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Total Synthesis of Beauvericin Using Dehydroamino Acid Residues to Facilitate Cyclization

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Chemical and Materials Physics - Chemistry

by

Gregory Thomas Cizio

Thesis Committee:
Professor Vy Dong, Chair
Professor James Nowick
Professor Christopher Vanderwal

2014

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DEDICATION

To

the pursuit of knowledge

and being able to do so for the sake of learning and understanding

and also for satisfying curiosity.

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LIST OF ABBREVIATIONS

Ac	acetyl
aq	aqueous
Boc	*tert*-butoxycarbonyl
Bu	butyl
Cbz	carboxybenzyl
COD	cyclooctadiene
conv.	conversion
d	day(s)
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
d.r.	diastereoselective ratio
ee	enantiomeric excess
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
equiv	equivalent(s)
Et	ethyl
•HCl	hydrochloride salt
•H_2O	hydrate
h	hour(s)
HOBt	hydroxybenzotriazole
HRMS	high resolution mass spectrometry

i	*iso*
Im	imidazole
imid.	imidazole
IR	infrared spectroscopy
L*	chiral ligand
LC-MS	liquid chromatography–mass spectrometry
μ	micro
m	milli
M	molar
min	minute(s)
Me	methyl
n	*normal*
NBD	norbornadiene
NMR	nuclear magnetic resonance
Pfp	pentafluorophenyl
Ph	phenyl
ppm	parts per million
Pr	propyl
quant.	quantitative
SPPS	solid phase peptide synthesis
•TFA	trifluoroacetate salt
t	*tert*
TAS-F	tris(dimethylamino)sulfonium difluorotrimethylsilicate

TBAF	tetra-*n*-butylammonium fluoride
TBAT	tetra-*n*-butylammonium difluorotriphenylsilicate
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

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ABSTRACT OF THE THESIS

Total Synthesis of Beauvericin Using Dehydroamino Acid Residues to Facilitate Cyclization

By

Gregory Thomas Cizio

Master of Science in Chemical and Materials Physics - Chemistry

University of California, Irvine, 2014

Professor Vy Dong, Chair

A unique strategy that helps facilitate macrocyclization has been developed and utilized in the synthesis of a cyclic hexadepsipeptide natural product. By using dehydrophenylalanine amino acids in the synthesis of beauvericin, the precursor to the macrocycle is folded in such a way that the *C*- and the *N*- termini of the depsipeptide are closer together and cyclization is facilitated. Two strategies towards construction of the macrocycle have been investigated: (1) a cyclotrimerization approach which utilizes a monomeric dipeptide oxazolone and (2) a linear approach which cyclizes a hexadepsipeptide oxazolone. The macrocycle then underwent a global *N*-methylation followed by rhodium-catalyzed asymmetric hydrogenation which reduced the unsaturated amino acid residues to their desired saturated forms to give rise to the natural product. With the cyclotrimerization route, beauvericin was afforded in 10% yield over 8 steps, and with the linear route, beauvericin was afforded in 19% yield over 12 steps.

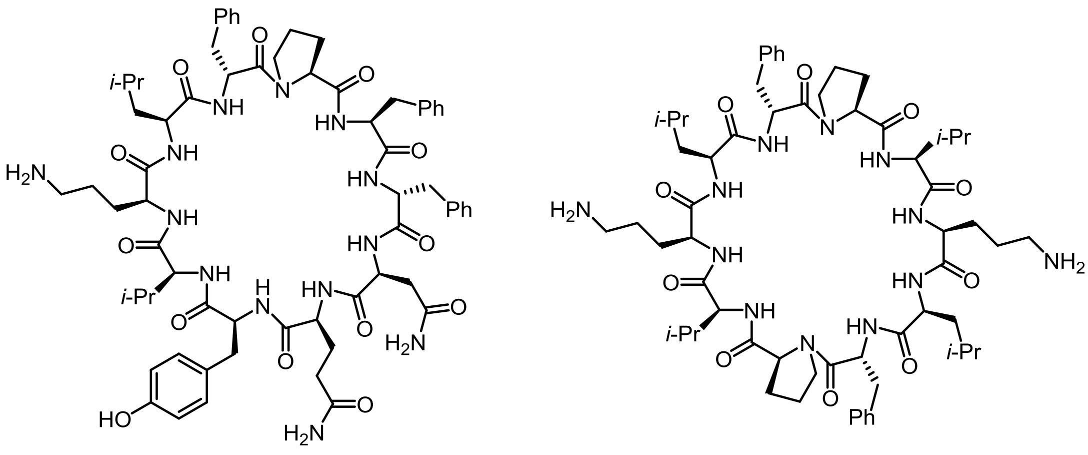
Chapter 1: First Generation Synthesis of Cyclic Hexadepsipeptide Beauvericin via a Dehydropeptide Cyclotrimerization Approach

1.1 Introduction

Cyclic peptides and depsipeptides are of interest to organic chemists for a variety of reasons. Perhaps most importantly, these compounds are valued for their use as reliable medicines ranging from antibiotic to antitumor, antimalarial, and immunosuppressant agents.[1-3] Aside from practicality, the synthesis of cyclic peptides and depsipeptides is of interest to the organic chemistry community due to the challenge that lies in cyclization of these compounds. Recently, it has been shown that dehydroamino acid residues can help fold the shape of these cyclization precursors, which could aid in the cyclization process.

1.1.a Cyclic Peptide and Depsipeptide Therapeutics

In 1939, American microbiologist Rene Dubos discovered and isolated the first cyclic peptides that showed medicinal activity.[4] He isolated tyrothricin, a mixture of two cyclic decapeptides: tyrocidine and gramicidin, from the soil microbe, *Bacillus brevis*. Tyrothricin is a potent antibiotic against gram-positive microorganisms, and tyrocidine was the first



tyrocidine

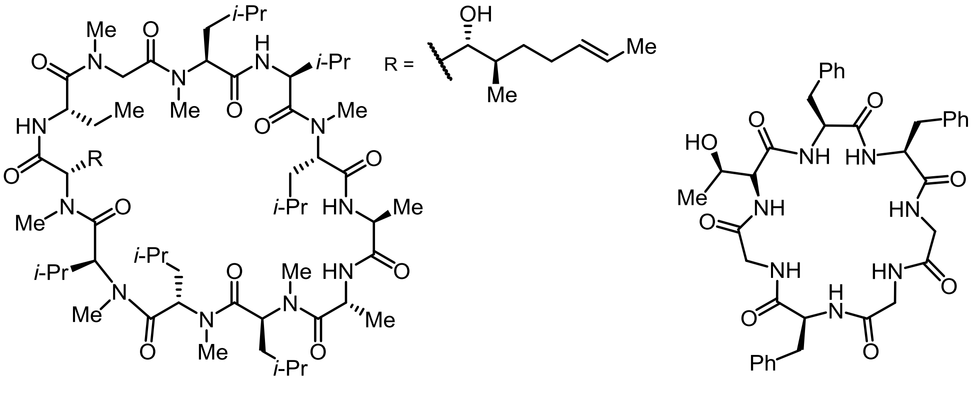
gramicidin S

Figure 1. Structures of tyrocidine and gramicidin S

commercially available cyclic peptide antibiotic and is still used today.[5]

In 1944, Russian microbiologists Gause and Brazhnikova discovered and isolated gramicidin S, a classical example of a medicinal cyclic peptide.[6] They found that gramicidin S was effective in the treatment of bullet wounds, lacerations, empyema (a condition in which pus and fluid from infected tissue collects in a body cavity), and osteomyelitis (infection and inflammation of bone or bone marrow). It wasn't until 1978 for the structure of gramicidin S to be determined, which was solved by Dodson and coworkers using x-ray crystallography.[7]

Other examples of medicinally-active cyclic peptides include the cyclic undecapeptide immunosuppressant cyclosporin A, isolated from the fungus *Tolypocladium inflatum,* as well as the cyclic heptapeptide antimalarial agent mahafacyclin B, isolated from the plant *Jatropha mahafalensis*.[8,9]

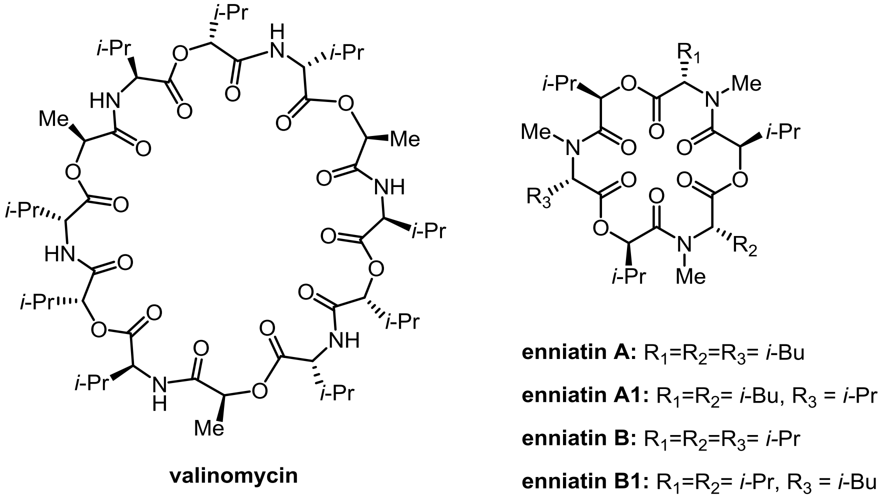


cyclosporin A **mahafacyclin B**

Figure 2. Structures of cyclosporin A and mahafacyclin B

With regards to medicinal cyclic depsipeptides, one of the most well-known examples is the cyclic dodecadepsipeptide antibiotic valinomycin, which is an ionophore that binds and transports potassium ions and selectively does so over sodium ions within the cell membrane.[10]

An interesting feature of valinomycin is that its structure alternates between amide and ester bonds.



valinomycin

enniatin A: $R_1=R_2=R_3=$ *i*-Bu

enniatin A1: $R_1=R_2=$ *i*-Bu, $R_3 =$ *i*-Pr

enniatin B: $R_1=R_2=R_3=$ *i*-Pr

enniatin B1: $R_1=R_2=$ *i*-Pr, $R_3 =$ *i*-Bu

Figure 3. Structures of valinomycin and enniatins

In fact, this pattern of alternation between amide then ester then amide then ester, and so on, is surprisingly prevalent in cyclic depsipeptides. Cyclic hexadepsipeptide enniatins A and B, as well as the topic of this thesis: beauvericin, follow this pattern even more so, with complete alternation of amino acid and hydroxy acid residues.

Important physiological properties that make cyclic peptides and depsipeptides desirable as medicinal agents include high cell membrane permeability, good target recognition, and improved metabolic stability compared to their linear analogs.

High cell membrane permeability arises from the fact that these cyclic compounds can exist in a conformation where they project their various side chains outward, creating a hydrophobic shell, while their carbonyls pointing inward, creating a hydrophilic core. The reverse can also occur, where the carbonyls are projected outward to create a hydrophilic shell, while the side chains are pointing inward to create a hydrophobic core. With these two conformations, cyclic peptides and depsipeptides are able to diffuse through cell membranes, such as phospholipid bilayers, by having a polar surface with their carbonyls pointing outward to bypass the phosphate part of the membrane, and then having a non-polar surface with their various side chains pointing outward to travel through the lipid part of the membrane.

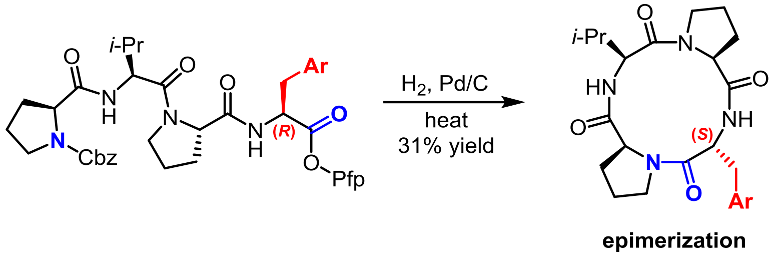
Good target recognition results from the limited number of conformations that cyclic peptides and depsipeptides can adopt. Because the degrees of freedom for these compounds are limited because the peptide or depsipeptide sequence is restricted to a ring, cyclic peptides and depsipeptides have fewer dominant conformations, resulting in greater specificity for targets.

Improved metabolic stability compared to their linear counterparts, again, is a result of these compounds being cyclic. Linear peptides and depsipeptides often degrade quickly once exposed to a biological system due to the ease of which cleaving enzymes, such as proteases, can recognize the amino or the carboxylic acid end of these compounds. However, when no terminal end is present, as is the case with cyclic peptides and depsipeptides, these enzymes take longer to identify and cleave amide and ester, resulting in a longer time that these cyclic compounds are intact.

Together, these three physiological properties: high cell membrane permeability, good target recognition, and improved metabolic stability compared to their linear counterparts, make cyclic peptides and depsipeptides desirable compounds to be used as therapeutic drugs.

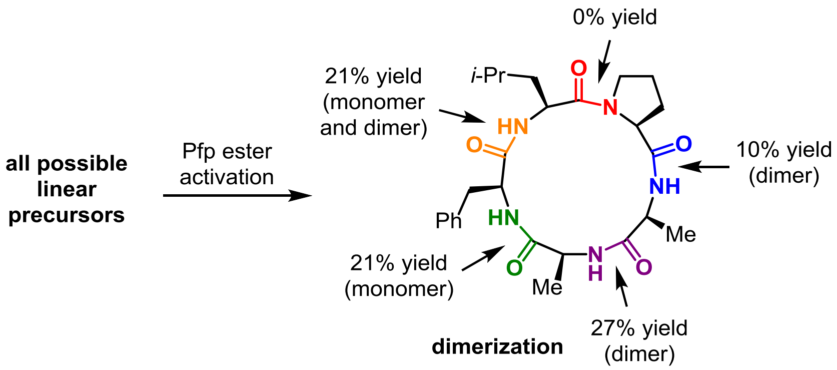
1.1.b Challenges with Cyclization

Two major problems that occur with the cyclization of cyclic peptides and depsipeptides are epimerization and dimerization. In 2007, Schmidt and coworkers showed that the cyclization of a proline-rich tetrapeptide, achieved by hydrogenolysis of the Cbz-protecting group while heating, resulted in significant epimerization of one of the tetrapeptide’s stereocenters.[11]


i-Pr
O
O
O
N
H
N
Cbz
N
N
H
(R)
Ar
O
O
Pfp
H2, Pd/C
heat
31% yield
i-Pr
O
N
HN
O
O
NH
(S)
N
O
Ar
epimerization

Scheme 1. Schmidt's proline-rich tetrapeptide cyclization

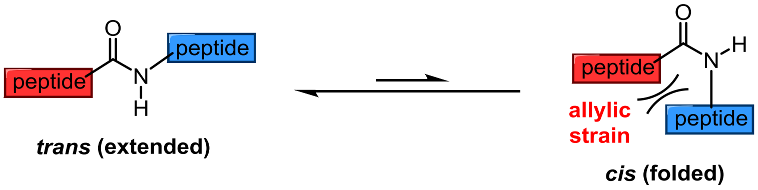
Additionally, all of the possible disconnections for the synthesis of a cyclic pentapeptide were evaluated, and it was found that depending on the amide bond chosen for cyclization, significant amounts of dimerization can occur, both by itself and together with the desired monocyclization.


0% yield
21% yield
(monomer
and dimer)
i-Pr
O
N
HN
O
all possible
linear
precursors
Pfp ester
activation
O
10% yield
(dimer)
NH
Ph
HN
Me
21% yield
(monomer)
O
N
H
O
Me
27% yield
(dimer)
dimerization

Scheme 2. Schmidt's assessment of a pentapeptide cyclization

The issue of epimerization can be suppressed with a quick cyclization, which results in a limited time that the cyclizing peptide is experiencing basic conditions from the deprotected amino group, ultimately reducing the amount of epimerization that can occur from basic conditions.

The issue of dimerization can be suppressed with dilute conditions for the cyclization. This is due to the fact that when assessing the amide bonds present in both peptides and depsipeptides, there is a preference for amide bonds to exist in the *trans* form, which avoids allylic strain that occurs in the *cis* form for the amide bonds. This preference for *trans*-amide bonds leads to extended conformations of the linear precursors, which make it difficult for the two reactive termini, the amino group and the carboxylic acid, to come close to one another for an intramolecular cyclization.



Scheme 3. *Trans*- vs. *cis*-amide bonds

Instead, intermolecular reactivity can result from these extended linear conformations, leading to dimerization. To prevent intermolecular reactivity, high dilution is employed which essentially helps isolate individual linear precursors so that there is less of a chance for reaction with another linear precursor, and instead intramolecular cyclization can occur. A drawback to high dilution for the suppression of dimerization is the excessive amount of solvent required for this effect.

1.1.c Folding from Dehydroamino Acids

Fortunately, there are ways to prevent linear precursors from existing in completely extended conformations. One way is through the use of β-turn inducers, residues that, as the name suggests, provide a β-turn in the chain. One prominent example of a β-turn inducer is proline, which has an overwhelming preference for the forming *cis*-amide bonds with respect to other amino acids. Another example of a β-turn inducer in peptides was shown in a 2005 report by Singh and coworkers, which showed that dehydrophenylalanine residues could exert conformational changes in peptides.[12]

In the report by Singh and coworkers, a tetrapeptide was shown to have undergone a conformational change as a result of the two internal amino acid residues being dehydrophenylalanine. Singh and coworkers were able to get an x-ray structure of the dehydropeptide, and it was clear that the *N*- and the *C*-termini are close together in space, which would not have occurred had the peptide been completely saturated and extended.

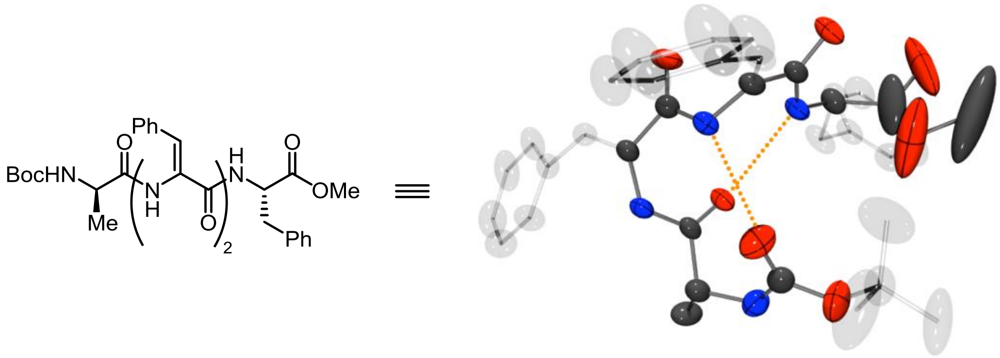
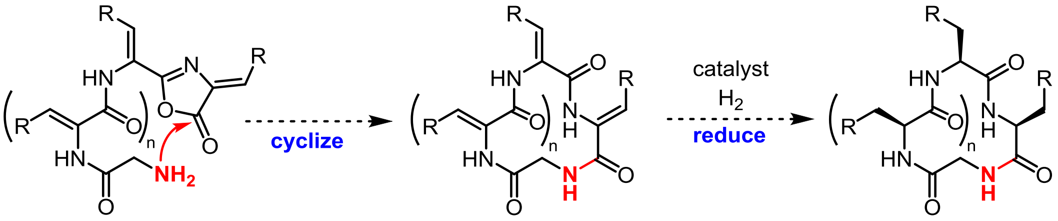


Figure 4. Singh's x-ray structure of tetradehydropeptide

By utilizing dehydroamino acid residues in peptides and depsipeptides, the reactive termini needed for cyclization can be brought closer together in space as a result of the unsaturated residues acting as *β*-turn inducers, and this can help facilitate cyclization and limit dimerization. Furthermore, by using dehydroamino acid residues, there would be no enolizable proton present, instead there would be a quaternary *α*-carbon as a result of the olefin, so epimerization would be reduced significantly. Thus, a dehydro approach can improve cyclization of cyclic peptides and depsipeptides by addressing the issues of epimerization and dimerization.



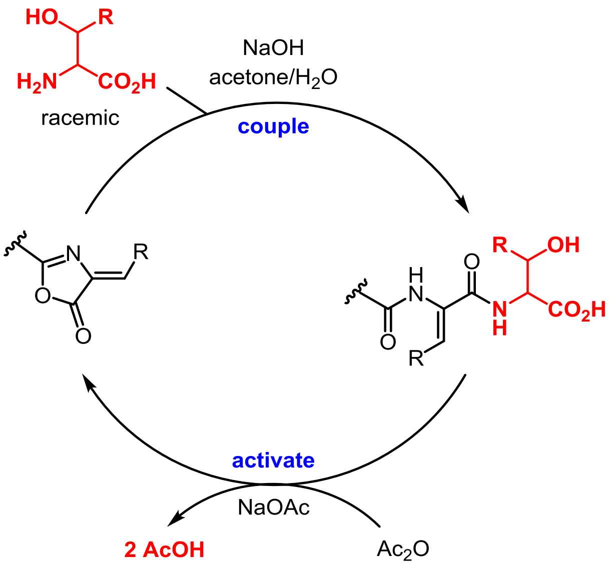
Scheme 4. Dong lab's dehydropeptide approach

To then get to the final, saturated form of the cyclic peptide or depsipeptide, a late-stage asymmetric hydrogenation could then be employed to reduce the olefins to the correct saturated amino acid residues.

1.2 Previous Dehydropeptide Approaches

1.2.a General Strategy

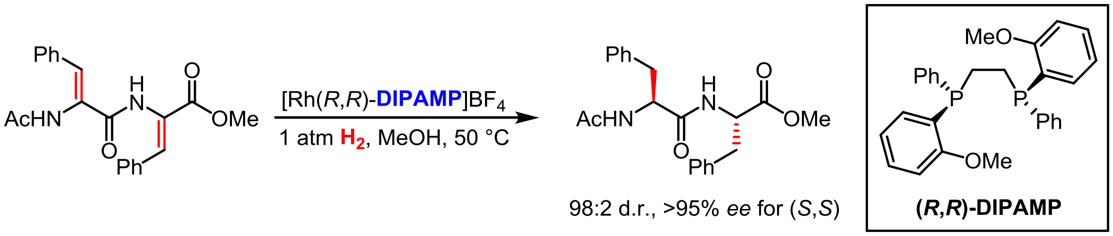
In 1943, Bergmann and coworkers showed that dehydropeptides could be efficiently synthesized through the coupling of oxazolones, five-membered rings, which act as activated forms of esters. Upon treatment with *β*-hydroxy amino acids in the presence of a base, such as triethylamine, a dehydroamino acid residue results (from the oxazolone), followed by an amide bond and the newly appended *β*-hydroxy amino acid.[13] Then, upon treatment with sodium acetate and acetic anhydride, the terminal *β*-hydroxy amino acid residue is then activated at the *C*-terminus via acetylation. This allows for the carbonyl of the adjacent dehydroamino acid residue to cyclize forming a new five-membered ring oxazolone which releases a first equivalent of acetic acid as a byproduct. Upon acetylation of the hydroxy group that is present in the *β*-hydroxy amino followed by dehydration, a second equivalent of acetic acid is generated as a byproduct. This process can repeat in an iterative manner to add on a new *β*-hydroxy amino acid residue, form the oxazolone, and elongation can continue until the desired length of dehydropeptide is achieved.



Scheme 5. Bergmann's method for dehydropeptide synthesis

An advantage of Bergmann's approach towards making dehydropeptides is the relatively benign side product that results from the coupling: acetic acid. Furthermore, when compared to more traditional forms of peptide coupling in which peptides are built from the *C*-terminus to the *N*-terminus direction, not as many protecting groups are required. To elaborate, with *C* to *N* direction peptide synthesis, the growing end of the peptide is the amino end, and to couple exclusively through the carboxylic acid of the newly added amino acid, that newly added amino acid must have its nitrogen protected. Thus, *C* to *N* direction peptide elongation involves coupling each new amino acid in the form of a *N*-protected amino acid, then once coupled, deprotection of the *N*-protecting group, followed by coupling of a new *N*-protected amino acid, and so forth. With Bergmann's dehydro approach, instead the peptide is built by taking the oxazolone which is a protecting group free C-terminus, and introducing β-hydroxy amino acids that do not have protecting groups.

In 1982, Kagan and coworkers showed that dehydrophenylalanine resides could be asymmetrically hydrogenated through the use of a chiral catalyst containing chiral bisphosphine ligands.[14] The report by Kagan and coworkers showed that an *N*-acetyl protected dehydrophenylalanine dipeptide methyl ester could be asymmetrically hydrogenated with a DIPAMP-ligated rhodium (I) catalyst to provide a dipeptide with high levels of diastereoselectivity.



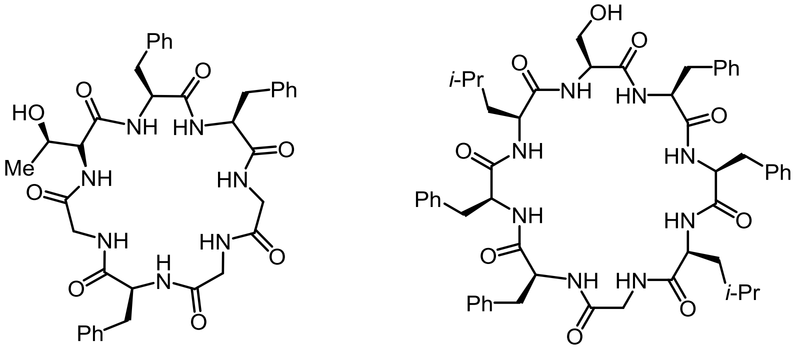
Scheme 6. Kagan's asymmetric hydrogenation of dehydrophenylalanine

With precedence from both Bergmann and Kagan, the idea of building dehydropeptides and late-stage asymmetrically hydrogenating them is a feasible one.

1.2.b Cyclic Peptide and Depsipeptide Targets

Beginning our investigation into using dehydroamino acid units in the synthesis of cyclic peptides and depsipeptides, the Dong lab started with the synthesis of a model cyclic pentapeptide. The pentapeptide consists of one residue being glycine, while the others are all dehydrophenylalanine. Cyclization was found to occur in relatively concentrated conditions, and hydrogenation occurred selectively.

With the results from the model cyclic pentapeptide serving as a proof of concept, the Dong lab then began to look into utilizing this strategy for the synthesis of two cyclic peptide natural products: mahafacyclin B and clausenain B.



mahafacyclin B **clausenain B**

Figure 5. Dong lab's cyclic peptide targets

One important thing to note with regards to these cyclic peptide natural products is that the targets do not contain *N*-methyl groups, an important and common moiety in both cyclic peptides and depsipeptides. Thus, for this dehydro strategy approach to be broadly applicable for the synthesis of cyclic peptides and depsipeptides, the late-stage asymmetric hydrogenation must be able to appropriately reduce *N*-methyl dehydroamino acid residues.

1.3 Background Information

1.3.a *N*-Methyl Groups

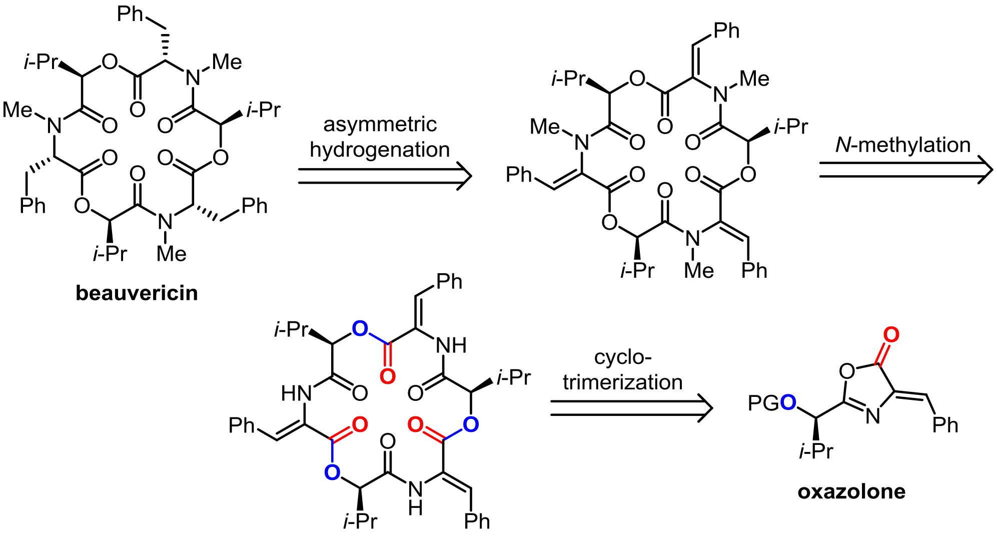
By incorporating methyl groups onto the nitrogens of peptides and depsipeptides, hydrophobicity is enhanced through reducing the number of hydrogen-bond donors and preventing the formation of both inter- and intramolecular hydrogen bonds.[15,16] In addition, *N*-methylation affects the backbone conformation of cyclic peptides and depsipeptides by allowing for the formation of *cis*-peptide bonds.[17] Steric interaction between the carbonyl oxygens or *α*-substitutions of constituent amino acids with the *N*-methyl group dictates cyclic peptide/depsipeptide conformation. As a result, more rigid scaffolds can be achieved when cyclic peptides and depsipeptides are *N*-methylated, which reduces conformational space of the peptide or depsipeptide.[18] Additionally, the increased hydrophobic nature increases the interaction of the cyclic peptides and depsipeptides with cellular membranes, resulting in improved intestinal permeability. *N*-Methylation of peptides and depsipeptides is a powerful technique for increasing the potential for using peptides and depsipeptides as pharmaceutical drugs, mainly due to its effects of increasing metabolic stability and bioavailability.[19] Due to the conformational constraint that *N*-methylation provides, cyclic peptide and depsipeptide populations can exist as a single conformer for a desired specific biological activity.

1.3.b Beauvericin

Beauvericin is an 18-membered cyclic hexadepsipeptide composed of alternating **D**-hydroxyisovaleric acid and *N*-methyl-phenylalanine residues, which gives rise to its C3 symmetry. As a cyclic depsipeptide, beauvericin has been found to have antibacterial and antifungal activity, and has even been reported for its use as an insecticide. In addition, beauvericin behaves as an ionophore and is known to coordinate and carry Ca^{2+} and Ba^{2+} cations across cell membranes, through coordination of its carbonyls to the metal ion. It was first isolated in 1969 by Hamill and coworkers from the fungus *Beauveria bassiana*, and has since been isolated from other natural sources.[20-23] In 1971, beauvericin was synthesized by Ovchinnikov and coworkers using the acid chloride method for coupling and cyclization.[24] Biosynthetically, beauvericin is made from by *Beauveria bassiana* through a repetitive process involving the couplings of a common dipeptidol intermediate.[25]

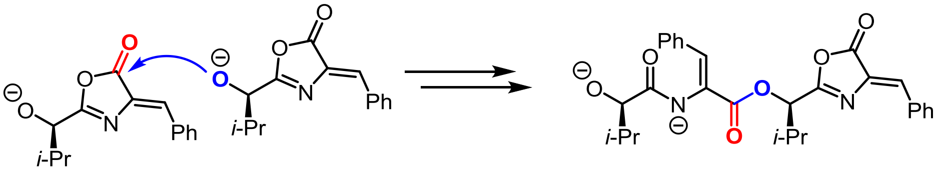
1.4 Retrosynthetic Analysis

With knowledge that dehydroamino acids can help fold peptides, investigation of the use of dehydrophenylalanine residues towards the synthesis of beauvericin was carried out. It was envisioned that beauvericin could be synthesized through a late-stage, asymmetric hydrogenation of an *N*-methyl dehydro macrocycle, of which the *N*-methyl groups could be installed through a global *N*-methylation.


beauvericin
asymmetric hydrogenation
N-methylation
cyclo-trimerization
oxazolone

Scheme 7. Retrosynthesis utilizing cyclotrimerization

The dehydromacrocycle could then be disconnected through a cyclotrimerization reaction to utilize the inherent C3 symmetry present in beauvericin.



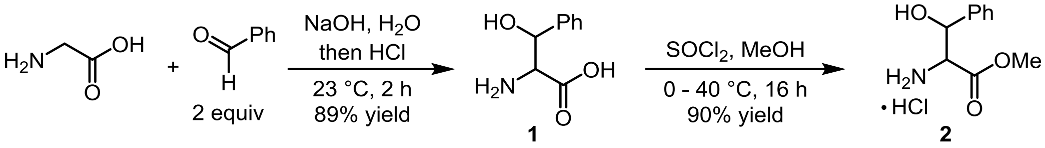
Scheme 8. Alkoxide/oxazolone coupling mechanism

By using a protected-hydroxy dipeptide oxazolone as the monomer, the cyclotrimerization could quickly and convergently achieve the synthesis of the macrocycle and in an additional two steps beauvericin could be reached.

1.5 Results

1.5.a Synthesis of Dipeptide Oxazolone

For our syntheses of oxazolones, β-hydroxy amino acids are required. For the particular synthesis of dehydrophenylalanine oxazolones, the β-hydroxy amino acid needed can be obtained from the condensation between glycine and benzaldehyde. Two equivalents of benzaldehyde are used, the first to condense with the amino group of glycine to form a Schiff base (which lowers the pK_a of the enolizable proton), and the second for the condensation with glycine to form β-hydroxy phenylalanine.

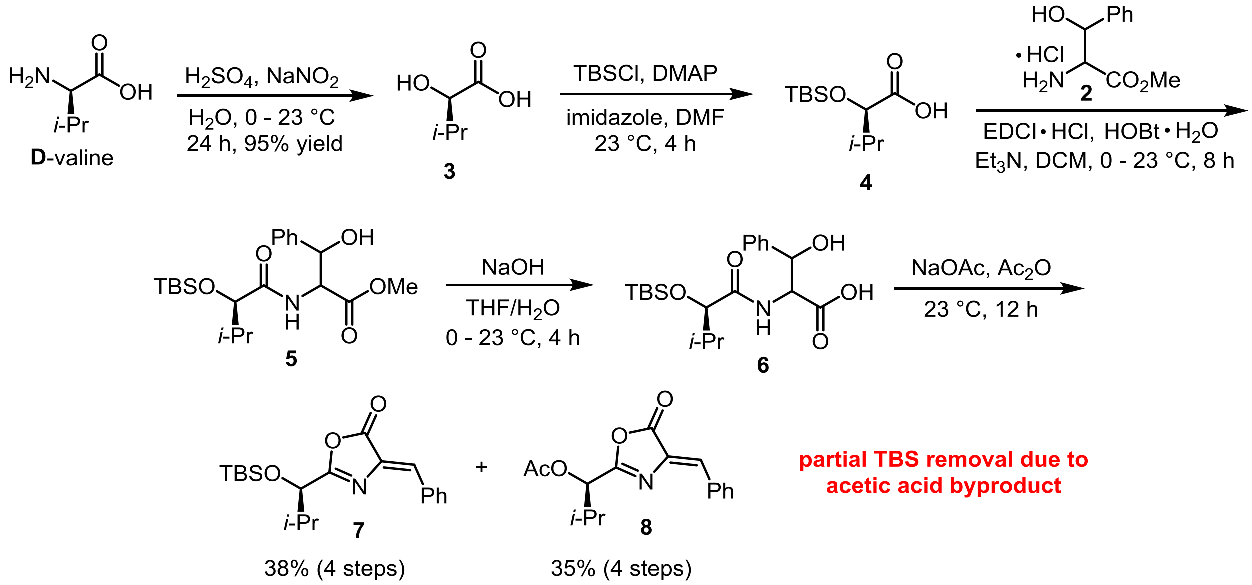


Scheme 9. Synthesis of β-hydroxy phenylalanine

This condensation between glycine and benzaldehyde formed β-hydroxy phenylalanine **1** in 89% yield. To obtain the methyl ester of β-hydroxy phenylalanine, the carboxylic acid was treated with thionyl chloride and methanol to provide compound **2** in 90% yield. Since amino acids **1** and **2** are common building blocks in the Dong lab, bulk synthesis is desirable and I was able to synthesize 80 grams of β-hydroxy phenylalanine **1** and 56 grams of β-hydroxy phenylalanine methyl ester **2**.

The first attempt at making the monomeric protected-hydroxy dipeptide oxazolone began with TBS as the choice for protecting group. The forward synthesis began with **D**-valine, which was converted to **D**-hydroxyisovaleric acid **3** in 95% yield through the formation of a diazonium intermediate obtained upon treatment of the amino acid with sodium nitrite and sulfuric acid.

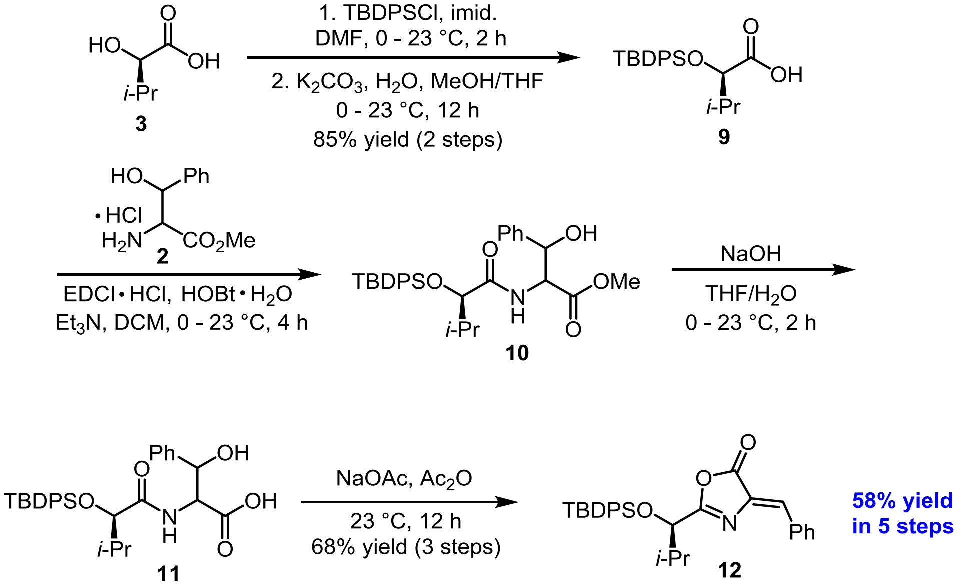
The diazonium intermediate undergoes an intramolecular displacement to release nitrogen gas, and then the resulting 3-membered ring is opened with attack from water, resulting in a double inversion of stereochemistry to retain the absolute configuration from amino acid to hydroxy acid (determined by optical rotation).


