Supporting Information

Synthesis and Evaluation of Vancomycin Aglycon Analogues that Bear Modifications in the *N*-terminal D-Leucyl Amino Acid

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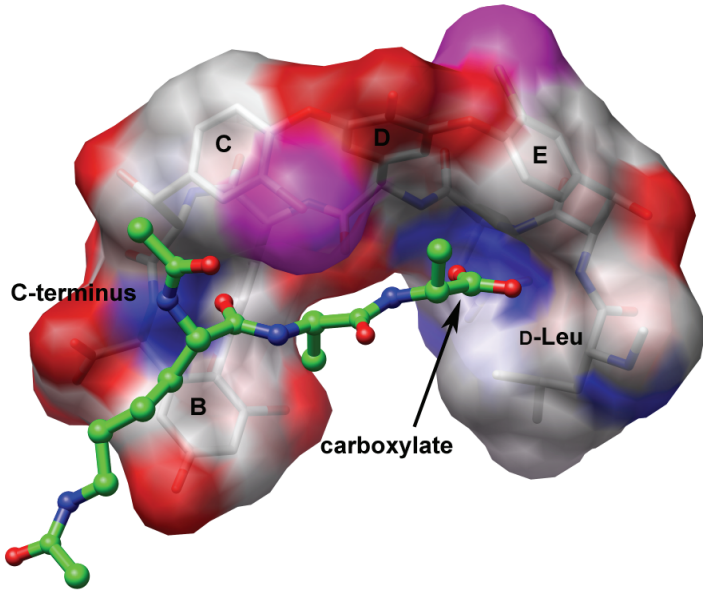


Figure 1SI. Surface representation of a single complex of the vancomycin aglycon (**5**) (surface area 767.16 Å^2) co-crystallized with *N*,*N*′-Ac$_2$-L-Lys-D-Ala-D-Ala (**2**, PDB Code: 1FVM) as determined by X-ray crystallography to 1.8 Å-resolution.[2] The surface area at the *N*-terminal region of **5** is made up of the residue 3 asparagine and the residue 1 D-leucyl moieties, which generate a "concave" non-polar surface for carboxylate binding, thereby potentially reducing its desolvation penalty. Color code: ligand skeleton: green; vancomycin skeleton: grey; atoms: O: red; N: blue; H: white. Surface color code: by element (above). Figure and surface area calculations were generated with the molecular graphics program Chimera.[3]

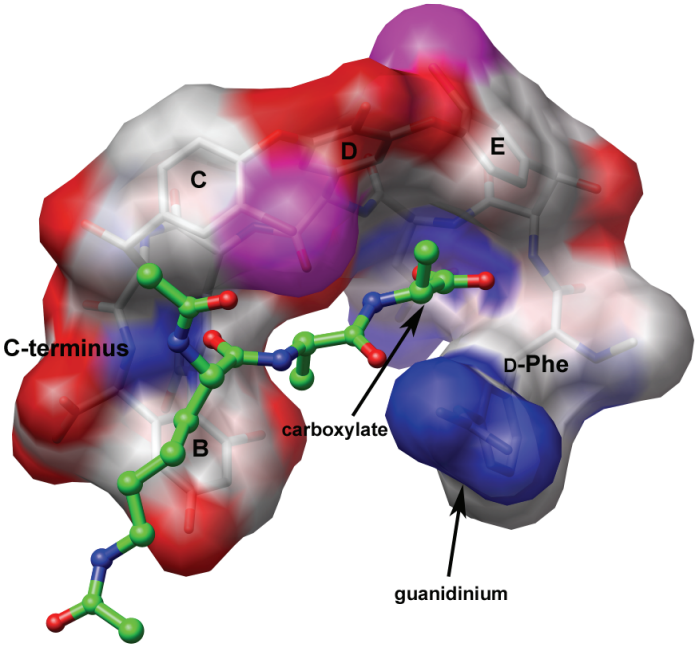


Figure 2SI. Surface representation of **11** (surface area 767.16 Å^2) with bound *N*,*N*′-Ac$_2$-L-Lys-D-Ala-D-Ala (**2**) as determined by molecular modeling using the program MOLOC.[1] Molecular modeling was preformed using the X-ray crystal structure of **5** co-crystallized with **2** (PDB Code: 1FVM).[2] The *N*-terminal region of **11** is made up of the residue 3 asparagine and the residue 1 3-guanidyl-D-Phe. Unlike **5**, residues 3 and 1 do not interact to generate a "concave" shaped non-polar surface, rather the D-Phe has moved away from the aspargine, allowing the guanidine substiuent to H-bond to the residue 2 carbonyl of **2**. Color code: ligand skeleton: green; vancomycin skeleton: grey; atoms: O: red; N: blue; H: white. Surface color code: by element (above). Figure and surface area calculations were generated with the molecular graphics program Chimera.[3]

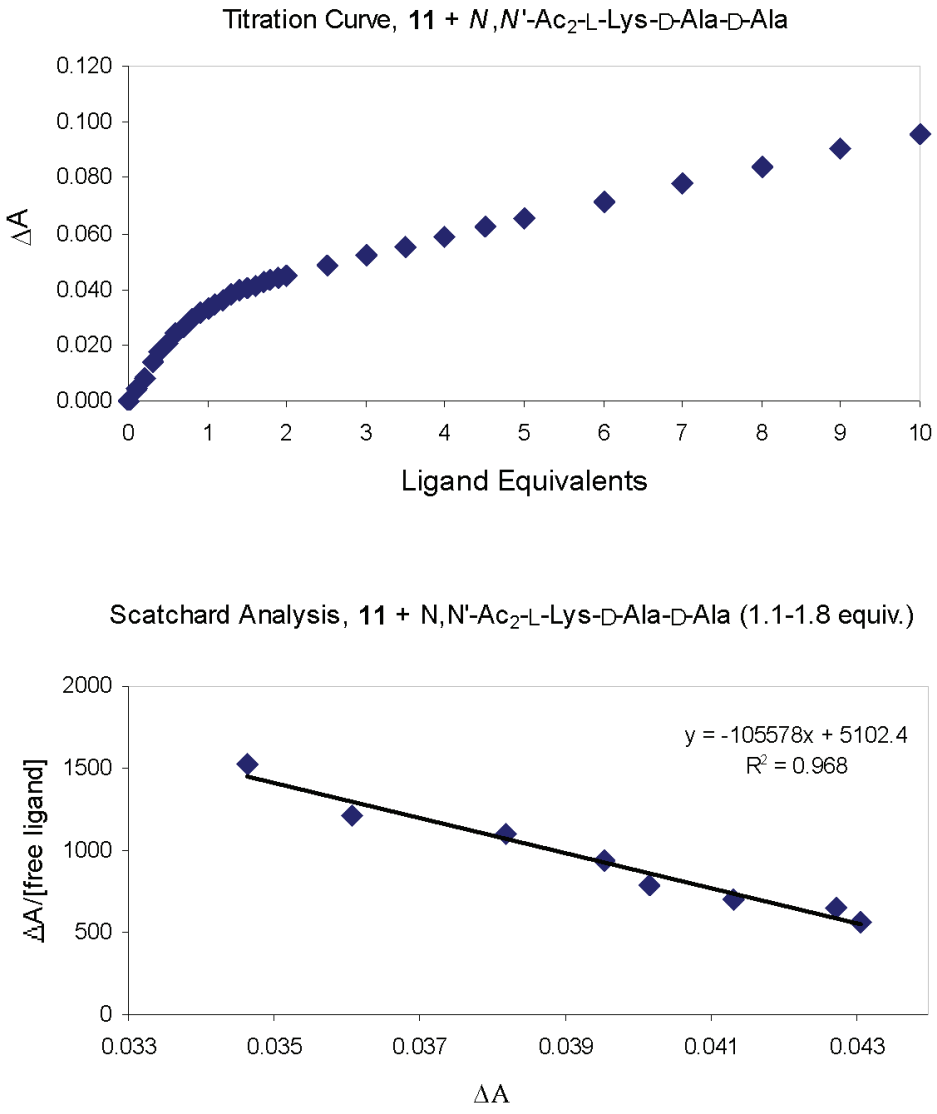
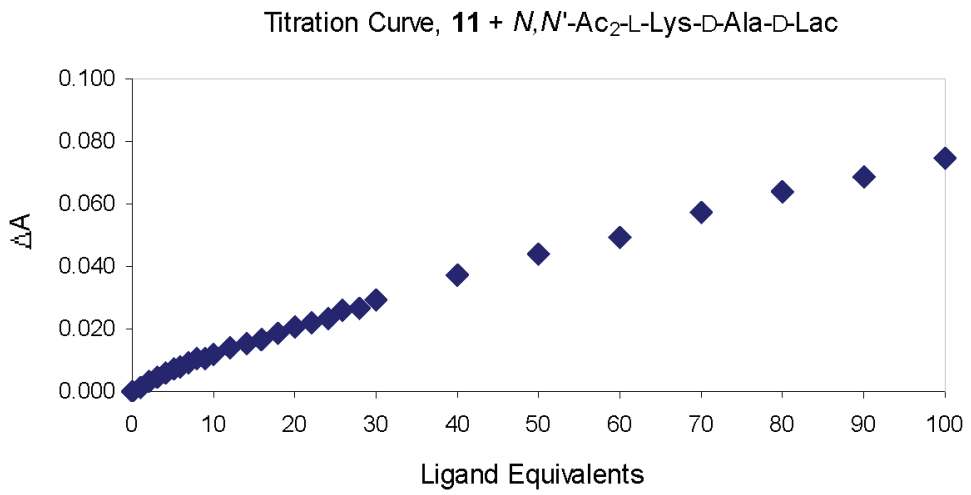


Figure 3SI. Representatice titration curve for the determination of the association constant (K_a) for the complex of **11** (1.1×10^{-4} M) + *N*,*N*′-Ac$_2$-L-Lys-D-Ala-D-Ala (**2**) in 20 mM sodium citrate buffer, pH 5.1. (a) Saturation curve (0–10 equiv). (b) Scatchard analysis (1.1–1.8 equiv).



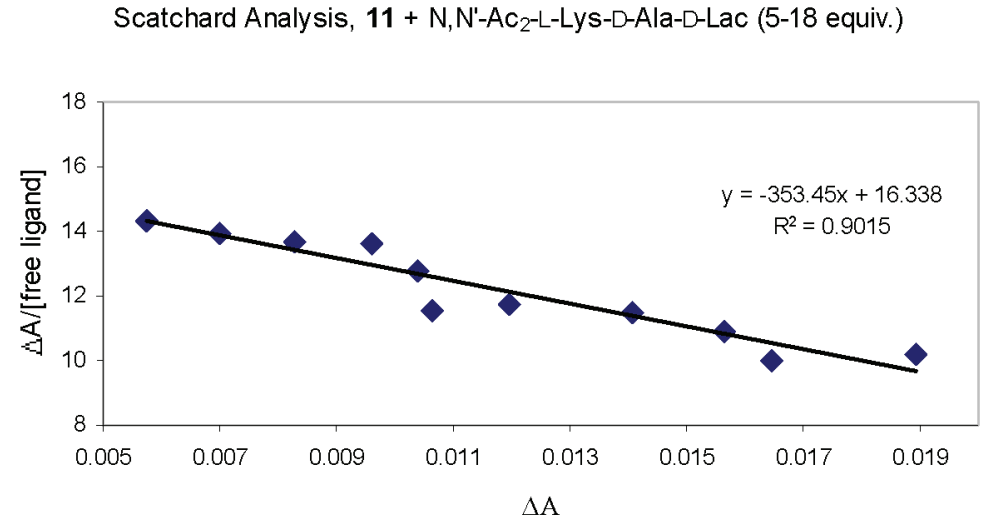


Figure 4SI. Representative titration curve for the determination of the association constant (K_a) for the complex of **11** (1.1×10^{-4} M) + *N*,*N*′-Ac$_2$-L-Lys-D-Ala-D-Lac (**3**) in 20 mM sodium citrate buffer, pH 5.1. (a) Saturation curve (0–100 equiv). (b) Scatchard analysis (5–18 equiv).

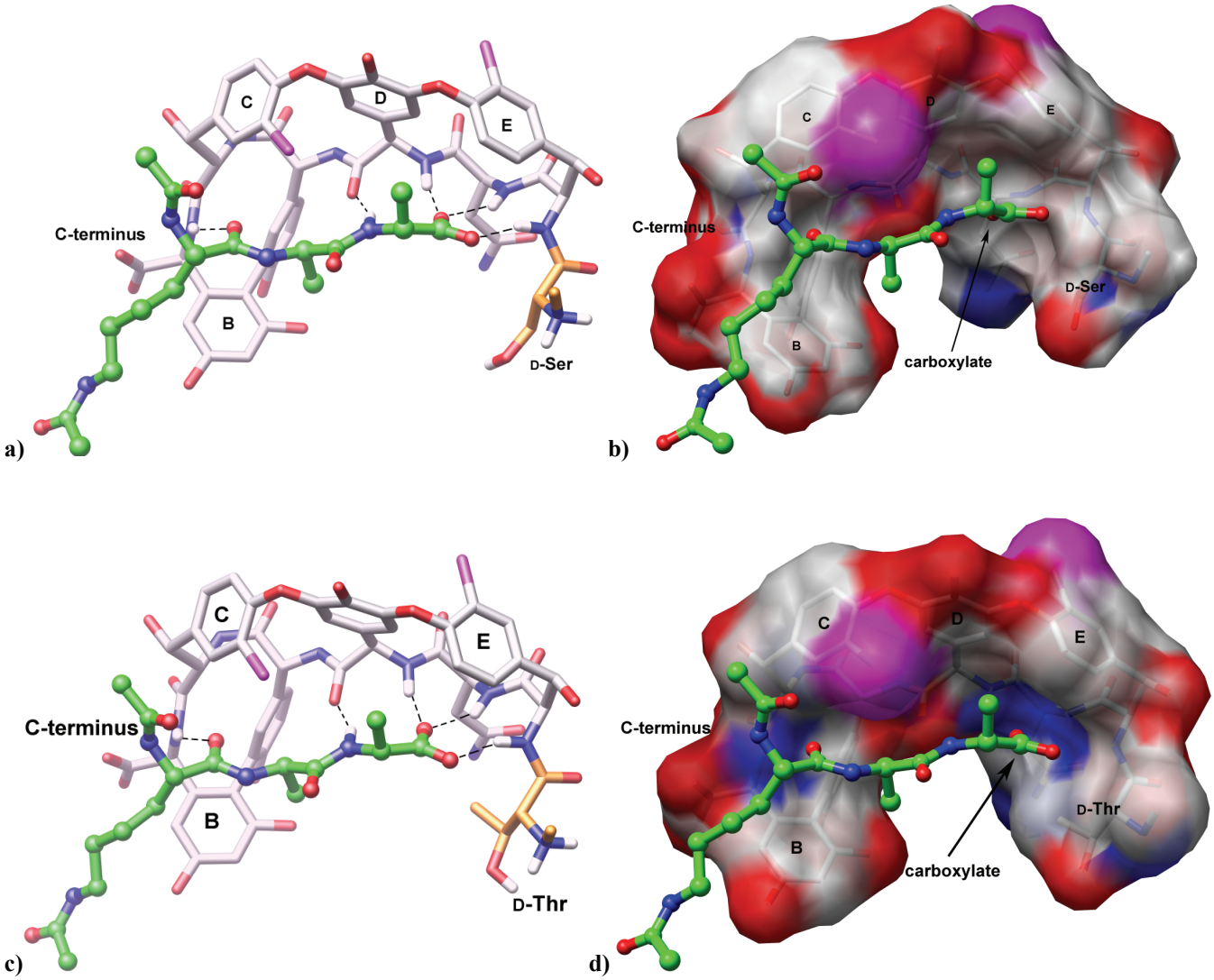


Figure 5SI. Proposed binding mode of target molecules **6** and **7** with ligand *N*,*N*′-Ac$_2$-L-Lys-D-Ala-D-Ala (**2**) as determined by molecular modeling using the program MOLOC.[2] Molecular modeling was preformed using the X-ray crystal structure of **5** co-crystallized with **2** (PDB Code: 1FVM).[2] The *N*-terminal region of **6** and **7** are made up of the residue 3 asparagine and the residue 1 D-Ser or D-Thr, respectively. Unlike **5**, residues 3 and 1 in the former do not generate a "concave" shaped non-polar surface (b), rather the additional methyl substituent of the latter compensates (d), thereby potentially reducing the desolvation penalty for carboxylate binding. (a) Ball and stick representation of **6**. (b) Surface representation of **6** (surface area 763.33 Å^2) with bound **2**. (c) Ball and stick representation **7**. (d) Surface representation of **7** (surface area 766.22 Å^2) with bound **3**. Black dashed lines represent

potential H-bonds. Color code: ligand skeleton: green; vancomycin skeleton: grey/orange; Cl: pink; amino acid skeleton: O: red; N: blue; H: white. Surface color code: by element above. All figures and surface area calculations were generated with the molecular graphics program Chimera.[3]

