Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

6″-Thioether Tobramycin Analogues: Towards Selective Targeting of Bacterial Membranes**

Ido M. Herzog, Keith D. Green, Yifat Berkov-Zrihen, Mark Feldman, Roee R. Vidavski, Anat Eldar-Boock, Ronit Satchi-Fainaro, Avigdor Eldar, Sylvie Garneau-Tsodikova, and Micha Fridman**

anie_201200761_sm_miscellaneous_information.pdf

Content

1. Bacterial strains, plasmids, materials, and instrumentation.

The bacterial strains utilized in this study were obtained from various sources. *E. coli* BL21 (DE3) (**M**) and *S. epidermidis* ATCC12228 (**A**) were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). All other *E. coli* BL21 (DE3) strains (**N**-**Q**) were

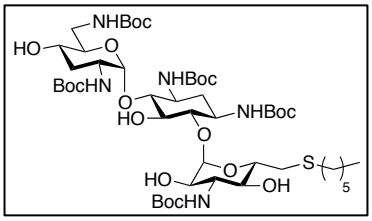
constructed in the Garneau-Tsodikova laboratory. *B. subtilis* 168 (**F**) utilized for preparation of the *B. subtilis* containing the AAC(6')/APH(2") (**G**) was obtained from the Bacillus Genetic Stock Center (Columbus, OH, USA). *S. aureus* NorA (**B**), methicillin-resistant *S. aureus* (MRSA) (**C**), vancomycin-resistant *Enterococcus* (VRE) (**J**), and *E. coli* TolC (**R**) were a gift from Prof. David H. Sherman (University of Michigan). *S. pyogenes* serotype M12 (strain MGAS9429) (**D**) was a gift from Prof. Itzhak Ofek (Faculty of Medicine, Tel Aviv University). *S. mutans* UA159 (**E**) was a gift from Prof. Doron Steinberg (Faculty of Dental Medicine, The Hebrew University of Jerusalem). The *Shigella* clinical isolate 6831 (**T**) was a gift from Prof. Dani Cohen (School of Public Health, Tel Aviv University). *B. anthracis* 34F2 Sterne strain (**I**) was a gift from Prof. Philip C. Hanna (University of Michigan). *B. cereus* ATCC1178 (**H**), *E. faecalis* ATCC29212 (**K**), *L. monocytogenes* ATCC19115 (**L**), *E. coli* MC1061 (**S**), and *S. enterica* ATCC14028 (**U**) were a gift from Prof. Paul J. Hergenrother (University of Illinois at Urbana-Champaign).

B. subtilis containing AAC(6')/APH(2") was prepared as previously reported.[1] The AAC(3)-IV[2] and AAC(6')/APH(2"),[2] AAC(6')-Ib',[3] AAC(6')-IId, Eis,[4] AAC(2')-Ic,[4] and ANT(4')[5] enzymes were purified as previously described. 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), ATP, acetyl-CoA, and inorganic pyrophosphatase were bought from Sigma-Aldrich and used without any further purification. TOB was bought from Tzamal D-Chem Laboratories Ltd. All thiols were purchased from Alfa Aesar. Compound **2** was prepared as previously described by Tor and co-workers.[6] MTT was purchased from TCI America (Portland, OR, USA). Spectrophotometric and colorimetric assays were performed on a multimode SpectraMax M5 plate reader using 96-well plates (Fisher Scientific). Chemical reactions were monitored by TLC (Merck, Silica gel 60 F_{254}).

Visualization was achieved using a cerium-molybdate stain ($(NH_4)_2Ce(NO_3)_6$ (5 g), $(NH_4)_6Mo_7O_{24}\bullet 4H_2O$ (120 g), H_2SO_4 (80 mL), H_2O (720 mL)). Compounds were purified by SiO_2 flash chromatography (Merck, Kieselgel 60). ^1H NMR spectra (including 1D-TOCSY) and ^{13}C NMR spectra were recorded on Bruker Avance™ 400 and 500 spectrometers. High-resolution electron spray ionization (HR-ESI) mass spectra were measured on a Waters Synapt instrument.

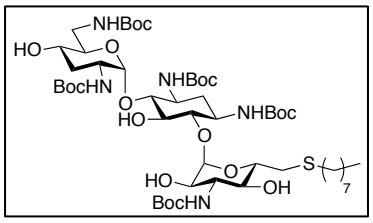
2. Chemical methods.

2.1. Synthesis of Boc-protected 6"-thioether TOB derivatives 3a-r.

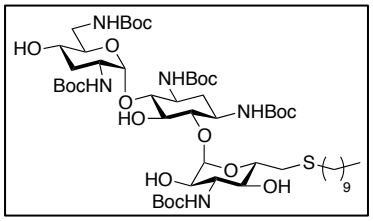


Boc-protected 6"-thioether TOB derivative 3a. To a solution of compound **2** (402 mg, 0.32 mmol) and Cs_2CO_3 (160 mg, 0.49 mmol) in dry DMF (3 mL), 1-hexanethiol (0.230 mL, 1.63 mmol) was added. The reaction mixture was stirred at 55 °C overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.44). The reaction mixture was diluted with EtOAc (10 mL) and the organic layer was washed twice with brine (2x5 mL). The aqueous layer was extracted again with EtOAc (10 mL) and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Further purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3a** (250 mg, 72%) as a white solid: ^1H NMR (400 MHz, CD_3OD) δ 5.09 (br s, 1H, H-1'), 5.04 (br d, J = 2.4 Hz, 1H, H-1"), 4.07 (ddd, J_1 = 9.2 Hz, J_2 = 6.8 Hz, J_3 = 2.2 Hz, 1H, H-5"), 3.74-3.28 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 2.99 (br dd, J_1 = 14.4 Hz, J_2 = 2.1 Hz, 1H, H-6"), 2.62 (m, 1H, H-6"), 2.59 (t, J = 7.4 Hz, 2H, $SC\underline{H}_2(CH_2)_4CH_3$), 2.15 (m, 1H, H-2eq), 2.01 (m, 1H, H-3'eq), 1.60-1.27 (m, 55H, H-2ax, H-3'ax, $5xCO_2C(C\underline{H}_3)_3$, $SCH_2(C\underline{H}_2)_4CH_3$), 0.91 (t, J =

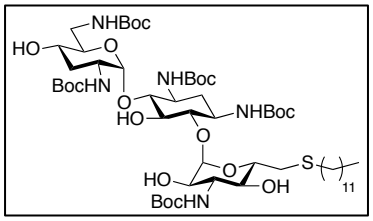
6.6 Hz, 3H, $SCH_2(CH_2)_4C\underline{H}_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 157.9, 157.7 (2C), 100.0 (anomeric C), 99.6 (anomeric C), 84.3, 82.6, 80.7, 80.5, 80.4, 80.2, 77.2, 73.9, 73.5, 72.1, 66.4, 57.1, 51.5, 51.2, 51.0, 41.9, 35.8, 34.7, 34.3, 34.1, 33.0, 32.6, 31.8, 30.8, 30.7, 30.4, 29.6, 28.8, 23.7, 14.5; HRESI-MS *m/z* calc'd for $C_{49}H_{89}N_5O_{18}SNa$ 1090.5821, found 1090.5822 $[M+Na]^+$.



Boc-protected 6"-thioether TOB derivative 3b. Compound **3b** was prepared as **3a** using compound **2** (502 mg, 0.41 mmol), Cs_2CO_3 (200 mg, 0.64 mmol), dry DMF (5 mL), and 1-octanethiol (0.353 mL, 2.03 mmol) at rt overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.53). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3b** (390 mg, 87%) as a white solid: ^1H NMR (400 MHz, CD_3OD) δ 5.09 (br s, 1H, H-1'), 5.04 (br s, 1H, H-1"), 4.07 (ddd, J_1 = 9.2 Hz, J_2 = 6.8 Hz, J_3 = 2.1 Hz, 1H, H-5"), 3.73-3.28 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 2.99 (br dd, J_1 = 14.4 Hz, J_2 = 2.0 Hz, 1H, H-6"), 2.62 (m, 1H, H-6"), 2.59 (t, J = 7.3 Hz, 2H, $SC\underline{H}_2(CH_2)_6CH_3$), 2.15 (m, 1H, H-2eq), 2.01 (m, 1H, H-3'eq), 1.68-1.28 (m, 59H, H-2ax, H-3'ax, 5x$CO_2C(C\underline{H}_3)_3$, $SCH_2(C\underline{H}_2)_6CH_3$), 0.91 (t, J = 6.9 Hz, 3H, $SCH_2(CH_2)_6C\underline{H}_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 157.9, 157.7 (2C), 100.0 (anomeric C), 99.6 (anomeric C), 84.4, 82.6, 80.7, 80.4, 80.2, 77.2, 73.9, 73.5, 72.1, 66.4, 57.1, 51.5, 51.2, 51.0, 42.0, 35.7, 34.7, 34.3, 34.1, 33.0, 30.8, 30.7, 30.4, 30.0, 28.9, 23.7, 14.5; HRESI-MS *m/z* calc'd for $C_{51}H_{93}N_5O_{18}SNa$ 1118.6134, found 1118.6130 $[M+Na]^+$.

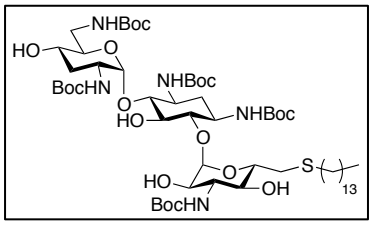


Boc-protected 6"-thioether TOB derivative 3c. Compound **3c** was prepared as **3a** using compound **2** (306 mg, 0.25 mmol), Cs_2CO_3 (145 mg, 0.45 mmol), dry DMF (2 mL), and 1-decanethiol (0.360 mL, 1.74 mmol) at rt overnight. Completion of the reaction was observed by TLC (MeOH:CH_2Cl_2/0.6:9.4, R_f 0.42). Purification by flash column chromatography (SiO_2, MeOH:CH_2Cl_2) gave **3c** (262 mg, 94%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 5.09 (br s, 1H, H-1'), 5.03 (br d, J = 2.7 Hz, 1H, H-1"), 4.07 (ddd, J_1 = 9.2 Hz, J_2 = 6.7 Hz, J_3 = 2.2 Hz, 1H, H-5"), 3.72-3.29 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 2.99 (br dd, J_1 = 14.2 Hz, J_2 = 2.1 Hz, 1H, H-6"), 2.62 (m, 1H, H-6"), 2.59 (t, J = 7.4 Hz, 2H, $SC\underline{H}_2(CH_2)_8CH_3$), 2.16 (m, 1H, H-2eq), 2.01 (m, 1H, H-3'eq), 1.64 (app. q, $J_1 = J_2 = J_3$ = 12.0 Hz, 1H, H-3'ax), 1.61-1.29 (m, 62H, H-2ax, 5x$CO_2C(C\underline{H}_3)_3$, $SCH_2(C\underline{H}_2)_8CH_3$), 0.91 (t, J = 6.6 Hz, 3H, $SCH_2(CH_2)_8C\underline{H}_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 157.9, 157.7 (2C), 100.1 (anomeric C), 99.6 (anomeric C), 84.4, 82.6, 80.7, 80.44, 80.37, 80.2, 77.2, 73.9, 73.6, 72.2, 66.5, 57.1, 51.5, 51.2, 51.0, 42.0, 35.7, 34.7, 34.3, 34.2, 33.1, 30.9, 30.7, 30.4, 30.5, 30.4, 30.0, 28.87, 28.84, 28.80, 23.7, 14.5; HRESI-MS *m/z* calc'd for $C_{53}H_{97}N_5O_{18}SNa$ 1146.6447, found 1146.6449 $[M+Na]^+$.



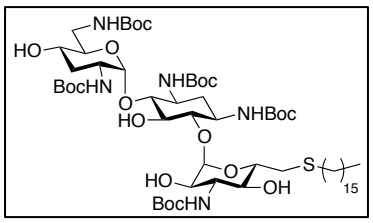
Boc-protected 6"-thioether TOB derivative 3d. Compound **3d** was prepared as **3a** using compound **2** (303 mg, 0.25 mmol), Cs_2CO_3 (144 mg, 0.44 mmol), dry DMF (2 mL), and 1-dodecanethiol (0.414 mL, 1.72 mmol) at rt overnight. Completion of the reaction was observed by TLC (MeOH:CH_2Cl_2/0.6:9.4, R_f 0.42). Purification by flash column chromatography (SiO_2, MeOH:CH_2Cl_2) gave **3d** (260 mg, 92%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 5.09

(br s, 1H, H-1'), 5.04 (br d, J = 2.8 Hz, 1H, H-1"), 4.07 (ddd, J_1 = 9.2 Hz, J_2 = 6.7 Hz, J_3 = 2.2 Hz, 1H, H-5"), 3.71-3.33 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 2.97 (dd, J_1 = 14.2 Hz, J_2 = 2.2 Hz, 1H, H-6"), 2.61 (m, 1H, H-6"), 2.59 (t, J = 7.3 Hz, 2H, $SC\underline{H}_2(CH_2)_{10}CH_3$), 2.16 (m, 1H, H-2eq), 2.00 (m, 1H, H-3'eq), 1.66-1.27 (m, 67H, H-2ax, H-3'ax, $5xCO_2C(C\underline{H}_3)_3$, $SCH_2(C\underline{H}_2)_{10}CH_3$), 0.90 (t, J = 6.6 Hz, 3H, $SCH_2(CH_2)_{10}C\underline{H}_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.2, 157.9, 157.7 (2C), 100.0 (anomeric C), 99.6 (anomeric C), 84.3, 82.5, 80.6, 80.5, 80.4, 80.3, 80.2, 77.1, 73.9, 73.5, 72.1, 66.4, 57.1, 51.5, 51.2, 51.0, 41.9, 35.7, 34.7, 34.3, 34.1, 33.0, 30.9, 30.7, 30.5, 30.4, 29.9, 28.8, 28.7, 23.7, 14.4; HRESI-MS *m/z* calc'd for $C_{55}H_{101}N_5O_{18}SNa$ 1174.6760, found 1174.6757 $[M+Na]^+$.

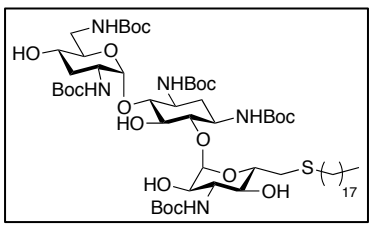


Boc-protected 6"-thioether TOB derivative 3e. Compound **3e** was prepared as **3a** using compound **2** (150 mg, 0.12 mmol), Cs_2CO_3 (72 mg, 0.22 mmol), dry DMF (1 mL), and 1-tetradecanethiol (0.230 mL, 0.84 mmol) at 55 °C overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.53). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3e** (90 mg, 63%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 5.09 (br s, 1H, H-1'), 5.04 (br d, J = 2.8 Hz, 1H, H-1"), 4.07 (ddd, J_1 = 9.2 Hz, J_2 = 7.1 Hz, J_3 = 2.3 Hz, 1H, H-5"), 3.72-3.29 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 2.99 (dd, J_1 = 14.4 Hz, J_2 = 2.0 Hz, 1H, H-6"), 2.61 (m, 1H, H-6"), 2.59 (t, J = 7.3 Hz, 2H, $SC\underline{H}_2(CH_2)_{12}CH_3$), 2.16 (m, 1H, H-2eq), 2.00 (m, 1H, H-3'eq), 1.64 (app. q, $J_1 = J_2 = J_3$ = 12.1 Hz, 1H, H-3'ax), 1.62-1.28 (m, 70H, H-2ax, $5xCO_2C(C\underline{H}_3)_3$, $SCH_2(C\underline{H}_2)_{12}CH_3$), 0.90 (t, J = 7.0 Hz, 3H, $SCH_2(CH_2)_{12}C\underline{H}_3$); ^{13}C NMR (125 MHz, CD_3OD) δ 159.5, 159.3, 158.0, 157.8 (2C), 100.0 (anomeric C), 99.6

(anomeric C), 84.3, 82.6, 80.7, 80.5, 80.4, 80.2, 77.2, 73.9, 73.6, 72.2, 66.5, 57.1, 51.5, 51.2, 51.1, 42.0, 35.7, 34.8, 34.3, 34.2, 33.1, 30.9, 30.80, 30.77, 30.5, 30.4, 30.0, 28.9, 28.84, 28.80, 23.7, 14.5; HRESI-MS *m/z* calc'd for $C_{57}H_{105}N_5O_{18}SNa$ 1202.7073, found 1202.7075 $[M+Na]^+$.

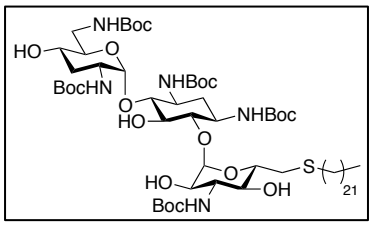


Boc-protected 6"-thioether TOB derivative 3f. Compound **3f** was prepared as **3a** using compound **2** (308 mg, 0.25 mmol), Cs_2CO_3 (105 mg, 0.30 mmol), dry DMF (2 mL), and hexadecanethiol (0.229 mL, 0.75 mmol) at 60 °C overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.61). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3f** (190 mg, 63%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 5.09 (br s, 1H, H-1'), 5.04 (br s, 1H, H-1"), 4.07 (ddd, J_1 = 9.2 Hz, J_2 = 6.7 Hz, J_3 = 2.3 Hz, 1H, H-5"), 3.73-3.30 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 2.99 (dd, J_1 = 14.1 Hz, J_2 = 1.7 Hz, 1H, H-6"), 2.61 (m, 1H, H-6"), 2.59 (t, J = 7.3 Hz, 2H, $SC\underline{H}_2(CH_2)_{14}CH_3$), 2.17 (m, 1H, H-2eq), 2.03 (m, 1H, H-3'eq), 1.64 (app. q, $J_1 = J_2 = J_3$ = 12.0 Hz, 1H, H-3'ax), 1.61-1.29 (m, 74H, H-2ax, $5xCO_2C(C\underline{H}_3)_3$, $SCH_2(C\underline{H}_2)_{14}CH_3$), 0.90 (t, J = 7.0 Hz, 3H, $SCH_2(CH_2)_{14}C\underline{H}_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.4, 159.2, 157.8, 157.6 (2C), 100.0 (anomeric C), 99.5 (anomeric C), 84.1, 82.5, 80.6, 80.3, 80.2, 77.1, 73.9, 73.5, 72.1, 66.4, 57.1, 51.4, 51.2, 50.1, 42.0, 35.7, 34.7, 34.3, 34.1, 33.0, 30.8, 30.4, 30.0, 28.89, 28.86, 28.82, 23.7, 14.5; HRESI-MS *m/z* calc'd for $C_{59}H_{109}N_5O_{18}SNa$ 1230.7386, found 1230.7390 $[M+Na]^+$.



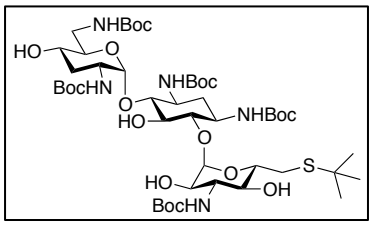
Boc-protected 6"-thioether TOB derivative 3g. Compound **3g** was prepared as **3a** using compound **2** (150 mg, 0.12 mmol),

Cs_2CO_3 (48 mg, 0.15 mmol), dry DMF (1 mL), and 1-octadecanethiol (105 mg, 0.36 mmol) at 55 °C overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.53). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3g** (96 mg, 64%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 5.09 (br s, 1H, H-1'), 5.04 (br d, J = 2.6 Hz, 1H, H-1"), 4.07 (ddd, J_1 = 9.4 Hz, J_2 = 6.8 Hz, J_3 = 2.5 Hz, 1H, H-5"), 3.71-3.29 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 2.99 (dd, J_1 = 14.1 Hz, J_2 = 2.1 Hz, 1H, H-6"), 2.61 (m, 1H, H-6"), 2.59 (t, J = 7.3 Hz, 2H, $SC\underline{H}_2(CH_2)_{16}CH_3$), 2.16 (m, 1H, H-2eq), 2.01 (m, 1H, H-3'eq), 1.64 (app. q, $J_1 = J_2 = J_3$ = 12.0 Hz, 1H, H-3'ax), 1.61-1.28 (m, 78H, H-2ax, $5xCO_2C(C\underline{H}_3)_3$, $SCH_2(C\underline{H}_2)_{16}CH_3$), 0.90 (t, J = 7.0 Hz, 3H, $SCH_2(CH_2)_{16}C\underline{H}_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 157.9, 157.7 (2C), 100.0 (anomeric C), 99.6 (anomeric C), 84.4, 82.6, 80.7, 80.43, 80.37, 80.2, 77.2, 73.9, 73.6, 72.2, 66.5, 57.1, 51.5, 51.2, 51.0, 42.0, 35.7, 34.8, 34.3, 34.1, 33.1, 30.8, 30.4, 30.0, 28.88, 28.85, 28.81, 23.7, 14.5; HRESI-MS m/z calc'd for $C_{61}H_{113}N_5O_{18}SNa$ 1258.7699, found 1258.7695 $[M+Na]^+$.

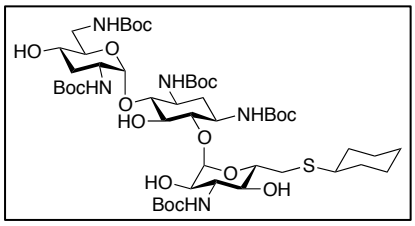


Boc-protected 6"-thioether TOB derivative 3h. Compound **3h** was prepared as **3a** using compound **2** (330 mg, 0.27 mmol), Cs_2CO_3 (105 mg, 0.32 mmol), dry DMF (2 mL), and 1-docosamethiol (275 mg, 0.80 mmol) at 55 °C overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.62). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3h** (197 mg, 57%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 5.09 (br s, 1H, H-1'), 5.04 (br d, J = 3.1 Hz, 1H, H-1"), 4.07 (ddd, J_1 = 9.4 Hz, J_2 = 7.2 Hz, J_3 = 2.5 Hz, 1H, H-5"), 3.71-3.30 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 2.99 (dd, J_1 = 13.9 Hz, J_2 =

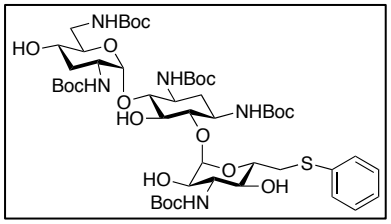
2.0 Hz, 1H, H-6"), 2.61 (m, 1H, H-6"), 2.59 (t, J = 7.3 Hz, 2H, $SC\underline{H}_2(CH_2)_{20}CH_3$), 2.16 (m, 1H, H-2eq), 2.02 (m, 1H, H-3'eq), 1.64 (app. q, $J_1 = J_2 = J_3$ = 12.0 Hz, 1H, H-3'ax), 1.61-1.26 (m, 86H, H-2ax, $5xCO_2C(C\underline{H}_3)_3$, $SCH_2(C\underline{H}_2)_{20}CH_3$), 0.90 (t, J = 7.0 Hz, 3H, $SCH_2(CH_2)_{20}C\underline{H}_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 157.9, 157.7 (2C), 100.1 (anomeric C), 99.6 (anomeric C), 84.4, 82.6, 80.7, 80.44, 80.37, 80.2, 77.2, 73.9, 73.6, 72.2, 66.5, 57.1, 51.5, 51.2, 51.1, 42.0, 35.8, 34.8, 34.3, 34.2, 33.1, 30.8, 30.5, 30.0, 28.89, 28.86, 28.82, 23.7, 14.5; HRESI-MS m/z calc'd for $C_{61}H_{121}N_5O_{18}SNa$ 1314.8325, found 1314.8319 $[M+Na]^+$.



Boc-protected 6"-thioether TOB derivative 3i. Compound **3i** was prepared as **3a** using compound **2** (320 mg, 0.26 mmol), Cs_2CO_3 (157 mg, 0.48 mmol), dry DMF (2 mL), and 2-methyl-2-propanethiol (0.205 mL, 1.81 mmol) at rt overnight. Completion of the reaction was observed by TLC ($MeOH:CH_2Cl_2$/0.6:9.4, R_f 0.44). Purification by flash column chromatography (SiO_2, $MeOH:CH_2Cl_2$) gave **3i** (254 mg, 94%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 5.11 (br s, 1H, H-1'), 5.01 (br d, J = 2.7 Hz, 1H, H-1"), 4.04 (m, 1H, H-5"), 3.70-3.25 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 3.07 (dd, J_1 = 13.4 Hz, J_2 = 2.3 Hz, 1H, H-6"), 2.62 (dd, J_1 = 13.4 Hz, J_2 = 7.7 Hz, 1H, H-6") 2.15 (m, 1H, H-2eq), 1.99 (m, 1H, H-3'eq), 1.65 (app. q, $J_1 = J_2 = J_3$ = 12.0 Hz, 1H, H-3'ax), 1.47-1.43 (m, 46H, H-2ax, $5xCO_2C(C\underline{H}_3)_3$), 1.32 (s, 9H, $SC(C\underline{H}_3)_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 158.0, 157.8 (2C), 100.4 (anomeric C), 99.5 (anomeric C), 84.7, 82.5, 80.7, 80.4, 80.2, 77.2, 74.6, 73.6, 72.7, 72.2, 66.5, 57.1, 51.6, 51.2, 43.1, 42.0, 35.6, 35.5, 34.4, 31.5, 31.2, 30.8, 28.9, 28.8, 25.1, 23.9; HRESI-MS m/z calc'd for $C_{47}H_{85}N_5O_{18}SNa$ 1062.5508, found 1062.5505 $[M+Na]^+$.

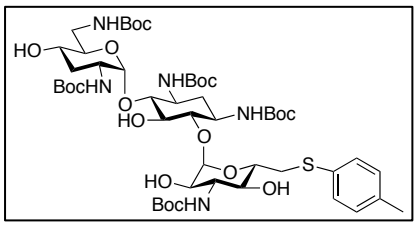


Boc-protected 6"-thioether TOB derivative 3j. Compound **3j** was prepared as **3a** using compound **2** (304 mg, 0.25 mmol), Cs_2CO_3 (144 mg, 0.44 mmol), dry DMF (2 mL), and cyclohexanethiol (0.211 mL, 1.72 mmol) at rt overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.36). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3j** (250 mg, 95%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 5.08 (br s, 1H, H-1'), 5.02 (br d, J = 2.8 Hz, 1H, H-1"), 4.04 (br ddd, J_1 = 9.2 Hz, J_2 = 7.4 Hz, J_3 = 1.8 Hz, 1H, H-5"), 3.71-3.27 (m, 13H, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 3.05 (dd, J_1 = 14.2 Hz, J_2 = 2.4 Hz, 1H, H-6"), 2.76 (m, 1H, $SC_6\underline{H}_{11}$), 2.58 (dd, J_1 = 14.0 Hz, J_2 = 7.4 Hz, 1H, H-6"), 2.15 (m, 1H, H-2eq), 2.00 (m, 3H, H-3'eq, $SC_6\underline{H}_{11}$), 1.76 (m, 2H, $SC_6\underline{H}_{11}$), 1.69-1.25 (m, 53H, H-2ax, H-3'ax, 5x$CO_2C(C\underline{H}_3)_3$, $SC_6\underline{H}_{11}$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 158.0, 157.8, 100.3 (anomeric C), 99.7 (anomeric C), 84.6, 82.7, 80.7, 80.5, 80.4, 80.2, 77.2, 74.2, 73.6, 72.5, 72.2, 66.4, 57.1, 51.6, 51.2, 51.0, 45.2, 42.0, 35.7, 35.0, 34.7, 34.4, 32.8, 28.9, 28.8, 27.0, 26.9; HRESI-MS *m/z* calc'd for $C_{49}H_{87}N_5O_{18}SNa$ 1088.5665, found 1088.5667 $[M+Na]^+$.



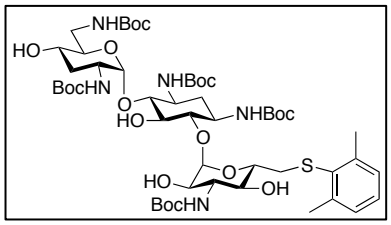
Boc-protected 6"-thioether TOB derivative 3k. Compound **3k** was prepared as **3a** using compound **2** (502 mg, 0.41 mmol), Cs_2CO_3 (200 mg, 0.61 mmol), dry DMF (5 mL), and thiophenol (0.210 mL, 2.03 mmol) at rt overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.36). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3k** (308 mg, 71%) as a white solid:

^{1}H NMR (400 MHz, CD_3OD) δ 7.40 (d, J = 6.0 Hz, 2H, aromatic), 7.30 (app. t, $J_1 = J_2$ = 6.0 Hz, 2H, aromatic), 7.17 (t, J = 5.8 Hz, 1H, aromatic), 5.07 (br s, 1H, H-1'), 5.02 (br s, 1H, H-1"), 4.10 (m, 1H, H-5"), 3.67-3.31 (m, 14H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4", H-6"), 3.00 (br dd, J_1 = 10.1 Hz, J_2 = 5.8 Hz, 1H, H-6"), 2.14 (m, 1H, H-2eq), 2.04 (m, 1H, H-3'eq), 1.67 (app. q, $J_1 = J_2 = J_3$ = 9.6 Hz, 1H, H-3'ax), 1.50-1.39 (m, 46H, H-2ax, 5x$CO_2C(C\underline{H}_3)_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 158.0, 157.8, 157.7,137.9, 130.1, 129.9, 126.9, 100.3 (anomeric C), 99.6 (anomeric C), 84.6, 82.4, 80.7, 80.5, 80.4, 80.2, 77.4, 73.6, 73.2, 72.4, 72.0, 66.5, 57.1, 51.5, 51.2, 51.0, 42.0, 36.3, 35.7, 34.4, 33.1, 32.0, 31.8, 30.7, 30.5, 28.9, 28.8; HRESI-MS *m/z* calc'd for $C_{49}H_{81}N_5O_{18}SNa$ 1082.5195, found 1082.5193 $[M+Na]^+$.

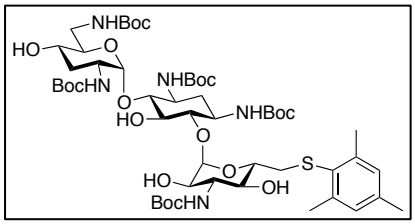


Boc-protected 6"-thioether TOB derivative 3l. Compound **3l** was prepared as **3a** using compound **2** (309 mg, 0.25 mmol), Cs_2CO_3 (146 mg, 0.45 mmol), dry DMF (2 mL), and 4-methylbenzenethiol (218 mg, 1.75 mmol) at rt overnight. Completion of the reaction was observed by TLC (MeOH:CH_2Cl_2/0.6:9.4, R_f 0.42). Purification by flash column chromatography (SiO_2, MeOH:CH_2Cl_2) gave **3l** (247 mg, 92%) as a white solid: ^{1}H NMR (500 MHz, CD_3OD) δ 7.30 (d, J = 6.5 Hz, 2H, aromatic), 7.13 (d, J = 6.1 Hz, 2H, aromatic), 5.09 (br s, 1H, H-1'), 5.01 (br s, 1H, H-1"), 4.05 (m, 1H, H-5"), 3.64-3.31 (m, 14H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4", H-6"), 2.93 (br dd, J_1 = 10.5 Hz, J_2 = 5.6 Hz, 1H, H-6"), 2.30 (s, 3H, $SC_6H_4(C\underline{H}_3)$), 2.17 (m, 1H, H-2eq), 2.03 (m, 1H, H-3'eq), 1.68 (app. q, $J_1 = J_2 = J_3$ = 9.5 Hz, 1H, H-3'ax), 1.48-1.44 (m, 46H, H-2ax, 5x$CO_2C(C\underline{H}_3)_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.4, 159.3, 158.0, 157.8 (2C), 137.1, 134.0,

130.8 (2C), 130.6 (2C), 129.1, 100.4 (anomeric C), 99.6 (anomeric C), 84.7, 82.3, 80.7, 80.5, 80.4, 80.2, 77.4, 73.6, 73.2, 72.4, 72.05, 66.5, 57.1, 51.5, 51.3, 51.0, 42.0, 37.0, 35.7, 34.4, 33.0, 30.7, 30.4, 28.9, 28.8, 21.0; HRESI-MS *m/z* calc'd for $C_{50}H_{83}N_5O_{18}SNa$ 1096.5352, found 1096.5350 $[M+Na]^+$.

