1, 28.3, 25.7, 24.8, 19.5, 16.9; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -75.1 (d, *J* = 6.6 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{16}H_{27}N_2O_4F_3Na^+$ $(M + Na)^+$ 391.1821, found 391.1814.



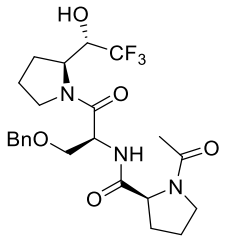
Cbz-Asp(OBn)-Val-Prolinolyl trifluoromethane (**4.57**)**.** Compound **4.56** (965 mg, 2.50 mmol, 1 equiv) was dissolved in 10 mL of DCM and cooled to 0 °C then 10 mL of TFA was added and the reaction was stirred for 1 hour at rt until complete by TLC then concentrated to dryness. The residue was dissolved in 25 mL of DCM and DIPEA (871 µL, 5.00 mmol, 2 equiv) was added followed by Cbz-Asp(OBn)-OPfp (1.57 g, 3 mmol, 1.2 equiv). The reaction was stirred for one

hour until complete as determined by a negative ninhydrin test. The mixture was washed with 25 mL of 0.5 M HCl then 25 mL of brine then the organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by flash chromatography, gradient elution: 25 to 60% EtOAc in hexane, to give **4.57** 1.35 g of a white foam (89% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31 (m, 10H), 7.23 (d, 1H, *J* = 8.8 Hz), 6.02 (d, 1H, *J* = 8.9 Hz), 5.30 (d, 1H, *J* = 7.4 Hz), 5.10 (m, 4H), 4.61 (m, 2H), 4.42 (t, 1H, *J* = 6.9 Hz), 4.31 (m, 1H), 3.81 (m, 1H), 3.41 (m, 1H), 3.05 (dd, 1H, *J* = 16.9, 4.3 Hz), 2.74 (dd, 1H, *J* = 17.1, 5.8 Hz), 2.03 (m, 4H), 1.78 (m, 1H), 0.92 (d, 3H, *J* = 6.7 Hz), 0.85 (d, 3H, *J* = 6.7 Hz); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 172.0, 171.5, 170.3, 156.1, 135.9, 135.3, 128.6, 128.4, 128.3, 128.25, 128.20, 124.9 (q, *J* = 283.9 Hz), 70.3 (q, *J* = 28.8 Hz), 67.4, 66.9, 59.1, 56.2, 51.3, 48.4, 36.2, 31.2, 25.9, 24.7, 19.5, 17.1; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -75.1 (d, *J* = 7.3 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{30}H_{36}N_3O_7F_3^+$ $(M + H)^+$ 608.2578, found 608.2577.



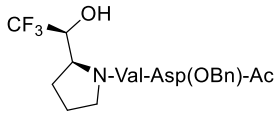
.Ac-Asp-Val-Prolinolyl trifluoromethane (**4.58**). Compound **4.57** (930 mg, 1.53 mmol, 1 equiv) was dissolved in 50 mL of MeOH and $Pd(OH)_2$ 20 wt% on carbon (93 mg, 0.13 mmol, 0.086 equiv) was added. The mixture was stirred under a hydrogen atmosphere for 3 hours then was filtered through celite and concentrated. The residue was dissolved in 20 mL of DMF and K_2CO_3 (422 mg, 3.06 mmol, 2 equiv) and p-nitrophenyl acetate (554 mg, 3.06 mmol, 2 equiv) were added. The mixture was stirred for 1 hour then was concentrated. The residue was purified by flash chromatography with 1% AcOH, 10% MeOH and 89% EtOAc to give **4.58** as 437 mg of white foam (67%). ^{1}H NMR (D_2O, 300 MHz): δ 4.58 (dd, 1H, *J* = 7.4, 6.0 Hz), 4.48 (qd, 1H, *J* = 8.0, 1.4 Hz), 4.3 3(d, 1H, *J* = 7.7 Hz), 4.19 (t, 1H, *J* = 5.7 Hz), 3.74 (m, 1H), 3.40 (m, 1H), 3.20

(s, 2H), 2.74 (dd, 1H, J = 16.9, 5.7 Hz), 2.64 (dd, 1H, J = 16.8, 7.8 Hz), 1.9 (m, 8H), 0.80 (d, 6H, J = 6.6 Hz); ^{13}C {^{1}H} NMR (D_2O, 75 MHz): δ 74.0, 173.8, 172.2, 171.6, 124.6 (q, J = 282.8 Hz), 67.4 (q, J = 29.3 Hz), 57.3, 56.8, 50.0, 48.8, 48.5, 35.3, 30.2, 24.6, 23.6, 21.6, 18.1, 17.3; ^{19}F NMR (D_2O, 282 MHz): δ -76.66 (d, J = 7.2 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{17}H_{27}N_3O_6F_3^+$ $(M + H)^+$ 426.1847, found 426.1845.



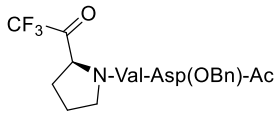
Ac-Asp-Val-Prolinyl trifluoromethane (**4.59**)**.** Alcohol **4.55** (610 mg, 1.23 mmol, 1.00 equiv) was added to a solution of Dess Martin reagent (1.30 g, 3.08 mmol, 2.50 equiv) in 13 mL of DCM. The clear solution was stirred at room temperature for 3 hours then quenched with 10 mL of sat. sodium thiosulfate. 50 mL of EtOAc was added and the mixture was washed twice with 50 mL of sat. $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography using 40% acetone in DCM to give 380 mg (62% yield). NMR showed that this existed as a mixture of isomers, approximately 3:7. These did not coalesce upon heating in DMSO and may result from epimerization of the carbonyl α-carbon. ^{1}H NMR (D_2O, 300 MHz): δ 8.07-7.70 (m, 1H), 7.36 (s, 5H), 4.9 (dd, 0.3H, J = 7.6, 4.6 Hz), 4.83 (ap t, 0.7H, J = 5.8 Hz), 4.55 (s, 2H), 4.51-4.47 (m, 1H), 4.38-4.25 (m, 1H), 3.81-3.38 (m, 6H), 2.17-1.79 (m, 11H); ^{13}C {^{1}H}NMR (4:1CD_3CN:D2O, 75 MHz): δ (major isomer) 173.3, 172.9, 171.5, 137.8, 128.4, 127.9, 127.8, 123.5 (q, J = 288.0 Hz), 73.0, 68.9, 62.7, 59.7, 51.5, 48.8, 48.2, 29.4, 25.9, 24.3, 24.0, 21.5; (minor isomer) 173.1, 172.8, 171.6, 137.7, 128.4, 127.9, 127.8, 123.5 (q, J = 288.0 Hz), 72.9, 68.8, 62.6, 61.2, 51.6, 48.8, 46.8, 31.7, 26.0, 24.1, 22.5, 21.4 (the carbonyl carbon was not visible due to keto/hydrate interconversion and $^{2}J_{CF}$ coupling). ^{19}F

NMR (4:1CD_3CN:D2O, 283 MHz): δ Hydrate (Major isomer-80.8), (Minor isomer)-80.9. Minor peaks (<5%) are also observed at -76.4 and -77.5 for the keto form.

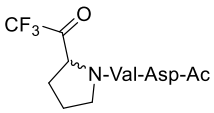


Ac-Asp(OBn)-Val-Prolinolyl trifluoromethane (**4.61**). Compound **4.56** (153 mg, 0.42 mmol, 1 equiv) was dissolved in 4 mL of 4 M HCl in dioxane and stirred for 1 hour then concentrated. A mixture of Ac-Asp(OBn)-OH (222 mg, 0.84 mmol, 2.0 equiv), HOAt (114 mg, 0.84 mmol, 2.0 equiv), DIPEA (61 mg, 0.84 mmol, 2.0 equiv) and DIC (106 mg, 0.84 equiv, 2.0 equiv) were added as a solution of 10 mL of DCM. The mixture was stirred overnight then the unreacted HOAt ester was destroyed by the addition of 270 μL of tris(2-aminoethyl)amine. After stirring a further 10 minutes the mixture was diluted with 100 mL of EtOAc and was washed twice with 50 mL of 10% citric acid and twice with 50 mL of 10% Na_2CO_3 then the organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography, 70 to 100% EtOAc in hexane, to give 106 mg of **4.61** as a white foam (49% yield). ^{1}H NMR (CD_3CN, 300 MHz): δ 7.35 (s, 5H), 7.19-7.09 (m, 1H), 6.98-6.92 (m, 1H), 5.09 (s, 2H), 4.78-4.68 (m, 1H), 4.61-4.50 (m, 3H), 4.22 (t, 1H, *J* = 7.1 Hz), 3.73 (m, 1H), 3.38 (dd, 1H, *J* = 16.6, 7.8 Hz), 2.82 (dd, 1H, *J* = 16.4, 6.4 Hz), 2.71 (dd, 1H, *J* = 16.6, 6.5 Hz), 2.13-1.74 (m, 8H), 0.91 (d, 3H, *J* = 6.8 Hz), 0.85 (d, 3H, *J* = 6.7 Hz); ^{13}C {^{1}H} NMR (CD_3CN, 75 MHz): δ (major rotamer)170.4, 170.3, 170.0, 169.9, 136.0, 125.0 (q, *J* = 283.0 Hz), 128.2, 127.8, 127.6, 67.7 (q, *J* = 28.5 Hz), 65.8, 56.8, 55.6, 49.4, 47.5, 35.2, 30.6, 24.5, 23.6, 21.6, 18.5; (minor rotamer) 170.4, 170.3, 170.0, 169.9, 136.0, 124.9 (q, *J* = 282.7 Hz), 128.2, 127.8, 127.7, 67.7 (q, *J* = 28.4 Hz), 65.8, 56.8, 55.7, 49.4, 47.5, 35.6, 30.5, 24.5, 23.6, 21.6, 18.5, 16.5; ^{19}F{1H}NMR (CD_3CN, 283

MHz): δ major rotamer -76.96 minor rotamer -76.98 HRMS-ESI$^+$ (*m/z*) calcd for $C_{24}H_{33}F_3N_3O_6^+$ $(M + H)^+$ 516.2316, found 516.2317.

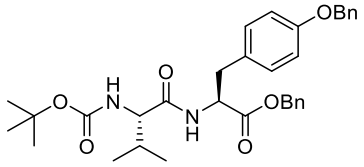


Ac-Asp(OBn)-Val-Prolinyl trifluoromethane (**4.62**). Alcohol **4.61** (350 mg, 0.67 mmol, 1 equiv) was dissolved in 3.5 mL of DCM and then Dess Martin reagent (0.2 M in DCM, 5.9 mL, 1.18 mmol, 1.75 equiv) was added. The mixture was stirred for 2 hours until complete by ^{19}F NMR. Then 25 mL of DCM was added and the mixture was washed twice with 25 mL of 5% Na_2CO_3. The organic layer was dried over $MgSO_4$ and the residue was purified by flash chromatography with 100 EtOAc to give 260 mg of a colorless oil (75% yield). ^1H NMR (2:1 Acetone-d_6:D_2O, 300 MHz): δ 7.28 (s, 5H), 5.05 (s, 2H), 4.79 (m, 1H), 4.5 (t, 1H, *J* = 8.0 Hz), 4.37 (m, 1H), 3.96 (m, 1H), 3.44 (m, 1H), 2.87 (dd, 1H, *J* = 16.1, 6.3 Hz), 2.72 (dd, 1H, *J* = 16.3, 8.6 Hz), 2.03-1.89 (m, 7H), 1.75 (m, 1H), 0.88 (d, 3H, *J* = 6.5 Hz), 0.83 (d, 3H, *J* = 6.6 Hz); ^{13}C {^1H}NMR (2:1 Acetone-d_6:D_2O, 75 MHz): δ 175.1, 171.8, 171.1, 170.7, 135.9, 123.7 (q, *J* = 289.6 Hz), 128.5, 128.0, 95.0 (q, *J* = 29.7 Hz), 66.4, 62.4, 56.6, 49.7, 49.0, 35.9, 30.5, 26.0, 24.1, 21.8, 18.6, 17.3; ^{19}F NMR (2:1 Acetone-d_6:D_2O, 283 MHz): δ -82.1 HRMS-ESI$^+$ (*m/z*) calcd for $C_{24}H_{31}F_3N_3O_6^+$ $(M + H)^+$ 514.2159, found 514.2161.



Ac-Asp-Val-Prolinyl trifluoromethane (**4.60**). Compound **4.62** (260 mg, 0.5 mmol) was dissolved in 9.5 mL of MeOH and 0.5 mL of AcOH. 26 mg of $Pd(OH)_2$, 20 wt % on carbon was added and the mixture was stirred under a hydrogen atmosphere for 16 hours. Then the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated and then the residue

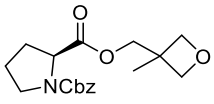
was purified by preparative TLC using 1% AcOH in EtOAc. The product was collected and lyophilized to give 96 mg of a white powder (44% yield). ^{19}F NMR indicated that the compound existed primarily as a hydrate in D_2O and as a 1:1 mixture of two isomers, additionally a small amount, <10%, of the ketone was present. ^{1}H NMR (D_2O, 500 MHz): δ 4.64 (m, 1H), 4.48 (m, 2H), 3.91 (m, 1H), 3.5 (m, 1H), 2.79-2.70 (m, 2H), 2.05-2.00 (m, 7H), 1.79 (m, 1H), 0.91-0.81 (m, 6H); ^{13}C {^{1}H} NMR (D_2O, 125 MHz): δ 175.01, 174.98, 174.1, 172.45, 123.02 (q, J = 288.6 Hz), 94.8 (q, J = 30.9 Hz), 61.68 and 61.63, 57.21 and 57.17, 50.18 and 50.16, 49.17, 35.9 and 35.5, 30.26 and 30.25, 25.62, 23.86, 21.66 and 21.61, 18.26 and 18.25, 17.06 and 17.04; ^{19}F NMR (D_2O, 471 MHz): δ (Hydrate) Mixture of isomers -82.21, -82.23 (Ketone) -75.8; HRMS-ESI$^+$ (*m/z*) calcd for $C_{17}H_{27}F_3N_3O7^+$ $(M + H_2O + H)^+$ 442.1796, found 442.1796.



