EFFORTS TOWARD THE SYNTHESIS OF NA22598A_1 AND THE INVESTIGATION OF ITS MECHANISM OF ACTION

by

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ABSTRACT

NA22598A_1 is a unique carbamoylated cyclic guanidine with a diol skipped diamine dipeptide. It was shown to have antimetastatic activity against DLD-1 colon cancer cells with an IC_{50} of 0.32 μM, but the absolute stereochemistry has yet to be confirmed. Guadinomine B is also a carbamoylated cyclic guanidine with a diol skipped diamine dipeptide. It was shown to inhibit the T3SS virulence system of Gram-negative bacteria with an IC_{50} of 0.014 μM. We endeavored to synthesize NA22598A_1 and determine if it was mischaracterized at isolation. In addition, we investigated a potential mechanism of action that could produce both of the phenotypes exhibited by NA22598A_1 and guadinomine B.

To my family, I never could have done it without you.

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LIST OF ABBREVIATIONS

AcOH	acetic acid
AgOAc	silver (I) acetate
$AgNO_3$	silver (I) nitrate
Bn	benzyl
Bu_3SnH	tributyltin hydride
Bu_3B	tributyl borane
Boc_2O	*tert*-butylcarbonyl anhydride
Cbz	benzyloxycarbonyl
$CDCl_3$	deuterated chloroform
CD_3OD	deuterated methanol
CH_2Cl_2	dichloromethane
CuBr	copper(I) bromide
DIAD	diisopropylcarbodiimide
DIBAL	diisobutylaluminum hydride
DMAP	4-(dimethylamino)-pyridine
DMBA	*N,N*-dimethylbarbaturic acid
DMB	dimethoxybenzyl
DMDO	dimethyldioxarane
DMF	*N*,*N*-dimethylformamide

D_2O	deuterium oxide
EDCI	ethyl-1-dimethylaminopropylcarbodiimide hydrochloride
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
HgO	mercury (II) oxide
HOBt	hydroxybenzotiazole
IBX	1-iodoxybenzoic acid
*I*PrMgCl	isopropylmagnesium chloride
iPr_2NEt	diisopropylethylamine
I_2	iodine
$InCl_3$	indium chloride
K_2CO_3	potassium carbonate
KHMDS	potassium bis(trimethylsilyl)amide
LDA	lithium diisopropylamine
LHMDS	lithium bis(trimethylsilyl)amide
LiOH	lithium hydroxide
MeCN	acetonitrile
MeOH	methanol
$MgSO_4$	magnesium sulfate
MsCl	methanesulfonyl chloride
NaOH	sodium hydroxide
Na_2SO_4	sodium sulfate
$NaBH_4$	sodium borohydride

*n*BuLi *normal* butyl lithium

NBS *N*-bromosuccinamide

NEt_3 triethylamine

NaH sodium hydride

NaN_3 sodium azide

NH_4Cl ammonium chloride

NH_4OH ammonium hydroxide

$MeCOONH_4$ ammonium acetate

$(NH_4)_6Mo_7O_{24}$ ammonium molybdate

NsCl 4-Nitrobenzenesulfonyl chloride

Pd/C palladium on carbon

$Pd(OH)_2/C$ palladium (II) hydroxide on carbon

Pt/C platinum on carbon

Ph phenyl

PhMe toluene

PMP *p*-methoxyphenyl

PPh_3 triphenylphosphine

Pyr pyridine

Rh/Al rhodium on alummina

$SOCl_2$ thionyl chloride

*t*Bu *tert*-butyl

TFA trifuoroacetic acid

TESCl triethylsiyl

TBAF tetra-n-butylammonium floride

TBS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
THF	tetrahydrofuran
$TMSN_3$	trimethylsilyl azide
TsCl	tosyl chloride
$VO(acac)_2$	vanadyl acetylacetonate
$Zn(OTF)_2$	zinc triflate
ZnCl	zinc chloride

CHAPTER 1

SYNTHESIS OF THE CARBAMOYLATED CYCLIC GUANIDINE FRAGMENT OF GUADINOMINE B/NA22598A$_1$

1.1 Isolation of NA22598A$_1$ and Guadinomine B

In 1997, Fukazawa and coworkers identified a new natural product, NA22598A$_1$, during a screen for antitumor compounds (Figure 1.1).[1] NA22598A$_1$ was isolated from *Streptomyces* species NA22598, and initial tests demonstrated that it inhibited anchorage-independent growth of a DLD-1 human colon cancer cell line. NA22598A$_1$ has a unique structure showcasing a fully reduced cyclic guanidine with an N5' attached carbamoyl group, and a diol skipped diamine moiety attached to a dipeptide. The initial identification of NA22598A$_1$ did not include the determination of the absolute stereochemistry, and no further structural elucidation has been reported.

The interesting biological activity as well as the stereochemical ambiguity prompted our interest in the natural product. The sixteen possible stereochemical combinations called for a modulated synthesis that would quickly allow for access to multiple diastereomers.

In 2007, Omura and coworkers isolated guadinomines A-E from *Streptomyces* sp. K01-0509 (Figure 1.1).[2] These compounds also contain a fully saturated carbamoylated cyclic guanidine. Guadinomines A and B both showed potent activity against the type three secretion system (T3SS) of bacteria. Guadinomine B and NA22598A$_1$ bear a striking

resemblance to each other, with the only structural difference being the connectivity of the carbamoyl group to the cyclic guanidine (Figure 1.1).

While the NMR data for guadinomine B and NA22598A$_1$ were not completely congruous, they were similar enough that we hypothesized the structure of NA22598A$_1$ was incorrectly assigned and these two compounds were in fact the same. One possible reason for the discrepancy in NMR data was the difference in NMR sample conditions. NA22598A$_1$ was characterized in D_2O, while guadinomine B was characterized in D_2O/1% TFA. To investigate how the carbamoyl connectivity affected the NMR data, Dr. Vasudev Bhonde, synthesized two simple model substrates, **X** and **Y** (Figure 1.2).[3] He then characterized the models and compared their respective ^{13}C NMR data.

The ^{13}C chemical shifts (δ) for these compounds were very similar. Therefore, he looked at the difference in chemical shifts ($\Delta\delta$) between **X** and **Y** as well as the difference between the $\Delta\delta$ of the natural products (Figure 1.2). The graphical representation shows that the differences between **X** and **Y** did not correlate to the differences between the natural products. The differences between the natural products were a consistent positive one ppm shift which supported the idea that these were spectra of the same compound that had been referenced differently.

1.2 Omura's Total Synthesis of Guadinomine B

The initial characterization of the guadinomines did not reveal the absolute stereochemistry of the molecules. In order to determine the absolute stereochemistry, Omura and coworkers undertook the total synthesis of guadinomines B and C_2.[4] The Omura lab had previously completed the synthesis of guadinomic acid, which confirmed a *syn* relationship between the cyclic guanidine and the alcohol at C_2.[5]

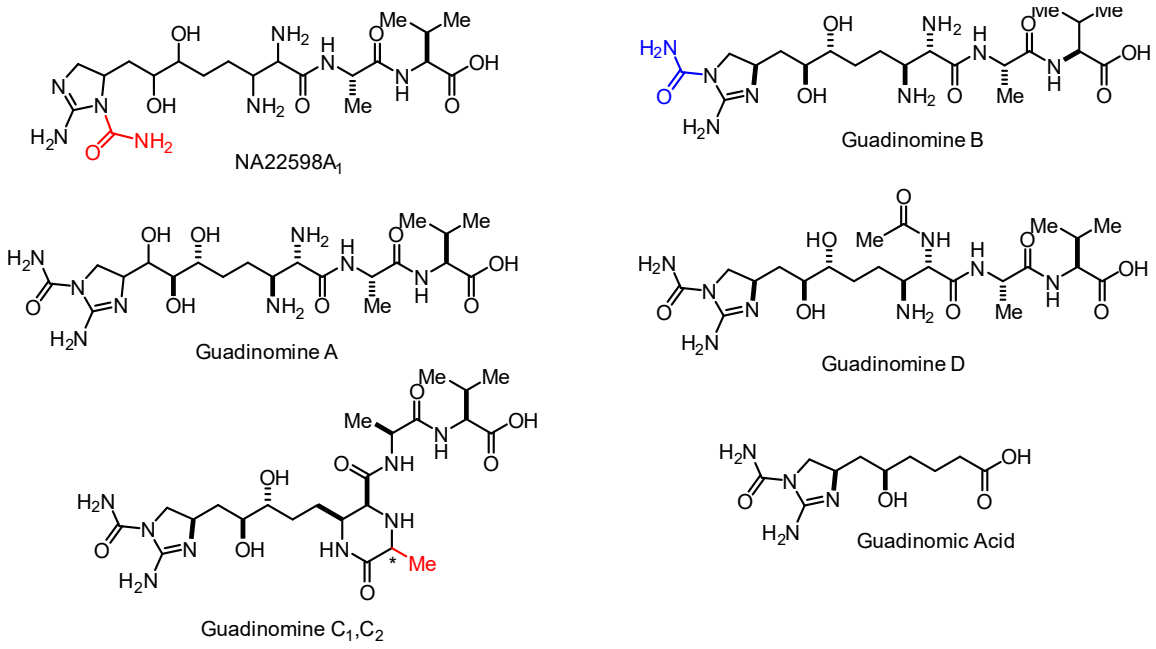


Figure 1.1: Carbamoylated cyclic guanidine natural products NA22598A$_1$, and guadinomines A-D and guadinomic acid.

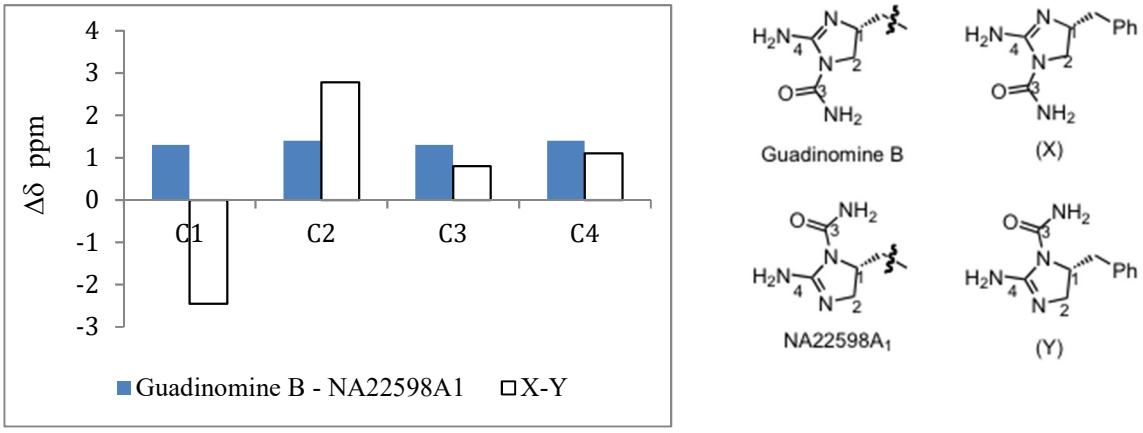


Figure 1.2: Comparison of the chemical shift differences between guadinomine B and NA22598A$_1$ and the model substrates with different carbamoyl connectivity.

Determining the stereochemistry of the alcohol at C3 and the diamine at C6 and C7 was initilly undertaken with guadinomine C_2. The cyclic nature of the diamine fragment in guadinomine C_2 allowed for a truncated model system to be synthesized and compared to the natural product (Figure 1.3). While the stereochemistry of the diamine was unknown, an NOE correlation revealed a *syn*-relationship between C6 and the CH_3-3" of guadinomine C_2. Two model systems, the *cis*-diamine and the *trans*-diamine, were then synthesized.

The synthesis of the simplified piprazinone models began with (*R*)-oxazolidinone **1**, which underwent an Evans aldol reaction with valeraldehyde and subsequent removal of the chiral auxiliary to give α-chloro-β-hydroxy acid **2**. The dipeptide fragment (NH_2-L-Ala-L-Val-O*t*Bu) was introduced through a peptide coupling to yield the chlorohydrin **3**. Epoxide formation was achieved under mildly basic conditions to form *cis*-epoxide **4** as a single diastereomer. Azidolysis followed by reduction of the azide provided the 1,2-hydroxylamine **5**. Treatment of hydroxylamine **5** with NsCl afforded the *N,O*-bis-Ns intermediate, which was promptly converted to the *cis*-Ns-aziridine **6** as a single diastereomer. Aziridine **6** was opened with NaN_3, which afforded the β-azide **7**. A Staudinger reduction followed by condensation with (*R*)-2-bromopropionic acid gave the piperadinone ring **8**. Elimination of the Ns group and deprotection of the *t*-butyl group yielded the *trans* model compound **9** (Scheme 1.1).

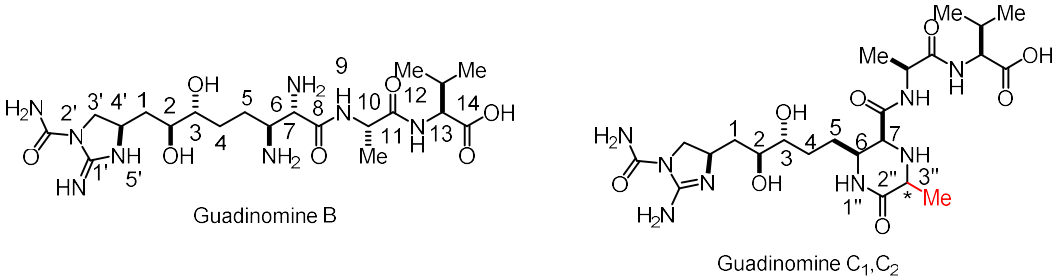
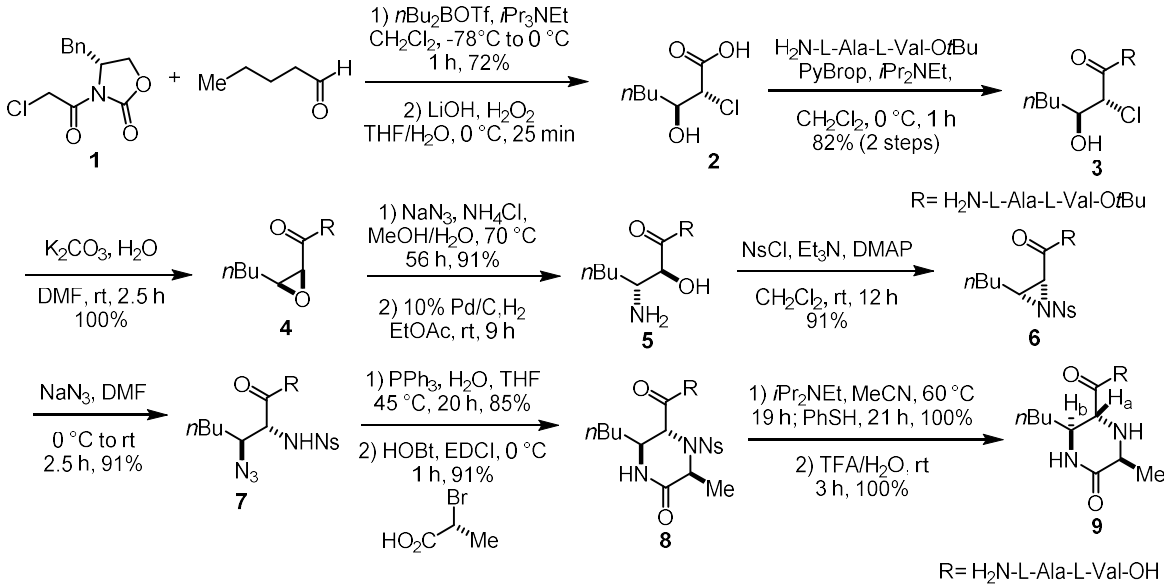
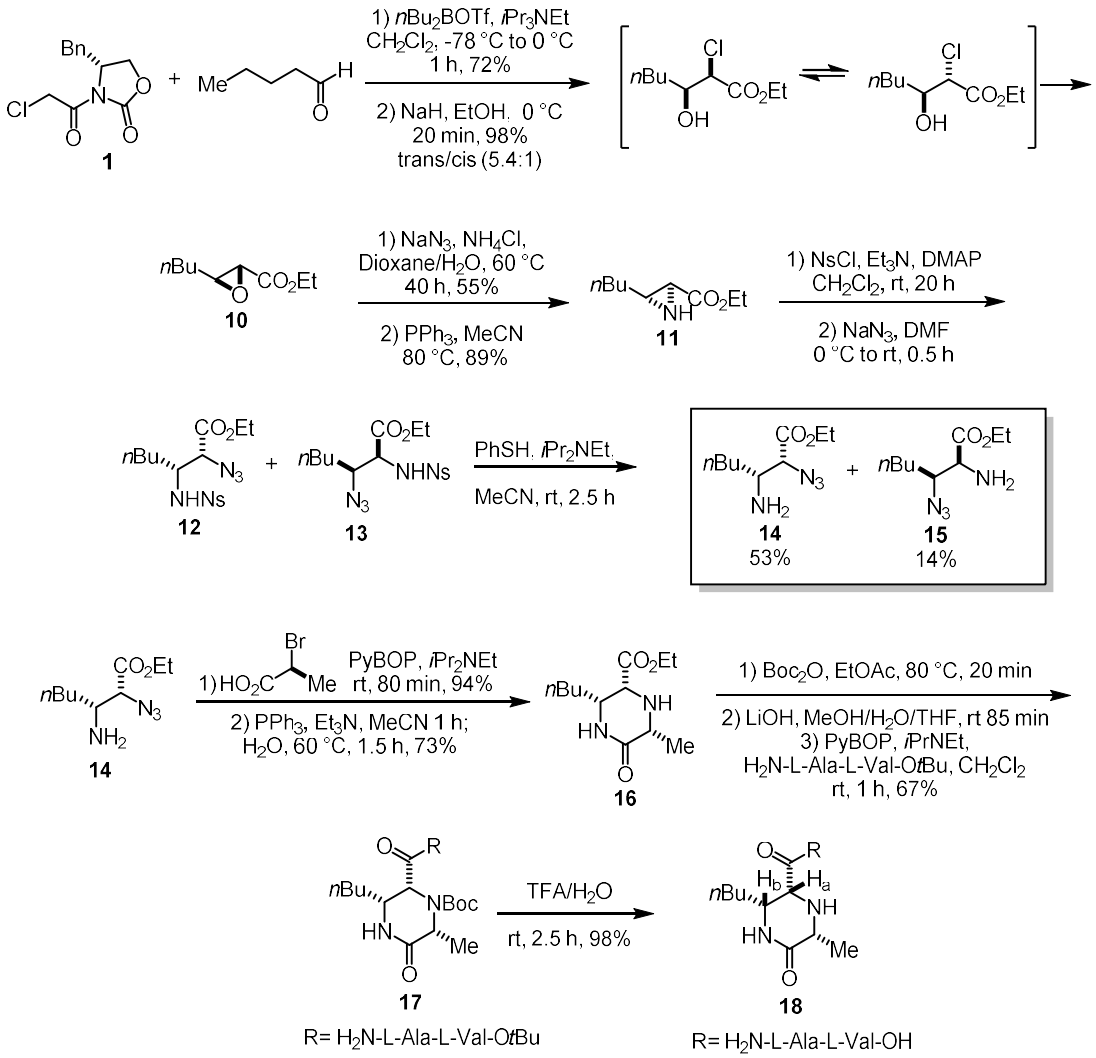


Figure 1.3: Guadinomines B and C_1, C_2.



Scheme 1.1: Synthesis of the trans piperadinone model of guadinomine C.

Synthesis of the *syn* piperazinone utilized the same Evans aldol auxillary as the *trans* piperazinone synthesis. Rather than removing the chiral auxilary with LiOH, a modified Azerad protocol was used to convert the ethyl ester as well as epimerize the alpha position, which facilitated the ring closing to the trans epoxide **10**. The epoxide **10** was converted to the aziridine **11** *via* an azidolysis followed by a Staudinger reduction. Aziridine **11** was activated with NsCl and opened with NaN_3; the azidolysis resulted in an inseperable mixture of regioisomers **12** and **13**. Removal of the Ns group allowed for the separation of the regioisomers **14** and **15** with the desired regioisomer **14** being formed with modest selectivity **14**/**15** = 3.7:1. (*S*)-2-bromopropionic acid and azido **14** were condensed followed by Staudinger reduction of the azide and S_N2 cyclization to give piperainone **16**. Boc protection of **16** was achived prior to hydrolysis and condensation with the peptide to give the protected piperizanone model **17**. Treatment of **17** with acid provided the deprotected model compound **18** (Scheme 1.2).



Scheme 1.2: Synthesis of the *syn* piperazinone model of guadinomine C.

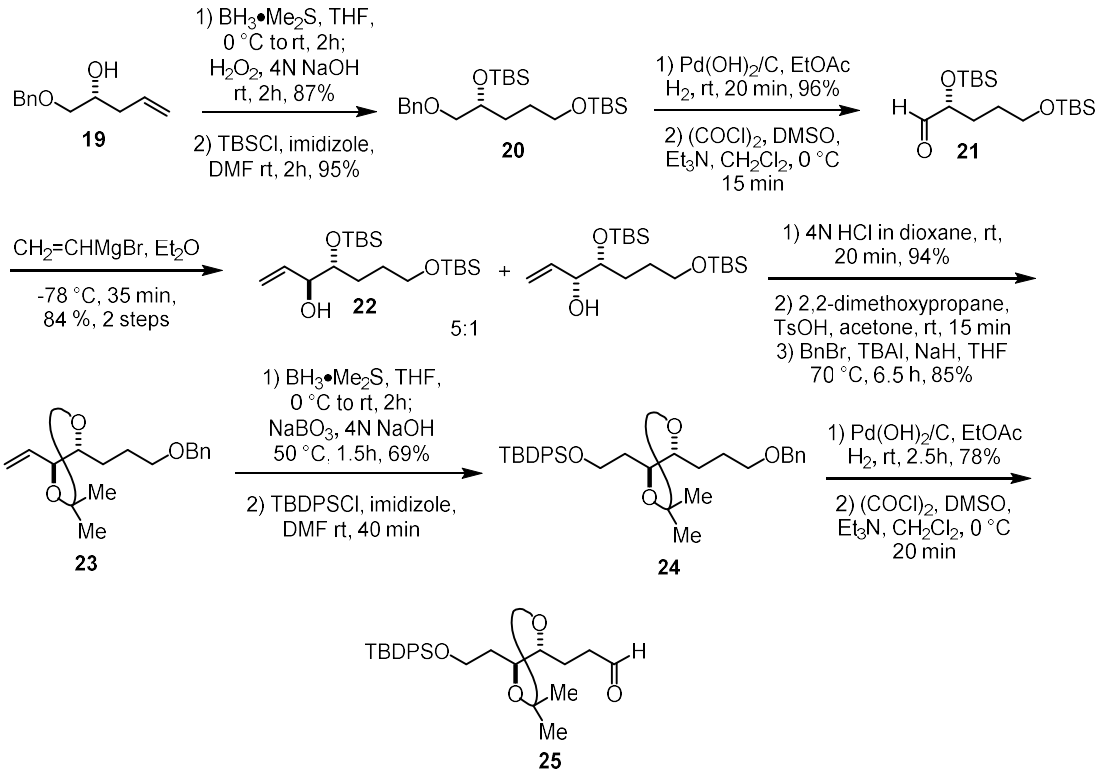
The NMR spectra of both model compounds **9** and **18** were collected in 1% TFA/D_2O. Comparing the coupling constants between H_a and H_b, it was determined that the *syn* piperazinone, $J(H_aH_b)$= 4.5 Hz, was a closer match to the guadinomine Cs $J(H_aH_b)$= 4.2 Hz, than the trans piperazinone $J(H_aH_b)$= 9.0 Hz. This led to the conclusion that the diamine in the natural product had a *syn*-relationship. However, the model system did not confirm the absolute stereochemistry in the natural product. To determine if the piperazinone in the natural product was the *(R,R)* or *(S,S)*, Omura and coworkers modified the synthesis of model

piperazinine **18** to incorporate the NH-$_D$-Ala-$_D$-Val-OH dipeptide.

The spectra of the piperazinone with the D peptide showed better agreement to the natural product than the piperazinone with L peptide. This confirmed the stereochemistry at the C6 and C7 positions to be *S*. This observation combined with the work the Omura group had previously done on guadinomic acid left only one unconfirmed stereocenter at C3. In order to confirm this stereocenter, both the *syn*-diol and *anti*-diol were synthesized. The spectra of the *anti*-diol was a closer match to the natural product than that of the *syn*-diol. The stereochemical information from the model systems was then translated into a complete synthesis of guadinomine C_2, which upon characterization matched the natural product. Having confirmed the stereochemistry of all the functionality in guadinomine C_2, the Omura group turned its focus to guadinomine B.

The synthesis of guadinomine B began with the construction of the *anti*-diol. The chiral alcohol **19** was hydroborated and the resultant alcohol TBS protected to give elongated triol **20**. The benzyl protecting group was selectively removed and the primary alcohol oxidized to the aldehyde **21**. A diastereoselective vinylation was utilized to form the diol unit **22** in a 5:1 distereomeric ratio. While the initial mixture was inseperable, removal of the TBS groups followed by cyclic acetal protection of the diol and benzyl protection of the primary alcohol allowed for the isolation of the acetonide **23** as a single diastereomer. Subsequent hydroborylation oxidation followed by protection of the alcohol yielded the *anti*-diol **24**. Removal of the benzyl group and oxidation to the aldehyde afforded the fully functionalized *anti*-diol subunit **25** (Scheme 1.3).

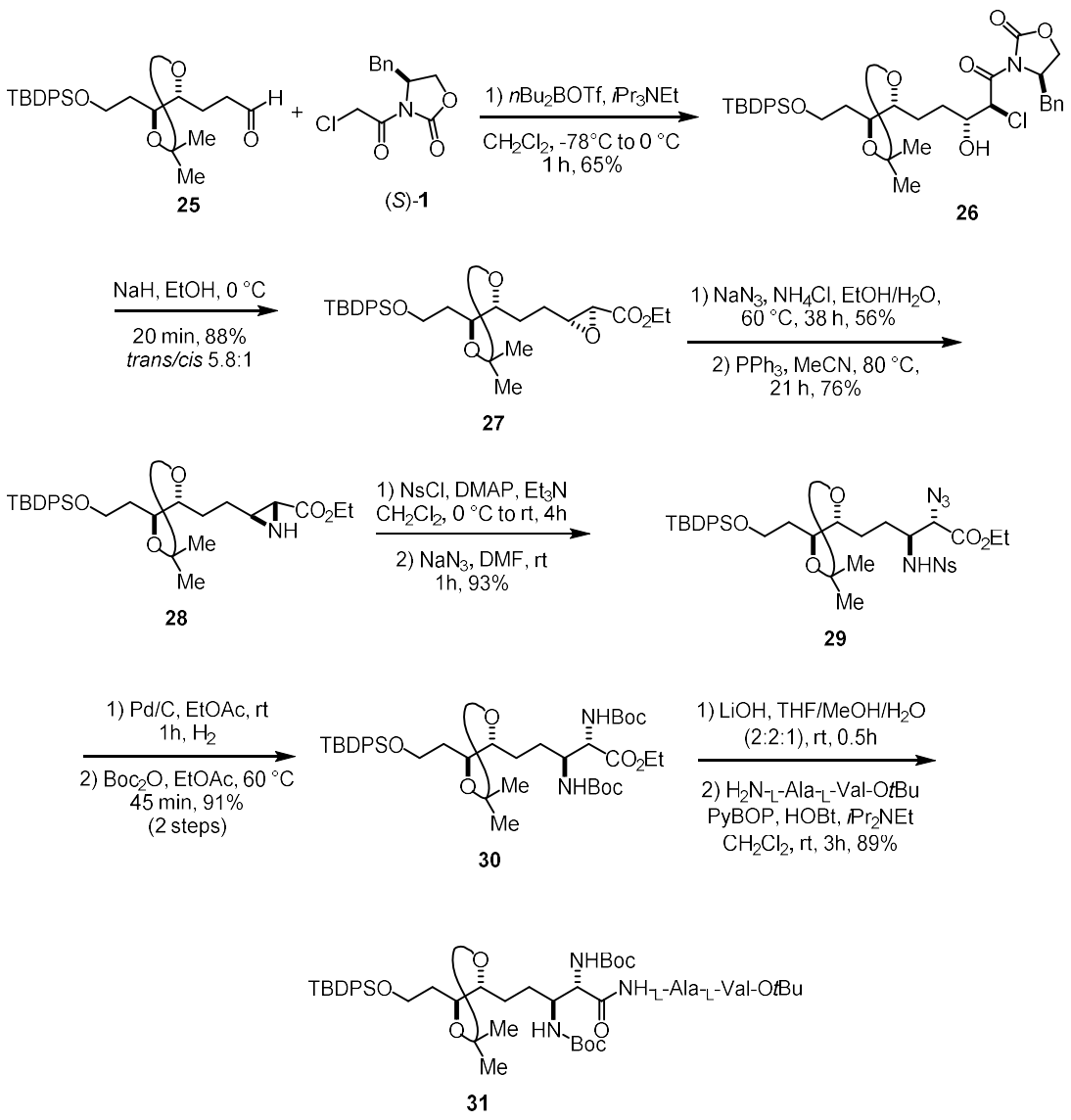
The methods used for the construction of the piperazinone unit in guadinomine C transferred well to the diol unit. (*S*)-**1** was utilized for the Evans aldol to generate the chlorohydrin **26**, which was converted to the predominantly *trans*-epoxide **27**. Azidolysis



Scheme 1.3: Synthesis of the *anti*-diol subunit of guadinomine B.

followed by Staudinger reduction gave exclusively the *trans*-aziridine **28**. NsCl was used to activate **28** and it was opened with NaN_3 at the alpha position to afford *trans*-aza amino **29**. Reduction of the azide and Boc protection of the diamine yielded **30**. Saponification of the ethyl ester followed by peptide coupling to form the diamine dipeptide **31** completed the installation of all the functionality save for the cyclic guanidine (Scheme 1.4).

The introduction of the cyclic guanidine began with the deprotection of the TBDPS group followed by oxidation to the aldehyde **32**. A modified Yamada nitro aldol using (*R*-*R*)-salen-cobalt catalyst **33** afforded nitro alcohol **34** in 90% diastereomeric excess. Reduction of the nitro group followed by guanylation gave the acyclic guanidine **35**. Treatment of **35** with PPh_3 and I_2 generated the phosphonium intermediate, which was intramolecularly displaced by the guanidine to form the cyclic guanidine **36**. This reaction proved very effective with no



Scheme 1.4: Introduction of the diamine to the diol on guadinomine B.

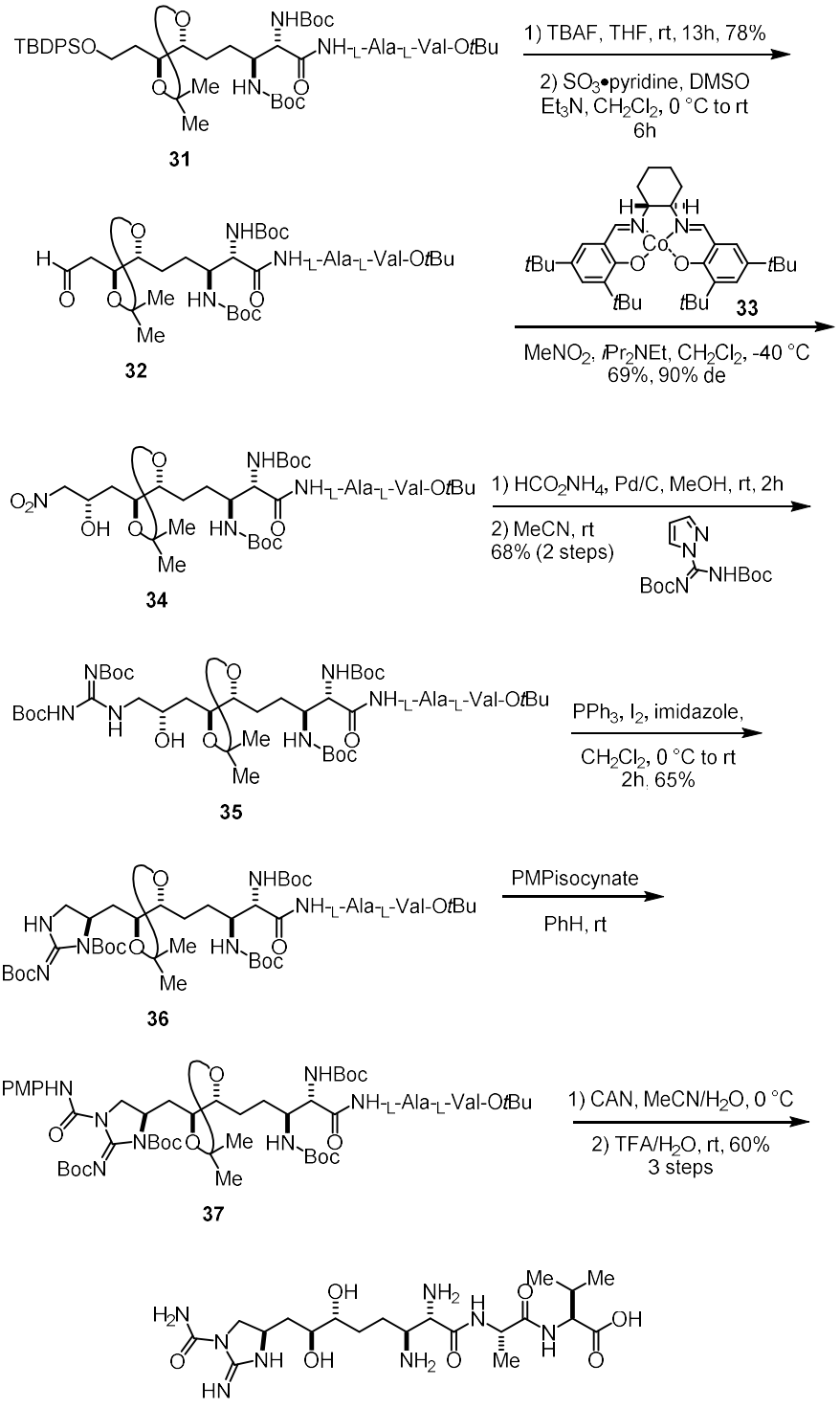
discernable side reactions. The final functionality to be introduced was the carbamoyl group, which was accomplished *via* the addition of the unprotected guanidine nitrogen into a PMP isocyanate to give the carbamoylated cyclic guanidine **37**. Removal of the PMP group with ceric ammonium nitrate followed by treatment with acid yielded guadinomine B (Scheme 1.5).

1.3 Comparison of NA22598A_1 and Guadinomine B

The model system used to evaluate the connectivity of the carbamoyl group on the cyclic guanidine suggested that the carbamoyl group of NA22598A_1 was assigned incorrectly after isolation. While the results of the model system were promising, we wanted to directly compare NA22598A_1 and guadinomine B. Fukazawa generously provided us with a sample of the natural product as well as the producing bacteria.

The sample of NA22598A_1 that we obtained from Fukazawa, however, was not pure by ^{1}H NMR spectroscopy or LCMS analysis. We endeavored to purify the sample with methods described in the original isolation. However, we were limited to an analytical C30 column, the molecule had no fluorophore, it did not ionize well, and we were unable to obtain any pure natural product. In order to obtain the natural product, we attempted to isolate it from the producing bacteria. We scaled down the initial isolation conditions, from 20 L to 6 L, and with the natural product being produced at approximately 1 mg/L, we hoped to obtain approximately 6 mg of NA22598A_1.[1,2]

The initial growth culture was done in a 100 mL seed culture of 2.4% starch, 0.1% glucose, 0.3% peptone, 0.3% meat extract, 0.5% yeast extract, and 0.3% $CaCO_3$ adjusted to pH 7.0. The growth culture was incubated for 3 days at 27 °C. An aliquot of the growth culture (10 mL) was then transferred to 1 L of the production media (6 flasks) containing 2.0% galactose, 2.0% dextrin, 0.5% corn steep liquor, 1.0% Bacto-soytone, 0.2% $(NH_4)_2SO_4$, and



Scheme 1.5: Completion of the total synthesis of guadinomine B

the fermentation carried out for 9 days. The media was then centrifuged and the supernatant transferred to evaporation trays. When the total volume of the supernatant was reduced to 2 L, it was transferred to a previously activated Dowex 50W x2 [H^+] column. After washing with 1 L of H_2O, the active material was eluted with 1 L of 1.5 N NH_4OH. The active fraction was neutralized with HCl and concentrated *in vacuo*.

The concentrated active fraction was then further purified on Dowex 50W x2 activated with 0.05M $MeCOONH_4$ buffer, adjusted to pH 3.7. After washing with 0.05, 0.1, and 0.2M $MeCOONH_4$, the active material was eluted with 0.4 and 0.6 M $MeCOONH_4$. The fractions were neutralized with NH_4OH and lyophilized to dryness. The crude material was then dissolved in a minimal amount of water and purified by HPLC on a Phenomenex Proteo C_{12} column (10 i.d. x 250 mm) over a gradient of 5% $MeCN/H_2O$ to 15% $MeCN/H_2O$ with 0.1% TFA, at 4 mL/min detected at UV 214 nm. The fractions collected from 13 minutes to 20 minutes were analyzed by LCMS and showed traces of the NA22598A_1 mass. However, after the fractions were concentrated, there was no discernible trace of the desired mass. It is unclear if this was due to a lack of natural product or our inability to detect it because of its poor ionization.

Our inability to isolate the natural product from the fermentation broth led us back to the impure natural product provided by Fukazawa. With the help of Dr. Mark Petersen, we explored various HPLC conditions. To that end, the crude natural product was dissolved in H_2O and purified by HPLC on a Phenomenex Proteo C_{12} column (10 i.d. x 250 mm) over a gradient of 5% $MeCN/H_2O$ to 15% $MeCN/H_2O$ with 0.1% TFA at 4 mL/min, monitored at 412 nm. The fractions from 12.5 minutes to 16 minutes were collected and concentrated. LCMS analysis showed a mixture of the desired NA22598A_1 and the decarbamoylated cyclic guanidine **38** (Figure 1.4). The LC trace for both the natural product [M+H] = 503.1 and the

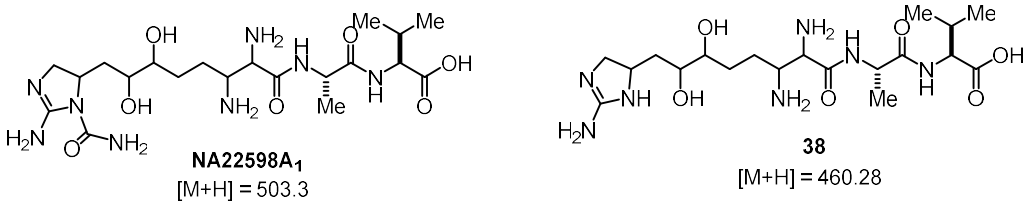


Figure 1.4: The compounds isolated from the crude natural product mixture.

decarbamoylated guanidine [M+H] = 460.3 were identical; therefore, we were unable to determine if the sample contained both compounds or if the natural product was fragmenting in the mass spectrometer.

The next step was to take the ^{1}H and ^{13}C NMR spectra of NA22598A$_1$ in 1% TFA/D_2O and compare the results with the guadinomine B spectra (Figure 1.5).[3] Under these conditions, the proton spectra of NA22598A$_1$ and guadinomine B were almost identical; however, the NA22598A$_1$ spectrum had an additional triplet at 3.3 ppm that was not seen in the guadinomine B spectrum. We collected the NA22598A$_1$ spectra on a 500 MHz instrument, which provided higher resolution than the original spectrum taken on a 300 MHz instrument, (Figure 1.5).[2]

The carbon NMR spectra were less conclusive; the upfield region of the NA22598A$_1$ spectrum was convoluted and we were unable to conclusively match it to the guadinomine B spectrum. The downfield region of spectrum, however, matched guadinomine B with the exception of an extra carbon at 162.5 ppm. If the carbamoyl group of NA22598A$_1$ had a different connectivity than the carbamoyl group of guadinomine B, we would have expected to see more variation in the carbon spectrum as predicted by the model substrates. While the data was not definitive, we felt the similarities were enough to conclude that NA22598A$_1$ and guadinomine B are the same compound.

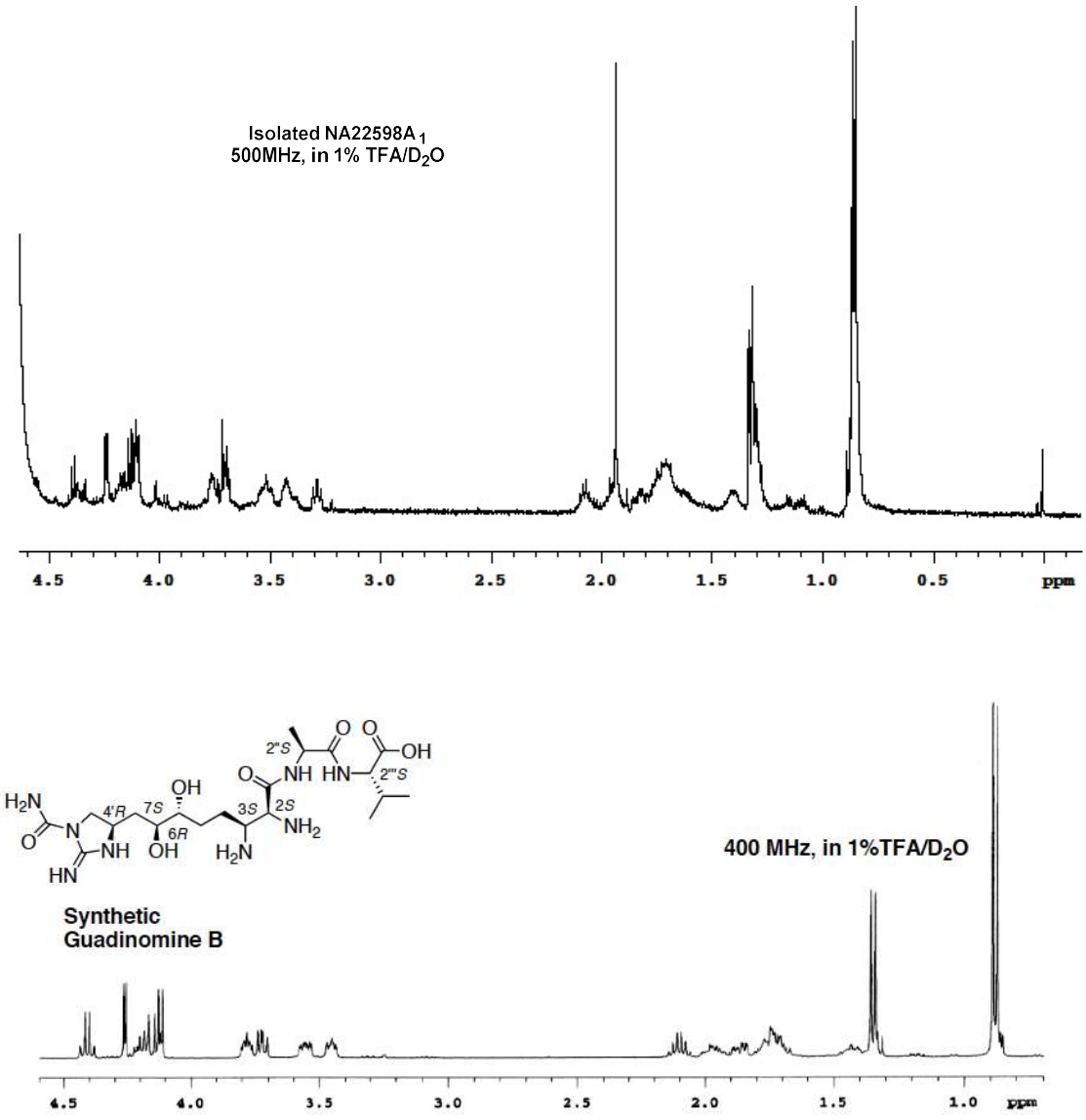


Figure 1.5: The NMR spectra of NA22598A$_1$ and guadinomine B.

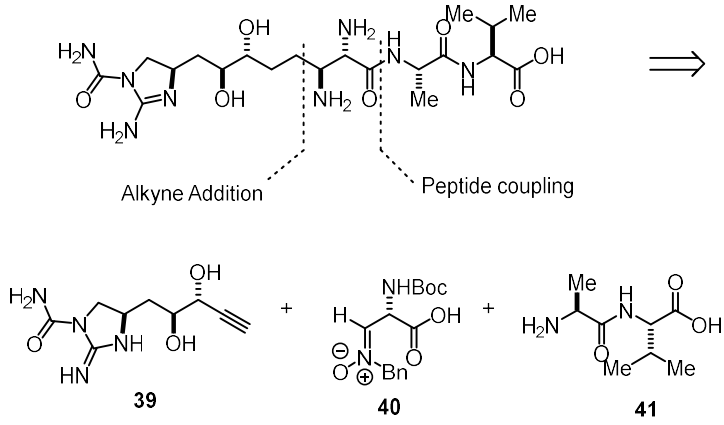
1.4 The First Generation Synthesis of Guadinomine B/NA22598A$_1$

The structures of guadinomine B and NA22586A$_1$ present a number of potential pharmacophores that could be responsible for the observed biological activity. When developing our synthetic strategy, we were mindful of the potential pharmacophores and endeavored to construct the molecule in such a manner that the carbamoylated guanidine and the diamine could be achieved independently and assessed for biological activity. Our initial synthetic strategy utilized the synthesis of three separate pieces, the carbamoylated cyclic guanidine diol, the diamine, and the dipeptide, which could then be coupled at a late stage during the synthesis (Scheme 1.6).

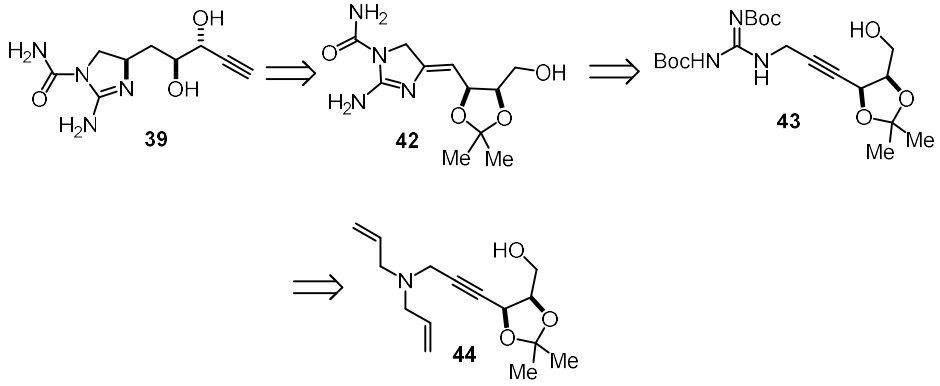
The synthesis of carbamoylated cyclic guanidine **39** was addressed first. We envisioned that the alkyne **39** would be generated from the stereoselective reduction of **42**. The cyclic ene-guanidine **42** would be furnished from the hydroaminiation of propargyl guanidine **43**. Propargyl guanidine **43** would come from the deprotection and guanylation of propargyl amine **44**, which in turn would come from a three-component coupling with an alkyne generated from D-isoascorbic acid (Scheme 1.7).

Due to a lack of literature precedent for a stereoselective reduction of an ene-guanidine, Dr. Vasudev Bhonde developed a simplified model substrate to study this reaction in more depth. The synthesis began with *D*-mannitol acetonide **45**, which was oxidatively cleaved to form glyceraldehyde acetonide **46**.[6] Treatment of the aldehyde with Bestmann-Ohira reagent afforded the alkyne **47**, which was subjected to a CuBr catalyzed three component coupling (3CC) with formaldehyde and diallylamine to give the propargyl amine **48**.[7]

A Pd-catylized di-deallylation was carried out to generate the free amine **49** followed by guanylation with *N'N'*-Di-Boc-*S*-methylisothiourea to produce the propargyl guanidine **50**.



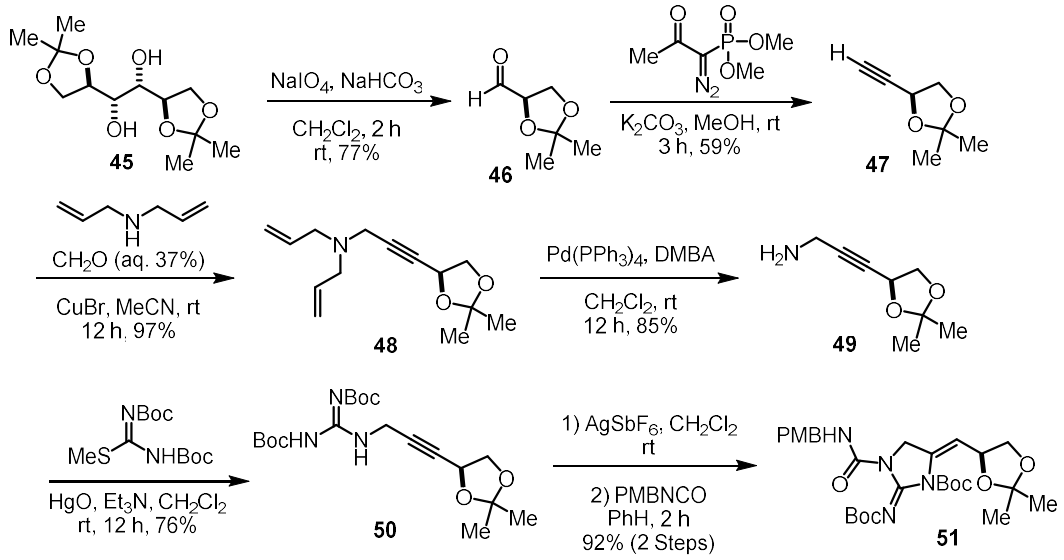
Scheme 1.6: Retrosynthetic analysis of guadinomine B.



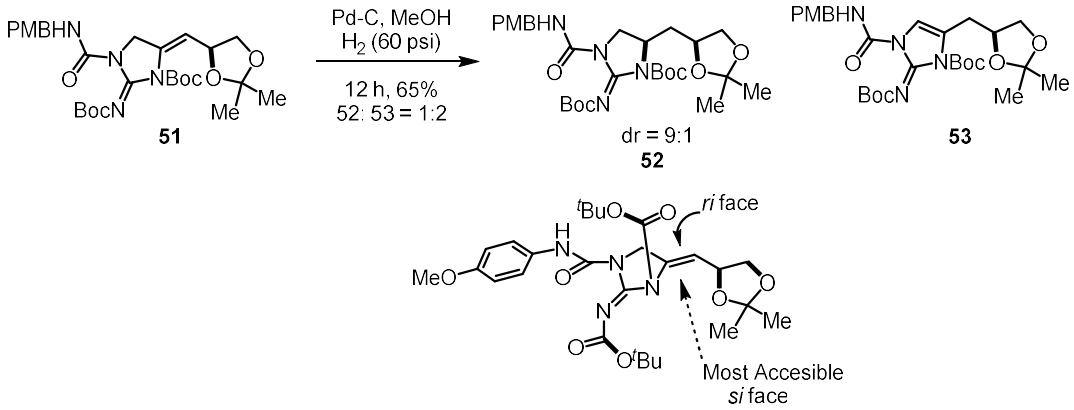
Scheme 1.7: Retrosynthetic analysis of the left-hand portion of guadinomine B.

[8] The hydroamination was completed with catalytic $AgSbF_6$ and the ene-guanidine was treated with PMBNCO to install the carbamoyl group and furnish the model substrate **51** (Scheme 1.8).

With the ene-guanidine in hand, focus shifted to the stereoselective reduction. A heterogeneous hydrogenation using Pd/C in MeOH produced a mixture of reduced product **52** in 9:1 dr and the isomerized 2-aminoimidizole **53** (Scheme 1.9). The absolute stereochemistry of the reduced product could not be verified by spectral analysis, but it was predicted that the *si*-face of the molecule would be blocked by the bulky Boc group favoring addition from the *re*-face.[3]



Scheme 1.8: Synthesis of the ene-guanidine **51**.



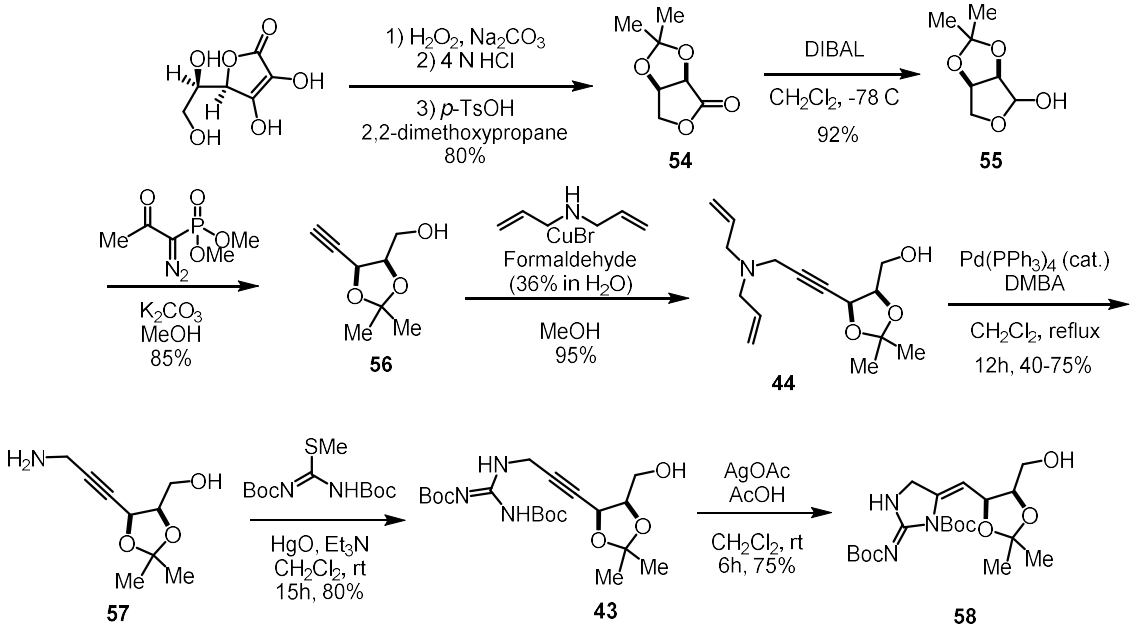
Scheme 1.9: Reduction of ene-guanidine **51**.

While the formation of the reduced guanidine was promising, the reaction needed to be optimized to increase the yield and minimize the formation of 2-aminoimidazole. Catalyst, solvent, and pressure were all analyzed and it was determined that Rh/Alumina in EtOAc at 60 psi of H_2 were the ideal conditions and provided the desired product in 80% yield with a 9:1 dr. The optimized reduction conditions were then applied to a compound that had a

functional handle alpha to the acetonide. D-isoascorbic acid was oxidized and protected to give lactone **54** in an 80% yield over two steps.[9] This precursor set the stereochemistry for what will be the diol in the final product. The lactone **54** was reduced to the lactol **55** and converted to alkyne **56** through a Bestmann-Ohira reaction.[10] A copper catalyzed 3CC with alkyne **56**, diallyl amine, and formaldehyde afforded the protected propargyl amine **44**.[7] Production of the amine **57** was achieved with apalladium catalyzed deprotection of **44**. The free amine **57** was converted to the propargyl guanidine **43** through a mercury (II) oxide guanylation with *N, N*'-Di-Boc-*S*-methylisothiourea. The propargyl guanidine was then regioselectively cyclized to the ene-guanidine **58** using silver acetate (Scheme 1.10).[8]

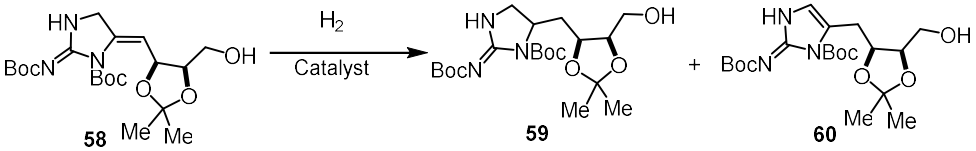
When ene-guanidine **58** was subjected to the optimized conditions developed with the model substrate, no reaction occurred. A number of common hydrogenation catalysts were then scanned, but never succeeded in generating more than trace amounts of the reduced guanidine **59** (Table 1.1). While the addition of the terminal alcohol seemed to stymie the typical hydrogenation catalysts, we hoped it could be used to direct the hydrogenation with Crabtree's catalyst; unfortunately, the starting material persisted under these conditions. Transfer hydrogenation catalysts only produced the 2-aminoimidazole **60**.

The only catalyst that showed potential for optimization was platinum on carbon, which led to the investigation of other platinum catalysts and conditions, shown in Table 1.2. Unfortunately, altering the catalyst and solvent had no effect on the yield. At high pressure, the internal Boc group at N5' migrated to the N2' nitrogen, giving **61**. We hypothesized that the migration ocurred because while on N5', the Boc group is forced out of conjugation with the guanidine due to steric strain; migration to the N2' position relieves the steric strain and allows conjugation. In order to mitigate the Boc group migration, the terminal alcohol was TBS protected furnishing **62** and the carbamoyl group was introduced *via* a PMB isocyanate



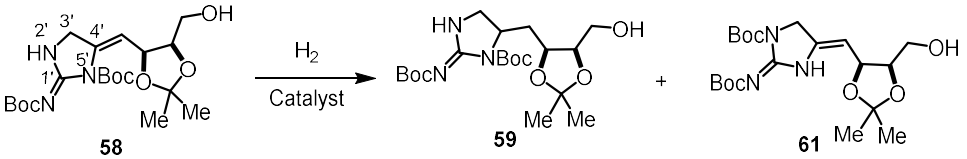
Scheme 1.10: Synthesis of cyclic ene-guanidine **58**.

Table 1.1: Catalyst screen for ene-guanidine reduction.



Conditions	Catalyst	Pressure	Results
EtOAc rt, 2d	Rh/Al	100 psi	NR
CH_2Cl_2/THF 1.5 h, rt	Crabtree	100 psi	NR
EtOAc rt, 2d	Pt/C	100 psi	Trace
EtOAc rt, 2d	$Pd(OH)_2$	100 psi	NR
EtOAc rt, 2d	Pd	100 psi	NR
MeOH rt, 10 h	Pd/C NH_4COOH	NA	Isomerized
THF sealed tube 24h, 70°C	$TsNHNH_2$ Et_3N	NA	Isomerized

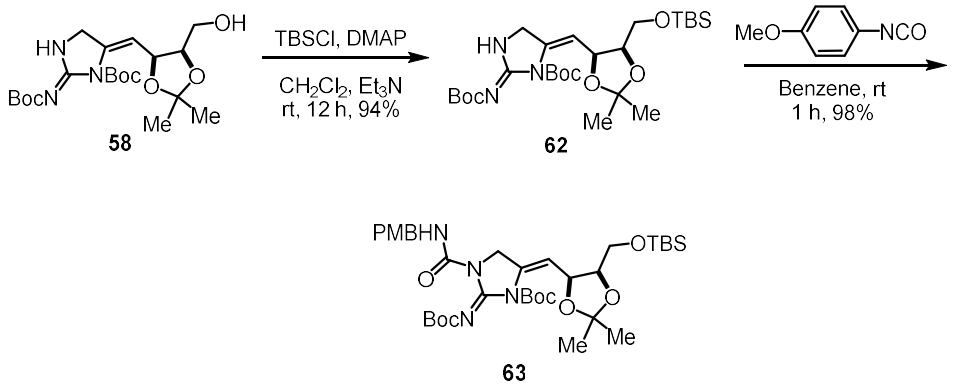
Table 1.2: Exploration of platinum catalysts.



Conditions	Catalyst	Pressure	Results
EtOAc rt, 18h	PtO_2	1 atm	NR
EtOH rt, 18h	PtO_2	100 psi	NR
MeOH rt, 18h	Pt/C	100 psi	Trace
cyclohexane rt, 18h	Pt/C	100 psi	Boc group migration
EtOAc rt, 4d	PtO_2	1200 psi	Boc group migration/ isomerization
EtOAc rt, 4d	Pt/C	1200 psi	Boc group migration/ isomerization
EtOAc rt, 4d	Rh/Al	1200 psi	Trace

forming the carbamoyl guanidine **63** (Scheme 1.11). The introduction of the electron withdrawing carbamoyl group also addressed the potential electronic differences between the model substrate and this system.

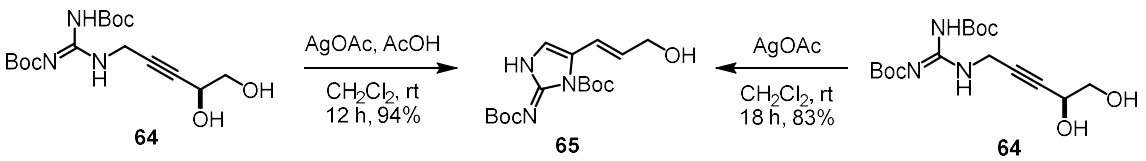
Carbamoyl ene-guanidine **63** was submitted to an exhaustive screen of reduction conditions including PtO_2, Rh/Alumina, and Pt/Carbon, in various solvents and at various pressures. To our dismay, the carbamoyl ene-guanidine **63** was unaffected by all of the hydrogenation conditions evaluated and only starting material was recovered. Having eliminated electronic differences between the model system and the substrate of interest, we concluded that the hydroxymethyl group blocked the *si* face making the alkene inaccessible from either side.



Scheme 1.11: Addition of the carbamoyl group.

In order to address the problem of steric bulk while also providing a functional handle that could direct hydrogenation, selectively removing the acetonide was explored. In the synthesis of amine **49**, it was determined that the acetonide was removed during an acidic work up, generating the diol, which was subsequently converted to the guanidine **64**. Cyclization to afford the ene-guanidine would provide a model system to study the alcohol assisted reduction and its potential stereochemical ramifications. However, when guanidine **64** was treated with AgOAc and AcOH, the ene-guanidine diol was not recovered. Rather, the secondary alcohol was eliminated and vinyl-2-aminoimidazole **65** was formed (Scheme 1.12). Hypothesizing that the acetic acid was involved in catalyzing the elimination of the secondary alcohol, the reaction was performed under neutral conditions, but the result was still the same vinyl-2-aminoimidazole.

While the result of the free diol cyclization was not as expected, the vinyl-2-aminoimidazole provided an interesting substrate. If the 2-aminoimidazole could be reduced, then a new synthetic avenue to reach the reduced guanidine would be available. When the vinyl-2-aminoimidazole **65** was submitted to another extensive screen of catalysts, conditions, and pressures including 1200 psi H_2, the alkene was reduced in every case, but the 2-



Scheme 1.12: Formation of the vinyl-2-aminoimidazole.

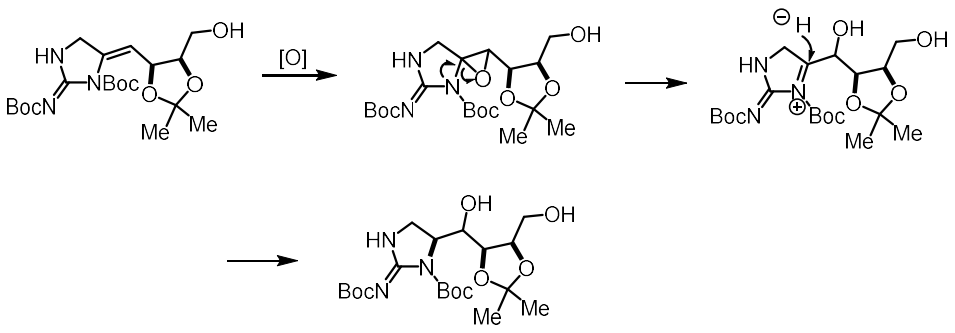
aminoimidazole persisted. The difficulties involved with reducing the ene-guanidine led to a strategy where rather than reducing the double bond, it would be functionalized.

1.5 Functionalization of the Ene-Guanidine

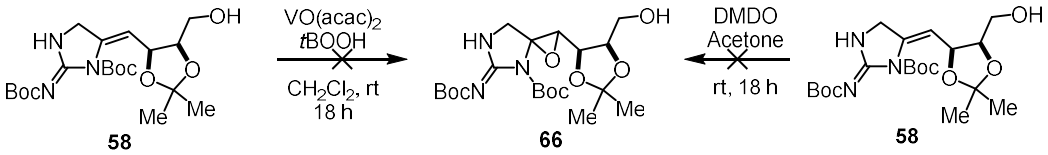
Functionalizing the ene-guanidine also offered an opportunity to access guadinomine A, which still contained an ambiguous stereocenter at the alcohol alpha to the cyclic guanidine. We developed a strategy to epoxidize the double bond with the hope that it could be opened through the formation of an iminium ion which could then be reduced *in situ* (Scheme 1.13).

The initial epoxidation conditions that were explored attempted to use the terminal alcohol to direct the epoxide formation. A vanadyl acetylacetonate catalyst and *t*BOOH was used as a directed electrophilic oxygen source; however, the epoxide **66** was not isolated. Cognizant of the steric congestion, a smaller oxygen source, DMDO, was used, but this smaller electrophile also failed to produce the epoxide **66** (Scheme 1.14). [11]

Failure to epoxidize the ene-guanidine led to the exploration of other functionalization options beginning with a hydrobromination. When the ene-guanidine **58** was treated with NBS in CH_2Cl_2, the cyclic ether **67** was generated. While there is precedence for forming a furan in this fashion, it was a surprise that the hemiaminal was stable enough to be isolated. [12] Hoping that the stability of the hemiaminal would extend to an intermolecular nucleophile,



Scheme 1.13: Mechanism for the epoxide opening.

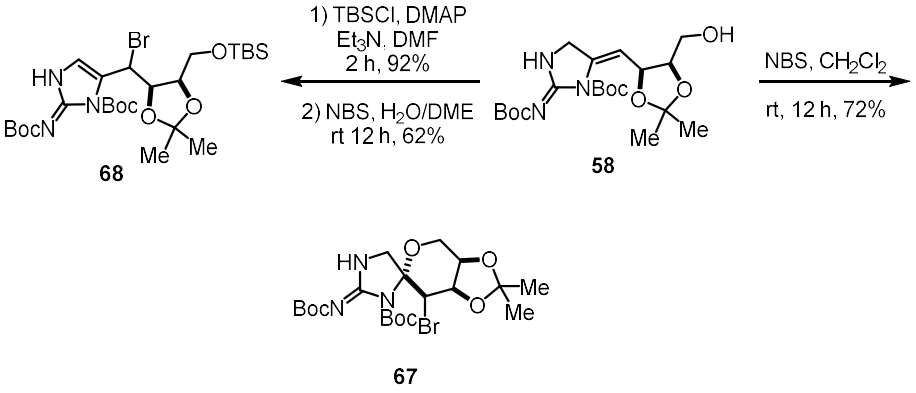


Scheme 1.14: Attempted epoxidation of the ene-guanidine.

the alcohol was TBS protected and the solvent changed to H_2O and DME (1:1). [13] This bromination did not lead to a stable hemiaminal and only produced the 2-aminoimidazole **68** (Scheme 1.15).

1.6 Second Generation Synthesis of the Left-Hand Fragment

The persistence of the ene-guanidine **58** forced us to retool our synthesis of the cyclic guanidine diol fragment. We drew inspiration from the synthesis of saxitoxin, and its use of a stereoselective iodoguanylation. [14] In order to utilize an electrophilic induced ring closure, the propargyl guanidine needed to be converted to the allylic guanidine to achieve the desire oxidation state of the cyclized product. The propargyl guanidine proved to be resistant to typical Lindlar reduction conditions. When the propargyl guanidine **42** was treated with $Pd/CaCO_3$ and 5% Pb, the majority remained unreacted, but a minor product was cyclized



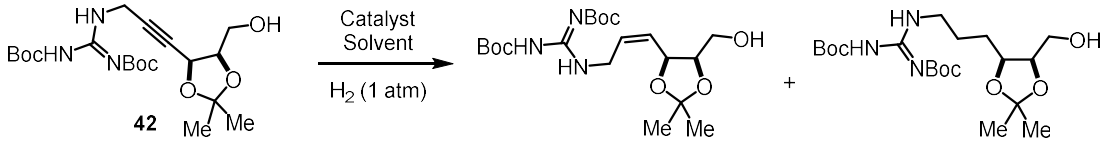
Scheme 1.15: Bromination of the ene-guanidine **58**.

through the terminal alcohol, likely due to the lead. To avoid the cyclic ether formation, the alcohol was TBS protected, and resubjected to Lindlar hydrogenation conditions. Even in more polar aprotic solvent, EtOAc, the alkyne persisted.

The unreacted propargylamine under typical Lindlar conditions led to the use of a stronger hydrogenation catalyst, Pd/C. Initially, an aprotic solvent was used because of the increased strength of the catalyst; when that failed to produce any change in the substrate, polar protic solvents were explored. Initial experiments with polar protic solvents produced either no change or complete reduction to the alkane. Adjustments to the solvent and additives did not produce the alkene, which led to a change in the catalyst. $Pd(OH)_2/C$ was scanned in a number of solvents from polar aprotic to polar protic; while it is a stronger reducing agent than Pd/C, it produced the same results (Table 1.3).

Failing to reduce the propargyl guanidine, it became necessary to introduce the alkene at an earlier stage. There was precedent for the reduction of a free propargyl amine to the allyl amine; therefore, the propargyl amine **57** was treated with $Pd/BaSO_4$ and ethylene diamine in DMF under a hydrogen atmosphere; to our delight, this furnished the allyl amine **70** (Scheme 1.16).[15] Initially, this appeared to be a low yielding reaction, with less than 50% product

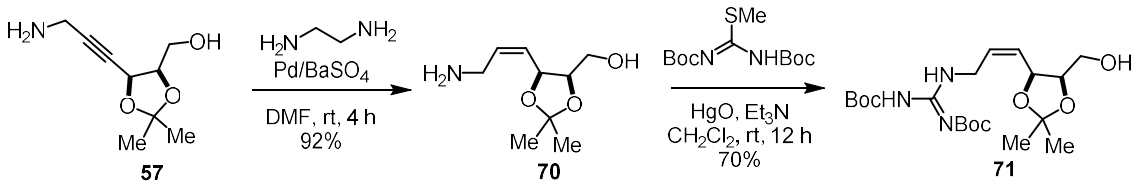
Table 1.3: Attempted reduction of the propargyl guanidine.



Conditions	Reagents	Results
PhMe	Lindlar ($Pb/CaCO_3$)	cyclized through alcohol/ NR
iPrOH	Pd/C	NR
EtOH	Pd/C	NR
MeOH	Pd/C	Alkane
MeOH	Pd/C Quinoline (1eq)	NR
EtOAc	$Pd(OH)_2$	NR
iPrOH	$Pd(OH)_2$	NR
EtOH	$Pd(OH)_2$	Alkane

recovered; however, the product was simply lost in the work up and when the water wash was eliminated, the yields were greatly improved. The guanylation of the allyl amine **70** was accomplished smoothly and provided the allylic guanidine **71** for an electrophilic cyclization. While both the free alcohol and the guanidine had the potential to be nucleophiles, previous work with the propargyl guanidine suggested that the alcohol would not be a competitive nucleophile. The first electrophilic cyclization utilized NBS to generate the bromonium ion, and the product isolated appeared to be the cyclic guanidine as a single diastereomer.

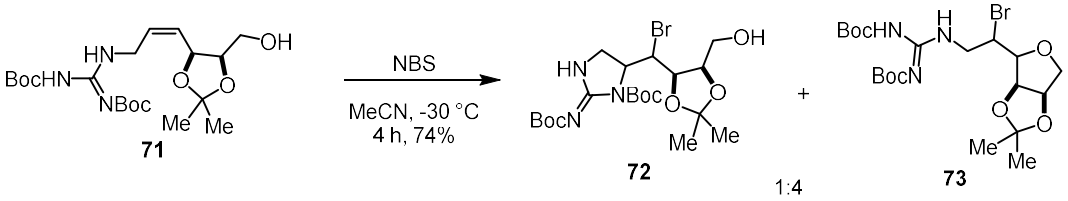
A minor product was detected when the reaction was run at room temperature; however, the negligible quantity prevented isolation. In an attempt to minimize the formation of the minor product, the reaction was cooled to –30 °C; however, the reduced temperature decreased the selectivity to 4:1 and allowed for the isolation of the minor product. Upon analysis of both products, it was determined that the major product was the cyclic ether **73**



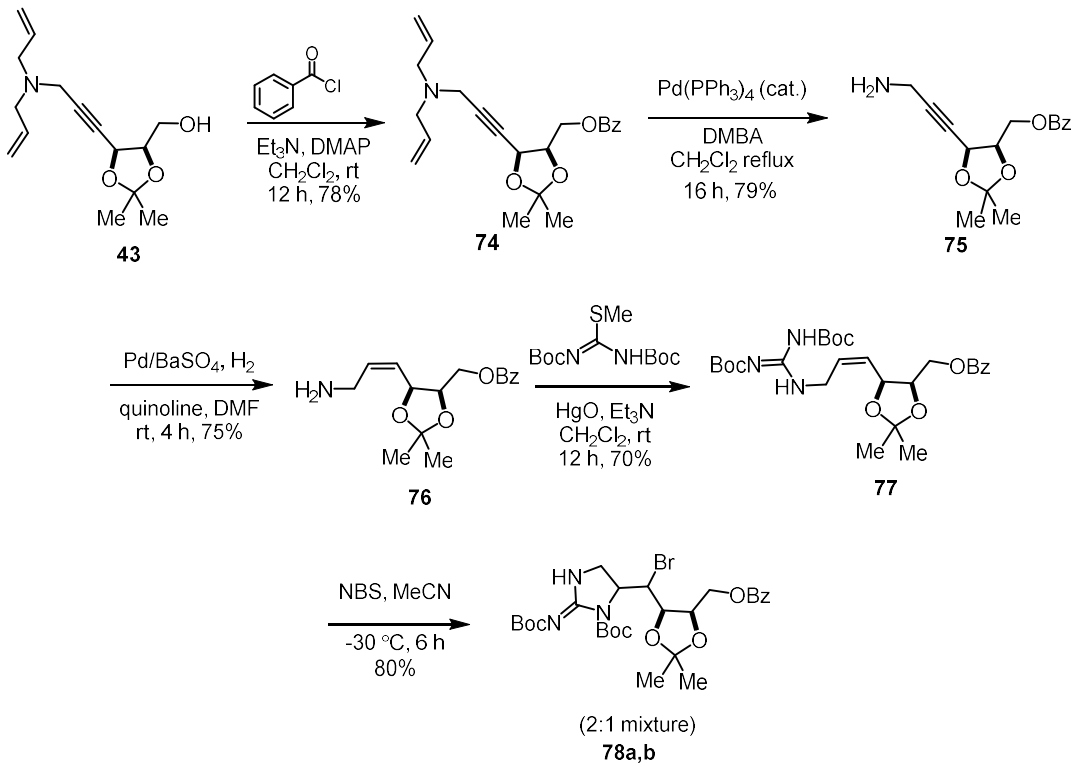
Scheme 1.16: Reduction of the propargyl amine **57**.

rather than the cyclic guanidine **72** (Scheme 1.17). The formation of the cyclic ether prompted the protection of the terminal alcohol; an alcohol protecting group was selected so that it could be selectively cleaved in the presence of the Boc groups. A base labile benzoyl group was introduced after the 3-CC in an effort to increase the yields of subsequent reactions by decreasing the polarity of the products, which allowed for easier purification.

The protected amine **43** was converted to the benzoate **74**, and the allyl groups were subsequently removed to form the propargyl amine **75**. Reduction of the alkyne presented a challenge; previously, ethylenediamine had been used to poison the catalyst, but its basicity had the potential to cleave the benzoyl group. To mitigate the problem, the less nucleophilic amine, quinoline, was used as an additive. This change produced the alkene **76** in good yield without cleaving the benzoyl group. The guanylation proceeded smoothly to give allylic guanidine **77**, which was treated with NBS in MeCN at -30 °C to produce a 2:1 mixture of diastereomers **78a,b** (Scheme 1.18).



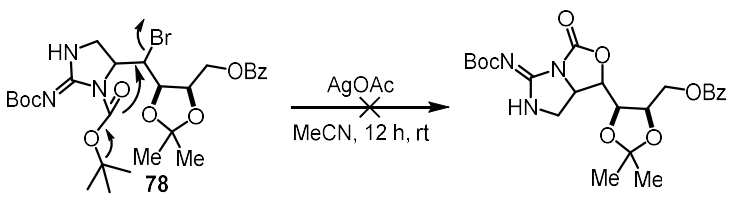
Scheme 1.17: Cyclization of the allylic guanidine.



Scheme 1.18: Protection of the terminal alcohol an NBS cyclization.

The 2:1 mixture of the cyclic guanidine was separable, but because neither of the compounds were crystalline, determination of the absolute stereochemistry of either compound was not determined. However, the functionalization alpha to the cyclic guanidine offered a unique opportunity to access guadinomine A. The adjacent Boc group could be cyclized onto the bromide to form the cyclic carbamate, which could then be opened to reveal the alpha alcohol.[14] The cyclic guanidine **78** was treated with AgOAc in MeCN with the intention of cyclizing the boc group onto the bromine forming the cyclic carbamate. Unfortunately, the cyclization was unsuccessful and the bromine persisted (Scheme 1.19).

The inability to form the cyclic carbamate led to an exploration of more traditional dehalogenation methods. Initial efforts focused on Bu_3SnH reductions, assuming that such a well-studied reaction would produce the dehalogenated compound. Bromoguanidine **78** was

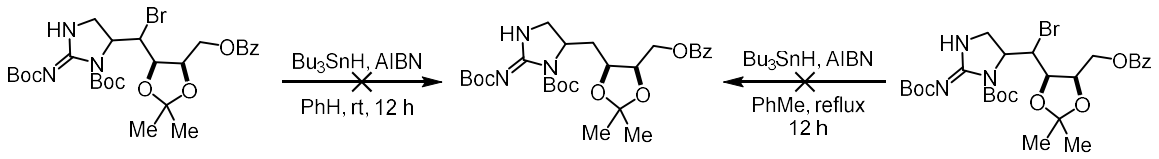


Scheme 1.19: Attempted cyclization of *t*butyl carbamoyl group on **78**.

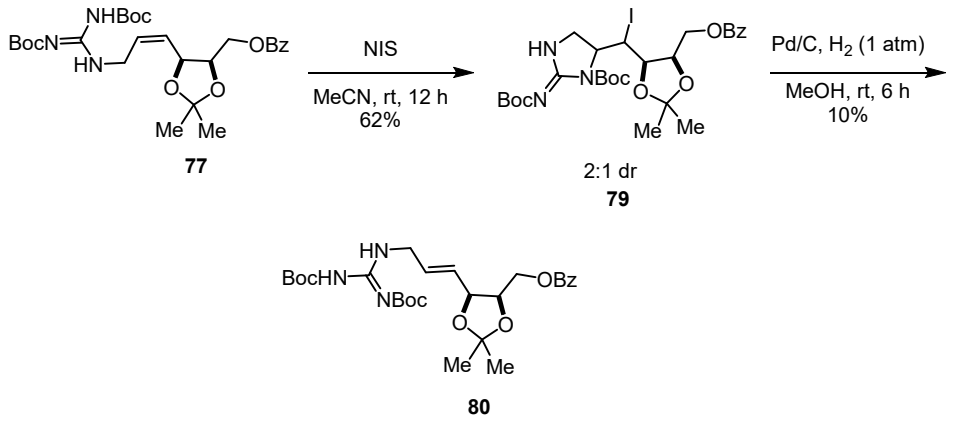
subjected to Bu_3SnH and AIBN in benzene at room temperature for 12 hours; after work up, no product was formed and the bromide remained.[16] No formation of product led to a change in solvent, from benzene to toluene, and the temperature was increased to 110 °C. Even at reflux temperatures in toluene, no dehalogenated product was recovered (Scheme 1.20).

The failure of Bu_3SnH to have any effect on the bromoguanidine suggested that steric incompatibility was playing a role in hindering the reaction. Unable to remove the bromide, iodine was utilized as electrophile. The initial iodine source NIS furnished the cyclic guanidine in moderate yield and a 2:1 mixture of diastereomers. The carbon iodide bond offered a number of new possibilities to dehalogenate the molecule.

The first dehalogenation reaction applied to the iodoguanidine **79** was a palladium catalyzed hydrogenation. We were disappointed to see that starting material remained largely unreduced under these conditions. There was a minor product that initially resembled a reversion to the *Z* alkene, but upon closer analysis, it proved to be the *E* alkene **80** (Scheme 1.21). While the NIS cyclization did provide the cyclic guanidine, it was in moderate yield and



Scheme 1.20: Tributyl tin hydride reduction of the bromoguanidine.

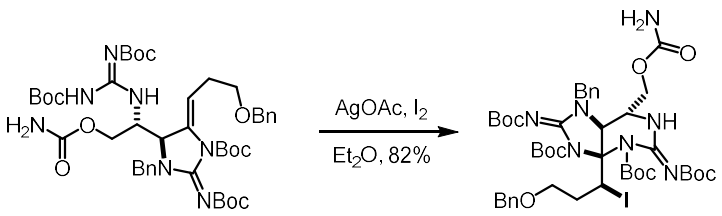


Scheme 1.21: Iodocyclization and hydrogenation.

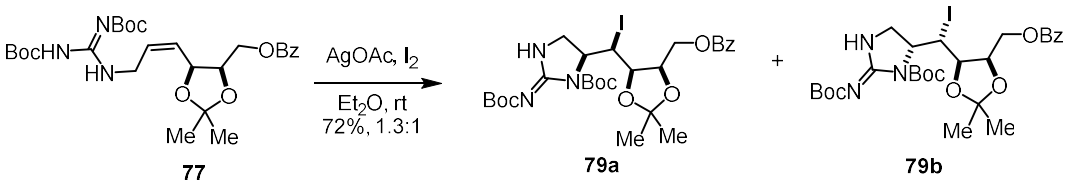
unreliable reproducibility. Exploring other sources of electrophilic iodine led back to the synthesis of saxitoxin, which utilized AgOAc to generate a more electrophilic iodine source. Additionally, the iodocyclization in saxitoxin produced a single diastereomer in 82% yield (Scheme 1.22). The rigid nature of saxitoxin contributed to the single diastereomer formation; however, the high yield suggested a reversible iodonium ion formation that we hoped to be able to capitalize on.[14]

Allylic guanidine **77** was treated with AgOAc and I_2 in Et_2O at room temperature and stirred for 3 hours. We were delighted to see the yield increased from 62% to 89%, but the diastereoselectivity was poor, furnishing a 1.3:1 mixture (Scheme 1.23). The selectivity of this cyclization will be more completely addressed in Chapter 2. Settling on conditions that provided the iodoguanidine in good yield, I focused on optimizing conditions to dehalogenate the molecule.

The synthesis of saxitoxin also utilized a Boc group cyclization onto an iodide to form a cyclic carbamate; while the bromoguanidine was unresponsive to such a cyclization, the iodoguanidine had more literature precedent. Initially, iodoguanidine **79a** was dissolved in MeCN and AgOAc was added; after 12 hours, there was no change to the starting material.



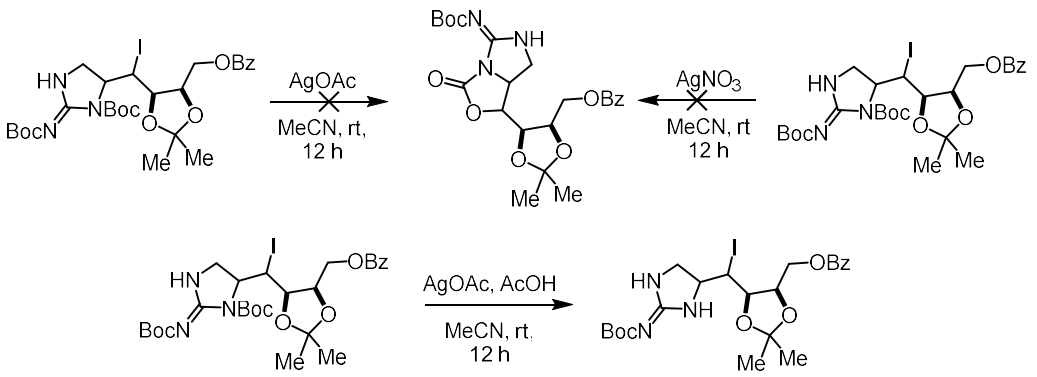
Scheme 1.22: Iodocyclization utilized in the synthesis of saxitoxin.



Scheme 1.23: AgOAc, I_2 cyclization of the allylic guanidine.

To determine if the solubility of the AgOAc was limiting the cyclization, the silver salt was changed to $AgNO_3$. This change had no effect on either diasteromer and no cyclic carbamate was observed. Increasing the temperature from room temperature to 60 °C did not facilitate the cyclization with either AgOAc or $AgNO_3$. In the synthesis of saxitoxin, the addition of AcOH helped to facilitate the cyclization of the cyclic carbamate, which I hoped would translate to the cyclization of iodoguanidine **79a/b**. Monitoring the reaction by TLC, a loss of starting material was observed; however, upon NMR analysis, it was clear the internal Boc group was simply cleaved rather than cyclized (Scheme 1.24).

The inability to form the cyclic carbamate led back to the optimization of intermolecular dehalogenation reactions. The alpha elimination seen in the palladium catalyzed hydrogenation prompted an exploration of radical dehalogenation reactions. Cognizant of the steric constraints, I looked for a smaller radical dehalogenation reagent. The Wood laboratory used tributyl borane rather than tributyl tin to cleave carbon iodine bonds, which seemed far better suited for this substrate. [17]

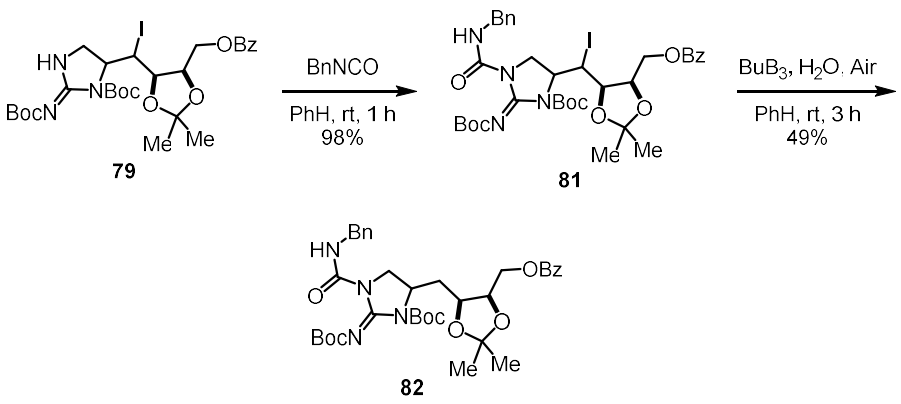


Scheme 1.24: Attempted formation of the cyclic carbamate.

The attempted carbamate formation with AcOH and AgOAc created concerns about the stability of the internal Boc group, so prior to the dehalogenation, the carbamoyl group was introduced. Iodoguanidine **79** was dissolved in benzene, and benzyl isocyanate was added; the reaction was then stirred for 1 hour, providing the carbamoyl guanidine **81** in 98% yield. The carbamoyl guanidine **81** was dissolved in benzene, and water was added to the reaction mixture followed by tributyl borane; air was then bubbled into the solution at a rate of 1 mL/h to produce the dehalogenated **82** (Scheme 1.25). The dehalogenated cyclic guanidine **82** proceeded with minimal formation of side products. However, the low yield prompted an optimization of this reaction. I found that if the reaction was simply open to air and the solvent was not degassed, the yield increased considerably to 61%. Additionally, adjusting the work up to use a weaker base minimized the loss of the Boc groups.

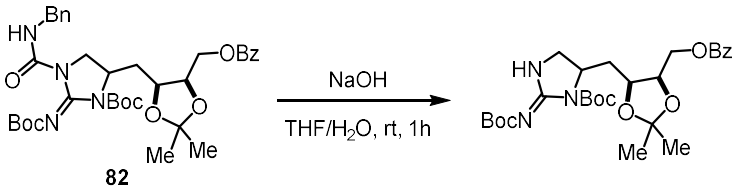
1.7 Deprotection of the Left-Hand Fragment

One of the highlights of the proposed route was the separation of potential pharmacophores so that each could be analyzed individually. To that end, a global deprotection of the left-hand fragments was undertaken. The first target was the benzoyl

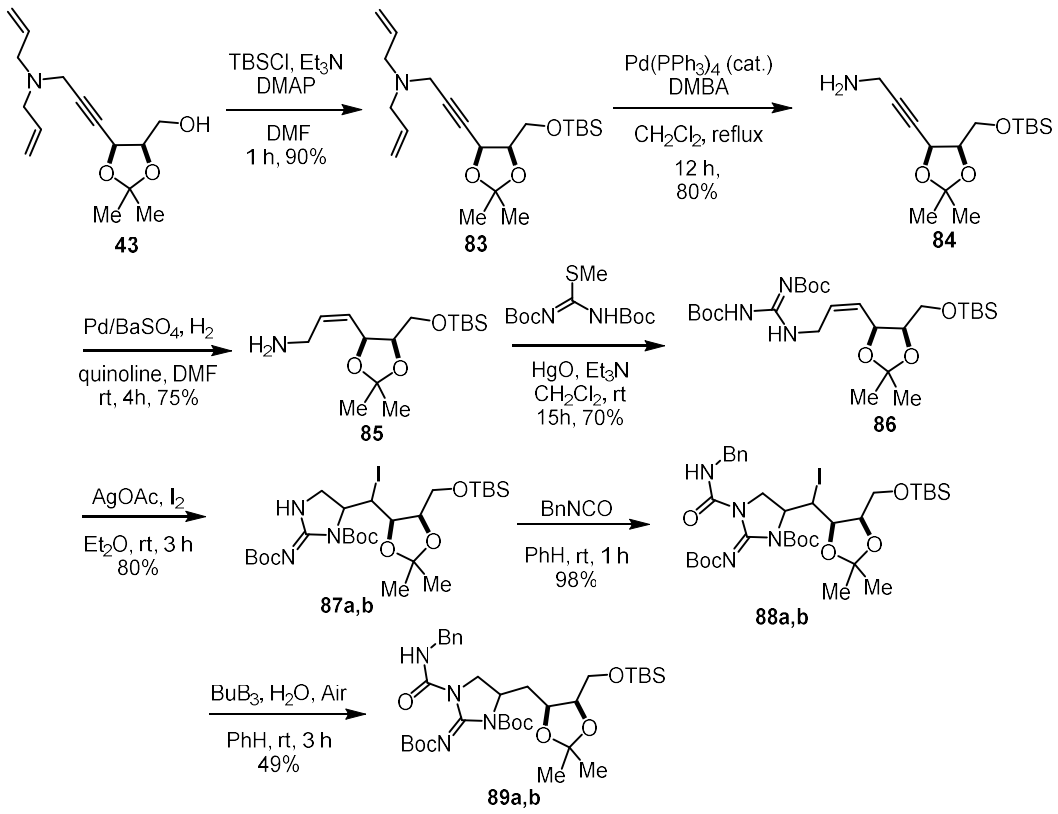


Scheme 1.25: Dehalogenation of the carbamoylated guanidine.

group, specifically because it would have to be selectively deprotected to further elongate the molecule. The cyclic guanidine **82** was treated with NaOH in THF and H_2O at room temperature and stirred for 1 hour. Upon work up, it was clear that the benzoyl group persisted while the carbamoyl group had been cleaved (Scheme 1.26). The selective cleavage of the carbamoyl group was concerning and led to a shift in the protecting group strategy. Keeping in mind that the terminal alcohol still needed to be selectively deprotected, a TBS protection was employed. The reaction sequence that produced the benzyl protected cyclic guanidine was repeated to produce the TBS protected cyclic guanidines **89a,b** (Scheme 1.27). The TBS protected guanidine was treated with TBAF in THF, which removed the Boc protecting groups rather than the TBS group, generating **90a,b**. The loss of the Boc groups was unexpected but did not hinder the global deprotection of the left-hand fragment for

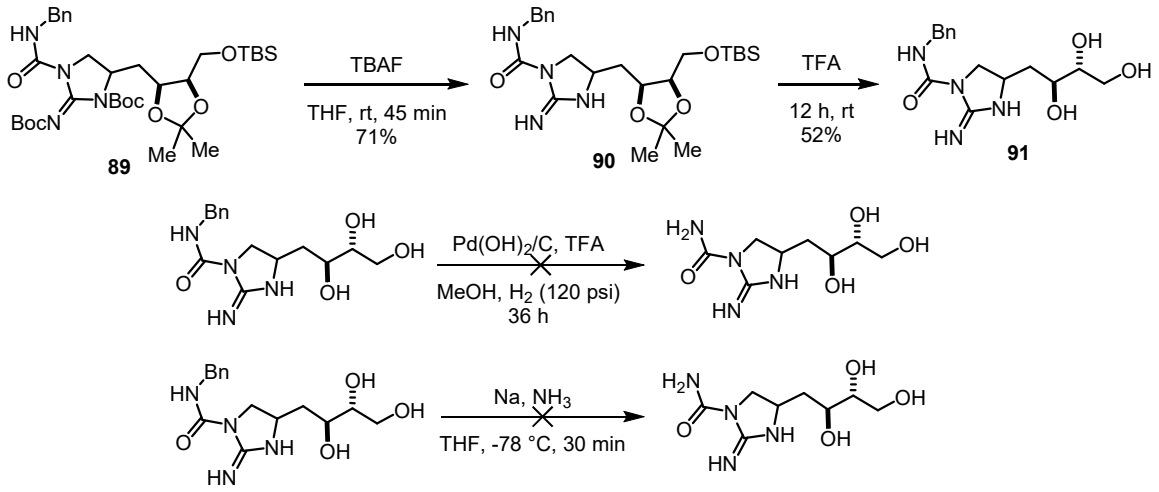


Scheme 1.26: Cleavage of the carbamoyl group.



Scheme 1.27: Synthesis of the TBS protected cyclic guanidine **89a,b**.

biological evaluation. The carbamoyl guanidine **90a,b** was dissolved in TFA and stirred overnight, which successfully removed the TBS group and the ketal, to provide the triol **91a,b**. The removal of the benzyl group appeared to be straightforward, and the triol **91a** was submitted to hydrogenation conditions with a $Pd(OH)_2$ catalyst. The hydrogenation was unsuccessful and the pressure of H_2 was increased to 60 psi, and when that produced no reaction, the pressure was increased to 120 psi (Scheme 1.28). When the hydrogenation failed to remove the benzyl group, a radical debenzylation method was implimented. Ammonia was condensed and sodium was added, followed by the triol **91a** in THF; the reaction was stirred for 30 minutes (Scheme 1.28). This method also failed to remove the benzyl group.



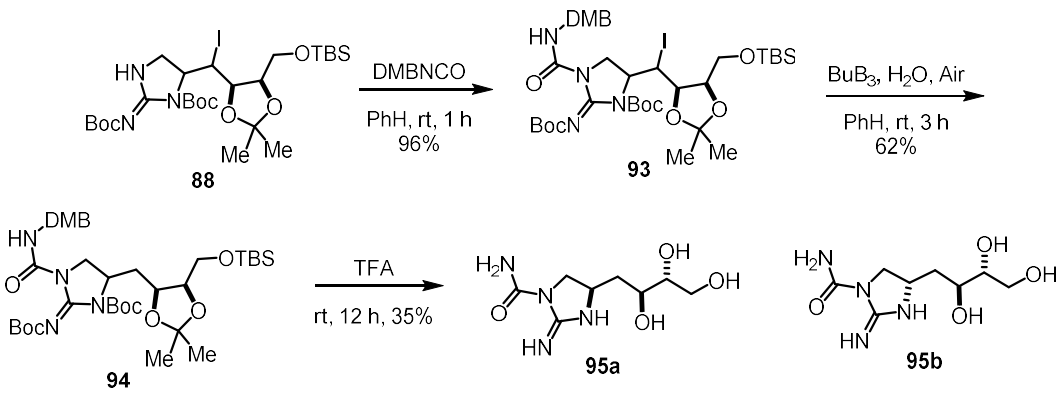
Scheme 1.28: Attempted total deprotection of cyclic guanidine **90**.

The inability to remove the benzyl group forced a shift in the protecting group on the carbamoyl guanidine. In order to change the protecting group, a new isocyanate was synthesized. 2,4-Dimethoxybenzyl amine was dissolved in CH_2Cl_2 and a saturated $NaHCO_3$ solution, then cooled to 0 °C; triphosgene was then added and the reaction stirred for 30 minutes, which furnished the 2,4-dimethoxyisocyanate **92** (DMBCNO) in 93% yield. The cyclic guanidine **88** was then converted to the carbamoyl guanidine **93**, which was dehalogenated to provide the fully protected guanidine **94**. When the fully protected guanidine **94** was submitted to TFA, the fully deprotected triols **95a** and **95b** were obtained (Scheme 1.29). The deprotected fragments not only provided the potential pharmacophore for biological testing, they also allowed us to confirm the stereochemistry of the cyclic guanidine.

The biological results will be discussed in Chapter 4.

1.8 Efforts Toward the Further Functionalization of the Left-Hand Fragment

Having a robust synthesis to create the left-hand fragment, I turned my attention to elongating the molecule. The challenge this presented was finding a way to create the two

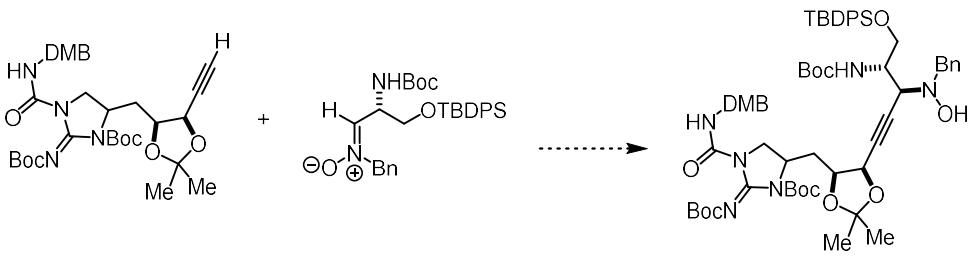


Scheme 1.29: Total deprotection of the left-hand fragment.

unfunctionalized carbons between the diol and the diamine. The initial synthetic plan was to use the diastereoselective addition of an alkyne into a nitrone developed by Merino (Scheme 1.30).[18] In order to apply that to our synthesis, we needed to convert the terminal alcohol to an alkyne.

The first hurdle we encountered was the selective deprotection of the terminal alcohol. The attempts to remove the TBS group inevitably led to the removal of the boc protecting groups as well. This led to yet another change in the alcohol protecting group, to the TBDPS silyl ether. The same synthetic route was used to reach the TBDPS cyclic guanidine **102** as was used to generate both the benzoyl and TBS derivatives (Scheme 1.31).

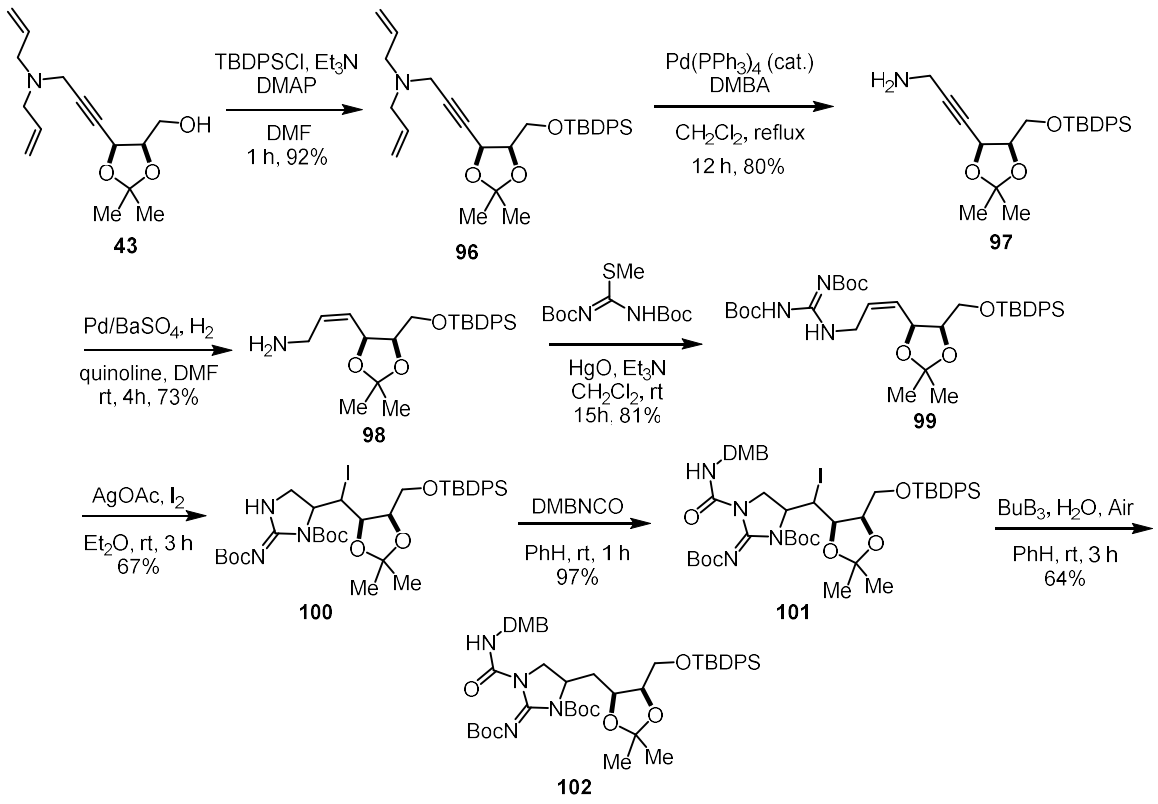
The TBDPS group was removed with a mixture of TBAF and AcOH in THF; the reaction was stirred for 18 hours and produced the free alcohol **103** in 91% yield. The alcohol was then converted to the aldehyde **104** using Dess-Martin periodinate (Scheme 1.32). This reaction proceeded smoothly; however, residual Dess-Martin reagent remained after the reaction was filtered. I chose not to purify aldehyde **104** with column chromatography because of the potential epimerization of the stereocenter alpha to the aldehyde; also the excess Dess-Martin did not pose a problem in the subsequent transformation to the alkyne.



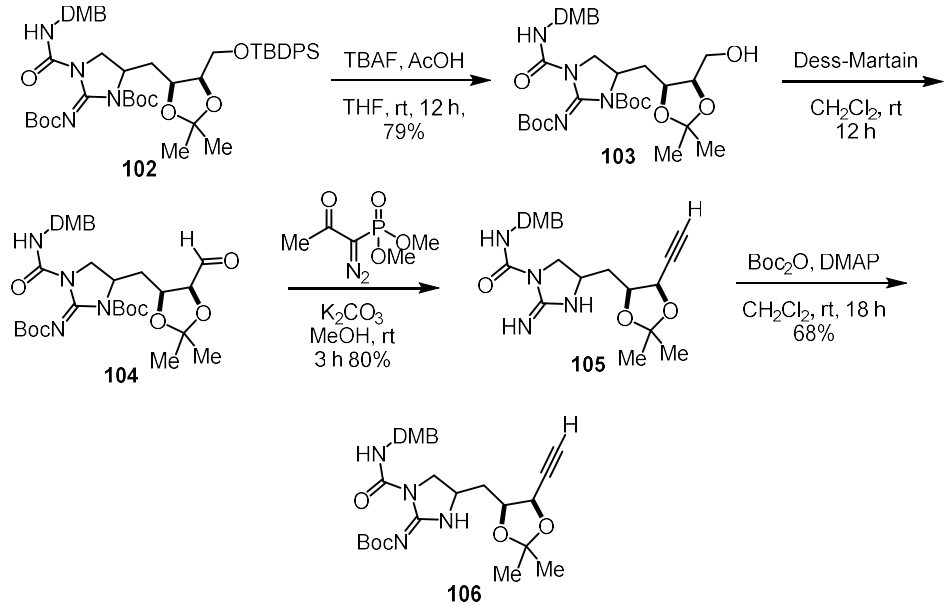
Scheme 1.30: The desired coupling of the left-hand fragment and the diamine.

The aldehyde **104** was dissolved in MeOH and Bestmann-Ohira reagent was added to the reaction followed by K_2CO_3 added in portions. This furnished the alkyne **105** in moderate yield but it also cleaved both Boc groups. The unprotected guanidine posed a problem because the addition into the nitrone required the alkynyl anion. The lower pKa of the guanidine protons, as well as the carbamoyl NH, suggested that it would be difficult to generate the alkynyl anion species. Attempting to reprotect the guanidine, the alkyne **105** was dissolved in CH_2Cl_2 and Boc_2O was added followed by DMAP and the reaction was stirred 18 hours. The exocyclic nitrogen was Boc protected but the internal nitrogen remained unprotected **106**; this was attributed to the steric constraints at that position (Scheme 1.32). The alkynyl addition into the nitrone will be discussed in Chapter 3.

Despite the challenges encountered in the synthesis of the carbamoylated cyclic guanidine diol fragment **106**, it was completed in 11 steps with a highly reproducible route. The dense functionality and the need for orthogonal protecting groups added to the complexity of creating this fragment. This route also provided the opportunity to create the carbamoyl guanidine triol fragment that was used to explore biological activity.



Scheme 1.31: Synthesis of the TBDPS protected derivative.



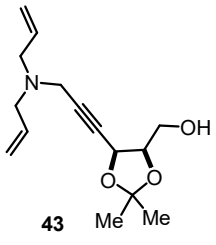
Scheme 1.32: Formation of the alkyne **106**

1.9 Supporting Information

Unless otherwise noted, all starting materials were either known compounds or were obtained from commercial sources and used without purification. All reactions requiring anhydrous conditions were performed under a positive pressure of argon using flame-dried glassware. Dichloromethane (CH_2Cl_2), and triethylamine (Et_3N), were distilled from CaH_2 immediately prior to use. Tetrahydrofuran (THF), diethylether (Et_2O), toluene (PhMe), and dimethylformamide (DMF) were degassed with argon and passed through a solvent purification system (J.C. Meyer of Glass Contour) containing either alumina or molecular sieves. Flash chromatography was performed on Merk silica gel Kieselgel 60 (230-400 mesh) from EM science with the indicated solvent. ^{1}HNMR spectra were recorded on a Varian VXR-500 MHz spectrometer. The chemical shifts (d) of proton resonances are reported relative to $CHCl_3$, DMSO-d_5, HOD, or HD_2COD using the following format: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app = apparent), coupling constant(s) (*J* in Hz), integral].[19,20] ^{13}CNMR spectra were recorded at 75, 100, or 125 MHz. The chemical shifts of carbon resonances are reported relative to the deuterated solvent peak.

Infrared spectra were recorded on a Nicolet 380-FT IR spectrometer fitted with a SmartOrbit sample system. All absorptions are reported in cm^{-1} relative to polystyrene (1601 cm^{-1}).

