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TOTAL SYNTHESIS OF NOMINAL 4*S*,7*R*,11*S*,14*R* CYCLOCINAMIDE B AND ANALYSIS OF THREE STEREOISOMERS OF CYCLOCINAMIDE A

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Stephanie Maureen Stepp Curzon

June 2012

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Tyrus Miller
Vice Provost and Dean of Graduate Studies

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2012

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Abstract

Title: Total Synthesis of Nominal 4*S*,7*R*,11*S*,14*R* Cyclocinamide B and analysis of stereoisomers of Cyclocinamide A

Author: Stephanie Maureen Stepp Curzon

The marine natural product cyclocinamide A was isolated from a *Psammocinia* sponge found in the waters of Papua New Guinea. The very similar natural product cyclocinamide B was isolated from the sponge *Cortium sp*. in Fijian waters. The cyclic tetrapeptide core of both natural products consist of two α-amino acids (5-bromotryptophan and asparagine) and two β- amino acids (1,2-diaminopropionic acid and isoserine). Both have a glycine chlorinated *N*-methyl pyrrole dipeptide side chain. The isolation and two previous total synthetic efforts are discussed in chapter 1. Original research resulting in the total synthesis of (4*S*,7*R*,11*S*,14*R*) cyclocinamide A and B is presented in chapters 2 and 3. Cyclocinamide is disconnected into three dipeptides: glycyl-4,5-dichloro-*N*-methylpyrrole, isoseryl-5-bromotryptophan and diaminopropionyl-(cyclo)asparagine. The synthesis of these three dipeptides is highlighted in chapter 2. The formation of the cyclic core and completion of the synthesis of the 4*R*,7*S*,11*R*,14*S* isomer of cyclocinamide A and B is detailed in chapter 3. The full NMR analysis in chapter 4 of the 4*R*,7*S*,11*R*,14*S*, 4*S*,7*S*,11*S*,14*S* and 4*S*,7*S*,11*R*,14*S* reveals that the assignment of both cyclocinamide A and B to be incorrect and further experimentation is needed to determine the actual stereochemistry of the natural products.

This dissertation is dedicated to my family for all the support I received to pursue my dreams.

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Copyright Notices

The text of this dissertation contains reprints of the following material:

Chapters 2 and 4:

Garcia, J.M., Curzon, S.S., Watts, K.M., Konopelski, J.P. "Total Synthesis of Nominal (11*S*)- and (11*R*)-Cyclocinamide A" *Org. Lett.,* **2012**, *14,* 2054-2057

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Chapter 1 – Introduction to small cyclic peptides and a review of the cyclocinamides

1.1) Overview of Cyclic Peptides

Protein-protein interactions occur through the recognition of the secondary structural conformations of the peptides. The two most important secondary structures in proteins are α-helices and β-sheets. In a β-sheet the structural motif that allows the peptide to fold back on itself thereby allowing the strands to form an anti-parallel arrangement is known as a β-turn. The β-turn is used as a recognition site between peptides and has become an important motif in drug targeting and mimicking. A β-turn consists of four amino acids with at least one turn promoting residue. Two of the major classes of β-turns that meet the criteria are called type I and type II as shown in Figure 1.1. Type I turns are the most prevalent in naturally occurring proteins. The *i* position has an amino acid with hydrogen bond accepting side chains capable of forming a hydrogen bond to the N-H of the *i*+2 residue. In the *i*+2 a proline is not seen because of the constrain of the ring. In the type II turn, the *i*+1 position can accommodate an L-residue; proline is common in this position. The *i*+2 position favors a glycine, small polar L-residue, or a D-residue due to steric clash with a side chain in the L-configuration. Proline in position *i*+1 is a strong sequence

determinant for either a type I or type II turn because of the restriction on the angle from the cyclic side chain.[1]

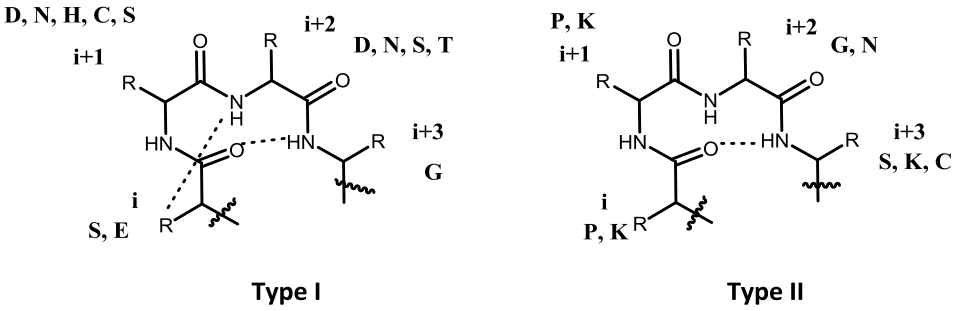


Figure 1.1 Types of β-turns

Small molecules that can mimic β-turn interactions are useful as probes for biologically active conformations. In order to properly mimic these interactions, a variety of scaffolds have been developed to carefully place the ligands in the three dimensional space occupied by the amino acid side chains on the β-turn. The most noteworthy turn mimics are small cyclic peptides.[2] Small cyclic peptides offer the ability to better mimic the natural state of the peptide with greater efficiency.

Naturally occurring cyclic peptides are increasingly being discovered. A majority have at least one turn promoting residue present including, but not limited to, D-Pro, D-pipecolic acid (Pip), or an Aib-type (α-methylalanine) residue in the cyclic system.[3] Cyclic peptides offer an advantage over linear peptides because the cyclization of a linear peptide restricts the conformational space that can be occupied which can increase potency and selectivity to a given receptor. In addition

to constraining the system cyclic peptides are more difficult to degrade. Even with those advantages, the use of peptides as drugs has not been widely embraced by the pharmaceutical industry. Only a few such compounds have become drugs, such as integrilin **1.1**, octreotide **1.2**, and cyclosporine A **1.3**, shown in Figure 1.2.[3] Most of the FDA approved peptide drugs are larger rings and are flexible with the ability to adopt multiple conformations in solution. This appears to allow for easy passage through the cell membrane and conformational stability in water.[4]

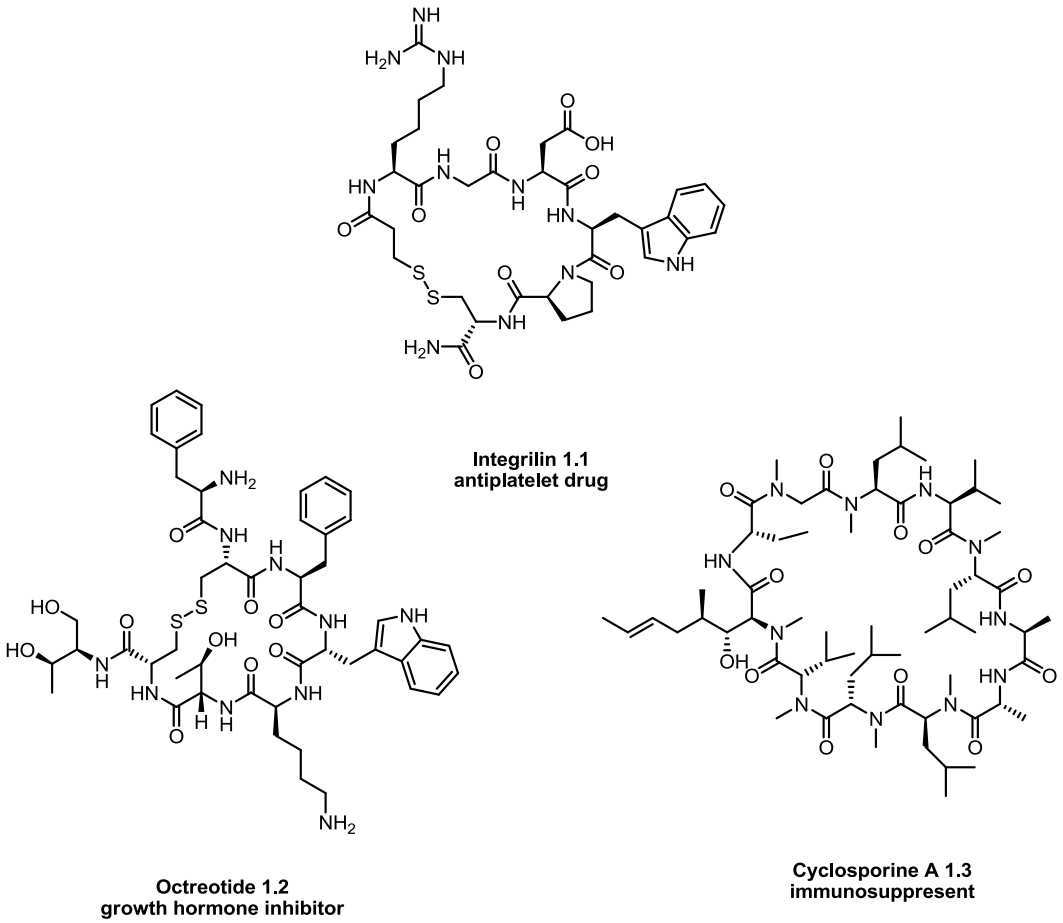


Figure 1.2 Cyclic peptides used as FDA approved drugs

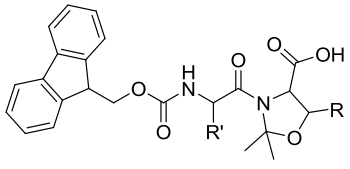
The lack of interest and pursuit of peptides as therapeutics is due to the fact that most do not obey Lipinski's rules.[5] Lipinski rules state that for a molecule to be a good candidate for a drug it must have five or less hydrogen bond donors and up to ten hydrogen bond acceptors. The molecule cannot have a molecular mass of more than 500 daltons and an octanol-water partition coefficient not greater than five. The main rule that is violated by cyclic peptides is the molecular mass which is typically over 500 daltons. Other factors are poor solubility, the high cost associated with reagents required for synthesis, and numerous stereocenters. There is also the question of differences in yield and ease of cyclization in the head-to-tail manner. Cyclic peptides that are smaller than seven amino acids are difficult to cyclize and success is highly dependent on the linear sequence chosen. The ability to cyclize small peptides is highly variable as shown in the following examples.

1.1.1) Brief overview of 12-membered rings and 15-membered peptide rings

The use of cyclic tetrapeptides has not been extensively explored even though their size and shape offer an attractive mimic to β-turns. In the literature there are very few examples of cyclic 12-membered tetrapeptides.[6] The lack of utilization of these systems is largely due to the great difficulties in synthesizing the highly strained 12-membered ring with four trans amide bonds in the backbone. The

demanding cyclization of these systems frequently causes the terminal residue to undergo epimerization in order to successfully form cyclic material. The strain can be eased by the introduction of a D-amino acid, an *N*-alkylated amino acid or a turn inducing amino acid such as proline.[6] The later two modifications allow access to the cisoid conformation of the amide bond which is more likely to permit cyclization without epimerization.

A notable investigation into the synthesis of 12-membered ring peptides was by the Jolliffe group.[7] They used the turn inducing pseudoprolines to successfully cyclize all-*S* tetrapeptides (Figure 1.3). The pseudoproline constrained the amide backbone in a cis conformation. The pseudoproline was used as a temporary protecting group for serine, threonine or cysteine (See Section 2.4.1 for discussion). Since its discovery a number of derivatives have become commercially available and are used as a protection group and turn inducer.



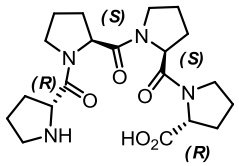
R = H or Me
R' = amino acid side chain

1.4

Figure 1.3 Commercially available pseudoproline Fmoc-Aaa-Ser/Thr($\psi^{Me,Me}$Pro)-OH

Along with the cis/trans nature of the amide bond, the peptide sequence is important and taken into consideration when forming the linear system. An example

of sequence dependent cyclization was demonstrated by Rothe *et al*.[8] They prepared a series of linear tetraprolines, where D- or L-prolines were placed in various locations of the sequences. The only sequence to successfully cyclize was the DLLD-Pro_4 **1.5**; all others failed to give the desired cyclic peptide. The result indicated that the sequence of the linear peptide played an essential role in the success of the cyclization. (Figure 1.4)



1.5

Figure 1.4 Sequence to form cyclic tetraproline

A notable investigation by Reissmann *et al.* into the probability of tetrapeptide cyclization demonstrated that the conformational space occupied by the linear system is critical to the success of the cyclization.[9] The authors examined the rate of cyclization of tetrapeptides containing various amounts of turn inducers in the system. In order to determine the rate of cyclization, the formation of the cyclic peptide as well as the disappearance of the starting material were taken into account and was monitored by HPLC. This allowed for the side reaction of oligomerization as well as cyclodimerization to be accounted for in the rate. It was

found that the sequence of the backbone was extremely important to the success and yield of the reaction.

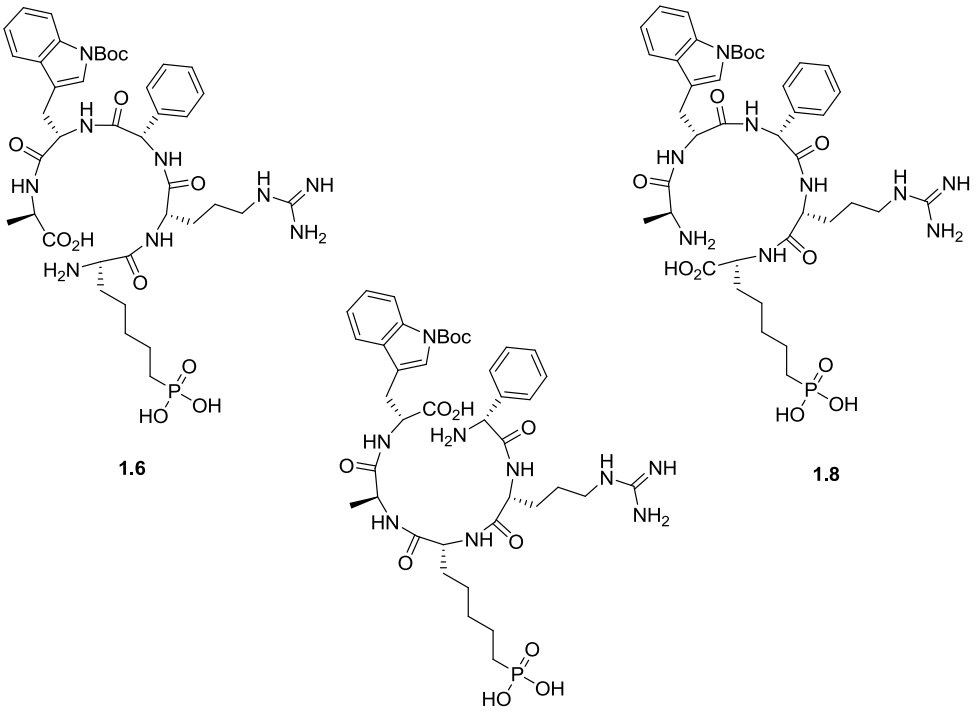


Figure 1.5 Three different linear pentapeptides tested for cyclization

The addition of a single residue increases the ring's flexibility and lowers the strain. An example of pentapeptide cyclization (15 atom ring) is by Kutscher *et al.*, in which they carried out extensive work on cyclization conditions and tendencies.[10] The authors demonstrated that having a small sized residue at the C-terminus was advantageous for cyclization (**1.6** and **1.8**) while a large side chain hindered ring formation and sometimes failed to cyclize (**1.7** shown in Figure 1.5). The *N*-terminus

was not affected by the size of the residue's side chain; instead the configuration of the residue had the most effect. The number and sequence of D-amino acids also had a significant effect on the cyclization. The peptides with a single D-amino acid were easier to synthesize than those containing two or more sequential D residues. The choice and quantity of coupling reagent had a large effect on the cyclization yield. The best reagent for the cyclizations was EDCI/DMAP, giving a 32% yield, followed by DEPBT with 22% and HBTU/DMAP at 12%. The results demonstrated that the success of pentapeptide cyclization is highly depended on multiple variables and is not a trivial event.

Pentapeptides are easier to cyclize than tetrapeptides but they are still difficult. It is only when one gets to the heptapepitde that macrocyclization becomes less residue and sequence dependent and therefore less prone to racemization. Racemization is a problem with the smaller peptide rings. The stability of cyclic heptapeptides is achieved through stabilizing β-turn conformations with multiple internal hydrogen bonds.

1.1.2) Inclusion of a β-amino acids to form 13-membered peptide rings

Another way to ease the difficult cyclization of tetrapeptide rings is to include a β-amino acid into the backbone thereby expanding the size of the ring by one carbon unit. It's been shown that with the inclusion of just one β-amino acid, a

cyclic system is easier to synthesize and chemically more stable than the 12-membered ring counterparts.

There are several different kinds of β-amino acids with either one or two stereocenters present; examples are shown in Figure 1.6. The β-amino acids with one stereocenter are classified as β^2 or β^3 depending on the location of the stereocenter. The inclusion of this kind of β-amino acid generally aids in the cyclization by adding conformational flexibility to the backbone of the peptide. The second category has two stereocenters present known as $\beta^{2,3}$. This type of β-amino acids can either help or hinder the cyclization of peptides based on the stereochemical configuration of the two chiral centers. The following examples focus on the β^2- or β^3-amino acids.

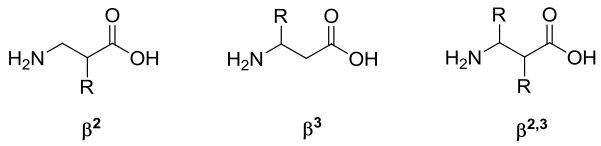


Figure 1.6 β-amino acids

The Fairlie group explored the influence that a β-amino acid had on a cyclic tetrapeptide.[11] The peptide sequence chosen included the lipo amino acid, 2-aminosuberic acid (Asu). In order to do this they synthesized the peptides: H_2N-Phe-(*R*)-Pro-Asu-Phe-OH **1.9**, H_2N-βPhe-(*R*)-Pro-Asu-Phe-OH **1.10**, and H_2N-Phe-(*R*)-Pro-Asu-βPhe-OH **1.11** (cyclic forms shown in Figure 1.7). The cyclization of **1.9**, which

forms a 12-membered ring, gave a mixture of diastereomers indicating racemization with ~50% yield as well as the presence of octapeptide. By contrast the inclusion of a β-amino acid in **1.10** and **1.11** gave highly improved yields, greater than 95% under the same conditions. They also found that there was a cooperative effect when the turn promoting proline and the β-amino acid were separated as shown by the sequence **1.11**. The authors demonstrated that cyclic tetrapeptides can be synthesized with great efficiency when the ring was expanded and care was taken in the design of the peptide backbone configuration.

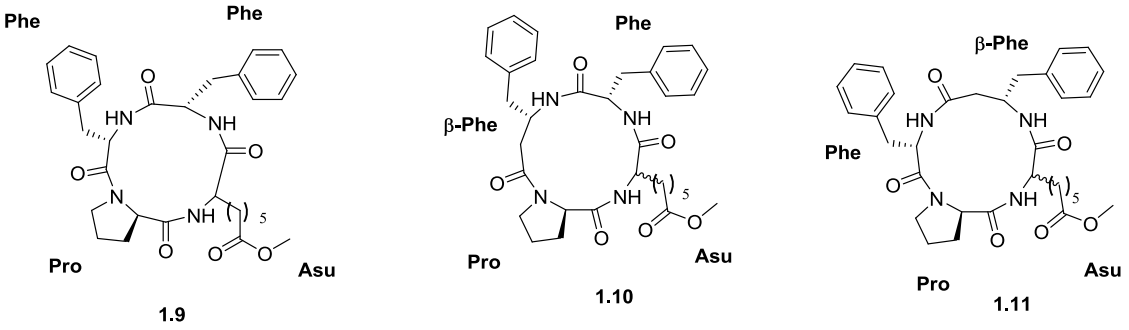


Figure 1.7 Sequences of various peptides synthesized by Farlie *et al.*

Another notable investigation into cyclic peptides came from the Ghadiri group, who found that 13-membered cyclic peptide can serve as potent HDAC (histone deacetylases) inhibitors.[12] The HDACs are a family of enzymes found in bacteria, fungi, plants and animals that affect cellular function. HDACs catalyzed the removal of acetyl groups from ε-N-acetylated lysine residues of various protein substrates: including histones, transcription factors, R-tubulin and nuclear importers.

HDACs are sometimes called lysine deacetylases to better describe the function of the enzyme. The cyclic peptides synthesized by Ghadiri are based on a family of HDAC inhibitors from the nonribosomal cyclic tetrapeptides including apicidins **1.12**, azumamide A **1.13**, and trapoxin **1.14** shown in Figure 1.8.[12] The unusual lipoamino acids in position 1 are isosteric approximations of an acetylated lysine. (Figure 1.8)

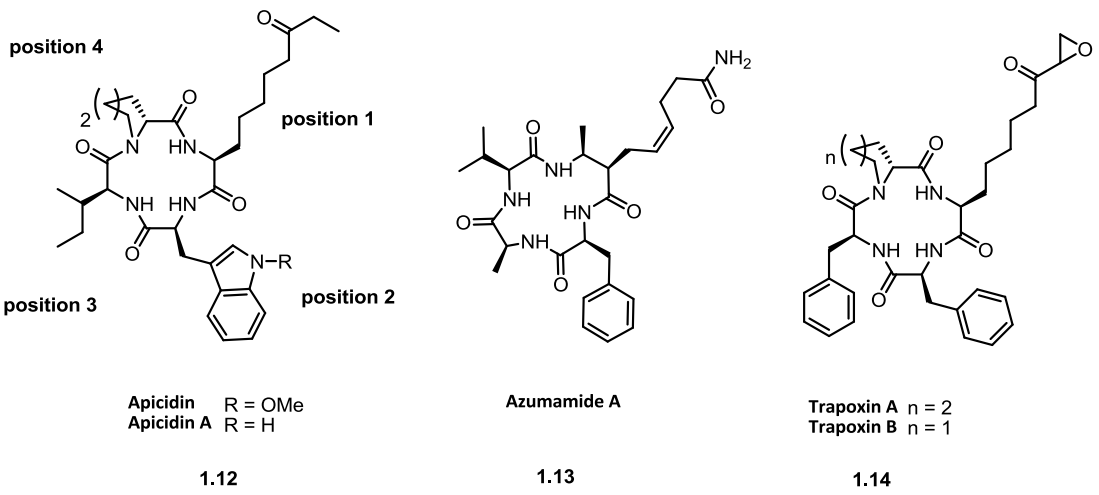


Figure 1.8 Naturally occurring cyclic peptides that are HDAC inhibitors

In an attempt to increase selectivity among the different HDAC proteins a SAR (structure activity relationship) study was conducted.[13] One focus of this effort was an examination into whether changes in the stereochemistry of the tetrapeptide ring would increase selectivity versus the natural products. The natural products are indiscriminate in their activity against the various HDACs. The authors changed the ring size from a 12- to a 13-membered ring by incorporating a β-amino acid (boxed in Figure 1.9) in the tetrapeptide ring system, for improved cyclization of

the peptides. The author tested the effect of location of the β-amino acid on the selectivity by changing the location of the β-amino acid. The β-amino acids β-leucine **1.15** and β-alanine **1.16** are two examples shown in Figure 1.9. The authors also tested the addition of two β-amino acids as represented cyclic peptide **1.17**. They found that when the β-amino acid was β-leucine (compound **1.15**) it showed the best activity of any of the peptides tested.

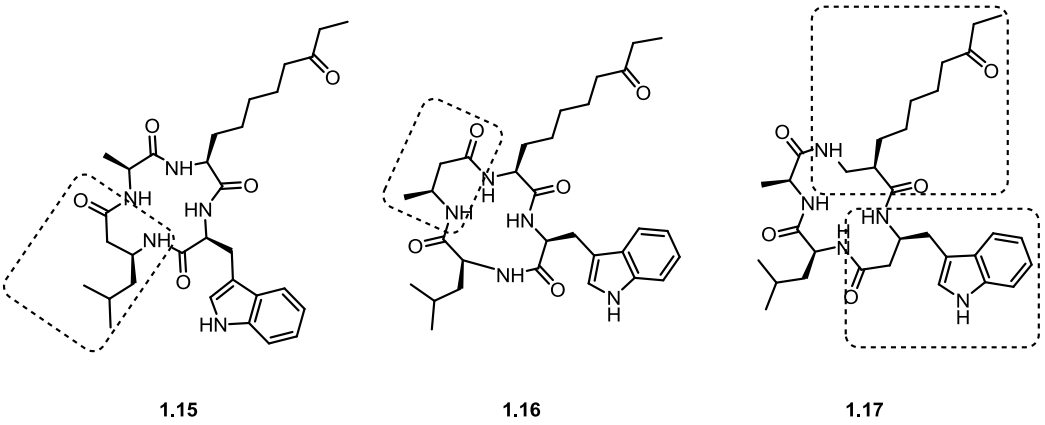


Figure 1.9 Different locations for incorporation of the β-amino acid

After choosing the most active of the series in Figure 1.9, the authors investigated the effects of stereochemistry, methylation and ring size.[14] It was found that when just a single stereocenter was changed the activity changed, due to orientation of the ring. When the 14-membered rings (2 α-amino acid and 2 β-amino acid) were tested, they noted that the location of the β-amino acids changed the activity drastically.

In another SAR study Ghardiri used the one-bead-one-compound method to examine the effect that amino acid substitution in positions 2-4 had on the selective binding of HDAC (Figure 1.10).[15] It was found that in position 2 an aromatic residue was needed for potency against class-1 HDAC but a substitution to Asp favored $HDAC_6$. In position 3, the β-amino acid location, an aliphatic side chain increased potency and a tyrosine residue enhances selectivity against $HDAC_6$. Basic amino acids such as Arg and Lys increased activity when in position 4. They were able to show that modifications to the ring had a profound effect and were able to increase the selectivity of activity to particular HDACs, demonstrating that the three dimensional orientation of the ring and side chains had a profound effect on activity.

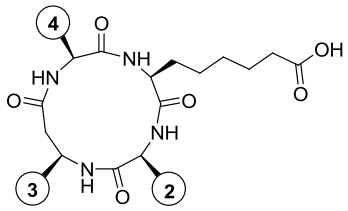


Figure 1.10 Template used by Ghardiri for SAR study

1.1.3) 14-membered tetrapeptide rings

A 14-membered tetrapeptide can be formed by the inclusion of a γ-amino acid or two β-amino acids. Both of these standard motifs add two carbons to the backbone of the peptide. The larger ring is slightly more flexible than the 12- or 13-

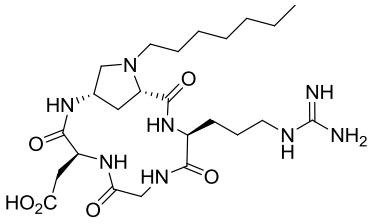
membered ring which allows for easier cyclizations and more variety in the sequence compared to the smaller rings. It is still difficult to cyclize these rings and the inclusion of turn inducers are commonly employed in successful syntheses. Several examples of both types of 14-membered tetrapeptides are examined below.

1.1.3.1) Inclusion of a γ-amino acid in cyclic tetrapeptides

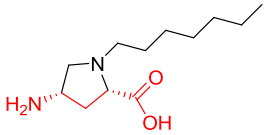
The cyclization of 14-membered tetrapeptide rings is easier than the 12-membered rings because of the larger ring size. The inclusion of a γ-amino acid adds flexibility to the backbone relieving some strain of the ring and allowing for an easier cyclization to occur.

A class of integrin binders ($\alpha_v\beta_3/\alpha_v\beta_5$) with the Arg-Gly-Asp (RGD) sequence has been integrated in a cyclic peptide **1.18** in order to constrain the sequence. The Casiraghi group incorporated the RDG sequence into a 14-membered ring using 4-aminoproline and cyclizing with the γ-nitrogen.[16] The Amp (4-aminopyrrolidine-2-carboxylic acid) **1.19** combines the structural features of a α,γ-bridged GABA motif (red) within the proline ring itself (Figure 1.11). The cyclization event was achieved under moderate dilution (3.5mM) in DMF in a good yield of 75%. This is structurally similar to a pentapeptide. Shrinking the size of the ring from 15 to 14 they were still able to mimic the integrin binding capabilities of the sequence. It adopted an inverse γ-turn centered at the Asp residue as determined by CD spectroscopy. This

was found to be highly beneficial to efficient binding when docking simulations were performed using the cyclic peptides.



1.18



1.19

Figure 1.11 AMP with GABA-like motif (red) and its incorporation into a cyclic integrin binder

An older example is the Goodman's lab synthesis of the cyclic peptides **1.20-1.22,** which includes the γ-amino acid.[17] Altering the structure, including the γ-amino acids, changes the requirements for the cyclization by allowing for the increased conformation flexibility of the amino acids. The synthesis of eight different cyclic peptides was completed, each with the D-isomer of the A_2Bu (2,4-diaminobutyric acid) amino acid. Three of the eight cyclic peptides synthesized (shown in Figure 1.12) have an amide bond in the cisoid conformation while the other five peptides (not shown) had all transoid conformation around the amide bonds as determined by NMR. The inclusion of at least one D-isomer and the flexible γ-amino acid permits for the success of cyclization because it allows the cis isomer to exist.

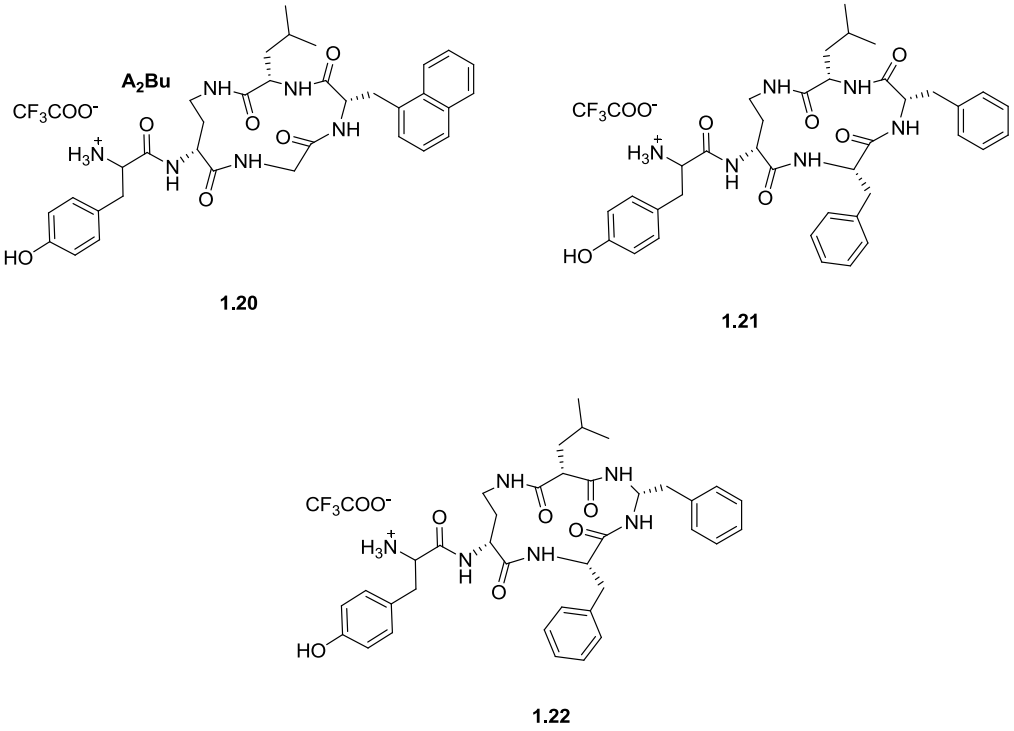


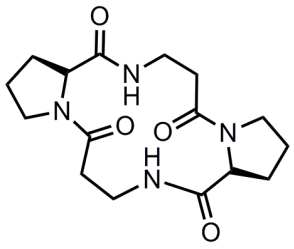
Figure 1.12 Tetrapeptides with the inclusion of a γ-amino acid

1.1.3.2) Inclusion of two β-amino acids

The $\alpha^2\beta^2$ tetrapeptides are 14-membered rings that contain two α-amino acids and two β-amino acids. The alternating α-amino acids and β-amino acids offer an interesting motif in cyclic peptides. The larger size of the ring due to the β-amino acid residues is easier to cyclize than the corresponding 12-membered ring; yet they still pose a challenge. These ring configurations are rare in nature and even rarer in

synthetic work because they are difficult to successfully cyclize; below are a few examples of this motif.

In 1996 the Muramatsu group was investigating reverse turn mimics; modeling γ-turns more specifically.[18] To achieve the γ-turns they synthesized a 14-membered ring containing two β-alanines and two prolines compound **1.24** (Figure 1.13). The two amino acids were chosen because prolines are good turn inducers and the β-alanines have more flexible backbones due to the lack of a chiral center. Upon NMR analysis the amide bonds were shown to be in a trans conformation and the protons of the amides were participating in hydrogen bonds. They also found that the cyclic peptide existed in a single conformation as a γ-turn structure showing the rigidity of the cyclic peptides.



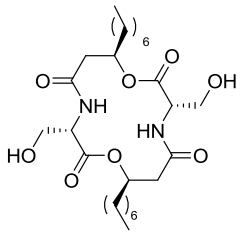
1.24

Figure 1.13 Cyclic tetrapeptide containing alternating β-alanine and proline

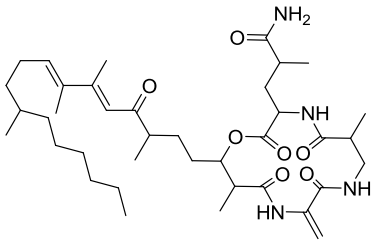
The cyclodepsipeptide serratamolide **1.25** was first isolated in 1961 by Wasserman *et al.* from a bacterial culture of *Serratia marcescens*.[19] It was found to possess a wide-range of biological activities, including antibacterial and anticancer

properties. Serratamolide is composed of alternating *R*-β-hydroxydecanoic acid units and *L*-serine units. A number of total syntheses of serratamolide have been published.[20] In a solid phase synthesis of **1.25**, macrocyclization was carried out via lactone bond formation with the coupling reagent PyAOP.

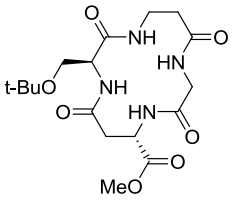
Fusaristatin A **1.26**, a lipophilic cyclodepsipeptide, was isolated from rice cultures of *Fusarium sp.* and showed cytotoxicity towards lung cancer (LU 65).[21] The stereochemistry was never defined for Fusaristatin A; to date no additional work has been published regarding the stereochemical assignment. The third cyclic peptide **1.27** depicted in Figure 1.14 was synthesized in an effort to explore cylindrical peptides.[22] The design was based on the structure of the depsipeptide serratamolide. Examination of the x-ray crystal structure showed that the ring contained four planar segments with the amide bonds in a trans configuration. Cyclization was achieved by forming the activated ester using the nitrophenyl group. Other than the $\alpha^2\beta^2$ configuration, compound **1.27** does not contain exceptionally fascinating structural features with only two stereocenters. The cyclocinamides are natural products with the $\alpha^2\beta^2$ configuration and four stereogenic centers present and are described in detail later.



Serratamolide 1.25



Fusaristatin A 1.26



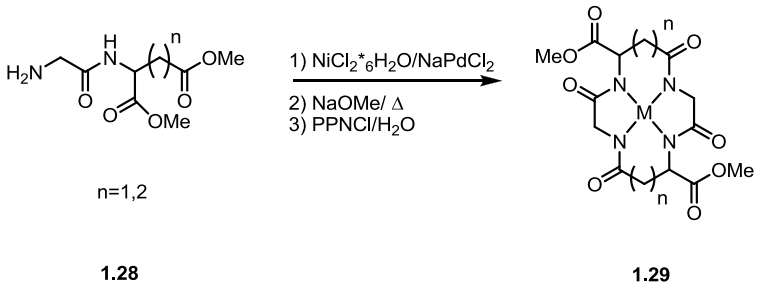
1.27

Figure 1.14 Various cyclic $\alpha^2\beta^2$ peptides

The Schnapp group was able to synthesize tetrapeptides through an unconventional method using a metal center including nickel (II) and palladium (II), in a half sandwich complex to perform a cyclocondensation. They synthesized cyclic peptides with two glycine residues and two β-amino acids that had various functionalities.[23] The 4 amide nitrogens were shown to coordinate to the metal center which occupies the central cavity of the cyclic peptide.

The example highlighted in Scheme 1.1 uses a dipeptide **1.28** composed of glycine and using the side chain of Asp and Glu. The authors demonstrated other functionalities, including a lysine side chain (not shown). Condensation using nickel gave the cyclic peptide **1.29**. The metal was then removed under acidic conditions to give the cyclic peptide. The structures of the cyclic peptides were verified by 2D

NMR as well as x-ray crystallography. This method illustrates a novel method to synthesize cyclic peptides.



Scheme 1.1 Condensation using metal to form a cyclic tetrapeptide

1.1.3.3) Other 14-membered ring natural products

There are several other 14-membered rings that contain a mix of amino acids and other non-peptide structures. A select few examples are highlighted in the examples below including both peptide as well as ones that are a mix of amino acids and other organic functional groups. The mixture allows for new conformational space to be accessed and can incorporate turns using structures other than a select few amino acids.

The apicidins **1.12** are cyclic 12-membered tetrapeptides that have an unusual lipoamino acid residue and the presence of one turn inducing Pro or Pip and a D-amino acid. The microsporin A-B **1.30** (only one structure shown) are similar in that they containing a turn inducing amino acid D-Pip. The azumamides A-E **1.13** are believed to be biosynthesized by a sponge-associated fungus present in the sponge extract. They do not contain a turn inducer amino acids such as that found in the apicidins, however they do contain a β-amino acid and three D-amino acids. As seen, these examples do have similarities in the structures of a lipoamino acid residue and at least one D-amino acid.[25] (Figure 1.15)

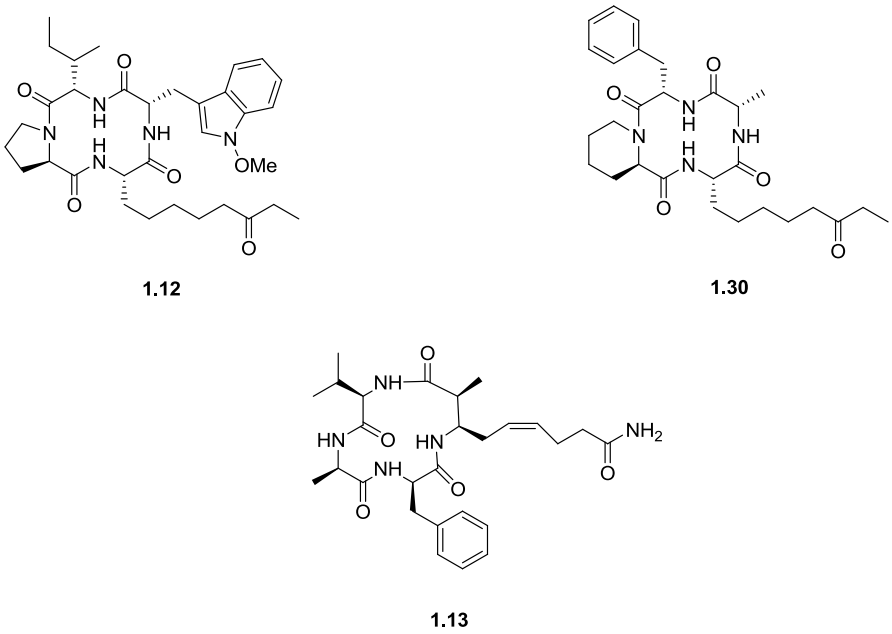
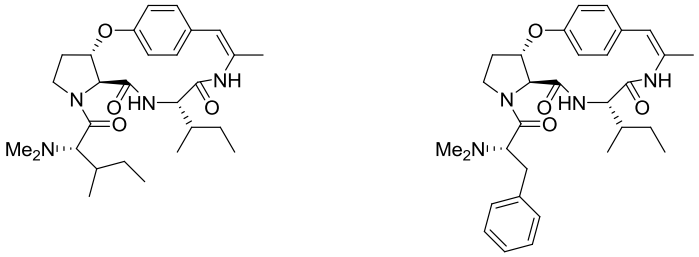


Figure 1.15 Cyclic tetrapeptides containing an unusual lipoamino acid

The cyclopeptide alkaloid mucronine **1.31** and lotusines **1.32** possess the 14-membered ring moiety of amphibine-β-type alkaloids which are formed using a mix

of peptide and non-peptide components.[26] The para connection of the aromatic ring offers a different kind of rigidity and conformation space occupied by the ring. This can reduce the strain associated with the amide bonds. It also reduces the number of amino acids in the ring, and the amount of stereocenters. (Figure 1.16)



1.31 1.32

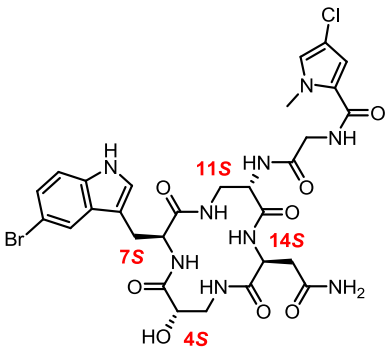
Figure 1.16 Cyclic compounds mucronine and lotusine

1.2) Isolation and structure elucidation of cyclocinamide A and B

The cyclocinamides containing 14-membered cyclic tetrapeptides have been shown to have a complex structure in the ring system, which is in contrast to the examples just seen. The $\alpha^2\beta^2$ ring system has typically been observed with simpler amino acid residues or turn inducing amino acids present in the molecules. The complexity of the cyclocinamides has warranted a closer look at the structure and an examination of previous synthetic efforts.

1.2.1) Cyclocinamide A

Cyclocinamide A **1.33** was isolated in 1997 by Professor Phillip Crews from the marine sponge *Psammocinia sp.* in the Milne Bay Province of Papua New Guinea (Figure 1.17).[27] The structure of this marine natural product was elucidated using extensive NMR analysis. It has a highly unusual skeletal structure, containing a 14-membered cyclic tetrapeptide core and a dipeptide side chain. The cyclic core is comprised of the two α-amino acids: 5-bromotryptophan and asparagine, as well as the two β-amino acids: isoserine and diaminopropionic acid. The side chain contains a glycine and a monochloroinated *N*-methylpyrrole, which is believed to be derived from proline. Of the four chiral centers in the cyclic core the stereochemistry of only the two α-amino acid centers were determined as being in the *S*-configuration at the time of the original publication. The stereochemistry was determined using the chiral TLC technique. They suspected the absolute configurations at C4 and C11 were in a *S*-configuration as well; "on the basis of biogenetic arguments."



1.33

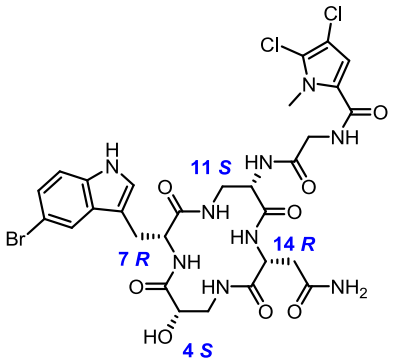
Figure 1.17 Cyclocinamide A

The cytotoxicity profile of cyclocinamide A aided in the isolation and purification; the soft agar disk diffusion assay was against C38, a colon cancer cell line. The purified material exhibited robust activity similar to the crude fractions. Unfortunately, for follow up studies a small amount was available for *in vivo* testing. A mouse with early stage M-16 (mammary adenocarcinoma no. 16) was selected for testing and cyclocinamide A proved to be completely inactive and non-toxic at a dose of 20 mg/kg.

A decade later the Crews group was resurveying sponge sources and collected fresh sponge specimens hoping to be able to fully assign the stereocenters of cyclocinamide A.[28] A small amount of cyclocinamide A was obtained and a full assignment of the absolute stereochemistry was undertaken using the Marfey's method. The natural product was digested using 6 N HCl for 4 h at 110^{o}C, then derivatized with L-FDAA (*N*-α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide). The same procedure was done with the standards and using LC-MS the stereochemistry was assigned as (4*S*,7*S*,11*S*,14*S*). Further evaluation of the biological activity revealed modest solid tumor selectivity against HCT-125 and murine CFU-GM in disk diffusion assays. It was also tested against HCT-116 and had an IC_{50} of >10 μg/mL. The results illustrate that the potency of cyclocinamide A, first observed in the C-38 cell line, was not seen the HCT cell lines. Due to a lack of abundance of the natural product a full biological evaluation was not undertaken.

1.2.2) Cyclocinamide B

A decade after the discovery of cyclocinamide A, Ireland's team isolated a very similar compound which was dubbed cyclocinamide B **1.34** (Figure 1.18).[29] Cyclocinamide B was isolated from the distantly related marine sponge *Cortium sp*. in Fijian waters. The fact that such similar natural products were isolated from different species of sponges suggests that cyclocinamide B is most likely biosynthesized by the microorganisms that reside in both species of sponges.



1.34

Figure 1.18 Cyclocinamide B

Cyclocinamide B consists of the same amino acids as cyclocinamide A, with a minor change of a dichlorinated side chain. Using a top-down, bottom-up approach involving 1D and 2D NMR as well as mass spectroscopy, six partial structures of the individual amino acids were established. Its similarity to cyclocinamide A led the Ireland group to believe that they found a similar structure. The Ireland group wished to establish the absolute stereochemistry of cyclocinamide B, since at the

time the absolute stereochemistry of the C4 and C11 centers of cyclocinamide A was not known. To accomplish this they employed Marfey's analysis in the same manner as the second publication of the Crews group. However, the Ireland group had difficulty establishing the stereochemistry of the isoserine fragment since the glycine and D-isoserine had overlapping retention times. This problem was circumvented by switching the Marfey's reagent to the L-FDVA [N_{α}-(2,4-dinitro-5-flurorphenyl)-l-valinamide] which has a greater lipophilicity than the L-FDAA and led to better separation of the fragments. The analysis indicated the assignment of (4*S*,7*R*,11*S*,14*R*) giving it a different stereochemistry from cyclocinamide A. Cyclocinamide B was tested against HCT-116 using a soft agar disk diffusion assay and was found to have no significant cytotoxicity. However due to the small amount of material further tested was unable to be undertaken.

1.3) Previous syntheses of cyclocinamide A

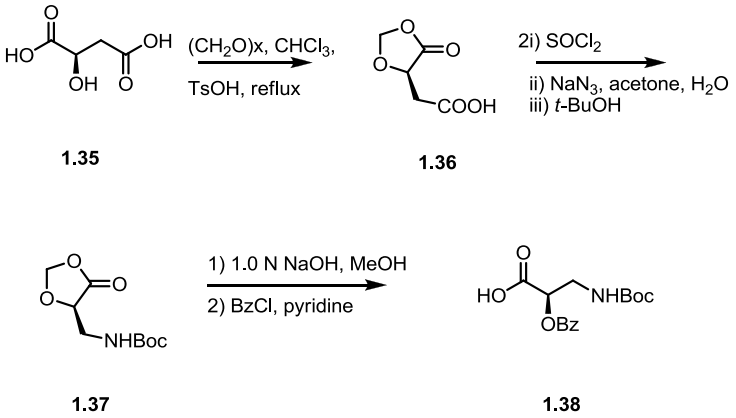
The synthesis of cyclocinamide A has been attempted several times in the past. In 1998, Rielly and Greico attempted to synthesize cyclocinamide A when the stereocenters of the β-amino acids was still unknown.[30] Of the 4 possibilities [(4S,11S), (4S,11R), (4R,11S), (4R,11R)] they chose to synthesize the last of the possible isomers. However, the synthetic sample was declared by Greico to not be the same as the natural product. In the mid 2000's, Postema and Lui attempted to

synthesize a stereoisomer library of the three remaining isomers in hopes of determining the absolute stereochemistry.[31] They were successful at synthesizing only one of the isomers (4R,11S) and again the claim was made that this isomer was not the natural product. These synthetic efforts are detailed below.

1.3.1) Grieco's synthesis of the 4*R*,7*S*,11*R*,14*S* cyclocinamide A

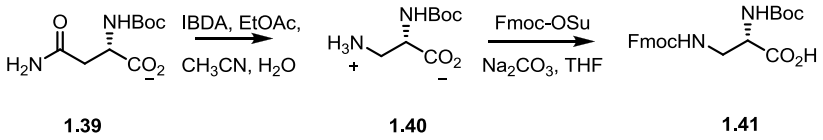
The novel structure of cyclocinamide A and the unknown stereochemistry of C4 and C11 lead to early interest in synthesizing cyclocinamide A. The synthetic scheme that Grieco[27] chose was to synthesize the core linear tetrapeptide, cyclize, and have a late stage attachment of the side chain dipeptide. The tetrapeptide was broken into its individual amino acid components (not shown).

The isoserine fragment was synthesized via a six step sequence starting from *R*-malic acid **1.35**. *R*-Malic acid was converted into dioxolone **1.36** in quantitative yield, and then via Curtius rearrangement was converted to the *N*-Boc-dioxolone **1.37**. Ring opening (1.0N NaOH, MeOH) followed by benzoylation protection of the free hydroxyl gives the *R*-isoserine fragment **1.38** with the desired protection scheme. (Scheme 1.2)



Scheme 1.2 Synthesis of isoserine

The *R*-2,3-diaminopropionic acid fragment is prepared from the corresponding sodium salt of Boc-*R*-asparagine **1.39** by a Hoffman rearrangement to give **1.40** and subsequent protection of the free amine with Fmoc. The *R*-2,3-diaminopropionic acid moiety **1.41** is obtained with an overall yield of 75%. (Scheme 1.3)

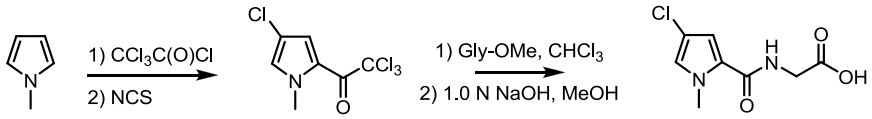


Scheme 1.3 Synthesis of diaminopropionic acid

The tryptophan is synthesized via Fischer indole synthesis from the corresponding pyroglutamic acid using a known literature procedure.[32] After successful synthesis of the tryptophan, it is converted from the methyl ester to the

corresponding fluorenylmethyl ester via a two step procedure of hydrolysis followed by esterification.

The side chain dipeptide was synthesized from the commercially available *N*-methylpyrrole. Reacting with trichloroacetyl chloride followed by a selective chlorination using *N*-chlorosuccinamide gave compound **1.42**. Glycine methyl ester was coupled with **1.42** and followed by hydrolysis to the corresponding carboxylic acid as compound **1.43**. (Scheme 1.4)



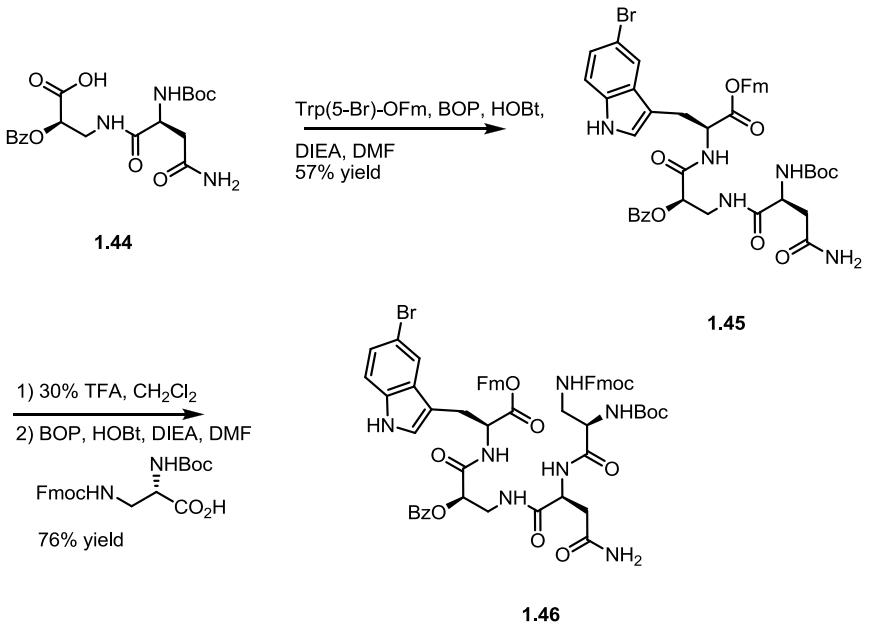
1.42 **1.43**

Scheme 1.4 Synthesis of the side chain

After preparing the necessary amino acids, the construction of the linear tetrapeptide for the assembly of the cyclic core of cyclocinamide A commenced. The isoserine fragment was first deprotected, giving the crude trifluoroacetate salt, which was then coupled to the 4-nitrophenyl activated ester of *S*-asparagine moiety using HOBT coupling protocols to give **1.44** in 65% yield.

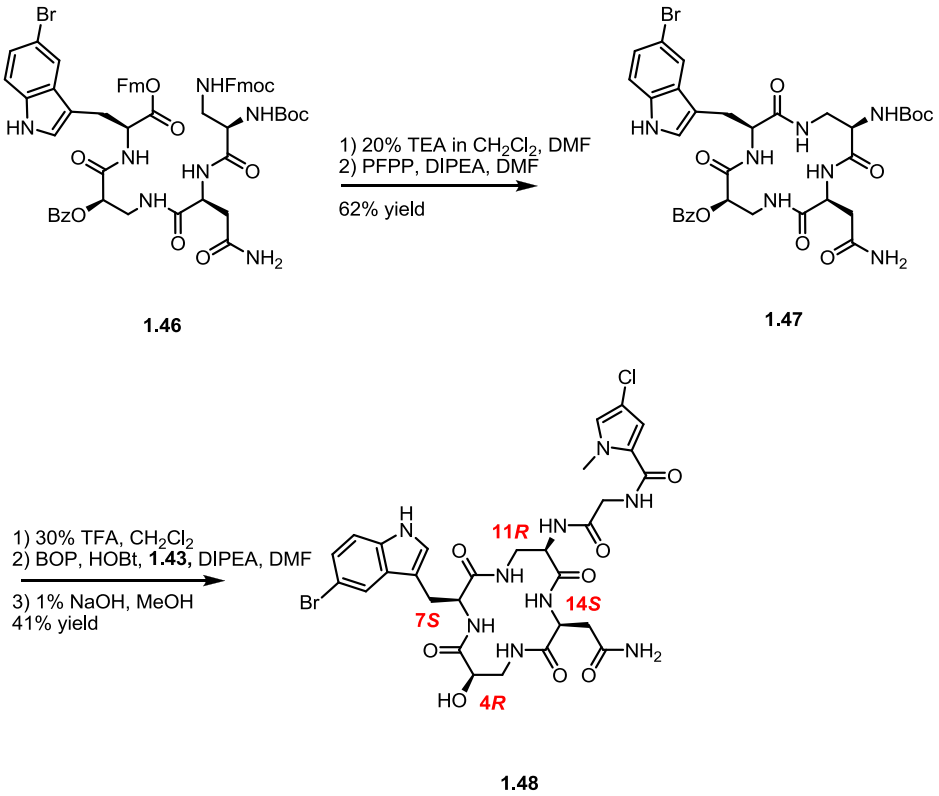
The dipeptide **1.44** was then coupled to tryptophan using conventional BOP promoted coupling protocols to afford the tripeptide **1.45** with a 57% yield (Scheme 1.5). Deprotection of the N-terminus of the tripeptide using TFA gave the crude

trifluoroacetate salt which was then coupled to the *R*-2,3-diaminopropanoic acid fragment to prepare the tetrapeptide **1.46** in 76% yield.



Scheme 1.5 Synthesis of the linear tetrapeptide

With the synthesis of the linear tetrapeptide **1.46** in hand the ring closure to form the cyclic core could be commenced. Deprotection of both ends was achieved using 20% TEA over the course of two hours followed by evaporation *in vacuo*. Using the coupling reagent pentafluorophenylphosphinate (PFPP), DIPEA in DMF afforded the cyclized tetrapeptide core **1.47** with a 62% yield. (Scheme 1.6)



Scheme 1.6 Cyclization of the peptide and completion of the synthesis

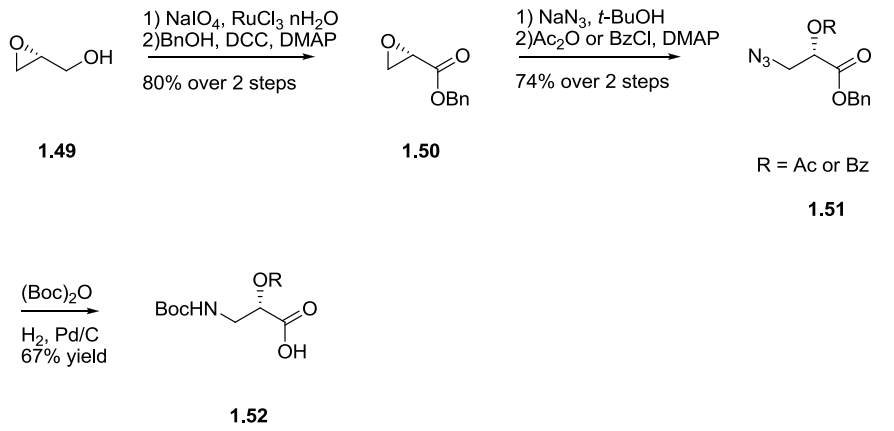
Completion of the total synthesis started with the deprotection of the Boc protection group and coupling of that product to the dipeptide side chain using standard BOP protocol, afforded the hexapeptide. The final deprotection of the benzyl ester at the C4 position was achieved using 1% sodium hydroxide in methanol and gave the (4*R*,7*S*,11*R*,14*S*)-cyclocinamide A with 41% yield. After comparison with the NMR of the natural product (both in methanol-d_4) Greico determined that the synthesized stereoisomer was not the same as the natural product.[30] (Scheme 1.6)

1.3.2) Postema's library synthesis

The absolute stereochemistry of two of the four ring amino acids was unknown at the time of isolation. With the synthesis of a non-natural isomer of cyclocinamide A by Greico, Postema set out to synthesize a library of the three remaining diastereomers. They were successful with synthesis of only one of the isomers (4*R*,7*S*,11*S*,14*S*). In the case of the (4*S*,7*S*,11*R*,14*S*) isomer, the cyclization was successful but the researchers were unable to achieve success in the last step of the synthesis.[31]

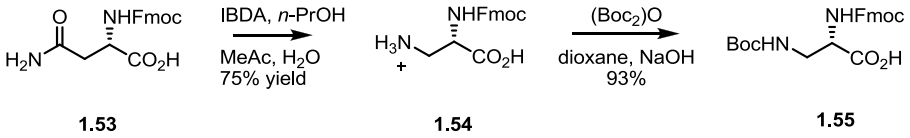
The retrosynthesis follows similar disconnections to those chosen by Greico. The core tetrapeptide was assembled from the individual amino acids. The cyclization in the macrolactamization occurs at a different location than Greico's, between the 1 and 2 positions for the present synthesis. Side chain attachment was followed by conversion of the aspartic acid side chain to the asparagine to give the final cyclocinamide A.

The isoserine fragment was synthesized from *S*-glycidol **1.49**, which is oxidized using sodium periodate. A DCC-mediated esterification yielded compound **1.50** which was followed by a ring opening with sodium azide and protection of the resulting secondary alcohol **1.51**. Reduction of the azide and Boc protection of the amine gives the *R*-isoserine **1.52**. (Scheme 1.7)



Scheme 1.7 Synthesis of isoserine from *S*-glycidol

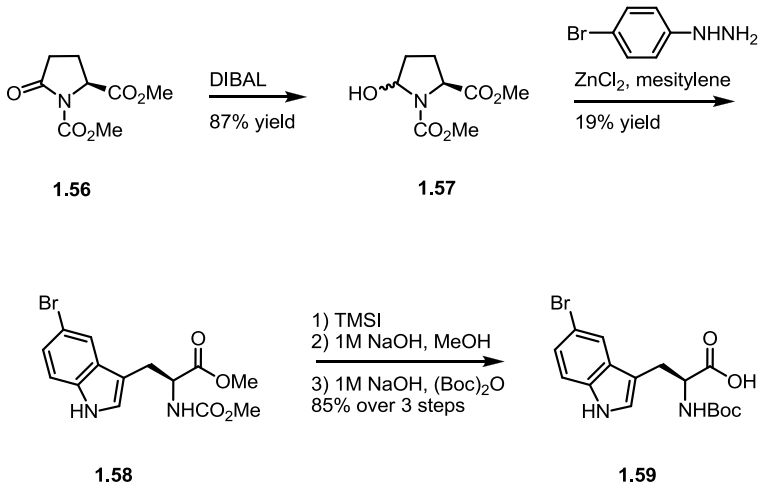
The diaminopropionic acid was synthesized starting from Fmoc-asparagine **1.53**. A Hoffman rearrangement of the amide side chain gives the β-amino group **1.54**, followed by Boc protection of the β-amine to give compound **1.55**. (Scheme 1.8)



Scheme 1.8 Synthesis of diaminopropionic acid

The bromotryptophan was synthesized from methoxycarbonylpyroglutamate methyl ester **1.56,** which was reduced using DIBAL to yield **1.57**, followed by a Fischer indole synthesis with 4-bromophenylhydrazine in a low yield (Scheme 1.9). Deprotection of the methyl carbamate, hydrolysis and reprotection with Boc gave

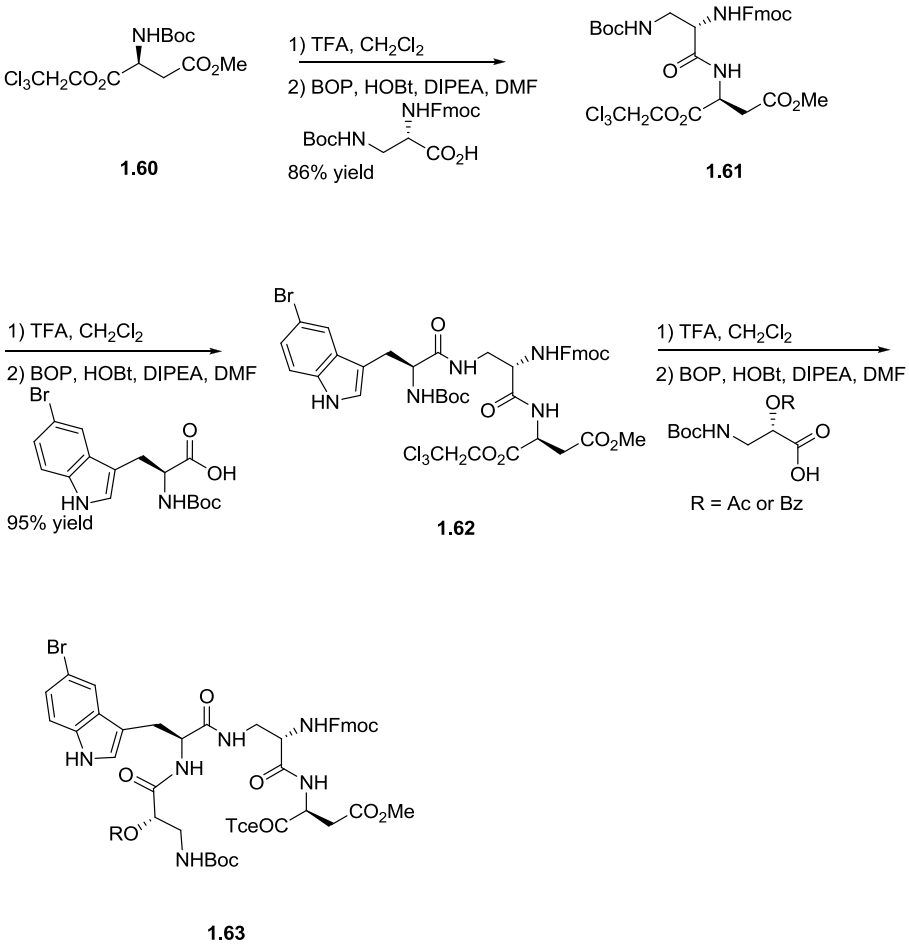
the desired protected 5-bromotryptophan. The asparagine fragment was derived from an aspartic acid residue with methyl ester protection on the side chain, which was converted to an amide in a late stage transformation.



1.56 **1.57** **1.58** **1.59**

Scheme 1.9 Synthesis of tryptophan via Fischer indole

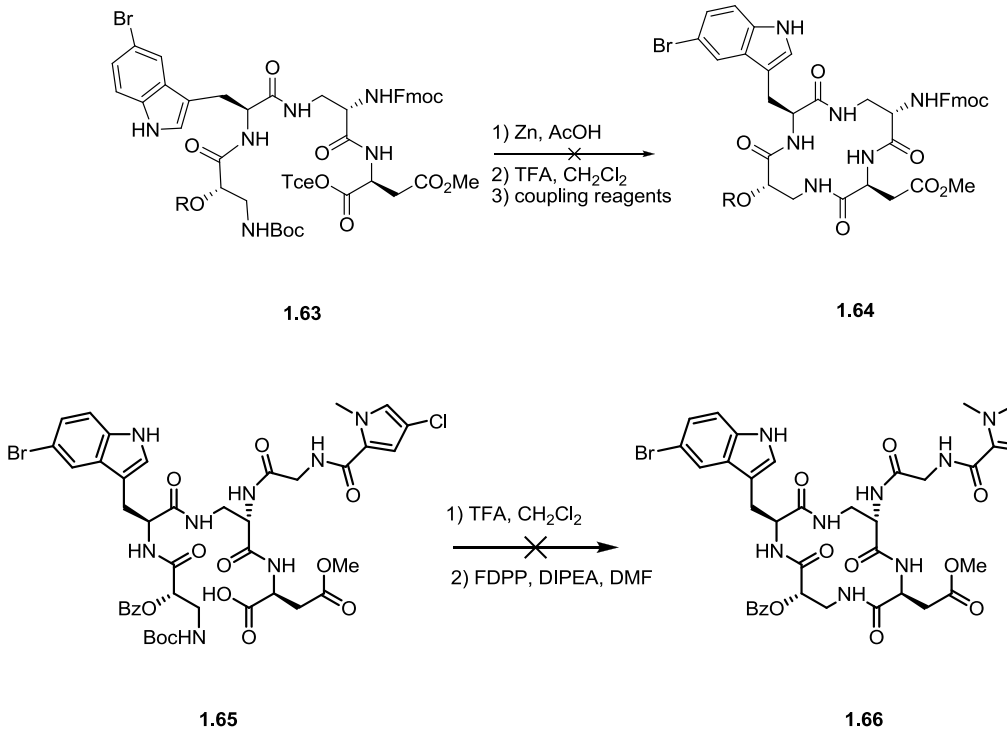
The linear tetrapeptide containing the cyclic core amino acids was synthesized through standard Boc deprotection followed by HOBt/BOP peptide coupling (Scheme 1.10). After the linear system was assembled the Tce (trichloroethanol) group was removed using Zn/AcOH.



Scheme 1.10 Synthesis of the 4*S*,7*S*,11*S*,14*S* linear tetrapeptide

A variety of coupling reagents were surveyed for the difficult macrolactamization of the compound **1.63** (4*S*,7*S*,11*S*,14*S*) all of which failed to give the desired product. It appeared that the cyclization was successful but was followed by decomposition (Scheme 1.11). It was thought that the Fmoc group was the cause of the cyclization problem. To circumvent the trouble with the Fmoc, the

side chain was attached earlier in the synthesis **1.65**. The result was the same: an apparent cyclization followed by decomposition.

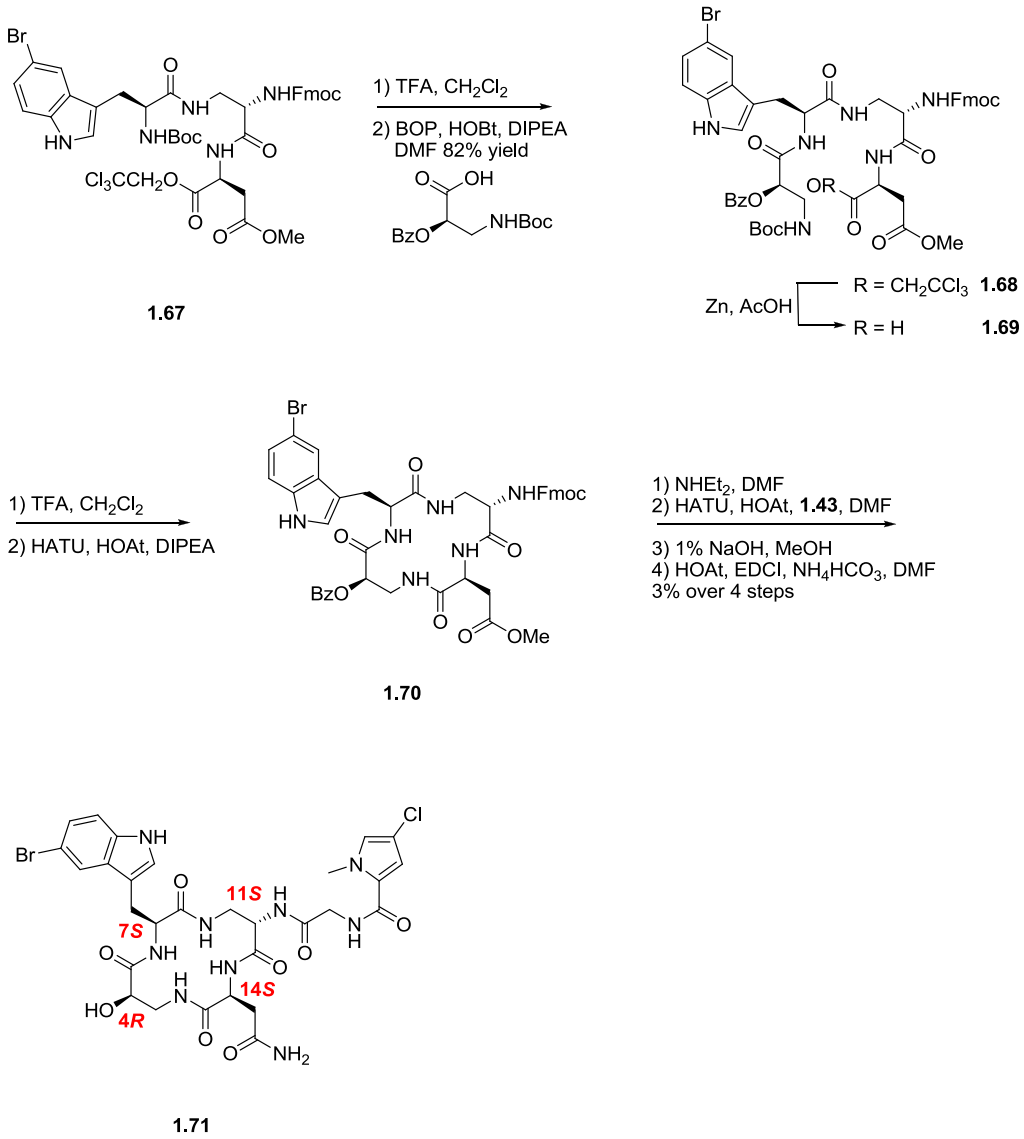


1.63 **1.64**

1.65 **1.66**

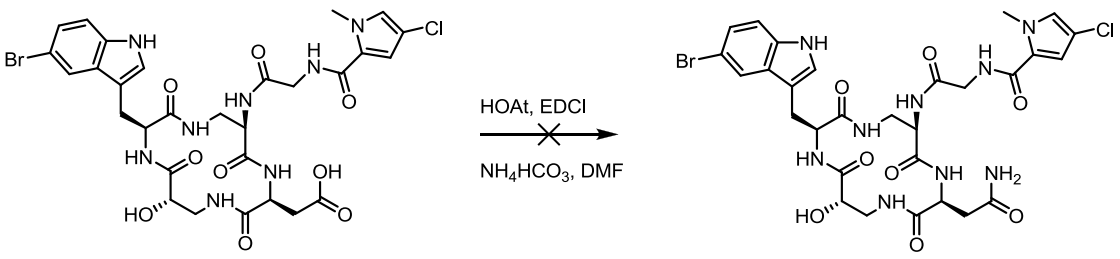
Scheme 1.11 Attempts at cyclization of the all-*S* linear peptide

Because of the inability to cyclization the all-*S* isomer it was reasoned that the other isomers should be examined. The 4*R* isomer was synthesized in a similar fashion to that shown in Scheme 1.12. Cyclization using HATU/HOAt was successful with an 80% yield; however the late stage conversion of the aspartic acid side chain to the asparagine side chain gave a very poor yield of 3% over four steps. Upon analysis of the ^{1}H NMR they discovered that the (4*R*,7*S*,11*S*,14*S*) isomer was not the same as the natural product.



Scheme 1.12 Completion of the synthesis of the 4*R*,7*S*,11*S*,14*S* isomer

With the 4*R* isomer being different than the natural product their attention turned to the last of the three possible isomers (4*S*,7*S*,11*R*,14*S*) in order to determine the structure of the natural product. The 11*R* synthesis followed the same route as the 4*R* isomer. The cyclization using HATU/HOAt was successful with an 80% mass recovery. The deprotection of the Fmoc, coupling of the side chain followed by deprotection of the benzoyl group gave a 12% yield over the three steps. The last remaining step was the conversion of the aspartic acid to the asparagine side chain. However it failed to give the desired free amide product. (Scheme 1.13)



1.72 **1.73**

Scheme 1.13 Failure to convert the aspartic acid to asparagine of the 4*S*,7*S*,11*R*,14*S* isomer

Postema and Lui were able to cyclize two of the isomers (4*R*,7*S*,11*S*,14*S*) and (4*S*,7*S*,11*R*,14*S*) and complete the synthesis of the (4*R*,7*S*,11*S*,14*S*) cyclocinamide A isomer. The presence of a D-amino acid allowed for the successful cyclization. The failure of the all-*S* isomer to cyclize lends evidence to the fact that D-amino acids help in the cyclization event. The lack of confirmation of the natural product

structure via this synthesis leaves open the question of the absolute stereochemistry of the natural product.

1.4) Conclusion

Small cyclic peptides are scarce in nature and even rarer in synthetic work. This is due to the difficulty of cyclizing strained systems containing all trans amide bonds. As a result of this difficulty, therapeutics derived from cyclic peptides is rare, despite the reported activity of the natural products. The inclusion of a turn inducer eases the strain and allows for successful cyclization of the peptide. Cyclocinamide A and B are complex cyclic tetrapeptides containing 4 stereocenters without the presence of a turn inducing residue. Due to this they have thus far eluded synthetic efforts, despite valiant attempts at defining the correct absolute stereochemistry for cyclocinamide A. The closely related structures, with the main difference being the stereochemical configuration of the chiral centers, has left open the need to synthesize the reported structures of cyclocinamide A and B.

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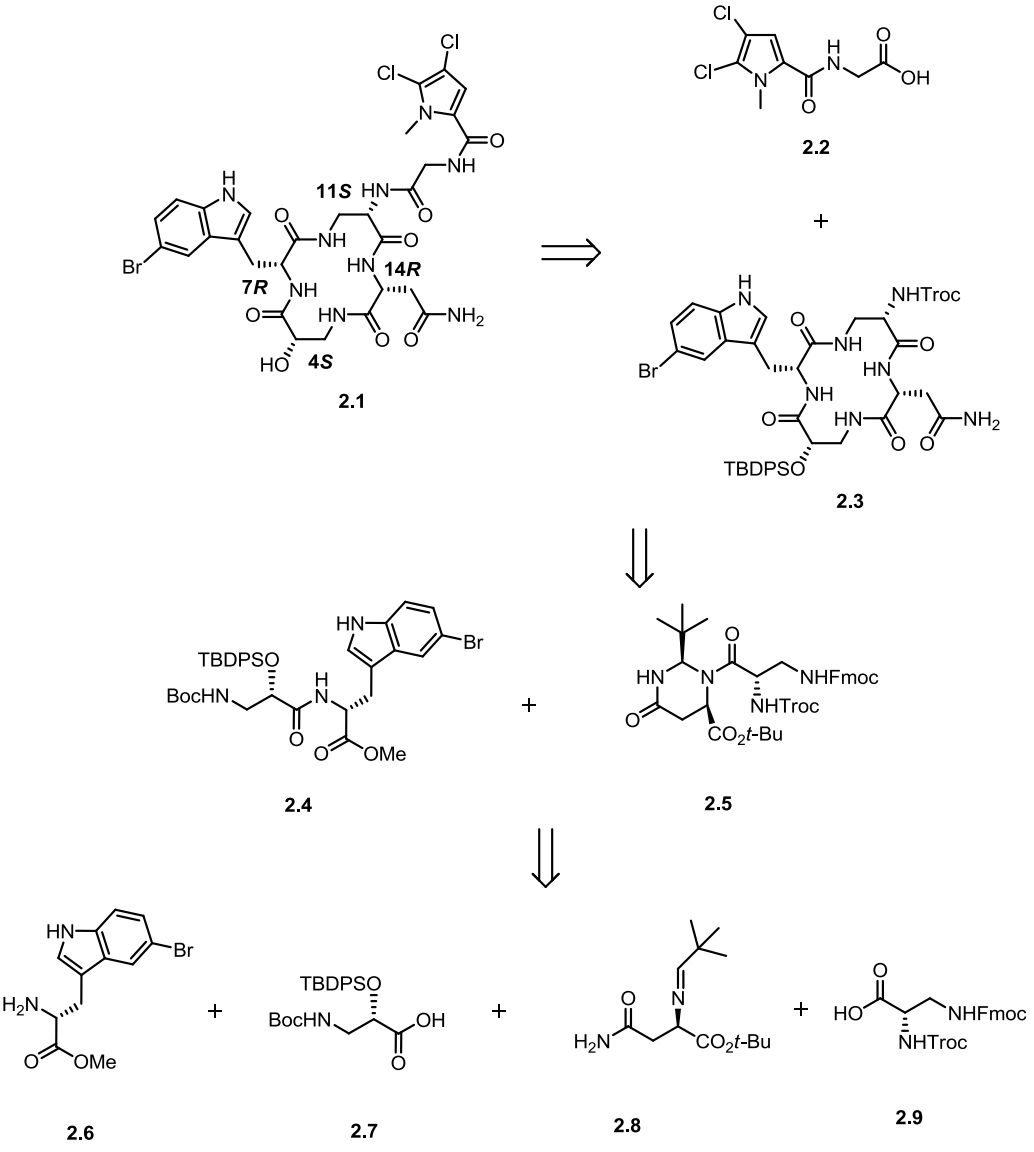
Chapter 2- Synthesis of the three dipeptides for cyclocinamide A and B

2.1) Introduction

When the synthesis of cyclocinamide A commenced there was no prior knowledge of either the synthetic efforts of Lee Lui[1] or the existence of cyclocinamide B; these were discovered during the beginning stages of our synthesis. The presence and alternately assigned stereochemistry of cyclocinamide B added interest to our project and it was decided at this time that pursuit of cyclocinamide B was warranted. Initally, the syntheses of cyclocinamide A and B followed the same synthetic route, with the appropriate stereoisomers of the requisite amino acids.

The retrosynthesis of cyclocinamide A and B was based on disconnecting the amide bonds in stages. First, cyclocinamide B **2.1** was disconnected into the cyclic core **2.3** and the glycine-pyrrole dipeptide side chain **2.2** is shown in Scheme 2.1. The cyclic core was further disconnected into the two corresponding dipeptides: diaminopropionyl-cyclo(Asn) **2.5** and isoseryl-tryptophan **2.4**. The two dipeptides **2.4** and **2.5** were synthesized from the corresponding amino acids. The incorporation of the cyclo(Asn) was the key step in the synthesis of cyclocinamide. It was believed that the cyclo(Asn) acting as a proline turn mimic would aid in the difficult cyclization

of the 14-membered ring and allow access to different stereoisomers of cyclocinamide A and B.



Scheme 2.1 Retrosynthesis of cyclocinamide B **2.1**

2.2) Synthesis of glycine-pyrrole dipeptide

With the presence of halogenated pyrroles being well-known, there are numerous synthetic strategies in the literature.[4,5] A few examples of halogenated pyrroles include: (*Z*)-hymenialdisine **2.10,** oridian **2.11** and tetrabromostyloguanidin **2.12** shown in Figure 2.1.[2,3] The most common halogenated positions include the 4 or 5 positions (pyrrole numbering). Bromine is the most commonly observed in these systems with chlorine being second.

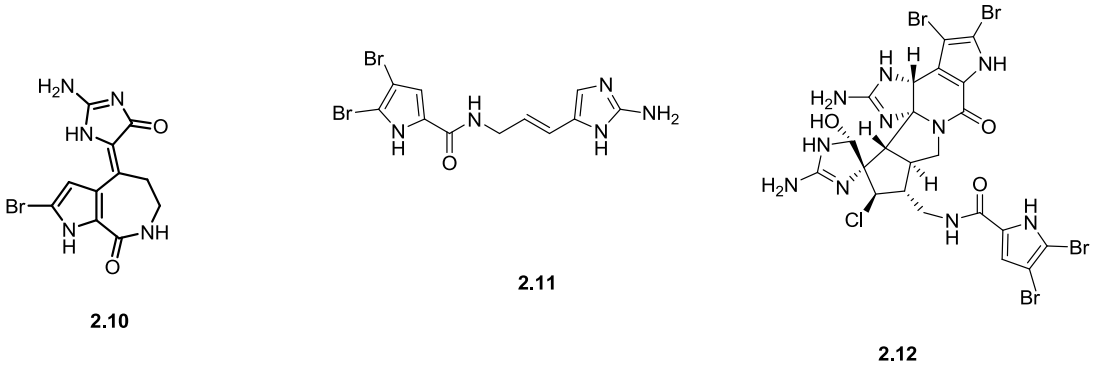
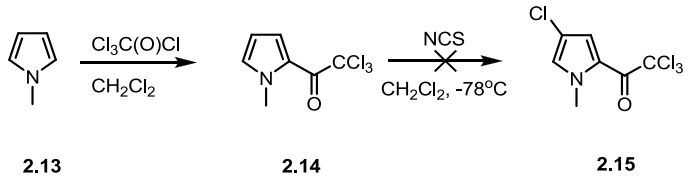


Figure 2.1 Examples of halogenated pyrroles

The synthesis of the side chain started with the *N*-methylpyrrole **2.13** and, following a known procedure,[6] trichloroacetyl chloride was added via Freidel-Crafts reaction to give compound **2.14**. The regioselective chlorination to form the cyclocinamide A side chain was first attempted with *N*-chlorosucinamide at -78 ^{o}C,

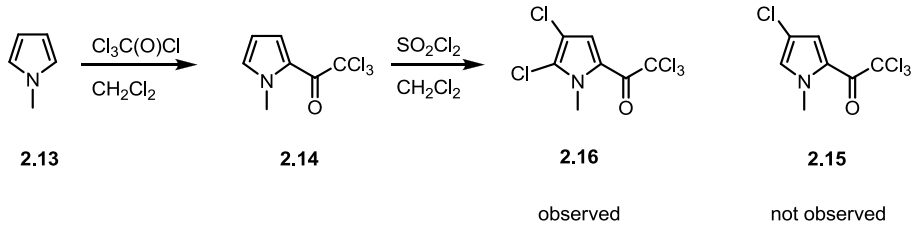
which gave no chlorination product **2.15**; instead the starting material was recovered (Scheme 2.2).[7] The reaction was then attempted at 0 ^{o}C and again there was no chlorinated product detected.[8] Changing the solvent from chloroform to dichloromethane still yielded no desired product.



Scheme 2.2 First attempt at synthesis of chlorinated pyrrole

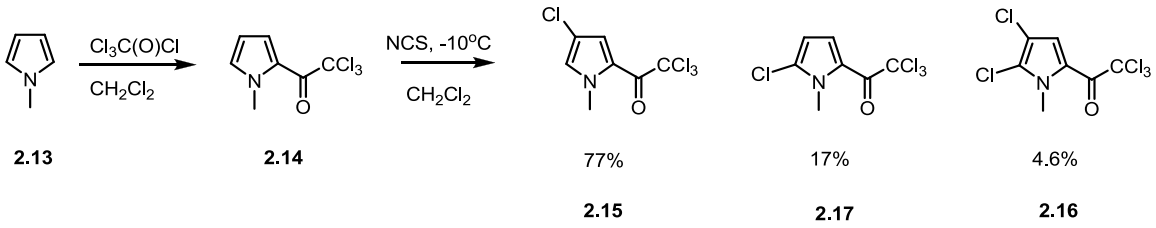
At this point it was worth investigating different chlorinating reagents. Sulfuryl chloride was selected because of the literature precedence for the selective chlorination at the 4 position. The sulfuryl chloride was reacted at 0 ^{o}C and gave only starting material. With the lack of reactivity at 0 ^{o}C, the temperature was raised to refluxing in dichloromethane and upon workup there was a positive result. The resulting oil was analyzed and indicated that bis-chlorination had occurred. Comparison to other halogenated pyrroles in the literature indicated that compound **2.16** was chlorinated at the 4 and 5 positions (Scheme 2.3).[8,9] This discovery was fortunate in that the dichlorinated product was found on the cyclocinamide B side chain. It was thought that the difference in chlorination from the literature arose from the presence of the *N*-methyl group in our system. In order to access the cyclocinamide A side chain the equivalents of sulfuryl chloride was reduced. Even

when the equivalents were reduced to 0.25:1.0 sulfuryl chloride:pyrrole the only product was the dichlorinated material along with the starting material. Since this route gave us easy access to the necessary cyclocinamide B fragment, our attention turned back to the cyclocinamide A side chain.



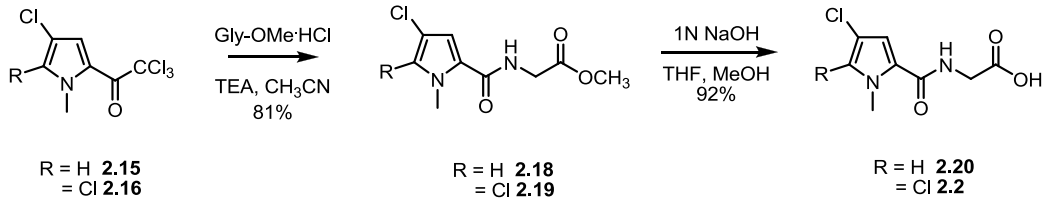
Scheme 2.3 Synthesis of the 4,5-dichlorinated pyrrole

The use of *N*-chlorosucinamide (NCS) as a chlorinating agent was re-examined. A literature procedure showed the bromination of the same starting pyrrole with NBS gave hope to the utility of NCS for our chlorination.[2] The conditions used were a slight variation from previously attempts with the biggest different being the reaction duration. The reaction was stirred at -10 ^{o}C and allowed to slowly warm to room temperature overnight in the presence of light. With the new conditions the correct chlorinated product **2.15** was observed along with the undesired 5-chloropyrrole **2.17** and 4,5-dichlorinatedpyrrole **2.16** as shown in Scheme 2.4. Initially it was difficult to separate the three compounds by flash column chromatography; however successful separation was achieved using hexanes/CH_2Cl_2 solvent system.