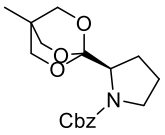
Boc-Val-Phe-Tyr(Bn)OBn (**4.67**). Boc-Tyr(Bn)-OBn (507 mg, 1.10 mol, 1.10 equiv) was dissolved in 10 mL of TFA. 1 mL of anisole was added as a *t*-butyl scavenger. This was stirred for 30 minutes until complete by TLC. The reaction was concentrated than redissolved in 30 mL of 10% Na_2CO_3 and extracted three times with 30 mL of EtOAc. The combined organic layers were dried over $MgSO_4$s, filtered, and concentrated. The residue was dissolved in 10 mL of DCM and Boc-Phe-OPfp (431 mg, 1.00 mmol, 1.00 equiv) was added followed by 10 mL of 5% $NaHCO_3$ the mixture was stirred for 2 hours. Then unreacted Pfp ester was destroyed by the addition of tris(2-aminoethyl)amine (1 mmol, 150 µL, 1.00 equiv) and stirring for 5 minutes. The organic layer was separated, and the aqueous layer was extracted with 20 mL of EtOAc. The combined organic layers were washed with 10 mL of 5% $NaHCO_3$ and 10 mL of 0.1 M HCl then

were dried over $MgSO_4$, filtered, and concentrated. This yielded crude Boc-Phe-Tyr(Bn)-OBn which was used without further purification.

The crude dipeptide was dissolved in 10 mL of TFA. 1 mL of anisole was added as a t-butyl scavenger. This was stirred for 30 minutes until complete by TLC then was concentrated then redissolved in 30 mL of 10% Na_2CO_3 a extracted three times with 30 mL of EtOAc. The combined organic layers were dried over $MgSO_4$s, filtered and concentrated. This was redissolved in 10 mL of DCM and Boc-Val-OPfp (421 mg, 1.1 mmol, 1.1 equiv) was added followed by 10 mL of 5% $NaHCO_3$. The reaction was stirred for 16 hours then unreacted Pfp ester was destroyed by the addition of Tris(2-aminoethyl)amine (1 mmol, 150 μL, 1.00 equiv) and stirring for 5 minutes. The reaction was diluted with 100 mL of EtOAc and then washed 3 times with 100 mL of 1 M HCl and 3 times with 10% Na_2CO_3. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, 20 to 40% EtOAc in hexane, to give 460 mg of **4.67** as a white solid (65% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.3 (m, 15H), 6.76 (s, 4H), 6.45 (d, 1H, *J* = 4.9 Hz), 6.09 (d, 1H, *J* = 5.0 Hz), 5.09 (s, 2H), 5.00 (s, 2H), 4.88 (d, 1H, *J* = 7.2 Hz), 4.71 (dd, 1H, *J* = 13.2, 6.1 Hz), 4.61 (dd, 1H, *J* = 13.3, 6.6 Hz), 3.88 (t, 1H, *J* = 6.8 Hz), 3.1 (dd, 1H, *J* = 13.6, 5.8 Hz), 2.96 (m, 2H), 2.09 (m, 1H), 1.44 (s, 9H), 0.88 (d, 3H, *J* = 6.8 Hz), 0.79 (d, 3H, *J* = 6.3 Hz); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): 171.6, 170.7, 170.2, 157.9, 155.9, 137.0, 136.3, 135.1, 130.3, 129.4, 128.7, 128.6, 128.5, 128.0, 127.7, 127.5, 127.0, 114.9, 80.0, 69.9, 67.2, 60.0, 54.2, 53.6, 38.3, 37.1, 30.8, 28.4, 19.3, HRMS-ESI$^+$ (*m/z*) calcd for $C_{42}H_{49}N_3O_7Li^+$ $(M + Li)^+$ 714.3725, found 714.3721.

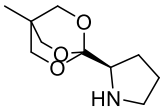


Cbz-proline (3-methyloxetan-3-yl)methyl ester (**4.70**). (3-methyloxetan-3-yl)methanol (490 mg, 4.80 mmol, 1.2 equiv) and DMAP (5 mg, 0.04 mmol, 0.01 equiv) were dissolved in 6 mL of DCM and cooled to 0 °C. DIC (555 mg, 475 µL, 4.40 mmol, 1.10 equiv) was added followed by Cbz-Pro-OH (1.00 g, 4.00 mmol, 1 equiv). After stirring for 2 hours, an additional 150 µL of DIC was added. After stirring for 3 hours in total the reaction was diluted with 50 mL of DCM and washed with 50 mL of 0.1 M HCl then 50 mL of brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography, 40 to 60% EtOAc in hexane to give **4.70** as a colorless oil, 1.00 g, (75% yield). 1:1 mixture of rotamers. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 (m, 5H), 5.13 (m, 2H), 4.53 (d, 0.5H, *J* = 6.0 Hz), 4.50 (d, 0.5H, *J* = 6.0 Hz), 4.45 (dd, 0.5H, *J* = 8.6, 3.5 Hz), 4.40 (dd, 0.5H, *J* = 8.7, 3.6 Hz), 4.36 (m, 2H), 4.28 (dd, 1H, *J* = 5.8, 3.8 Hz), 4.25 (d, 0.5H, *J* = 11.2 Hz), 4.21 (d, 0.5H, *J* = 11.1 Hz), 4.10 (d, 0.5H, *J* = 11.1 Hz), 4.00 (d, 0.5H, *J* = 11.1 Hz), 3.58 (m, 2H), 2.3 (m, 1H), 1.98 (m, 3H), 1.33 (s, 1.5H), 1.22 (s, 1.5H); ^{13}C {^{1}H} NMR ($CDCl_3$, 125 MHz): δ 172.9, 172.7, 154.8, 154.2, 136.7, 136.5, 128.5, 128.4, 128.0, 128.0, 128.0, 127.9, 79.4, 79.4, 79.2, 79.2, 68.9, 68.9, 67.1, 67.0, 59.3, 58.9, 46.9, 46.4, 39.2, 39.0, 31.0, 30.0, 24.4, 23.5, 21.1, 20.9; HRMS-ESI$^+$ (*m/z*) calcd for $C_{18}H_{24}O_5N^+$ $(M + H)^+$ 334.1649, found 334.1648.

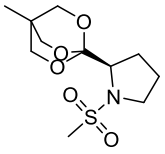


Cbz-Pro-OBO (**4.71**). Ester **4.70** (5.93 g, 17.8 mmol, 1.00 equiv) was dissolved in 40 mL of DCM and $BF_3{\cdot}OEt_2$ (252 mg, 220 µL, 1.78 mmol, 0.1 equiv) was added. The reaction was stirred at room temperature for 6 hours then was quenched by adding 400 µL of triethyl amine and stirring an additional 30 minutes. The solution was concentrated then dissolved in 120 mL of EtOAc and washed twice with 100 mL of sat. NH_4Cl, twice with 100 mL of sat. $NaHCO_3$ and

once with 100 mL of brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give 5.41 g of a white solid that was used without further purification (91% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.39 (d, 2H, *J* = 7.3 Hz), 7.33 (t, 2H, *J* = 7.4 Hz), 7.28 (m, 1H), 5.21 (broad doublet, 1H, *J* = 8.5 Hz), 5.07 (d, 1H, *J* = 12.4 Hz), 4.18 (m, 1H), 3.86 (s, 6H), 3.55 (m, 1H), 3.39 (m, 1H), 2.08 (m, 2H), 1.76 (m, 2H), 0.77 (s, 3H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz):, δ, 155.7, 137.2, 128.3, 127.8, 127.7, 109.8, 72.6, 66.7, 60.4, 47.1, 30.5, 26.4, 23.8, 14.5; HRMS-ESI$^+$ (*m/z*) calcd for $C_{18}H_{24}NO_5^+$ $(M + H)^+$ 333.1649, found 334.1646.

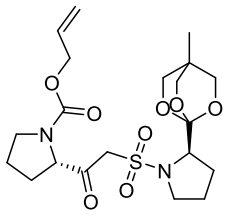


Pro-OBO (**4.72**). Compound **4.71** (260 mg, 0.78 mmol) was dissolved in 18 mL of MeOH and 42 mg of Pd 10 wt% on carbon was added. The suspension was stirred under a hydrogen atmosphere for 16 hours then was filtered through a pad of celite and concentrated. The residue was purified by flash chromatography, 0 to 10% triethylamine in EtOAc to give 127 mg of a yellow solid (82% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.75 (s, 6H), 3.06 (t, 1H, *J* = 6.9 Hz), 2.84 (m, 1H), 2.7 (m, 1H), 1.95 (s, 1H), 1.56 (m, 4H), 0.64 (s, 3H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 109.5, 72.6, 61.5, 46.9, 30.5, 26.0, 25.5, 14.4 HRMS-ESI$^+$ (*m/z*) calcd for $C_{10}H_{18}NO_3^+$ $(M + H)^+$ 200.1281, found 200.1282.



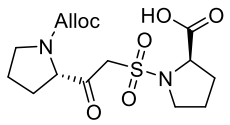
Mesyl-Pro-OBO (**4.73**). Compound **4.72** (1.95 g, 9.80 mmol, 1.00 equiv) was dissolved in 50 mL of DMF and DIPEA (3.10 g, 4.30 mL, 24 mmol, 2.40 equiv) was added followed by methanesulfonyl chloride (1.14 g, 930 µL, 12.0 mmol, 1.2 equiv) the reaction was stirred for 90 minutes until complete as judged by TLC then was concentrated. The residue was purified by

flash chromatography, 60% EtOAc in hexane to give 2.4 g of light brown solid (89% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ4.07 (dd, 1H, *J* = 8.2, 2.5 Hz), 3.86 (s, 6H), 3.66 (m, 1H), 3.2 (m, 1H), 2.97 (s, 3H), 1.92 (m, 4H), 0.78 (s, 3H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 109.4, 72.6, 62.2, 48.7, 40.9, 30.6, 27.4, 24.8, 14.4; HRMS-ESI^{+} (*m/z*) calcd for $C_{11}H_{19}NO_5S$ $(M + H)^{+}$ 278.1058, found 278.1057.

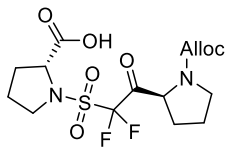


allyl (S)-2-(2-(((R)-2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)pyrrolidin-1-yl)sulfonyl)-acetyl)pyrrolidine-1-carboxylate (**4.74**) Compound **4.73** (1.22 g, 4.40 mmol, 2.10 equiv) was dissolved in 30 mL of THF and cooled to – 78 °C. BuLi (2.5 M in hexane, 1.76 mL, 4.40 mmol, 2.1 equiv) was added dropwise and the mixture was stirred for 30 minutes before adding alloc-Pro-OMe (446 mg, 2.09 mmol, 1.00 equiv) as a solution in 5 mL of THF over a period of 5 minutes. The reaction was stirred for an additional 40 minutes until complete by TLC then was quenched with sat. NH_4Cl, 50 mL. The reaction was extracted 3 times with 50 mL of DCM then the combined organic layers were dried over $MgSO_4$, filtered, and concentrated, flash chromatography, 40 to 50% EtOAc in hexane gave 775 mg **4.74** as a colorless oil (81% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.93 (m, 1H), 5.3 (d, 1H, *J* = 17.4 Hz), 5.21 (d, 1H, *J* = 10.4 Hz), 4.62 (m, 4H), 4.33 (m, 1H), 4.18 (m, 1H), 3.92 (s, 6H), 3.73 (dd, 1H, *J* = 15.1, 8.2 Hz), 3.58 (m, 2H), 3.35 (m, 1H), 2.21 (m, 3H), 1.96 (m, 5H), 0.81 (s, 3H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ (Major Rotamer) 199.2, 154.6, 132.7, 117.1, 109.1, 72.3, 65.7, 64.8, 62.3, 49.0, 46.7, 30.4, 28.7, 27.1, 24.4, 24.3, 14.1 (Minor Rotamer) 198.4 153.6 132.7 117.4 109.1 72.3 65.8 64.9 61.5 49.1

47.1 30.4 29.5 27.1 24.5 23.3 14.1 HRMS-ESI$^+$ (*m/z*) calcd for $C_{20}H_{31}N_2O_8S^+$ $(M + H)^+$ 459.1796, found 459.1773.

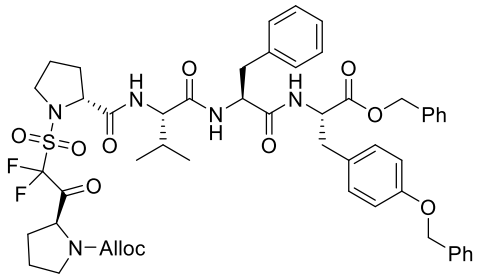


N-(alloc-prolinyl)methanesulfonyl proline (**4.76**). Compound **4.74** (652 mg, 1.42 mmol) was dissolved in a mixture of 24 mL of DCM, 600 µL of water and 150 µL of TFA. The reaction was stirred at room for 30 minutes until complete by TLC then was concentrated. The residue was dissolved in 6 mL of THF and 18 mL of 0.5 M NaOH was added. This was stirred at room temperature for 1 hour until complete by TLC and then was diluted with 15 mL of 1 M HCl and extracted three times with 15 mL of 3:1 $CHCl_3$:*i*PrOH. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, 80% EtOAc, 19% hexane, 1%AcOH, to give 478 mg of a white solid (89% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.75 (s, 1H), 5.8 (m, 1H), 5.14 (m, 2H), 4.36 (m, 6H), 3.44 (m, 4H), 2.04 (m, 8H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ (major rotamer) 198.8 175.6 155.2 132.4 117.5 66.3 65.7 60.9 59.3 48.5 46.8 30.8 28.2 24.7 24.2 (minor rotamer) 198.6, 175.6, 154.4, 132.4, 118.0, 66.4, 65.6, 60.9, 58.9, 48.6, 47.2, 30.9, 29.2, 24.7, 23.4; HRMS-ESI$^+$ (*m/z*) calcd for $C_{15}H_{23}N_2O_7S_1^+$ $(M + H)^+$ 375.1221, found 375.1217.



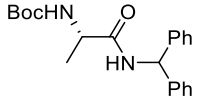
N-(alloc-prolinyl)difluoromethanesulfonyl proline (**4.66**). Compound **4.74** (400 mg, 0.87 mmol, 1 equiv) was dissolved in 16 mL of DCM then 400 µL of water and 100 µL of TFA were added. The mixture was stirred for 30 minutes until complete by TLC and then evaporated to dryness. The residue was dissolved in 4 mL of THF and 12 mL of 0.5 M NaOH was added. The reaction

was stirred for 1 hour and then acidified to pH 1 and diluted with 40 mL of brine. This was extracted three times with 40 mL of 3:1 $CHCl_3$;*i*PrOH. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved in 20 mL of DMF and cesium fluoride (800 mg, 5.2 mmol, 6.0 equiv) was added followed by Selectfluor (1.85 g, 5.2 mmol, 6.0 equiv). After stirring 1 hour the reaction was diluted with 100 mL of EtOAc and washed twice with 100 mL of 0.1 M HCl. The combined aqueous layers were back extracted with 100 mL of 3:1 $CHCl_3$;*i*PrOH, then the combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography, 47.5:2.5:50 EtOAc:AcOH:hexane to give 278 mg of a white solid (78% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.21 (broad singlet, 1H), 5.87 (m, 1H), 5.23 (m, 2H), 4.94 (dd, 1H, *J* = 8.3, 4.7 Hz), 4.54 (m, 3H), 3.63 (m, 4H), 2.15 (m, 8H); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 194.6 (t, *J* = 26.0 Hz), 174.7, 154.1 (m), 132.5, 117.4, 66.3, 61.4, 61.2, 49.6, 46.6, 30.8, 29.2, 24.2, 23.3; ^{19}F NMR ($CDCl_3$, 282 MHz): δ (major rotamer) -106.7 (d, *J* = 250.6 Hz), -108.6 (d, *J* = 251.2 Hz); (minor rotamer) -107.7 (apparent singlet); HRMS-ESI$^+$ (*m/z*) calcd for $C_{15}H_{21}N_2O_7SF_2^+$ $(M + H)^+$ 411.1032, found 411.1018.