D-valine
H_2SO_4, $NaNO_2$
H_2O, 0 - 23 °C
24 h, 95% yield
3
TBSCl, DMAP
imidazole, DMF
23 °C, 4 h
4
•HCl
2
EDCI•HCl, HOBt•H_2O
Et_3N, DCM, 0 - 23 °C, 8 h
5
NaOH
THF/H_2O
0 - 23 °C, 4 h
6
NaOAc, Ac_2O
23 °C, 12 h
7
38% (4 steps)
8
35% (4 steps)
partial TBS removal due to acetic acid byproduct

Scheme 10. Synthesis of TBS-protected dipeptide oxazolone

It should be noted that **D**-hydroxyisovaleric acid is also commercially available. Upon TBS-protection of the hydroxy group to afford TBS-protected acid **4**, coupling with *β*-hydroxy phenylalanine methyl ester **2** through the use of EDCI and HOBt provided dipeptide methyl ester **5**, which was then hydrolyzed to carboxylic acid **6** using NaOH. Note: compounds **5** and **6** are diastereomeric mixtures. Dipeptide acid **6** was then subjected to oxazolone formation conditions to form TBS-protected dipeptide oxazolone **7** (*Z*-isomer only) in 38% yield over 4 steps. Unfortunately, the acetic acid that was generated in the oxazolone formation step deprotected some of the TBS group and, in the presence of acetic anhydride, replaced it with an acetyl group to give acetyl-protected dipeptide oxazolone **8** in 35% yield over 4 steps. Because of this, efforts were shifted towards the synthesis of a less acid-labile silyl protecting group that could withstand the acetic acid generated in the oxazolone formation step.

Upon switching from a TBS to a TBDPS protecting group, it was found that the generation of acetic acid in the oxazolone formation step was no longer an issue.



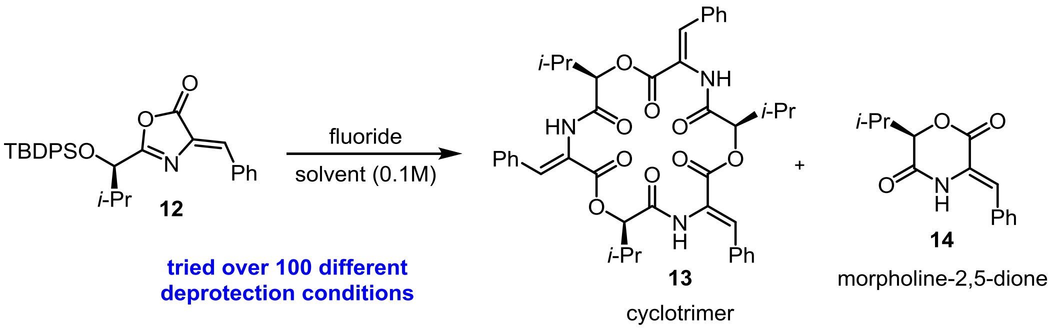
3
1. TBDPSCl, imid.
DMF, 0 - 23 °C, 2 h
2. K2CO3, H2O, MeOH/THF
0 - 23 °C, 12 h
85% yield (2 steps)
9
2
EDCI • HCl, HOBt • H2O
Et3N, DCM, 0 - 23 °C, 4 h
10
NaOH
THF/H2O
0 - 23 °C, 2 h
11
NaOAc, Ac2O
23 °C, 12 h
68% yield (3 steps)
12
58% yield
in 5 steps

Scheme 11. Synthesis of TBDPS-protected dipeptide oxazolone

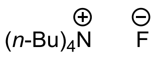
Thus, starting from **D**-hydroxyisovaleric acid **3** and protecting with TBDPS chloride and hydrolyzing the resulting silyl ester afforded TBDPS-protected hydroxy acid **9** in 85% yield over 2 steps. The TBDPS-protected hydroxy acid was then subjected to the same steps from the TBS-protected oxazolone synthesis: EDCI coupling with *β*-hydroxy phenylalanine methyl ester **2** to afford dipeptide methyl ester **10**, followed by hydrolysis to give dipeptide acid **11** (note: compounds **10** and **11** are diastereomeric mixtures) which then underwent oxazolone formation resulting in TBDPS-protected dipeptide oxazolone **12** (*Z*-isomer only) in an overall yield of 58% across 5 steps, with no deprotection of the TBDPS group occurring in the oxazolone formation step. This TBDPS synthesis was scaled up and 31 grams of TBDPS-protected dipeptide oxazolone **12** was synthesized.

1.5.b Cyclotrimerization and *N*-Methylation

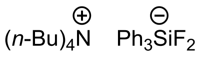
Initial efforts towards investigating the cyclotrimerization reaction began with TBS-protected dipeptide oxazolone **7**, but due to the improved oxazolone synthesis from the switch to the TBDPS group, the investigation of the cyclotrimerization reaction primarily occurred by looking at a variety of different fluoride sources and conditions for the deprotection of the TBDPS group of TBDPS-protected dipeptide oxazolone **12**. Over 100 different reactions were evaluated for the cyclotrimerization, with many of the conditions giving no desired cyclotrimer formation. Ultimately, the best conditions for cyclotrimerization to occur were found with basic, nucleophilic forms of fluoride.


TBDPSO
i-Pr
Ph
12
fluoride
solvent (0.1M)
tried over 100 different deprotection conditions
i-Pr
NH
i-Pr
HN
Ph
N H
i-Pr
Ph
Ph
13
cyclotrimer
+
i-Pr
N H
Ph
14
morpholine-2,5-dione

Entry	Solvent	Fluoride	Temp. (°C)	Time (h)	% cyclotrimer	% dione
1	THF	TBAF	23	48	7	41
2	MeCN	TBAT	80	24	18	22
3	**MeCN**	**TAS-F**	**80**	**18**	**23**	**26**
4	MeCN	$Et_3N \bullet 3HF$	80	24	0	94


$(n\text{-Bu})_4\overset{\oplus}{N}\ \ \overset{\ominus}{F}$

TBAF


$(n\text{-Bu})_4\overset{\oplus}{N}\ \ Ph_3\overset{\ominus}{Si}F_2$

TBAT

$\mathbf{(Me_2N)_3\overset{\oplus}{S}\ \ Me_3\overset{\ominus}{Si}F_2}$

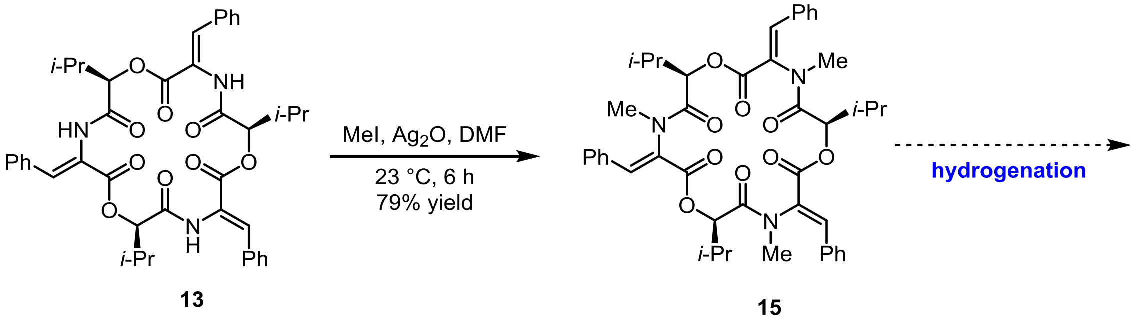
TAS-F

Table 1. Best results for cyclotrimerization of oxazolone

The initial hit for the cyclotrimerization investigation was obtained with treatment of oxazolone **12** with TBAF, furnishing cyclotrimer **13** in 7% yield and dione **14** in 41% yield. Switching from TBAF to TBAT, a similar but more anhydrous form of basic fluoride, the yield of cyclotrimer **13** increased nearly two and a half fold from 7% to 18%, and dione **14** was formed in 22% yield. Upon switching from TBAT to TAS-F, the cyclotrimerization improved as the yield of cyclotrimer **13** increased further to 23% yield, presumably due to the presence of the less coordinating tris(dimethylamino)sulfonium cation, and dione **14** was produced in 26% yield.

Unfortunately, whenever the cyclotrimerization worked, the byproduct formation of undesired dione **14** accompanied in greater yield. The formation of dione **14** could be a result of the monomeric oxazolone cyclizing on itself, or it could be a result of a tetra- or hexadepsipeptide (the dimer or trimer intermediates in the cyclotrimerization reaction) undergoing a deletion pathway to clip off and form dione units. Interestingly enough, when less basic forms of fluoride source were investigated, such as buffered triethylamine trihydrofluoride, exclusive formation of dione **14** was achieved in 94% yield.

Having optimized the cyclotrimerization reaction with TAS-F as the fluoride source giving 23% yield of cyclotrimer **13**, the rest of the synthesis towards beauvericin ensued.

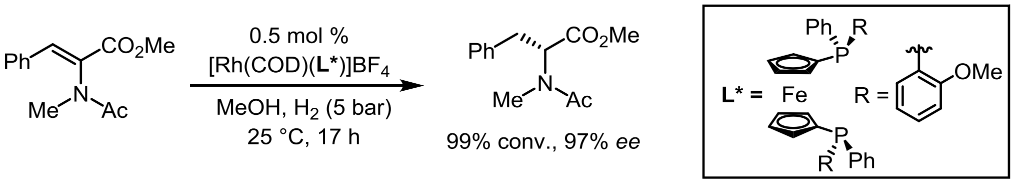


Scheme 12. Global *N*-methylation of macrocycle

Upon treatment of cyclotrimer **13** with silver oxide and methyl iodide, global *N*-methylation of the macrocycle went smoothly and formed macrocycle **15** in 79% yield. The last step remaining for the synthesis of beauvericin was the asymmetric hydrogenation of macrocycle **15**. Before trying the hydrogenation on the macrocycle, however, a single *N*-methyl dehydrophenylalanine residue was investigated first for optimization of the asymmetric hydrogenation conditions.

1.5.c Model Asymmetric Hydrogenation

It is well documented that dehydrophenylalanine can be asymmetrically reduced to phenylalanine, but with the *N*-methyl analog, it is a more difficult process.[26-28] To the best of my knowledge, the only successful solution to this problem of *N*-methyl dehydrophenylalanine asymmetric hydrogenation was the work done by Mezzetti and coworkers in 1999, which resulted in a rhodium-catalyzed asymmetric hydrogenation of the substrate with high enantioselectivity.[29] The catalyst used consists of a Rh(I) center with both a cyclooctadiene ligand and a ferrocenyl bisphosphine chiral ligand ligated to the metal center.

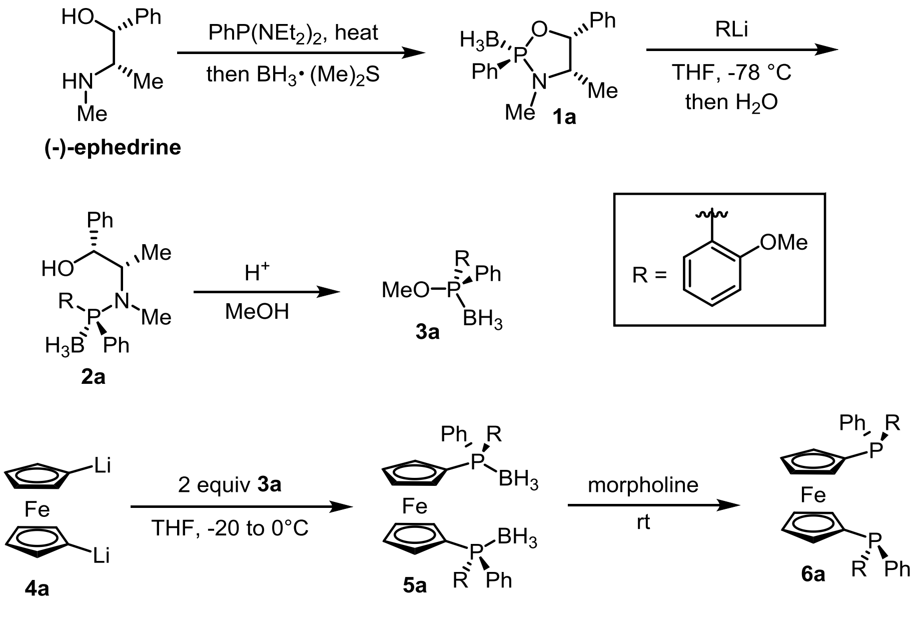


Scheme 13. Mezzetti's asymmetric hydrogenation of *N*-Me dehydrophenylalanine

The authors think that the high enantioselectivity they can achieve is due to the fact that ferrocenyl phosphines have large bite angles, which makes the ferrocene unit a good bridge between the two phosphorous atoms of the ligand. The ferrocenyl bisphosphine chiral ligand is not commercially available, and can be synthesized in five steps from commercially available starting materials.

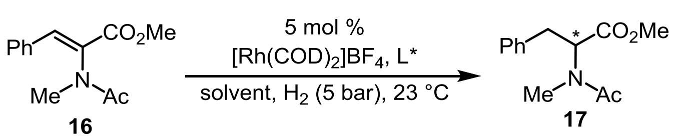
To obtain a chiral phosphorous center, (-)-ephedrine forms chiral oxazaphospholidine **1a** upon treatment with bis(diethylamino)phenylphosphine and then borane dimethyl sulfide. Oxazaphospholidine **1a** is then cleaved at the P-O bond in the presence of an aryllithium reagent followed by water, which furnishes aminophosphine borane **2a**. Under acidic methanolysis conditions, (-)-ephedrine is then cleaved (and can be reused) from aminophosphine borane **2a** to provide phosphinite borane **3a**, which then reacts with 1,1'-dilithioferrocene **4a** to form ferrocenyl bisphosphine borane **5a**. Deboronation is then achieved with morpholine to arrive at ferrocenyl bisphosphine ligand **6a**.

Before synthesizing and investigating the use of ferrocenyl bisphosphine ligand **6a** in the synthesis of beauvericin, I decided to first look at the various commercially-available bisphosphine ligands we had in our glove box as a means for a quick evaluation of the model *N*-methyl dehydrophenylalanine asymmetric hydrogenation.


HO
Ph
HN
Me
Me
(-)-ephedrine
PhP(NEt2)2, heat
then BH3• (Me)2S
H3B
O
Ph
P
Ph
N
Me
Me
1a
RLi
THF, -78 °C
then H2O
Ph
HO
Me
R
P
N
Me
H3B
Ph
2a
H+
MeOH
MeO
P
R
Ph
BH3
3a
R =
OMe
Li
Fe
Li
4a
2 equiv 3a
THF, -20 to 0°C
Ph
R
P
BH3
Fe
P
BH3
R
Ph
5a
morpholine
rt
Ph
R
P
Fe
P
R
Ph
6a

Scheme 14. Synthesis of Mezzetti's ferrocenyl bisphosphine ligand

Ligands that were similar in structure to ferrocenyl bisphosphine ligand **6a** were initially investigated for the hydrogenation of *N*-Me dehydrophenylalanine **16** to *N*-Me phenylalanine **17**, including DIPAMP and a variety of ferrocenyl bisphosphine ligands (JosiPhos, MandyPhos, TaniaPhos, WalPhos) which gave enantioselectivities ranging from 9 to 51% *ee*. Upon heading away from ferrocenyl bisphosphine ligands and instead looking at more rigid bisphosphine ligands containing carbon bridges such as DuanPhos, QuinoxP, and BenzP, it was found that a significant increase in enantioselectivity resulted (from 9–51% to 84–93% *ee*). By switching from DCM to MeOH and then finally to acetone, I determined that both DuanPhos and BenzP resulted in complete enantioselectivity in the hydrogenation, meaning that no detectable minor enantiomer was observed by SFC analysis.


Ph
CO$_2$Me
Me
N
Ac
5 mol %
[Rh(COD)$_2$]BF$_4$, L*
solvent, H$_2$ (5 bar), 23 °C
Ph
*
CO$_2$Me
Me
N
Ac

16

17

full conversion

Entry	Ligand	Solvent	% *ee*
1	(-)-DIPAMP	DCM	26
2	JosiPhos	DCM	9
3	MandyPhos	DCM	15
4	WalPhos	DCM	45
5	TaniaPhos	DCM	51
6	(*R*,*R*,*S*,*S*)-DuanPhos	DCM	84
7	(*R*,*R*)-QuinoxP	DCM	87
8	(*R*,*R*)-BenzP	DCM	93
9	(*R*,*R*,*S*,*S*)-DuanPhos	MeOH	94
10	(*R*,*R*)-QuinoxP	MeOH	89
11	(*R*,*R*)-BenzP	MeOH	87
12	**(*R*,*R*,*S*,*S*)-DuanPhos**	**acetone**	**>99**
13	**(*R*,*R*)-BenzP**	**acetone**	**>99**

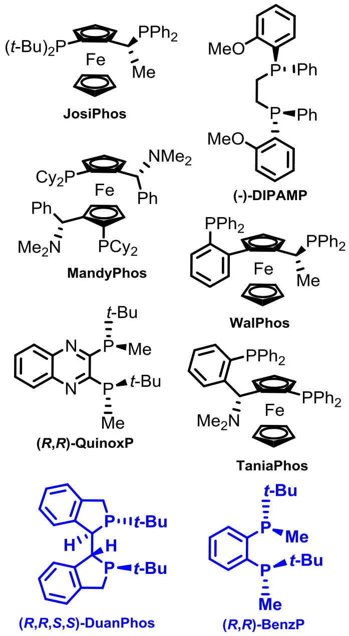

(*t*-Bu)$_2$P
Fe
PPh$_2$
Me
JosiPhos
MeO
P
Ph
P
Ph
MeO
(-)-DIPAMP
Cy$_2$P
Fe
NMe$_2$
Ph
Ph
Me$_2$N
PCy$_2$
MandyPhos
PPh$_2$
Fe
PPh$_2$
Me
WalPhos
t-Bu
P
Me
N
N
P
t-Bu
Me
(*R*,*R*)-QuinoxP
PPh$_2$
Me$_2$N
Fe
PPh$_2$
TaniaPhos
P
t-Bu
H
H
P
t-Bu
(*R*,*R*,*S*,*S*)-DuanPhos
t-Bu
P
Me
P
t-Bu
Me
(*R*,*R*)-BenzP

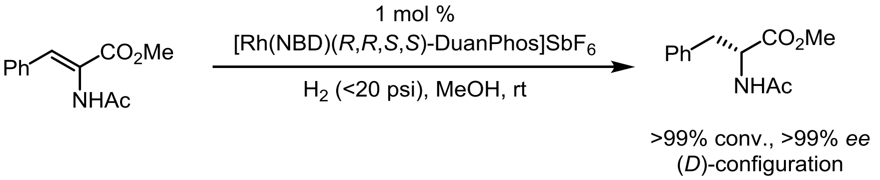
Table 2. Optimization of *N*-Me dehydrophenylalanine asymmetric hydrogenation

1.5.d Late-stage Asymmetric Hydrogenation

Once optimized, the conditions for asymmetric hydrogenation of the single dehydrophenylalanine residue were ready to be applied to the asymmetric hydrogenation of *N*-

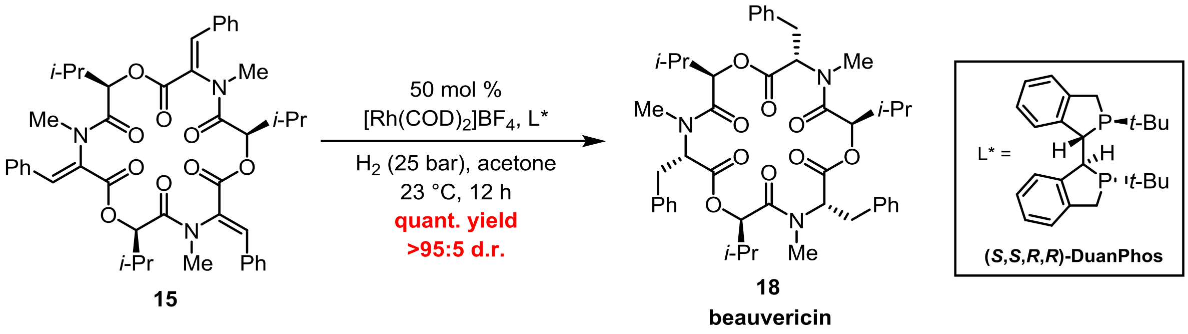
methyl dehydromacrocycle **15**. But first, the issue of which enantiomer of chiral ligand to be used needed to be addressed, otherwise it would end up being a trial-and-error process of finding the right chiral ligand.

In a report by Zhang in 2005, it was found that the (*R*,*R*,*S*,*S*)-DuanPhos enantiomer with cationic rhodium provided hydrogenation of dehydrophenylalanine to *D*-phenylalanine with complete enantioselectivity.[30] For the synthesis of beauvericin, since the desired stereochemical outcome of the hydrogenation is *L*-phenylalanine, it was hypothesized that the opposite enantiomer, the (*S*,*S*,*R*,*R*)-DuanPhos enantiomer would give the desired beauvericin product.


Ph
CO2Me
NHAc
1 mol %
[Rh(NBD)(R,R,S,S)-DuanPhos]SbF6
H2 (<20 psi), MeOH, rt
Ph
CO2Me
NHAc
>99% conv., >99% ee
(D)-configuration

Scheme 15. Zhang's use of DuanPhos for asymmetric hydrogenation

Upon hydrogenating *N*-methyl macrocycle **15** with my optimized conditions using 50 mol % catalyst and ligand loading, it was found that the hydrogenation worked and formed beauvericin **18** in quantitative yield and with greater than 95:5 diastereoselectivity (determined by ^{1}H and ^{13}C NMR, which showed no minor diastereomers).


15
50 mol %
[Rh(COD)2]BF4, L*
H2 (25 bar), acetone
23 °C, 12 h
quant. yield
>95:5 d.r.
18
beauvericin
L* =
(S,S,R,R)-DuanPhos

Scheme 16. Hydrogenation to afford beauvericin

Of the eight possible diastereomers (3 alkenes being reduced to 3 chiral centers so $2^3 = 8$), the only observed diastereomer was beauvericin. The identity was confirmed by comparison of the products ^{1}H and ^{13}C NMR spectra to the spectra reported in the isolation papers of beauvericin. Thus, it was shown that a key cyclotrimerization reaction and a late-stage asymmetric hydrogenation utilizing dehydrophenylalanine residues was a successful way towards synthesizing beauvericin.

In agreement with Zhang's report on hydrogenation of single dehydroamino acids with a Rh(I)/DuanPhos catalyst, the opposite enantiomer of the bisphosphine ligand that Zhang used to give *D*-phenylalanine induced the desired stereochemistry (*L*-phenylalanine) for our system.

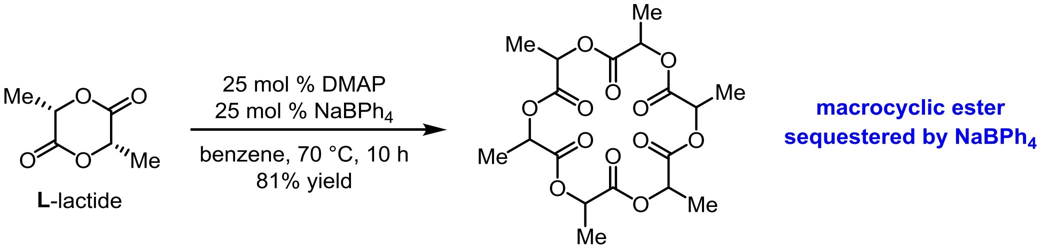
1.6 Conclusions and Future Work

In conclusion, I have developed a convergent synthesis of beauvericin that utilizes a key cyclotrimerization transformation. The cyclotrimerization reaction is a direct and quick way to build structural complexity, allowing the monomeric oxazolone to essentially undergo three reactions in one (dimerization, trimerization, then cyclization). Overall, I was able to synthesize beauvericin in 10% yield across 8 steps.

Further investigation towards reducing formation of dione byproduct that occurs in the cyclotrimerization step should be pursued. For this to occur, I think a more detailed understanding of the cyclotrimerization is warranted, through mechanistic understanding of this transformation (via NMR experiments and/or computational chemistry).

One interesting aspect of this project to synthesize beauvericin through a key cyclotrimerization reaction is that the dione byproduct is always prevalent in greater yield than the desired macrocycle. Thus, if there were enough effort put into developing a ring-opening polymerization strategy of the 6-membered ring dione, then perhaps the cyclotrimerization route would gain an extra step and become much higher yielding (during the course of cyclotrimerization investigation, I found conditions that give dione in 94% isolated yield). That, of course, depends on if ring-opening polymerization can occur with the strained dione system.

Ring-opening polymerization have been shown to work with glycolides and lactides from the work done by Chisholm and Gallucci in 2007, so maybe that could be extended to the morpholine-2,5-dione system.[31]



Scheme 17. Chisholm’s ring-opening polymerization of *L*-lactide

1.7 References

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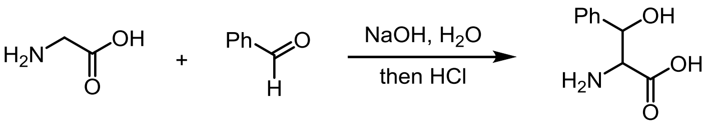
1.8 Supporting Information

1.8.a General Methods

Amino acids and peptide coupling reagents were purchased from Anaspec and Chem-Impex and used without purification. All reactions were carried out under nitrogen or argon atmosphere unless otherwise indicated. Reactions were monitored using thin-layer chromatography (TLC) on EMD Silica Gel 60 F254 plates (visualization of the developed chromatogram was performed by fluorescence quenching or KMnO4 stain) or via analysis on a Waters 2795 Separations Module equipped with a Waters 2996 Photodiode Array and a Waters Micromass ZQ mass spectrometer. Organic solutions were concentrated under reduced pressure using a Büchi rotary evaporator. ^{1}H and ^{13}C NMR spectra were recorded on any of the four spectrometers: Bruker AV600, Bruker GN500, Bruker CRYO500, or Bruker DRX400 NMR instrument. NMR spectra were internally referenced to residual proton solvent signals. Data for ^{1}H NMR were recorded at ambient temperature unless noted otherwise and are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), integration, coupling constant (Hz), and assignment. Data for ^{13}C NMR are reported in terms of chemical shift (δ ppm).

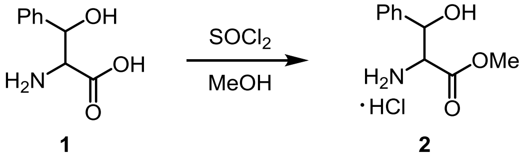
High-resolution mass spectra (HRMS) were acquired on a Waters LCT Premier quadrupole time-of-flight spectrometer and were obtained by peak matching. Infrared (IR) spectra were obtained on a Thermo-Scientific Nicolet iS5 equipped with an iD5 Diamond ATR and are reported in terms of frequency of absorption (cm^{-1}). Chiral analysis was performed on an Agilent 1200 series HPLC equipped with an Aurora SFC module. Optical rotations were measured on a Rudolph Research Analytical Autopol III Automatic Polarimeter. Column chromatography was performed using SiliCycle Silia-P Flash Silica Gel, using either glass columns, a Biotage SP1 system, or a Teledyne CombiFlash Isco system. All salts were purchased from Aldrich and used without purification. Achiral and chiral bisphosphine ligands were purchased from Strem. Unless otherwise noted, all reactions were carried out under an atmosphere of nitrogen or argon in flame-dried glassware.

1.8.b Synthesis of Substrates

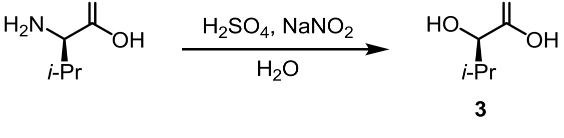


1

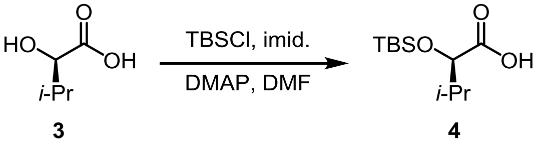
2-Amino-3-hydroxy-3-phenylpropanoic acid (1). To a stirring solution of NaOH (30 g, 0.75 mol) in water (125 mL) at room temperature was added glycine (37.5 g, 0.5 mol). The solution was stirred for 10 min and then benzaldehyde (107 mL, 1.05 mol) was added, and the solution was stirred for 20 min. As the mixture began to solidify and harden, the resulting solid was broken up into small pieces and 12M aqueous HCl (65 mL) was added and the mixture was stirred for 2 h (while continuing to break up the bigger pieces into smaller pieces and sonicating the mixture). The mixture was cooled at 0 °C for 1 h while precipitation occurred. The solid was collected via vacuum filtration and washed with hexanes (3 times). The resulting off-white solid was dried *in vacuo* to give 80.8 g (89% yield, 2.5:1 d.r.) of compound **1**. ^{1}H NMR (500 MHz, D_2O) δ 7.55–7.22 (m, 2H + 5H), 5.35 (d, *J* = 3.5 Hz, 0.4H), 5.29 (d, *J* = 4.2 Hz, 1H), 4.07 (d, *J* = 4,1 Hz, 0.4H), 3.90 (d, *J* = 4.3 Hz, 1H). ^{13}C NMR (126 MHz, D_2O) δ 174.3, 173.4, 141.1, 139.1, 131.2, 131.1, 131.1, 130.8, 128.6, 128.1, 73.6, 73.4, 63.2, 62.7. All spectral data are in agreement with reported literature values.[S1]



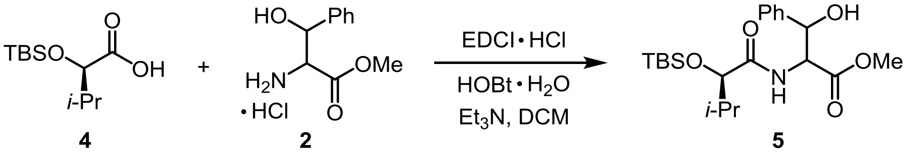
Methyl 2-amino-3-hydroxy-3-phenylpropanoate hydrochloride (2). To a stirring solution of compound **1** (48.7 g, 268.8 mmol) in MeOH (336 mL) at 0 °C was added thionyl chloride (42.9 mL, 591.4 mmol) dropwise. The reaction was warmed to room temperature and then heated at 40 °C for 16 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The reaction was evaporated under reduced pressure, dissolved in DCM (400 mL), evaporated under reduced pressure a second time, dissolved in DCM (400 mL), then evaporated under reduced pressure a third time to afford 56.1 g (90% yield, 2.5:1 d.r.) of compound **2**. ^{1}H NMR (500 MHz, CD_3OD) δ 7.53–7.13 (m, 2H + 5H), 5.30 (d, *J* = 3.8 Hz, 0.4H), 5.25 (d, *J* = 4.5 Hz, 1H), 4.37 (d, *J* = 3.9 Hz, 0.4H), 4.25 (d, *J* = 4.6 Hz, 1H), 3.79 (s, 3H), 3.70 (s, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 169.2, 168.3, 140.4, 139.7, 129.9, 129.9, 129.6, 129.6, 129.5, 129.3, 129.2, 128.2, 127.3, 127.2, 72.2 72.1, 60.5, 60.1, 53.6, 53.2. All spectral data are in agreement with reported literature values.[S2]



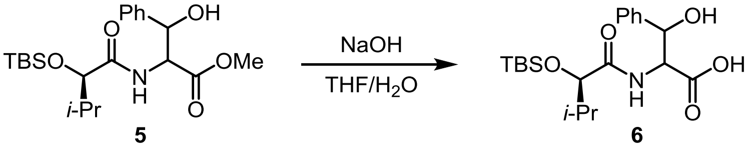
(*R*)-2-Hydroxy-3-methylbutanoic acid (3). To a stirring solution of **D**-valine (4.39 g, 37.5 mmol) in 1M aqueous H_2SO_4 (75 mL) at 0 °C was added a solution of $NaNO_2$ (15.53 g, 225 mmol) in H_2O (45 ml) dropwise. The reaction was warmed to room temperature and reacted for 24 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was extracted with Et_2O (3 times) and the combined organic layers dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford 4.2 g (95% yield) of compound **3**. Further purification was not necessary. ^1H NMR (500 MHz, CD_3OD) δ 4.94 (s, 2H), 3.93 (d, *J* = 4.2 Hz, 1H), 2.05 (m, 1H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 177.4, 76.2, 33.2, 19.3, 16.9. $[\alpha]_D$ (c = 1.0 in $CHCl_3$, T = 23 ºC): -15.7º. Sigma-Aldrich: -17 ± 1º (c = 1.0 in $CHCl_3$). All spectral data are in agreement with reported literature values.[S3]



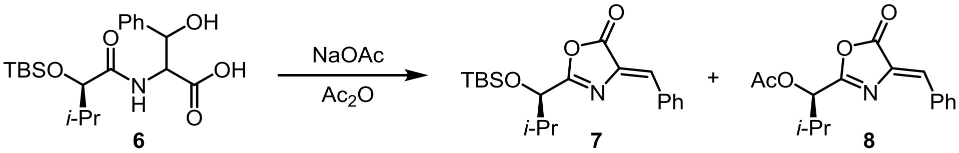
(*R*)-2-((*tert*-Butyldimethylsilyl)oxy)-3-methylbutanoic acid (4). To a stirring solution of compound **3** (0.84 g, 7.1 mmol) in DMF (3.6 mL) was added TBSCl (2.36 g, 15.6 mmol), imidazole (1.06 g, 15.6 mmol), and DMAP (0.26 g, 2.1 mmol). The reaction was stirred at room temperature for 4 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was acidified with 1M HCl (aq) and extracted with Et_2O (3 times). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford crude compound **4**. Crude material was used without further purification in the next step (contained TBS-alcohol byproduct). ^1H NMR (600 MHz, $CDCl_3$) δ 3.91 (d, *J* = 4.5 Hz, 1H), 2.02 (m, 1H), 0.95 (dd, *J* = 5.0, 2.0 Hz, 4H), 0.94 (s, 8H) 0.91 (s, 9H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.27 (d, *J* = 5.9 Hz, 6H), 0.06 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.9, 77.7, 32.9, 25.9, 25.7, 19.3, 17.0, -2.8, -4.8, -4.7, -5.4. All spectral data are in agreement with reported literature values.[S4]



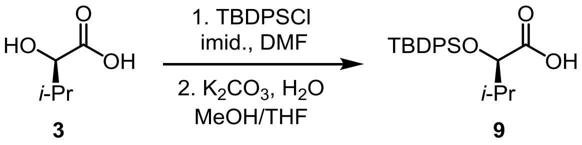
Methyl 2-((*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-methylbutanamido)-3-hydroxy-3-phenylpropanoate (5). To a stirring solution of crude compound **4** (2.4 g, 7.1 mmol) in DCM (71 mL) at 0 °C was added compound **2** (1.98 g, 8.5 mmol), HOBt•H_2O (1.15 g, 8.5 mmol), Et_3N (1.2 mL, 8.5 mmol) and lastly, EDCI•HCl (1.64 g, 8.5 mmol). The reaction was warmed to room temperature and reacted for 8 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was acidified with 10% wt/v $KHSO_4$ (aq) and extracted with DCM (3 times). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford crude compound **5**. Crude material is a diastereomeric mixture (see ^{1}H and ^{13}C NMR spectra in Appendix A), and was used without NMR analysis and without further purification in the next step. IR (in $CDCl_3$): 3413, 2957, 2930, 2858, 1743, 1656, 1520, 1254, 1060, 862, 835, 780, 708 cm^{-1}.



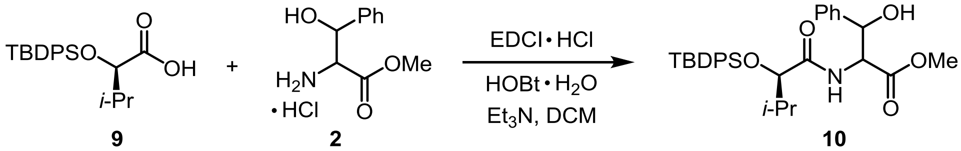
2-((*R*)-2-((*tert*-Butyldimethylsilyl)oxy)-3-methylbutanamido)-3-hydroxy-3-phenylpropanoic acid (6). To a stirring solution of compound **5** (2.9 g, 7.1 mmol) in THF (71 mL) at 0 °C was added 1M aqueous NaOH (14.3 ml) dropwise. The reaction was warmed to room temperature and reacted for 4 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was acidified with 10% wt/v $KHSO_4$ (aq) and extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford crude compound **6**. Crude material is a diastereomeric mixture (see ^{1}H and ^{13}C NMR spectra in Appendix A), and was used without NMR analysis and without further purification in the next step. IR (in DCM): 3394, 2962, 1727, 1651, 1527, 1264, 1059, 836, 735, 701 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{20}H_{33}NO_5Si$ [M-H]$^-$ 394.2050, found 394.2050.



