Solid-Phase Synthesis of Dihydrovirginiamycin S_1, a Streptogramin B Antibiotic

Alex Shaginian,[a] Marissa C. Rosen,[a] Brock F. Binkowski,[b] and Peter J. Belshaw*[a, b]

Abstract: We describe the first solid-phase synthesis of dihydrovirginiamycin S_1, a member of the streptogramin B family of antibiotics, which are nonribosomal-peptide natural products produced by *Streptomyces*. These compounds, along with the synergistic group A components, are "last line of defense" antimicrobial agents for the treatment of life-threatening infections such as vancomycin-resistant enterococci. The synthesis features an on-resin cyclization and is designed to allow production of streptogramin B analogues with diversification at positions 1′, 1, 2, 3, 4, and 6. Several synthetic challenges known to hinder the synthesis of this class of compounds were solved, including sensitivity to acids and bases, and epimerization and rearrangements, through the judicious choice of deprotection conditions, coupling conditions, and synthetic strategy. This work should enable a better understanding of structure–activity relationships in the streptogramin B compounds, possible identification of analogues that bypass known resistance mechanisms, and perhaps the identification of analogues with novel biological activities.

Keywords: antibiotics · cyclic peptides · depsipeptides · solid-phase synthesis · virginiamycins

Introduction

The streptogramin (or synergimycin) family of antibiotics consist of two components, A and B, that synergistically inhibit protein synthesis by binding to distinct sites on the 50S subunit of the prokaryotic ribosome. The group A components are polyunsaturated macrolactones and the group B components are N_1^{α}-acylated cyclic hexadepsipeptides (Figure 1). Although the virginiamycins have been used for many years as a growth-promoting additive in livestock feed, limited water solubility has hampered the clinical utility of streptogramin antibiotics. Recently, the semisynthetic water-soluble derivatives quinupristin and dalfopristin (marketed as Synercid TM) were approved for the treatment of Gram-positive coccal infections including vancomycin-resistant enterococci (Figure 1).[1] Here we describe the first solid-phase synthesis of a group B streptogramin. Our synthesis is designed to enable the synthesis of streptogramin B analogues at positions 1′, 1, 2, 3, 4, and 6.

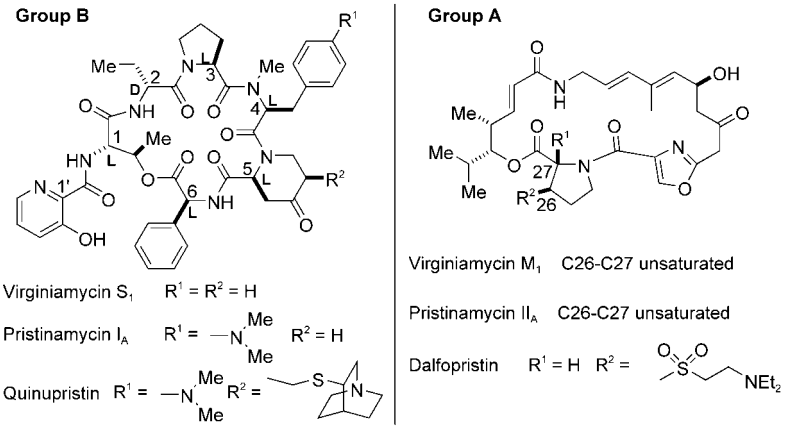

Figure 1. Structures of streptogramin antibiotics. Dihydrovirginiamycin S_1 has a *trans*-hydroxyl group replacing the carbonyl of residue 5.

The genes responsible for the biosynthesis of pristinamycin I in *Streptomyces pristinaespiralis* and virginiamycin S_1 in

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Streptomyces virginiae have been sequenced, and the homologous nonribosomal peptide synthetases (NRPS) that assemble these natural products in a template-directed fashion have been identified.[2] Recent work has shown that it is possible to re-engineer the substrate specificity of NRPSs, thus enabling the possibility of cost-effective production of analogues through engineered biosynthesis in microorganisms.[3] Although significant progress has been made in the combined chemo-enzymatic synthesis of libraries,[4] the exclusive biosynthetic production of nonribosomal peptide libraries remains a challenge.

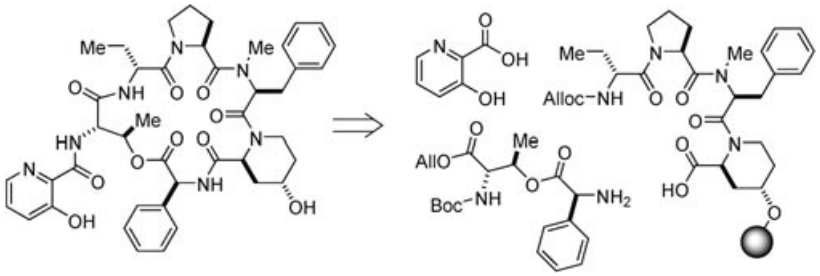


Figure 2. Retro-synthetic disconnections for $dhVS_1$. All = allyl, Alloc = allyloxycarbonyl, Boc = *tert*-butyloxycarbonyl.

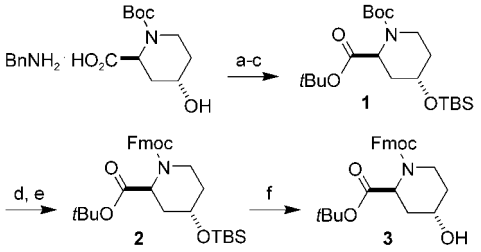
We have developed a solid-phase synthesis of dihydrovirginiamycin S_1 ($dhVS_1$)[5] to enable the synthesis of streptogramin B analogues for investigation of structure–activity relationships in these antibiotics. The hexapeptide macrocycle of the streptogramin B antibiotics has many features that make it a favorable scaffold for the identification of analogues with new biological activities: the parent compounds are cell permeable; the macrocycle is rigidified by a *trans*-annular hydrogen bond between the carbonyl oxygen atom of Pro and the amide N–H group of Phg; the conformation of the macrocycle directs the side chains (potential diversity elements) in a radial manner and could provide a diverse set of three-dimensional structures;[6] and the side chains are projected over a relatively large surface area, a property that may be beneficial for binding to protein–protein interaction surfaces. Protein–protein interactions are of great interest as new pharmacological targets, yet difficult to modulate with small molecules.[7]

Previous syntheses of streptogramin B natural products[8] and analogues[9] have been conducted in solution with closure of the macrocycle through formation of an amide bond from a linear depsipeptide precursor. Along with these total syntheses, efforts toward the production of semi/hemisynthetic analogues revealed several challenges:[10] compounds containing homochiral *N*-alkyl amino acid triads are sensitive to strong acids, the Phg ester is sensitive to both epimerization and elimination, cyclization is nearly always accompanied by epimerization, and the *des*-*N*-hydroxypicolinic acid derivatives are susceptible to rearrangements giving inactive macrolactam and oxazoline byproducts.