((4S,5R)-5-(3-(diallylamino)prop-1-yn-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (43)

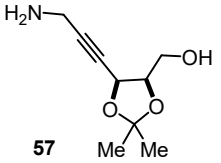


43

To a solution of alkyne **56** (3.00 g, 19.22 mmol) in methanol was added diallylamine (2.37 mL, 19.22 mmol), formaldehyde (1.51 mL, 19.22 mmol), and CuBr (275 mg, 1.92 mmol). The reaction was stirred for 16

hours at room temperature. The mixture was filtered through a pad of Celite and concentrated under reduced pressure and purified by flash chromatography, (1:1 Hex/EtOAc) to yield **10**, as a pale yellow oil. R_f = 0.71 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +9.1 (c= 1.13, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 5.80 (ddt, J = 6.5 Hz, 10.5 Hz, 17.0 Hz, 2H) 5.20 (dd, J = 1.5 Hz, 17 Hz, 2 H) 5.14 (d, J = 10.5 Hz, 2H) 4.59 (dt, J = 1.5 Hz, 8 Hz, 1H) 4.13-4.10 (m, 1H) 3.86 (dd, J = 3 Hz, 12.5 Hz, 1H) 3.65 (dd, J = 4.5 Hz, 12.5 Hz, 2H) 3.40 (d, J = 2 Hz, 2H) 3.09 (d, J = 7 Hz, 4H) 1.48 (s, 3H) 1.41 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 135.15, 118.42, 110.43, 82.38, 82.08, 81.68, 66.75, 60.95, 56.53, 41.71, 26.91, 26.33 ppm. IR (neat): 3409, 2931, 1723, 1259, 1058, 923, 714 cm^{-1}.

((4S,5R)-5-(3-aminoprop-1-yn-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (11)

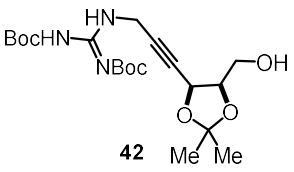


57

To a solution of alkyne **43** (2.96 g, 11.15 mmol) in CH_2Cl_2 was added $Pd(PPh_3)_4$ (644 mg, 0.587 mmol), and 1,3-dimethyl barbutiric acid (10.57 g, 66.89 mmol). The solution was heated to reflux for 12 hours then concentrated under reduced pressure and redissolved in Et_2O; the organic layer was washed with H_2O (4 x 100 ml). The aqueous layer was basified with saturated $NaHCO_3$ and NaCl (500 mg) was added. The aqueous layer was concentrated under reduced pressure to give an off white solid. $CHCl_3$ (300 ml) and Na_2SO_4 were added to the flask and the mixture stirred for 4 hours. The mixture was then filtered with suction and the filtrate was concentrated under reduced pressure to give **57** (1.96 g, 95%) as an orange oil, which was used without further purification. R_f = 0.11 (10:1 CH_2Cl_2/MeOH), $[\alpha]^{20}_D$ = +12.9 (c= 0.8, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 4.59 (d, J = 8 Hz, 1H), 4.10-4.08 (m, 1H), 3.87 (dd, J = 3.5, 12 Hz, 1H), 3.66 (dd, J = 4, 12 Hz, 1H) 3.47 (s, 2H) 1.49 (s, 3H) 1.42 (s, 3H) ppm, ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 110.37, 87.96, 82.03, 78.74, 66.72, 60.77, 31.76, 26.94, 26.49 ppm. IR (neat): 3295,

2930, 2360, 2339, 1669, 1653, 1583, 1055 cm^{-1}.

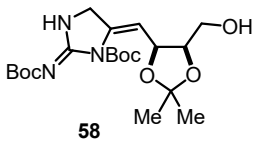
Propargyl guanidine (42)



42

To a solution of **57** (437 mg, 2.36 mmol) in CH_2Cl_2 (25 ml) at room temperature was added N,N'-Di-Boc-S-methylisothiourea (685 mg, 2.36 mmol), Et_3N (0.329 mL, 2.36 mmol), and HgO (511.4 mg, 2.36 mmol). The reaction was stirred 12 hours then filtered through a pad of celite. The pad of celite was washed with an additional 15 mL of CH_2Cl_2 and the combined organics were concentrated under reduced pressure. Purification by flash chromatography (4:1 Hex/EtOAc) gave **42** (725 mg, 72%) as a colorless foam . R_f = 0.62 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +15.72 (c= 1.4, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 11.39 (s, 1H), 8.41 (s, 1H), 4.56 (d, J = 7.5 Hz, 1H), 4.24 (d, J = 5.5 Hz, 2H) 4.11-4.05 (m, 2H) 3.83 (dd, J = 3, 12 Hz, 1H), 3.63 (d, J = 11.5 Hz, 1H), 1.46-1.45 (m, 21 H), 1.38 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 163.26, 155.68, 153.03, 110.52, 83.47, 80.08, 81.87, 80.50, 79.64, 66.52, 70.77, 60.45, 30.95, 28.29, 28.08, 28.03, 26.83, 26.27 ppm.

(5E)-tert-butyl 2-((tert-butoxycarbonyl)imino)-5-(((4R,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methylene)imidazolidine-1-carboxylate (58)

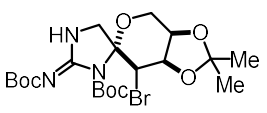


58

To a solution of **42** (1.18 g, 2.5 mmol) in CH_2Cl_2 (50 mL) at room temperature was added AgOAc (84 mg, 0.5 mmol) and AcOH (0.429 ml, 7.5 mmol). The reaction was stirred in the dark for 4 hours. The mixture was concentrated under reduced pressure. Purification by flash chromatography (20:1 CH_2Cl_2/MeOH) afforded **58** (952 mg, 80%) as a yellow foam. R_f = 0.5 (20:1 CH_2Cl_2/MeOH), $[\alpha]^{20}_D$ = +25.72 (c= 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 4.95

(dt, *J* = 2, 8 Hz, 1H) 4.63 (t, *J* = 8.5 Hz, 1H), 4.29 (s, 2H), 3.82-3.76 (m, 2H), 3.65-3.61 (m, 1H), 1.57 (s, 9H), 1.48 (s, 3H), 1.46 (s, 9H), 1.38 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 150.25, 109.25, 108.33, 86.063, 81.74, 73.65, 61.52, 28.16, 27.94, 27.21, 27.09 ppm. IR (neat): 3344, 2981, 2361, 1756, 1656, 1613, 1456, 1250, 1143 cm^{-1}.

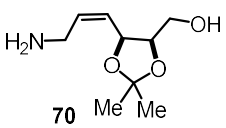
(3a*R*,4'*R*,7*R*,7a*R*)-*tert*-butyl 7-bromo-2'-((*tert*-butoxycarbonyl)imino)-2,2-dimethyltetrahydrospiro[[1,3]dioxolo[4,5-*c*]pyran-6,4'-imidazolidine]-3'-carboxylate (67)



67

To a solution of **58** (50 mg, 0.106 mmol) in CH_2Cl_2 (5 mL) was added *N*-bromosuccinimide (22.5 mg, 0.127 mmol) in portions and the reaction was stirred for 5 hours. The solution was concentrated under reduced pressure and purified by flash chromatography (1:1 Hex/EtOAc) to give **67** as a pale yellow oil. R_f = 0.91 (20:1 CH_2Cl_2/MeOH), ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 10.45 (s, 1H), 9.36 (s, 1H), 4.27 (dd, *J* = 4, 16 Hz, 1H), 4.11-4.09 (m, 2H), 3.86-3.84 (m, 1H), 2.98 (dd, *J* = 3.5, 15.5 Hz, 1H), 2.78 (dd, *J* = 5, 16 Hz, 1H), 1.96-1.83 (m, 2H), 1.51 (s, 9 H), 1.48 (s, 9H), 1.42 (s, 6H) ppm.

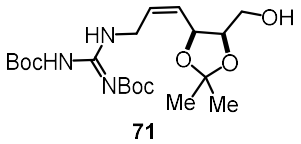
((4S,5R)-5-((Z)-3-aminoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (70)



70

To a solution of alkyne **57** (53 mg, 0.286 mmol) in DMF (5 mL) was added $Pd/BaSO_4$ (24 mg, 0.015 mmol), and ethylenediamine (0.02 ml, 0.344 mmol). The reaction was flushed with H_2 and stirred under an atmosphere of H_2 for 2 ½ hours. The solution was filtered through a pad of celite which was washed with toluene (3 x 30 mL) and the filtrate was concentrated under reduced pressure. The amine **70** was used without further purification.

Allylic Guanidine (71)

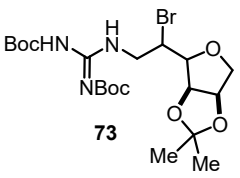


71

To a solution of **70** (492 mg, 1.70 mmol) in CH_2Cl_2 (25 mL) at room temperature was added N,N'-Di-Boc-S-methylisothiourea (492 mg, 1.70 mmol), Et_3N (0.236 mL, 1.70 mmol), and HgO (367 mg, 2.36 mmol). The reaction was stirred for 12 hours then filtered through a pad of Celite. The pad of celite was washed with an additional 15 mL of CH_2Cl_2 and the combined organics were concentrated under reduced pressure. Purification by flash chromatography (4:1 Hex/EtOAc) gave **71** (508 mg, 63%) as a colorless foam. R_f = 0.53 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 11.46 (s, 1H), 8.47 (t, J = 5 Hz, 1H), 5.70-5.65 (m, 1H), 5.58 (t, J = 9.5 Hz, 1H), 4.83 (t, J = 8.5 Hz, 1H), 4.44 (p, J = 7 Hz, 1H), 3.94-3.88 (m, 1H), 3.82 (dd, J = 3.5, 12 Hz, 1H), 3.78-3.75 (m, 1H), 3.70 (dd, J = 3.5, 12 Hz, 1H) 1.49 (s, 9 H), 1.48 (s, 9H), 1.44 (s, 3H), 1.43 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 163.33, 156.17, 153.19, 149.12, 130.59, 129.73, 109.32, 83.36, 81.34, 79.61, 73.14, 60.52, 53.51, 38.05, 28. 27, 28.08, 27.17, 27.02 ppm.

Cyclic guanidine (72)/ Furan (73)

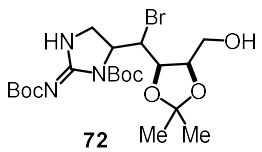
A solution of **71** (300 mg, 0.633 mmol) in MeCN (10 mL) was cooled to –30 °C. *N*-bromosuccinimide (124 mg, 0.696 mmol) was added in portions and the reaction was stirred for 6 hours. The solution was then concentrated under reduced pressure and purification by flash chromatography (4:1 Hex:EtOAc) gave **73** (202 mg, 60%) as a colorless oil and **74** (80 mg, 25%) as a colorless oil.



73

73: R_f = 0.21 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 9.26(s, 1H), 4.66-4.63 (m, 1H), 4.54 (d, J = 4.5 Hz, 1H), 4.13 (dd, J = 3, 11.5 Hz, 1H), 4.04-4.00 (m, 1H), 3.96 (d, J = 7.5 Hz, 1H), 3.74-3.64 (m,

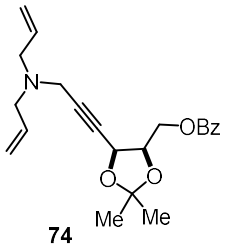
3H), 1.48 (s, 9H), 1.44 (s, 9H), 1.43 (s, 3H), 1.3 (s, 1H) ppm.



72

72: R_f = 0.07 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 4.57 (s, 1H), 4.24 (d, J =9.5 Hz, 1H), 4.11-4.07 (m, 1H), 4.03-4.4.00 (m, 1H), 3.96-3.91 (m, 3H), 3.76-3.72 (m, 1H), 1.53 (s, 9H), 1.46 (s, 9H), 1.33 (s, 3H), 1.32 (s, 3H) ppm.

((4S,5R)-5-(3-(diallylamino)prop-1-yn-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl benzoate (74)

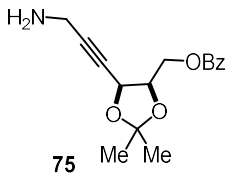


74

To a solution of **43** (291 mg, 1.10 mmol) in CH_2Cl_2 (50 mL) was added DMAP (67 mg, 0.548 mmol), Et_3N (0.46 ml, 3.3 mmol), and benzoyl chloride (0.225 ml, 2.2 mmol). The solution was stirred at room temperature for 3 hours. The reaction was then washed with H_2O (2 x 30 mL) and brine (30 mL); the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography (6:1 Hex:EtOAc) gave **74** (319 mg, 78%) as a yellow oil. R_f = 0.91 (4:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +17.7 (c= 1.646, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.07 (d, J = 8.5 Hz, 2H), 7.57 (t, J = 8 Hz, 1H), 7.44 (t, J = 8 Hz, 2H), 5.80 (ddt, J = 6.5, 10.5, 17 Hz, 2H), 5.21 (dd, J = 1.5, 17 Hz, 2H), 5.14 (d, 10 Hz, 2H), 4.67 (dt, J = 2, 7.5 Hz, 1H), 4.57 (dd, J = 4.5, 11.5 Hz, 1H), 4.43 (dd, J = 5, 11.5 Hz, 1H), 4.40-4.36 (m, 1H), 3.41 (d, J = 1 Hz, 2H), 3.10 (d, J = 7 Hz, 4H), 1.53 (s, 3H), 1.45 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 166.12, 135.20, 133.36, 129.89, 128.57, 118.43, 111.04, 82.11, 81.77, 79.94, 67.99, 63.46, 56.54, 41.76, 27.04, 26.48 ppm. IR (neat): 2985, 1723, 1451, 1381, 1269, 1110, 711 cm^{-1}

((4S,5R)-5-(3-aminoprop-1-yn-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl benzoate

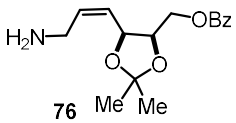
(75)



75

To a solution of alkyne **74** (438 mg, 1.18 mmol) in CH_2Cl_2 was added $Pd(PPh_3)_4$ (68.14 mg, 0.059 mmol), and 1,3-dimethyl barbutiric acid (651 mg, 4.72 mmol). The solution was heated to reflux and stirred for 12 hours then concentrated under reduced pressure and redissolved in Et_2O; the organic layer was washed with H_2O (4 x 100 mL). The aqueous layer was basified with saturated $NaHCO_3$. The aqueous layer was extracted with chloroform (4 x 35 mL) and the combined chloroform extracts were dried over Na_2SO_4 and concentrated under reduced pressure to yield **75** (270 mg, 79%) as an orange oil, which was used without further purification. R_f = 0.06 (10:1 CH_2Cl_2/MeOH), $[\alpha]^{20}_D$ = +23.9 (c= 1.67, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.06 (d, J = 6.5 Hz, 2H), 7.57 (t, J = 7.5 Hz, 1H), 7.44 (t, 7.5 Hz, 1H), 4.61 (d, J = 7 Hz, 1H), 4.56 (dd, J = 5, 11.5 Hz, 1H), 4.44 (dd, J = 5.5, 11.5 Hz, 1H), 4.34-4.30 (m, 1H), 3.41 (s, 2H), 1.50 (s, 3H), 1.42 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 166.26, 133.39, 129.85, 128.56, 110.88, 88.35, 79.41, 78.19, 68.04, 63.25, 31.73, 26.98, 26.60 ppm. IR (neat): 2987, 1721, 1381, 1272, 1070, 712 cm^{-1}.

((4S,5R)-5-((Z)-3-aminoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl benzoate (76)

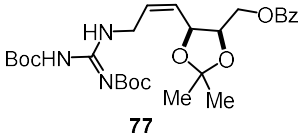


76

To a solution of alkyne **75** (270 mg, 0.938 mmol) in DMF (10 mL) was added $Pd/BaSO_4$ (79 mg, 0.037 mmol), and quinoline (0.144 mL, 1.21 mmol). The reaction was flushed with H_2 and stirred under an atmosphere of H_2 for 4 hours. The solution was filtered through a pad of Celite, which was washed with toluene (3 x 30 mL) and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (20:1 CH_2Cl_2:MeOH) gave **76** (204 mg, 75%) as an orange oil. R_f = 0.11

(10:1 CH_2Cl_2/ MeOH), $[\alpha]^{20}_D$ = -3.1 (c= 1.37, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.04 (d, J = 8 Hz, 2H), 7.57 (t, J = 6 Hz, 1H), 7.44 (t, J = 8 Hz, 2H), 5.81 (dt, J = 7, 11 Hz, 1H), 5.49 (t, J = 11.5 Hz, 1H), 4.71 (t, J = 9 Hz, 1H), 4.52 (dd, J = 4, 11.5 Hz, 1H), 4.36 (dd, J = 5, 12.5 Hz, 1H) , 4.00 (p, 4.5 Hz, 1H), 3.49-3.44 (m, 1H), 3.30 (dd, J = 6.5, 14 Hz, 1H), 1.69 (s, 2H), 1.46 (s, 3H), 1.44 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 166.43, 137.10, 133.40, 128.81, 128.60, 126.53, 109.88, 79.05, 78.74, 73.58, 63.61, 63.20, 39.14, 27.27, 27.22, 27.04 ppm. IR (neat): 2985, 1720, 1371, 1275, 1070, 712 cm^{-1}

((4S,5R)-5-((1Z)-3-(2,3-bis(tert-butoxycarbonyl)guanidino)prop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl benzoate (77)

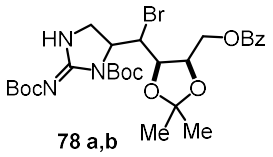


77

To a solution of **76** (500 mg, 1.71 mmol) in CH_2Cl_2 (50 mL) at room temperature was added N,N'-Di-Boc-S-methylisothiourea (508 mg, 1.71 mmol), Et_3N (0.22 ml, 1.71 mmol), and HgO (372 mg, 1.71 mmol). The reaction was stirred for 12 hours then filtered through a pad of celite. The pad of celite was washed with an additional 15 mL of CH_2Cl_2 and the combined organics were concentrated under reduced pressure. Purification by flash chromatography (4:1 Hex:EtOAc) gave **77** (645.6 mg, 70.1%) as a colorless foam. R_f = 0.54 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = -1.8 (c= 1.57, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 11.43, (s, 1H), 8.38 (s, 1H), 8.04 (d, 8.5 Hz, 2H), 7.55 (t, J = 7.5 Hz, 1H), 7.44 (t, J = 6.5 Hz, 2H), 5.84-5.79 (m, 1H), 5.64 (t, J = 9 Hz, 1H), 4.78 (t, J = 9 Hz, 1H), 4.55 (dd, J = 3.5, 11.5 Hz, 1H), 4.38 (dd, J = 5, 12 Hz, 1H), 4.28-4.23 (m, 1H), 4.13-4.07 (m, 1H), 4.04 (p, J = 5 Hz, 1H), 1.48 (s, 9H), 1.47 (s, 9H), 1.45 (s, 3H), 1.44 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 166.28, 163.58, 156.07, 153.26, 133.24, 131.51, 129.85, 129.31, 128.54, 110.06, 83.31, 79.46, 79.103, 73.64, 63.44, 38.143, 31.714, 28.40, 28.17, 27.25, 27.06 ppm. IR (neat): 3330, 1722, 1638, 1614, 1271,

1131, 712 cm^{-1}

Cyclic guanidine (78a, b)

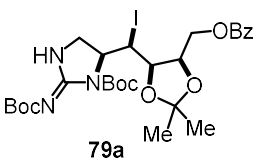


78 a,b

To a solution of **77** (280 mg, 0.47 mmol) in MeCN (10 mL) cooled to 0 °C was added *N*-bromosuccinimide (92 mg, 0.517 mmol) in one portion and the reaction was stirred for 3 hours. The solution was then concentrated under reduced pressure and purified by flash chromatography (1:1 Hex:EtOAc) to give **78a,b** (192mg 66%) as a mixture of diastereomers (2:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.04 (d, J = 8 Hz, 2H), 7.53 (t, J = 7 Hz, 1H), 7.42 (t, J = 7.5 Hz, 2H), 4.76-4.73 (m, 1H), 4.55 (d, J = 4.5 Hz, 1H), 4.41-4.37 (m, 2H), 4.29-4.25 (m, 1H), 4.17 (dd, J = 3, 12 1H), 3.90 (d, J = 10 Hz, 1H) 3.82 (s, 1H), 1.45 (s, 21H), 1.34 (s, 3H) ppm.

Cyclic guanidine (79 a, b)

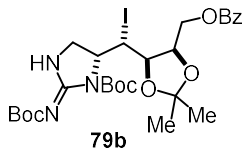
To a solution of **77** (645.6 mg, 1.21 mmol) in Et_2O (25 mL) was added I_2 (243.5 mg, 1.46 mmol) and AgOAc (370.2 mg, 1.46 mmol). The reaction was stirred for 6 hours then filtered through a pad of celite and rinsed with EtOAc (2 x 30 mL); the combined organic layers were transferred to a separatory funnel and washed with saturated $Na_2S_2O_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography (1:1 Hex:EtOAc) gave **79a** (354.1 mg, 44.3%) and **79b** (327.2 mg, 41%) as colorless foams.



79a

79a: R_f = 0.32 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +7.4 (c= 1.06, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.04 (d, J = 8 Hz, 2H), 7.53 (t, J = 7 Hz, 1H), 7.42 (t, J = 7.5 Hz, 2H), 4.79-4.75 (m, 2H), 4.42, (dd, J = 5, 12, Hz, 1H), 4.36 (dd, J = 4.5, 11.5 Hz, 1H), 4.13-4.06 (m, 2H), 3.77 (s, 1H), 3.16 (d, J = 8 Hz,

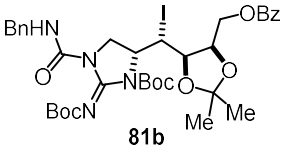
1H), 1.50 (s, 3H), 1.46 (s, 18H), 1.33 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 166.24, 133.43, 129.95, 129.52, 128.70, 110.86, 80.33, 73.89, 63.83, 61.29, 32.83, 31.71, 28.18, 28.14, 27.30, 27.10 ppm. IR (neat): 3063, 2860, 1716, 1451, 1265, 1095, 933, 708 cm^{-1}.



79b

79b: R_f = 0.05 (1:1 Hex/EtOAc). $[\alpha]^{20}_D$ = -15.5 (c= 1.61, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.04 (d, J = 8 Hz, 2H), 7.53 (t, J = 7 Hz, 1H), 7.42 (t, J = 7.5 Hz, 2H), 4.75 (dd, J = 2, 12.5 Hz, 1H), 4.66 (d, J = 9.8 Hz, 1H), 4.44-4.40 (m, 2H), 4.30-4.22 (m, 2H), 3.96-3.92 (m, 1H), 3.85 (d, J = 14.5 Hz, 1H), 1.51 (s, 9H), 1.45 (s, 9H), 1.35 (s, 3H), 1.30 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 166.42, 133.25, 129.93, 128.54, 111.11, 84.04, 81.49, 76.82, 65.89, 61.38, 33.78, 28.36, 28.19, 27.67, 27.31 ppm. IR (neat): 3315, 2978, 1706, 1531, 1367, 1271, 1144, 850, 712 cm^{-1}.

Carbamoyl cyclic guanidine (79b)

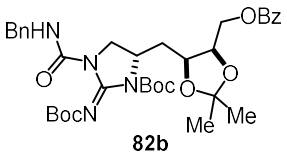


81b

To a stirring solution of **79b** (327.2 mg, 0.496 mmol) in benzene (10 mL) was added benzyl isocyanate (0.065 mL, 0.537 mmol). The reaction was stirred for 3 hours then concentrated under reduced pressure. Purification by flash chromatography (1:1 Hex:EtOAc) gave **81b** (354.5 mg, 89.9%) as a colorless oil. R_f = 0.57 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 9.31 (t, J = 6 Hz, 1H), 8.06 (d, J = 8.5 Hz, 2H), 7.54 (t, J = 7 Hz, 1H), 7.42-7.28 (m, 6H), 4.79 (d, J = 10 Hz, 1H), 4.68 (d, J = 8.5 Hz, 1H), 4.56-4.53 (m, 2H), 4.49 (s, 2H), 4.46-4.43 (m, 1H), 4.38 (s, 1H), 4.34 (s, 2H), 4.23 (d, J = 12.5 Hz, 1H). 3.88 (dd, J = 9.5, 12 Hz, 1H), 1.53 (s, 9H), 1.48 (s, 12 Hz), 1.37 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm), 166.43, 156.25, 152.74, 149.65, 146.25, 138.50, 133.24, 129.94, 128.98, 128.64, 128.52, 128.09, 127.24, 127.09, 126.85, 111.64, 84.8, 80.65, 80.45, 77.51, 65.67, 58.42, 45.12, 44.04,

36.01, 28.24, 27.45, 27.02 ppm.

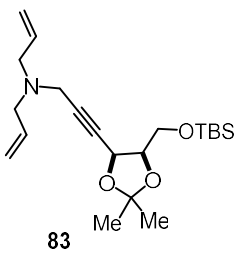
Carbamoyl guanidine (82b)



82b

To a solution of **81b** (154 mg, 0.195 mmol) in benzene (3mL) and water (0.035 mL, 1.95 mmol) was added tibutylborane (0.19 mL, 0.78 mmol). Immediately following the addition, air (15.88 mL, 0.156 mmol O_2) was bubbled through the solution *via* syringe pump over 16 hours. The reaction was quenched with NaOH (2 mL, 3 M) and H_2O_2 (2 mL) at the same time and stirred for 10 minutes. The mixture was then extracted with EtOAc (3 x 25 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography (4:1 Hex:EtOAc) gave **82b** (77.9 mg, 60%) as a colorless oil. R_f = 0.56 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 9.34 (s, 1H), 8.04 (d, J = 8.5 Hz, 2H), 7.55 (t, J = 7.5 Hz, 1H), 7.45-7.42 (m, 2H), 7.33-7.26 (m, 7H), 4.56-4.42 (m, 5H), 4.36-4.32 (m, 1H), 4.11 (d, J = 11 Hz, 1H), 4.04-3.96 (m, 2H), 3.85-3.79 (m, 1H), 2,17-2.13 (m, 1H), 1.94-1.88 (m, 1H), 1.46 (s, 9H), 1.45 (s, 9H), 1.43 (s, 3H), 1,40 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm), 166.3, 157.3, 152.9, 149.5, 147.5, 138.4, 133.3, 129.8, 128.6, 127.3, 110.2, 84.1, 80.5, 79.1, 77.2, 75.7, 63.7, 54.0, 47.1, 44.1, 38.2, 28.1 ppm.

N-allyl-N-(3-((4S,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)prop-2-yn-1-yl)prop-2-en-1-amine (83)



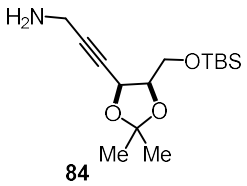
83

To a solution of **43** (2.04 g, 7.69 mmol) in CH_2Cl_2 was added TBSCl (2.32 g, 15.38 mmol), Et_3N (3.0 mL, 23.07 mmol), and DMAP (469 mg, 3.84 mmol). The solution was stirred at room temperature for 18 hours, then washed with H_2O (2 x 200 mL) and brine (200 mL), the

organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was then purified by flash chromatography (4:1 Hex/EtOAc) to yield **83** in 90% yield. R_f = 0.95 (4:1 Hex/EtOAc) $[\alpha]^{20}_D$ = +6.1 (c= 1.35, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 5.83-5.75 (m, 2H), 5.20 (d, J = 17.5 Hz, 2H), 5.12 (d, J = 9.5 Hz, 2H), 4.63 (d, J = 9 Hz, 1H), 4.08-4.04 (m, 1H), 3.75 (qd, J = 4.5 Hz, 11 Hz, 2H), 3.40 (s, 2 H), 3.09 (d, J = 6.5 Hz, 4H), 1.47 (s, 3H), 1.39 (s, 3H), 0,88 (s, 9H), 0.06 (s, 6H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 135.4, 118.2, 110.3, 82.7, 81.2, 67.3, 62.2, 56.5, 41.8, 26.9, 26.5, 26.0, 25.9, 25.8, 18.5 ppm. IR (neat): 3079, 2985, 2928, 2857, 2815, 1644, 1472, 1462, 1380, 1371, 1322, 1252, 1212, 1145, 1076, 1028, 955, 919, 834, 775 cm^{-1}

3-((4S,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)prop-2-yn-1-amine (84)

To a solution of **83** (2.93 g, 7.73 mmol) in CH_2Cl_2 was added $Pd(PPh_3)_4$ (440 mg. 0.386 mmol), and dimethyl barbateric acid (4.27 g, 30.92 mmol). The solution was heated to reflux and stirred for 18 hours. It was then cooled to room temperature washed with $NaHCO_3$ (3 x 250 ml), the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was passed through a plug of silica (10:1 CH_2Cl_2/MeOH) and used without further purification.

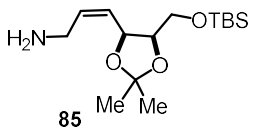


84

(Z)-3-((4S,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)prop-2-en-1-amine (85)

Amine **84** (1.79 g, 5.88 mmol) was dissolved in DMF, followed by the addition of quinolone (0.91 mL, 7.64 mmol) and $Pd/BaSO_4$ (500 mg, 0.24 mmol); the solution was then

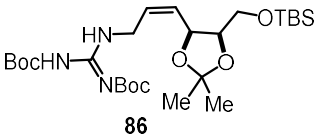
flushed with H_2. The reaction was stirred under and H_2 atmosphere for 6 hours, then filtered over a bed of Celite, washed with toluene, and concentrated under reduced pressure. The crude material was passed through a plug of silica (5:1 CH_2Cl_2/MeOH) and used without further purification.



85

Allylic Guanidine (86)

The allyl amine **86** (554 mg. 1.83 mmol) was dissolved in CH_2Cl_2, followed by the addition of Et_3N (0.26 mL, 1.83 mmol), HgO (398 mg, 1.83 mmol), and N,N'-Di-Boc-S-methylisothiourea (553.6 mg, 1.83 mmol) and the reaction was stirred for 18 hours. The crude mixture was filtered through a pad of Celite, and concentrated under reduced pressure. The crude compound was purified by flash chromatography (10:1 Hex/EtOAc), to give **86** in 70% yield as a white foam. R_f = 0.83 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +3.9 (c= 2.71, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 11.45 (s, 1H), 8.31 (t, J = 5.2 Hz, 1H), 5.76-5.69 (m, 1H), 5.62-5.55 (m, 1H), 4.72 (t, 8.2 Hz, 1H), 4.21-4.07 (m, 2H), 3.79-3.73 (m, 1H), 3.71-3.66 (m, 2H), 1.48 (s, 9H), 1.47 (s, 9H), 1.40 (s, 3H), 1.39 (s, 3H), 0.87 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H). ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 163.5, 155.9, 153.1, 130.1, 109.2, 88.0, 83.1, 81.5, 79.2, 73.0, 61.8, 38.1, 28.3, 28.0, 27.1, 26.9, 25.9, 18.3, -5.3 ppm. IR (neat): 3335, 2931, 1721, 1614, 1412, 1367, 1311, 1251, 1227, 1130, 1081, 1057, 835, 776 cm^{-1}

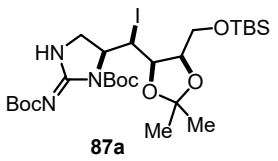


86

Cyclic Guanidine (87a,b)

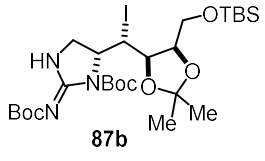
To a solution of **86** (2.2 g, 4.05 mmol) in Et_2O (100 mL) was added I_2 (1.23 g, 4.86 mml) and AgOAc (811 mg, 4.86 mmol). The reaction was stirred for 6 hours then filtered through a pad of Celite and rinsed with EtOAc (2 x 100 mL); the combined organic layers

were transferred to a separatory funnel and washed with saturated $Na_2S_2O_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography (1:1 Hex:EtOAc) gave **87a** (587 mg, 21.9%) and **87b** (327.2 mg, 22.9%) as colorless foams.



87a

87a: R_f = 0.80 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = -40.5 (c= 2.82, MeOH). ^{1}H NMR (500 MHz, CD_3OD): 4.78-4.75 (m, 2H), 4.49 (dd, J = 1.5 Hz, 10.5 Hz, 1H), 4.28-4.25 (m, 1H), 3.99-3.85 (m, 4H), 3.75 (dd, J = 2.5 Hz, 14 Hz, 1H), 1.56 (s, 9H), 1.49 (s, 9H), 1.34 (s, 3H), 1.29 (s, 3H), 0.93 (s, 9H), 0.11 (s, 3H), 0.09 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, CD_3OD): δ (ppm): 109.8, 83.9, 80.8, 78.2, 75.3, 64.2, 61.2, 33.2, 27.4, 27.2, 26.7, 26.3, 25.3, 18.0, 6.1 ppm. IR (neat): 3317, 2930, 1764, 1706, 1651, 1613, 1530, 1457, 1367, 1322, 1252, 1143, 1035, 836, 749 cm^{-1}

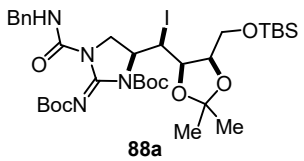


87b

87b: R_f = 0.67 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +41.4 (c= 1.94, MeOH). ^{1}H NMR (500 MHz, CD_3OD): 4.75-4.74 (m, 1H), 4.63 (d, J = 4 Hz, 1H), 4.07 (dd, J = 3 Hz, 13 Hz, 1H), 3.79-3.69 (m, 4H), 3.22 (d, J = 7.5 Hz, 1H), 1.55 (s, 9H), 1.48 (s, 9H), 1.45 (s, 3H), 1.31 (s, 3H), 0.91 (s, 9H), 0.08 (s, 6H) ppm. ^{13}C {^{1}H} NMR (125 MHz, CD_3OD): δ (ppm): 156.4, 109.6, 83.9, 82.0, 80.2, 78.1, 61.4, 54.4, 33.2, 27.2, 26.9, 26.3, 26.1, 25.2, 17.9, 6.4 ppm. IR (neat): 2979, 2931,1767, 1709, 1654, 1622, 1463, 1367, 1298, 1252, 1137, 1101, 837, 810, 766 cm^{-1}

tert-butyl (R,Z)-3-(benzylcarbamoyl)-2-((tert-butoxycarbonyl)imino)-5-((R)-((4R,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)imidazolidine-1-carboxylate (88a)

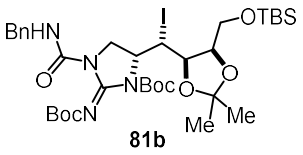
To a stirring solution of **87a** (587 mg, 0.877 mmol) in benzene (20 mL) was added benzyl isocyanate (0.1 mL, 0.887 mmol). The reaction was stirred for 3 hours then



88a

concentrated under reduced pressure. Purification by flash chromatography (1:1 Hex:EtOAc) gave **88a** (589 mg, 83.6%) as a colorless oil. R_f = 0.83 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): 9.27 (t, *J* = 6 Hz, 1H), 7.32-7.27 (m, 4H), 7.26-7.23 (m, 1H), 4.67-4.63 (m, 2H), 4.56 (dd, *J* = 2, 11.5 Hz, 1 H), 4.50 (ddd, *J* = 6, 15, 21 Hz, 2H), 3.87-3.80 (m, 2H), 3.79-3.64 (m, 2H), 3.30 (d, *J* = 7.5 Hz, 1H), 1.51 (s, 9H), 1.49 (s, 9H), 1.48 (s, 3H), 1.36 (s, 3H), 0.90 (s, 10H), 0.09 (s, 3H), 0.08 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): 156.9, 152.5, 149.3, 138.6, 128.6, 127.6, 109.7, 84.6, 82.6, 80.5, 77.2, 75.9, 64.1, 59.7, 45.8, 44.1, 34.7, 28.2, 27.0, 26.6, 26.2 ppm.

tert-butyl (S,Z)-3-(benzylcarbamoyl)-2-((tert-butoxycarbonyl)imino)-5-((S)-((4R,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)imidazolidine-1-carboxylate (88b)

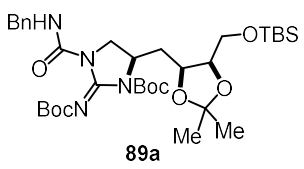


81b

To a stirring solution of **87b** (623 mg, 0.931 mmol) in benzene (20 mL) was added benzyl isocyanate (0.12 mL, 0.931 mmol). The reaction was stirred for 3 hours then concentrated under reduced pressure. Purification by flash chromatography (1:1 Hex:EtOAc) gave **88b** (650 mg, 87.0%) as a colorless oil. R_f = 0.82 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): 9.30 (t, *J* = 5.5 Hz, 1H), 7.30 (d, *J* = 5 Hz, 4H), 7.25-7.24 (m, 1H), 4.67-4.65 (m, 1H), 4.57-4.49 (m, 2H), 4.29-4.20 (m, 2H), 4.16 (d, *J* = 12 Hz, 1H), 4.06-4.02 (m, 1H), 3.94 (dd, *J* = 2, 11 Hz, 1H), 3.87-3.80 (m, 2H), 1.53 (s, 9H), 149 (s, 9H), 1.43 (s, 3H), 1.35 (s, 3H), 0.90 (s, 9H), 0.09 (s, 3H), 0.08(s, 3H) ppm.

tert-butyl (R,Z)-3-(benzylcarbamoyl)-2-((tert-butoxycarbonyl)imino)-5-(((4S,5R)-5-

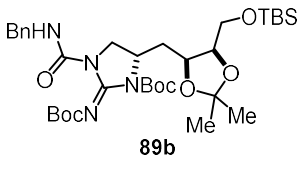
(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)imidazolidine-1-carboxylate (89a)



89a

To a solution of **88a** (589 mg, 0.734 mmol) in benzene (7mL) and water (0.13 mL, 7.34 mmol) was added tibutylborane (0.72 mL, 2.93 mmol). Immediately following the addition of tributylborane, air (60 mL, 0.58 mmol O_2) was bubbled through the solution *via* syringe pump over 16 hours. The reaction was quenched with NaOH (2 mL, 3 M) and H_2O_2 (2 mL) at the same time and stirred for 10 minutes. The mixture was then extracted with EtOAc (3 x 25 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography (4:1 Hex:EtOAc) gave **89a** (221.4 mg, 44.5%) as a colorless oil. R_f = 0.76 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): 9.34 (t, *J* = 6 Hz, 1H), 7.32-7.23 (m, 5H), 4.50 (d, *J* = 5.5 Hz, 2H), 4.44-4.39 (m, 1H), 3.97 (d, *J* = 11.5 Hz, 1H), 3.93-3.89 (m, 1H), 3.80-3.74 (m, 2H), 3.72-3.69 (m, 1H), 3.66-3.63 (m, 1H), 1.97 (t, *J* = 6 Hz, 2H), 1.50 (s, 9H), 1.48 (s, 9H), 1.35 (s, 3H), 1.31 (s, 3H), 0.86 (s, 9H), 0.04 (s, 6H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): 157.3, 152.9, 149.7, 147.6, 138.5, 128.6, 127.3, 109.3, 83.8, 81.0, 76.9, 74.7, 63.5, 52.7, 45.7, 44.0, 37.3, 28.2, 27.4, 26.9, 26.0, 18.4 ppm.

tert-butyl (S,Z)-3-(benzylcarbamoyl)-2-((tert-butoxycarbonyl)imino)-5-(((4S,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)imidazolidine-1-carboxylate (89b)

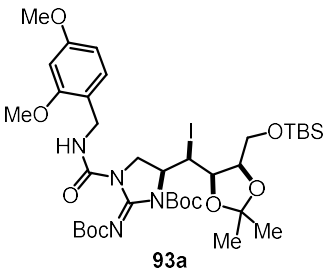


89b

To a solution of **88b** (600 mg, 0.747 mmol) in benzene (7mL) and water (0.13 mL, 7.47 mmol) was added tibutylborane (0.72 mL, 2.93 mmol). Immediately following the addition, air (60 mL, 0.58 mmol O_2) was bubbled through the solution *via* syringe pump over 16 hours. The reaction

was quenched with NaOH (2 mL, 3 M) and H_2O_2 (2 mL) at the same time and stirred for 10 minutes. The mixture was then extracted with EtOAc (3 x 25 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography (4:1 Hex:EtOAc) gave **89b** (198.4 mg, 39.2%) as a colorless oil. R_f = 0.76 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): 9.35 (t, *J* = 5.5 Hz, 1H), 7.33-7.22 (m, 6H), 4.51 (ddd, *J* = 5.5, 15.5, 21 Hz, 2H), 4.32-4.30 (m, 1H), 4.11 (d, *J* = 11.5 Hz, 1H), 4.00-3.96 (m, 1H), 3.77 (dd, *J* = 3, 8 Hz, 1H), 3.71 (d, *J* = 4 Hz, 2H), 3.67-3.63 (m, 1H), 2.08 (dt, *J* = 3, 14 Hz, 1H), 1.85 (dt, *J* = 9, 9 Hz, 1H), 1.49 (s, 9H), 1.47 (s, 9H), 1.37 (s, 3H), 1.34 (s, 3H), 0.87 (s, 9H), 0.05 (s, 6H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): 157.2, 152.9, 149.6, 147.5, 138.5, 128.6, 127.3, 109.4, 83.9, 81.7, 80.4, 77.4, 75.9, 62.9, 54.0, 47.1, 44.1, 38.4, 28.2, 27.4, 27.1, 26.0, 18.5 ppm.

tert-butyl (S,Z)-2-((tert-butoxycarbonyl)imino)-5-(((4S,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-3-((2,4-dimethoxybenzyl)carbamoyl)imidazolidine-1-carboxylate (93a)

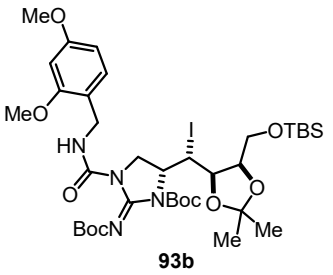


93a

To a stirring solution of **88a** (275 mg, 0.411 mmol) in benzene (20 mL) was added DMB isocyanate (74 mg, 0.411 mmol). The reaction was stirred for 3 hours, then concentrated under reduced pressure. Purification by flash chromatography (1:1 Hex:EtOAc) gave **93a** (174 mg, 49.1%) as a colorless oil. R_f = 0.82 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): 9.20 (t, *J* = 5.5 Hz, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 6.39-6.35 (m, 2H), 4.62-4.57 (m, 2H), 4.51 (dd, *J* = 1.5, 11 Hz, 1H), 4.37 (ddd, *J* = 6, 14, 20.5 Hz, 2H), 3.83-3.82 (m, 2H), 3.80 (s, 3H), 3.77-3.76 (m, 2H), 3.75 (s, 3H), 3.66-3.61 (m, 2H), 3.26 (dd, *J* = 1.5, 7.5 Hz, 1H), 1.48 (s, 18H), 1.24 (s, 3H), 0.92 (s, 3H), 0.88 (s,

9H), 0.07 (s, 3H), 0.06 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): 173.1, 160.5, 158.7, 157.1, 152.1, 149.2, 130.1, 119.5, 109.5, 103.6, 98.5, 84.3, 82.5, 80.1, 75.8, 64.1, 59.5, 55.5, 55.3, 45.8, 39.6, 34.6, 28.3, 28.1, 26.8, 26.1 ppm.

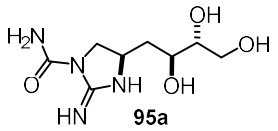
tert-butyl (R,Z)-2-((tert-butoxycarbonyl)imino)-5-(((4S,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-3-((2,4-dimethoxybenzyl)carbamoyl)imidazolidine-1-carboxylate (93b)



93b

To a stirring solution of **88b** (329.1 mg, 0.492 mmol) in benzene (20 ml) was added DMB isocyanate (88.1 mg, 0.492 mmol). The reaction was stirred for 3 hours then concentrated under reduced pressure. Purification by flash chromatography (1:1 Hex:EtOAc) gave **93b** (204 mg, 47.9%) as a colorless oil. R_f = 0.82 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): 9.22 (t, *J* = 6 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.39-6.35 (m, 2H), 4.60 (dt, *J* = 1.5, 10.5 Hz, 1H), 4.38 (ddd, *J* = 5.5, 14.5, 19 Hz, 2H), 4.11-4.07 (m, 1H), 4.00-3.98 9m 1H), 3.91 (dd, *J* = 3, 11 Hz, 1H), 3.82 (s, 3H), 3.81-3.76 (m, 2H), 3.75 (s, 3H), 1.49 (s, 18H), 1.38 (s, 3H), 1.28 (s, 3H), 0.88 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): 160.4, 158.6, 156.5, 152.3, 149.7, 145.9, 129.6, 119.4, 110.8, 103.7, 98.4, 84.4, 83.3, 80.1, 76.8, 64.6, 57.8, 55.4, 45.6, 39.6, 37.0, 28.3, 28.2, 27.3, 27.2, 26.1, 18.6 ppm.

(R)-2-imino-4-((2S,3R)-2,3,4-trihydroxybutyl)imidazolidine-1-carboxamide (95a)

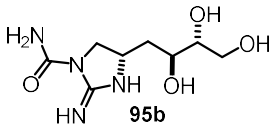


95a

94a (39.5 mg, 0.054 mmol) was dissolved in TFA and stirred for 18 hours. The reaction was concentrated and tritrated with Et_2O to give the TFA salt of **95a** (9 mg, 72%) as a white solid. ^{1}H NMR (500 MHz, D_2O): 4.17-4.06 (m, 2H), 3.67 (dd, *J* = 6.5, 2.5 Hz, 1H), 3.62 (dt, *J* = 3, 10 Hz, 1H), 3.53-3.49 (m, 1H), 3.45-

3.41 (m, 2H), 1.78 (ddd, *J* = 6.5, 10, 5.5 Hz, 1H), 1.68 (ddd, *J* = 3, 6, 9 Hz, 1H) ppm. ^{13}C {^{1}H} NMR (125 MHz, D_2O): 155.9, 74.3, 68.8, 62.5, 51.2, 50.5, 37.2 ppm.

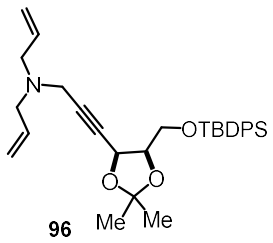
(S)-2-imino-4-((2S,3R)-2,3,4-trihydroxybutyl)imidazolidine-1-carboxamide (95b)



95b

94a (66.0 mg, 0.089 mmol) was dissolved in TFA and stirred for 18 hours. The reaction was concentrated and tritrated with Et_2O to give the TFA salt of **95a** (8.4 mg, 41%) as a white solid. ^{1}H NMR (500 MHz, D_2O): 4.14-4.07 (m, 2H), 3.68-3.59 (m, 2H), 3.52-3.39 (m, 4H), 1.73-1.68 (m, 2H) ppm. ^{13}C {^{1}H} NMR (125 MHz, D_2O): 155.9, 127.4, 127.6, 74.4, 68.2, 62.6, 51.2, 37.9 ppm.

N-allyl-N-(3-((4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)prop-2-yn-1-yl)prop-2-en-1-amine (96)

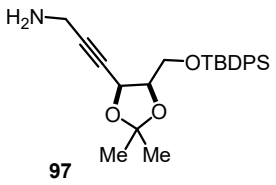


96

To a solution of **43** (1.59 g, 6.2 mmol) in CH_2Cl_2 was added TBDPSCl (2.4 mL, 9.3 mmol), Et_3N (1.72 mL, 12.4 mmol), and DMAP (379 mg, 3.1 mmol). The solution was stirred at room temperature for 18 hours, then washed with H_2O (2 x 200 mL) and brine (200 mL), the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was then purified by flash chromatography (4:1 Hex/EtOAc) to yield **96** in 85% yield. R_f = 0.95 (4:1 Hex/EtOAc). $[\alpha]^{20}_D$ = +19.5 (c= 1.25, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.73-7.67 (m, 5H), 7.44-7.35 (m, 7H), 5.85-5.77 (m, 2H), 5.21 (dd, *J* = 2.5 Hz, 17 Hz, 2H), 5.14 (dd, *J* = 0.5 Hz, 10 Hz, 2H), 4.80 (d, *J* = 7.5 Hz, 1H), 4.15 (dt, *J* = 4 Hz, 7 Hz, 1H), 3.81 (qd, *J* = 4.5 Hz, 11.5 Hz, 2H), 3.42 (d, *J* = 2 Hz, 2H), 3.10 (d, *J* = 6.5 Hz, 4H), 1.51 (s, 3H), 1.44 (s, 3H), 1.07 (s, 9H) ppm. . ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$):

δ (ppm) 135.8, 135.7, 135.4, 134.9, 133.3, 133.2, 129.9, 129.7, 127.8, 118.3, 110.4, 82.8, 82.6, 81.3, 67.5, 62.9, 56.6, 41.8, 27.1, 26.9, 26.7, 26.5, 19.4 ppm. IR (neat): 3071, 2984, 2930, 2857, 2360, 2342, 1472, 1427, 1380, 1371, 1323, 1239, 1212, 1112, 1028, 922, 701 cm^{-1}

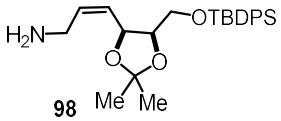
3-((4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)prop-2-yn-1-amine (97)



97

To a solution of **96** (2.65 g, 5.26 mmol) in CH_2Cl_2 was added $Pd(PPh_3)_4$ (304 mg. 0.26 mmol), and dimethyl barbateric acid (3.31 g, 21.04 mmol). The solution was heated to reflux and stirred for 18 hours. It was then cooled to room temperature, washed with $NaHCO_3$ (3 x 50 mL); the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was passed through a plug of silica (10:1 CH_2Cl_2/MeOH) and used without further purification.

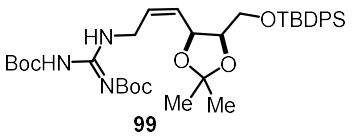
((4R,5S)-5-((Z)-3-aminoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (98)



98

Amine **97** (1.80 g, 4.25 mmol) was dissolved in DMF, followed by the addition of quinolone (0.55 mL, 4.25 mmol) and $Pd/BaSO_4$ (181 mg, 0.17 mmol); the solution was then flushed with H_2. The reaction was stirred under and H_2 atmosphere for 6 hours; it was then filtered over a bed of Celite, washed with toluene, and concentrated under reduced pressure. The crude material was passed through a plug of silica (5:1 CH_2Cl_2/MeOH) and used without further purification.

Allylic Guanidine (99)



99

The allyl amine **98** (665 mg. 1.56 mmol) was dissolved in CH_2Cl_2, followed by the addition of Et_3N (0.22 mL, 1.56 mmol), HgO (339 mg, 1.56 mmol), and N,N'-Di-Boc-S-methylisothiourea (454 mg, 1.56 mmol), the reaction was stirred for 18 hours. The crude mixture was filtered through a pad of Celite, and concentrated under reduced pressure. The crude compound was purified by flash chromatography (10:1 Hex/EtOAc), to give **99** in 81% yield as a white foam. R_f = 0.91 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = -40.6 (c= 2.96, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 11.47 (s, 1H), 8.33 (t, *J* = 6 Hz, 1H), 7.72-7.67 (m, 4H), 7.45-7.35 (m, 6H), 5.76-5.67 (m, 1H), 5.62-5.53 (m, 1H), 4.81 (t, *J* = 10.5 Hz, 1H), 4.23-4.09 (m, 2H), 3.81 (dd, *J* = 4.5 Hz, 9 Hz,1H), 3.78-3.75 (m,1H), 3.70 (dd, *J* = 4 Hz, 13.5 Hz, 1H), 1.50 (s, 9H), 1.49 (s, 9H), 1.44 (s, 3H), 1.43 (s, 3H), 1.07 (s, 9H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 163.6, 155.9, 153.1,135.7, 133.1, 130.2, 130.0, 129.7, 127.7, 127.6, 110.0, 109.3, 87.9, 83.1, 81.6, 79.1, 73.2, 62.6, 38.3, 28.3, 28.0, 27.2, 26.9, 26.8, 19.2 ppm. IR (neat): 3330 (w), 2980, 2931, 2859, 1720, 1638, 1614, 1412, 1367, 1311, 1250, 1227, 1155, 1130, 1078, 1058, 755, 701 cm^{-1}

Cyclic Guanidine (100 a,b)

To a solution of **99** (623 mg, 0.93 mmol) in Et_2O (20 mL) was added I_2 (331 mg, 1.3 mml) and AgOAc (220 mg, 1.3 mmol). The reaction was stirred for 6 hours then filtered through a pad of Celite and rinsed with EtOAc (2 x 100 mL); the combined organic layers were transferred to a separatory funnel and washed with saturated $Na_2S_2O_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography (1:1 Hex:EtOAc) gave **100a** (260.8 mg, 35%) and **100b** (327.2 mg, 44.3%) as colorless foams.

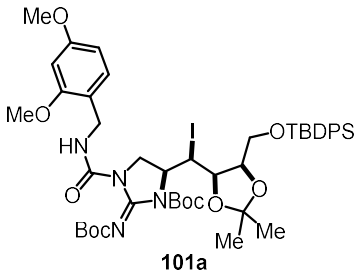
100a: R_f = 0.88 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = -15.6 (c= 2.30, MeOH). ^{1}H NMR (500 MHz,

CD_3OD): 7.70 (dd, *J* = 1.5 Hz, 7.5 Hz, 4H), 7.42-7.34 (m, 6H), 4.65 (d, *J* = 4 Hz, 1H), 4.41-4.40 (m, 1H), 4.28-4.23 (m, 1H), 4.02-3.98 (m, 1H), 3.96 (dd, *J* = 2.5 Hz, 11Hz, 1H), 3.83 (dd, *J* = 5.5 Hz, 11.5 Hz, 2H), 1.52 (s, 9H), 1.47 (s, 9H), 1.35 (s, 3H), 1.29(s, 3H), 1.06 (s, 9H) ppm. ^{13}C {^1H} NMR (125 MHz, CD_3OD): δ (ppm): 135.4, 133.0, 129.5, 127.5, 109.8, 83.9, 78.1, 75.3, 64.7, 61.4, 32.7, 27.3, 27.2, 26.6, 26.4, 26.2, 18.8 ppm. IR (neat): 3314, 2979, 2931, 1763, 1706, 1652, 1613, 1530, 1367, 1321, 1254, 1216, 1144, 746, 701 cm^{-1}

100b: R_f = 0.77 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +73.4 (c= 1.10, MeOH). ^1H NMR (500 MHz, CD_3OD): 7.68 (d, *J* = 6.5 Hz, 4H), 7.44-7.37 (m, 5H), 4.79-4.73 (m, 2H), 4.58 (s, 1H), 4.08 (d, *J* = 12 Hz, 1H), 3.87-3.83 (m, 1H), 3.79-3.69 (m, 3H), 3.26 (d, *J* = 8 Hz, 1H), 1.46 (s, 3H), 1.45 (s, 9H), 1.41 (s, 9H), 1.32 (s, 3H), 1.05 (s, 9H) ppm. ^{13}C {^1H} NMR (125 MHz, CD_3OD): δ (ppm): 135.4, 132.8, 129.7, 127.5, 109.9, 83.9, 82.4, 78.0, 61.4, 33.5, 27.2, 27.1, 26.5, 26.2, 18.7 ppm. IR (neat): 3310, 2979, 2931, 2858, 1763, 1709, 1652, 1617, 1528, 1473, 1428, 1368, 1319, 1276, 1246, 1137, 997, 749, 701 cm^{-1}

tert-butyl (R,Z)-2-((tert-butoxycarbonyl)imino)-5-((R)-((4R,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)-3-((2,4-dimethoxybenzyl)carbamoyl)imidazolidine-1-carboxylate (101a)

To a stirring solution of **100a** (385 mg, 0.48 mmol) in benzene (20 mL) was added DMB isocyanate (148 mg, 0.97 mmol). The reaction was stirred for 3 hours then concentrated under reduced pressure. Purification by flash chromatography (1:1 Hex:EtOAc) gave **101a** (229 mg, 54%) as a colorless oil. R_f = 0.90 (1:1 Hex/EtOAc). ^1H NMR (500 MHz, $CDCl_3$): 9.23 (t, *J* =6.5 Hz, 1H), 7.69-7.67 (m, 4H), 7.42-7.30 (m, 6H), 7.18 (d, *J* = 8 Hz, 1H), 6.40-6.37(m, 2H), 4.65-4.63 (m, 2H), 4.55 (d, *J* = 12 Hz, 1H), 4.38 (ddd, *J*

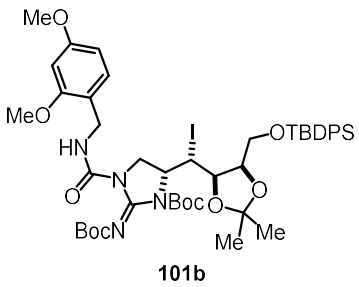


101a

= 6, 14, 20.5 Hz, 2H), 3.94-3.89 (m,1H), 3.87-3.85 (m, 1H), 3.84 (s, 1H), 3.76 (s, 3H), 3.73-3.67 (m, 2H), 3.36 (d, *J* =8 Hz, 1H), 1.50 (s, 9H), 1.45 (s, 9H), 1.27 (s, 3H), 1.07 (s, 9H), 0.93 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, CD_3OD): δ (ppm): 160.4, 158.6, 157.1, 152.0, 149.0, 148.0, 135.6, 133.2, 132.8, 129.9, 129.7, 129.6, 127.8, 119.4, 109.4, 103.6, 98.4, 84.3, 82.6, 79.9, 75.7, 64.7, 59.6, 55.4, 55.3, 45.6, 39.5, 34.5, 28.2, 28.0, 27.9, 27.0, 26.7, 25.9, 19.2 ppm.

tert-butyl (S,Z)-2-((tert-butoxycarbonyl)imino)-5-((S)-((4R,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)-3-((2,4-dimethoxybenzyl)carbamoyl)imidazolidine-1-carboxylate (101b)

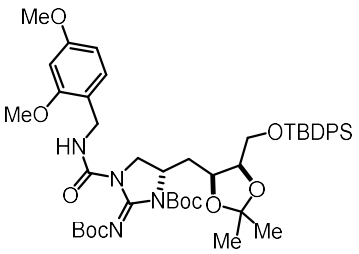
To a stirring solution of **100b** (260.8 mg, 0.33 mmol) in benzene (20 mL) was added DMB isocyanate (74 mg, 0.40 mmol). The reaction was stirred for 3 hours then concentrated under reduced pressure. Purification by flash chromatography (1:1 Hex:EtOAc) gave **101b** (207 mg, 49.1%) as a colorless oil.



101b

R_f = 0.90 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): 9.24 (t, *J* = 6Hz, 1H), 7.73-7.69 (m, 4H), 7.42-7.36 (m, 6H), 7.16 (d, *J* = 8 Hz, 1H), 6.42 (s, 1H), 6.37 (dd, *J* = 1.5, 8 Hz, 1H), 4.61 (d, *J* = 10 Hz, 1H), 4.41 (ddd, *J* = 6.5, 15.5, 22 Hz, 2H), 4.36 (dd, *J* = 14.5, 13.5 Hz, 1H), 4.25 (dd, *J* = 3, 10.5 Hz, 1H), 4.17 (dd, *J* = 1.5, 11.5 Hz, 1H), 4.07-4.05 (m, 1H), 3.98 (dd, *J* = 2.5, 11.5 Hz, 1H), 3.89-3.85 (m, 1H), 3.84 (s, 3H) 3.80 (dd, *J* = 9, 3 Hz, 1H), 3.76 (s, 3H), 1.50 (s, 9H), 1.49 (s, 9H), 1.42 (s, 3H), 1.34 (s, 3H), 1.06 (s, 9H) ppm. ^{13}C {^{1}H} NMR (125 MHz, CD_3OD): δ (ppm): 158.8, 152.5, 149.8, 146.0, 136.1, 133.5, 129.9, 127.9, 119.6, 110.9, 103.9, 98.6, 84.4, 83.4, 76.9, 64.9, 60.6, 58.4, 55.6, 45.5, 39.8, 36.2, 28.4, 28.3, 27.4, 27.3, 27.1, 19.5, 14.4 ppm.

tert-butyl (S,Z)-2-((tert-butoxycarbonyl)imino)-5-(((4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-3-((2,4-dimethoxybenzyl)carbamoyl)imidazolidine-1-carboxylate (102b)



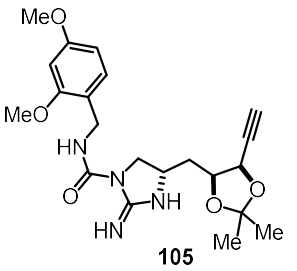
102b

To a solution of **101b** (712 mg, 0.720 mmol) in benzene (7mL) and water (0.13 mL, 7.20 mmol) was added tibutylborane (0.75 mL, 2.88 mmol). Immediately followed by the addition, air (73 mL, 0.71 mmol O_2) was bubbled through the solution *via* syringe pump over 16 hours. The reaction was quenched with NaOH (2 mL, 3 M) and H_2O_2 (2 mL) at the same time and stirred for 10 minutes. The mixture was then extracted with EtOAc (3 x 25 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography (4:1 Hex:EtOAc) gave **102b** (198.4 mg, 39.2%) as a colorless oil. R_f = 0.76 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): 9.33 (t, J = 7 Hz, 1H), 7.69-7.61 (m, 4H), 7.42-7.34 (m, 6H), 7.18 (d, J = 10 Hz, 1H), 6.44-6.36 (m, 2H), 4.42-4.37 (m, 2H), 4.28-4.23 (m 1H), 4.03-4.98 (m, 1H), 3.86 (s, 3H), 3.77 (s, 3H), 3.75-3.67 (m, 4H), 2.01-1.93 (m 1H), 1.81-1.75 (m 1H), 1.48 (s, 9H), 1.43 (s, 9H), 1.37 (s, 3H), 1.36 (s, 3H), 1.03 (s, 9H) ppm. ^{13}C {^{1}H} NMR (125 MHz, CD_3OD): δ (ppm): 160.4, 158.7, 157.3, 152.5, 149.6, 147.2, 135.6, 133.0, 129.8, 127.7, 119.3, 109.4, 103.6, 98.4, 83.6, 81.8, 80.0, 76.9, 75.4, 63.6, 55.4, 53.9, 47.0, 39.7, 38.2, 28.2, 28.0, 27.3, 27.0, 26.8, 19.1 ppm.

(S)-N-(2,4-dimethoxybenzyl)-4-(((4S,5R)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-2-iminoimidazolidine-1-carboxamide (105)

Aldehyde **104** (210 mg, 0.34 mmol) was dissolved in MeOH and cooled to 0 °C, followed by the addition of Bestmann-Ohira reagent (97 mg, 0.5 mmol). K_2CO_3 (94 mg, 0.68

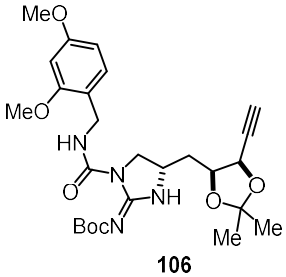
mmol) was added in portions and the reaction stirred for 12 hours. The reaction was then quenched with NH_4Cl (20 mL), and then washed with EtOAc (3 x 30 mL) the organic layers were dried over Na_2SO_4 and concentrated to yield **105** in 53%. R_f = 0.40 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): 7.02 (d, *J* = 10.5 Hz, 1H), 6.41-6.37 (m, 2H), 5.00-4.97 (m, 5H), 4.27-4.11 (m, 4H), 3.88-3.84 (m, 2H), 3.82 (s, 3H), 3.75 (s, 3H), 2.32 (d, *J* = 17 Hz, 1H), 1.85-1.79 (m, 1H), 1.43 (s, 3H), 1.38 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, CD_3OD): δ (ppm): 160.1, 158.1, 156.1, 1.49.1, 128.4, 117.3, 110.9, 104.0, 98.4, 79.6, 78.5, 75.5, 70.2, 55.3, 51.5, 48.6, 39.6, 37.7, 27.1, 26.1 ppm.



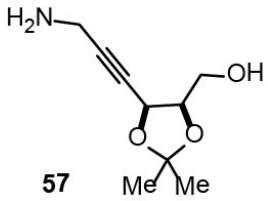
105

tert-butyl ((S,E)-1-((2,4-dimethoxybenzyl)carbamoyl)-4-(((4S,5R)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)imidazolidin-2-ylidene)carbamate (106)

Alkyne **105** (77.1 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (20 mL), followed by the addition of DMAP (3 mg, 0.018 mmol) and Boc_2O (121 mg, 0.5 mmol), the reaction stirred for 18 hours. The reaction was concentrated and purification by flash chromatography (1:1 Hex:EtOAc) gave **106** (72 mg, 77%) as a colorless oil. R_f = 0.54 (1:1 Hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): 7.10 (d, *J* = 8.5 Hz, 1H), 6.40 (s, 1H), 6.36 (d, *J* = 2.5 Hz, 1H), 5.03 (d, *J* = 6 Hz, 2H), 4.53 (t, *J* = 9.5 Hz, 1H), 4.20 (d, *J* = 7 Hz, 1H), 4.09-3.98 (m, 3H), 3.79 (s, 3H), 3.75 (s, 3H), 2.32 (d, *J* = 14.5 Hz, 1H), 1.94-1.86 (m, 1H), 1.56 (s, 9H), 1.40 (s, 3H), 1.37 (s, 3H) ppm. . ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 160.5, 158.5, 155.6, 154.2, 149.1, 129.7, 116.9, 111.4, 104.2, 98.7, 85.5, 79.0, 77.0, 75.9, 70.5, 55.7, 55.6, 54.8, 45.8, 40.3, 36.0, 28.2, 27.2, 26.4 ppm.



106


H2N
OH
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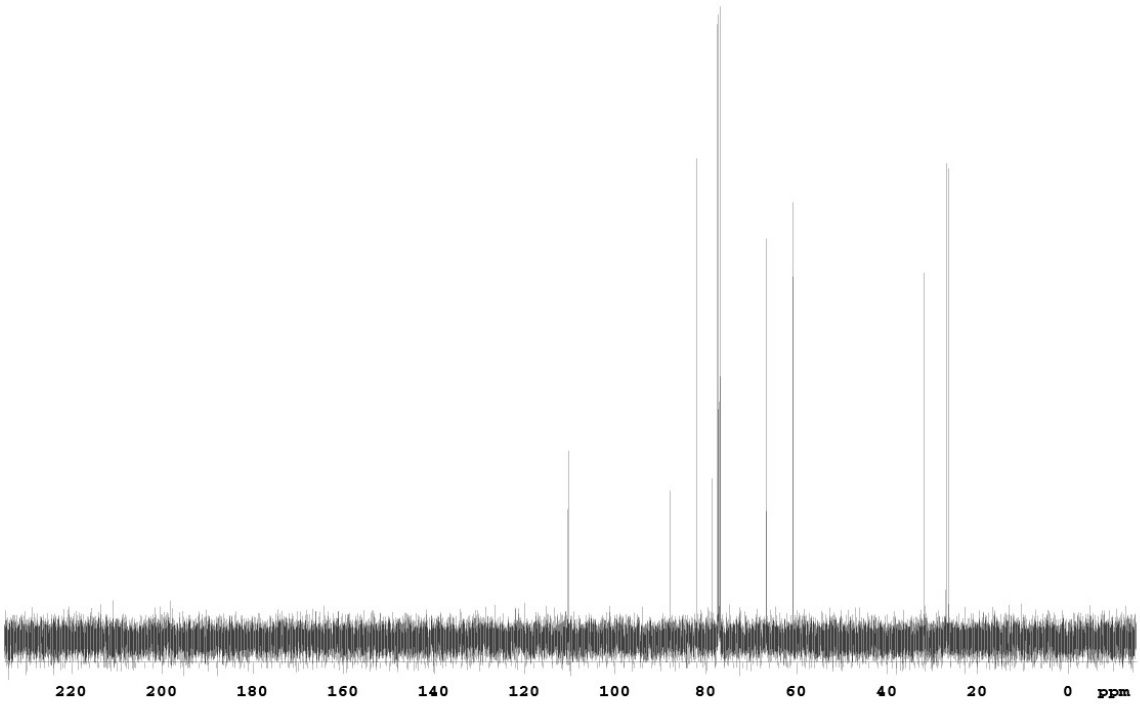
O
Me
Me

57

9
8
7
6
5
4
3
2
1
ppm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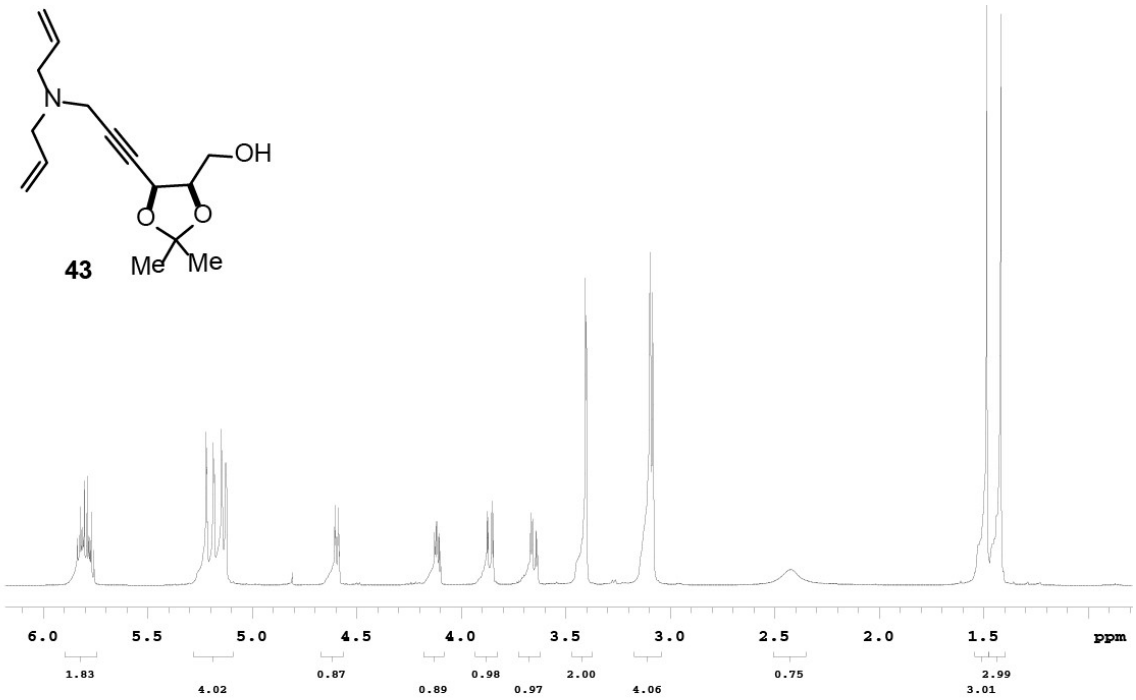
80
60
40
20
0
ppm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OH
O
O
Me
Me
43
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5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
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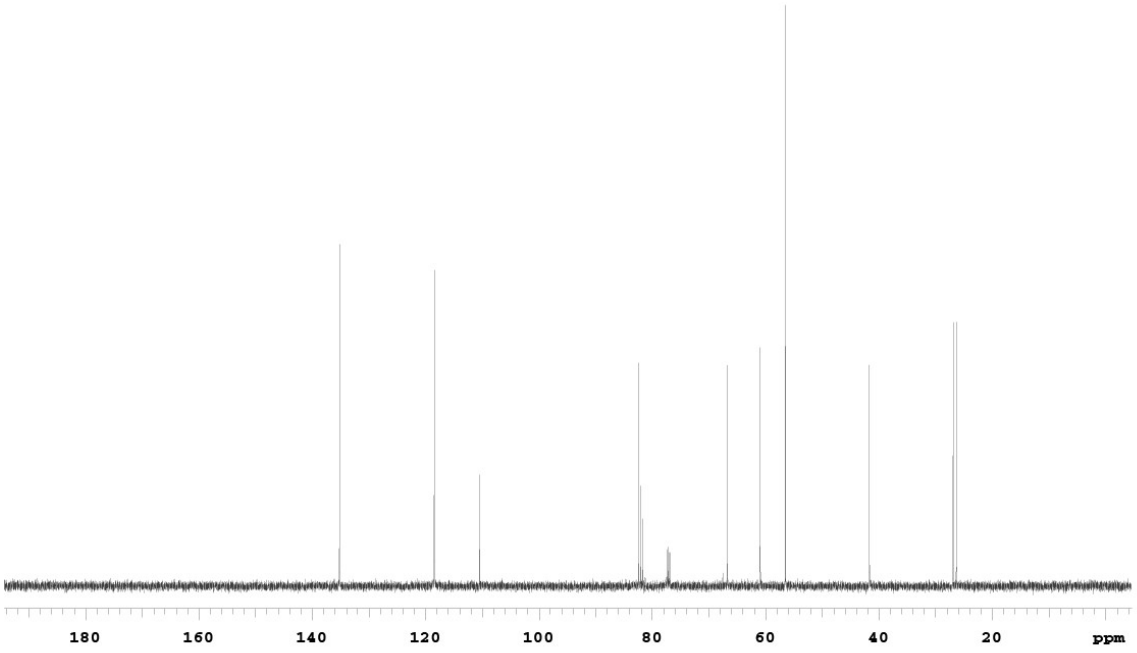
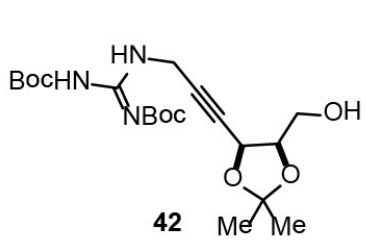
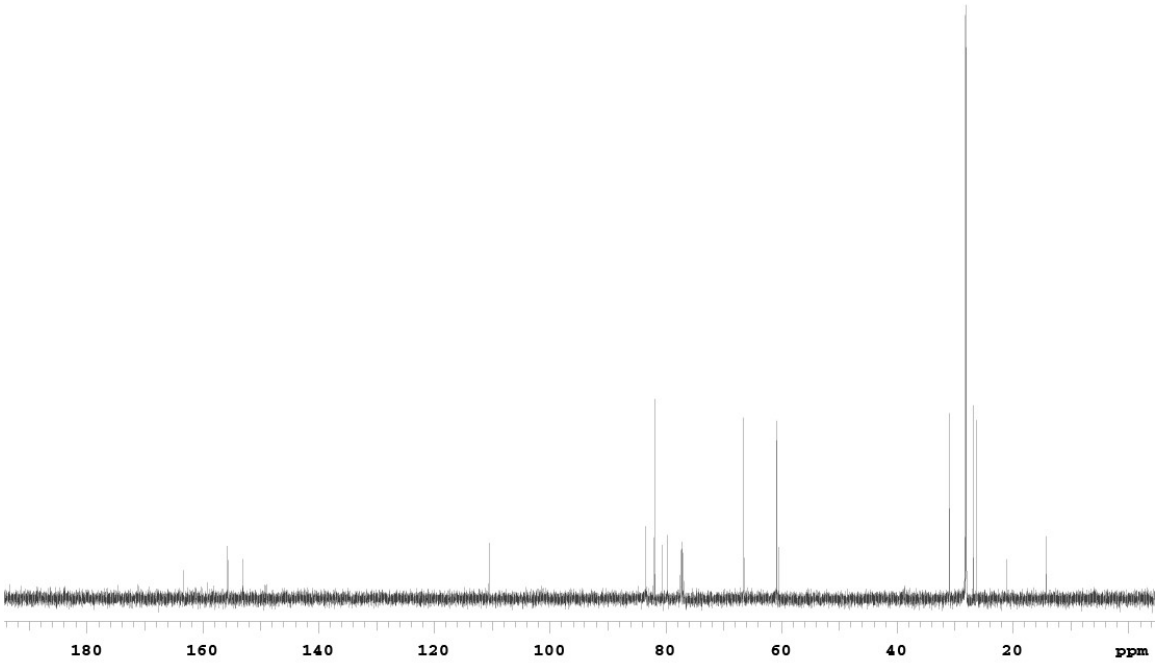
4.02
0.87
0.89
0.98
0.97
2.00
4.06
0.75
2.99
3.01


180
160
140
120
100
80
60
40
20
ppm


HN
BocHN
NBoc
OH
Me
Me

42

12
10
8
6
4
2
0
-2 ppm
0.83
1.09
0.99
2.24
1.07
2.26
1.08
3.59
32.70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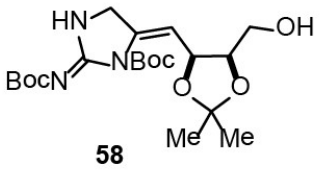
140
120
100
80
60
40
20
ppm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OH
NBoc
BocN
O
O
Me
Me

58

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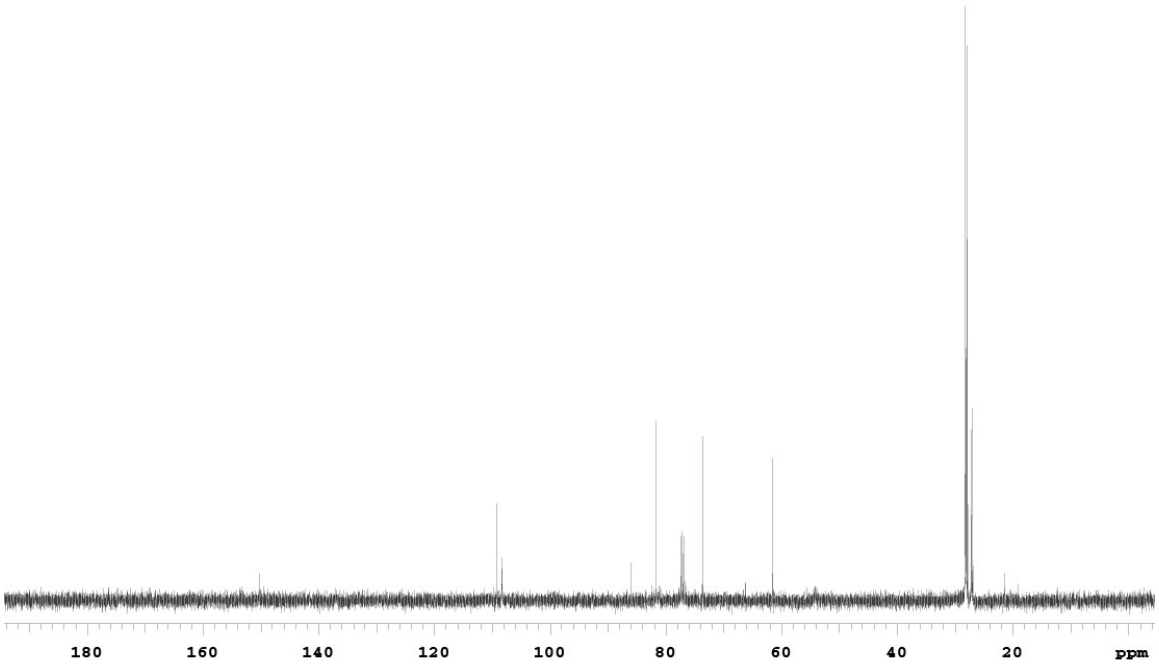
10
8
6
4
2
0
-2
ppm
1.01
2.13
0.98
0.94
2.14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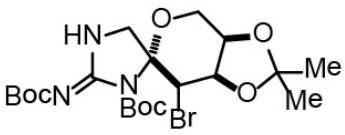
140
120
100
80
60
40
20
ppm


HN
O
O
Me
Me
BocN
N
Boc
Br
O

67

14
13
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm
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5.05
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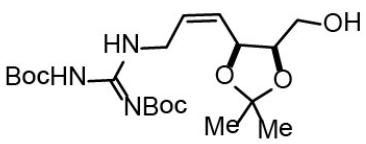
3.02
2.54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NBoc
O
O
Me
Me

71

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10
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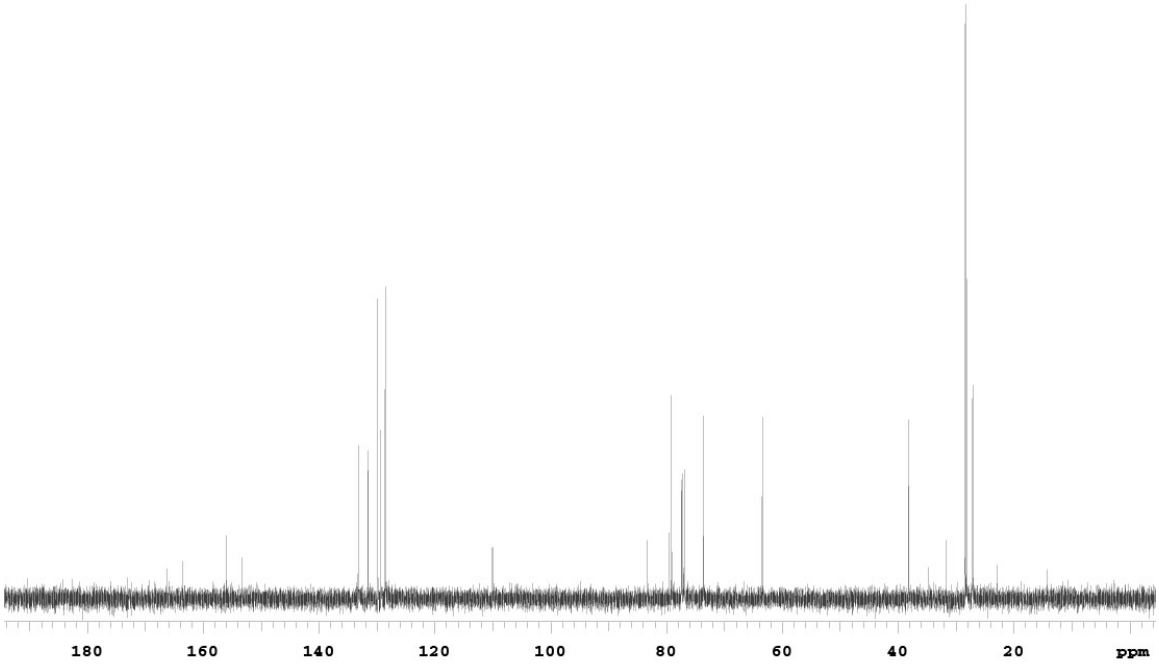
8
7
6
5
4
3
2
1
ppm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160
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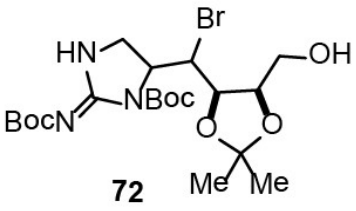
120
100
80
60
40
20
ppm


Br
HN
OH
NBoc
BocN
O
O
Me
Me

72

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8
7
6
5
4
3
2
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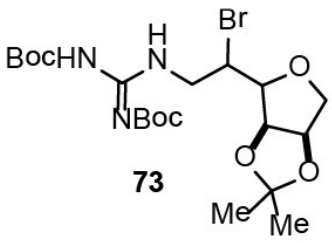
ppm
0.96
9.18


Br
H
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BocHN
NBoc
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O
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Me

73

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6
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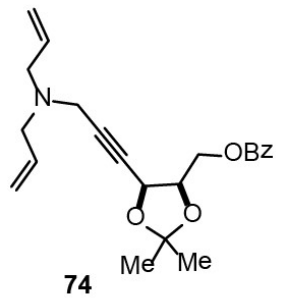
5.93
12.76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OBz
O
O
Me
Me

74

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10
8
6
4
2
0
-2
ppm
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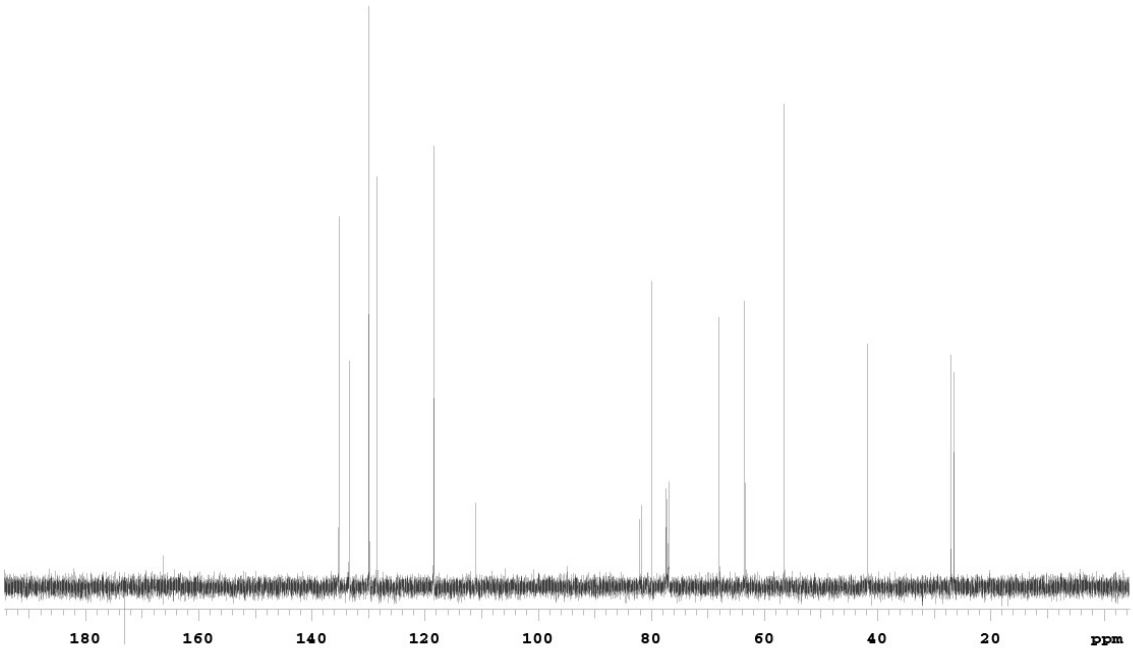
0.91
1.83
2.10
2.00
0.99
1.96
1.90
4.00
2.92
2.93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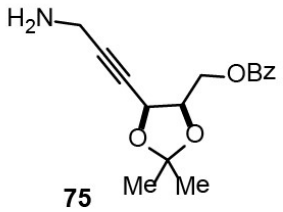
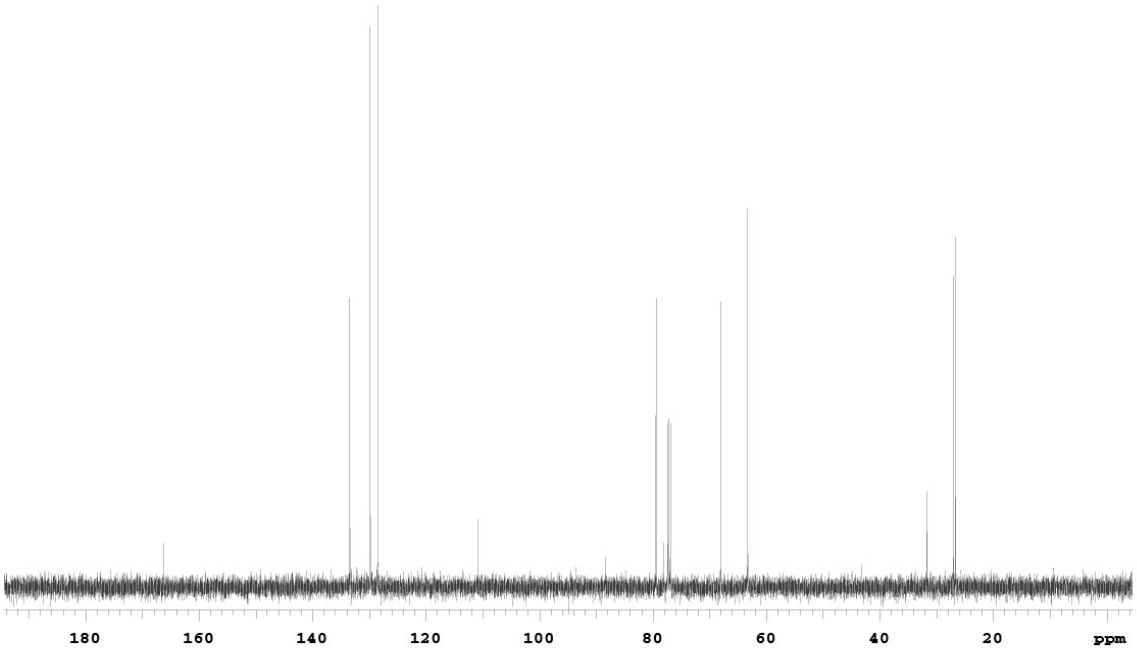
160
140
120
100
80
60
40
20
ppm


H2N
OBz
O
O
Me
Me

75

12
10
8
6
4
2
0
-2
ppm
1.91
2.15
0.99
1.92
3.27
3.69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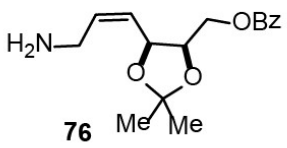
160
140
120
100
80
60
40
20
ppm


H2N
OBz
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O
Me
Me

76

12
10
8
6
4
2
0
-2
ppm
2.25
2.34
1.13
0.98
0.95
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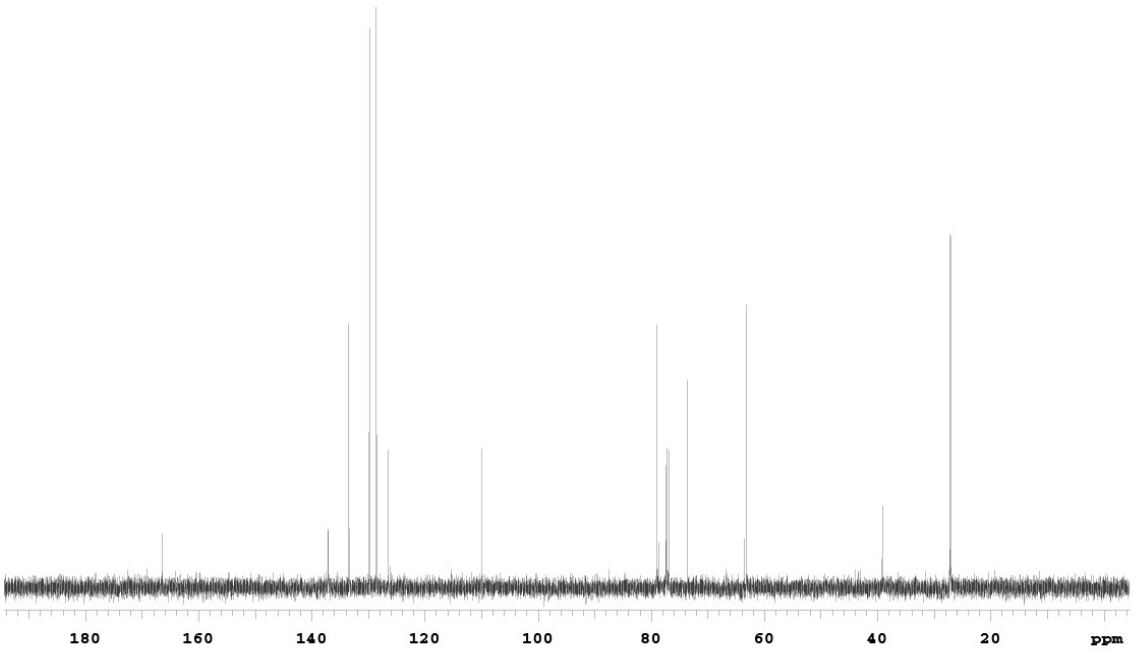
1.19
0.17
0.98
1.37
1.01
1.41
2.47
3.42
3.56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20
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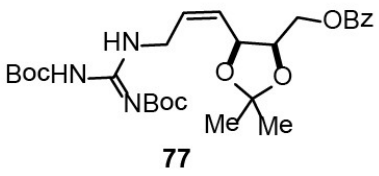
HN
OBz
BocHN
O
O
NBoc
Me
Me

77

12
10
8
6
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2
0
-2
ppm
0.93
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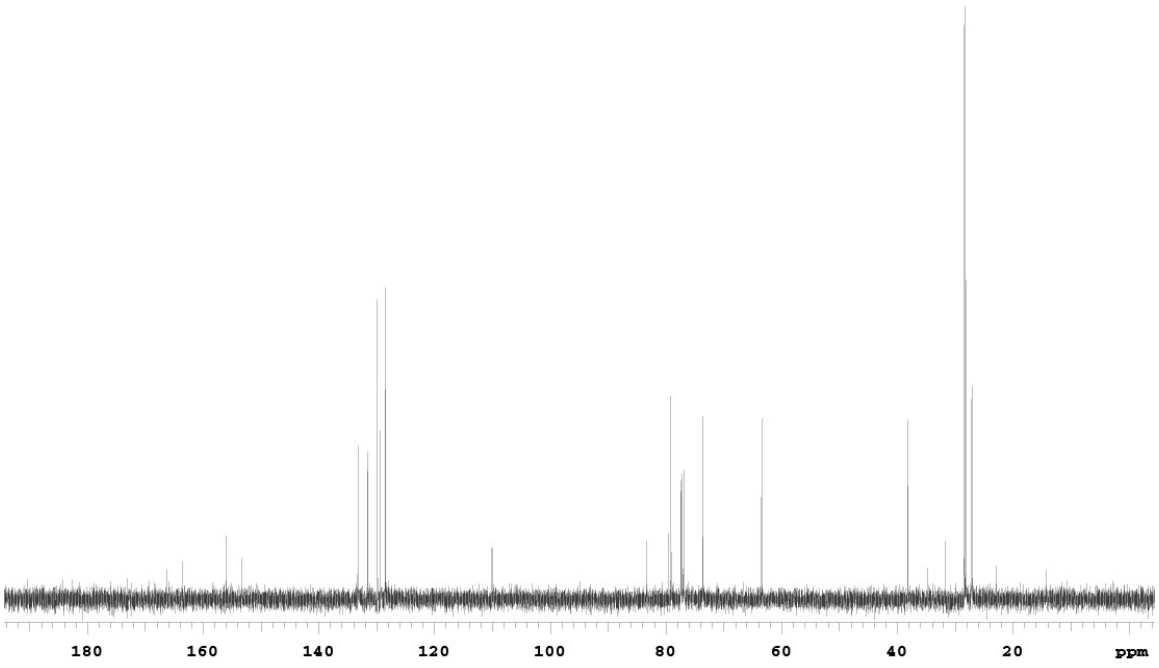
1.92
1.00
1.01
0.88
0.97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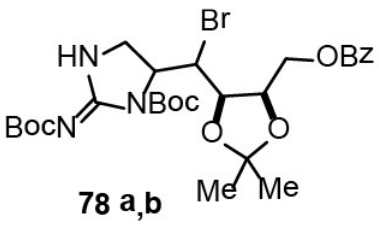
Br
HN
OBz
NBoc
BocN
O
O
Me
Me

78 a,b

9
8
7
6
5
4
3
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1
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1.99
2.07
1.00
0.99
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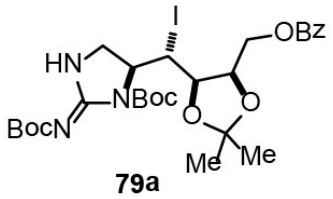
2.08
0.96
1.03
2.75
21.52


I
OBz
HN
NBoc
BocN
O
O
Me
Me

79a

9
8
7
6
5
4
3
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0.82 1.90
3.30 0.97
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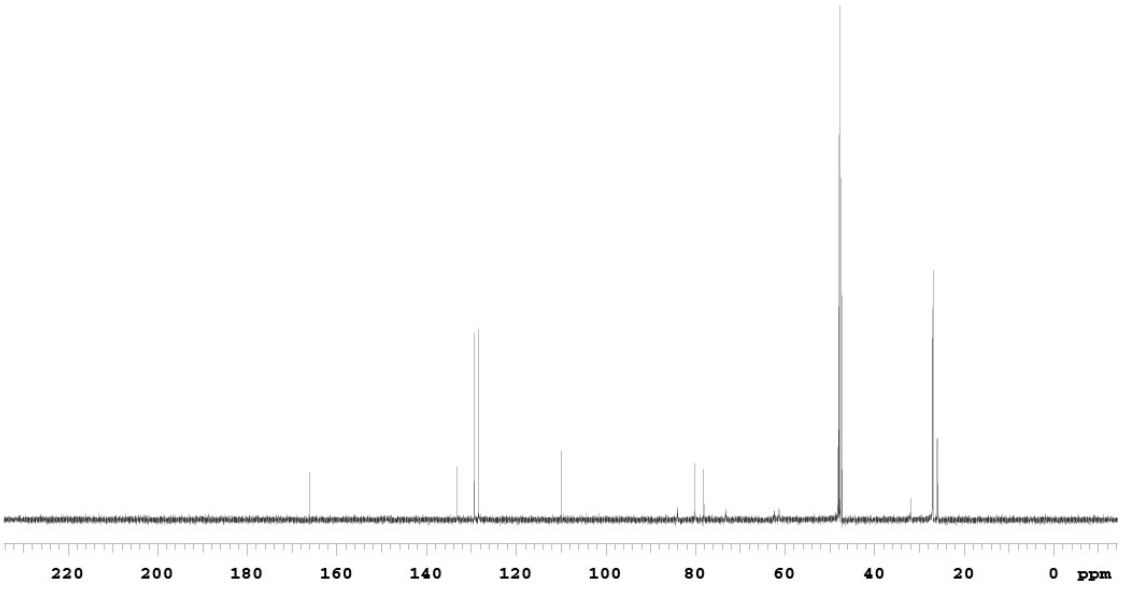
1.00
0.83
0.67
9.17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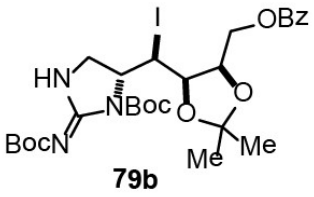
I
OBz
HN
NBoc
O
O
BocN
Me
Me

79b

11
10
9
8
7
6
5
4
3
2
1
ppm
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1.55 1.94
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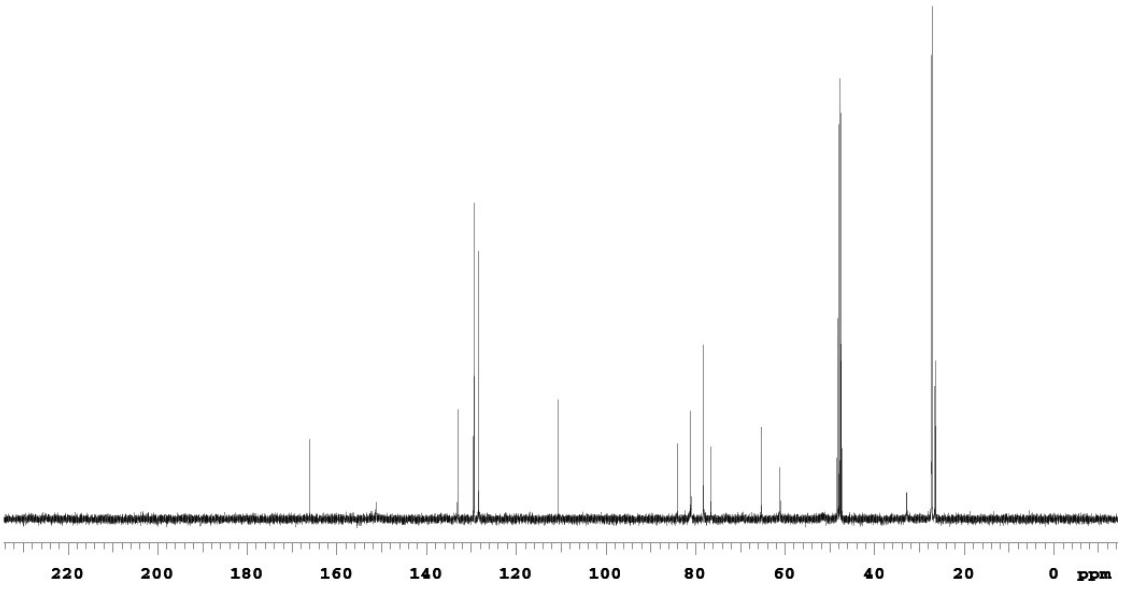
2.06 4.03 1.00
8.85 8.88
8.20 8.78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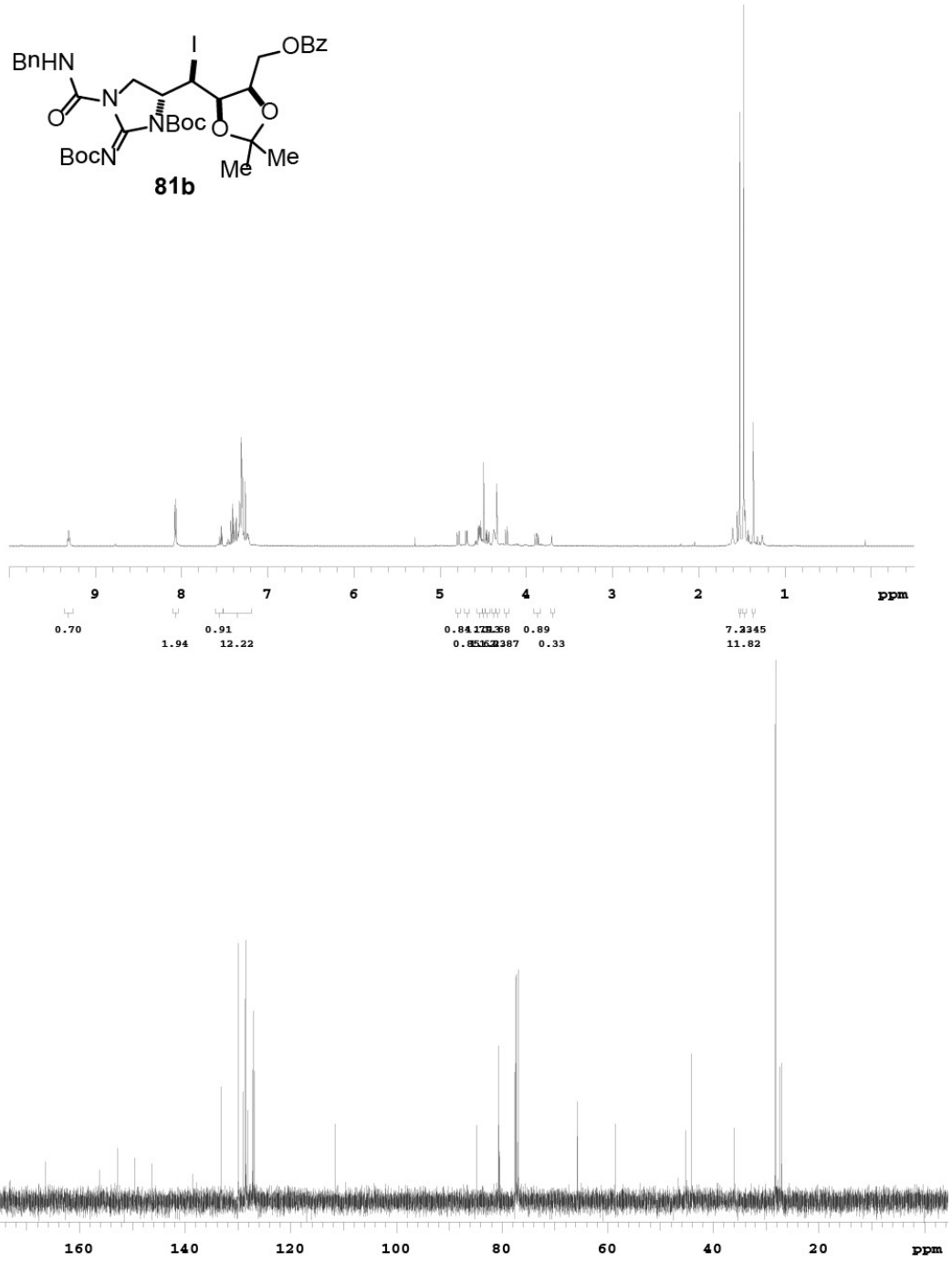
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I
OBz
N
O
NBoc
O
O
BocN
Me
Me
81b
9
8
7
6
5
4
3
2
1
ppm
0.70
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0.89
0.33
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60
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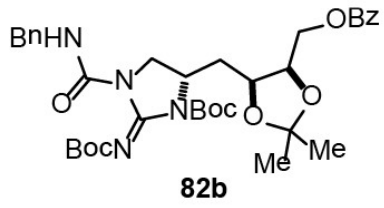
20
ppm


BnHN
OBz
N
O
NBoc
O
O
BocN
Me
Me

82b

9
8
7
6
5
4
3
2
1
ppm
0.76
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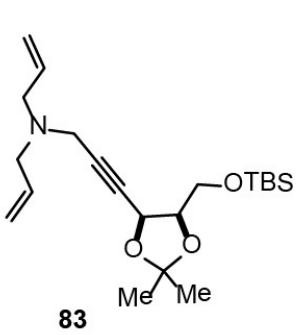
0.97
7.02
2.35
1.95
1.96
4.26
1.42
1.10
0.76
0.74
28.97


N
OTBS
O
O
Me
Me

83

9
8
7
6
5
4
3
2
1
ppm
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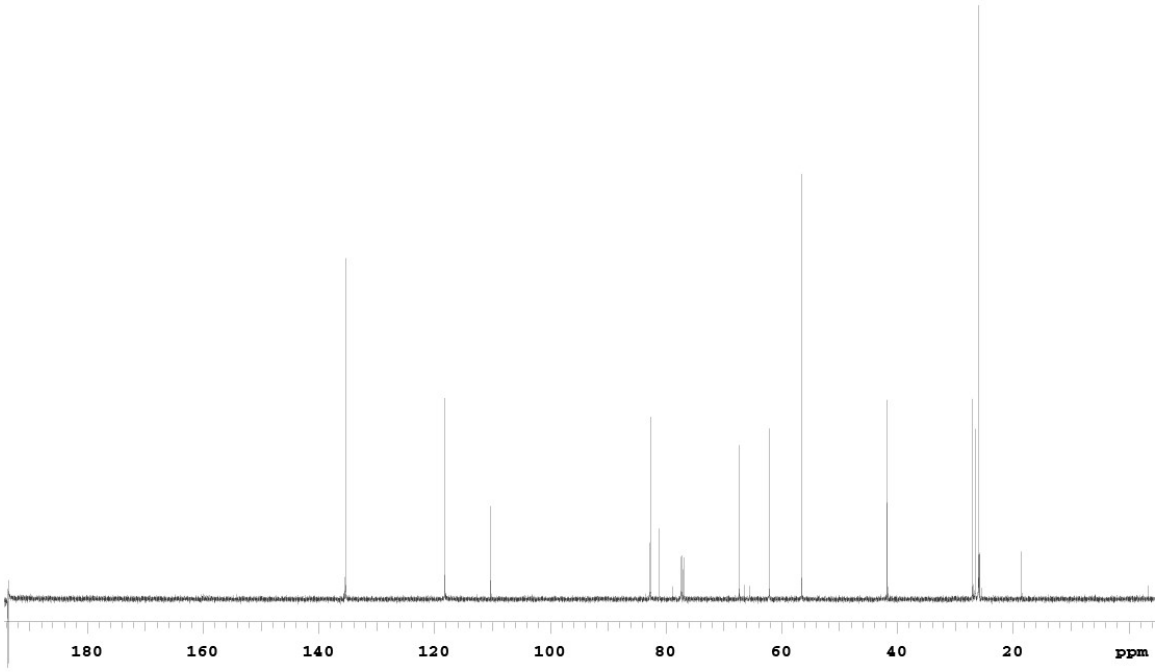
2.10
2.11
0.92
1.05
2.08
2.13
4.29
2.99
3.02
10.31
5.98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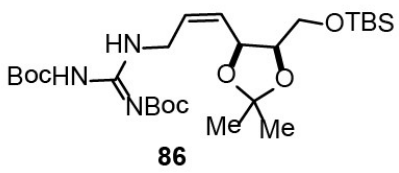
100
80
60
40
20
ppm


