Supporting Information

Carbacaprazamycins: Chemically Stable Analogues of Caprazamycin Nucleoside Antibiotics

Satoshi Ichikawa,[a,b,*] Mayumi Yamaguchi,[a] Lee Shang Hsuan,[a] Yuta Kato,[a] and Akira Matsuda.[a]

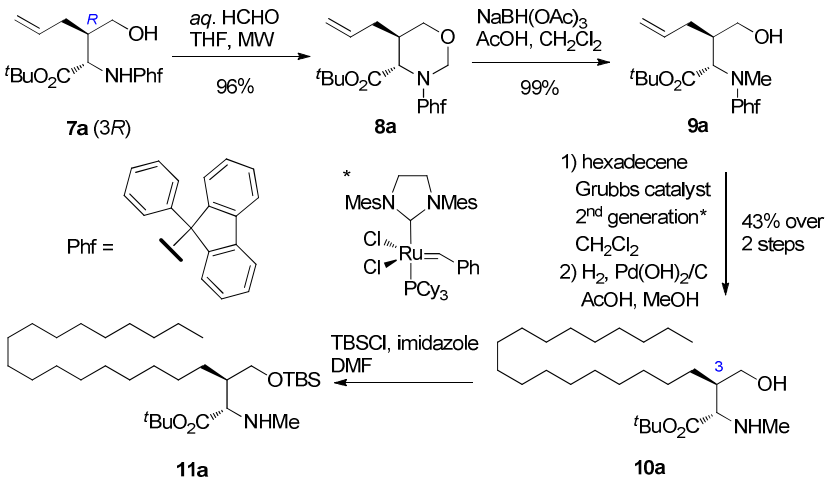
[a] Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan.
[b] Center for Research and Education on Drug Discovery, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

Contents

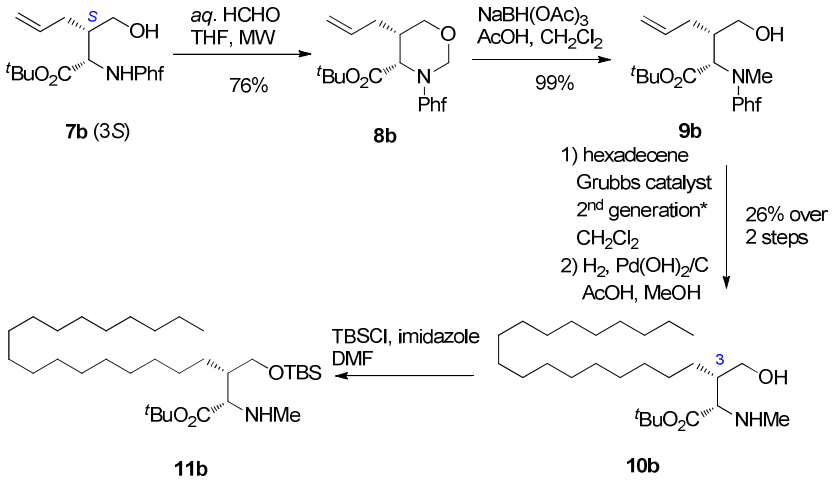
1. Preparation of compounds

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 NMR spectra. MS data were obtained on a JEOL JMS-HX101 or JEOL JMS-700TZ. Purity of all the compounds tested for biological evaluation was confirmed to be >90% by ^{1}H NMR analysis.

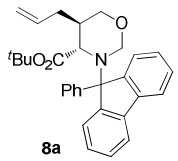
Scheme S1. Preparation of 11a



Scheme S2. Preparation of 11b



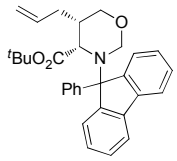
(4*S*,5*R*)-4-*tert*-Butoxycarbonyl-3-(1-phenylfluorenyl)-1,3-oxadinane (8a)



8a

A mixture of (2*S*,3*R*)-*tert*-butyl-3-hydroxymethyl-2-[*N*-methyl-(1-phenylfluorenyl)amino]hex-5-enoate[1] (**7a**, 250 mg, 0.549 mmol) and 37% aqueous HCHO (260 μL, 1.97 mmol) in THF (1.5 mL) was irradiated at 150 °C for 1 h (9 bar). The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (3 x 11 cm, 10% AcOEt–hexane) to afford **8a** (246 mg, 96%) as a colorless syrup. $[\alpha]^{22}_{D}$ +4.6 (c 5.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.56 (dd, 2H, Ar, J = 7.5, 13.8 Hz,), 7.43 (d, 2H, Ar, J = 6.9 Hz), 7.38 (d, 1H, Ar, J = 8.0 Hz), 7.30-7.07 (m, 8H, Ar), 5.45 (dddd, 1H, H-5, $J_{5,4a}$ = 3.5, $J_{5,4b}$ = 6.9, $J_{5,6a}$ = 9.8, $J_{5,6b}$ = 17.2 Hz), 5.08 (d, 1H, H-6a, $J_{6a,5}$ = 9.8 Hz), 4.82 (m, 3H, H-6b, -NCH_2O-), 3.71 (dd, 1H, CH_2OH-a, $J_{CH2OH\text{-}a,3}$ = 2.3, $J_{CH2OH\text{-}a,CH2OH\text{-}b}$ = 11.5 Hz), 3.58 (d, 1H, CH_2OH-b, $J_{CH2OH\text{-}b,CH2OH\text{-}a}$ = 11.5 Hz), 3.10 (s, H-2), 2.05 (m, 1H, H-4a), 1.89 (m, 1H, H-4b), 1.53 (m, H-3), 1.18 (s, 9H, *tert*-Bu); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.9, 148.4, 147.7, 144.5, 140.3, 140.1, 136.7, 128.6, 128.5, 128.4, 127.9, 127.7, 127.4, 127.0, 126.7, 126.3, 120.1, 120.0, 116.8, 80.6, 78.3, 67.1, 58.9, 36.2, 35.7, 28.0; ESIMS-LR *m/z* 490 [(M+Na)$^+$]; ESIMS-HR calcd for $C_{31}H_{33}NNaO_3$ 490.2353, found 490.2345.

