STUDIES TOWARD THE TOTAL SYNTHESIS OF LYDIAMYCIN A

Submitted by
Tatyana C. Sabodash
Department of Chemistry

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Master’s Committee:
Advisor: Robert M. Williams
Amy L. Prieto
Julia M. Inamine
ABSTRACT

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The isolation of lydiamycin A from *Streptomyces lydicus* (strain HKI0343) was reported by Sattler and coworkers in 2006. Lydiamycin A showed potent activity against slow-growing and pathogenic mycobacteria, suggesting a novel mode of action in comparison with existing therapeutics. In addition to having no assigned absolute configuration at C-2 and C-3, the interesting structural complexity of the thirteen–membered cyclodepsipeptide makes it an attractive synthetic target. To this date there has been two synthesis reported—lydiamycin B (Ma 2009) and lydiamycin A (Xu and Ye 2010) but none of the synthetic compounds matched the reported isolation data suggesting incorrect assignment of stereocenter(s) in the ring portion of the lydiamycins.

Current work has been focused on closing the macrocyclic core of lydiamycin A using standard coupling conditions. Once the method for effective cyclization is developed, synthesis of diastereomers would be approached to aid in the correct assignment of natural lydiamycin A. Once we have identified the correct structure of natural lydiamycin A, we plan to use the macrocyclic core to make a series of side-chain analogs of the lydiamycins with the goal of improving potency against drug-resistant strains of *Mycobacteria tuberculosis* in collaboration with Prof. McNeil’s laboratory. The ultimate goal is to synthesize dozens of analogs of lydiamycin to be
assayed for in vitro activity against non-pathogenic, pathogenic and drug-resistant strains of *Mycobacteria* sp.
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CHAPTER 1

INTRODUCTION

1.1 Isolation and Significance

In 2006 Sattler and coworkers published their isolation of Lydiamycins A–D (1–4, Figure 1).¹ The natural products were isolated from *Streptomyces lydicus* (strain HKI0343) by physico-chemical analysis of its metabolites.

**Figure 1.** Lydiamycins A–D.

<table>
<thead>
<tr>
<th></th>
<th>R¹</th>
<th>R²</th>
<th>X-Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>-CH₂-NH-</td>
</tr>
<tr>
<td>2</td>
<td>OH</td>
<td>H</td>
<td>-CH₂-NH-</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>H</td>
<td>-CH=N-</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>OH</td>
<td>-CH₂-NH-</td>
</tr>
</tbody>
</table>

The lydiamycins are cycldepsipeptides, which are a class of cyclic peptides with a presence of at least one ester linkage.² The thirteen-membered cyclic core contains piperazic acid (PBB1), with a dehydropiperazic acid moiety (PBB2) located peripheral to the ring that is connected with the 2-pentylsuccinic acid (PSA) moiety. The absolute
configuration at C-19 of PBB2, (PSA) C-25 and C-29 could not be assigned using NMR studies and are currently unknown.

There are several hundred cyclodepsipeptides that have been identified, and only 34 thirteen-membered cyclic depsipeptides of four families are known. These depsipeptides, including the lydiamycins, display no significant cytotoxicity, which is a very common characteristic of larger cyclodepsipeptides. Other than the smaller ring size, the other interesting structural feature of the lydiamycins is the presence of the piperazic acid building blocks, which are not found in any of the other small congeners. However, two piperazic acid moieties are the major characteristic of the 19-membered cyclodepsipeptides like azinothricin (6), aurantimycins A (8) and verucopeptin (7), (Figure 2). Another common feature of the azinothricin family and other cyclodepsipeptides, as well as lydiamycins, is the presence of one polyketide chain of varying complexity and length.
In biological profiling, lydiamycins A–C (1–3) were shown to exhibit potent antibacterial activity by selectively inhibiting *Mycobacterium smegmatis* SG 987, *M. aurum* SB66, and *M. vaccae* IMET 10670 in a panel of Gram-positive and Gram-negative bacteria, yeasts and fungi (Table 1).
Table 1. Antibacterial activities of lydiamycins A–C (1–3).

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC [µg mL⁻¹][a]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> ATCC 6633</td>
<td>100.1</td>
</tr>
<tr>
<td><em>Mycobacterium smegmatis</em> SG 987</td>
<td>25.0</td>
</tr>
<tr>
<td><em>Mycobacterium aurum</em> SB66</td>
<td>6.3</td>
</tr>
<tr>
<td><em>Mycobacterium vaccae</em> IMET 10670</td>
<td>3.1</td>
</tr>
<tr>
<td><em>Mycobacterium fortuitum</em> B.</td>
<td>100.1</td>
</tr>
</tbody>
</table>

[a]MIC = minimal inhibitory concentration; mean of three independent experiments.

Further studies on the antibiotic activity of lydiamycin A against slow-growing and pathogenic mycobacteria, including the *M. tuberculosis* standard strain (H37Rv) and multi-drug resistant clinical isolate, confirmed its antimycobacterial properties (Table 2). Activity against the multi-drug resistant strain of *M. tuberculosis* suggests a mechanism of action different from that of available therapeutics (isoniazid, rifampicine, etambutol, and streptomycin).

Table 2. Activity of lydiamycin A (1) against *M. tuberculosis* strains.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Cmpds.</th>
<th>MIC [µg mL⁻¹]</th>
<th>MIC [µg mL⁻¹][a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>H37Rv</td>
<td>1</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td># 246[b]</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>H37Rv</td>
<td>Control[c]</td>
<td>1.17</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td># 246</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

[a]Minimal bacterial concentration. [b]Clinical isolate resistant against main antituberculosis drugs. [c]Isoniazid.

According to the World Health Organization, about one-third of the world’s population is currently infected with the TB bacillus. There have been documented strains of TB that are resistant to a single drug in every country surveyed and multi-drug resistant TB continues to emerge. While drug-resistant TB is generally treatable, it requires extensive chemotherapy (up to
two years of treatment) with second-line anti-TB drugs which are more costly than first-line drugs, and which produce adverse drug reactions that are more severe. While current tools can control TB, there is a growing need for more effective therapies for the multi-drug resistant TB infections.

1.2 Chemistry of Piperazic Acids

Piperazic acids are nonproteinogenic amino acids with a cyclic hydrazine skeleton having the general structures 10–12 (Figure 3). Hassall was the first to discover members of the family with the structure of 10 in the 1960s from degradation of monamycins. Since then, many piperazic acid containing natural products have been isolated, and that number continues to grow. Some examples (Figure 2) include antrimycin A (5), which acts as a tuberculostatic agent, and azinothricin (6), an anti-tumor antibiotic. Also, the prescription drug Cilazopril® (9), although much simpler in structure than the natural peptides, also possess the essential piperazic acid core and is used to treat hypertension.

Numerous synthetic strategies have been developed to access piperazic acids and their derivatives. The framework is usually accessed from
cyclization of α-hydrazino acid precursors, which can be obtained through N-amination or delivery of the complete hydrazine subunit to the suitable substrate. There are a few challenges with the construction of these small molecules, which include establishing the stereocenter at the C-3 carbon and introducing the nitrogen-nitrogen bond (Figure 4). In other derivatives it might be also required to asymmetrically functionalize C-4 or C-5 centers; lydiamycin B features such example.

**Figure 4. Challenges in the synthesis of piperazic acids.**

Schmidt used the typical approach to chiral piperazic acids with the cyclization and protection of L-glutamic acid 13 (Scheme 1). Reduction of protected lactam 14 with sodium borohydride gave alcohol 15, which was subsequently mesylated. Standard N-nitrosation followed by *in situ* reduction and acylation gave acyclic precursor 16. Treatment of 16 with sodium hydride provided the orthogonally protected piperazic acid 17. Three-step protecting group manipulation yielded the desired piperazic acid derivative 18 in 61% with 92% ee. While synthesis starts with enantiomerically pure amino acid 13.
and the authors give no explanation to the source of the degradation of the stereocenter at C-3.