(*R*,*Z*)-4-Benzylidene-2-(1-((*tert*-butyldimethylsilyl)oxy)-2-methylpropyl)oxazol-5(4*H*)-one (7) and (*R*,*Z*)-1-(4-benzylidene-5-oxo-4,5-dihydrooxazol-2-yl)-2-methylpropyl acetate (8). To a stirring solution of compound **6** (2.8 g, 7.1 mmol) in Ac_2O (27 mL, 284 mmol) was added NaOAc (1.17 g, 14.2 mmol). The reaction was stirred at room temperature for 12 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was quenched with saturated aqueous $NaHCO_3$ (until bubbling stopped) and then extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford a mixture of compounds **7** and **8**. The products were purified by column chromatography (0 to 10% EtOAc in hexanes) to provide 0.96 g (38% yield, 4 steps) of compound **7** and 0.71 g (35% yield, 4 steps) of compound **8**. Compound **7:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (m, 2H), 7.44 (m, 3H), 7.21 (s, 1H), 4.34 (d, *J* = 5.9 Hz, 1H), 2.17 (m, 1H), 1.00 (dd, *J* = 8.7, 6.8 Hz, 6H), 0.94 (s, 9H), 0.09 (d, *J* = 15.5 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 167.8, 133.3, 132.9, 132.6, 132.1, 131.4, 129.0, 74.4, 33.1, 25.8, 18.9, 18.4, 17.6, -4.7, -5.1. IR (in $CDCl_3$): 2958, 2929, 2857, 1807, 1778, 1657, 1252, 1162, 1140, 1070, 895, 836, 768, 688 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{20}H_{29}NO_3Si$ $[M+Na]^+$ 382.1814, found 382.1806. Compound **8:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12–8.02 (m, 2H), 7.47–7.39 (m, 3H), 7.22 (s, 1H), 5.39 (d, *J* = 5.9 Hz, 1H), 2.36 (m, 1H), 2.19 (s, 3H), 1.07 (d, *J* = 6.9, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4, 167.1, 165.3, 133.7, 133.0, 132.7, 131.6, 129.0, 73.7, 30.8, 20.7, 18.6, 17.7. IR (neat): 2971, 1779, 1744, 1655, 1371, 1224, 1162, 1032, 893, 864, 767, 688 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{16}H_{17}NO_4$ $[M+Na]^+$ 310.1055, found 310.1045.

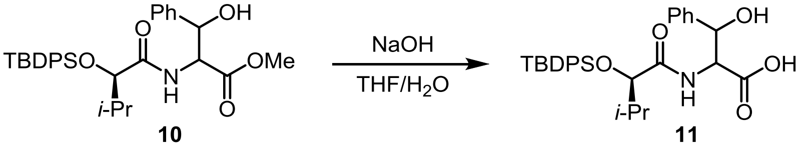


(*R*)-2-((*tert*-Butyldiphenylsilyl)oxy)-3-methylbutanoic acid (9). To a stirring solution of compound **3** (100 mg, 0.85 mmol) in DMF (0.35 mL) at 0 °C was added a solution of TBDPSCl (0.7 mL, 2.7 mmol) and imidazole (612.7 mg, 9 mmol) in DMF (0.55 mL). The reaction was warmed to room temperature and reacted for 2 h, at which monitoring by LC-MS showed absence of starting material and presence of mono and disilylated material. The mixture was diluted with cold brine and extracted with 1:3 Et_2O/hexanes (5 times). The combined organic layers were washed with water, dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford a crude oil. The crude oil was then dissolved in 3:2 MeOH/THF (7.5 mL), cooled to 0 °C, and a solution of K_2CO_3 (264 mg, 1.9 mmol) in water (1.75 mL) was added dropwise to the stirring solution. The reaction was warmed room temperature and reacted for 12 h, at which monitoring by LC-MS showed absence of disilylated material and presence of monosilylated material. The mixture was diluted with brine, cooled to 0 °C, acidified with H_2SO4, and then extracted with 1:3 Et_2O/hexanes (4 times). The combined organic layers were extracted with 2% aqueous NaOH (4 times), and the combined aqueous layers were cooled to 0 °C, acidified with H_2SO_4, and then extracted with DCM (5 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford 257.9 mg (85% yield) compound **9**. ^1H NMR (500 MHz, $CDCl_3$) δ 7.65 (dd, *J* = 11.8, 7.0 Hz, 4H), 7.49–7.33 (m, 6H), 4.12 (d, *J* = 3.9 Hz, 1H), 1.94 (m, 1H), 1.13 (s, 9H), 0.90 (dd, *J* = 10.2, 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.6, 136.1, 136.0, 134.9, 132.9, 132.6, 130.2, 130.2, 129.8, 127.9, 77.5, 33.4, 27.1, 26.7, 19.6, 18.0, 17.5. All spectral data are in agreement with reported literature values.[S5]

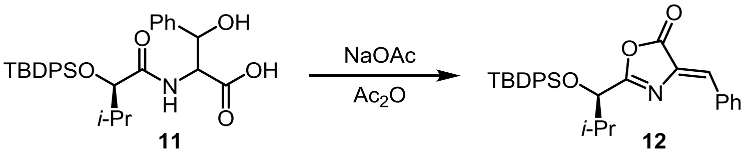


Methyl 2-((*R*)-2-((*tert*-butyldiphenylsilyl)oxy)-3-methylbutanamido)-3-hydroxy-3-phenyl-propanoate (10). To a stirring solution of crude compound **9** (33.65 g, 94.4 mmol) in DCM (944 mL) at 0 °C was added compound **2** (26.24 g, 113.3 mmol), HOBt•H_2O (15.29 g, 113.3 mmol), Et_3N (15.9 mL, 113.3 mmol) and lastly, EDCI•HCl (21.71 g, 113.3 mmol). The reaction was warmed to room temperature and reacted for 4 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was acidified with 10% wt/v $KHSO_4$ (aq) and extracted with DCM (5 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford crude compound **10**. Crude material is a diastereomeric mixture (see ^1H and ^{13}C NMR spectra in

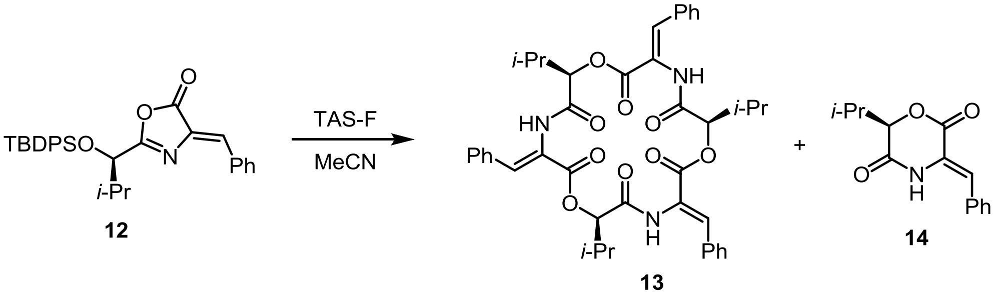
Appendix A), and was used without NMR analysis and without further purification in the next step. IR (in DCM): 3413, 2959, 2858, 1742, 1658, 1516, 1428, 1111, 1061, 848, 820, 738, 700, 608 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{31}H_{39}NO_5Si$ $[M+Na]^+$ 556.2495, found 556.2488.



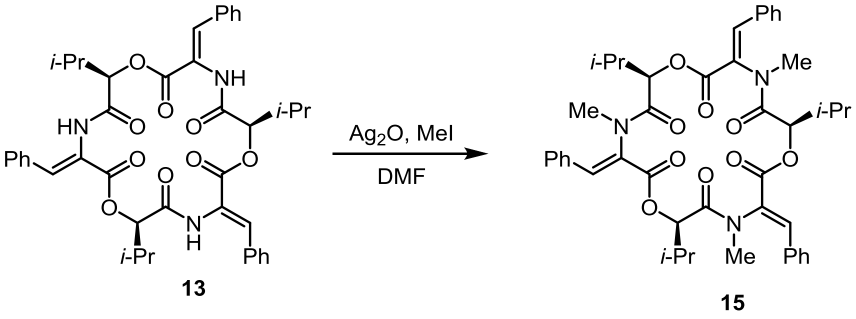
2-((*R*)-2-((*tert*-Butyldiphenylsilyl)oxy)-3-methylbutanamido)-3-hydroxy-3-phenylpropanoic acid (11). To a stirring solution of compound **10** (94.4 mmol) in THF (944 mL) at 0 °C was added 1M aqueous NaOH (189 mL) dropwise. The reaction was warmed to room temperature and reacted for 2 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was acidified with 10% wt/v $KHSO_4$ (aq) and extracted with EtOAc (5 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford crude compound **11**. Crude material is a diastereomeric mixture (see ^{1}H and ^{13}C NMR spectra in Appendix A), and was used without NMR analysis and without further purification in the next step. IR (in DCM): 3411, 2929, 2857, 1731, 1657, 1520, 1427, 1264, 1111, 1060, 847, 821, 738, 700, 611 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{30}H_{37}NO_5Si$ $[M+Na]^+$ 542.2339, found 542.2328.



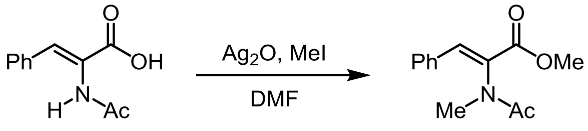
(*R*,*Z*)-4-Benzylidene-2-(1-((*tert*-butyldiphenylsilyl)oxy)-2-methylpropyl)oxazol-5(4*H*)-one (12). To a stirring solution of compound **11** (94.4 mmol) in Ac_2O (178 mL, 1.89 mol) was added NaOAc (15.48 g, 188.8 mmol). The reaction was monitored by LC-MS, which showed a slow progression. After 5 days, additional NaOAc (15.48 g, 188.8 mmol) was added. The reaction stirred for an additional day, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was quenched with both solid and saturated aqueous $NaHCO_3$ (until bubbling stopped) and then extracted with EtOAc (4 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford a crude oil, which was purified by column chromatography (0 to 10% EtOAc in hexanes) to provide 31.2 g (68% yield, 3 steps) of compound **12**. Note: smaller scale reaction (2.3 mmol) required no additional NaOAc and was completed in 12 hours. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 7.4 Hz, 2H), 7.66 (d, *J* = 7.1 Hz, 4H), 7.43–7.24 (m, 9H), 6.98 (s, 1H), 4.41 (d, *J* = 6.2 Hz, 1H), 2.20 (m, 1H), 1.13 (s, 9H), 1.10 (d, *J* = 6.9 Hz, 3H), 0.97 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.8, 167.5, 136.1, 133.1, 133.1, 132.9, 132.6, 132.5, 131.9, 131.2, 130.0, 130.0, 128.9, 127.8, 127.6, 75.2, 33.7, 27.1, 19.7, 18.5, 18.2. IR (neat): 3068, 2964, 2931, 2858, 1806, 1781, 1658, 1428, 1157, 1136, 1112, 700, 610 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{30}H_{33}NO_3Si$ $[M+Na]^+$ 506.2127, found 506.2122.



(6*R*,12*R*,18*R*)-3,9,15-Tri((*Z*)-benzylidene)-6,12,18-triisopropyl-1,7,13-trioxa-4,10,16-triaza-cyclooctadecane-2,5,8,11,14,17-hexaone (13) and (*R*,*Z*)-3-benzylidene-6-isopropyl-morpholine-2,5-dione (14). To a stirring solution of compound **12** (96.7 mg, 2 mmol) in MeCN (2 mL) was added TAS-F (67.4 mg, 1.2 mmol). The reaction was heated at 80 °C for 18 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was quenched with brine and then extracted with EtOAc (3 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford a crude oil, which was purified by column chromatography (first 0 to 25% EtOAc in hexanes, then 25% isocratic) to provide 11.4 mg (23% yield) of compound **13** and 12.9 mg (26% yield) of compound **14**. Compound **13:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 3H), 7.65 (s, 3H), 7.48–7.37 (m, 15H), 5.36 (d, *J* = 4.1 Hz, 3H), 2.37 (m, 3H), 1.07 (dd, *J* = 6.1 Hz, 18H).^{13}C NMR (126 MHz, $CDCl_3$) δ 165.1, 133.3, 130.3, 130.2, 128.9, 30.8, 29.8, 19.0, 17.9. IR (in $CDCl_3$): 3270, 2963, 2926, 1722, 1449, 1371, 1254, 1200, 1095, 1025, 696 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{42}H_{45}N_3O_9$ $[M+Na]^+$ 758.3054, found 758.3035. Compound **14:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.46 (t, *J* = 7.5 Hz, 2H), 7.39 (dd, *J* = 12.6, 7.3 Hz, 3H), 7.11 (s, 1H), 4.78 (d, *J* = 3.9 Hz, 1H), 2.46 (m, 1H), 1.17 (d, *J* = 7.0 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.6, 159.7, 132.2, 129.7, 129.6, 128.7, 122.1, 120.7, 83.5, 32.6, 18.6, 16.4. IR (in DCM): 3232, 2967, 1737, 1689, 1639, 1373, 1243, 1200, 1141, 1019, 860, 768, 691 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{14}H_{15}NO_3$ $[M+Na]^+$ 268.0950, found 268.0956.

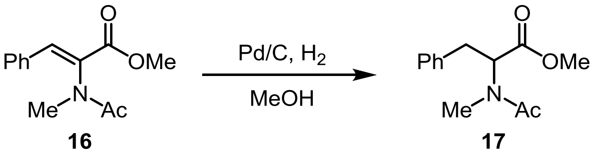


(6*R*,12*R*,18*R*)-3,9,15-Tri((*Z*)-benzylidene)-6,12,18-triisopropyl-4,10,16-trimethyl-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexaone (15). To a stirring solution of compound **13** (16.6 mg, 0.02 mmol) in DMF (90 μL) at room temperature was added Ag_2O (17.3 mg, 0.07 mmol) and then MeI (8.5 μL, 0.14 mmol). The reaction was stirred at room temperature for 6 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was eluted through a plug of silica gel using EtOAc and then evaporated under reduced pressure to afford 13.9 mg (79% yield) of compound **15**. Further purification was not necessary. Note: compound **15** exists in multiple conformations which made NMR characterization complicated and NMR analysis was not performed (see ^{1}H and ^{13}C NMR spectra in Appendix A). IR (in $CDCl_3$): 2966, 2930, 2874, 1721, 1674, 1448, 1396, 1247, 1192, 1089, 776, 732, 693 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{45}H_{51}N_3O_9$ $[M+Na]^+$ 800.3523, found 800.3511.



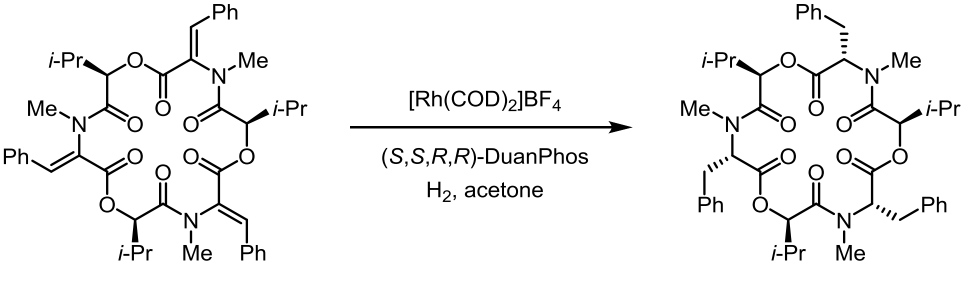
16

Methyl (*Z*)-2-(*N*-methylacetamido)-3-phenylacrylate (16). To a stirring solution of *α*-acetamidocinnamic acid (1.03 g, 5 mmol) in DMF (20 mL) at room temperature was added silver oxide (2.32 g, 10 mmol) and then methyl iodide (0.93 mL, 15 mmol). The reaction was stirred at room temperature for 18 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was eluted through a plug of silica gel using EtOAc and then evaporated under reduced pressure to afford a crude oil which was purified by column chromatography (40 to 50% EtOAc in hexanes) to provide 1.05 g (90% yield) of compound **16**. Note: compound **16** exists as a mixture of *cis*- and *trans*-amide conformers (^{1}H NMR ratio changes from 5:1 in DMSO to 12:1 in $CDCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.51 (m, 2H), 7.42 (m, 3H), 3.86 (s, 3H), 3.06 (s, 3H), 1.88 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.1, 165.7, 138.0, 132.2, 131.2, 131.2, 130.4, 129.4, 52.9, 33.9, 21.4. All spectral data are in agreement with reported literature values.[S6]



16 **17**

To a stirring solution of compound **16** (46.7 mg, 0.2 mmol) in MeOH (2 mL) was purged with nitrogen and then 10% Pd/C (21.3 mg, 0.02 mmol) was added through transfer in water. The reaction was purged with H_2 (3 times) and then sealed under a H_2 balloon. The reaction was stirred at room temperature for 5 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was eluted through a plug of celite using EtOAc, evaporated under reduced pressure to afford an oil, then passed through a another plug of celite using EtOAc and evaporated under reduced pressure to give 47.1 mg (quantitative yield) of compound **17**. Note: compound **17** exists as a mixture of *cis*- and *trans*-amide conformers. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32–7.11 (m, 6H), 5.27 (dd, J = 10.7, 5.5 Hz, 1H), 3.72 (s, 3H), 3.35 (dd, J = 14.6, 5.5 Hz, 1H), 3.03 (dd, J = 14.6, 10.8 Hz, 1H), 2.81 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5, 171.3, 137.2, 128.8, 128.6, 126.8, 58.2, 52.4, 34.8, 33.8, 21.8. All spectral data are in agreement with reported literature values.[S7]



15 **18**

(3*S*,6*R*,9*S*,12*R*,15*S*,18*R*)-3,9,15-Tribenzyl-6,12,18-triisopropyl-4,10,16-trimethyl-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexaone (18). Inside a glove box, to a vial containing (*S*,*S*,*R*,*R*)-DuanPhos (1.5 mg, 3.7 μmol) was added $[Rh(COD)_2]BF_4$ (1.4 mg, 3.7 μmol) with degassed acetone (0.7 mL). The resulting solution was stirred at room temperature for 5 min. The mixture was then added to a HEL reactor vial containing compound **15** (5.7 mg, 7.4 μmol). The vial was placed inside a HEL reactor and pressurized to the desired hydrogen pressure (25 bar). The hydrogenation was performed at room temperature for 12 h. After slowly releasing the hydrogen, the reaction mixture was analyzed by ^{1}H NMR and LC-MS which determined full conversion from starting material to product. Purification was achieved by eluting the mixture through a plug of silica gel with EtOAc to afford 5.8 mg (quantitative yield) of compound **18.** Alternatively, purification can be carried out with column chromatography or preparative TLC. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56–7.27 (m, 7H), 7.24–7.03 (m, 8H), 5.52 (d, *J* = 10.8 Hz, 3H), 4.88 (d, *J* = 8.9 Hz, 3H), 3.35 (s, 3H), 3.01 (s, 6H), 1.99 (s, 3H), 1.63 (s, 8H), 1.25 (s, 12H), 0.90–0.77 (m, 9H), 0.40 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.1, 129.0, 128.7, 127.0, 110.1, 34.9, 32.1, 29.9, 29.5, 22.9, 18.5, 17.5, 14.4. All spectral data are in agreement with reported literature values.[S8]

1.8.c References

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Chapter 2: Second Generation Synthesis of Cyclic Hexadepsipeptide Beauvericin via a Linear Dehydrodepsipeptide Approach

2.1 Introduction

Having shown that beauvericin can be synthesized using a cyclotrimerization approach, I then wondered if the natural product could be synthesized through a linear route as well. To my knowledge, all of the recent literature for synthesizing cyclic depsipeptides proceeds through a single bond formation in the cyclization step (and not three like the cyclotrimerization; one for the dimerization, another for the trimerization, and a third for the cyclization). Most of these cyclization steps are carried out through the use of peptide coupling reagents to make an amide bond for the final connection, however there are examples of the cyclization occurring with the formation of an ester bond, or in rarer cases: the formation of a disulfide bond or an olefin (via ring closing metathesis). The previously reported synthesis of beauvericin, achieved by Ovchinnikov and coworkers in 1971, accomplished the cyclization through the formation of an amide bond.

2.1.a Methods of Cyclizing Depsipeptides

Of the ways to cyclize depsipeptides, the most reported method is through the formation of an amide bond. In a recent review on the synthesis of cyclic depsipeptides, 11 out of the 15 total syntheses that were described were cyclized via macrolactamization.[1] Since macrolactamization requires the use of peptide coupling reagents, there are many different specific peptide coupling reagents to achieve the amide bond formation. Featured in the review were examples that used HATU, PyBOP, PyAOP, as well as EDCI with HOBt as the peptide coupling reagents (the first three have the peptide coupling additive built into the reagent, EDCI is what initially activates the carboxylic acid and then the additive HOBt attacks).

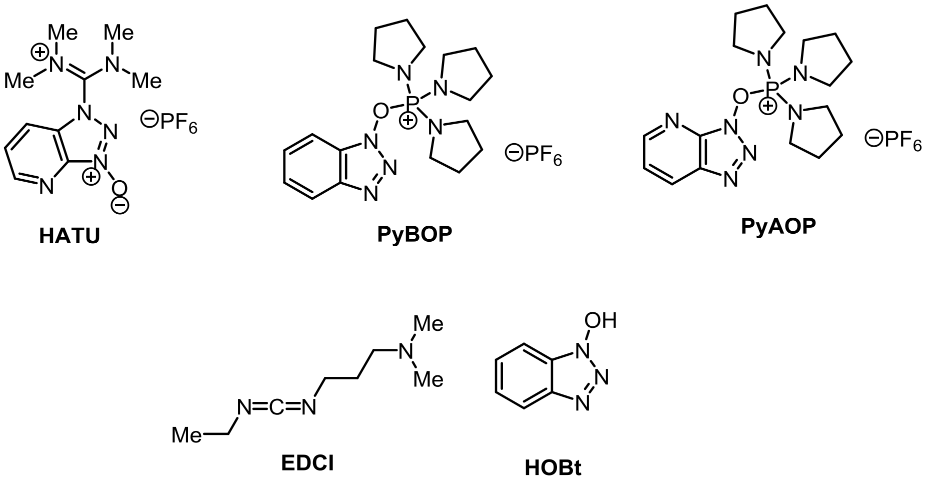
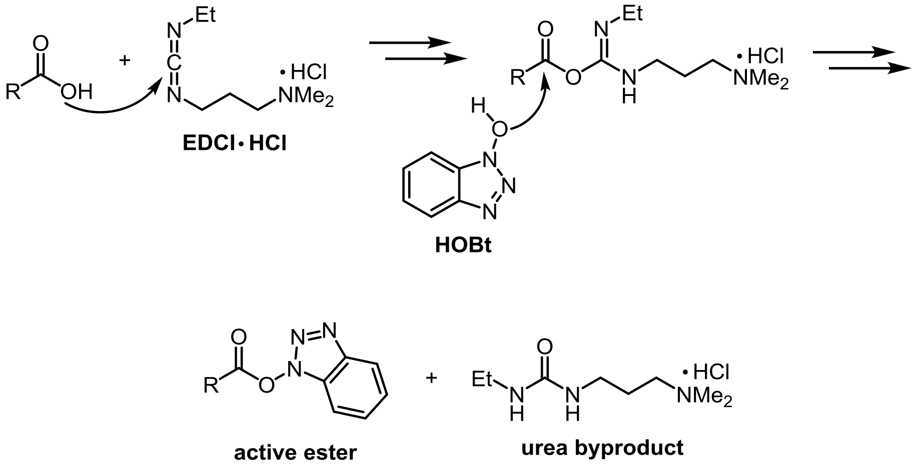


Figure 6: Examples of peptide coupling reagents

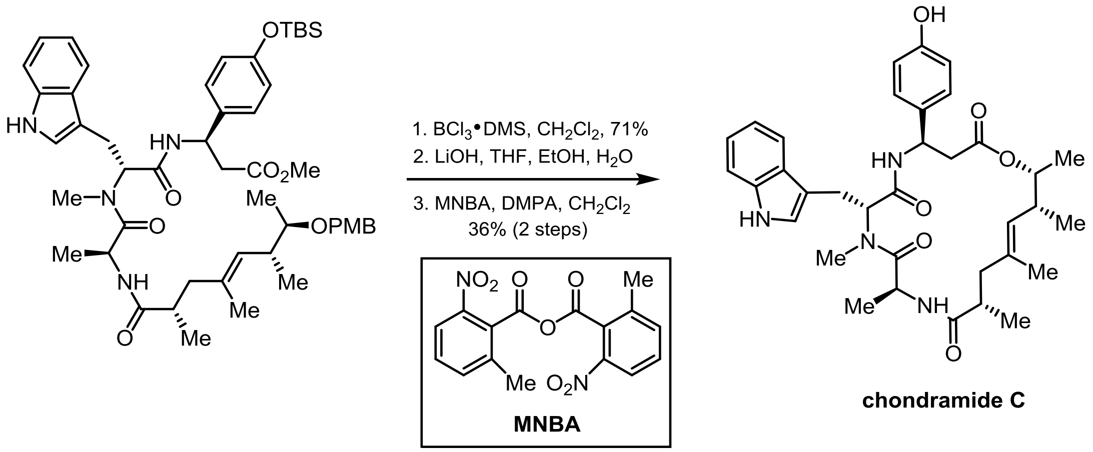
Each of these reagents achieves the same intermediate upon reaction with the carboxylic acid of the coupling reaction: formation of an active ester. For the desired coupling to occur, the amine nucleophile then attacks the active ester, and in the case of peptide coupling using EDCI and HOBt, an amide bond is formed and HOBt is released as a leaving group.



Scheme 18. Formation of active ester using EDCI and HOBt

One of the downfalls in peptide coupling chemistry is the possibility of racemization or epimerization of the *C*-terminus α-stereocenter before the active ester is formed. For some instances, avoiding this can be reason enough to cyclize the depsipeptide through other means. In addition, depending on the substrate, lactamization can be more difficult if the nitrogens are all very sterically hindered.

Macrolactonization can be a useful alternative when epimerization might be an issue, depending on the particular cyclic depsipeptide. One way for macrolactonization to occur, featured in the review, is through the use of the reagent MNBA with DMAP (the MNBA forms a mixed anhydride by reacting with a carboxylic acid and the DMAP serves as an acyl-transfer reagent), as shown in the work done by Kalesse and coworkers in the synthesis of the 18-membered cyclic depsipeptide chondramide C.[2]



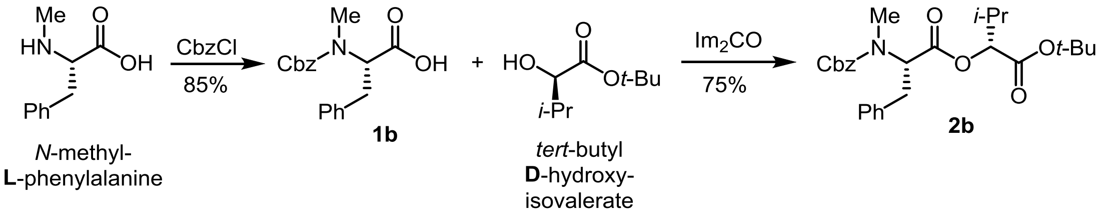
Scheme 19. Macrolactonization using MNBA

Another method featured in the review, which worked when other methods (condensative macrolactonizations using Yamaguchi, Shiina, Mukaiyama, Corey-Nicolaou, and Mitsunobu conditions) failed, consists of an S_N2 displacement of an alkyl halide by a carboxylate (achieved upon treatment of a carboxylic acid with K_2CO_3). In the example, a primary alkyl chloride was used.

Also shown in the review for ways to synthesize cyclic depsipeptides were through the formation of a disulfide bond, or through the formation of olefins (via RCM). These methods are much more substrate dependent, and since the definition of a depsipeptide is a peptide that contains at least one ester bond, the first two methods (macrolactamization and macrolactonization) are more common.

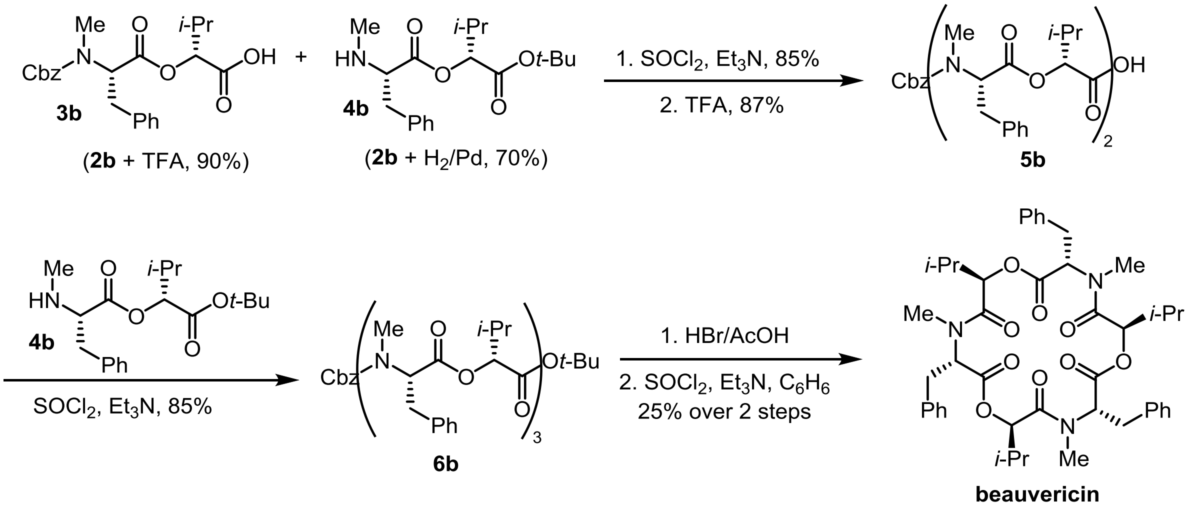
2.1.b Previous Synthesis of Beauvericin

Ovchinnikov and coworkers synthesized beauvericin in 1971 through a non-dehydroamino acid, linear route.[3] They began by taking *N*-methyl-*L*-phenylalanine and Cbz-protecting the nitrogen, which proceeded in 85% yield to furnish protected amino acid **1b**. Then, the authors report coupling of protected amino acid **1b** with *tert*-butyl **D**-hydroxyisovalerate through the use of a diimidazole carbonyl, affording didepsipeptide **2b** in 75% yield (protected at both the *N*- and the *C*-terminus).



Scheme 20. Ovchinnikov's synthesis of common didepsipeptide building block

From common didepsipeptide **2b**, they then diverge in their synthesis. For the coupling to form tetradepsipeptide **5b**, they take common didepsipeptide **2b** and on one hand, treat with TFA to remove the *tert*-butyl group and make didepsipeptide acid **3b** in 90% yield, and on the other hand, treat with hydrogenolysis conditions via H_2/Pd to deprotect the Cbz group and make didepsipeptide ester **4b** in 70% yield. Coupling of didepsipeptide acid **3b** with didepsipeptide ester **4b** ensues through the use of thionyl chloride and triethylamine to form an acid chloride *in situ*, and the resulting tetradepsipeptide ester (not shown) is obtained in 85% yield. Upon treatment with TFA, tetradepsipeptide acid **5b** is synthesized in 87% yield. Elongation by coupling with the didepsipeptide ester **4b** then follows, again through the acid chloride method obtained upon treatment with thionyl chloride and triethylamine to furnish hexadepsipeptide acid **6b** in 85% yield. Deprotection of the Cbz group is carried out with HBr in acetic acid, and the subsequent cyclization is done with thionyl chloride and triethylamine in "highly dilute benzene conditions" (again, the paper does not supply detailed information) to provide beauvericin in 25% yield over the last two steps.


3b
(2b + TFA, 90%)
4b
(2b + H2/Pd, 70%)
1. SOCl2, Et3N, 85%
2. TFA, 87%
5b
4b
SOCl2, Et3N, 85%
6b
1. HBr/AcOH
2. SOCl2, Et3N, C6H6
25% over 2 steps
beauvericin

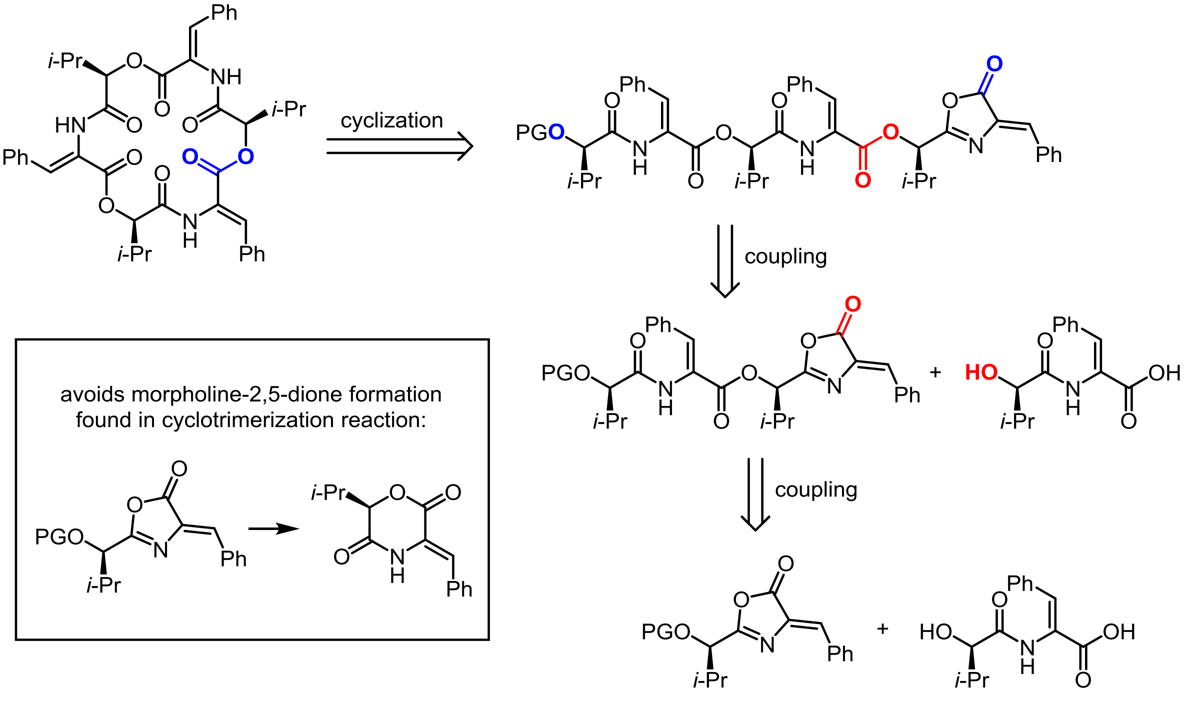
Scheme 21. Ovchinnikov's total synthesis of beauvericin

Overall, the synthesis carried out by Ovchinnikov and coworkers is a somewhat convergent one (didepsipeptide coupling) and affords beauvericin in 7% yield across 8 steps.

2.2 Retrosynthetic Analysis

One of the promising features to a linear synthesis of beauvericin, I thought, could be the fact that the dione formation that was present in the cyclotrimerization reaction could be suppressed due to the fact that a linear route would not involve the deprotection of the protected dipeptide oxazolone.

Retrosynthetically, I envisioned formation of the macrocycle through a cyclization of a linear hexadepsipeptide oxazolone. The hexadepsipeptide oxazolone could be disconnected by means of coupling a tetradepsipeptide oxazolone with a hydroxy dipeptide acid. The tetradepsipeptide could be made in a similar manner by coupling the same hydroxy dipeptide acid with a protected dipeptide oxazolone.


cyclization
coupling
coupling
avoids morpholine-2,5-dione formation found in cyclotrimerization reaction:
PGO
i-Pr
Ph
HO
OH

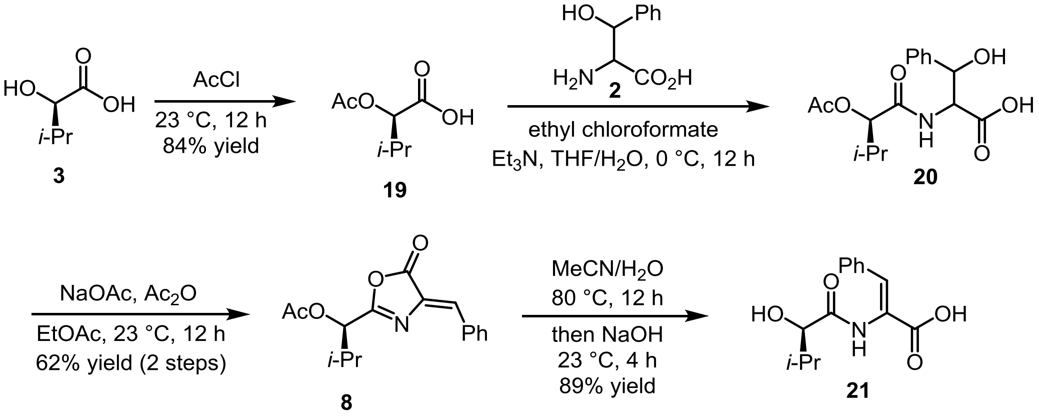
Scheme 22. Retrosynthesis of beauvericin via linear route

In fact, the only new piece needed to begin this linear route would be the hydroxy dipeptide acid, since the same TBDPS-protected oxazolone used for the cyclotrimerization could be used for this linear route.