(4*S*,5*S*)-4-*tert*-Butoxycarbonyl-3-(1-phenylfluorenyl)-1,3-oxadinane (8b)

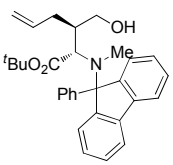


8b

In a manner similar to the synthesis of **8a**, **8b** (66 mg, 76%) was prepared as a white solid from (2*S*,3*S*)-*tert*-butyl-3-hydroxymethyl-2-[*N*-methyl-(1-phenylfluo-renyl)amino]hex-5-enoate[1] (**7b**, 85 mg, 0.18 mmol). $[\alpha]^{22}_{D}$ +99.3 (c 2.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.65 (m, 3H, Ar), 7.39 (m, 4H, Ar), 7.33 (m, 2H, Ar), 7.20 (m, 4H, Ar), 5.43 (dddd, 1H, H-5, $J_{5,4a}$ = 5.2, $J_{5,4b}$ = 6.9, $J_{5,6a}$ = 10.4, $J_{5,6b}$ = 17.2 Hz), 5.25 (d, 1H, -NCH_2O-a, $J_{NCH2O\text{-}a,NCH2O\text{-}b}$ = 11.8 Hz), 4.87 (d, 1H, -NCH_2O-b, $J_{NCH2O\text{-}b,NCH2O\text{-}a}$ = 11.8 Hz), 4.84 (dd, 1H, H-6a, $J_{6a,3}$ = 1.2, $J_{6a,5}$ = 10.4 Hz), 4.79 (dd, 1H, H-6b, $J_{6b,3}$ = 1.7, $J_{6b,5}$ = 17.2 Hz), 3.74 (dd, 1H, CH_2OH-a, $J_{CH2OH\text{-}a,3}$ = 11.5, $J_{CH2OH\text{-}a,CH2OH\text{-}b}$ = 17.3 Hz), 3.71 (dd, 1H, CH_2OH-b, $J_{CH2OH\text{-}b,3}$ = 4.6, $J_{CH2OH\text{-}b,CH2OH\text{-}a}$ = 17.3 Hz), 3.26 (d, 1H, H-2, $J_{2,3}$ = 5.2 Hz), 1.74 (m, 2H, H-4), 1.60 (m, H-3), 1.32 (s, 9H, *tert*-Bu); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.9, 148.7, 147.7, 143.8, 140.5, 139.9, 135.4, 128.6, 128.5, 128.0, 127.7, 127.7, 127.5, 127.0, 126.9, 120.0, 119.9, 116.4, 80.6, 78.0, 67.6, 58.0, 35.4, 32.8, 28.1; ESIMS-LR *m/z* 490 [(M+Na)$^+$]; ESIMS-HR calcd for $C_{31}H_{33}NNaO_3$ 490.2353, found 490.2343.

(2*S*,3*R*)-*tert*-Butyl 3-Hydroxymethyl-2-[*N*-methyl-(1-phenylfluorenyl)amino]hex-5-

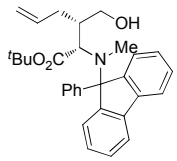
enoate (9a)



9a

A solution of **8a** (170 mg, 0.364 mmol) and AcOH (61 μL, 3.64 mmol) in CH_2Cl_2 (4 mL) was stirred at room temperature for 15 min. Sodium triacetoxyborohydride (227 mg, 1.09 mmol) was then added to the mixture, which was stirred at room temperature for 12 h. The mixture was diluted with AcOEt and washed with saturate aqueous $NaHCO_3$ and saturate aqueous NaCl. The organic layers was dried with Na_2SO_4, filtered, and concentrated in vacuo, and the residue was purified by silica gel column chromatography (2 x 9 cm, 20% AcOEt–hexane) to afford **9a** (169 mg, 99%) as a colorless syrup. $[\alpha]^{21}_D$ –329.3 (c 2.23, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (d, 1H, Ar, J = 7.4 Hz), 7.65 (d, 1H, Ar, J = 7.5 Hz), 7.47 (m, 3H, Ar), 7.32-7.16 (m, 8H, Ar), 5.59 (br m, 1H, H-5), 4.95 (dd, 2H, H-6, $J_{6,4}$ = 1.2, $J_{6,5}$ = 12.6 Hz), 4.01 (dd, 1H, OH), 3.87 (m, 1H, CH_2OH-a), 3.76 (m, 1H, CH_2OH-b), 3.16 (d, 1H, $J_{2,3}$ = 10.9 Hz), 2.83 (s, 3H, NCH_3), 2.26 (m, 1H, H-4a), 1.82 (m, 1H, H-4b), 1.62 (m, H-3), 1.02 (s, 9H, *tert*-Bu); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2, 148.0, 146.1, 143.5, 142.4, 139.7, 135.7, 129.0, 128.7, 128.5, 128.1, 127.8, 127.6, 127.4, 126.8, 125.6, 120.4, 120.1, 117.0, 80.7, 78.7, 64.7, 63.7, 39.4, 32.5, 32.3, 27.7; ESIMS-LR *m/z* 492 [(M+Na)$^+$]; ESIMS-HR calcd for $C_{31}H_{35}NNaO_3$ 492.2509, found 492.2501.

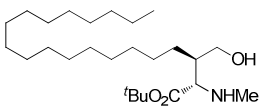
(2*S*,3*S*)-*tert*-Butyl 3-Hydroxymethyl-2-[*N*-methyl-(1-phenylfluorenyl)amino]hex-5-enoate (9b)



