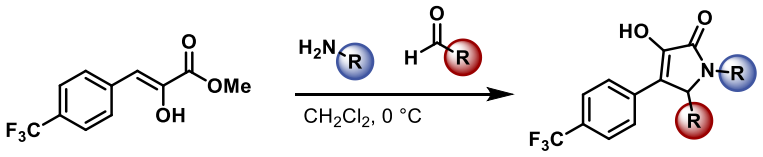
ABSTRACT

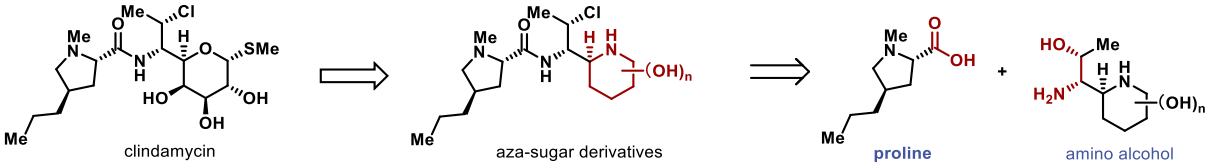
NIE, MINHUA. Design and Synthesis of Novel Aza-sugar Clindamycin Antibiotics (Under the direction of Dr. Joshua G. Pierce).

Chapter 1: Herein we report a new library of 2,3-pyrrolidinedione analogues that expands on our previous report on the antimicrobial studies of this heterocyclic scaffold. The novel 2,3-pyrrolidinediones reported herein have been evaluated against methicillin-resistant *S. aureus* (MRSA) and *Staphylococcus aureus* (MSSA) biofilms, and this work constitutes our first report about the antibiofilm properties of this class of compounds. The antibiofilm activity of these 2,3-pyrrolidinediones has been assessed through minimum biofilm eradication concentration (MBEC) and minimum biofilm inhibition concentration (MBIC) assays. The compounds displayed antibiofilm properties and represent intriguing scaffolds for further optimization and development.



Chapter 2: Lincomycin is an antimicrobial natural product isolated from *Streptomyces lincolnensis* and clindamycin results from the chemical modification of lincomycin. Clindamycin exhibits improved antibacterial activities and pharmacokinetics; however, it's not effective against resistant bacteria possessing the *erm* gene and *crf* gene. Due to the increase in drug resistance, developing new clindamycin derivatives with improved potency and a broad antibacterial spectrum of activity becomes critical. Many groups have focused on modifications at the proline side chain and the C_7 position. However, deep-seated analogs such as aza-sugar clindamyicn derivatives have not been explored until now. Since aza-sugars possess many different biological properties, we plan to synthesize aza-sugar clindamycin derivatives and explore the structure-

activity relationships of lincosamide antibiotics. Based on the SAR study and computer-aided molecular design, we report the model study towards the monohydroxylated aza-sugar clindamycin derivative and progress toward the trihydroxylated aza-sugar clindamycin derivative.



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Design and Synthesis of Noval Aza-sugar Clindamycin Antibiotics

by

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BIOGRAPHY

Minhua Nie was born and raised in Henan province, China. She received her Bachelor of Pharmaceutical Engineering and Master of Pharmacy from Shenyang Pharmaceutical University, performing research in Prof. Ping Gong's lab, where she focused on the synthesis of small molecules as c-Met Kinase inhibitors and process study of Apitolisib as PI3K/mTOR dual inhibitor. Afterwards, she did a one-year research in Shanghai Institute of Materia Medica in Prof. Hong Liu's group, where she mainly focused on the design and synthesis of mGluR2 PAMs and fluorescent molecular probes of antianxiety agents. Then she moved to the United States and pursued her PhD in organic chemistry. Under the guidance of Prof. Joshua G. Pierce and co-workers, she has strengthened her skills in organic synthesis and gained knowledge in chemical biology.

ACKNOWLEDGMENTS

Prof. Pierce

Thank you for giving me the opportunity to join your group! I think I would not have survived the past six years without your generosity and support. I'm grateful for your guidance and inspiration and I will never forget the experience here being a member of the Pierce group family. Lastly, thanks for helping me find a postdoc position.

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Thank you for being on my committee and for your helpful advice for the prelim and final defense. I also benefited a lot from the course you taught in the past six years.

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LIST OF ABBREVIATIONS

AMR: Antimicrobial resistance

AR: Antibiotic resistance

CDC: Centers for disease control and prevention

GBS: Group B *Streptococcus*

LCM: Lincomycin

MTL: Methylthiolincosamide

PPL: *N*-methyl-4-propyl-L-proline

CLDM: Clindamycin

MRSA: Methicillin-resistant *Staphylococcus aureus*

PTC: Peptidyl transferase center:

A: Adenine

G: Guanine

C: Cytosine

U: Uracil

erm: *Erythromycin ribosome methylase*

cfr: Chloramphenicol-florfenicol resistance

rRNA: Ribosomal ribonucleic acid

GDP: Guanosine diphosphate

L-DOPA: L-3,4-dihydroxyphenylalanine

VPCA: 3-Vinyl-2,3-pyrroline-5-carboxylic acid

PCP: Peptidyl carrier protein

MSH: Mycothiol

EGT: Ergothioneine

SAR: Structure-activity relationship

QSAR: Quantitative structure-activity relationship

MIC: Minimum inhibitory concentration

PDB: Protein data bank

SP: Standard precision

XP: Extra precision

RNA: Ribonucleic acid

Boc: *tert*-butoxycarbonyl

DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene

M: Molar

LCMS: Liquid chromatography mass

PMB: *N*-4-methoxybenzyl

DMF: *N,N*-Dimethylformamide

NMR: Nuclear magnetic resonance

dr: Diastereomer ratio

ee: Enantiomeric excess

RCM: Ring closing metathesis

THF: Tetrahydrofuran

Cbz: Benzyloxycarbonyl

Ac_2O: Acetic anhydride

SM: Starting material

SAM: *S*-Adenosylmethionine

GTP: Guanosine triphosphate

SAH: S-Adenosylhomocysteine

HG-II: Hoveyda-Grubbs 2nd generation

MIC: Minimum inhibitory concentration

TA: Tethered aminohydroxylation

E. coli: *Escherichia coli*

Chapter 1: Expanded Library of Novel 2,3-Pyrrolidinedione Analogues Exhibit Gram-positive Anti-biofilm Activity

Portions of this Chapter are reprinted with permission from, Minhua Nie†, M. Alejandro Valdes-Pena†, Bram H. Frohock, Emma Smitts, Jennifer C. Daiker, Jessica M. Gilbertie, Lauren V. Schnabel, and Joshua G. Pierce* Expanded Library of Novel 2,3-Pyrrolidinedione Analogues Exhibit Gram-positive Anti-biofilm Activity. *Bioorg. & Med. Chem. Lett.* **2024**, *99*, 129609.

1.1 Background

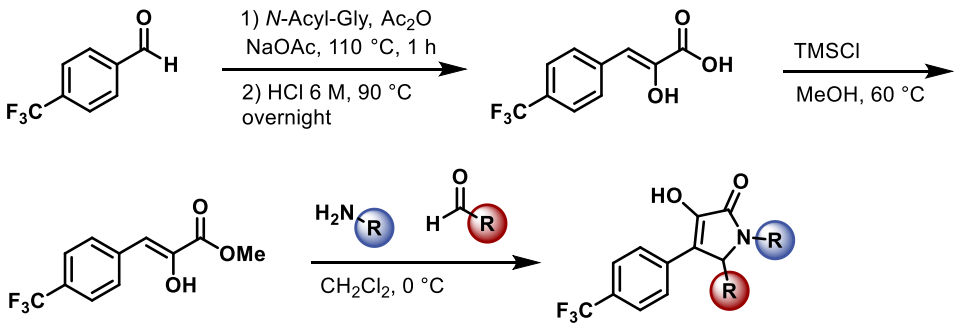
Since the introduction in 1937 of the first effective antimicrobials, the sulfonamides, the development of specific mechanisms of resistance has plagued their therapeutic use. The misuse and overuse of antimicrobials in humans, animals and plants are the main drivers in the development of drug-resistant pathogens. Antimicrobial resistance (AMR) is a growing public health threat[1] that has been made worse by the lack of novel antimicrobial agents and novel antimicrobial classes[2], and also by the rise in antimicrobial-resistant infections[3]. AMR claimed more than 35,000 lives in the U.S.[4] and caused around 4.95 million deaths worldwide in 2019[3]. Besides, AMR has significant economic costs. In addition to resistance, antimicrobial tolerance due to biofilms is a growing issue in the clinical treatment of bacterial infections[5–8]. Novel classes of antimicrobials are thus in dire need, and we have built a number of programs focused on the synthesis and antimicrobial evaluation of novel heterocyclic natural products and their analogues[9–12]. In particular, the antimicrobial activity of the 2,3-pyrrolidinedione heterocycle has been of particular interest. This motif is present in terrestrial and marine natural products such as leopolic acid A[13,14], phenopyrrozyn[15] and *p*-hydroxyphenopyrrozin[16], which have been already reported as antimicrobial agents[17,18].

1.2 Synthesis of Novel 2,3-Pyrrolidinedione Analogues and Biological Evaluation

Our group has previously reported the synthesis of a library of novel 2,3-pyrrolidinediones, which displayed promising antimicrobial activity (2 – 8 μg/mL) against several strains of *S. aureus* and *S. epidermis sp*[19–21]. As a follow-up to this broader body of work we looked to expand the library of 2,3-pyrrolidinedione analogues of the most active compound previously reported and additionally explore the antibiofilm properties of this class of small molecules.

Herein we report the synthesis of 15 novel 2,3-pyrrolidinedione analogues with the goal of building on compounds in which the 4-position of the 2,3-pyrrolidinedione core is substituted with an electron-deficient aryl group, with the *p*-trifluoromethyl phenyl substituent providing optimal antimicrobial activity in previous efforts[19]. Herein we report the synthesis of 15 novel 2,3-pyrrolidinedione analogues bearing different *N*-substituted and 5-substituted 4-trifluoromethylphenyl- 2,3-pyrrolidinedione substituents.

For the synthesis of this new library of compounds, we used a versatile multicomponent reaction that we developed previously in our lab, which allows one-pot access to the target 2,3-pyrrolidinedione analogues when employing a phenyl pyruvic methyl ester derivative, an aldehyde and amine (**Scheme 1.1**).



Scheme 1.1. Multicomponent reaction used for the 2,3-pyrrolidiendione analogue synthesis.

We initially examined the effects of different substituents in the 5-position of the 2,3-pyrrolidinedione core, maintaining the N-phenyl substituent present in the lead compound (**1**) we reported in our prior publication[19]. We limited our query to small 5-alkyl substituents and encountered very little tolerance for modifications in this position. As previously reported, the 5-ethyl analogue **1** displayed the best activity, while the 5-methyl analogue **2** retained modest levels of antimicrobial activity; however, the 5-unsubstituted analogue **3** or the 5-isopropyl analogue **4** resulted in almost complete loss of the antimicrobial activity of these scaffolds (**Figure 1.1**).

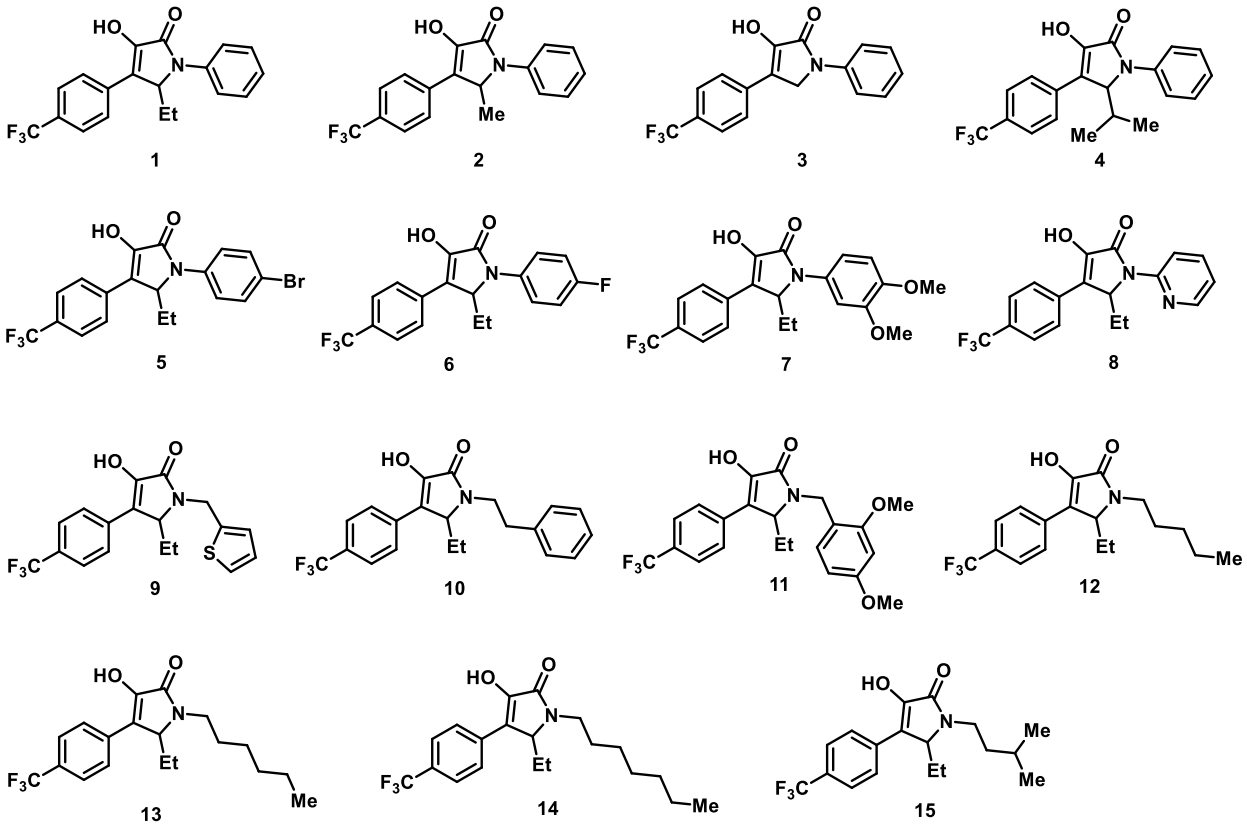


Figure 1.1. 2,3-Pyrrolidinedione analogues synthetized.

To further assess the antimicrobial activity of the 2,3-pyrrolidinediones, we performed studies on *S. aureus* bacterial biofilms. We assessed the antibiofilm properties through two different assays: Minimum Biofilm Inhibition Concentration (MBIC) and Minimum Biofilm Eradication

Concentration (MBEC). With the MBIC we measured the inhibitory effect that different concentrations (5 and 40 μM) of our 2,3- pyrrolidinediones would have on these biofilms, while the MBEC experiment informs at which concentration the 2,3- pyrrolidinediones would eliminate preformed biofilms. Although analogues **1** and **2** displayed modest antimicrobial activity, they displayed remarkable biofilm inhibition properties at 40 μM (**Table 1.1**). A number of compound classes have been demonstrated to possess such biofilm inhibition properties without complete killing[22–29], and we have also validated that bacteria are still viable upon treatment in the MBIC assay. In the more robust MBEC assays looking at the treatment of preformed biofilms very little activity was observed for **1** and **2**. Compounds 3 and 4 were inactive for both antibiofilm properties, even though 4 itself had an MIC of 16 μg/mL against the biofilm forming strain, thereby highlighting the disconnect between MIC and MBIC. Considering the most promising activity was found for analog 1, we continued to explore the effects of the *N*-substitution while retaining the 5-ethyl substituent on the heterocycle.

Table 1.1. Biological data.

Microbiology assay / Compounds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MIC[a] (MSSA ATCC 25923) μg/mL	16	16	>256	16	32	16	64	32	32	64	>256	32	32	32	64
MIC[b] (MRSA ATCC 33591) μg/mL	16	32	>128	>128	32	16	>128	>128	>128	>128	>128	16	16	64	>128
% Biofilm Inhibition[c] (5 μM)	5	-26	26	-5	34	22	17	4	54	23	63	58	59	64	47
% Biofilm Inhibition[c] (40 μM)	85	72	8	3	72	81	70	31	79	45	57	63	85	59	43
MBEC[d] (MSSA ATCC 25923) μg/mL	128	256	512	512	128	128	>512	128	128	256	>512	256	512	>512	>512

(a) MIC data was collected using cation adjusted MHBII adjusted to pH 9.0 for the media. (b) MIC data was collected using MHB for the media with no pH adjustments. (c) Biofilm inhibition data was collected using MRSA (ATCC BAA-44). (d) MBEC data was collected using MHB instead of PBS and the pH was adjusted to 9.0 to increase solubility of the analogs.

Further functionalization of the *N*-phenyl moiety with electron-withdrawing groups such as -Br and -F (compounds **5** and **6**) caused retention of the antimicrobial and antibiofilm activity, while the presence of a π-deficient heterocycle (**8**) eliminated the antimicrobial and antibiofilm activity completely.

It was unexpected that the presence of electron donating groups on the *N*-phenyl moiety (**7**) retained most of the biofilm inhibition properties at 40 μM while negatively affecting the antimicrobial activity and the antibiofilm properties. The presence of other aromatic groups linked to the heterocyclic core through one or two methylene groups resulted in reduction of antibiofilm properties (compounds **9**, **10** and, **11**), with the thiophene analog displaying relevant biofilm inhibition properties at 40 μM.

Medium chain *N*-alkyl substituted 2,3-pyrrolidinediones resulted in activity, with antimicrobial activities comparable to lead compound 1 for *N*-pentyl (**12**) and *N*-hexyl (**13**) compounds. Compounds **12** and **13** also displayed promising biofilm inhibition properties, even at a low 5 μM concentration. This bioactivity was also found for the *N*-heptyl analogue **14**, although the antimicrobial activity was diminished with this analogue. Branched-chain alkyl chains seemed to affect the activity as the *N*-isopentyl analog **15** displayed a decrease in all activity compared with the *N*-pentyl analog **12.**

Among all of the analogues synthesized, compounds **1**, **6,** and **13** were the ones that displayed more than 80% biofilm inhibition at 40 μM while displaying modest planktonic antibacterial potency (MIC ~ 16 μg/mL). These antibiofilm properties indicate that these compounds have the potential to impact biofilm formation and may not have lethal effects at effective concentrations. This is particularly relevant when developing adjuvants for antibiotic therapies that have already lost effectiveness due to the antibiotic tolerance. Considering that biofilm-forming ability of

bacterial pathogens is among the risk factors involved in antibiotic resistance generation[30–32], antibiofilm agents may play a key role in developing effective antimicrobial treatment strategies going forward.

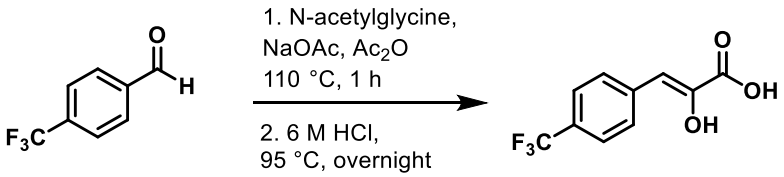
In conclusion, this work has identified *N*-aryl, *N*-p-fluoro-aryl and, *N*-pentyl analogues as the most promising compounds due to their antimicrobial and antibiofilm properties. Further studies are needed to improve the antibiofilm activity of these compounds, alternatively explore paths to enhance the planktonic antibacterial properties and biofilm eradication properties of these scaffolds, and ultimately uncover the mechanism of action of this family of compounds.

1.3 Experimental Details

Synthesis: Dichloromethane (DCM) was purified using an alumina filtration system before use. Aldehydes were purchased from a commercial chemical company and used as received unless otherwise noted. Test reactions were monitored by TLC analysis (pre-coated silica gel 60 F_{254} plates, 250 mm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a $KMnO_4$ solution (1.5 g of $KMnO_4$, 10 g of K_2CO_3, and 1.25 mL of a 10% NaOH solution in 200 mL of water). Test reactions were also monitored by LC-MS (2.6 mm C18 50 x 2.10 mm column) using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). A Biotage® flash chromatography system was used to purify all of the compounds. Melting points were determined using a DigiMelt apparatus. Infrared spectra were determined on a Bruker Alpha spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a 500, or 600 MHz instrument in $CDCl_3$ or DMSO-d_6 as indicated. Chemical shifts were reported as observed in parts per million (ppm) with the residual solvent peak used as an internal standard ($CDCl_3$ = 7.26 ppm for ^{1}H and 77.16 ppm for ^{13}C; DMSO-d_6 = 2.50 ppm for ^{1}H and 39.52 ppm for ^{13}C). ^{1}H NMR spectra were run at 500 or 600 MHz and are tabulated as follows: chemical shift,

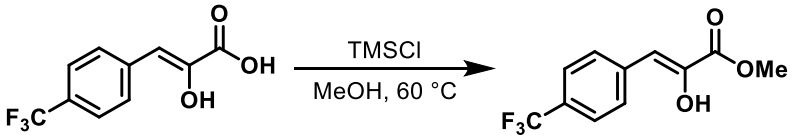
multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, brs = broad singlet, dt = doublet of triplet, tt = triplet of triplet), number of protons, and coupling constant(s). ^{13}C NMR spectra were run at 125 or 150 MHz using a proton-decoupled pulse sequence with a d1 of 1 second unless otherwise noted, and are tabulated by observed peak. High resolution mass spectra were obtained on an ion trap mass spectrometer using heated electrospray ionization (HESI).

Preparation of 2-oxa-3-arylpropanoic acid:



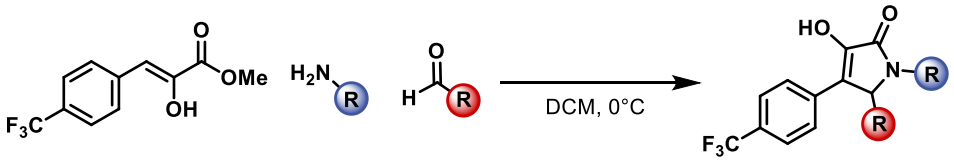
4-(Trifluoromethyl)benzaldehyde (5.00 g, 3.92 mL, 28.71 mmol), sodium acetate (2.59 g, 31.59 mmol), *N*-acetylglycine (3.36 g, 28.72 mmol) and acetic anhydride (8.14 mL, 86.15 mmol) were added to a 250 mL round bottom (RB) flask. The contents of the flask were stirred vigorously and refluxed at 110 °C for 1 h. The reaction mixture was pulled out of the oil bath and added 50 mL of 6 M HCl and the suspension was stirred for 24 h at 95 °C. The resulting reaction mixture was filtered and washed with small amount of cold deionized water. The resulting dark yellow solid was transferred to an Erlenmeyer flask and triturated with dichloromethane (50 mL), filtered and washed with additional dichloromethane to obtain 4.49 g of a bright yellow solid[33]. The dried solid was used without further purification.

Preparation of methyl (*Z*)-2-hydroxy-3-(4-(trifluoromethyl)phenyl)acrylate:

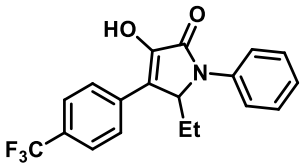


In a round bottom flask, (*Z*)-2-Hydroxy-3-(4-(trifluoromethyl)phenyl)acrylic acid (4.49 g, 19.34 mmol) was dissolved in dry methanol (65.00 mL) and chlorotrimethylsilane (8.87 mL, 7.59 g, 58.02 mmol) was added dropwise. The mixture was stirred and heated at 65 ºC overnight and reaction was followed by TLC. Upon reaction completion, the solvent was evaporated using a rotary evaporator and the mixture was dry loaded on silica, and the mixture was purified using flash chromatography (2-8% EtOAc:hexane). Product was obtained as a white semisolid in 97% yield (4.65 g). Analytical data was consistent with data previously reported[21].

General procedure: Synthesis of 3-hydroxy-1,5-dihydro-2*H*-pyrrol-2-ones



To a solution of the (Z)-2-hydroxy-3-(4-(trifluoromethyl)phenyl)acrylate (0.40 mmol) in DCM (0.05 M) at 0 °C was added aldehyde (1.05 mmol), followed by amine (1.05 mmol). The reaction was stirred at 0 °C for 0.5 - 24 hours, being monitored by TLC and/or HPLC-MS. Upon consumption of the acrylate, solvent was removed *in vacuo*. The crude products were then purified by column chromatography and/or trituration with acetonitrile.

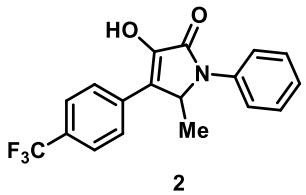


1

5-Ethyl-3-hydroxy-1-phenyl-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (1): According to general procedure, **1** was synthesized in 13% yield (18.3 mg) as a light yellow solid: NMR data was consistent with data previously reported[19];

mp: 160.4-167.2 °C;

HRMS (HESI) *m*/*z* calcd for $C_{19}H_{17}F_3NO_2$ $[M+H]^+$ 348.1206, found 348.1204.



2

3-Hydroxy-5-methyl-1-phenyl-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (2): According to general procedure, **2** was synthesized in 31% yield (41.9 mg) as an orange solid:

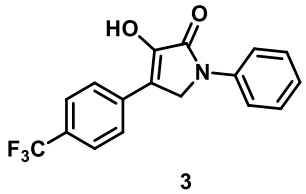
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, *J* = 9.8 Hz, 2H), 7.70 (d, *J* = 9.9 Hz, 2H), 7.62-7.57 (m, 2H), 7.50-7.44 (m, 2H), 7.29-7.23 (m, 1H), 5.13 (q, *J* = 7.9 Hz, 1H), 1.38 (d, *J* = 7.9 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 164.91, 142.64, 136.26, 134.82, 129.57 (q, *J* = 27.7 Hz), 129.50, 127.69, 125.98, 125.80 (q, *J* = 3.2 Hz), 124.17 (q, *J* = 231.8 Hz), 122.41, 121.44, 54.68, 18.85;

IR ν_{max} (cm^{-1}): 3187, 2928, 1667, 1616, 1597, 1498, 1378, 1323, 847, 778, 758, 695;

mp: decomposed at 178 °C;

HRMS (HESI) *m*/*z* calcd for $C_{18}H_{15}F_3NO_2$ $[M+H]^+$ 334.1049, found 334.1045.



3

3-Hydroxy-1-phenyl-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (3): According to general procedure, **3** was synthesized in 30% yield (38.9 mg) as a white solid:

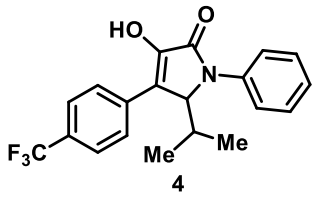
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.1 Hz, 2H), 7.80 (d, *J* = 7.9 Hz, 2H), 7.69 (d, *J* = 8.2 Hz, 2H), 7.44 (t, *J* = 8.0 Hz, 2H), 7.21 (t, *J* = 7.4 Hz, 1H), 6.92 (s, 1H), 4.67 (s, 2H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 165.94, 143.51, 138.90, 135.31, 130.03 (q, *J* = 32.1 Hz), 129.75, 126.83, 126.13 (q, *J* = 3.7 Hz), 125.37, 125.09, 118.93, 114.72, 48.65; (reported as observed)

IR ν_{max} (cm^{-1}): 3169, 2923, 2852, 1687, 1615, 1502, 1462, 1392, 1325, 1112, 833, 767, 688;

mp: decomposed at 189.1 °C;

HRMS (HESI) *m*/*z* calculated for $C_{17}H_{13}F_3NO_2$ $[M+H]^+$ 320.0893, found 320.0890.



4

3-Hydroxy-5-isopropyl-1-phenyl-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (4): According to general procedure, **4** was synthesized in 23% yield (33.7 mg) as a white solid:

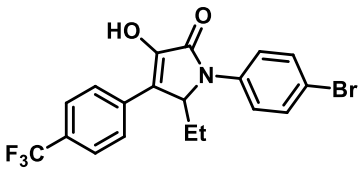
^{1}H NMR (600 MHz, DMSO) δ 10.55 (s, 1H), 7.89 (d, *J* = 11.3 Hz, 2H), 7.81 (d, *J* = 8.3 Hz, 2H), 7.61 (d, *J* = 7.6 Hz, 2H), 7.47 (t, *J* = 7.9 Hz, 2H), 7.26 (t, *J* = 7.4 Hz, 1H), 5.67 (d, *J* = 2.1 Hz, 1H), 2.00 (m, 1H), 0.58 (t, *J* = 7.6 Hz, 6H);

^{13}C NMR (150 MHz, DMSO) δ 164.79, 144.53, 137.67, 136.71, 128.84, 128.41, 127.16 (q, *J* = 31.5 Hz), 125.46, 125.19 (q, *J* = 3.6 Hz), 123.93, 123.37, 119.95, 61.35, 30.00, 18.03, 16.28; (reported as observed)

IR ν_{max} (cm^{-1}): 3175, 2963, 2927, 1663, 1616, 1597, 1501, 1428, 1383, 1321, 1115, 839, 776, 689;

mp: 214.5-217.4 °C;

HRMS (HESI) m/z calculated for $C_{20}H_{19}F_3NO_2$ $[M+H]^+$ 362.1362, found 362.1357.



5

1-(4-Bromophenyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (5): According to general procedure, **5** was synthesized in 26% yield (45.0 mg) as a colorless crystalline solid:

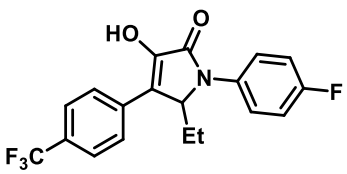
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 2H), 7.69 (d, *J* = 8.2 Hz, 2H), 7.60-7.56 (m, 2H), 7.51-7.46 (m, 2H), 7.25 (s, 1H), 5.24 (t, *J* = 3.5 Hz, 1H), 1.97-1.89 (m, 1H), 1.88-1.81 (m, 1H), 0.42 (t, *J* = 7.3 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 165.50, 143.16, 135.12, 134.50, 132.46, 129.68 (q, *J* = 32.4 Hz), 127.55, 125.73 (q, *J* = 3.7 Hz), 123.99 (q, *J* = 270.4 Hz), 123.76, 118.97; 58.14, 21.82, 5.07;

IR ν_{max} (cm^{-1}): 3194, 2970, 2936, 2879, 1667, 1616, 1589, 1492, 1384, 1322, 849, 828;

mp: 182.0-185.4 °C;

HRMS (HESI) *m/z* calculated for $C_{19}H_{16}BrF_3NO_2$ $[M+H]^+$ 426.0311, found 426.0316.



6

5-Ethyl-1-(4-fluorophenyl)-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (6): According to general procedure, **6** was synthesized in 42% yield (62.3 mg) as a white solid:

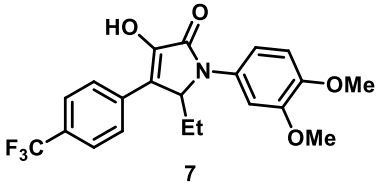
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 2H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.57-7.48 (m, 2H), 7.17 (t, *J* = 8.6 Hz, 2H), 5.22 (t, *J* = 3.4 Hz, 1H), 1.94-1.80 (m, 2H), 0.44 (t, *J* = 7.3 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 165.44, 160.44 (d, *J* = 210.0 Hz), 143.17, 134.61, 132.04 (d, *J* = 2.6 Hz), 129.58 (q, *J* = 27.8 Hz), 127.50, 125.71 (q, *J* = 3.2 Hz), 124.42 (d, *J* = 7.0 Hz), 124.00 (q, *J* = 231.4 Hz), 118.73, 116.28 (d, *J* = 19.4 Hz), 58.63, 21.85, 5.13;

IR ν_{max} (cm^{-1}): 3196, 2970, 2932, 1665, 1616, 1509, 1435, 1387, 1323, 837;

mp: decomposed at 147.0-155.0 °C;

HRMS (HESI) *m*/*z* calculatedd for $C_{19}H_{16}F_4NO_2$ $[M+H]^+$ 366.1112, found 366.1111.



7

1-(3,4-Dimethoxyphenyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (7): According to general procedure, **7** was synthesized in 13% yield (21.5 mg) as a colorless crystalline solid:

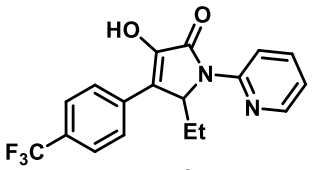
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.2 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.14 (d, *J* = 2.3 Hz, 1H), 6.99 (s, 1H), 6.94-6.83 (m, 2H), 5.11 (t, *J* = 3.5 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 1.91-1.81 (m, 1H), 1.81-1.72 (m, 1H), 0.39 (t, *J* = 7.3 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 165.48, 149.45, 147.39, 143.45, 134.84, 129.39 (q, *J* = 33.0 Hz); 129.25, 127.45, 125.64 (q, *J* = 3.0 Hz), 124.05 (q, *J* = 271.5 Hz), 118.52, 115.15, 111.39, 107.39, 58.96, 56.16, 56.12, 22.04, 5.32;

IR ν_{max} (cm^{-1}): 3210, 3178, 2971, 2938, 1665, 1615, 1516, 1457, 1389, 1325, 848;

mp: 203.5-204.8 °C;

HRMS (HESI) *m*/*z* calculated for $C_{21}H_{21}F_3NO_4$ $[M+H]^+$ 408.1417, found 408.1424.



8

5-Ethyl-3-hydroxy-1-(pyridin-3-yl)-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (8): According to general procedure, **8** was synthesized in 22% yield (31.1 mg) as an orange solid:

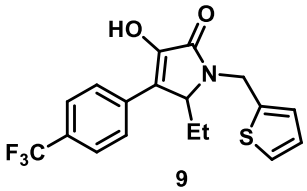
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.83 (d, *J* = 2.2 Hz, 1H), 8.52 (d, *J* = 4.0 Hz, 1H), 8.13-8.08 (m, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.71 (d, *J* = 8.1 Hz, 2H), 7.45-7.41 (m, 1H), 5.34 (t, *J* = 3.4 Hz, 1H), 2.00-1.88 (m, 2H), 0.45 (t, *J* = 7.3 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 165.76, 146.47, 143.16, 142.96, 134.39, 133.06, 129.48, 127.60, 125.76 (q, *J* = 3.8 Hz), 123.98, 119.48, 57.75, 21.78, 5.04; (reported as observed)

IR ν_{max} (cm^{-1}): 2928, 2524, 1680, 1616, 1574, 1489, 1382, 1323, 849;

mp: decomposed at 218.0-220.0 °C;

HRMS (HESI) *m*/*z* calculated for $C_{18}H_{16}F_3N_2O_2$ $[M+H]^+$ 349.1158, found 349.1159.



9

5-Ethyl-3-hydroxy-1-(thiophen-2-ylmethyl)-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (9): According to general procedure, **9** was synthesized in 39% yield (58.2 mg) as a white solid:

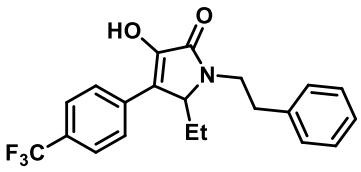
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.9 Hz, 2H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 5.0 Hz, 1H), 7.04 (d, *J* = 2.6 Hz, 1H), 6.99 (t, *J* = 4.2 Hz, 1H), 5.38 (d, *J* = 15.7 Hz, 1H), 4.55 (t, *J* = 3.0 Hz, 1H), 4.36 (d, *J* = 15.6 Hz, 1H), 2.11-1.98 (m, 1H), 1.91-1.79 (m, 1H), 0.51 (t, *J* = 7.3 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 166.53, 143.38, 138.67, 134.85, 129.31, 127.43, 127.10, 126.97, 125.91, 125.50 (q, *J* = 3.7 Hz), 119.28, 56.58, 38.71, 21.36, 5.34; (reported as observed)

IR ν_{max} (cm^{-1}): 3147, 2965, 2925, 2854, 1663, 1615, 1452, 1389, 1323, 1290, 848;

mp: 182.0-183.0 °C;

HRMS (HESI) *m*/*z* calculated for $C_{18}H_{17}F_3NO_2S$ $[M+H]^+$ 368.0927, found 368.0923.



10

5-Ethyl-3-hydroxy-1-phenethyl-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (10): According to general procedure, **10** was synthesized in 30% yield (45.7 mg) as a grey solid:

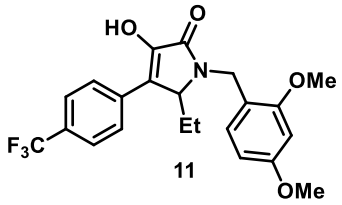
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88-7.77 (brs, 1H), 7.64 (dd, *J* = 12.4, 8.7 Hz, 4H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 3H), 4.34 (t, *J* = 3.3 Hz, 1H), 4.23-4.16 (m, 1H), 3.34-3.25 (m, 1H), 3.05-2.92 (m, 2H), 1.97-1.87 (m, 1H), 1.83-1.75 (m, 1H), 0.46 (t, *J* = 7.3 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 166.80, 143.72, 138.41, 135.04, 129.07 (q, *J* = 32.2 Hz), 128.75, 128.71, 127.30, 126.77, 125.53 (q, *J* = 3.7 Hz), 124.08 (q, *J* = 270.6 Hz), 118.68, 57.57, 41.93, 34.84, 21.39, 5.37;

IR ν_{max} (cm^{-1}): 3176, 2968, 2931, 1659, 1614, 1455, 1390, 1322, 849;

mp: 196.0-198.0 °C;

HRMS (HESI) *m*/*z* calculated for $C_{21}H_{21}F_3NO_2$ $[M+H]^+$ 376.1519, found 376.1515.