Scheme 2.4 Synthesis of different chlorinated pyrroles

The coupling to glycine methyl ester was straightforward with the trichloroacetyl group acting as the activated acid derivative.[9] This allowed for the coupling to proceed smoothly with similarly good yield for the mono- and di-chlorinated pyrrole. The dipeptide side chain compounds **2.18** & **2.19** were stored at this stage until needed, at which time the methyl ester was hydrolyzed. The removal of the methyl ester was easily accomplished by using an excess of sodium hydroxide in methanol and THF as shown in Scheme 2.5. With the side chain synthesis complete, our attention turned to the synthesis of the other two dipeptides **2.4** and **2.5**.



Scheme 2.5 Synthesis of the chlorinated dipeptide side chain

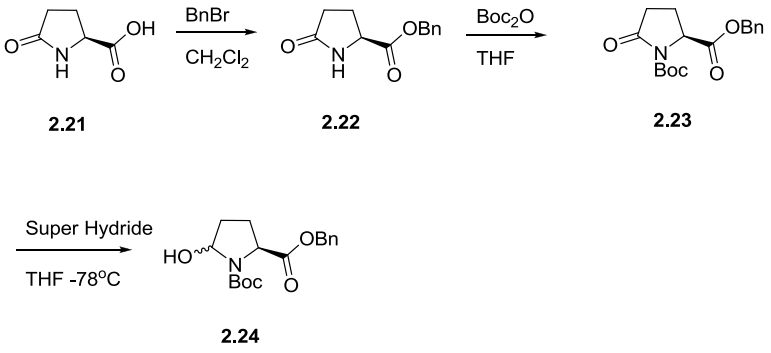
2.3) Synthesis of isoserine-5-bromo-tryptophan dipeptide fragment

The two dipeptides that form the 14-membered ring of cyclocinamide A and B are isoserine-5-bromotryptophan **2.4** and (cyclo)asparagyl-diaminopropionic acid **2.5**. The two dipeptides were connected to form a linear tetrapeptide that cyclized to form the cyclocinamide core (see Chapter 3). The first dipeptide synthesized was the isoseryl-5-bromotryptophan, which was formed from the corresponding amino acids. As shown in Chapter 1, Grieco synthesized the isoserine from the *R*-malic acid and the tryptophan via Fischer indole synthesis.[10,11] Postema synthesized the isoserine from *S*-glycidiol and the tryptophan via Fischer indole synthesis.[1]

2.3.1) Synthesis of 5-bromortryptophan via Fischer indole synthesis

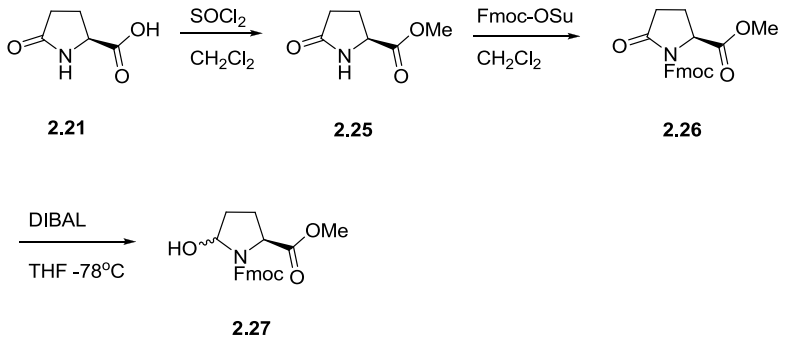
The Fischer indole synthesis begins with the corresponding pyroglutamic acid **2.21**. After the acid and amine are protected, reduction of the carbonyl to the hemi-aminal allowed for the key Fischer indole reaction. Two different protection group schemes were envisioned to allow for versatility in the latter stages of the synthesis. The first synthetic scheme involves the protection of the carboxylic acid with benzyl bromide to yield compound **2.22**. Protection of the nitrogen as the Boc (*tert*-butoxycarboxylate) gave the fully protected desired pyroglutamate **2.23**.[12] Reduction of the amide to form the hemi-aminal **2.24** was performed using super

hydride (lithium triethylborohydride) and gave a 1:1 mixture of stereoisomers at the newly formed hydroxyl[13] (Scheme 2.6).



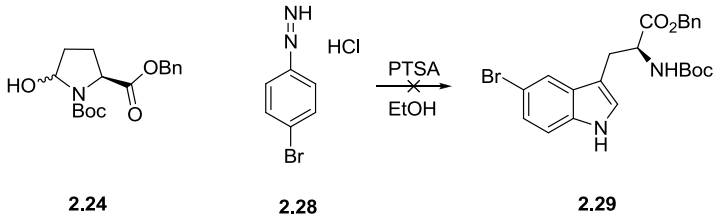
Scheme 2.6 Synthesis of reduced pyroglutamate **2.24**

The second protection scheme was commenced with the protection of pyroglutamic acid **2.21** as a methyl ester **2.25** using standard conditions.[14] This was followed by Fmoc protection of the nitrogen forming compound **2.26**.[15] The reduction using super hydride proved to be unsuccessful and gave no desired product. The reducing reagent was switched to DIBAL which allowed for the reaction to proceed smoothly as shown in Scheme 2.7.[16]



Scheme 2.7 Synthesis of the reduced methyl pyroglutamate **2.27**

With both reduced pyroglutamate in hand, the key step was performed. The Fischer indole reaction was first performed using compound **2.24** and the 4-bromophenylhydrazone **2.28** in the presence of *p*-toluenesulfonic acid while refluxing in ethanol.[17] After 6 hours the reaction was cooled and there was no desired product seen. It was then decided to have the reaction proceed for a longer period of time; even after three days there was no desired product.



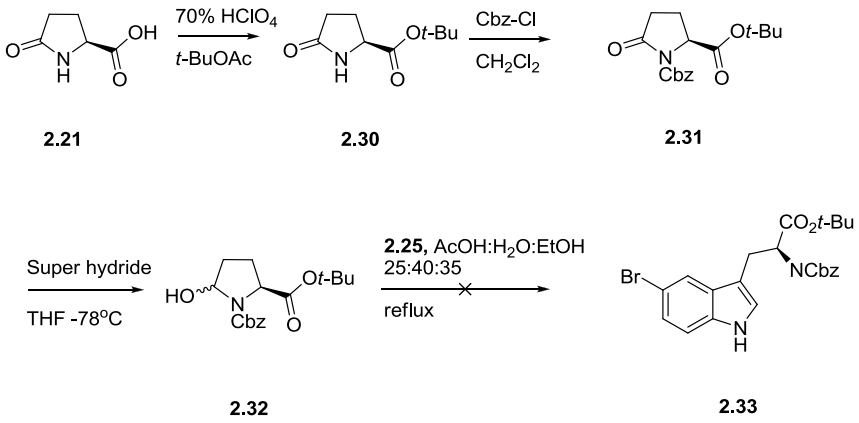
Scheme 2.8 First attempt at the Fischer indole synthesis reaction using **2.24**

We thought that the protection groups were detrimental to the success of the reaction due to the fact that the Boc was not surviving the acidic conditions. The next attempt employed compound **2.27**; it was thought that this material would be

able to survive the acidic conditions.[18] The switch of protection groups proved to be unsuccessful as well. Even after various reaction times were explored no desired indole product was ever detected.

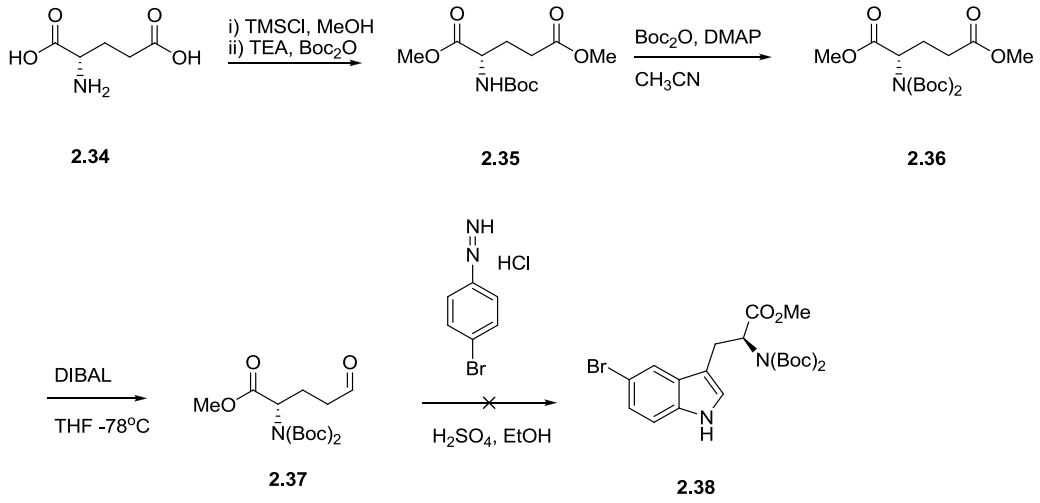
Our next effort was to change the acid used in the reaction. Following the work by Moloney *et al*. the PTSA was switched with sulfuric acid. The hydrazone was dissolved in a 10% solution of sulfuric acid and added to the pyroglutamate **2.27**.[19] The mixture was then refluxed and worked up and unfortunately gave no desired product. Different reaction lengths were tested with the sulfuric acid, but the desired indole was never observed. Based on analysis by thin layer chromatography, no tryptophan was detected and a complex mixture was observed.

The Cbz-pyrogultamate *tert*-butyl ester **2.31** was prepared using literature procedure.[18,19] Following reduction with super hydride the aminal **2.32** was subjected to the prior conditions for Fischer indole with no desired product detected.[20] Attempts with both sulfuric acid and *p*-tolunensulfonic acid gave no desired product. The reaction conditions were switched to the solvent reaction mixture of acetic acid/water/ethanol (25:40:35) at reflux temperature.[21] These conditions did not give the desired tryptophan **2.33** product, even when the reaction was allowed to proceed for several days. Another revision of the substrate was needed in order to have a successful Fischer indole reaction (Scheme 2.9).



Scheme 2.9 Attempt to synthesize tryptophan using Z-pyroglutamate-O*t*-Bu

It was thought that the masked aldehyde was not in a favorable equilibrium for the Fischer indole synthesis. To push the material into the aldehyde over the hemiaminal structure the synthesis was redesigned. Using *S*-glutamic acid **2.34** as the starting amino acid, the acids were protected as the methyl esters, followed by Boc protection in one pot to give compound **2.35**. The nitrogen was then bis-protected with Boc giving **2.36**. The selective reduction of the side chain ester was performed following the work done by Martin *et al.* using DIBAL. The reaction was allowed to run for 5 minutes then quenched to give compound **2.37**.[22] The selectivity is believed to come from the congestion that occurs around the main chain carbonyl. With the aldehyde in hand the Fischer indole synthesis reaction was attempted and after several attempts using the previously described conditions, no desired indole **2.38** was ever detected. (Scheme 2.10)



Scheme 2.10 Attempt to synthesize tryptophan from glutamic acid

In order to determine which part of the mechanism might be the cause of failure for the reaction it was split into two parts. It was envisioned that first the hydrazone would be formed then the formation of the ring would take place separately. The formation of the hydrazone was attempted by letting the hydrazone react with the hemi-aminal, the reaction was then simply concentrated. Analysis of the material indicated that the hydrazone was not easily formed. The reaction gave a complex mixture and all attempts to isolate the hydrazone failed to yield the desired compound even with various workup conditions. It appeared that the formation of the hydrazone was not a favorable reaction.

With the failure of the reaction, an examination of the work performed by Dr. Lee Lui showed that the reaction was poorly behaved.[1] The reaction was performed using a methylcarbamate and methyl ester protected pyroglutamate. The

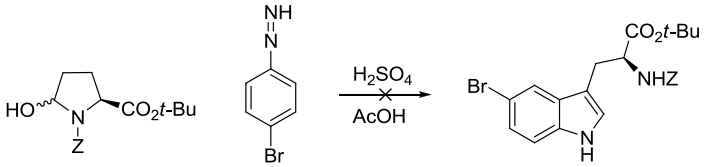
Fischer indole synthesis step was performed using zinc(II) chloride and heating to 140 ^{o}C. These conditions gave a very low yield of 19% indicating that this reaction is intrinsically difficult.

2.3.2) Fischer indole using microwave reactions

With the failure of the reaction using conventional heating our attention turned to the use of microwave heating in order to speed up the reaction times and see if it can lead to a successful reaction. The use of microwaves has been shown previously to decrease lengthy reaction times from days to hours. The reactions have also been shown to be cleaner and typically higher yielding by quickening the reaction times. With the advantages of using a microwave heating the Fischer indole synthesis reaction was attempted.[23]

Table 2.1, entry 1 shows the first attempts used 100W and a temperature of 150 ^{o}C failed to give the desired product. Even with various run times there was no product formed. The starting material was still seen in all reactions. Analysis by thin layer chromatography showed that the only change was for a spot to form at the base line. Reducing the temperature to 140 ^{o}C (Table 2.1, entry 2) gave promise when a new compound appeared by TLC. However there was no indole present in the NMR analysis of the material. The temperature was again lowered this time to 130^{o}C. This however proved to be unsuccessful when only starting material was

observed. The temperature was brought back up to 140°C (Table 2.1, entry 4) and the wattage was increased to 125W, however the reaction appeared the same as entry 2. Increasing the wattage again (Table 2.1, entry 5) gave a change in the reaction with multiple compounds as shown by TLC. With the disappointing results it was concluded that the Fischer indole was not a viable route to the synthesis of 5-bromotryptophan.



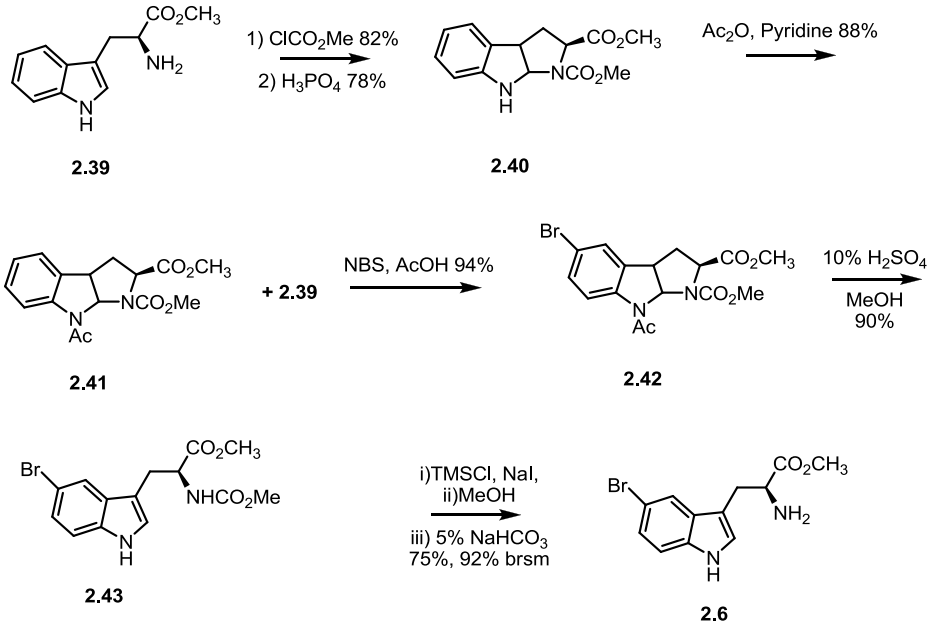
Entry	Temperature (°C)	Wattage (W)	Result
1	150	100	No indole
2	140	100	No indole
3	130	100	No indole
4	140	125	No indole
5	140	150	No indole

Table 2.1 Attempts at Fischer indole via microwave heating

2.3.3) Synthesis via Hino's cyclic tautomer method

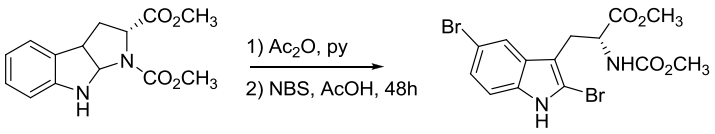
Another route to synthesize the 5-bromotryptophan presented itself via Hino's cyclic tautomer method.[24] The synthesis commenced with the protection of tryptophan as the methyl ester **2.39** in 85% yield. The material was recrystallized and taken on to the protection of the α-nitrogen with methylchloroformate.

Tautomerization was facilitated using 85% phosphoric acid in 78% yield to give compound **2.40**.[25] The purification by flash column chromatography at this stage was cumbersome. In order to circumvent the difficult purification a small amount was taken crude into the next step. Fortunately, the acyl protection with acetic anhydride allowed for the selective protection of the indole nitrogen of the cyclic tautomer; thus the tricyclic material was aceylated to give **2.41** while the indole compound **2.39** was left untouched. The two compounds had very different Rf values, allowing for easy separation by flash column chromatography. Compound **2.41** could also be successfully recrystallized from the mixture which eliminated the need for column purification. The starting tryptophan could be recycled to yield more tautomer compound. (Scheme 2.11)



Scheme 2.11 Synthesis of 5-bromotryptophan via Hino's cyclic tautomer method

The selective electrophilic aromatic substitution preceded cleanly using *N*-bromosuccinamide in acetic acid.[26] It was discovered that allowing the reaction to run longer than 24 h resulted in additional bromination events, resulting in compound **2.44** shown in Scheme 2.12. Compound **2.41** was again recrystallized at this point, removing any additional free bromine.



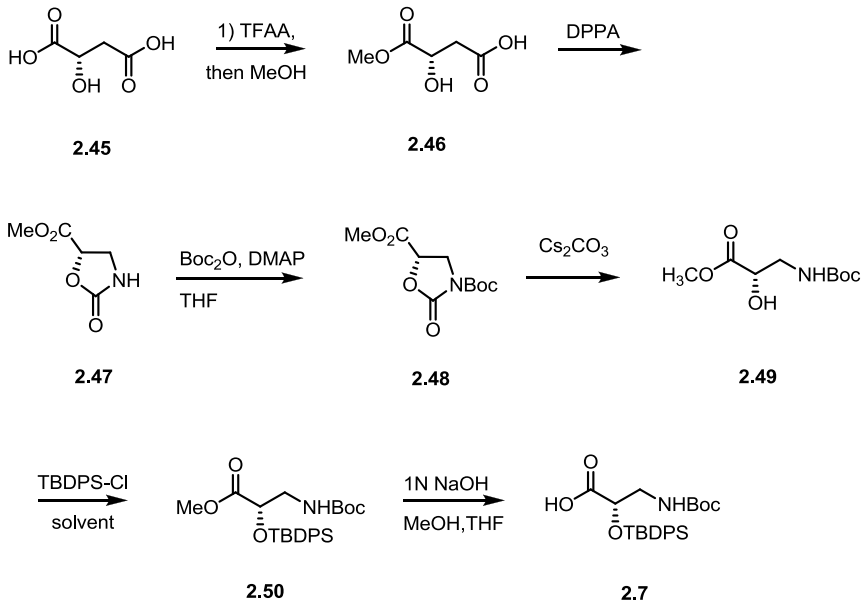
2.40 **2.44**

Scheme 2.12 Double bromination events using tricyclic form of tryptophan

The tricyclic compound was opened using 10% sulfuric acid in methanol in excellent yield.[27] The *N*-methylcarbamate deprotection preceded smoothly with in-situ generation of trimethylsilyliodide from trimethylsilylchloride and sodium iodide. This method was chosen over using performed trimethylsilyliodide due to hazards of the reagent. Neutralization of the iodo salt with sodium bicarbonate gave the free amine **2.6** with a 78% yield.[28] Analysis by NMR indicated that no further purification was necessary. The starting material **2.43** could be recovered from the reaction and utilized again to yield more free amine **2.6**.

2.3.4) Synthesis of isoserine from malic acid

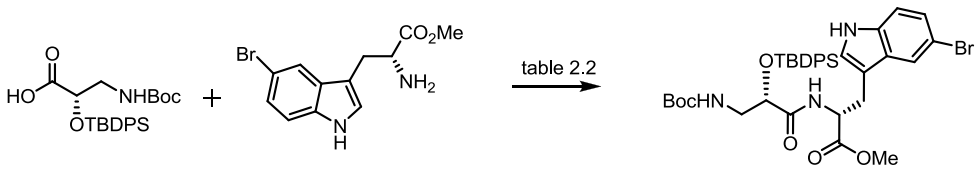
The β-amino acid isoserine was synthesized from the corresponding malic acid. The synthesis commenced with the selective methylation of **2.45** by treatment with trifluoroacetic anhydride to form the cyclic anhydride. Subsequent regioselective ring opening using methanol gave the mono-methyl malate **2.46** in good yield. This was followed by a Curtius rearrangement facilitated by DPPA; the intermediate isocyanate was trapped intramolecularly by the free secondary hydroxyl giving the cyclic carbamate **2.47**.[29] The resulting nitrogen was then protected with Boc to give compound **2.48**. The cyclic carbamate was opened using cesium carbonate to reveal the β-amino acid **2.49**. Protection of the alcohol with *tert*-butyldiphenylsiliyl chloride gave the desired compound **2.50** with a high yield of 91%.[30] Hydrolysis of the methyl ester with 1 equivalent of sodium hydroxide gave the free acid **2.7**.



Scheme 2.13 Synthesis of (*S*)-isoserine from malic acid

2.3.5) Formation of the isoseryl-5-bromotryptophan dipeptide

With the isoserine and tryptophan synthesized the peptide coupling reaction proceeded smoothly with multiple reagents. A summary of different reagents in Table 2.2 shows that the coupling reactions preceded well using 200 mg of the starting acid. There were no significant differences between the yields using the different reagents. When the amounts of starting amino acids were increased the yields started to decline and the reaction became more complex by TLC. The reaction was run at the 200 mg scale with multiple vessels running in parallel to stockpile the dipeptide **2.4**.



2.7 2.6 2.4

Entry	Coupling reagents	Base	Solvent	Yield %
1	EDCI/HOBt	DIPEA	CH_2Cl_2	70
2	EDCI/HOAt	DIPEA	CH_2Cl_2	68
3	EDCI/HOAt	DIPEA	DMF	79
4	HATU/HOAt	DIPEA	DMF	75
5	COMU	collidine	DMF	73

Table 2.2 Survey of coupling reagents for the synthesis of dipeptide **2.4**

2.4) Synthesis of removable turn inducer

The ability of small peptides with all-*S* configurations to cyclize has been shown to require the presence of turn inducers. These turn inducers can be a residue that is permanently present such as proline. It can also be a removable turn inducer, where a residue can be temporarily constrained for the cyclization event. The removable turn inducer would have to be easy to access and should be easily removed. Two important turn inducers discussed in the following sections are the pseudoprolines and cycloasparagine. Both compounds can be temporarily present during a synthesis and then removed upon acid treatment; thereby making them ideal for the synthesis of small cyclic peptides.

2.4.1) Pseudoproline as turn inducers

In order to synthesize highly strained cyclic peptides the Jolliffe group developed a strategy using pseudoprolines as a removable turn inducer. The pseudoprolines are oxazolidine or thiazolidine derivatives of serine, threonine and cysteine amino acid residues, which form by the cyclocondensation of the amino acid with an aldehyde or ketone. The pseudoproline was first used as a temporary protecting group to help with solubility and synthetic problems of linear polypeptides because they were found to have a significant effect on the peptide backbone conformation. The pseudoproline derivatives induce a cisoid amide bond into the linear peptide which facilitates cyclization of short peptide sequences. The bold lines in Figure 2.2 indicate the trans **2.51** and cis **2.52** conformation, with the cis being the more favorable conformation. The technology of pseudoprolines has been used in many different applications with many variations that are commercially available. Two examples are shown below: first their use in the synthesis of all-L cyclic tetrapeptides, second in the synthesis of cyclogossine B.

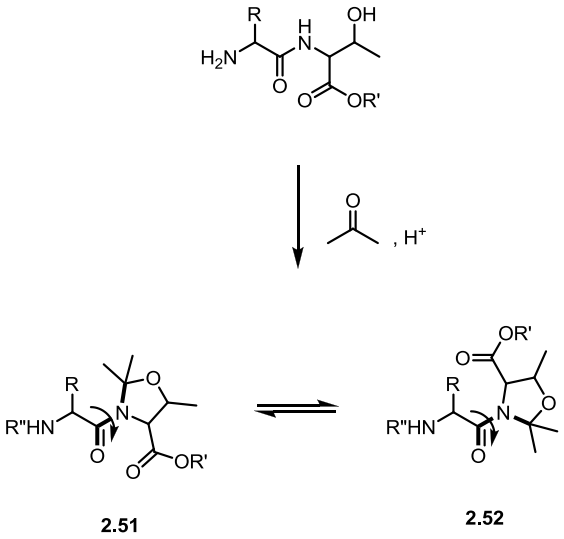
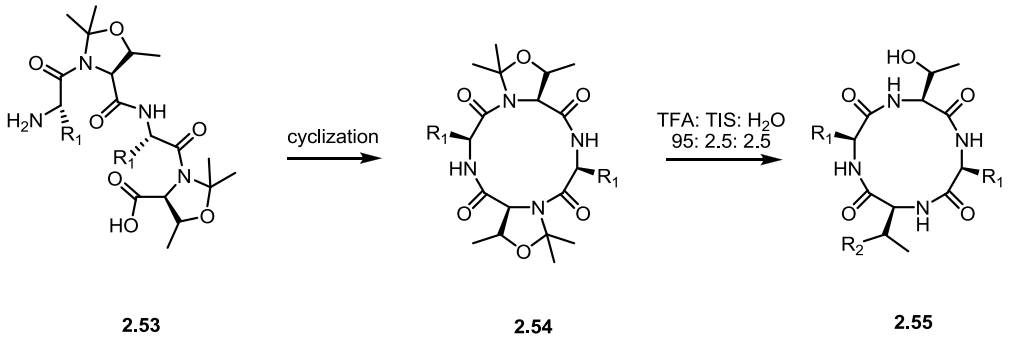


Figure 2.2 Interconversion of cis-trans isomers of pseudoproline from threonine

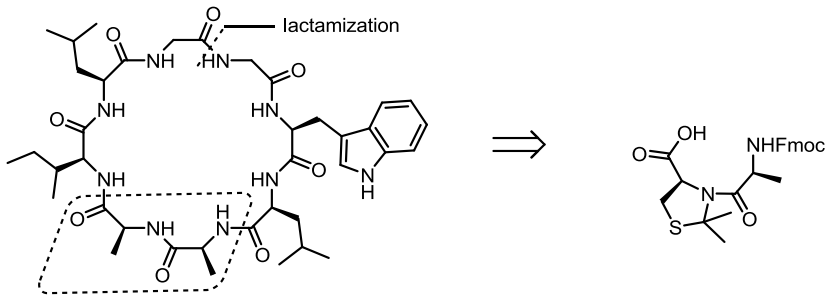
The cyclization of tetrapeptides frequently yields cyclic dimers rather then the cyclic monomers. The Jolliffe lab incorporated two pseudoproline (derived from threonine) residues into a series of tetrapeptides **2.53** with alternating spacer amino acids (Phe, Ile, Leu, and Val). The alternating turn inducers cause the ends of the peptide to be closer in space as was determined with x-ray crystallography. Cyclization occurred with the use of FDPP (pentafluorophenyl diphenylphosphinate) or DMTMM BF_4 [4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate] as the coupling reagent with yields ranging from 20-73% of structure **2.54**. Deprotection of the pseudoproline using TFA gave the cyclic peptide without a turn inducing amino acid present in the final structure **2.55**. With this sequence the synthesis of various cyclic tetrapeptides can be achieved when there is no turn inducer present in the final compounds.



2.53 2.54 2.55

Scheme 2.14 Synthesis of cyclic tetrapeptides that use threonine as a pseudoproline turn inducer

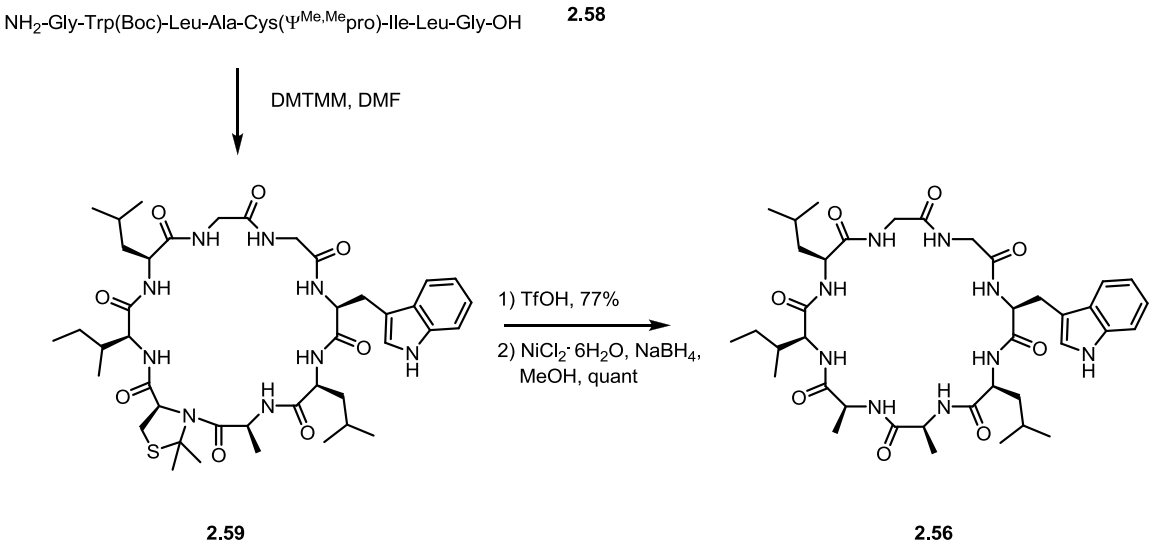
The Jolliffe group took this technology and applied it to the total synthesis of cyclogossine B **2.56**; a cyclic octapeptide isolated from the latex extract of *Jatropha gossypifolia*. The peptide showed no turn inducing structure present and there was also no serine or threonine present to form the pseudoproline. Looking at the retrosynthesis, the alanine was derived from cysteine derivative **2.57** which can undergo desulfurization to give the natural product.



2.56 2.57

Scheme 2.15 Retrosynthesis for cyclogossine B **2.56**

The linear octapeptide **2.58** was synthesized by standard solid phase peptide synthesis techniques using Fmoc/HBTU. The use of the 2-chlorotritylchloride resin allowed for cleavage from the resin and retention of the pseudoproline. The cyclization occurred in quanitive yield using DMTMM. They also found that the protected peptide **2.59** displayed much higher solubility properties in organic solvents than the unprotected peptides. The presence of the pseudoproline had two useful functions; as a turn inducer and to aid in peptide solubility.



Scheme 2.16 Cyclization and synthesis of cyclogossine B

After cyclization the pseudoproline was removed using triflic acid to reveal the cysteine. The desulfurization occurred rapidly using nickel boride (Ni_2B) which was formed in situ using sodium borohydride and nickel(II) chloride hexahydrate.

The synthetic material **2.56** was identical to the natural product, showing the utility of the methodology in cyclic peptide synthesis.

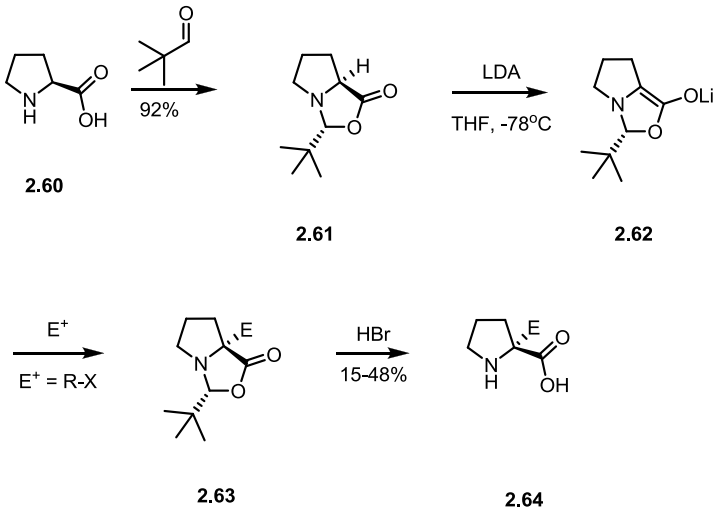
2.4.2) (Cyclo)asapragine

Another type of removable turn inducer is derived from the amino acid asparagine. The use of asparagine as a proline-like turn inducer arose from its ability to constrain the side chain amide into forming a tetrapyrimidinone. This form of asparagine was dubbed (cyclo)asparagine. In addition to initiating a turn it also constrains the side chain amide. The cyclo(Asn) allows for easier handling of peptides that contain asparagine by giving it increased solubility through constraining the side chain.

2.4.2.1) Seebach's work of self replication of chirality

The seminal work of Seebach *et al.*[35] in the area of peptide chemistry, and more importantly α-alkylation of amino acids, was the inspiration for the cyclo(Asn) chemistry. The term "self-replication of chirality" was used to describe the chemistry as demonstrated with proline in Scheme 2.17. *L*-Proline **2.60** is condensed with pivalaldehyde to yield a single diastereomer of the bicyclic aminal **2.61**. The stereogenic α-carbon of proline directs the formation of the *S*-conformation at the

newly formed stereogenic center. The aminal is then deprotonated to form the enolate **2.62**, thereby erasing the stereochemistry at proline's α-carbon **2.63**. However, upon nucleophilic attack by the enolate to an electrophlie, the new stereocenter directs the reproduction of the original *S*-configuration at the proline's α-carbon. Removal of the aminal with acid allows for α-alkyl amino acid **2.64**.



Scheme 2.17 Seebach's addition of an electrophile to proline

Although the chirality of the original stereogenic center is lost in enolate formation, the stereocenter is reformed to its original conformation. This methodology can be used to synthesize other, acyclic amino acid derivatives besides proline.[35] This work influenced the asparagine derived tetrapyrimidinones used by the Konopelski lab.

2.4.2.2) Previous work by the Konopelski lab

The Konopelski lab became interested in the synthesis of a cyclic form of asparagine in order to synthesize the enatiomerically pure β-amino acid β-tyrosine for jaspakinolide **2.65**, as shown in Figure 2.3**.** Jaspakinolide is a marine natural product isolated simultaneously by the Crews and Ireland-Falkner groups in 1986 and has shown to have activity against actin binding protein.[36]

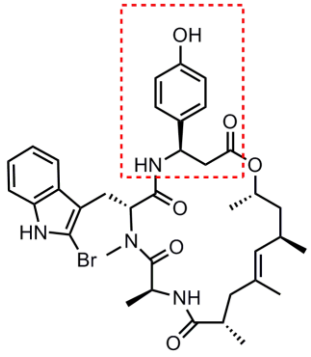
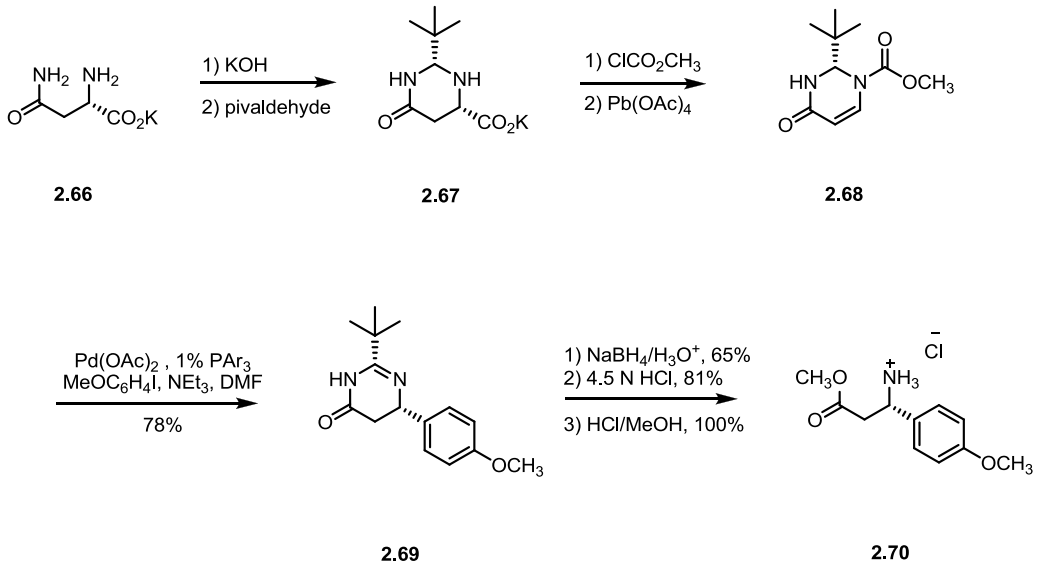


Figure 2.3 Structure of jaspakinolide

Following the work of Seebach, the Konopelski lab used asparagine as the starting amino acid for the synthesis of the β-tyrosine, highlighted in the red box of Figure 2.3.[37] The asparagine was prepared in two different ways. First, the potassium salt of asparagine was formed in a separate step, and then cyclization occured with an aldehyde in boiling methanol. The second method was to form the potassium salt in the methanol or water and aldehyde (e.g. pivaldehyde) was added to the mixture

without need to isolate the asparagine salt. For the synthesis of jaspakinolide, the asparagine was dissolved in aqueous KOH followed by the addition of pivaldehyde to give the desired tetrahydropyrimidine **2.67**.[38] Following the work of Seebach on the "self-reproduction of chirality," decarboxylation after protection of the amine nitrogen gave **2.68**. The addition of the aromatic side chain of tyrosine gave enatiomerically pure material **2.69**. Hydrolysis of the aminal with 3N HCl afforded β-tyrosine **2.70**. (Scheme 2.18)



2.66 **2.67** **2.68**

2.69 **2.70**

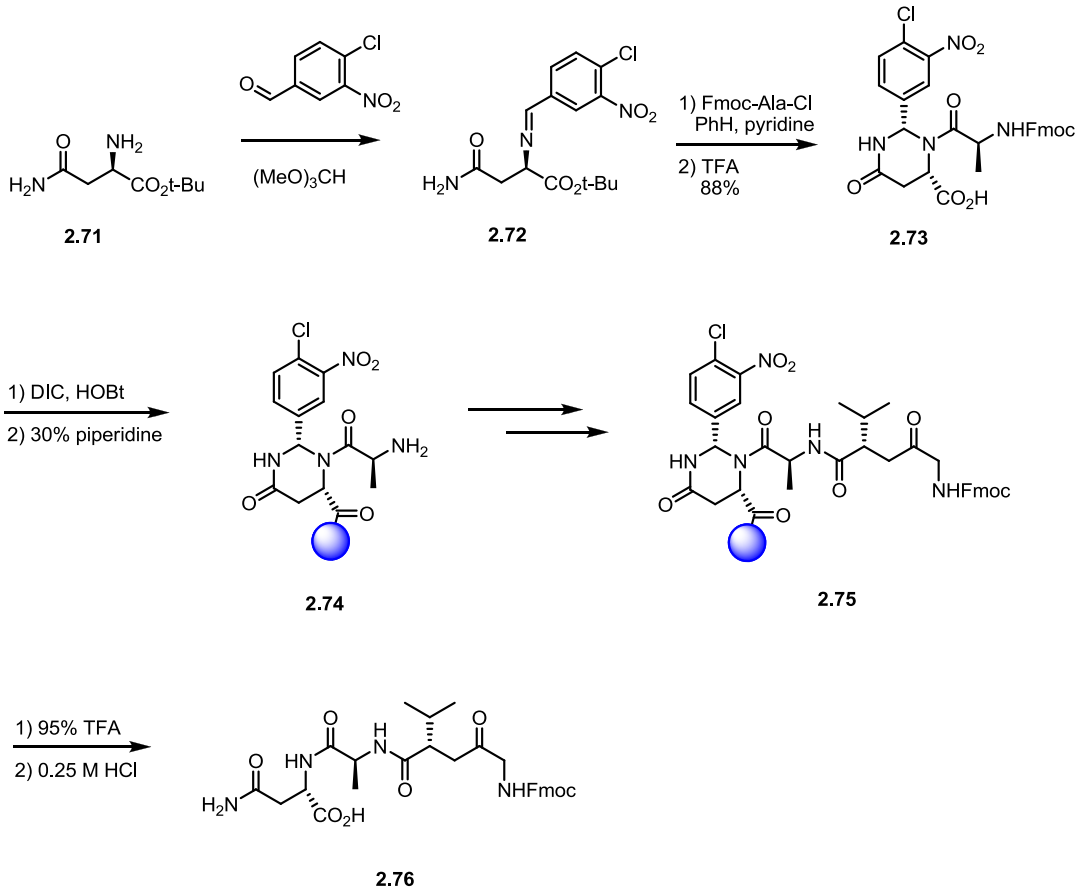
Scheme 2.18 Synthesis of β-tyrosine for jaspakinolide

Another utility of the (cyclo)Asn chemistry found in the Konopelski lab was in the form of dipeptide surrogates for use in building peptides. They functioned as protected organic soluble asparagine. The Konopelski lab became interested in the use of a cyclic form of asparagine for peptide synthesis. The tetrahydropyrimidines

were attractive because unlike other more conventional forms of amide protection, the absolute configuration and conformation was known. The incorporation of a turn inducer adds additional rigidity to a peptide system. It was shown that the (cyclo)asparagine conformation was similar to proline. Proline is an important residue in the type I and type II β-turns, most often occurring at the *i* + 1 position. (see Chapter 1)

The synthesis of the cyclo(Asn) was modified from the previously described method. The imine **2.72** was formed by reaction asparagine *tert*-butyl ester **2.71** and one equivalent of aldehyde (4-chloro-3-nitrobenzaldehyde shown) in the dehydrating solvent of trimethylorthoformate. The imine was then reacted with an acid chloride activated α-amino acid in the presence of pyridine, affording **2.73**. A wide range of amino acids were employed in the initial study, indicating that the reaction was flexible to the presence of a variety of functionalilties.[39] When an x-ray structure of the (cyclo)Asn was obtained it showed that the six membered ring was in a boat conformation and the R group from the imine was blocking one face of the molecule. This allowed for the retention of stereochemistry at the original site of chirality in the event of deprotanation ("self-replication of chirality"). The utility of this methodology was demonstrated when the cyclo(Asn) was attached to a resin at the carboxylic acid end. The synthesis of a tetrapeptide was accomplished while keeping the cycloasparagine residue intact. The tetrapeptide was removed form the

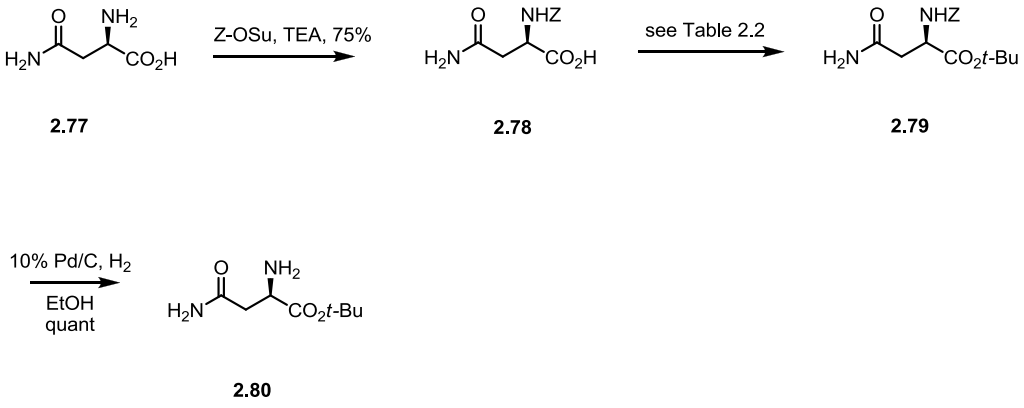
resin and the (cyclo)asparagine was easily removed with mild acid treatment to give the linear tetrapeptide **2.76** as shown in Scheme 2.19.[40]



Scheme 2.19 Synthesis of a linear tetrapeptide on resin utilizing cyclo(Asn)

2.4.3) Synthesis of the starting amino acids for the (cyclo)asparagine

The (cyclo)aspargyl-diaminopropionic acid dipeptide fragment was synthesized from the corresponding amino acids. The asparagine *tert*-butyl ester was synthesized from the starting asparagine hydrate. The asparagine was protected with Cbz-OSu to give Z-Asn-OH **2.78** in an excellent yield. The acid was protected as the *tert*-butyl ester using isobutene with sulfuric acid. Then removal of the Z-protection group by hydrogenolysis gave the desired *tert*-butyl asparagine. (Scheme 2.20)



2.77 **2.78** **2.79** **2.80**

Scheme 2.20 Synthesis of *tert*-butyl asparagine

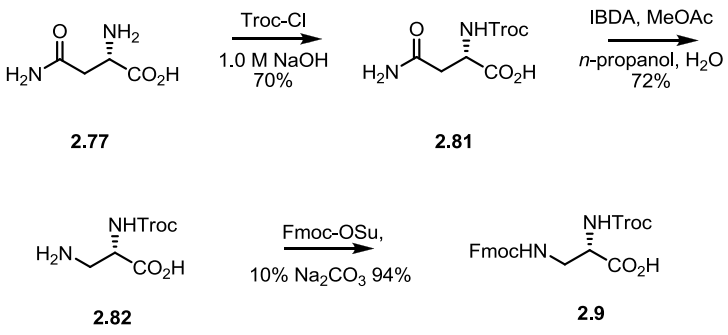
The protection with the isobutene was slow, cumbersome and gave a low yield (Table 2.3, entry 1). Because of the poor yield we decided to examine alternative esterification reagents. Attempts that used basic conditions with DMAP and Boc_2O proved unsuccessful and gave no desired product. Attention turned to *tert*-butyl acetate, and transesterification with perchloric acid gave much improved

results with a cleaner reaction and a higher yield (Table 2.3, entry 3). Increasing the amount of acid gave an improved result, however the use of perchloric acid was undesirable due to the risk of forming perchlorate salts (Table 2.3, entry 4). The acid was changed to sulfuric acid and the yield was reduced (Table 2.3, entry 5). When the time was reduced the amount of side reactions was reduced as well (determined by TLC) and the yield of the desired product was higher. Reducing the time any further gave reduced yields. The new route gives quicker access to the *tert*-butyl ester with the use of cheaper reagents and shorter reaction times compared to more classical methods.

Entry	*tert*-butyl source	Catalysis	Time (hour)	% Yield of 2.79
1	Isobutene	2.0 eq H_2SO_4	16	30
2	Boc_2O	DMAP	24	0
3	*t*-BuOAc	0.35 eq. 70% $HClO_4$	18	55
4	*t*-BuOAc	1.0 eq 70% $HClO_4$	18	62
5	*t*-BuOAc	1.0 eq H_2SO_4	18	45
6	*t*-BuOAc	1.0 eq H_2SO_4	2	73
7	*t*-BuOAc	1.0 eq H_2SO_4	1	44

Table 2.3 Survey of different *tert*-butyl sources

With the synthesis of the asparagine completed, the synthesis of diaminopropionic acid was under taken. Scheme 2.21 depicts the synthesis starting with asparagine, which was first protected using 2,2,2-trichlorethoxycarbonyl chloride (Troc-Cl)[41] in good yields to afford **2.81**. The β-nitrogen was installed via Hoffman rearrangement in a mixed solvent system of methyl acetate: *n*-propanol: water.[42] The product **2.82** was highly insoluble in the solvent system and would crash out as a pure solid. The β-nitrogen of the newly formed diaminopropionic acid fragment was protected with Fmoc to give compound **2.9**. The β-amino acid has two acid stable and orthogonal protection groups for the key (cyclo)asparagine reaction.

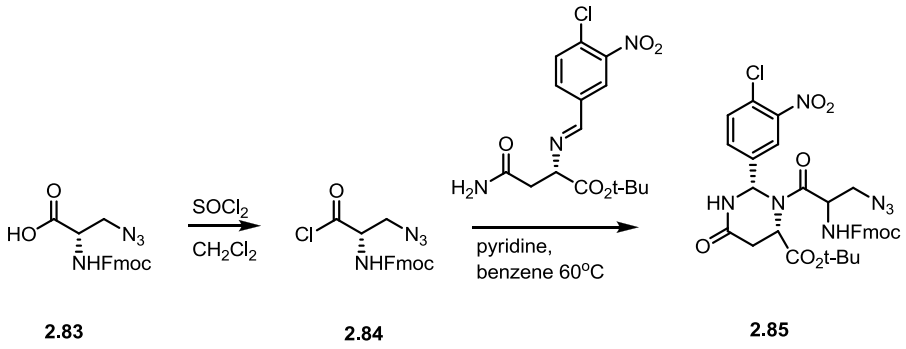


Scheme 2.21 Synthesis of the diaminopropionic acid β-amino acid

2.4.4) Jessica Garcia's synthesis and trials with (cyclo)asparagine used to form cyclocinamide A

The synthesis of the (cyclo)asparagine fragment was first undertaken by Dr. Jessica Garcia in her synthesis of cyclocinamide A. It was at this time that the

synthesis of cyclo(Asn) **2.85** had shown to be more difficult than anticipated. During the initial synthesis the use of an azido group was chosen as shown in compound **2.84**. However the reaction did not proceed smoothly and gave a very complex mixture with an unacceptably low yield of 15% for the desired material **2.85**. This route was quickly abandoned because the yields were unacceptably low and there was great difficulty in the purification process.[30] (Scheme 2.22)

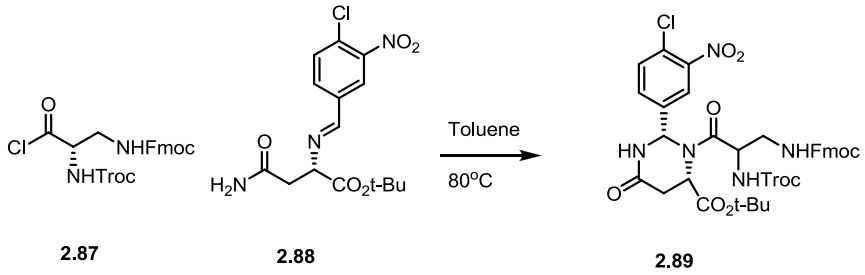


2.83 **2.84** **2.85**

Scheme 2.22 Synthesis of cyclo(Asn) β-azido-Fmoc-alanine

A revision of the Dap fragment synthesis led to the removal of the azido group since it was thought to be the cause of the poor results. The β-amino acid had the Fmoc group and, after an extensive research into different protection groups, Troc was chosen. Troc like Fmoc is orthogonal to the acid conditions used in the formation of the acid chloride. In addition, Troc was stable to the basic conditions used in the removal of the Fmoc group, which was thought to be

advantageous. With the revised Dap fragment the cyclo(Asn) reaction proceeded with more success at an initial yield of 30%.[30] (Scheme 2.23)



Scheme 2.23 Synthesis of cyclo(asn) using aryl group

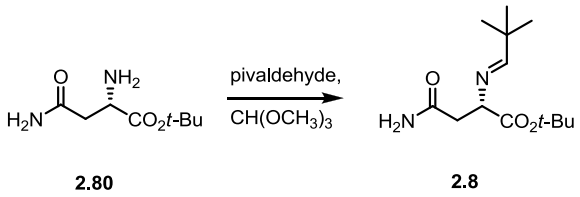
Optimization of the reaction was undertaken after the initial results were obtained. Solvent conditions were first examined because of the poor solubility of the imine. The different solvents examined include: dimethylformamide, toluene, dichloromethane, and acetonitrile. Of those listed toluene showed an improvement to 38% yield over the previously reported solvent, benzene.[42] With the new solvent, a survey of bases was undertaken including: collidine, DBU, DABCO, proton sponge and quinoline. Of these, proton sponge had a detrimental effect and yielded < 5% yield. Quinoline and collidine gave significantly improved yields of 46%. When the reaction was performed without a base present, the reaction was much cleaner and had an improved yield of 51%. Different temperature conditions at the start of the reaction were also examined. The best results came when the acid chloride and the imine were stirred at room temperature for 30 minutes followed by heating to 80

^{o}C for 6 hours. The product formed in 51% yield was a cis-trans mixture of rotamers around the new amide bond that coalesced at 100 ^{o}C, as seen in the ^{1}H NMR in DMSO-d_6.[30]

2.4.5) Synthesis of 11*S*,14*R* (cyclo)asparagine

The extensive optimization required to obtain the yields of **2.89** indicated that the reaction to form the corresponding 11*S*,14*R* isomer was going to be difficult. The choice of imine was changed in the synthesis of the cyclocinamide B route, because at the time we began a library of different isomers was proposed. We initially proposed the simultaneous synthesis of a number of isomers of cyclocinamide A and B in a single synthetic sequence utilizing **2.89** (*S*-Asn used aryl aldehyde) and **2.5** (*R*-Asn used pivaldehyde) simultaneously, then separating isomers on the basis of polarity (Ar vs *t*-Bu). However, this approach was never executed.

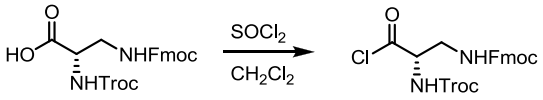
With the modified synthesis of *tert*-butyl asparagine, the synthesis of the imine was undertaken. Following a previously reported procedure,[43] asparagine **2.80** was mixed with pivaldehyde in trimethylorthoformate overnight (Scheme 2.24). The resulting imine **2.8** was highly sensitive to moisture and hydrolytically unstable, in contrast to the aromatic imine which proved to be highly stable and could be stored for months at a time.



2.80 **2.8**

Scheme 2.24 Synthesis of the imine of asparagine with pivaldehyde

The Troc-Dap(Fmoc)-OH **2.9** was converted to the acid chloride by using thionyl chloride in the presence of a catalytic amount of DMF at room temperature (Scheme 2.25).[44] Concentration of the reaction and drying under high vacuum gave the crude acid chloride as a white solid. The acid was found to initially have poor solubility in dichloromethane and conversion to the acid chloride greatly increased solubility. It was found that the presence of the DMF was not needed for conversion to the acid chloride.

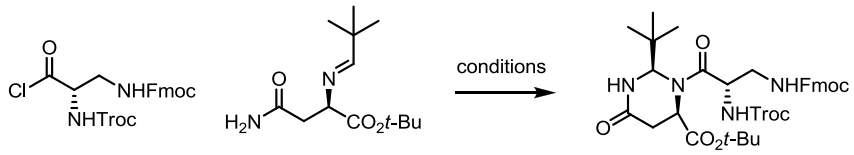


2.9 **2.87**

Scheme 2.25 Synthesis of acid chloride **2.87**

With the imine and acid chloride formed, the synthesis of the (cyclo)asparagine commenced (Scheme 2.26). The first reactions were performed according to the previously reported literature procedures.[43] Adding the acid chloride to the imine followed by refluxing in benzene for 6 h gave a very poor yield

of 12%. The poor yield led to a change of solvent, following Jessica's procedure (described in Section 2.5.3), toluene was chosen for the experiments going forward. The new solvent improved the yield slightly to 18%. Extending the reaction time from 6 to 12 hours also led to an increase in the yield, however the reaction product mixture became a great deal more complex.

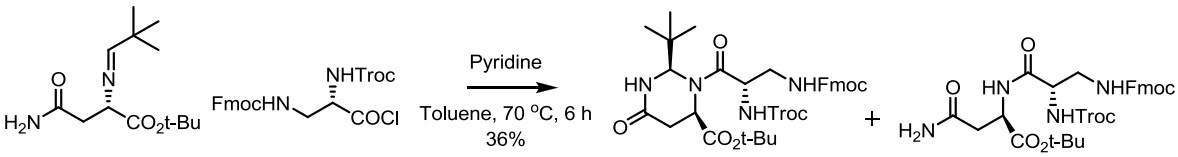


2.87 **2.8** **2.5**

Scheme 2.26 Formation of *tert*-butyl cyclo(Asn)

The base was the next component of the reaction to be examined, as shown in Table 2.4. Pyridine was the first base used in the reaction and only gave a yield of 18%, leaving much to be desired. Changing the base from pyridine to collidine showed an improvement of the yield to 24% (Table 2.4, entry 2). This was an encouraging result leading to continued examination. The next entry was performed without any base present using different bases unfortunately gave no desired product. This result was in contrast to the synthesis of the 11*S*,14*S* (cyclo)asparagine **2.89,** where the lack of base gave remarkably improved yields. It was at this time that the use of polyvinyl pyridine was attempted. The idea was to have a base present but not in the solution. However, at the end of the reaction no

desired product was seen. The dipeptide **2.90** was seen after the reaction (Table 2.4, entry 3). This indicated that the presence of a base was a necessity for the success of the reaction. The best yielding base was collidine, as was observed in the aryl cyclo(Asn) **2.89**.



2.87 **2.8** **2.5** **2.90**

Entry	Base	Solvent	Time (hours)	% Yield	Comment
1	Pyridine	Toluene	12	18	Complex mixture
2	Collidine	Toluene	12	24	Complex mixture
3	No base	Toluene	12	0	Formed **2.90**
4	Polyvinyl pyridine	Toluene	12	0	Formed **2.90**

Table 2.4 Survey of bases for cyclo(Asn) reaction

With the solvent and the base selected the next step was to examine the effects of the reaction times. Due to the success of the aromatic cyclo(Asn) reaction times were not investigated by Dr. Garcia. However, in the 11*S*,14*R* system it was found that the reaction length did have a effect on the yield. Starting by doubling of the reaction time to 12 hours showed an improved yield of 25% (Table 2.5, entry 1).

When the reaction time was again doubled to 24 hours the yield was markedly lower at 8%. When the reaction time was shortened to 5 hours, the yield showed a slight decrease. When the time was shortened to 3 hours the yield showed a slight improvement to 25% (Table 2.5, entry 4). The reaction was also much cleaner than the previous attempts. The main product was the dipeptide **2.90** without the cyclo(Asn) present, with the desired product present as well. With the possibility that shortened reaction time would increase the yield the reaction was next allowed to heat for 2 hours. The yield, however, was quite low at 16% (Table 2.5, entry 5). The best time was found to be either 3 hours or 12 hours, both having a disappointing yield of 25%.

Entry	Temperature (^{o}C)	Time (hour)	% Yield
1	65	12	25
2	65	6	28
3	65	5	22
4	65	3	25
5	65	2	16

Table 2.5 Survey of reaction length for cyclo(Asn)

The next parameter examined was the temperature at which the reaction was performed. When the reaction was performed at reflux the yields were always

variable. The temperature was then held at various points to determine if it was an important factor in the reaction. Starting at 60 ^{o}C the yield was low at 12% (Table 2.6, entry 1). Raising the temperature five degrees the yield improved to 17%. Another increase of 5 ^{o}C gave a significantly improved yield. It wasn't until the temperature reached 80 ^{o}C (Table 2.6, entry 5) that the yield started to decline. When the temperature was tested at 90 ^{o}C, there was no desired product detected. It was concluded that the best temperature for the reaction was 75 ^{o}C, not the reflux that was previously used. Thus, the best conditions found to that point were to heat for 3 hours at 75 ^{o}C, and to use collidine as a base, giving the best yield of 36%.

Entry	Temperature (^{o}C)	Time (Hour)	% Yield
1	60	12	12
2	65	12	17
3	70	12	25
4	75	12	36
5	80	12	5
6	90	12	0
7	65	12	15

Table 2.6 Survey of temperatures for the cyclo(Asn) formation

Testing the addition order of the reaction components was thought to be a parameter worth investigating. The slow addition of acid chloride to the stirring

solution of imine gave cleaner reactions and a slightly higher yield. When the addition was reversed and the imine was added to the acid chloride, the yield of the desired product decreased significantly. Simultaneous addition of the imine and acid chloride to the preheated pot gave lower yields as well. These results indicated that the addition order was important to the success of the reaction.

With the best order established the length of time for the addition was examined. During the previous experiments the addition time was held steady at 5 minutes. It was thought that a longer reaction time would allow for more product to be formed continuously, thus giving a higher yield. When the addition time was lengthened to 20 minutes, the reaction was indeed cleaner; however the yield did not improve. When the acid chloride was added extremely slowly, over a period of hours, the reaction was markedly cleaner but the majority of the compound formed was the dipeptide **2.90**.

During the synthesis the stability of the *tert*-butyl cyclo(Asn) was called into question. It was observed that upon standing at room temperature for a week the purified cyclo(Asn) started to decompose. Upon repurification the main decomposed material was the dipeptide with the free amide side chain. After this discovery was made the purified cyclo(Asn) was stored cold in order the prevent the adventitious loss of the *tert*-butyl group. Even with cold storage, the *tert*-butyl group showed poor stability. It was at this point that the plan for the cyclo(Asn) changed and stockpiling material was abandoned in favor of pushing material

through until a point of stability could be reached. This finding is in stark contrast to both the aromatic cyclo(Asn) and the earlier experiments in the Konopelski Lab with *t*-butyl cyclo(Asn), where there was excellent stability observed even after months of storage at room temperature.

After extensive testing on the cyclo(Asn) as conducted by Jessica Garcia and this author, it was concluded that the optimized conditions were found. Dr. Garcia optimized conditions were heating to 80 ^{o}C for 6 h and without base present. This author's conditions were heating to 75 ^{o}C for 3 h and adding the acid chloride over 20 minutes using collidine as a base. The yield for Dr. Garcia was a modest 51% after all the optimizations, while the yield for the *tert*-butyl system was a poor 36%. The significantly different outcomes can be attributed to the use of different aldehydes in the imine formation. The aromatic ring with electron withdrawing groups adds to the stability of the imine which appears to add more stability to the (cyclo)asparagine.

2.5) Conclusion

The synthesis of the three dipeptides that comprise cyclocinamide A and B have been shown. The side chain was successfully chlorinated regioselectively giving cyclocinamide A side chain. It was also dichlorinated using sulfuryl chloride to give the cyclocinamide B side chain. The isoseryl-5-bromotryptophan was synthesized using Hino's cyclic tautomer method to selectively brominate an indole derivative. The arduous synthesis of (cyclo)asparagine has allowed for the incorporation of a turn-inducer for easier access to the 14-membered core. With the three dipeptides in hand our attention turns toward the synthesis of the cyclic core and completion of the synthesis of cyclocinamide B.

2.6) Experimental Details

All reactions were performed in oven- or flame-dried glassware under an inert atmosphere of nitrogen with the utilization of standard syringe techniques, unless otherwise noted. Microwave reactions were carried out in a CEM Discovery 1 instrument at the indicated power and temperature. Anhydrous acetonitrile (CH_3CN), dichloromethane (CH_2Cl_2), methanol (CH_3OH), tetrahydrofuran (THF) and toluene were obtained from a Pur-Solv 400 solvent purification system manufactured by Innovative Technology. All reagents were used as purchased without further purification, with the following exceptions: benzyl alcohol was fractionally distilled and stored over 4Å molecular sieves; imidazole was recrystallized from benzene; diethylamine, diisopropylamine and triethylamine were distilled from calcium hydride and stored over sodium hydroxide pellets; 2,4,6-trimethylpyridine (collidine) was distilled from calcium hydride; trimethylsilyl chloride (TMSCl) was distilled and stored over polyvinylpyridine.

Reactions were monitored by thin layer chromatography on Whatman polyester-backed plates (250 μm layer of silica) and visualized by ultraviolet light at 254 nm or chemical stain (ninhydrin, potassium permanganate, phosphomolybdic acid or vanillin). Crude products were purified using flash-grade silica gel from Dynamic Adsorbents, Inc. (32-63 μm).

Proton and carbon (^{1}H and ^{13}C) NMR spectra were obtained on either 500 or 600 (cold probe) MHz Varian spectrometers in $CDCl_3$, CD_3OD and DMSO-d_6. Abbreviations used for multiplicity are as follow: s = singlet, d = doublet, t = triplet, sep = septet, m = multiplet. Melting points were determined on a Mel Temp II device and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer OneSource FTIR instrument as thin films; ν_{max} values are reported in inverse centimeters. High resolution mass spectra were obtained at Berkeley Mass Spectroscopy Facilities. Optical rotation measurements were found on a Jasco DIP-310 digital polarimeter; concentration is given in g/100 mL.

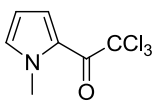
The shorthand amino acid nomenclature **PG_1-AA(PG_2)-X** is defined as follows:

PG_1: the protecting group on the nitrogen in the α-position of the α-amino acids tryptophan (Trp), asparagine (Asn) and diaminopropionic acid (Dap), and the β-nitrogen of isoserine (Ise)

AA: 3-letter common abbreviation of the amino acid. Exceptions are isoserine (Ise), diaminopropionic acid (Dap) and (cyclo)asparagine [(cyclo)Asn].

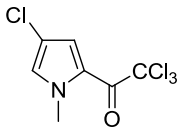
PG_2: the protecting group on the heteroatom located on the side chain of the amino acids Asn, Dap (β-nitrogen) and Ise (α-hydroxyl).

X: can be another amino acid, “OH” (free acid) or “OR” (an acid protected as its corresponding ester.



2-Trichloroacetyl-1-methylpyrrole. (2.14)

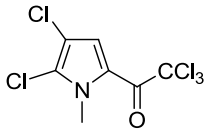
In a two-neck round bottom flask equipped with an addition funnel, a solution of *N*-methylpyrrole (2.5 g, 30.8 mmol, 1.0 equiv) in dichloromethane (12.3 mL) was added to a solution trichloroacetylchloride (3.5 mL, 30.8 mmol, 1.0 equiv) in dichloromethane (18.4 mL) over the course of 3 h. The solution was stirred vigorously overnight with nitrogen being swept over the reaction. The solvent was removed and the resulting residue was dissolved in chloroform and filtered through a silica plug. The solvent was evaporated *in vacuo* to yield the product as a white solid (75% yield, 5.2 g). Experimental data matched the literature.[7]



4-Chloro-2-trichloroacetyl-1-methylpyrrole. (2.15)

Using a modified literature procedure, a solution of 2-trichloroacetyl-1-methylpyrrole (0.5 g, 2.2 mmol, 1.0 equiv) was dissolved in THF (6.5 mL), *N*-chlorosucinamide (0.3 g, 2.2 mmol, 1.0 equiv) was added to the solution and the resulting mixture was allowed to stir at -10 °C in the dark for 2 h. The solution was removed from the dark and allowed to warm to room temperature with continued stirring for 16 h. The solvent was evaporated *in vacuo* and the crude residue was purified by flash column chromatography (9:1 Hexanes: Ethyl acetate) to afford a white solid (75% yield, 0.42 g).

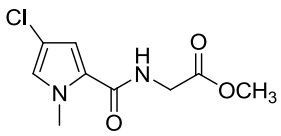
Mp 92-94 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 (d, *J* = 1.5 Hz, 1H), 6.92 (d, *J* = 1.5 Hz, 1H), 3.94 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 130.6, 121.9, 121.0, 112.1, 95.7, 38.7; IR (CH_2Cl_2) 3944, 3688, 3133, 1680, 1420, 1366, 1191 cm^{-1}; HRMS [M+H] for $C_7H_6Cl_4NO$, calcd., 259.9125 found, 259.9058



4,5-Dichloro-2-trichloroacetyl-1-methylpyrrole. (2.16)

In a two-neck round bottom flask, a solution of 2-trichloroacetyl-1-methylpyrrole (3.0 g, 13.2 mmol, 1.0 equiv) was dissolved in dichloromethane (25.0 mL). Sulfuryl chloride (3.4 mL, 13.2 mmol, 1.0 equiv) was added slowly dropwise and allowed to stir at 50 °C for 2 h in the dark. The solution was carefully poured into a cooled saturated solution of $NaHCO_3$ (30.0 mL). The aqueous layer was extracted with dichloromethane. The organic layer was filtered through a silica plug and was evaporated *in vacuo* to yield the product as a yellow oil (62% yield, 2.4 g).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49 (s, 1H), 3.98 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 121.5, 120.3, 111.1, 35.2, 25.7; IR (neat) 3146, 2961, 1766, 1681, 1396, 1205, 976, 908, 630 cm^{-1}; HRMS [M+H] for $C_7H_4Cl_5NO$, calcd., 293.8736 found, 293.8688

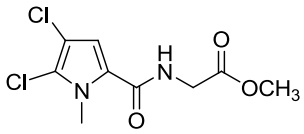


2-[(4-Chloro-1-methyl-pyrrole-2-carbonyl)-amino]-ethanonic acid methyl ester. (2.18)

In a three-neck round bottom flask, a solution of 4-chloro-2-trichloroacetyl-1-methylpyrrole (0.5 g, 1.7 mmol, 1.0 equiv) was dissolved in CH_3CN (8.3 mL). Glycine

methyl ester hydrochloride (0.2 g, 1.7 mmol, 1.0 equiv) was added along with triethylamine (0.2 mL, 1.7 mmol, 1.0 equiv). The reaction was stirred at room temperature for 24 h. The solvent was removed *in vacuo* and the residue was dissolved in dichloromethane. The organic layer was washed with water, 0.5 N HCl, followed by a solution of saturated sodium chloride. The organic layer was dried with Na_2SO_4 and evaporated. The crude product was recrystallized using EtOAc and hexanes to give a white solid (89% yield, 0.39 g).

Mp 81-83 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.68 (d, *J* = 1.4 Hz, 1H), 6.54 (d, *J* = 1.4 Hz, 1H), 6.26 (bs, 1H), 4.15 (d, *J* = 5.0 Hz, 2H), 3.88 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (125 MHz, CDCl3) δ 170.5, 160.9, 125.2, 111.5, 110.9, 52.4, 40.9, 36.7; IR (CH_2Cl_2) 3439, 2956, 1748, 1662, 1545, 1505, 1217, 946, 896 cm^{-1}; HRMS [M+H] for $C_9H_{12}ClN_2O_3$, calcd., 231.0458 found, 231.0526

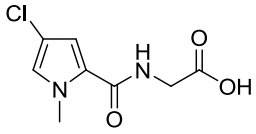


2-[(4,5-dichloro-1-methyl-pyrrole-2-carbonyl)-amino]-ethanonic acid methyl ester. (2.19)

Following the same procedure used for the synthesis of 2-[(4-chloro-1-methyl-pyrrole-2-carbonyl)-amino]-ethanonic acid methyl ester, a solution of 4,5-dichloro-2-trichloroacetyl-1-methylpyrrole (0.25 g, 0.85 mmol, 1.0 equiv) was dissolved in CH_3CN (4.5 mL). Glycine methyl ester hydrochloride (0.11 g, 0.85 mmol, 1.0 equiv) was added along with triethylamine (0.11 mL, 0.85 mmol, 1.0 equiv). The reaction was stirred at room temperature for 48 h. The solvent was removed and the residue

was dissolved in dichloromethane. The organic layer was washed with water, 0.5 N HCl, filtered through a silica plug, dried and evaporated in vacuo to yield an orange solid (81% yield, 0.18 g).

Mp 84-86 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.86 (t, *J* = 5.5 Hz, 1H), 6.59 (s, 1H), 4.05 (d, *J* = 4.8 Hz, 2H), 3.81 (s, 3H), 3.73 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 160.6, 123.6, 120.6, 111.2, 108.9, 52.4, 40.8, 33.5; IR (CH_2Cl_2) 3146, 2961, 1766, 1681, 1506, 1396, 976, 908, 630 cm^{-1}; HRMS [M+H] for $C_9H_{11}Cl_2N_2O_3$, calcd., 265.0141 found 264.0144

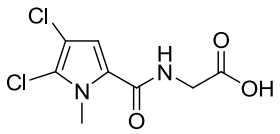


2-[(4-Chloro-1-methyl-pyrrole-2-carbonyl)-amino]-ethanonic acid. (2.20)

2-[(4-Chloro-1-methyl-pyrrole-2-carbonyl)-amino]-ethanonic acid methyl ester (0.1 g, 0.43 mmol, 1.0 equiv) was dissolved in THF (3.7 mL) and MeOH (0.7 mL) and cooled to 0 °C. An excess of NaOH (1N, 0.62 mL) was slowly added dropwise and the reaction was warmed to room temperature and allowed to stir overnight. Water and EtOAc were added, followed by quenching with potassium hydrogen sulfate (1M) until the pH reached 3. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with a saturated solution of sodium chloride, dry over Na_2SO_4 and the solvent was removed to afford a white solid (92% yield, 80.0 mg).

Mp 161-164 °C (decomp); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.93 (bs, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 6.72 (d, *J* = 2.0 Hz, 1H), 3.76 (s, 3H), 3.59 (d, *J* = 5.0 Hz, 2H); ^{13}C NMR δ

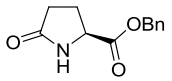
(125 MHz): 171.5, 160.6, 125.0, 111.8, 108.7, 40.4, 36.3; IR (thin film) 3563, 2946, 1747, 1682, 1578, 1505, 1217, 946, 896 cm^{-1}; HRMS [M+Na] for $C_8H_9ClN_2O_3Na$, calcd., 239.0197 found, 239.0184



2-[(4,5-dichloro-1-methyl-pyrrole-2-carbonyl)-amino]-ethanonic acid (2.2)

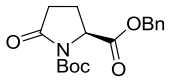
The methyl ester **2.19** (0.5 g, mmol, 1.0 equiv) was dissolved in THF:MeOH (13.2 mL: 2.8 mL) and cooled to 0 °C. Sodium hydroxide (1.0 N, 2.4 mL) was slowly added dropwise and the reaction was warmed to room temperature and allowed to stir overnight. Water (8.0 mL) and EtOAc (15.0 mL) were added to dilute the reaction, followed by quenching with potassium hydrogen sulfate (1M) until the pH reaches 3. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with a saturated solution of sodium chloride, dried over $NaSO_4$ and the solvent was removed to afford a pale yellow solid. (92% yield, 0.43 g).

Mp 172 °C (decomp); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.57 (s, 1H), 8.53 (t, J = 5.5 Hz, 1H), 6.99 (s, 1H), 3.84 (s, 3H), 3.84 (d, J = 5.5 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.2, 160.0, 124.5, 118.6, 111.2, 107.3, 40.5, 33.3; IR (thin film) 3566, 2939, 1745, 1679, 1576, 1501, 946, 896 cm^{-1}; HRMS [M+Na] for $C_8H_8Cl_2N_2O_3Na$, calcd., 272.9804 found, 272.9796



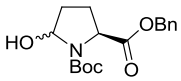
H-Pyr-OBn (2.22)

Compound was prepared via commercially available *S*-pyroglutamic acid according to literature procedure. Analytical data match reported values.[12]



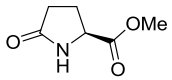
Boc-Pyr-OBn (2.23)

Compound was prepared according to literature procedure from **2.22**. Analytical data match reported values.[12]



Boc-Pyr(5-OH)-OBn (2.24)

Compound was prepared according to literature procedure starting with **2.23**. The crude material was isolated with quantitative mass recovery and used without further purification. Analytical data match reported values.[13]



H-Pyr-OMe (2.25)

Compound was prepared via commercially available *S*-pyroglutamic acid according to literature procedure. Analytical data match reported values.[14]

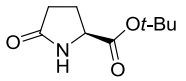
Fmoc-Pyr-OMe (2.26)

Compound was prepared according to literature procedure from **2.25**

Analytical data match reported values.[15]

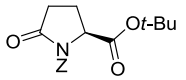
Fmoc-Pyr(5-OH)-OMe (2.27)

Compound was prepared according to literature procedure starting with **2.26**. The crude material was isolated with quantitative mass recovery and used without further purification. Analytical data match reported values.[16]



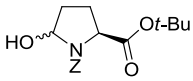
H-Pyr-O*t*-Bu (2.30)

Compound was prepared via commercially available *S*-pyroglutamic acid according to literature procedure. Analytical data match reported values.[18]



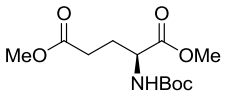
Z-Pyr-O*t*-Bu (2.31)

Compound was prepared according to literature procedure from **2.30**. Analytical data match reported values.[19]



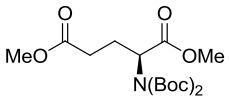
Z-Pyr(5-OH)- O*t*-Bu (2.32)

Compound was prepared according to literature procedure starting with **2.31**. The crude material was isolated with quantitative mass recovery and used without further purification. Analytical data match reported values.[19]



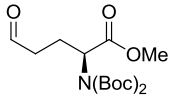
Boc-Glu(OMe)-OMe (2.35)

Compound was prepared from the commercially available *S*-glutamic acid according to literature procedure. Analytical data match reported values.[22]



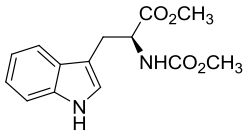
Boc_2-Glu(OMe)-OMe (2.36)

Compound was prepared according to literature procedure using compound **2.35**. Analytical data match reported values.[22]



(S)-methyl-2-(bis(tert-butoxycarbonyl)amino)-5-oxopentanoate (2.37)

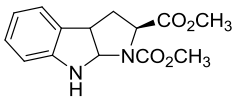
Compound was prepared according to literature procedure from compound **2.36**. The crude material was isolated with quantitative mass recovery and used without further purification. Analytical data match reported values.[22]



N_α-Methylcarbamate-tryptophan methyl ester.

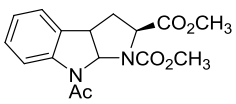
(*S*)-Tryptophan methyl ester (1.0 g, 3.9 mmol, 1.0 equiv) was dissolved in dichloromethane (20 mL) and cooled to 0 ^{o}C. Sodium hydroxide (2 M, 14.4 mL, 7.9 mmol, 2.0 equiv) and methyl chloroformate (0.6 mL, 7.9 mmol, 2.0 equiv) were added and the reaction was allowed to stir overnight, coming to room temperature. The aqueous layer was then extracted with dichloromethane. The

combined organic layers were washed with water and a solution of saturated sodium chloride, dried over Na_2SO_4 and evaporated. The residue was recrystallized in methanol and diethyl ether giving a white solid (78% yield, 0.98 g). Analytical data matched literature values.[25] The *R* isomer was prepared in the same manner.



(2*S*)-Dimethyl 3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1,2(2H)-dicarboxylate. (2.40)

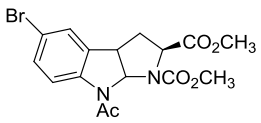
N_α-Methoxycarbonyl-tryptophan methyl ester (0.8 g, 2.7 mmol, 1.0 equiv) was dissolved in H_3PO_4 (85%, 8.3 mL) at room temperature and stirred for 5 h. The reaction was carefully poured into a cool solution of Na_2CO_3 (10 %, 200 mL) and then extracted with dichloromethane. The organic layer was washed with water and a solution of saturated sodium chloride, then dried over Na_2SO_4 and evaporated. The crude product was a white solid and was used without further purification (78% yield, 0.62 g). Analytical data matched literature values.[25] The *R* isomer was prepared in the same manner.



(2*S*)-dimethyl 8-acetyl-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1,2(2H)-dicarboxylate (2.41)

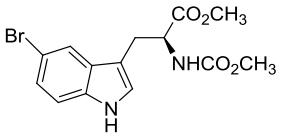
In a two-neck round bottom flask **2.40** (2.9 g, 10.4 mmol, 1.0 equiv) was dissolved in pyridine (58.0 mL), acetic anhydride (28.0 mL) was added and the reaction was allowed to stir overnight. The solvent was removed *in vacuo* and the resulting

residue was dissolved in dichloromethane and washed with a 5% HCl, saturated $NaHCO_3$, and water. The organic layer was washed with solution of saturated sodium chloride, and dried over Na_2SO_4 and evaporated. The residue was purified by flash column chromatography using EtOAc:Hexanes, (4:1) resulting in a white solid (60 % yield, 2.0 g). Analytical data matched literature values.[25] The *R* isomer was prepared in the same manner.



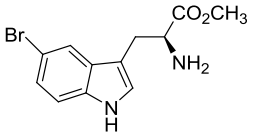
(2*S*)-dimethyl8-acetyl-5-bromo-3,3a,8,8a-tetrahydropyrrolo [2,3-b]indole-1,2(2H)-dicarboxylate. (2.42)

In a round bottom flask (2*S*)-dimethyl 8-acetyl-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1,2(2H)-dicarboxylate (1.58 g, 4.98 mmol, 1.0 mmol) was dissolved in acetic acid (27.0 mL) and freshly recrystallized *N*-bromosuccinamide (1.15 g, 6.48 mmol, 1.3 equiv) was added to the reaction, and was allowed to stir at room temperature for 24 h. The solvent was removed and the residue was dissolved in dichloromethane and washed with water. The organic layer was washed with a solution of saturated sodium chloride, and dried over Na_2SO_4 and evaporated. The crude material was recrystallized in MeOH forming a white solid (95% yield, 1.86 g). Analytical data matched literature values. [26] The *R* isomer was prepared in the same manner.



5-bromo-N_α-methoxycarbonyl-tryptophan methyl ester. (2.43)

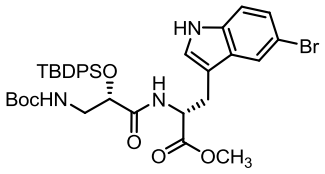
Compound **2.42** (1.7 g, 4.3 mmol, 1.0 equiv) was dissolved in 10% H_2SO_4 in MeOH (83.2 mL) and stirred at room temperature for 5 h. The reaction was carefully poured into cool water (380 mL) and extracted with dichloromethane. The organic layer was washed with water, saturated $NaHCO_3$, a solution of saturated sodium chloride, dried over Na_2SO_4 and evaporated giving a white solid (94% yield, 1.43 g). Analytical data matched literature values.[26] The *R* isomer was prepared in the same manner.



5-bromo-tryptophan methyl ester (H-Trp(5-Br)-OMe). (2.6)

In a two-neck round bottom flask *S*-5-bromo-N_α-methoxycarbonyltryptophan methyl ester (0.5 g, 1.4 mmol, 1.0 equiv) was dissolved in CH_3CN (8.1 mL). Sodium iodide (0.4 g, 2.8 mmol, 2.0 equiv), followed by TMSCl (0.35 mL, 2.8 mmol, 2.0 equiv), was added to the reaction. The resulting mixture was stirred at reflux for 2 h and was allowed to cool to room temperature then quenched with MeOH (5.0 mL) and allowed to stir for an additional 30 min. The solvent was removed leaving an orange residue. The residue was washed with $CHCl_3$ leaving behind the product as the pale yellow colored salt. The free base was obtained by partitioning the salt between dichloromethane and a solution of 5% $NaHCO_3$, and extracting the aqueous layer with dichloromethane. The organic layer

was washed with a solution of saturated sodium chloride and dried over $MgSO_4$ and evaporated forming a pale yellow solid (75% yield, 0.31 g). Analytical data matched literature values.[27] The *R* isomer was prepared in the same manner.



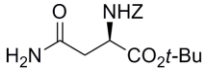
Boc-*S*-Ise(TBDPS)-*R*-Trp(5-Br)-OMe (2.4)

In a flame-dried flask Boc-Ise(TBDPS)-OH[31] **2.7** (0.43 g, 0.98 mmol, 1.0 equiv) was dissolved in dry dichloromethane (12.0 mL) and cooled to 0 °C. HOAt (0.14 g, 1.08 mmol, 1.1 equiv), HATU (0.41 g, 1.08 mmol, 1.1 equiv) and DIPEA (0.22 mL, 1.96 mmol, 2.0 equiv) were added and the mixture was allowed to stir for one hour. In a separate flask, tryptophan **2.6** (0.3 g, 1.08 mmol 1.1 equiv) was dissolved in dichloromethane (12.0 mL) was added. The mixture was stirred at room temperature overnight. Water (18.0 mL) was added and the solution was washed with dilute HCl, a solution of saturated $NaHCO_3$, and a solution of saturated sodium chloride. The organic layer was dried over $MgSO_4$ and evaporated. The crude residue was purified by flash column chromatography (Ethyl Acetate: Hexanes 2:3) resulting in a white solid (90% yield, 0.63 g).

Mp = 75-76 °C (decomp); $[\alpha]^{27}_D$ = -27.7, (c 3.6, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.65 (s, 1H), 7.14-7.53 (m, 12H), 6.56 (d, *J* = 2.0 Hz, 1H), 5.15 (t, J = 5.0 Hz, 1H), 4.80 (dd, *J* = 7.0, 13.0 Hz, 1H),4.18 (t, *J* = 4.5 Hz, 1H), 3.74 (s, 3H), 3.44-3.35 (m, 2H), 3.25 (dd, *J* = 5.5, 15.0 Hz, 1H), 3.12 (dd, *J* = 7.0, 15.0 Hz, 1H), 1.41 (s, 9H),

1.01 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 172.1, 155.9, 135.7, 135.6, 135.0, 132.8, 131.9, 130.4, 130.3, 128.9, 128.0, 125.2, 124.2, 121.2, 112.9, 112.8, 109.2, 79.0, 73.1, 52.6, 52.1, 44.5, 28.3, 27.3, 26.7, 19.1; IR (CH_2Cl_2) 3413, 3053, 2955, 2932, 2860, 1741, 1711, 1675, 1461, 1440, 1428, 1366, 1265, 1169, 1112, 1048, 738, 703 cm^{-1}; HRMS [M+H] for $C_{36}H_{45}BrN_3O_6Si$ calcd., 722.2250 found, 722.2256

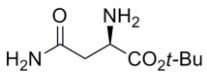
(*R*)-Carbobenzyloxy-asparagine *tert*-butyl ester (2.79)



Compound **2.78** (2.0 g, 7.51 mmol 1.0 equiv) was added to the *tert*-butylacetate (20 mL) and stirred vigorously. Sulfuric acid (conc., 0.8 mL, 15.03 mmol 2.0 equiv) was added dropwise and stirred at room temperature for 2 h. The reaction was carefully poured into a cool solution of saturated $NaHCO_3$ (80 mL), which was then extracted with EtOAc. The organic layer was washed with water, a solution of saturated sodium chloride and dry over Na_2SO_4. The solvent was removed and the resulting residue was recrystallized with ethyl acetate and hexanes to give a white solid (73% yield, 1.76 g).

Analytical data matched the reported literature values.[41]

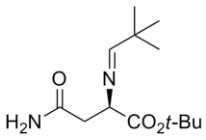
(*R*)-Asparagine *tert*-butyl ester (2.80)



A solution of compound **2.79** (3.0 g, 9.3 mmol, 1.0 equiv) dissolved in ethanol (50.0 mL) containing Pd/C (10%, 1.0g), was subjected to hydrogenation (50 psi) at room temperature overnight. The reaction mixture was filtered through a

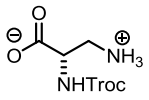
pad of celite and washed with methanol. The filtrate was evaporated concentrated leaving behind pure material as a wax-like solid (quant. 1.75 g).

