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Progress Toward Theonellamide F

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ABSTRACT

PROGRESS TOWARD THE SYNTHSIS OF THEONELLAMIDE F

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Theonellamide is a bicyclic peptide isolated from a marine sponge, which shows interesting biological activity. It contains several unnatural amino acids, among which are (2*S*,3*R*)-3-hydroxyasparagine (L-erythro-β-hydroxyasparagine) (β-OHAsn) and τ-L-histindino-D-alanine (τ-HAL). Although there were previous synthetic efforts toward each of these unnatural amino acids, the efforts were not ideal due to expensive starting material, time-consuming steps, and poor regioselectivity. The presented work demonstrates an inexpensive, enantioselective synthesis of β-OHAsn, which can be completed in a matter of weeks, as well as several attempts at a novel regiospecific approach toward τ-HAL, including work on a model study.

Keywords: Theonellamide, bicyclic peptide, β-OHAsn, τ-HAL

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List of Abbreviations

AAA	α-aminoadipic acid
Aboa	(3*S*,4*S*,5*E*,7*E*)-3-amino-4-hydroxy-6-methyl-8-(*p*-bromophenyl)-5,7-octadienoic acid
OAc	acetate
Ahad	(2*S*,4*R*)-2-amino-4-hydroxyadipic acid
Ala	alanine
Apoa	(3*S*,4*S*,5*E*,7*E*)-3-amino-4-hydroxy-6-methyl-8-phenyl-5,7-octadienoic acid
Ar	argon
Ara	arabinose
Asn	asparagine
BMPA	β-metyl-*p*-bromophenylalanine
Bn	benzyl
Boc	*tert*-butoxycarbonyl
β-OHAsn	(2*S*,3*R*)-3-hydroxyasparagine
BPA	*p*-bromophenylalanine
brs	broad singlet
Cbz	benzyloxycarbonyl
d	doublet
dd	doublet of doublets
$(DHQD)_2PHAL$	Hydroquinidine 1,4-phthalazinediyl diether

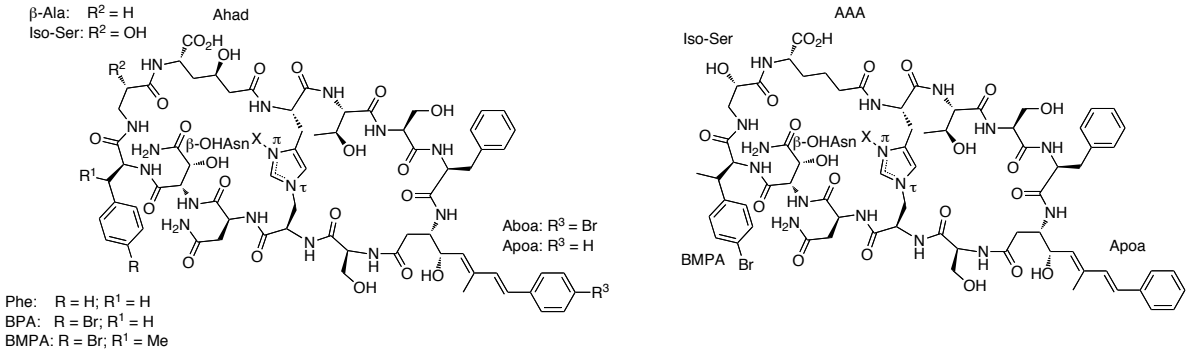
DIAD	diisopropyl azodicarboxylate
DMAP	4-dimethylaminopyridine
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ESI	electrospray ionization
Et	ethyl
Gal	galactose
h	hour
His	histadine
HOBt	1-hydroxybenzotriazole hydrate
HRMS	high-resolution mass spectrometry
Hz	hertz
IR	infrared
J	coupling constant
KHMDS	potassium bis(trimethylsilyl)amide
m	multiplet
Me	methyl
mg	milligram(s)
μg	microgram(s)
MHz	megahertz
min	minutes
mL	milliliter(s)
mmol	millimole(s)

NMR	nuclear magnetic resonance
nPr	propyl
p	para
Ph	phenyl
Phe	phenylalanine
PMP	paramethoxyphenyl
rt	room temperature
s	singlet
Ser	serine
t	triplet
t-Bu	*tert*-butyl
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TEA	triethylamine
TES	triethylsilyl
Tf	triflate
τ-HAL	τ-L-histindino-D-alanine
THF	tetrahydrofuran
TMS	trimethylsilyl
Ts	tosyl

1. Introduction

1.1 Theonellamide F

Theonellamide F (**1, Figure 1**) was isolated from the marine sponge *Theonella* sp.[1] Later other family members were also identified: Theonellamides A-E[2] (**2-6**), Theopalauamide[3] (**7**), and Theonegramide[4] (**8**). It was suggested[2-4] these compounds could actually be metabolites of bacteria that are symbionts of the sponge, as they are not consistently found in all *Theonella* sp.



Theonellamide	R	R^1	R^2	R^3	X
A (2):	Br	Me	OH	H	β-D-Gal
B (3):	Br	Me	OH	H	NA
C (4):	H	H	H	Br	NA
D (5):	Br	H	H	Br	β-L-Ara
E (6):	Br	H	H	Br	β-D-Gal
F (1):	Br	H	H	Br	NA

Theopalauamide (7): X = β-D-Gal
Theonegramide (8): X = β-D-Ara

Figure 1: Theonellamide Family

1.2 Biological Activity

Theonellamide F was found to be biologically active, inhibiting growth of three types of pathogenic fungi at 3-12 μg/ml, and was also found to be cytotoxic against L1210 and P388 leukemia cell lines, with IC_{50} values of 3.2 and 2.7 μg/ml[1] respectively. When the biological activity was researched further, it was found that the compound induced extensive large vacuole formation[5] by a unique mechanism[6] that surprisingly did

not cause cell death, as is typical for this phenomenon. It was demonstrated, however, to inhibit cellular autophagy,[5] which is the cell's ability to breakdown and reuse its components. Its cytotoxicity was attributed in part to its binding with 17β-hydroxysteroid dehydrogenase IV, which plays a role in intracellular transport, although it also was found to bind glutamate dehydrogenase, which plays a role in the citric acid cycle.[7]

1.3 Structure

Dodecapeptide **1** was found to contain several unusual amino acids[1] (Figure 1). Those of interest include (2*S*,3*R*)-3-hydroxyasparagine (L-erythro-β-hydroxyasparagine) (β-OHAsn), (3*S*,4*S*,5*E*,7*E*)-3-amino-4-hydroxy-6-methyl-8-(*p*-bromophenyl)-5,7-octadienoic acid (Aboa), (2*S*,4*R*)-2-amino-4-hydroxyadipic acid (Ahad), and τ-L-histindino-D-alanine (τ-HAL).

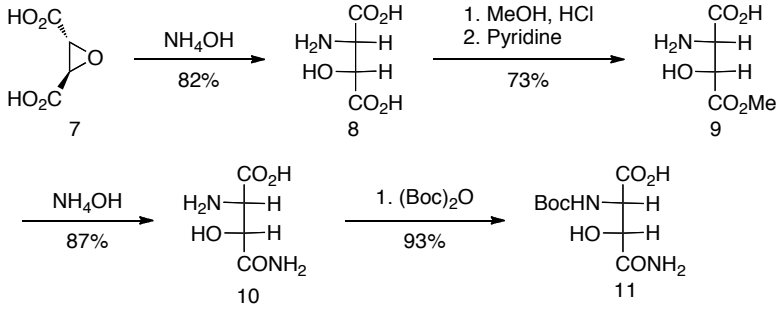
1.4 Previous Synthetic Work

The Shioiri group attempted a total synthesis of Theonellamide F,[8] designing conditions for the synthesis of Aboa,[9] Ahad,[10] and β-OHAsn and τ-HAL.[8b] As the scope of this thesis only covers β-OHAsn and τ-HAL, the discussion here shall be limited to these amino acids.

1.4.1 β-OHAsn Syntheses

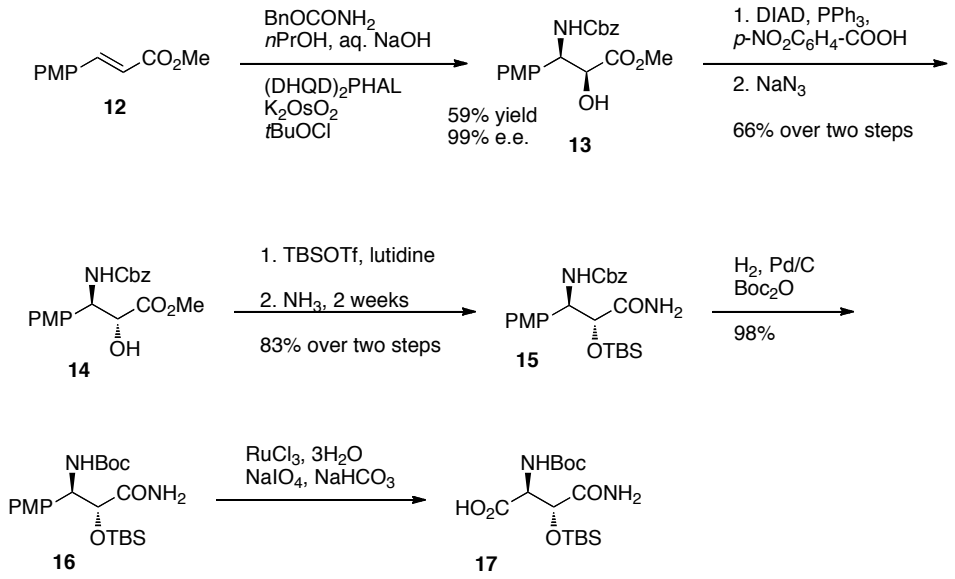
Their efforts toward protected β-OHAsn were based on previously published work,[8b, 11] shown in Scheme 1. Epoxysuccinic acid **7** was treated with ammonium hydroxide, which opens the strained three-membered ring, yielding **8**. This dicarboxylic acid is selectively converted to methyl ester **9**, which is exchanged for amide **10**. This β-OHAsn is then N-protected with a Boc group to yield **11** in a 48% overall yield.