**Scheme 1. Schmidt’s enantioselective synthesis of piperazic acid.**

To introduce chirality, Hughes used a Sharpless asymmetric epoxidation reaction (**Scheme 2**). Treatment of aldehyde 20 with Horner-Wadsworth-Emmons reagent 19 gave the α,β-unsaturated ester 21. DIBAL reduction afforded alcohol 22, which was employed directly in an asymmetric epoxidation to give 23. Oxidation of the alcohol with ruthenium tetroxide, followed by methylation with diazomethane, then gave methyl ester 24. Saponification of the ester provided the corresponding potassium salt, which underwent epoxide opening with aqueous hydrazine to afford the air-sensitive intermediate 25. Treatment of 25 with TFA then gave the desired C-4 oxygenated piperazic acid 26.
Scheme 2. Hughes’ enantioselective synthesis of piperazic acid derivative.

Hale used Evans auxiliary followed by electrophilic hydrazination of enolate to access piperazic acids enantioselectively (Scheme 3).\textsuperscript{12} N-Acylation of oxazolidinone 27 with 5-bromovaleryl chloride 28 followed by diastereoselective α-hydrazination with di-t-butylazodicarboxylate (DBAD) gave 30. Lithium hydroxide mediated removal of the auxiliary then gave free acid 31 with non-differentially protected nitrogens. The TFA salt 32 was formed in good yield by treatment of 31 with trifluoroacetic acid.

Scheme 3. Hale’s enantioselective synthesis of piperazic acid.

These examples represent a small sampling of available methods for synthesizing piperazic acids. Each rout has its flaws, which include working
with highly unstable intermediates (i.e. 25), protecting group differentiation (31), and lack of complete optical purity (18).

1.3 Ma’s Synthesis of the Proposed Structure of Lydiamycin B

In 2009 Ma and coworkers reported the first total synthesis of the proposed structure of lydiamycin B (2). Their retrosynthetic approach began with the first disconnect shown in Scheme 4 arriving at macrocyclic core 33 and the side chain moiety 34. Macrolactamization at D-Leu-L-Ser site was envisioned to enable the construction of macrocycle 33. To establish the stereochemistry at C(19) and C(25), four possible isomers of 34 were necessary to be synthesized, thereby providing four corresponding diastereomers of compound 2.
Scheme 4. Retrosynthetic analysis of proposed structure of Lydiamycin B.

In the synthetic direction, the lithium enolate of \( N \)-acyl oxazolidinone 35 was reacted with tert-butyl bromoacetate to provide ester 36 (Scheme 5). The chiral auxiliary was then removed to afford ally ester 37, which upon treatment with TFA gave acid 38; this was then converted to acid chloride 39 using oxalyl chloride.
Scheme 5. Construction of 2-n-pentyl succinic acid chloride.

Side-chain formation began by selectively protecting the primary alcohol of known diol 40 as TBDPS ether 41 (Scheme 6). This was then treated with triflic anhydride and tert-butyl carbazate to afford hydrazide 42. Acylation of the secondary nitrogen using acid chloride 39 then provided compound 43. Removal of the TBDPS group was accomplished with TBAF and AcOH to arrive at alcohol 44, which was oxidized to its aldehyde derivative using IBX/DMSO. These conditions provided a mixture of hemiaminal and aldehyde (not shown), which were treated together with TFA to give hydrazone 34. The three other diastereomers were prepared using a similar approach.

The macrocyclic core was constructed starting from known lactone 45, which was oxidized to give alcohol 46 (Scheme 7). Hydrazine 47 was then accessed via the triflate of alcohol 46. Acylation of 47 with Cbz-L-Ala, followed by silyl deprotection, gave dipeptide 48. After triflation and treatment with TFA, intramolecular N-alkylation occurred spontaneously to give an intermediate piperazide (not shown), which was condensed with 1-aminopiperidine and then silylated with TBSOTf to afford protected dipeptide 49. Hydrogenolysis facilitated deprotection of alanine N-terminus, which in turn was coupled with Fmoc-D-Leu to provide tripeptide 50. Upon treatment with N-bromosuccinimide and pyridine to remove the 1-aminopiperidine group, partial oxidation at the piperazic ring occurred, the resulting mixture was condensed with Z-Ser-OAllyl to furnish a mixture of esters 51 and 52 in a ratio of 1 : 3. Hydrazone 51 could be converted to 52 with NaBH$_3$CN. The
allyl and Fmoc protecting groups were cleaved with Pd chemistry and diethylamine treatment, respectively. After deprotection the amino acid was then subjected to macrolactamization in the presence of HATU/HOAt/i-Pr$_2$NEt to afford the cyclized product 53.Cbz group was removed using Adam’s catalyst to provide the free amine 54.

**Scheme 7. Synthesis of the macrocyclic core.**
Finally, the macrocyclic core 54 was coupled with each of the isomers of 34 to provide the corresponding protected cores of natural product 55 (Scheme 8). Global deprotection of 55 with TAS-F and Pd(Ph₃P)₄/NMA then gave all four possible stereoisomers of the proposed structure of lydiamycin B (2).

Scheme 8. Final steps.

None of these isomers matched the NMR data reported for the natural product. The major difference arose from discrepancies in the proton signals at C-8 position. The four synthetic isomers of 2 exhibited chemical shifts of 1.27 ppm for these protons, while the natural product’s corresponding signal was 1.47 ppm. The authors implied that the stereochemistry of the surrounding amino acid residues might have been misassigned.
1.4 Xu and Ye’s Synthesis of the Proposed Structure of Lydiamycin A

In 2010, after Ma’s report, the synthesis of the proposed isomers of lydiamycin A was reported by Xu and Ye.\(^\text{14}\)

Scheme 9. Retrosynthesis of lydiamycin A.

The retrosynthetic approach, in a familiar method seen in Ma’s synthesis, includes disconnect between L-Ser and the dehydropiperazic acid unit to provide the macrocyclic core \(56\) and the side chain moiety \(57\) (Scheme 9). Macrolactonization at L-Ser and internal piperazic acid was used to construct the macrocyclic core \(56\). Likewise to establish stereochemistry at C(19) and C(25) four possible isomers of \(57\) were
necessary to be synthesized and coupled to the macrocyclic core 56 to confirm the stereochemistry of the natural lydiamycin A.

Starting with a known acetal 58, dehydropiperazic acid fragment was constructed (Scheme 11). The lithium enolate derived from N-acyl oxazolidinone 58 was reacted with dibenzyl azodicarboxylate (CbzN=NCBz) to provide hydrazine 59. The chiral auxiliary was transesterified with methanol to give methyl ester 60. Both Cbz groups were removed by hydrogenolysis and regioselectively Boc-protected in situ to furnish compound 61. Acylation with 39, followed by TFA/H₂O treatment and LiOH assisted saponification afforded hydrozone 62.

Scheme 11. Synthesis of the side chain.

Hydrogenolysis of the known bis-Cbz protected (R)-tetrahydropyridazine 63, followed by a selective Cbz-Cl protection gave 64 (Scheme 12). Silver cyanide-mediated coupling reaction between Fmoc-Ala-Cl and 64 afforded peptide 65. Cleavage of the Fmoc group followed by coupling with L-Cbz-Ser-(OTBDPS)-d-Leu-OH yielded the desired tetrapeptide 66. The TBS protecting group of the acycle 66 was removed and the exposed alcohol was then oxidized in a two-step process using Dess-Martin and Pinnik oxidations.
providing the corresponding acid. The TBDPS group on the serine alcohol was removed with TBAF to afford the hydroxy acid 67 which upon treatment with DEAD and PPh\textsubscript{3} cyclized to give the macrocycle 68. Both Cbz groups were removed using to give the free amine 69, which in turn was coupled with acid 62 to give adduct 70. Allyl deprotection gave the final natural product isomer 1. All four isomers of the proposed structure of lydiamycin A were synthesized in a similar fashion.

