Supporting Information

Synthesis and Biological Evaluation of Muraymycin Analogues Active against Anti-Drug-Resistant Bacteria

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List of contents

1. *Experimental Procedures*

General experimental methods. NMR spectra were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as internal standard otherwise noted. Coupling constant (*J*) was reported in herz (Hz). Abbreviations of multiplicity were as follows; s: singlet, d; doublet, t: triplet, q: quartet, m: multiplet, br: broad. Data were presented as follows; chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^{1}H–^{1}H COSY, HMBC and HMQC NMR spectra. Purity of all compounds tested for MraY inhibitory and antibacterial activity was checked by HPLC analysis resulting >97%.

8-(Biphenyl-4-yl)octanol (10). A solution of **9** (1.0 g, 5.56 mmol) and 7-octen-1-ol (5.0 mL, 33 mmol) in CH_2Cl_2 (60 mL) was treated with Grubbs catalyst 2nd generation (235 mg, 0.028 mmol) at 40 °C and stirred at the same temperature for 8 h. The reaction was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (10×10 cm, 25% AcOEt–hexane) to afford a mixture of olefins (1.2 g, 4.28 mmol, 13:1 mixture of geometric isomers) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.58 (d, 2H, H-2′-biphenyl and H-6′-biphenyl, J = 6.9 Hz), 7.52 (d, 2H, H-3 and H-5-biphenyl, J = 8.6 Hz), 7.41 (m, 4H, H-2, H-6, H-3′ and H-5′-biphenyl), 7.32 (t, 1H, H-4′, J = 7.5 Hz), 6.40 (d, 1H, H-8, $J_{8,7}$ = 15.4 Hz), 6.26 (dt, 1H, H-7, $J_{7,6a}$ = 6.9, $J_{7,6b}$ = $J_{7,8}$ = 15.4 Hz), 3.65 (m, 2H, H-1), 2.23 (m, 2H, H-6), 1.59 (m, 2H, H-2), 1.50 (m, 2H, H-3), 1.40 (m, 4H, H-4 and H-5); EIMS-LR *m/z* 280 [M^+]. A mixture of the olefins and 10% Pd/C (400 mg) in MeOH (100 mL) was vigorously stirred under H_2 atmosphere at room temperature for 6 h. The insoluble was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10×5 cm, 11% AcOEt–hexane) to afford **10** (1.22 g, 77% over 2 steps) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.58 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 7.5 Hz), 7.51 (d, 2H, H-3-biphenyl and H-5-biphenyl, J = 8.6 Hz), 7.42 (t, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 7.5 Hz), 7.32 (t, 1H, H-4′-biphenyl, J = 7.5 Hz), 7.25 (m, 2H, H-2′-biphenyl and H-6′-biphenyl), 3.64 (t, 2H, H-1, $J_{1a,1b}$ = $J_{1,2}$ = 6.3 Hz), 2.64 (t, 2H, H-8, $J_{8,7}$ = $J_{8a,8b}$ = 8.0 Hz), 1.65 (m, 2H, H-2), 1.55 (m, 2H, H-7), 1.35 (m, 8H, H-3, H-4, H-5 and H-6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.1, 141.3, 138.6, 128.9, 128.8, 128.1, 63.2, 35.7, 32.9, 31.6, 29.6, 29.4, 25.8; EIMS-LR *m/z* 282 [M^+]; EIMS-HR calcd for $C_{20}H_{26}O$ 282.1983, found 282.1985.

8-(Biphenyl-4-yl)octanal (3c). To a solution of **10** (84.6 mg, 0.30 mmol) and Et_3N (167 μL, 1.20 mmol) in DMSO (1.5 mL) and CH_2Cl_2 (1.5 mL) was added sulfur trioxide pyridine complex (95.5 mg, 0.6 mmol) at room temperature, and the mixture was stirred at the same temperature for 1 h. The organic phase was diluted with AcOEt and washed

with 1 M *aq.* HCl, saturated *aq.* $NaHCO_3$, H_2O, saturated *aq.* NaCl. The organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to afford **3c** (85.0 mg, quant.) as a white solid. This compound was used to the next reaction without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.75 (s, 1H, formyl), 7.58 (d, 2H, H-2′-biphenyl and H-6′-biphenyl, J = 7.5 Hz), 7.50 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.0 Hz), 7.43 (t, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 7.5 Hz), 7.31 (t, 1H, H-4′-biphenyl, J = 7.5 Hz), 7.25 (d, 2H, H-3-biphenyl and H-5-biphenyl, J = 8.0 Hz), 2.63 (t, 2H, H-8, J = 7.2 Hz), 2.42 (m, 2H, H-2), 1.64 (m, 4H, H-7 and H-3), 1.37 (m, 6H, H-4, 5 and H-6); ESIMS-LR *m/z* 335 [$(M + MeOH + Na)^+$].

Ethyl 3-(4′-butoxybiphenyl-4-yl)propanoate (12). Diethyl ethoxycarbonylmethyl phosphonate (1.17 mL, 5.8 mmol) was added dropwise to a suspension of 60% NaH (234 mg, 5.8 mmol) in THF (20 mL) at 0 °C, and the mixture was stirred at the same temperature for 30 min. 4′-Butoxybiphenyl-4-carbaldehyde[19] (**11**, 990 mg, 3.9 mmol) in THF (30 mL) was added dropwise to the mixture at 0 °C over 10 min, and the resulting suspension was stirred at the same temperature for 24 h. The suspension was poured onto 0.3 M *aq.* HCl at 0 °C, and the resulting mixture was diluted with AcOEt. The organic phase was washed with saturated *aq.* $NaHCO_3$, H_2O, saturated *aq.* NaCl, dried (Na_2SO_4) and concentrated *in vacuo*. A mixture of the residue and 10 % Pd/C (400 mg) in MeOH (100 mL) was vigorously stirred under H_2 atmosphere at room temperature for 30 min. The insoluble was filtered off through a thiosilica gel pad and a Celite pad, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10×5 cm, 11% AcOEt–hexane) to afford **12** (1.1 g, 82% over 2 steps) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.47 (m, 4H, H-2, 3, 5 and H-6-biphenyl), 7.24 (m, 2H, H-2′-biphenyl and H-6′-biphenyl), 6.93 (m, 2H, H-3′-biphenyl and H-5′-biphenyl), 4.14 (m, 2H, $CO_2CH_2CH_3$), 3.97 (m, 2H, $OCH_2CH_2CH_2CH_3$), 2.95 (m, 2H, H-4), 2.63 (m, 2H, H-2), 1.77 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.49 (m, 2H, H-3), 1.33 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.24 (m, 3H, $CO_2CH_2CH_3$), 0.98 (dd, 3H, $OCH_2CH_2CH_2CH_3$, J = 6.9, 12.1 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.0, 158.7, 139.0, 133.3, 128.8, 128.0, 126.8, 114.8, 67.8, 60.5, 36.0, 31.4, 30.7, 19.4, 16.4, 14.3, 14.0; EIMS-LR *m/z* 326 [M^+]; EIMS-HR calcd for $C_{21}H_{26}O_3$ 326.1882, found 326.1882.

3-(4′-Butoxybiphenyl-4-yl)propanal (3d). To a solution of **12** (97.8 mg, 0.3 mmol) in CH_2Cl_2 (3 mL) was added 1 M DIBAL solution in toluene (300 μL, 0.3 mmol) at −78 °C, and the mixture was stirred at the same temperature for 1 h. The reaction was quenched with MeOH (1 mL), and the resulting mixture was partitioned between AcOEt and 0.3 M aquous HCl. The organic layer was washed with saturated *aq.*

$NaHCO_3$, H_2O, saturated *aq*. NaCl, dried (Na_2SO_4) and concentrated *in vacuo* to afford **3d** (84.6 mg, quant.) as a white solid. This compound was used to the next reaction without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.84 (s, 1H, formyl), 7.48 (m, 4H, H-2, 3, 5 and H-6-biphenyl), 7.23 (d, 2H, H-2′-biphenyl and H-6′-biphenyl, J = 10.3 Hz), 6.94 (d, 2H, H-3-biphenyl and H-5-biphenyl, J = 10.3 Hz), 3.99 (t, 2H, $OCH_2CH_2CH_2CH_3$, J = 8.8 Hz), 2.98 (t, 2H, H-4, J = 9.5 Hz), 2.82 (t, 2H, H-2, J = 9.8 Hz), 1.78 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.51 (m, 4H, H-3 and $OCH_2CH_2CH_2CH_3$), 0.98 (t, 3H, $OCH_2CH_2CH_2CH_3$, J = 9.7 Hz).

4′-Hexyloxybiphenyl-4-carbaldehyde (3e). A solution of **13**[20] (1.0 g, 3.36 mmol) in THF (40 mL) was treated with 1 M borane-THF complex in THF (8.4 mL) at 0 °C for 8 h. The reaction was quenched carefully by MeOH (10 mL) at 0 °C, and the resulting mixture was concentrated *in vacuo*. The residue was partitioned between AcOEt and 1 M *aq*. HCl, and the organic layer was washed with saturated *aq.* $NaHCO_3$, H_2O, saturated *aq.* NaCl, dried (Na_2SO_4) and concentrated *in vacuo* to afford the alcohol (910 mg, 95%). The alcohol (910 mg, 3.2 mmol) and MnO_2 (3.64 g, 64 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature for 8 h. The insoluble was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5×10 cm, 12% AcOEt–hexane) to afford **3e** (3.64 g, quant.) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.0 (s, 1H, formyl), 7.91 (d, 2H, H-3 and H-5-biphenyl, J = 8.6 Hz), 7.72 (d, 2H, H-2 and H-6-biphenyl), 7.57 (d, 2H, H-2′-biphenyl and H-6′-biphenyl, J = 6.8 Hz), 6.99 (d, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 6.8 Hz), 4.00 (t, 2H, $OCH_2CH_2CH_2CH_2CH_2CH_3$, J = 6.8 Hz), 1.81 (dt, 2H, $OCH_2CH_2CH_2CH_2CH_2CH_3$, J = 6.8, 14.3 Hz), 1.48 (m, 2H, $OCH_2CH_2CH_2CH_2CH_2CH_3$, J = 6.8 Hz), 1.35 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2CH_3$), 0.91 (t, 3H, $OCH_2CH_2CH_2CH_2CH_2CH_3$, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.9, 159.8, 146.9, 134.7, 131.8, 130.4, 128.5, 127.0, 115.1, 68.2, 31.7, 29.3, 25.9, 22.7, 14.2; ESIMS-LR *m/z* 305 [$(M + Na)^+$].

Compounds 7b and 8b (General procedure of the preparation of 7 and 8 by the U4CR). Carboxylic acid **2** (42.7 mg, 0.073 mmol), **3b** (89.8 mg, 0.37 mmol) and **4** (53.9 μL, 0.37 mmol) were dissolved in EtOH (1 mL), and the solution was concentrated *in vacuo*. The residue was coevaporated with EtOH three times. The residue and the isonitrile **5** (56.9 mg, 0.073 mmol) in EtOH (1 mL) were concentrated *in vacuo*, and the resulting syrup was kept at room temperature for 48 h. The mixture was diluted with AcOEt, and the solution was washed with H_2O, saturated *aq.* NaCl and dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by neutral silica gel column chromatography (0.5×2 cm, 66% AcOEt–hexane) to afford the U-4CR