We targeted the synthesis of $dhVS_1$, since this analogue is known to be active, the hydroxyl group on the hydroxypipecolic acid residue provides a handle for attachment to the resin, and this residue has been extensively modified with many analogues retaining activity.[10c] Our retrosynthesis (Figure 2) disconnects the macrocycle into three subfragments: a linear tetrapeptide anchored to the resin through the hydroxypipecolic acid, a fragment containing Phg with an ester bond to the side chain of Thr, and the exocyclic 3-hydroxypicolinic acid. These disconnections were chosen to allow cyclization at the Thr1-D-Abu2 peptide bond by means of activation of a carbamate-protected Thr to prevent epimerization during cyclization. This strategy also allows the Phg ester to be constructed in solution and introduced late in the synthesis to minimize complications with this sensitive functionality.

Results and Discussion

The synthesis of Fmoc-*trans*-4-hydroxypipecolic acid *tert*-butyl ester **3** is outlined in Scheme 1. The commercially available benzylamine salt of Boc-*trans*-4-hydroxypipecolic acid was silylated and converted to the *tert*-butyl ester (**1**).

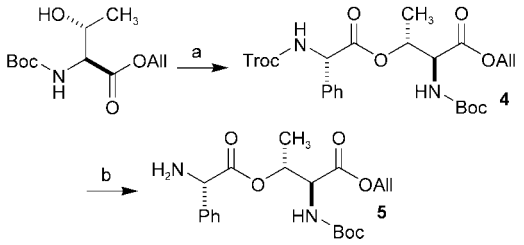


Scheme 1. Synthesis of Fmoc-hyPip-O*t*Bu. Reagents and conditions: a) 0.5 M $HCl_{(aq)}$ in $NaCl_{(sat)}$, EtOAc, 0 °C, 20 min; b) TBS-Cl, imidazole, 2,6-lutidine, THF, 48 h, 98% for two steps; c) *t*BuOH, DIC, $DMAP_{(cat)}$, CH_2Cl_2, 94%; d) TMS-OTf (1 equiv), CH_2Cl_2/toluene, 4 h; e) Fmoc-Cl, pyridine, CH_2Cl_2, 15 h, 77% for two steps; f) HF/pyridine, THF, 2 h, 96%. hyPip = (*2S*,*4S*)-4-hydroxypiperidine-2-carboxylic acid, Bn = benzyl, *t*Bu = *tert*-butyl, TBS = *tert*-butyldimethylsilyl, Fmoc = 9-fluorenylmethyloxycarbonyl, THF = tetrahydrofuran, DIC = 1,3-diisopropylcarbodiimide, DMAP = 4-(dimethylamino)pyridine, TMS = trimethylsilyl, Tf = trifluoromethanesulfonyl.

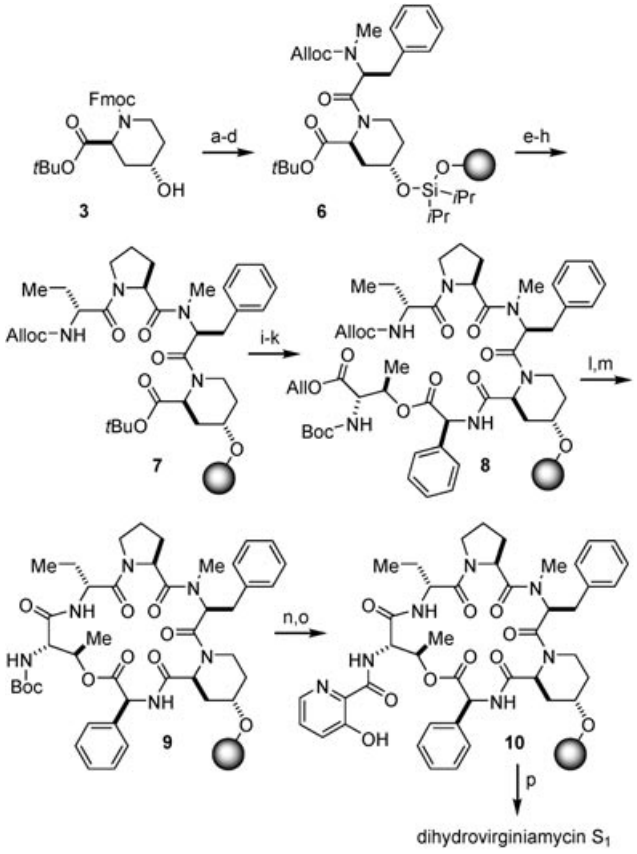
Selective Boc cleavage with one equivalent of TMS-OTf,[11] followed by Fmoc protection afforded **2**. Selective removal of the TBS group in the presence of the *tert*-butyl ester with HF/pyridine afforded **3**.[12]

The synthesis of the dipeptide amine **5** is shown in Scheme 2. Boc-Thr-OAll[13] was condensed with Troc-Phg-OH[14] at −25 °C in the presence of 1,3-diisopropylcarbodiimide (DIC) and a catalytic amount of DMAP to afford ester **4** without epimerization. Troc deprotection with zinc in acetic acid afforded amine **5**.

Our synthesis of $dhVS_1$ is outlined in Scheme 3. We chose Danishefsky's bis(silyl ether) linker[15] for attachment of *trans*-4-hydroxypipecolic (hyPip) to the resin as it is stable to mild bases and palladium cleavage conditions, yet readily



Scheme 2. Synthesis of dipeptide ester **5**. a) Troc-Phg-OH, DIC, $DMAP_{(cat)}$, CH_2Cl_2, 2 h, −25 °C, 95 %; b) Zn, AcOH, 2 h, 88 %. Troc = 2,2,2-trichloroethoxycarbonyl.