2.3 Results

2.3.a Synthesis of Dipeptide Acid

Synthesis of hydroxy dipeptide acid **21** was straightforward. Similar to my previous oxazolone syntheses, we took **D**-hydroxyisovaleric acid **3** and protected the hydroxy group, but this time with an acetyl protecting group through the use of acetyl chloride to furnish acetyl protected hydroxy acid **19** in 93% yield. Then, acetyl protected hydroxy acid **19** was coupled to *β*-OH phenylalanine through formation of a mixed anhydride by reacting the carboxylic acid with ethyl chloroformate to afford dipeptide acid **20**, which was then subjected to sodium acetate and acetic anhydride to form oxazolone **8** in 62% yield over the past two steps**.**



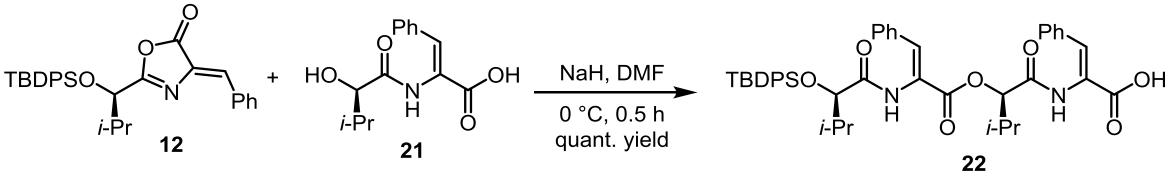
AcCl
23 °C, 12 h
84% yield
3
19
2
ethyl chloroformate
Et3N, THF/H2O, 0 °C, 12 h
20
NaOAc, Ac2O
EtOAc, 23 °C, 12 h
62% yield (2 steps)
8
MeCN/H2O
80 °C, 12 h
then NaOH
23 °C, 4 h
89% yield
21

Scheme 23. Synthesis of hydroxy dipeptide acid

At this point, I would like to highlight the useful reaction that Nathan Bennett discovered with respect to the hydrolysis of the oxazolone using a one to one mixture of acetonitrile and water at elevated temperature. This has shown to be a mild way to hydrolyze oxazolones, and once complete, sodium hydroxide was then added in the same pot to hydrolyze and remove the acetyl protecting group without potentially racemizing the dipeptide (since the pK_a of the α-proton of hydroxyisovaleric acid is significantly lowered when the oxazolone is present). Thus, we were able to arrive at hydroxy dipeptide acid **21** from oxazolone **8** in 89% yield.

2.3.b Linear Elongation and Cyclization

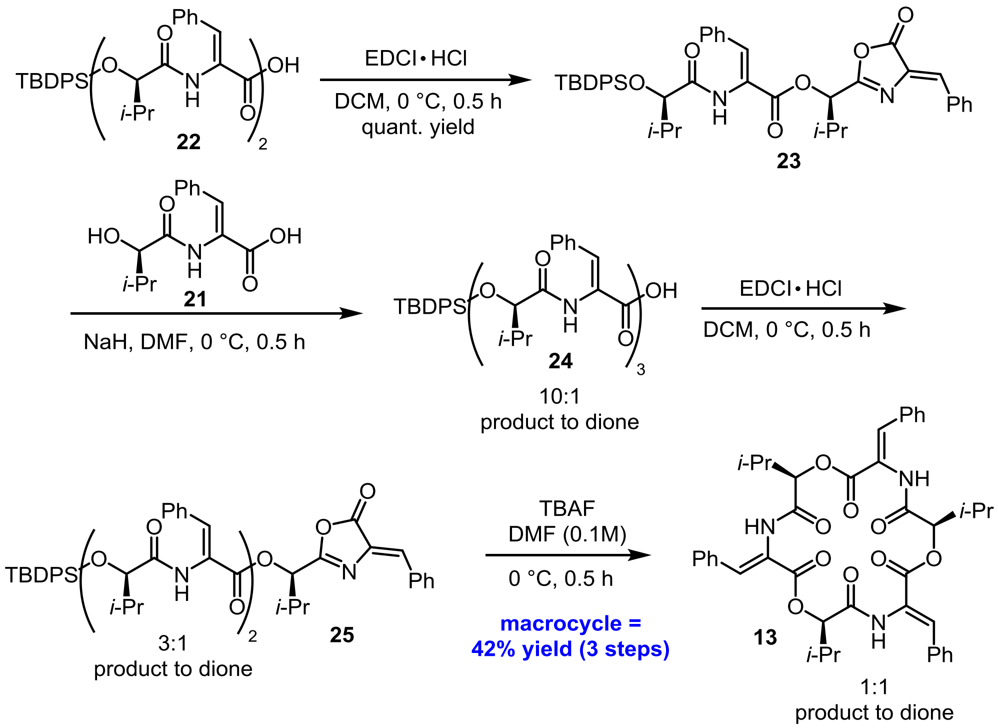
With hydroxy dipeptide acid **21** at hand, I was ready to begin the forward synthesis of the hexadepsipeptide oxazolone needed for the cyclization to afford the macrocyclic precursor to beauvericin. By treating hydroxy dipeptide acid **21** with 2.2 equivalents of sodium hydride, I discovered that the resulting alkoxide dipeptide carboxylate coupled extremely well with dipeptide oxazolone **12** to afford tetradepsipeptide acid **22** in quantitative yield. It should be noted that milder bases such as NaOH or Et_3N did not work in the esterification of oxazolone **12** with alcohol **21**.



Scheme 24. Synthesis of tetradepsipeptide acid

To our knowledge, oxazolones had been used as activated esters for coupling with nitrogen nucleophiles to result in dehydroamino acid amide bonds, but this was the first case of using an oxygen nucleophile to make a dehydroamino acid ester bond!

Upon treatment of tetradepsipeptide acid **22** with EDCI, I was then able to make tetradepsipeptide oxazolone **23** in quantitative yield. Coupling of tetradepsipeptide oxazolone **23** with the alkoxide dipeptide carboxylate obtained from hydroxy dipeptide acid **21** (again, with 2.2 equiv NaH), however, did result in some dione formation (9%). But more importantly, the major product hexadepsipeptide acid **24** was the desired product, which was carried forward as a mixture and subjected to EDCI conditions to synthesize hexadepsipeptide oxazolone **25** (1H NMR of product showed mixture containing 25% dione, so 16% formed from hexadepsipeptide acid **24** to hexadepsipeptide oxazolone **25**).


22
EDCI • HCl
DCM, 0 °C, 0.5 h
quant. yield
23
21
NaH, DMF, 0 °C, 0.5 h
24
10:1
product to dione
EDCI • HCl
DCM, 0 °C, 0.5 h
25
3:1
product to dione
TBAF
DMF (0.1M)
0 °C, 0.5 h
macrocycle =
42% yield (3 steps)
13
1:1
product to dione

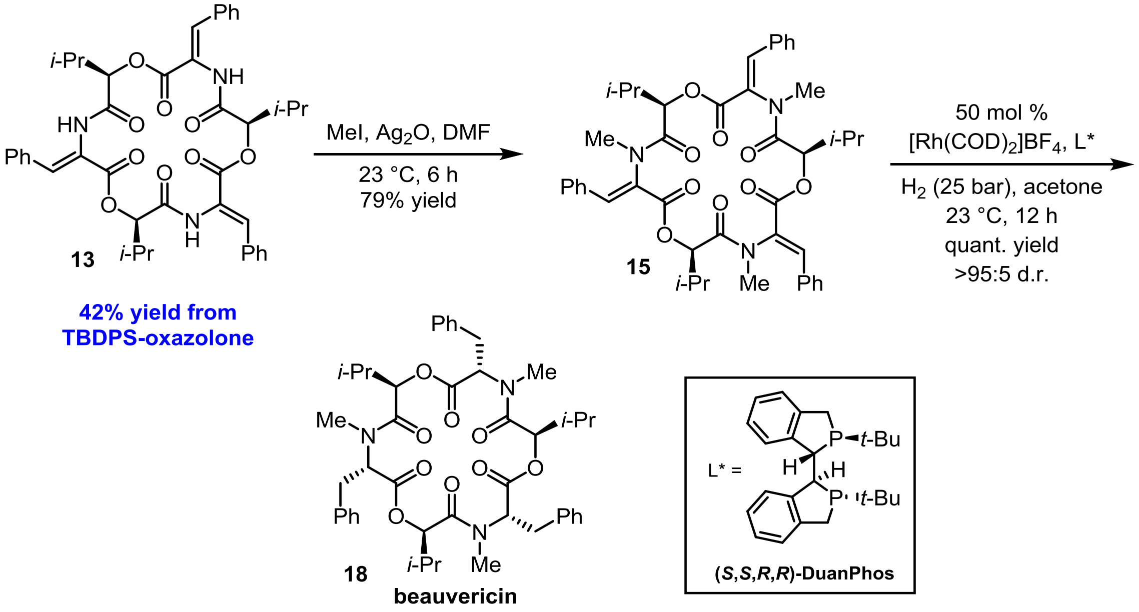
Scheme 25. Linear synthesis towards beauvericin

Having made hexadepsipeptide oxazolone **25** (as a mixture containing 25% dione), I was then ready for the cyclization to form macrocycle **13**. Deprotection of the TBDPS group of hexadepsipeptide oxazolone **25** was carried out with TBAF, and I was pleased to discover that the cyclization worked! Upon purifying the macrocycle, I obtained an isolated yield of 42% over the last three steps (and when considering the steps to synthesize tetradepsipeptide acid **22** and tetradepsipeptide oxazolone **23** were quantitative, the linear sequence starting with dipeptide oxazolone **12** and hydroxy dipeptide acid **21** is two steps away from beauvericin and at 42% yield over five steps).

With regards to the unexpected dione formation, I suspect that this could be a result of hydrolysis of the internal ester bond of the tetra and hexadepsipeptide, as well as a result of deletion that could be occurring at each of these steps. In my efforts to discover an alternative linear route before finding the sodium hydride method to work so well, I synthesized a tetradepsipeptide methyl ester and tried to selectively hydrolyze the terminal ester (in the presence of the internal ester) using 1 equiv LiOH. Unfortunately, the tetradepsipeptide methyl ester fragmented at the internal ester position, suggesting that the internal esters are susceptible in both the cyclotrimer and the linear routes towards beauvericin.

2.3.c *N*-Methylation and Asymmetric Hydrogenation

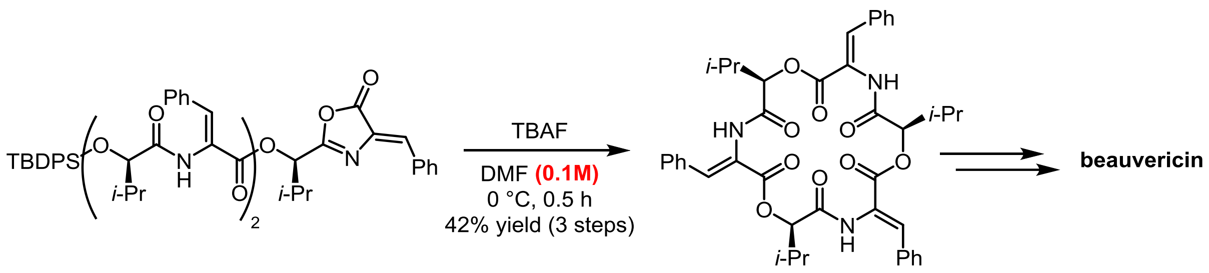
Having procured macrocycle **13** in 42% yield from TBDPS-protected dipeptide oxazolone **12** using the iterative approach that the linear route offers, the second generation synthesis completed the same as before.


13
42% yield from
TBDPS-oxazolone
MeI, Ag$_2$O, DMF
23 °C, 6 h
79% yield
15
50 mol %
[Rh(COD)$_2$]BF$_4$, L*
H$_2$ (25 bar), acetone
23 °C, 12 h
quant. yield
>95:5 d.r.
18
beauvericin
L* =
(*S*,*S*,*R*,*R*)-DuanPhos

Scheme 26. Linear route endgame

2.4 Conclusions and Future Work

Having synthesized beauvericin in a total of 12 steps from commercially available **D**-hydroxyisovaleric acid with an overall yield of 19%, the linear route is an efficient way to synthesize beauvericin (to date, it is the highest yielding synthesis of beauvericin, but also the longest).

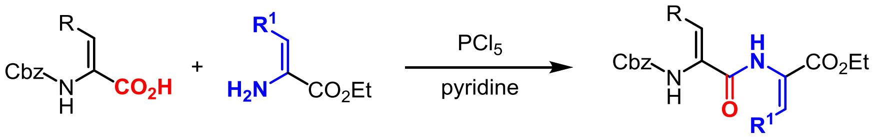


Scheme. 27. Cyclization to afford macrocycle in linear route

One idea that I would still like to try if time permits is a one-pot linear synthesis starting from the TBDPS-protected dipeptide oxazolone and ending at the macrocycle. In theory, this process would be like a controlled, stepwise cyclotrimerization. Starting with the oxazolone, I would add alkoxide dipeptide carboxylate to elongate to the tetradepsipeptide acid in the first part, and the second part would involve adding EDCI to form the tetradepsipeptide oxazolone *in situ*, and then these two parts would be repeated to make the hexadepsipeptide acid and then the hexadepsipeptide oxazolone, and upon treatment of TBAF would lead to cyclization. Essentially, this condenses five transformations into one pot, but if stoichiometry is carefully controlled and there is no cross reactivity with any byproducts, the idea should work.

Additional future work that I would like to look into is to reduce formation of dione byproduct. Perhaps temperatures lower than 0 °C would effectively slow down the rate of formation of dione but not affect the rate of coupling as much. After all, oxazolones are 5-membered ring activated esters, so less energy should be required for esterification with them than in comparison to a typical, aliphatic ester.

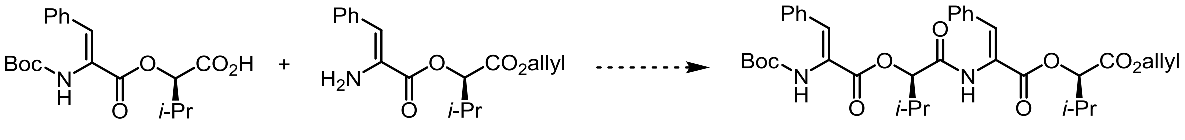
Lastly, I think investigating an alternative method for the linear route should be done. Upon searching for instances of "dehydropeptide coupling" I was fortunate enough to come across the work of Shin and coworkers from 1979, in which dehydroamino acid coupling is shown to proceed through an acid chloride generated *in situ* with modest yields.[3]


R
Cbz
N
H
CO2H
+
R1
H2N
CO2Et
PCl5
pyridine
R
Cbz
N
H
H
N
O
R1
CO2Et

R	R^1	% yield
Me	Et	41
i-Pr	Et	43
Et	*n*-Pr	51
Me	*i*-Pr	65

Scheme 28. Shin's dehydroamino acid coupling

With literature precedence for the direct coupling of dehydroamino acids through the nitrogen and not the carbon (enamine), it is possible to extend this concept towards the synthesis of beauvericin that still uses dehydrophenylalanine residues, but with nitrogen as the nucleophile for elongation and cyclization instead of oxygen. That way, the synthesis would be very similar to the work done by Ovchinnikov and coworkers, except instead of a saturated system, it would be unsaturated (which would improve cyclization).


Ph
Boc
N
H
O
O
CO2H
i-Pr
+
Ph
H2N
O
O
CO2allyl
i-Pr
Ph
Boc
N
H
O
O
i-Pr
Ph
O
N
H
O
O
CO2allyl
i-Pr

Scheme 29. Plans for synthesizing a new dehydro tetradepsipeptide

Thus, a linear, dehydro route towards beauvericin which uses nitrogen as the nucleophile should provide a facile synthesis of beauvericin (assuming that the dehydrophenylalanine unit can be easily prepared and that the synthesis of the "common building block" is straightforward, since it contains an internal ester bond).

2.5 References

1. Li, W.; Schlecker, A.; Ma, D.. *Chem. Commun*. **2010**, *46*, 5403-5420.

2. Eggert, U.; Diestel, R.; Sasse, F.; Jansen, R.; Kunze, B.; Kalesse, M.. *Angew. Chem., Int. Ed.* **2008**, *47*, 6478-6482.

3. Shin, C.; Yonezawa, Y.; Yoshimura, J. *Tetrahedron Lett*. **1979**, *42*, 4085-4086.

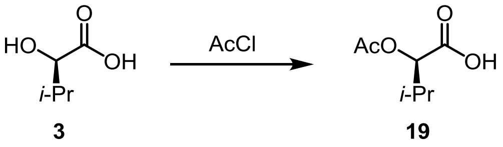
2.6 Supporting Information

2.6.a General Methods

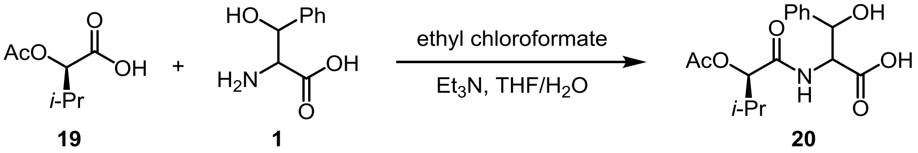
Amino acids and peptide coupling reagents were purchased from Anaspec and Chem-Impex and used without purification. All reactions were carried out under nitrogen or argon atmosphere unless otherwise indicated. Reactions were monitored using thin-layer chromatography (TLC) on EMD Silica Gel 60 F254 plates (visualization of the developed chromatogram was performed by fluorescence quenching or KMnO4 stain) or via analysis on a Waters 2795 Separations Module equipped with a Waters 2996 Photodiode Array and a Waters Micromass ZQ mass spectrometer. Organic solutions were concentrated under reduced pressure using a Büchi rotary evaporator. ^{1}H and ^{13}C NMR spectra were recorded on any of the four spectrometers: Bruker AV600, Bruker GN500, Bruker CRYO500, or Bruker DRX400 NMR instrument. NMR spectra were internally referenced to residual proton solvent signals. Data for ^{1}H NMR were recorded at ambient temperature unless noted otherwise and are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), integration, coupling constant (Hz), and assignment. Data for ^{13}C NMR are reported in terms of chemical shift (δ ppm).

High-resolution mass spectra (HRMS) were acquired on a Waters LCT Premier quadrupole time-of-flight spectrometer and were obtained by peak matching. Infrared (IR) spectra were obtained on a Thermo-Scientific Nicolet iS5 equipped with an iD5 Diamond ATR and are reported in terms of frequency of absorption (cm^{-1}). Column chromatography was performed using SiliCycle Silia-P Flash Silica Gel, using either glass columns, a Biotage SP1 system, or a Teledyne CombiFlash Isco system. All salts were purchased from Aldrich and used without purification. Achiral and chiral bisphosphine ligands were purchased from Strem. Unless otherwise noted, all reactions were carried out under an atmosphere of nitrogen or argon in flame-dried glassware.

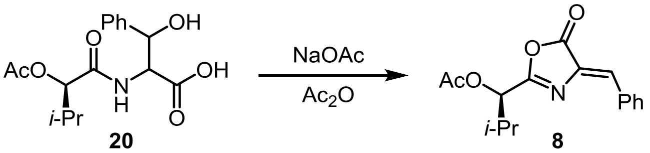
2.6.b Synthesis of Substrates



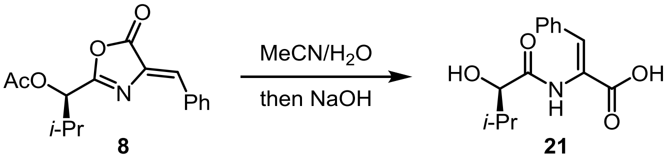
(*R*)-2-Acetoxy-3-methylbutanoic acid (19). Acetyl chloride (0.64 mL, 9 mmol) was added to compound **3** (0.59 g, 5 mmol) and stirred at room temperature. After 4 hours, monitoring by LC-MS showed absence of starting material and presence of product. The mixture was evaporated under reduced pressure, diluted with 1M HCl (aq) and brine, and extracted with DCM (3 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford 0.67 g (84% yield) of compound **19.** Further purification was not necessary. ^1H NMR (400 MHz, $CDCl_3$) δ 9.50 (s, 1H), 4.88 (d, *J* = 4.3 Hz, 1H), 2.26 (m, 1H), 2.14 (s, 3H), 1.02 (dd, *J* = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.7, 171.1, 76.41, 30.0, 20.6, 18.9, 17.2. All spectral data are in agreement with reported literature values.[S1]



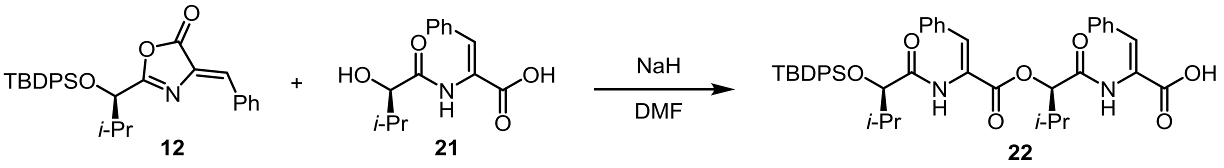
2-((*R*)-2-Acetoxy-3-methylbutanamido)-3-hydroxy-3-phenylpropanoic acid (20). To a stirring solution of compound **19** (320 mg, 2 mmol) in THF (4 mL) at 0 °C was added Et_3N (0.3 mL, 2.1 mmol), the reaction was allowed to stir for 5 min, then ethyl chloroformate (0.2 mL, 2.1 mmol) was added dropwise. The mixture was stirred for 15 min, at which monitoring by LC-MS showed absence of starting material and presence of mixed anhydride. To the mixture, compound **1** (435 mg, 2.4 mmol) with Et_3N (0.36 mL, 2.6 mmol) in a solution of THF (2.4 mL) and H_2O (1.8 mL) was added (note: an additional 0.5 ml of both THF and H_2O were used to dissolve compound **1**). The reaction was stirred for 12 h, at which monitoring by LC-MS showed absence of both starting material and mixed anhydride and presence of product. The mixture was acidified with 10% wt/v $KHSO_4$ (aq) and extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford crude compound **20**. Crude material is a diastereomeric mixture (see ^1H and ^{13}C NMR spectra in Appendix A), and was used without NMR analysis and without further purification in the next step. IR (in $CDCl_3$): 3306, 2969, 1728, 1651, 1526, 1372, 1230, 1030, 910, 731, 700 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{16}H_{21}NO_6$ $[M-H]^-$ 322.1291, found 322.1293.



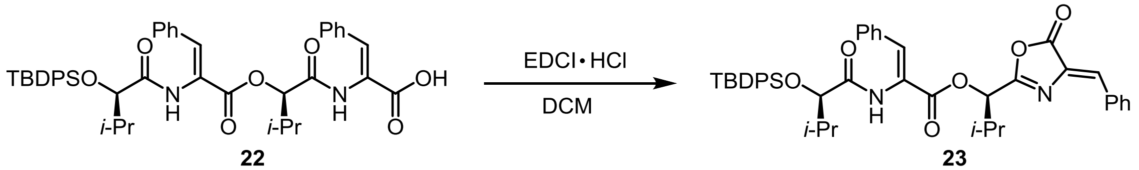
(*R*,*Z*)-1-(4-Benzylidene-5-oxo-4,5-dihydrooxazol-2-yl)-2-methylpropyl acetate (8). Note: the following reaction was done by Nathan Bennett. To a stirring solution of compound **20** (1 equiv) in EtOAc (0.5 M) was added Ac_2O (5 equiv) and NaOAc (2 equiv). The reaction was stirred at room temperature for 16 h, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was quenched with saturated aqueous $NaHCO_3$ (until bubbling stopped) and then extracted with EtOAc (3 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford a crude oil which was purified by column chromatography (EtOAc/hexanes) to provide compound **8**. Note: compound **8** has already been characterized in Chapter 1.



(*R*,*Z*)-2-(2-Hydroxy-3-methylbutanamido)-3-phenylacrylic acid (21). To a stirring solution of compound **8** in MeCN/H_2O was heated to reflux at 80 °C. The reaction was refluxed for 2 h, at which monitoring by LC-MS showed absence of starting material and presence of acetyl-protected hydrolyzed oxazolone. The reaction was cooled to room temperature and then NaOH was added and the reaction was stirred at room temperature for 6 h, at which monitoring by LC-MS showed absence of acetyl-protected hydrolyzed oxazolone and presence of product. The mixture was acidified with 10% wt/v $KHSO_4$ (aq) and extracted with EtOAc (3 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford a crude oil which was purified to give compound **21**. ^1H NMR (400 MHz, CD_3OD) δ 7.60 (m, 2H), 7.52 (s, 1H), 7.41–7.26 (m, 3H), 3.96 (d, *J* = 3.7 Hz, 1H), 2.13 (m, 1H), 1.04 (d, *J* = 6.9 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.4, 168.0, 135.4, 135.1, 131.0, 130.6, 129.6, 126.3, 77.4, 33.2, 19.7, 16.5. IR (neat): 3334, 2966, 2873, 1669, 1626, 1483, 1289, 1185, 1029, 934, 765, 687 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{14}H_{17}NO_4$ $[M+Na]^+$ 286.1055, found 268.1048.



(5*R*,11*R*)-8,14-Di((*Z*)-benzylidene)-5,11-diisopropyl-2,2-dimethyl-6,9,12-trioxo-3,3-diphenyl-4,10-dioxa-7,13-diaza-3-silapentadecan-15-oic acid (22). To a flame-dried Schlenk flask under nitrogen was added 60% oil dispersion NaH (48 mg, 1.2 mmol) and DMF (1 mL). Upon stirring and cooling to 0 °C, a solution of compound **21** (142.8 mg, 0.54 mmol) in DMF (2 mL) was added and stirred at 0 °C for 30 min (while venting the evolved H_2). To the reaction was added a solution of compound **12** (262.3 mg, 0.54 mmol) in DMF (2 mL) at 0 °C, and the mixture was stirred at 0 °C for 30 min, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was acidified with 10% wt/v $KHSO_4$ (aq) and extracted with EtOAc (3 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford 434.9 mg (quantitative yield) of compound **22**. Further purification was not necessary. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.71 (s, 1H), 7.78–7.49 (m, 8H), 7.39 (m, 9H), 7.21 (m, 3H), 5.31 (d, *J* = 3.2 Hz, 1H), 4.18 (d, *J* = 2.8 Hz, 1H), 2.47 (m, 1H), 1.70 (m, 1H), 1.06–0.98 (m, 11H), 0.68 (dd, *J* = 15.4, 7.0 Hz, 5H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 169.5, 169.3, 164.3, 136.1, 135.9, 135.8, 133.5, 133.0, 132.8, 131.9, 130.7, 130.6, 130.4, 130.4, 129.8, 129.5, 129.2, 128.4, 128.2, 128.0, 125.1, 124.1, 79.8, 78.7, 33.8, 30.7, 27.2, 19.6, 19.3, 17.7, 17.2, 16.9. IR (in DCM): 3376, 3235, 2963, 1681, 1480, 1263, 1201, 1111, 737, 700, 612 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{44}H_{50}N_2O_7Si$ $[M+Na]^+$ 769.3285, found 769.3304.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N
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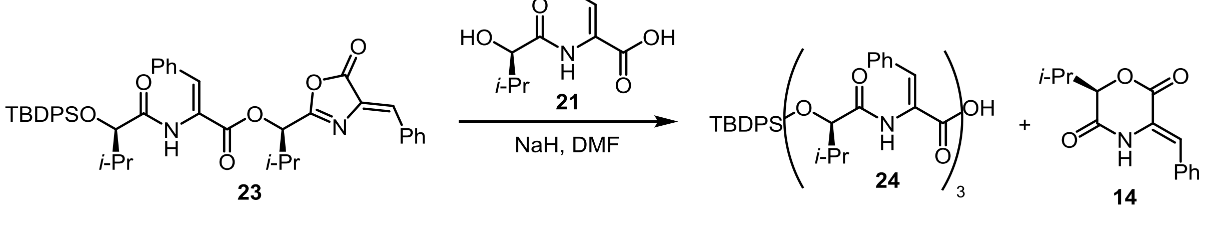
i-Pr
O
O
i-Pr
Ph
O
N
H
OH
O
22
EDCI•HCl
DCM
TBDPSO
Ph
O
N
H
i-Pr
O
O
i-Pr
O
O
N
Ph
23

(*R*)-1-(4-((*Z*)-Benzylidene)-5-oxo-4,5-dihydrooxazol-2-yl)-2-methylpropyl (*Z*)-2-((*R*)-2-((*tert*-butyldiphenylsilyl)oxy)-3-methylbutanamido)-3-phenylacrylate (23). To a stirring solution of compound **22** (0.54 mmol) in DCM (5.4 mL) at 0 °C was added EDCI•HCl (124.8 mg, 0.65 mmol). The reaction was stirred at 0 °C for 30 min, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was acidified with 10% wt/v $KHSO_4$ (aq) and extracted with EtOAc (3 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford 405.9 mg (quantitative yield) of compound **23**. Further purification was not necessary. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.10 (d, *J* = 6.9 Hz, 2H), 7.68 (d, *J* = 14.3, 6.9 Hz, 4H), 7.51 (s, 1H), 7.48–7.28 (m, 16H), 5.69 (d, *J* = 5.8 Hz, 1H), 4.27 (d, *J* = 3.1 Hz, 1H), 2.50 (m, 1H), 1.85 (m, 1H), 1.20–1.15 (m, 6H), 1.12 (s, 9H), 0.85 (d, *J* = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6, 167.1, 164.9, 164.3, 136.0, 135.9, 134.0, 133.9, 133.0, 133.0, 132.8, 132.5, 131.6, 131.5, 131.3, 130.3, 129.7, 129.5, 129.0, 128.6, 128.0, 127.9, 123.4, 78.9, 74.7, 33.9, 30.9, 27.2, 19.5, 18.6, 17.8, 17.8, 17.4. IR (in $CDCl_3$): 3391, 2963, 2931, 1809, 1698, 1658, 1471, 1105, 732, 701, 690 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{44}H_{48}N_2O_6Si$ $[M+Na]^+$ 751.3179, found 751.3180.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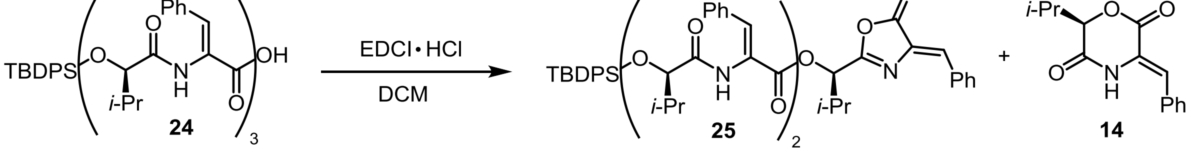
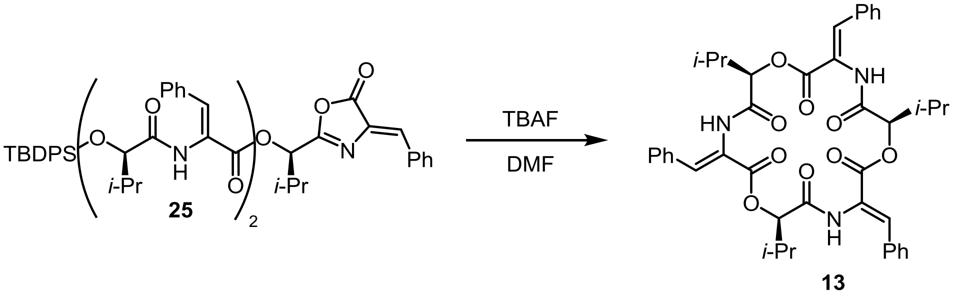
Ph
23
Ph
HO
O
N
H
OH
i-Pr
O
21
NaH, DMF
TBDPS
O
Ph
O
N
H
i-Pr
O
OH
24
3
+
i-Pr
O
O
O
N
H
Ph
14

(5*R*,11*R*,17*R*)-8,14,20-Tri((*Z*)-benzylidene)-5,11,17-triisopropyl-2,2-dimethyl-6,9,12,15,18-pentaoxo-3,3-diphenyl-4,10,16-trioxa-7,13,19-triaza-3-silahenicosan-21-oic acid (24). To a flame-dried Schlenk flask under nitrogen was added 60% oil dispersion NaH (17.6 mg, 0.44 mmol) and DMF (0.5 mL). Upon stirring and cooling to 0 °C, a solution of compound **21** (52.6 mg, 0.2 mmol) in DMF (0.75 mL) was added and stirred at 0 °C for 30 min (while venting the evolved H_2). To the reaction was added a solution of compound **23** (145.7 mg, 0.2 mmol) in DMF (0.75 mL) at 0 °C, and the mixture was stirred at 0 °C for 30 min, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was acidified with 10% wt/v $KHSO_4$ (aq) and extracted with EtOAc (3 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford 194.5 mg (quantitative yield) of a mixture containing compound **24** (91%) and compound **14** (9%). The mixture containing compound **24** was not purified and was used directly in the next step. ^1H NMR (500 MHz, $CDCl_3$) δ 9.67 (s, 1H), 9.02 (s, 1H), 8.81 (s, 1H), 7.61–7.27 (m, 41H), 7.20–7.14 (m, 2H), 7.04–6.96 (m, 3H), 5.34 (d, *J* = 3.1, 1H), 5.28 (d, *J* = 3.6 Hz, 1H), 3.92 (d, *J* = 2.5 Hz, 1H), 2.44 (m, 2H), 2.36 (m, 1H) 1.50 (m, 1H), 1.18–0.96 (m, 36H), 0.63 (d, *J* = 7.0 Hz, 3H), 0.54 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.5, 171.1, 169.8, 169.2, 164.4, 163.9, 136.4, 136.1, 135.9, 135.8, 135.6, 135.2, 133.5, 133.3, 132.8, 132.6, 131.7, 130.8, 130.7, 130.5, 130.4, 130.4, 129.9, 129.6, 129.6, 129.5, 129.2, 128.5, 128.2, 127.9, 124.7, 124.3, 124.0, 79.8, 79.2, 78.6, 33.4, 30.8, 30.5, 27.1, 19.6, 19.2, 19.1, 17.8, 17.0, 16.9. IR (in DCM): 3369, 3222, 3054, 2965, 1723, 1665, 1484, 1264, 1201, 1106, 735, 691 cm^{-1}. HRMS (ESI: *m*/*z* calculated for $C_{58}H_{65}N_3O_{10}Si$ $[M+Na]^+$ 1014.4337, found 1014.4343.



24 **25** **14**

(*R*)-1-(((*Z*)-3-((*R*)-1-(4-((*Z*)-Benzylidene)-5-oxo-4,5-dihydrooxazol-2-yl)-2-methylpropoxy)-3-oxo-1-phenylprop-1-en-2-yl)amino)-3-methyl-1-oxobutan-2-yl (*Z*)-2-((*R*)-2-((tert-butyldiphenylsilyl)oxy)-3-methylbutanamido)-3-phenylacrylate (25). To a stirring solution of a mixture containing 91% compound **24** (144.5 mg, 0.15 mmol) in DCM (1.5 mL) at 0 °C was added EDCI•HCl (33.8 mg, 0.18 mmol). The reaction was stirred at 0 °C for 30 min, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was acidified with 10% wt/v $KHSO_4$ (aq) and extracted with EtOAc (3 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford 147.7 mg (quantitative yield) of a mixture containing compound **25** (75%) and compound **14** (25%). The mixture containing compound **25** was not purified and was used directly in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.72 (s, 1H), 8.10 (2H), 7.72–7.31 (m, 44H), 7.22–6.97 (m, 7H), 5.60 (d, *J* = 5.9 Hz, 1H), 5.33 (d, *J* = 3.1 Hz, 1H), 4.16 (d, *J* = 3.0 Hz, 1H), 2.45 (m, 3H), 1.66 (m, 2H), 1.18–0.94 (m, 43H), 0.65 (dd, *J* = 15.2, 7.0 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.9, 169.2, 167.3, 165.1, 164.3, 164.2, 163.0, 153.8, 134.7, 133.7, 133.2, 133.1, 132.8, 132.8, 131.9, 131.7, 131.5, 130.7, 130.6, 130.3, 129.8, 129.5, 129.4, 129.2, 129.0, 128.4, 128.2, 128.0, 125.1, 124.3, 79.8, 78.7, 74.6, 33.7, 31.0, 30.6, 29.9, 27.2, 19.6, 19.4, 18.6, 17.9, 17.5, 17.4, 16.9. IR (in $CDCl_3$): 3377, 3241, 2964, 2931, 1808, 1781, 1728, 1682, 1481, 1370, 1277, 1202, 1111, 702, 691 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $C_{58}H_{63}N_3O_9Si$ [M+Na]$^+$ 996.4232, found 996.4241.