11

1-(2,4-Dimethylbenzyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (11): According to general procedure, **11** was synthesized in 54% yield (85.4 mg) as an orange solid:

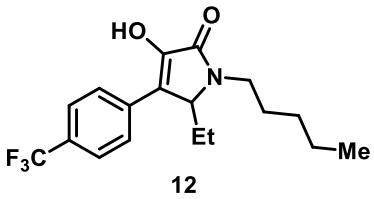
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91-7.78 (brs, 1H), 7.68 (d, *J* = 9.8 Hz, 2H), 7.61 (d, *J* = 10.1 Hz, 2H), 7.19 (d, *J* = 9.8 Hz, 1H), 6.49-6.42 (m, 2H), 5.06 (d, *J* = 17.9 Hz, 1H), 4.40 (t, *J* = 4.1 Hz, 1H), 4.25 (d, *J* = 17.9 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 2.14-2.05 (m, 1H), 1.85-1.69 (m, 1H), 0.49 (t, *J* = 8.8 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 166.94, 160.87, 158.55, 143.90, 135.36, 131.13, 129.03 (q, *J* = 30.7 Hz), 127.50, 125.54, 124.22 (q, *J* = 226.5 Hz), 118.89, 117.23, 104.57, 98.73, 56.98, 55.63, 55.57, 38.49, 21.31, 5.41; (reported as observed)

IR ν_{max} (cm^{-1}): 3111, 2967, 2937, 1661, 1614, 1508, 1455, 1389, 1323, 1120, 848, 797, 733;

mp: 173.0-178.5 °C ;

HRMS (HESI) m/z calculated for $C_{22}H_{23}F_3NO_4$ $[M+H]^+$ 422.1574, found 422.1578.



12

5-Ethyl-3-hydroxy-1-pentyl-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (12): According to general procedure, **12** was synthesized in 59% yield (81.8 mg) as a white solid:

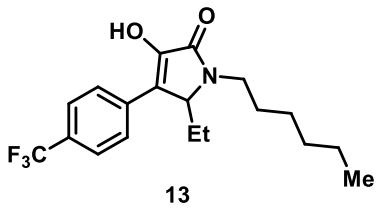
^{1}H NMR (600 MHz, $CDCl_3$) δ 9.55-8.65 (brs, 1H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.66 (d, *J* = 8.2 Hz, 2H), 4.64-4.59 (m, 1H), 4.00-3.88 (m, 1H), 3.15-2.96 (m, 1H), 2.04-1.94 (m, 1H), 1.94-1.83 (m, 1H), 1.75-1.57 (m, 2H), 1.47-1.22 (m, 4H), 0.93 (t, *J* = 6.9 Hz, 3H), 0.50 (t, *J* = 7.2 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 167.24, 144.69, 135.37, 128.76 (q, *J* = 31.1 Hz), 127.25, 125.41 (q, *J* = 3.7 Hz), 124.15 (q, *J* = 270.4 Hz), 118.51, 56.95, 40.25, 29.05, 28.16, 22.36, 21.46, 13.98, 5.33;

IR ν_{max} (cm^{-1}): 3147, 2962, 2933, 2874, 1658, 1614, 1455, 1390, 1321, 1112, 847;

mp: 121.2-123.6 °C;

HRMS (HESI) m/z calculated for $C_{18}H_{23}F_3NO_2$ $[M+H]^+$ 342.1675, found 342.1674.



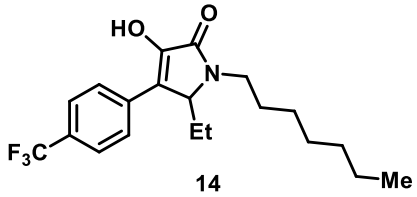
13

5-Ethyl-1-hexyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (13): According to general procedure, **13** was synthesized in 87% yield (125.6 mg) as a light yellow solid:

NMR data was consistent with data previously reported;

mp: 113-115.6 °C;

HRMS (HESI) m/z calculated for $C_{19}H_{25}F_3NO_2$ $[M+H]^+$ 356.1832, found 356.1830.



14

5-Ethyl-1-heptyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (14): According to general procedure, **14** was synthesized in 64% yield (96.0 mg) as a light yellow solid:

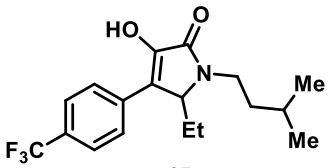
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.73 (d, *J* = 9.8 Hz, 2H), 7.64 (d, *J* = 10.0 Hz, 2H), 4.59 (t, *J* = 4.0 Hz, 1H), 3.96-3.87 (m, 1H), 3.10-2.99 (m, 1H), 2.03-1.91 (m, 1H), 1.91-1.81 (m, 1H), 1.70-1.53 (m, 2H), 1.39-1.23 (m, 8H), 0.88 (t, *J* = 8.3 Hz, 3H), 0.48 (t, *J* = 8.8 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 166.77, 143.92, 135.15, 128.98 (q, *J* = 27.7 Hz), 127.30, 125.51 (q, *J* = 3.2 Hz), 124.11 (q, *J* = 231.8 Hz), 118.39, 56.98, 40.31, 31.72, 28.95, 28.50, 26.88, 22.59, 21.45, 14.06, 5.33;

IR ν_{max} (cm^{-1}): 3123, 2960, 2931, 2859, 1661, 1615, 1456, 1391, 1324, 1125, 849;

mp: 125.8-130.9 °C;

HRMS (HESI) m/z calculated for $C_{21}H_{26}F_3NO_2$ $[M+H]^+$ 370.1988, found 370.1988.



15

5-Ethyl-3-hydroxy-1-isopentyl-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2*H*-pyrrol-2-one (15): According to general procedure, **15** was synthesized in 47% yield (65.2 mg) as an orange solid:

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.40-7.95 (brs, 1H), 7.74 (d, *J* = 8.1 Hz, 2H), 7.65 (d, *J* = 8.2 Hz, 2H), 4.63-4.56 (m, 1H), 4.02-3.91 (m, 1H), 3.11-3.00 (m, 1H), 2.02-1.92 (m, 1H), 1.92-1.82 (m, 1H), 1.66-1.57 (m, 1H), 1.57-1.48 (m, 2H), 0.97 (t, *J* = 6.0 Hz, 3H), 0.49 (t, *J* = 7.3 Hz, 3H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 166.86, 144.24, 135.26, 128.89 (q, *J* = 32.5 Hz), 127.26, 125.48 (q, *J* = 3.6 Hz), 124.13 (q, *J* = 270.1 Hz), 118.33, 56.84, 38.60, 37.21, 26.03, 22.74, 22.21, 21.44, 5.35;

IR ν_{max} (cm^{-1}): 3124, 2961, 2929, 2875, 1659, 1614, 1456, 1390, 1323, 1126, 849;

mp: 141.5-143.5 °C;

HRMS (HESI) m/z calculated for $C_{18}H_{23}F_3NO_2$ $[M+H]^+$ 342.1675, found 342.1677.

General information - Biological Assays

Methicillin-resistant and methicillin sensitive Staphylococcus aureus (MRSA and MSSA respectively) strains were obtained from the Laboratory of Professor Christian Melander (NCSU) (ATCC BAA 44 and 33591) and Dr. Jessica Gilbertie (ATCC 25923). Bacteria were kept in frozen stocks on glycerol at - 80 °C until use. Bacteria was streaked onto tryptic-soy agar for colony isolation. Mueller-Hinton broth (MHB, 211443-BD), tryptic soy broth (TSB, Remel: R455052) and *D*-glucose (CAS: 492-62-6) were purchased from Fisher Scientific. Tryptic soy agar (TSA, cat. # 22091) and Linezolid (cat. # P70014) were purchased from Sigma-Aldrich. Bacteria for biofilm inhibition were cultured overnight in TSB-G (tryptic soy broth with 0.5% glucose supplement) in 96 well plates. All assays were run in triplicate and repeated at least two separate times for MIC assays and at least three separate times for biofilm inhibition. All compounds were dissolved in molecular biology grade DMSO as 10 mM stock solutions. Optical densities were measured using a Thermo Scientific Genesys 20 spectrophotometer. Data for biofilm inhibition, MBEC, and MIC assays were collected using a BioTek ELx808 Microplate Reader. All graphs were generated and analyzed using GraphPad Prism 7.

Determination of the inhibitory effect of test compounds on MRSA biofilm formation (MBIC)

Inhibition assays[34] were performed by subculturing an overnight culture of MRSA (ATCC BAA 44) to an OD_{600} of 0.01 in TSB-G (tryptic soy broth with a 0.5% glucose supplement). Stock solutions of predetermined concentrations of the test compound were then made using the inoculated TSB-G. These stock solutions were aliquoted (200 μL) into the top wells of the 96-well

microtiter plate and 100 uL of the inoculated broth in all other wells. After a serial dilution from the top well was completed, sample plates were sealed then incubated for 24 h at 37 °C. After incubation, the medium was discarded from the wells and the plates were washed 2x with PBS. Prior to staining, plates were left to dry at ambient temperature for 2-3 h. Plates were then stained with 0.1% solution of crystal violet (CV, 125 μL) and then incubated at ambient temperature for 30 min. Plates were washed with PBS again and the remaining stain was solubilized with 99% ethanol (200 μL). A sample of solubilized CV stain (110 μL) from each well was transferred to the corresponding wells of a polystyrene microtiter dish. Biofilm biomass was quantified by measuring the OD540 of each well and inhibition was calculated as a percentage of the control (no compound); a negative control lane wherein no biofilm was formed served as a background and was subtracted out. Percent inhibition was then plotted against concentration in Prism 7. Each of the four experiments were plotted separately and S15 analyzed by a normalized nonlinear regression. The graphs on the following pages were generated from an average of the total data set. Biofilm data represent four separate experiments, with each experiment performed in duplicate (average of 8 data points for each concentration tested, unless otherwise noted).

Determination of the Minimum Biofilm Eradication Concentrations (MBEC) using the Calgary Biofilm Device (CBD) on MSSA (ATCC 25923) biofilms

Biofilm eradication experiments[35] were performed using MSSA (ATCC 25923) and the Calgary Biofilm Device (CBD) to determine MBEC values for various compounds of interest (Innovotech, product code: 19111). The Calgary device is a 96-well plate with a lid containing 96 pegs that sit in the media contained in the bottom well. Biofilm are established on the individual pegs. The established biofilm (contained on the individual peg) can then be transferred to a new base well for MBEC testing. For the MBEC assay, an overnight culture of MSSA (ATCC 25923) was

adjusted to 0.5 McFarland in MHB-G. The CBD was inoculated with 100 µL of the 0.5 McFarland and incubated at 37 °C for 24 hours to establish biofilms. The CBD lid containing the established biofilms on individual pegs was removed, washed 3x with PBS and transferred to another 96-well plate containing serial dilutions of the test compounds (the "challenge plate") and incubated at 37 °C for 24 hours. The CBD lid was then removed from the challenge plate, washed 3x with PBS to remove any residual compound and placed into a new 96-well base plate containing fresh MHB. The plate was then sonicated for sonicated for 30 minutes to disperse biofilms on the pegs into the fresh MHB in the base well. After sonication, the plate was incubated for 24 hours at 37 °C. MBEC values were determined as the lowest test concentration that resulted in no growth in the sonicate fluid confirmed by absorbance at 600 nm in a plate reader.

Broth microdilution method for determination of minimum inhibitory concentrations (MIC) at pH 9.0

As prescribed by the Clinical and Laboratory Standards Institute (CLSI) M07-A8, Vol. 29 (2) MSSA (ATCC 25923) and MRSA (ATCC BAA 44 and 33591) was grown in MHB for 6-8 h; this culture was used to inoculate fresh cation adjusted MHBII which was previously adjusted to a pH of 9.0 using NaOH and a pH meter (5×10^5 CFU/mL). The resulting bacterial suspension was aliquoted (0.5 mL) into 1.5 mL Eppendorf tubes and compound was added from a 10 mM DMSO stock to achieve the desired initial starting concentration (typically 128 µg/mL). Linezolid (from a 10 mM DMSO stock) was used as a positive control. Inoculated media not treated with compound served as the negative control. The MIC was determined by microbroth dilution following the CLSI guidelines. The MIC was defined as the lowest concentration of antibiotic with no visible growth. The plate was sealed and incubated under stationary conditions at 37 °C. After 16 h, MIC values were recorded as the lowest concentration of compound at which no visible

growth of bacteria was observed and was also confirmed via absorbance readings from a plate reader.

Determination of the Minimum Biofilm Eradication Concentrations (MBEC) using the Calgary Biofilm Device (CBD) on MSSA (ATCC 25923) biofilms at pH 9.0

Biofilm eradication experiments[35] were performed using MSSA (ATCC 25923) and the Calgary Biofilm Device (CBD) to determine MBEC values for various compounds of interest (Innovotech, product code: 19111). The Calgary device is a 96-well plate with a lid containing 96 pegs that sit in the media contained in the bottom well. Biofilm are established on the individual pegs. The established biofilm (contained on the individual peg) can then be transferred to a new base well for MBEC testing. For the MBEC assay, an overnight culture of MSSA (ATCC 25923) was adjusted to 0.5 McFarland in MHB-G. The CBD was inoculated with 100 µL of the 0.5 McFarland and incubated at 37 °C for 24 hours to establish biofilms. The CBD lid containing the established biofilms on individual pegs was removed, washed 3x with PBS and transferred to another 96-well plate containing serial dilutions of the test compounds (the "challenge plate") and incubated at 37 °C for 24 hours. This challenge plate was made using fresh cation adjusted MHBII which was previously adjusted to a pH of 9.0 using NaOH and a pH meter. The CBD lid was then removed from the challenge plate, washed 3x with PBS to remove any residual compound and placed into a new 96-well base plate containing fresh MHB. The plate was then sonicated for sonicated for 30 minutes to disperse biofilms on the pegs into the fresh MHB in the base well. After sonication, the plate was incubated for 24 hours at 37 °C. MBEC values were determined as the lowest test concentration that resulted in no growth in the sonicate fluid confirmed by absorbance at 600 nm in a plate reader.

1.4 References

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Chapter 2: Design and Synthesis of Novel Aza-sugar Clindamycin Antibiotics

2.1 General Introduction

2.1.1 A brief history of antibiotics and the desperate need for new antibiotics

Antibiotics are used to treat bacterial infections by targeting the pathogen's essential pathways, such as the biosynthesis of cell walls or proteins. Many antibiotics are small molecules produced by living organisms. The first synthetic antibiotic, salvarsan, was used clinically in 1910. Later penicillin (β-lactam) was discovered by Alexander Fleming in 1928 and became widely available after World War II. Antibiotics have drastically changed modern medicine over the last 100 years. Waksman's work initiated the Golden Age of antibiotic discovery from the 1940s to the 1960s[1,2] (**Figure 2.1**). Antibiotics are classified into 3 classes based on mechanism of action: (1) cell wall or membrane synthesis inhibitors, such as penicillin, vancomycin (glycopeptide) and polymyxins; (2) protein synthesis inhibitors, such as macrolides, clindamycin (lincosamide), linezolid (oxazolidinone) and chloramphenicol; and (3) nucleic acid synthesis inhibitors, such as the quinolones and rifampicin (ansamycin)[3].

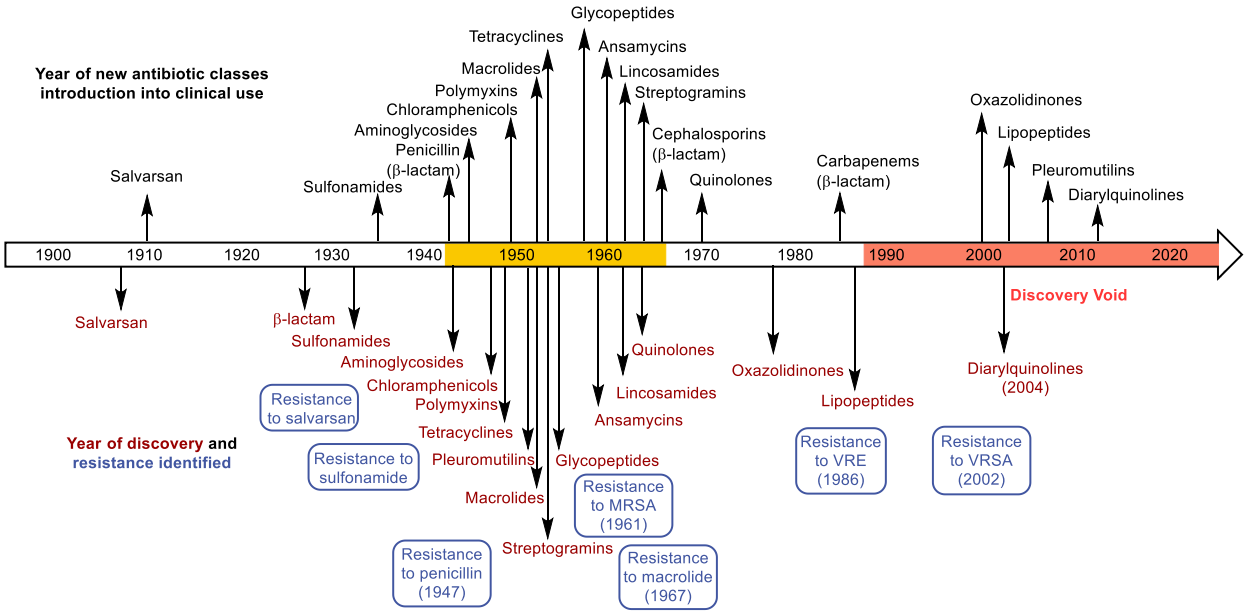


Figure 2.1. The timeline of antibiotic discovery and identified resistance.

Most of these antibiotics are still in clinical use but their effectiveness has been eroded by the rapid rise of antimicrobial resistance (AMR). Bacteria resist antibiotics in mainly three ways: (1) through target-site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target, (2) through efflux of the antibiotic, and (3) by drug inactivation[4]. No major classes of antibiotics have been introduced since 1987. According to the 2019 AR Threats Report released by the CDC[5], more than 2.8 million antibiotic-resistant infections occur in the U.S. each year, and more than 35,000 people die as a result. Hence, there is a constant need for new antibacterial drugs owing to the inevitable development of resistance. In the report, clindamycin-resistant Group B *Streptococcus* (**Figure 2.2**) is one of the threats that are concerning. Group B *Streptococcus* (GBS) is a type of bacteria that can cause severe illnesses in people of all ages, including bloodstream, infections, pneumonia, meningitis, and skin infections. Overall, about 31,000 severe GBS infections occurred in 2016, causing 1,700 deaths. Clindamycin-resistant strains have caused more than 40% of GBS infections.

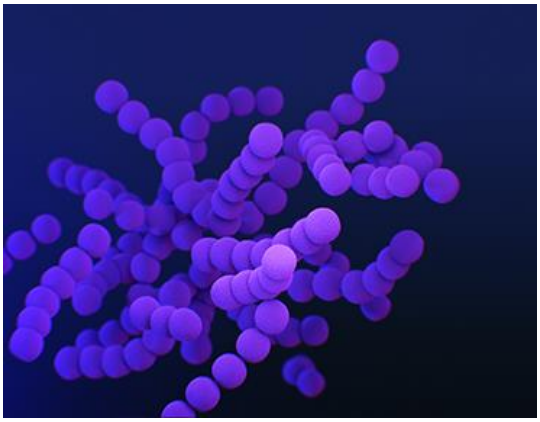


Figure 2.2. Clindamycin-resistant Group B *Streptococcus*.

2.1.2 Introduction of lincosamides

Lincosamides are a small class of antibiotics exhibiting biological activity against Gram-positive bacteria, characterized by an unusual octose joined with an amino acid by an amide bond[6]. The naturally occurring lincosamide compounds mainly include lincomycin (LCM), celesticetin and Bu-2545 (**Figure 2.3**). Lincomycin A (**1**) consists of an *N*-methyl-4-propyl-L-proline (PPL, **2**) and a methylthiolincosamide (MTL, **3**). It is the first antibiotic of the lincosamide family, which was isolated from *Streptomyces lincolnensis* var *lincolnesis* in 1962 and was made available for clinical studies in January 1963 by Upjohn[7,8]. Lincomycin B (**4**) is a minor product in *S. lincolnensis* culture. Celesticetin (**5**) was isolated from the culture broth of *Streptomyces caelestis* in 1955. Desalicetin (**6**) is the hydrolysis product of *celesticetin*[9] and Bu-2545 (**7**) is a structural hybrid between lincomycin and celesticetin, isolated from *Streptomyces* strain H230-5 in 1980[10].

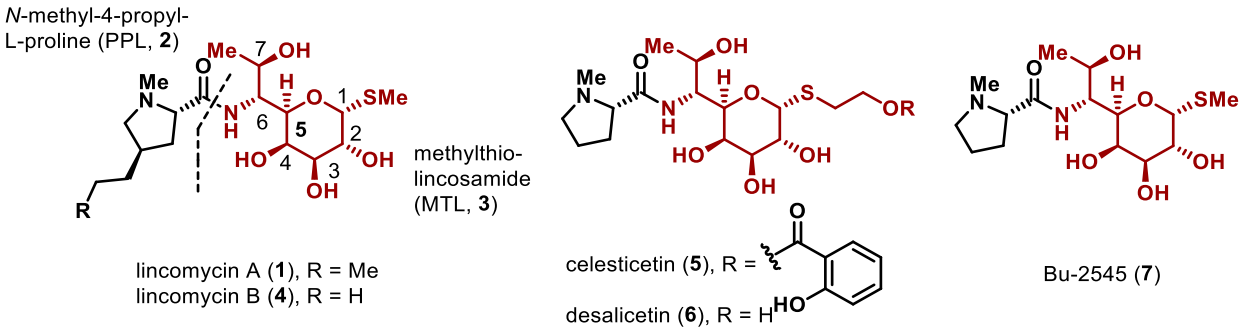


Figure 2.3. Naturally occurring lincosamides.

Many semisynthetic derivatives of LCM have been prepared (**Figure 2.4**). Clindamycin (CLDM, **8**) was prepared by replacing the 7-hydroxyl group with a chlorine atom with the inversion of chirality. It exhibited improved antibacterial activity compared to LCM and was released for medical use in September 1964. Pirlimycin (**9**), which was a 4'-substituted pipecolamide derivative, was used in mastitis therapy for cattle in Europe and the United States[11]. VIC-105555 (**10**) was selected as a clinical candidate, exhibiting good *in vitro* antibacterial activities against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis*[12]. Mirincamycin (U24729A, **11**), possessing a n-pentyl group at 4' position, was a potential clinical candidate as an antimalarial drug[13]. In 2014, it was reported that its combinations may be promising candidates in the treatment and prophylaxis of multidrug-resistant falciparum malaria or in combination with 4 or 8- aminoquinolines for the treatment and relapse prevention of vivax malaria[14].

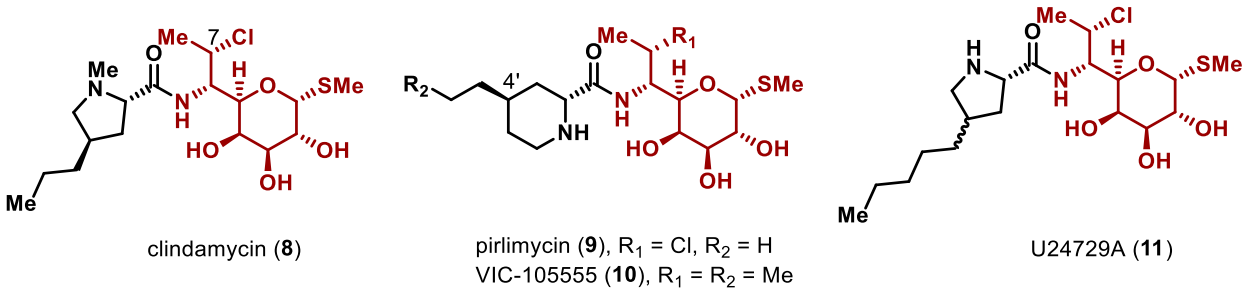


Figure 2.4. Semisynthetic lincosamides.

LCM and CLDM are clinically important antibiotics that are frequently used to treat infections. LCM is effective against Gram-positive bacteria, but it is no longer widely used. CLDM, which has an improved antibacterial activity compared with LCM, is active against many aerobic Gram-positive cocci and a range of anaerobic Gram-positive and Gram-negative bacteria. Clindamycin prevents peptide-bond formation by binding to the peptidyl transferase center (PTC) of the 50S ribosomal subunit. A crystal structure of *E. coli* 50S ribosomal subunit complexed (**Figure 2.5**) with clindamycin documented this binding[15,16]. The 2OH of clindamycin appears to form an H bond with N1 of nucleotide A2058. The 3OH group can interact with N6 of A2058 and with the non-bridging phosphate-oxygens of G2505. The 4OH group of clindamycin could form an H bond with N6 of A2059 and with 2'OH of A2503. In addition, the bridging amine of clindamycin could form an H bond with the ribose O4' of G2505. Additionally, the nucleotides C2452 and U2506 pack tightly against the propyl group of clindamycin via van der Waals interactions. Resistance to the lincosamides is commonly caused by the activity of a methyltransferase enzyme encoded by *erm* (*erythromycin ribosome methylase*) gene. This enzyme methylates 23S rRNA at the N6 position of adenosine A2058. Besides, a distinct rRNA methyltransferase which encoded by the transferrable gene *cfr* (chloramphenicol-florfenicol resistance) methylates C8 of the 23S rRNA residue A2503[17,18].

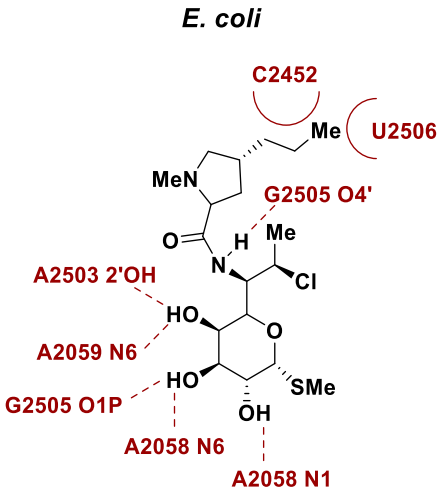


Figure 2.5. Clindamycin bound to the *E. coli* ribosome.

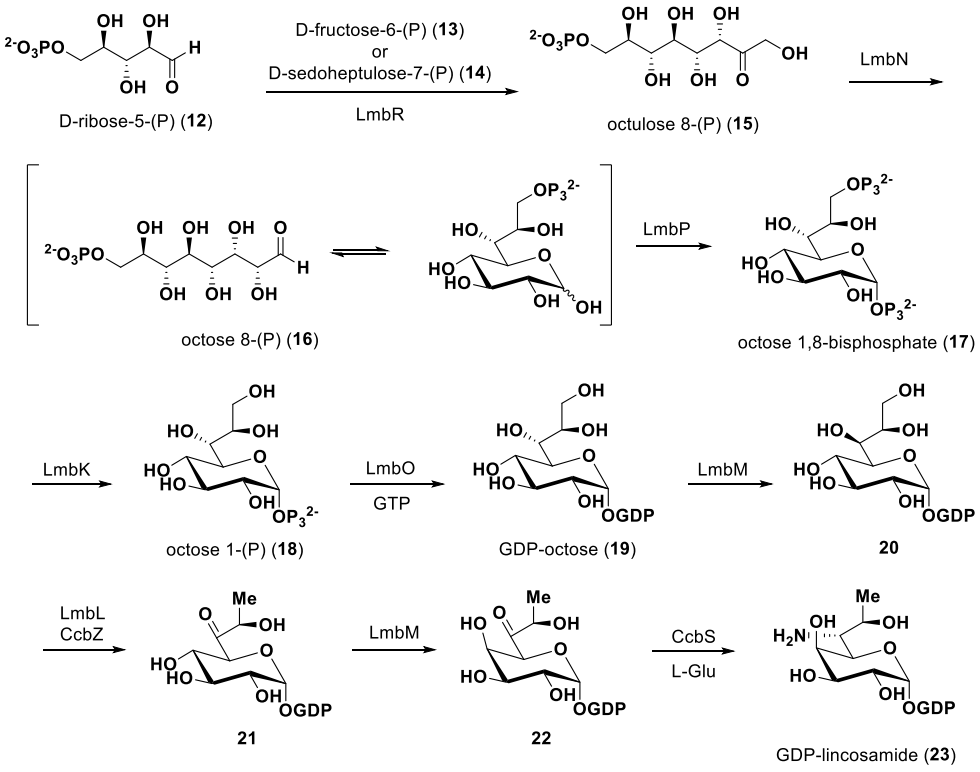
2.1.3 Biosynthesis of lincomycin

Lincosamides are comprised of a naturally rare amino-octose, to which an amino acid is attached via an amide bond. Specifically, lincomycin A is composed of an MTL core and a PPL moiety. Earlier biosynthetic studies on lincomycin by Upjohn demonstrated that the PPL residue came from the rearrangement of tyrosine[19]. Afterward, Hurley's group reported the first insight into how the MTL unit is assembled from glucose[20]. Later, the lincomycin biosynthetic gene cluster (lmb) was identified and sequenced in *S. lincolnesis* strains 78-11 and ATCC 25466[21,22]. Subsequently, the biosynthetic pathway has been deeply explored over the past decades.

2.1.3.1 Biosynthesis of the sugar moiety

As shown in **Scheme 2.1**, the moiety MTL is formed via a transaldol reaction catalyzed by LmbR using D-fructose 6-phosphate (**13**) or D-sedoheptulose 7-phosphate (**14**) as the C_3 donor and D-ribose 5-phosphate (**12**) as the C_5 acceptor. Subsequent 1,2-isomerization catalyzed by LmbN converts the resulting octulose 8-phosphate (**15**) to octose 8-phosphate (**16**), which can be anomerically phosphorylated by the kinase LmbP to yield octose 1,8-biphosphate (**17**)[23]. The

octose 1,8-bisphosphate intermediate is first converted to octose 1-phosphate (**18**) by a phosphatase, LmbK. The subsequent conversion of **18** to GDP-D-α-D-octose (**19**) is catalyzed by the octose 1-phosphate guanylyltransferase, LmbO[24]. At last, GDP-octose (**19**) is epimerized to **20** catalyzed by LmbM, followed by dehydration catalyzed by LmbL/CcbZ to **21**. Epimerization of **21** to also catalyzed by LmbM. Finally, the CcbS catalyzed transamination of **22** to GDP-lincosamide (**23**) take place[25].

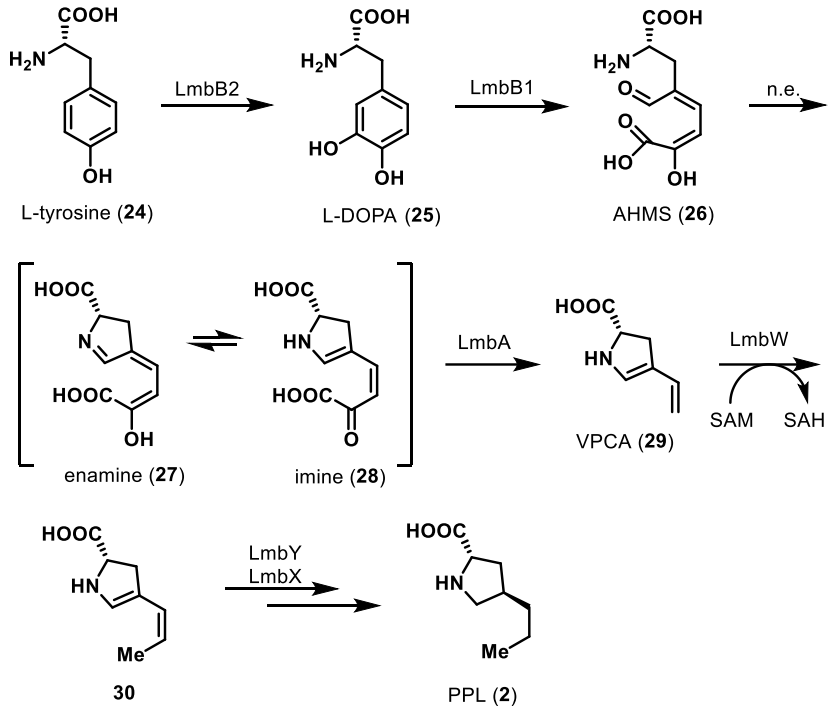


Scheme 2.1. Biosynthesis of GDP-lincosamide.

2.1.3.2 Biosynthesis of the amino acid moiety

The biosynthesis of PPL (**2**) begins with ortho-hydroxylation of L-tyrosine (**24**) catalyzed by LmbB2 to produce L-3,4-dihydroxyphenylalanine (L-DOPA, **25**)[26,27] (**Scheme 2.2**). Subsequently, an L-DOPA 2,3-dioxygenase LmbB1 conducts oxidative cleavage for benzoic ring opening and

produces the semialdehyde intermediate AHMS (**26**), which is immediately cyclized to yield an unstable pyrroline product in the enamine (**27**) or imine (**28**) form. The hydrolase LmbA is responsible for the generation of 3-vinyl-2,3-pyrroline-5-carboxylic acid (VPCA, **29**) by removal of the terminal two-carbon unit through C−C bond cleavage. Further functionalization, including LmbW-catalyzed C-methylation, LmbY-catalyzed hydrogenation, and LmbX-catalyzed epimerization is required to prepare PPL (**2**).

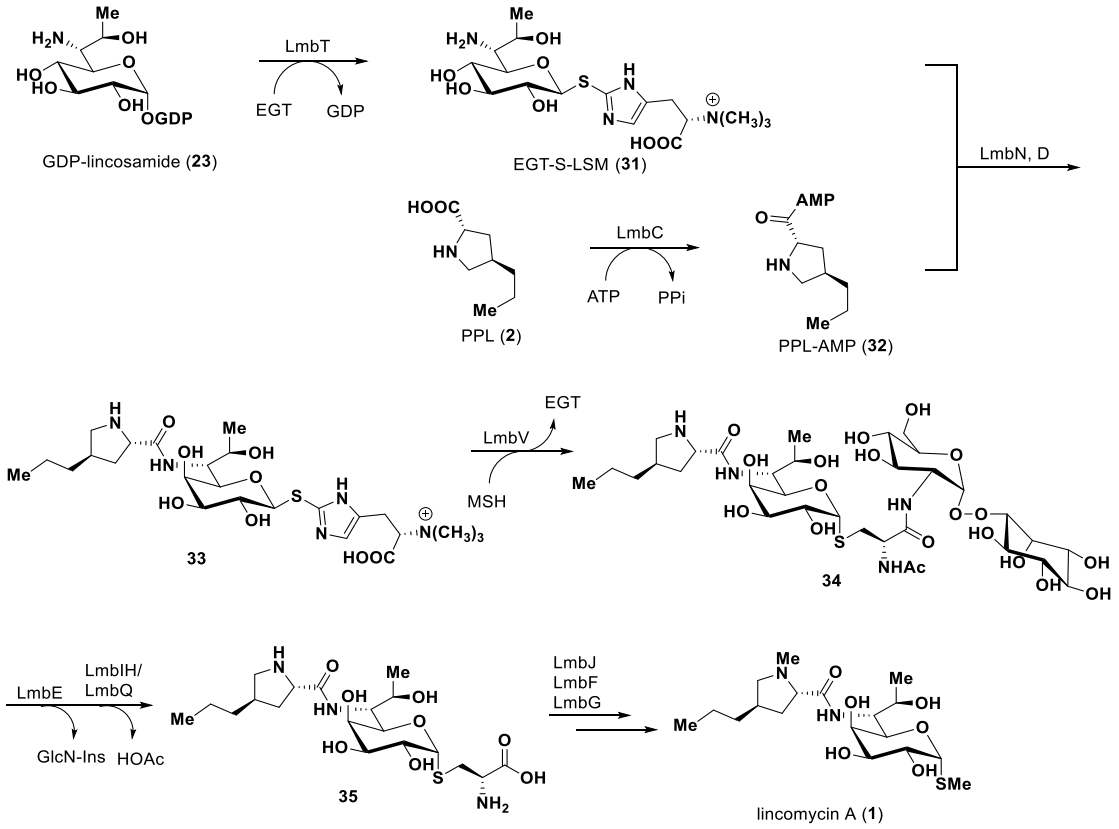


Scheme 2.2. Biosynthesis of PPL.

