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A concise route to virginiamycin M2

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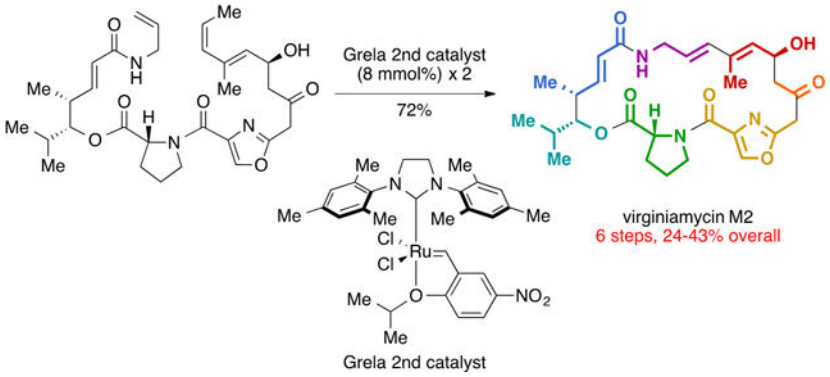
Abstract

Modular, fully synthetic routes to structurally complex natural products provide useful avenues to access chemical diversity. Herein we report a concise route to virginiamycin M2, a member of the group A streptogramin class of natural products that inhibits bacterial protein synthesis. Our approach features a longest linear sequence of six steps from 7 simple building blocks, and is the shortest and highest yielding synthesis of any member of the streptogramin class reported to date. We believe this route will enable access to unexplored structural diversity and may serve as a useful tool to improve the therapeutic potential of the streptogramin class of antibiotics.

Graphical Abstract



Keywords

Virginiamycin M2; Antibiotics; Bacterial resistance; Total synthesis; Ring-closing metathesis

1. Introduction

Millions of lives have been saved since the advent of antibiotics in the last century.[1] Due to the use and misuse of antibiotics in humans and animals, antibiotic resistance is becoming a

critical worldwide health issue.[2] There is an urgent need for the development of new antibiotics to overcome infections caused by multidrug-resistant, life-threatening pathogens.

The first pair of streptogramin antibiotics was isolated from *Streptomyces pristinaperaelis* by Charney in 1953.[3] This class was divided into group A members featuring macrocyclic polyketide/nonribosomal peptide hybrids (e.g., **1**-**5**, Fig. 1) and group B featuring 19-membered macrocyclic depsipeptides (e.g., virginiamycin S1 (**6**)).[3,4] The activities and mechanisms of action of streptogramins have been well defined by biochemical and structural studies. Group A and group B streptogramins work in concert to inhibit protein synthesis by binding to adjacent sites in the bacterial ribosome.[4] Although this synergy results in a potent antibacterial activity and has led to promising clinical candidates,[5] only one streptogramin combination has been approved by the US Food and Drug Administration. Synercid is a 70:30 combination of dalfopristin (group A) and quinupristin (group B), which are derived semisynthetically from virginiamycin M1 (**2**) and virginiamycin S1 (**6**).[6]

Due to their potent antibacterial activities and uncommon chemical architectures, streptogramin A antibiotics have received much attention from the chemistry community. In 1996, Schlessinger and colleagues published the first total synthesis of virginiamycin M2 (**1**) featuring vinylogous urethane chemistry.[7a] This report emerged concomitantly with the first total synthesis of madumycin II by the Meyers group,[7b] who also disclosed the first total synthesis of griseoviridin (**5**) in 2000.[8] Recently, Panek reported an elegant route to virginiamycin M2 (**1**) from an allylsilane precursor in 6% overall yield in 10 linear steps by means of alkyne–alkyne reductive cross coupling.[9] Other syntheses of these and related streptogramins have also emerged.[10] In 2017, we reported a synthesis of four natural group A streptogramin antibiotics that proceeded in 7 linear steps (longest linear sequence) from 7 simple building blocks and featured an intramolecular Stille coupling reaction to construct the 23-membered macrocycle (Fig. 2A).[11] Our strategy represented the shortest synthesis of virginiamycin M2 (**1**) and madumycin II (**4**), and also provided the first fully synthetic access to virginiamycin M1 (**2**) and madumycin I (**3**). Despite its brevity, our first generation route suffers from contextual limitations including acid sensitivity of intermediates, reliance on toxic organotin reagents (and generation of organotin byproducts), and a moderate-yielding Stille macrocyclization. Herein we report a shorter, higher-yielding, fully synthetic route to virginiamycin M2 (**1**) that overcomes these limitations and we anticipate it will serve to optimize this scaffold for improved therapeutic utility.

2. Results and discussion

A retrosynthetic analysis for our revised route is described in Fig. 2B. We envisioned that the conjugated diene of **1** could be constructed by ring closing metathesis (RCM), which would not require organotin reagents and would shorten the overall strategy. RCM was first reported by Didier Villemin in 1980[12] and was expanded upon by R. H. Grubbs, R. R. Schrock, and Y. Chauvin to become a powerful method for the construction of alkene-containing ring systems.[13,14] RCM precursor **8** could be assembled by peptide bond formation from amine **9** and acid **10** which we anticipated could be prepared using the framework of our first synthesis as a guideline.[11]

Our initial efforts toward a second generation route to viginiamycin M2 (**1**) are depicted in Scheme 1. The synthesis of the left half of the molecule (**9)** commenced with an oxazaborolidine-catalyzed, Mukaiyama-type vinylogous aldol reaction between isobutyraldehyde (**11**) and silyl dienol ether **12** to provide enoate **14** in 94% yield and 87% ee.[11,15] Trimethylaluminum-mediated amidation of **14** with allyl amine (**15**) produced **16** in 90% yield. Coupling of **16** with Fmoc-D-Pro-OH (**17**) in presence of DCC and catalytic DMAP followed by removal of the Fmoc-group with diethylamine in one pot provided amine **9** in 96% yield. The synthesis of the left half (**9**) proceeds from building blocks **11** and **12** in three steps in 81% overall yield.

For the right half of the molecule (**10**), we initially targeted acid **10a**, which contains a diene with minimal substitution (R = H). We found that the conditions used in our previous synthesis ($TiCl_4$ and $^{i}Pr_2EtN$)[11,16] were unsuitable for the coupling of dienal **18a** with acetyl thiazolidinethione **19**, as they led to retro-aldol reaction after quenching with water or pH = 7 buffer. When $Sn(OTf)_2$ and *N*-ethylpiperidine were used, **20a** was produced in 74% yield as a single diastereomer.[17] The minimal toxicity of tin(II) salts compared to organotin compounds[18] made this an acceptable concession in the current route. Silylation of **20a** with *tert*-butyldimethylsilyl trifluoromethanesulfonate in the presence of 2,6-lutidine delivered **21a** in 93% yield. Cleavage of the thiazolidinethione auxiliary by dianion of oxazole **22** proceeded in 70% yield to provide the right half **10a**. The route to the right half (**10a**) proceeds in 48% yield over three steps from **18a** and **19**. Then the left half (**9**) and the right half (**10a**) are coupled by means of HATU and $^{i}Pr_2EtN$, providing RCM precursor **8a** yield. Using the same general strategy, we also prepared precursor **8b**, which contains an additional *trans*-methyl group on the diene function.

Efforts to cyclize the 23-membered ring of virginiamycin M2 are summarized in Table 1. Attempted cyclization of **8a** with Grubbs I/II and Hoveyda-Grubbs I/II (**[Ru]-1–4**) catalysts at 23 °C resulted no conversion by ^{1}H NMR analysis (entry 1). At higher temperature (70 °C), **8a** was consumed but no product was detected (entry 2). *Trans*-methyl-substituted precursor **8b** produced similar results with **[Ru]-2** (entries 3 and 4). Our first promising result came when RCM precursor **8b** was exposed to more active, phosphine-free Hoveyda-Grubbs II catalyst (**[Ru]-4**), resulting in 15% yield of macrocycle **23** at ambient temperature determined by ^{1}H NMR with 1,4-dinitrobenzene as an internal standard.

During preliminary experiments, batches of *trans*-methyl diene precursor **8b** were contaminated with small amounts of the corresponding *cis*-methyl precursor (data not shown). ^{1}H-NMR analysis of crude RCM reaction mixtures indicated this contaminant was completely consumed, even when the majority of **8b** remained unreacted. Thus, we explored the possibility that the *cis*-*trans* diene might serve as a more effective precursor for macrocyclization than the *trans*-methyl diene in **8b**. We were readily able to obtain significant quantities of *cis*-methyl precursor **8c** using stereoisomerically pure dienal **18c** as a starting material (Scheme 2). Following the same strategy as depicted in Scheme 1, we produced macrocycle precursor **8c** in 41% from building block **18c** or in 73% yield from building block **11** over 4 steps.