**Scheme 12. Final steps.**
None of the synthesized diastereomers of 1 matched the $^1$H or $^{13}$C spectra of the isolated natural product. The main discrepancies were apparent in the $^1$H data in the 4-5 ppm region. The authors also speculated that the misassignment of the stereochemistry is somewhere in the macrocycle.

1.5 Research Objectives

This research project started with the objective to synthesized the proposed isomers of the natural product lydiamycin A—to be able to determine the stereochemistry of the natural product. Mentioned publications have shown that the proposed structures are incorrect and none of which match the isolated natural product.

Identifying the structure of lydiamycin A will give an access to a new structural class of anti-Mycobacterial agents. We plan to use the macrocyclic core to make a series of side-chain analogs of the lydiamycins with the goal of improving potency against drug-resistant strains of *Mycobacteria tuberculosis*. 


CHAPTER 2

PROGRESS TOWARD THE TOTAL SYNTHESIS OF LYDIAMYCIN A

2.1 Retrosynthetic Analysis

In a depsipeptide, the majority of retrosynthetic disconnections are made at amide and ester bonds. Despite the straightforward nature of such disconnections, several challenges and roadblocks remain in devising a concise synthetic approach. The first task usually considered is the sequence of the disconnections followed by careful choice of appropriate protecting groups.

Lydiamycin A 1 can be broken down into six distinct pieces: piperazic acid precursor 70, L-alanine 71, D-leucine 72, L-serine 73, dehydropiperazic acid 76 and 2-pentylsuccinic acid 75 (Scheme 13). We chose to focus on early-stage macrocycle 69 formation, which comes from coupling of piperazic acid 70, L-alanine 71, D-leucine 72, and L-serine 73. The dehydropiperazic acid 76 and succinic acid derivative 75 can then be coupled to the macrocycle at the end stages of the synthesis. After global deprotection we should be able to arrive at lydiamycin A. Our approach is very similar to already published strategies by Ma and Xu but we hope to improve the amount of synthetic steps, yields and determine the correct stereochemistry.
It was also important to pick an appropriate strategy to make the piperazic acid moiety. Yutaka Aoyagi, a visiting professor, developed a scalable route toward both enantiomers of the piperazic acid using lipase TL mediated kinetic resolution of (±)-5-benzyloxy-1-tert-butyldimethylosilyloxy-2-pentanol \(^{15}\). We planned to access the piperazic acid derivatives (S)-77 and (R)-77 from hydrazino alcohols (S)-78 and (R)-78 via an intramolecular Mitsunobu reaction (Scheme 14). Introduction of hydrazine to compound 79 would occur via a reductive amination. Enantiomerically enriched alcohol 80 and acetate 79 would be obtained from lipase TL-mediated kinetic resolution of the racemic alcohol 80, which can be accessed from commercially available 4-penten-1-ol 81 in three steps.
Scheme 14. Retrosynthetic analysis of piperazic Acid (R)-77 and (S)-77.

2.2 Piperazic Acid Synthesis

Piperazic acid methyl ester (R)-77 (Scheme 15) was synthesized starting with benzylation 1-pentene-ol 81 followed by dihydroxylation with catalytic osmium tetroxide and N-methylmorpholine-N-oxide (NMO) to give diol 83. Selective protection of the primary alcohol with tert-butyldimethylsilyl chloride (TBSCI) and imidazole gave a racemic alcohol 80 that underwent kinetic resolution with lipase TL. Resolved acetate (R)-84 underwent hydrogenolysis in the presence of 10% palladium on carbon to give alcohol 85. Oxidation of 85 with Dess-Martin periodinane gave aldehyde 86, which in turn was reductively aminated with tert-butyl carbazate and sodium cyanoborohydride to give the corresponding hydrazine 87. Carboxybenzyl (Cbz) protection of 52 followed by transesterification using K₂CO₃/MeOH gave 78, which was the precursor for the intramolecular Mitsunobu reaction to give
At this point, straightforward conversion of the silyl ether 89 into a methyl ester by removal of the TBS protecting group with tetra-$n$-butylammonium fluoride (TBAF), followed by Jones oxidation and methylation with trimethylsilyldiazomethane gave 90. $N$-Boc deprotection with trifluoroacetic acid (TFA) gave the free amine $(R)$-77 that is ready for the first peptide coupling. The other enantiomer $(S)$-77 can be accessed in the same fashion after the $(S)$-80 alcohol is acylated.

**Scheme 15: Asymmetric synthesis of piperazic acid.**

2.3 Coupling of piperazic acid and L-Ala

After piperazic acid derivative 77 was synthesized, efforts toward the first coupling with L-alanine to give 56 (**Scheme 16**) were undertaken following standard peptide coupling conditions and literature precedents.
First, typical coupling conditions were explored (Table 3) using HOBr with EDCI, PyBOP and HOAt with DIC. These conditions did not give new products and starting material was fully recovered. Next, Mukaiyama’s reagent was tested using reflux and microwave conditions, which once again gave no product and complete decomposition respectively.

Looking to the literature it became evident that much more activated acids needed to be explored.\textsuperscript{16} Both acid chlorides and acid fluorides were tested, and in all cases no product was formed and starting material was recovered. It should also be noted that in all literature precedents the nitrogen on the coupling amino acid was either methylated or completely absent.\textsuperscript{17} Protecting the nitrogen as phthalamide was intended to mimic the \textit{N}-methylated-\textit{L}-alanine by making the nitrogen less nucleophilic. Unfortunately, even in the phthalamide case the reaction would not go to completion in addition to giving a complex mixture of products. After these failed attempts Fmoc-\textit{N}-methylated \textit{L}-alanine was tested to make sure that the coupling could be done using literature specific substrates. For the first time coupling of (\textit{R})-\textit{77} with \textit{L}-alanine worked using Mukaiyama reagent under reflux conditions. Unfortunately this strategy could not be utilized since lydiamycin A does not have \textit{N}-methylated \textit{L}-alanine moiety and there is no efficient strategy for the removal of methyl groups from amide nitrogens. It is still unclear to why is \textit{N}-methylated Fmoc-\textit{L}-alanine couples to the piperazic acid (\textit{R})-\textit{77} and the Fmoc-\textit{L}-alanine does not.
Scheme 16. Coupling of piperazic acid (R)-77 and L-alanine.

Table 3: Coupling conditions.

<table>
<thead>
<tr>
<th>R, R' R''</th>
<th>Coupling Conditions</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>-OH, -H, -Boc</td>
<td>HOBt, EDCI, Et$_3$N, DMF/DCM rt, 20 h</td>
<td>starting material</td>
</tr>
<tr>
<td></td>
<td>PyBOP, DIEA, DCM/MeCN rt, 20 h</td>
<td>starting material</td>
</tr>
<tr>
<td></td>
<td>HOAt, DIC, DCM rt, 20 h</td>
<td>starting material</td>
</tr>
<tr>
<td>-OH, -H, -Fmoc</td>
<td>Mukaiyama, DIEA, DCM reflux, 20 h</td>
<td>starting material</td>
</tr>
<tr>
<td></td>
<td>Mukaiyama, DIEA, DCM microwave, 100 °C, 15 min.</td>
<td>decomposition</td>
</tr>
<tr>
<td>-Cl, -H, -Fmoc</td>
<td>AgCN, PhH reflux, 20 h</td>
<td>starting material</td>
</tr>
<tr>
<td>-Cl, -H, -Fmoc</td>
<td>10% NaHCO$_3$(aq), DCM, 20 h</td>
<td>starting material</td>
</tr>
<tr>
<td>-F, -H, -Boc</td>
<td>DIEA, DCM rt, 20 h</td>
<td>starting material</td>
</tr>
<tr>
<td>-OH, -phthalimide</td>
<td>Mukaiyama, DIEA, DCM reflux, 20 h</td>
<td>decomposition</td>
</tr>
<tr>
<td>-OH, -Me, -Fmoc</td>
<td>Mukaiyama, DIEA, DCM reflux, 20 h</td>
<td>starting material consumed product formed</td>
</tr>
</tbody>
</table>

2.4 Other strategies to make piperazic acid L-Ala adduct

After a number of failed attempts at direct coupling of L-alanine and piperazic acid derivative 77, new strategies were considered (Scheme 17). One new approach involved a Mitsunobu reaction of 94, which has undergone peptide coupling prior to cyclization. Reductive amination of 86 with
allyloxycarbonyl (Alloc)\(^{18}\) protected hydrazine gave 92. N-Cbz protection followed by Alloc deprotection gave the free amine 93.\(^{19}\) After coupling 58 with N-Boc-L-alanine, the acetate group was removed giving the desired acyclic precursor 94.