Table 1SI. Selected conditions examined for the reaction between hydrazine derivatives **13**–**15** and *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Lac (**3**).[a]

Entry	**Compound**	**Ligand** (equiv)	**[Compound]**	**Solvent**	**Temp** (°C)	**Time** (h)	**result**
1	**13**	20	0.13 mM	H_2O	20	18	No product
2	**13**	20	0.13 mM	A[b]	37	18	No product
3	**13**	20	0.13 mM	H_2O	20	18	No product
4	**13**	20	0.13 mM	A[b]	37	18	No product
5	**13**	2	7 mM	MeOH	20	18	No product
6	**13**	2	6 mM	EtOH	20	72	No product
7	**13**	2	6 mM	EtOH	65	48	No product
8	**13**+$NaHCO_3$ (2 equiv)	2	7 mM	EtOH	20	20	No product
9	**13**+Et_3N (1 equiv)	1	0.02 M	EtOH/EtSH (99:1)	0-75	18	No product
10	**13**+Et_3N (10 equiv)	1	0.02 M	EtOH/EtSH (99:1)	0-75	18	No product
11	**14**+Et_3N(8 equiv)	0.8	0.07 M	H_2O	20-75	24 48	No product
12	**15**	0.8	0.07 M	H_2O	20-75	24 48	No product
13	**14**	0.9	0.06 M	H_2O	20	336	[**14**+**3**] complex
14	**14**+$NaHCO_3$ (10 equiv)	1.1	0.06 M	H_2O	20	72	No product
15	**12**+$NaHCO_3$ (20 equiv)	1.0	16 mM	*i*PrOH	20	72	No product
16	**12**+$NaHCO_3$ (20 equiv)	1.0	16 mM	MeOH	20	72	No product
17	**12**+$NaHCO_3$ (20 equiv)	1.0	16 mM	H_2O	20-75	72-72h	[**12**+**3**] complex

[a] Crude reactions were monitored by LC–MS. [b]20 mM sodium citrate buffer (pH 5.1)

Experimental Section

General. Vancomycin aglycon (**1**) and desleucylvancomycin aglycon (**16**) were prepared according to literature procedures.[4,5] Reagents and solvents were purchased reagent-grade and used without further purification. Amino acid building blocks Boc-D-Thr and Boc-D-HoSer(OBn) were purchased from from Chem-Impex International, *N*-Boc-D-*O*-*tert*-butylserine from Novabiochem, and Boc-D-3-CN-Phe from Peptech. For the antimicrobial assays: Muller Hinton broth and Bacto™ Brain heart infusion were purchased from Becton, Dickinson and Company. For the UV-difference titration assays: the ligands **2** and **3** were purchased from Bachem; and the buffer 0.2 mM sodium citrate (100 mL) was prepared from citric acid (0.131 g) and sodium citrate tribasic (0.388 g) and the pH was adjusted to 5.1 with 5 N NaOH. THF was freshly distilled from sodium benzophenone ketyl. All reactions were performed in oven-dried glassware under an Argon atmosphere, unless otherwise stated. Evaporation and concentration in vacuo was performed at 20 °C and ca. 760 m Hg. Further drying of the new compounds was carried out at ca. 5 millibar. TLC: pre-coated SiO_2 60 F_{254} glass plates from EMD, visualization by UV light (254 or 366 nm) or by staining with a solution of ninhydrin or bromocresol green. Column chromatography (FC): SiO_2 60 (0.04-0.063 mm) from Fluka with a head pressure of ca. 0.2 bar (FC). Analytical and Preparative reverse-phase HPLC was performed using a Waters HPLC on a Waters Nova-Pac® HR C18 6 μm, 60 Å column (25 x 100 mm) using a gradient of MeCN/water with 0.07% TFA at flow rate of 5 mL min^{-1} and with UV detection at λ = 254 and 280 nm. Uncorrected melting points (M.p.) were determined in an open capillary using a Mel-Temp II apparatus. NMR (^1H or ^{13}C): Varian Inova-400, Bruker DRX-500 and Bruker DRX-600 NMR spectrophotometers at 298 K. Residual solvent peaks were used as an internal reference. Coupling constants (*J*) *(*H,H*)* are given in Hz. Coupling patterns are designated as singlet (s), doublet (d), triplet (t), quadruplet (q), quintuplet (qt), multiplet (m), or broad signal (br). IR spectra: Thermo Scientific Nicolet 380 FT-IR spectrophotometer measured neat. High resolution mass spectral data were acquired on an Agilent Technologies High Resolution LC/MSD-TOF and the detected masses are given as *m/z* with *M* representing the molecular ion.

***In vitro* Assays.** One day before experiments were run fresh cultures of vancomycin-sensitive *Staphlococcus aureus* (strain ATCC 25923) in Mueller-Hinton broth and vancomycin-resistant *Enterococcus faecalis* (VanA, BM4166) in Brain-Heart Infusion were inoculated and warmed in an orbital shaker at 37 °C. After 24 h, the bacterial stock solutions were serial diluted with the culture medium (Muller–Hinton broth for *S. aureus* and Brain-Heart Infusion for *E. faecalis*-VanA) to achieve the turbidity equivalent of 1:100 dilution of 0.5 M Macfarland solution. This diluted bacterial stock solution was then inoculated into a well of a V-shaped 96-well microtiter plate, supplemented with serial diluted aliquots of the antibiotic solution in water or DMSO (1–5 μL), to achieve a total assay volume of 0.1 mL. The plate was then incubated at 37 °C for 24 h, minimal inhibitory concentrations (MICs) were determined by monitoring the cell growth (observed as a pellet) in the wells. The lowest concentration of antibiotic (in μg/mL) capable of eliminating the cell growth, in the wells, is reported as the MIC. The reported MIC values for the new antibiotics were determined against vancomycin or vancomycin aglycon as a standard in the first well, which have well-established MIC values.[6,7]

UV-difference assay for the determination of association constants for the complexes of the natural product analogues and 2 and 3. The association constants (K_a) of the complexes between compounds **6–15** and the model ligands *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Ala (**2**) and *N*,*N*′-Ac_2-L-Lys-D-Ala-D-Lac (**3**) were determined according to literature procedures.[6-8] UV-difference experiments were carried out on a CARY 3E UV/Vis spectrometer in 1.5 mL quartz cuvettes (path length = 1 cm), and measured from λ = 200 to 345 nm. The UV absorbance scans were run after a baseline correction (0.02 M sodium citrate buffer) from λ = 200 to 345 nm. The initial absorbance (A_o) of a 1 mL solution of the antibiotic (1.1×10^{-4} M in 0.02 M sodium citrate buffer) was recorded (λ = 200 to 345 nm) with respect to reference cell containing only 1 mL of 0.02 M sodium citrate buffer. UV spectra were recorded after the

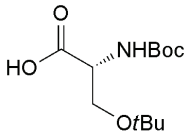
addition of 1 μL of either **2** (0.011 M, 0.1–10 equivalents) or **3** (0.11 M, 1–100 equivalents) in 0.02 M sodium citrate buffer to each cell. The absorbance value at the λ_{max} (279 mm) was recorded and the running change in absorbance ΔA, at x-equivalents (A_x) of ligand, where $\Delta A = (A_o - A_x$ equiv), was measured. The number of ligand equivalents was plotted versus ΔA to provide a ligand saturation curve for each complex. The break point of this curve is the saturation point ($\Delta A_{saturation}$) of the system and its *x*,*y*-coordinates were determined by establishing the point intersection of the linear functions for the pre- and post-saturation parts of the titration curve. The concentration of free ligand ([free ligand]) in solution at each titration step was then calculated from $\Delta A_{saturation}$. A Scatchard plot of ΔA at x-equivalents versus ΔA/[free ligand] gave a linear function, the slope of which provides the association constant (K_a).

General Procedures (*GP*)

***GP1* for the selective *N*-methylation of amino acids.** NaH (3.3 equiv) was added portionwise to a stirred solution of the corresponding amino acid (1.0 equiv) in anhydrous THF (0.3 M) at 0 °C. After stirring for 10 min, MeI (3.0 equiv) and DMF (10% v/v) were added, and the mixture was allowed to stir for an additional hour at 0 °C and was then warmed to 20 °C. After 18 h, the reaction was quenched by the addition of water and extracted with EtOAc. The aqueous phase was then acidified to pH of 2–3 with aqueous 0.2 N $NaHSO_4$ and extracted with EtOAc, which was washed with aqueous 5% NaS_2O_4, and dried over Na_2SO_4 (see the experimental details).

***GP2* for ester hydrolysis.** An aqueous solution of 0.2 N LiOH (1.0–3.0 equiv) was added portionwise to a solution of the corresponding methyl ester (1.0 equiv) in THF (0.35 M) at 0 °C. After 2–4 h at 0 °C, the solution was acidified to a pH of 2–3 with aqueous 0.2 N $NaHSO_4$ and extracted with EtOAc (3 ×), and the combined organics layers were dried over Na_2SO_4, filtered and concentrated in vacuo.

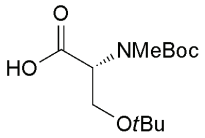
***GP3* for DCC mediated anhydride coupling.** A solution of dicyclohexylcarbodiimide (2.0 equiv) in anhydrous CH_2Cl_2 was added to a solution of the corresponding amino acid (4.0 equiv) in anhydrous CH_2Cl_2 (0.14 M) at 0 °C. After 1 h at 0 °C, the volatiles were removed with a gentle stream on N_2. The residue was re-dissolved in anhydrous DMF (0.15 M) and added dropwise to a solution of desleucylaglucovancomycin (**16**[4,5], 1.0 equiv) and $NaHCO_3$ (4.0 equiv) in anhydrous DMF (0.04 M) at 0 °C. After 1 h at 0 °C, the mixture was warmed to 20 °C. After 18 h, the volatiles were removed with a gentle stream of N_2 gas and the crude residue was purified by reverse-phase HPLC using a gradient of MeCN/water with 0.07% TFA. The fractions that contained product were combined and lyophilized. The resulting protected intermediates were isolated and directly carried to the next step without full characterization. The intermediates were treated with 10–50% TFA/CH_2Cl_2 at 0–20 °C for 5–40 min, the volatiles were removed with a gentle stream of N_2 and purified by reverse-phase HPLC (see the experimental details).



(*R*)-3-*tert*-Butoxy-2-(*tert*-butoxycarbonylamino)propanoic Acid (17)

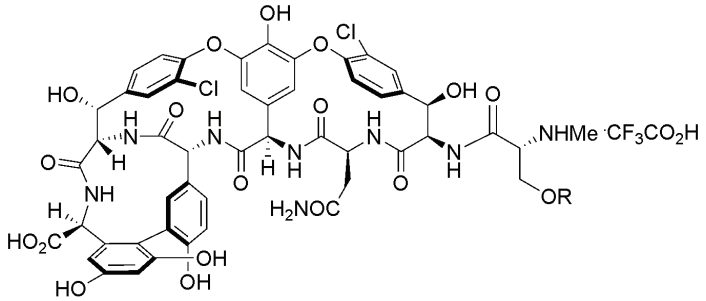
Aqueous NaOH (3.6 mL, 0.47 mmol) followed by di-*tert*-butyl dicarbonate (390 μL, 1.70 mmol) were added to a solution of H-D-Ser(tBu)-OH (250 mg, 1.55 mmol) in dioxane (3.6 mL) at 0 °C. After 1 h, the solution was warmed to 25 °C. After 24 h, the mixture was acidified to pH of 2–3 with aqueous 0.2 N $NaHSO_4$ and extracted with EtOAc (2 × 100 mL), which was dried over Na_2SO_4, filtered and concentrated. Column chromatography (26 g SiO_2, 0–10% MeOH/EtOAc) afforded **17** as a pale yellow oil (195 mg, theoretical 0.406 g, 48%): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.08 (br s, 1H), 5.36 (br s, 1H),

4.39 (br s, 1H), 3.87 (d, 1H, J = 4.2 Hz), 3.56 (dd, 1H, J = 4.2, 8.4 Hz), 1.46 (s, 9H), 1.18 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.3, 155.8, 80.2, 74.2, 61.7, 53.8, 28.3 (3C), 27.3 (3C); IR (neat) ν_{max} 2976*m*, 2361*w*, 1719*s*, 1506*m*, 1393*m*, 1366*m*, 1165*s*, 1103*w*, 1068*w* cm^{-1}; HR ESI-TOF *m/z* 284.1473 (M + Na^+, $C_{12}H_{23}NNaO_5^+$, requires 284.1468); $[\alpha]^{25}_D$ –21 (*c* 0.5, CH_2Cl_2).