9b

In a manner similar to the synthesis of **9a**, **9b** (260 mg, 99%) was prepared as a colorless syrup from **8b** (260 mg, 0.56 mmol). $[\alpha]^{24}_D$ –187.6 (c 5.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (d, 1H, Ar, J = 7.5 Hz), 7.63 (d, 1H, Ar, J = 8.0 Hz), 7.51 (d, 1H, Ar, J = 7.5 Hz), 7.43 (dt, 1H, Ar, J = 1.2, 7.5 Hz), 7.38-7.31 (m, 4H, Ar), 5.85 (dddd, 1H, H-5, $J_{5,4b}$ = 6.9, $J_{5,4a}$ =9.2, $J_{5,6b}$ = 10.3, $J_{5,6a}$ = 16.6 Hz), 5.19 (dd, 1H, H-6a, $J_{6a,5}$ = 1.2, $J_{6a,6b}$ = 16.6 Hz), 3.54 (m, CH_2OH-a), 3.39 (d, 1H, CH_2OH-b, $J_{CH2OH\text{-}b,CH2OH\text{-}a}$ = 12.6 Hz), 3.23(d, 1H, $J_{2,3}$ = 10.4 Hz), 2.84 (s, 3H, NCH_3), 2.24 (dt, 1H, H-4a, $J_{4a,3}$ = $J_{4a,5}$ = 9.2, $J_{4a,4b}$ = 14.3 Hz), 2.01 (ddd, 1H, H-4b, $J_{4b,3}$ = 3.5, $J_{4b,5}$ = 6.9, $J_{4b,4a}$ = 14.3 Hz), 1.28 (m, H-3), 1.02 (s, 9H, *tert*-Bu); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.3, 148.0, 146.9, 144.7, 142.0, 139.5, 137.5, 128.6, 128.5, 128.3, 127.8, 127.5, 127.5, 127.2, 126.8, 126.1, 120.3, 120.0, 116.7, 80.9, 78.5, 61.3, 61.1, 41.9, 32.3, 31.9, 31.7, 27.7, 14.3; ESIMS-LR *m/z* 492 [(M+Na)$^+$]; ESIMS-HR calcd for $C_{31}H_{35}NNaO_3$ 492.2509, found 492.2502.

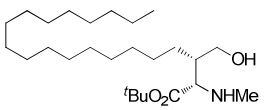
(2*S*,3*R*)-*tert*-Butyl 3-Hydroxymethyl-2-*N*-methylaminoheneicosanate (10a)



10a

A mixture of **9a** (47 mg, 0.1 mmol), hexadecene (260 μL), and Gubbs 2nd catalyst (8.6 mg, 0.01 mmol) in CH_2Cl_2 (1 mL) was heated under reflux for 1.5 h. The mixture was cooled to room temperature and concentrated in vacuo. The residue was passed through a silica gel pad with 50% AcOEt in hexane as an eluent to give a crude heneicosanate, which was used to the next step. A mixture of the heneicosanate, AcOH (0.5 mL) and $Pd(OH)_2$ (10%, 8 mg) in MeOH (1 mL) was vigorously stirred under H_2 atmosphere at room temperature for 2 h. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1 x 10 cm, 75% AcOEt–hexane) to afford **10a** (19 mg, 43%) as a pale yellow syrup. $[\alpha]^{21}_D$ +8.8 (c 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.74 (dd, 1H, C*H*$_2$OH-a, $J_{CH2OH\text{-}a,3}$ = 2.9, $J_{CH2OH\text{-}a,CH2OH\text{-}b}$ = 10.9 Hz), 3.76 (dd, 1H, C*H*$_2$OH-b, $J_{CH2OH\text{-}b,3}$ = 9.2, $J_{CH2OH\text{-}b,CH2OH\text{-}a}$ = 10.9 Hz), 2.97 (d, 1H, $J_{2,3}$ = 9.8 Hz), 2.35 (s, 3H, NC*H*$_3$), 1.76 (m, H-3), 1.49 (s, 9H, *tert*-Bu), 1.36-1.12 (m, 32H, -(C*H*$_2$)$_{16}$CH$_3$), 0.87 (t, 3H, -(CH$_2$)$_{16}$C*H*$_3$, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 81.9, 70.4, 67.3, 42.8, 35.0, 32.1, 30.1, 29.8, 29.8, 29.8, 29.6, 29.5, 28.9, 28.3, 26.8, 22.8, 14.3; ESIMS-LR *m/z* 450 [(M+Na)$^+$]; ESIMS-HR calcd for $C_{26}H_{53}NNaO_3$ 450.3912, found 450.3915.

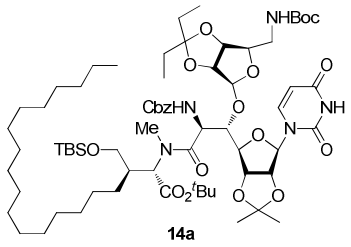
(2*S*,3*S*)-*tert*-Butyl 3-Hydroxymethyl-2-*N*-methylaminoheneicosanate (10b)



10b

In a manner similar to the synthesis of **10a**, **10b** (59 mg, 26%) was prepared as a colorless syrup from **9b** (250 mg, 0.53 mmol). $[\alpha]^{23}_D$ –22.9 (c 0.62, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.62 (br s, 2H, O*H*, N*H*), 3.83 (dd, 1H, C*H*$_2$OH-a, $J_{CH2OH\text{-}a,3}$ = 2.1, $J_{CH2OH\text{-}a,CH2OH\text{-}b}$ = 11.5 Hz), 3.71 (dd, 1H, C*H*$_2$OH-b, $J_{CH2OH\text{-}b,3}$ = 5.2, $J_{CH2OH\text{-}b,CH2OH\text{-}a}$ = 11.5 Hz), 3.31 (d, 1H, H-2, $J_{2,3}$ = 3.5 Hz), 2.37 (s, 3H, NC*H*$_3$), 1.88 (m, H-3), 1.53-1.41 (m, 10H, *tert*-Bu, H-4a), 1.29-1.01 (m, 33H, H-4b, -(C*H*$_2$)$_{16}$CH$_3$), 0.86 (t, 3H, -(CH$_2$)$_{16}$C*H*$_3$, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.0, 82.0, 68.1, 65.1, 41.3, 35.4, 32.1, 29.8, 29.7, 29.6, 29.5, 28.2, 27.5, 24.8, 22.8, 14.3; ESIMS-LR *m/z* 428 [(M+H)$^+$]; ESIMS-HR calcd for $C_{26}H_{54}NO_3$ 428.4098, found 428.4093.