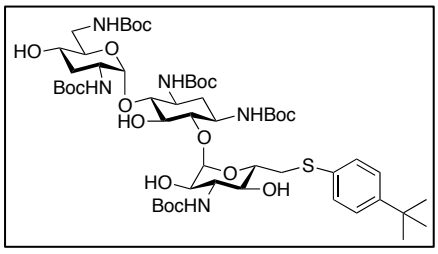


Boc-protected 6"-thioether TOB derivative 3m. Compound **3m** was prepared as **3a** using compound **2** (404 mg, 0.33 mmol), Cs_2CO_3 (160 mg, 0.49 mmol), dry DMF (4 mL), and 2,6-dimethylbenzenethiol (0.217 mL, 1.64 mmol) at rt overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.44). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3m** (275 mg, 77%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.07 (m, 3H, $SC_6\underline{H}_3(CH_3)_2$), 5.13 (br s, 1H, H-1'), 5.06 (br s, 1H, H-1"), 4.19 (m, 1H, H-5"), 3.73-3.27 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 3.05-2.91 (m, 2H, H-6"), 2.54 (s, 6H, $SC_6H_3(C\underline{H}_3)_2$), 2.13 (m, 1H, H-2eq), 1.98 (m, 1H, H-3'eq), 1.64 (m, 1H, H-3'ax), 1.48-1.25 (m, 46H, H-2ax, $5xCO_2C(C\underline{H}_3)_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 157.9, 157.7 (2C), 143.9, 135.8, 129.2, 129.1, 100.0 (anomeric C), 99.3 (anomeric C), 83.7, 82.3, 80.7, 80.4, 80.2, 77.3, 73.9, 73.5, 72.1, 66.5, 57.1, 51.7, 51.1, 51.0, 41.9, 39.0, 35.9, 35.5, 34.3, 33.0, 32.0, 31.8, 30.7, 30.4, 28.8, 22.6; HRESI-MS *m/z* calc'd for $C_{51}H_{85}N_5O_{18}SNa$ 1110.5508, found 1110.5507 $[M+Na]^+$.



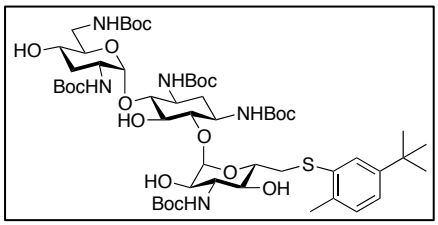
Boc-protected 6"-thioether TOB derivative 3n. Compound **3n** was prepared as **3a** using compound **2** (411 mg, 0.33 mmol), Cs_2CO_3 (163 mg, 0.50 mmol), dry DMF (4 mL), and

2,4,6-trimethylbenzenethiol (0.251 mL, 1.66 mmol) at rt overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.44). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3n** (306 mg, 83%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 6.90 (s, 2H, aromatic), 5.13 (br s, 1H, H-1'), 5.06 (br s, 1H, H-1"), 4.18 (m, 1H, H-5"), 3.73-3.30 (m, 13H, H-1, H-3, H-4, H-5, H-6, H-2' H-4', H-5', H-6' (2H), H-2", H-3", H-4"), 2.98 (dd, J_1 = 12.6 Hz, J_2 = 2.7 Hz, 1H, H-6"), 2.92 (dd, J_1 = 12.6 Hz, J_2 = 5.6 Hz, 1H, H-6"), 2.50 (s, 6H, $SC_6H_2(C\underline{H}_3)_3$), 2.22 (s, 3H, $SC_6H_2(C\underline{H}_3)_3$), 2.15 (m, 1H, H-2eq), 1.99 (m, 1H, H-3'eq), 1.63 (app. q, $J_1 = J_2 = J_3$ = 12.0 Hz, 1H, H-3'ax), 1.46-1.39 (m, 46H, H-2ax, 5x$CO_2C(C\underline{H}_3)_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 158.0, 157.8 (2C), 143.8 (2C), 139.1, 132.4, 129.9 (2C), 100.4 (anomeric C), 99.3 (anomeric C), 83.9, 82.2, 80.7, 80.4, 80.2, 77.4, 74.0, 73.5, 72.1, 66.5, 57.1, 51.7, 51.1, 50.0, 42.0, 39.2, 35.9, 35.5, 34.3, 33.1, 31.8, 30.7, 30.5, 28.9, 28.8, 28.7, 22.5, 21.0; HRESI-MS *m/z* calc'd for $C_{52}H_{87}N_5O_{18}SNa$ 1124.5665, found 1124.5669 $[M+Na]^+$.

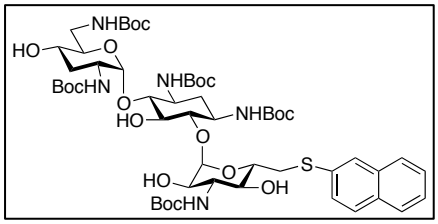


Boc-protected 6"-thioether TOB derivative 3o. Compound **3o** was prepared as **3a** using compound **2** (505 mg, 0.41 mmol), Cs_2CO_3 (200 mg, 0.61 mmol), dry DMF (5 mL), and 4-*tert*-butylbenzenethiol (0.344 mL, 2.04 mmol) at rt overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.38). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3o** (410 mg, 90%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.35 (s, 4H, aromatic), 5.11 (br s, 1H, H-1'), 5.0 (br d, J = 2.2 Hz, 1H, H-1"), 4.07 (m, 1H, H-5"), 3.68-3.29 (m, 14H, H-1, H-3, H-4, H-5, H-6, H-2' H-4', H-5', H-6' (2H), H-2", H-3", H-4", H-6"), 2.96 (br dd, J_1 = 13.7 Hz, J_2 = 7.5

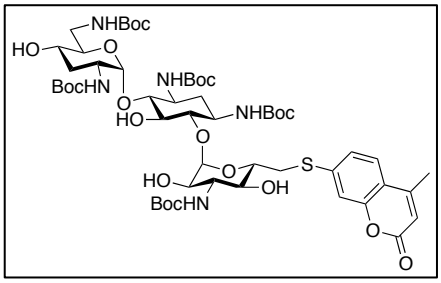
Hz, 1H, H-6"), 2.15 (m, 1H, H-2eq), 2.03 (m, 1H, H-3'eq), 1.68 (app. q, $J_1 = J_2 = J_3 = 11.8$ Hz, 1H, H-3'ax), 1.47-1.29 (m, 55H, H-2ax, 5x$CO_2C(C\underline{H}_3)_3$, $SC_6H_4(C\underline{H}_3)_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.4, 159.3, 158.0, 157.8, 157.7, 150.4, 134.2, 130.4 (2C), 127.1 (2C), 100.4 (anomeric C), 99.6 (anomeric C), 84.8, 82.3, 80.7, 80.44, 80.38, 80.2, 77.4, 73.6, 73.3, 72.0, 72.4, 72.0, 66.5, 57.1, 51.4, 51.2, 51.0, 42.0, 35.7, 35.3, 34.4, 33.0, 31.8, 30.7, 30.4, 28.9, 28.8; HRESI-MS *m/z* calc'd for $C_{53}H_{89}N_5O_{18}SNa$ 1138.5821, found 1138.5823 $[M+Na]^+$.



Boc-protected 6"-thioether TOB derivative 3p. Compound **3p** was prepared as **3a** using compound **2** (331 mg, 0.27 mmol), Cs_2CO_3 (157 mg, 0.48 mmol), dry DMF (2 mL), and 5-*tert*-butyl-2-methylbenzenethiol (0.344 mL, 1.88 mmol) at rt overnight. Completion of the reaction was observed by TLC (MeOH:CH_2Cl_2/0.6:9,4, R_f 0.44). Purification by flash column chromatography (SiO_2, MeOH:CH_2Cl_2) gave **3p** (284 mg, 94%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.44 (d, J = 1.6 Hz, 1H, aromatic), 7.11 (br dd, J_1 = 8.0 Hz, J_2 = 1.3 Hz, 1H, aromatic), 7.07 (br d, J = 8.0 Hz, 1H, aromatic), 5.04 (br s, 2H, H-1', H-1"), 4.20 (m, 1H, H-5"), 3.74-3.29 (m, 14H, H-1, H-3, H-4, H-5, H-6, H-2' H-4', H-5', H-6' (2H), H-2", H-3", H-4", H-6"), 3.06 (dd, J_1 = 12.9 Hz, J_2 = 6.2 Hz, 1H, H-6"), 2.33 (s, 3H, $C\underline{H}_3$), 2.17 (m, 1H, H-2eq), 2.02 (m, 1H, H-3'eq), 1.64 (app. q, $J_1 = J_2 = J_3 = 12.1$ Hz, 1H, H-3'ax), 1.46-1.29 (m, 55H, H-2ax, 5x$CO_2C(C\underline{H}_3)_3$, $C(C\underline{H}_3)_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 158.0, 157.7 (2C), 150.6, 136.6, 135.6, 130.7, 126.7, 123.9, 100.3 (anomeric C), 99.8 (anomeric C), 84.5, 82.8, 80.7, 80.4, 80.2, 77.2, 73.7, 72.2, 72.0, 66.4, 57.1, 51.5, 51.3, 51.1, 41.9, 36.3, 35.5, 34.3, 31.9, 31.8, 28.9, 28.8, 20.3; HRESI-MS *m/z* calc'd for $C_{54}H_{91}N_5O_{18}SNa$ 1152.5978, found 1152.5970 $[M+Na]^+$.



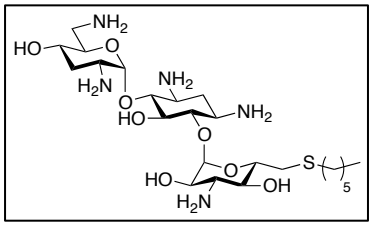
Boc-protected 6"-thioether TOB derivative 3q. Compound **3q** was prepared as **3a** using compound **2** (406 mg, 0.33 mmol), Cs_2CO_3 (160 mg, 0.49 mmol), dry DMF (4 mL), and 2-naphthalenethiol (263 mg, 1.64 mmol) at rt overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/3:2, R_f 0.38). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3q** (347 mg, 95%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.85-7.77 (m, 4H, aromatic), 7.48-7.39 (m, 3H, aromatic), 5.03 (br s, 2H, H-1', H-1"), 4.28 (m, 1H, H-5"), 3.72-3.29 (m, 14H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4", H-6"), 3.12 (br dd, J_1 = 12.9 Hz, J_2 = 6.0 Hz, 1H, H-6"), 2.15-2.03 (m, 2H, H-2eq, H-3'eq), 1.69 (br app. q, $J_1 = J_2 = J_3$ = 11.8 Hz, 1H, 3'ax), 1.60-1.36 (m, 46H, H-2ax, 5x$CO_2C(C\underline{H}_3)_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 159.5, 159.3, 157.9, 157.7, 135.3, 133.1, 129.5, 128.7, 128.2, 127.7, 127.2, 126.6, 100.2 (anomeric C), 99.7 (anomeric C), 84.5, 82.6, 80.7, 80.5, 80.4, 80.2, 77.3, 73.6, 73.2, 72.4, 72.0, 66.4, 57.1, 5 51.3, 51.1, 41.9, 35.9, 35.7, 34.4, 33.1, 31.8, 30.7, 30.5, 28.9, 28.8; HRESI-MS *m/z* calc'd for $C_{53}H_{83}N_5O_{18}SNa$ 1132.5352, found 1132.5354 $[M+Na]^+$.



Boc-protected 6"-thioether TOB derivative 3r. Compound **3r** was prepared as **3a** using compound **2** (222 mg, 0.18 mmol), Cs_2CO_3 (70 mg, 0.21 mmol), dry DMF (2 mL), and 7-mercapto-4-methylcoumarin (104 mg, 0.54 mmol) at 55 °C overnight. Completion of the reaction was observed by TLC (EtOAc:petroleum ether/7:3, R_f 0.33). Purification by flash column chromatography (SiO_2, EtOAc:petroleum ether) gave **3r** (136

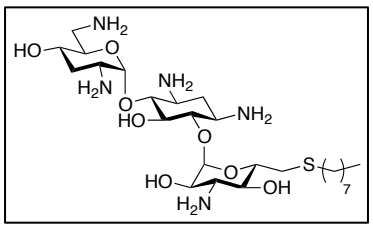
mg, 66%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.66 (d, J = 8.4 Hz, 1H, coumarin ring), 7.36 (s, 1H, coumarin ring), 7.32 (d, J = 8.4 Hz, 1H, coumarin ring), 6.25 (s, 1H, coumarin ring), 5.07 (br s, 1H, H-1'), 4.89 (br s, 1H, H-1"), 4.26 (m, 1H, H-5"), 3.73-3.30 (m, 14H, H-1, H-3, H-4, H-5, H-6, H-2', H-4', H-5', H-6' (2H), H-2", H-3", H-4", H-6"), 3.13 (m, 1H, H-6"), 2.25 (s, 3H, C$\underline{H}_3$ of coumarin), 2.04 (m, 2H, H-2eq, H-3'eq), 1.65 (br app. q, $J_1 = J_2 = J_3$ = 11.8 Hz, 1H, H-3'ax), 1.60-1.25 (m, 46H, H-2ax, 5x$CO_2C(C\underline{H}_3)_3$); ^{13}C NMR (125 MHz, CD_3OD) δ 162.9, 159.5, 159.4, 157.9, 157.7, 155.3, 155.0, 145.2, 126.4, 124.5, 118.4, 115.1, 114.2, 99.7 (anomeric C), 99.4 (anomeric C), 83.1, 82.3, 80.7, 80.5, 80.4, 80.2, 77.1, 73.5, 72.8, 72.4, 71.9, 66.5, 57.1, 52.1, 51.5, 51.1, 51.0, 42.0, 35.9, 34.8, 34.3, 33.1, 30.8, 30.5, 28.9, 28.8, 18.7; HRESI-MS *m/z* calc'd for $C_{53}H_{83}N_5O_{20}SNa$ 1164.5250, found 1164.5251 $[M+Na]^+$.

2.2. Synthesis of 6"-thioether TOB derivatives 4a-r.



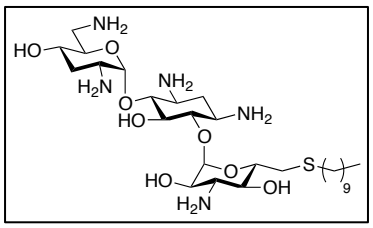
6"-thioether TOB derivative 4a. Compound **3a** (70 mg, 0.07 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4a** (68 mg, 91%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S1) δ 5.60 (d, J = 3.5 Hz, 1H, H-1'), 4.94 (d, J = 3.7 Hz, 1H, H-1"), 3.89-3.77 (m, 4H, H-4, H-5', H-2", H-5"), 3.74 (app. t, J_1 = J_2 = 9.0 Hz, 1H, H-5), 3.63 (app. t, $J_1 = J_2$ = 10.1 Hz, 1H, H-6), 3.60-3.36 (m, 5H, H-1, H-3, H-2', H-4', H-4"), 3.34-3.25 (m, 2H, H-6', H-3"), 3.11 (dd, J_1 =13.6 Hz, J_2 = 6.9 Hz, 1H, H-6'), 2.89 (dd, J_1 = 14.1 Hz, J_2 = 2.4 Hz, 1H, H-6"), 2.62 (dd, J_1 = 14.0 Hz, J_2 = 7.8 Hz, 1H, H-6"), 2.47 (app. t, $J_1 = J_2$ = 7.4 Hz, 2H, S$C\underline{H}_2(CH_2)_4CH_3$), 2.41 (app. dt, J_1 = 12.6 Hz, $J_2 = J_3$ = 4.2 Hz, 1H, H-2eq), 2.15 (app. dt, J_1 = 12.1 Hz, $J_2 = J_3$ = 4.4 Hz, 1H, H-3'eq), 1.88 (app. q, $J_1 = J_2 = J_3$ = 12.2

Hz, 1H, H-3'ax), 1.79 (app. q, $J_1 = J_2 = J_3 = 12.8$ Hz, 1H, H-2ax), 1.47-1.39 (m, 2H, $SCH_2(C\underline{H}_2)_4CH_3$), 1.26-1.07 (m, 6H, $SCH_2(C\underline{H}_2)_4CH_3$), 0.70 (t, 3H, $J = 6.9$ Hz, $SCH_2(CH_2)_4C\underline{H}_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S2) δ 162.3 (q, $J = 35$ Hz, $CF_3\underline{C}O_2H$), 116.3 (q, $J = 290$ Hz, $\underline{C}F_3CO_2H$), 100.6 (anomeric C), 94.5 (anomeric C), 83.9, 77.6, 74.2, 72.6, 70.3, 68.1, 67.9, 64.4, 54.6, 49.1, 48.3, 47.9, 39.8, 32.4, 32.3, 30.6, 29.3, 28.7, 27.8, 27.6, 21.8, 13.3; HRESI-MS *m/z* calc'd for $C_{24}H_{50}N_5O_8S$ 568.3380, found 568.3384 $[M+H]^+$.

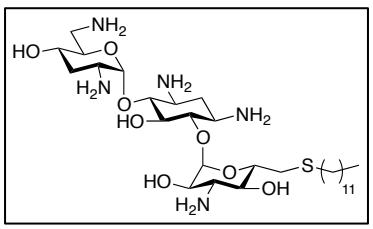


6"-thioether TOB derivative 4b. Compound **3b** (70 mg, 0.06 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4b** (62 mg, 83%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S3) δ 5.59 (d, $J = 3.5$ Hz, 1H, H-1'), 4.94 (d, $J = 3.6$ Hz, 1H, H-1"), 3.90-3.77 (m, 4H, H-4, H-5', H-2", H-5"), 3.74 (app. t, $J_1 = J_2 = 9.0$ Hz, 1H, H-5), 3.64 (app. t, $J_1 = J_2 = 9.5$ Hz, 1H, H-6), 3.61-3.37 (m, 5H, H-1, H-3, H-2', H-4', H-4"), 3.34-3.25 (m, 2H, H-6', H-3"), 3.11 (dd, $J_1 = 13.6$ Hz, $J_2 = 7.1$ Hz, 1H, H-6'), 2.89 (dd, $J_1 = 14.1$ Hz, $J_2 = 2.2$ Hz, 1H, H-6"), 2.62 (dd, $J_1 = 14.1$ Hz, $J_2 = 7.8$ Hz, 1H, H-6"), 2.46 (app. t, $J_1 = J_2 = 7.4$ Hz, 2H, $SC\underline{H}_2(CH_2)_6CH_3$), 2.41 (app. dt, $J_1 = 12.6$ Hz, $J_2 = J_3 = 4.2$ Hz, 1H, H-2eq), 2.15 (app. dt, $J_1 = 12.2$ Hz, $J_2 = J_3 = 4.3$ Hz, 1H, H-3'eq), 1.88 (app. q, $J_1 = J_2 = J_3 = 12.2$ Hz, 1H, H-3'ax), 1.80 (app. q, $J_1 = J_2 = J_3 = 12.6$ Hz, 1H, H-2ax), 1.44 (br p, $J = 7.4$ Hz, 2H, $SCH_2(C\underline{H}_2)_6CH_3$), 1.25-1.08 (m, 10H, $SCH_2(C\underline{H}_2)_6CH_3$), 0.70 (t, $J = 6.8$ Hz, 3H, $SCH_2(CH_2)_6C\underline{H}_3$); ^{13}C NMR (125 MHz, D_2O) (Fig. S4) δ 162.5 (q, $J = 35$ Hz, $CF_3\underline{C}O_2H$), 116.0 (q, $J = 290$ Hz, $\underline{C}F_3CO_2H$), 100.2 (anomeric C), 94.1 (anomeric C), 83.5, 77.3, 73.8, 72.2, 69.9,

67.7, 67.6, 64.0, 54.3, 49.0, 47.9, 47.5, 39.4, 32.1, 31.9, 30.7, 28.9, 28.4, 27.9, 27.8, 27.5, 27.4, 21.6, 13.0; HRESI-MS *m/z* calc'd for $C_{26}H_{54}N_5O_8S$ 596.3693, found 596.3696 $[M+H]^+$.

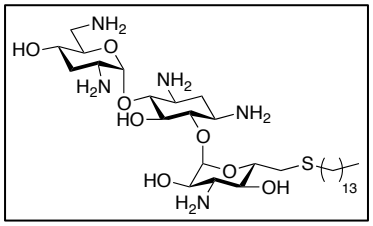


6"-thioether TOB derivative 4c. Compound **3c** (76 mg, 0.07 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4c** (74 mg, 92%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S5) δ 5.60 (d, J = 3.6 Hz, 1H, H-1'), 4.95 (d, J = 3.7 Hz, 1H, H-1"), 3.90-3.78 (m, 4H, H-4, H-5', H-2", H-5"), 3.75 (app. t, J_1 = J_2 = 9.1 Hz, 1H, H-5), 3.65 (app. t, J_1 = J_2 = 9.4 Hz, 1H, H-6), 3.61-3.37 (m, 5H, H-1, H-3, H-2', H-4', H-4"), 3.36-3.26 (m, 2H, H-6', H-3"), 3.11 (dd, J_1 = 13.6 Hz, J_2 = 7.1 Hz, 1H, H-6'), 2.90 (dd, J_1 = 14.1 Hz, J_2 = 2.4 Hz, 1H, H-6"), 2.63 (dd, J_1 = 14.1 Hz, J_2 = 7.8 Hz, 1H, H-6"), 2.48 (t, J = 7.4 Hz, $SC\underline{H}_2(CH_2)_8CH_3$), 2.41 (app. dt, J_1 = 12.6 Hz, J_2 = J_3 = 4.2 Hz, 1H, H-2eq), 2.17 (app. dt, J_1 = 12.1 Hz, J_2 = J_3 = 4.4 Hz, 1H, H-3'eq), 1.89 (app. q, J_1 = J_2 = J_3 = 12.2 Hz, 1H, H-3'ax), 1.80 (app. q, J_1 = J_2 = J_3 = 12.6 Hz, 1H, H-2ax), 1.49-1.40 (m, 2H, $SCH_2(C\underline{H}_2)_8CH_3$), 1.26-1.10 (m, 14H, $SCH_2(C\underline{H}_2)_8CH_3$), 0.71 (t, J = 7.0 Hz, 3H, $SCH_2(CH_2)_8C\underline{H}_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S6) δ 162.6 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 116.0 (q, J = 289 Hz, $\underline{C}F_3CO_2H$), 100.3 (anomeric C), 94.2 (anomeric C), 83.6, 77.4, 73.9, 72.3, 70.0, 67.8, 67.7, 64.1, 54.4, 49.1, 48.0, 47.6, 39.5, 32.2, 32.0, 30.9, 29.0, 28.5, 28.4, 28.3, 28.2, 27.9, 27.6, 21.7, 13.1; HRESI-MS *m/z* calc'd for $C_{28}H_{58}N_5O_8S$ 624.4006, found 624.4005 $[M+H]^+$.



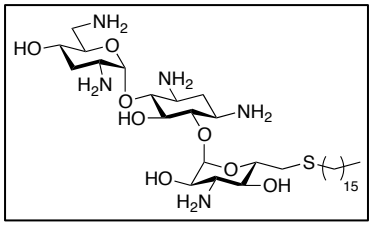
6"-thioether TOB derivative 4d. Compound **3d** (78 mg, 0.07 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The

TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4d** (79 mg, 95%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S7) δ 5.57 (d, J = 3.4 Hz, 1H, H-1'), 4.90 (d, J = 3.6 Hz, 1H, H-1"), 3.87-3.74 (m, 4H, H-4, H-5', H-2", H-5"), 3.71 (app. t, $J_1 = J_2$ = 9.0 Hz, 1H, H-5), 3.61 (app. t, $J_1 = J_2$ = 9.6 Hz, 1H, H-6), 3.58-3.34 (m, 5H, H-1, H-3, H-2', H-4', H-4"), 3.32-3.22 (m, 2H, H-6', H-3"), 3.07 (dd, J_1 = 13.6 Hz, J_2 = 7.2 Hz, 1H, H-6'), 2.86 (dd, J_1 = 14.0 Hz, J_2 = 2.3 Hz, 1H, H-6"), 2.58 (dd, J_1 = 14.0 Hz, J_2 = 7.8 Hz, 1H, H-6"), 2.43 (t, J = 7.4 Hz, 2H, $SC\underline{H}_2(CH_2)_{10}CH_3$), 2.39 (app. dt, J_1 = 12.7 Hz, $J_2 = J_3$ = 4.2 Hz, 1H, H-2eq), 2.12 (app. dt, J_1 = 12.1 Hz, $J_2 = J_3$ = 4.4 Hz, 1H, H-3'eq), 1.85 (app. q, $J_1 = J_2 = J_3$ = 11.9 Hz, 1H, H-3'ax), 1.77 (app. q, $J_1 = J_2 = J_3$ = 12.6 Hz, 1H, H-2ax), 1.45-1.36 (m, 2H, $SCH_2(C\underline{H}_2)_{10}CH_3$), 1.22-1.05 (m, 18H, $SCH_2(C\underline{H}_2)_{10}CH_3$), 0.68 (t, J = 7.0 Hz, 3H, $SCH_2(CH_2)_{10}C\underline{H}_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S8) δ 163.3 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 116.7 (q, J = 289 Hz, $\underline{C}F_3CO_2H$), 101.0 (anomeric C), 94.9 (anomeric C), 84.3, 78.0, 74.6, 73.0, 70.7, 68.5, 68.3, 64.8, 55.0, 49.8, 48.6, 48.3, 40.2, 32.9, 32.7, 31.6, 29.7, 29.20, 29.15, 29.1, 29.0, 28.9, 28.7, 28.3, 28.2, 22.4, 13.8; HRESI-MS *m/z* calc'd for $C_{30}H_{61}N_5O_8SNa$ 674.4139, found 674.4141 $[M+Na]^+$.



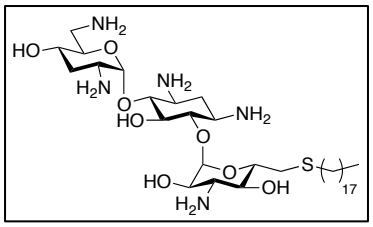
6"-thioether TOB derivative 4e. Compound **3e** (52 mg, 0.04 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4e** (52 mg, 95%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S9) δ 5.57 (d, J = 3.5 Hz, 1H, H-1'), 4.93 (d, J = 3.7 Hz, 1H, H-1"), 3.88-3.76 (m, 4H, H-4, H-5', H-2", H-5"), 3.73 (app. t, $J_1 = J_2$ = 9.1 Hz, 1H, H-5), 3.62 (app. t, $J_1 = J_2$ = 9.9 Hz, 1H, H-6), 3.58-3.33 (m, 5H, H-1, H-3, H-2',

H-4', H-4"), 3.30 (app. t, $J_1 = J_2 = 10.6$ Hz, 1H, H-3"), 3.27 (dd, $J_1 = 13.6$ Hz, $J_2 = 3.4$ Hz, 1H, H-6'), 3.10 (dd, $J_1 = 13.6$ Hz, $J_2 = 7.0$ Hz, 1H, H-6'), 2.89 (dd, $J_1 = 14.0$ Hz, $J_2 = 2.1$ Hz, 1H, H-6"), 2.61 (dd, $J_1 = 14.0$ Hz, $J_2 = 7.8$ Hz, 1H, H-6"), 2.46 (t, $J = 7.4$ Hz, 2H, $SC\underline{H}_2(CH_2)_{12}CH_3$), 2.38 (app. dt, $J_1 = 12.5$ Hz, $J_2 = J_3 = 4.1$ Hz, 1H, H-2eq), 2.14 (app. dt, $J_1 = 12.1$ Hz, $J_2 = J_3 = 4.3$ Hz, 1H, H-3'eq), 1.86 (app. q, $J_1 = J_2 = J_3 = 12.0$ Hz, 1H, H-3'ax), 1.76 (app. q, $J_1 = J_2 = J_3 = 12.6$ Hz, 1H, H-2ax), 1.47-1.38 (m, 2H, $SCH_2(C\underline{H}_2)_{12}CH_3$), 1.24-1.08 (m, 22H, $SCH_2(C\underline{H}_2)_{12}CH_3$), 0.69 (t, $J = 6.7$ Hz, 3H, $SCH_2(CH_2)_{12}C\underline{H}_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S10) δ 162.6 (q, $J = 35$ Hz, $CF_3\underline{C}O_2H$), 116.0 (q, $J = 290$ Hz, $\underline{C}F_3CO_2H$), 100.3 (anomeric C), 94.3 (anomeric C), 83.7, 77.6, 74.0, 72.3, 70.0, 67.8, 67.7, 64.1, 54.4, 49.2, 48.0, 47.6, 39.5, 32.2, 32.1, 30.9, 29.1, 28.53, 28.46, 28.40, 28.3, 28.2, 28.0, 27.7, 27.6, 21.7, 13.1; HRESI-MS *m/z* calc'd for $C_{32}H_{65}N_5NaO_8S$ 702.4452, found 702.4453 $[M+Na]^+$.



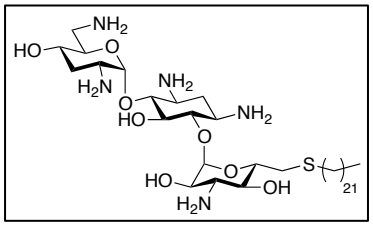
6"-thioether TOB derivative 4f. Compound **3f** (34 mg, 0.03 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4f** (35 mg, 98%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S11) δ 5.58 (d, $J = 3.4$ Hz, 1H, H-1'), 4.93 (d, $J = 3.6$ Hz, 1H, H-1"), 3.87-3.76 (m, 4H, H-4, H-5', H-2", H-5"), 3.73 (app. t, $J_1 = J_2 = 9.1$ Hz, 1H, H-5), 3.63 (app. t, $J_1 = J_2 = 9.7$ Hz, 1H, H-6), 3.59-3.31 (m, 5H, H-1, H-3, H-2', H-4', H-4"), 3.30 (app. t, $J_1 = J_2 = 10.6$ Hz, 1H, H-3"), 3.28 (dd, $J_1 = 13.5$ Hz, $J_2 = 3.5$ Hz, 1H, H-6'), 3.09 (dd, $J_1 = 13.5$ Hz, $J_2 = 7.0$ Hz, 1H, H-6'), 2.89 (dd, $J_1 = 14.0$ Hz, $J_2 = 2.1$ Hz, 1H, H-6"), 2.60 (dd, $J_1 = 14.0$ Hz, $J_2 = 7.8$ Hz, 1H, H-6"), 2.45 (t, $J = 7.3$ Hz, 2H, $SC\underline{H}_2(CH_2)_{14}CH_3$), 2.39 (app. dt, $J_1 = 12.6$ Hz, $J_2 = J_3 = 4.1$ Hz, 1H, H-2eq), 2.15 (app. dt, $J_1 = 12.1$ Hz, $J_2 = J_3 = 4.4$ Hz,

1H, H-3'eq), 1.86 (app. q, $J_1 = J_2 = J_3 = 11.9$ Hz, 1H, H-3'ax), 1.76 (app. q, $J_1 = J_2 = J_3 = 12.6$ Hz, 1H, H-2ax), 1.47-1.38 (m, 2H, $SCH_2(C\underline{H}_2)_{14}CH_3$), 1.24-1.05 (m, 26H, $SCH_2(C\underline{H}_2)_{14}CH_3$), 0.70 (t, $J = 6.3$ Hz, 3H, $SCH_2(CH_2)_{14}C\underline{H}_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S12) δ 162.6 (q, $J = 35$ Hz, $CF_3\underline{C}O_2H$), 116.0 (q, $J = 290$ Hz, $\underline{C}F_3CO_2H$), 100.3 (anomeric C), 94.2 (anomeric C), 83.6, 77.4, 73.9, 72.3, 70.0, 67.8, 67.6, 64.1, 54.3, 49.1, 48.0, 47.6, 39.5, 32.2, 32.1, 30.9, 29.0, 28.5, 28.4, 28.3, 28.1, 28.0, 27.7, 27.5, 21.8, 13.1; HRESI-MS *m/z* calc'd for $C_{34}H_{70}N_5O_8S$ 708.4945, found 708.4948 $[M+H]^+$.