However, succinic acid **7** must be harvested from bacteria, which is labor intensive, or purchased for $400 per gram, making this synthesis impractical.



Scheme 2: β-OHAsn synthesis of the Shioiri group[8b,11]

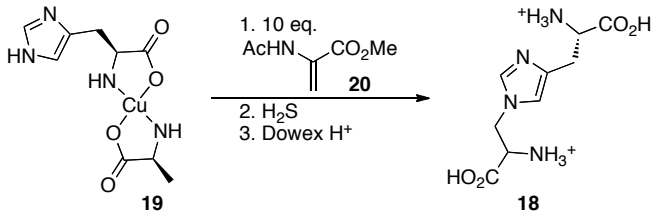
Later, Wong and Taylor[12] constructed β-OHAsn as well, using a Sharpless asymmetric aminohydroxylation reaction to form **13**, with excellent enantiomeric excess. A Mitsunobu reaction was then used with *p*-nitrobenzoic acid as the inverting nucleophile to form the ester, which was then removed by azidolysis to give **14**. The newly formed hydroxyl group was then protected as a TBS ether, and the methyl ester was converted to the amide. The latter process took two weeks. At this point, Wong and Taylor had trouble oxidizing the PMP-protecting group to the acid in the presence of the Cbz-protected amine, and therefore had to do some protecting group shuffling before finally constructing **17**. The overall yield was about 23%, and the route took several weeks to complete.



Scheme 3: β-OHAsp synthesis of the Taylor[12] group

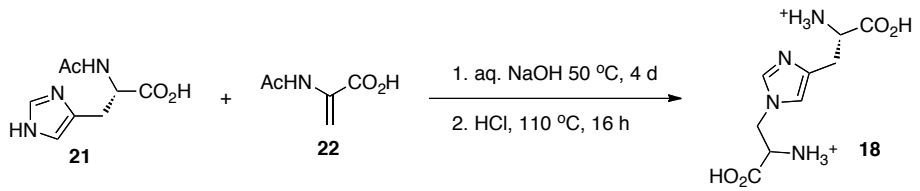
1.4.2 τ-HAL Syntheses

The previous attempts toward τ-HAL **18** have been extensive and varied.[13] The Friedman group[14] used a copper-complexed histidine **19** to undergo a conjugate addition with a large excess of protected amino acrylate **20** (**Scheme 3**).



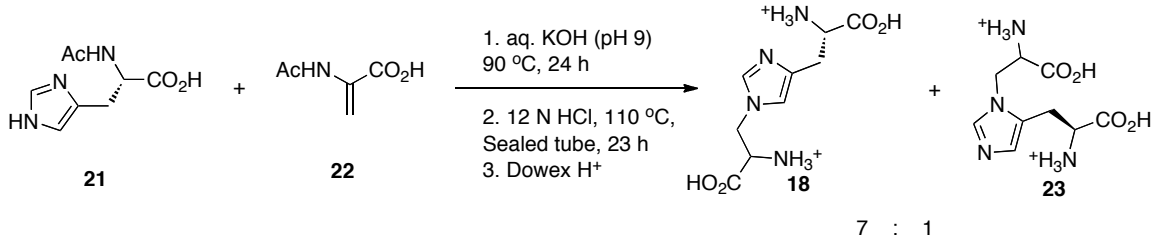
Scheme 3: Friedman's synthesis of τ-HAL[14]

The Fujimoto group[15] applied a similar approach, using protected histidine 2**1** with α-amino acrylic acid **22** and strong base (**Scheme 4**).



Scheme 4: Fujimoto's synthesis of τ-HAL[14]

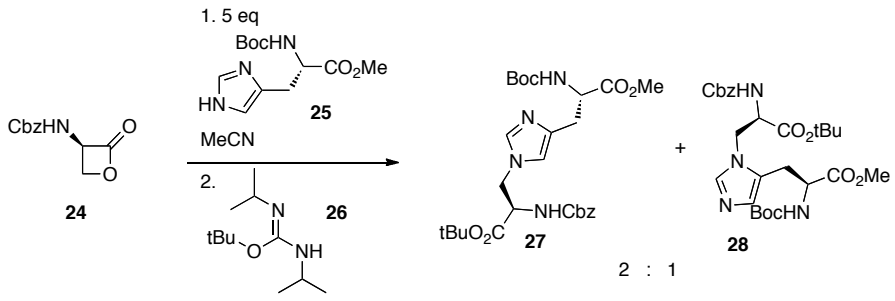
These syntheses were only used to confirm structural data, so the full experimental details were not reported. However, the Henle group[16] later published full experimental details showing a modest 50% yield for similar conditions (**Scheme 5**). This conjugate addition approach was also demonstrated to generate a 7:1 mixture of isomers in favor of the desired τ isomer. The Boschin group[17] later optimized the Henle conditions to a 94% yield by increasing the time exposed to base to 10 days and decreasing the temperature to room temperature. However, this resulted in a lower regioselectivity of only 5:1.



Scheme 5: Henle's synthesis of τ-HAL[16]

In an effort to maintain both stereo- and regiocontrol of this peptide coupling for their work toward the total synthesis of Theonellamide F, the Todho group[8b] used a different approach.[18] They utilized strained β-lactone **24** as an electrophile for an excess of protected histidine **25**, and quenched the reaction with a *tert*-butyl source to yield the *tert*-butyl esters **27** and **28**. While this approach was stereoselective, it was only mildly regioselective, generating a 2:1 ratio of the desired τ regioisomer to its π counterpart.

Furthermore, the yield was only 61% combined, with only 40% of the desired regioisomer. Other problems arise when the syntheses of this reaction's starting materials are considered. Lactone **24** is a product of two reactions with only moderate yields.[18-19] Likewise, protected histidine **25** is used in large excess, which is also undesirable.



Scheme 6: Tohdo's synthesis of τ-HAL[8b]

1.5 Summary

Theonellamide F (**1**) is a natural bicyclic dodecapeptide with interesting biological activity.[1, 5-7] Due to its limited natural abundance and its interesting structure, a total synthesis would be valuable.[8-10] _ENREF_9 Previous attempts toward β-OHAsn were based on an impractical starting material[8b] or suffered from excessive length.[12] The syntheses of the central τ-HAL moiety (**12**) have been lacking in stereo- and regioselectivity, leaving room for improvement.[8b, 13-17]

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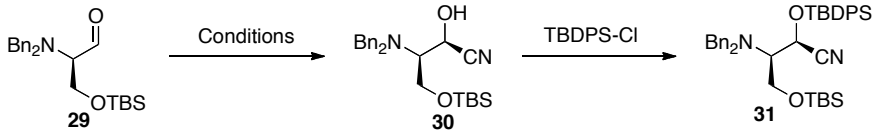
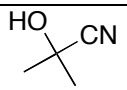
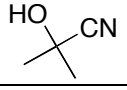
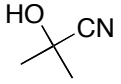
2. β-OHAsn

For our synthesis of β-OHAsn (**10**), we recognized a similarity in our proposed L-histidine pathway for our τ-HAL (**12**) synthesis, and took advantage of the potentially shared intermediate (**29**) to create a divergent synthesis. Commercially available D-serine methyl ester was transformed into **29**[1] in four steps, with an overall yield of 89%. Conditions employed with related amino aldehydes[2] were screened (**Table 1**) for the best yielding and most diastereoselective cyanide addition to **29** (**Scheme 1**) by a coworker and myself.



Scheme 4: Protected β-OHAsn formation[2]

Table 1: Conditions screened for the diastereoselective addition of cyanide

Lewis Acid	–CN Source	Time (Hours)	Temp (^{o} C)	dr	Calculated Yield
$AlMe_3$		4.5	0	2:1	38%
$AlMe_3$		6	0	3.3:1	46%
$AlMe_3$		7	0	3.3:1	78%
$ZnBr_2$	TMSCN	6	–20	>19:1	39%
$ZnBr_2$	TMSCN	5	–20	>19:1	55%

$AlMe_3$ and $ZnBr_2$ were shown to preferentially form the *syn*-diastereomer by both NMR studies using Mosher esters[2a] and X-ray cystallography.[2b] This trend may be explained using the Felkin-Ahn model of this reaction (**Scheme 2**). The nucleophilic attack will be more likely to happen over the less sterically hindered side of the molecule, giving the *syn* stereochemistry.