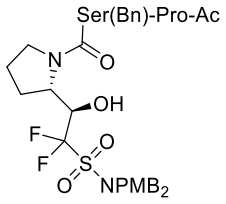


N-(alloc-prolinyl)difluoromethanesulfonyl Pro-Val-Phe-Tyr(Bn)-OBn (**4.65**). Tripeptide **4.67** (50 mg, 0.071 mmol, 1 equiv) was dissolved in 3 mL of 4 M HCl in dioxane with 1 mL of anisole added as a t-butyl scavenger. The reaction was stirred for 30 minutes and then concentrated and redissolved in 10 mL of Na_2CO_3 and extracted three times with 10 mL of 1:3 *i*PrOH:$CHCl_3$. The

combined organic layers were dried over $MgSO_4$, filtered, and concentrated then redissolved in 3 mL of DMF. HOAt (50 mg, 0.37 mmol, 5.2 equiv) and **4.66** (30 mg, 0.73 mmol, 1.0 equiv) were added and the mixture cooled to 0 °C. DCC (50 mg, 0.364 mmol, 5.1 equiv) was added and then the mixture was stirred overnight. The suspension was then filtered through a pad of celite, diluted with 50 mL of EtOAc, then washed with 50 mL of water then 50 mL of brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated then the residue was purified by flash chromatography, 50 to 70% EtOAc in hexane, to give 58 mg of **4.65** as a white solid (81% yield). This was judged to be >95% pure by analytical RP-HPLC. HRMS-ESI$^+$ (*M/z*): calcd for $C_{52}H_{60}O_{11}N_5F_2S^+$ $[M+H]^+$, 1000.3973; found 1000.3975. See Appendix Figure A21 and A22 for copies of data.

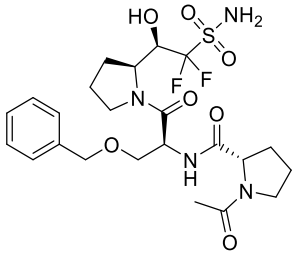


Boc-Ala-diphenylmethylamide (**4.81**). Sulfonamide **4.80** (50 mg, 0.1 mmol, 1 equiv) was dissolved in 4.5 mL of toluene and 500 μL of TFA was added. The reaction was stirred for 1 hour until complete by TLC then 5 mL of 10% Na_2CO_3 was added. Boc-Ala-OPfp (50 mg, 0.13 mmol, 1.1 equiv) was added dissolved in 1 mL of toluene. The reaction was stirred for 90 minutes at room temperature then diluted with 20 mL of EtOAc and washed twice with 20 mL of saturated $NaHCO_3$ and twice with 20 mL of 0.05 M HCl. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography to give **4.81** as a white solid (22 mg, 63%). NMR spectra were identical to those reported in the literature.[236]

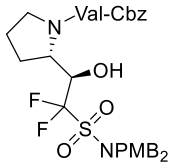


N,N-bis(4-methoxybenzyl)-1-(ac-Pro-O^3-benzyl-Ser-prolinyl)-1,1-difluoromethanesulfonamide (**4.84**). Sulfonamide **4.82** (305 mg, 0.42 mmol, 1 equiv) and 30 mg of palladium 10 wt% on carbon were placed in 1 mL of DCM, 4 mL of MeOH and 1 mL of TES. The mixture is cooled to 0 °C and TFA (77 µL, 114 mg, 1.00 mmol, 2.4 mmol) was added dropwise. The mixture was stirred for 30 minutes until complete by TLC and then filter with a plug of celite. 10 mL of 10% Na_2CO_3 was added and then the reaction was extracted three times with DCM. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was dissolved in 10 mL of DMF and DIPEA (261 µL, 1.5 mmol, 3.6 equiv) and Ac-Pro-Ser(Bn)-OH (258 mg, 0.75 mmol, 1.8 equiv) were added. The mixture was cooled to – 40 °C and then DPPA (160 µL, 0.75 mmol, 1.8 equiv) was added. The reaction was stirred for 16 hours while warming slowly to 0 °C. The reaction was quenched with 20 mL of 10% citric acid and the mixture was extracted 5 times with 20 mL of EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, 10 to 90% EtOAc in hexane to give 280 mg of a white solid (83% yield). ^{19}F NMR showed that a minor rotamer accounted for approximately 10% of the mixture. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.44 (d, 1H, *J* = 7.2 Hz), 7.29 (s, 5H), 7.09 (d, 4H, *J* = 8.6 Hz), 6.79 (d, 4H, *J* = 8.6 Hz), 5.1 (d, 1H, *J* = 7.5 Hz), 4.9 (m, 1H), 4.78 (m, 1H), 4.49 (m, 3H), 4.32 (s, 4H), 4.11 (m, 1H), 3.77 (s, 6H), 3.55 (m, 6H), 2.02 (m, 11H); ^{13}C {^{1}H}NMR ($CDCl_3$, 75 MHz): δ 173.0, 171.4, 170.7, 159.2, 137.6, 130.2, 128.4, 127.7, 127.7, 127.0, 122.6 (dd, *J* = 292.4, 287.6 Hz), 113.8, 73.7 (dd, *J* = 23.7, 21.3 Hz), 73.4, 70.0, 59.6, 58.0, 55.2, 51.2, 50.5, 48.2, 47.5, 28.2, 27.8, 24.9, 24.1, 22.4; ^{19}F NMR ($CDCl_3$, 481 MHz):

δ (major rotamer) -104.3 (d, 1F, J = 241.4 Hz), -114.8 (dd, 1F, J = 241.5, 18.0 Hz); (minor rotamer) -104.8 (dd, 1F, J = 242.0, 20.0 Hz), -113.2 (dd, 1F, J = 243.2, 18.5 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{39}H_{49}F_2N_4O_9S^+$ $(M + H)^+$ 787.3183, found 787.3192.

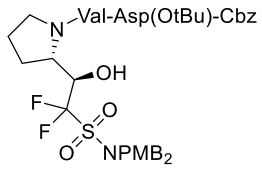


(Ac-Pro-O^3-benzyl-Ser(Bn)-Prolinolyl)difluoromethanesulfonamide (**4.86**). Sulfonamide **4.84** (78 mg, 0.1 mmol, 1.0 equiv) was dissolved in 10 mL of CH_3CN and cooled to -20 °C then CAN (438 mg, 0.80 mmol, 8.0 equiv) was added as a solution in 4 mL of water. The mixture was stirred overnight at 0 °C then was quenched with 50 mL of brine and 50 mL of 10% citric acid. The product was extracted 3 times with 50 mL of EtOAc then the combine organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography to give 51 mg of a white foam (93% yield). This was judged to be > 90% pure by analytical RP-HPLC HRMS-ESI$^+$ (*m/z*) calcd for $C_{23}H_{31}F_2N_4O_7S^+$ $(M + H)^+$ 545.1876, found 545.2045. See Appendix Figures A23 and A24 for copies of data.



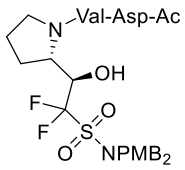
N,N-bis(4-methoxybenzyl)-1-(Val-prolinyl)-1,1-difluoromethanesulfonamide (**4.87**). Sulfonamide **4.82** (710 mg, 1 mmol, 1 equiv) was dissolved in 10 mL of DCM, 10 mL of MeOH and 4 mL of triethylsilane were added followed by 71 mg Pd 10 wt% on carbon. The mixture was cooled in an ice bath and TFA (150 µL, 2 mmol, 2 equiv) was added dropwise (caution, effervescence) the

mixture was then stirred at room temperature for 30 minutes until complete by TLC (20% EtOAc in hexane) and filtered using a pad of celite. 20 mL of 5% Na_2CO_3 was added and the mixture was extracted three times with 10 mL of DCM. The combined organic layers were dried over $MgSO_4$ filtered and concentrated yielding solid which consisted of the desired free amine and 9-phenylfluorene. Separately Z-Val-OH (3 mmol, 753 mg, 3 equiv) and HOAt (408 mg, 3 mmol, 3 equiv) were dissolved in 30 mL of DCM and EDC·HCl (576 mg, 3 mmol, 3 equiv) was added the mixture was stirred for 15 minutes at room temperature yielding a clear colorless solution which was added to the free amine. This was stirred until complete by TLC (1h) then 400 µL of Tris(2-aminoethyl)amine was added and stirred for 15 minutes to destroy excess activated ester. The mixture was then diluted with 200 µL of EtOAc and washed with 100 mL of 10% citric acid, 100 mL of 5% Na_2CO_3 and 100 mL of brine then dried over $MgSO_4$ filtered and concentrated. The residue was purified by flash chromatography using a gradient of 20 to 80% EtOAc in hexane to give 410 mg of a white foam (58% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.35 (m, 5H), 7.12 (d, 4H, *J* = 8.7 Hz), 6.82 (d, 4H, *J* = 8.7 Hz), 5.50 (d, 1H, *J* = 8.7 Hz), 5.08 (m, 3H), 4.80 (m, 1H), 4.35 (m, 5H), 4.15 (m, 1H), 3.82, (m, 1H), 3.80 (s, 6H), 3.59 (m, 1H), 2.04 (bs, 5H), 1.03 (d, 3H, *J* = 6.7 Hz), 0.96 (d, 3H, *J* = 6.7Hz); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ ν(F1) [ppm] 175.3, 159.3, 156.5, 136.4, 130.2, 128.5, 128.1, 127.9, 126.9, 12.6 (dd, *J* = 292.9, 288.0 Hz), 113.9, 74.0 (dd, *J* = 23.1, 21.7 Hz), 66.8, 57.9, 57.8, 55.2, 50.5, 47.6, 31.3, 27.8, 27.8, 24.3, 19.3, 17.7; ^{19}F NMR ($CDCl_3$, 282 MHz): δ -114.0 (dd, 1F, *J* = 241.0Hz, 17.7 Hz), -104.5 (d, 1F, *J* = 241.1 Hz); HRMS-ESI$^+$ (*m/z*) calcd for $C_{35}H_{44}N_3O_8SF_2^+$ $(M + H)^+$ 704.2812, found 704.2782.



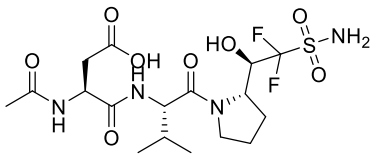
N,N-bis(4-methoxybenzyl)-1-(Cbz-Asp(OtBu)-Val-prolinyl)-1,1-difluoromethanesulfonamide (**4.88**). Dipeptide **4.87** (251 mg, 0.357 mmol, 1 equiv) was dissolved in 10 mL of MeOH and 25 mg of $Pd(OH)_2$ 20 wt% on carbon was added. The mixture was stirred under hydrogen atmosphere for one hour, then was filtered with a pad of celite and concentrated. Separately, Cbz-Asp(OtBu)-OH (345 mg, 1.07 mmol, 3 equiv) and HOAt (145 mg, 1.07 mmol, 3 equiv) were placed in 15 mL of DCM and cooled to 0 °C. EDC·HCl (205 mg, 1.07 mmol, 3 equiv) and the mixture was stirred for 15 minutes, then added to the free amine. This reaction was stirred for 2 hours at room temperature then 250 µL of Tris (2-aminoethyl)amine was added and stirred 5 minutes to destroy any unreacted HOAt ester. The reaction was diluted with 100 mL of EtOAc and washed with 50 mL of 10% citric acid, 50 mL of 10% Na_2CO_3, 50 mL of brine, then the organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, 50 to 100% EtOAc in hexane. To give **4.88** as 125 mg of a white solid (40% yield). A minor epimer or rotamer was observed by ^{19}F NMR which comprised 6% of the mixture. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38 (m, 5H), 7.14 (m, 5H), 6.84 (d, 4H, *J* = 8.7 Hz), 6.06 (d, 1H, *J* = 8.7 Hz), 5.16 (s, 2H), 5.11 (d, 1H, *J* = 8.1 Hz), 4.8 (m, 1H), 4.59 (m, 2H), 4.41 (d, 2H, *J* = 15.0 Hz), 4.32 (d, 2H, *J* = 15.1 Hz), 4.17 (m, 1H), 3.81 (s, 6H), 3.60 (m, 1H), 2.96 (dd, 1H, *J* = 17.1, 4.3 Hz), 2.63 (dd, 2H, *J* = 17.1, 5.8 Hz), 2.07 (m, 2H), 1.44 (s, 9H), 1.01 (d, 3H, *J* = 6.7 Hz), 0.94 (d, 3H, *J* = 6.7 Hz); ^{13}C {^{1}H} NMR ($CDCl_3$, 75 MHz): δ 174.6, 171.1, 170.5, 159.3, 156.1, 136.0, 130.2, 128.6, 128.3, 128.2, 126.9, 122.5 (dd, *J* = 292.7, 287.7 Hz), 113.9, 81.8, 74.3 (apparent triplet, *J* = 22.3 Hz), 67.3, 57.7, 56.0, 55.2, 51.4, 50.4, 47.6, 37.1, 31.5, 27.9, 27.9, 24.3, 19.3, 17.5; ^{19}F NMR ($CDCl_3$, 282 MHz): δ (Major)-104.4 (d, 1F, *J* = 240.9 Hz), -114.2 (dd, 5F, *J* = 240.8, 18.0 Hz); (minor) -106.3 (dd, 1F, *J* = 241.7, 6.2 Hz), -

112.0 (dd, 3F, J = 242.7, 16.5 Hz), HRMS-ESI$^+$ (*m/z*) calcd for $C_{43}H_{56}F_2N_4NaO_{11}S^+(M + Na)^+$ 897.3527, found 897.3504.