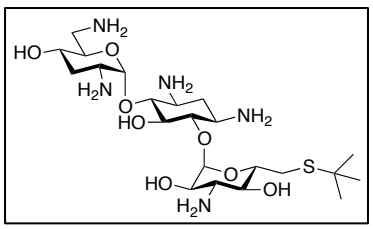


6"-thioether TOB derivative 4g. Compound **3g** (37 mg, 0.03 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4g** (38 mg, 96%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S13) δ 5.58 (d, $J = 3.1$ Hz, 1H, H-1'), 4.87 (d, $J = 3.0$ Hz, 1H, H-1"), 3.85-3.73 (m, 4H, H-4, H-5', H-2", H-5"), 3.70 (app. t, $J_1 = J_2 = 9.0$ Hz, 1H, H-5), 3.59 (app. t, $J_1 = J_2 = 9.2$ Hz, 1H, H-6), 3.49-3.31 (m, 5H, H-1, H-3, H-2', H-4', H-4"), 3.31-3.24 (m, 2H, H-6', H-3"), 2.98 (br dd, $J_1 = 12.2$ Hz, $J_2 = 7.9$ Hz, 1H, H-6'), 2.85 (br d, $J_1 = 12.1$ Hz, 1H, H-6"), 2.52 (br dd, $J_1 = 13.4$ Hz, $J_2 = 6.9$ Hz, 1H, H-6"), 2.37 (m, 3H, H-2eq, $SC\underline{H}_2(CH_2)_{16}CH_3$), 2.11 (m, 1H, H-3'eq), 1.84 (app. q, $J_1 = J_2 = J_3 = 11.5$ Hz, 1H, H-3'ax), 1.77 (app. q, $J_1 = J_2 = J_3 = 12.6$ Hz, 1H, H-2ax), 1.37 (m, 2H, $SCH_2(C\underline{H}_2)_{16}CH_3$), 1.19-1.05 (m, 30H, $SCH_2(C\underline{H}_2)_{16}CH_3$), 0.69 (t, $J = 6.8$ Hz, 3H, $SCH_2(CH_2)_{16}C\underline{H}_3$); ^{13}C NMR (125 MHz, D_2O) (Fig. S14) δ 162.4 (q, $J = 35$ Hz, $CF_3\underline{C}O_2H$), 116.0 (q, $J = 290$ Hz, $\underline{C}F_3CO_2H$), 100.2 (anomeric C), 93.8 (anomeric C), 83.6, 77.2, 73.9, 72.5, 69.9, 67.8, 67.7, 64.4, 54.5, 49.1, 48.0,

47.6, 39.8, 32.6, 32.2, 31.4, 29.2, 29.0, 28.8, 28.6, 28.1, 27.6, 22.1, 13.4; HRESI-MS *m/z* calc'd for $C_{36}H_{74}N_5O_8S$ 736.5258, found 736.5259 $[M+H]^+$

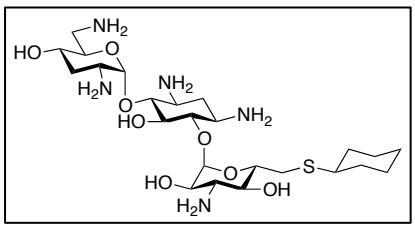


6"-thioether TOB derivative 4h. Compound **3h** (98 mg, 0.08 mmol) was treated at rt with 95% TFA (1.5 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4h** (76 mg, 74%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S15) δ δ 5.61 (d, J = 2.8 Hz, 1H, H-1'), 4.87 (d, J = 2.6 Hz, 1H, H-1"), 3.89-3.73 (m, 4H, H-4, H-5', H-2", H-5"), 3.72 (app. t, $J_1 = J_2$ = 9.0 Hz, 1H, H-5), 3.61 (app. t, $J_1 = J_2$ = 9.4 Hz, 1H, H-6), 3.49-3.34 (m, 5H, H-1, H-3, H-2', H-4', H-4"), 3.33-3.26 (m, 2H, H-6', H-3"), 2.96 (br dd, J_1 = 13.3 Hz, J_2 = 8.2 Hz, 1H, H-6'), 2.86 (br d, J_1 = 12.1 Hz, 1H, H-6"), 2.51 (br dd, J_1 = 13.3 Hz, J_2 = 6.8 Hz, 1H, H-6"), 2.39 (m, 3H, H-2eq, $SC\underline{H}_2(CH_2)_{20}CH_3$), 2.12 (m, 1H, H-3'eq), 1.89-1.79 (m, 2H, H-2ax, H-3'ax), 1.37 (m, 2H, $SCH_2(C\underline{H}_2)_{20}CH_3$), 1.15-1.07 (m, 38H, $SCH_2(C\underline{H}_2)_{20}CH_3$), 0.72 (t, J = 6.6 Hz, 3H, $SCH_2(CH_2)_{20}C\underline{H}_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S16) δ 162.4 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 116.0 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 100.1 (anomeric C), 93.6 (anomeric C), 83.4, 76.8, 73.8, 72.5, 70.2, 67.8, 67.7, 64.5, 54.5, 49.0, 48.0, 47.5, 39.8, 32.7, 32.4, 31.6, 29.4, 29.2, 29.1, 28.8, 28.3, 27.4, 22.2, 13.5; HRESI-MS *m/z* calc'd for $C_{40}H_{81}N_5O_8SNa$ 814.5704, found 814.5706 $[M+Na]^+$.



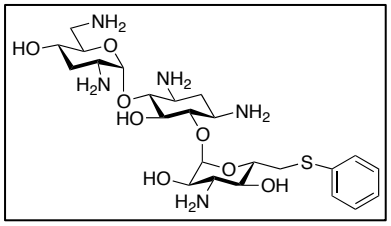
6"-thioether TOB derivative 4i. Compound **3i** (41 mg, 0.06 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford

4i (41 mg, 94%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S17) δ 5.57 (d, J = 3.4 Hz, 1H, H-1'), 4.92 (d, J = 3.6 Hz, 1H, H-1"), 3.89-3.77 (m, 4H, H-4, H-5', H-2", H-5"), 3.73 (app. t, J_1 = J_2 = 9.0 Hz, 1H, H-5), 3.63 (app. t, J_1 = J_2 = 10.2 Hz, 1H, H-6), 3.58-3.34 (m, 5H, H-1, H-3, H-2', H-4', H-4"), 3.31 (app. t, J_1 = J_2 = 10.6 Hz, 1H, H-3"), 3.27 (dd, J_1 = 13.6 Hz, J_2 = 3.3 Hz, 1H, H-6'), 3.13 (dd, J_1 = 13.6 Hz, J_2 = 6.9 Hz, 1H, H-6'), 2.93 (dd J_1 = 13.0 Hz, J_2 = 2.0 Hz, 1H, H-6"), 2.67 (dd, J_1 = 13.2 Hz, J_2 = 8.0 Hz, 1H, H-6"), 2.41 (app. dt, J_1 = 11.4 Hz, J_2 = J_3 = 4.1 Hz, 1H, H-2eq), 2.16 (app. dt, J_1 =12.3 Hz, J_2 = J_3 = 4.1 Hz, 1H, H-3'eq), 1.88 (app. q, J_1 = J_2 = J_3 = 12.6 Hz, 1H, H-3'ax), 1.79 (app. q, J_1 = J_2 = J_3 = 12.6 Hz, 1H, H-2ax), 1.18 (s, 9H, SC(C$\underline{H}_3$)$_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S18) δ 162.3 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 115.5 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 100.1 (anomeric C), 94.1 (anomeric C), 83.4, 77.2, 73.6, 72.2, 69.9, 67.9, 67.4, 63.8, 54.1, 48.9, 47.8, 47.4, 42.3, 39.2, 29.4, 28.7, 28.4, 27.3; HRESI-MS *m/z* calc'd for $C_{22}H_{46}N_5O_8S$ 540.3067, found 540.3068 $[M+H]^+$.



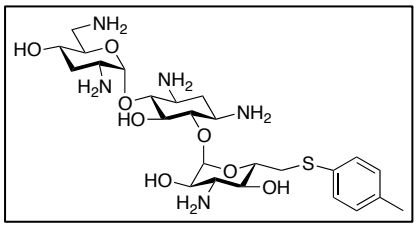
6"-thioether TOB derivative 4j. Compound **3j** (53 mg, 0.05 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4j** (52 mg, 92%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S19) δ 5.54 (d, J = 3.3 Hz, 1H, H-1'), 4.89 (d, J = 3.5 Hz, 1H, H-1"), 3.86-3.73 (m, 4H, H-4, H-5', H-2", H-5"), 3.69 (app. t, J_1 = J_2 = 9.1 Hz, 1H, H-5), 3.59 (app. t, J_1 = J_2 = 10.1 Hz, 1H, H-6), 3.55-3.33 (m, 5H, H-3, H-1, H-2', H-4', H-4"), 3.26 (app. t, J_1 = J_2 = 10.6 Hz, 1H, H-3"), 3.23 (dd, J_1 = 13.4 Hz, J_2 = 3.3 Hz, 1H, H-6'), 3.07 (dd, J_1 = 13.6 Hz, J_2 = 7.1 Hz, 1H, H-6'), 2.89 (dd, J_1 = 13.8 Hz, J_2 = 2.0 Hz, 1H, H-6"), 2.63-2.57 (m, 2H, H-6", $SC_6\underline{H}_{11}$), 2.37 (app. dt, J_1 = 12.6 Hz, J_2 = J_3 = 4.0 Hz, 1H,

H-2eq), 2.11 (app. dt, J_1 = 12.2 Hz, J_2 = J_3 = 4.3 Hz, 1H, H-3'eq), 1.84 (app. q, J_1 = J_2 = J_3 = 12.0 Hz, 1H, H-3'ax), 1.80-1.71 (m, 3H, H-2ax, $SC_6\underline{H}_{11}$), 1.57-1.49 (m, 2H, $SC_6\underline{H}_{11}$), 1.44-1.36 (m, 1H, $SC_6\underline{H}_{11}$), 1.17-0.97 (m, 5H, $SC_6\underline{H}_{11}$); ^{13}C NMR (125 MHz, D_2O) (Fig. S20) δ 162.7 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 116.0 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 100.4 (anomeric C), 94.3 (anomeric C), 83.7, 77.3, 73.9, 72.3, 70.2, 67.9, 67.7, 64.1, 54.4, 49.2, 48.0, 47.7, 44.2, 39.5, 33.1, 32.9, 30.2, 29.0, 27.5, 25.4, 25.3, 24.9; HRESI-MS *m/z* calc'd for $C_{24}H_{48}N_5O_8S$ 566.3224, found 566.3226 $[M+H]^+$.

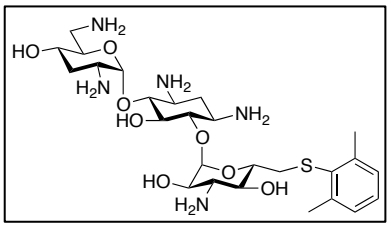


6"-thioether TOB derivative 4k. Compound **3k** (45 mg, 0.04 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4k** (40 mg, 83%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S21) δ 7.27 (d, J = 7.5 Hz, 2H, aromatic), 7.20 (dd, J_1 = J_2 = 7.5 Hz, 2H, aromatic), 7.12 (t, J = 7.4 Hz, 1H, aromatic), 5.54 (d, J = 3.5 Hz, 1H, H-1'), 4.92 (d, J = 3.6 Hz, 1H, H-1"), 3.94 (ddd, J_1 = 9.7 Hz, J_2 = 8.0 Hz, J_3 = 2.2 Hz, 1H, H-5"), 3.87-3.79 (m, 2H, H-4, H-2"), 3.77 (ddd, J_1 = 9.0 Hz, J_2 = 7.5 Hz, J_3 = 3.6 Hz, 1H, H-5'), 3.68 (app. t, J_1 = J_2 = 9.0 Hz, 1H, H-5), 3.61 (app. t, J_1 = J_2 = 9.8 Hz, 1H, H-6), 3.58-3.51 (m, 2H, H-4', H-4"), 3.48 (app. dt, J_1 = 11.8 Hz, J_2 = J_3 = 3.9 Hz, 1H, H-2'), 3.44-3.32 (m, 3H, H-1, H-3, H-6"), 3.30 (app. t, J_1 = J_2 = 10.6 Hz, 1H, H-3"), 3.24 (dd, J_1 = 13.6 Hz, J_2 = 3.4 Hz, 1H, H-6'), 3.08 (dd, J_1 = 13.5 Hz, J_2 = 7.2 Hz, 1H, H-6'), 3.02 (dd, J_1 = 14.2 Hz, J_2 = 7.8 Hz, 1H, H-6"), 2.38 (app. dt, J_1 = 12.6 Hz, J_2 = J_3 = 4.2 Hz, 1H, H-2eq), 2.10 (app. dt, J_1 = 12.2 Hz, J_2 = J_3 = 4.4 Hz, 1H, H-3'eq), 1.84 (app. q, J_1 = J_2 = J_3 = 12.0 Hz, 1H, H-3'ax), 1.77 (app. q, J_1 = J_2 = J_3 = 11.6 Hz, 1H, H-2ax); ^{13}C NMR (100 MHz, D_2O) (Fig. S22) δ 162.5 (q, J = 35 Hz,

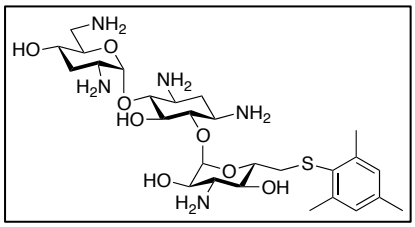
$CF_3\underline{C}O_2H$), 134.9, 129.2 (2C), 128.7 (2C), 126.6, 116.0 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 100.4 (anomeric C), 94.0 (anomeric C), 83.5, 77.2, 74.0, 72.0, 70.2, 67.9, 67.7, 64.1, 54.3, 49.2, 48.0, 47.6, 39.5, 34.6, 29.0, 27.5; HRESI-MS *m/z* calc'd for $C_{24}H_{41}N_5O_8SNa$ 582.2574, found 582.2575 $[M+Na]^+$.



6"-thioether TOB derivative 4l. Compound **3l** (42 mg, 0.04 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4l** (42 mg, 95%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S23) δ 7.19 (d, J = 8.1 Hz, 2H, aromatic), 7.20 (app. t, $J_1 = J_2$ = 7.5 Hz, 2H, aromatic), 5.53 (d, J = 3.5 Hz, 1H, H-1'), 4.92 (d, J = 3.6 Hz, 1H, H-1"), 3.93 (ddd, J_1 = 9.5 Hz, J_2 = 8.0 Hz, J_3 = 2.4 Hz, 1H, H-5"), 3.86-3.79 (m, 2H, H-4, H-2"), 3.76 (m, 1H, H-5'), 3.69 (app. t, $J_1 = J_2$ = 9.0 Hz, 1H, H-5), 3.61 (app. t, $J_1 = J_2$ = 9.9 Hz, 1H, H-6), 3.58-3.51 (m, 2H, H-4', H-4"), 3.48 (m, 1H, H-2'), 3.45-3.32 (m, 2H, H-1, H-3), 3.31-3.21 (m, 3H, H-6', H-3", H-6"), 3.08 (dd, J_1 = 13.6 Hz, J_2 = 7.0 Hz, 1H, H-6'), 2.99 (dd, J_1 = 14.1 Hz, J_2 = 7.8 Hz, 1H, H-6"), 2.38 (app. dt, J_1 = 12.6 Hz, $J_2 = J_3$ = 4.2 Hz, 1H, H-2eq), 2.13 (s, 3H, $SC_6H_4C\underline{H}_3$), 2.10 (app. dt, J_1 = 11.9 Hz, $J_2 = J_3$ = 4.0 Hz, 1H, H-3'eq), 1.83 (app. q, $J_1 = J_2 = J_3$ = 11.9 Hz, 1H, H-3'ax), 1.77 (app. q, $J_1 = J_2 = J_3$ = 12.6 Hz, 1H, H-2ax); ^{13}C NMR (125 MHz, D_2O) (Fig. S24) δ 162.7 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 137.5, 131.1, 129.9 (2C), 129.6 (2C), 116.1 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 100.4 (anomeric C), 94.3 (anomeric C), 83.6, 77.4, 74.1, 72.2, 70.2, 67.9, 67.7, 64.1, 54.4, 49.2, 48.0, 47.7, 39.5, 35.5, 29.0, 27.5, 19.8; ESI-MS *m/z* calc'd for $C_{25}H_{44}N_5O_8S$ 574.2911, found 574.2910 $[M+H]^+$.

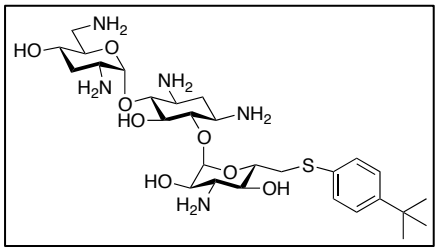


6"-thioether TOB derivative 4m. Compound **3m** (71 mg, 0.06 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4m** (65 mg, 86%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S25) δ 7.05 (m, 3H, aromatic), 5.54 (d, J = 3.5 Hz, 1H, H-1'), 4.90 (d, J = 3.6 Hz, 1H, H-1"), 3.96 (ddd, J_1 = 9.2 Hz, J_2 = 6.0 Hz, J_3 = 3.0 Hz, 1H, H-5"), 3.85-3.79 (m, 2H, H-4, H-2"), 3.75 (m, 1H, H-5'), 3.70 (app. t, $J_1 = J_2$ = 9.1 Hz, 1H, H-5), 3.62-3.57 (m, 2H, H-6, H-4"), 3.55 (m, 1H, H-4'), 3.47 (app. dt, J_1 = 11.7 Hz, $J_2 = J_3$ = 3.8 Hz, 1H, H-2'), 3.43-3.33 (m, 2H, H-1, H-3), 3.29 (app. t, $J_1 = J_2$ = 10.6 Hz, 1H, H-3"), 3.23 (dd, J_1 = 13.6 Hz, J_2 = 3.5 Hz, 1H, H-6'), 3.08 (dd, J_1 = 13.6 Hz, J_2 = 7.0 Hz, 1H, H-6'), 2.91 (dd, J_1 = 13.5 Hz, J_2 = 6.3 Hz, 1H, H-6"), 2.85 (dd, J_1 = 13.4 Hz, J_2 = 3.0 Hz, 1H, H-6"), 2.38 (m,1H, H-2eq), 2.35 (s, 6H, $SC_6H_3(C\underline{H}_3)_2$), 2.09 (app. dt, J_1 = 12.2 Hz, $J_2 = J_3$ = 4.1 Hz, 1H, H-3'eq), 1.83 (app. q, $J_1 = J_2 = J_3$ = 11.9 Hz, 1H, H-3'ax), 1.75 (app. q, $J_1 = J_2 = J_3$ = 12.6 Hz, 1H, H-2ax); ^{13}C NMR (125 MHz, D_2O) (Fig. S26) δ 162.5 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 142.5, 132.3, 128.5, 127.8 (2C), 116.0 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 100.3, 93.9, 83.2, 77.1, 73.8, 71.9, 69.9, 67.5, 67.3, 63.8, 54.2, 49.0, 47.8, 47.3, 39.2, 35.6, 28.7, 27.3, 20.7 (2C); HRESI-MS *m/z* calc'd for $C_{26}H_{46}N_5O_8S$ 588.3067, found 588.3063 $[M+H]^+$.



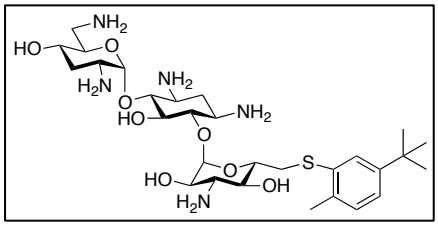
6"-thioether TOB derivative 4n. Compound **3n** (53 mg, 0.05 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4n** (55 mg, 98%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S27) δ 6.90 (s, 2H,

aromatic), 5.56 (d, J = 3.6 Hz, 1H, H-1'), 4.90 (d, J = 3.7 Hz, 1H, H-1"), 3.96 (ddd, J_1 = 9.4 Hz, J_2 = 6.2 Hz, J_3 = 3.2 Hz, 1H, H-5"), 3.87-3.82 (m, 2H, H-4, H-2"), 3.77 (ddd, J_1 = 8.8 Hz, J_2 = 7.1 Hz, J_3 = 3.6 Hz, 1H, H-5'), 3.72 (app. t, $J_1 = J_2$ = 9.0 Hz, 1H, H-5), 3.65-3.53 (m, 3H, H-6, H-4', H-4"), 3.49 (app. dt, J_1 = 11.4 Hz, $J_2 = J_3$ = 4.0 Hz, 1H, H-2'), 3.45-3.36 (m, 2H, H-1, H-3), 3.32 (app. t, $J_1 = J_2$ = 10.6 Hz, 1H, H-3"), 3.25 (dd, J_1 = 13.6 Hz, J_2 = 3.6 Hz, 1H, H-6'), 3.10 (dd, J_1 = 13.6 Hz, J_2 = 7.0 Hz, 1H, H-6'), 2.89 (dd, J_1 = 13.4 Hz, J_2 = 6.3 Hz, 1H, H-6"), 2.83 (dd, J_1 = 13.4 Hz, J_2 = 3.3 Hz, 1H, H-6"), 2.40 (app. dt, J_1 = 12.6 Hz, $J_2 = J_3$ = 4.2 Hz 1H, H-2eq), 2.32 (s, 6H, $SC_6H_2(C\underline{H}_3)_3$), 2.11 (ddd, J_1 = 12.2 Hz, J_2 = 8.8 Hz, J_3 = 4.4 Hz, 1H, H-3'eq), 2.08 (s, 3H, $SC_6H_2(C\underline{H}_3)_3$), 1.85 (app. q, $J_1 = J_2 = J_3$ = 12.1 Hz, 1H, H-3'ax), 1.80 (app. q, $J_1 = J_2 = J_3$ = 12.6 Hz, 1H, H-2ax); ^{13}C NMR (100 MHz, D_2O) (Fig. S28) δ 162.5 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 142.9, 139.6, 129.3, 129.0 (2$\underline{C}$), 116.0 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 100.8, 94.3, 83.7, 77.5, 74.2, 72.4, 70.4, 68.0, 67.8, 64.3, 54.7, 49.6, 48.3, 47.8, 39.7, 36.2, 29.2, 27.8, 21.1, 19.9; HRESI-MS *m/z* calc'd for $C_{27}H_{48}N_5O_8S$ 602.3224, found 602.3227 $[M+H]^+$.



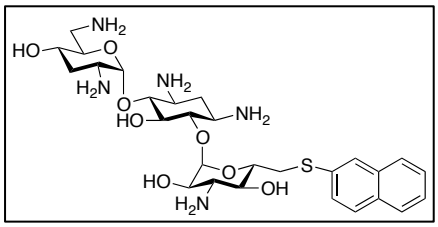
6"-thioether TOB derivative 4o. Compound **3o** (79 mg, 0.07 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4o** (73 mg, 84%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S29) δ 7.30 (d, J = 8.5 Hz, 2H, aromatic), 7.24 (d, J = 8.4 Hz, 2H, aromatic), 5.54 (d, J = 3.5 Hz, 1H, H-1'), 4.92 (d, J = 3.6 Hz, 1H, H-1"), 3.93 (ddd, J_1 = 9.7 Hz, J_2 = 7.9 Hz, J_3 = 2.2 Hz, 1H, H-5"), 3.86-3.80 (m, 2H, H-4, H-2"), 3.76 (ddd, J_1 = 9.2 Hz, J_2 = 7.2 Hz, J_3 = 3.6 Hz, 1H, H-5'), 3.70 (app. t, $J_1 = J_2$ = 9.1 Hz, 1H, H-5), 3.61 (app. t, $J_1 = J_2$ = 9.2 Hz, 1H, H-6), 3.58-3.51 (m, 2H, H-

4', H-4"), 3.47 (app. dt, J_1 = 11.9 Hz, J_2 = J_3 = 4.0 Hz, 1H, H-2'), 3.44-3.33 (m, 3H, H-1, H-3, H-6"), 3.32-3.26 (m, 2H, H-3", H-6"), 3.23 (dd, J_1 = 13.6 Hz, J_2 = 3.5 Hz, 1H, H-6'), 3.08 (dd, J_1 = 13.6 Hz, J_2 = 7.1 Hz, 1H, H-6'), 3.00 (dd, J_1 = 14.0 Hz, J_2 = 7.8 Hz, 1H, H-6"), 2.37 (app. dt, J_1 = 12.6 Hz, J_2 = J_3 = 4.2 Hz, 1H, H-2eq), 2.10 (app. dt, J_1 = 12.2 Hz, J_2 = J_3 = 4.3Hz, 1H, H-3'eq), 1.83 (app. q, J_1 = J_2 = J_3 = 12.0 Hz, 1H, H-3'ax), 1.77 (app. q, J_1 = J_2 = J_3 = 12.6 Hz, 1H, H-2ax), 1.10 (s, 9H, $SC_6H_4(C\underline{H}_3)_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S30) δ 162.5 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 151.0, 131.7, 129.5 (2C), 126.6 (2C), 116.3 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 100.7 (anomeric C), 94.5 (anomeric C), 83.8, 77.6, 74.2, 72.4, 70.4, 68.1, 67.9, 64.3, 54.6, 49.4, 48.2, 47.9, 39.7, 35.5, 33.9, 30.3 (3C), 29.2, 27.7; HRESI-MS *m/z* calc'd for $C_{28}H_{50}N_5O_8S$ 616.3380, found 616.3381 $[M+H]^+$.



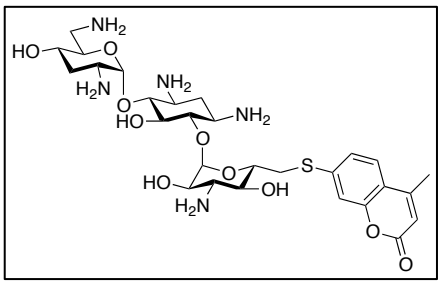
6"-thioether TOB derivative 4p. Compound **3p** (26 mg, 0.02 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4p** (26 mg, 94%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S31) δ 7.40 (d, J = 4.1 Hz, 1H, aromatic), 7.19 (dd, J_1 = 7.9 Hz, J_2 = 1.8 Hz, 1H, aromatic), 7.11 (d, J = 8.0 Hz, 1H, aromatic), 5.60 (d, J = 3.5 Hz, 1H, H-1'), 4.95 (d, J = 3.6 Hz, 1H, H-1"), 4.01 (ddd, J_1 = 9.5 Hz, J_2 = 7.1 Hz, J_3 = 2.4 Hz, 1H, H-5"), 3.90-3.84 (m, 2H, H-4, H-2"), 3.80 (ddd, J_1 = 12.1 Hz, J_2 = 8.2 Hz, J_3 = 3.6 Hz, 1H, H-5'), 3.72 (app. t, J_1 = J_2 = 9.0 Hz, 1H, H-5), 3.68-3.57 (m, 3H, H-6, H-4', H-4"), 3.52 (app. dt, J_1 = 11.6 Hz, J_2 = J_3 = 3.9 Hz, 1H, H-2'), 3.49-3.25 (m, 5H, H-1, H-3, H-6', H-3", H-6"), 3.13 (dd, J_1 = 13.7 Hz, J_2 = 7.2 Hz, 1H, H-6"), 3.08 (dd, J_1 = 13.6 Hz, J_2 = 6.9 Hz, 1H, H-6'), 2.42 (app. dt, J_1 = 12.6 Hz, J_2 = J_3 = 4.0 Hz, 1H, H-2eq), 2.21 (s,

3H, C<u>H</u>$_3$), 2.15 (app. dt, J_1 = 12.3 Hz, J_2 = J_3 = 4.0 Hz, 1H, H-3'eq), 1.88 (app. q, J_1 = J_2 = J_3 = 11.9 Hz, 1H, H-3'ax), 1.80 (app. q, J_1 = J_2 = J_3 = 12.6 Hz, 1H, H-2ax), 1.15 (s, 9H, C(C<u>H</u>$_3$)$_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S32) δ 162.8 (q, J = 35 Hz, CF_3<u>C</u>O_2H), 150.5, 135.3, 134.1, 130.2, 126.1, 124.0, 116.3 (q, J = 290 Hz, <u>C</u>F_3CO_2H), 100.8 (anomeric C), 94.1 (anomeric C), 83.8, 77.2, 74.1, 72.3, 70.6, 67.9, 64.2, 54.6, 49.6, 48.3, 47.8, 39.7, 34.4, 34.0, 30.4, 29.2, 27.7, 19.0; HRESI-MS *m/z* calc'd for $C_{29}H_{52}N_5O_8S$ 630.3537, found 630.3534 $[M+H]^+$.



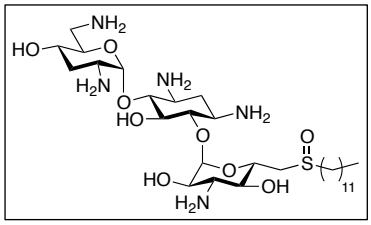
6"-thioether TOB derivative 4q. Compound **3q** (53 mg, 0.05 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4q** (55 mg, 97%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S33) δ 7.78-7.70 (m, 4H, aromatic), 7.45-7.37 (m, 3H, aromatic), 5.48 (d, J = 3.5 Hz, 1H, H-1'), 4.94 (d, J = 3.7 Hz, 1H, H-1"), 4.05 (ddd, J_1 = 9.8 Hz, J_2 = 7.8 Hz, J_3 = 2.4 Hz, 1H, H-5"), 3.86 (dd, J_1 = 10.8 Hz, J_2 = 3.6 Hz, 1H, H-2"), 3.84-3.79 (m, 1H, H-4), 3.75 (ddd, J_1 = 8.8 Hz, J_2 = 7.2 Hz, J_3 = 3.5 Hz, 1H, H-5'), 3.69 (app. t, J_1 = J_2 = 8.9 Hz, 1H, H-5), 3.63-3.52 (m, 3H, H-6, H-4', H-4"), 3.50 (dd, J_1 = 14.2 Hz, J_2 = 2.4 Hz, 1H, H-6"), 3.45-3.32 (m, 4H, H-1, H-3, H-2', H-3"), 3.25 (dd, J_1 = 13.6 Hz, J_2 = 3.5 Hz, 1H, H-6'), 3.17 (dd, J_1 = 14.2 Hz, J_2 = 7.7 Hz, 1H, H-6"), 3.07 (dd, J_1 = 13.6 Hz, J_2 = 7.2 Hz, 1H, H-6'), 2.38 (app. dt, J_1 = 12.6 Hz, J_2 = J_3 = 4.2 Hz, 1H, H-2eq), 2.10 (app. dt, J_1 = 12.2 Hz, J_2 = J_3 = 4.3 Hz, 1H, H-3'eq), 1.82 (m, 1H, H-3'ax), 1.76 (app. q, J_1 = J_2 = J_3 = 12.6 Hz, 1H, H-2ax); ^{13}C NMR (100 MHz, D_2O) (Fig. S34) δ 162.7 (q, J = 35 Hz, CF_3<u>C</u>O_2H), 133.2, 132.8, 131.3, 128.6, 127.5, 126.9, 126.7, 126.5, 126.1, 115.5 (q, J = 290 Hz, <u>C</u>F_3CO_2H), 100.4 (anomeric C), 93.9 (anomeric C), 83.4, 77.0, 74.0, 72.1, 70.2, 68.0, 67.7, 64.1,

54.4, 49.2, 48.1, 47.5, 39.5, 34.3, 29.0, 27.5; HRESI-MS *m/z* calc'd for $C_{28}H_{44}N_5O_{10}S$ 610.2911, found 610.2912 $[M+H]^+$.



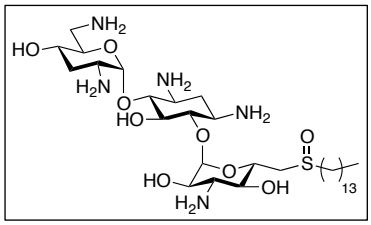
6"-thioether TOB derivative 4r. Compound **3r** (37 mg, 0.03 mmol) was treated at rt with 95% TFA (1 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **4r** (36 mg, 93%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S35) δ 7.52 (d, J = 8.5 Hz, 1H, coumarin ring), 7.26 (d, J = 1.6 Hz, 1H, coumarin ring), 7.20 (dd, J_1 = 8.4 Hz, J_2 = 1.5 Hz, 1H, coumarin ring), 6.16 (s, 1H, coumarin ring), 5.59 (d, J = 3.5 Hz, 1H, H-1'), 4.86 (d, J = 3.6 Hz, 1H, H-1"), 4.01 (ddd, J_1 = 9.4 Hz, J_2 = 6.8 Hz, J_3 = 2.8 Hz, 1H, H-5"), 3.84-3.78 (m, 2H, H-4, H-2"), 3.74 (m, 1H, H-5'), 3.67 (app. t, $J_1 = J_2$ = 9.1 Hz, 1H, H-5), 3.63-3.52 (m, 4H, H-6, H-2', H-4', H-4"), 3.46 (dd, J_1 = 14.8 Hz, J_2 = 2.7 Hz, 1H, H-6"), 3.43-3.29 (m, 3H, H-1, H-3, H-3"), 3.25 (dd, J_1 = 13.8 Hz, J_2 = 3.5 Hz, H-6"), 3.18 (dd, J_1 = 14.6 Hz, J_2 = 6.6 Hz, 1H, H-6'), 3.08 (dd, J_1 = 13.7 Hz, J_2 = 7.1 Hz, 1H, H-6'), 2.36 (app. dt, J_1 = 12.6 Hz, $J_2 = J_3$ = 4.1 Hz, 1H, H-2eq), 2.28 (s, 3H, C<u>H</u>$_3$, coumarin ring), 2.14 (app. dt, J_1 = 12.3 HZ, $J_2 = J_3$ = 4.0 Hz, 1H, H-3'eq), 1.85 (app. q, $J_1 = J_2 = J_3$ = 12.1 Hz, 1H, H-3'ax), 1.74 (app. q, $J_1 = J_2 = J_3$ = 12.6 Hz, 1H, H-2ax); ^{13}C NMR (125 MHz, D_2O) (Fig. S36) δ 163.7, 162.8 (q, J = 35 Hz, CF_3<u>C</u>O_2H), 155.8, 152.5, 141.8, 125.1, 123.2, 117.4, 115.5 (q, J = 290 Hz, <u>C</u>F_3CO_2H), 113.9, 112.1, 100.5 (anomeric C), 93.7 (anomeric C), 83.4, 76.6, 74.0, 71.8, 70.1, 67.6, 67.4, 63.9, 54.2, 49.1, 47.9, 47.4, 39.4, 32.6, 28.9, 27.3, 17.5; HRESI-MS *m/z* calc'd for $C_{28}H_{44}N_5O_{10}S$ 642.2809, found 642.2804 $[M+H]^+$.