(*R*)-3-*tert*-Butoxy-2-(*tert*-butoxycarbonyl(methyl)amino)propanoic Acid (19)

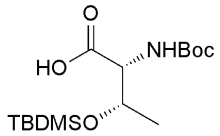
The title compound was prepared according to *GP1* with **17** (150 mg, 0.576 mmol), NaH (76 mg, 1.9 mmol), MeI (107 μL, 1.73 mmol), THF (1.92 mL), and DMF (97 μL). Column chromatography (10 g SiO_2, 10% MeOH/CH_2Cl_2) provided **19** (143 mg, theoretical 157 mg, 92%) as a pale yellow oil (rotomer ratio in $CDCl_3$ A/B = 10:9): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.96 (br s, 2H), 4.65 (t, 1H_A, J = 5.4 Hz), 4.35 (t, 1H_B, J = 6.0 Hz), 3.83–3.71 (m, 4H), 2.95 (s, 3H_B), 2.92 (s, 3H_A), 1.46 (s, 9H_A), 1.43 (s, 9H_B), 1.19 (s, 18H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 175.1/174.6, 157.2/156.1, 81.6/81.4, 75.1, 61.4/61.2, 61.5/60.1, 34.6, 29.20/29.16 (3C), 28.2 (3C); IR (neat) ν_{max} 2973*m*, 2928*m*, 1693*s*, 1452*m*, 1390*m*, 1366*m*, 1327*w*, 1148*s*, 1088*m*, 868*m* cm^{-1}; HR ESI-TOF *m/z* 276.1799 (M + H^+, $C_{13}H_{26}NO_5^+$, requires 276.1805); $[\alpha]^{25}_D$ –20 (*c* 0.5, CH_2Cl_2).



1-(D-Serinyl)desleucylaglucovancomycin Trifluoroacetate (6) and 1-(*O-tert*-Butyl- D-serine)desleucylaglucovancomycin Trifluoroacetate (7)

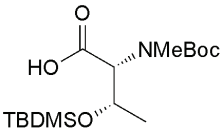
The title compounds were prepared starting from **19** (7.80 mg, 0.028 mmol), and dicyclohexyl carbodiimide (2.92 mg, 0.014 mmol) in CH_2Cl_2 (0.2 mL), followed by treatment with $NaHCO_3$ (2.38 mg, 0.028 mmol) and desleucylaglucovancomycin (**16**, 8.00 mg, 0.007 mmol) in DMF (0.4 mL) according to *GP3*. The protected intermediate was isolated as a white solid (6.60 mg, 73%) by reverse-phase HPLC using a gradient of 35–95 % MeCN/water with 0.07% TFA (t_R = 12.4 min), and lyophilized, and taken directly to the next step without further characterization. The intermediate (6.60 mg, 0.0051 mmol) was taken up in 50% TFA in CH_2Cl_2 (0.2 mL) and stirred at 20 °C. After 20 min, the volatiles were removed with a gentle stream of N_2 and residual TFA was azetroped with CH_2Cl_2 (3 × 5 mL). The residue was purified reverse-phase HPLC using a gradient of 5–40% MeCN/water with 0.07% TFA to provide **6** (34%) and **7** (65%). Characterization of the TFA salt of **6** (R = H, t_R = 23.8 min, 2.20 mg, 34%): white powder; ^{1}H NMR (CD_3OD, 600 MHz) δ 7.72 (br s, 2H), 7.63 (s, 1H), 7.58 (d, 1H, J = 8.4 Hz), 7.47 (br s, 1H), 7.19 (d, 1H, J = 8.4 Hz), 7.01 (s, 1H), 6.71 (br s, 2H), 6.52 (s, 1H), 6.40 (d, 1H, J = 2.4 Hz), 5.95 (br s, 1H), 5.83 (br s, 1H), 5.38–5.32 (m, 4H), 4.70 (s, 1H), 4.64 (br s, 1H), 4.34 (br s, 1H), 4.11 (s, 1H), 3.98 (t, 1H, J = 5.4 Hz), 3.83 (br s, 2H), 2.89 (d, 1H, J = 15.0 Hz), 2.79 (s, 3H), 2.16 (br s, 1H); HR ESI-TOF *m/z* 1117.2342 (M + H^+, $C_{50}H_{47}Cl_2N_8O_{18}^+$, requires 1117.2380); $[\alpha]^{25}_D$ +33 (*c* 0.2, CH_3OH). Characterization for the TFA salt of **7** (R = *t*Bu, t_R = 26.9 min, 4.40 mg, 65%): white powder; ^{1}H NMR (CD_3OD, 600 MHz) δ 7.82 (br s, 2H), 7.73 (br s, 1H), 7.63 (s,

1H), 7.57 (br s, 1H), 7.57 (br s, 1H), 7.55 (d, 1H, J = 8.4 Hz), 7.17 (d, 1H, J = 8.4 Hz), 7.04 (s, 1H), 6.67–6.64 (m, 2H), 6.44 (s, 1H), 6.40 (s, 1H), 6.06 (br s, 1H), 5.94 (br s, 1H), 5.37 (s, 1H), 5.33 (s, 1H), 5.27 (s, 1H), 4.75 (br s, 1H), 7.74 (s, 1H), 4.26 (d, 1H, J = 9.0 Hz), 4.15 (s, 1H), 4.10 (t, 1H, J = 6.6 Hz), 3.73 (d, 2H, J = 7.8 Hz), 2.95 (d, 1H, J = 15.6 Hz), 2.83 (s, 3H), 1.99 (br s, 1H), 1.2 (s, 9H); HR ESI-TOF *m/z* 1173.2975 ($M + H^+$, $C_{54}H_{55}Cl_2N_8O_{18}^+$, requires 1173.3006); $[\alpha]^{25}_D$ +49 (*c* 0.2, CH_3OH).



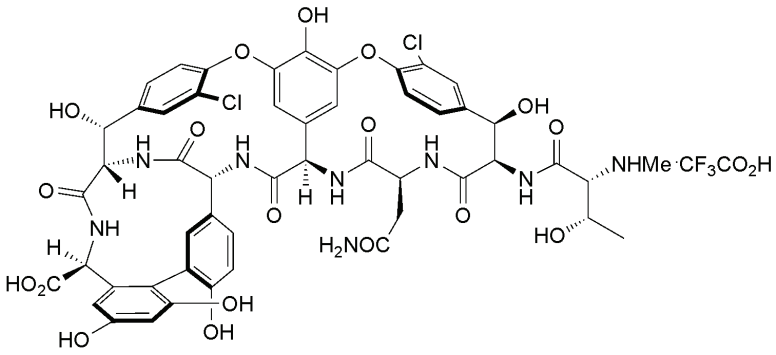
(2*R*,3*S*)-2-(*tert*-Butoxycarbonylamino)-3-(*tert*-butyldimethylsilyloxy)butanoic Acid (18)

A chilled solution of Boc-D-Thr (521 mg, 2.38 mmol) in anhydrous DMF (4.7 mL) at 0 °C was treated with *tert*-butyldimethylsilyl chloride (466 mg, 3.09 mmol) followed by imidazole (486 mg, 7.14 mmol), and the mixture was stirred for 1 h at 0 °C, and then warmed to 20 °C. After 18 h, the mixture was partitioned between aqueous 1 N HCl and Et_2O, and aqueous phase was washed Et_2O (2 × 40 mL). The combined organics layers were dried over Na_2SO_4, filtered, and the volatiles were removed in vacuo. Column chromatography (50 g SiO_2, 60% EtOAc/hexanes) provided **18** (636 mg, theoretical 648 mg, 98%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.71 (br s, 1H), 5.23 (d, J = 9.0 Hz, 1H), 4.44 (dd, J = 2.4, 6.6 Hz, 1H), 4.25 (dd, J = 2.4, 9.0 Hz, 1H), 1.46 (s, 9H), 1.19 (d, J = 6.0 Hz, 3H), 0.85 (s, 9H), 0.073 (s, 3H), 0.05 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 175.3, 156.1, 80.1, 68.7, 59.0, 28.3, 25.6, 20.0, 17.8, –4.6, –5.2; IR (neat) ν_{max} 3454*w*, 2954*m*, 1721*s*, 1694*s*, 1510*m*, 1366*w*, 1311*w*, 1253*s*, 1163*s*, 1129*s*, 1100*m*, 1040*m*, 833*s*, 773*s* cm^{-1}; HR ESI-TOF *m/z* 356.1866 ($M + Na^+$, $C_{15}H_{31}NNaO_5Si^+$, requires 356.1864); $[\alpha]^{25}_D$ –14 (*c* 0.5, CH_2Cl_2).



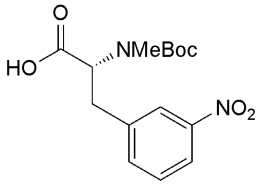
(2*R*,3*S*)-2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(*tert*-butyldimethylsilyloxy)butanoic Acid (20)

The title compound was prepared according to *GP1* from **18** (366 mg, 1.09 mmol), NaH (145 mg, 3.63 mmol), MeI (206 μL, 3.32 mmol), THF (3.6 mL), and DMF (175 μL). Column chromatography (30 g, SiO_2, 0–50% EtOAc/hexanes) provided **20** (231 mg, theoretical 381 mg, 60%) as an off-white crystalline solid (rotomer ratio in $CDCl_3$ A/B = 2:1): mp 87 °C (decomp.); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.41 (br s, 2H), 4.75 (br s, 2H), 4.63–4.52 (m, 1H), 3.02 (s, $3H_B$), 3.01 (s, $3H_A$), 1.48 (s, $9H_A$), 1.44 (s, $9H_B$), 1.20 (s, $3H_A$), 1.9 (s, $3H_B$), 0.85 (s, 18H), 0.08 (s, 6H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 175.4/175.3, 157.1/155.7, 80.5/80.4, 68.9/68.8, 64.1, 34.1/33.2, 28.3 (3C), 25.6 (3C), 20.7/20.4, 17.8, –4.43/–4.46/–5.26 (2C); IR (neat) ν_{max} 2956*m*, 2932*m*, 1695*s*, 1475*w*, 1369*m*, 1315*m*, 1255*m*, 1151*s*, 1069*m*, 940*m*, 835*m*, 776*m* cm^{-1}; HR ESI-TOF *m/z* 348.2206 ($M + H^+$, $C_{16}H_{34}NO_5Si^+$, requires 348.2201); $[\alpha]^{25}_D$ –20 (*c* 0.5, CH_2Cl_2).



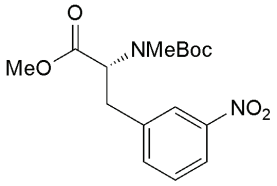
1-(D-Threonyl)desleucylaglucovancomycin Trifluoroacetate (8)

The title compound was prepared from **20** (5.30 mg, 0.014 mmol), and dicyclohexylcarbodiimide (1.46 mg, 0.007 mmol) in CH_2Cl_2 (0.1 mL), followed by treatment with $NaHCO_3$ (1.19 mg, 0.015 mmol), desleucylaglucovancomycin (**16**, 4.00 mg, 0.004 mmol) in DMF (0.2 mL) according to *GP3*. The protected intermediate was isolated as a white solid (3.30 mg, theoretical 4.70 mg, 70%) by reverse-phase HPLC using a gradient of 35–95 % MeCN/water with 0.07% TFA (t_R = 20.0 min), and lyophilized, and taken directly to the next step without further characterization. The intermediate (3.3 mg, 0.0025 mmol) was taken up in 50% TFA in CH_2Cl_2 (0.2 mL) and stirred at 20 °C. After 10 min, the volatiles were removed with a gentle stream of N_2 and residual TFA was azetroped with CH_2Cl_2 (3 × 5 mL). The residue was purified by reverse-phase HPLC using a gradient of 5–40% MeCN/water with 0.07% TFA (t_R = 24.4 min) and afforded the TFA salt of **7** (3.3 mg, theoretical 4.3 mg, 76%) as white powder after lyophilization: ^{1}H NMR (CD_3OD, 600 MHz) δ 8.94 (d, 1H, NH, J = 6.0 Hz), 8.78 (d, 1H, NH, J = 5.4 Hz), 7.75 (br s, 1H), 7.71 (br s, 1H), 7.66 (s, 1H), 7.57 (d, 2H, J = 8.4 Hz), 7.19 (d, 1H, J = 8.4 Hz), 7.04 (s, 1H), 6.67 (br s, 2H), 6.42 (dd, 2H, J = 1.8, 14.4 Hz), 6.02 (br s, 1H), 5.91 (br s, 1H), 5.35 (s, 1H), 5.33 (s, 1H), 5.27 (d, 1H, J = 1.8 Hz), 4.75–4.74 (m, 2H), 4.28 (d, 1H, J = 8.4 Hz), 4.22 (br s, 1H), 4.14 (s, 1H), 3.88 (d, 1H, J = 6.0 Hz), 2.94 (d, 1H, J = 15.6 Hz), 2.79 (s, 3H), 2.05 (br s, 1H), 1.06 (br s, 3H) (two signals buried under residual water); HR ESI-TOF m/z 1131.2484 (M + H^+, $C_{51}H_{49}Cl_2N_8O_{18}^+$, requires 1131.2536); $[\alpha]^{25}_D$ +47 (c 0.2, CD_3OD).



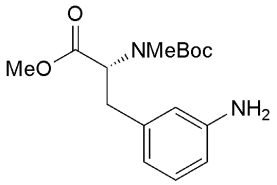
(*R*)-2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-nitrophenyl)propanoic Acid (22)

The title compound was prepared starting from commercially available Boc-D-3-nitrophenylalanine (**21**, 0.50 g, 1.62 mmol), NaH (0.214 g, 5.32 mmol), MeI (0.30 mL, 4.84 mmol), THF (5.4 mL), and DMF (0.26 mL) according to *GP1*. Column chromatography (50 g SiO_2, 10% MeOH/CH_2Cl_2) afforded **22** (423 mg, theoretical 518 mg, 82%) as a peach powder (rotomer ratio in $CDCl_3$ A/B = 3:4): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.07 (br s, 2H), 8.08 (d, 4H, J = 11.0 Hz), 7.59–7.47 (m, 4H), 4.90 (dd, $1H_B$, J = 5.0, 11.0 Hz), 4.70 (dd, $1H_B$, J = 4.0, 10.0 Hz), 3.45–3.41 (m, 2H), 3.24–3.13 (m, 2H), 2.76 (s, $3H_A$), 2.73 (s, $3H_B$), 1.36 (s, $9H_B$), 1.34 (s, $9H_A$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.3/175.2, 156.0/154.9, 148.4/148.2, 139.5/139.4, 135.3/135.2, 129.5/129.4, 123.9/123.7, 121.9/121.8, 81.8/81.0, 60.8/59.8, 34.9/34.3, 32.7/32.5, 28.1 (3C); IR (neat) ν_{max} 2977*w*, 1688*m*, 1528*m*, 1480*w*, 1449*w*, 1350*m*,1264*m*, 1144*m*, 732*s* cm^{-1}; HR ESI-TOF m/z 347.1219 (M + Na^+, $C_{15}H_{20}N_2NaO_6^+$, requires 347.1213); $[\alpha]^{25}_D$ +56 (c 0.5, $CHCl_3$).