2.1.3.3 Condensation and final modifications

In the condensation step (**Scheme 2.3**), two small-molecule thiols, mycothiol (MSH) and ergothioneine (EGT) are involved, which are known for their redox-relevant roles in protection against various endogenous and exogenous stresses[28]. Firstly, the *S*-glycosyltransferase LmbT converts GDP-lincosamide (**23**) to EGT *S*-conjugated lincosamide through a S_N2 displacement.

Then LmbC activates PPL with ATP and transfers it onto a peptidyl carrier protein (PCP) of LmbN, followed by LmbD-catalyzed condensation with EGT *S*-conjugated lincosamide (**31**) to afford EGT *S*-conjugated intermediate **33**. Next, thiol exchange converts **33** into MSH *S*-conjugate **34** in the presence of the second *S*-glycosyltransferase LmbV. Subsequently, hydrolysis of the resulting **34** and *N'*-deacetylation leads to a mercapturic acid derivative **35**[29]. Finally, lincomycin A (**1**) is synthesized by *N*-methylation, C-S bond cleavage, and *S*-methylation.



Scheme 2.3. Condensation and final modifications.

2.1.4 Structure-activity relationships (SAR) of lincosamides

During the past several decades, a variety of lincomycin analogs have been prepared by modification at both the sugar moiety and proline moiety. Among these analogs, CLDM is the most significant and has been widely used in clinical practice as it shows increased potency and

enhanced absorption compared to LCM. At the same time, the analogs provide a good opportunity for the development of the structure-activity relationships of lincosamide antibiotics.

2.1.4.1 Modification at sugar moiety

Almost all the modifications of either substituent or stereochemistry at positions 1, 2, 3, or 4 of the sugar moiety resulted in drastic reduction of antibacterial activity[30–32]. Increasing the degree of oxidation of the sulfur atom was an unfavorable change[33]. The 1-β-anomer was less active than the α-anomer. S- and C_1-substituted analogs, reported by Vasella's group, also proved less active than LCM[34]; however, 1-demethylthio-1-ethylthiolincomycin had at least equivalent activity to lincomycin. Markedly decreased activity was observed for 2-deoxylincomycin and 2-*O*-methyllincomycin. The 4-epilincomycin had no antibacterial activity. Additionally, introducing a C_6-C_7 double bond by dehydration also led to inactive compounds. To improve the taste properties of the compounds, a series of 2-, 3- or 7-monoesters, 2,3-dicarbonate ester, and 2,7-dialkyl carbonate esters were prepared, but they proved less active[35,36].

Unlike the previous modifications, modification at the C_7 position of the sugar moiety does not result in a complete loss of activity[37,38]. Antibacterial activities are affected by the substituent's configuration and the structure at the C_7 position. For example, clindamycin, which has a 7(*S*)-configuration, is highly active compared to lincomycin. 7(*R*)-7-*O*-methyllincomycin showed improved potency, whereas 7(*S*)-7-*O*-methyllincomycin was 3.5x more active than LCM against *Sarcina lutea*. Unfortunately, both larger alkoxy groups and substituted alkoxy groups lead to weaker antibacterial activities than those of LCM[39]. 7(*R*)-azido-7-deoxylincomycin was more potent than lincomycin, but less active than clindamycin[40]. However, its amine derivative (**36**, **37**) and urea derivatives (**38**, **39**) had decreased activities[41] (**Figure 2.6**).

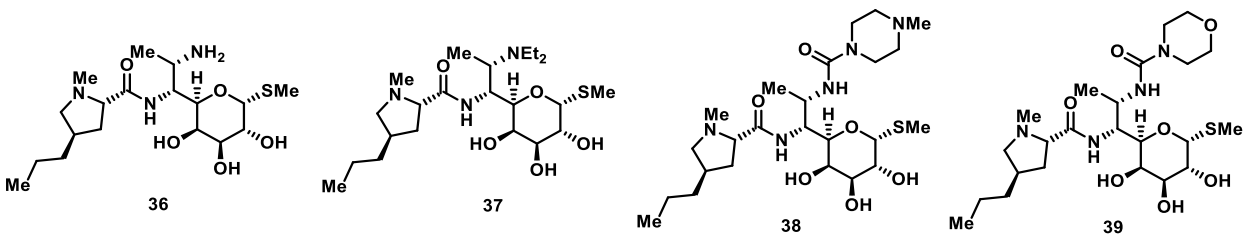


Figure 2.6. LCM amine and urea derivatives by modifying at the C_7 position.

On the other hand, 7(*S*)-7-deoxy-7-thiolincomycin and 7(*R*)-7-deoxy-7-thiolincomycin showed only 10% activity compared with LCM. 7(*S*)-7-alkylthio-7-deoxylincomycin and 7(*S*)-7-substituted alkylthio-7-deoxylincomycin were more active. Sztaricskai *et al*. reported a series of 7(*S*)-7-heteroarylthio-7-deoxylincomycin derivatives, but only compound **40** (**Figure 2.7**) was almost as active as LCM, but less effective than CLDM. Most recently, researchers of Japan's Meiji Seika Pharma Co. prepared a variety of lincomycin derivatives possessing a substituted phenyl, heteroaryl or substituted azetidine moiety via sulfur atom with 7(*S*)-configuration[42–50]. Many of these compounds, such as compounds **42**-**47**, showed potent activities against a variety of *Streptococcus pneumoniae* possessing *erm* genes.

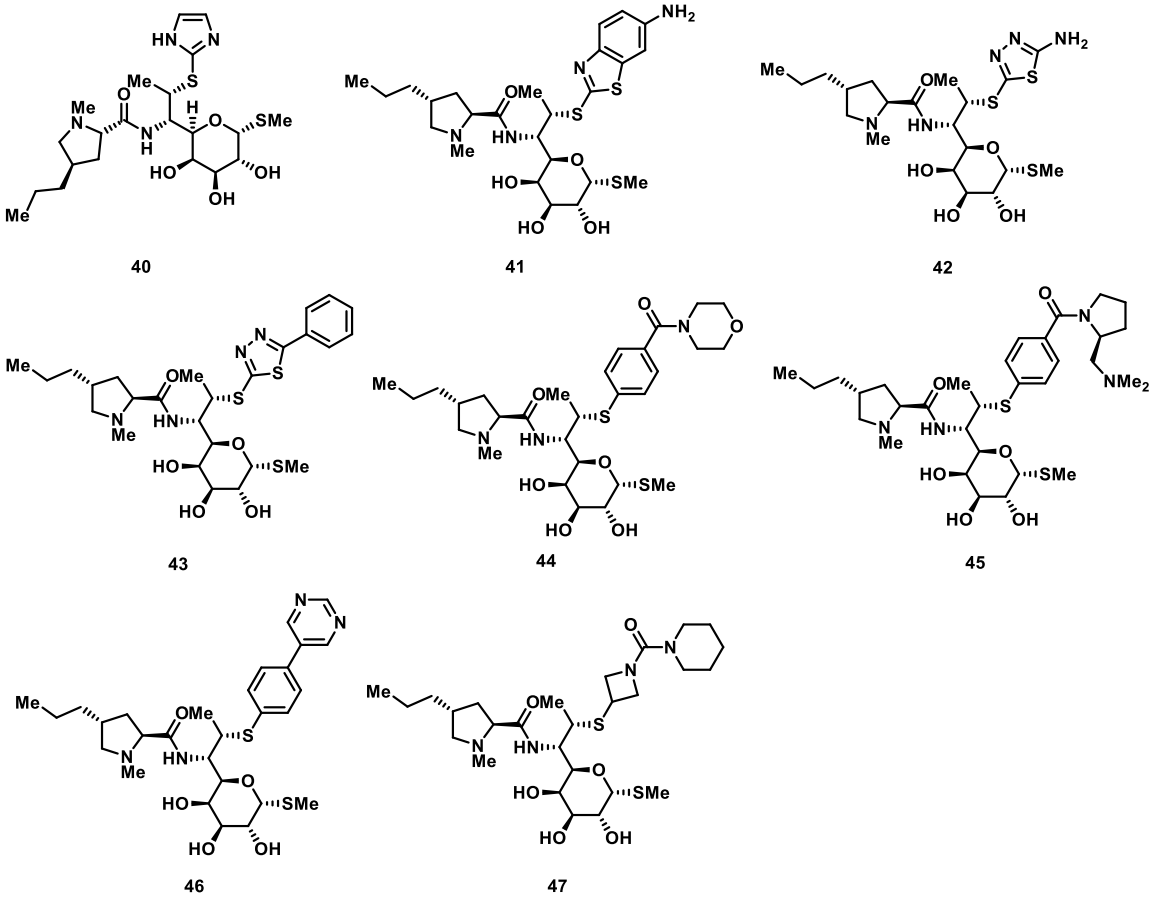


Figure 2.7. LCM derivatives by modifying at the C_7 position with thio substituents.

3-Dimensional analysis of **44** and the peptidyl transferase was investigated. Based on the docking results and SAR analysis, filling a space around the C_7 position of LCM has an important role in enhancing antibacterial activities by hydrogen bonding, π-π stacking or CH–π interaction to an undefined binding site on 23S rRNA. The location of a basic group in the C_7 side chain is an important factor to enhance antibacterial activities. It is important for a substituent to keep a specific size, length, and three-dimensional direction for appropriate binding to rRNA. Importantly, 7(*S*)-configuration of lincomycin derivatives is found to be necessary for enhancing antibacterial activity.

2.1.4.2 Modification at the proline moiety

Since 1,2,3-triazoles are known amide bond isosteres resistant to hydrolytic cleavage and oxidative transformations, Vasella and co-workers reported[51] several 1,2,3-triazole analogs (**48**) (**Figure 2.8**), but all of them proved inactive against the wild-type and A2058G mutant *M. smegmatis* cells. A hydrogen atom and a methyl group at the 1'-position of the proline moiety could maintain potency. Antibacterial activity increased as the number of carbon atoms increased at the 4'-position, but the maximum activity was achieved when pentyl or hexyl moieties were present, such as U24279A (**11**). 4'-*cis*-isomers were roughly one-half as active as the corresponding *trans*-isomer. The introduction of hetero atoms to the 4'-position resulted in lost activity. Expanding the five-membered ring to a six- or seven-membered ring and simultaneously optimizing the side chain at 4' position was a good strategy to improve the potency, such as pirlimycin (**9**) and VIC-105555 (**10**). Conversely, analogs with 4'-*cis*-piperidine moieties showed greater antibacterial activities than those with 4'-*trans*-pyrrolidine moieties.

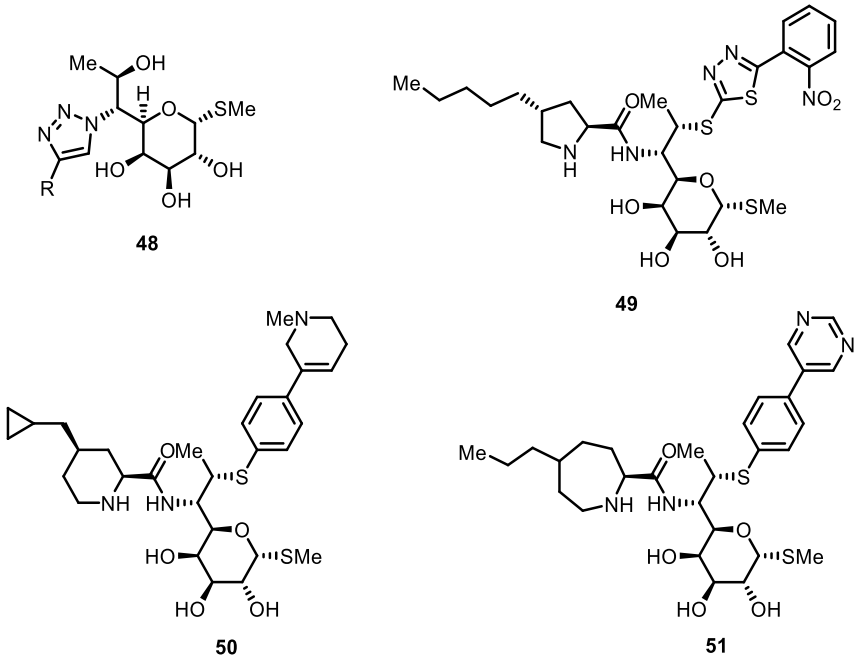


Figure 2.8. LCM derivatives obtained by modifying at the proline moiety.

Researchers of Japan's Meiji Seika Pharma Co. also synthesized a series of lincomycin derivatives, such as compound **49-51** (**Figure 2.8**), by modifications at the proline moiety and C_7 positions, and they all improved antibacterial activities against *S. pneumoniae* possessing the *erm* gene.

In 2021, Myer's group reported[52] a rigid oxepanoproline which was linked to MTL (**3**) to produce an antibiotic of exceptional potency and spectrum of activity, which was named iboxamycin (**Figure 2.9**, **52**). Iboxamycin is effective against ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*) including strains expressing *erm* and *cfr* genes. Soon after, they reported[53] a bridged macrobicyclic antibiotic cresomycin (**53**) which exhibited in vitro and in vivo efficacy against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

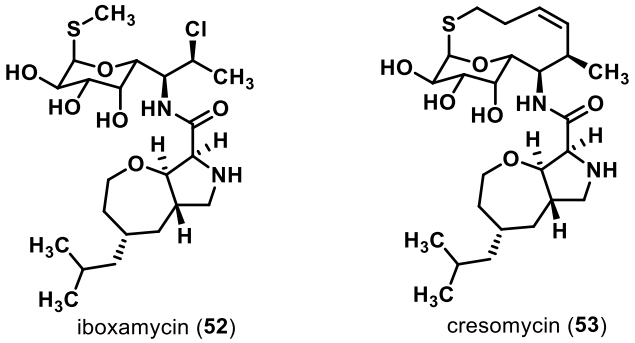


Figure 2.9. Iboxamycin and cresomycin.

2.2 Computer-aided Molecular Design

In molecular modeling, docking has been employed as a fast way to estimate the binding pose of a given compound within a specific target (protein or RNA) and predict binding affinity. In this case, the lincosamide antibiotics bind to the 23S rRNA of the 50S ribosomal subunit to prevent protein synthesis. Hence, we collaborated with Dr. Fourches' group identify some potential compounds by developing a QSAR model followed by screening a virtual library of analogs.

2.2.1 Molecular design

Azasugars are sugars with the ring oxygen replaced by nitrogen, which occur widely in plants and microorganisms. Recent studies have shown that azasugars possess many different biological properties and can be used to treat a wide range of conditions, including diabetes, viral infections, tumor metastasis, lysosomal storage disorders, and cystic fibrosis[54–57] (**Figure 2.10**, **54-60**).

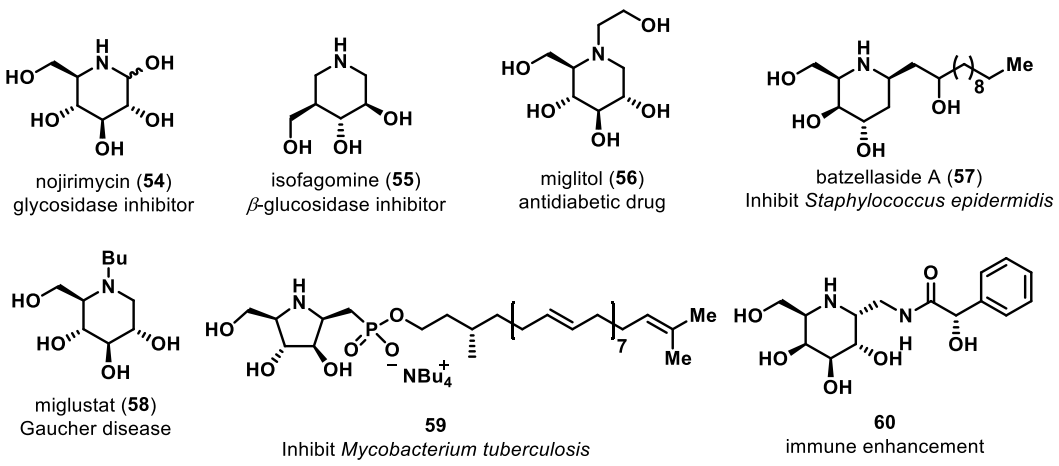


Figure 2.10. Structures of representative azasugars with biological activities.

Although it's difficult to improve the antibacterial activity by modifying the sugar moiety due to the H-bonding, we envision that the aza-sugar clindamycin derivatives by replacing the oxygen atom with a nitrogen atom could improve antibiotic potency or expand the antibacterial spectrum. As shown in **Figure 2.11**, we used different piperidines with varying numbers of hydroxyl groups to replace the pyranose. Some highly optimized sulfur-aromatic motifs from the literature were selected to replace the chlorine atom. Analogs that removed the methyl group at the C_7 position were also designed to simplify the molecular structure. Amide isosteres are generally introduced to modulate polarity and bioavailability, while ester isosteres are used to improve metabolism. Therefore, some small amide bioisosteres were adopted to replace the amide, such as cyclopropane, oxetane, trifluoromethyl, monofluoro-substituted double bond and ester. Moreover, we also selected some optimized ring-expansion motifs from the literature and designed some proline structures with amide bond at the end of the side chain to replace the pyrrolidine part. After systematic enumeration and 4 generations, 629 theoretical analogs were designed in total.

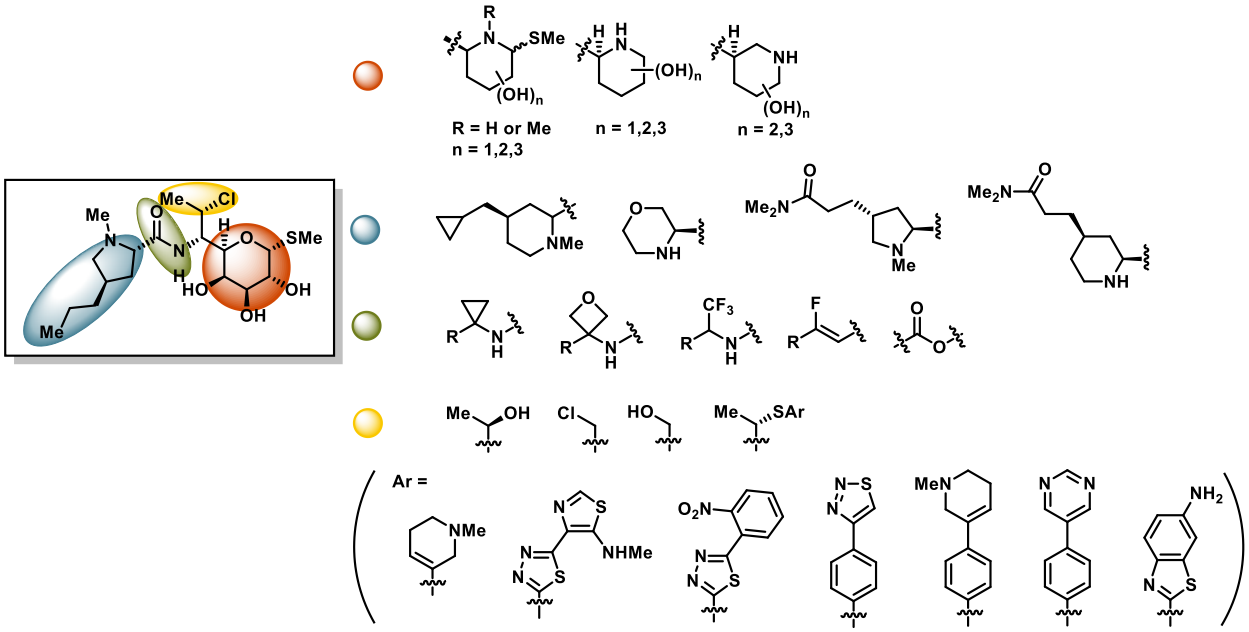


Figure 2.11. Molecular design of our virtual library.

2.2.2 Docking results

To develop a QSAR model, 207 CLDM analogs with their MIC data were collected from the literature and *E. coli* ribosome (PDB code 4V7V) was selected as receptor to perform docking calculations. At first, the receptor was processed with Protein Preparation Wizard to add hydrogen atoms, remove water molecules and metal ions, and cut atoms that are further than 50Å from the native clindamycin ligand. Next, 207 ligands were prepared with LigPrep, and then docked in *E. coli* receptor with the Glide®. Once pose generation was completed, the resulting ligand binding pose was evaluated by both standard precision (SP) and extra precision (XP) scoring functions. SP and XP are scoring options for sampling ligand position and conformation in the binding pocket of the protein/RNA to find the best pose with the best interaction.

The docking results showed that the best docked compounds were in the protonated states. It's reported that there are 6 hydrogen bonds and 2 van der Waals interactions between the native

clindamycin and *E. coli* receptor. The best docking poses of CLDM are shown in **Figure 2.12**. Scoring calculations showed that the best binding free energy of native clindamycin was -8.85 kcal/mol in SP and -10.58 kcal/mol in XP and the second-best docking poses in SP (-8.62 kcal/mol) and XP (-10.49 kcal/mol) had 6 hydrogen bonds in common (**Figure 2.13**). Although SP is better adapted to RNA than XP[58] and in this case SP option is better to recognize the most susceptible compounds, it is good to have both scores. All reported analogs with improved MICs had better docking scores than the native clindamycin, which demonstrated that there was a good correlation between docking score and MIC value in the developed QSAR model and gave us increased confidence for our virtual library screen.

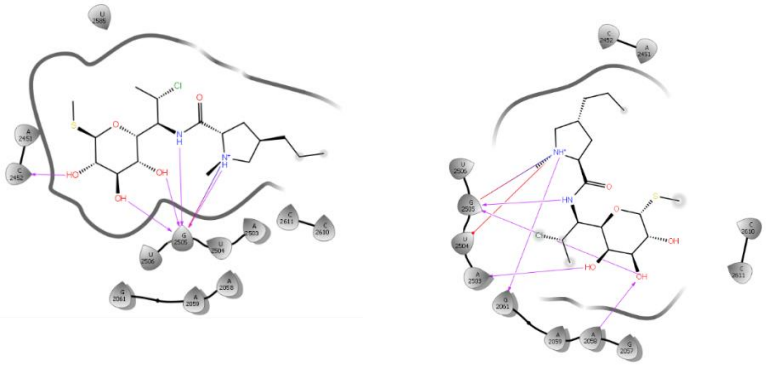


Figure 2.12. The best docking pose of CLDM in SP (left) and XP (right).

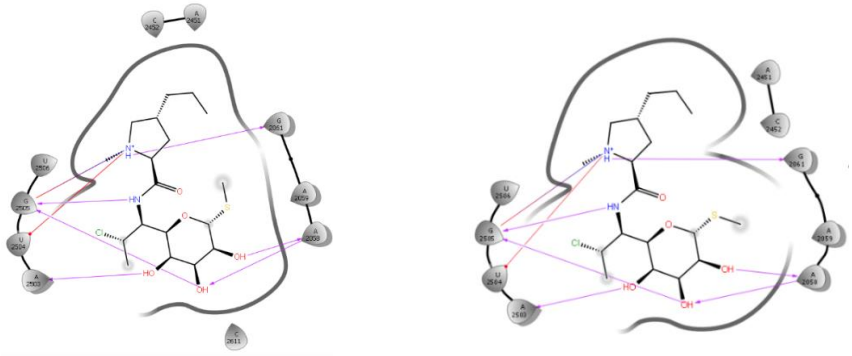


Figure 2.13. Docking pose of CLDM in SP (left) and XP (right) with highest number (6) of hydrogen bonds.

With QASR model in hand, 629 theoretical analogs were docked. The docking results showed that 241 ligands have better SP docking score than native clindamycin (<-8.85 kcal/mol) and 165 ligands with XP option (<-10.58 kcal/mol). For example, SP score and XP score of T583 (**61**) were -7.67 kcal/mol and -8.38 kcal/mol. However, aza-CLDM (T49, **62**) has a poor docking scores (SP >-8.715 kcal/mol and XP = -10.151 kcal/mol) (**Figure 2.14**).

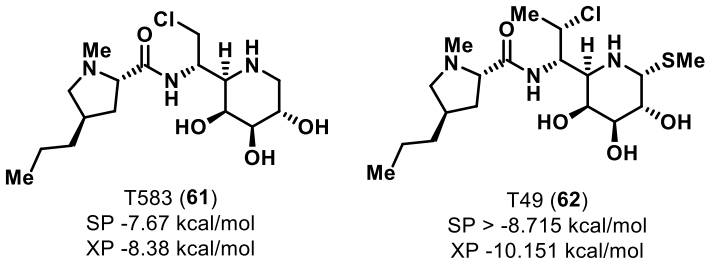


Figure 2.14. Docking results of T583 (**61**) and aza-CLDM (T49, **62**).

Although those theoretical compounds do not have better SP docking scores than native clindamycin, they still have good scores in the same range as the native pose which is around -8 kcal/mol and the differences are almost within 1 kcal/mol. Besides, as we can see from the selected docking pose of T584 and T585 (**Figure 2.15**), azasugar interactions with *E. coli* receptor have one extra H bond between N atom of piperidine and *E. coli* receptor. Hence, we will propose that aza-sugar clindamycin derivatives could have good, yet unique, binding affinity with the *E. coli* ribosome.

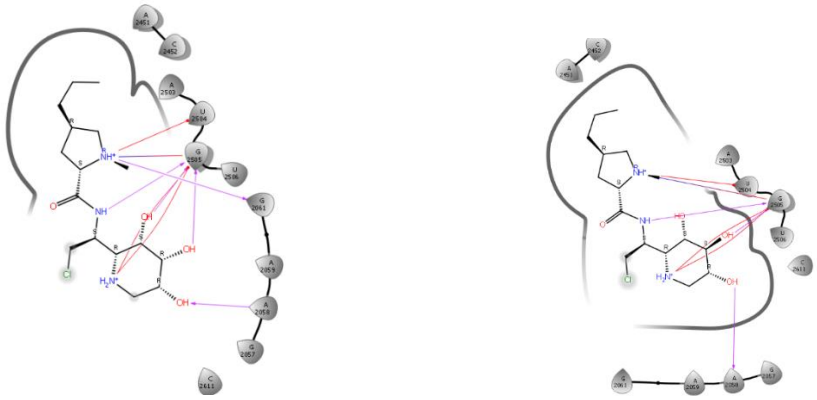
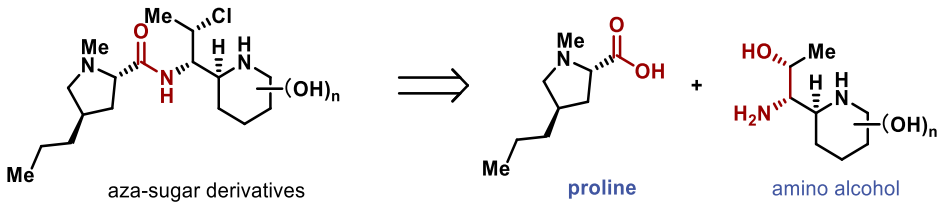


Figure 2.15. Docking poses of T584 (left) and T585 (right) in SP option.

Next, we will focus on the synthesis. Apparently, the aza-sugar derivatives could be divided into two moieties, the proline moiety and the amino alcohol moiety (**Scheme 2.4**).



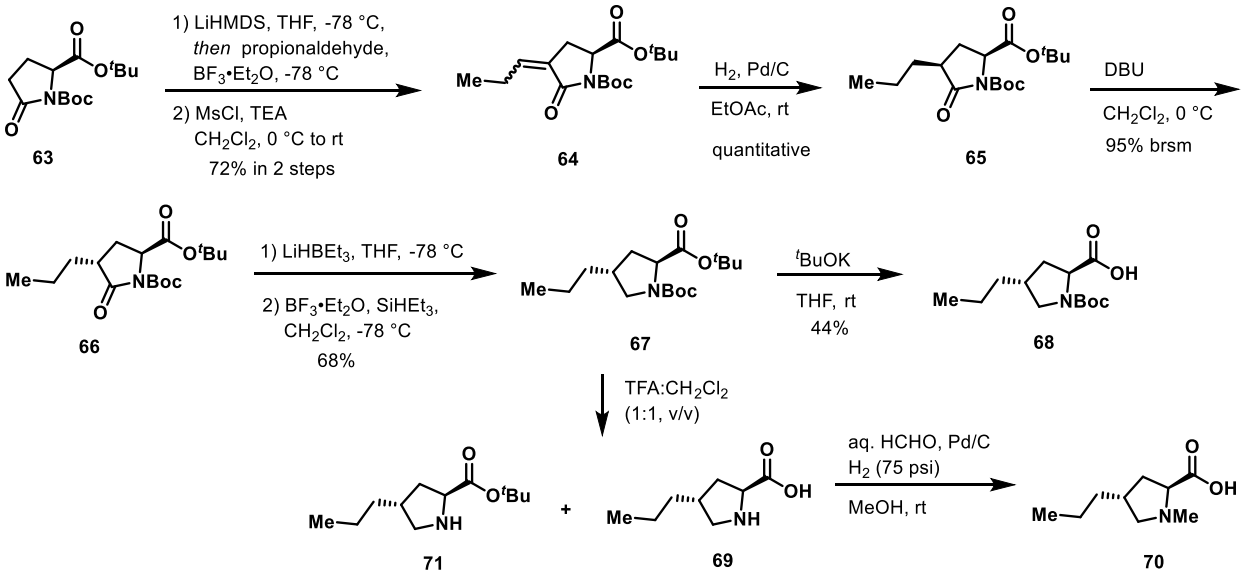
Scheme 2.4. Retrosynthetic analysis towards the aza-sugar CLDM derivatives.

2.3 Attempts towards the Proline Moiety

2.3.1 Original synthetic route toward the proline moiety

The proline moiety was synthesized by our previous group member Dr. Bram Frohock, who used the commercially available *tert*-butyl *N*-Boc pyroglutamate (**63**) as the starting material (**Scheme 2.5**). Aldol condensation with propionaldehyde and subsequent dehydration[59] resulted in a mixture of the 4-propylidenepyroglutamates **64** in 72% yield over two steps. The mixture of **64** was hydrogenated on Pd/C affording the *cis* 4-propyl pyroglutamate **65** in quantitative yield. The inversion of the configuration was achieved by treatment with DBU in 95% yield (brsm).

Afterwards, the chemoselective two-steps reduction[60] of **66** afforded *N*-Boc 4-propyl proline **67** in 68% yield in 2 steps. The subsequent treatment with TFA of **67** led to the *N*-Boc proline **68**. However, when this route was revisited, less than 20% yield was obtained in the first two steps with the aldol condensation product recycled. Besides, in the last step, when **67** was treated with TFA at rt for 12 h, both **71** and **69** were formed which suggested the Boc group fell off first. However, the tert-butyl group could be selectively removed by ^{t}BuOK in 44% yield. In 2019, Bates group[61] reported a method to convert **69** to **70** via reductive amination in the presence H_2 at 75 psi and the product was purified by ion exchange chromatography which increases the synthetic difficulty. Additionally, it will also take a few steps to convert the **67** to the desired product **70**. Therefore, we decided to revise the synthetic route.

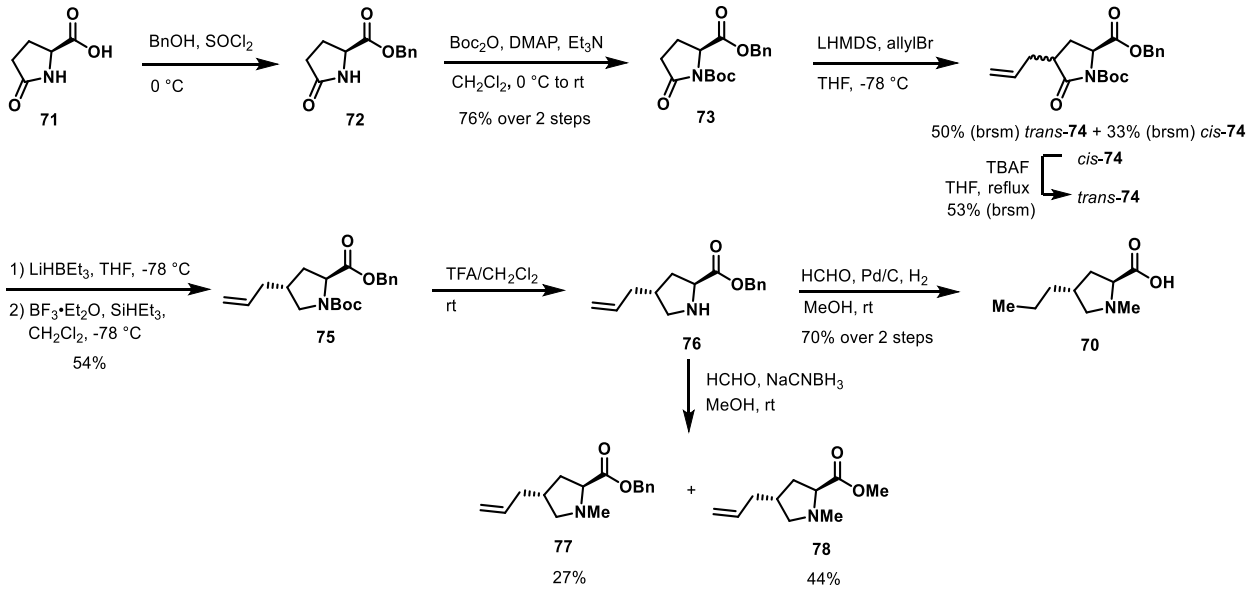


Scheme 2.5. The original synthetic route toward the proline moiety.

2.3.2 Revised synthetic route toward the proline moiety

Considering the low yield of the aldol condensation and the difficulty of selective deprotection of the Boc group, we proposed that switching the *tert*-butyl ester to benzyl ester could make the late-

stage modification easier and the propyl side chain could be installed by alkylation. In 2000, Dr. Llinàs-Brunet and her co-workers were able to access the 4-allyl substituted *N*-Boc pyroglutamic acid benzyl ester **75**, but no procedure and data were reported in the paper[62]. Thus, we started from the cheap L-pyroglutamic acid **71**, which underwent benzyl ester formation and *N*-Boc protection to afford the commercially available **73** in 76% yield over two steps (**Scheme 2.6**). Next, we attempted allylation according to the procedure of Ezquerra *et al*.[63], which resulted in a mixture of *trans*/*cis* isomers **74** in good yield. Afterwards, the *cis*-**74** could be epimerized in the presence of TBAF to afford *trans*-**74** in 53% yield after recycling of the *cis* isomer. Then, *trans*-**74** underwent chemoselective reduction and Boc deprotection to generate the intermediate **76**. With **76** in hand, we proposed that the desired product **70** could be accessed by reductive amination and hydrogenation. Unfortunately, a mixture of **77** and **78** was obtained after reductive amination with $NaCNBH_3$ as the reducing reagent. To our surprise, when replacing $NaCNBH_3$ with Pd/C in presence of H_2, the desired product **70** could be prepared in one step in 70% yield over two steps. Overall, we could access the desired proline moiety in 5 steps from benzyl *N*-Boc pyroglutamate or 7 steps from L-pyroglutamic acid.

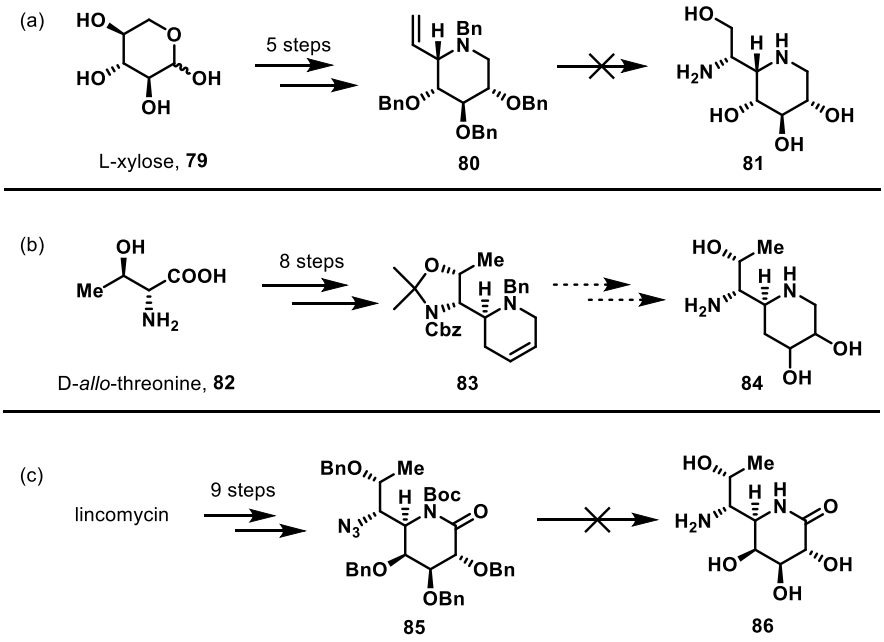


Scheme 2.6. Revised synthetic route toward the proline moiety.