To our delight, when **8c** was treated with **[Ru]-4** in tolene at 23 °C, the yield was greatly improved (38% ^{1}H NMR yield, Table 2, entry 4). Unsurprisingly, we observed as a byproduct the *trans*-methyl macrocyclization precursor **8b**, which results from isomerization of **8c**. Such isomerizations are well-documented for olefin metathesis,[19] and we next sought conditions that would favor the cyclization pathway over the unproductive isomerization pathway. Catalysts with pyridyl coordinating groups (e.g., **[Ru] −5**[20] and **[Ru] −6**,[21] Table 2, entries 5 and 6) or with smaller carbene ligands (e.g., **[Ru]-7**,[22] entry 7) did not improve the yield of macrocycle **23**. We found that batchwise addition of catalyst **[Ru]-4** (2 × 8 mol %) and a more polar solvent (benzenetrifluoride) led to small improvements (entries 8 and 9). Finally, a screen of metathesis catalysts that are known to increase initial reaction rate (entries 10–14)[23] revealed that catalyst **[Ru]-11**, which was developed by Grela and coworkers,[24] provided the highest yield of macrocycle **23** from precursor **8c** (49% isolated yield). Interestingly, we found that removal of the silyl groups in prior to macrocizliation (**8c** → **8d**) greatly increased the yield of the macrocyclization, providing virginiamycin M2 (**1**) in 72% isolated yield (Table 2, entry 14). While the cause of this improvement is unclear, it is possible that it arises from catalyst coordination to the exposed allylic alcohol[25] or from favorable conformational bias in the desilylated macrocyclic precursor **8d** relative to **8c**. It is worth noting that this desilylation strategy did not improve the yield of the Stille macrocyclization in our 1st generation route.[11]

The interesting reactivity patterns of the four macrocycle precursors (**8a–d**) merit further discussion. Z alkenes have been shown to improve the yield and stereoselectivity of ring-closing metatheses to provide Z-alkene-containing macrocycles.[26] In the present context, however, metathesis of the Z alkene precursors **8c** and **8d** selectively deliver the E macrocycles **23** and **1**. To the best of our knowledge this is the first example of a Z alkene serving as a more effective RCM precursor than an E alkene or a monosubstituted alkene to provide an E-alkene-containing macrocycle. This pattern of reactivity may be due in part to the conformational rigidity imparted by the abundance of sp^2 atoms in the macrocycle precursors herein, or from the differing reactivity of dienes compared to monoenes in metatheses reactions. We believe, however, that these results may prove to be more general, and that inclusion of Z alkene precursors should be considered during the optimization of challenging RCM reactions.

Scheme 3 provides a comparison of our two routes to virginiamycin M2 (**1**). By leveraging olefin metathesis to close the 23-membered macrocycle, we were able to reduce the longest linear sequence (LLS) from 7 to 6 steps. This resulted in improvement of the yields of each half and in a higher yield for macrocyclization (59% → 72%). Moreover, the new route is not reliant on organotin reagents and does not produce organotin byproducts, although it does require tin(II) salts. The absense of vinyltin intermediates in the current route also expands the scope of reagents that may be used during the synthesis of streptogramin analogs (e.g., acidic reagents that would protodestannylate vinyltin compounds). It is worth noting that both routes required extensive optimization of the macrocyclization step,[11] which is not uncommon in the synthesis of macrocycles.

3. Conclusion

In summary, we have develeped a short, efficient route to virginiamycin M2 (**1**) that proceeds in 6 steps from building blocks **11** and **18c** in 24–43% overall yield. This route features a ring closing metathesis to form the 23-membered ring from an enediene system. Three precursors, terminal diene, *trans*-methyldiene and *cis*-methyldiene were designed to perform ring-closing metathesis and various Ru-carbene catalysts were screened to facilitate this transformation. The current route is one step shorter than our first generation route and proceeds in higher yield. We believe it will serve as a useful platform for the synthesis of group A streptogramin analogs with improved properties for therapeutic use.

Experimental section

3.1. General

All reactions were performed in flame- or oven-dried glassware fitted with rubber septa under a positive pressure of nitrogen or argon, unless otherwise noted. All reaction mixtures were stirred throughout the course of each procedure using Teflon-coated magnetic stir bars. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula. Solutions were concentrated by rotary evaporation below 35 °C. Analytical thin- layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25-mm, 60-Å pore size, 230–400 mesh, SILICYCLE INC) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV), and then were stained by submersion in a basic aqueous solution of potassium permanganate or with an acidic ethanolic solution of anisaldehyde, followed by brief heating.

DCM and tetrahydrofuran (THF) to be used in anhydrous reaction mixtures were dried by passage through activated alumina columns immediately prior to use. Anhydrous toluene and benzotrifluoride (BTF) used were purchased from Sigma-Aldrich. Hexanes used were ≥85% *n*-hexane. Other commercial solvents and reagents were used as received, unless otherwise noted.

Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on 400 MHz Bruker Avance III HD 2-channel instrument NMR spectrometers at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, 03B4 scale) and are referenced to residual protium in the NMR solvent ($CHC1_3$: δ 7.26). Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonance of the NMR solvent ($CDC1_3$: δ 77.0). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, sxt = sextet, m = multiplet, br = broad, app = apparent), integration, and coupling constant (J) in hertz (Hz). Optical rotations were measured using a JASCO P-2000 polarimeter. High-resolution mass spectra were obtained on a Waters Acquity UPLC/Xevo G2-XS QTOF mass spectrometer with ESI ionization (special thanks to Ziyang Zhang in the Shokat Laboratory for assistance). Melting points were recorded on an Electrothermal IA6304 Melting Point Apparatus.

3.2. Experimental procedures and data for synthetic compounds

4.2.1 Mukaiyama aldol product 14—A 250-mL round-bottom flask was charged with phenylboronic acid (1.22 g, 10.0 mmol, 0.5 equiv) and (S)-diphenyl(pyrrolidin-2-yl)methanol (2.53 g, 10.0 mmol, 0.5 equiv). The vessel was equipped with a reflux condenser and a Dean-Stark apparatus, evacuated and flushed with nitrogen (the process of nitrogen exchange was repeated a total of 3 times). Toluene (50 mL) was added, and the resulting clear solution was brought to reflux by means of a 145 °C oil bath. After 12 h, the reaction mixture was allowed to cool to 23 °C and was concentrated. The resulting white solid was dried at ≤1 Torr for 1 h. The vessel was flushed with nitrogen and DCM (80 mL) was added. The resulting colorless solution was cooled to −78 °C and TfOH (0.80 mL, 8.99 mmol, 0.45 equiv) was added dropwise over 5 min by means of glass syringe (CAUTION: TfOH rapidly corrodes most plastic syringes!). Some of the TfOH freezes upon contact with the solution. After 1 h, the solids had dissolved, and a mixture of isobutyraldehyde (**11**, 1.82 mL, 20.0 mmol, 1 equiv), silyl dienol ether **12** (5.70 g, 25.0 mmol, 1.25 equiv), and isopropanol (1.68 mL, 22.0 mmol, 1.1 equiv) in DCM (20 mL) was added dropwise over 2 h by syringe pump. The mixture was stirred at −78 °C for another 1.5 h and saturated aqueous $NaHCO_3$ solution (50 mL) was added in one portion. The vessel was removed from the cooling bath and the system was allowed to warm to 23 °C while it was rapidly stirred. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL). The organic layers were combined and the resulting solution was dried (Na_2SO_4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:10 to 1:6) to afford Mukaiyama aldol product **14** (3.48 g, 94%) as a colorless oil. **TLC** (EtOAc:hexanes = 1:6): R_f = 0.25 (UV, $KMnO_4$). **$[\alpha]^{23}_D$** = + 23.5 (*c* = 1.0, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.92 (dd, *J* = 15.7, 8.1 Hz, 1H), 5.86 (dd, *J* = 15.7, 1.2 Hz, 1H), 3.72 (s, 3H), 3.26 (t, *J* = 5.8 Hz, 1H), 2.59 – 2.39 (m, 1H), 1.78–1.64 (m, 1H), 1.59 (br s, 1H), 1.09 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 167.1, 152.2, 120.4, 80.0, 51.4, 39.9, 30.9, 19.6, 16.5, 13.9. **HRMS-EI** m/z calcd for C H +10 O $^+$19 3 $[M + H]^+$ 187.1329, found 187.1331.

Determination of enantiomeric excess: To a solution of **14** (20 mg, 0.11 mmol, 1 equiv) in DCM (2 mL) at 23 °C was added successively Et_3N (0.12 mL, 0.86 mmol, 8.0 equiv), DMAP (18 mg, 0.15 mmol, 1.4 equiv) and (*S*) or (*R*)- Mosher acid chloride (80 μL, 0.43 mmol, 4.0 equiv). After 2 h, the mixture was diluted with EtOAc (15 mL). The mixture was transferred to a separatory funnel and washed successively with 1 M aqueous $KHSO_4$ solution (3 × 5 mL), 1 M aqueous NaOH solution (5 mL) and saturated aqueous $NaHCO_3$ solution (3 × 5 mL). The organic phase was dried over $MgSO_4$, the dried solution was filtered, and the filtrate concentrated. The crude residue was analyzed by ^{1}H-NMR.

For (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride: The enantiomeric excess was calculated from integration of the peaks at 5.82 ppm (major) and 5.84 ppm (minor). The ee was 87%.

For (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride: The enantiomeric excess was calculated from integration of the peaks at 5.84 ppm (major) and 5.82 ppm (minor). The ee was 87%.

4.2.2 Amide 16—A 250-mL round-bottom flask was charged with allylamine (**15**, 3.2 mL, 43.0 mmol, 4.0 equiv) and dry DCM (72 mL) under nitrogen. The resulting colorless solution was cooled to 0 °C by means of an ice-water bath. A solution of $AlMe_3$ in heptane (1 M, 43.0 mL, 43.0 mmol, 4.0 equiv) was added dropwise over 30 min (CAUTION: Gas evolution!). The mixture was allowed to warm to 23 °C. After stirring for 30 min, a solution of **14** (2.00 g, 10.7 mmol, 1 equiv) in DCM (20 mL) was added over 10 min (CAUTION: Gas evolution!). The vessel was equipped with a reflux condenser and the solution was brought to reflux by means of a 50 °C oil bath. After 3 h, the mixture was cooled to 0 °C by means of ice-water bath and MeOH (10 mL) was added (CAUTION: Gas evolution!). Once gas evolution ceased, saturated aqueous potassium sodium tartrate solution (100 mL) was added. After stirring for 1 hour, the biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL) and the washed solution was dried (Na_2SO_4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:1) to afford amide **16** (2.05 g, 90%) as a white solid. **TLC** (EtOAc:hexanes = 1:1): R_f = 0.20 (UV, $KMnO_4$).**^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.79 (dd, *J* = 15.4, 7.8 Hz, 1H), 5.91 (br t, *J* = 5.6 Hz, 1H), 5.89 – 5.77 (m, 2H), 5.18 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.12 (dq, *J* = 10.2, 1.4 Hz, 1H), 3.92 (tt, *J* = 5.8, 1.6 Hz, 2H), 3.23 (q, *J* = 5.6 Hz, 1H), 2.47 (dddd, *J* = 8.1, 7.0, 5.7, 1.3 Hz, 1H), 1.98 (d, *J* = 5.3 Hz, 1H), 1.71 (dq, *J* = 13.1, 6.6 Hz, 1H), 1.06 (d, *J* = 6.7 Hz, 3H), 0.91 (d, *J* = 2.5 Hz, 3H), 0.89 (d, *J* = 2.8 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 165.9, 147.7, 134.1, 123.1, 116.5, 79.1, 41.9, 39.5, 30.8, 19.7, 16.9, 13.7. **HRMS-ESI** m/z calcd for $C_{12}H_{21}NNaO_2^+$ $[M + Na]^+$ 234.1464, found 234.1473.