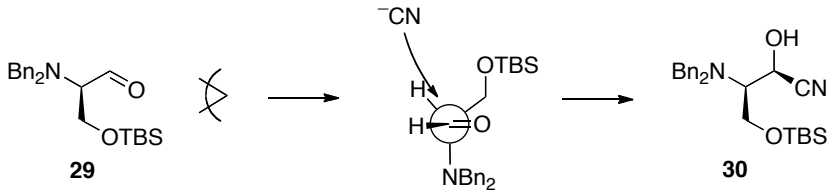


Figure 1: Felkin-Ahn model of 29

While the $AlMe_3$ and acetone cyanohydrin method afforded a higher yield of the desired isomer (60%) as calculated by NMR, the diastereomers were difficult to purify, lowing the obtained yield to 51%. The best conditions found for this cyanohydrin formation were using $ZnBr_2$ at –20 ^oC for 5 hours to give a 55% yield with a dr that was greater than 19:1, as this is the limit of detection using NMR.

The cyanohydrin was then protected to yield TBDPS ether **31**. Application of the previously used[1] silylation conditions of the TBS group using TBDPSCl and imidazole yielded incomplete silylation, and 60% of the starting material was recovered. Other conditions[3] using DMAP and triethylamine produced **31** in 75% yield.

2.1 Summary

While the previous syntheses of β-OHAsn (**10**) (**Chapter 1**) were accomplished at a reported overall 48% yield[4] and 23% yield,[5] they were impractical for our use. The Shiori group's synthesis[4] was based on a starting material only available through harvesting from bacteria or purchasing for $400 a gram, while the Taylor synthesis[5] had an amidation step that took two weeks and involved protecting group shuffling. Our overall yield was 37%, although from common intermediate **29** of the proposed τ-HAL synthesis (**Chapter 3**) it was 42%. Importantly, the conversion of D-Ser-OMe into **31** can be completed in one week.

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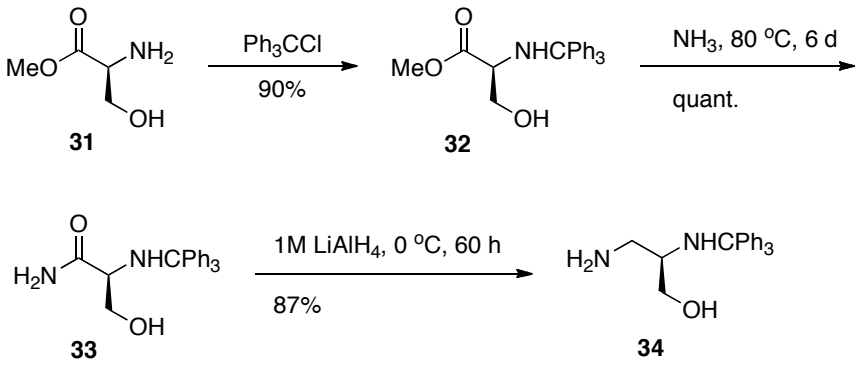
3. Progress Toward τ-HAL

As previously shown (**Chapter 1**), the prior work creating τ-HAL (**12**) relied heavily on the nucleophilicity of the imidazole nitrogens of histidine, which leads to the undesired π-regioisomer also forming.[1] We propose first creating the linear frame of the dipeptide and then designing a cyclization reaction to create the histidine imidazole. This would eliminate the problems of regio- and stereocontrol.

3.1 Synthesis of the D-Ala Moiety

For the D-alanine portion of the HAL subunit (**Scheme 1**), we started with commercially available L-serine methyl ester (**31**), which was trityl protected[2] by a coworker to afford **32**. I then used the Rapoport group's conditions[3] for the ammonolysis to provide **33** and the following reduction to produce **34**. This afforded the free amine used for the peptide coupling to the L-His portion in a 78% overall yield.



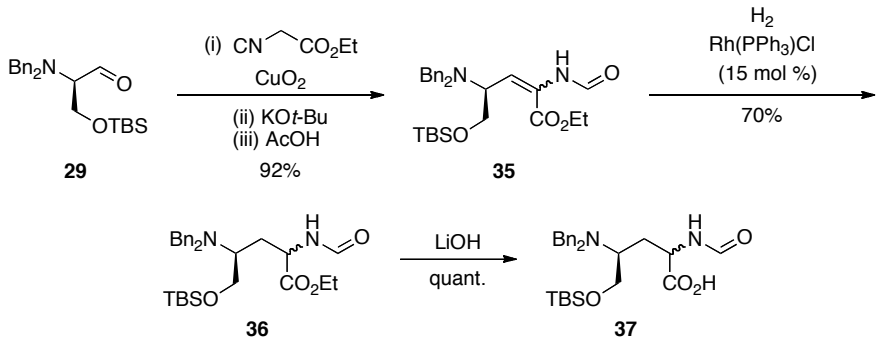
Scheme 5: Synthesis of the D-Ala portion of τ-HAL

3.2 Synthesis of the L-His Moiety

The L-histidine portion was created using previously mentioned aldehyde **29** (**Chapter 2**). This aldehyde underwent a condensation reaction[4] with ethyl isocyanoacetate to yield diastereomers **35** (**Scheme 2**). At this point the mixture of

diastereomers was not separated, as the configuration is unimportant and does not impact later reactions.

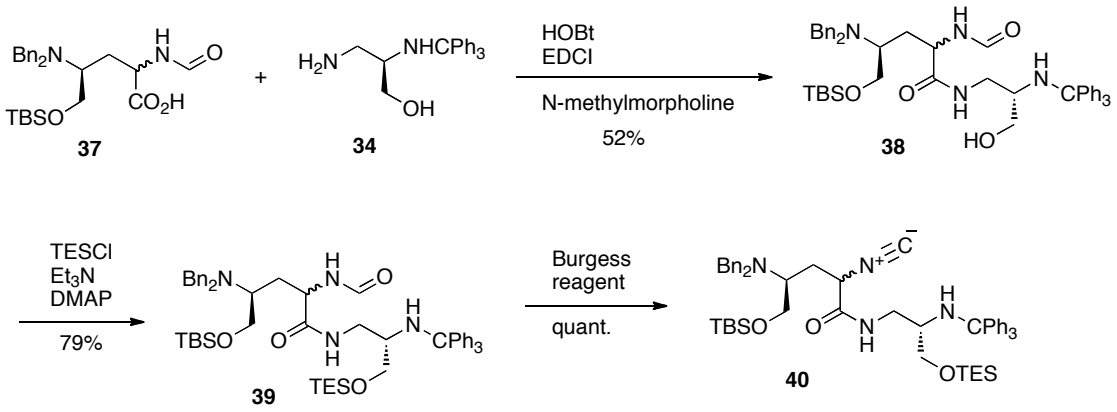
Scheme 6: Synthesis of the D-His portion of τ-HAL



This mixture was then subjected to hydrogenation.[4-5] After an inability to reproduce the yields reported with conditions previously used,[6] we found that changing the solvent from CH_2Cl_2 to 1:1 THF:MeOH[4] and the loading of Wilkinson's catalyst from 10 mol % to 15 mol %,[4] with an additional quantity introduced on the second day, made the yields higher and consistent. Afterward, **36** was hydrolyzed[6] to **37** in quantitative yields.

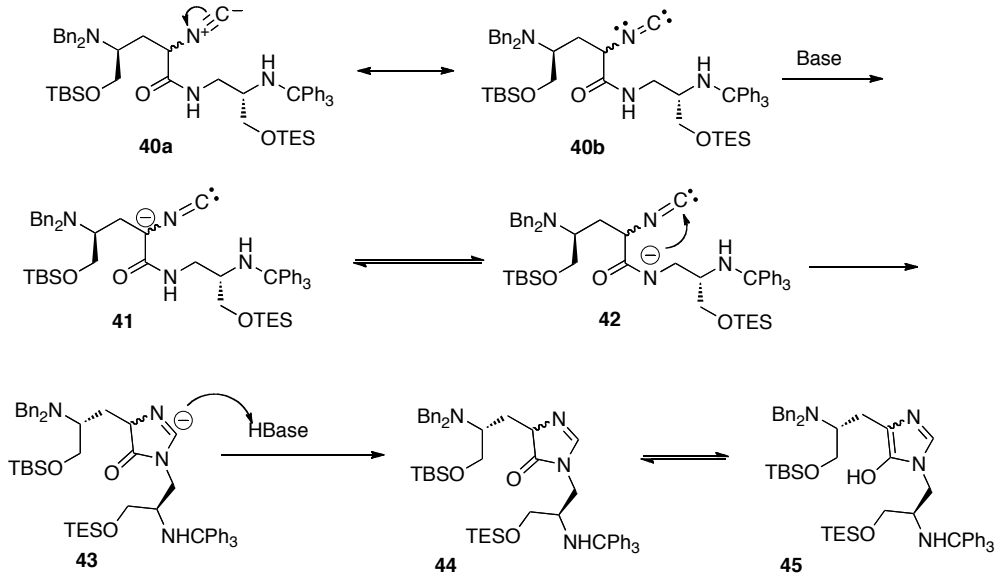
3.3 Progress Toward τ-HAL

From this point, **34** and **37** were joined in a peptide coupling (**Scheme 3**).[6] Protecting[3] the free hydroxyl group of dipeptide **38** yielded **39**. From this point, we used the Burgess reagent,[7] which is a zwitterionic carbamate, to dehydrate the formamide to an isonitrile.