HN
BocHN
NBoc
OTBS
O
O
Me
Me

86

10
9
8
7
6
5
4
3
2
1
ppm
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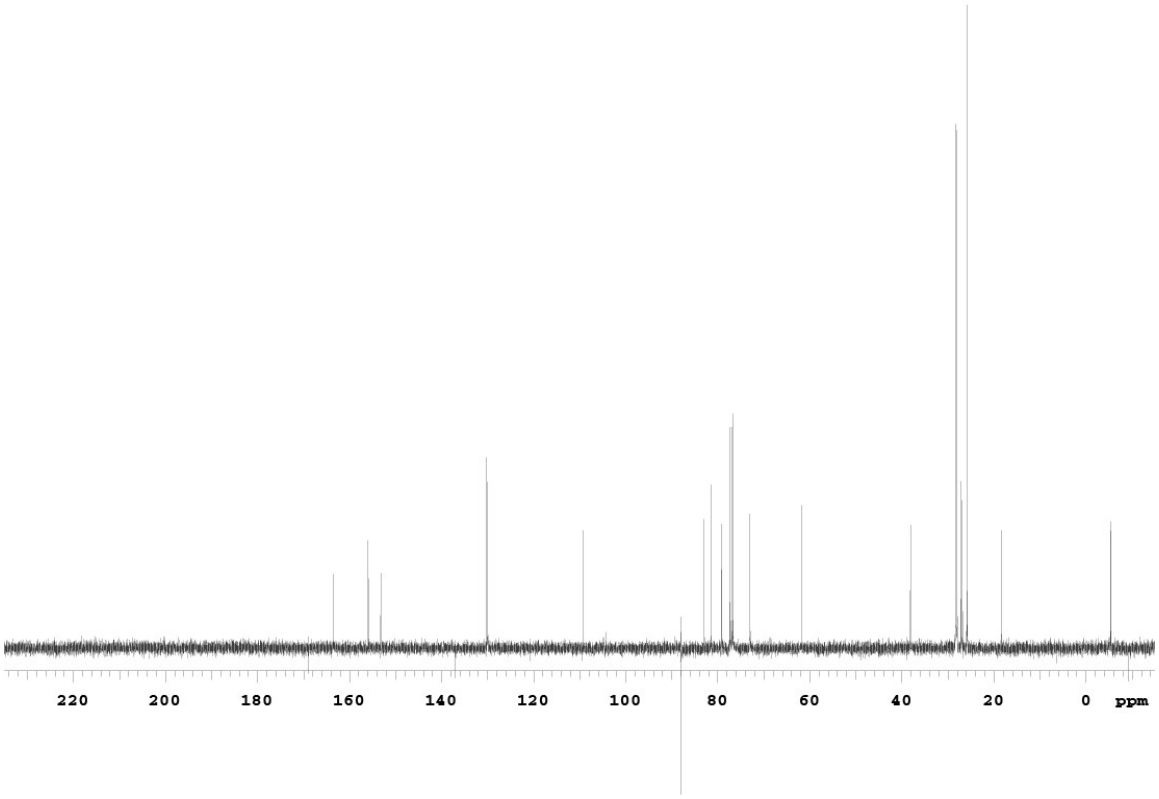
1.10
0.99
1.00
2.19
3.30
22.30
6.85
10.44
6.29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120
100
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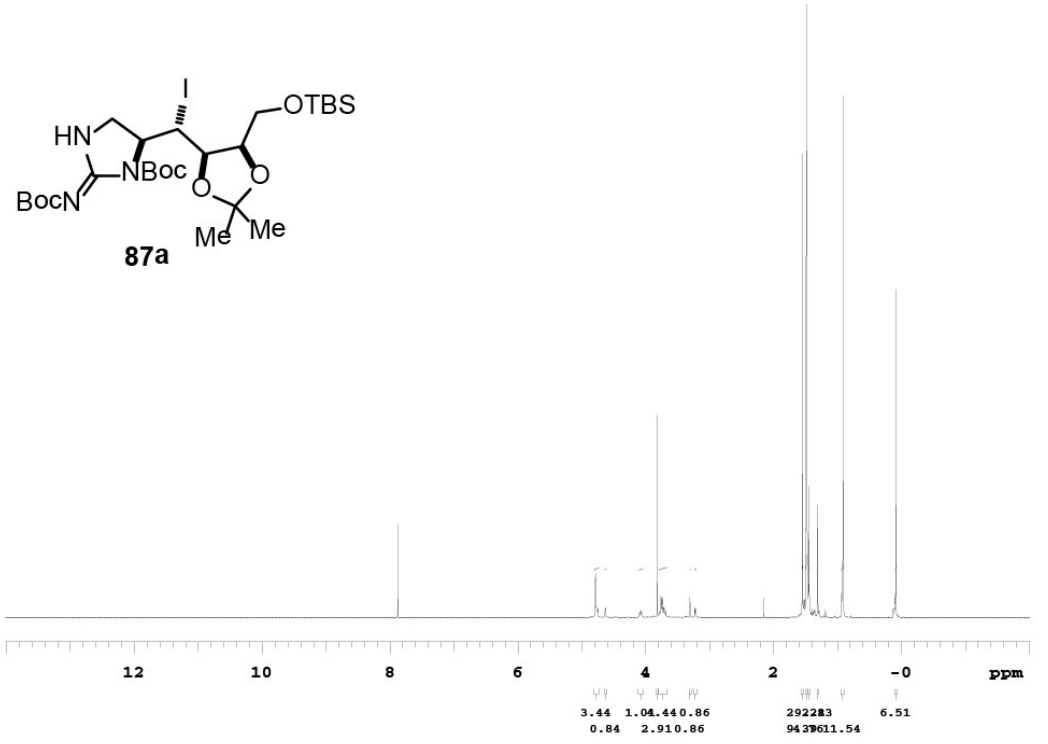
0
ppm


I
OTBS
HN
NBoc
BocN
O
O
Me
Me
87a
12
10
8
6
4
2
-0
ppm
3.44
1.04
4.44
0.86
6.51
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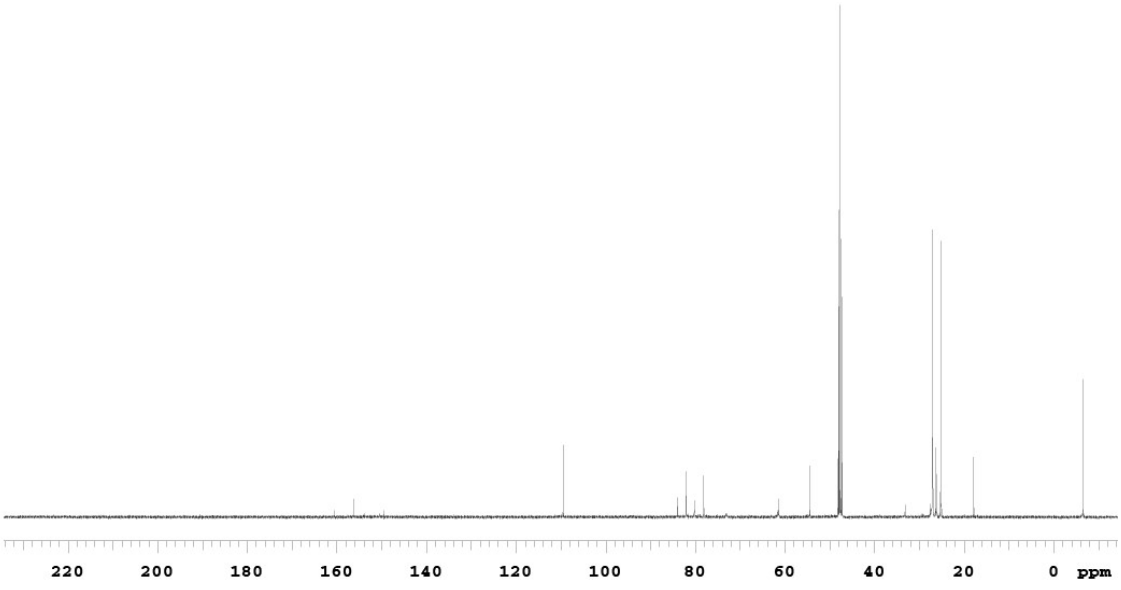
2.91
0.86
11.54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120
100
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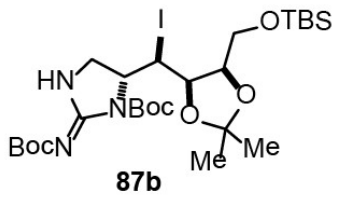
ppm


I
OTBS
HN
NBoc
O
O
BocN
Me
Me

87b

9
8
7
6
5
4
3
2
1
ppm
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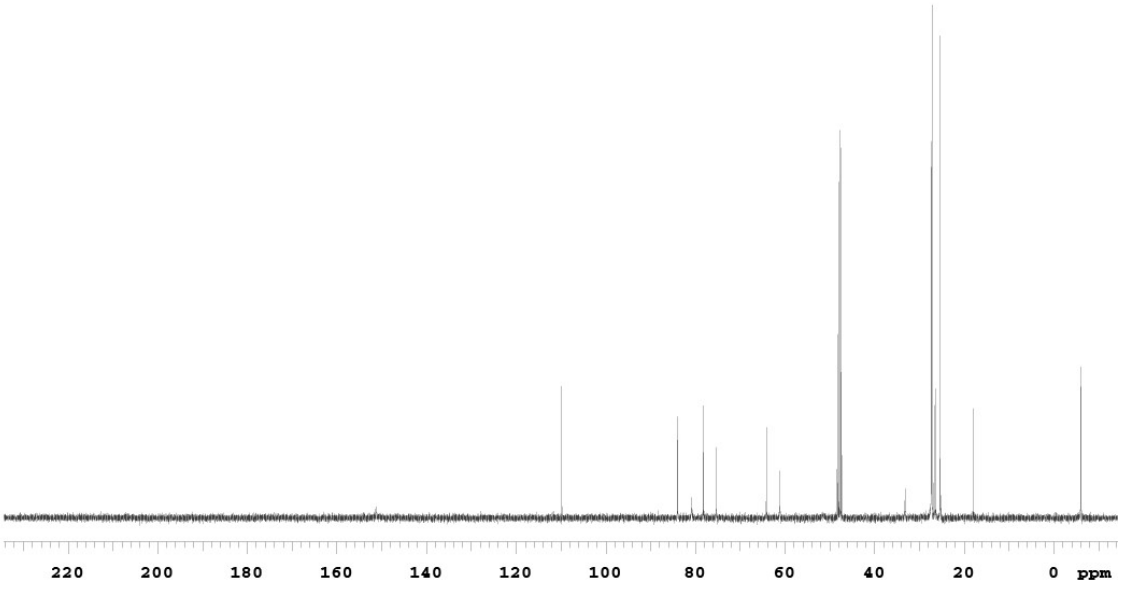
1.13
0.99
4.18
0.36
8.85
5.01
9.34
9.55
1.04
5.67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100
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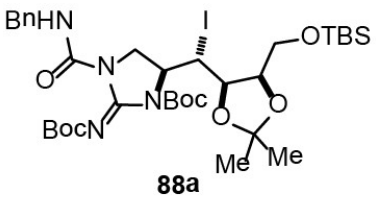
20
0
ppm


BnHN
I
OTBS
O
N
NBoc
O
O
BocN
Me
Me

88a

9
8
7
6
5
4
3
2
1
ppm
0.99
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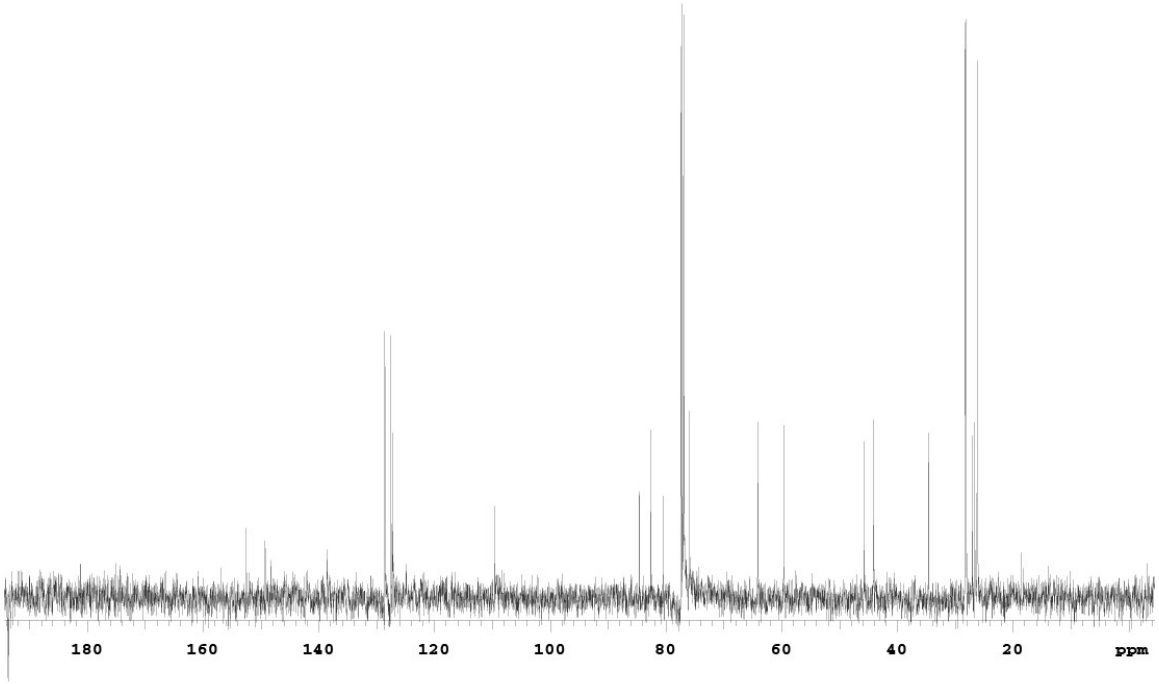
2.18
2.13
2.30
1.00
9.3654
9.75
10.45
2.97
6.51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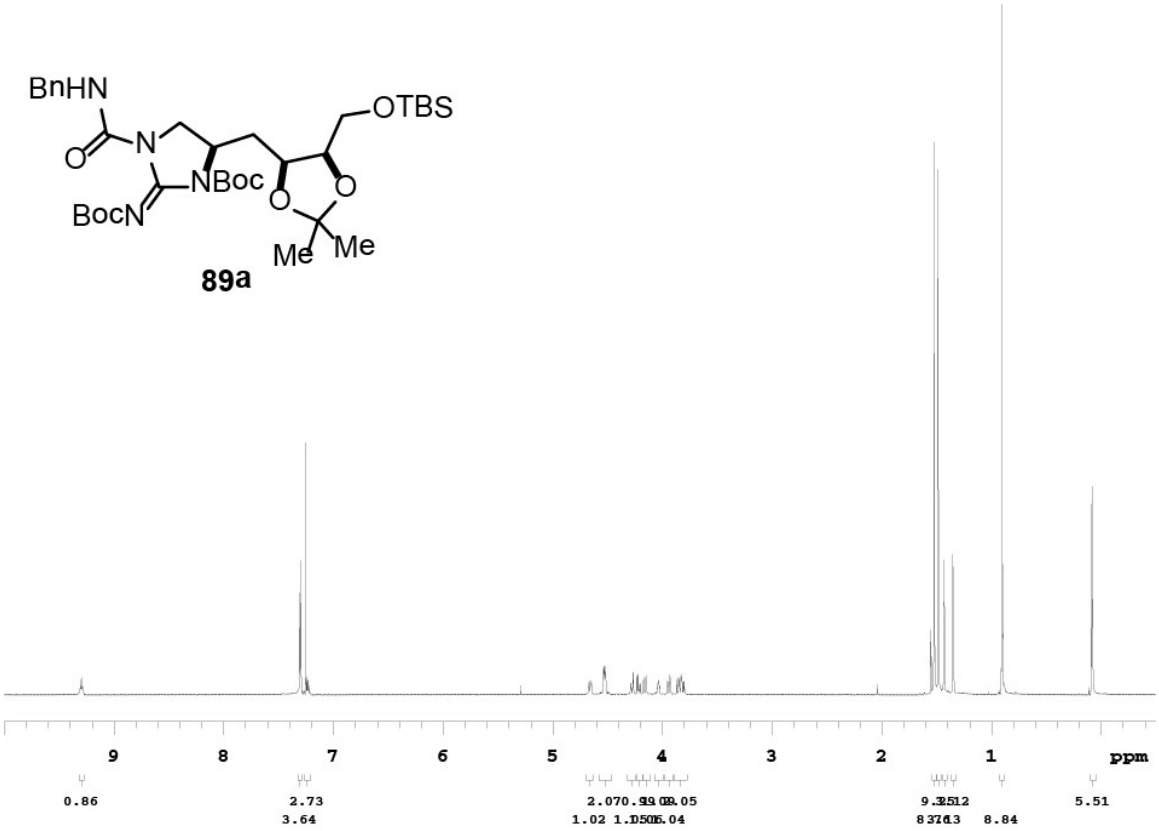
140
120
100
80
60
40
20
ppm


BnHN
O
N
NBoc
BocN
O
O
OTBS
Me
Me
89a
9
8
7
6
5
4
3
2
1
ppm
0.86
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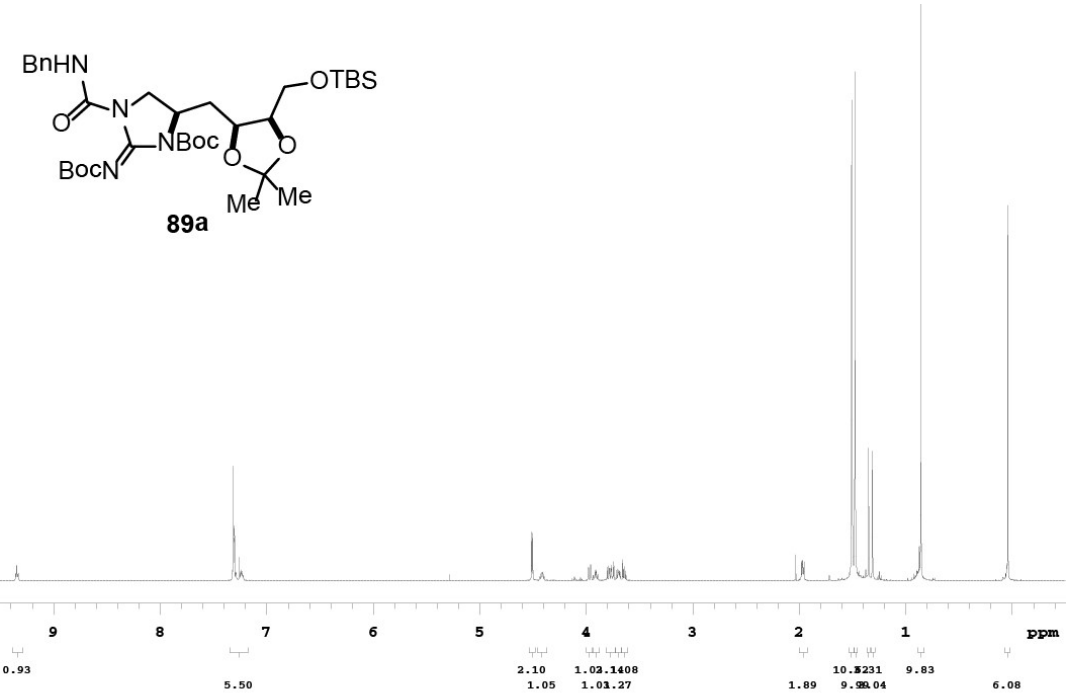
3.64
2.07
1.02
5.51
8.84


BnHN
O
N
NBoc
BocN
OTBS
O
O
Me
Me
89a
9
8
7
6
5
4
3
2
1
ppm
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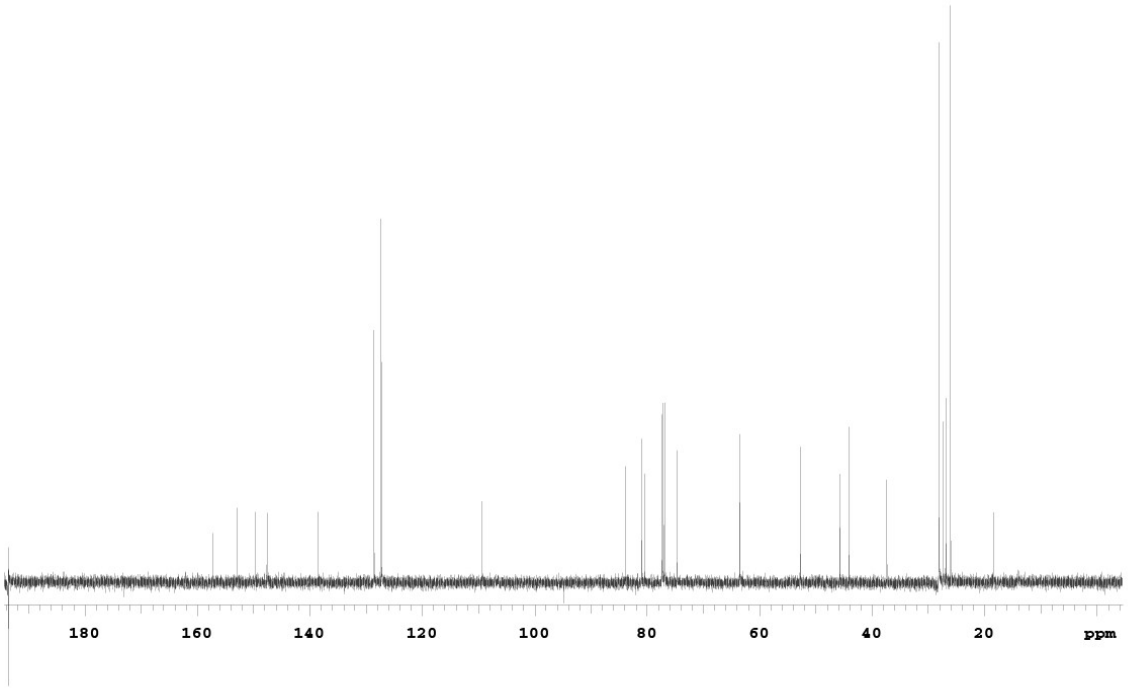
2.10
1.05
1.02
1.01
1.14
1.27
1.89
10.00
9.83
6.08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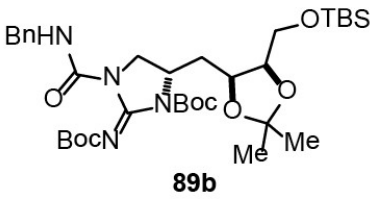
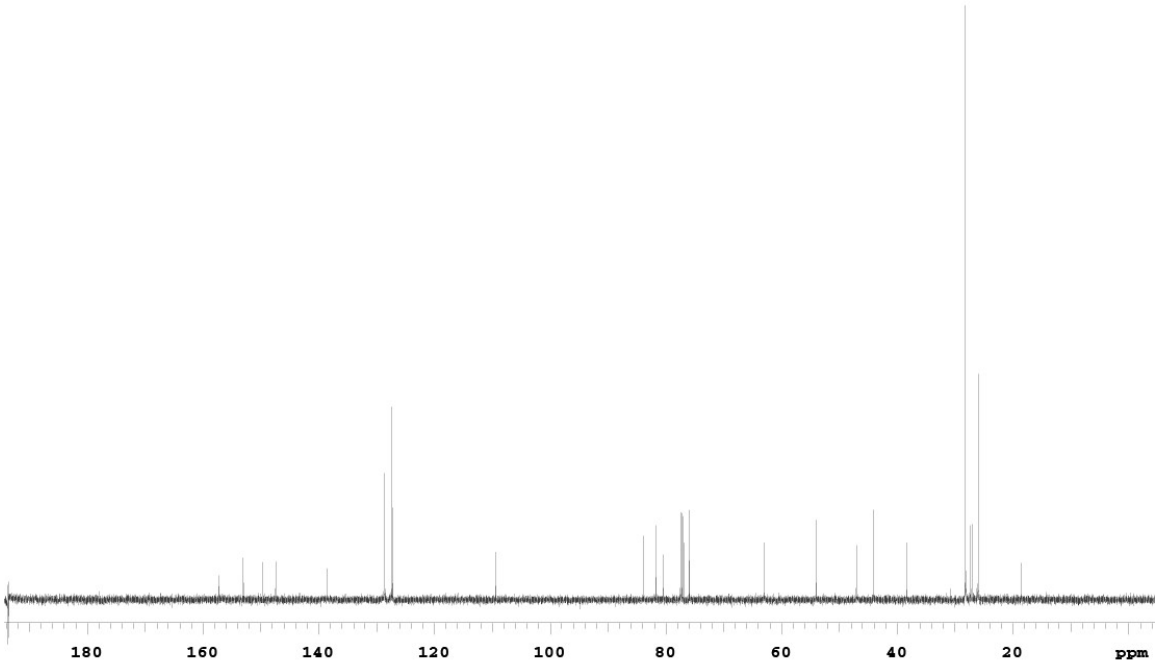
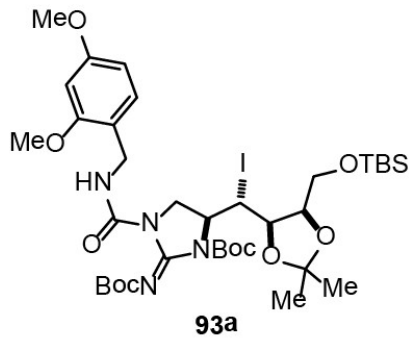
120
100
80
60
40
20
ppm


BnHN
N
O
NBoc
BocN
OTBS
O
O
Me
Me

89b

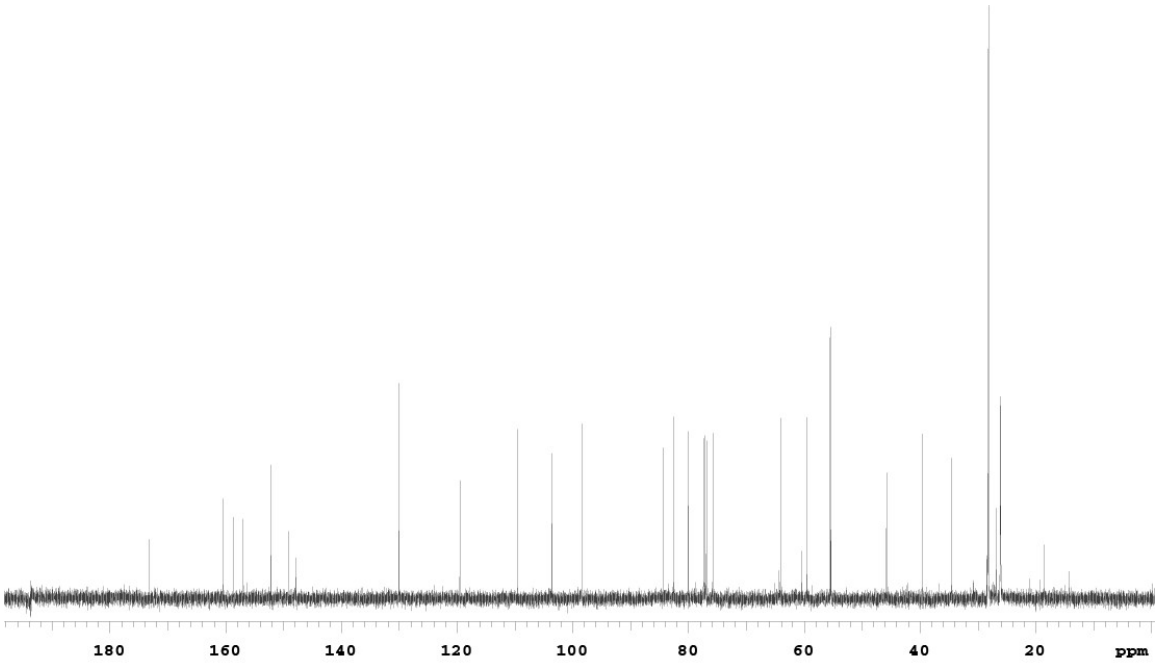
9
8
7
6
5
4
3
2
1
ppm
0.91
6.30
2.38
1.10
1.04
23.68
11.22
1.01
7.43
6.85

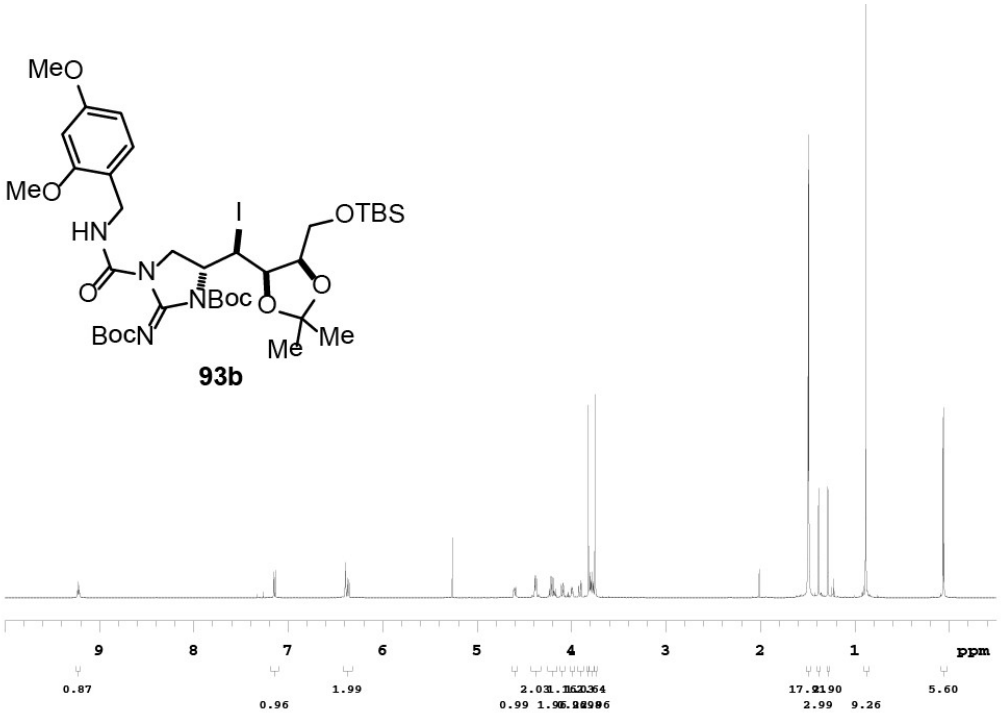

180
160
140
120
100
80
60
40
20
ppm


MeO
MeO
HN
O
N
I
OTBS
NBoc
O
O
BocN
Me
Me

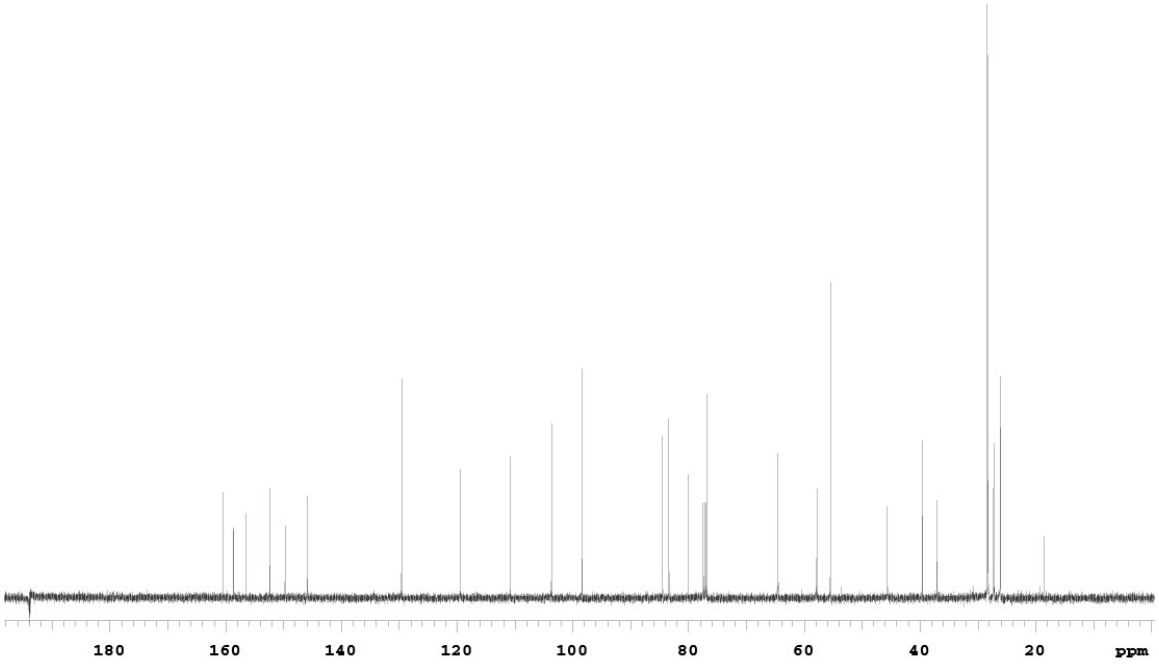
93a

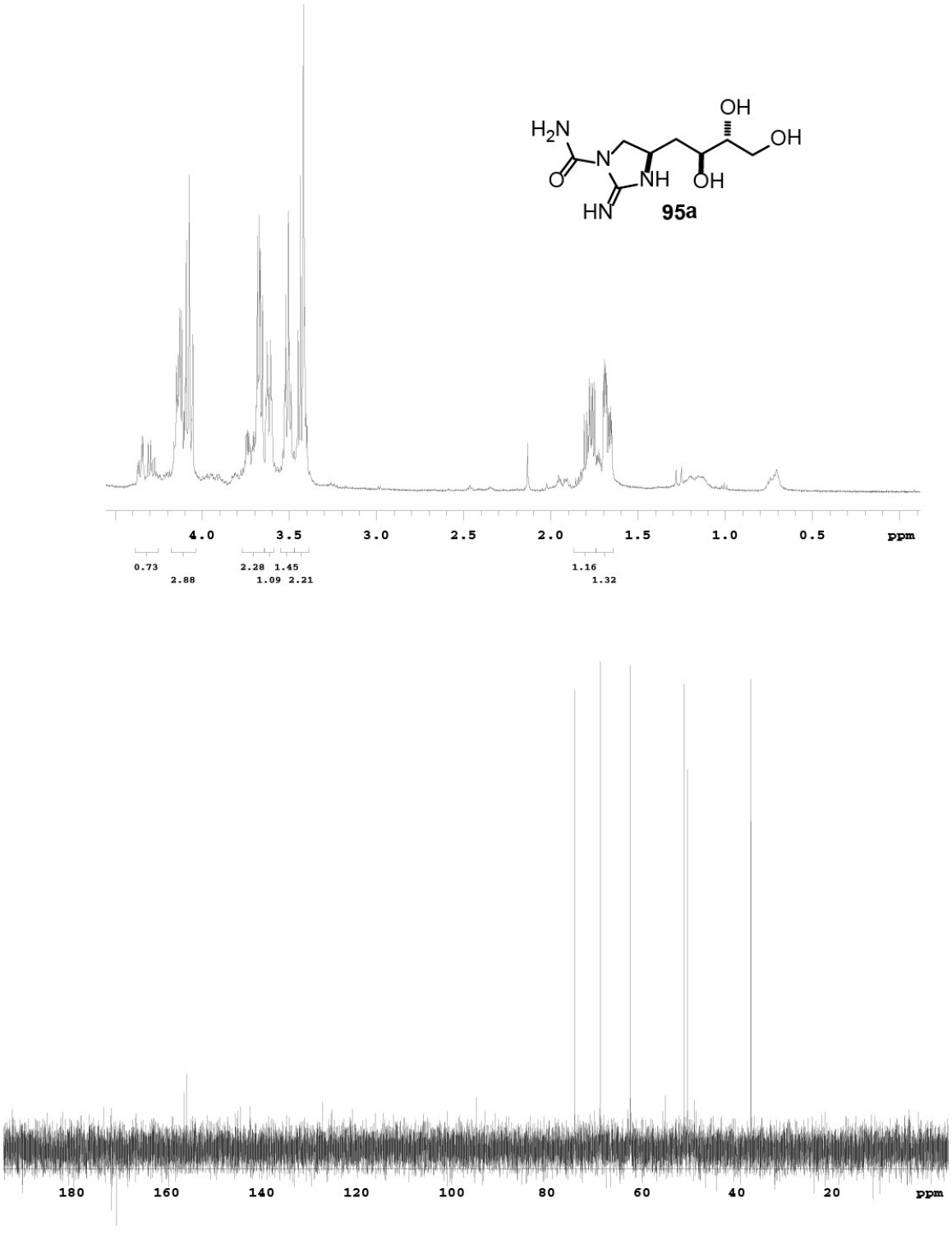
9
8
7
6
5
4
3
2
1
ppm
1.03
1.15
2.46
1.25
1.22
5.60
0.79
2.58
4.73
0.55
5.74
0.90
30.99
15.44
6.32
9.12

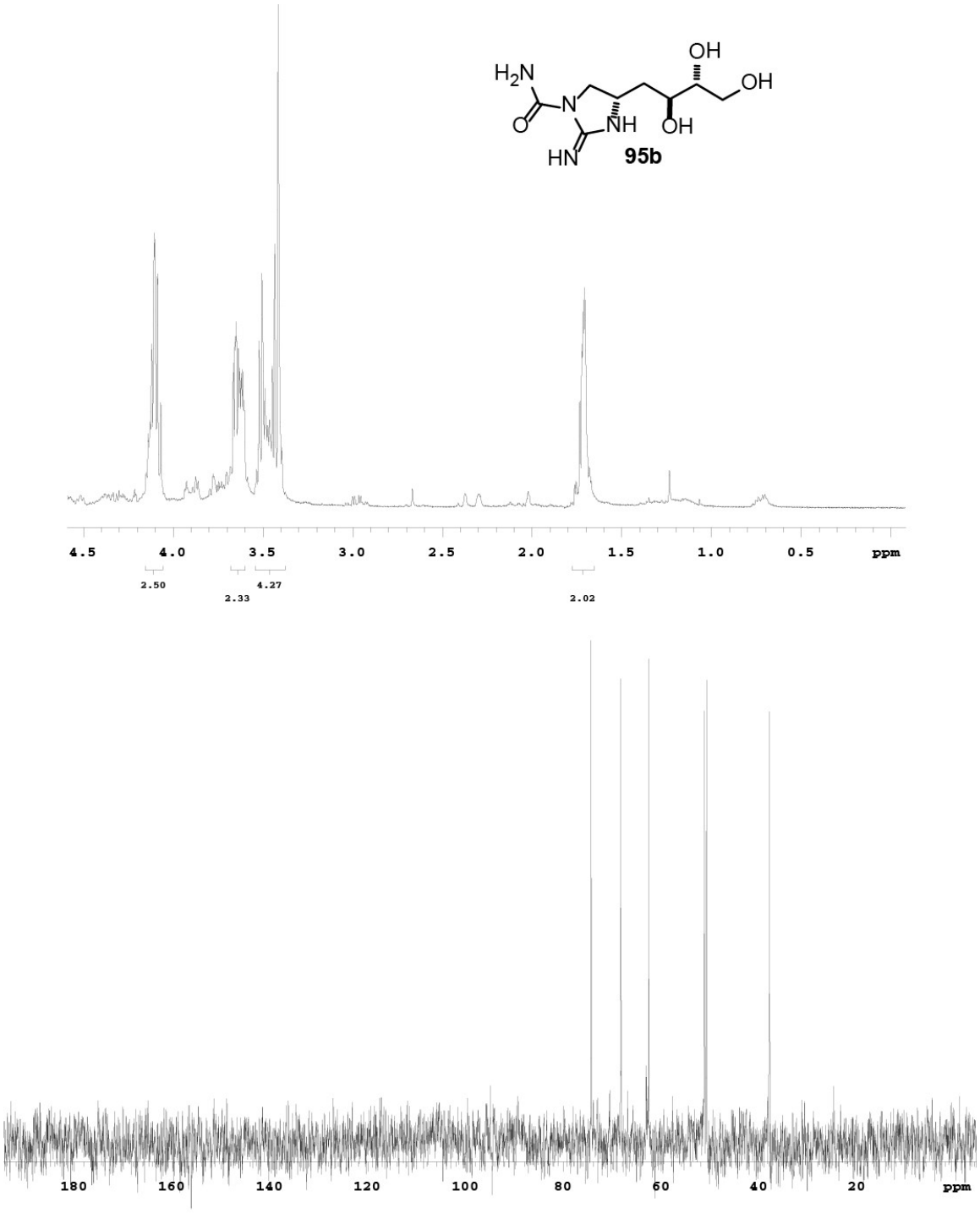

180
160
140
120
100
80
60
40
20
ppm

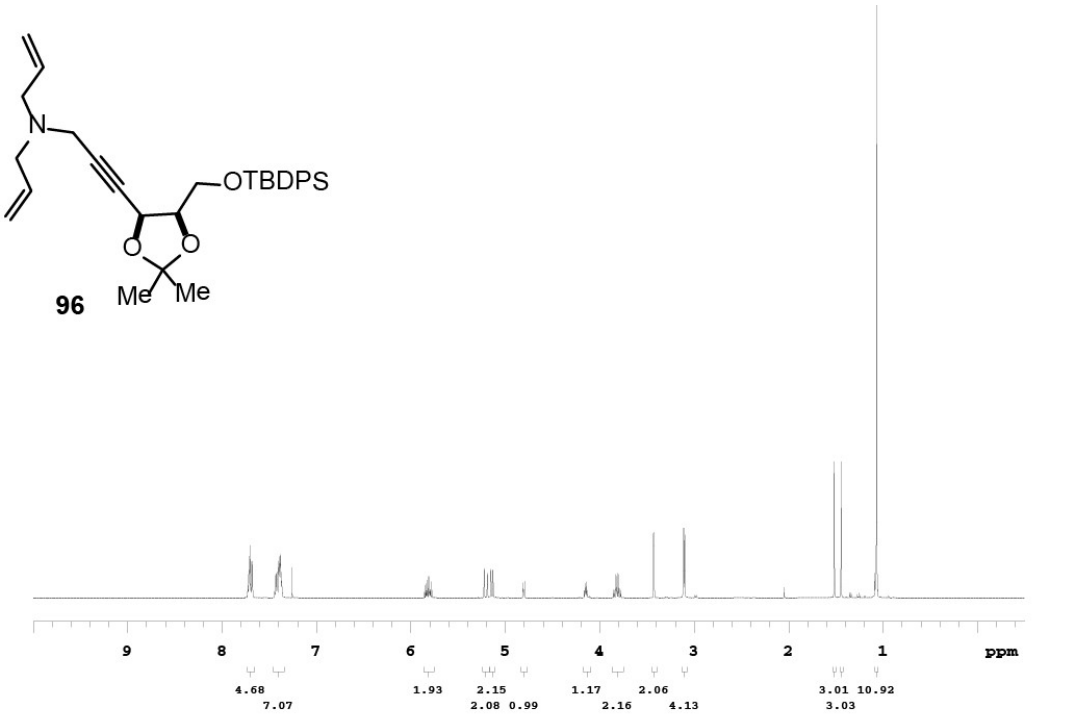

MeO
MeO
HN
O
N
I
OTBS
O
NBoc
O
BocN
Me
Me
9
8
7
6
5
4
3
2
1
ppm
0.87
0.96
1.99
2.99
9.26
5.60

93b

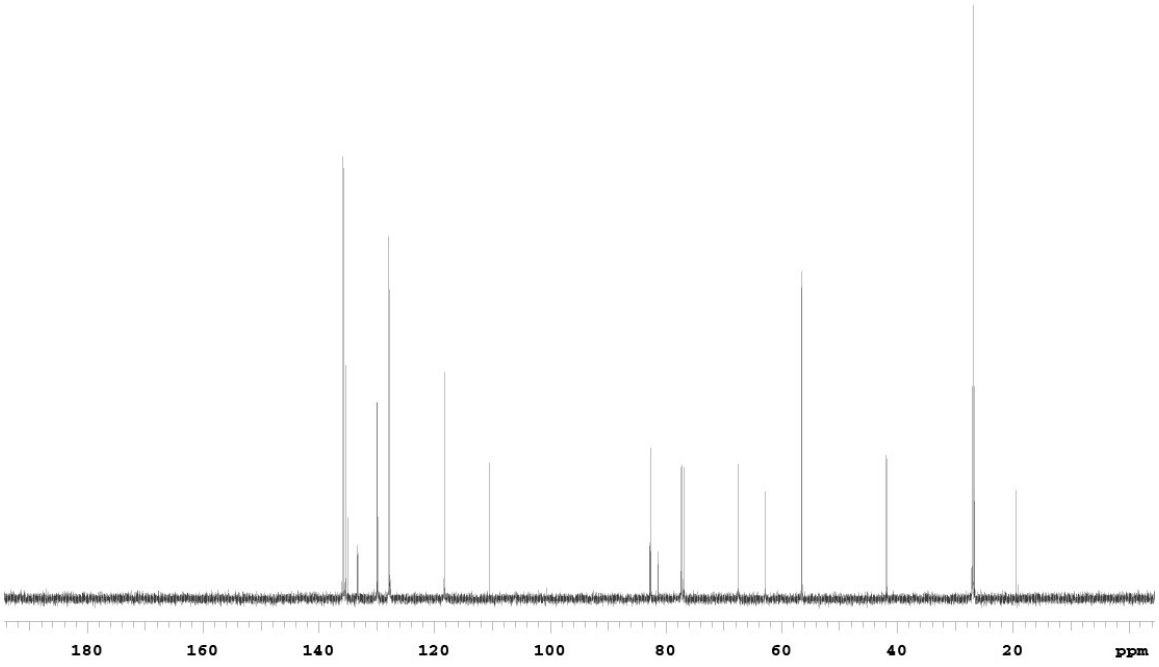

180
160
140
120
100
80
60
40
20
ppm

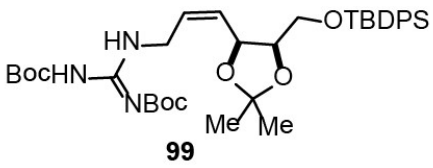

OH
H2N
OH
N
O
NH
OH
HN
95a
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.73
2.88
2.28
1.09
1.45
2.21
1.16
1.32
180
160
140
120
100
80
60
40
20
ppm


OH
H2N
OH
N
NH
OH
O
HN
95b
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.50
2.33
4.27
2.02
180
160
140
120
100
80
60
40
20
ppm


N
OTBDPS
O
O
Me
Me
9
8
7
6
5
4
3
2
1
ppm
4.68
7.07
1.93
2.15
2.08
0.99
1.17
2.16
2.06
4.13
3.01
10.92
3.03

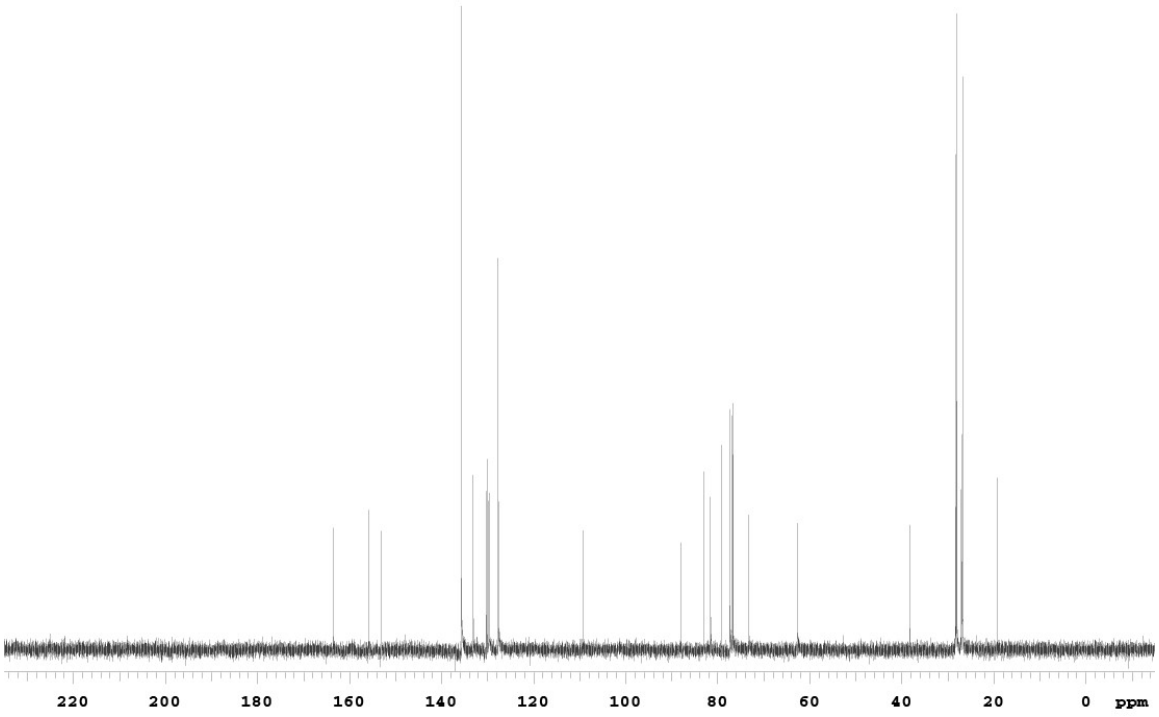
96

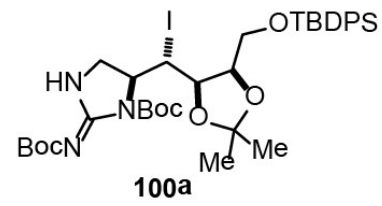

180
160
140
120
100
80
60
40
20
ppm


HN
OTBDPS
BocHN
NBoc
O
O
Me
Me

99

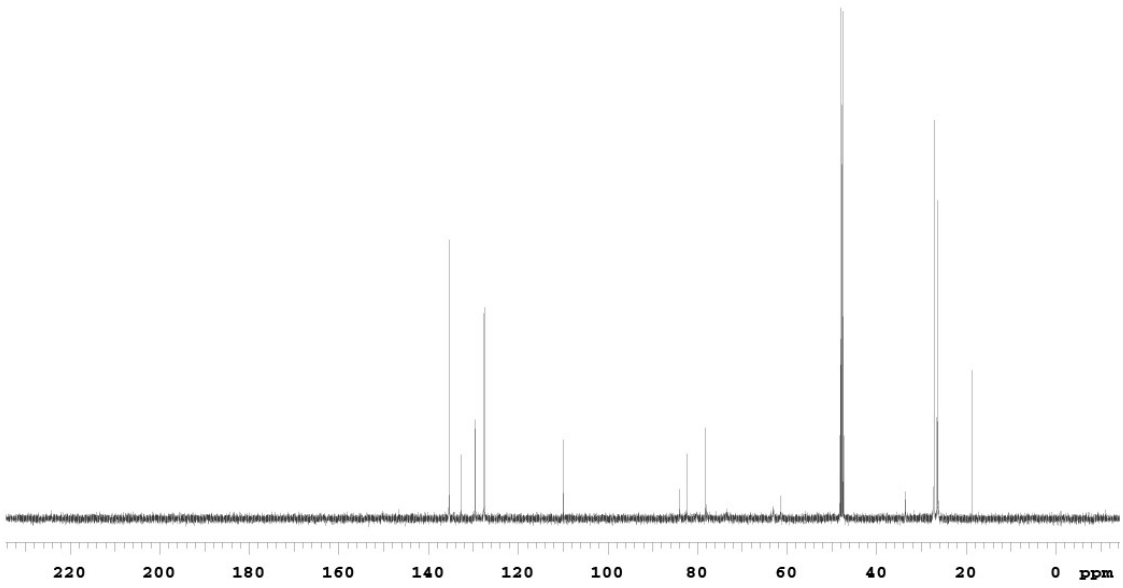
10
9
8
7
6
5
4
3
2
1
ppm
0.97
6.54
4.37
1.03
0.99
1.01
2.11
1.04
2.17
6.40
19.92
9.56


220
200
180
160
140
120
100
80
60
40
20
0
ppm


I
OTBDPS
HN
NBoc
O
O
BocN
Me
Me

100a

9
8
7
6
5
4
3
2
1
ppm
1.25
7.37
4.92
1.11
2.43
1.32
1.17
3.65
0.43
1.00
11.23


220
200
180
160
140
120
100
80
60
40
20
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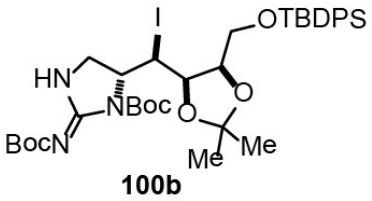
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100b

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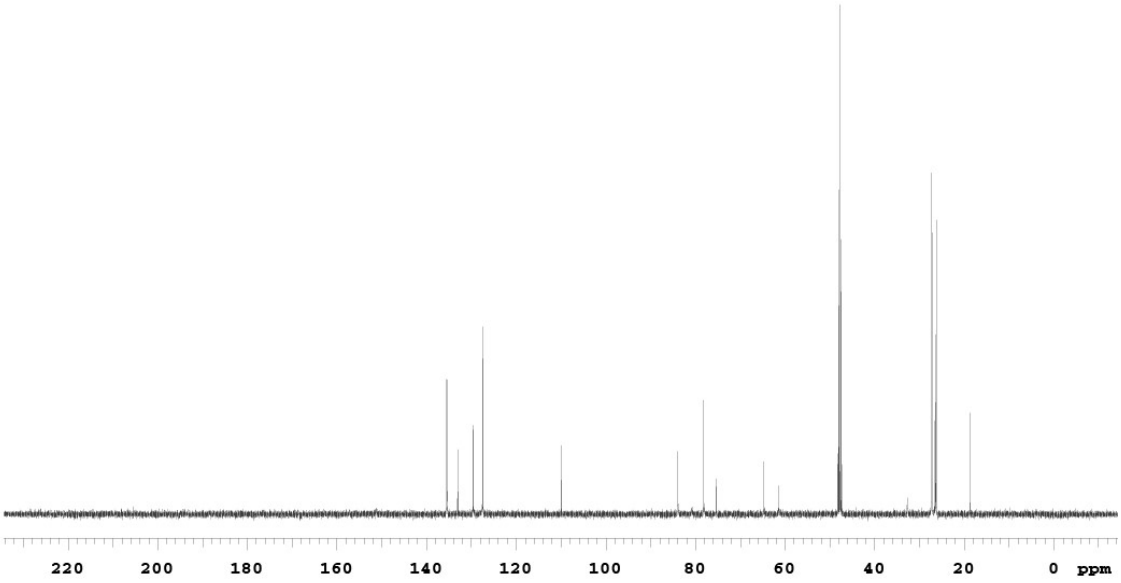
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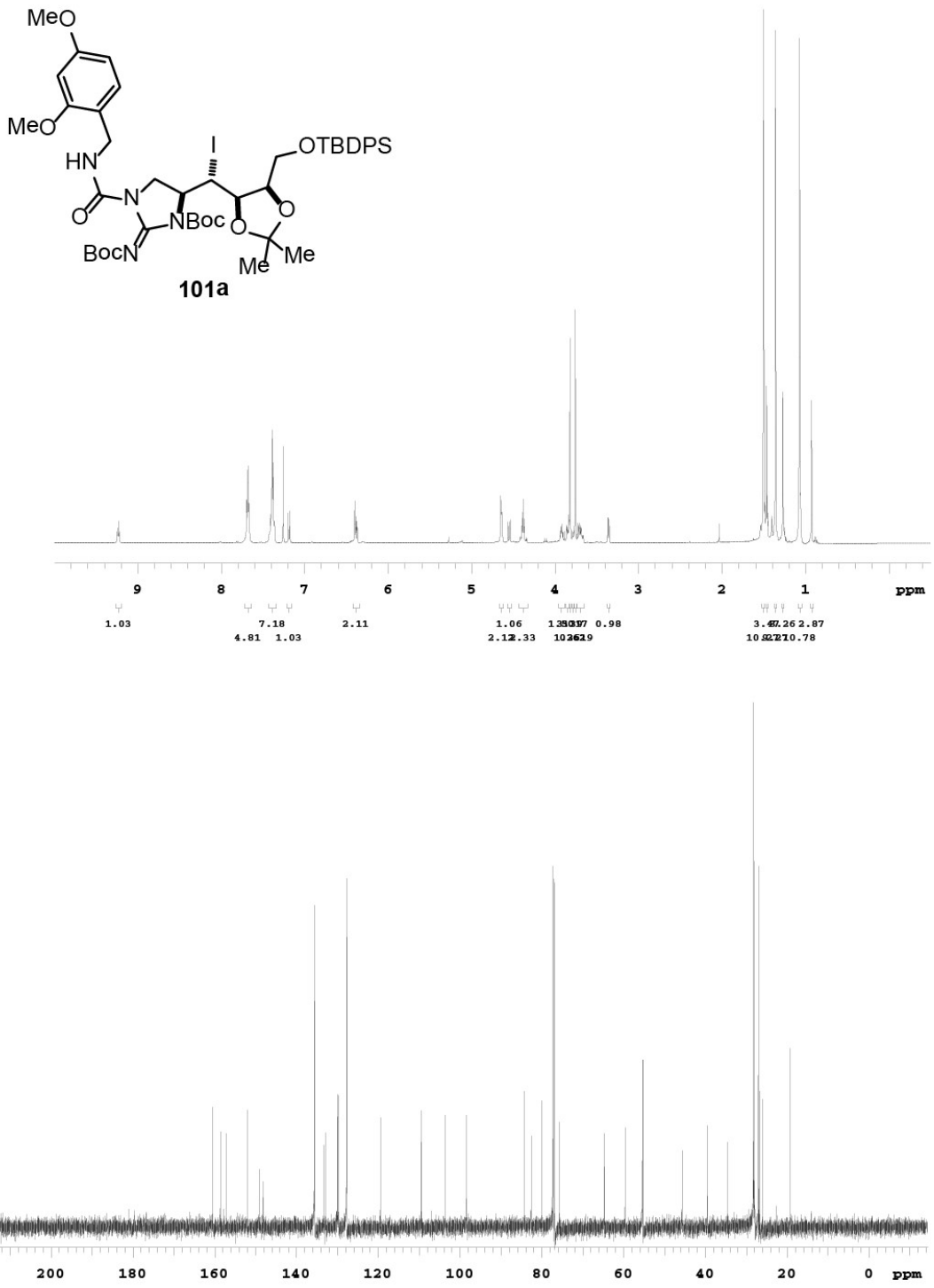
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1
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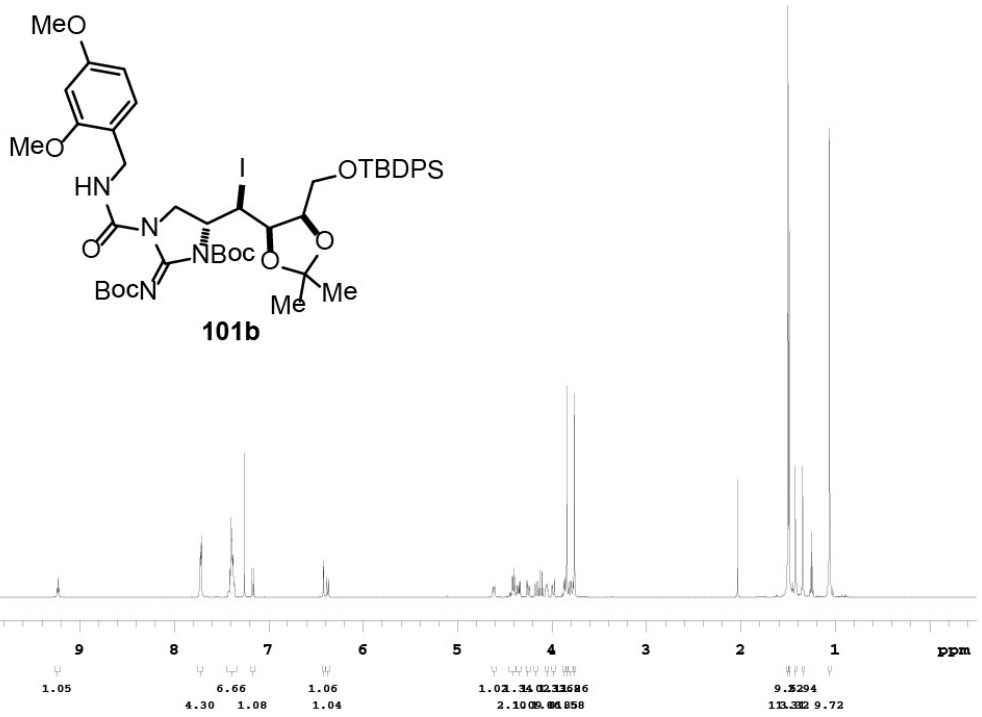
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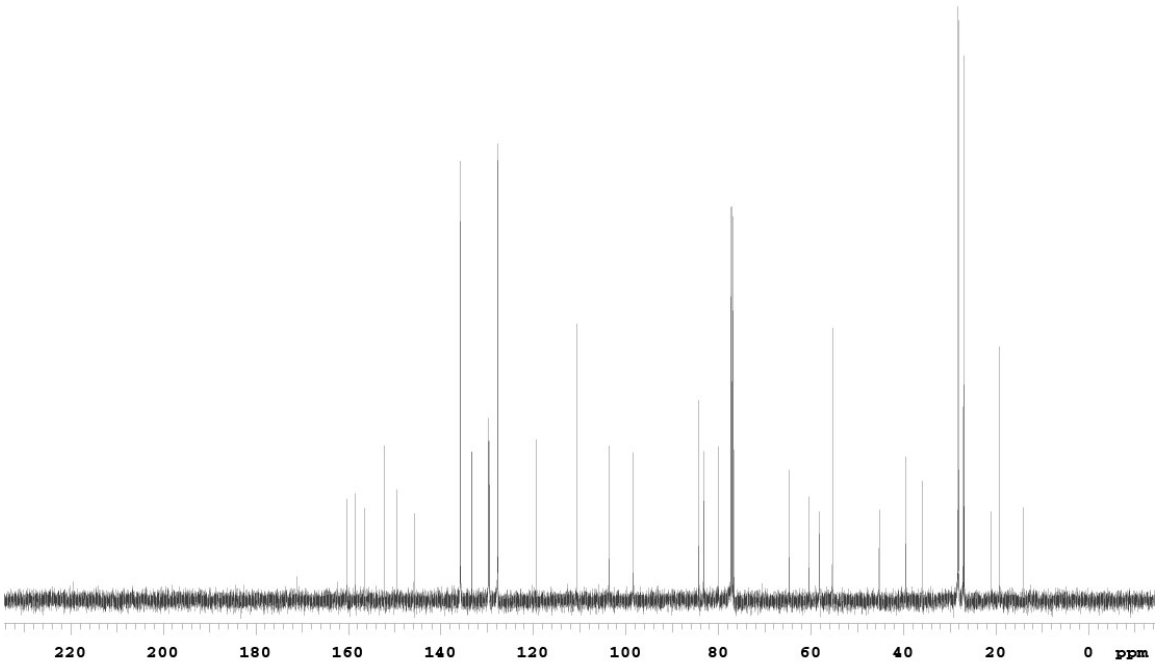
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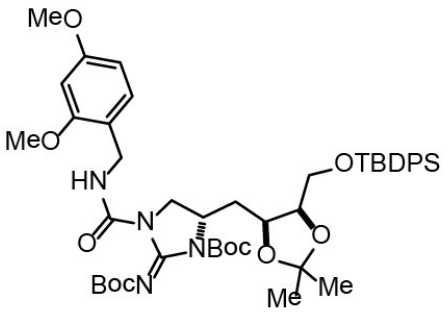
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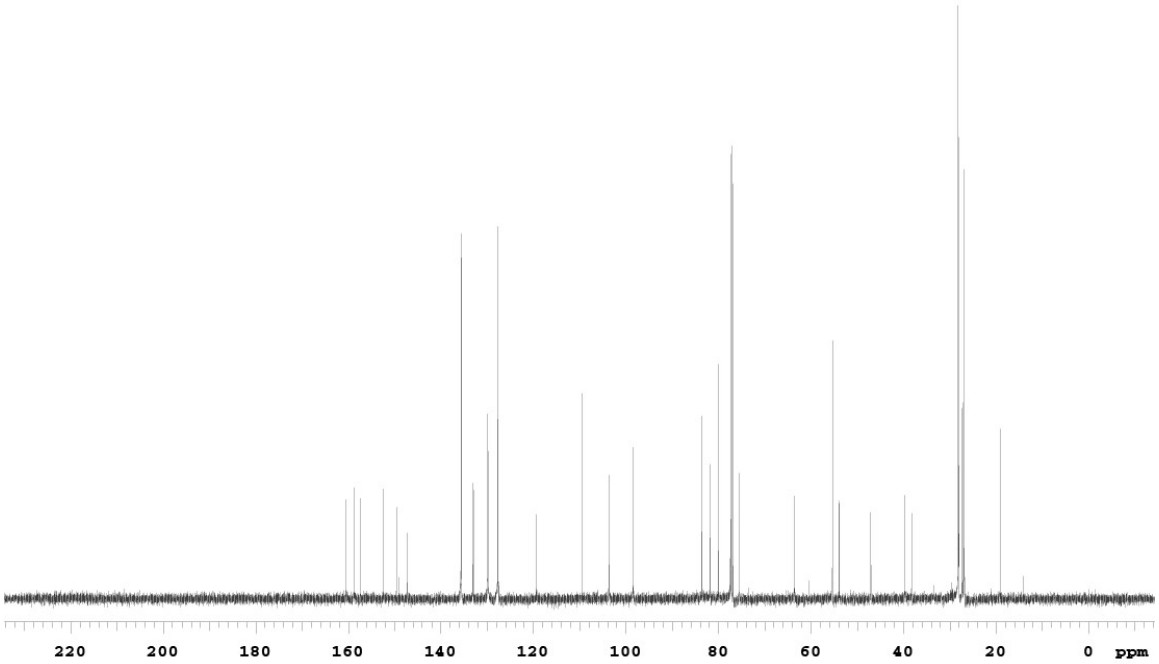
1.91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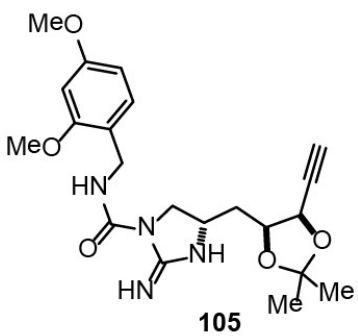
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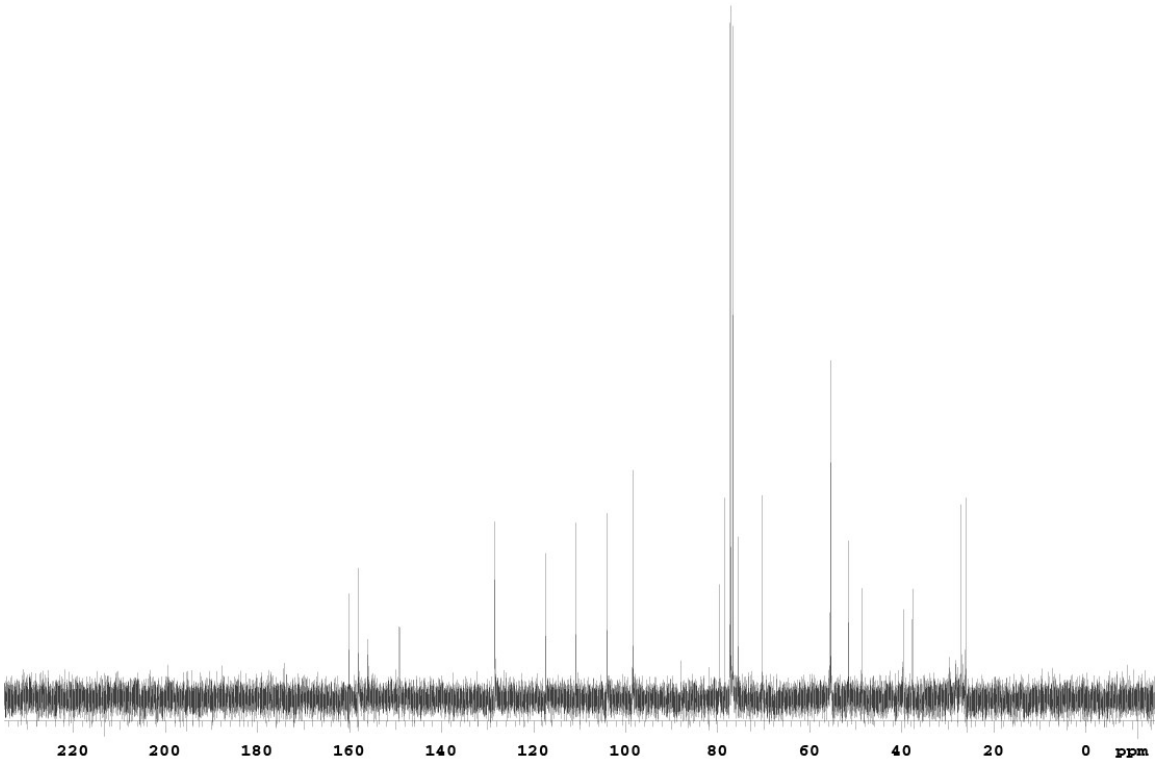
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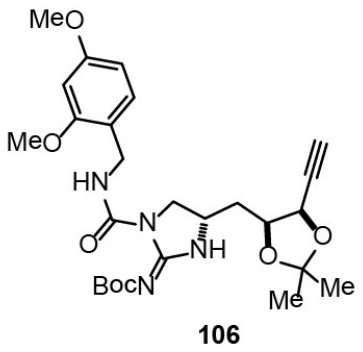
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ppm
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1.03
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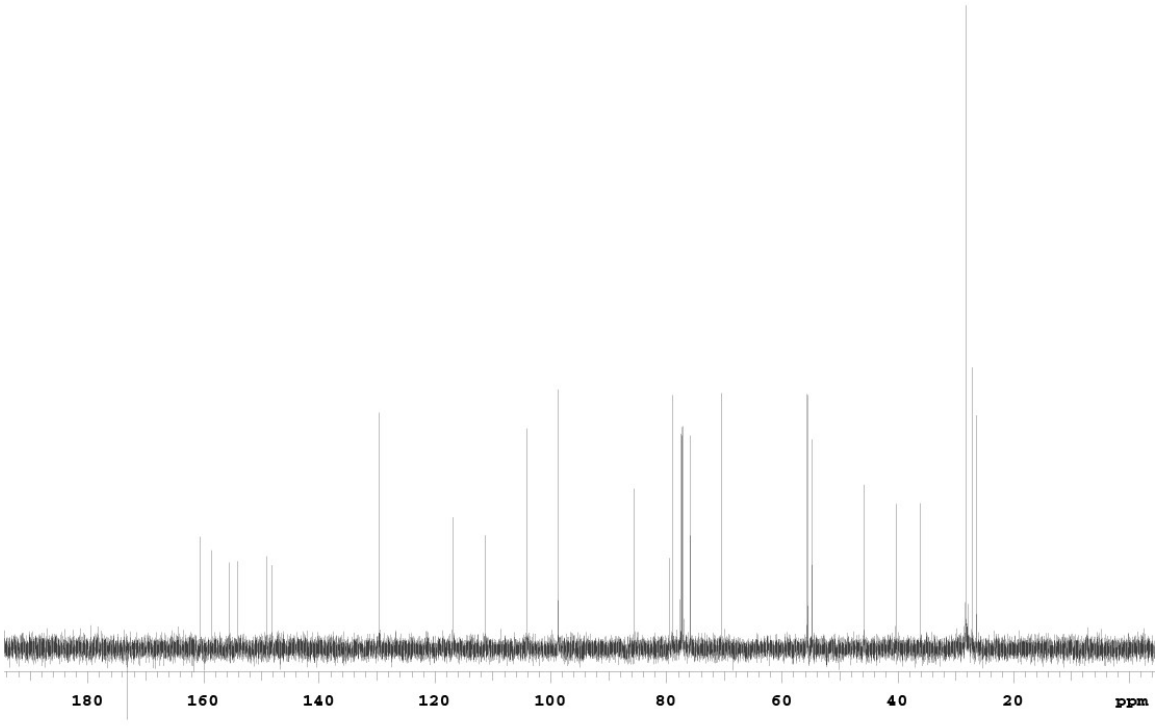
10.47
3.21
79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ppm

1.10 References

1. Kuwahara, A.; Nishikiori, T.; Shimada, N.; Nakagawa, T.; Fukazawa, H.; Mizuno, S.; Uehara, Y. NA22598A1, a Novel Antitumor Substance Produced by Streptomyces sp. NA22598. *J. Antibiot.* **1997,** *50,* 712-713.

2. Iwatsuki, M.; Uchida, R.; Yoshijima, H.; Ui, H.; Shiomi, K.; Matsumoto, A.; Takahashi, Y.; Abe, A.; Tomoda, H.; Omura, S. Guadinomines, Type III Secretion System Inhibitors, Produced by Streptomyces sp. K01-0509. *J. Antibiot.* **2008,** *61,* 222-229.

3. Bhonde, V. R. *Propargylguanidine Hydroamination Application to the Total Synthesis of Bis-guanidine Natural Products, Ph.D. Dissertation;* University of Utah: Salt Lake City, UT, 2013.

4. Hirose, T.; Sunazuka, T.; Tsuchiya, A.; Tanaka, T.; Kojima, Y.; Mori, R.; Iwatsuki, M.; Omura, S. Total Synthesis and Determination of the Absolute Configuration of Guadinomines B and C2. *Chem. Euro. J.* **2008,** *14,* 8220-8238.

5. Tsuchiya, S.; Sunazuka, T.; Hirose, T.; Mori, R.; Tanaka, T.; Iwatsuki, M.; Omura, S. Asymmetric Total Synthesis of (+)-K01-0509 B: Determination of Absolute Configuration. *Org. Lett.* **2006,** *8,* 5577-5580.

6. Pietruszka, J.; Witt, A. Enantiomerically Pure Cyclopropylboronic Esters: Auxiliary - Versus Substrate Control. *J. Chem. Soc., Perkin Trans.* **2000,** *1,* 4293-4300.

7. Koradin, C.; Gommermann, N.; Polborn, K.; Knochel, P. Synthesis of the Enantiomerically Enriched Propargylamines by Copper-Catalyzed Adition of Alkynes to Enamines. *Chem. Eur. J.* **2003,** *9,* 2797-2811.

8. Gainer, M. J.; Bennett, N. R.; Takahashi, Y.; Looper, R. E. Regioselective Rhodium (II)-Catalyzed Hydroaminations of Propargylguanidines. *Angew. Chem. Int. Ed.* **2011,** *50,* 684-687.

9. Cohen, N.; Banner, B. L.; Lopresti, R. J.; Wong, F.; Rosenberger, M.; Liu, Y.; Thom, E.; Liebman, A. A. Enantiospecific Synthesis of Leukotrienes C4. D4, and E4, and [14,15-3H2]Leukotriene E4 Dimethyl Ester. *J. Am. Chem. Soc.* **1983,** *105,* 3661-3672.

10. Pandey, G.; Bharadwaj, K. C.; Khan, M. I.; Sashidhara, K. S.; Purnaik, V. G. Synthesis of Polyhydroxy Piperidines and Their Analogues: A Novel Approach Towards Selective Inhibitors of Glucosidase. *Org. Biomol. Chem.* **2008,** *6,* 2587-2595.

11. Hashimoto, N.; Kanda, A. Practical and Enviormentally Friendly Epoxidation of Olefins Using Oxone. *Org. Process Res. Dev.* **2002,** *6,* 405-406.

12. Cook, C. H.; Cho, Y. S.; Jew, S. S.; Jung, Y. H. Bromocycloetherification Using NBS-Acetonitrile. *Arch. Pharmacal. Res.* **1985,** *8,* 39-41.

13. Corey, E. J.; Das, J. A New Method for Stereospecific cis Hydroxylation of Olefins. *Tetrahedron Lett.* **1982,** *23,* 4217-4220.

14. Bhonde, V. R.; Looper, R. E. A Stereocontrolled Synthesis of (+)-Saxitoxin. *J. Am. Chem. Soc.* **2011,** *133,* 20172-20174.

15. Campos, K. R.; Cai, D.; Journet, M.; Kowel, J. J.; Larsen, R. D.; Reider, P. J. Controlled Semihydrogenation of Aminoalkynes Using Ethylenediamine as a Poison for Lindlar's Catalyst. *J. Org. Chem.* **2001,** *66,* 3634-3635.

16. Ragahavan, S.; Mustafa, S.; Sridhar, B. A Versatile Route to (E)-and (Z)-2-Hydroxy-3,4-unsaturated Disubstituted Sulfilimines and Thier Haloamidation Reaction. *J. Org. Chem.* **2009,** *74,* 4499-4507.

17. Medeiros, M. R.; Schacherer, L. N.; Spiegel, D. A.; Wood, J. L. Expanding the Scope of Trialkylborane/Water-Mediated Radical Reactions. *Org. Lett.* **2007,** *9,* 4427-4429.

18. Merino, P.; Lanaspa, A.; Merchan, F. L.; Tejero, T. Stereocontrolled Synthesis of 2.3-Diaminobutanoic Acids. *Tetrahedron Lett.* **1997,** *38,* 1813-1816.

19. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities *J. Org. Chem.* **1997**; *62*(21); 7512-7515.

20. Hoye, T.R.; Hansen, P.R.; Vyvyan, J.R. A Practical Guide to First-Order Multiplet Analysis in 1H NMR Spectroscopy *J. Org. Chem.* **1994**; *59*(15); 4096-4103.

CHAPTER 2

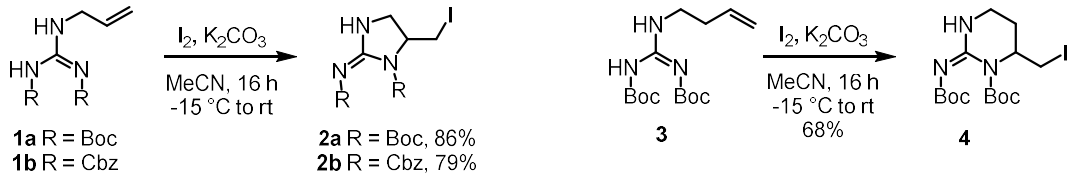
SUBSTRATE CONTROLLED STEREOSELECTIVE IODOGUANYLATION

2.1 Iodoguanylations in the Literature

In Chapter 1, the efforts to find an alcohol protecting group that could be removed selectively, without deprotecting the guanidine, led to a number of acyclic precursors. While the synthesis of saxitoxin produced a single diastereomer, both possible diastereomers of the cyclic guanidine were observed upon cyclization of the precursors.[1] The literature contained limited examples of this transformation on a less rigid system, many using a terminal alkene.[2] [3]

The most comprehensive study of acyclic guanidine iodocyclization came from the Murphy laboratory, as they explored the formation of five and six membered rings using this methodology.[4] [5] [6] When *N*-allylguanidines **1a** and **1b** were treated with K_2CO_3 and I_2 in MeCN, they were converted to the 5-*exo*-cyclic guanidines **2a** and **2b** in high yield. Additionally, the homoallylic guanidine **3** also cyclized in a 6-*exo* fashion with the standard conditions and proceeded in good yield to give **4** (Scheme 2.1).

When the more substituted dimethylallyl guanidine **5** was subjected to standard conditions, the major compound recovered was the 6-*endo* cyclization product **6**; however, the 5-*exo* cyclization product **7** was also recovered. As this was an unexpected result, they attempted to increase the ratio of the 5-*exo*-cyclization product. To that end, they varied the



Scheme 2.1: Iodoguanylations of allyl and homoallyl guanidines.

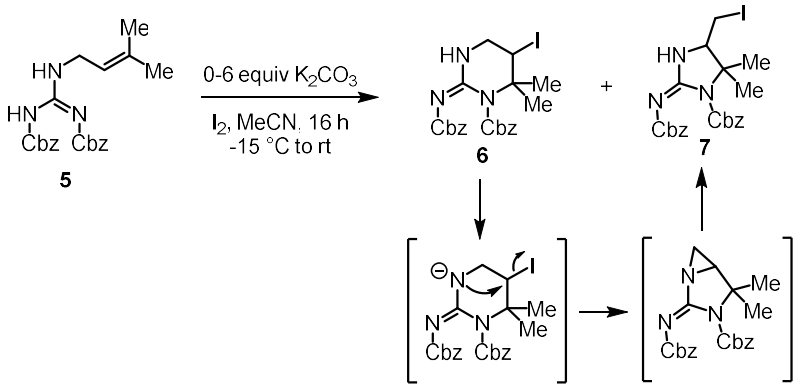
amount of base in the reaction and observed that the absence of base led solely to the 6-*endo* product, but more than 4 equiv. of base produced the 5-*exo* product in greater yields (Scheme 2.2). The formation of the 5-*exo* product led them to conclude that a ring contraction occurred and the resultant aziridine was opened with I^- yielding the 5-*exo*-product. Unfortunately, they did not address the stereochemical outcome of the 6-*endo* cyclization.

2.2 Optimizing Iodination Reagents

Allylic guanidine **8** was subjected to the conditions developed by Murphy and coworkers intitally. The acyclic guanidine **8** was dissolved in MeCN and cooled to –15 °C, followed by addition of I_2 and K_2CO_3. After stirring the reaction for 16 hours the desired cyclic guanidine **9** was not observed (Scheme 2.3). I believe that the electron-withdrawing nature of both the adjacent guanidine and the ketal decreased the electron density of the alkene rendering it incapable of forming the iodonium ion with just I_2.

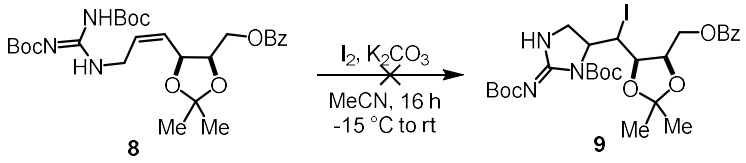
The synthesis of saxitoxin functionalized an electron poor ene-guanidine by generating a more electrophilic iodine source with AgOAc. [1] Therefore, the allylic guanidine **8** was dissolved in Et_2O followed by the addition of AgOAc and I_2, and the reaction was stirred for 12 hours. These conditions did produce the cyclic guanidine in a mixture of diastereomers **9a** and **9b**; however, the reaction did not go to completion (Scheme 2.4).

The initial cyclization gave a 1.3:1 ratio of diastereomers; however, it was unclear which

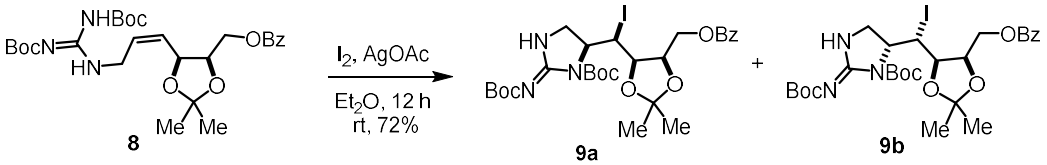


K_2CO_3 (equiv)	Ratio
0.0	100:0
1.0	100:0
2.0	100:0
3.0	100:0
4.5	78:22
6.0	46:54

Scheme 2.2: Formation of the 6-*endo* and 5-*exo* cyclizations.



Scheme 2.3: Initial cyclization conditions.



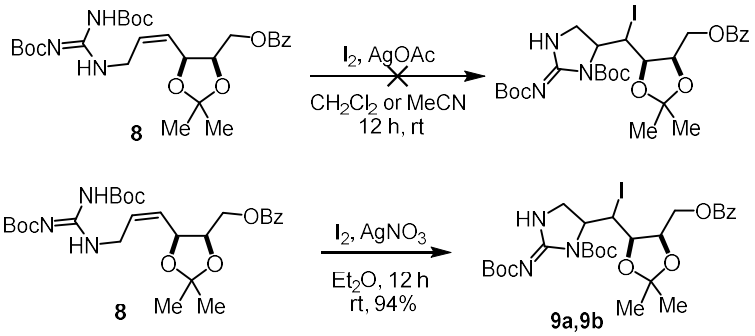
Scheme 2.4: Successful cyclization of the allylic guanidine.

compound was the desired diastereomer. Although determining the stereoselectivity was a high priority, initial efforts were focused on optimizing the reaction to consume all the starting material. The solubility of the AgOAc in Et_2O was minimal, and in an effort to increase solubility, more polar solvents were employed. Both MeCN and CH_2Cl_2 were used as solvents; unfortunately, in these more polar solvents, neither diastereomer of the cyclic guanidine was recovered (Scheme 2.5). The clean conversion of acyclic guanidine **8** in Et_2O prompted a reversion to that solvent and an alteration of the silver salt to increase solubility. Substituting $AgNO_3$ for AgOAc decreased the reaction time, but a small amount of starting material persisted. I was pleased to see that an increase in the amount of I_2 and $AgNO_3$ from 1.2 equiv. to 1.4 equiv. led to the complete conversion of the starting material **8** (Scheme 2.5).

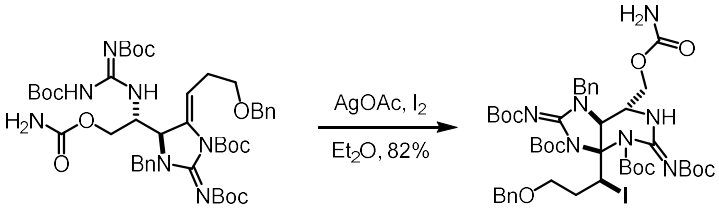
2.3 Optimization of the Stereoselectivity

Having optimized the solvent and silver source for this transformation probing the stereoselectivity became the primary focus. Considering the synthesis of saxitoxin, we determined that the formation of the iodonium ion must be reversible for such a high yield of a single diastereomer (Scheme 2.6). Additionally, the different alcohol protecting groups showed various selectivity under the same conditions, which led to the exploration of various conditions to manipulate the selectivity.

The previous optimization of both the solvent and the silver salt limited the variables that could be adjusted in the effort to manipulate selectivity; varying the reaction temperature was the obvious starting place. The benzoyl protected guanidine **8** was treated with I_2 and $AgNO_3$ in Et_2O at varying temperatures (Table 2.1). The variation in selectivity was minor, but the change in the major product was promising. The greatest diastereomeric ratio was seen at higher temperatures; to be able to increase the temperature, the solvent was changed



Scheme 2.5: Optimization of the solvent and silver source.

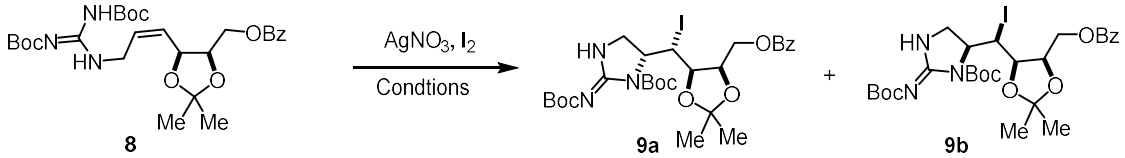


Scheme 2.6: Formation of a single diastereomer from the iodoguanylation in saxitoxin.

to THF, because it had a higher boiling point. While the previous deviation from Et_2O had produced a complicated mixture of products, I hoped that an ethereal solvent would minimize the formation of side products. The allylic guanidine **8** was treated with I_2 and $AgNO_3$ in THF at the same temperatures as in Et_2O. Unfortunately, this change in solvent had very little effect on the diastereomeric ratio (Table 2.1).

At the time of cyclization, it was unclear which diastereomer matched the stereochemistry of the natural product. Because of that ambiguity, we hoped to maximize the formation of each diastereomer in varying conditions. The benzoyl substrate fluctuated between 2:1 and 1:2; in order to determine the effect of different protecting groups, various protected guanidines were subjected to the same conditions. The protecting groups were initially chosen for the specific aim of selectively deprotecting the terminal alcohol. However,

Table 2.1: Cyclization of the acyclic guanidine **8**.



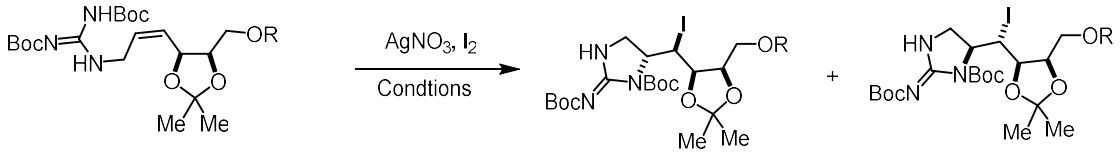
Solvent	Temp	Ratio (A:B)
Et_2O	Reflux	2:1
	Room Temp	NA
	-10 °C	1.3:1
	-20 °C	1.5:1
	-40 °C	1.1:1
THF	Reflux	1.5:1
	Room Temp	1:1.1
	-10 °C	1:1.4
	-20 °C	1:1.4
	-40 °C	1:2

when exploring the selectivity, it was important to vary the size of the protecting group to determine if it had an effect on selectivity. To that end, four protecting groups were explored, TBDPS, TBS, Bz, and Ac, which created a range of protecting group sizes to assess the substrate effect on the cyclization.

All three additional substrates were synthesized in the method described in Chapter 1, Scheme 1.27, and subjected to the cyclization conditions optimized with the benzoyl guanidine **8** at various temperatures (Table 2.2). The Ac protected guanidine **10** produced a synthetically useful range of **13a:13b** shifting from 3:1 to 1:4. The TBS protected guanidine **11** showed a very similar ratio of **14a:14b** as was seen in the benzoyl protected **8** cyclization. While the TBDPS protected guanidine **12** showed surprisingly high selectivity of **15a:15b** in Et_2O.

In order to functionalize the cyclic guanidines for further elaboration into the natural product, the cyclic guanidines **9**,**13-15a** and **9,13-15b** were each treated with *p*-methoxyphenyl isocyanate to form the carbamoyl guanidines **16-19a** and **16-19b**. To assess the absolute

Table 2.2: The cyclization of various guanidines.



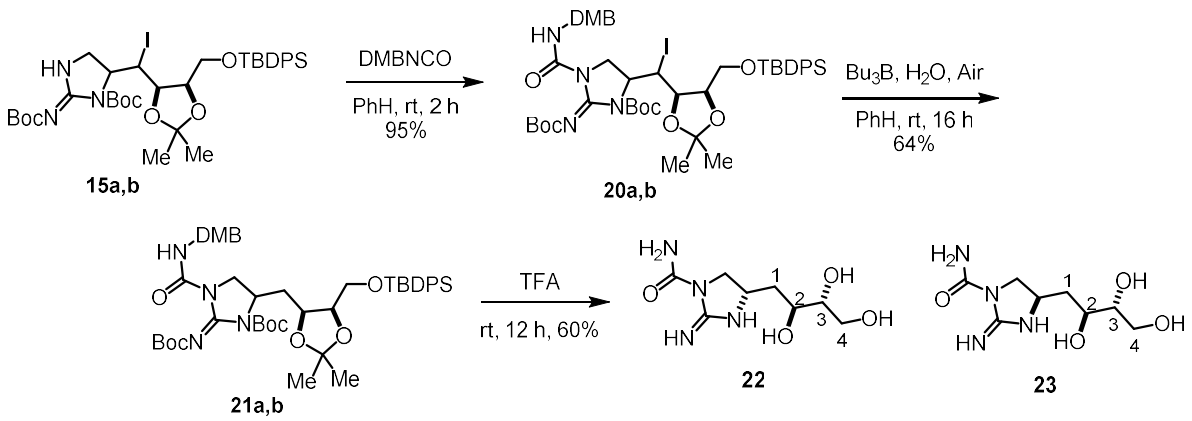
10,11,12 **13,14,15a** **13,14,15b**

R	Solvent	Temp	Ratio (A:B)
Ac	Et_2O	Reflux	2.6:1
		Room Temp	2.6:1
		-10 °C	1.6:1
		-20 °C	1.8:1
		-40 °C	1:3.8
Ac	THF	Reflux	3:1
		Room Temp	2:1
		-10 °C	1.2:1
		-20 °C	1.1:1
		-40 °C	1:1.1
TBS	Et_2O	Reflux	3:1
		Room Temp	3.3:1
		-10 °C	1.8:1
		-20 °C	1.4:1
		-40 °C	1:2
TBS	THF	Reflux	ND
		Room Temp	2:1
		-10 °C	1.6:1
		-20 °C	1.3:1
		-40 °C	ND
TBDPS	Et_2O	Reflux	ND
		Room Temp	10:1
		-10 °C	2.3:1
		-20 °C	1.8:1
		-40 °C	ND
TBDPS	THF	Reflux	ND
		Room Temp	2.2:1
		-10 °C	1.6:1
		-20 °C	1.1:1
		-40 °C	1:3

stereochemistry, each diastereomer needed to be globally deprotected. To achieve a clean global deprotection, the cyclic guanidines **15a** and **15b** were treated with DMB isocyanate. This produced carbamoylated guanidines **20a** and **20b**, which were then dehalogenated to give **21a** and **21b**. A simple, one-step acidic deprotection afforded the guanidines **22** and **23** (Scheme 2.7).

To determine the stereochemistry of the deprotected guanidines **22** and **23**, the proton NMR spectra of each compound were compared to the NMR spectrum of guadinomine B. The most diagnostic protons were the C_1 protons, which in the natural product were both doublet of doublet of doublets at 1.70 and 1.83 ppm. The guanidine triol **23** had the same splitting pattern at 1.67 and 1.77 ppm while the other guanidine triol **22** had a single multiplet at 1.7 ppm. It was disappointing to note that the best diastereomeric ratio, observed at room temperature with the TBDPS protecting group, favored the undesired diastereomer. However, the 4:1 ratio of the desired product that was displayed by the Ac protecting group at -40 °C was promising enough to utilize this method moving forward.

The significant difference between the selectivity with the Ac protecting group and the TBDPS protecting group led to a more in-depth consideration of the mechanism. The conformation of the alkene was governed by $A^{1,3}$ strain; in order to minimize the strain, the ketal was perpendicular to the alkene. This conformation positioned the protected alcohol behind the alkene; the larger protecting groups created a larger steric barrier to that face of the alkene. The formation of the iodonium ion on the less hindered face of the molecule was favored. The favored iodonium led to the desired product; however, the cyclization of the guanidine was slow step. In order for the guanidine to cyclize on the favored iodonium ion, it had to overcome the steric hindrance of the alcohol protecting group. The smaller

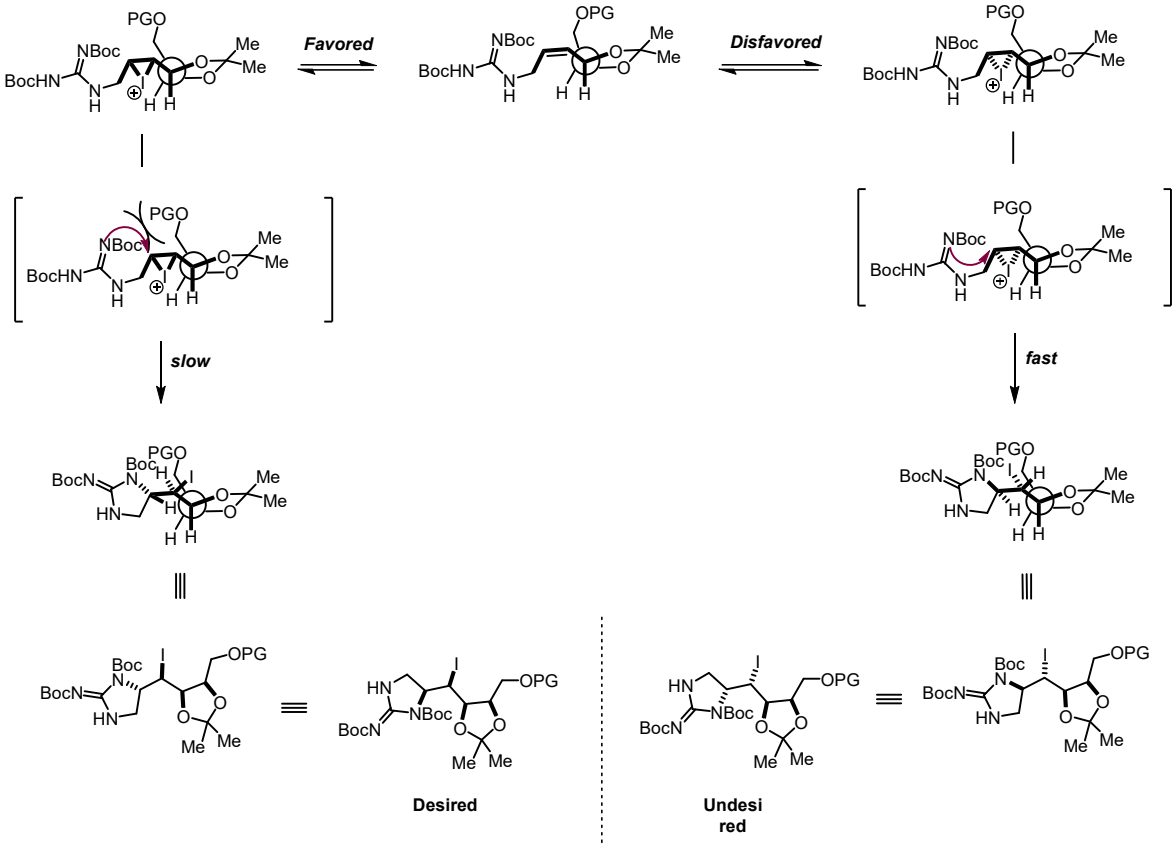


Scheme 2.7: An example of the final steps to form the deprotected guanidines **22** and **23**.

protecting groups offered less steric hindrance, which allowed for the increased distereomeric ratio seen at cold temperatures with the Ac group. The temperature contributed to the selectivity because at colder temperatures the reversibility of the iodonium ion was slower, allowing the guanidine more time to cyclize. However, at higher temperatures, the faster reversibility of the iodonium ion creates a higher population of the slower iodonium formation and the faster cyclization of the guanidine leads to the undesired product (Scheme 2.8).

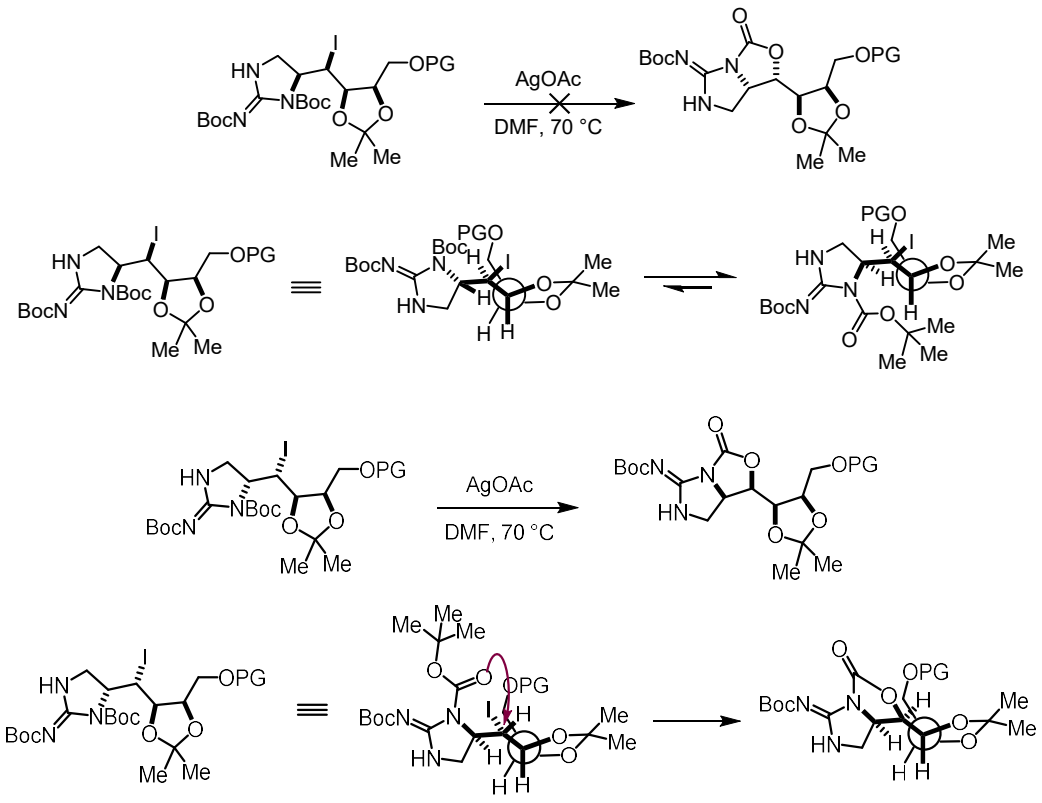
The more in-depth probe of the iodoguanylation prompted us to revisit the Boc cyclization to form the cyclic carbamate and gain access to guadinomine A. The steric congestion, which minimized the formation of the desired diastereomer, could slow the formation of the cyclic carbamate with the benzoyl group in place. Additionally, Srinivas Paladugu, another lab member, had been working on a similar cyclization and found that increasing the equiv. of AgOAc to 2 equiv. and changing the solvent to DMF led to increased yields.

The TBDPS protected guanidines **20a** and **20b** were dissolved in DMF; AgOAc was added and the reaction heated to 60 °C and stirred for 12 hours. The guanidine **20b** showed no cyclic guanidine formation, which was consistent with our expectations because of the



Scheme 2.8: Rationalization of the stereoselectivity for the iodocyclization.

steric clash. The guanidine **20a** was then transformed to the cyclic carbamate **24** in 81% yield (Scheme 2.9). Even with the smaller Ac protecting group on **16b**, no cyclic carbamate was formed. The lowest energy conformation of the cyclic guanidine **16b** positions the Boc group too far away from the iodide to form the cyclic carbamate. The limitation of accessing the desired diastereomer was not ideal, but being able to manipulate the stereoselectivity of an iodoguanylation was a promising step. As far as we know, this is the first example of a stereoselective iodoguanylation. Understanding the substrate control of the stereoselctivity derived from $A^{1,3}$ strain could be useful for predicting the stereoselectivity of this cyclization in other substrates. We believed that a 4:1 mixture of the desired to undesired stereoisomer was significant enough to be a key step in our synthesis.



Scheme 2.9: Cyclic carbamate formation from guanidine **20a**.

2.4 Supporting Information

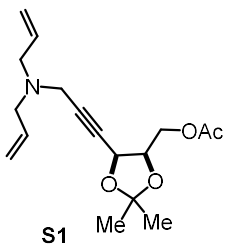
Unless otherwise noted, all starting materials were either known compounds or were obtained from commercial sources and used without purification. All reactions requiring anhydrous conditions were performed under a positive pressure of argon using flame-dried glassware. Dichloromethane (CH_2Cl_2), and triethylamine (Et_3N), were distilled from CaH_2 immediately prior to use. Tetrahydrofuran (THF), diethylether (Et_2O), toluene (PhMe), and dimethylformamide (DMF) were degassed with argon and passed through a solvent purification system (J.C. Meyer of Glass Contour) containing either alumina or molecular

sieves. Flash chromatography was performed on Merk silica gel Kieselgel 60 (230-400 mesh) from EM science with the indicated solvent. ^{1}HNMR spectra were recorded on a Varian VXR-500 MHz spectrometer. The chemical shifts (d) of proton resonances are reported relative to $CHCl_3$, DMSO-d_5, HOD, or HD_2COD using the following format: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app = apparent), coupling constant(s) (*J* in Hz), integral].[7,8] ^{13}CNMR spectra were recorded at 75, 100, or 125 MHz. The chemical shifts of carbon resonances are reported relative to the deuterated solvent peak.

Infrared spectra were recorded on a Nicolet 380-FT IR spectrometer fitted with a SmartOrbit sample system. All absorptions are reported in cm^{-1} relative to polystyrene (1601 cm^{-1}).

((4R,5S)-5-(3-(diallylamino)prop-1-yn-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl acetate (S1)

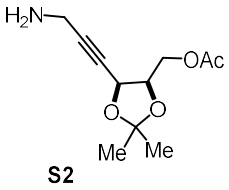
To a solution of **1.43** (3.94 g, 15.4 mmol) in CH_2Cl_2 was added AcCl (1.6 mL, 23.1 mmol), Et_3N (4.3 mL, 30.7 mmol), and DMAP (940 mg, 7.7 mmol). The solution was stirred at room temperature for 18 hours, then washed with H_2O (2 x 20 mL) and brine (20 mL); the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was then purified by flash chromatography (4:1 Hex/EtOAc) to yield **S1** in 79% yield. R_f = 0.75 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +35.1 (*c*= 1.5, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 5.79-5.74 (m, 2H), 5.17 (d, *J* = 17 Hz, 2H), 5.10 *d, *J* = 10 Hz, 2H), 4.47 (d, *J* = 7.5 Hz, 1H), 4.31 (d, *J* = 12 Hz, 1H), 4.20 (s, 1H), 4.07 (dd, *J* = 5, 12 Hz, 1H), 3.38 (s, 2H), 3.06 (d, *J* = 6 Hz, 4H), 2.06 (s, 3H), 1.46 (s, 3H), 1.38 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 135.3, 118.3,



S1

110.9, 82.1, 81.5, 79.8, 67.7, 63.1, 56.6, 41.7, 26.9, 26.4, 20.9 ppm.

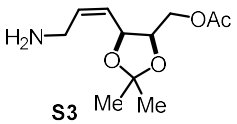
((4R,5S)-5-(3-aminoprop-1-yn-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl acetate (S2)



S2

To a solution of **S1** (3.42 g, 11.12 mmol) in CH_2Cl_2 was added $Pd(PPh_3)_4$ (1.2 g. 1.12 mmol), and dimethyl barbateric acid (7.03 g, 44.5 mmol). The solution was heated to reflux and stirred for 18 hours. It was then cooled to room temperature, washed with $NaHCO_3$ (3 x 50 mL); the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was passed through a plug of silica (10:1 CH_2Cl_2/MeOH) and used without further purification.

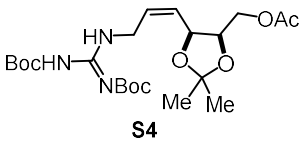
((4R,5S)-5-((Z)-3-aminoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl acetate (S3)



S3

Amine **S2** (1.66 g, 7.3 mmol) was dissolved in DMF, and the reaction was flushed with N_2. Quinolone (1 mL, 7.3 mmol) and $Pd/BaSO_4$ (310 mg, 0.29 mmol) were added and the solution was flushed with H_2. The reaction was then stirred under an H_2 atmosphere for 6 hours; it was filtered over a bed of Celite, washed with toluene, and concentrated under reduced pressure. The crude material was passed through a plug of silica (5:1 CH_2Cl_2/MeOH) and used without further purification.