**Scheme 17. Alternative route to piperazic acid and L-alanine adduct.**

Cyclization attempts using either tributylphosphine or triphenylphosphine in the presence of DEAD gave a new product, which was unstable to column chromatography. The isolated products appeared to be acyclic according to the NMR. Comparing isolated product to the cyclic analogs 90 and 62 (vide \textit{infra}) there were large discrepancies in the NMR data. The unidentified compound was also submitted for Mass Spec experiments, which gave incorrect mass (plus water molecule) for the cyclic compound. This analytical data led us to believe that this cyclization strategy was not giving the desired product.

### 2.5 Solving the Coupling Problem

Literature search gave us new ideas of how to solve our coupling problem. Piperazic acids occur in nature only as components of peptide
natural products, and in all known cases, they are found as N-2 acyl derivatives. Interestingly, N-2 acylation of a free piperazic acid is problematic. In 1979 Hassall observed that 95 reacts selectively at N-1 with various acylation agents, and that N-2 acylation of the resultant 96 is possible only with acid chlorides (Scheme 18).20

Scheme 18. Nucleophilicity of piperazic acids.

![Scheme 18]

In 1997 Ciufolini showed that dehydropiperazic acids with structure 98 are even further deactivated toward the N-2 acylation (Figure 4).21 The abnormal reluctance of piperazic acids to undergo N-2 acylation seems to be due to an electronic affect, not a steric/conformational problem.

![Figure 4]

Once the acid was reduced to the corresponding alcohol, compound 100 underwent facile N-2 acylation even with weak acylating reagents as 4-nitrophenyl esters in the presence of HOBT (Scheme 19). Also when 100 is reacted with Ac₂O/pyridine, only N-2 acylation occurs and there is no evidence of N-1 acylation and consequent enamide formation.
In analogous situation we were unable to perform the coupling on the ester 77 and in other attempts to cyclize the alternative acyclic precursor 94, so the coupling of the silyl ether 64 was tested (Scheme 20).

Scheme 20. Coupling of piperazic acid silyl ether derivative and L-Ala.

Thus the silyl ether 89 was treated with TMSOTf and 2,6-lutidine\textsuperscript{22} to remove the Boc group to give the free amine 64. To our delight coupling proceeded using Mukaiyama’s reagent and Fmoc-L-alanine and gave dipeptide 65 45-75% yield. The reaction time could be cut down to 8 hours if five instead of three equivalents of the coupling reagent and Fmoc-L-alanine were used.

2.6 Making the Acycle

Dipeptide 65, was treated with diethyl amine to remove Fmoc protecting group, and the crude material was then taken on to the next
coupling step with N-Boc-D-leucine to give tripeptide 63 (Scheme 21). At this point, the silyl group was removed with TBAF and the resultant alcohol was oxidized first with DMP to give the aldehyde 64 followed by Pinnick oxidation to give the acid 65. Esterification of the acid 103 with serine 106 gave the acyle 104. Trichloroethyl (Tce) acid protecting group was removed with Zn In AcOH and H₂O, and the Boc group was removed with TFA. Unfortunately all cyclization attempts were unsuccessful, resulting in decomposition or the Fmoc protecting group and no observed product formation.

Scheme 21. Attempts at macrocyclization.

2.7 Improving Synthesis

Since the Fmoc protection group in 105 seemed to fall off during the attempted cyclizations we decided to replaced it with the Cbz group
(Schemen 22). This strategy would also allow us to reduced number of deprotection steps to get to 110. During this time we also improved some steps to make the synthesis more efficent. Esterification of 103 using EDCI as acid activating agent proved to be problematic for this synthesis because the reaction was unreliable and not always reproducible, at times giving absolutely no product. EDCI was replaced with analogous less expensive DCC, the yields were only slightly improved from 15-25% to 25-40% but reaction always worked.

**Scheme 22. Final attempts at macrocyclization.**

The next challenge we encountered is the removal of the trichloroethyl (Tce) group in 107. We utilized standard conditions with Zn, AcOH, H2O, but once again the reaction gave deprotected product inconsistently. Different concentrations and zinc batches were tested, until it was discovered that only fresh Zn from a new Strem container gave full conversion in this reaction. Using TFA Boc group was removed and the crude material 108 was
subjected to different coupling conditions (Table 5). Unfortunately none of these conditions gave the desired macrocyclic core 110, instead result was decomposition or inseparable mixture of multiple unidentifiable products.

Table 5. Coupling conditions.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Time</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>HATU, DIEA</td>
<td>16 h, 1 day.</td>
<td>DCM</td>
</tr>
<tr>
<td>HATU, HOAt, DIEA</td>
<td>16 h, 1 day, 2 days, 3 days</td>
<td>DCM</td>
</tr>
<tr>
<td>HATU, HOAt, DIEA</td>
<td>16 h, 1 day, 2 days, 3 days</td>
<td>DMF</td>
</tr>
<tr>
<td>DCC, DMAP</td>
<td>16 h, 1 day, 2 days, 3 days</td>
<td>DCM</td>
</tr>
</tbody>
</table>

2.8 Future Investigations

Both macrolactamization and macrolactonization strategies should be further investigated (Scheme 23). Once the macrocycle 110 is complete coupling with 118 followed by global deprotection will give lydiamycin A (1).

Scheme 23. Macrolactonization and macrolactamization.

Also efforts are underway to make the side chain moieties (dehydropiperazic derivative 113 and 2-pentysuccinic acids 75). Professor Yutaka Aoyagi has already shown synthetic advances toward these compounds. Silyl ether 89 can be treated with Pd catalyst to remove Cbz group and give amine 111 which in turn can be oxidized to give
dehydropiperazic acid derivative 112 (Scheme 25). Sill ether 113 could be accessed from 112 by removal of Boc group using TMSOTf and 2,6-lutidine.

Scheme 25. Making dehydropiperazic acid precursor 113.

\[
\begin{align*}
\text{TBSO} & \quad \text{Boc} \\
\text{N} & \quad \text{N} \\
\text{Cbz} & \quad \text{Boc} \\
\text{89} & \quad \text{10\% Pd/C, H}_2 \\
\text{TBSO} & \quad \text{111} \\
\text{N} & \quad \text{TB} \\
\text{SO} & \quad \text{112} \\
\end{align*}
\]

2-pentylsuccinic acid derivative 116 can be obtained enantioselectively by using Evans alkylation (Scheme 26). Acylation of 114 will give 115 followed by stereoselective alkylation to provide 116 in with the desired stereochemistry.