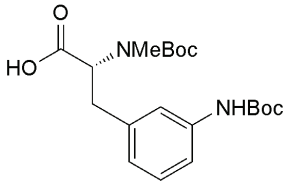
(*R*)-Methyl 2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-nitrophenyl)propanoate (23)

Crushed and vacuum-oven dried $KHCO_3$ (260 mg, 2.74 mmol) followed by MeI (112 μL, 1.83 mmol) was added to a solution of **22** (423 mg, 1.30 mmol) in anhydrous DMF (4.3 mL) at 0 °C. After the addition was complete, the mixture was allowed to warm to 25 °C. After 12 h, the mixture was acidified to a pH of 2–3 with aqueous 1 M $NaHSO_4$ and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with water (20 mL), saturated aqueous $NaHCO_3$ (20 mL), water (20 mL), then dried over $MgSO_4$, filtered, and concentrated in vacuo. Column chromatography (50 g SiO_2, 20% EtOAc/hexanes) afforded **23** (416 mg, theoretical 442 mg, 94%) as a yellow oil (rotomer ratio in $CDCl_3$ A/B = 1:1): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.05 (d, 4H, J = 12.5 Hz), 7.56–7.42 (m, 4H), 4.87 (dd, 1H$_A$, J = 5.0, 10.5 Hz), 4.59 (dd, 1H$_B$, J = 4.5, 10.0 Hz), 3.74 (s, 3H$_B$), 3.73 (s, 3H$_A$), 3.38 (dd, 2H, J = 5.0, 14.0 Hz), 3.16–3.09 (m, 2H), 2.70 (s, 6H), 1.33 (s, 9H$_A$), 1.32 (s, 9H$_B$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.0/170.8, 155.63/155.61, 148.3/148.2, 139.8/139.6, 135.3/135.2, 129.4/129.2, 123.9/123.7, 121.8/121.7, 80.6/80.3, 60.9/59.4, 52.3, 35.1/34.5, 32.4/32.2, 28.1 (3C); IR (neat) ν_{max} 2976*w*, 1741*m*, 1690*s*, 1527*s*, 1479*m*, 1436*m*, 1391*m*, 1348*s*, 1224*m*, 1141*s*, 734*w* cm^{-1}; HR ESI-TOF *m/z* 361.1375 (M + Na$^+$, $C_{16}H_{22}N_2NaO_6^+$, requires 361.1370); $[\alpha]^{25}_D$ +56 (*c* 0.5, $CHCl_3$).



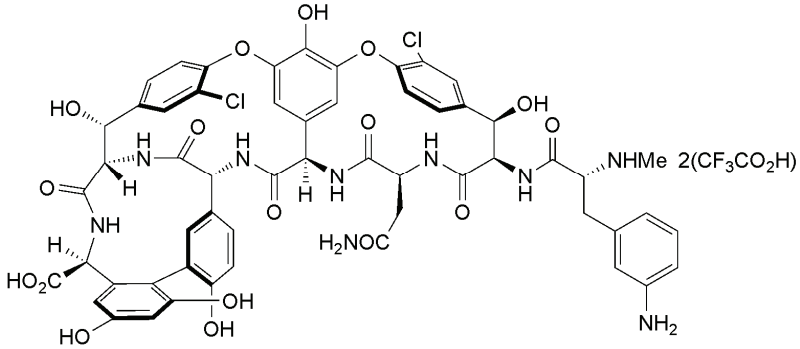
(*R*)-Methyl 3-(3-Aminophenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)propanoate (20)

A vacuum-degassed and N_2 flushed solution of **23** (400 mg, 1.18 mmol) in MeOH (11.8 mL) was treated with 10% Pd/C (0.1% wt., 0.040 g), and the mixture was again vacuum-degassed and flushed with N_2 (3 ×). The mixture was then placed under an atmosphere of H_2 (balloon) at 20 °C. After 13 h, the solution was filtered through a short plug of Celite and the volatiles were removed in vacuo. Column chromatography (50 g SiO_2, 50% EtOAc/hexanes) afforded **20** (358 mg, theoretical 365 mg, 98%) as a dark brown oil (rotomer ratio in $CDCl_3$ A/B = 3:4): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.06–7.04 (m, 2H), 6.60–6.48 (m, 6H), 4.90 (dd, 1H$_A$, J = 4.5, 9.5 Hz), 4.49 (dd, 1H$_B$, J = 4.0, 10.0 Hz), 3.73 (s, 3H$_B$), 3.71 (s, 3H$_A$), 3.62 (br s, 4H), 3.21–3.15 (m, 2H), 2.93–2.88 (m, 2H), 2.73 (s, 3H$_B$), 2.71 (s, 3H$_A$), 1.39 (s, 9H$_A$), 1.35 (s, 9H$_B$); ^{13}C NMR ($CDCl_3$, 150MHz) δ 171.9/171.6, 155.8/154.9, 146.5/146.4, 138.8/138.4, 129.3/129.1, 119.1, 115.6/115.5, 113.33/113.27, 80.1/79.9, 61.6/59.3, 52.1, 35.5/34.9, 32.7/31.9, 28.2/28.1 (3C); IR (neat) n_{max} 3459*w*, 3366*w*, 1738*m*, 1684*s*, 1605*m*, 1435*m*, 1392*m*, 1329*m*, 1256*m*, 1221*m*, 1142*s*, 1030*w*, 774*m* cm^{-1}; HR ESI-TOF *m/z* 309.1807 (M + H$^+$, $C_{16}H_{25}N_2O_4^+$, requires 309.1809); $[\alpha]^{25}_D$ +62 (*c* 0.5, CH_2Cl_2).



(*R*)-2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-(*tert*-butoxycarbonylamino)phenyl)propanoic Acid (24)

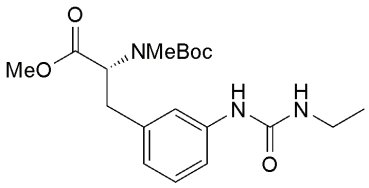
The title compound was prepared in two steps of which the first step was conducted according to *GP2* from **20** (100 mg, 0.32 mmol), aqueous 0.2 N LiOH (3.24 mL, 0.648 mmol), in THF (4.62 mL) at 0 °C for 2 h. Column chromatography (10 g SiO_2, 0–5% MeOH/EtOAc) provided the intermediate carboxylic acid (58.4 mg, 62%) as a brown oil, which was carried to the next step without full characterization. Boc_2O (50 μL, 0.22 mmol) was added to a solution of the carboxylate acid (58.4 mg, 0.198 mmol) in 1:1 THF/water (0.5 mL) at 20 °C. After 18 h, the reaction mixture was acidified to a pH of 2–3 with aqueous 3 N HCl and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and the volatiles were removed in vacuo. Column chromatography (7g SiO_2, 2–10% MeOH/CH_2Cl_2) afforded **24** (58.8 mg, theoretical 78.3 mg, 75%) as a yellow oil (rotomer ratio in $CDCl_3$ A/B = 1:1): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.31–7.15 (m, 8H), 6.88 (d, 1H, *J* = 6.6 Hz), 6.85 (d, 1H, *J* = 6.6 Hz), 6.60 (s, 2H), 4.82 (d, 1H, *J* = 5.4 Hz), 4.63 (d, 1H, *J* = 7.8 Hz), 3.3–3.28 (m, 2H), 3.07 (t, 1H, *J* = 12.0 Hz), 2.99 (t, 1H, *J* = 12.0 Hz), 2.76 (s, 3H), 2.70 (s, 3H), 1.51 (s, 18H), 1.40 (s, 9H), 1,35 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 175.6/175.2, 156.4/155.1, 152.8/152.6 (br), 138.52/138.48, 138.0, 129.1/129.0, 123.7/123.6, 119.0 (br), 116.9 (br), 80.7 (2C), 61.3/60.5, 35.4/34.7, 32.9/32.6, 28.3 (3C), 28.2/28.1 (3C); IR (neat) ν_{max} 2980*w*, 1717*m*, 1610*w*, 1523*w*, 1489*w*, 1441*w*, 1392*w*, 1368*w*, 1264*m*, 1156*m*, 702*s* cm^{-1}; HR ESI-TOF *m/z* 395.2177 ($M + H^+$, $C_{20}H_{31}N_2O_6^+$, requires 395.2177); $[\alpha]^{25}_D$ +47 (*c* 0.5, CH_2Cl_2).



1-[(3-Amino)-D-phenylalanyl]desleucylaglucovancomycin Bis(trifluoroacetate) (9)

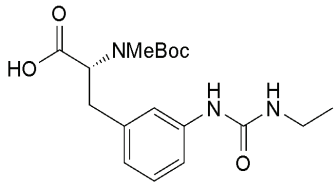
The title compound was prepared from **24** (5.58 mg, 0.015 mmol), and dicyclohexylcarbodiimide (1.46 mg, 0.007 mmol) in CH_2Cl_2 (100 μL), followed by treatment with $NaHCO_3$ (1.19 mg, 0.015 mmol), desleucylaglucovancomycin (**16**, 4.00 mg, 0.004 mmol) in DMF (200 μL) according to *GP3*. The protected intermediate was isolated as a white solid (2.70 mg, theoretical 4.90 mg, 55%) by reverse-phase HPLC with a gradient of 10–80 % MeCN/water with 0.07% TFA (t_R = 29.2 min) and lyophilized, and taken directly to the next step without further characterization. The intermediate (2.70 mg, 0.002 mmol) was taken up in 50% TFA in CH_2Cl_2 (0.2 mL) and stirred at 20 °C. After 15 min, the volatiles were removed with a gentle stream of N_2 and residual TFA was azetroped with CH_2Cl_2 (3 × 5 mL). The residue was purified by reverse-phase HPLC using a gradient of 5–40% MeCN/water with 0.07% TFA (t_R = 24.1 min) and afforded the bis-TFA salt of **9** (2.3 mg, theoretical 2.5 mg, 92%) as white powder after lyophilization: ^{1}H NMR ($(CD_3)_2SO$, 600 MHz) δ 9.55 (s, 1H), 9.42 (s, 1H), 9.13 (s, 1H), 9.04–9.00 (m, 2H), 8.65 (s, 1H), 8.55 (d, 3H, *J* = 3.6 Hz), 7.84 (s, 1H), 7.57 (d, 1H, *J* = 8.4 Hz), 7.45 (d, 3H, *J* =

8.4 Hz), 7.25 (t, 2H, J = 8.4 Hz), 7.18 (s, 1H), 7.13 (s, 1H), 7.10 (s, 1H), 7.04–6.97 (m, 3H), 6.76 (dd, 1H, J = 1.8, 8.4 Hz), 6.70 (d, 3H, J = 8.4 Hz), 6.50 (br s, 4H), 6.38 (d, 1H, J = 1.8 Hz), 6.25 (d, 1H, J = 1.8 Hz), 5.94 (br s, 1H), 5.88 (br s, 1H), 5.69 (d, 1H, J = 7.8 Hz), 5.63 (br s, 1H), 5.17 (s, 1H), 5.15 (s, 1H), 5.11 (br s, 1H), 4.88 (br s, 1H), 4.44–4.42 (m, 2H), 4.27 (br s, 2H), 4.16 (d, 1H, J = 11.4 Hz), 3.08 (dd, 1H, J = 5.4, 14.4 Hz), 2.81 (dd, 1H, J = 7.8, 13.8 Hz), 2.54 (s, 3H), 2.22 (dd, 1H, J = 7.8, 15.6 Hz); HR ESI-TOF *m/z* 1192.2870 (M + H^+, $C_{56}H_{52}Cl_2N_9O_{17}^+$, requires 1192.2853); $[\alpha]^{25}_D$ +25 (*c* 0.2, CH_3OH).



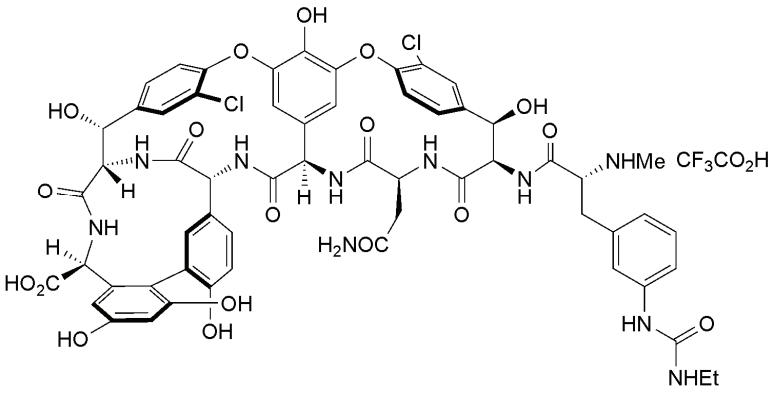
(*R*)-Methyl 2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-(3-ethylureido)phenyl)propanoate (25)

Ethylisocyanate (74 μL, 0.94 mmol) was added dropwise to a stirred solution of **20** (0.15 g, 0.47 mmol) in anhydrous CH_2Cl_2 (5.8 mL). After 15 h, the volatiles were removed in vacuo. Column chromatography (18 g SiO_2, 50% EtOAc/hexanes) provided **25** (0.17 g, theoretical 0.18 mg, 97%) as a yellow oil (rotomer ratio in $CDCl_3$ A/B = 3:4): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.21–7.13 (m, 8H), 6.98 (br s, 1H), 6.83–6.62 (m, 1H), 5.65 (br s, 1H), 5.32 (br s, 1H), 5.02 (dd, $1H_B$, J = 4.8. 10.8 Hz), 4.50 (d, $1H_A$, J = 7.2 Hz), 3.73 (s, $3H_A$), 3.72 (s, $3H_B$), 3.27–3.20 (m, 6H), 2.96–2.88 (m, 2H), 2,71 (s, 6H), 1.31 (s, 18H), 1.15–1.10 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.5/171.4, 156.2/156.1, 155.9/155.1, 139.4/138.8, 138.7/138.1, 129.2, 124.2/123.6, 121.5/120.7, 119.3/118.3, 80.4/80.2, 61.6/59.0, 52.23/52.20, 35.5/35.0, 34.95/34.91, 32.7/31.4, 28.2/28.1 (3C), 15.4; IR (neat) ν_{max} 3333*w*, 1741*w*, 1651*s*, 1610*w*, 1555*m*, 1482*m*, 1436*m*, 1392*w*, 1225*m*, 1143*s*, 910*m* cm^{-1}; HR ESI-TOF *m/z* 380.2183 (M + H^+, $C_{19}H_{30}N_3O_5^+$, requires 380.2180); $[\alpha]^{25}_D$ +70 (*c* 0.5, CH_2Cl_2).