product (47.6 mg, 37%) as a white foam. The product (7.0 mg, 0.0040 mmol) in THF (1 mL) and 1 M *aq.* NaH_2PO_4 (500 μL) was treated with Zn (4.3 mg, 0.080 mmol) for 40 h. After the resulting mixture was concentrated *in vacuo*, the residue was suspended in AcOEt. The insoluble was filtered off through a short silica gel pad, and the filtrate was concentrated *in vacuo*. The residue was treated with 80% *aq.* TFA (2 mL) for 8 h, and the resulting mixture was concentrated *in vacuo*. The residue was purified by HPLC (YMC J′sphere ODS M80, 4.6×150 mm, 0.1% TFA, a linear gradient with MeOH from 73 to 80% for 20 min, 7.09 min for **8b**, 9.53 min for **7b**) to afford **7b** (1.5 mg, 35%) and **8b** (1.5 mg, 35%) as a white foam. Data for **7b**; $[\alpha]^{22}_{D}$ +294.3 (*c* 0.053, MeOH); ^{1}H NMR (D_2O containing CD_3OD, 500 MHz) δ 7.78 (br s, 1H, H-6), 5.91 (br s, 1H, H-5), 5.85 (br s, 1H, H-1′), 5.25 (br s, 1H, H-1″), 4.65 (br s, 1H, H-5′), 4.44 (br s, H, H-2′, H-2-*epi*-Cpm), 4.35 (m, 2H, H-3′ and 4′), 4.20 (m, 4H, H-2″, 3″, 4″ and C*H*$CH_2(CH_2)_{13}CH_3$), 4.09 (br s, 1H, H-2-Val), 3.01 (m, 2H, H-6′ and H-3-*epi*-Cpm), 3.37 (m, 4H, H-10′ and H-5-*epi*-Cpm), 3.23 (m, 4H, H-8′ and 5″), 2.17 (m, 1H, H-3-Val), 2.00 (m, 4H, H-9′ and H-4-*epi*-Cpm), 1.72 (m, 2H, -CHC*H*$_2(CH_2)_{13}CH_3$), 1.26 (m, 26H, -$CHCH_2(CH_2)_{13}CH_3$), 0.97 (m, 3H, H-4-Val), 0.93 (br s, 3H, H-4-Val), 0.86 (s, 3H, -$CHCH_2(CH_2)_{13}CH_3$); ^{13}C NMR (D_2O, 125 MHz) δ 177.2, 172.2, 166.6, 159.8, 154.9, 152.1, 143.2, 141.9, 109.8, 103.1, 92.6, 85.5, 80.4, 76.8, 75.8, 73.7, 72.8, 70.2, 64.9, 59.7, 56.7, 55.1, 47.6, 47.0, 43.4, 37.1, 32.8, 30.7, 30.6, 30.3, 26.6, 23.5, 20.0, 18.1, 14.7, 9.13; ESIMS-LR *m/z* 1071 [(M + H)$^+$]; ESIMS-HR calcd for $C_{48}H_{84}N_{11}O_{16}$ 1070.6098, found 1070.6069. Data for **8b**; $[\alpha]^{22}_{D}$ +297.8 (*c* 0.059, MeOH); ^{1}H NMR (D_2O, 600 MHz) δ 7.90 (br s, 1H, H-6), 6.04 (br s, 1H, H-5), 6.00 (br s, 1H, H-1′), 5.38 (br s, 1H, H-1″), 4.80 (br s, 1H, H-5′), 4.68 (br s, 1H, H-2′), 4.49 (m, 3H, H-3′,4′ and H-2-*epi*-Cpm), 4.33 (m, 4H, H-2″, 3″, 4″ and C*H*$CH_2(CH_2)_{13}CH_3$), 4.16 (br s, 1H, H-2-Val), 4.00 (m, 2H, H-6′ and H-3-*epi*-Cpm), 3.52 (m, 4H, H-10′ and H-5-*epi*-Cpm), 3.37 (m, 4H, H-8′ and H-5″), 2.29 (m, 1H, H-3-Val), 2.10 (m, 4H, H-9′ and H-4-*epi*-Cpm), 1.80 (m, 2H, -CHC*H*$_2(CH_2)_{13}CH_3$), 1.37 (m, 26H, -$CHCH_2(CH_2)_{13}CH_3$), 1.08 (br s, 3H, H-4-Val), 1.03 (br s, 3H, H-4-Val), 0.98 (s, 3H, -$CHCH_2(CH_2)_{13}CH_3$); ^{13}C NMR (D_2O containing CD_3OD, 150 MHz) δ 177.8, 173.9, 167.2, 160.4, 155.5, 152.6, 143.8, 142.5, 110.4, 103.7, 93.2, 86.1, 80.6, 77.4, 76.4, 74.3, 73.4, 70.7, 65.5, 60.2, 57.3, 55.7, 48.2, 47.6, 43.9, 37.7, 33.4, 31.3, 31.2, 30.9, 27.2, 24.0, 20.6, 18.7, 15.3, 9.7; ESIMS-LR *m/z* 1070 [(M + H)$^+$]; ESIMS-HR calcd for $C_{48}H_{84}N_{11}O_{16}$ 1070.6098, found 1070.6073.

Compounds 7c and 8c. According to the general procedure, **7c** (5.1 mg, 27%) and **8c** (5.1 mg, 27%) were obtained as a white foam from **2** (17.4 mg, 0.03 mmol), **3c** (85.0 mg, 0.30 mmol), **4** (45.0 μL, 0.3 mmol), and **5** (23.1 mg, 0.03 mmol) after purification

by HPLC (YMC J′sphere ODS M80, 10×150 mm, 0.1% TFA, 62% MeOH–H_2O, 8.6 min for **8c**, 14.0 min for **7c**). Data for **7c**; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.64 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.56 (d, 2H, H-1′-biphenyl and H-6′-biphenyl, J = 8.0 Hz), 7.49 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.0 Hz), 7.39 (dd, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 7.5, 8.0 Hz), 7.28 (t, 2H, H-4′-biphenyl, J = 7.5 Hz), 7.22 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.0 Hz), 5.75 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.17 (s, 1H, H-1″), 4.56 (d, 1H, H-5′, $J_{5',4'}$ = 5.2 Hz), 4.31 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 7.4 Hz), 4.28 (dd, 1H, H-2′, $J_{2',1'}$ = 2.3, $J_{2',3'}$ = 5.2 Hz), 4.23 (m, 2H, H-3′ and H-4′), 4.15 (m, 1H, H-2-8-(biphenyl-4-yl)heptylglycine), 4.12 (d, 1H, H-2-Val, $J_{2,3}$ = 4.6 Hz), 4.08-4.02 (m, 3H, H-2″, 3″ and 4″), 3.96 (s, 1H, H-6′), 3.87 (dd, 1H, H-3-*epi*-Cpm, $J_{3,2}$ = 4.6, $J_{3,4}$ = 12.3 Hz), 3.40 (m, 1H, H-5a-*epi*-Cpm), 3.29 (m, 2H, H-10′a and H-5b-*epi*-Cpm), 3.18 (m, 4H, H-5″, H-8′a and H-10′b), 3.04 (m, 1H, H-8′b), 2.62 (t, 2H, H-9-8-(biphenyl-4-yl)heptylglycine, J = 8.0 Hz), 2.18 (m, 1H, H-3-Val), 1.86 (m, 5H, H-9′, H-4-*epi*-Cpm and H-3a-8-(biphenyl-4-yl)heptylglycine), 1.64 (m, 3H, H-3b-8-(4-biphenylyl-4-yl)heptylglycine and H-4-8-(biphenyl-4-yl)heptylglycine), 1.34 (m, 8H, H-5, H-6, H-7 and H-8-8-(biphenyl-4-yl)heptylglycine), 0.98 (d, 3H, H-4a-Val, $J_{4a,3}$ = 6.9 Hz), 0.94 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.9 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 176.4, 175.2, 172.5, 166.3, 166.0, 160.4, 155.6, 152.1, 143.3, 143.1, 142.4, 139.9, 130.1, 129.9, 129.8, 128.0, 127.8, 127.8, 110.2, 103.1, 93.9, 85.7, 80.4, 77.6, 76.4, 74.4, 73.9, 73.8, 71.1, 60.1, 59.2, 56.9, 55.7, 43.9, 37.5, 36.7, 36.4, 32.6, 32.4, 31.5, 30.7, 30.6, 30.4, 30.2, 27.2, 22.5, 19.8, 18.2; ESIMS-LR *m/z* 1110 [$(M + H)^+$]; ESIMS-HR calcd for $C_{52}H_{76}N_{11}O_{16}$ 1110.5466, found 1110.5465. Data for **8c**; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.64 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.55 (d, 2H, H-1′ and 6′-biphenyl, J = 8.0 Hz), 7.49 (d, 2H, H-2 and 6-biphenyl, J = 8.0 Hz), 7.39 (dd, 2H, H-3′ and 5′-biphenyl, J = 7.5, 8.0 Hz), 7.28 (t, 2H, H-4′-biphenyl, J = 7.5 Hz), 7.22 (d, 2H, H-2 and 6-biphenyl, J = 8.0 Hz), 5.74 (d, 1H, H-1′, $J_{1',2'}$ = 2.8 Hz), 5.71 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.18 (s, 1H, H-1″), 4.58 (d, 1H, H-5′, $J_{5',4'}$ = 4.9 Hz), 4.43 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 5.5 Hz), 4.30 (dd, 1H, H-2′, $J_{2',1'}$ = 2.8, $J_{2',3'}$ = 5.5 Hz), 4.24 (m, 2H, H-3′ and H-4′), 4.16 (d, 2H, H-2-8-(biphenyl-4-yl)heptylglycine and H-2-Val, $J_{2,3}$ = 5.2 Hz), 4.08-4.00 (m, 3H, H-2″, 3″ and 4″), 3.93 (s, 1H, H-6′), 3.70 (dd, 1H, H-3-*epi*-Cpm, $J_{3,2}$ = 5.5, $J_{3,4}$ = 13.6 Hz), 3.40 (m, 2H, H-5a-*epi*-Cpm), 3.29 (m, 2H, H-10′a), 3.18 (m, 4H, H-5″, 8′a and H-10′b), 3.06 (m, 1H, H-8′b), 2.62 (t, 2H, H-9-8-(biphenyl-4-yl)heptylglycine, J = 8.1 Hz), 2.15 (m, 1H, H-3-Val), 1.93 (m, 4H, H-9′ and H-4-*epi*-Cpm), 1.69 (m, 3H, H-3-8-(biphenyl-4-yl)heptylglycine and H-4-8-(biphenyl-4-yl)heptylglycine), 1.34 (m, 8H, H-5, 6, 7 and H-8-8-(biphenyl-4-yl)heptylglycine), 0.95 (d, 3H, H-4a-Val, $J_{4a,3}$ = 6.9 Hz), 0.91 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.9 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 189.2,

176.4, 173.1, 166.8, 161.0, 156.4, 152.9, 144.2, 143.9, 143.2, 140.6, 130.7, 130.6, 128.8, 128.6, 128.6, 111.0, 104.0, 98.5, 94.8, 93.6, 86.6, 81.2, 77.3, 75.1, 74.6, 72.0, 57.5, 56.7, 53.0, 44.7, 38.2, 37.8, 37.2, 33.5, 33.4, 32.6, 31.2, 31.0, 27.9, 23.1, 20.5, 18.8; ESIMS-LR *m/z* 1110 [(M + H)$^+$]; ESIMS-HR calcd for $C_{52}H_{76}N_{11}O_{16}$ 1110.5466, found 1110.5466.

Compounds 7d and 8d. According to the general procedure, **7d** (4.8 mg, 24%) and **8d** (4.8 mg, 24%) were obtained as a white foam from **2** (17.4 mg, 0.03 mmol), **3d** (84.6 mg, 0.30 mmol), **4** (45.0 μL, 0.3 mmol), and **5** (23.1 mg, 0.03 mmol) after purification by HPLC (YMC J′sphere ODS M80, 10×150 mm, 0.1% TFA, 60% MeOH–H_2O, 5.8 min for **8d**, 10.2 min for **7d**). Data for; **7d**; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.65 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.48 (m, 4H, H-3, 5, 2′ and H-6′-biphenyl), 7.24 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.6 Hz), 6.94 (d, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 6.9 Hz), 5.76 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.16 (s, 1H, H-1″), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 5.7 Hz), 4.27 (dd, 1H, H-2′, $J_{2',1'}$ = 2.3, $J_{2',3'}$ = 5.2 Hz), 4.24 (m, 1H, H-2-*epi*-Cpm), 4.23 (dd, 1H, H-4′, $J_{4',5'}$ = 5.7, $J_{4',3'}$ = 9.8 Hz), 4.21 (m, 1H, H-4′), 4.19 (m, 1H, H-2-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 4.12 (d, 1H, H-2-Val, $J_{2,3}$ = 5.2 Hz), 4.06 (m, 1H, H-4″), 4.04 (m, 1H, H-3″), 4.02 (m, 1H, H-2″), 3.97 (t, 2H, OC*H*$_2$CH$_2$CH$_2$CH$_3$, J = 7.4 Hz), 3.92 (s, 1H, H-6′), 3.90 (m, 1H, H-3-*epi*-Cpm), 3.39 (m, 1H, H-10′a), 3.32 (m, 3H, H-5″a and H-5a-*epi*-Cpm), 3.22 (m, 1H, H-5″b), 3.16 (m, 2H, H-8′a and H-10′b), 3.04 (m, 1H, H-8′b), 2.75 (m, 1H, H-4a-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 2.64 (m, 1H, H-4b-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 2.18 (m, 2H, H-3-Val and H-3a-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 2.01 (m, 1H, H-3b-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 1.87 (m, 4H, H-9′ and H-4-*epi*-Cpm), 1.75 (m, 2H, OCH$_2$C*H*$_2$CH$_2$CH$_3$), 1.51 (m, 2H, OCH$_2$CH$_2$C*H*$_2$CH$_3$), 0.99 (d, 3H, H-4a-Val, $J_{4a,3}$ = 10.6 Hz), 0.96 (m, 3H, OCH$_2$CH$_2$CH$_2$C*H*$_3$), 0.95 (d, 3H, H-4b-Val, $J_{4b,3}$ = 8.8 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 175.8, 173.6, 161.3, 160.9, 156.3, 152.9, 144.2, 141.3, 140.8, 135.2, 130.8, 129.5, 128.4, 116.7, 111.1, 104.0, 94.6, 86.6, 81.3, 78.6, 77.3, 75.2, 74.5, 71.9, 69.6, 57.9, 56.0, 52.2, 48.7, 47.6, 44.7, 38.2, 37.6, 34.6, 33.7, 33.3, 32.3, 27.8, 23.4, 21.1, 20.6, 19.0, 15.0, 10.0; ESIMS-LR *m/z* 1111 [(M + H)$^+$]; ESIMS-HR calcd for $C_{51}H_{74}N_{11}O_{17}$ 1111.5259, found 1111.5271. Data for; **8d**; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.62 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz), 7.46 (m, 4H, H-3, H-5, H-2′ and H-6′-bihenyl), 7.23 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.6 Hz), 6.94 (d, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 9.2 Hz), 5.73 (d, 1H, H-1′, $J_{1',2'}$ = 2.8 Hz), 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.1 Hz), 5.17 (s, 1H, H-1″), 4.57 (d, 1H, H-5′, $J_{5',4'}$ = 5.7 Hz), 4.45 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 8.6 Hz), 4.30 (dd, 1H, H-2′, $J_{2',1'}$ = 2.8, $J_{2',3'}$ = 5.2 Hz),