Scheme 26. 2-pentylsuccinic acid and dehydropiperazic acid.

\[
\begin{align*}
\text{Me} & \quad \text{Me} \\
\text{HN} & \quad \text{HN} \\
\text{O} & \quad \text{O} \\
\text{114} & \quad 1. \text{n-BuLi} \\
\text{} & \quad 2. \text{octanoyl chloride} \\
\text{Me} & \quad \text{Me} \\
\text{HN} & \quad \text{HN} \\
\text{O} & \quad \text{O} \\
\text{115} & \quad \text{NaN(TMS)$_2$} \\
\text{} & \quad \text{BrCH$_2$CO$_2$allyl, -78 \degree C} \\
\text{Me} & \quad \text{Me} \\
\text{HN} & \quad \text{HN} \\
\text{O} & \quad \text{O} \\
\text{116} & \quad \text{TBSO} \\
\text{} & \quad \text{N} \\
\text{CO$_2$} & \quad \text{CO$_2$} \\
\text{117} & \quad \text{113} \\
\text{Me} & \quad \text{Me} \\
\text{Me} & \quad \text{Me} \\
\text{118} & \quad \text{TBSO} \\
\end{align*}
\]

After the removal of the auxiliary the acid 117 can be coupled to the dehydropiperazic moiety 113 to give the adduct 118. After TBS deprotection...
and oxidation can be converted to a coupling partner. This method would of course give us access to all four diastereomers of the acid.

2.9 Conclusion.

Three strategies of making piperazic acid and L-alanine adduct were tested. First, piperazic acid methyl ester was used in coupling with L-alanine, using variety of coupling reagents and activated acids. All the attempts proved to be futile and highlighted poor nucleophilicity of the nitrogen. Next, cyclization strategy was explored using L-alanine coupled precursor. Unfortunately, this attempt also did not give the desired product. Finally, the of the silyl ether was nucleophilic enough to give the desired coupled product. Using this approach we were able to access the advanced acyclic intermediate and unfortunately all efforts were futile in the contraction of the macrocyclic core. Future efforts will be devoted to the completion of the macrocyclic core of lydiamycin A and the synthesis of dehydropiperazic acid side chain moiety. There is also a great need to determine the correct stereochemistry of the natural lydiamycins since neither Ma nor Xe obtained structures that matched spectroscopically to the published isolation data. We hope that identifying the structure of lydiamycin A will give an access to a new structural class of anti-Mycobacterial agents. The ultimate future of this project lies in making analogs of lydiamycins with manipulation to the side chain. The goal is to
test dozens of analogs and improve drug potency against drug-resistant strains of *Mycobacterium tuberculosis*. 
CHAPTER 3

EXPERIMENTS

General Methods.

Unless otherwise specified, all materials were obtained from commercial sources and used without purification. Reactions required anhydrous conditions were performed under atmospheric pressure of argon using flame-dried glassware. Dichloromethane, tetrahydrofuran, methanol, dimethylsulfoxide, triethylamine, and acetonitrile were degassed with argon and dried through a solvent purification system (J.C. Meyer of Glass Contour). Flash chromatography was performed on Merck silica gel Kieselgel 60 (230-400 mesh) from EM science with the indicated solvent. $^1$H NMR and $^{13}$C NMR spectra were recorded on Varian 300 and 400 MHz spectrometers as indicated. Mass spectra were obtained at the Colorado State University CIF on a Fisons VG Autospec.
1-benzyl oxy-4-pentene (82).

Under Ar atmosphere NaH (60%, 10.4 g, 260 mmol) was added to dry THF (230 mL) and the suspension was cooled to 0 °C, followed by slow addition of 4-penten-1-ol (20 g, 24 mL, 233 mmol). The resulting mixture was warmed to room temperature and stirred for 2 h, followed by a second cooling to 0 °C and addition of BnBr (39.0 g, 27.3 mL, 230 mmol). The resulting mixture was stirred at room temperature for 20 h. The reaction was then diluted with EtOAc (300 mL) and poured into 500 mL of ice water. The layers were separated and the aqueous phase was extracted with Et₂O (200 mL X 2). All organic layers were then combined and dried over Na₂SO₄, filtered and concentrated to give a light yellow oil. The oil was purified using silica gel column chromatography (Hexanes: Et₂O, 19 : 1) to yield 1-benzyl oxy-4-pentene 82 (39.0 g, 96%) as a light colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 7.39-7.28 (m, 5H), 5.89-5.75 (m, 1H), 5.06-4.93 (m, 2H), 4.51 (s, 2H), 3.511-3.46 (t, J=6.5 Hz, 2H), 2.19-2.12 (m, 2H), 1.77-1.67 (m, 2H).
5-(benzyloxy)pentane-1,2-diol (83).

Under Ar atmosphere, 4% osmium tetroxide in H$_2$O (25 mL, 4 mmol) was added to a mixture of 1-benzyloxy-4-pentene 82 (37.0 g, 210 mmol), MeCN (153 mL), H$_2$O (77 mL), and N-methylmorpholine-N-oxide (54.0, 460 mmol) at 0 °C. After reaction mixture was stirred for 24 h at room temperature, sat. Na$_2$SO$_3$ (500 mL) was added to the mixture and stirred for 20 min. The mixture was then diluted with EtOAc (200 mL) and the layers were separated. Aqueous layer was then extracted with EtOAc (500 mL X 2). Combined organic layers were washed with 1 N HCl (100 mL X 3) and sat. NaCl (100 mL X 2), then dried with Na$_2$SO$_4$, filtered and concentrated to give light-brown oil. The crude material was then purified using silica gel column chromatography (CHCl$_3$ : MeOH = 10 : 1) to give diol 83 as light-yellow oil (44 g, quant. yield). $^1$H NMR (300 MHz, CDCl$_3$) δ 7.38-7.28 (m, 5H), 4.52 (s, 2H), 3.72-3.41 (m, 5H), 3.11 (br.s. 1H), 2.05 (br.s. 1H), 1.81-1.7 (m, 2H), 1.62-1.45 (m, 4H).
5-(benzyloxy)-1-((tert-butyldimethylsilyl)oxy)pentan-2-ol ((±)-80).

Under Ar atmosphere a mixture of diol 83 (44 g, 210 mmol), imidazole (29.0 g, 420 mmol), TBSCI (35 g, 230 mmol) and dry DMF (220 mL) was stirred at room temperature for 24 hours. The reaction mixture was then poured into ice-water (500 mL), diluted with EtOAc (200 mL) and separated. The aqueous phase was then extracted EtOAc (100 mL X 2). Combined organic fractions were then washed with sat. NaCl (100 mL X 3), dried with Na₂SO₄, filtered and concentrated to give a yellow oil. The crude material was purified using silica gel column chromatography (Hexanes: EtOAc = 9: 1 to 4: 1) to get ((±)-80 as clear oil (54 g, 80%). ^1H NMR (300 MHz, CDCl₃) δ 7.34-7.28 (m, 5H), 4.51 (s, 2H), 3.67-3.38 (m, 7H), 2.65 (d, J=3.3, 1H), 1.82-1.43 (m, 8H), 0.89 (s, 9H), 0.06 (s, 6H).
**Kinetic Resolution of 5-(benzyloxy)-1-((tert-butyldimethylsilyl)oxy)pentan-2-ol (±-80).**

Lipase TL (1.25 g) was added to a solution of (±)-80 (0.5 g, 1.54 mmol), vinyl acetate (2.58 g, 30 mmol), dry pyridine (0.64 g, 8.0 mmol) and dry hexanes (25 mL) at -5 °C and stirred under Ar atmosphere for 48 hours. Lipase TL was filtered off with the help of Celite 454. The filtrate was washed with cooled 1N HCl (30 mL X 3), sat. NaHCO₃ (30 mL X 3) and sat. NaCl (30 mL X 3), dried with Na₂SO₄, filtered, and concentrated to give crude oil. The mixture was purified using silica gel column chromatography (Hexanes: EtOAc = 8 : 1) to give (R)-84 (0.27 g, 47%, 98% ee) and (S)-80 (0.25 g, 50%, 90% ee) as colorless oils. (R)-84 ¹H NMR (300 MHz, CDCl₃) δ 7.35-7.30 (m, 5H), 4.92 (br s, 1H), 4.49 (s, 2H) 3.63 (d, J=5.1 MHz, 2H), 3.49-3.45 (m, 2H), 2.04 (s, 3H), 1.73-1.61 (br m, 5H), 0.87 (s, 9H), 0.03 (s, 6H). (S)-80 ¹H NMR (300 MHz, CDCl₃) δ 7.34-7.28 (m, 5H), 4.51 (s, 2H), 3.67-3.38 (m, 7H), 2.65 (d, J=3.3, 1H), 1.82-1.43 (m, 8H), 0.89 (s, 9H), 0.06 (s, 6H).
BnO

\((R)\) OTBS

\((R)\)-84
5-(benzyloxy)-1-(((tert-butyldimethylsilyl)oxy)pentan-2-yl-\((S)\)-acetate ((S)-79).