2.4 Attempts towards the Amino Alcohol Moiety

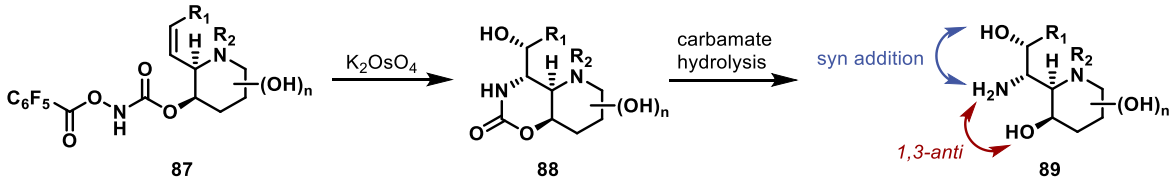
2.4.1 Synthetic strategies towards the amino alcohol moiety and model study

At the very beginning, we proposed that the amino alcohol moiety could be synthesized through aminohydroxylation from alkene **79** (**Scheme 2.7a**) which was prepared from L-xylose in 5 steps. However, the Sharpless asymmetric aminohydroxylation condition didn't work. Besides, several attempts have been made, such as haloazidation, aziridination, oxaziridination, epoxidation, and dihydroxylation, but we still couldn't convert the alkene to the amino alcohol moiety. At the same time, we tried to synthesize the des-hydroxylated aza-sugar through chiral pool synthesis with D-*allo*-threonine **82** as the starting material (**Scheme 2.7b**), which underwent eight steps to provide the intermediate **83**. However, this synthetic method hasn't been explored due to the low yield and expensive starting material. Afterward, we planned to synthesize the aza-clindamycin through semisynthesis based on Vasella's method[64] (**Scheme 2.7c**). Unfortunately, in the debenzylation step, we failed to completely remove all the benzyl groups of the azide intermediate **85**.



Scheme 2.7. Previously synthetic strategies towards the amino alcohol moiety.

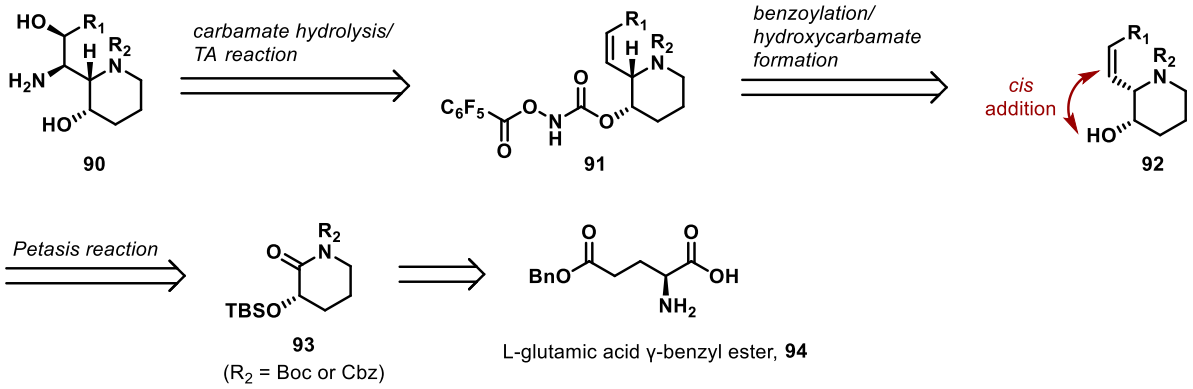
Finally, we assumed that the amino alcohol moiety could be constructed by a tethered aminohydroxylation (TA) reaction developed by Donohoe's group[65–69] in which the homoallylic alcohol derivatives could provide the vicinal amino alcohol with good diastereoselectivity (**Scheme 2.8**).



Scheme 2.8. Attempts towards the amino alcohol moiety by tethered-aminohydroxylation.

To explore the feasibility of tethered aminohydroxylation, we planned to synthesize the amino alcohol moiety **90** with one hydroxyl group on the piperidine (**Scheme 2.9**). Amino alcohol **90** could be prepared from benzoylated hydroxylamine **91** through TA reaction followed by carbamate hydrolysis. **91** was easily constructed via benzoylation and hydroxycarbamate

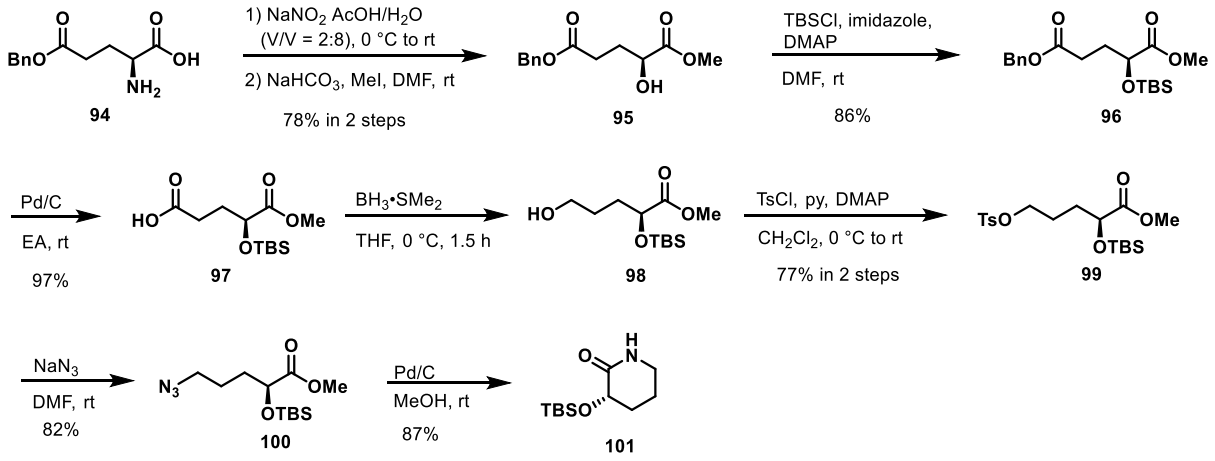
formation from *Z*-alkene **92**, which could be provided from the lactam **93** via a Petasis reaction. Access to lactam **93** has been reported with L-glutamic acid γ-benzyl ester **94** as the starting material.



Scheme 2.9. Retrosynthetic analysis of monohydroxylated piperidine.

2.4.2 Lactam synthesis

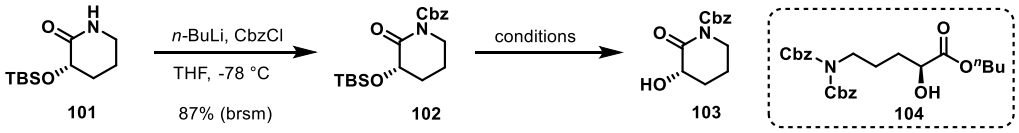
The synthesis of know δ-lactam[70] **101** is shown in **Scheme 2.10**. Starting from L-glutamic acid γ-benzyl ester **94**, the amino group was successfully converted to hydroxyl group with the retention of the stereocenter in the aqueous solution of $NaNO_2$ under acidic conditions, followed by in situ methylation to afford the ester **95** in 78% yield over two steps. TBS protection of secondary alcohol afforded the silyl ether **96** in good yield. Debenzylation proceeded smoothly over palladium on carbon under hydrogen gas in ethyl acetate instead of methanol in which dimethyl ester was observed. Subsequently, the acid **97** was reduced to alcohol **98** with borane dimethyl sulfide complex solution as reducing reagent, followed by tosylation and azide substitution to afford the azide **100** in good yield. Upon hydrogenation of azide **100**, spontaneous cyclization occurred to give the known δ-lactam **101** in moderate yield.



Scheme 2.10. Forward synthesis of the known δ-lactam **101** based on reported method.

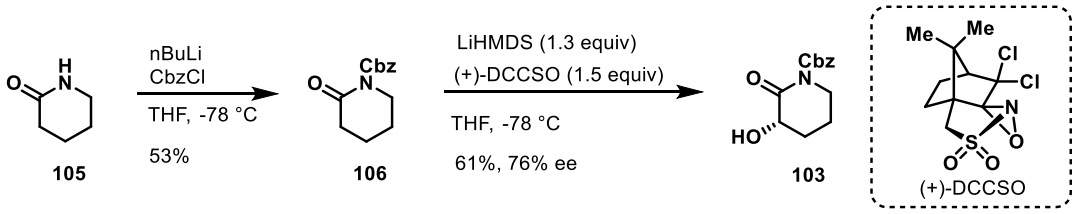
With the known δ-lactam **101** in hand, we were able to convert it to imide **102** in 88% yield with the treatment of *n*-butyl lithium and CbzCl. Here, the Cbz protection step depended on the quality of *n*-butyl lithium. When the *n*-butyl lithium solution was cloudy, indicating lithium hydroxide's presence, side product **104** was observed after TBS deprotection. In the removal of the TBS protecting group, TBAF solution gave the product **103** in low yield due to the basicity and adding acetic acid to the system resulted in a lower yield. Several different reagents have been screened (**Table 2.1**, entries 3-6), but none was better than TBAF. To our delight, the yield increased greatly when doing Cbz protection followed by TBS deprotection without purification. However, as noted, the yield of Cbz protection depended on the quality of *n*-butyl lithium and the starting material couldn't be entirely consumed in this step, which resulted in subsequent issues. Finally, HF•pyridine solution could provide the product **103** in 75% yield. Overall, the intermediate **103** was obtained in 10 steps.

Table 2.1. TBS deprotection.



entry	conditions	results
1	TBAF/THF	39%
2	TBAF/HOAc/THF	30%
3	TFA/CH_2Cl_2	13%
4	3 M HCl/MeCN	0
5	TBAT/THF	<10%
6	TASF/DMF	<10%
7	TBAF/THF (Cbz protection then deTBS without purification)	65% in 2 steps
8	HF·pyr/THF	75%

Due to the long synthetic route and inspiration from literature reports of the asymmetric α-hydroxylation[71], we explored the asymmetric synthesis from Cbz protected 2-piperidinone **106** with (+)-8,8-dichlorocamphoryl-sulfonyl (DCCSO) as oxidant (**Scheme 2.11**), which provided the product **103** in 61% yield with 76% ee (based on optical rotation results).

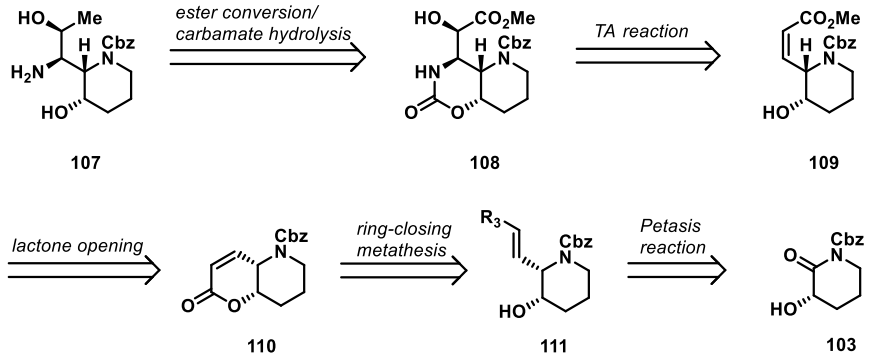


Scheme 2.11. Asymmetric synthesis of Cbz protected lactam **103**.

2.4.3 *Z*-Alkene installation by alkenyl addition

With compound **103** in hand, we envisioned that the amino alcohol **107** could be traced back to carbamate ester **108** through carbamate hydrolysis and conversion of methyl ester to methyl group (**Scheme 2.12**). A tethered aminohydroxylation reaction could provide carbamate ester **108** from *Z*-alkene **109**, which could be constructed through ring-closing metathesis followed by lactone

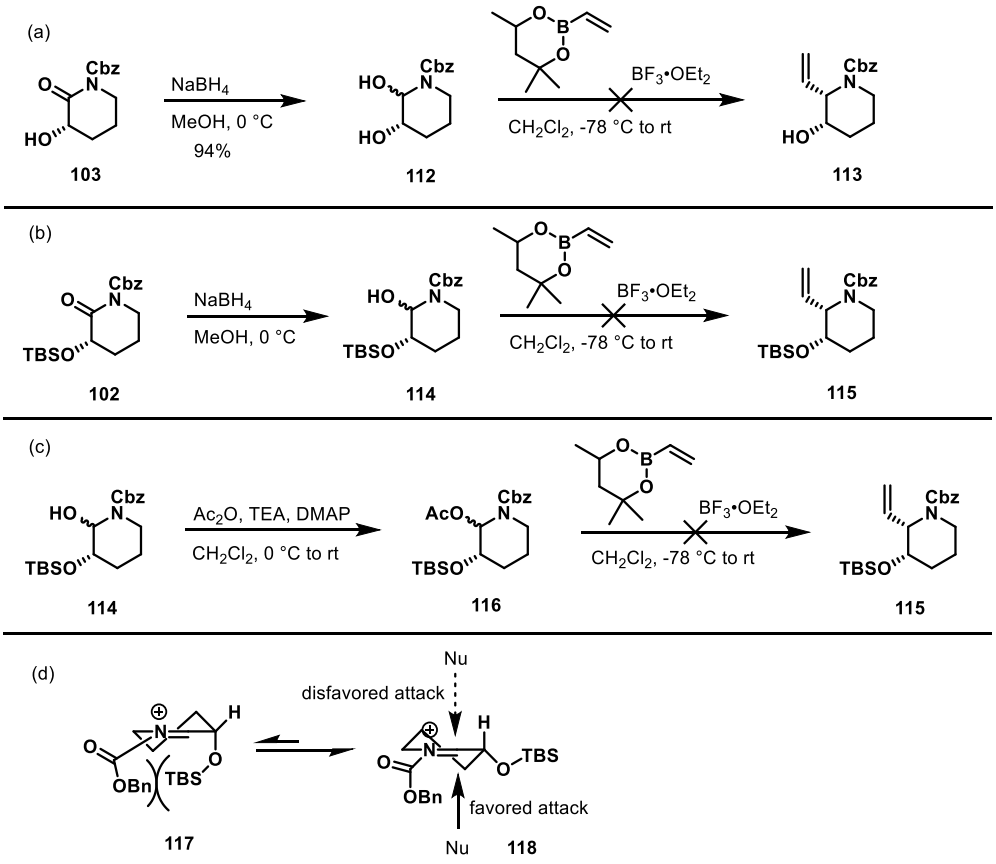
opening. Here, the biggest challenge is to install the alkene **111** from compound **103** via Petasis reaction.



Scheme 2.12. Retrosynthetic analysis of amino alcohol.

2.4.3.1 Attempts of alkenyl installation with vinylboronic acid pinacol ester

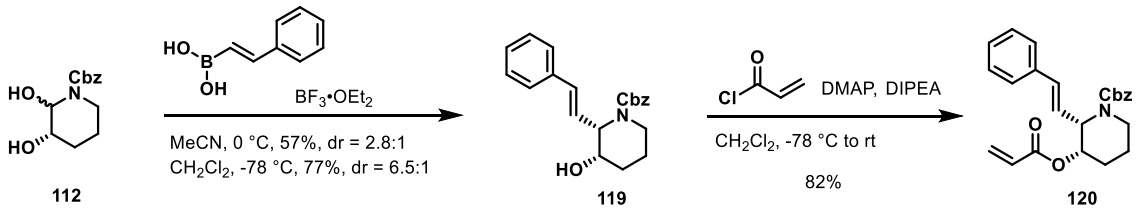
Compound **103** was reduced to hemiaminal **112** with $NaBH_4$ in methanol. Disappointingly, after the generation of *N*-acyliminium ion by in-situ dehydration in the presence of BF_3•OEt_2, the *cis* vinyl addition product **113** was not observed (**Scheme 2.13a**). To get the *cis* addition product, we also tested the TBS-protected hemiaminal **114** (**Scheme 2.13b**) and its acetylated form **116** (**Scheme 2.13c**). Here, the cis selectivity came from the two competitive conformations of **117** and **118** in the transition state (**Scheme 2.13d**). Due to the 1,3-interaction of the Cbz group with the sterically bulky TBS group, the more stable transition state **118** should be the favored conformation. An axial attack on **118** would occur from the same face of TBS group to generate a chair conformation which resulting in the *cis* addition product **57**. However, no product was detected in both reactions.



Scheme 2.13. Attempts of alkenyl addition with vinylboronic acid pinacol ester.

2.4.3.2 Attempts of alkenyl addition with (*E*)-styreneboronic acid

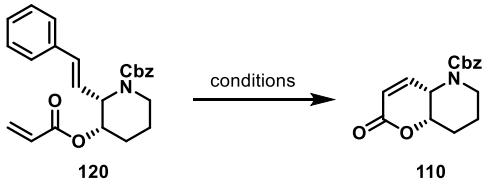
Given the failure of addition with vinylboronic acid pinacol ester, we reconsidered that our goal was to get the lactone. Therefore, what kind of reagent was used was not critical. Thus, the (*E*)-styreneboronic acid was tested, and to our surprise (**Scheme 2.14**), the *cis* addition product **119** was obtained in moderate yield with a 2.8:1 diastereomeric ratio when MeCN was used as the solvent. An increased yield with a 6.5:1 diastereomeric ratio was also observed when using CH_2Cl_2 as the solvent. Afterward, treating **119** with acryloyl chloride in the presence of DIPEA with a catalytic amount of DMAP provided the compound **120** in an 82% yield.



Scheme 2.14. Attempts of alkenyl addition with (*E*)-styreneboronic acid.

Next, we explored the ring-closing metathesis under different conditions (**Table 2.2**). To our disappointment, trace product was observed when using 7 mol % HG-II in DCE at 83 °C with compounds **119** and **120** recovered (entry 1). When increasing the amount of HG-II catalyst and the temperature to 110 °C, it was almost the same result (entry 2). Changing the catalyst to Grubbs I or II in CH_2Cl_2 or toluene didn't improve the outcone (entries 3-5). No promising results were detected even if a catalytic amount of $Ti(O^iPr)_4$ was added to the reaction system (entries 6-7).

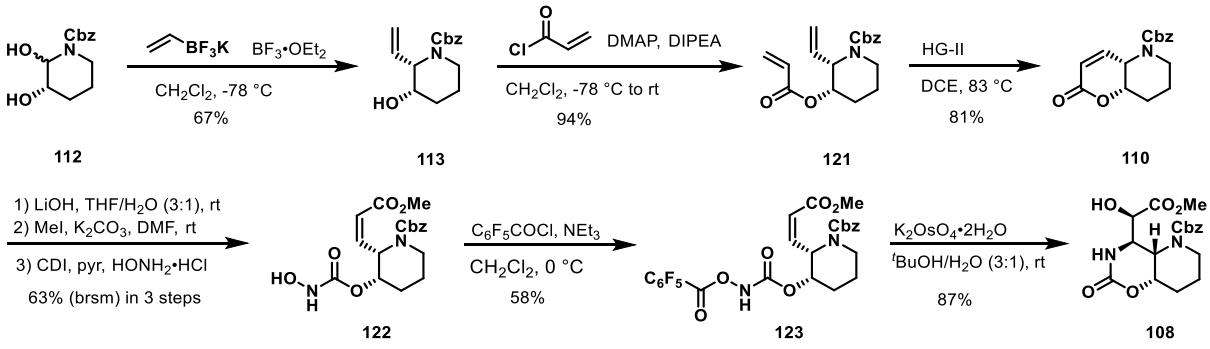
Table 2.2. Ring-closing metathesis.



entry	catalyst	solvent	temp.	results
1	7 mol % HG-II	DCE	83 °C	trace product, **119** and **120** recovered
2	20 mol % HG-II	PhMe	110 °C	trace product, SM left
3	8 mol % Grubbs I	CH_2Cl_2	45 °C	NR
4	17 mol % Grubbs I	PhMe	110 °C	NR
5	10 mol % Grubbs II	PhMe	110 °C	trace product, SM left and deacryloyed product formed
6	10 mol % Grubbs II, 0.2 eq $Ti(O^iPr)_4$	PhMe	80 °C	SM left, deacryloyed product formed
7	10 mol % HG-II, 0.2 eq $Ti(O^iPr)_4$	PhMe	110 °C	SM left, deacryloyed product formed

2.4.3.3 Alkenyl addition with vinyl BF_3K

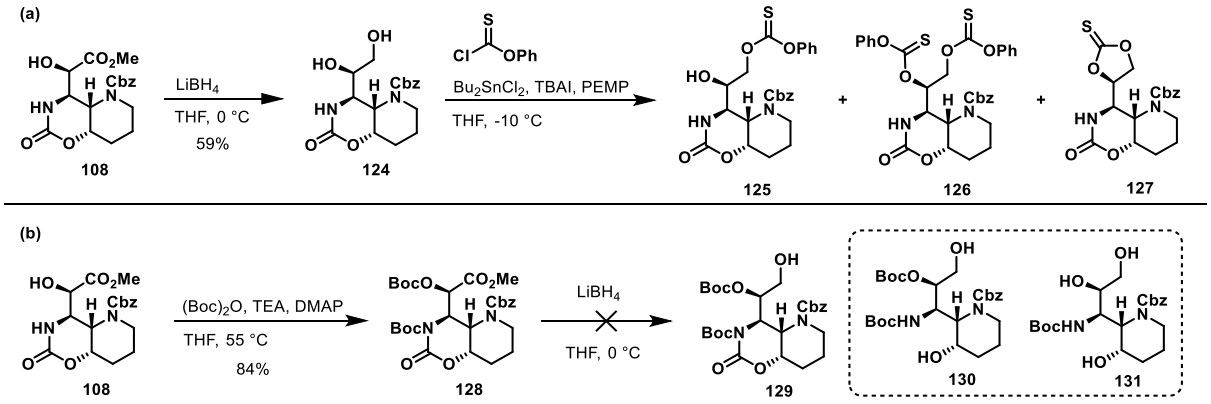
Due to the steric hindrance in the ring-closing metathesis step, we had to reconsider the installation of a small alkenyl group. To our delight, potassium vinyltrifluoroborate worked well and provided the *cis* addition product **113** in 67% yield in one single diastereomer (**Scheme 2.15**). Compound **113** was then treated with acryloyl chloride to provide **121** in good yield[72]. Then RCM with catalytic amount of HG-II in DCE at 83 °C yielded the lactone **110** in 81% yield. Hydrolysis of the lactone **110** with LiOH in the THF/H_2O mixed solvent and then methylation with MeI in the presence of K_2CO_3 afforded the *Z*-α,β-unsaturated ester, in which the hydroxyl group was converted to the *N*-hydroxy carbamate **122** in 63% overall yield. In preparation of the TA reaction precursor, **122** was acylated by slow addition of diluted penfluorobenzoyl chloride to give **123** in moderate yield; otherwise, the disubstituted product would form. Subsequently, compound **123** was subjected to the aminohydroxylation condition to afford the oxazinanone **108** in 87% yield in one single diastereomer.



Scheme 2.15. Forward synthesis of TA product **108**.

With the TA product **108** in hand, we attempted to convert the methyl ester group to methyl group. At the beginning, we explored the Barton-McCombie deoxygenation as shown in **Scheme 2.16**. TA product **108** was treated with $LiBH_4$ in THF at 0 °C to afford the 1,2-diol **124** in moderate

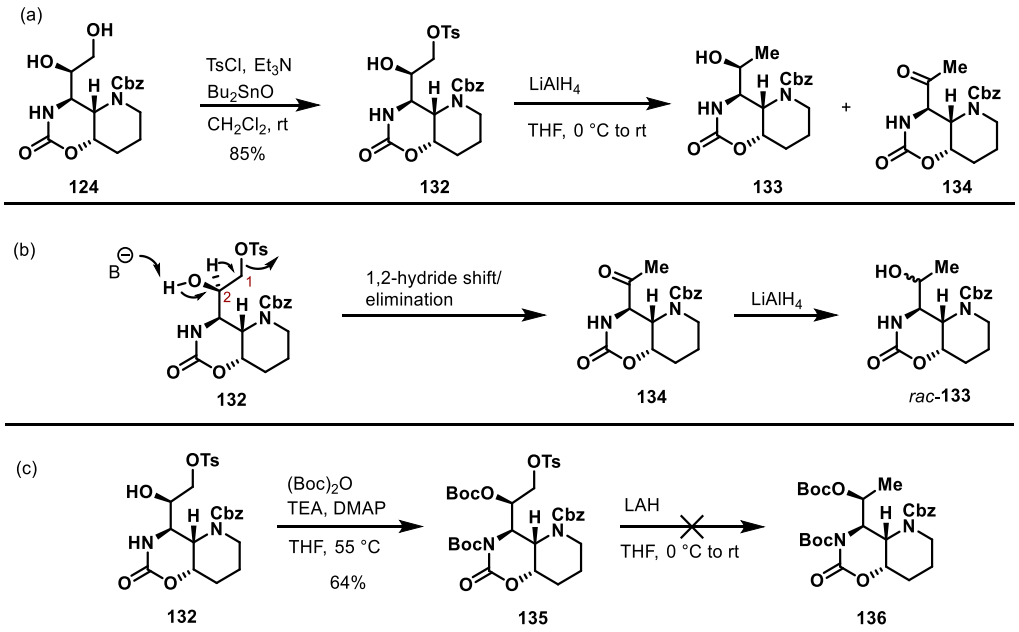
yield. To regioselective thiocarbonylate the primary alcohol in diol **124**, Bu_2SnCl_2 was used in the presence of tetrabutylammonium iodide (TBAI) and 1,2,2,6,6-pentamethylpiperidine (PEMP) at -10 °C[73]. However, a mixture of monothiocarbonate **125**, dithiocarbonate compound **126** and 1,3-dioxolane-2-thione **127** was observed and the monothiocarbonate **125** was easily converted into **127** once warming to rt. Next, we attempted to protect the secondary alcohol followed by methyl ester reduction. Unfortunately, the 6-membered carbamate opened during the $LiBH_4$ reduction of **128** which yielded a mixture of compounds **130** and **131**.



Scheme 2.16. Attempts of Barton McCombie deoxygenation.

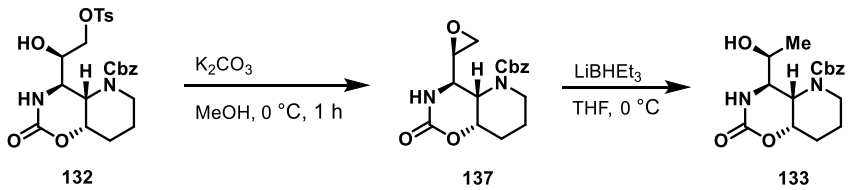
Given the difficulty in preparation of the Barton McCombie deoxygenation precursor, we anticipated that tosylate reduction would give a good result. Dibutyltin oxide catalyzed selective sulfonylation of 1,2-diol **124**, resulted in the complete sulfonylation at the primary alcohol in good yield[74] (**Scheme 2.17a**). In the $LiAlH_4$ reduction step, the mass peaks of desired product **133** and ketone **134** were detected by LC-MS. We assumed that $LiAlH_4$ (LiOH) could play a role of base rather than a reducing reagent which promoted an elimination to provide the enol which tautomerized to the ketone **134**. However, the proton at C2 was less acidic than that of C1 and was not expected to be abstracted initially. Later, we found that Dr. Rüedi and co-workers reported a 1,2-hydride shift followed by an elimination process during the tosylate reduction process in

2003[75]. The proposed mechanism was shown in **Scheme 2.17b**. Switching $LiAlH_4$ to an alternate reducing reagent, $LiBHEt_3$, didn't work well either. Therefore, we attempted to protect the secondary alcohol followed by $LiAlH_4$ reduction; unfortunately, a complex mixture was obtained, and no desired product was detected (**Scheme 2.17c**).



Scheme 2.17. Attempts of tosylated reduction.

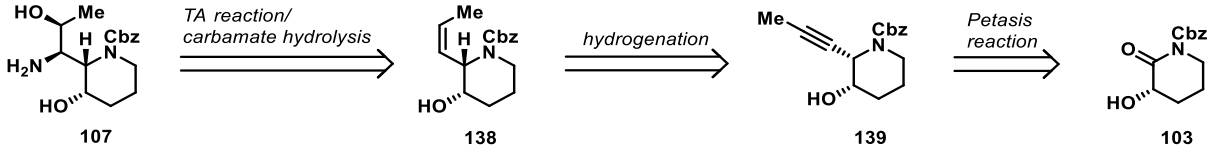
Finally, the tosylate **132** was converted to epoxide **137** in the presence of K_2CO_3 in methanol at 0 °C for 1 h; otherwise, the nucleophilic methoxide would attack the epoxide to yield the epoxide opening product (**Scheme 2.18**). Epoxide **137** was treated with $LiBHEt_3$ in THF at 0 °C to furnish the desired product **133**. Overall, it required 13 steps to access compound **133** from the Cbz protected lactam **103**.



Scheme 2.18. Epoxide reduction.

2.4.4 *Z*-Alkene installation by alkynyl addition

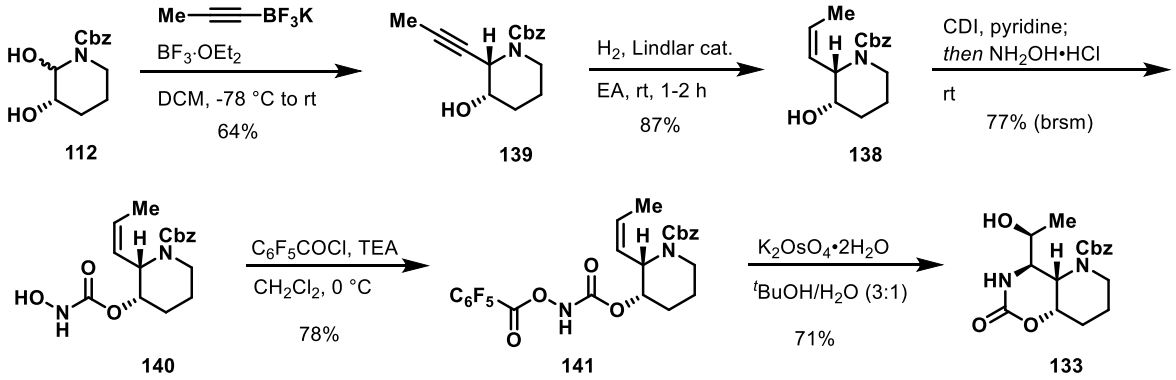
Due to the long linear synthetic route and the low overall yield, we revised our synthetic route (**Scheme 2.19**) and proposed that the *Z*-alkene **138** could be installed by alkyne hydrogenation in the presence of Lindlar catalyst. Moreover, conversion of the methyl ester to methyl group could be avoided by installation of a 1-propynyl group. Petasis reaction product **139** could be achieved with potassium trifluoro(prop-1-yn-1-yl)borate in the presence of Lewis acid.



Scheme 2.19. Revised retrosynthetic analysis towards amino alcohol.

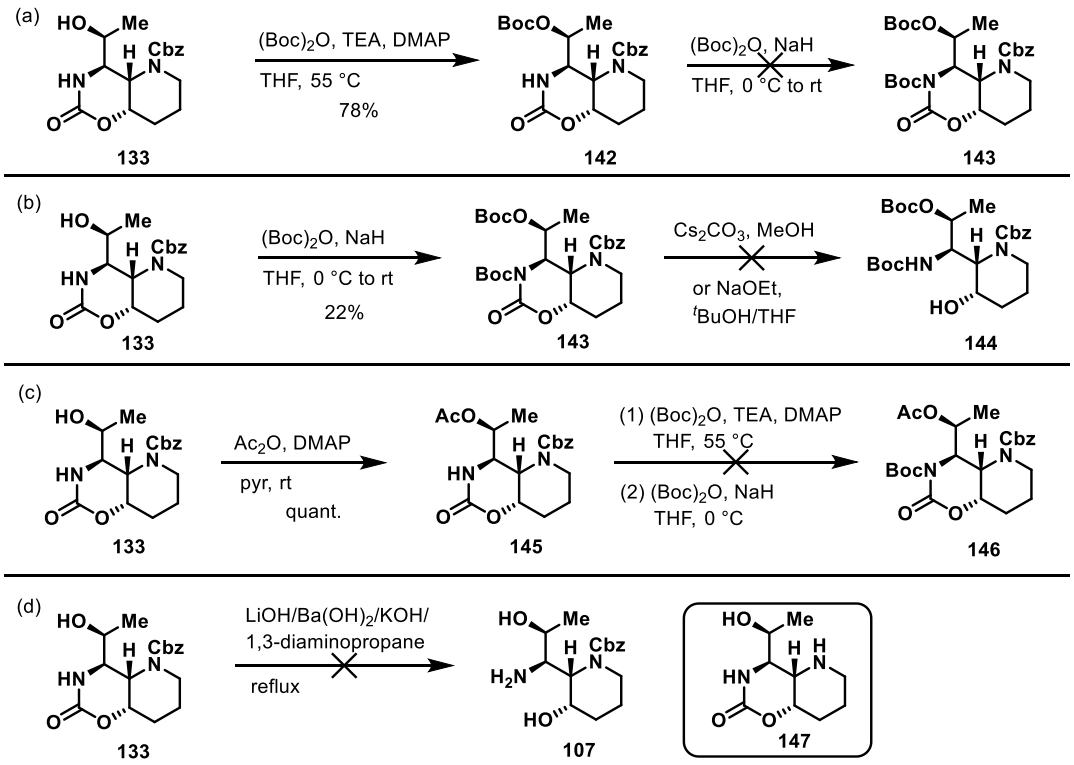
In 2009, Pyne and co-workers reported the Petasis reaction with the usage of potassium 1-alkynyltrifluoroborates in the presence of BF_3•Et_2O which resulted in *cis* adducts with good diastereoselectivity and in moderate to good yield[76].To our delight, hemiaminal **112** was reacted with potassium trifluoro(prop-1-yn-1-yl)borate to provide the *cis* adduct **139** in one single diastereomer and in moderate yield (**Scheme 2.20**). Hydrogenation with Lindlar catalyst under hydrogen gas in ethyl acetate at room temperature furnished the Z-alkene **138** in good yield. The reaction was monitored by LC-MS since **139** and **138** had almost the same Rf value and retention time, and the yield decreased greatly when extending the reaction time because **138** could be over

hydrogenated to the corresponding alkane. After formation *N*-hydroxyl carbamate, **140** was treated with diluted penfluorobenzoyl chloride to afford the TA reaction precursor **141**, which was then subjected to TA reaction system to give the product **133** in good yield.



Scheme 2.20. Forward synthesis towards the TA product **133**.

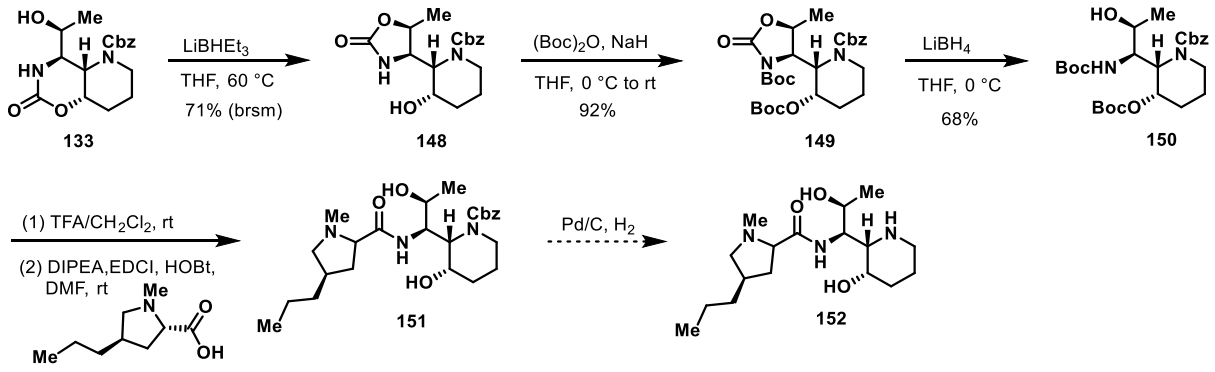
With the TA product **133** in hand, we attempted to open the 6-membered carbamate. Generally, activation of the *N* atom of carbamate with a Boc group, followed by the treatment with a catalytic amount of Cs_2CO_3, could make the hydrolysis easier[77]. However, it was difficult during the Boc protection process (**Scheme 2.21a**) as only the alcohol was protected in the presence of triethylamine with a catalytic amount of DMAP at 55 °C. Besides, no reaction was observed when the mono-Boc protected **142** was treated with excess NaH and $(Boc)_2O$. Subjecting **133** directly to excess NaH and $(Boc)_2O$ gave the desired product **143** in a very low yield (**Scheme 2.21b**). Moreover, in the subsequent carbamate hydrolysis step, the reaction proceeded slowly and accompanied by side products once heating up. Replacing the mild base Cs_2CO_3 with NaOEt removed the Cbz group simultaneously. And protection of the alcohol with a small acetyl group followed by *N*-Boc protection with triethylamine or NaH didn't work well either (**Scheme 2.21c**). Lastly, only the Cbz removal product **147** was observed when the TA product **133** was treated with strong bases, such as LiOH, $Ba(OH)_2$, KOH or 1,3-diaminopropane.



Scheme 2.21. Attempts towards 6-membered carbamate opening.