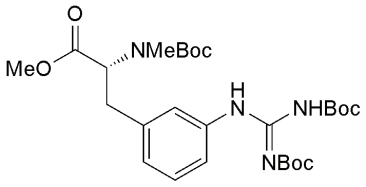
(*R*)-2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-(3-ethylureido)phenyl)propanoic Acid (26)

An aqueous solution of 0.2 N LiOH (3.95 mL) was added to a solution of **25** (100 mg, 0.263 mmol) in THF (3.75 mL) according to *GP2*. Column chromatography (10 g SiO_2, 5–10% MeOH/EtOAc with 1% AcOH) provided **26** (84 mg, theoretical 96 mg, 88%) as an off-white foam (rotomer ratio in acetone-D_6 A/B = 3:4): ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 7.90 (br s, $1H_B$), 7.88 (br s, $1H_A$), 7.42 (br s, 1H), 7.35–7.32 (m, 3H), 7.15–7.12 (m, 2H), 6.83 (d, 2H, J = 7.2 Hz), 5.86 (br s, $1H_A$), 5.80 (br s, $1H_B$), 4.94 (dd, 1H, J = 4.8, 11.4 Hz), 4.72 (dd, 1H, J = 3.6, 10.8 Hz), 3.26–3.22 (m, 6H), 3.03–2.96 (m, 2H), 2.75 (s, $3H_A$), 2.72 (s, $3H_B$), 1.34 (s, $9H_A$), 1.31 (s, $9H_B$), 1.11–1.08 (m, 6H) (CO_2H not observed); ^{13}C NMR ($(CD_3)_2CO$, 150 MHz) δ 174.0/173.9, 157.4/157.3, 157.2/156.5, 142.6/142.4, 140.8/140.5, 130.4/130.3, 124.1/124.0, 120.9/120.7, 118.5/118.2, 81.0/80.9, 62.8/61.3, 37.2/36.3, 36.2, 33.4/33.3, 29.4/29.3 (3C), 16.9; IR (neat) ν_{max} 3348*w*, 1654*s*, 1609*w*, 1557*s*, 1484*m*, 1442*m*, 1392*m*, 1243*s*, 1162*s* cm^{-1}; HR ESI-TOF *m/z* 366.2026 (M + H^+, $C_{18}H_{28}N_3O_5^+$, requires 366.2023); $[\alpha]^{25}_D$ +63 (*c* 0.5, CH_3OH).



1-[3-(3-Ethylureido)-D-phenylalanyl]desleucylaglucovancomycin Trifluoroacetate (10)

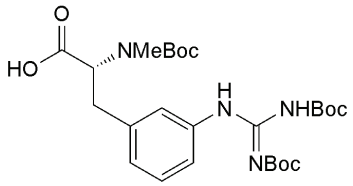
The title compound was prepared from **26** (5.17 mg, 0.015 mmol), and dicyclohexylcarbodiimide (1.46 mg, 0.007 mmol) in CH_2Cl_2 (100 μL), followed by treatment with $NaHCO_3$ (1.19 mg, 0.015 mmol), desleucylaglucovancomycin (**16**, 4.00 mg, 0.004 mmol) in DMF (200 μL) according to *GP3*. The protected intermediate was isolated as a white solid (3.3 mg, theoretical 5.6 mg, 59%) by reverse-phase HPLC with a gradient of 35–95% MeCN/water with 0.07% TFA (t_R = 19.9 min), and lyophilized, and was taken directly to the next step without further characterization. The intermediate (3.30 mg, 0.002 mmol) was taken up in 50% TFA in CH_2Cl_2 (0.2 mL) and stirred at 20 °C. After 10 min, the volatiles were removed with a gentle stream of N_2 and residual TFA was azetroped with CH_2Cl_2 (3 × 5 mL). The residue was purified by reverse-phase HPLC using a gradient of 10–60% MeCN/water with 0.07% TFA (t_R = 20.6 min) and afforded the TFA salt of **10** (2.50 mg, theoretical 3.30 mg, 75%) as white powder after lyophilization: ^{1}H NMR (CD_3OD, 600 MHz) δ 7.82 (br s, 2H), 7.65 (s, 1H), 7.62 (br s, 2H), 7.56 (d, 1H, *J* = 8.4 Hz), 7.28 (t, 1H, *J* = 7.8 Hz), 7.19 (d, 1H, *J* = 6.6 Hz), 7.07 (s, 1H), 7.03 (d, 2H, *J* = 6.6 Hz), 6.57 (br s, 3H), 6.40 (d, 1H, *J* = 1.2 Hz), 6.12 (br s, 1H), 5.93 (br s, 1H), 5.41 (s, 1H), 5.38 (s, 1H), 5.30 (d, 1H, *J* = 2.4 Hz), 4.80 (br s, 2H), 4.26–4.22 (m, 2H), 4.14 (s, 1H), 3.19–3.14 (m, 4H), 2.97 (d, 1H, *J* = 15.6 Hz), 2.88–2.83 (m, 1H), 2.64 (s, 3H), 2.01 (br s, 1H), 1.11 (t, 3H, *J* = 7.2 Hz); ESI-TOF HRMS *m*/*z*: 1263.3225 (M + H$^+$, $C_{59}H_{57}Cl_2N_{10}O_{18}^+$, requires 1263.3224); $[\alpha]^{25}_D$ +45 (*c* 0.1, CH_3OH).



(*R*)-Methyl 3-(3-(2,3-Bis(*tert*-butoxycarbonyl)guanidino)phenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)propanoate (28)

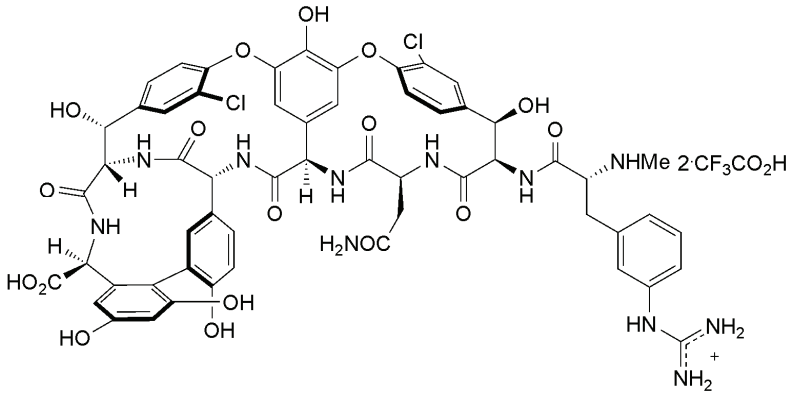
Amine **20** (100 mg, 0.325 mmol) was added to a solution of *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine (**35**, 100 mg, 0.325 mmol) in anhydrous CH_3CN (1.63 mL) and the mixture was warmed at 80 °C. After 2 h, the volatiles were removed in vacuo. Column chromatography (20 g SiO_2, 0–10 % MeOH/CH_2Cl_2) provided **28** (85.0 mg, theoretical 179 mg, 47%) as a white foam (rotomer ratio in $CDCl_3$ A/B = 10:11): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 11.63 (s, 2H), 10.33 (d, 2H, *J* = 8.4 Hz), 7.57 (d, 1H$_A$, *J* = 7.8 Hz), 7.54 (d, 1H$_B$, *J* = 7.8 Hz), 7.43 (s, 1H$_B$), 7.37 (s, 1H$_A$), 7.29–7.25 (m, 2H), 6.99 (d, 1H$_A$, *J* = 7.2 Hz), 6.94 (d, 1H$_B$, *J* = 7.8 Hz), 4.89 (dd, 1H$_A$, *J* = 4.8, 10.2 Hz), 4.51 (d, 1H$_B$, *J* = 6.0 Hz), 3.76 (s, 6H), 3.30 (dd, 2H, *J* = 4.8, 13.8 Hz), 3.04 (dd, 2H, *J* = 10.8, 13.4 Hz), 2.75 (s, 6H), 1.55–1.50 (m, 36H), 1.39 (s, 18H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.8/171.6, 163.5, 155.8/154.9, 153.4, 153.3, 138.4/138.1, 137.0/136.8, 129.02/128.96, 125.3/125.2, 122.6/122.5, 120.39/120.36, 83.7, 80.3/80.0, 79.5, 61.6/59.5, 35.5/34.8, 33.1/32.2, 29.7/29.2, 28.2 (3C), 28.1 (3C), 28.0 (3C); IR (neat) 3270*w*, 2977*w*, 2361*w*, 1699*m*, 1634*m*, 1394*m*, 1367*m*, 1334*m*, 1300*m*, 1246*m*, 1149*s*, 1109*m*, 1057*w* cm^{-1}; HR ESI-TOF *m/z* 573.2895 (M +

Na^+, $C_{27}H_{42}N_4NaO_8^+$, requires 573.2895) ; $[\alpha]^{25}_D$ +36 (*c* 0.5, CH_2Cl_2).



(*R*)-3-(3-(2,3-Bis(*tert*-butoxycarbonyl)guanidino)phenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)propanoic Acid (29)

An aqueous solution of 0.2 N LiOH (1.08 mL) was added to a solution of **28** (80 mg, 0.07 mmol) in THF (1.0 mL) according to *GP2*. Column chromatography (5 g SiO_2, 100% EtOAc) provided **29** (52 mg, theoretical 78 mg, 66%) as a clear oil (rotomer ratio in acetone-D_6 A/B = 3:4): ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 11.75 (br s, 2H), 10.29 (d, 2H, *J* = 9.6 Hz), 7.70–7.67 (m, 2H), 7.52–7.48 (m, 2H), 7.31–7.26 (m, 2H), 7.08 (d, 2H, *J* = 7.2 Hz), 4.90 (d, $1H_A$, *J* = 7.8 Hz), 4.76 (d, $1H_B$, *J* = 8.4 Hz), 3.32–3.30 (m, 2H), 3.12–3.05 (m, 2H), 2.79 (s, $3H_A$), 2.74 (s, $3H_B$), 1.53–1.47 (m, 36H), 1.34 (s, 18H) (CO_2H protons not observed); ^{13}C NMR ($(CD_3)_2CO$, 150 MHz) δ 174.4, 157.4, 156.5, 155.3, 153.2, 141.3/141.1, 139.1/139.0, 130.5/130.4, 127.3/127.2, 124.5/124.4, 122.0/121.9, 83.8, 80.9, 80.7, 62.8/61.5, 37.1/36.4, 33.6, 29.5/29.4 (3C), 29.2 (3C), 29.1 (3C); IR (neat) ν_{max} 3270*w*, 2977*w*, 1718*w*, 1696*w*, 1638*s*, 1480*w*, 1394*m*, 1368*m*, 1331*m*, 1299*m*, 1247*m*, 1149*s*, 1109*m*, 1058*w*, 777*w* cm^{-1}; HR ESI-TOF *m/z* 537.2924 ($M + H^+$, $C_{26}H_{41}N_4O_8^+$, requires 537.2919); $[\alpha]^{25}_D$ +33 (*c* 0.5, CH_3OH).

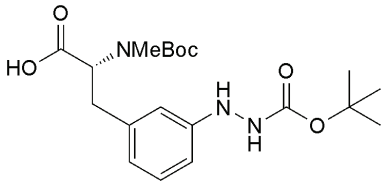


1-[(3-Guanudyl)-D-phenylalanyl]desleucylaglucovancomycin Bis(trifluoroacetate) (11)

The title compound was prepared starting from **29** (7.60 mg, 0.015 mmol), and dicyclohexylcarbodiimide (1.46 mg, 0.007 mmol) in CH_2Cl_2 (100 μL), followed by treatment with $NaHCO_3$ (1.19 mg, 0.015 mmol), desleucylaglucovancomycin (**16**, 4.00 mg, 0.004 mmol) in DMF (200 μL) according to *GP3*. The protected intermediate was isolated as a white solid (2.70 mg, theoretical 5.40 mg, 50%) by reverse-phase HPLC with a gradient of 35–95 % MeCN/water with 0.07% TFA (t_R = 19.7 min), and lyophilized, and was taken directly to the next step without further characterization. The residue was taken up in 50% TFA in CH_2Cl_2 (0.2 mL) and stirred at 20 °C. After 15 min, the volatiles were removed with a gentle stream of N_2 and residual TFA was azetroped with CH_2Cl_2 (3 × 5 mL). Purification by reverse-phase HPLC using a gradient of 5–40% MeCN/water with 0.07% TFA (t_R = 24.2 min) and afforded the bis-TFA salt of **11** (2.70 mg, theoretical 5.10 mg, 53%) as a white powder: ^{1}H NMR (CD_3OD, 600 MHz) δ 8.99 (d, 1H, NH, *J* = 4.8 Hz), 8.84 (d, 1H, NH, *J* = 5.4 Hz), 8.81 (br s, 1H), 7.82 (br s, 1H), 7.78 (s, 1H), 7.63 (br s, 1H), 7.56 (s, 1H), 7.47 (d, 1H, *J* = 7.8 Hz), 7.35 (t, 1H, *J* = 7.2 Hz), 7.21–7.12 (m, 5H), 6.92 (br s, 1H), 6.86 (br s, 1H), 6.72 (d, 1H, *J* = 8.4 Hz), 6.44 (d, 1H, *J* = 2.4 Hz), 6.42 (d, 1H, *J* = 1.8 Hz), 6.08 (br s, 1H), 5.97 (br s, 1H), 5.45 (s, 1H), 5.31 (s, 1H), 5.16 (s, 1H), 4.80 (d, 1H, *J* = 6.0 Hz), 4.73 (s, 1H), 4.30–4.18 (m, 3H), 3.23–3.21 (m, 1H), 2.84 (d, 1H, *J* = 16.2 Hz),

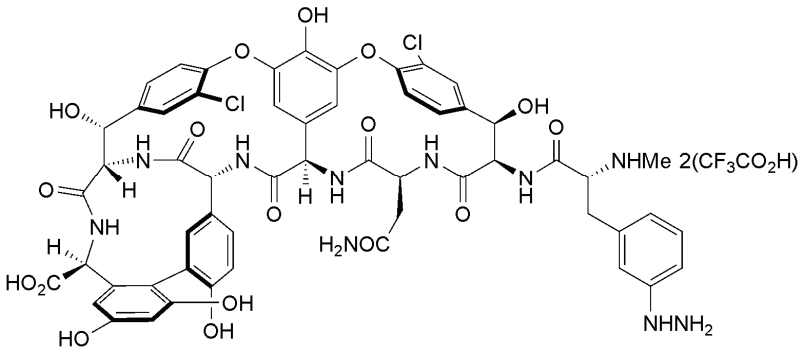
2.77 (s, 3H), 1.84 (br s, 1H); ESI-TOF HRMS *m/z* 1234.3011 (M + H^+, $C_{57}H_{54}Cl_2N_{11}O_{17}^+$, requires 1234.3071); $[\alpha]^{25}_D$ +22 (*c* 0.5, CH_3OH).