Analytical data matched the reported literature values.[41]



(*R*)-*tert*-Butyl-4-amino-2-(2,2-dimethylpropylideneamino) -4-oxobutanoate (2.8)

In a flame-dried round bottom flask compound **2.80** (0.2 g, 1.1 mmol, 1.0 equiv) was added to trimethylorthoformate (5.0 mL) followed by pivaldehyde (0.1 mL, 1.1 mmol, 1.0 equiv). The reaction was stirred at room temperature under an atmosphere of nitrogen for 24 h. The solvent was removed and the resulting residue was washed with dichloromethane and dried under reduced pressure and used without further purification.

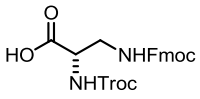


H-Dap(Troc)-OH. (2.82)

This procedure is based on a procedure performed on similar compounds in the literature.[44] Troc-Asn-OH (1.0 g, 3.25 mmol, 1.0 equiv) was dissolved in 8.6 mL of a mixture of ethyl acetate, CH_3CN and water (2:2:1) in a round bottomed flask. This solution was cooled in an ice-water bath to approx. 10 ^{o}C and iodobenzene diacetate (1.26 g, 3.91 mmol, 1.2 equiv) was added in one portion. The mixture was allowed to stir 0.5 h before allowing the temperature to rise to 20 ^{o}C. The reaction mixture was left to stir at this temperature for 5 h forming a white

precipitate. The flask was warmed to 70 °C until a solids dissolved then allowed to slowly cool to room temperature. The flask was then cooled in an ice bath, the solid filtered and washed with ethyl acetate to give a white solid. (77% yield, 0.72g).

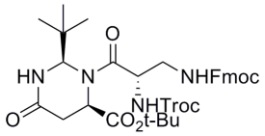
Mp 178-181 °C (decomp.); $[\alpha]_D^{26}$ = +26.3 (*c* 0.32, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.32 (bs, 2H), 7.16 (d, *J* = 6.5 Hz, 1H), 4.83 (d, *J* = 12.5 Hz, 1H), 4.78 (d, *J* = 12.5 Hz, 1H), 3.79 (ddd, *J* = 5.0, 6.5, 10.0 Hz, 1H), 3.05 (dd, *J* = 5.0, 12.0 Hz, 1H), 2.87 (dd, *J* = 10.0, 12.0, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.0, 153.9, 95.9, 73.4, 51.7, 40.1; IR (KBr pellet): 3317, 3025, 2922, 1943, 1704, 1267 cm^{-1}; HRMS [M+H] for $C_6H_{10}N_2O_4Cl_3$, calcd. 278.9701, found 278.9624.



Fmoc-Dap(Troc)-OH (2.9).

Troc-Dap-OH (1.4 g, 5.0 mmol, 1.0 equiv) was dissolved in 13 mL of a 10% Na_2CO_3 aq. Solution and was cooled in an ice bath. Fmoc-OSu (1.7 g, 5.04 mmol, 1.01 equiv) was dissolved in 13 mL of THF prior to addition. Upon complete addition of the Fmoc-Su solution to the amino acid solution, the flask was warmed to room temperature and stirred overnight. The THF was removed by rotary evaporation and the resulting aqueous layer was acidified to pH 2 with aq. HCl. The cloudy solution was extracted with ethyl acetate and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered and concentrated via rotary evaporation. The crude foam was recrystallized from $CHCl_3$ and hexanes to give the desired product as a white solid (94% yield, 2.42g).

Mp 95-97 °C; $[\alpha]_D^{27}$ = -7.4 (*c* 0.2, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6): 7.93 (d, *J* = 8.0, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.69 (dd, *J* = 3.0, 7.5 Hz, 2H), 7.41 (dd, *J* = 7.5, 7.5 Hz, 3H), 7.34 (ddd, *J* = 1.0, 7.5, 7.5 Hz, 2H), 4.80 (d, *J* = 1.0, 2H), 4.32 (dd, *J* = 8.0, 10.5 Hz, 1H), 4.28 (dd, *J* = 7.0, 10.5 Hz, 1H), 4.22 (dd, *J* = 7.0, 8.0 Hz, 1H), 4.18 (ddd, *J* = 5.5, 7.5, 7.5 Hz, 1H), 3.45 (ddd, *J* = 5.0, 5.0, 14.0, 1H), 3.38 (ddd, *J* = 7.0, 7.0, 14.0, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.6, 156.2, 154.5, 143.9, 140.8. 127.7, 127.1, 125.2, 120.1, 95.9, 73.6, 65.6, 54.1, 46.6, 41.4; IR (KBr pellet): 3378, 3081, 3025, 2923, 2849, 1942, 1748, 1697, 1601, 1492, 757 cm^{-1}; HRMS [M+H] for $C_{21}H_{20}N_2O_6Cl_3$, calcd. 501.0382, found 501.0372.



Fmoc-Dap(Troc)-*R*-(cyclo)Asn(*t*-Bu)-O*t*-Bu (2.5)

The imine **2.8** (0.1 g from **2.80**, 0.53 mmol, 1.0 equiv) was dissolved in toluene (3.0 mL) and warmed to 65 °C. To this collidine (0.09 mL, 0.69 mmol, 1.3 equiv) was added. The acid chloride **2.87** (0.3 g, 0.69 mmol, 1.3 equiv) dissolved in toluene (3.0 mL) was slowly added drop wise to the preheated solution of imine. The solution was stirred at 65 ^{o}C for 3 h, and was allowed to cool to room temperature, then filtered and washed with additional toluene. Solvent was removed in vauco. And the residue was purified by column chromatography (20% CH_2Cl_2 in diethyl ether) to give a white solid (36% yield, 0.14 g).

Mp = 78 °C (decomp); $[\alpha]^{28}_{D}$ = 2.7 (c 1.1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.58 (t, *J* = 7.5 Hz, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.33 (td, *J* = 3.5, 7.5 Hz, 2H), 5.34 (d, *J* = 8.0 Hz, 1H), 5.21 (dd, *J* = 5.5, 7.5 Hz, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.56 (d, *J* = 12.0 Hz, 1H), 4.55 (dd, *J* = 6.0, 9.5 Hz, 1H), 4.29 (d, *J* = 13.0 Hz, 1H), 4.17 (m, 3H), 3.73 (m, 2H), 3.55 (dt, *J* = 4.5, 15.5 Hz, 1H), 3.21 (d, *J* = 18.0 Hz, 1H), 2.58 (d, *J* = 18.0 Hz, 1H), 1.53 (s, 9H), 0.98 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.5, 169.4, 157.4, 153.5, 143.7, 143.6, 141.3, 127.8, 127.1, 125.1, 124.9, 120.0, 97.7, 96.0, 95.1, 85.4, 74.6, 67.3,65.8, 54.0, 47.0, 42.8, 32.8, 27.8, 25.1, 15.1; IR (CH_2Cl_2) 3437, 1698, 1651, 1634, 1526, 1423, 1153, 1045 cm^{-1}; HRMS [M+H] for $C_{34}H_{42}Cl_3N_4O_8$, calcd., 738.2062 found, 739.2063

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Chapter 3 – Synthesis of the linear peptide and macrocyclization to form nominal cyclocinamide B

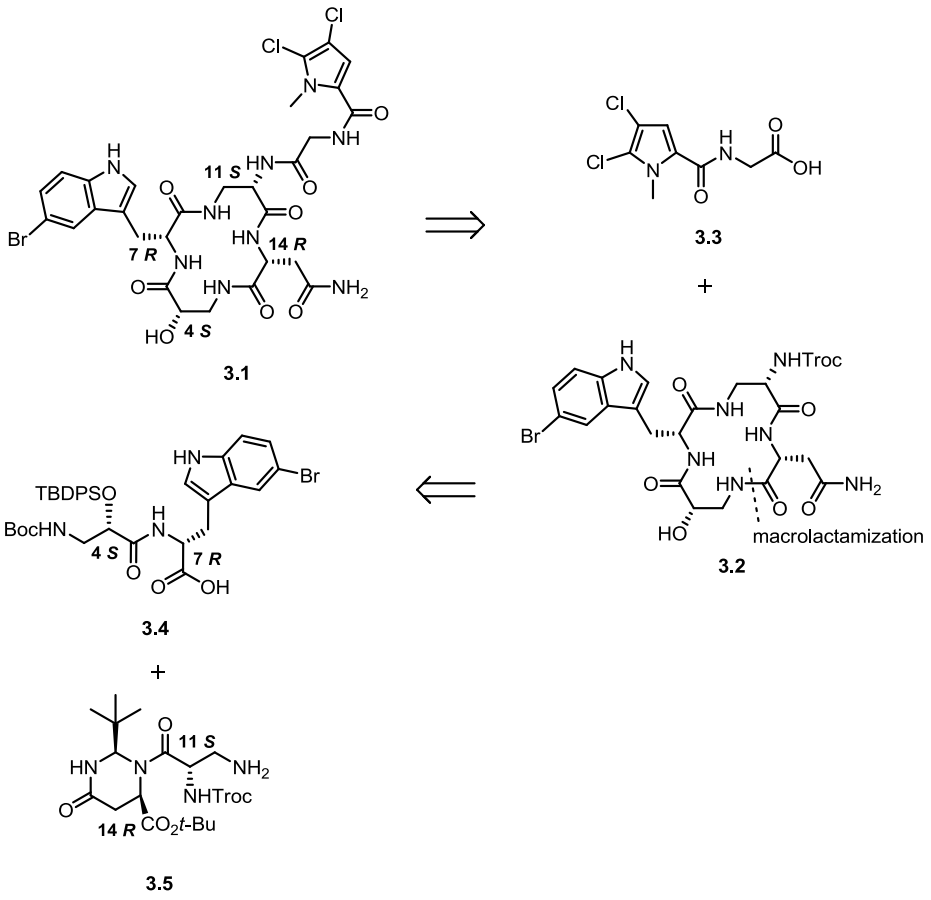
3.1) Introduction

As seen in Chapter 1, the cyclization of tetrapeptides is a difficult process prone to failure due to the transoid nature of the amide bonds and small ring size. This can be circumvented by the presence of a turn inducer.[1] The location of macrolactamization as well as the presence of turn inducers are important factors in the success of the cyclization. These factors were kept in mind moving forward in the synthesis of the linear system and the cyclization to form the cyclocinamide core and completion of the synthesis of cyclocinamide B.[2] With the synthesis of the isoserine-5-bromotryptophan dipeptide **3.4** and the (cyclo)Asn-diaminopropionic acid dipeptide **3.5** fragments completed, our attention turned to the synthesis of the tetrapeptide and cyclization. A late stage attachment of the side chain will complete the synthesis of cyclocinamide B.

3.2) Route A synthesis

The retrosynthesis shown in scheme 3.1 portrays cyclocinamide B **3.1** depicting disconnection of the side chain **3.3** and the cyclic core **3.2**. It was

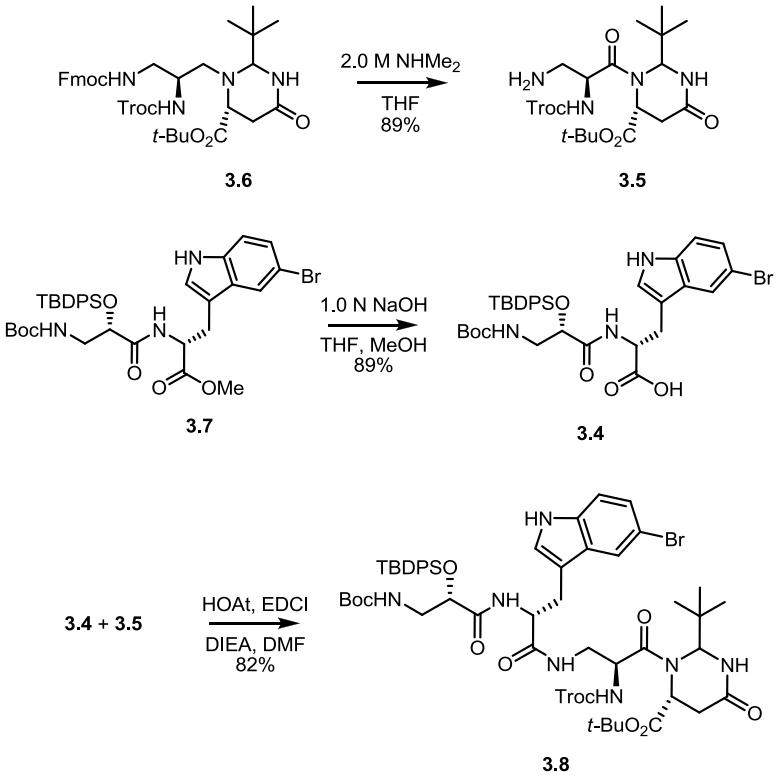
envisioned that the cyclic core would be synthesized from the corresponding linear tetrapeptide with closure at the 1-2 position (cyclocinamide numbering). The linear tetrapeptide is disconnected to the two equally complex dipeptide fragments: isoserine-5-bromotryptophan dipeptide **3.4** and (cyclo)Asn-diaminopropionic acid **3.5**.



Scheme 3.1 Retrosynthesis of cyclocinamide B

3.2.1) Synthesis of the linear tetrapeptide

With the three dipeptides in hand, synthesis of the linear tetrapeptide commenced. The (cyclo)Asn fragment **3.6** was treated with a 2.0 M solution of dimethylamine in THF. A variety of secondary amines of decreasing bulk were investigated including: diisopropylamine, diethylamine and dimethylamine. It was found that the reaction proceeded quicker using the less hindered amine base; from overnight with diisopropylamine to two hours using dimethylamine. In addition, fewer side products were observed when the less hindered amine was used. With the free amine **3.5** obtained, the work turned towards methyl ester deprotection of the isoseryl-tryptophan dipeptide **3.7**. Using standard conditions of 1.0 N NaOH in a solvent mixture of THF and MeOH the free acid **3.4** was obtained in good yields as a very clean reaction product.



Scheme 3.2 Synthesis of the linear tetrapeptide

After the successful deprotection of the two dipeptides, (cyclo)Asn-diaminopropionic acid **3.5** and isoseryl-5-bromotryptophan **3.4**, formation of the linear tetrapeptide was undertaken. The first coupling to form the tetrapeptide **3.7** was preformed with the commonly used carbodiimide-based coupling reagent, EDC and additive HOBt, which gave a decent yield of 72%. The reaction also gave a multitude of unidentifiable side products, so other coupling reagents (see Figure 3.1 for structures) were employed in hope of improving the reaction yields and giving

cleaner reactions. Using HOAt instead of HOBt gave markedly improved yields and cleaner reactions, while still using EDC. Switching to the uronium based coupling reagent HATU, and inclusion of HOAt gave the highest yield of 82% and was used for all further tetrapeptide couplings as shown in Scheme 3.2. There was also no adventitious formation of secondary hydroxyl, from TBDPS deprotection, present in the freshly prepared tetrapeptide. This was in contrast to the all-*S* tetrapeptide **3.9**, which showed liberation of the secondary hydroxyl by removal of the TBDPS.

When comparing the deprotection of the 4*R*,7*S* dipeptide **3.4** to the 4*S*,7*S* dipeptide synthesized by Dr. Jessica Garcia for cyclocinamide A[3], there was a clear difference in the reactivity. The 4*S*,7*S* dipeptide had an appreciable amount of free secondary alcohol present at the end of the reaction and thus a depressed yield of the desired product. This result was in contrast to the 4*S*,7*R* dipeptide where there was no observable deprotection of the TBDPS group.

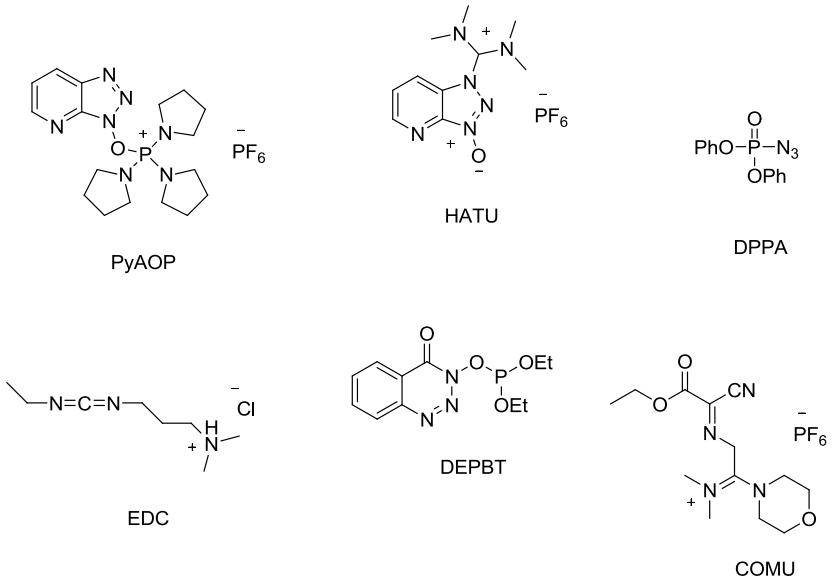


Figure 3.1 Various coupling reagents used for peptide coupling[4]

After the successful synthesis of the linear tetrapeptide we became aware that the tetrapeptide was not stable; similar to our previous observations of the (cyclo)Asn fragment (see Chapter 2 for discussion). Upon synthesis of the tetrapeptide it was noticed that decomposition of the material was occurring before and after purification. If the tetrapeptide was not purified immediately after synthesis (within 24 h of work up), new compounds were detected causing purification to become much more burdensome and resulted in a significantly lower yield. The new compounds included: loss of the TBDPS group, the opening of the (cyclo)Asn (identified by mass spectroscopy) and other products that were not identified. In an attempt to stop the decomposition that occurred, the peptide was stored in the freezer. Even when the pure tetrapeptide was stored in our freezer the same compounds were observed after a week. With the very discouraging findings

of the tetrapeptide storage and stability, stockpiling of the tetrapeptide was abandoned and instead would be used immediately. The instability seen with the 4*S*,7*R*,11*S*,14*R* **3.8** was in contrast to the stability seen the 4*S*,7*S*,11*S*,14*S* system **3.9**, which could stay at room temperature without showing signs of decomposition.[3] The difference was thought to be due to the use of the *tert*-butyl group for the (cyclo)Asn instead of the aryl group seen in Figure 3.2. Another factor in the lower stability could be the difference in stereochemistry of the two peptides.

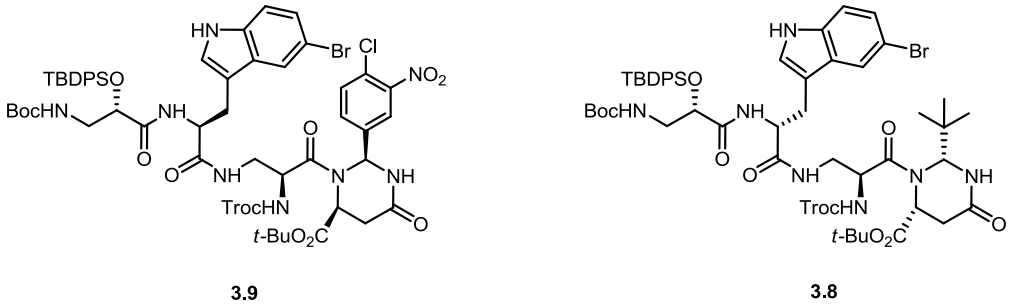


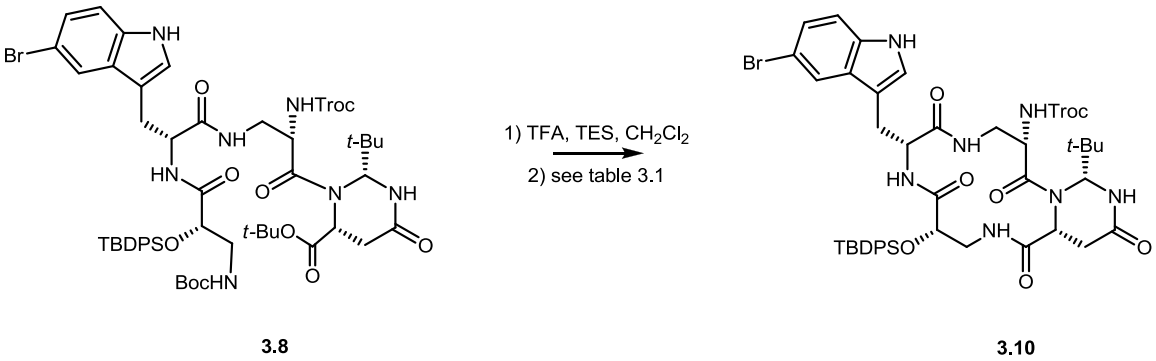
Figure 3.2 Comparison of the linear tetrapeptide for cyclocinamide A and B

3.2.2) Cyclization

After the synthesis of the linear peptide our attention turned to cyclization of the peptide, while keeping in mind the inherent difficulties of cyclization of tetrapeptides. The bis-deprotection of the linear tetrapeptide **3.8** was undertaken using trifloroacetic acid and triethylsilane in a solution of dichloromethane to give the free carboxylic acid and the TFA ammonium salt of the *N*-terminal amine. The

presence of the triethylsilane allows for the preservation of the *tert*-butyldiphenylsiloxy group, and the deprotection proceeds cleanly as indicated by mass spectroscopy.

The cyclization of the tetrapeptide shown in Scheme 3.3 were all performed under dilute conditions (1×10^{-4} M) in order to prevent the formation of dimers and oligomers.[4] The cyclization was first attempted with EDC and HOAt (Table 3.1, entry 1) and after three days gave a complex mixture. There was no desired product formed as established by mass spectroscopy. With the disappointing results a survey of coupling reagents was undertaken in order to determine if cyclization of the system was possible. The next attempt was the use of PyAOP[5], which was successful in the synthesis of the cyclic all *S* compound (63% yield). When the 4*S*,7*R*,1*S*,14*R* isomer was subjected to the same conditions as used for the all *S* isomer there was no desired product seen, indicating that the two diastereomers were in different conformations.



3.8 **3.10**

Scheme 3.3 Cyclization of the linear tetrapeptide

Turning to the HATU/HOAt system that had the best yield in the synthesis of the linear tetrapeptide, encouraging results were observed where the desired cyclic peptide **3.10** was obtained with a low yield of 12% but with the cleanest reaction of all the coupling reagents attempted thus far (Table 3.1, entry 3). The new uronium coupling reagent COMU[6] was tested and after 2 days the yields were improved from the previous results. However, they were still unacceptably low. Letting the reaction run longer in the presence of HOAt gave a sight increase in the yield. Next our attention turned to the use of DPPA which gave good yields of 60% for the 4*S*,7*S*,11*S*,14*S* system. After several attempts using DPPA the desired product was never detected.

With the preliminary results, it was determined that the tetrapeptide can be cyclized successfully, albeit at much lower yields than the all *S* isomer. Optimization of the reaction was then undertaken using both the COMU and the HATU/HOAt

system because of the cleaner reactions that were obtained. The two systems were also the only ones that showed positive results in the macrolactamization.

Entry	Coupling Reagent	Temperature (^{o}C)	Time (Days)	Yield %
1	EDCI/ HOAt	RT	3	0
2	PyAOP	RT	3	0
3	HATU/ HOAt	RT	3	12
4	COMU	RT	2	31
5	COMU/HOAt	RT	3	37
6	DPPA	RT	3	0

Table 3.1 Survey of coupling reagents used in cyclization

After the best coupling reagent was chosen, other factors were investigated for optimization. The next step was to run the cyclization reaction at elevated temperatures as shown in Table 3.2. With the HATU/HOAt system the first attempt was to increase the temperature to 30 ^{o}C (Table 3.2, entry 2). Encouraging results were seen after two days, with a slight increase in the yield. With an increase of 5 ^{o}C more the yield again rose (Table 3.2, entry 3). The HATU/HOAt system benefited greatly from increasing the temperature. There was an increase in the yield by letting the reaction run for three days as opposed to two days (Table 3.2, entry 5). After three days there was not an appreciable increase in the yield; so this became the length of time for the cyclization event. It was only when the temperature was

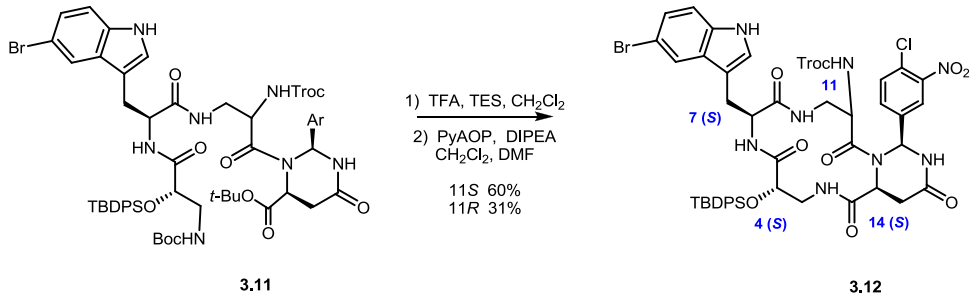
45 ^{o}C (Table 3.2, entry 6) that the yield decreases to 21% from the best yield of 54%. This indicates that the material was decomposing when the temperature was too high. The reaction with COMU/HOAt which proved successful at room temperature gave a very poor yield at the elevated temperature of 40 ^{o}C, and was not pursued further (Table 3.2, entry 7). With the optimization of coupling reagent, time and temperature the cyclization reaction gave an optimized yield of 54%.

Entry	Coupling reagents	Temperature (^{o}C)	Time (days)	Yield %
1	HATU/ HOAt	RT	3	12
2	HATU/ HOAt	30	2	16
3	HATU/ HOAt	35	2	24
4	HATU/ HOAt	40	2	42
5	HATU/ HOAt	40	3	54
6	HATU/ HOAt	45	2	21
7	COMU/ HOAt	40	2	10

Table 3.2 Survey of temperatures used in cyclization

The results found here are in contrast to what was found with the 4*S*,7*S*,11*S*,14*S* diastereomer used in cyclocinamide A (Scheme 3.4). With that isomer the optimal conditions were with the use of PyAOP at room temperature for 3 days, giving a 63% yield of cyclic material **3.12**. The 4*S*,7*S*,11*S*,14*S* cyclization, no additional heat was required, which stands in contrast to the necessity for heating the

4*S*,7*R*,11*S*,14*R* isomer in order to obtain a decent yield. The results indicate that the conformation induced by the presence of the (cyclo)Asn moiety in the all *S* linear tetrapeptide allows for improved cyclization, while the 4*S*,7*R*,11*S*,14*R* linear peptide is just at the range that allows for cyclization. Heating the linear peptide allows for sampling of more conformations and this leads to a successful cyclization.



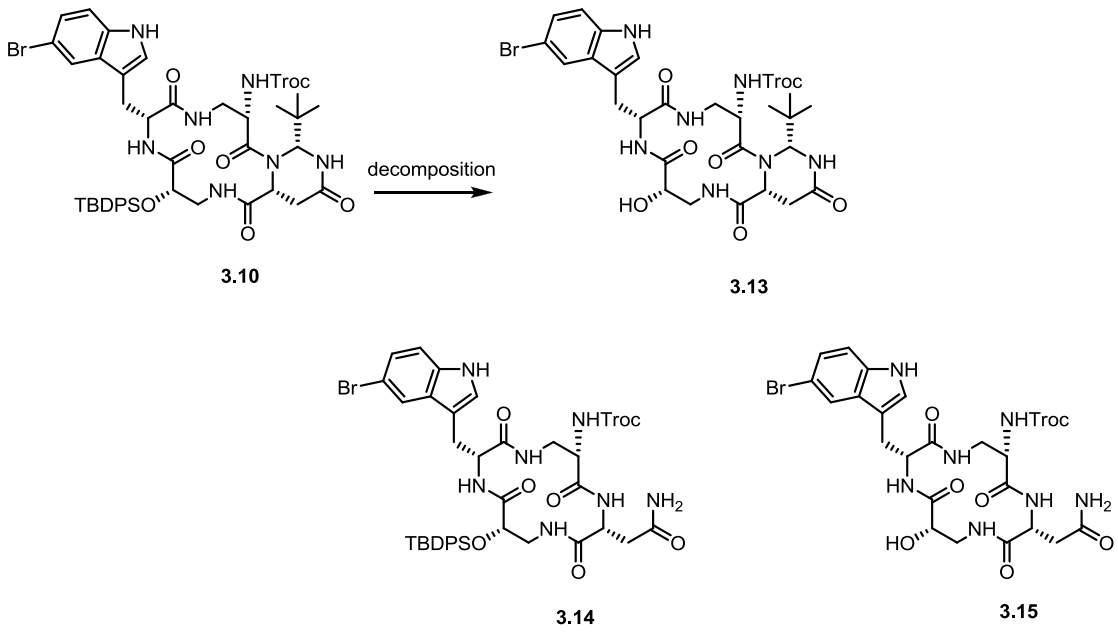
3.11 3.12

Scheme 3.4 Cyclization of the 11*S* and 11*R* cyclocinamide A core

3.2.3) Attempts to finish the synthesis – removal of (cyclo)asparagine

With the successful cyclization of the tetrapeptide, attention turned to an unexpected result found when the material was allowed to stand at room temperature. It was discovered that the cyclic material **3.10** would decompose upon standing and that this was occurring almost immediately after the reaction had been purified. It was seen by TLC that the pure material was isolated as a single compound and after 24 hours, 3 new compounds appeared. When the material was

stored at lower temperatures the results were the same. The principle degradation products included: free alcohol **3.13**, (cyclo)Asn hydrolysis product **3.14**, and free hydroxyl/free asparagine compound **3.15** (Scheme 3.5). The structures were analyzed using ESI-MS and NMR the same day of purification in order to minimize mixtures from forming because of further decomposition. Analysis of the cyclic compounds became difficult due to the instability. Therefore mass spectroscopy was relied upon for analysis of the samples.



Scheme 3.5 Decomposition products of the cyclic material upon standing

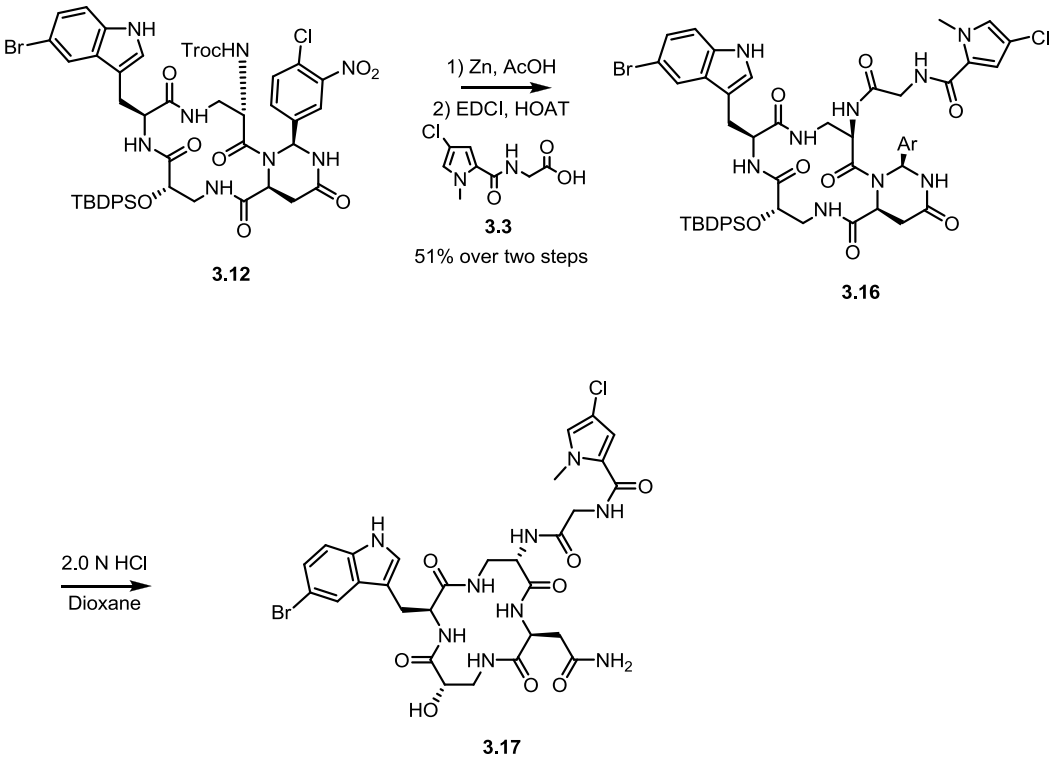
The decomposition products were pushed to compound **3.15** by stirring the crude product from the cyclization in 0.25 N HCl overnight which gave a quantitative yield. This result indicated that the protection groups are exceedingly acid labile in the cyclic peptide, much more so than when the peptide is in its linear form. The loss of the (cyclo)Asn moiety was believed to be advantageous due to the extreme difficulty in hydrolysis of the aryl *N,N*-acetal used in the (cyclo)Asn of the 4*S*,7*S*,11*S*,14*S* diastereomer (see Scheme 3.6 and discussion in next section). With **3.15** in hand we moved forward with the Troc deprotection and addition of the side chain to complete the synthesis of cyclocinamide B.

3.2.4) Attempts to complete the synthesis – removal of Troc

A look at the completion of the synthesis of cyclocinamide A shown in Scheme 3.6 is warranted at this time because of the divergence of the syntheses at this point. Dr. Jessica Garcia used the aryl group in forming the (cyclo)Asn; this proved to be quite advantageous in the stability of the cyclic material that formed. In the removal of the Troc protection group it proved to be the key factor in the success of that reaction. Using standard conditions allowed for the liberation of the amine in a clean reaction. What was also observed was the reduction of the aromatic nitro group to the corresponding amine. The presence of the second amine was thought to cause issues during the coupling of the side chain **3.3** such as double

addition or coupling to the undesired amine. The coupling proceeded slowly with modest yields and the formation of two products **3.16**. The exact nature of these two products could not be determined, even after extensive 2D NMR analysis, beyond the fact that the side chain coupled to the correct nitrogen and the amine of aromatic ring was left untouched.

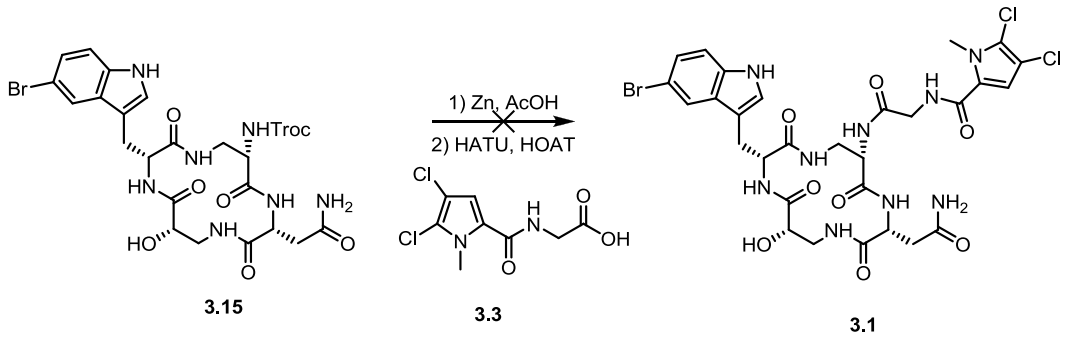
The removal of the (cyclo)Asn was thought to be straight forward and using previous methods developed in our laboratory yielded only starting material. It was after extensive testing that the asparagine side chain was liberated using 2.0 N HCl in dioxane in very poor yields. Despite the poor yield enough material was purified to obtain complete NMR analysis of the nominal structure of cyclocinamide A **3.17**. (discussion of the analytical data will occur in chapter 4)



Scheme 3.6 Completion of the synthesis of cyclocinamide A

In this total synthesis program, the *tert*-butyl derived (cyclo)Asn has proven to be extremely labile in comparison to the corresponding aryl group derived system, something that had not been observed in our previous studies with these systems. The *tert*-butyl system also has the advantage of not having a group that can be reduced, unlike the nitro of the aryl group system. These two things led us to believe that despite the lower yield in cyclization and lower stability of the peptides leading up to the cyclization, the *tert*-butyl was a superior choice for the end game of the synthesis.

With the free asparagine side chain material **3.15**, the removal of the Troc protection group was undertaken. We first employed standard conditions of zinc dust in acetic acid for the removal and worked the reaction up by filtration through celite and concentration.[8] The crude material was taken on to the coupling of **3.3**; however no desired product was detected (Scheme 3.7).



Scheme 3.7 Attempted completion of the synthesis of cyclocinamide B

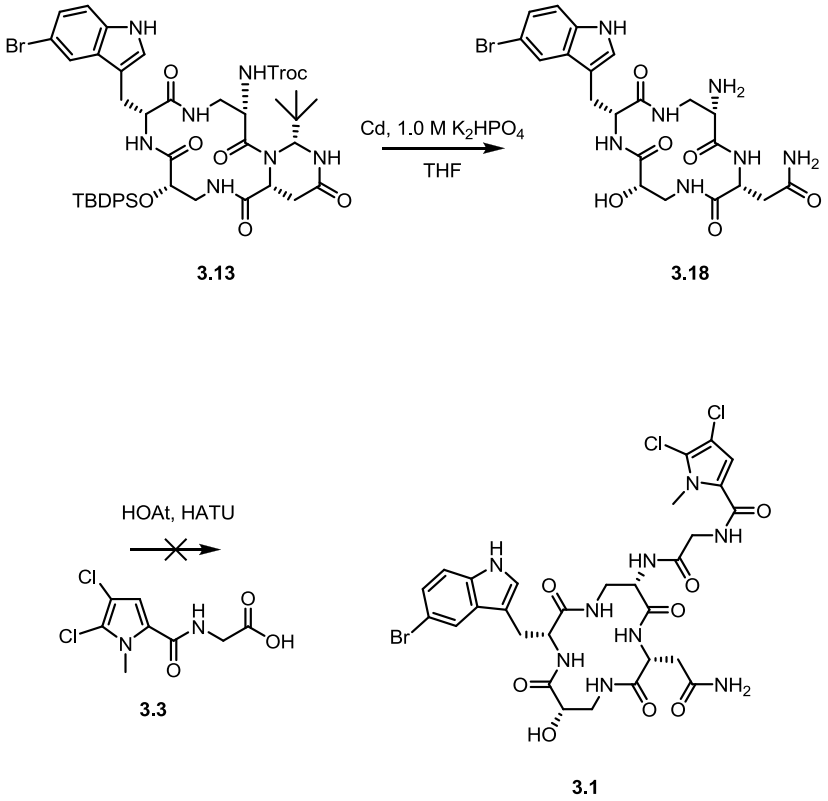
Stepping back and examining the reaction after the attempt at Troc removal using zinc, the crude material was examined by mass spectroscopy and showed no desired product and only decomposed material. Mass spectroscopy was chosen as the primary analytical tool because of the ability to quickly determine if the deprotected material was present. The poor result indicated a need to consider milder conditions for the removal of the Troc protection group.

The next set of reaction conditions involved the replacement of acetic acid for a milder solvent.[9] This was achieved using zinc that was preactivated in THF: DMF

(1:1); however there still was no desired product formed. Again using preactivated zinc a buffered solution was attempted utilizing 1.0 M KH_2PO_4 in THF.[10] After 4 h the reaction was checked and the mass of the desired deprotected cyclic material **3.18** was seen by mass spectroscopy. The reaction was filtered through celite, concentrated and the resulting crude material was taken on and attempted to couple to the dipeptide side chain **3.3**. However no desired product was detected.

After repeated attempts at the two step procedure, the lack of desired material was discouraging and the deprotection step was reexamined. It was thought that concentrating the reaction left an abundant amount of salts in the sample which were taken on to the side chain coupling reaction. This salt contamination was thought to have contributed to the failures that occurred previously.

In order to remove the salts different workup methods were examined. Instead of concentration after filtering through celite, the reaction was washed with water. The wash proved to give a slightly cleaner end product by TLC. However after examination of the weight of crude material, there was an abundance of salts still present. The next attempt was to wash the organic layer with a 0.5 M solution of EDTA, which removed most metals present in the solution. Chelation of small cyclic peptides to metals has been seen before as shown in Chapter 1. Even after changing the work up the coupling still did not proceed successfully. The poor outcome led us to reexamine the reaction itself.



Scheme 3.8 Second attempt at completion of the synthesis of Cyclocinamide B

Our attention turned to other metals to remove the Troc protection group and led to the use of cadmium metal in the next experiments seen in Table 3.3.[11] Using cadmium in varying ratios of acetic acid and DMF gave positive results. After 4 h the reaction showed a mixture of desired product, starting material and additional components by mass spectroscopy. Letting the reaction run longer showed that all the starting material were consumed; however there were more unknown components present. The amount of acetic acid was decreased from 5:1 or 10:1

ratio of DMF to acetic acid, and gave better results with three compounds seen instead of four as determined by thin layer chromatography. When this mixture was taken on to the finally coupling step a complex mixture was observed and no desired product was detected. When the cadmium was preactivated and used in a buffered solution previously used with zinc there was a seemingly positive result. The cyclic material free of any protection groups **3.18** was indicated by mass spectroscopy. This material was taken on crude into the side chain dipeptide **3.3** coupling but the coupled product was not seen (Scheme 3.8).

Reagents	Time	Result*
Cd 1:1 AcOH : DMF	4 hours	Absence of Troc seen and SM
Cd 1:1 AcOH : DMF	7 hours	Mixture of compounds
Cd 1:5 AcOH : DMF	7 hours	Slightly cleaner than previous entry
Cd 1M KH_2PO_4	4 hours	Mixture of **3.14** and other compounds

***All reactions were analyzed by ESIMS**

Table 3.3 Use of cadmium for Troc deprotection

It was thought that the free asparagine side chain was contributing to the decomposition of the material in the Troc deprotection step by coordinating to the metal and causing undesired activity. Thus, examination of the fully protected compound **3.10** was warranted at this time, as a way to both have the asparagine and isoserine protected. This protection scheme brought the current sequence more

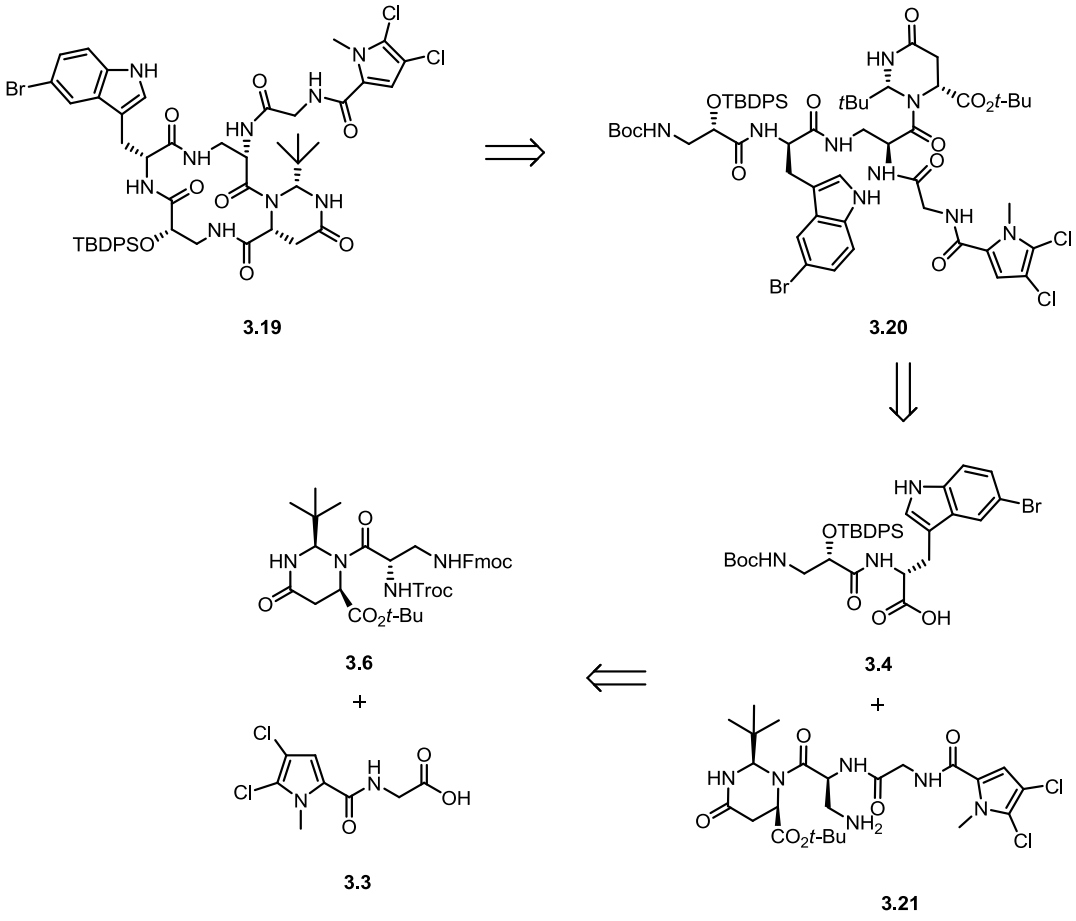
in line with the successful deprotection and coupling sequence preformed by Dr. Jessica Garcia. When the material was reacted in a 5:1 DMF: acetic acid solvent system for 6h, there were multiple compounds present with the Troc removed, as determined by the isotopic pattern in the mass spectroscopy. When this material was taken on to the final step a complex mixture formed again with no desired material detected by mass spectroscopy. With the very disappointing results it was decided that the cyclic material was not stable to the Troc deprotection. A reexamination of the synthetic scheme became warranted.

3.3) Route B cyclization

With the inability to couple the side chain in the late stages of the synthesis and the extreme labiality of the protections groups, our attention turned to addition of the side chain earlier. This change would circumvent the problem of the late stage Troc deprotection that was quite unsuccessful. It was believed that addition of the side chain before cyclization would allow for a successful synthesis. It was already shown that the (cyclo)Asn utilizing the *tert*-butyl group was highly labile and prone to spontaneous deprotection. The location chosen for the attachment of the side chain was to the fully protected cycloasparagine fragment **3.6**.

A revision to the retrosynthesis in Scheme 3.9; the cyclic hexapeptide **3.19** would arise from macrolactamization of the linear hexapeptide **3.20** at the same

position as the previous route. It was envisioned that the hexapeptide would be synthesized from the previously used dipeptide **3.4** and the new tetrapeptide **3.21**. The tetrapeptide was disconnected to give the previously synthesized dipeptides **3.6** and **3.3**, with the belief that it wouldl circumvent the issues previously observed.

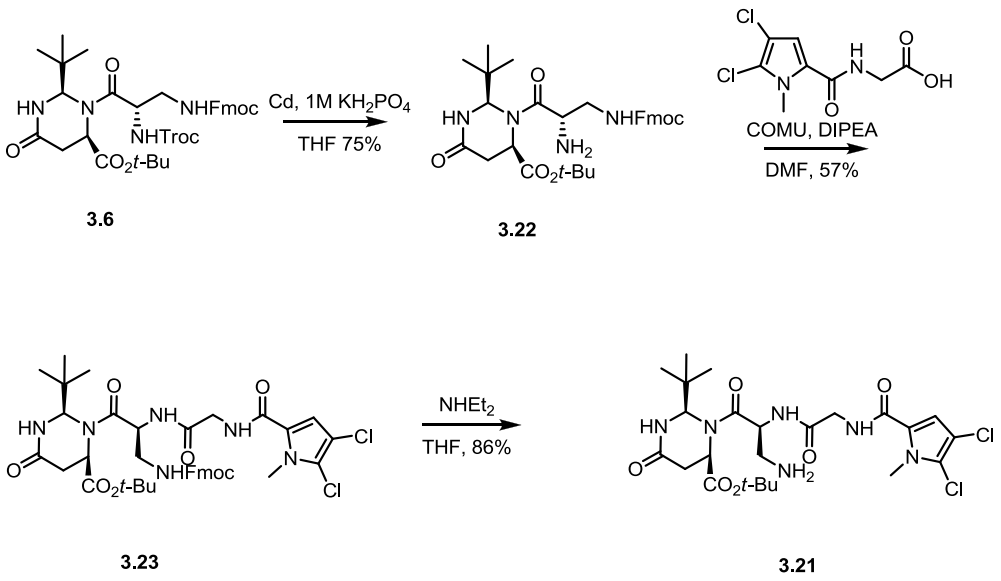


Scheme 3.9 Revised retrosynthesis utilizing earlier attachment of the side chain

3.3.1) Synthesis of the linear system

With the revised synthetic scheme the same (cyclo)Asn fragment **3.6** was deprotected at the α-nitrogen by removing the Troc. The use of zinc and cadmium where considered, as well as the use of a buffered solution. When zinc was used in acetic acid, the dipeptide decomposed and there was no desired product detected. When the reaction was conducted in a buffered solution of 1.0 M KH_2PO_4 in THF, no product was obtained.[11]

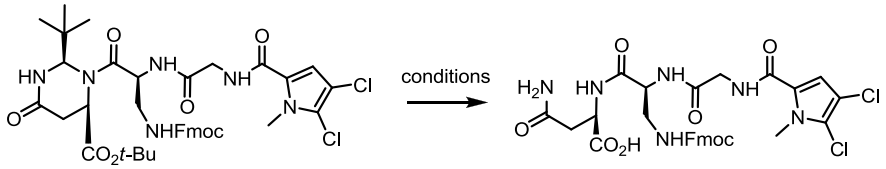
The use of cadmium in a buffered solution gave the desired free amine **3.22** in a clean reaction. The coupling of the side chain was sluggish and gave only moderate yields, even with extended reaction times (Scheme 3.10). The results would indicate that the α-nitrogen is either sterically crowded or deactivated due to the neighboring groups and therefore difficult to access. After synthesis of the tetrapeptide **3.23** an advantageous discovery was made. It appeared that this system was far more stable that the dipeptide (cyclo)Asn **3.6**. The shelf life of the dipeptide was only a few days before decomposition started to occur. With the side chain attached the tetrapeptide was shelf stable for over one week. The removal of the Fmoc group was facilitated with diethylamine in a clean reaction to give the free β-nitrogen of the tetrapeptide **3.21**.



Scheme 3.10 Synthesis of the alternative tetrapeptide

The extra stability seen in the newly formed tetrapeptide gave us pause. The ability to remove the (cyclo)Asn was brought into question, due to the difficulty in the removal that arose with the aryl (cyclo)Asn protection group in the cyclocinamide A synthesis.[3] A look into this hydrolysis was warranted before the synthesis was continued. The first attempt to remove the (cyclo)Asn group was using the same conditions (Table 3.4, entry 1) that liberated the amide side chain in the cyclic peptide from the previous route using hydrochloric acid.[12] However, only the starting material was seen by mass spectroscopy even after 4 days of reaction time. The acid concentration was increased to 0.5 N HCl with no product detected even after a week. It was when the HCl was at 1.0 N that there was any change; the

starting material was completely consumed. Unfortunately, there was no product detected by either mass spectroscopy or NMR.



3.23 **3.24**

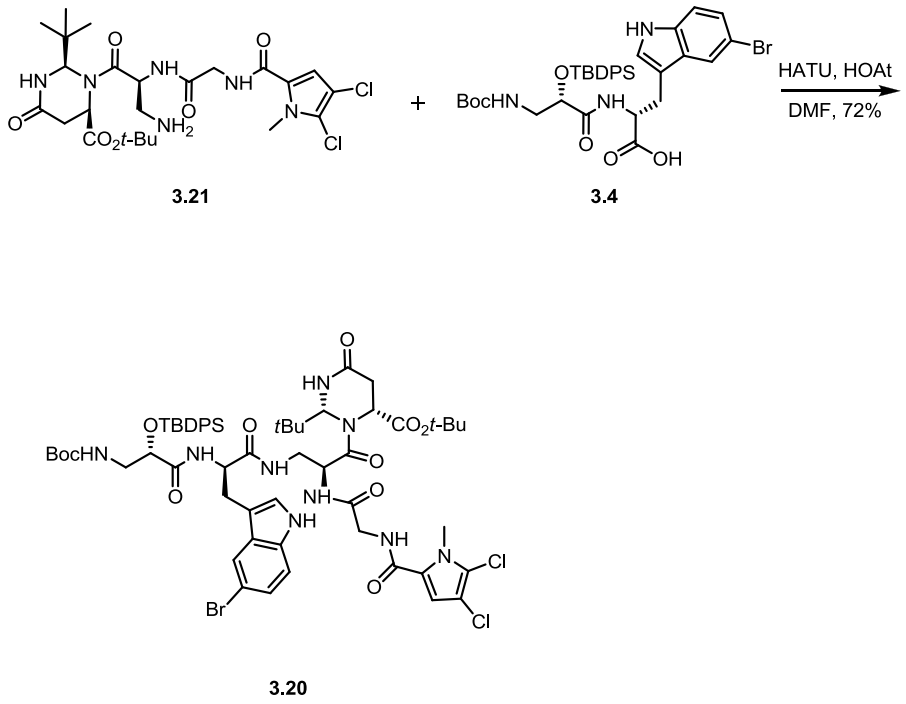
Entry	Conditions	Result
1	0.25 N HCl	SM
2	0.5 N HCl	SM
3	1.0 N HCl	Decomp
4	90:5:5 TFA: TES: ethanedithiol	4 hours- SM 1 d - Decomp
5	90: 5: 5 TFA: TES: H_2O	6 hours- SM 2 d –Decomp
6	90: 5: 2.5: 2.5 TFA:TES: H_2O: ethanedithiol	4 h –SM o/n –product

Table 3.4 Opening of the (cyclo)Asn **3.23**

A different acid was examined after the poor results with HCl. When the tetrapeptide was subjected to aqueous TFA there was no change in the reaction after six hours, and letting the reaction run for two days led to decomposition. The next set of conditions used a sulfur nucleophile in an aqueous TFA solution.[13] The

addition of a sulfur nucleophile was thought to help aid with the removal by adding stability to the open form of (cyclo)Asn, and the addition of water was thought to facilitate the removal based on the work of others. These conditions are similar to those used to liberate the pseudoprolines[14] and other temporary turn inducers employed for the synthesis of cyclic peptides (see Chapter 2). As seen in Table 3.4, entry 6, this route was successful. With the knowledge that the (cyclo)Asn can be removed and the peptide would stay intact, attention turned to completing the synthesis.

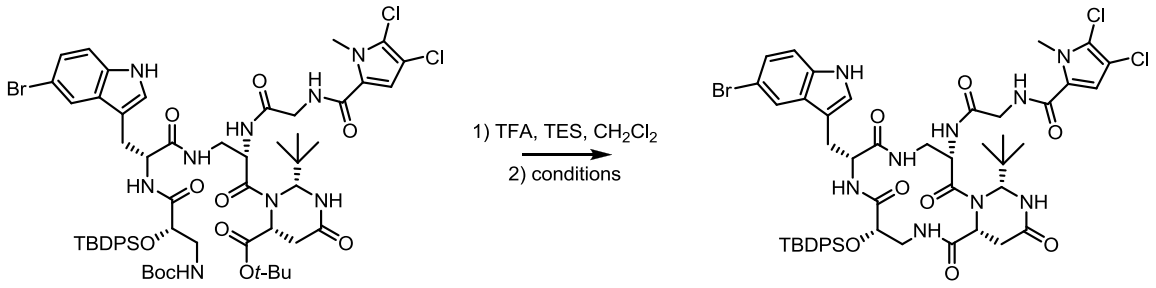
The tetrapeptide **3.21** was coupled to the isoserine-5-bromotryptophan dipeptide **3.4** using the same conditions as previously used for the synthesis of the tetrapeptide **3.8**, and gave the desired linear hexapeptide **3.20** in a decent yield of 72% shown in Scheme 3.11. It was at this time that the linear peptide was found to be very unstable. Decomposition occurred within a day at room temperature after purification. Attempts to extend the shelf life by storage in the freezer did not work. This instability was similar to that of the tetrapeptide **3.8** in the previous route. Due to this fact the linear hexapeptide was used the same day it was made.



Scheme 3.11 Synthesis of the linear hexapeptide

3.3.2) Cyclization

The bis-deprotection of hexapeptide **3.20** was carried out in the same manner as was used for the deprotection of **3.8**, 9:1 DCM:TFA mixture solvent system to provide the ammonium salt and the free acid of the hexapeptide, which is ready to cyclize. Cyclization was undertaken using the same conditions that worked for the previous route, using HOAt/HATU under high dilution conditions and a slightly elevated temperature. This resulted in no desired product detected; complex mixtures were formed instead.



3.20 3.19

Entry	Amount of hexapeptide	Coupling reagent	Yield (MR)	Conditions
1	20	HATU/HOAt	0	2 d, 30 °C
2	120	EDC/Pfp	20	2 d, 30 °C
3	80	EDC/Pfp	0	2 d, 30 °C
4	80	EDC/Pfp	12	3 d, 30 °C
5	80	EDC/Pfp	4	3 d, 30 °C
6	30	EDC/Pfp	40	3 d, 30 °C
7	78	COMU	25	3 d, 30 °C
8	78	COMU	13	2 d, 30 °C
9	200	EDC/Pfp	10	3 d, 30 °C
10	30	EDC/Pfp	0	4 d, 30 °C
11	30	COMU	0	4 d, 30 °C
12	30	DPPA	0	4 d, 30 °C

***All mass recovery (MR) reported in mg**

Table 3.5 Cyclization of the hexapeptide **3.20**

With these very discouraging results attention was turned to surveying a range of coupling reagents in order to obtain cyclic material. The next attempt was to use the method that was deployed in our lab by Dr. Maximillian Mahoney in the

cyclization of a pentapeptide.[15] In this method, the EDC and pentaflorophenol (Pfp) were allowed to react for 20 minutes while the nitrogen was left as the TFA salt, which provided an *in situ* protecting group to prevent the N-terminus from reacting prematurely.[16] The amine is then released by the addition of a base to the solution allowing for coupling to take place.

At first the results looked promising as there was what looked like product by NMR of the crude material recovered from the reaction (Table 3.5, entry 2). However, all attempts to purify the material failed to yield the desired cyclic product. The reaction was repeated with three flasks running in parallel, to build a stockpile of material. The three reactions (Table 3.5, entries 3-5) showed varying results on the yield of crude material collected after work up. This result indicated that the reaction with EDC/Pfp was not repeatable and there was something else happening. Next we tried the coupling reagent COMU, which provided the same results as with the previous coupling reagents. There was what appeared to be product detected by movement of a significant amount of material by TLC and loss of starting material. No pure material could be isolated by any attempts, indicating that either the analytical data of the crude material was faulty or the cyclic material was not stable to the purification conditions. Similar results were observed by Dr. Lui in the attempts to synthesize different isomers for their library.[17]

The work up and purification of the reaction were thought to be the cause of the problems. This led to an investigation of different work up conditions. First the

work up was eliminated; the reaction was simply concentrated and purified by flash column chromatography. Neither standard work up nor simple concentration of the reaction, however promising the initial results were, led to desired product isolation. Second, the column conditions were investigated including different solvent mixtures (5%-10% MeOH in CH_2Cl_2, 5% MeOH in EtOAc, EtOAc/CH_2Cl_2 mixtures). When this failed to yield cyclic material, the normal phase silica gel column was replaced. Attempts were made to use a reverse-phase silica gel column using methanol and water as eluents. No desired material was ever seen during the extensive testing of work up conditions.

In order to understand what was happening in the reaction and to determine if the product was formed in the course of the reaction a series of experiments were run. The starting material was separated into three equal portions so to remove the possibility that any difference in the starting material would affect the outcome of the reactions. Then three different coupling reagents were used as indicated by entries 10-12. The reaction were treated the same and after completion of the reaction the solvent was removed and the crude material was examined using an LC/MS. The traces and mass spectroscopy results are shown in Figure 3.3. The LC/MS traces show that there are multiply compounds and a complex mixture. They demonstrate that there are no compounds in the mixture that are close to the weight of the hexapeptide and there are only lower weight compounds indicating that decomposition occurred in the deprotection and cyclization sequence. The

peaks were examined in both positive and negative ionization modes, nothing of value was seen. This led us to the conclusion that the instability seen in the fully protected linear material was causing problems in the cyclization event and therefore failure in the synthesis of the cyclic peptide.

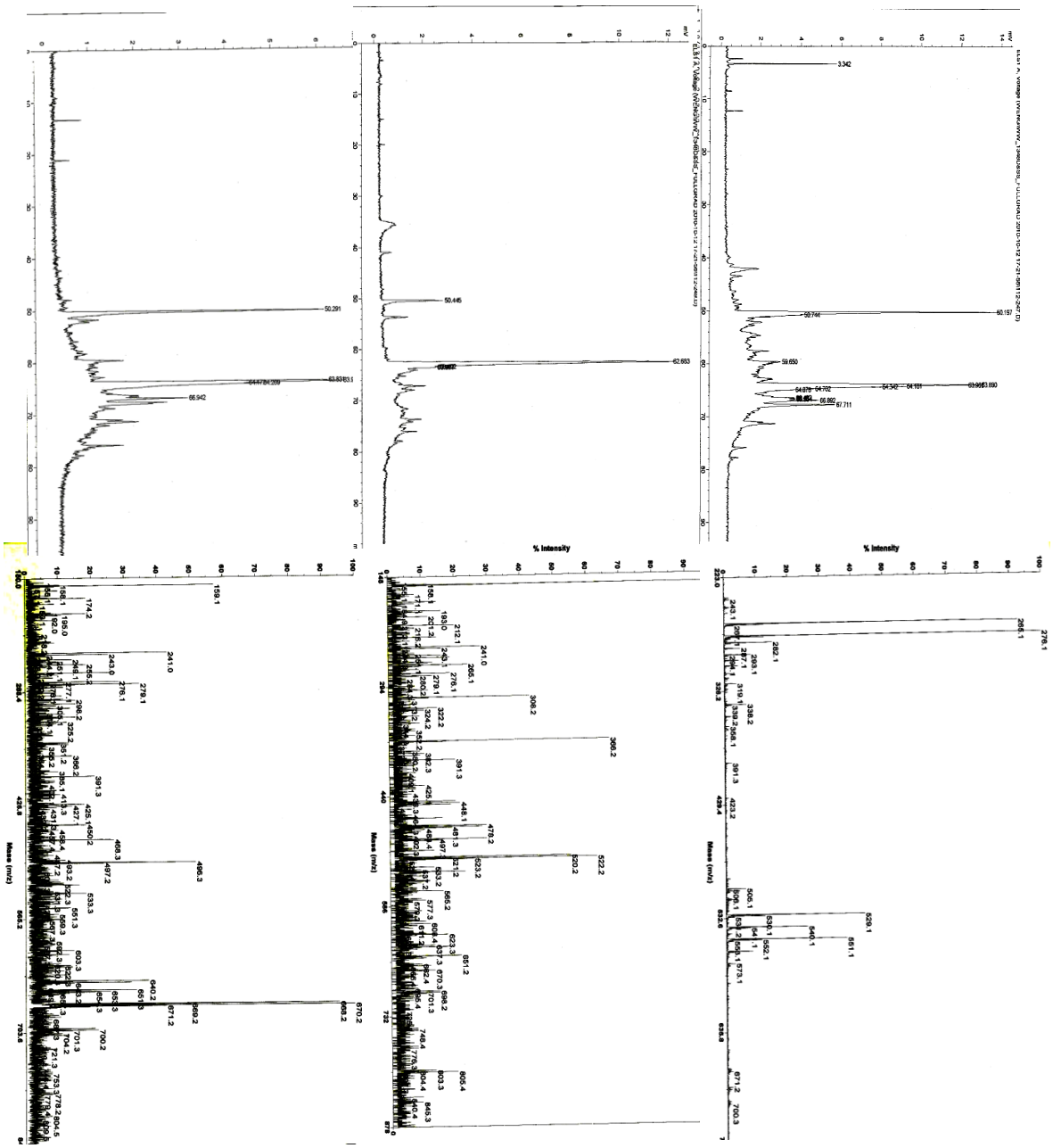
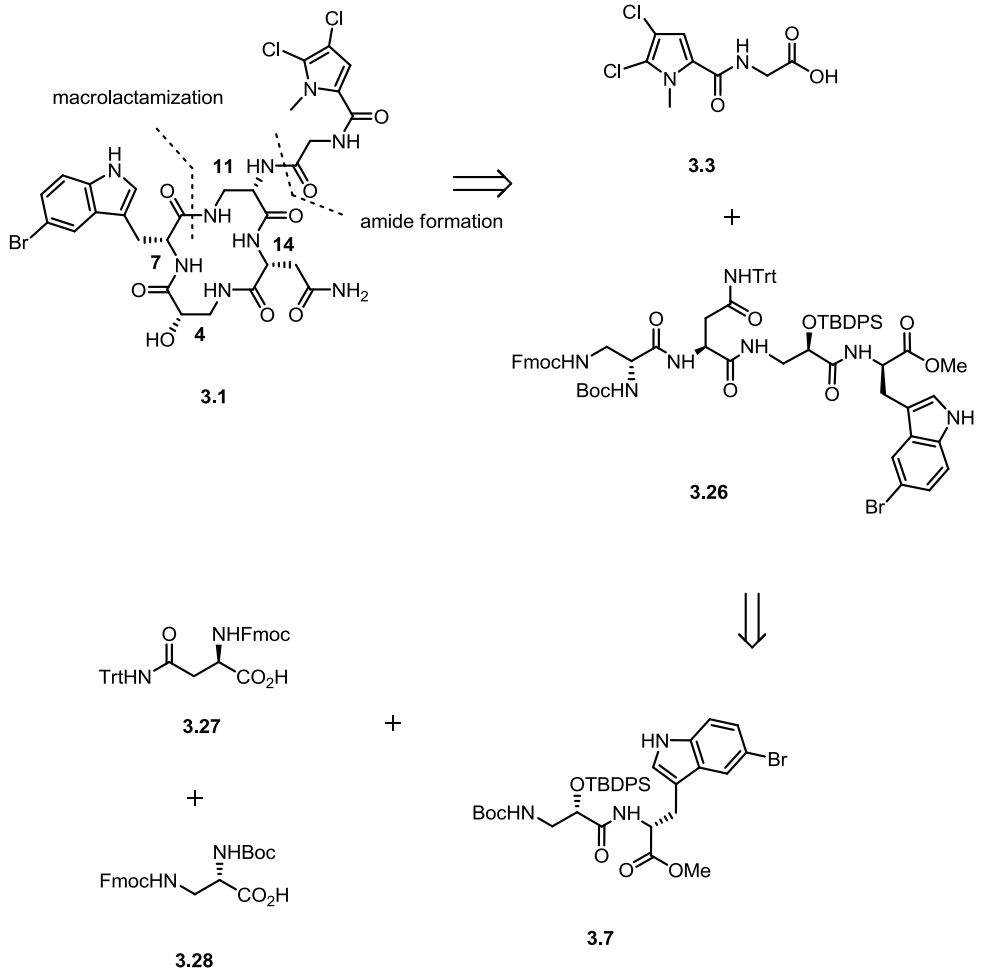


Figure 3.3 From left to right LC/MS traces and mass spec for cyclization 10-12 in table 3.5

3.4) Route C

Our thinking was that the (cyclo)Asn was interfering in the cyclization event despite the enormous success it showed in the cyclization of the all-*S* and 11*R* cyclocinamide A isomers. Attention was now turned to a new synthetic route. The new route chosen would follow that of Grieco[18] and Postema[17] more closely and not use the (cyclo)Asn turn inducer. Another change was switching the Troc for a Boc which was thought to simplify the late stage deprotection.

Again a revision to the retrosynthesis became warranted. The Fmoc protected asparagine **3.27** would be coupled to the isoseryl-tryptophan dipeptide fragment **3.7** to form a tripeptide and the diaminopropionic acid **3.28** was added to make the tetrapeptide **3.26** which would be cyclized at the 8-9 position. Following Boc deprotection, the side chain **3.3** would be added followed by global deprotection to give cyclocinamide B **3.1**. (Scheme 3.12)

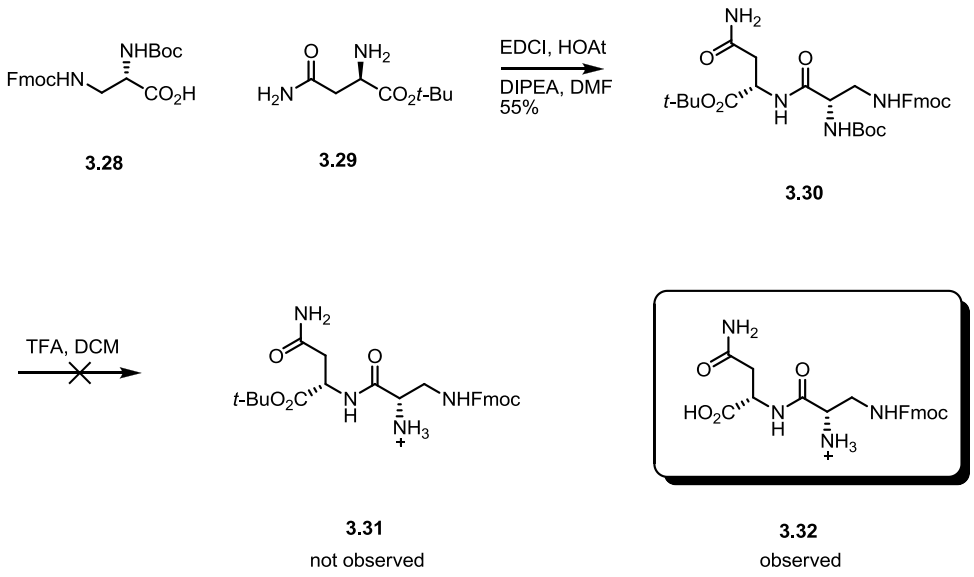


Scheme 3.12 Revised retrosynthesis without cyclo(Asn) present

3.4.1) Synthesis of the linear tetrapeptide without (cyclo)asparagine

The synthesis utilized the material already stockpiled from the previous routes. Two routes were examined. The first was to form two dipeptides then couple to form a tetrapeptide. The second was to stepwise build the linear tetrapeptide

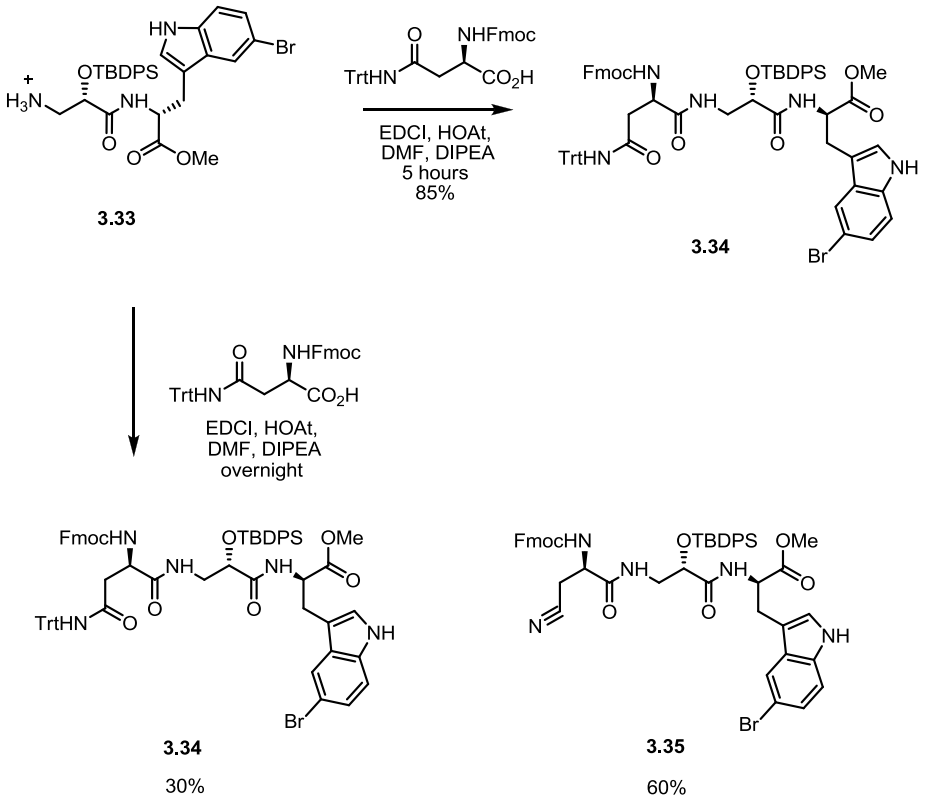
from the starting dipeptide **3.7**. The first route started with the construction of an aspargyl-diaminopropionic acid dipeptide **3.32** and coupling it to the isoseryl-tryptophan dipeptide to form the linear tetrapeptide with the same amino acid sequence as was previously being used. In an attempt to use the asparagine *tert*-butyl ester **3.29** that was previously synthesized, it was coupled to the diaminopropionic acid **3.28**.[19] The reaction proved to be messy and only had a 55 % yield of the dipeptide **3.30** (Scheme 3.13).



Scheme 3.13 Attempted synthesis of aspargyl-diaminopropionic acid fragment

The selective deprotection of the Boc over the *tert*-butyl ester to form the dipeptide **3.31** proved to be problematic and was never successful. Instead the bis-

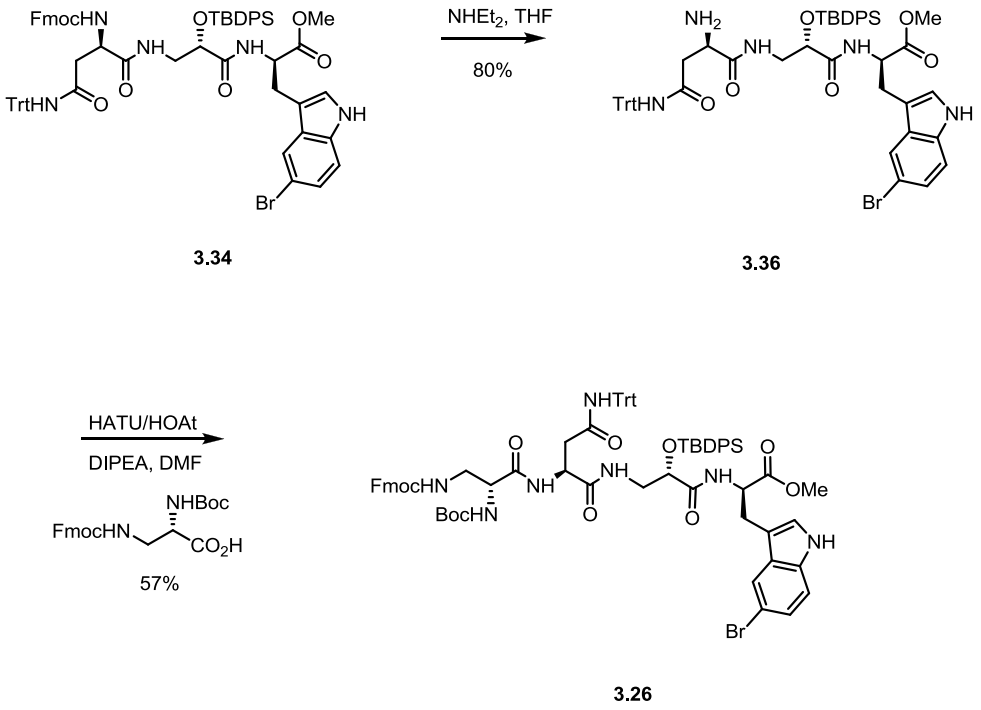
deprotection product **3.32** occurred. Selective reprotection of the nitrogen proved to be problematic and a very messy reaction. The peptide was difficult to handle because of poor solubility in organic solvent, likely due to the free asparagine side chain. Due to the difficulties in the sequence this route was quickly abandoned in favor of building the tetrapeptide piecewise off the nitrogen end of the isoseryl-tryptophan dipeptide.



Scheme 3.14 Synthesis of the linear tripeptide and unexpected nitrile formation

The Boc protection group of **3.7** was selectively deprotected using TFA and TES, in a clean reaction (leaving the TBPDS intact) and producing dipeptide **3.33** in a good yield of 90%. This was coupled to the side chain trityl protected Fmoc asparagine, forming the tripeptide **3.34** in an excellent yield. It was noticed that when the reaction was allowed to stir overnight or for an extended period of time the trityl group would start to disappear. Upon examination of the side product the trityl was indeed gone as well as the carbonyl peak for the asparagine in the ^{13}C NMR. After looking at the IR the presence of a large peak at 2300 cm^{-1} indicated that the asparagine side chain amide was being converted to the nitrile **3.35**.[20] To overcome this side product the reaction was run for a shorter time of 5 h and this minimized the undesired nitrile formation shown in Scheme 3.14.

The tripeptide **3.34** was selectively deprotected at the nitrogen terminal using diethylamine. The tetrapeptide **3.26** was formed by coupling diaminopropionic acid using HATU/HOAt in 57% yield, a much lower yield than the other couplings. This is thought to occur because of the particular amino acid, which has proven to be a difficult amino acid to work with. The reaction has shown to be extremely sluggish and required longer reaction times. The formation of nitrile was not seen in this or subsequent reaction. It appeared that nitrile formation only occurred in the synthesis of the tripeptide (Scheme 3.15).

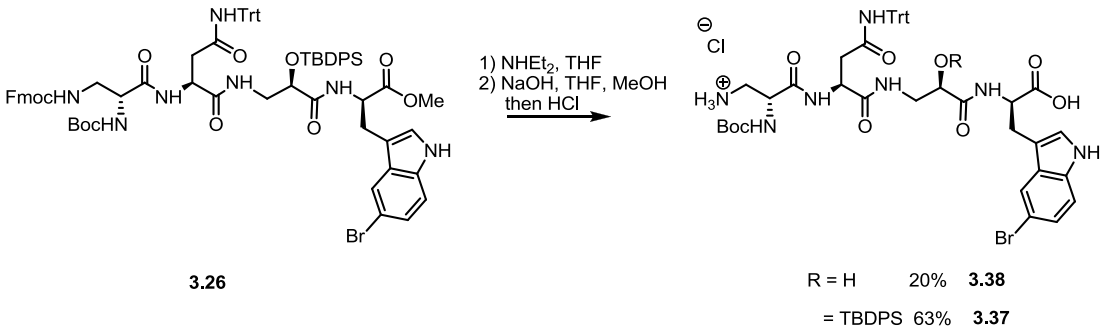


Scheme 3.15 Synthesis of the linear tetrapeptide without cyclo(Asn)

3.4.2) Cyclization

With the linear tetrapeptide **3.26** in hand, cyclization was the next step to be examined (Scheme 3.16). It was believed to be a difficult step due to the previous issues that had occurred with other routes. It was discovered that the deprotection would not occur as a single step. Therefore the nitrogen terminal was first deprotected followed by hydrolysis of the methyl ester. The deprotection of the methyl ester took longer than previously experienced with a reaction time of 48

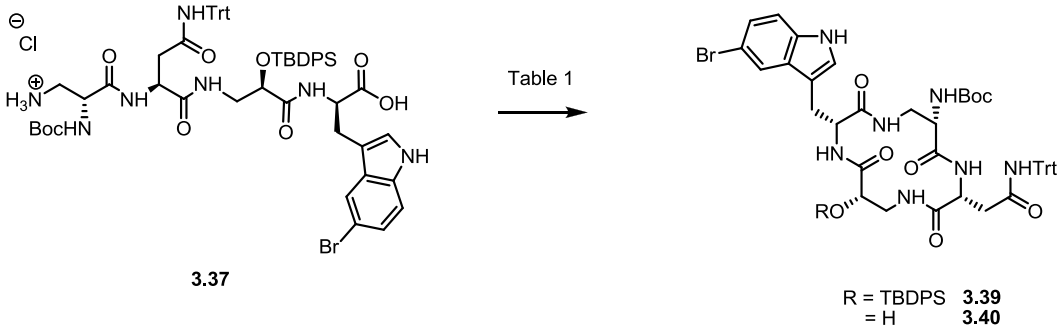
hours. When the deprotection sequence was reversed none of the desired bis-deprotected material was seen, indicated that the order was important. In the process of handling the material it was discovered that the bis-deprotected material showed more stability than the previous routes. It could be stored for days and did not show much in the way of deterioration, and the stability allowed for the material to be purified prior to cyclization.



Scheme 3.16 Bis-deprotection of the linear tetrapeptide

After the cyclization reaction shown in Scheme 3.17, the crude material was a complex mixture and difficult to purify. When the linear material was rigorously purified before cyclization the crude material after cyclization was much cleaner and easier to purify and allowed for a higher reaction yield. It was found that the linear material was a mixture of free secondary hydroxyl **3.38** as well as the TBDPS

protected **3.37**. The two compounds were separable and the protected material was taken forward.



Scheme 3.17 Cyclization of the linear material without cyclo(Asn)

The cyclization was undertaken with the knowledge that it was a difficult reaction in previous routes although this was the first attempt at cyclization of a tetrapeptide with the S,R,S,R stereochemistry. The purified linear tetrapeptide was cyclized with HATU and HOAt since that reagent combination was successful in the first route. However, the material seen after the cyclization was extremely messy and didn't yield any of the desired cyclic material. None of the linear material was seen after the reaction. After changing the reaction length and temperature several times it was soon realized that the peptide would not cyclized using HATU/HOAt. The reagent COMU was tried with the same result of a highly messy reaction and no detectable product.

With this, our attention was turned to using the EDCI/pentaflorophenol which had been shown to work with the cyclization of Dr. Maximillian Mahoney's pentapeptide.[15] After allowing the reaction to stir for three days the reaction was worked up using the standard washing procedure and the reaction was extremely complex by TLC analysis. However, the desired cyclic material was seen by mass spectroscopy. After the extensive purification that was required the yield was undesirably low at 12% yield.

Amine Terminus	Entry	Coupling Reagent	Time (days)	Result
NH_2-R	1	HATU/HOAt	3	Complex mixture/ no product
	2	COMU	3	Complex mixture
	3	EDCI/Pfp	3	Complex mixture ~12% yield
	4	DEPBT	3	Complex mixture 23% yield
Cl NH_3-R	5	DEPBT	3	Much cleaner, most **3.40** 40% yield
	6	DEPBT	3	Purified SM, Cleanest , 22% **3.39** 44 % **3.40**

Table 3.6 Survey of coupling reagents for cyclization of tetrapeptide **3.47**

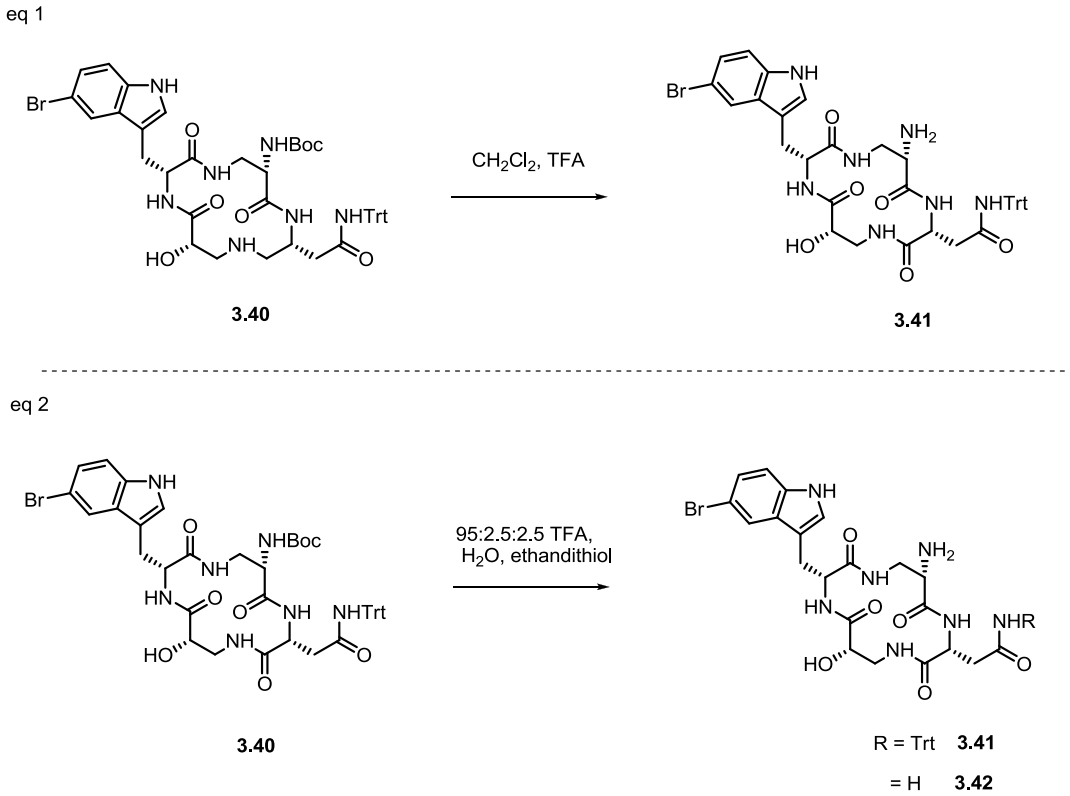
With the low yield a search of the literature for coupling reagents was undertaken. It was found that the reagent DEPBT (Figure 3.1) was said to be a good reagent for difficult cyclizations and leads to a low rate of epimerization.[21] The

reagent was utilized by Dr. Jessica Garcia in the cyclization of her tetrapeptide and found that, although it was successful, the yield was undesirable at 19% after two days.[3] With the low yield the reagent was abandoned in the synthesis of the 4*S*,7*S*,11*S*,14*S* isomer. Even with the low yields observed by Dr. Jessica Garcia, DEPBT was the next to be attempted for the cyclization. A small amount of purified bis-deprotected linear peptide **3.37** was reacted using DEPBT under the same conditions that were used for the peptide in route A. Upon workup the crude material was significantly cleaner with the cyclic material as the major spots by TLC. With the success of the cyclization, more material was cyclized in 50 mg quantities running in parallel as to allow more material to be cyclized and to avoid any issues that may arise due to scale up.

The purification was troublesome and after extensive trials a purification sequence was developed. After a standard workup the crude material was chromatographed using silica gel with a gradient of 0-7% methanol in dichloromethane. This produced the cyclic material as a mixture of compounds **3.39** and **3.40** along with some impurities. The semi-pure material was then purified by another flash column first using the gradient 80-100% ethyl acetate in hexanes, and then flushing the column with of ethyl acetate, followed by a gradient of 0-10% methanol in ethyl acetate. This eluted the pure cyclic material as two compounds **3.39** and **3.40**.