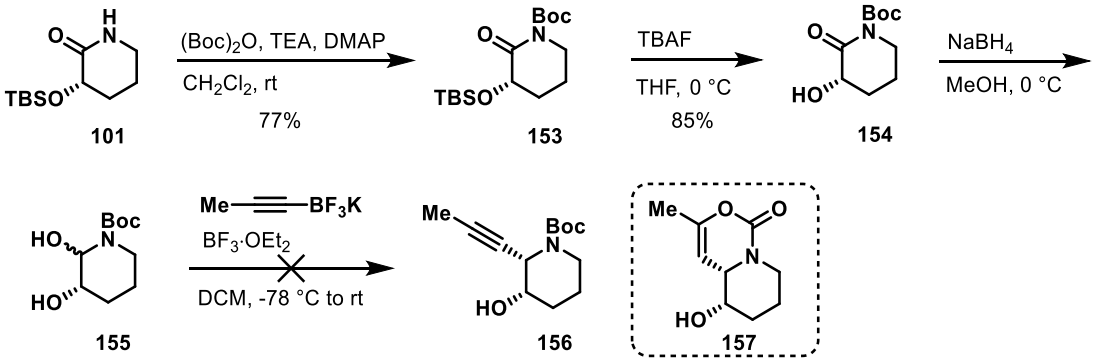
To this end, we revisited the related paper and found that the carbamate could migrate in the presence of reducing reagent $LiAlH_4$[78]. Therefore, we envisioned that transformation of a fused bicyclic compound **133** to compound **148** via carbamate migration would alleviate the rigidity and the freely rotating bond between the two rings in **148** would decrease the steric hindrance caused by the following di-Boc protection. Successfully, 5-membered carbamate **148** could be obtained in moderate yield and then it furnished the di-Boc protected product **149** in good yield when treated with excess NaH in THF (**Scheme 2.22**). Similarly, a complex mixture was observed when using Cs_2CO_3 to open the carbamate. At this time, we realized that the $LiBH_4$ could lead to the opening of carbamate ring of the compound **128**. Similarly, it worked well with compound **149** and the desired product **150** was obtained in good yield. After Boc deprotection with TFA, the amino

alcohol moiety was coupled with proline moiety using EDCI/HOBt in presence of DIPEA to afford product **151**. Removal of Cbz group is still being explored.



Scheme 2.22. Carbamate migration and coupling.

At the same time, considering the impact of the Cbz group in 6-membered carbamate opening process[79], we explored the Petasis reaction with Boc-protected hemiaminal **155** as substrate (**Scheme 2.23**). The known lactam **101** was protected with a Boc group with triethylamine and a catalytic amount of DMAP in CH_2Cl_2 to furnish compound **153** in 77% yield. In the removal of the TBS group, compound **153** was stable in TBAF solution and converted to **154** in good yield. To our surprise, $NaBH_4$ reduction followed by Petasis reaction afforded a bicyclic compound **157** instead of **156**. But this cyclization process has been reported by Lin's group recently[80,81]. The difference was that they observed the cyclization between the Boc protected hemiaminal with terminal alkynes or ynamides in the presence of BF_3•Et_2O.

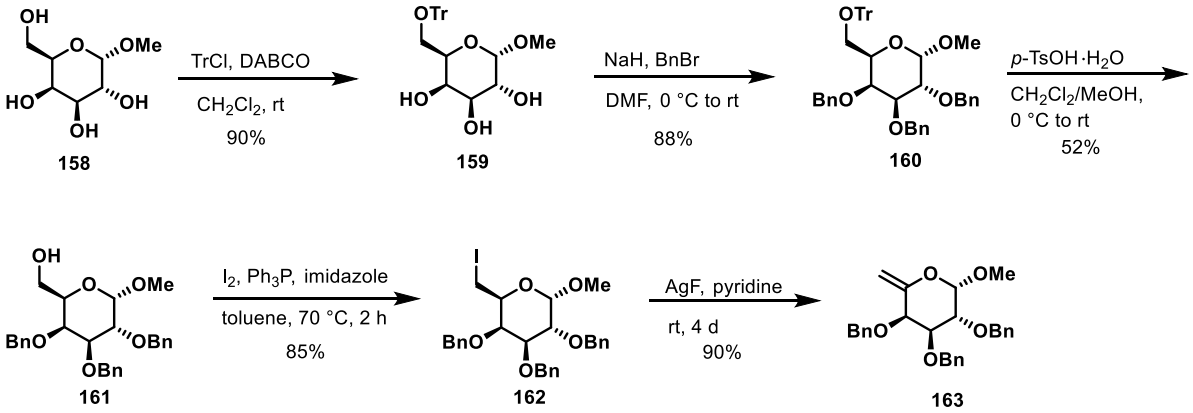


Scheme 2.23. Cyclization process.

2.5 Progress Towards the Trihydroxyl Aza-sugar Clindamycin Derivatives

2.5.1 Synthesis of *N*-substituted δ-lactam

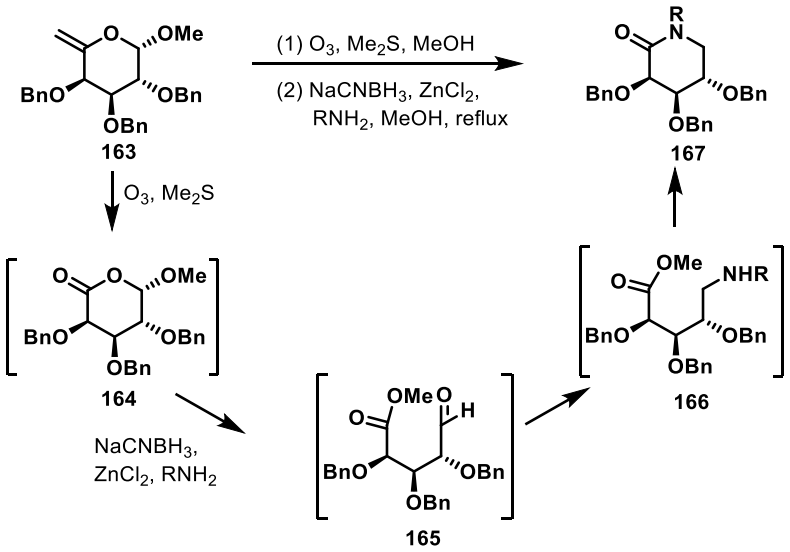
To obtain the target *N*-substituted δ-lactams, we attempted to follow Ye's method[82] from galactose alkene **163**. The galactose alkene **163** was easily obtained from commercially available methyl α-D-galactopyranoside **158** through five steps in high overall yield[83,84] (**Scheme 2.24**).



Scheme 2.24. Synthesis of galactose alkene **163**.

The challenge of this approach was that the key step of Ye's method was a one-pot tandem procedure with lactone **164** as intermediate which was achieved through ozonolysis at -78 °C (**Scheme 2.25**). Then without separation, the resulting methoxyl acetal lactone was directly treated

with $NaCNBH_3$, amines, and $ZnCl_2$ in the same pot to provide *N*-substituted δ-lactams **167** in high yield. It was proposed that lactone **164** underwent a tandem reaction in which it was first subjected to methanolysis to provide **165**, followed by reductive amination and cyclization yielding lactams **167**.

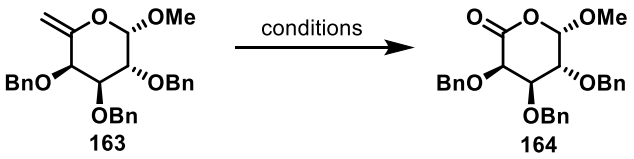


Scheme 2.25. Ye's one-pot reaction to synthesize *N*-substituted δ-lactam **167**.

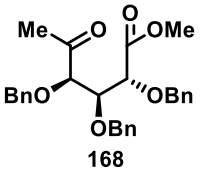
However, since ozone is not available in our lab, we attempted to access the target intermediate **167** in a stepwise manner. The first step was to convert the galactose alkene **163** to lactone **164**. Using $KMnO_4$ and *m*CPBA as oxidants, the desired product was detected by LC-MS but the reaction mixture was complex and hard to purify (entries 1-2, **Table 2.3**). When PCC was tested in dichloromethane at rt, no desired product was detected, and the SM could not be consumed completely (entry 3). Besides, a ring opening side product **168** was isolated in 41% yield and its structure was confirmed by ^1H and ^{13}C NMR. In the end, oxidative cleavage using ruthenium trichloride with sodium periodate/oxone to generate ruthenium tetroxide in situ was employed (entries 4-6). When the condition was buffered with 1.5 equiv. $NaHCO_3$ to prevent the build-up of

significant acidic side products, the best isolated yield was achieved using a mixed solvent system (MeCN/CCl_4/H_2O, 1:2:1).

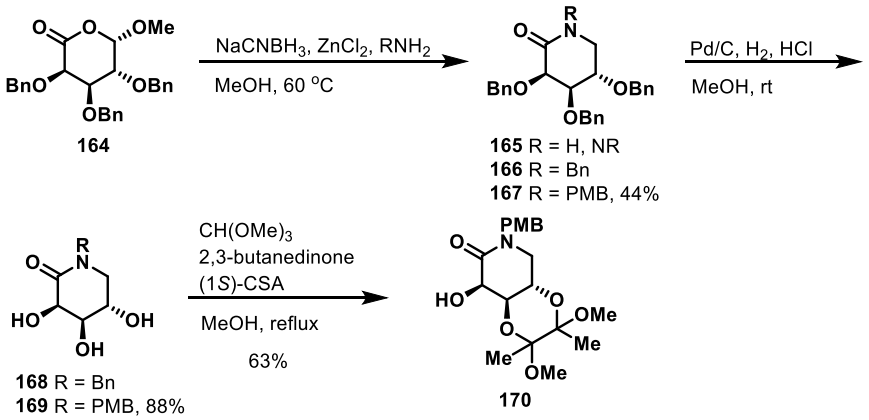
Table 2.3. Galactose alkene oxidation to lactone.



entry	conditions	results
1	$KMnO_4$, $NaIO_4$, K_2CO_3, acetone/H_2O, 12 h	DP detected, complex
2	*m*CPBA, CH_2Cl_2, 5 h	DP detected, complex
3	PCC, CH_2Cl_2, 24 h	no DP, **168** isolated (41%)
4	$RuCl_3$, $NaIO_4$,MeCN/CCl_4/H_2O, 2.5 h	13%
5	$RuCl_3$, oxone, $NaHCO_3$, MeCN/H_2O	26%
6	$RuCl_3$, $NaHCO_3$, $NaIO_4$,MeCN/CCl_4/H_2O	48%



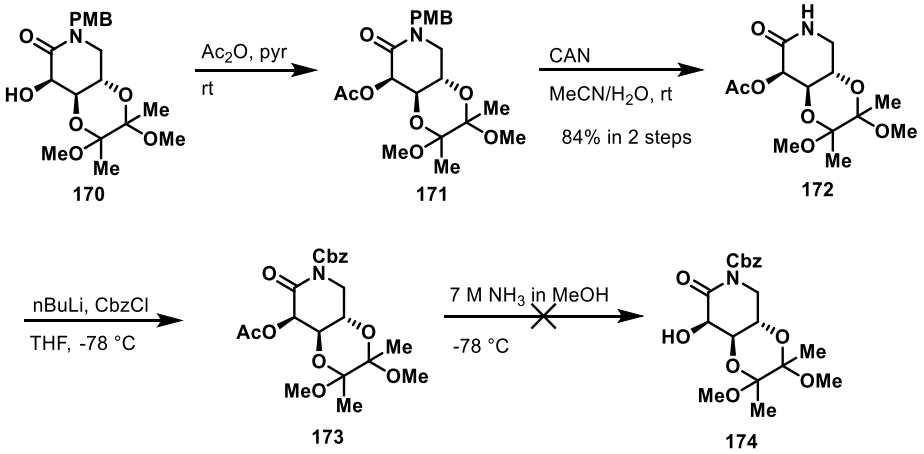
With the lactone **164** in hand, we explored the reductive amination and cyclization with different amines (**Scheme 2.26**). No reaction was observed when using 7 M ammonia in methanol solution. To our delight, the *N*-benzyl lactam **166** and *N*-PMB lactam 1**67** could be isolated. Considering the difficulty of removing *N*-benzyl group, *N*-PMB lactam 1**67** was used in the subsequent steps, which underwent debenzylation in the presence hydrogen with palladium on carbon in acidic condition and transdiol protection to afford the butane diacetal 1**70** in good yield.



Scheme 2.26. Attempts towards butane diacetal **170**.

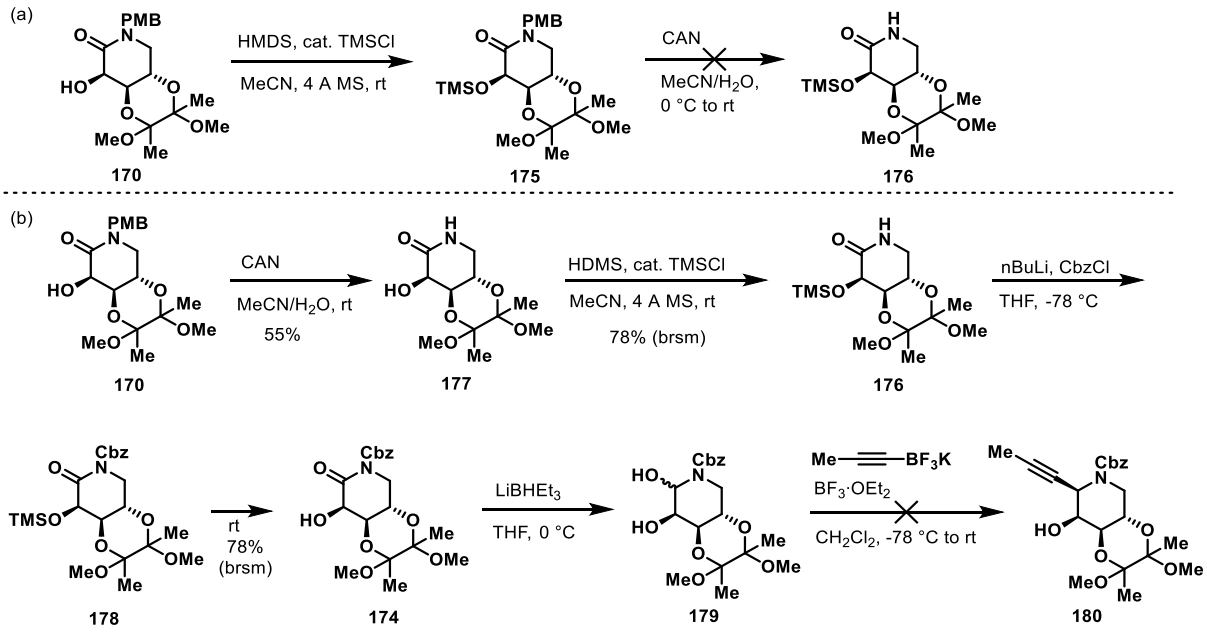
2.5.2 Strategies towards *N*-Cbz δ-lactam and attempt of Petasis reaction

With the butane diacetal protected lactam **170** in hand, we planned to protect the free alcohol first, followed by conversion of the PMB group to the Cbz group since the *N*-PMB lactam could not be reduced to a hemiaminal. However, neither TBS nor TES worked in the presence of base no matter at rt or reflux conditions. Fortunately, we were able to access the acetylated lactam 1**71** under the condition of Ac_2O/pyridine at rt (**Scheme 2.27**). Afterwards, 1**71** underwent PMB removal with CAN and Cbz protection to yield 1**73**. But the intermediate 1**74** could not be obtained by deprotection using saturated ammonia in methanol because the Cbz group fell off simultaneously.



Scheme 2.27. Attempts towards *N*-Cbz δ-lactam **174** through acetyl protection.

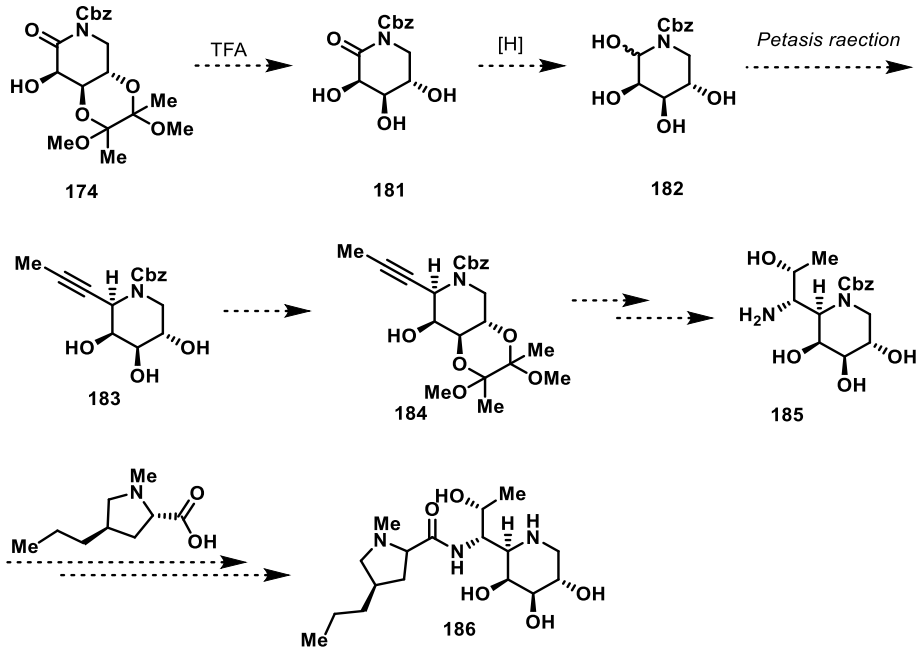
Finally, we chose TMS to protect the free alcohol. Due to the instability of TMS in acidic conditions when oxidizing with CAN (**Scheme 2.28a**), the PMB group was removed first, followed by TMS protection with HMDS in the presence of cat. TMSCl at rt to afford 1**76** in moderate yield (**Scheme 2.28b**). To our surprise, after Cbz protection, *N*-Cbz lactam **178** was unstable and could convert to **174** over time at rt on the bench top. With intermediate 1**74** in hand, we attempted the Petasis reaction after lactam reduction with super hydride. Unfortunately, no desired product was observed. We proposed that the butane diacetal protecting group could lock the conformation making the Petasis reaction difficult to through a chair-like transition state.



Scheme 2.28. Attempts towards *N*-Cbz δ-lactam **174** through TMS protection and Petasis reaction.

2.5.3 Future plan

Our future plan is to remove the butane diacetal protecting group first (**Scheme 2.29**), and then intermediate **181** could undergo reduction and Petasis reaction to produce the alkynyl addition product **184**. **184** is converted to the amino alcohol **185** via a TA reaction and carbamate opening, and subsequently coupling with proline generates the product **186**.



Scheme 2.29. Proposed synthetic route towards the trihydroxyl aza-sugar clindamycin derivative.

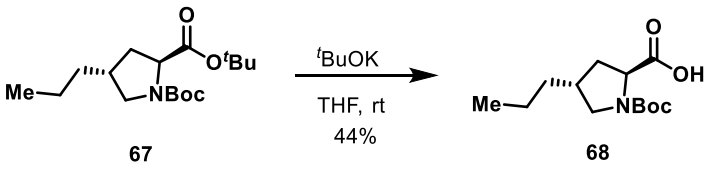
2.6 Experimental Details

General experimental procedures: All reactions were carried out under an inert nitrogen/argon atmosphere with anhydrous solvents under anhydrous conditions unless otherwise stated. Organic solvents were removed under reduced below 33 °C. Degassed solvents or reagents were degassed by sparging with argon for 20 minutes in an ultrasound bath at 25 °C. All reactions, except those conducted in the presence of water were carried out in flame-dried apparatus. Yields refer to chromatographically purified yield, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) analysis (pre-coated silica gel 60 F254 plates, 250 mm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with $KMnO_4$ solution (1.5 g of $KMnO_4$, 10 g of K_2CO_3, and 1.25 mL of a 10% NaOH solution in 200 mL of water) with heating or iodine (1 g of I_2 in 15 g of SiO_2). Reactions were also monitored by LC-MS (2.6 mm C18 50 x 2.10 mm column). Flash chromatography on SiO_2 was used to purify

the crude reaction mixtures and performed on a Biotage Isolera utilizing Biotage cartridges and linear gradients.

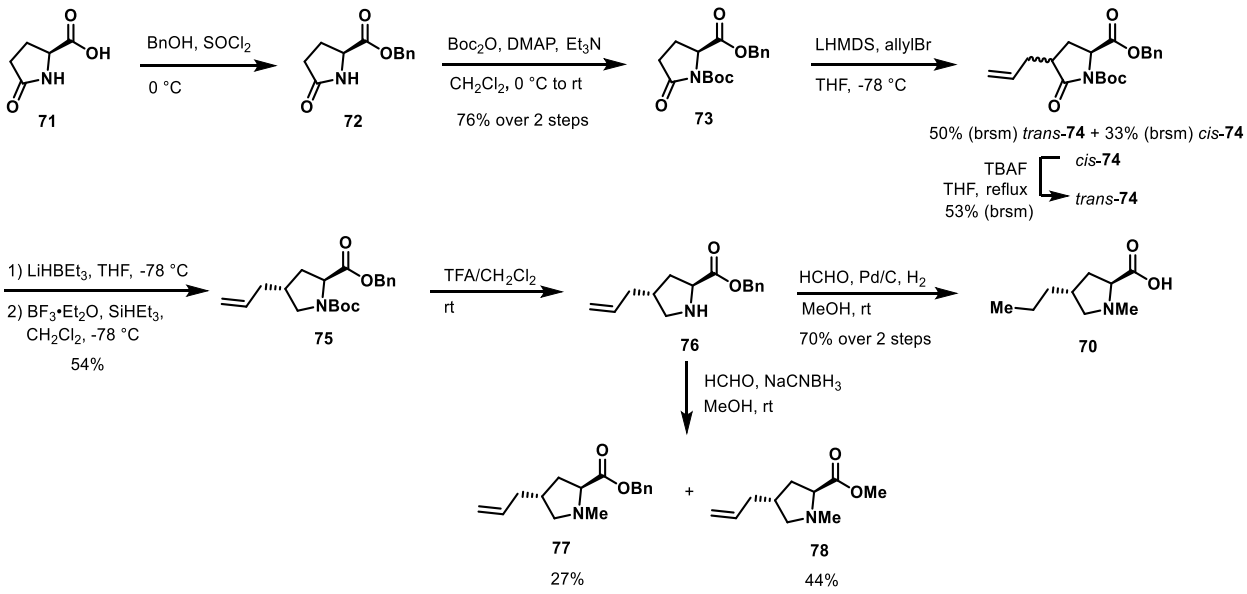
Materials: Tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) were obtained by passing the previously degassed solvents through activated alumina columns under nitrogen atmosphere. Triethylamine and *N*,*N*-diisopropylethylamine were distilled over CaH_2. Deuterated solvents (containing 0.03 to 0.05 vol % tetramethylsilane, TMS) were purchased from Cambridge Isotope Laboratories. All other reagents were purchased from commercial chemical companies and used without further purification, unless otherwise stated.

Instrumentation: Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus. Infrared spectra were determined on the Agilent Cary 630 FTIR spectrometer and data are represented as frequency of absorption (cm^{-1}). Optical rotation was measured on Jasco P-2000 polarimeter. ^{1}H and ^{13}C NMR spectra were obtained on a 500, 600 or 700 MHz instrument in $CDCl_3$ or CD_3OD unless otherwise noted. COSY, HSQC, and where necessary NOESY and HMBC spectra were used to aid structure assignments. Chemical shifts (δ) were reported in parts per million (ppm) with the residual solvent peak used as an internal standard ($CDCl_3$ ^{1}H NMR = 7.26 ppm, ^{13}C NMR = 77.16 ppm; CD_3OD ^{1}H NMR = 3.31, 4.87 ppm, ^{13}C NMR = 49.0 ppm; TMS ^{1}H NMR = 0 ppm, ^{13}C NMR = 0 ppm) and multiplicities are reported as observed. The following abbreviations were used to report NMR peak multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad. Low resolution mass spectra were obtained using electrospray ionization (ESI). High-resolution mass spectra were obtained on a high-resolution mass spectrometer – the Thermo Fisher Scientific Exactive Plus MS, a benchtop full-scan Orbitrapä mass spectrometer – using Heated Electrospray Ionization (HESI).



(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-propylpyrrolidine-2-carboxylic acid (68): To a solution of **67** (60.9 mg, 0.19 mmol) in THF (2 mL) was added potassium *tert*-butoxide (0.39 mL, 0.39 mmol, 1 M in THF) in N_2 atmosphere, and stirred at room temperature for 12 hours. Ammonium chloride (15 mL) was added to the reaction. The aqueous phase was extracted with EtOAc (15 mL x 3) and the combined organic phases were washed with brine (15 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography to afford the product **68** (22.2 mg, 44%).

MS (ESI$^-$): *m/z* 256 ([M-H]$^-$)



Benzyl (*S*)-5-oxopyrrolidine-2-carboxylate (72): To a solution of L-pyroglutamic acid (0.50 g, 3.87 mmol) in benzyl alcohol (3.50 mL, 34.1 mmol) at 0 °C was added thionyl chloride (0.56 mL,

7.74 mmol) dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. The reaction was quenched by the slow addition of saturated aq. $NaHCO_3$ (40 mL) and extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. Benzyl alcohol was removed by vacuum distillation and the crude mixture was used in the next step without purification.

2-Benzyl 1-(*tert*-butyl) (*S*)-5-oxopyrrolidine-1,2-dicarboxylate (73): To a solution of crude **72** in CH_2Cl_2 (19.4 mL, 0.2 M) at 0 °C was added DMAP (0.047 g, 0.39 mmol), triethylamine (0.54 mL, 3.87 mmol), and di-*tert*-butyl dicarbonate (1.02 g, 4.65 mmol). The reaction mixture was maintained at 0 °C for 1 h and then allowed to warm to room temperature and stirred for 16 h. The reaction was diluted with CH_2Cl_2 (50 mL), washed with saturated aq. NH_4Cl (2 x 40 mL), brine (40 mL), dried (Na_2SO_4), and concentrated in vacuo. The crude was purified by column chromatography to afford 0.96 g (78%) of **73** as a white solid. Compound characterization data was consistent with previous reports.

R_f = 0.18 (Hex:EtOAc = 3:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.25 (m, 5H), 5.20 (d, *J* = 12.2 Hz, 1H), 5.17 (d, *J* = 12.1 Hz, 1H), 4.62 (dd, *J* = 9.5, 2.9 Hz, 1H), 2.58 (dt, *J* = 17.5, 9.9 Hz, 1H), 2.45 (ddd, *J* = 17.5, 9.4, 3.5 Hz, 1H), 2.29 (dq, *J* = 13.4, 9.7 Hz, 1H), 1.99 (ddt, *J* = 13.0, 9.6, 3.2 Hz, 1H), 1.39 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 171.2, 149.2, 135.1, 128.7, 128.7, 128.6, 83.6, 67.4, 59.0, 31.1, 27.8, 21.5.

MS (ESI^+): *m/z* 337 ($[M+NH_4]^+$), 383 ($[M+MeCN+Na]^+$), 656 ($[2M+NH_4]^+$), 661 ($[2M+Na]^+$)

2-Benzyl 1-(*tert*-butyl) (2*S*)-4-allyl-5-oxopyrrolidine-1,2-dicarboxylate (74): To a solution of benzyl *N*-Boc pyroglutamate **73** (4.65 g, 14.6 mmol) was added a solution of LiHMDS (16.7 mL,

16.7 mmol, 1 M in THF) in THF (36 mL) at -78 °C. The stirring was continued for 1 h at -78 °C prior to the addition of a solution of allyl bromide (1.45 mL) in THF solution (14 mL). Upon more stirring for 1 h at -78 °C, the reaction was quenched with 100 mL saturated NH_4Cl solution and extracted with ethyl ether (100 mL x 3). The combined organic phases were dried over Na_2SO_4, filtered, and evaporated to dryness. The crude mixture was purified by column chromatography to afford 1.48 g of *trans*-**74** (50% brsm) and 0.96 g of *cis*-**74** (33% brsm).

2-Benzyl 1-(*tert*-butyl) (2*S*,4*R*)-4-allyl-5-oxopyrrolidine-1,2-dicarboxylate (*trans*-74): To a solution of the separated *cis*-**74** (0.21 g, 0.58 mmol) in THF (2.54 mL) stirred at room temperature was then added a TBAF solution (0.62 mL, 0.62 mmol., 1 M in THF) before refluxing the mixture at 85 °C for 1.5 h. The reaction was then quenched with saturated ammonium chloride solution (30 mL) and the aqueous layer was extracted with EtOAc (30 mL x 3). The combined organic layers were then washed with water and brine (30 ml), dried over Na_2SO_4, filtered, and finally evaporated to dryness. The *trans*-**74** (76 mg, 53% brsm) was purified by column chromatography to afford a white crystalline solid with 67.8 mg of *cis*-**74** recovered.

cis-**74**:

R_f = 0.3 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34-7.23 (m, 5H), 5.65-5.57 (m, 1H), 5.14 (d, *J* = 12.2 Hz, 1H), 5.10 (d, *J* = 12.2 Hz, 1H), 4.95 (dd, *J* = 10.2, 1.7 Hz, 1H), 4.89 (dd, *J* = 17.1, 1.5 Hz, 1H), 4.47 (dd, *J* = 9.1, 6.4 Hz, 1H), 2.59-2.48 (m, 2H), 2.38 (dt, *J* = 13.5, 9.3 Hz, 1H), 2.13-2.05 (m, 1H), 1.68-1.61 (m, 1H), 1.36 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 174.6, 171.4, 149.4, 135.1, 134.6, 128.8, 128.7, 128.7, 117.8, 83.8, 67.4, 57.5, 42.2, 35.2, 27.9, 26.6.

MS (ESI^+): *m/z* 377 ($[M+NH_4]^+$), 423 ($[M+MeCN+Na]^+$), 736 ($[2M+NH_4]^+$), 741 ($[2M+Na]^+$)

trans-**74**:

R_f = 0.43 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33-7.21 (m, 5H), 5.63 (ddt, *J* = 17.1, 10.2, 7.0 Hz, 1H), 5.14 (d, *J* = 12.1 Hz, 1H), 5.10 (d, *J* = 12.1 Hz, 1H), 5.02-4.95 (m, 2H), 4.51 (dd, *J* = 9.6, 1.6 Hz, 1H), 2.63 (dtd, *J* = 11.5, 8.8, 4.2 Hz, 1H), 2.56-2.49 (m, 1H), 2.15-2.05 (m, 2H), 1.93 (ddd, *J* = 13.4, 11.4, 9.7 Hz, 1H), 1.34 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 174.30, 171.15, 149.35, 135.09, 134.31, 128.69, 128.64, 128.49, 117.76, 83.56, 67.35, 57.13, 41.14, 34.37, 27.81, 27.74.

MS (ESI^+): *m/z* 377 ($[M+NH_4]^+$), 423 ($[M+MeCN+Na]^+$), 736 ($[2M+NH_4]^+$), 741 ($[2M+Na]^+$)

2-Benzyl 1-(*tert*-butyl) (2*S*,4*R*)-4-allylpyrrolidine-1,2-dicarboxylate (75): To a solution of *trans*-**74** (0.533 g, 1.48 mmol) in THF (7.4 mL) was added a solution of lithium triethylborohydride (1.78 mL, 1.78 mmol, 1 M in THF) at -78 °C under argon atmosphere. After 30 min, the reaction was quenched with saturated aq. $NaHCO_3$ solution (0.76 mL) and warmed to 0 °C. H_2O_2 (5 drops, 30% w/w) was added, and the mixture was stirred at 0 °C. After 20 min, the organic solvent was removed in vacuo, and the aqueous layer was extracted with CH_2Cl_2 (25 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude reaction mixture was dissolved in CH_2Cl_2 (7.4 mL) and used without further purification. After the addition of triethylsilane (0.24 mL, 3.0 mmol), the mixture was cooled to -78 °C. Boron trifluoride etherate (0.21 mL, 1.63 mmol) was dropwise added under argon atmosphere. Additional triethylsilane (0.24 mL, 3.0 mmol) and boron trifluoride etherate (0.21 mL, 1.63 mmol) were added after 30 min and the mixture was stirred at -78 °C for 2 h. The reaction mixture was quenched with

saturated aqueous $NaHCO_3$ (15 mL), allowed to reach room temperature, extracted with CH_2Cl_2 (30 mL x 3), and dried over Na_2SO_4. Evaporation of the solvent and purification by column chromatography yielded *N*-Boc proline *tert*-butyl ester **75** (0.275 g, 54 %).

R_f = 0.68 (Hex:EtOAc = 3:1)

^{1}H NMR (500 MHz, $CDCl_3$, rotamers with a ratio of 3:2) δ 7.46-7.24 (m, 5H), 5.72 (ddt, *J* = 17.2, 10.3, 7.0 Hz, 1H), 5.30-4.95 (m, 4H), 4.43 (dd, *J* = 9.0, 2.5 Hz, 0.4H), 4.31 (dd, *J* = 9.0, 2.8 Hz, 0.6H), 3.73 (dd, *J* = 10.5, 7.5 Hz, 0.6H), 3.65 (dd, *J* = 10.4, 7.6 Hz, 0.4H), 3.06 (dd, *J* = 10.6, 8.3 Hz, 0.6H), 3.00 (dd, *J* = 10.4, 8.4 Hz, 0.4H), 2.43-2.29 (m, 1H), 2.18-2.00 (m, 3H), 1.95-1.80 (m, 1H), 1.46 (s, 3.6H), 1.34 (s, 5.4H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 172.7, 154.3, 153.7, 135.8, 135.8, 135.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 116.5, 116.5, 79.8, 79.8, 66.6, 59.1, 58.8, 51.5, 51.3, 37.1, 36.9, 36.1, 36.0, 35.3, 28.4, 28.2.

MS (ESI^+): *m/z* 346 ($[M+H]^+$), 409 ($[M+MeCN+Na]^+$)

Benzyl (2*S*,4*R*)-4-allylpyrrolidine-2-carboxylate (76): To a solution of **75** (239.3 mg, 0.69 mmol) in CH_2Cl_2 (4.8 mL, 20 v/m) was added TFA (4.8 mL, 20 v/m) at rt and stirred for 3 h. Evaporate the solvent to afford a slurry, which was used for next step without purification.

MS (ESI^+): *m/z* 246 ($[M+H]^+$), 287 ($[M+MeCN+H]^+$)

(2*S*,4*R*)-1-Methyl-4-propylpyrrolidine-2-carboxylic acid (70): To a solution of **76** (0.69 mmol) in methanol (1.2 mL) was added 40% aqueous formaldehyde solution (84.9 µL, 0.762 mmol). This was followed by the addition of a 10% palladiumon-charcoal catalyst (23 mg) and the resulting slurry was stirred in a hydrogen atmosphere overnight. The slurry was then filtered through a Celite

pad to remove the catalyst. The pad was washed with methanol, and the combined filtrates were concentrated under reduced pressure to afford a yellow solid (83.4 mg, 70% over 2 steps), which is pure enough for the next step.

^{1}H NMR (500 MHz, CD_3OD) δ 3.83 (dd, *J* = 10.4, 4.2 Hz, 1H), 3.77-3.68 (m, 1H), 2.91 (s, 3H), 2.75 (t, *J* = 10.9 Hz, 1H), 2.35-2.20 (m, 2H), 2.16-2.04 (m, 1H), 1.49-1.27 (m, 4H), 0.94 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (150 MHz, CD_3OD) δ 171.4, 69.1, 62.5, 41.4, 37.7, 35.7, 35.3, 22.1, 14.2.

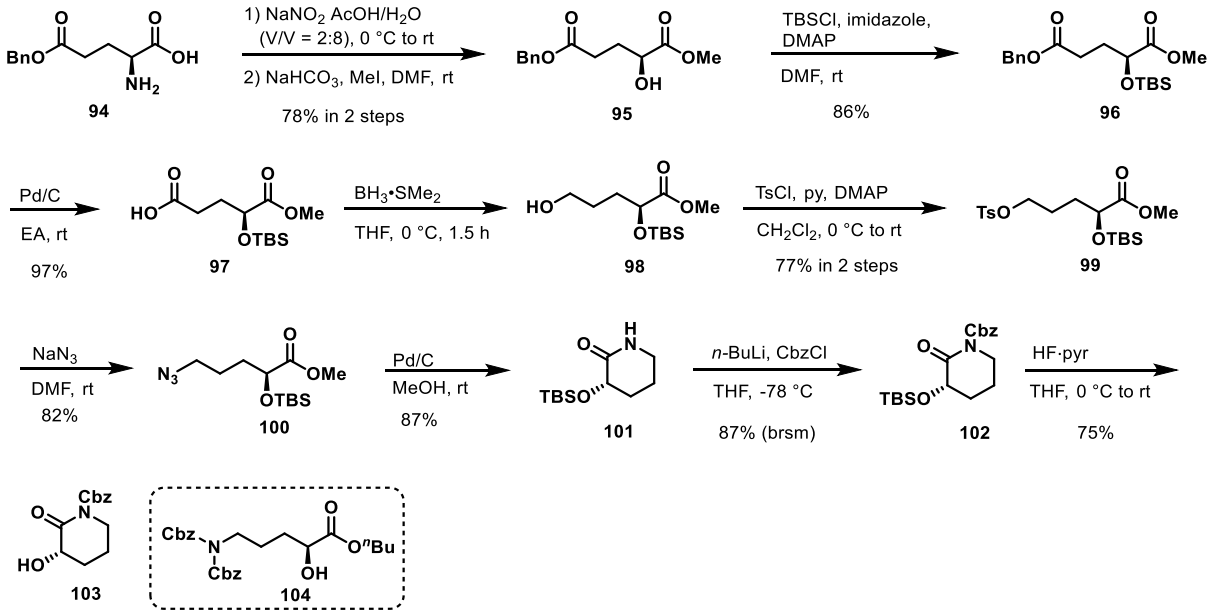
MS (ESI^+): *m/z* 172 ($[M+H]^+$), 343 ($[2M+H]^+$)

Benzyl (2*S*,4*R*)-4-allyl-1-methylpyrrolidine-2-carboxylate (**77**): To a stirring solution of **76** (286 mg, 1.17 mmol) in MeOH (8.1 mL) was added formaldehyde (0.3 mL, 3.5 mmol) and AcOH (0.4 mL, 7.0 mmol) at rt. After being stirred for 1 h, $NaCNBH_3$ (221 mg, 3.5 mmol) was added. The reaction mixture was stirred for 24 h. Volatiles were evaporated under pressure. The crude viscous oil was diluted with ice water (30 mL) and extracted with EA (30 mL x 3). The organic layer was washed with $NaHCO_3$ solution (30 mL) and brine solution (30 mL). Evaporation of the solvent and purification by column chromatography yielded **77** (83 mg, 27%) and **78** (94 mg, 44%).