2.3. Oxidation of 6"-thioether TOB derivatives 3d-e into 6"-sulfoxide TOB derivatives 5d-e.



6"-sulfoxide TOB derivative 5d. Compound **3d** (61 mg, 0.05 mmol) dissolved in $CHCl_3$ (1.5 mL) was treated with *m*-chloroperbenzoic acid (70-75%) (12 mg, ~0.07 mmol). The reaction mixture was stirred at rt and progress of the reaction was monitored by ESI-MS by following the disappearance of the starting material ($[M+H]^+$, *m/z* 1153.20) and the formation of the corresponding sulfoxide ($[M+H]^+$, *m/z* 1169.19). Upon completion, the reaction mixture was diluted with $CHCl_3$ (10 mL), washed with 1 M KOH (2 mL), concentrated under reduced pressure, and treated with 95% TFA (0.4 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **5d** (34 mg, 55%) as a white foam. (*Note*: A mixture of 2 diastereomers (~4:1 ratio) was obtained. The major and minor diastereomers are designated as (i) and (ii), respectively): ^{1}H NMR (400 MHz, D_2O) (Fig. S37) δ 5.69 (d, J = 3.5 Hz, 1H, H-1'(i)), 5.63 (d, J = 3.5 Hz, 1H, H-1'(ii)), 4.96 (d, J = 3.6 Hz, 1H, H-1"(ii)), 4.95 (d, J = 3.6 Hz, 1H, H-1"(i)), 4.26 (app. td, $J_1 = J_2$ = 9.2 Hz, J_3 = 2.2 Hz, 1H, H-5"(i)), 4.09 (app. td, $J_1 = J_2$ = 9.7 Hz, J_3 = 2.4 Hz, 1H, H-5"(ii)), 3.89-3.69 (m, 8H, H-4(i), H-4(ii), H-5(i), H-5(ii), H-5'(i), H-5'(ii), H-2"(i), H-2"(ii)), 3.65-3.35 (m, 14H, H-1(i), H-1(ii), H-3(i), H-3(ii), H-6(i), H-6(ii), H-2'(i), H-2'(ii), H-4'(i), H-4'(ii), H-3"(i), H-3"(ii), H-4"(i), H-4"(ii)), 3.31-3.24 (m, 3H, H-6'(i), H-6"(i), H-6"(ii)), 3.20 (dd, J_1 = 13.7 Hz, J_2 = 2.4 Hz, 1H, H-6'(ii)), 3.10 (dd, J_1 = 13.6 Hz, J_2 = 7.1 Hz, 2H, H-6'(i), H-6'(ii)), 2.97-2.88 (m, 6H, H-6"(i), H-6"(ii), $SC\underline{H}_2(CH_2)_{10}CH_3$(i), $SC\underline{H}_2(CH_2)_{10}CH_3$(ii)), 2.41 (app. dt, J_1 = 12.6 Hz, $J_2 = J_3$ = 4.2 Hz, 2H, H-2eq(i), H-2eq(ii)), 2.15 (app. dt, J_1 = 12.1 Hz, $J_2 = J_3$ = 4.4 Hz, 2H, H-3'eq(i), H-3'eq(ii)), 1.92-1.74 (m, 4H, H-2ax(i), H-2ax(ii), H-3'ax(i), H-3'ax(ii)), 1.57 (m, 4H, $SCH_2(C\underline{H}_2)_{10}CH_3$(i), $SCH_2(C\underline{H}_2)_{10}CH_3$ (ii)), 1.30 (m, 4H, $SCH_2(C\underline{H}_2)_{10}CH_3$(i), $SCH_2(C\underline{H}_2)_{10}CH_3$(ii)),

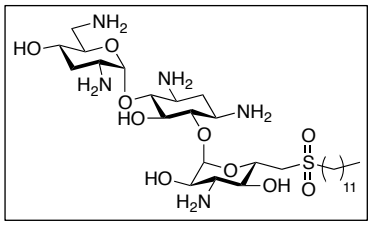
1.22-1.10 (m, 32H, $SCH_2(C\underline{H}_2)_{10}CH_{3(i)}$, $SCH_2(C\underline{H}_2)_{10}CH_{3(ii)}$), 0.71 (t, 6H, J = 6.9 Hz, $SCH_2(CH_2)_{10}C\underline{H}_{3(i)}$, $SCH_2(CH_2)_{10}C\underline{H}_{3(ii)}$); ^{13}C NMR (100 MHz, D_2O) (Fig. S38) δ 162.9 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 116.3 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 101.2 (anomeric $C_{(ii)}$), 100.7 (anomeric $C_{(i)}$), 94.4 (anomeric $C_{(ii)}$), 93.9 (anomeric $C_{(i)}$), 84.8(ii), 84.4(i), 77.2(ii), 76.5(i), 74.4(i), 74.2(ii), 70.1(i), 69.0(i), 68.4(ii), 68.3(i), 68.1(ii), 67.9(i), 66.8(ii), 64.5(i), 54.7(i), 54.6(ii), 51.8, 51.1(ii), 51.0(ii), 49.7(ii), 49.5(i), 49.4(i), 48.3(i), 48.2(ii), 47.9(ii), 47.8(i), 39.9(i), 31.2(i), 29.6, 29.3, 28.7, 28.6, 28.5, 28.4, 28.2, 28.1, 27.7, 27.6, 22.0(i), 21.7(ii), 13.4; HRESI-MS *m/z* calc'd for $C_{30}H_{62}N_5O_9S$ 668.4268, found 668.4271 $[M+H]^+$.



6"-sulfoxide TOB derivative 5e. Compound **3e** (130 mg, 0.11 mmol) dissolved in $CHCl_3$ (3.5 mL) was treated with *m*-chloroperbenzoic acid (70-75%) (26 mg, ~0.15 mmol). The reaction mixture was stirred at rt and progress of the reaction was monitored by ESI-MS by following the disappearance of the starting material ($[M+H]^+$, *m/z* 1181.22) and the formation of the corresponding sulfoxide ($[M+H]^+$, *m/z* 1197.22). (*Note*: small quantities of the corresponding sulfone ($[M+H]^+$, *m/z* 1213.21) and of the starting material ($[M+H]^+$, *m/z* 1181.22) were in the mixture that was further processed). Upon near completion, the reaction mixture was diluted with $CHCl_3$ (15 mL), washed with 1 M KOH (2 mL), and concentrated under reduced pressure. Further purification by flash column chromatography (SiO_2, MeOH:CH_2Cl_2) gave the Boc-protected diastereomeric mixture of the sulfoxide (50 mg, 38%) that was then treated with 95% TFA (0.4 mL) for 3 min. The TFA was removed under reduced pressure, the residue was dissolved in a minimal volume of H_2O and freeze-dried to afford **5e** (46 mg, 87%) as a white foam. (*Note*: A mixture of 2 diastereomers (~4:1 ratio) was obtained. The major and minor

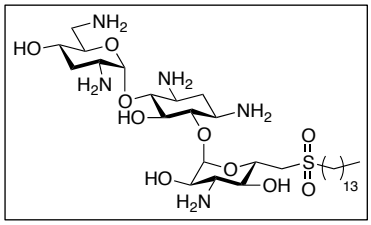
diastereomers are designated as (i) and (ii), respectively): ^{1}H NMR (400 MHz, D_2O) (Fig. S39) δ 5.69 (d, J = 3.5 Hz, 1H, H-1'(i)), 5.63 (d, J = 3.4 Hz, 1H, H-1'(ii)), 4.95-4.92 (m, 2H, H-1"(i), H-1"(ii)), 4.26 (br app. td, $J_1 = J_2$ = 9.2 Hz, J_3 = 1.7 Hz, 1H, H-5"(i)), 4.07 (m, 1H, H-5"(ii)), 3.88-3.69 (m, 8H, H-4(i), H-4(ii), H-5(i), H-5(ii), H-5'(i), H-5'(ii), H-2"(i), H-2"(ii)), 3.65-3.35 (m, 14H, H-1(i), H-1(ii), H-3(i), H-3(ii), H-6(i), H-6(ii), H-2'(i), H-2'(ii), H-4'(i), H-4'(ii), H-3"(i), H-3"(ii), H-4"(i), H-4"(ii)), 3.31-3.18 (m, 4H, H-6'(i), H-6'(ii), H-6"(i), H-6"(ii)), 3.10 (m, 2H, H-6'(i), H-6'(ii)), 2.96-2.74 (m, 6H, H-6"(i), H-6"(ii), $SC\underline{H}_2(CH_2)_{12}CH_3$(i), $SC\underline{H}_2(CH_2)_{12}CH_3$(ii)), 2.41 (app. dt, J_1 = 12.0 Hz, $J_2 = J_3$ = 4.2 Hz, 2H, H-2eq(i), H-2eq(ii)), 2.15 (app. dt, J_1 = 12.1 Hz, $J_2 = J_3$ = 4.3 Hz, 2H, H-3'eq(i), H-3'eq(ii)), 1.93-1.74 (m, 4H, H-2ax(i), H-2ax(ii), H-3'ax(i), H-3'ax(ii)), 1.55 (m, 4H, $SCH_2(C\underline{H}_2)_{12}CH_3$(i), $SCH_2(C\underline{H}_2)_{12}CH_3$ (ii)), 1.29 (m, 4H, $SCH_2(C\underline{H}_2)_{12}CH_3$(i), $SCH_2(C\underline{H}_2)_{12}CH_3$(ii)), 1.20-1.10 (m, 40H, $SCH_2(C\underline{H}_2)_{12}CH_3$(i), $SCH_2(C\underline{H}_2)_{12}CH_3$(ii)), 0.71 (t, 6H, J = 7.0 Hz, $SCH_2(CH_2)_{12}C\underline{H}_3$(i), $SCH_2(CH_2)_{10}C\underline{H}_3$(ii)); ^{13}C NMR (100 MHz, D_2O) (Fig. S40) δ 163.6 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 117.0 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 101.9 (anomeric C(ii)), 101.4 (anomeric C(i)), 95.0 (anomeric C(ii)), 94.5 (anomeric C(i)), 85.5(ii), 85.2(i), 77.8(ii), 77.1(i), 75.1(i), 74.9(ii), 70.8(i), 69.6(i), 69.2(ii), 69.0(i), 68.8(ii), 68.6(i), 67.4(ii), 65.2(i), 55.4(i), 55.3(ii), 52.5(i), 51.9(ii), 50.3(ii), 50.1(i), 49.0(i), 48.9, 48.6, 48.5(i), 40.6(i), 32.0(ii), 30.0, 29.6, 29.3, 29.0, 28.4, 22.8(i), 22.5(ii), 14.2; HRESI-MS m/z calc'd for $C_{32}H_{66}N_5O_9S$ 696.4581, found 696.4584 $[M+H]^+$.

2.4. Oxidation of 6"-thioether TOB derivatives 3d-e into 6"-sulfone TOB derivatives 6d-e.



6"-sulfone TOB derivative 6d. Compound **6d** was prepared as **5d** using compound **3d** (37 mg, 0.03 mmol), $CHCl_3$ (1 mL), *m*-chloroperbenzoic acid (70-75%) (20 mg, ~0.11 mmol) at rt. ESI-MS indicated the disappearance of the starting material ($[M+H]^+$,

m/z 1153.20) and the formation of the corresponding sulfone ($[M+H]^+$, *m/z* 1185.02). Deprotection using 95% TFA (0.4 mL) for 3 min gave **6d** (32 mg, 85%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S41) δ 5.54 (d, *J* = 3.6 Hz, 1H, H-1'), 5.00 (d, *J* = 3.7 Hz, 1H, H-1"), 4.29 (app. td, $J_1 = J_2 = 9.7$ Hz, $J_3 = 1.2$ Hz, 1H, H-5"), 3.89-3.77 (m, 4H, H-4, H-5, H-5', H-2"), 3.66 (dd, $J_1 = 10.2$ Hz, $J_2 = 8.6$ Hz, 1H, H-6), 3.60-3.36 (m, 8H, H-1, H-3, H-2', H-4', H-3", H-4", H-6"(2H)), 3.28 (dd, J_1 =13.6 Hz, $J_2 = 3.5$ Hz, 1H, H-6'), 3.18 (m, 2H, $SC\underline{H}_2(CH_2)_{10}CH_3$), 3.10 (dd, $J_1 = 13.6$ Hz, $J_2 = 6.9$ Hz, 1H, H-6'), 2.43 (app. dt, $J_1 = 12.6$ Hz, $J_2 = J_3 = 4.2$ Hz, 1H, H-2eq), 2.16 (app. dt, $J_1 = 11.9$ Hz, $J_2 = J_3 = 4.4$ Hz, 1H, H-3'eq), 1.86 (app. q, $J_1 = J_2 = J_3 = 11.3$ Hz, 1H, H-3'ax), 1.80 (app. q, $J_1 = J_2 = J_3 = 12.6$ Hz, 1H, H-2ax), 1.65 (m, 2H, $SCH_2(C\underline{H}_2)_{10}CH_3$), 1.30 (m, 2H, $SCH_2(C\underline{H}_2)_{10}CH_3$),1.20-1.10 (m, 16H, $SCH_2(C\underline{H}_2)_{10}CH_3$), 0.71 (t, 3H, *J* = 7.0 Hz, $SCH_2(CH_2)_{10}C\underline{H}_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S42) δ 162.9 (q, *J* = 35 Hz, $CF_3\underline{C}O_2H$), 116.3 (q, *J* = 290 Hz, $\underline{C}F_3CO_2H$), 100.5 (anomeric C), 95.0 (anomeric C), 84.3, 77.7, 74.6, 69.9, 67.7, 67.65, 67.5, 64.5, 54.5, 54.1, 52.3, 49.2, 48.1, 48.0, 39.9, 31.2, 29.6, 29.4, 28.7, 28.6, 28.5, 28.4, 28.0, 27.7, 27.4, 22.0, 21.1, 13.4; HRESI-MS *m/z* calc'd for $C_{30}H_{62}N_5O_{10}S$ 684.4217, found 684.4216 $[M+H]^+$.



6"-sulfone TOB derivative 6e. Compound **6e** was prepared as **5e** using compound **3e** (63 mg, 0.05 mmol), $CHCl_3$ (1.5 mL), *m*-chloroperbenzoic acid (70-75%) (35 mg, ~0.20 mmol) at rt. ESI-MS indicated the disappearance of the starting material ($[M+H]^+$, *m/z* 1181.22) and the formation of the corresponding sulfone ($[M+H]^+$, *m/z* 1213.21). Deprotection using 95% TFA (0.4 mL) for 3 min gave **6e** (44 mg, 70%) as a white foam: ^{1}H NMR (400 MHz, D_2O) (Fig. S43) δ 5.54 (d, *J* = 3.6 Hz, 1H, H-1'), 5.00 (d, *J* = 3.7 Hz, 1H, H-1"), 4.29 (app. td, $J_1 = J_2 = 9.6$ Hz, $J_3 = 1.2$ Hz, 1H,

H-5"), 3.89-3.77 (m, 4H, H-4, H-5, H-5', H-2"), 3.66 (dd, J_1 = 10.3 Hz, J_2 = 8.4 Hz, 1H, H-6), 3.60-3.35 (m, 8H, H-1, H-3, H-2', H-4', H-3", H-4"H-6"(2H)), 3.28 (dd, J_1 =13.6 Hz, J_2 = 3.6 Hz, 1H, H-6'), 3.18 (m, 2H, $SC\underline{H}_2(CH_2)_{12}CH_3$), 3.10 (dd, J_1 = 13.6 Hz, J_2 = 6.9 Hz, 1H, H-6'), 2.42 (app. dt, J_1 = 12.6 Hz, $J_2 = J_3$ = 4.2 Hz, 1H, H-2eq), 2.16 (app. dt, J_1 = 11.9 Hz, $J_2 = J_3$ = 4.2 Hz, 1H, H-3'eq), 1.85 (app. q, $J_1 = J_2 = J_3$ = 11.0 Hz, 1H, H-3'ax), 1.79 (app. q, $J_1 = J_2 = J_3$ = 12.5 Hz, 1H, H-2ax), 1.65 (m, 2H, $SCH_2(C\underline{H}_2)_{12}CH_3$), 1.30 (m, 2H, $SCH_2(C\underline{H}_2)_{12}CH_3$),1.20-1.10 (m, 20H, $SCH_2(C\underline{H}_2)_{12}CH_3$), 0.71 (t, 3H, J = 7.0 Hz, $SCH_2(CH_2)_{12}C\underline{H}_3$); ^{13}C NMR (100 MHz, D_2O) (Fig. S44) δ 163.7 (q, J = 35 Hz, $CF_3\underline{C}O_2H$), 117.1 (q, J = 290 Hz, $\underline{C}F_3CO_2H$), 101.2 (anomeric C), 95.8 (anomeric C), 85.0, 78.5, 75.3, 70.7, 68.5, 68.3, 68.2, 65.3, 55.2, 54.8, 53.1, 49.9, 48.8, 48.7, 40.7, 31.9, 30.3, 30.1, 29.5, 29.4, 29.3, 29.2, 29.1, 28.8, 28.5, 28.1, 22.8, 21.8, 14.1; HRESI-MS *m/z* calc'd for $C_{32}H_{65}N_5O_{10}SNa$ 734.4350, found 734.4348 $[M+H]^+$.

3. Biochemical methods

3.1. Determination of MIC values of 6"-thioether TOB derivatives 4a-r.

MIC values were determined against a variety of Gram-positive bacterial strains: *S. epidermidis* ATCC12228 (**A**), *S. aureus* NorA (**B**), MRSA (**C**), *S. pyogenes* serotype M12 (strain MGAS9429) (**D**), *S. mutans* UA159 (**E**), *B. subtilis* 168 (**F**), *B. subtilis* 168 with AAC(6')/APH(2")-pRB374 (**G**), *B. cereus* ATCC11778 (**H**), *B. anthracis* 34F2 Sterne strain (**I**), VRE (**J**), *E. faecalis* ATCC29212 (**K**), and *L. monocytogenes* ATCC19115 (**L**). MIC values were also determined against a variety of Gram-negative bacterial strains: *E. coli* BL21 (DE3) (**M**), *E. coli* BL21 (DE3) with pET22b (**N**), *E. coli* BL21 (DE3) with AAC(6')/APH(2")-pET22b (**O**), *E. coli* BL21 (DE3) with AAC(3)-IV-Int-pET19b-pps (**P**), *E. coli* BL21 (DE3) with Eis (**Q**), *E. coli* TolC (**R**), *E. coli* MC1061 (**S**), *Shigella* clinical isolate 6831 (**T**), and *S. enterica*

ATCC14028 (**U**). Strains were tested using a double-dilution of 6"-thioether TOB derivatives **4a-r** starting at 150 μg/mL (Fig. S45A). All experiments were performed in two separate sets of duplicate or triplicate experiments. MIC values were confirmed by the addition of MTT (50 μL of a 1 mg/mL solution in H_2O).

3.2. Prokaryotic protein translation inhibition test (luciferase assay system).

The prokaryotic *in vitro* translation inhibition by TOB (**1**) and the 6"-thioether TOB derivative **4e** was quantified in coupled transcription/translation assays[7] by using *E. coli* S30 extract for circular DNA with the pBEST*luc* plasmid (Promega), according to the manufacturer's protocol. Translation reactions (10 μL) containing various concentrations of the tested compounds (0.1, 0.5, 1, 5, 10, 25, 50, and 100 ng/mL) were incubated at 37 °C for 90 min, cooled on ice for 5 min, and diluted with a dilution reagent (tris-phosphate buffer (25 mM, pH 7.8 adjusted at rt), DTT (2 mM), 1,2-diaminocyclohexanetetraacetate (2 mM), glycerol (10%), Triton X-100 (1%), and BSA (1 mg/mL)) into 96-well plates. The luminescence was measured immediately after the addition of the luciferase assay reagent (50 μL, Promega), and the light emission was recorded with a FLx800 Fluorescence Microplate Reader (Biotek). The half-maximal inhibition concentration (IC_{50}) values were obtained from fitting concentration-response curves to the data of at least two independent experiments by using Grafit 5 software.

3.3. Time-kill kinetic study of TOB (1) and 4e against *S. mutans* UA159 and *S. pyogenes* serotype M12 (strain MGAS9429).

To determine the time-kill kinetic against *S. mutans* UA159 and *S. pyogenes* serotype M12 (strain MGAS9429), the bacteria were grown aerobically (37 °C, 5% CO_2, overnight) from a

frozen glycerol stock kept at -80 °C in Brain Heart Infustion (BHI) broth (BBL Microbiology Systems, Cockeysville, MD, USA) (25 mL) until they reached stationary phase. The overnight culture was then diluted into BHI broth (1 mL of overnight culture into 250 mL of fresh broth). The diluted *S. mutans* UA159 and *S. pyogenes* cultures were then aliquoted (1 mL/well) into 12-well plates (Nunc, Rochester, NY, USA) and incubated in the presence of TOB (**1**) (75 μg/mL for *S. mutans* and 18.8 μg/mL for *S. pyogenes* = 1× MIC) or of the 6"-thioether TOB derivative **4e** (2.3 μg/mL = 1× MIC) and grown aerobically (37 °C, 5% CO_2) for a total of 5 h. A positive control consisted of aliquots (1 mL) of *S. mutans* UA159 or *S. pyogenes* cultures incubated in the absence of antibiotic. Immediately (time 0), and after 10 min, 1, 2, 3, 4, and 5 h, an aliquot (5 μL) was diluted (10-, 10^3-, and 10^6-fold) into BHI broth and 10 μL of each dilution (50 μL total) were plated in duplicate on BHI agar (BBL Microbiology Systems) plates. After exactly 24 h (starting from each tested time point) of incubation, the number of colonies on each plate was counted using a colony counter. Time-kill assays were analyzed by determining the reductions in viable count (CFU/mL) at the above time points by TOB (**1**) and compound **4e** as compared with the positive control (Fig. S45B). All duplicate experiments were performed 3 times.

3.4. Epi-fluorescence microscopy using the 6"-thioether TOB derivative 4e.

To verify if compound **4e** targets the bacterial membrane, *B. subtilis* PY79 cells carrying YFP under an inducible IPTG promoter[8] were used. This bacterial strain was constructed by cloning of the constitutive *trpE* promoter into the *B. subtilis* PY79 integration plasmid AEC127 designed to integrate into the *sacA* position and carrying a *yfp* gene. Cloning was done using standard techniques in *E. coli* DH5α. *B. subtilis* PY79 cells carrying YFP under an inducible IPTG promoter from a freshly streaked plate were grown (37 °C, ~14 h) in Luria-Bertani (LB) broth (3

mL) supplemented with tetracycline (10 μg/mL) and IPTG (1 mM). The overnight culture (0.1 mL) was diluted into fresh LB broth (10 mL) containing IPTG (1 mM) and grown to an OD_{600} of 0.5. The cells were then treated with either TOB (**1**) (2.3 μg/mL (2× MIC) and 9.4 μg/mL (8× MIC)) or with the 6"-thioether TOB derivative **4e** (4.7 μg/mL (2× MIC) and 18.8 μg/mL (8× MIC)) and continued to grow at 37 °C. After 1 h in the presence of **1** or **4e**, aliquots (1 μL) were put on agar slabs made of PBS. Snapshots fluorescence images were taken with a 100× lens of an inverted epi-fluorescence microscope (Nikon TiE, Nikon, Japan).

3.5. Determination of AME activity on the 6"-thioether TOB derivatives 4a-r.

To determine the activity of the modified TOB AGs (**4a-r**, **5d-e**, and **6d-e**) with various AMEs, several previously developed assays were utilized to monitor the transformation of the AGs (Figs. 3 (in the main text) and S46).

Acetylation:

The acetylation activity of several AACs (AAC(6')/APH(2"),[1; 2] AAC(6')-Ib',[3] AAC(6')-IId, AAC(3)-IV,[1; 2] AAC(2')-Ic,[4] and Eis[4]) was monitored using the Ellman's method where the reaction of the CoASH released during acetylation is reacted with DTNB and monitored at 412 nm. Briefly, reactions (200 μL) containing AG (100 μM) and AcCoA (500 μM for Eis or 150 μM for the remaining AACs) were incubated with AAC enzyme (0.125 μM AAC(2')-Ic and AAC(3)-IV or 0.5 μM for all remaining AACs) in the presence of DTNB (2 mM) and the appropriate buffer (50 mM MES pH 6.6 for AAC(6')/APH(2") and AAC(3)-IV, 50 mM Tris pH 7.5 for AAC(6')-Ib' and AAC(6')-IId, 50 mM Tris pH 8.0 for Eis, and 100 mM sodium phosphate pH 7.4 for AAC(2')-Ic). The reactions were monitored at 37 °C (AAC(6')/APH(2")) or 25 °C

(AAC(6')-Ib', AAC(6')-IId, AAC(3)-IV, AAC(2')-Ic, and Eis) on a SpectraMax M5 microplate reader, taking measurements every 30 s for 1 h. The initial rates of the reactions were calculated using the first 2 min of the reaction (AAC(3)-IV) or the first 10 min (all remaining AACs) and normalized to TOB (**1**). All experiments were performed in triplicate.

Nucleotidylation:

The nucleotidyltransferase activity of the ANT(4') was monitored at 600 nm through the complex formation of molybdate in malachite green and the P_i generated by inorganic pyrophosphatase cleavage of the released PP_i during the ANT catalyzed reaction.[5; 9] To analyze the activity of ANT(4') on the 6"-thioether TOB derivatives (**4a-r**) as well as the sulfoxide and sulfone derivatives **5d-e** and **6d-e**, reactions (160 μL) containing Tris-HCl (50 mM, pH 7.5), $MgCl_2$ (10 mM), KCl (50 mM), inorganic pyrophosphatase (0.2 U/mL), AG (100 μM), and ATP (0.5 mM) were performed at 25 °C. The reactions were initiated by addition of ANT(4') (1 μM), incubated for 15, 30, 45, 60, and 75 s, and quenched with the molybdate/malachite green reagent (40 μL) to terminate the reaction. After 15 min of color development, the liberated P_i concentration was measured by absorbance at 600 nm. The initial rates were determined using the first 60 sec of reaction time. All experiments were performed in triplicate.

3.6. Red blood cells (RBC) lysis assay.

Rat or human RBC solution (2% w/w) was incubated with 6"-thioether TOB analogues **4b-h** (18.8 μg/mL as well as 75 μg/mL, 1 h, 37 °C). Negative controls were PBS and Dextran (MW ~70,000 Da) while positive controls were 1% w/v solution of Triton X100 (100% lysis). Following centrifugation (2,000 rpm, 10 min, rt), the supernatant was drawn off and its

absorbance measured at 550 nm using a microplate reader (Genios, TECAN). The results were expressed as percentage of hemoglobin released relative to the positive control (Triton X100).

4. Abbreviations.

AAC, aminoglycoside acetyltransferase; AG, aminoglycoside; AME, aminoglycoside-modifying enzyme; ANT, aminoglycoside nucelotidyltransferase; APH, aminoglycoside phosphotransferase; BHI, brain heart infusion; BOC, di-*tert*-butyl dicarbonate; BSA, bovine serum albumin; DMF, dimethylformamide; DTT, dithiothreitol; EtOAc, ethyl acetate; IPTG, Isopropyl β-D-1-thiogalactopyranoside; MIC, minimum inhibitory concentration; MTT, thiazolyl blue tetrazolium bromide; PBS, phosphate buffered saline; P_i, inorganic phosphate; PP_i, inorganic pyrophosphate; RBC, red blood cells; rt, room temperature; TFA, trifluoroacetic acid; TLC, thin layer chromatography; TOB, tobramycin; XDR, extensively-drug resistant; YFP, yellow fluorescent protein.

5. Supporting information references.

1. Shaul, P., Green, K. D., Rutenberg, R., Kramer, M., Berkov-Zrihen, Y., Breiner-Goldstein, E., Garneau-Tsodikova, S. & Fridman, M. (2011). Assessment of 6'- and 6'''-N-acylation of aminoglycosides as a strategy to overcome bacterial resistance. *Organic & biomolecular chemistry* **9**, 4057-63.
2. Green, K. D., Chen, W., Houghton, J. L., Fridman, M. & Garneau-Tsodikova, S. (2010). Exploring the substrate promiscuity of drug-modifying enzymes for the chemoenzymatic generation of N-acylated aminoglycosides. *Chembiochem : a European journal of chemical biology* **11**, 119-26.
3. Green, K. D., Chen, W. & Garneau-Tsodikova, S. (2011). Effects of altering aminoglycoside structures on bacterial resistance enzyme activities. *Antimicrobial agents and chemotherapy* **55**, 3207-13.
4. Chen, W., Biswas, T., Porter, V. R., Tsodikov, O. V. & Garneau-Tsodikova, S. (2011). Unusual regioversatility of acetyltransferase Eis, a cause of drug resistance in XDR-TB. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 9804-8.

5. Porter, V. R., Green, K. D., Zolova, O. E., Houghton, J. L. & Garneau-Tsodikova, S. (2010). Dissecting the cosubstrate structure requirements of the Staphylococcus aureus aminoglycoside resistance enzyme ANT(4'). *Biochemical and biophysical research communications* **403**, 85-90.
6. Michael, K., Wang, H. & Tor, Y. (1999). Enhanced RNA binding of dimerized aminoglycosides. *Bioorganic & medicinal chemistry* **7**, 1361-71.
7. Greenberg, W. A., Priestley, E. S., Sears, P. S., Alper, P. B., Rosenbohm, C., Hendrix, M., Hung, S.-C. & Wong, C.-H. (1999). Design and synthesis of new aminoglycoside antibiotics containing neamine as an optimal core structure: correlation of antibiotic activity with in vitro inhibition of translation. *Journal of the American Chemical Society* **121**, 6527-41.
8. Eldar, A., Chary, V. K., Xenopoulos, P., Fontes, M. E., Loson, O. C., Dworkin, J., Piggot, P. J. & Elowitz, M. B. (2009). Partial penetrance facilitates developmental evolution in bacteria. *Nature* **460**, 510-4.
9. McQuade, T. J., Shallop, A. D., Sheoran, A., Delproposto, J. E., Tsodikov, O. V. & Garneau-Tsodikova, S. (2009). A nonradioactive high-throughput assay for screening and characterization of adenylation domains for nonribosomal peptide combinatorial biosynthesis. *Analytical biochemistry* **386**, 244-50.

6. Supporting information Figs. S1-S45.

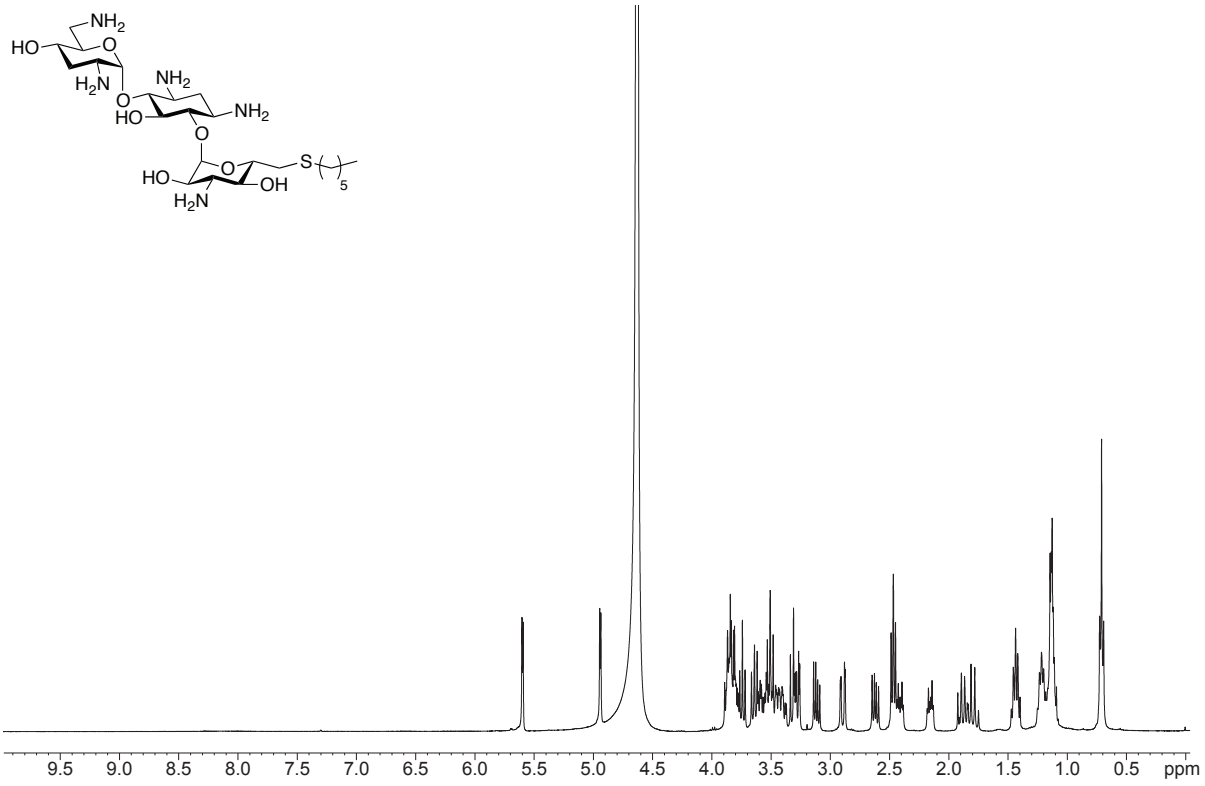


Fig. S1. ^{1}H NMR for 6"-thioether TOB derivative **4a**.