3.4.3) Attachment of the side chain and completion of the synthesis

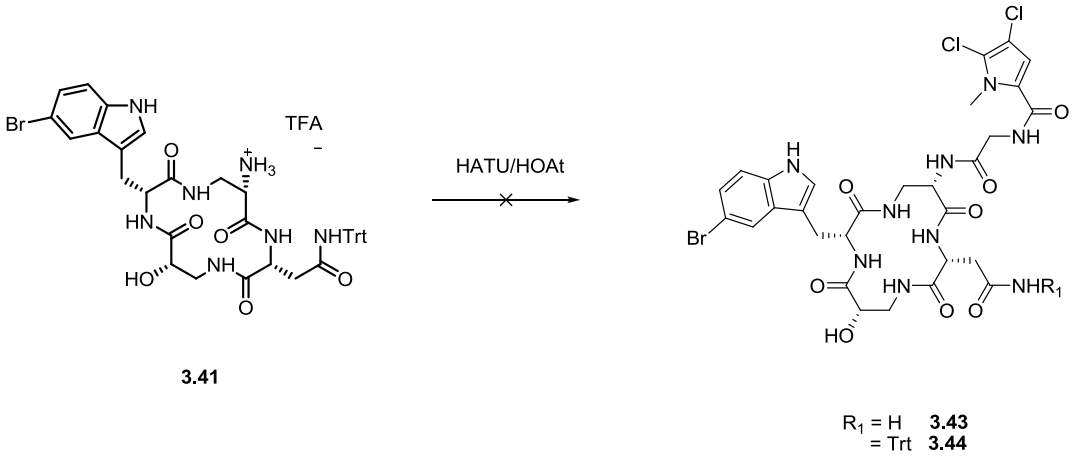
With the cyclization optimized attention turned to the deprotection and coupling of the side chain and the global deprotection. Most of the isolated cyclic compound was free hydroxyl; the TBDPS was very labile in this compound. This material was used going forward. With the belief that the coupling reaction would be selective for the nitrogen over the free hydroxyl, we moved forward by selectively deprotecting the Boc over the trityl protection group to afford **3.41**. The



Scheme 3.18 Deprotection of the cyclic tetrapeptide by two different reagents

deprotection was accomplished using a 10:1 ratio of CH_2Cl_2:TFA. This allowed for the trityl to stay intact and the free amine material was taken on crude to the coupling (Scheme 3.18, eq 1). This proved to be an unfortunate choice as the crude material was extremely messy and proved unsuccessful in forming the hexapeptide. After varying both the equivalents of side chain added to the coupling and the reaction time, the result did not improve. The material was extremely messy and still failed to show any desired product.

With the disappointing results we thought that having the large trityl group next to the reacting amine was causing the coupling reaction to be too sterically hindered. Thus, we decided to remove the trityl. The cocktail mixture commonly found in removal of peptides from solid phase resins of 90:5:5 TFA:H_2O:ethanedithiol was used for 3 h. This allowed for the removal of all the protection groups on the cyclic peptide **3.42**. However, the reaction was somewhat complex, with some decomposition of the material. The crude mixture was taken on to the side chain coupling, using 1.0 equivalent of the side chain to help facilitate the selective coupling to the nitrogen and not the free hydroxyl. After several attempts the desired material was not seen and the reaction was extremely messy. This disappointing result led us to believe that the trityl was not the cause of the failure for the coupling (Scheme 3.19).

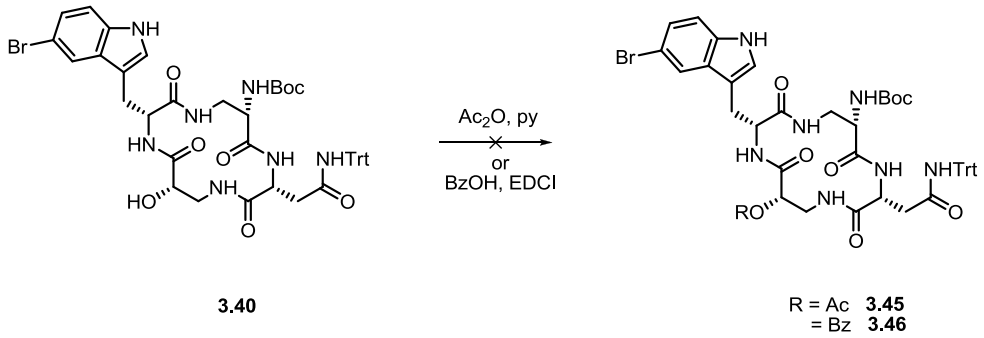


Scheme 3.19 Attempt at side chain coupling on cyclic peptide with free alcohol

Since we have tested both the trityl and free asparagine in the coupling reaction and neither proved to be successful, the presence of the free alcohol was examined. This group became suspect since it has been present in all unsuccessful coupling reactions of route A and route C. In order to determine if the free alcohol was the cause and interfering in the coupling it was decided to reprotect the alcohol. Two different protection groups were looked at; Ac **3.45** and Bz **3.46**. Both of these groups are stable to the acid deprotection of the Boc and easily removed with base. The protection was undertaken using standard conditions shown in Scheme 3.20.

The acyl group (Ac)[21] was first attempted and the resulting product was contaminated with an unknown byproduct. All attempts to purify the product failed to give pure material and the slightly contaminated material was low yielding, so this route was abandoned. The next attempt was with the benzoyl group (Bz).[22] The protection was undertaken using EDCI coupling to freshly recrystallized benzoic acid.

This resulted in a very complex mixture of products. The desired material was not seen by either mass or NMR spectroscopy. So this protection group was abandoned quickly. The difficulty in the reprotection of the hydroxyl has been seen before and was not unexpected.



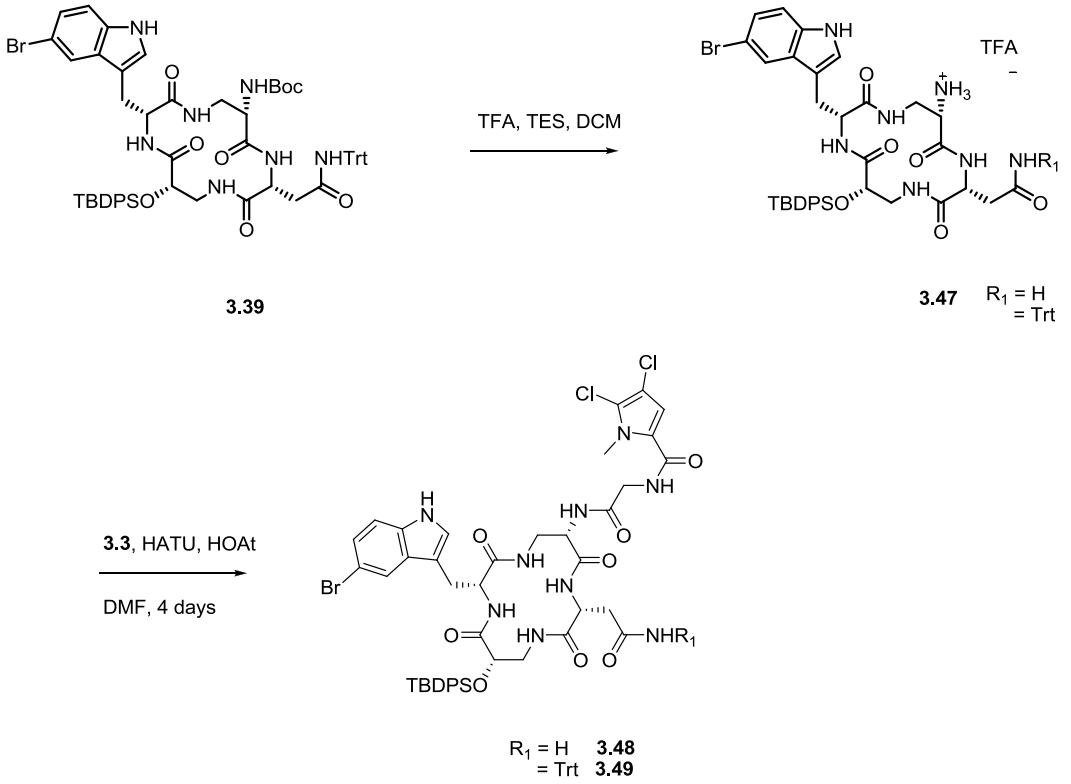
3.40

R = Ac **3.45**
= Bz **3.46**

Scheme 3.20 Attempt to reprotect the alcohol with acyl and benyl

With the insights into the chemistry that was occurring with the functional groups of the ring, our attention turned to the minor product from the cyclization reaction. The fully protected cyclic material **3.39** was looked at to selectively deprotect the Boc protection group and leave the TBDPS intact. This was accomplished by using the same conditions used to deprotect the dipeptide **3.7**. The crude material, which looked clean by TLC, was taken into the side chain coupling reaction. After allowing the reaction to run for four days, the reaction was worked up using standard procedures. Two main compounds were seen; the major product being the fully protected cyclic material with the side chain attached **3.49** and the other had the free asparagine **3.48**. The partial trityl deprotection most likely

occurred during the TFA deprotection step. It appeared as though the trityl is more labile when the alcohol is protected. These interesting results became evidence for the fact that the trityl does not interfere with the side chain coupling and that the free alcohol was causing the interference (Scheme 3.21).

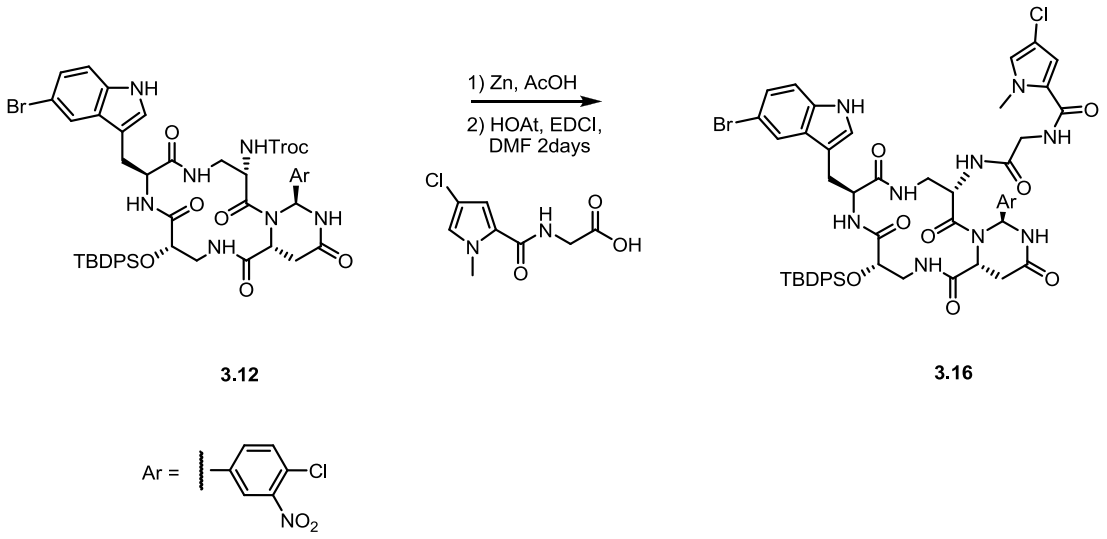


Scheme 3.21 Addition of the side chain starting with the cyclic peptide **3.39**

3.4.4) Synthesis and cyclization of the linear hexapeptide

During the synthesis of the linear tetrapeptide it was seen that the side chain attachment had prove problematic again. The difficulties observed in the coupling to the cyclic material were seen in route A. In that route the side chain coupling to compound **3.15** failed and after the observations of route C it can be concluded that the free alcohol caused the failure.

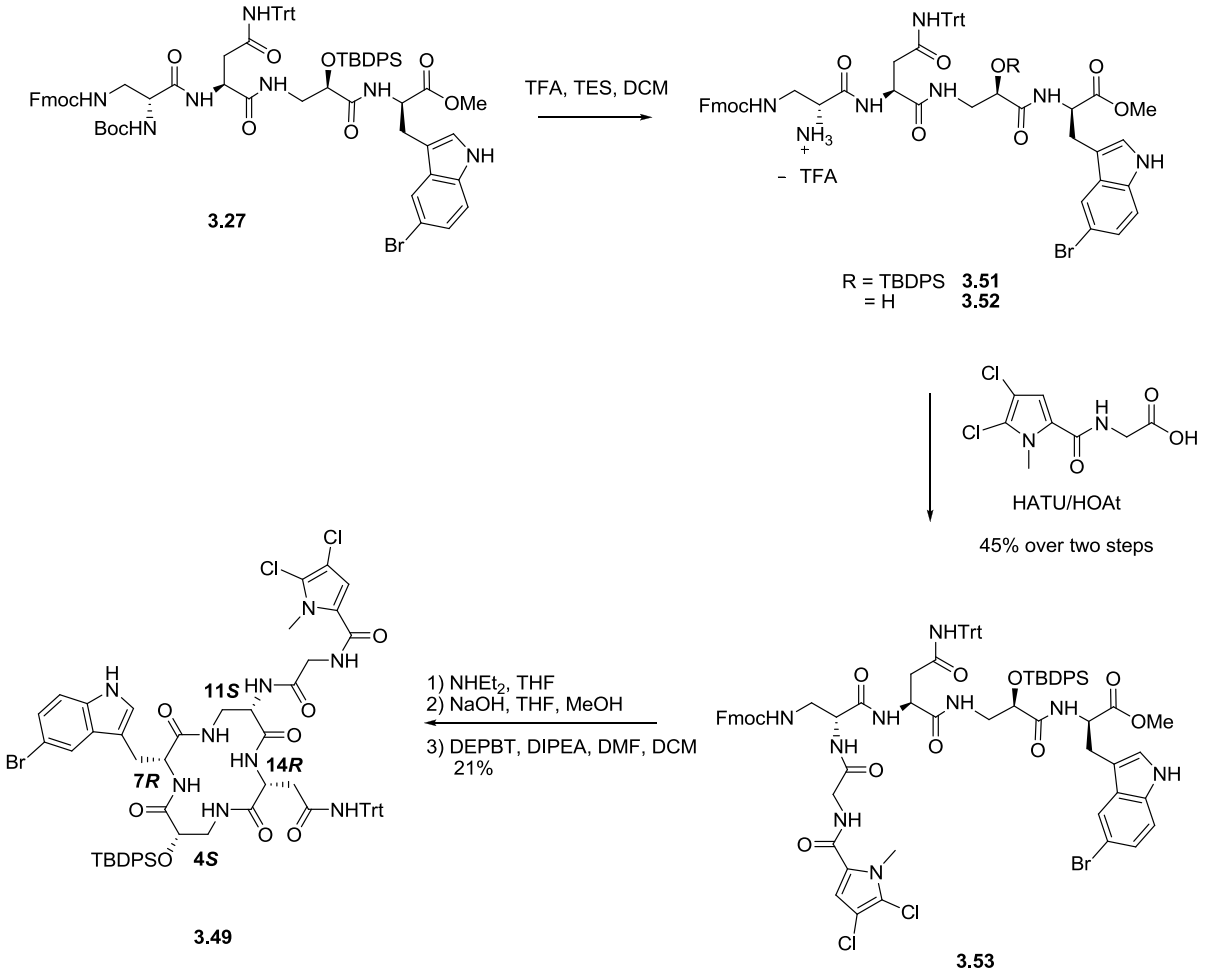
It should be noted at this time that the coupling of the side chain on the cyclocinamide A core **3.12** preformed by Dr. Jessica Garcia encountered many difficulties.[3] The reaction was sluggish and resulted in multiple products with a low yield as shown in Scheme 3.22. Even after extensive experimentation to improve the yield, 51% was the highest obtain and it was quite common to have a yield even lower. With the difficulties observed in the side chain coupling of cyclocinamide A and cyclocinamide B, it was thought that a different substrate for side chain coupling would improve yields.



Scheme 3.22 Side chain coupling of 4*S*,7*S*,11*S*,14*S* cyclocinamide A

In order to see what conditions would allow for the successful coupling the linear material was tested. The peptide **3.27** was selectively Boc deprotected to yield the TFA salt **3.51** as well as free OH **3.52**. Since, some of the free alcohol was observed and the material was rigorously purified. Coupling the tetrapeptide to the side chain was slow for the TBDPS **3.51** and failed to yield the desired material for the free OH material **3.52**. After 4 days the reaction was complete and had a low yield of 45% over the two steps giving the desired hexapeptide **3.53** shown in Scheme 3.23.

This was still a success because the Boc was selectively deprotected while not disturbing the TBDPS (which has proven to be quite labile previously). Similarly low yields were observed in route B where the coupling had a low yield of 57%. The sluggish reaction was seen in all the various substrates used and would indicate that the α position was deactivated. This result was taken into consideration when the side chain was to be coupled to the cyclic material **3.39**.



3.27 **3.49** **3.53**

Scheme 3.23 Synthesis and cyclization of the linear hexapeptide

The hexapeptide was then taken on to the cyclization in hopes of improved yields. Bis deprotection of the hexapeptide followed the same procedure as was preformed on the tetrapeptide **3.26**. Cyclization under the optimized conditions found for the tetrapeptide gave a poor yield of 21% over the three steps. The different yields can be explained by the presence of the side chain. The side chain appeared to cause the peptide to be in a less favorable conformation. The low yield led to the previously used tetrapeptide route being chosen (Scheme 3.23).

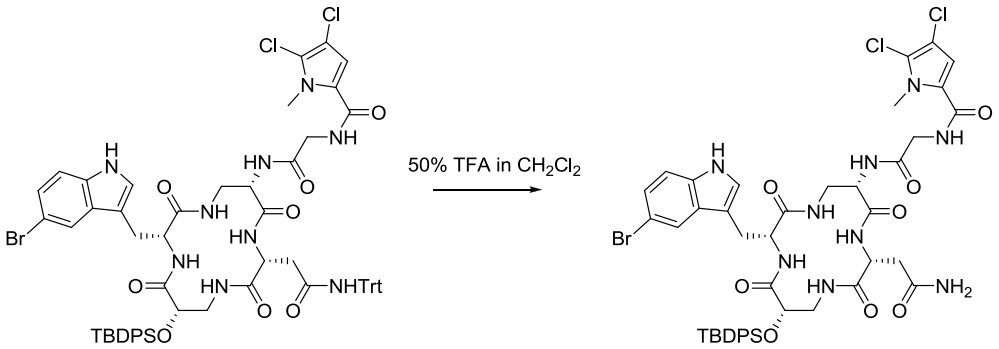
These two different substrates for the side chain coupling, as well as the coupling involved in cyclocinamide A, indicate that the reaction is slow. The reaction is low yield for all the different attempts made by our laboratory.

3.4.5) Completion of the synthesis of 4*S*,7*R*,11*S*,14*R* cyclocinamide B

With the formation of the hexapeptides **3.48** and **3.49** successful, the last step was global removal of the protection groups to obtain cyclocinamide B. Looking at the previous attempts to remove the trityl the solvent mixture of 90:5:5 TFA:H_2O:ethanedithiol was employed. However, this mixture didn't produce the final product of cyclocinamide B, therefore milder conditions were tried. The use of 50% TFA in DCM was next attempted; after a few hours the material appeared to be partially deprotected. It was decided to let the reaction go overnight in order to allow for fully removal. The next day the solvent was removed and the material was

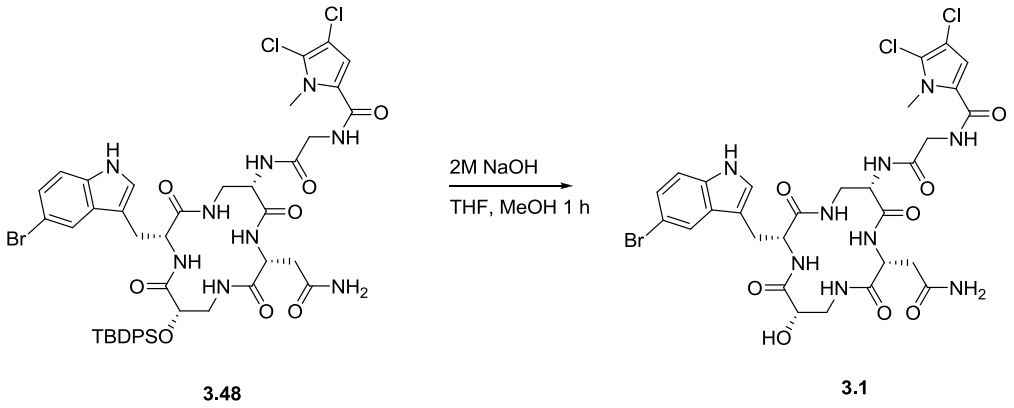
examined. It was found that the trityl was removed but the TBDPS was still present in the cyclic material **3.48**. This was an unexpected result because the silyl has been extremely labile during the entire synthesis. The TBDPS has shown adventitious removal at most steps of the synthesis.

A search for ways to remove the TBDPS that were both mild and relatively quick led to sodium hydroxide.[24] Using THF and MeOH as the solvent an excess of 2.0N NaOH was added. Monitoring by TLC showed that the reaction was complete after just 1 h. During work up it was observed that the material had poor solubility and crashed out as a white solid in between the organic and aqueous layers. The material was collected in the organic layer and looked at by mass spectroscopy and the desired product was seen (Figure 3.4).



Scheme 3.24 Removal of the trityl protection group from the cyclic peptide **3.47**

With the extremely encouraging results from the mass spectroscopy the sample was purified by HPLC in order to obtain the natural product as a pure compound for analytical testing. At this time some difficulty was observed with the fact that the sample was insoluble in both methanol and acetonitrile.[25] In order to solublize the sample a solvent mixture of 3:1:1 CH_3CN:H_2O:MeOH was used, followed by sonication before injection. Using a C18 semi prep reverse phase column and eluting over 20 minutes with 25-75% acetonitrile allowed for the collection of 2.0 mg of pure cyclocinamide B.



3.48 **3.1**

Scheme 3.25 Completion of the synthesis of cyclocinamide B **3.1**

As seen in Figure 3.4 the low resolution mass spectroscopy showed the appropriate mass as well as the M+Na peak. It also showed the correct isotopic pattern for the presence of the three halogens $BrCl_2$. After this confirmation of the desired product, the pure material was examined by ^1H and ^{13}C NMR. In order to

obtain the important 2D NMRs: COSY, HSQC, and HMBC the sample was sent to the 900 MHz facility at University of California, Berkeley.[26] With the help of Dr. Jeffrey G. Pelton, the NMR spectra were successfully obtained and used to verify the structure of the synthetic sample of cyclocinamide B.

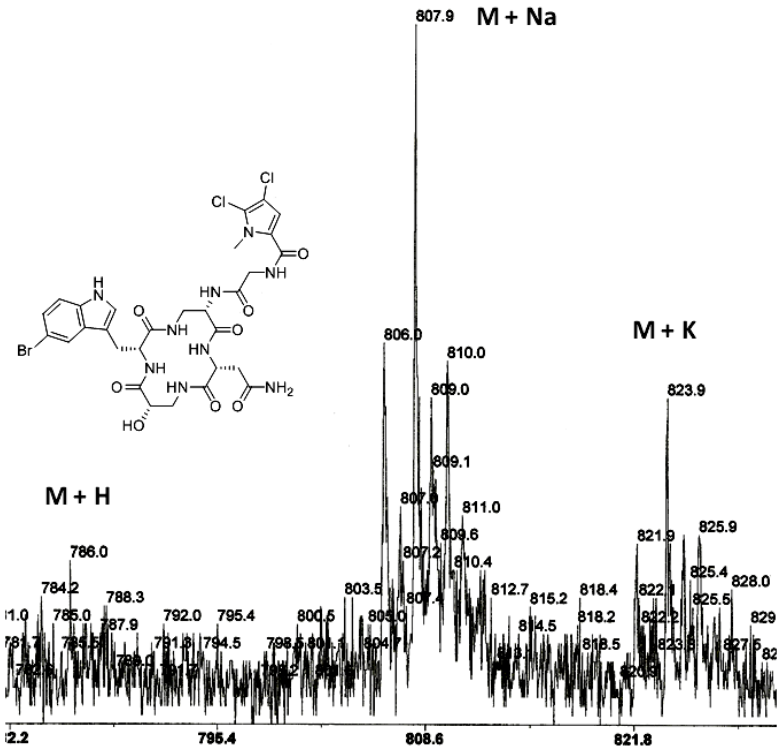


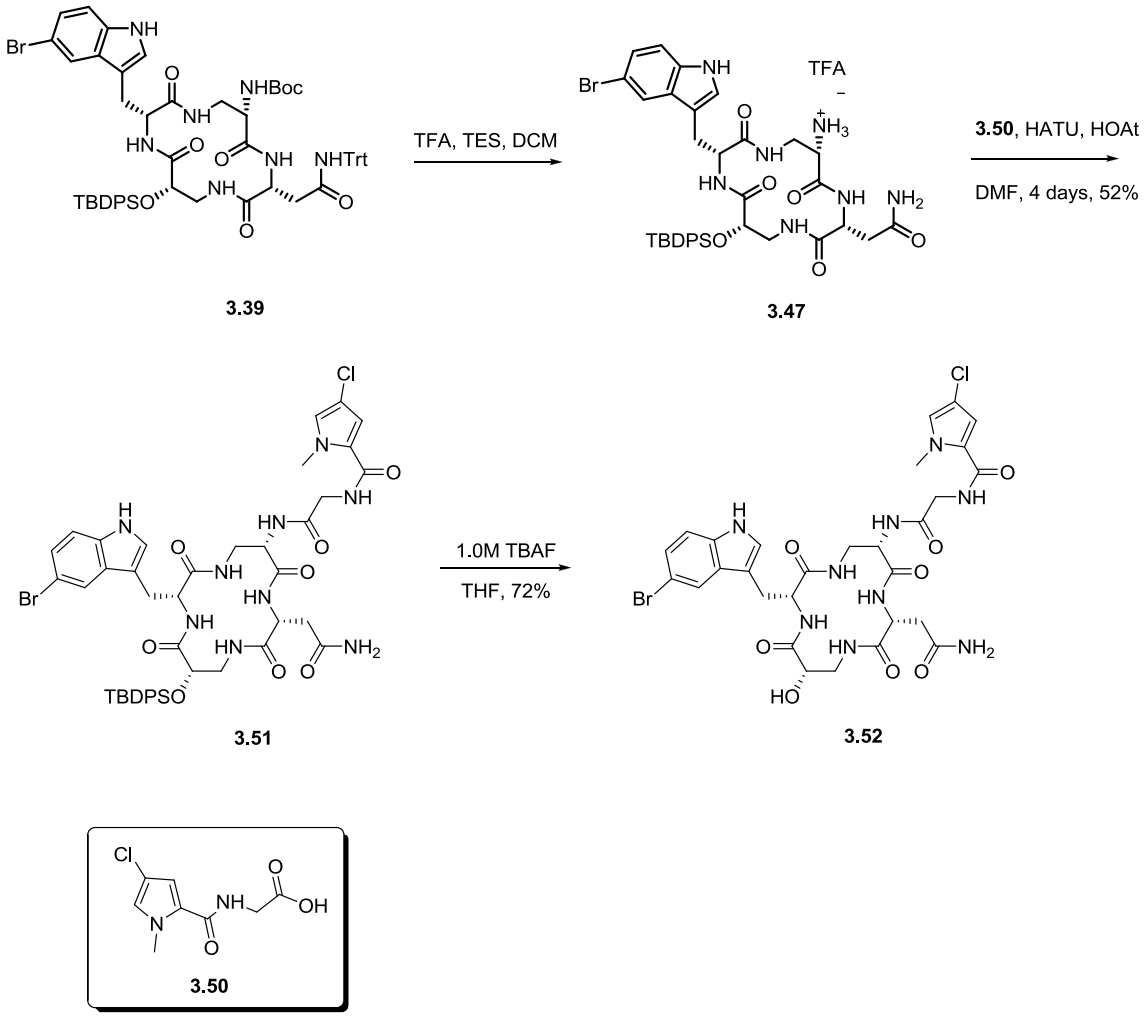
Figure 3.4 Mass spectroscopy of cyclocinamide B **3.1**

3.4.6) Attachment of the monochlorinated side chain

With the successful completion of the reported structure of cyclocinamide B, it came to our attention that synthetic scheme would allow for attachment of the cyclocinamide A monochlorinated side chain in a late stage event. This would allow us to obtain the enantiomer of the isomer synthesized by Grieco and have another isomer of cyclocinamide A for analysis.

The synthetic route shown in Scheme 3.26, started with cyclic compound **3.39** which was deprotected using TFA in dichloromethane to afford the cyclic compound **3.47**. The reaction was run for six hours instead of an hour to deprotect the trityl as well, since it was shown earlier that the absence of the trityl group did not affect the reaction. The monochlorinated side chain **3.50** was then coupled using the same procedure as was used for the dichlorinated side chain **3.2**. The cyclic hexapeptide **3.51** was obtained from that procedure in a decent yield. This compound was then TBDPS deprotected using the same sodium hydroxide procedure as was used for the synthesis of cyclocinamide B **3.1**. However, the deprotection did not yield the desired compound. At this point a different base, tetrabutylammonium fluoride was examined as found to be more effective. The result was a clean reaction in high yield providing compound **3.52**. An NMR of the crude material showed that the silyl group was removed cleanly with no other peptide compound present; however there was a significant amount of the

tetrabutylammonium salt contaminating the product. It was found that this contaminate was easily removed by triturating the cyclocinamide A isomer with methanol. The result was a clean cyclocinamide A isomer that did not need to be purified any further.



Scheme 3.26 Synthesis of the 4*S*,7*R*,11*S*,14*R* cyclocinamide A isomer

3.5) Conclusion

The successful synthesis of the reported structure of cyclocinamide B has been completed. Although the presence of the turn inducing cyclo(Asn) was essential to the success of the synthesis of cyclocinamide A it proved to be a detriment to the synthesis of cyclocinamide B. It was then found that the side chain could be added earlier in the synthesis, however the cyclization became unsuccessful. The removal of the cyclo(Asn) and minor changes to protection groups used for the synthesis of the tetrapeptide led to a successful cyclization. Attachment of the side chain and completion proved to be less problematic after the modifications to the synthetic route were made. Analysis of final product and comparison to the isomers synthesized by Dr. Jessica Garcia will allow us to determine the correct stereoisomers of cyclocinamides A and B.

3.6) Experimental Detail

All reactions were performed in oven- or flame-dried glassware under an inert atmosphere of nitrogen with the utilization of standard syringe techniques, unless otherwise noted. Microwave reactions were carried out in a CEM Discovery 1 instrument at the indicated power and temperature. Anhydrous acetonitrile (CH_3CN), dichloromethane (CH_2Cl_2), methanol (CH_3OH), tetrahydrofuran (THF) and toluene were obtained from a Pur-Solv 400 solvent purification system manufactured by Innovative Technology. All reagents were used as purchased without further purification, with the following exceptions: benzyl alcohol was fractionally distilled and stored over 4Å molecular sieves; imidazole was recrystallized from benzene; diethylamine, diisopropylamine and triethylamine were distilled from calcium hydride and stored over sodium hydroxide pellets; 2,4,6-trimethylpyridine (collidine) was distilled from calcium hydride; trimethylsilyl chloride (TMSCl) was distilled and stored over polyvinylpyridine.

Reactions were monitored by thin layer chromatography on Whatman polyester-backed plates (250 μm layer of silica) and visualized by ultraviolet light at 254 nm or chemical stain (ninhydrin, potassium permanganate, phosphomolybdic acid or vanillin). Crude products were purified using flash-grade silica gel from Dynamic Adsorbents, Inc. (32-63 μm).

Proton and carbon (^{1}H and ^{13}C) NMR spectra were obtained on either 500, 600 (cold probe) or 900 MHz Varian spectrometers in $CDCl_3$, CD_3OD or DMSO-*d*6. Abbreviations used for multiplicity are as follow: s = singlet, d = doublet, t = triplet, sep = septet, m = multiplet. Melting points were determined on a Mel Temp II device and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer OneSource FTIR instrument as thin films; ν_{max} values are reported in inverse centimeters. High resolution mass spectra were obtained at Berkeley Mass Spec Facility. Optical rotation measurements were found on a Jasco DIP-310 digital polarimeter; concentration is given in g/100 mL.

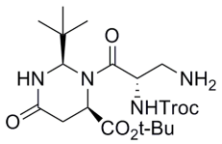
The shorthand amino acid nomenclature **PG_1-AA(PG_2)-X** is defined as follows:

PG_1: the protecting group on the nitrogen in the α-position of the α-amino acids tryptophan (Trp), asparagine (Asn), and the β-nitrogen of isoserine (Ise) and diaminopropionic acid (Dap)

AA: 3-letter common abbreviation of the amino acid. Exceptions are isoserine (Ise), diaminopropionic acid (Dap) and (cyclo)asparagine [(cyclo)Asn].

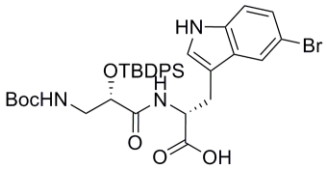
PG_2: the protecting group on the heteroatom located on the side chain of the amino acids Asn, Dap (α-nitrogen) and Ise (α-hydroxyl).

X: can be another amino acid, “OH” (free acid) or “OR” (an acid protected as its corresponding ester.



Troc-Dap(H)-*R*-(cyclo)Asn(*t*-Bu)-O*t*-Bu (3.5)

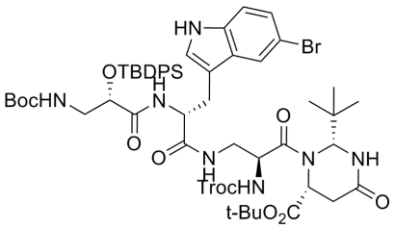
The dipeptide **3.6** (0.28 g, 0.38 mmol, 1.0 equiv) was dissolved in a 2M solution of dimethylamine in THF (9.6 mL). The solution was allowed to stir for 2h at room temperature, followed by removal of the solvent in vauco. The crude material was washed with hexanes and dried to yield a semi-crude white solid, 89% mass recovery.



Boc-Ise(TBDPS)-*R*-Trp(5-Br)-OH (3.4)

The dipeptide **3.7** (0.15 g, 0.208 mmol, 1.0 equiv) was dissolved in THF: MeOH (3:1, 1.32 mL: 0.26 mL) and cooled to 0 °C. Sodium hydroxide (1N, 0.25 mL) was slowly added dropwise and the reaction was warmed to room temperature and allowed to stir overnight. Water (1.5 mL) and EtOAc (5.0 mL) were added to dilute the reaction, followed by quenching with 1M potassium hydrogen sulfate until the pH reaches 3. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with a saturated solution of sodium chloride, dry over $NaSO_4$ and the solvent was removed to yield the product as a white foam (92% yield, 0.181 g). Mp = 51-53 °C (decomp); $[\alpha]^{27}_D$ = -7.2, (c 4.6, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.49 (s, 1H); 7.71 (m, 3H), 7.58 (d, *J* = 6.0 Hz, 1H), 7.40 (m, 5H), 7.20 (d, *J* = 7.0 Hz, 1H), 7.12 (d, *J* = 7.0 Hz, 1H), 6.96 (s, 1H), 5.45 (s, 1H), 4.78 (d, *J* = 5.0 Hz, 1H), 4.13 (s, 1H), 3.53 (d, *J* = 11.0 Hz, 1H), 3.21 (m, 3H), 1.35 (s, 9H), 1.07 (s 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.3,

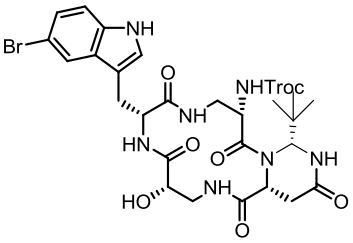
173.0,; 158.7, 135.3, 134.9, 129.6, 129.1, 124.8, 124.6, 121.1, 112.9, 109.1, 80.8, 72.8, 52.5, 44.3, 28.1, 27.0, 26.6, 26.4, 20.9, 18.9; IR (CH_2Cl_2): 3392, 2931, 2858, 1718, 1664, 1520, 1460, 1440, 1367, 1163, 1113, 1048, 856, 821, 605 cm^{-1}; HRMS [M-H] for $C_{35}H_{41}BrN_3O_6Si$ calcd., 706.1942 found, 706.1953



Boc-Ise(TBDPS)-*R*-Trp(5-Br)-Troc-Dap-*R*-(cyclo)Asn(*t*-Bu)-O*t*-Bu (3.8)

The dipeptide **3.4** (134.2 mg, 0.19 mmol, 1.0 equiv) was dissolved in DMF (10.5 mL) and HOAt (51.6 mg, 0.37 mmol, 2.0 equiv) , EDCI (72.6 mg, 0.37 mmol, 2.0 equiv) and DIPEA (0.13 mL, 0.76 mmol, 4.0 equiv) were added in sequential order followed by cooling to 0 °C. The dipeptide free amine derived from **3.5** (98.0 mg, 0.19 mmol, 1.0 equiv), dissolved in DMF (2.0 mL), was added and the mixture was allowed to stir for 5 min at 0 °C, then allow to warm to room temperature and stirred overnight. EtOAc and water were added to the reaction. The organic layer was washed with a 5% solution of HCl, a saturated solution of $NaHCO_3$, and a saturated solution of sodium chloride. The organic layer was dried over $NaSO_4$, and the solvent was removed leaving behind a yellow residue. The crude material was purified by flash column chromatography (5% MeOH in $CHCl_3$) to yield the product as a white solid (72 % yield, 164.6 mg).

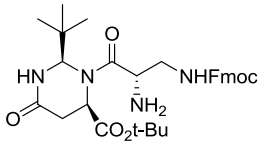
Mp = 64 °C (decomp) $[\alpha]^{25}_{D}$ = -10.7 (c 1.8, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (s, 1H), 7.19-7.71 (m, 12H), 7.12 (m, 1H), 7.06 (d, *J* = 2.0 Hz, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 6.91 (s, 1H), 5.07 (m, 2H), 5.02 (bs, 2H), 4.88 (m, 2H), 4.43 (m, 2H), 4.26 (m, 1H), 4.19 (m, 3H), 3.78 (m, 1H), 3.59 (m, 2H), 3.44 (m, 4H), 3.37 (m, 1H), 3.30 (m, 2H), 3.28 (m, 2H), 2.98 (m, 3H), 1.55 (s, 9H), 1.42 (s, 9H), 1.12 (s, 9H), 0.96 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.2, 167.0, 166.8, 166.3, 162.0, 155.9, 135.4, 134.9, 134.8, 129.6, 129.1, 128.5, 128.0, 127.7, 127.5, 127.1, 125.9, 125.3, 125.2, 124.9, 124.7, 124.4, 121.4, 121.0, 120.0, 113.2, 113.1, 112.9, 112.7, 107.6, 94.1, 80.5, 74.7, 74.6, 73.1, 54.6, 52.5, 52.0, 44.5, 41.8, 28.2, 28.1, 27.8, 26.4, 18.9; IR (CH_2Cl_2): 3459, 2931, 1736, 1673, 1668, 1651, 1646, 1549, 1508, 1438, 1369, 1113, 951 cm^{-1}; HRMS [M+Na] for $C_{54}H_{71}BrCl_3N_7O_{11}SiNa$, calcd., 1228.3122 found, 1228.3179



Cyclo[Ise(OH)-*R*-Trp(5-Br)-Troc-Dap-*R*-(cyclo)Asn(*t*-Bu)] (3.13)

In a flamed-dried round bottom flask the tetrapeptide **3.8** (70.0 mg, 0.05 mmol, 1.0 equiv) was dissolved in dichloromethane (9.6 mL). TFA (1.0 mL) and $HSiEt_3$ (0.5 mL) was added and the reaction was stirred for 2 h and the solvent was removed in vacuo and dried under reduced pressure. The crude residue was dissolved in DMF (11.2 mL) and dichloromethane (56.2 mL) followed by the addition of HOAt (23.7 mg, 0.17 mmol, 3.0 equiv), HATU (66.3 mg, 0.17 mmol, 3.0 equiv), DIPEA (0.05 mL, 0.29 mmol, 5.0

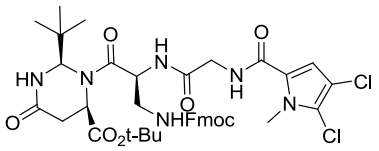
equiv). The reaction was heated to 40 °C for 48 h. The reaction was quenched with water and extracted with a 10% solution of HCl, a saturated solution of $NaHCO_3$, a saturated solution of sodium chloride, and dried over $NaSO_4$ and evaporated to give a yellow residue. The crude material was purified by flash column chromatography (5% MeOH in CH_2Cl_2) to yield the product as an off white foam (31% yield, 14.2 mg). ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.10, (d, *J* = 1.8 Hz, 1H), 9.45 (t, *J* = 6.0 Hz, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 2.4 Hz, 1H), 7.31 (dd, *J* = 0.6, 8.4 Hz, 1H), 7.17 (s, 1H), 7.16 (dd, *J* = 1.8, 8.4 Hz, 1H), 6.11 (d, *J* = 6.0 Hz, 1H), 4.94 (m, 2H), 4.48 (d, *J* = 4.8 Hz, 1H), 4.40 (t, *J* = 6.0 Hz, 1H), 4.11 (m, 1H), 4.05 (ddd, *J* = 4.2, 5.4, 9.0 Hz, 1H), 3.48 (dt, *J* = 4.8, 13.2 Hz, 1H), 3.42 (dd, *J* = 5.4, 10.8 Hz, 1H),3.36 (dd *J* = 6.0, 10.8 Hz, 2H), 3.29 (m, 2H), 3.21 (ddd, *J* = 6.0, 8.4, 15.0 Hz, 1H), 3.03 (dd, *J* = 7.2, Hz,1H), 3.00 (dd, *J* = 7.2, 10.2 Hz, 1H), 1.24 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 182.4, 175.4, 170.3, 160.8, 159.8, 154.8, 134.6, 134.4, 129.1, 125.5, 123.3, 120.4, 113.3, 112.9, 111.0, 108.9, 107.3, 95.5, 93.9, 83.7, 72.4, 69.3, 63.0, 53.2, 42.9, 36.4, 35.1; IR (CH_2Cl_2): 3522, 3455, 2931, 1726, 1663, 1658, 1651, 1646, 1542, 1438, 1369, 1113, 951 cm^{-1}; HRMS [M+H] for $C_{29}H_{35}BrCl_3N_7O_8$, calcd., 793.0796 found, 793.0226



H-Dap(Fmoc)-*R*-(cyclo)Asn(*t*-Bu)-O*t*-Bu (3.22)

Compound **3.6** (140.0 mg, 0.18 mmol, 1.0 equiv) was dissolved in THF (7.0 mL) and potassium hydrogen sulfate (1.0 M, 9.0 mL) was added to the solution. Activated Zn (1.01 g, 15.44 mmol, 85.0 equiv) was added and the reaction

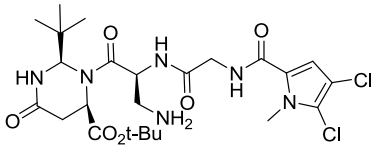
was allowed to stir for 4 h at which time the reaction was completed as indicated by TLC. The solvent was removed and the resulting residue was dissolved in EtOAc. The organic layer was washed with a saturated solution of sodium bicarbonate, water and a saturated solution of sodium chloride. The organic layer was dried over Na_2SO_4 and the solvent was removed to obtain a white solid (73% yield, 78.1 mg).



(Gly-N-Me-pyrrole-4,5-Cl)-Dap(Fmoc)-*R*-(cyclo)Asn(*t*-Bu)-O*t*-Bu (3.23).

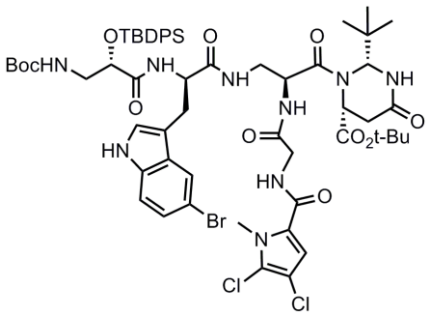
The dipeptide **3.22** (156.0 mg, 0.276 mmol, 1.0 equiv) was dissolved in DMF (3.0 mL). The solution was cooled to 0 °C and the side chain 2-[(4,5-dichloro-1-methyl-pyrrole-2-carbonyl)-amino]-ethanonic acid **3.3** (76.0 mg, 0.304 mmol, 1.1 equiv), dissolved in DMF (1.0 mL), was added to the reaction. To the cooled solution COMU (118.0 mg, 0.304 mmol, 1.1 equiv) was added and the reaction was allowed to stir for 30 min after which time DIPEA (0.074 mL, 0.552 mmol, 2.0 equiv) was added and stirred for an additional 30 min. The reaction was warmed to room temp and stirred for 2 days. The solvent was removed and EtOAc was added to the residue. The organic layer was washed with 1 M HCl, a solution of saturated $NaHCO_3$, and solution of saturated sodium chloride. The organic layer was dried over $MgSO_4$ and evaporated. The crude residue was purified by flask column chromatography (5% MeOH in CH_2Cl_2) to yield the product as a pale yellow solid (51% yield, 112.2 mg). $[\alpha]^{25}_D$ = 10.7, (c 1.8, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88 (t, *J* = 6.5 Hz, 1H), 7.77

(dd, *J* = 7.5, Hz, 1H), 7.73 (d, *J* = 7.5 Hz, 2H), 7.52 (d, *J* = 7.5 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.28 (d, *J* = 6.0 Hz, 2H), 6.83 and 6.89 (bs, 1H), 6.63 and 6.65 (s, 1H), 5.84 (t, *J* = 5.0, 1H), 4.66 (t, *J* = 6.5 Hz, 1H), 4.58 (t, *J* = 5.0 Hz, 1H), 4.18 (m, 2H), 3.99 (dd, *J* = 6.0, 11.0 Hz, 2H), 3.72 and 3.73 (s, 3H), 3.61 (m, 2H), 2.96 (m, 1H), 2.93 (dd, *J* = 4.5, 6.5 Hz, 1H), 2.80 (s, 9H), 1.44 and 1.46 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 170.4, 167.7, 167.6, 161.5, 161.4, 158.3, 143.6, 143.6, 141.2, 140.9, 127.7, 127.1, 125.1, 123.2, 120.0, 116.8, 116.7, 116.4, 111.9, 109.2, 109.1, 84.4, 84.3, 67.3, 55.1, 54.9, 49.4, 49.3, 46.8, 43.3, 42.1, 38.5, 33.4, 29.6, 27.7, 20.7, 20.6; IR ($CHCl_3$): 3304, 1735, 1696, 1648, 1545, 1451, 1371, 1350, 1257, 1157, 847 cm^{-1}; HRMS [M+H] for $C_{39}H_{47}Cl_2N_6O_8$ calcd., 797.2754 found, 797.2066



(4,5-dichloro-1-methyl-1H-pyrrole-2-carboxamido)-Gly)-Dap(H)-*R*-(cyclo)Asn(*t*-Bu)-O*t*-Bu (3.21)

The tetrapeptide **3.23** (200.0 mg, 0.25 mmol, 1.0 equiv) was dissolved in THF (12.0 mL) and diethylamine (2.4 mL, 23.1 mmol, 100.0 equiv) was added. The reaction was allowed to stir at room temperature for 4 h at which time all starting material was consumed as indicated by TLC. The solvent was removed and the resulting residue was washed with cold diethyl ether giving a pale orange solid. (80 % yield, 115.3 mg) The solid was dried under vacuum and used without further purification.

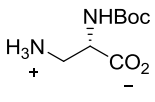


Boc-Ise(TBDPS)-*R*-Trp(5-Br)-(4,5-dichloro-1-methyl-1H-pyrrole-2-carboxamido)-Gly-Dap-*R*-(cyclo)Asn(*t*-Bu)-O*t*-Bu (3.24)

In a flame dried round bottom flask dipeptide **3.4** (161.0 mg, 1.0 equiv, 0.23 mmol) was dissolved in of DMF (2.0 mL) and cooled to 0 ^{o}C, followed by the addition of COMU (97.2 mg, 1.0 equiv, 0.23 mmol) and stirred for 20 min. The tetrapeptide **3.21** (128.0 mg, 1.0 equiv, 0.23 mmol) dissolved in DMF (1.0 mL) was added to the reaction along with DIPEA (0.08 mL, 2.0 equiv, 0.45 mmol). The reaction was warmed to room temperature and stirred overnight. The solvent was removed and the residue was dissolved in EtOAc. The organic layer was washed with 1% HCl, a saturated solution of $NaHCO_3$, water, and a saturated solution of sodium chloride. The organic layer was dried over $MgSO_4$ and evaporated to give a yellow residue. The crude material was purified by flash column chromatography (5% MeOH in CH_2Cl_2) to give an off white solid as a mixture of inseparable rotamers (58% yield, 161 mg).

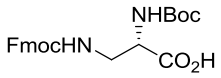
$[\alpha]^{26}_{D}$ = -13.3 (c 1.6, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.0 (bs, 1H), 8.63 (t, *J* =5.4 Hz, 1H), 7.75 (d, *J* = 10.2 Hz, 1H), 7.67 (d, *J* = 2.4 Hz, 1H), 7.28-7.47 (m, 11H), 7.27 (d, *J* = 10.4 Hz, 1H), 7.15 (d, *J* = 2.4 Hz, 1H), 6.95 and 6.96 (s, 1H), 6.52 and 6.53 (s, 1H), 5.72, (d, *J* = 7.2 Hz, 1H), 4.85 (dd, *J* = 7.2 Hz, 1H), 4.56 (m, 1H), 4.46 (m, 2H), 4.24 (m, 2H), 4.14 (m, 1H), 3.95 (dd, *J* =3.6, 6.0 Hz, 2H), 3.82 and 3.83 (s, 3H), 3.73

(m, 2H), 3.54 (t, *J* = 6.0 Hz, 2H), 3.26 (dd, *J* =5.4, 7.2 Hz, 2H), 3.16 (dd, *J* = 6.0, 7.8 Hz, 2H), 2.95 (dd, *J* = 6.3, 17.4 Hz, 2H), 1.35 and 1.37 (s, 9H), 1.34 (s, 9H), 1.22 and 1.24 (s, 9H), 0.86 and 0.94 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 171.3, 170.1,170.0, 160.0, 156.3, 155.6, 135.4, 135.2, 134.7, 134.5, 132.7, 129.9, 127.7, 127.5, 125.5, 124.7, 123.4, 120.5, 118.4, 113.8, 113.3, 111.2, 111.1, 109.1, 107.2, 94.2, 86.2, 77.7, 70.4, 65.8, 59.8, 48.6, 46.9, 44.2, 43.8, 40.9, 37.8, 33.2, 28.1, 27.9, 27.5, 27.3, 27.1, 26.4, 13.8, 12.5; IR (CH_2Cl_2): 3309, 3019, 1734, 1648, 1612, 1532, 1453, 1369, 1215, 1159, 777, 669 cm^{-1}



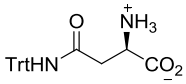
Boc-Dap-OH

Desired compound accessed from the commercially available Boc-asparagine by modified a literature procedure. Boc-Asn-OH (1.3 g, 5.59 mmol, 1.0 equiv) was dissolved in a mixture of MeOAc, n-propanol and water. To the asparagine iodobenzene diaceate (1.4 g, 4.34 mmol, 1.3 equiv) was added and the reaction was stirred at room temperature for 6 h at which time the mixture become cloudy as the product formed. The reaction was then heated to 70 ^{o}C for one hour then allowed to cool slowly to room temperature, after which the reaction was cooled to 0 ^{o}C. The solid was filtered and washed with EtOAc and dried under vacuum. The desired *S*-Boc-Dap-OH was isolated in 76% yield (0.86 g, 4.21 mmol). Analytical data matched the reported.[27]



Boc-Dap(Fmoc)-OH (3.29)

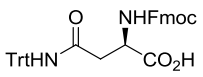
Boc-Dap-OH (5.6 g, 27.51 mmol, 1.0 equiv) was suspended in Na_2CO_3 (10%, 42.3 mL) and cooled to 0 ^{o}C. A solution of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (9.28 g, 27.51 mmol, 1.0 equiv) in THF (39.0 mL) was added to the reaction. The solution was allowed to warm to room temperature and stirred overnight. The organic layer was removed and the aqueous layer was washed with EtOAc. The aqueous layer was then acidified to pH 2 with conc. HCl and the resulting cloudy solution was extracted with EtOAc. The organic layer was washed with water and a saturated solution of sodium chloride, and dried over $MgSO_4$. Solvent was removed by rotary evaporation and the remaining colorless foam was used without further purification. The desired *S*-Boc-Dap(Fmoc)-OH was isolated in 98% yield (11.47 g, 26.91 mmol). Analytical data match literature values.[28]



H-*R*-Asn(Trt)-OH

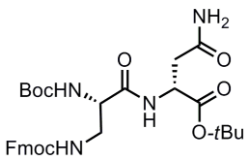
Following a modification of the known literature procedure,[29] in an oven dried two neck round bottom flask asparagine (1.32 g, 1.0 equiv, 1.0 mmol) was dissolved in the solvent mixture of acetic acid (3.0 mL), acetic anhydride (1.89 mL), and concentrated sulfuric acid (0.61 mL). To the mixture triphenylmethanol (0.52 g, 2.0 equiv, 2.0 mmol) was added and the reaction was heated to 60 ^{o}C for 2 hours. The reaction was cooled to room temperature and was carefully added to

cold water. The reaction was cooled in an ice bath and the pH was adjusted to 6 using concentrated NaOH and allowed to stir for 1 h at 0 ^{o}C. The solids were filtered off and washed first with cold water and then toluene and dried to give a white solid (72% yield, 1.4 g.) Analytical data matched the literature values.



***R*-Fmoc-Asn(Trt)-OH (3.30)**

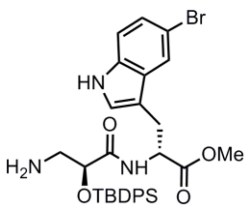
H-Asn(Trt)-OH (1.4 g, 3.57 mmol, 1.0 equiv) was dissolved of a aqueous solution of Na_2CO_3 (10%, 32.5 mL). The flask was cooled in an ice bath while Fmoc-succinimide (1.05 g, 3.57 mmol, 1.0 equiv) was dissolved in 80 mL of CH_3CN. The solution was then warmed to room temperature and stirred overnight. The CH_3CN was removed by rotary evaporation and the resulting aqueous layer was acidified to pH 2 with 1.0M $KHSO_4$ and extracted EtOAc. The organic layer was with water, a saturated solution of sodium chloride and dried over $MgSO_4$. The organic layer was concentrated to give the desired compound Fmoc-Asn(Trt)-OH in 87% yield (1.85 g, 3.10 mmol). Analytical data matched the literature values.



***S*-Fmoc-Dap(Boc)-*R*-Asn-O*t*-Bu (3.30)**

In a flame dried round bottom flask compound **3.29** (200.0 mg, 1.06 mmol, 1.0 equiv) was dissolved in DMF (5.0 mL) and cooled to 0 ^{o}C. To this COMU (453.0 mg, 1.06 mmol, 1.0 equiv) was added followed by the Asn-O*t*-Bu **3.28** (452.0 mg, 1.06 mmol, 1.0 equiv) and DIPEA (0.4 mL, 2.12

mmol, 2.0 equiv) was added and stirred at 0 ^{o}C for one hour. The reaction was warmed to room temperature and stirred for 5 h. The solvent was removed and EtOAc was added. The organic layer was washed with 1M HCl, a saturated solution of $NaHCO_3$, water, and a saturated solution of sodium chloride. The organic layer was dried over $MgSO_4$. The crude material was purified by flash column chromatography (5% MeOH in CH_2Cl_2) to give an off white solid. (55% yield, 348 mg) ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.10 (d, *J* = 8.0 Hz, 1H) , 7.38 (bs, 1H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.65 (d, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.31 (dt, *J* = 7.5, 1.0 Hz, 2H), 7.22 (t, *J* = 5.5 Hz, 1H), 6.89 (bs, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 4.43 (dd, *J* = 5.5, 13.5 Hz, 1H), 4.24 (d, *J* = 7.0 Hz, 2H), 4.19 (t, *J* = 6.5, 7.0 Hz, 1H), 4.05 (dd, *J* = 8.0, 13.0 Hz, 1H), 3.28 (dt, *J* = 5.0, 14.0 Hz, 1H), 3.18 (dd, *J* = 7.5, 7.0 Hz, 1H)1.35 (s, 9H), 1.34 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.3, 170.1, 169.8, 156.3, 155.2, 143.8, 143.8, 140.7, 127.6, 127.1, 125.2, 120.1, 80.5, 78.3, 65.5, 54.5, 49.2, 46.5, 42.1, 36.5, 28.0, 27.4; HRMS [M+H] for $C_{31}H_{40}N_4O_8$ calcd., 596.2846 found, 596.3451

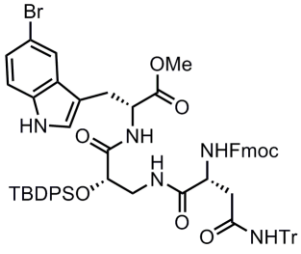


H-Ise(TBDPS)-*R*-Trp(5-Br)-OMe (3.34).

The dipeptide **3.4** (300.0 mg, 1.0 equiv, 0.41 mmol) was dissolved in CH_2Cl_2 (10.0 mL). To this solution was added TFA (0.5 mL) and Et_3SiH (0.5 mL) and the resulting reaction mixture was stirred until all the starting material was consumed as monitored by TLC (typically 3 h). The reaction

was then concentrated and dried under vacuum to yield the TFA salt as a pale yellow solid (233.0 mg, 90 % yield).

$[\alpha]^{27}_{D}$ = -16.7 (c 1.5, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.76 (d, *J* = 7.0, 1H), 7.70 (d, *J* = 1.5 Hz, 1H), 7.46 (m, 2H), 7.35 (t, *J* = 7.5 Hz, 2H), 7.31 (m, 3H), 7.25 (m, 3H), 7.18 (d, *J* = 8.5 Hz, 1H), 7.09 (t, *J* = 7.5, 2H), 6.48 (bs, 1H), 4.73 (m, 1H), 4.25 (s, 1H), 3.78 (s, 3H), 3.68 (dd, *J* = 4.5, 5.0 Hz, 1H), 3.36 (dd, *J* = 4.0, 15.0 Hz, 1H), 3.24 (dd, *J* = 3.0, 4.5 Hz, 1H), 3.12-3.18 (m, 2H), 3.04 (d, *J* = 9.5 Hz, 1H), 2.85 (s, 2H), 1.01 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.7, 135.8, 135.7, 135.2, 131.9, 130.9, 130.8, 130.5, 128.6, 128.3, 128.2, 128.0, 125.3, 125.2, 121.1, 113.1, 113.0, 109.1, 70.4, 66.6, 53.3, 52.8, 47.2, 44.0, 38.5, 28.2, 26.4, 26.2, 19.0; IR (CH_2Cl_2): 3405, 2575, 1730, 1692, 1674, 1512, 1472, 1448, 1265, 1107, 1036, 738, 702 cm^{-1}; HRMS [M+H] for $C_{31}H_{37}BrN_3O_4Si$ calcd., 622.6247 found, 622.1731

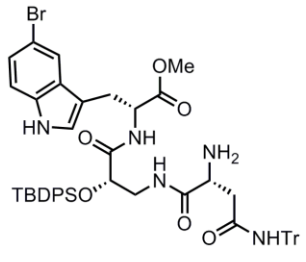


Fmoc-*R*-Asn(Tr)-Ise(TBDPS)-*R*-Trp(5-Br)-OMe (3.35)

In a flame dried round bottom flask Fmoc-*R*-Asn(Trt)-OH (320.0 mg, 1.0 equiv, 0.53 mmol) was dissolved in DMF (3.0 mL) and cooled to 0 ^{o}C. HATU (413.0 mg, 2.0 equiv, 1.08 mmol), HOAt (147.0 mg, 2.0 equiv, 1.08 mmol) was added and the solution was allowed to stir for 10 min. To the solution the dipeptide **3.34** (0.35 mg, 1.0 equiv, 0.53 mmol) was added, followed by DIPEA (0.2 mL, 4.0 equiv, 2.16 mmol). The mixture was stirred at 0 ^{o}C for 1 h then allowed to warm to room temperature and

stirred for an additional 3 h. Water and EtOAc were added and the reaction was extracted with additional EtOAc. The organic layer was washed with 1.0 N HCl, a saturated solution of $NaHCO_3$, water, and a saturated solution of sodium chloride. The organic layer was dried over $MgSO_4$ and concentrated to give the crude material as light orange oil. The crude material was purified by flash column chromatography eluting with 5% methanol in dichloromethane to give the tripeptide as a white solid (91 % yield, 580 mg).

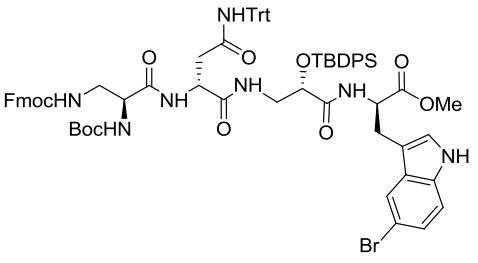
$[\alpha]^{24}_D$ = 9.1 (c 0.24, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07 (bs, 1H), 7.78 (dd, *J* = 6.5, 5.5 Hz, 2H), 7.55-7.61 (m, 4H), 7.07-7.45 (m, 31H), 6.71 (bs, 1H), 6.26 (d, *J* = 8.0 Hz, 1H), 4.66 (dd, *J* = 7.0, 6.0 Hz, 1H), 4.40 (m, 2H), 4.23 (t, *J* = 7.0 Hz, 1H), 4.17 (m, 1H), 3.60 (s, 3H), 3.55 (dd, *J* = 8.0, 6.0 Hz, 1H), 3.36, (dd, *J* = 12.0, Hz, 1H), 3.12 (d, *J* = 5.5 Hz, 1H), 2.78 (dd, *J* = 15.0, 6.0 Hz, 1H), 2.65 (dd, *J* = 15.0, 4.5 Hz, 1H), 0.92 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.3; 172.0, 170.7, 169.7, 156.6, 144.5, 143.9, 143.8, 141.4, 135.9, 135.7, 134.9, 132.7, 131.8, 130.1, 130.5, 128.9, 128.8, 128.1, 128.0, 127.2, 125.3, 124.3, 121.2, 120.1, 113.0, 109.1, 72.9, 70.8, 67.6, 52.8, 54.4, 52.0, 47.1, 43.0, 39.2, 26.7, 26.4; IR (CH_2Cl_2): 3405, 3055, 2953, 2585, 1720, 1674, 1514, 1495, 1448, 1265, 1112, 1038, 738, 702 cm^{-1}; HRMS [M+H] for $C_{69}H_{67}BrN_5O_8Si$ calcd., 1200.3937 found, 1200.3975.



_R_-Asn(Tr)-Ise(TBDPS)-_R_-5-Trp(5-Br)-OMe (3.36).

The tripeptide **3.35** (150.0 mg, 0.12 mmol, 1.0 equiv) was dissolved in THF (7.0 mL) and diethylamine (0.43 mL) was added. The solution was allowed to stir for 3 h at room temperature, followed by removal of the solvent in vauco. The residue was washed with toluene and dried. The crude material was pasted through a silica plug eluting first with the gradient of 0-10% methanol in dichloromethane to yield the product as an off white solid (80% yield, 93.7 mg).

$[\alpha]^{27}_{D}$ = 3.1 (c 0.10, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.86 (s, 1H); 7.86 (s, 1H), 7.61 (d, *J* = 1.5 Hz, 1H), 7.51-7.56 (m, 5H), 7.40-7.47 (m, 3H), 7.23-7.35 (m, 23H), 7.14 (d, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 9.0 Hz, 1H), 6.77 (d, *J* = 2.0 Hz, 1H), 4.77 (dd, *J* = 6.0, 7.0 Hz, 1H), 4.24 (dd, *J* = 3.0, 5.0 Hz, 1H), 3.67 (s, 3H), 3.61 (dd, *J* = 3.5, 9.0 Hz, 1H), 3.50 (dt, *J* = 6.0, 14.5 Hz, 1H), 3.44 (ddd, *J* = 3.0, 5.0, 14.0 Hz, 1H), 3.22 (dd, *J* = 6.0, 15.0 Hz, 1H), 3.15 (dd, *J* = 5.5, 15.0 Hz, 1H), 2.71 (dd, *J* = 3.5, 14.5 Hz, 1H), 2.38 (dd, *J* = 9.5, 15.0 Hz, 1H), 0.99 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.9, 172.9, 171.9, 170.6, 144.6, 135.8, 135.7, 135.0, 132.5, 132.0, 130.5, 130.4, 129.1, 128.3, 128.2, 128.1, 127.1, 125.2, 124.2, 121.1, 113.3, 112.9, 108.6, 72.8, 70.6, 53.5, 53.0, 52.8, 51.8, 42.7, 41.4, 26.8, 26.7, 19.1; IR (CH_2Cl_2): 3411, 3055, 2953, 2585, 1720, 1651, 1517, 1495, 1265, 1112, 1038, 738, 700 cm^{-1}; HRMS [M+H] for $C_{54}H_{57}BrN_5O_6Si$ calcd., 978.3250 found, 978.3256

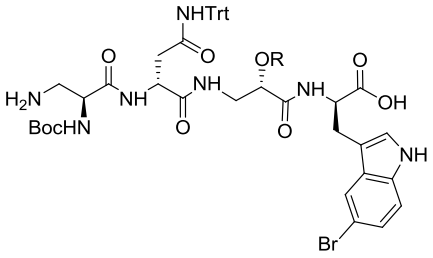


***S*-Boc-Dap(Fmoc)-*R*-Asn(Tr)-Ise(TBDPS)-*R*-Trp(5-Br)-OMe (3.26).**

In a flame dried round bottom flask *S*-Boc-Dap(Fmoc)-OH **3.31** (108.0 mg, 1.0 equiv, 0.26 mmol) was dissolved in DMF (6.0 mL) and cooled to 0 ^{o}C. HOAt (34.4 mg, 1.0 equiv, 0.26 mmol) and HATU (96.9 mg, 1.0 equiv, 0.26 mmol) were added and the reaction was allowed to stir for 1 h. To the mixture the tripeptide free amine **3.36** (250.0 mg, 1.0 equiv, 0.26 mmol) was added followed by DIPEA (0.06 mL, 2.0 equiv, 0.52 mmol). The reaction was allowed to warm to room temperature and was stirred for 48 h after which the reaction was concentrated. Ethyl acetate was added to the reaction and the organic layer was washed with 1.0 N HCl, a saturated solution of sodium carbonate, water and a saturated solution of sodium chloride. The organic layer was dried over $MgSO_4$ and concentrated to give an orange residue. The crude material was purified by flash column chromatography eluting with EtOAc: Hexanes (1:1) to give the pure material as a white solid (52% yield, 187 mg).

$[\alpha]^{27}_{D}$ = -4.6 (c 1.6 , MeOH); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.53 (bs, 1H), 7.71-7.77 (m, 2H), 7.34-7.62 (m, 17H), 7.08-7.32 (m, 21H), 6.94 (s, 1H), 6.81 (s, 1H), 6.14 (s, 1H), 5.81 (d, *J* = 4.2 Hz, 1H), 4.69 (d, *J* = 5.4 Hz, 1H), 4.61 (m, 1H), 4.27 (m, 2H), 4.15 (dd, *J* = 6.0, 2.4 Hz, 1H), 3.61 (s, 3H), 3.53 (m, 2H), 3.39 (dd, *J* = 13.8, 4.8 Hz, 1H), 3.29 (d, *J* = 12.0 Hz, 1H), 3.24 (dd, *J* = 13.8, 6.0 Hz, 1H), 3.15 (dt, *J* = 14.4, 5.4 Hz, 1H), 3.07 (dt, *J* = 15.0, 4.8 Hz, 1H), 2.63 (d, *J* = 11.4 Hz, 1H), 2.51 (d, *J* = 12.6 Hz, 1H), 1.41 (s, 9H),

0.96 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.4, 172.2, 170.9, 170.1, 169.7, 157.8, 156.0, 148.5, 144.4, 144.1, 141.4, 135.9, 135.8, 135.0, 132.5, 131.9, 130.4, 130.3, 129.0, 128.8, 128.6, 128.3, 128.0, 127.8, 127.2, 127.1, 125.4, 125.2, 124.3, 113.3, 112.6, 108.6, 80.4, 72.6, 67.2, 67.1, 55.6, 52.9, 51.8, 50.6, 47.1, 43.2, 43.1, 38.1, 29.7, 28.3, 26.6; IR (CH_2Cl_2): 3414, 3054, 2987, 1712, 1676, 1503, 1438, 1422, 1264, 1158, 1112, 1018, 896, 740, 701 cm^{-1}; HRMS [M+H] for $C_{76}H_{79}BrN_7O_{11}Si$ calcd., 1386.4941 found, 1386.4937



_S_-Boc-Dap(H)-_R_-Asn(Trt)-_S_-Ise(R)-_R_-Trp(5-Br)-OH

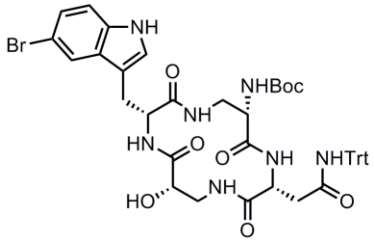
In a flame-dried round bottom flask **3.27** (200.0 mg, 1.0 equiv, 0.15 mmol) was dissolved in THF (10.2 mL) and diethylamine (1.02 mL, 65.7 equiv, 9.85 mmol) was added slowly. The reaction was stirred for 3 h, after which time the solvent was removed and the residue was washed with toluene then dried under vacuum. The crude material was dissolved in MeOH (0.15 mL) and THF (0.75 mL) and NaOH (1N, 0.15 mL, 1.0 equiv, 0.15 mmol) was added slowly and allowed to stir at room temperature for 24 h. Water (1.5 mL) and EtOAc (5.0 mL) was added followed by acidification to pH = 3 with 1.0 N HCl. The aqueous layer was extracted with EtOAc. The organic layer was washed with water and a saturated solution of sodium chloride and was dried over magnesium sulfate, followed by removal of the solvent to give the crude material as

a yellow residue. The material was purified by flash column chromatography eluting with 7% MeOH in CH_2Cl_2 to give two compounds (compound A, 63% yield, 104.5 mg; compound B, 20% yield, 26.4 mg).

Compound A 3.37. (R=TBDPS). $[\alpha]^{25}_D$ = 5.9 (c 4.2 , MeOH); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.08 (s, 1H); 10.97 (s, 1H), 8.94, 8.82, 7.92 (d, *J* = 4.8 Hz, 1H), 7.71, 7.67, 7.13-7.46 (m, 30H), 6.98 (s, 1H),4.72 (td, *J* = 4.2, 9.6 Hz, 1H), 4.46 (td, *J* = 3.6, 9.0 Hz, 1H),4.40 (td, *J* = 3.6, 7.8 Hz, 1H), 4.17 (ddd, *J* = 3.6, 4.2, 9.0 Hz, 1H), 3.89 (t, *J* = 3.2 Hz, 1H), 3.59-3.63 (m, 2H), 3.37-3.43 (m, 2H), 3.27 (dd, *J* = 3.6, 15.6 Hz, 1H), 3.17 (d, *J* = 11.2 Hz, 1H), 3.13 (dd, *J* = 4.2, 14.4 Hz, 1H), 3.00-3.06 (m, 3H), 2.91-2.99 (m, 4H), 2.60 (dd, *J* = 10.2, 13.8 Hz, 1H), 1.41 (s, 9H), 0.90 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 176.3; 173.6, 171.1, 170.9, 169.5, 168.4, 156.5, 155.6, 144.8, 135.5, 135.1, 135.0, 134.7, 132.2, 132.0, 130.1, 130.0, 129.1, 128.9, 128.6, 128.2, 127.7, 127.5, 126.3, 125.5, 124.4, 123.3, 120.5, 120.4, 113.4, 113.3, 111.2, 111.1, 110.9, 110.1, 78.7, 72.6, 69.3, 54.9, 54.2, 52.7, 51.3, 51.1, 42.7, 40.9, 28.1, 27.4, 26.4; IR (thin film): 3272, 3053, 2931, 1707, 1669, 1588, 1520, 1492, 1392, 1265, 1165, 1112, 1025, 884, 822, 738, 702 cm^{-1}; HRMS [M+H] for $C_{61}H_{69}BrN_7O_9Si$ calcd., 1150.4104 found, 1150.4121

Compound B 3.38. (R=H). $[\alpha]^{26}_D$ = -6.1 (c 0.6 , MeOH); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.14 (bs, 1H), 8.70 (s, 1H), 8.41 (bd, *J* = 7.2 Hz, 1H), *7*.87 (t, *J* = 6.0 Hz, 1H), 7.82 (t, *J* = 7.8 Hz, 1H), 7.69 (d, *J* = 1.2 Hz, 1H), 7.24-7.32 (m, 12H), 7.13-7.22 (m, 18H), 5.87 (d, *J* = 5.4 Hz, 1H), 4.59 (dd, *J* = 7.8, 12.6 Hz, 1H), 4.51 (dd, *J* = 13.2, 6.6 Hz, 1H), 4.31

(dd, *J* = 7.8, 13.2 Hz, 1H), 3.94 (m, 1H), 3.47 (m, 1H), 3.17 (m, 3H), 3.09 (m, 2H), 2.94 (dd, *J* = 7.0, 12.6 Hz, 1H), 2.71 (dd, *J* = 8.4, 15.0 Hz, 1H), 2.65 (dd, *J* = 4.2, 15.0 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.1, 171.8, 170.8, 169.3, 168.9, 155.3, 148.9, 144.6, 134.7, 129.1, 128.9, 128.5, 128.2, 127.5, 126.3, 125.4, 123.4, 113.4, 111.1, 109.3, 94.3, 79.0, 70.2, 69.3, 63.0, 52.2, 51.9, 50.3, 43.1, 28.0, 26.5, IR (CH_2Cl_2): 3273, 3049, 2941, 1712, 1672, 1576, 1520, 1491, 1395, 1259, 1165, 1112, 1025, 884, 741, 702 cm^{-1}; HRMS [M+H] for $C_{45}H_{51}BrN_7O_9$ calcd., 912.2932 found, 912.2729



Cyclo[Ise(R)-*R*-Trp(5-Br)-Boc-Dap-*R*-Asn(Tr)].

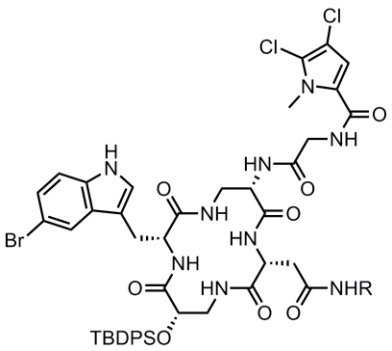
In a flame dried round bottom flask the above linear peptide **3.37** (72.0 mg, 1.0 equiv, 0.062 mmol) was dissolved in DMF (5.0 ml) and added to CH_2Cl_2 (25.0 mL). The solution was cooled to 0 ^{o}C and DEPBT (37.1 mg, 2.0 equiv, 0.124 mmol) was added. The solution was stirred for 30 min after which time DIPEA (0.08 mL, 10.0 equiv, 0.620 mmol) was added and stirred for an additional 30 min. The reaction was warmed to room temperature and stirred for 3 days. The solvent was removed and the resulting residue was dissolved in EtOAc and washed with 1.0 M HCl, a saturated solution of sodium bicarbonate, water and a solution of saturated sodium chloride. The organic layer was dried with magnesium sulfate and the concentrated to yield a yellow residue. The crude material was purified by flash column chromatography with a

gradient 0-5% MeOH in CH_2Cl_2, which yielded a mixture of compounds **A** and **B**. The two compounds were then separated by flash column chromatography using 80-100% EtOAc in Hexanes, followed by flushing the column with EtOAc, followed by the gradient 0-10% MeOH in EtOAc. Compound **A** was isolated as a white solid (10% yield, 5.6 mg) and compound **B** was a white solid (69% yield, 48.8 mg).

Compound A 3.40 (R = H). $[\alpha]^{26}_D$ = -5.4 (c 0.8, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.06 (s, 1H), 11.03 (s, 1H), 8.70 (s, 1H), 8.13 (d, *J* = 9.0 Hz, 1H), 7.92 (d, *J* = 9.0 Hz, 1H), 7.81 (d, *J* = 7.2 Hz, 1H), 7.69 (s, 1H), 7.36-7.48 (m, 3H), 7.24-7.31 (m, 8H), 7.14-7.20 (m, 15H), 7.13 (s, 1H), 6.05 (bs, 1H), 4.59 (m, 2H), 4.16 (d, *J* = 6.0 Hz, 1H), 4.12 (s, 1H), 4.04 (s, 1H), 3.39 (m, 2H), 3.23 (d, *J* = 13.8 Hz, 1H), 3.12 (dd, *J* = 6.6, 14.4 Hz, 1H), 2.90 (dd, *J* = 6.0, 14.4 Hz, 1H), 2.61 (dd, *J* = 5.6, 15.6 Hz, 1H), 2.57 (dd, *J* = 6.0, 15.6 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.9, 173.6, 173.1, 172.7, 170.6, 170.5, 156.3, 148.8, 144.5, 139.8, 135.6, 135.4, 135.2, 132.2, 131.8, 130.0, 129.9, 129.0, 128.6, 128.1, 127.9, 127.7, 127.6, 127.2, 126.3, 120.6, 119.4, 112.6, 112.4, 111.5, 109.3, 108.9, 80.0, 73.1, 71.3, 70.1, 55.8, 52.6, 49.9, 43.0, 41.4, 36.6, 27.1, 26.2, 25.9; IR (CH_2Cl_2): 3273, 2941, 1712, 1672, 1621, 1576, 1520, 1491, 1395, 1259, 1165, 1112, 1025, 884, 741, 702 cm^{-1}; HRMS [M+H] for $C_{45}H_{49}BrN_7O_8$ calcd., 916.2645 found, 916.2104

Compound B 3.39 (R = TBDPS). $[\alpha]^{25}_D$ = 7.5 (c 0.4, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.03 (d, *J* = 1.8 Hz, 1H), 8.74 (s, 1H), 8.14 (t, *J* = 5.4 Hz, 1H), 8.03 (d, *J* = 8.4 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.66 (d, *J* = 7.2 Hz, 2H), 7.52 (d, *J*

= 7.2 Hz, 2H), 7.44 (m, 5H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.14-7.26 (m, 26H), 7.10 (s, 1H), 4.65 (dd, *J* = 7.8, 15.0 Hz, 1H), 4.51 (dd, *J* = 6.6, 14.4 Hz, 1H), 4.15 (m, 1H), 4.04 (dd, *J* = 4.2, 6.6 Hz, 1H), 3.48 (m, 2H), 3.30 (m, 1H), 3.21 (m, 1H), 3.17 (dd, *J* = 6.6, 14.4 Hz, 1H), 2.80 (dd, *J* = 6.6, 15.0 Hz, 1H), 2.54 (m, 1H), 1.40 (s, 9H), 0.99 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6); δ 172.7, 170.9, 170.2, 169.4, 155.1, 144.8, 135.5, 135.4, 135.0, 132.5, 132.2, 130.0, 129.0, 128.6, 127.8, 127.8, 127.4, 126.3, 125.2, 123.4, 120.6, 113.4, 111.0, 109.9, 79.1, 73.2, 69.2, 64.9, 56.0, 54.9, 52.4, 50.2, 42.2, 41.4, 38.0, 28.0, 26.7, 18.7; IR (CH_2Cl_2); 3273, 3049, 2941, 1713, 1672, 1586, 1520, 1495, 1395, 1259, 1165, 1112, 1025, 884, 749, 702 cm^{-1}; HRMS [M+H] $C_{61}H_{66}BrN_7O_8Si$ for calcd. 1132.3998 found, 1132.4033



Cyclic peptide with side chain 3.49 (R = Tr) and 3.48 (R = H).

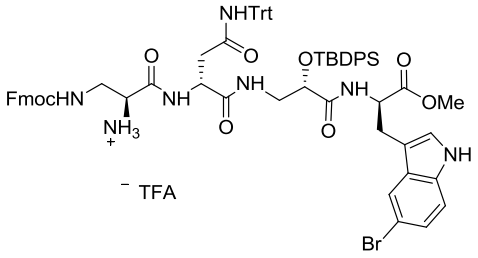
Method A. In a flame dried round bottom flask the above cyclic peptide **3.39** (30.0 mg, 1.0 equiv, 0.026 mmol) was dissolved in CH_2Cl_2 (1.0 mL) and TFA (0.1 mL) and Et_3SiH (0.1 mL) were added to the peptide. The reaction was stirred for 2 h at room temperature after which time the solvent was removed. The crude mixture was dried and used in the next step without further purification. The peptide was then dissolved in DMF and cooled to 0 ^{o}C. To this was added **3.3** [from the corresponding free acid (4.26 mg, 1.0 equiv, 0.026 mmol), HATU (9.91 mg, 1.0 equiv,

0.026 mmol) and HOAt (3.32 mg, 1.0 equiv, 0.026 mmol), allowed to stir 1 h before being added to the cyclic peptide]. To the reaction was added DIPEA (2 μL, 2.0 equiv, 0.052 mmol) and the resulting mixture was further stirred for 30 min at 0 ^{o}C. The reaction was warmed up and allowed to stir for 4 days, after which time it was concentrated. The crude mixture was dissolved in EtOAc and the organic layer with 1.0 M HCl, a saturated solution of sodium bicarbonate, water and a solution of saturated sodium chloride solution. The organic layer was dried with magnesium sulfate and the concentrated to yield a yellow residue. The material was purified by flash column chromatography (5% MeOH in CH_2Cl_2) to yield the products: compound **3.48** as a white solid (21% yield, 5.6 mg) and compound **3.49** as a white solid (51% yield, 17.0 mg).

Compound 3.49, (R = Tr) $[\alpha]^{26}_{D}$ = -25.4 (c 0.37, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.02 (bs, 1H); 8.66 (s, 1H), 8.62 (t, *J* = 4.8 Hz, 1H), 8.50 (bd, *J* = 5.4 Hz, 1H), 8.38 (t, *J* = 5.4 Hz, 1H), 8.12 (bs, 1H), 7.76 (d, *J* = 1.2 Hz, 1H), 7.64 (d, *J* = 7.2 Hz, 1H), 7.37-7.47 (m, 4H), 7.23-7.31 (m, 8H), 7.16-7.19 (m, 11H), 7.08 (s, 1H), 7.03 (d, *J* = 1.8 Hz, 1H), 4.62 (dd, *J* = 7.2, 8.4 Hz, 1H), 4.37 (m, 2H), 4.00 (dd, *J* = 4.2, 4.8 Hz, 1H), 3.81 (s, 3H), 3.79 (d, *J* = 6.0 Hz, 2H), 3.53 (dt, *J* = 7.8, 13.8 Hz, 1H), 3.43 (m, 2H), 3.37 (dd, *J* = 5.4, 10.8 Hz, 1H), 3.42 (dt, *J* = 5.4, 7.2 Hz, 1H), 3.20 (dt, *J* = 4.8, 14.4 Hz, 1H), 3.08 (dd, *J* = 6.0, 13.8 Hz, 1H), 3.01 (dd, *J* = 6.6, 14.4 Hz, 1H), 2.96 (dd, *J* = 7.2, 15.6 Hz, 1H), 2.41 (dd, *J* = 6.0, 15.0 Hz, 1H), 0.97 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.7, 170.8, 170.2, 169.2, 168.9, 168.9, 160.4, 144.8, 144.7, 135.5, 135.3, 135.0, 132.2,

132.0, 130.0, 128.9, 128.6, 127.8, 127.4, 126.2, 125.2, 124.5, 123.4, 120.6, 118.6, 113.4, 111.6, 111.0, 109.6, 107.3, 72.7, 72.4, 69.2, 63.0, 53.5, 52.5, 51.3, 50.3, 42.7, 42.4, 40.7, 37.8, 33.3, 31.2, 28.9, 28.6, 26.4; IR (thin film): 3296, 3054, 2931, 2858, 1736, 1686, 1668, 1533, 1515, 1448, 1264, 1223, 1112, 1050, 1026, 1007, 883, 822, 736, 702 cm^{-1}; HRMS [M+Na] for $C_{64}H_{64}BrCl_2N_9O_8SiNa$ calcd., 1286.3105 found, 1286.3120

Compound 3.48, (R = H) $[\alpha]^{25}_D$ = -21.8 (c 0.21, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6): δ 0.97 (s, 9H), 2.56 (dd, *J* = 4.8, 6.6 Hz, 1H), 2.76 (dd, *J* = 7.8, 15.0 Hz, 1H), 3.11 (dd, *J* = 6.6, 14.4 Hz, 1H), 3.17 (dd, *J* = 3.6, 10.2 Hz, 1H), 3.42 (m, 1H), 3.48 (dt, *J* = 5.4, 13.8 Hz, 1H), 3.81 (m, 2H), 3.84 (s, 3H), 3.86 (dd, *J* = 4.8, 5.4 Hz, 1H), 3.91 (dd, *J* = 6.0, 11.4 Hz, 1H), 3.98 (dd, *J* = 3.6, 4.2 Hz, 1H), 4.35-4.40 (m, 3H), 4.62 (dd, *J* = 7.2, 7.8 Hz, 1H), 6.88 (s, 1H), 6.94 (s, 1H), 7.12 (bs, 1H), 7.17 (s, 1H), 7.18 (m, 3H) 7.29 (m, 2H), 7.37 (m, 3H), 7.42-7.47 (m, 2H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.72 (s, 1H), 8.35 (t, *J* = 5.4 Hz, 1H), 8.40 (d, *J* = 7.2 Hz, 1H), 8.65 (d, *J* = 7.8 Hz, 1H), 8.68 (t, *J* = 6.0 Hz, 1H), 11.01 (bs, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 26.4, 26.6, 28.5, 33.3, 36.2, 40.9, 42.4, 42.6, 50.8, 52.5, 53.8, 63.0, 72.5, 107.3, 109.6, 111.0, 111.7, 113.4, 1118.5, 120.6, 123.4, 124.6, 125.1, 127.8, 127.9, 128.5, 128.9, 130.0, 131.9, 132.2, 134.9, 135.3, 135.4, 160.3, 168.9, 169.1, 170.0, 170.9, 171.8, 172.6; IR (thin film): 3351, 3296, 3054, 2936, 2878, 1736, 1676, 1659, 1533, 1518, 1442, 1264, 1223, 1112, 1050, 1007, 883, 822, 736, 702 cm^{-1}; HRMS [M+Na] for $C_{45}H_{50}BrCl_2N_9O_8SiNa$ calcd. 1021.2112 found 1021.2247

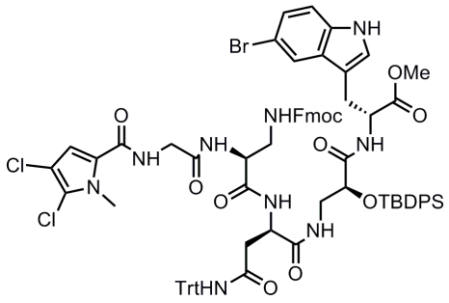


H-Dap(Fmoc)-*R*-Asn(Tr)-*S*-Ise(TBDPS)-*R*-Trp(5-Br)-OMe (3.51)

In a flamed-dried round bottom flask the tetrapeptide **3.27** (100.0 mg, 0.078 mmol, 1.0 equiv) was dissolved in dichlrormethane (5.0 mL). Trifloroacetic acid (0.3 mL) and $HSiEt_3$ (0.3 mL) was added and the reaction was stirred for 1 h and the solvent was removed *in vacuo* and dried under reduced pressure to give a yellow residue (80% yield, 74.8 mg).