4.23 (m, 3H, H-3′, H-4′ and H-2-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 4.18 (d, 1H, H-2-Val, $J_{2,3}$ = 4.5 Hz), 4.04 (m, 3H, H-2″, H-3″ and H-4″), 3.99 (t, 2H, OCH_2CH$_2$CH$_2$CH$_3$, J = 6.9), 3.93 (s, 1H, H-6′), 3.75 (dd, 1H, H-3-*epi*-Cpm, $J_{3,4}$ = 5.7, $J_{3,2}$ = 8.6 Hz), 3.44 (m, 1H, H-5a-*epi*-Cpm), 3.36 (m, 1H, H-5b-*epi*-Cpm), 3.35 (m, 1H, H-10′a), 3.28 (m, 1H, H-10′b), 3.21-3.15 (m, 4H, H-8′a, 10′b and H-5″), 3.06 (m, 1H, H-8′b), 2.78 (m, 1H, H-4a-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 2.66 (m, 1H, H-4b-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 2.16 (m, 1H, H-3-Val), 2.07 (m, 2H, H-3-4-(4′-butoxybiphenyl-4-yl)ethylglycine), 1.96 (m, 2H, H-4-*epi*-Cpm), 1.88 (m, 2H, H-9′), 1.75 (m, 2H, OCH$_2$CH_2CH$_2$CH$_3$), 1.51 (m, 2H, OCH$_2$CH$_2$CH_2CH$_3$), 0.99 (m, 6H, H-4a-Val and OCH$_2$CH$_2$CH$_2$CH_3), 0.92 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.8 Hz); ^{13}C NMR (CD$_3$OD, 150 MHz) δ 176.7, 176.1, 173.3, 166.8, 161.0, 160.9, 156.4, 152.9, 144.3, 141.2, 140.9, 135.3, 130.8, 129.6, 128.4, 116.7, 111.1, 104.0, 94.5, 86.6, 81.2, 78.6, 77.3, 75.0, 74.5, 72.0, 69.6, 60.5, 57.7, 56.1, 52.9, 48.0, 44.7, 38.2, 35.2, 33.5, 33.3, 32.6, 28.0, 23.2, 21.1, 20.5, 18.8, 15.0; ESIMS-LR *m/z* 1111 [(M + H)$^+$]; ESIMS-HR calcd for $C_{51}H_{74}N_{11}O_{17}$ 1111.5259, found 1111.5272.

Compounds 7e and 8e. According to the general procedure, **7e** (4.8 mg, 32%) and **8e** (4.8 mg, 32%) were obtained as a white foam from **2** (17.4 mg, 0.03 mmol), **3e** (85.2 mg, 0.30 mmol), **4** (45.0 μL, 0.3 mmol), and **5** (23.1 mg, 0.03 mmol) after purification by HPLC (YMC J′sphere ODS M80, 10×150 mm, 0.1% TFA, 60% MeOH–H_2O, 10.6 min for **8e**, 18.6 min for **7e**). Data for **7e**; ^{1}H NMR (CD$_3$OD, 500 MHz) δ 7.62 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.58 (d, 2H, H-3-biphenyl and H-5-biphenyl, J = 8.6 Hz), 7.51 (d, 2H, H-2′-biphenyl and H-6′-biphenyl, J = 9.1 Hz), 7.45 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.6 Hz), 6.96 (d, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 9.1 Hz), 5.78 (d, 1H, H-1′, $J_{1',2'}$ = 2.3 Hz), 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.30 (s, 1H, H-2-4′-hexyloxybiphenyl-4-ylglycine), 5.17 (s, 1H, H-1″), 4.56 (d, 1H, H-5′, $J_{5',4'}$ = 5.2 Hz), 4.45 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 7.0 Hz), 4.28 (dd, 1H, H-2′, $J_{2',1'}$ = 2.3, $J_{2',3'}$ = 5.7 Hz), 4.25 (dd, 1H, H-4′, $J_{4',5'}$ = 5.2, $J_{4',3'}$ = 5.7 Hz), 4.20 (t, 1H, H-3′, $J_{3',2'}$ = $J_{3',4'}$ = 5.7 Hz), 4.15 (d, 1H, H-2-Val, $J_{2,3}$ = 5.2 Hz), 4.05-4.00 (m, 3H, H-2″, 3″ and H-4″), 3.97 (d, 2H, OCH_2CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$, J = 6.3 Hz), 3.93 (s, 1H, H-6′), 3.87 (dd, 1H, H-3-*epi*-Cpm, $J_{3,2}$ = 7.0, $J_{3,4}$ = 12.3 Hz), 3.35 (m, 3H, H-5-*epi*-Cpm and H-10′a), 3.26 (m, 4H, H-8′a, 10′b and H-5″), 3.14 (m, 1H, H-8′b), 2.17 (dd, 1H, H-3-Va, $J_{3,2}$ = 5.2, $J_{3,4}$ = 12.3 Hz), 1.95 (m, 1H, H-4a-*epi*-Cpm), 1.85 (m, 3H, H-9′ and H-4b-*epi*-Cpm), 1.77 (dt, 2H, OCH$_2$CH_2CH$_2$CH$_2$CH$_2$CH$_3$, J = 6.3, J = 14.9 Hz), 1.48 (m, 2H, OCH$_2$CH$_2$CH_2CH$_2$CH$_2$CH$_3$), 1.35 (m, 4H, OCH$_2$CH$_2$CH$_2$CH_2CH_2CH$_3$), 0.99 (d, 3H, H-4a-Val, $J_{4a,3}$ = 6.9 Hz), 0.95 (d, 3H, H-4b-Val, $J_{4b,3}$ = 6.9 Hz), 0.91 (d, 3H, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH_3, J = 18.6 Hz); ^{13}C NMR (CD$_3$OD, 150 MHz) δ 174.1, 172.8,

161.3, 156.3, 152.9, 144.0, 143.3, 136.8, 134.6, 130.2, 129.8, 128.8, 116.8, 111.1, 104.0, 94.6, 86.5, 81.3, 78.5, 77.3, 75.2, 74.6, 71.9, 69.9, 60.3, 57.6, 52.5, 47.8, 44.7, 38.1, 37.8, 33.5, 32.4, 31.2, 28.1, 27.6, 24.4, 23.0, 20.6, 19.0, 15.1; ESIMS-LR *m/z* 1111 [(M + H)$^+$]; ESIMS-HR calcd for $C_{51}H_{74}N_{11}O_{17}$ 1111.5259, found 1111.5259. Data for **8e**; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.66 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.59 (d, 2H, H-3-biphenyl and H-5-biphenyl, J = 8.0 Hz), 7.51 (d, 2H, H-2′-biphenyl and H-6′-biphenyl, J = 8.0 Hz), 7.45 (d, 2H, H-2-biphenyl and H-6-biphenyl, J = 8.0 Hz), 6.96 (d, 2H, H-3′-biphenyl and H-5′-biphenyl, J = 8.0 Hz), 5.77 (s, 1H, H-1′), 5.73 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.31 (s, 1H, H-2-4′-hexyloxybiphenyl-4-ylglycine), 5.17 (s, 1H, H-1″), 4.58 (d, 1H, H-5′, $J_{5',4'}$ = 4.6 Hz), 4.48 (d, 1H, H-2-*epi*-Cpm, $J_{2,3}$ = 8.1 Hz), 4.31 (s, 1H, H-2′), 4.25 (m, 2H, H-3′ and H-4′), 4.20 (d, 1H, H-2-Val, $J_{2,3}$ = 4.6 Hz), 4.07-4.02 (m, 3H, H-2″, 3″ and H-4″), 3.98 (t, 2H, OC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$, J = 5.7 Hz), 3.92 (s, 1H, H-6′), 3.74 (m, 1H, H-3-*epi*-Cpm), 3.43 (m, 3H, H-5-*epi*-Cpm and H-10′a), 3.20 (m, 4H, H-8′a, 10′b and H-5″), 3.07 (m, 1H, H-8′b), 2.14 (m, 1H, H-3-Va), 1.98 (t, 2H, H-4-*epi*-Cpm, $J_{4,3}$ = $J_{4,5}$ = 3.8 Hz), 1.89 (m, 2H, H-9′), 1.77 (m, 2H, OCH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.49 (m, 2H, OCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.36 (m, 4H, OCH$_2$CH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_3$), 0.91 (m, 9H, H-4-Val and OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (CD_3OD, 150 MHz) δ 174.3, 172.7, 166.8, 161.3, 161.0, 156.4, 153.0, 144.3, 143.3, 136.4, 130.2, 129.8, 128.8, 118.1, 116.8, 111.1, 104.1, 94.7, 86.8, 81.3, 78.6, 77.3, 75.2, 74.5, 72.0, 69.9, 60.4, 57.4, 53.0, 48.7, 48.0, 44.7, 38.1, 33.8, 33.5, 32.7, 31.5, 31.2, 28.1, 27.6, 24.4, 23.1, 20.6, 18.9, 15.2, 10.0; ESIMS-LR *m/z* 1111 [(M + H)$^+$]; ESIMS-HR calcd for $C_{51}H_{74}N_{11}O_{17}$ 1111.5259, found 1111.5263.

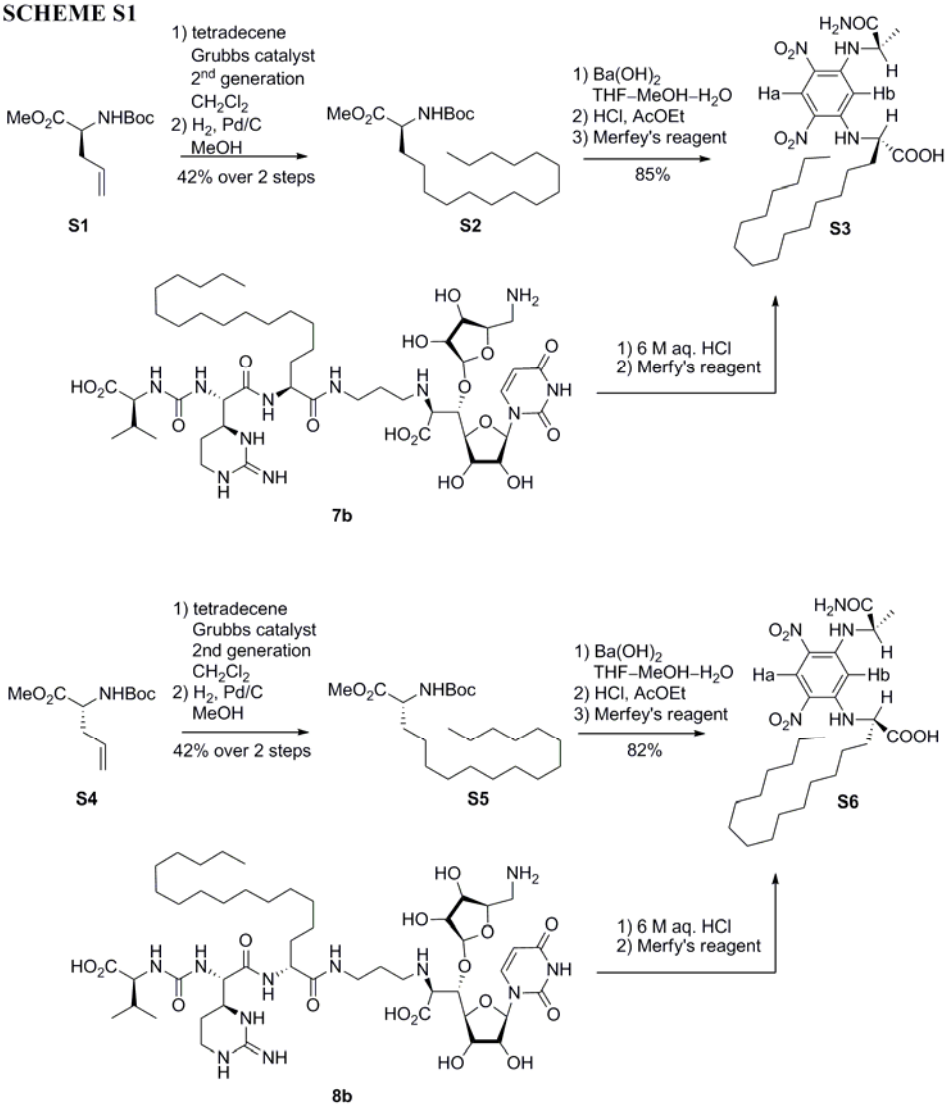
***tert*-Butyl 5-*O*-[5-*tert*-butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-6-deoxy-6-dodecylamino-2,3-*O*-isopropylidene-1-(uracil-1-yl)-β-D-*glycero*-L-*talo*-heptofuranuronate (16).** A mixture of **15**[12] (85 mg, 0.1 mmol) and 10% Pd/C (10 mg) in MeOH (3 mL) was vigorously stirred for 1 h under H_2 atmosphere. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to give the free amine **16**. The amine and dodecanal (24 μL, 0.1 mmol) in CH_2Cl_2 (1 mL) was treated with AcOH (28 μL) and $NaBH(OAc)_3$ (84 mg, 0.40 mmol) at room temperature for 1 h. The reaction mixture was diluted with AcOEt (50 mL), which was washed with saturated *aq.* $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (2×10 cm, 50% AcOEt/hexane) to give **16** (71 mg, 80% over 2 steps) as a white foam: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.70 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.70 (d, 1H, H-1′, $J_{1'',2''}$ = 1.7 Hz), 5.66 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.21 (dd, 1H, H-2′, $J_{2',1'}$ = 1.7, $J_{2',3'}$ = 6.3 Hz), 5.04 (s, 1H, H-1″), 4.83 (dd, 1H, H-3′, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 4.0 Hz),