Analogues modified at the *N*-terminus for covalent attachment to Ac_2-L-Lys-D-Ala-D-Lac (3)



(*R*)-2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-(2-(*tert*-butoxycarbonyl)hydrazinyl)phenyl)propanoic Acid (30)

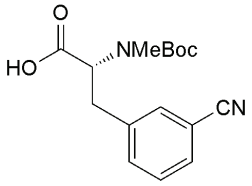
A solution of oxaziridine **31**[15] (0.234 g, 0.811 mmol) in anhydrous CH_2Cl_2 (2.35 mL) was added dropwise to a solution of **20** (0.250 g, 0.811 mmol) in CH_2Cl_2 (2.35 mL) at 20 °C. After 18 h, the volatiles were removed in vacuo. Column chromatography (15 g SiO_2, 50% Et_2O/hexanes) provided the crude hydrazine **32** (80 mg, 23%) as a pale yellow foam. The residue (70 mg, 0.16 mmol) was taken up in THF (2.35 mL) at 0 °C and treated with a solution of aqueous 0.2 N LiOH (2.4 mL, 0.48 mmol) and was allowed to stir for 1.75 h at 0 °C according to *GP2*. Column chromatography (7 g SiO_2, 5–10% MeOH/CH_2Cl_2 with 0.1% CH_3CO_2H) afforded **30** (43 mg, 66 mg theoretical, 65%) as a pale yellow foam (rotomer ratio A/B = 6:5): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.15 (t, 2H, *J* = 7.8 Hz), 6.74–6.66 (m, 6H), 6.50 (br s, 2H), 5.77 (br s, 4H), 4.72 (br s, $1H_B$), 4.52 (br s, $1H_A$), 3.24 (br s, 2H), 3.11–2.95 (m, 2H), 2.75 (s, $3H_B$), 2.68 (s, $3H_A$), 1.46 (s, 18H), 1.42 (s, 9H), 1.37 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 175.4/175.1, 156.4, 155.0, 148.54/148.47, 138.7/138.3, 129.4/129.2, 121.4, 113.6, 111.4, 81.44/81.42, 80.69/80.64, 61.6/60.9, 35.5/34.8, 33.2/32.9, 28.4 (6C); IR (neat) ν_{max} 3309*w* (br), 2977*m*, 2931*w*, 1679*s*, 1610*m*, 1480*m*, 1452*m*, 1392*m*, 1368*m*, 1250*s*, 1157*s* cm^{-1}; HR ESI-TOF *m/z* 410.2287 (M + H^+, $C_{20}H_{32}N_3O_6^+$, requires 410.2286); $[\alpha]^{25}_D$ +56 (*c* 0.5, CH_2Cl_2).



1-[(3-Hydrazino)-D-phenylalanyl]desleucylaglucovancomycin Bis(trifluoroacetate) (13)

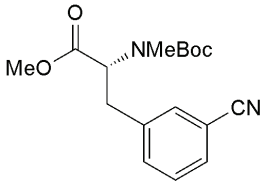
The title compound was prepared starting from **30** (11.7 mg, 0.029 mmol), and dicyclohexyl carbodiimide (2.95 mg, 0.014 mmol) in CH_2Cl_2 (205 μL), followed by treatment with $NaHCO_3$ (2.41 mg, 0.029 mmol), desleucylaglucovancomycin (**16**, 11.0 mg, 0.009 mmol) in DMF (0.514 mL) according to *GP3*. The protected intermediate was isolated as a white solid (9.80 mg, theoretical 12.6 mg, 77%) by reverse-phase HPLC with a gradient of 10–80% MeCN/water with 0.07% TFA (t_R = 28.5 min), and lyophilized, and was taken directly to the next step without further characterization. The intermediate (2.50 mg, 0.002 mmol) was taken up in 10% TFA in CH_2Cl_2 (0.1 mL) and stirred at 0 °C. After 30 min, the volatiles were removed with a gentle stream of N_2 and residual TFA was azetroped with CH_2Cl_2 (3 × 5 mL). The residue was purified by reverse-phase HPLC using a gradient of 5–40%

MeCN/water with 0.07% TFA (t_R = 23.7 min) and afforded the bis-TFA salt of **13** (1.6 mg, theoretical 2.5 mg, 64%) as an off-white powder: ^{1}H NMR (CD_3OD, 600 MHz) δ 8.98 (d, 1H, NH, *J* = 3.0 Hz), 8.88 (br s, 1H, NH), 7.76 (br s, 2H), 7.66 (br s, 1H), 7.57–7.54 (m, 2H), 7.31 (t, 1H, *J* = 7.8 Hz), 7.21 (d, 1H, *J* = 7.8 Hz), 7.10 (s, 1H), 7.03 (d, 1H, *J* = 7.2 Hz), 6.84 (dd, 1H, *J* = 1.8, 8.4 Hz), 6.76–6.70 (m, 3H), 6.47 (s, 1H), 6.44 (d, 1H, *J* = 1.8 Hz), 6.01 (br s, 1H), 5.87 (br s, 1H), 5.38 (s, 1H), 5.36 (s, 1H), 5.28 (d, 1H, *J* = 2.4 Hz), 4.79–4.77 (m, 1H), 4.33 (br s, 1H), 4.27 (d, 1H, *J* = 8.4 Hz), 4.18 (br s, 1H), 3.22 (dd, 1H, *J* = 6.0, 13.8 Hz), 2.95 (d, 1H, *J* = 15.6 Hz), 2.70 (s, 3H), 2.08 (br s, 1H) (three signals buried); ESI-TOF HRMS *m*/*z* 1207.2907 (M + H$^+$, $C_{56}H_{53}Cl_2N_{10}O_{17}^+$, requires 1207.2962); $[\alpha]^{25}_D$ +18 (*c* 0.1, CH_3OH).



(*R*)-2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-cyanophenyl)propanoic Acid (34)

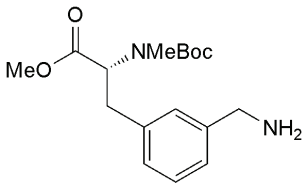
The title compound was prepared from Boc-D-3-cyanophenylalanine (**33**, 1.00 g, 3.44 mmol) with NaH (454 mg, 11.4 mmol), iodomethane (643 μL, 10.3 mmol), and anhydrous THF (11.5 mL), and DMF (546 μL) according to *GP1*. Column chromatography (10 g SiO_2, 0–0.2% AcOH/EtOAc) provided **34** (1.01 g, theoretical 1.05 g, 97%) as a colorless opaque oil (rotomer ratio in $CDCl_3$ A/B = 8:5): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.55–7.50 (m, 6H), 7.43–7.41 (m, 2H), 4.80 (dd, 1H, *J* = 3.6, 9.0 Hz), 4.64 (d, 1H, *J* = 7.2 Hz), 3.37–3.34 (m, 2H), 3.16 (t, 1H, *J* = 12.0 Hz), 3.08 (t, 1H, *J* = 12.0 Hz), 2.77 (s, $3H_B$), 2.71 (s, $3H_A$), 1.41 (s, $9H_A$), 1.36 (s, $9H_B$) (CO_2H not observed); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.9/174.7, 156.2/154.7, 139.0/138.8, 133.6/133.5, 132.5, 130.6, 129.5/129.3, 118.7/118.6, 112.7/112.6, 81.2, 60.8/60.3, 34.9/34.3, 33.1/32.5, 28.2 (3C); IR (neat) ν_{max} 2976*w*, 2230*w*, 1684*s*, 1481*m*, 1447*m*, 1391*m*, 1367*m*, 1322*m*, 1252*m*, 1142*s*, 1074*m* cm^{-1}; ESI-TOF HRMS *m*/*z* 327.1312 (M + Na$^+$, $C_{16}H_{20}N_2NaO_4^+$, requires 327.1315); $[\alpha]^{25}_D$ +65 (*c* 0.5, CH_2Cl_2).



(*R*)-Methyl 2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-cyanophenyl)propanoate (35)

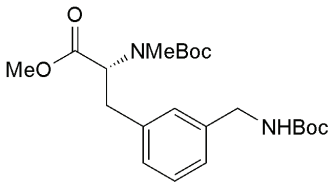
A solution of **34** (600 mg, 1.97 mmol) in anhydrous DMF (6.6 mL) at 0 °C was treated with oven-dried $KHCO_3$ (374 mg, 3.94 mmol) followed by iodomethane (171 μL, 2.76 mmol). After 1 h at 0 °C, the mixture was gently warmed to ambient temperature. After 18 h, the reaction was quenched with water (10 mL), and the mixture was extracted with EtOAc (100 mL), the organic was discarded. The aqueous phase was acidified to pH of 2–3 with aqueous 0.2 N $NaHSO_4$ and extracted with EtOAc (3 × 200 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated. Column chromatography (75 g SiO_2, 20–60% hexanes/EtOAc) provided **35** (617 mg, theoretical 626 mg, 99%) as a white solid (rotomer ratio in $CDCl_3$ A/B = 6:5): mp 83–84 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.54–7.39 (m, 6H), 7.41–7.39 (m, 2H), 4.90 (dd, $1H_A$, *J* = 5.4, 10.2 Hz), 4.55 (dd, $1H_B$, *J* = 3.6, 9.6 Hz), 3.77 (s, $3H_B$), 3.75 (s, $3H_A$), 3.36–3.32 (m, 2H), 3.08–3.03 (m, 2H), 2.73 (s, $3H_B$), 2.71 (s, $3H_A$), 1.38 (s, $9H_A$), 1.35 (s, $9H_B$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.2/171.9, 155.7/154.7, 139.3/139.1, 133.7/133.6, 132.5, 130.5/130.4, 129.3/129.2, 118.8/118.6, 112.7/112.4, 80.7/80.5, 61.1, 59.3, 35.1/34.6, 32.5/32.0, 28.2 (3C); IR (neat) ν_{max} 2976*w*, 2230*w*, 1741*s*, 1689*s*, 1481*m*, 1436*m*, 1391*m*,

1367*m*, 1322*m*, 1223*m*, 1141*s*, 734*s* cm^{-1}; ESI-TOF HRMS *m/z* 341.1476 ($M + Na^+$, $C_{17}H_{22}N_2NaO_4^+$, requires 341.1472); $[\alpha]^{25}_D$ +68 (*c* 1.0, CH_2Cl_2).



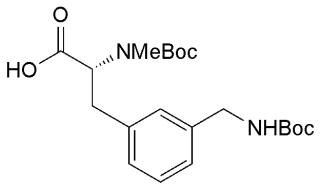
(*R*)-Methyl 3-(3-(Aminomethyl)phenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)propanoate (36)

An aqueous solution of Raney-Ni (cat.) was added to an oxygen degassed solution of **35** (300 mg, 0.940 mmol) in MeOH (9.4 mL) and aqueous ammonium hydroxide (1.4 mL). The mixture was vigorously stirred and placed under an atmosphere of H_2 gas (balloon). After 18 h, the solids were removed by filtration over Celite, and washed with MeOH. The filtrate was concentrated in vacuo. The residue was taken up in water (10 mL) and extracted with EtOAc (3 × 200 mL). The combined organics were dried over Na_2SO_4, filtered, and the volatiles removed in vacuo providing **36** (293 mg, theoretical 303 mg, 97%) as a deep yellow oil (rotomer ratio in $CDCl_3$ A/B = 10:11): ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 7.26–7.05 (m, 8H), 4.89 (dd, $1H_A$, *J* = 4.5, 10.0 Hz), 4.58 (dd, $1H_B$, *J* = 4.0, 10.5 Hz), 3.84 (br s, 4H), 3.73 (s, $3H_B$), 3.71 (s, $3H_A$), 3.26 (d, 2H, *J* = 14.5 Hz), 2.99 (dd, 2H, *J* = 11.0, 14.5 Hz), 2.68 (s, 6H), 1.69 (br s, 4H), 1.35 (s, $9H_A$), 1.30 (s, $9H_B$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.0/172.8, 157.6/156.9, 141.0/140.5, 139.8/139.6, 130.0/129.9, 129.7, 129.57/129.55, 127.3, 81.9/81.5, 63.2/61.5, 52.84/52.77, 45.9/45.8, 36.3/35.7, 33.7/33.1, 28.6/28.5 (3C); IR (neat) ν_{max} 2975*w*, 1740*m*, 1688*s*, 1608*w*, 1480*w*, 1437*m*, 1391*m*, 1324*m*, 1222*m*, 1140*s*, 773*m*, 733*m*, 701*m* cm^{-1}; HR ESI-TOF *m/z* 323.1965 ($M + H^+$, $C_{17}H_{27}N_2O_4^+$, requires 323.1965); $[\alpha]^{25}_D$ +56 (*c* 1.0, CH_2Cl_2).



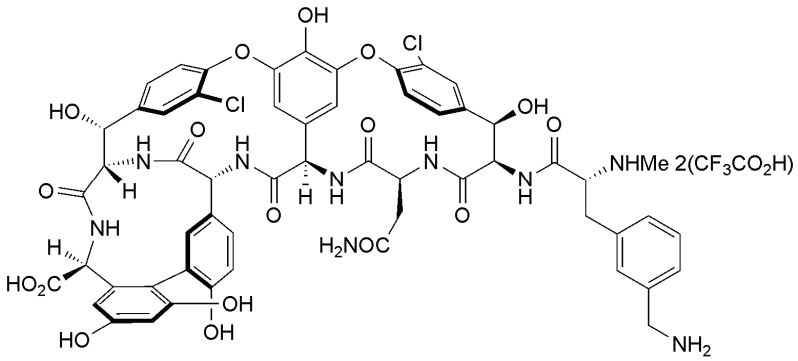
(*R*)-Methyl 2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-((*tert*-butoxycarbonylamino)methyl)phenyl)propanoate (37)

A solution of di-*tert*-butyl dicarbonate (30 μL, 0.13 mmol) in $CHCl_3$ (250 μL) was added to a solution of **36** (41 mg, 0.13 mmol) in $CHCl_3$ (520 μL) and the solution was stirred at 20 °C. After 24 h, the solution was diluted with $CHCl_3$ (5 mL) and washed with 0.2 N $NaHSO_4$, saturated aqueous $NaHCO_3$, water, and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. Column chromatography (10 g SiO_2, 30–100% hexanes/EtOAc) provided **37** (rotomer ratio in $CDCl_3$ A/B = 10:11) as a pale yellow oil (47 mg, theoretical 55 mg, 86%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.25–7.23 (m, 2H), 7.15–7.06 (m, 6H), 4.93 (dd, $1H_A$, *J* = 5.5, 10.5 Hz), 4.82 (br s, 2H), 4.52 (dd, $1H_B$, *J* = 4.0, 10.0 Hz), 4.28 (br s, 4H), 3.74 (s, $3H_B$), 3.73 (s, $3H_A$), 3.31–3.24 (m, 2H), 2.99 (dd, 2H, *J* = 11.0, 14.5 Hz), 2.71 (s, $3H_B$), 2.69 (s, $3H_A$), 1.45 (s, 18H), 1.36 (s, $9H_A$), 1.33 (s, $9H_B$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.8/171.5, 155.8 (2C), 139.2/138.9, 138.1/137.8, 128.8/128.7 (2C), 128.1/128.0, 125.8, 80.3/80.0, 79.4, 61.5/59.4, 52.2, 44.6, 35.5/34.9, 32.6/31.9, 28.4 (3C), 28.2 (3C); IR (film) ν_{max} 3366w, 2975w, 2361w, 1742w, 1691s, 1512w, 1450w, 1392w, 1366w, 1328w, 1248m, 1164s, 1046w, 776w cm^{-1}; HRESI-TOF *m/z* 423.2496 ($M + H^+$, $C_{22}H_{35}N_2O_6^+$, requires 423.2490); $[\alpha]^{25}_D$ +46 (*c* 1.0, CH_2Cl_2).