MS of **77** (ESI^+): *m/z* 260 ($[M+H]^+$)

^{1}H NMR of **78** (500 MHz, $CDCl_3$) δ 5.84-5.62 (m, 1H), 5.08-4.90 (m, 2H), 3.78-3.64 (m, 3H), 3.28-3.16 (m, 1H), 3.08-2.96 (m, 1H), 2.44-2.32 (m, 4H), 2.16-2.05 (m, 3H), 2.05-1.96 (m, 1H), 1.86-1.73 (m, 1H).

MS of **78** (ESI^+): *m/z* 184 ($[M+H]^+$)



5-Benzyl 1-methyl (*S*)-2-hydroxypentanedioate (95): To a suspension of L-glutamic acid γ-benzyl ester **94** (10.0 g, 42.1 mmol) in a mixture of H_2O and acetic acid (210 mL, ratio 8/2) was dropwise added a 2 mol/L aqueous solution of a $NaNO_2$ (5.8 g, 84.0 mmol, 42 mL) over 30 min at 0 °C. The reaction mixture was then warmed to rt and stirred for 4 h, after which it became homogeneous. Water (100 mL) was then added to the mixture and the title compound was extracted by ethyl acetate (3 × 100 mL). The organic layers were combined, washed with water and brine, and dried over sodium sulfate. The solvent was removed by evaporation to give a viscous oil which was used for the next step without purification. To a solution of crude hydroxyacid in dry DMF (211 mL) was added sodium bicarbonate (14.2 g, 169 mmol) at 0°C. The solution stirred at 0°C for 30 min before the addition of iodomethane (5.78 mL, 92.7 mmol). The mixture was warmed to rt and stirred overnight. Then the mixture was added 150 mL water and extracted with ethyl acetate (150 mL x 3), washed with water (150 mL x 3), 5% aq. LiCl (150 mL x 3) and brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification by column

chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) afforded the product **95** (8.09 g, 76% in 2 steps).

$\boldsymbol{R_f}$ = 0.27 (Hex:EtOAc = 3:1)

MS (ESI^+): *m/z* 253 ($[M+H]^+$), 275 ($[M+Na]^+$), 316 ($[M+MeCN+Na]^+$)

5-Benzyl 1-methyl (*S*)-2-((*tert*-butyldimethylsilyl)oxy)pentanedioate (96): To a solution of compound **95** (3.15 g, 3.96 mmol) with imidazole (1.30 g, 19.1 mmol) and DMAP (152 mg, 1.2 mmol) in DMF (62 mL) was added *tert*-butyldimethylsilyl chloride (2.80 g, 18.6 mmol) at rt and stirred for 5 - 20 h. When the starting material was consumed, water (100 mL) was added and the reaction was extracted with ethyl acetate (100 mL x 3), washed with water (150 mL x 3), 5% aq. LiCl (150 mL x3) and brine (150 mL x 3), dried with Na_2SO_4, concentrated and purified by flash chromatography (SiO_2, gradient elution: 0 to 10% EtOAc in hexane) to afford the desired compound **96** (3.92 g, yield 86%)

$\boldsymbol{R_f}$ = 0.42 (Hex:EtOAc = 10:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42-7.28 (m, 5H), 5.12 (s, 2H), 4.29 (dd, *J* = 7.5, 4.5 Hz, 1H), 3.71 (s, 3H), 2.56-2.42 (m, 2H), 2.16-2.08 (m, 1H), 2.06-1.94 (m, 1H), 0.89 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H).

MS (ESI^+): *m/z* 367 ($[M+H]^+$)

(*S*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-methoxy-5-oxopentanoic acid (97): To a solution of compound **96** (3.92 g, 10.7 mmol) dissolved in wet ethyl acetate (107 mL) was added 0.39 g 10% Pd/C. The mixture was stirred at rt under H_2 gas until complete consumption of the SM. The

reaction was filtered and concentrated to afford the crude product **97** (2.87 g, yield 97%) which was pure enough and used for the next step without purification.

R_f = 0.13 (Hex:EtOAc = 3:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.30 (dd, *J* = 7.2, 4.7 Hz, 1H), 3.72 (s, 3H), 2.56-2.41 (m, 2H), 2.14-2.06 (m, 1H), 2.05-1.96 (m, 1H), 0.90 (s, 9H), 0.08 (s, 3H), 0.05 (s, 3H).

MS (ESI^+): *m/z* 277 ($[M+H]^+$)

Methyl (*S*)-2-((*tert*-butyldimethylsilyl)oxy)-5-hydroxypentanoate (98): To a solution of acid **97** (1.5 g, 5.4 mmol) in THF (54 mL) was added BH_3•SMe2 (5.4 mL, 10.9 mmol, 2 M THF) at 0 °C. The solution was stirred at 0 °C for 1.5 h. The reaction was quenched via the slow addition of saturated $NaHCO_3$. The mixture was extracted with ethyl acetate (60 mL x 3). The organic phases were then combined and washed with brine (100 mL x 3), dried over Na_2SO_4, and concentrated in vacuo to afford the product as a colorless oil which was used for the next step without purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.23 (t, *J* = 5.8 Hz, 1H), 3.68 (s, 3H), 3.64-3.55 (m, 2H), 2.21 (brs, 1H), 1.82-1.74 (m, 2H), 1.68-1.53 (m, 2H), 0.87 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H).

MS (ESI^+): *m/z* 263 ($[M+H]^+$)

Methyl (*S*)-2-((*tert*-butyldimethylsilyl)oxy)-5-(tosyloxy)pentanoate (99): To a solution of crude **98** in CH_2Cl_2 (108 mL) was added TsCl (1.56 g, 8.2 mmol), followed by Et_3N (1.5 mL, 10.9 mmol) and DMAP (66 mg, 0.54 mmol) at 0 °C and then warmed to room temperature and stirred overnight until the SM disappeared. The reaction was quenched by ice water, extracted with CH_2Cl_2 (100 mL x 3), washed with brine (100 mL x 3), dried over Na_2SO_4, concentrated, and purified by column

chromatography (SiO_2, gradient elution: 0 to 10% EtOAc in hexane) to afford the desired product **99** (1.73 g, yield 77% in 2 steps).

$\boldsymbol{R_f}$ = 0.25 (Hex:EtOAc = 10:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78, (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 4.19-4.13 (m, 1H), 4.08-4.00 (m, 2H), 3.69 (s, 3H), 2.44 (s, 3H), 1.78-1.67 (m, 4H), 0.86 (s, 9H), 0.04 (s, 3H), 0.00 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8, 144.9, 133.2, 130.0, 128.0, 71.5, 70.4, 52.0, 31.2, 25.8, 24.9, 21.8, 18.4, -4.9, -5.3.

MS (ESI^+): *m/z* 439 ($[M+H]^+$), 480 ($[M+MeCN+Na]^+$)

Methyl (*S*)-5-azido-2-((*tert*-butyldimethylsilyl)oxy)pentanoate (100): To a solution of compound **99** (1.32 g, 3.2 mmol) in DMF (32 mL) was added NaN_3 (0.31 g, 4.8 mmol) at 0 °C under N_2 gas and stirred at rt overnight. The reaction was quenched by water, extracted with ethyl acetate (50 mL x 3), washed with water (100 mL x 3), 5% aq. LiCl (100 mL x 3) and brine (100 mL x 3), dried over Na_2SO_4, concentrated, and purified by column chromatography (SiO_2, gradient elution: 0 to 10% EtOAc in hexane) to afford the desired product **100** as a colorless oil (0.75 g, yield 82%).

$\boldsymbol{R_f}$ = 0.42 (Hex:EtOAc = 10:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.24 (dd, *J* = 6.8, 4.8 Hz, 1H), 3.72 (s, 3H), 3.35-3.25 (m, 2H), 1.86-1.75 (m, 2H), 1.72-1.64 (m, 2H), 0.90 (s, 9H), 0.08 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 173.9, 71.8, 52.0, 51.3, 32.3, 25.8, 24.8, 18.4, -4.8, -5.3.

(*S*)-3-((*tert*-Butyldimethylsilyl)oxy)piperidin-2-one (101): To a solution of compound **100** (0.85 g, 3.0 mmol) in MeOH (59 mL) was added 85 mg 10% Pd/C . The mixture was stirred under H_2 gas at room temperature until the reaction was complete, filtered, concentrated, and purified by column chromatography (SiO_2, gradient elution: 0 to 50% EtOAc in hexane) to afford the desired product **101** as a colorless oil (0.59 g, yield 87%).

$\boldsymbol{R_f}$ = 0.38 (Hex:EtOAc = 1:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66 (brs, 1H), 4.09 (dd, *J* = 7.6, 4.5 Hz, 1H), 3.35-3.29 (m, 1H), 3.27-3.20 (m, 1H), 2.04-1.96 (m, 2H), 1.90-1.80 (m, 1H), 1.79-1.69 (m, 1H), 0.90 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H).

MS (ESI^+): *m/z* 230 ($[M+H]^+$), 293 ($[M+MeCN+Na]^+$)

Benzyl (*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2-oxopiperidine-1-carboxylate (102): To a solution of compound **101** (0.70 g, 3.05 mmol) in THF (30.5 mL) at -78 ^oC was added nBuLi (5.2 mL, 7.6 mmol, 1.47 M in THF). After 1 h, CbzCl (0.81 mL, 5.5 mmol) was added to the reaction. The reaction was stirred at this temperature for 2 h and then was quenched by the addition of a sat. $NaHCO_3$ solution, extracted with CH_2Cl_2 (50 mL x 3), washed with brine (100 mL x 3), dried with Na_2SO_4, and purified by column chromatography (SiO_2, gradient elution: 0 to 50% EtOAc in hexane) to afford the SM (130 mg) and product **102** (784 mg, yield 87% brsm).

$\boldsymbol{R_f}$ = 0.22 (Hex:EtOAc = 10:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.12 (m, 5H), 5.11 (s, 2H), 4.04 (dd, *J* = 8.2, 5.7 Hz, 1H), 3.66-3.48 (m, 2H), 1.95-1.87 (m, 1H), 1.86-1.76 (m, 1H), 1.75-1.60 (m, 2H), 0.74 (s, 9H), 0.00 (s, 3H), -0.05 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 154.2, 135.6, 128.6, 128.3, 128.1, 71.6, 68.5, 45.8, 30.4, 25.8. 19.9, 18.4, -4.5, -5.4.

MS (ESI^+): *m/z* 364 ($[M+H]^+$), 427 ($[M+MeCN+Na]^+$)

$[\alpha]_D^{22}$: -19.3°, (c = 0.01, MeOH)

Benzyl (*S*)-3-hydroxy-2-oxopiperidine-1-carboxylate (103): To a stirred solution of compound **102** (672 mg, 1.85 mmol) in THF (18.5 mL) was added HF•pyridine (1.4 mL, 55.5 mmol, 70% in pyridine, 40 M) dropwise at 0 °C. The resultant mixture was warmed to room temperature and stirred at room temperature overnight. The reaction mixture was cooled to 0 °C, quenched with sat. $NaHCO_3$ solution, and extracted with CH_2Cl_2 (30 mL x 3). The combined organic layers were washed with sat. $NaHCO_3$ (100 mL x 1), 1 M HCl solution (100 mL x 2), dried over Na_2SO_4, and concentrated in vacuo. The crude mixture was purified by flash column chromatography (SiO_2, gradient elution: 0 to 50% EtOAc in hexane) to afford the product **103** as a colorless oil (347 mg, yield 75%).

R_f = 0.33 (Hex:EtOAc = 1:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.27 (m, 5H), 5.30 (d, *J* = 12.4 Hz, 1H), 5.26 (d, *J* = 12.4 Hz, 1H), 4.15 (dd, *J* = 11.5, 7.1 Hz, 1H), 3.83 (ddd, *J* = 13.4, 8.4, 5.2 Hz, 1H), 3.69 (dddd, *J* = 12.9, 6.3, 5.1, 1.1 Hz, 1H), 2.38-2.30 (m, 1H), 1.96-1.83 (m, 2H), 1.69 (dddd, *J* = 12.9, 11.4, 9.8, 6.6 Hz, 1H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 174.5, 153.4, 135.1, 128.6, 128.5, 128.2, 69.4, 68.9, 45.7, 28.1, 20.0.

MS (ESI^+): *m/z* 250 ($[M+H]^+$), 313 ($[M+MeCN+Na]^+$), 499 ($[2M+H]^+$), 521 ($[2M+Na]^+$)

[α] $_{D}^{21}$: -23.7°, (c = 0.01, MeOH)

Enantiomer of **103**:

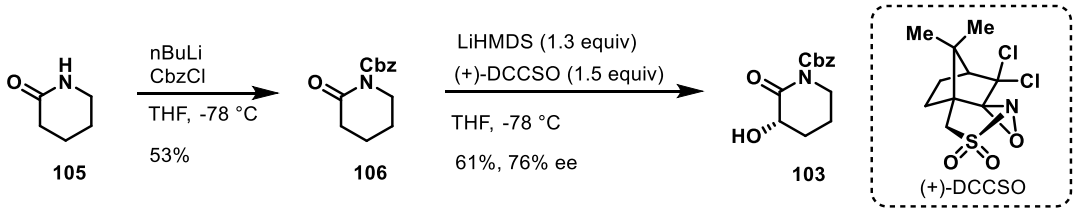
[α] $_{D}^{20}$: +24.2°, (c = 0.01, MeOH)

Side product **104**:

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.28 (m, 10H), 5.24 (s, 4H), 4.18-4.09 (m, 3H), 3.76 (t, *J* = 7.0 Hz, 2H), 2.86 (brs, 1H), 1.81-1.69 (m, 3H), 1.64-1.57 (m, 3H), 1.41-1.31 (m, 2H), 0.93 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 153.6, 135.3, 128.7, 128.4, 128.2, 70.1, 68.7, 65.7, 46.5, 31.4, 30.6, 24.5, 19.1, 13.7.

MS (ESI^+): *m/z* 458 ($[M+H]^+$), 480 ($[M+Na]^+$)



Benzyl 2-oxopiperidine-1-carboxylate (106): To a solution of compound **105** (0.50 g, 5.04 mmol) in THF (50 mL) at -78 ^{o}C was added nBuLi (4.1 mL, 6.05 mmol, 1.47 M in THF). After 1 h, CbzCl (0.74 mL, 5.04 mmol) was added to the reaction and the reaction was stirred at this temperature for 2 h. The reaction was quenched by the addition of a sat. $NaHCO_3$ solution, extracted with CH_2Cl_2, washed with brine, dried with Na_2SO_4, and purified by column

chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the product **106** (0.62 g, 53%).

$\boldsymbol{R_f}$ = 0.36 (Hex:EtOAc = 1:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46-7.26 (m, 5H), 5.28 (s, 2H), 3.73 (s, 2H), 2.53 (s, 2H), 1.83 (s, 4H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 171.2, 154.2, 135.5, 128.6, 128.3, 128.1, 88.5, 46.6, 35.0, 22.7, 20.5.

MS (ESI^+): *m/z* 234 ($[M+H]^+$), 297 ($[M+MeCN+Na]^+$), 489 ($[2M+Na]^+$)

Benzyl (*S*)-3-hydroxy-2-oxopiperidine-1-carboxylate (103): To a solution of compound **106** (50 mg, 0.21 mmol) in THF (4.3 mL) was added LiHMDS (0.28 mL, 0.28 mmol, 1 M in THF) at -78 ^oC. The reaction was stirred at this temperature for 2.5 h when a solution of (-)-(8,8-dichlorocamphorylsulfonyl) oxaziridine (96 mg, 0.32 mmol) in THF (1.6 mL) was added dropwise. The clear solution was stirred at -78°C for 15 h. Then the reaction was quenched with aqueous NH_4Cl (10 mL) and diluted with CH_2Cl_2 (20 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (20 mL x 3). The combined organic phases were washed with brine (25 mL) and dried (Na_2SO_4), concentrated, and purified by column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to yield the desired product **103** (32.6 mg, 61% yield, ee = 76%) as a colorless oil.

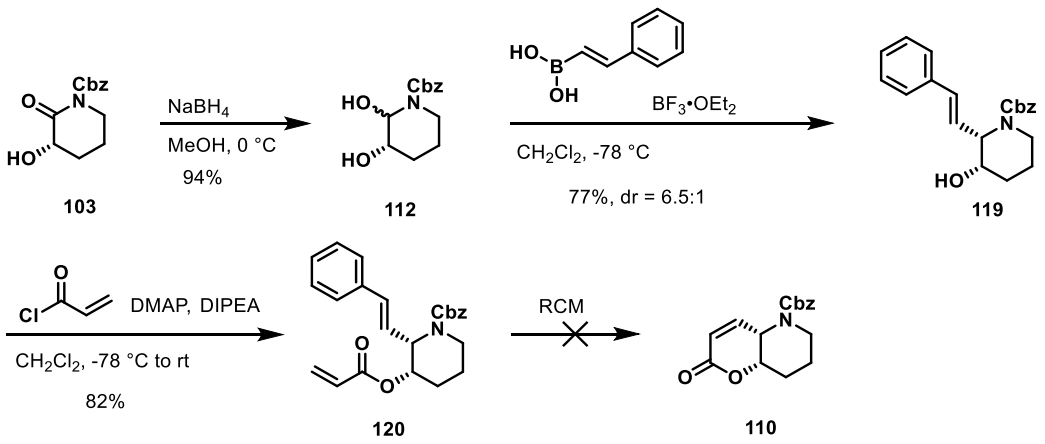
$\boldsymbol{R_f}$ = 0.19 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49-7.31 (m, 5H), 5.32 (d, *J* = 12.3 Hz, 1H), 5.28 (d, *J* = 12.5 Hz, 1H), 4.17 (dd, *J* = 11.5, 7.1 Hz, 1H), 3.86 (ddd, *J* = 13.5, 8.3, 5.3 Hz, 1H), 3.77-3.59 (m, 2H), 2.37 (dq, *J* = 12.3, 6.0 Hz, 1H), 2.00-1.86 (m, 2H), 1.71 (tdd, *J* = 12.2, 9.7, 6.8 Hz, 1H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 174.6, 153.5, 135.2, 128.8, 128.6, 128.3, 69.5, 69.0, 45.8, 28.2, 20.2.

MS (ESI^+): *m/z* 250 ($[M+H]^+$), 313 ($[M+MeCN+Na]^+$), 521 ($[2M+Na]^+$)

$[\alpha]_D^{22}$: -17.9°, (c = 0.01, MeOH)



Benzyl (3*S*)-2,3-dihydroxypiperidine-1-carboxylate (112): To a solution of compound **103** (42 mg, 0.17 mmol) in methanol (2.2 mL) was added $NaBH_4$ (7.6 mg, 0.2 mmol) at 0 °C. After stirring at this temperature for 1 h, the reaction was quenched with sat. $NaHCO_3$ (10 mL). The reaction mixture was extracted with EA (10 mL x 3). The combined organic layers were washed with brine (15 mL x 3), dried over Na_2SO_4, concentrated in vacuo and purified by flash column chromatography (SiO_2, gradient elution: 0 to 50% EtOAc in hexane) to afford the product **112** as colorless oil (39.9 mg, 94% yield).

R_f = 0.21 (Hex:EtOAc = 1:1)

MS (ESI^+): *m/z* 274 ($[M+Na]^+$), 315 ($[M+MeCN+Na]^+$)

Benzyl (2*S*,3*S*)-3-hydroxy-2-((*E*)-styryl)piperidine-1-carboxylate (119): To a stirred suspension of compound **112** (24 mg, 0.0955 mmol) and (*E*)-styreneboronic acid (15.3 mg, 0.10 mmol) in CH_2Cl_2 (2.0 mL) was added $BF_3 \cdot OEt_2$ (76 µL, 0.29 mmol) at –78 °C under N_2 atmosphere. The reaction mixture was warmed to rt over 4 h and stirred overnight and then quenched with saturated aqueous NH_4Cl (10 mL). The reaction mixture was extracted with CH_2Cl_2 (10 mL x 3). The combined organic phases were washed with brine (10 mL x 3), dried over Na_2SO_4, concentrated in vacuo and purified by flash column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford 21.5 mg **119** and 3.3 mg diastereomer (77% yield, dr = 6.5:1).

R_f = 0.2 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44-7.19 (m, 10H), 6.56 (d, *J* = 15.9 Hz, 1H), 6.39 (dd, *J* = 16.1, 6.3 Hz, 1H), 5.18 (d, *J* = 12.4 Hz, 1H), 5.14 (d, *J* = 12.4 Hz, 1H), 5.11 (s, 1H), 4.04 (d, *J* = 13.7 Hz, 1H), 3.83 (dt, *J* = 10.4, 4.8 Hz, 1H), 2.93 (td, *J* = 13.1, 3.2 Hz, 1H), 1.91-1.85 (m, 1H), 1.77-1.70 (m, 1H), 1.60-1.48 (m, 2H).

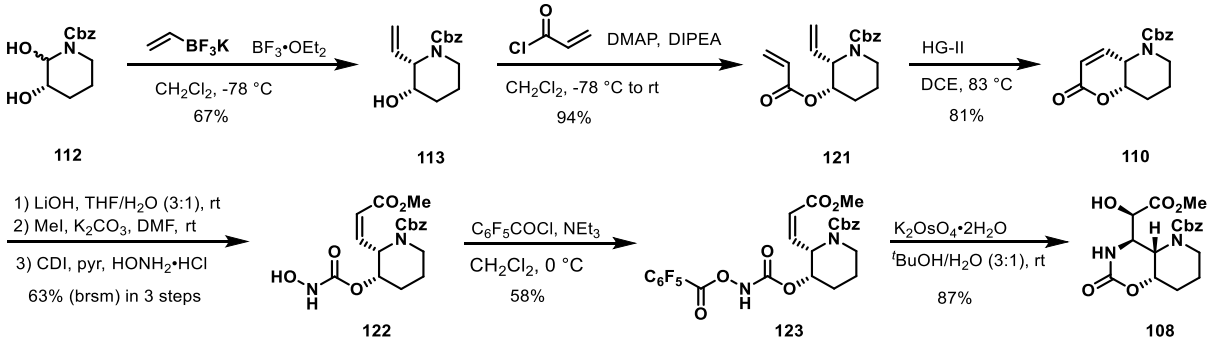
MS (ESI^+): *m/z* 338 ($[M+H]^+$)

Benzyl (2*S*,3*S*)-3-(acryloyloxy)-2-((*E*)-styryl)piperidine-1-carboxylate (120): To a solution of compound **119** (14.4 mg, 0.043 mmol) in CH_2Cl_2 (0.9 mL) was added DIPEA (39 µL, 0.3 mmol) and DMAP (0.3 mg, 0.0025 mmol) and the reaction was cooled to -78 °C. The acryloyl chloride (17.2 µL, 0.21 mmol) was added to the reaction. After stirring at −78 °C for 1 h, the reaction was warmed to rt over 0.5 h and then quenched with 1 M HCl (10 mL). The mixture was transferred to a separatory funnel, and the organic layer was removed. The aqueous portion was extracted with

CH_2Cl_2 (10 mL x 3). The organic portions were combined, washed with $NaHCO_3$ (20 mL x 1), dried over Na_2SO_4, filtered, and concentrated in vacuo, and purified by flash column chromatography (SiO_2, gradient elution: 0 to 10% EtOAc in hexane) to afford the product **120** (13.6 mg, 82% yield).

R_f = 0.6 (Hex:EtOAc = 3:1)

MS (ESI^+): *m/z* 392 ($[M+H]^+$), 414 ($[M+Na]^+$), 455 ($[M+MeCN+Na]^+$)



Benzyl (2*S*,3*S*)-3-hydroxy-2-vinylpiperidine-1-carboxylate (113): To a stirred suspension of compound **112** (323 mg, 1.29 mmol) and vinylBF_3K (266 mg, 1.93 mmol) in CH_2Cl_2 (13 mL) was added $BF_3{\cdot}OEt_2$ (1.01 mL, 3.86 mmol) at –78 °C under N_2 atmosphere. The reaction mixture was warmed to rt over 4 h and stirred overnight and then quenched with saturated $NaHCO_3$ (30 mL). The reaction mixture was extracted with CH_2Cl_2 (30 mL x 3). The combined organic phases were washed with brine (30 mL x 3), dried over Na_2SO_4, concentrated in vacuo, and purified by flash column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the desired product **113** as a colorless oil (224.7 mg, 67% yield).

R_f = 0.27 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33-7.19 (m, 5H), 5.94 (ddd, *J* = 17.4, 10.6, 5.8 Hz, 1H), 5.29 (dt, *J* = 10.6, 1.7 Hz, 1H), 5.17 (d, *J* = 17.4 Hz, 1H), 5.07 (d, *J* = 12.5 Hz, 1H), 5.05 (d, *J* = 12.5 Hz, 1H), 4.86 (s, 1H), 3.96-3.85 (m, 1H), 3.69 (ddd, *J* = 11.6, 5.8, 4.1 Hz, 1H), 2.78 (td, *J* = 13.2, 3.1 Hz, 1H), 1.82-1.72 (m, 1H), 1.62 (dt, *J* = 13.1, 3.0 Hz, 1H), 1.50-1.42 (m, 1H), 1.38-1.29 (m, 1H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 155.9, 136.7, 130.4, 128.6, 128.1, 127.9, 119.5, 69.0, 67.4, 58.3, 38.9, 28.9, 24.3.

MS (ESI^+): *m/z* 262 ($[M+H]^+$)

Benzyl (2*S*,3*S*)-3-(acryloyloxy)-2-vinylpiperidine-1-carboxylate (121): To a solution of compound **113** (0.1 g, 0.38 mmol) in CH_2Cl_2 (7.7 mL) was added DIPEA (0.33 mL, 1.91 mmol) and DMAP (2.3 mg, 0.02 mmol) and the reaction was cooled to -78 °C. The acryloyl chloride (93 μL, 1.15 mmol) was added to the reaction. After stirring at −78 °C for 1 h, the reaction was warmed to rt over 0.5 h and then quenched with 1 M HCl (20 mL). The mixture was transferred to a separatory funnel, and the organic layer was removed. The aqueous portion was extracted with CH_2Cl_2 (20 mL x 3). The organic portions were combined, washed with $NaHCO_3$ (20 mL x 1), dried over Na_2SO_4, filtered, concentrated in vacuo, and purified by flash column chromatography (SiO_2, gradient elution: 0 to 10% EtOAc in hexane) to afford the desired product **121** as a colorless oil (113 mg, 94% yield).

$\boldsymbol{R_f}$ = 0.61 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.27 (m, 5H), 6.40 (dd, *J* = 17.3, 1.4 Hz, 1H), 6.10 (dd, *J* = 17.3, 10.5 Hz, 1H), 5.95 (ddd, *J* = 17.3, 10.7, 5.0 Hz, 1H), 5.84 (dd, *J* = 10.4, 1.4 Hz, 1H), 5.32 (d, *J* = 10.7 Hz, 1H), 5.24-5.05 (m, 4H), 4.94 (qd, *J* = 5.7, 4.2 Hz, 1H), 4.03 (brs, 1H), 2.98-2.84 (m, 1H), 1.97-1.87 (m, 1H), 1.78-1.70 (m, 1H), 1.67-1.57 (m, 2H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 165.1, 155.8, 136.7, 131.3, 130.6, 128.6, 128.5, 128.1, 127.9, 118.8, 71.0, 67.5, 55.2, 38.9, 25.6, 24.0.

MS (ESI^+): *m/z* 316 ($[M+H]^+$), 338 ($[M+Na]^+$), 379 ($[M+MeCN+Na]^+$)

Benzyl (4a*S*,8a*S*)-2-oxo-2,4a,6,7,8,8a-hexahydro-5*H*-pyrano[3,2-*b*]pyridine-5-carboxylate (110): To a solution of compound **121** (103 mg, 0.33 mmol) in anhydrous 1,2-dichloroethane (6.5 mL) at room temperature under N_2 atmosphere was added Hoveyda-Grubbs-II catalyst (30.7 mg, 0.049 mmol). After refluxing at 83 °C until the SM disappeared, the reaction was concentrated and purified by flash column chromatography (SiO_2, gradient elution: 0 to 10% EtOAc in hexane) afforded the desired product **110** as a brown solid (76.3 mg, 81% yield).

R_f = 0.42 (Hex:EtOAc = 1:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43-7.29 (m, 5H), 6.60 (d, *J* = 10.0 Hz, 1H), 6.06 (dd, *J* = 10.0, 2.8 Hz, 1H), 5.28 (s, 1H), 5.15 (s, 2H), 4.54 (dddd, *J* = 11.7, 6.8, 5.0, 1.8 Hz, 1H), 4.13-4.02 (m, 1H), 2.82-2.64 (m, 1H), 2.09-2.04 (m, 1H), 1.87 (tdd, *J* = 12.9, 11.6, 4.4 Hz, 1H), 1.76-1.69 (m, 1H), 1.55-1.43 (m, 1H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 161.4, 155.2, 146.7, 136.0, 128.7, 128.4, 128.1, 122.5, 74.4, 68.0, 49.2, 40.5, 25.8, 23.2.

MS (ESI^+): *m/z* 288 ($[M+H]^+$)

Benzyl (2*S*,3*S*)-3-((hydroxycarbamoyl)oxy)-2-((*Z*)-3-methoxy-3-oxoprop-1-en-1-yl)piperidine-1-carboxylate (122): To a solution of lactone **110** (76.3 g, 0.266 mmol) in THF (2.1 mL) and H_2O (0.7 mL) was added $LiOH·H_2O$ (28.0 mg, 0.664 mmol) at rt. After stirring for 5 h, the reaction mixture was transferred to a separatory funnel and partitioned between 0.2 M HCl

(20 mL) and EtOAc (20 mL). The organic layer was removed, and the aqueous layer was extracted with additional EtOAc (10 mL x 4). The organic portions were combined, washed with brine (40 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting oil was dissolved in DMF (2.7 mL) at rt, and K_2CO_3 (92 mg, 0.664 mmol) and MeI (60 µL, 0.96 mmol) were added. After stirring for 1 h, the reaction mixture was transferred to a separatory funnel and diluted with a brine (20 mL) and 1.0 M HCl solution (6 mL) and extracted with $CHCl_3$ (10 mL). The organic layer was removed, and the aqueous layer was extracted with $CHCl_3$ (4 × 10 mL). The organic layers were combined, dried (Na_2SO_4), and concentrated in vacuo. The resulting oil was dissolved in pyridine (0.6 mL), and CDI (86 mg, 0.53 mmol) was added in one portion. After stirring for 12 h at rt, the reaction was cooled to 0 °C and $H_2NOH{\cdot}HCl$ (72 mg, 1.0 mmol) was added. The reaction was then allowed to warm slowly to rt over 5 h. The reaction mixture was diluted with 0.5 M HCl (20 mL), transferred to a separatory funnel, and extracted with EtOAc (20 mL x 2). The combined organic portions were washed with brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 66% EtOAc in hexane) to afford the desired *N*-hydroxy carbamate **122** (62.6 mg, 63% brsm over 3 steps) as a colorless oil and free hydroxyl ester (23.5 mg).

$\boldsymbol{R_f}$ = 0.34 (Hex:EtOAc = 1:2)

MS (ESI^+): *m/z* 379 ($[M+H]^+$)

Benzyl (2*S*,3*S*)-2-((*Z*)-3-methoxy-3-oxoprop-1-en-1-yl)-3-((((perfluorobenzoyl)oxy)carbamoyl)oxy)piperidine-1-carboxylate (123): To a solution of *N*-hydroxy carbamate **122** (37.1 mg, 0.098 mmol) in CH_2Cl_2 (1 mL) and triethylamine (20.4 µL, 0.147 mmol, 1.1 equiv) at 0 °C was dropwise added a diluted pentafluorobenzoyl chloride (14.4 µL, 0.098 mmol) in CH_2Cl_2 (1 mL) solution. Once the SM disappeared, the reaction was diluted

with sat. aq. NH_4Cl (10 mL) and transferred to a separatory funnel. The organic portion was removed, and the aqueous portion was extracted with additional CH_2Cl_2 (15 mL x 3). The combined organic fractions were washed with sat. aq. $NaHCO_3$ (30 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the desired compound **123** (32.8 mg, 58% yield) as a sticky oil.

R_f = 0.58 (Hex:EtOAc = 1:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.36-7.26 (m, 5H), 6.27 (dd, *J* = 11.6, 9.6 Hz, 1H), 6.18 (dd, *J* = 9.8, 5.9 Hz, 1H), 5.95 (d, *J* = 11.6 Hz, 1H), 5.14 (d, *J* = 12.4 Hz, 1H), 5.08-5.03 (m, 2H), 4.09-4.02 (m, 1H), 3.60 (s, 3H), 3.02-2.94 (m, 1H), 1.98-1.93 (m, 1H), 1.87-1.81 (m, 1H), 1.69-1.61 (m, 2H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 166.0, 158.2, 155.5, 154.9, 146.8, 145.1, 143.5, 138.8, 137.8, 137.1, 136.3, 128.6, 128.1, 128.0, 124.3, 104.9, 77.2, 67.7, 51.7, 50.6, 38.7, 25.5, 23.3.

MS (ESI^+): *m/z* 573 ($[M+H]^+$)

Benzyl (4*R*,4a*S*,8a*S*)-4-((*R*)-1-hydroxy-2-methoxy-2-oxoethyl)-2-oxooctahydro-5*H*-pyrido[2,3-*e*][1,3]oxazine-5-carboxylate (108): To a solution of compound **123** (32.8 mg, 0.136 mmol) in *t*-BuOH/water solution (3:1, 2.4 mL) was dropwise added a solution of $K_2OsO_4 \cdot H_2O$ (0.53 mg, 2.5 mol %) in water (0.3 mL) over 10 min. After stirring at rt under N_2 for 1.5 h, the reaction was quenched with addition of Na_2SO_3 (30 mg, 200 mg/mmol) and stirred for an additional 0.5 h. The solvent was azeotropically removed with toluene and chloroform and concentrated in vacuo. The resulting residue was purified by flash chromatography (SiO_2, gradient

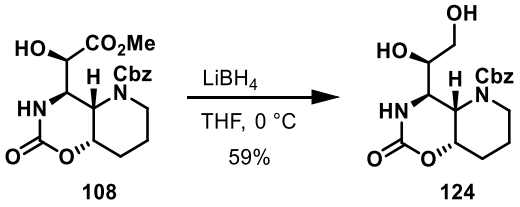
elution: 0 to 5% MeOH in CH_2Cl_2) to afford the desired product **108** (18.9 mg, 87% yield) as a white solid.

R_f = 0.18 (CH_2Cl_2:MeOH = 20:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47-7.28 (m, 5H), 6.84 (s, 1H), 5.13 (s, 2H), 4.95 (dd, *J* = 10.3, 5.7 Hz, 1H), 4.35 (s, 1H), 4.31-4.23 (m, 1H), 4.19 (d, *J* = 10.1 Hz, 1H), 4.11-3.95 (brs, 1H), 3.66 (s, 3H), 2.74 (td, *J* = 13.5, 2.9 Hz, 1H), 2.16-2.10 (m, 1H), 1.87-1.68 (m, 2H), 1.58-1.45 (m, 1H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 155.4, 153.4, 135.8, 128.8, 128.6, 128.5, 73.2, 68.5, 53.6, 53.0, 51.2, 45.1, 31.1, 26.5, 23.8.