To a mixture of \((S)-80\) (5.0 g, 15.4 mmol), DMAP (0.19 g, 1.54 mmol), dry pyridine (1.82 g, 18.5 mmol) and dry DCM (100 mL), Ac\(_2\)O (1.89 g, 18.5 mmol) was added dropwise at 0 °C under Ar atmosphere. The reaction was stirred at room temperature for 14 hours, then poured into ice water (100 mL), diluted with EtOAc (100 mL) and separated layers. The aqueous phase was extracted with EtOAc (50 mL X 2). Combined organic phases were washed with sat. NaCl (100 mL X 3), dried with Na\(_2\)SO\(_4\), filtered and concentrated to give oil residue. The crude material was purified using silica gel column chromatography (Hexanes : EtOAc = 6 : 1) to give a clear oil (S)-79 (5 g, 92 %). NMR is identical to the racemic analog.
**(R)-1-((tert-butyldimethylsilyl)oxy)-5-hydroxypentan-2-yl acetate (85).**

In the presence of 10% Pd/C (0.16 g) a MeOH solution (60 mL) of 79 (1.6 g, 4.4 mmol) was stirred at room temperature for 16 hours under H₂ atmospheric pressure. It is very important to note that palladium catalyst had to be bread new and purchased from Strem, any other catalyst of the shelf would not give any product with up to 60% by weight catalyst loading, under increased pressure (75 psi) and over a prolonged time (3-4 days). The catalyst was then filtered off using Celite 545 and the filtrate was concentrated to give oily residue. The crude material was purified using silica gel column chromatography (Hexanes : EtOAc = 1 : 3) to give 85 (1.2, 95%) as a colorless oil. $^{1}$H NMR (300 MHz, CDCl₃) δ 4.95-4.88 (m,1H), 3.68-3.63 (m, 4H), 2.05 (s, 3H), 1.71-1.43 (br m, 6H), 0.87 (s, 9H), 0.04 (s, 6H).
DMP oxidation to give (R)-1-((tert-butyldimethylsilyl)oxy)-5-oxopentan-2-yl acetate (86).

To a solution of 85 (1.58 g, 5.7 mmol) in dry DCM (80 mL) was added Dess-Martin-periodinane (3.6 g, 8.6 mmol) in three portions at 0 °C. The reaction mixture was stirred at room temperature for 2 hours, followed by a quench with sat. Na₂S₂O₃ (40 mL) and sat. NaHCO₃ (40 mL). The reaction mixture was stirred for 30 minutes, diluted with EtOAc (80 mL) and then the layers were separated. The organic layer was washed with sat. NaCl (40 mL X 3), dried over Na₂SO₄, filtered and concentrated to give a light yellow oil. The crude mixture was purified using silica gel column chromatography (Hexanes : EtOAc = 5 : 1) to give 86 as a colorless oil (1.56, quantitative yield). ¹H NMR (300 MHz, CDCl₃) δ 9.76 (t, J=1.4 MHz, 1H), 4.89 (m, 1H), 3.65 (dd, J=2.1, 5.1, 2H), 2.50 (t, J=7.5, 2H), 2.04 (s, 3H), 1.55 (br s, 1H), 0.87 (s, 9H), 0.04 (s, 6H).
Swern oxidation to give (R)-1-((tert-butyldimethylsilyl)oxy)-5-oxopentan-2-yl acetate (86).

This procedure was designed to oxidize alcohol 85 to the corresponding aldehyde for large scale, because DMP became inefficient once the amount of alcohol reached 2-3 g.

Under Ar atmosphere, in a flame dried round bottom flask, dry DMC (320 mL) and (COCl)$_2$ (6.05 mL, 69.43 mmol) were combined and cooled to -78 °C. DMSO (9.85 mL, 138.84 mmol) was added to the reaction mixture and stirred for 10 minutes. Alcohol 85 (9.59 g, 34.71 mmol) was added to the reaction mixture in dry DCM (80 mL) and stirred for another 15 min. NEt$_3$ (19.35 mL, 138.84 mmol) was then added to the mixture and stirred for additional 10 minutes. The reaction mixture was then warmed to 0 °C and stirred for another 15 minutes. The reaction mixture was then diluted with H$_2$O (300 mL), layers separated, and the aqueous layer was then extracted with DCM (300 mL X 3). Combined organic layers were dried over Na$_2$SO$_4$, filtered and concentrated to give crude light yellow oil. The material was purified using silica gel column chromatography (Hexanes : EtOAc = 6 : 1) to give aldehyde 86 (8.9 g, 93%). All the spectra matched the DMP oxidation procedure.
(R)-tert-butyl 2-(4-acetoxy-5-((tert-butyldimethylsilyl)oxy)pentyl) hydrazinecarboxylate (87).

To a solution of tert-butylcarbazte (0.97 g, 7.3 mmol) in dry MeOH (10 mL) was added 86 (1.0 g, 3.7 mmol) in dry MeOH (8 mL) was added under Ar atmosphere. The reaction mixture was stirred at room temperature for 1 hour and then cooled to 0 °C. AcOH (0.36 mL, 6.3 mmol) was added and the mixture was stirred for 10 minutes NaBH₃CN (1.3 g, 21.0 mmol) was then added and the mixture was stirred at room temperature for 1 hour. The solvents were then evaporated and the yellow oil was diluted with EtOAc (50 mL) and quenched with phosphate buffer (pH 7.2, 50 mL). The layers were then separated and the aqueous layer was then extracted with EtOAc (30 mL X 2). Combined organic layers were then washed with sat. NaCl (30 mL X 3), dried over Na₂SO₄, filtered and concentrated to give a viscous yellow oil. The crude product was then purified using silica gel chromatography (Hexanes : EtOAc = 95 : 5, 1 : 1) to give 87 (0.96 g, 67%) as a colorless viscous oil. ¹H NMR (300 MHz, CDCl₃) δ 6.14 (br s, 1H), 4.90 (m, 1H), 3.64 (d, J=5.1, 2H), 2.83 (t, J=6.9, 2H), 2.05 (s, 3H), 1.70-1.55 (br m, 2H), 1.45 (s, 9H), 0.87 (s, 9H), 0.03 (s, 6H).
(R)-1-benzyl 2-tert-butyl 1-(4-acetoxy-5-((tert-butyldimethylsilyl)oxy)pentyl)hydrazine-1,2-dicarboxylate (88).

To a mixture of 87 (6.3 g, 16.5 mmol), DCM (25 mL), and 1 N NaHCO$_3$ (25 mL) was added CbzCl (3.5 g, 21 mmol) dropwise at 0 °C. After the reaction mixture was stirred vigorously at 0 °C for 1.5 hours, the organic layer was separated. The aqueous layer was extracted with DCM (30 mL X 3). The combined organic layers were washed with sat. NaCl (30 mL X 3), dried over Na$_2$SO$_4$, filtered and concentrated. The crude material was purified using silica gel column chromatography (Hexanes : EtOAc = 5 : 1) to give 88 (8.2 g, 97 %) as a viscous clear oil. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.33 (br s, 5H), 6.42 (br s, 1H), 5.13 (br s, 1H), 4.89 (br s, 1H), 3.52 (br m, 4H), 2.04 (s, 3H), 1.62 (br d, 4H), 1.40 (br m, 9H), 0.87 (s, 9H), 0.03 (s, 6H).
(R)-1-benzyl 2-tert-butyl 1-(5-((tert-butyldimethylsilyl)oxy)-4-hydroxypentyl)hydrazine-1,2-dicarboxylate (78).