Scheme 7: The synthesis of τ-HAL precursor

We then proposed a novel cyclization (**Scheme 4**) based on a similar previous approach[8] that would result in the formation of the imidazole ring of the histidine. As seen in the carbene resonance form of the isonitrile, **40b**, there is an empty *p* orbital on the isocyano carbon. While treatment with base deprotonates the alpha carbon to make **41**, an equilibrium also forms with the amide nitrogen deprotonated (**42**), which can add into the *p* orbital of the isocyanide carbon creating the anion **43**, which may then protonate to give the keto-enol tautomers **44** and **45**.



Scheme 8: Proposed τ-HAL cyclization

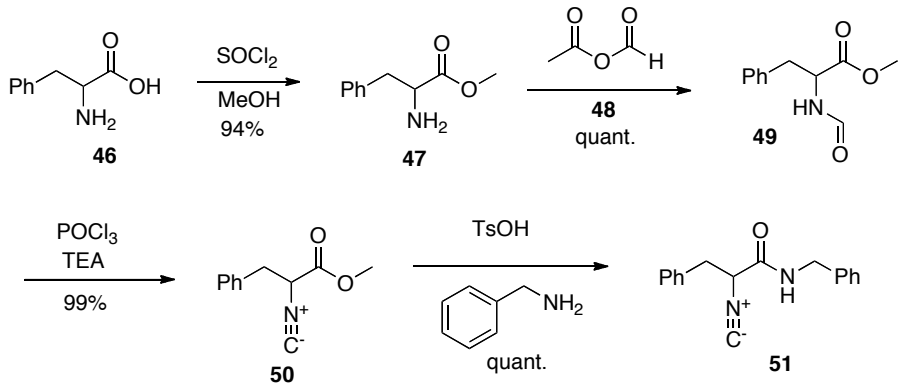
While there was evidence obtained by a coworker supporting this cyclization,[6] it was reported at an 8% yield and was not reproducible. After screening several bases and various conditions in an attempt to elucidate more suitable conditions for this cyclization (**Table 1**), it became clear a model study would be beneficial in preserving the precious material and also to simplify the NMR to deduce potential side reactions.

Table 1: τ-HAL cyclization attempts

Conditions	**Results**
1 eq. tBuOK, 0 ^{o}C, 30 min, warmed to rt	No reaction
1 eq. tBuOK, 0 ^{o}C, overnight	Decomposition
2 eq. nBuLi, –78 ^{o}C, 1 h	Decomposition
Excess LDA, –20 ^{o}C, 20 min	No product isolated
3.6 eq. NaH, rt, 1 h	Decomposition
2.5 eq KHMDS, 0 ^{o}C, 1 h	No product isolated
2 eq. NaOMe, 0 ^{o}C, 2 h	Decomposition

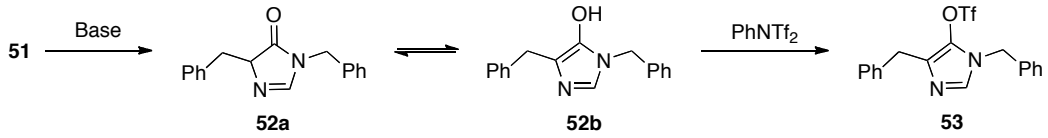
3.4 τ-HAL Model Study

For the model study, we employed a published[9] procedure to synthesize the isocyanide precursor (**Scheme 5**). Using racemic phenylalanine **46**, thionyl chloride, and methanol, methyl ester **47** was made. Acetic anhydride and formic acid were combined to make mixed anhydride **48**, and **47** was added to create formamide **49**.



Scheme 9: Model τ-HAL cyclization precursor synthesis

Previously, for the isocyanide formation of **40** the Burgess reagent was used because we were concerned about the potential of the other nitrogens reacting as well. However, in the model system there is less functionality, so we followed literature precedent,[9] applying phosphoryl chloride to create isocyanide **50** in nearly quantitative yield. Following this, an amidation reaction[8a] provided **51** in quantitative yields after recrystallization.



Scheme 10: Model cyclization

Attempting cyclization[8b] of **51** with various bases yielded results similar to those previously obtained, which lead to postulating possible instability of tautomers **52**. We then proceeded to trap enol **52b** as triflate **53**. This was desirable, as we hoped to remove the oxygen later to yield the unsubstituted imidazole, and this made for a one-pot method. Several bases and two triflating agents were screened (**Table 2**) for this reaction. We were able to see the two sets of benzylic hydrogens in the ^{1}H NMR spectra of **51**, doublet of doublets centered at 3.26 and 4.43, collapse into two singlets at 4.06 and 5.55 ppm respectively in the ^{1}H NMR spectra of **53**. However, upon later analysis, the product was

coeluting with the excess triflating agents during purification, making exact yields unknown. Subsequent preparative thin plate chromatography yielded the di- and monotriflated triflating agents, but was unable to produce the cyclized product, suggesting it was unstable in those purification conditions. However, using the NMR spectra of these coeluted species, calculated yields were made using molar ratios.

Table 2: τ-HAL model cyclization attempts

Base	Triflating agent	Result	Equivalents of Base
nBuLi	$NPhTf_2$	Decomposition	1
KHMDS	$NPhTf_2$	Decomposition	1
tBuOK in THF	$NPhTf_2$	ca. 8% yield*	1
Phosphazine Base P1	$NPhTf_2$	Decomposition	1
NaH	$NPhTf_2$	Decomposition	1.5
tBuOK in THF	Comins' reagent	ca. 10% yield*	1
tBuOK in tBuOH	Comins' reagent	ca. 7% yield*	1

*These yields were calculated using NMR-derived molar ratios

3.5 Application of the Cyclization Conditions on τ-HAL

Returning to the cyclization of the τ-HAL precursor, the conditions developed in the model study (**Chapter 3.4**), were applied to **40**. Unfortunately, however, this again yielded an unpromising crude ^1H NMR spectrum and the column was fruitless. Additionally, the cyclized material was not detected by mass spectrometry in the crude mixture.

3.6 Summary

The D-Ala and L-His components were synthesized[2-6] then joined to form the τ-HAL precursor **40**.[3, 6-9] As the actual cyclization was troublesome, a model study was undertaken. This produced conditions[8b] that brought about triflate trapped model τ-HAL

53, albeit in low yield and contaminated with excess triflating reagent. However, when these conditions were applied to our dipeptide precursor, it again was not fruitful.

3.7 Conclusions and Suggested Future Work

While the NMR and mass spectral data suggest we were successful in synthesizing a triflate trapped model τ-HAL, we were unsuccessful in purifying the cyclized product from the triflating agents and in applying those conditions to the dipeptide **40**. The low yields were also undesirable, and perhaps had many contributing factors. It is possible that side products could form from the starting material through intermolecular additions or from another anion in equilibrium during the cyclization conditions, as no starting material remained. There is also the evidence of decomposition on preparative thin layer chromatography that suggests the cyclized product's instability. Although molar ratios of the triflating agent to **53** were found to be similar when crude ^{1}H NMR spectra were compared to the purified ones, indicating decomposition was not occurring during the column, it is possible that during the reaction time the cyclized product went on to further react. In support of this, one side product isolated periodically suggested an unexplained debenzylation of the τ nitrogen, although its spectra otherwise matched that of cyclized product **53**. It would be beneficial for future work to address identifying possible side products and improving the reaction conditions to eliminate them. Future work should also focus on the purification of **53** from the triflating agents. The next steps are removal of the triflate group to yield the unsubstituted imidazole and further optimizing both reactions for use on the τ-HAL precursor **40**.

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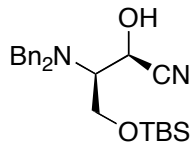
4. Experimental

4.1 General Experimental Details

All anhydrous solvents were dried by passage through a Glass Contour solvent drying system containing cylinders of activated alumina. All equipment was dried in an oven and cooled over desiccant prior to use. Reagents were purchased from Sigma-Aldrich and Alfa Aesar, and used without further purification. Flash chromatography was carried out using 60-230 mesh silica gel. ^{1}H NMR spectra were acquired on 500 MHz spectrometers with tetramethylsilane (0.00 ppm) as internal reference. Signals are reported as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), brs (broad singlet), m (multiplet). Coupling constants are reported in hertz (Hz). ^{13}C NMR specta were acquired on spectrometers operating at 125 MHz with solvent (by lock) as internal reference. Mass spectral data were obtained using ESI techniques.