$[\alpha]^{25}_D$ = -3.2 (c 1.4 , MeOH); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.05 (bd, *J* = 2.4 Hz, 1H), 8.65 (s, 1H), 8.25 (bd, *J* = 6.0 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 2H), 7.81 (d, *J* = 7.2 Hz, 1H), 7.69 (d, *J* = 7.8 Hz, 2H), 7.61 (d, *J* = 2.4 Hz, 1H), 7.56 (dd, *J* = 1.2, 7.8 Hz, 2H), 7.53 (t, *J* = 6.0 Hz, 2H), 7.48 (dd, *J* = 1.2, 7.8 Hz, 1H), 7.39-7.42 (m, 5H), 7.29-7.36 (m, 9H), 7.22-7.25 (m, 9H), 7.15-7.19 (m, 15H), 7.04 (d, *J* = 1.8, 1H), 4.50 (q, *J* = 7.2 Hz, 2H), 4.31 (d, *J* = 7.2 Hz, 2H), 4.20 (t, *J* = 6.6 Hz, 1H), 4.13 (t, *J* = 5.4 Hz, 1H), 3.52 (s, 3H), 3.38 (m, 2H), 3.27 (dd, *J* = 9.0, 4.2 Hz, 1H), 3.17 (m, 1H), 3.16 (dd, *J* = 5.4, 13.8 Hz, 1H), 3.08 (m, 1H), 3.04 (t, *J* = 7.8 Hz, 1H), 3.01 (dd, *J* = 7.2, 10.8 Hz, 1H), 2.97 (dd, *J* = 6.0, 9.0 Hz, 1H), 2.71 (dd, *J* = 8.4, 15.0 Hz, 1H), 2.56 (dd, *J* = 4.8, 15.0 Hz, 1H), 0.94 (s, 9H), ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.9, 171.9, 170.9, 170.7, 169.3, 156.5, 144.7, 140.7, 143.9, 135.4, 135.3, 134.9, 132.5, 132.3, 129.9, 128.9, 128.8, 128.5, 128.2, 127.7, 127.6, 127.4, 127.0, 126.3, 125.4, 125.2, 123.4, 121.4, 120.3, 120.1, 120.0,

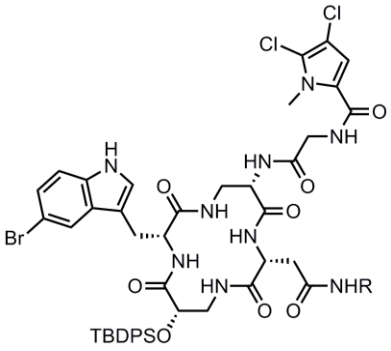
113.5, 111.1, 108.7, 72.1, 69.3, 65.3, 54.9, 52.5, 51.9, 49.8, 46.6, 44.8, 42.9, 26.7, 26.4; IR (thin film): 3296, 3054, 2931, 2858, 1736, 1686, 1668, 1533, 1515, 1448, 1264, 1223, 1112, 1050, 1026, 1007, 883, 822, 736, 702 cm^{-1}; HRMS [M+H] for $C_{72}H_{73}BrN_7O_9Si$ calcd., 1285.4344 found, 1285.4



Linear hexapeptide (3.53)

The dipeptide **3.3** (5.4 mg, 1.0 equiv, 0.022 mmol) was dissolved in DMF (1.0 mL) and cooled to 0 ^{o}C. To the dipeptide HATU (9.1 mg, 1.1 equiv, 0.023 mmol) and HOAt (3.23 mg, 1.1 equiv, 0.023 mmol) and the reaction was stirred for 30 min. To the reaction the tetrapeptide **3.52** (30.0 mg, 1.0 equiv, 0.022 mmol) dissolved in DMF (0.5mL) was added followed by DIPEA (0.007 mL, 2.0 equiv, 0.047 mmol) then it was allowed to stir for an additional 20 minutes at 0^{o}C. The reaction was warmed to room temperature and stirred for 3 days. The solvent was removed and the residue was dissolved in EtOAc and washed with 1.0 N HCl, saturated sodium bicarbonate, water and a saturated solution of sodium chloride. The organic layer was dried over magnesium sulfate and removed to give the crude material as an orange residue. The residue was purified by flash column chromatography using 5% methanol in dichloromethane as an eluent to give the pure material as a white solid (64% yield, 23 mg).

^{1}H NMR (600 MHz, DMSO-d_6): δ 11.02 (bs, 1H), 8.61 (s, 1H), 8.45 (t, *J* = 5.4, Hz, 1H), 8.20 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.78 (d, *J* = 7.2, 1H), 7.62 (d, *J* = 7.2, 2H), 7.59 (d, *J* = 1.2 Hz, 1H), 7.55 (m, 3H), 7.48 (d, *J* =7.2, 2H), 7.13-7.42 (m, 29H), 7.02 (d, *J* = 2.4 Hz, 1H), 6.98 (s, 1H), 5.32 (t, *J* = 4.8, 1H), 4.55 (dd, *J* = 7.8, 13.8 Hz, 1H), 4.43 (m, 1H), 4.18 (dd, *J* = 3.6, 7.8 Hz, 1H), 4.15 (dd, *J* = 5.4, 7.2 Hz, 1H), 3.79 (dd, *J* = 4.2, 4.8 Hz, 1H), 3.74 (s, 3H), 3.49 (s, 3H), 3.38,(dd, *J* = 7.8, 15.0 Hz, 1H), 3.24 (dd, *J* = 7.8, 14.4 Hz, 1H), 3.13 (dd, *J* = 7.2, 12.6 Hz, 1H), 3.02 (dd, *J* = 6.0, 8.4 Hz, 1H), 2.64 (m, 1H), 2.45 (m, 1H), 0.94 (s, 9H), ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.9, 170.8, 170.6, 169.2, 169.1, 160.2, 154.0, 144.7, 143.8, 140.7, 135.2, 132.5, 132.4, 129.9, 128.5, 127.7, 127.6, 127.0, 126.3, 125.2, 123.4, 120.3, 120.1, 113.5, 111.2, 111.1, 108.7, 72.1, 69.3, 65.6, 63.2, 53.2, 52.5, 51.8, 49.8, 46.5, 33.2, 28.9, 28.6, 26.4; IR (CH_2Cl_2): 3309, 3019, 1734, 1648, 1612, 1532, 1453, 1369, 1215, 1159, 777, 669 cm^{-1}

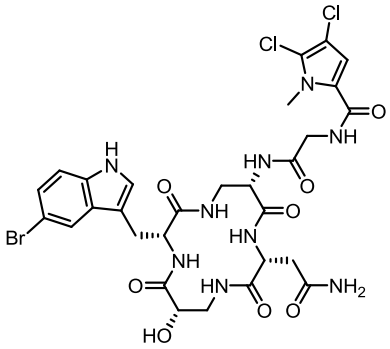


Cyclic peptide with side chain 3.49 (R = Tr) and 3.48 (R = H).

Method B. In a flame-dried round bottom flask **3.27** (20.0 mg, 1.0 equiv, 0.015 mmol) was dissolved in THF (1.2 mL) and diethylamine (0.1 mL, 65.7 equiv, 0.98 mmol) was added slowly. The reaction was stirred for 3 h, after which time the solvent was removed and the residue was washed with toluene then dried under vacuum. The crude material was dissolved in MeOH (0.15 mL) and THF (0.75 mL) and

NaOH (1N, 0.015 mL, 1.0 equiv, 0.015 mmol) was added slowly and allowed to stir at room temperature for 24 h. Water (1.5 mL) and EtOAc (5.0 mL) was added followed by acidification to pH = 3 with 1.0 N HCl. The aqueous layer was extracted with EtOAc. The organic layer was washed with water and a saturated solution of sodium chloride and was dried over magnesium sulfate, followed by removal of the solvent to give the crude material as a yellow residue. Cyclization: In flame dried round bottom flask the above linear peptide **3.54** (13.0 mg, 1.0 equiv, 0.012 mmol) was dissolved in DMF (1.6 mL) and added to CH_2Cl_2 (8.3 mL). The solution was cooled to 0 ^{o}C and DEPBT (8.16 mg, 2.0 equiv, 0.027 mmol) was added. The solution was stirred for 30 min after which time DIPEA (0.01 mL, 10.0 equiv, 0.054 mmol) was added and stirred for an additional 30 min. The reaction was warmed to room temperature and stirred for 3 days. The solvent was removed and the resulting residue was dissolved in EtOAc and washed with 1.0 M HCl, a saturated solution of sodium bicarbonate, water and a solution of saturated sodium chloride. The organic layer was dried with magnesium sulfate and the concentrated to yield a yellow residue. The crude material was purified by flash column chromatography with a gradient 0-5% MeOH in CH_2Cl_2, which yielded a mixture of compounds **3.48** and **3.49**. The two compounds were then separated by flash column chromatography using 80-100% EtOAc in Hexanes, followed by flushing the column with EtOAc, followed by the gradient 0-10% MeOH in EtOAc. Compound **3.49** was isolated as a

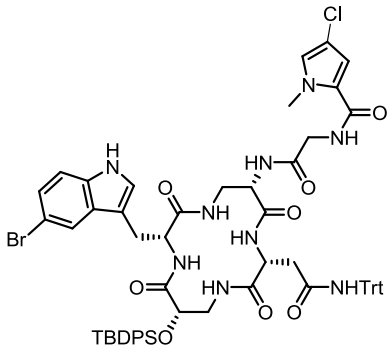
white solid (10% yield, 2.6 mg) and compound **3.48** was a white solid (29% yield, 2.0 mg). Analytical data matched that described in method A.



Nominal Cyclocinamide B 3.1

Method A. Compound **3.49** from the above reaction (6.0 mg, 1.0 equiv, 4.74 μmol) was dissolved in a 50% TFA in CH_2Cl_2 (2.0 mL). The reaction was left open to the atmosphere and stirred for 2 h and the solvent was removed. At this time the TBDPS group was still present. The crude material was dissolved in a solvent mixture of THF (0.9 mL) and MeOH (0.3 mL). To the reaction NaOH (2.0 M, 0.1 mL) was added and the reaction was allowed to stir for 1 h. Ethyl Acetate was added, followed by the addition of water. The resulting mixture was neutralized by the addition of 1.0 M HCl until the pH reached 4. The aqueous layer was extracted with EtOAc. The organic layer was washed with water and a saturated solution of sodium chloride and dried over magnesium sulfate. The organic layer was then concentrated to yield a yellow residue. The residue was dissolved in a mixture of MeOH:CH_3CN:H_2O (3:1:1) and purified by reverse phase HPLC. (2.0 mg, 53% yield).

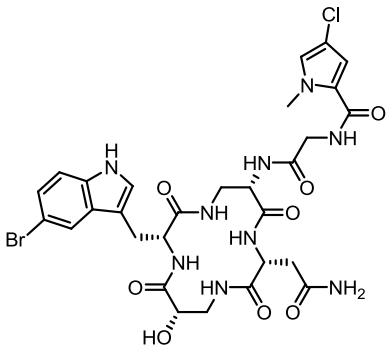
HRMS [M+Na] for $C_{29}H_{32}Br\ Cl_2N_9O_8Na$ calcd., 806.0828 found, 806.0826



cyclic hexapeptide (3.51)

In a flame dried round bottom flask the above cyclic peptide **3.39** (15.0 mg, 1.0 equiv, 0.013 mmol) was dissolved in CH_2Cl_2 (0.5 mL) and TFA (0.05 mL) and Et_3SiH (0.05 mL) were added to the peptide. The reaction was stirred for 4 h at room temperature after which time the solvent was removed. The crude mixture was dried and used in the next step without further purification. The peptide was then dissolved in DMF and cooled to 0 ^oC. To this was added **3.50** [from the corresponding free acid (2.13 mg, 1.0 equiv, 0.013 mmol), HATU (4.96 mg, 1.0 equiv, 0.013 mmol) and HOAt (1.66 mg, 1.0 equiv, 0.013 mmol), allowed to stir 1 h before being added to the cyclic peptide]. To the reaction was added DIPEA (1.0 μL, 2.0 equiv, 0.026 mmol) and the resulting mixture was further stirred for 30 min at 0 ^oC. The reaction was warmed up and allowed to stir for 4 days, after which time it was concentrated. The crude mixture was dissolved in EtOAc and the organic layer with 1.0 M HCl, a saturated solution of sodium bicarbonate, water and a solution of saturated sodium chloride solution. The organic layer was dried with magnesium sulfate and the concentrated to yield a yellow residue. The material was purified by flash column chromatography (5% MeOH in CH_2Cl_2) to yield the products: compound **3.52** as a pale yellow solid (8.46 mg, 52% yield).

$[\alpha]^{27}_{D}$ = -15.1 (c 0.79, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.03 (bs, 1H); 8.50 (d, *J* = 5.4 Hz, 1H), 8.41 (m, 1H), 8.21 (m, 1H), 7.96 (s, 1H), 7.78 (s, 1H), 7.76 (m, 1H), 7.31-7.64 (m, 24H), 7.23-7.31 (m, 7H), 7.16-7.19 (m, 8H), 7.09 (d, *J* = 1.8 Hz, 1H), 7.03 (s, 1H), 6.96 (s, 1H), 6.96 (s, 1H), 6.86 (m, 1H), 5.87 (m, 1H), 4.89 (m, 1H), 4.71 (m, 2H), 4.00 (t, *J* = 8.4 Hz, 1H), 3.80 (d, *J* = 3.0 Hz, 2H), 3.78 (s, 3H), 3.62 (dt, *J* = 7.8, 13.8 Hz, 1H), 3.55 (m, 2H), 3.15 (m, 1H), 3.09 (m, 2H), 3.04 (dd, *J* = 6.0, 11.4 Hz, 1H), 2.96 (dd, *J* = 9.6, 15.6 Hz, 1H),), 2.69 (m, 1H), 2.44 (m, 1H), 0.97 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.9, 170.3, 169.6, 169.2, 168.6, 160.9, 135.5, 135.3, 134.9, 132.2, 132.1, 130.1, 128.9, 127.9, 127.8, 127.5, 127.4, 125.3, 124.9, 123.4, 120.7, 118.3, 113.4, 111.8, 111.5, 111.0, 109.6, 108.7, 72.7, 59.7, 52.9, 52.6, 50.0, 42.4, 36.3, 35.7, 26.5, 20.7, 18.6, 18.5; IR (thin film): 3296, 3054, 2931, 2858, 1736, 1686, 1668, 1533, 1515, 1448, 1264, 1223, 1112, 1050, 1026, 1007, 883, 822, 736, 702 cm^{-1}; HRMS [M+H] for $C_{64}H_{65}BrClN_9O_8Si$ calcd., 1229.3597 found, 1229.3678



4S,7R,11S,14R cyclocinamide A 3.53

Method B. Compound **3.52** from the above reaction (15.0 mg, 1.0 equiv, 12.2 μmol) was dissolved in a 50% TFA in CH_2Cl_2 (2.0 mL). The reaction was stirred for 2 h and the solvent was removed. At this time the TBDPS group was still present. The crude material was dissolved in 1.0 M TBAF in THF and the reaction was stirred overnight. Ethyl Acetate was added and the reaction was washed with a saturated solution of NH_4Cl. The organic layer was

washed with water and a saturated solution of sodium chloride and dried over magnesium sulfate. The organic layer was then concentrated to yield a yellow residue. The residue was (6.5 mg, 72% yield).

HRMS [M+Na] for $C_{29}H_{32}Br\ Cl_2N_9O_8Na$ calcd., 806.0828 found, 806.0826

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Chapter 4 – Analytical evidence for the absolute stereochemistry of cyclocinamide A and B

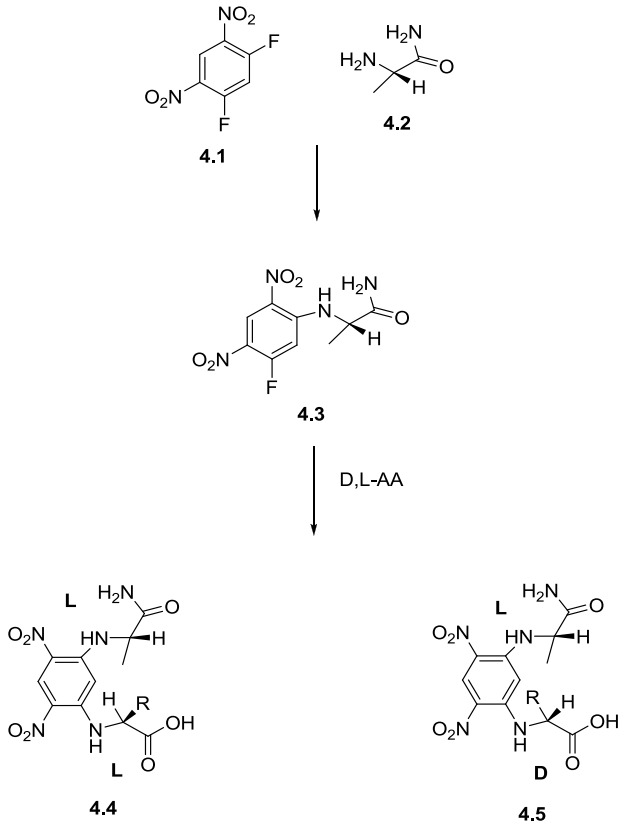
4.1) Introduction

With the successful synthesis of the reported structures for both cyclocinamide A and B, as well as other stereoisomers, our attention turned to the analysis of the cyclic core by Marfey's analysis, rotation and NMR. The analysis of the three stereoisomers synthesized by Dr. Garcia and this author proved critically important to understanding both cyclocinamide A and B. Marfey's analysis was important to determine the success of our syntheses and to provide evidence that epimerization did not take place during the critical cyclization event. This is particularly important for the all *S* isomer because similar systems have been shown to easily epimerize.[1] The rotations were important as a quick tool for analysis of the correct stereochemistry.

The NMR analysis proved to be vital to the determination of the correct identity of cyclocinamide A and B. By analyzing the proton and carbon shifts, the differences between the isomers synthesized can be determined and compared to the natural products. This allowed us to establish which isomer is closest to the natural product.

4.2) Marfey's analysis

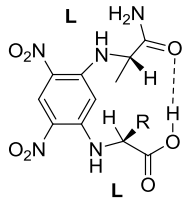
In 1984 Marfey's reagent was developed in order to differentiate enantiomeric amino acids.[2] Marfey was the first to synthesize the reagent FDAA (**4.3**, 1-flouro-2,4-dinitrophenyl-5-L-alaninamide, now known as one of Marfey's reagents). The synthesis of FDAA starts with the reaction of 1,5-diflouro-2,4-dinitrobenzene **4.1** with L-alaninamide **4.2**. The L-alaninamide displaces one of the fluorine atoms to form compound **4.3**, without racemization of the alanine stereocenter. This leaves one fluorine still present which is available for further substitution. Marfey's reagent can then be derivatized with an amino acid which produces a diastereomer referred to as 2,4-dinitrophenyl-5-L-alaninamide amino acid or DNPA-amino acid. As shown in Scheme 4.1 the use of a L,D-alanine mixture produces two products when derivatived with Marfey's reagent.



Scheme 4.1 Synthesis of Marfey's reagent

The two derivatized products of the Marfey's reagent generally have different retention times which allows for separation of the diastereomers. The differences arise from the H-bonding patterns. In the L,L-diastereomer **4.4**, the carboxyl group is located close to the carboxamide of the reagent and forms an intramolecular hydrogen bond shown in Figure 4.1. In the D,L diastereomer **4.5**, the carboxylic acid and carboxamide group have less ability to form hydrogen bonds due to steric interaction between the R group and the alanine methyl group. It was thought that the less symmetrical form of the molecule causes the stronger

interaction of the alkyl side chains in reverse phase HPLC. This increases the retention times, causing the D,L diastereomer to elute later than the L,L-diastereomer in general.[3]



4.4

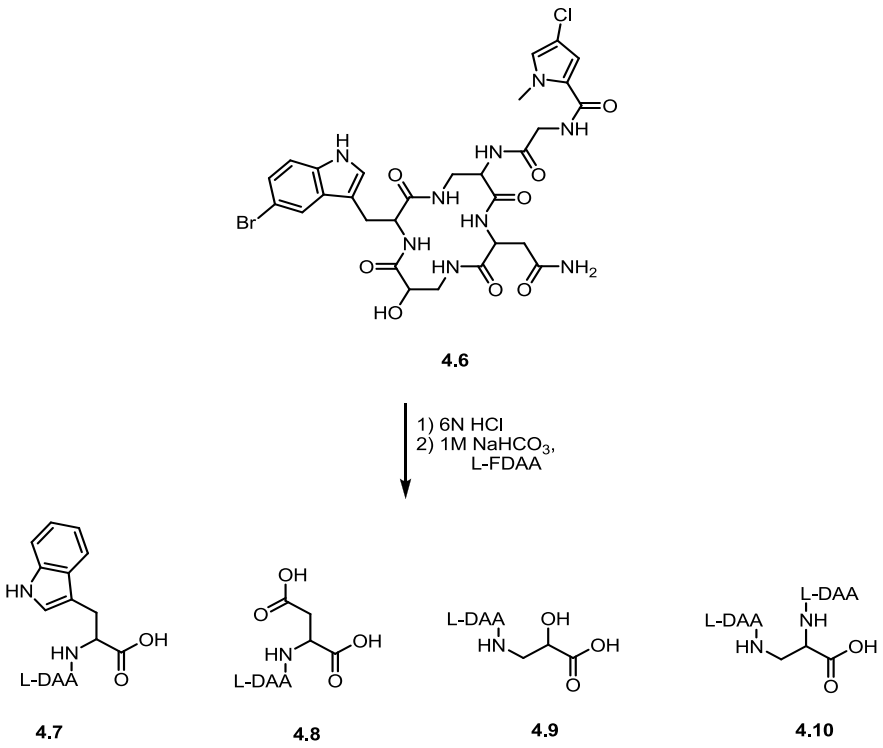
Figure 4.1 Internal Hydrogen bonding in L,L-diastereomer

For compounds that have a relatively small difference in retention times the use of more hydrophobic amino acids in the synthesis of the Marfey's reagent allows for better separation. The most common amino acids derivatives used are valine, luecine, and isoleucine with the most hydrophobic compound having the largest retention times.

There are limitations to this technique. The analysis of amino acids with additional amino groups such as histadine, lysine and ornithine can prove problematic by forming either a mono- or di- derivitized product. This doubles the number of peaks seen in the chromatograph. This problem can be circumvented by the addition of an excess of Marfey's reagent to the reaction, thereby converting all to the double derivitized product and thus eliminating the mixture of compounds. The use of Marfey's analysis for β-amino acids can cause the diastereomers to elute

with very similar retention times or even in reverse order.[3] This can lead to mistaken assignment if a standard sample of both enantiomers is not tested first to establish the retention times.

4.2.1) Previous Marfey's analysis of cyclocinamide A and B



Scheme 4.2 Advanced Marfey's method on cyclocinamide A

When cyclocinamide A was first isolated in 1998, advanced Marfey's analysis was not widely used.[4] The stereochemistry was determined using chiral TLC instead, which permitted the assignment of the two α stereocenters. The β stereocenters were not assigned at the time. The reisolation of cyclocinamide A in 2008 allowed

for the determination of the absolute configuration of the stereocenters by Marfey's technique (Scheme 4.2).[5] The alanine derivitized Marfey's reagent was used in the assignment of *S* for all four stereocenters. However there was some difficulty in the assignment of the isoserine amino acid. The D and L isomers had poor separation in the initial attempt. To circumvent this issue the buffer was changed from 0.1% formic acid to 0.1M NH_4OAc (pH = 6). The order of elution was reversed with the D isomer eluting first compared to the rest of the amino acids where the L isomer was the first eluted. (Figure 4.2)

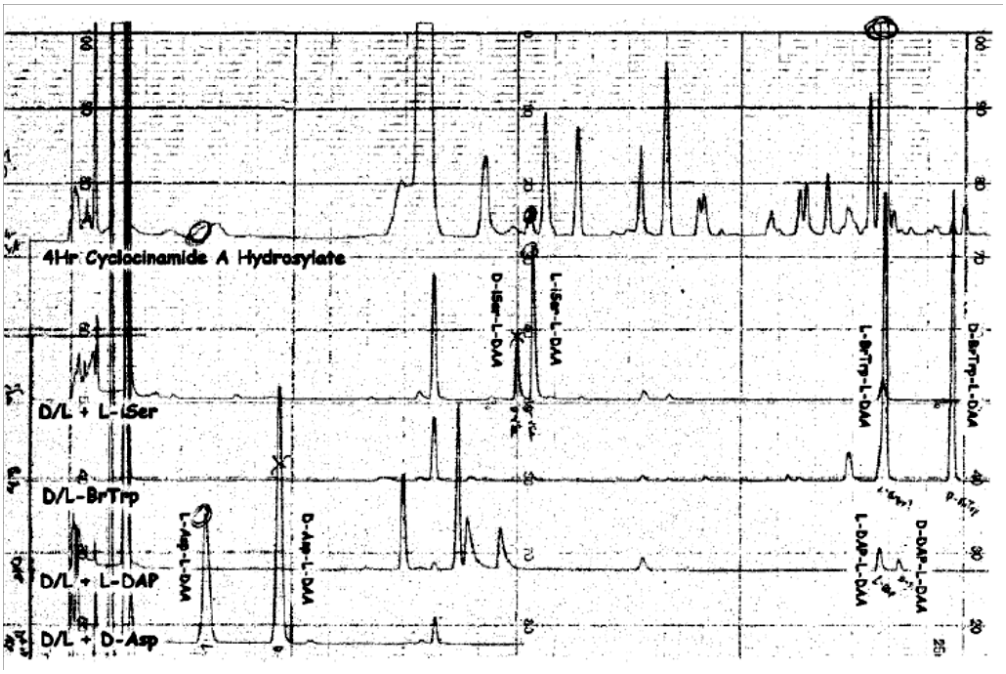


Figure 4.2 HPLC chromatograph from the thesis of Dr. Rubio[4] of Marfey's analysis of natural cyclocinamide A

The absolute stereochemistry of Cyclocinamide B was determined by advanced Marfey's technique as well.[6] Marfey's analysis was undertaken using the

alanine derivative and three of the four amino acids were unambiguously assigned: D-Asp, L-Dap, D-5-Br-Trp. The L-*i*Ser and glycine derivative gave overlapping peaks in HPLC analysis making assignment of the fourth stereocenter not possible using the alanine derived Marfey reagent. To circumvent this problem the more lipophilic L-FDVA-derivatized amino acid [N_α-(2,4-dinitro-5-fluorophenyl)-L-valinamide] was employed. The result showed an enhancement of the L-*i*Ser over D-*i*Ser confirming the alternating stereochemistry as 4*S*,7*R*,11*S*,14*R*-cyclocinamide B.

4.2.2) Marfey's analysis of synthetic cyclocinamide A and B

With the completion of the syntheses of the all *S* and 4*S*,7*S*,11*R*,14*S* isomers conformation of the stereochemistry was needed.[7] Marfey's analysis was performed on the all *S* isomer which allowed us to establish if any of the stereocenters were epimerized during the synthesis. The analysis was carried out using the same conditions as the L-FDAA for the reisolated cyclocinamide A. When the alanine derivatived Marfey's reagent was used, it caused ambiguity in the isoserine fragment. The two diastereomers were extremely close and difficult to distinguish. To overcome the lack of separation the more lipophilic leucine derived Marfey's reagent was used. With the retention times of the standards determined (Table 4.1), analysis of the all *S* cyclocinamide A was undertaken. The same hydrolysis procedure that was outlined for the natural product gave the LC/MS chromatographs shown in

Figure 4.3.[8] The results showed that the cyclo(Asn) chemistry allowed for the closure of the all *S* 14-membered ring without racemization of any of the stereocenters. Due to a limitation of material the 4*S*,7*S*,11*R*,14*S* isomer was not analyzed by Marfey's. It was inferred that if no racemization took place with the all *S* system, which is more difficult to cyclize, then there should not be any racemization occurring in the 11*R* isomer.

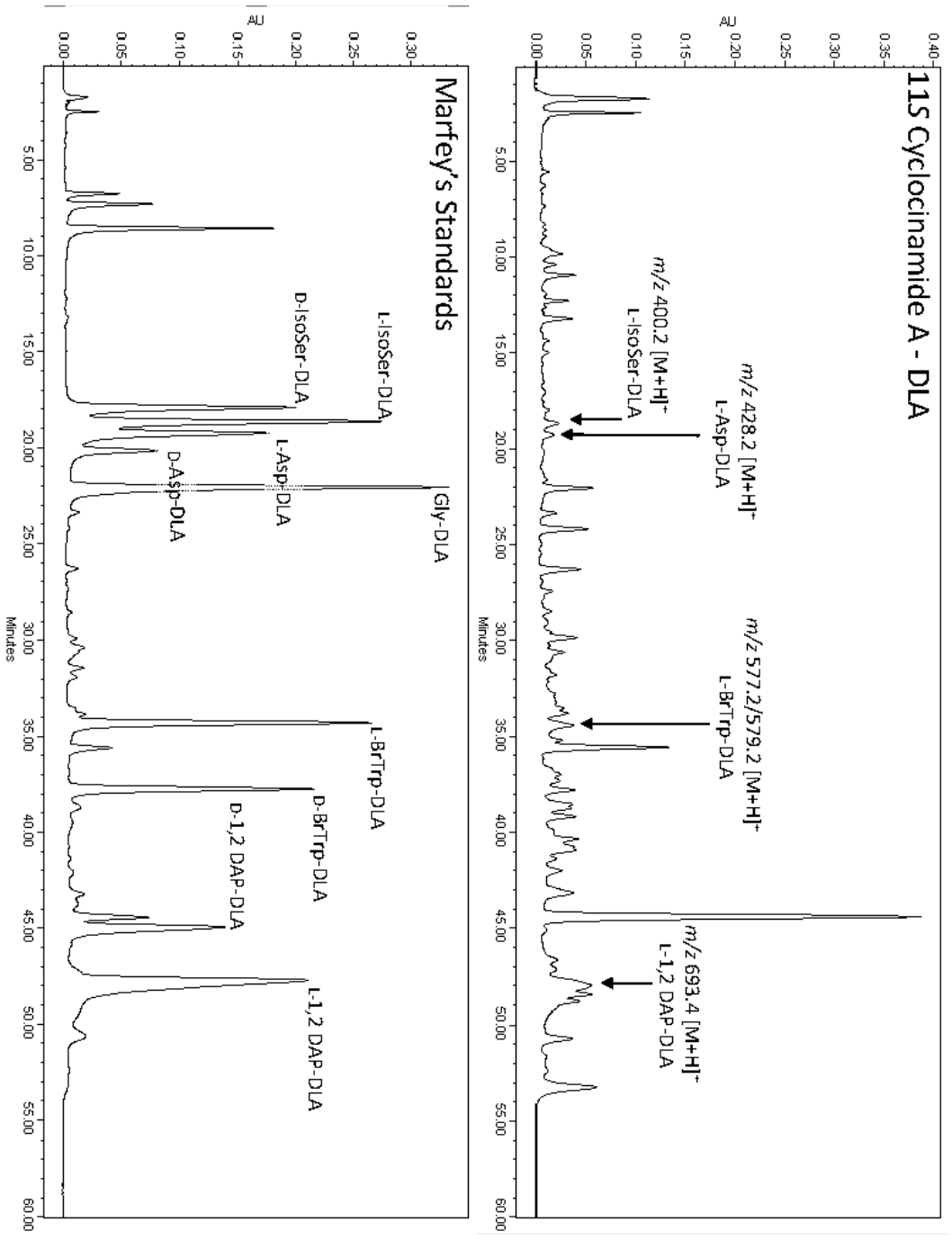


Figure 4.3 Stacked LC/MS chromatograms of L-DLA derivatives of 11*S*-cyclocinamide A and standard derivatives.[8]

Amino Acid Standards	RT(min)	m/z [M+H]+
D-*i*Ser-DLA	17.6	400.2
L-*i*Ser-DLA	18.3	400.2
L-Asp-DLA	18.7	428.2
D-Asp-DLA	19.8	428.2
L-5-Br-Trp-DLA	33.9	577.2/579.2
L-5-Br-Trp-DLA	37.4	577.2/579.2
D-Dap-DLA	43.9	639.4
L-Dap-DLA	45.7	639.4

Table 4.1 Retention times and masses for the standards of the Marfey's reagent

The Marfey analysis was carried out on the synthetic 4*S*,7*R*,11*S*,14*R* cyclocinamide B core since the synthetic path differed from that of the all *S* and 4*S*,7S,11*R*,14*S* isomers. By using the same conditions as those employed for the all *S* isomer it was found that the synthetic cyclocinamide B core did not undergo racemization during the cyclization event. These results demonstrate that the alternating stereochemistry allows for cyclization without a turn inducer present as opposed to the cyclocinamide A core for which a turn inducer was required.

4.3) Comparison of the rotations

Conformation that the stereocenters did not epimerize led us to the next step in the determination of the correct stereochemistry of cyclocinamide A and B. The rotations were examined for all isolations of natural product. The two isolations

of cyclocinamide A from the Crews laboratory are designated Clark for the 1996 isolation and Rubio for the 2008 isolation. Table 4.2 demonstrates that the rotations for the two compounds are very similar at +29 and +32 respectively. The rotation of Cyclocinamide B (Ireland) was much smaller than either cyclocinamide A rotation, at +9.6. This data would appear to show that there is a difference in structures between the two natural cyclocinamides.

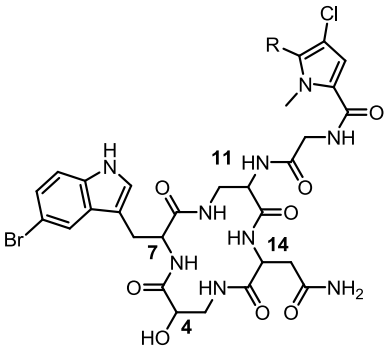
The two previous syntheses of cyclocinamide A were performed between the two isolation of cyclocinamide A. In 1998 Grieco synthesized the alternating stereochemistry as show in Table 4.2; however there was no reported rotation data.[9] In a 2002 thesis from the Postema group the successful synthesis of the 4*R*,7*S*,11*S*,14*S* stereoisomer of cyclocinamide A was reported.[10] However there was very little analytical data for the compound and no rotation reported. The lack of rotation data for the two compounds is disappointing and makes a full comparison more difficult.

The syntheses of the last two isomers of cyclocinamide A were performed in our laboratory. The all *S* isomer, designated Garcia 1 in Table 4.2, had a rotation of the +14.2, which is smaller than either cyclocinamide A isolation. It is in between the rotations of cyclocinamide A and B.

The last rotations examined are the Curzon compounds, which include the nominal cyclocinamide B structure and the enantiomer of the isomer synthesized by Grieco. This allowed us to obtain the rotation for a third isomer of cyclocinamide A.

The rotations for compounds Curzon A and B established that the addition of a chlorine atom on the side chain pyrrole does not affect the rotation substantially. The rotations for Curzon A and B are within a few degrees of each other indicating that the same stereocenters are present. However they have the opposite sign to both natural products.

The results found that the rotations for both natural products are different than the synthetic isomers. The rotation for both isolations of cyclocinamide A is around +30 but the rotation for the synthetic all *S* isomer is +14.2 which is much smaller. The same can be seen with the cyclocinamide B isomer. The rotation for the natural isomer is +9.6 while both synthetic isomers of the alternating stereochemistry are negative.



Compound	Source	Configuration					[α] (in MeOH)
		R=	4	7	11	14	
Clark	*Psammocinia sp.*	H	*S*	--	*S*	--	29
Rubio	*Psammocinia aff.*	H	*S*	*S*	*S*	*S*	32
Ireland	*Corticium sp.*	Cl	*S*	*R*	*S*	*R*	9.6
Grieco	synthetic	H	*R*	*S*	*R*	*S*	--
Postema	synthetic	H	*R*	*S*	*S*	*S*	--
Garcia 11*S*	synthetic	H	*S*	*S*	*S*	*S*	14.2
Garcia 11*R*	synthetic	H	*S*	*S*	*R*	*S*	--
Curzon B	synthetic	Cl	*S*	*R*	*S*	*R*	-15.9
Curzon A	synthetic	H	*S*	*R*	*S*	*R*	-12.5

Table 4.2 Rotations for all the isolations and syntheses of cyclocinamide A and B

4.4) NMR comparisons

A complete comparison of the natural material and the three isomers synthesized in our laboratory will give further insight into the actual structure of the natural product. Due to the similarities between all isomers careful analysis of the proton, carbon and 2D NMRs became necessary. The initially isolated material (Clark) and the reisolated material (Rubio) showed that they had identical ^{1}H and ^{13}C

NMR spectra. For the purpose of comparison to the natural cyclocinamide A these two samples are used interchangeably.

A comparison of both natural cyclocinamide A and B was the starting point since they were reported to have different stereochemistry. This was then followed by comparing the natural products to the three synthetic isomers prepared in this laboratory: [4*S*,7*S*,11*S*,14*S* (Garcia 11S), 4*S*,7*S*,11*R*,14*S* (Garcia 11R), 4*S*,7*R*,11*S*,14*R* (mono Cl-Curzon A and di Cl-Curzon B)]. (Figure 4.4)

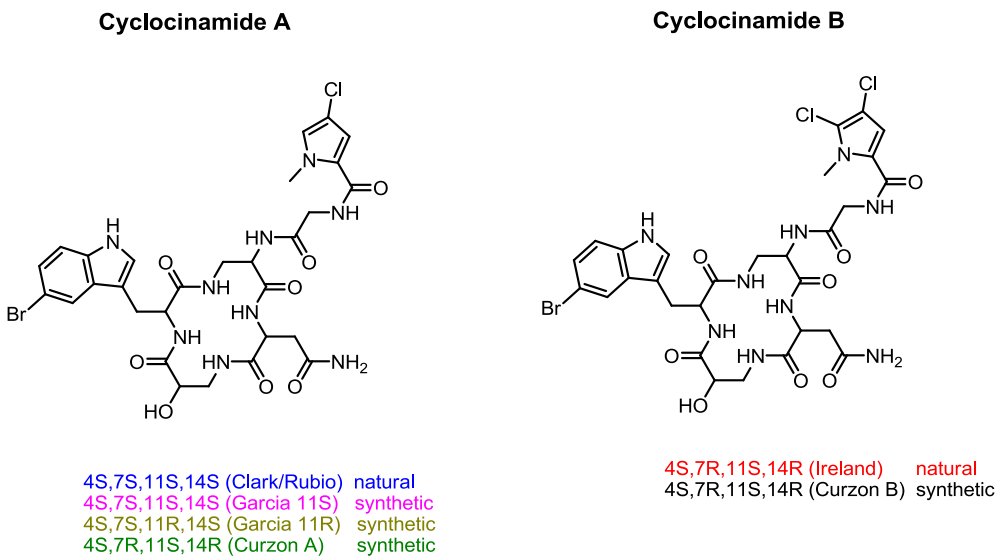


Figure 4.4 Isomers of Cyclocinamide A and B

The previous isomers 4*R*,7*S*,11*R*,14*S* (Grieco) and 4*R*,7*S*,11*S*,14*S* (Postema) were lacking complete analytical data with only ^{1}H NMR spectra in deuterated methanol obtained. The natural product spectra were obtained in DMSO-d_6 so a direct comparison is difficult to obtain.

4.4.1) Comparison of cyclocinamide A to cyclocinamide B

A close examination of the NMR data became vital, since the previous syntheses of two isomers of cyclocinamide A claimed to not be the same as the natural product and cyclocinamide B was claimed to have a unique stereochemical array. The initially reported data for cyclocinamide A and B suggest that the two are closely related but slightly different (Table 4.3). The proton shifts were close with an average difference between all the ring hydrogens of 0.06 ppm. A striking difference was in the ^{13}C NMR where all the carbons showed a difference of 0.6-0.7 ppm. This consistent difference warranted closer look at the actual spectra of both compounds.

The original Clark FID data was not available for analysis but the information from the paper and subsequent table therein was used.[4] The reisolated cyclocinamide A from Rubio was used to examine the physical spectra because the FID files were available.[5] After the original files were obtained from both the Crews group 2008 isolation (cyclocinamide A) and the Ireland group (cyclocinamide B), the

reason for the differences was discovered. Upon closer examination it was revealed that the Ireland NMR spectra were not referenced to the same standard as the Crews material.

Position		δ ^{1}H natural (Clark)	δ ^{13}C natural (Clark)	δ ^{1}H natural (Ireland)	δ ^{13}C natural (Ireland)	Δ δ ^{1}H (Clark/ Ireland)	Δ δ ^{13}C (Clark/ Ireland)
C=O	1		170.5		171.1		-0.6
N-H	2	7.14		7.05		0.09	0.0
C-H	3	3.5	42.8	3.48	43.5	0.02	-0.7
C-H	3'	3.36		3.35		0.01	
C-H *	4	4.04	69.7	4.04	70.4	0.00	-0.7
C=O	5		170.9		171.6		-0.7
N-H	6	7.90		7.87		0.03	
C-H *	7	4.57	53.3	4.52	54.0	0.05	-0.7
C=O	8		172.7		173.5		-0.8
N-H	9	8.00		7.98		0.02	
C-H	10	3.42	40.3		40.8		-0.5
C-H	10'	3.33		3.34		-0.01	
C-H *	11	4.33	54.3	4.27	55.2	0.06	-0.9
C=O	12		168.8		169.4		-0.6
N-H	13	8.00		7.89		0.11	
C-H *	14	4.57	49.5	4.56	50.0	0.01	-0.5
C-H	15	2.49	36.5	2.45	37.1	0.04	-0.6
C-H	15'	2.31		2.26		0.05	
C=O	16		171.9		172.6		-0.7
N-H	17	7.28		7.28		0.00	
N-H	17'	6.80		6.8		0.00	
C-H	18	3.03	27.8	2.95	28.5	0.08	-0.7
C-H	18'	2.99					
C=O	19		109.6		110.2		-0.6
C-H	20	7.17	125.3	7.15	126.0	0.02	-0.7
N-H	21	11.10		11.1		0.00	
C-H	22		134.8		135.5		-0.7
C-H	23	7.30	113.4	7.28	114.1	0.02	-0.7
C-H	24	7.17	123.4	7.14	124.1	0.03	-0.7
C-H	25		111.1		111.8		-0.7
C-H	26	7.67	120.7	7.63	121.4	0.04	-0.7
C-H	27		129.1		129.8		-0.7
N-H	28	8.18		8.09		0.09	
C=O	29		169.2		169.7		-0.5
C-H	30	3.84	42.3	3.83	42.9	0.01	-0.6
C-H	30'	3.78		3.75		0.03	
N-H	31	8.42		8.57		-0.15	
C=O	32		160.9		161.0		-0.1
	33		124.9		125.2		-0.3
C-H	34	6.87	111.8	7.02	112.2	-0.15	-0.4
C-Cl	35		108.6		107.9		0.7
C-H	36	7.06	124.9		119.2		5.7
	38	3.77	36.4	3.76	34.1	0.01	2.3
OH				5.97			

Table 4.3 Comparison of the reported NMR data form cyclocinamide A and B (red highlights the ring sp^3 carbon)

When the solvent references were changed to 2.50 ppm for ^{1}H and 39.52 ppm for ^{13}C spectra, there was a dramatic effect in the data for cyclocinamide B. The differences in the proton were much smaller and a dramatic adjustment occurred in the carbon spectra. Initially the peaks were almost all shifted by 0.7 ppm in the ^{13}C spectrum, now the peaks are almost all identical with only a few shifted in ^{1}H and ^{13}C NMRs. The main area that is different now is the side chain portion of the molecule which one would expect when the extra chlorine atom was taken into account. The stacked spectra (Figure 4.5) of cyclocinamide A and B show that nearly all peaks are in the same positions. These changes are also highlighted in Table 4.4, the new cyclocinamide B data along with the cyclocinamide A data revealed that the two compounds are much closer than initially thought. A careful reevaluation of the NMR data obtained from Ireland led to the conclusion that there were incorrect assignments in the published NMR data table and a reexamination of all the Ireland spectra were needed.

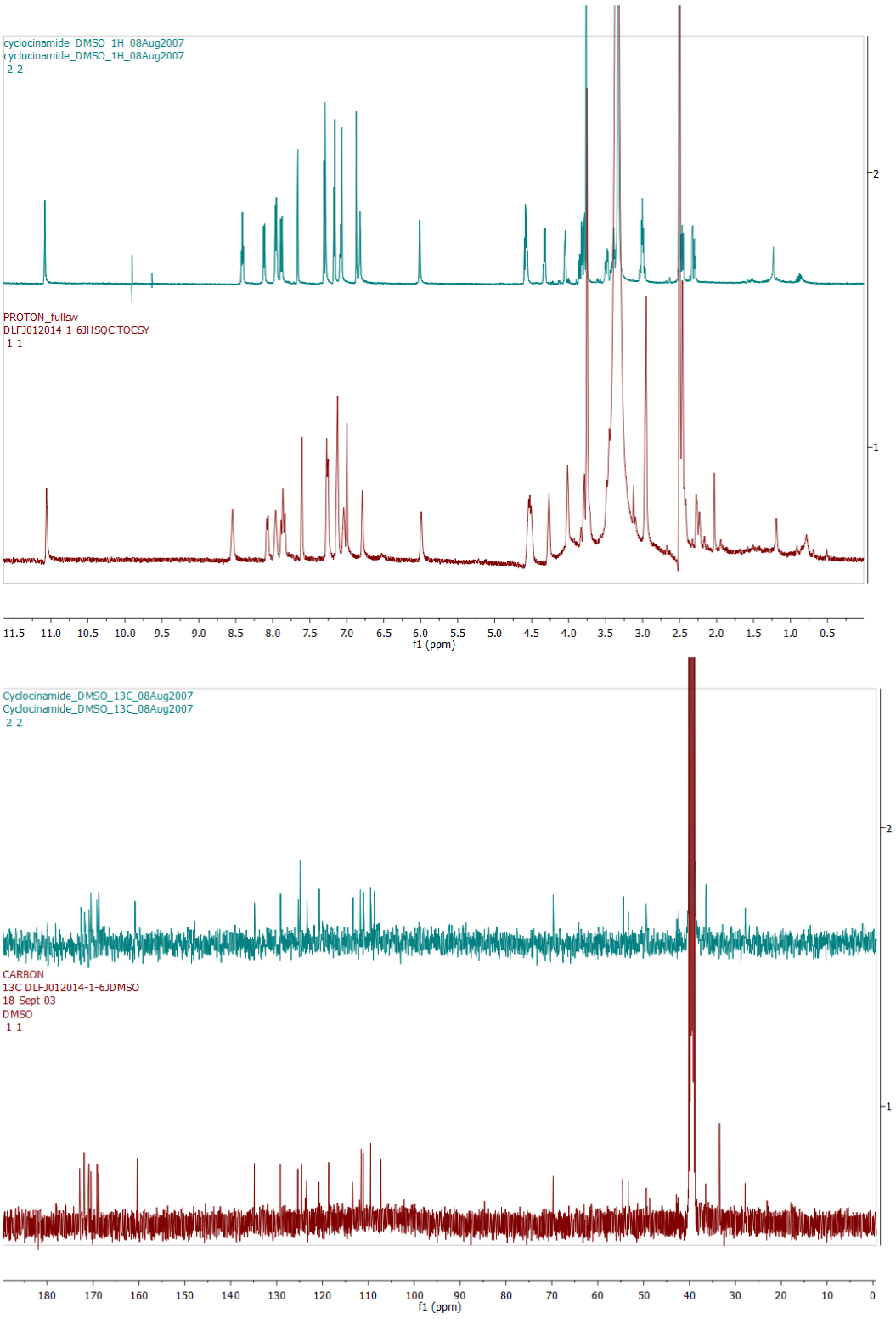


Figure 4.5 Comparison of ^{1}H and ^{13}C spectra for cyclocinamide A (blue) from Rubio and cyclocinamide B (red) from Ireland

Position		δ ^{1}H natural (Clark)	δ ^{13}C natural (Clark)	δ ^{1}H natural (Ireland)	δ ^{13}C natural (Ireland)	Δ δ ^{1}H (Clark/ Ireland)	Δ δ ^{13}C (Clark/ Ireland)
C=O	1		170.5		170.5		0.0
N-H	2	7.14		7.03		0.11	
C-H	3	3.50	42.8	3.46	42.8	0.04	0.0
C-H	3’	3.36		3.31		0.05	
C-H *	4	4.04	69.7	4.00	69.7	0.04	0.0
C=O	5		170.9		170.9		0.0
N-H	6	7.90		7.83		0.07	
C-H *	7	4.57	53.3	4.50	53.3	0.07	0.0
C=O	8		172.7		172.9		-0.2
N-H	9	8.00		7.94		0.06	
C-H	10	3.42	40.3		40.8		-0.5
C-H	10’	3.33		3.31		0.02	
C-H *	11	4.33	54.3	4.25	54.6	0.08	-0.3
C=O	12		168.8		168.8		0.0
N-H	13	8.00		7.86		0.14	
C-H *	14	4.57	49.5	4.52	49.4	0.05	0.1
C-H	15	2.49	36.5	2.41	36.5	0.08	0.0
C-H	15’	2.31		2.23		0.08	
C=O	16		171.9		171.9		0.0
N-H	17	7.28		7.23		0.05	
N-H	17'	6.80		6.77		0.03	
C-H	18	3.03	27.8	2.93	27.9	0.10	-0.1
C-H	18’	2.99					
C=O	19		109.6		109.5		0.1
C-H	20	7.17	125.3	7.11	125.4	0.06	-0.1
N-H	21	11.10		11.04		0.06	
C-H	22		134.8		134.8		0.0
C-H	23	7.30	113.4	7.25	113.4	0.05	0.0
C-H	24	7.17	123.4	7.10	123.4	0.07	0.0
C-H	25		111.1		111.1		0.0
C-H	26	7.67	120.7	7.59	120.7	0.08	0.0
C-H	27		129.1		129.4		-0.3
N-H	28	8.18		8.06		0.12	
C=O	29		169.2		169.1		0.1
C-H	30	3.84	42.3	3.80	42.5	0.04	-0.2
C-H	30’	3.78		3.80		-0.02	
N-H	31	8.42		8.52		-0.10	
C=O	32		160.9		160.4		0.5
	33		124.9		124.5		0.4
C-H	34	6.87	111.8	6.98	111.6	-0.11	0.2
C-Cl	35		108.6		107.3		1.3
C-H	36	7.06	124.9	C-Cl	118.6		6.3
	38	3.77	36.4	3.73	33.4	0.04	3.0
OH				5.97			

Table 4.4 Comparison of the revised Ireland (cyclocinamide B) data to cyclocinamide A (red highlights the ring sp^3 carbon)

A comparison of the proton and carbon sp^3 hybridized C-H shifts of the cyclic cores (see highlighted numbers in Table 4.4) of cyclocinamide A and B is illustrated in the graphs of Figure 4.6. In the comparison of the carbon shifts all except for three of the six carbons have identical shifts and the largest difference is 0.5 ppm which occurs at the C10 position. These comparisons show how close both natural products are to each other.

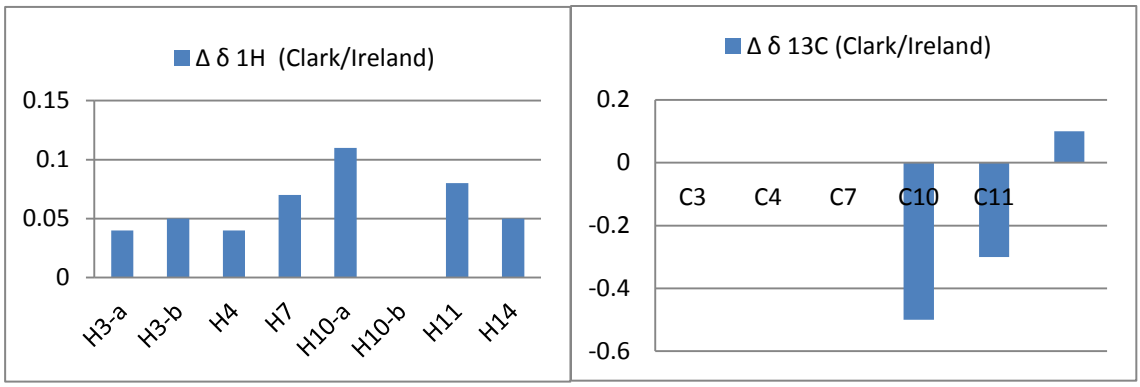


Figure 4.6 Differences in the proton and carbon spectra of cyclocinamide A and B

With this new discovery a careful comparison (in subsequent sections) of the COSY, HMBC, and HSQC showed the two compounds had the same correlations. This adds to the evidence that the initially assigned stereochemistry for the two natural products is erroneous for one or both compounds. In addition, it seems reasonable based on the close similarity of the ^{1}H and ^{13}C spectra of the two natural products, that the arrays of stereocenters in each are the same. Such a conclusion would be

suspected based on a biosynthetic basis, arguing that such similar structures would likely arise from the same biosynthetic machinery and possess identical stereochemistry. It also shows the importance of a careful examination of the three isomers synthesized in our laboratory in order to establish the correct stereochemistry of the natural products.

An accurate comparison of the natural material to the three isomers synthesized in our laboratory provided further insight into the actual structure of the natural product. For this purpose the cyclocinamide B data from the Ireland group was used as the standard for the natural product because there were no available data files for the Clark isolation, the only visual spectra obtained were paper copies. Additionally, during the reisolation in 2008 there was no 2D NMR data obtained. This left the only complete set of raw data files available for the natural product from the Ireland isolation. Since the two natural products were so similar the 2D NMRs from the Ireland group were used as the natural product standard for comparison.

4.4.2) Comparison of the ^{1}H and ^{13}C spectra

The detailed comparison commenced with the 1D NMRs: ^{1}H and ^{13}C. For ease of comparison and to keep the differences in structures of the synthetic samples to a minimum, the 4*S*,7*R*,11*S*,14*R* cyclocinamide A isomer (Curzon A) was used unless otherwise stated. A comparison of the proton spectra can be made across all

isomers synthesized, However the ^{13}C was only obtained for the 4*S*,7*R*,11*S*,14*R* isomer. There was insufficient material for the Garcia 11*S* and Garcia 11*R* to obtain 1D carbon spectra. The carbon shifts for the latter two isomers were obtained through the use of 2D NMR data.

There is inherent variation in NMRs based on the sample purity, concentration, pH as well as variation in the machines used to obtain the spectra. Before a full comparison was made the baseline variability needed to be established. The two samples that are used are Curzon A and B since they were made at the same time from the same synthetic pathway and only differ by the chlorination of the pyrrole side chain. The ring centers, proton and carbon, were examined and differences were found between the two compounds. The average difference in the proton spectra between the two Curzon compounds is 0.04 ppm which is slightly lower than the average difference between Clark and Ireland. The largest difference is 0.08 ppm at the C3 position. Since we know that the two compounds are the same based on the synthetic route, the difference observed can be considered the baseline variability of the cyclocinamides. The differences are similar to those found in the natural cyclocinamide A and B, reinforcing the hypothesis that these two natural products posses the same core structure.

The ^{13}C spectra were also examined to determine what the baseline variability was. The sp^3 carbons had an average difference of 0.2 ppm which is comparable to the two natural isomers. The largest difference is 0.4 ppm at C14

position which is comparable to the natural isomers which had a difference of 0.5 ppm. The differences observed with the two natural isomers (Clark and Ireland) and the two Curzon samples are used as the baseline variability. That variability is that the average difference in the proton can be as high as 0.05 ppm with individual protons varying as much as 0.08 ppm. In the carbon spectra the average difference can be as much as 0.2 ppm with individual peaks as much as 0.5 ppm.

An inspection of the ^{1}H NMR found two areas where differences were easily observed; the amide hydrogens and the aliphatic ring C-H. This can be seen in the stacked proton spectra shown in Figure 4.7. The top two natural cyclocinamide B (red) and natural cyclocinamide A (blue), have near identical shifts. Comparing these to Curzon A (green) with the alternating stereochemistry shows that they are very similar. Differences were seen with the stereotopic protons in the region of 4.5 ppm. In the natural samples the two peaks are overlapped and with the synthetic sample they are easily distinguishable. A similar observation was made with the protons at 3.0 ppm which correspond to the C18 protons. These protons are overlapping in the natural samples where they are easily distinguishable in the synthetic sample. A comparison of the isomer Garcia 11*R* (yellow) with the natural product showed a significant shift in the most shielded of the stereotopic protons as well as the same distinction of protons in the 4.5 ppm region. The same shifts are observed with Garcia 11*S* (purple) isomer. The two Garcia compounds show the largest shifts in the observed protons indicating that they are less likely to be the natural isomer.

The spectra for all the isomers are similar and a closer look at the shifts of the ring hydrogens can give a better view of the differences. The sp^3 carbons and hydrogens were chosen for two reasons. First, the amide protons are known to vary based on solvent, concentration and pH and therefore are less diagnostic. Secondly, the sp^3 carbons are known to not vary widely with solvent or concentration. Table 4.5 shows the ring sp^3 protons for both natural products and the three synthetic isomers of cyclocinamide A. The amide protons (not shown) vary in their shifts across all the isomers, even between the natural products as expected. The greatest interest is with the sp^3 protons since the only differences are in the stereogenic centers. It was believed that these protons would be the most diagnostic. In Garcia 11*R* and Garcia 11*S* the stereogenic protons between the isomers show different shifts, which is evident with H4, H7, and H11 in. To a lesser extent H7 in Curzon A shows a different shift, however all the other stereogenic centers show shifts that are very similar to the natural product. It is the diastereotopic hydrogens of Garcia 11*S*, and Garcia 11*R* that show significantly different shifts from the natural product.

The green bars (Garcia 11*S*) on Table 4.5 show two shifts that are over 0.5 ppm difference from the natural isomer for H3 and H10. The purple bars (Garcia 11*R*) shows one diastereotopic proton with a difference of 0.5 ppm from natural cyclocinamide. The difference in shift of Garcia 11*R* and the natural product at C10 is now much smaller when compared to the difference shown by Garcia 11*S* and the natural product.

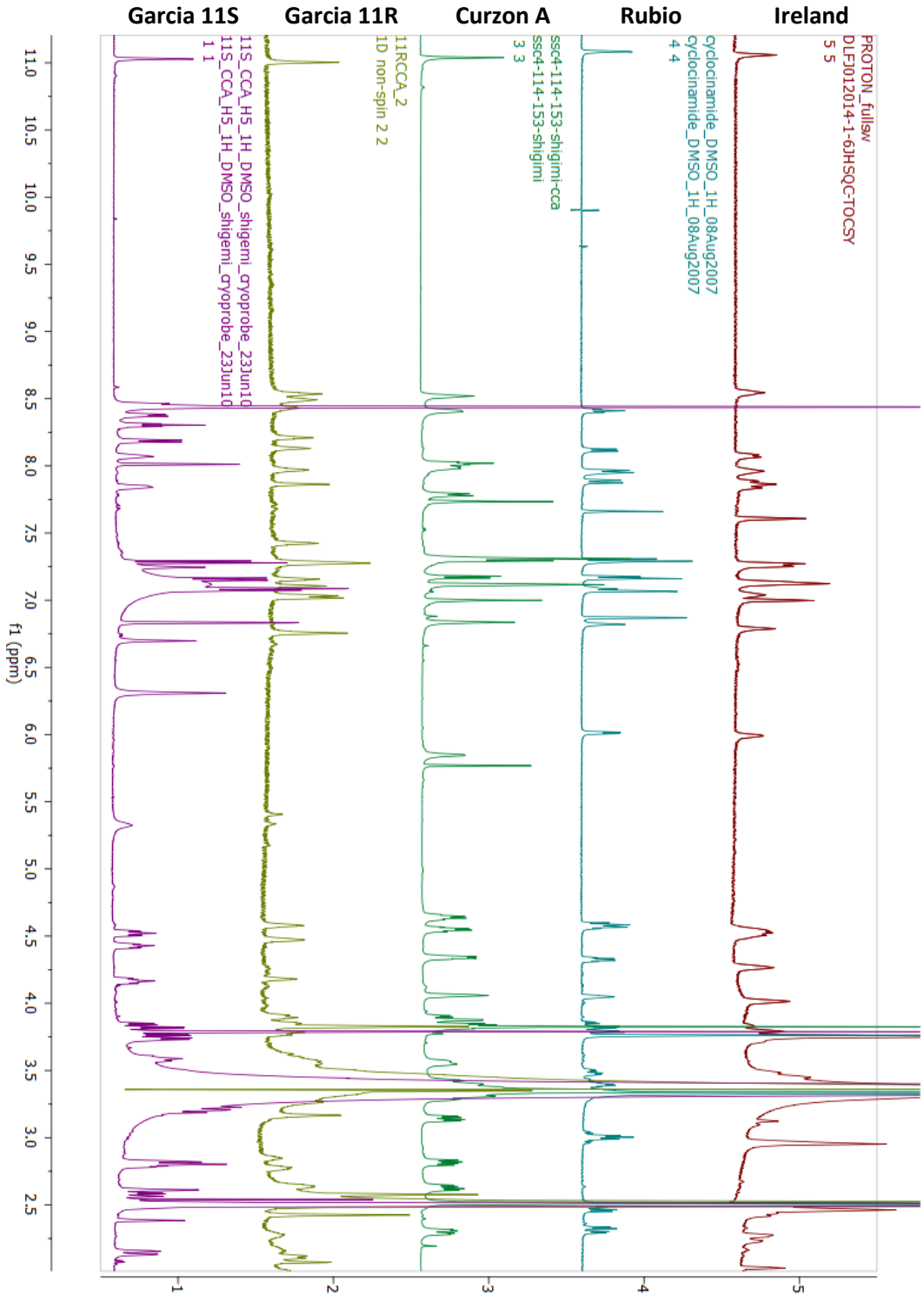


Figure 4.7 Stacked ^{1}H spectra for the five isomers of cyclocinamide A and B

Position		δ ^{1}H natural (Ireland)	δ ^{1}H natural (Clark)	δ ^{1}H synthetic (Curzon A)	δ ^{1}H synthetic (Garcia-11*R*)	δ ^{1}H synthetic (Garcia-11*S*)
C-H	3	3.46	3.50	3.51	3.56	3.59
C-H	3’	3.31	3.36	3.29	2.85	2.81
C-H *	4	4.00	4.04	4.03	3.87	3.75
C-H *	7	4.50	4.57	4.61	4.47	4.43
C-H	10	3.31	3.42	3.36	3.56	3.45
C-H	10’		3.33		3.22	2.81
C-H *	11	4.25	4.33	4.34	4.19	4.17
C-H *	14	4.52	4.57	4.54	4.58	4.52

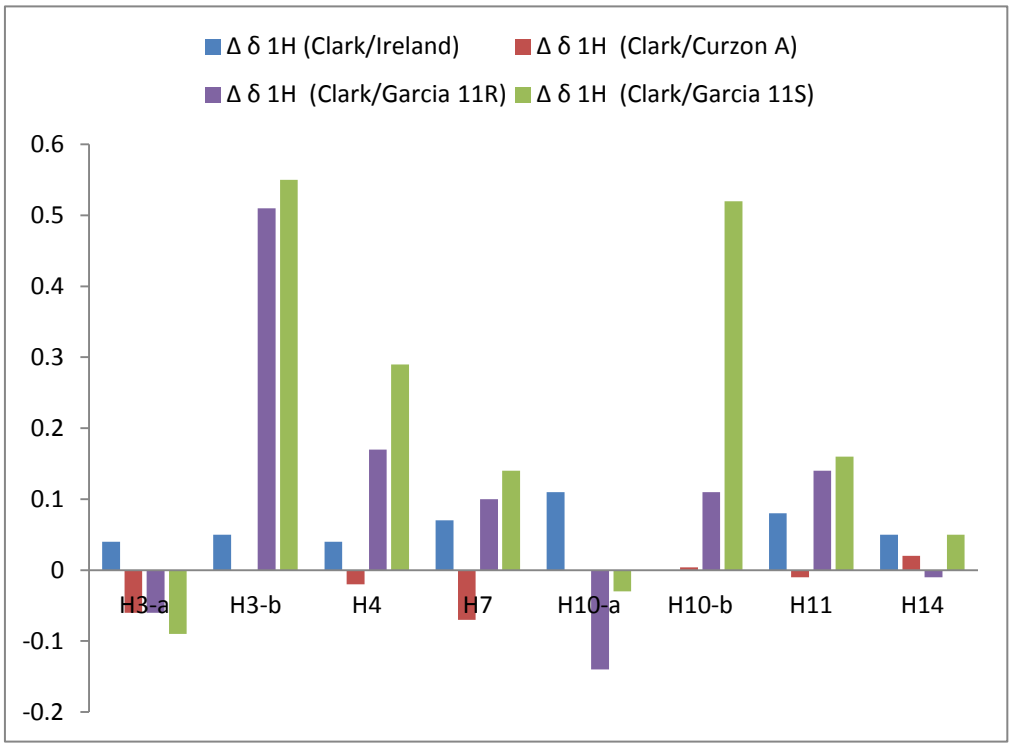


Table 4.5 Ring protons and graph showing the differences between the ring C-H of the natural and synthetic material

It can be surmised from the data in Table 4.5 that the protons with difference of 0.5 ppm or greater are in a very different chemical environment when compared to the natural product. Comparing Garcia 11*R* and 11*S* where the stereochemistry is known and the difference between the two isomers is one stereogenic center can give a clearer indication of the effect stereochemistry has on the chemical shift. The proton shifts for the stereogenic C11 are very similar with both showing just over 0.1 ppm difference to the natural isomer. When the diastereotopic protons at the C10 position for Garcia 11*R* and 11*S* are examined a large difference can be found between the two synthetic samples. One of the diastereotopic protons at C10 shows a difference of 0.41 ppm between the two isomers. All the other shifts of the ring protons were within approximately 0.1 ppm of each other indicating that there is a baseline variability of the shifts. This demonstrates that the difference in stereoisomers can be observed in diastereotopic protons of the ^{1}H spectra. The difference observed in the C10 proton is significantly higher than what appears to be the baseline variability. The proton spectral comparisons indicate that the Garcia 11*S* and 11*R* isomer have significant differences from the natural cyclocinamide and therefore are less likely to be the correct stereochemistry. The alternating stereochemistry of Curzon A appears to be the closest to the natural cyclocinamide.

The next comparison carried out was with the carbon shifts. This proved to be more difficult do to the fact that a 1D ^{13}C spectra was not obtained for the Garcia 11*S* and Garcia 11*R* isomers due to insufficient quantity of material. The quaternary

carbons for Garcia 11*R* were not detectable due to the severely limited quantity of material. A complete analysis across all isomers cannot be made, however the alternating stereochemistry of Curzon A and Garcia 11*S* can be compared to the natural products.

The stacked ^{13}C spectra for both natural cyclocinamide B (red) top and cyclocinamide A (blue) and both 4*S*,7*R*,11*S*,14*R* synthetic isomers Curzon A (green) and Curzon B (purple) are shown in Figure 4.8. Upon first observation of the spectra the shifts look very similar across all spectra. The easily observed differences are the shifts of the methyl groups between the cyclocinamide A and B isomers of the synthetic material. (Figure 4.8)

The ^{13}C shifts for the ring carbons of all five cyclocinamide compounds are shown in Table 4.6. The carbon shifts for the Garcia 11*R* and 11*S* isomers were obtained using 2D NMR techniques. The differences between the Clark and Ireland samples are nearly nonexistent. There are four carbons that are not identical and they are different by less than 0.5 ppm. The differences center on the C10 carbon with 0.5 ppm between the two natural products. There are also differences on either side of the C10 but they are smaller.

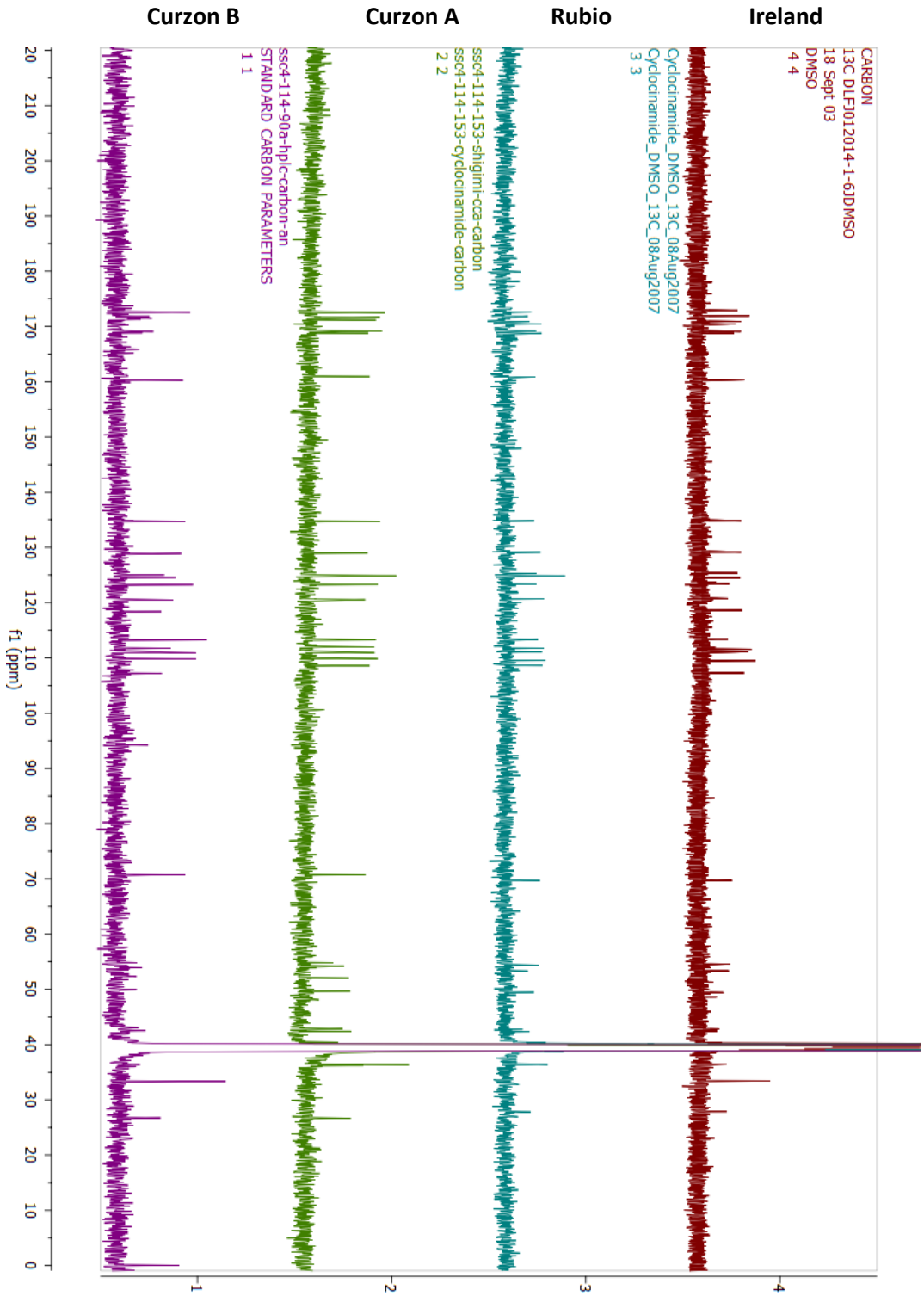


Figure 4.8 Stacked ^{13}C spectra for the isomers of cyclocinamide A and B

Position		δ ^{13}C natural (Ireland)	δ ^{13}C natural (Clark)	δ ^{13}C synthetic (Curzon A)	δ ^{13}C synthetic (Garcia-11*R*)	δ ^{13}C synthetic (Garcia-11*S*)
C=O	1	170.5	170.5	171.4		169.3
N-H	2					
C-H	3	42.8	42.8	42.9	42.3	42.4
C-H *	4	69.7	69.7	70.8	69.1	69.2
C=O	5	170.9	170.9	171.8		172.6
N-H	6					
C-H *	7	53.3	53.3	52.1	53.2	53.1
C=O	8	172.9	172.7	172.7		169.4
N-H	9					
C-H	10	40.8	40.3	40.5	40.4	40.3
C-H *	11	54.6	54.3	54.3	55.5	51.6
C=O	12	168.8	168.8	169.0		169.9
N-H	13					
C-H *	14	49.4	49.5	49.7	49.0	49.0

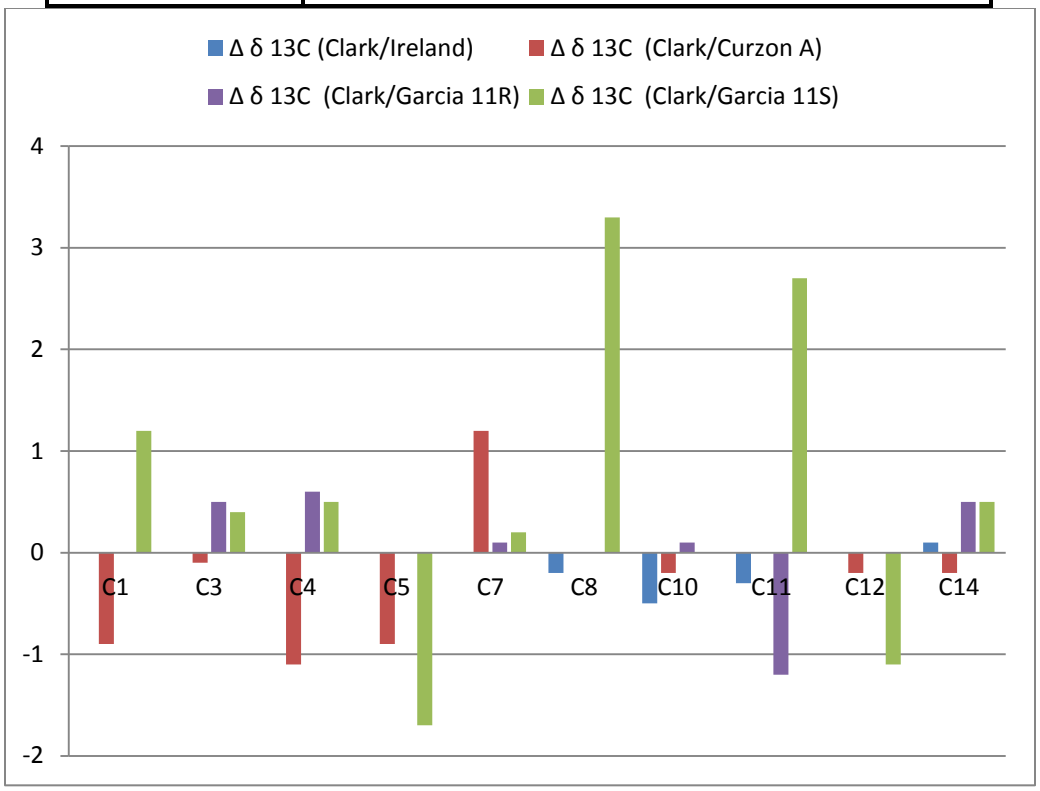


Table 4.6 Ring carbon and graph showing the differences between the ring carbons of the natural and synthetic material

The first sample compared to the natural product is Garcia 11*R* (Table 4.6, purple) and the sp^3 carbons were used for comparison. The C4 and C11 have the most significant difference between the natural product and synthetic material. The C11 shifted is over 1.0 ppm from the natural product. This would indicate that the carbon is in a different chemical environment from the natural product. The average difference between the sp^3 carbons is 0.4 ppm which is higher than the 0.1 ppm difference observed between Clark and Ireland.

The next comparisons were performed between Clark and Garcia 11*S* (green) as can be seen in Table 4.6. All the ring carbons were observed and assigned through the use of the 2D NMRs: HSQC and HMBC. There are two peaks that show large differences at 2.7 ppm and 3.3 ppm for C8 and C11 respectively. A comparison of Garcia 11*S* to 11*R* reveals a difference of 3.9 ppm for C11. It can easily be observed that this isomer is not the natural isomer due to the multiple peaks that are over 1.0 ppm shifted from the natural product. This would indicate that if there is a different stereocenter present there would be an observable in the carbon shifts of the sp^3 carbons.

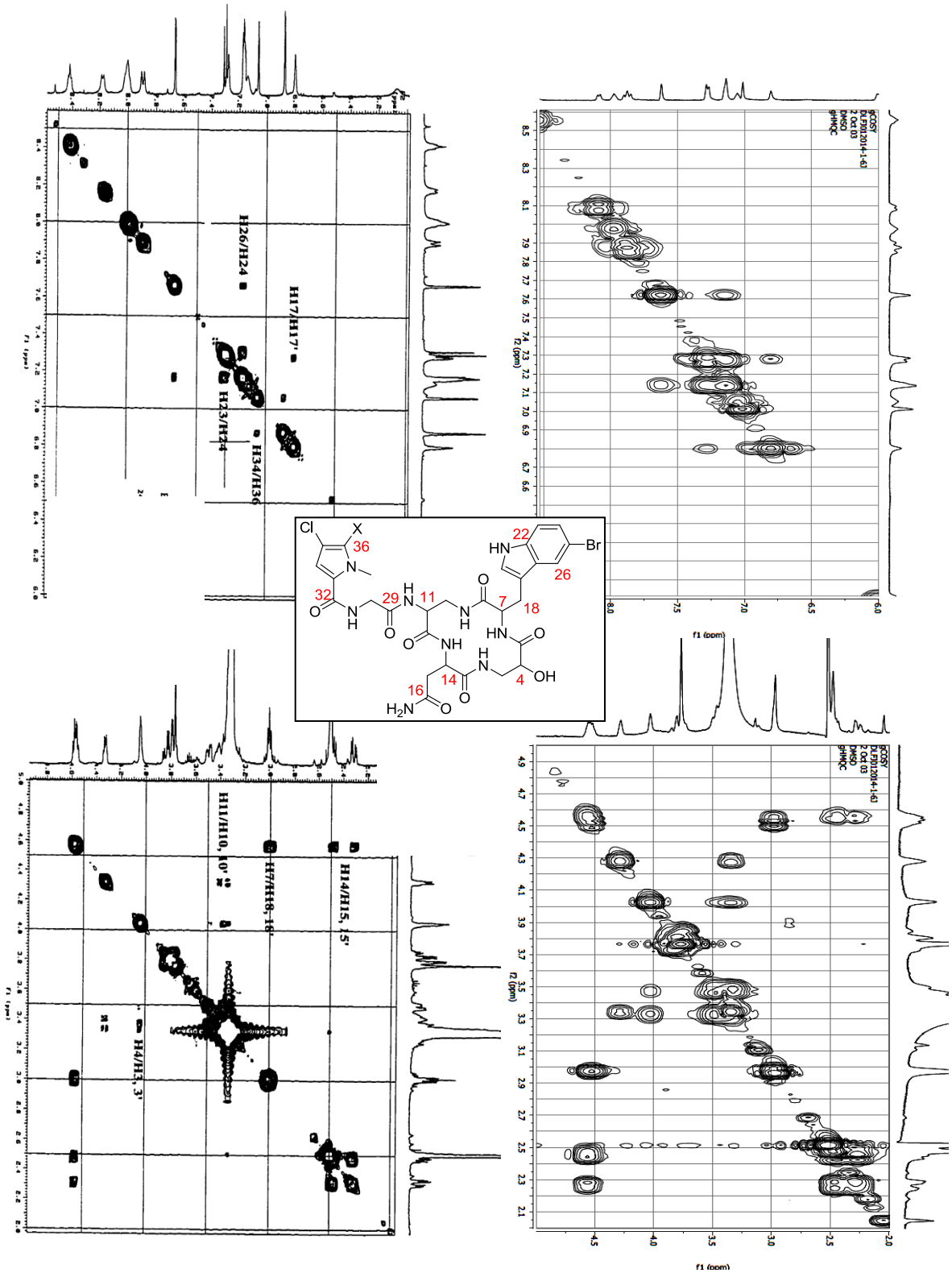
The last synthetic comparison to Clark is with Curzon A (red) and it was found that it is not as closely related to the natural isomer as was seen in the proton comparison. As can be seen from the graph in Table 4.6 the isoserine and tryptophan region of the cyclic core shows the largest difference from the natural product with several carbons that are shifted more than 1.0 ppm from natural

cyclocinamide. This is in contrast to the diaminopropionic acid and asparagine half of the ring which is extremely close to the natural isomer. The overall discrepancy between sp^3 carbons of the natural cyclocinamide A and Curzon A is 0.4 ppm which is the same magnitude as was observed with Garcia 11*R*.

The fact that none of the synthetic isomers closely match the natural isomer in the carbon spectra is very disappointing. The average differences of the sp^3 carbons show that Garcia 11*R* and Curzon A both have an average difference of 0.4 ppm and Garcia 11*S* has the largest average difference at 1.0 ppm. It can be concluded that the 11*S* isomer is not the natural product and that the assignment of cyclocinamide A needs to be reexamined. The assignment made for cyclocinamide B by the Ireland group is still in question because the carbon spectrum is not conclusive. Therefore an examination of the 2D NMR spectra was in order.

4.4.3) Comparison of the COSY spectra

The first of the 2D NMRs examined was the COSY spectra. The COSY is a homonuclear correlation spectroscopy and is used to identify spin systems that are coupled to each other.[11] This allowed for verification of the correct assignment of the protons in all isomers. It also allowed for the differences between the synthetic isomer and the natural products to be seen.


H26/H24
H17/H17'
H23/H24
H34/H36
H11/H10, 10'
H7/H18, 18'
H14/H15, 15'
H4/H3, 3'
f1 (ppm)
f2 (ppm)

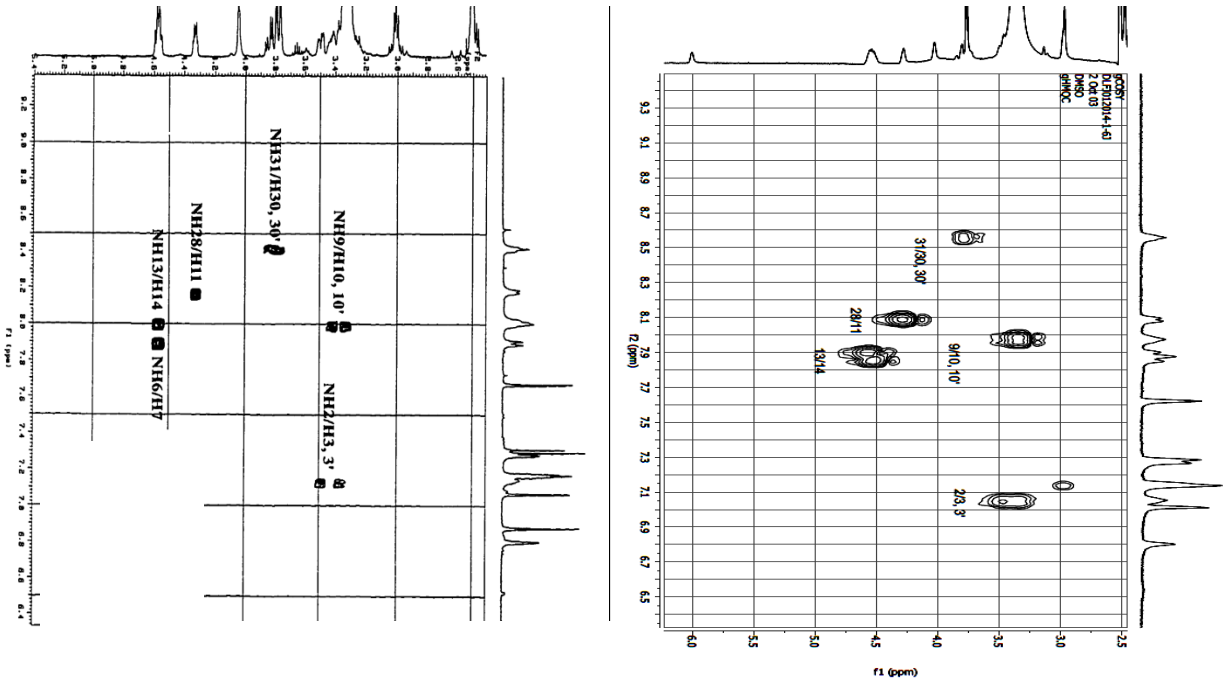


Figure 4.9 COSY of cyclocinamide A (Clark isolation) on previous and current pages left and cyclocinamide B (Ireland isolation) on right

Making a visual comparison of the COSY for cyclocinamide A and B indicates that these are identical in the spectra. Figure 4.9 is the enlargements of three different regions of both natural cyclocinamides and indicates that the two natural products have the same correlations. As can be observed the two spectra appear to be identical. For comparison to the three synthetic isomers the Ireland data was used since its data file exists unlike cyclocinamide A which are reproduction of images from Dr. Clark's thesis.[4]

The next task was to compare the natural cyclocinamide COSY to the three synthetic isomers. The comparison was done on three different regions of the COSY

spectra. The COSY close up on the region 6-9 ppm showed the correlations found in the aromatic systems. As can be seen in Figure 4.10, all the isomers show the same correlations at similar resonances. This indicated that the aromatic rings have the correct chemistry and assignment.

A close-up of the sp^3 region (2-5 ppm) illustrates differences in all the spectra shown in Figure 4.11. The cross peak correlations are the same indicating that the amino acids were correctly identified. A closer look shows that the diastereotopic protons in the natural product do not show significant splitting in the COSY. Most of the peaks appear to have only one correlation even though splitting is actually occurring between both protons with just an overlapped peak. A comparison of the natural product to the three synthetic isomers in this region shows significant differences in the locations of the cross peaks. However, they all have similar correlations indicating that the cores are similar. None of the synthetic samples are an exact match due to the splitting that is occurring in the diastereotopic proton. Curzon A has the most similar correlations because the protons are the closest to the natural product. The other two isomers, Garcia 11*S* and 11*R* had cross peaks in very different locations due to the large differences observed in the proton spectra but still have the same crosspeaks.

The next comparison of the region to be preformed is for the stereotopic protons and nitrogen of the peptide core (Figure 4.12). There is greater similarity between all the correlations shown. Upon more rigorous inspections there is an

extra correlation seen in the synthetic samples. The 6/7 correlations is observed in two synthetic samples Garcia 11*S* and Curzon A, but not in the natural sample or Garcia 11*R*. None of the peaks in these close-ups show a significant amount of overlap and this is due to the fact that amide proton shifts are dependent on the pH and concentration of the solutions and therefore are less indicative to the stereochemistry.

After examining the COSY spectra the most important correlations observed in the COSY are in Figure 4.11 which shows the sp^3 protons. The location of the cross peaks show that the Curzon A isomer is the closest to the natural product. The main difference comes from the diastereotopic protons have distinct splitting while in the natural product does not. Garcia 11*S* has a very different pattern in the cross peaks when compared to the natural product. This would indicate that it is less likely to be the natural isomer. Garcia 11*R* does not have a similar pattern when compared to the natural product. These observations indicate that the alternating stereochemistry of Curzon A is the closest to the natural product. The COSY adds an additional level of confirmation to the observations made in with 1D ^{1}H NMR, indicated that the alternating stereocenters were closest to the observed natural product. The next step is to examine the HBMC because this would indicate carbon correlation since this is where the greatest differences were observed for all synthetic isomers.