4.2.3 Amine 9—A oven-dried 250-mL round-bottom flask was charged with amide **16** (2.00 g, 9.46 mmol, 1.0 equiv), Fmoc-D-Pro-OH (**17**, 4.79 g, 14.2 mmol, 1.5 equiv) and DMAP (0.23 g, 1.89 mmol, 0.2 equiv). DCM (95 mL) was added, resulting in a colorless solution. DCC (3.12 g, 15.1 mmol, 1.6 equiv) was added in one portion by briefly removing the septum, resulting in a white suspension. After 5 h, the alcohol was entirely consumed as indicated by TLC analysis (eluent: EtOAc:hexanes = 1:3) and diethyl amine (48 mL) was added. After additional 3 h, the mixture was filtered through a pad of celite and the filter cake was washed with DCM (2 × 20 mL). The filtrate was concentrated and the crude residue was purified by flash chromatography (silica gel, eluent: NH_4OH:MeOH:DCM = 0.2:1:100 to 0.2:1:50) to afford proline ester **9** (2.80 g, 96 %) as a light yellow, waxy solid. **TLC** (MeOH:DCM = 1:20): R_f = 0.30 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.68 (dd, *J* = 15.5, 7.7 Hz, 1H), 5.91 2013 5.67 (m, 3H), 5.18 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.15 – 5.06 (m, 1H), 4.80 (t, *J* = 6.2 Hz, 1H), 3.98 – 3.88 (m, 2H), 3.78 (dd, *J* = 8.5, 5.7 Hz, 1H), 3.07 (ddd, *J* = 10.3, 7.4, 6.2 Hz, 1H), 2.91 (dt, *J* = 10.3, 6.7 Hz, 1H), 2.72 – 2.58 (m, 1H), 2.52 (br s, 1H), 2.20 – 2.05 (m, 1H), 1.95 – 1.80 (m, 2H), 1.80 – 1.65 (m, 2H), 1.02 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.3 Hz, 3H), 0.86 (d, *J* = 6.4 Hz, 3H). **^{13}C NMR** (100 MHz,

$CDCl_3$) δ 175.0, 165.4, 145.3, 134.1, 123.7, 116.5, 80.5, 59.8, 46.8, 41.9, 38.1, 30.4, 29.8, 25.3, 19.5, 16.9, 14.5. **HRMS-ESI** m/z calcd for $C_{17}H_{29}N_2O_3^+$ $[M + H]^+$ 309.2173, found 309.2180.

4.2.2 General procedures for preparation of aldol products 20a–c—A round-bottom flask charged with $Sn(OTf)_2$ (1.3 equiv) was exposed to vacuum (≤ 1 Torr). The vessel was heated with a heat gun for 1 minute, and was allowed to cool for 2 minutes. This process was repeated 5 times, after which the vessel was flushed with nitrogen and sealed with a rubber septum. Dry DCM was added (10 mL for each mmol substrate 18), resulting in a white suspension and the vessel was cooled to −40 °C by means of a dry ice-acetonitrile bath. *N*-ethylpiperidine (1.3 equiv) was added dropwise, resulting in a deep yellow solution. After 5 min, a solution of 19 (1.2 equiv) in dry DCM (1.0 M) was added dropwise, and the resulting yellow solution was stirred for 5 hous at −40 °C. The reaction mixture was cooled to −78 °C by means of a dry ice- acetone bath. A solution of aldehyde 18 (1.0 equiv) in DCM (10 mL per mmol 18) was added via syringe pump over 30 min. After 3 hours (monitored by TLC plate), pH = 7.0 phosphate buffer (100 mL) was added. The vessel was removed from the cooling bath and the system was allowed to warm to 23 °C while the mixture was rapidly stirred. The biphasic mixture was filtered, the filtrate was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL) and the washed solution was dried (Na_2SO_4). The dried solution was filtered and the filtrate was concentrated. The crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:6 to 1:2.5) to afford aldol product 20.

4.2.2.1 Aldol product 20a: Aldol product **20a** was prepared according to the general procedure from $Sn(OTf)_2$ (0.54 g, 1.30 mmol, 1.3 equiv), *N*- ethylpiperidine (0.18 mL, 1.30 mmol, 1.3 equiv), **19** (0.24 g, 1.2 mmol, 1.2 equiv) and **18a** (0.096 g, 1.0 mmol, 1 equiv). **20a** (0.22 g, 74%) was obtained as a yellow oil. **TLC** (EtOAc:hexanes = 1:2.5): R_f = 0.40 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.36 (dd, *J* = 17.4, 10.7 Hz, 1H), 5.53 (d, *J* = 8.5 Hz, 1H), 5.23 (d, *J* = 17.4 Hz, 1H), 5.15 (ddd, *J* = 7.6, 6.2, 1.0 Hz, 1H), 5.07 (d, *J* = 10.8 Hz, 1H), 5.07 – 4.97 (m, 1H), 3.58 (ddd, *J* = 17.7, 3.1, 1.0 Hz, 1H), 3.53 (dd, *J* = 11.5, 8.0 Hz, 1H), 3.35 (dd, *J* = 17.7, 8.7 Hz, 1H), 3.04 (dd, *J* = 11.5, 1.1 Hz, 1H), 2.78 (dd, *J* = 4.1, 2.2 Hz, 1H), 2.44 – 2.29 (m, *J* = 6.8 Hz, 1H), 1.83 (d, *J* = 1.2 Hz, 3H), 1.07 (d, *J* = 6.8 Hz, 3H), 0.99 (d, *J* = 6.9 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 202.9, 172.4, 140.6, 136.16, 132.0, 113.6, 71.4, 65.1, 45.3, 30.8, 30.62, 19.1, 17.8, 12.3. **HRMS-ESI** m/z calcd for $C_{14}H_{20}NOS_2^+$ $[M – OH]^+$ 282.0981, found 282.0989.

4.2.2.2 Aldol product 20b: Aldol product **20b** was prepared according to the general procedure from $Sn(OTf)_2$ (1.60 g, 3.90 mmol, 1.3 equiv), *N*- ethylpiperidine (0.53 mL, 3.90 mmol, 1.3 equiv), **19** (0.73 g, 3.60 mmol, 1.2 equiv) and **18b** (0.33 g, 3.0 mmol, 1 equiv). **20b** (0.60 g, 64%) was obtained as a yellow oil. **TLC** (EtOAc:hexanes = 1:2.5): R_f = 0.40 (UV, $KMnO_4$). H NMR (400 MHz, $CDCl_3$) δ 6.05 (ddt, *J* = 15.5, 2.1, 1.4 Hz, 1H), 5.72 (dq, *J* = 15.5, 6.6 Hz, 1H), 5.39 (d, *J* = 8.6 Hz, 1H), 5.14 (ddd, *J* = 8.0, 6.1, 1.1 Hz, 1H), 5.00 (td, *J* = 8.7, 3.1 Hz, 1H), 3.57 – 3.48 (m, 2H), 3.34 (dd, *J* = 17.6, 8.8 Hz, 1H), 3.02 (dd, *J* = 11.5, 1.1 Hz, 1H), 2.74 (br s, 1H), 2.36 (dq, *J* = 13.6, 6.8 Hz, 1H), 1.80 (d, *J* = 1.3 Hz, 3H), 1.76

(dd, *J* = 6.6, 1.5 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 202.9, 172.5, 136.0, 135.0, 129.1, 125.3, 71.4, 65.0, 45.5, 30.8, 30.6, 19.0, 18.2, 17.7, 13.0. **HRMS-ESI** m/z calcd for $C_{15}H_{22}NOS^{+}{}_{2}$ $[M - OH]^+$ 296.1137, found 296.1133.

4.2.2.3 Aldol product 20c: Aldol product **20c** was prepared according to the general procedure from $Sn(OTf)_2$ (5.9 g, 14.20 mmol, 1.3 equiv), *N*- ethylpiperidine (1.95 mL, 14.20 mmol, 1.3 equiv), **19** (2.66 g, 13.1 mmol, 1.2 equiv) and **18c** (1.20 g, 10.9 mmol, 1 equiv). **20c** (2.40 g, 70%) was obtained as a yellow oil. **TLC** (EtOAc:hexanes = 1:2.5): R_f = 0.40 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.81 (d, *J* = 11.7 Hz, 1H), 5.50 (dq, *J* = 11.5, 7.2 Hz, 1H), 5.40 (d, *J* = 8.5 Hz, 1H), 5.18 – 5.10 (m, 1H), 4.97 (td, *J* = 8.7, 2.9 Hz, 1H), 3.58 (dd, *J* = 17.6, 2.7 Hz, 1H), 3.52 (dd, *J* = 11.5, 8.0 Hz, 1H), 3.37 (dd, *J* = 17.6, 8.7 Hz, 1H), 3.03 (d, *J* = 11.5 Hz, 1H), 2.74 (br s, 1H), 2.45 – 2.30 (m, *J* = 6.8 Hz, 1H), 1.85 (s, 3H), 1.79 (dd, *J* = 7.2, 1.8 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.99 (d, *J* = 6.9 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 202.9, 172.5, 135.4, 132.7, 129.9, 125.6, 71.4, 65.1, 45.4, 30.8, 30.6, 19.1, 17.8, 17.4, 14.7. **HRMS-ESI** m/z calcd for $C_{15}H_{22}NOS^{+}{}_{2}$ $[M - OH]^+$ 296.1137, found 296.1133.