4.2 Synthesis of β-OHAsn

(2*R*,3*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-3-(dibenzylamino)-2-hydroxybutanenitrile (30)

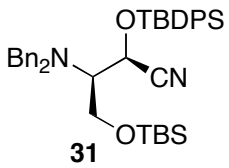


30

Aldehyde **29** (62.8 mg, 0.163 mmol) was suspended in anhydrous CH_2Cl_2 (1.4 mL) and cooled to –20 ^{o}C under Ar. TMSCN (0.055 mL, 0.412 mmol) was added, followed by $ZnBr_2$ (55.4 mg, 0.246 mmol). The resulting mixture was stirred at –20 ^{o}C for 5 h. It was then warmed to rt and treated with 10% citric acid in MeOH (1.5 mL) and stirred for 15 min. H_2O (3 mL) was added and the aqueous layer was extracted with EtOAc (3 × 6

mL), washed with brine (5 mL), dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (10% EtOAc in hexanes elution) produced **30** (36.7 mg, 55%) as a yellow oil: $[\alpha]^{25}_D$ –40.9 (*c* 1.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35–7.24 (m, 10H), 4.5 (d, *J* = 8.5 Hz, 1H), 4.11 (dd, *J* = 11.0, 4.5 Hz, 1H), 3.95 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.92 (d, *J* = 13.5 Hz, 2H), 3.65 (d, *J* = 13.5 Hz, 2H), 3.14–3.12 (m, 1H), 0.93 (s, 9H), 0.14 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.2, 129.1, 128.7, 127.7, 119.7, 60.2, 59.7, 54.9, 54.5, 26.6, 25.8, –5.6; HRMS (ESI) *m/z* 411.2418 (MH^+, $C_{24}H_{34}N_2O_2SiH^+$ requires 411.2418); IR (film) ν_{max} 3430, 30.28, 2928, 2856, 2359, 1494, 1454, 1361, 1258, 1101, 837 cm^{-1}.

(2*R*,3*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-2-((*tert*-butyldiphenylsilyl)oxy)-3-(dibenzylamino)butanenitrile (31)



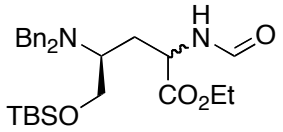
31

Cyanohydrin **30** (36.0 mg, 0.0877 mmol) was suspended in anhydrous CH_2Cl_2 (0.8 mL) under Ar. NEt_3 (0.032 mL, 0.230 mmol) and DMAP (6.8 mg, 0.0557 mmol) were added, and the reaction mixture was cooled to 0 ^oC. TBDPSCl (0.046 mL, 0.177 mmol) was added, the mixture was warmed to rt and stirred for 20 h. It was then diluted with EtOAc (6 mL), washed with saturated aqueous NH_4Cl (2 mL), H_2O (1 mL), saturated aqueous $NaHCO_3$ (2 mL), and brine (2 mL). It was then dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (2.5–5% EtOAc in hexanes gradient elution) produced **31** (41.1 mg, 72%) as a yellow oil: $[\alpha]^{25}_D$ –7.5 (*c* 0.56, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.64–7.20 (m, 20H), 4.40 (d, *J* = 8.5 Hz, 1H), 3.89 (dd, *J* = 15.0, 5.0 Hz, 1H),

3.83 (dd, J = 22.0, 13.5 Hz, 4H), 3.65 (dd, J = 10.5, 7.5 Hz, 1H), 3.33–3.28 (m, 1H), 1.09 (s, 9H), 0.86 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 136.1, 135.8, 128.9, 128.3, 127.0, 119.3, 63.7, 63.0, 60.6, 55.7, 26.9, 25.9, 19.3, 18.0, –5.5, –5.6; HRMS (ESI) *m/z* 649.3583 (MH^+, $C_{40}H_{52}N_2O_2Si_2H^+$ requires 649.3583); IR (film) ν_{max} 2929, 2857, 1471, 1427, 1256, 1106, 838 cm^{-1}.

4.3 Synthesis of Model τ-HAL

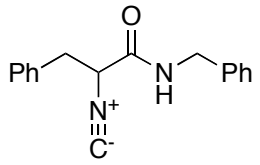
(4*S*)-Ethyl-5-((*tert*-butyldimethylsilyl)oxy)-4-(dibenzylamino)-2-formamidopentanoate[1]



36

Allylformamide **35** (1.28 g, 2.58 mmol) was suspended in anhydrous THF:MeOH (1:1, 25 mL) and placed under Ar. Wilkinson's catalyst (0.34 g, 0.387 mmol) was added and the flask was set into a bomb. The chamber was flushed (3 × 25 bar) with H_2, and then filled with H_2 (25 bar). The reaction was stirred at rt overnight for 2 d. The chamber was vented and two small spatula scoops of catalyst were added. The chamber was flushed and repressurized as before, stirring for another 2 d. The chamber was vented and the reaction mixture was filtered and concentrated in vacuo. Flash chromatography (5–10% EtOAc in hexanes gradient elution) afforded **36** (0.90 g, 70%). Spectral data were identical to those previously collected.[1]

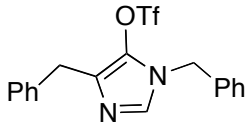
***N*-Benzyl-2-isocyano-3-phenylpropanamide (51)**



51

Isocyanide **50** (5.95 g, 31.5 mmol), benzylamine (3.45 mL, 31.5 mmol), and *p*-toluenesulfonic acid (31.5 mg, 0.183 mmol) were mixed together neat at 60 °C for 35 min under Ar, at which point an off-white precipitate was formed. The precipitate was filtered, rinsed with cold MeOH and recrystallized with *i*-PrOH to afford **51** (8.42 g, 100%) as an ivory powder: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34–7.29 (m, 6H), 7.27 (s, 1H), 7.25 (s, 1H), 7.14 (s, 1H), 7.13 (s, 1H), 6.49 (brs, 1H), 4.50–4.45 (m, 3H), 4.39 (dd, *J* = 15.0, 5.0 Hz, 1H), 3.31 (dd, *J* = 15.0, 5.0 Hz, 1H), 3.22 (dd, *J* = 15.0, 5.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.6, 136.7, 134.4, 129.6, 128.8, 128.7, 127.9, 127.8, 127.7, 110.0, 44.0, 38.6; HRMS (ESI) *m/z* 265.1361 (MH^+, $C_{17}H_{16}N_2OH^+$ requires 264.3217); IR (film) ν_{max} 3298, 3064, 3030, 2930, 2142, 1666, 1604, 1534, 1496, 1454, 1286, 1240, 1081, 1029 cm^{-1}.

1,4-Dibenzyl-1*H*-imidazol-5-yl trifluoromethanesulfonate (53)



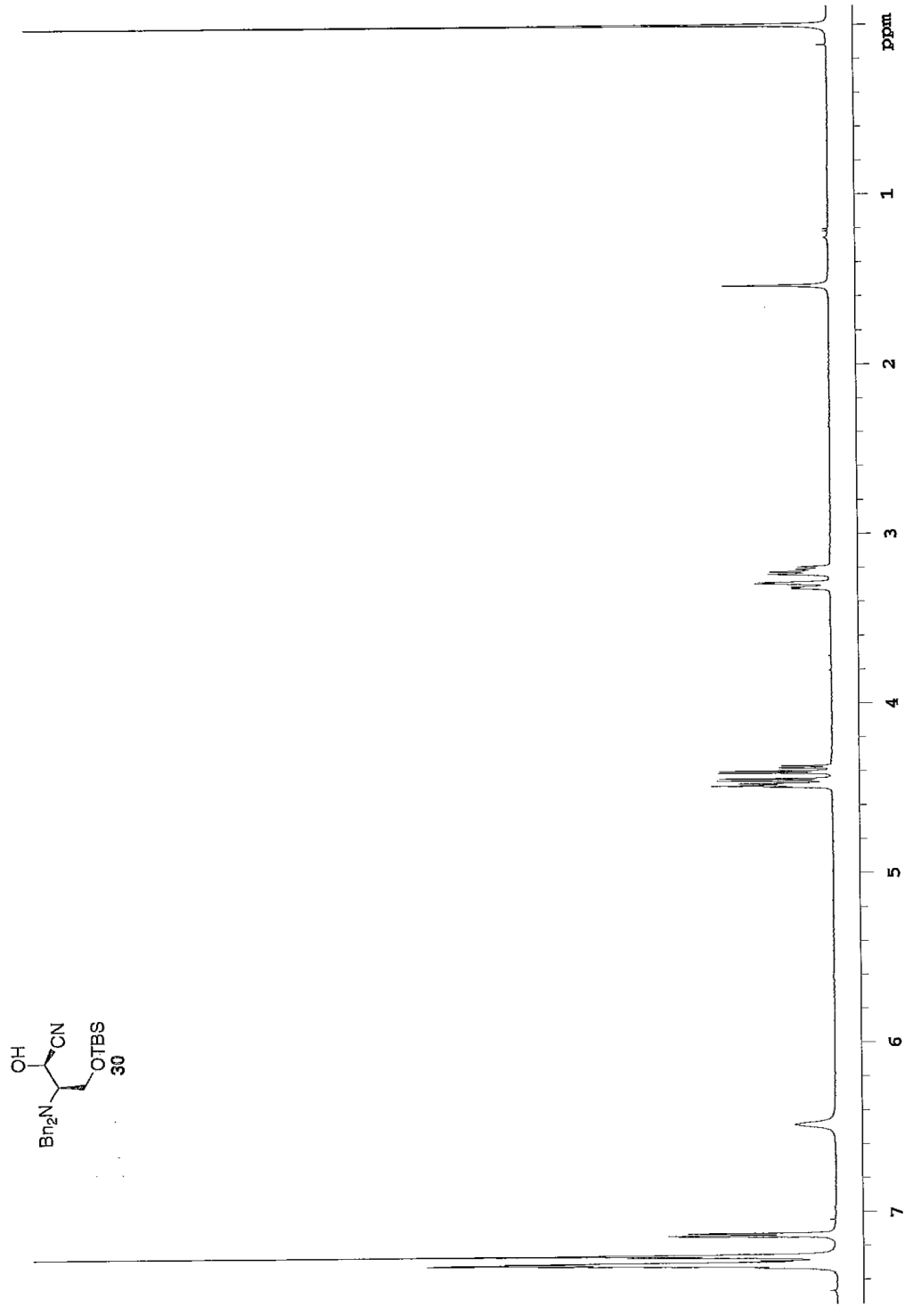
53

Isocyanide **51** (0.7092 g, 2.68 mmol) was suspended in anhydrous THF (26.8 mL) and cooled to –78 °C under Ar. Potassium *tert*-butoxide (1M in *tert*-butanol) (2.68 mL) was added dropwise. The reaction was warmed to –20 °C for 20 minutes and then cooled to –78 °C. Comins' reagent (2.1 g, 5.37 mmol) in THF (1.8 mL) was added, and the resulting mixture was stirred at –78 °C for 1 hour, then warmed to rt and stirred