***N*-[(1*S*,2*R*)-1-*tert*-Butoxycarbonyl-2-*tert*-butyldimethylsilyloxymethyleicosanyl]-*N*-methyl-6-benzyloxycarbonylamino-1-(3-benzyloxymethyluracil-1-yl)-5-*O*-[5-*tert*-butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-6-deoxy-2,3-*O*-isopropylidene-β-D-*glycero*-L-*talo*-heptofuranuronamide (14a)**

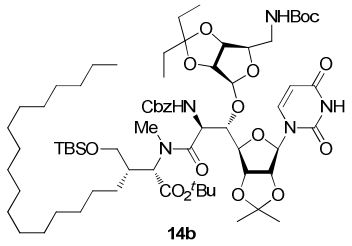


14a

A solution of **10a** (30 mg, 0.068 mmol) and imidazole (14 mg, 0.2 mmol) in CH_2Cl_2 (1 mL) was treated with TBSCl (15 mg, 0.1 mmol) at room temperature for 30 min. Few drops of MeOH was added to the mixture, which was further stirred for 5 min. The mixture was diluted with AcOEt, which was washed with 0.1 M aqueous HCl, saturate aqueous $NaHCO_3$ and saturate aqueous NaCl. The organic layers was dried with Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude **11a**, which was used to the next step without further purification. A mixture of the crude **11a** and **12**[2] (53 mg, 0.068 mmol) in THF (1 mL) was treated sequentially with $NaHCO_3$ (8.5 mg, 0.10 mmol) and DEPBT (30 mg, 0.10 mmol) at 0 °C for 1 h, which was allowed to room temperature and stirred for additional 48 h. The reaction mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The organic phase was washed with saturated aqueous NaCl, dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1 x 11 cm, 33% AcOEt–hexane) to afford **14a** (46 mg, 51%) as a white foam. $[\alpha]^{23}_{D}$ –25.9 (c 0.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.50 (d, 1H, H-6, $J_{6,5}$ = 8.2 Hz), 7.33 (m, 5H, Ph), 5.92 (br s, 1H, N*H*Boc), 5.80 (s, 1H, H-1′), 5.77 (d, 1H, H-5, $J_{5,6}$ = 8.2 Hz), 5.18 (d, 1H, PhC*H*a, J = 12.0 Hz), 5.04 (s, 1H, H-1″), 4.88 (d, 1H, PhC*H*b, J = 12.0 Hz), 4.91 (m, 2H, H-2‴, H-6′), 4.80 (br s, 2H, H-2′, H-3′), 4.58 (d, 1H, H-2″, $J_{2'',3''}$ = 6.3 Hz), 4.48 (d, 1H, H-3″, $J_{3'',2''}$ = 6.3 Hz), 4.23 (m, 1H, H-4′), 4.18 (t, 1H, H-4″, J= 5.2 Hz), 4.09 (t, 1H, H-5′, J = 4.6 Hz), 3.51 (dd, 1H, H-4‴a, $J_{4'''a,3'''}$ = 4.0, $J_{4'''a,4'''b}$ = 10.3 Hz), 3.40 (dd, 1H, H-4‴b, $J_{4'''b,3'''}$ = 5.2, $J_{4'''b,4'''a}$ = 10.3 Hz), 3.22 (m, 1H, H-5″a), 3.12 (s, 3H, NCH_3), 3.07 (m, 1H, H-5″b), 2.10 (m, 1H, H-3‴), 1.58 (m, 4H, C(CH_2CH$_3$)$_2$), 1.51 (s, 3H, acetonide), 1.48-1.43 (m, 13H, C(CH_2CH$_3$)$_2$, *tert*-Bu), 1.41 (m, 13H, C(CH_2CH$_3$)$_2$, *tert*-Bu), 1.30 (s, 3H, acetonide), 1.25 (m, 41H, -(CH_2)$_{16}$CH$_3$, *tert*-Bu), 0.86 (m, 6H, C(CH$_2$CH_3)$_2$), 0.80 (t, 3H, -(CH$_2$)$_{16}$CH_3, J = 6.7 Hz), –0.01 (s, 3H, CH_3), –0.02 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.3, 169.6, 162.8, 156.5, 156.3, 150.1, 141.7, 136.3, 128.6, 128.5, 116.9, 115.1, 112.5, 103.1, 92.8, 86.9, 86.1, 85.5, 84.0, 82.1, 81.8, 80.6, 79.6, 79.2, 67.3, 61.4, 59.5, 51.2, 43.1, 39.8, 32.1, 30.1, 29.8, 29.7, 29.5, 29.4, 28.6, 28.2, 28.0, 27.3, 27.0, 26.0, 25.5, 22.8, 18.4, 14.3, 8.5, 7.4, –5.4, –5.6; ESIMS-LR *m/z* 1337 [(M+Na)$^+$]; ESIMS-HR calcd for $C_{69}H_{115}N_5NaO_{17}Si$ 1336.7949, found 1336.7923.

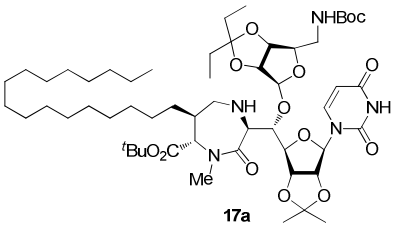
_N_-[(1_S_,2_S_)-1-_tert_-Butoxycarbonyl-2-_tert_-butyldimethylsilyloxymethyleicosanyl]-_N_-methyl-6-benzyloxycarbonylamino-1-(3-benzyloxymethyluracil-1-yl)-5-_O_-[5-_tert_-bu

toxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-6-deoxy-2,3-*O*-isopropylidene-β-D-*glycero*-L-*talo*-heptofuranuronamide (14b)