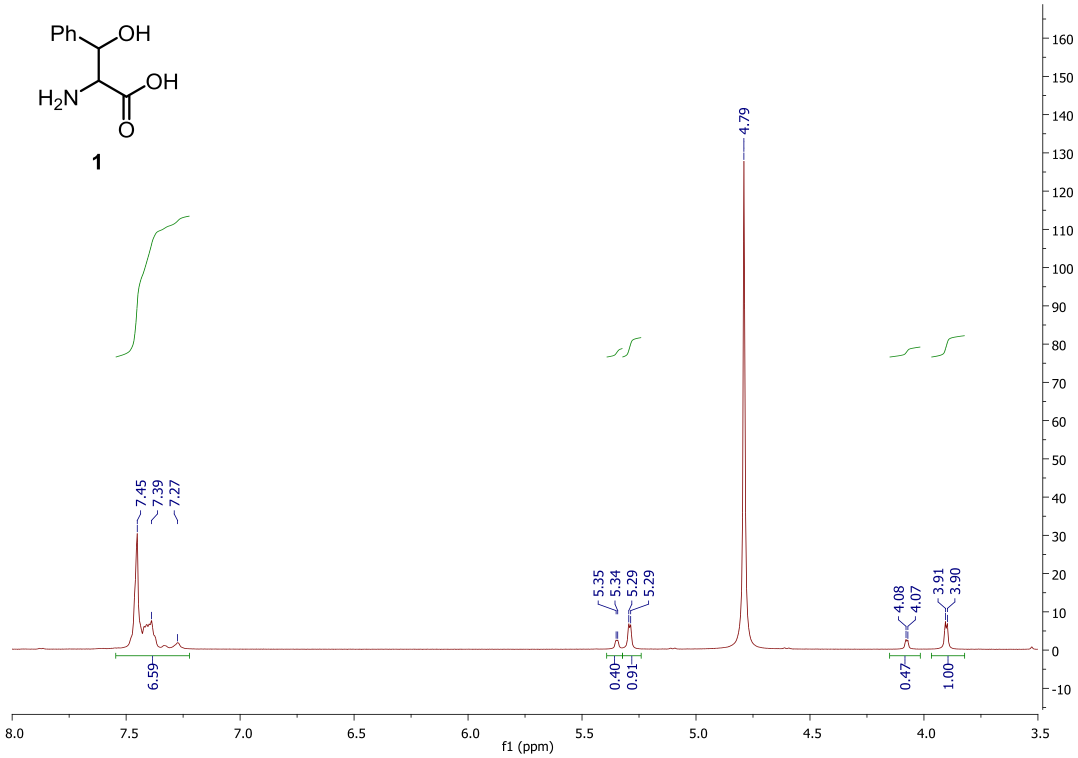


(6*R*,12*R*,18*R*)-3,9,15-Tri((*Z*)-benzylidene)-6,12,18-triisopropyl-1,7,13-trioxa-4,10,16-triaza-cyclooctadecane-2,5,8,11,14,17-hexaone (13). To a stirring solution of a mixture containing 75% compound **25** (63.8 mg, 0.06 mmol) in DMF (0.6 mL) at 0 °C was added TBAF (1M in THF, 0.08 mL). The reaction was stirred at 0 °C for 30 min, at which monitoring by LC-MS showed absence of starting material and presence of product. The mixture was quenched with 10% wt/v $KHSO_4$ (aq) and then extracted with EtOAc (3 times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and then evaporated under reduced pressure to afford a crude oil, which was purified by preparative TLC chromatography (3% MeOH and 1% Et_3N in MeOH) to provide 19.5 mg (42% yield, 3 steps) of compound **13** (dione was not isolated; ^{1}H NMR showed 1:1 so an increase from 25 to 42%). Note: compound **13** has already been characterized in Chapter 1.

2.6.c References

S1. Athanasellis, G.; Detsi, A.; Prousis, K.; Igglessi-Markopoulou, O.; Markopoulos, J. *Synthesis* **2003**, *13*, 2015–2022.

Appendix A: ^{1}H and ^{13}C NMR Spectral Data



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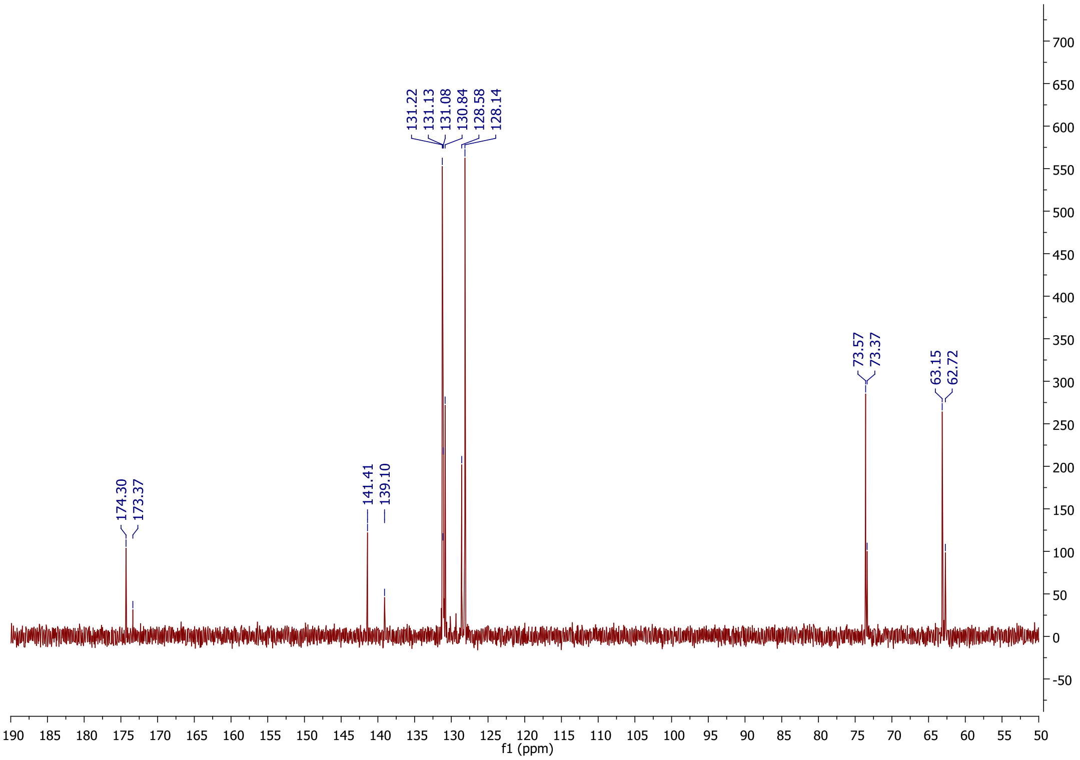

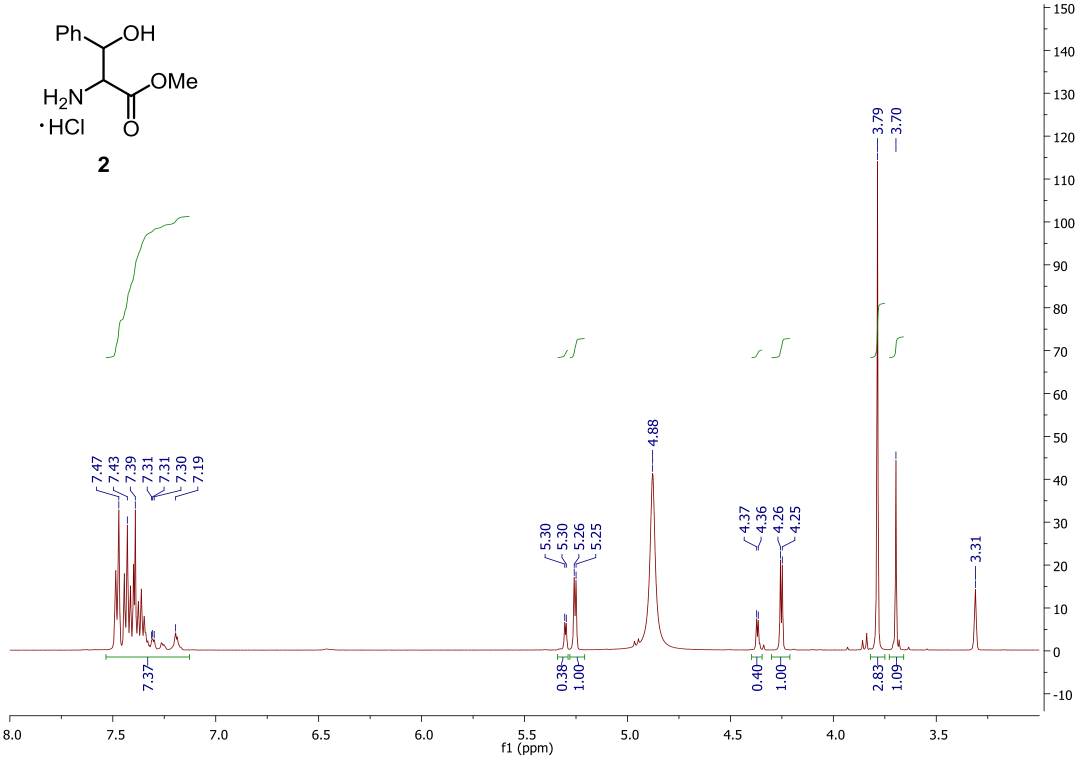
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5.26
5.25
4.88
4.37
4.36
4.26
4.25
3.79
3.70
3.31
7.37
0.38
1.00
0.40
1.00
2.83
1.09
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
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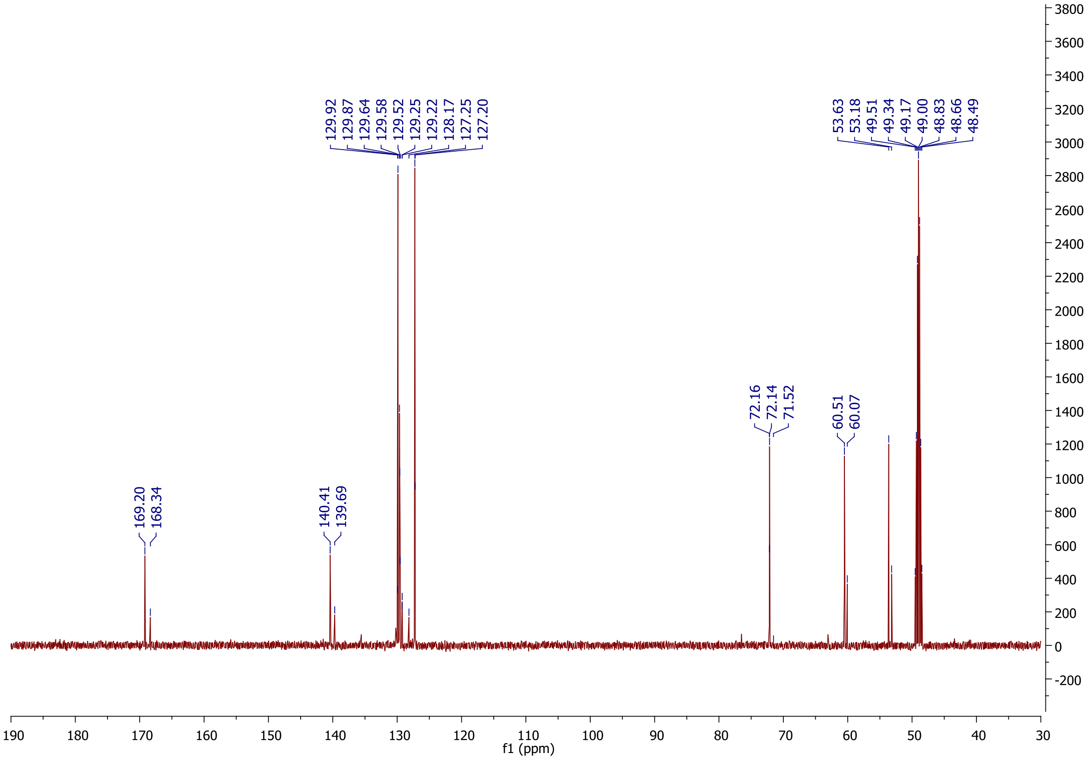
6.5
6.0
5.5
5.0
4.5
4.0
3.5
f1 (ppm)

2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49.34
49.17
49.00
48.83
48.66
48.49
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72.14
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3200
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2600
2400
2200
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1800
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1400
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1000
800
600
400
200
0
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80
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40
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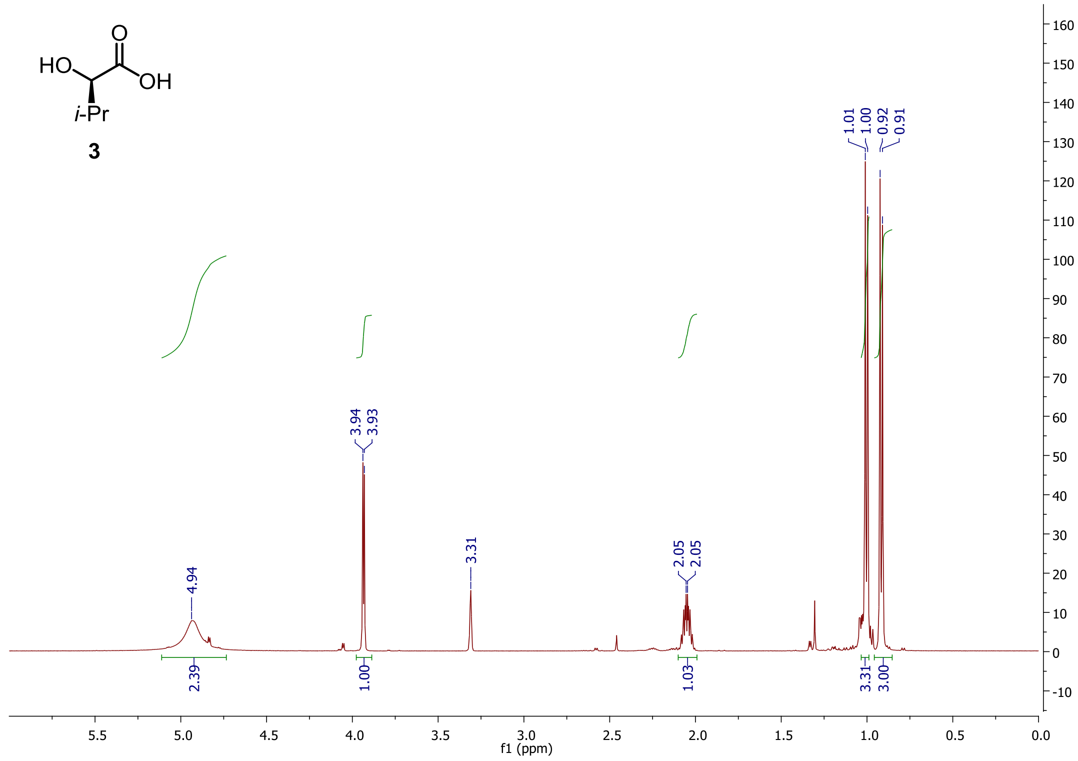
f1 (ppm)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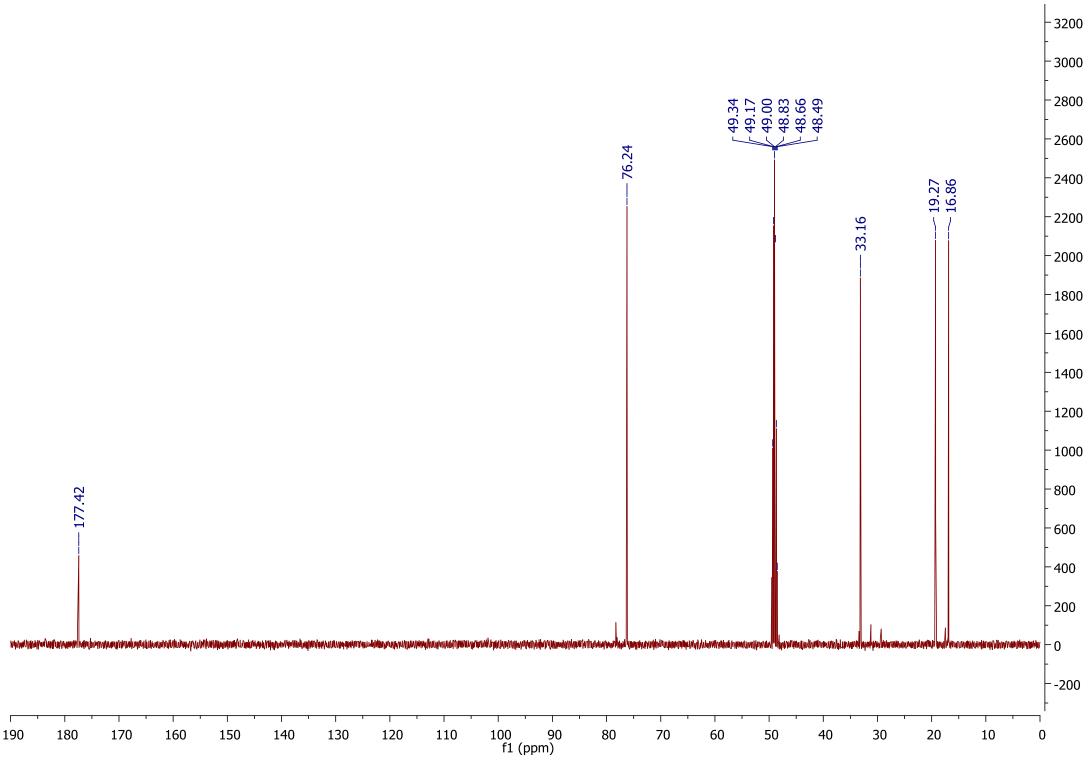
f1 (ppm)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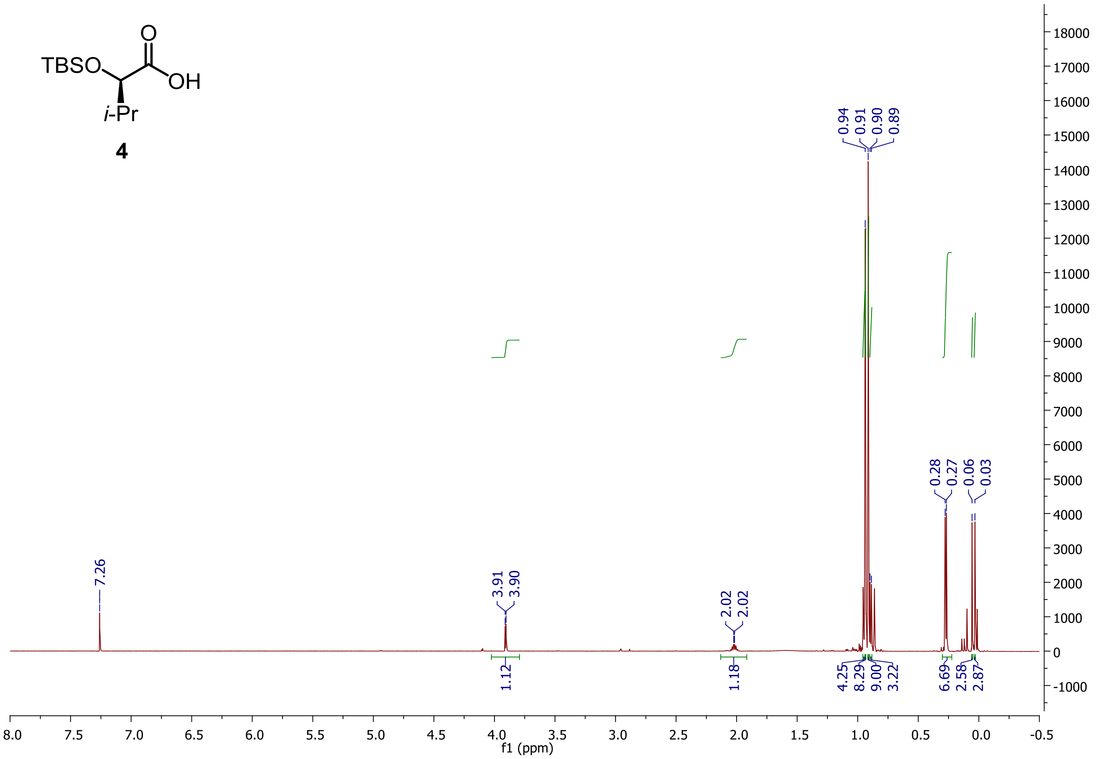
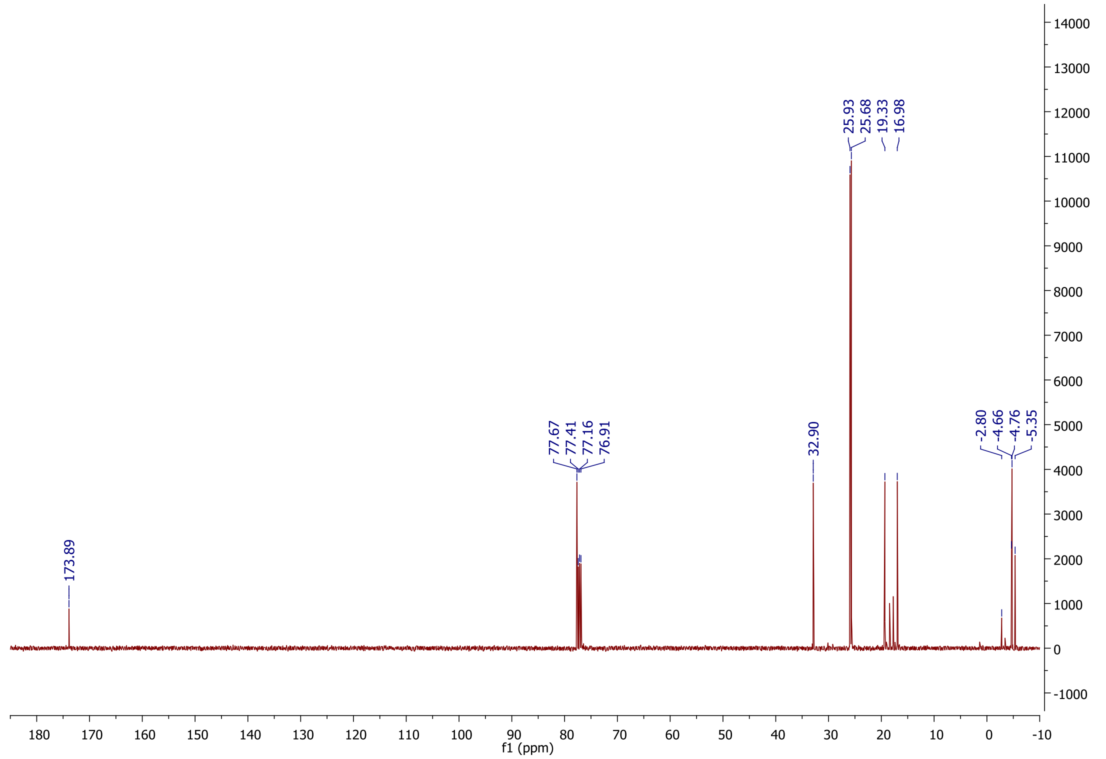
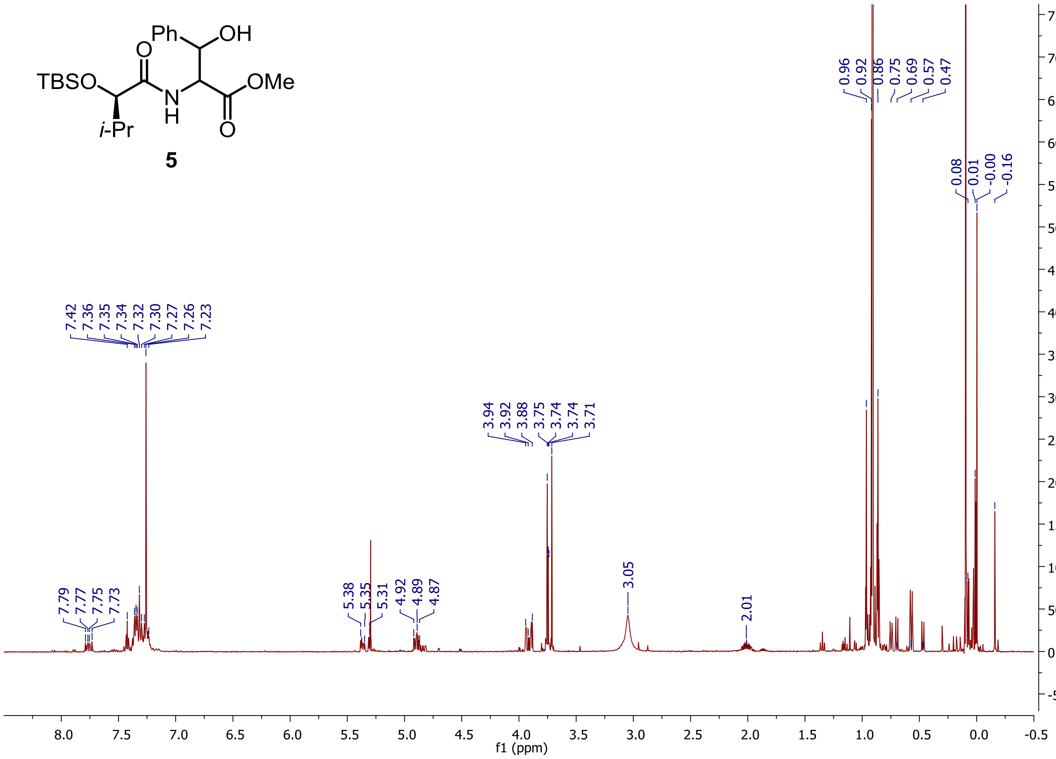
19.27
16.86
f1 (ppm)

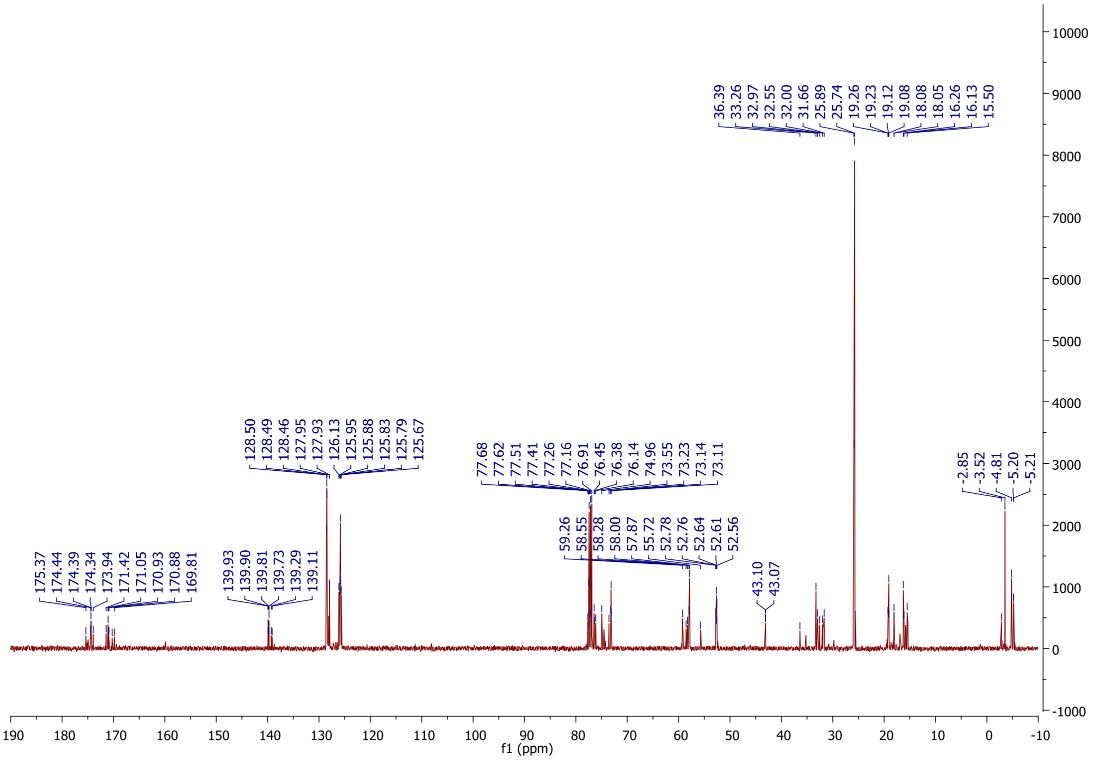
TBSO
O
OH
i-Pr

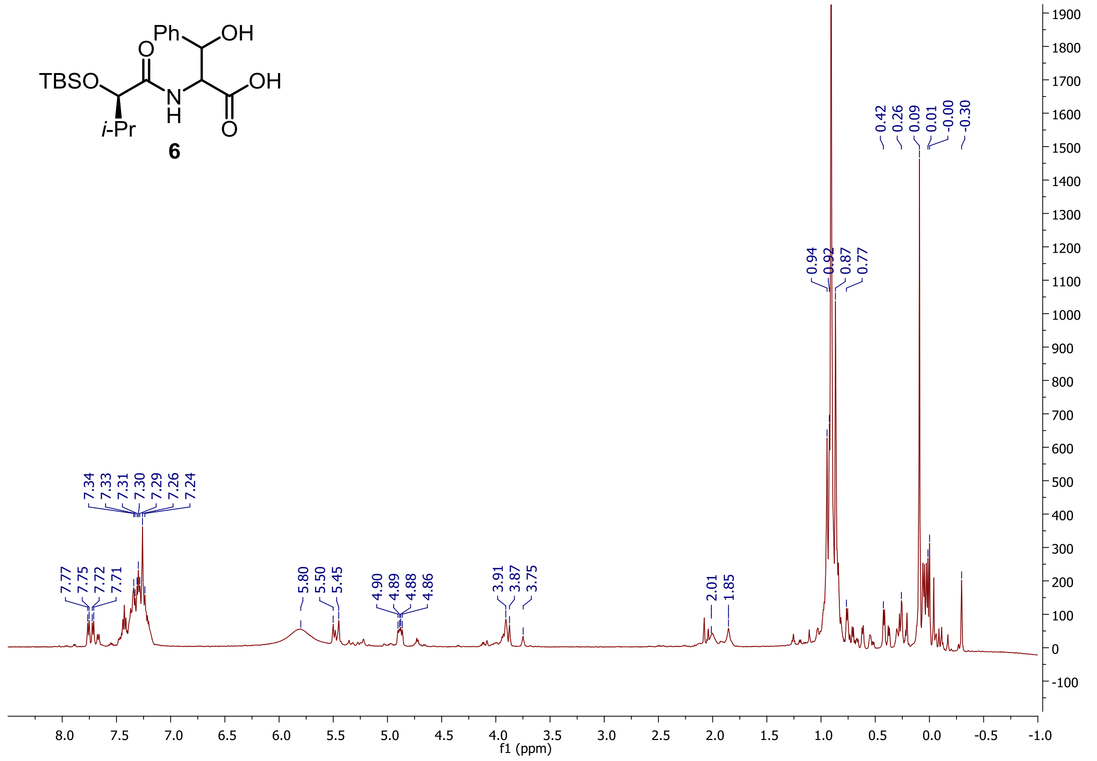
4


7.26
3.91
3.90
2.02
2.02
0.94
0.91
0.90
0.89
0.28
0.27
0.06
0.03
1.12
1.18
4.25
8.29
9.00
3.22
6.69
2.58
2.87
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

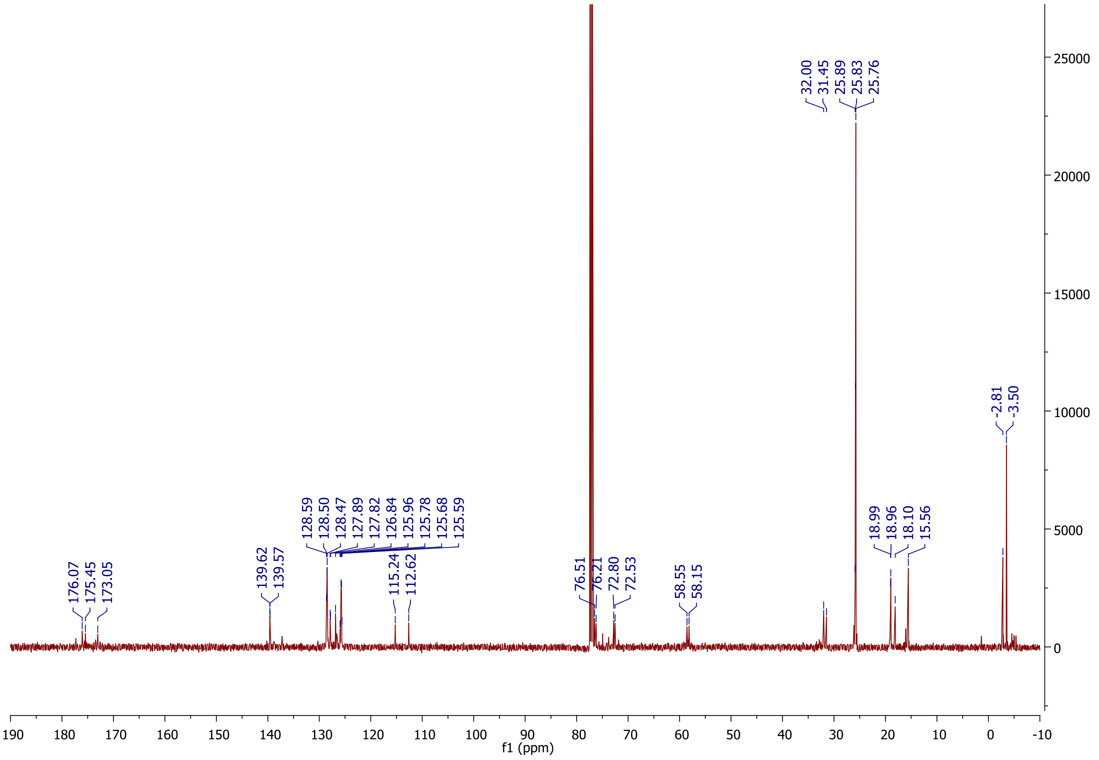

173.89
77.67
77.41
77.16
76.91
32.90
25.93
25.68
19.33
16.98
-2.80
-4.66
-4.76
-5.35
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

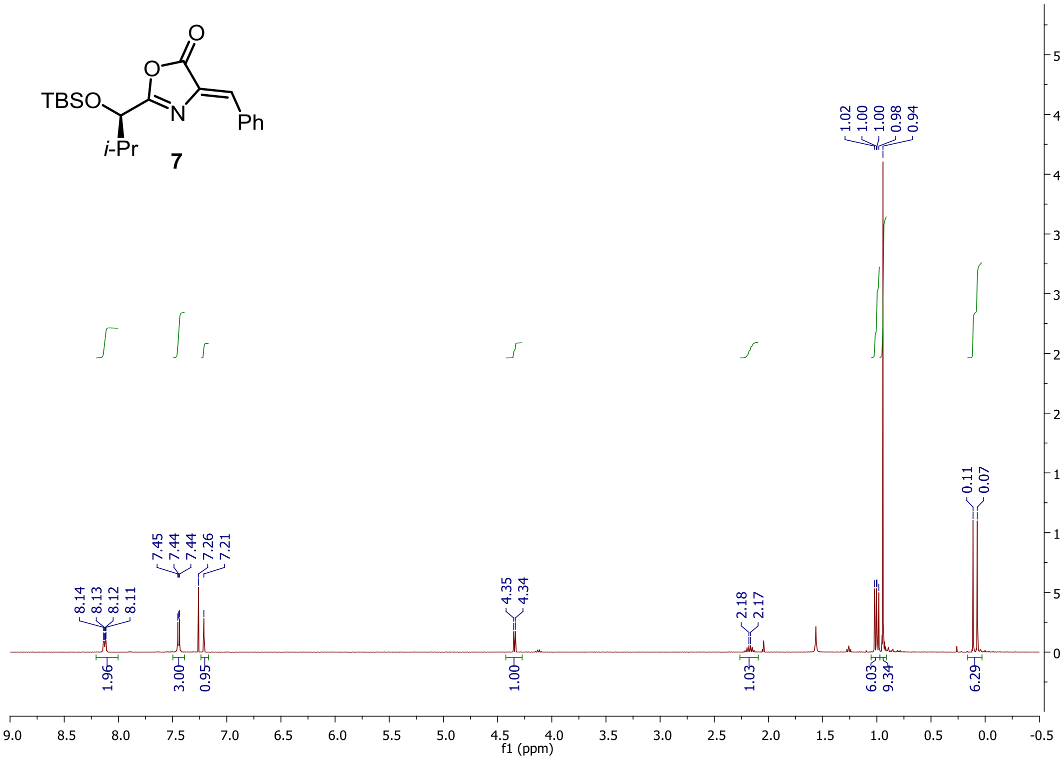

TBSO
i-Pr
Ph
OH
O
N
H
OMe
O
5
7.42 7.36 7.35 7.34 7.32 7.30 7.27 7.26 7.23
7.79 7.77 7.75 7.73
5.38 5.35 5.31
4.92 4.89 4.87
3.94 3.92 3.88 3.75 3.74 3.74 3.71
3.05
2.01
0.96 0.92 0.86 0.75 0.69 0.57 0.47
0.08 0.01 -0.00 -0.16
f1 (ppm)


175.37 174.44 174.39 174.34 173.94 171.42 171.05 170.93 170.88 169.81
139.93 139.90 139.81 139.73 139.29 139.11
128.50 128.49 128.46 127.95 127.93 126.13 125.95 125.88 125.83 125.79 125.67
77.68 77.62 77.51 77.41 77.26 77.16 76.91 76.45 76.38 76.14 74.96 73.55 73.23 73.14 73.11
59.26 58.55 58.28 58.00 57.87 55.72 52.78 52.76 52.64 52.61 52.56
43.10 43.07
36.39 33.26 32.97 32.55 32.00 31.66 25.89 25.74 19.26 19.23 19.12 19.08 18.08 18.05 16.26 16.13 15.50
-2.85 -3.52 -4.81 -5.20 -5.21
f1 (ppm)