overnight. The solvent was removed, and CH_2Cl_2 (35 mL) was added. The organics were washed with H_2O (2 × 35 mL), dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (0–5% EtOAc in hexanes gradient elution) produced **53** (0.9712 g of 14:1 mixture with Comins' reagent, ca. 7%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39–7.28 (m, 6H), 7.24–7.17 (m, 3H), 7.13–7.06 (m, 2H), 5.56 (s, 2H), 4.05 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 136.5, 135.0, 133.2, 139.2, 129.0, 128.6, 128.5, 127.9, 127.3, 126.9, 126.6, 49.5, 32.2; HRMS (ESI) *m/z* 397.0877 (MH^+, $C_{18}H_{15}F_3N_2O_3SH^+$ requires 397.0877).

References

1. Robinson, J. W., MS Thesis; Brigham Young University. **2010**.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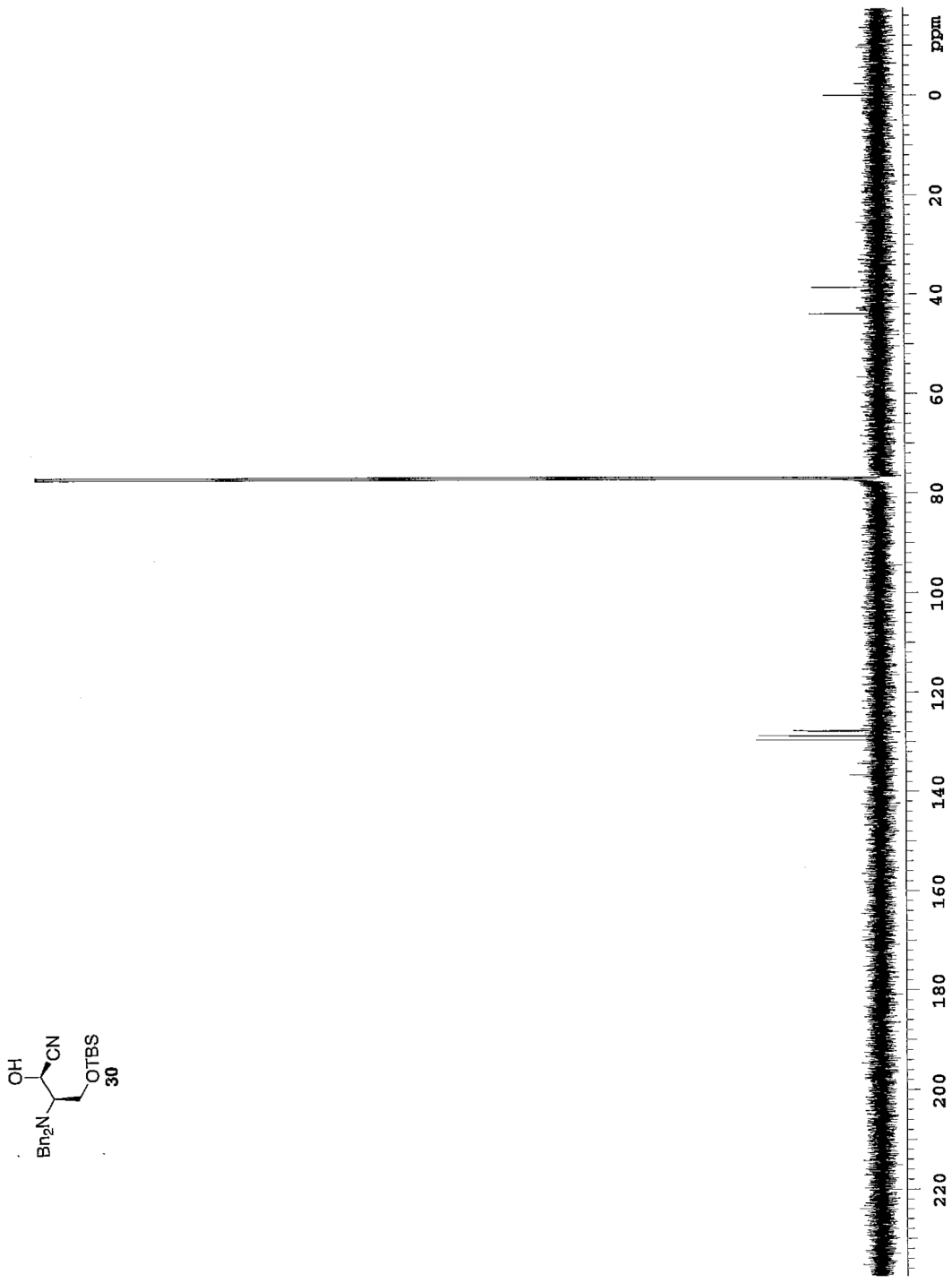
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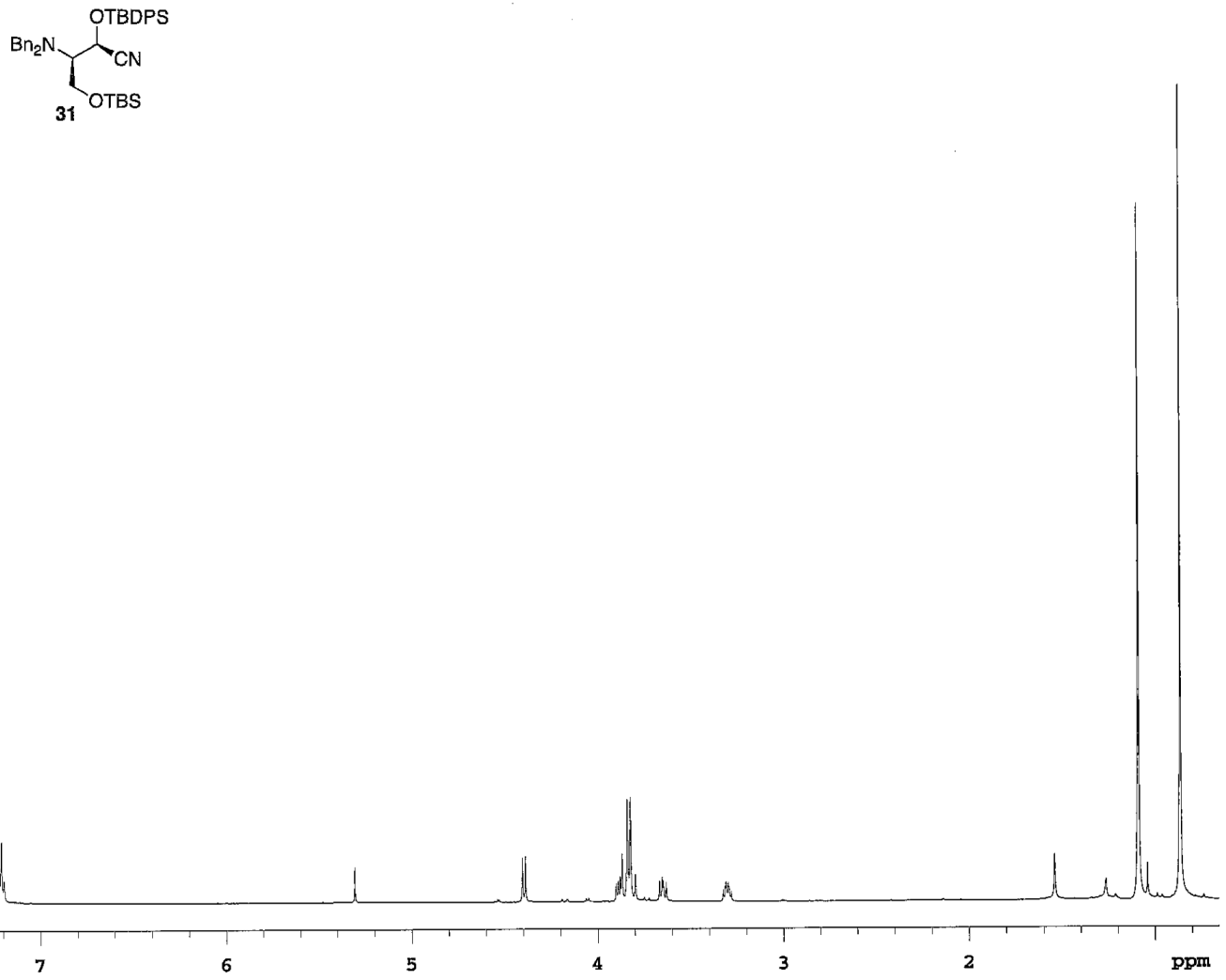
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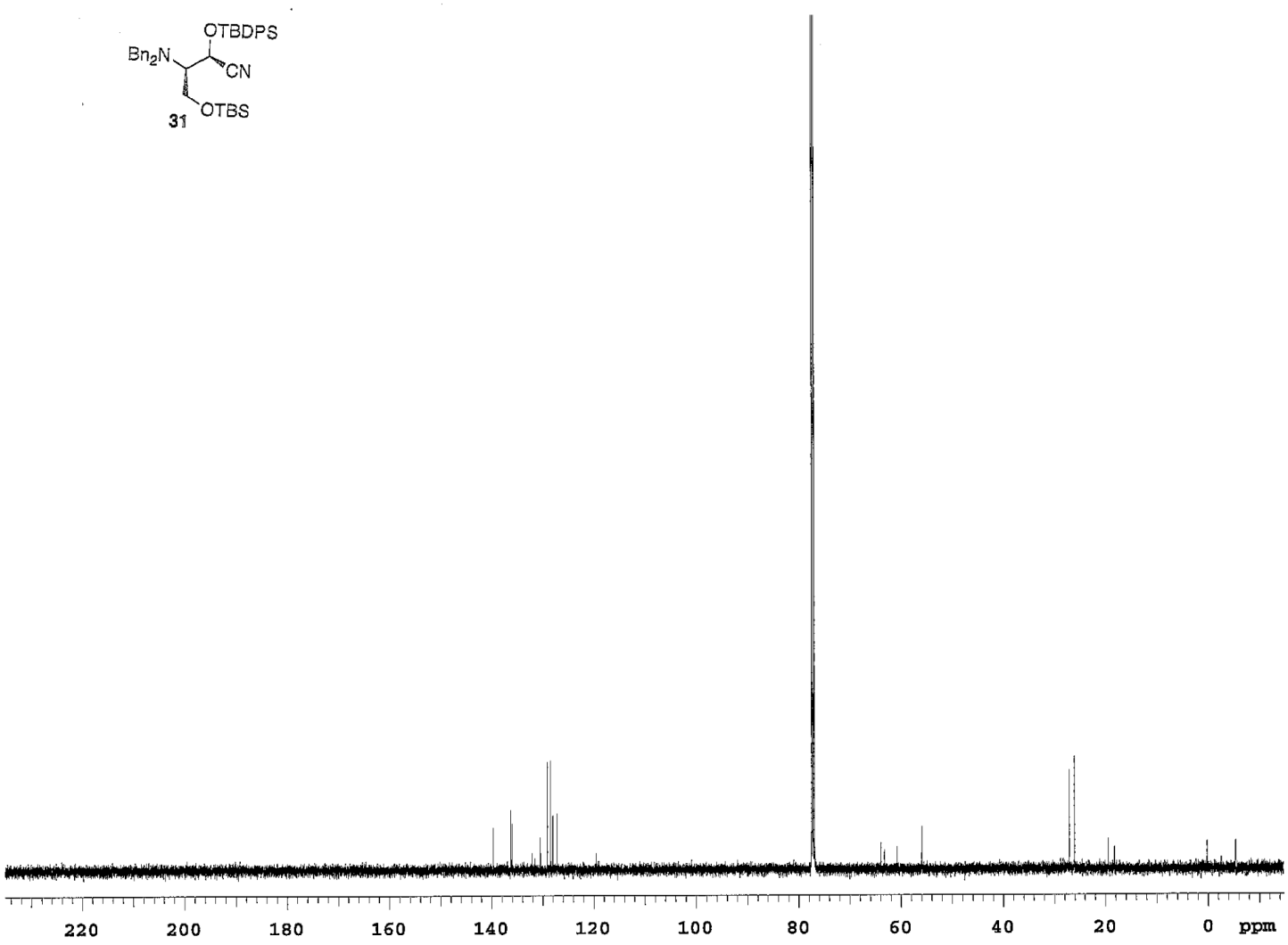
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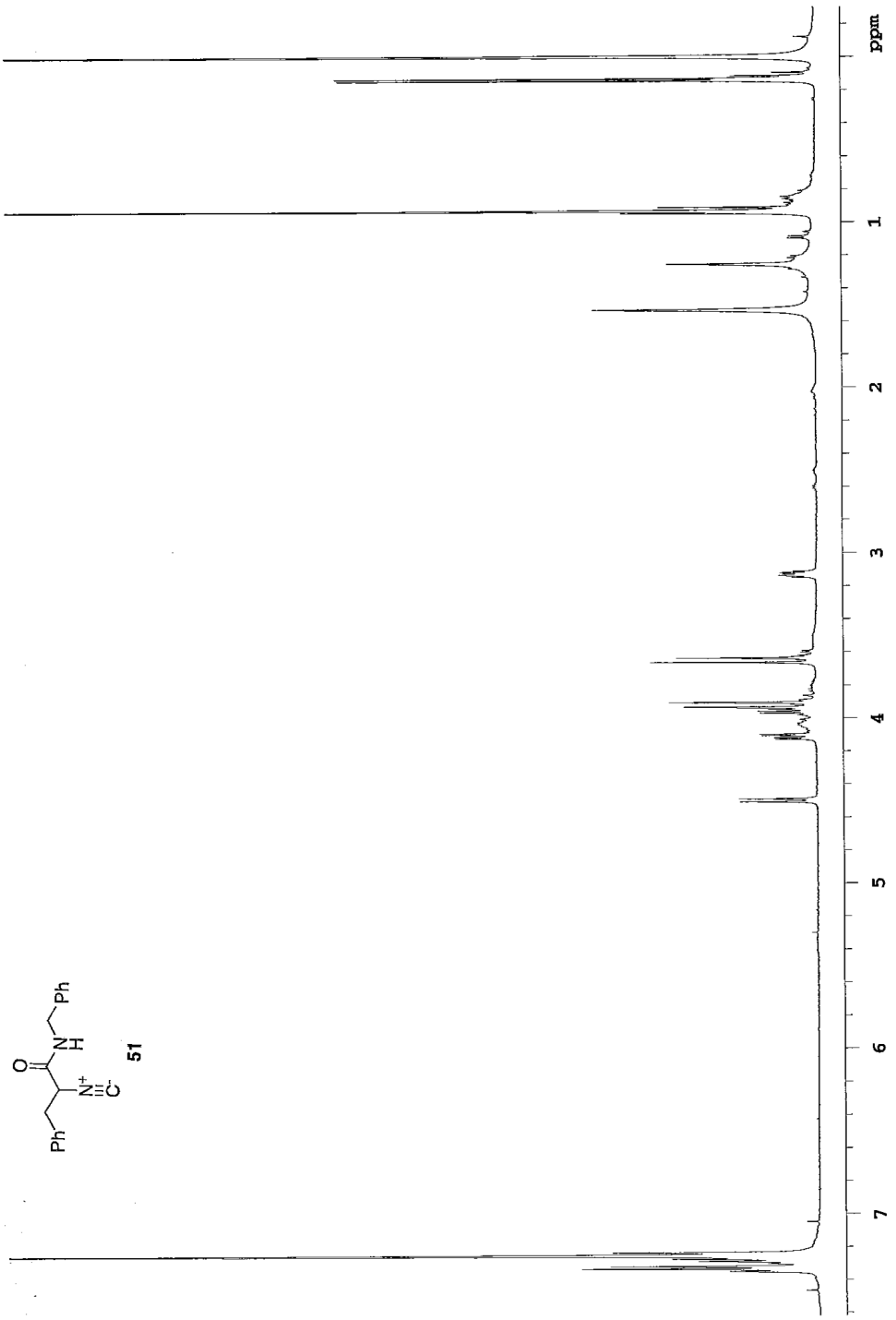
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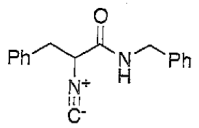