In a manner similar to the synthesis of **14a**, **14b** (22 mg, 14%) was prepared as a colorless glass from **10b** (48 mg, 0.12 mmol) and **12** (94 mg, 0.12 mmol). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.52 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 7.33 (m, 5H, Ph), 5.85 (s, 1H, H-1′), 5.80 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.72 (br s, 1H, N*H*Boc), 5.18 (d, 1H, PhC*H*a, J = 12.1 Hz), 5.12 (d, 1H, H-6′, J = 9.7 Hz), 5.04 (s, 1H, H-1″), 5.01 (d, 1H, PhC*H*b, J = 12.1 Hz), 4.90 (m, 1H, H-2‴), 4.78 (br s, 2H, H-2′, H-3′), 4.59 (d, 1H, H-2″, $J_{2'',3''}$ = 6.3 Hz), 4.44 (d, 1H, H-3″, $J_{3'',2''}$ = 6.3 Hz), 4.27 (m, 1H, H-4′), 4.15 (m, 2H, H-4″, H-5′), 3.67 (dd, 1H, H-4‴a, $J_{4'''a,3'''}$ = 3.5, $J_{4'''a,4'''b}$ = 11.0 Hz), 3.62 (dd, 1H, H-4‴b, $J_{4'''b,3'''}$ = 4.0, $J_{4'''b,4'''a}$ = 11.0 Hz), 3.21 (m, 1H, H-5″a), 3.09 (s, 3H, NCH_3), 3.06 (m, 1H, H-5″b), 2.04 (m, 1H, H-3‴), 1.60 (m, 4H, C(CH_2CH$_3$)$_2$), 1.52 (s, 3H, acetonide), 1.48-1.43 (m, 13H, C(CH_2CH$_3$)$_2$, *tert*-Bu), 1.42 (m, 9H, *tert*-Bu), 1.31 (s, 3H, acetonide), 1.24 (m, 41H, -(CH_2)$_{16}$CH$_3$, *tert*-Bu), 0.89 (m, 6H, C(CH$_2$CH_3)$_2$), 0.81 (t, 3H, -(CH$_2$)$_{16}$CH_3, J = 6.7 Hz), 0.02 (s, 6H, CH_3 x 2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.6, 169.7, 162.6, 156.3, 150.0, 141.2, 136.3, 128.6, 128.4, 117.3, 115.0, 112.5, 103.3, 92.6, 86.6, 85.9, 85.2, 84.0, 82.0, 81.7, 80.9, 80.2, 79.5, 79.0, 67.2, 61.3, 58.4, 51.4, 43.0, 39.7, 32.0, 30.1, 29.8, 29.5, 28.6 (C2), 28.1, 27.9, 27.3, 26.9, 26.0, 25.4, 22.8, 18.4, 14.3, 8.5, 7.5, 0.1, −5.4, −5.5; ESIMS-LR *m/z* 1337 [(M+Na)$^+$]; ESIMS-HR calcd for $C_{69}H_{115}N_5NaO_{17}Si$ 1336.7949, found 1336.7930.

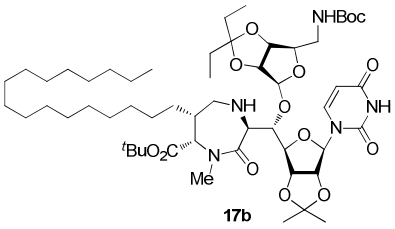
Compound 17a



A solution of **14a** (49 mg, 0.037 mmol) in MeCN (2 mL) was treated with 3HF·Et$_3$N (62 μL, 0.37 mmol) at room temperature for 6 h. The mixture was diluted with AcOEt, which was washed with saturated aqueous NaCl and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give a crude alcohol. A solution of the alcohol in CH_2Cl_2 (2 mL) was treated with Dess-Martin periodinane (44 mg, 0.11 mmol) at room temperature for 1 h. The mixture was diluted with AcOEt, and a mixture of saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ (4:1 5 mL) was added. The whole mixture was vigorously stirred for 15 min, and the organic phase was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give a crude aldehyde. A mixture of the aldehyde and Pd black (20 mg) in

i-PrOH (4 mL) was vigorously stirred under a H_2 atmosphere at room temperature for 1 h. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo*. The residue in CH_2Cl_2 (4 mL) was treated with AcOH (33 μL) and $NaBH(OAc)_3$ (14 mg, 0.11 mmol), and the reaction mixture was stirred at room temperature for 12 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1x11 cm, 33–50% AcOEt–hexane) to afford **17a** (32 mg, 73%) as a white foam: $[\alpha]^{23}_D$ +10.4 (c 1.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.30 (br s, 1H, N*H*-3), 7.34 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.91 (br s, 1H, N*H*Boc), 5.71 (m, 2H, H-1′, H-5), 5.37 (s, 1H, H-1″), 4.85 (dd 1H, H-3′, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 2.3 Hz), 4.60 (m, 2H, H-2′, H-2″), 4.56 (dd, 1H, H-4′, $J_{4',3'}$ = 2.3, $J_{4',5'}$ = 8.0 Hz), 4.48 (d, 1H, H-3″, $J_{3'',2''}$ = 5.8 Hz), 4.39 (dd, 1H, H-4′, $J_{4',5'b}$ = 5.7, $J_{4',5'a}$ = 8.0 Hz), 4.25 (dd, 1H, H-5′, $J_{5',6'}$ = 4.0, $J_{5',4'}$ = 8.0 Hz), 3.87 (d, 1H, H-2″, $J_{2'',3''}$ = 4.6 Hz), 3.44 (m, 1H, H-6′), 3.29 (br s, 1H, H-4‴a), 3.05 (m, 4H, NCH_3, H-5″a), 2.95 (m, 1H, H-4‴a), 2.84 (dd, 1H, H-5″b, $J_{5''b,4''}$ = 2.9, $J_{5''b,5''a}$ = 14.3 Hz), 2.22 (m, 1H, H-3‴), 1.62 (m, 4H, C(CH_2CH$_3$)$_2$), 1.52 (s, 3H, acetonide), 1.48-1.42 (m, 13H, C(CH_2CH$_3$)$_2$, *tert*-Bu), 1.28 (s, 3H, acetonide), 1.25 (m, 41H, -(CH_2)$_{16}$CH$_3$, *tert*-Bu), 0.87 (t, 3H, -(CH$_2$)$_{15}$CH_3, *J* = 6.9 Hz), 0.82 (m, 6H, C(CH$_2$CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.8, 169.7, 162.7, 156.3, 149.7, 141.7, 116.7, 115.0, 111.1, 102.7, 92.8, 87.2, 86.8, 86.6, 84.6, 82.9, 82.3, 81.3, 79.2, 79.1, 67.5, 60.9, 49.4, 43.6, 39.9, 39.2, 32.1, 29.8, 29.8, 29.7, 29.5, 29.3, 29.1, 28.6, 28.1, 28.0, 27.4, 25.6, 22.8, 14.3, 8.48, 7.53; ESIMS-LR *m/z* 1070 [(M+Na)$^+$]; ESIMS-HR calcd for $C_{55}H_{93}N_5NaO_{14}$ 1070.6611, found 1070.6610.