N,N-bis(4-methoxybenzyl)-1-(Ac-Asp-Val-prolinyl)-1,1-difluoromethanesulfonamide (**4.89**). Tripeptide **4.88** (251 mg, 0.287 mmol, 1 equiv) was dissolved 10 mL of MeOH and 25 mg of 10 wt % $Pd(OH)_2$ on carbon was added. The mixture was stirred under an atmosphere of hydrogen until complete by TLC then was filtered through a pad of celite and concentrated. The residue was dissolved in 10 mL of DCM and 200 µL of acetic anhydride. After two hours no reaction had occurred as judged by TLC and an additional 200 µL of acetic anhydride was added followed by 300 µL of Et_3N. The mixture was stirred overnight and then the reaction mixture was placed on a silica column and eluted with EtOAc and concentrated to give a mixture of mono- and di- acylated material as judged by LRMS. (the second acylation occurring on the hydroxyl group which is ß-to the sulfonamide). The undesired acylation was removed by treatment with 10 mL of a 0.1 M solution of LiOH in 1:1 H_2O:THF for 6 hours at room temperature. The crude material was then neutralized with minimal 1 M HCl and concentrated. The residue was dissolved in 5 mL of 95:5:5 TFA:TIPS:H_2O at 0 °C and stirred for 1 hour then was diluted in 50 mL of heptane and concentrated. The residue was subjected to flash chromatography, 0 to 10% MeOH in EtOAc (1% AcOH). Fractions containing product were further purified by RP-HPLC using a gradient of 30 to 70% CH_3CN in 0.1% TFA:H_2O over 50 minutes (retention time = 35 min). This gave 103 mg of **4.89** as a white solid (49% yield). A minor rotamer or epimer could be observed in the ^{19}F NMR which comprised approximately

12% of the mixture. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.15 (d, 1H, *J* = 6.1 Hz), 6.78 (d, 1H, *J* = 5.7 Hz), 6.62 (d, 4H, *J* = 6.4 Hz), 6.4 (d, 4H, *J* = 6.4 Hz), 4.95 (m, 1H), 4.81 (m, 1H), 4.67 (t, 1H, *J* = 6.1 Hz), 4.5 (m, 4H), 4.14 (m, 1H), 4.08 (s, 6H), 3.93 (bs, 1H), 3.4 (dd, 1H, *J* = 12.7, 3.5 Hz), 3.28 (dd, 1H, *J* = 12.6, 4.5 Hz), 2.74 (m, 8H), 1.92 (d, 3H, *J* = 5.0 Hz), 1.89 (d, 3H, *J* = 4.9 Hz); ^{13}C {^{1}H} NMR ($CDCl_3$, 125 MHz): δ 174.2, 174.1, 171.2, 171.0, 159.3, 130.2, 126.9, 122.8 (dd, *J* = 293.4, 287.1 Hz), 113.9, 72.2 (dd, *J* = 23.6, 20.8 Hz), 58.3, 56.6, 55.3, 50.5, 49.7, 47.8, 36.5, 31.1, 27.1, 24.1, 22.9, 19.2, 18.0; ^{19}F NMR ($CDCl_3$, 483 MHz): δ (major rotamer) -104.6 (d, 1F, *J* = 240.3 Hz), -114.6 (dd, 1F, *J* = 240.4, 18.9 Hz); (minor rotamer) -104.8 (dd, 1F, *J* = 239.6, 26.9 Hz), -111.9 (dd, 1F, *J* = 242.7 Hz); HRMS-ESI^{+} (*m/z*) calcd for $C_{33}H_{45}F_2N_4O_{10}S^{+}$ $(M + H)^{+}$ 727.2819, found 727.2800.



1-(Ac-Asp-Val-prolinyl)-1,1-difluoromethanesulfonamide (**4.90**). Tripeptide **4.89** (103 mg, 0.1412 mmol, 1 equiv) was dissolved in 10 mL of acetonitrile and cooled to –20 °C then CAN (621 mg, 1.13 mmol, 8 equiv) was added as a solution in 4 mL of water. The mixture was stirred overnight at 0 °C then 25 mL of 10% citric acid and 25 mL of brine were added. The mixture was extracted 5 times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by RP-HPLC, 10 to 50% CH_3CN in water over 50 minutes, retention time = 28 min, yielding 55 mg of a white solid (78% yield). A minor rotamer or epimer was observed in ^{19}F NMR which comprised approximately 20% of the mixture. Experimental procedure. ^{1}H NMR (MeOD, 300 MHz): δ 7.4 (m, 2H), 4.98 (d, 1H, *J* = 6.8 Hz), 4.71 (m, 2H), 4.44 (m, 1H), 3.9 (m, 1H), 3.55 (m, 1H), 2.84 (m, 1H), 2.68 (dd, 1H, *J* =

16.5, 7.4 Hz), 2.05 (m, 8H), 0.97 (m, 6H); ^{13}C {^{1}H} NMR (MeOD, 75 MHz): δ (major rotamer) 172.9, 172.4, 172.0, 171.6, 121.1 (dd, *J* = 291.7, 286.3 Hz), 70.4 (dd, *J* = 24.5, 20.4 Hz), 58.0, 56.5, 50.0, 44.9, 35.1, 30.5, 26.6, 23.5, 21.0, 18.4, 16.8, (minor rotamer) 172.5, 172.3, 172.1, 170.8, 120.8 (ap t, *J* = 287.1 Hz), 70.9 (t, *J* = 23.1 Hz), 56.9, 56.4, 50.2, 42.5,, 35.2, 32.6, 28.7,21.0, 20.5, 17.9, 17.0; ^{19}F NMR (MeOD, 282 MHz): δ (major rotamer) -106.6 (d, 1F, *J* = 242.3 Hz), -119.0 (dd, 1F, *J* = 242.3, 20.6 Hz); (minor rotamer) -101.9 (2, 1F, *J* = 254.3, 11.6 Hz), -111.7 (2, 1F, *J* = 255.5, 6.9 Hz); HRMS-ESI^{+} (*m/z*) calcd for $C_{17}H_{29}N_4O_8SF_2^{+}$ $(M + H)^{+}$ 487.1669, found 487.1685.

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Appendix — HPLC/HRMS Data of compounds 4.32-4.41, 4.65, and 4.86.

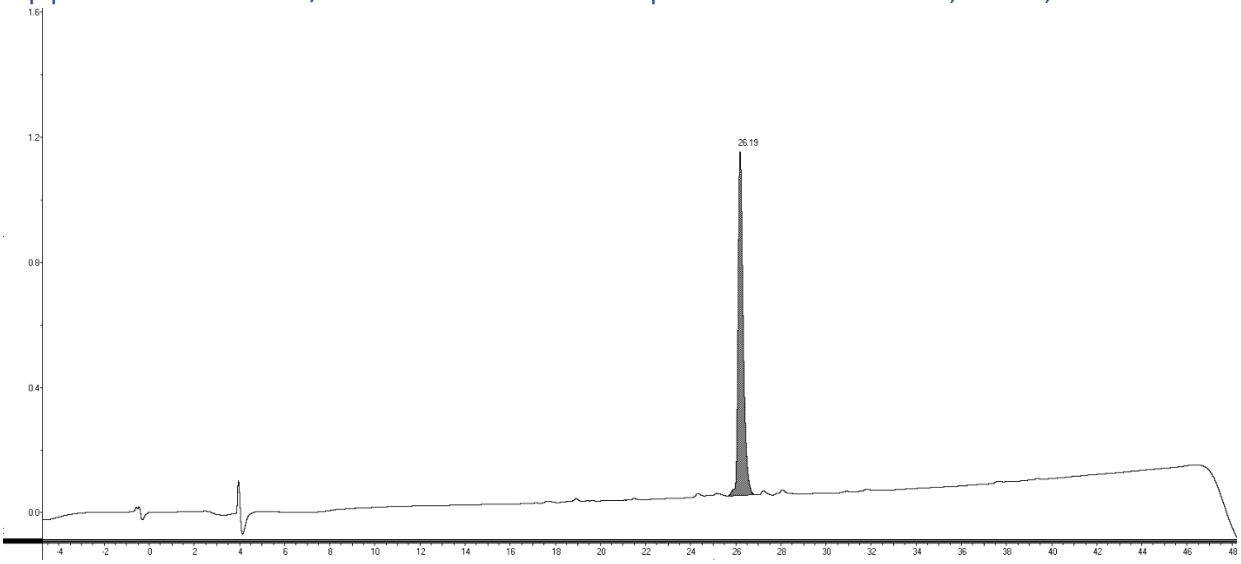

Figure A1. HPLC Chromatogram of Ac-Pro-Ser(Bn)-Pro-pNA (**4.32**). Gradient 5:95 CH_3CN:H_2O (0.1% TFA) to 95:5 CH_3CN:H_2O (0.1% TFA) over 40 min. (λ = 220 nm).

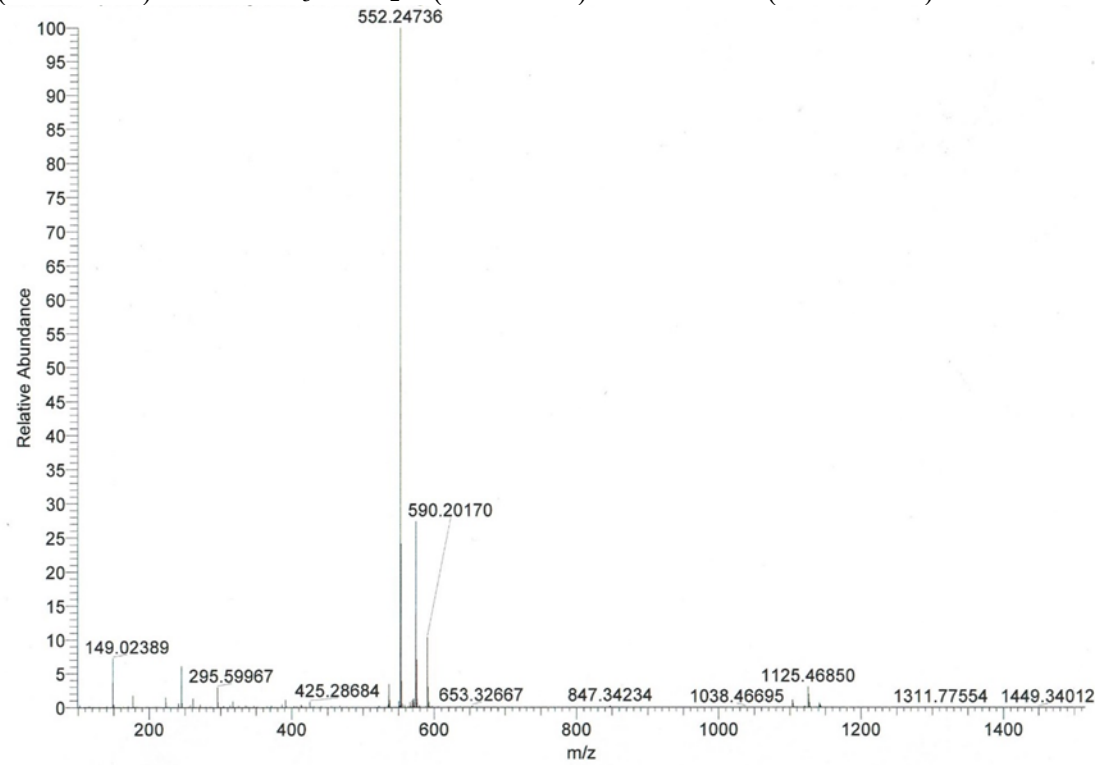

Figure A2. HRMS data for Ac-Pro-Ser(Bn)-Pro-pNA (**4.32**). The peak at *m/z* = 552.24736 corresponds to the M + H species (*m/z*) calcd for $C_{28}H_{34}N_5O_7^+$ = 552.2453. The peak at *m/z* 590.20170 corresponds to the M+K species (*m/z*) calcd for $C_{28}H_{33}KN_5O_7^+$ = 590.2012.

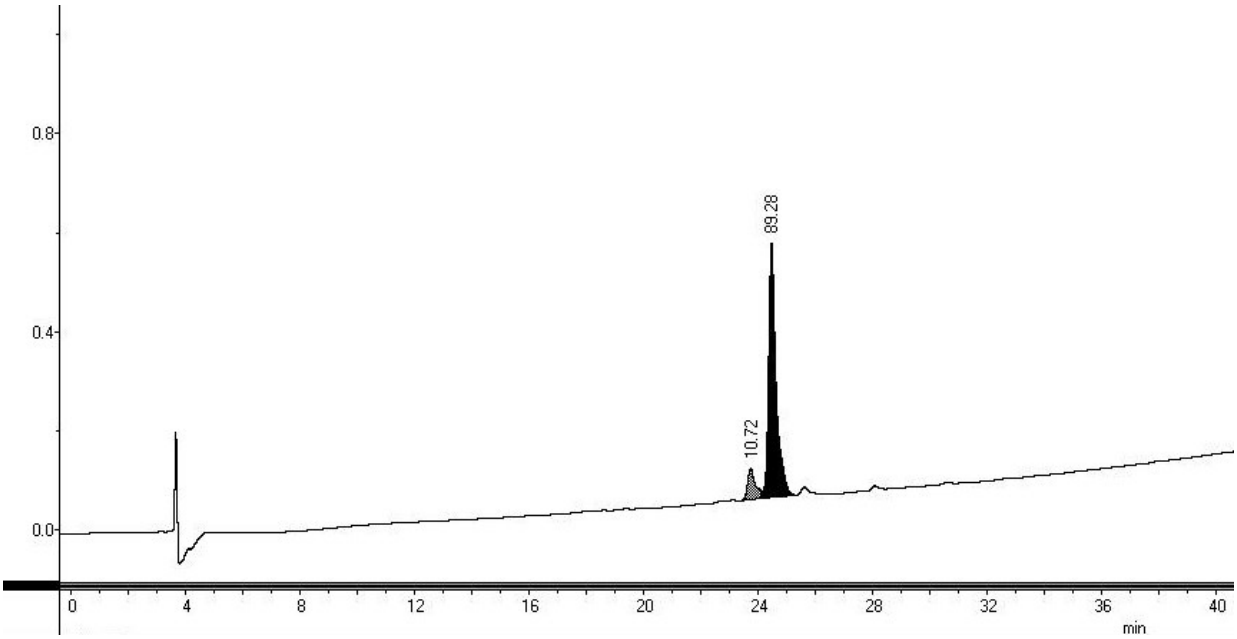

Figure A3. HPLC Chromatogram of Ac-Thr-Pro-Ser(Bn)-Pro-pNA (**4.33**). Gradient 5:95 CH_3CN:H_2O (0.1% TFA) to 95:5 CH_3CN:H_2O (0.1% TFA) over 40 min. (λ = 220 nm).