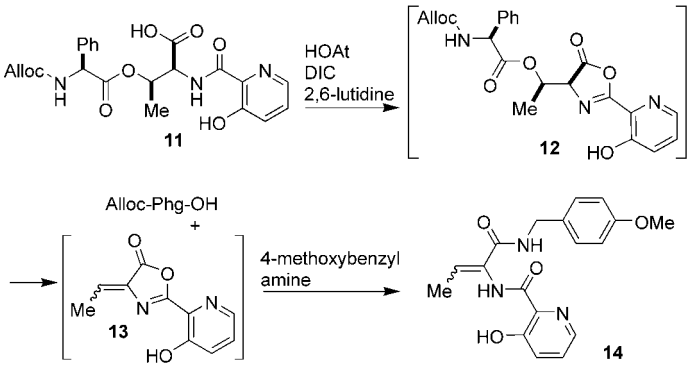


Scheme 3. Solid-phase synthesis of $dhVS_1$. a) diisopropyldichlorosilane, imidazole, 30 min; b) hydroxymethyl-polystyrene resin, 48 h; c) 30 % piperidine, CH_2Cl_2, 30 min; d) Alloc-MePhe-OH, HOAt, DIC, 2,6-lutidine, DMF, 48 h; e) $Pd(PPh_3)_{4(cat)}$, Bu_3SnH, 5 % AcOH, CH_2Cl_2, 3 h, f) Fmoc-Pro-OH, HOAt, DIC, 2,6-lutidine, DMF, 48 h (×3); g) 30 % piperidine, CH_2Cl_2, 30 min; h) Alloc-D-Abu-OH, HOAt, DIC, 2,6-lutidine, DMF, 24 h; i) TMS-OTf, 2,6-lutidine, CH_2Cl_2, 5 h; j) MeOH, 20 min; k) **5**, HOAt, DIC, 2,6-lutidine, DMF, 6 h; l) $[Pd(PPh_3)_4]_{(cat)}$, $PhSiH_3$, CH_2Cl_2, 5 h; m) PyAOP, 2,6-lutidine, CH_2Cl_2, 48 h; n) TMS-OTf, 2,6-lutidine, CH_2Cl_2, 2 h, 23 °C, then 20 min, 4 °C; o) 3-hydroxypicolinic acid, PyAOP, 2,6-lutidine, THF, 4 h, 4 °C, then 10 h, 23 °C; p) HF/pyridine, THF, 1 h, 15 % overall. HOAt = 1-hydroxy-7-azabenzotriazole, DMF = *N*,*N*-dimethylformamide, Abu = 2-aminobutyric acid, PyAOP = 7-aza-benzotriazole-1-yloxytris(pyrrolidinophosphonium) hexafluorophosphate.

resin-bound intermediate **6**. Removal of the Alloc protecting group with palladium under mild acidic conditions, and subsequent coupling with Fmoc-Pro-OH using DIC/HOAt/2,6-lutidine yielded the tripeptide resin. Fmoc deprotection and coupling to Alloc-D-Abu-OH yielded resin-bound tetrapeptide **7**. Subsequently, the *tert*-butyl ester was deprotected under neutral conditions (TMS-OTf/lutidine),[17] and then coupled with amine **5** to yield linear hexapeptide resin **8**. Simultaneous deprotection of the allyl and Alloc groups with palladium, followed by PyAOP-mediated cyclization afforded the Boc-protected macrocycle **9**. Boc removal was accomplished under neutral conditions with TMS-OTf/lutidine, and PyAOP/lutidine-mediated coupling with 3-hydroxypicolinic acid afforded **10**. HF/pyridine-mediated cleavage from resin yielded $dhVS_1$ in 15 % overall isolated yield after purification by silica gel chromatography. LC/MS analyses of synthetic intermediates cleaved from the resin indicated that the on-resin cyclization largely determined the final yield of $dhVS_1$.

Several issues encountered during the solid-phase synthesis of $dhVS_1$ are worth noting. In our initial experiments we attempted to employ the allyl ester of hyPip in place of *tert*-butyl ester **3**. However, upon deprotection of Fmoc-MePhe-hyPip(O-resin)-OAll with piperidine, the free amine rapidly cyclized to form the 2,5-diketopiperazine (DKP). The combination of the bulky *tert*-butyl ester on pipecolic acid, Alloc deprotection under acidic conditions, and DIC/HOAt/lutidine coupling under neutral conditions were required to allow successful peptide extension without any DKP formation.

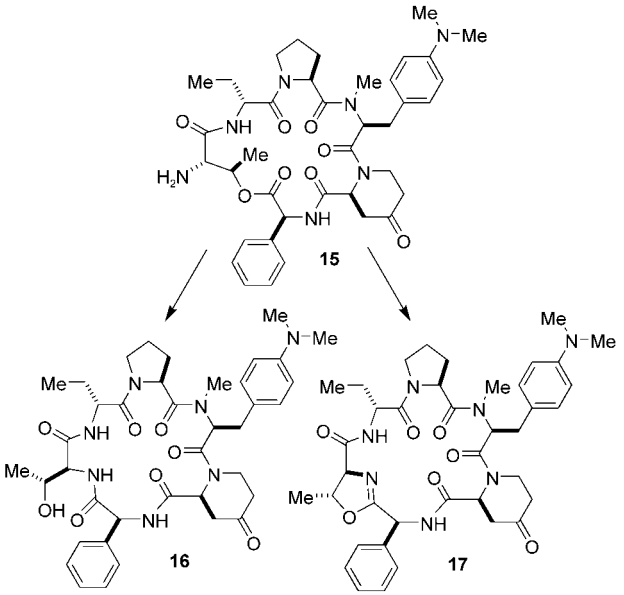
We also investigated approaches to $dhVS_1$ involving cyclization with 3-hydroxypicolinic acid installed on the exocyclic amine of Thr1. However, solution-phase experiments with model compound **11** revealed a rapid elimination immediately upon activation of Thr, resulting in the cleavage of the ester bond (Scheme 4). This possibly occurs via formation of



Scheme 4. Elimination of N^a-protected Phg during activation of side-chain acylated Thr.

removed with HF/pyridine. The synthesis commences with loading of Fmoc-hyPip-O*t*Bu (**3**) onto the hydroxymethyl polystyrene resin. Fmoc removal followed by DIC/1-hydroxy-7-azabenzotriazole (HOAt)/2,6-lutidine-mediated coupling[16] of Alloc-MePhe-OH resulted in the formation of a 5(4*H*)-oxazolone **12** that eliminates to give the unstable intermediate **13**, which was trapped with 4-methoxybenzyl amine to yield **14** (data not shown). Protection of the Thr exocyclic amine as a urethane during cyclization avoided this elimination pathway.