To a mixture of 88 (1.0 g, 1.9 mmol), MeOH (22 mL), and H₂O (11 mL) was added powdered K₂CO₃ (4.0 g, 28.6 mmol) at room temperature. After the reaction mixture was stirred at room temperature for 6 hours EtOAc (100 mL) was added and the layers separated. The organic layer was washed with sat. NaCl (50 mL X 3), dried over Na₂SO₄, filtered and concentrated. The crude material was purified using silica gel column chromatography (Hexanes : EtOAc = 4 : 1) to give 78 (0.67 g, 73 %) as a clear oil. ¹H NMR (300 MHz, CDCl₃) δ 7.33 (br s, 5H), 6.47 (br s, 1H), 5.14 (br s, 2H), 3.55 (br m, 4H), 3.35 (br s, 1H), 1.84-1.58 (br m, 11H), 0.89 (s, 9H), 0.06 (s, 6H).
(R)-1-benzyl 2-tert-butyl 3-(((tert-butyldimethylsilyl)oxy)methyl) tetrahydropyridazine-1,2-dicarboxylate (89).

To a THF (330 mL) solution of 78 (0.8 g, 1.66 mmol) and PPh$_3$ (1.70 g, 6.64 mmol) was added DEAD (1 M solution of Toluene, 5.0 mmol) at reflux and under Ar atmosphere. The reaction mixture was refluxed for 0.5 h. After the solvents were evaporated, the oily residue was purified using silica gel column chromatography (Hexanes : EtOAc = 99 : 1 (to remove PPh$_3$), 3 : 1) to give 89 (0.67 g, 86%) as a clear oil. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.34 (br m, 5H), 5.12 (d, $J$=5.7 MHz, 1H), 4.31 (br s, 0.7H), 4.1 (qt, $J$=6.9, 14.1, 3H), 3.7 (br m, 1H), 3.56 (qt, $J$=9.8, 18.6, 1H), 2.04 (s, 3H), 1.60-1.30 (br m, 13H), 0.86-0.84 (m, 9H), 0.06-0.01 (m, 6H).
(R)-1-benzyl 2-tert-butyl 3-methyl tetrahydropyridazine-1,2,3-tricarboxylate (90).

To a solution of 89 (0.20 g, 0.43 mmol) in dry THF (8.6 mL) was added 1.0 M TBAF in THF (0.9 mL, 0.9 mmol) at room temperature under an Ar atmosphere. The reaction mixture was stirred at the same temperature for 10 minutes and then diluted with EtOAc (30 mL). The organic solvent was washed with NaCl (20 mL X 3), dried over Na$_2$SO$_4$, filtered and concentrated. The crude oily residue was purified using silica gel column chromatography (Hexanes : EtOAc = 1 : 1) to give the correspond alcohol as colorless oil (0.18 g, quantitative yield). To a solution of the corresponding alcohol (0.18 g, 0.43 mmol) in acetone (9 mL) was added Jones reagent (0.7 mL, 1.8 mmol), prepared from CrO$_3$ (2.67 g), sulfuric acid (2.3 mL), and H$_2$O (5.8 mL). The mixture was stirred at room temperature for 1 hour then quenched with iPrOH (3 mL), and the green insoluble solids were removed by filtration through Celite 545. The filtrate was diluted with EtOAc (30 mL) and washed with sat. NaCl (20 mL X 3), dried over Na$_2$SO$_4$, filtered and concentrated to give crude corresponding acid (0.2 g), which was used in the next step without further purification. To a solution of crude acid (0.2 g) in dry MeOH (4 mL) was added 2.0 M TMSCHN$_2$ in hexanes (0.4 mL, 0.8 mL) at room temperature.
After 30 minutes the reaction was then concentrated to give an oily residue, which was purified using silica gel chromatography (Hexanes : EtOAc = 3 : 1) to give 90 (0.13 g, 81% over 3 steps). All proton and carbon NMR matched published material.
(R)-1-benzyl 3-methyl tetrahydropyridazine-1,3(2H)-dicarboxylate ((R)-77).

To a solution of 90 (0.13 g, 0.35 mmol) in DCM (1.2 mL) was added TFA (0.6 mL) at 0 °C. After the reaction mixture had been stirred at the same temperature for 3 hours, the solvents were evaporated to give an oily residue. The concentrate was diluted with EtOAc (50 mL) and washed with sat. NaHCO₃ (20 mL X 3) and sat. NaCl (20 mL X 3), dried over Na₂SO₄, filtered and concentrated to give an oily residue. The crude material was purified using silica gel column chromatography (Hexanes : EtOAc = 2 : 1) to give (R)-77 (0.08 g, 84 %) as a colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 7.35 (m, 5H), 5.18 (s, 2H), 4.05 (br d, J=14.1, 1H), 3.72 (s, 3H), 3.55 (m, 1H), 3.13 (m, 1H), 2.04 (br m 1H), 1.78-1.59 (br m, 2H), 1.56 (s, 1H).
(R)-benzyl 3-((tert-butyldimethylsilyl)oxy)methyl)tetrahydropyridazine-1 (2H)-carboxylate (64).

To a solution of 89 (2.7 g, 5.8 mmol) in DCM (18 mL) under Ar atmosphere was added 2,6-lutidine (1.6 mL, 11.6 mmol) at 0 °C. The reaction mixture was then cooled to -78 °C and fuming TMSOTf (1.6 mL, 8.72 mmol) was added dropwise. The reaction mixture was then warmed to room temperature over an hour and quenched with sat. NH₄Cl (20 mL). The aqueous layer was extracted with Et₂O (20 mL X 3). Combined organic layers were washed with sat. NaCl (80 mL X 3), dried over Na₂SO₄, filtered and concentrated. The crude material was purified column chromatography (Hexanes : EtOAc = 6 : 1) to give 64 (2.1 g, quantitative yield) as a clear oil. ¹H NMR (300 MHz, CDCl₃) δ 7.36-7.30 (m, 5H), 5.17 (s, 2H), 3.64 (dd, J=3.6, 9.9 MHz, 1H), 3.45 (m, 1H), 3.00 (br m, 1H), 2.89 (br m, 1H), 1.71-1.5 (br m, 4H), 1.3-1.28 (br m, 1H), 0.87 (s, 9H), 0.03 (s, 6H).
(R)-benzyl 2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoyl)-3-(((tert-butyl(dimethyl)silyl)oxy)methyl)tetrahydropyridazine-1(2H)-carboxylate (65).