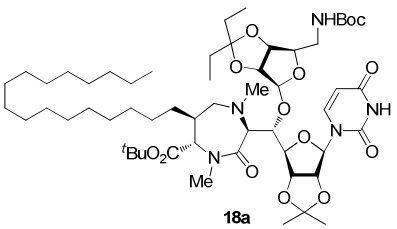
Compound 17b



In a manner similar to the synthesis of **17a**, **17b** (10 mg, 70%) was prepared as a colorless glass from **14b** (18 mg, 0.14 mmol). $[\alpha]^{27}_D$ +12.3 (c 0.99, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.19 (br s, 1H, N*H*-3), 7.35 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.91 (br s, 1H N*H*Boc), 5.71 (m, 2H, H-1′, H-5), 5.37 (s, 1H, H-1″), 4.86 (dd 1H, H-2′, $J_{2',1'}$ = 2.3, $J_{2',3'}$ = 6.3 Hz), 4.62 (m, 2H, H-3′, H-2″), 4.56 (dd, 1H, H-4′, $J_{4',3'}$ = 2.3, $J_{4',5'}$ = 8.1 Hz), 4.48 (d, 1H, H-3″, $J_{3'',2''}$ = 6.3 Hz), 4.37 (dd, 1H, H-4‴, $J_{4'',5''b}$ = 5.8, $J_{4'',5''a}$ = 7.5 Hz), 4.26 (dd, H-5′, $J_{5',6'}$ = 4.0, $J_{5',4'}$ = 8.1 Hz), 3.87 (d, 1H, H-2‴, $J_{2''',3'''}$ = 4.0 Hz), 3.44 (m, 1H, H-6′), 3.29 (m, 1H, H-4‴a), 3.05 (s, 3H, NCH_3), 3.03 (m, 1H, H-5″a), 2.94 (m, 1H, H-4‴b), 2.84 (dd, H-5″b, $J_{5''b,4''}$ = 2.9, $J_{5''b,5''a}$

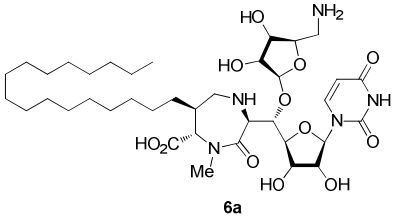
= 14.9 Hz), 2.21 (m, 1H, H-3‴), 1.62 (m, 4H, C(CH_2CH$_3$)$_2$), 1.52 (s, 3H, acetonide), 1.47 (s, 9H, *tert*-Bu), 1.46 (m, 2H, C(CH_2CH$_3$)$_2$), 1.43 (s, 9H, *tert*-Bu), 1.28 (s, 3H, acetonide), 1.25 (m, 32H, -(CH_2)$_{16}$CH$_3$), 0.87 (t, 3H, -(CH$_2$)$_{16}$CH_3, J = 6.9 Hz), 0.81 (m, 6H, C(CH$_2$CH_3)$_2$); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 174.8, 169.7, 162.6, 156.3, 149.7, 141.8, 116.7, 115.0, 111.1, 102.6, 92.9, 87.3, 86.8, 86.6, 86.2, 84.6, 82.9, 81.3, 79.1, 67.5, 60.9, 49.4, 43.6, 39.9, 39.2, 32.1, 30.0, 29.8, 29.7, 29.5, 29.3, 29.1, 28.6, 28.1, 27.4, 25.6, 22.8, 14.3, 8.48, 7.53; ESIMS-LR *m/z* 1070 [(M+Na)$^+$]; ESIMS-HR calcd for $C_{55}H_{93}N_5NaO_{14}$ 1070.6611, found 1070.6612.

Compound 18a



A solution of **17a** (10 mg, 9.5 μmol) in AcOEt (1 mL) was sequentially treated with paraformaldehyde (1.5 mg, 48 μmol), AcOH (20 μL), and NaBH(OAc)$_3$ (8 mg, 38 μmol) at room temperature for 72 h. Saturated aqueous NaHCO$_3$ was added to the mixture, which was extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried (Na$_2$SO$_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1 x 11 cm, 50% AcOEt–hexane) to afford **18a** (7.9 mg, 78%) as a white foam. [α]$^{21}_D$ −121.9 (c 0.72, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ 7.93 (br s, 1H, N*H*-3), 7.70 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 6.68 (br s, 1H, N*H*Boc), 5.92 (d, 1H, H-1′, $J_{1',2'}$ = 3.4 Hz), 5.69 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.24 (s, 1H, H-1″), 4.83 (dd, 1H, H-3′, $J_{3',4'}$ = 4.0, $J_{3',2'}$ = 6.3 Hz), 4.69 (dd, 1H, H-2′, $J_{2',1'}$ = 3.4, $J_{2',3'}$ = 6.3 Hz), 4.56 (d, 1H, H-2″, $J_{2'',3''}$ = 6.3 Hz), 4.49 (m, 2H, H-4′, H-3″), 4.46 (m, 1H, H-5′), 4.40 (m, 1H, H-4″), 3.72 (d, 1H, H-2‴, $J_{2''',3'''}$ = 4.0 Hz), 3.62 (d, 1H, H-6′, $J_{6',5'}$ = 8.6 Hz), 3.24-3.20 (m, 3H, H-5″, H-4‴a), 3.09 (s, 3H, NCH_3), 2.66 (m, 1H, H-4‴b), 2.38 (s, 3H, NCH_3), 2.31 (m, 1H, H-3‴), 1.65 (m, 2H, C(CH_2CH$_3$)$_2$), 1.56 (s, 9H, *tert*-Bu), 1.51 (m, 4H, C(CH_2CH$_3$)$_2$), 1.45 (s, 3H, acetonide), 1.40 (s, 9H, *tert*-Bu), 1.34 (s, 3H, acetonide), 1.30-1.20 (m, 32H, -(CH_2)$_{16}$CH$_3$), 0.89-0.83 (m, 9H, C(CH$_2$CH_3)$_2$, -(CH$_2$)$_{16}$CH_3); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 169.4, 162.5, 156.5, 149.8, 140.7, 116.8, 115.2, 111.8, 10.25, 86.9, 85.8, 84.1, 82.7, 82.5, 80.6, 79.3, 67.2, 60.5, 57.7, 50.0, 43.4, 39.6, 38.5, 32.1, 31.7, 29.8, 29.7, 29.6, 29.5, 29.0, 28.7, 28.6, 28.5, 28.4, 28.1, 27.9, 27.4, 25.4, 22.8, 21.2, 14.3, 14.3, 8.67, 7.57; ESIMS-LR *m/z* 1084 [(M+Na)$^+$]; ESIMS-HR calcd for $C_{56}H_{95}N_5NaO_{14}$ 1084.6768, found 1084.6756.