Another potential complication was the instability of 1-(*des*-3-hydroxypicolinoyl) pristinamycin I_A (**15**), as reported by Barriere and co-workers,[10e] which can rearrange to lactam **16** and oxazoline **17** (Scheme 5). Expecting that basic conditions would promote these rearrangements in the relat-



Scheme 5. Rearrangements of 1-(*des*-3-hydroxypicolinoyl) pristinamycin I_A.

ed compound in our synthesis, we chose to protect this amine with a Boc group (intermediate **9**, Scheme 3), as it can be cleaved under neutral conditions with TMS-OTf/lutidine.[17] Moreover, in our initial studies we noticed that TMS-OTf could promote epimerization of the sensitive Phg residue. To minimize competing epimerization, O→N acyl shift, and oxazoline formation, the Boc deprotection was carried out in CH_2Cl_2 for 2 h at room temperature. Shorter reaction times at room temperature and longer reaction times (5–6 h) at 4 °C resulted in incomplete deprotection or epimerization of Phg, respectively. Acylation with 3-hydroxypicolinic acid was then achieved in THF with PyAOP/lutidine. Other solvents such as CH_2Cl_2 and DMF and coupling agents such as DIC/HOAt gave less satisfactory results. This strategy of utilizing neutral deprotection and coupling conditions with HOAt-active esters minimizes exposure of the free amine and may prove generally useful for difficult amide bond formations in which undesired side reactions of the free amine compete. These strategies enabled successful acylation of MePhe4 and the exocyclic amine of Thr1, thereby allowing both of these positions to be sites for diversification in our synthetic route.

The conditions for cleavage from resin were also critical to avoid acidic endopeptolysis of the Pro3-MePhe4-hyPip5 triad of *N*-alkyl amino acids.[10b] Cleavage with HF/pyridine in dichloromethane led exclusively to endopeptolysis from rupture of the MePhe4-hyPip5 peptide bond. Interestingly, the endopeptolysis was solvent-dependent and is completely avoided in THF or can be suppressed with an excess of pyridine.

To confirm the identity of our synthetic $dhVS_1$, we prepared semisynthetic $dhVS_1$ from the natural product virginiamycin S_1 isolated from Stafac,[18] by reduction with sodium borohydride.[5] Synthetic $dhVS_1$ displayed identical ^1H NMR spectral data to the compound prepared from the natural product. To confirm the biological activity of $dhVS_1$, we assayed our synthetic and semi-synthetic $dhVS_1$ for activity against *B. subtilis*, both alone and in combination with virginiamycin M (Table 1). Both samples displayed equal activ-

Table 1. Growth inhibition assays. The growth of *B. subtilis* strain BR151 (ATCC 33677) in liquid culture was monitored in the presence of varying concentrations of compound(s).

Compound(s)[a]	MIC[b] [$\mu g\,mL^{-1}$]
synthetic $dhVS_1$	50
natural $dhVS_1$	50
VS_1	2
VM	3
synthetic $dhVS_1$/VM	1/1.5
natural $dhVS_1$/VM	1/1.5
VS_1/VM	0.4/0.6

[a] VS_1/VM compound mixtures were present in a 3:7 molar ratio. $dhVS_1$ = dihydrovirginiamycin S_1, VS_1 = virginiamycin S_1, VM = virginiamycin M. [b] MIC = minimal concentration required to completely inhibit growth after a 10 h incubation from a starting inoculum of ~2000 cfu mL^{-1}.

ity and a synergistic increase in activity with virginiamycin M, albeit with a slight decrease in potency compared to the parent natural product, consistent with previous reports.[5]

Conclusion

We have developed a rapid and efficient solid-phase synthesis of dihydrovirginiamycin S_1 that should enable the preparation of novel streptogramin B analogues. These compounds could be used to identify structure–activity relationships in this important class of antibiotics, possibly identifying new compounds with increased potency, the ability to bypass resistance mechanisms,[19] or completely new biological activities. Having determined conditions for the successful acylation of MePhe4 and the exocyclic amine of Thr1 in solid-phase synthesis, our synthetic route should allow the facile construction of analogues at positions 1′, 2, 3, and 4, and variations at Thr1 and Phg6, which could be readily accomplished through the incorporation of appropriate ester-containing fragments.

Experimental Section

General procedures: 1-Hydroxy-7-azabenzotriazole (HOAt) and (7-azabenzotriazole-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP) were obtained from Applied Biosystems, (Foster City, CA). Amino acids were obtained from Advanced ChemTech (Louisville, KY)

and EMD Biosciences (San Diego, CA). Diisopropyldichlorosilane was obtained from Fluka (Milwaukee, WI). The benzylamine salt of (*2S,4S*)-*N*-Boc-4-hydroxypiperidine-2-carboxylic acid was obtained from Chirotech Technology Ltd (Cambridge, UK). Hydroxymethyl polystyrene resin SS, 100–200 mesh, 1% DVB, 1.0 mmol g^{-1} was obtained from Advanced ChemTech (Louisville, KY). All other reagents were obtained from Aldrich Chemical (Milwaukee, WI) and used without further purification. Solid-phase reactions were conducted inside 50 mL round-bottomed flasks or 10 mL polypropylene poly-prep chromatography columns obtained from Bio-Rad Laboratories (Hercules, CA) and agitated on a Labquake shaker. Tetrahydrofuran (THF) and toluene were distilled from sodium benzophenone ketyl; dichloromethane (CH_2Cl_2) was distilled from phosphorous pentoxide. Analytical thin-layer chromatography (TLC) was carried out on EM Science TLC plates precoated with silica gel 60 F_{254} (250 μm layer thickness). TLC visualization was accomplished by using a UV lamp and/or charring solutions of either ninhydrin or phosphomolybdic acid (PMA). Flash column chromatography (FCC) was performed on Silicycle silica gel 60 (230–400 mesh). ^{1}H NMR spectra were recorded in deuterated solvents on a Bruker AC-250 (250 MHz), Bruker AC-300 (300 MHz) or a Varian UNITY-500 (500 Mhz) spectrometer. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (TMS, δ 0.00) or relative to residual solvent signals ($CDCl_3$ 7.27 (1) or CD_3CN 1.94 (5)). Coupling constants (J values) are given in Hz, and peak multiplicities are denoted by s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dq (doublet of quartets), dt (doublet of triplets), m (multiplet), q (quartet), and t (triplet). ^{13}C NMR spectra were recorded in deuterated solvents on a Bruker AC-250 (62.5 MHz), Bruker AC-300 (75 MHz) or a Varian UNITY-500 (125 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm, δ) relative to residual solvent signals ($CDCl_3$ 77.23 (3) or CD_3CN 1.39 (7)). For compounds with multiple rotamers all observed signals are reported. Optical rotations were obtained on a Perkin–Elmer 241 digital polarimeter at room temperature with an Na lamp. Concentrations (c) are reported in g per 100 mL. Fourier transform infrared (FT-IR) spectra were obtained on a Mattson Polaris instrument. High-resolution electrospray ionization mass spectra (HRESI-MS) were obtained on a Micromass LCT.