4.67 (d, 1H, H-2″, $J_{2'',3''}$ = 5.7 Hz), 4.55 (d, 1H, H-3″, $J_{3'',2''}$ = 5.7 Hz), 4.48 (dd, 1H, H-4′, $J_{4',3'}$ = 9.7, $J_{4',5'}$ = 4.6 Hz), 4.42 (d, 1H, H-5′, $J_{5',4'}$ = 4.6 Hz), 4.14 (t, 1H, H-4″, $J_{4'',5''}$ = 6.3 Hz), 3.25 (br s, 1H, H-6′), 3.21 (dd, 1H, H-5″a, $J_{5''a,4''}$ = 5.7, $J_{5''a,5''b}$ = 14.3 Hz), 3.05 (dd, 1H, H-5″b, $J_{5''b,4''}$ = 7.5, $J_{5''b,5''a}$ = 14.3 Hz), 2.68 (m, 1H, $CH_3(CH_2)_{10}CH_2NH$), 2.42 (m, 1H, $CH_3(CH_2)_{10}CH_2NH$), 1.58-1.32 (m, 39H, $CH_3(CH_2)_{10}CH_2NH$, *t*-butyl, CH_2CH_3×2, and acetonide), 0.93 (t, 3H, $CH_3(CH_2)_{10}CH_2NH$, J = 6.9 Hz), 0.83, 0.81 (each t, each 3H, CH_2CH_3, J = 7.4 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.0, 166.4, 158.2, 152.2, 146.3, 117.1, 115.4, 114.2, 102.7, 97.3, 89.1, 87.7, 87.4, 86.2, 83.7, 83.6, 83.4, 83.3, 80.2, 62.9, 44.4, 33.1, 30.8, 30.7, 30.6, 30.5, 30.4, 29.7, 28.8, 28.6, 28.5, 28.3, 27.6, 25.7, 23.8, 14.5, 8.9, 7.6; ESIMS-HR *m*/*z* calcd for $C_{45}H_{77}N_4O_{13}$ 881.5409, found 881.5480.

5-*O*-(5-Amino-5-deoxy-β-D-ribofuranosyl)-6-deoxy-6-dodecylamino-1-(uracil-1-yl)-β-D-*glycero*-L-*talo*-heptofuranuronic acid trifluoroacetic salt (17). Compound **16** (50 mg, 0.057 mmol) was treated with 80% *aq.* TFA (1 mL) at room temperature for 6 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by C18 reverse phase column chromatography (1.5×10 cm, 80% *aq.* MeOH containing 0.5% TFA) to afford **17** (33 mg, 94%) as a TFA salt: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.69 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.77 (s, 1H, H-1′), 5.76 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.23 (s, 1H, H-1″), 4.61 (br s, 1H, H-5′), 4.33 (br s, 1H, H-2′), 4.27 (m, 2H, H-6′ and H-3″), 4.21 (br s, 1H, H-4′), 4.14-4.06 (m, 3H, H-3′, H-2″, and H-4″), 3.24 (br s, 2H, H-5″a and H-5″b), 3.22-3.11 (m, 2H, $CH_3(CH_2)_9CH_2CH_2NH$), 1.75 (m, 2H, $CH_3(CH_2)_9CH_2CH_2NH$), 1.36-1.32 (m, 18H, $CH_3(CH_2)_9CH_2CH_2NH$), 0.93 (t, 3H, $CH_3(CH_2)_9CH_2CH_2NH$, J = 6.9 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 170.9, 165.9, 152.0, 143.5, 110.1, 103.1, 94.5, 85.3, 80.3, 77.7, 76.3, 74.2, 73.7, 71.2, 63.8, 44.0, 33.1, 30.7, 30.6, 30.5, 30.2, 28.2, 27.6, 26.8, 23.7, 14.4; ESIMS-HR *m*/*z* calcd for $C_{28}H_{49}N_4O_{11}$ 617.3320, found 617.3381.

2. *Determination of the stereochemistry of 7b and 8b*

SCHEME S1



L-Boc-pentadecylglycine-OMe (S2). A mixture of tetradecene (777 μL, 3.1 mmol), L-Boc-allylglycine-OMe (**S1**, 70 mg, 0.31 mmol) and Grubbs catalyst 2nd generation (26 mg, 0.031 mmol) in CH_2Cl_2 (5 mL) were stirred at room temperature for 3 h. The mixture was concentrated *in vacuo*, and the residue and 10 % Pd/C (30 mg) in MeOH (5 mL) were vigorously stirred under H_2 atmosphere at room temperature for 24 h. The insoluble was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (2×10 cm, 6.3% AcOEt–hexane) to afford **S2** (52 mg, 42%) as a colorless oil. $[\alpha]^{22}_{D}$ +9.14 (*c* 1.71, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.99 (d, 1H, NH-2, $J_{NH,2}$ = 8.0 Hz), 4.26 (dd, 1H,

H-2, $J_{2,NH}$ = 8.0, $J_{2,3}$ = 13.2 Hz), 3.71 (s, 3H, OMe), 1.75 (m, 2H, $CH_3(CH_2)_{12}CH_2CH_2$CH-), 1.58 (m, 2H, $CH_3(CH_2)_{12}CH_2$ CH_2CH-), 1.42 (s, 9H, *t*-butyl), 1.26 (m, 24H, $CH_3(CH_2)_{12}CH_2CH_2$CH-), 0.86 (t, 3H, $CH_3(CH_2)_{12}CH_2CH_2$CH-, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 155.4, 79.8, 53.5, 52.2, 32.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.3, 22.8, 14.2; ESIMS-LR *m/z* 422 [$(M + Na)^+$]; ESIMS-HR calcd for $C_{23}H_{45}NNaO_4$ 422.3246, found 422.3255.

L-Ala-NH_2-DNP-L-pentadecylglycine (S3). Barium hydroxide octahydrates (9.46 mg, 0.030 mmol) were added to a solution of **S2** (10.0 mg, 0.025 mmol) in THF–MeOH–H_2O (3:1:1, 1 mL) at 0 °C, and the resulting reaction mixture was stirred at 0 °C for 20 min. The mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was partition between AcOEt and 1 M *aq*. HCl, and the organic phase was washed with saturated *aq.* NaCl, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was treated with 4 M HCl in AcOEt (1 mL) and stirred at room temperature for 1 h. The resulting mixture was concentrated *in vacuo*, and the residue in acetone (1 mL) and saturated aq. $NaHCO_3$ (1 mL) was treated with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (6.80 mg, 0.025 mmol) at 40 °C for 2 h. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (2× 3 cm, 0.1% TFA, 17% MeOH–CH_2Cl_2) to afford **S3** (11.4 mg, 85%) as a yellow powder. $[\alpha]^{22}_D$ +3.57 (*c* 0.23, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.11 (br s, 1H, Ha), 8.65 (br s, 1H, N*H*-2-pentadecylglycine), 8.48 (br s, N*H*-2-Ala), 1H, Hb), 6.74 (br s, 2H, CONH_2), 5.55 (br s, 1H, Hb), 4.09 (br s, 2H, H-2-Ala, pentadecylglycine), 1.98 (br s, 2H, $CH_3(CH_2)_{13}CH_2$CH-), 1.59 (br s, 3H, H-3-Ala), 1.23 (m, 26H, $CH_3(CH_2)_{13}CH_2$CH-), 0.87 (br s, 3H, $CH_3(CH_2)_{13}CH_2CH_2$CH-); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.6, 175.2, 147.8, 147.3, 128.9, 125.0, 124.2, 93.1, 56.6, 53.7, 32.0, 29.8, 29.5, 25.7, 22.8, 18.5, 14.2; ESIMS-LR *m/z* 538 [$(M - H)^-$]; ESIMS-HR calcd for $C_{26}H_{42}N_5O_7$ 536.3084, found 536.3062.

D-Boc-pentadecylglycine-OMe (S5). According to the procedure described for the preparation of **S2**, **S5** (52 mg, 42%) was obtained as a colorless oil. $[\alpha]^{22}_D$ –9.20 (*c* 4.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.99 (d, 1H, NH-2, $J_{NH,2}$ = 8.0 Hz), 4.26 (dd, 1H, H-2, $J_{2,NH}$ = 8.0, $J_{2,3}$ = 13.2 Hz), 3.71 (s, 3H, OMe), 1.75 (m, 2H, $CH_3(CH_2)_{12}CH_2CH_2$CH-), 1.58 (m, 2H, $CH_3(CH_2)_{12}CH_2CH_2$CH-), 1.42 (s, 9H, *t*-butyl), 1.26 (m, 24H, $CH_3(CH_2)_{12}CH_2CH_2$CH-), 0.86 (t, 3H, $CH_3(CH_2)_{12}CH_2CH_2$CH-, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 155.4, 79.8, 53.5, 52.2, 32.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 25.3, 22.8, 14.2; ESIMS-LR *m/z* 422 [$(M + Na)^+$]; ESIMS-HR calcd for $C_{23}H_{45}NNaO_4$ 422.3246, found 422.3255.

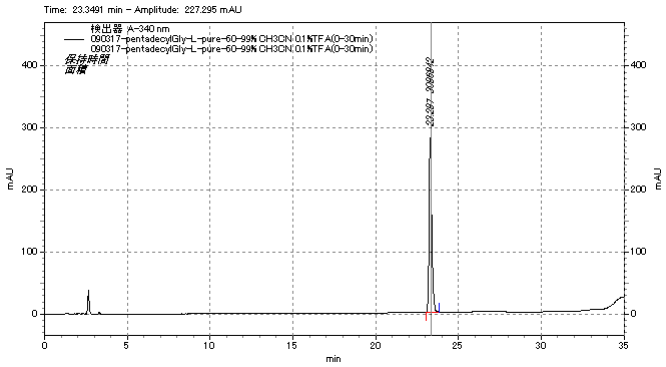
L-Ala-NH_2-DNP-D-pentadecylglycine (S6). According to the procedure described for

the preparation of **S3**, **S6** (11.0 mg, 82%) was obtained from **S5** (10.0 mg, 0.025 mmol) as a yellow powder. $[\alpha]^{22}_{D}$ +23.3 (*c* 0.05, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.20 (br s, 1H, Ha), 8.55 (br s, 1H, N*H*-2-pentadecylglycine), 8.44 (br s, N*H*-2-Ala), 6.57 (br s, 2H, CONH_2), 5.48 (br s, 1H, Hb), 4.03 (br s, 2H, H-2-Ala, pentadecylglycine), 2.01 (br s, 2H, $CH_3(CH_2)_{13}CH_2$CH-), 1.67 (br s, 3H, H-3-Ala), 1.24 (m, 26H, $CH_3(CH_2)_{13}CH_2$CH-), 0.87 (br s, 3H, $CH_3(CH_2)_{13}CH_2CH_2$CH-); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.4, 176.7, 148.1, 147.0, 129.2, 125.1, 124.5, 93.0, 57.0, 53.8, 32.1, 31.9, 29.7, 29.5, 29.4, 29.1, 25.8, 22.7, 18.8, 14.1; ESIMS-LR *m/z* 538 $[(M - H)^-]$; ESIMS-HR calcd for $C_{26}H_{42}N_5O_7$ 536.3084, found 536.3071.

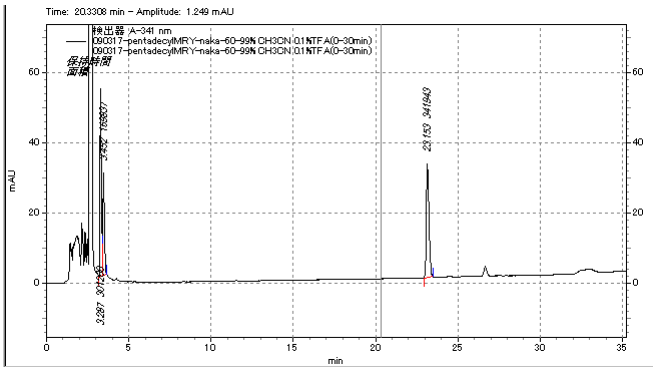
Amino acid analysis of 7b using Marfey's reagent. Compound **7b** (2.60 mg, 2.84 μmol) was treated with 6 M *aq.* HCl (1 mL) at 100 °C for 24 h. The resulting mixture was concentrated *in vacuo*. The residue in acetone (1 mL) and saturated *aq.* $NaHCO_3$ (20 μL) was treated with Marfey's reagent (4.4 mg, 0.016 mmol) at 40 °C for 2 h. The crude mixture was dissolved in DMSO (1 mL) and analyzed by HPLC (YMC J′sphere ODS M80, 4.6×150 mm, 0.1% TFA, a linear gradient of MeOH from 60 to 99% for 30 min, 23.2 min). HPLC analysis of a) the authentic **S3**, b) the reaction mixture of hydrolysis products of **7b** with Marfey's reagent, and c) double injection of a) and b) were shown in Figure S1.