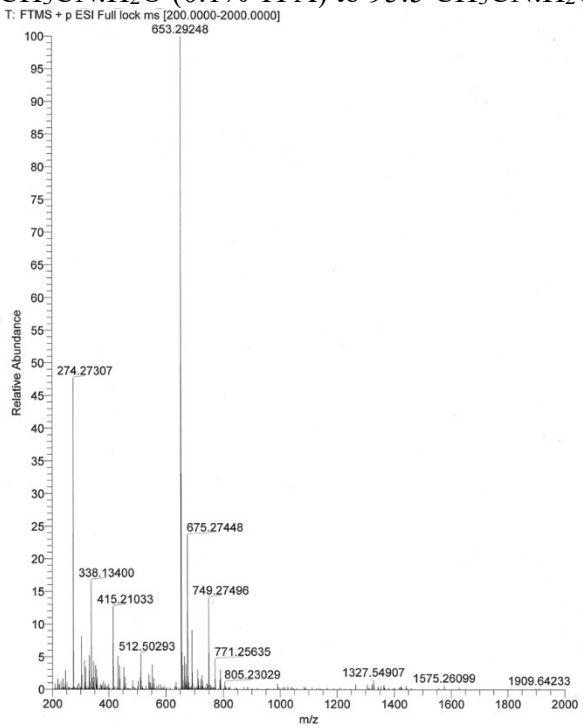

Figure A4. HRMS data for Ac-Thr-Pro-Ser(Bn)-Pro-pNA (**4.33**). The peak at *m/z* = 653.29248 corresponds to the M+H species (*m/z*) calcd for $C_{32}H_{41}N_6O_9^+$ = 653.2930. The peak at *m/z* = 675.27448 corresponds to the M+Na species (*m/z*) calcd for $C_{32}H_{40}N_6NaO_9^+$ = 675.2749.

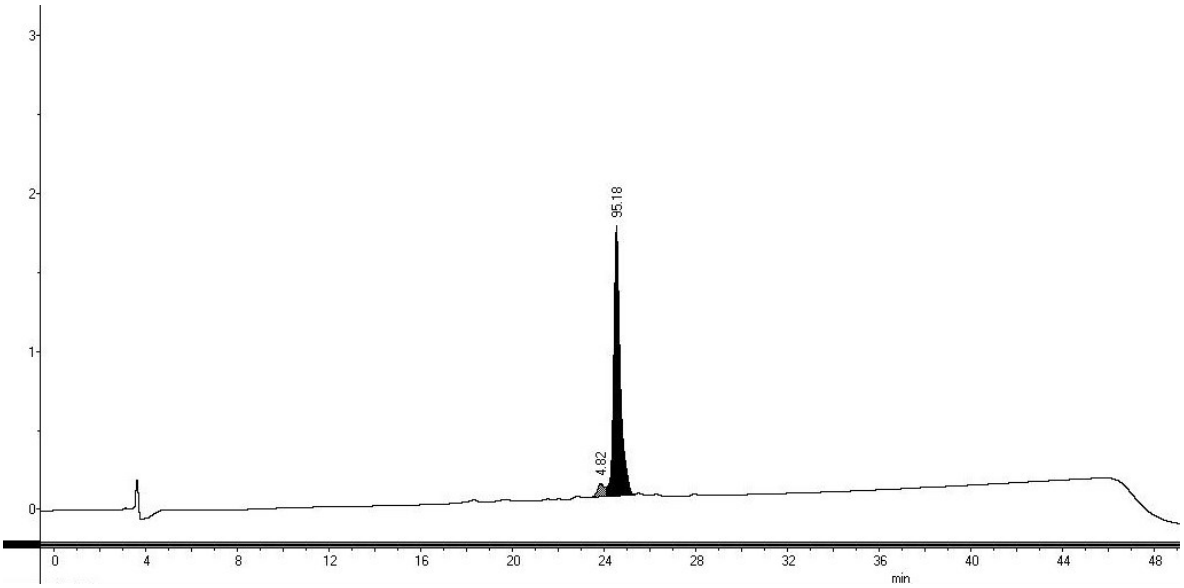

Figure A5. HPLC Chromatogram of Ac-Pro-Thr-Pro-Ser(Bn)-Pro-pNA (**4.34**). Gradient 5:95 CH_3CN:H_2O (0.1% TFA) to 95:5 CH_3CN:H_2O (0.1% TFA) over 40 min. (λ = 220 nm).

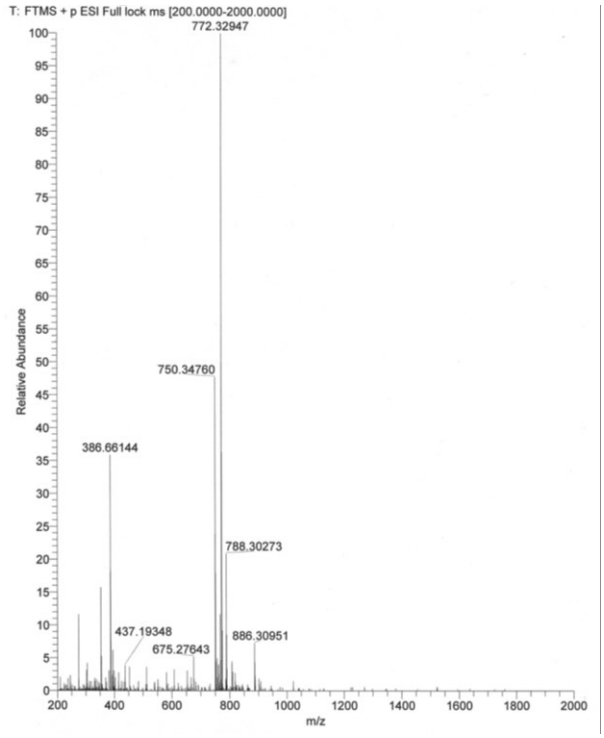

Figure A6. HRMS data for Ac-Pro-Thr-Pro-Ser(Bn)-Pro-pNA (**4.34**). The peak at *m/z* = 750.34760 corresponds to the M+H peak (*m/z*) calcd for $C_{37}H_{48}N_7O_{10}^+$ = 750.3457. The peak at *m/z* = 772.32947 corresponds to the M + Na species (*m/z*) calcd for $C_{37}H_{47}N_7NaO_{10}^+$ = 772.3277. The peak at *m/z* = 788.30273 corresponds to the M+K species (*m/z*) calcd for $C_{37}H_{47}N_7KO_{10}^+$ = 788.3016.

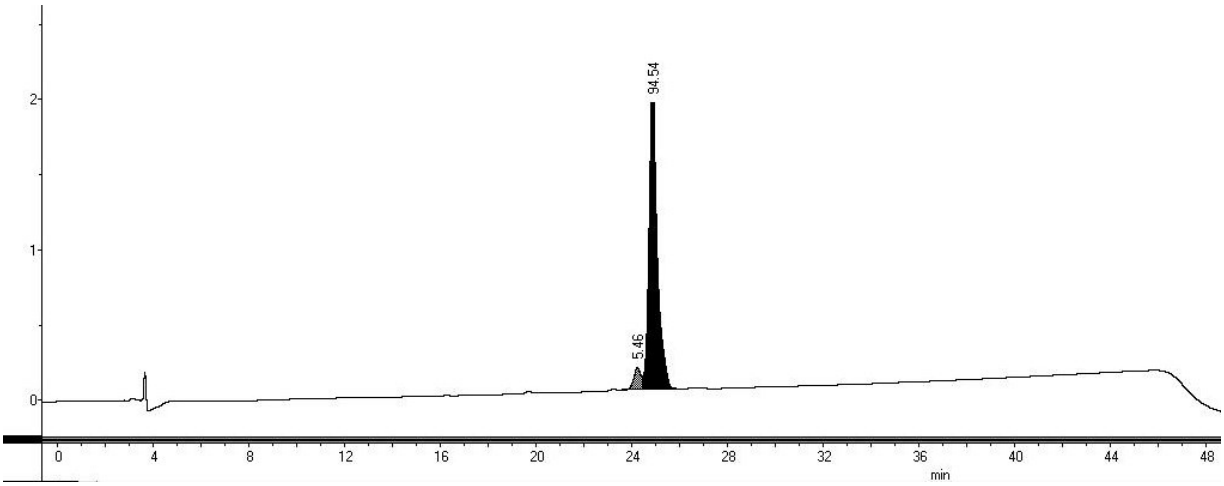

Figure A7. HPLC Chromatogram of Ac-Pro-Pro-Thr-Pro-Ser(Bn)-Pro-pNA (**4.35**). Gradient 5:95 CH_3CN:H_2O (0.1% TFA) to 95:5 CH_3CN:H_2O (0.1% TFA) over 40 min. (λ = 220 nm).

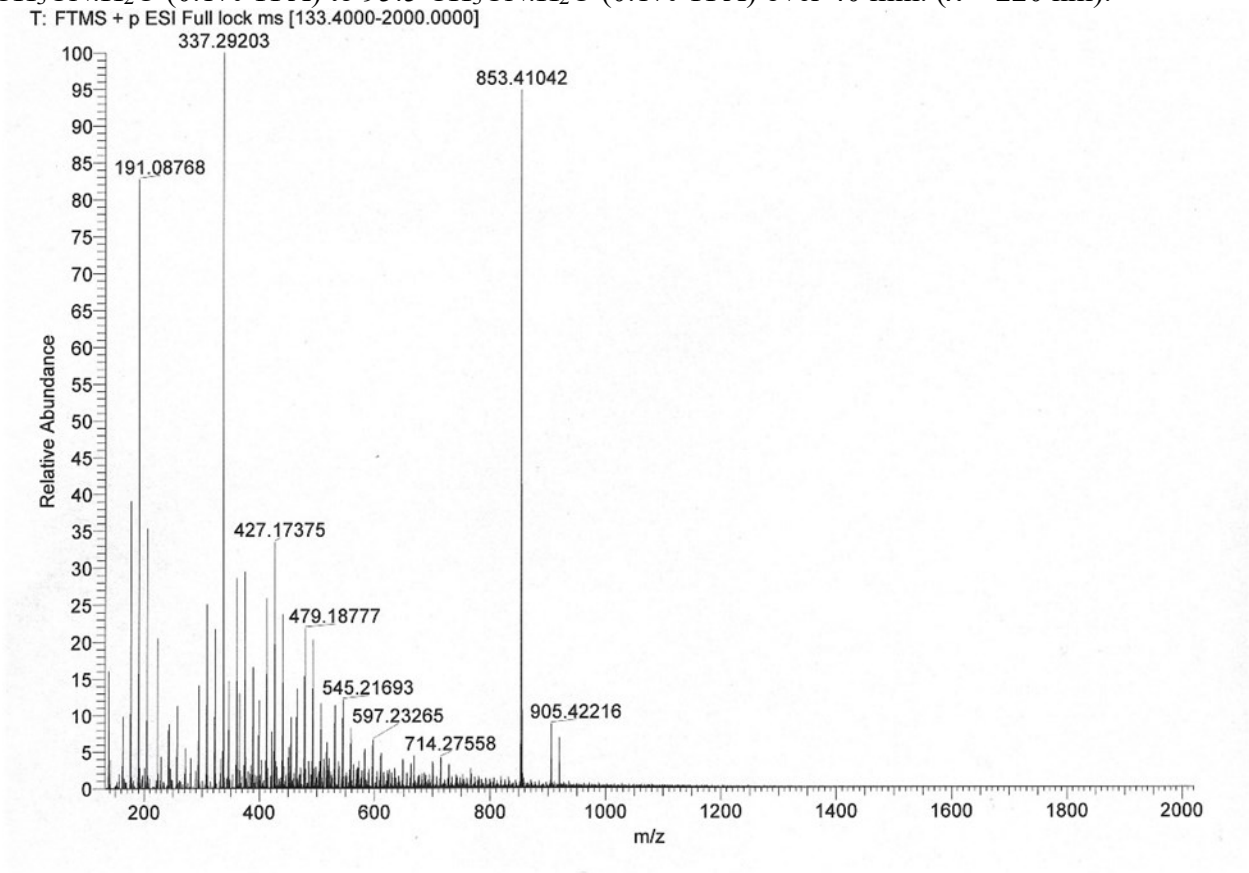

Figure A8. HRMS data for Ac-Pro-Pro-Thr-Pro-Ser(Bn)-Pro-pNA (**4.35**). The peak at *m/z* = 853.41042 corresponds to the M+Li species (*m/z*) calcd for $C_{42}H_{54}LiN_8O_{11}^+$ = 853.4067.

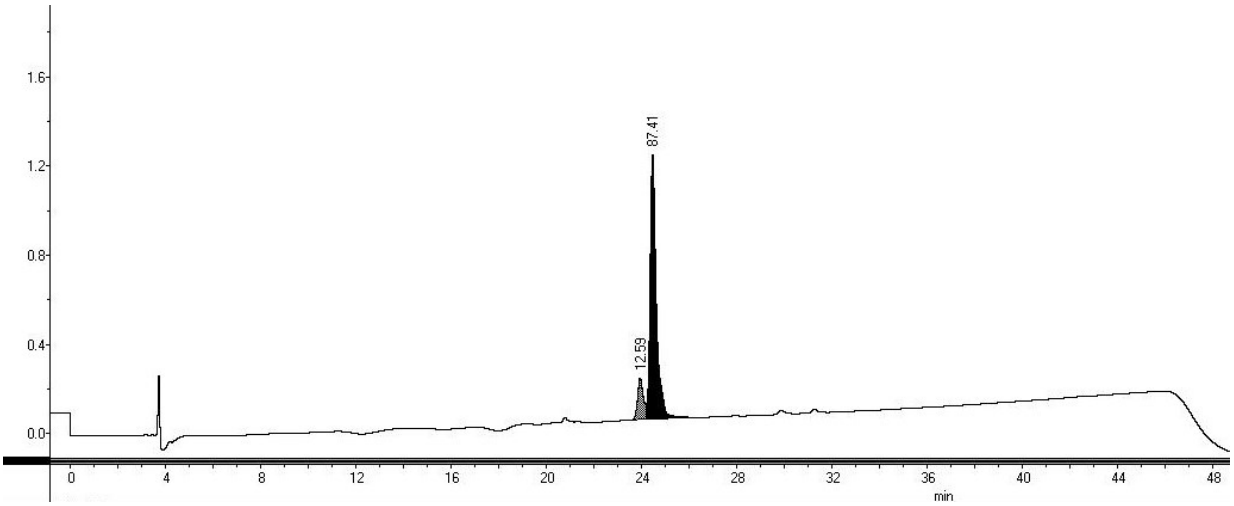

Figure A9. HPLC Chromatogram of Ac-Thr-Pro-Pro-Thr-Pro-Ser(Bn)-Pro-pNA (**4.36**). Gradient 5:95 CH_3CN:H_2O (0.1% TFA) to 95:5 CH_3CN:H_2O (0.1% TFA) over 40 min. (λ = 220 nm).