MS (ESI^+): *m/z* 379 ($[M+H]^+$)



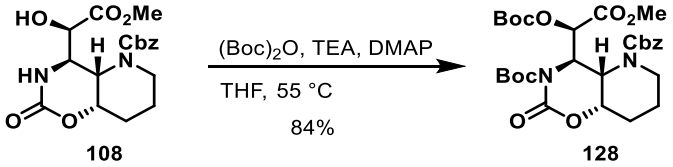
108 124

Benzyl (4*R*,4a*S*,8a*S*)-4-((*R*)-1,2-dihydroxyethyl)-2-oxooctahydro-5*H*-pyrido[2,3-*e*][1,3]oxazine-5-carboxylate (124): To a solution of compound **108** (31.8 mg, 0.084 mmol) in THF (0.84 mL) was added $LiBH_4$ (5.6 mg, 0.252 mmol) at 0 °C. The reaction was warmed to rt and stirred overnight. The resulting mixture was diluted with sat. aq. NH_4Cl (10 mL) and brine (10 mL) and extracted with CH_2Cl_2 (10 mL x 3). The combined organic portions were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. This residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 10% MeOH in CH_2Cl_2) to afford the desired product **124** (17.4 mg, 59% yield) as a yellow oil.

***R*f** = 0.35 (CH_2Cl_2:MeOH = 10:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52-7.27 (m, 5H), 6.75 (s, 1H), 5.15 (d, *J* = 12.2 Hz, 1H), 5.10 (d, *J* = 12.2 Hz, 1H), 4.64 (dd, *J* = 9.7, 5.3 Hz, 1H), 4.28 (dt, *J* = 10.7, 4.7 Hz, 1H), 4.06 (d, *J* = 13.4 Hz, 1H), 3.93 (d, *J* = 9.4 Hz, 1H), 3.78 (s, 1H), 3.71 (s, 2H), 2.91 (t, *J* = 13.1 Hz, 1H), 2.11-1.98 (m, 1H), 1.88-1.70 (m, 2H), 1.60-1.46 (m, 1H).

MS (ESI^+): *m/z* 351 ($[M+H]^+$)

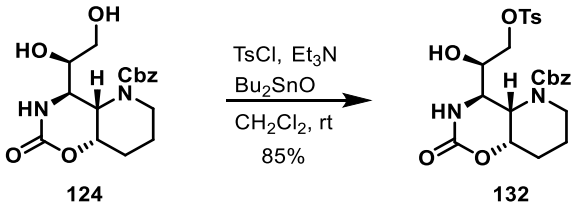


5-Benzyl 3-(*tert*-butyl) (4*R*,4a*S*,8a*S*)-4-((*R*)-1-((*tert*-butoxycarbonyl)oxy)-2-methoxy-2-oxoethyl)-2-oxohexahydro-2*H*-pyrido[2,3-*e*][1,3]oxazine-3,5-dicarboxylate (128): To a solution of **108** (18.9 mg, 0.05 mmol) in THF (1 mL) was added triethylamine (21 μL, 0.15 mmol), di-*tert*-butyl carbonate (32.7 mg, 0.15 mmol), and DMAP (0.6 mg, 0.005 mmol). The reaction was stirred at 55 °C for 24 h. The reaction was concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, gradient elution: 0 to 10% EtOAc in hexane) to afford the compound **128** (24.2 mg, 84% yield) as a white solid.

***R*f** = 0.57 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44-7.27 (m, 5H), 5.58 (d, *J* = 2.6 Hz, 1H), 5.18 (d, *J* = 12.2 Hz, 1H), 5.05 (d, *J* = 12.2 Hz, 1H), 5.01 (t, *J* = 6.4), 4.83 (dd, *J* = 6.5, 2.7 Hz, 1H), 4.35 (dt, *J* = 11.2, 5.6 Hz, 1H), 4.16-4.08 (m, 1H), 3.64 (s, 3H), 2.84 (ddd, *J* = 14.6, 12.6, 3.1 Hz, 1H), 2.08-2.04 (m, 1H), 1.83-1.78 (m, 1H), 1.77-1.70 (m, 1H), 1.62-1.59 (m, 1H), 1.54 (s, 3H), 1.48 (s, 3H).

MS (ESI$^+$): *m/z* 601 ([M+Na]$^+$), 642 ([M+MeCN+Na]$^+$)



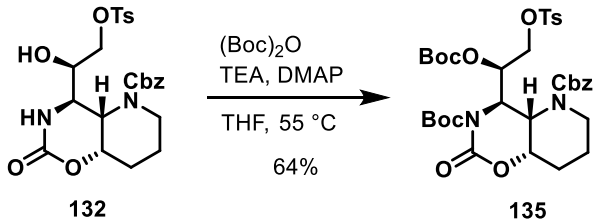
124 132

Benzyl (4*R*,4a*S*,8a*S*)-4-((*R*)-1-hydroxy-2-(tosyloxy)ethyl)-2-oxooctahydro-5*H*-pyrido[2,3-*e*][1,3]oxazine-5-carboxylate (132): To a solution of diol **124** (30.7 mg, 0.088 mmol) in CH_2Cl_2 (1.8 mL) at 0 °C was added *p*-toluenesulfonyl chloride (17.5 mg, 0.092 mmol), dry Et_3N (22 µL, 0.158 mmol) and a catalytic amount of Bu_2SnO (2.2 mg, 0.0088 mmol). The reaction mixture was stirred vigorously at room temperature and progress was monitored by TLC. When the starting material was consumed completely, the reaction mixture was diluted with water (10 mL) and the organic layer separated. The aqueous layer was extracted with CH_2Cl_2 (10 mL x 3), and the combined organic layers were washed with brine solution followed by drying over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, gradient elution: 0 to 5% MeOH in CH_2Cl_2) to afford the compound **132** (42 mg, 85% yield).

R_f = 0.3 (CH_2Cl_2:MeOH = 20:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.9 Hz, 2H), 7.40-7.29 (m, 7H), 6.37 (s, 1H), 5.16-5.05 (m, 2H), 4.62 (dd, *J* = 9.8, 5.7 Hz, 1H), 4.25 (dt, *J* = 10.5, 4.8 Hz, 1H), 4.22-4.14 (m, 1H), 4.13-4.02 (m, 2H), 3.99-3.91 (m, 2H), 2.86 (t, *J* = 12.6 Hz, 1H), 2.42 (s, 3H), 2.12-2.04 (m, 1H), 1.83-1.71 (m, 2H), 1.58-1.47 (m, 1H).

MS (ESI$^+$): *m/z* 505 ([M+H]$^+$)

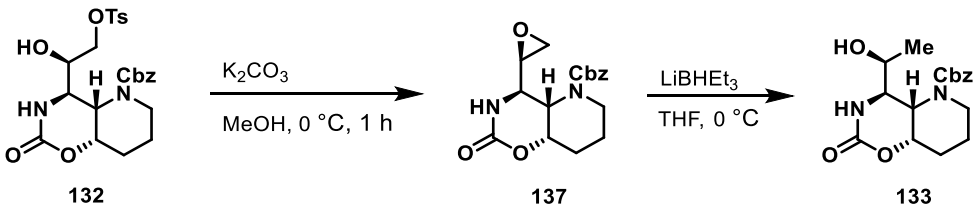


5-Benzyl 3-(*tert*-butyl) (4*R*,4a*S*,8a*S*)-4-((*R*)-1-((*tert*-butoxycarbonyl)oxy)-2-(tosyloxy)ethyl)-2-oxohexahydro-2*H*-pyrido[2,3-*e*][1,3]oxazine-3,5-dicarboxylate (135): To a solution of **132** (8.5 mg, 0.017 mmol) in THF (0.2 mL) was added triethylamine (7 µL, 0.051 mmol), di-*tert*-butyl carbonate (11 mg, 0.051 mmol), and a catalytic among of DMAP (0.2 mg, 0.0017 mmol). The mixture was stirred at 55 °C for 12 h. The reaction was concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the compound **135** (7.6 mg, 64% yield).

R_f = 0.21 (Hex:EtOAc = 3:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.1 Hz, 2H), 7.42-7.28 (m, 7H), 5.32 (td, *J* = 6.8, 2.7 Hz, 1H), 5.18 (d, *J* = 12.2 Hz, 1H), 5.13 (d, *J* = 12.2 Hz, 1H), 5.06-4.96 (m, 1H), 4.45 (dd, *J* = 6.2, 2.9 Hz, 1H), 4.34-4.22 (m, 2H), 4.19-4.05 (m, 2H), 2.86-2.73 (m, 1H), 2.43 (s, 3H), 2.12-2.01 (m, 1H), 1.83-1.67 (m, 2H), 1.60-1.53 (m, 1H), 1.52 (s, 9H), 1.43 (s, 9H).

MS (ESI$^+$): *m/z* 727 ([M+Na]$^+$)



Benzyl (4*R*,4a*S*,8a*S*)-4-((*R*)-oxiran-2-yl)-2-oxooctahydro-5*H*-pyrido[2,3-*e*][1,3]oxazine-5-carboxylate (137): To a solution of the tosylate **132** (2.7 mg, 0.0054 mmol) in methanol (0.1 mL) was added solid K_2CO_3 (2.2 mg, 0.016 mmol) at 0 °C, and the mixture was stirred for 1 h. The solids were removed by filtration. The filtrate was diluted with water and extracted with diethyl ether (5 mL x 3). The combined organic extracts were washed with brine, dried with Na_2SO_4, and concentrated under reduced pressure to afford the crude mixture which was used for the next step without purification.

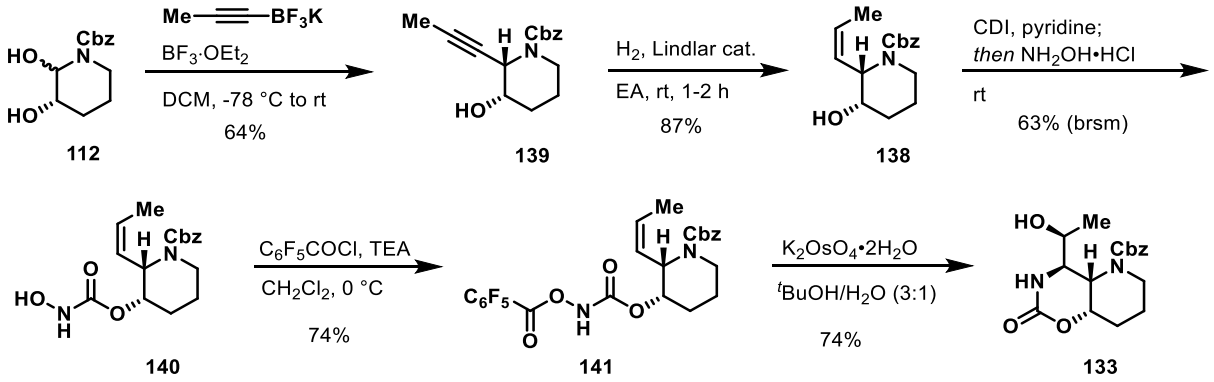
R_f = 0.19 (CH_2Cl_2:MeOH = 40:1)

MS (ESI^+): *m/z* 333 ($[M+H]^+$), 665 ($[2M+H]^+$)

Benzyl (4*R*,4a*S*,8a*S*)-4-((*S*)-1-hydroxyethyl)-2-oxooctahydro-5*H*-pyrido[2,3-*e*][1,3]oxazine-5-carboxylate (133): To a stirred solution of crude **137** in THF (0.1 mL) at 0 °C under nitrogen was added lithium triethylborohydride (21.4 µL, 0.0214 mmol, 1 M in THF) dropwise. The reaction was stirred at 0 °C for 30 min and then warmed to room temperature. The reaction mixture was cooled to 0 °C and quenched with water, diluted with ethyl acetate, and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo to afford the product.

R_f = 0.27 (CH_2Cl_2:MeOH = 20:1)

MS (ESI^+): *m/z* 335 ($[M+H]^+$), 357 ($[M+Na]^+$), 398 ($[M+MeCN+Na]^+$)



Benzyl (2*S*,3*S*)-3-hydroxy-2-(prop-1-yn-1-yl)piperidine-1-carboxylate (139): To a stirred suspension of compound **112** (323 mg, 1.29 mmol) and potassium 1-alkynyltrifluoroborates (266 mg, 1.93 mmol) in CH_2Cl_2 (13 mL) was added $BF_3{\cdot}OEt_2$ (1.01 mL, 3.86 mmol) at –78 °C under N_2 atmosphere. The reaction mixture was warmed to rt over 4 h and stirred overnight. Quenching with saturated $NaHCO_3$ (30 mL), the aqueous phase was extracted with CH_2Cl_2 (30 mL x 3). The combined organic phases were washed with brine (30 mL x 3), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the product **139** as a colorless oil (224.7 mg, 64% yield).

$\boldsymbol{R_f}$ = 0.15 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48-7.27 (m, 5H), 5.25 (brs, 1H), 5.13 (s, 2H), 3.94 (brs, 1H), 3.56 (dt, *J* = 10.5, 4.6 Hz, 1H), 2.98 (brs, 1H), 2.17 (s, 1H), 1.93-1.81 (m, 4H), 1.72-1.57 (m, 2H), 1.44 (brs, 1H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 155.0, 136.5, 128.6, 128.1, 128.0, 83.6, 73.1, 68.1, 67.5, 50.8, 39.4, 29.5, 23.7, 3.7.

IR (Diamond-ATR, neat) ν_{max} (cm^{-1}): 3414, 2937, 2863, 1677, 1416, 1342, 1252, 1118, 1073, 1029, 947, 738, 693.

MS (ESI^+): *m/z* 274 ($[M+H]^+$)

$[\alpha]_D^{22}$: 55.9°, (c = 0.01, MeOH)

Benzyl (2*S*,3*S*)-3-hydroxy-2-((*Z*)-prop-1-en-1-yl)piperidine-1-carboxylate (138): To a solution of compound **139** (90.6 mg, 0.33 mmol) in EtOAc (6.6 mL) was added Lindlar catalyst (41 mg). The reaction mixture was exposed to atmospheric hydrogen pressure for 2.5 hours. The reaction was filtered through a pad of Celite fiber, concentrated, and purified by flash column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the product **138** (80 mg, 87%) as a colorless oil.

***R*f** = 0.15 (Hex:EtOAc = 3:1)

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.42-7.27 (m, 5H), 5.90 (dq, *J* = 13.7, 7.1 Hz, 1H), 5.63 (tt, *J* = 9.4, 1.8 Hz, 1H), 5.23 (s, 1H), 5.15 (d, *J* = 12.3 Hz, 1H), 5.10 (d, *J* = 12.4 Hz, 1H), 3.98 (d, *J* = 13.7 Hz, 1H), 3.74 (dt, *J* = 10.8, 5.0 Hz, 1H), 2.83 (td, *J* = 13.2. 3.0 Hz, 1H), 1.90-1.84 (m, 1H), 1.83-1.60 (m, 5H), 1.56-1.43 (m, 1H).

^{13}C NMR (175 MHz, $CDCl_3$) δ 155.3, 136.7, 132.4, 128.6, 128.1, 128.0, 121.7, 69.0, 67.4, 52.5, 38.9, 28.9 24.2, 13.8.

IR (Diamond-ATR, neat) ν_{max} (cm^{-1}): 3414, 3027, 2937, 2863, 1670, 1424, 1245, 1059, 991, 738, 693.

MS (ESI^+): *m/z* 276 ($[M+H]^+$)

$[\alpha]_D^{22}$: 23.6°, (c = 0.01, MeOH)

Benzyl (2*S*,3*S*)-3-((hydroxycarbamoyl)oxy)-2-((*Z*)-prop-1-en-1-yl)piperidine-1-carboxylate (140): To a solution of compound **138** (325 mg, 1.18 mmol) in pyridine (2.4 mL) was added CDI (383 mg, 2.36 mmol) in one portion. After stirring for 12 h at rt, the reaction was cooled to 0 °C, and added $H_2NOH{\cdot}HCl$ (328 mg, 4.72 mmol). Then the reaction was allowed to warm slowly to rt over 5 h. The reaction mixture was diluted with 0.5 M HCl (20 mL), transferred to a separatory funnel, and extracted with EtOAc (20 mL x 2). The combined organic portions were washed with brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 66% EtOAc in hexane) to afford the desired *N*-hydroxy carbamate **140** (234 mg, 63% brsm) as a colorless oil and recovered **138** (21 mg).

$\boldsymbol{R_f}$ = 0.18 (Hex:EtOAc = 1:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46-7.27 (m, 6H), 5.76 (dq, *J* = 13.5, 7.1 Hz, 1H), 5.60 (ddd, *J* = 10.8, 9.0, 2.0 Hz, 1H), 5.39 (dd, *J* = 9.0, 5.6 Hz, 1H), 5.15 (d, *J* = 12.5 Hz, 1H), 5.10 (d, *J* = 12.2 Hz, 1H), 4.79 (dt, *J* = 11.2, 5.0 Hz, 1H), 3.98 (d, *J* = 13.6 Hz, 1H), 2.84 (td, *J* = 13.3, 3.0 Hz, 1H), 1.96-1.83 (m, 1H), 1.79-1.47 (m, 6H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 158.4, 155.2, 136.6, 131.3, 128.6, 128.1, 128.0, 121.8, 72.6, 67.6, 49.9, 38.9, 25.3, 24.0, 13.5.

IR (Diamond-ATR, neat) ν_{max} (cm^{-1}): 3273, 3027, 2945, 2870, 2363, 1670, 1424, 1342, 1305, 1245, 1103, 872, 738, 693.

MS (ESI^+): *m/z* 334 ($[M+H]^+$)

$[\alpha]_D^{22}$: 8.1°, (c = 0.01, MeOH)

Benzyl (2*S*,3*S*)-3-((((perfluorobenzoyl)oxy)carbamoyl)oxy)-2-((*Z*)-prop-1-en-1-yl)piperidine-1-carboxylate (141): To a solution of *N*-hydroxy carbamate **140** (232 mg, 0.694 mmol) in CH_2Cl_2 (14 mL) and NEt_3 (106.2 µL, 0.763 mmol, 1.1 equiv) at 0 °C was added a diluted pentafluorobenzoyl chloride (102 µL, 0.694 mmol) in CH_2Cl_2 (2 mL) solution dropwise. Once the starting material was consumed by TLC, the reaction was diluted with sat. aq. NH_4Cl (20 mL) and transferred to a separatory funnel. The organic portion was removed, and the aqueous portion was extracted with additional CH_2Cl_2 (20 mL x 3). The combined organic fractions were washed with sat. aq. $NaHCO_3$ (30 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the desired product **141** (270 mg, 74% yield) as a sticky oil.

R_f = 0.34 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.39-7.28 (m, 5H), 5.74 (dq, *J* = 13.8, 7.2 Hz, 1H), 5.61 (ddd, *J* = 11.0, 9.0, 2.0 Hz, 1H), 5.44 (dd, *J* = 9.1, 5.7 Hz, 1H), 5.18 (d, *J* = 12.3 Hz, 1H), 5.09 (d, *J* = 12.3 Hz, 1H), 4.87 (dt, *J* = 10.3, 5.2 Hz, 1H), 4.00 (dd, *J* = 13.4, 4.4 Hz, 1H), 2.85 (td, *J* = 13.3, 2.8 Hz, 1H), 1.97 (dt, *J* = 12.4, 3.8 Hz, 1H), 1.80-1.54 (m, 6H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 158.3, 155.1, 146.7, 145.0, 143.4, 138.7, 137.0, 136.5, 131.4, 128.5, 128.0, 127.9, 121.3, 104.8, 73.7, 67.5, 49.6, 38.7, 25.1, 23.8, 13.4.

^{19}F NMR (470 MHz, $CDCl_3$) δ -135.23 (m, 2F), -145.19 (t, *J* = 21.1 Hz, 1F), -159.22 (dd, *J* = 21.4, 15.1 Hz, 2F).

IR (Diamond-ATR, neat) ν_{max} (cm^{-1}): 3198, 3027, 2945, 2870, 2363, 2333, 1782, 1752, 1677, 1498, 1424, 1327, 1245, 1178, 1096, 1066, 991, 909, 798 ,738, 693, 604.

MS (ESI^+): *m/z* 529 ($[M+H]^+$)

$[\alpha]_D^{21}$: 3.6°, (c = 0.01, MeOH)

(4*R*,4a*S*,8a*S*)-4-((*S*)-1-hydroxyethyl)-2-oxooctahydro-5*H*-pyrido[2,3-*e*][1,3]oxazine-5-carboxylate (133): To a solution of compound **141** (260 mg, 0.49 mmol) in *t*-BuOH/water solution (3:1, 10 mL) was added a solution of $K_2OsO_4 \cdot H_2O$ (4.5 mg, 5 mol %) in water (2.5 mL) dropwise over 10 min under N_2 atmosphere at rt. After stirring for 1.5 h, the reaction was quenched with addition of Na_2SO_3 (98.4 mg, 200 mg/mmol) and stirred for an additional 0.5 h. The solvent was azeotropically removed with toluene and chloroform and concentrated in vacuo. The resulting residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 5% MeOH in CH_2Cl_2) to afford the desired product **133** (122 mg, 74% yield) as a white solid.

***R*f** = 0.27 (CH_2Cl_2:MeOH = 20:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46-7.27 (m, 5H), 6.66 (s, 1H), 5.16 (d, *J* = 12.0 Hz, 1H), 5.09 (d, *J* = 12.1 Hz, 1H), 4.49 (brs, 1H), 4.25 (dt, *J* = 10.9, 5.1 Hz, 1H), 4.10 (s, 1H), 3.94-3.74 (m, 2H), 3.06-2.78 (m, 2H), 2.14-2.06 (m, 1H), 1.88-1.70 (m, 2H), 1.60-1.47 (m, 1H), 1.36-1.00 (m, 3H).

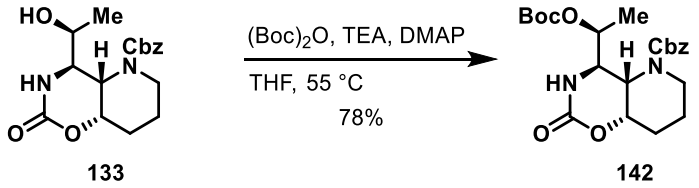
^{13}C NMR (150 MHz, $CDCl_3$) δ 155.4, 153.8, 135.8, 128.8, 128.6, 128.4, 73.5, 68.2, 67.4, 52.8, 46.8, 38.8, 26.3, 23.8, 16.1.

IR (Diamond-ATR, neat) ν_{max} (cm^{-1}): 3317, 2937, 2363, 2363, 1677, 1424, 1357, 1252, 1148, 1126, 1073, 760, 693, 604.

mp: 148-150 °C.

MS (ESI^+): *m/z* 334 ($[M+H]^+$)

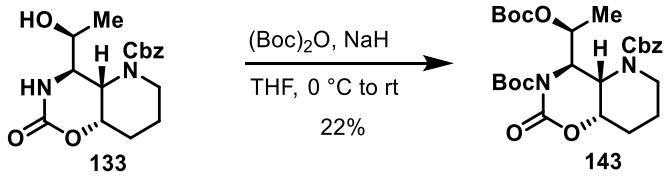
$[\alpha]_D^{23}$: 7.3°, (c = 0.01, MeOH)



Benzyl (4*R*,4a*S*,8a*S*)-4-((*S*)-1-((*tert*-butoxycarbonyl)oxy)ethyl)-2-oxooctahydro-5*H*-pyrido[2,3-*e*][1,3]oxazine-5-carboxylate (142): To a solution of **133** (19.7 mg, 0.059 mmol) in THF (1.2 mL) was added Et_3N (33 µL, 0.236 mmol), DMAP (0.7 mg, 0.0059 mmol), and Boc_2O (38.6 mg, 0.177 mmol). The solution was stirred at 55 °C overnight. The clear orange reaction mixture was cooled to rt, diluted with H_2O (10 mL), and extracted with EtOAc (10 mL x 3). The organic layer was washed with 1 M HCl (15 mL x1), brine, dried over Na_2SO_4, concentrated in vacuo and purifiied by flash chromatography (SiO_2, gradient elution: 0 to 5% MeOH in CH_2Cl_2) to afford the compound **142** (20 mg, 78% yield).

^{13}C NMR (125 MHz, $CDCl_3$) δ 155.3, 152.4, 152.1, 135.8, 128.8, 128.6, 128.2, 83.1, 73.3, 72.8, 68.3, 50.5, 46.6, 38.8, 27.9, 26.5, 23.7, 12.6.

MS (ESI^+): *m/z* 435 ($[M+H]^+$), 457 ($[M+Na]^+$), 869 ($[2M+H]^+$)

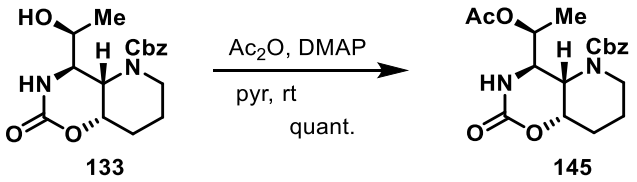


5-Benzyl 3-(*tert*-butyl) (4*R*,4a*S*,8a*S*)-4-((*S*)-1-((*tert*-butoxycarbonyl)oxy)ethyl)-2-oxohexahydro-2*H*-pyrido[2,3-*e*][1,3]oxazine-3,5-dicarboxylate (143): To a solution of **133** (15.3 mg, 0.046 mmol) in tetrahydrofuran (0.92 mL) was added sodium hydride (15 mg, 0.366 mmol, liquid paraffin 60%) under nitrogen atmosphere at 0 °C, and the mixture was stirred for 30

min. Then $(Boc)_2O$ (40 mg, 0.183 mmol) was added to the obtained solution. Then the mixture was warmed to room temperature and stirred for 20 hrs. Then, water (10 mL) was added to the obtained solution, and the solution was extracted with ethyl acetate (10 mL x 3). The combined organic extracts were washed with water (20 mL), brine (20 mL) and dried over Na_2SO_4. After filtration and concentrated in vacuo, the crude was purified by flash chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the product **143** (5.5 mg, 22% yield).

$\boldsymbol{R_f}$ = 0.35 (Hex:EtOAc = 3:1)

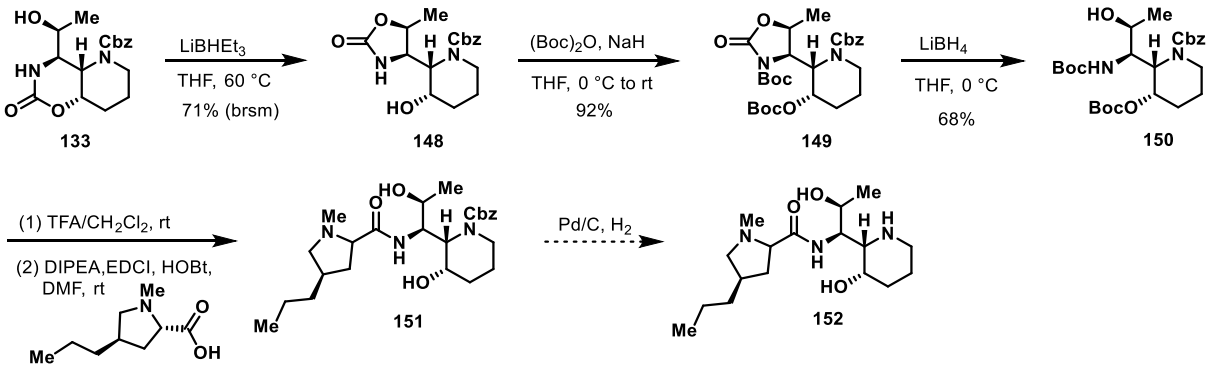
MS (ESI^+): *m/z* 552 ($[M+NH_4]^+$), 557 ($[M+Na]^+$), 598 ($[M+MeCN+Na]^+$)



Benzyl (4*R*,4a*S*,8a*S*)-4-((*S*)-1-acetoxyethyl)-2-oxooctahydro-5*H*-pyrido[2,3-*e*][1,3]oxazine-5-carboxylate (145): To a solution of **133** (15 mg, 0.045 mmol) in pyridine (0.6 mL) was added Ac_2O (0.1 mL) at room temperature under nitrogen atmosphere. The reaction was stirred for 15 h and then quenched by the addition of MeOH. The resulting mixture was concentrated, extracted with EtOAc (10 mL x 3). The combined organic phases were washed with 2 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, gradient elution: 0 to 5% MeOH in CH_2Cl_2) to afford the product **145** in quantitative yield.

$\boldsymbol{R_f}$ = 0.3 (CH_2Cl_2:MeOH = 10:1)

MS (ESI$^+$): *m/z* 377 ([M+H]$^+$), 753 ([2M+H]$^+$)



Benzyl (2*S*,3*S*)-3-hydroxy-2-((4*R*,5*S*)-5-methyl-2-oxooxazolidin-4-yl)piperidine-1-carboxylate (148): To a solution of compound **133** (20 mg, 0.06 mmol) in THF (1.2 mL) was added a solution of $LiBHEt_3$ (0.18 mL, 0.18 mmol, 1 M in THF) at room temperature. The reaction mixture was stirred at 60 °C for 5 h. The reaction was quenched by addition of MeOH (1 mL), filtered through a pad of Celite, and concentrated in vacuo. The resulting residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 3% MeOH in CH_2Cl_2) to afford the desired product **148** as a white solid (11.6 mg, 71% brsm) and recovered SM (3.6 mg).

R_f = 0.34 (CH_2Cl_2:MeOH = 20:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53-7.27 (m, 5H), 6.11 (s, 1H), 5.26-5.04 (m, 2H), 4.74-4.60 (m, 1H), 4.58-4.36 (m, 2H), 4.16-3.81 (m, 2H), 3.48 (s, 1H), 2.69 (s, 1H), 2.00-1.86 (m, 1H), 1.80-1.69 (m, 2H), 1.51 (s, 1H), 1.35-1.16 (m,3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 159.1, 155.4, 136.3, 128.6, 128.4, 128.0, 75.9, 69.1, 67.9, 53.6, 51.7, 40.2, 28.4, 24.4, 14.7.

IR (Diamond-ATR, neat) ν_{max} (cm^{-1}): 3384, 2937, 2878, 2244, 1737, 1692, 1446, 1416, 1290, 1245, 1163, 1141, 1044, 1014, 954, 917, 760, 731, 693.

mp: 57-59 °C.

MS (ESI^+): *m/z* 334 ($[M+H]^+$)

$[\alpha]_D^{23}$: 18.9°, (c = 0.01, MeOH)

***tert*-Butyl (4*R*,5*S*)-4-((2*S*,3*S*)-1-((benzyloxy)carbonyl)-3-((*tert*-butoxycarbonyl)oxy)piperidin-2-yl)-5-methyl-2-oxooxazolidine-3-carboxylate (149):** To a solution of **148** (7.3 mg, 0.022 mmol) in tetrahydrofuran (1.1 mL) was added sodium hydride (20 mg, 0.327 mmol, liquid paraffin 60%) under nitrogen atmosphere at 0 °C. The mixture was stirred for 30 min and then added $(Boc)_2O$ (28.6 mg, 0.13 mmol). The resulting mixture was warmed to room temperature and stirred for 20 hrs. The reaction was quenched with water (10 mL) and extracted with ethyl acetate (10 mL x 3). The combined organic extracts were washed with water (20 mL), brine (20 mL) and dried over Na_2SO_4. After filtration and concentrated in vacuo, the residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the product **149** (10.8 mg, 92%) as a white solid.

R_f = 0.31 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$, rotameric mixture) δ 7.56-7.08 (m, 5H), 5.20-4.97 (m, 2H), 4.92-4.22 (m, 4H), 3.74 (s, 1H), 3.09 (s, 1H), 2.32-2.03 (m, 1H), 1.80-1.62 (m, 2H), 1.54-1.13 (m, 22H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 155.6, 152.8, 151.3, 150.3, 150.0, 136.5, 136.0, 128.6, 128.3, 128.0, 84.0, 83.9, 82.6, 75.9, 73.0, 68.2, 67.7, 56.4, 55.8, 28.0, 27.8, 25.8, 23.8, 13.8.

IR (Diamond-ATR, neat) ν_{max} (cm^{-1}): 2974, 2930, 2356, 1797, 1729, 1700, 1454, 1342, 1275, 1245, 1155, 1111, 1073, 1036, 977, 939, 865, 768, 693.

mp: 69-71 °C.

MS (ESI^+): *m/z* 552 ($[M+NH_4]^+$), 1086 ($[2M+NH_4]^+$)

$[\alpha]_D^{24}$: -12.7°, (c = 0.015, MeOH)

Benzyl (2*S*,3*S*)-2-((1*R*,2*S*)-1-((*tert*-butoxycarbonyl)amino)-2-hydroxypropyl)-3-((*tert*-butoxycarbonyl)oxy)piperidine-1-carboxylate (150): To a solution of compound **149** (14.9 mg, 0.028 mmol) in THF (1.4 mL) was added $LiBH_4$ (3.7 mg, 0.17 mmol) at 0 °C. The reaction was stirred at this temperature until the SM disappeared. The resulting mixture was diluted with water (10 mL) and extracted with CH_2Cl_2 (10 mL x 3). The combined organic portions were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. This residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 50% EtOAc in hexane) to afford the desired product **150** as a white solid (9.6 mg, 68% yield).

R_f = 0.33 (Hex:EtOAc = 10:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46-7.26 (m, 5H), 5.23-5.05 (m, 2H), 5.03-4.79 (m, 1H), 4.76-4.61 (m, 1H), 4.59-4.37 (m, 1H), 4.34-4.15 (m, 1H), 4.12-3.92 (m, 1H), 3.76 (s, 1H), 2.91 (s, 1H), 2.01-1.85 (m, 2H), 1.80-1.71 (m, 1H), 1.67-1.39 (m, 19H), 1.24-0.88 (m, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 156.5, 155.5, 152.5, 136.2, 128.6, 128.2, 128.0, 82.5, 79.9, 74.4, 69.0, 67.8, 54.3, 52.6, 39.3, 28.4, 27.8, 25.0, 23.9, 18.3

IR (Diamond-ATR, neat) ν_{max} (cm^{-1}):

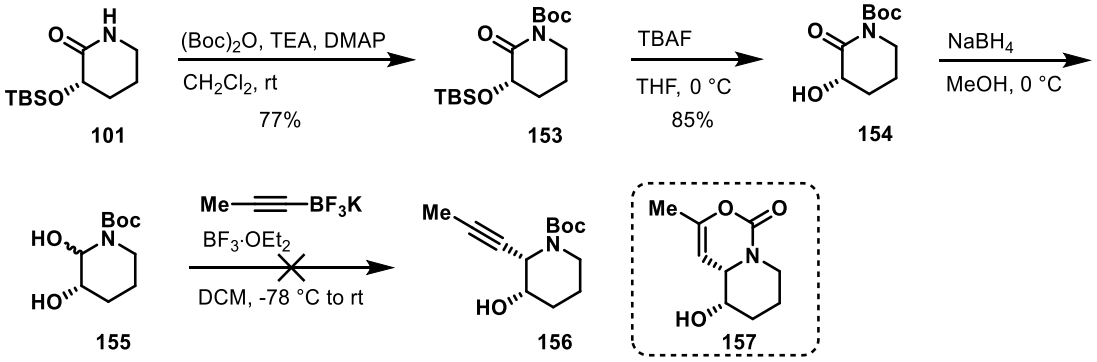
mp: 59-61 °C.

MS (ESI^+): *m/z* 509 ($[M+H]^+$)

$[\alpha]_D^{21}$: -25.6°, (c = 0.005, MeOH)

Benzyl (2*S*,3*S*)-3-hydroxy-2-((1*R*,2*S*)-2-hydroxy-1-((4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido)propyl)piperidine-1-carboxylate (151): To a solution of **150** (4.1 mg, 00081 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.4 mL) at room temperature. After stirring for 1 h, the reaction was concentrated in vacuo. To a solution of the crude mixture in DMF (0.4 mL) was added DIPEA (14 uL, 0.081 mmol), a solution of proline (1.4 mg, 0.008 mmol) in DMF (0.4 mL), EDCI (1.7 mg, 0.009 mmol) and HOBt (1.4 mg, 0.009 mmol) and stirred at room temperature overnight. The reaction mixture was quenched with water (5 mL) and extracted with EtOAc (5 mL x 3). The combined organic layers were washed with 5% LiCl solution (10 mL x 3), bine (10 mL) and concentrated in vacuo to afford a crude mixture of product **151**.

MS (ESI^+): *m/z* 462 ($[M+H]^+$), 945 ($[2M+Na]^+$)



***tert*-Butyl (*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2-oxopiperidine-1-carboxylate (153):** To a solution of compound **101** (112.7 mg, 0.49 mmol) in dry CH_2Cl_2 (5 mL) was added Et_3N (0.34 mL, 2.46 mmol), a catalytic amount of DMAP (6 mg, 0.049 mmol), and $(Boc)_2O$ (332 mg, 1.47

mmol) at room temperature under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 15 hours. Then the solvent was removed under reduced pressure. The resulting residue was diluted with water, extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, concentrated in vacuo and purified by flash column chromatography (SiO_2, gradient elution: 0 to 10% EtOAc in hexane) to afford the product **153** as a colorless oil (125 mg, 77% yield).