4.2.3 General procedures for preparation of TBS ether 21a–c—A round-bottom flask charged with **20** (1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. DCM (10 mL per mmol of **20**) was added followed by 2,6-lutidine (2.0 equiv), resulting a yellow solution. The vessel was cooled to 0 °C by means of ice-water bath. TBSOTf (1.25 equiv) was added dropwise over 10 min. After stirring for 30 min, DCM (10 mL per mmol of **20**) was added and the mixture was transferred to a separatory funnel and washed with water (2 × 100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtered and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:40) to afford TBS ether **20**.

4.2.3.1 TBS ether 21a: TBS ether **21a** was prepared according to the general procedure from **20a** (0.86 g, 2.90 mmol, 1 equiv), 2,6-lutidine (0.67 mL, 5.70 mmol, 2.0 equiv) and TBSOTf (0.82 mL, 3.6 mmol, 1.25 equiv). **21a** (1.10 g, 93%) was obtained as a yellow oil. **TLC** (EtOAc:hexanes = 1:10): R_f = 0.30 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.33 (dd, *J* = 17.4, 10.7 Hz, 1H), 5.45 (d, *J* = 8.9 Hz, 1H), 5.18 (d, *J* = 17.4 Hz, 1H), 5.12 (td, *J* = 8.8, 3.8 Hz, 1H), 5.04 (d, *J* = 10.1 Hz, 1H), 5.03 – 4.98 (m, 1H), 3.74 (dd, *J* = 16.2, 8.8 Hz, 1H), 3.46 (dd, *J* = 11.4, 7.8 Hz, 1H), 3.07 – 3.02 (m, 1H), 3.01 (s, 1H), 2.44 – 2.30 (m, *J* = 6.9 Hz, 1H), 1.80 (d, *J* = 1.2 Hz, 3H), 1.05 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 7.0 Hz, 3H), 0.83 (s, 9H), 0.03 (s, 3H), 0.00 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 202.8, 171.1, 140.9, 134.5, 133.5, 112.9, 71.7, 66.8, 45.8, 30.9, 30.8, 25.7, 19.1, 18.0, 17.8, 12.2, −4.4, −5.0. **HRMS-ESI** m/z calcd for $C_{20}H_{35}NNaO_2S_2Si^+$ $[M + Na]^+$ 436.1771, found 436.1783.

4.2..2 TBS ether 21b: TBS ether **21b** was prepared according to the general procedure from **20b** (0.60 g, 1.90 mmol, 1 equiv), 2,6-lutidine (0.45 mL, 3.80 mmol, 2.0 equiv) and TBSOTf (0.55 mL, 2.4 mmol, 1.25 equiv). **21b** (0.71 g, 87%) was obtained as a yellow oil. **TLC** (EtOAc:hexanes = 1:10): R_f = 0.30 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ

6.03 (ddd, J = 15.6, 1.7, 0.8 Hz, 1H), 5.67 (dtd, J = 15.5, 6.8, 6.2 Hz, 1H), 5.32 (ddd, J = 9.0, 1.3, 0.7 Hz, 1H), 5.10 (td, J = 8.9, 3.8 Hz, 1H), 5.00 (ddd, J = 7.6, 6.2, 1.1 Hz, 1H), 3.74 (dd, J = 16.1, 8.8 Hz, 1H), 3.46 (dd, J = 11.4, 7.8 Hz, 1H), 3.02 (dd, J = 11.4, 1.1 Hz, 1H), 3.00 (dd, J = 16.2, 3.8 Hz, 1H), 2.45 – 2.31 (m, 1H), 1.77 (d, J = 1.2 Hz, 3H), 1.76 (dd, J = 5.7, 1.2 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 0.97 (d, J = 7.0 Hz, 3H), 0.83 (s, 9H), 0.03 (s, 3H), 0.00 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 202.8, 171.2, 135.3, 133.3, 131.7, 124.6, 71.8, 66.9, 46.0, 30.9, 30.8, 25.8, 19.1, 18.2, 18.0, 17.8, 13.0, −4.3, −5.0. **HRMS-ESI** m/z calcd for $C_{21}H_{37}NNaO_2S_2Si^+$ $[M + Na]^+$450.1927, found 450.1911.

4.2.3.3 TBS ether 21c: TBS ether **21c** was prepared according to the general procedure from **20c** (2.30 g, 7.34 mmol, 1 equiv), 2,6-lutidine (1.70 mL, 14.70 mmol, 2.0 equiv) and TBSOTf (2.11 mL, 0.17 mmol, 1.25 equiv). **21c** (3.08 g, 98%) was obtained as a yellow oil. **TLC** (EtOAc:hexanes = 1:10): R_f = 0.30 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.76 (dp, J = 11.6, 1.7 Hz, 1H), 5.47 (dq, J = 11.7, 7.2 Hz, 1H), 5.33 (dt, J = 8.9, 1.5 Hz, 1H), 5.12 – 4.93 (m, 2H), 3.76 (dd, J = 16.2, 8.7 Hz, 1H), 3.46 (dd, J = 11.4, 7.8 Hz, 1H), 3.11 – 2.96 (m, 2H), 2.46 – 2.32 (m, J = 6.9 Hz, 1H), 1.82 (d, J = 1.4 Hz, 3H), 1.78 (dd, J = 7.2, 1.8 Hz, 3H), 1.06 (d, J = 6.8 Hz, 3H), 0.97 (d, J = 6.9 Hz, 3H), 0.84 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 202.8, 171.2, 132.9, 132.6, 132.3, 125.1, 71.8, 66.9, 46.0, 31.0, 30.8, 25.8, 19.1, 18.0, 17.8, 17.3, 14.7, −4.4, −4.9. **HRMS-ESI** m/z calcd for $C_{21}H_{37}NNaO_2S_2Si^+$ $[M + Na]^+$ 450.1927, found 450.1911.

4.2.4 General procedures for preparation of the right halves 10a–c—A round-bottom flask charged with oxazolyl carboxylic acid **22** (2.0 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. THF (10 mL per mmol **21**) was added, resulting in a light yellow solution and the vessel and its contents were cooled to −78°C in a dry ice-acetone bath. A solution of *n*-butyllithium in hexanes (4.0 equiv) was added dropwise over 15 min, resulting in a deep red solution. After 30 min, a solution of **21** (1.0 equiv) in THF (10 mL per mmol **22**) was added over 30 min by syringe pump. After an additional 30 min, water (100 mL) was added, followed by 1 M aqueous $KHSO_4$ solution (8.0 equiv). The system was allowed to warm to 23 °C while the mixture was rapidly stirred. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL) and the washed solution was dried (Na_2SO_4). The dried solution was filtered and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: AcOH:EtOAc:hexanes = 0.5:50:50) to afford carboxylic acid **10**.

4.2.4.1 Acid 10a: Acid **10a** was prepared according to the general procedure from TBS ether **21a** (0.83 g, 2.00 mmol, 1 equiv), acid **22** (0.80 g, 4.00 mmol, 2.0 equiv) and ^{n}BuLi (2.5 M, 3.20 mL, 8.00 mmol, 4.00 equiv). **10a** (0.63 g, 70%) was obtained as a yellow solid. **TLC** (MeOH:DCM = 1:20): R_f = 0.30 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.30 (dd, J = 17.4, 10.7 Hz, 1H), 5.40 (d, J = 8.8 Hz, 1H), 5.18 (d, J = 17.4 Hz, 1H), 5.04 (d, J = 10.7 Hz, 1H), 4.99 (dt, J = 8.7, 4.3 Hz, 1H), 4.14 (d, J = 17.1 Hz, 1H), 4.06 (d, J = 17.1 Hz, 1H), 2.85 (dd, J = 14.9, 8.6 Hz, 1H), 2.51 (dd, J = 14.9, 4.2 Hz, 1H),1.76 (s, 3H), 0.84 (s,

9H), 0.36 (s, 9H), 0.03 (s, 3H), −0.01 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 201.1, 165.4, 165.3, 161.3, 140.7, 140.6, 134.0, 133.7, 113.3, 66.6, 50.1, 43.9, 25.8, 18.0, 12.1, −2.2, −4.5, −5.1. **HRMS-ESI** m/z calcd for $C_{21}H_{36}O_3Si_2^-$ $[M - HCO_2]^-$ 406.2239, found 406.2339.