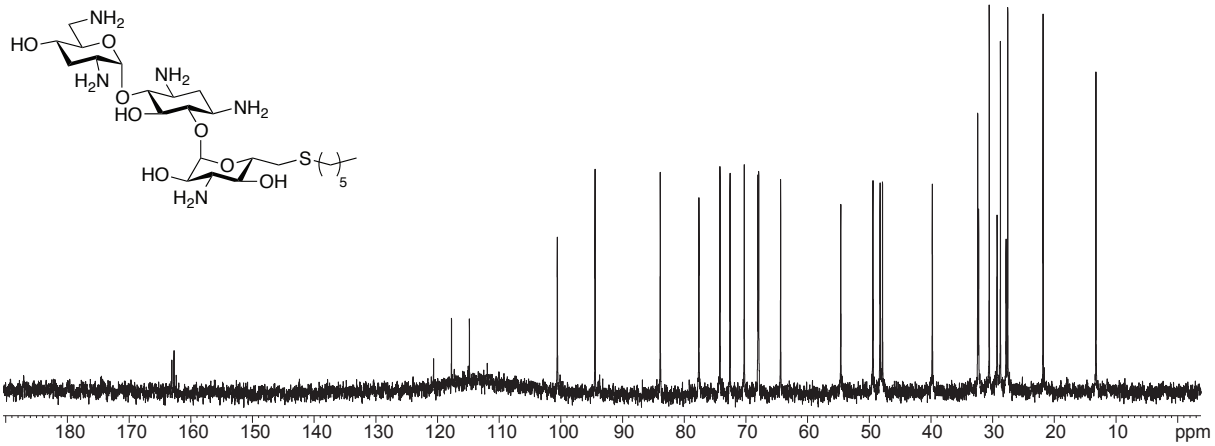


Fig. S2. ^{13}C NMR for 6"-thioether TOB derivative **4a**.

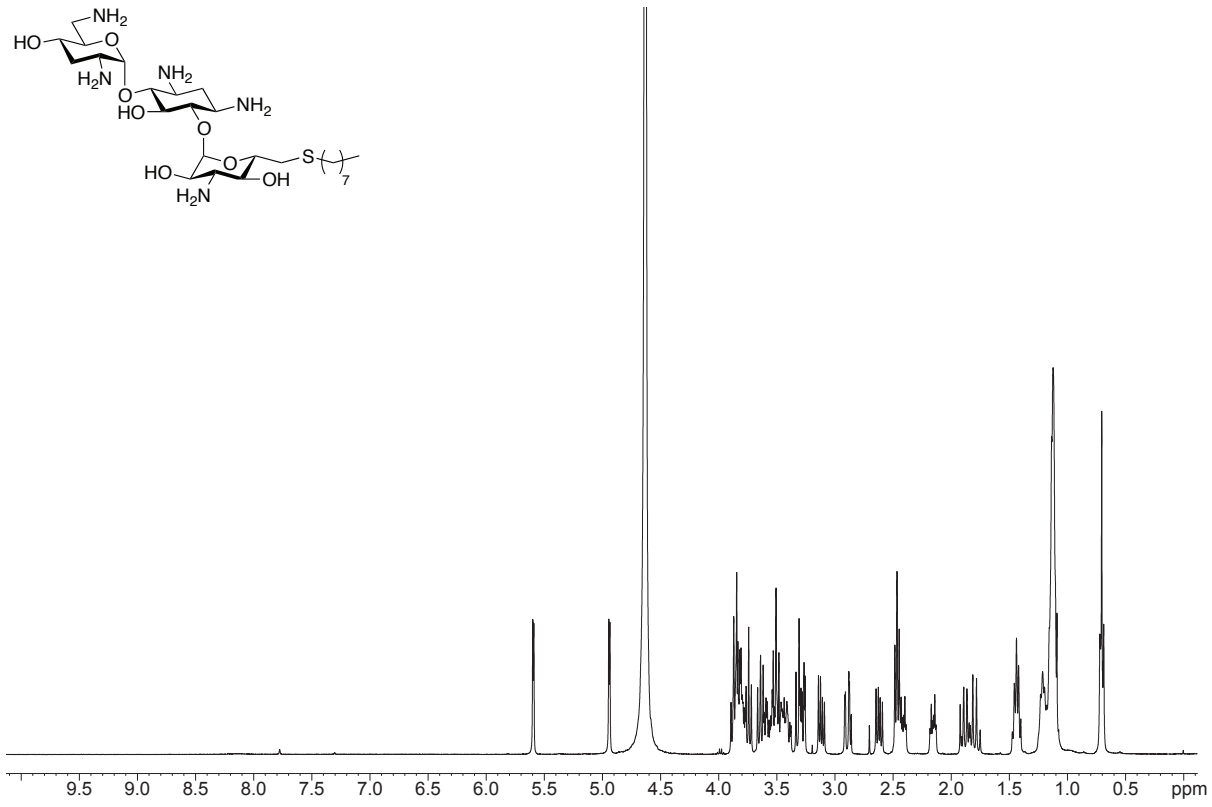


Fig. S3. [1]H NMR for 6"-thioether TOB derivative **4b**.

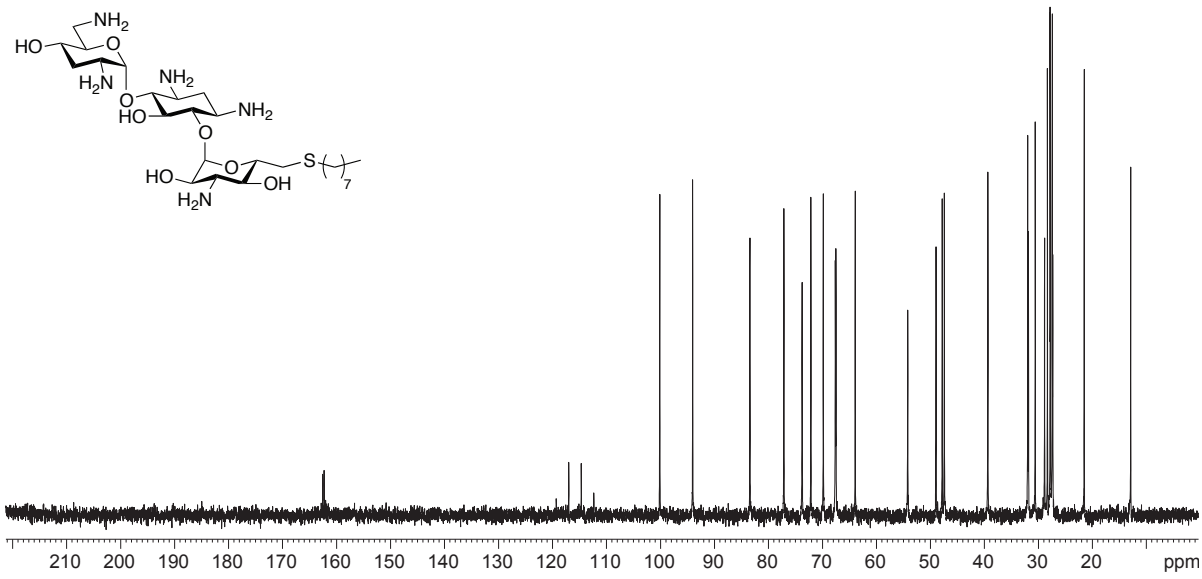


Fig. S4. [13]C NMR for 6"-thioether TOB derivative **4b**.

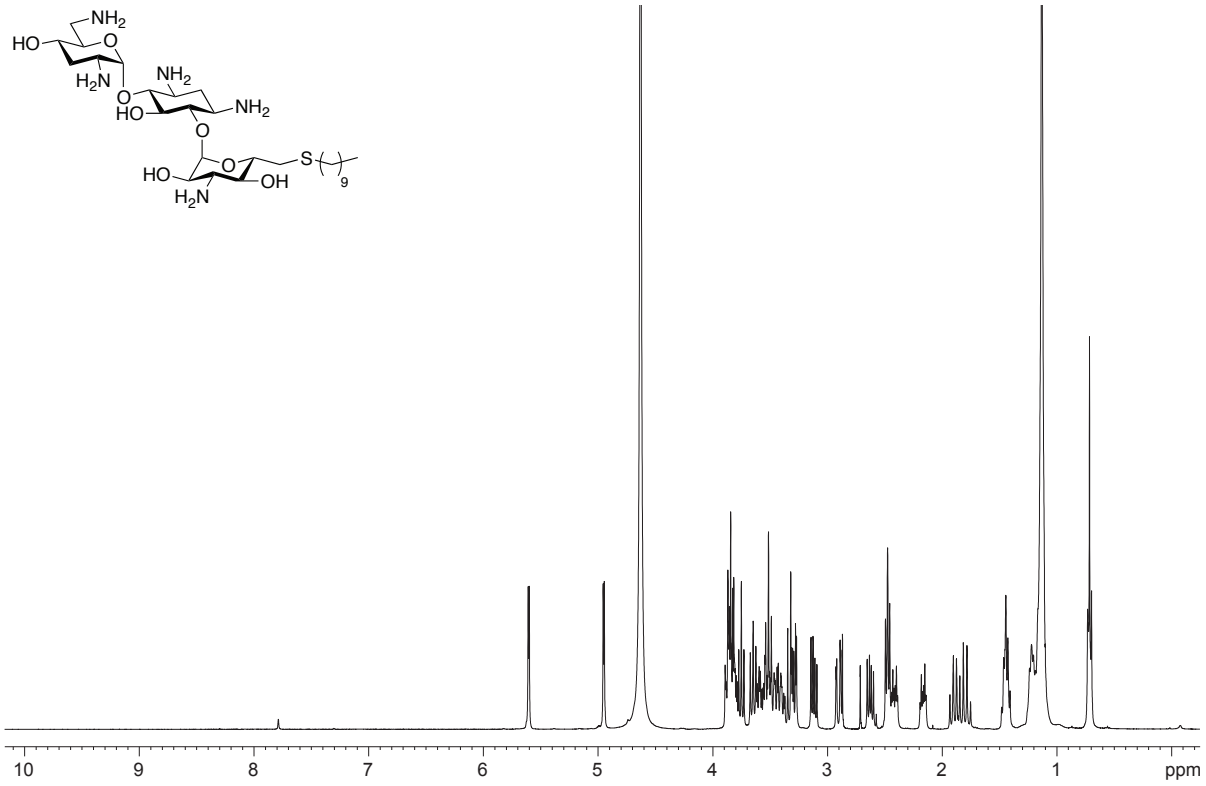


Fig. S5. [1]H NMR for 6"-thioether TOB derivative **4c**.

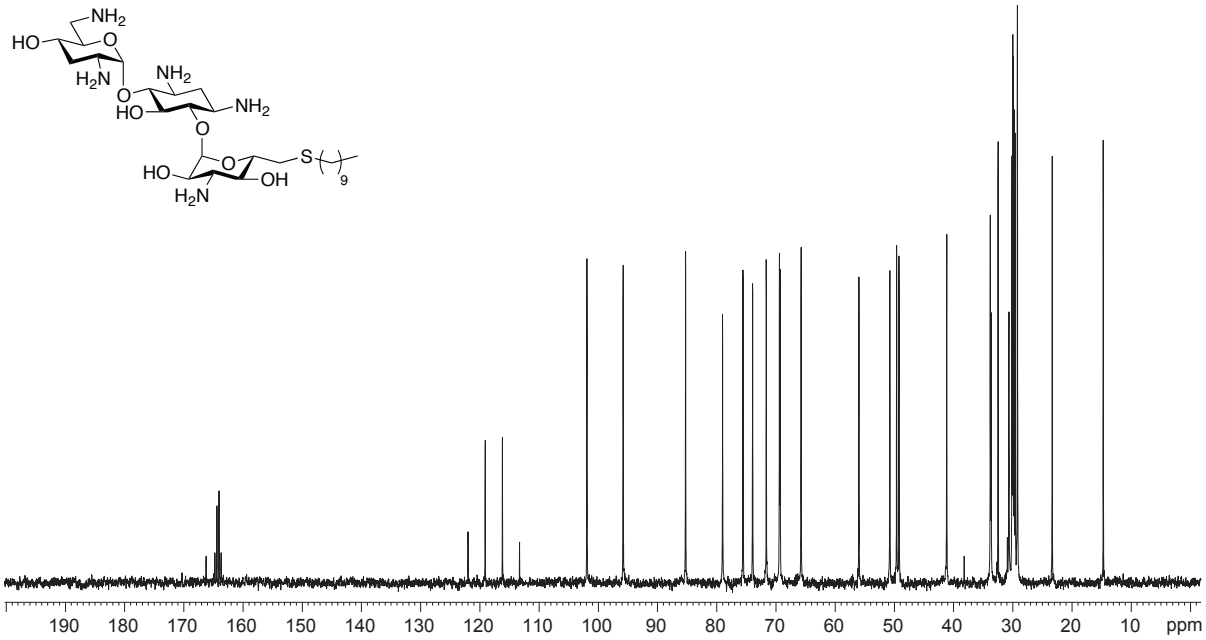


Fig. S6. [13]C NMR for 6"-thioether TOB derivative **4c**.

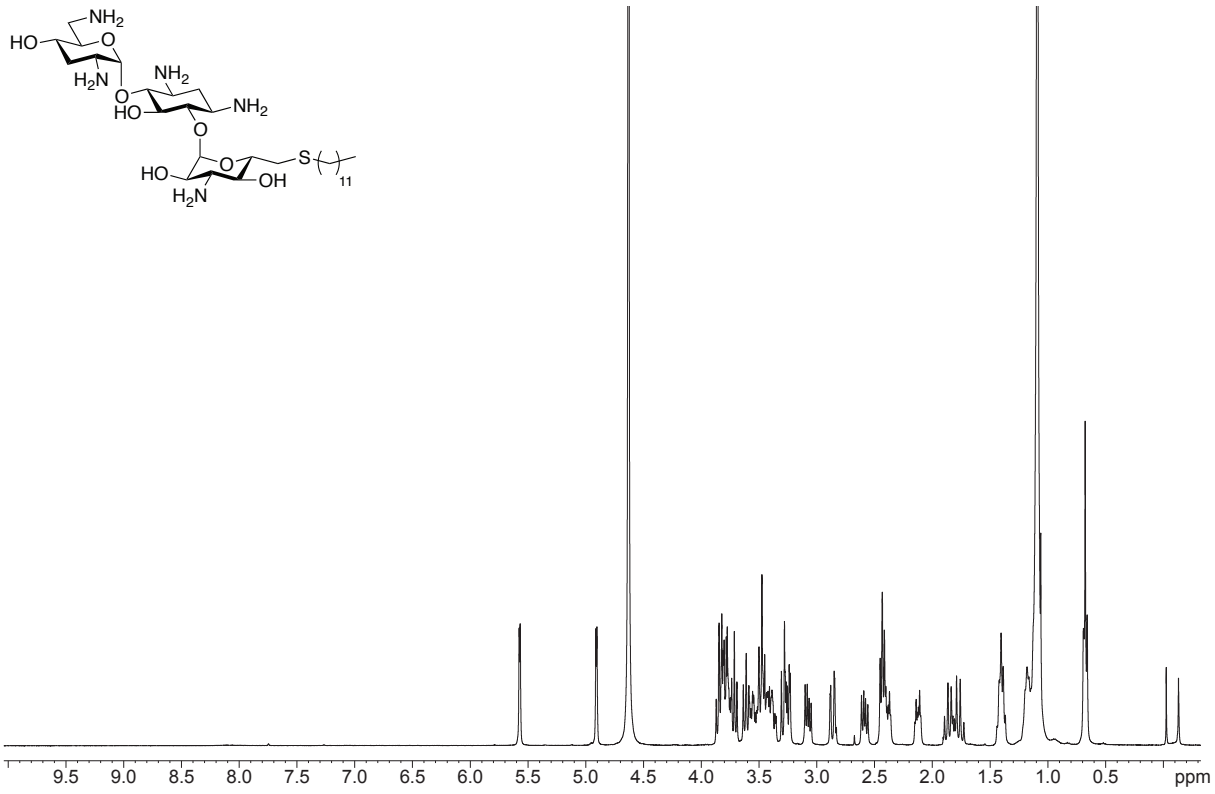


Fig. S7. [1]H NMR for 6"-thioether TOB derivative **4d**.

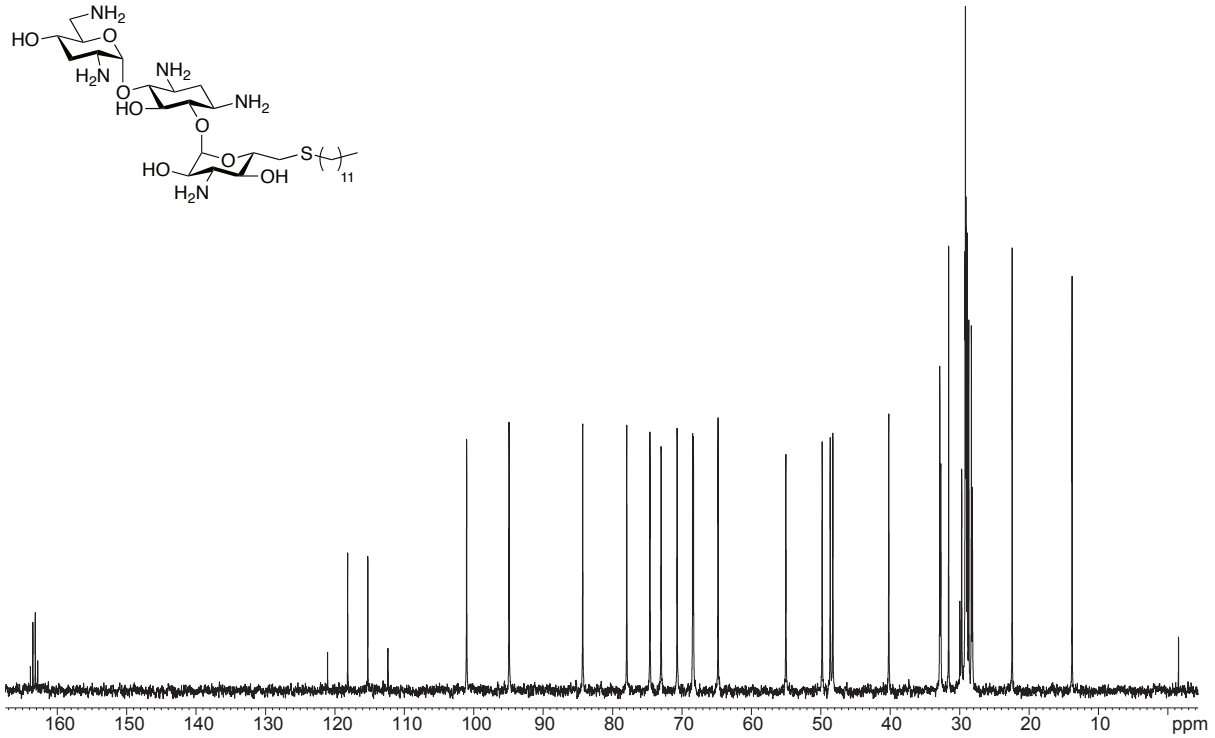


Fig. S8. [13]C NMR for 6"-thioether TOB derivative **4d**.

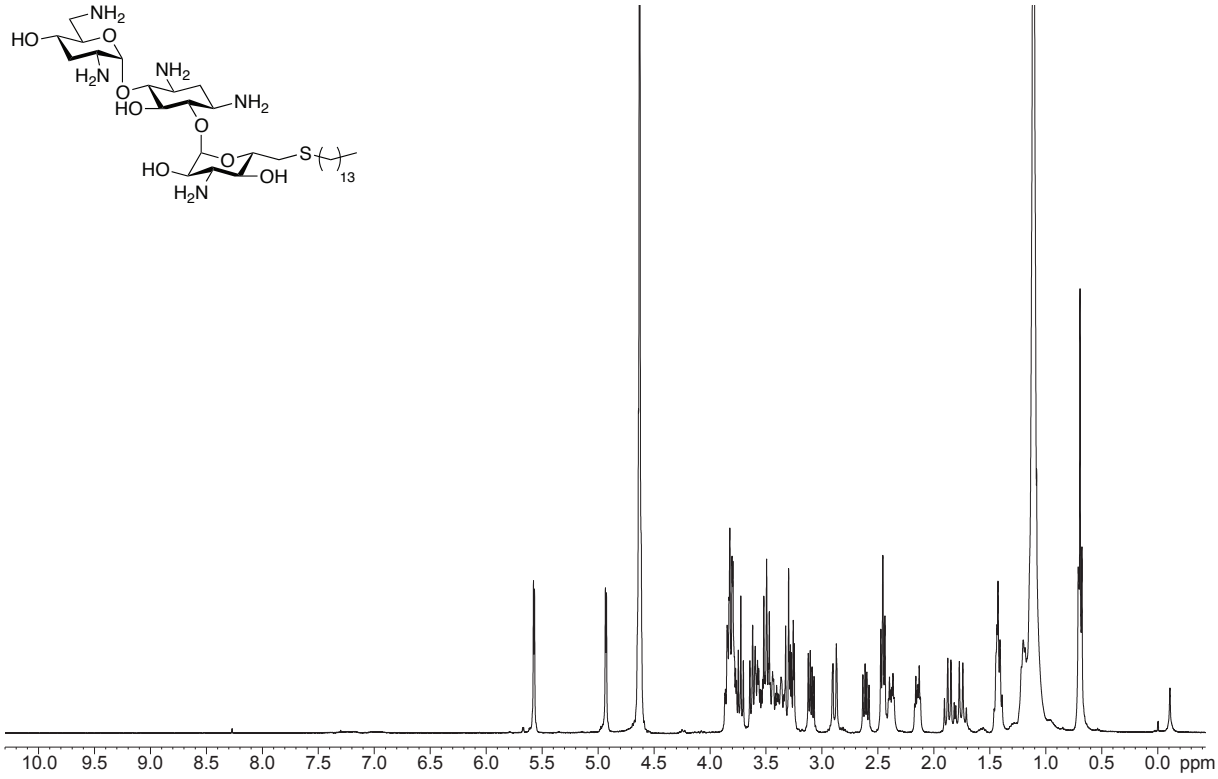


Fig. S9. [1]H NMR for 6"-thioether TOB derivative **4e**.

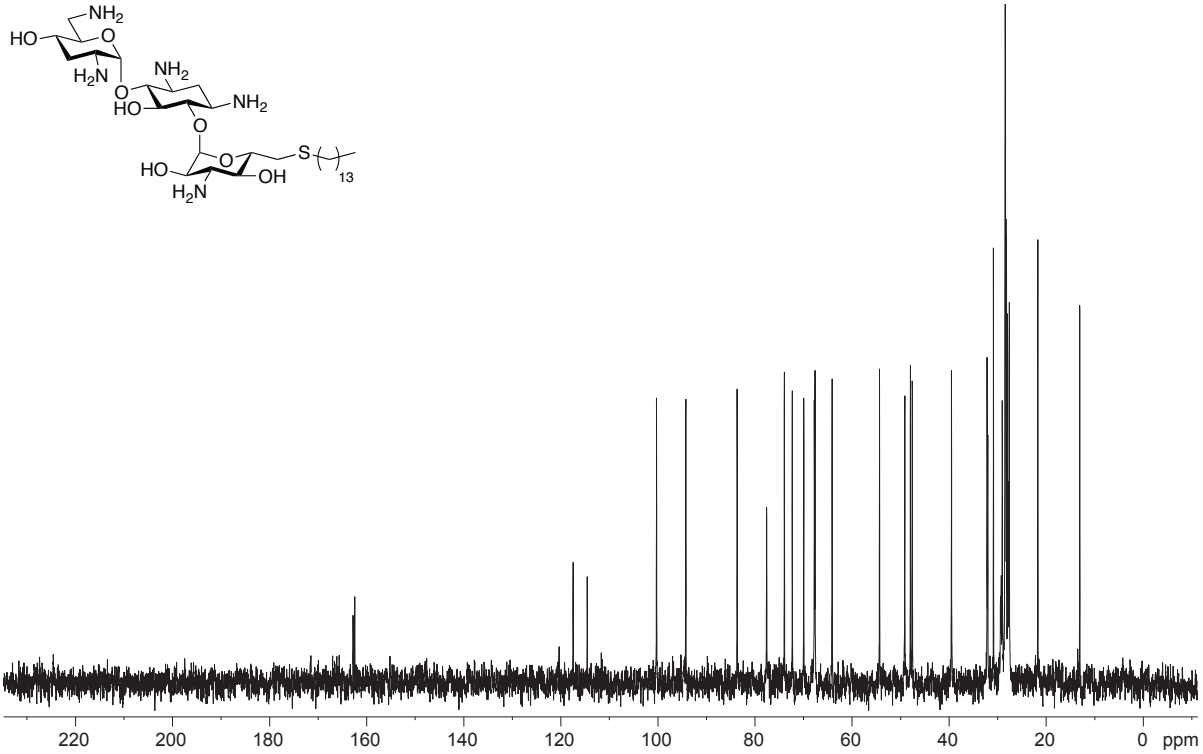


Fig. S10. [13]C NMR for 6"-thioether TOB derivative **4e**.

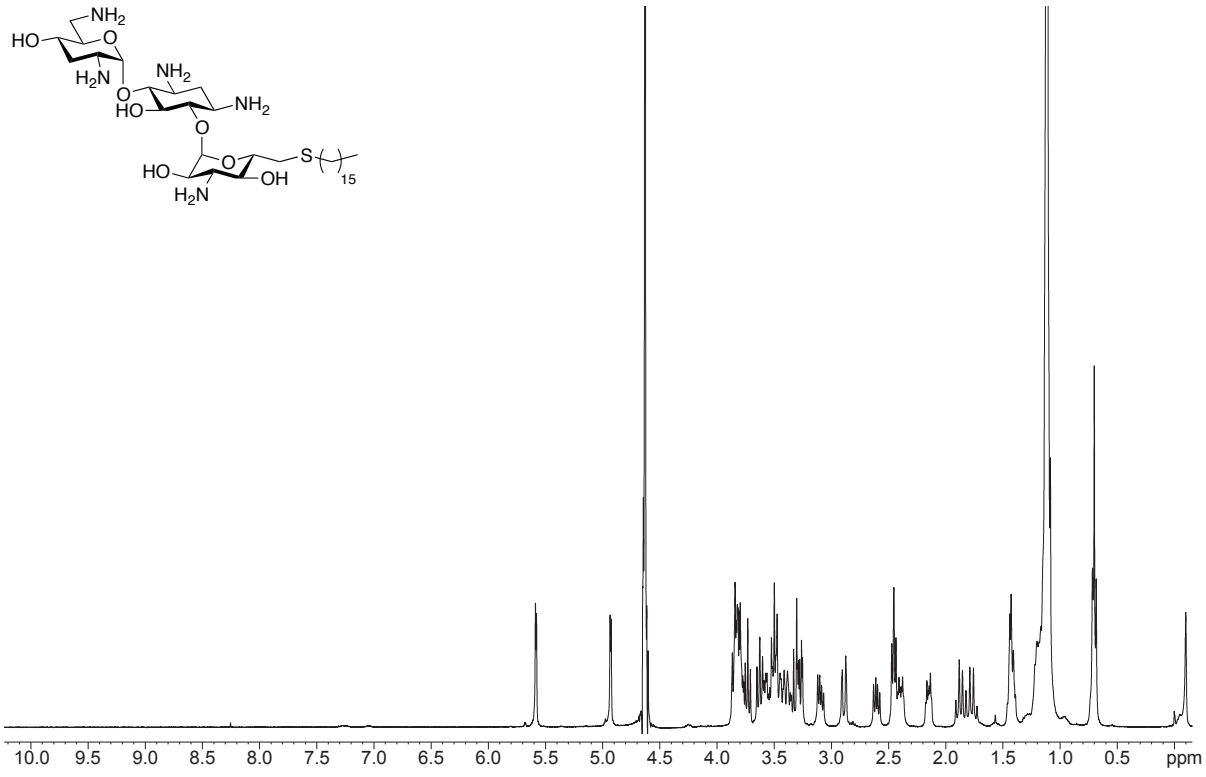


Fig. S11. ^{1}H NMR for 6"-thioether TOB derivative **4f**.

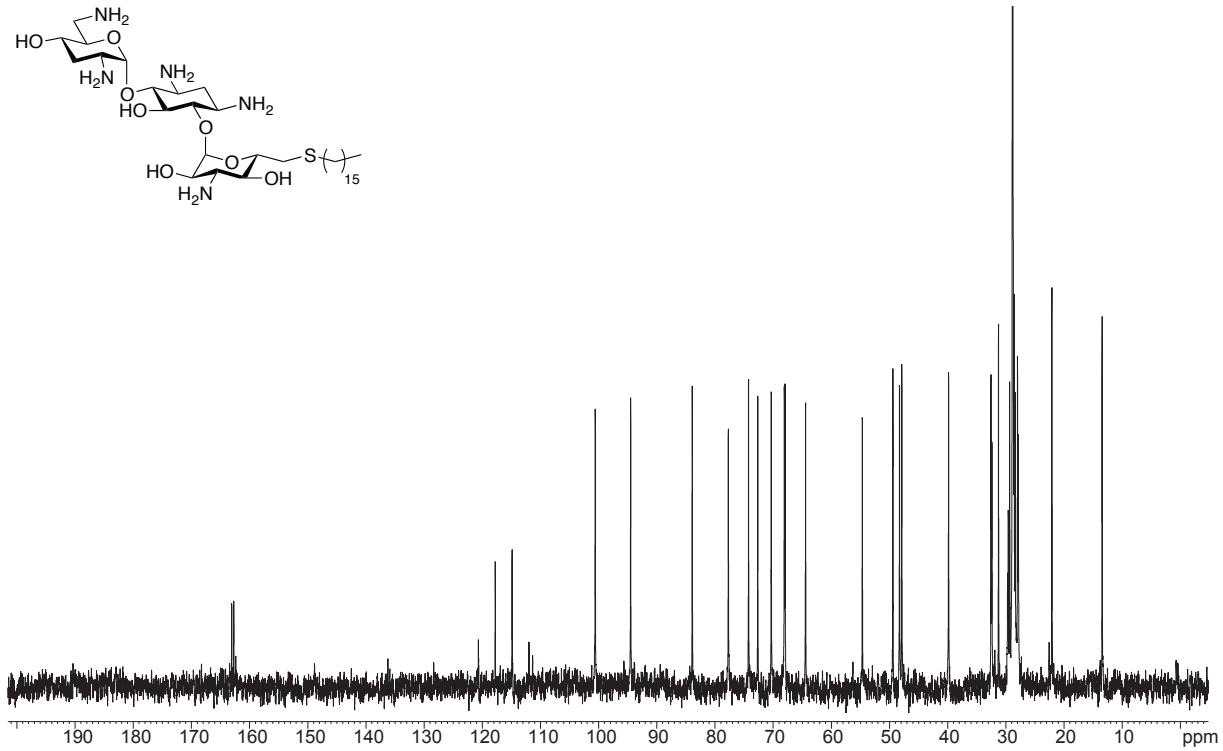


Fig. S12. ^{13}C NMR for 6"-thioether TOB derivative **4f**.