R_f = 0.68 (Hex:EtOAc = 3:1)

^{1}H NMR (700 MHz, $CDCl_3$) δ 4.13 (dd, *J* = 8.4, 5.7 Hz, 1H), 3.63 (ddd, *J* = 13.0, 8.2, 4.7 Hz, 1H), 3.58 (ddd, *J* = 12.4, 6.7, 4.9 Hz, 1H), 2.05-1.99 (m, 1H), 1.96-1.89 (m, 1H), 1.85-1.72 (m, 2H), 1.47 (s, 9H), 0.86 (s, 9H), 0.93-0.78 (m, 9H), 0.12 (s, 3H), 0.07 (s, 3H).

^{13}C NMR (175 MHz, $CDCl_3$) δ 172.0, 152.7, 82.7, 71.6, 45.6, 30.5, 28.0, 25.8, 19.9, 18.3, -4.6, -5.5.

MS (ESI^+): *m/z* 393 ($[M+MeCN+Na]^+$), 681 ($[2M+Na]^+$)

***tert*-Butyl (*S*)-3-hydroxy-2-oxopiperidine-1-carboxylate (154):** To a solution of **153** (123.8 mg, 0.38 mmol) in anhydrous THF (3.8 mL) was added a 1 M THF solution of TBAF (0.56 mL, 0.56 mmol, 1 M in THF) under nitrogen atmosphere at 0 °C. The mixture was stirred at 0 °C for 1 h and then quenched with water (10 mL). The aqueous layer was extracted with DCM (10 mL x 3). The combined organic phases were washed with brine (15 mL) and dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, the residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the product **154** as a white solid (68.4 mg, 85% yield).

R_f = 0.21 (Hex:EtOAc = 3:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.12 (dd, *J* = 11.5, 7.2 Hz, 1H), 3.78 (ddd, *J* = 13.4, 8.2, 5.3 Hz, 1H), 3.72 (s, 1H), 3.62-3.56 (m, 1H), 2.37-2.29 (m, 1H), 1.96-1.84 (m, 2H), 1.72-1.61 (m, 1H), 1.51 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 174.6, 152.0, 83.7, 69.3, 45.3, 28.2, 28.1, 20.1

***tert*-Butyl (3*S*)-2,3-dihydroxypiperidine-1-carboxylate (155):** To a solution of imide **154** (68 mg, 0.32 mmol) in MeOH (3.2 mL) at 0 °C was added $NaBH_4$ (6 mg, 0.16 mmol) in one portion. After stirring for 1 h at 0 °C, the reaction was quenched with sat. $NaHCO_3$ (20 mL). The mixture was transferred to a separatory funnel and extracted with DCM (20 mL x 3). The organic portions were combined, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, gradient elution: 0 to 50% EtOAc in hexane) to afford the product **155** as white solid.

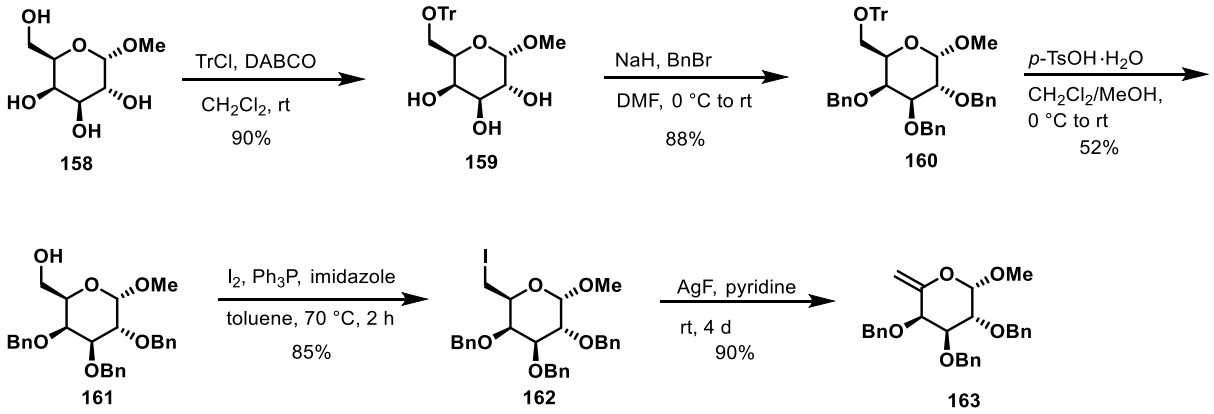
R_f = 0.22 (Hex:EtOAc = 1:1)

(4a*S*,5*S*)-5-Hydroxy-3-methyl-4a,5,7,8-tetrahydro-1*H*,6*H*-pyrido[1,2-*c*][1,3]oxazin-1-one (157): To a solution of compound **155** (0.32 mmol) and potassium trifluoro(prop-1-yn-1-yl)borate (138 mg, 0.95 mmol) in CH_2Cl_2 (3.2 mL) at -78 °C was added $BF_3{\cdot}Et_2O$ (0.33 mL, 1.26 mmol) dropwise. The solution was warmed to rt over 4 h and stirred at room temperature for an additional 3 h. The reaction was quenched with sat. $NaHCO_3$ (10 mL), and the mixture was transferred to a separatory funnel. The organic layer was removed, and the aqueous layer was extracted with CH_2Cl_2 (10 mL x 3). The organic fractions were combined and washed with brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting yellow oil was purified by flash column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the product **157** as a white solid.

R_f = 0.32 (100% EtOAc)

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.64 (d, *J* = 2.5 Hz, 1H), 4.45 (ddt, *J* = 13.3, 4.2, 2.0 Hz, 1H), 3.92 (dt, *J* = 3.3, 1.6 Hz, 1H), 3.68-3.61 (m, 1H), 2.71 (td, *J* = 13.2, 2.9 Hz, 1H), 2.09-2.03 (m, 1H), 2.02-1.95 (m, 1H), 1.90 (s, 3H), 1.63-1.59 (m, 1H), 1.52-1.46 (m, 1H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 150.6, 150.2, 95.3, 66.7, 59.0, 45.0, 30.0, 18.8, 18.6.



(2*S*,3*R*,4*S*,5*R*,6*R*)-2-Methoxy-6-((trityloxy)methyl)tetrahydro-2*H*-pyran-3,4,5-triol (159): To a solution of methyl-α-D-galactopyranoside **158** (2 g, 10.3 mmol) in CH_2Cl_2 (40 mL) was added DABCO (2.3 g, 20.5 mmol), followed by 98% TrCl (5.9 g, 20.6 mmol). The reaction mixture was stirred at room temperature for 4 h. The solvent was removed under vacuum. After purification by column chromatography (SiO_2, gradient elution: 0 to 10% MeOH in CH_2Cl_2), the desired product **159** was obtained as a white solid (4.0 g, 90%).

R_f = 0.3 (100% EtOAc)

MS (ESI$^+$): *m/z* 500 ([M+MeCN+Na]$^+$), 895 ([2M+Na]$^+$), 481 ([M+FA-H]$^-$)

(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-tris(Benzyloxy)-2-methoxy-6-((trityloxy)methyl)tetrahydro-2*H*-pyran (160): To a solution of **159** (2.12 g, 4.9 mmol) in anhydrous DMF (49 mL) was added portion-wise a suspension of NaH (60% in mineral oil, 1.4 g, 40.0 mmol) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C, followed by a slowly addition of benzyl chloride (3.46 mL, 29.1 mmol). The resulting suspension was stirred for 5 min at 0 °C, then warmed to rt, and stirred for 16 h. The reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. After purification by column chromatography (SiO_2, gradient elution: 0 to 10% EtOAc in hexane), the desired product **160** was obtained as a viscous yellow oil (3.1 g, 90%).

MS (ESI^+): *m/z* 729 ($[M+Na]^+$)

((2*R*,3*S*,4*S*,5*R*,6*S*)-3,4,5-tris(Benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methanol (161): To a solution of **160** (12.5 g, 17.7 mmol) in CH_2Cl_2/MeOH (1:2, 177 mL) was added *p*-TsOH monohydrate (0.34 g, 1.77 mmol) at 0 °C. The resulting solution was warmed to rt, stirred overnight, and concentrated under reduced pressure. The residue was dissolved in DCM (100 mL), washed with water (100 mL × 3), aqueous 10% Na_2CO_3 solution (100 mL × 2), aqueous saturated NaCl solution (100 mL × 3). The organic layer was dried over Na_2SO_4, filtered, concentrated in vacuo, and purified by column chromatography (SiO_2, gradient elution: 0 to 50% EtOAc in hexane) to afford the product **161** as a light syrup (4.3 g, 52%).

MS (ESI^+): *m/z* 487 ($[M+Na]^+$)

(2*S*,3*R*,4*S*,5*R*,6*S*)-3,4,5-tris(Benzyloxy)-2-(iodomethyl)-6-methoxytetrahydro-2*H*-pyran (162): To a solution of **161** (1.12 g, 2.4 mmol) in toluene (24 mL) at rt was added PPh_3 (1.27 g, 4.8 mmol), imidazole (0.82 g, 12.1 mmol) and molecular iodine (1.23 g, 4.8 mmol). The reaction

was stirred at 70 °C for 2 h. After completion of the reaction, the reaction mixture was quenched with $Na_2S_2O_3 \cdot 5H_2O$ and extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, concentrated in vacuo, and purified by flash column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the product **162** as a light oil (1.18 g, 85%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.14 (m, 15H), 4.96 (d, *J* = 11.3 Hz, 1H), 4.82 (d, *J* = 11.7 Hz, 1H), 4.76 (d, *J* = 12.1 Hz, 1H), 4.69 (d, *J* = 11.7 Hz, 1H), 4.61 (d, *J* = 12.1 Hz, 1H), 4.58-4.53 (m, 2H), 4.00-3.91 (m, 2H), 3.86 (dd, *J* = 10.0, 2.9 Hz, 1H), 3.77 (t, *J* = 6.9 Hz, 1H), 3.34 (s, 3H), 3.15 (dd, *J* = 10.1, 7.6 Hz, 1H), 3.00 (dd, *J* = 10.1, 6.2 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 138.8, 138.5, 138.4, 128.6, 128.5, 128.5, 128.3, 128.0, 127.9, 127.8, 127.7, 99.0, 79.2, 76.1, 75.9, 75.2, 73.8, 71.4, 55.9, 3.7.

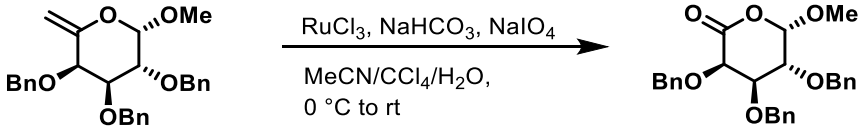
MS (ESI^+): *m/z* 592 ($[M+NH_4]^+$), 597 ($[M+Na]^+$), 638 ($[M+MeCN+Na]^+$)

(2*S*,3*R*,4*S*,5*R*)-3,4,5-tris(Benzyloxy)-2-methoxy-6-methylenetetrahydro-2*H*-pyran (163): To a solution of **162** (0.616 g, 1.07 mmol) in dry pyridine (10.7 mL) was added AgF (0.544 g, 4.29 mmol) under an atmosphere of nitrogen. The reaction mixture was stirred in the dark for 96 hours at room temperature, then concentrated under reduced pressure, and purified by flash column chromatography (SiO_2, gradient elution: 0 to 10% EtOAc in hexane) to afford the product **163** as a light oil (0.43 g, 90%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42-7.28 (m, 15H), 4.92 (d, *J* = 12.1 Hz, 1H), 4.82-4.69 (m, 5H), 4.62 (d, *J* = 11.9 Hz, 1H), 4.48 (s, 1H), 4.19 (dd, *J* = 9.9, 3.5 Hz, 1H), 4.08 (d, *J* = 3.5 Hz, 1H), 3.98 (dd, *J* = 9.9, 3.5 Hz, 1H), 3.44 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 151.7, 138.7, 138.7, 138.0, 128.5, 128.4, 128.4, 128.2, 128.1, 127.8, 127.8, 127.7, 127.6, 102.0, 100.0, 76.7, 75.7, 74.9, 74.1, 72.6, 69.4, 55.8.

MS (ESI^+): *m/z* 464 ($[M+NH_4]^+$), 469 ($[M+Na]^+$)



163 164

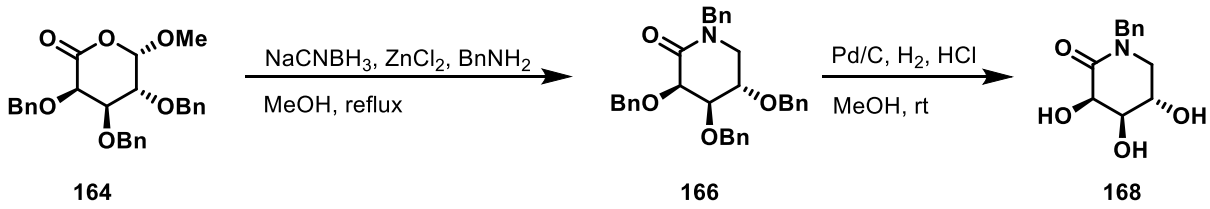
(3*R*,4*R*,5*R*,6*S*)-3,4,5-tris(Benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-one (164): To a solution of enol ether **163** (1.94 g, 4.3 mmol) in a mixed solvent of $MeCN/CCl_4/H_2O$ (1:2:1, 11 mL/22 mL/11 mL) was added $RuCl_3$ (0.18 g, 0.87 mmol), followed by $NaIO_4$ (3.72 g, 17.4 mmol) and $NaHCO_3$ (0.55 g, 6.5 mmol) at 0 °C. The reaction was warmed to rt and stirred for 5 h. The reaction was diluted with water (20 mL) and dichloromethane (20 mL). The mixture was filtered through a pad of Celite and then extracted with dichloromethane (20 mL x 3). The combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford product **164** as a colorless oil (0.94 g, 48%).

R_f = 0.41 (Hex:EtOAc = 3:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.16 (m, 15H), 5.16 (d, *J* = 3.0 Hz, 1H), 4.98 (d, *J* = 11.9 Hz, 1H), 4.75 (d, *J* = 11.9 Hz, 1H), 4.59 (d, *J* = 12.1 Hz, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 4.51 (d, *J* = 11.9 Hz, 1H), 4.48-4.43 (m, 2H), 3.91 (t, *J* = 4.1 Hz, 1H), 3.77 (dd, *J* = 4.4, 3.0 Hz, 1H), 3.50 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 159.31, 137.82, 137.55, 137.39, 128.65, 128.59, 128.54, 128.23, 128.15, 128.10, 128.03, 127.99, 102.28, 76.62, 75.94, 74.60, 73.86, 73.69, 73.55, 58.05.

MS (ESI^+): *m/z* 466 ($[M+Na]^+$), 512 ($[M+MeCN+Na]^+$), 919 ($[2M+Na]^+$)



164 166 168

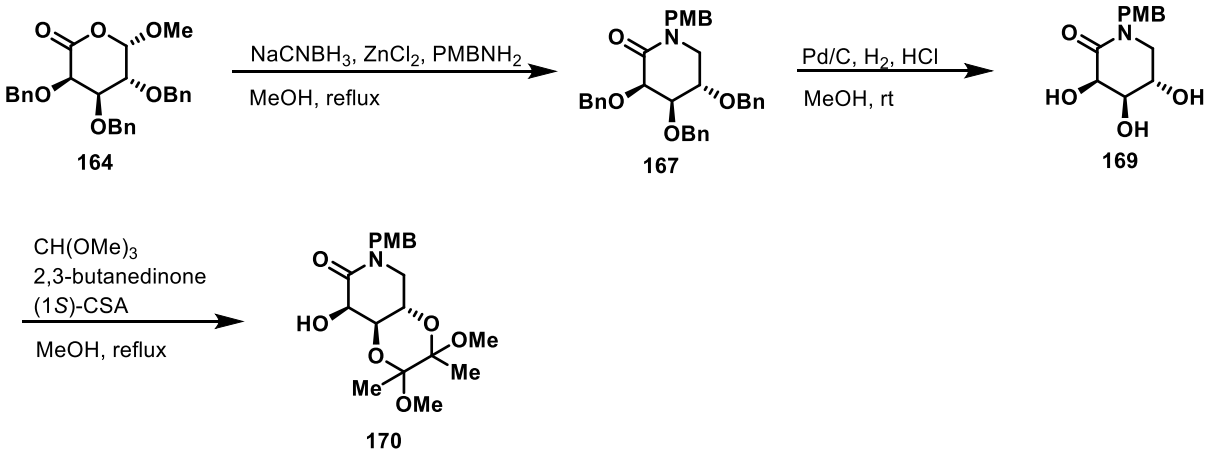
(3*R*,4*R*,5*S*)-1-Benzyl-3,4,5-tris(benzyloxy)piperidin-2-one (166): To a solution of lactone **164** (23 mg, 0.051 mmol) in MeOH (0.51 mL) was added benzylamine (11.2 µL, 0.103 mmol), $NaCNBH_3$ (6.5 mg, 0.103 mmol) and $ZnCl_2$ (1 drop, 0.5 M in THF). The mixture was allowed to reflux for 3 h, followed by quenching with saturated $NaHCO_3$ (2 mL) at 0 °C. After removal of the solvent, the mixture was dissolved in water, extracted with ethyl acetate (10 ml x 3) and washed with brine (20 ml × 2). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford product 1**66**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.10 (m, 20H), 5.09 (d, *J* = 12.2 Hz, 1H), 4.79 (d, *J* = 12.0 Hz, 1H), 4.71 (d, *J* = 6.7 Hz, 1H), 4.69 (d, *J* = 3.9 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.33 (d, *J* = 15.0 Hz, 1H), 4.31 (s, 2H), 4.28 (d, *J* = 2.9 Hz, 1H), 3.93, (dd, *J* = 4.9, 2.9 Hz, 1H), 3.72, (td, *J* = 4.7, 2.8 Hz, 1H), 3.46 (dd, *J* = 13.2, 4.6 Hz, 1H).

MS (ESI^+): *m/z* 508 ($[M+H]^+$)

(3*R*,4*R*,5*S*)-1-Benzyl-3,4,5-trihydroxypiperidin-2-one (168): To a solution of **166** (10 mg, 0.02 mmol) in MeOH (0.45 mL) was added hydrogen chloride solution (1 drop, 3 M in methanol) and Pd/C catalyst (10% wt, 3 mg). The reaction mixture was stirred under the atmosphere of hydrogen gas for 40 h. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure to afford the crude product **168**.

MS (ESI^+): *m/z* 238 ($[M+H]^+$)



(3*R*,4*R*,5*S*)-3,4,5-tris(Benzyloxy)-1-(4-methoxybenzyl)piperidin-2-one (167): To a solution of lactone **164** (651 mg, 1.45 mmol) in MeOH (15 mL) were added 4-methoxylbenzylamine (0.38 mL, 2.9 mmol), $NaCNBH_3$ (183 mg, 2.9 mmol) and $ZnCl_2$ (40 mg, 0.29 mmol). The mixture was allowed to reflux for 3h, followed by quenching with saturated $NaHCO_3$ (5 mL) at 0 °C. After removal of the solvent, the mixture was dissolved in water, extracted with ethyl acetate (15 ml x 3) and washed by brine (30 ml x 2). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford product 1**67** (347 mg, 44%).

***R*f** = 0.27 (Hex:EtOAc = 3:1)

MS (ESI^+): *m/z* 538 ($[M+H]^+$)

(3*R*,4*R*,5*S*)-1-Benzyl-3,4,5-trihydroxypiperidin-2-one (169): To a solution of **167** (269 mg, 0.02 mmol) in MeOH (10 mL) was added hydrogen chloride solution (1.25 mL, 3 M in methanol) and Pd/C catalyst (10% wt, 27 mg). The reaction mixture was stirred under the atmosphere of hydrogen gas for 15 h. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, gradient elution: 0 to 10% MeOH in CH_2Cl_2) to afford product **169** (132 mg, 88%).

^{1}H NMR (500 MHz, CD_3OD) δ 7.22 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 4.59 (d, *J* = 14.7 Hz, 1H), 4.47 (d, *J* = 14.7 Hz, 1H), 4.38 (d, *J* = 3.3 Hz, 1H), 4.08 (dd, *J* = 4.6, 3.3 Hz, 1H), 3.98 (td, *J* = 4.3, 2.7 Hz, 1H), 3.78 (s, 3H), 3.60, (dd, *J* = 13.2, 4.2 Hz, 1H), 3.12 (dd, *J* = 13.1, 2.7 Hz, 1H).

^{13}C NMR (125 MHz, CD_3OD) δ 171.5, 159.2, 128.8, 128.3, 113.6, 71.2, 67.8, 66.6, 54.3, 50.2, 48.9.

MS (ESI^+): *m/z* 268 ($[M+H]^+$)

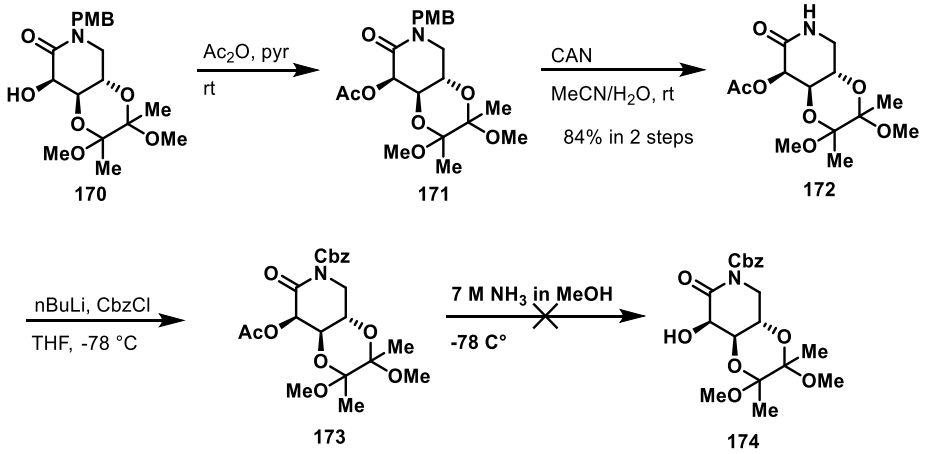
(4a*S*,8*R*,8a*S*)-8-Hydroxy-2,3-dimethoxy-6-(4-methoxybenzyl)-2,3-dimethylhexahydro-[1,4]dioxino[2,3-*c*]pyridin-7(4a*H*)-one (170): To a solution of 1**69** (94.2 mg, 0.35 mmol) in dry MeOH (7.1 mL) under nitrogen atmosphere was added trimethyl orthoformate (0.32 mL, 2.89 mmol), butane-2, 3-dione (0.08 mL, 0.88 mmol) and CSA (10.6 mg, 0.046 mmol). The reaction was allowed to reflux for 20 h and then neutralized with Et_3N (6.4 μL, 0.046 mmol). The resulting mixture was concentrated in vacuo and purified by flash column chromatography (SiO_2, gradient elution: 0 to 5% MeOH in CH_2Cl_2) to afford product **170** (85 mg, 63%) as a white solid.

***R*f** = 0.23 (CH_2Cl_2:MeOH = 20:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 4.72 (d, *J* = 14.9 Hz, 1H), 4.40 (d, *J* = 14.9 Hz, 1H), 4.28 (t, *J* = 4.5 Hz, 1H), 4.13 (d, *J* = 4.6 Hz, 1H), 3.99 (dt, *J* = 4.4, 2.2 Hz, 1H), 3.79 (d, *J* = 2.5 Hz, 1H), 3.77 (s, 3H), 3.26 (s, 3H), 3.19 (s, 3H), 3.03 (d, *J* = 12.9 Hz, 1H), 1.33 (s, 3H), 1.26 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 168.3, 158.9, 129.0, 128.9, 114.0, 99.1, 99.1, 66.5, 65.9, 65.8, 55.4, 50.6, 49.5, 49.4, 48.3, 18.0, 17.8.

MS (ESI^+): *m/z* 381 ($[M+H]^+$), 445 ($[M+MeCN+Na]^+$), 763 ($[2M+H]^+$), 785 ($[2M+Na]^+$)



(4a*S*,8*R*,8a*R*)-2,3-Dimethoxy-6-(4-methoxybenzyl)-2,3-dimethyl-7-oxooctahydro-[1,4]dioxino[2,3-*c*]pyridin-8-yl acetate (171): To a solution of **170** (20.9 mg, 0.049 mmol) in pyridine (0.27 mL) was added acetic anhydride (0.27 mL). The mixture was stirred at room temperature for 24 h and then concentrated under reduced pressure. The residue was co-evaporated three times with toluene. The crude product was used in the next step without purification.

***R*f** = 0.4 (CH_2Cl_2:MeOH = 20:1)

(4a*S*,8*R*,8a*R*)-2,3-Dimethoxy-2,3-dimethyl-7-oxooctahydro-[1,4]dioxino[2,3-*c*]pyridin-8-yl acetate (172): To a solution of **171** (0.049 mmol) in CH_3CN/H_2O (4:1, 1.0 mL/0.25 mL) was added cerium (IV) ammonium nitrate (90.2 mg, 0.15 mmol). The mixture was stirred for 3 h at rt and then diluted with water (10 mL). The aqueous layer was extracted with ethyl acetate (10 mL x 3). The combined organic layers were washed successively with saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The resulting residue was purified by flash column chromatography (SiO_2, gradient elution: 0 to 5% MeOH in CH_2Cl_2) to afford product **172** (14 mg, 84% in 2 steps).

R_f = 0.26 (CH_2Cl_2:MeOH = 20:1)

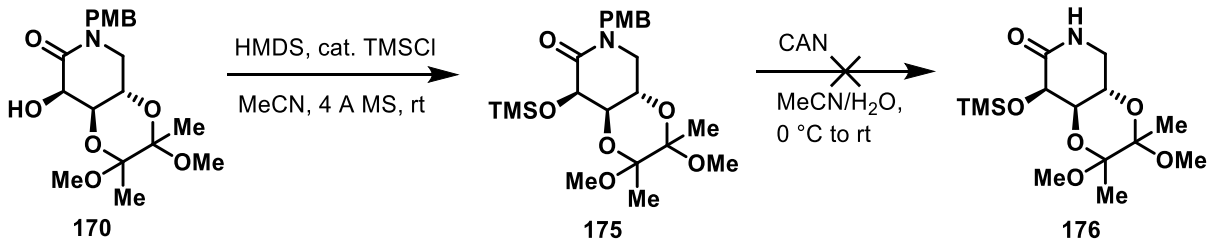
^{1}H NMR (600 MHz, $CDCl_3$) δ 6.36 (d, *J* = 3.8 Hz, 1H), 5.07 (dq, *J* = 4.0, 2.0 Hz, 1H), 4.31 (td, *J* = 4.5, 1.2 Hz, 1H), 3.99 (d. *J* = 4.7 Hz, 1H), 3.88-3.82 (m, 1H), 3.32-3.29 (m, 1H), 3.29 (s, 3H), 3.24 (s, 3H), 2.07 (s, 3H), 1.34 (s, 3H), 1.28 (s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 169.9, 99.3, 99.2, 68.5, 65.3, 63.5, 50.6, 48.6, 41.8, 21.1, 17.9, 17.6.

MS (ESI^+): *m/z* 607 ($[2M+H]^+$), 629 ($[2M+Na]^+$)

Benzyl (4a*S*,8*R*,8a*R*)-8-acetoxy-2,3-dimethoxy-2,3-dimethyl-7-oxohexahydro-[1,4]dioxino[2,3-*c*]pyridine-6(5*H*)-carboxylate (173): To a solution of compound **172** (3.2 mg, 0.011 mmol) in THF (0.2 mL) at -78 °C was added nBuLi (14.4 µL, 0.021 mmol, 1.47 M). After 1 h, CbzCl (1.9 µL, 0.013 mmol) was added to the reaction. The reaction was stirred at -78 °C for 2 h and then quenched by sat. $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4. The dried solution was filtered, and the filtrate was concentrated to afford the product **173** which was used in the next step without purification.

MS (ESI$^+$): *m/z* 438 ([M+H]$^+$), 501 ([M+MeCN+Na]$^+$), 892 ([2M+NH$_4$]$^+$), 897 ([2M+Na]$^+$)



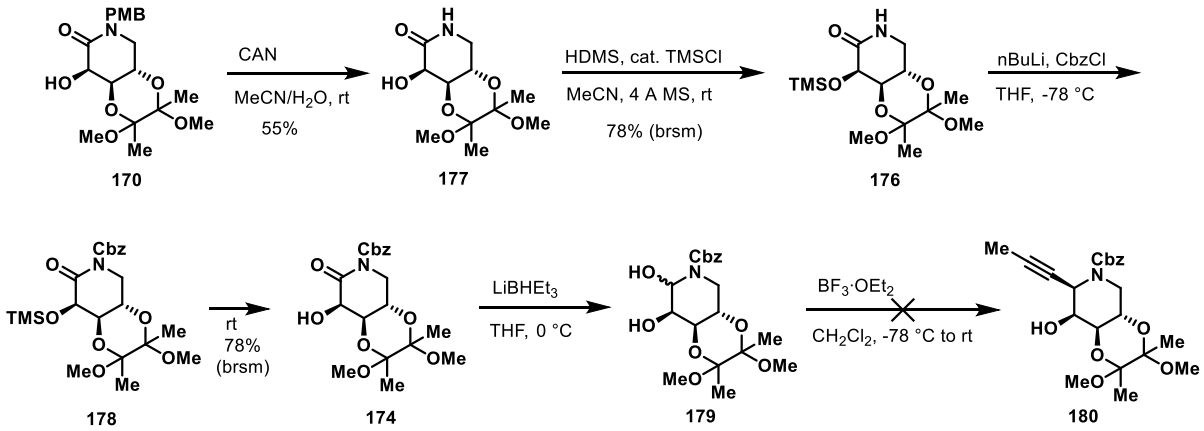
(4a*S*,8*R*,8a*R*)-2,3-Dimethoxy-6-(4-methoxybenzyl)-2,3-dimethyl-8-((trimethylsilyl)oxy)hexahydro-[1,4]dioxino[2,3-*c*]pyridin-7(4a*H*)-one (175): To a solution of **170** (20 mg, 0.052 mmol) in acetonitrile (1.1 mL) was added 4 Å MS, HMDS (55 µL, 0.26 mmol) and a catalytic amount of TMSCl (0.7 µL, 0.005 mmol) at room temperature. The reaction mixture was stirred for 24 h and then diluted with absolute ethanol. The reaction mixture was filtered through a pad of Celite, concentrated under reduced pressure, and purified by flash column chromatography (SiO_2, gradient elution: 0 to 5% MeOH in CH_2Cl_2) to afford product **175** as a white solid.

R_f = 0.51 (CH_2Cl_2:MeOH = 20:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 4.59 (q, *J* = 15.1 Hz, 2H), 4.16-4.10 (m, 2H), 3.91-3.87 (m, 1H), 3.78 (s, 3H), 3.73 (dd, *J* = 12.7, 2.2 Hz, 1H), 3.27 (s, 3H), 3.23 (s, 3H), 2.91-2.85 (m, 1H), 1.35 (s, 3H), 1.28 (s, 3H), 0.04 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 167.9, 158.8, 129.1, 128.9, 113.9, 99.1, 99.1, 67.0, 66.4, 66.0, 55.4, 50.6, 49.5, 49.2, 48.2, 18.0, 17.8, 0.2.

MS (ESI$^+$): *m/z* 454 ([M+H]$^+$), 907 ([2M+H]$^+$), 929 ([2M+Na]$^+$)



(4a*S*,8*R*,8a*S*)-8-Hydroxy-2,3-dimethoxy-2,3-dimethylhexahydro-[1,4]dioxino[2,3-*c*]pyridin-7(4a*H*)-one (177): To a solution of 1**70** (42.6 mg, 0.117 mmol) in CH_3CN/H_2O (4:1, 4.4 mL/1.1 mL) was added cerium (IV) ammonium nitrate (183.7 mg, 0.335 mmol). The mixture was stirred for 3 h at rt and then diluted with water (20 mL). The aqueous layer was extracted with ethyl acetate (20 mL x 3). The combined organic layers were washed successively with saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, gradient elution: 0 to 10% MeOH in CH_2Cl_2) to afford the product **177** (16 mg, 55% in 2 steps).

$\boldsymbol{R_f}$ = 0.3 (CH_2Cl_2:MeOH = 10:1)

MS (ESI^+): *m/z* 523 ($[2M+H]^+$), 545 ($[2M+Na]^+$)

(4a*S*,8*R*,8a*R*)-2,3-Dimethoxy-2,3-dimethyl-8-((trimethylsilyl)oxy)hexahydro-[1,4]dioxino[2,3-*c*]pyridin-7(4a*H*)-one (176): a solution of **177** (11.8 mg, 0.045 mmol) in acetonitrile (0.9 mL) was added 4 Å MS, HMDS (66 µL, 0.32 mmol) and a catalytic amount of TMSCl (0.3 µL, 0.002 mmol) at room temperature. The reaction mixture was stirred for 24 h and then diluted with absolute ethanol. The reaction mixture was filtered through a pad of Celite, concentrated under reduced pressure, and purified by flash column chromatography (SiO_2,

gradient elution: 0 to 5% to 10% MeOH in CH_2Cl_2) to afford the recovered **177** (1.2 mg) and the product **176** (10 mg, 78% brsm) as a white solid.

R_f = 0.4 (CH_2Cl_2:MeOH = 20:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 6.03-5.93 (m, 1H), 4.18-4.13 (m, 1H), 4.05 (d, *J* = 4.6 Hz, 1H), 3.97 (dq, *J* = 3.9, 1.9 Hz, 1H), 3.77 (dd, *J* = 12.6, 2.2 Hz, 1H), 3.29 (s, 3H), 3.25 (s, 3H), 3.10-3.04 (m, 1H), 1.34 (s, 3H), 1.27 (s, 3H), 0.12 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 170.0, 99.2, 67.4, 66.2, 65.5, 50.6, 48.3, 44.9, 18.0, 17.7, 0.0.

MS (ESI^+): *m/z* 667 ($[2M+H]^+$), 689 ($[2M+Na]^+$)

Benzyl (4a*S*,8*R*,8a*S*)-8-hydroxy-2,3-dimethoxy-2,3-dimethyl-7-oxohexahydro-[1,4]dioxino[2,3-*c*]pyridine-6(5*H*)-carboxylate (174): To a solution of compound **176** (20.9 mg, 0.063 mmol) in THF (3.1 mL) at -78 °C was added nBuLi (0.11 µL, 0.16 mmol, 1.47 M). After 1h, CbzCl (13.8 µL, 0.094 mmol) was added to the reaction. The reaction was stirred at -78 °C for 2 h and then quenched by sat. $NaHCO_3$ solution. The resulting mixture was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, filtered, concentrated in vacuo, and purified by flash column chromatography (SiO_2, gradient elution: 0 to 25% EtOAc in hexane) to afford the recovered **176** (6.7 mg) and the product **178** which was decomposed overtime at rt on the bench top to provide **174** (13.1 mg, 78% brsm).

Compound **178**:

R_f = 0.5 (Hex:EtOAc = 3:1)

MS (ESI^+): *m/z* 468 ($[M+H]^+$), 952 ($[2M+NH_4]^+$), 957 ($[2M+Na]^+$)

Compound **174**:

***R*f** = 0.26 (Hex:EtOAc = 1:1)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50-7.28 (m, 5H), 5.32 (d, *J* = 12.6 Hz, 1H), 5.27 (d, *J* = 12.5 Hz, 1H), 4.28 (td, *J* = 4.6, 1.2 Hz, 1H), 4.15 (d, *J* = 4.9 Hz, 1H), 4.14-4.11 (m, 1H), 4.03 (ddd, *J* = 13.6, 2.9, 1.2 Hz, 1H), 3.97 (dd, *J* = 13.5, 2.1 Hz, 1H), 3.28 (s, 3H), 3.13 (s, 3H), 1.33 (s, 3H), 1.26 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 168.8, 154.3, 135.5, 128.7, 128.3, 128.0, 99.2, 99.0, 68.7, 67.1, 66.2, 65.1, 50.7, 48.5, 48.0, 17.9, 17.4.

MS (ESI^+): *m/z* 396 ($[M+H]^+$), 459 ($[M+MeCN+Na]^+$), 808 ($[2M+NH_4]^+$), 813 ($[2M+Na]^+$)

Benzyl (4a*S*,8*R*,8a*S*)-7,8-dihydroxy-2,3-dimethoxy-2,3-dimethylhexahydro-[1,4]dioxino[2,3-*c*]pyridine-6(5*H*)-carboxylate (179): To a solution of **174** (5 mg, 0.013 mmol) in THF (0.27 ml) was added dropwise with $LiBHEt_3$ solution (0.06 ml, 0.063 mmol, 1 M in THF) at 0 °C. The reaction was stirred for 1 h, and then quenched with sat. $NaHCO_3$ solution. The resulting mixture was extracted with CH_2Cl_2. (5 mL x 2). The combined organic layers were dried (Na_2SO_4) and evaporated to afford product **179**, which was used for the next step without further purification.

MS (ESI^+): *m/z* 461 ($[M+MeCN+Na]^+$), 817 ($[2M+Na]^+$)

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