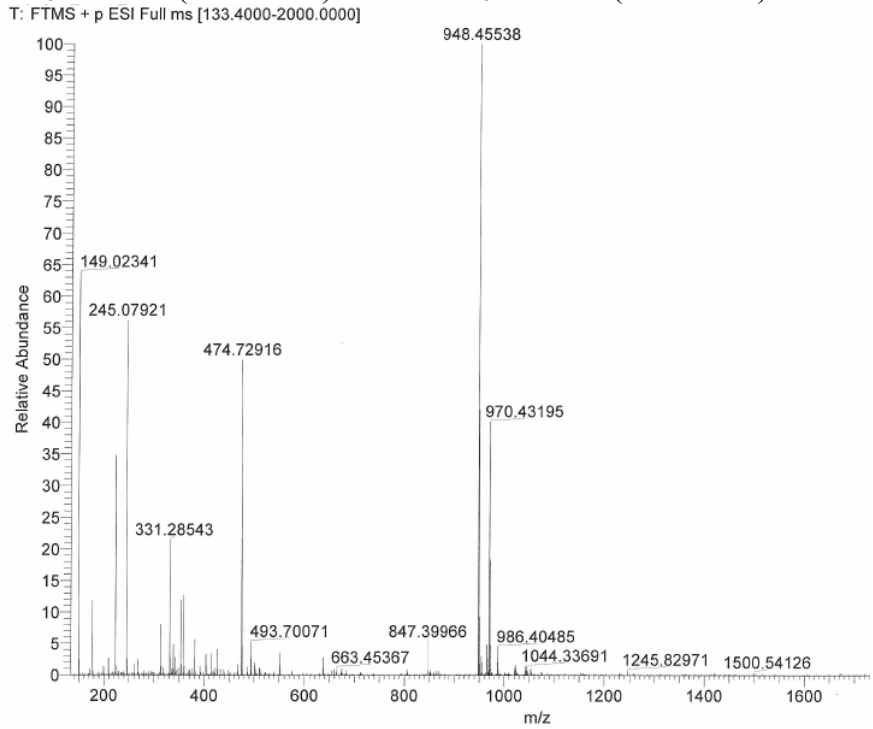

Figure A10. HRMS data for Ac-Thr-Pro-Pro-Thr-Pro-Ser(Bn)-Pro-pNA (**4.36**). The peak at *m/z* = 948.45538 corresponds to the M+H species (*m/z*) calcd for $C_{46}H_{62}N_9O_{13}^+$= 948.4462. The peak at *m/z* = 970.43195 corresponds to the M+Na species (*m/z*) calcd for $C_{46}H_{61}N_9NaO_{13}^+$= 970.4281.

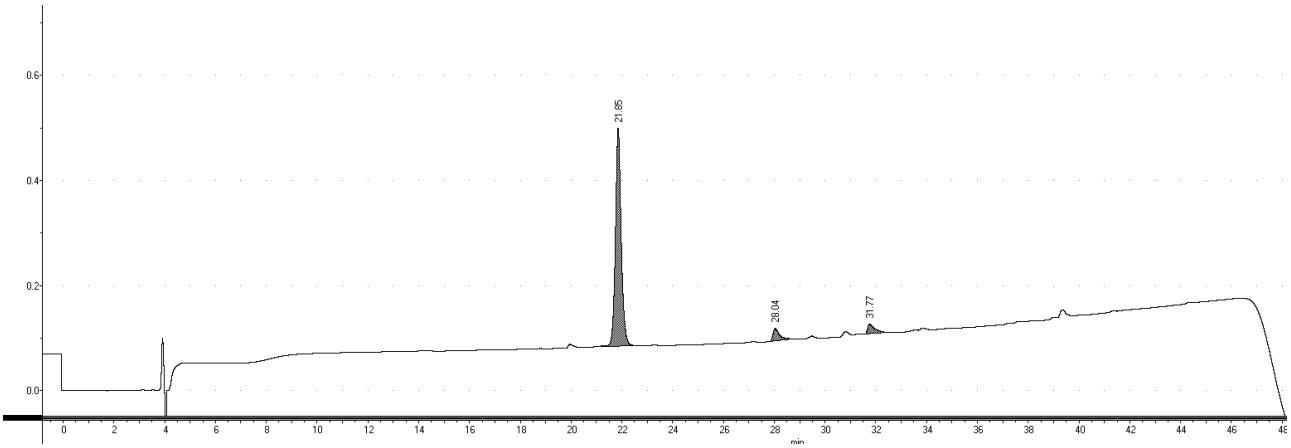

Figure A11. HPLC Chromatogram of Ac-Asp-Val-Pro-pNA (**4.37**). Gradient 5:95 CH_3CN:H_2O (0.1% TFA) to 95:5 CH_3CN:H_2O (0.1% TFA) over 40 min. (λ = 220 nm).

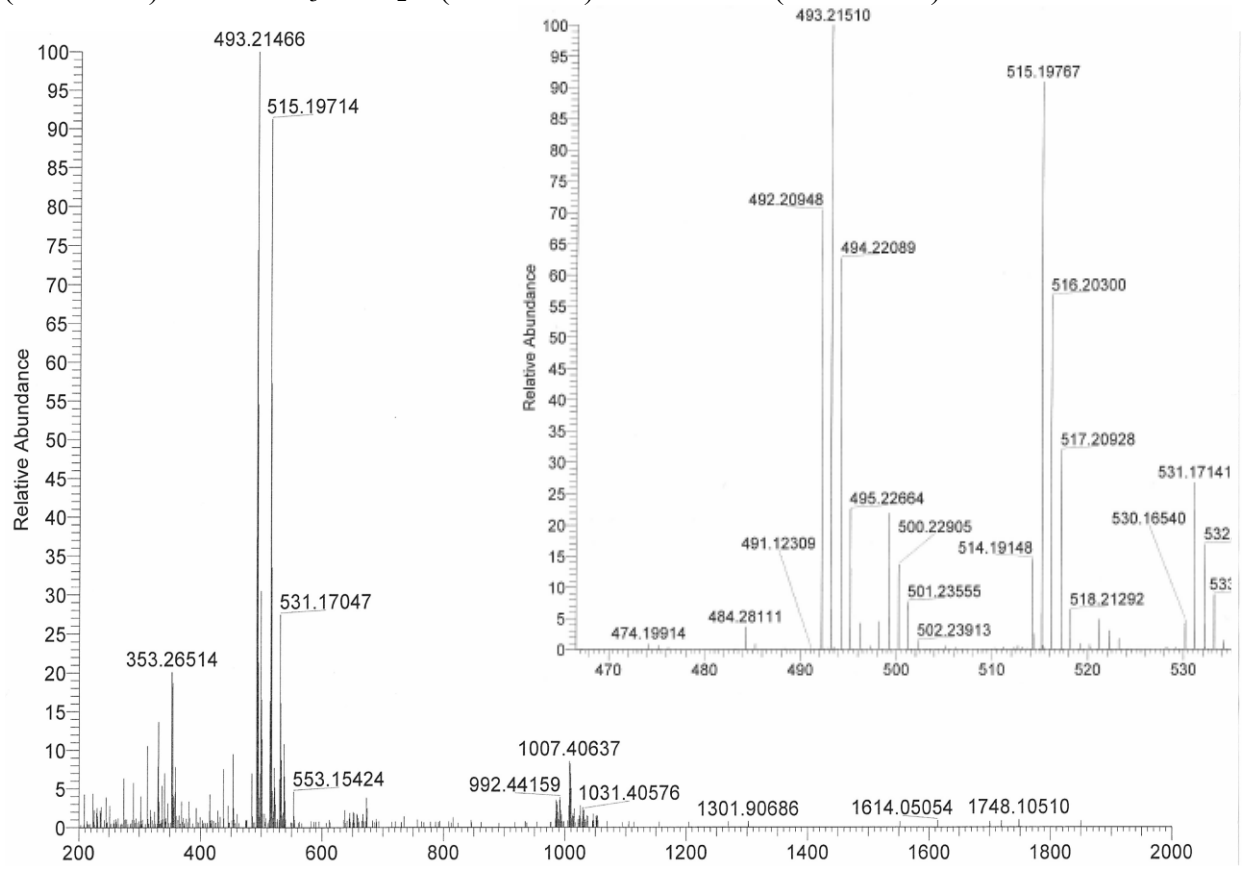

Figure A12. HRMS data for Ac-Asp-Val-Pro-pNA (**4.37**). The peak at *m/z* = 492.20948 corresponds to the M+H species (*m/z*) calcd for $C_{22}H_{30}N_5O_8^+$ = 492.2089. The peak at *m/z* = 493.2146 corresponds to the M+D species (*m/z*) calcd for C22H29DN5O8+ = 493.2152. The peak at *m/z* = 514.195 corresponds to the M+Na species (*m/z*) calcd for $C_{22}H_{29}N_5NaO_8^+$ = 514.1908. The peak at 515.19714 corresponds to the M-H+D+Na species (*m/z*) calcd for $C_{22}H_{28}DN_5NaO_8^+$ = 515.1971

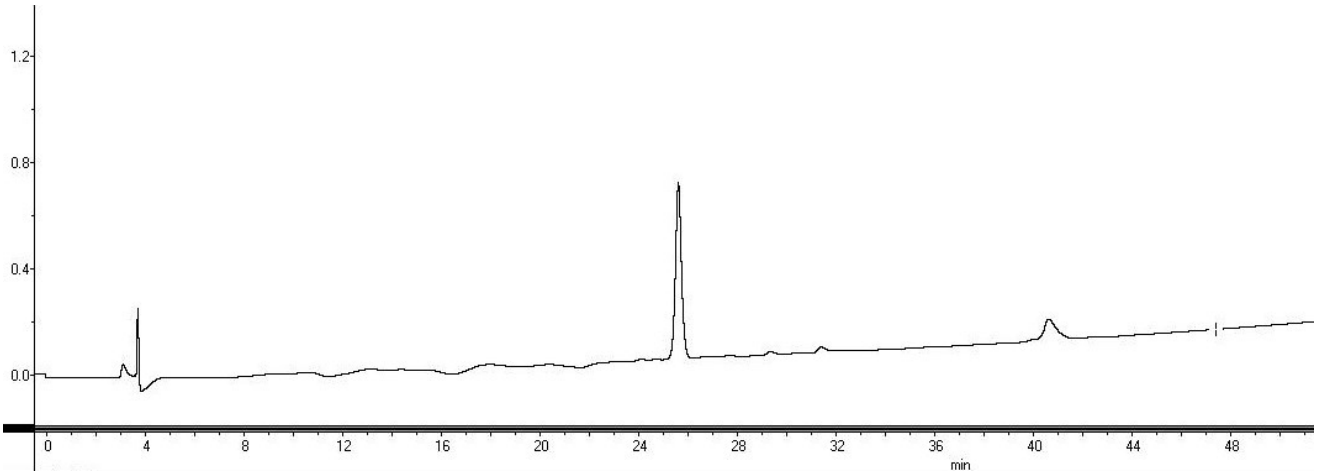

Figure A13. HPLC Chromatogram of Ac-Ala-Asp-Val-Pro-pNA (**4.38**). Gradient 5:95 $CH_3CN:H_2O$ (0.1% TFA) to 95:5 $CH_3CN:H_2O$ (0.1% TFA) over 40 min. (λ = 220 nm).

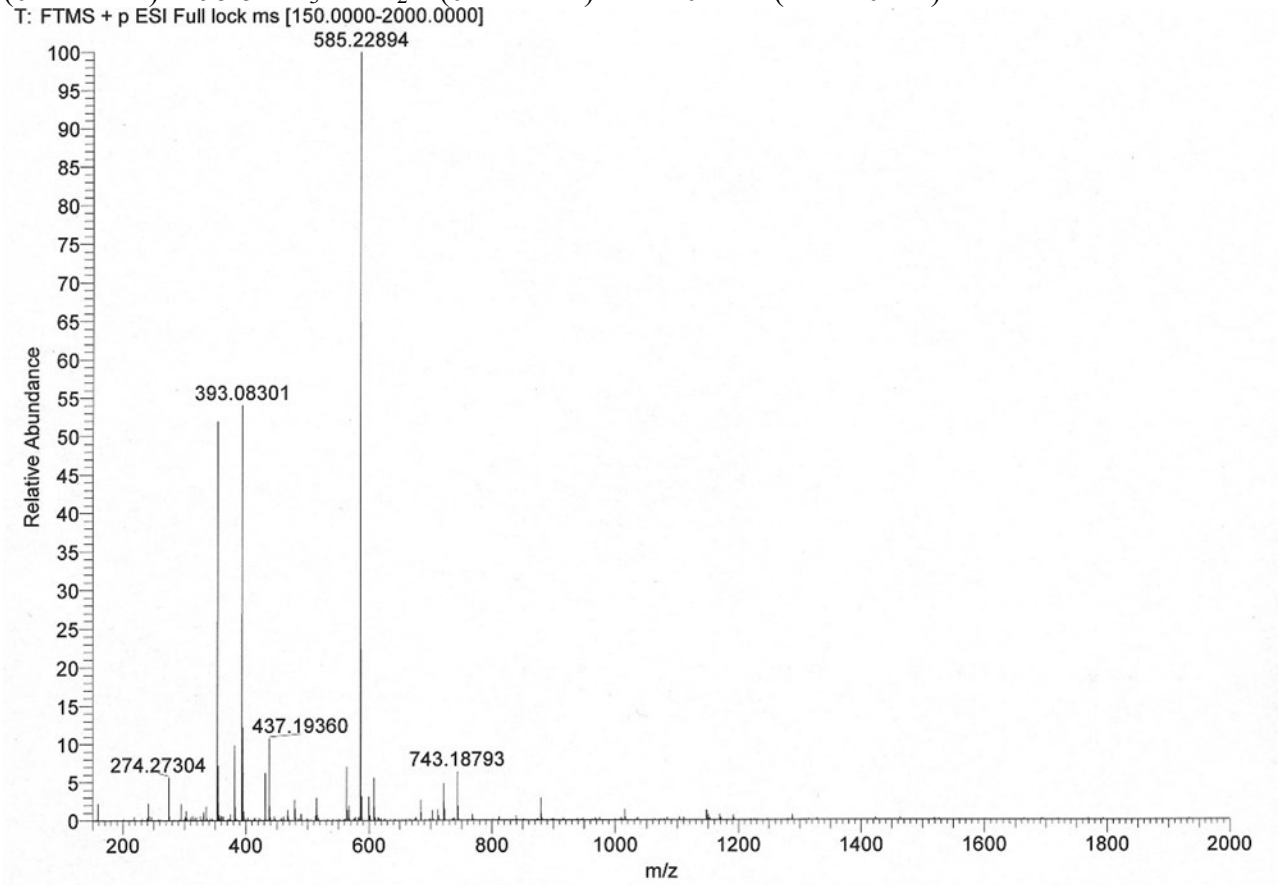

Figure A14. HRMS data for Ac-Ala-Asp-Val-Pro-pNA (**4.38**). The peak at *m/z* = 585.22894 corresponds to the M+Na species (*m/z*) calcd for $C_{25}H_{34}N_6NaO_9^+$ = 585.2279.