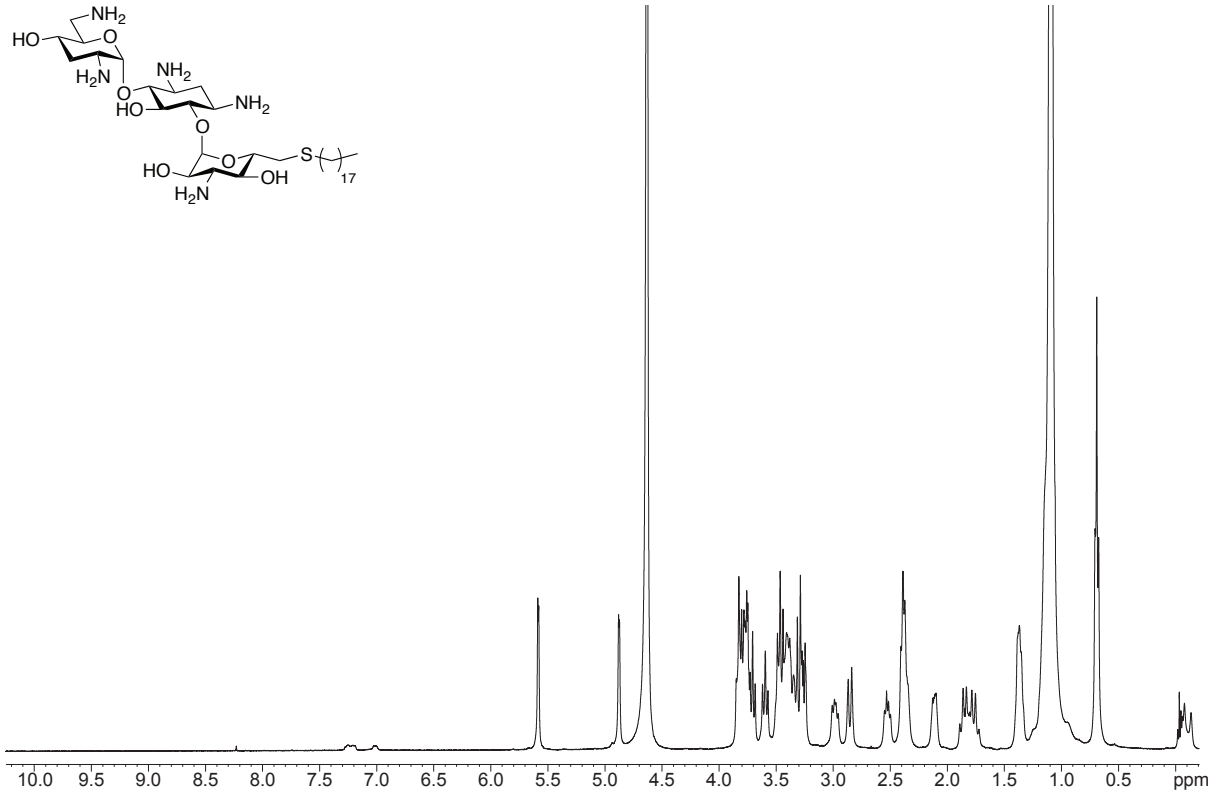


Fig. S13. ^{1}H NMR for 6"-thioether TOB derivative **4g**.

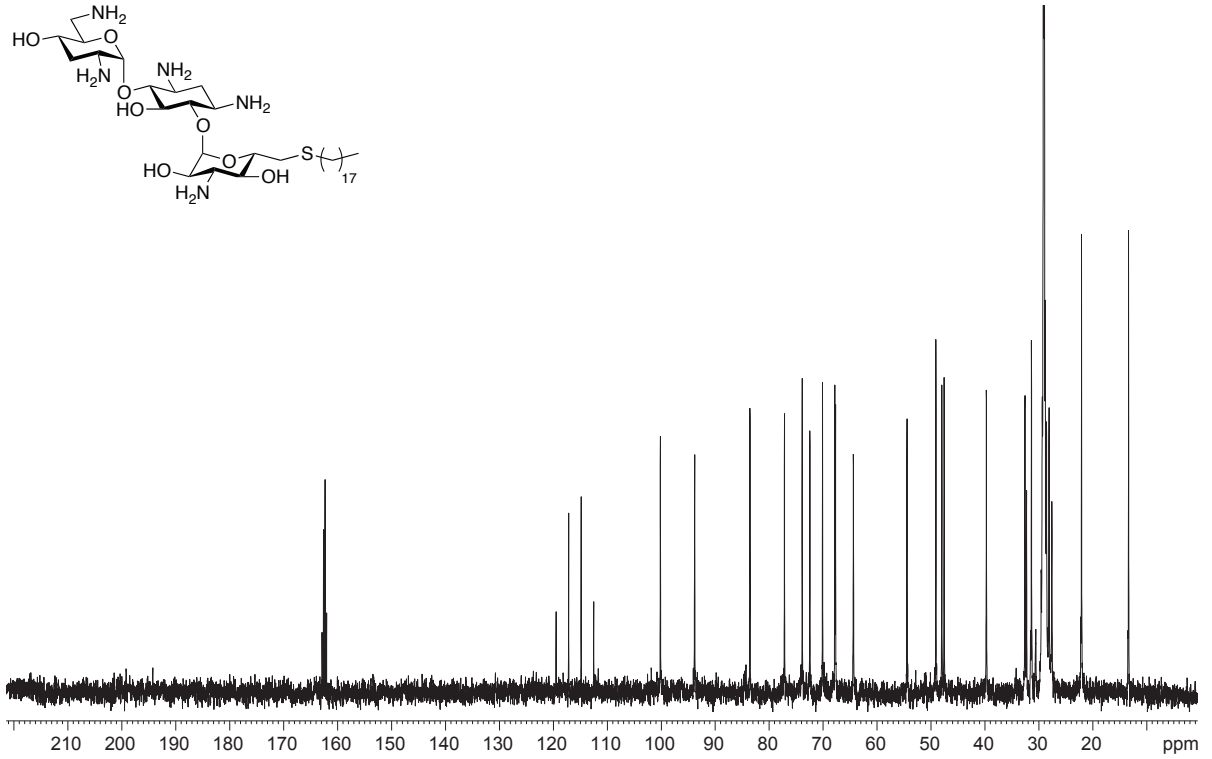


Fig. S14. ^{13}C NMR for 6"-thioether TOB derivative **4g**.

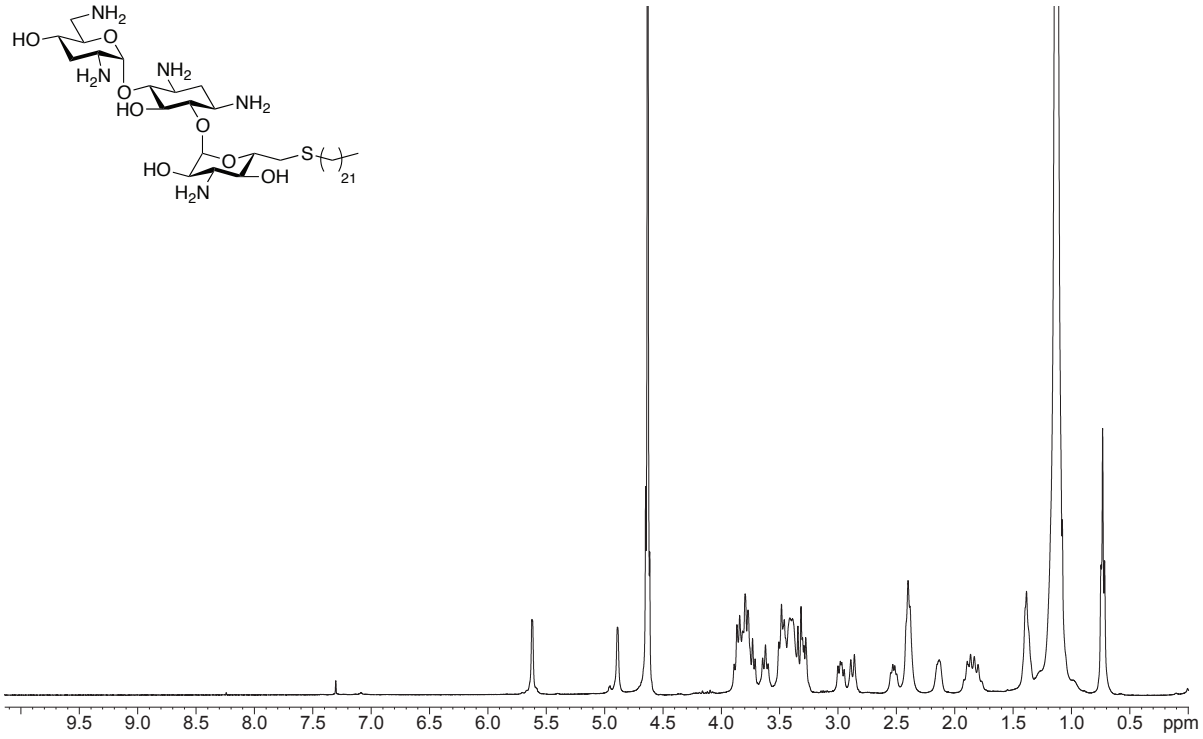


Fig. S15. [1]H NMR for 6"-thioether TOB derivative **4h**.

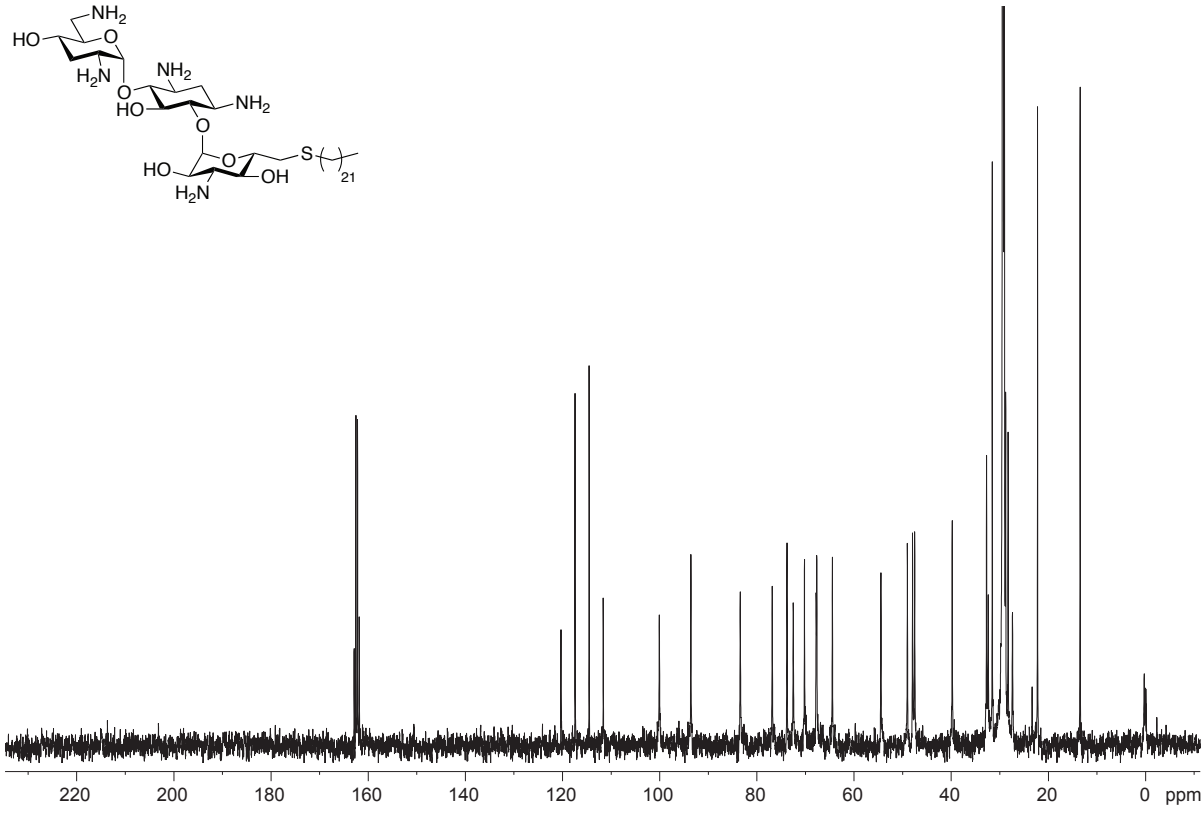


Fig. S16. [13]C NMR for 6"-thioether TOB derivative **4h**.

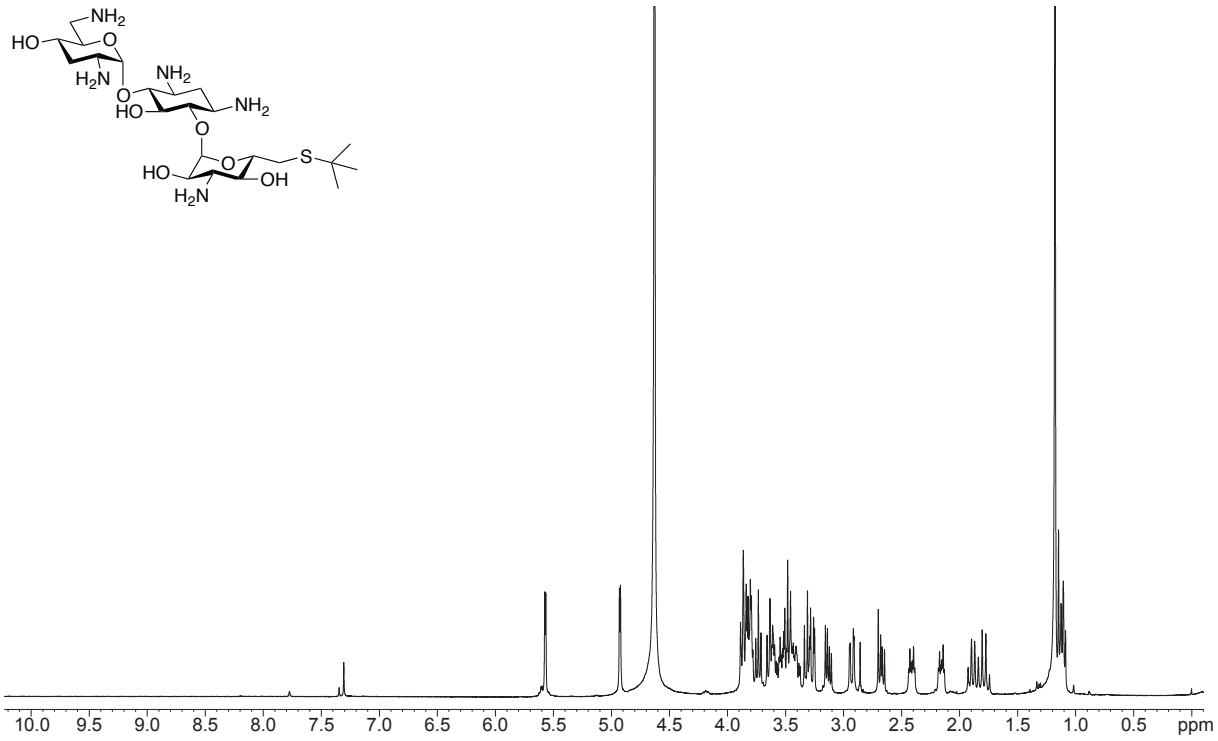


Fig. S17. [1]H NMR for 6"-thioether TOB derivative **4i**.

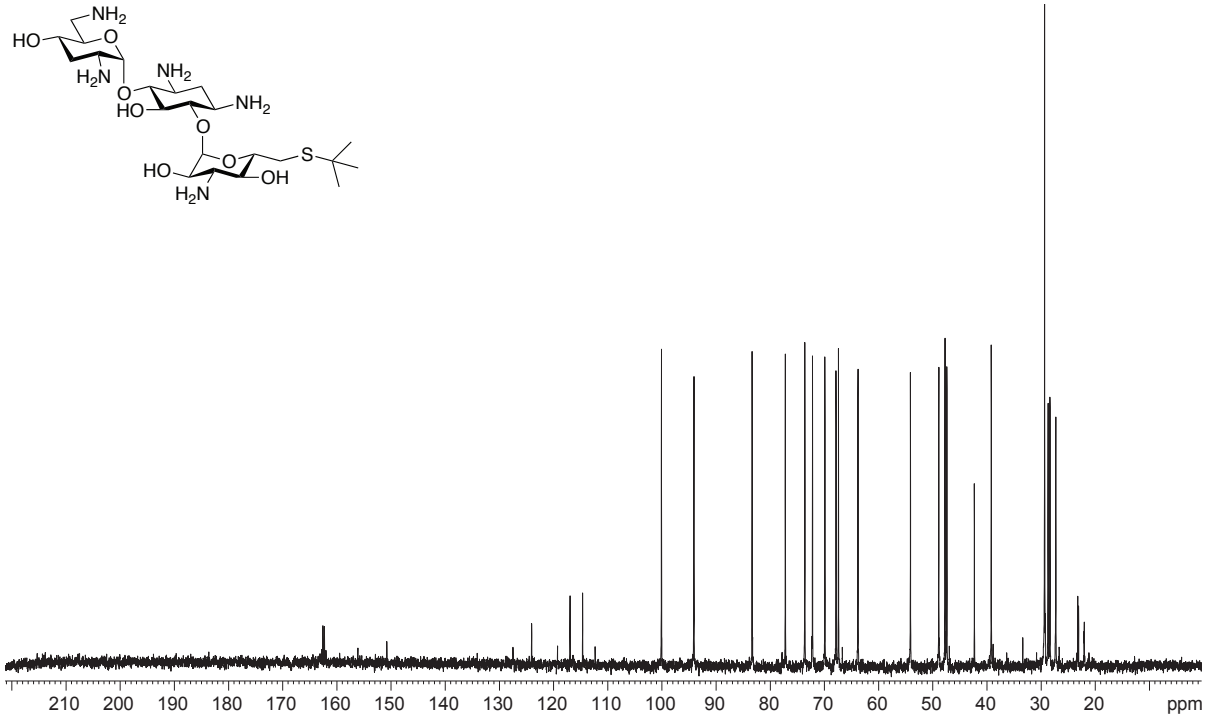


Fig. S18. [13]C NMR for 6"-thioether TOB derivative **4i**.

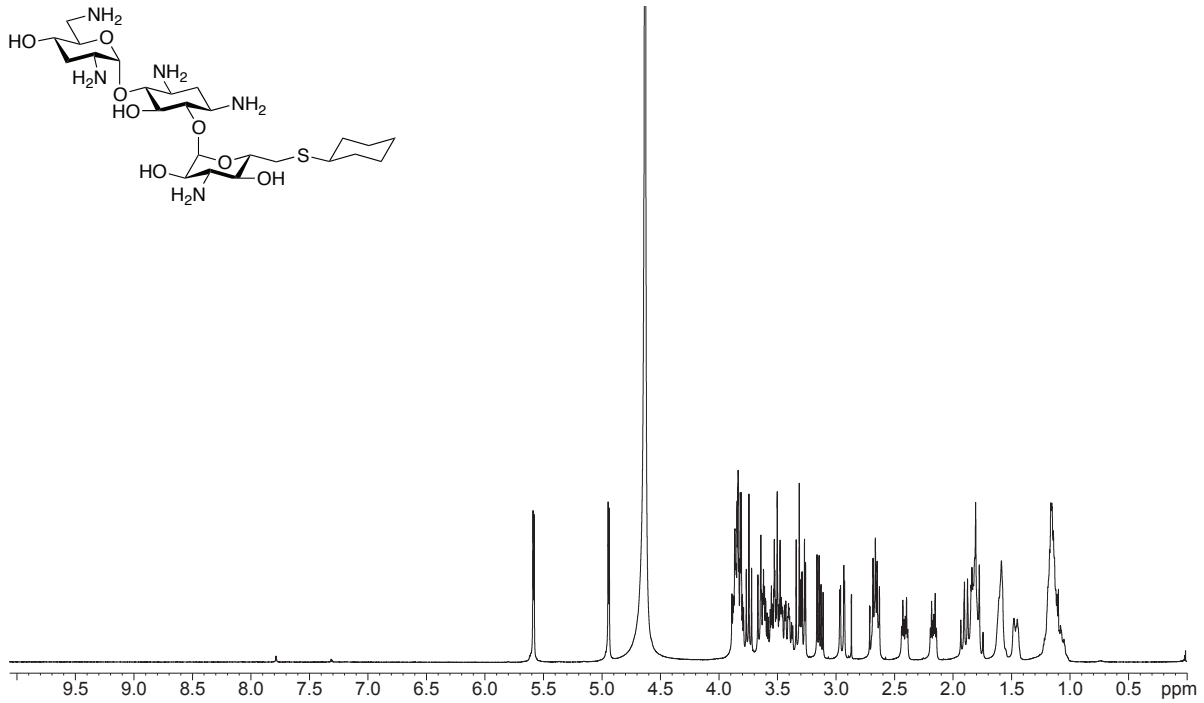


Fig. S19. [1]H NMR for 6"-thioether TOB derivative **4j**.

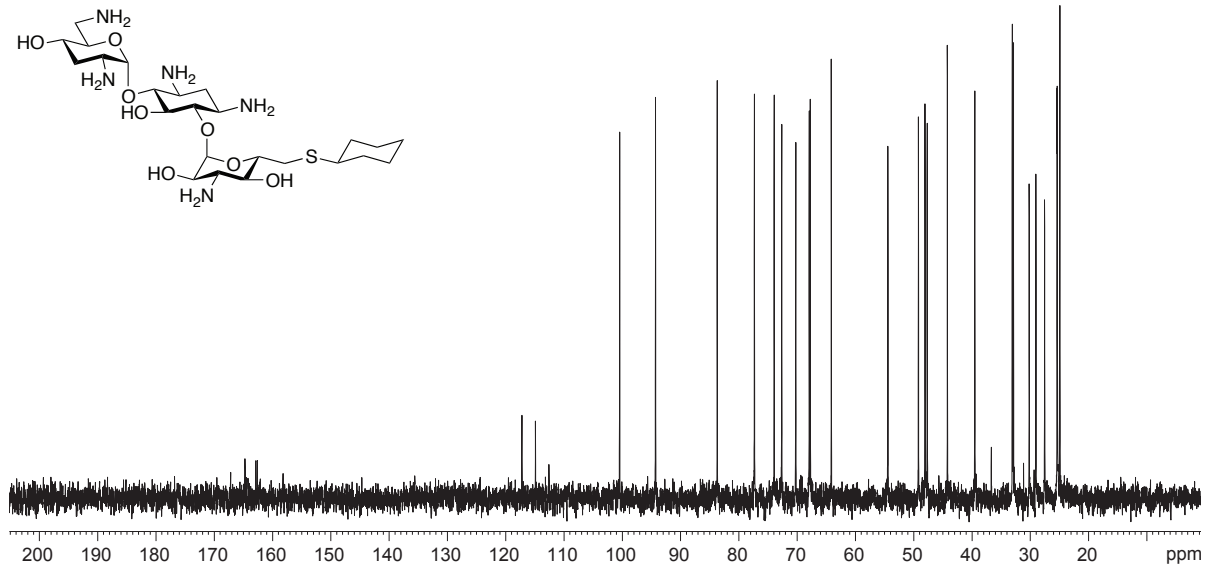


Fig. S20. [13]C NMR for 6"-thioether TOB derivative **4j**.

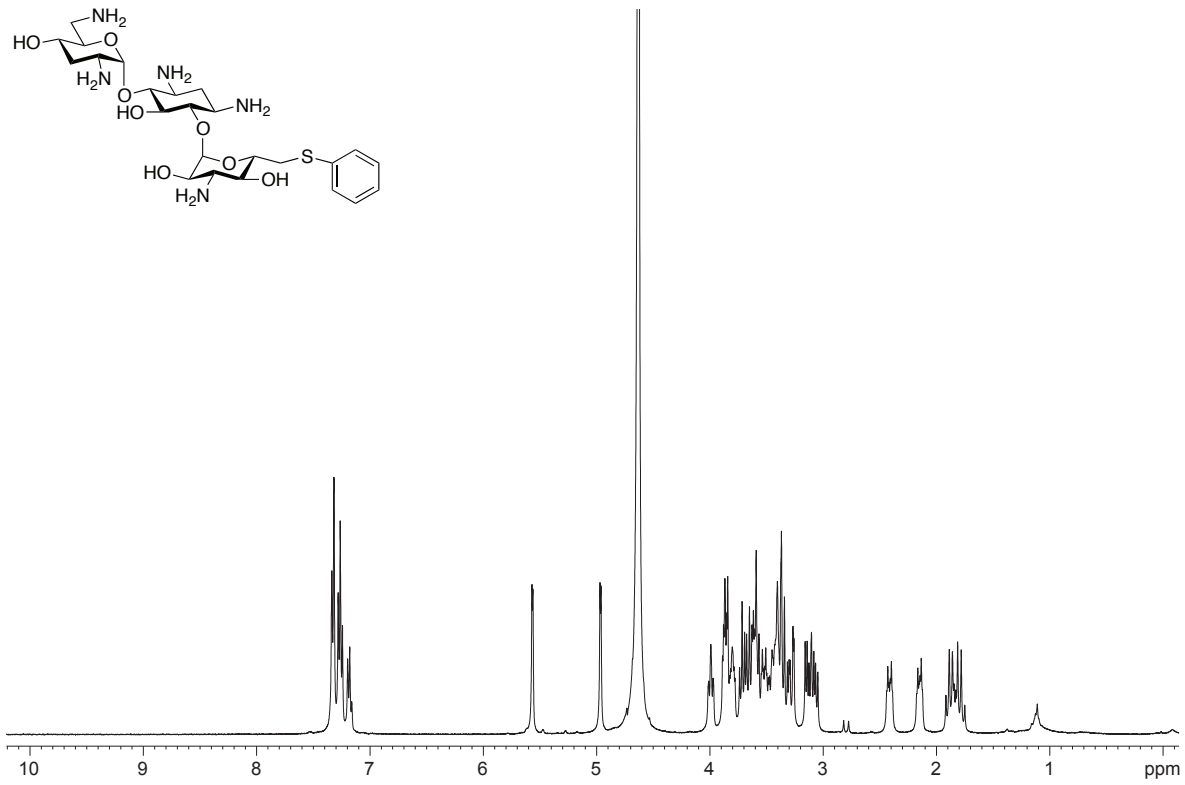


Fig. S21. [1]H NMR for 6"-thioether TOB derivative **4k**.

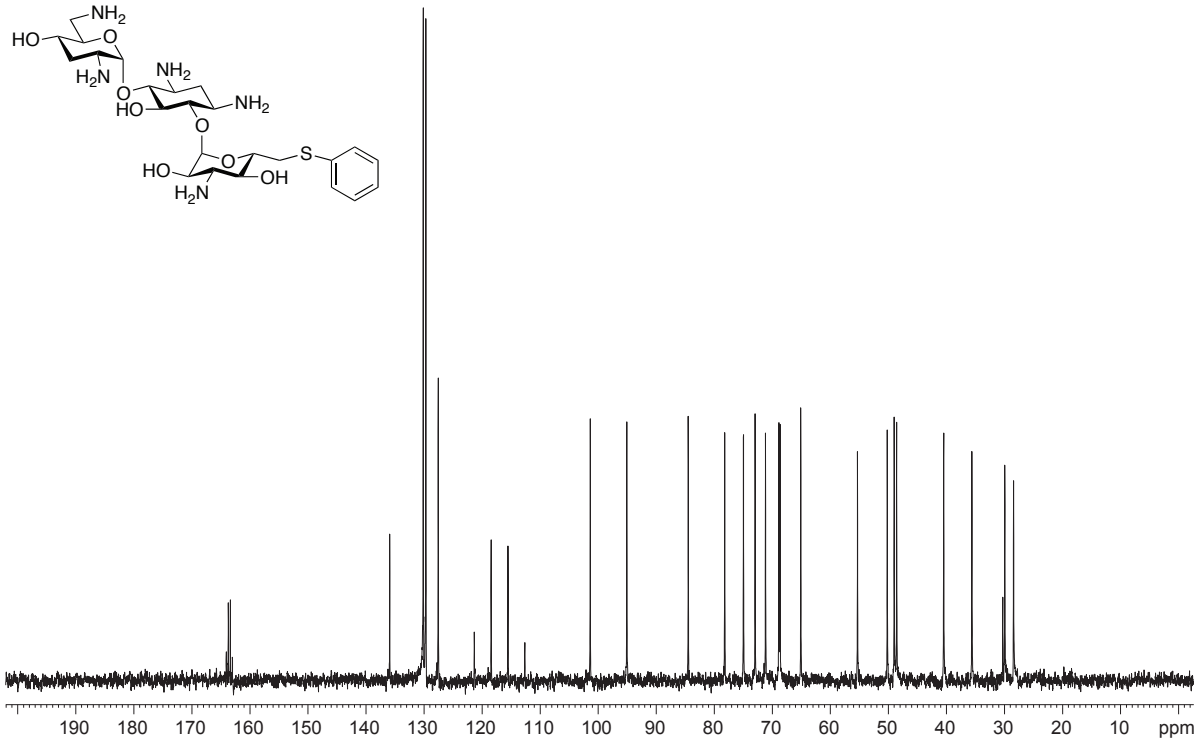


Fig. S22. [13]C NMR for 6"-thioether TOB derivative **4k**.

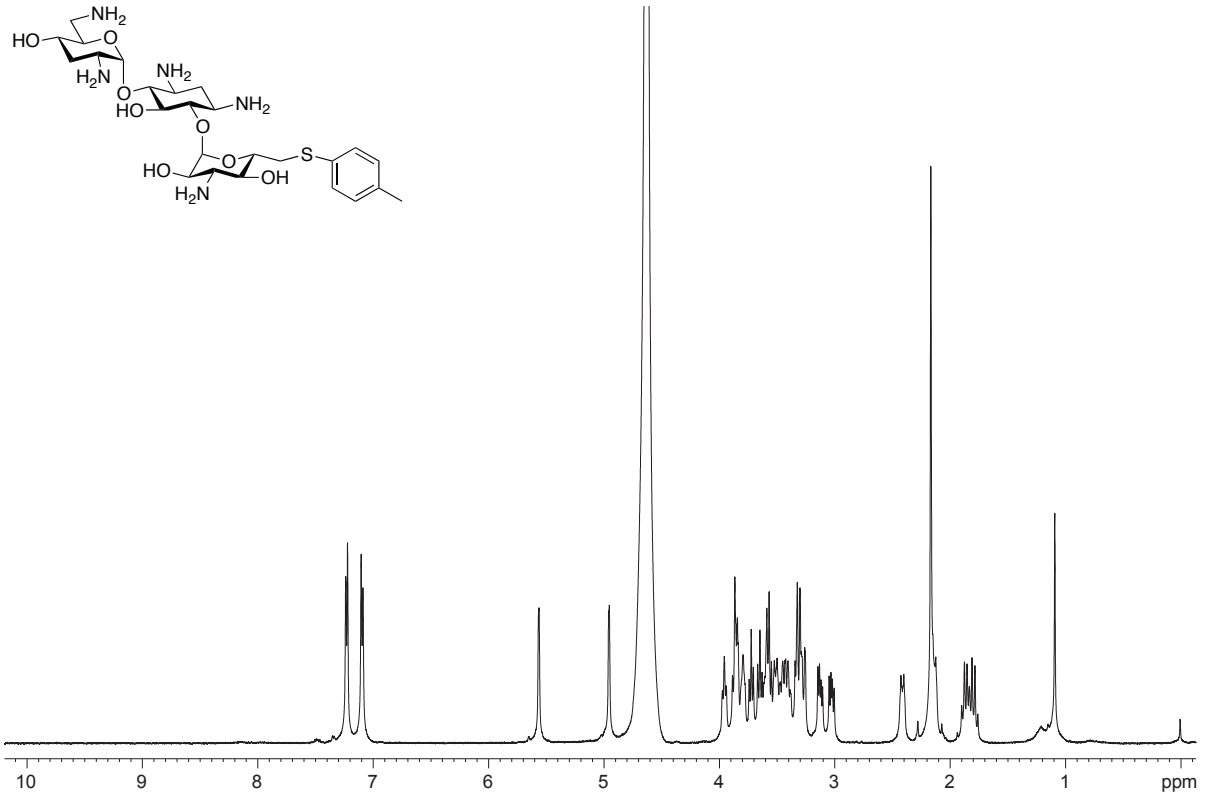


Fig. S23. [1]H NMR for 6"-thioether TOB derivative **4l**.

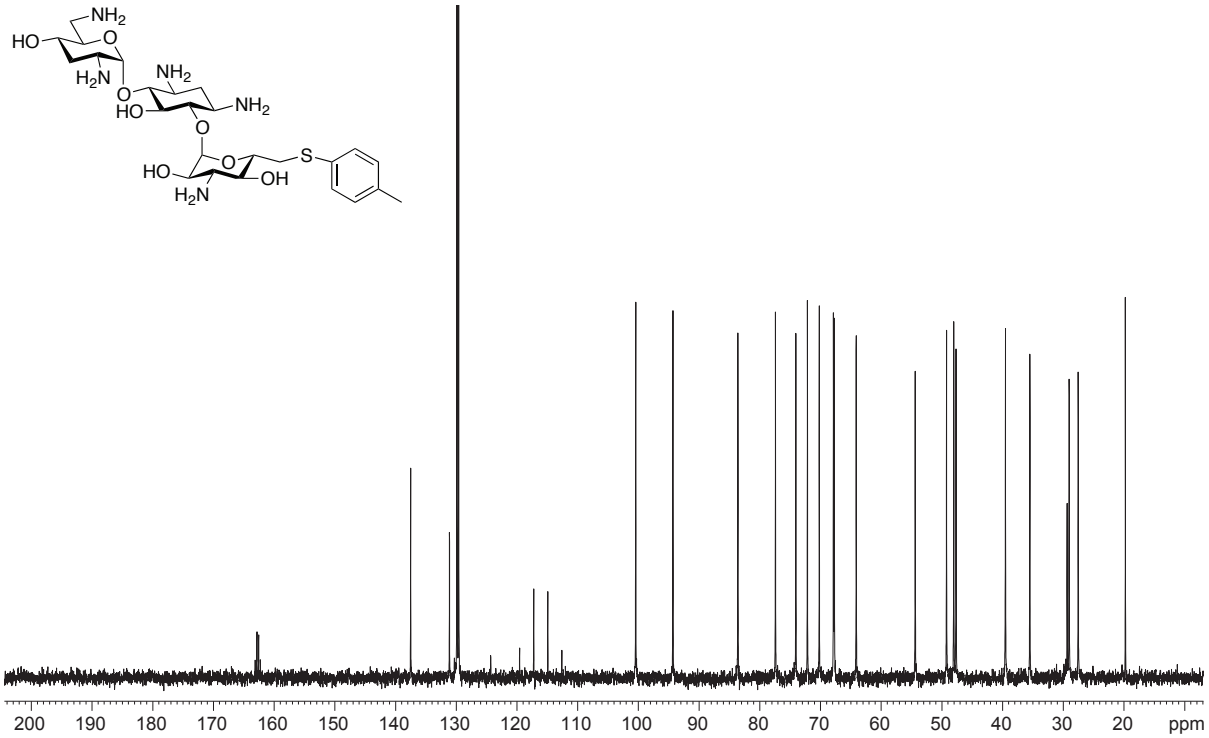


Fig. S24. [13]C NMR for 6"-thioether TOB derivative **4l**.