4.2.4.2 Acid 10b: Acid **10b** was prepared according to the general procedure from TBS ether **21b** (0.40 g, 0.94 mmol, 1.0 equiv), acid **22** (0.37 g, 1.87 mmol, 2.0 equiv) and ^{n}BuLi (2.5 M, 1.50 mL, 3.74 mmol, 4.00 equiv). **10b** (0.30 g, 69%) was obtained as a yellow solid. **TLC** (MeOH:DCM = 1:20): R_f = 0.30 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.97 (br s, 1H), 5.99 (dd, *J* = 15.6, 0.9 Hz, 1H), 5.66 (dq, *J* = 15.6, 6.6 Hz, 1H), 5.25 (d, *J* = 8.8 Hz, 1H), 4.96 (td, *J* = 8.7, 4.2 Hz, 1H), 4.16 (d, *J* = 17.1 Hz, 1H), 4.07 (d, *J* = 17.2 Hz, 1H), 2.82 (dd, *J* = 14.8, 8.5 Hz, 1H), 2.49 (dd, *J* = 14.7, 4.3 Hz, 1H), 1.75 (dd, *J* = 6.7, 1.5 Hz, 3H), 1.73 (d, *J* = 1.3 Hz, 3H), 0.83 (s, 9H), 0.35 (s, 9H), 0.01 (s, 3H), −0.02 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 201.2, 165.4, 165.1, 161.4, 140.8, 135.1, 133.47, 131.2, 124.9, 66.7, 50.3, 43.9, 25.8, 18.2, 13.0, 12.8, −2.2, −4.4, −5.1. **HRMS-ESI** m/z calcd for $C_{22}H_{38}O_3Si^2$ $[M - HCO_2]^-$ 420.2393.

4.2.4.3 Acid 10c: Acid **10c** was prepared according to general procedure from TBS ether **21c** (0.66 g, 1.54 mmol, 1.0 equiv), acid **22** (0.62 g, 3.09 mmol, 2.0 equiv) and ^{n}BuLi (2.5 M, 2.47 mL, 6.17 mmol, 4.00 equiv). **10c** (0.48 g, 67%) was obtained as a yellow solid. **TLC** (MeOH:DCM = 1:20): R_f = 0.30 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.75 (dt, *J* = 11.7, 1.6 Hz, 1H), 5.48 (dq, *J* = 11.7, 7.2 Hz, 1H), 5.28 (dt, *J* = 8.7, 1.4 Hz, 1H), 4.93 (td, *J* = 8.6, 4.2 Hz, 1H), 4.15 (d, *J* = 17.2 Hz, 1H), 4.06 (d, *J* = 17.2 Hz, 1H), 2.86 (dd, *J* = 14.7, 8.5 Hz, 1H), 2.53 (dd, *J* = 14.7, 4.2 Hz, 1H), 1.78 (d, *J* = 1.4 Hz, 3H), 1.77 (dd, *J* = 7.2, 1.8 Hz, 3H), 0.85 (s, 9H), 0.36 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 201.2, 165.4, 165.3, 161.3, 140.6, 132.8, 132.7, 131.8, 125.3, 66.8, 50.4, 44.0, 25.8, 18.0, 17.3, 14.6, −2.2, −4.5, −5.0. **HRMS-ESI** m/z calcd for $C_{22}H_{38}O_3Si_2^-$ $[M - HCO_2]^-$ 420.2396, found 420.2393.

4.2.5 General procedures for preparation of ring closing metathesis (RCM) precursors 8a–c—A 100-mL round-bottom flask was charged with amine **9** (1 equiv), carboxylic acid **10** (1.05 equiv) and ^{i}Pr$_2$EtN (2.0 equiv). DCM (10 mL per mmol **9**) was added, resulting in a clear, colorless solution and HATU (1.25 equiv) was added to this solution in one portion at 23 °C. After stirring for 5 h, the mixture was diluted with DCM (100 mL). The solution was transferred to a separatory funnel and was washed with water (2 × 100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtered and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:6 to 1:4) to afford RCM precursor **8**.

4.2.5.1 RCM precursor 8a: RCM precursor **8a** was prepared according to the general procedure from amine **9** (0.40 g, 1.30 mmol, 1 equiv), acid **10a** (0.62 g, 1.40 mmol, 1.05 equiv), ^{i}Pr$_2$EtN (0.45 mL, 2.60 mmol, 2.0 equiv), and HATU (0.62 g, 1.60 mmol, 1.25 equiv). **8a** (0.86 g, 93%) was obtained as a yellow, waxy solid. **TLC** (EtOAc:hexanes = 1.2.5): R_f = 0.30 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.73 – 6.56 (m, 1H), 6.35 – 6.25 (m, 1H), 5.98 – 5.70 (m, 3H), 5.47 – 5.35 (m, 1H), 5.24 – 5.01 (m, 4H), 5.00 – 4.90 (m,

1H), 4.83 – 4.68 (m, 1H), 4.65 – 4.55 (m, 1H), 4.11 – 3.80 (m, 5H), 3.81 – 3.64 (m, 1H), 2.88 – 2.75 (m, 1H), 2.65 – 2.35 (m, 2H), 2.29 – 2.12 (m, 2H), 2.11 – 1.81 (m, 3H), 1.76 (s, 3H), 1.05 – 0.88 (m, 6H), 0.82 (m, 12H), 0.33 (s, 4.5H), 0.27 (s, 4.5H), 0.018 (s, 1.5H), 0.011 (s, 1.5H), −0.014 (s, 1.5H), −0.019 (s, 1.5H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 201.7, 201.2, 172.35, 172.32, 165.7, 165.4, 163.1, 162.2, 161.57, 161.55, 159.32, 159.28, 145.34, 145.22, 145.17, 145.1, 140.56, 140.54, 134.12, 134.08, 134.06, 134.01, 133.6, 123.89, 123.72, 116.5, 116.3, 113.3, 80.9, 80.5, 66.8, 66.6, 60.4, 59.9, 50.01, 49.96, 48.8, 47.0, 44.4, 44.2, 41.91, 41.87, 38.6, 38.3, 38.0, 31.5, 29.9, 29.7, 28.9, 25.73, 25.66, 25.2, 21.5, 19.6, 19.4, 18.0, 17.2, 17.0, 14.7, 14.2, 12.1, −1.79, −1.81, −4.5, −5.10, −5.12. **HRMS- ESI** m/z calcd for $C_{39}H_{64}N_3O_7Si_2^+$ $[M + H]^+$ 742.4277, found 742.4292.

4.2.5.2 RCM precursor 8b: RCM precursor **8b** was prepared according to the general procedure from amine **9** (0.26 g, 0.84 mmol, 1 equiv), acid **10b** (0.41 g, 0.89 mmol, 1.05 equiv), $^{i}Pr_2EtN$ (0.29 mL, 1.70 mmol, 2.0 equiv), and HATU (0.40 g, 1.10 mmol, 1.25 equiv). **8b** (0.51 g, 80%) was obtained as a yellow, waxy solid. **TLC** (EtOAc:hexanes = 1.2.5): R_f = 0.30 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.74 – 6.54 (m, 1H), 6.04 – 5.95 (m, 1H), 5.95 – 5.57 (m, 4H), 5.29 – 5.05 (m, 3H), 5.00 – 4.90 (m, 1H), 4.81 – 4.56 (m, 1H), 4.12 – 3.81 (m, 5H), 3.81 – 3.63 (m, 1H), 2.85 – 2.71 (m, 1H), 2.68 – 2.48 (m, 1H), 2.48 – 2.38 (m, 1H), 2.29 – 2.10 (m, 2H), 2.11 – 1.81 (m, 3H), 1.82 – 1.65 (m, 6H), 1.07 – 0.87 (m, 6H), 0.87 – 0.67 (m, 12H), 0.33 (s, 4H), 0.27 (s, 5H), 0.01 (s, 1.5H), 0.01 (s, 1.5H), −0.01 (s, 1.5H), −0.02 (s, 1.5H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 201.9, 201.4, 172.4, 172.3, 165.7, 165.4, 163.0, 162.2, 161.6, 159.39, 159.35, 145.34, 145.22, 145.16, 145.07, 135.04, 135.02, 134.13, 134.02, 133.5, 131.27, 131.24, 125.03, 125.02, 123.9, 123.7, 116.5, 116.3, 80.8, 80.5, 67.9, 66.9, 66.8, 60.4, 59.9, 50.3, 50.2, 48.8, 47.0, 44.4, 44.2, 41.91, 41.87, 38.6, 38.3, 38.0, 31.5, 29.9, 29.7, 28.9, 25.8, 25.7, 25.6, 25.2, 21.5, 19.6, 19.4, 18.2, 18.0, 17.2, 17.0, 14.7, 14.2, 12.9, −1.79, −1.80, −4.4, −5.09, −5.11. **HRMS- ESI** m/z calcd for $C_{40}H_{66}N_3O_7Si_2^+$ $[M + H]^+$ 756.4434, found 756.4435.

4.2.5.3 RCM precursor 8c: RCM precursor **8c** prepared according to the general procedure from amine **9** (0.50 g, 1.60 mmol, 1 equiv), acid **10c** (0.79 g, 1.70 mmol, 1.05 equiv), $^{i}Pr_2EtN$ (0.57 mL, 3.200 mmol, 2.0 equiv), and HATU (0.77 g, 2.0 mmol, 1.25 equiv). **8c** (1.10 g, 90%) was obtained as a yellow, waxy solid. **TLC** (EtOAc:hexanes = 1.2.5): R_f = 0.30 (UV, $KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.74 – 6.56 (m, 1H), 5.97 – 5.65 (m, 4H), 5.55 – 5.40 (m, 1H), 5.35 – 4.99 (m, 3H), 4.98 – 4.58 (m, 3H), 4.17 – 3.84 (m, 5H), 3.84 – 3.63 (m, 1H), 2.90 −2.75 (m, 1H), 2.70 – 2.38 (m, 2H), 2.32 – 2.13 (m, 2H), 2.13 – 1.83 (m, 3H), 1.83 – 1.72 (m, 6H), 1.07 – 0.89 (m, 6H), 0.89 – 0.79 (m, 12H), 0.34 (s, 4H), 0.29 (s, 5H), 0.05 (s, 1.5H), 0.04 (s, 1.5H), 0.03 (s, 1.5H), 0.02 (s, 1.5H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 201.9, 201.4, 172.4, 165.7, 165.4, 163.1, 162.2, 161.6, 159.38, 159.35, 145.5, 145.3, 145.2, 145.1, 134.1, 134.0, 132.7, 131.90, 131.88, 125.3, 123.9, 123.7, 116.5, 116.4, 80.9, 80.5, 67.0, 66.8, 65.8, 60.5, 59.9, 50.3, 50.2, 48.8, 47.0, 44.4, 44.2, 41.93, 41.89, 38.3, 38.0, 31.6, 29.9, 29.8, 28.9, 25.8, 25.7, 25.24, 25.18, 22.6, 21.5, 19.6, 19.4, 18.0, 17.2, 17.02, 15.2, 14.7, 14.2, 14.1, 11.4, −1.78, −1.79, −4.5, −4.6, −5.04, −5.06. **HRMS-ESI** m/z calcd for $C_{40}H_{66}N_3O_7Si_2^+$ $[M + H]^+$ 756.4434, found 756.4435.