Boc-(*2S,4S*)-hyPip(OTBS)-OH: Aqueous HCl (0.5 M) saturated with sodium chloride (10 mL) was slowly added to the suspension of the benzylamine salt of (*2S,4S*)-*N*-Boc-4-hydroxypiperidine-2-carboxylic acid (1.0 g, 2.84 mmol) in EtOAc (10 mL) at 0 °C. The mixture was stirred at 0 °C for 20 min, diluted with EtOAc, and extracted with saturated aq NaCl. The organic layer was collected, dried (Na_2SO_4), and concentrated, affording acid as a solid. The obtained acid was dissolved in THF (30 mL) and imidazole (1.2 g, 17.04 mmol), 2,6-lutidine (1.3 mL, 11.36 mmol), and TBS-Cl (1.3 g, 8.52 mmol) were added at 0 °C. The reaction mixture was stirred at room temperature under an atmosphere of N_2 for 48 h. The mixture was diluted with EtOAc and extracted with aq HCl (0.1 M, ×2). The organic layer was collected and concentrated. The resultant residue was suspended in MeOH (20 mL), stirred for 5 h, after which the mixture was concentrated and purified by chromatography on silica gel (50:50:1 hexanes/Et_2O/AcOH) to give the side-chain TBS-protected acid as a solid (1.0 g, 98%) that was homogeneous by TLC analysis. R_f=0.32 (hexanes/Et_2O/HOAc 25:25:1); $[\alpha]=-23.3$ (c=0.18 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ=0.060 (d, J=1.3 Hz, 6H), 0.87 (s, 9H), 1.45 (s, 9H), 1.72 (m, 3H), 2.36 (m, 1H), 2.98 (m, 1H), 3.65 (m, 1H), 4.01 (m, 1H), 4.92 (m, 1H), 11.35 ppm (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta=-4.8$, 17.9, 25.7, 28.2, 34.3, 35.4, 39.8, 40.5, 53.6, 54.5, 66.7, 80.6, 155.2, 155.7, 176.4, 176.6 ppm; FT-IR (KBr pellet): $\tilde{\nu}$=2954, 2856, 1750, 1630, 1439 cm^{-1}; MS (HRESI-MS) calcd for $[C_{17}H_{32}NO_5Si]^-$: 358.2050; found: 358.2045.

Boc-(*2S,4S*)-hyPip(OTBS)-O*t*Bu 1: Boc-(*2S,4S*)-hyPip(OTBS)-OH (0.92 g, 2.56 mmol) was dissolved in CH_2Cl_2 (5 mL) and anhydrous 2-methyl-2-propanol (0.72 mL, 7.68 mmol) was added, followed by DMAP (94 mg, 0.77 mmol). The solution was cooled to 0 °C, and DIC (0.80 mL, 5.12 mmol) in CH_2Cl_2 (5 mL) was injected dropwise over 15 min. The reaction mixture was stirred at 0 °C under an atmosphere of N_2 for 15 min and then at room temperature for 5 h. The mixture was diluted with Et_2O and extracted with aq HCl (0.1 M, ×2) and H_2O (×1). The organic layer was collected, concentrated, and purified by chromatography on silica gel (9:1 hexanes/Et_2O) to give **1** as a solid (1.0 g, 94%) that was homogeneous by TLC analysis. R_f=0.66 (hexanes/Et_2O 7:3); $[\alpha]=-17.2$ (c=0.087 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ=0.065 (d, J=0.7 Hz, 6H), 0.88 (s, 9H), 1.44 (s, 9H), 1.47 (s, 10H), 1.60 (m, 1H), 1.79 (m, 1H), 2.30 (m, 1H), 2.99 (m, 1H), 3.58 (m, 1H), 3.99 (m, 1H), 4.74 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta=-4.6$, 18.2, 25.9, 28.1, 28.4, 34.5, 34.8, 36.1, 40.2, 40.7, 54.5, 55.6, 67.0, 79.9, 81.4, 155.5, 155.6, 170.6, 170.8 ppm; FT-IR (thin film): $\tilde{\nu}$=2976, 2930, 2857, 1739, 1704, 1473 cm^{-1}; MS (HRESI-MS) calcd for $[C_{21}H_{41}NO_5Si+Na]^+$: 438.2652; found: 438.2656.

Fmoc-(*2S,4S*)-hyPip(OTBS)-O*t*Bu 2: Boc-(*2S,4S*)-hyPip(OTBS)-O*t*Bu **1** (30 mg, 0.072 mmol) was dissolved in CH_2Cl_2 (0.7 mL) and treated with a solution of TMS-OTf in toluene (0.292 M, 0.25 mL, 0.073 mmol) at room temperature. The reaction mixture was stirred at room temperature under an atmosphere of N_2 for 4 h and was quenched with MeOH (1 mL), concentrated and purified by chromatography on silica gel (50:1 Et_2O/MeOH) to give the α-amino ester as a liquid that was homogeneous by TLC analysis. The product was dissolved in CH_2Cl_2 (2 mL), pyridine (50 μL, 0.62 mmol) was added, and the system was cooled to 0 °C. Fmoc-Cl (26 mg, 0.1 mmol) was added and the reaction mixture was stirred at room temperature under an atmosphere of N_2 for 15 h. The mixture was concentrated and purified by chromatography on silica gel (9:1 hexanes/Et_2O) to give **2** as a solid (30 mg, 77%) that was homogeneous by TLC analysis. R_f=0.40 (hexanes/Et_2O 7:3); $[\alpha]=-11.4$ (c=0.70 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ=0.12 (s, 6H), 0.93 (s, 9H), 1.46 (m, 1H), 1.50 (s, 9H), 1.66 (m, 1H), 1.87 (m, 1H), 2.40 (m, 1H), 3.17 (m, 1H), 3.67 (m, 1H), 4.14 (m, 1H), 4.35 (m, 3H), 4.89 (m, 1H), 7.36 (m, 4H), 7.60 (m, 2H), 7.78 ppm (d, J=7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta=-4.5$, 18.3, 26.0, 28.2, 34.7, 34.8, 36.3, 40.6, 40.7, 47.4, 47.5, 55.2, 55.3, 66.9, 68.0, 82.0, 82.2, 120.1, 125.3, 127.3, 127.8, 141.5, 144.1, 144.3, 155.9, 156.3, 170.4 ppm; FT-IR (thin film): $\tilde{\nu}$=3067, 2953, 2929, 1736, 1708, 1451 cm^{-1}; MS (HRESI-MS) calcd for $[C_{31}H_{43}NO_5Si+Na]^+$: 560.2808; found: 560.2815.