Amino acid analysis of 8b using Marfey's reagent. According to the procedure described for the amino acid analysis of **7b**, **8b** was hydrolyzed, reacted with Marfey's reagent, and analyzed by HPLC (YMC J′sphere ODS M80, 4.6×150 mm, 0.1% TFA, a linear gradient of MeOH from 60 to 99% for 30 min, 26.3 min). HPLC analysis of a) the authentic **S6**, b) the reaction mixture of hydrolysis products of **8b** with Marfey's reagent, and c) double injection of a) and b) were shown in Figure S2.

a) Authentic **S3**



b) The reaction mixture of hydrolysis products of **7b** with Marfey's reagent



c) Double injection of authentic **S3** and the reaction mixture obtained from **7b**

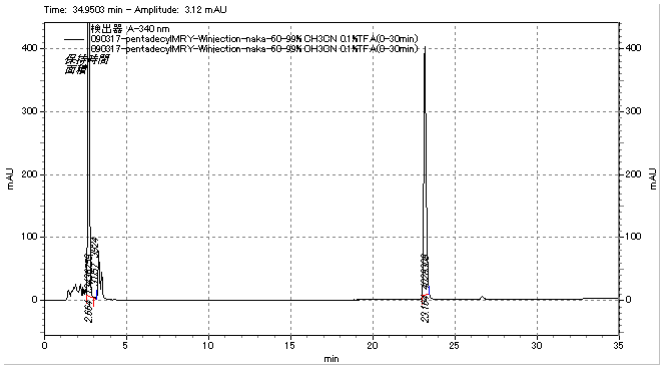
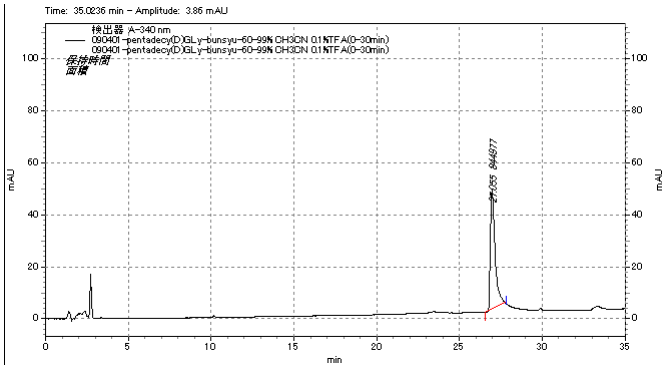
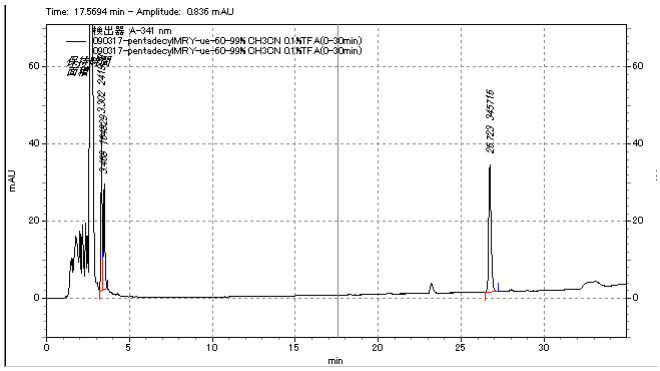


Figure S1. HPLC chromatograms of Amino acid analysis of **7b**

a) Authentic **S6**



b) The reaction mixture of hydrolysis products of **8b** with Marfey's reagent



c) Double injection of the authentic **S6** and the reaction mixture obtained from **8b**

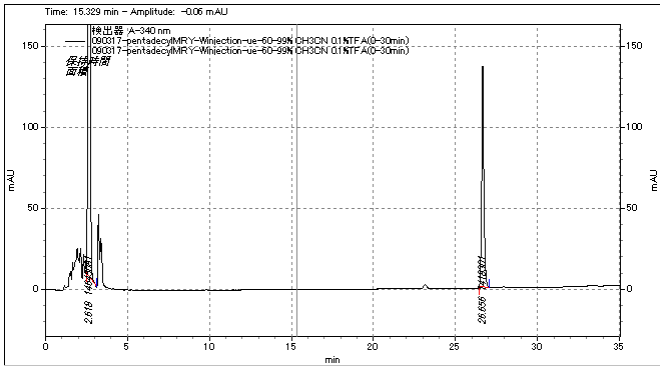
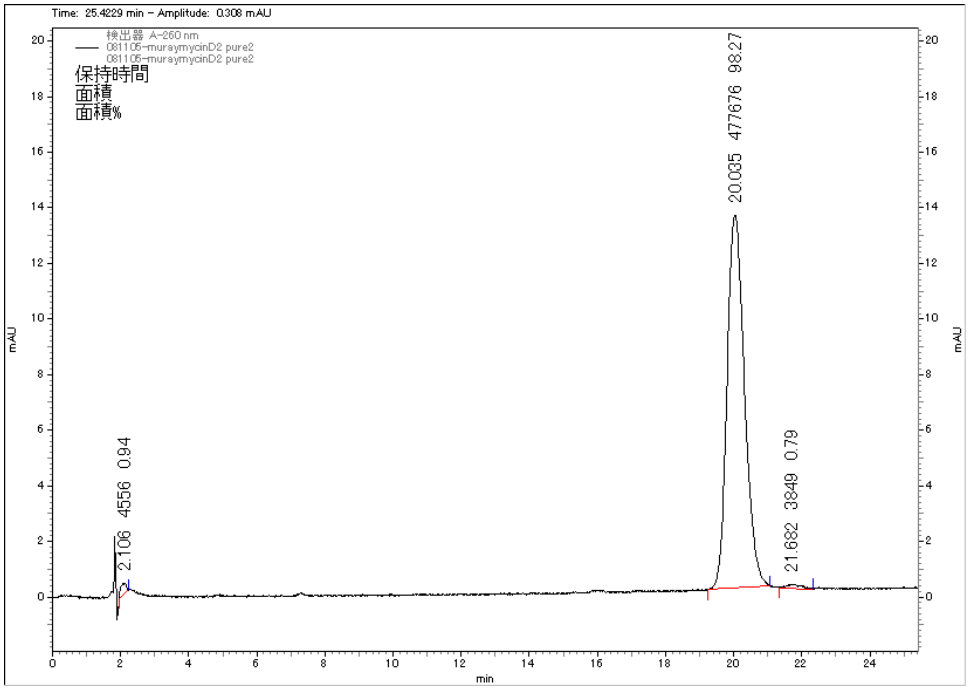


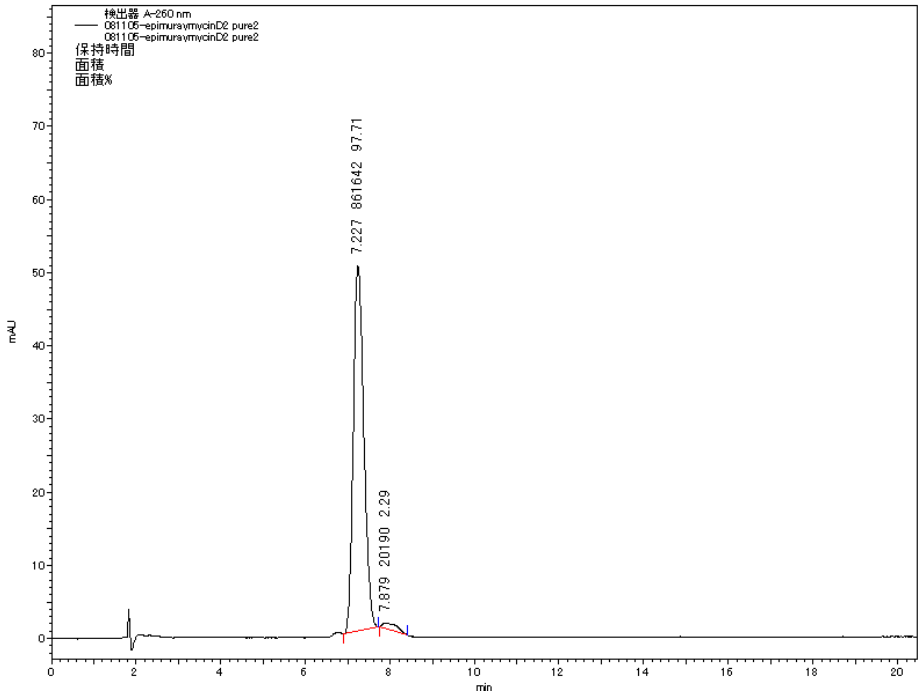
Figure S2. HPLC chromatograms of Amino acid analysis of **8b**