Under Ar atmosphere Fmoc-L-ala (5.25 g, 20.57 mmol) and Mukiyama reagent (5.25 g, 20.57 mmol) were dissolved in dry DMC (55 ml). DIEA (3.6 mL, 20.57 mmol) was added to the reaction mixture and stirred for 10 minutes. Dissolved in dry DCM (12 mL) 64 (1.5 g, 4.11 mmol) was added to the reaction mixture and refluxed for 6 hours. The reaction was then cooled to room temperature and quenched with sat. NaHCO₃ (70 mL). The layers were separated and the organic layer was washed with sat. NaHCO₃ (70 mL X 2) and sat. NaCl (70 mL X 1), dried over Na₂SO₄, filtered and concentrated. The crude oil was purified using silica gel chromatography (Hexanes : EtOAc = 9 : 1, 2 : 1) to give 65 (2.12 g, 75 %) as a white crispy foam. ¹H NMR (300 MHz, CDCl₃) δ 7.79 (d, J=7.1 MHz, 2H), 7.63 (d, J=6.9, 2H) 7.42-7.29 (m, 6H), 5.90 (br s, 0.2H), 5.73-5.58 (m, 0.6H), 5.21-5.02 (m, 2H), 4.71 (m, 0.6H), 4.57 (br s, 1H), 4.37-4.32 (br m, 2H), 4.22-4.15 ( br m, 1H), 4.13-4.04 (br m, 1H), 3.50 (br m, 0.6H), 3.41 (t, J=9.9 MHz, 1H), 2.99 (br m, 1H), 2.04 (s, 1H), 1.73-1.66 (br m, 1H), 1.63-1.58 (br m, 0.5H), 1.53-1.49 (br m, 1H), 1.42-1.23 (br m, 4H), 0.83 (br s, 9H), 0.086-0.057 (br m, 6H).
$^{13}$C NMR (75 MHz, CDCl$_3$) δ 5.35, 18.38, 19.90, 22.71, 22.74, 26.03, 30.86, 46.76, 47.37, 52.08, 60.07, 60.23, 64.58, 67.07, 69.10, 120.17, 125.47, 127.27, 127.39, 128.80, 141.49, 144.15.
To a solution of 65 (0.59 g, 0.867 mmol) in dry DCM (30 mL) under Ar atmosphere was added NHEt₂ (3 mL) dropwise. The reaction mixture was stirred at room temperature for 30 min and then concentrate in vacuo. The crude material was then took onto the next coupling step. Deprotected adduct (0.402 g, 0.867 mmol), N-Boc-d-lue (0.40 g, 1.73 mmol) and PyBoP (0.902 g, 1.734 mmol) were dissolved in dry DMF (12 mL). After the mixture was stirred for 10 minutes, DIEA (0.3 mL, 1.73 mmol) was added. After the reaction mixture was stirred for 5 hours, it was diluted with EtOAc (70 mL), washed with sat. NaHCO₃ (50 mL X 2), sat. NaCl (50 mL X 3), dried over Na₂SO₄, filtered and concentrated in vacuo to give an oily foam. The crude material was purified using silica gel column chromatography (Hexanes : EtOAc = 19 : 1, 2: 1) to give 101 (0.47 g, 83%) as white crispy foam. 

³¹H NMR (400 MHz, CDCl₃) δ 7.30 (br m, 5H), 6.40 (br m, 1H), 5.24-5.06 (br m, 2H), 4.78 (br m, 1H), 4.33-3.90 (br m, 3H), 3.70-3.27 (br m, 3H), 1.81 (br m, 2H), 1.62 (s, 3H), 1.41 (s, 12 H), 1.24 (dd, J=6.8, 58, 2H), 0.91 (d, J=6, 6H), 0.84-
0.81 (m, 9H), 0.038-0.66 (m, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 5.42, 5.28, 17.44, 17.68, 18.25, 18.45, 19.24, 22.10, 22.61, 23.22, 24.84, 25.95, 28.47, 41.75, 44.75, 46.22, 47.39, 52.57, 53.17, 60.91, 68.53, 79.58, 128.18, 128.56, 128.79, 135.18, 135.98, 155.75, 156.55, 172.80, 175.47.
To a solution of 101 (0.72 g, 1.105 mmol) in dry THF (11 mL) under Ar atmosphere was added 1 M TBAF in THF (2.2 mL, 2.2 mmol). The reaction was stirred for 1 hour and then diluted with EtOAc (15 mL) and washed with sat. NaCl (25 mL X 3), dried over Na₂SO₄, filtered and concentrated. The crude foam was purified using silica gel column chromatography to give corresponding alcohol (0.47 g, 80%). To a solution of alcohol (0.45 g, 0.843 mmol) in DCM (9 mL), DMP (0.54 g, 1.26 mmol) was added. The reaction mixture was stirred at room temperature for 3 hours, followed by a quench with sat. Na₂S₂O₃ (10 mL) and sat. NaHCO₃ (10 mL). The reaction mixture was stirred for 30 minutes, diluted with EtOAc (40 mL) and then the layers were separated. The organic layer was washed with sat. NaCl (40 mL X 3), dried over Na₂SO₄, filtered and concentrated. The crude material was put through a short silica gel plug (Pet. Ether : EtOAc = 3 : 2) to give the corresponding aldehyde (0.34 g, 76%) as acrispy foam. The aldehyde was dissolved in MeCN (10 mL) and cooled to 0 °C, followed by the addition of 2-
methyl-2-butene. An aueous solution (10 mL) of NaClO₂ (0.519 g, 5.74 mmol) and NaH₂PO₄ (0.731 g, 5.74 mmol) was added portion wise over 15 minutes. After 20 minutes the reaction mixture was diluted and extracted with DCM (30 mL X 3). Combined organic fractions were washed with 0.5 M HCl (90 mL X 3), sat. NaCl (90 mL X 2), dried over Na₂SO₄, filtered and concentrated. The crude material was then purified using silica gel column chromatography (CHCl₃ : MeOH : AcOH = 97.5 : 2.5 : 0.02) to give 103 (0.31 g, 90%) as a white crispy foam. $^{13}$C (75 MHz, CDCl₃) d-17.15, 22.03, 23.14, 24.87, 28.45, 41.38, 44.79, 53.11, 68.97, 77.03, 77.45, 77.65, 77.88, 80.16, 81.46, 128.71, 135.39, 156.63, 172.33, 173.61, 176.22.
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(S)-1-benzyl 3-((S)-2-(((benzyloxy)carbonyl)amino)-3-oxo-3-(2,2,2-trichloroethoxy)propyl) 2-((S)-2-((R)-2-((tert-butoxycarbonyl)amino)-4-methylpentanamido)propanoyl)tetrahydropyridazine-1,3(2H)-dicarboxylate (107).

To a solution of 103 (0.030 g, 0.54 mmol) in dry DCM (1.5 mL), N-Cbz-ser (0.24 g, 0.064 mmol), and DMAP (0.003 g, 0.027 mmol) were added. The dissolved in dry DCM (0.5 mL) DCC (0.013 g, 0.064) was added dropwise at 0 °C. The reaction was then warmed to room temperature and stirred for 16 hours. The reaction was then cooled again to 0 °C, urea was filtered, and the filtrated was concentrated. The crude material was purified using silica gel column chromatography (CH$_3$Cl : MeOH = 95 : 5) to give 107 (0.041g, 95%) as a crispy white foam. $^1$H (300 MHz, CDCl$_2$) δ 7.32 (br m, 10H) 6.52 (br s, 1H) 5.28 (br m, 1H), 5.16 (br m, 2H), 5.16 (br s, 1H), 4.76 (br m, 6H), 4.29 (br m, 1H), 4.11 (br m, 2H), 3.47 (m, 2H), 1.94 (br m, 6H), 1.63 (br m, 6H), 1.44 (br s, 9H), 1.39-1.0 (br m, 12H), 0.92 (d, $J$=5.1, 8H).
To a solution of 107 (0.003 g, 0.004 mmol) in 90% AcOH/H$_2$O (0.3 mL), Zinc dust (0.03g, 0.411 mmol) was added. After the reaction mixture was stirred for 16 hours, Zn was filtered off using cotton and the crude material was azeotroped with Toluene to give a light oil. The mixture was purified using silica gel column chromatography (CHCl$_3$ : MeOH : AcOH = 97.5 : 2.5 : 0.1 mL) giving the corresponding acid (0.003 g). The acid was dissolved in DCM (0.3 mL) and TFA (30 µL) was added to the mixture. After the reaction was stirred for 3 hours, it was concentrated in vacuo and taken on crude onto macrocyclization. Unfortunately, that was where the progress ended. We were never able to observe any cyclization product.