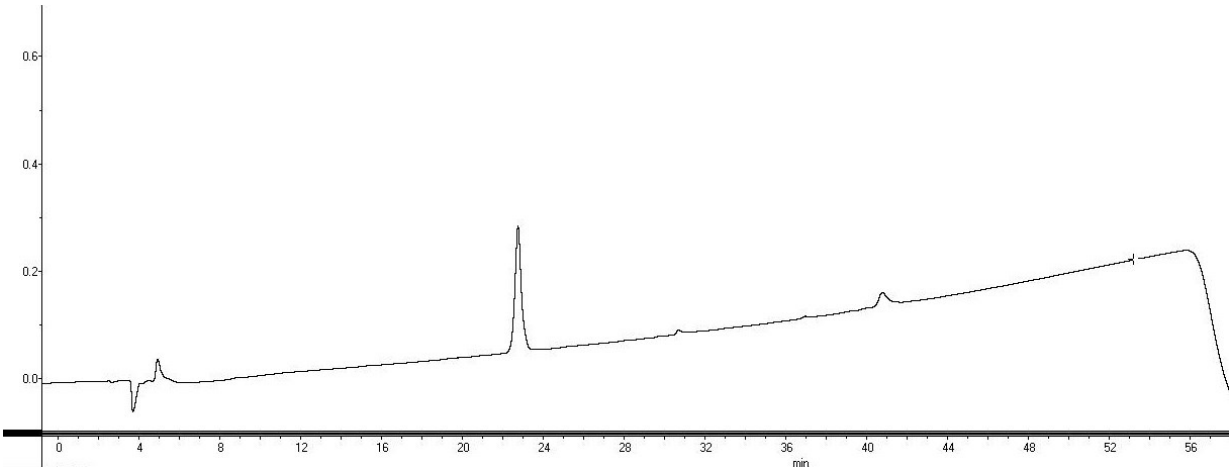

Figure A15. HPLC Chromatogram of Ac-Gln-Ala-Asp-Val-Pro-pNA (**4.39**). Gradient 5:95 CH_3CN:H_2O (0.1% TFA) to 95:5 CH_3CN:H_2O (0.1% TFA) over 40 min. (λ = 220 nm).

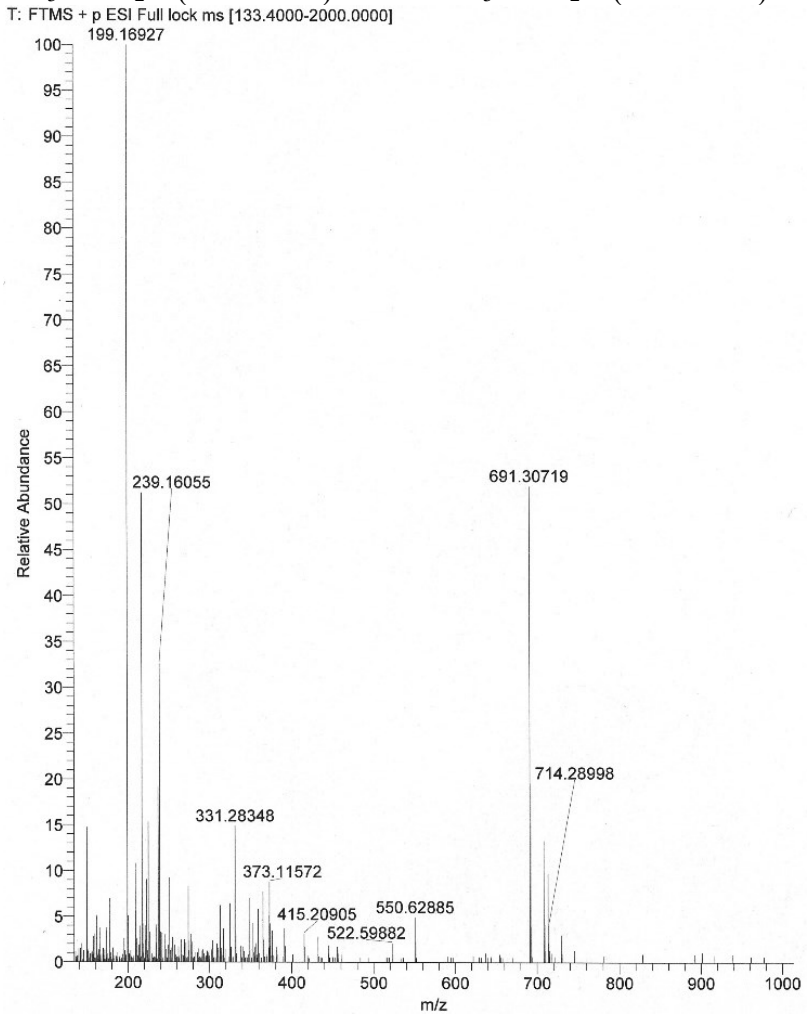

Figure A16. HRMS data for Ac-Gln-Ala-Asp-Val-Pro-pNA (**4.39**). The peak at *m/z* = 691.30719 corresponds to the M+H species (*m/z*) calcd for $C_{30}H_{43}N_8O_{11}^+$ = 691.3046.

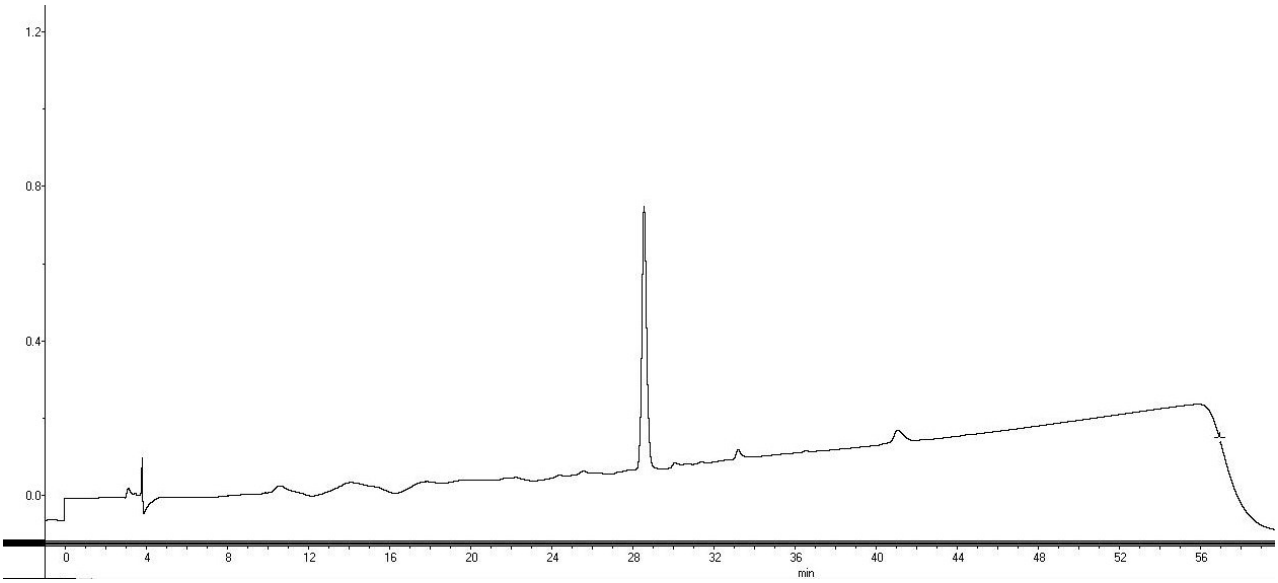

Figure A17. HPLC Chromatogram of Ac-Ile-Gln-Ala-Asp-Val-Pro-pNA (**4.40**). Gradient 5:95 CH_3CN:H_2O (0.1% TFA) to 95:5 CH_3CN:H_2O (0.1% TFA) over 40 min. (λ = 220 nm).

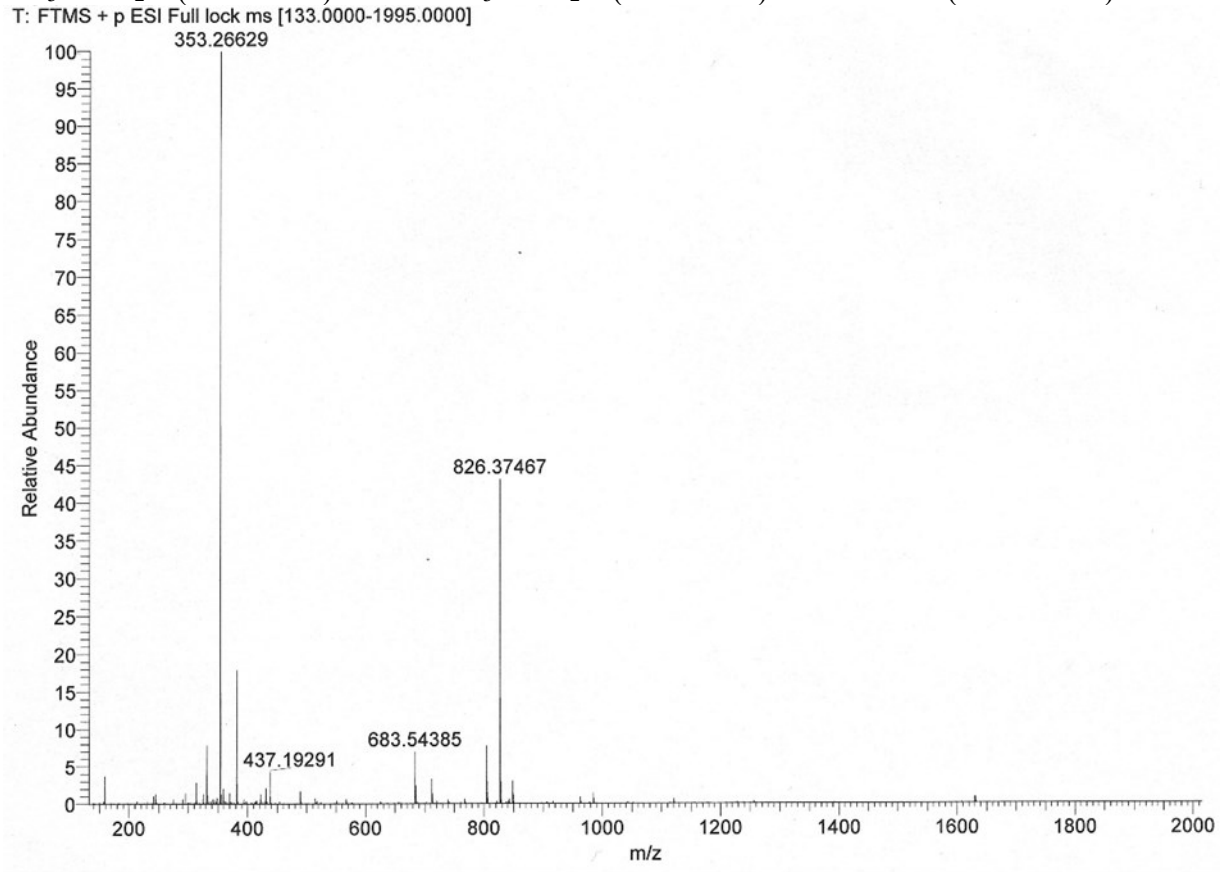

Figure A18. HRMS data for Ac-Ile-Gln-Ala-Asp-Val-Pro-pNA (**4.40**). The peak at *m/z* = 826.37467 corresponds to the M+Na species (*m/z*) calcd for $C_{36}H_{53}N_9NaO_{12}^+$ = 826.3706.

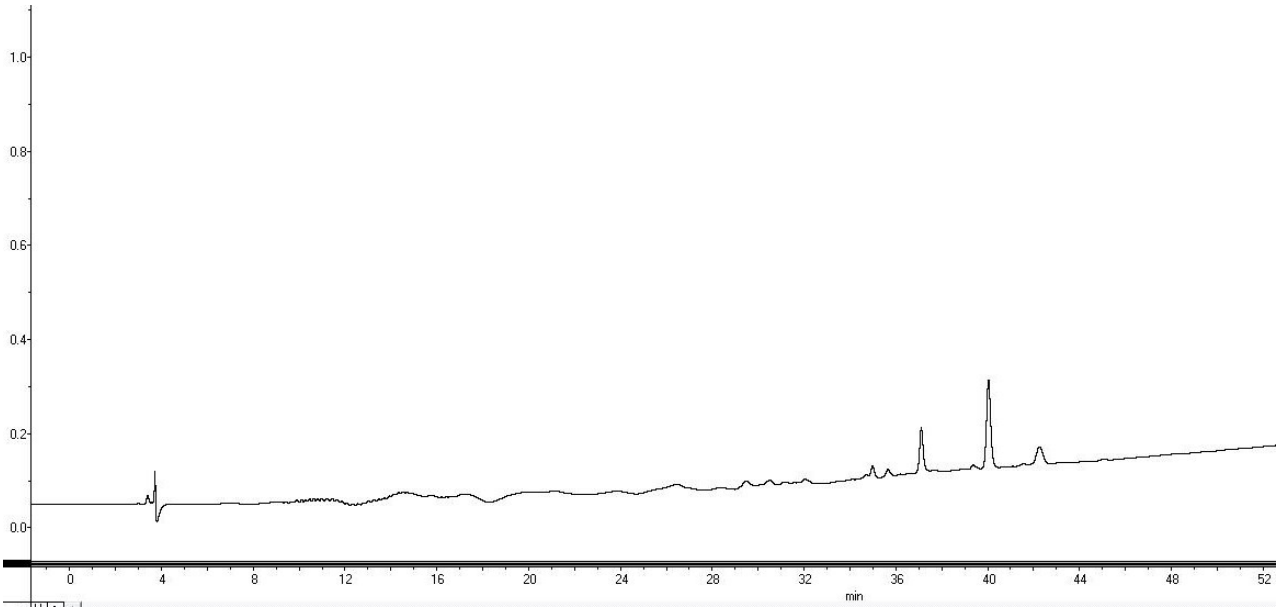

Figure A19. HPLC Chromatogram of the attempted synthesis of Ac-Asn-Ile-Gln-Ala-Asp-Val-Pro-pNA (**4.41**). Gradient 5:95 CH_3CN:H_2O (0.1% TFA) to 95:5 CH_3CN:H_2O (0.1% TFA) over 40 min. (λ = 220 nm).