(*R*)-2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-((*tert*-butoxycarbonylamino)methyl)phenyl)propanoic Acid (38)

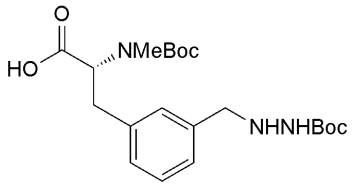
The title compound was prepared from **37** (58.0 mg, 0.136 mmol), 0.2 N aqueous LiOH (1.36 ml), and THF (1.94 mL) according to *GP2*. The mixture was stirred for 3 h at 0 °C. Column chromatography (5 g SiO_2, 50% EtOAc/hex) afforded **38** (rotomer ratio in acetone-D_6 A/B = 10:12) as a white foam (49.8 mg, theoretical 55.5 mg, 88%): ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 7.25–7.15 (m, 10H), 6.40 (br s, 1H), 6.38 (br s, 1H), 4.91 (dd, 1H_A, J = 3.6, 10.2 Hz,), 4.71 (d, 1H_B, J = 10.2 Hz), 4.25 (br s, 4H), 3.30–3.26 (m, 2H), 3.10–3.03 (m, 2H), 2.72 (s, 3H_A), 2.70 (s, 3H_B), 1.42 (s, 18H), 1.36 (s, 9H_A), 1.32 (s, 9H_B); ^{13}C NMR ($(CD_3)_2CO$, 150 MHz) δ 173.6/173.5, 157.8/157.3, 156.4, 142.2/142.0, 140.3/140.1, 130.2/130.1, 129.9/129.8, 129.4/129.3, 127.12/127.09, 81.0/80.8, 79.7, 62.8/61.3, 45.8, 37.0/36.4, 33.6/33.5, 29.6 (3C), 29.5/29.4 (3C); IR (film) ν_{max} 2976*w*, 1686*s*, 1515*w*, 1480*w*, 1450*m*, 1392*m*, 1366*m*, 1251*m*, 1162*s* cm^{-1}; ESI-TOF HRMS *m*/*z* 409.2337 (M + H$^+$, $C_{21}H_{33}N_2O_6^+$, requires 409.2333); $[\alpha]^{25}_D$ +45 (*c* 0.5, CH_2Cl_2).



1-[(3-Aminomethyl)-D-phenylalanyl]desleucylaglucovancomycin Bis(trifluoroacetate) (12)

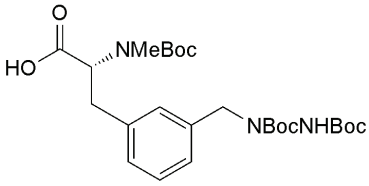
The title compound was prepared starting from **38** (8.65 mg, 0.021 mmol), and dicyclohexylcarbodiimide (2.18 mg, 0.014 mmol), in CH_2Cl_2 (0.150 mL), followed by treatment with $NaHCO_3$ (1.78 mg, 0.021 mmol), desleucylaglucovancomycin (**16**, 10.0 mg, 0.009 mmol) in DMF (0.30 mL) according to *GP3*. The protected intermediate was isolated as a white solid (9.80 mg, theoretical 12.6 mg, 77%) by reverse-phase HPLC with a gradient of 35–95% MeCN/water with 0.07% TFA (t_R = 13.9 min), and lyophilized, and was taken directly to the next step without further characterization. The intermediate (9.80 mg, 0.007 mmol) was taken up in 10% TFA in CH_2Cl_2 (0.2 mL) and stirred at 0 °C. After 30 min, the volatiles were removed with a gentle stream of N_2 and residual TFA was azetroped with CH_2Cl_2 (3 × 5 mL). The residue was purified by reverse-phase HPLC using a gradient of 5–40% MeCN/water with 0.07% TFA (t_R = 23.3 min) and afforded the bis-TFA salt of **12** (7.8 mg, theoretical 12.6 mg, 62%): ^{1}H NMR (CD_3OD, 600 MHz) δ 9.04 (br s, 1H, NH), 8.91 (d, 1H, NH, J = 5.4 Hz) 8.72 (br s, 1H, NH), 7.77 (br s, 1H), 7.70 (s, 1H), 7.66 (s, 1H), 7.53 (d, 1H, J = 7.8 Hz), 7.40–7.31 (m, 4H), 7.20 (t, 2H, J = 7.8 Hz), 7.12 (s, 1H), 6.81 (br s, 1H), 6.72 (d, 1H, J = 8.4 Hz), 6.46 (s, 1H), 6.44 (d, 1H, J = 2.4 Hz), 6.12 (br s, 1H), 5.92 (br s, 1H), 5.41 (s, 1H), 5.34 (s, 1H), 5.21 (d, 1H, J = 2.4 Hz), 4.84 (d, 1H, J = 6.0 Hz), 4.77 (d, 1H, J = 4.8 Hz) 4.30 (t, 1H, J = 7.8 Hz), 4.25 (s, 1H), 4.23 (s, 1H), 4.10 (s, 2H), 3.33–3.27 (m, 1H), 3.16–3.12 (m, 1H), 2.84 (d, 1H, J = 16.2 Hz), 2.75 (s, 3H), 1.98 (br s, 1H) (one signal buried below residual solvent); ESI-TOF HRMS *m*/*z* 1206.2988 (M + H$^+$, $C_{57}H_{54}Cl_2N_9O_{17}^+$,

requires 1206.3009); $[\alpha]^{25}_{D}$ +33 (*c* 0.2, CH_3OH).



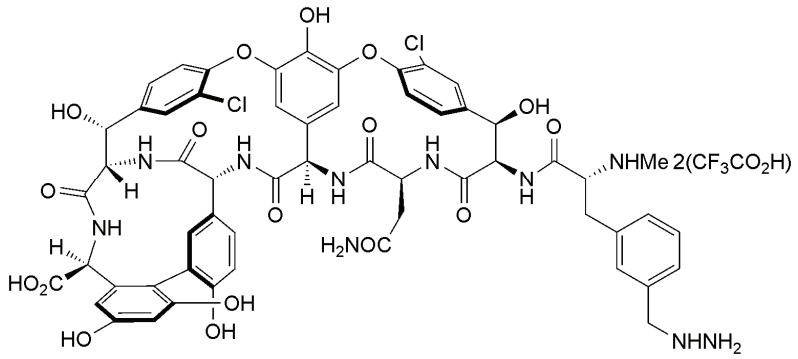
(*R*)-2-(*tert*-Butoxycarbonyl(methyl)amino)-3-(3-((2-(*tert*-butoxycarbonyl)hydrazinyl)methyl)phenyl)propanoic Acid (40)

A solution of oxaziridine **31**[15] (67.0 mg, 0.232 mmol) in anhydrous toluene (0.8 mL) was added dropwise over a 15 min period to a solution of **36** (75.0 mg, 0.232 mmol) in anhydrous toluene (0.8 mL) at 0 °C. After stirring for a total of 30 min at 0 °C, the volatiles were removed in vacuo. Column chromatography (15 g SiO_2, 50-60% EtOAc/hexanes) afforded **39** (58 mg, theoretical 100 mg, 58%) as a yellow oil. The residue (58 mg, 0.133 mmol) was taken up in THF (1.9 mL) and treated with aqueous 0.2 N LiOH (2.0 mL, 0.44 mmol) at 0 °C, according to *GP2*. Column chromatography (7 g, SiO_2 50–100% EtOAc/hexanes) provided **40** (35.0 mg, theoretical 56.3 mg, 62%) as a white foam (rotomer ratio in acetone-D_6 A/B = 3:4): ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 7.31 (s, 2H), 7.27–7.14 (m, 8H), 4.93 (dd, $1H_A$, *J* = 4.8, 11.4 Hz), 4.73 (dd, $1H_B$, *J* = 4.2, 11.4 Hz), 3.97–3.91 (m, 4H), 3.31–3.24 (m, 2H), 3.12–3.04 (m, 2H), 2.71 (s, $3H_B$), 2.71 (s, $3H_A$), 2.08 (d, 2H, *J* = 0.6 Hz), 2.07–2.06 (m, 2H), 1.41 (s, 18H), 1.34 (s, $9H_A$), 1.29 (s, $9H_B$); ^{13}C NMR ($(CD_3)_2CO$, 150 MHz) δ 173.7/173.5, 158.5, 157.2/156.6, 140.6/140.4, 140.1/139.8, 131.1, 130.0/129.9, 129.6/129.5, 128.6/128.5, 81.0/80.8, 80.6/80.5, 61.5/61.3, 56.7/56.5, 36.9/36.3, 33.5, 29.5 (3C), 29.4/29.3 (3C); IR (film) ν_{max} 1687*s*, 1479*m*, 1450*m*, 1391*m*, 1366*s*, 1251*m*, 1246*s*, 1061*w*, 863*w*, 769*m*, 735*m* cm^{-1}; ESI-TOF HRMS *m*/*z* 424.2446 (M + H^+, $C_{21}H_{34}N_3O_6^+$, requires 424.2442); $[\alpha]^{25}_{D}$ +33 (*c* 0.5, CH_2Cl_2).



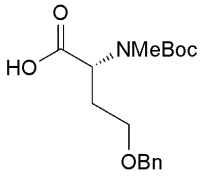
(*R*)-3-(3-((1,2-Bis(*tert*-butoxycarbonyl)hydrazinyl)methyl)phenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)propanoic Acid (41)

$NaHCO_3$ (0.027 g, 0.318 mmol) and di-*tert*-butyl dicarbonate (0.026 mL, 0.117 mmol) were added to a solution of **40** (0.045 g, 0.106 mmol) in 1:1 THF/H_2O (0.265 mL) at 0 °C. The mixture was slowly warmed to room temperature and stirred for 18 h. The solution was then quenched with the addition of water (2 mL) and then acidified to a pH of 2 with aqueous 0.2 N $NaHSO_4$. The mixture was extracted with EtOAc (6 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography (5 g SiO_2 50–100% EtOAc/hexanes) provided **41** (48.0 mg, theoretical 55.5 mg, 87%) as a pale yellow oil (rotomer ratio in CD_3OD A/B = 3:1): ^{1}H NMR (CD_3OD, 500 MHz) δ 7.28–7.14 (m, 8H), 4.60 (dd, 2H, *J* = 3.5, 11.0 Hz), 4.55 (br s, 2H), 3.27 (dd, 4H, *J* = 4.5, 14.5 Hz), 3.05 (dd, 2H, *J* = 11.5, 13.5 Hz,), 2.71 (s, $3H_B$), 2.68 (s, $3H_A$), 1.53–1.30 (m, 54H); ^{13}C NMR ($(CD_3)_2CO$, 150 MHz) δ 173.6/173.4, 157.5, 157.3/156.8, 140.4/140.2, 139.8/139.6, 130.7, 130.5/130.4, 129.8/129.6, 128.1/127.9, 82.3/81.8, 81.5/81.4, 81.2/80.9, 62.9, 56.0/55.9, 54.3/54.3, 36.9/36.4, 33.8/33.6, 29.5 (3C), 29.4 (6C); IR (film) ν_{max} 1679*s*, 1479*m*, 1452*m*, 1392*s*, 1367*s*, 1252*m*, 1150*s*, 1053*w*, 1024*w*, 858*w*, 761*m*, 736*m* cm^{-1}; ESI-TOF HRMS *m*/*z* 524.2961 (M + H^+, $C_{26}H_{42}N_3O_8^+$, requires 524.2966); $[\alpha]^{25}_{D}$ +42 (*c* 0.5, CH_2Cl_2).



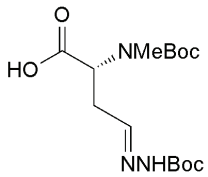
1-[(3-Methylhydrazino)-D-phenylalanyl]desleucylaglucovancomycin Bis(trifluoroacetate) (15)

The title compound was prepared from **41** (11.1 mg, 0.021 mmol), and dicyclohexylcarbodiimide (2.18 mg, 0.011 mmol) in CH_2Cl_2 (0.15 mL), followed by treatment with $NaHCO_3$ (1.78 mg, 0.021 mmol), desleucylaglucovancomycin (**16**, 10.0 mg, 0.009 mmol) in DMF (0.3 mL) according to *GP3*. The protected intermediate was isolated as a white solid (7.50 mg, theoretical 13.8 mg, 55%) by reverse-phase HPLC with a gradient of 35–95% MeCN/water with 0.07% TFA (t_R = 23.0 min), and lyophilized, and was taken directly to the next step without further characterization. The intermediate (3.0 mg, 0.002 mmol) was taken up in 40% TFA in CH_2Cl_2 (0.2 mL) at 0 °C and slowly allowed to room temperature. After 45 min, the volatiles were removed with a gentle stream of N_2 and residual TFA was azetroped with CH_2Cl_2 (3 x 5 mL). The residue was purified by reverse-phase HPLC using a gradient of 5–40% MeCN/water with 0.07% TFA (t_R = 23.6 min) and afforded the bis-TFA salt of **15** (2.60 mg, theoretical 2.75 mg, 94%): ^{1}H NMR (CD_3OD, 600 MHz) δ 8.99 (br s, 1H, NH), 8.23 (br s, 1H, NH), 7.76 (br s, 1H) 7.71 (s, 1H), 7.62 (s, 1H), 7.54–7.50 (m, 2H), 7.35 (d, 1H, *J* = 7.2 Hz), 7.32 (s, 2H), 7.19 (d, 1H, *J* = 7.2 Hz), 7.14 (br s, 1H), 7.10 (s, 1H), 6.86 (br s, 1H), 6.73 (d, 1H, *J* = 6.0 Hz), 6.46 (s, 1H), 6.44 (s, 1H), 6.05 (br s, 1H), 5.88 (br s, 1H), 5.41 (s, 1H), 5.34 (s, 1H), 5.20 (s, 1H), 4.84–4.81 (m, 2H), 4.73 (br s, 1H), 4.33–4.23 (m, 3H), 4.15–4.10 (m, 2H), 2.87 (d, 1H, *J* = 15.0 Hz), 2.77 (s, 3H), 2.01 (br s, 1H) (two signals buried below residual solvent/water peak); ESI-TOF HRMS *m*/*z* 1221.3107 (M + H^+, $C_{57}H_{55}Cl_2N_{10}O_{17}^+$, requires 1221.3118); $[\alpha]^{25}_D$ +17 (*c* 0.2, CH_3OH).