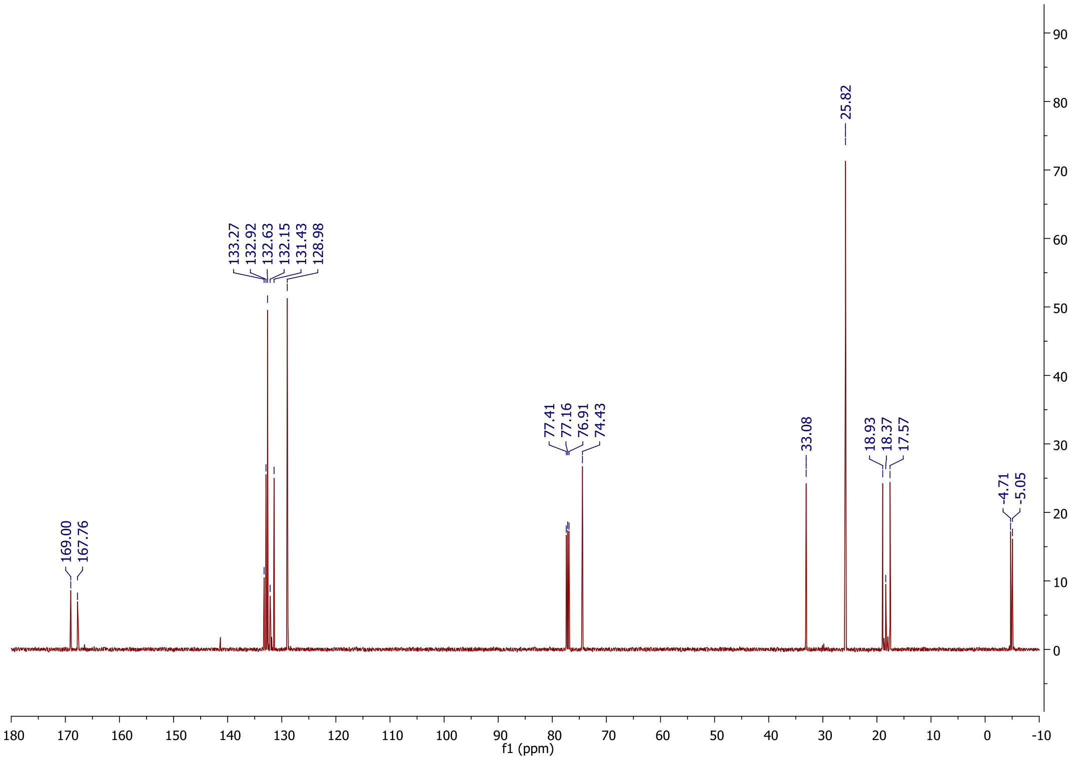

TBSO
Ph
OH
O
N
H
OH
O
i-Pr
7.77
7.75
7.72
7.71
7.34
7.33
7.31
7.30
7.29
7.26
7.24
5.80
5.50
5.45
4.90
4.89
4.88
4.86
3.91
3.87
3.75
2.01
1.85
0.94
0.92
0.87
0.77
0.42
0.26
0.09
0.01
-0.00
-0.30
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

6

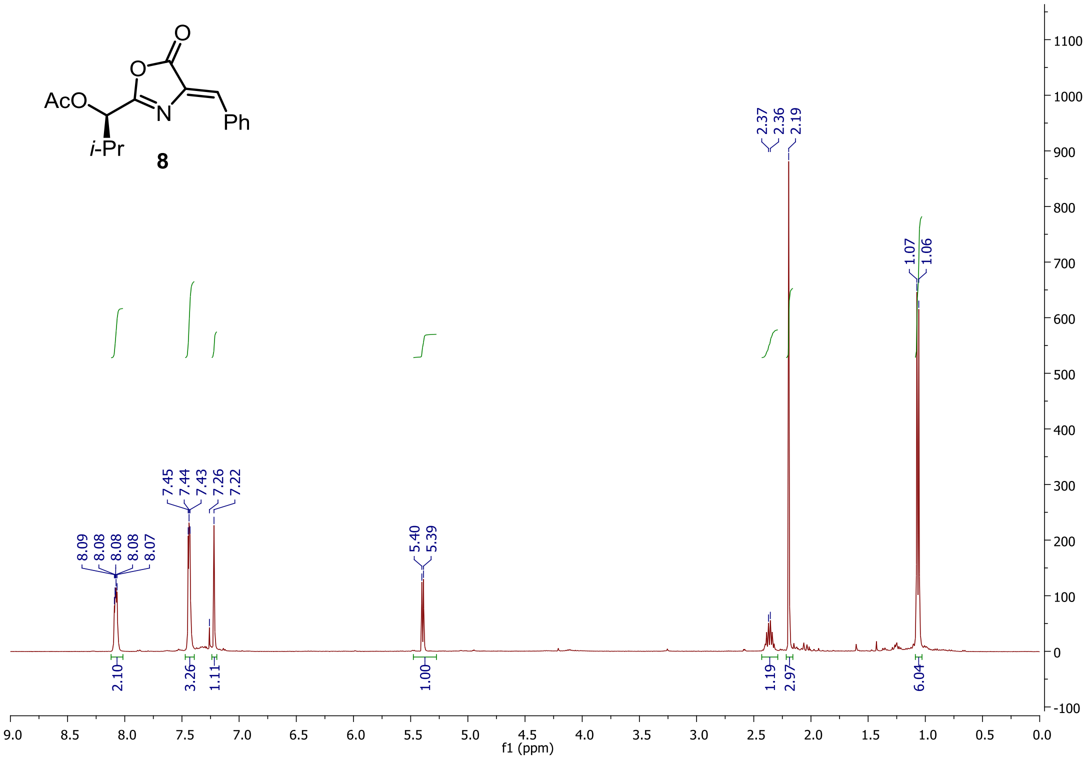

176.07
175.45
173.05
139.62
139.57
128.59
128.50
128.47
127.89
127.82
126.84
125.96
125.78
125.68
125.59
115.24
112.62
76.51
76.21
72.80
72.53
58.55
58.15
32.00
31.45
25.89
25.83
25.76
18.99
18.96
18.10
15.56
-2.81
-3.50
25000
20000
15000
10000
5000
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

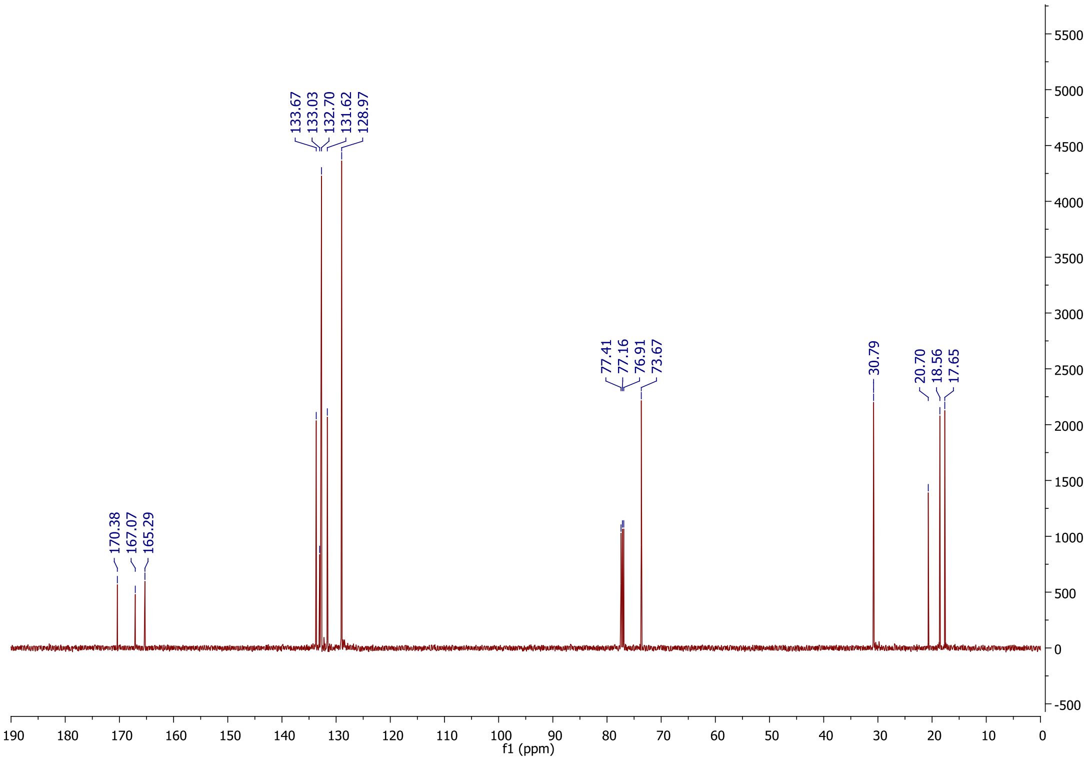

7

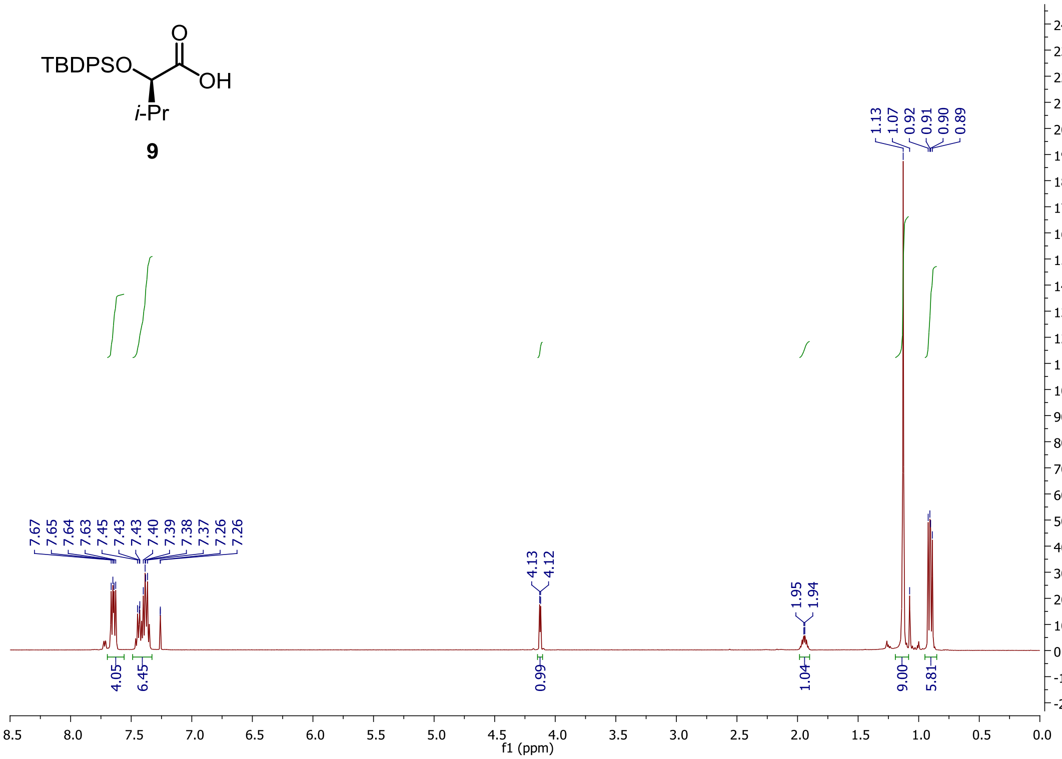
TBSO
O
N
Ph
i-Pr
8.14
8.13
8.12
8.11
7.45
7.44
7.44
7.26
7.21
4.35
4.34
2.18
2.17
1.02
1.00
1.00
0.98
0.94
0.11
0.07
1.96
3.00
0.95
1.00
1.03
6.03
9.34
6.29
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0



169.00
167.76
133.27
132.92
132.63
132.15
131.43
128.98
77.41
77.16
76.91
74.43
33.08
25.82
18.93
18.37
17.57
-4.71
-5.05
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0

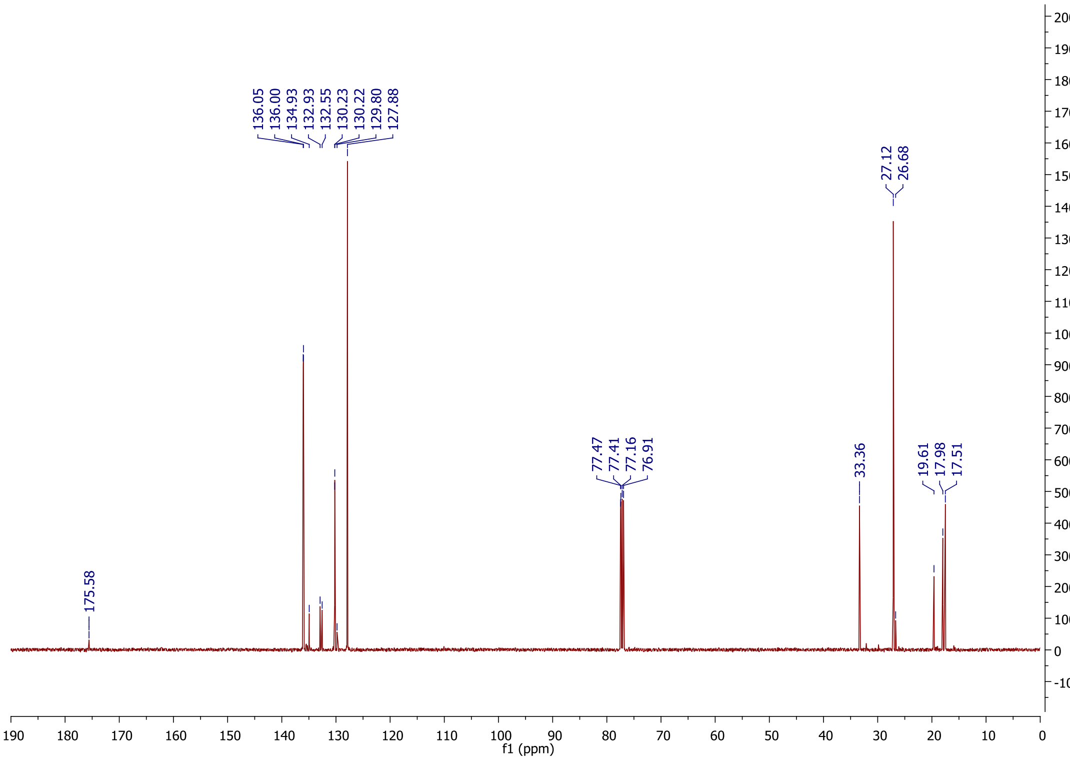

AcO
O
O
N
Ph
i-Pr
8
8.09
8.08
8.08
8.08
8.07
7.45
7.44
7.43
7.26
7.22
5.40
5.39
2.37
2.36
2.19
1.07
1.06
2.10
3.26
1.11
1.00
1.19
2.97
6.04
f1 (ppm)


170.38
167.07
165.29
133.67
133.03
132.70
131.62
128.97
77.41
77.16
76.91
73.67
30.79
20.70
18.56
17.65
f1 (ppm)

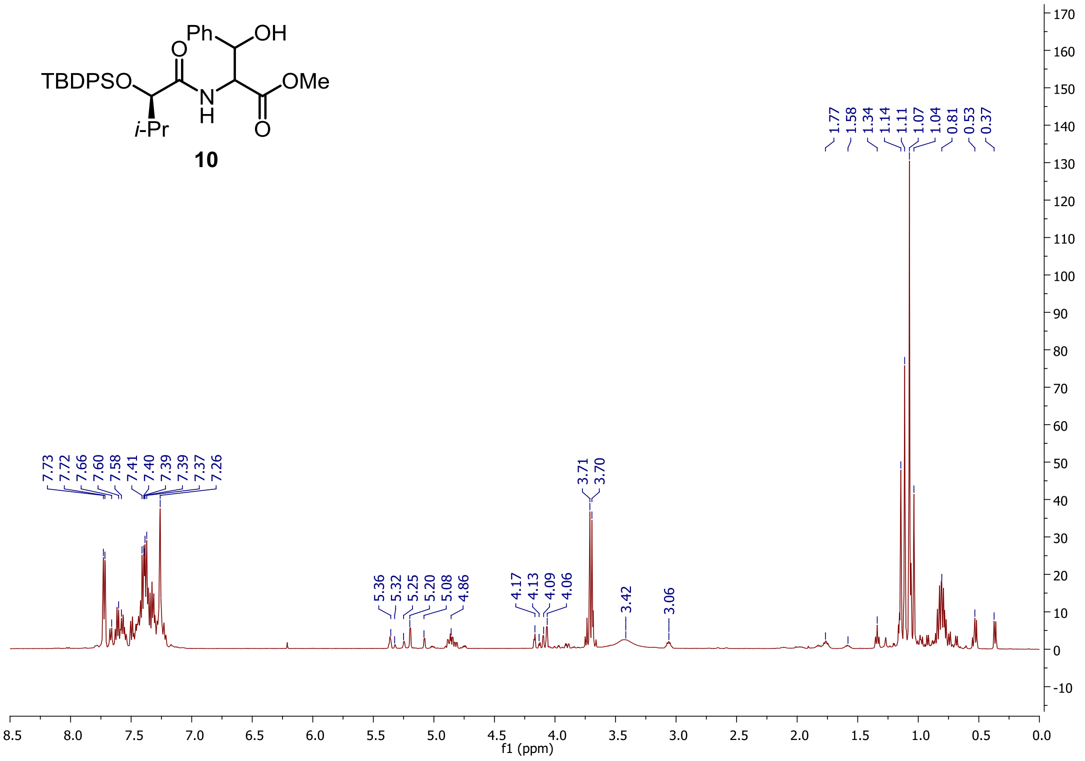


TBDPSO
O
OH
i-Pr
7.67
7.65
7.64
7.63
7.45
7.43
7.43
7.40
7.39
7.38
7.37
7.26
7.26
4.13
4.12
1.95
1.94
1.13
1.07
0.92
0.91
0.90
0.89
4.05
6.45
0.99
1.04
9.00
5.81
f1 (ppm)

9

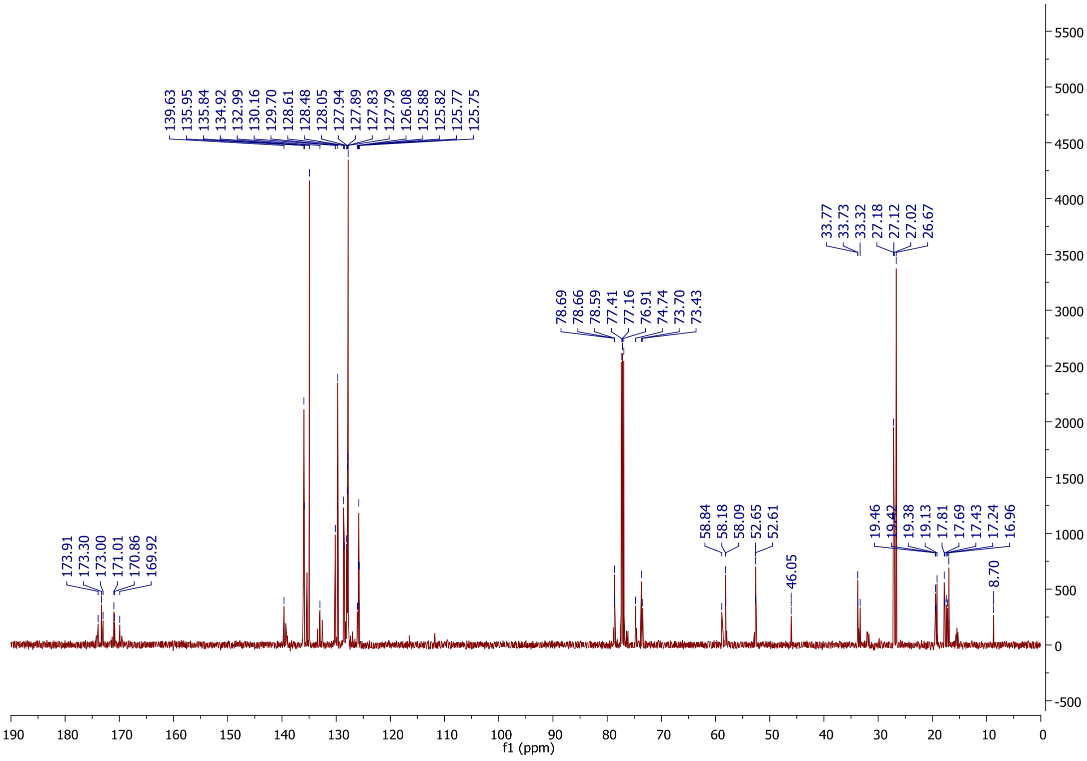


136.05
136.00
134.93
132.93
132.55
130.23
130.22
129.80
127.88
175.58
77.47
77.41
77.16
76.91
33.36
27.12
26.68
19.61
17.98
17.51
f1 (ppm)

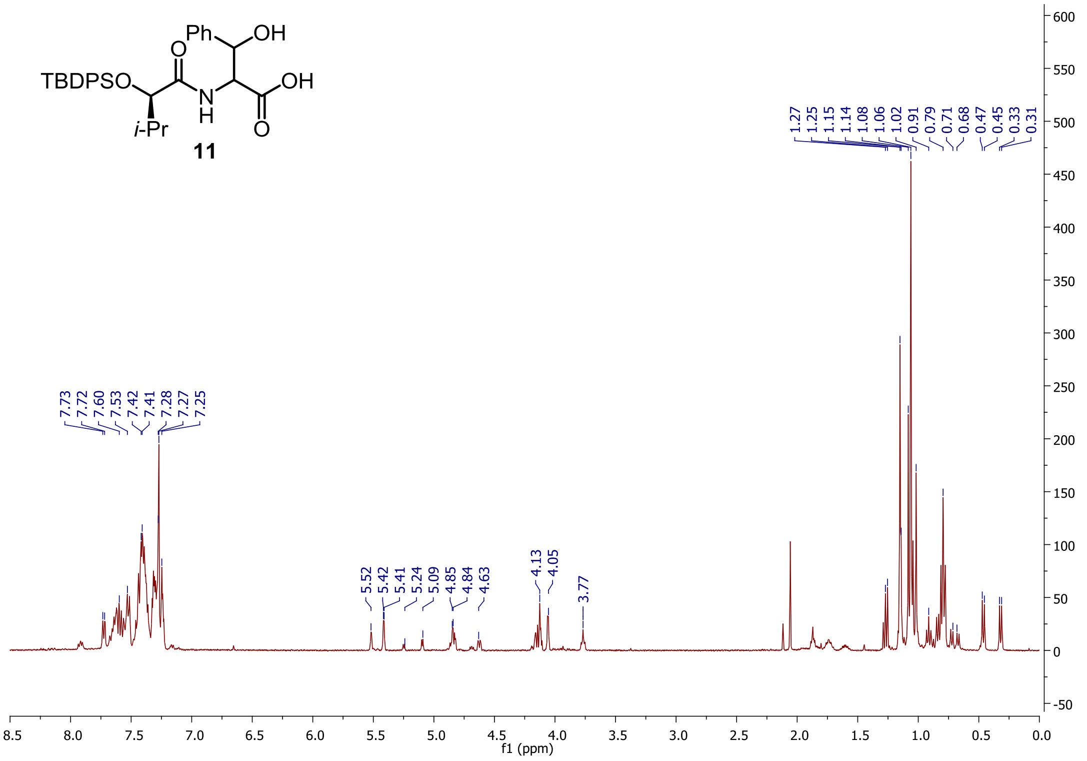


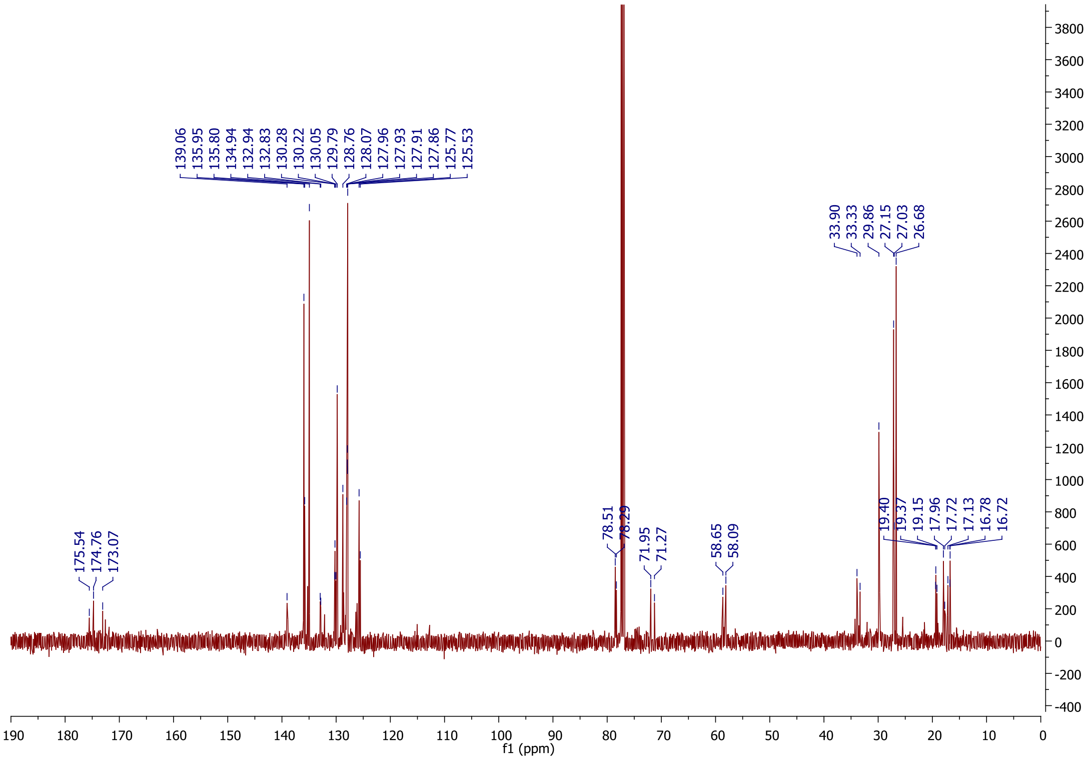
10

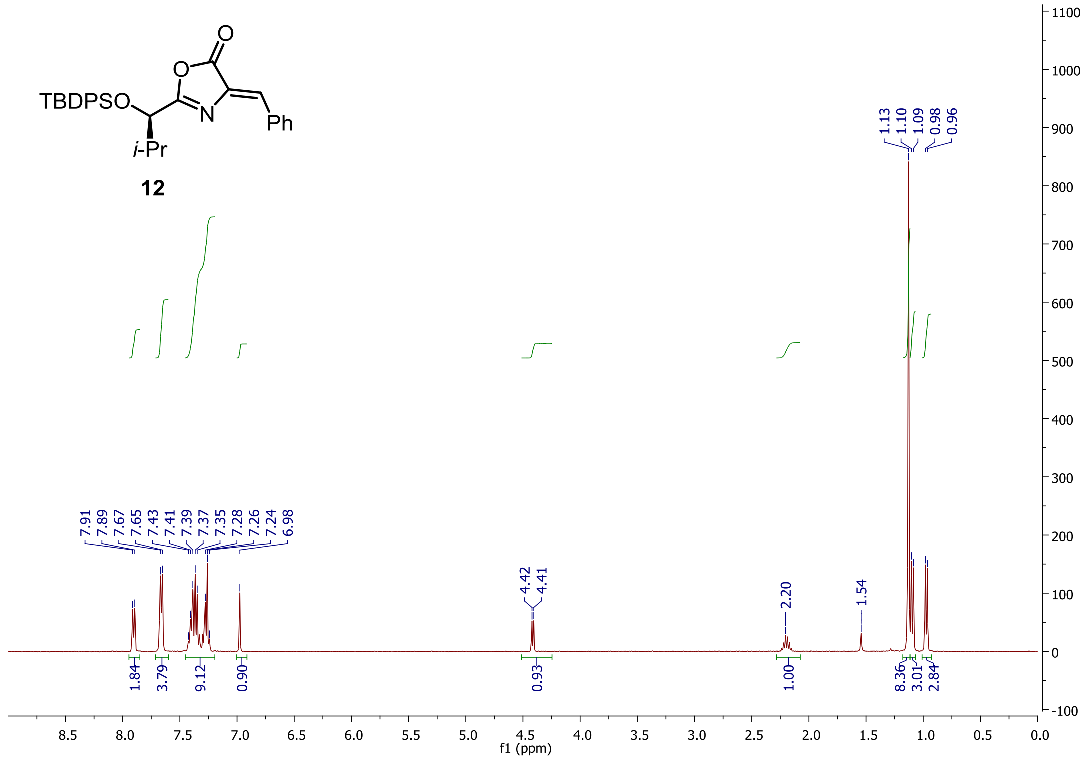
TBDPSO
i-Pr
Ph
OH
O
N
H
OMe
O
7.73
7.72
7.66
7.60
7.58
7.41
7.40
7.39
7.39
7.37
7.26
5.36
5.32
5.25
5.20
5.08
4.86
4.17
4.13
4.09
4.06
3.71
3.70
3.42
3.06
1.77
1.58
1.34
1.14
1.11
1.07
1.04
0.81
0.53
0.37
f1 (ppm)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

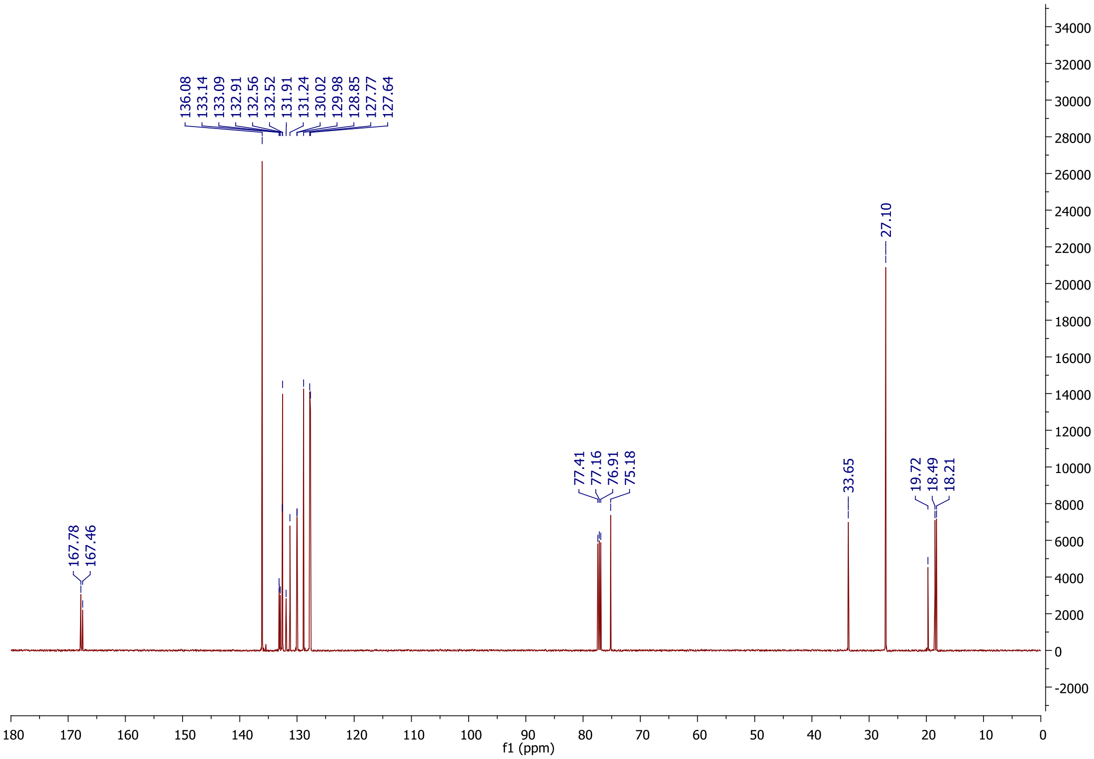


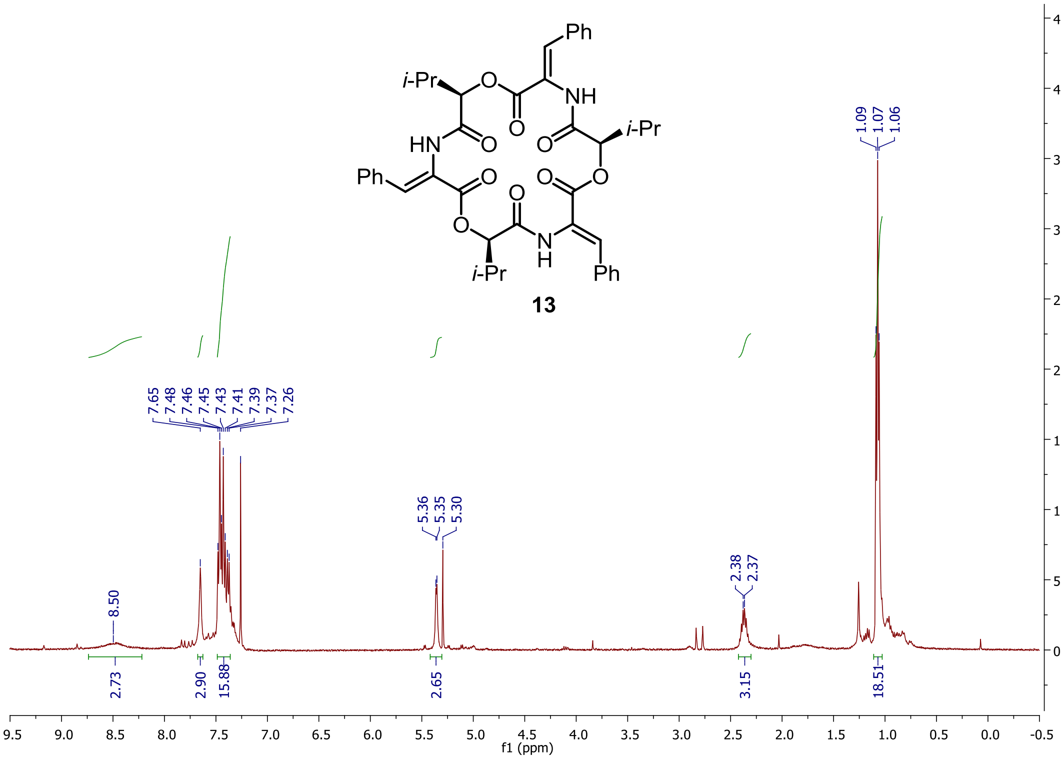
139.63
135.95
135.84
134.92
132.99
130.16
129.70
128.61
128.48
128.05
127.94
127.89
127.83
127.79
126.08
125.88
125.82
125.77
125.75
173.91
173.30
173.00
171.01
170.86
169.92
78.69
78.66
78.59
77.41
77.16
76.91
74.74
73.70
73.43
58.84
58.18
58.09
52.65
52.61
46.05
33.77
33.73
33.32
27.18
27.12
27.02
26.67
19.46
19.42
19.38
19.13
17.81
17.69
17.43
17.24
16.96
8.70
f1 (ppm)
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500


Ph
OH
O
TBDPSO
N
H
OH
O
i-Pr
11
7.73
7.72
7.60
7.53
7.42
7.41
7.28
7.27
7.25
5.52
5.42
5.41
5.24
5.09
4.85
4.84
4.63
4.13
4.05
3.77
1.27
1.25
1.15
1.14
1.08
1.06
1.02
0.91
0.79
0.71
0.68
0.47
0.45
0.33
0.31
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


139.06
135.95
135.80
134.94
132.94
132.83
130.28
130.22
130.05
129.79
128.76
128.07
127.96
127.93
127.91
127.86
125.77
125.53
175.54
174.76
173.07
78.51
78.29
71.95
71.27
58.65
58.09
33.90
33.33
29.86
27.15
27.03
26.68
19.40
19.37
19.15
17.96
17.72
17.13
16.78
16.72
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
-400
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