4.2.6 TBS-TMS-viginiamycin M2 (23)—A 50-mL round-bottom flask was charged with RCM precursor **8c** (30 mg, 40 μmol, 1 equiv) and Grela II catalyst (2.1 mg, 3.2 μmol, 0.08 equiv). The vessel was evacuated and filled with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. Benzentrifluoride (8 mL) was added, resulting in a green solution. A stream of argon was passed through the solution for 30 min. After 12 hours, another portion of Grela II catalyst (2.1 mg, 3.2 μmol, 0.08 equiv) was added. The mixture was stirred for another 12 hours and concentrated under vacuum. The resulting residue was purified by flash chromatography (silica gel, eluent: EtOAc: Hexanes = 1:3 to 1:1.5) to afford TBS-TMS- viginiamycin M2 (**23**, 14 mg, 49%) as a white solid. **TLC** (EtOAc:hexanes = 1:2): R_f = 0.20 (UV). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.49 (dd, *J* = 16.3, 4.2 Hz, 1H), 6.19 – 6.10 (m, 1H), 6.07 (dd, *J* = 9.2, 3.2 Hz, 1H), 5.77 (dd, *J* = 16.4, 2.0 Hz, 1H), 5.57 (ddd, *J* = 15.5, 9.4, 4.2 Hz, 1H), 5.42 (d, *J* = 8.9 Hz, 1H), 5.00 (ddd, *J* = 8.9, 7.0, 5.9 Hz, 1H), 4.85 – 4.72 (m, 2H), 4.57 – 4.43 (m, 1H), 3.89 (d, *J* = 17.2 Hz, 1H), 3.78 – 3.69 (m, 3H), 3.39 (ddd, *J* = 14.8, 9.5, 3.3 Hz, 1H), 2.92 (dd, *J* = 15.9, 7.0 Hz, 1H), 2.79 – 2.68 (m, 2H), 2.18 – 2.04 (m, 1H), 1.90 (dddd, *J* = 24.9, 15.9, 11.3, 6.8 Hz, 3H), 1.77 – 1.68 (m, 1H), 1.66 (d, *J* = 1.2 Hz, 3H), 1.08 (d, *J* = 6.9 Hz, 3H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.85 (s, 9H), 0.30 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 201.0, 172.1, 166.4, 161.8, 161.3, 159.6, 145.1, 144.8, 136.7, 134.7, 132.4, 124.9, 123.7, 81.1, 65.4, 58.7, 50.6, 48.4, 43.7, 41.3, 36.7, 29.3, 28.2, 25.7, 24.8, 19.9, 18.6, 18.1, 12.67, 9.9, −1.8, −4.5, −5.0. **HRMS-ESI** m/z calcd for $C_{37}H_{60}N_3O_7Si_2$ $[M + H]^+$ 714.3964, found 714.3968.

4.2.7 viginiamycin M2 (1)—A 100-mL round-bottom flask charged with **23** (58 mg, 81 μmol, 1 equiv) was evacuated and filled with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. THF (1.6 mL) was added, resulting in a light yellow solution. In a separate flask, Im·HCl (84 mg, 0.81 mmol, 10.0 equiv) was added to a solution of TBAF in THF (1 M, 0.81 mL, 0.81 mmol, 10.0 equiv). The resulting colorless solution was added dropwise to the solution of **23**. After 12 h, the mixture was concentrated and the residue was dissolved in DCM (50 mL). The resulting solution was transferred to a separatory funnel and was washed with water (3 × 30 mL) and brine (30 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtered and the filtrate was concentrated. The resulting crude residue was purified by flashed chromatography (silica gel, eluent: MeOH:DCM = 1:40) to afford virginiamycin M2 (**1**, 35 mg, 82%) as a light yellow solid. **m. p**. 120 – 125 °C (DCM). **TLC** (MeOH:DCM = 1:20): R_f = 0.30 (UV). **$[\alpha]^{25}_D$** = - 67.4 (*c* = 0.3, DCM). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 6.47 (dd, *J* = 16.4, 5.0 Hz, 1H), 6.39 (dd, *J* = 9.0, 3.7 Hz, 1H), 6.11 (m, *J* = 15.6 Hz, 1H), 5.78 (dd, *J* = 16.4, 1.9 Hz, 1H), 5.69 (ddd, *J* = 15.6, 9.2, 4.6 Hz, 1H), 5.41 (d, *J* = 8.8 Hz, 1H), 4.90 (dt, *J* = 8.9, 5.6 Hz, 1H), 4.73 (dd, *J* = 10.1, 2.0 Hz, 1H), 4.70 (dd, *J* = 8.9, 3.2 Hz, 1H), 4.45 (ddd, *J* = 13.9, 8.9, 4.6 Hz, 1H), 4.00 – 3.92 (m, 1H), 3.82 (s, 2H), 3.79 – 3.70 (m, 1H), 3.39 (ddd, *J* = 14.0, 9.2, 3.6 Hz, 1H), 3.05 (dd, *J* = 17.0, 6.0 Hz, 1H), 2.89 (dd, *J* = 17.0, 5.2 Hz, 1H), 2.74 (ddt, *J* = 6.9, 4.9, 2.0 Hz, 1H), 2.60 (br s, 1H), 2.24 – 2.08 (m, 1H), 2.01 – 1.88 (m, 3H), 1.88 – 1.75 (m, 1H), 1.71 (d, *J* = 1.2 Hz, 3H), 1.03 (d, *J* = 6.9 Hz, 3H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 202.1, 171.6, 166.5, 160.2, 156.9, 144.5, 143.9, 136.92, 136.86, 134.3, 132.7, 125.2, 124.0, 81.4, 65.0, 59.6,

48.9, 48.4, 43.3, 40.9, 36.6, 29.4, 28.3, 25.0, 19.7, 18.7, 12.6, 10.4. **HRMS-ESI** m/z calcd for $C_{28}H_{38}N_3O_7$ $[M + H]^+$ 528.2704, found 528.2703.

4.2.8 RCM precursor 8d—An 100-mL round-bottom flask charged with **8c** (0.38 g, 0.50 mmol, 1 equiv) was evacuated and filled with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. THF (20 mL) was added, resulting in a light yellow solution. In a separate flask, Im·HCl (0.52 mg, 5.00 mml, 10.0 equiv) was added to a solution of TBAF (5.0 mL, 5.00 mml, 10.0 equiv). The resulting colorless solution was added dropwise to the above solution of **8c**. After 12 h, the mixture was concentrated and the residue was dissolved in DCM (100 mL). The resulting solution was transferred to a separatory funnel and was washed with water (4 × 100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtered and the filtrate was concentrated. The resulting crude residue was purified by flashed chromatography (silica gel, eluent: acetone:hexanes = 1:3 to 1:1.5) to RCM precursor **8d** (0.23 g, 83%) as a light yellow solid. **TLC** (acetone:hexanes = 1:1.5): R_f = 0.40 (UV, $KMnO_4$).**^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.21 (s, 0.4H), 8.16 (s, 0.6H), 6.65 – 6.50 (m, 1H), 6.35 (t, *J* = 5.8 Hz, 0.6H), 5.96 – 5.69 (m, 3.4H), 5.60 – 5.40 (m, 2H), 5.35 – 5.26 (m, 1H), 5.23 – 5.06 (m, 2H), 5.00 – 4.85 (m, 1H), 4.81 – 4.59 (m, 2H), 4.05 (t, *J* = 6.5 Hz, 1H), 4.01 – 3.84 (m, 4H), 3.83 – 3.62 (m, 1H), 2.88 – 2.73 (m, 1H), 2.73 – 2.45 (m, 2H), 2.33 – 2.19 (m, 2H), 2.13 – 1.85 (m, 3H), 1.84 – 1.71 (m, 6H), 1.00 – 0.81 (m, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 202.74, 202.72, 172.2, 171.7, 166.1, 165.3, 160.08, 160.05, 157.2, 157.1, 145.3, 144.7, 144.5, 144.2, 137.3, 137.1, 135.4, 135.3, 134.3, 134.0, 132.6, 132.5, 130.0, 129.9, 125.7, 125.6, 124.3, 123.6, 116.4, 116.2, 81.2, 80.8, 64.74, 64.71, 60.5, 60.2, 49.4, 49.3, 48.74 47.1, 43.2, 43.1, 41.91, 41.88, 38.2, 37.6, 31.5, 29.9, 29.8, 28.8, 25.3, 21.4, 19.6, 19.3, 17.6, 17.4, 17.3, 14.70, 14.2, 13.9. **HRMS-ESI** m/z calcd for $C_{31}H_{44}N_3O_7^+$ $[M + H]^+$ 570.3174, found 570.3181.