Fmoc-(*2S,4S*)-hyPip-O*t*Bu 3: Fmoc-(*2S,4S*)-hyPip(OTBS)-O*t*Bu **2** (90 mg, 0.167 mL) was dissolved in THF (5 mL) and HF/pyridine (3.0 mL) was injected at 0 °C. The reaction mixture was stirred at room temperature for 2 h and diluted with Et_2O. The mixture was extracted with saturated aq $NaHCO_3$ (×1), aq HCl (0.1 M, ×2), and H_2O (×1). The organic layer was collected, concentrated, and purified by chromatography on silica gel (1:4 hexanes/Et_2O) to give **3** as a solid (68 mg, 96%) that was homogeneous by TLC analysis. R_f=0.45 (Et_2O); $[\alpha]=-23.2$ (c=1.10 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ=1.26 (m, 1H), 1.47 (s, 9H), 1.60 (m, 1H), 1.97 (m, 1H), 2.08 (s, 1H), 2.50 (m, 1H), 3.14 (m, 1H), 3.70 (m, 1H), 4.12 (m, 1H), 4.36 (m, 3H), 4.90 (m, 1H), 7.35 (m, 4H), 7.58 (m, 2H), 7.78 ppm (d, J=7.6 Hz, 2H) ; ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ=28.2, 34.2, 35.7, 40.4, 40.6, 47.4, 47.5, 55.0, 55.1, 66.0, 66.1, 68.0, 82.2, 82.4, 120.2, 125.2, 127.2, 127.9, 141.4, 144.0, 144.2, 155.9, 156.3, 170.2 ppm; FT-IR (thin film): $\tilde{\nu}$=3435, 3066, 2975, 2931, 1733, 1704, 1451 cm^{-1}; MS (HRESI-MS) calcd for $[C_{25}H_{29}NO_5+Na]^+$: 446.1943; found: 446.1921.

Alloc-L-MePhe-OH: A solution of allyl chloroformate (1.5 mL, 13.9 mmol) in dioxane (20 mL) and aq NaOH (1 M, 20 mL) were simultaneously added dropwise at 0 °C to a solution of H-L-MePhe-OH·HCl (2.0 g, 9.27 mmol), H_2O (30 mL), aq NaOH (1 M, 20 mL), and Et_2O (20 mL). The ice-bath was removed, and the reaction mixture was stirred for 12 h at room temperature. The mixture was diluted with EtOAc and extracted with saturated aq $NaHCO_3$. The aqueous layer was collected, acidified with concentrated HCl, and extracted with EtOAc. The organic layer was collected, dried (Na_2SO_4), and concentrated affording a solid (1.95 g, 80%) that was homogeneous by TLC analysis. R_f=0.35 (hexanes/Et_2O/HOAc 30:20:1); $[\alpha]=-65.4$ (c=2.09 in MeOH); ^{1}H NMR (250 MHz, CD_3CN, 67 °C): δ=2.81 (s, 3H), 3.10 (dd, J=10.7, 14.5 Hz, 1H), 3.33 (dd, J=5.2, 14.4 Hz, 1H), 4.51 (d, J=5.2 Hz, 2H), 4.88 (dd, J=5.2, 10.5 Hz, 1H), 5.20 (m, 2H), 5.89 (m, 1H), 7.28 (m, 5H), 8.24 ppm (s, 1H); ^{13}C NMR (62.5 MHz, CD_3CN, 67 °C): δ=33.0, 36.1, 61.9, 67.2, 117.8, 127.9, 129.8, 130.3, 134.7, 139.3, 157.5, 172.8 ppm; FT-IR (thin film): $\tilde{\nu}$=3078, 3029 2938, 1743, 1700, 1653, 1401 cm^{-1}; MS (HRESI-MS) calcd for $[C_{14}H_{16}NO_4]^-$: 262.1079; found: 262.1069.

Alloc-D-Abu-OH: (D)-Aminobutyric acid (1.0 g, 9.7 mmol) was dissolved in a mixture of H_2O (15 mL), aq NaOH (1 M, 10 mL), and Et_2O (10 mL) and the system cooled to 0 °C. A solution of allyl chloroformate (1.4 mL, 12.9 mmol) in dioxane (13 mL) was added slowly and simultaneously

with aq NaOH (1 M, 13 mL). The ice-bath was removed, and the reaction mixture was stirred for 16 h at room temperature. The mixture was diluted with EtOAc and extracted with saturated aq $NaHCO_3$. The aqueous layer was collected, acidified with concentrated HCl, and extracted with EtOAc. The organic layer was collected, dried (Na_2SO_4), and concentrated, affording a liquid (1.85 g, 100%) that was homogeneous by TLC analysis. $R_f = 0.50$ (EtOAc/HOAc 50:1); $[\alpha] = -10.0$ ($c = 0.70$ in $CHCl_3$); ^{1}H NMR (300 MHz, CD_3CN, 25 °C): $\delta = 0.94$ (t, $J = 7.4$ Hz, 3H), 1.78 (m, 2H), 4.10 (m, 1H), 4.53 (d, $J = 5.0$ Hz, 2H), 5.18 (dd, $J = 1.4$, 10.5 Hz, 1H), 5.29 (d, $J = 17.3$ Hz, 1H), 5.90 (m, 1H), 6.02 (d, $J = 7.0$ Hz, 1H), 9.61 ppm (s, 1H); ^{13}C NMR (75 MHz, CD_3CN, 25 °C): $\delta = 10.5$, 25.7, 56.1, 66.3, 118.3, 134.2, 157.4, 174.9 ppm; FT-IR (thin film): $\tilde{\nu} = 3323$, 3085, 2973, 2882, 1718, 1533, 1235 cm^{-1}; MS (HRESI-MS) calcd for $[C_8H_{12}NO_4]^-$: 186.0764; found: 186.0771.