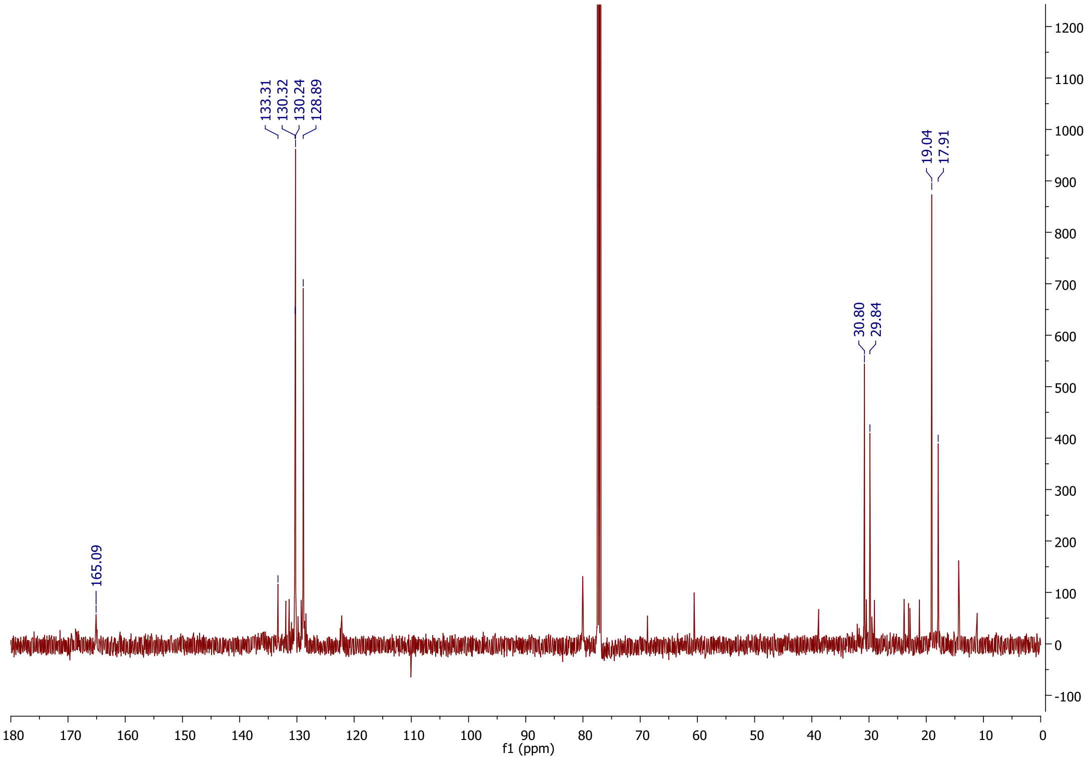

12
TBDPSO
O
O
N
Ph
i-Pr
7.91
7.89
7.67
7.65
7.43
7.41
7.39
7.37
7.35
7.28
7.26
7.24
6.98
4.42
4.41
2.20
1.54
1.13
1.10
1.09
0.98
0.96
1.84
3.79
9.12
0.90
0.93
1.00
8.36
3.01
2.84
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


136.08
133.14
133.09
132.91
132.56
132.52
131.91
131.24
130.02
129.98
128.85
127.77
127.64
167.78
167.46
77.41
77.16
76.91
75.18
33.65
27.10
19.72
18.49
18.21
34000
32000
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

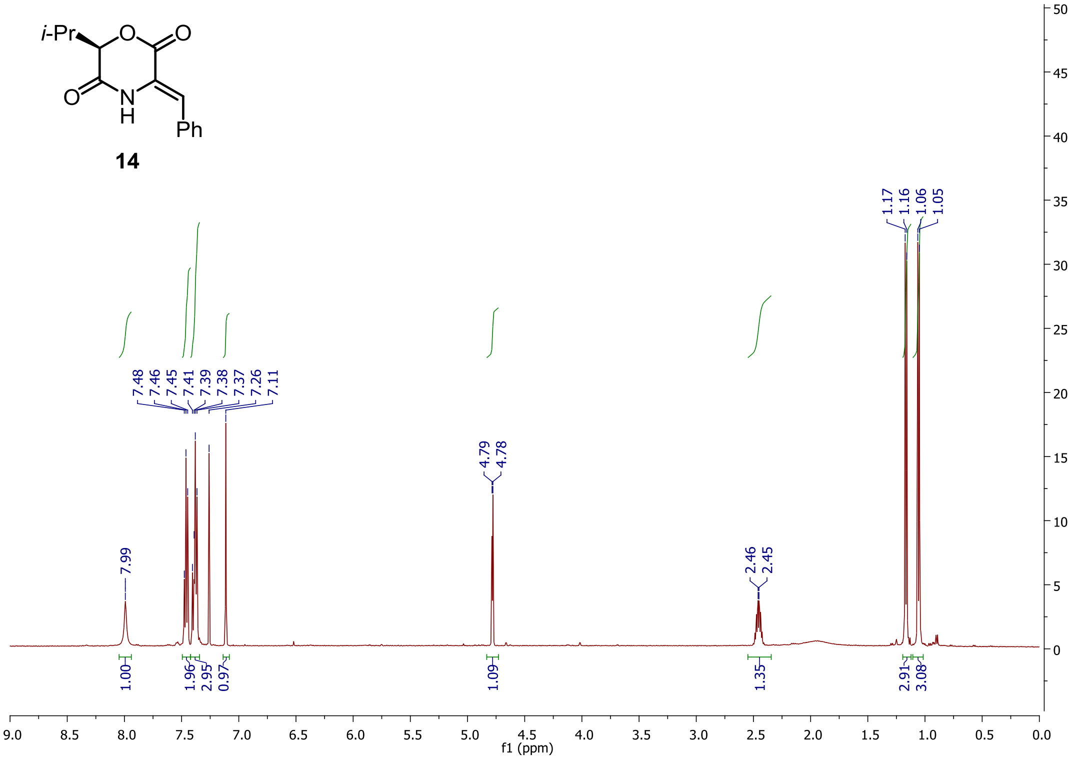


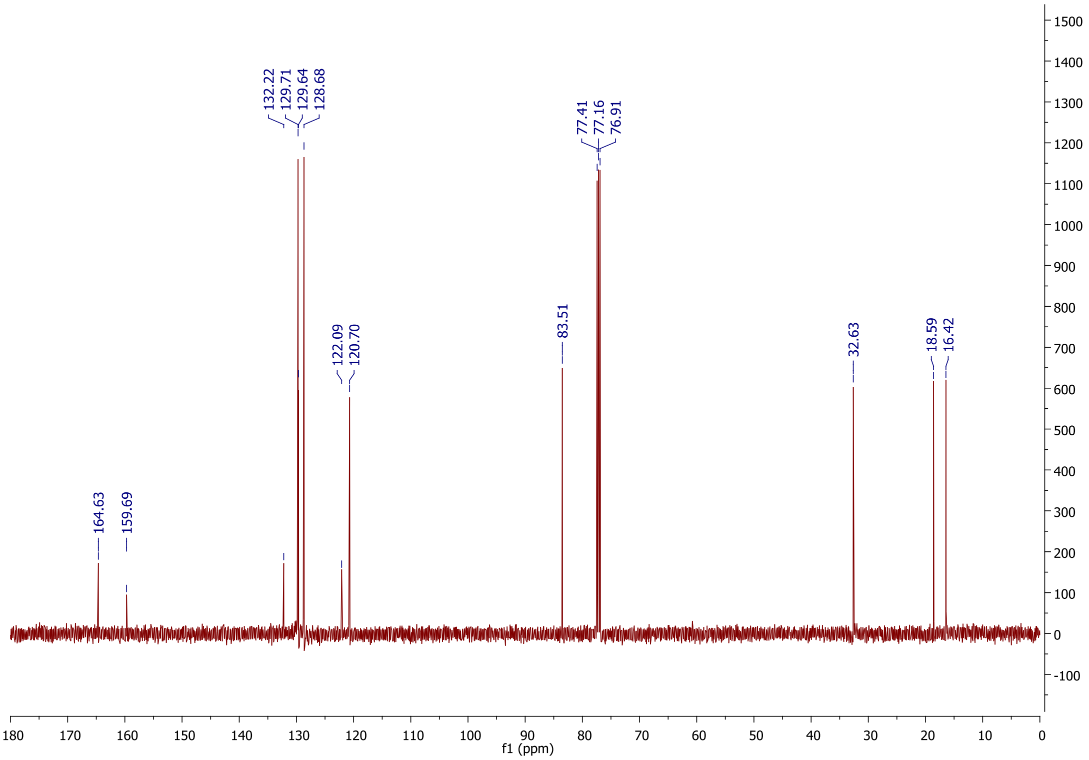
13

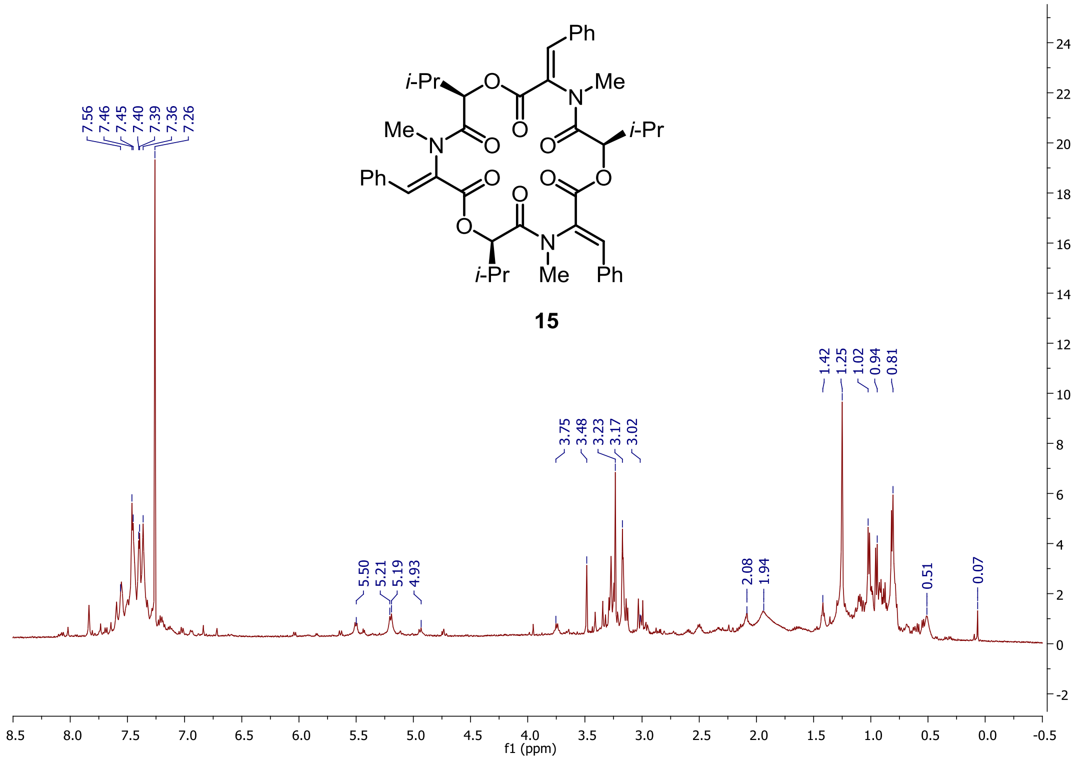
Ph
i-Pr
O
NH
O
HN
O
O
i-Pr
Ph
O
O
O
O
O
N
H
i-Pr
Ph
7.65
7.48
7.46
7.45
7.43
7.41
7.39
7.37
7.26
8.50
5.36
5.35
5.30
2.38
2.37
1.09
1.07
1.06
2.73
2.90
15.88
2.65
3.15
18.51
f1 (ppm)



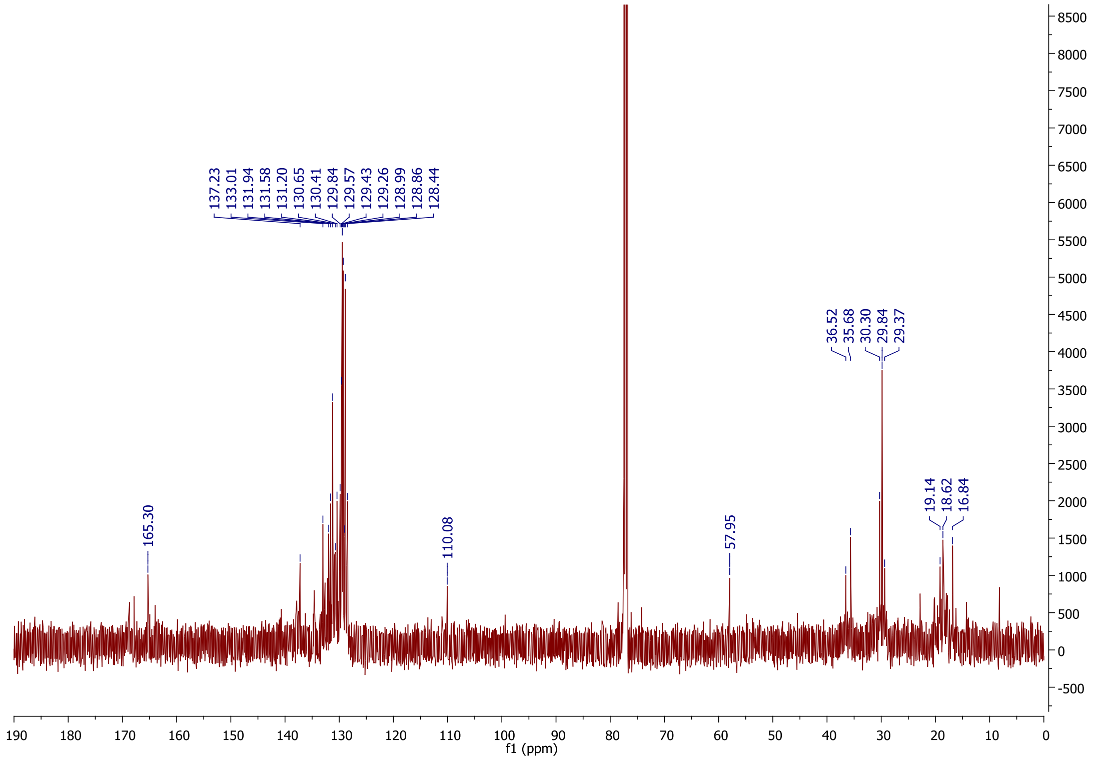
165.09
133.31
130.32
130.24
128.89
30.80
29.84
19.04
17.91
f1 (ppm)

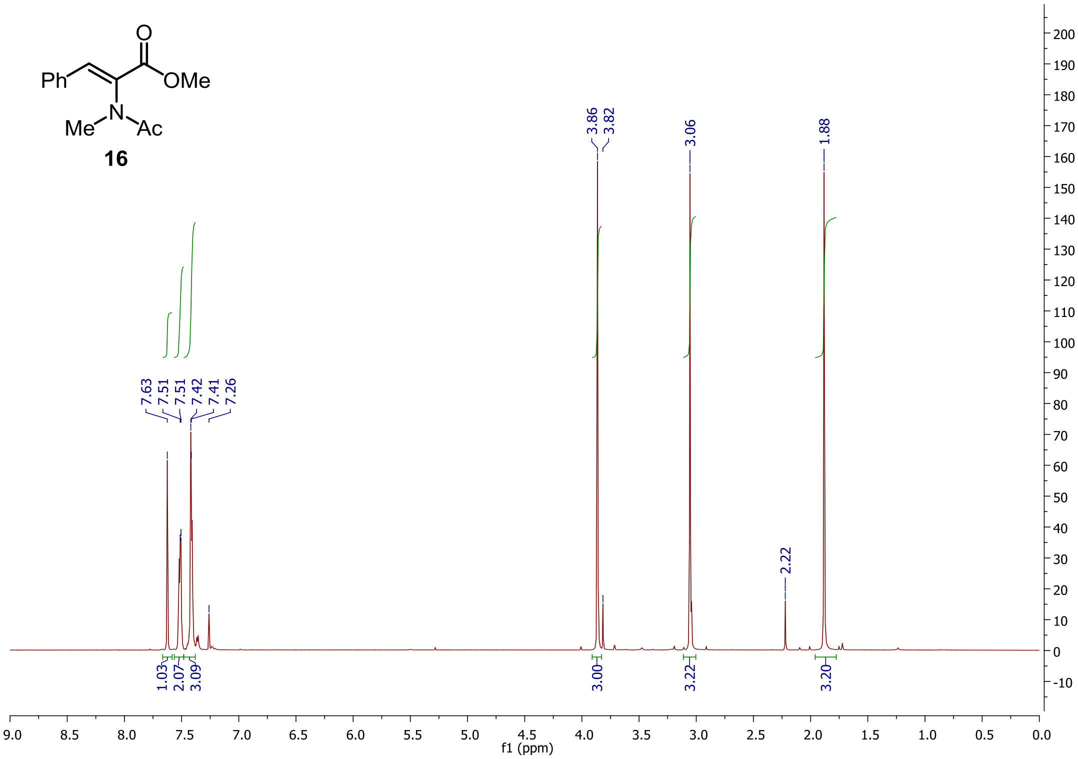

14
i-Pr
O
O
O
N
H
Ph
7.99
7.48
7.46
7.45
7.41
7.39
7.38
7.37
7.26
7.11
4.79
4.78
2.46
2.45
1.17
1.16
1.06
1.05
1.00
1.96
2.95
0.97
1.09
1.35
2.91
3.08
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
50
45
40
35
30
25
20
15
10
5
0

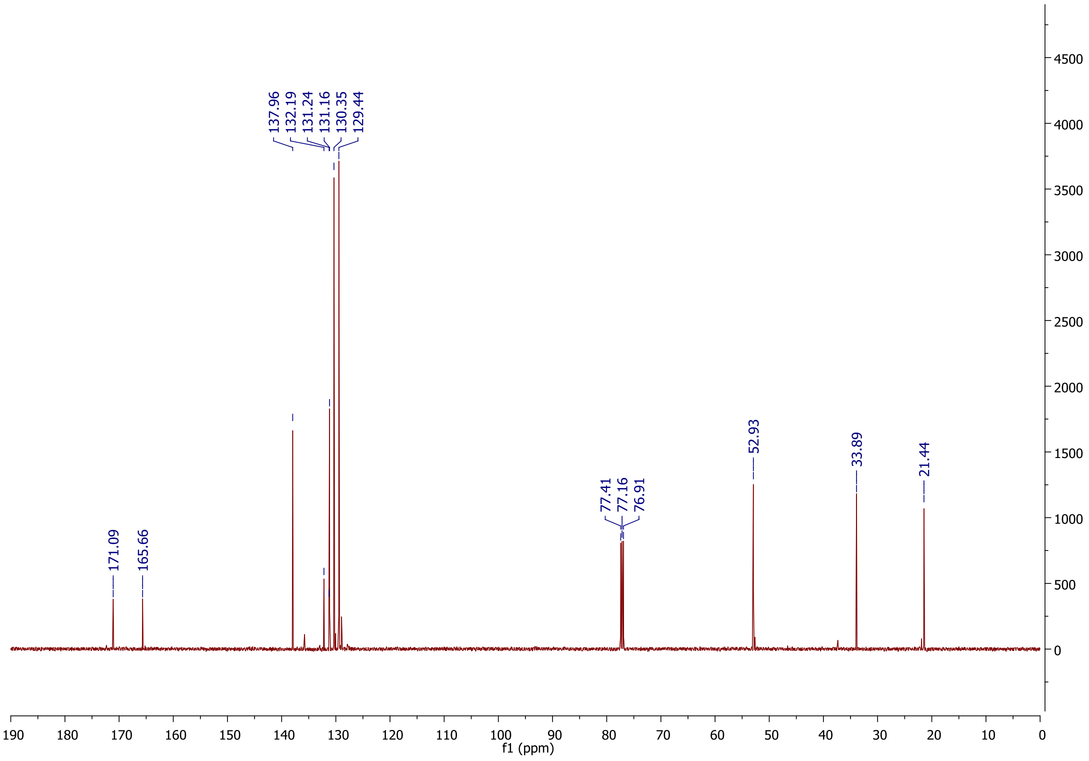

164.63
159.69
132.22
129.71
129.64
128.68
122.09
120.70
83.51
77.41
77.16
76.91
32.63
18.59
16.42
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100

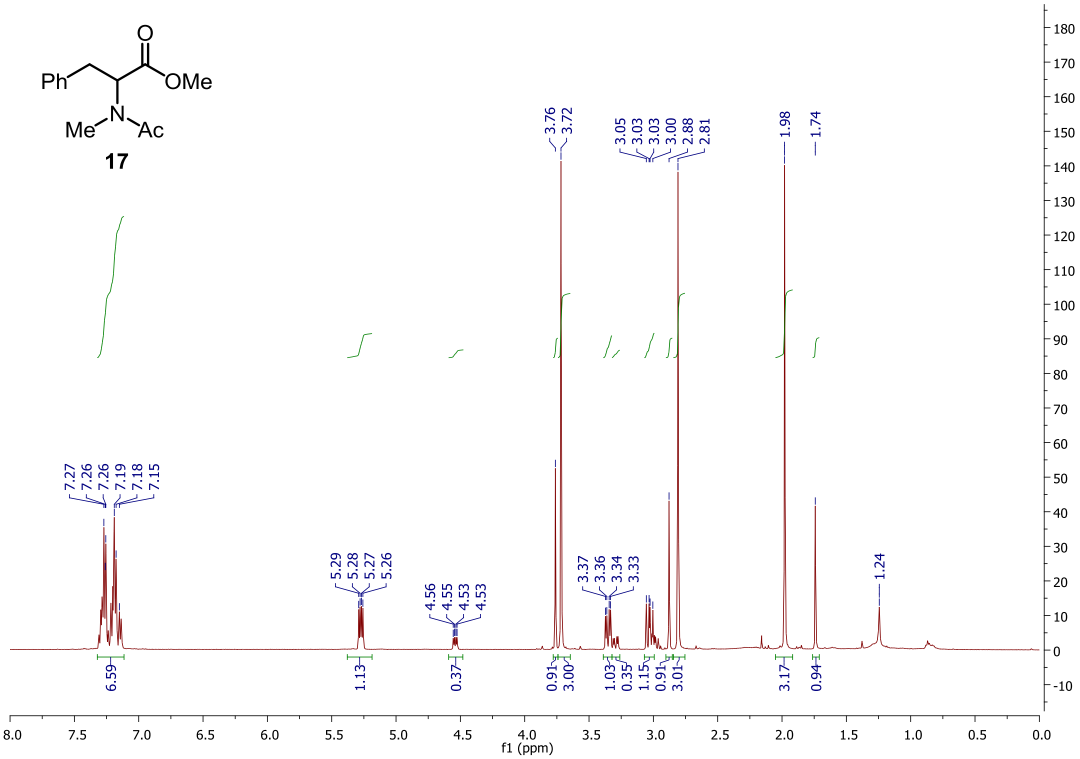

7.56
7.46
7.45
7.40
7.39
7.36
7.26
i-Pr
O
Ph
N
Me
Me
O
Ph
O
O
i-Pr
i-Pr
Me
Ph
5.50
5.21
5.19
4.93
3.75
3.48
3.23
3.17
3.02
2.08
1.94
1.42
1.25
1.02
0.94
0.81
0.51
0.07
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)
24
22
20
18
16
14
12
10
8
6
4
2
0
-2

15

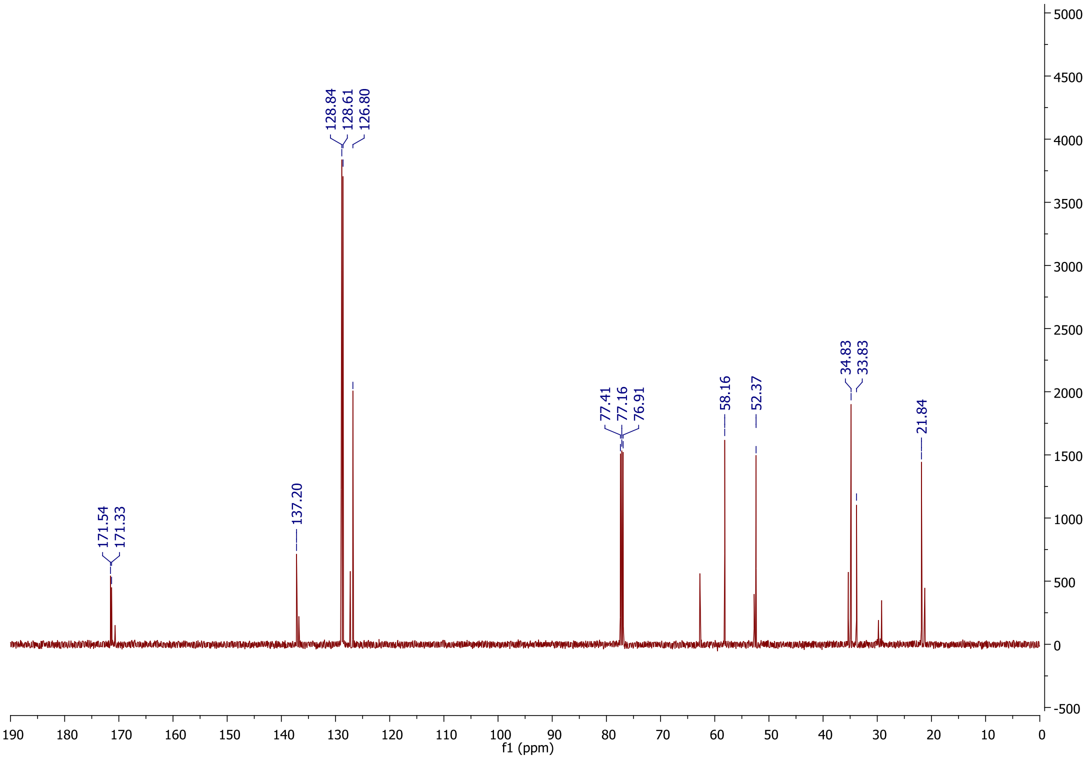

137.23
133.01
131.94
131.58
131.20
130.65
130.41
129.84
129.57
129.43
129.26
128.99
128.86
128.44
165.30
110.08
57.95
36.52
35.68
30.30
29.84
29.37
19.14
18.62
16.84
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500


Ph
O
OMe
Me
N
Ac
16
7.63
7.51
7.51
7.42
7.41
7.26
3.86
3.82
3.06
2.22
1.88
1.03
2.07
3.09
3.00
3.22
3.20
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

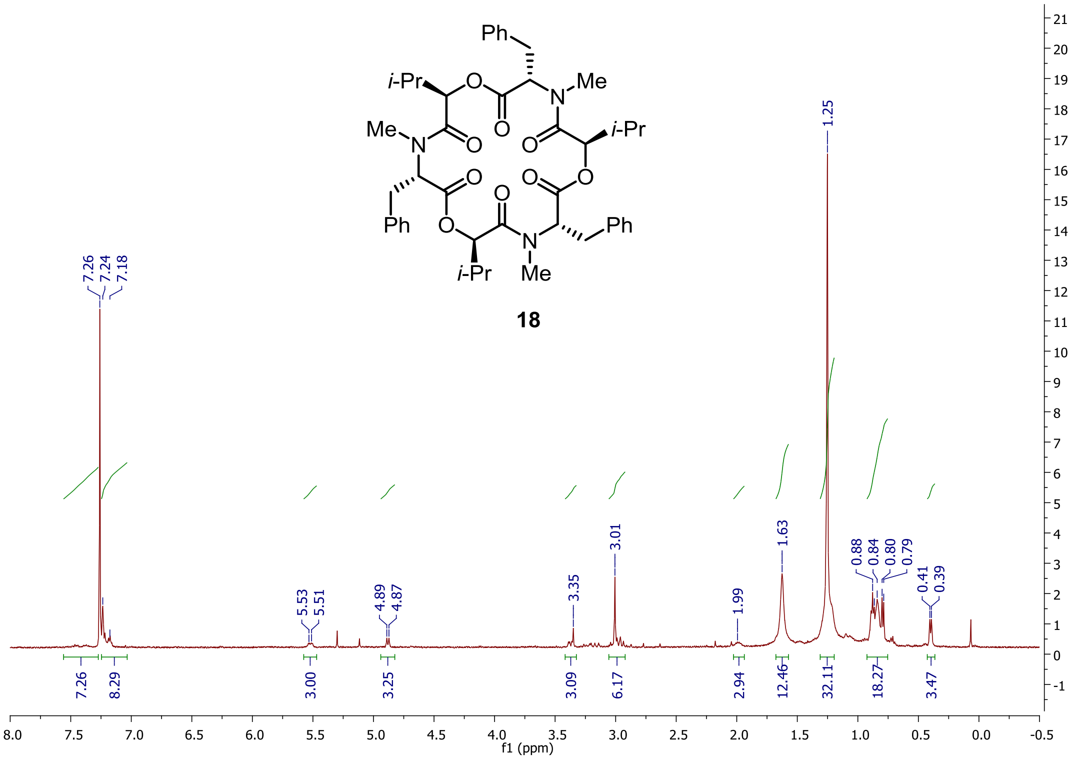

137.96
132.19
131.24
131.16
130.35
129.44
171.09
165.66
77.41
77.16
76.91
52.93
33.89
21.44
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)
4500 4000 3500 3000 2500 2000 1500 1000 500 0


17

Ph
O
OMe
N
Me
Ac
7.27
7.26
7.26
7.19
7.18
7.15
5.29
5.28
5.27
5.26
4.56
4.55
4.53
4.53
3.76
3.72
3.37
3.36
3.34
3.33
3.05
3.03
3.03
3.00
2.88
2.81
1.98
1.74
1.24
6.59
1.13
0.37
0.91
3.00
1.03
0.35
1.15
0.91
3.01
3.17
0.94
f1 (ppm)

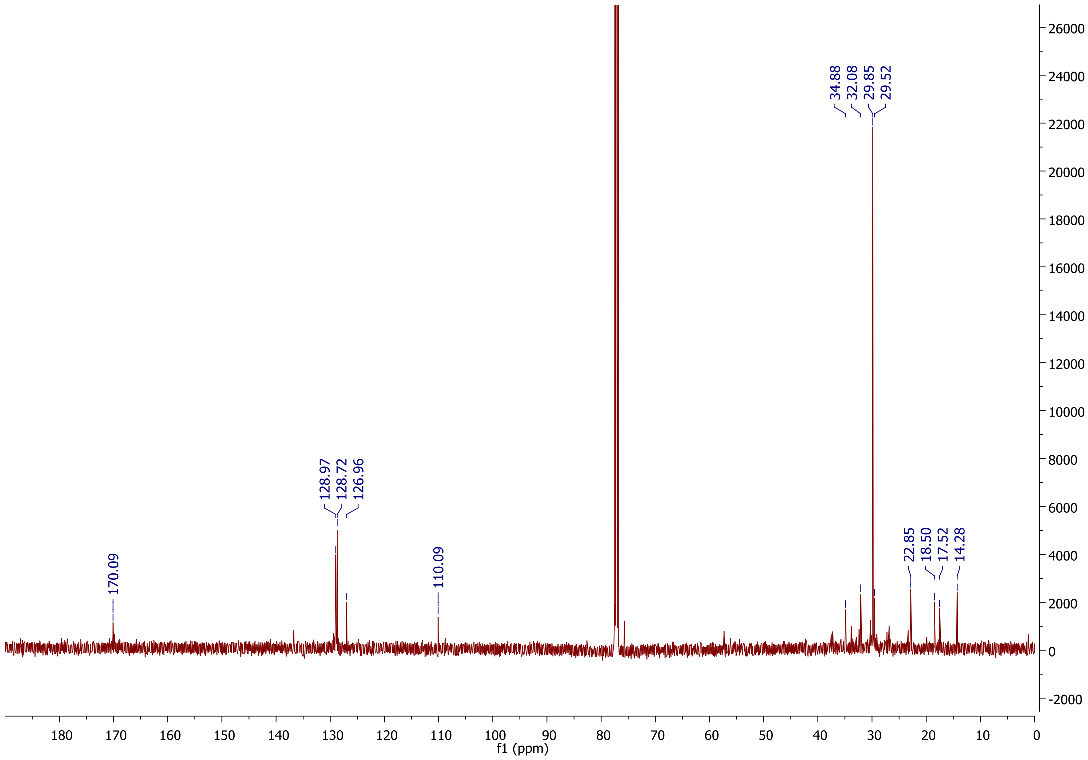


171.54
171.33
137.20
128.84
128.61
126.80
77.41
77.16
76.91
58.16
52.37
34.83
33.83
21.84
f1 (ppm)

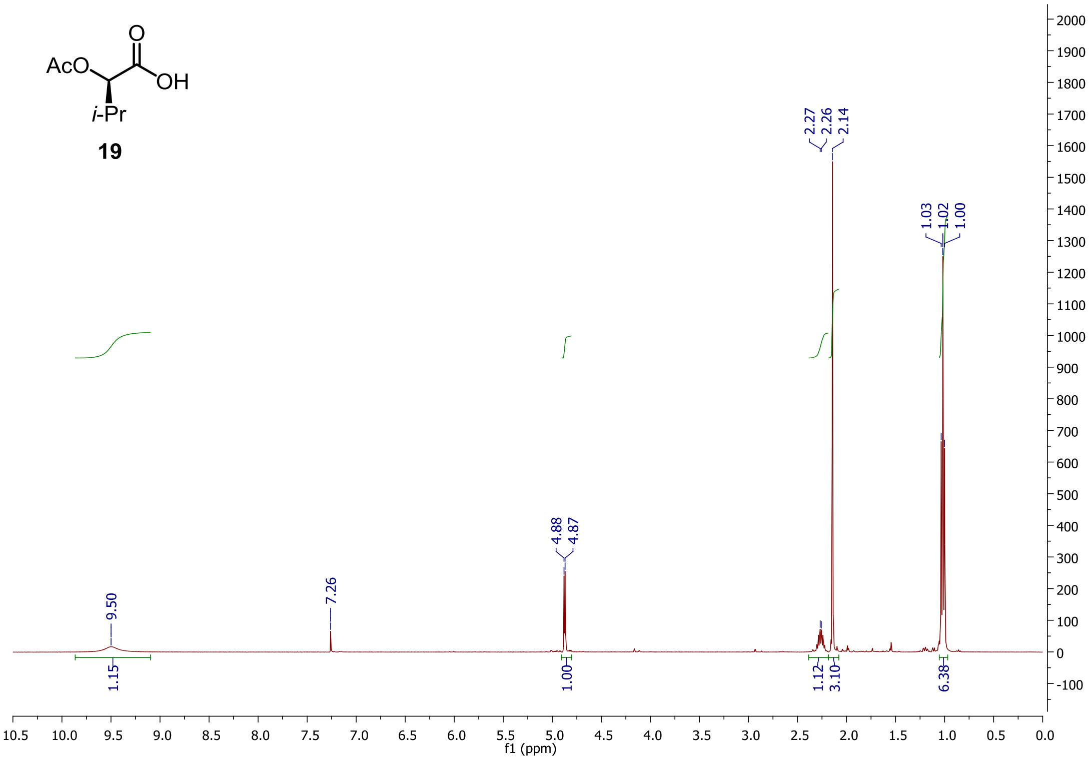


18

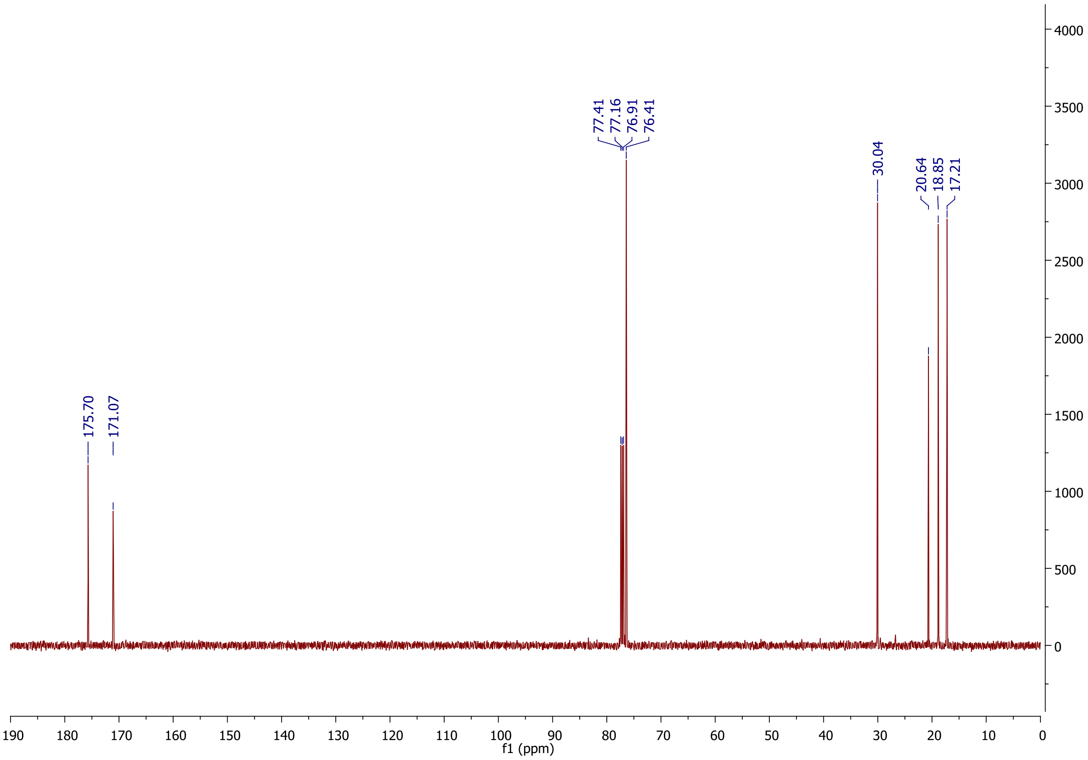
i-Pr
O
N
Me
Ph
7.26
7.24
7.18
5.53
5.51
4.89
4.87
3.35
3.01
1.99
1.63
1.25
0.88
0.84
0.80
0.79
0.41
0.39
7.26
8.29
3.00
3.25
3.09
6.17
2.94
12.46
32.11
18.27
3.47
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5