4.2.9 viginiamycin M2 (1) from RCM precursor 8d—An 50-mL round-bottom flask was charged with RCM precursor **8d** (30 mg, 53 mol, 1 equiv) and Grela II catalyst (2.8 mg, 4.2 mmol, 0.08 equiv). The vessel was evacuated and filled with nitrogen (this process was repeated a total of 3 times) and was sealed with a rubber septum. DCM (10 mL) was added, resulting in a green solution. A stream of argon was passed through the solution for 30 min at 0 °C, then the reaction mixture was warmed to 23 °C. After 12 h, another portion of Grela II catalyst (Grela II catalyst (2.8 mg, 4.2 mmol, 0.08 equiv) was added. After the next 12 hours, the solvent was removed under vacuum. The resulting residue was purified by flash chromatography (silica gel, eluent: acetone:hexanes = 1:2 to 1:1) to afford virginianmycin M2 (**1**, 20 mg, 72%) as a white solid. The spectral data were consistent with those reported above.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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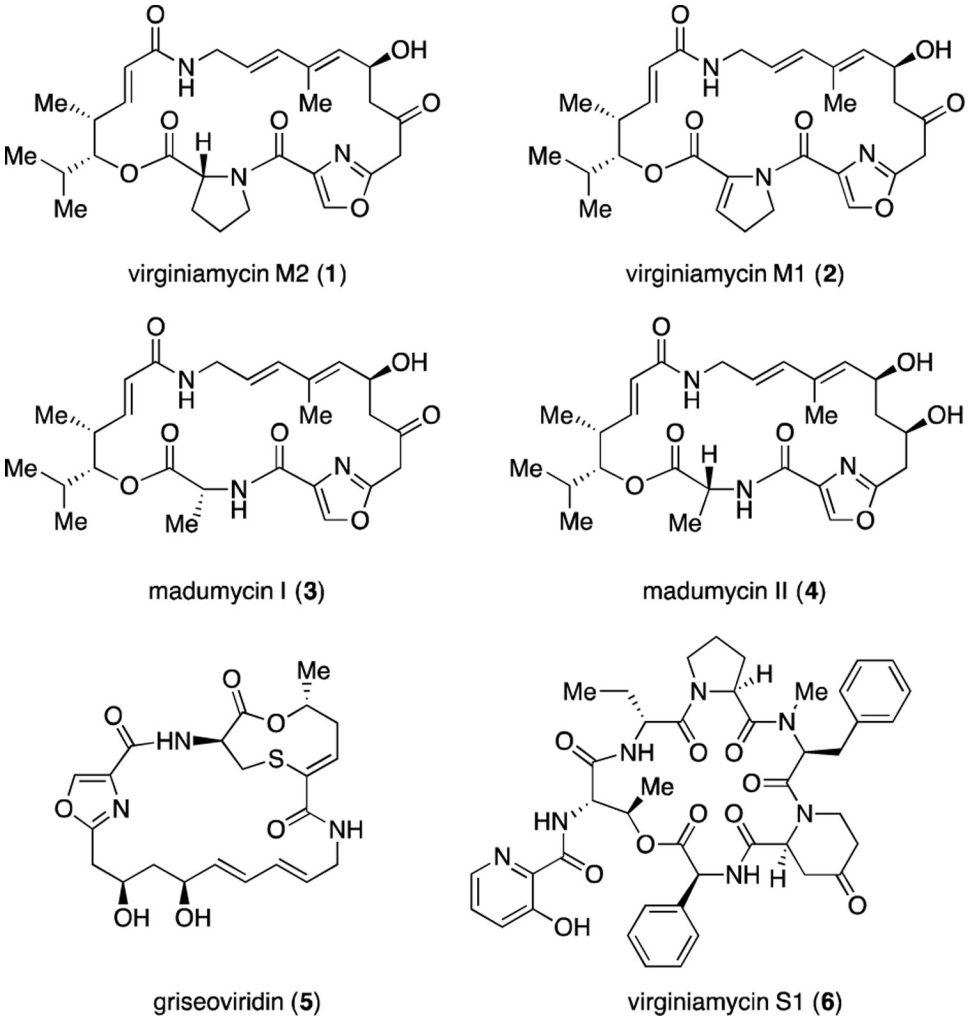


Fig. 1.
Representive steptogramin antibiotics.

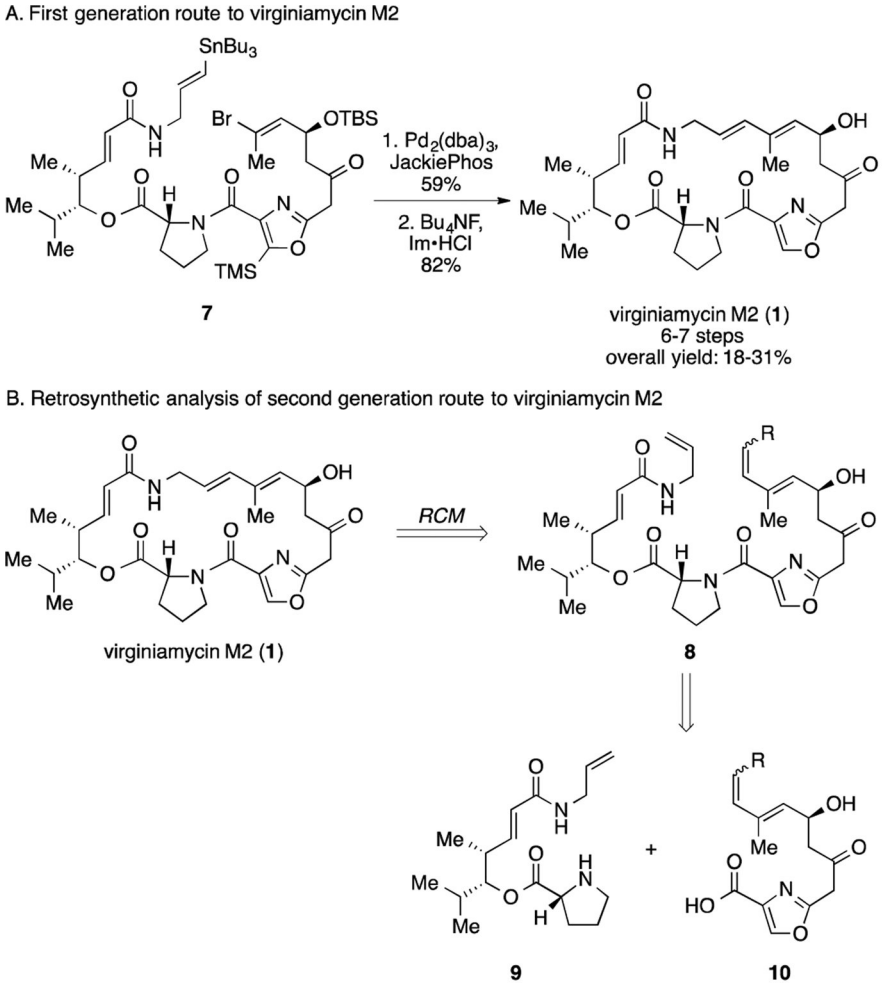
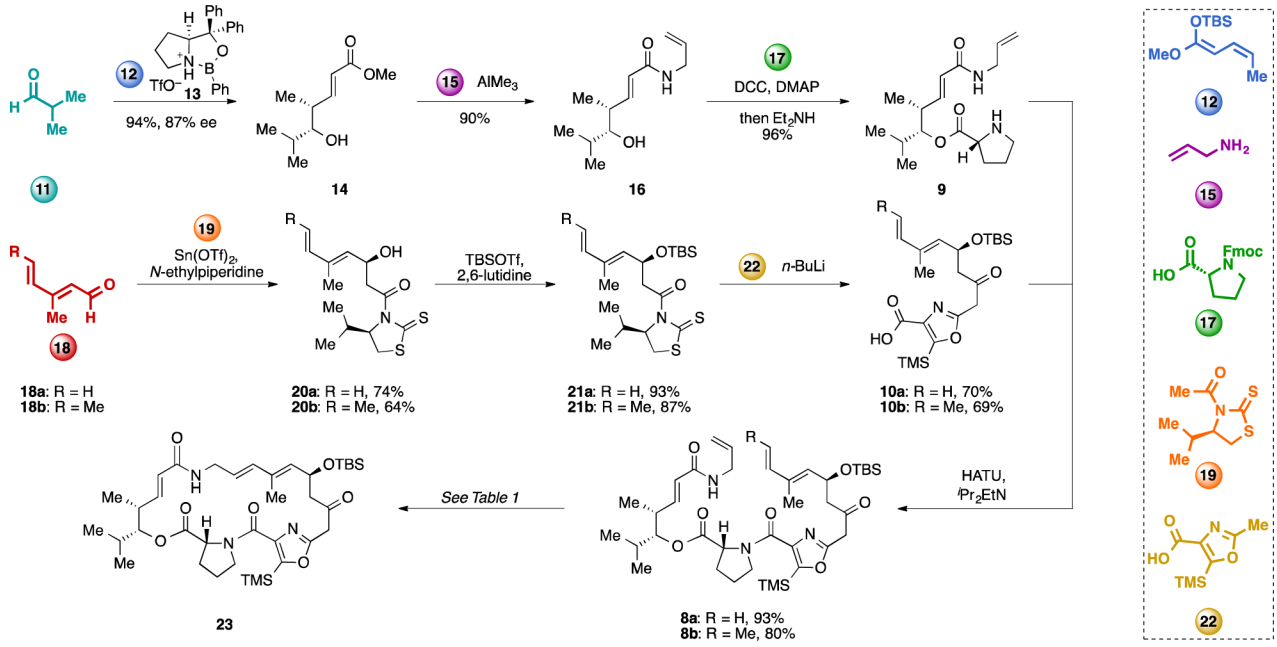


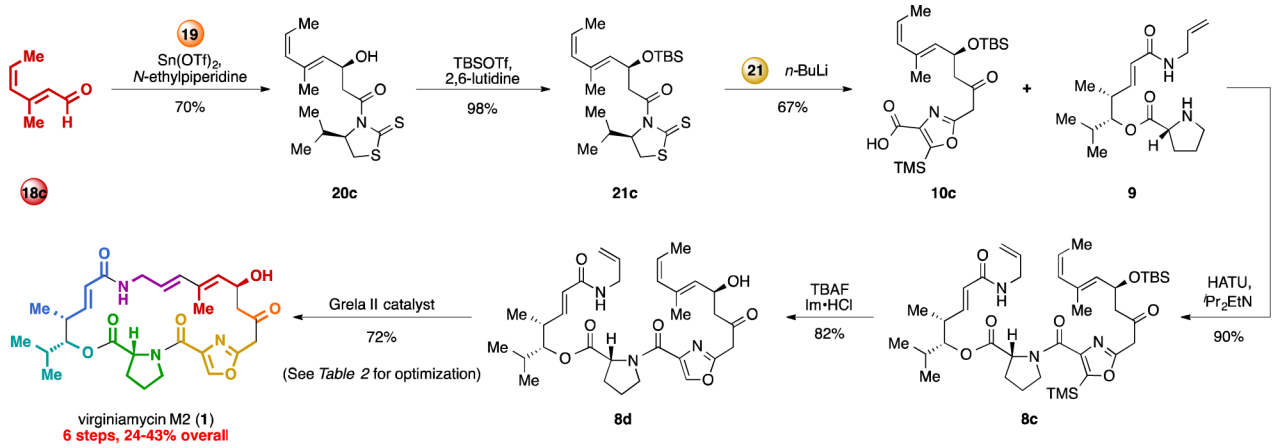
Fig. 2.