3. *HPLC purity data*

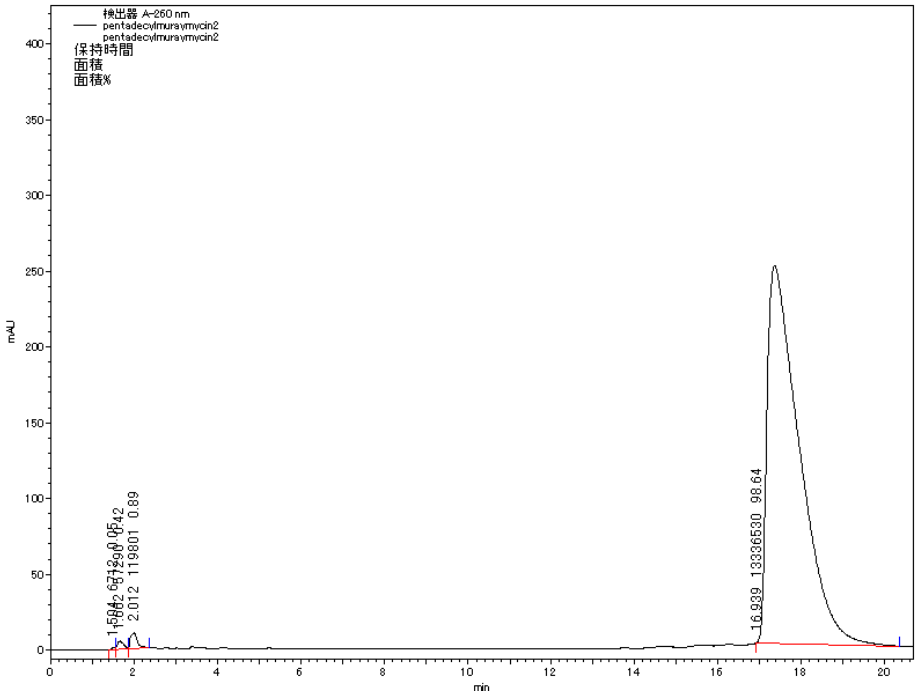
7a: purity 98.3%



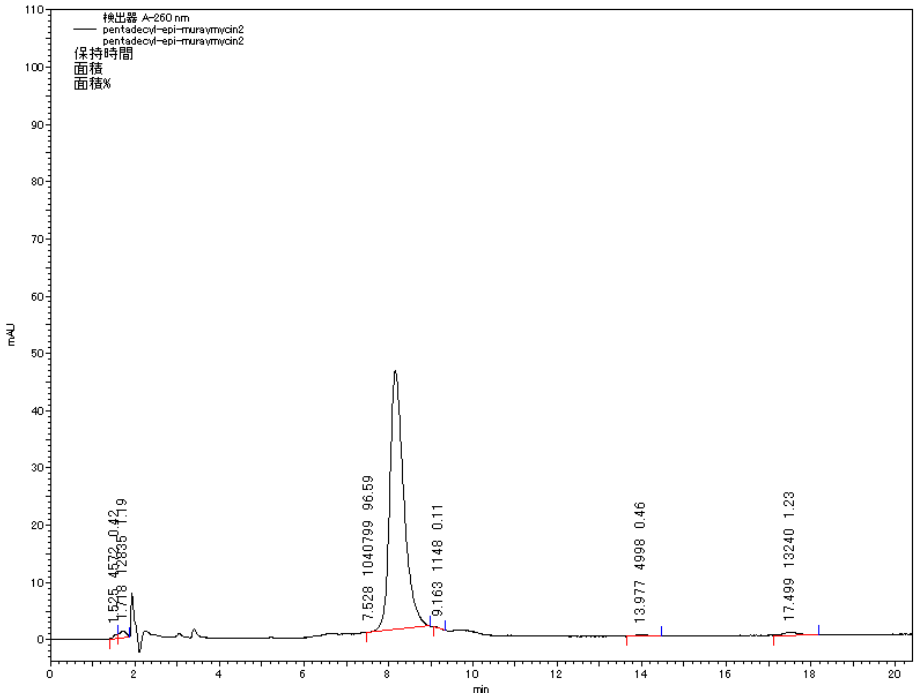
8a: purity 97.7%



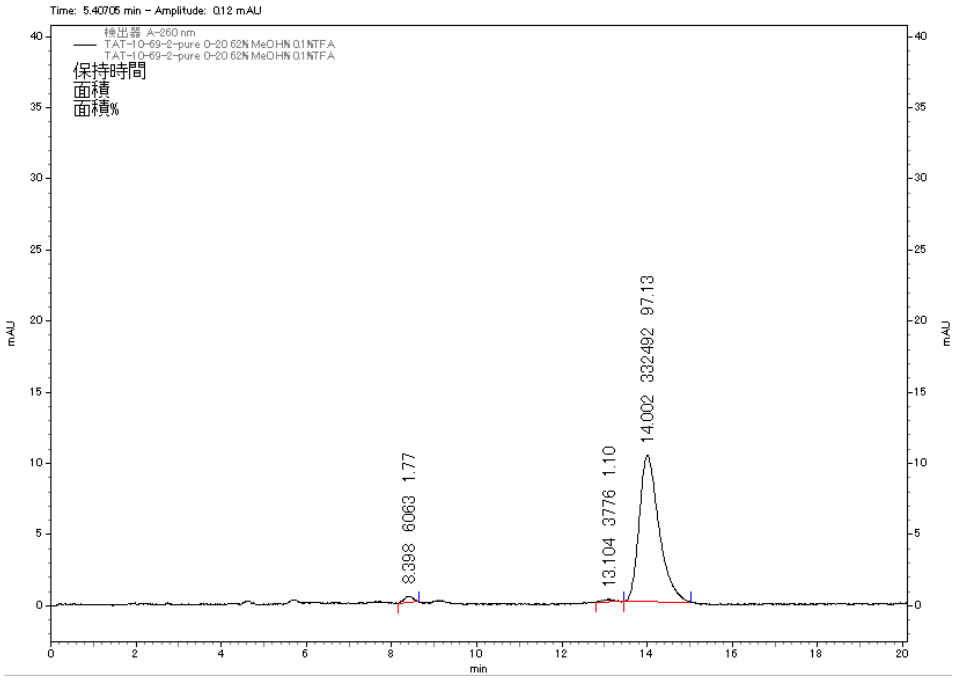
7b: purity 98.6%



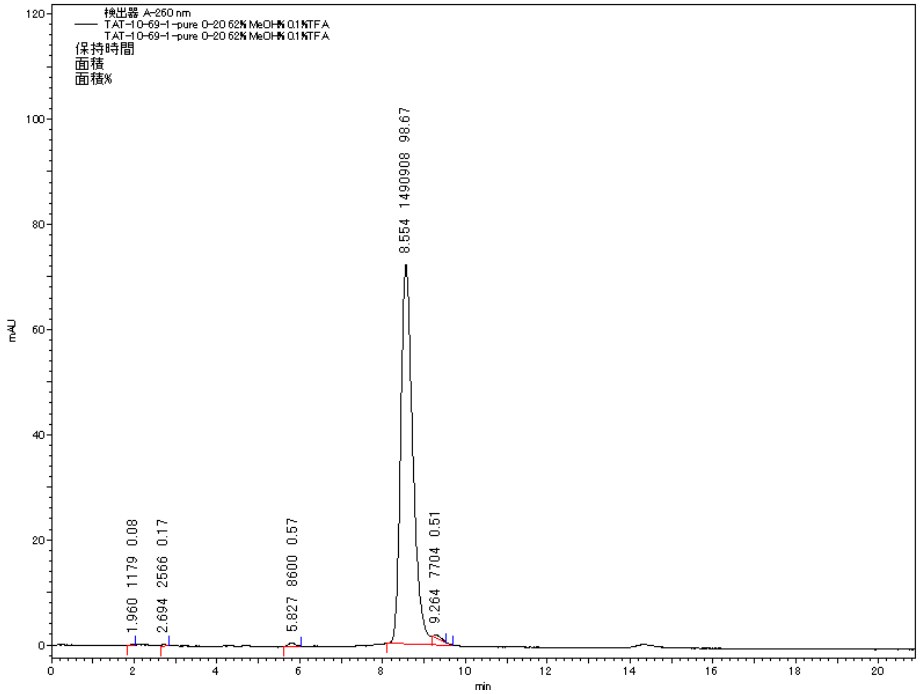
8b: purity 96.6%



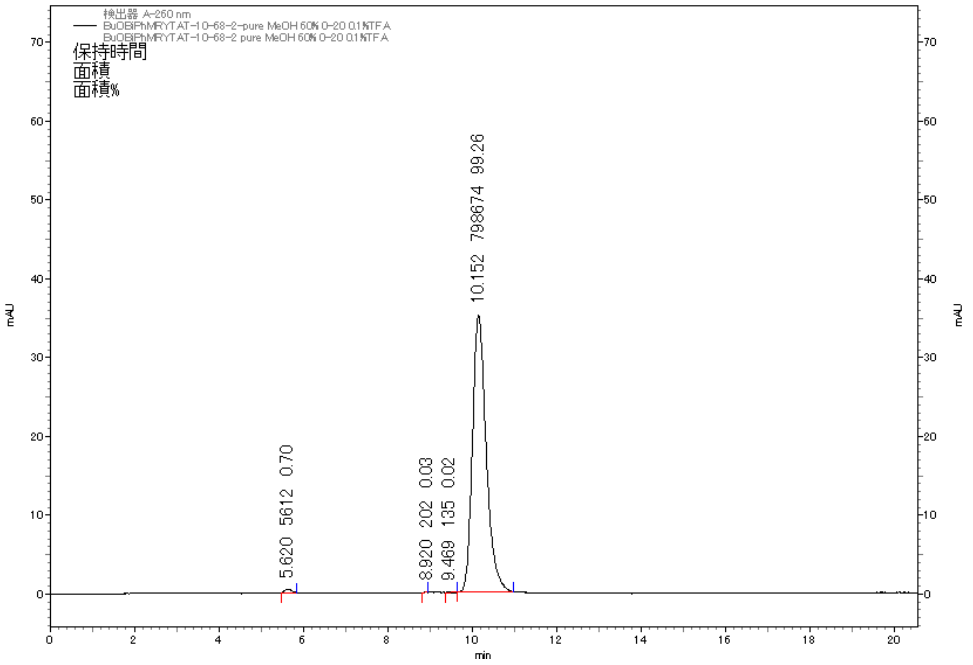
7c: purity 97.1%



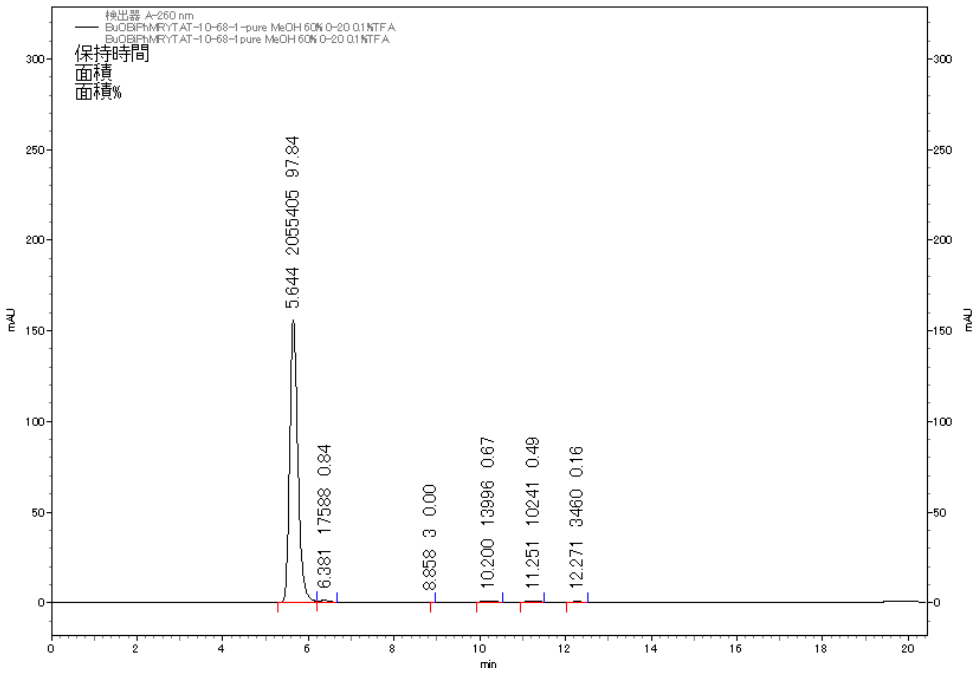
8c: purity 98.7%



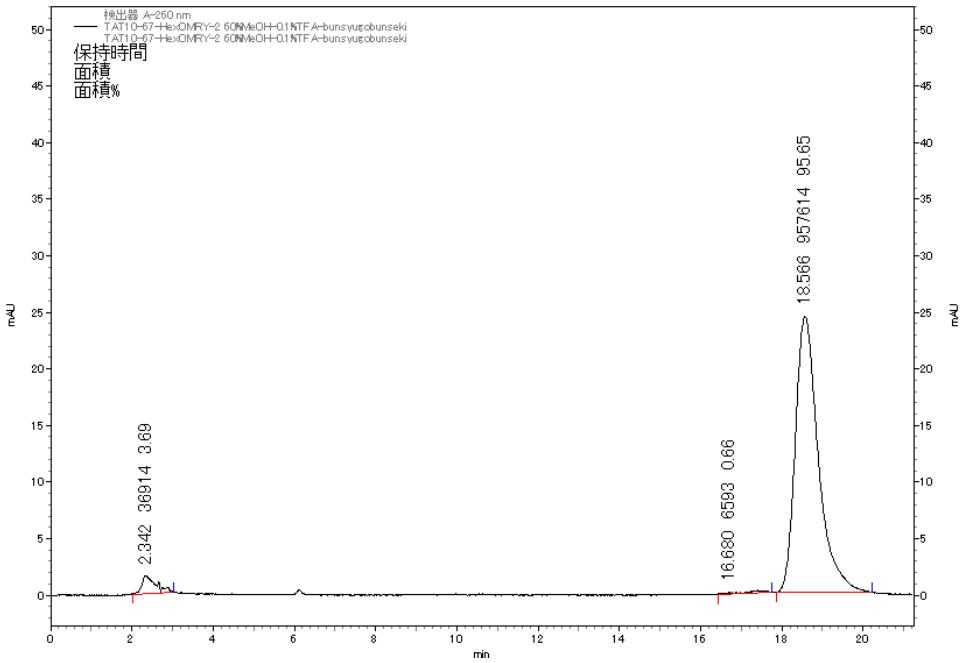
7d: purity 99.3%



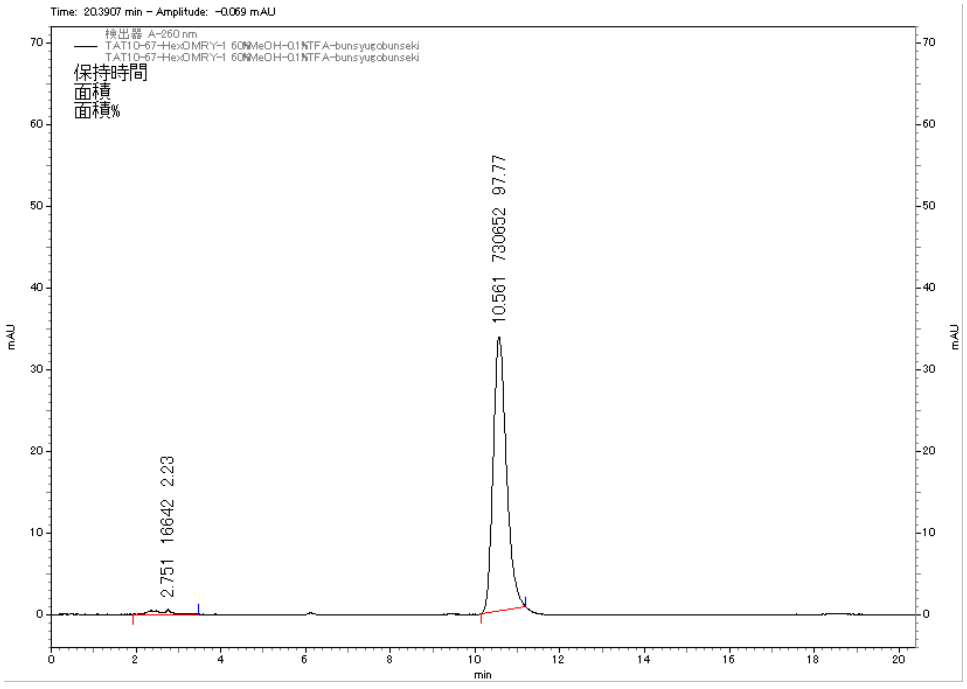
8d: purity 97.8%



7e: purity 99.3%



8e: purity 97.8%



4. Enzymatic Evaluation. The activities of the compounds were tested against purified MraY from *B. subtilis*.[15] The assay was performed in a reaction mixture (10 μL) containing, in final concentrations, 100 mM Tris-HCl, pH 7.5, 40 mM $MgCl_2$, 1.1 mM C_{55}-P, 250 mM NaCl, 0.25 mM UDP-Mur*N*Ac-[^{14}C]pentapeptide (337 Bq), and 8.4 mM *N*-lauroyl sarcosine. The reaction was initiated by the addition of MraY enzyme, and the mixture was incubated for 30 min at 37 °C under shaking with a thermomixer (Eppendorf). The reaction was stopped by heating at 100 °C for 1 min. The radiolabeled substrate UDP-Mur*N*Ac-pentapeptide and reaction product (lipid I, product of MraY) were separated by TLC on silica gel plates LK6D (Whatman) using 2-propanol/conc. ammonium hydroxide/water (6:3:1; v/v/v) as a mobile phase. The radioactive spots were located and quantified with a radioactivity scanner (model Multi-Tracemaster LB285; EG&G Wallac/Berthold). IC_{50} values were calculated with respect to a control assay without the inhibitor. Data represent the mean of independent triplicate determinations.