((4R,5S)-5-((Z)-3-((E)-2,3-bis(tert-butoxycarbonyl)guanidino)prop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl acetate (S4)



S4

The allylic amine **S3** (303.6 mg, 1.32 mmol) was dissolved in CH_2Cl_2. Et_3N (0.2 mL, 1.32 mmol), HgO (287 mg, 1.32 mmol), and N,N'-Di-Boc-S-methylisothiourea (384 mg, 1.32 mmol) were

added and the reaction was stirred for 18 hours. The crude mixture was filtered through a pad of Celite, and concentrated under reduced pressure. The crude compound was purified by flash chromatography (10:1 Hex/EtOAc), to give **S4** in 61% yield as a white foam.

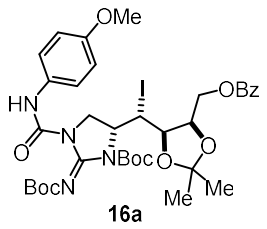
R_f = 0.93 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = -0.5 (c= 1.01, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 11.42 (s, 1H), 8.33 (t, J = 5 Hz, 1H), 5.80-5.70 (m, 1H), 5.60-5.50 (m, 1H), 4.63 (dt, J = 0.5 Hz, 8.5 Hz, 1H), 4.28 (dd, J = 3.5 Hz, 12 Hz, 1H), 4.22-4.14 (m, 1H), 4.08-4.00 (m, 2H), 3.88-3.83 (m, 1H), 2.10 (s, 3H), 1.45 (s, 9H), 1.44 (s, 9H), 1.40 (s, 3H), 1.39 (s, 3H) ppm. . ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 170.8, 163.7, 156.2, 153.4, 131.4, 129.6, 110.1, 83.5, 79.6, 79.0, 73.7, 63.2, 38.3, 28.5, 28.4, 28.3, 27.3, 27.1, 21.0, 19.3 ppm. IR (neat): 3331, 2981, 1744, 1721, 1637, 1614, 1565, 1411, 1368, 1323, 1226, 1157, 1129, 1057cm^{-1}

Cyclic Guanidines (9, 13, 14, 15)

A solution of **8, 10, 11,** or **12** in THF or diethyl ether (0.06M) was adjusted to the desired temperature (see Table 2.1), and the flask covered to minimize exposure to light. I_2 (1.4 eqiv.) and $AgNO_3$ (1.4 eqiv.) were added and the reaction was stirred until complete by TLC, 3-10 hours. The reaction was filtered through a pad of Celite and washed with EtOAc. The combined organics were washed with $Na_2S_2O_3$ (2 x 20 mL) and dried over Na_2SO_4. The diastereomers were separated by column chromatography (see Table 2.1).

tert-butyl (S,Z)-5-((S)-((4R,5R)-5-((benzoyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)-2-((tert-butoxycarbonyl)imino)-3-((4-methoxyphenyl)carbamoyl)imidazolidine-1-carboxylate (16a)

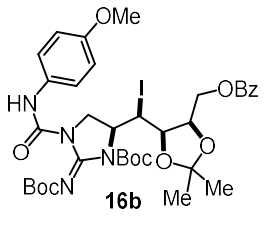
General Procedure A: The cyclic guanidine **9a** (75.9 mg, 0.12 mmol) was dissolved in benzene (10 mL) followed by the addition of a 1M solution of PMP isocyanate in benzene (0.14 mL, 0.14 mmol); the reaction was stirred for 3 hours. The reaction was concentrated and purified by column chromatography (4:1 Hex/EtOAc) to give **16a** in 67% yield as a colorless oil. R_f = 0.92 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +57.2 (c= 0.50, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$):10.91 (s, 1H), 8.08-8.06 (m, 2H), 7.55 (tt, J = 1 Hz, 7.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 7.37 (dt, J = 3 Hz, 9 Hz, 2H), 6.87 (dt, J = 3.5 Hz, 9 Hz, 2H), 4.72-4.65 (m, 3H), 4.45 (d, J = 5 Hz, 2H), 4.16-4.12 (m, 1H), 3.79 (s, 3H), 3.51 (d, J = 8 Hz, 1H), 1.46 (s, 3H), 1.45 (s, 9H), 1.43 (s, 9H), 1.23 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 166.2, 156.7, 156.2, 149.7, 148.7, 148.3, 133.1, 130.7, 129.8, 129.7, 128.4, 121.8, 114.2, 110.4, 84.8, 80.9, 80.6, 73.9, 63.8, 59.3, 55.4, 45.4, 32.8, 28.0, 27.9, 26.9, 26.5 ppm. IR (neat): 2980, 1757, 1711, 1603, 1556, 1512, 1453, 1369, 1311, 1269, 1234, 1129, 712 cm^{-1}



16a

tert-butyl (R,Z)-5-((R)-((4R,5R)-5-((benzoyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)-2-((tert-butoxycarbonyl)imino)-3-((4-methoxyphenyl)carbamoyl)imidazolidine-1-carboxylate (16b)

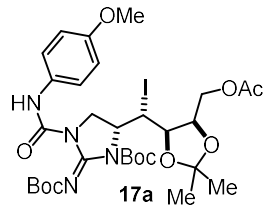
Prepared according to General Procedure A to give **16b** in 61% yield. R_f = 0.92 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = -38.6 (c= 1.46, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 10.95 (s, 1H), 8.05 (dd, J = 1 Hz, 8 Hz, 2H), 7.52 (tt, J = 1 Hz, 7.5 Hz, 1H), 740-7.36 (m, 3H), 6.86 (dt, J = 3,5 Hz, 9 Hz, 2H), 4.80-4.78 (m, 1H), 4.72 (d, J = 9 Hz, 1H), 4.45 (dd, J = 4.5 Hz, 12 Hz, 1H), 4.37 (s, 3H), 4.27 (dd, J = 1.5 Hz, 12 Hz, 1H), 1.53 (s, 9H), 1.48 (s, 3H), 1.36 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 166.2, 156.3, 155.8, 150.0, 149.4, 146.3, 133.0, 130.7,



16b

129.8, 129.7, 128.3, 122.1, 114.2, 111.5, 84.8, 80.5, 80.4, 77.3, 65.4, 58.3, 55.5, 44.8, 35.5, 28.1, 28.0, 27.3, 26.9 ppm. IR (neat): 2979, 1757, 1716, 1693, 1639, 1602, 1555, 1512, 1452, 1369, 1306, 1272, 1230, 1149, 1125, 1037, 828, 756, 711cm^{-1}

tert-butyl (S,Z)-5-((S)-((4R,5R)-5-(acetoxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)-2-((tert-butoxycarbonyl)imino)-3-((4-methoxyphenyl)carbamoyl)imidazolidine-1-carboxylate (17a)

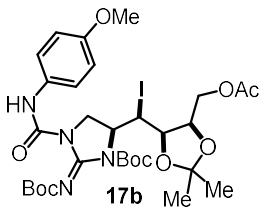


17a

Prepared according to General Procedure A to give **17a** in 63% yield. R_f = 0.90 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +103.5 (c= 1.13, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 10.9 (s, 1H), 7.37 (dt, J = 3.5 Hz, 9 Hz, 2H), 6.86 (dt, J = 3.5 Hz, 9 Hz, 2H), 4.69-4.61 (m, 3H), 4.29 (dd, J = 4.5 Hz, 12 Hz, 1H), 4.13 (dd, J = 4.5 Hz, 12.5 Hz, 1H), 3.99-3.95 (m, 1H), 3.79 (s, 3H), 3.78-3.76 (m, 1H), 3.42 (dd, J = 1.5 Hz, 8 Hz, 1H), 2.14 (s, 3H), 1.54 (s, 9H), 1.52 (s, 9H), 1.41 (s, 3H), 1.20 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 170.7, 156.9, 156.2, 149.7, 148.7, 148.4, 130.7, 121.8, 114.2, 110.2, 84.8, 80.9, 80.5, 72.8, 62.3, 59.4, 55.4, 45.4, 32.7, 28.1, 27.9, 26.8, 26.3, 20.8 ppm. IR (neat): 3314, 2979, 2931, 1763, 1706, 1652, 1613, 1530, 1367, 1321, 1254, 1216, 1144, 746, 701 cm^{-1}

tert-butyl (R,Z)-5-((R)-((4R,5R)-5-(acetoxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)-2-((tert-butoxycarbonyl)imino)-3-((4-methoxyphenyl)carbamoyl)imidazolidine-1-carboxylate (17b)

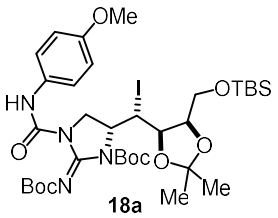
Prepared according to General Procedure A to give **17b** in 65% yield. R_f = 0.90 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = -49.1 (c= 1.50, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): 10.95 (s, 1H), 7.37 (dt, J = 3.5 Hz, 9 Hz, 2H), 6.85 (dt, J = 3.5 Hz, 9 Hz, 2H), 4.69 (dt, J = 2 Hz, 9 Hz, 1H), 4.56 (dd, J = 2 Hz, 11.5 Hz, 1H), 4.30 (dd, J = 2.5 Hz, 11 Hz, 1H), 4.22-4.19 (m, 3H), 4.15 (dd, J = 5.5 Hz, 12 Hz, 1H), 3.90 (dd, J = 9.5 Hz, 2.5 Hz, 1H), 2.09 (s, 3H), 1.54 (s, 9H), 1.52 (s, 9H), 1.45 (s, 3H), 1.35 (s, 3H) ppm. ^{13}C {^1H} NMR (125 MHz, $CDCl_3$): δ (ppm): 170.5, 156.3, 155.8, 155.0, 149.4, 146.3, 130.6, 122.0, 114.2, 111.4, 84.8, 80.4, 80.3, 65.2, 58.2, 55.4, 44.8, 35.5, 28.1, 28.0, 27.2, 26.9, 20.8 ppm. IR (neat): 2979,1750, 1714, 1639, 1555, 1512, 1368, 1305, 1229, 1149, 1127,1036, 828, 753 cm^{-1}



17b

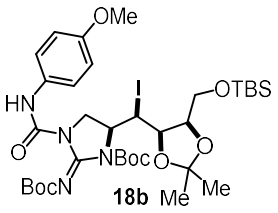
tert-butyl (R,Z)-2-((tert-butoxycarbonyl)imino)-5-((R)-((4R,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)-3-((4-methoxyphenyl)carbamoyl)imidazolidine-1-carboxylate (18a)

Prepared according to General Procedure A to give **18a** in 65% yield. R_f = 0.91 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +54.3 (c= 0.88, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): 10.93 (s, 1H), 7.37 (d, J = 9 Hz, 2H), 6.85 (d, J = 8.5 Hz, 2H), 4.70-4.64 (m, 3H), 3.88-3.81 (m, 2H), 3.78 (s, 4H), 3.75-3.73 (m, 1H), 3.67 (dd, J = 7 Hz, 3 Hz, 1H), 3.35 (d, J = 7 Hz, 1H), 1.52 (s, 18H), 1.39 (s, 3H), 1.15 (s, 3H), 0.91 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H) ppm. ^{13}C {^1H} NMR (125 MHz, $CDCl_3$): δ (ppm): 162.0, 159.9, 156.5, 156.1, 149.7, 148.9, 148.5, 148.3, 130.8, 121.7, 114.1, 109.5, 84.6, 82.5, 80.8, 80.5, 75.8, 63.9, 59.6, 55.7, 55.4, 45.6, 34.3, 28.1, 27.9, 26.8, 26.5, 26.0, 18.4, 5.4, 5.3 ppm. IR (neat): 2979, 2930, 1759, 1710, 1622, 1556, 1513, 1464, 1368, 1298, 1243, 1137, 836, 778 cm^{-1}



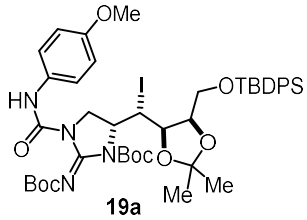
18a

tert-butyl (S,Z)-2-((tert-butoxycarbonyl)imino)-5-((S)-((4R,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)-3-((4-methoxyphenyl)carbamoyl)imidazolidine-1-carboxylate (18b)



Prepared according to General Procedure A to give **18b** in 65% yield. R_f = 0.91 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = -40.2 (c= 0.43, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): 10.95 (s, 1H), 7.37 (dt, J = 3 Hz, 9 Hz, 2H), 6.85 (dt, J = 3.5 Hz, 9 Hz, 2H), 4.69 (dt, J = 2 Hz, 9 Hz, 1H), 4.29 (dd, J = 3 Hz, 10.5 Hz, 1H), 4.25 (dd, J = 5.5 Hz, 10.5 Hz, 1H), 4.19 (dd, J = 1.5 Hz, 12 Hz, 1H), 4.04-4.01 (m, 1H), 3.93 (dd, J = 3 Hz, 8.5 Hz, 1H), 3.88 (dd, J = 3 Hz, 9 Hz, 1H), 3.82 (dd, J = 4Hz, 11 Hz, 1H), 3.77 (s, 3H), 1.53 (s, 9H), 1.52 (s, 9H), 1.43 (s, 3H), 1.34 (s, 3H), 0.89 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H) ppm. ^{13}C {^1H} NMR (125 MHz, $CDCl_3$): δ (ppm): 156.2, 155.8, 149.9, 149.5, 146.2, 130.7, 122.1, 114.1, 110.8, 84.6, 83.3, 80.3, 76.5, 64.3, 58.0, 55.4, 45.2, 36.5, 28.2, 28.1, 27.3, 27.1, 25.9, 18.4, 5.2, 5.1 ppm. IR (neat): 2930, 2856, 1758, 1695, 1640, 1556, 1512, 1472, 1369, 1305, 1244, 1138, 1037, 835, 777cm^{-1}

tert-butyl (R,Z)-2-((tert-butoxycarbonyl)imino)-5-((R)-((4R,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)-3-((4-methoxyphenyl)carbamoyl)imidazolidine-1-carboxylate (19a)

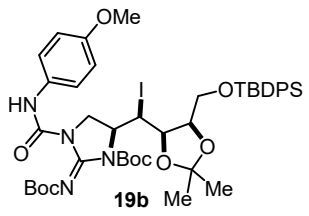


Prepared according to General Procedure A to give **19a** in 93% yield. R_f = 0.91 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = +73.1 (c= 1.50, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): 10.97 (s, 1H), 7.73-7.68 (m, 4H), 7.44-7.37 (m, 8H), 6.87 (dt, J = 3.5 Hz, 9Hz, 2H), 4.73-4.68 (m, 3H), 4.00-3.96 (m, 1H), 3.88 (dd, J = 4.5 Hz, 10.5 Hz, 1H), 3.79 (s, 3H), 3.78-3.74 (m, 2H), 3.44 (d, J = 7.5 Hz, 1H), 1.55 (s, 9H), 1.40 (s, 12H), 1.14 (s, 3H), 1.10 (s, 9H) ppm. ^{13}C {^1H}

NMR (125 MHz, $CDCl_3$): δ (ppm): 156.7, 156.2, 149.8, 149.0, 148.6, 135.6, 135.5, 133.2, 132.8, 130.8, 129.8, 129.6, 127.8, 127.7, 121.7, 114.2, 109.6, 84.7, 82.6, 80.5, 75.9, 64.7, 59.7, 55.4, 45.6, 34.4, 28.2, 27.9, 27.0, 26.9, 26.5, 19.2 ppm. IR (neat): 2979, 2931, 2858, 1755, 1709, 1614, 1556, 1512, 1471, 1368, 1307, 1233, 1123, 756, 702 cm^{-1}

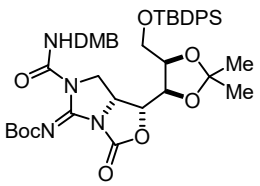
tert-butyl (S,Z)-2-((tert-butoxycarbonyl)imino)-5-((S)-((4R,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)iodomethyl)-3-((4-methoxyphenyl)carbamoyl)imidazolidine-1-carboxylate (19b)

Prepared according to General Procedure A to give **19a** in 80% yield. R_f = 0.91 (1:1 Hex/EtOAc), $[\alpha]^{20}_D$ = -18.8 (c= 1.39, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): 10.97 (s, 1H), 7.76-7.70 (m, 4H), 7.44-7.36 (m, 8H), 6.87 (dt, J = 3.5 Hz, 9 Hz, 2H), 4.70 (dt, J = 1.5 Hz, 9 Hz, 1H), 4.42 (dd, J = 6 Hz, 10.5 Hz, 1H), 4.32 (dd, J = 3 Hz, 10.5 Hz, 1H), 4.27 (dd, J = 1.5 Hz, 11.5 Hz, 1H), 4.10-4.07 (m, 1H), 4.00 (dd, J = 2 Hz, 11 Hz, 1H), 3.91-3.87 (m, 2H), 3.79 (s, 3H), 1.54 (s, 9H), 1.53 (s, 9H), 1.48 (s, 3H), 1.40 (s, 3H), 1.07 (s, 9H) ppm. ^{13}C {^1H} NMR (125 MHz, $CDCl_3$): δ (ppm): 156.4, 156.0, 150.2, 149.6, 146.4, 136.0, 135.9, 133.5, 133.4, 130.9, 129.9, 129.8, 127.9, 127.8, 122.3, 114.3, 110.9, 84.7, 83.4, 80.5, 76.6, 64.8, 58.6, 55.6, 45.1, 42.9, 35.8, 28.3, 28.2, 27.4, 27.2, 27.0, 19.4 ppm. IR (neat): 2977, 2931, 1758, 1695, 1606, 1556, 1512, 1472, 1369, 1305, 1229, 1135, 1037, 756, 702 cm^{-1}

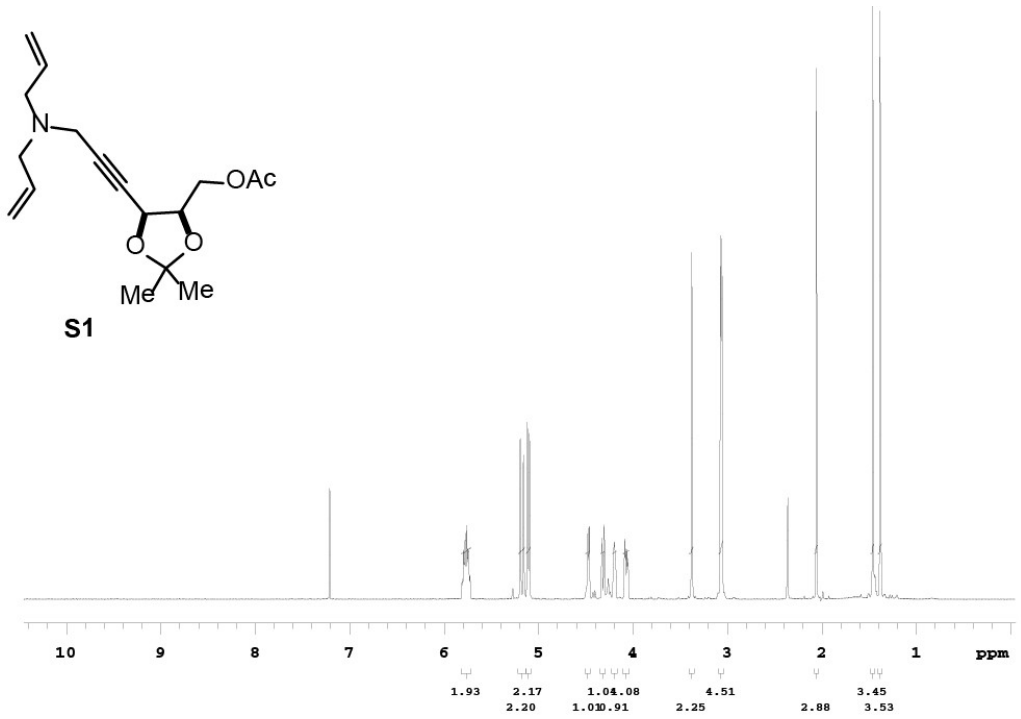


19b

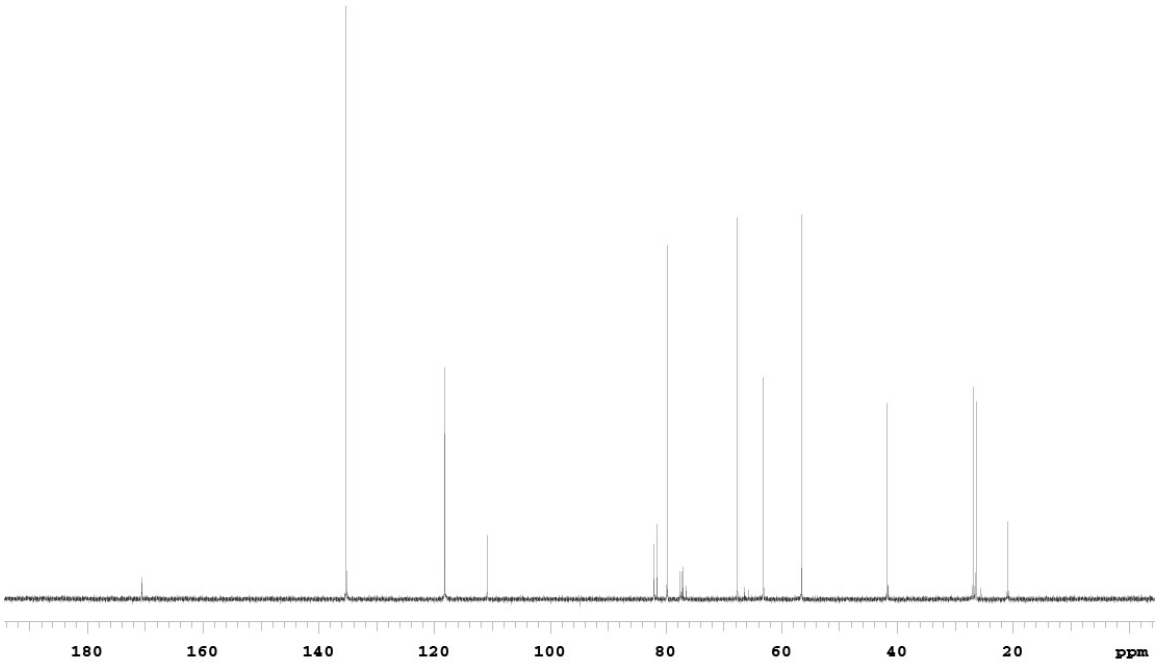
tert-butyl ((1R,7aR,Z)-1-((4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-6-((3,4-dimethylbenzyl)carbamoyl)-3-oxotetrahydro-3H,5H-imidazo[1,5-c]oxazol-5-ylidene)carbamate (23)

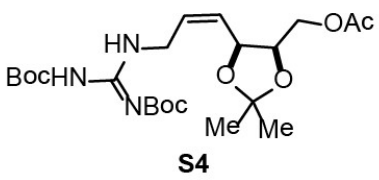


Cyclic guanidine **19a** (396.4 mg, 0.46 mmol) was dissolved in DMF, followed by the addition of AgOAc (107 mg, 0.91 mmol) and the reaction was heated to reflux for 24 hours. The reaction was cooled to room temperature and diluted with Et_2O and H_2O. The aqueous layer was washed with Et_2O (3 x 50 mL) and the organic layers were combined and dried over Na_2SO_4, then concentrated and purification by flash chromatography (4:1 Hex/EtOAc) gave **23** (130 mg, 35%) as a colorless oil. R_f = 0.62 (1:1 Hex/EtOAc) ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.72-7.67 (m, 5H), 7.43-7.36 (m, 7H), 6.99 (d, J = 8 Hz, 1H), 6.39 (s, 1H), 6.29 (d, J = 10.5 Hz, 1H), 5.06-5.07 (m, 3H), 4.61 (d, J = 11.5 Hz, 1H), 4.42 (dd, J = 6, 11.5 Hz, 1H), 4.24 (dd, J = 2, 12 Hz, 1H), 4.10-4.03 (m, 1H), 4.02-3.95 (m, 2H), 3.90-3.83 (m, 2H), 3.79 (s, 3H), 3.71 (s, 3H), 1.56 (s, 9H), 1.53 (s, 3H), 1.48 (s, 3H), 1.03 (s, 9H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 160.1, 158.1, 155.6, 154.5, 148.7, 148.4, 135.8, 133.1, 129.8, 127.8, 127.7, 116.8, 111.3, 103.9, 98.5, 85.4, 83.6, 76.4, 64.6, 61.2, 55.4, 41.6, 39.9, 30.9, 28.3, 28.1, 28.0, 27.4, 27.1, 26.8, 19.3 ppm.


N
OAc
O
O
Me
Me
10
9
8
7
6
5
4
3
2
1
ppm
1.93
2.17
2.20
1.01
1.04
0.91
4.08
2.25
4.51
2.88
3.45
3.53

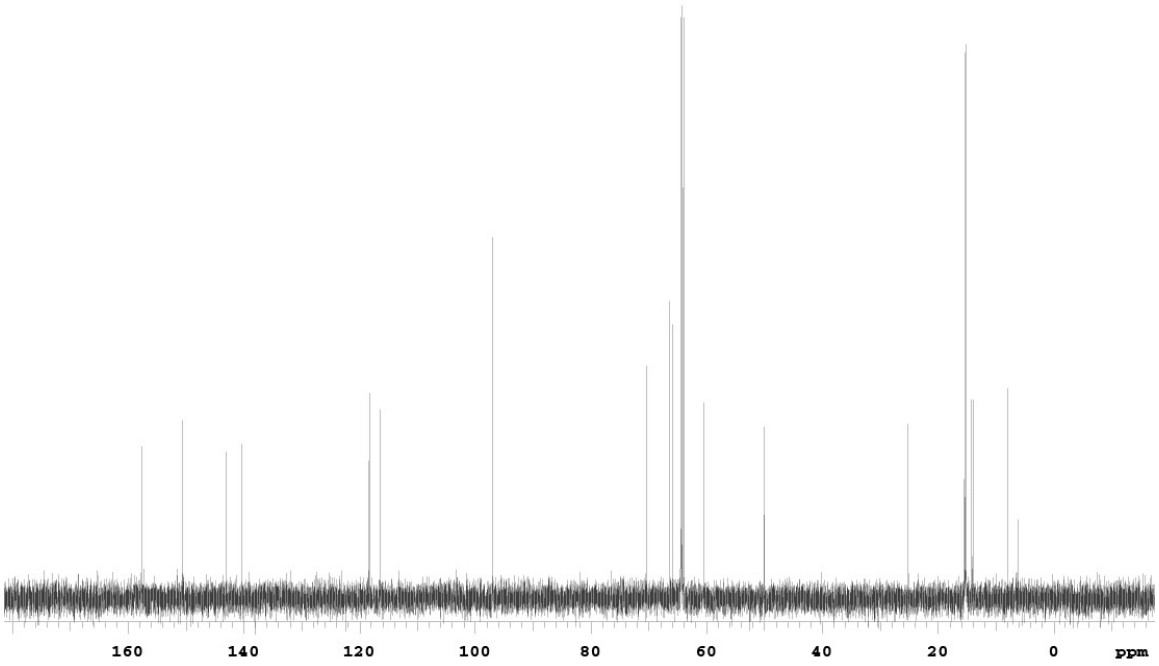
S1

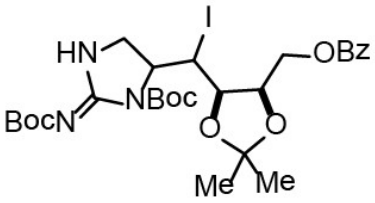

180
160
140
120
100
80
60
40
20
ppm


HN
OAc
BocHN
NBoc
O
O
Me
Me

S4

9
8
7
6
5
4
3
2
1
ppm
1.03
1.04
1.00
1.03
1.13
1.04
2.83
6.47
19.82

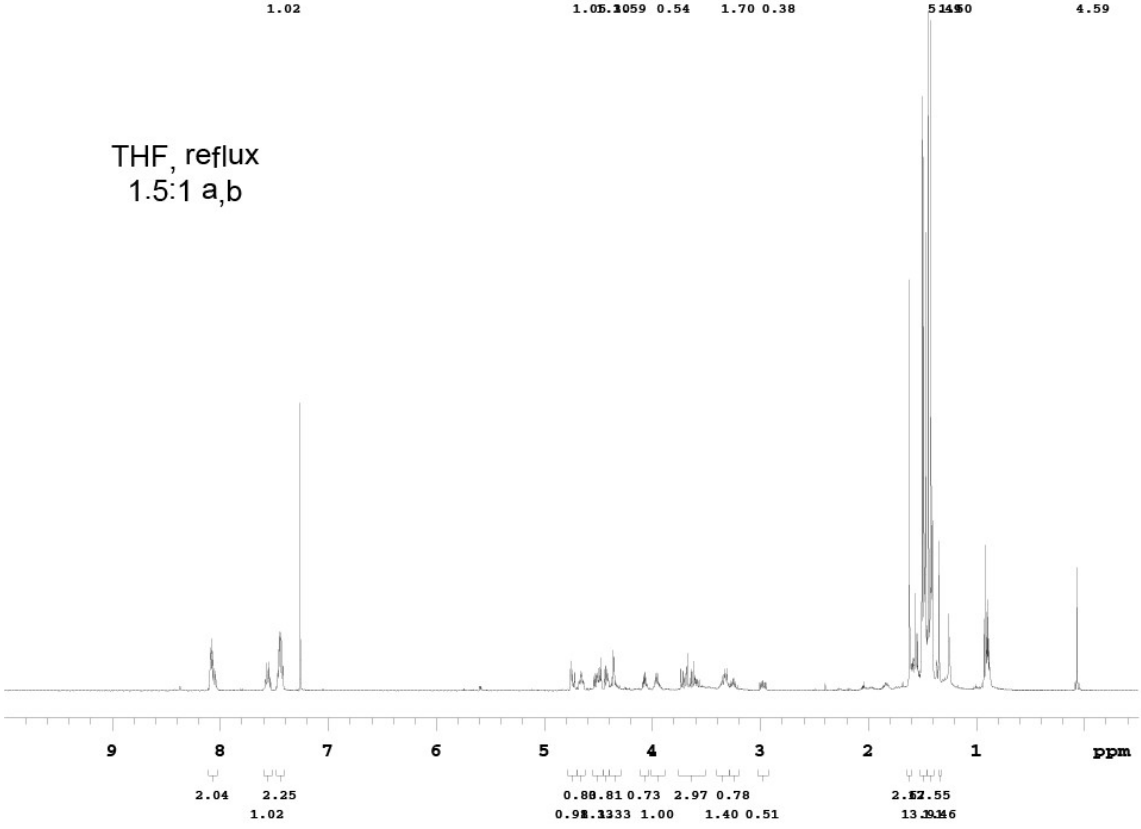

160
140
120
100
80
60
40
20
0
ppm

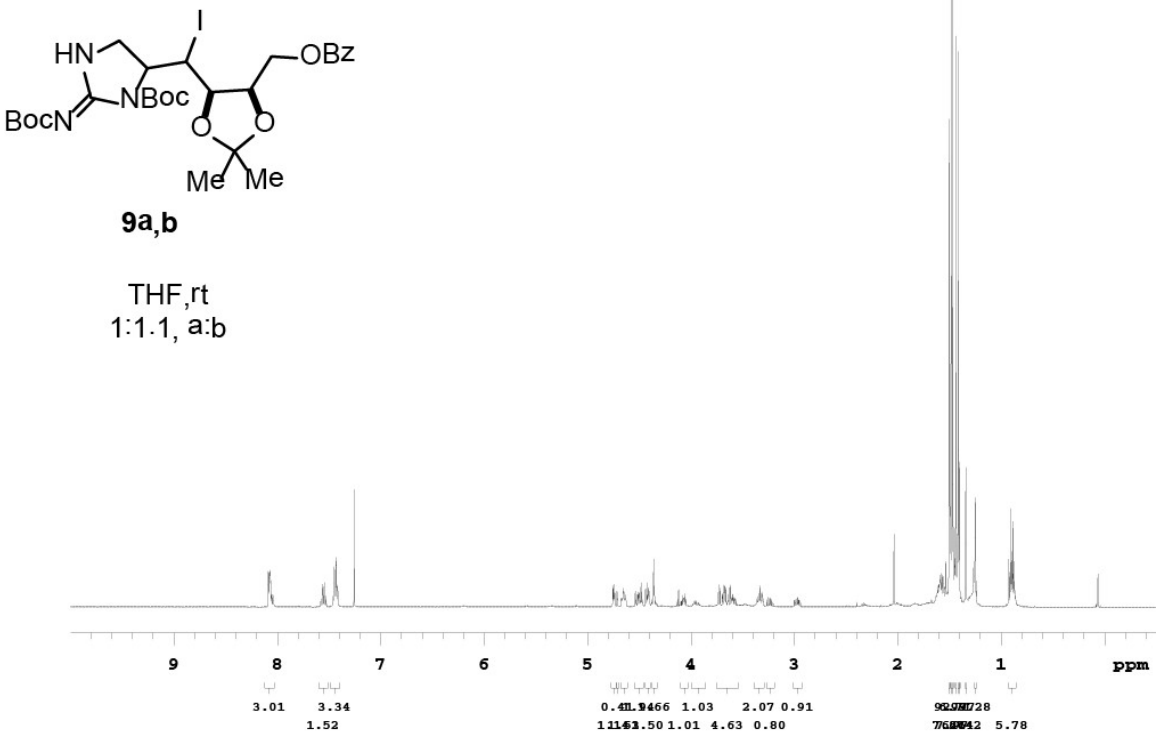


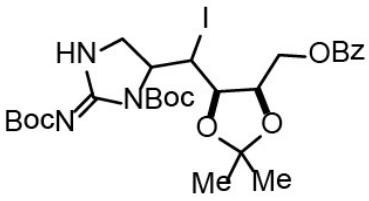
9a,b

Et_2O, reflux
2:1 a,b

THF, reflux
1.5:1 a,b



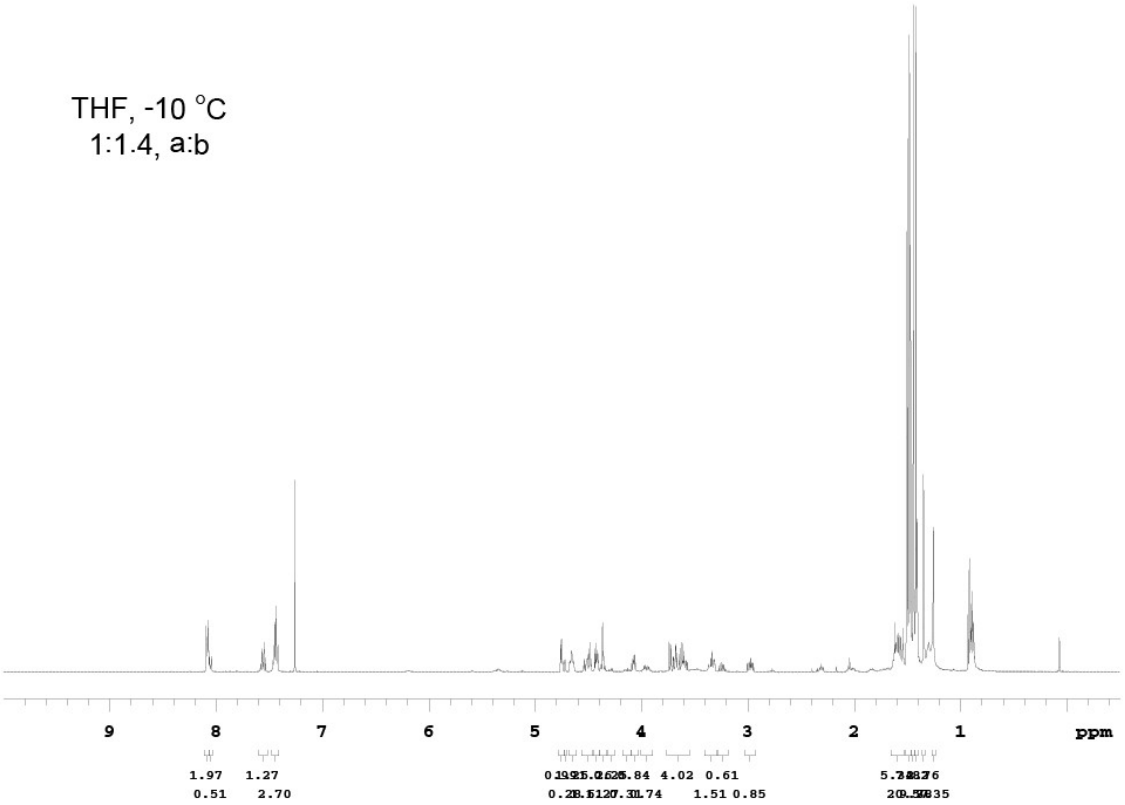

I
HN
OBz
NBoc
BocN
O
O
Me
Me
9a,b
THF,rt
1:1.1, a:b
9
8
7
6
5
4
3
2
1
ppm
3.01
3.34
1.52
1.03
2.07
0.91
1.01
4.63
0.80
5.78

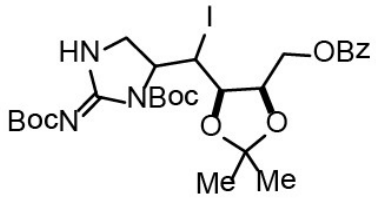


9a,b

Et_2O, -10 °C
1.3:1, a:b

THF, -10 °C
1:1.4, a:b

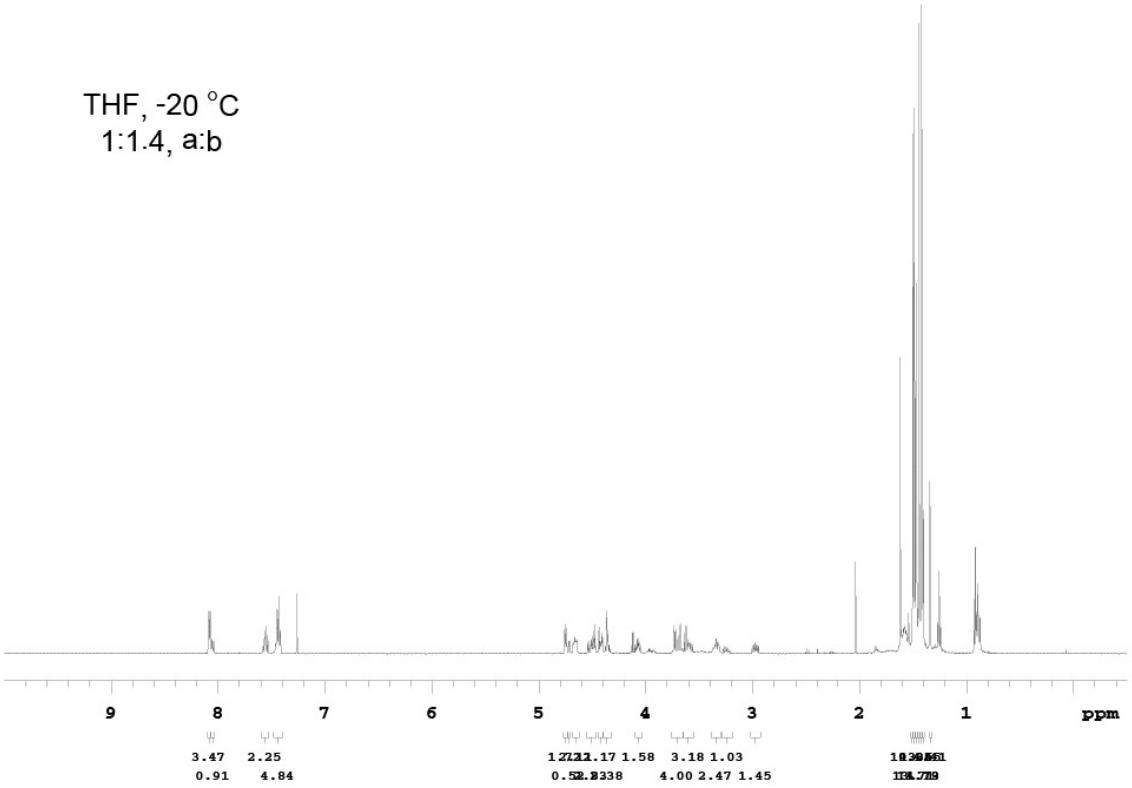


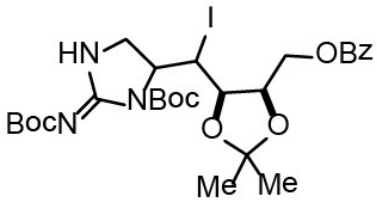


9a,b

Et_2O, -20 °C
1.5:1, a:b

THF, -20 °C
1:1.4, a:b

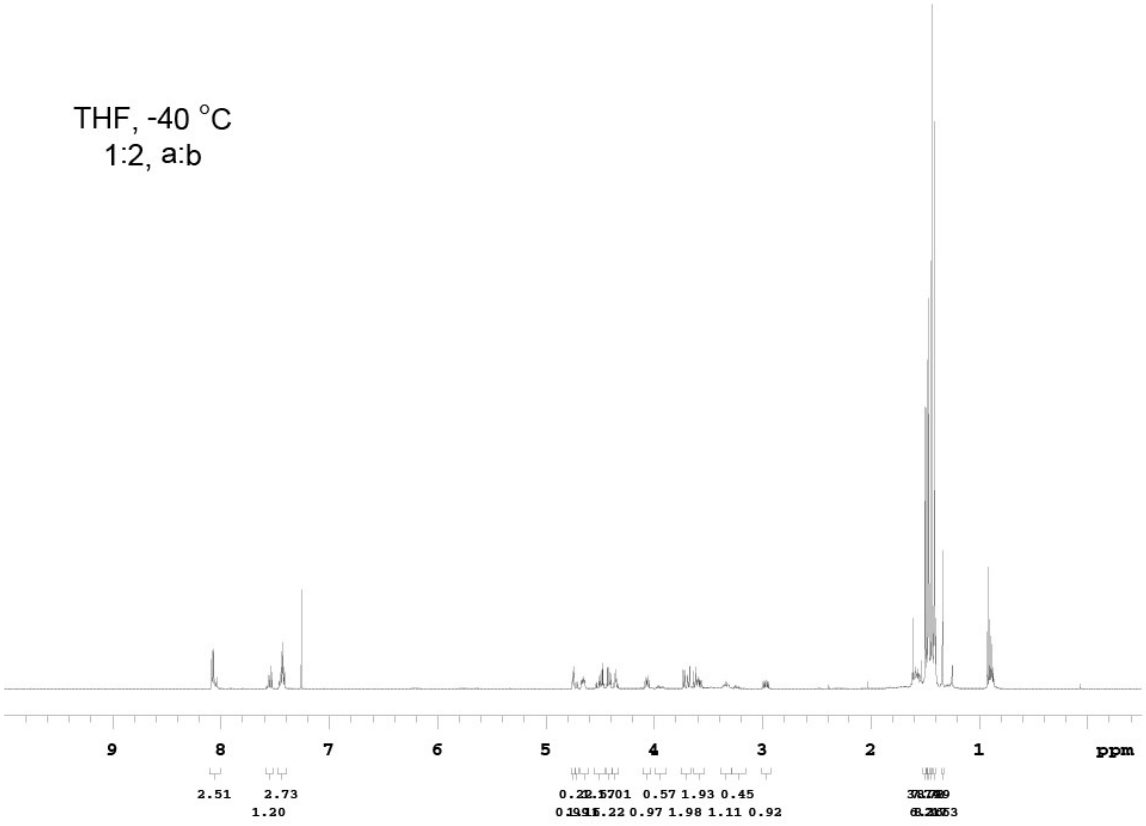


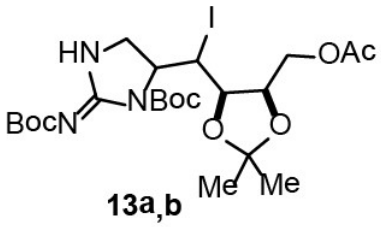


9a,b

Et_2O, -40 °C
1.1:1, a:b

THF, -40 °C
1:2, a:b

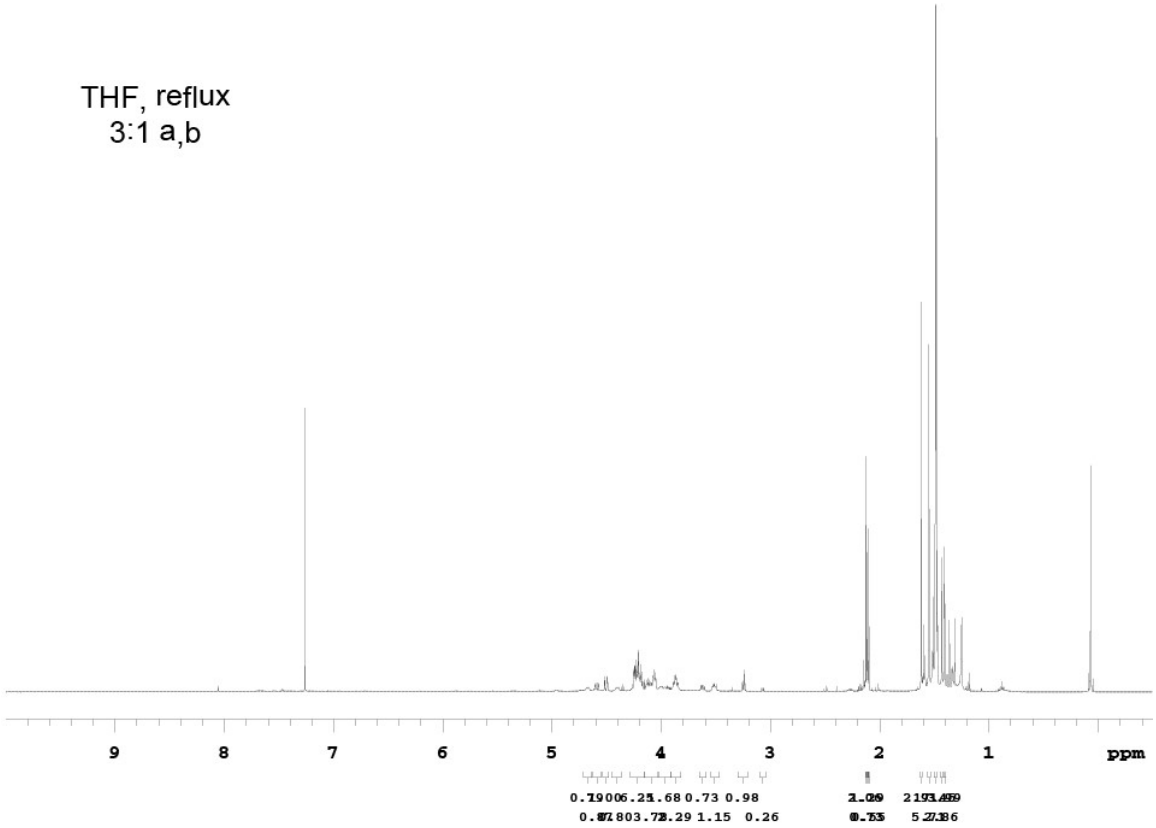


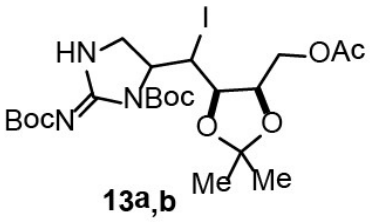


13a,b

Et_2O, reflux
2.6:1 a,b

THF, reflux
3:1 a,b

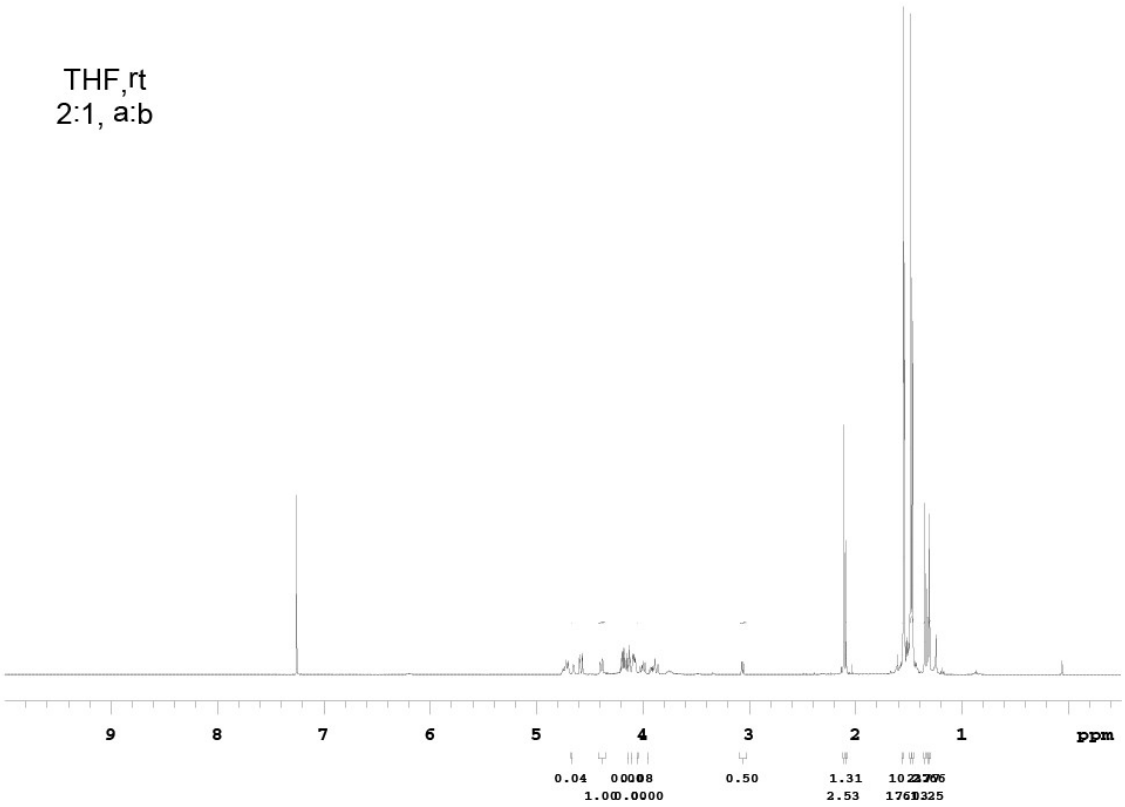


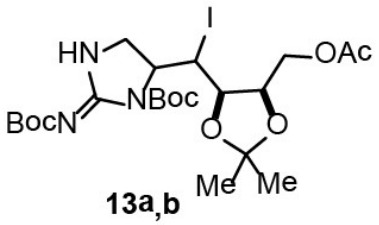


13a,b

Et_2O,rt
2.6:1, a:b

THF,rt
2:1, a:b

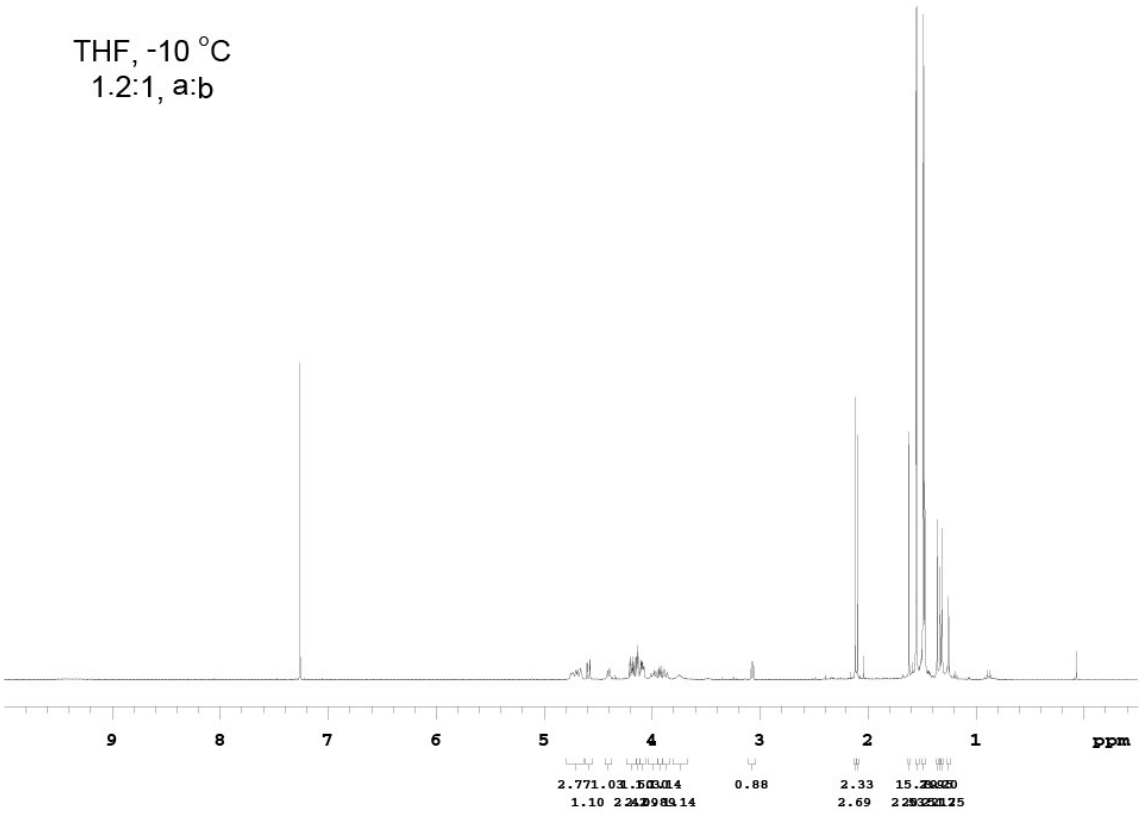


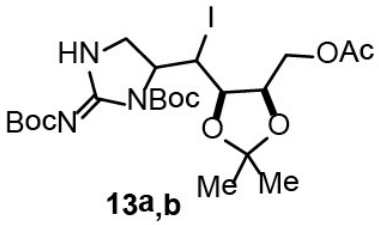


13a,b

Et_2O, -10 °C
1.6:1, a:b

THF, -10 °C
1.2:1, a:b

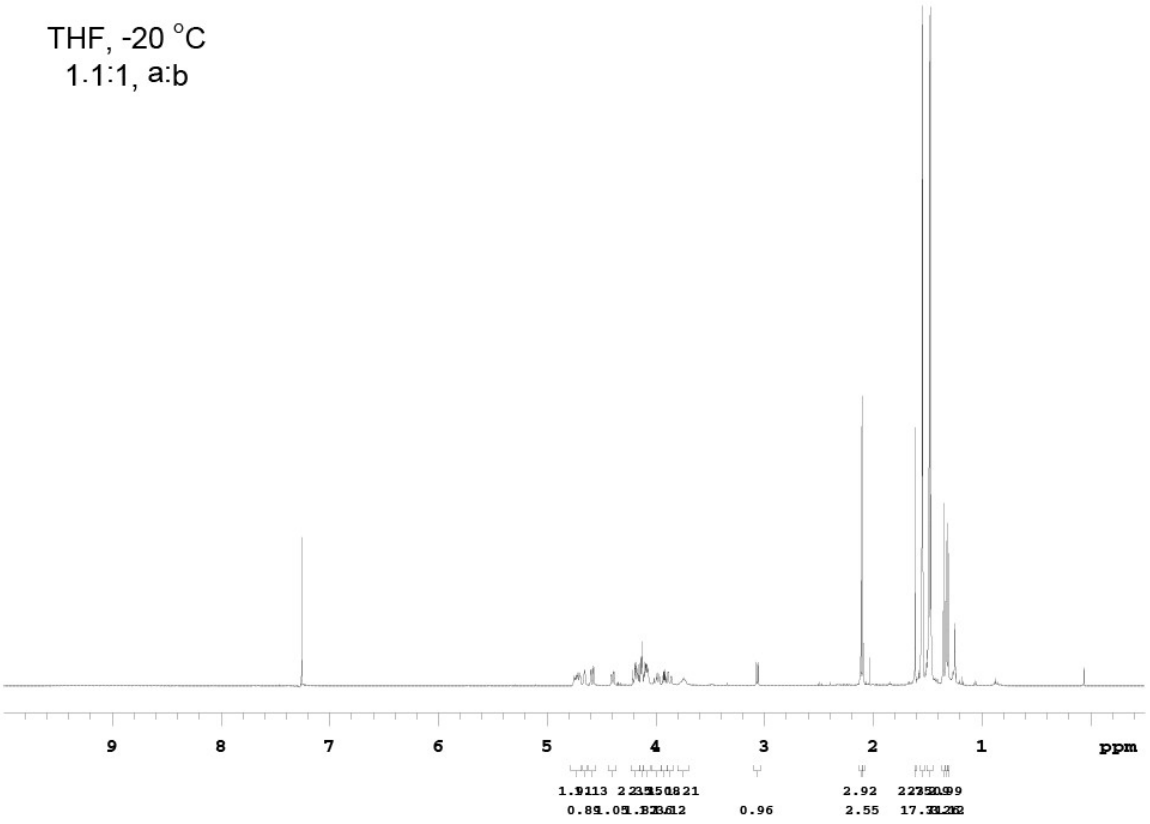


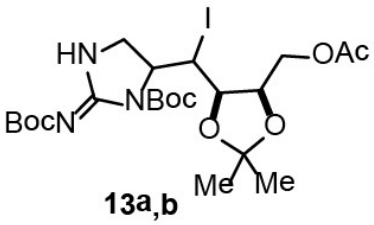


13a,b

Et_2O, -20 °C
1.8:1, a:b

THF, -20 °C
1.1:1, a:b

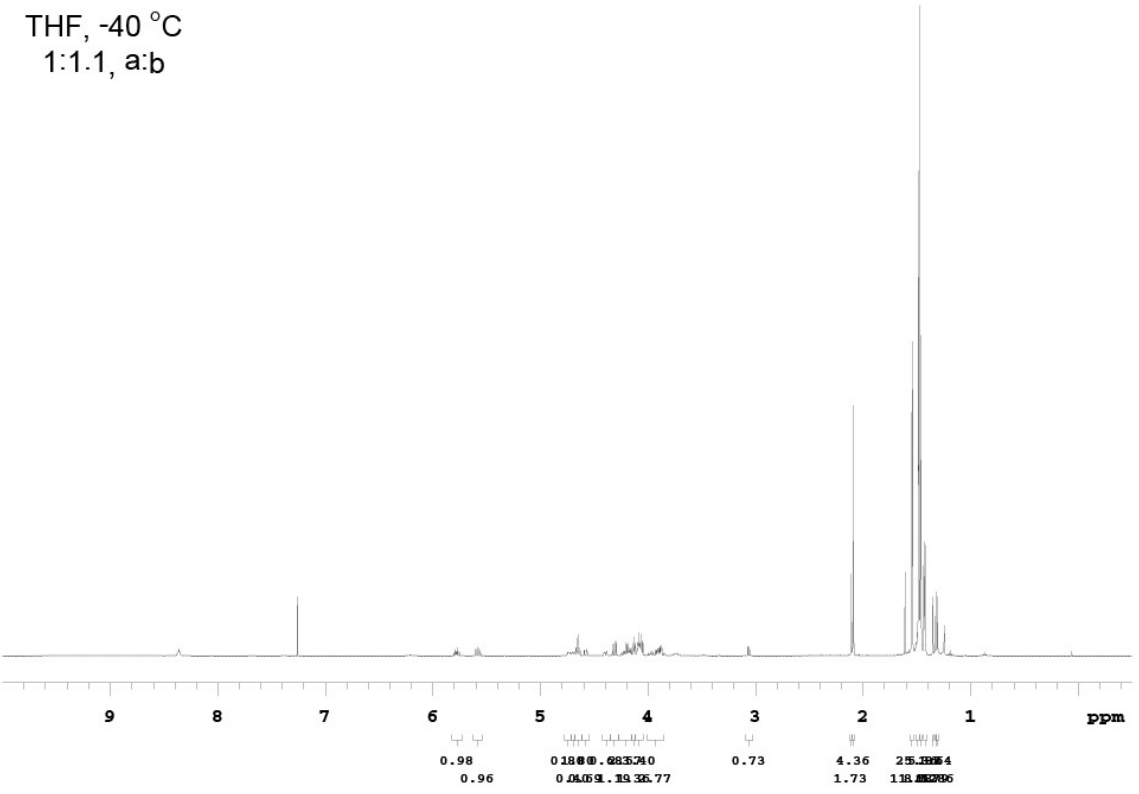


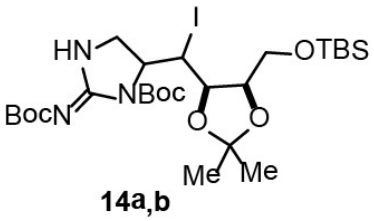


13a,b

Et_2O, -40 °C
1:3.8, a:b

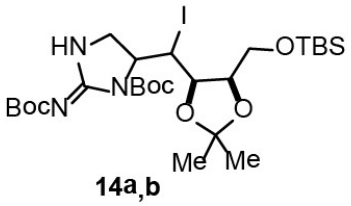
THF, -40 °C
1:1.1, a:b





14a,b

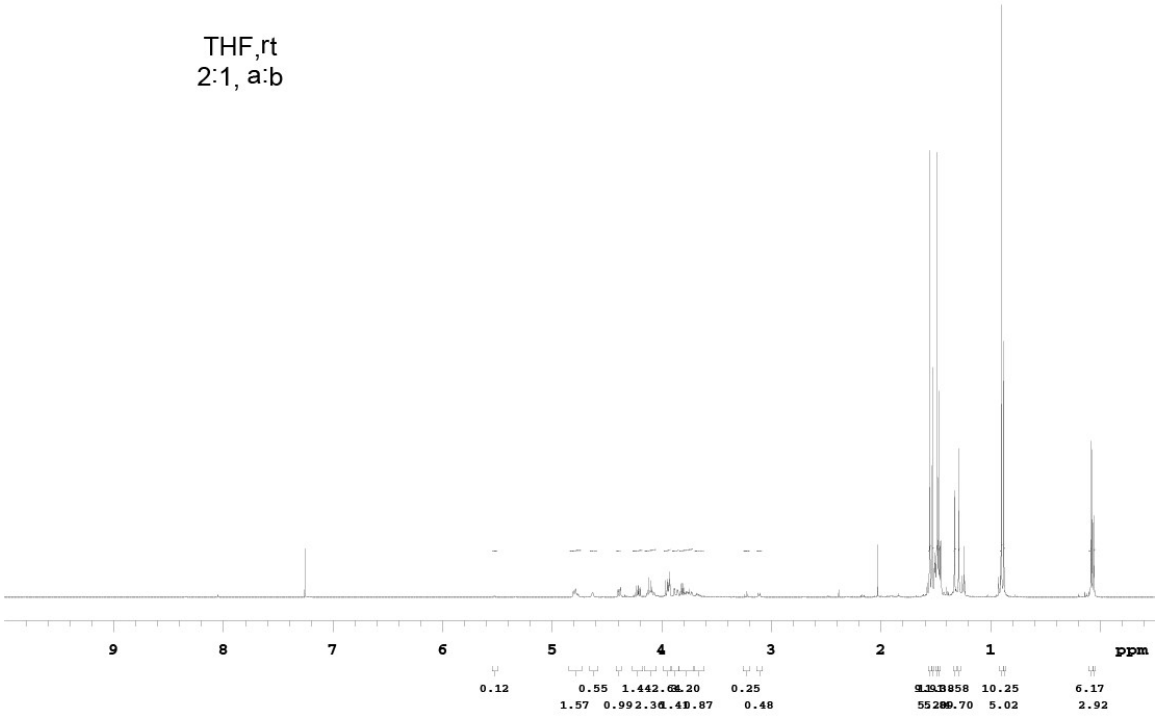
Et_2O, reflux
3:1 a,b

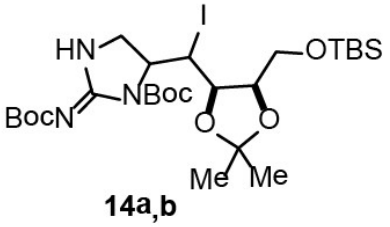


14a,b

Et_2O,rt
3.3:1, a:b

THF,rt
2:1, a:b

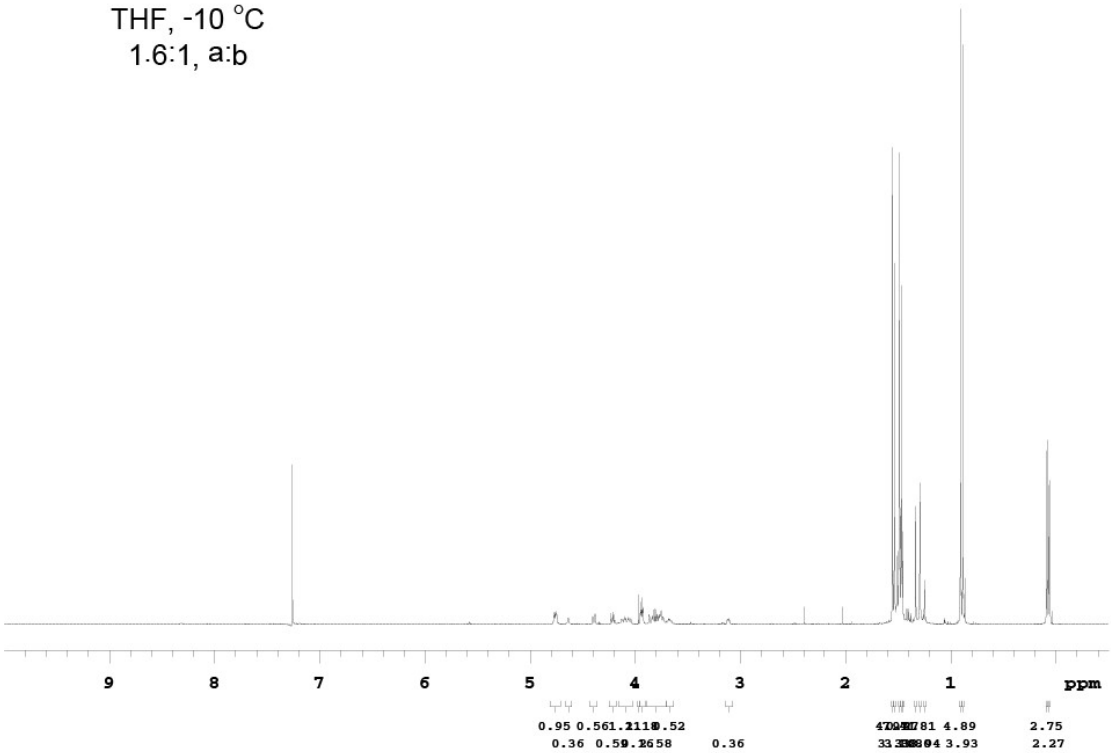


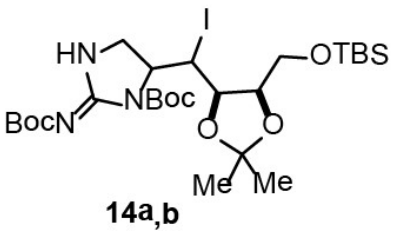


14a,b

Et_2O, -10 °C
1.8:1, a:b

THF, -10 °C
1.6:1, a:b

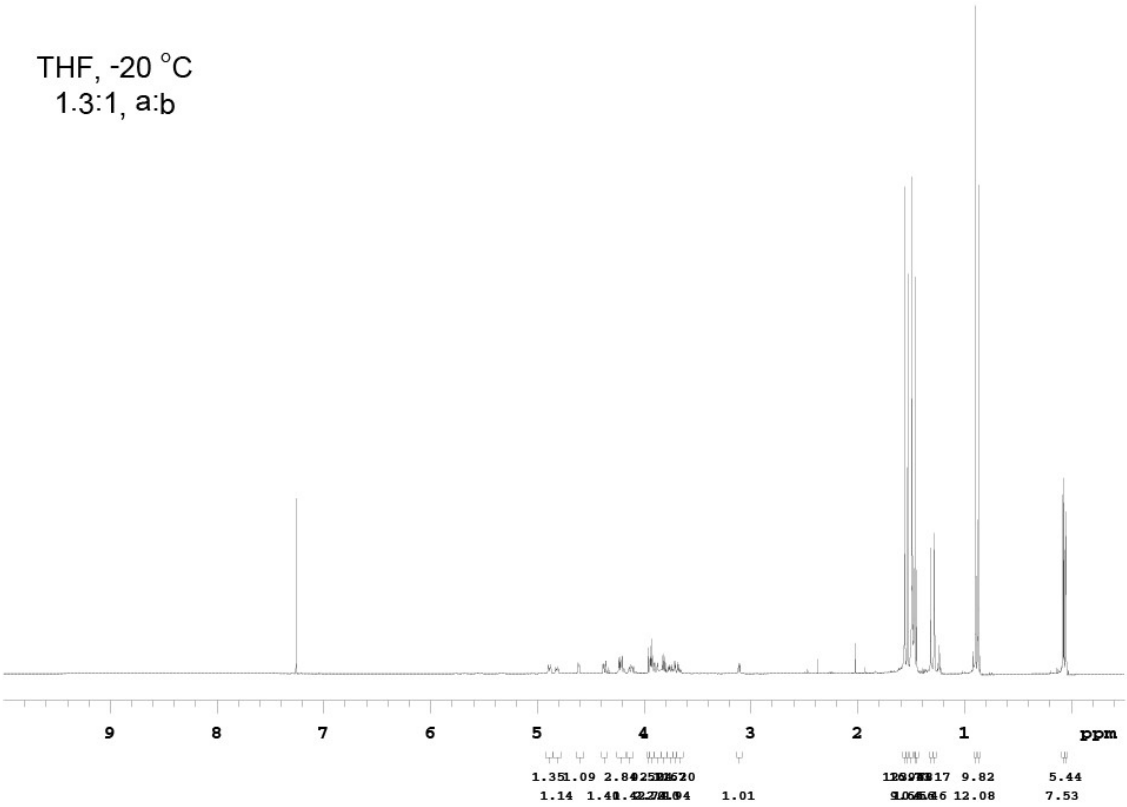


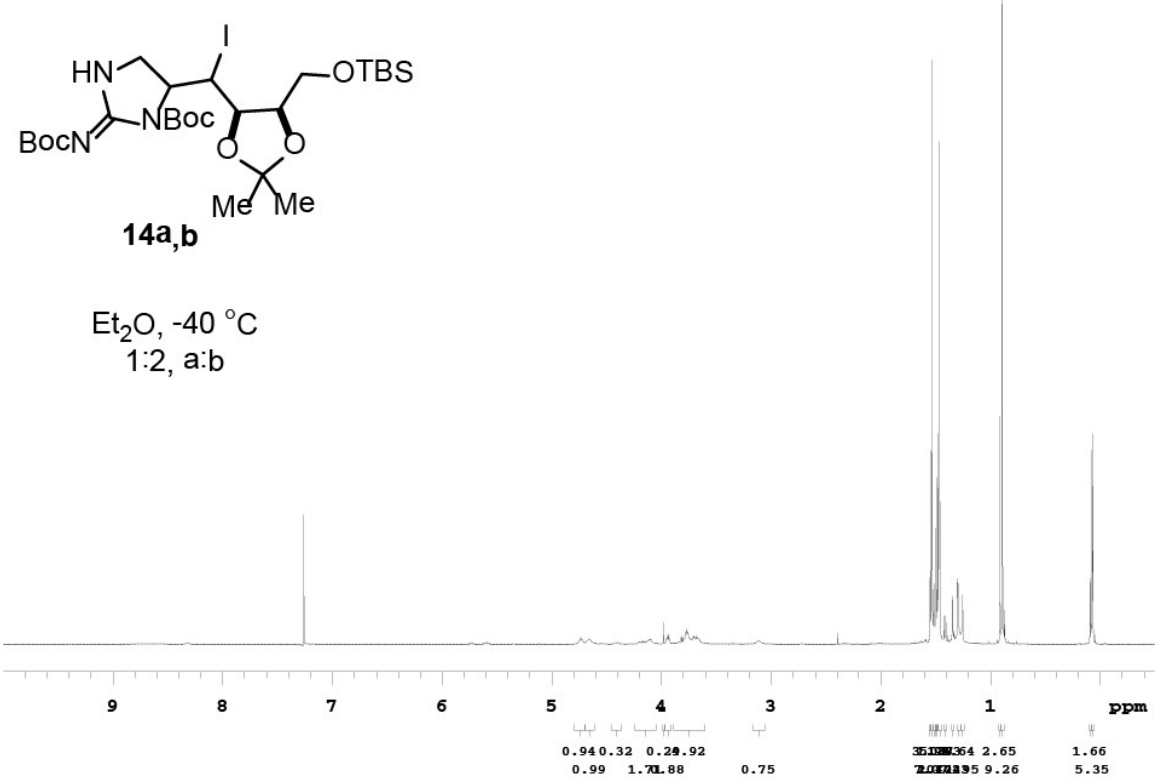


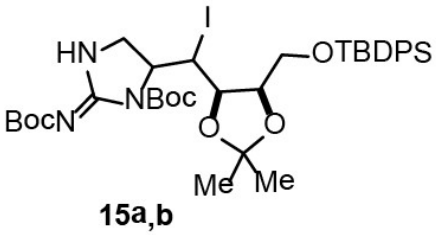
14a,b

Et_2O, -20 °C
1.4:1, a:b

THF, -20 °C
1.3:1, a:b



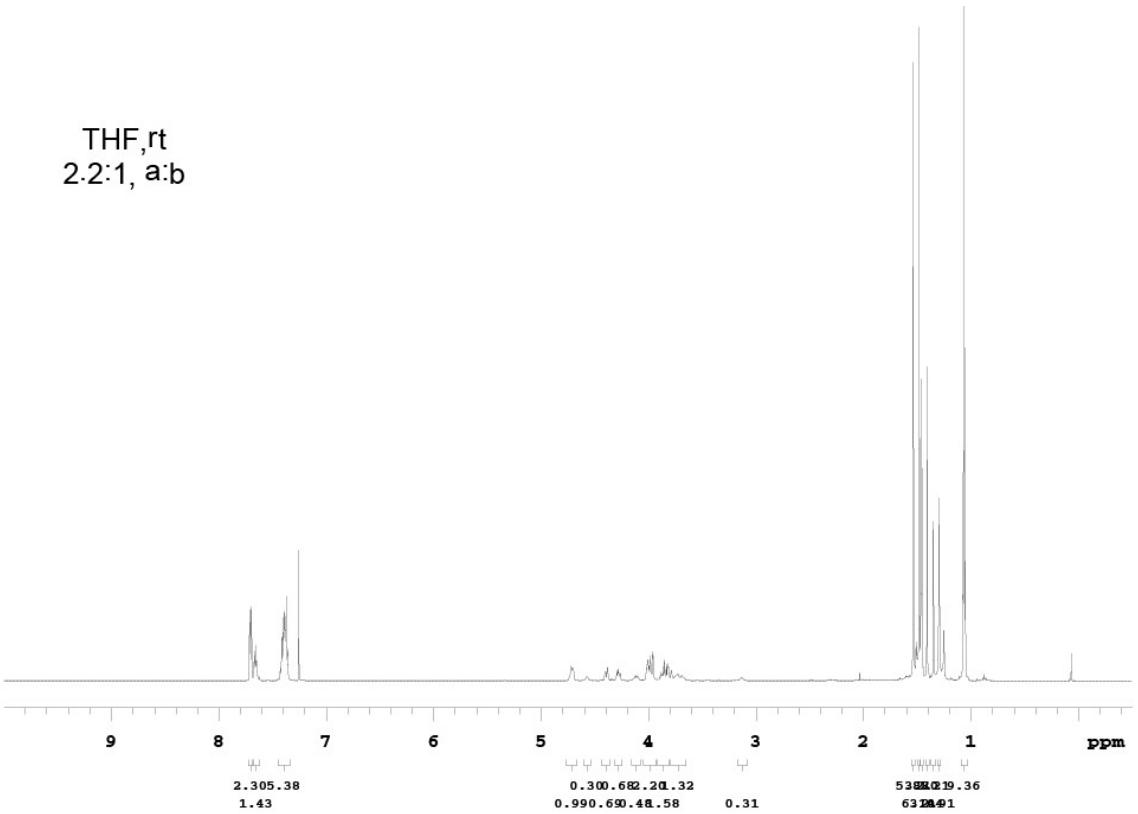

I
HN
OTBS
NBoc
BocN
O
O
Me
Me
14a,b
Et2O, -40 °C
1:2, a:b
9
8
7
6
5
4
3
2
1
ppm
0.94 0.32 0.29 0.92
0.99 1.70 1.88 0.75
2.65
9.26
1.66
5.35

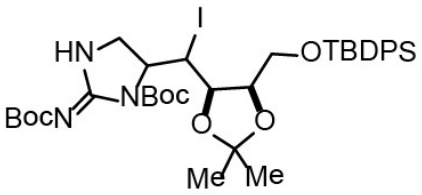


15a,b

Et_2O,rt
10:1, a:b

THF,rt
2.2:1, a:b

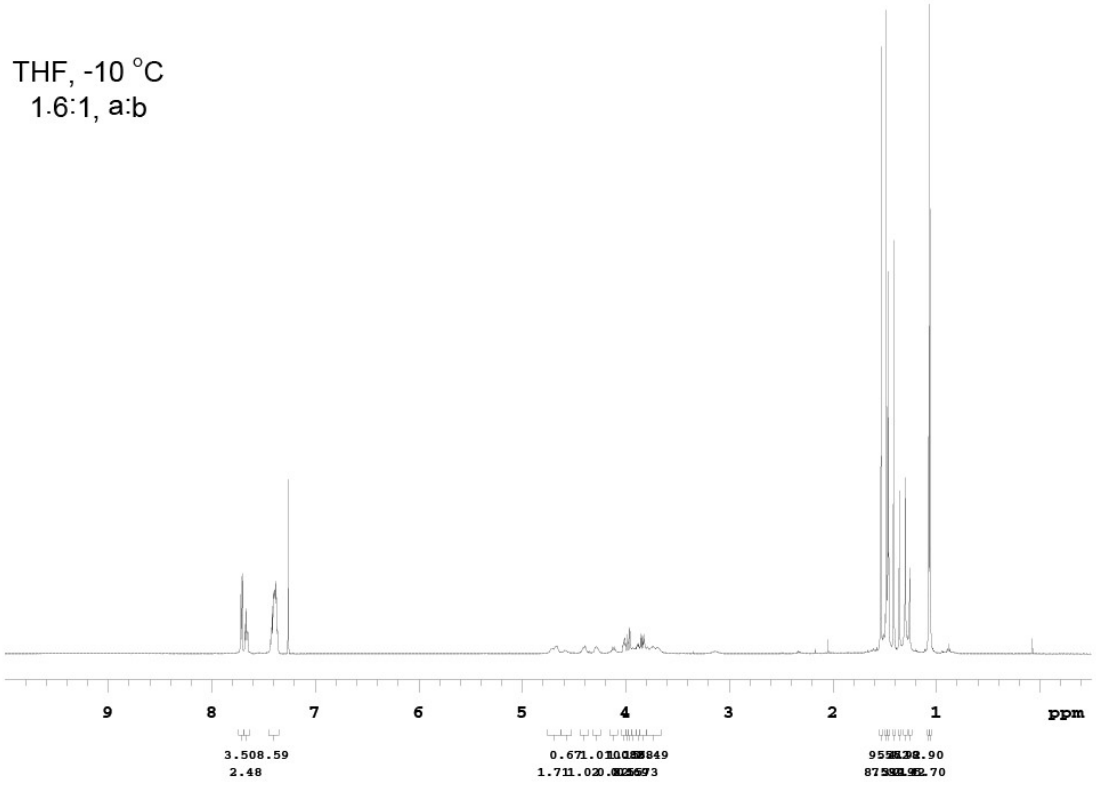


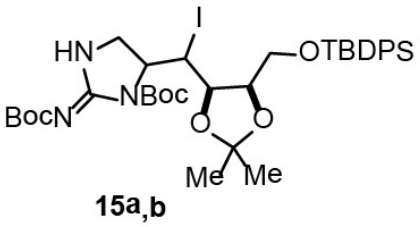


15a,b

Et_2O, -10 °C
2.3:1, a:b

THF, -10 °C
1.6:1, a:b

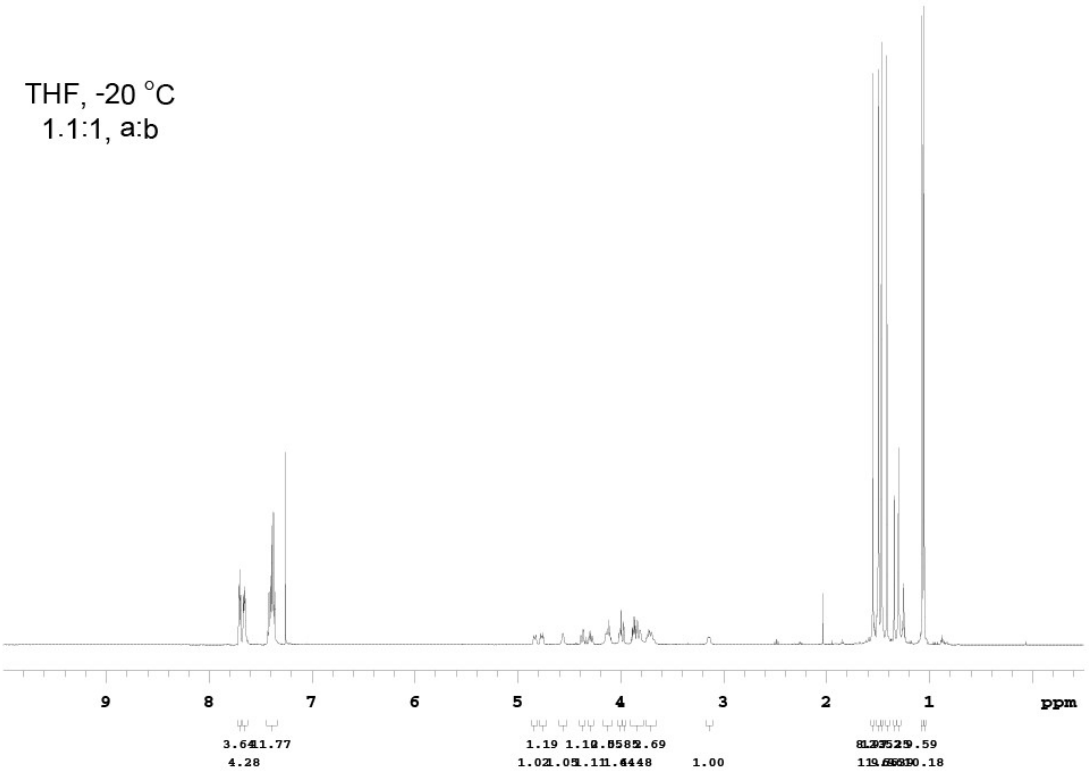


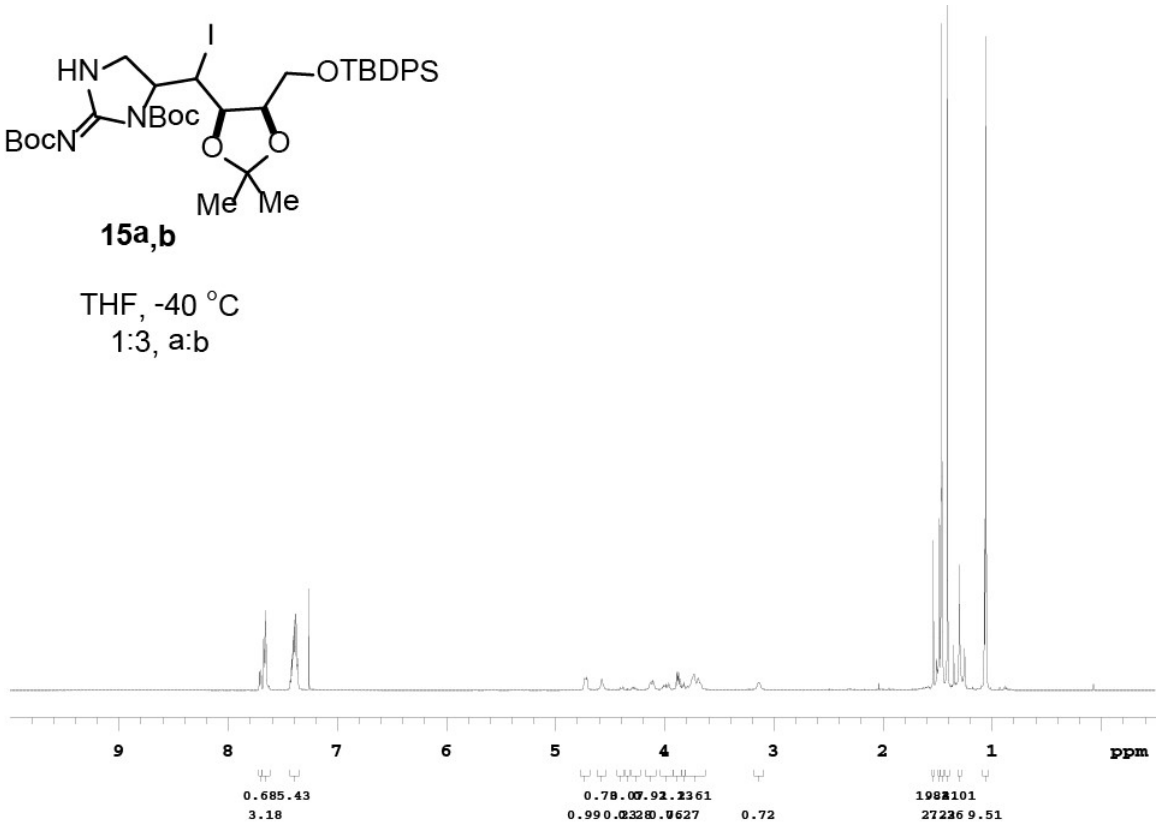


15a,b

Et_2O, -20 °C
1.8:1, a:b

THF, -20 °C
1.1:1, a:b

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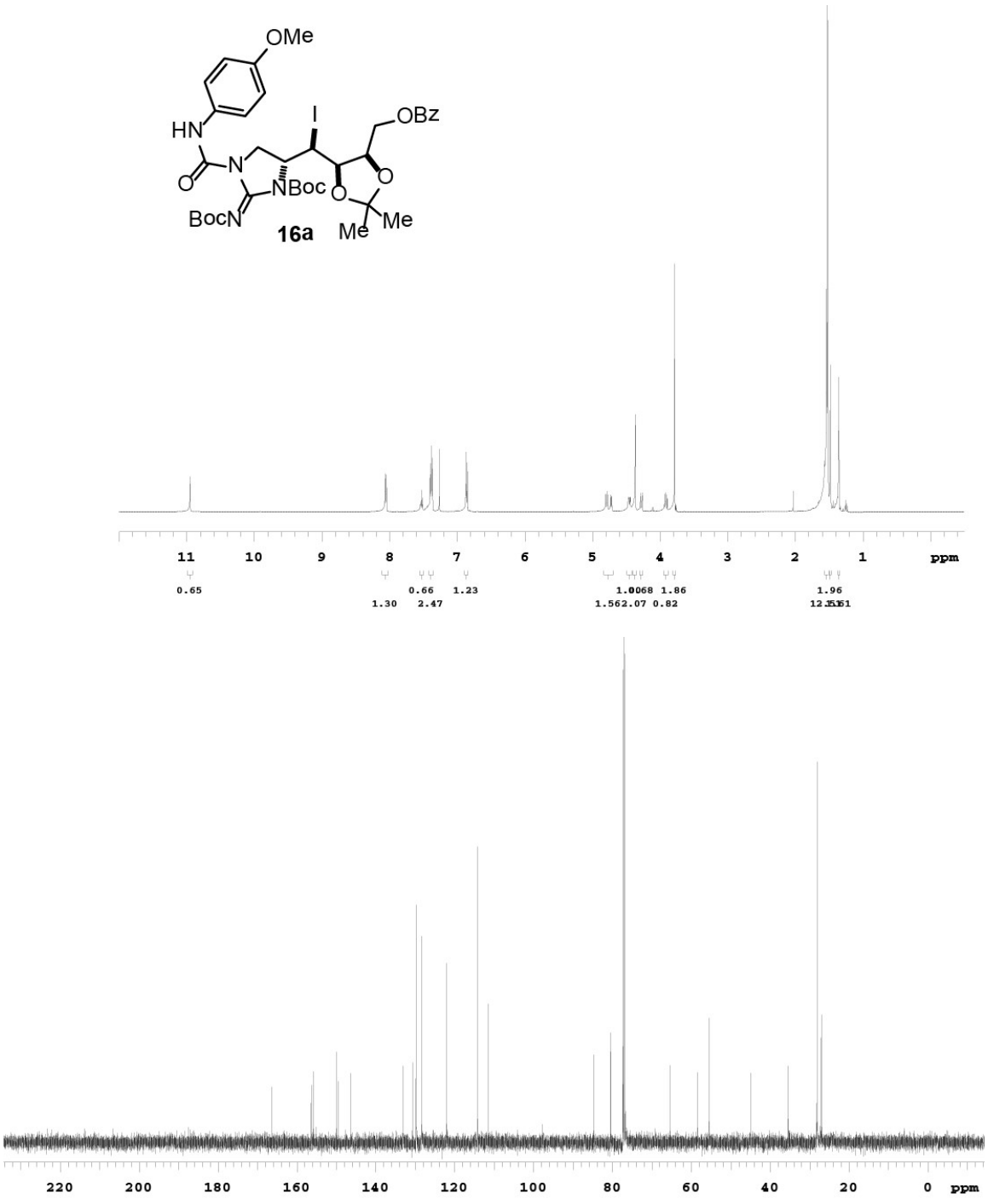
HN
OTBDPS
NBoc
BocN
O
O
Me
Me
15a,b
THF, -40 °C
1:3, a:b
9
8
7
6
5
4
3
2
1
ppm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OBz
N
O
NBoc
O
O
BocN
Me
Me
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10
9
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7
6
5
4
3
2
1
ppm
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0.66
2.47
1.23
1.00
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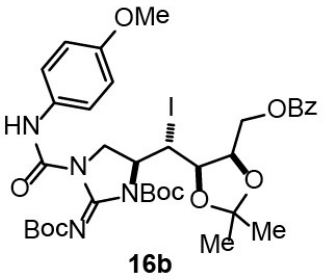
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BocN
Me
Me

16b

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5
4
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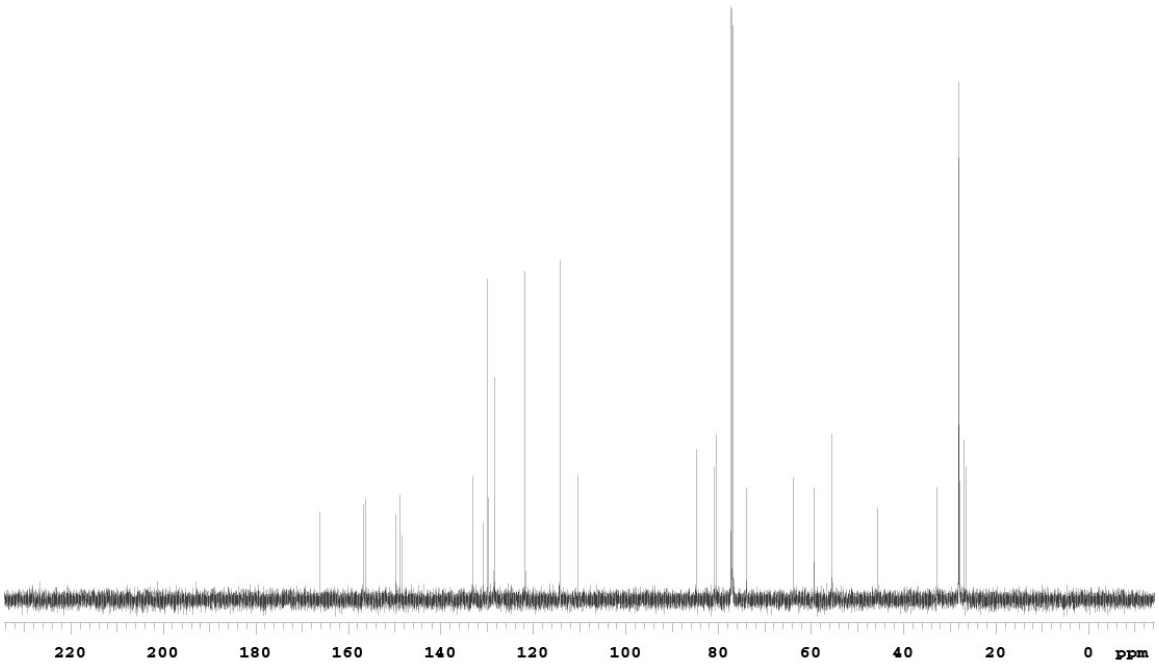
11.06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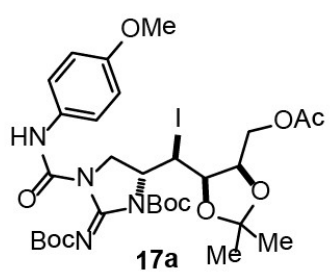
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HN
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O
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O
O
BocN
Me
Me

17a

11
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9
8
7
6
5
4
3
2
1
ppm
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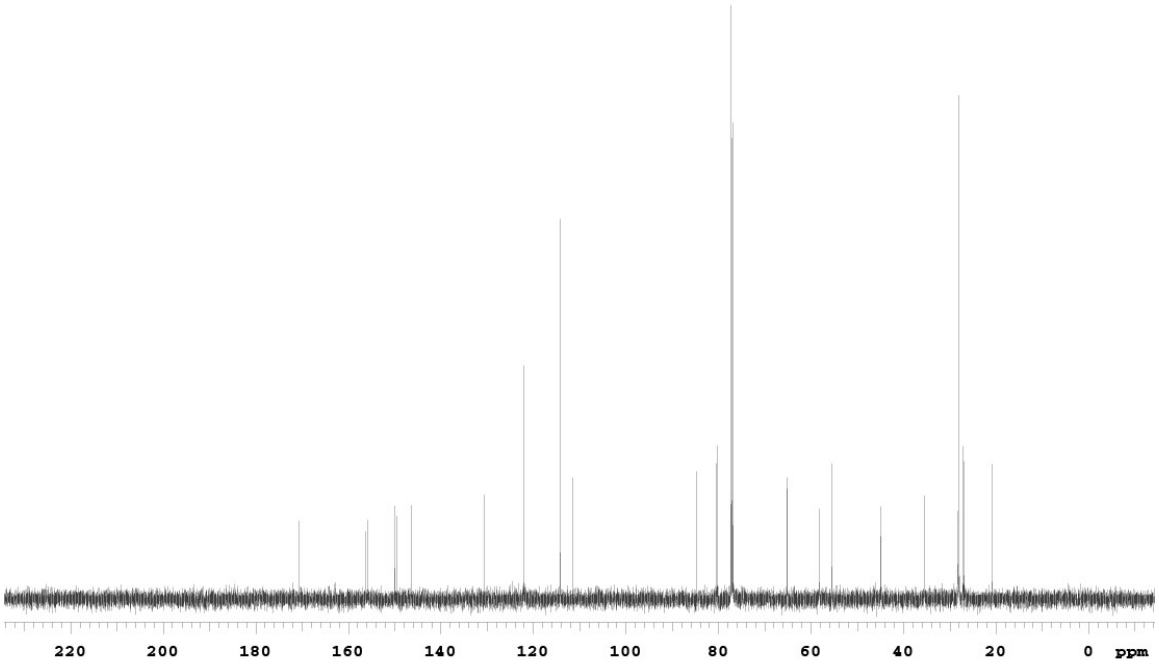
1.89
2.64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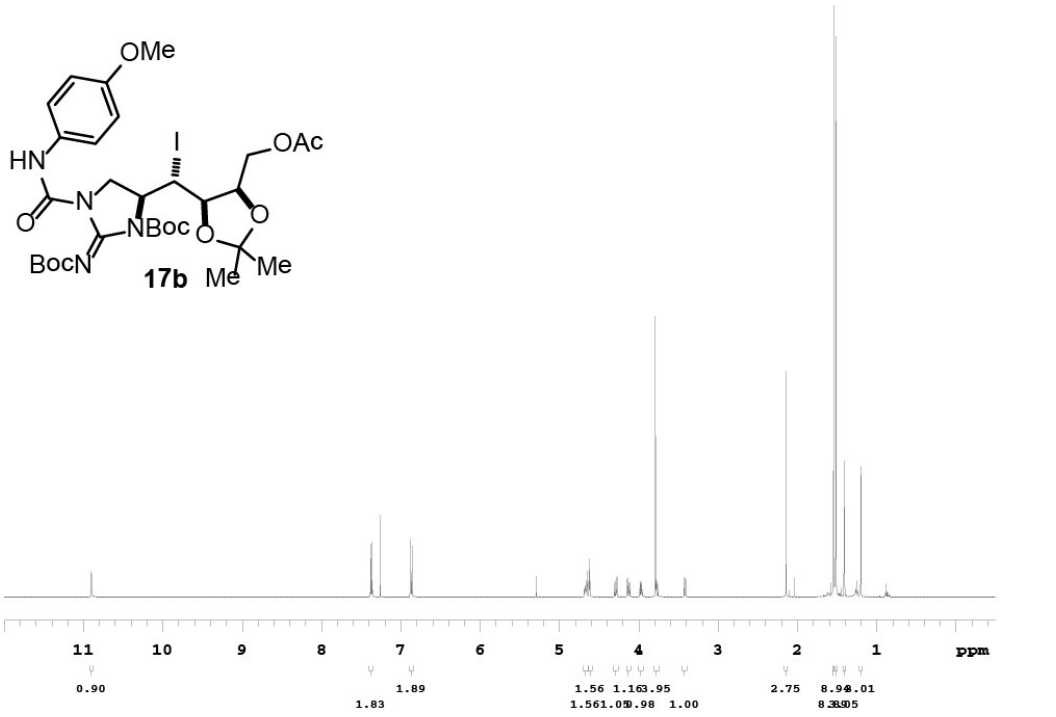
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HN
I
OAc
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NBoc
O
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BocN
Me
Me
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10
9
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6
5
4
3
2
1
ppm
0.90
1.83
1.89
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8.01
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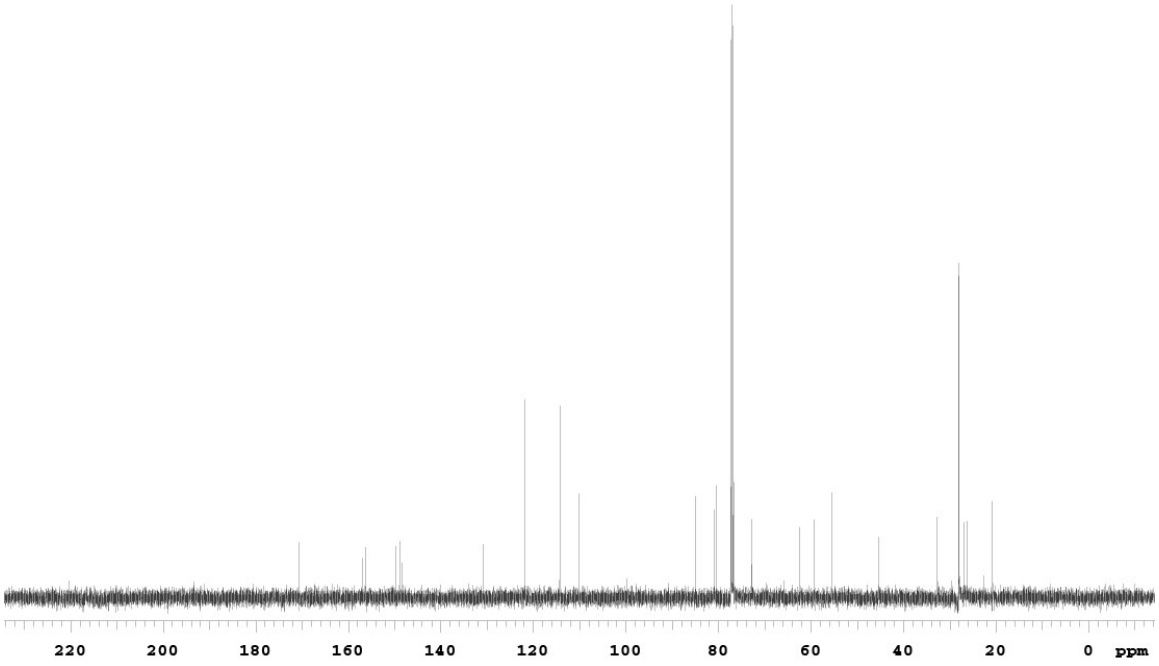
8.05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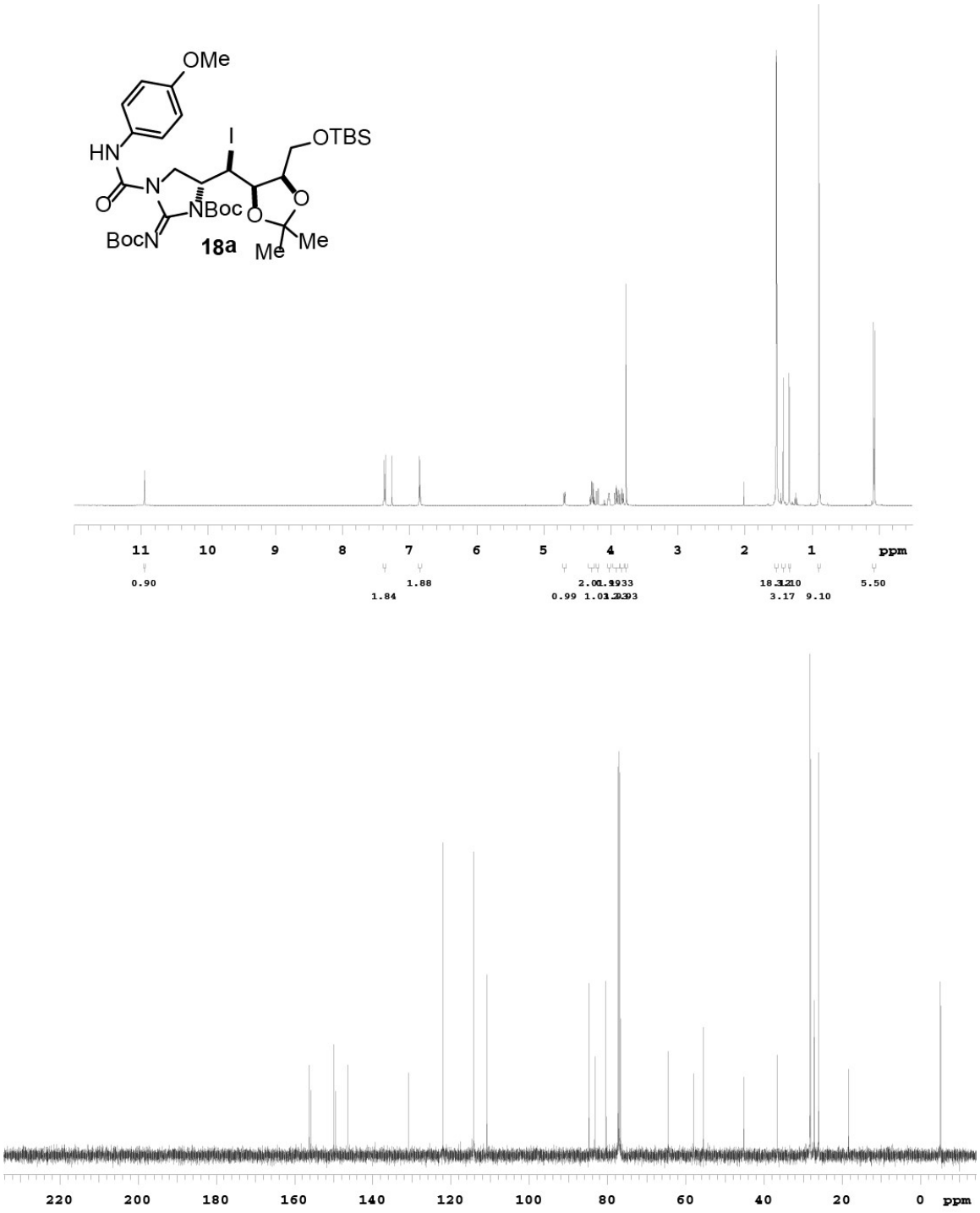
ppm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I
OTBS
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NBoc
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BocN
Me
Me
18a
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10
9
8
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5
4
3
2
1
ppm
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1.84
1.88
0.99
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3.17
9.10
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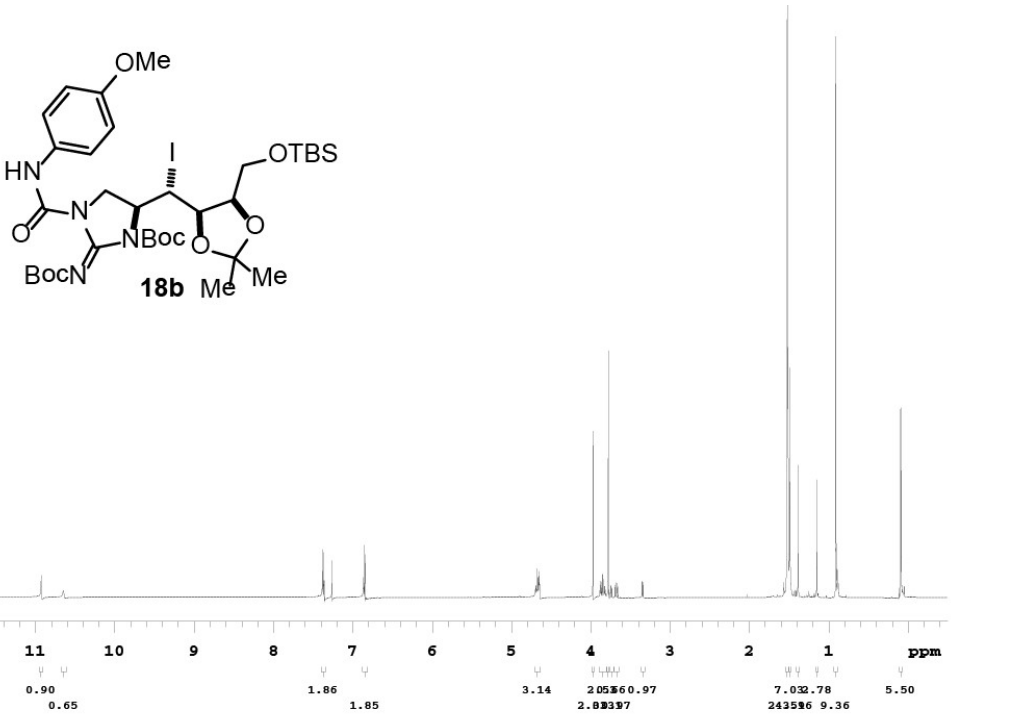
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Me
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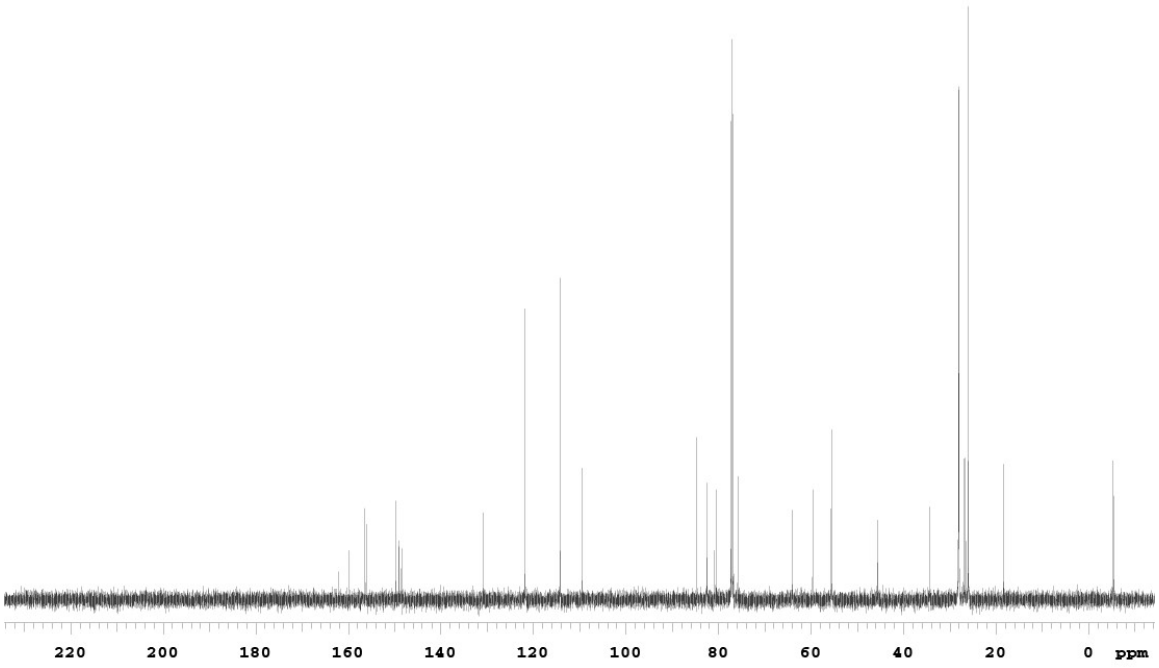
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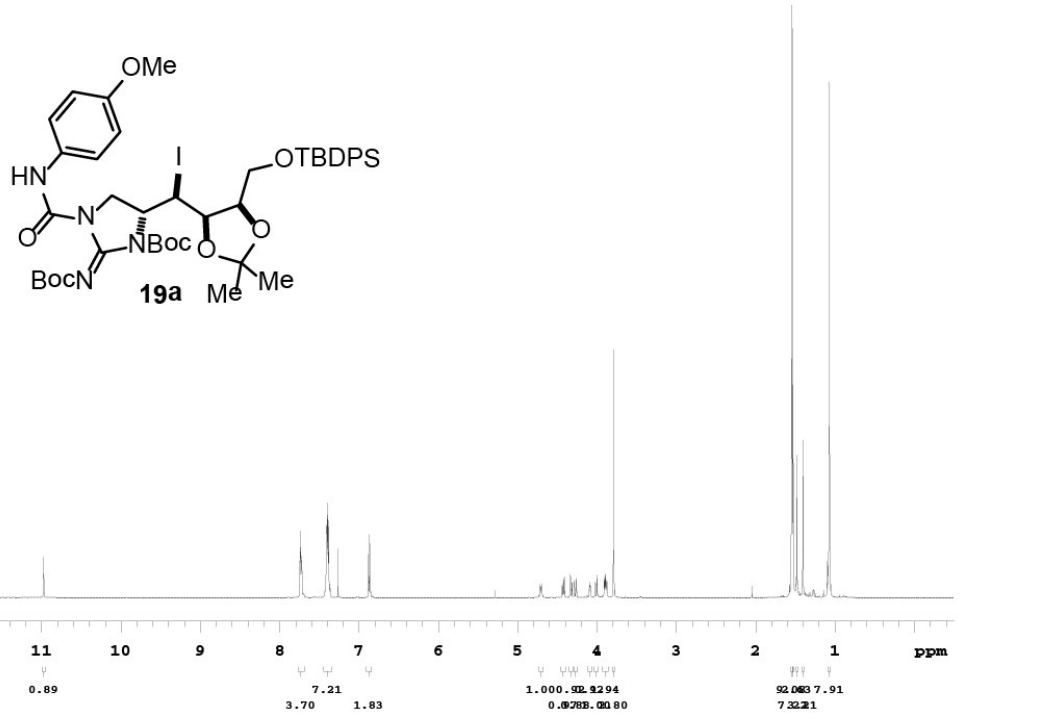
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NBoc
O
O
BocN
Me
Me
19a
11
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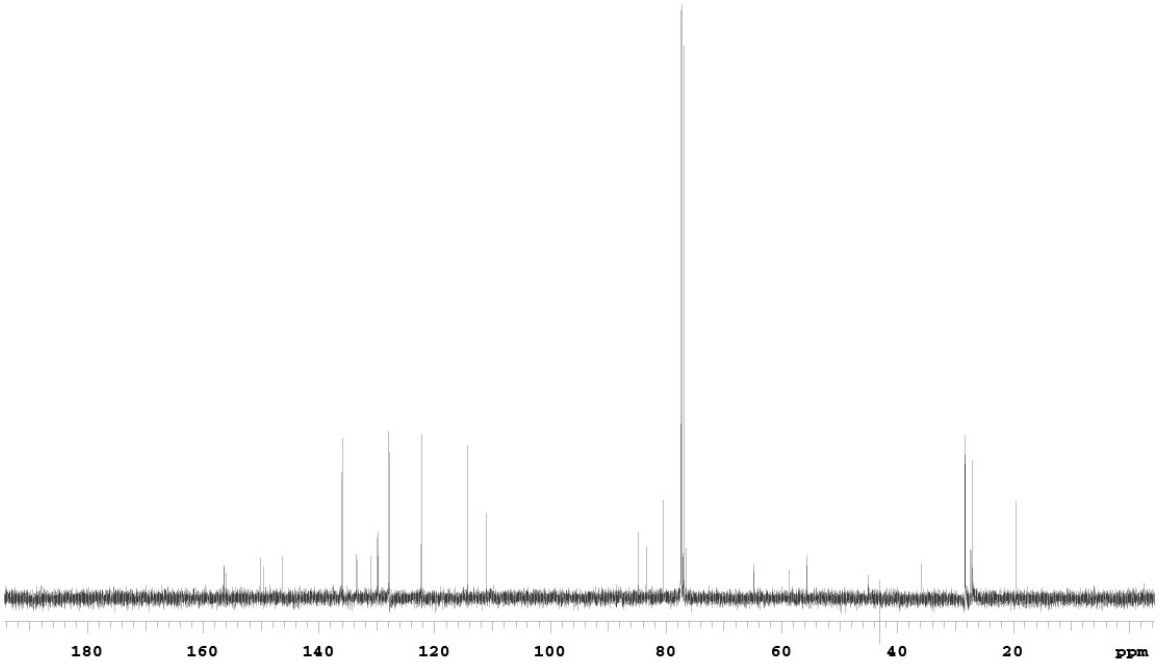
3.70
1.83
1.000
7.91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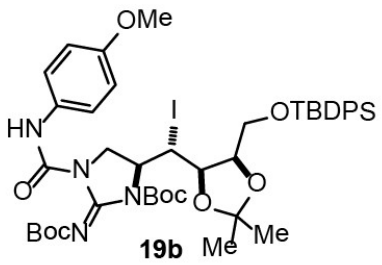
ppm


OMe
HN
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O
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Me
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19b

11
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6
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3
2
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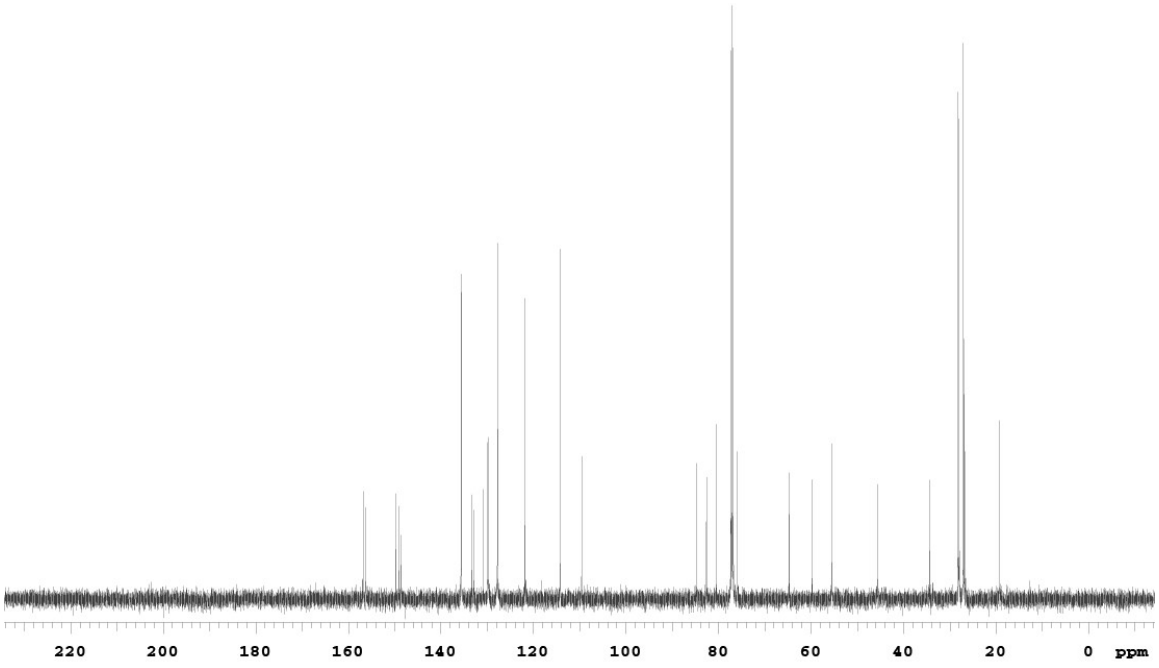
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140
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100
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60
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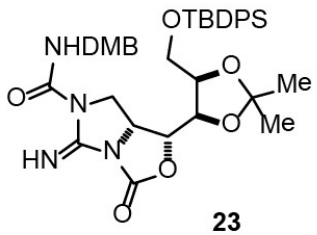
20
0
ppm


OTBDPS
NHDMB
O
N
HN
N
O
O
O
Me
O
Me
O

23

9
8
7
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5
4
3
2
1
ppm
5.06
0.91
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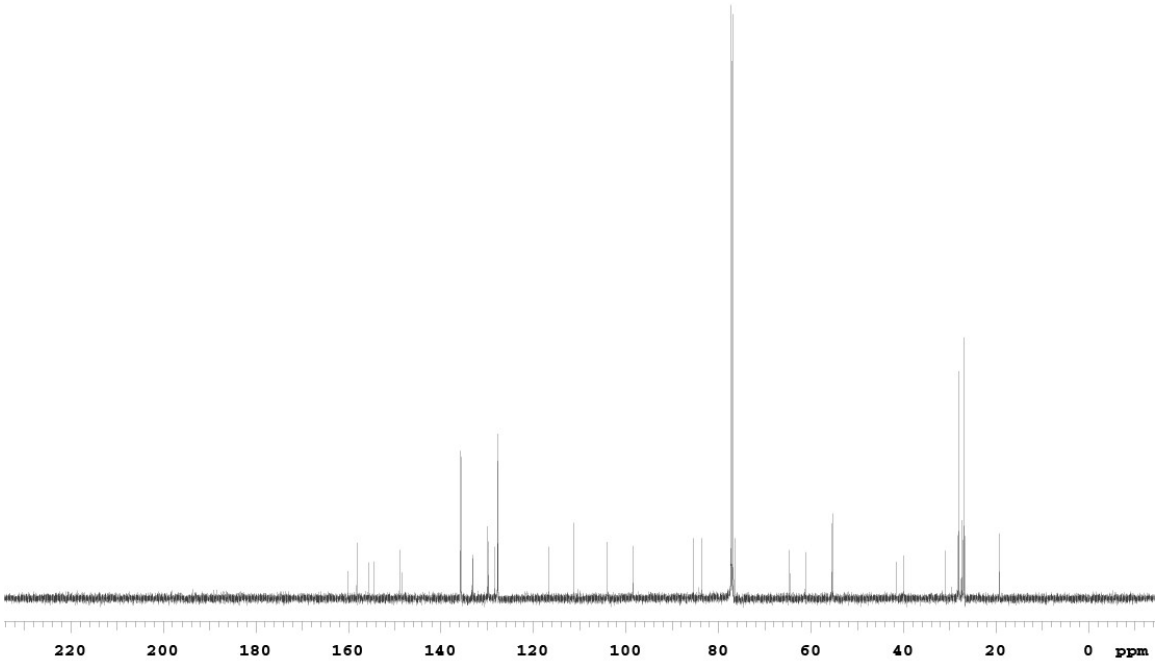
7.55
0.94
3.04
1.02
1.23


220
200
180
160
140
120
100
80
60
40
20
0
ppm

2.5 References

1. Bhonde, V. R.; Looper, R. E. A Stereocontrolled Synthesis of (+)-Saxitoxin. *J. Am. Chem. Soc.* **2011,** *133,* 20172-20174.