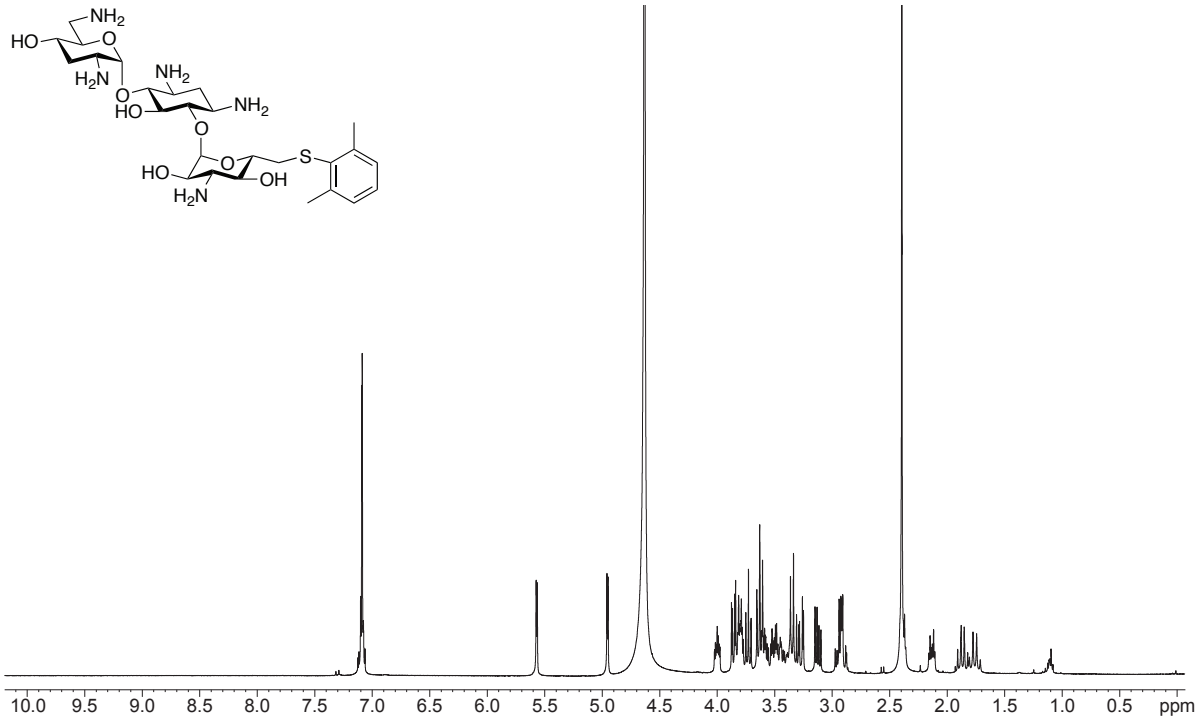


Fig. S25. [1]H NMR for 6"-thioether TOB derivative **4m**.

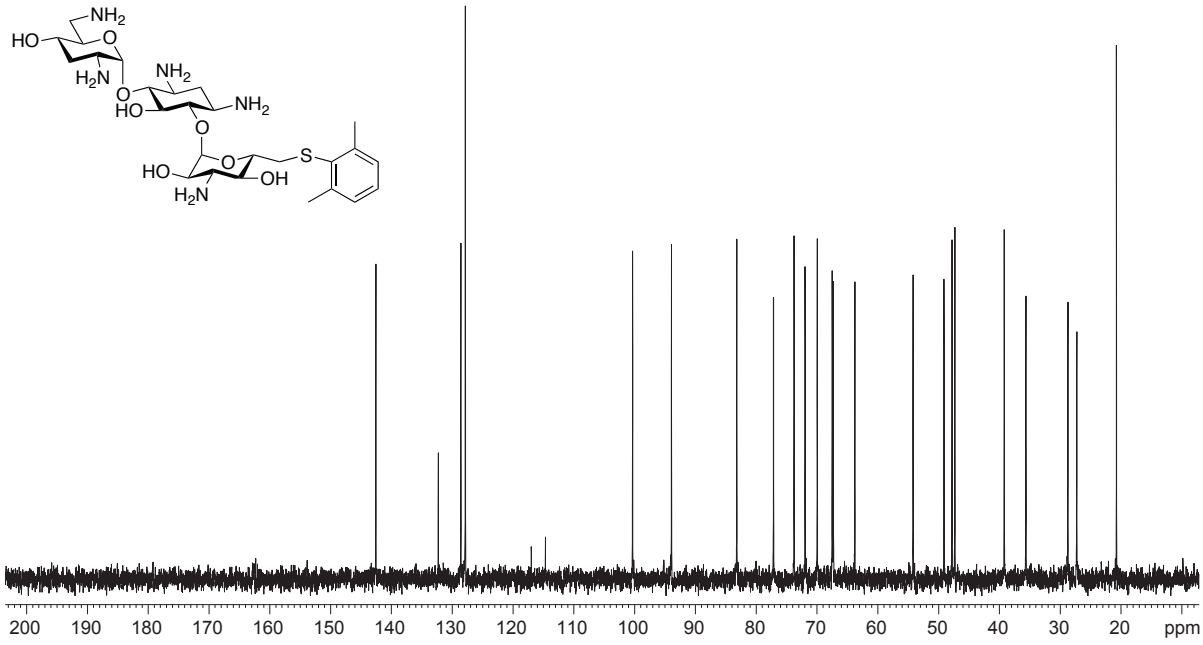


Fig. S26. [13]C NMR for 6"-thioether TOB derivative **4m**.

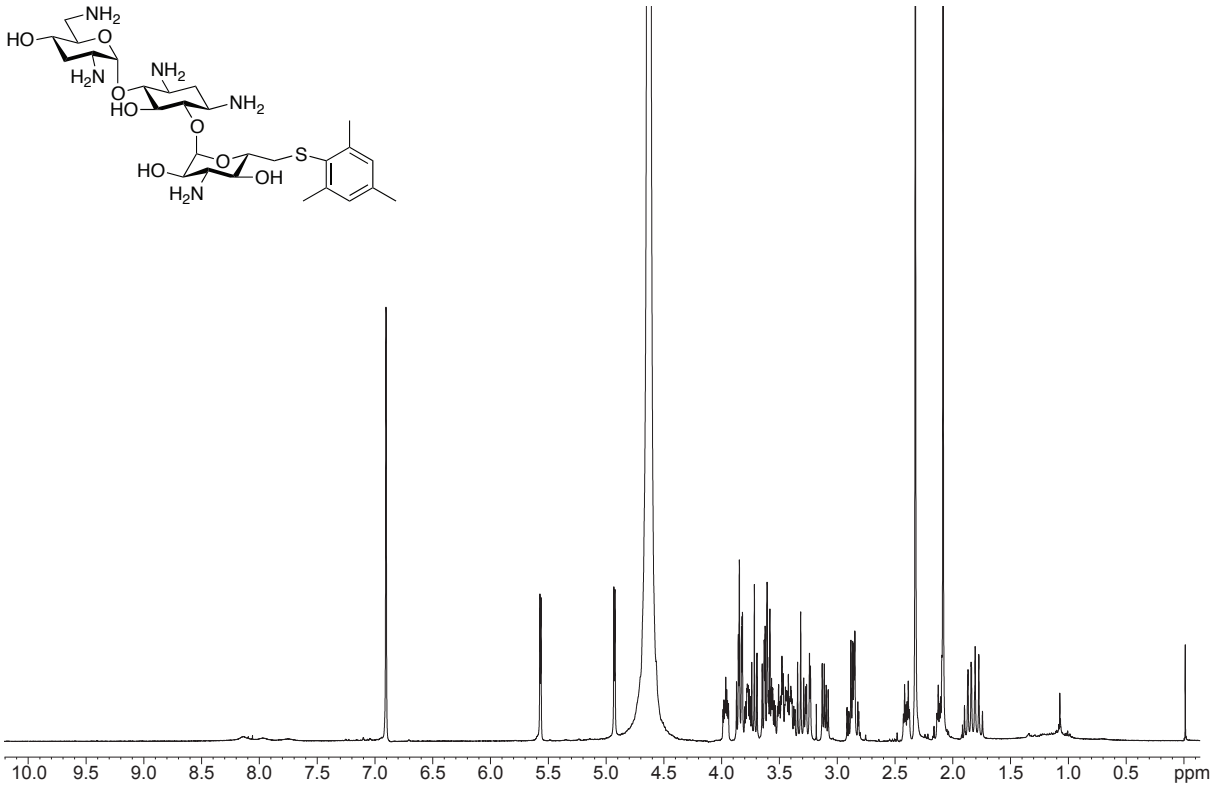


Fig. S27. [1]H NMR for 6"-thioether TOB derivative **4n**.

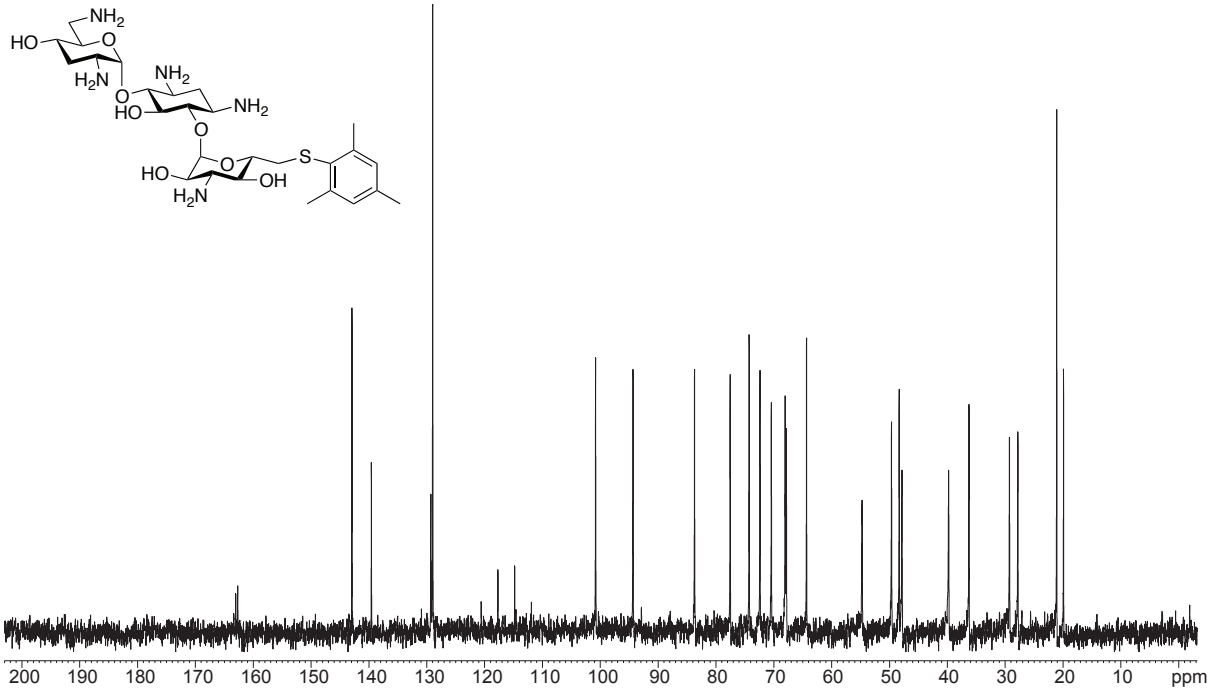


Fig. S28. [13]C NMR for 6"-thioether TOB derivative **4n**.

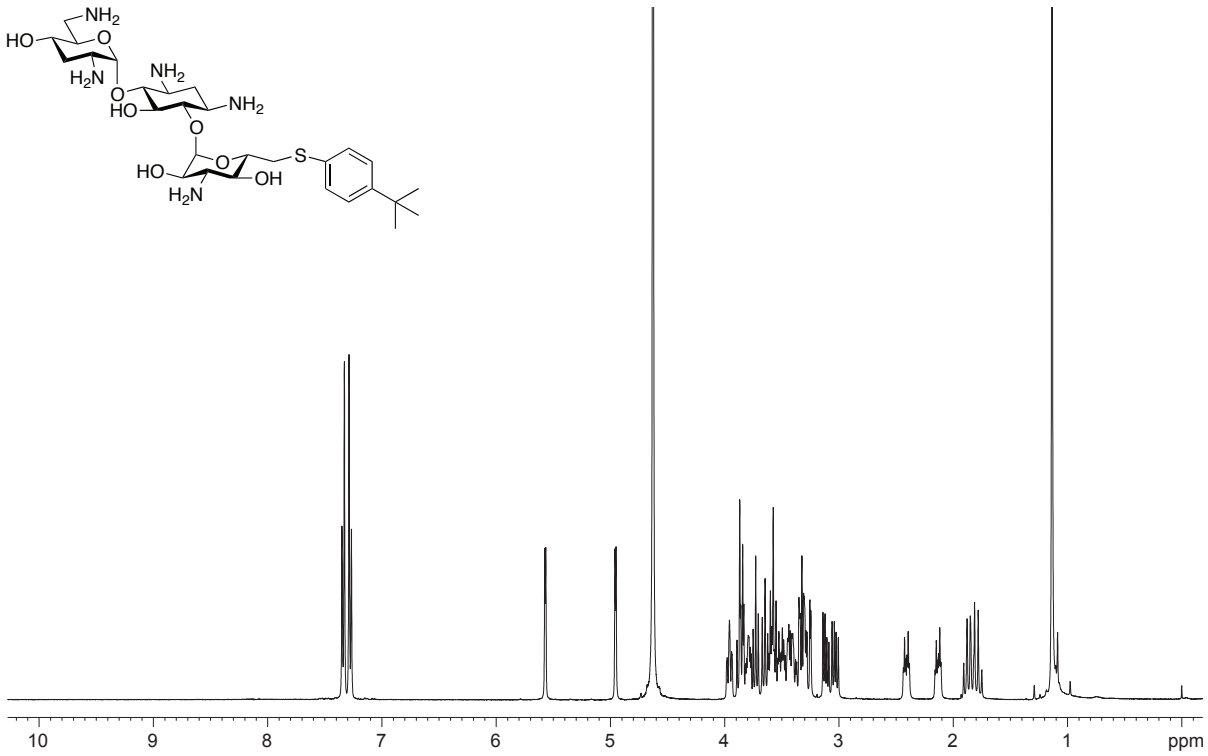


Fig. S29. [1]H NMR for 6"-thioether TOB derivative **4o**.

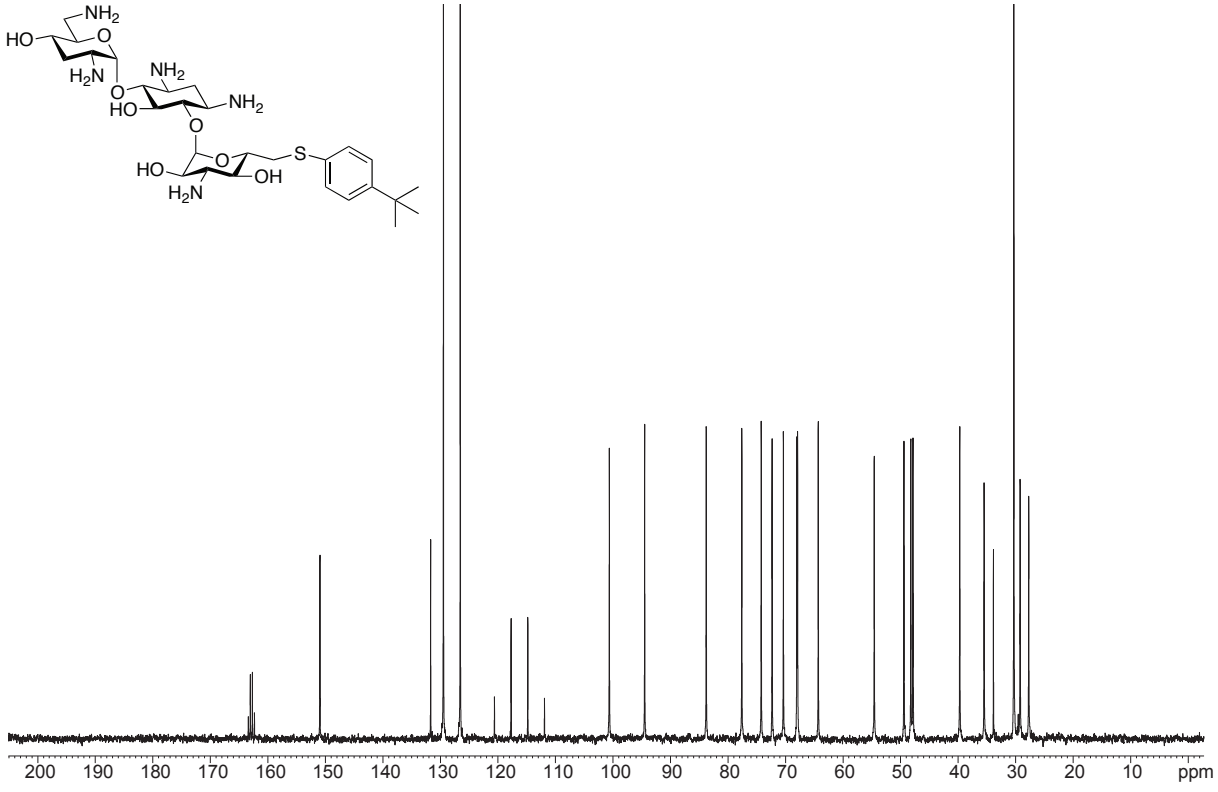


Fig. S30. [13]C NMR for 6"-thioether TOB derivative **4o**.

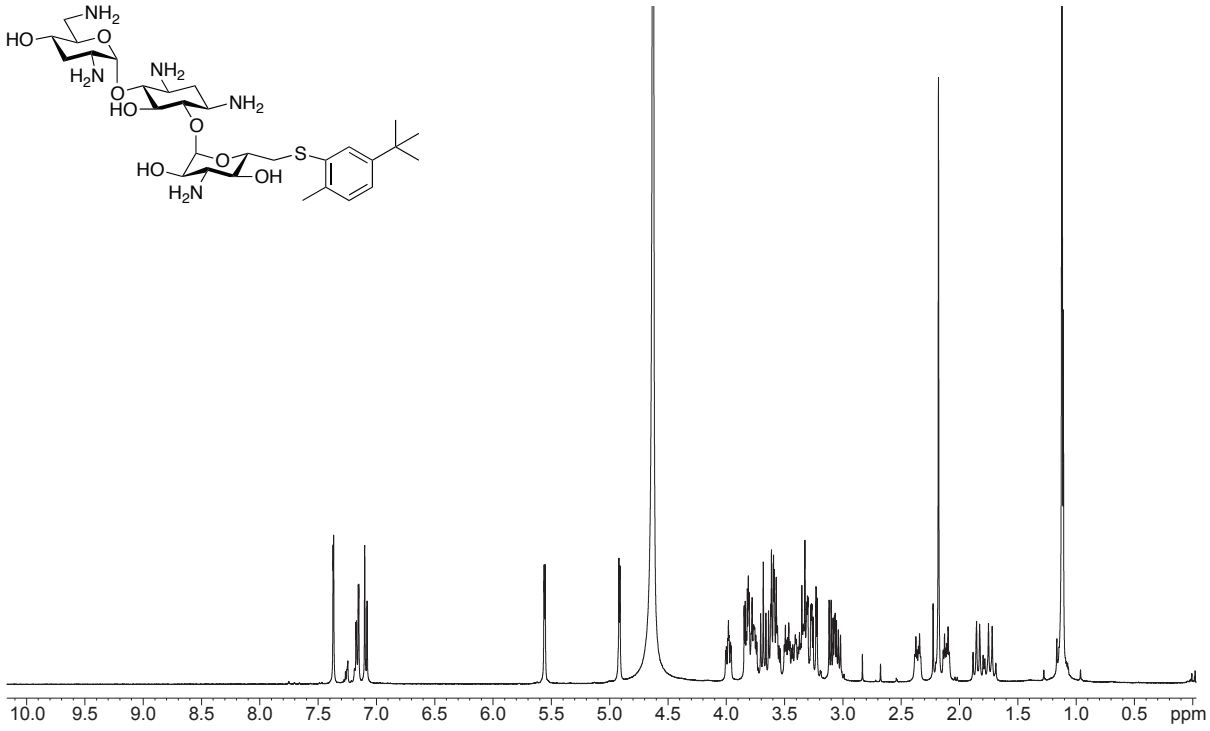


Fig. S31. ^{1}H NMR for 6"-thioether TOB derivative **4p**.

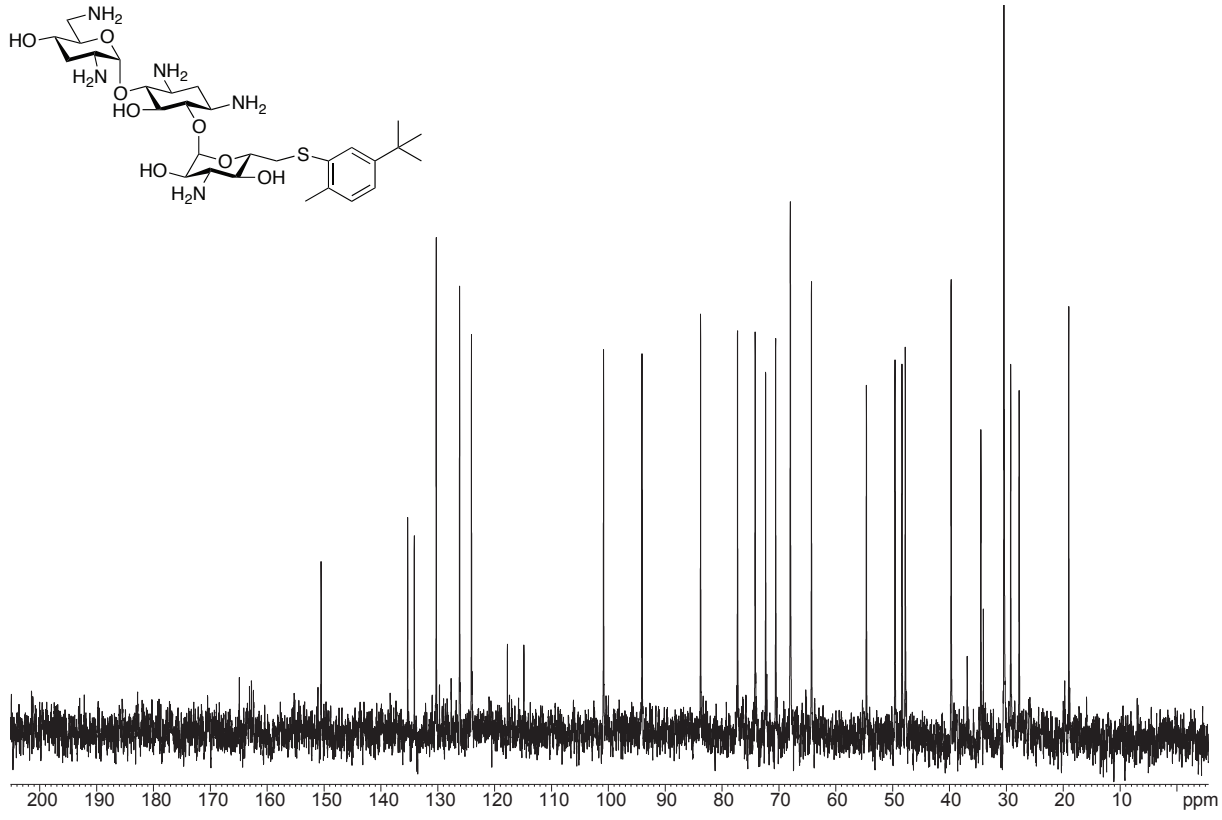


Fig. S32. ^{13}C NMR for 6"-thioether TOB derivative **4p**.

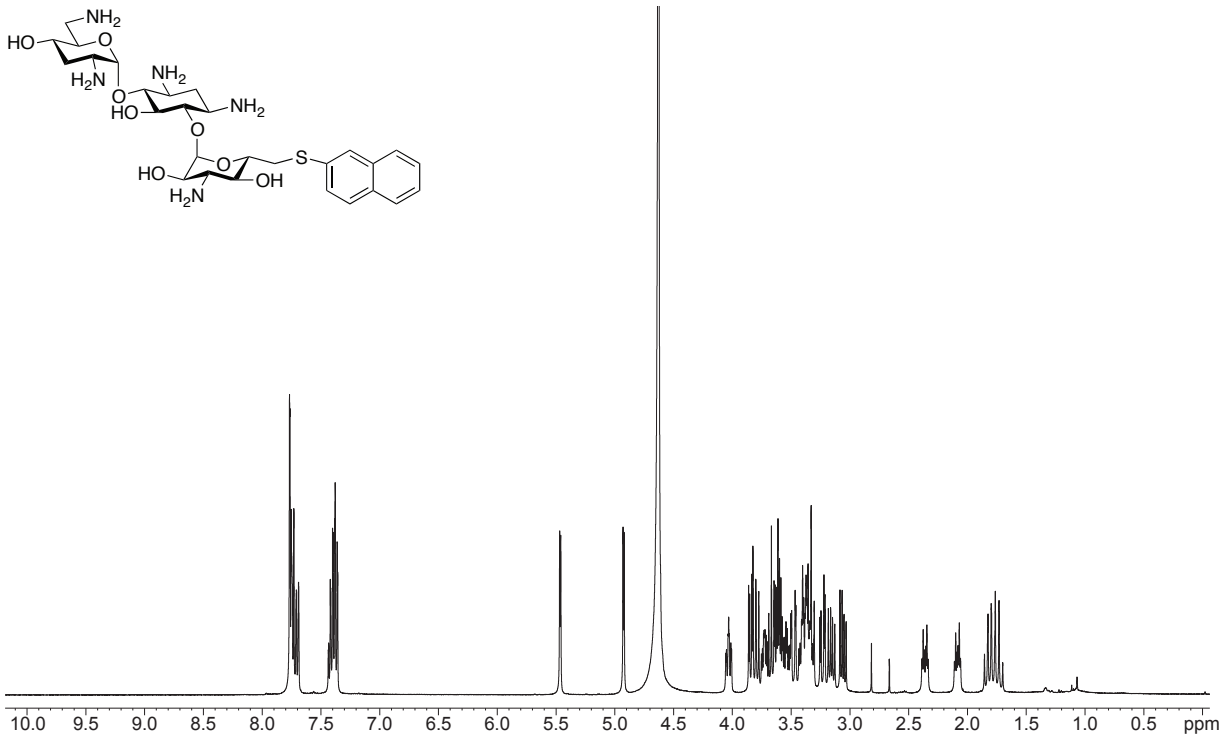


Fig. S33. [1]H NMR for 6"-thioether TOB derivative **4q**.

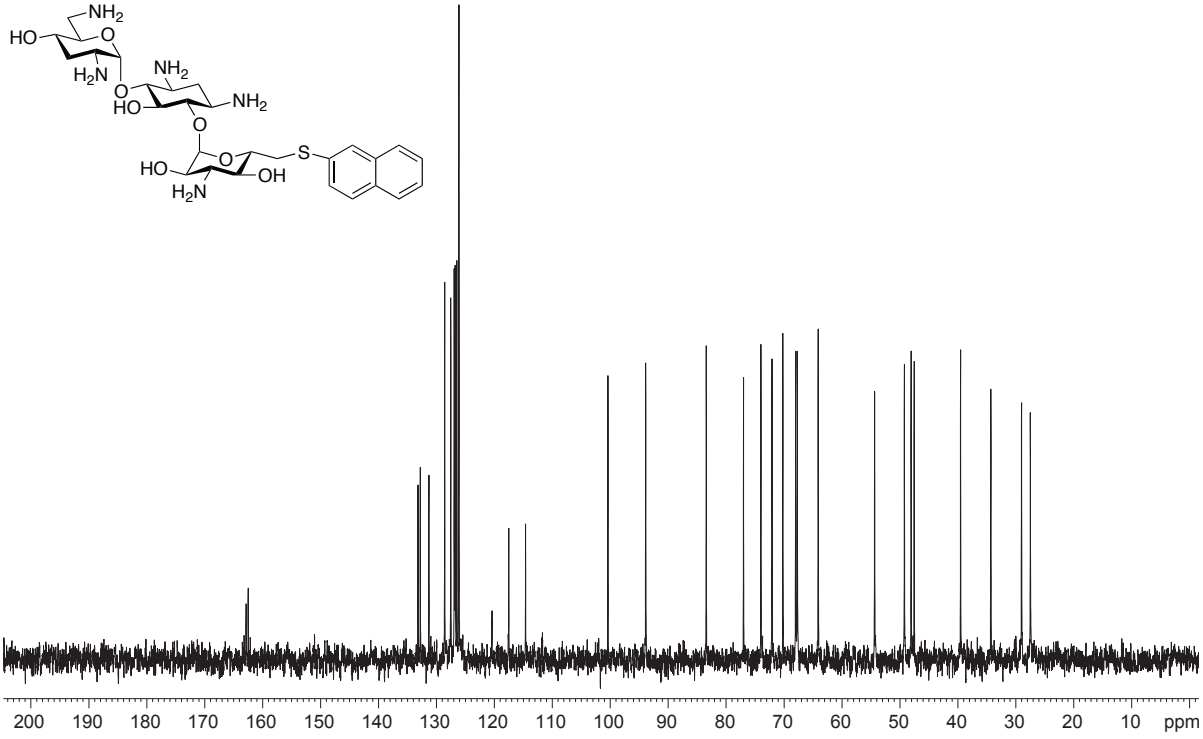


Fig. S34. [13]C NMR for 6"-thioether TOB derivative **4q**.

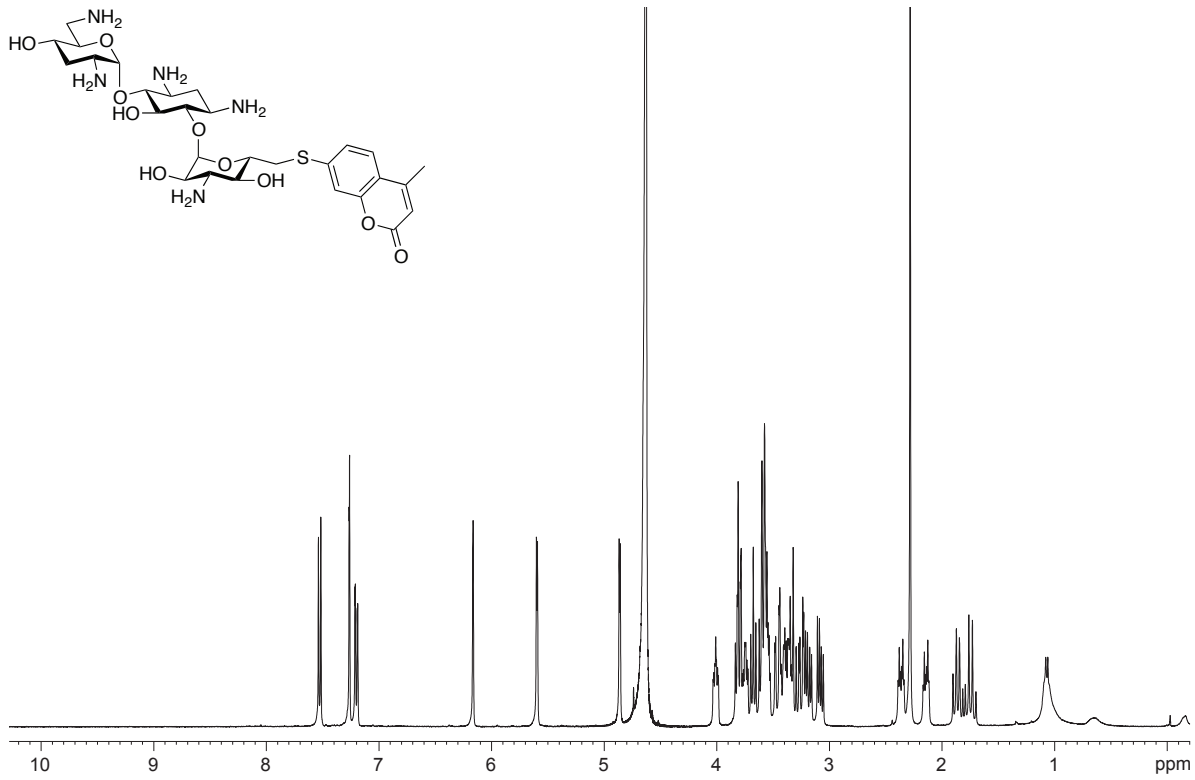


Fig. S35. [1]H NMR for 6"-thioether TOB derivative **4r**.