(A) The first generation route to virginiamycin M2. (B) Retrosynthetic analysis of the second generation route to virginiamycin M2.



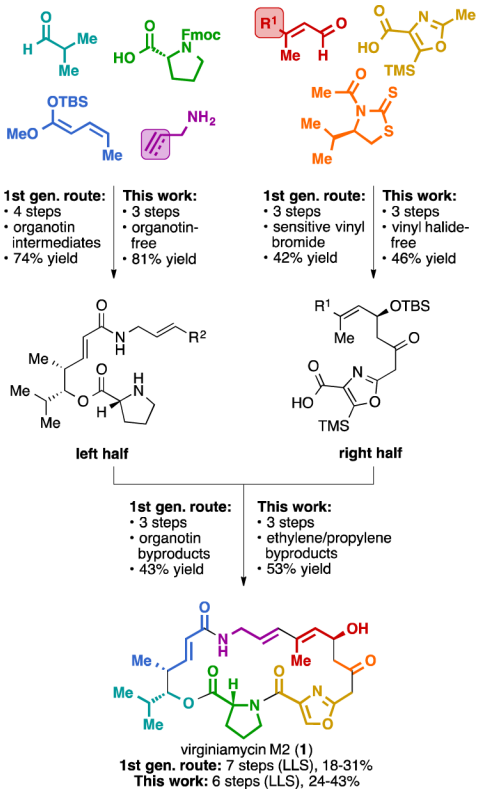
Scheme 1.

Initial efforts to synthesize virginiamycin M2 (**1**) by ring closing metathesis. DCC = dicyclohexylcarbodiimide, DCM = dichloromethane, DMAP = 4- dimethylaminopyridine, Fmoc = 9-fluorenylmethoxycarbonyl, HATU = 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium-3- oxidhexafluorophosphate, Im = imidazole, TBS = *tert*-butyldimethylsilyl, TfO = trifluoromethanesulfonate, TBAF = tetra-*n*-butylammonium fluoride, TMS = trimethylsilyl.



Scheme 2.

Optimized synthesis of virginiamycin M2 (**1**) by means of a macrocycle precursor containing a Z alkene. DCC = dicyclohexylcarbodiimide, DCM = dichloromethane, DMAP = 4- dimethylaminopyridine, Fmoc = 9-fluorenylmethoxycarbonyl, HATU = 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5- *b*]pyridinium-3-oxidhexafluorophosphate, Im = imidazole, TBS = *tert*-butyldimethylsilyl, TfO = trifluoromethanesulfonate, TBAF = tetra-*n*-butylammonium fluoride, TMS = trimethylsilyl.

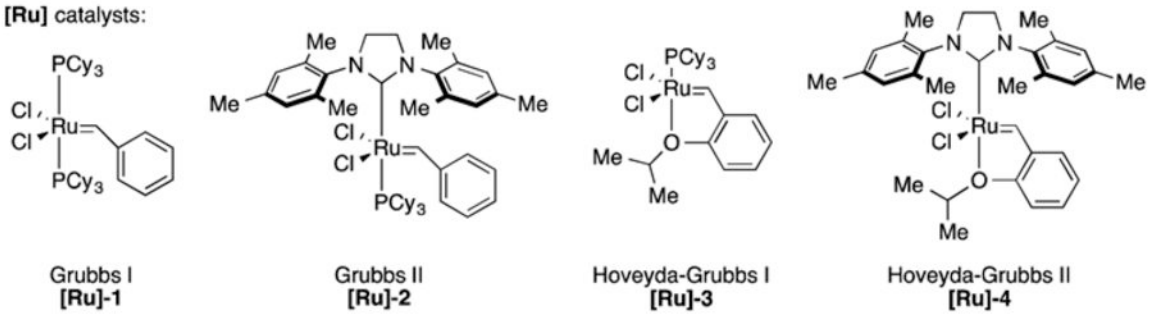


Scheme 3.

Comparison of the two routes to the group A streptogramin virginiamycin M2 (**1**).

Table 1.

Preliminary investigations into the ring closing metathesis of macrocycle precursors **8a** and **8b**.



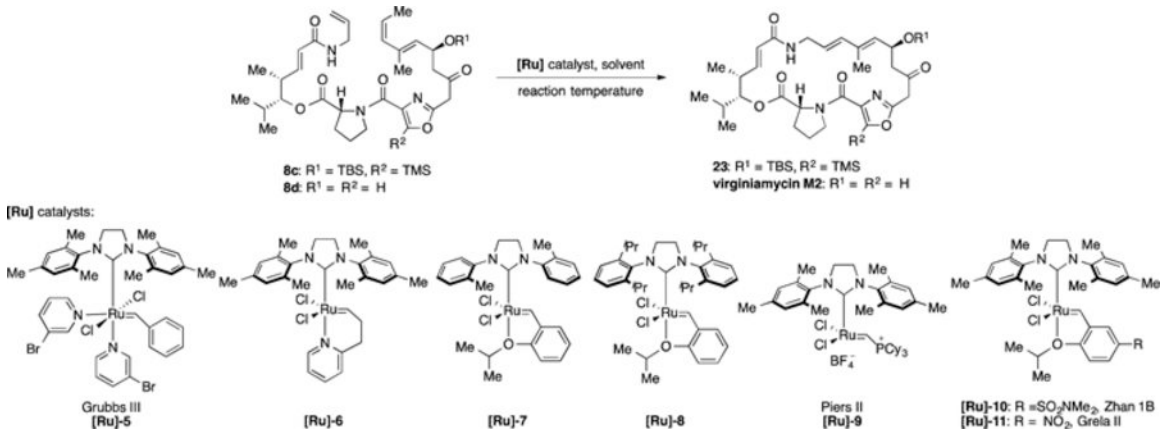
Entry	RCM precursor	Catalyst	Solvent	T(°C)	Yield
1	**8a**	**[Ru]-1, [Ru]-2, [Ru]-3 or [Ru]-4** (20 mmol%)	Tol	23	-[a]
2	**8a**	**[Ru]-1, [Ru]-2, [Ru]-3 or [Ru]-4** (20 mmol%)	Tol	70	-[a]
3	**8b**	**[Ru]-2** (20 mmol%)	Tol	23	-[a]
4	**8b**	**[Ru]-2** (20 mmol%)	Tol	70	-[a]
5	**8b**	**[Ru]-4** (20 mmol%)	Tol	23	15%[b]

[a] No product detected.

[b] Yield determined by ^{1}H NMR analysis of the crude reaction mixture using 1,4-dinitrobenzene as an internal standard. Tol = toluene.

Table 2.

Optimization of ring closing metathesis on macrocycle precursors **8c** and **8d**, which contain Z alkenes.



Entry	RCM precursor	Catalyst	Solvent	T(°C)	Yield
1	**8c**	**[Ru]-1** (20 mmol%)	Tol	23	-[a]
2	**8c**	**[Ru]-2** (20 mmol%)	Tol	23	25%[b]
3	**8c**	**[Ru]-3** (20 mmol%)	Tol	23	-[a]
4	**8c**	**[Ru]-4** (20 mmol%)	Tol	23	38%[b]
5	**8c**	**[Ru]-5** (20 mmol%)	Tol	23	-[a]
6	**8c**	**[Ru]-6** (20 mmol%)	Tol	23	-[a]
7	**8c**	**[Ru]-7** (20 mmol%)	Tol	23	27%[b]
8	**8c**	**[Ru]-4** (8 mmol%) × 2	Tol	23	40%[b]
9	**8c**	**[Ru]-4** (8 mmol%) × 2	BTF	23	42%[b]
10	**8c**	**[Ru]-8** (8 mmol%) × 2	BTF	23	33%[b]
11	**8c**	**[Ru]-9** (8 mmol%) × 2	BTF	0	-[a]
12	**8c**	**[Ru]-10** (8 mmol%) × 2	BTF	23	28%[b]

Entry	RCM precursor	Catalyst	Solvent	T(°C)	Yield
13	**8c**	**[Ru]-11** (8mmol%) × 2	BTF	23	49%[c]
14	**8d**	**[Ru]-11** (8 mmol%) × 2	DCM	23	72%[c]

[a] No product detected.

[b] Yield determined by ^{1}H NMR analysis of the crude reaction mixture using 1,4-dinitrobenzene as an internal standard.

[c] isolated yield after column chromatography. BTF = benzotrifluoride, DCM = dichloromethane, Tol = toluene.