2. Noguchi, M.; Okada, H.; Watanabe, M.; Okuda, K.; Nakamura, O. Iodocyclization of 3-Alkynyl and 3-Allenyl-2-(Substituted Amino)-1-imidazolin-4-ones. *Tetrahedron* **1996,** *52,* 6581-6590.

3. Butters, M.; Davies, C. D.; Elliott, M. C.; Cousins, J. H.; Kariuki, B. M.; Ooi, L. L.; Wood, J. L.; Wordingham, S. V. Synthesis and Stereochemical Determination of Batzelladine C Methyl Ester. *Org. Biomol. Chem.* **2009,** *7,* 5001-5009.

4. Albrecht, C.; Barnes, S.; Bockemeier, H.; Davies, D.; Dennis, M.; Evans, D. M.; Fletcher, M. D.; Jones, I.; Leitmann, V.; Murphy, P. J.; Rowles, R.; Nash, R.; Stephenson, R. A.; Horton, P. N.; Hursthouse, M. B. Cyclisation Reactions of Bis-protected Guanidines. *Tetrahedron Lett.* **2008,** *49,* 185-188.

5. Davies, D.; Fletcher, M. D.; Franken, H.; Hollinshead, J.; Kahm, K.; Murphy, P. J.; Nash, R.; Potter, D. M. Iodocyclisation an Rearrangement Reactions of Mono-protected Allyl Substituted Guanidines. *Tetrahedron Lett.* **2010,** *51,* 6825-6829.

6. Shuhaib, Z. A.; Davies, D. H.; Dennis, M.; Evans, D. M.; Fletcher, M. D.; Franken, H.; Hancock, P.; Hollinshead, J.; Jones, I.; Kahm, K.; Murphy, P. J.; Nash, R.; Potter, D.; Rowles, R. Iodocyclisations Reactions of Boc- and Cbz-protected N-allylguanidines. *Tetrahedron* **2014,** *70,* 4412-4419.

7. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities *J. Org. Chem.* **1997**; *62*(21); 7512-7515.

8. Hoye, T.R.; Hansen, P.R.; Vyvyan, J.R. A Practical Guide to First-Order Multiplet Analysis in 1H NMR Spectroscopy *J. Org. Chem.* **1994**; *59*(15); 4096-4103.

CHAPTER 3

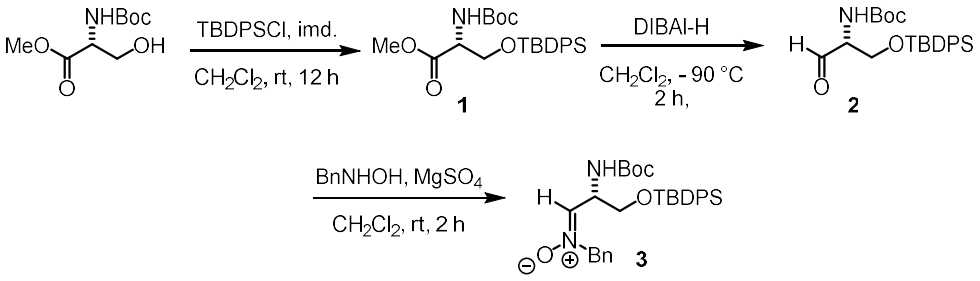
FORMATION OF THE DIAMINE

3.1 Alkyne Addition into the Serine Derived Nitrone

In the original vision for the synthesis of guadinomine B, the carbamoylated guanidine diol fragment would be coupled with the diamine fragment through a stereoselective alkynyl addition to a nitrone. The nitrone was synthesized by Dr. Vasudev Bhonde, from D-methylserine; the terminal alcohol was TBDPS protected to form **1**, which was then treated with DIBAl-H to form the aldehyde **2**. Aldehyde **2** was then converted to the nitrone **3** by the addition of *N*-benzylhydroxylamine (Scheme 3.1).

In Chapter 1, the synthesis of the alkyne **1.106** was described; despite the loss of the internal Boc group, I chose to attempt the alkynyl addition. The alkyne nitrone coupling was selected based on the work of the Merino group, which optimized the *anti*-selective addition of an alkyne into a nitrone by the introduction of the TBDPS group on the terminal alcohol of the nitrone.[1] The use of *i*PrMgCl as the base serves a two-fold purpose; it deprotonates the alkyne and it can also coordinate the nitrogen of the alpha amine and the oxygen of the nitrone, which stabilizes the nitrone in a confirmation that leads to a single diastereomer (Figure 3.1).

The alkyne **4** was dissolved in THF and cooled to 0 °C; *i*PrMgCl was added and the reaction was stirred for 10 minutes before the addition of the nitrone. The reaction was then



Scheme 3.1: Synthesis of the nitrone **3**.

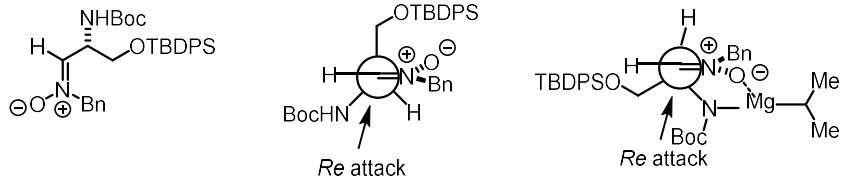
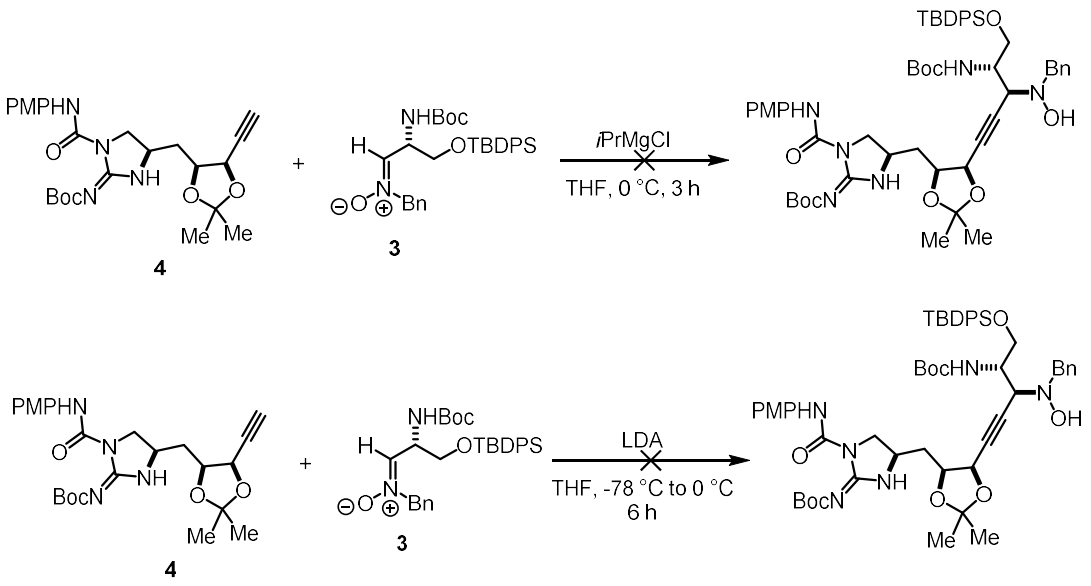


Figure 3.1: Rationalization for the selectivity into the nitrone.

stirred for 3 hours and warmed to room temperature. Upon work up, there was no change in either the nitrone or the alkyne; this was attributed to the acidic protons on the guanidine and the carbamoyl group (Scheme 3.2). Considering that the *i*PrMgCl could be coordinating to the guanidine or the carbamoyl group and promoting the deprotonation of those groups over the alkyne, LDA was employed, hoping that the extra bulk would favor the alkyne deprotonation. The alkyne in THF was cooled to -78 °C and the LDA was added, the reaction was stirred for 15 minutes before the nitrone was added and then warmed to 0 °C and stirred for 6 hours. This reaction was also unsuccessful; in an effort to force the deprotonation of the alkyne, the reaction was performed with 5 equiv. of LDA, but this also failed to produce the product (Scheme 3.2).

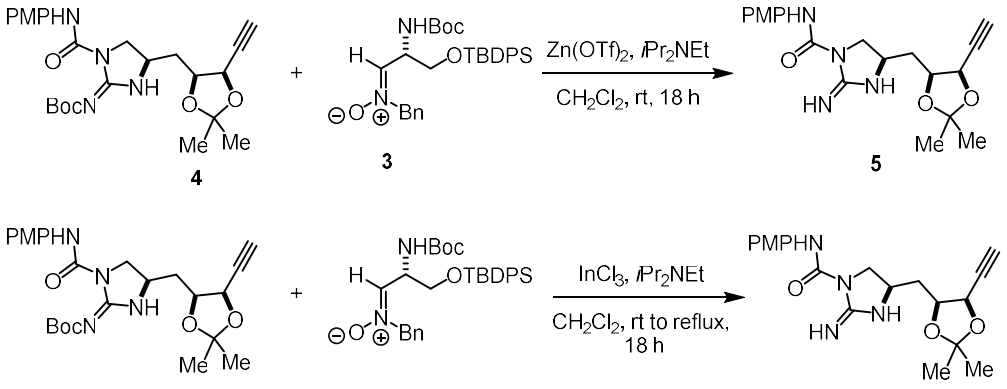
Facing the problem of deprotonating the alkyne selectively, a Lewis acid coupling strategy was explored. Using literature precedence, $Zn(OTf)_2$ was the first Lewis acid employed. The alkyne **4** was dissolved in CH_2Cl_2 followed by the addition of $Zn(OTf)_2$, and



Scheme 3.2: Attempted alkynyl addition into the nitrone.

iPr_2Net; after 20 minutes, the nitrone was added and the reaction was stirred for 18 hours. [2] The consumption of the starting material by TLC was promising, but after purification, the NMR spectrum showed no product but rather the deprotection of the guanidine **5** (Scheme 3.3). The failure of this reaction was attributed to the potential of the carbamoyl guanidine to coordinate the zinc ion, keeping it from forming the zinc-alkyne bond. In an effort to mitigate the sequestration of the Lewis acid, $InCl_3$, which is larger than zinc and less likely to form a tight bond with the carbamoyl guanidine, was used. [3] The alkyne **4** was dissolved in CH_2Cl_2 followed by $InCl_3$ and *i*Pr_2Net; after 5 minutes, the nitrone was added and stirred for 18 hours. Observing no consumption of starting material, the reaction was heated to reflux for 3 hours. This reaction also failed to produce product and cleaved the guanidine Boc group (Scheme 3.3).

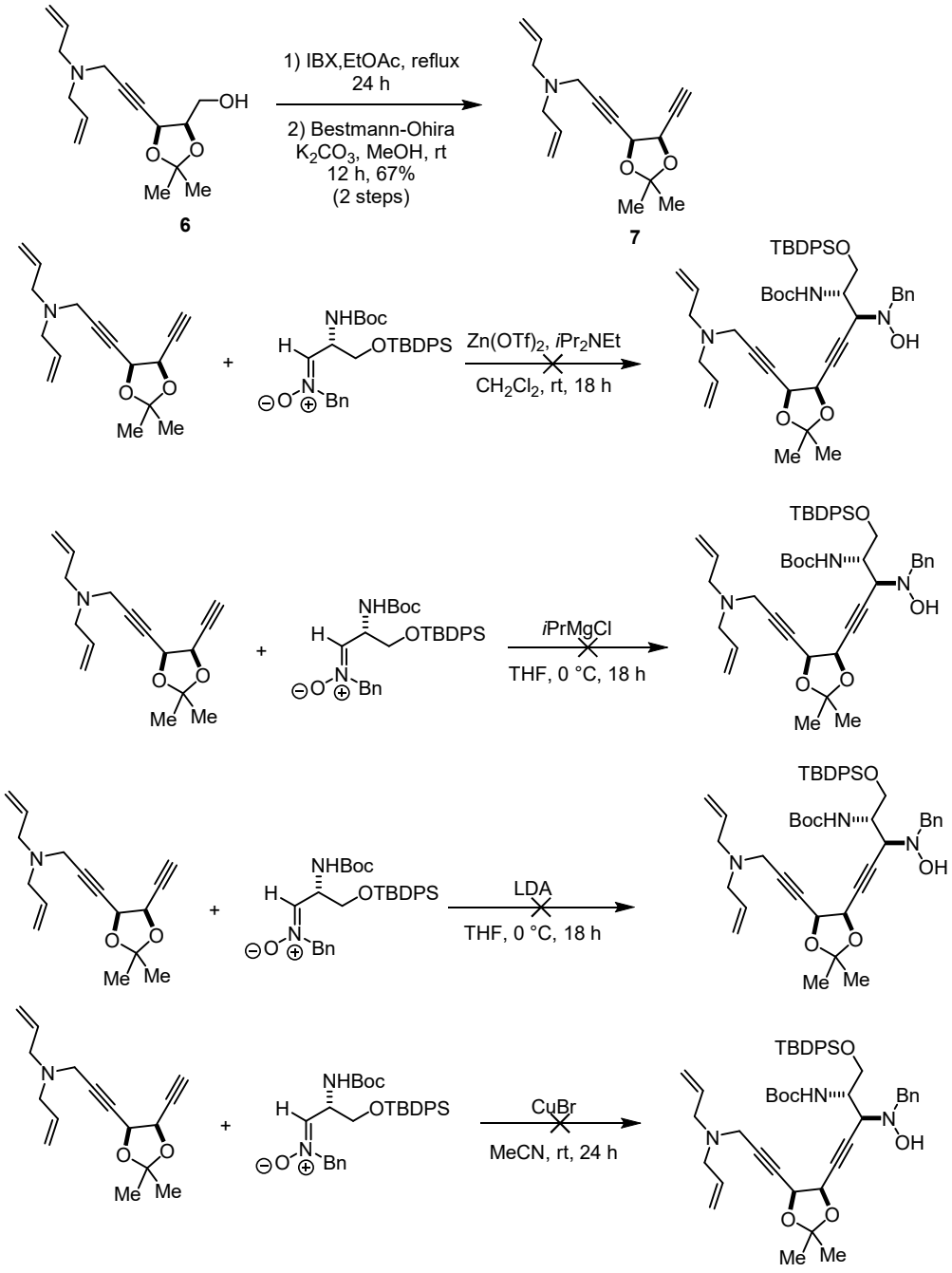
To eliminate the problem of deprotonating the guanidine, the protected amine **6** was transformed to the bisalkyne **7** (Scheme 3.4). The bisalkyne was then dissolved in CH_2Cl_2



Scheme 3.3: Attempted Lewis acid addition of the alkyne into the nitrone.

followed by the addition of $Zn(OTf)_2$ and *i*Pr_2Net; this was stirred for 20 minutes, the nitrone was added, and the reaction stirred overnight. Unfortunately, this reaction produced no appreciable product. By utilizing the bisalkyne, the problematic acidic protons were also eliminated, allowing for the reexamination of strong bases. The bisalkyne **7** was dissolved in THF and cooled to 0 °C and *i*PrMgCl was added; the reaction stirred for 30 minutes before addition of the nitrone. The reaction was then stirred 18 hours; upon work up, the starting material was recovered. Similarly, when bisalkyne **7** was dissolved in THF and cooled to 0 °C followed by the addition of LDA and the nitrone, no product was recovered (Scheme 3.4). Finally, a copper catalyzed alkyne addition into the nitrone was attempted.[4] Bisalkyne **7** was dissolved in MeCN, followed by the CuBr and the nitrone; this reaction was stirred overnight, but even after 24 hours, there was no reaction (Scheme 3.4).

Our inability to couple the alkyne to the nitrone forced us to rethink the coupling strategy; this led to the idea of using the aldehyde formed prior to the alkyne **7** as the functional handle to join the guanidine diol and the diamine.



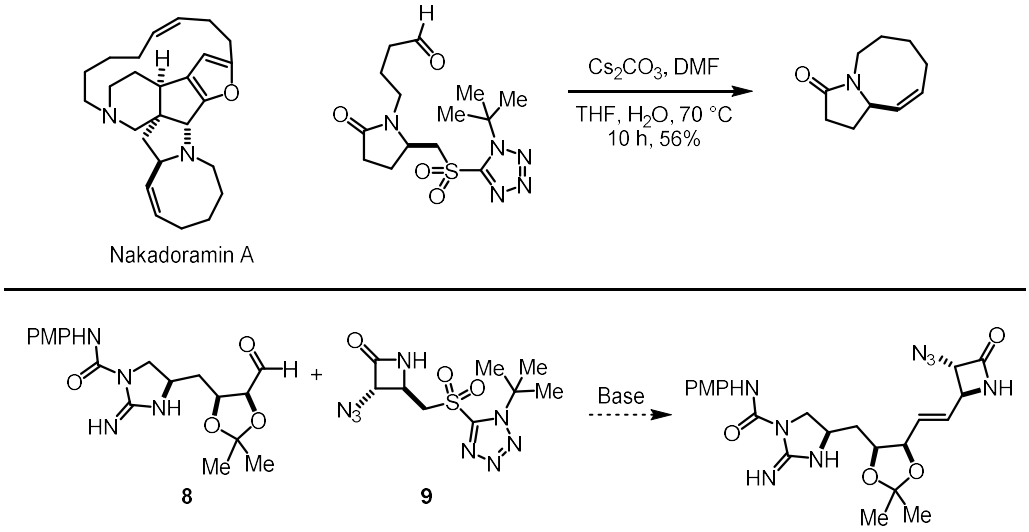
Scheme 3.4: Attempted couplings of the bisalkyne **7** and the nitrone.

3.2 β-lactam Diamine Formation

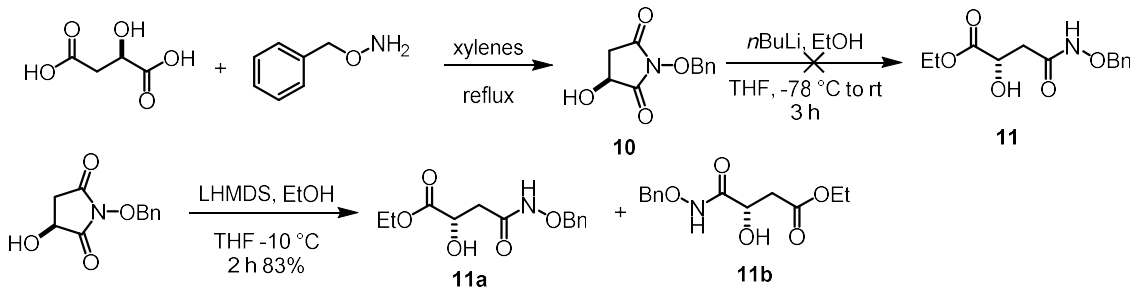
While the proposed synthetic scheme had the advantage of separating the potential pharmacophores, it posed the significant challenge of installing the two-carbon linker between the diol and the diamine at a late stage. When the initial synthetic plan of the alkyne addition into the nitrone failed, other synthetic strategies that could produce the two-carbon linker while maintaining stereochemical fidelity were considered. A metal catalyzed metathesis was discounted because the dense functionality of the substrates would be incompatible with the catalyst.

The total synthesis of nakadomarin A utilized a Julia olefination on a pyrrolidinone, which could be a useful transformation in installing the diamine (Scheme 3.5).[5] The diamine could come from a precursor functionalized β-lactam **8** which could be coupled with aldehyde **9** (Scheme 3.5).

The synthesis of the β-lactam began with L-malic acid, which was suspended in xylenes; O-benzylhydroxylamine was then added and the reaction was heated to reflux for 6 hours. The reaction was cooled to 0 °C overnight and the succinamide **10** was collected as colorless crystals.[6] In the literature, succinamide **10** was transformed to the ethyl ester **11** by treating it with *n*BuLi and EtOH in THF. However, these conditions only produced the butyl addition (Scheme 3.6). This led to the optimization of the base, to identify a base that would not be a competitive nucleophile. A number of bases were substituted into the reaction, but the best results were observed with LHMDS. A solution of LHMDS in THF was cooled to –10 °C and EtOH was added; the reaction mixture was stirred for 20 minutes before the succinamide **10** was added. The reaction was then stirred for 2 hours and quenched with NH_4Cl (Scheme 3.6). While the literature only reported a single regioisomer, a mixture of both ethyl ester **11a** and **11b** were isolated from the optimized base.



Scheme 3.5: The inspiration and for the β-lactam and the envisioned coupling.



Scheme 3.6: Formation of the succinamide **10** and subsequent opening.

The regioisomers were easily separated by column chromatography, but distinguishing them from one another with simple spectroscopic analysis was difficult. Both ethyl esters **11a** and **11b** were subjected to Mitsunobu conditions to form the β-lactam, assuming that the undesired regioisomer would not react. This assumption was correct and the desired regioisomer, **11b**, was identified and formed the β-lactam **12** in good yield (Scheme 3.7). The purification of this compound proved to be difficult, as it coeluted with the DIAD.

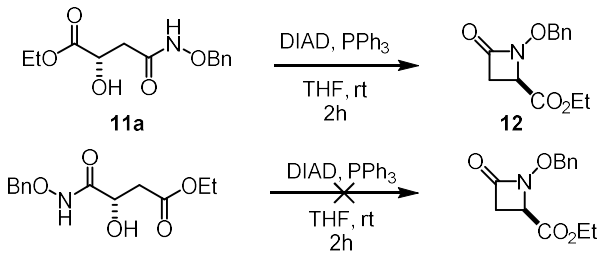
A closely monitored palladium catalyzed hydrogenation removed the benzyl group from β-lactam **12** without cleaving the N-O bond; however, prolonged exposure to the

hydrogenation did produce small amounts of the free β-lactam.[7] This hydrogenation was also not affected by the residual DIAD from the Mitsunobu reaction and the debenzylated β-lactam **13** was much easier to separate (Scheme 3.8). The β-lactam **13** was then treated with TsCl and Et_3N in CH_2Cl_2, which produced the tosylated β-lactam **14** (Scheme 3.8).

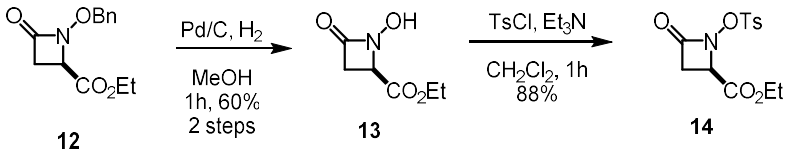
The tosylated β-lactam **14** was similar to substrates developed by Miller and coworkers that underwent C3 functionalization and N-O bond reduction.[7] Utilizing the known methodology, an azide was introduced at the C3 position in a stereoselective manner. The tosylated β-lactam **14** was dissolved in MeCN, followed by *i*PrNEt$_2$ and $TMSN_3$, and the reaction was stirred for 24 hours. The azide substituted β-lactam **15** was formed in modest yield (Scheme 3.9). The introduction of the azide generated the precursor to the diamine. The ethyl ester was then successfully reduced to the alcohol with $NaBH_4$ in MeOH to form **16** (Scheme 3.9).

The next step was to introduce the thiotetrazole **17**, which was generated from *t*butyl isothiocyanate and NaN_3; this reaction proceeded in high yield.[8] To install the thiotetrazole, the alcohol of the β-lactam **16** needed to be converted to a leaving group. While this step could be eliminated by a Mitsunobu reaction, the Staudinger reduction that would likely occur under those conditions led to an S_N2 strategy. The alcohol **16** was treated with TsCl in pyridine to generate the tosylate **18** (Scheme 3.10). To form the thioether **19**, the tetrazole **17** was dissolved in THF and cooled to 0 °C; NaH was then added and reaction was stirred for 10 minutes before the addition of the β-lactam **19**; the reaction was then heated to reflux and stirred for 12 hours (Scheme 3.10). The final conversion to the sulfone **20** was achieved with an ammonium molybdate oxidation.

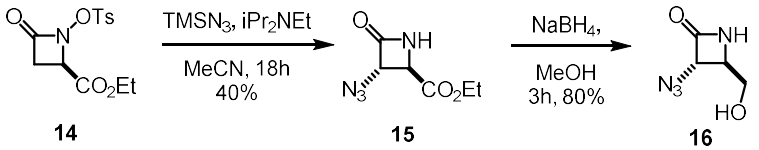
Before attempting the Julia olefination on the complex aldehyde **8,** a model system was employed to explore this reaction. The sulfone **20** and benzaldehyde were dissolved in



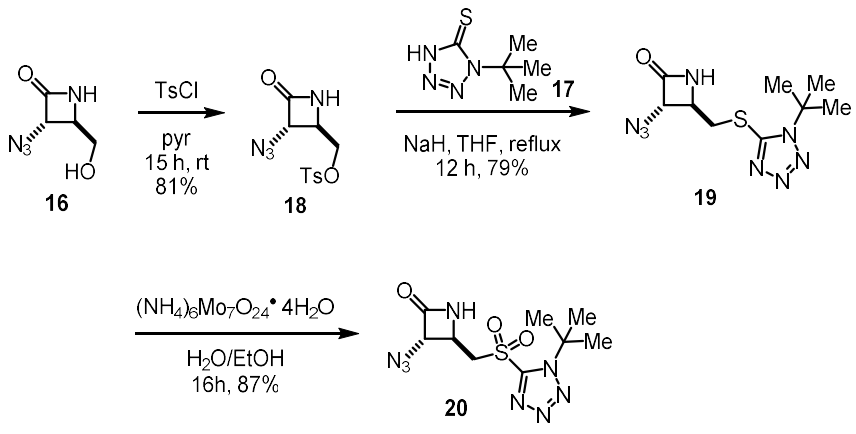
Scheme 3.7: Formation of the β-lactam **12**.



Scheme 3.8: Formation of the tosylated β-lactam **14**.



Scheme 3.9: Formation of the azide substituted β-lactam **16**.

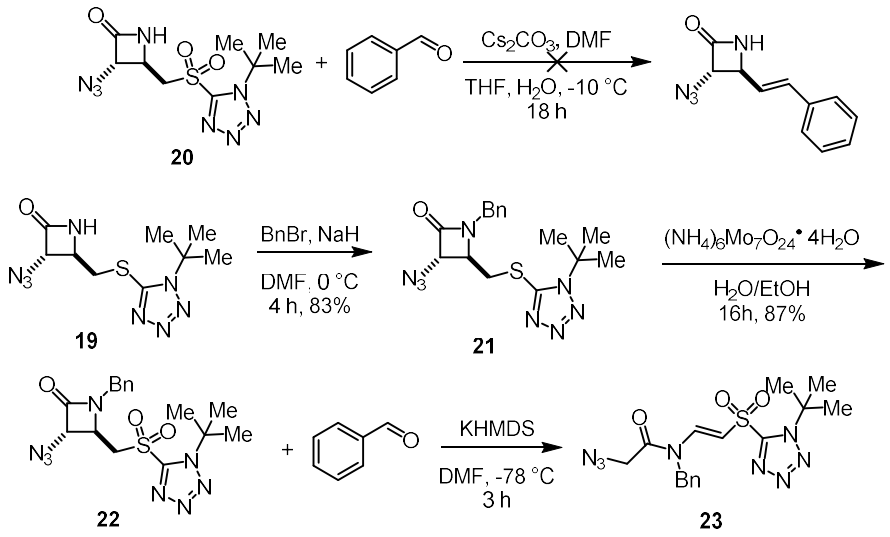


Scheme 3.10: Formation of the sulfone **20**.

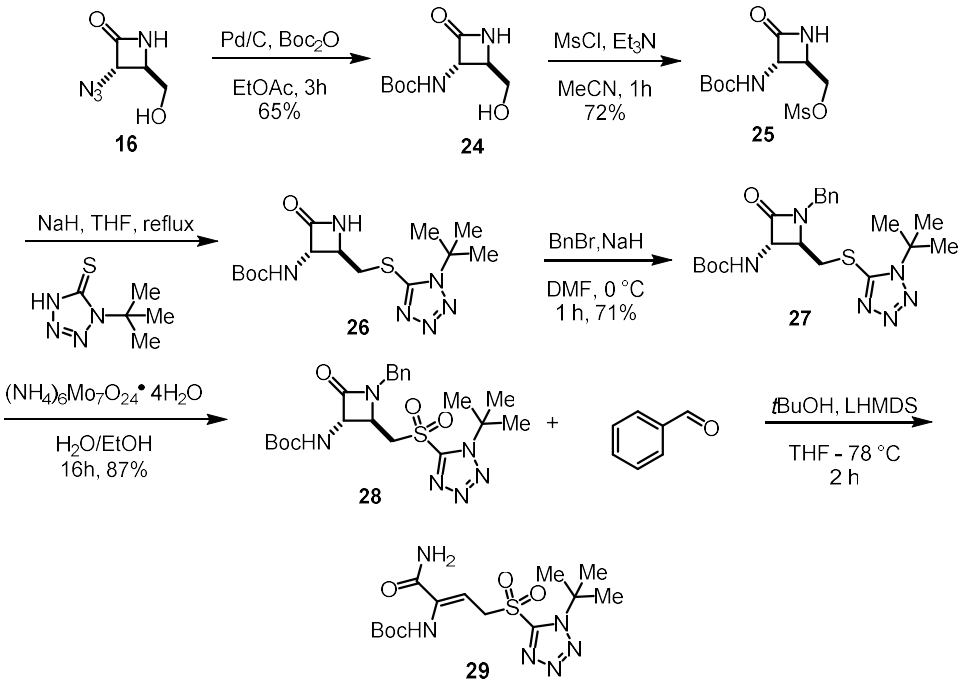
THF/DMF and cooled to -10 °C, then Cs_2CO_3 was added in portions and reaction was stirred 18 hours, but no reaction occurred (Scheme 3.11).[5] Comparing the sulfone **20** and the pyrrolidinone used in the synthesis of Nakadomarin A, the most notable difference was the N-alkylation; therefore, the sulfone **20** was transformed into the N-benzyl sulfone **22**. The thioether **19** was treated with BnBr and NaH in DMF to produce the N-benzylthioether **21**, which was then oxidized to the sulfone **23** (Scheme 3.11). When sulfone **22** was subjected to the same conditions as sulfone **20**, again no reaction occurred. Unsure if the sulfone was being deprotonated by the Cs_2CO_3, a stronger base used. Sulfone **22** was dissolved in DMF and cooled to –78 °C; KHMDS and benzaldehyde were then added and the reaction was stirred for 3 hours. This reaction led to a complex mixture of products, but one notable product was the enamine **23** (Scheme 3.11).

The formation of the enamine **23** was a surprising result. It suggested that the initial deprotonation occurred alpha to the sulfone, but rather than adding into the aldehyde, it eliminated to the enolate leading to the enamine **23**. I believed that the azide was contributing to the stabilization of the favored enolate and hoped that converting azide to the protected amine would favor the deprotonated sulfone. The attempts to reduce the azide on sulfone **22** were unsuccessful, which led to the reduction of the azide earlier in the synthesis.

In an attempt to reduce the azide as cleanly as possible, the β-lactam **16** was reduced with a palladium catalyzed hydrogenation; the *in situ* Boc protection of the newly formed amine **24** was accomplished with the addition of Boc_2O (Scheme 3.12). The conversion of the azide to the Boc protected amine added steric bulk to the β-lactam **24** and the subsequent tosylation was unsuccessful. When the β-lactam **24** was treated with MsCl, in MeCN, the mesylate **25** was formed in good yield (Scheme 3.12). The mesylate **25** was then converted to the thioether **26** by the addition of the thiotetrazole. The β-lactam was benzyl protected to form **27** and



Scheme 3.11: Julia olefination model reaction.



Scheme 3.12: Formation of the elimination product **29**.

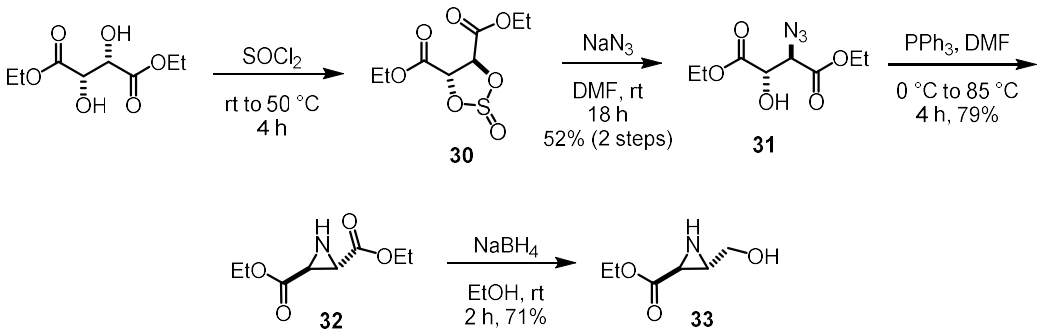
ammonium molybdate oxidation furnished the sulfone **28**.

We chose to treat this sulfone with an alkoxide base hoping that the reversibility of the deprotonation would increase the population of the sulfone anion. *t*BuOH was added to THF and cooled to –78 °C; LHMDS was then added and the reaction was stirred for 40 minutes before the addition of the sulfone **28** and benzaldehyde. The reaction was stirred for 2 hours and the majority of the sulfone remained unreacted; however, the eliminated product **29** was also recovered (Scheme 3.12).

3.3 Aziridine Strategy to Form the Diamine

The fragmentation of both the azide substituted β-lactam **23** and the protected amine derivative **28** led to a new strategy for the formation of the diamine. Looking to Omura's synthesis of guadinomine B, an aziridine was considered as a synthon for generating the diamine. Still considering the Julia olefination as a method to couple the guanidine diol **8** and the diamine, a synthetic route that would allow the instillation of the sulfone adjacent to the aziridine was developed. Hoping to avoid an elimination similar to the β-lactam, the aziridine would be opened prior to attempting the Julia olefination.

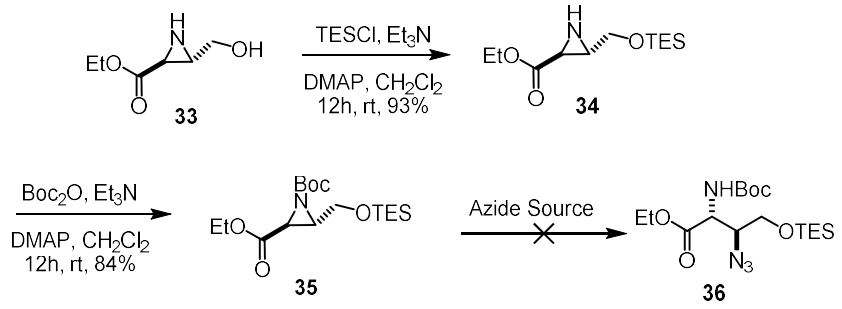
The synthesis of the aziridine began with D-diethyltartrate, which was treated with $SOCl_2$ to generate the dioxathiolane **30**.[9] NaN_3 was then used to produce the azahydrin **31**; the meso nature of the dioxathiolane eliminated any regioisomer concerns with the azahydrin. To form the aziridine **31**, the azahydrin **31** was dissolved in DMF and cooled to 0 °C and PPh_3 was added in portions. The reaction was then warmed to 85 °C and stirred for 12 hours (Scheme 3.13). The bisester aziridine **32** was dissolved in EtOH and cooled to 0 °C; $NaBH_4$ was then added and the reaction stirred for 18 hours to form the hydroxyaziridine ethyl ester **33** (Scheme 3.13). In order to open the aziridine, it needed to be activated, which could be



Scheme 3.13: Synthesis of the hydroxyaziridine ethyl ester **33**.

achieved with a Boc protection.

However, to eliminate a competitive electrophile in the form of a carbonate, the alcohol was protected. The hydroxyaziridine **33** was dissolved in DMF and treated with TESCl, DMAP, and Et_3N to furnish the protected hydroxyaziridine **34** (Scheme 3.14). The aziridine was then Boc protected to give the activated aziridine **35**. Various azide sources were then used in an attempt to open the aziridine. The protected aziridine **35** was dissolved in MeOH; $TMSN_3$ was added and the reaction heated to 70 °C for 3 days; unfortunately, only starting material was recovered. Attempting to increase the electrophilicity of the aziridine, $BF_3{\cdot}OEt_2$ was employed in conjunction with $TMSN_3$, but the starting material persisted. Moving on to a different azide source, the protected aziridine **34** was dissolved in DMF and NaN_3 was added; the reaction was stirred for 2 days. This reaction also failed to produce the product **36** (Scheme 3.14). Because of the inability to open the aziridine and the concern that a Julia olefination with an adjacent strained ring system would be unsuccessful, this route was abandoned.

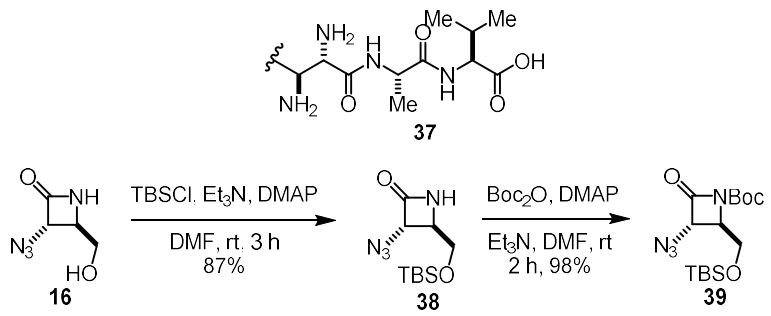


Scheme 3.14: Failure to produce the diamine **35**.

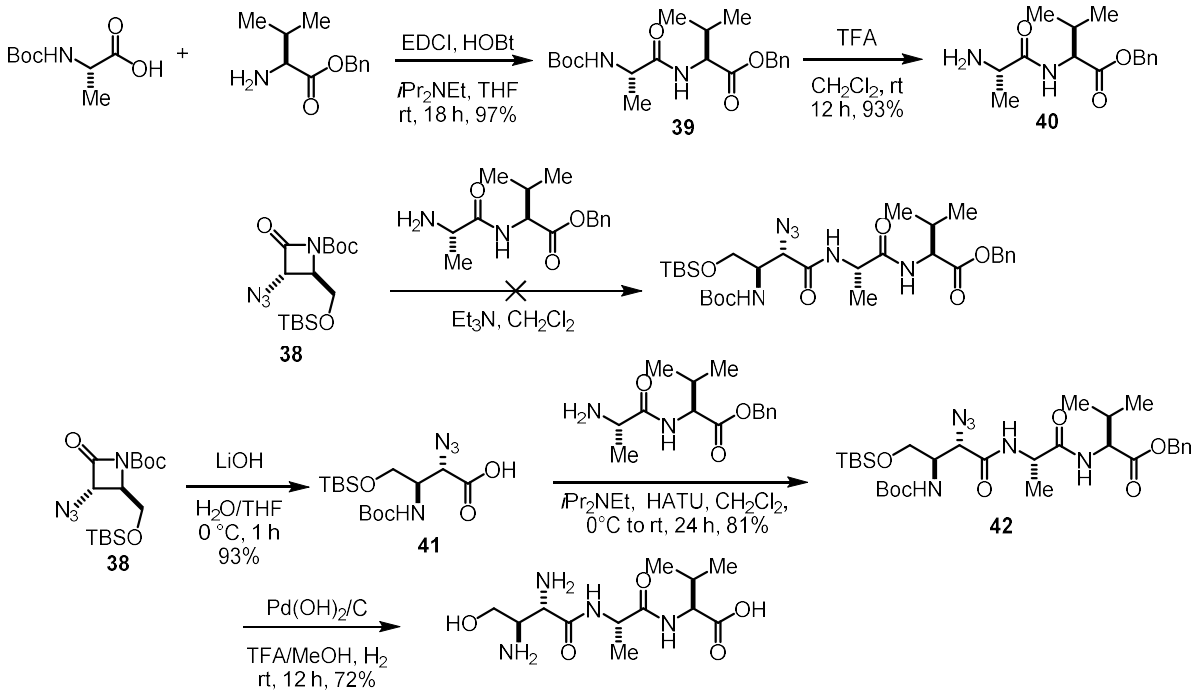
3.4 Completion of the Right-Hand Fragment of $NA22598A_1$

The difficulty of coupling the guanidine diol **8** and the diamine fragment caused a shift in our aims, and I chose to synthesize the diamine dipeptide fragment **37**. To accomplish this, the β-lactam strategy was employed again. Before activating the β-lactam, the alcohol was protected as the TBS ether to provide the protected β-lactam **38**. Treatment with Boc_2O and DMAP in CH_2Cl_2 provided the substrate **39** which would form the diamine dipeptide upon opening (Scheme 3.15).

The dipeptide **40** which would act as the nucleophile for opening the β-lactam **39** was formed through a peptide coupling in high yield (Scheme 3.16). The removal of the Boc group was achieved cleanly with a TFA deprotection and formation of the TFA salt **41**. The dipeptide **41** was stirred with Et_3N in CH_2Cl_2 followed by the addition of the β-lactam **39**, and the reaction was stirred for 18 hours (Scheme 3.16). This reaction did not produce the diamine dipeptide; taking steric congestion into account, the β-lactam **39** was opened with LiOH to form the carboxylic acid **42**; this reaction proceeded in very good yield.[10] A subsequent peptide coupling was utilized to form the protected diamine dipeptide **43** (Scheme 3.16). Deprotection was achieved with a $Pd(OH)_2/C$ catalyzed hydrogenation in a 1:1 mixture of MeOH and TFA (Scheme 3.16).



Scheme 3.15: Formation of the activated β-lactam **39**.



Scheme 3.16: Synthesis of the right-hand fragment of NA22598A$_1$.

While it was disappointing to be unable to couple the guanidine diol **8** and the diamine fragment, we were happy to see the formation of the diamine dipeptide fragment. This fragment allowed us to explore the biological potential of the diamine dipeptide, discussed in Chapter 4.

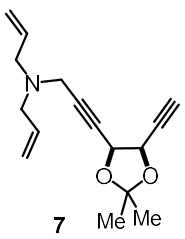
3.5 Supporting Information

Unless otherwise noted, all starting materials were either known compounds or were obtained from commercial sources and used without purification. All reactions requiring anhydrous conditions were performed under a positive pressure of argon using flame-dried glassware. Dichloromethane (CH_2Cl_2), and triethylamine (Et_3N), were distilled from CaH_2 immediately prior to use. Tetrahydrofuran (THF), diethylether (Et_2O), toluene (PhMe), and dimethylformamide (DMF) were degassed with argon and passed through a solvent purification system (J.C. Meyer of Glass Contour) containing either alumina or molecular sieves. Flash chromatography was performed on Merk silica gel Kieselgel 60 (230-400 mesh) from EM science with the indicated solvent.

^{1}HNMR spectra were recorded on a Varian VXR-500 MHz spectrometer. The chemical shifts (d) of proton resonances are reported relative to $CHCl_3$, DMSO-d_5, HOD, or HD_2COD using the following format: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app = apparent), coupling constant(s) (*J* in Hz), integral].[11,12] ^{13}CNMR spectra were recorded at 75, 100, or 125 MHz. The chemical shifts of carbon resonances are reported relative to the deuterated solvent peak.

Infrared spectra were recorded on a Nicolet 380-FT IR spectrometer fitted with a SmartOrbit sample system. All absorptions are reported in cm^{-1} relative to polystyrene (1601 cm^{-1}).

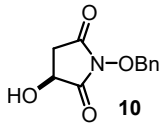
N-allyl-N-(3-((4S,5R)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)prop-2-yn-1-yl)prop-2-en-1-amine (7)



7

The protected propargyl **6** (1.00 g, 3.9 mmol) was dissolved in EtOAc (100 mL); IBX (2.19 g, 7.8 mmol) was then added and the reaction heated to reflux for 18 hours. It was then cooled to room temperature and filtered through a pad of Celite. The resulting aldehyde was used without further purification. The aldehyde (1.12 g, 4.39 mmol) was dissolved in MeOH, Bestmann-Ohira reagent was then added, K_2CO_3 (1.82 g, 13.17 mmol) followed in portions, and the reaction was stirred for 4 hours. The reaction was quenched with AcOH; the methanol was removed and the reaction mixture was diluted with EtOAc and washed with H_2O (3 x 100 mL); the organic layer was dried over Na_2SO_4 and purified by column chromatography (1:1 Hex/EtOAc) to give **7** in 54% yield as a yellow oil. R_f = 0.81 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 5.84 (ddd, J = 5.5, 11.5, 17 Hz, 2H), 5.31-5.19 (m, 4H), 4.72 (dt, J = 2.5, 11.5 Hz, 1H), 4.62 (dd, J = 3.5, 11 Hz, 1H), 3.50 (d, J = 2.5 Hz, 2H), 3.19 (d, J = 11 Hz, 4H), 2.58 (d, J = 3.5 Hz, 1H), 2.07 (s, 3H), 1.49 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 135.1, 118.3, 111.6, 109.9, 79.3, 75.1, 71.3, 56.4, 41.6, 26.6, 26.5 ppm.

(S)-1-(benzyloxy)-3-hydroxypyrrolidine-2,5-dione (10)

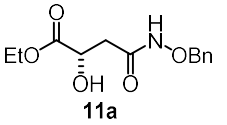


10

O-benzylhydroxylamine (23.12 g, 187.7 mmol) was added to xylenes (1 L) and L-malic acid was added. The reaction flask was fitted with a Dean-Stark trap and reflux condenser and the reaction was heated to reflux and stirred for 4 hours. The reaction was then cooled to 0 °C for 18 hours. The precipitate formed was filtered and washed with toluene (3 x 50 mL). **10** was collected as colorless crystals (85 % yield, 16.69g) and was used without further purification. R_f = 0.81 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 7.47-7.45

(m, 2H), 7.38-7.37 (m, 3H), 5.11 (s, 2H), 4.53 (dd, J = 4, 8.5 Hz, 1H), 3.02 (dd, J = 8.5, 18 Hz, 1H), 2.60 (dd, J = 4.5, 18 Hz, 1H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 168.8, 133.1, 130.1, 129.7, 128.7, 79.0, 64.5, 35.3 ppm.

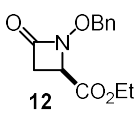
Ethyl (*S*)-4-((benzyloxy)amino)-2-hydroxy-4-oxobutanoate (11a)



11a

A solution of LHMDS (12.5 mL, 1M) and EtOH (0.5 mL, 8.7 mmol) in THF (200 mL) was cooled to –10 °C. The succinamide (1.30 g, 5.8 mmol) was added in portions and the reaction was stirred for 1 hour. The reaction was then quenched with NH_4Cl (aq) (50 mL) and the THF removed by rotary evaporation. The remaining slurry was then diluted with EtOAc and water. The aqueous layer was extracted with EtOAc (2 x 100 mL) and the organic layers were combined and dried with Na_2SO_4. The solvent was removed by rotary evaporation and the crude material was purified by column chromatography (1:1 Hex/EtOAc) to give **11a** in 74% yield. R_f = 0.61 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 7.37-7.32 (m, 5H), 4.87 (s, 2H), 4.46-4.41 (m, 1H), 4.21 (q, J = 7 Hz, 2H), 2.59 (dd, J = 2.5, 14.5 Hz, 1H), 2.47 (dd, J = 7.5, 14.5 Hz, 1H), 1.27 (t, J = 7 Hz, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 173.4, 167.9, 135.3, 129.36, 128.9, 128.8, 78.4, 67.5, 62.2, 37.7, 14.2 ppm.

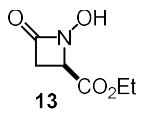
Ethyl (*R*)-1-(benzyloxy)-4-oxoazetidine-2-carboxylate (12)



12

To a solution of **11a** (5.00 g, 18.7 mmol) in THF was added DIAD (3.7 mL, 18.7 mmol) and PPh_3 (4.9 g, 18.7 mmol). The reaction was stirred for 1 hour. The solvent was removed by rotary evaporation and the crude material was purified by column chromatography (1:1 Hex/EtOAc). The material recovered was a mixture of **12** and DIAD; this mixture was used in the next reaction.

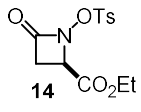
Ethyl (*R*)-1-hydroxy-4-oxoazetidine-2-carboxylate (13)



13

To a solution of **12** and DIAD in MeOH was added Pd/C (1.95 g, 1.84 mmol). The solution was flushed with H_2 and the reaction was stirred under an atmosphere of H_2 for 1 hour. The reaction was filtered through a pad of Celite and washed with CH_2Cl_2. The filtrate was concentrated under reduced pressure and crude material was purified by column chromatography (1:1 Hex/EtOAc). **13** was isolated, in 76% yield over 2 steps, as a colorless oil (2.22 g). R_f = 0.51 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 4.36 (dd, *J* = 2, 5.5 Hz, 1H), 4.26 (dq, 2, 7.5 Hz, 2H), 3.01 (dd, *J* = 5.5, 13.5 Hz, 1H), 2.76 (dd, *J* = 2.5, 13.5 Hz, 1H), 1.30 (t, *J* = 7 Hz, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 169.5, 164.6, 62.3, 57.4, 36.9, 14.2 ppm.

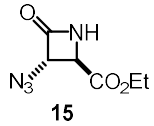
Ethyl (*R*)-4-oxo-1-(tosyloxy)azetidine-2-carboxylate (14)



14

To a solution of **13** (1.34 g, 8.4 mmol) in CH_2Cl_2 was added TsCl (1.6 g, 8.4 mmol) and Et_3N (1.17 mL, 8.4 mmol). The reaction was stirred for 1 hour and the solvent was removed under reduced pressure. The crude material was purified by column chromatography (4:1 Hex/EtOAc). The product was isolated as a yellow oil in 81% yield (2.14 g). R_f = 0.91 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 7.88 (d, *J* = 8 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 2H), 4.44 (dd, *J* = 3.5, 6.5 Hz, 1H), 4.32-4.25 (m, 2H), 3.03 (dd, *J* = 6.5, 14.5 Hz, 1H), 2.92 (dd, *J* = 3, 14 Hz, 1H), 2.46 (s, 3H), 1.34 (t, *J* = 7 Hz, 3H) ppm.

Ethyl (2*R*,3*S*)-3-azido-4-oxoazetidine-2-carboxylate (15)

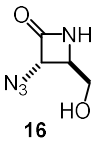


15

To a solution of **14** (2.14 g, 6.8 mmol) in MeCN was added Hunig's base (3.26 mL, 18.7 mmol) and $TMSN_3$ (1.8 mL, 13.6 mmol). The reaction was stirred for 16 hours and turned dark red. The solvent was removed under reduced

pressure and the crude material was purified by column chromatography (1:1 Hex/EtOAc). The product was isolated as yellow oil in 42 % yield (528 mg). R_f = 0.87 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 6.91 (s, 1H), 4.67 (d, *J* = 2 Hz, 1H), 4.25 (dq, *J* = 3, 7.5 Hz, 2H), 4.06 (d, *J* = 2.5 Hz, 1H), 1.29 (t, *J* = 7.5 Hz, 3H), ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 169.1, 163.8, 69.9, 62.5, 55.8, 14.2 ppm

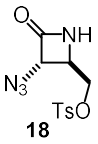
(3*S*,4*R*)-3-azido-4-(hydroxymethyl)azetidin-2-one (16)



16

$NaBH_4$ (108 mg, 2.86 mmol) was added to a solution of **16** (528 mg, 2.86 mmol) in MeOH. The reaction was stirred for 1 hour and quenched with NH_4Cl_{aq}. Solvent was removed by rotary evaporation; the resulting solid was suspended in 3:1 $CHCl_3$/MeOH. The slurry was filtered through a pad of Na_2SO_4 and the filtrate was concentrated. It was purified by column chromatography (1:1 Hex/EtOAc) to give **16** as a colorless oil in 74% yield (303 mg). R_f = 0.05 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, CD_3OD): 4.52 (s, 1H), 3.89 (dd, *J* =3.5, 12 Hz, 1H), 3.74 (dd, *J*= 4.5, 12 Hz, 1H), 3.70-3.68 (m, 1H) ppm. ^{13}C {^{1}H} NMR (125 MHz, CD_3OD): δ (ppm): 66.1, 61.6, 57.4, 50.9 ppm.

((2*R*,3*S*)-3-azido-4-oxoazetidin-2-yl)methyl 4-methylbenzenesulfonate (17)

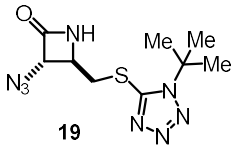


18

To a solution of **16** (303 mg, 2.13 mmol) in pyridine (1 mL) was added TsCl (609 mg, 3.2 mmol); the reaction was stired for 3 hours. Pyridine was removed under reduced pressure, the crude reaction mixture was purified by column chromatography (1:1 Hex/EtOAc) and the product was isolated as a colorless oil in 60% yield (380 mg). R_f = 0.42 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 7.78 (d, *J* = 8 Hz, 2H), 7.37 (d, *J* = 8.5 Hz, 2H), 4.37 (t, *J* = 2 Hz, 1H), 4.25 (dd, *J* = 4, 11.5 Hz, 1H), 4.14 (dd, *J* = 5.5, 11 Hz, 1H), 3.76-3.73 (m, 1H), 2.46 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm):

163.5, 145.9, 130.3, 128.0, 68.3, 68.9, 54.3, 21.8, 14.3 ppm.

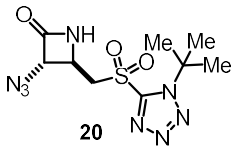
(3S,4R)-3-azido-4-(((1-(tert-butyl)-1H-tetrazol-5-yl)thio)methyl)azetidin-2-one (19)



19

The tetrazole **17** (216 mg, 1.36 mmol) was dissolved in THF (20 mL) followed by the addition of NaH (55 mg, 1.36 mmol); the reaction was stirred for 10 minutes before the azide **18** (202 mg, 0.68 mmol) was added; the reaction was heated to reflux and stirred for 18 hours. It was then quenched with NH_4Cl and washed with EtOAc (3 x 50 mL); the organic layers were combined and dried over Na_2SO_4. Purification by column chromatography yielded **19** in 47% yield as a pale yellow oil. R_f = 0.39 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 6.55 (s, 1H), 4.50 (s, 1H), 4.05 (t, *J* = 6 Hz, 1H), 3.74 (dd, *J* = 1.5, 6 Hz, 1H), 3.64 (dd, *J* = 5, 15 Hz, 1H), 1.73 (s, 9H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 163.3, 151.5, 69.5, 55.6, 35.5, 28.9 ppm.

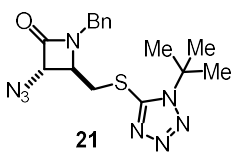
(3S,4R)-3-azido-4-(((1-(tert-butyl)-1H-tetrazol-5-yl)sulfonyl)methyl)azetidin-2-one (20)



20

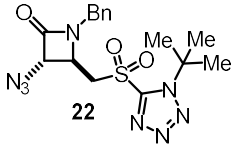
The thioether **19** (75 mg, 0.25 mmol) was dissolved in EtOH (2 mL) and cooled to 0 °C and a solution of $Mo_7O_{24}(NH_4)_6$ (27.8 mg, 0.023 mmol) in H_2O_2 (0.3 mL) was added; the reaction was warmed to room temperature and stirred for 18 hours. The reaction was then washed with EtOAc and the sulfone **20** was obtained in 98% yield without further purification. R_f = 0.72 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 4.67-4.66 (m, 1H), 4.36 (dd, *J* = 4.5, 14.5 Hz, 1H), 4.23-4.21 (m, 1H), 4.14 (dd, *J* = 7.5, 14.5 Hz, 1H), 1.85 (s, 9H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 163.2, 153.8, 70.6, 66.2, 59.5, 50.8, 29.0 ppm.

(3S,4R)-3-azido-1-benzyl-4-(((1-(tert-butyl)-1H-tetrazol-5-yl)thio)methyl)azetidin-2-one (21)



The thioether **19** (61 mg, 0.21 mmol) was dissolved in DMF (3 mL) and cooled to 0 °C. BnBr (0.1 mL, 0.63 mmol) was added followed by NaH (103 mg, 0.26 mmol) and the reaction was stirred for 3 hours. It was then quenched with NH_4Cl and the aqueous layer was extracted with Et_2O (3 x 25 mL); the organic layers were combined and dried over Na_2SO_4 and purified by column chromatography (1:1 Hex/EtOAc) to give **21** in 83% yield. R_f = 0.52 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 7.39-7.27 (m, 5H), 4.55 (d, *J* = 24.5 Hz, 1H), 4.32 (d, *J* = 25 Hz, 1H), 3.85-3.82 (m, 1H), 3.67 (dd, *J* = 6, 24 Hz, 1H), 3.36 (dd, *J* = 13, 24 Hz, 1H), 1.70 (s, 9H) ppm.

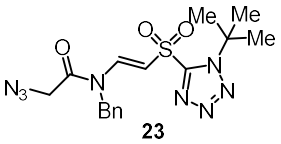
(3S,4R)-3-azido-1-benzyl-4-(((1-(tert-butyl)-1H-tetrazol-5-yl)sulfonyl)methyl)azetidin-2-one (22)



The thioether **21** (48 mg, 0.13 mmol) was dissolved in EtOH and cooled to 0 °C; a solution of $Mo_7O_{24}(NH_4)_6$ (14 mg, 0.011 mmol) in H_2O_2 was added and the reaction warmed to room temperature and stirred for 18 hours. The reaction was then diluted with CH_2Cl_2 and washed with H_2O; the organic layer was dried over Na_2SO_4 and concentrated to give **22** in 83% yield. R_f = 0.82 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 7.39-7.31(m, 5H), 4.83 (d, *J* = 2Hz, 1H), 4.57 (d, *J* = 15.5 Hz, 1H), 4.36 (d, *J* = 15.5 Hz, 1H), 4.22 (dd, *J* = 3.5, 15 Hz, 1H), 4.01-3.98 (m, 1H), 3.81 (dd, *J* = 9.5, 15 Hz, 1H), 1.80 (s, 9H) ppm.

(E)-2-azido-N-benzyl-N-(2-((1-(tert-butyl)-1H-tetrazol-5-yl)sulfonyl)vinyl)acetamide

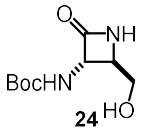
(23)



23

The sulfone **22** (26 mg, 0.064 mmol) was dissolved in DMF and cooled to -78 °C. KHMDS (0.18mL, 0.77 mmol) was added followed by benzaldehyde (0.01 mL, 0.077 mmol) and the reaction was stirred for 3 hours. It was then quenched with NH_4Cl and diluted with H_2O and Et_2O, the aqueous layer was extracted with Et_2O (3 x 25 mL), and the organic layers were combined and washed with brine. The organic layer was then dried over Na_2SO_4 and purified by column chromatography (1:1 Hex/EtOAc) to give **23** in 61% yield. R_f = 0.63 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 9.32 (d, *J* = 6 Hz, 1H), 8.18 (d, *J* = 5.5 Hz, 1H), 7.38 (d, *J* = 4Hz, 5H), 4.73 (d, *J* =6 Hz, 2H), 2.04 (d, 1Hz, 1H), 1.72 (s, 9H) ppm.

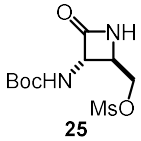
tert-butyl ((2R,3S)-2-(hydroxymethyl)-4-oxoazetidin-3-yl)carbamate (24)



24

The azide **16** (248 mg, 1.75 mmol) was dissolved in EtOAc (20 mL) followed by Boc_2O (1.14g, 5.2 mmol) and Pd/C (18.6 mg). The reaction flask was then flushed with H_2 and stirred under an atmosphere of hydrogen for 2 hours. The reaction was then filtered through a pad of Celite and purified with column chromatography (EtOAc) to give **24** in 77% yield. R_f = 0.05 (EtOAc), ^{1}H NMR (500 MHz, CD_3OD): 4.41 (s, 1H), 3.75 (dd, *J* = 3, 11 Hz, 1H), 3.70-3.63 (m, 2H), 1.44 (s, 9 H) ppm.

((2R,3S)-3-((tert-butoxycarbonyl)amino)-4-oxoazetidin-2-yl)methyl methanesulfonate (25)

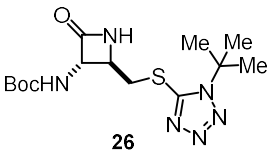


25

The β-lactam **24** (100 mg, 0.46 mmol) was dissolved in MeCN, Et_3N (0.13 mL, 0.92 mmol) and MsCl (0.06 mL, 0.69 mmol) were then added, and the reaction was stirred for 1 hour. The reaction was then concentrated and purified

by column chromatography (1:1 Hex/EtOAc) to give **25** in 72% yield. R_f = 0.20 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 6.78 (s, 1H), 5.58 (d, *J* = 7.5 Hz, 1H), 4.53-4.49 (m, 1H), 4.34-4.30 (m, 1H), 3.94-3.93 (m, 1H), 3.07 (s, 3H), 1.43 (s, 9H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): *δ* (ppm): 166.8, 155.3, 81.0, 69.2, 60.7, 55.5, 37.8, 28.4 ppm.

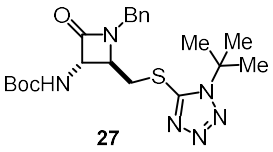
tert-butyl ((2R,3S)-2-(((1-(tert-butyl)-1H-tetrazol-5-yl)thio)methyl)-4-oxoazetidin-3-yl)carbamate (26)



26

The tetrazole **17** (73 mg, 0.46 mmol) was dissolved in THF, followed by NaH (18 mg, 0.46 mmol), and the reaction was stirred for 10 minutes before the addition of the β-lactam **25** (98 mg, 0.33 mmol). The reaction was then heated to reflux and stirred for 15 hours. It was quenched with NH_4Cl, and diluted with EtOAc; the aqueous layer was washed with EtOAc (3 x 20 mL). The organic layers were combined and washed with brine before being dried over Na_2SO_4 and purified with column chromatography (1:1 Hex/EtOAc) to give **26** in 71% yield. R_f = 0.31 (EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 6.47 (s, 1H), 5.34 (d, *J* = 7 Hz, 1H), 4.59 (d, *J* = 6.5 Hz, 1H), 4.10-4.07 (m, 1H), 3.86 (dd, *J* = 2, 16.5 Hz, 1H), 3. 58 (d, *J* = 14.5 Hz, 1H), 1.72 (s, 9H), 1.43 (s, 9H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): *δ* (ppm): 166.2, 155.2, 80.9, 63.2, 61.6, 60.6, 56.8, 35.9, 28.9, 28.4, 21.2, 14.4ppm.

tert-butyl ((2R,3S)-1-benzyl-2-(((1-(tert-butyl)-1H-tetrazol-5-yl)thio)methyl)-4-oxoazetidin-3-yl)carbamate (27)

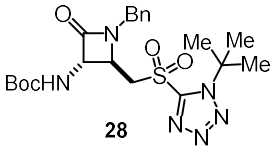


27

The thioether **26** (93 mg, 0.26 mmol) was dissolved in DMF (3 mL) followed by BnBr (0.18 mL, 1.04 mmol); the reaction was cooled to 0 °C and NaH (22 mg, 0.55 mmol) was added. The reaction

was stirred for 1 hour. The reaction was quenched with NH_4Cl and diluted with EtOAc; the organic layer was washed with H_2O (3 x 20 mL) and brine then dried over Na_2SO_4 and purified by column chromatography (1:1 Hex/EtOAc) to give **27** in 81% yield. R_f = 0.42 (EtOAc), ^1H NMR (500 MHz, $CDCl_3$): 7.28-7.24 (m, 5H), 4.26 (d, *J* = 16 Hz, 1H), 3.85 (s, 1H), 3.42 (dd, *J* = 4.5, 4Hz, 1H), 1.63 (s, 9H), 1.42 (bs, 9H) ppm. ^{13}C {^1H} NMR (125 MHz, $CDCl_3$): δ (ppm): 128.9, 128.8, 128.6, 128.0, 127.6, 68.1, 61.3, 34.7, 28.9, 28.5 ppm.

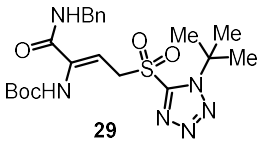
tert-butyl ((2R,3S)-1-benzyl-2-(((1-(tert-butyl)-1H-tetrazol-5-yl)sulfonyl)methyl)-4-oxoazetidin-3-yl)carbamate (28)



28

The thioether **27** (106 mg, 0.198 mmol) was dissolved in EtOH and cooled to 0 °C; a solution of $Mo_7O_{24}(NH_4)_6$ (22 mg, 0.018 mmol) in H_2O_2 was added and the reaction warmed to room temperature and stirred for 18 hours. The reaction was then diluted with CH_2Cl_2 and washed with H_2O; the organic layer was dried over Na_2SO_4 and concentrated to give **28** in 83% yield. R_f = 0.72 (1:1 Hex/EtOAc), ^1H NMR (500 MHz, $CDCl_3$): 7.28 (bs, 2H), 7.19 (bs, 3H), 4.96-4.45 (m, 2H), 4.43-4.19 (m, 1H), 4.12 (bs, 1H), 3.83 (bs, 1H), 1.80 (s, 9H), 1.46 (bs, 9H) ppm. ^{13}C {^1H} NMR (125 MHz, $CDCl_3$): δ (ppm): 128.9, 127.6, 69.7, 65.8, 58.6, 29.9, 28.5 ppm.

tert-butyl (Z)-(1-(benzylamino)-4-((1-(tert-butyl)-1H-tetrazol-5-yl)sulfonyl)-1-oxobut-2-en-2-yl)carbamate (29)



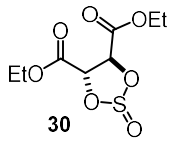
29

The sulfone **28** (35 mg, 0.06 mmol) was dissolved in DMF and cool to -78 °C KHMDS (0.17 mL, 0.77 mmol) was added followed by benzaldehyde (0.01 mL, 0.077 mmol) and the reaction was stirred for 3 hours. It was then quenched with NH_4Cl and diluted with H_2O and Et_2O, the aqueous layer

was extracted with Et_2O (3 x 25 mL), and the organic layers were combined and washed with brine. The organic layer was then dried over Na_2SO_4 and purified by column chromatography (1:1 Hex/EtOAc) to give **29** in 61% yield. R_f = 0.63 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 7.28-7.25 (m, 5H), 5.83 (t, *J* = 7.5 Hz, 1H), 5.05 (d, *J* = 7.5Hz, 2H), 4.33 (d, *J* = 5.5 Hz, 2H), 1.84 (s, 9H), 1.39 (s, 9H) ppm.

diethyl (4S,5S)-1,3,2-dioxathiolane-4,5-dicarboxylate 2-oxide (30)

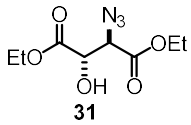
Diethyl tartrate (2 mL, 11.7 mmol) was cooled to 0 °C; $SOCl_2$ (0.98 mL, 13.56 mmol) was added dropwise over 5 minutes followed by 5 drops of DMF. The reaction was warmed to room temperature, then heated to 50 °C for 30 minutes. The reaction was cooled to room temperature and N_2 was bubbled through the reaction for 30 minutes to neutralize the excess $SOCl_2$; the reaction was concentrated and the **30** was used crude.



30

diethyl (2R,3S)-2-azido-3-hydroxysuccinate (31)

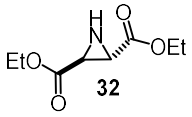
Dioxathiolane **30** (1.28 g, 4.8 mmol) was dissolved in DMF and NaN_3 (9.37 g, 14.4 mmol) was added and the reaction was stirred for 24 hours. CH_2Cl_2 (5 mL) and H_2O (5 mL) were added and the reaction was stirred an additional 2 hours. The aqueous layer was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4 to yield **31** in 77% yield. R_f = 0.85 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 4.26-4.16 (m, 4H), 2.86-2.83 (m, 2H), 1.32-1.26 (m, 6H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 170.3, 62.5, 61.9, 36.4, 35.5, 14.3 ppm.



31

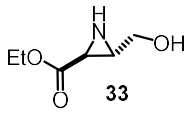
diethyl (2S,3S)-aziridine-2,3-dicarboxylate (32)

The azido alcohol **31** (870 mg, 3.7 mmol) was dissolved in DMF and cooled to 0 °C and PPh_3 was added in portions. The reaction was allowed to warm to room temperature and was stirred for 1.5 hours; it was then warmed to 85 °C and stirred until complete by TLC. The reaction was concentrated and purified by column chromatography (6:1 Hex/EtOAc) to give **32** in 63% yield. R_f = 0.85 (4:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 4.30 (q, *J* = 7.5 Hz, 2H), 4.17 (q, *J* = 4 Hz, 2H), 1.34 (t, *J* = 7 Hz, 3H), 1.28 (t, *J* = 7 Hz, 3H) ppm.



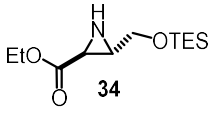
ethyl (2S,3S)-3-(hydroxymethyl)aziridine-2-carboxylate (33)

The aziridine **32** (1.30 g, 6.9 mmol) was dissolved in EtOH (25 mL) and the reaction was cooled to 0 °C; $NaBH_4$ (210 mg, 5.5 mmol) was added and the reactionwas stirred for 12 hours. It was then quenched with NH_4Cl and diluted with CH_2Cl_2 and washed with H_2O (10 mL) and the organic layer was dried over Na_2SO_4 then purified by column chromatography (1:1 Hex/EtOAc) to give **33** in 71% yield. R_f = 0.55 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 4.25-4.18 (m, 2H), 3.80 (dd, *J* = 3.5, 12 Hz, 1H), 3.52 (dd, *J* = 5, 12.5 Hz, 1H), 1.29 (t, *J* = 7.5 Hz, 3H) ppm.



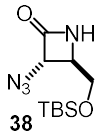
ethyl (2S,3S)-3-(((tert-butyldimethylsilyl)oxy)methyl)aziridine-2-carboxylate (34)

The aziridine **33** (327.8 mg, 2.26 mmol) was dissolved in DMF, followed by TESCl (511 mg, 3.39 mmol), DMAP (137.8 mg, 1.13 mmol), and Et_3N (0.63 mL, 4.52 mmol). The reaction was stirred for 1 hour, then diluted with H_2O and Et_2O; the organic layer was extracted with brine and dried over Na_2SO_4. Purification was done by column chromatography (4:1 Hex/EtOAc) to give **34** in 93% yield. R_f = 0.75 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 4.24-4.18 (m, 2H), 3.62 (d, *J* = 46.5 Hz, 1H), 2.42



(d, J = 27.5 Hz, 1H), 1.29 (t, J = 7 Hz, 3H), 0.95 (t, J = 8 Hz, 9H), 0.60 (q, J = 8Hz, 6H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 61.8, 59.0, 37.5, 31.0, 11.6, 4.1, 1.8 ppm.

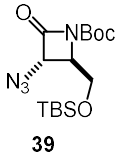
(3S,4R)-3-azido-4-(((tert-butyldimethylsilyl)oxy)methyl)azetidin-2-one (38)



38

β-lactam **16** (58 mg, 0.4 mmol) was dissolved in DMF, followed by TBSCl (133 mg, 0.6 mmol) DMAP (25 mg, 0.2 mmol) and Et_3N (0.12 mL, 0.8 mmol). The reaction was stirred for 1 hour, then diluted with H_2O and Et_2O; the organic layer was extracted with brine and dried over Na_2SO_4. Purification was done by column chromatography (4:1 Hex/EtOAc) to give **38** in 91% yield. R_f = 0.76 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 4.41 (s, 1H), 3.80-3.73 (m, 2H), 3.65-3.62 (m, 1H), 0.89 (s, 9H), 0.07 (s, 3H), 0.6 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 164.5, 154.4, 66.7, 62.7, 57.4, 25.9 ppm.

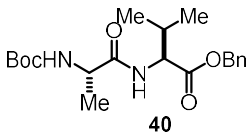
tert-butyl (2R,3S)-3-azido-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-oxoazetidine-1-carboxylate (39)



39

The TBS β-lactam **38** (83.6 mg, 0.32 mmol) was dissolved in CH_2Cl_2 followed by Boc_2O (106.7 mg, 0.5 mmol), DMAP (19.5 mg, 0.16 mmol), and Et_3N (0.1 mL, 0.64 mmol). The reaction was stirred for 24 hours then concentrated and purified by column chromatography (4:1 Hex/EtOAc) to give **39** in 98% yield. R_f = 0.86 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 4.66 (d, J = 2.5 Hz, 1H), 4.11 (dd, J = 3, 11.5 Hz, 1H), 3.88 (dd, J = 1.5, 11 Hz, 1H), 3.85-3.83 (m, 1H), 1.52 (s, 9H), 0.88 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 154.4, 84.3, 64.8, 60.3, 58.9, 28.2, 25.9, 18.4 ppm.

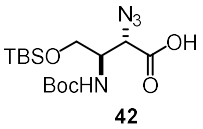
benzyl (tert-butoxycarbonyl)-L-alanyl-L-valinate (40)



40

benzyl L-valinate (1.54 g, 6.3 mmol) and *i*Pr_2NEt (2 mL, 11.4 mmol) were added to THF followed by EDCI (984 mg, 6.3 mmol), HOBt (971 mg, 6.3 mmol), and N-Boc-alanine (1.00 g, 5.2 mmol). The reaction was stirred for 18 hours, then diluted with CH_2Cl_2 and H_2O; the organic layer was washed with brine and dried over Na_2SO_4. The crude mixture was purified by column chromatography (1:1 Hex/EtOAc) to give **40** in 97% yield. R_f = 0.86 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 7.36-7.33 (m, 5H), 6.63 (bs, 1H), 5.15 (q, 12.5 Hz, 2H), 4.96 (bs, 1H), 4.57 (dd, *J* = 5, 9 Hz, 1H), 4.17 (bs, 1H), 2.21-2.17 (m, 1H), 1.44 (s, 9H), 1.34 (d. *J* = 7 Hz, 3H), 0.90 (d, *J* = 7 Hz, 3H), 0.85 (d, *J* = 7 Hz, 3H) ppm.

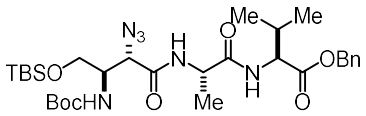
(2S,3R)-2-azido-3-((tert-butoxycarbonyl)amino)-4-((tert-butyldimethylsilyl)oxy)butanoic acid (42)



42

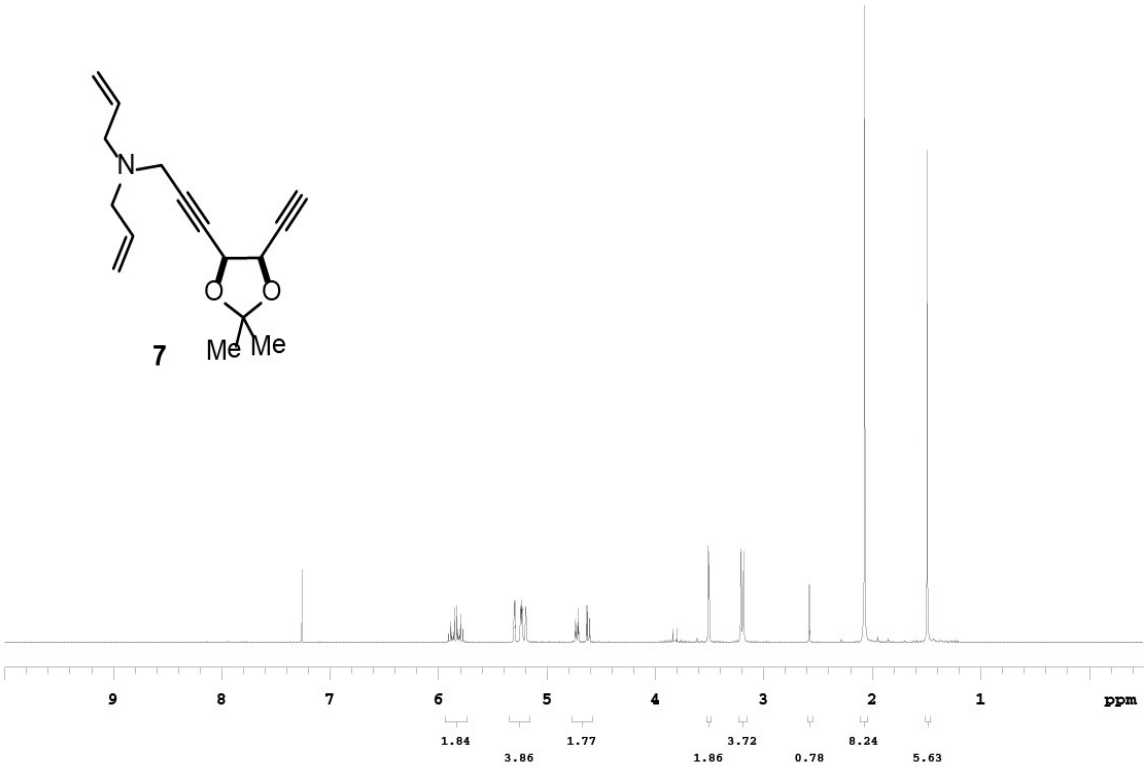
The β-lactam **39** (600 mg, 1.68 mmol) was dissolved in THF/H_2O (1:1) and a 1M solution of LiOH (1.68 mL, 1.68 mmol) was added. The reaction was stirred for 1 hour and quenched with NH_4Cl. It was then diluted with EtOAc, and the organic layer was washed with H_2O then dried over Na_2SO_4 and concentrated to give **42** in 93% yield without further purification. R_f = 0.52 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 5.53 (d, *J* = 4 Hz, 1H), 4.08-4.-2 (m, 1H), 3.86-3.82 (m, 1H), 1.90 (m, 1H), 1.43 (s, 9H), 0.88 (s, 9H), 0.05 (s, 6H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 98.6, 68.7, 67.6, 60.6, 33.3, 28.5, 27.9, 26.0, 23.6, 22.4 ppm.

benzyl ((2S,3R)-2-azido-3-((tert-butoxycarbonyl)amino)-4-((tert-butyldimethylsilyl)oxy)butanoyl)-L-alanyl-L-valinate (43)



43

The carboxylic acid **42** (39 mg, 0.1 mmol) was dissolved in CH_2Cl_2, followed by the addition of *i*Pr_2Net (0.03 ml, 0.14 mmol), HATU (51 mg, 0.14 mmol), and **41** (53 mg, 0.14 mmol) and the reaction was stirred for 18 hours. It was then washed with H_2O and the organic layer was dried over Na_2SO_4. Purification was done by column chromatography (1:1 Hex/EtOAc) to give diamine dipeptide **43** in 91% yield. R_f = 0.71 (1:1 Hex/EtOAc), ^{1}H NMR (500 MHz, $CDCl_3$): 7.38-7.23 (m, 5 H), 7.12 (bs, 1H), 6.93 (d, *J* = 6.5 Hz, 1H), 6.43 (d, *J* = 8 Hz, 1H), 6.27 (d, *J* = 8.5 Hz, 1H), 5.23-5.19 (m, 1H), 5.15-5.11 (m, 2H), 4.59-4.52 (m, 2H), 4.46-4.43 (m, 1H), 4.25 (d, *J* = 6.5 Hz, 1H), 4.04-4.01 (m, 1H), 3.81-3.76 (m, 1H), 3.66 (dd, *J* = 5.5, 10.5 Hz, 1H), 2.24-2.16 (m, 1H), 1.42 (s, 9H), 1.37 (d, *J* = 7 Hz, 3H), 1.25 (s, 6H), 0.92-0.85 (m, 12 H), 0.06 (s, 6H) ppm. ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm): 168.9, 126.0, 64.7, 64.5, 59.3, 54.7, 46.7, 28.6, 27.1, 25.7, 23.2, 16.3, 14.9, 8.0 ppm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O
O
Me
Me
9
8
7
6
5
4
3
2
1
ppm
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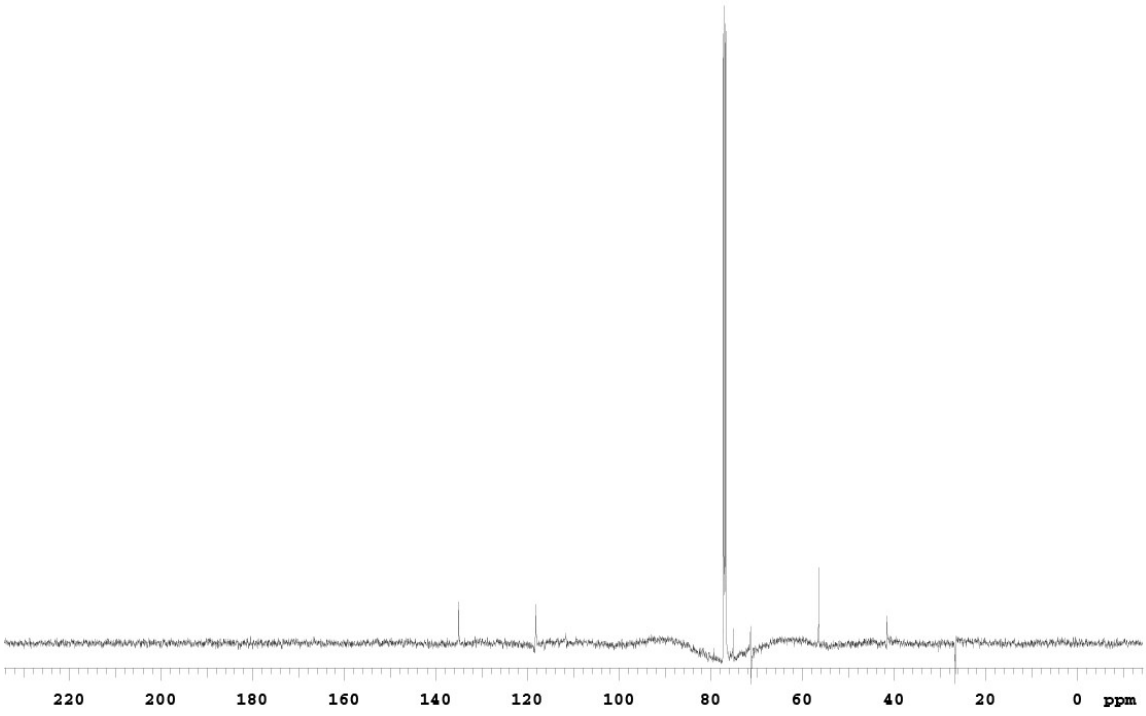
3.86
1.77
1.86
3.72
0.78
8.24
5.63

7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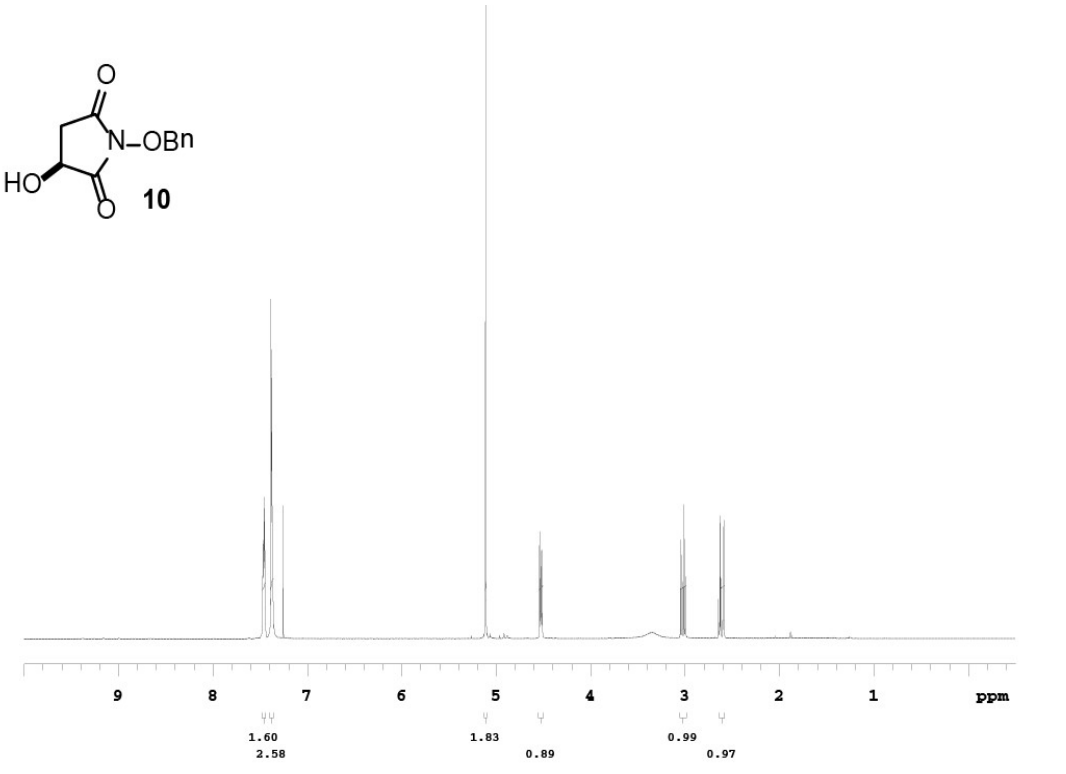
200
180
160
140
120
100
80
60
40
20
0
ppm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N–OBn
HO
O
10
9
8
7
6
5
4
3
2
1
ppm
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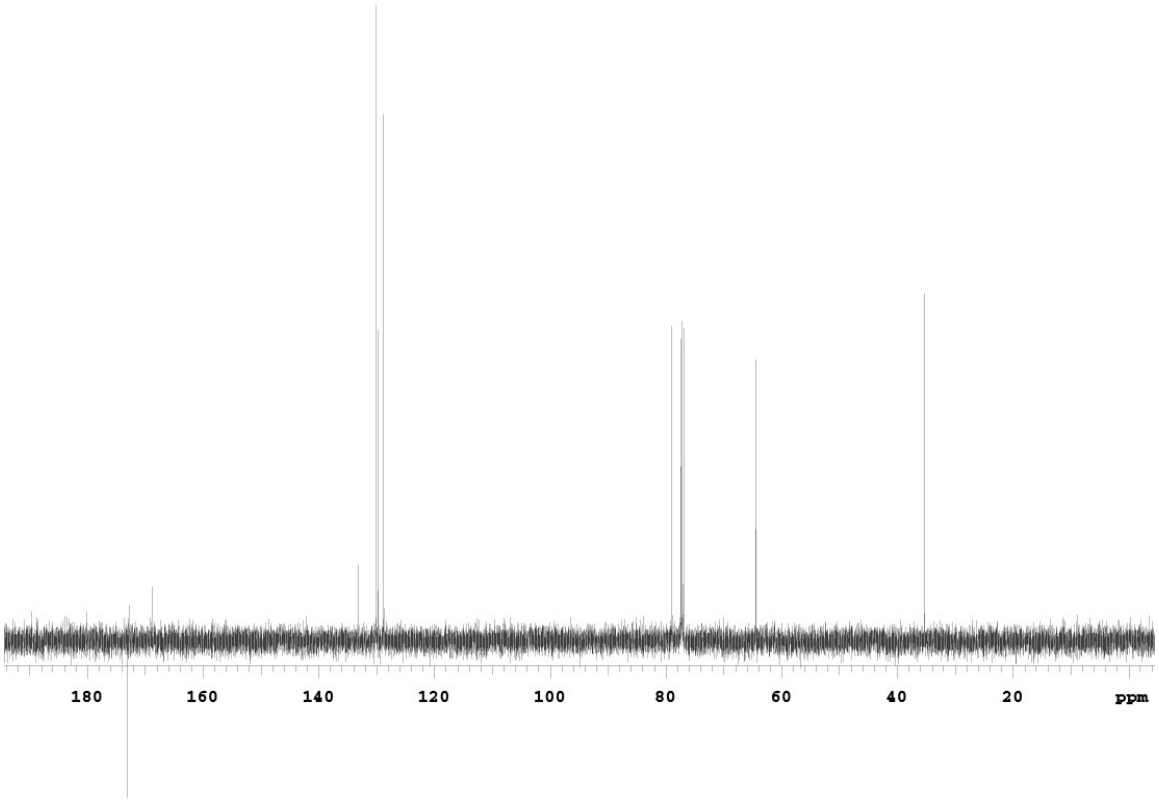
1.83
0.89
0.99
0.97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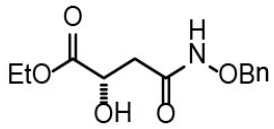
160
140
120
100
80
60
40
20
ppm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H
N
EtO
OBn
OH
O

11a

9
8
7
6
5
4
3
2
1
ppm
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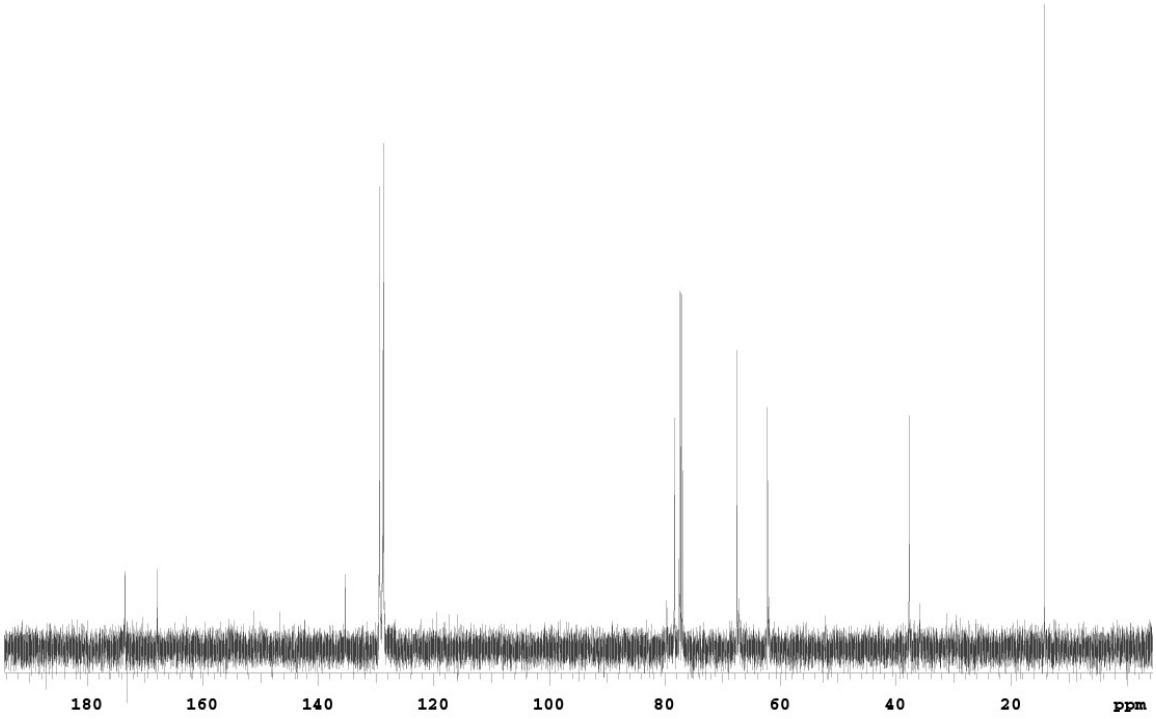
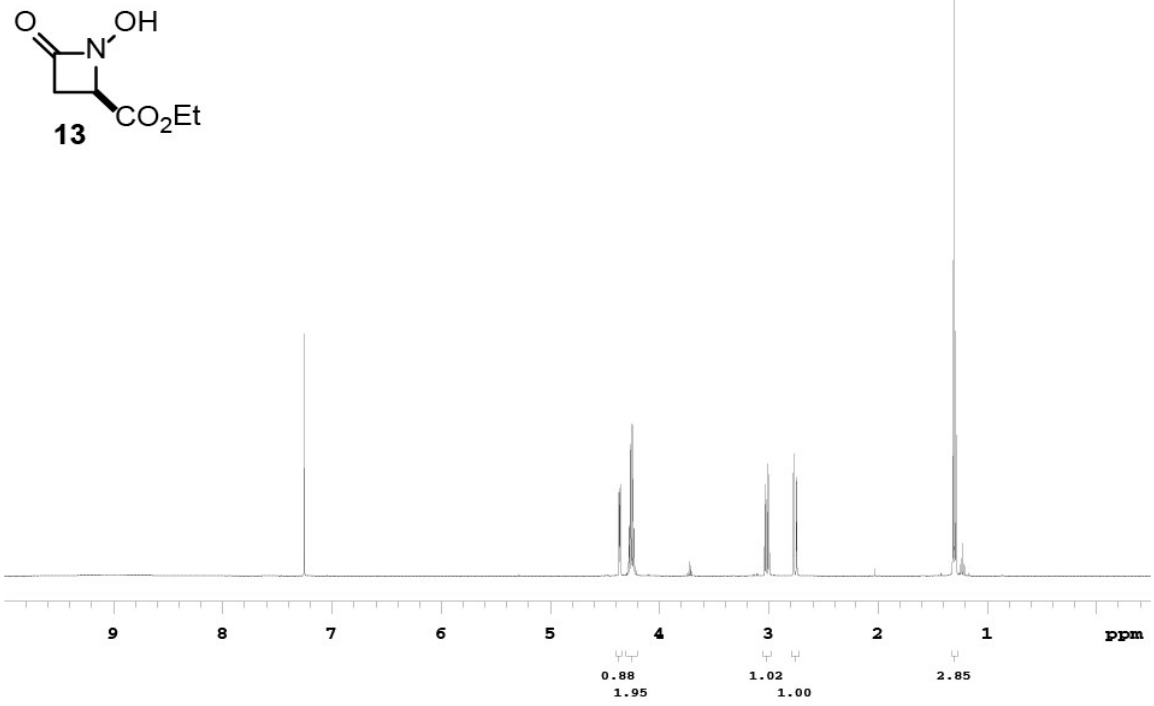
6.02
2.66
1.29
2.58
0.88
0.58
0.99
1.00
3.77