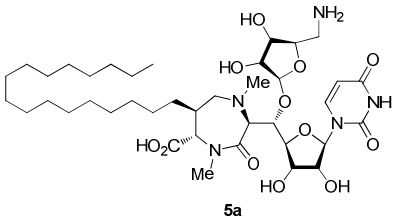
Carbacaprazamycin 6a



6a

A solution of **17a** (3.0 mg, 2.86 μmol) in 80% aqueous TFA (1 mL) was stirred at room temperature for 7 h. The volatiles were removed *in vacuo* to afford synthetic **6a** (2.4 mg, quant., 2 trifluoroacetic acid salts) as a white solid: $[\alpha]^{23}_{D}$ +4.8° (*c* 0.18, MeOH); ^{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-5, $J_{6,5}$ = 8.0 Hz), 5.70 (s, 1H, H-1′), 5.39 (s, 1H, H-1″), 4.62 (br s, 1H, H-2‴), 4.28 (d, 1H, *J* = 4.6 Hz), 4.20-4.18 (m, 2H), 4.11-4.06 (m, 3H), 4.02 (dd, 1H, *J* = 1.7, 4.6 Hz), 3.33-2.76 (overlap, H-5″a,b, H-4‴a,b), 3.16 (s, 3H, CONCH_3), 2.57 (m, 1H, H-3‴), 1.36-1.29 (m, 32H, -(CH_2)$_{16}$CH$_3$), 0.90 (t, 3H, -(CH$_2$)$_{16}$CH_3, *J* = 6.7 Hz); ESIMS-LR (negative mode) *m/z* 782 [(M-Na)$^-$]; ESIMS-HR (NBA, negative mode) calcd for $C_{38}H_{64}N_5O_{12}$ 782.4557, found 782.4578.

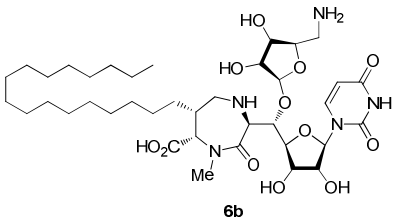
Carbacaprazamycin 5a



5a

In a manner similar to the synthesis of **6a**, **5a** (2.9 mg, quant., 2 trifluoroacetic acid salts) was prepared as a white solid from **18a** (3.0 mg, 2.86 μmol). $[\alpha]^{20}_{D}$ +13.9° (*c* 0.60, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.75 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.71 (d, 1H, H-5, $J_{6,5}$ = 8.0 Hz), 5.61 (d, 1H, H-1′, $J_{1',2'}$ = 1.2 Hz), 5.29 (s, 1H, H-1″), 4.60 (br s, 1H, H-2‴), 4.22 (m, 1H), 4.18 (dd, 1H, *J* = 1.7, 5.2 Hz), 4.14-4.07 (m, 3H), 4.04-4.00 (m, 3H), 3.32-3.28 (overlap, H-5″a,b, H-4‴a,b, NCH_3), 3.19 (s, 3H, CONCH_3), 2.57 (m, 1H, H-3‴), 1.34-1.28 (m, 32H, -(CH_2)$_{16}$CH$_3$), 0.90 (t, 3H, -(CH$_2$)$_{16}$CH_3, *J* = 7.5 Hz); ESIMS-LR *m/z* 798 [(M+H)$^+$]; ESIMS-HR (NBA) calcd for $C_{39}H_{68}N_5O_{12}$ 798.4859, found 798.4877.

Carbacaprazamycin 6b



6b

In a manner similar to the synthesis of **6a**, **6b** (2.8 mg, quant., 2 trifluoroacetic acid salts) was prepared as a white solid from **17b** (3.0 mg, 2.86 μmol). $[\alpha]^{20}_{D}$ +9.6° (*c* 0.03, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.71 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 5.71 (d, 1H, H-5, $J_{6,5}$ = 8.0 Hz), 5.70 (d, 1H, H-1′, $J_{1',2'}$ = 1.2 Hz), 5.37 (d, 1H, H-1″, $J_{1'',2''}$ = 1.3 Hz), 4.63 (br s, 1H, H-2‴), 4.25 (d, 1H, *J* = 5.2 Hz), 4.20-4.18 (m, 2H), 4.12-4.06 (m, 4H), 4.01 (dd, 1H, *J* = 1.2, 4.6 Hz),

3.33-2.76 (overlap, H-4‴a,b), 3.21 (dd, 2H, H-5″a,b, J = 7.5, 14.9 Hz), 3.11 (s, 3H, CONCH_3), 2.53 (m, 1H, H-3‴), 1.31-1.29 (m, 32H, -(CH_2)$_{16}$CH$_3$), 0.90 (t, 3H, -(CH$_2$)$_{16}$CH_3, J = 6.9 Hz); ESIMS-LR (negative mode) *m/z* 782 [(M-Na)$^-$].

2. Fluorescence based MraY assay[3]

Reactions were carried out in 384-well microplate. Reaction mixtures contained, in a final volume of 20 μL, 50 mM Tris-HCl (pH 7.6), 50 mM KCl, 25 mM $MgCl_2$, 0.2% Triton X-100, 8% glycerol, 100 μM C_{55}-P and 100 μM UDP-MurNAc-dansylpentapeptide. The reaction was initiated by the addition of *Staphylococcus aureus* MraY enzyme (11 ng/5 μL/well). After 3-4 h incubation at room temperature, the formation of dansylated lipid I was monitored by fluorescence enhancement (excitation at 355 nm, emission at 535 nm) by using the EnVision^{TM} 2103 Multilabel Plate Reader. The inhibitory effects of the each compound were determined in the MraY assays described above. The mixtures contained 2% dimethyl sulfoxide in order to increase the solubility of the compounds.

3. 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).[4] 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 0.1 mL Plates were incubated at 35 °C for 20 h and then MICs were scored.