Ester 4: Boc-L-Thr-OAll (0.95 g, 3.66 mmol) was dissolved in CH_2Cl_2 (15 mL), and Troc-L-Phg-OH (1.32 g, 4.03 mmol) was added. The system was cooled to −30 °C, and DIC (0.63 mL, 4.03 mmol) was injected, followed by DMAP (4.5 mg, 0.0366 mmol). The reaction mixture was maintained between −30 and −20 °C under an atmosphere of N_2 for 2 h, diluted with Et_2O, and quenched with aq HCl (0.1 M). The mixture was extracted with aq HCl (0.1 M, ×2), saturated aq $NaHCO_3$ (×2), and H_2O (×1). The organic layer was collected and concentrated. The crude residue was purified by chromatography on silica gel (2.3:1 hexanes/Et_2O) to give **4** as a solid (1.98 g, 95%) that was homogeneous by TLC analysis. $R_f = 0.58$ (hexanes/Et_2O 1:1); $[\alpha] = +43.9$ ($c = 0.62$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 1.16$ (d, $J = 6.5$ Hz, 3H), 1.45 (s, 9H), 4.48 (dd, $J = 2.5$, 9.5 Hz, 1H), 4.61 (d, $J = 6.0$ Hz, 2H), 4.68 (d, $J = 12.0$ Hz, 1H), 4.76 (d, $J = 12.2$ Hz, 1H), 5.25 (m, 4H), 5.48 (m, 1H), 5.85 (m, 1H), 5.98 (d, $J = 7.1$ Hz, 1H), 7.36 ppm (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 16.4$, 28.4, 57.2, 58.3, 66.7, 72.8, 74.9, 80.6, 95.4, 119.5, 127.3, 129.1, 129.3, 131.5, 135.7, 153.7, 155.9, 169.4, 169.7 ppm; FT-IR (thin film): $\tilde{\nu} = 3336$, 2980, 1746, 1720, 1508 cm^{-1}; MS (HRESI-MS) calcd for $[C_{23}H_{29}Cl_3N_2O_8+Na]^+$: 589.0887; found: 589.0867.

Amine 5: Ester **4** (1.8 g, 3.17 mmol) was dissolved in AcOH (5 mL) and zinc dust (2 g) was added. The reaction mixture was stirred at room temperature for 1 h, after which additional AcOH (1.5 mL) and zinc dust (2 g) were added. The mixture was stirred at room temperature for another hour and purified by chromatography on silica gel (10:1 Et_2O/EtOAc) affording **5** as a solid (1.1 g, 88%) that was homogeneous by TLC analysis. $R_f = 0.52$ (Et_2O/MeOH 20:1); $[\alpha] = +66.7$ ($c = 0.75$ in $CHCl_3$); ^{1}H NMR (300 MHz, CD_3CN, 25 °C): $\delta = 1.11$ (d, $J = 6.4$ Hz, 3H), 1.42 (s, 9H), 1.95 (s, 2H), 4.42 (dd, $J = 2.3$, 9.5 Hz, 1H), 4.54 (m, 2H), 4.75 (s, 1H), 5.20 (dd, $J = 1.1$,10.4 Hz, 1H), 5.30 (ddd, $J = 1.5$, 3.1, 17.4 Hz, 1H), 5.40 (m, 1H), 5.86 (m, 1H), 5.89 (d, $J = 10.5$ Hz, 1H), 7.38 ppm (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 17.0$, 28.4, 57.1, 58.4, 66.8, 73.1, 80.4, 119.1, 127.3, 129.3, 129.4, 131.8, 136.9, 156.3, 170.9, 171.0 ppm; FT-IR (thin film): $\tilde{\nu} = 3313$, 2981, 2936, 1745, 1716, 1506 cm^{-1}; MS (HRESI-MS) calcd for $[C_{20}H_{28}N_2O_6+Na]^+$: 415.1845; found: 415.1837.

Resin-bound dipeptide 6: Imidazole (0.30 g, 4.43 mmol) was dissolved in CH_2Cl_2 (4.0 mL) and diisopropyldichlorosilane (0.16 mL, 0.89 mmol) was injected dropwise. The mixture was stirred at room temperature under an atmosphere of N_2 for 5 min, and a solution of alcohol **3** (0.375 g, 0.885 mmol) in CH_2Cl_2 (3.0 mL) was added slowly dropwise over 15 min with a canula. The mixture was stirred at room temperature under an atmosphere of N_2 for 30 min, followed by addition of the hydroxymethyl polystyrene resin (0.65 g, 0.65 mmol). The mixture was agitated at room temperature for 48 h, drained, and washed with MeOH (20 mL), CH_2Cl_2 (20 mL), and Et_2O (20 mL). The resin was dried under vacuum and subjected to Fmoc quantitation. The loading level was determined to be 0.50 mmol g^{-1}. The resin was then treated with a solution of 30% piperidine in CH_2Cl_2 (20 mL) for 30 min, drained, and washed with CH_2Cl_2 (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was dried under vacuum and treated with Alloc-L-MePhe-OH (0.70 g, 2.66 mmol), a solution of HOAt in DMF (0.5 M, 10 mL, 5.0 mmol), DIC (0.78 mL, 5.0 mmol), and 2,6-lutidine (1.16 mL, 10.0 mmol). The mixture was agitated for 48 h at room temperature, drained, and washed with CH_2Cl_2 (20 mL), MeOH (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was dried under vacuum.

Resin-bound tetrapeptide 7: The resin-bound dipeptide **6** was suspended in a 5% solution of AcOH in CH_2Cl_2 (10.0 mL) and treated with tributyltin hydride (1.43 mL, 5.31 mmol) and tetrakis(triphenylphosphine)palladium(0) (100 mg, 0.087 mmol). The mixture was agitated at room temperature in the dark for 3 h, drained, and washed with CH_2Cl_2 (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was dried under vacuum and treated with Fmoc-L-Pro-OH (0.90 g, 2.66 mmol), a solution of HOAt in DMF (0.5 M, 10 mL, 5.0 mmol), DIC (0.78 mL, 5.0 mmol), and 2,6-lutidine (1.16 mL, 10.0 mmol). The mixture was agitated for 48 h at room temperature, drained, and washed with CH_2Cl_2 (20 mL), MeOH (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The same coupling protocol was repeated again twice, after which the resin was washed and dried under vacuum. The resin was then treated with a solution of 30% piperidine in CH_2Cl_2 (20 mL) for 30 min, drained, and washed with CH_2Cl_2 (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was dried under vacuum, suspended in CH_2Cl_2 (5.0 mL), and treated with a solution of Alloc-D-Abu-OH in CH_2Cl_2 (0.48 M, 5.5 mL, 2.66 mmol), PyAOP (2.6 g, 5.0 mmol), and 2,6-lutidine (1.16 mL, 10.0 mmol). The mixture was agitated for 24 h at room temperature, drained, and washed with CH_2Cl_2 (20 mL), MeOH (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was dried under vacuum.