180
160
140
120
100
80
60
40
20
ppm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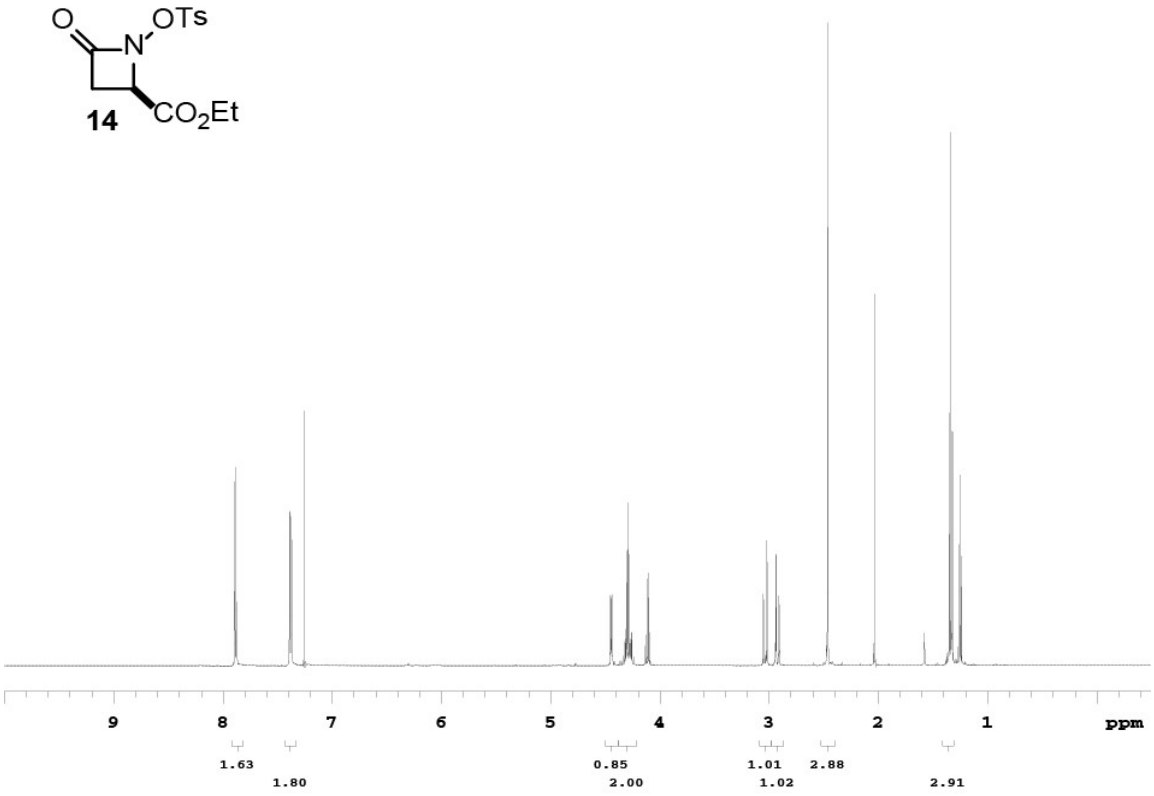
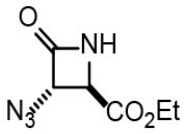
OH
CO2Et
13
9
8
7
6
5
4
3
2
1
ppm
0.88
1.95
1.02
1.00
2.85


O
N
OTs
CO2Et
14
9
8
7
6
5
4
3
2
1
ppm
1.63
1.80
0.85
2.00
1.01
1.02
2.88
2.91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N3
CO2Et

15

9
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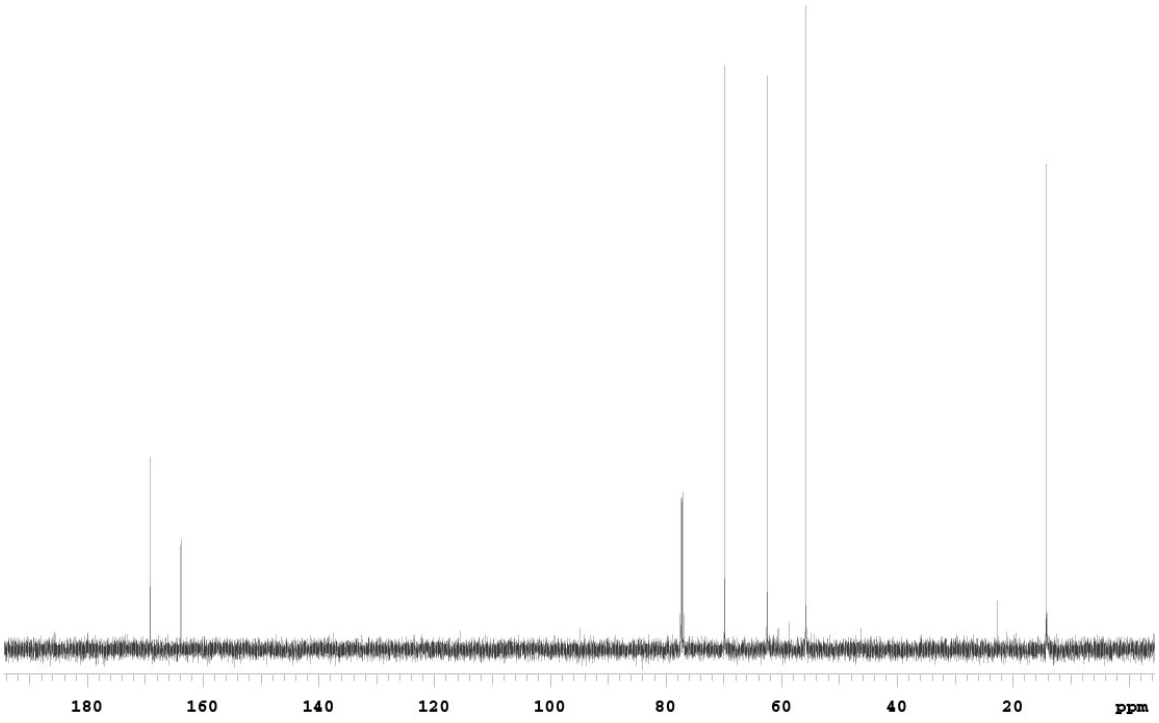
7
6
5
4
3
2
1
ppm
1.02
0.92
2.48
1.09
3.67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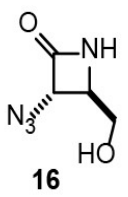
160
140
120
100
80
60
40
20
ppm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NH
N3
HO

16

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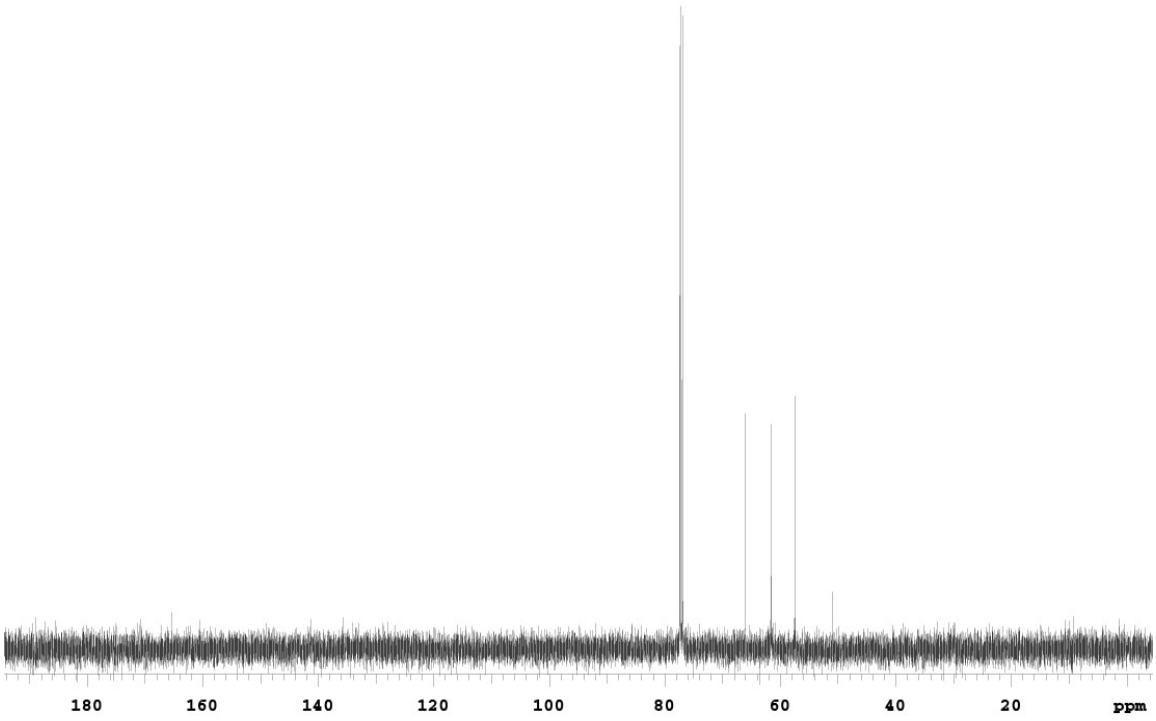
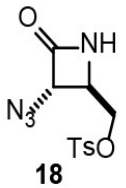
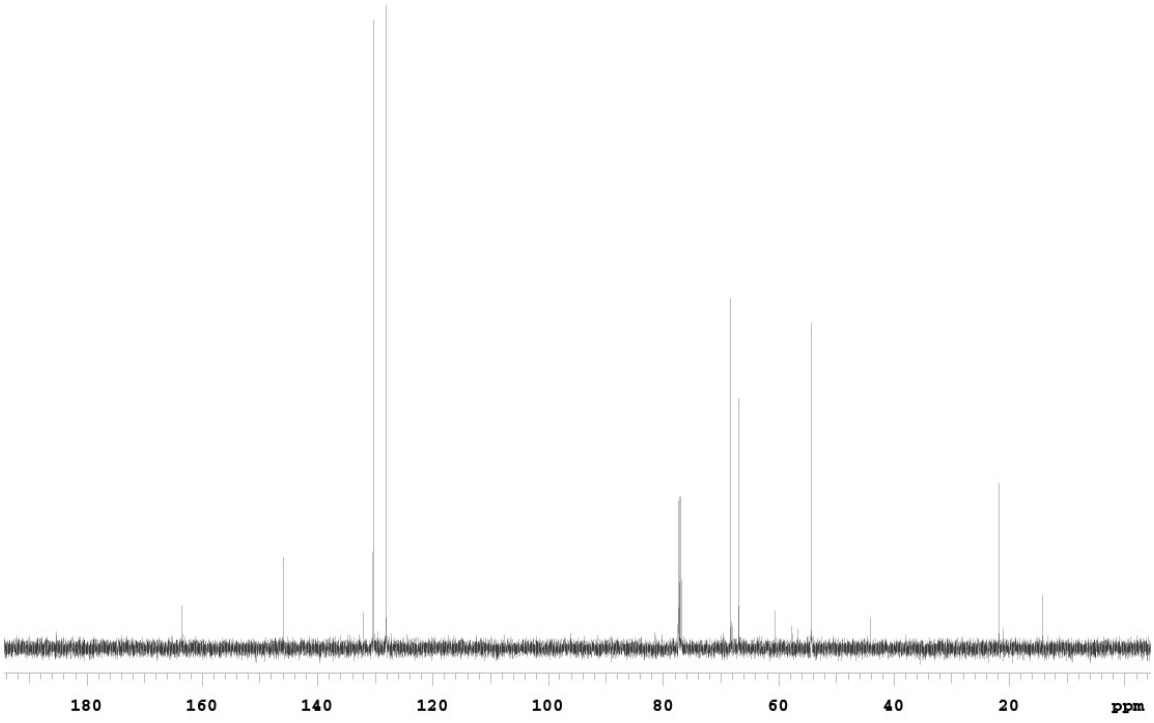
7
6
5
4
3
2
1
ppm
1.09
0.99
1.33
5.11
1.33
2.69


180
160
140
120
100
80
60
40
20
ppm


O
NH
N3
TsO

18

9
8
7
6
5
4
3
2
1
ppm
2.09
2.16
1.01
1.21
0.90
0.72
1.06
0.21
3.35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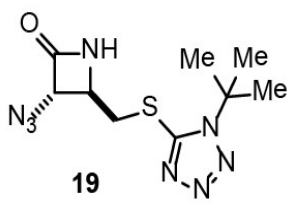
140
120
100
80
60
40
20
ppm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Me
N3
S
N
N
N
N

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4
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0.90
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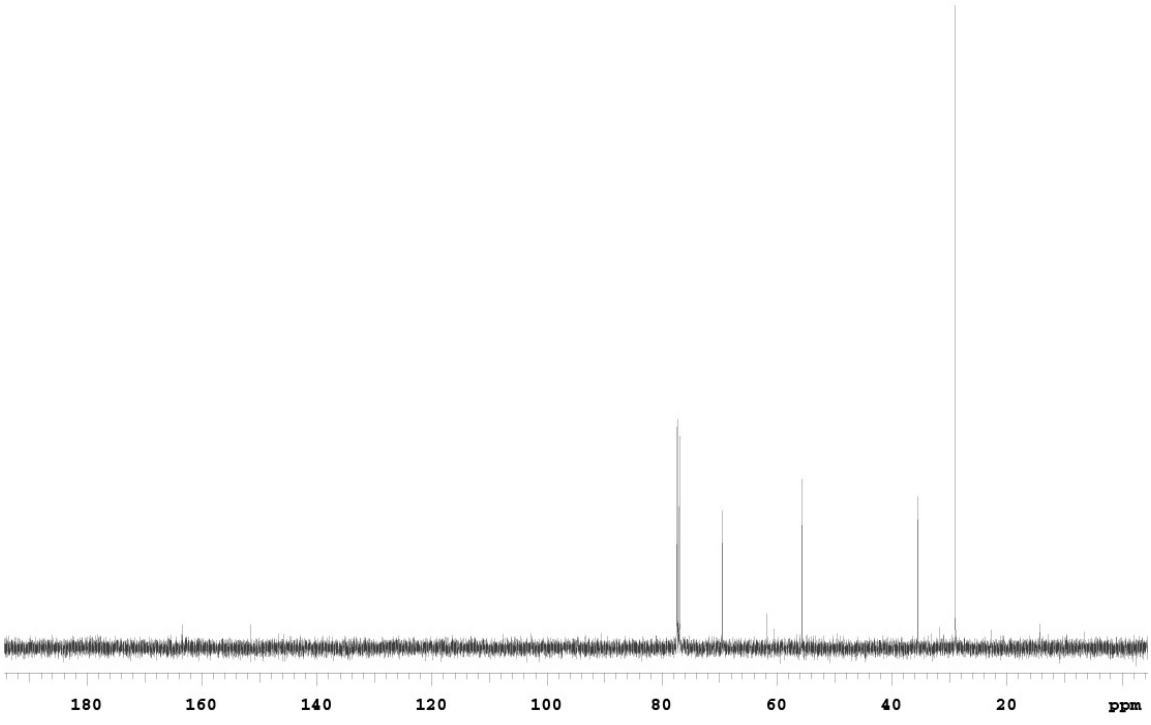
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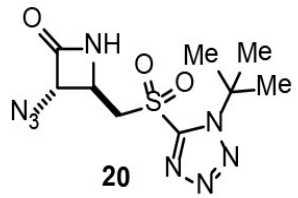
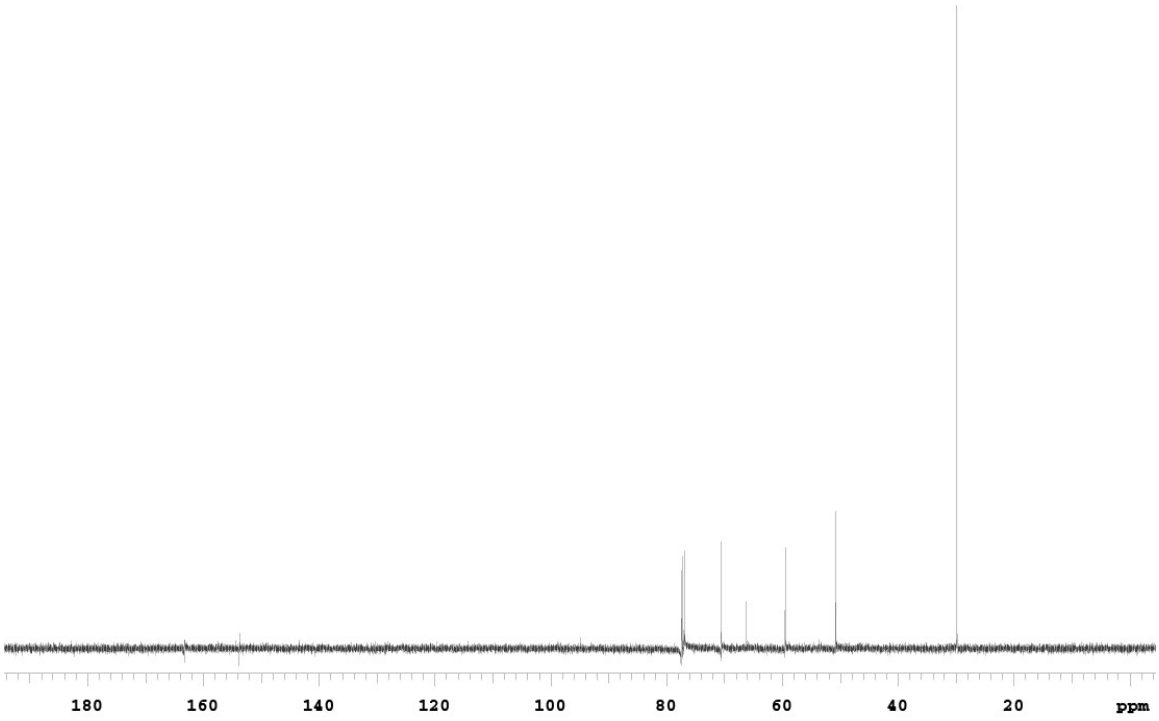
140
120
100
80
60
40
20
ppm


O
NH
O
Me
Me
Me
O
S
N
N
N
N
N3

20

9
8
7
6
5
4
3
2
1
ppm
1.00
0.97
1.05
1.13
9.61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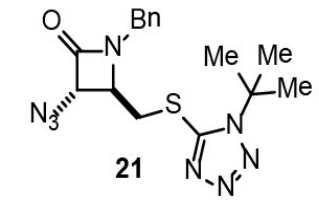
160
140
120
100
80
60
40
20
ppm


O
Bn
N
Me Me
Me
N3
S
N
N
N
N

21

9
8
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6
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2
1
ppm
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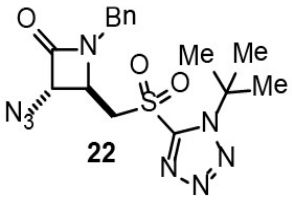
0.29 1.07
0.99
1.07
1.05
9.37


O
Bn
N
O
Me Me
O
S
Me
N3
N
N
N

22

9
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5
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3
2
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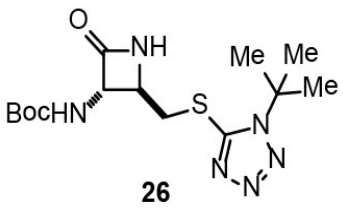
0.93 1.11 1.01
10.11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N
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26

9
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ppm
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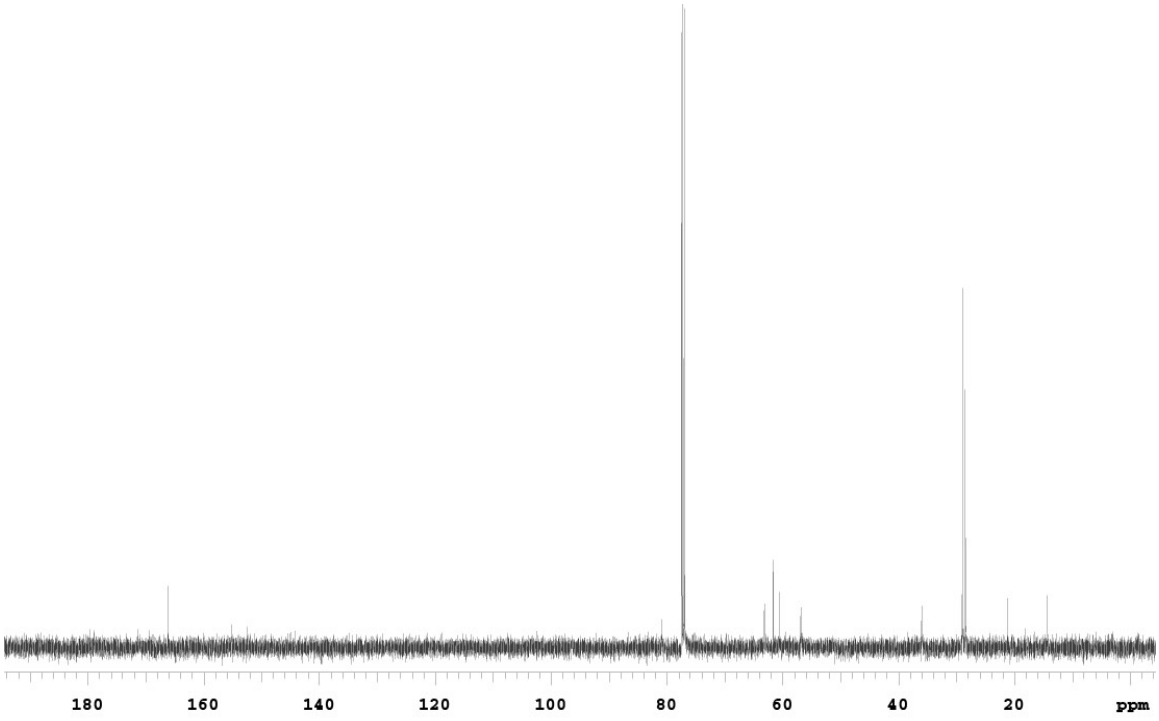
10.59
9.75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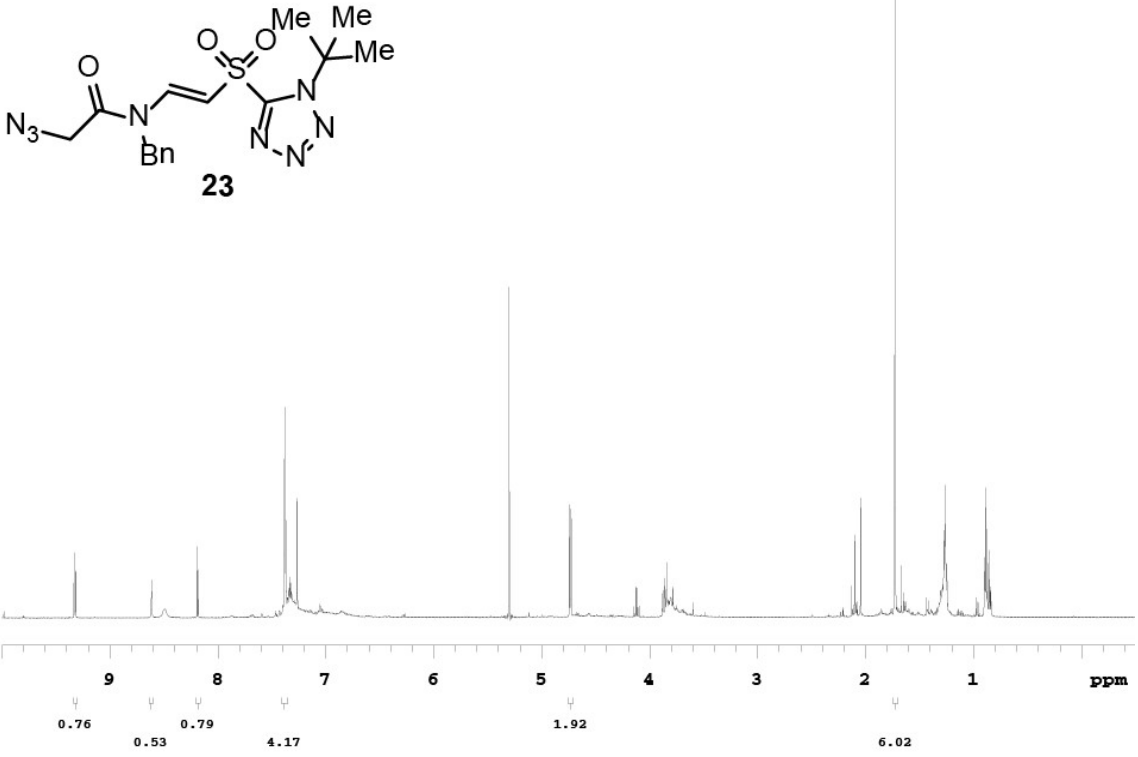
140
120
100
80
60
40
20
ppm


Me
Me
Me
O
O
S
O
N
N
N
N
N
N_3
Bn
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8
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4
3
2
1
ppm
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0.79
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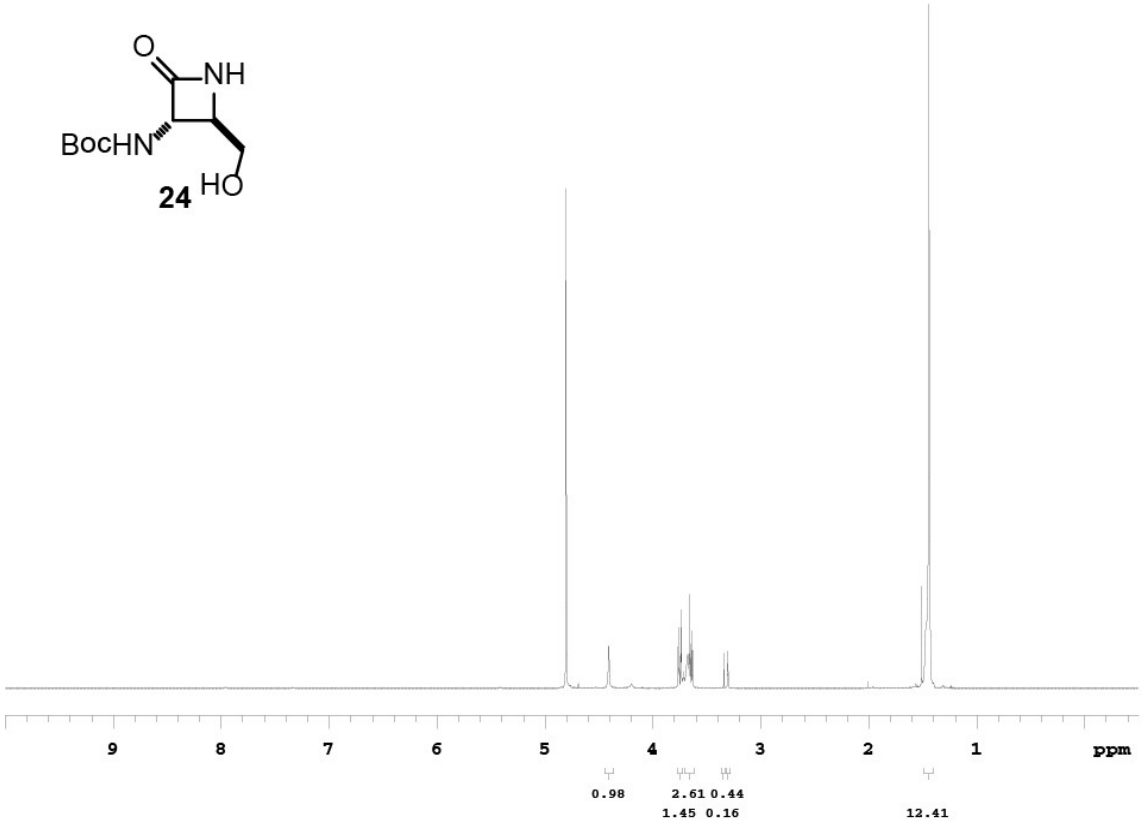
1.92
6.02

23


O
NH
BocHN
HO
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8
7
6
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4
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2
1
ppm
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0.44
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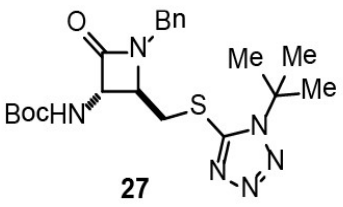
0.16
12.41

24


O
Bn
N
Me
Me
Me
BocHN
S
N
N
N
N

27

9
8
7
6
5
4
3
2
1
ppm
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1.71
3.00
0.95
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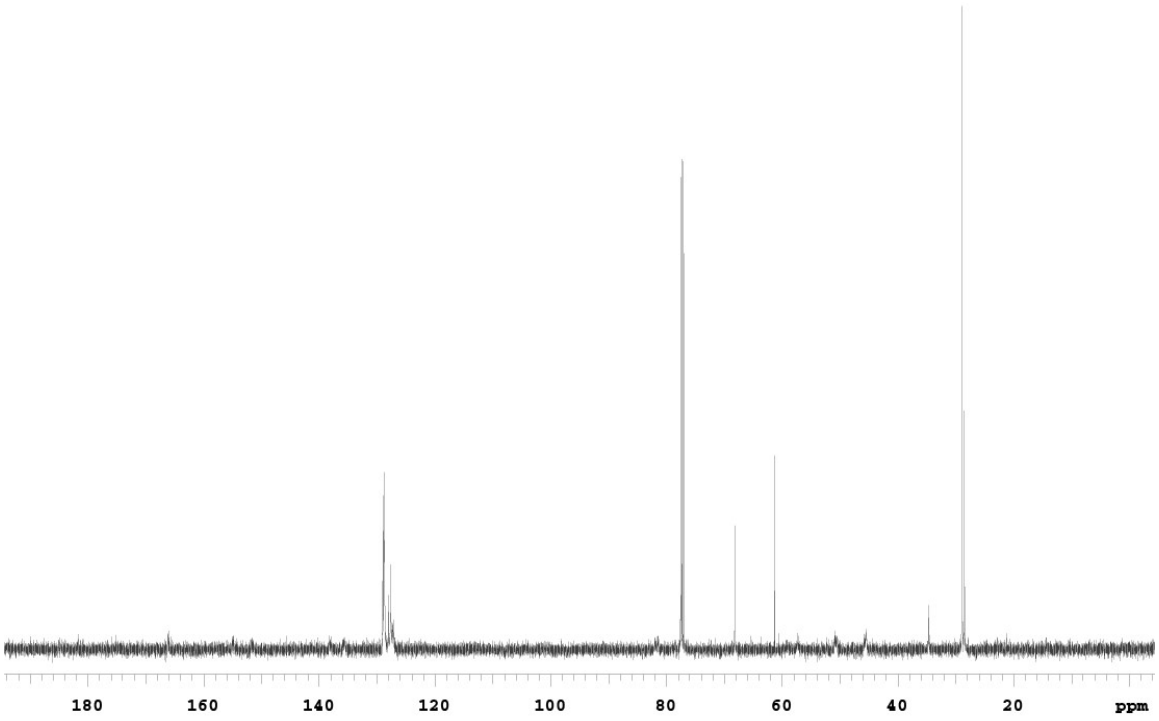
0.99
7.82
8.14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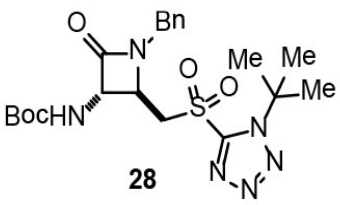
160
140
120
100
80
60
40
20
ppm


O
Bn
N
O
Me
Me
O
S
Me
BocHN
N
N
N

28

9
8
7
6
5
4
3
2
1
ppm
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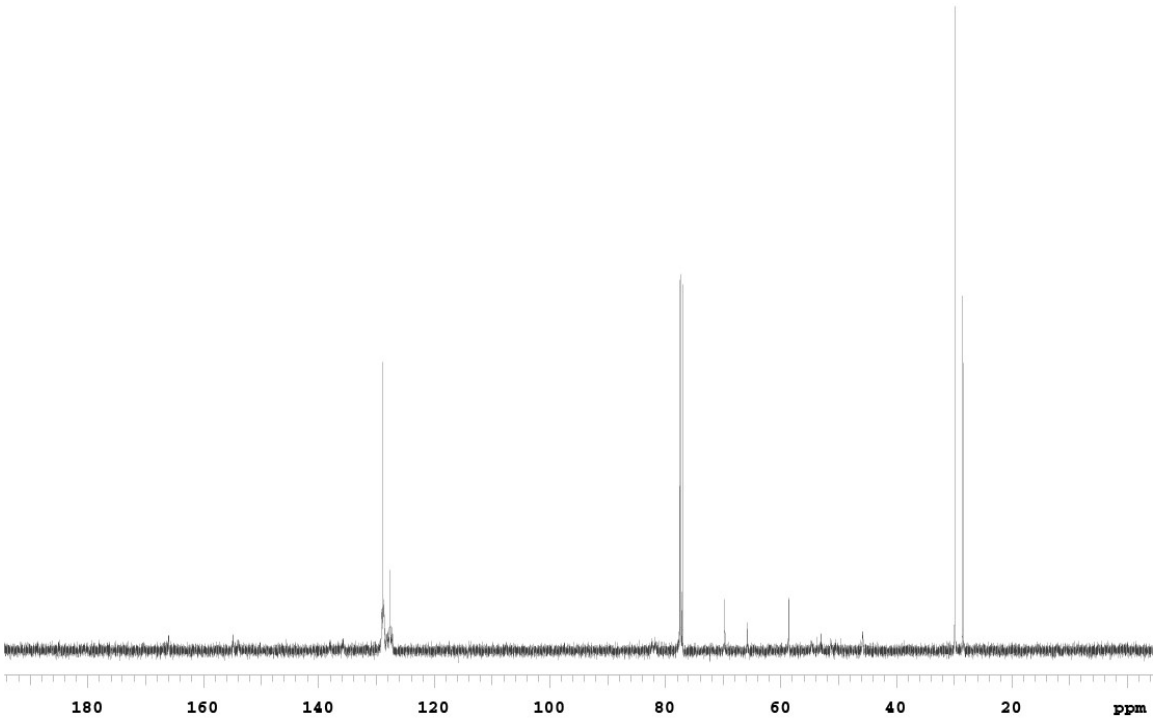
4.72
3.57
3.43
1.64
0.46
8.98
9.43


180
160
140
120
100
80
60
40
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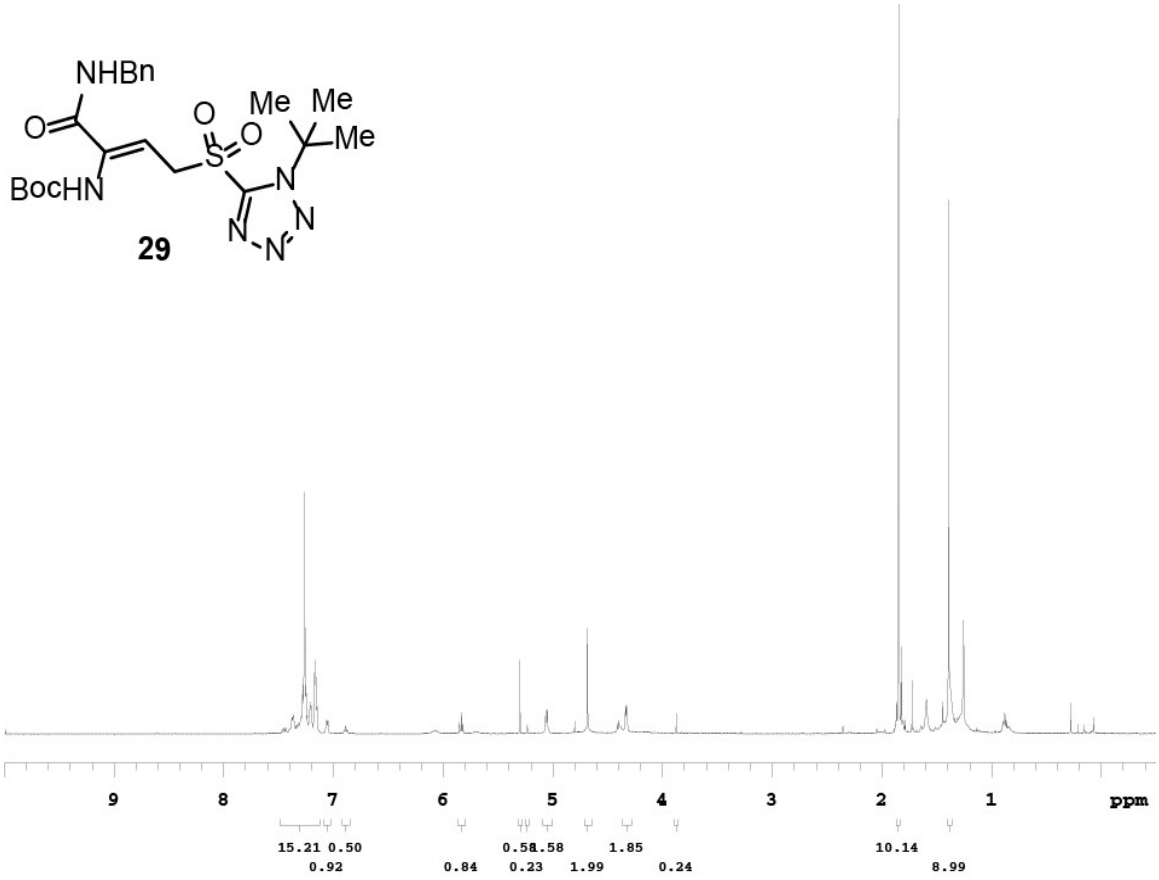
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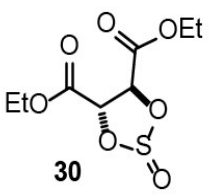
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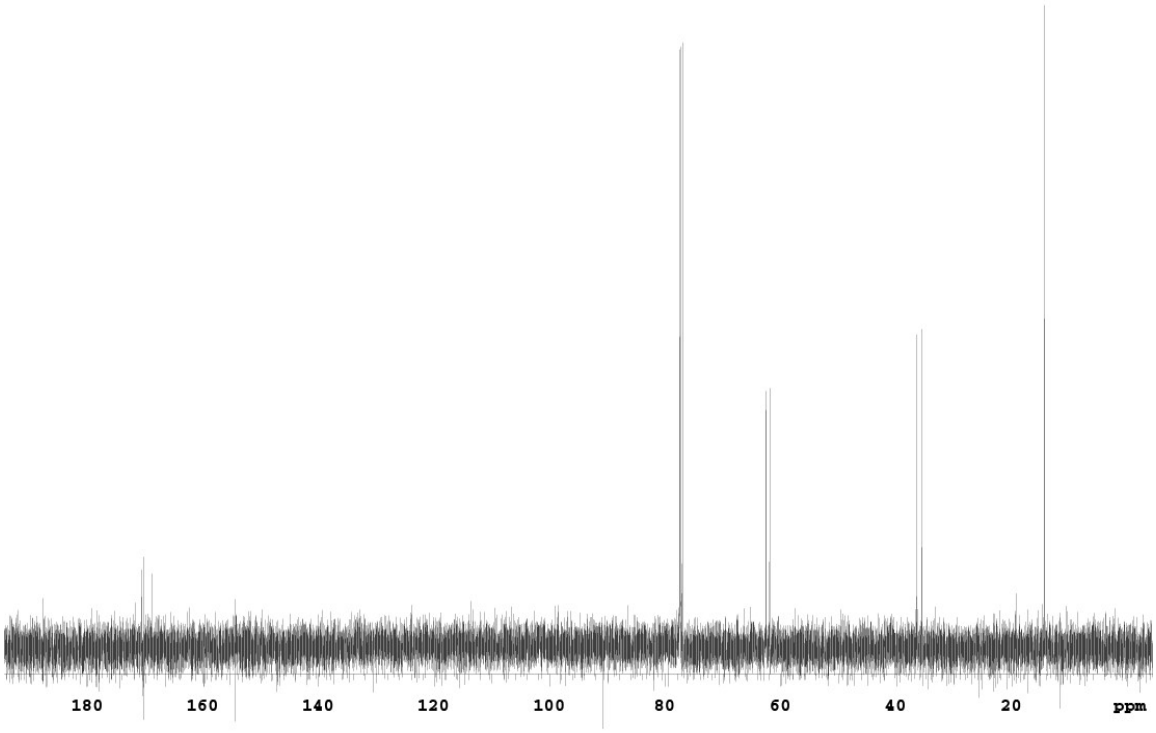
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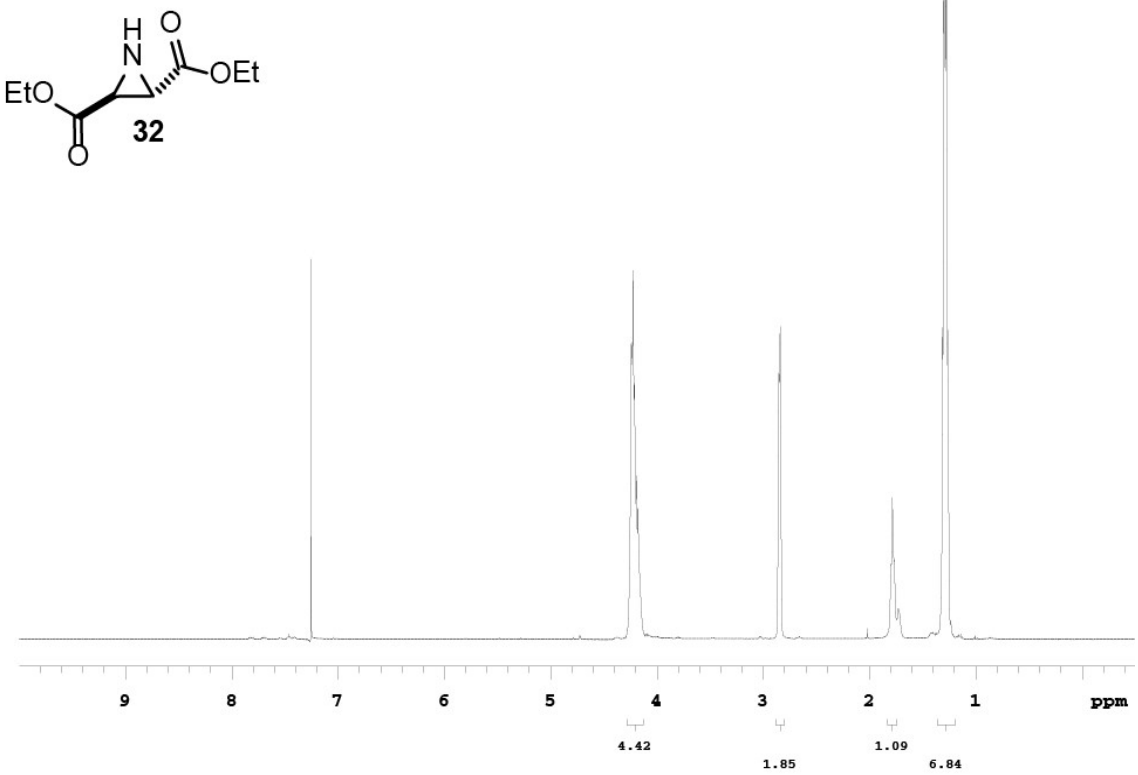
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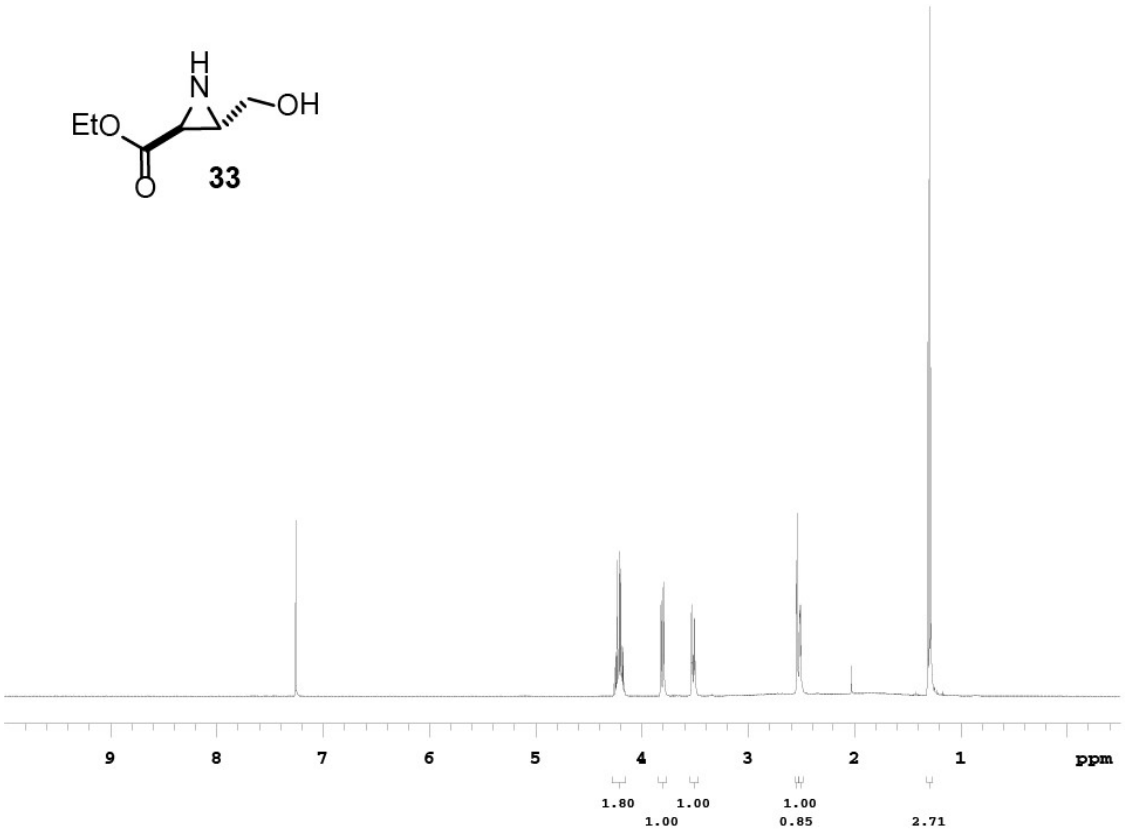
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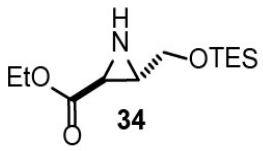
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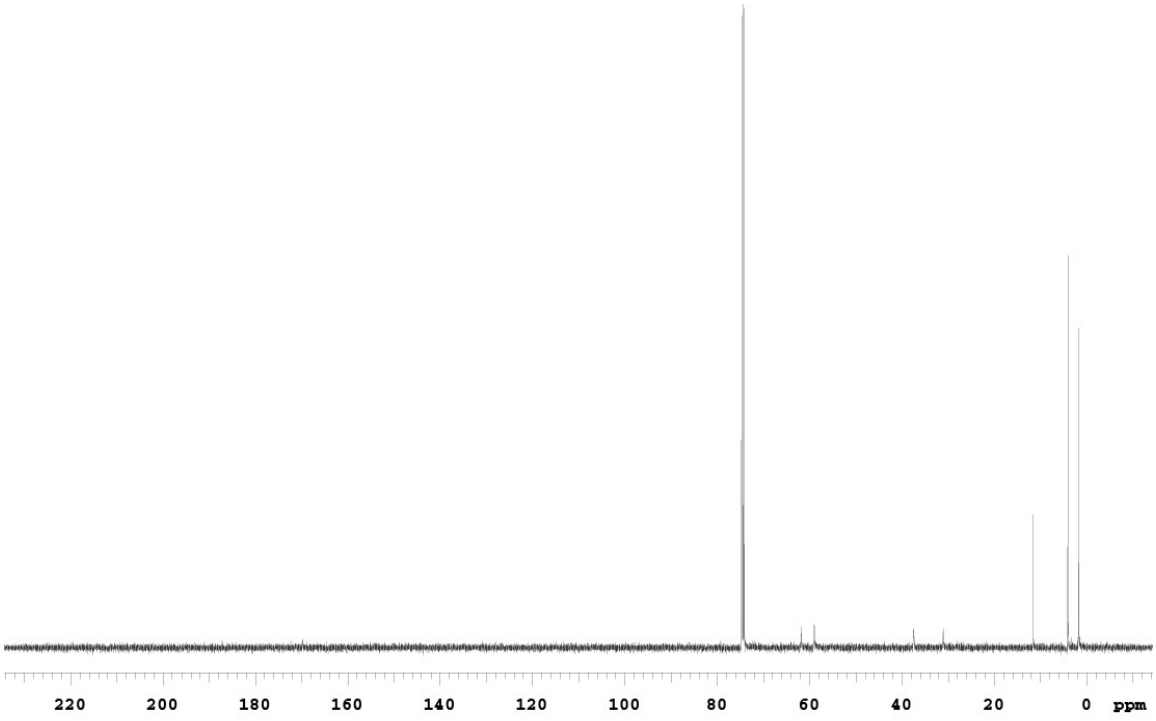
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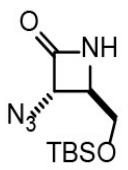
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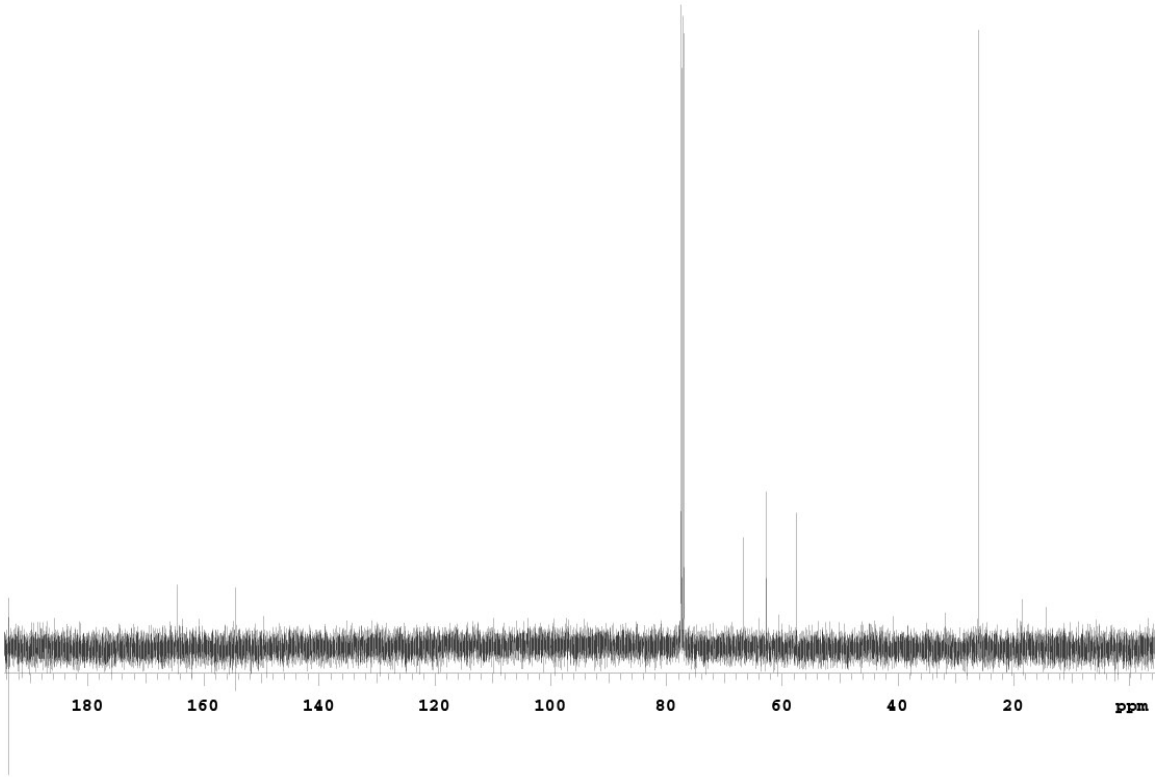
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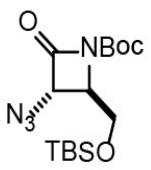
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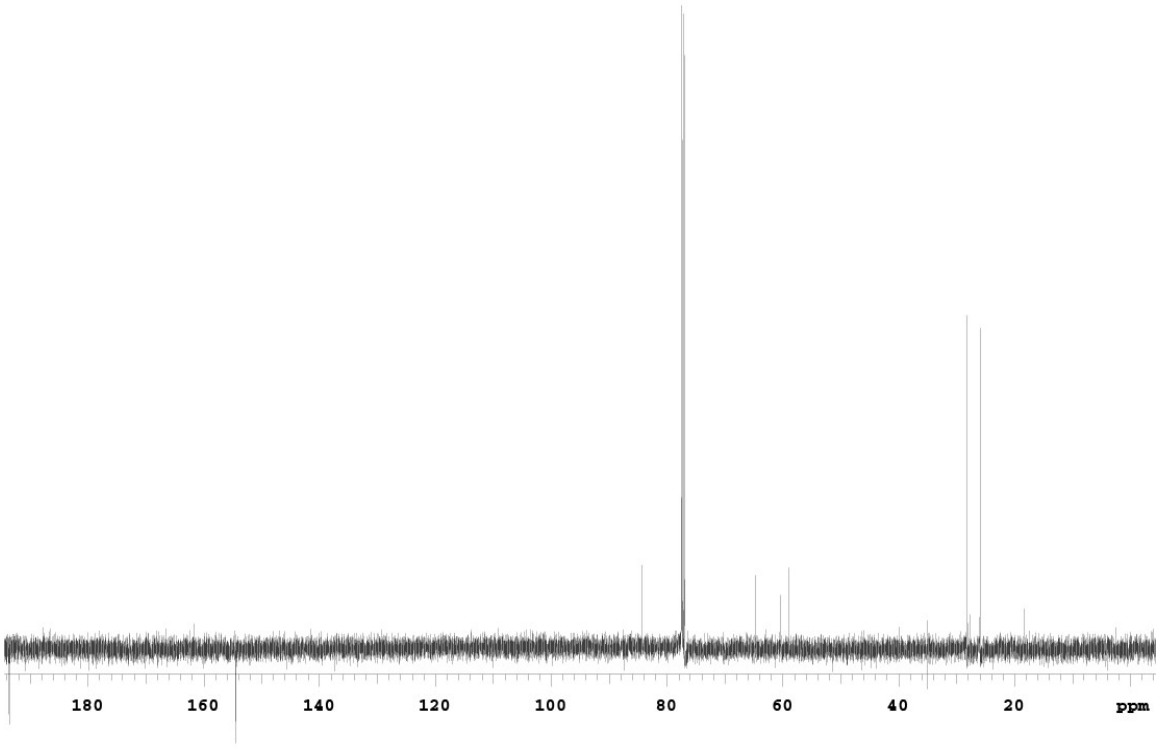
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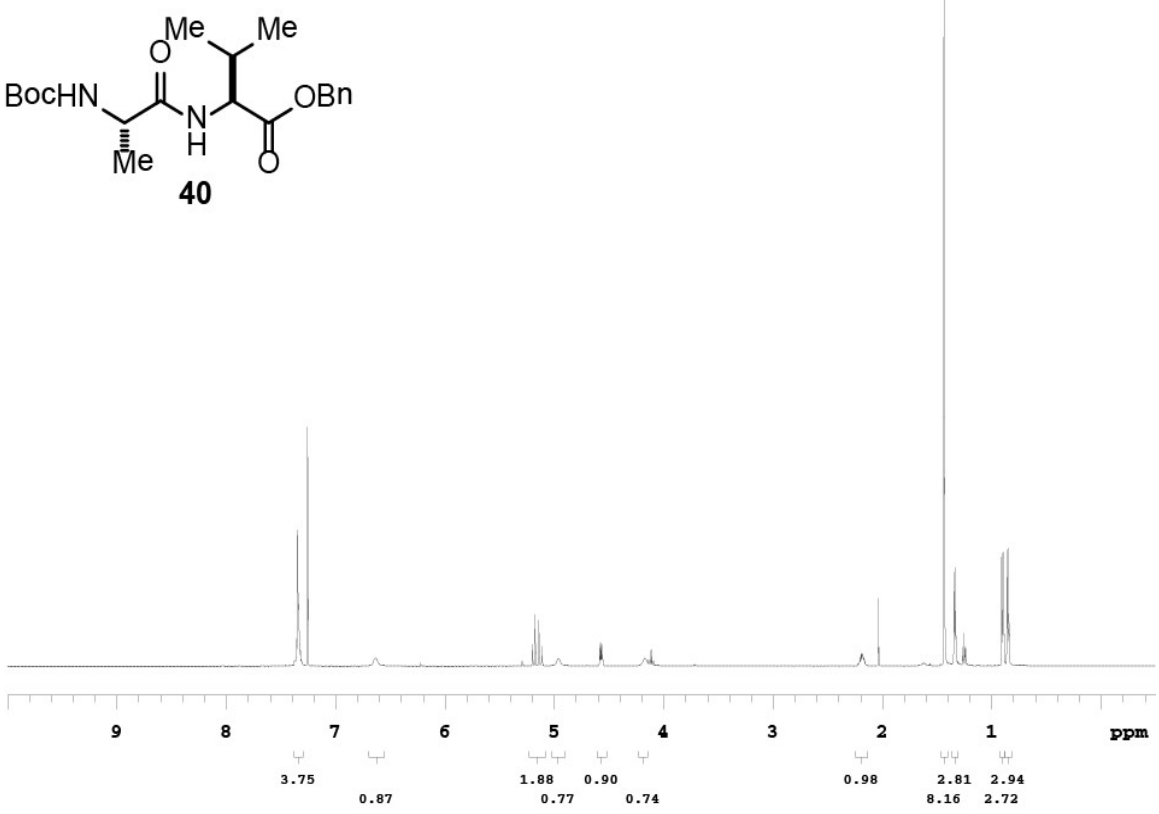
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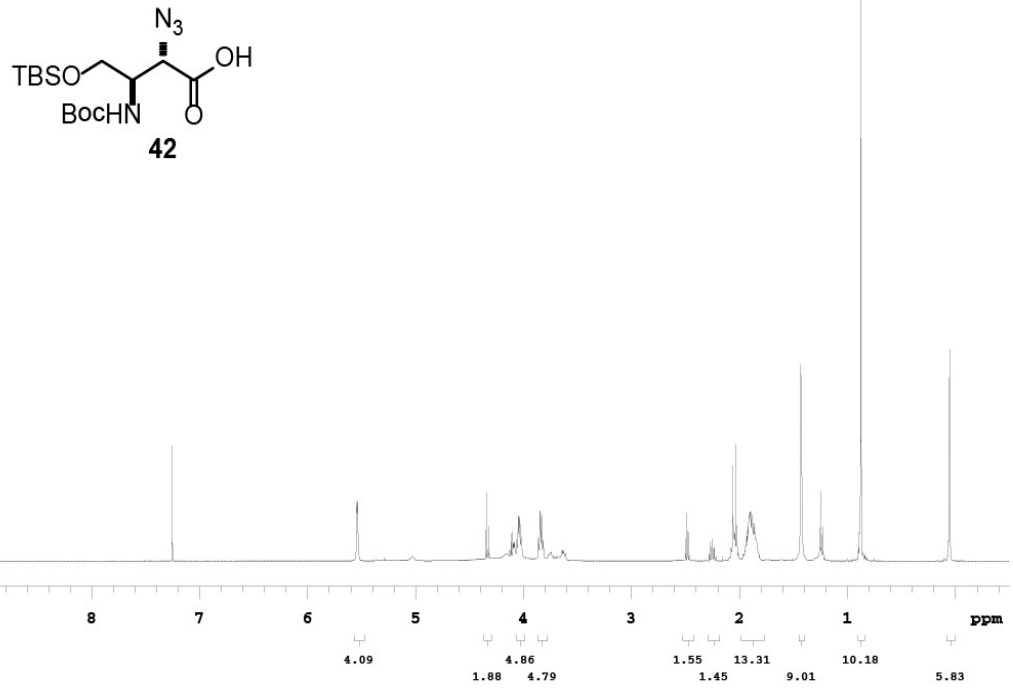
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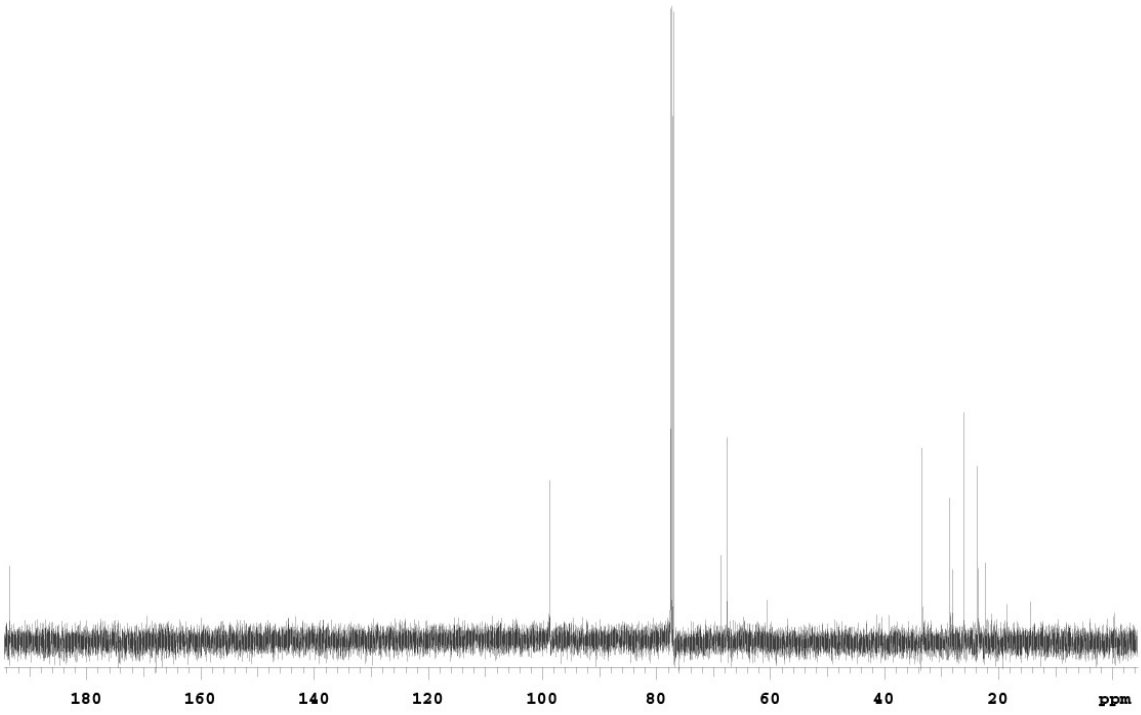
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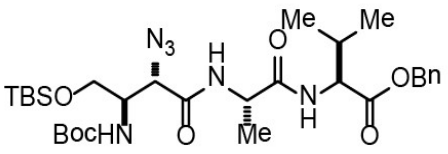
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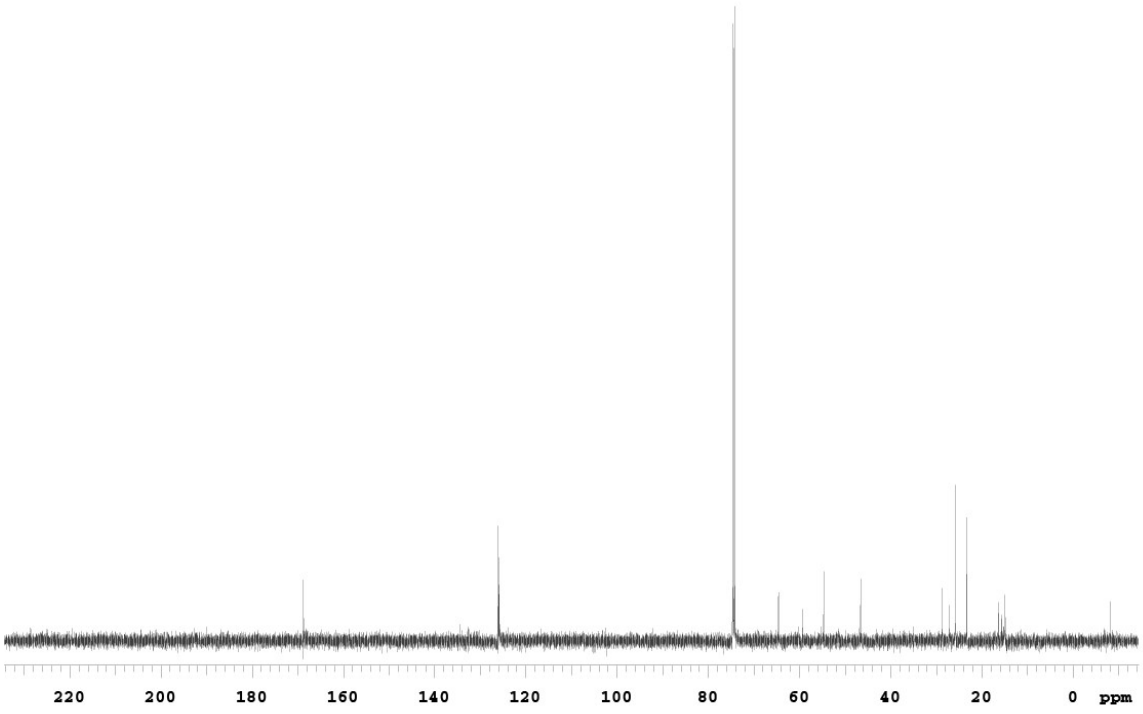
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3.6 References

1. Merino, P.; Lanaspa, A.; Merchan, F. L.; Tejero, T. Stereocontrolled Synthesis of 2.3-Diaminobutanoic Acids. *Tetrahedron Lett.* **1997,** *38,* 1813-1816.

2. Muraka, S.; Studer, A. Zinc Triflate Catalyzed Aerobic Cross-Dehydrogenative Coupling (CDC) of Alkynes with Nitrones: ANew Entry to Isoxazoles. *Org. Lett.* **2011,** *13,* 2746-2749.

3. Ji, D. M.; Xu, M. H. InBr3-catalyzed Direct Alkynylation of Nitrones with Terminal Alkynes: an Efficient Synthesis of N-hydroxy-propargyl Amines. *Tetrahedron Lett.* **2009,** *50,* 2952-2955.

4. Koradin, C.; Gommermann, N.; Polborn, K.; Knochel, P. Synthesis of the Enantiomerically Enriched Propargylamines by Copper-Catalyzed Adition of Alkynes to Enamines. *Chem. Eur. J.* **2003,** *9,* 2797-2811.

5. Jakubec, P.; Cockfield, D. M.; Dixon, D. J. Total synthesis of (-)-Nakadmarin A. *J. Am. Chem. Soc.* **2009,** *131,* 16632-16633.

6. Shibuya, M.; Jinbo, Y.; Kubota, S. Synthesis of 3-Acylamino-4-hydroxymethyl-2-oxo-1-sulfoazetidines and Related Compounds. *Chem. Pharm. Bull.* **1984,** *32,* 1303-1312.

7. Durham, T. B.; Miller, M. J. An Enantioselective Synthesis of Differentially Protected erythro-a,b-Diamino Acids and Its Application to the Synthesis of an Analogue of Rhodopeptin B5. *J. Org. Chem.* **2003,** *68,* 35-42.

8. Aissa, C. Improved Julia-Kocienski Conditions for the Methylenationof Aldehydes and Ketones. *J. Org.Chem.* **2006,** *71,* 360-363.

9. Hili, R.; Yudin, A. K. Readily Available Unprotected Amino Aldehydes. *J. Am. Chem. Soc.* **2006,** *128,* 14772-14773.

10. Nakayama, K.; Kawato, H. C.; Inagaki, H.; Nakajima, R.; Kitamure, A.; Someya, K.; Ohtu, T. Synthesis and Antifungal Activity of Rhodopeptin Analogues 2. Modification of the West Amino Acid Moiety. *Org. Lett.* **2000,** *2,* 977-980.

11. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities *J. Org. Chem.* **1997**; *62*(21); 7512-7515.

12. Hoye, T.R.; Hansen, P.R.; Vyvyan, J.R. A Practical Guide to First-Order Multiplet Analysis in 1H NMR Spectroscopy *J. Org. Chem.* **1994**; *59*(15); 4096-4103.

CHAPTER 4

BIOLOGICAL EVALUATION OF NA22598A$_1$ AND NATURAL PRODUCT FRAGMENTS

4.1 Hypothesis for a Common Mechanism of Action

The initial biological evaluations for both NA22598A$_1$ and guadinomine B were phenotypic assays. Guadinomine B exhibited inhibition of the type III secretion system (T3SS) of bacteria (IC_{50} = 0.0139 μM), which was assessed with a EPEC-induced hemolysis assay.[1] NA22598A$_1$ was shown to inhibit anchorage indepent growth (AIG) of a human colon-cancer cell line, DLD-1 (IC_{50} = 0.32 μM), but did not inhibit anchorage dependent growth at the same concentration.[2] Studies showed that NA22598A$_1$ arrests the cell cycle at the G1 phase and inhibits the synthesis of cyclin D1.[4]

The type III secretion system (T3SS) is a protein system unique to Gram-negative bacteria and is responsible for the virulence of a number of deadly strains including salmonella, meningitis, and pneumonia. T3SS are highly conserved multimolecular proteins that are responsible for delivering effector proteins into a host eukaryotic cell.[5] There are three distinctive parts of the T3SS, a basal body which spans the bacterial membrane periplasm, a needle structure which spans the bacterial membranes and the extracellular space, and a needle tip that links the needle and the host membrane allowing for insertion of the translocon.[6]

Once the T3SS comes into contact with the host cells, translocators are secreted and create a pore in the host cell membrane which provides access to the host cell's cytosol.

Bacteria that rely on the T3SS to deliver effector proteins to the host cell can be rendered almost nonpathogenic by the inhibition of the T3SS.[7] T3SS producing bacteria are linked to more serious infections and a worse prognosis, as demonstrated by El-Sohl and coworkers, who noted that patients with ventilator-associated pneumonia who had the T3SS-producing bacteria had a higher mortality rate (66%) than patients who were infected with non-T3SS producing bacteria (33%).[8] While the T3SS system is critical for virulence, it is not involved in a physiological processes of bacteria, making it an attractive target for inhibition because of the lower potential to develop resistance.[1]

The phenotypes associated with NA22598A_1 and guadinomine B and our conclusion that they are in fact the same compound led to the hypothesis that both molecules had the same mode of action that produced the different phenotypes. In order to identify a potential protein target, the key features of the molecules were evaluated. Due to the highly polar fuctional groups on NA22598A_1, it will likely be dicationic at physiological pH and therefore unlikely to permeate the cell membrane. The carbamoyl guanidine moiety is aligned such that it would form a six membered ring if it were to chelate a metal ion. This led to the hypothesis that NA22598A_1 would act on a surface protein containing a catalytic metal cation. Because metal cations often act as cofactors, we hypothesized that it was more likely to be a competetive inhibitor of its protein target rather than allosteric inhibitor. In addition, the dipeptide tail could mimic the amino acid backbone of a protein, a common motif in enzymatic recognition.

Through an investigation of the literature, matrix metalloproteinases (MMPs) emerged as a viable protein target for NA22598A_1. MMPs are zinc-based endopeptidases that are responsible for extracellular matrix remodeling through the cleavage of collegen and gelatine; the active site is highly conserved for all isoforms, which consists of three histidine residues

coordinated to a zinc ion. In addition, MMPs are also responsible for signalling through the liberation of a number of cytokines, chemokines, and growth factors.[9] From a structural standpoint, the MMP active site is well suited for binding NA22598A_1. An initial comparsion can be made between NA22598A_1 and tissue inhibitor of metalloproteinases (TIMP); TIMP uses an α-aminoacid functionality to chelate the zinc ion and NA22598A_1 has this same functionality in the right-hand portion of the molecule (Figure 4.1).

However, the impetus for the metal chelating hypothesis came from the carbamoyl guanidine, which led to an exploration of the literature for similar molecules that act as MMP inhibitors. Pyrimidine-triones are potent MMP inhibitors that not only chelate the zinc ion but also provide the necessary hydrogen bond to the glutamate residue disrupting the glutamate-H_2O hydrogen bond which is required for catalysis.[10] While these inhibitors are structurally different from NA22598A_1, the carbamoyl guanidine on NA22598A_1 could mimic their binding and not only chelate the metal cation but also provide additional hydrogen bonds to His 201 and Glu 202 (Figure 4.2). Structually MMPs are a viable target protein for NA22598A_1, and this led to an examination of the role of MMPs in the pathology of both T3SS producing bacteria and cancer cells capable of AIG.

The biggest concern with proposing MMP inhibition as a mode of action was the conserved nature of the active site across all 23 isoforms of human MMPs. However, we explored this family of proteases to see if inhibition of a specific MMP could result in the phenotypes seen for both NA22598A_1 and guadinomine B.

4.2 The Correlation between MMPs and Metastasis and T3SS Virulence

While exploring the literature, it became clear that the gelatinases MMP-2 and MMP-9 were implicated in tumor metastasis.[9] MMP-2 and MMP-9 are the only two members of

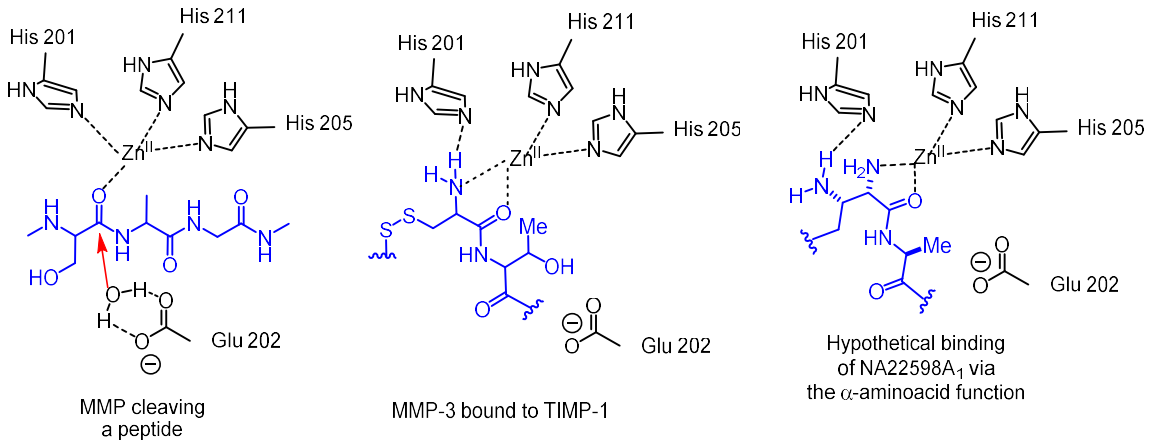


Figure 4.1: The potential binding of NA22598A$_1$ to the catalytic site of MMP 3.

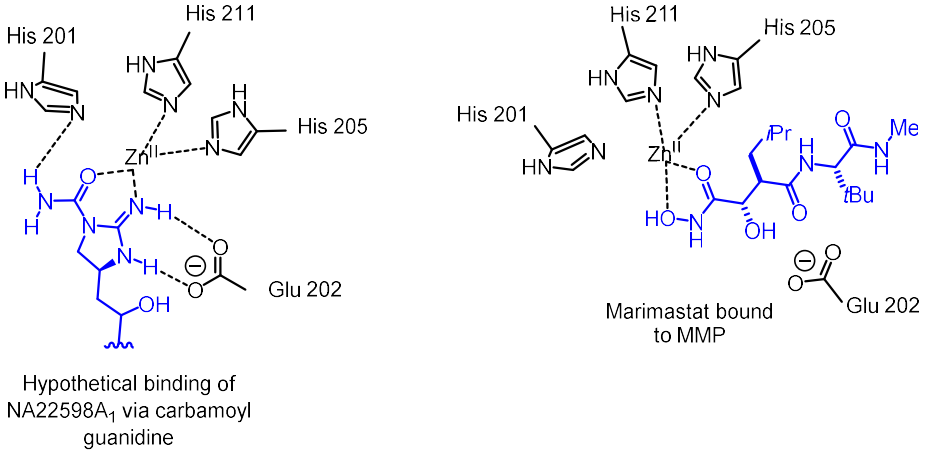


Figure 4.2: Hypothetical binding of NA22598A$_1$ via the carbamoyl guanidine.

the MMP subfamily known as the soluble gelatinases, which proteolytically degrade gelatin (denatured collagen). MMP-2 is ubiquitously expressed as a proenzyme (proMMP-2), which is activated by forming a complex with TIMP-2 generating a substrate for MMP-14, which is responsible for cleaving the prodomain to activate MMP-2. [9] MMP-2 degrades basal membrane, is involved in neovascularization, and directly involved in the migration of cells *via* degradation of the basement membrane. The role of MMP-2 in cell signaling may be much more significant than originally posited. [9] MMP-9 is a gelatinase produced primarily by

neutrophils, although it can be induced by stress in other cells. The greatest difference in selectivity between MMP-2 and MMP-9 is MMP-9's inability to directly proteolyze collagen I.

The overexpression of MMP-2 and MMP-9 have been related to malignance in a vast number of cancers. There is a positive correlation between gelatinase expression and the invasive potential of tumors in a number of cancers, implicating the gelatinases in metastasis. [9] We specifically wanted to assess the role of either MMP-2 or MMP-9 in colon cancer to determine if NA22598A$_1$'s activity could be explained by MMP inhibition. MMP-9 was identified as a noninvasive diagnostic marker for colorectal cancer because elevated levels were detected in the fecal matter of patients with cancer versus a control group. [11]

The relationship between MMPs and Gram-negative infection is not as clear cut as the relationship between MMPs and cancer. However, a number of the effects exerted by T3SS producing bacteria on the host cells can be traced to MMP containing pathways. T3SS bacteria induce an inflammatory response in host cells. The release of inflammatory cytokines can disrupt tight junctions and increase permeability promoting barrier damage and disease progression. [6] TNF-α is a cytokine that is elevated in pathogenic bacterial infection and leads to increased permeability of the epithelial monolayer. The suppression of MMP-2 showed a significant decrease in the secreted levels of TNF-α. [12] IL-1β is another cytokine that is upregulated in an immune response, and it was shown to induce MMP-2 expression; however, the inhibition of MMP-2 essentially eliminates the observed effects of increased IL-1β. [13]

The gelatinases MMP-2 and MMP-9 are indicated in both tumor metastasis and potentially in T3SS mediated infection. To determine if NA22598A$_1$ was a potential gelatinase inhibitor, a manual docking was performed with Biovia and a minimization of the ligand was performed using a Drieding-like forcefield to optimize the geometry in the MMP-9 active site (Figure 4.3). This theoretical model suggested that in addition to the carbamoyl guanidine

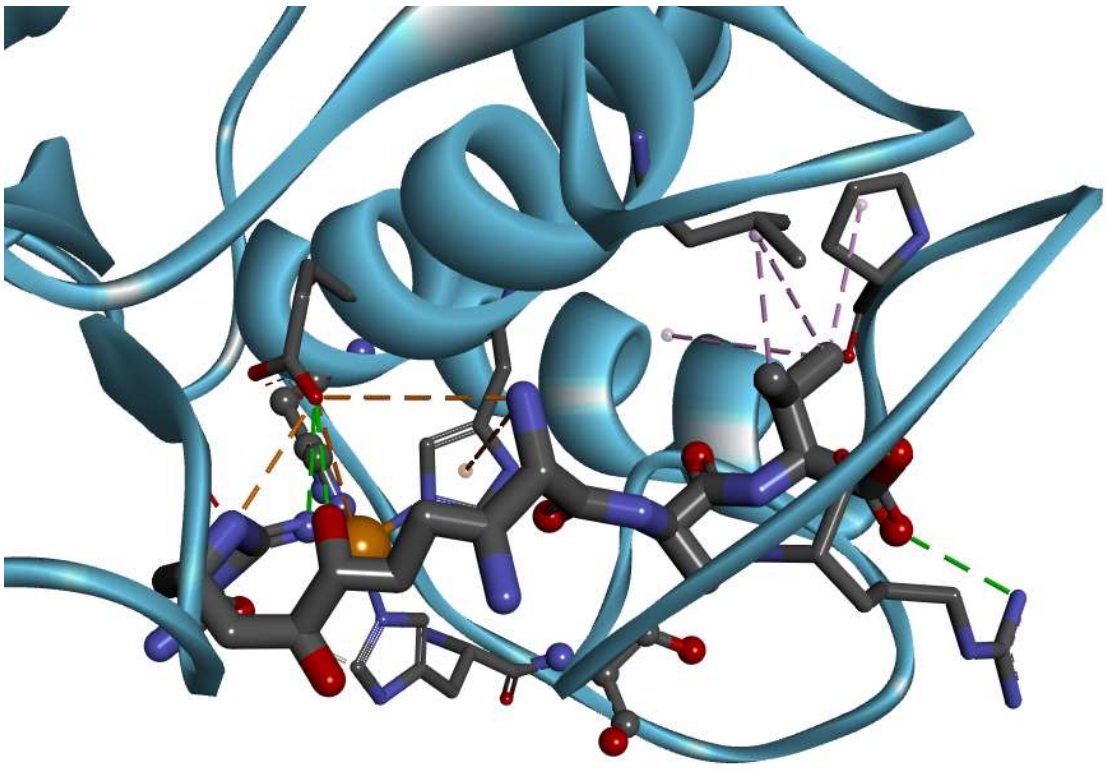


Figure 4.3: Model of NA22598A$_1$ docked to MMP-9.

binding the Zn^{2+} ion, the γ alcohol formed a hydrogen bond with the glutamate, as did the amine. Additionally, the valine residue provided hydrophobic interaction to increase binding affinity.

4.3 Evaluation of NA22598A$_1$ and Molecular Fragments as MMP Inhibitors

A hypothesis was generated that NA22598A$_1$ was a gelatinase inhibitor, and through this mechanism of action produced the anti-metastatic phenotype seen in DLD-1 colon cancer cells, and the T3SS inhibition seen with guadinomine B. To test the validity of this hypothesis, NA22598A$_1$ was tested *in vitro* against MMP-2 and MMP-9 using a colorimetric assay that measured the catalytic activity of the MMPs. In order to determine if there was any selectivity between the gelatinases and the other isoforms of soluble MMPs, NA22598A$_1$ was also tested

against MMP-3, a stromelysin, and MMP-8, a collagenase. In addition to NA22598A$_1$, we also wanted to evaluate the synthesized natural product fragments **2**-**4**; we had proposed Zn^{2+} binding by either the carbamoyl guanidine moiety or the diamine moiety; we hoped by testing the fragments we could determine which moiety contributed to NA22598A$_1$'s activity (Figure 4.4).

Colorimetric MMP assays were used to evaluate the potential inhibitors NA22598A$_1$ and fragments **2**-**4**. A range of concentrations, 100 μM, 10 μM, and 1 μM, for the fragments **2**-**4**, and 100 μg/ml, 10 μg/ml, and 1 μg/ml for the natural product, were incubated with the MMP at 37 °C for 1 hour before addition of the substrate. After the addition of the substrate, the assays were incubated for an additional hour before the inhibition was measured (Table 4.1).

The results of the MMP inhibition assays were suprising. All of the compounds showed inhibitory activity against MMP-2, but it was interesting to note that the 100-fold concentration range did not produce a range of inhibition. MMP-3 appeared to be activated by all of the compounds, but there was no correlation between the concentration of the compounds and the percentage of activation. MMP-8 and MMP-9 were largely unaffected by any of the compounds.

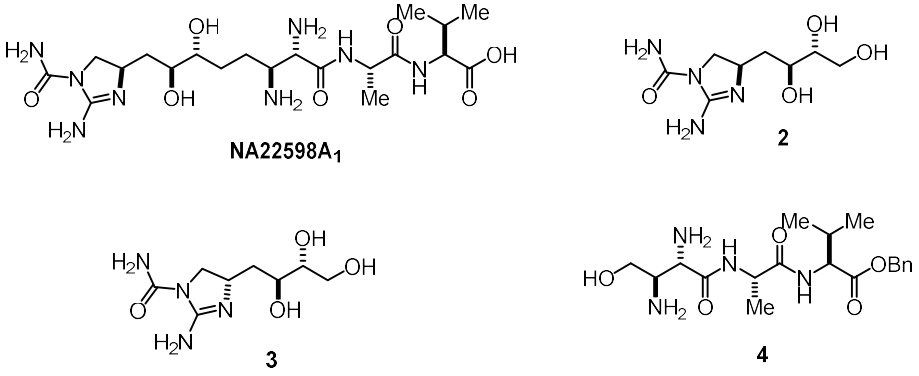


Figure 4.4: The NA22598A$_1$ fragments tested against MMPs.

Table 4.1: Inhibition of MMPs by NA2598A$_1$ and natural product fragments.

Compound	Concentration	% Inhibition			
		MMP-2	**MMP-3**	**MMP-8**	**MMP-9**
NA22598A$_1$	100 μg/ml	30.2 ± 11%	-11.5 ± 1.4%	-3.7 ± 0.6%	-9.7 ± 1.7%
	10 μg/ml	39.0 ± 7.5%	-11.6 ± 0.1%	-2.9 ± 0.7%	-4.9 ± 3.6%
	1 μg/ml	46.6 ± 3.8%	-97.0 ± 7.6%	-1.1 ± 0.5%	0.6 ± 1.9%
2	100 μM	47.8 ± 2.5%	-12.4 ± 3.5%	3.4 ± 0.2%	10.0 ± 0.2%
	10 μM	42.8 ± 1.2%	-14.9 ± 1.7%	0.1 ± 3.2%	9.9 ± 2.4%
	1 μM	7.5 ± 4.9%	ND	-0.9 ± 1.2%	13.8 ± 0.2%
3	100 μM	46.5 ± 5.0%	-7.0 ± 4.9%	4.8 ± 2.7%	4.3 ± 5.1%
	10 μM	53.8 ± 1.6%	-89.5 ± 1.5%	3.3 ± 4.9%	12.7 ± 2.1%
	1 μM	52.8 ± 1.2%	-10.1 ± 1.6%	1.1 ± 4.5%	11.9 ± 3.8%
4	100 μM	56.6 ± 0.3%	8.0 ± 1.0%	2.1 ± 0.5%	-3.8 ± 6.3%
	10 μM	51.9 ± 1.6%	-101.4 ± 7.0%	3.1 ± 0.8%	1.7 ± 4.2%
	1 μM	43.7 ± 0.3%	-17.2 ± 2.3%	2.6 ± 3.6%	2.5 ± 2.5%

The selective inhibition of MMP-2 over MMP-9 and the limited but consistent inhibition of MMP-2 at all of the tested concentrations prompted us to revisit our hypothesis of competitive inhibition. MMP-2 has broader substrate selectivity than MMP-9; therefore, a competitive inhibitor that acts on MMP-2 should act on MMP-9. The basis of our competitive inhibitor hypothesis was that the carbamoyl guanidine could bind zinc. To determine if the carbamoyl guanidine was a Zn^{2+} chelator, **3** was titrated with $ZnCl_2$ and it was discovered that the carbamoyl guanidine was not binding Zn^{2+}.[14] The inability of the carbamoyl guanidine to bind Zn^{2+} could be attributed to the protonation of the guanidine, as it was isolated as the

TFA salt. However, we predicted that at physiological pH, the guanidine would also be protonated and therefore unlikely to be able to chelate Zn^{2+} in the enzyme.

The inability of the carbamoyl guanidine to bind Zn^{2+} as well as the MMP-2 selectivity and non-dose-dependent inhibition led us to consider that NA22598A$_1$ may be a noncompetitive or uncompetitive inhibitor. To determine if the lack of dose response was simply due to the maximum inhibition being achieved at very low concentrations, NA22598A$_1$, **3**, and **4**, were tested against MMP-2 at lower concentrations (Table 4.2). The lower concentrations of NA22598A$_1$, **3**, and **4**, did display a measure of dose response activity against MMP-2.

Seeing approximately 50% inhibition at concentrations between 1μM and 0.1 μM was consistent with the phenotypic data collected for NA22598A$_1$ and guadinomine B which exhibited IC$_{50}$'s in that range.[1 2] One key piece of data that was not clearly elucidated by the MMP assays was which functional groups were responsible for the biological activity. While the diamine fragment **4** showed consistent inhibition down to lower concentrations, the carbamoyl guanidine **3** had similar potency to the natural product as low as 1 μM. The inhibition of MMP-2 by NA22598A$_1$ and the fragmented pharmacophores **3**, and **4** was very interesting and deserves further investigation.

4.4 Further Studies to Investigate the Inhibition of MMP-2 by NA22598A$_1$

The initial MMP inhibition exhibited by NA22598A$_1$ requires a more detailed hypothesis for the inhibitor-enzyme interaction. A Lineweaver-Burk plot was generated to determine the kind of inhibitor NA22598A$_1$ is, but the substrate used in the MMP assay had a very slow cleavage rate and the results were inconclusive. In addition to determining the type of inhibition exerted by NA22598A$_1$, we would like to explore its effect on proMMP2. If

Table 4.2: Inhibition of MMP-2 by NA22598A$_1$ and natural product fragments.

Compound	Concentration	% Inhibition
		MMP-2
NA22598A$_1$	1 μg/ml	40. ± 3.7%
	0.1 μg/ml	36.3 ± 7.0%
	0.01 μg/ml	10.6 ± 1.7%
	0.001 μg/ml	2.6 ± 9.6%
3	1 μM	58.2 ± 5.4%
	0.1 μM	18.4 ± 5.0%
	0.01 μM	2.9 ± 0.5%
4	1 μM	45.6 ± 5.4%
	0.1 μM	50.4 ± 2.1%
	0.01 μM	37.5 ± 1.7%
	0.001 μM	0.5 ± 0.5%

NA22598A$_1$ is in fact an allosteric inhibitor, it may bind to proMMP-2 and inhibit the MMP-14 cleavage to form the active enzyme. The basis for this hypothesis is that proMMP-2 must bind to TIMP-2 before it can be cleaved; this interaction occurs through the C-terminal domains of both proteins excluding an inhibitory interaction between TIMP-1 and MMP-2. [15] We can envision NA22598A$_1$ binding at the C-terminal of proMMP-2 and blocking the binding of TIMP-2, which would keep the enzyme from being activated.

We also want to examine the T3SS inhibition more thoroughly. Ideally, we would be able to find an assay that directly measures T3SS inhibition rather than measuring the effects on eukaryotic cells. This would allow us to determine if guadinomine B was acting on the

bacteria directly.

4.5 Supporting Information

4.5.1 MMP Inhibition Assays

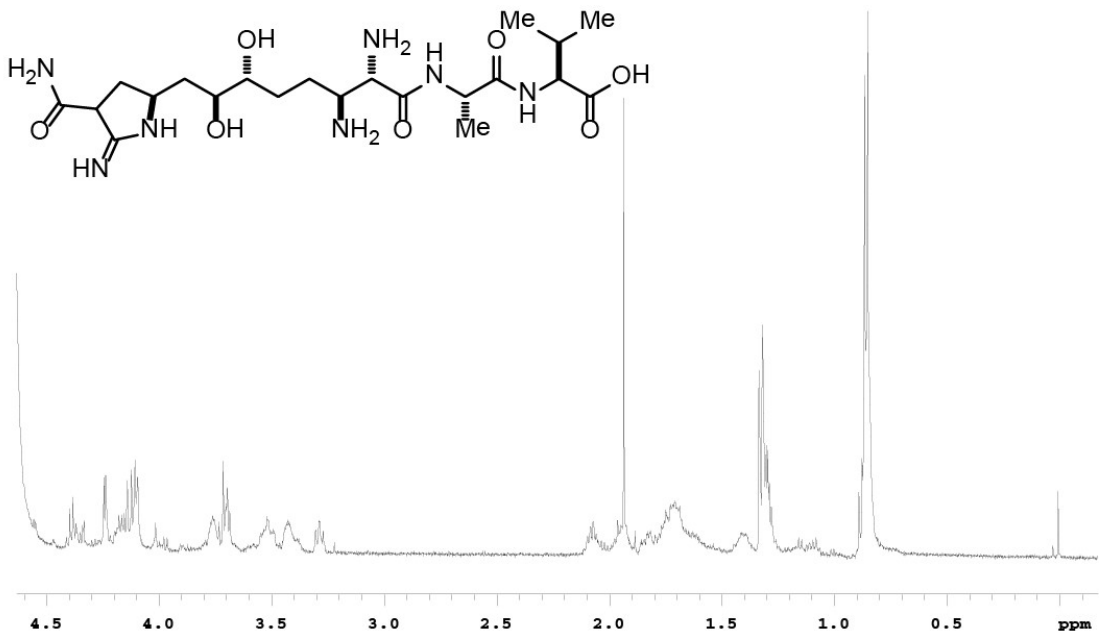
MMP inhibition assays were carried out using Enzo Life Sciences Colorimetric drug discovery kits. Each assay utilized a thiopeptide as a chromogenic substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-CO_2H_5), which upon cleavage produced a sulfhydryl group that reacted with DTNB to form 2-nitro-5-thiobenzoic acid. 2-nitro-5-thiobenzoic acid absorbance was then detected at 412 nm.

The compounds of interest (10 μl) were added to wells of a 96-well plate which each contained 60 μl of buffer. Twenty μl aliquots of enzyme solution were added to the wells and the plate was pre-incubated for 1 hour at 37 °C. Ten μl of the substrate solution was then added to each well and the plate was incubated at 37 °C for 1 hour. At 10 minute intervals, the absorption values were measured at 412 nm.

Each compound of interest was tested in duplicate and the absorption values were averaged. The absorption values measured at 60 minutes showed the maximum inhibition, and are the values reported.

4.5.2 Zn^{2+} Titration Experiment

Compound **3** was divided into six portions (39.8 μM each) in CD_3OD and treated with 0.0, 0.1, 1.0, and 2.0 equivalents of $ZnCl_2$. The ^1H NMR spectra of each solution was measured. The NMR data did not change with the increasing concentrations of $ZnCl_2$ as we would expect if it was a Zn^{2+} chelator.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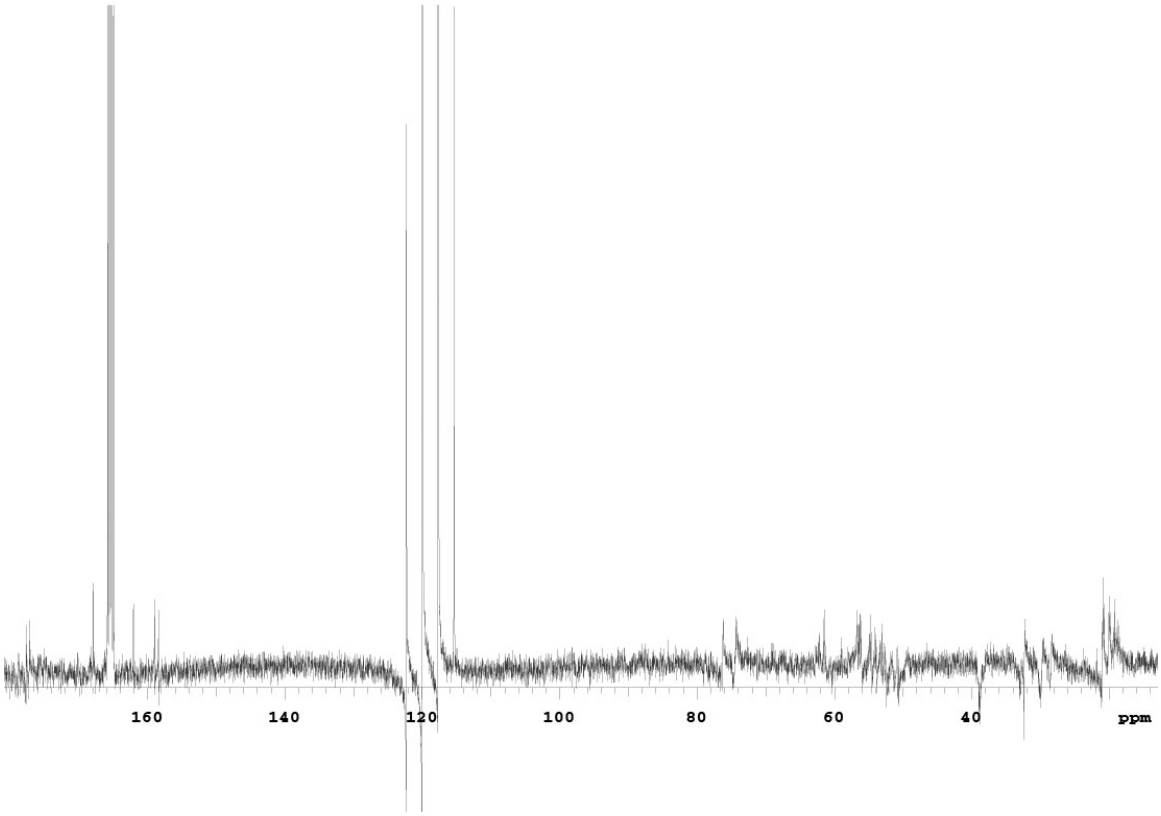
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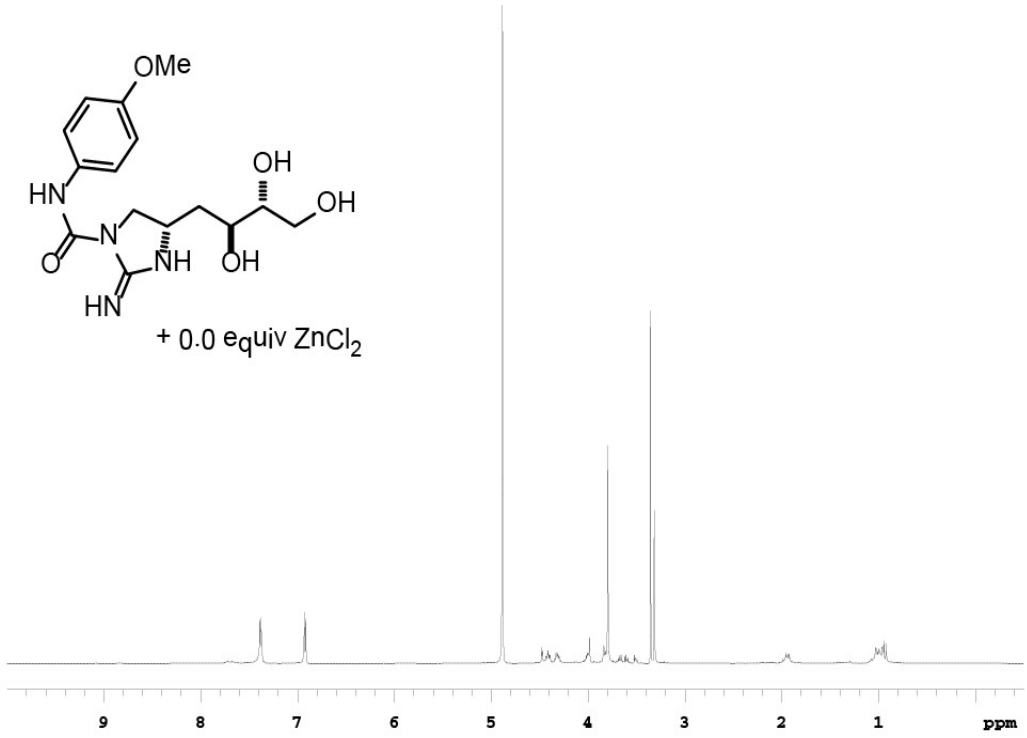
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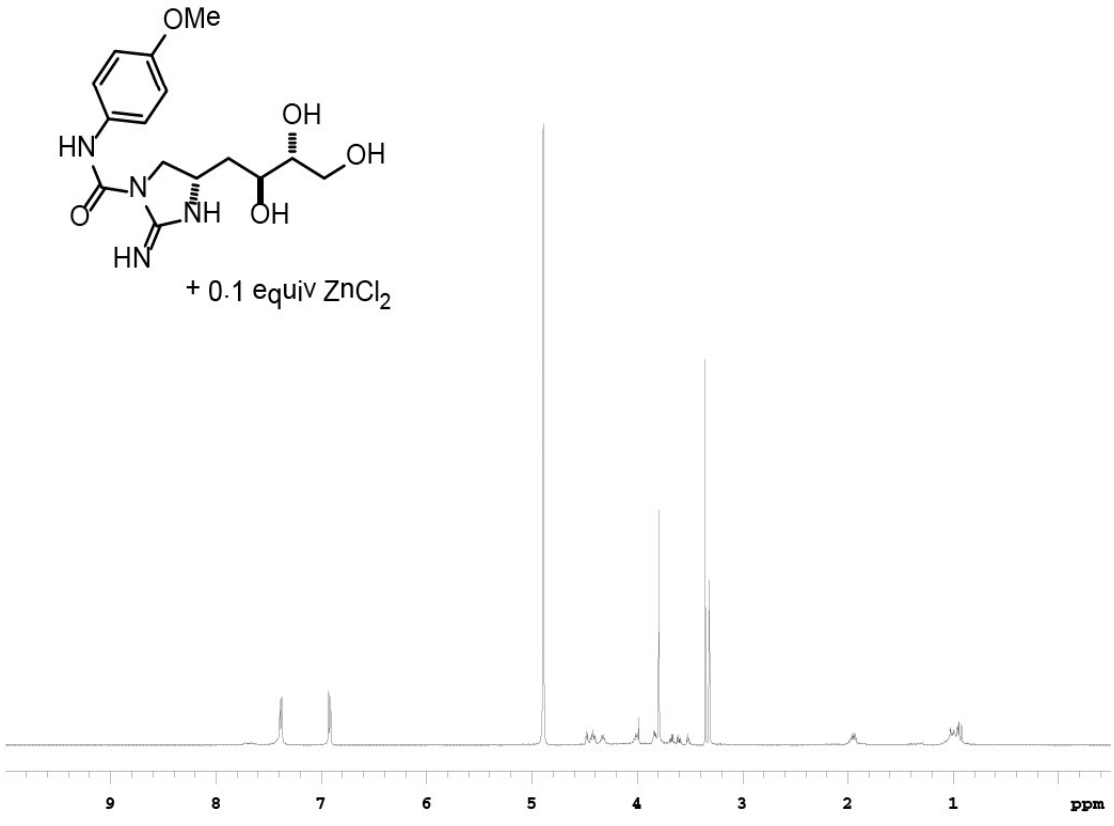
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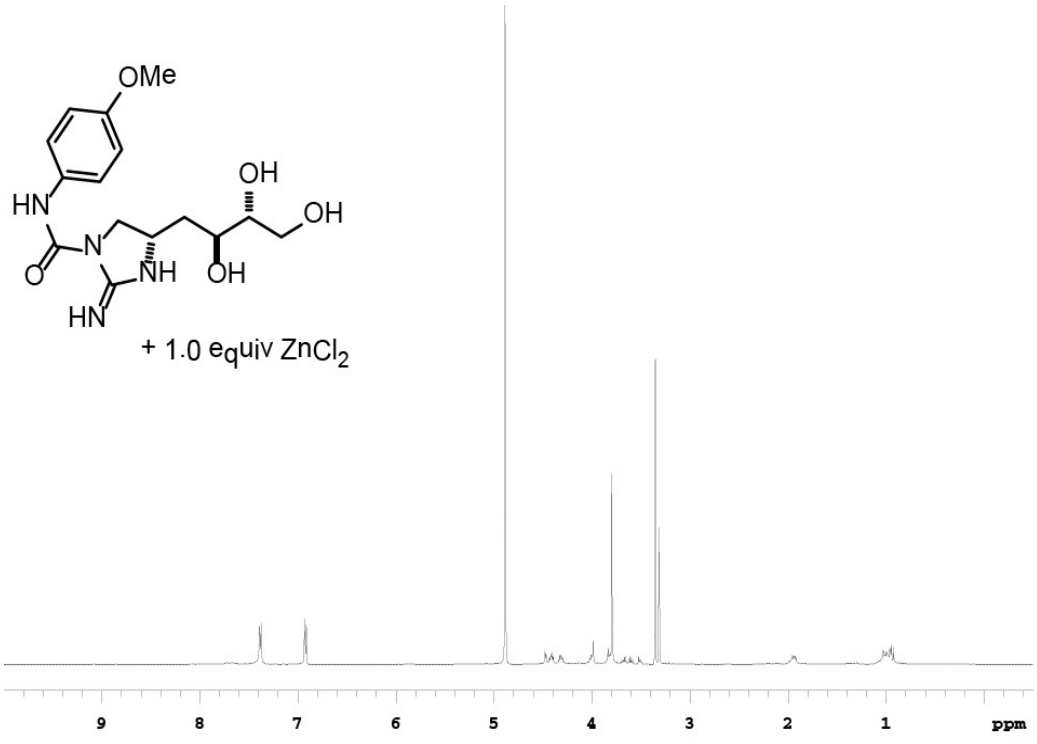
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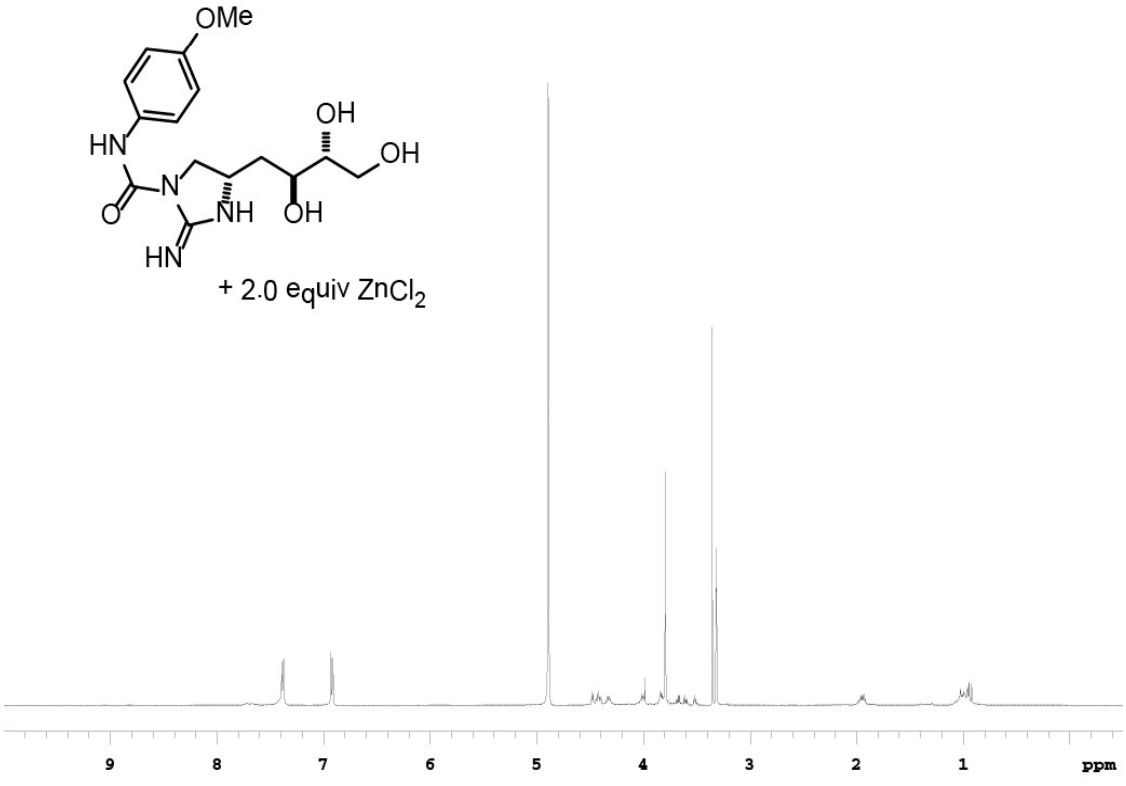
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4.6 References

1. Iwatsuki, M.; Uchida, R.; Yoshijima, H.; Ui, H.; Shiomi, K.; Matsumoto, A.; Takahashi, Y.; Abe, A.; Tomoda, H.; Omura, S. Guadinomines, Type III Secretion System Inhibitors, Produced by Streptomyces sp. K01-0509. *J. Antibiot.* **2008,** *61,* 222-229.