4. Conformational analysis

For simplifying the calculation, model compounds A for 3‴-R and B for 3‴-S, which have the reduced number of carbon atom of the lipophilic side chain were used for conformational analysis. The energy-minimized conformations of **A** and **B** were calculated by a conformational search by a Macro-Model program ver 9.2.[5] The ionization status in H_2O at pH 7±1 was first predicted by Epik,[6] which is an empirically based p*K*a predictor and ionization state generator based upon the Hammett and Taft methodologies. These structures were used for the following conformational analysis. Conformational searching was carried out using the Monte Carlo multiple minimum (MCMM) method[7] (100,000 steps), followed by Polak-Ribiere conjugate gradient (PRCG) minimization[8] with the OPLS 2005 force field. Water was chosen for a solvent with the GB/SA model.[9] The other settings were used as default. Structural analysis of energy-minimum conformers calculated for **A** and **B** falls into several conformers within 25 kJ/mol (6.2 kcal/mol). The lowest energy-minimum conformer of **A** or **B** is shown in Figure S1. Finally the calculated conformers were refined by density functional theory (DFT) quantum mechanical calculations at the BL3LYP/6-31G* level.[10] Structural comparison of the global energy-minimum conformers of each **A** (Figure S1c) and **B** (Figure S1d) revealed that the conformation of the diazepanone interconverted between **A** and **B** with the alkyl side chain positioned at

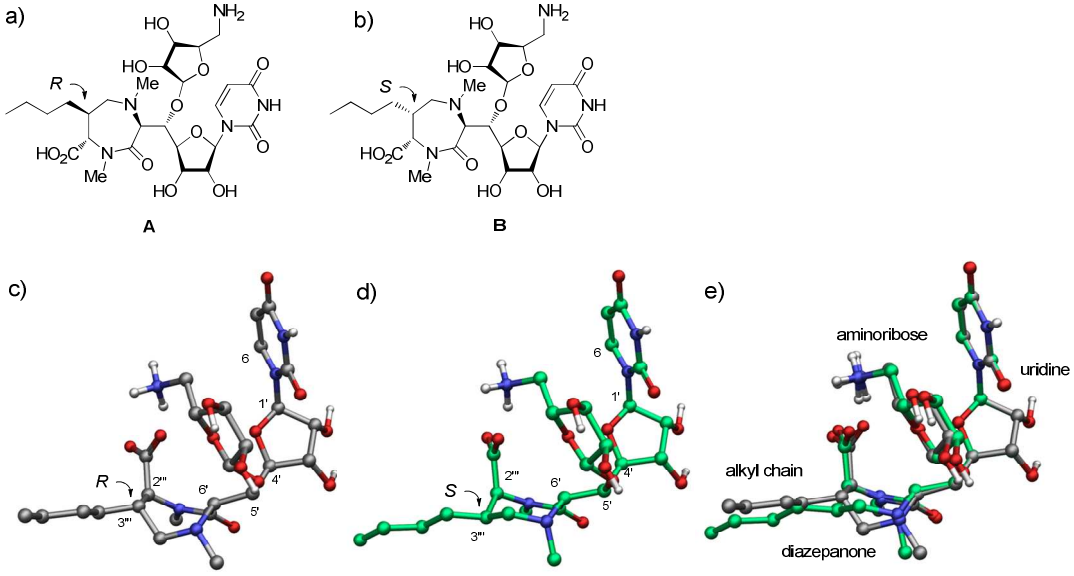


Figure S1. Conformational calculation. Chemical structures of model compounds a) **A** and b) **B**. The global energy-minimum conformations of c) **A** and d) **B**. e) A superposition of c) and d) by merging the uridine moiety. Non-polar hydrogens were undisplayed for clarity.

pseudo-equatorial orientation. As a result, relative spatial orientation of each the uridine,

the aminoribose, and the lipophilic moiety was quite similar (Figure S1e). Presumably this conformational adaptation of the diazepanone moiety could be one of the reasons why both diastereomers exhibited a similar MraY inhibitory and antibacterial activity.

5. Scanning electron microscope protocol

Single colonies of *S. aureus* ATCC29213 were picked into tryptic soy broth and shaken overnight at 30 °C. These cultures were then diluted 1/50 into 5 mL of fresh TSB and shaken at 30°C to OD~0.3. DMSO (negative control), **5a** (3.2 μg/mL, DMSO solution), or vancomycin (1.6 μg/mL, DMSO solution) was added to the cultures, which continued to shake at 30 °C for 2 h. Samples were spun down (7500 x *g*; 8 min) and the resulting pellets were resuspended in 0.25 mL TSB, and 0.25 mL glutaraldehyde fixative (2% formaldehyde and 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4) was added to the sample. After 30 min at room temperature, the fixed samples were spun down. The pellets were washed five times with H_2O. The dried pellets were coated with Pt/Pd, and the sample images were acquired on a JEOL JSM-7400F microscope and shown in Figure S2.

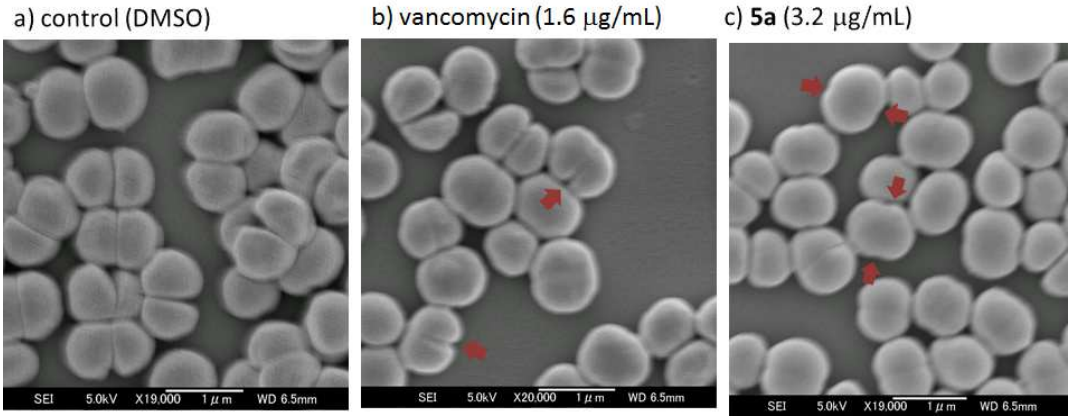


Figure S2. Images of scanning electron microscopy of S. aureus ATCC29213 treated with a) DMSO as a control, b) vancomycin or c) carbacaprazamycin (**5a**).

6. References

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