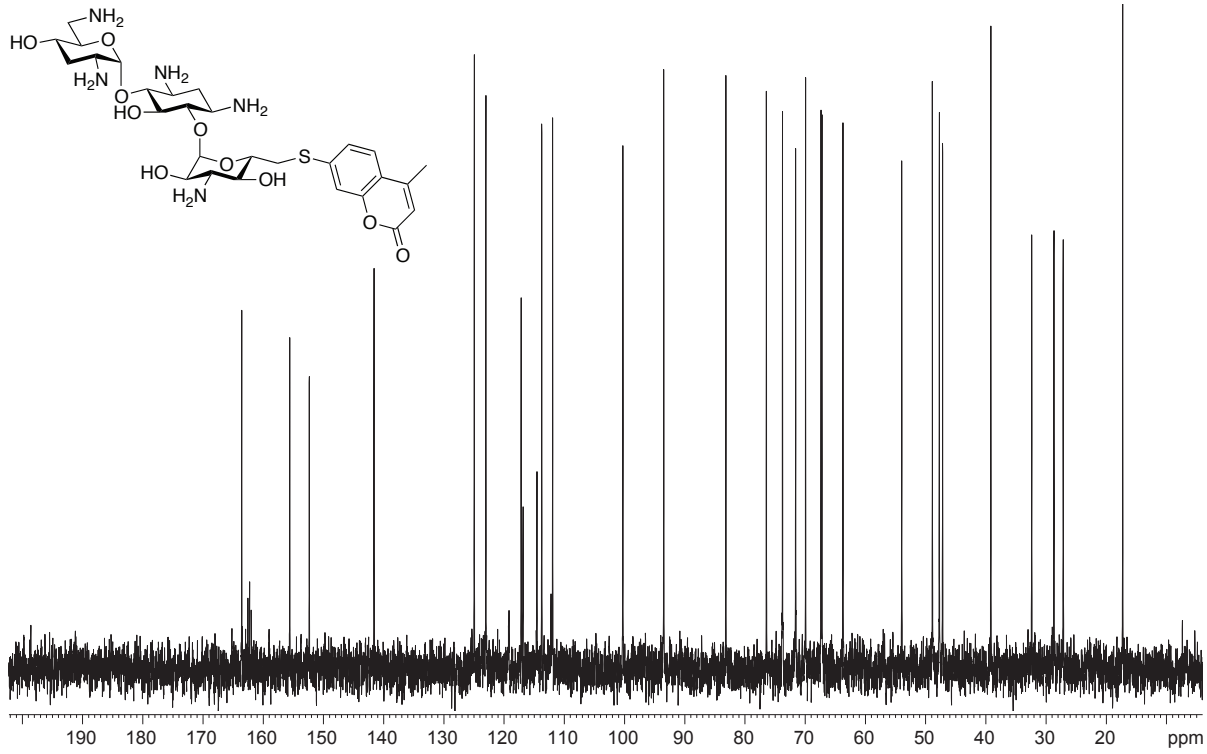


Fig. S36. [13]C NMR for 6"-thioether TOB derivative **4r**.

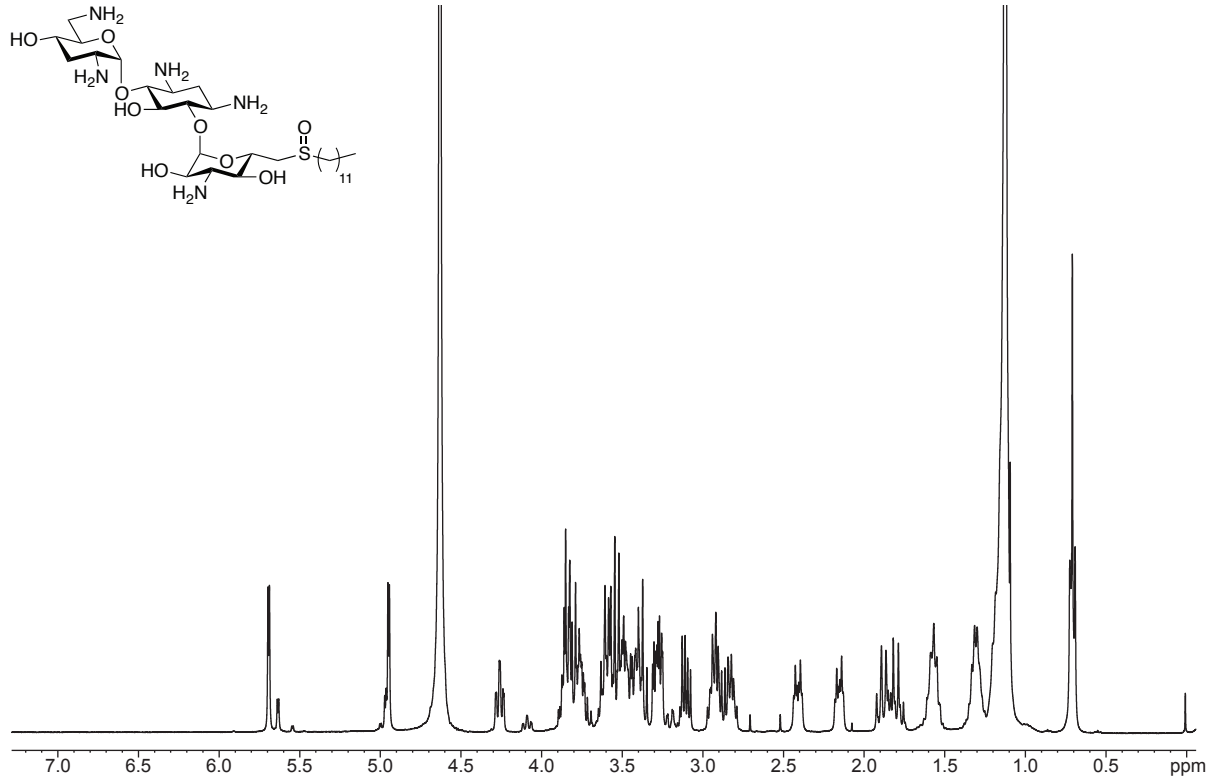


Fig. S37. [1]H NMR for 6"-sulfoxide TOB derivative **5d**.

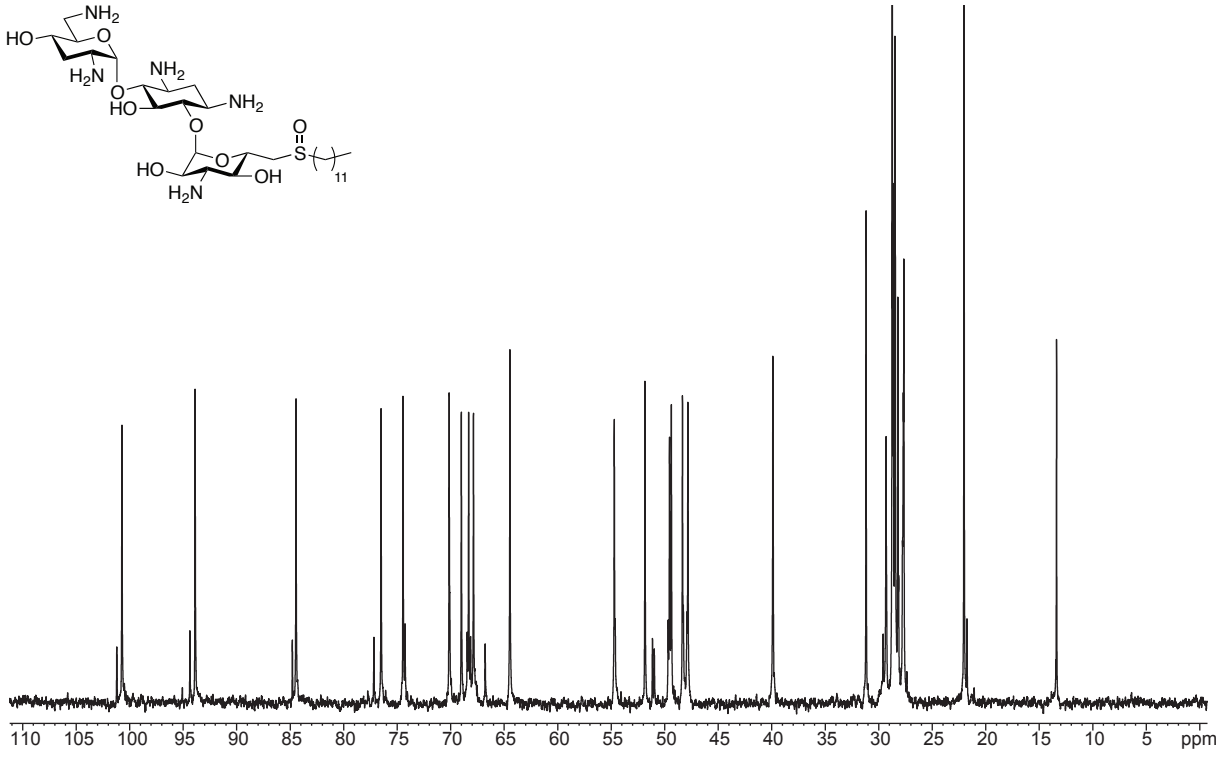


Fig. S38. [13]C NMR for 6"-sulfoxide TOB derivative **5d**.

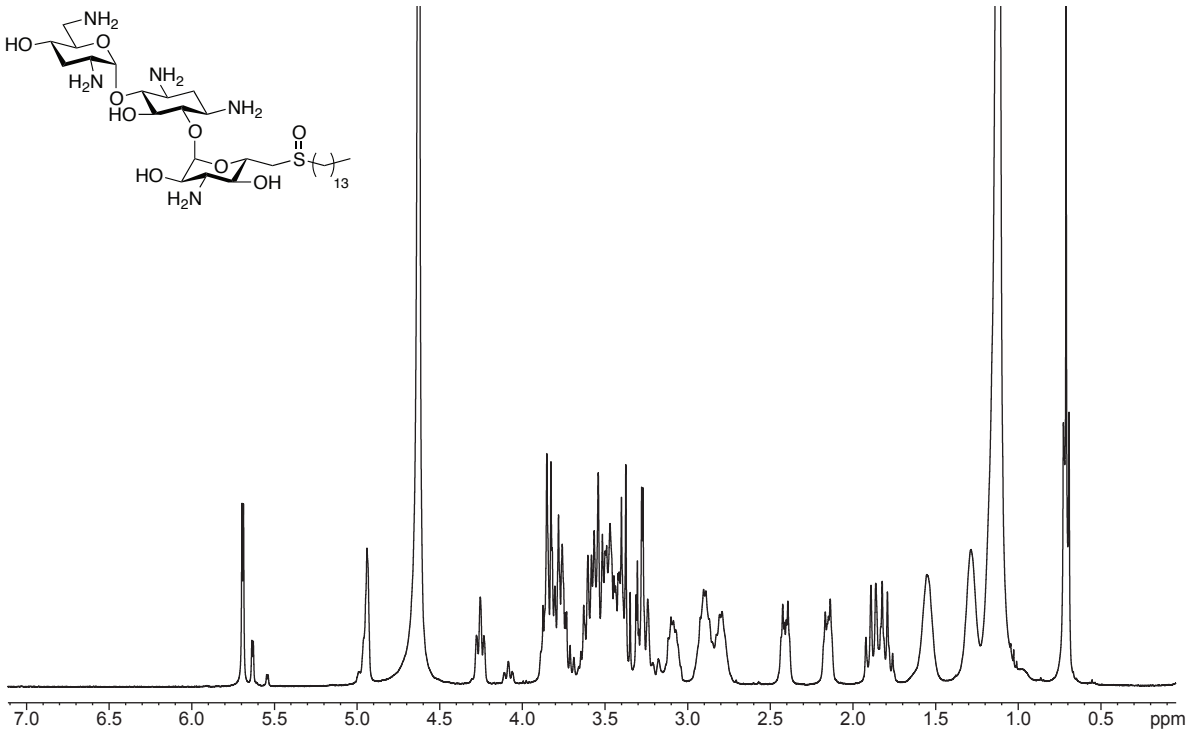


Fig. S39. [1]H NMR for 6"-sulfoxide TOB derivative **5e**.

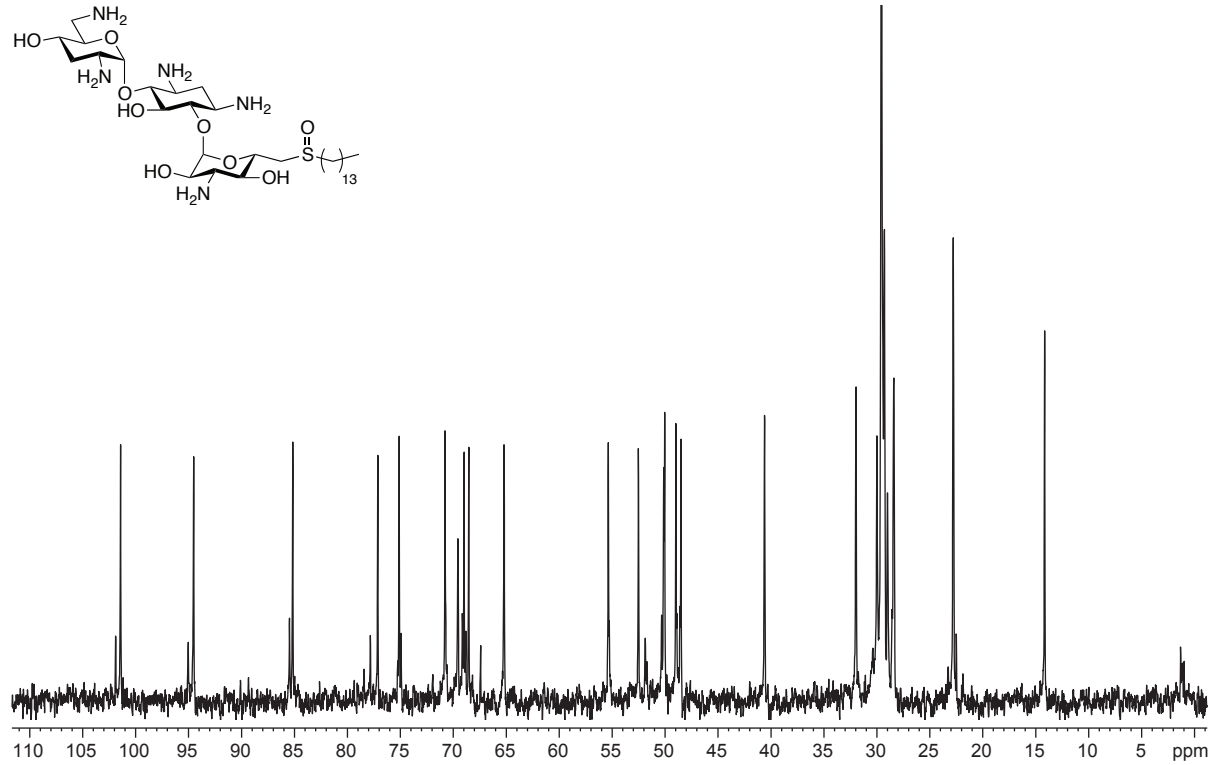


Fig. S40. [13]C NMR for 6"-sulfoxide TOB derivative **5e**.

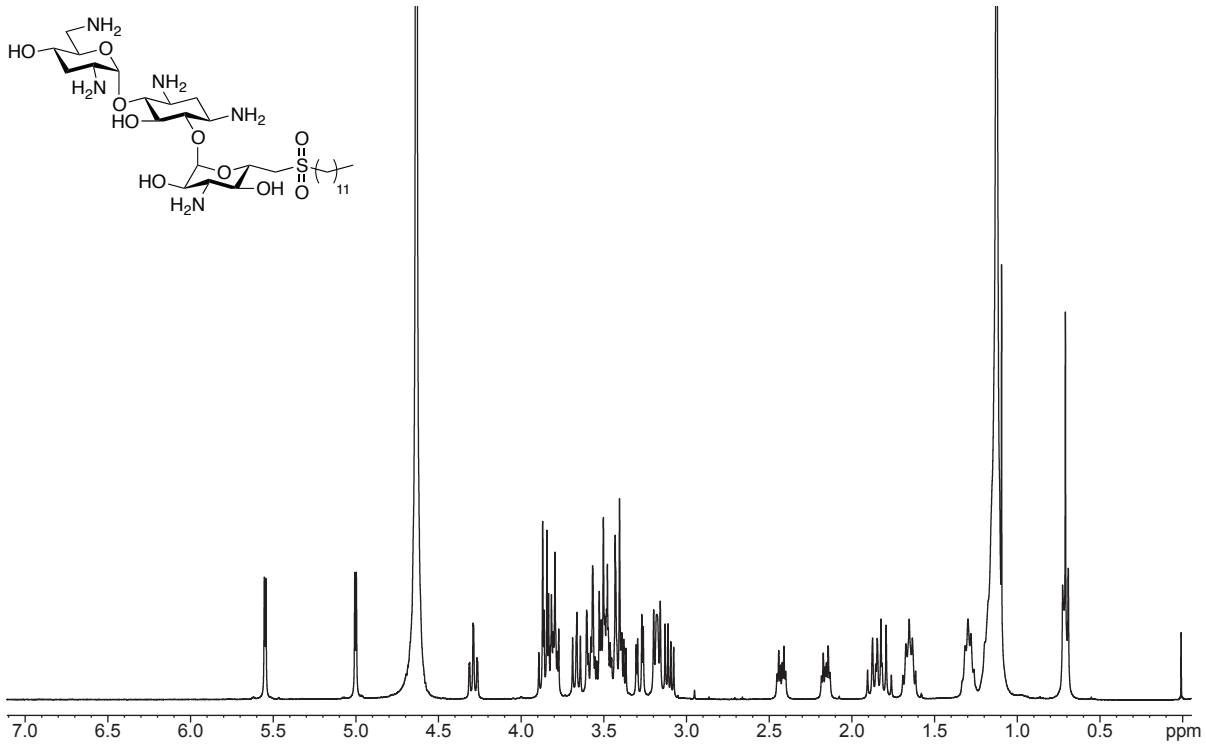


Fig. S41. ^{1}H NMR for 6"-sulfone TOB derivative **6d**.

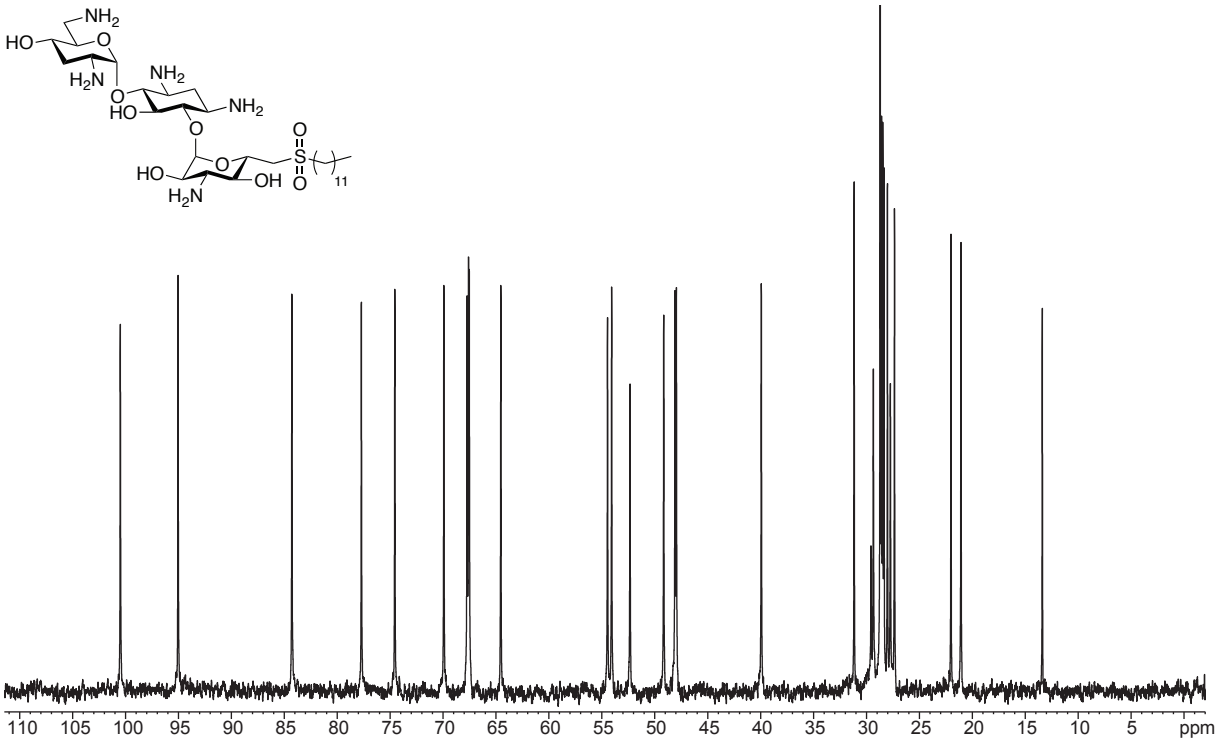


Fig. S42. ^{13}C NMR for 6"-sulfone TOB derivative **6d**.

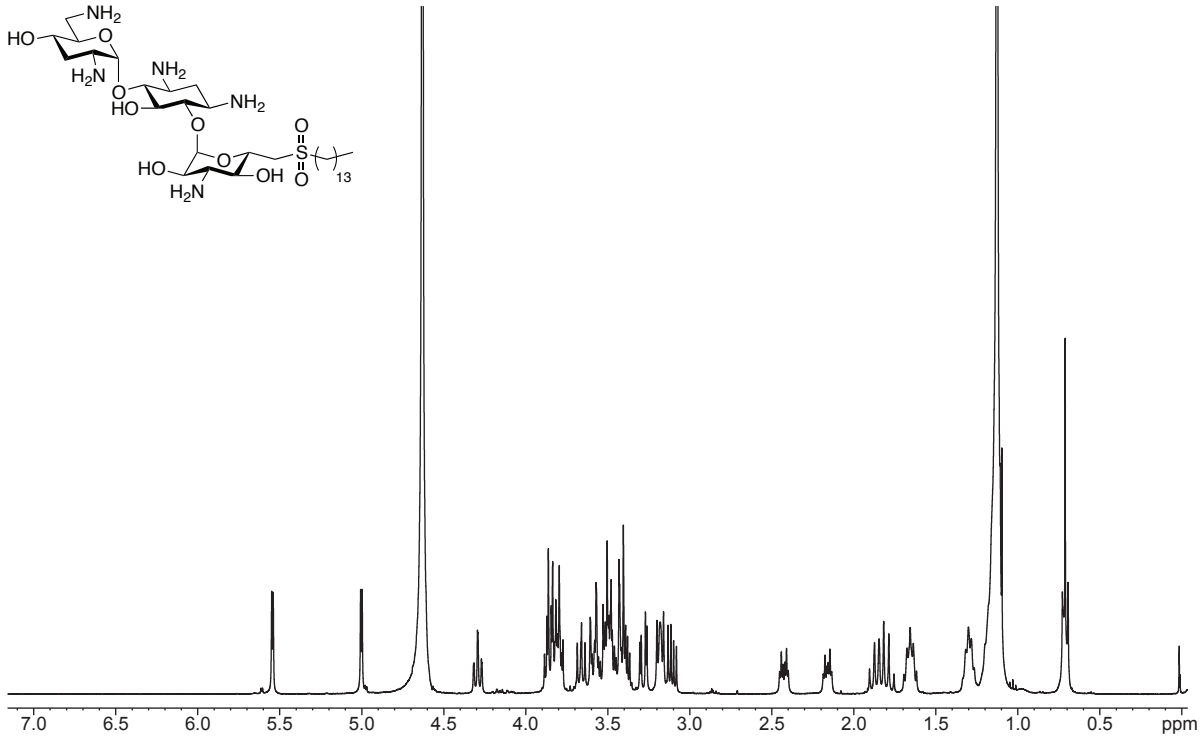


Fig. S43. [1]H NMR for 6"-sulfone TOB derivative **6e**.

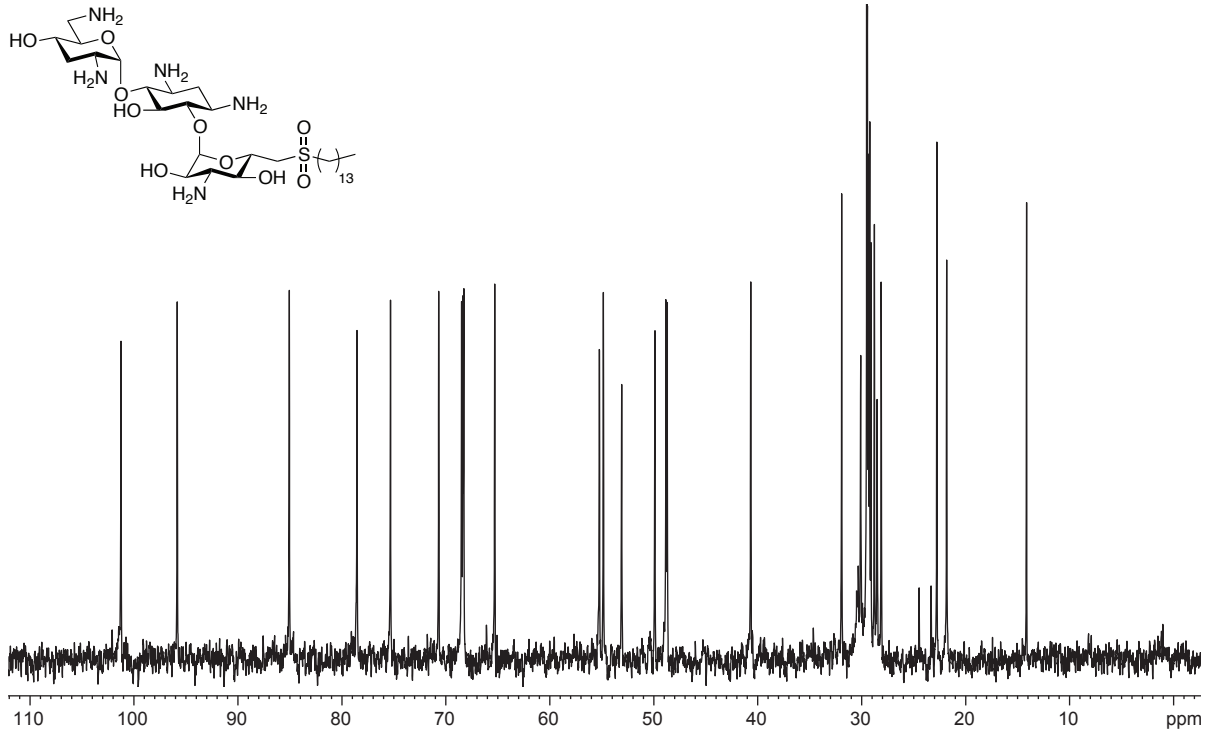


Fig. S44. [13]C NMR for 6"-sulfone TOB derivative **6e**.

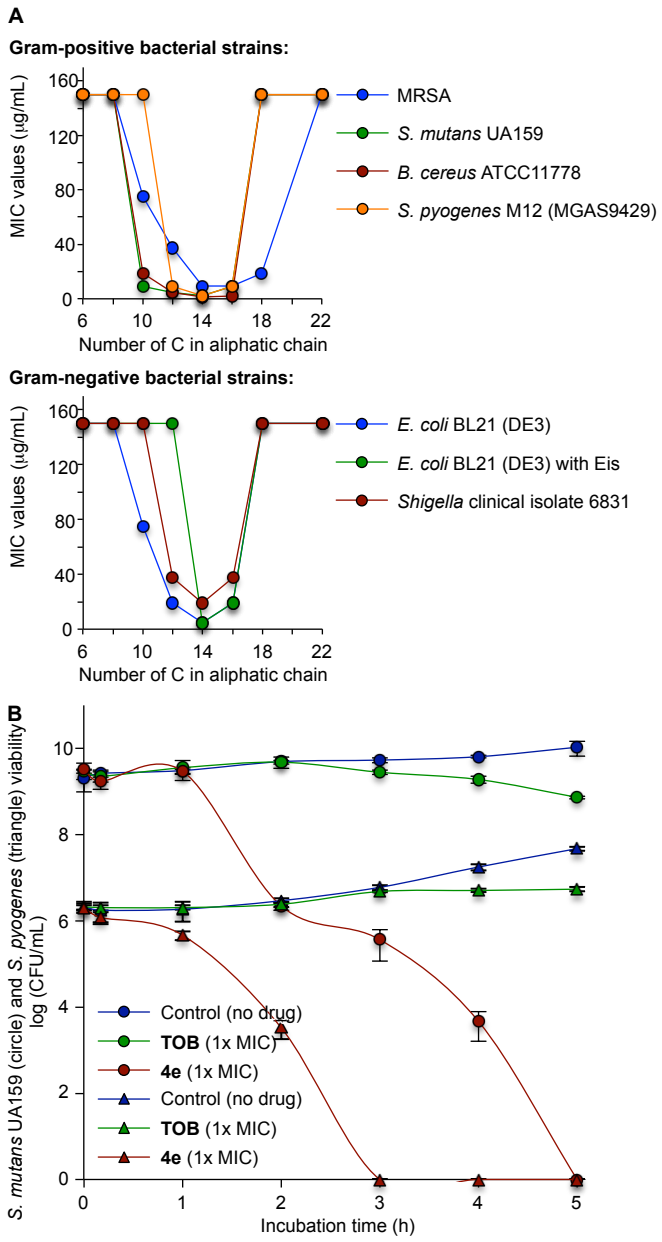


Fig. S45. A. The effect of aliphatic tail length on MIC values of Gram-positive and Gram-negative bacterial strains. **B.** Time-kill kinetics towards *S. mutans* UA159 (circle) and *S. pyogenes* serotype M12 (strain MGAS9429) (triangle) with TOB (**1**) or compound **4e** at 1× MIC (75 μg/mL of **1** (*S. mutans*) or 18.8 μg/mL of **1** (*S. pyogenes*)) and 2.3 μg/mL of **4e**). After 0, 10 min, 1, 2, 3, 4, and 5 h, an aliquot (5 μL) was diluted (10-, 10^3-, and 10^6-fold) into BHI broth and 10 μL of each dilution (50 μL total) were plated in duplicate on BHI agar plates. After exactly 24 h (starting from each tested time point) of incubation, the number of colonies on each plate was counted using a colony counter. Time-kill assays were analyzed by determining the reductions in viable count (CFU/mL). Triplicate experiments were repeated twice.

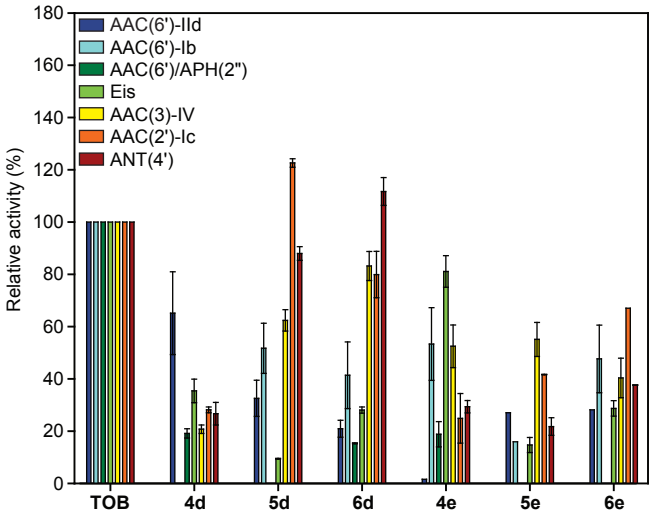


Fig. S46. Bar graph showing the relative initial rates of AME reactions with TOB and the 6"-thioether analogues **4d-e** and their corresponding sulfoxides (**5d-e**) and sulfones **(6d-e**). Rates are normalized to TOB and experiments were performed in triplicate. AMEs tested include AAC(6')-IId (blue), AAC(6')-Ib (cyan), AAC(6')/APH(2") (dark green), Eis (light green), AAC(3)-IV (yellow), AAC(2')-Ic (orange), and ANT(4') (red).