2. Kuwahara, A.; Nishikiori, T.; Shimada, N.; Nakagawa, T.; Fukazawa, H.; Mizuno, S.; Uehara, Y. NA22598A1, a Novel Antitumor Substance Produced by Streptomyces sp. NA22598. *J. Antibiot.* **1997,** *50,* 712-713.

3. Hirose, T.; Sunazuka, T.; Tsuchiya, A.; Tanaka, T.; Kojima, Y.; Mori, R.; Iwatsuki, M.; Omura, S. Total Synthesis and Determination of the Absolute Configuration of Guadinomines B and C2. *Chem. Euro. J.* **2008,** *14,* 8220-8238.

4. Kawada, M.; Kuwahara, A.; Nishikiori, T.; Mizuno, S.; Uehara, Y. NA22598, a Novel Antitumor Compound, Reduces Cyclin D1 Levels, Arrests Cell Cycle at G1 Phase, and Inhibits Anchorage Independent Growth of Human Tumor Cells. *Exp. Cell Res.* **1999,** *249,* 240-247.

5. Luo, W.; Donnenbrg, M. S. Interactions and Predicted Host Membrane Topology of the Enteropathogenic Escherichia coli Translocator Protein EspB. *J. Bacteriol.* **2011,** *193,* 2972-2980.

6. Ashida, H.; Ogawa, M.; Kim, M.; Mimuro, H.; Sasakawa, C. Bacteria and Host Interactions in the Gut Epithelial Barrier. *Nat. Chem. Biol.* **2012,** *8,* 36-45.

7. Galan, J. E.; Wolf-Watz, H. Proein Delivery into Eukaryotic Cells by Type III Secretion Machines. *Nature* **2006,** *444,* 567-573.

8. El-Sohl, A. A.; Amsterdam, D.; Alhajhusain, A.; Akinnusi, M. E.; Saliba, R. G.; Lynch, S. V.; Wiener-Kronish, J. P. Matrix Metalloproteinases in Bronchoalveolar Lavage Fluid of Patients with Type III Pseudomonas aeruginosa pneumonia. *Journal of Infection* **2009,** *59,* 49-55.

9. Klien, T.; Bischoff, R. Physiologyand Pathophysiology of Matix Metalloproteases. *Amino Acids* **2011,** *41,* 271-290.

10. Dunten, P.; Kammlott, U.; Crowther, R.; Levin, W.; Foley, L. H.; Wang, P.; R., P. X-Ray Structure of a Novel Matrix Metalloprotease Inhibitor Complexed to Stromelysin. *Protein Sci.* **2001,** *10,* 923-926.

11. Annahazi, A.; Abraham, S.; Farkas, K.; Rosztoczy, A.; Infczefi, O.; Foldesi, I.; Szucs, M.; Rutka, M.; Theodorou, V.; Eutamene, H.; Bueno, L.; Lazar, G.; Wittman, T.; Molnar, T.; Roka, R. A Pilot Study of Feacal MMP-9: A New Noninvasive Diagnostic Marker of Colorectal Cancer. *Br. J. Cancer* **2016,** *114,* 787-792.

12. Kesanakurti, D.; Chetty, C.; Bhoopathi, P.; Lakka, S.; Gorantla, B.; Tsung, A. J.; Rao, J. S. Suppressionof MMP-2 Attenuates TNFa Induced NF-kB Activation and Leads to JNK MEdiated Cell Death in Glioma. *PLoS ONE* **2011,** *6,* e19341.

13. Choi, Y. A.; Lee, D. J.; Lim, H. K.; Jeong, J. H.; Sonn, J. K.; Kang, S. S.; Baek, S. H. Interlukin-1b Stimulates Matrix Metalloproteinase-2 Expression via a Prostaglandin E2-dependent Mechanism in Human Chondrocytes. *Exp. Mol. Med.* **2004,** *36,* 226-232.

14. Fujita, M.; Nakao, Y.; Matsunaga, S.; Seiki, M.; Itoh, Y.; Yamashita, J.; van Soset, R. W. M.; Fusetani, N. Ageladine A: An Antiangiogenic Matrixmetalloproteinase Inhibitor from the Marine Sponge Agelas nakamurai. *J. Am. Chem. Soc.* **2003,** *125,* 15700-15701.

15. Morgunova, E.; Tuuttila, A.; U., B.; Tryggvason. Structrual Insight into the Complex Formation of Latent Matrix Metalloproteinase 2 with Tissue Inhibitor of Metalloproteinase 2. *PNAS* **2002,** *99,* 7414-7419.

CHAPTER 5

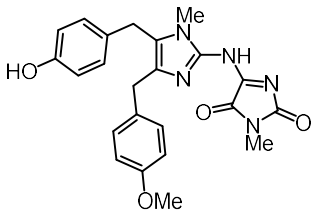
THE SYNTHESIS OF ZNA AND ANALOGS

5.1 Natural Product Inspiration

Marine sponges *Leucetta* and *Clathrina sp.* are a rich source of imidazole alkaloids, many of which have shown an interesting array of biological activity.[1] The compounds that were of most interest to us were the naamidines, particularly naamidine A (Figure 5.1). Naamidine A was tested in an EGF mitogenic assay and it was found to preferentially inhibit EGF dependent cell proliferation (IC_{50} = 11.3μM, NIH3T3) verses insulin dependent cell proliferation (IC_{50} = 242μM, NIH3T3).[2] This selectivity made naamidine A attractive for further investigation because EGF is frequently overexpressed in cancer cells, specifically its link to breast cancer.[2,3]

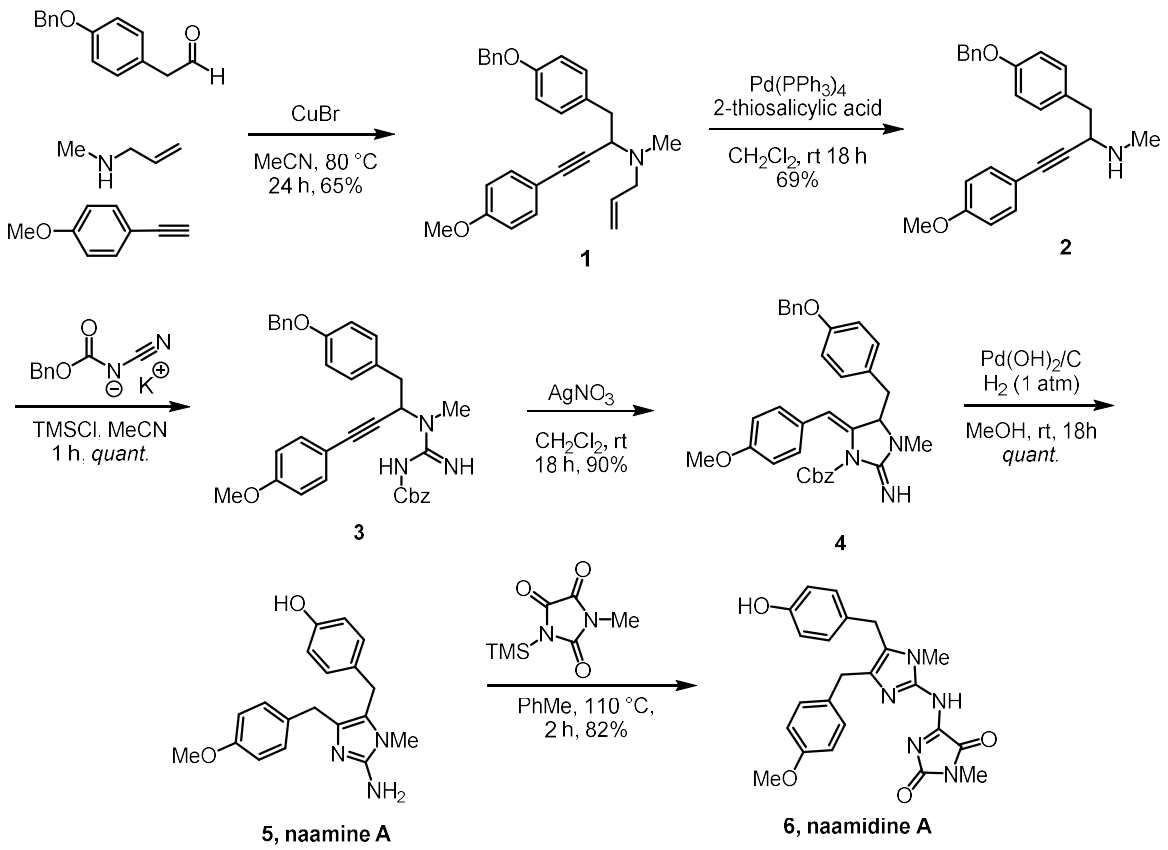
In addition to the interesting biology, naamidine A was appealing to our group due to its 2-aminoimidazole core, which could be constructed from the hydroamination of propargyl guanidines previously explored in our lab.[4] Dr. Joseph Gibbons, a former coworker, undertook the synthesis of naamidine A.[5] First, a Cu(I)-mediated 3CC of an aldehyde, a secondary amine, and an alkyne provided the protected amine **1** (Scheme 5.1).

This was a robust reaction that tolerated a range of substitution on all three components providing easy access to diversification. Deallylation with thiosalicylic acid and catalytic $Pd(PPh_3)_4$ gave the secondary propargyl amine **2** in good yield. The guanidine was installed with a TMSCl activated Cbz-cyanamide potassium salt which proceeded in



Naamidine A

Figure 5.1: Naamidine A, a biologically active compound isolated from *Leucetta sp.*



Scheme 5.1: Synthesis of naamidine A

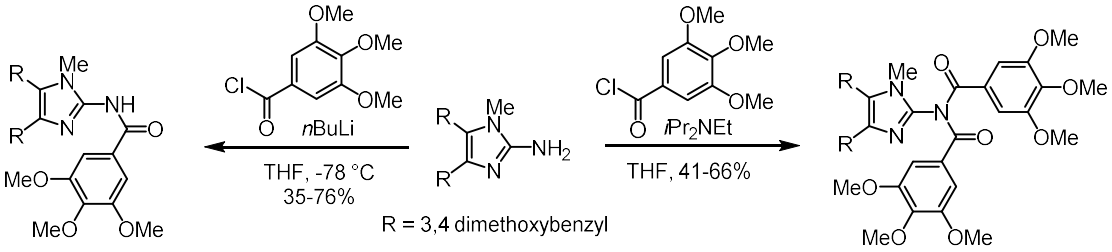
quantitative yield providing the monoacylguanidine **3**.[6] An $AgNO_3$ mediated cyclization yielded the 5-*exo*-trig cyclized guanidine **4** as a single regioisomer. A $Pd(OH)_2/C$ catalyzed hydrogentation performed multiple functions, as it cleaved the Cbz group, isomerized the ene-guanidine to the 2-aminoimidazole, and cleaved the benzyl ether to provide naamine a, **5**. Ohta's method was then used to install the hydantoin, and provided naamidine A, **6**, in good yield (Scheme 5.1).[7] This concise and high yielding synthetic route to access naamidine A was ideal for the synthesis of analogs.

5.2 Synthesis of Initial Naamidine A Analogs

When thinking about naamidine A analog design, altering the benzyl substituents could be easily done by changing the 3CC components, but altering the hydantoin presented a more significant challenge. However, an N^2-acyl-2-aminoimidazole would produce a bioisosteric amide that we hoped would mimic the heterocycle. The challenge in designing such a bioisostere was selectively generating the N^2-monoacyl-2-aminoimidazole. Jiang and coworkers encountered difficulty in their synthesis of naamidine A analogs with their attempt to acylate free 2-aminoimidazoles, resulting in a mixture of monoacylated and diacylated N^2 products (Scheme 5.2).[8]

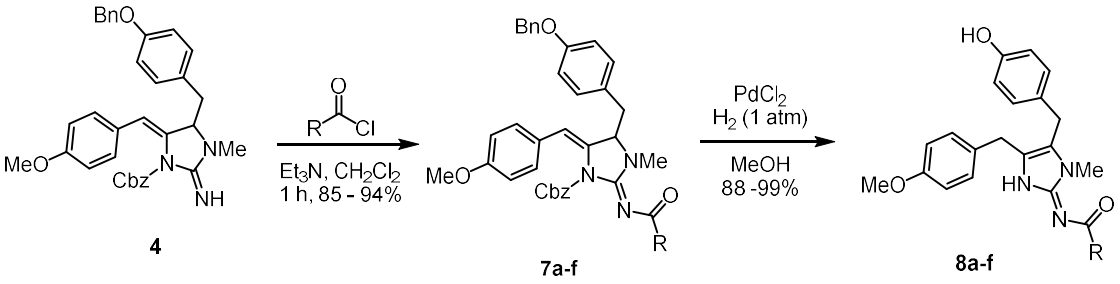
The intermediate **4** presented an interesting substrate for selective N^2-acylation because N^3 is protected, and the imino tautomer of the exocyclic N^2 should only undergo monoacylation. To test this hypothesis, ene-guanidine **4** was treated with a range of aryl and alkanyl chlorides; to our delight, this reaction produced a range of N^2-monoacylguanidines **7a-e** (Table 5.1). An alteration to the hydrogenation conditions provided the deprotected and isomerized 2-aminoimidazoles **8a-f**.

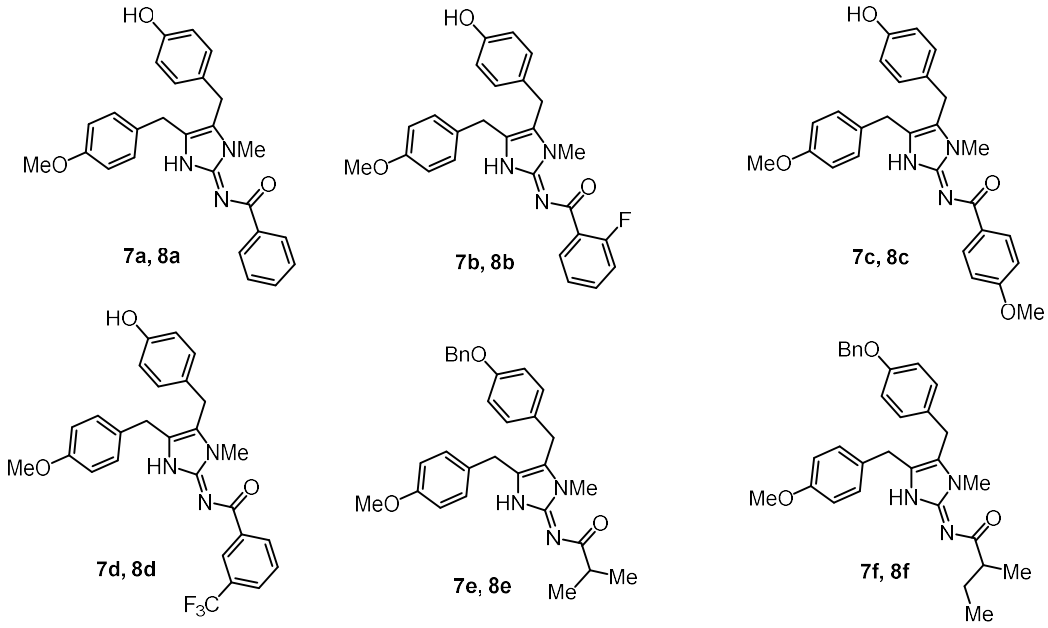
The successful formation of the N^2-acyl-2-aminoimidazoles from ene-guanidine **4**



Scheme 5.2: Jiang's synthesis of naamidine A derivatives

Table 5.1: Synthesis of naamidine A analogs

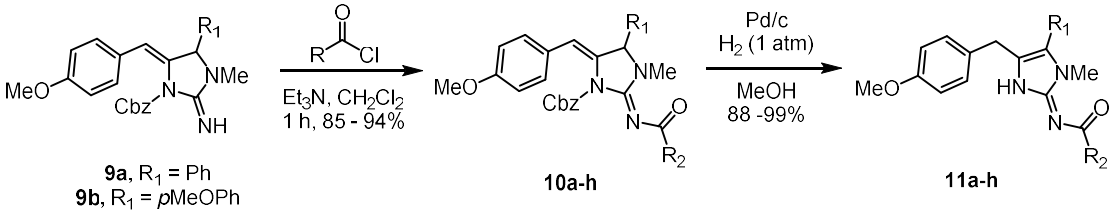


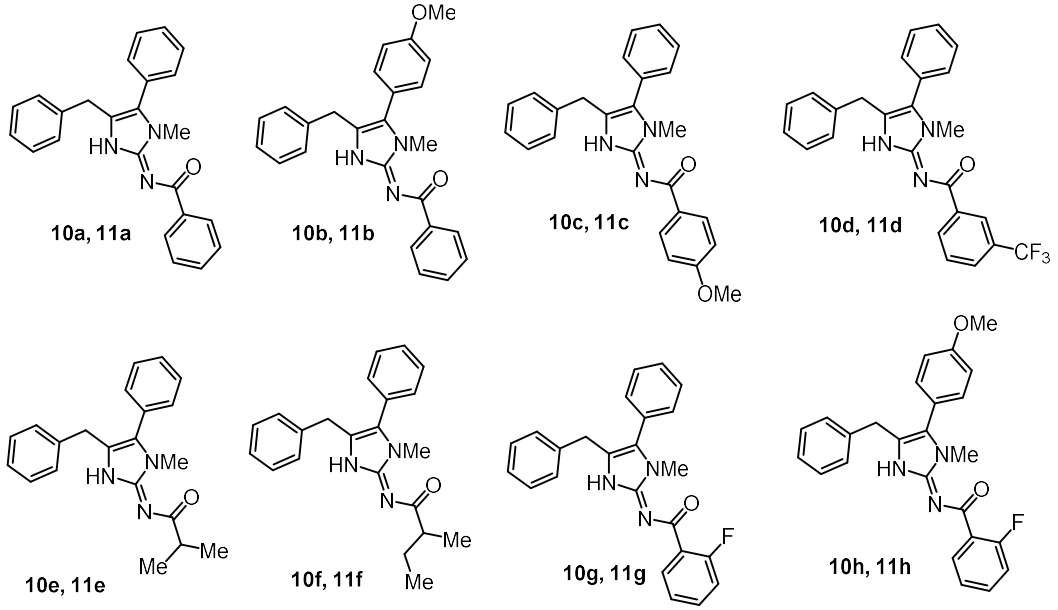


prompted an expansion of the analog library. The C^5-phenyl and C^4-benzyl analogs **9a** and **9b** were constructed with the same methodology that produced the ene-guanidine **4**. The selective N^2-monoacylations proceeded in excellent yield to afford **10a-i**. The 2- aminoimidazoles **11a-i** were then generated from a Pd/C hydrogenation at 60 psi H_2 (Table 5.2).

Naamidine A and all of the analogs synthesized were evaluated in a university-wide screen to identify new drug leads for the treatment of breast cancer.[9] The screen was run against primary metastatic tumor cells isolated from patient-derived malignant pleural effusions at the Huntsman Cancer Institute. These cells created a model to assess molecules against chemoresistance developed from current therapies. Additionally, compounds were

Table 5.2: Synthesis of N^2-Acyl-2-aminoimidazoles



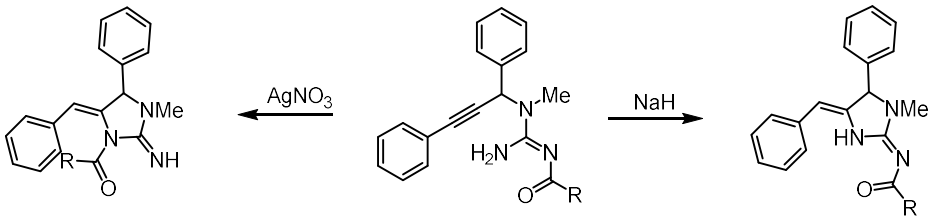


screened against normal epithelial cells from reduction mammoplasty to determine the therapeutic window.[9] Naamidine A was not identified as a viable lead due to its cytotoxicity; however, analog **11h**, known as zinaamidole (ZNA), displayed remarkable selectivity. ZNA showed cytotoxicity against all tested breast cancer cell lines regardless of estrogen receptor subtype (MCF-7, T47D, MDA-MB-231, and PE10005339), but showed almost no effect on untransformed tissues (MCF-10A, hTERT-HMEC). What set ZNA apart was not its EC_{50} against MCF-7 cells, which was comparable to naamidine A, 3.3 μM, but rather its lack of a measurable EC_{50} against MCF-10A cells, as opposed to naamidine A which had an EC_{50} = 8.1μM against MCF-10A cells. The significant change in selectivity between ZNA and naamidine A suggested that the phenotype displayed by ZNA was the product of a different mechanism of action than that of naamidine A. In order to explore the mechanism of action and the structure activity relationship of ZNA, the synthesis of an analog library was undertaken.

5.3 Synthesis of ZNA Analogs

The ideal synthetic route to ZNA and its analogs would be one in which the N^2-acyl-2-aminoimidizole could be formed directly from the propargyl guanidine. We hypothesized that the single regioisomer seen in the $AgNO_3$ cyclization was due to the preferred confirmation of the imino tautomer being in conjugation with the acyl group. If our hypothesis was correct, then treating the propargyl guanidine with base would deprotonate the N^3 nitrogen and promote cyclization through the anion, resulting in the N^2-acyl ene-guanidine (Scheme 5.3).[10]

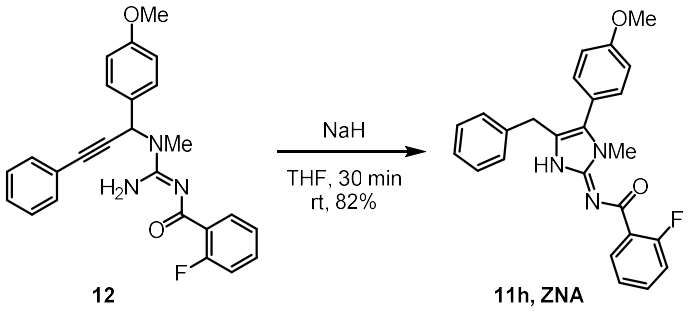
To test this hypothesis, propargylguanidine **12** was treated with one equivalent of NaH in THF and we were delighted to see that not only did the cyclization occur exclusively through



Scheme 5.3: Proposed mechanism for a base catalyzed of the monoacylated guanidine

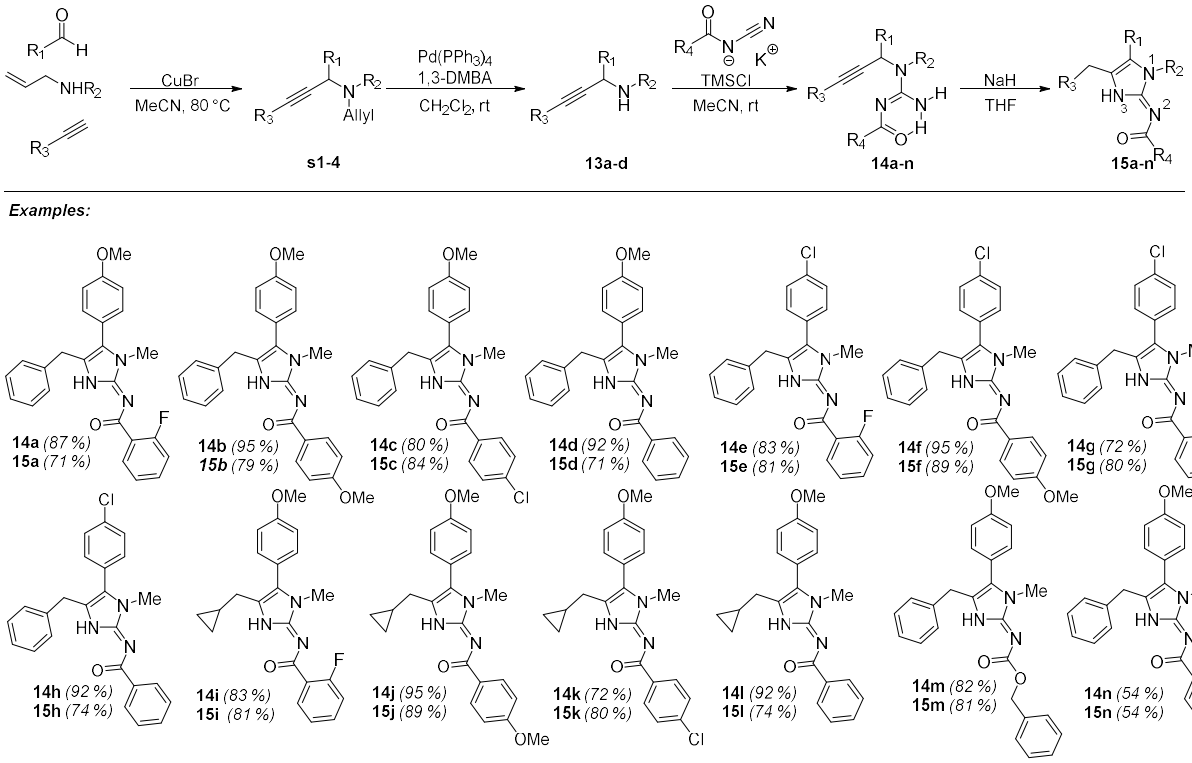
N^3 nitrogen, but the isomerization was induced *in situ* to provide the N^2-acyl-2-aminoimidazole **11h** in a single step from the propargylguanidine (Scheme 5.4). To ensure that this compound was the same as ZNA, the NMR spectra was compared and they appeared identical; a crystal structure of **11h** definitively confirmed that the base catalyzed cyclization product was identical to ZNA. This synthetic route allowed access to the N^2-acyl-2-aminoimidazoles in four steps without protecting groups.

To explore the versatility of this transformation, a series of mono-*N*-acylpropargylguanidines were prepared from potassium salts of *N*-cyanobenzamides activated by TMSCl and respective secondary propargylic amines (Table 5.3). All of the performed reactions proceeded in good yield at room temperature, usually reaching completion within 20



Scheme 5.4: NaH mediated cyclization of monoacylguanidine **12**

Table 5.3: Synthesis of mono-*N*-acylpropargylguanidines



minutes. As a trend, the more electron-deficient *N*-cyanobenzamides performed better in the guanidinylation step, presumably because of the increased reactivity of their *N*-silylcarbodiimide intermediates.

An investigation into the scope of the NaH mediated hydroamination of mono-*N*-acylpropargylguandidines proved that the reaction could tolerate electron-donating and withdrawing aryl substituents at the R^1 and R^4 positions. Introduction of a cyclopropyl group at the R^3 position gave comparable yields to aryl substituents, **15i-15l**. Carbamoyl guanidines also cyclized selectively under these conditions as illustrated by **15m**. The regiochemistry was further supported by the X-ray structure of **15m** (Figure 5.2). Interestingly, this N^2-carbamoyl derivative exists as the endocyclic N^3-imino tautomer in contrast to structures of the amides

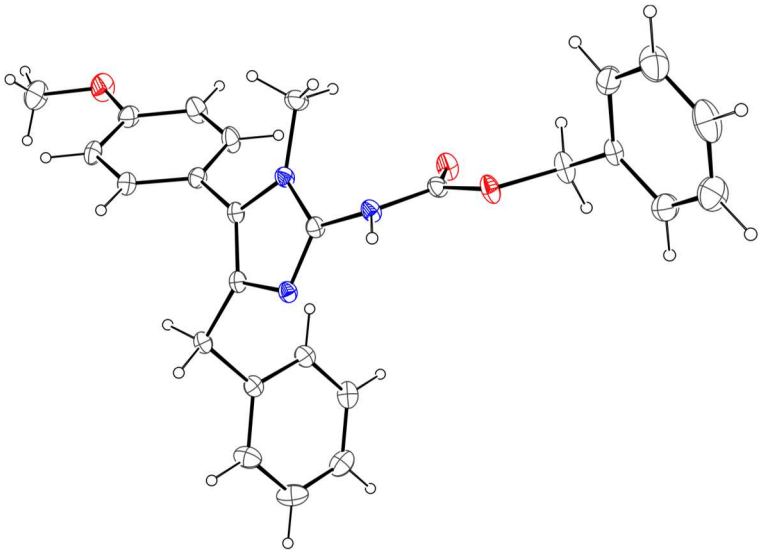


Figure 5.2: Crystal structure of **15m** [10]

at N^2, previously obtained in our laboratory, which exist predominantly as the exocyclic N^2-imino tautomers.

5.4 The Exploration of the Structure-Activity Relationship

The EC_{50} values for various analogs were determined by a firefly luciferase-mediated ATPlite assay, which was completed by Dr. Rachel Vaden. This assay was run with untransformed epithelial MCF-10A cells, and breast cancer MCF-7 cells. The limited range of EC_{50} values in the MCF-7 cell line suggested that the peripheral aryl rings did not play a critical role in selectivity, though **15e**, the 4-chlorophenyl, showed the best results (Figure 5.3). The cluster of EC_{50} values, all within 20 μM, prompted Justin Salvant, a fellow graduate student, to prepare a more diverse library of analogs.

In addition to optimizing the structure-activity relationship, our group, in collaboration with the Welm group, set out to determine the mechanism of ZNA's arrest of cellular proliferation. [11] It was determined that ZNA did not trigger the cleavage of pro-caspase

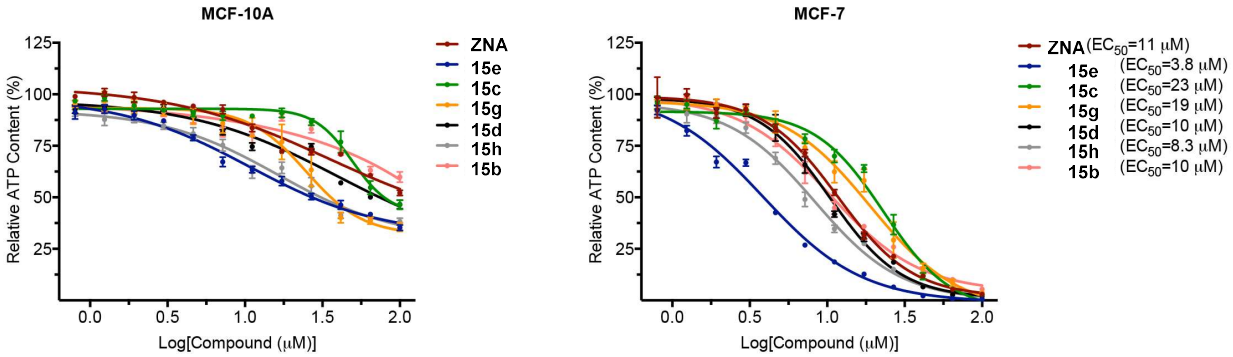


Figure 5.3: Dose-response curve of ZNA analogs

3/7, 8 or 9, which suggests a different biological target than naamidine A. Dr. Rachel Vaden performed a series of RNAseq profiling experiments to characterize the transcriptome changes induced by ZNA. The genes most upregulated by ZNA were metal trafficking metallothionein genes MT1F, MT1X, and MT2A. More specifically, the profiling of the transcriptome showed an increase in the Zn^{2+} transport genes SLC30A1 and SLC30A2.[11] The upregulation of these genes suggests that ZNA effects the intracellular Zn^{2+} concentration. In order to assess the intracellular Zn^{2+} concentration of cells treated with ZNA, a fluorescent Zn^{2+} indicator, Fluo-Zin 3 stain, was used. These assays showed a 13-fold increase of intracellular Zn^{2+} in MCF-7 cells after 48 hours, in contrast to the 2-fold increase of intracellular Zn^{2+} in MCF-10A cells in the same time frame.[11] The influx of Zn^{2+} into the cell generated a hypothesis that a ZNA bound to the metal ion might be the species responsible for cytotoxicity. The formation of the ZNA-Zn^{2+} complex was supported by the initial isolation of naamidine A, which was isolated as a zinc bound dimer; because ZNA was designed as a bioisostere, it was reasonable to think that it would act in the same way (Figure 5.4).[12] To confirm the structure of a ZNA-Zn^{2+} complex, ZNA was treated with $ZnSO_4$ in MeOH and the ZNA-Zn^{2+} dimer that was precipitated was crystalized (Figure 5.4).

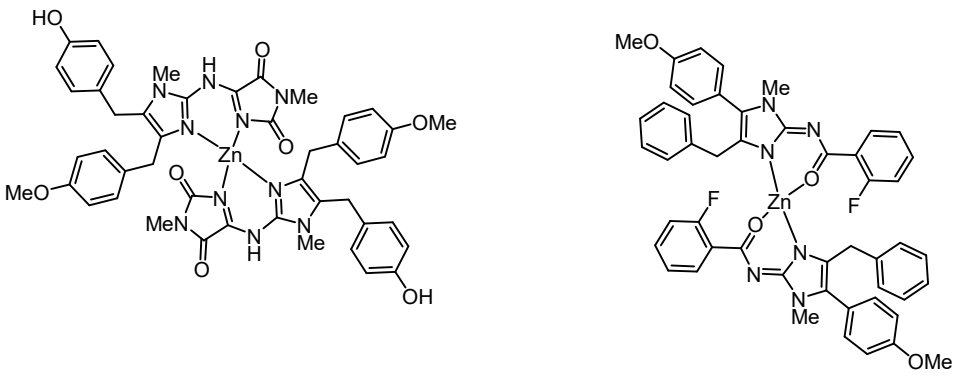


Figure 5.4: The naamidine A Zn dimer and the ZNA Zn dimer

To determine how Zn^{2+} affected ZNA's cytotoxicity, MCF-7 and MCF-10A cells were treated with 30 μM ZNA and 30 μM $ZnSO_4$ and intracellular Zn was measured after 3 hours. The MCF-7 cells treated with ZNA and $ZnSO_4$ showed a 170-fold increase in intracellular Zn compared to those treated with $ZnSO_4$ alone. This was drastically different from the MCF-10A cells which showed only a 9-fold increase when treated with ZNA and $ZnSO_4$ rather than $ZnSO_4$ alone. When additional $ZnSO_4$ was added to the ZNA dosing media, there was a synergistic reduction in the viability of MCF-7, T47D, and MDA-MB-231 breast cancer cell lines. These results demonstrate that ZNA has a unique ability to affect Zn^{2+} trafficking differentially between cancer cells and untransformed cells. [11]

The exact mechanism by which ZNA induces zinc dyshomeostasis has not yet been elucidated; however, this project is ongoing in our lab. The robust four step synthesis from commercially available starting materials provides access the N^2-acyl-2-aminoimidazoles without protecting groups. The ZNA analogs generated can be used to further probe the structure-activity relationship of the N^2-acyl-2-aminoimidazoles and understand the complex mechanism of zinc dyshomeostasis.

5.5 Supporting Information

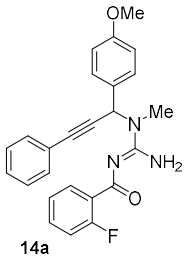
Unless otherwise noted, all starting materials were either known compounds or were obtained from commercial sources and used without purification. All reactions requiring anhydrous conditions were performed under a positive pressure of argon using flame-dried glassware. Dichloromethane (CH_2Cl_2), and triethylamine (Et_3N), were distilled from CaH_2 immediately prior to use. Tetrahydrofuran (THF), diethylether (Et_2O), toluene (PhMe), and dimethylformamide (DMF) were degassed with argon and passed through a solvent purification system (J.C. Meyer of Glass Contour) containing either alumina or molecular sieves. Flash chromatography was performed on Merk silica gel Kieselgel 60 (230-400 mesh) from EM science with the indicated solvent.

^{1}HNMR spectra were recorded on a Varian VXR-500 MHz spectrometer. The chemical shifts (d) of proton resonances are reported relative to $CHCl_3$, DMSO-d_5, HOD, or HD_2COD using the following format: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app = apparent), coupling constant(s) (*J* in Hz), integral].[13,14] ^{13}CNMR spectra were recorded at 75, 100, or 125 MHz. The chemical shifts of carbon resonances are reported relative to the deuterated solvent peak.

Infrared spectra were recorded on a Nicolet 380-FT IR spectrometer fitted with a SmartOrbit sample system. All absorptions are reported in cm^{-1} relative to polystyrene (1601 cm^{-1}).

Preparation of mono-N-acylguanidines from propargylamines:

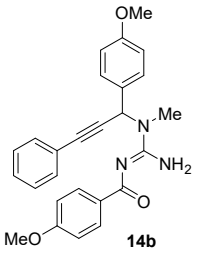
2-Fluoro-N-(N-(1-(4-methoxyphenyl)-3-phenylprop-2-yn-1-yl)-N-methylcarbamimidoyl)benzamide (14a).



14a

In a 50 mL round bottom flask containing a magnetic stir bar were added **2a** (97 mg, 0.48 mmol), chlorotrimethylsilane (64 μL, 0.50 mmol), and acetonitrile (10 mL) under N_2. The solution was stirred at room temperature for 10 minutes. A solution of **13a** (100 mg, 0.40 mmol) in acetonitrile (5 mL) was then added, and the reaction mixture was allowed to stir at room temperature for 1 hour. The solvent was then removed under reduced pressure and the crude product was re-dissolved in EtOAc (50 mL). The organic layer was washed with $NaHCO_3$ (20 mL) and brine (20 mL) and dried and filtered over Na_2SO_4. The crude product was purified *via* flash chromatography, (6:4 hexanes/EtOAc) to give **14a** as a foamy white oil (124 mg, 75% yield). R_f = 0.16 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.06 (t, J = 12.5 Hz, 1H), 7.65 (s, 1H), 7.58-7.51 (m, 4H), 7.38-7.32 (m, 4H), 7.18 (t, J = 13.0 Hz, 1H), 7.07 (t, J = 13.0 Hz, 1H), 6.92 (d, J = 14.5 Hz, 2H), 3.81 (s, 3H), 2.86 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.5, 162.0 (d, J_{CF} = 254.3 Hz), 160.7. 159.7. 148.0. 138.4. 132.2. 132.1 (2), 129.0, 128.8 (d, J_{CF} = 24.6 Hz), 128.6, 123.6 (d, J_{CF} = 3.9 Hz), 122.6, 116.8 (d, J_{CF} = 23.2 Hz), 114.7, 114.2 , 87.0, 75.4, 55.6, 50.9, 29.3 ppm. IR (thin film) 1673, 1588, 1560, 1533, 1509, 1452, 1423, 1355, 1304, 1247, 1218, 1173, 1153, 1030, 897, 845, 758, 732, 691, 590, 550 cm^{-1}. HRMS (ESI+) Calculated for $C_{25}H_{22}N_3O_2NaF$ *m/z* 438.1594 (M+Na), Obsd. 438.1599.

4-Methoxy-N-(N-(1-(4-methoxyphenyl)-3-phenylprop-2-yn-1-yl)-N-methylcarbamimidoyl)benzamide (14b).

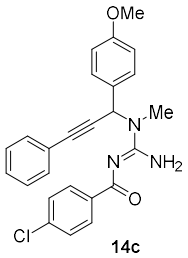


14b

Prepared by guanylation of **13a** (100 mg, 0.40 mmol) with **2b** (103 mg, 0.48 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (119.8 mg, 70% yield). R_f = 0.13 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.25 (d, J = 9.5 Hz,

2H), 7.65 (s, 1H), 7.58 (d, *J* = 9.0 Hz, 2H), 7.55-7.52 (m, 2H), 7.37-7.35 (m, 3H), 6.92-6.91 (m, 2H), 3.84 (s, 3H), 3.81 (s, 3H), 2.87 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 176.9, 162.3, 160.7, 159.7, 132.1, 131.8, 131.3, 129.0, 128.6, 122.7, 114.3, 113.3, 87.0, 85.5, 55.6, 55.5, 50.8, 29.4 ppm. IR (thin film) 1583, 1558, 1528, 1508, 1464, 1424, 1351, 1305, 1263, 1248, 1173, 1155, 1032, 893, 788, 730, 701, 653, 554 cm^{-1}. HRMS (ESI+) Calculated for $C_{26}H_{25}N_3O_3$ *m/z* (M+H) 450.1818, Obsd. 450.1799.

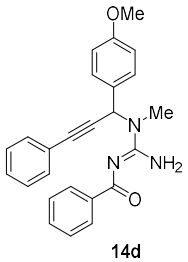
4-Chloro-N-(N-(1-(4-methoxyphenyl)-3-phenylprop-2-yn-1-yl)-N-methylcarbamimidoyl)benzamide (14c).



14c

Prepared by guanylation of **13a** (100 mg, 0.40 mmol) with **2c** (105 mg, 0.48 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (140 mg, 81%). R_f = 0.22 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.20 (d, *J* = 8.5 Hz, 2H), 7.65 (s, 1H), 7.57-7.51 (m, 4H), 7.38-7.33 (m, 5H), 6.92 (d, *J* = 8.5 Hz, 2H), 3.82 (s, 3H), 2.89 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 176.1, 160.9, 159.8, 137.5, 137.4, 132.1, 130.8, 129.4, 129.0, 128.9, 128.6, 128.3, 122.6, 114.3, 87.2, 85.2, 55.6, 50.9, 29.5 ppm. IR (thin film) 1583, 1582, 1528, 1508, 1488, 1464, 1421, 1350, 1304, 1246, 1171, 1116, 1111, 1086, 1058, 1034, 1012, 996, 974, 893, 853, 801, 772, 755, 733, 710, 689, 621, 587, 553 cm^{-1}. HRMS (ESI+) Calculated for $C_{25}H_{23}N_3O_2Cl$ *m/z* 432.1479 (M+H), Obsd. 432.1489.

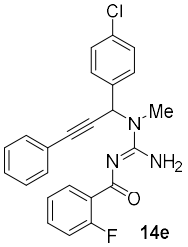
N-(N-(1-(4-Methoxyphenyl)-3-phenylprop-2-yn-1-yl)-N-methylcarbamimidoyl)benzamide (14d).



14d

Prepared by guanylation of **13a** (100 mg, 0.40 mmol) with **2c** (81 mg, 0.48 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (121.3 mg, 76% yield). R_f = 0.22 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.28 (d, *J* = 7 Hz, 2H), 7.71 (s, 1H), 7.58 (d, *J* = 9 Hz, 2H), 7.55-7.52 (m, 2H), 7.47-7.33 (m, 7H), 6.92 (d, *J* = 8 Hz, 2H), 3.82 (s, 3H), 2.89 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 177.2, 160.9, 159.7, 139.0, 132.1, 131.3, 129.6, 129.4, 129.0, 128.9, 128.6, 128.1, 122.6, 114.2, 87.1, 85.4, 55.6, 50.9, 29.4 ppm. IR (thin film) 1587, 1556, 1530, 1508, 1489, 1448, 1422, 1353, 1299, 1247, 1171, 1067, 1026, 891, 756, 733, 710, 689, 621, 587, 553 cm^{-1}. HRMS (ESI+) Calculated for $C_{25}H_{23}N_3O_2Na$ *m/z* 420.1688 (M+Na), Obsd. 420.1697.

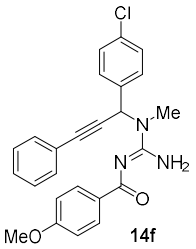
N-(N-(1-(4-Chlorophenyl)-3-phenylprop-2-yn-1-yl)-N-methylcarbamimidoyl)-2-fluorobenzamide (14e).



14e

Prepared by guanylation of **13b** (100 mg, 0.39 mmol) with **2a** (81 mg, 0.47 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (90 mg, 62% yield). R_f = 0.32 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.03 (t, *J* = 7.5 Hz, 1H), 7.69 (s, 1H), 7.58 (d, *J* = 8.5 Hz, 2H), 7.53-7.51 (m, 2H), 7.39-7.33 (m, 6H), 7.14 (t, *J* = 8.0, 1H), 7.13-7.05 (m, 1H), 2.87 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.6, 162.0 (d, J_{CF} = 254.6 Hz), 160.7, 136.1, 134.3, 132.2 (d, J_{CF} = 8.8 Hz), 132.0 (3), 128.1, 129.0 (2), 128.6, 126.6 (d, J_{CF} = 15.2 Hz), 122.3, 116.8 (d, J_{CF} = 23.3 Hz), 87.5, 84.5, 50.9, 29.4 ppm IR (thin film) 1683, 1589, 1560, 1531, 1488, 1452, 1426, 1353, 1327, 1290, 1263, 1218, 1175, 1152, 1130, 1091, 1062, 1032, 1014, 897, 844, 791, 756, 737, 691, 669, 539 cm^{-1}. HRMS (ESI+) Calculated for $C_{24}H_{19}N_3OFNaCl$ *m/z* 442.1098 (M+Na), Obsd. 442.1099.

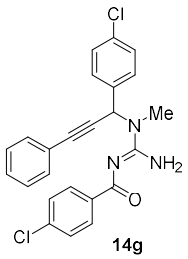
N-(N-(1-(4-Chlorophenyl)-3-phenylprop-2-yn-1-yl)-N-methylcarbamimidoyl)-4-methoxybenzamide (14f).



14f

Prepared by guanylation of **13b** (100 mg, 0.39 mmol) with **2b** (101 mg, 0.47 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (94 mg, 56% yield). R_f = 0.21 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.21 (d, *J* = 9.0 Hz, 2H), 7.73 (s, 1H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.53-7.51 (m, 2H), 7.36-7.33 (m, 5H), 6.89 (d, *J* = 9.0 Hz, 2H), 3.83 (s, 3H), 2.86 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 177.0, 162.4, 160.7, 136.3, 134.3, 132.1, 131.6, 131.3, 129.1, 128.7, 122.4, 113.3, 87.5, 84.7, 50.6, 50.8, 29.5 ppm. IR (thin film) 1654, 1603, 1455, 1383, 1300, 1273, 1257, 1169, 1125, 1069, 1035, 1011, 932, 914, 855, 769, 685, 639, 612 cm^{-1}. HRMS (ESI+) Calculated for $C_{25}H_{22}N_3O_2NaCl$ *m/z* 454.1298 (M+Na), Obsd. 454.1296.

4-Chloro-N-(N-(1-(4-chlorophenyl)-3-phenylprop-2-yn-1-yl)-N-methylcarbamimidoyl)benzamide (14g).

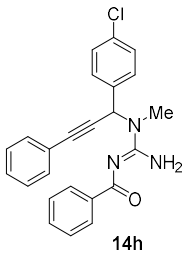


14g

Prepared by guanylation of **13b** (100 mg, 0.39 mmol) with **2c** (102 mg, 0.47 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (80 mg, 47% yield). R_f = 0.37 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.17 (d, *J* = 8.5 Hz, 2H), 7.68 (s, 1H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.54-7.52 (m, 2H), 7.39-7.35 (m, 7H), 2.89 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 176.2, 173.2, 160.9, 137.5, 137.3, 136.0, 134.5, 132.1, 130.8, 129.2, 129.1, 129.0, 128.7, 128.3, 122.3, 87.7, 84.4, 51.0, 29.6 ppm. IR (thin film) 3310, 3169, 1684, 1586, 1556, 1523, 1488, 1469, 1422, 1358, 1323, 1290, 1242, 1159, 1031, 1089, 1064, 1015, 997, 978, 896, 856, 792, 779, 755, 689, 629, 548 cm^{-1}. HRMS (ESI+) Calculated for

$C_{24}H_{20}N_3ONaCl_2$ *m/z* 436.0983 (M+H), Obsd. 436.0988.

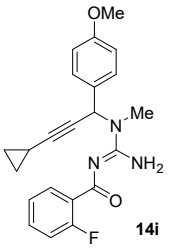
N-(N-(1-(4-Chlorophenyl)-3-phenylprop-2-yn-1-yl)-N-methylcarbamimidoyl)benzamide (14h).



14h

Prepared by guanylation of **13b** (100 mg, 0.39 mmol) with **2d** (81 mg, 0.47 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (90 mg, 57% yield). R_f = 0.27 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.26 (d, *J* = 8.0 Hz, 2H), 7.55 (s, 1H), 7.60 (d, *J* = 8.5 Hz, 2H), 7.58-7.52 (m, 2H), 7.48-7.33 (m, 8H), 2.88 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 177.3, 160.9, 138.8, 136.2, 134.4, 132.1, 131.4, 129.4, 129.1, 128.7, 128.1, 122.3, 87.6, 84.6, 50.9, 29.5 ppm. IR (thin film) 1585, 1580, 1509, 1490, 1456, 1356, 1329, 1250, 1172, 1156, 1101, 1032, 892, 850, 788, 758, 692 cm^{-1}. HRMS (ESI+) Calculated for $C_{24}H_{21}N_3OCl$ *m/z* 402.1373 (M+H), Obsd. 402.1374.

N-(N-(3-Cyclopropyl-1-(4-methoxyphenyl)prop-2-yn-1-yl)-N-methylcarbamimidoyl)-2-fluorobenzamide (14i).

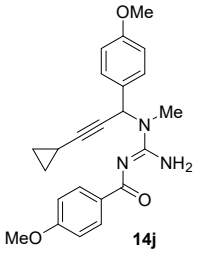


14i

Prepared by guanylation of **13c** (100 mg, 0.46 mmol) with **2a** (93 mg, 0.46 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (74% yield). R_f = 0.21 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.02 (dt, *J* = 1.8, 7.8 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.38-7.33 (m, 1H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.06 (dd, *J* = 1.0, 8.3 Hz, 1H), 6.87 (d, *J* = 7.3 Hz, 2H), 3.79 (s, 3H), 2.76 (s, 3H), 1.39-1.31 (m, 1H), 0.85-0.80 (m, 2H), 0.78-0.73 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.4, 162.9 (d, J_{CF} = 254.4 Hz), 160.5, 159.4, 132.2 (d, J_{CF} = 9.8 Hz), 132.0, 131.9, 128.8, 127.8 (d, J_{CF} = 9.0 Hz), 123.5 (d, J_{CF}

= 3.8 Hz), 116.8 (d, J_{CF} = 23.2 Hz), 113.9, 90.7, 71.2, 55.5, 50.4, 8.5 (2) ppm. IR (thin film) 3336, 2933, 1588, 1559, 1536, 1509, 1453, 1425, 1353, 1248, 1173, 1031, 897 cm^{-1}.HRMS (ESI+) Calculated for $C_{22}H_{22}N_3O_2NaF$ *m/z* 402.1594 (M+Na), Obsd. 402.1601.

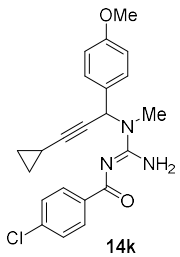
N-(N-(3-Cyclopropyl-1-(4-methoxyphenyl)prop-2-yn-1-yl)-N-methylcarbamimidoyl)-4-methoxybenzamide (14j).



14j

Prepared by guanylation of **13c** (100 mg, 0.46 mmol) with **2b** (100 mg, 0.46 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (83% yield). R_f = 0.16 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.2 (d, *J* = 6.0 Hz, 2H), 7.47 (d, *J* = 7.5 Hz, 2H), 6.87 (t, *J* = 7.5 Hz, 4H), 3.83 (s, 3H), 3.78 (s, 3H), 2.77 (s, 3H), 1.40-1.32 (m, 1H), 0.86-0.80 (m, 2H), 0.79-0.73 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 176.7, 162.0, 160.5, 159.5, 131.1, 128.7, 113.9, 113.0, 90.67, 71.2, 55.4, 8.6, 8.5 ppm. IR (thin film) 2933, 1585, 1528, 1507, 1462, 1349, 1346, 1152, 1029 cm^{-1}. HRMS (ESI+) Calculated for $C_{23}H_{26}N_3O_3$ *m/z* 392.1974 (M+H), Obsd. 392.1974.

4-Chloro-N-(N-(3-cyclopropyl-1-(4-methoxyphenyl)prop-2-yn-1-yl)-N-methylcarbamimidoyl)benzamide (14k).

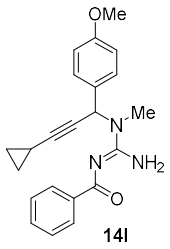


14k

Prepared by guanylation of **13c** (100 mg, 0.46 mmol) with **2c** (100 mg, 0.46 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (80% yield). R_f = 0.35 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.17 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 7.9 Hz, 2H), 7.43 (d, *J* = 9.1 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 3.79 (s, 3H), 2.78 (s, 3H), 1.40-1.33 (m, 1H), 0.86-0.82 (m, 2H), 0.79-0.74 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125

MHz): δ 175.9, 160.8, 159.5, 137.6, 137.3, 130.7, 128.7, 128.1, 114.2, 91.0, 71.1, 55.8, 50.5, 8.6, 8.5 ppm. IR (thin film) 3355, 2932, 1552, 1530, 1508, 1422, 1349, 1246, 1172, 1087, 1030, 1013 cm^{-1}. HRMS (ESI+) Calculated for $C_{22}H_{22}N_3O_2NaCl$ *m/z* 418.1303 (M+Na), Obsd. 418.1300.

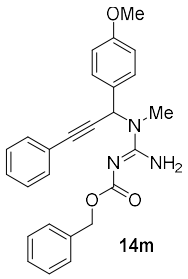
N-(N-(3-Cyclopropyl-1-(4-methoxyphenyl)prop-2-yn-1-yl)-N-methylcarbamimidoyl)benzamide (14l).



14l

Prepared by guanylation of **13c** (100 mg, 0.46 mmol) with **2d** (85 mg, 0.46 mmol), with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (65% yield). R_f = 0.26 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.25 (d, *J* = 7.2 Hz, 2H), 7.48 (d, *J* = 9.7 Hz, 2H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.39 (d, *J* = 7.9 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 3.79 (s, 3H), 2.78 (s, 3H), 1.4-1.33 (m, 1H), 0.86-0.81 (m, 2H), 0.79-0.75 (m, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 176.9, 160.8, 159.4, 139.1, 131.2, 129.3, 128.8, 127.9, 114.0, 90.7, 77.4, 71.2, 55.4, 55.4, 8.6, 8.5 ppm. IR (thin film) 3346, 2962, 1588, 1552, 1536, 1467, 1423, 1353, 1329, 1168, 1066, 893 cm^{-1}. HRMS (ESI+) Calculated for $C_{22}H_{23}N_3O_2Na$ *m/z* 384.1688 (M+Na), Obsd. 384.1693.

Benzyl (Z)-5-benzylidene-2-imino-4-(4-methoxyphenyl)-3-methylimidazolidine-1-carboxylate (3m).

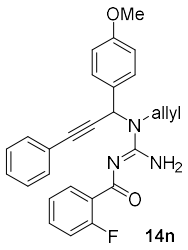


14m

Prepared by guanylation of **13a** with **2e**, with purification on silica gel eluting with 1:1 hexanes/EtOAc to give a dark orange oil (2.97 g, 82%). R_f = 0.48 (1:1 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.51-7.43 (m, 6H), 7.36-7.25 (m, 7H), 6.90 (d, *J* = 6.3 Hz, 2H), 5.18 (s, 2H), 3.80 (s, 3H), 2.80 (s, 3H). ^{13}C NMR ($CDCl_3$, 300MHz): δ 164.1, 160.9, 159.4, 137.6, 131.9, 129.0, 128.7, 128.6,

128.4, 128.0, 127.7, 122.2, 113.9, 86.6, 85.2, 66.9, 55.3, 50.6, 29.7 ppm. IR (thin film) 3403, 2932, 1646, 1584, 1532, 1508, 1488, 1440, 1376, 1273, 1246, 1121, 1150, 1110, 1027, 908, 845, 799, 775, 755, 729, 690, 647, 586, 552 cm^{-1}. HRMS (ESI+) Calculated for $C_{26}H_{26}N_3O_3$ *m/z* 428.1974 (M+Na), Obsd. 428.1979.

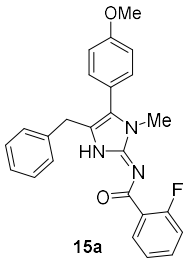
N-(N-Allyl-N-(1-(4-methoxyphenyl)-3-phenylprop-2-yn-1-yl)carbamimidoyl)-2-fluorobenzamide (3n).



Prepared by guanylation of **13d** with **2a,** with flash chromatography purification eluting with 6:4 hexanes/EtOAc to yield a foamy white oil (54% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.08 (dt, *J* = 8.0 Hz, 2.0 Hz, 1H), 7.64 (s, 1H), 7.61 (d, *J* = 9.0 Hz, 2H), 7.40-7.3 (m, 2H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.08 (dd, *J* = 11.0 Hz, 8.5 Hz, 1H), 6.91 (d, *J* = 9.0 Hz, 2H), 5.77-5.72 (m, 1H), 5.32 (d, *J* = 17.0 Hz, 1H), 5.25 (dd, *J* = 10.0 Hz, 1.5 Hz, 1H), 3.95 (ABq, *J* = 15 Hz, 38 Hz, 2H), 3.82 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.8, 162.0 (d, J_{CF} = 254.6 Hz), 161.0, 159.8, 134.0, 132.2 (d, J_{CF} = 8.3 Hz), 132.1 (d, J_{CF} = 1.5 Hz), 132.0, 129.7, 129.2, 128.9, 128.6, 127.8 (d, J_{CF} = 3.8 Hz), 123.6 (d, J_{CF} = 3.8 Hz), 122.6, 118.5, 114.2, 87.1, 85.7, 55.6, 50.8, 47.1 ppm. IR (thin film) 1586, 1560, 1510, 1452, 1327, 1248, 1219, 1173, 1152, 1096, 1031, 906, 836, 757, 726, 690, 668, 646 cm^{-1}. HRMS (ESI+) Calculated for $C_{27}H_{25}N_3O_2F$ *m/z* 442.1931 (M+H), Obsd. 442.1925.

NaH-mediated Cyclizations:

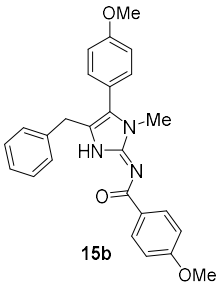
N-(4-Benzyl-5-(4-methoxyphenyl)-1-methyl-1H-imidazol-2(3H)-ylidene)-2-fluorobenzamide (15a).



15a

In a 25 mL round bottom flask containing a magnetic stir bar were added **14a** (402.5 mg, 0.97 mmol) and THF (30 mL) under N_2. The solution was stirred at room temperature, and NaH (22.5 mg, 0.97 mmol) was added, resulting in a bright yellow solution. The reaction was stirred for 30 minutes, after which the solvent was removed under reduced pressure and the crude product was re-dissolved in EtOAc (25 mL). The organic layer was washed with saturated aqueous NH_4Cl (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered, and concentrated. The resulting yellow solid required no further purification (330 mg, 82%). R_f = 0.41 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.07 (t, J = 8.0 Hz, 2H), 7.34-7.32 (m, 1H), 7.32-7.27 (m, 4H), 7.21 (t, J = 8.5 Hz, 1H), 7.19-7.16 (m, 3H), 7.08 (t, J = 9.5 Hz, 1H), 7.00 (d, J = 8.5 Hz, 2H), 3.86 (s, 3H), 3.81 (s, 2H), 3.44 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.8, 162.5 (d, J_{CF} = 253.4 Hz), 160.2, 148.7, 137.8, 131.8, 131.7 (d, J_{CF} = 1.9 Hz), 131.6, 128.8, 128.2, 126.8, 126.4, 124.8, 123.6 (d, J_{CF} = 3.8 Hz), 119.9, 116.5 (d, J_{CF} = 22.9 Hz), 114.5, 55.4, 31.0, 30.1 ppm. IR (thin film) 2929, 2360, 2340, 1684, 1569, 1511, 1494, 1455, 1401, 1339, 1290, 1248, 1176, 1032, 834, 815, 757, 731, 696, 667 cm^{-1}. HRMS (ESI+) Calculated for $C_{22}H_{25}N_3O$ m/z (M+H) 347.1998, Obsd. 438.1594.

N-(4-Benzyl-5-(4-methoxyphenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)-4-methoxybenzamide (15b).

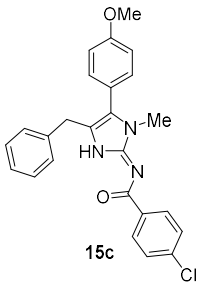


15b

Prepared *via* NaH-mediated cyclization of **14b** (31.5 mg, 0.074 mmol) in THF as a yellow foam (26.5 mg, 84%). R_f = 0.21 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.08 (d, J = 8.7 Hz, 2H), 7.30–7.21 (m, 5H), 7.14 (d, J = 8.7 Hz, 2H), 7.01 (d, J = 9.0 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.82 (s, 2H), 3.47 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$,

125 MHz): δ 161.8, 160.2, 137.7, 131.6, 130.5, 128.5, 128.2, 126.8, 124.4, 119.9, 114.5, 113.1, 55.4, 55.3, 30.7, 30.0 ppm. IR (thin film) 1671, 1603, 1567, 1508, 1454, 1414, 1398, 1349, 1308, 1289, 1246, 1175, 1163, 1108, 1028, 1005, 882, 835, 799, 765, 733, 697, 608 cm^{-1}. HRMS (ESI+) Calculated for $C_{26}H_{26}N_3O_2$ *m/z* 428.1974 (M+H), Obsd. 428.1971.

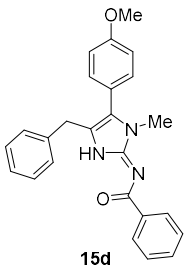
N-(4-Benzyl-5-(4-methoxyphenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)-4-chlorobenzamide (15c).



15c

Prepared *via* NaH-mediated cyclization of **14c** in THF as a yellow foam (42.2 mg, 82%). R_f = 0.38 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.19 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.30–7.21 (m, 5H), 7.12 (d, *J* = 7.5 Hz, 2H), 7.01 (d, *J* = 8.0 Hz, 2H), 3.86 (s, 3H), 3.82 (s, 2H), 3.47 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.7, 160.5, 150.6, 137.6, 137.2, 136.8, 131.8, 130.4, 129.2, 128.4, 128.2, 127.2, 124.5, 120.4, 119.8, 113.8, 55.6, 50.8, 30.1 ppm. IR (thin film) 1670, 1603, 1566, 1508, 1453, 1348, 1307, 1280, 1242, 1174, 1162, 1108, 1027, 835, 779, 733, 697, 607 cm^{-1}. HRMS (ESI+) Calculated for $C_{25}H_{23}N_3O_2Cl$ *m/z* 432.1479 (M+H), Obsd. 432.1480.

N-(4-Benzyl-5-(4-methoxyphenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)benzamide (15d).

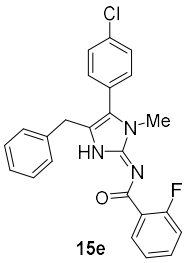


15d

Prepared *via* NaH-mediated cyclization of **14d** in THF as a yellow foam (55.4 mg, 63%). R_f = 0.47 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.27 (d, *J* = 7.0 Hz, 2H), 7.45-7.40 (m, 3H), 7.32-7.27 (m, 4H), 7.22-? (m, 1H), 7.15 (d, *J* = 7.5 Hz, 2H), 7.01 (d, *J* = 9.0 Hz, 2H), 3.86 (s, 3H), 3.83 (s, 2H), 3.49 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 160.5, 138.7, 131.9, 130.8, 129.2,

128.9, 128.4, 128.1, 127.2, 124.5, 120.0, 114.8, 55.7, 32.4, 31.0 ppm. IR (thin film) 3061, 2933, 1675, 1636, 1566, 1541, 1494, 1464, 1453, 199, 1350, 1288, 1246, 1174, 1108, 1025, 1004, 906, 832, 718, 709, 645, 593 cm^{-1}. HRMS (ESI+) calculated for $C_{25}H_{24}N_3O_2$ *m/z* (M+H) 398.1869, Obsd. 398.1869.

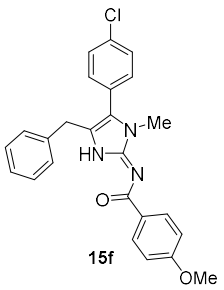
N-(4-Benzyl-5-(4-chlorophenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)-2-fluorobenzamide (15e).



15e

Prepared *via* NaH-mediated cyclization of **14e** in THF as a yellow foam (32.7 mg, 62%). R_f = 0.38 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.08 (t, *J* = 2.5 Hz, 1H), 7.46 (d, *J* = 8 Hz, 2H), 7.40 (m, 1H), 7.30-7.25 (m, 4H), 7.22-7.10 (m, 5H), 3.84 (s, 2H), 3.43 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.1, 161.4 (d, J_{CF} = 252.5 Hz), 147.9, 137.7, 135.2, 132.3 (d, J_{CF} = 8.8 Hz), 131.7 (d, J_{CF} = 2.5 Hz), 131.5, 129.3, 128.9, 128.2, 126.9, 126.5, 124.3, 123.8 (d, J_{CF} = 2.5 Hz), 116.6, 116.4, 31.2, 30.5 ppm. IR (thin film) 1682, 1567, 1490, 1352, 1221, 1091, 1010, 906 cm^{-1}. HRMS (ESI+) Calculated for $C_{24}H_{20}N_3OFCl$ *m/z* 420.1279 (M+H), Obsd. 420.1278.

N-(4-Benzyl-5-(4-chlorophenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)-4-methoxybenzamide (15f).

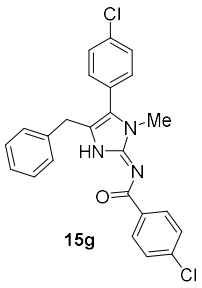


15f

Prepared *via* cyclization of **14f** in THF as an off-white foam (25.7 mg, 74%). R_f = 0.38 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.19 (d, *J* = 8.7 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.30-7.23 (m, 5H), 7.13 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 9.0 Hz, 2H), 3.84 (s, 2H), 3.83 (s, 2H), 3.48 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.0, 137.5, 135.2, 131.6, 131.4, 130.5, 129.5, 129.3, 128.9, 128.2, 128.1, 126.9, 126.5, 114.6, 113.5, 113.6, 55.3,

30.9, 30.2 ppm. IR (thin film) 1671, 1568, 1513, 1491, 1452, 1346, 1309, 1248, 1174, 1162, 1090, 881, 831, 779, 764, 728, 696, 607 cm^{-1}. HRMS (ESI+) calculated for $C_{25}H_{23}N_3O_2Cl$ (M+H) *m/z* 432.1479, Obsd. 432.1480.

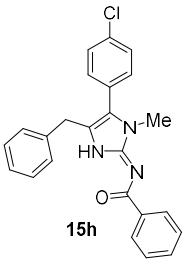
N-(4-Benzyl-5-(4-chlorophenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)-4-chlorobenzamide (15g).



15g

Prepared *via* NaH-mediated cyclization of **14g** in THF as a yellow foam (35.1 mg, 64%). R_f = 0.68 (6:4 hexanes/EtOAc); ^1H NMR ($CDCl_3$, 500 MHz): δ 8.18 (d, *J* = 8.5 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.30-7.25 (m, 5H), 7.12 (d, *J* = 7.0 Hz, 2H), 3.83 (s, 2H), 3.48 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 137.1, 136.7, 135.8, 131.7, 130.4, 129.7, 129.3, 128.3, 127.4, 126.2, 123.6, 30.8, 30.4 ppm. IR (thin film) 1571, 1492, 1397, 1350, 1091, 1012, 767 cm^{-1}. HRMS (ESI+) Calculated for $C_{24}H_{20}N_3OCl_2$ *m/z* 436.0983 (M+H), Obsd. 436.0984.

N-(4-Benzyl-5-(4-chlorophenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)benzamide (15h).

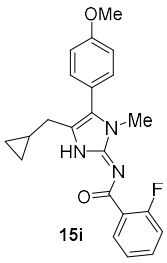


15h

Prepared *via* NaH-mediated cyclization of **14h** in THF as a white foam (39.3 mg, 77%). R_f = 0.40 (6:4 hexanes/EtOAc); ^1H NMR ($CDCl_3$, 500 MHz): δ 8.24 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.45-7.39 (m, 3H), 7.30-7.20 (m, 5H), 7.12 (d, *J* = 7.0 Hz, 2H), 3.83 (s, 2H), 3.48 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.1, 149.9, 137.8, 137.7, 135.4, 131.6, 131.1, 129.6, 129.1, 128.9, 128.3, 128.1, 127.1, 126.6, 124.0, 122.6, 31.1, 30.5 ppm. IR (thin film) 1678, 1566, 1492, 1467, 1453, 1396, 1353, 1304, 1280, 1169, 1092, 1025, 1011, 876, 831, 741, 711 cm^{-1}. HRMS

(ESI+) calculated for $C_{24}H_{20}N_3ONaCl$ (M+Na) m/z 423.1193, Obsd. 424.1203.

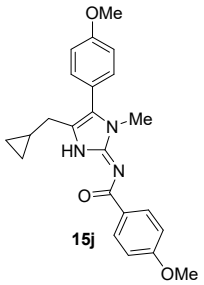
N-(4-(Cyclopropylmethyl)-5-(4-methoxyphenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)-2-fluorobenzamide (15i).



15i

Prepared *via* NaH-mediated cyclization of **14i** in THF as a yellow foam (45.6 mg, 86%). R_f = 0.3 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.09 (dt, J = 1.7, 6.1 Hz, 1H), 7.40-7.34 (m, 2H), 7.23 (d, J = 7.3 Hz, 2H), 7.16 (t, J = 8.4, 1H), 7.09 (dd, J = 3.0 Hz, 8.4 Hz, 1H), 6.99 (d, J = 9.0 Hz, 2H), 3.86 (s, 3H), 3.42 (s, 3H), 2.40 (d, J = 6.7 Hz, 2H), 0.97-0.88 (m, 1H), 0.56 (d, J = 8.3 Hz, 2H), 0.17 (d, J = 4.8 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.5, 161.5 (d, J_{CF} = 252.5 Hz), 160.1, 149.1, 131.7, 131.6 (d, J_{CF} = 2.5 Hz), 131.5 (d, J_{CF} = 8.8 Hz), 127.1 (d, J_{CF} = 8.8 Hz), 123.5 (d, J_{CF} = 3.8 Hz), 123.3, 122.4, 120.0, 116.5 (d, J_{CF} = 22.5 Hz), 114.3, 55.3, 29.9, 29.3, 10.2, 4.5 ppm. IR (thin film) 2934, 1566, 1510, 1480, 1353, 1247, 1174, 1031 cm^{-1}. HRMS (ESI+) calculated for $C_{22}H_{22}N_3O_2FNa$ (M+Na) m/z 402.1594, Obsd. 402.1598.

N-(4-(Cyclopropylmethyl)-5-(4-methoxyphenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)-4-methoxybenzamide (15j).

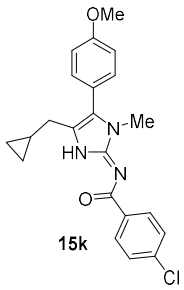


15j

Prepared *via* NaH-mediated cyclization of **14j** in THF as a yellow foam (63.2 mg, 84%). R_f = 0.27 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.27 (d, J = 8.2 Hz, 2H), 7.24 (d, J = 8.6 Hz, 2H), 7.00 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.2 Hz, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.45 (s, 3H), 2.39 (d, J = 6.8 Hz, 2H), 0.97-0.88 (m, 1H), 0.57 (d, J = 8.1 Hz, 2H), 0.17 (d, J = 4.7 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.6, 173.1, 161.9, 160.2, 131.8, 130.6, 122.9, 120.9, 114.5, 113.2, 95.1, 55.7, 29.9, 29.3, 10.3, 4.7 ppm. IR (thin film) 2931, 2836,

1568, 1509, 1463, 1348, 1290, 1246, 1163, 1100, 1030 cm^{-1}. HRMS (ESI+) Calculated for $C_{23}H_{25}N_3O_3Na$ *m/z* 414.1794 (M+Na), Obsd. 414.1794.

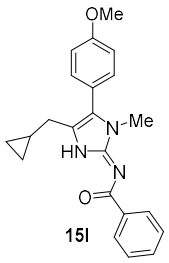
4-Chloro-N-(4-(cyclopropylmethyl)-5-(4-methoxyphenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)benzamide (15k).



15k

Prepared *via* NaH-mediated cyclization of **14k** in THF as a yellow foam (48.2 mg, 87%). R_f= 0.48 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.24 (d, *J* = 7.8 Hz, 2H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.23 (d, *J* = 8.2 Hz, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 3.86 (s, 3H), 3.45 (s, 3H), 2.40 (d, *J* = 6.8 Hz, 2H), 0.97-0.88 (m, 1H), 0.57 (d, *J* = 7.1 Hz, 2H), 0.18 (d, *J* = 5.5 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.8, 173.2, 160.3, 150.3, 137.3, 136.7, 131.8, 130.3, 128.1, 123.2, 121.7, 120.0, 114.5, 55.6, 29.9, 29.3, 10.2, 4.7 ppm. IR (thin film) 2929, 1822, 1725, 1569, 1512, 1466, 1348, 1289, 1248, 1162, 1087, 1013 cm^{-1}. HRMS (ESI+) Calculated for $C_{22}H_{22}N_3O_2NaCl$ *m/z* 418.1298 (M+Na), Obsd. 418.1302.

N-(4-(Cyclopropylmethyl)-5-(4-methoxyphenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)benzamide (15l).

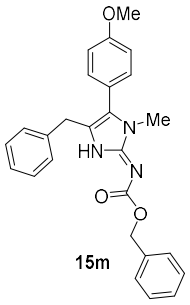


15l

Prepared *via* NaH-mediated cyclization of **14l** in THF as a yellow foam (52.3 mg, 91%). R_f= 0.55 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.31 (d, *J* = 7.3 Hz, 2H), 7.47-7.39 (m, 4H), 7.24 (d, *J* = 9.0 Hz, 3H), 7.01 (d, *J* = 8.7 Hz, 2H), 3.87 (s, 3H), 3.47 (s, 3H), 2.41 (d, *J* = 7.3 Hz, 2H), 0.97-0.89 (m, 1H), 0.58 (d, *J* = 7.8 Hz, 2H), 0.19 (d, *J* = 4.8 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.9, 173.2, 160.2, 138.8, 130.7, 128.9, 128.0, 123.1, 120.2, 114.5, 55.6, 29.9, 29.3, 10.3, 4.7 ppm. IR (thin film) 2979, 1821, 1724, 1569, 1511, 1464, 1349, 1287, 1248, 1138, 1023 cm^{-1}.

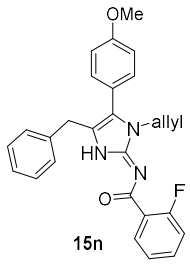
HRMS (ESI+) Calculated for $C_{22}H_{23}N_3O_2Na$ *m/z* 384.1688 (M+Na), Obsd. 384.1690.

Benzyl (4-benzyl-5-(4-methoxyphenyl)-1-methyl-1,3-dihydro-2H-imidazol-2-ylidene)carbamate (15m).



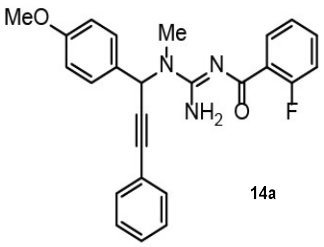
Prepared *via* NaH-mediated cyclization of **14m** in THF as a yellow foam (3.0 g, 81 %). R_f = 0.22 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.41 (d, *J* = 6.9 Hz, 2H), 7.29-7.19 (m, 8H), 7.11 (d, *J* = 6.9 Hz, 2H), 6.99 (d, *J* = 8.9 Hz, 2H), 5.15 (s, 2H), 3.84 (s, 3H), 3.77 (s, 2H), 3.32 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.9, 160.3, 150.2, 137.9, 137.8, 131.8, 129.1, 128.5, 128.3, 128.1, 127.6, 127.1, 124.6, 121.2, 120.0, 114.6, 66.9, 55.6, 31.0, 30.2 ppm. IR (thin film) 1724, 1590, 1508, 1298, 1244, 1210, 1175, 1059, 906 cm^{-1}. HRMS (ESI+) Calculated for $C_{26}H_{26}N_3O_3$ *m/z* 428.1974 (M+H), Obsd. 428.1974.

N-(1-allyl-4-benzyl-5-(4-methoxyphenyl)-1,3-dihydro-2H-imidazol-2-ylidene)-2-fluorobenzamide (15n).

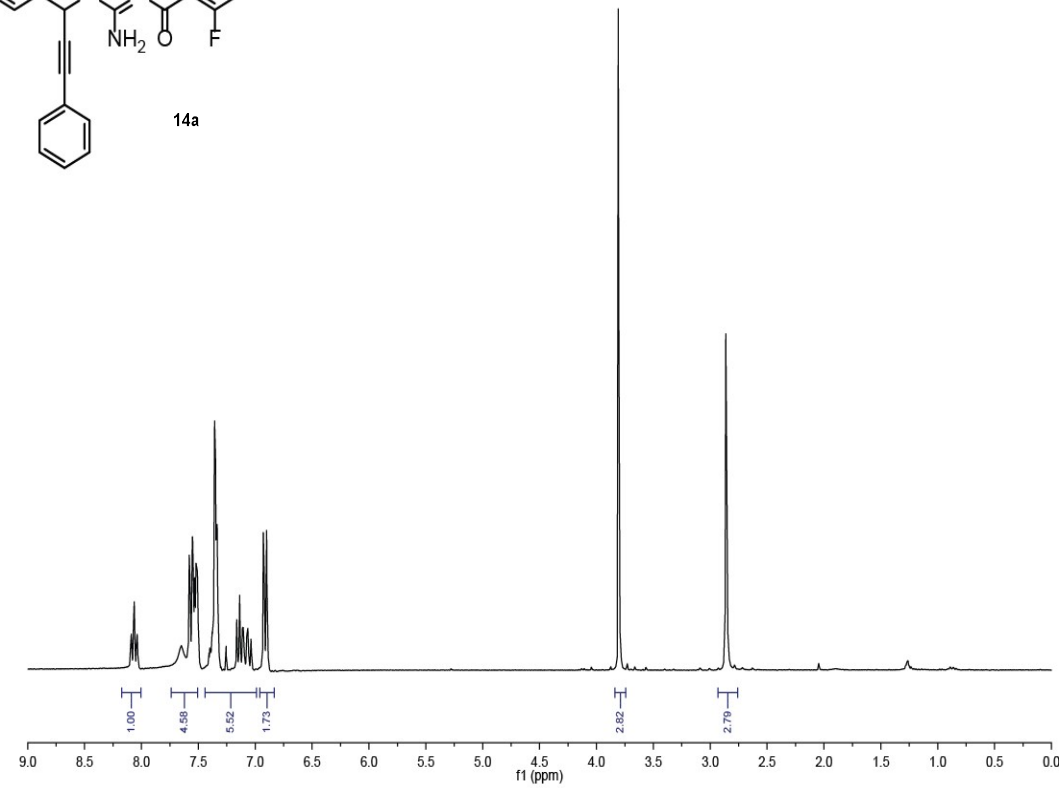


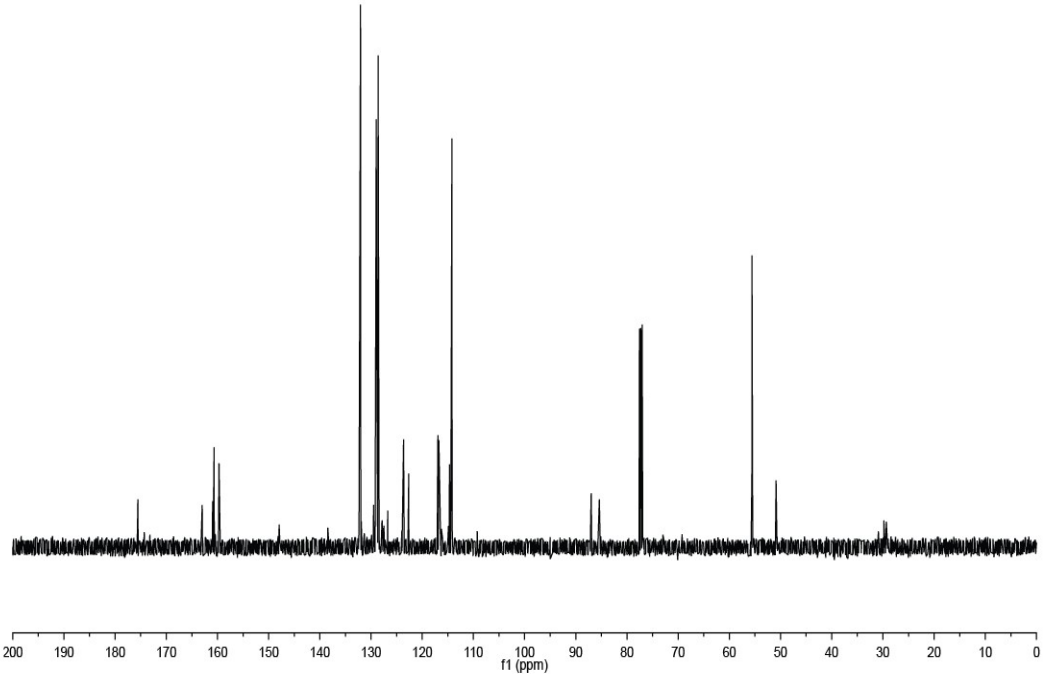
Prepared *via* NaH-mediated cyclization cyclization of **14n** in THF as a yellow foam (42.6 mg, 54%). R_f = 0.50 (6:4 hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.06 (dt, *J* = 1.5 Hz, 8.0 Hz, 1H), 7.56-7.53 (m, 1H), 7.29-7.26 (m, 4H), 7.23-7.20 (m, 1H), 7.15-7.12 (m, 2H), 7.09-7.05 (m, 1H), 6.98 (d, *J* = 9.0 Hz, 2H), 5.91-5.83 (m, 1H), 5.12 (dd, *J* = 1.0 Hz, 10.0 Hz, 1H), 4.96 (dd, *J* = 1.0 Hz, 17.5 Hz, 1H), 3.86 (s, 3H), 3.80 (s, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 161.9 (d, J_{CF} = 252.8 Hz), 160.5, 137.9, 132.9, 132.2, 132.1, 132.0 (3), 129.1, 128.4, 127.1, 123.7 (d, J_{CF} = 3.8 Hz), 120.0, 117.8, 116.7 (d, J_{CF} = 23.2 Hz), 114.5 55.6, 45.5, 31.1 ppm. IR (thin film)

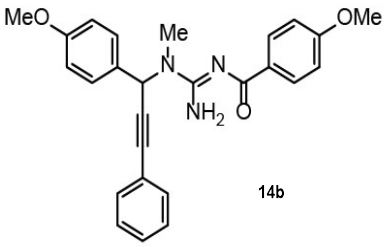
2924, 1567, 1512, 1364, 1290, 1252, 1176, 1032, 759, 687 cm^{-1}. HRMS (ESI+) Calculated for $C_{27}H_{25}N_3O_2F$ m/z 442.1931 (M+H), Obsd. 442.1929.


MeO
Me
N
N
NH2
O
F

14a

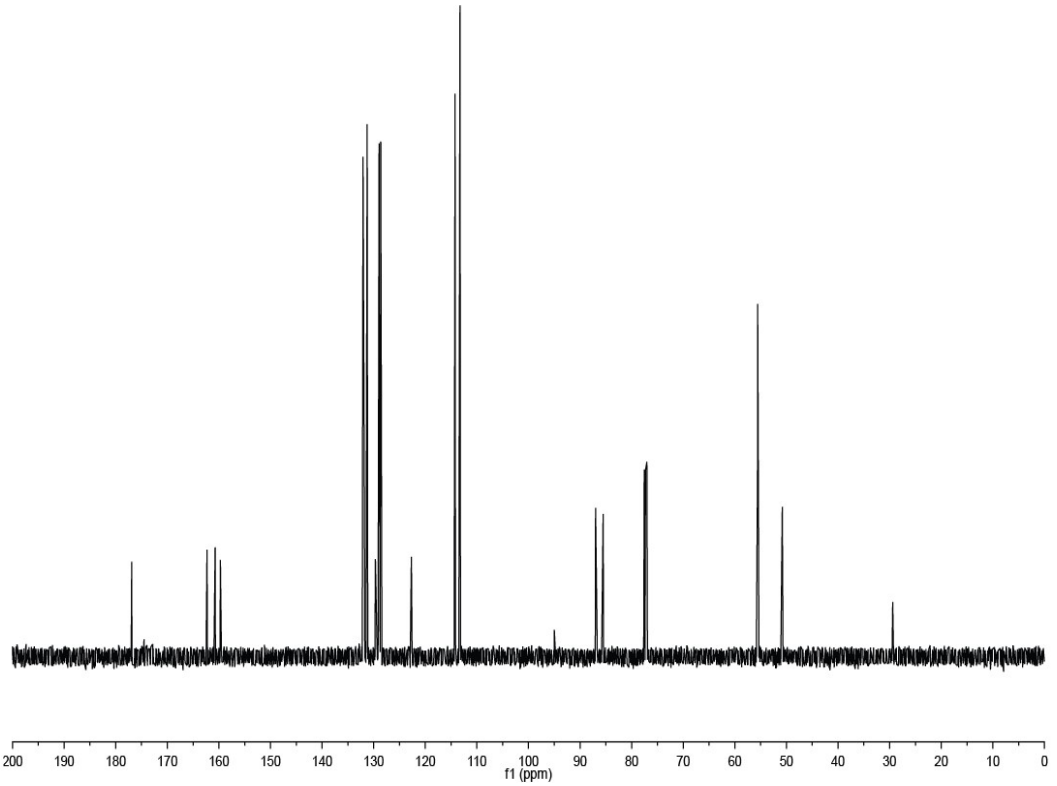

1.00
4.58
5.52
1.73
2.82
2.79
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

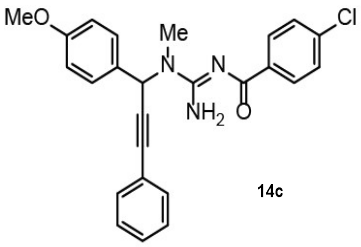

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
N
NH2
O
OMe

14b

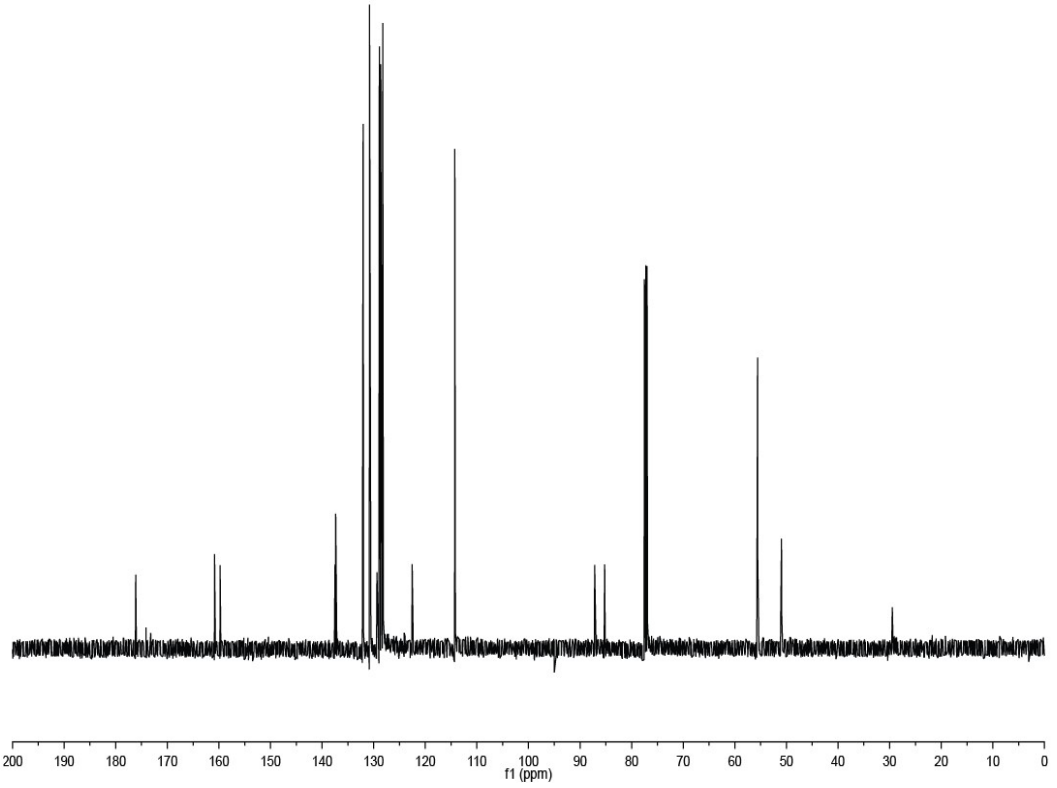
1.93
0.73
3.95
2.82
4.07
6.35
3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
NH2
O
Cl

14c

1.91
4.67
5.01
2.04
3.12
3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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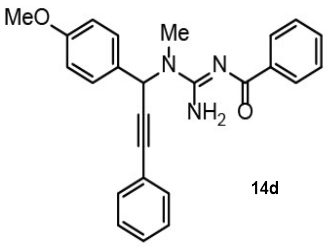
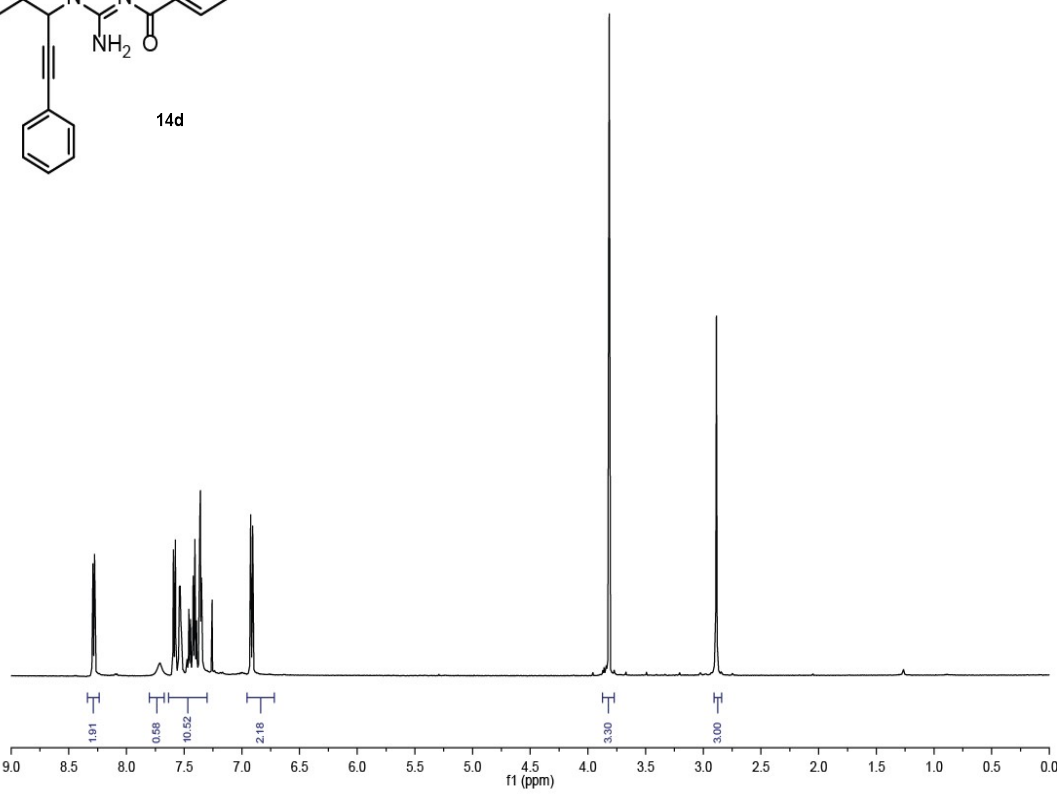
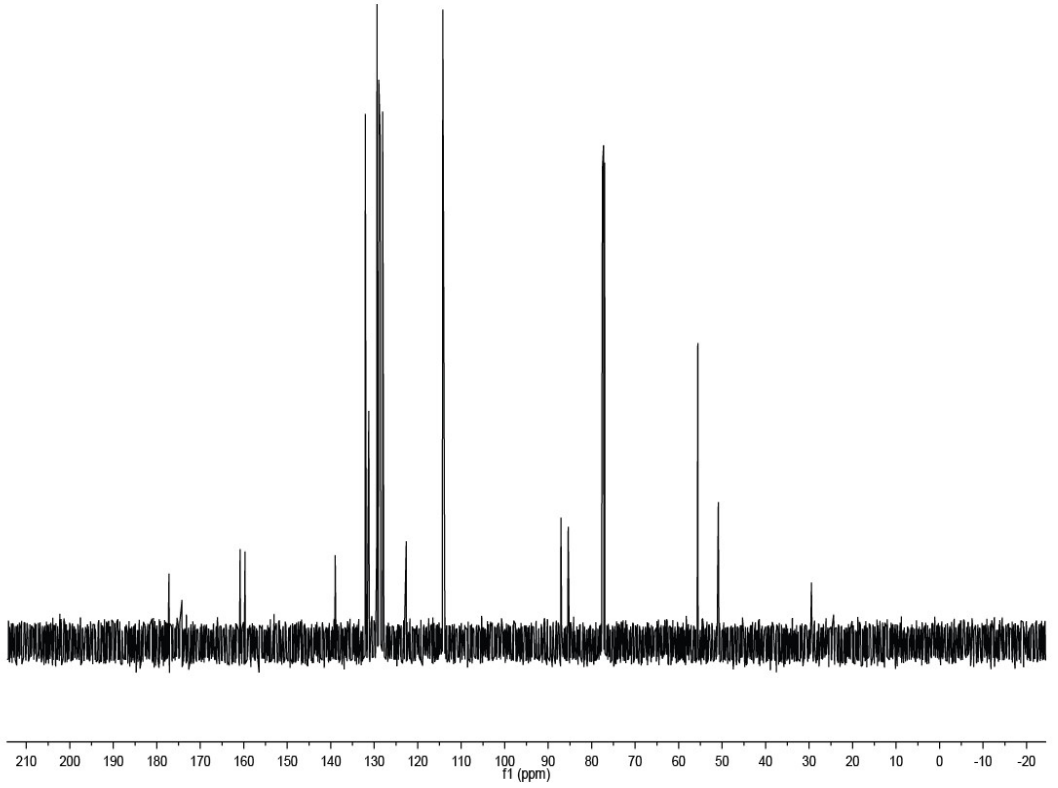
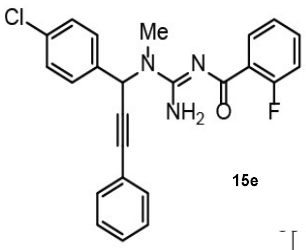
f1 (ppm)


MeO
Me
N
N
N
NH2
O

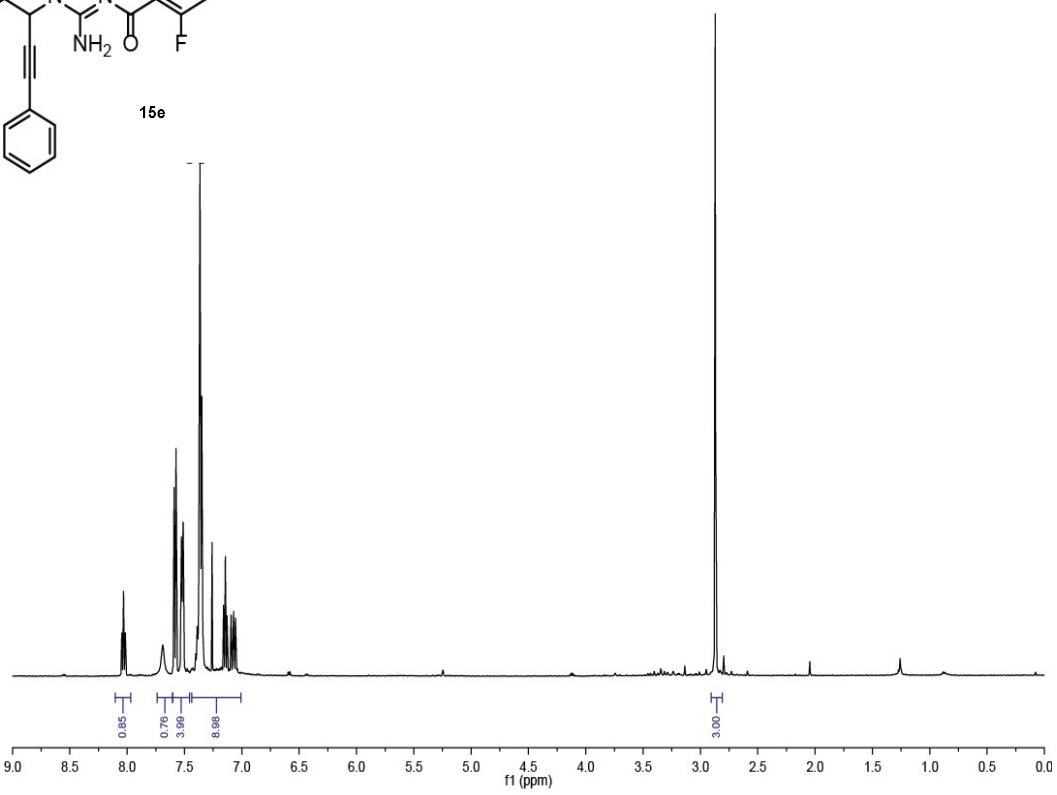
14d

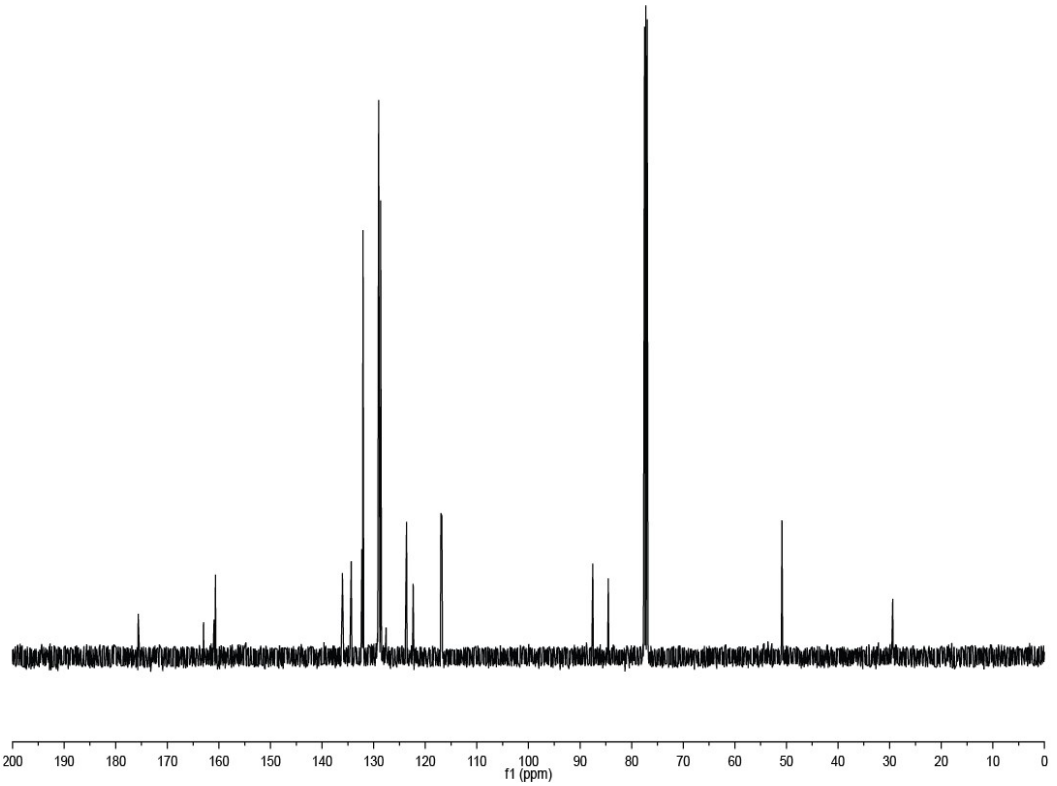

1.91
0.58
10.52
2.18
3.30
3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

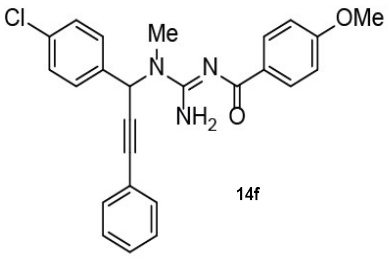

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


Cl
Me
N
N
NH2
O
F

15e


0.85
0.76
3.99
6.88
3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Cl
Me
N
N
OMe
NH2
O

14f

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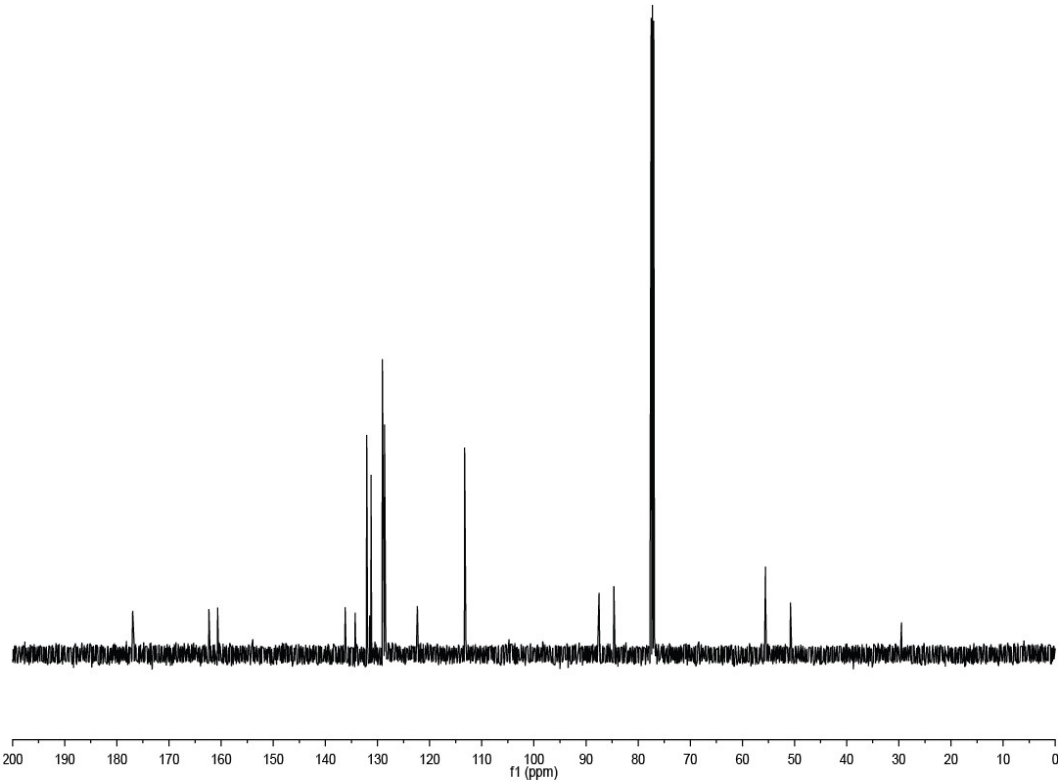
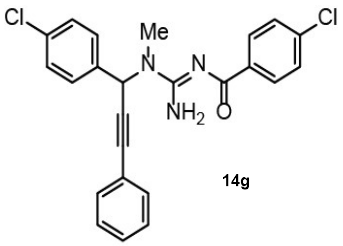
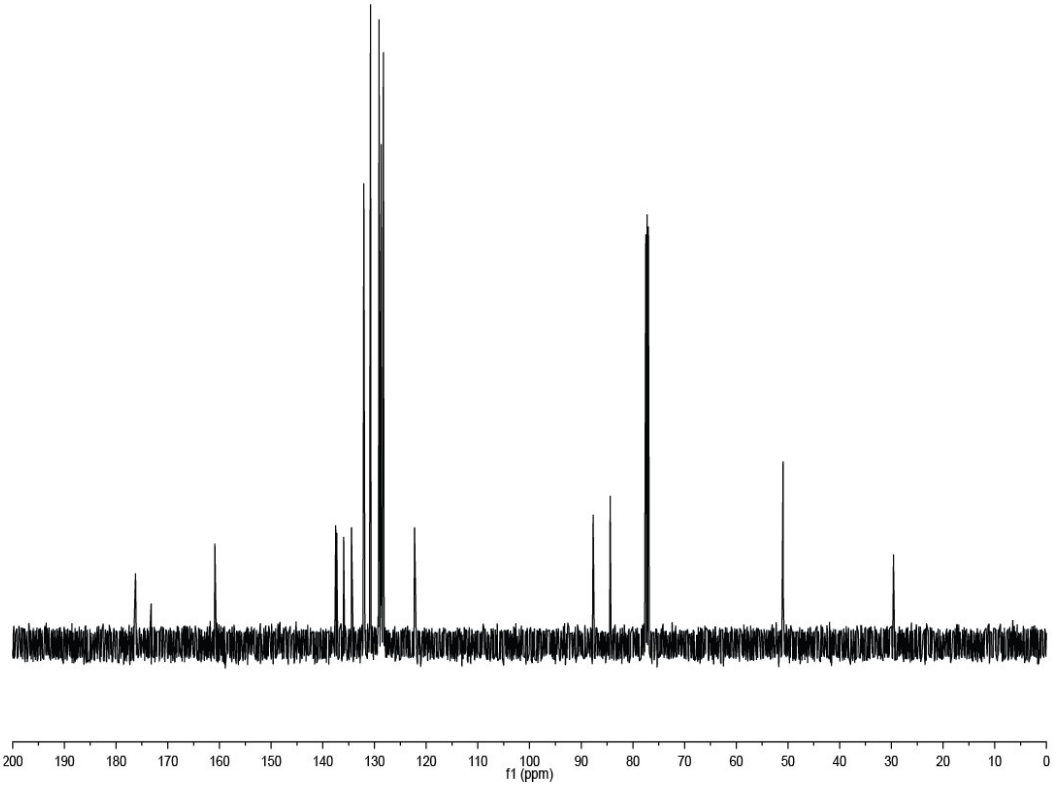
0.94
3.98
4.42
2.09
3.25
3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