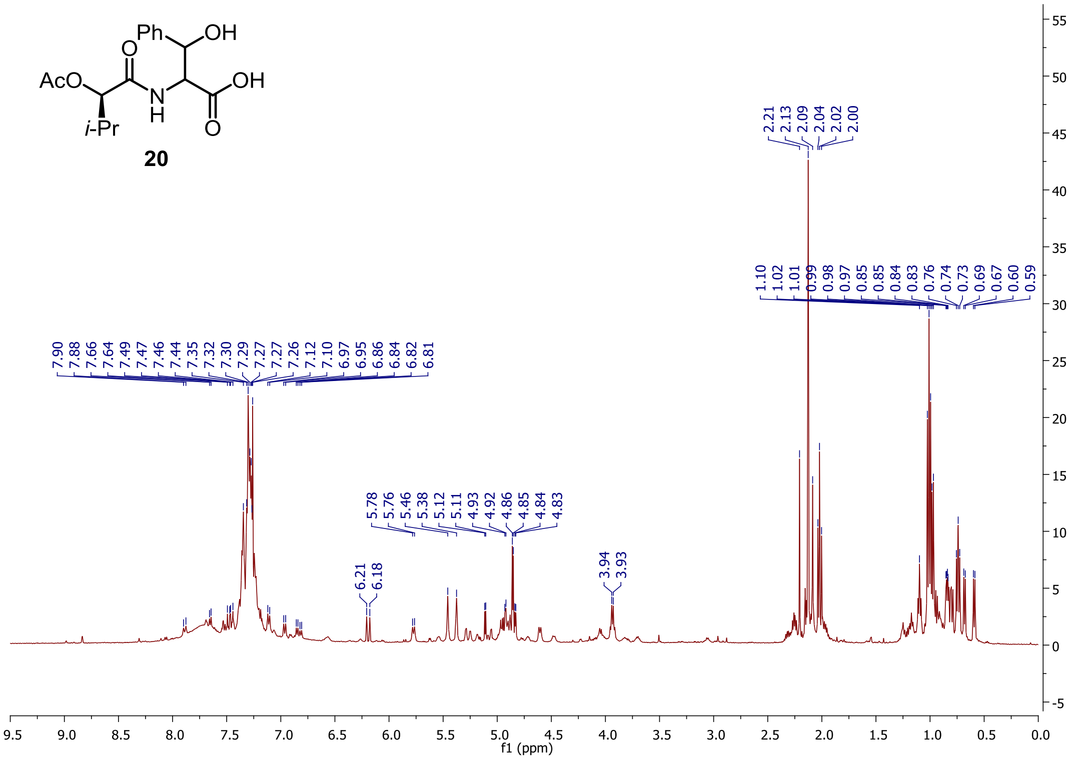


170.09
128.97
128.72
126.96
110.09
34.88
32.08
29.85
29.52
22.85
18.50
17.52
14.28
f1 (ppm)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000

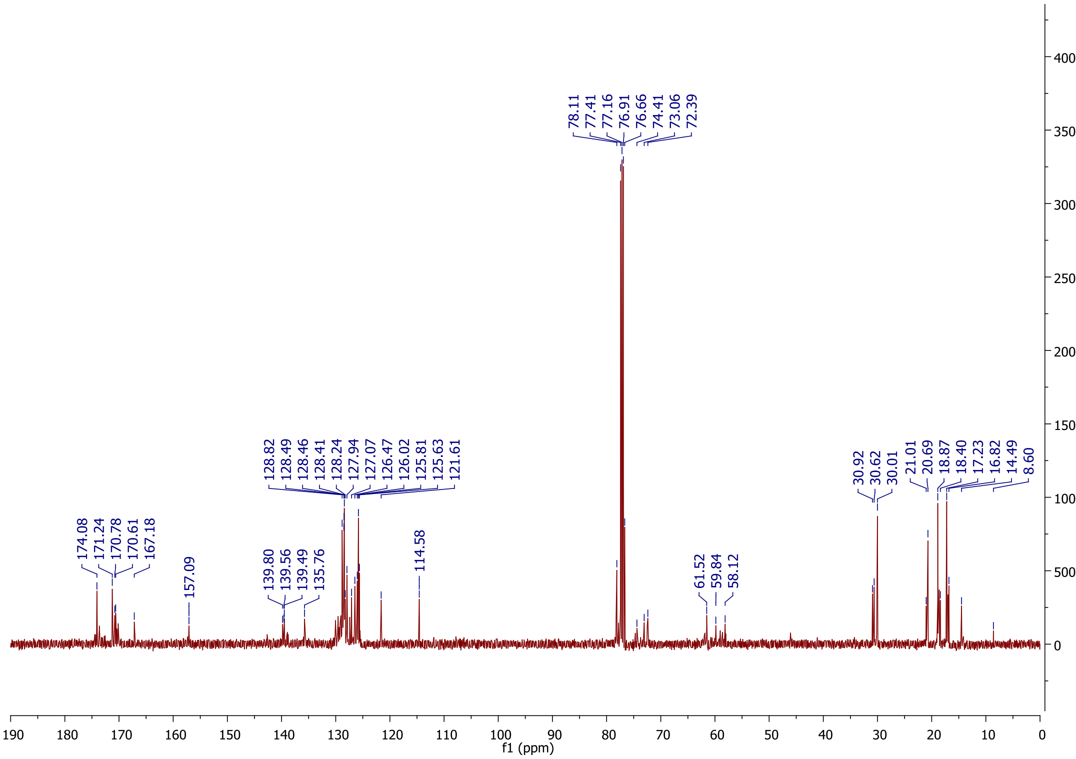

19

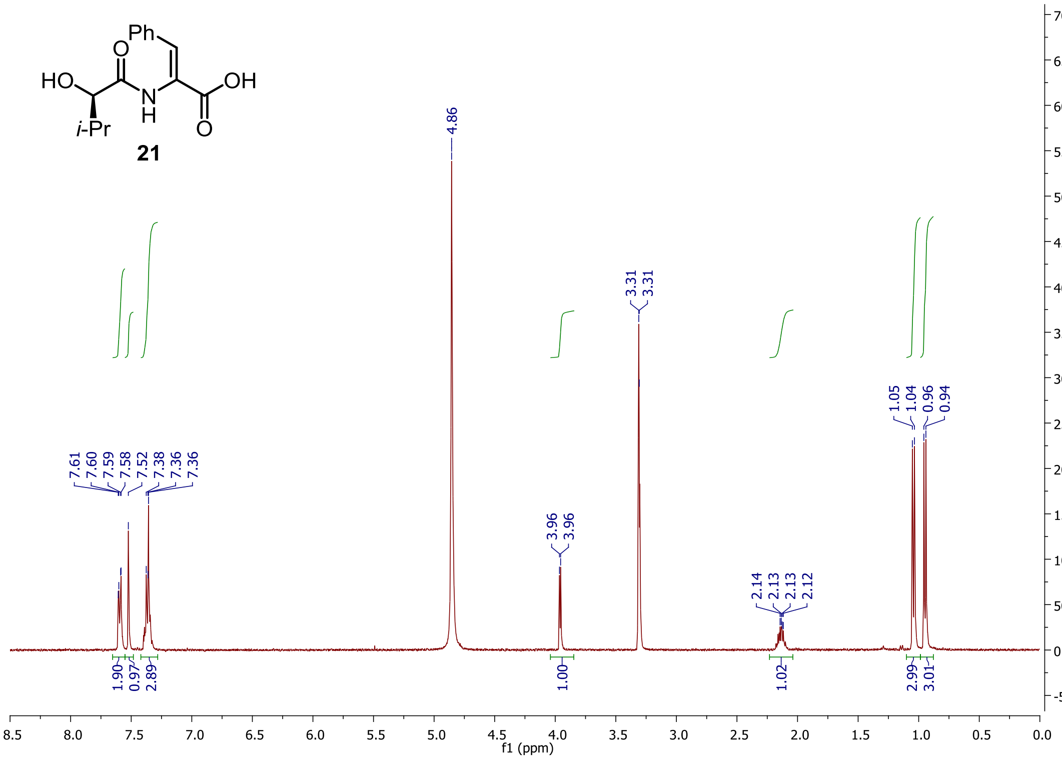
AcO
O
OH
i-Pr
9.50
7.26
4.88
4.87
2.27
2.26
2.14
1.03
1.02
1.00
1.15
1.00
1.12
3.10
6.38
f1 (ppm)
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
2000 1900 1800 1700 1600 1500 1400 1300 1200 1100 1000 900 800 700 600 500 400 300 200 100 0 -100

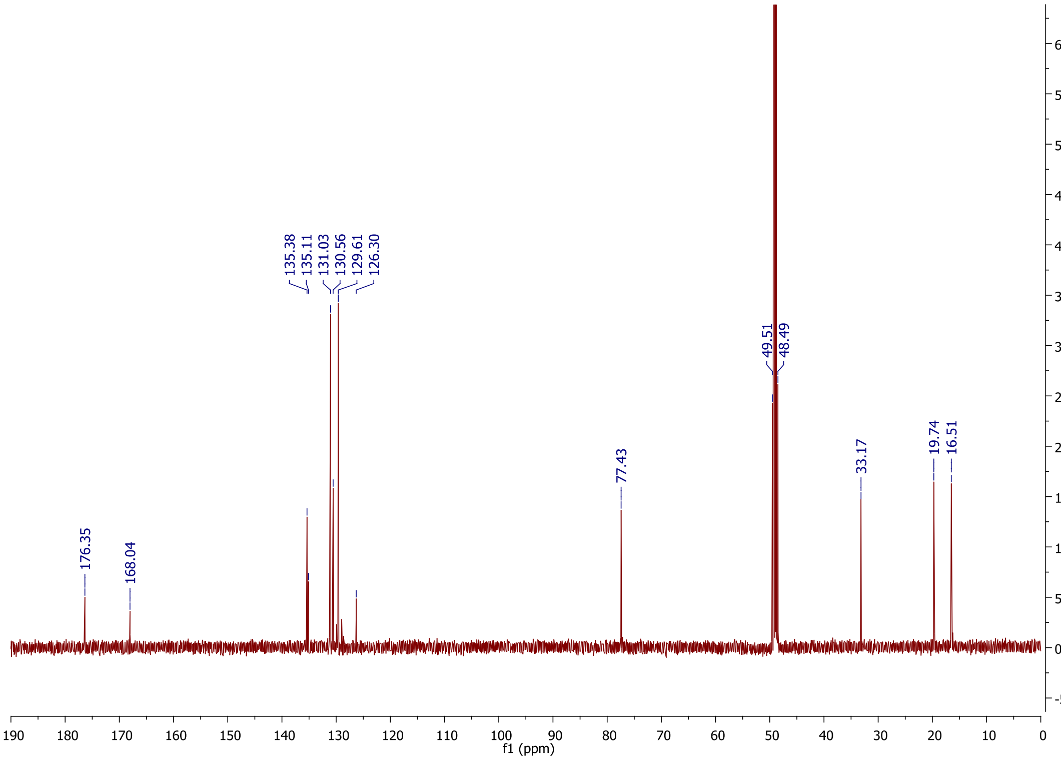

175.70
171.07
77.41
77.16
76.91
76.41
30.04
20.64
18.85
17.21
f1 (ppm)
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
4000 3500 3000 2500 2000 1500 1000 500 0

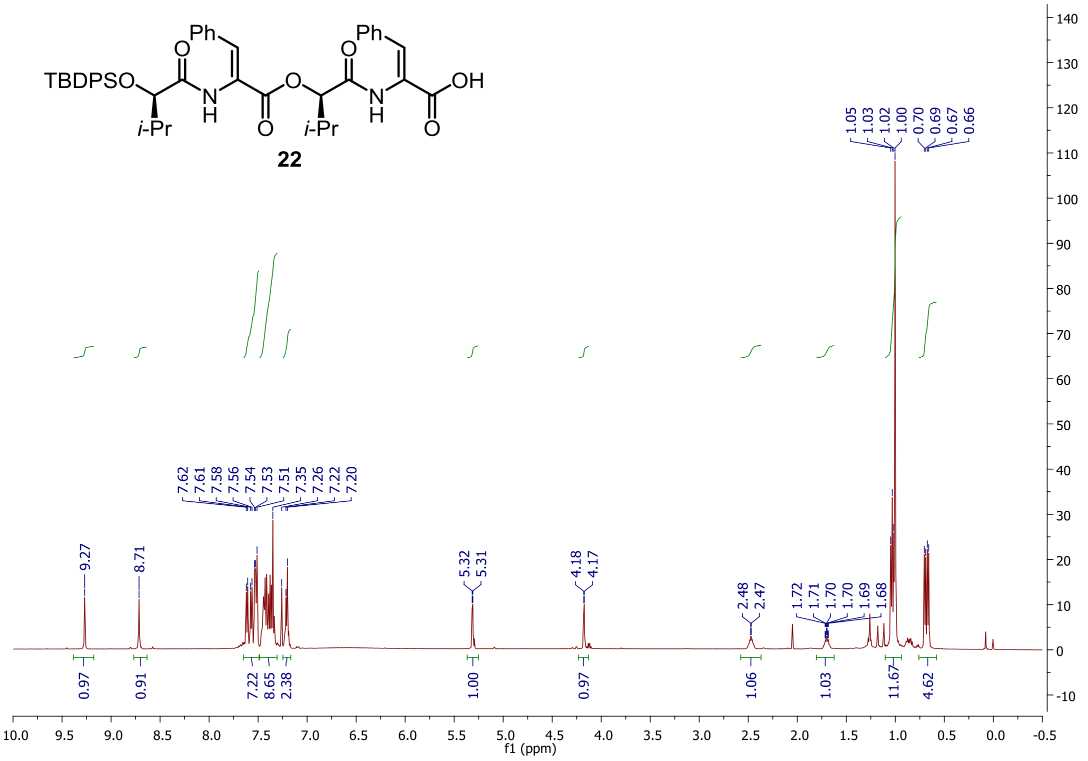

AcO
i-Pr
O
N
H
Ph
OH
OH
O
7.90 7.88 7.66 7.64 7.49 7.47 7.46 7.44 7.35 7.32 7.30 7.29 7.27 7.27 7.26 7.12 7.10 6.97 6.95 6.86 6.84 6.82 6.81
6.21 6.18
5.78 5.76 5.46 5.38 5.12 5.11 4.93 4.92 4.86 4.85 4.84 4.83
3.94 3.93
2.21 2.13 2.09 2.04 2.02 2.00
1.10 1.02 1.01 0.99 0.98 0.97 0.85 0.85 0.84 0.83 0.76 0.74 0.73 0.69 0.67 0.60 0.59
55 50 45 40 35 30 25 20 15 10 5 0 -5
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

20

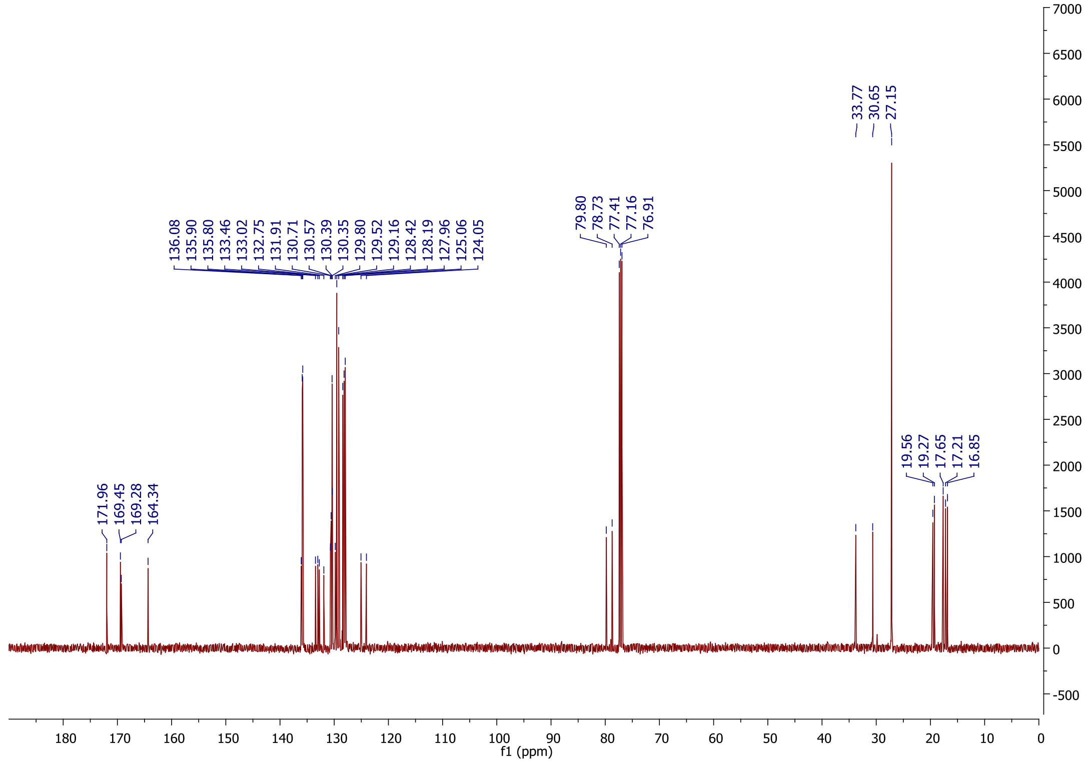

174.08 171.24 170.78 170.61 167.18
157.09
139.80 139.56 139.49 135.76
128.82 128.49 128.46 128.41 128.24 127.94 127.07 126.47 126.02 125.81 125.63 121.61
114.58
78.11 77.41 77.16 76.91 76.66 74.41 73.06 72.39
61.52 59.84 58.12
30.92 30.62 30.01
21.01 20.69 18.87 18.40 17.23 16.82 14.49 8.60
4000 3500 3000 2500 2000 1500 1000 500 0
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

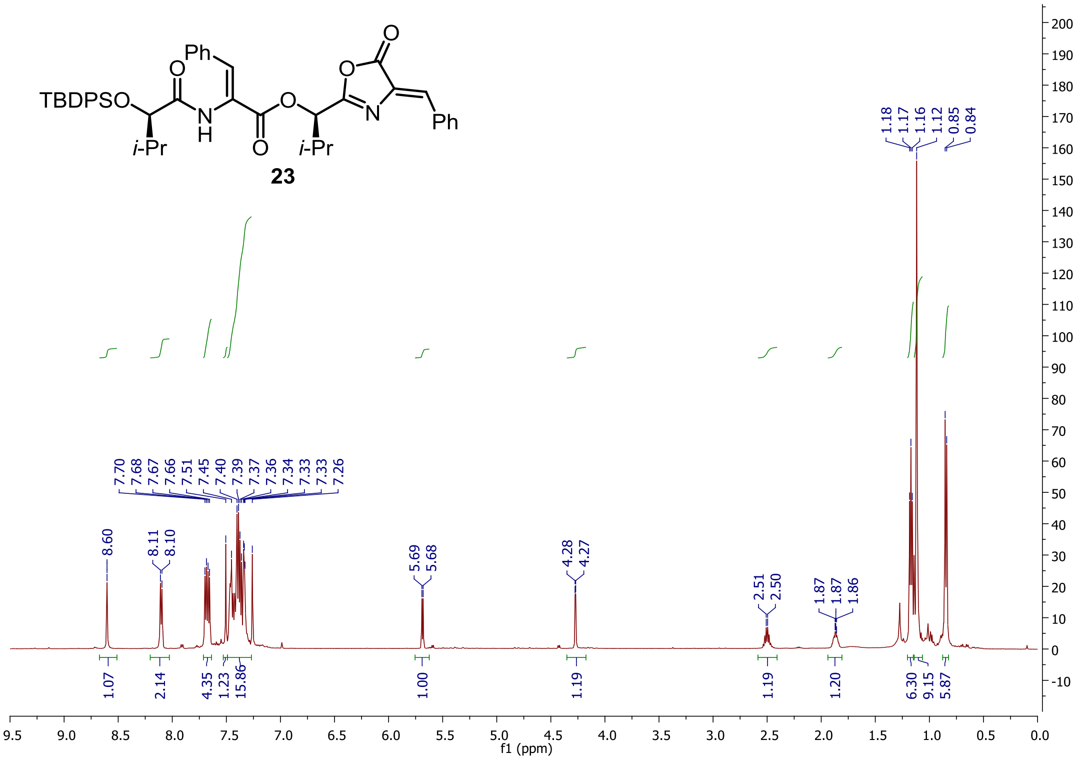

Ph
HO
O
N
H
OH
O
i-Pr
21
4.86
3.31
3.31
7.61
7.60
7.59
7.58
7.52
7.38
7.36
7.36
3.96
3.96
2.14
2.13
2.13
2.12
1.05
1.04
0.96
0.94
1.90
0.97
2.89
1.00
1.02
2.99
3.01
f1 (ppm)


176.35
168.04
135.38
135.11
131.03
130.56
129.61
126.30
77.43
49.51
48.49
33.17
19.74
16.51
f1 (ppm)

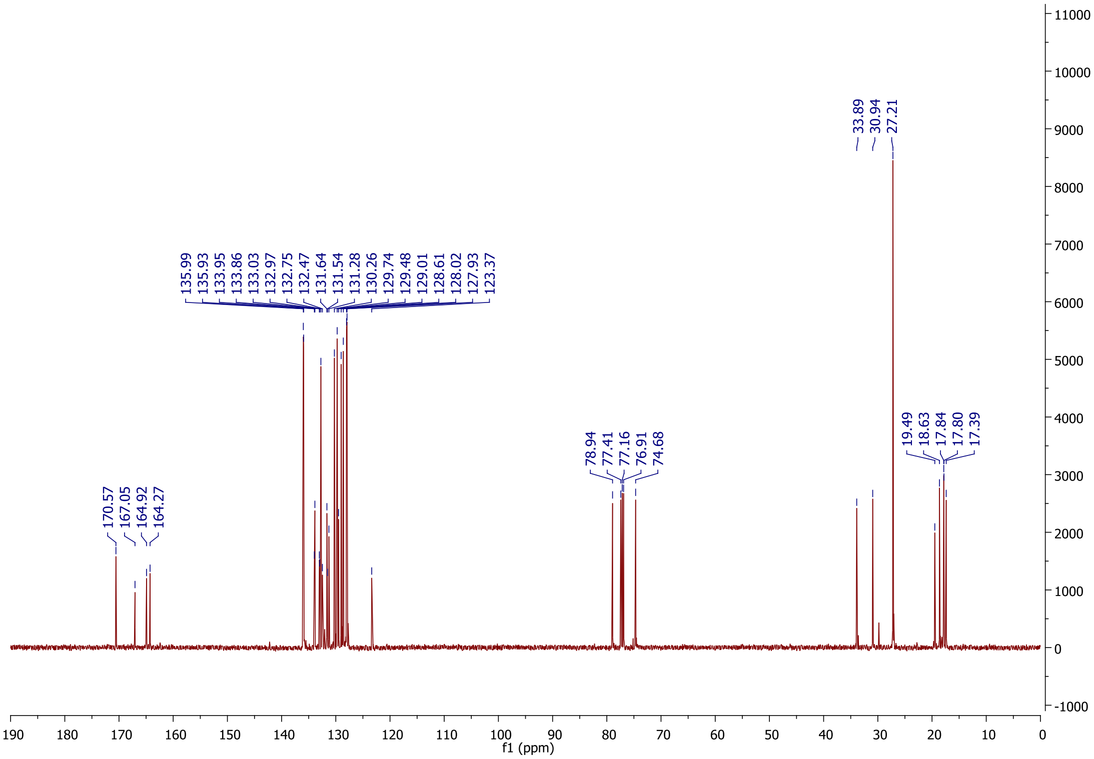

TBDPSO
i-Pr
O
H
N
Ph
O
O
i-Pr
O
H
N
Ph
O
OH
9.27
8.71
7.62
7.61
7.58
7.56
7.54
7.53
7.51
7.35
7.26
7.22
7.20
5.32
5.31
4.18
4.17
2.48
2.47
1.72
1.71
1.70
1.70
1.69
1.68
1.05
1.03
1.02
1.00
0.70
0.69
0.67
0.66
0.97
0.91
7.22
8.65
2.38
1.00
0.97
1.06
1.03
11.67
4.62
f1 (ppm)

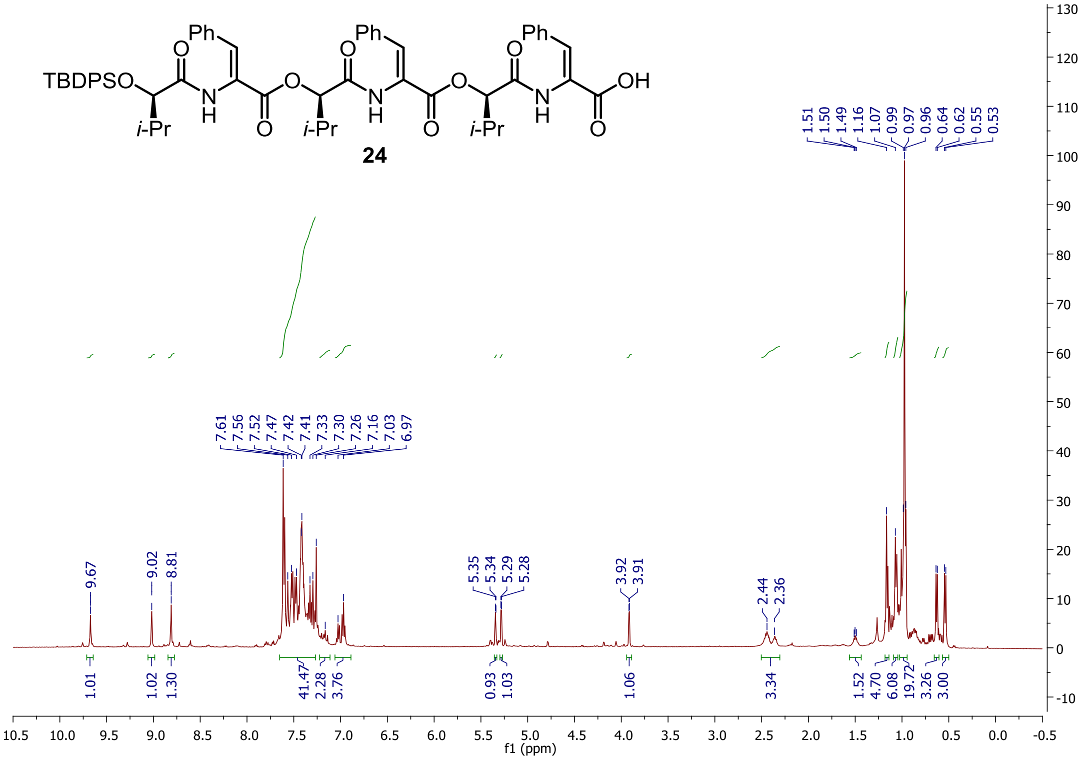
22


171.96
169.45
169.28
164.34
136.08
135.90
135.80
133.46
133.02
132.75
131.91
130.71
130.57
130.39
130.35
129.80
129.52
129.16
128.42
128.19
127.96
125.06
124.05
79.80
78.73
77.41
77.16
76.91
33.77
30.65
27.15
19.56
19.27
17.65
17.21
16.85
f1 (ppm)


TBDPSO
O
Ph
N
H
i-Pr
O
O
i-Pr
O
O
N
Ph
8.60
8.11
8.10
7.70
7.68
7.67
7.66
7.51
7.45
7.40
7.39
7.37
7.36
7.34
7.33
7.33
7.26
5.69
5.68
4.28
4.27
2.51
2.50
1.87
1.87
1.86
1.18
1.17
1.16
1.12
0.85
0.84
1.07
2.14
4.35
1.23
15.86
1.00
1.19
1.19
1.20
6.30
9.15
5.87
f1 (ppm)

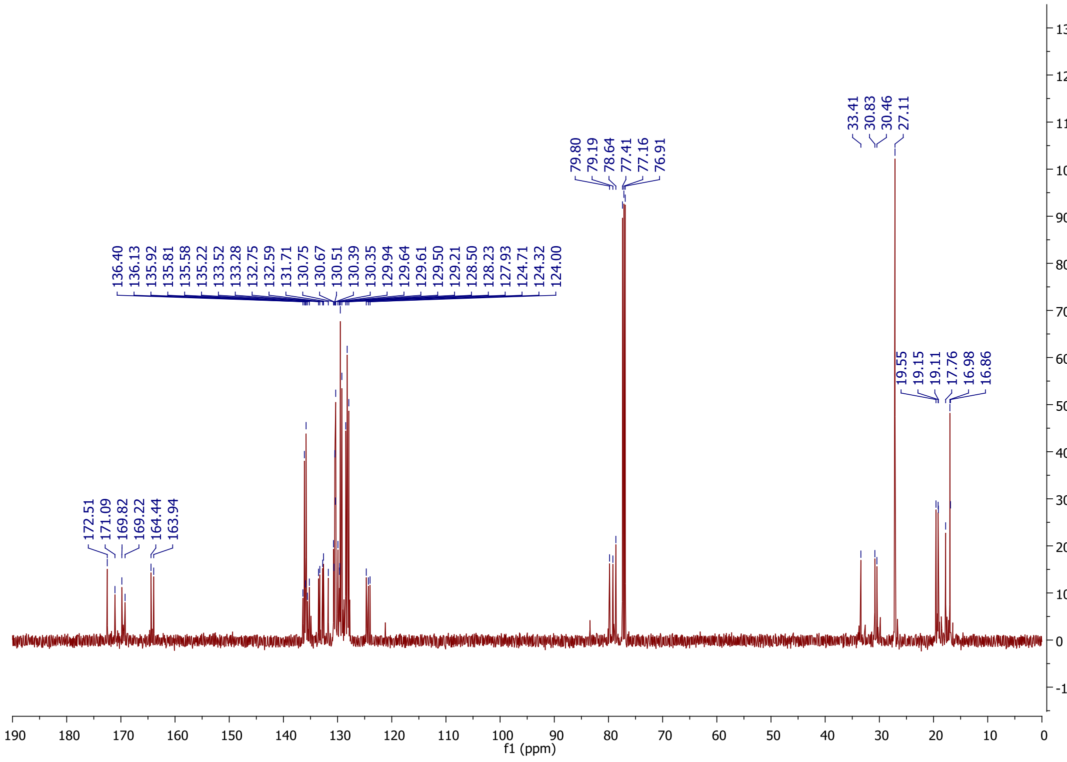
23


170.57
167.05
164.92
164.27
135.99
135.93
133.95
133.86
133.03
132.97
132.75
132.47
131.64
131.54
131.28
130.26
129.74
129.48
129.01
128.61
128.02
127.93
123.37
78.94
77.41
77.16
76.91
74.68
33.89
30.94
27.21
19.49
18.63
17.84
17.80
17.39
f1 (ppm)

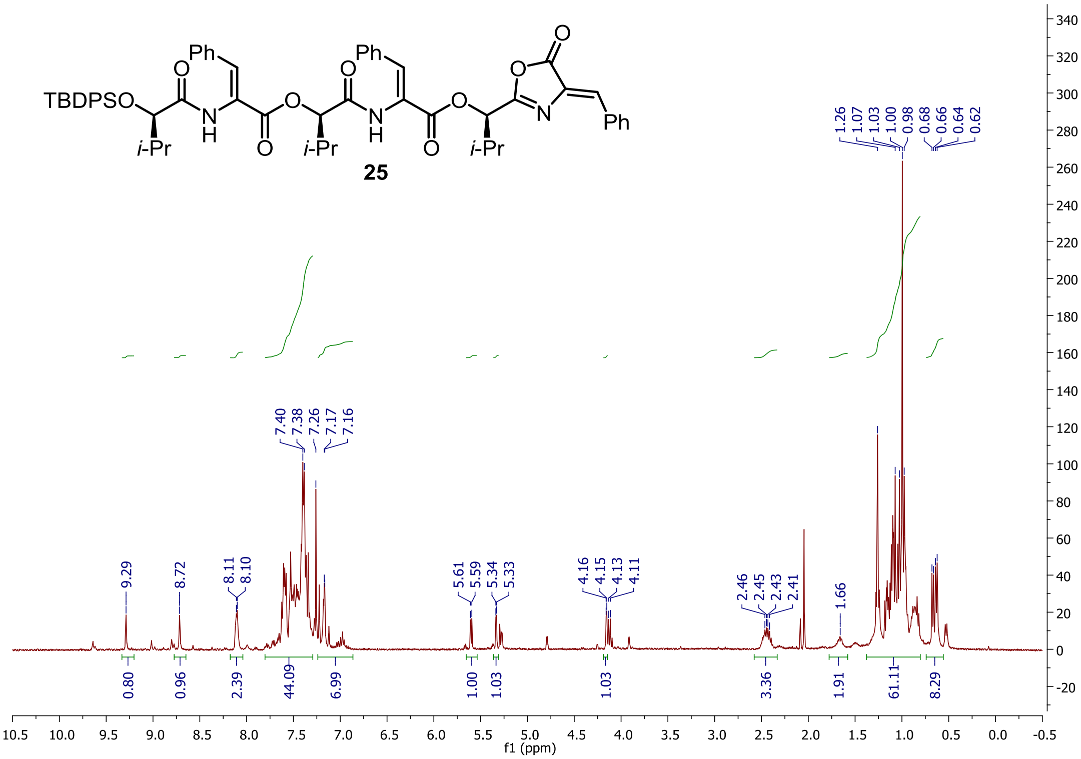


TBDPSO
i-Pr
Ph
O
N
H
OH
9.67
9.02
8.81
7.61
7.56
7.52
7.47
7.42
7.41
7.33
7.30
7.26
7.16
7.03
6.97
5.35
5.34
5.29
5.28
3.92
3.91
2.44
2.36
1.51
1.50
1.49
1.16
1.07
0.99
0.97
0.96
0.64
0.62
0.55
0.53
1.01
1.02
1.30
41.47
2.28
3.76
0.93
1.03
1.06
3.34
1.52
4.70
6.08
19.72
3.26
3.00
f1 (ppm)

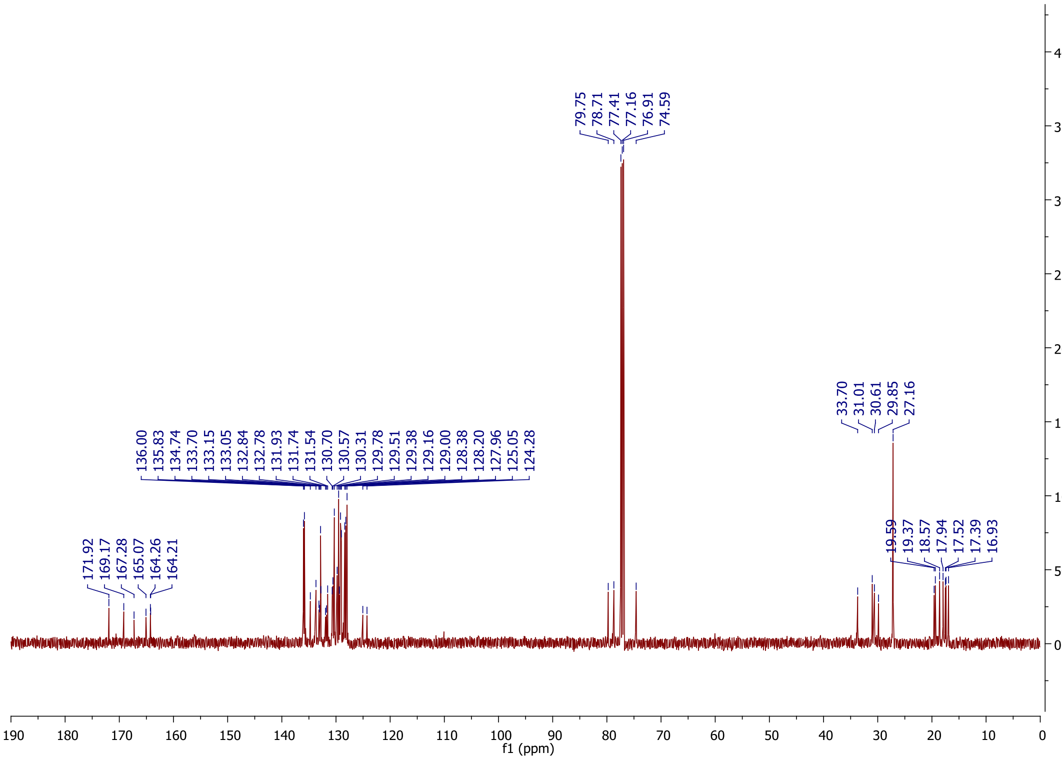
24



172.51
171.09
169.82
169.22
164.44
163.94
136.40
136.13
135.92
135.81
135.58
135.22
133.52
133.28
132.75
132.59
131.71
130.75
130.67
130.51
130.39
130.35
129.94
129.64
129.61
129.50
129.21
128.50
128.23
127.93
124.71
124.32
124.00
79.80
79.19
78.64
77.41
77.16
76.91
33.41
30.83
30.46
27.11
19.55
19.15
19.11
17.76
16.98
16.86
f1 (ppm)


TBDPSO
i-Pr
H
N
O
Ph
O
O
i-Pr
H
N
O
Ph
O
O
i-Pr
O
N
O
Ph
9.29
8.72
8.11
8.10
7.40
7.38
7.26
7.17
7.16
5.61
5.59
5.34
5.33
4.16
4.15
4.13
4.11
2.46
2.45
2.43
2.41
1.66
1.26
1.07
1.03
1.00
0.98
0.68
0.66
0.64
0.62
0.80
0.96
2.39
44.09
6.99
1.00
1.03
1.03
3.36
1.91
61.11
8.29
f1 (ppm)

25


171.92
169.17
167.28
165.07
164.26
164.21
136.00
135.83
134.74
133.70
133.15
133.05
132.84
132.78
131.93
131.74
131.54
130.70
130.57
130.31
129.78
129.51
129.38
129.16
129.00
128.38
128.20
127.96
125.05
124.28
79.75
78.71
77.41
77.16
76.91
74.59
33.70
31.01
30.61
29.85
27.16
19.59
19.37
18.57
17.94
17.52
17.39
16.93
f1 (ppm)