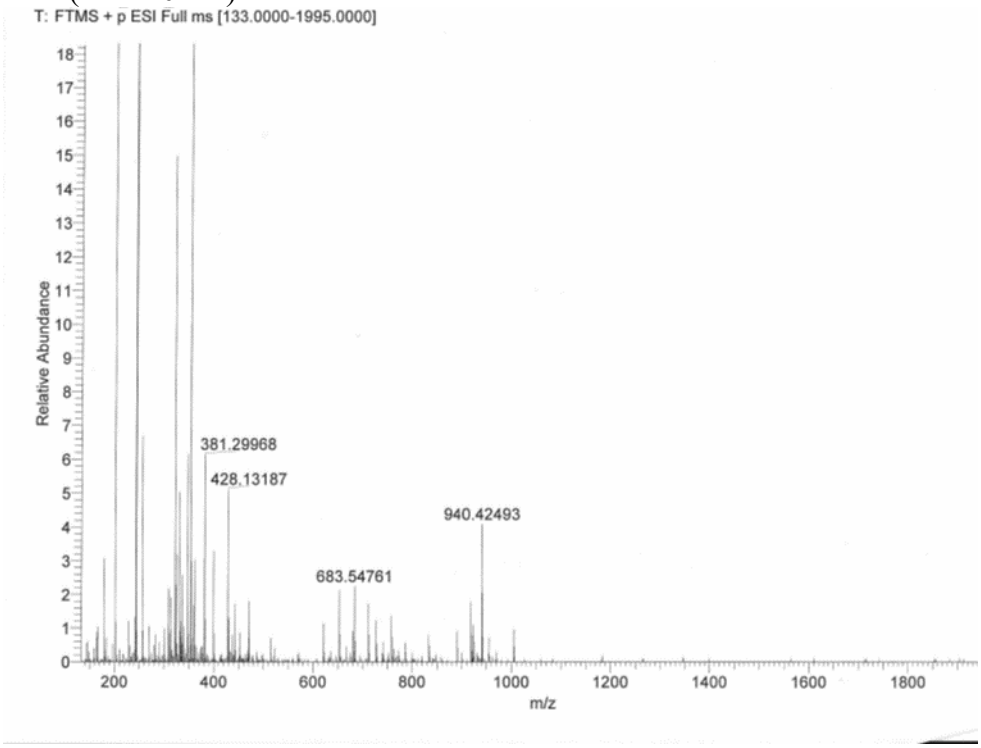

Figure A20. HRMS data for Ac-Asn-Ile-Gln-Ala-Asp-Val-Pro-pNA (**4.41**). The peak at 940.42493 corresponds to the M+Na species (*m/z*)calcd for $C_{40}H_{59}N_{11}NaO_{14}^{+}$ = 940.4135.

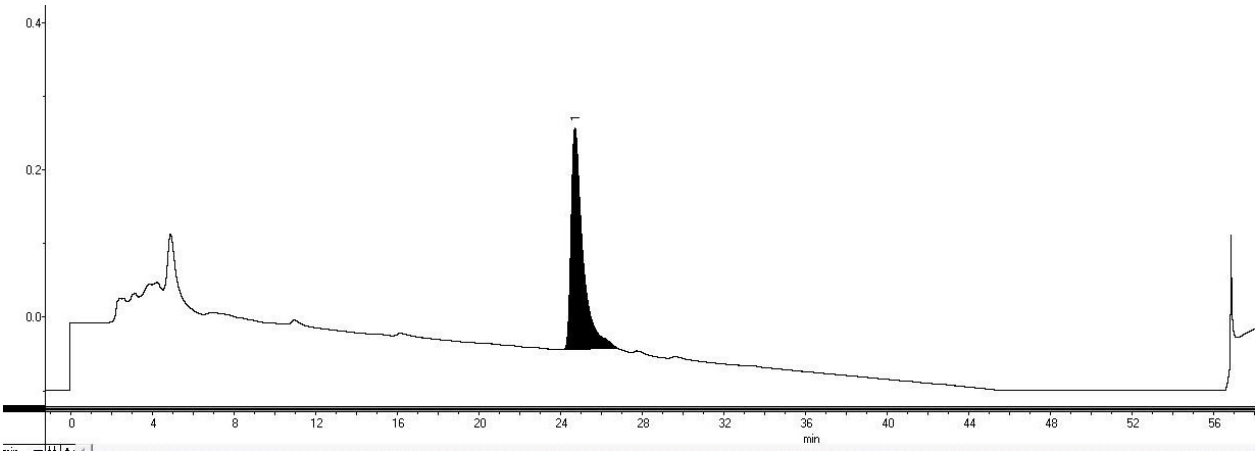

Figure A21. HPLC Chromatogram of compound 4.65. Gradient 50:50 $CH_3CN:H_2O$ (0.1% TFA) to 100:0 $CH_3CN:H_2O$ over 40 min. (λ = 220 nm).

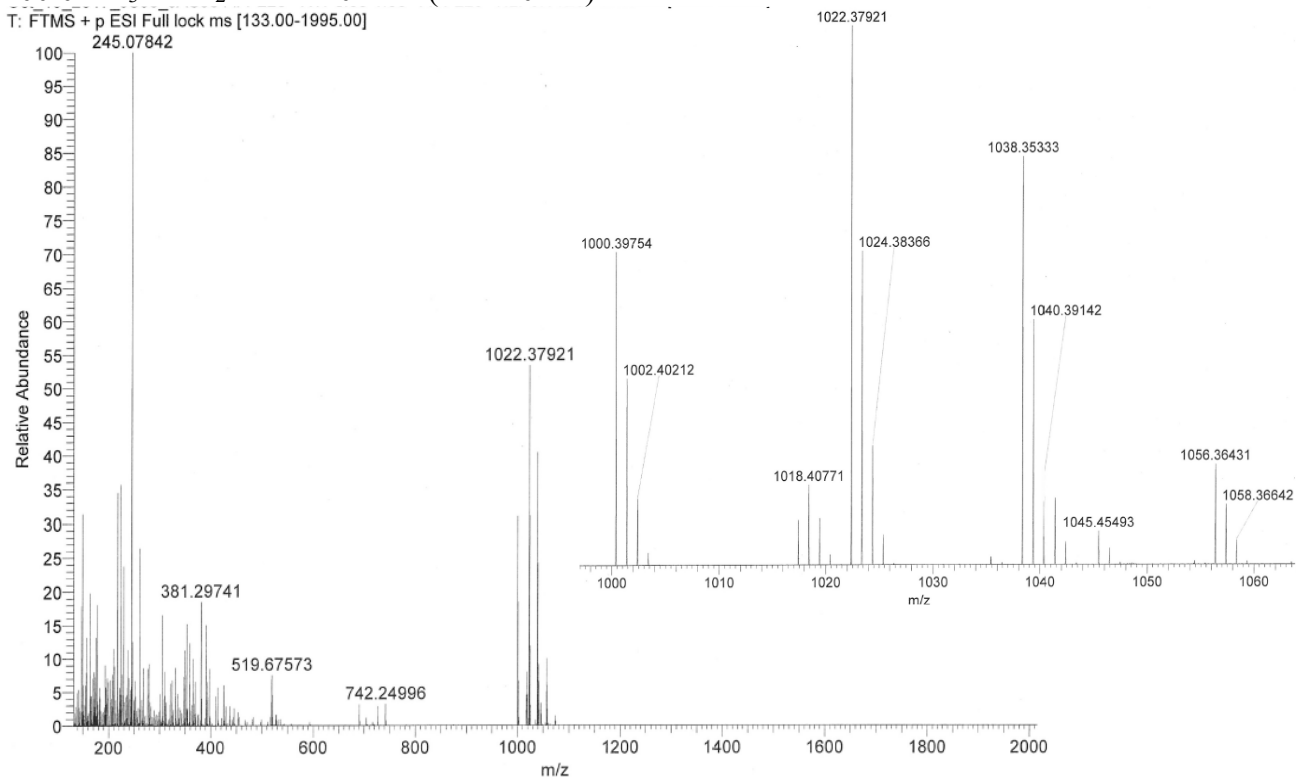

Figure A22. HRMS data for compound 4.65. The peak at *m/z* = 1000.39754 corresponds to the M+H species (*m/z*) calcd for $C_{52}H_{60}F_2N_5O_{11}S^+$ = 1000.3973. The peak at *m/z* = 1018.40771 corresponds to the M+H_2O+H species (*m/z*) calcd for $C_{52}H_{62}F_2N_5O_{12}S^+$ = 1018.4078. The peak at *m/z* = 1022.37921 corresponds to the M+Na species (*m/z*) calcd for $C_{52}H_{59}F_2N_5NaO_{11}S^+$ = 1022.3792. The peak at *m/z* = 1038.35333 corresponds to the M+K species (*m/z*) calcd for $C_{52}H_{59}F_2N_5O_{11}SK^+$ = 1038.3531. The peak at *m/z* = 1056.36431 corresponds to the M+H_2O+K species (*m/z*) calcd for $C_{52}H_{61}F_2N_5O_{12}SK^+$ = 1056.3637.

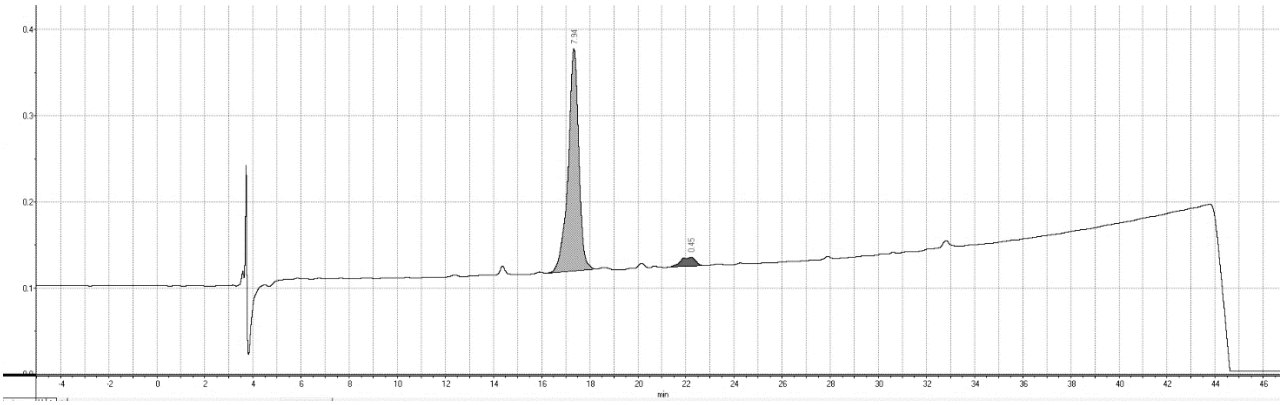

Figure A23. HPLC Chromatogram of compound **4.86**. Gradient 10:90 $CH_3CN:H_2O$ (0.1% TFA) to 90:10 $CH_3CN:H_2O$ (0.1% TFA) over 40 min. (λ = 220 nm).

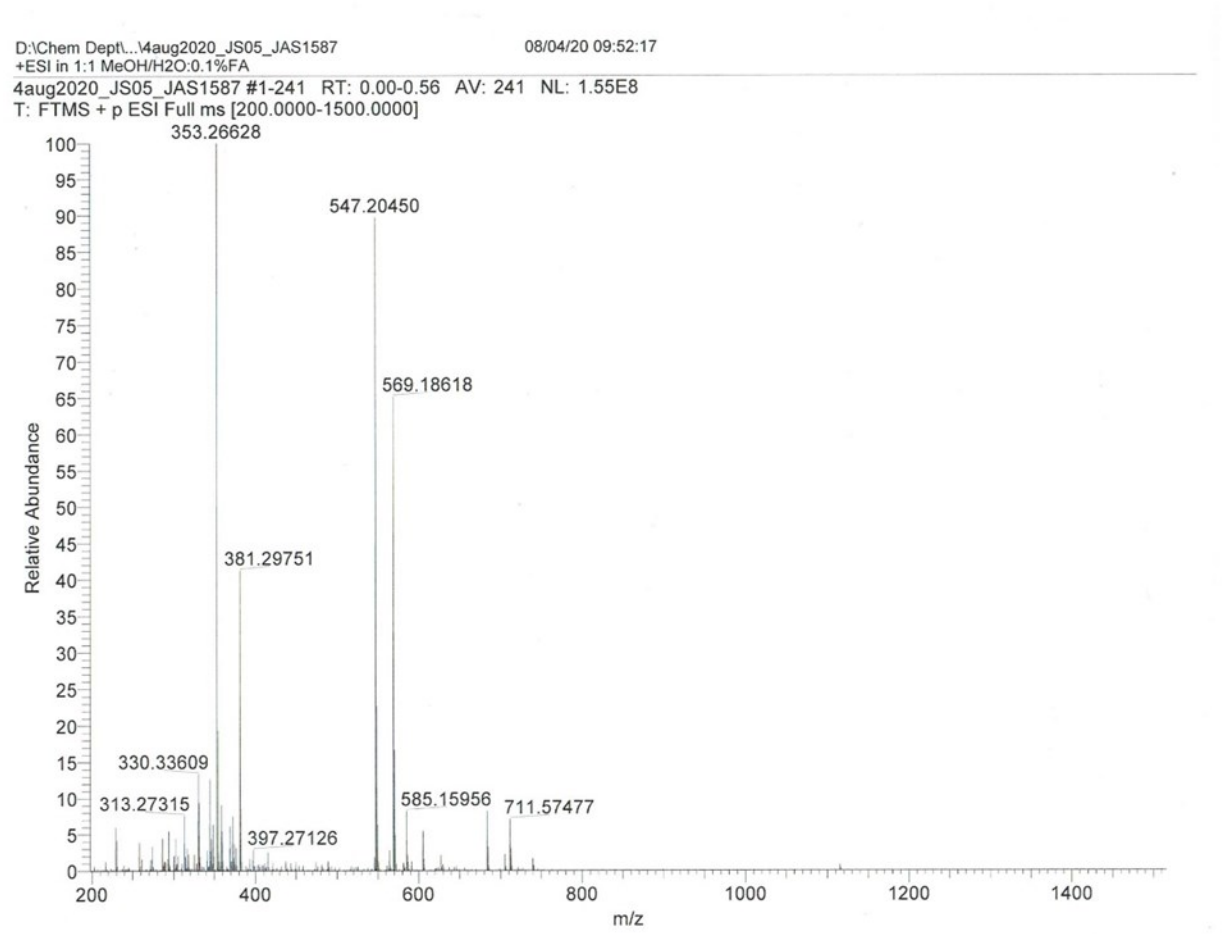

Figure A24. HRMS data for compound **4.86**. The peak at *m/z* = 547.20450 corresponds to the M+H species (*m/z*) calcd for $C_{23}H_{33}F_2N_4O_7S^+$= 547.2033. The peak at *m/z* = 459.18618 corresponds to the M+Na species (*m/z*) calcd for $C_{23}H_{32}F_2N_4O_7NaS^+$= 569.1852.