5. Antibacterial Activity Evaluation

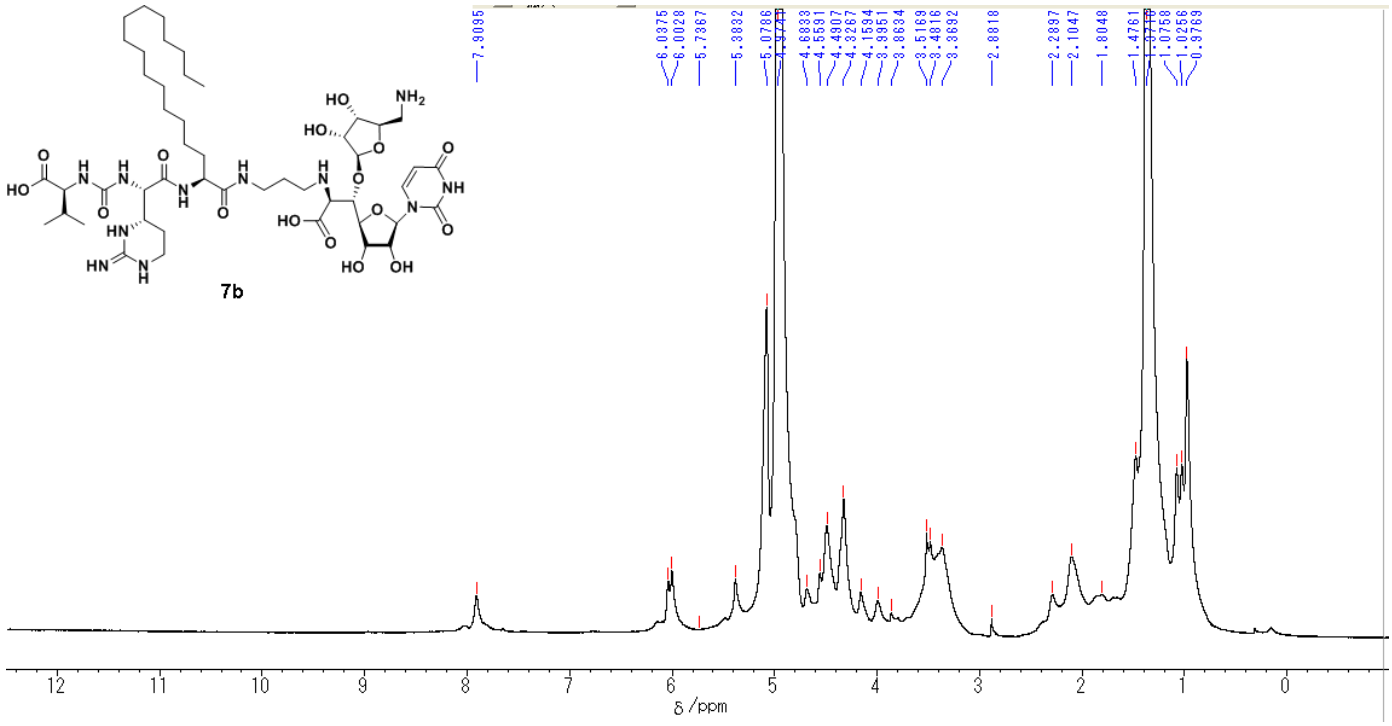
Vancomycin-resistant *Enterococcus faecalis* SR7914 (VanA) and *Entercoccus faecium* SR7917 (VanA), and methicillin-resistant *Staphylococcus aureus* SR3637 were clinical isolates collected from hospitals of Japan and kindly provided by Shionogi & Co., Ltd. (Osaka, Japan).[37] MICs were determined by a microdilution broth method as recommended by the NCCLS (National Committee for Clinical Laboratory Standards, **2000**, National Committee for Clinical Laboratory Standards, Wayne, Pa.) with cation-adjusted Mueller-Hinton broth (CA-MHB) (Becton Dickinson, Sparks, Md.). Serial two-fold dilutions of each compound were made in appropriate broth, and the plates were inoculated with 5×10^4 CFU of each strain in a volume of 0.1mL Plates were incubated at 35 °C for 20 h and then MICs were scored.

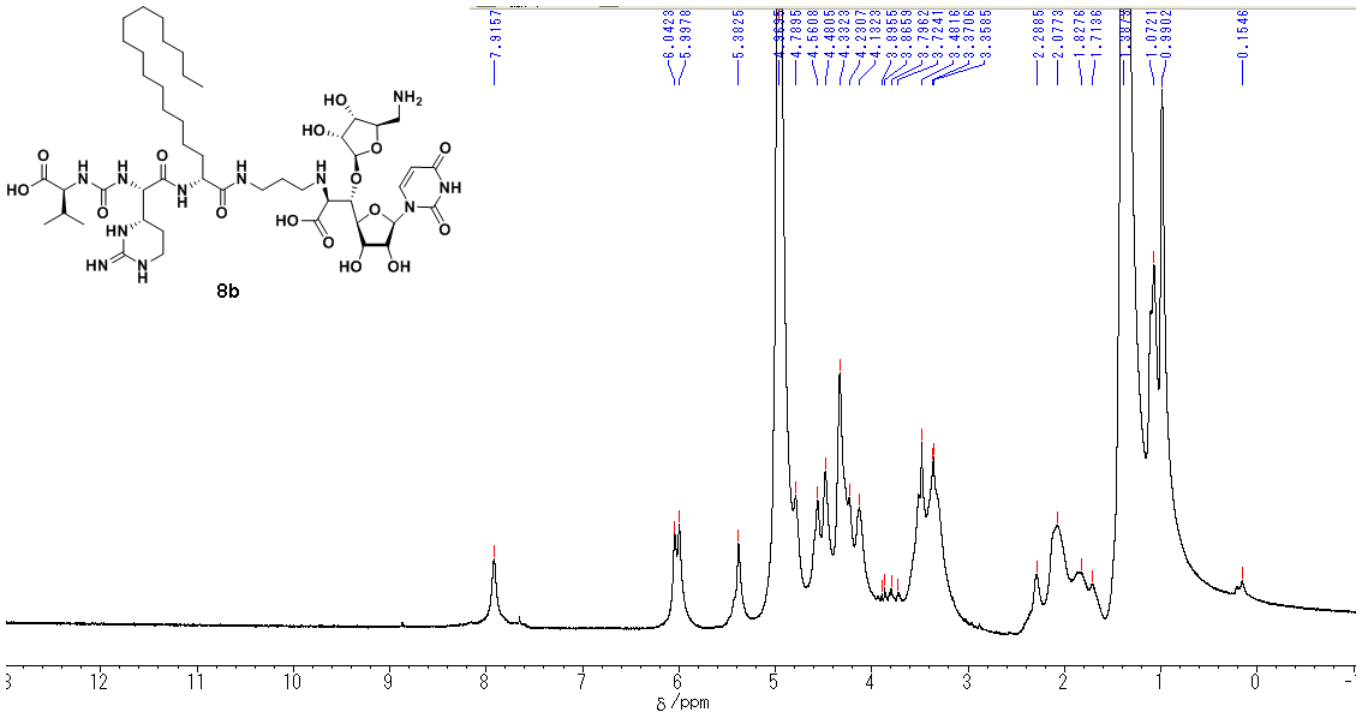
6. Assay of cytotoxicity

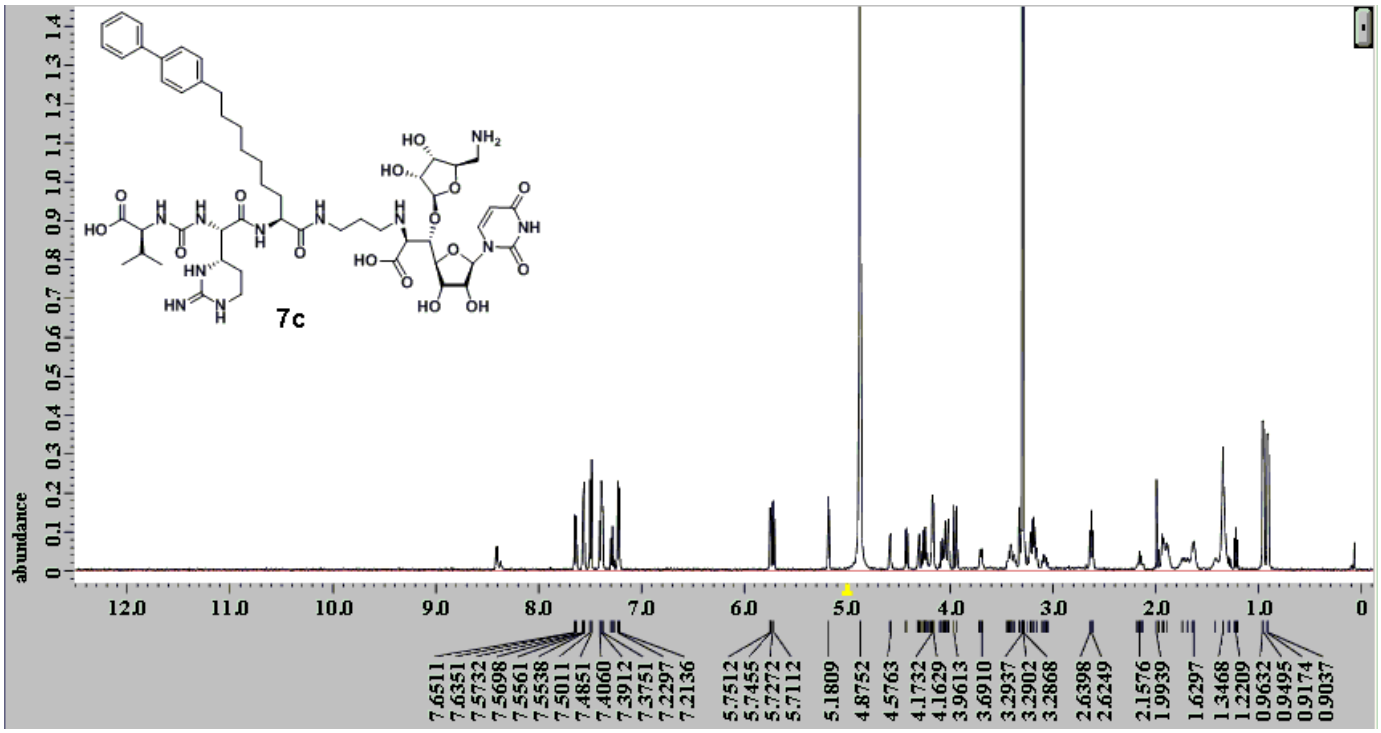
HepG2 cells were suspended in Dulbecco-modified Eagle's medium containing 10% fetal bovine serum, and then seeded in 96-well tissue culture plates at a $1x10^4$ cells/well. After 24 h, cells were treated with varying concentrations of **7b-e**, **8b-e** or tunicamycin (as a positive control) for 48 h. After the treatment, WST-8 reagent (Kishida Chemical) was added to each well, and cells were incubated for 1.5 h at 37 °C. The cell viability was measured as the absorbance at 450 nm, and percentage inhibition in growth was calculated against that of cells treated without those compounds. Tunicamycin exhibited cytotoxicity with the IC_{50} value of 26.8 μg/mL. IC_{50} values of

all the compounds tested were >100 μg/mL.