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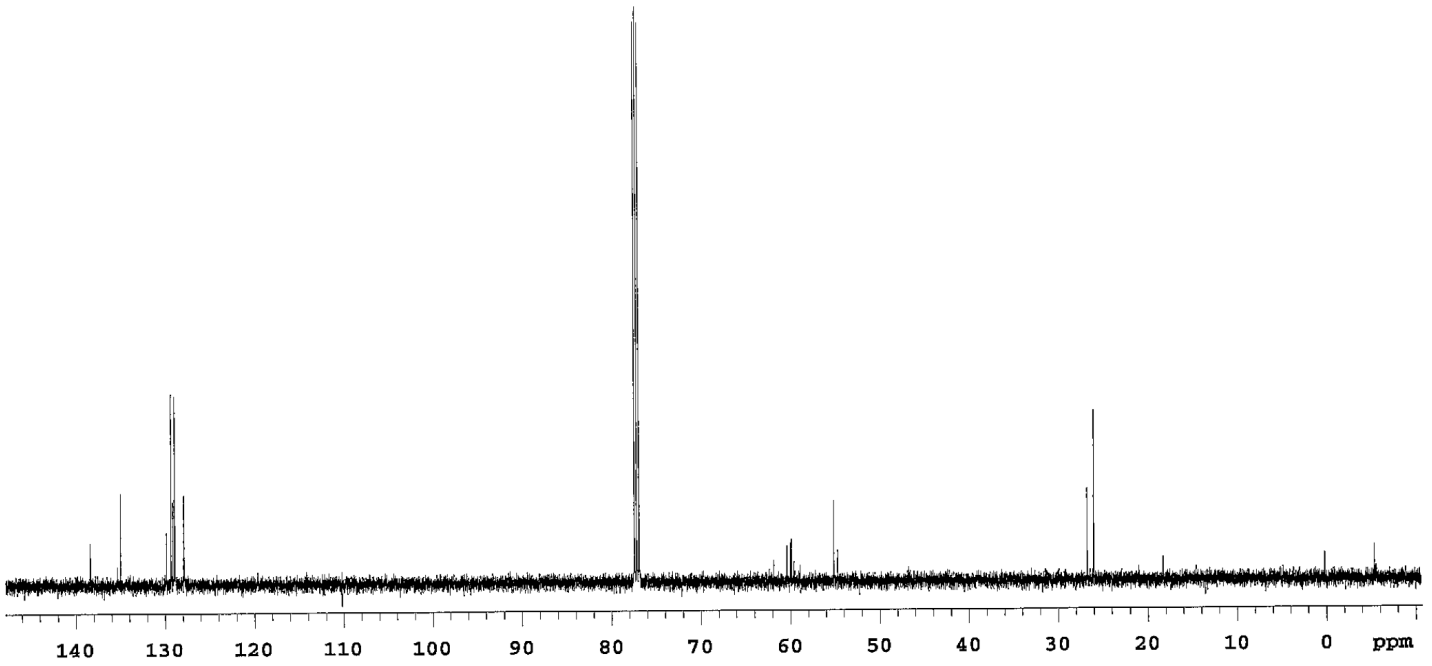

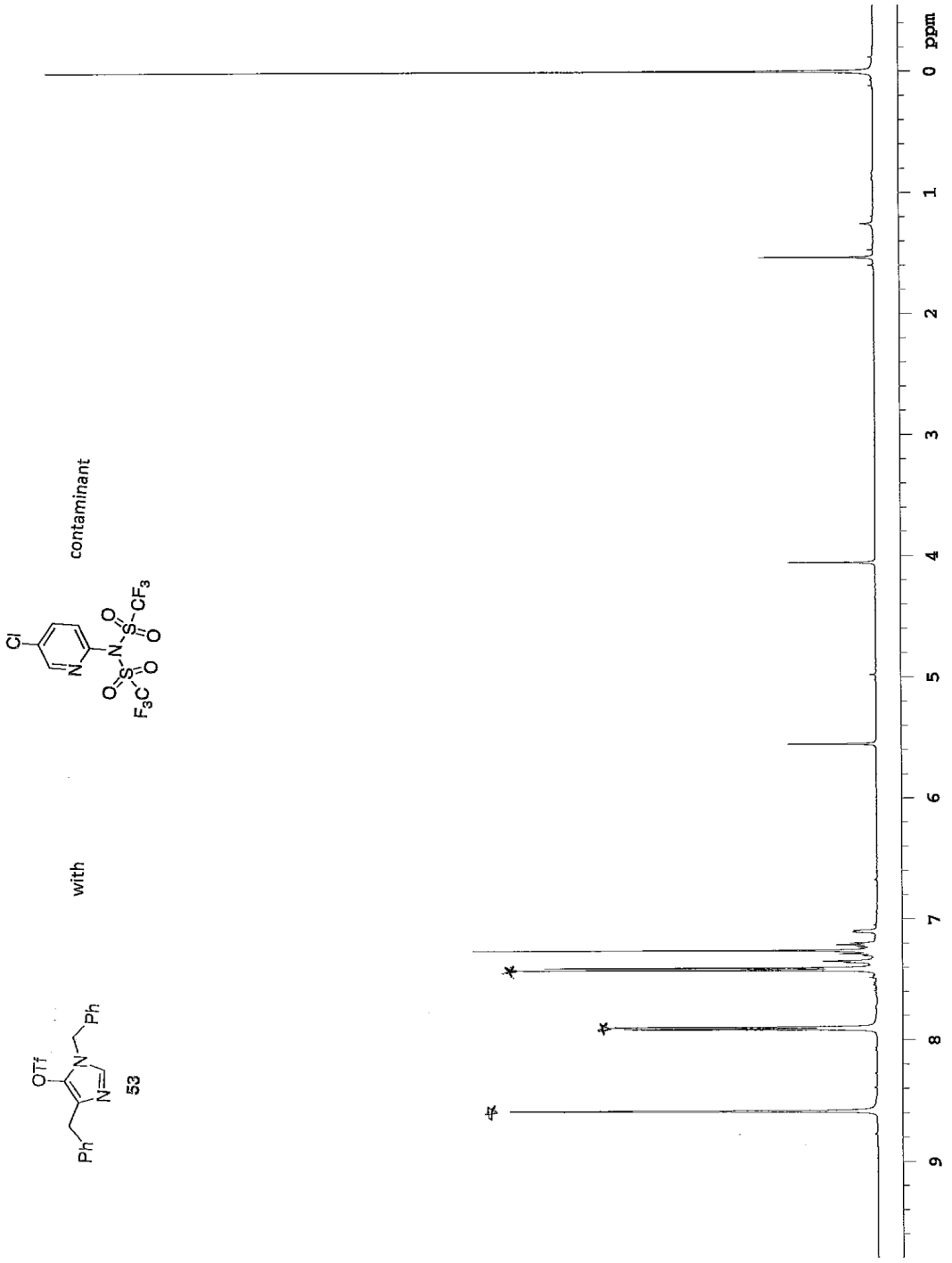
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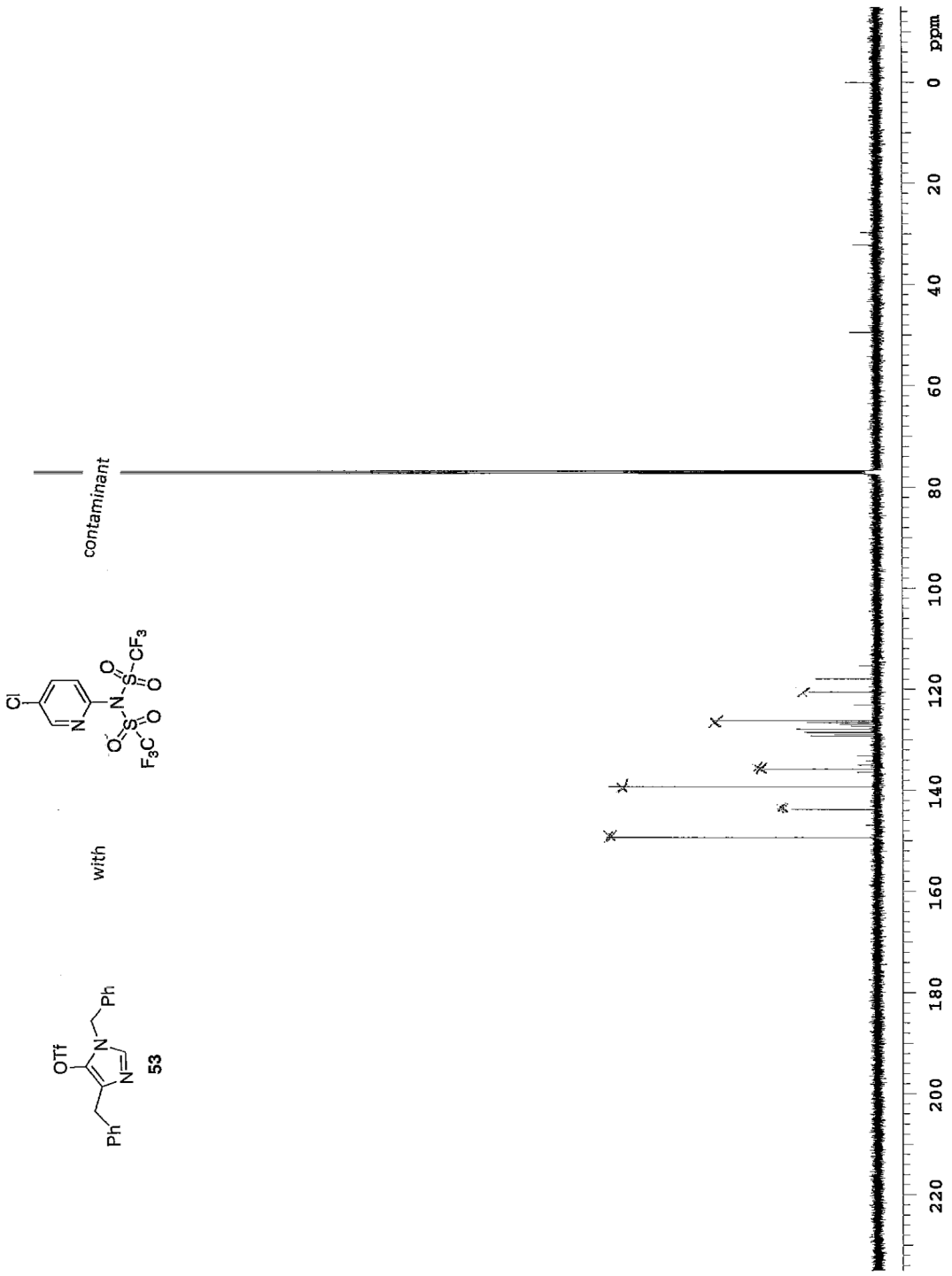
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