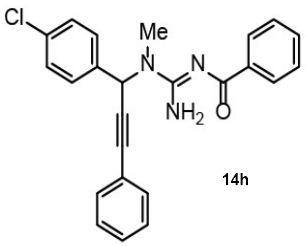

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Cl
Me
N
N
Cl
NH2
O

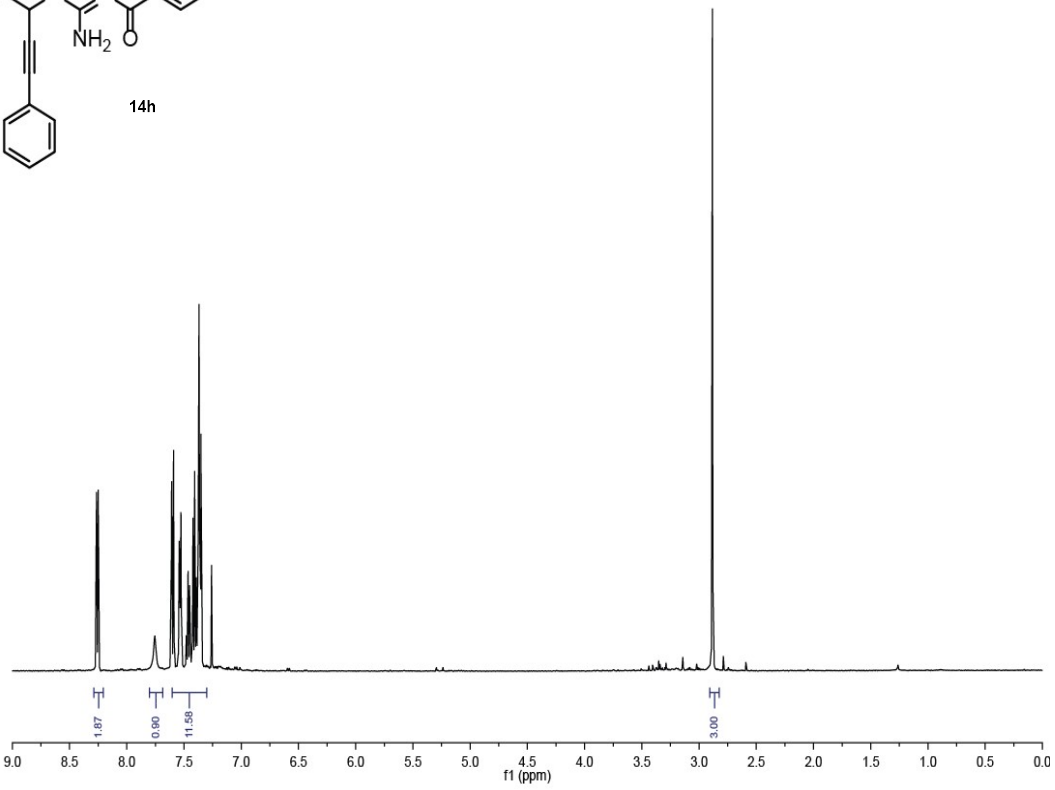
14g

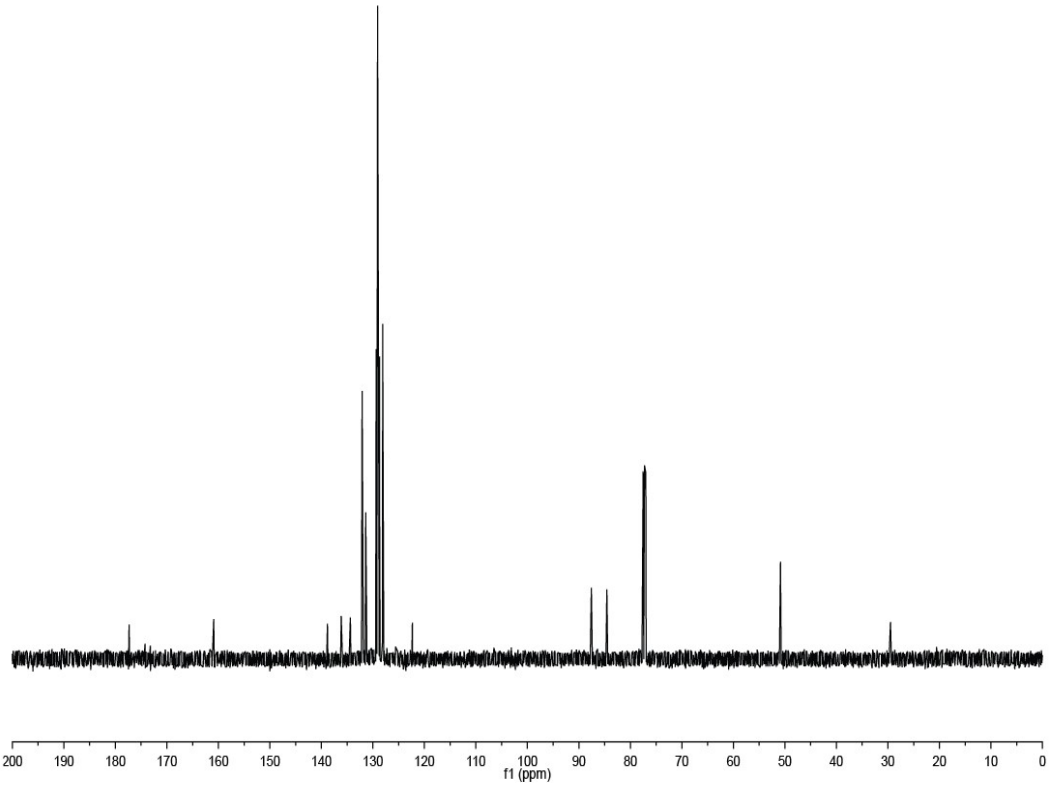
1.86
0.78
4.01
6.81
3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Cl
Me
N
N
N
NH2
O

14h


1.87
0.90
11.58
3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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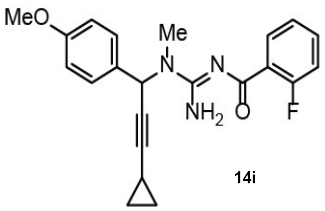
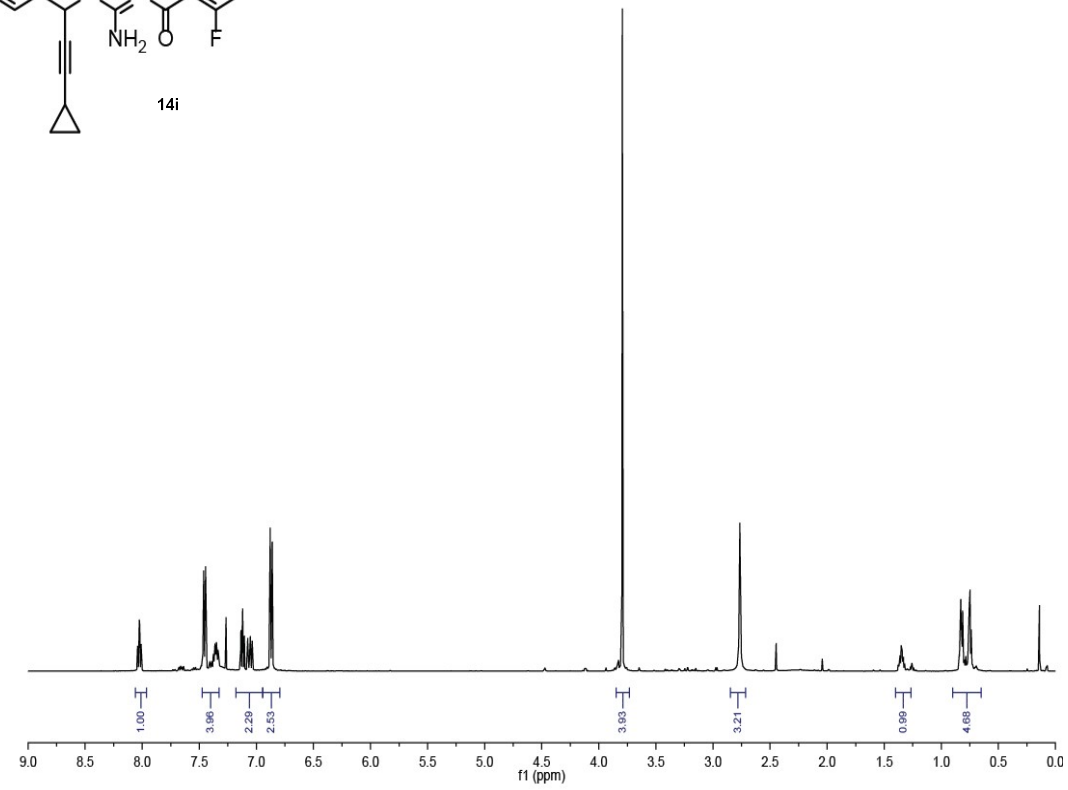
f1 (ppm)


MeO
Me
N
N
NH2
O
F

14i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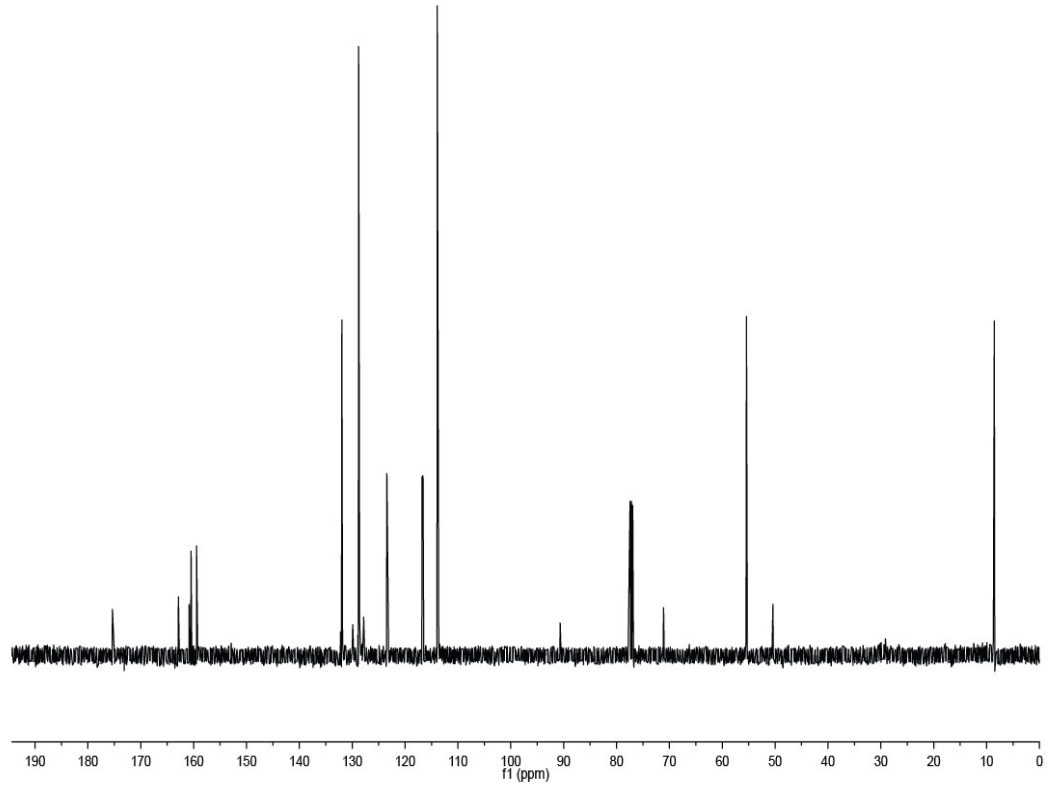
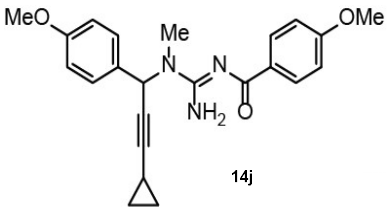
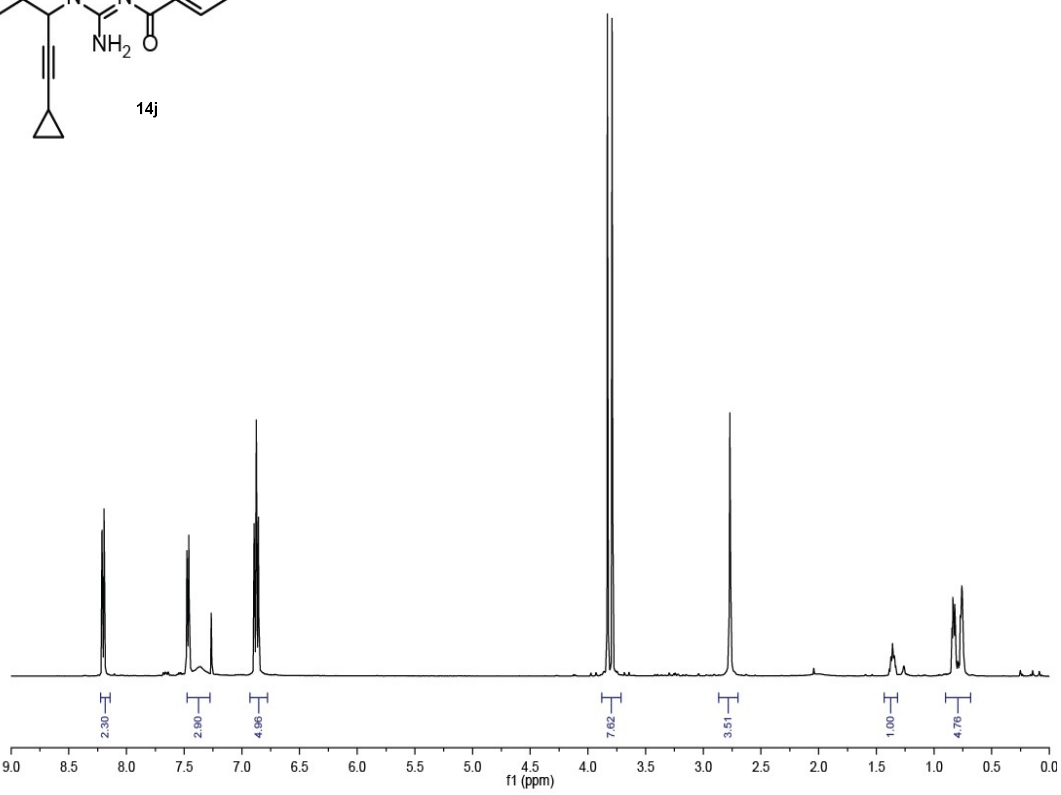
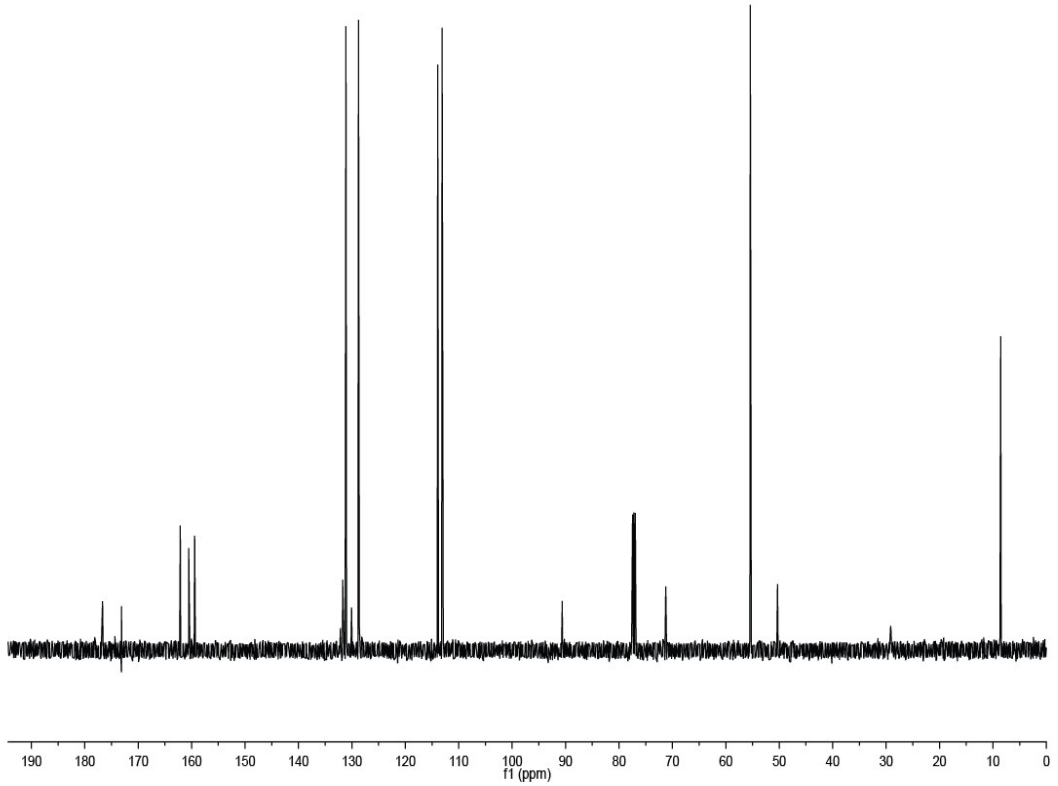
3.96
2.29
2.53
3.93
3.21
0.99
4.68
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

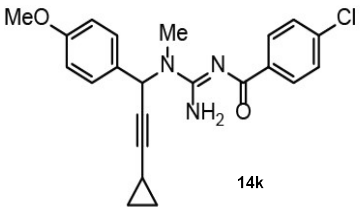

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
OMe
NH2
O

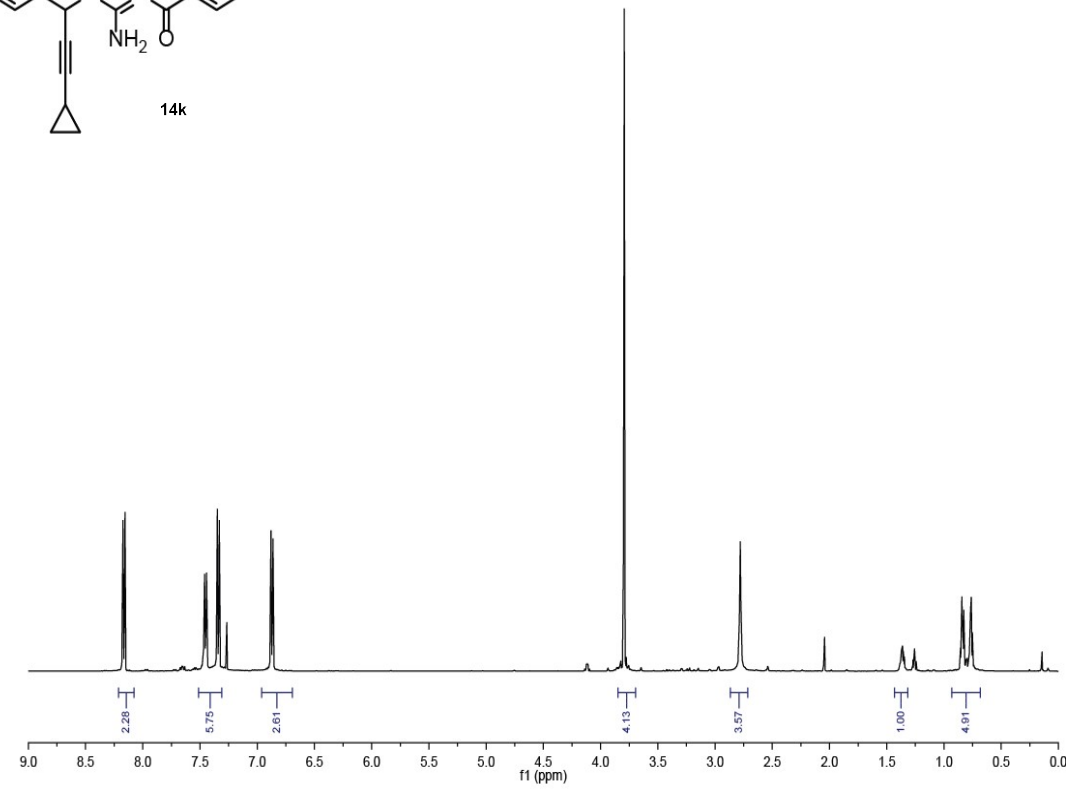
14j

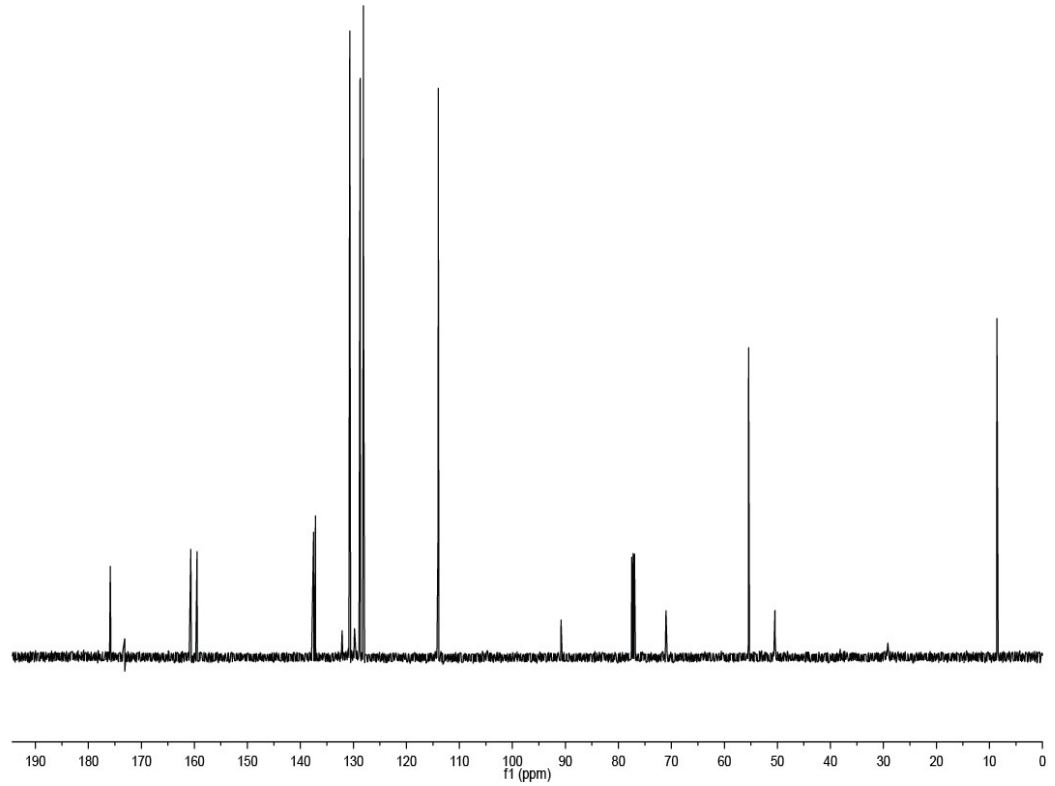

2.30
2.90
4.96
7.62
3.51
1.00
4.78
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

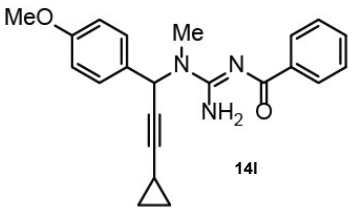

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
NH2
O
Cl

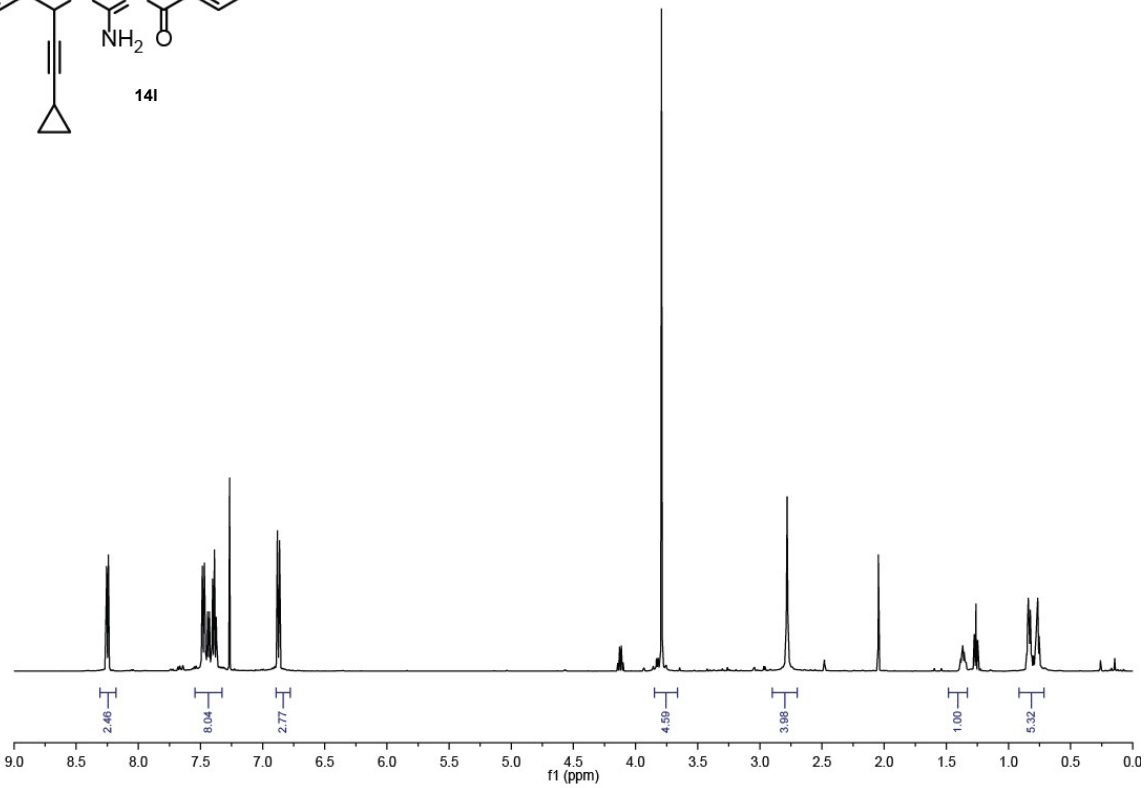
14k

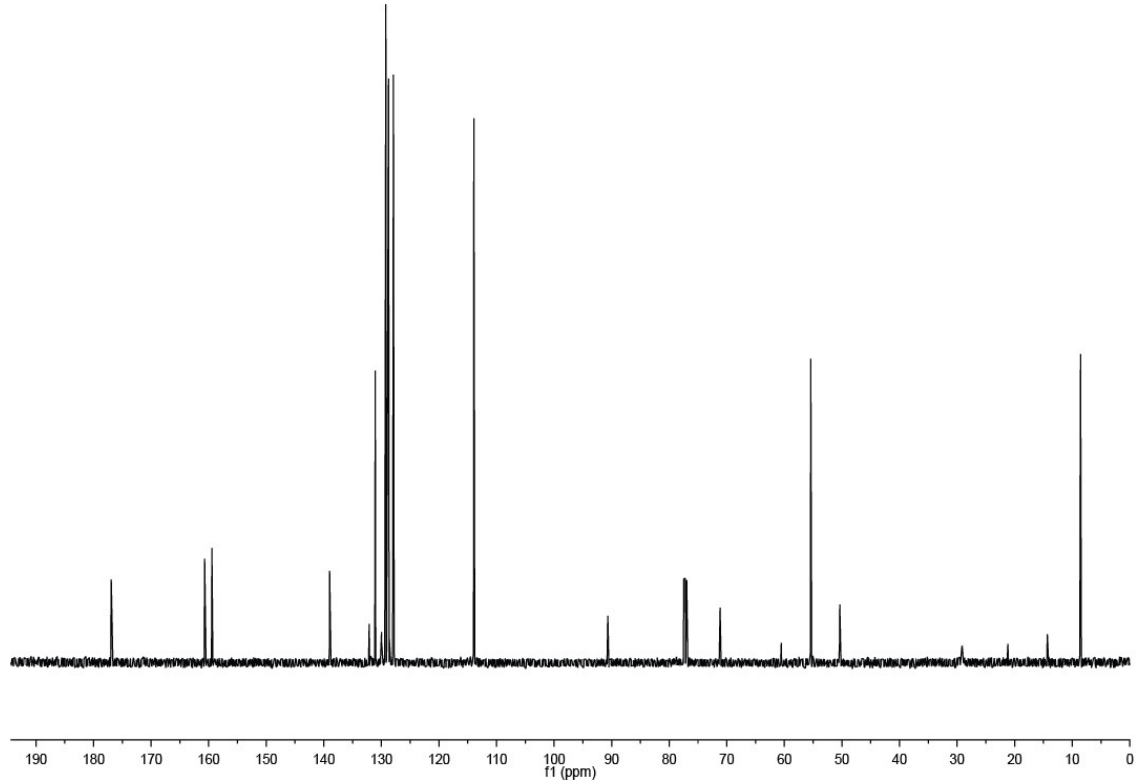

2.28
5.75
2.61
4.13
3.57
1.00
4.91
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

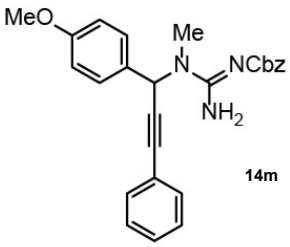

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
NH2
O

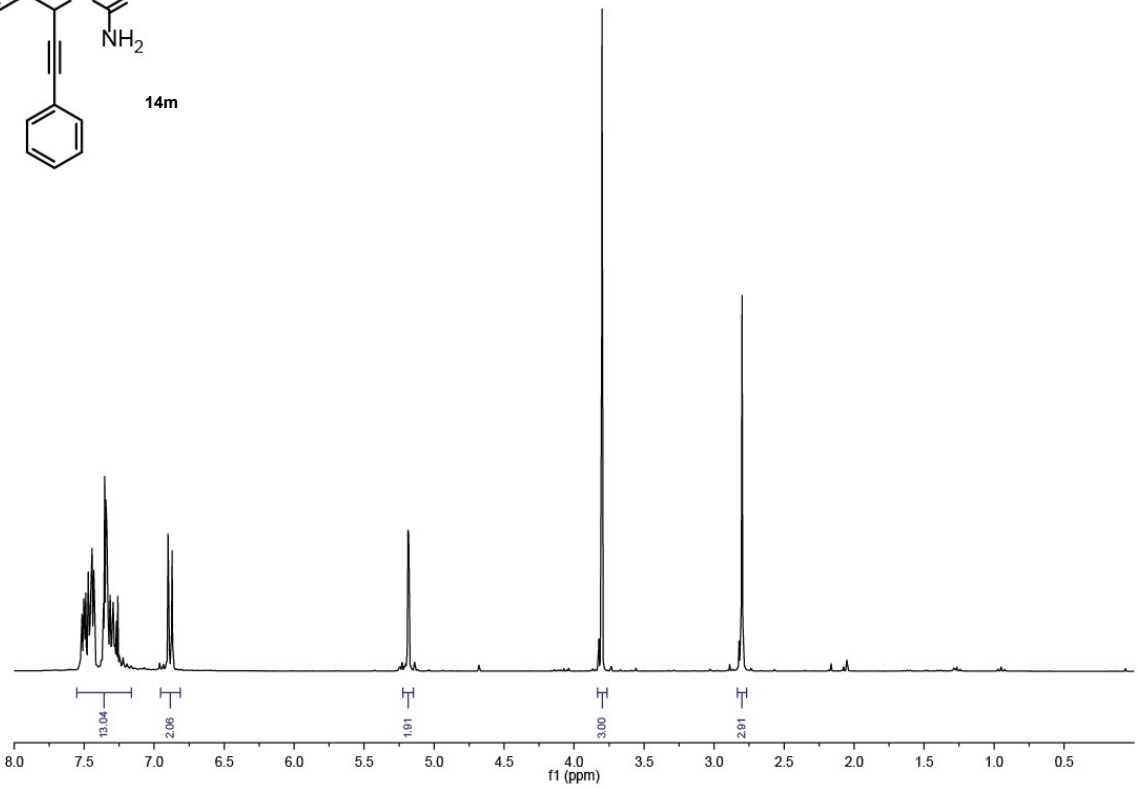
14l

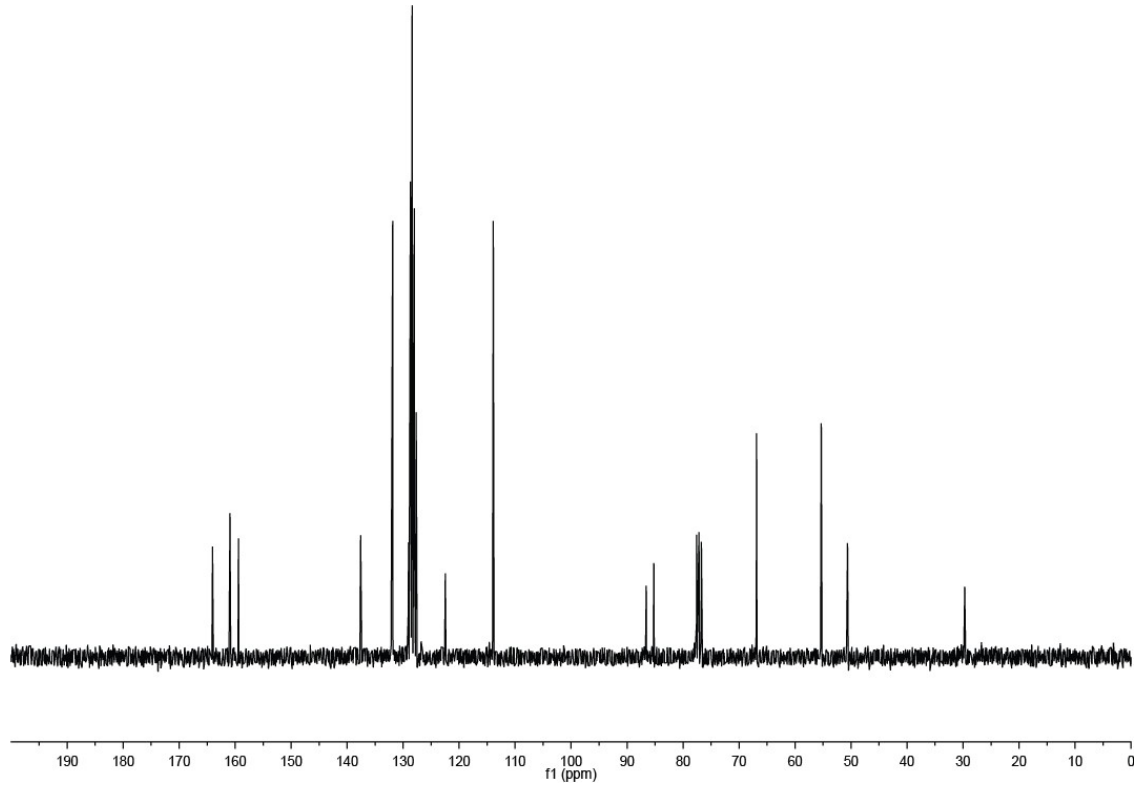

2.46
8.04
2.77
4.59
3.98
1.00
5.32
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

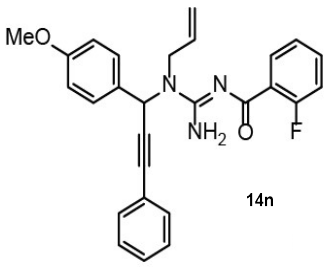

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
NCbz
NH2

14m

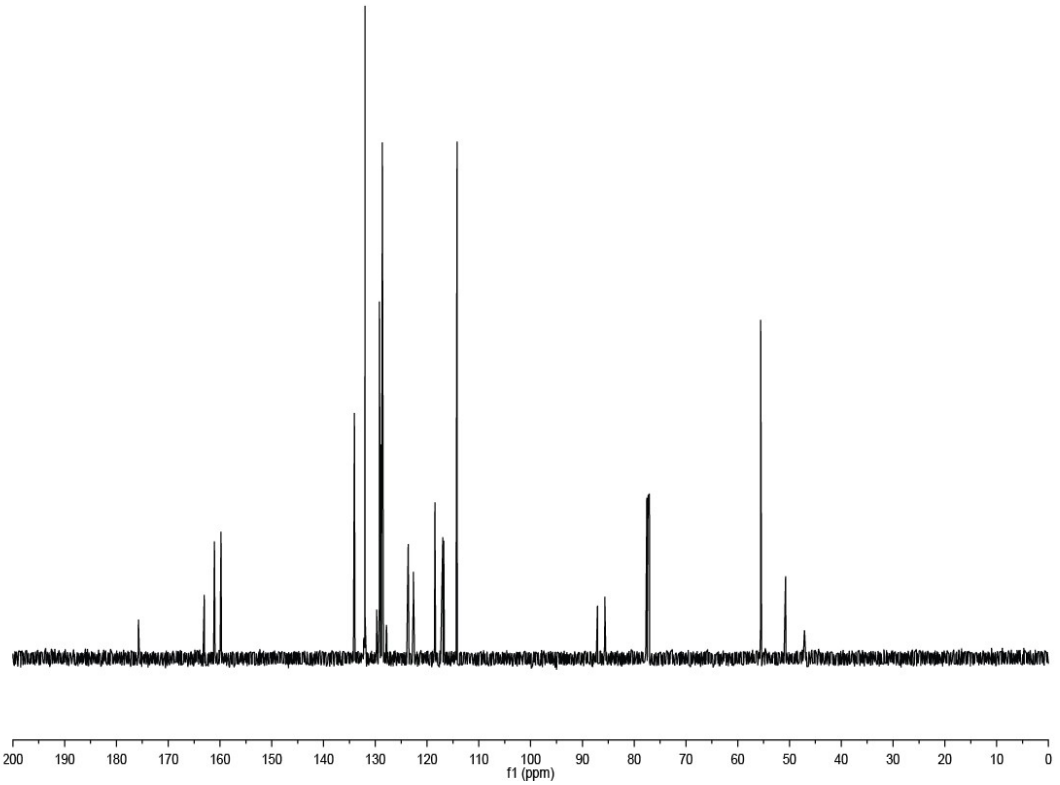

13.04
2.06
1.91
3.00
2.91
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

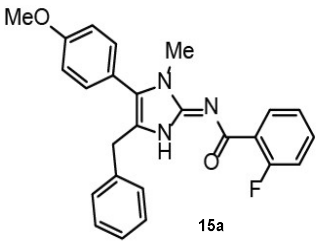

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


MeO
N
N
NH2
O
F

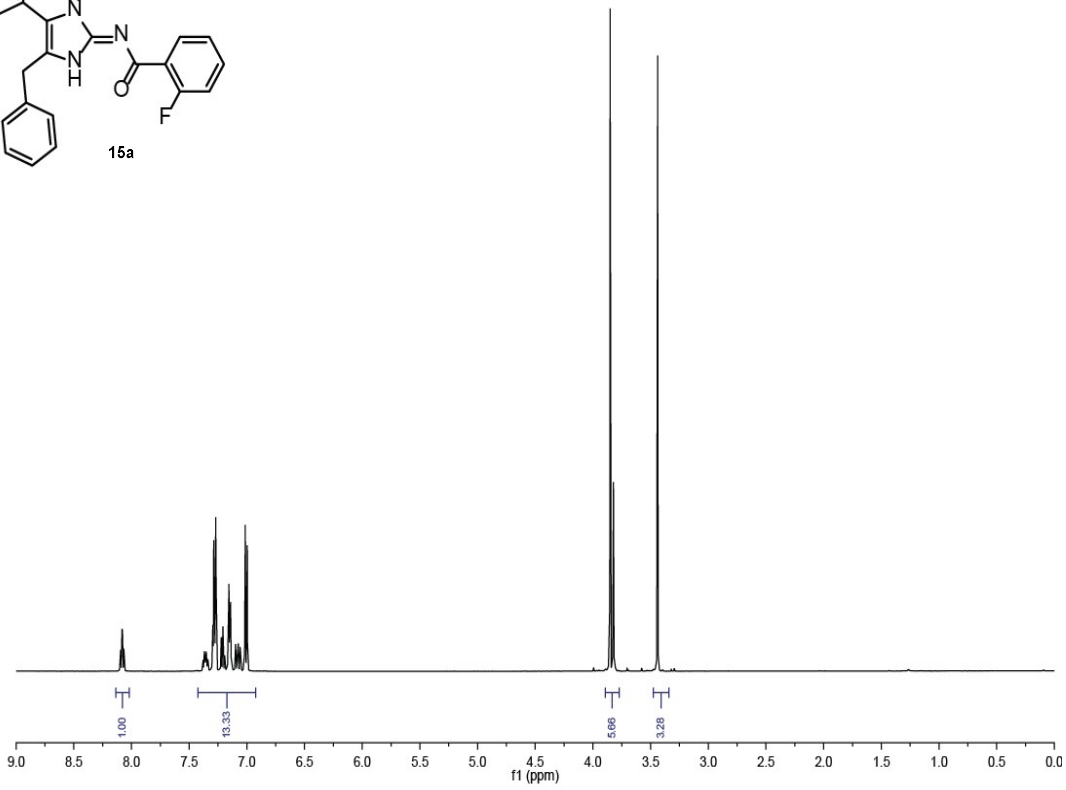
14n

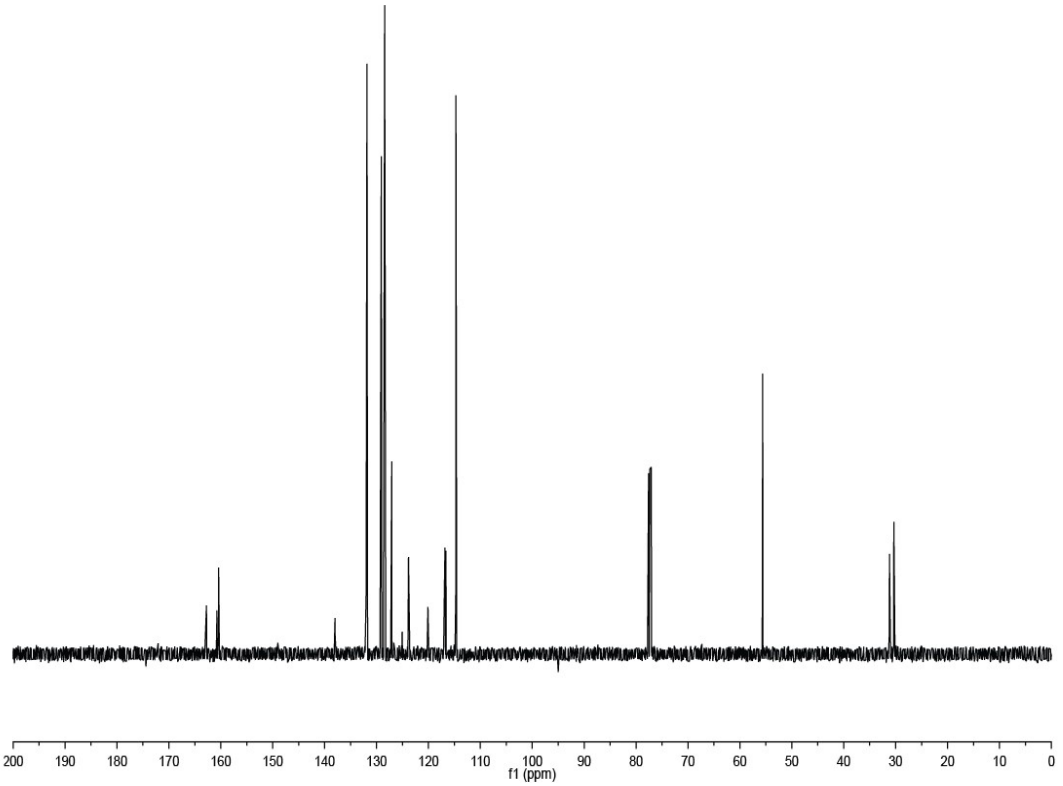
0.92
2.79
2.17
4.29
2.08
2.19
1.00
2.20
1.93
3.46
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

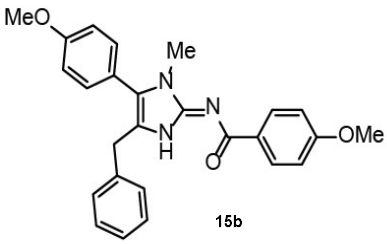

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
N
H
O
F

15a

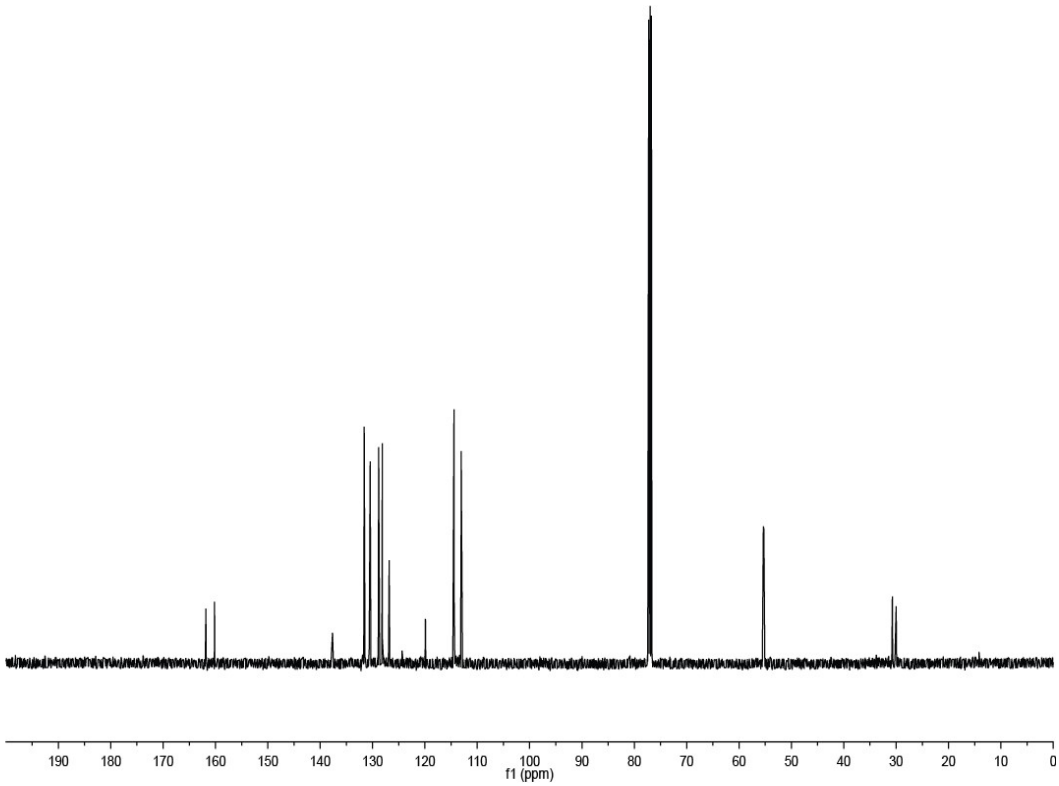

1.00
13.33
5.66
3.28
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

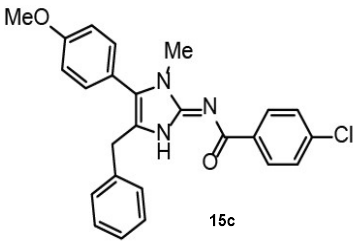

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
H
N
O
OMe

15b

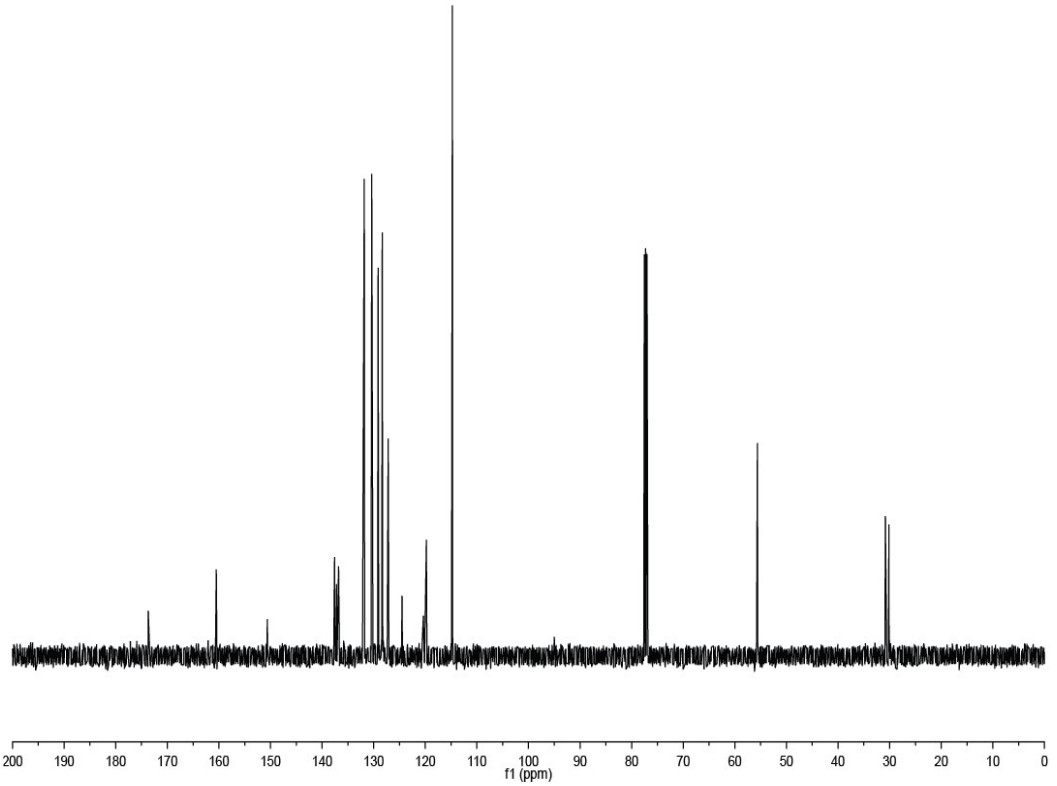
1.33
5.18
1.36
1.55
5.74
2.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)

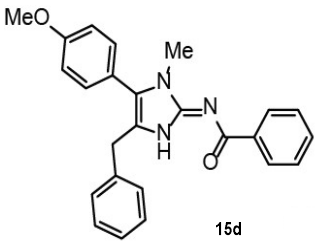

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
N
H
O
Cl

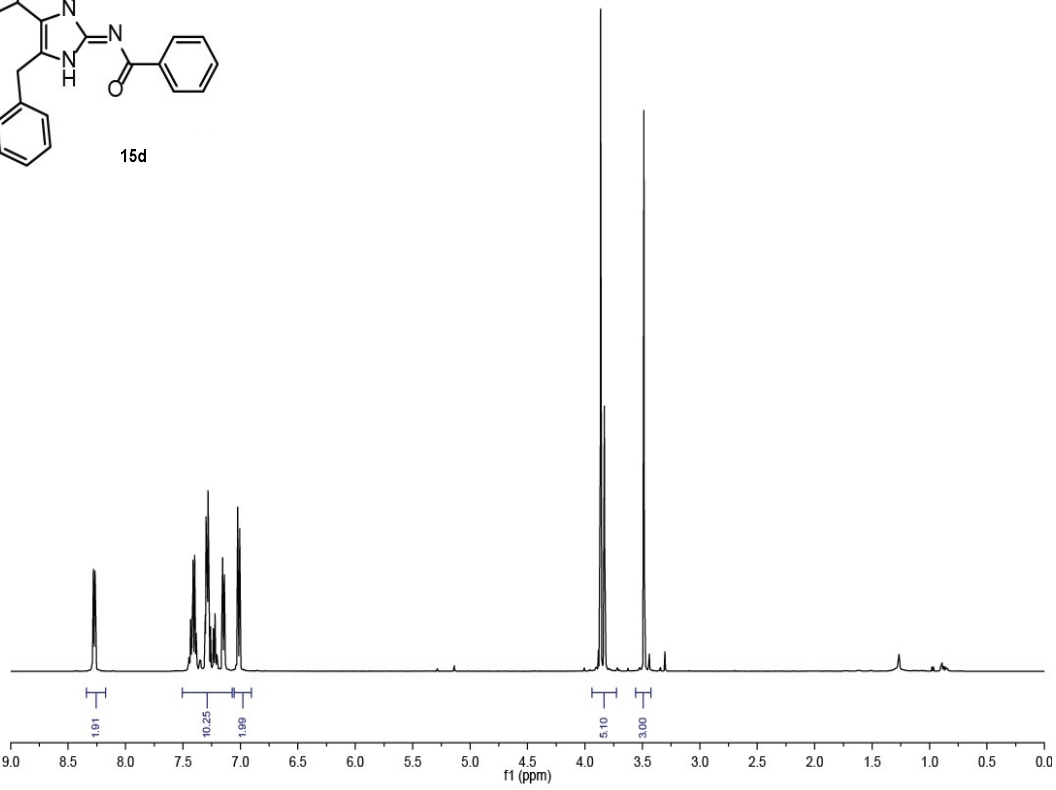
15c

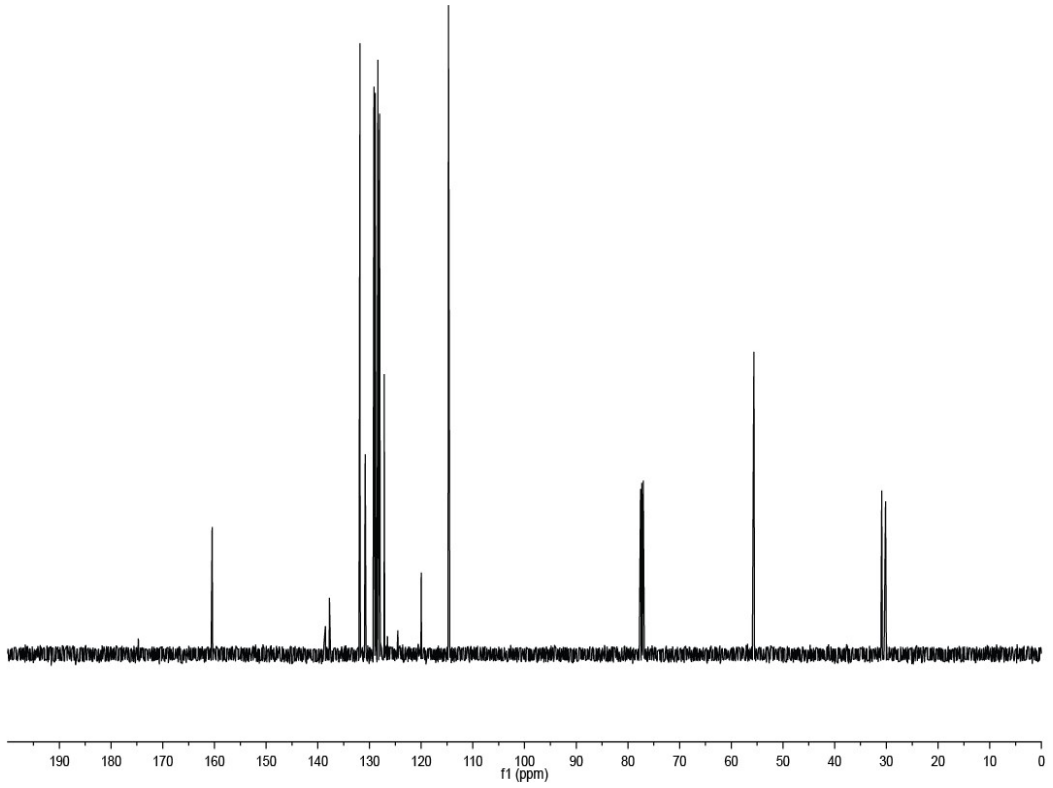
1.89
9.11
2.02
5.15
3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

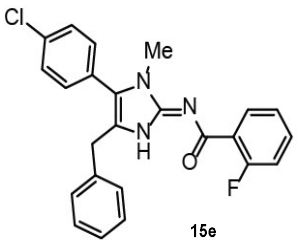

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
N
H
O

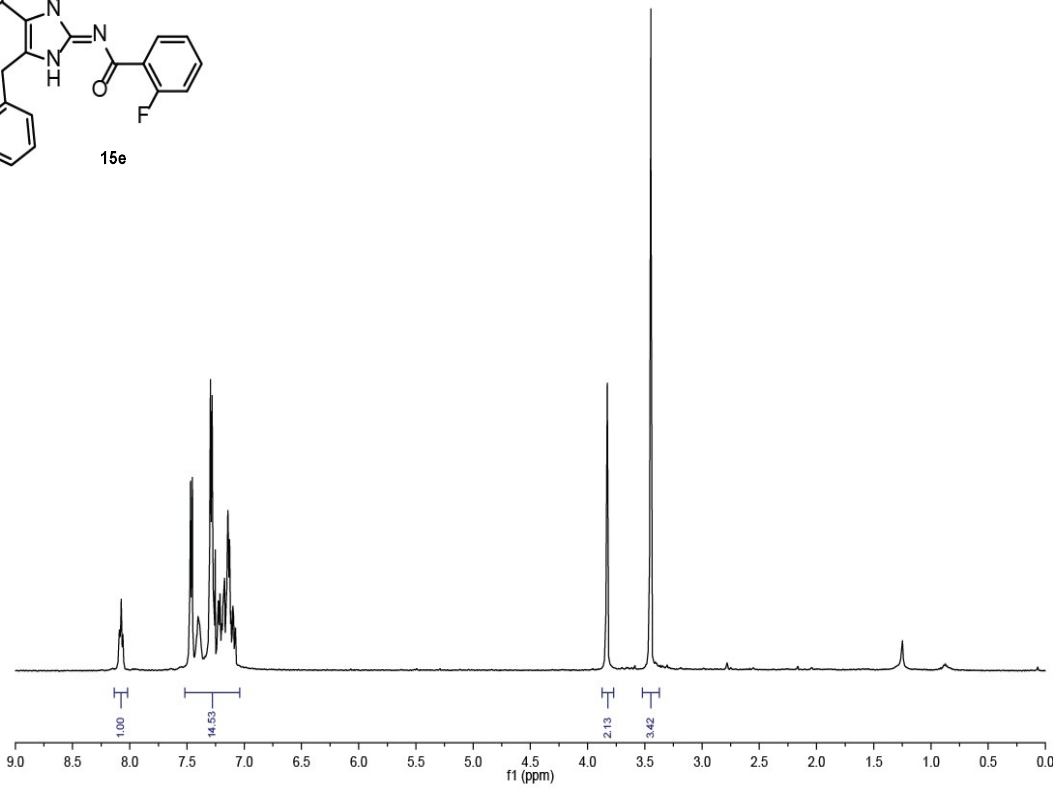
15d

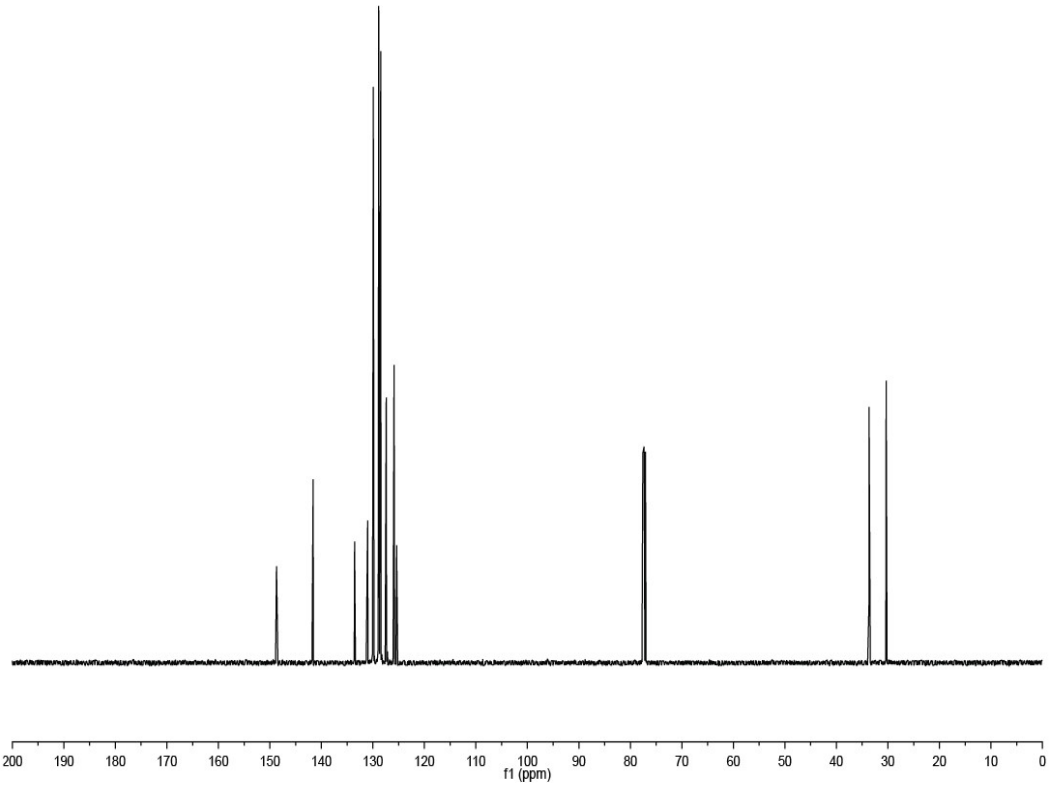

1.91
10.25
1.99
5.10
3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

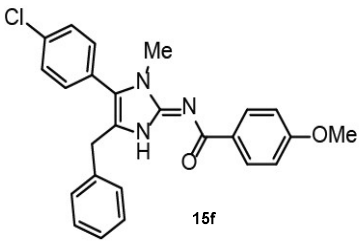

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Cl
Me
N
N
H
N
O
F

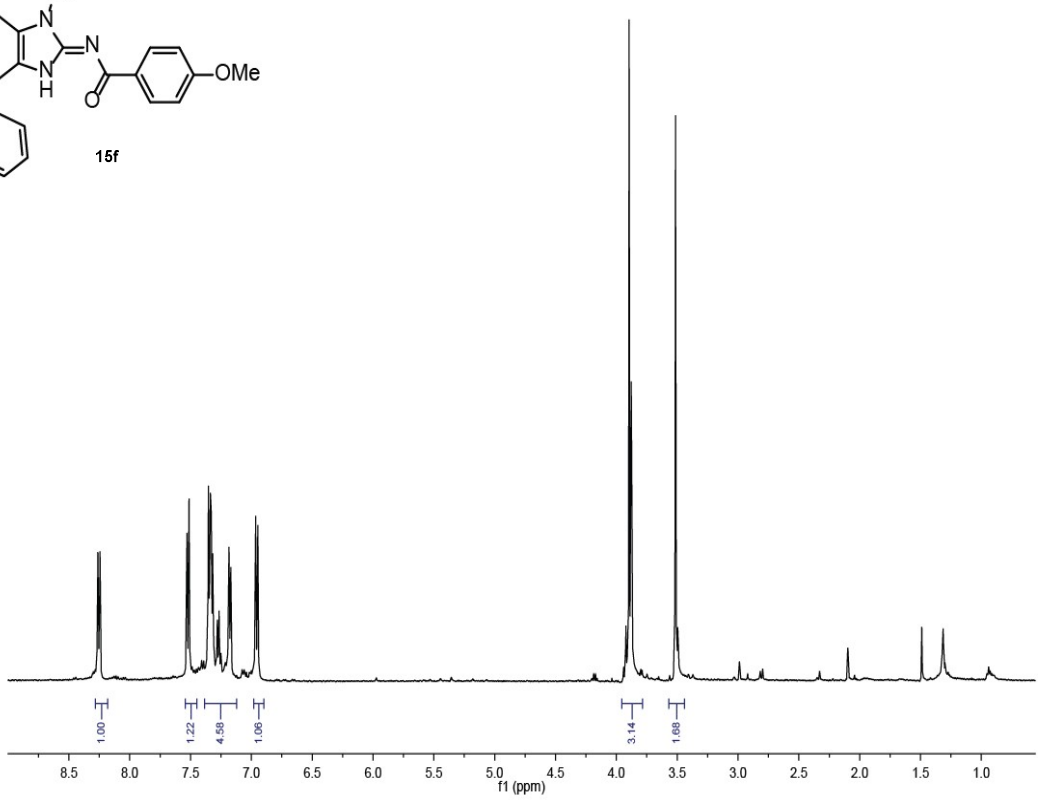
15e

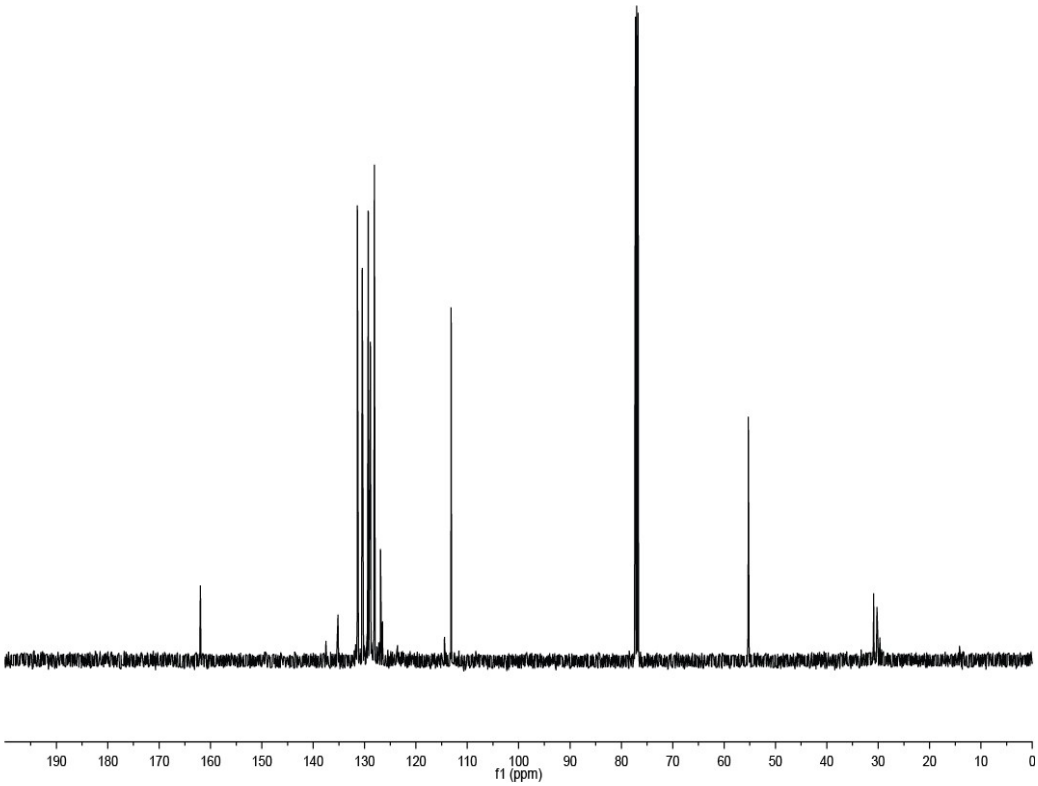

1.00
14.53
2.13
3.42
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

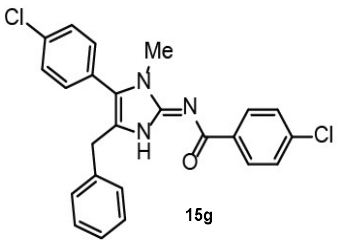

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Cl
Me
N
N
H
N
O
OMe

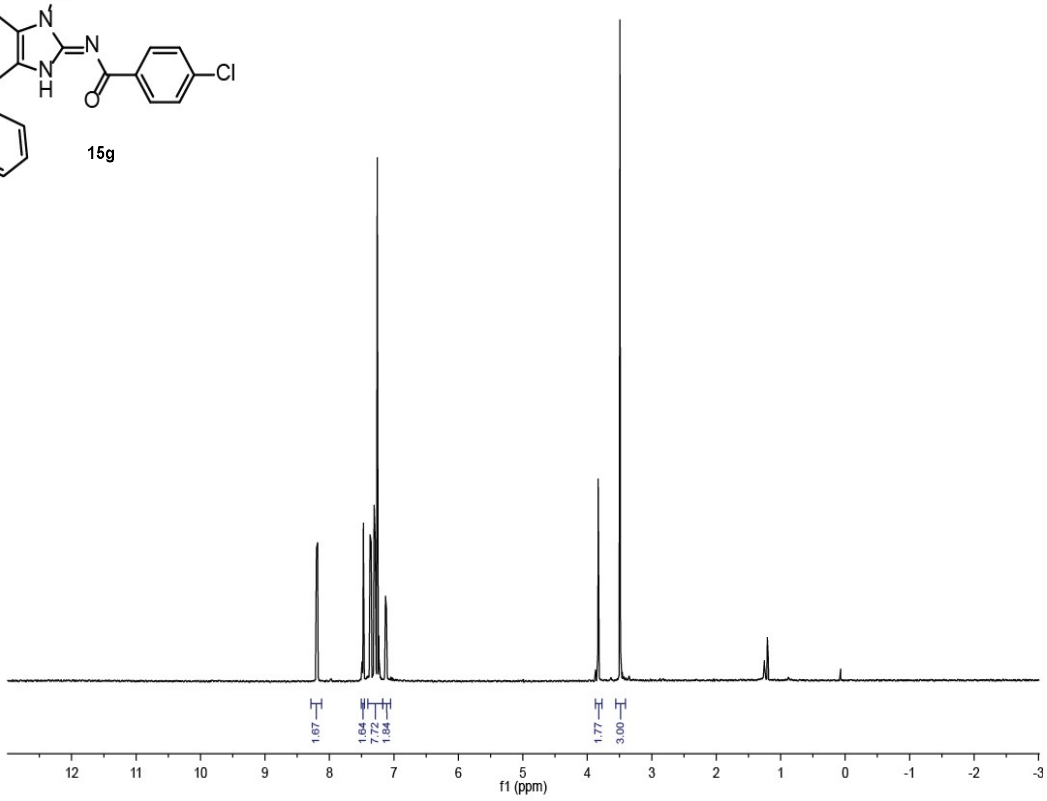
15f

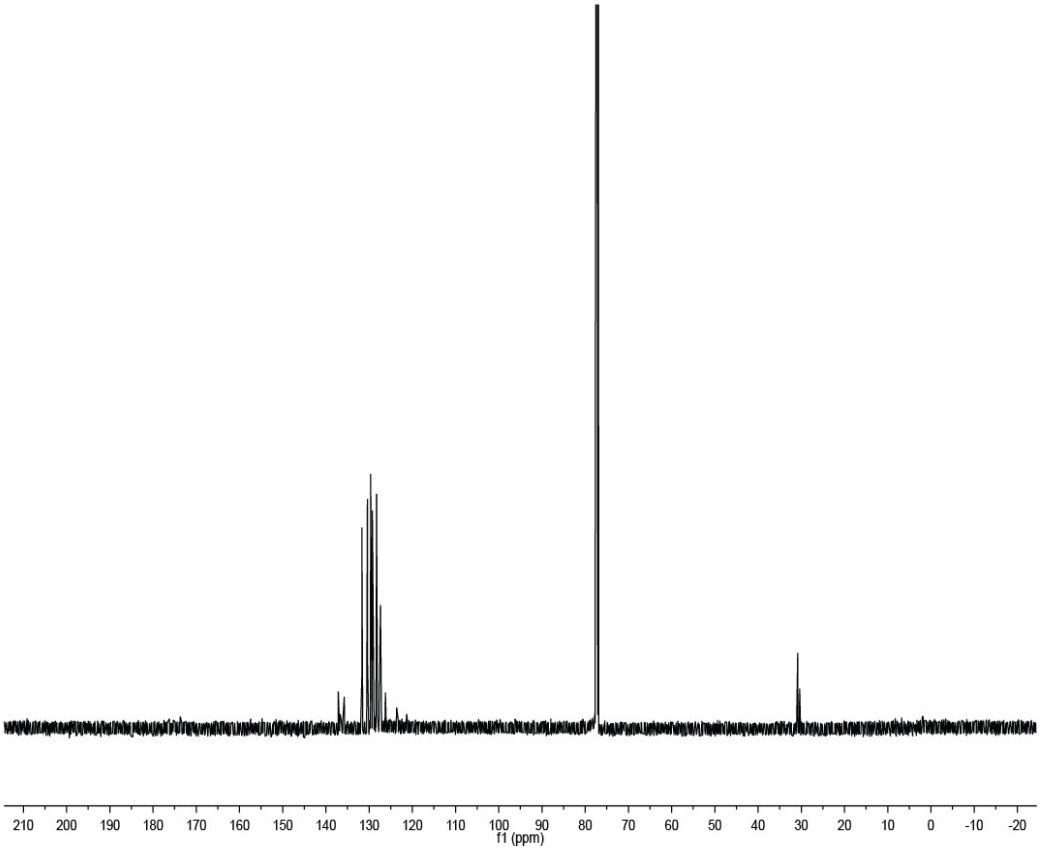

1.00
1.22
4.58
1.06
3.14
1.68
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

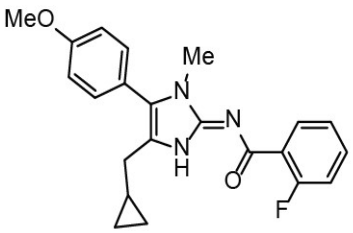

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


Cl
Me
N
N
N
H
O
Cl

15g

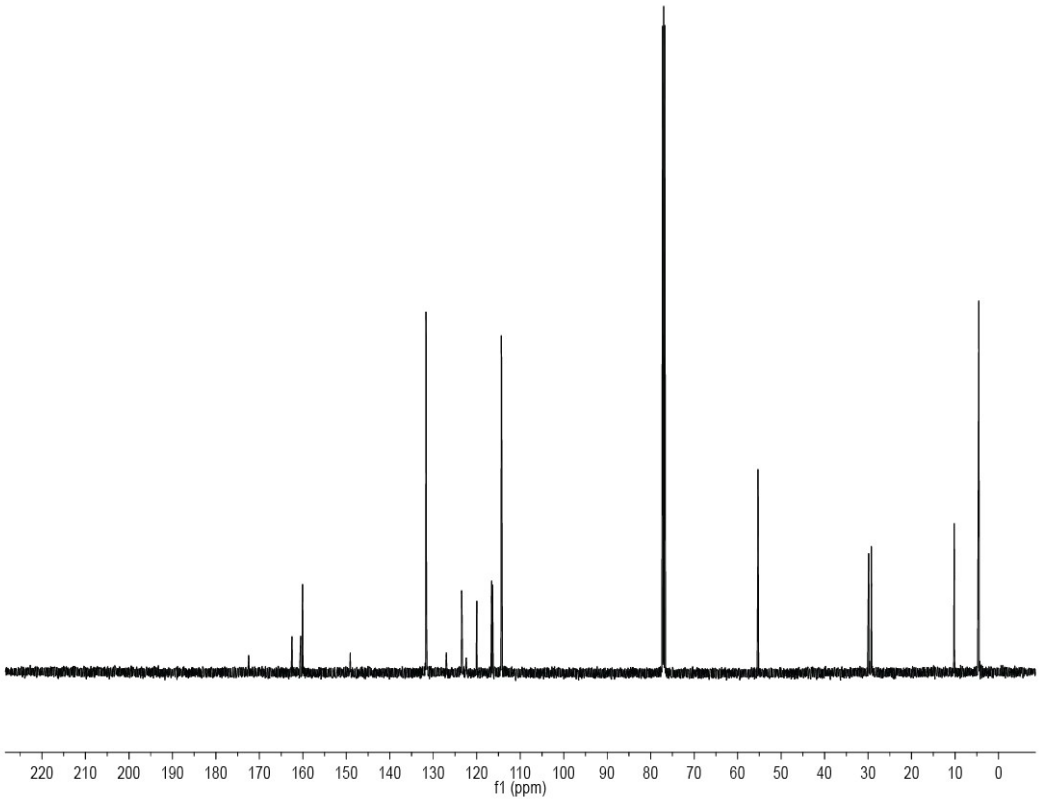

1.67
1.64
7.72
1.84
1.77
3.00
12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)

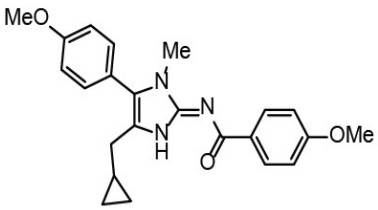

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


MeO
Me
N
N
H
N
O
F

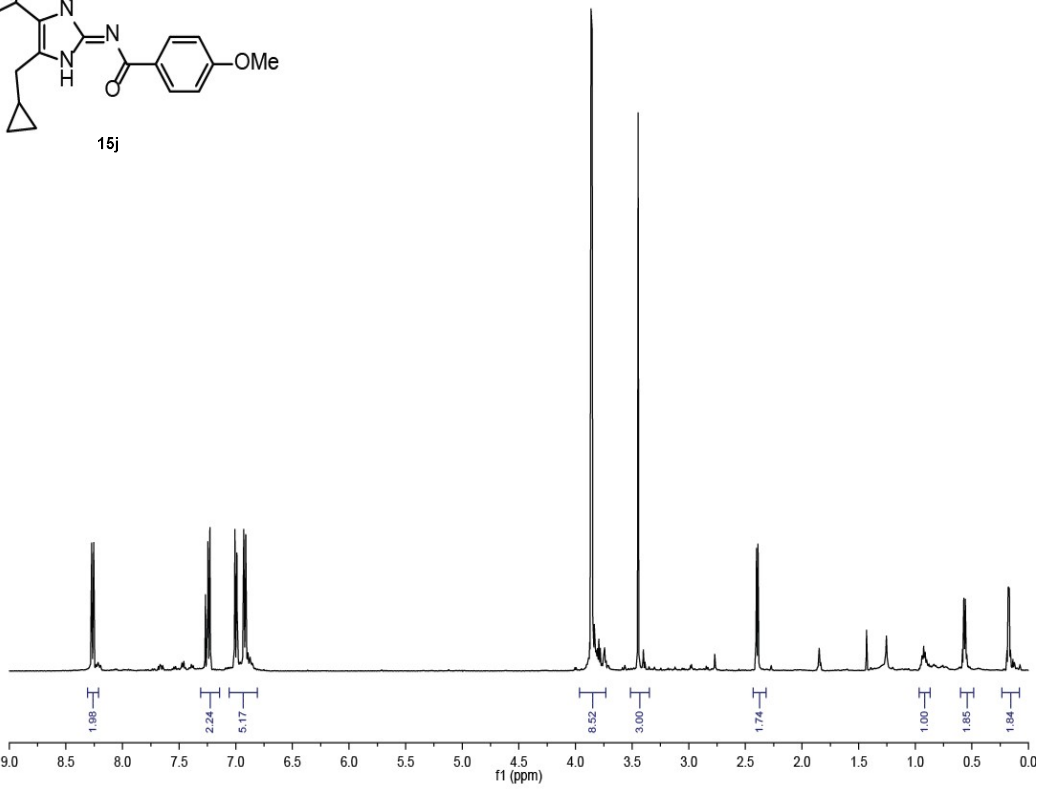
15i

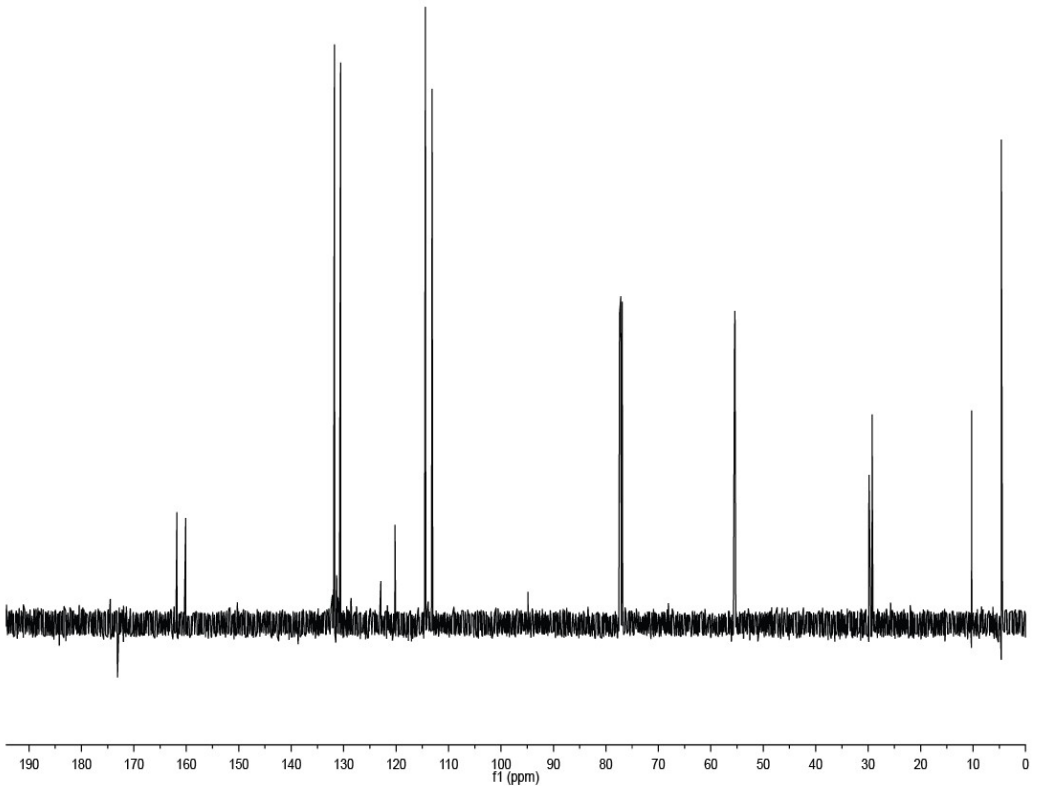
1.00
9.80
4.75
3.02
1.86
0.90
1.87
1.76
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

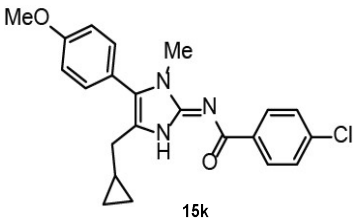

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
N
H
O
OMe

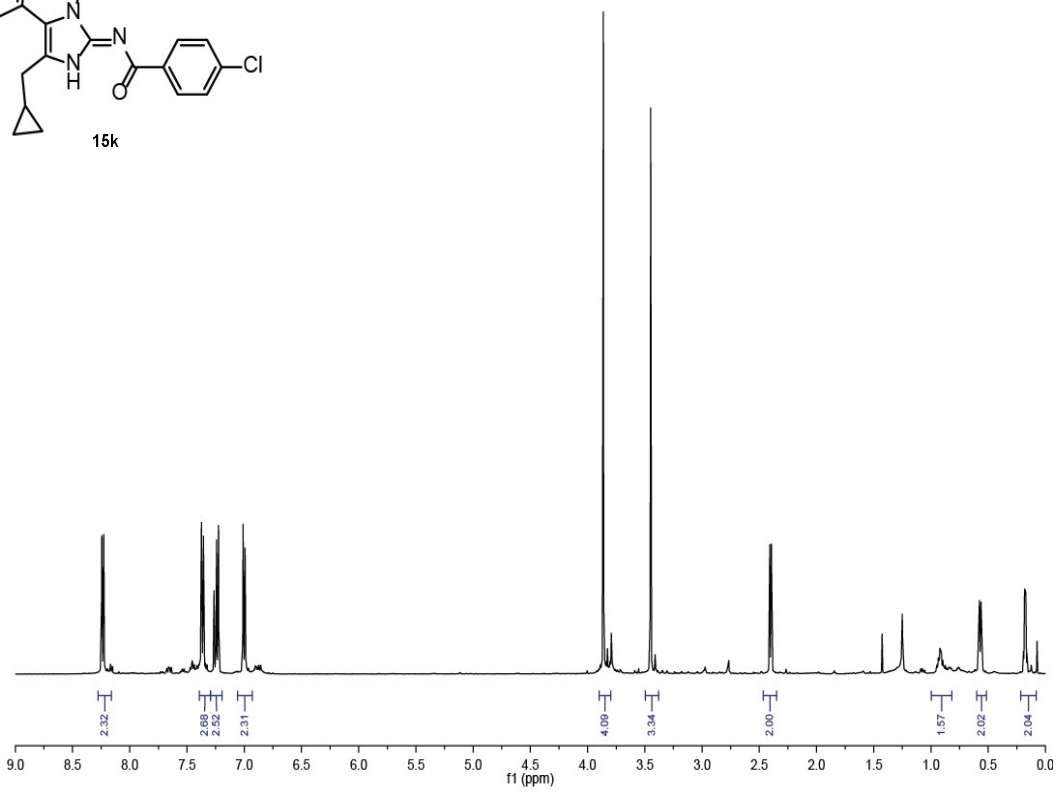
15j

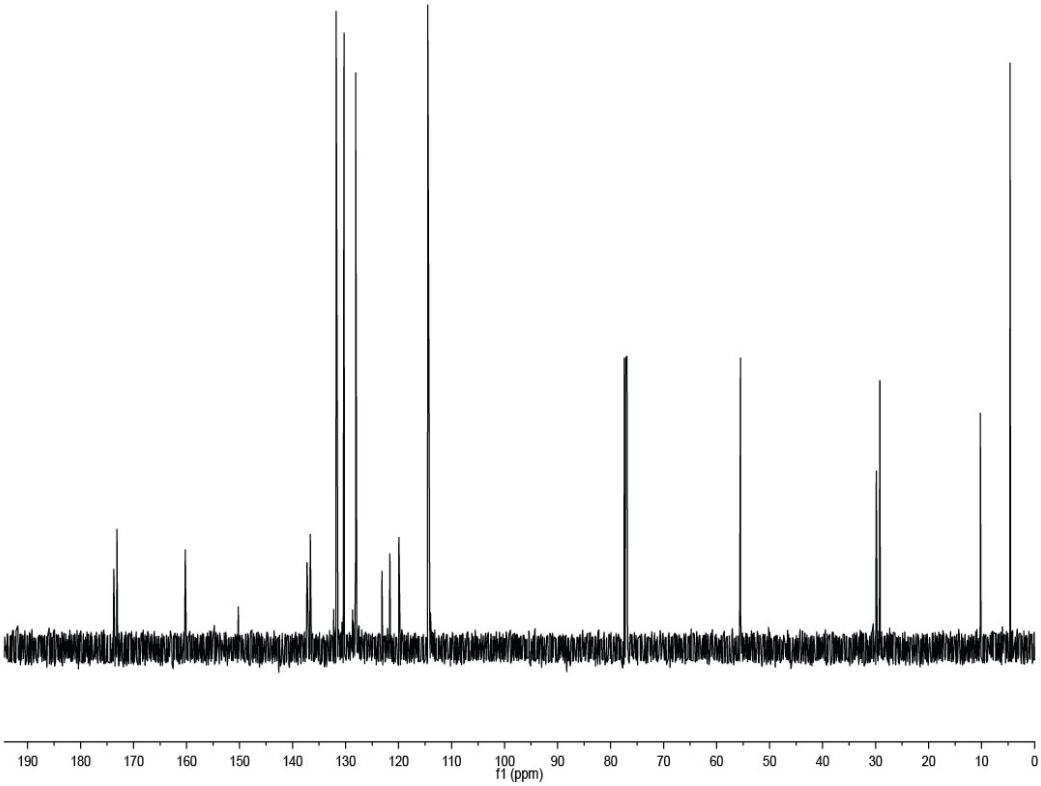

1.98
2.24
5.17
8.52
3.00
1.74
1.00
1.85
1.84
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

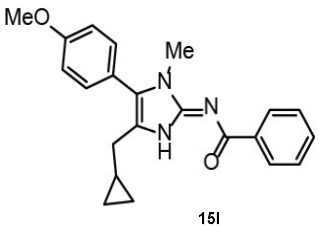

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
H
N
O
Cl

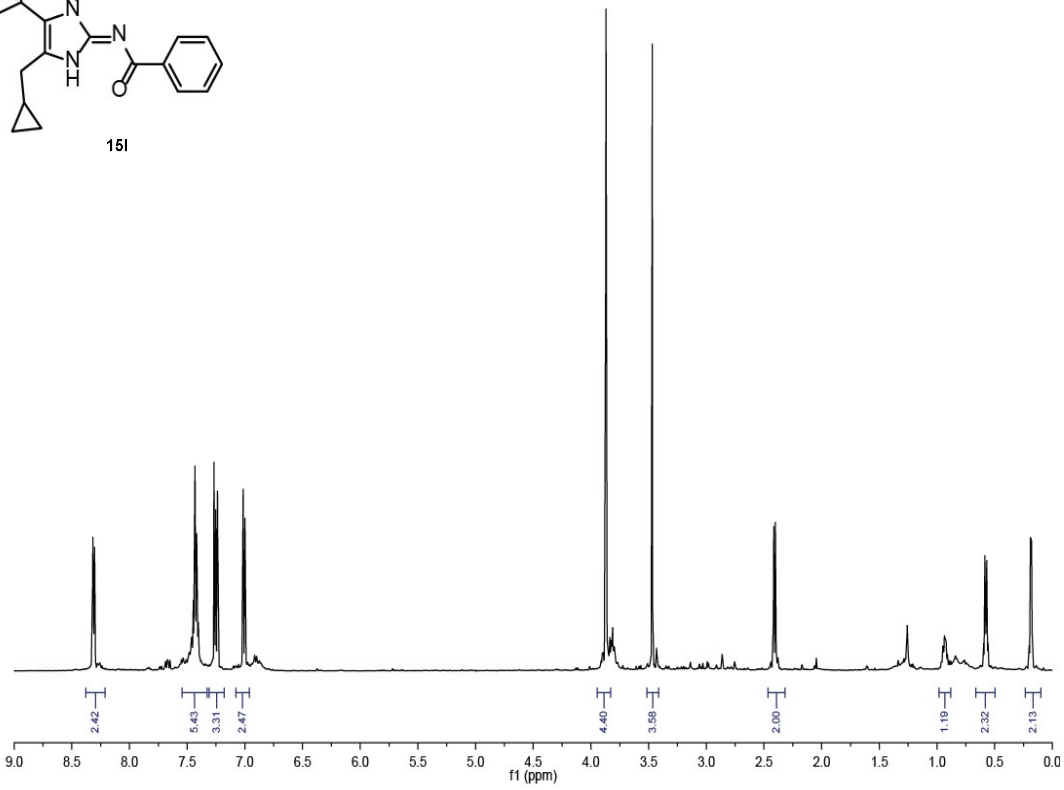
15k

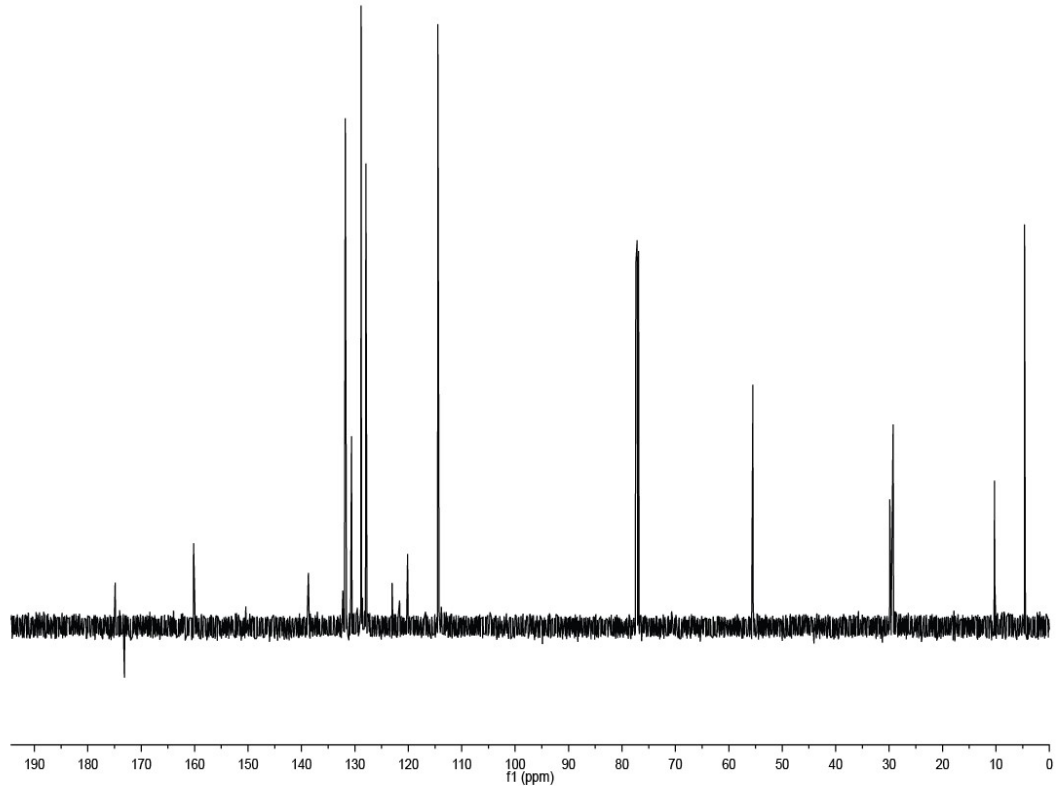

2.32
2.68
2.52
2.31
4.09
3.34
2.00
1.57
2.02
2.04
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

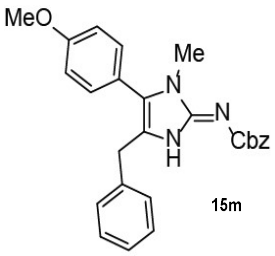

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
N
H
O

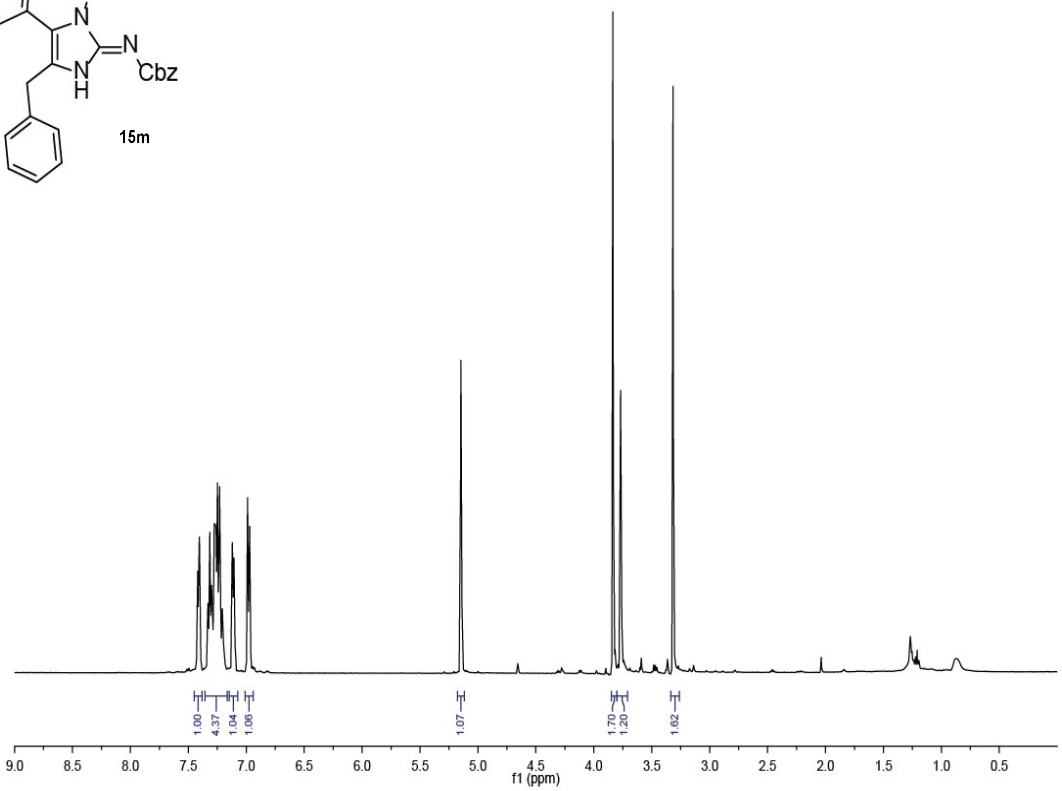
15l

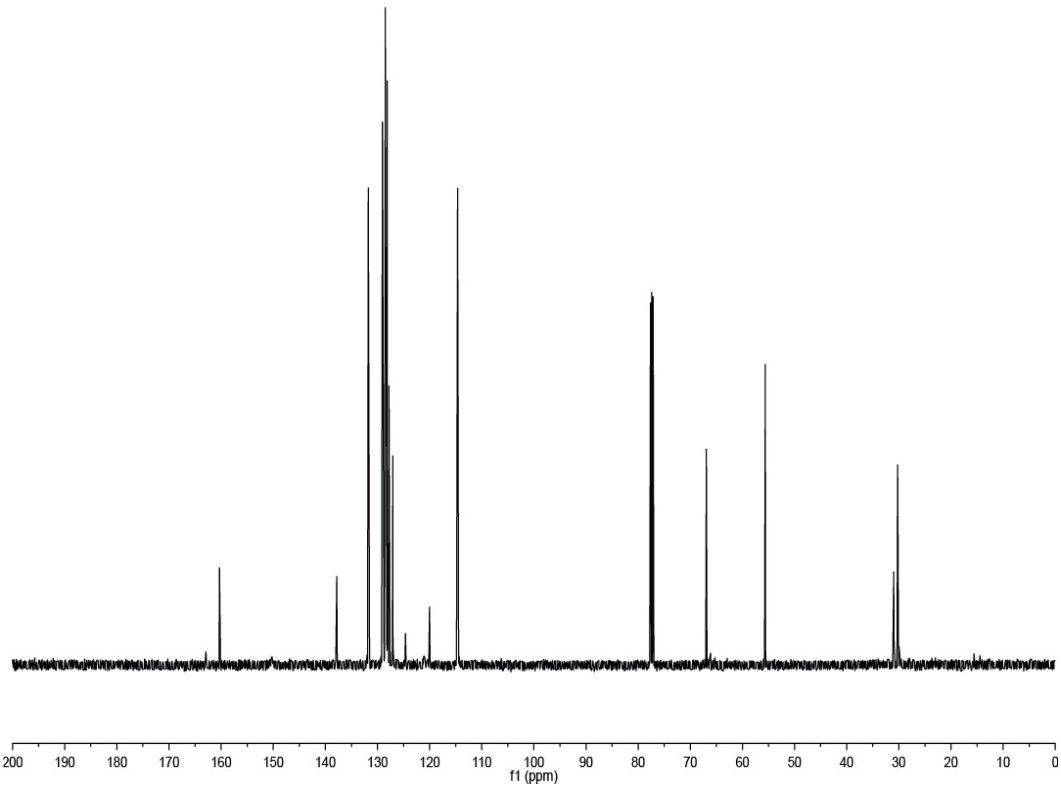

2.42
5.43
3.31
2.47
4.40
3.58
2.00
1.19
2.32
2.13
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

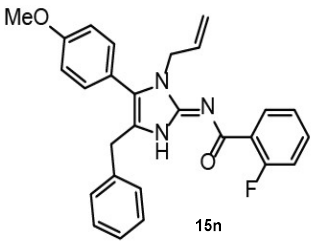

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
Me
N
N
N
H
Cbz

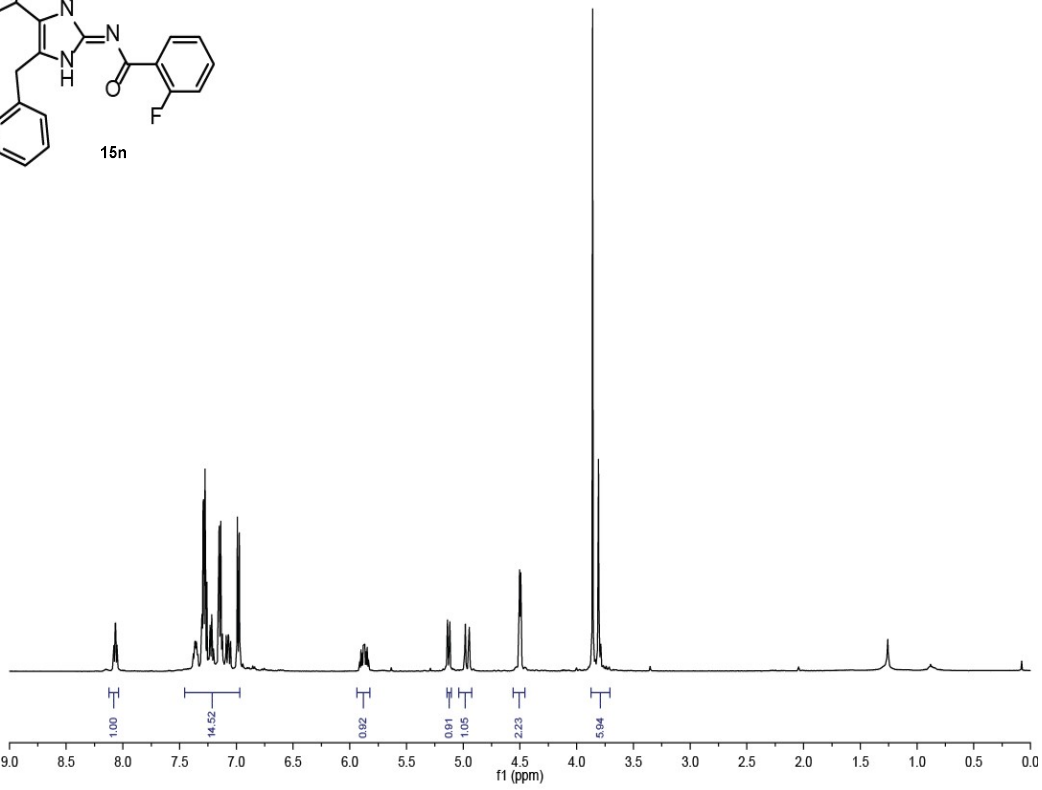
15m

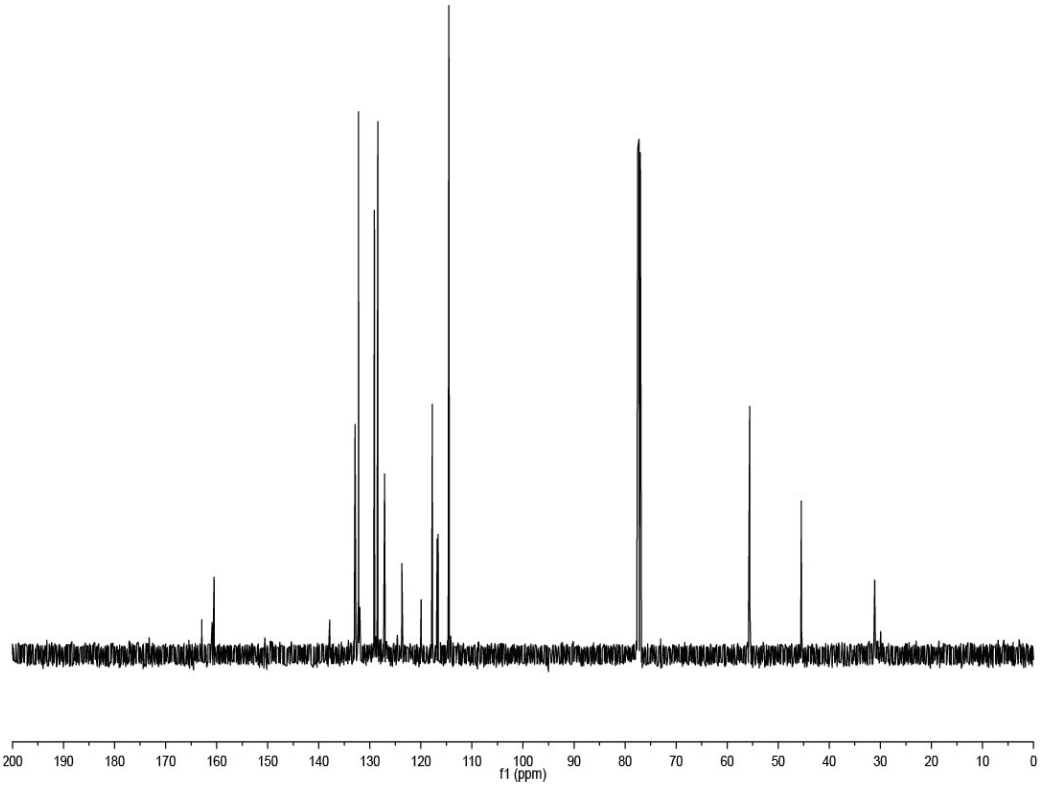

1.00
4.37
1.04
1.08
1.07
1.70
1.20
1.62
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


MeO
N
N
H
O
F

15n


1.00
14.52
0.92
0.91
1.05
2.23
5.94
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

5.6 References

1. Koswatta, P. B.; Lovely, C. J. Structure and Synthesis of 2-aminoimidazole Alkaloids from Leucetta and Clathrina Sponges. *Nat. Prod. Rep.* **2011,** *28,* 511-528.

2. Copp, B. R.; Fairchild, C. R.; Cornell, L.; Casazza, A. M.; Robinson, S.; Ireland, C. M. Naamidine A Is an Antagonist of the Epidermal Growth Factor Receptor and an in VIvo Active Antitumor Agent. *J. Med. Chem.* **1998,** *41,* 3909-3911.

3. Aaronson, S. Growth Factors and Cancer. *Science* **1991,** *254,* 1146-1153.

4. Gainer, M. J.; Bennett, N. R.; Takahashi, Y.; Looper, R. E. Regioselective Rhodium (II)-Catalyzed Hydroaminations of Propargylguanidines. *Angew. Chem. Int. Ed.* **2011,** *50,* 684-687.

5. Gibbons, J. B.; Salvant, J. M.; Vaden, R. M.; Kwon, K. H.; B.E., W.; Looper, R. E. Synthesis of Naamidine A and Selective Access ot N2-Acyl-2-aminoimidazole Analogues. *J. Org. Chem* **2015,** *80,* 10076-10085.

6. R.E., L.; Haussener, T. J.; Mack, J. B. Chlorotrimethylsilane Activation of Acylcyanamides for the Synthesis of Mono-N-acylguanidines. *J. Org. Chem.* **2011,** *76,* 6967-6971.

7. Ohta, S.; Tsuno, N.; Nakamura, S.; Taguchi, N.; Yamashita, M.; Kawasaki, I.; Fujieda, M. Total Synthesis of Naamine A and Naamidine A, Marine Imidazole Alkaloids. *Heterocycles* **2000,** *53,* 1939-1955.

8. Zhang, N.; Zhang, Z.; Wong, I.; Wan, S.; Chow, L.; Jiang, T. 4,5-Disubstituted Benzylimidazole-2-substituted Amines as he Structure Template for the Design and Synthesis of Reversal Agents Against P-gp-mediated Multidrug Resistance Breast Cancer Cells. *European Journal of Medicinal Chemistry* **2014,** *83,* 74-83.

9. Gligorich, M. K.; Vaden, R. M.; Shelton, D. N.; Wang, G.; Matsen, C. B.; Looper, R. E.; Sigman, M. S.; Welm, B. E. Development of a Screen to Identify Selective Small Molecules Active Against Patient-Derived Metastatic and Chemoresistant Breast Cancer Cells. *Breast Cancer Res.* **2013,** *15,* R58.

10. Salvant, J. M.; Edwards, A. V.; Gibbons, J. B.; Kuerk, D.; Looper, R. E. Regioselective Synthesis of N2-acyl-2aminoimidazoles via Base Catalyzed Cyclizations of Mono-N-acylpropargylguanidines. *Submitted to J. Org. Chem.*

11. M., V. R.; Santiago, C. B.; Arunachalim, S.; Gibbons, J. B.; Salvant, J. S.; Sigman, M. S.; Looper, R. E.; Welm, B. E. The Novel Small Molecule Zinaamidole Induces Intracellular Zn2+ Dyshomeostasis and Cancer-Selective Cell Death via a Caspase Independent Mechanism. *In Preperation for Nat. Chem. Biol*

12. Carmely, S.; Kashman, Y. Naamines and Naamidines, Novel Imidazole Alkaloids from the Calcareous Sponge Leucetta chagosensis. *Tetrahedron Lett.* **1987,** *28,* 3003-3006.

13. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities *J. Org. Chem.* **1997**; *62*(21); 7512-7515.

14. Hoye, T.R.; Hansen, P.R.; Vyvyan, J.R. A Practical Guide to First-Order Multiplet Analysis in 1H NMR Spectroscopy *J. Org. Chem.* **1994**; *59*(15); 4096-4103.