7. *Spectra of compounds*






7c
abundance
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7.6351
7.5732
7.5698
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7.5538
7.5011
7.4851
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7.3912
7.3751
7.2297
7.2136
5.7512
5.7455
5.7272
5.7112
5.1809
4.8752
4.5763
4.1732
4.1629
3.9613
3.6910
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3.2902
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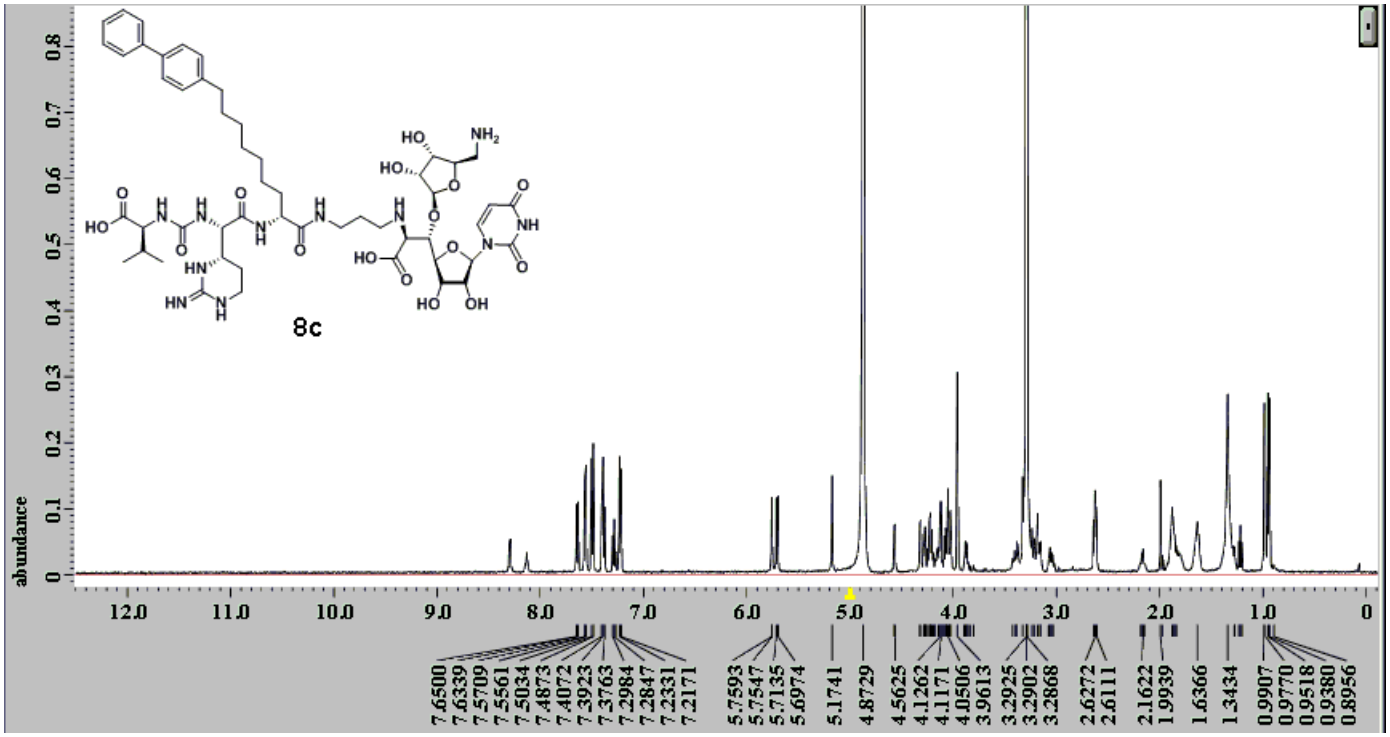
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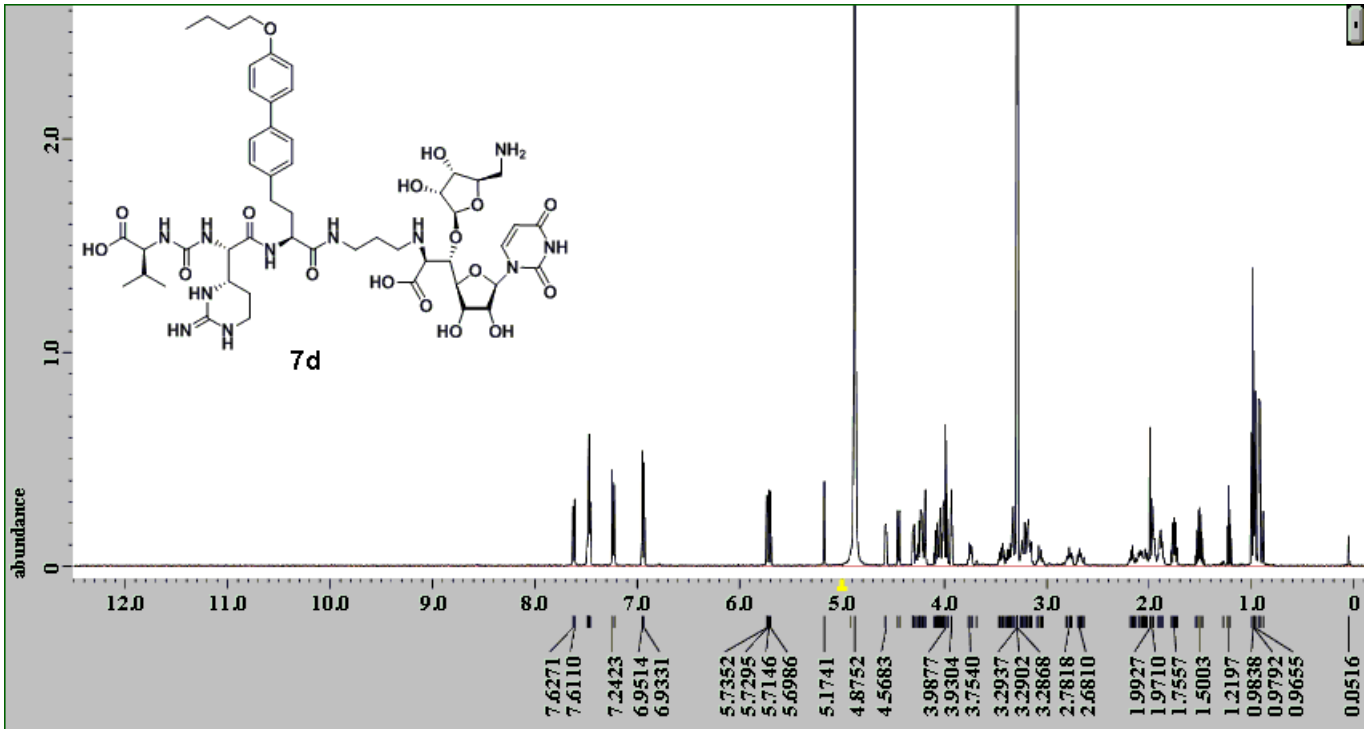
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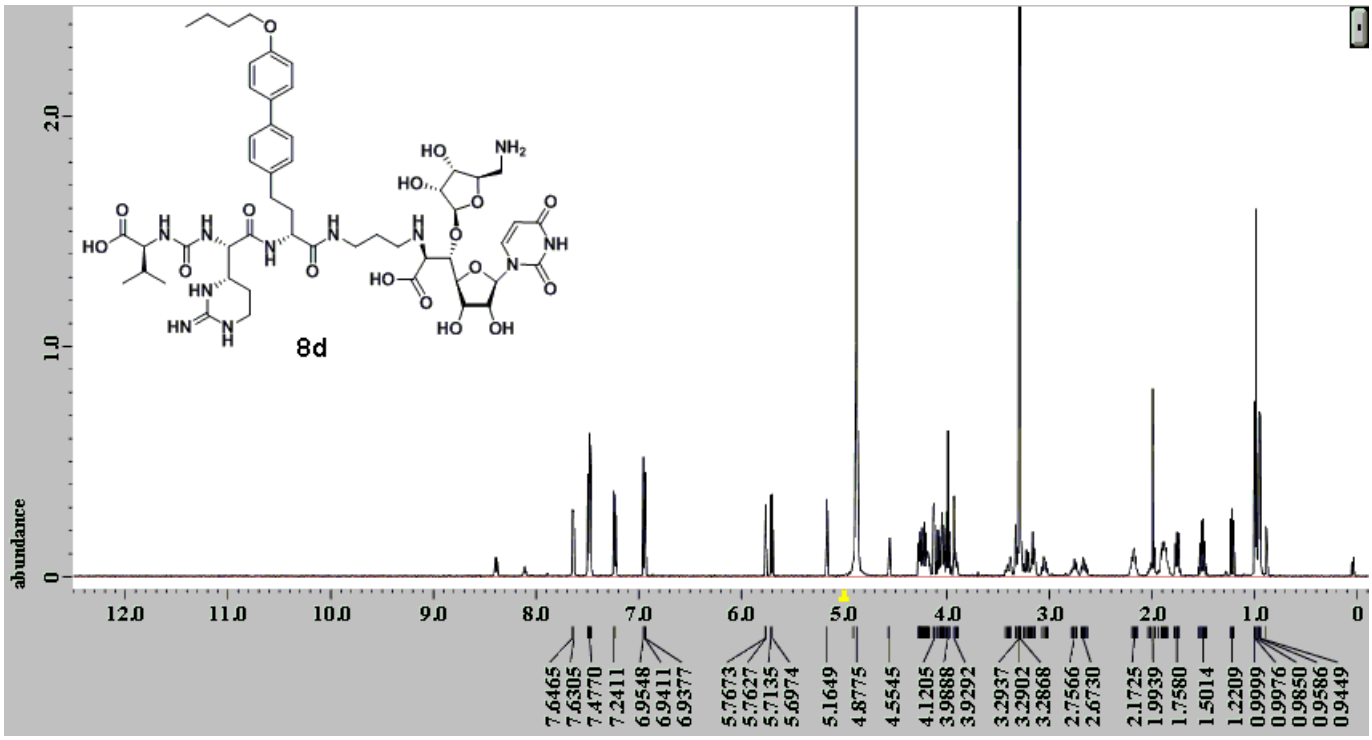
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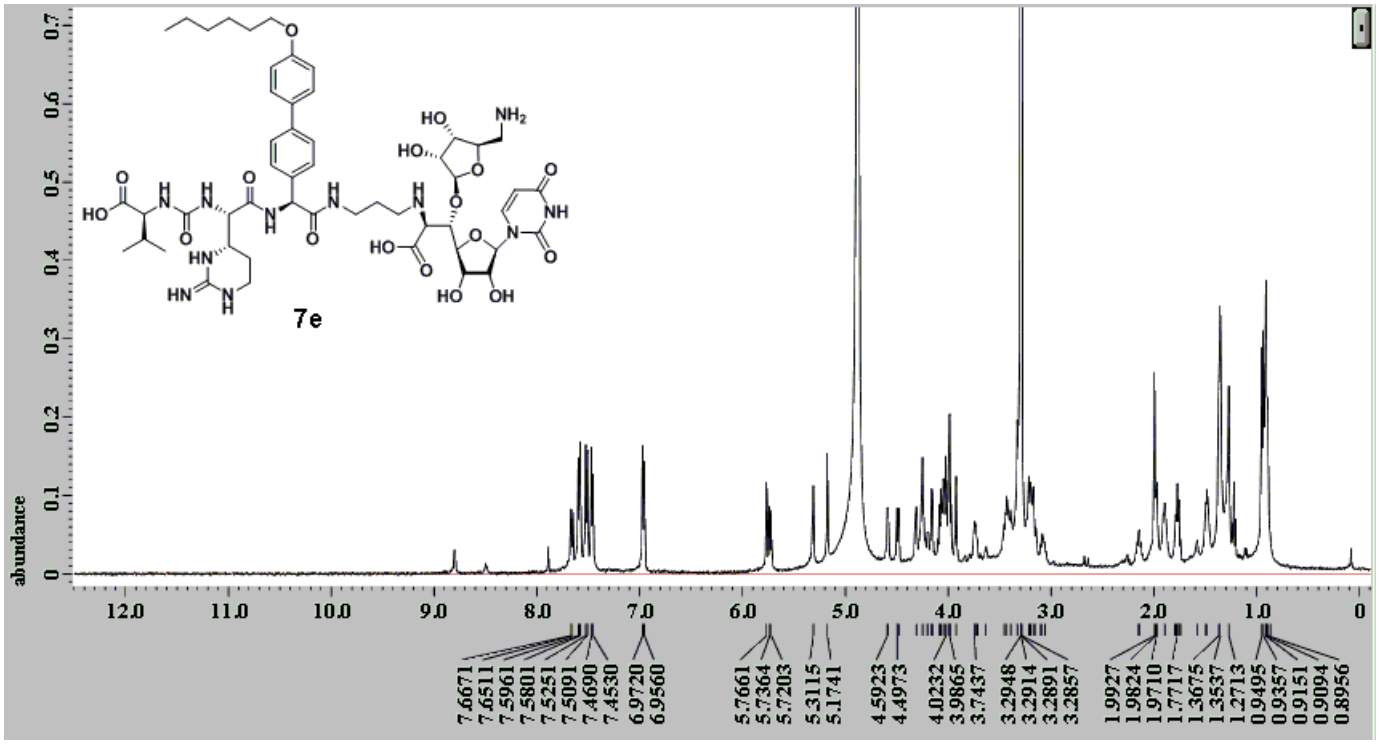
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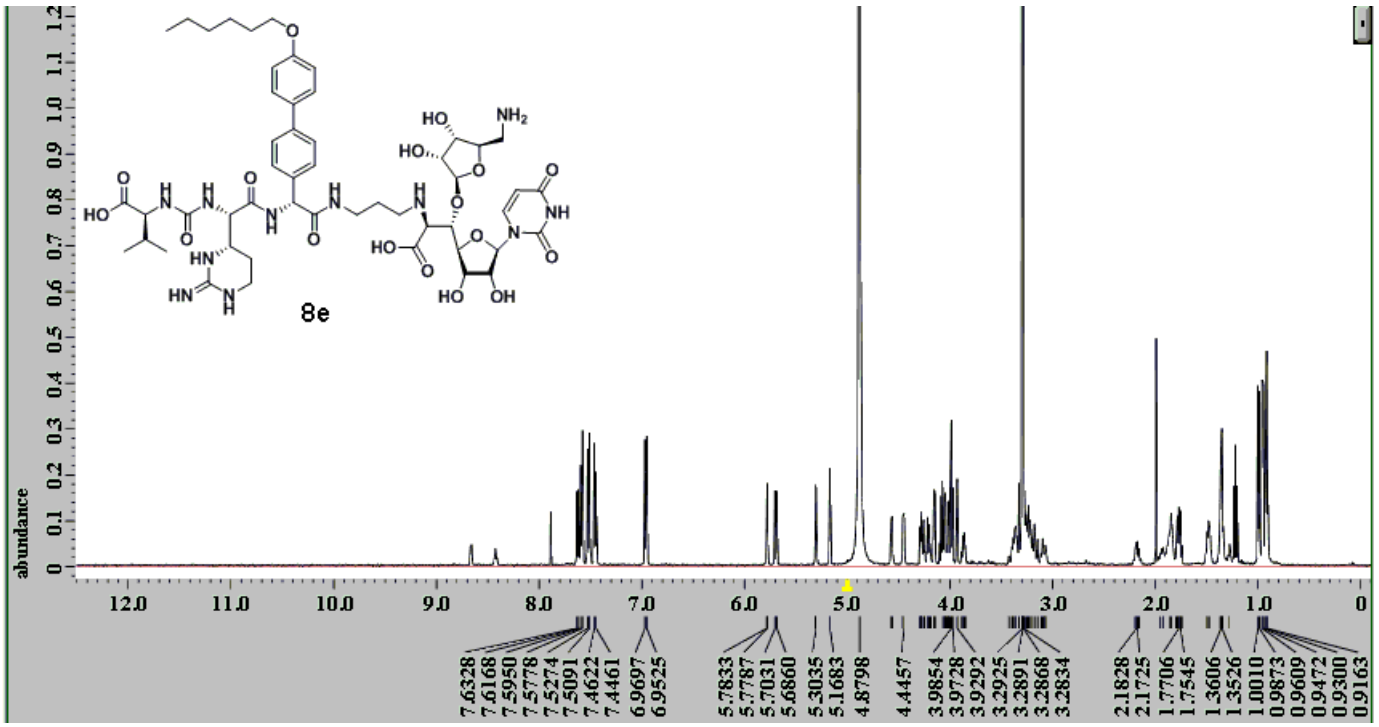
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