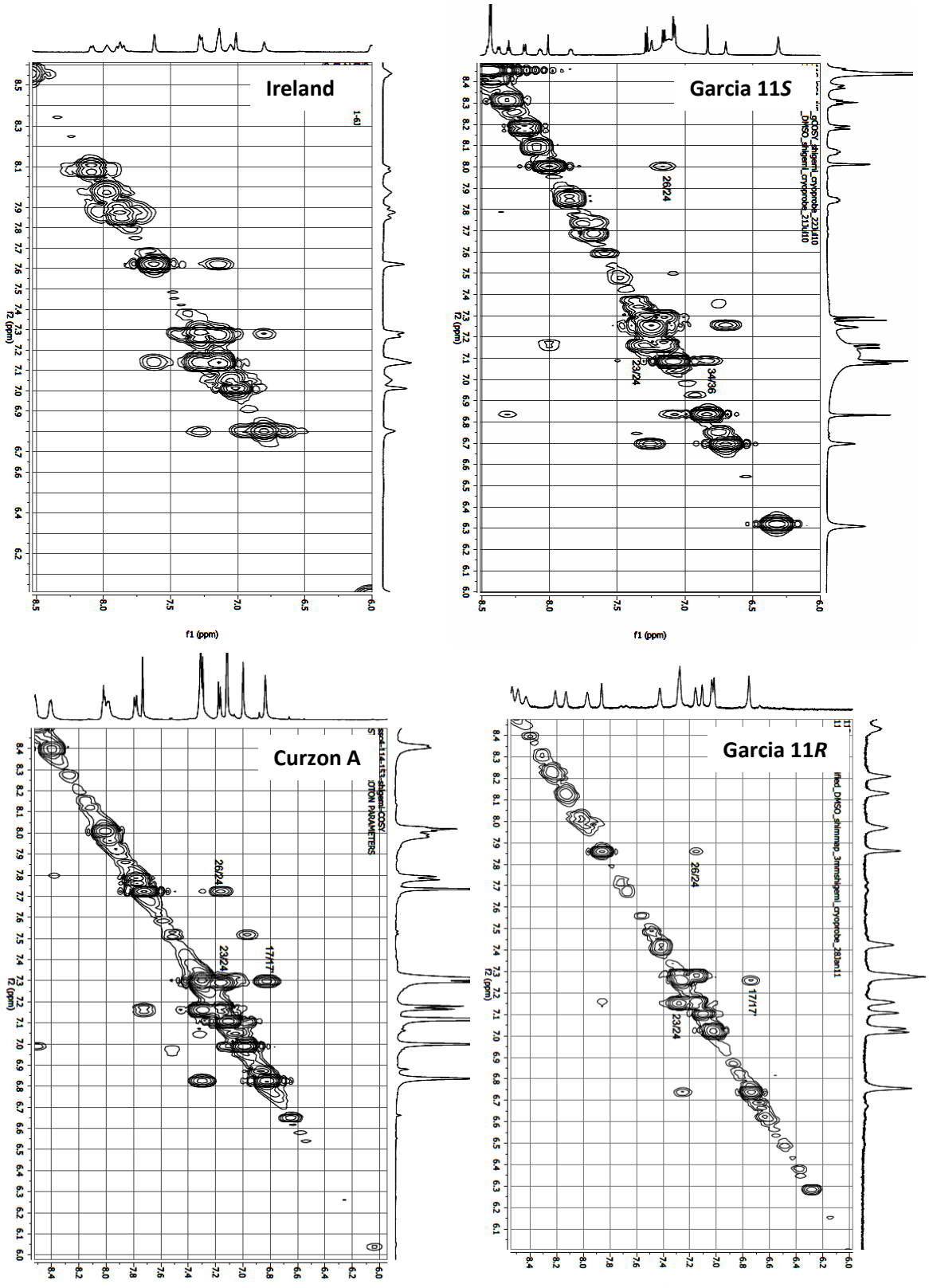


Figure 4.10 Comparison of the ^{1}H-^{1}H COSY spectra for the cyclocinamide isomers

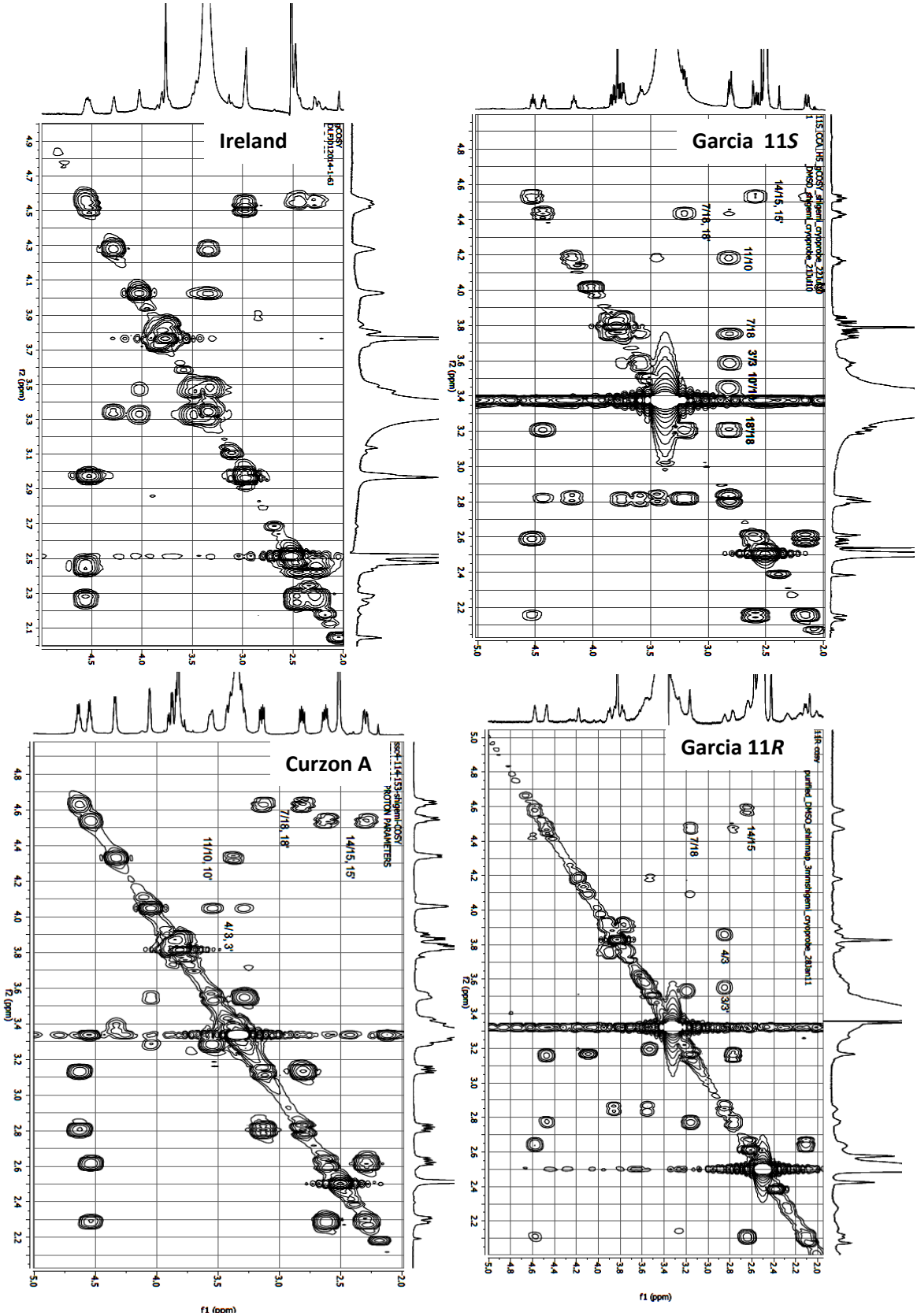


Figure 4.11 ^{1}H-^{1}H COSY sp^{3} proton correlations

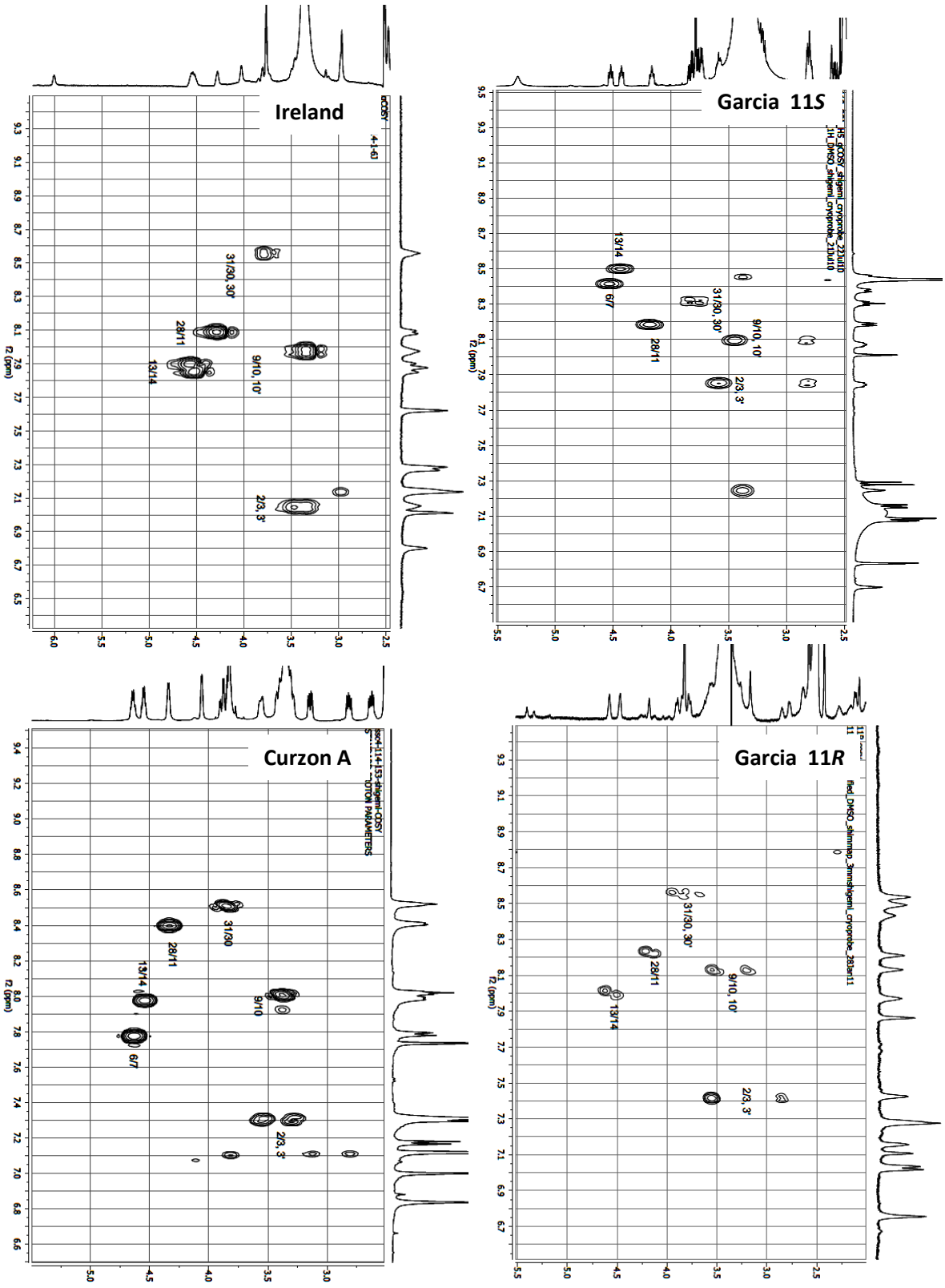


Figure 4.12 ^{1}H-^{1}H COSY sp^{3}-amide proton correlations

4.4.4) HMBC spectra comparison

An important 2D NMR is the HMBC (Heteronuclear Multiple Bond Correlation experiment), this gives information about weak proton-carbon *J*-couplings.[12] A weak proton-carbon *J*-coupling indicates that the proton is 2-4 bonds away from the carbon. This experiment can give an enormous amount of information about molecular structure, including information about which protons are near to (but not directly bonded to) different carbons. The long range proton-carbon correlations can include quaternary carbons, in addition to protonated carbons.

The HMBC for both the natural products in Figure 4.13 show the correlations indicating that the same amino acids were assigned. There are no long range cross peaks that would show any stereochemical differences between the two compounds. The differences observed can be due to the different sensitivities of instrumentation which affects the quality of data collected. Since the only HMBC data for cyclocinamide A (Clark isolation) was printed copies, comparisons of the synthetic isomers were made using the Ireland data.

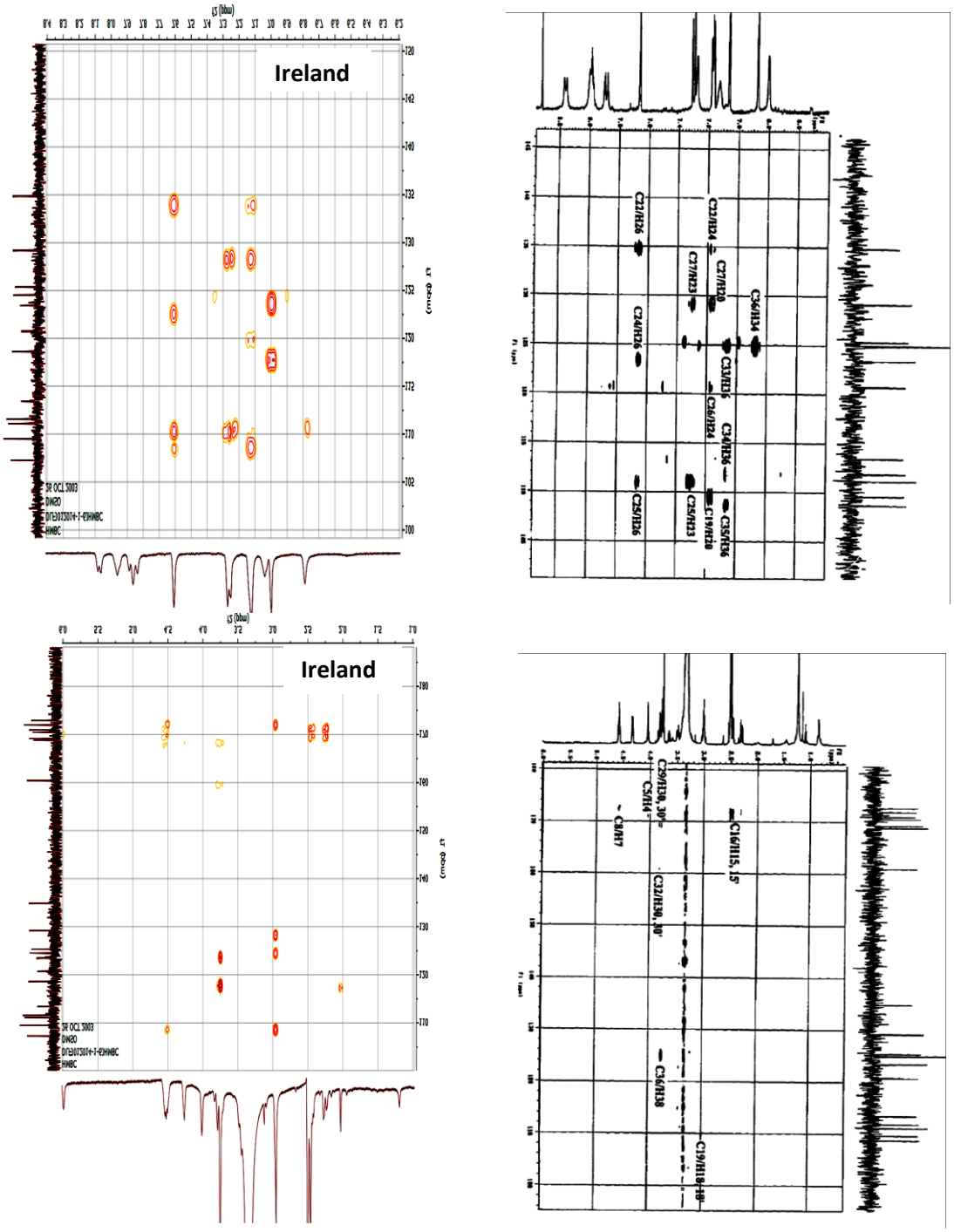


Figure 4.13 Comparison of HMBC for Ireland (left) and Clark (right)

Figure 4.14 shows a close up of the aromatic and amide proton region and the natural product shows multiple correlations. These correlations are seen in both Curzon A and Garcia 11*S* indicating that there is not a noticable difference in the long range correlations. Garcia 11*R* does not show the same peaks due to the fact that there was insufficent sample, which hampered a full comparison of the three synthetic isomers.

The next region examined was the sp^3 ring protons and the carbonyls as shown in Figure 4.15. Again when the comparison was made between the natural isomer and the synthetic Curzon A and Garcia 11*S* the spectra appear to have very similar correlations. Garcia 11*R* again doesn't have any strong correlation due to the dilute sample. With the two synthetic isomers (Curzon A and Garcia 11*S*) having very similar HMBC correlations, it can be concluded that the amino acid connectivity is correct and the sequence in the ring is correct as well. The HMBC comparison did not aid in the verification of the stereochemistry of the natural product.

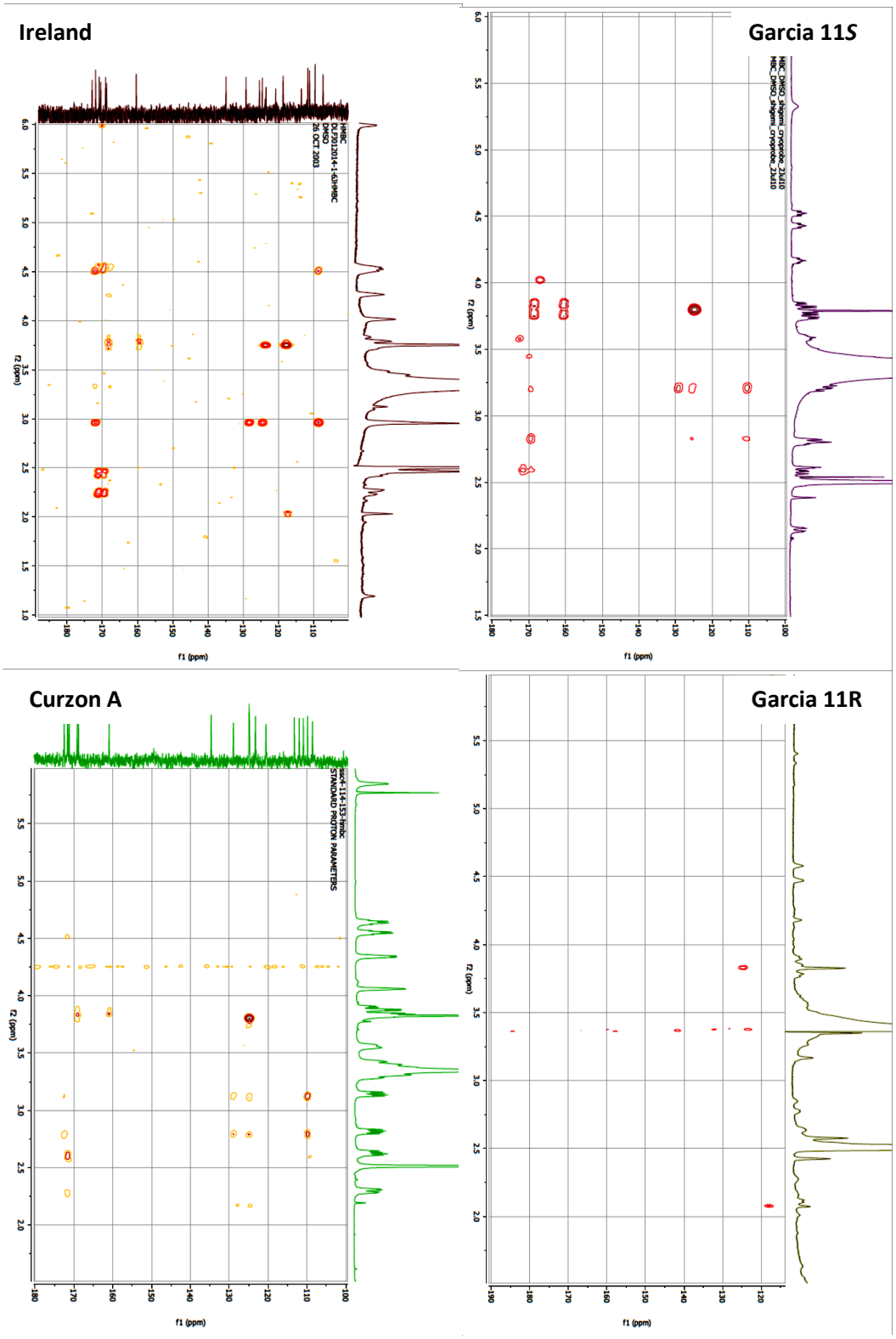


Figure 4.14 HMBC comparison of the natural cyclocinamide and the three isomers

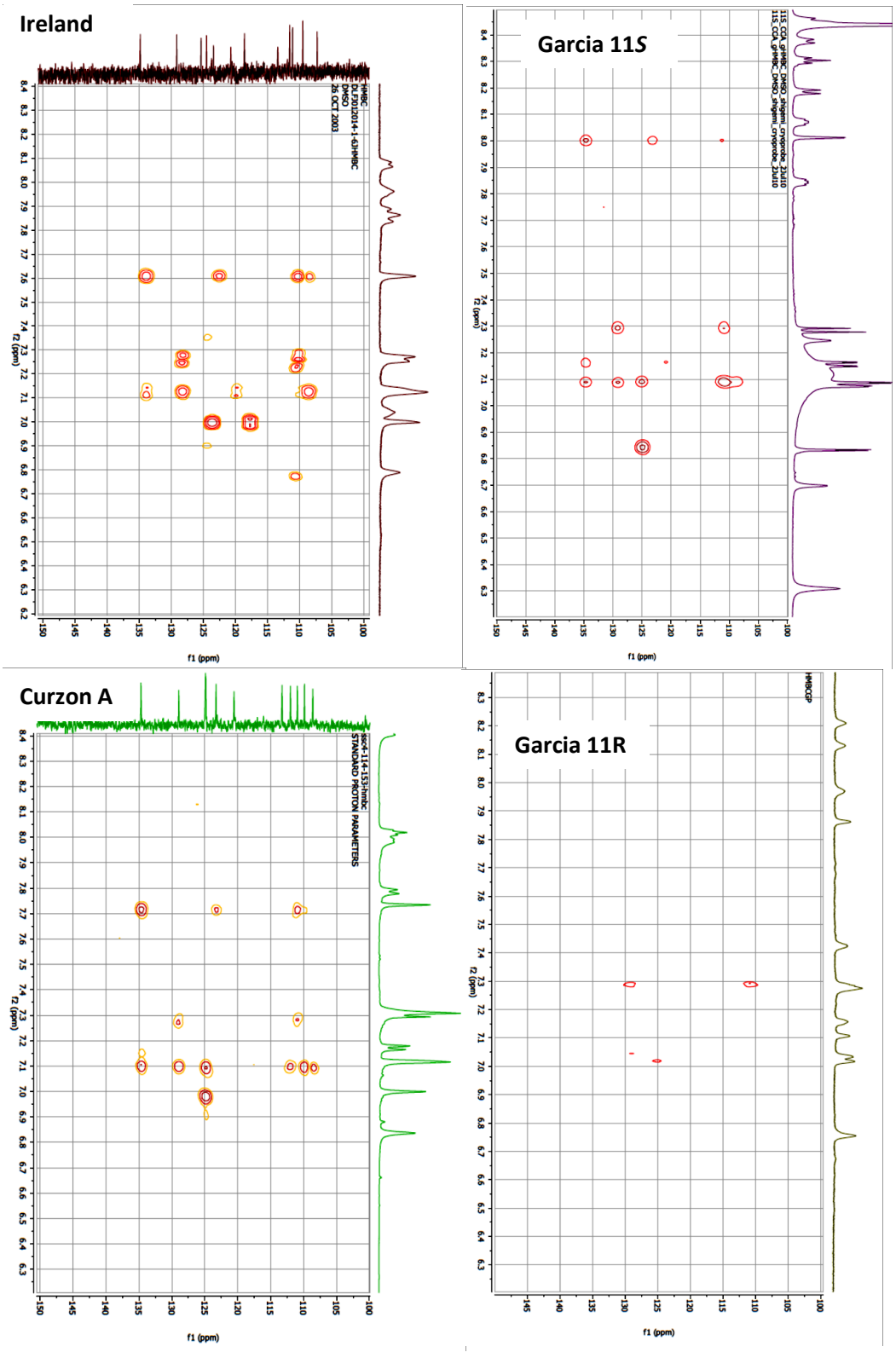


Figure 4.15 HMBC comparison of the natural cyclocinamide and the three isomers

With all the NMR data compared it can be concluded that none of the synthetic samples match up exactly to the natural isomer of cyclocinamide. Based on the proton spectra the alternating stereochemistry of Curzon A is the closest to the natural product. There is a very small average difference in the ring protons. The synthetic samples of Garcia 11*S* and 11*R* show significant shifts of more than 0.5 ppm in at least one proton. When comparing the sp^3 carbons the 11*S* isomer is the furthest from the natural isomers. The synthetic samples Garcia 11*R* and Curzon A both have about the same deviation in the carbon spectra. Due to the inability to determine the quaternary carbons a complete comparison is not possible. Comparison of the 2D NMRs confirmed the structure and sequence of the cyclic peptides.

4.5) Additives present in the sample

The lack of absolute verification of the stereochemistry of cyclocinamide A or B has brought into question the presence of other substances present in the solution that are not visible by NMR. The ability of cyclic peptides to coordinate ions has been documented in a few cases.[13] A notable case involved jaspakinolide which was shown to complex with lithium.[14] A titration of LiBr was observed by NMR with the peaks first broadening then at the end point the peaks again sharpened. The peaks also shifted positions leading to the conclusion that addition of an additive to the solution can affect the shifts of the proton.

The cyclic peptides are analogues to macrocyclic ploye thers where the amide nitrogens are present instead of oxygen. The cyclic polyethers are present in different size rings which coordinate specific cations.[15] A notable example by Gokel involved cyclic polyethers with appendages that add a coordination site to the soft cation.[16] The appendages are largely aromatic rings including indole and phenyl and coordinate through alkali metal cation-π interactions. This allows for smaller rings to coordinate larger ions by having the side appendage providing an additional coordination site. The structure of cyclocinamide is analogous to macrocyclic polyether systems with indole and pyrrole side chain comparable to the appendages seen in work by Gokel.[17]

With this possibility a purified sample of Curzon A was analyzed by NMR and found to match the other synthetic samples. Then to the NMR tube, one equivalent of KCl was added and the NMR was taken again. As shown in Figure 4.16, there are a few differences present from the two samples. The amide protons are very different in that the peaks have broadened and shifted positions. This would be expected since a soft ion was added. There is coordination occurring between the ions and the amides of the peptide. In a more interesting area of the spectrum 3.5-4.8 ppm there was a shift in the C14 proton. This is the stereogenic proton of asparagine and indicates that the coordinating capabilities of the asparagine side chain amide may be affecting the shift. The peak became more deshielded and overlapped with the isoserine stereogenic proton. This same overlap can be seen in the natural isomer.

There still are differences in the spectra but now it is more closely aligned with the natural product in the ^{1}H NMR.

In the ^{13}C spectrum there were a few differences observed between the KCl adduct and the pure sample. From the spectra in Figure 4.17 there aren't any dramatic differences in the shifts. Upon closer examination there are a few shifts that changed. The most notable differences are with the C4 and C11 stereogenic centers which showed shift differences of 1.5 ppm and 0.5 ppm respectively. These are the largest differences and they both occur with the β-amino acids indicating that the hydrogen bonding capabilities of side chains affect the shift.

These results show that there can be a significant shift in the C-H protons when salts are present in the sample. The most affected protons were on the asparagine side chain which could act as an additional coordination site for the potassium ion. The carbons most affected are those that were in close proximity to hydrogen bonding groups on the side chains of the β-amino acids. Cyclocinamide may act as an ionophore to soft cations. Though not covered in this work the addition of different salts can shift the protons in different positions. This could explain the difficulty of verifying the structure of the natural product. If there is an ion present then it could cause shifts of around 1 ppm in the carbon and 0.3 ppm in the proton spectra.

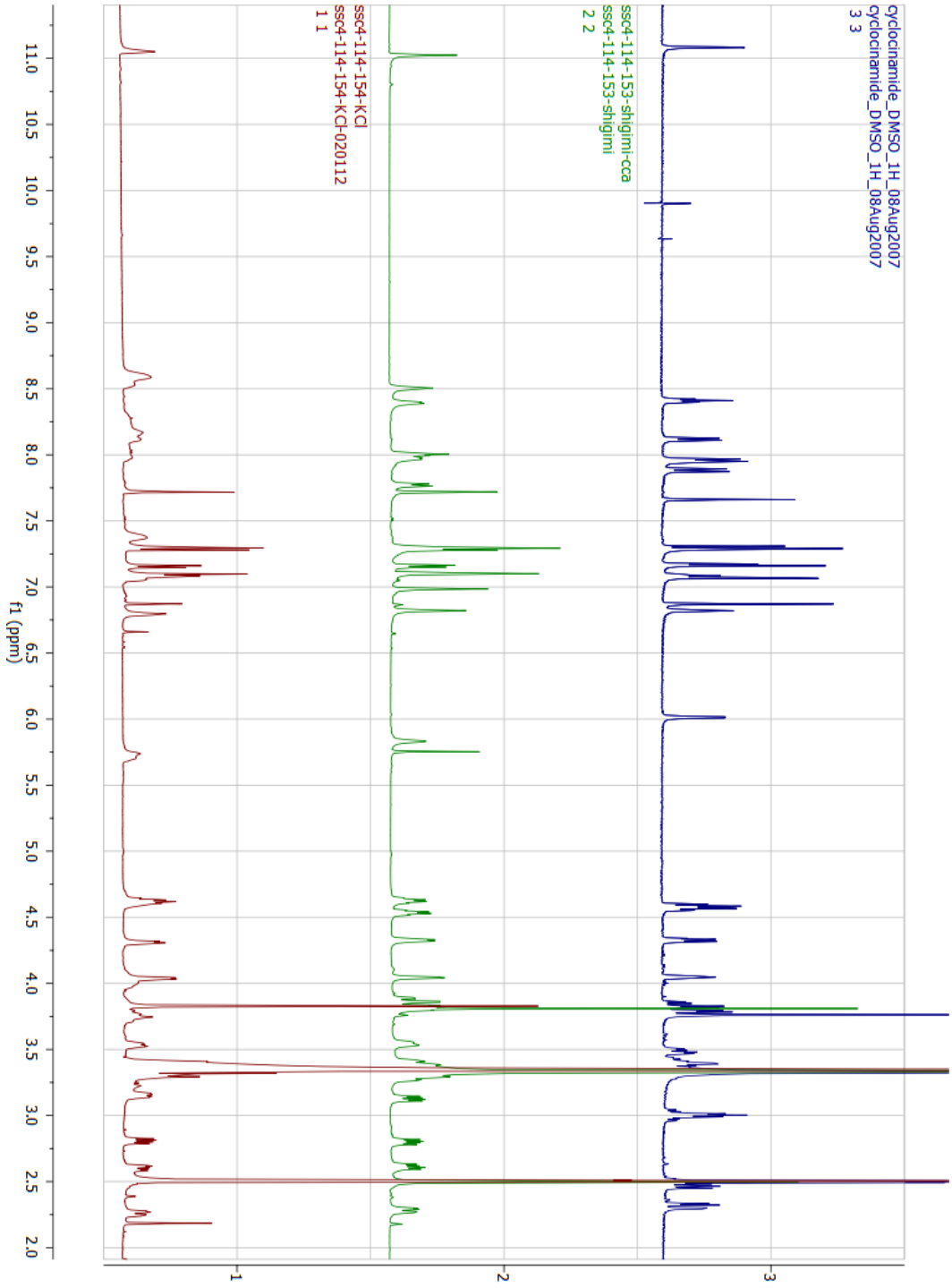


Figure 4.16 Stacked ^{1}H spectra of Ireland (blue), Curzon A (green) and KCl additive (red)

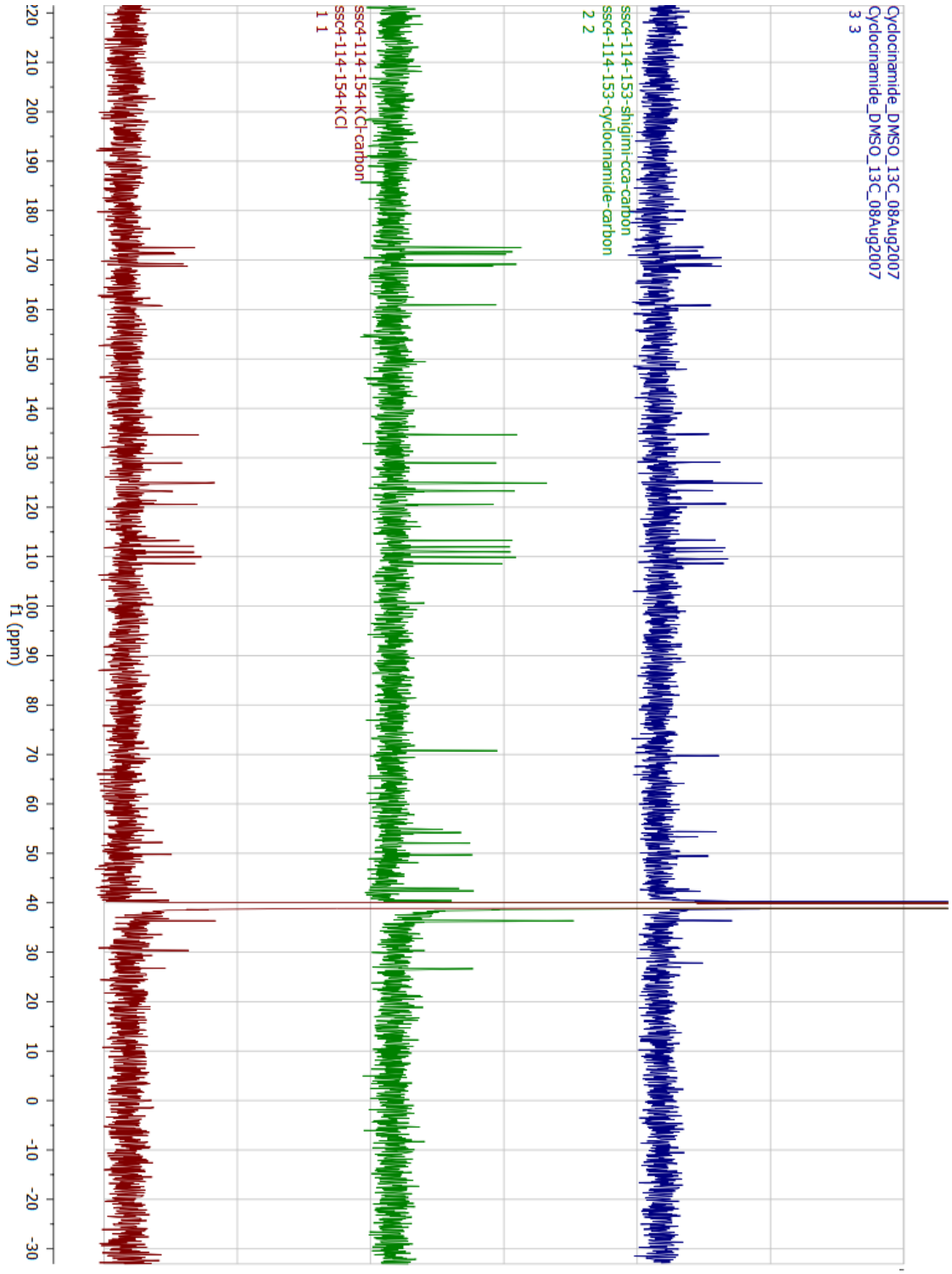


Figure 4.17 Stacked ^{13}C spectra of Ireland (blue), Curzon A (green) and KCl additive (red)

4.6) Conclusion

The successful syntheses of three isomers of cyclocinamide A and the synthesis of nominal cyclocinamide B have been detailed. During the synthesis it was discovered that the structure of natural cyclocinamide A and B are much closer than initially reported. The largest difference was 0.08 ppm and 0.5 ppm in the ^{1}H and ^{13}C respectively. This in combination with the Curzon A and B compounds were used as the baseline variability in comparison of the three isomers to the natural products. The differences in the two Curzon compounds were 0.08 ppm for the proton spectrum and 0.4 ppm for the carbon spectrum.

The analysis of the 2D NMRs showed that the connectivity of the amino acid in reported cyclocinamide was correct. The stereochemistry was more difficult to assign. The nominal structure of cyclocinamide A (4*S*,7*S*,11*S*,14*S*) showed the largest difference with the natural products, indicating that was not the correct structure. The differences in the cyclic core were over 0.5 ppm in the ^{1}H and 3.0 ppm in the ^{13}C. The 4*S*,7*S*,11*R*,14*S* cyclocinamide A isomer was closer to the natural product than the all *S* isomer. There were still significant differences in the proton spectra with a difference in the H3 proton of over 0.5 ppm. The carbon spectrum of the 11R isomer is much closer than the all-*S* isomer with the largest difference of over 1.0 ppm at the C11 position. The alternating stereoconfiguration of 4*S*,7*R*,11*S*,14*R* cyclocinamide was the closest when proton and carbon were compared together. The largest difference in the ^{1}H spectrum of less than 0.1 ppm and in the ^{13}C

spectrum there were a couple of peaks with differences of slightly larger than 1.0 ppm. The proton differences fall in the baseline variability established but the differences in the carbon are larger. The sample of Curzon A with the KCl additive had significant shifts from the nonadditive sample. This raised the possibility that any sample contaminated by an NMR transparent cation could exhibit resonance shifts that make it more difficult to discern the actual structure of the natural product.

4.7) Experimental Details

	δ ^{13}C synthetic	δ ^{1}H synthetic	*J* (Hz)	HMBC	COSY
1	169.4				
2		7.84	dd, 7.7, 4.3	1	3
3	42.4	3.59	m	4,5	3
		2.81	m		
4	69.2	3.75	m		3
5	172.4				
6		8.49	d, 7.3	5	7
7	53.0	4.43	ddd, 9.9, 7.5, 3.6		
8	169.5				
9		8.07	dd, 7.4, 4.6	8	10
10	40.3	3.45	m	11,12	
		2.81	m		
11	51.4	4.17	m		10
12	169.8				
13		8.38	d, 7.5	12	14
14	48.9	4.52	ddd, 10.4, 8.0, 3.6		
15	35.9	2.62	m	1,14,16	14,15
		2.14	dd 15.4, 3.3		
16	171.7				
17		6.70	s		
17'		6.30	s		
18	26.5	3.21	dd, 13.7, 9.9	7,8,19,20,27	7,18
		2.81	m		
19	111.2				
20	125.3	7.07	d, 2.1	19,22,27	
21		11.0	s	19,20,22,27	20
22	134.6				
23	113.4	7.29	d, 8.6	19,27	
24	123.1	7.15	dd, 8.6, 1.9	22,26	
25	110.8				
26	121.0	8.01	d, 1.5	22,24,25	
27	129.1				
28		8.19	d, 7.7	29	11
29	168.5				
30	41.4	3.83	dd, 16.6, 6.1	29,32	
		3.75	m		
31		8.31	t, 6.1	32	30
32	160.4				
33	124.9				
34	111.5	6.83	d, 2.0	36	
35	108.5				
36	124.7	7.09	d, 1.9	33,35	
38	36.4	3.79	s	33	

Table 4.7 4*S*,7*S*,11*S*,14*S* Cyclocinamide A

	δ ^{13}C synthetic	δ ^{1}H synthetic	*J* (Hz)	COSY
1				
2		7.42	dd, 5.5, 7.2	3
3	42.3	3.56	m	3
		2.85	m	
4	69.1	3.87	m	3, OH
5				
6		8.50	bs	
7	53.2	4.47	dd, 9.5, 6.8, 1.2	18
8				
9		8.14	m	10
10	40.4	3.56	m	10, 11
		3.22	m	
11	55.4	4.19	m	10, 28
12				
13		8.02	m	14
14	49.0	4.58	m	13, 15
15	35.8	2.64	dd, 9.0, 16.2	14,15
		2.10	dd 4.8, 16.2	
16				
17		7.26	bs	17'
17'		6.74	bs	17
18	26.5	3.17	m	7,18
		2.77	dd , 5.8, 14.1	
19				
20	125.4	7.03	bs	21
21		10.99	s	20
22				
23	113.1	7.28	d, 8.5	24
24	123.4	7.16	dd, 8.5, 1.8	23, 26
25				
26	120.9	7.86	d, 0.9	11
27				
28		8.25	d, 6.4	30, 31
29				
30	41.5	3.92	dd, 7.2, 16.6	
		3.76	dd, 11.8, 16.6	
31		8.58	bs	30
32				
33				
34	112.3	7.03	bs	36
35				
36	124.9	7.10	d 1.6	34
38	36.9	3.83	s	

Table 4.8 4*S*,7*S*,11*R*,14*S* Cyclocinamide A

	δ ^{13}C synthetic	δ ^{1}H synthetic	*J* (Hz)	HMBC	COSY
1	171.4				
2		7.20			3
3	43.2	3.51	m		2, 4
		3.29	m		
4	70.8	4.03	m	5	3, OH
5	171.9				
6		8.03			7
7	52.4	4.61	m		6,8
8	172.7				
9		8.23			10
10	40.4	3.36	m		9, 11
11	54.0	4.34	dd, 7.2, 3.6		10, 28
12	169.0				
13		7.82			14
14	50.1	4.54	m		13, 15
15	36.5	2.58	m	1, 16	14
		2.28			
16	171.6				
17		7.30	s	15, 16	17'
17'		6.81	s	15, 16	17
18	26.8	3.10	dd, 14.4, 6.0	7,8,19,20,27	7, 20
		2.79	dd, 14.4, 7.8		
19	110.0				
20	124.7	7.09	d, 2.4	19	18, 21
21		11.03	d, 1.8	19,20,22,27	20
22	134.8				
23	113.4	7.28	d, 8.4	25,27	24, 26
24	123.3	7.16	dd, 8.4, 1.8	22,26	23, 26
25	111.0				
26	120.6	7.71	s	22,24,25	23,24
27	129.0				
28		8.52			11
29	169.2				
30	42.6	3.91	dd, 15.0, 3.6	29,32, 38	31
		3.83	dd, 10.8, 6		
31		8.72			30, 38
32	160.4				
33	125.1				
34	111.9	7.16	s		36
35	107.5				
36	118.5				
38	33.4	3.86	s	33, 36	31, 34
OH		6.29			

Table 4.9 4*S*,7*R*,11*S*,14*R* Cyclocinamide B

	δ ^{13}C synthetic	δ ^{1}H synthetic	*J* (Hz)	HMBC	COSY
1	171.4				
2		7.29			3, 3'
3	42.9	3.56	dd 4.2, 13.8		4
		3.37			4
4	70.8	4.06	dd 2.4,		3, 3', OH
5	171.8				
6		7.77			12
7	52.1	4.64	dd 7.2, 8.4		
8	172.7				
9		8.42			11
10	40.5	3.42	dd, 4.8, 14.4		
		3.29	dd 13.2		28
11	54.3	4.34	dd 3.6, 7.2		10, 9
12	169				
13		7.97			14
14	49.7	4.55	dd 6.6, 7.2		15, 15'
15	36.4	2.63	dd 7.8, 15.6		14
		2.29	dd 4.8, 15.6	14	14
16	171.8				
17		7.29			
17'		7			
18	26.7	3.14	dd 6.6, 15.0	7	7
		2.81	dd 7.8, 15.0	7	7
19	110				
20	124.7	7.1		19, 22, 27	
21		11.04		19,22,27	20, 26
22	134.8				
23	113.4	7.28		25, 29	26, 24
24	123.4	7.17			26, 23
25	111.1				
26	120.7	7.73		22,24,25	23, 24
27	129.1				
28		8.1			10
29	169.3				
30	42.6	3.89	dd 5.4, 16.2		
		3.83	dd 6.0, 16.2		
31		8.51			30
32	161.1				
33	124.9				
34	112	6.98		33, 36, 35	
35	108.7				
36	125	7.11			30
38	36.4	3.82	s		
OH		5.85			4

Table 4.10 4*S*,7*R*,11*S*,14*R* Cyclocinamide A

		δ ^{1}H synthetic (Garcia-11*S*)	δ ^{1}H synthetic (Garcia-11*R*)	δ ^{1}H natural (Clark)	δ ^{1}H natural (Ireland)	δ 1H synthetic (Curzon)	δ 1H ent-Grieco (Curzon)	δ 1H Curzon KCl
C=O	**1**							
N-H	**2**	7.84	7.42	7.14	7.03	7.20	7.29	7.37
C-H	**3**	3.59	3.56	3.50	3.46	3.51	3.56	3.52
C-H		2.81	2.85	3.36	3.31	3.29	3.37	3.33
C-H *	**4**	3.75	3.87	4.04	4.00	4.03	4.06	4.03
C=O	**5**							
N-H	**6**	8.49	8.50	7.90	7.83	8.03	7.77	8.10
C-H *	**7**	4.43	4.47	4.57	4.50	4.61	4.64	4.64
	8							
N-H	**9**	8.07	8.14	8.00	7.94	8.23	8.42	8.53
C-H	**10**	3.45	3.56	3.42			3.42	3.41
C-H		2.81	3.22	3.33	3.31	3.36	3.29	3.31
C-H *	**11**	4.17	4.19	4.33	4.25	4.34	4.34	4.29
C=O	**12**							
N-H	**13**	8.38	8.02	8.00	7.86	7.82	7.97	7.97
C-H *	**14**	4.52	4.58	4.57	4.52	4.54	4.55	4.61
C-H	**15**	2.62	2.64	2.49	2.41	2.58	2.63	2.58
C-H		2.14	2.10	2.31	2.23	2.28	2.29	2.25
C=O	**16**							
N-H	**17**	6.70	7.26	7.28	7.23	7.30	7.29	7.08
N-H	**17'**	6.30	6.74	6.80	6.77	6.81	7.00	6.79
C-H	**18**	3.21	3.17	3.03	2.93	3.10	3.14	2.96
C-H		2.81	2.77	2.99	2.93	2.79	2.81	2.82
C=O	**19**							
C-H	**20**	7.07	7.03	7.17	7.11	7.09	7.10	7.10
N-H	**21**	11.00	10.99	11.10	11.04	11.03	11.04	11.15
C-H	**22**							
C-H	**23**	7.29	7.28	7.30	7.25	7.28	7.28	7.29
C-H	**24**	7.15	7.16	7.17	7.10	7.16	7.17	7.17
C-H	**25**							
C-H	**26**	8.01	7.86	7.67	7.59	7.71	7.73	7.71
C-H	**27**							
N-H	**28**	8.19	8.25	8.18	8.06	8.52	8.10	8.17
C=O	**29**							
C-H	**30**	3.83	3.92	3.84	3.80	3.91	3.89	4.12
C-H		3.75	3.76	3.78	3.80	3.83	3.83	3.69
N-H	**31**	8.31	8.58	8.42	8.52	8.72	8.52	8.58
C=O	**32**							
	33							
C-H	**34**	6.83	7.03	6.87	6.98	7.16	6.84	6.87
	35							
C-H	**36**	7.09	7.10	7.06	C-Cl	C-Cl	7.11	7.08
	38	3.79	3.83	3.77	3.73	3.86	3.82	3.83
OH					5.97	6.29	5.85	5.66

Table 4.11 Compilation of proton shifts for all cyclocinamides

	δ ^{13}C synthetic 11S (Garcia)	δ ^{13}C synthetic 11R (Garcia)	δ ^{13}C natural (Clark)	δ ^{13}C natural (Rubio)	δ ^{13}C natural (Ireland)	δ ^{13}C synthetic (Curzon)	δ ^{13}C ent-G (Curzon 2)	δ ^{13}C ent-G KCl(Curzon 2)
1	169.3		170.5	170.5	170.5	171.4	171.4	171.3
2								
3	42.4	42.3	42.8	42.7	42.8	43.2	42.9	42.9
4	69.2	69.1	69.7	69.7	69.7	70.8	70.8	69.3
5	172.6		170.9	170.9	170.9	171.9	171.8	171.5
6								
7	53.1	53.2	53.3	53.3	53.3	52.4	52.1	52.3
8	169.4		172.7	172.6	172.9	172.7	172.7	172.6
9								
10	40.3	40.4	40.3	40.3	40.8	40.4	40.5	40.5
11	51.6	55.5	54.3	54.4	54.6	54.0	54.3	54.8
12	169.9		168.8	168.8	168.8	169.0	169	168.9
13								
14	49.0	49.0	49.5	49.5	49.4	50.1	49.7	49.6
15	35.9	35.8	36.5	36.4	36.5	36.5	36.4	36.4
16	171.7		171.9	171.9	171.9	171.6	171.8	171.6
17								
17'								
18	26.5	26.5	27.8	27.8	27.9	26.8	26.7	26.8
19	111.0		109.6	109.5	109.5	110.0	110.0	110.0
20	125.5	125.4	125.3	125.3	125.4	124.7	125.0	125.0
21								
22	134.7		134.8	134.8	134.8	134.8	134.8	134.7
23	113.4	113.1	113.4	113.3	113.4	113.4	113.4	113.3
24	123.3	123.4	123.4	123.4	123.4	123.4	123.4	123.3
25	110.6		111.1	111.1	111.1	111.0	111.1	111.0
26	121.0	120.9	120.7	120.7	120.7	120.6	120.7	120.7
27	129.2		129.1	129.1	129.4	129.0	129.1	129.0
28								
29	168.6		169.2	169.2	169.1	169.2	169.3	169.3
30	41.4	41.5	42.3	42.3	42.5	42.6	42.6	42.3
31								
32	160.5		160.9	160.9	160.4	160.4	161.1	160.8
33	125.1		124.9	124.9	124.5	125.1	124.9	124.9
34	111.5	112.3	111.8	111.8	111.6	111.9	112.0	112.2
35	108.7		108.6	108.6	107.3	107.3	108.7	108.7
36	124.8	124.9	124.9	124.9	118.6	118.5	125.0	125.0
38	36.4	36.9	36.4	36.4	33.4	33.4	36.4	36.4

Table 4.12 Compilation of carbon shifts for all cyclocinamides

4.8) References

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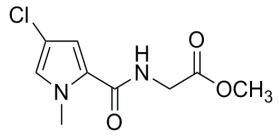
Appendix

Selected ^{1}H, ^{13}C and 2D NMR spectra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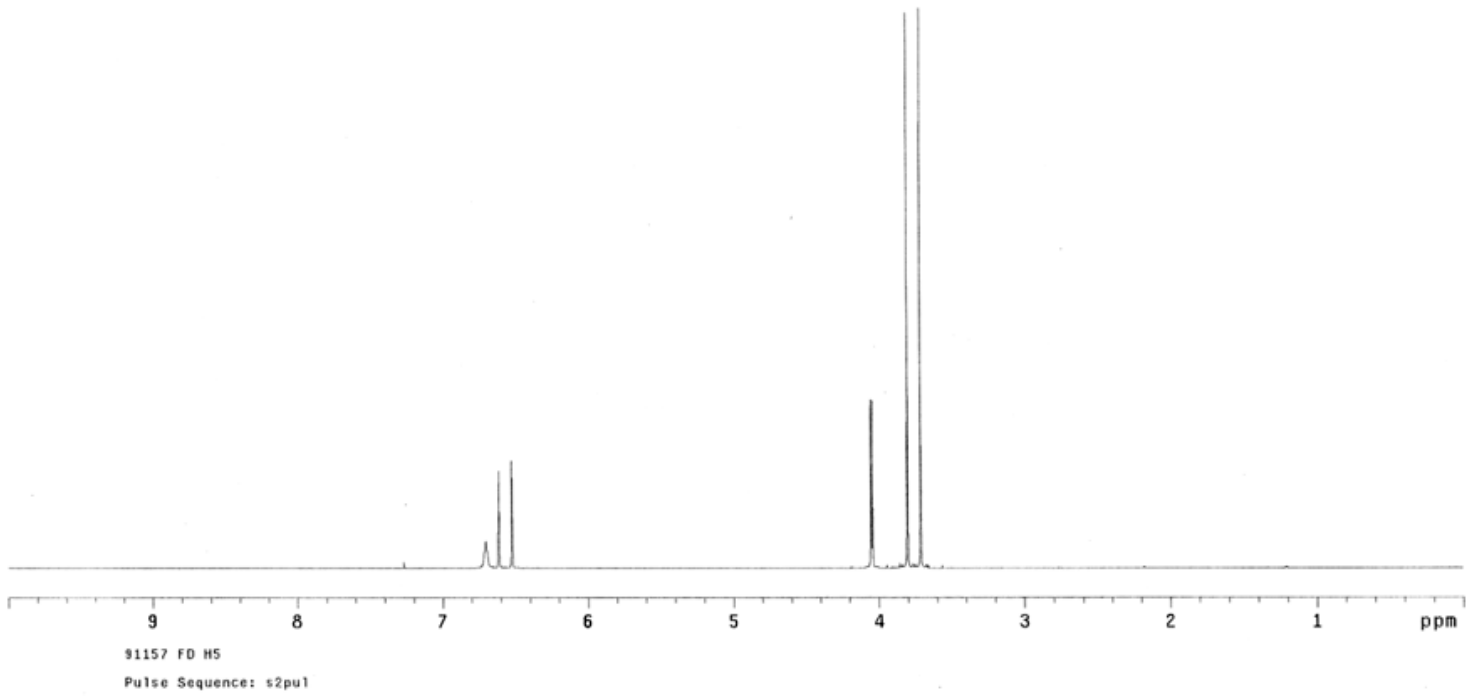
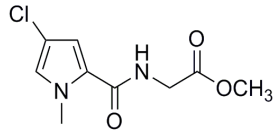
H
N
O
O
O
OCH3

2.18


9
8
7
6
5
4
3
2
1
ppm
91157 FD H5
Pulse Sequence: s2pul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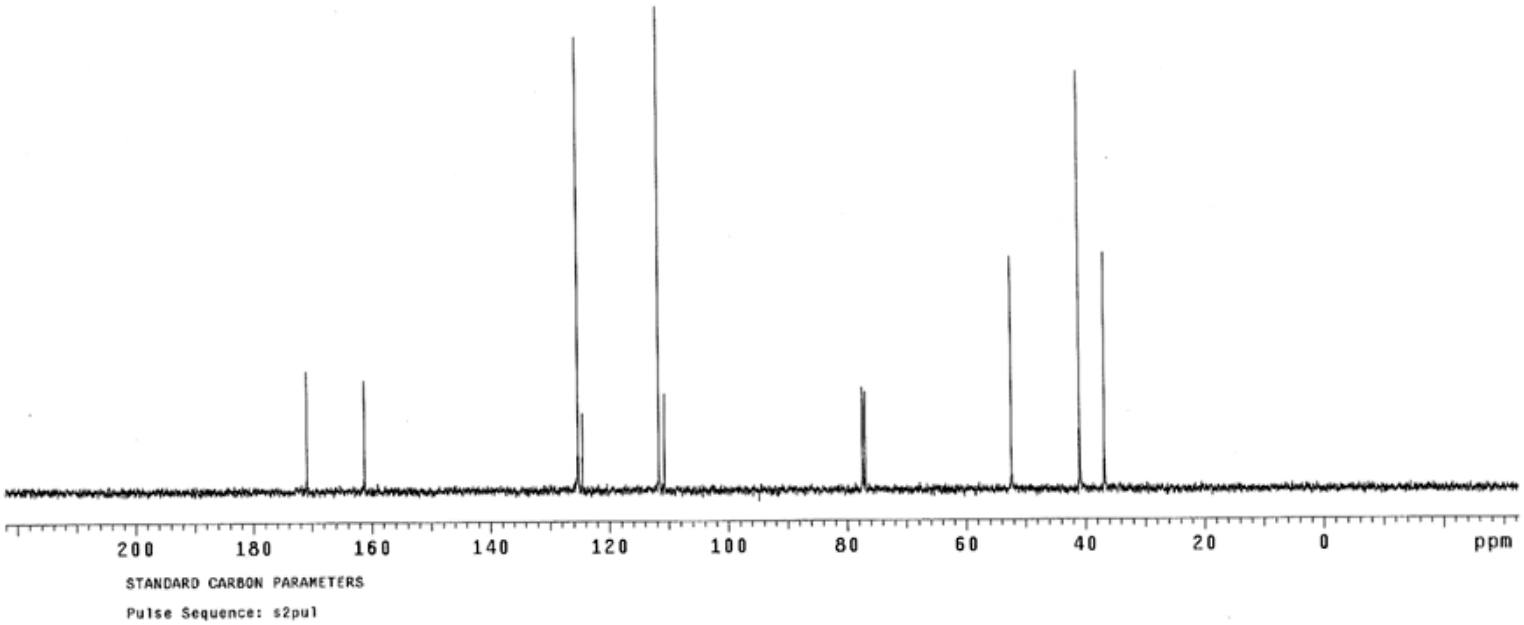
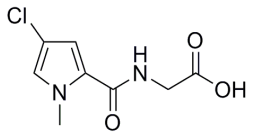
H
N
O
N
O
OCH3

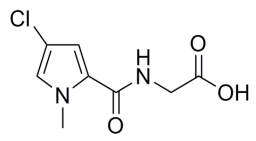
2.18


200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul


Cl
H
N
O
N
O
OH

2.20

14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
ssc4-114-20
Pulse Sequence: presat

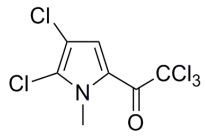

Cl
H
N
O
N
O
OH

2.20

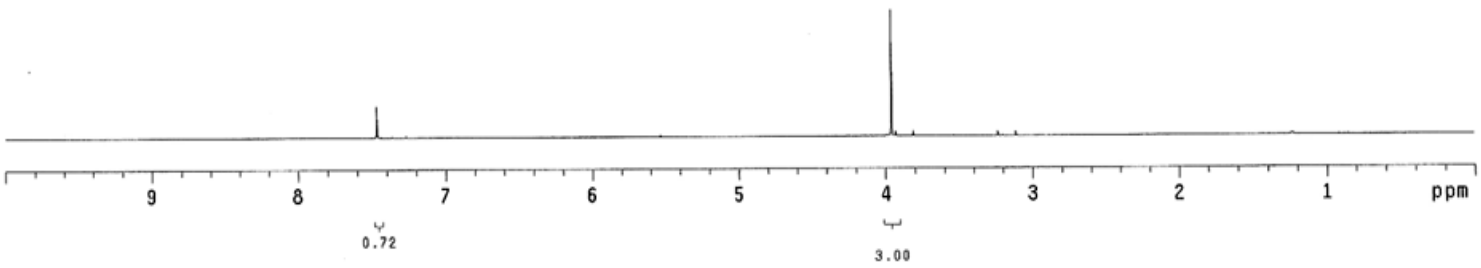
39.808
39.637
39.310
39.139
200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

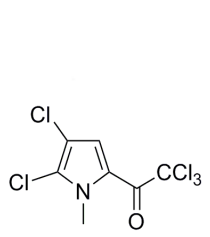
91157 FD H5

Pulse Sequence: s2pul



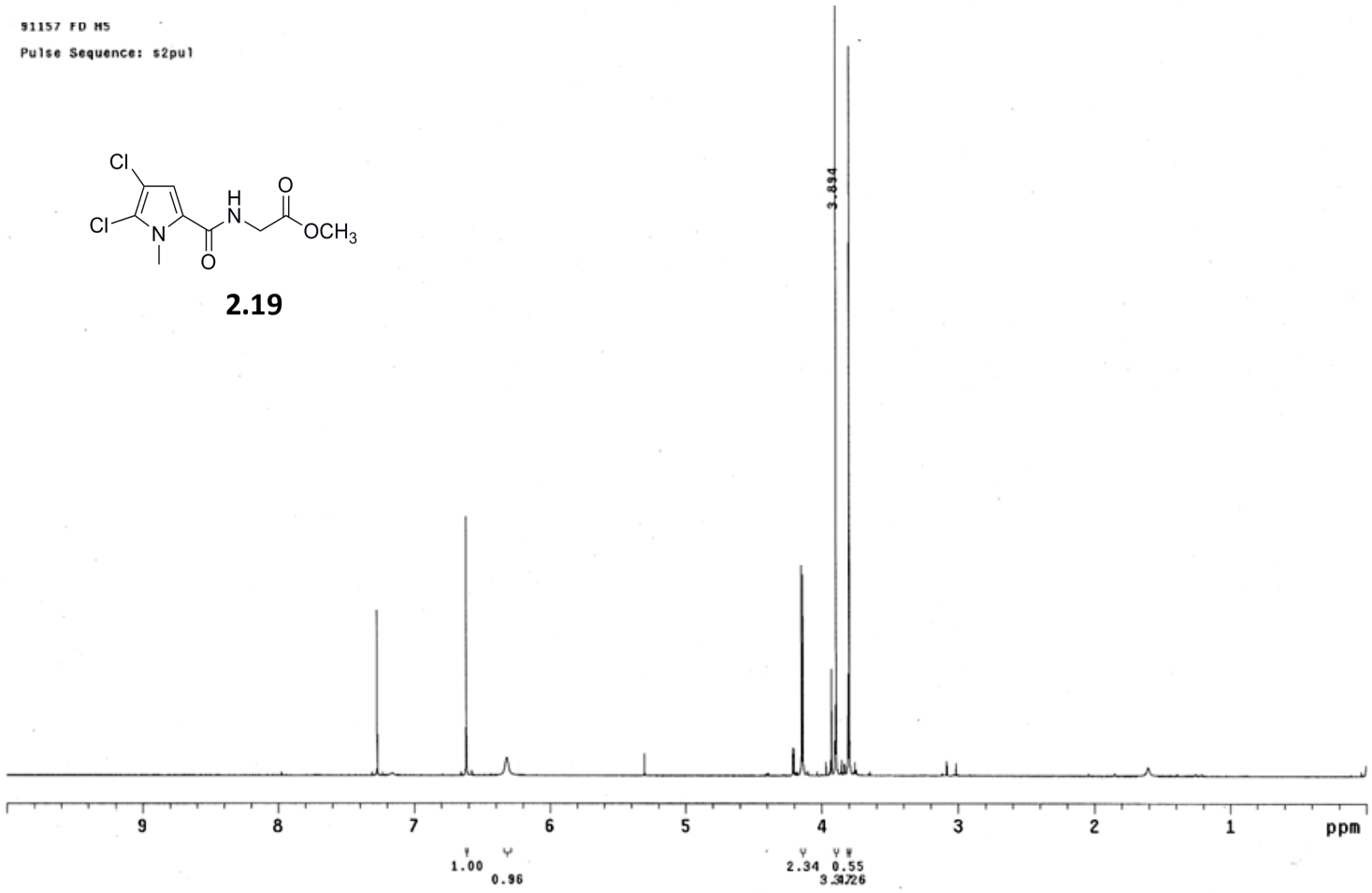
2.16




Cl
Cl
N
CCl3
O

2.16

200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul


91157 FD H5
Pulse Sequence: s2pul
Cl
Cl
N
H
N
O
O
O
OCH3
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8
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6
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3
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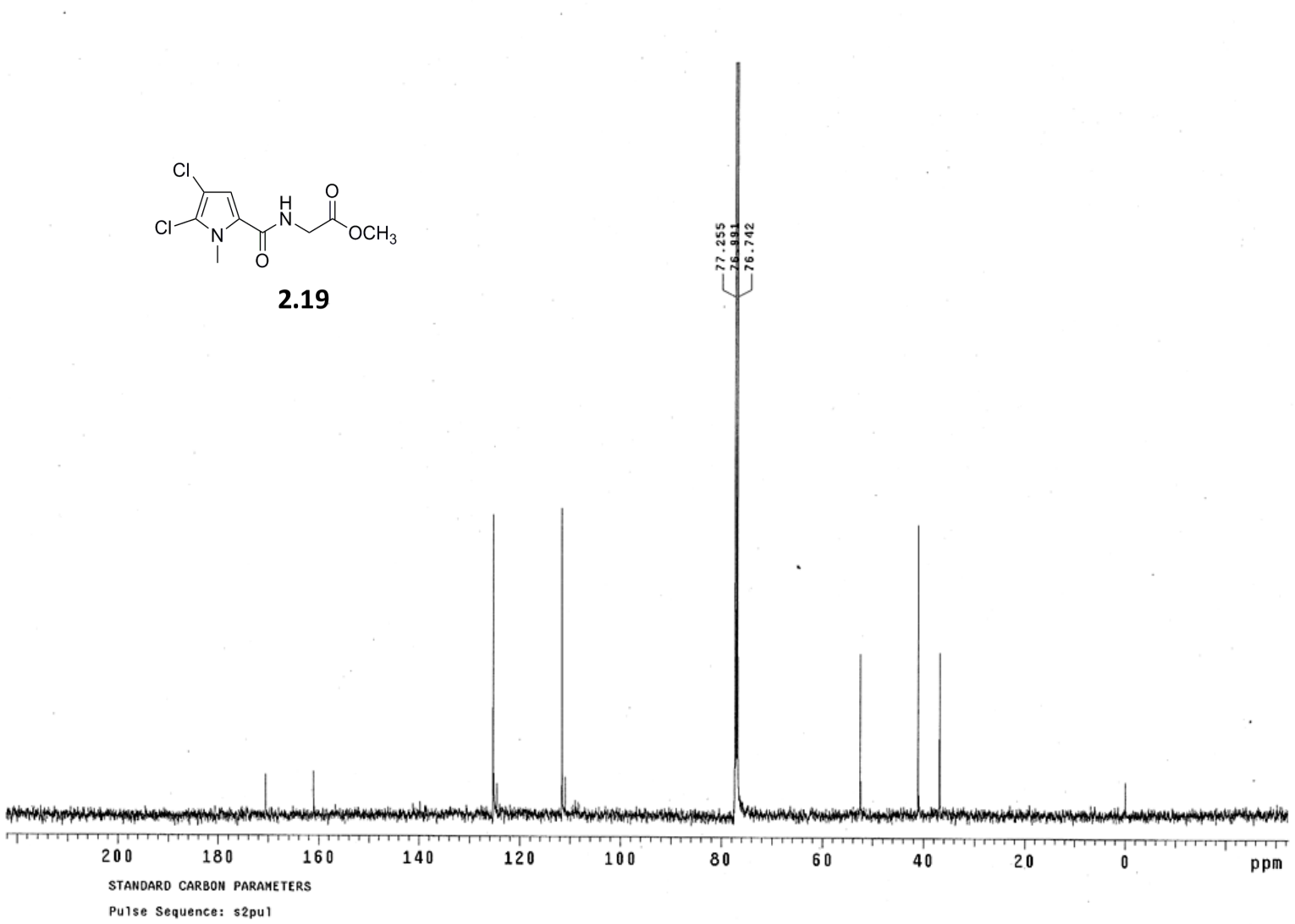
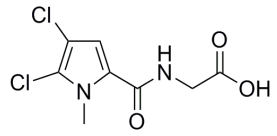
ppm
1.00
0.96
2.34
0.55

2.19

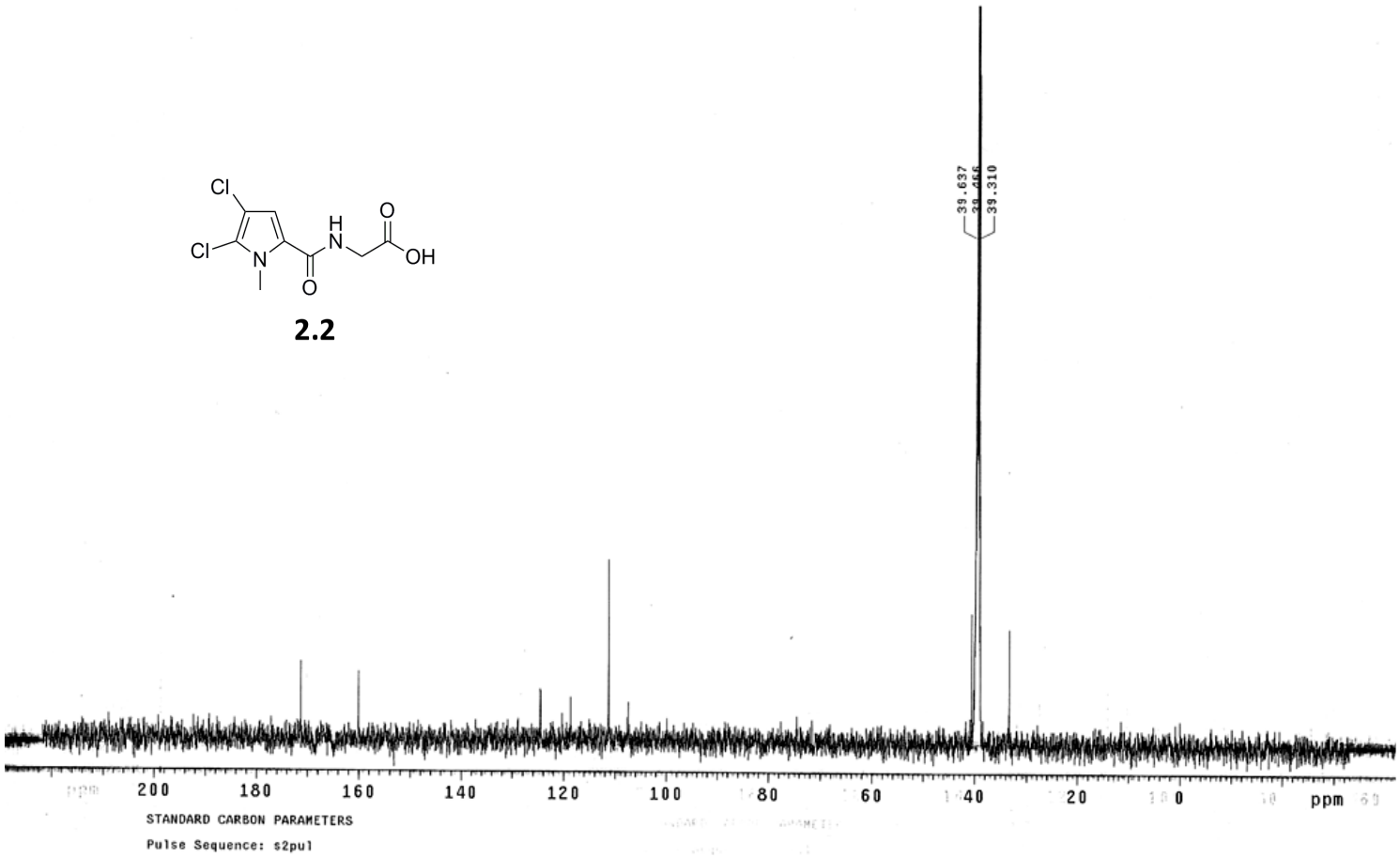

Cl
Cl
N
H
N
O
O
OCH3
77.255
76.891
76.742
200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

2.19

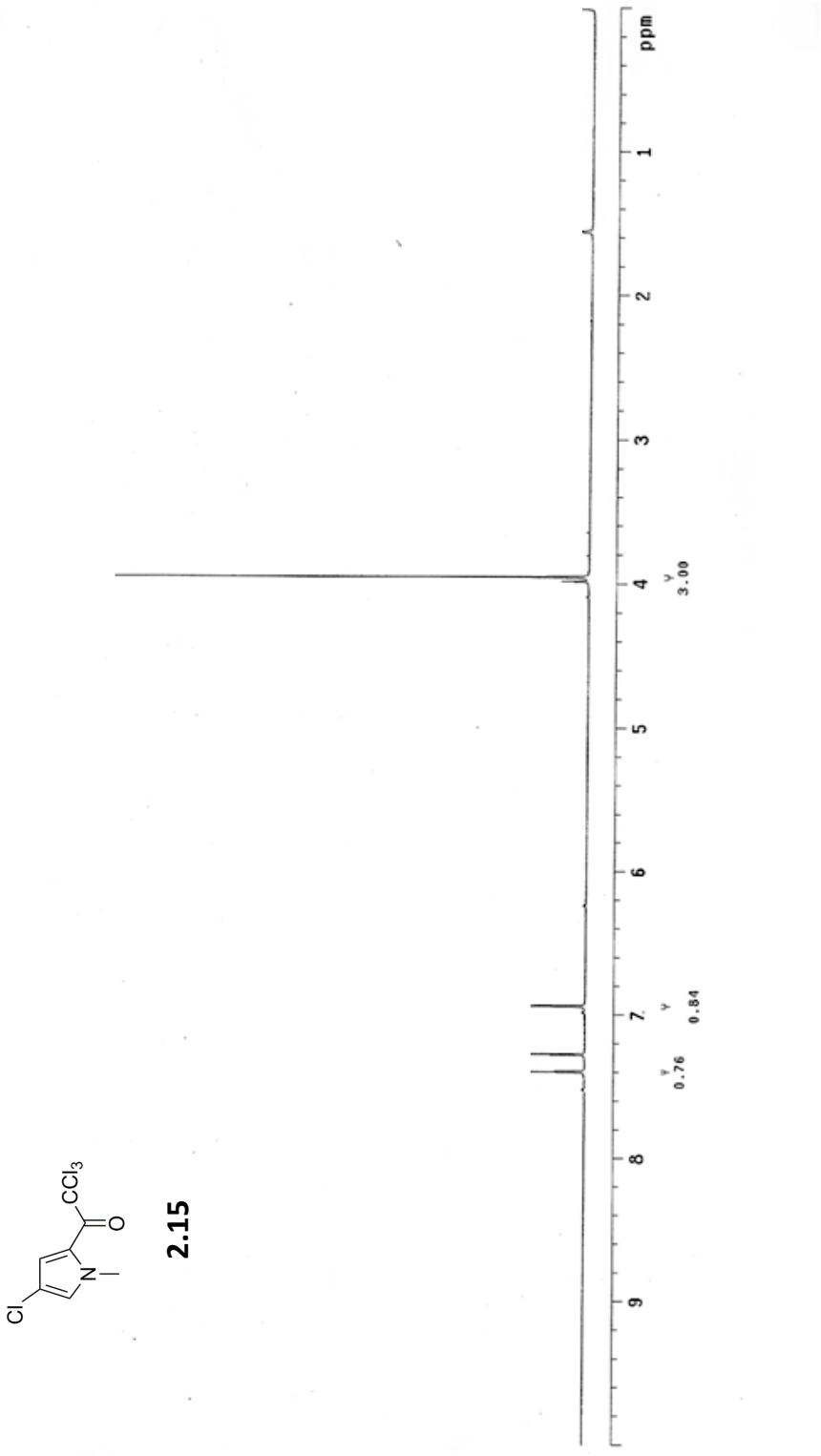

Cl
Cl
N
H
N
O
O
OH
O

2.2

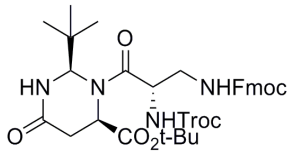
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
ssc4-114-19
Pulse Sequence: presat


Cl
Cl
N
H
N
O
O
OH
39.637
39.310
200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

2.2

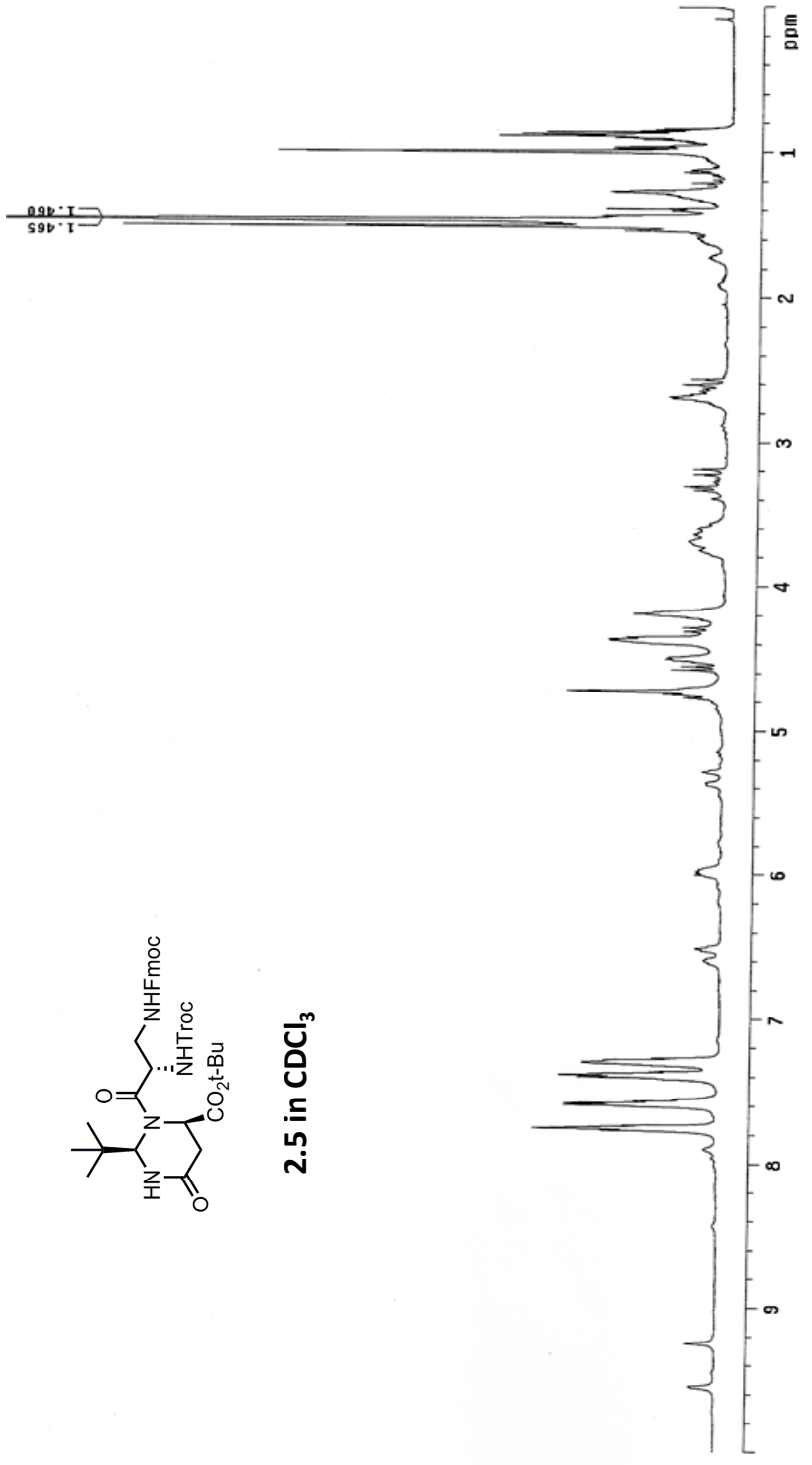


2.15

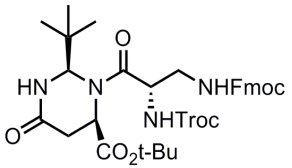

HN
N
O
O
NHFmoc
NHTroc
CO2t-Bu

2.5 in DMSO-d_6

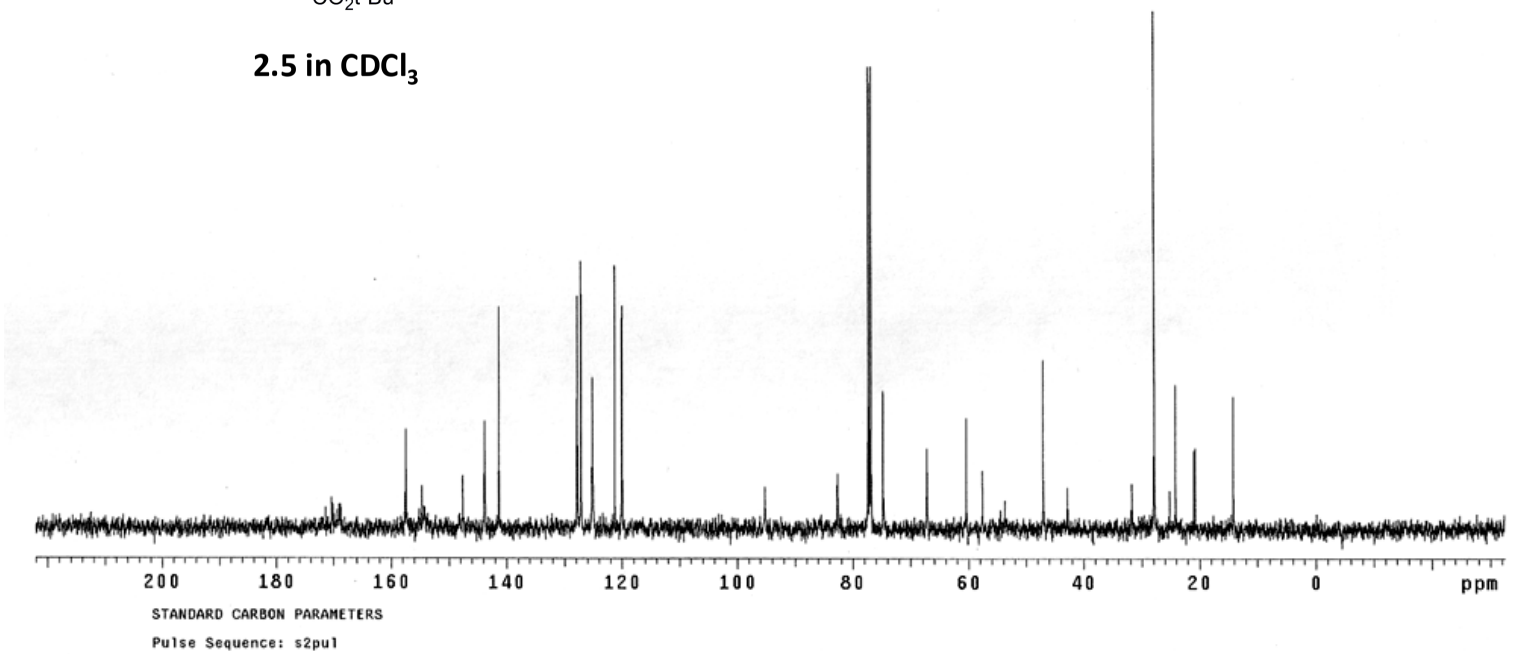
3.339
3.336
2.511
2.508
2.505
2.502
2.499
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc3-24
Archive directory:
Sample directory:
File: presat

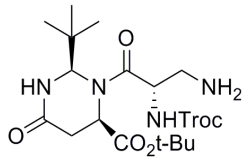


2.5 in $CDCl_3$


HN
N
O
O
NHFmoc
NHTroc
CO2t-Bu

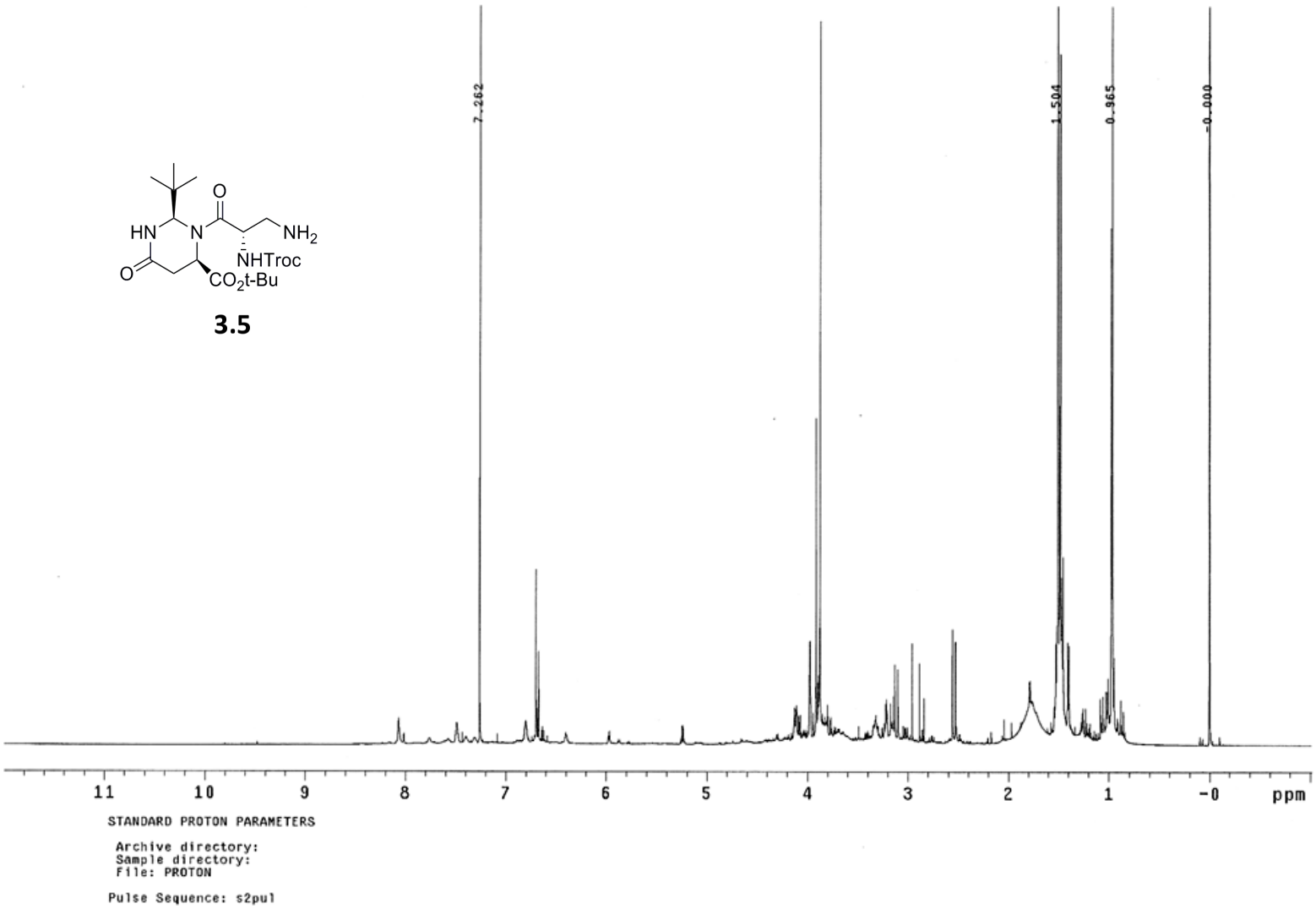
2.5 in $CDCl_3$


200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

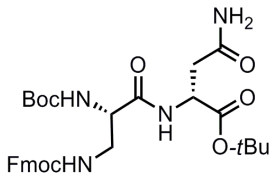

HN
N
O
O
NH2
NHTroc
CO2t-Bu

3.5

7.264
1.556
0.002
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc3-107-an
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat

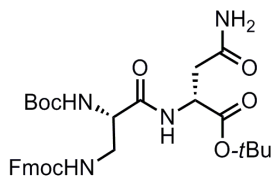

HN
N
O
O
NH2
NHTroc
CO2t-Bu
7.262
1.504
0.965
-0.000
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
STANDARD PROTON PARAMETERS
Archive directory:
Sample directory:
File: PROTON
Pulse Sequence: s2pul

3.5


NH2
O
O
BocHN
N
H
O
O-tBu
FmocHN

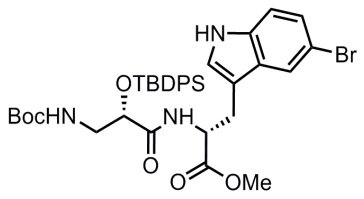
3.30

1.354
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm
ssc3-256-crude
Pulse Sequence: s2pul


NH2
O
O
BocHN
N
H
O
O-tBu
FmocHN

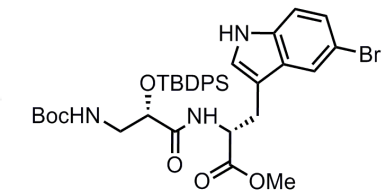
3.30

200
180
160
140
120
100
80
60
40
20
0
ppm
ssc3-256-an-carbon
Pulse Sequence: s2pul


HN
Br
OTBDPS
BocHN
NH
O
O
OMe

2.4

1.416
9
8
7
6
5
4
3
2
1
ppm
91157 FD H5
Pulse Sequence: s2pul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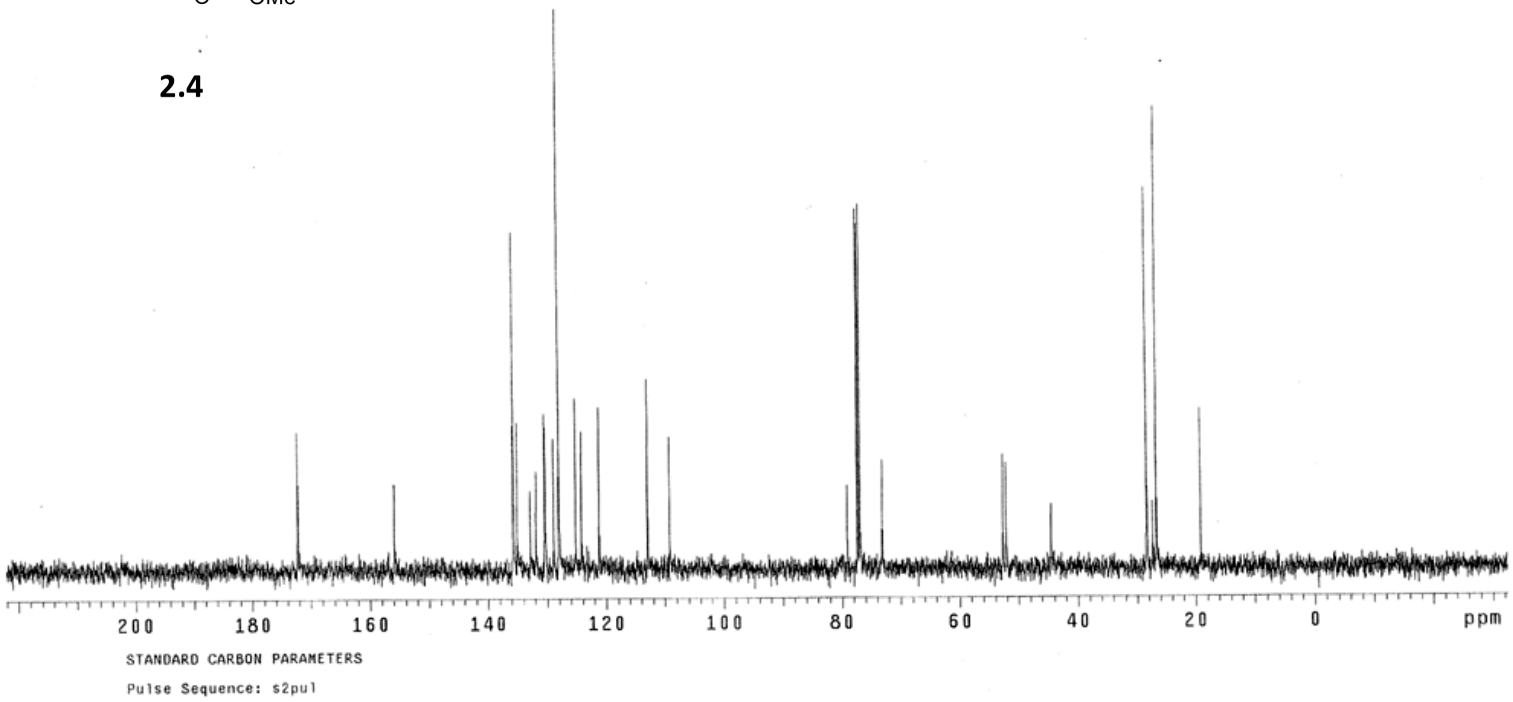
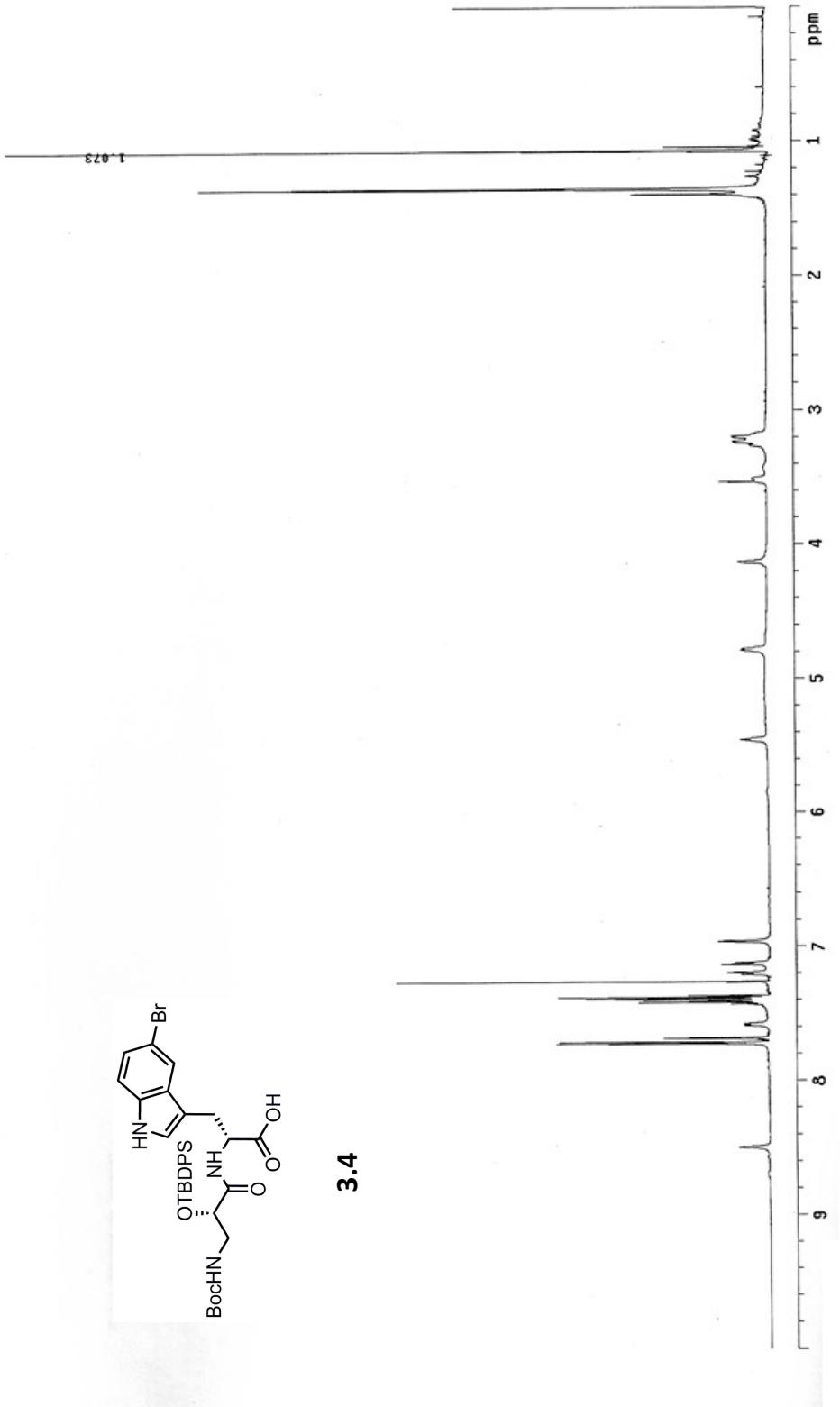
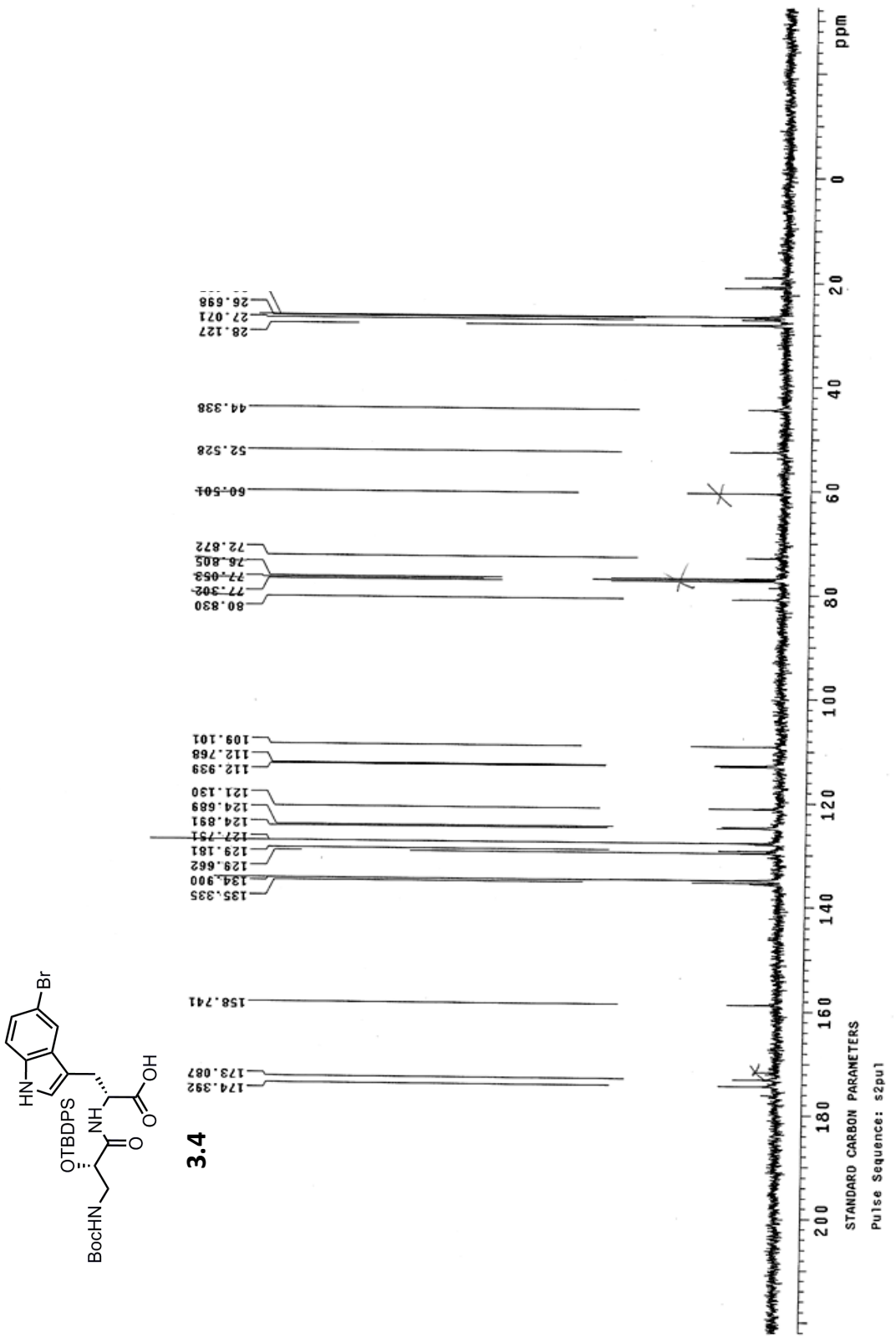
Br
OTBDPS
BocHN
NH
O
O
O
OMe

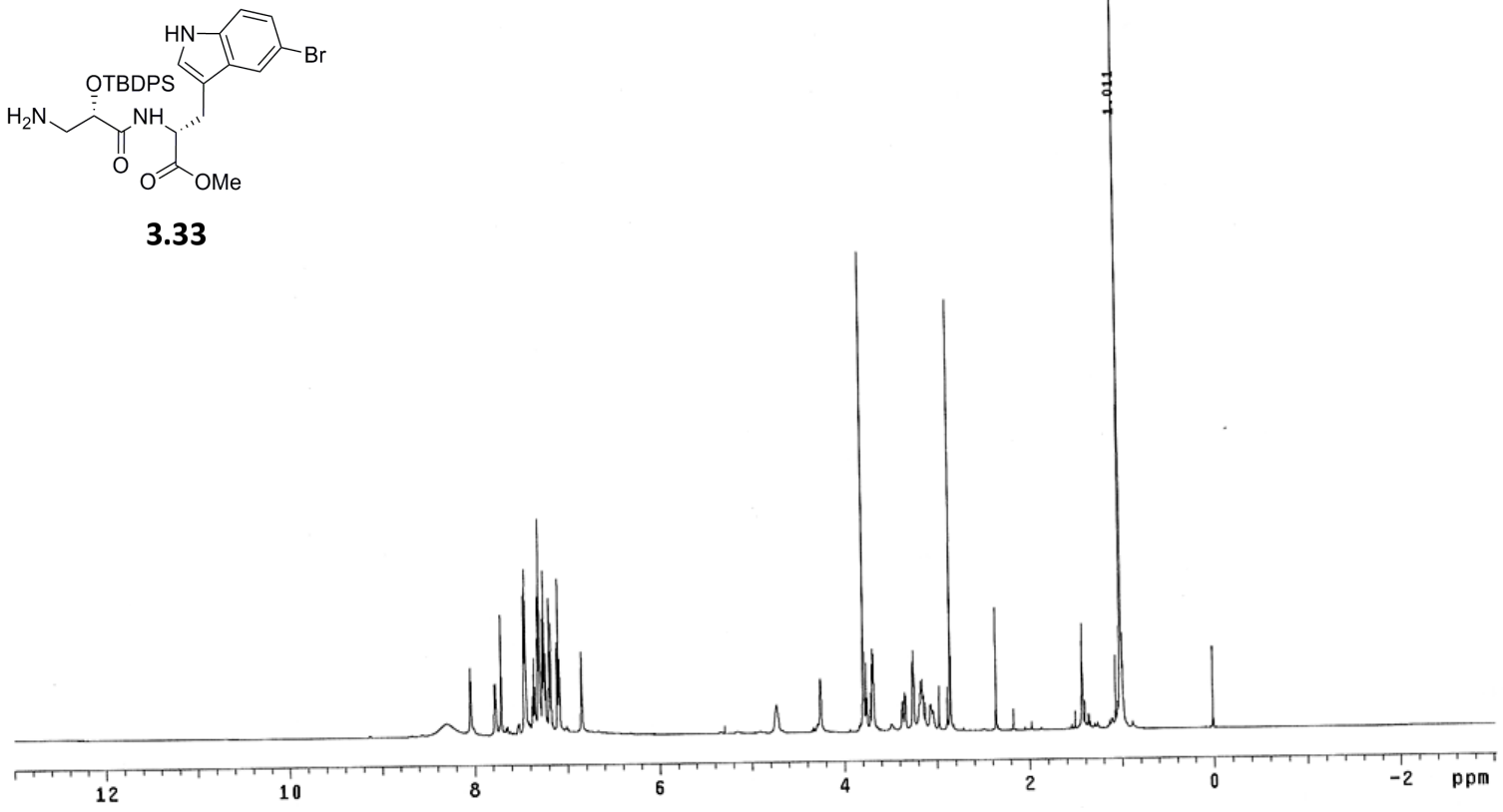
2.4

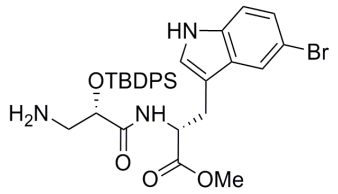

200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul



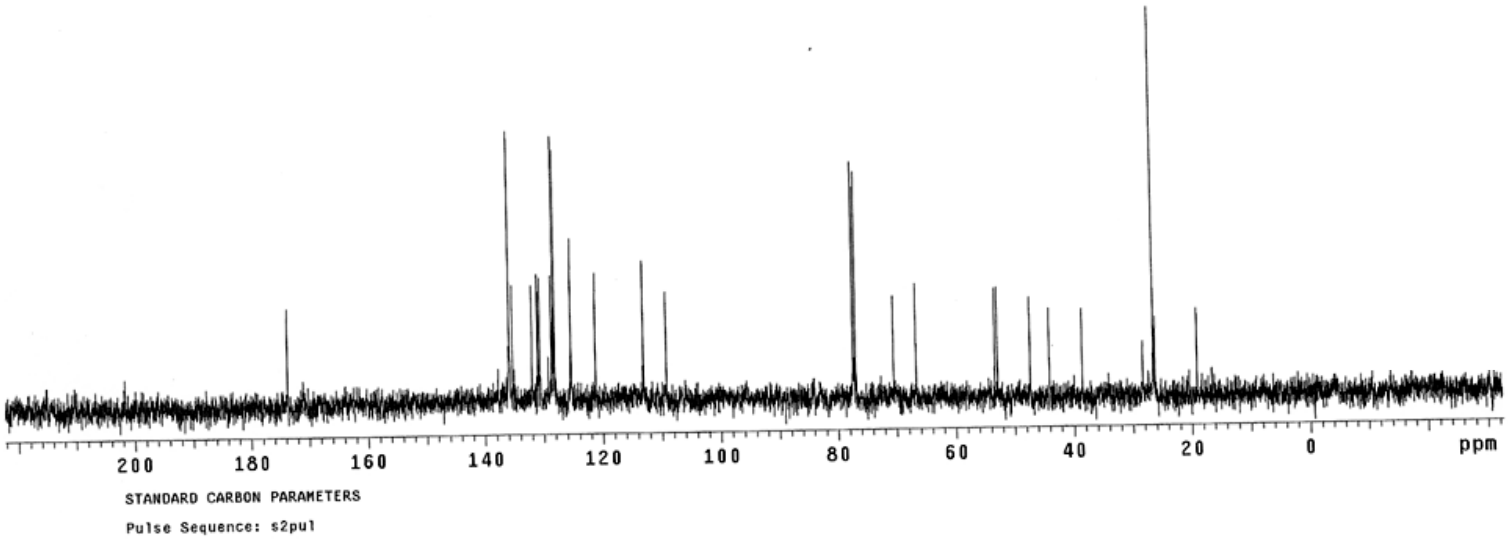
3.4

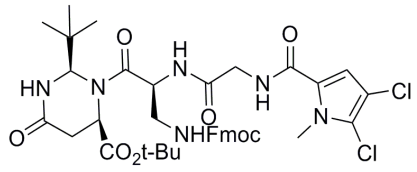

Br
HN
NH
OTBDPS
BocHN
O
OH
3.4
174.392
173.087
158.741
135.335
134.900
129.662
129.181
127.751
124.891
124.689
121.130
112.939
112.768
109.101
80.830
77.302
77.058
76.805
72.872
60.501
52.528
44.338
28.127
27.071
26.698
200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul


HN
Br
OTBDPS
H2N
NH
O
O
OMe
3.33
1.011
12
10
8
6
4
2
0
-2
ppm


HN
Br
OTBDPS
H2N
NH
O
O
O
OMe

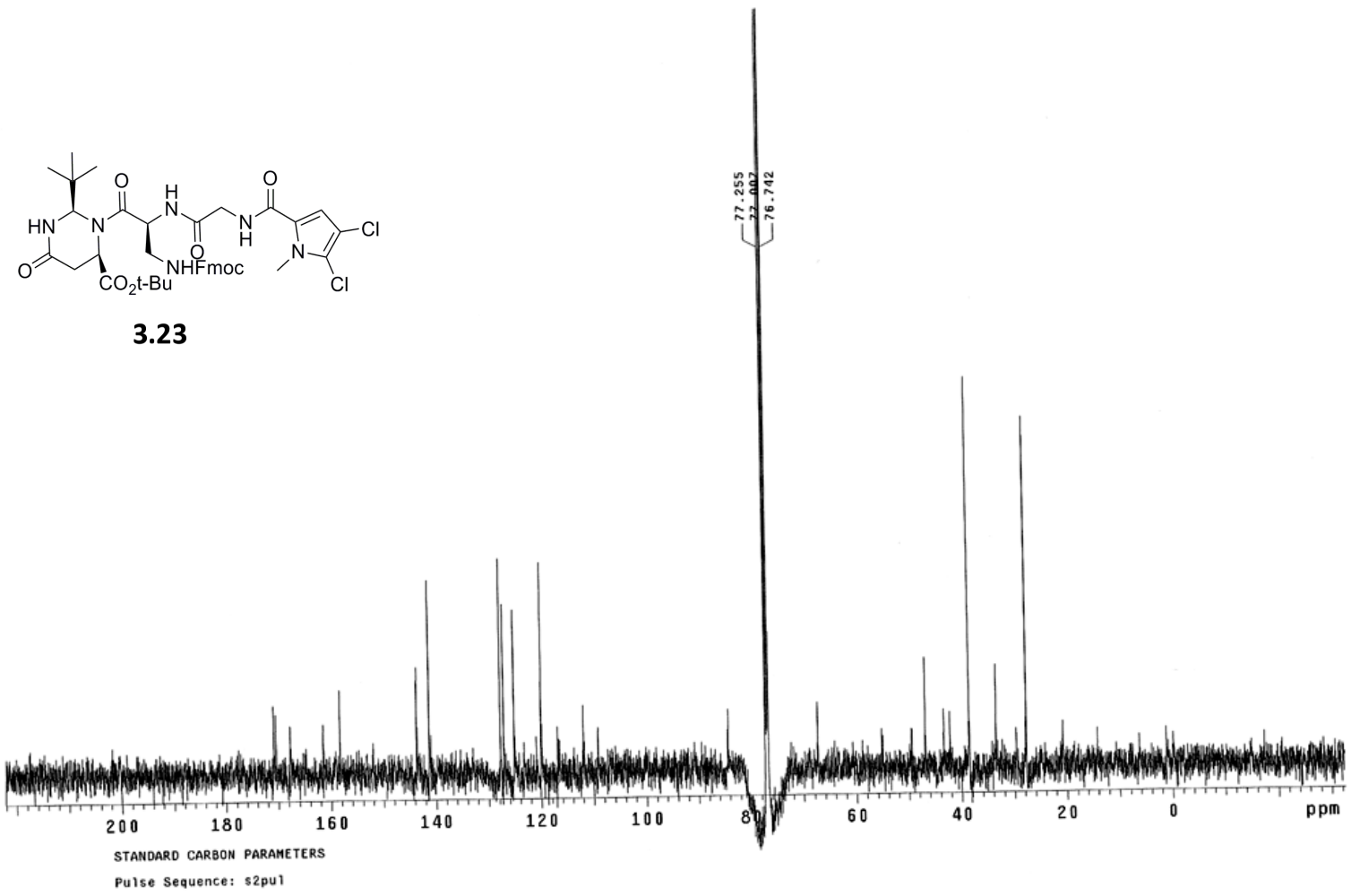
3.33


200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

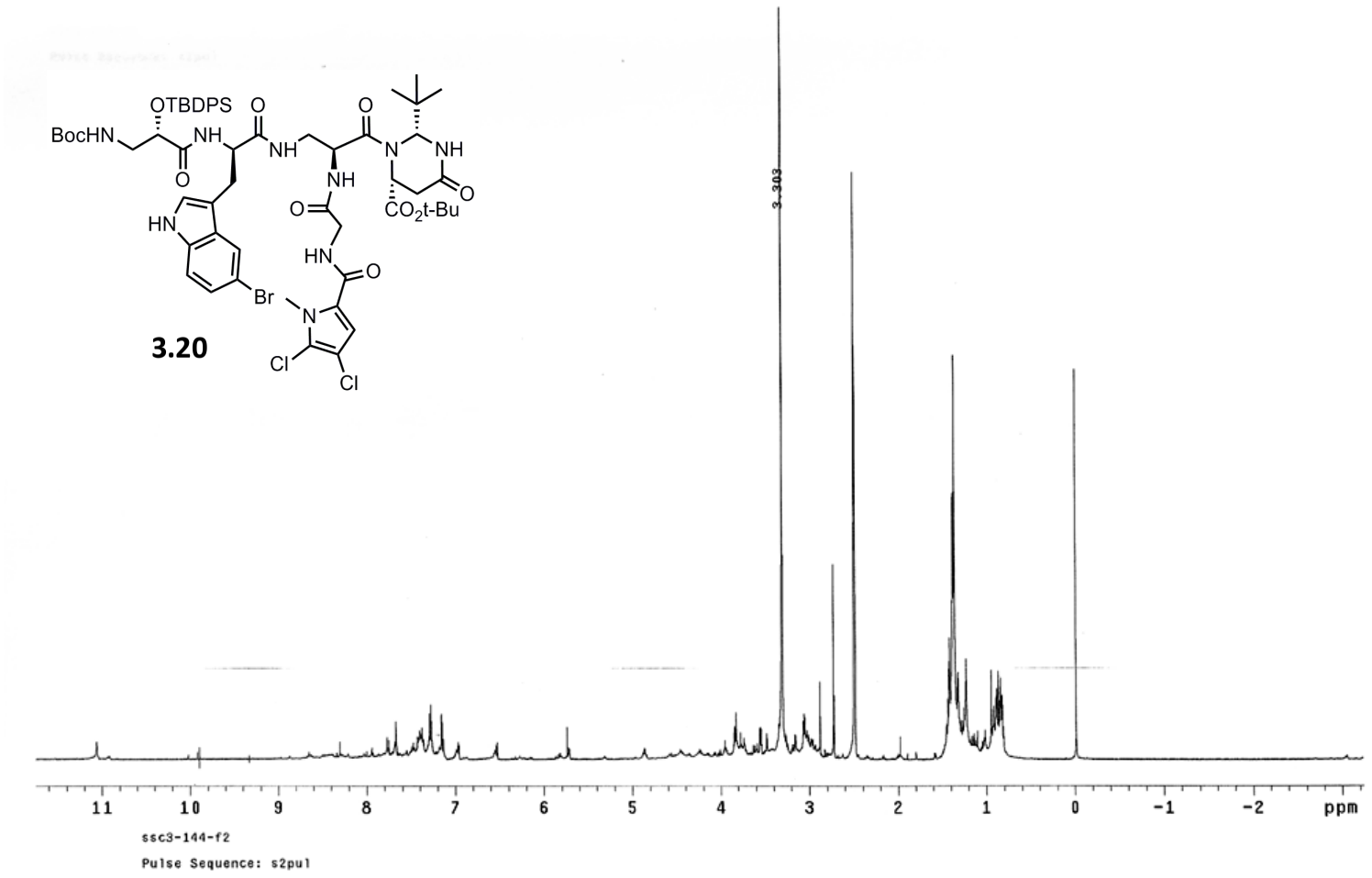

HN
N
O
H
N
O
N
H
O
Cl
N
Cl
O
NHFmoc
CO2t-Bu

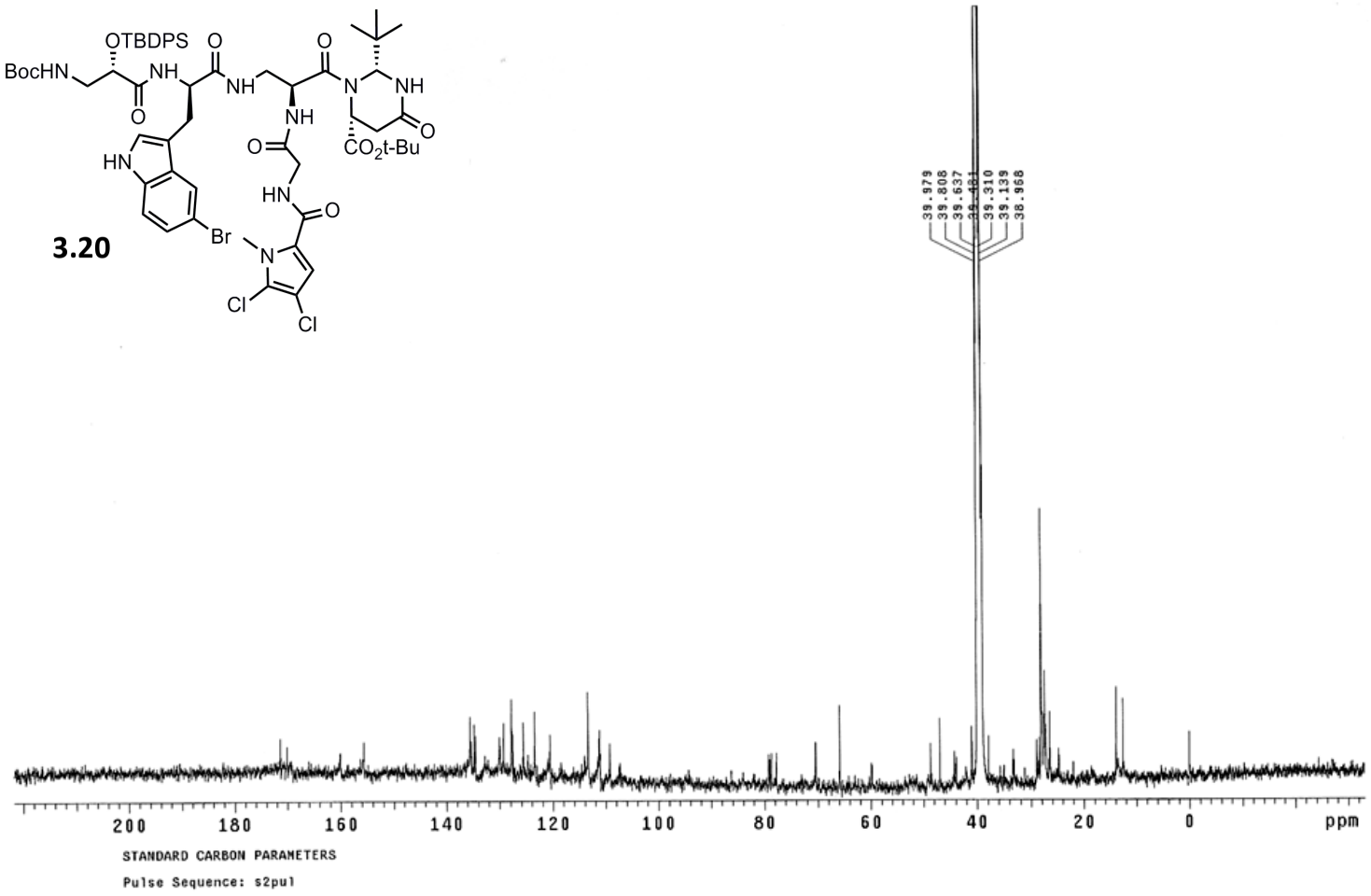
3.23

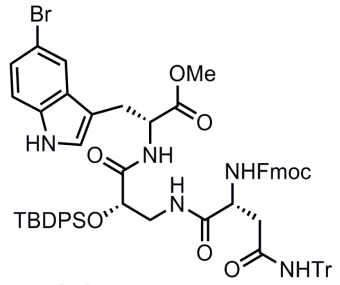
2.801
12
10
8
6
4
2
0
-2
ppm
91157 FD H5
Pulse Sequence: s2pul


77.255
77.007
76.742
200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

3.23

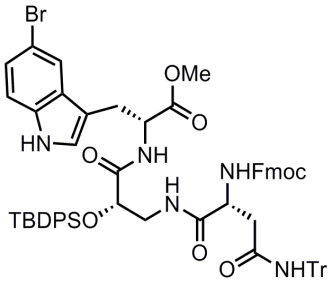

OTBDPS
BocHN
NH
NH
NH
N
NH
HN
HN
Br
N
Cl
Cl
CO_2t-Bu
3.20
3.303
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm
ssc3-144-f2
Pulse Sequence: s2pul


OTBDPS
BocHN
NH
NH
NH
N
NH
HN
HN
Br
N
Cl
Cl
CO_2t-Bu
3.20
39.979
39.808
39.637
39.481
39.310
39.139
38.968
200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

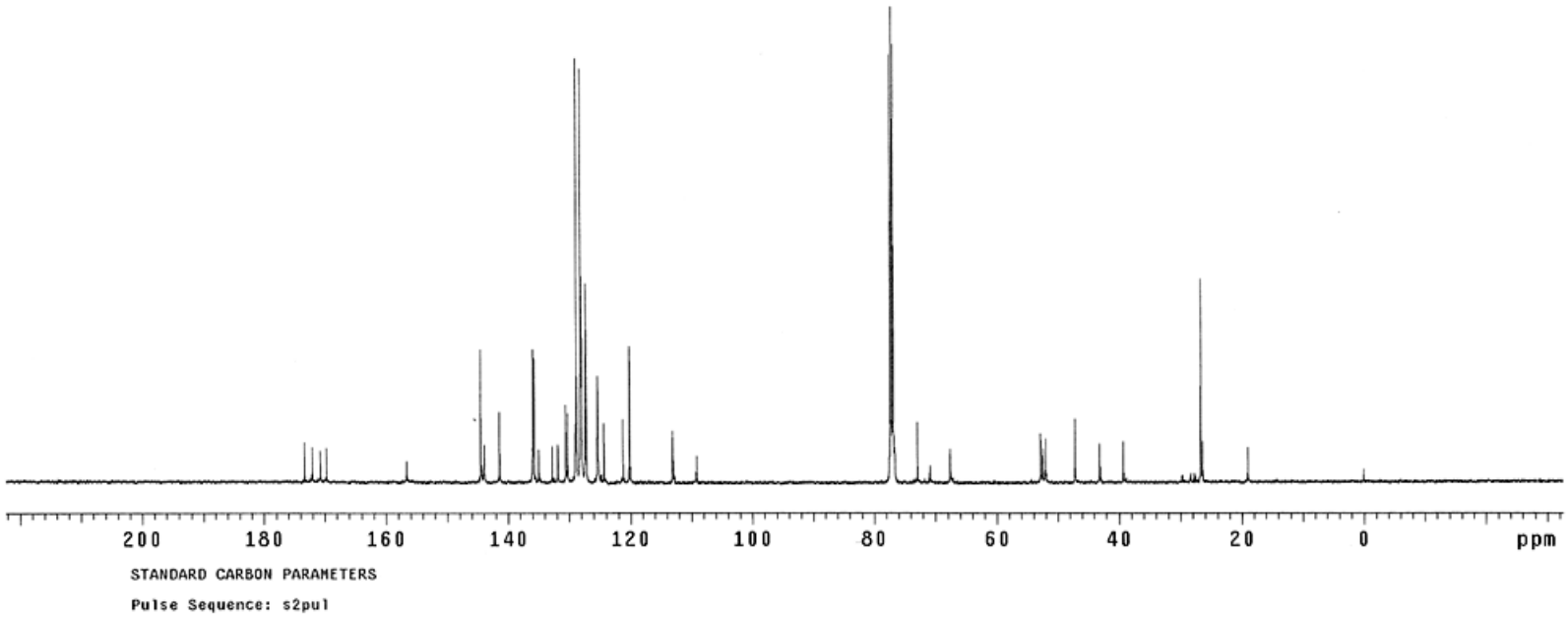

Br
OMe
O
HN
O
NH
NHFmoc
H
N
TBDPSO
O
O
NHTr

3.34

12
10
8
6
4
2
0
-2
ppm
ssc3-271-an
Pulse Sequence: s2pul

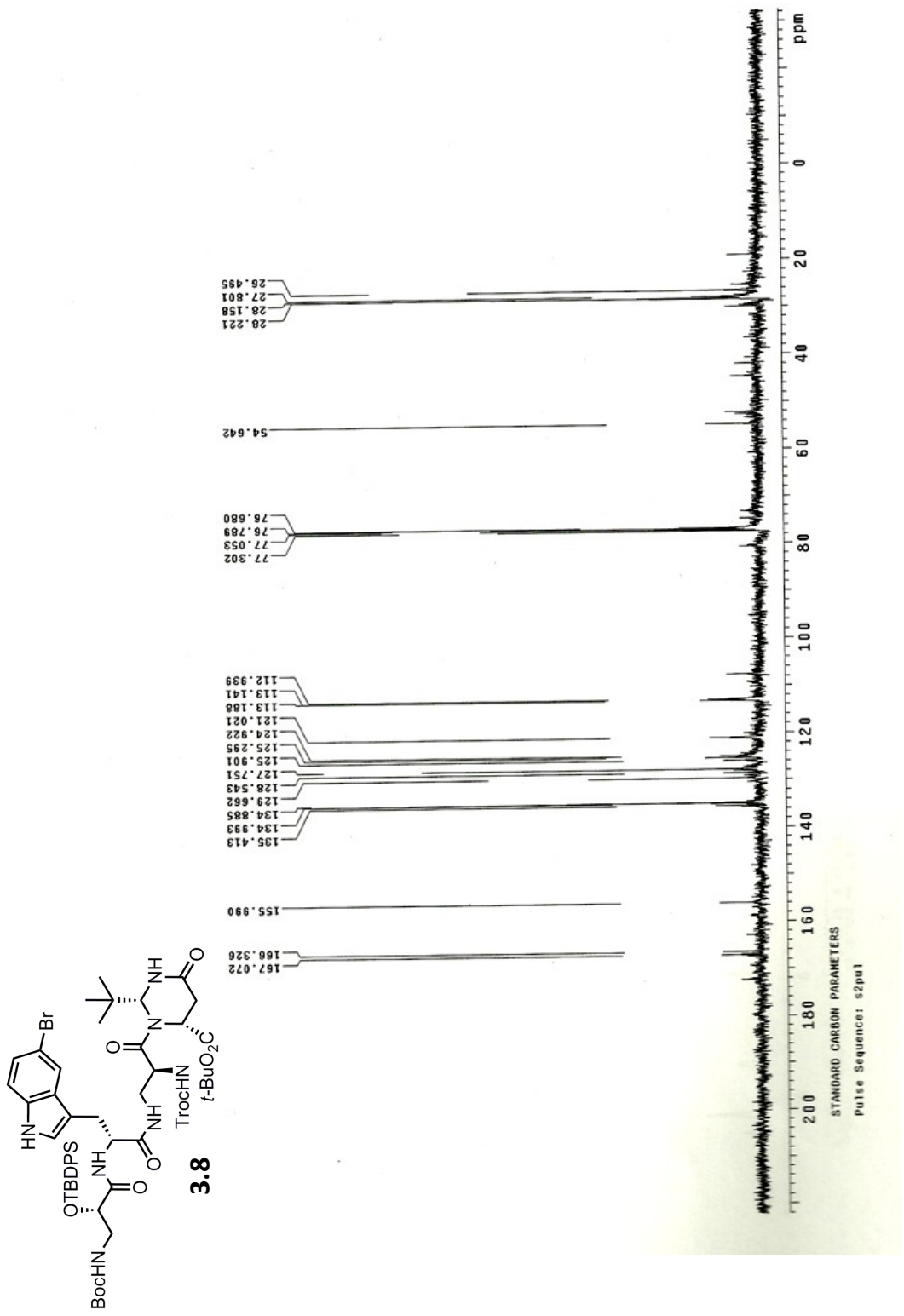

Br
OMe
O
HN
O
NH
NHFmoc
H
N
TBDPSO
O
O
NHTr

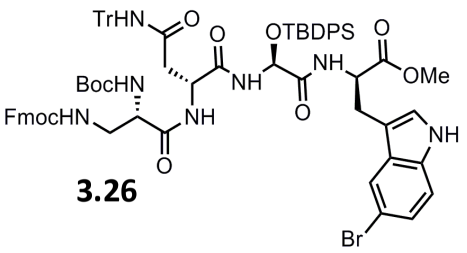
3.20


200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

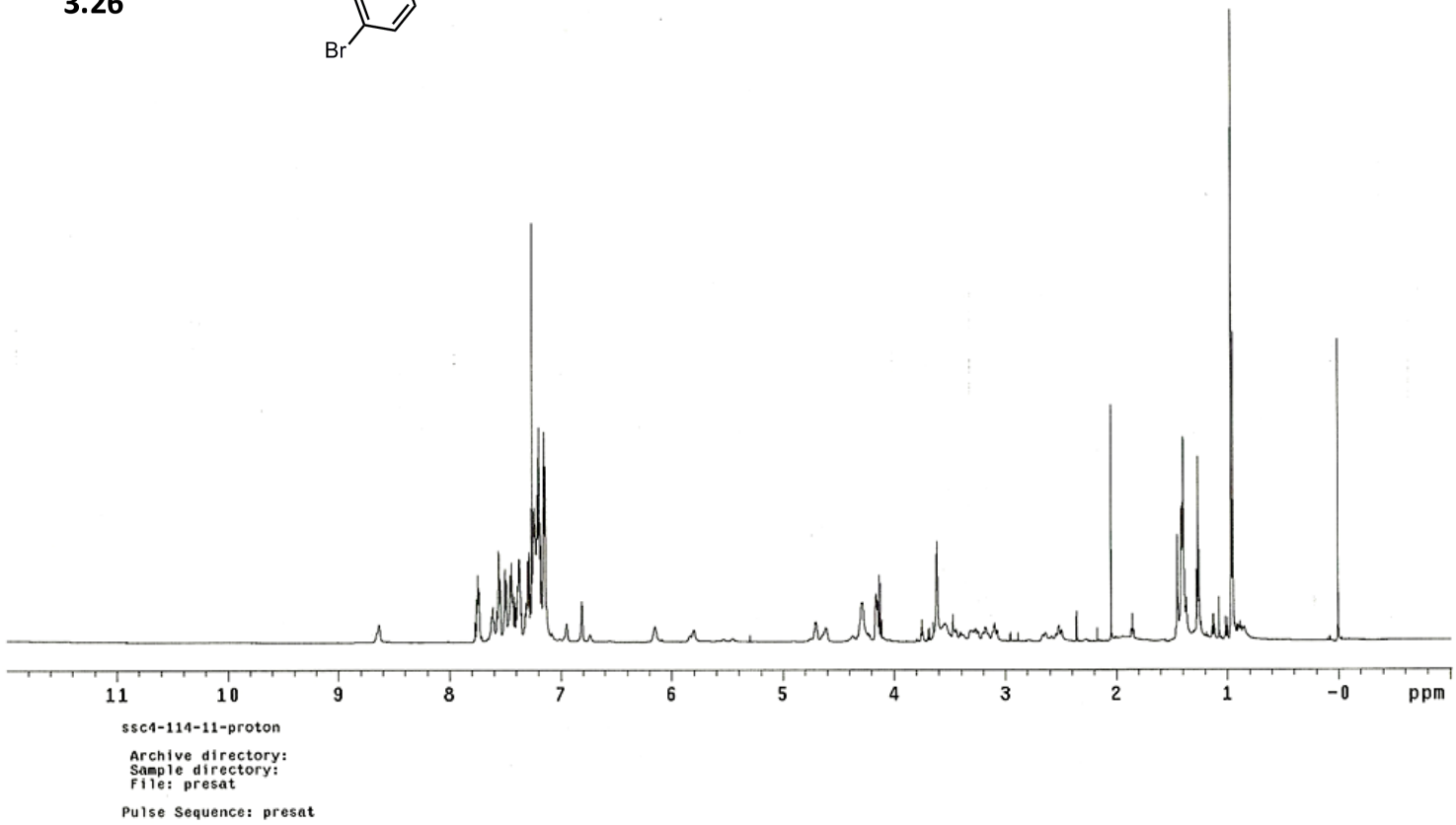

0.098
0.006
0.000
0.916
0.967
1.124
1.249
1.261
1.273
1.383
1.386
1.394
1.398
1.401
1.422
1.431
1.555
2.047
2.607
2.667
2.805
2.870
3.699
3.693
4.129
4.117
5.301
7.084
7.211
7.228
7.233
7.262
7.303
7.338
ppm
1
2
3
4
5
6
7
8
9
HN
Br
OTBDPS
NH
O
BocHN
O
NH
O
N
TrocHN
t-BuO_2C
NH
O

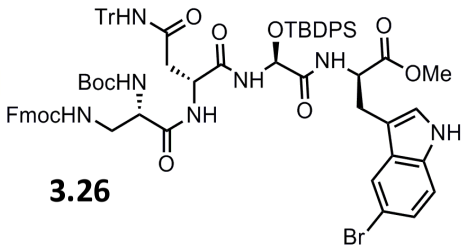
3.8


BocHN
OTBDPS
HN
Br
NH
O
NH
O
TrocHN
t-BuO2C
N
NH
O
3.8
167.072
166.326
155.990
135.413
134.993
134.885
129.662
128.543
127.751
125.901
125.295
124.922
121.021
119.188
113.141
112.939
77.302
77.053
76.769
76.680
54.642
28.221
28.158
27.801
26.495
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

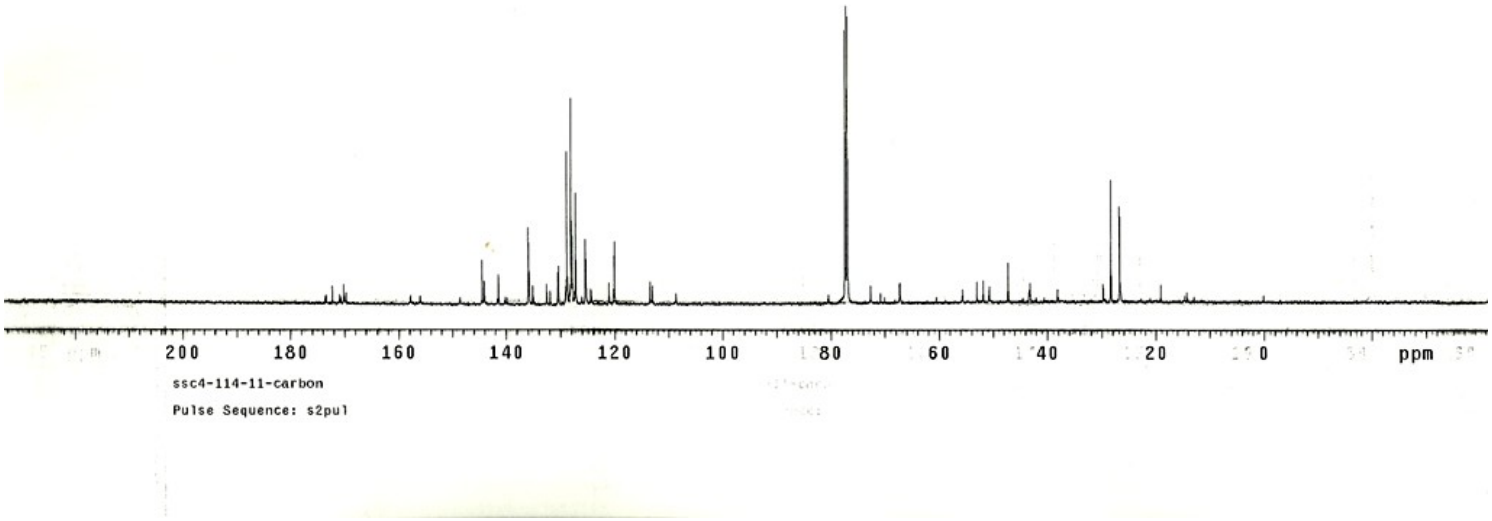

TrHN
O
O
OTBDPS
O
NH
NH
OMe
BocHN
FmocHN
NH
O
O
O
NH
Br

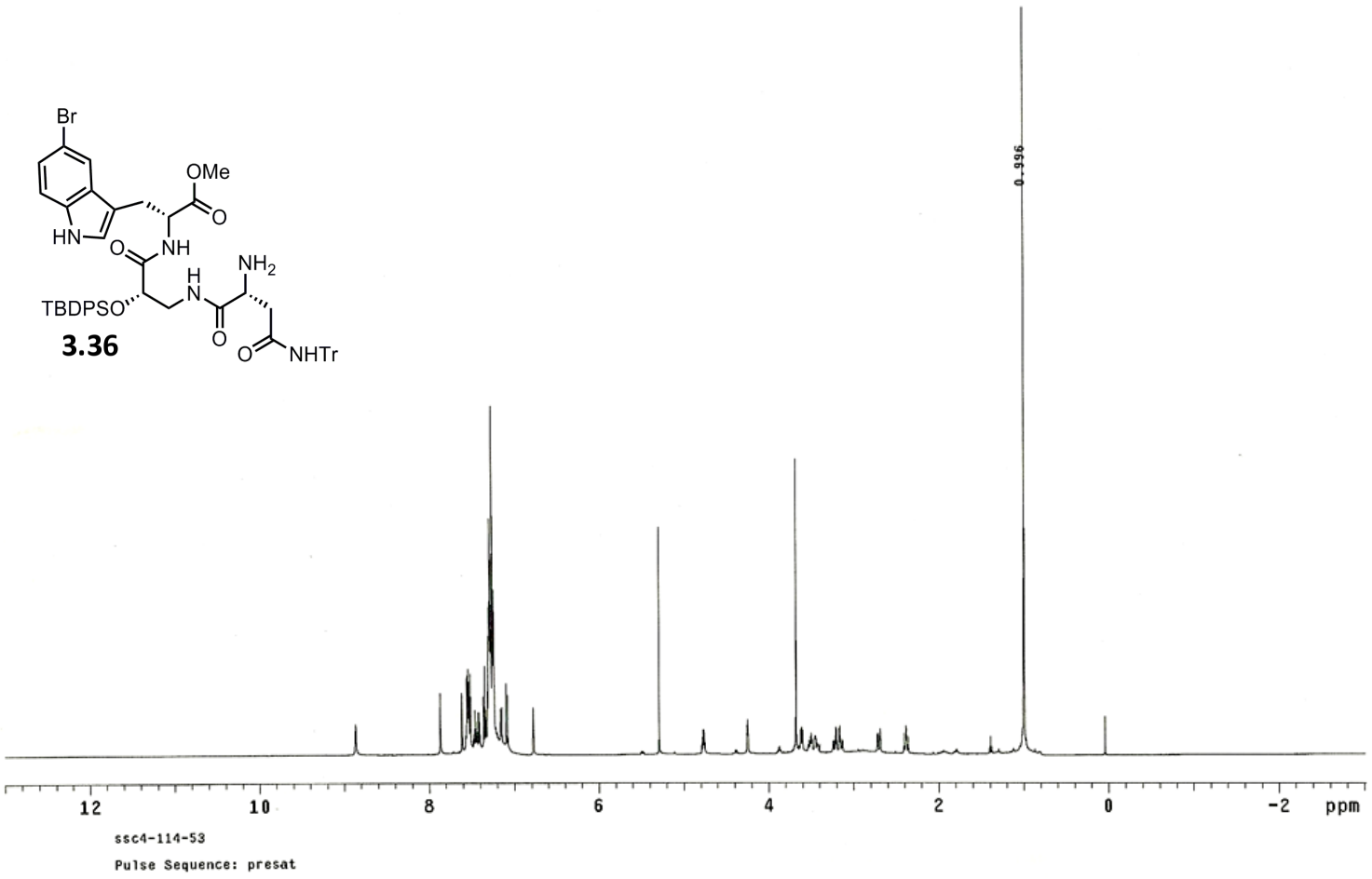
3.26

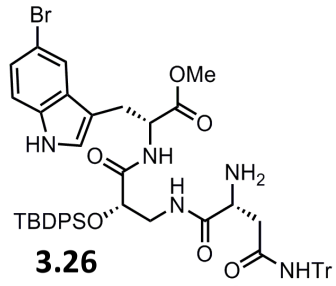

11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc4-114-11-proton
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat


TrHN
O
O
OTBDPS
O
NH
NH
OMe
BocHN
NH
FmocHN
O
O
NH
O
Br

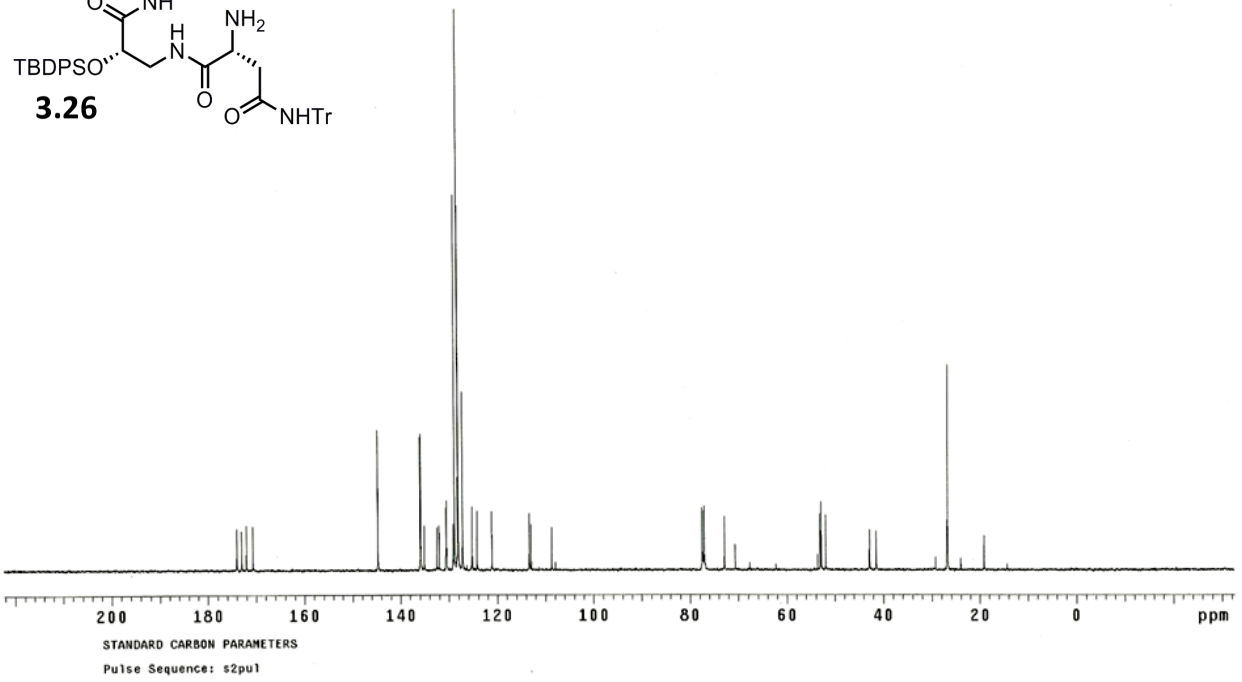
3.26

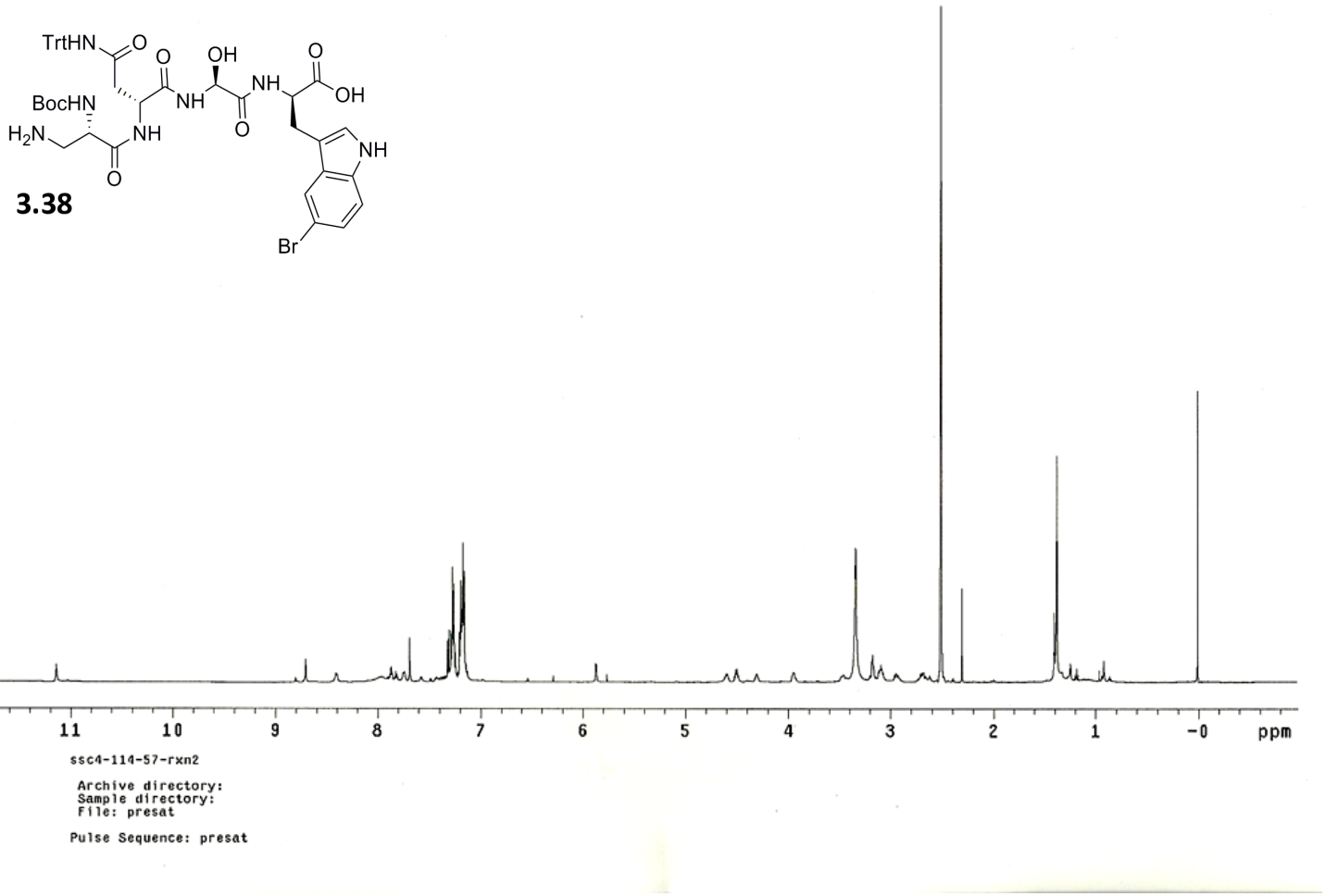

200
180
160
140
120
100
80
60
40
20
0
ppm
ssc4-114-11-carbon
Pulse Sequence: s2pul

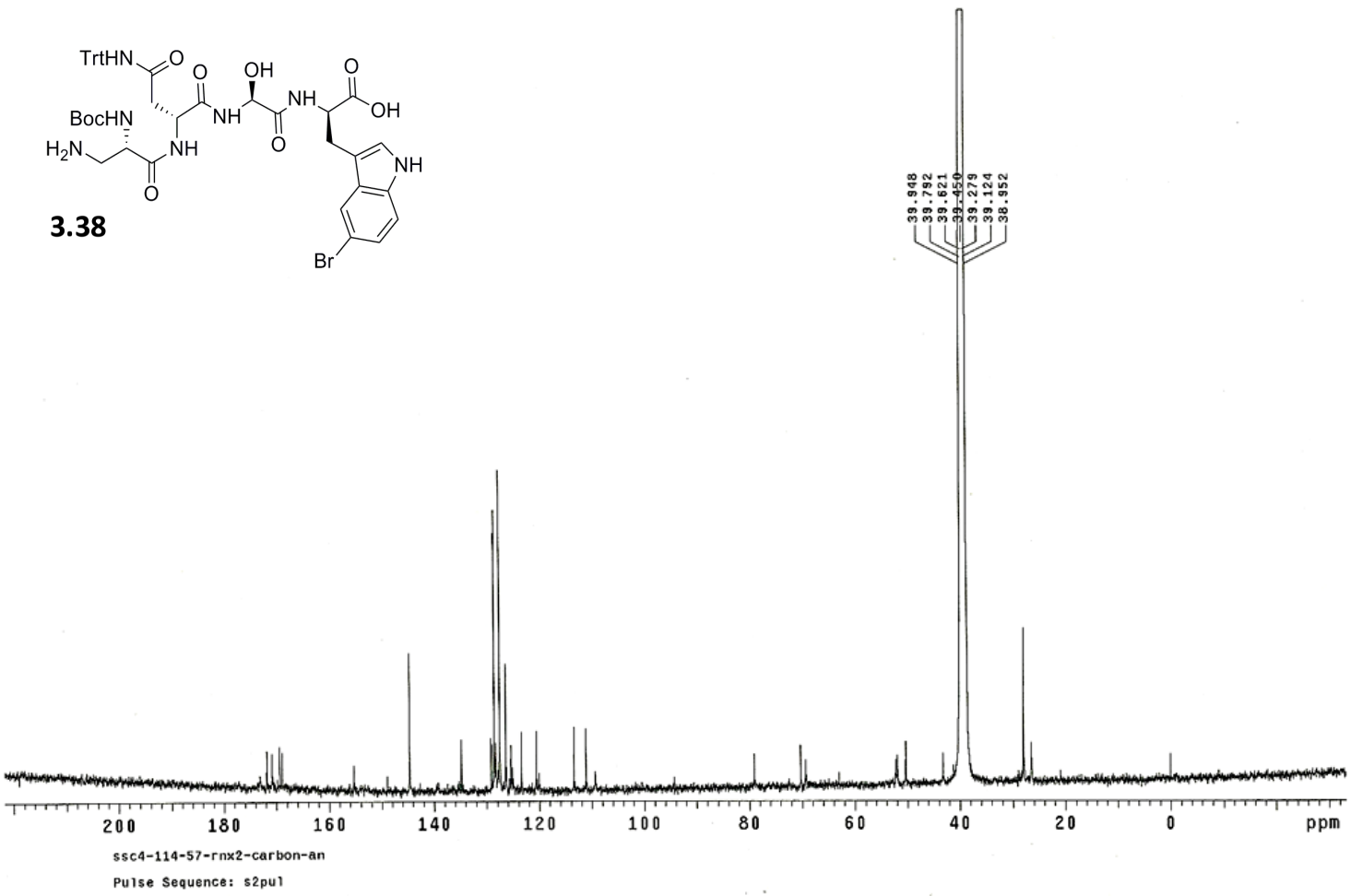

Br
OMe
O
HN
O
NH
NH2
H
N
TBDPSO
O
O
NHTr
3.36
0.996
12
10
8
6
4
2
0
-2
ppm
ssc4-114-53
Pulse Sequence: presat

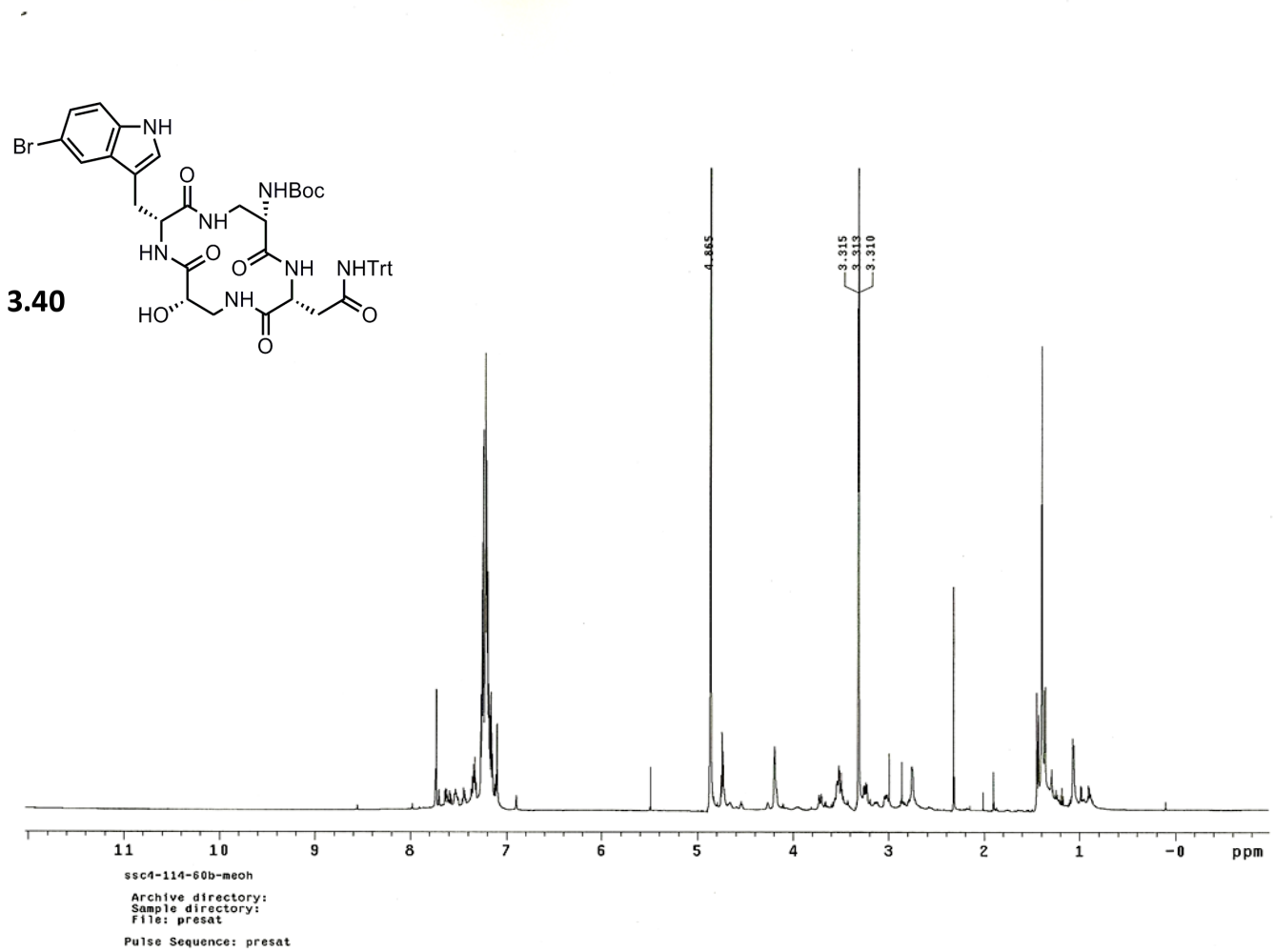

Br
OMe
O
HN
O
NH
H
N
NH2
TBDPSO
O
O
NHTr

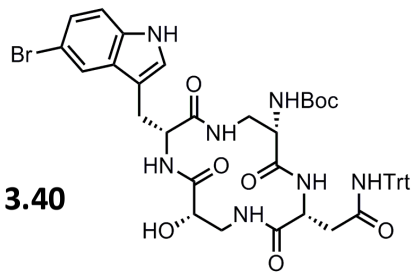
3.26


200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul


TrtHN
O
O
OH
NH
O
BocHN
NH
OH
H_2N
NH
O
O
NH
3.38
Br
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc4-114-57-rxn2
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat

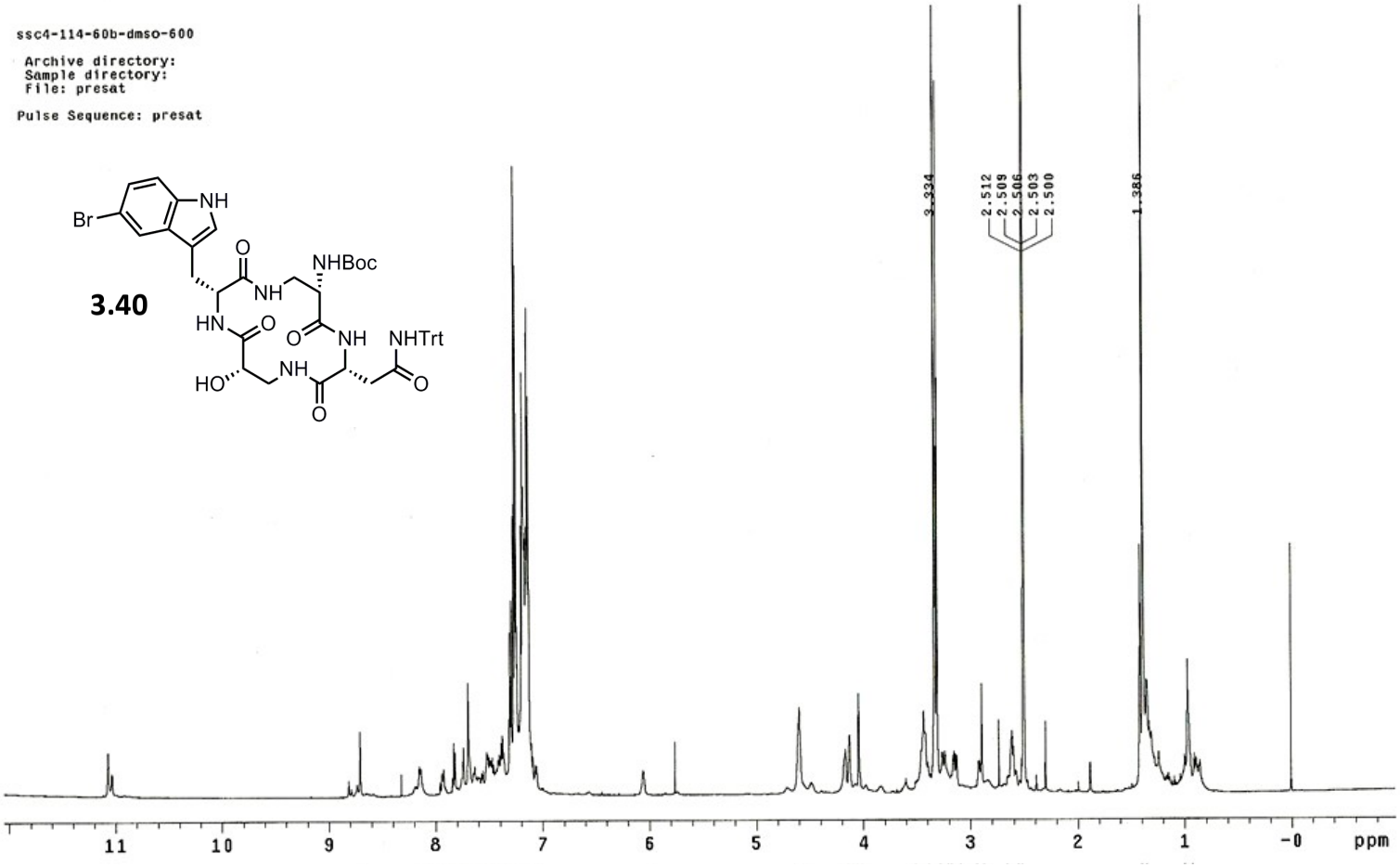

TrtHN
O
BocHN
H2N
NH
O
O
NH
OH
O
NH
O
OH
NH
Br
3.38
39.948
39.792
39.621
39.450
39.279
39.124
38.952
200
180
160
140
120
100
80
60
40
20
0
ppm
ssc4-114-57-rnx2-carbon-an
Pulse Sequence: s2pul

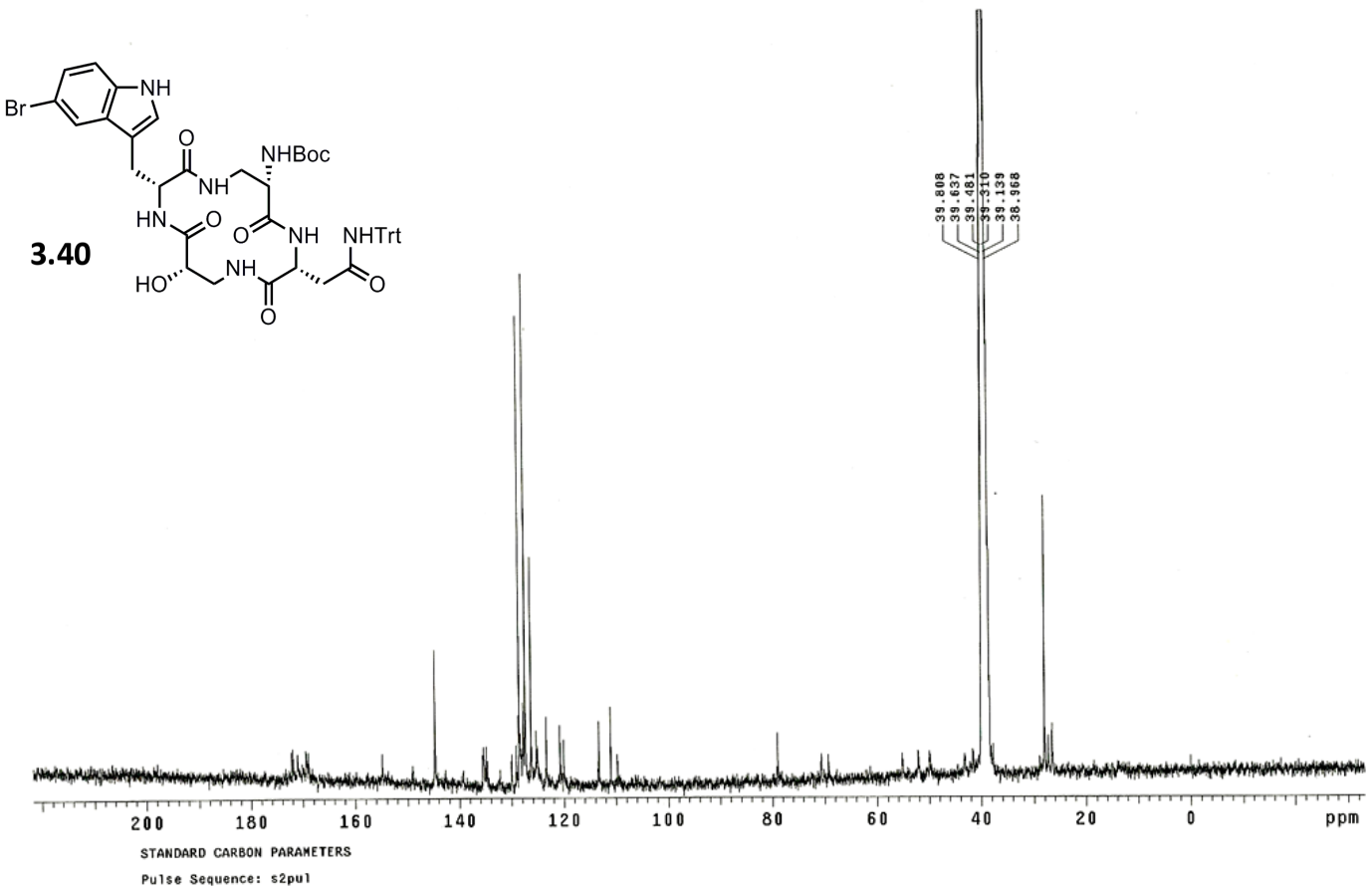

3.40
Br
NH
O
NHBoc
NH
HN
O
O
NH
NHTrt
HO
NH
O
O
4.865
3.315
3.313
3.310
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc4-114-60b-meoh
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat

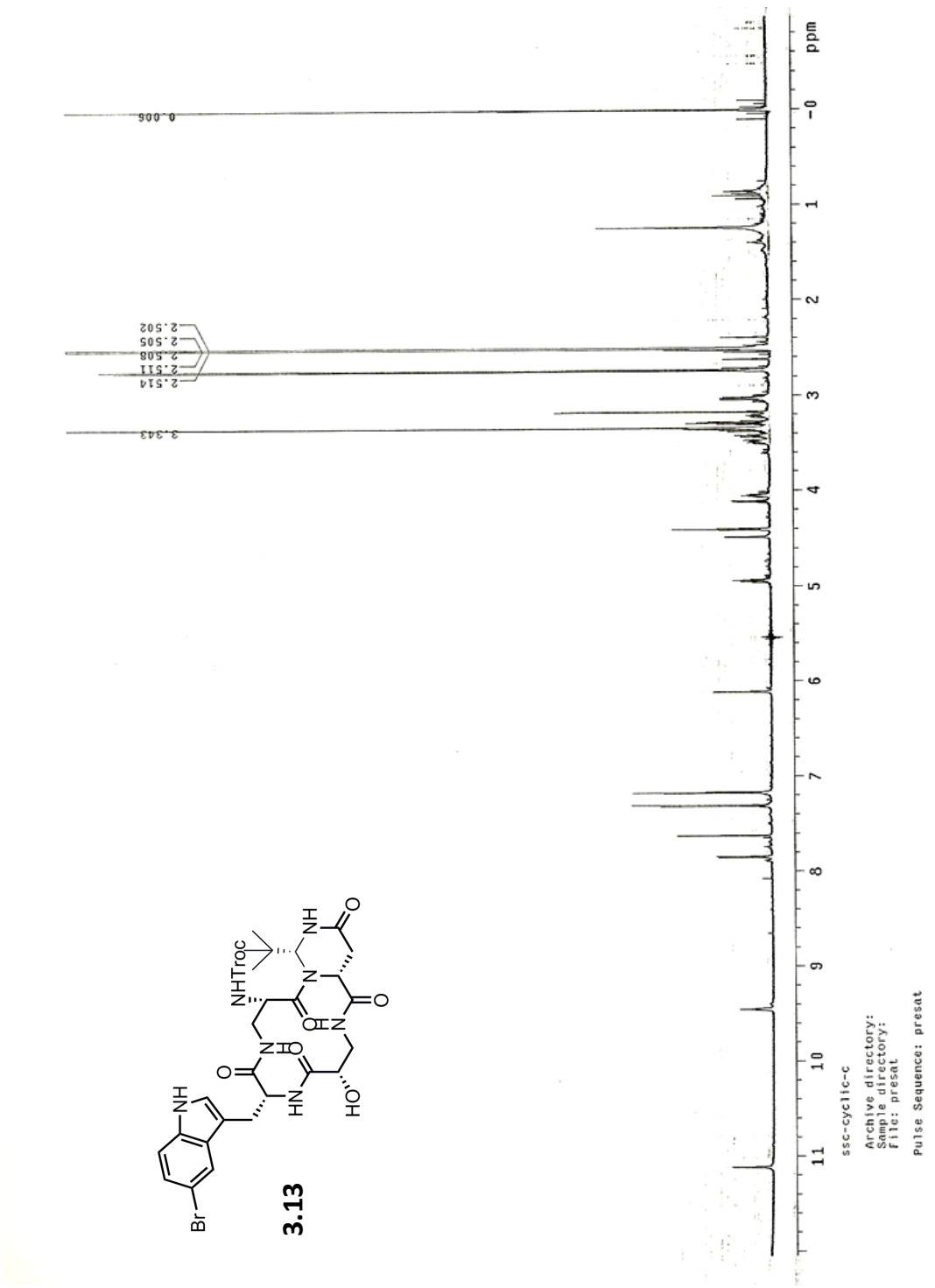

Br
NH
O
NH
NHBoc
HN
O
O
NH
NHTrt
HO
NH
O
O

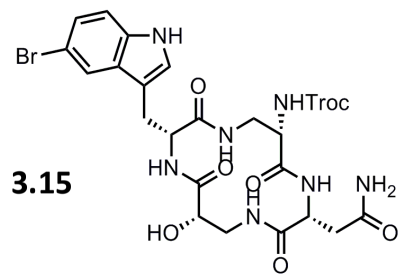
3.40

47.845
47.668
47.508
47.331
47.155
220
200
180
160
140
120
100
80
60
40
20
0
-20
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul


ssc4-114-60b-dmso-600
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat
3.40
Br
NH
O
NH
NHBoc
HN
O
O
NH
NHTrt
HO
NH
O
O
3.334
2.512
2.509
2.506
2.503
2.500
1.386
11
10
9
8
7
6
5
4
3
2
1
-0
ppm

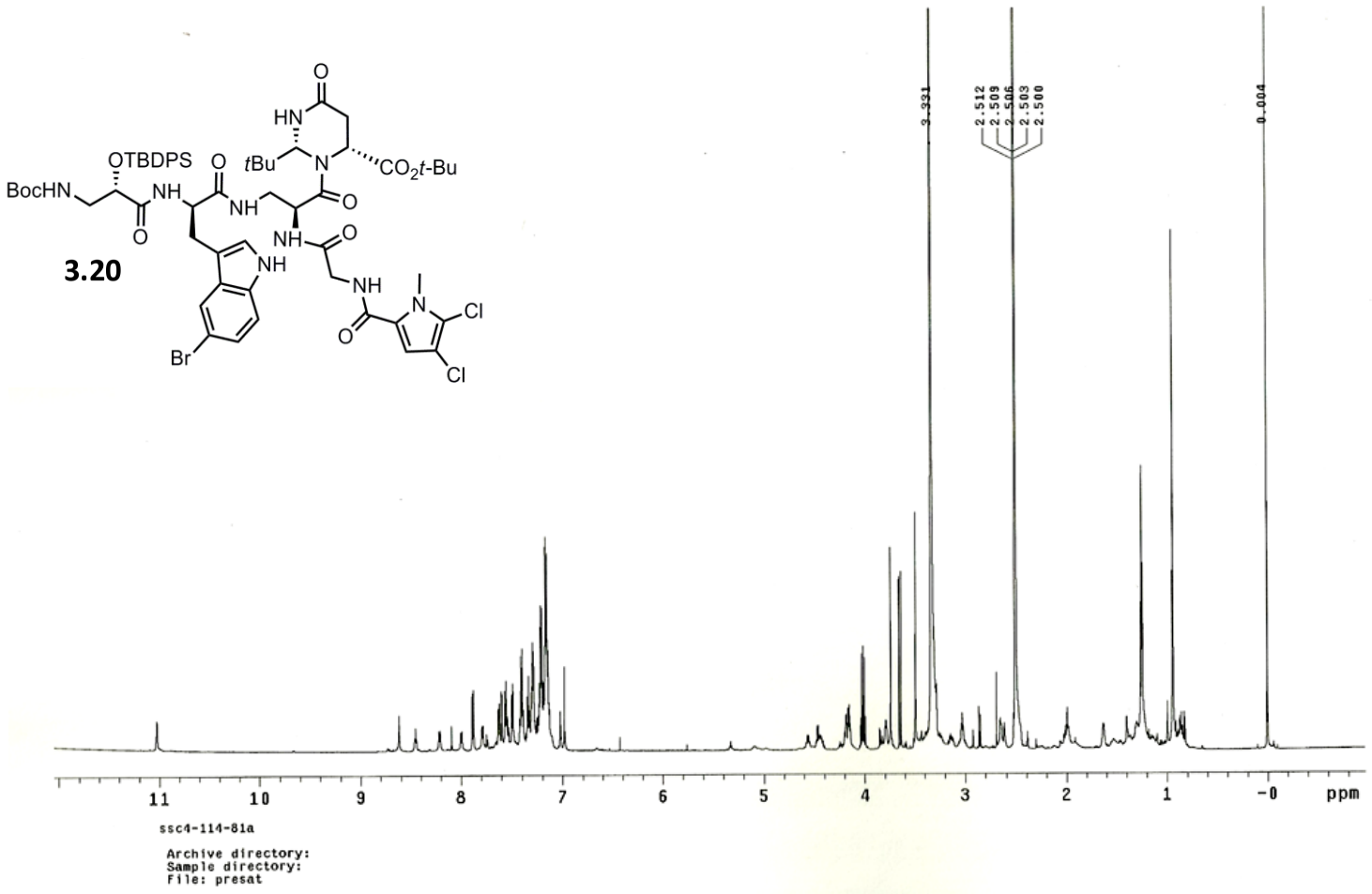

Br
NH
O
NHBoc
NH
HN
O
O
NH
NHTrt
3.40
HO
NH
O
O
39.808
39.637
39.481
39.310
39.139
38.966
200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul


NHTroc
NH
O
N
HN
HO
Br
3.13
0.006
3.343
2.514
2.511
2.508
2.505
2.502
ppm
ssc-cyclic-c
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat

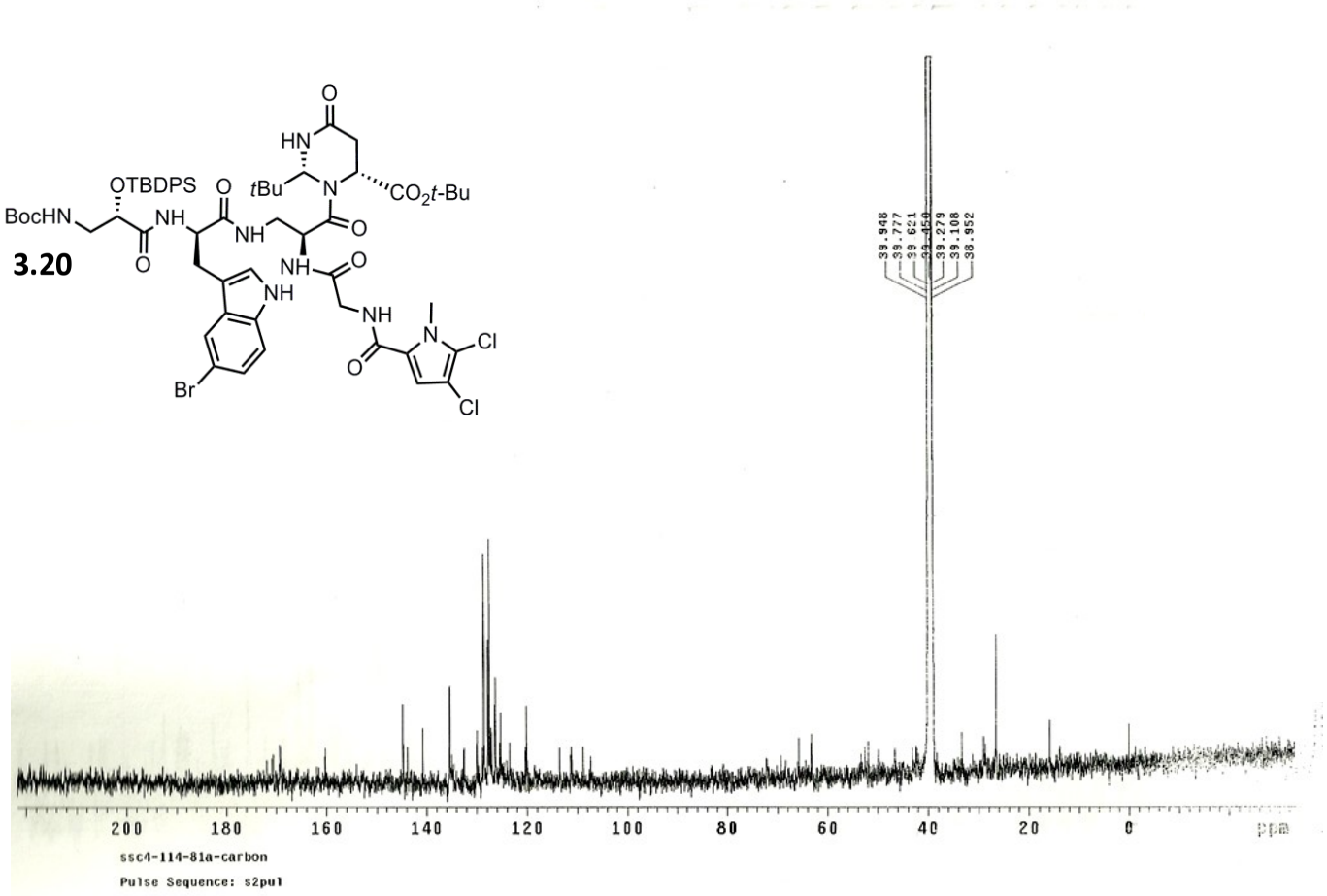

Br
NH
O
NHTroc
N
H
HN
O
O
NH
NH2
N
H
HO
O
O

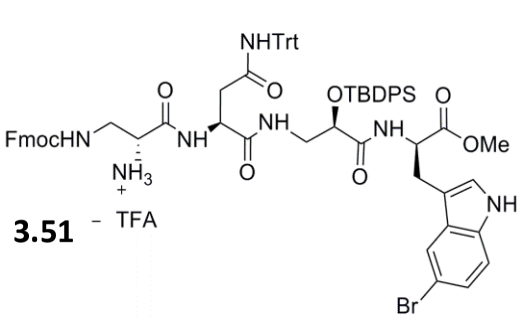
3.15

11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc-cyclic-a
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat


3.331
2.512
2.509
2.506
2.503
2.500
0.004
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc4-114-81a
Archive directory:
Sample directory:
File: presat

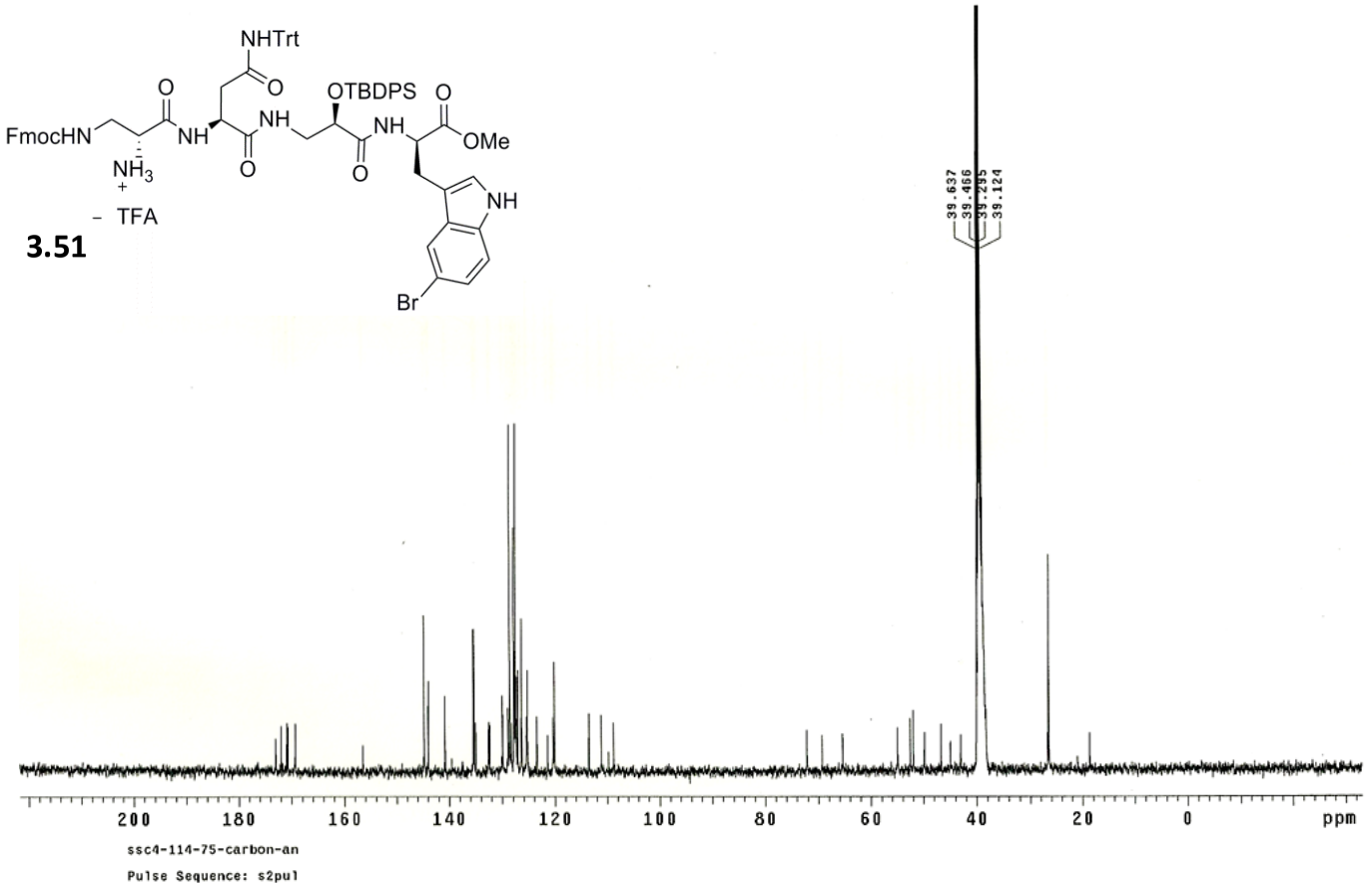
3.20

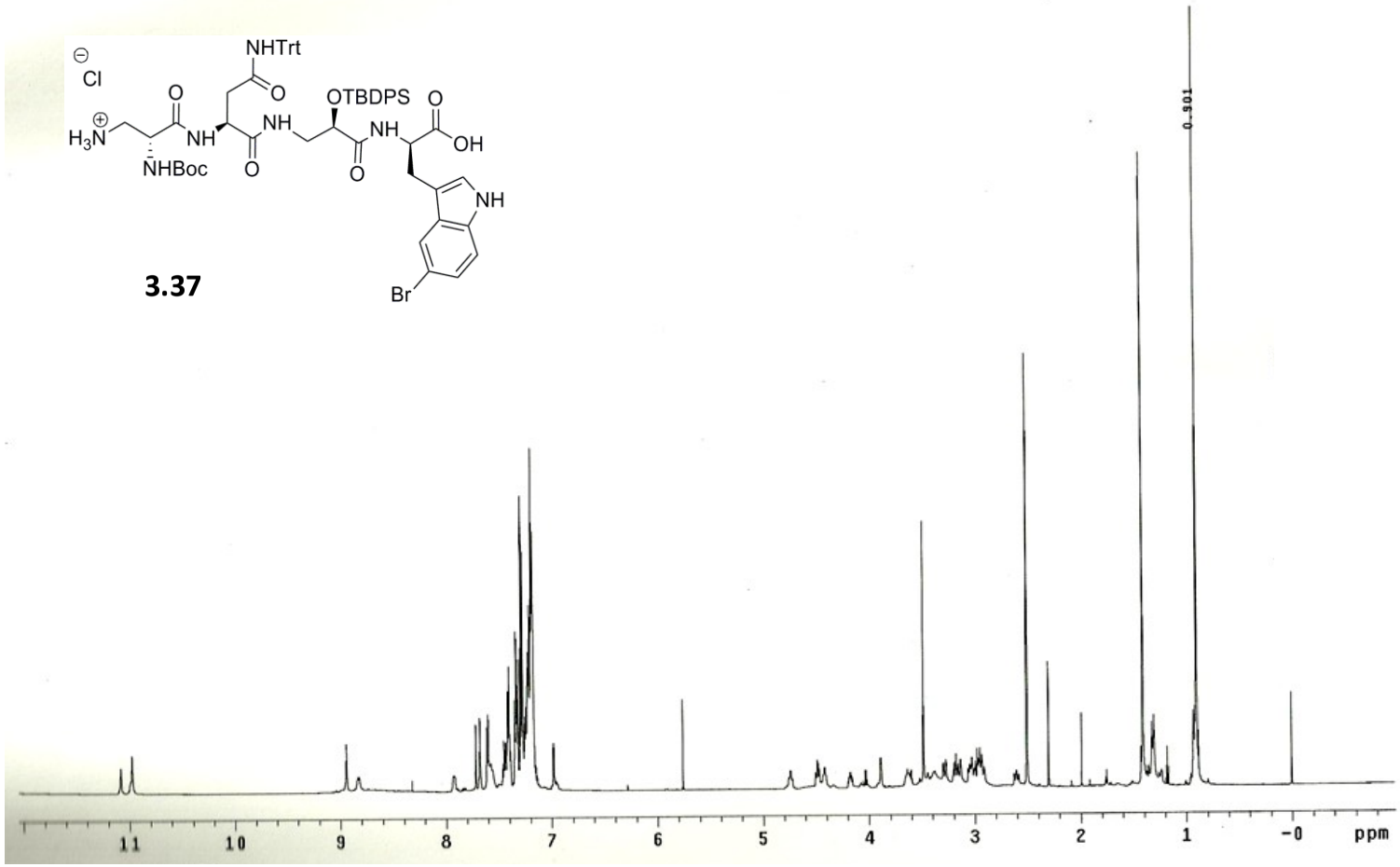

3.20
BocHN
OTBDPS
NH
O
NH
tBu
HN
N
CO2t-Bu
NH
NH
NH
N
Cl
Cl
Br
39.948
39.777
39.621
39.450
39.279
39.108
38.952
200
180
160
140
120
100
80
60
40
20
0
ppm
ssc4-114-81a-carbon
Pulse Sequence: s2pul

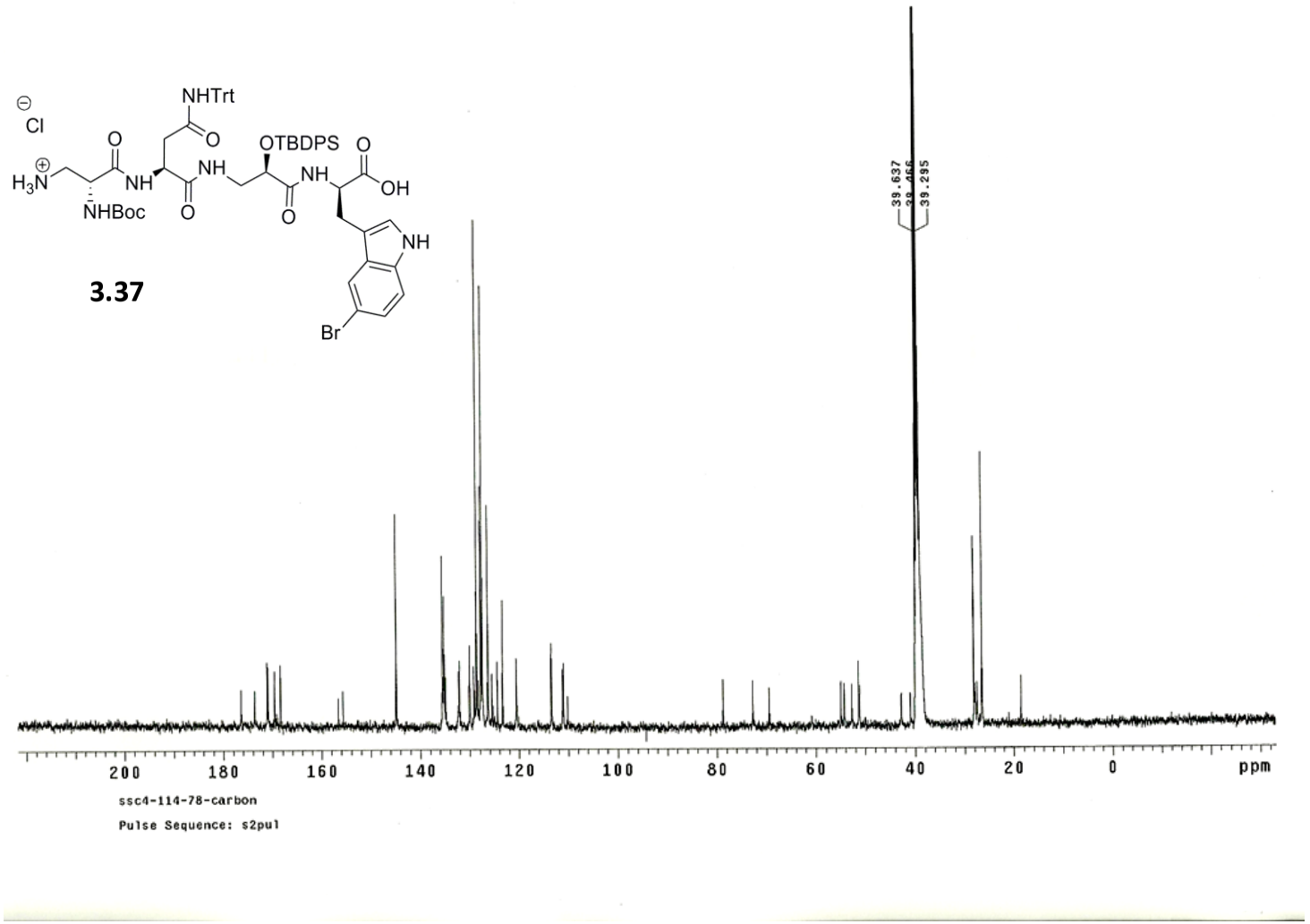

NHTrt
O
O
OTBDPS
O
FmocHN
NH
NH
NH
OMe
O
O
O
NH3
+
NH
Br

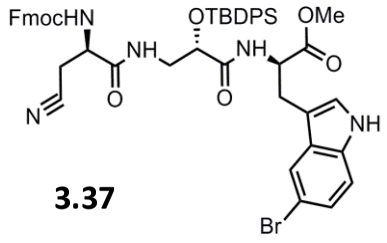
3.51 – TFA

11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc4-114-75
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat

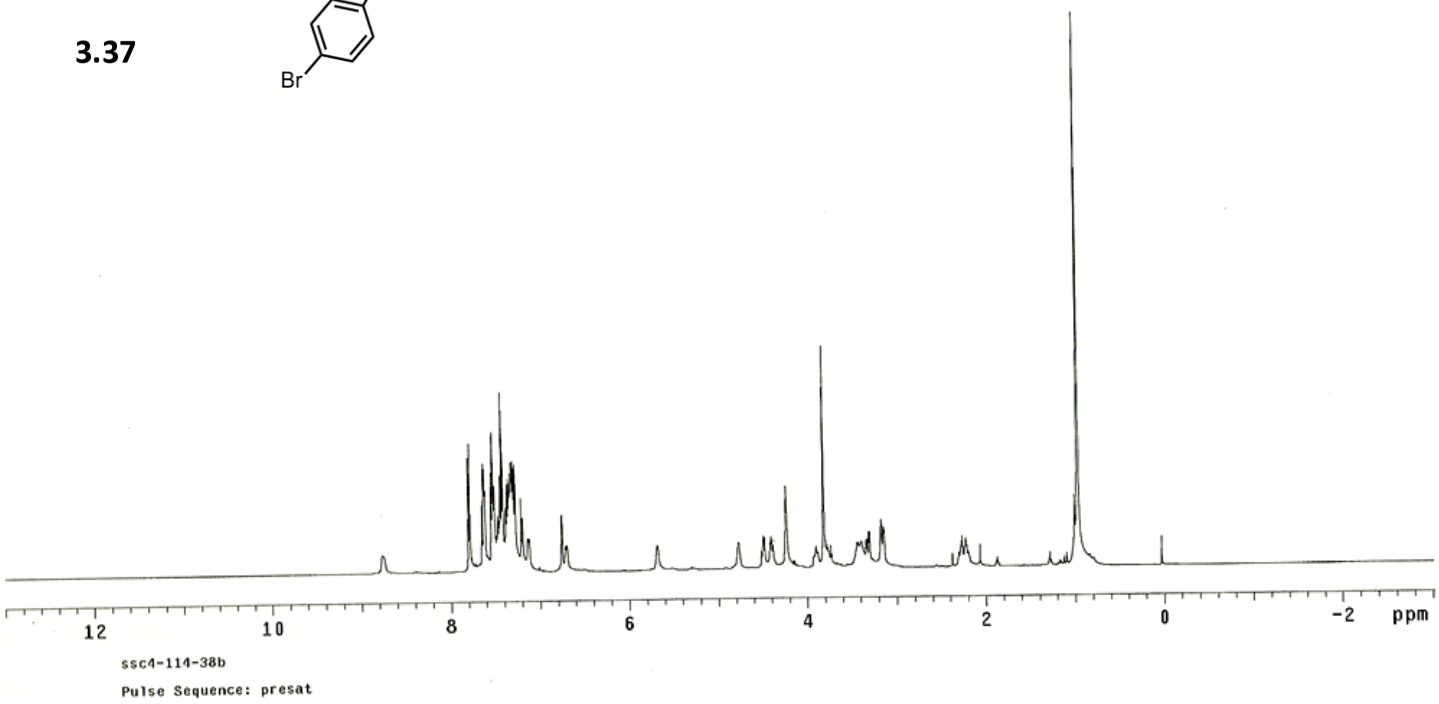

NHTrt
OTBDPS
FmocHN
NH
NH
NH
OMe
NH3
+
– TFA
3.51
NH
Br
39.637
39.466
39.295
39.124
200
180
160
140
120
100
80
60
40
20
0
ppm
ssc4-114-75-carbon-an
Pulse Sequence: s2pul

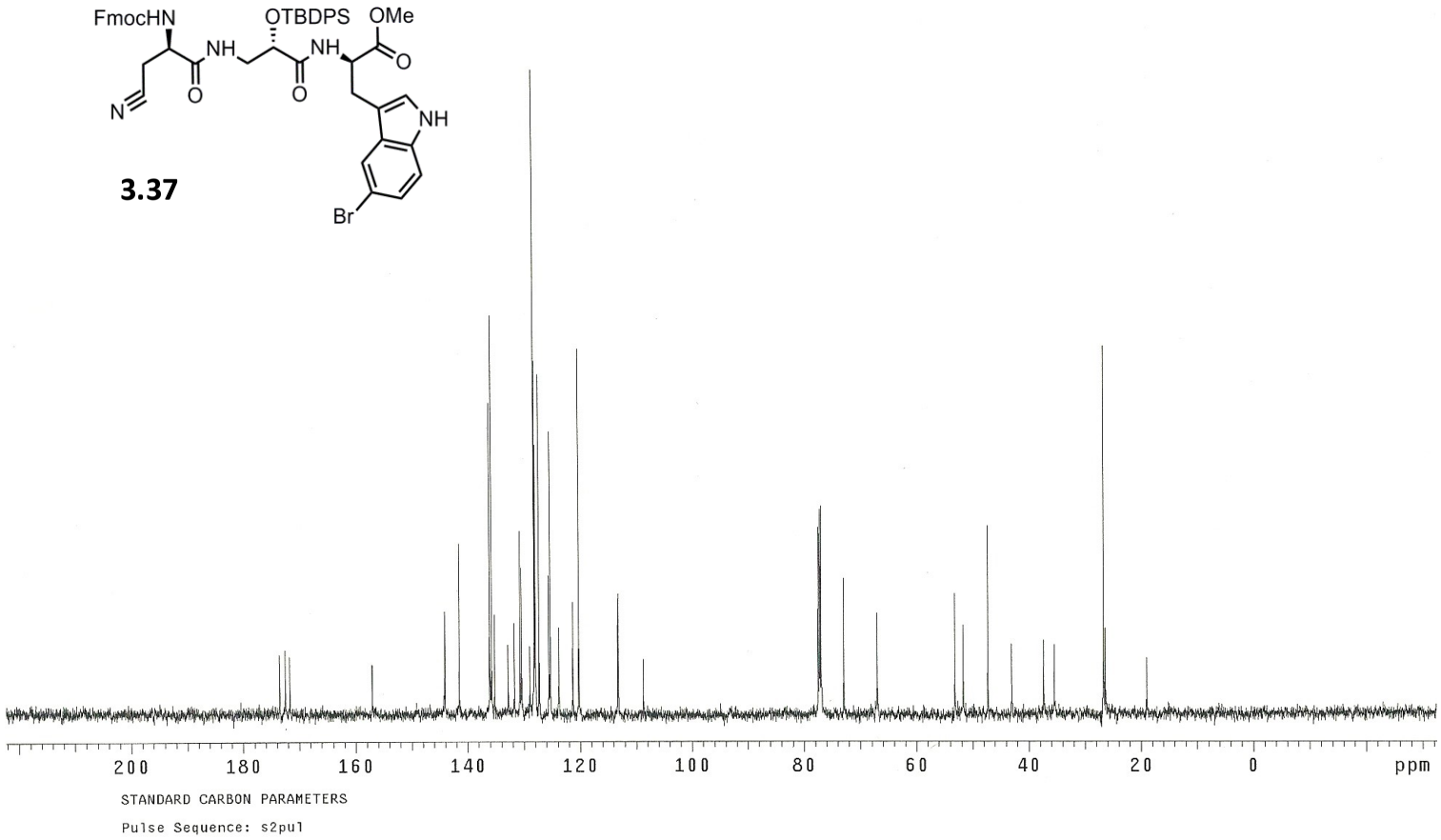

NHTrt
Cl
OTBDPS
OH
NHBoc
NH
Br
3.37
0.901
11
10
9
8
7
6
5
4
3
2
1
-0
ppm

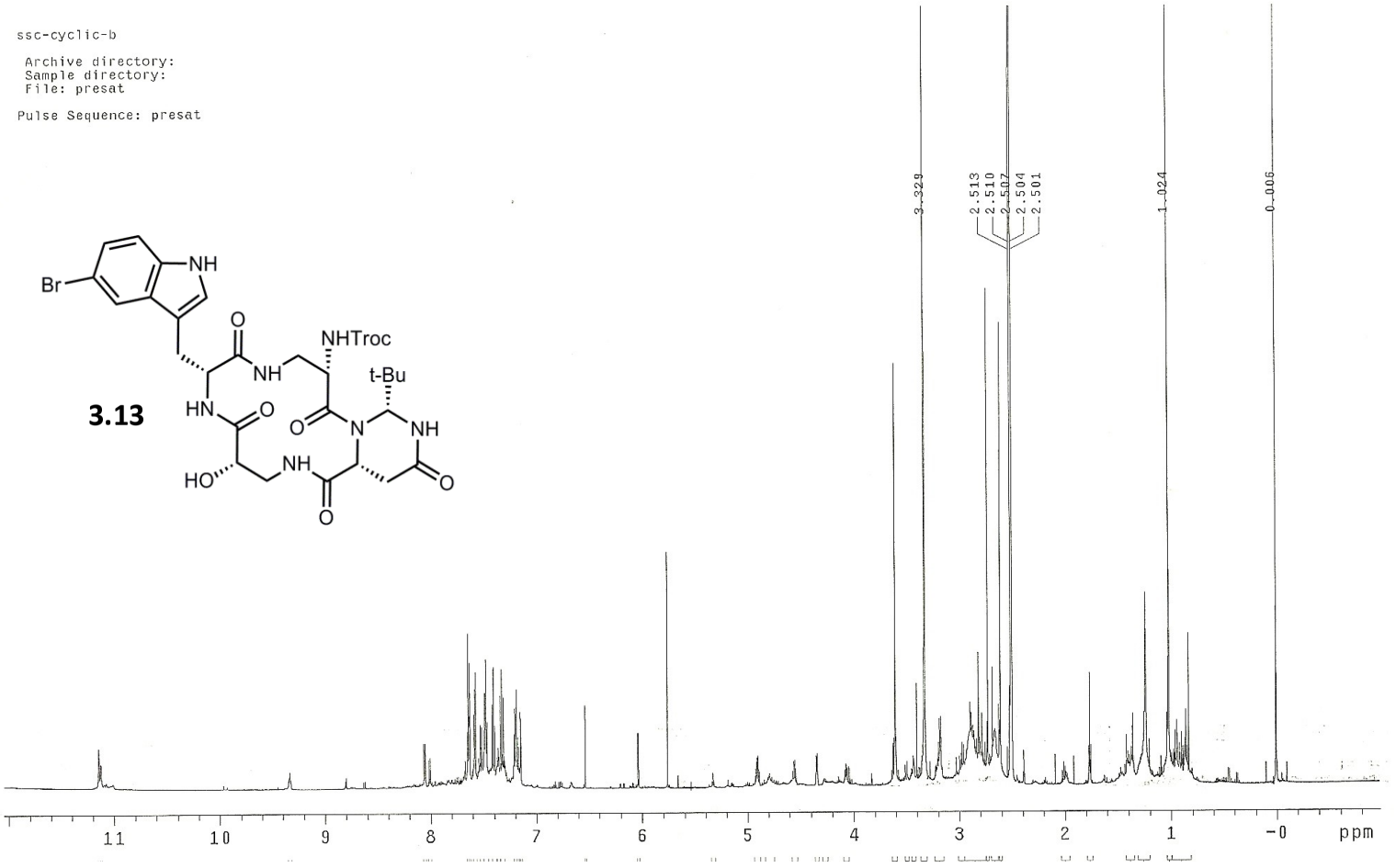

NHTrt
Cl
OTBDPS
OH
NHBoc
NH
Br
3.37
39.637
39.466
39.295
200
180
160
140
120
100
80
60
40
20
0
ppm
ssc4-114-78-carbon
Pulse Sequence: s2pul

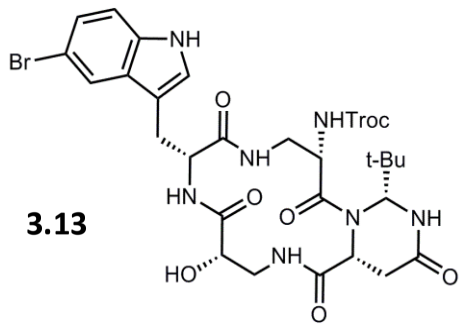

FmocHN
OTBDPS
OMe
NH
NH
O
O
O
N
NH
Br

3.37


12
10
8
6
4
2
0
-2
ppm
ssc4-114-38b
Pulse Sequence: presat

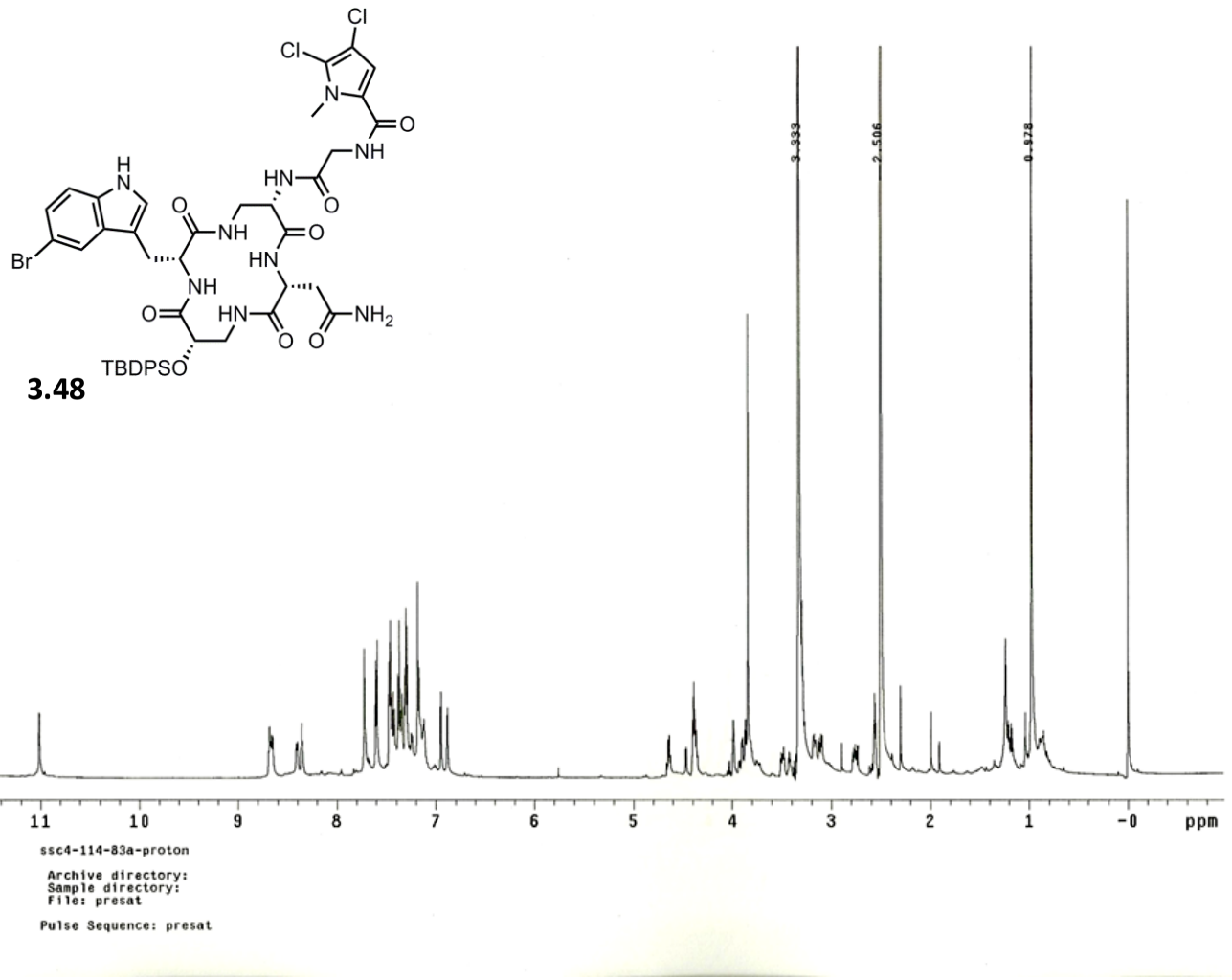

FmocHN
OTBDPS
OMe
NH
NH
O
O
O
N
NH
Br
3.37
200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

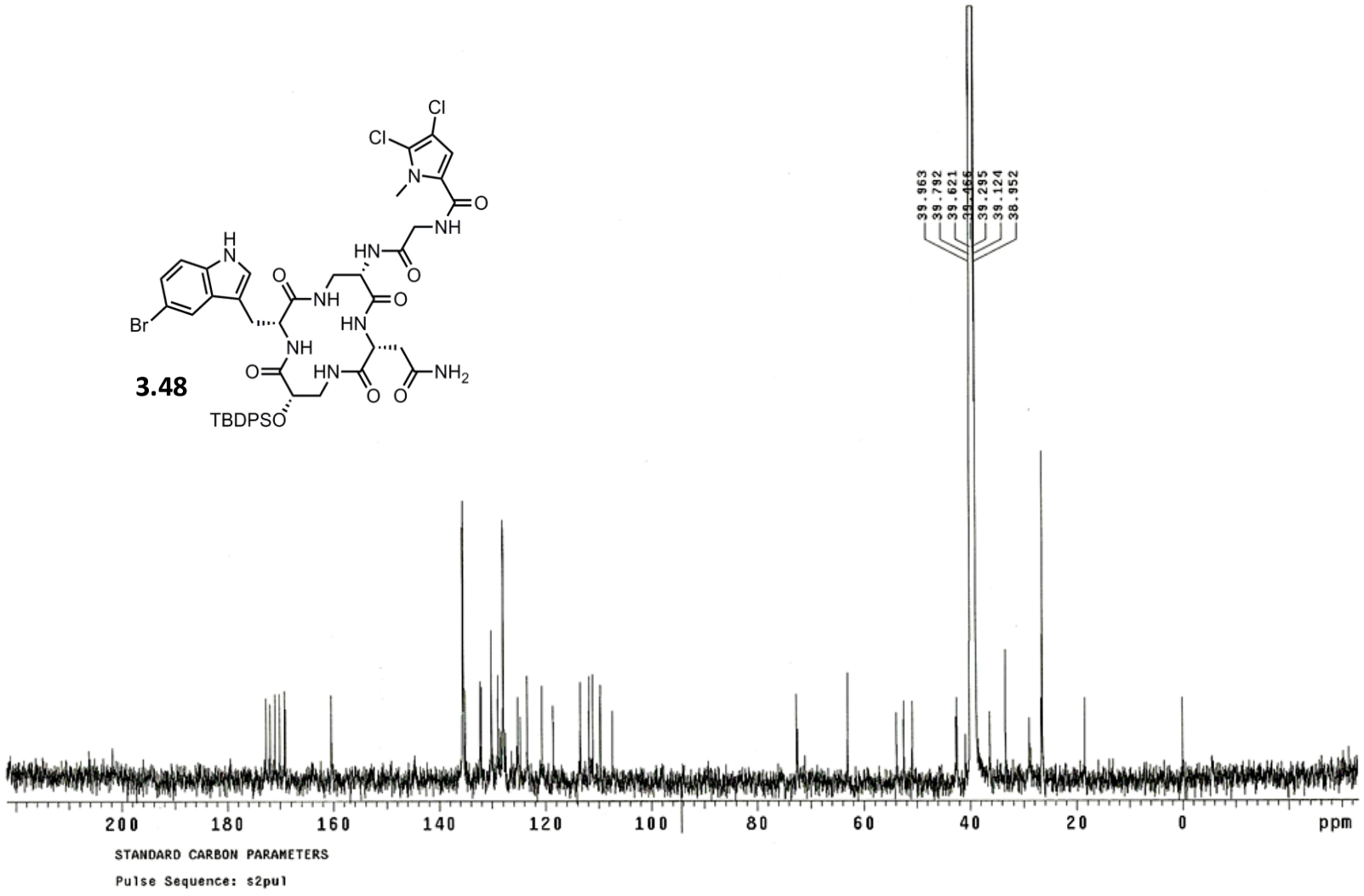

ssc-cyclic-b
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat
3.13
Br
NH
O
NH
NHTroc
t-Bu
HN
O
O
N
NH
HO
NH
O
O
3.329
2.513
2.510
2.507
2.504
2.501
1.024
0.006
11
10
9
8
7
6
5
4
3
2
1
-0
ppm

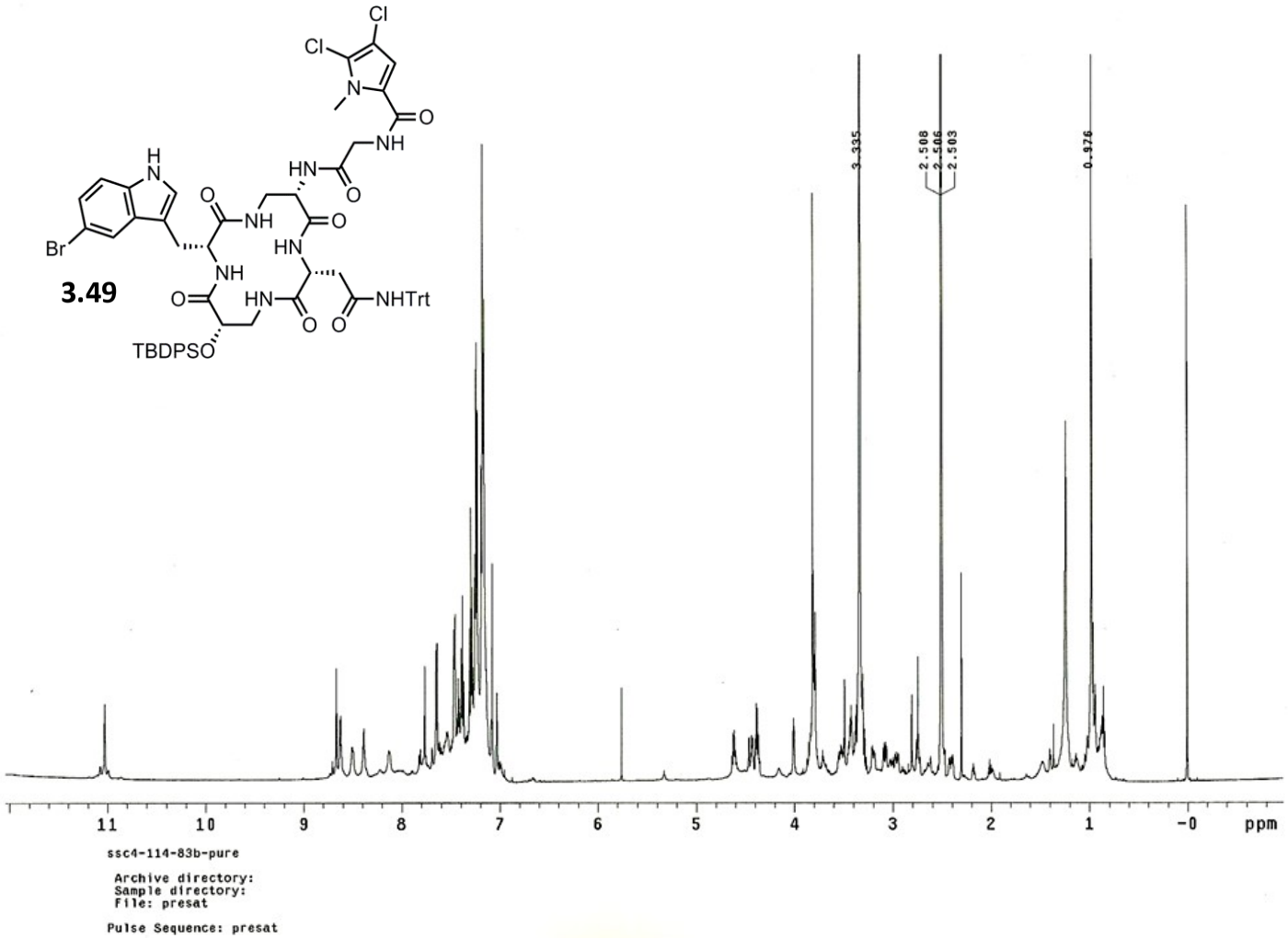

Br
NH
O
NH
NHTroc
t-Bu
HN
O
O
N
NH
HO
NH
O
O

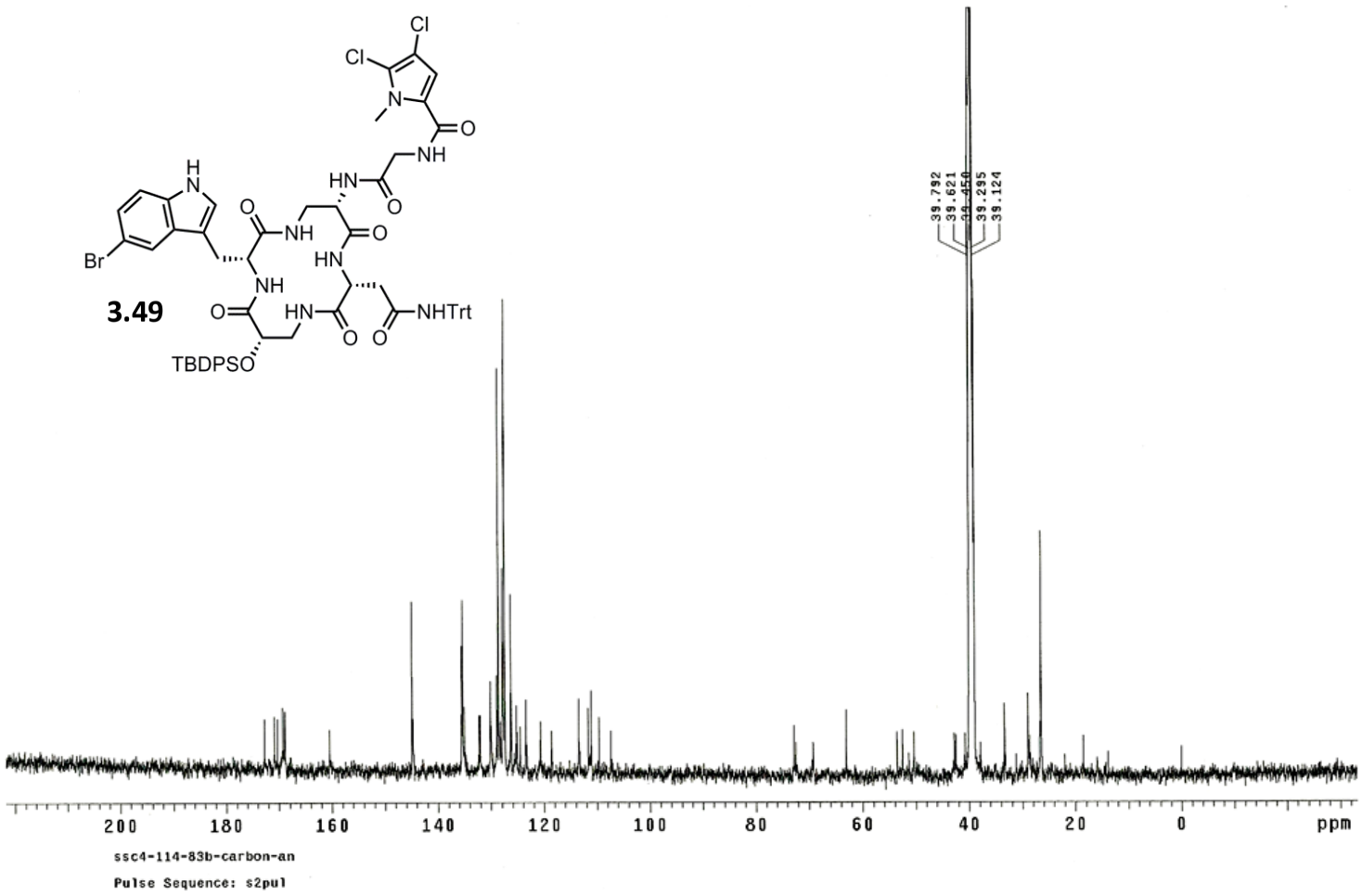
3.13

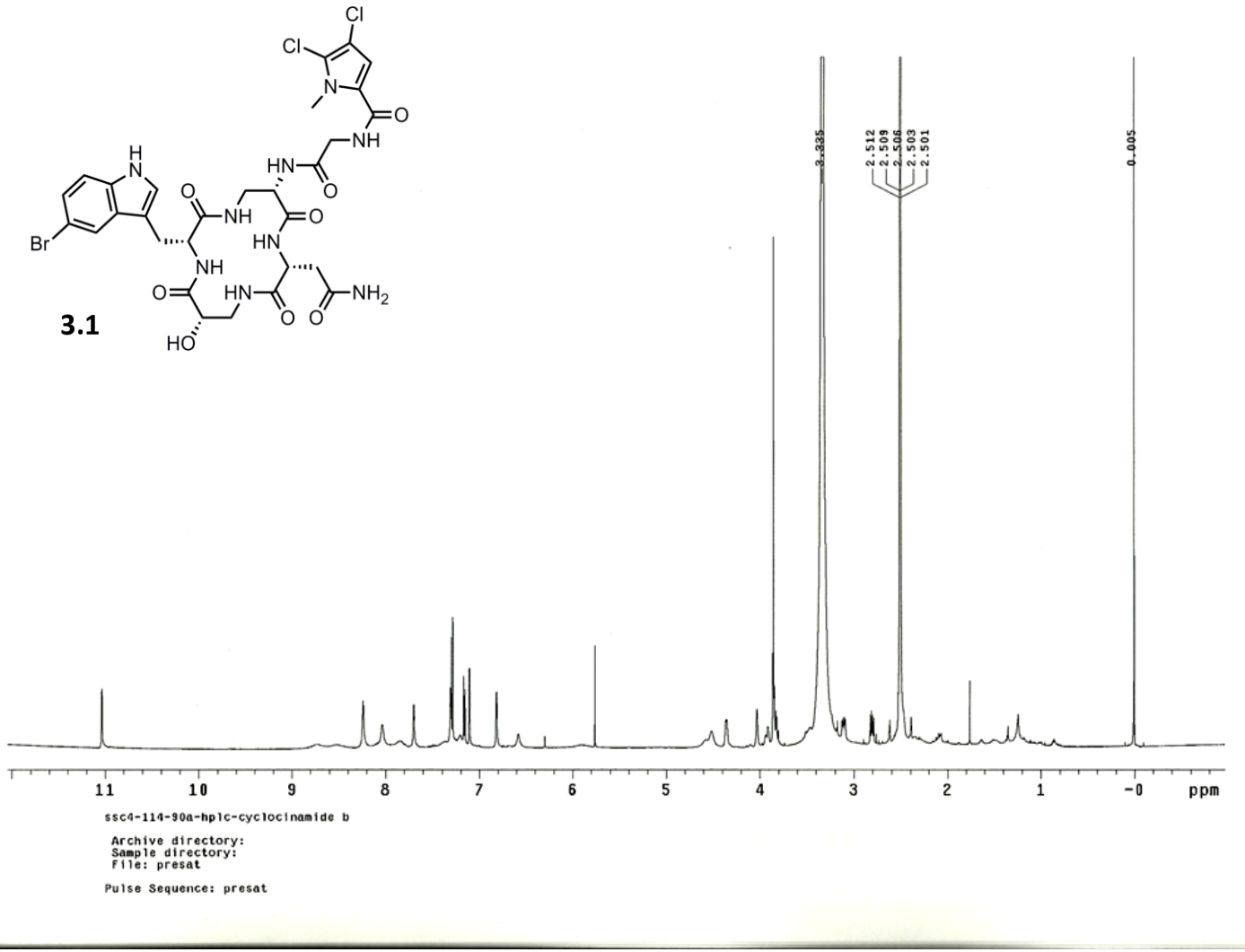
40.062
39.981
39.838
39.757
39.696
39.621
39.554
39.479
39.411
39.343
39.269
39.201
39.126
38.977
200
180
160
140
120
100
80
60
40
20
0
ppm
cyclic core with cycloasn
Pulse Sequence: s2pul

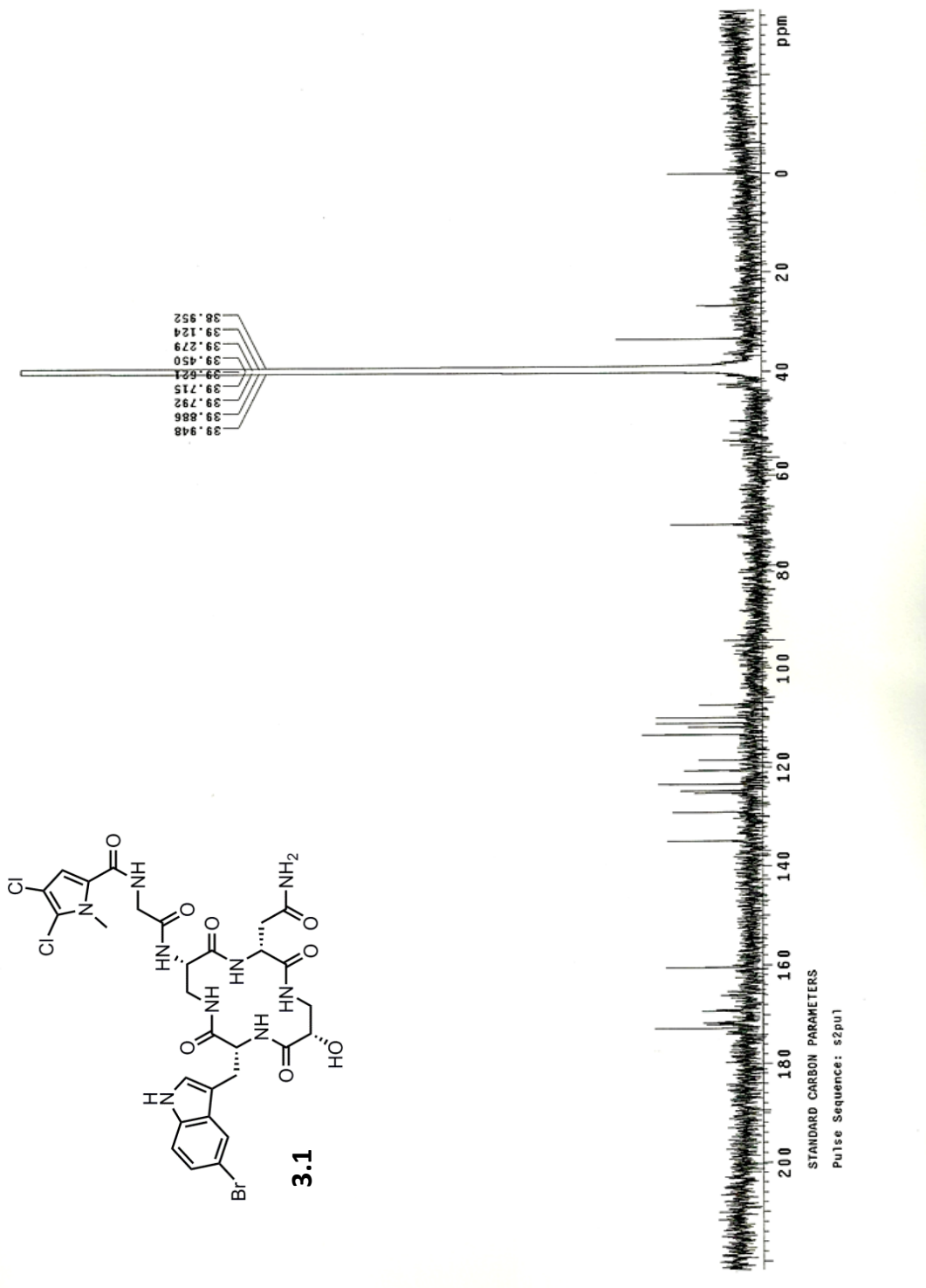

Cl
Cl
N
O
NH
HN
O
H
N
O
NH
O
HN
Br
NH
O
HN
NH2
O
O
TBDPSO
3.48
3.333
2.506
0.978
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc4-114-83a-proton
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat


Cl
Cl
N
O
NH
HN
O
H
N
O
NH
O
HN
Br
NH
O
HN
NH2
O
O
O
TBDPSO
3.48
39.963
39.792
39.621
39.456
39.295
39.124
38.952
200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

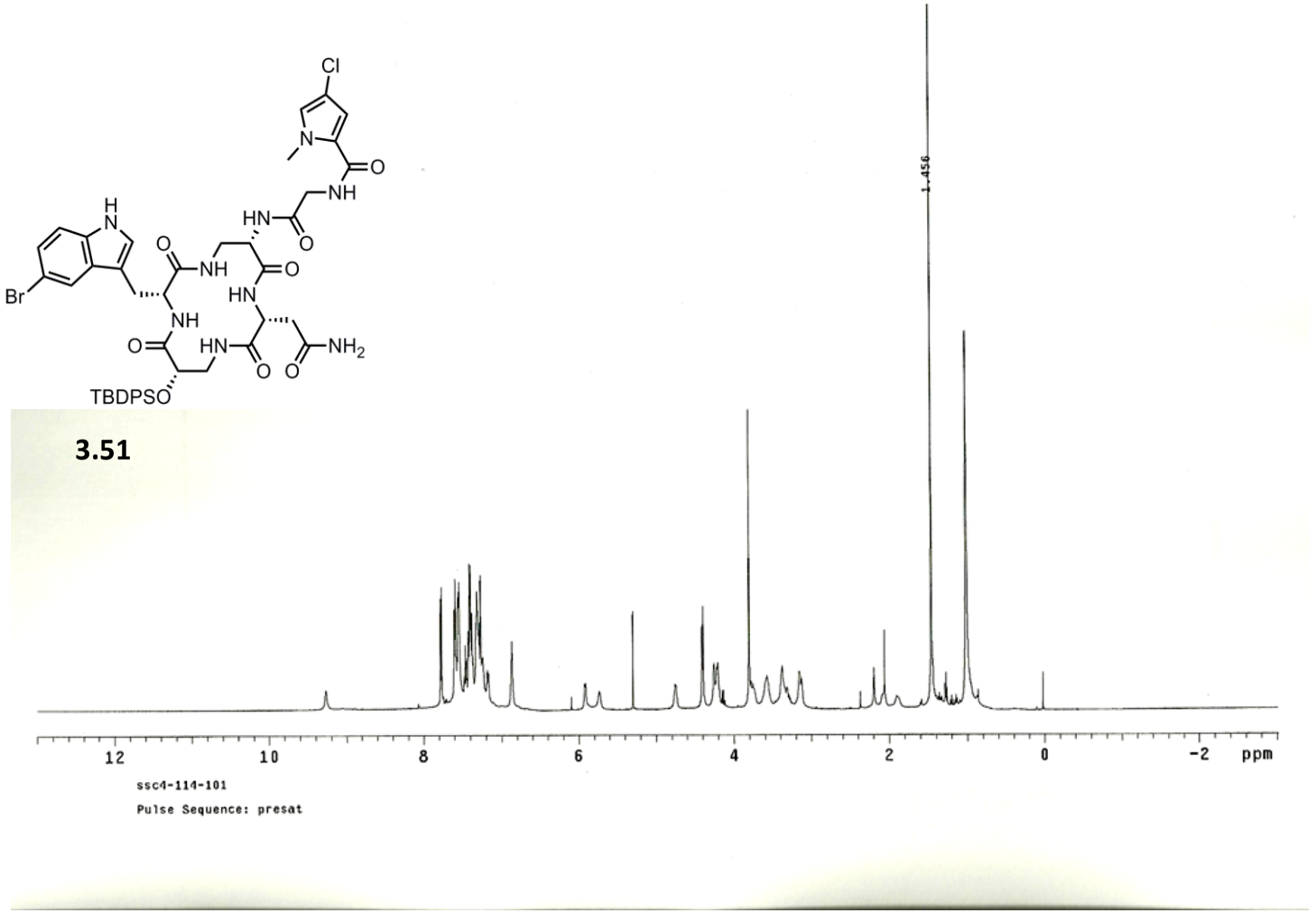

3.49
3.335
2.508
2.506
2.503
0.376
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc4-114-83b-pure
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat


3.49
Cl
Cl
N
O
NH
HN
O
H
N
Br
O
NH
O
HN
NH
O
HN
NHTrt
O
O
TBDPSO
39.792
39.621
39.450
39.295
39.124
200
180
160
140
120
100
80
60
40
20
0
ppm
ssc4-114-83b-carbon-an
Pulse Sequence: s2pul

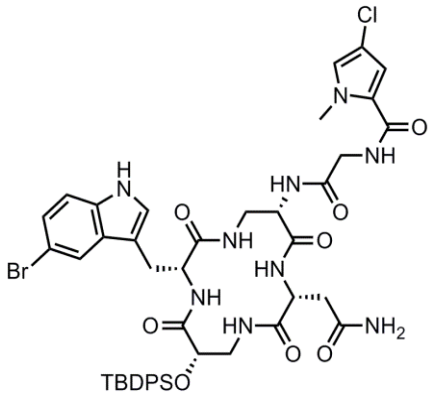

Cl
Cl
N
O
NH
HN
O
H
N
O
NH
O
HN
Br
NH
O
HN
O
NH2
O
O
HO
3.1
3.335
2.512
2.509
2.506
2.503
2.501
0.005
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc4-114-90a-hplc-cyclocinamide b
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat


39.948
39.886
39.792
39.715
39.621
39.450
39.279
39.124
38.952
ppm
0
20
40
60
80
100
120
140
160
180
200
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

3.1

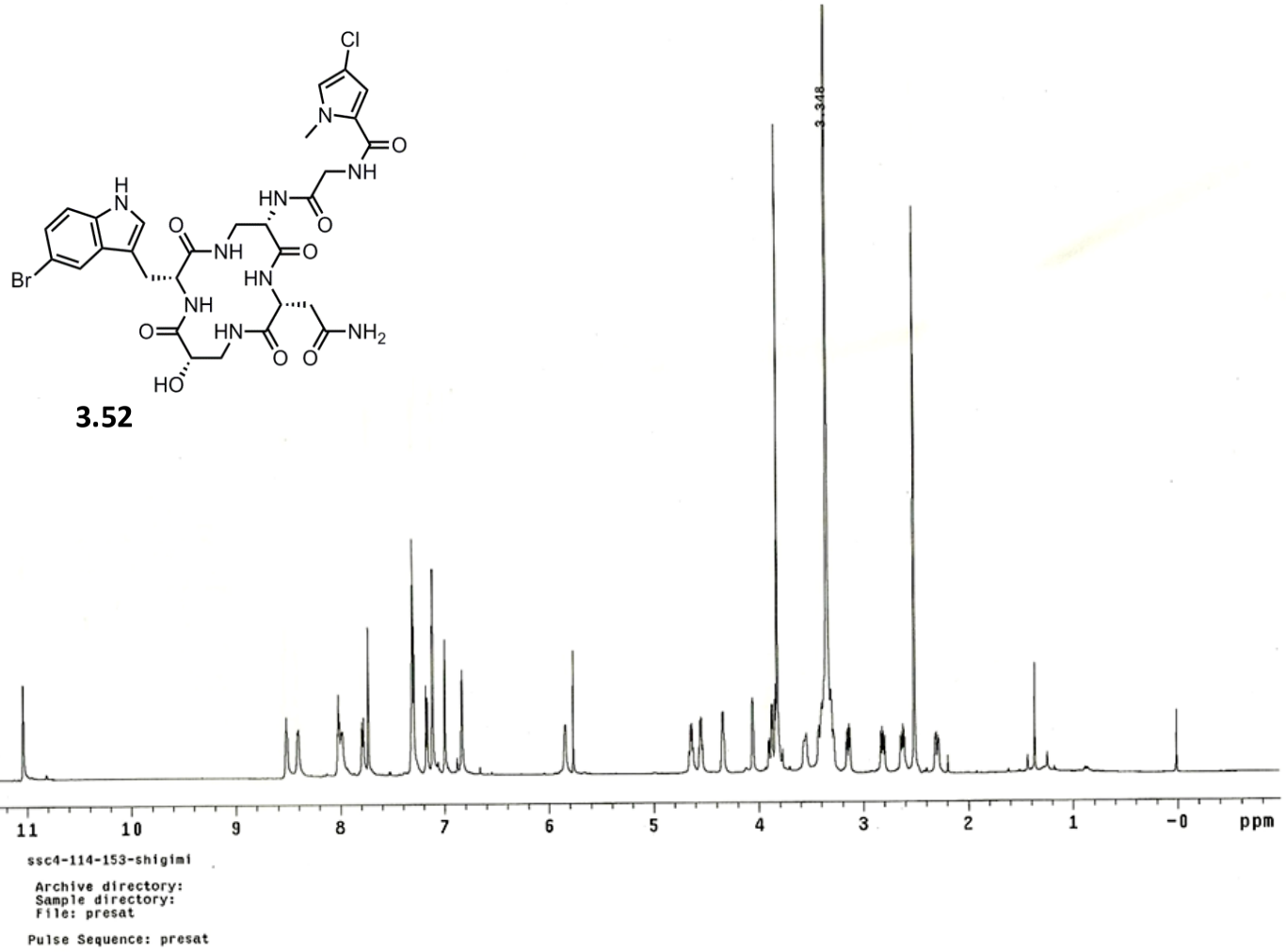

Cl
N
O
NH
HN
O
H
N
O
NH
O
HN
Br
NH
O
HN
O
NH2
O
O
TBDPSO
1.456
12
10
8
6
4
2
0
-2
ppm
ssc4-114-101
Pulse Sequence: presat

3.51

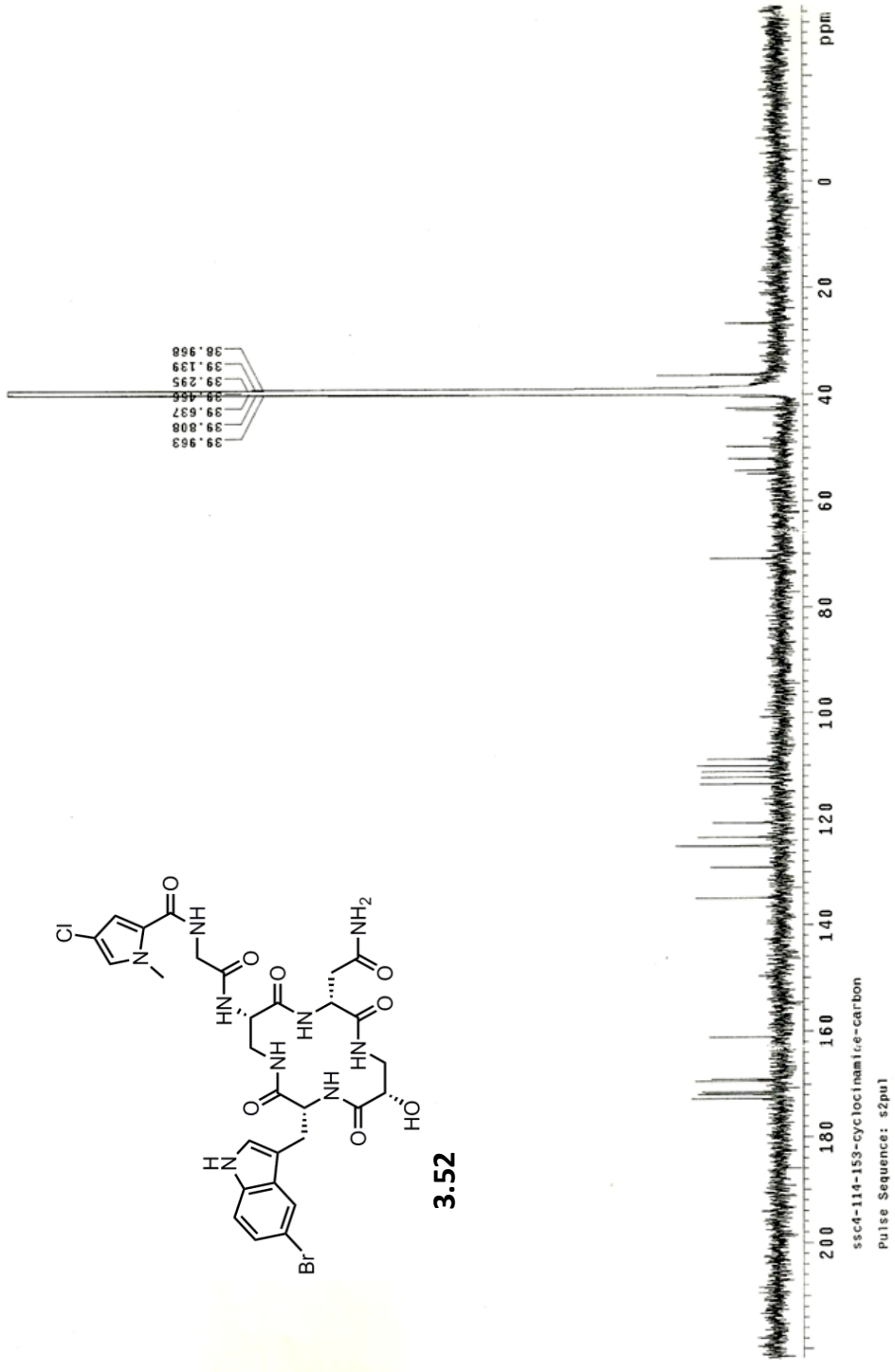

Cl
N
O
NH
HN
O
H
N
O
NH
O
HN
Br
NH
O
HN
O
NH2
O
O
TBDPSO

3.51

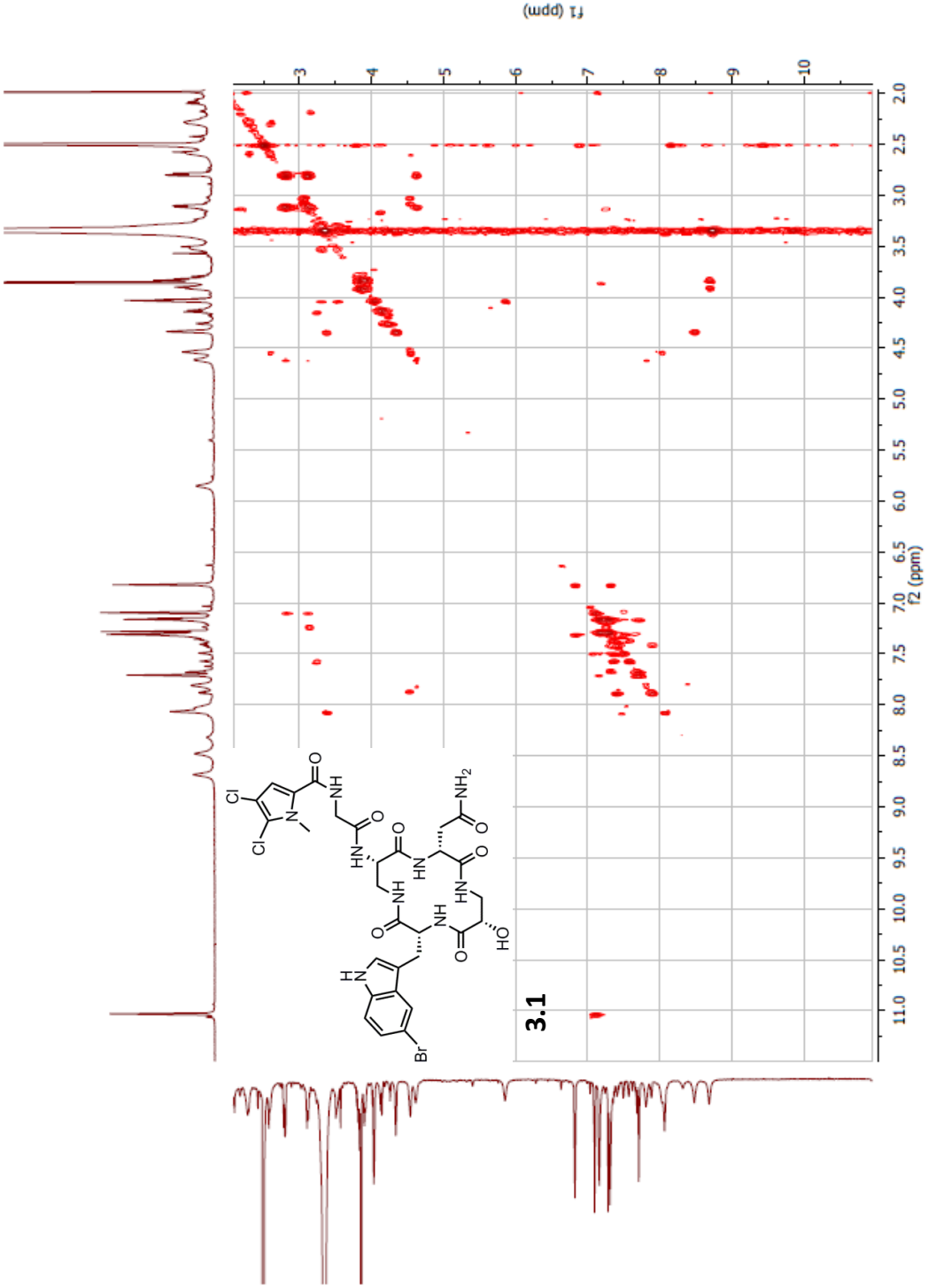
200
180
160
140
120
100
80
60
40
20
0
ppm
STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul

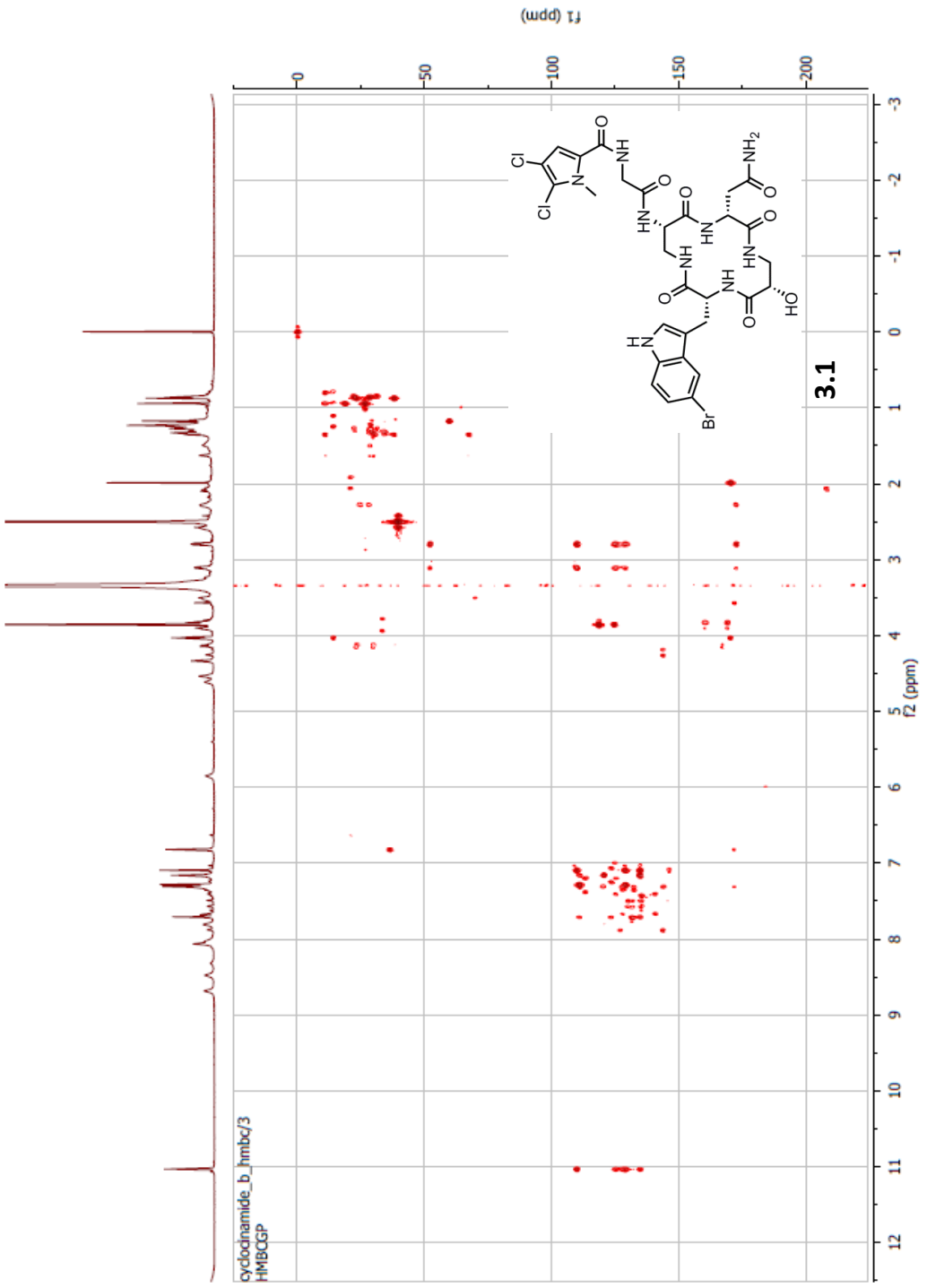

Cl
N
O
NH
HN
O
H
N
O
NH
O
HN
Br
NH
O
HN
O
NH2
O
O
HO
3.348
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
ssc4-114-153-shigimi
Archive directory:
Sample directory:
File: presat
Pulse Sequence: presat

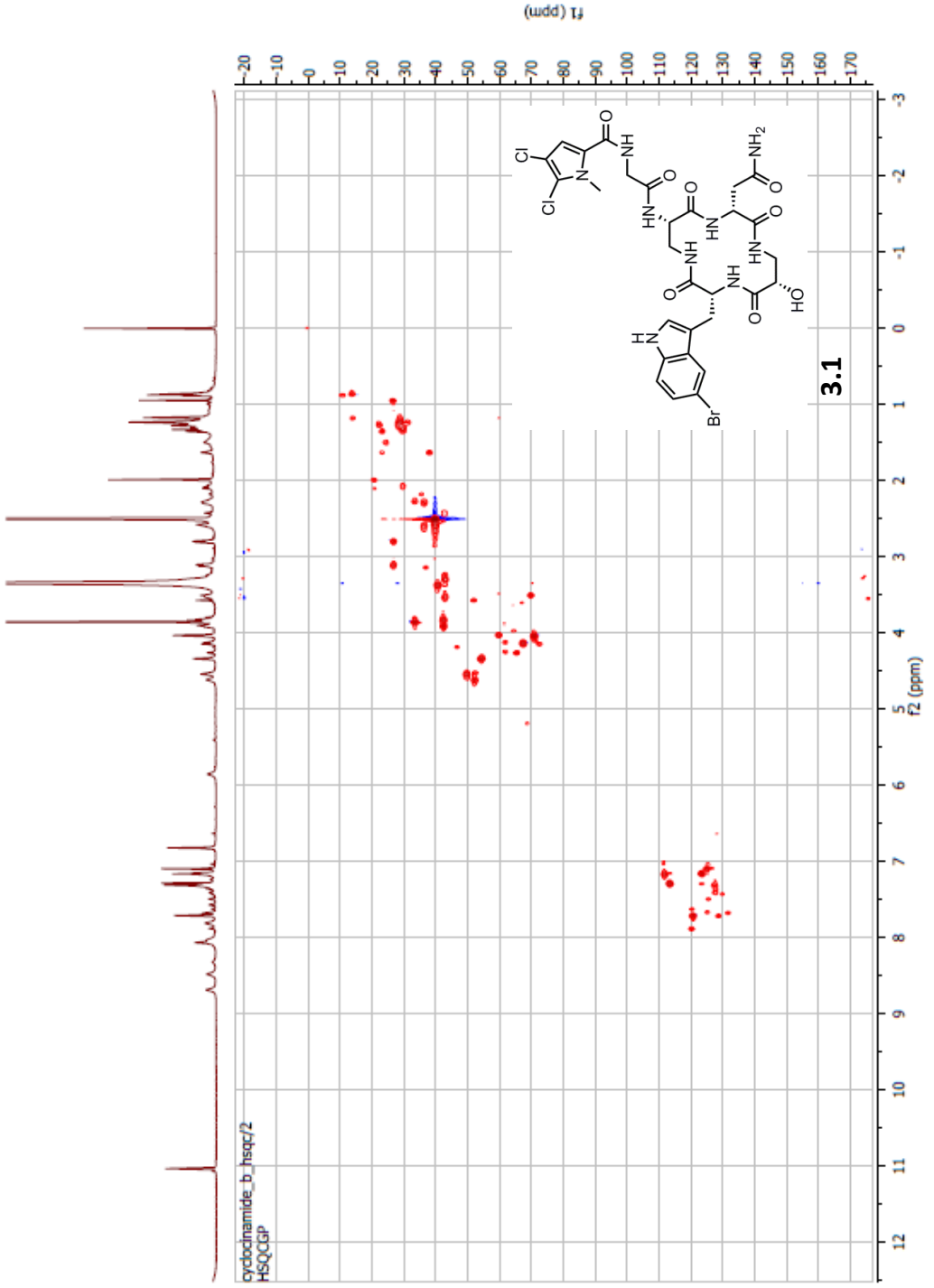
3.52

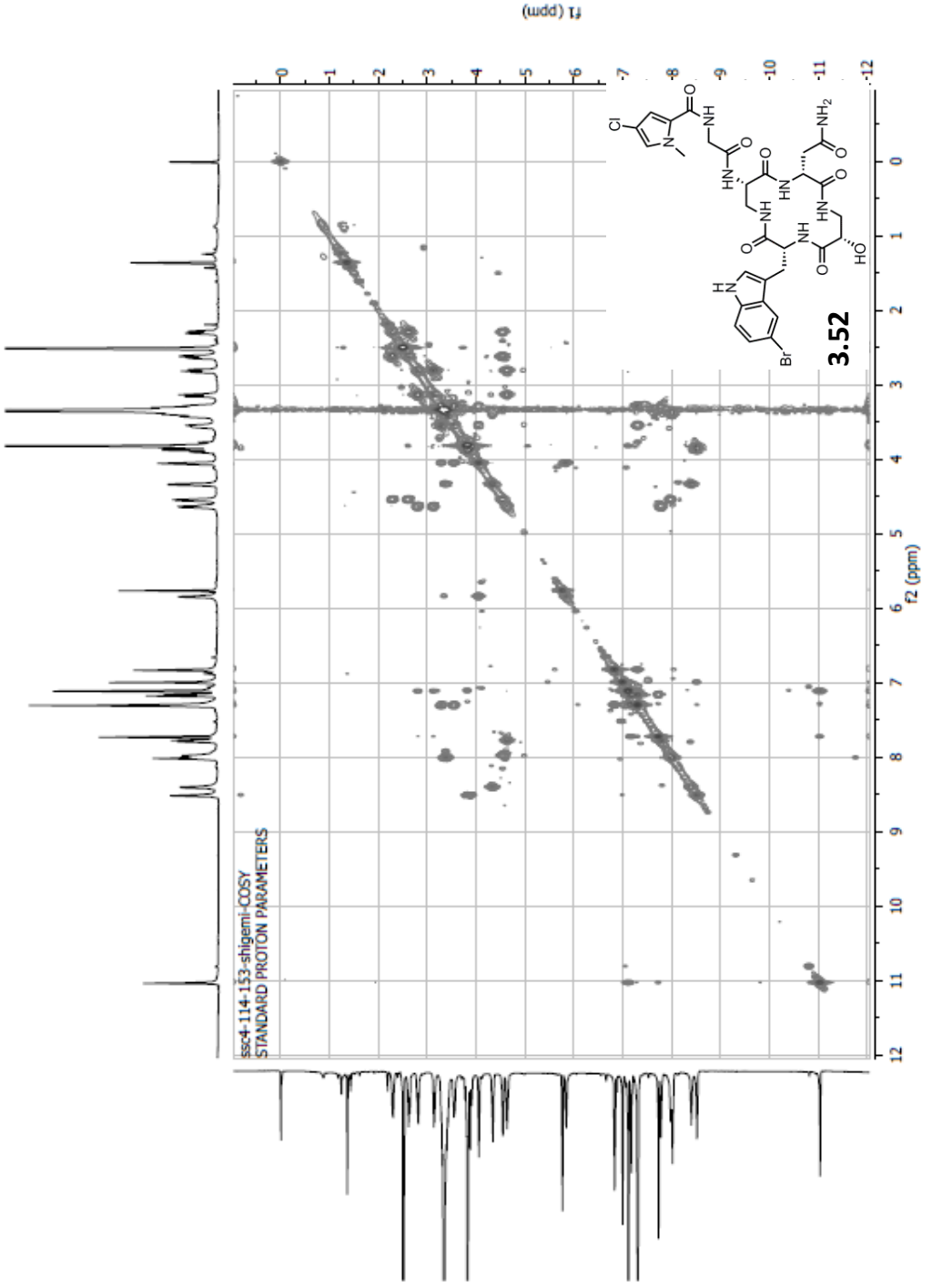

39.963
39.808
39.637
39.466
39.295
39.139
38.968
ppm
0
20
40
60
80
100
120
140
160
180
200
ssc4-114-153-cyclocinnamide-carbon
Pulse Sequence: s2pul

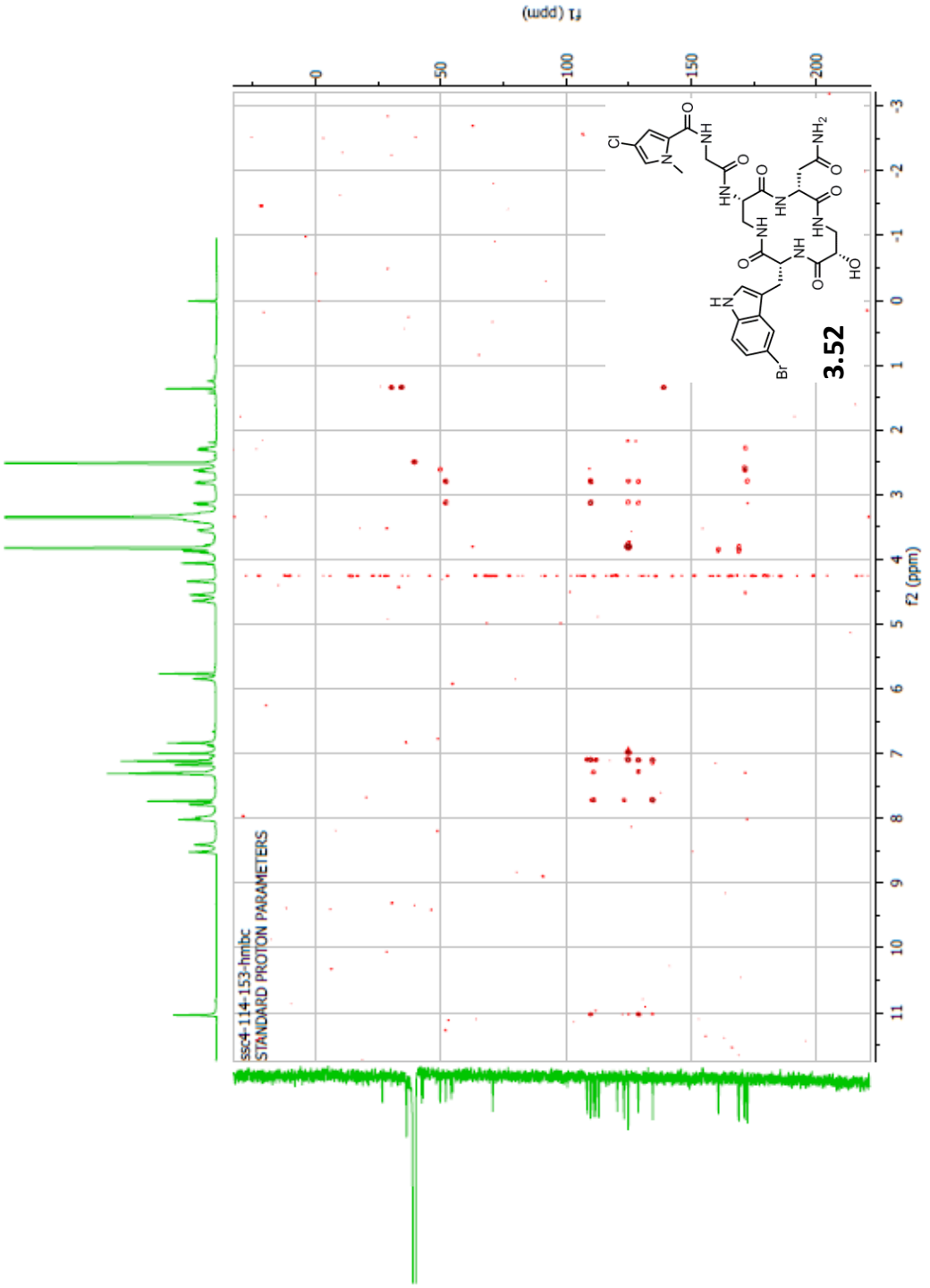
3.52

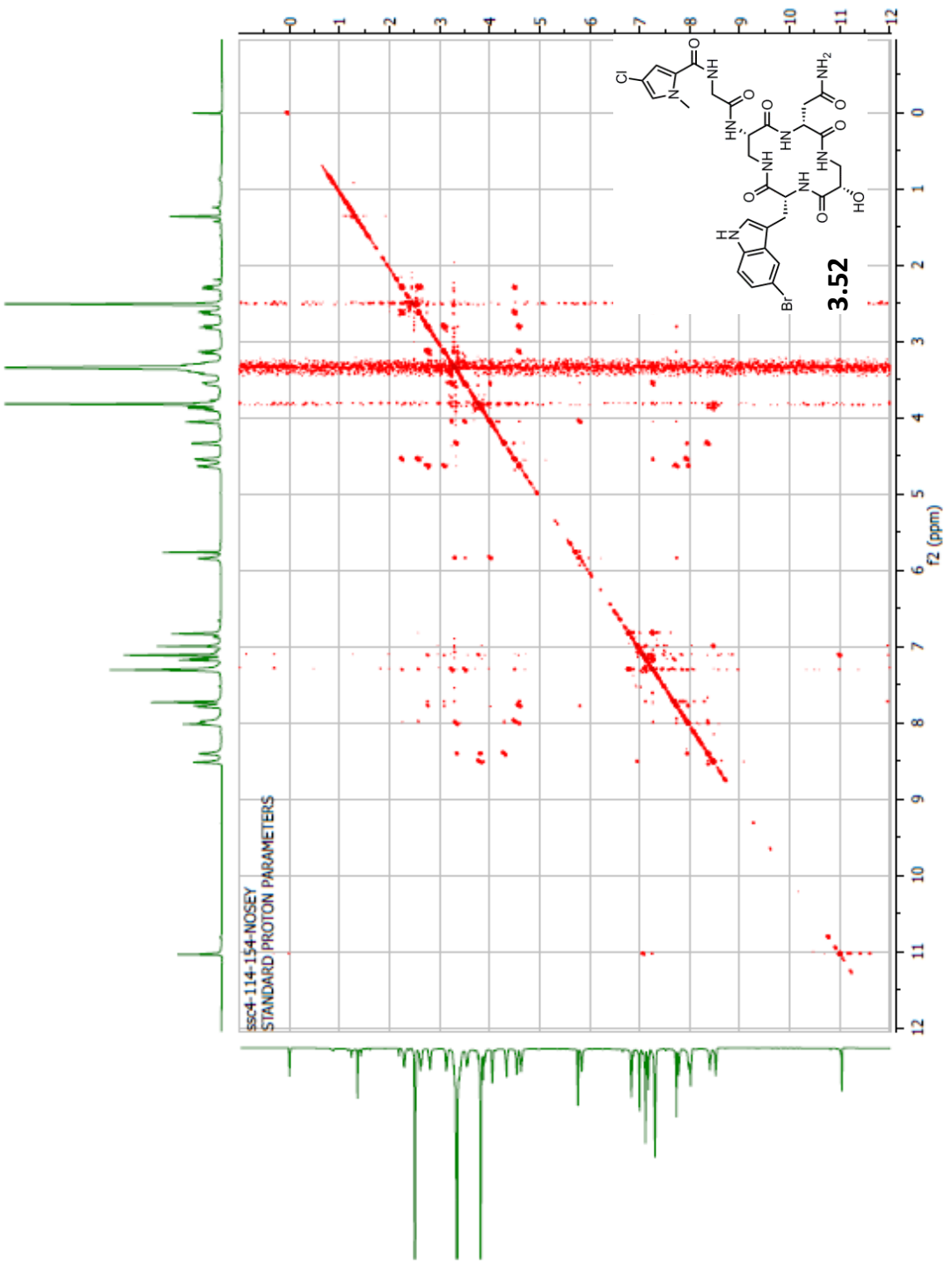

f1 (ppm)
f2 (ppm)
3.1


cyclodnamide_b_hmbc/3
HMBCGP
f1 (ppm)
f2 (ppm)
3.1


cyclocinamide_b_hsqc/2
HSQCGP
f1 (ppm)
f2 (ppm)
3.1


ssc4-114-153-shigemi-COSY
STANDARD PROTON PARAMETERS
f2 (ppm)
f1 (ppm)
3.52


ssc4-114-153-hmbc
STANDARD PROTON PARAMETERS
f1 (ppm)
f2 (ppm)
3.52


f1 (ppm)
f2 (ppm)
ssc4-114-154-NOSEY
STANDARD PROTON PARAMETERS
3.52

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