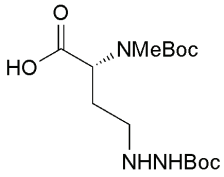
(*R*)-4-(Benzyloxy)-2-(*tert*-butoxycarbonyl(methyl)amino)butanoic Acid (43)

The title compound was prepared from Boc-D-*O*-benzylhomoserine (**42**, 1.00 g, 3.23 mmol), NaH (426 mg, 10.6 mmol), methyl iodide (0.603 mL, 9.69 mmol) in anhydrous THF (10.7 mL), and DMF (0.514 mL) according to *GP1*. Column chromatography (100 g SiO_2, 0–0.2 % AcOH/EtOAc) afforded **43** (1.00 g, theoretical 1.04 g, 96%) as a white solid (rotomer ratio in acetone-D_6 A/B = 1:1): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.36–7.28 (m, 10H), 4.70 (dd, 1H, *J* = 4.8, 8.4 Hz), 4.51 (s, 4H), 4.48 (dd, 1H, *J* = 7.8, 9.0 Hz), 3.60–3.56 (m, 2H), 3.52–3.46 (m, 2H), 2.89 (s, $3H_A$), 2.84 (s, $3H_B$), 2.36–2.30 (m, 2H), 2.10–2.01 (m, 2H), 1.46 (s, $9H_A$), 1.41 (s, $9H_B$) (CO_2H not observed); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 177.0/175.9, 156.5/155.4, 137.9, 128.4 (2C), 127.7 (2C), 127.6, 80.7/80.6, 73.2/73.1, 66.8/66.3, 57.3/57.0, 33.3/32.8, 29.8/29.0, 28.3/28.3 (3C); IR (neat) ν_{max} 1738*m*, 1696*s*, 1480*m*, 1453*m*, 1392*m*, 1367*m*, 1151*s*, 1103*m* cm^{-1}; ESI-TOF HRMS *m*/*z* 346.1625 (M + Na^+, $C_{17}H_{25}NNaO_5^+$, requires 346.1625); $[\alpha]^{25}_D$ +22 (*c* 1.0, CH_2Cl_2).



(*R*)-2-(*tert*-Butoxycarbonyl(methyl)amino)-4-(2-(*tert*-butoxycarbonyl)hydrazono)butanoic Acid (44)

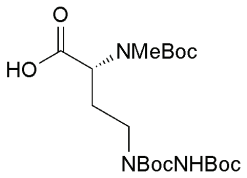
An O_2 degassed solution of **43** (500 mg, 1.54 mmol) in EtOAc (15.4 mL) with 10% Pd/C (100 mg) was placed under an atmosphere of H_2 gas (balloon) and the mixture was vigorously stirred at room temperature. After 18 h, the solution was filtered over Celite. The filtrate was concentrated in vacuo to afford the primary alcohol as a mixture with lactone (358 mg, theoretical 359 mg, 99%). The residue (300.0 mg, 1.28 mmol) was dissolved in anhydrous CH_2Cl_2 (12.8 mL) and Dess–Martin periodinane (600 mg, 1.41 mmol) was added and the mixture was vigorously stirred for 2 h at room temperature. The volatiles were removed in vacuo and the residue was diluted in CH_2Cl_2 (2 mL). The solid precipitate was removed by filtration, and the filtrate was concentrated in vacuo. The crude aldehyde was dissolved in anhydrous THF (1.35 mL) and *tert*-butyl carbazate (170 mg, 1.28 mmol) in anhydrous THF (1.35 mL) was added. After 16 h, the volatiles were removed in vacuo and the residue was partitioned between water (15 mL) and EtOAc (30 mL). The aqueous phase was acidified to a pH of 2 with aqueous 0.2 N $NaHSO_4$, and extracted with EtOAc (15 mL). The organic phase were dried over Na_2SO_4, filtered, and concentrated. Column chromatography (15 g SiO_2, 70–100% EtOAc/hexanes) provided **44** (210 mg, theoretical 441 mg, 48%) as a white foam (rotomer ratio in acetone-D_6 A/B = 1:1): ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 9.53 (br s, 2H), 7.44 (br s, $1H_A$), 7.38 (br s, $1H_B$), 4.86 (dd, $1H_A$, J = 4.8, 9.6 Hz), 4.63 (dd, $1H_B$, J = 4.2, 9.0 Hz), 2.89 (s, $3H_A$), 2.86 (s, $3H_B$), 2.78–2.69 (m, 4H), 1.44 (s, 18H), 1.42 (s, $9H_A$), 1.41 (s, $9H_B$) (CO_2H not observed); ^{13}C NMR ($(CD_3)_2CO$, 150 MHz) δ 173.3/173.2, 157.5/156.6, 154.2 (br), 145.1 (br), 81.3/81.1, 81.0, 59.7/58.3, 34.6, 33.9/33.3, 29.5 (3C), 29.4 (3C); IR (neat) ν_{max} 1678*s* (br), 1529*w*, 1480*w*, 1453*w*, 1391*m*, 1367*m*, 1328*w*, 1247*m*, 1148*s*, 1045*w*, 1017*w*, 859*w*, 770*w*, 734*s* cm^{-1}; HR ESI-TOF *m/z* 368.1797 (M + Na^+, $C_{15}H_{27}N_3NaO_6^+$, requires 368.1792); $[\alpha]^{25}_D$ +14 (*c* 0.5, CH_2Cl_2).



(*R*)-2-(*tert*-Butoxycarbonyl(methyl)amino)-4-(2-(*tert*-butoxycarbonyl)hydrazinyl)butanoic Acid (45)

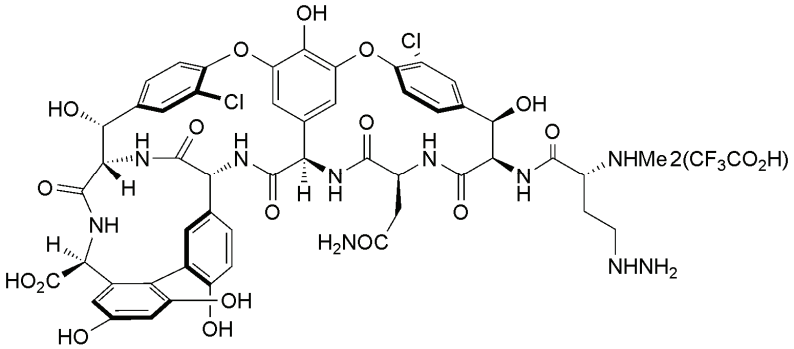
An O_2 degassed solution of **44** (104 mg, 0.301 mmol) in methanol (3.0 mL) was treated with *tert*-butylcarbazate (79.6 mg, 0.602 mmol). The solution was degassed and backfilled with N_2, and 5% Pt/C (50% wt., 52 mg) was added. The mixture was subsequently degassed and backfilled with N_2 then placed under an atmosphere of H_2 (balloon) and vigorously stirred. After 18 h, the solids were removed by filtration over Celite and the filtrate was concentrated in vacuo. Column chromatography (5 g SiO_2, 70–100% EtOAc/hexanes) provided **45** (79.8 mg, theoretical 104 mg, 76%) as a clear colorless oil (rotomer ratio in $(CD_3)_2CO$ A/B = 1:1): ^{1}H NMR ($(CD_3)_2CO$, 500 MHz) δ 4.81 (dd, $1H_A$, J = 4.5, 10.0 Hz), 4.55 (dd, $1H_B$ J = 4.5, 10.0 Hz), 2.93–2.78 (m, 12H), 2.14–2.05 (m, 6H), 1.94–1.87 (m, 2H), 1.45–1.42 (m, 36H) (CO_2H not observed); ^{13}C NMR (CD_3OD, 150 MHz) δ 175.0/174.9, 158.9, 158.0/157.6, 81.9/81.5, 80.9, 59.6/57.8, 33.6, 32.1, 28.7/28.6 (3C), 28.5/27.9 (3C), 20.8; IR (neat) ν_{max} 3293*w* (br),

1680*s*, 1478*m*, 1452*m*, 1391*m*, 1367*m*, 1250*m*, 1146*s*, 865*m*, 773*m* cm^{-1}; HR ESI-TOF *m/z* 348.2131 ($M + H^+$, $C_{15}H_{30}N_3O_6^+$, requires 348.2129); $[\alpha]^{25}_D$ +59 (*c* 0.5, CH_3OH).



(*R*)-4-(1,2-Bis(*tert*-butoxycarbonyl)hydrazinyl)-2-(*tert*-butoxycarbonyl(methyl)amino)butanoic Acid (46)

Di-*tert*-butyl dicarbonate (58.0 μL, 0.253 mmol) was added dropwise to a solution of **45** (79.8 mg, 0.229 mmol) in 1:1 THF/H_2O (0.573 mL) at 0 °C. The solution was warmed to room temperature and stirred for 16 h. The mixture was partitioned between water and EtOAc and the aqueous was washed with EtOAc (6 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Column chromatography (5 g SiO_2, 30–50% EtOAc/hexanes) provided **46** (49.2 mg, theoretical yield 102 mg, 48%) as a white foam (rotomer ratio in acetone-D_6 A/B = 1:1): mp 148–149 °C; ^{1}H NMR ($(CD_3)_2CO$, 600 MHz) δ 8.14–7.51 (br m, 2H), 4.67 (d, $1H_A$, J = 7.2 Hz), 4.38 (d, $1H_B$, J = 9.0 Hz), 3.61–3.30 (m, 4H), 2.87 (s, $3H_B$), 2.85 (s, $3H_A$), 2.29 (br s, 2H), 2.03 (br s, 2H), 1.46 (s, 18H), 1.45 (s, 18H) 1.42 (s, 18H); ^{13}C NMR ($(CD_3)_2CO$, 150 MHz) δ 174.0, 157.7, 157.2/157.0, 156.9/156.8/156.8/156.7/156.6, 82.1/81.7/81.6, 81.5, 81.11/81.07, 59.5/57.8, 50.1/50.0, 48.4/48.0, 34.2/32.9, 29.6 (3C), 29.5 (3C), 29.4 (3C); IR (neat) ν_{max} 3294*w*, 1693*s*, 1479*w*, 1453*w*, 1392*m*, 1367*s*, 1252*m*, 1150*s*, 1093*w*, 1019*w*, 856*w*, 775*w*, 736*m* cm^{-1}; ESI-TOF HRMS *m/z* 448.2651 ($M + H^+$, $C_{20}H_{38}N_3O_8^+$, requires 448.2651); $[\alpha]^{25}_D$ +17 (*c* 0.5, 1:1 EtOAc/EtOH).



1-[(*R*)-4-(Hydrazinyl-2-methylamino)butanoyl]desleucylaglucovancomycin Bis(trifluoroacetate) Salt (14)

The title compound was prepared from **46** (9.50 mg, 0.021 mmol), and dicyclohexylcarbodiimide (2.19 mg, 0.011 mmol) in CH_2Cl_2 (0.151 mL), followed by treatment with $NaHCO_3$ (1.78 mg, 0.021 mmol), desleucylaglucovancomycin (**16**, 10.0 mg, 0.009 mmol) in DMF (0.3 mL) according to *GP3*. The protected intermediate was isolated as a white solid (10.4 mg, theoretical 12.8 mg, 81%) by reverse-phase HPLC with a gradient of 35–75% MeCN/water with 0.07% TFA (t_R = 18.0 min), and lyophilization, and was taken directly to the next step without further characterization. The intermediate (5.00 mg, 0.004 mmol) was taken up in 40% TFA in CH_2Cl_2 (0.280 mL) and stirred at 0 °C for 5 min then gently warmed to room temperature. After 45 min, the volatiles were removed with a slowly stream of N_2 and residual TFA was azetroped with CH_2Cl_2 (3 × 5 mL). The residue was purified by reverse-phase HPLC using a gradient of 5–40% MeCN/water with 0.07% TFA (t_R = 23.4 min) and afforded the bis-TFA salt of **14** (2.90 mg, theoretical 4.80 mg, 60%): ^{1}H NMR (CD_3OD, 600 MHz) δ 9.01 (d, 1H, NH, J = 4.2 Hz), 8.79 (br s, 1H, NH), 7.67–7.57 (m, 4H), 7.38 (br s, 1H), 7.24 (d, 1H, J =

8.4 Hz), 7.05 (s, 1H), 6.86 (br s, 1H), 6.87 (d, 1H, J = 8.4 Hz), 6.46 (s, 1H), 4.32 (s, 1H), 5.90 (br s, 1H), 5.81 (br s, 1H), 5.38–5.34 (m, 4H), 4.74 (s, 1H), 4.60 (br s, 1H), 4.46 (br s, 1H), 4.15 (s, 1H), 4.10 (t, 1H, J = 6.3 Hz), 3.10–3.08 (m, 1H), 2.97 (d, 1H, J = 15.6 Hz), 2.81 (s, 3H), 2.27 (br s, 1H), 2.18 (br s, 2H) (one signal buried under residual solvent/water peak); ESI-TOF HRMS m/z 1145.2782 ($M + H^+$, $C_{51}H_{51}Cl_2N_{10}O_{17}^+$, requires 1145.2805); $[\alpha]^{25}_D$ +30 (c 0.2, CH_3OH).

Table 2SI. Purity Analysis **6**–**15**.[a]

Compound	Gradient (Solvent A/B)[b]	Percent purity
6	5-40% A/B(30 min)	94%
7	5-40% A/B(30 min)	94%
8	5-40% A/B(30 min)	94%
9	5-40% A/B(30 min)	93%
10	5-40% A/B(30 min)	96%
11	5-40% A/B(30 min)	99%
12	5-40% A/B(30 min)	96%
13	5-40% A/B(30 min)	90%
14	5-40% A/B(30 min)	93%
15	5-40% A/B(30 min)	86%

[a] Purity of each compound was determined on a preparative reverse-phase Waters™ Nova-Pac® HR C18 6 µm, 60 Å column (25 x 100 mm) using a Waters™ 600 HPLC with a Waters™ Photodiode Array Detector. A gradient of acetonitrile/water (containing 0.07% TFA) at flow rate of 5 mL min^{-1} was used for all samples with detection at 220, 254, 280 nm. [b] solvent A = acetonitrile, solvent B = water with 0.07% TFA.

Supporting Information References

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(3) (a) All molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081). For details see: (b) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera-A Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.* **2004**, *25*, 1605-1612; (c) Chimera home page can be found at http://www.cgl.ucsf.edu/chimera.

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(7) Antimicrobial assays were run as previously described: (a) McComas, C. C.; Crowley, B. M.; Hwang, I.; Boger, D. L. Synthesis and Evaluation of Methyl Ether Derivatives of Vancomycin, Teicoplanin, and Ristocetin Aglycon Methyl Ethers. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2933-2936. (b) McAtee, J. J.; Castle, S. L.; Jin, Q.; Boger, D. L. Synthesis and Evaluation of Vancomycin and Vancomycin Aglyon Analogues that Bear Modifications in the Residue 3 Asparagine. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1319-1322.

(8) UV-difference titration assays were run as previously described: (a) Nieto, M.; Perkins, H. R. The Specificity of Combination between Ristocetins and Peptides Related to Bacterial Cell Wall Mucopeptide Precursors. *Biochem J.* **1971**, *124*, 845-852. (b) Nieto, M.; Perkins, H. R. Physiochemical Properties of Vancomycin and Iodovancomycin and their Complexes with Diacetyl-L-lysyl-D-alanyl-D-alanine. *Biochem J.* **1971**, *123*, 773-787. (c) Perkins, H. R. Specificity of Combination between Mucopeptide Precursors and Vancomycin or Ristocetin. *Biochem J.* **1969**, *111*, 195-205.