Resin-bound linear hexapeptide 8: The resin-bound tetrapeptide **7** was suspended in CH_2Cl_2 (4.5 mL) and treated with 2,6-lutidine (4.0 mL, 34.5 mmol) and TMS-OTf (3.0 mL, 16.6 mmol). The mixture was agitated at room temperature for 5 h, drained, and washed with CH_2Cl_2 (20 mL), MeOH (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was suspended in MeOH (10 mL) and agitated at room temperature for 20 min, after which it was drained and washed with CH_2Cl_2 (20 mL), MeOH (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was dried under vacuum, suspended in a solution of HOAt in DMF (0.5 M, 10 mL, 5.0 mmol), and treated with amine **5** (0.69 g 1.77 mmol), DIC (0.78 mL, 5.0 mmol), and 2,6-lutidine (1.16 mL, 10.0 mmol). The mixture was agitated for 6 h at room temperature, drained, and washed with CH_2Cl_2 (20 mL), MeOH (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was dried under vacuum.

Resin-bound cyclic hexapeptide 9: The resin-bound linear hexapeptide **8** (690 mg, 0.226 mmol, based on 0.5 mmol g^{-1} initial loading and subsequent weight increase of the resin) was placed into a 10 mL polypropylene poly-prep chromatography column. The resin was suspended in CH_2Cl_2 (6.0 mL), treated with phenylsilane (2.0 mL, 16.2 mmol), H_2O (100 μL), and tetrakis(triphenylphosphine)palladium(0) (100 mg, 0.087 mmol). The mixture was agitated at room temperature in the dark for 5 h, drained, and washed with CH_2Cl_2 (20 mL), MeOH (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was dried under vacuum, resuspended in CH_2Cl_2 (8.0 mL), and treated with PyAOP (0.50 g, 0.96 mmol), and 2,6-lutidine (1.0 mL, 8.61 mmol). The mixture was agitated for 48 h at room temperature, drained, and washed with CH_2Cl_2 (20 mL), MeOH (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was dried under vacuum. All the subsequent reactions through the completion of the synthesis were conducted in the same poly-prep chromatography column.

Resin-bound acylated macrocycle 10: The resin-bound cyclic hexapeptide **9** was suspended in CH_2Cl_2 (4.0 mL) and treated with 2,6-lutidine (3.0 mL, 25.8 mmol) and TMS-OTf (2.0 mL, 11.1 mmol). The mixture was agitated at room temperature for 2 h and then for 25 min at 4 °C. The resin was washed with precooled-to-4 °C THF (20 mL), immediately suspended in precooled-to-4 °C THF (6.0 mL), and treated with 3-hydroxypicolinic acid (0.20 g, 1.44 mmol), PyAOP (0.50 g, 0.96 mmol), and 2,6-lutidine (0.60 mL, 5.17 mmol). The mixture was agitated at 4 °C for 4 h and then for 10 h at room temperature. The resin was drained and washed with CH_2Cl_2 (20 mL), MeOH (20 mL), THF (20 mL), DMF (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). The resin was dried under vacuum.

Dihydrovirginiamycin S_1: The resin-bound macrocycle **10** was suspended in THF (7.0 mL) and treated with HF/pyridine (1.0 mL). The resin was agitated at room temperature for 1 h. The resin was drained and washed with THF (20 mL), methoxytrimethylsilane (20.0 mL, 145.64 mmol), CH_2Cl_2 (20 mL), MeOH (20 mL), MeCN (20 mL), and again with CH_2Cl_2 (20 mL). All the washes were combined with the original filtrate, stirred at room temperature for 1 h and, concentrated. The residual amount of pyridine was removed under vacuum. The product was puri-

fied by chromatography on silica gel (25:1 CH_2Cl_2/MeOH) to give dihydrovirginiamycin S_1 (28 mg, 15%) as a solid that was homogeneous by TLC analysis. R_f=0.32 (CH_2Cl_2/MeOH 20:1); $[\alpha]=-4.4$ (c=0.54 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ=0.33 (dt, J=11.6, 5.1 Hz, 1H; 5β_2), 0.92 (t, J=7.3 Hz, 3H; 2γ), 1.25 (m, 1H; 5δ_2), 1.14 (m, 1H; 3β_1), 1.33 (d, J=6.5 Hz, 3H; 1γ), 1.31 (m, 1H; 3γ_2), 1.33 (d, J=6.5 Hz; 3H; 1γ), 1.58 (m, 1H; 3γ_1), 1.67 (m, 1H; 2β_2), 1.73 (m, 1H; 2β_1), 1.90 (m, 1H; 5δ_1), 1.97 (m, 1H; 3β_2), 2.38 (dd, J=12.3, 4.8 Hz, 1H; 5β_1), 2.45 (dt, J=13.4, 2.1 Hz, 1H; 5ε_2), 3.11 (s, 3H; 4N-Me), 3.13 (m, 1H; 4β_2), 3.25 (dd, J=14.6, 8.6 Hz, 1H; 4β_1), 3.33 (q, J=7.5 Hz, 1H; 3δ_2), 3.51 (m, 1H; 3δ_1), 4.27 (m, 1H; 5γ), 4.51 (t, J=6.7 Hz, 1H; 3α), 4.60 (d, J=13.7 Hz, 1H; 5ε_1), 4.78 (q, J=7.4 Hz, 1H; 2α), 4.85 (dd, J=9.6, 1.2 Hz, 1H; 1α), 5.12 (m, 1H; 5α), 5.43 (t, J=8.1 Hz, 1H; 4α), 5.61 (d, J=7.8 Hz, 1H; 6α), 5.89 (dq, J=6.7, 1.2 Hz, 1H; 1β), 6.60 (d, J=9.3 Hz, 1H; 2NH), 7.10 (m, 2H; aromatic), 7.17 (dd, J=8.5, 4.3 Hz, 1H; 1′H$_5$), 7.29 (m, 9H; aromatic), 7.70 (dd, J=4.4, 1.1 Hz, 1H; 1′H$_6$), 8.38 (d, J=9.5 Hz, 1H; 1′NH), 8.58 (d, J=7.9 Hz, 1H; 6NH), 11.67 ppm (s, 1H; 1′OH); ^{13}C NMR (125 MHz, $CDCl_3$, 25°C): δ=10.3, 16.7, 25.0, 25.3, 27.9, 31.0, 34.0, 35.5, 35.6, 38.1, 47.9, 51.8, 52.1, 56.3, 56.9, 57.7, 57.8, 65.0, 71.5, 127.0, 127.6, 128.2, 128.4, 129.0, 129.1, 129.3, 130.5, 135.7, 136.2, 139.9, 158.6, 167.6, 168.8, 169.7, 170.3, 170.5, 171.4, 173.0 ppm; FT-IR (thin film): $\tilde{\nu}$=3344, 3272, 2958, 2917, 2851, 1739, 1675, 1628, 1517, 1448 cm^{-1}; MS (HRESI-MS) calcd for $[C_{43}H_{51}N_7O_{10}+Na]^+$: 848.3595; found: 848.3575.

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