The effect of heteroatom substitution of sulfur for selenium in glucosidase inhibitors on intestinal α-glucosidase activities

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Supporting Information

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Experimental Section

General: Optical rotations were measured at 23 °C. ^{1}H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively. All assignments were confirmed with the aid of two-dimensional ^{1}H, ^{1}H (COSYDFTP) or ^{1}H, ^{13}C (INVBTP) experiments using standard pulse programs. Column chromatography was performed with Silica 60 (230-400 mesh). High resolution mass spectra were obtained by the electrospray ionization method, using an Agilent 6210 TOF LC/MS high resolution magnetic sector mass spectrometer.

Enzyme Inhibition Assays: Kinetic parameters were determined by measuring the amount of glucose produced upon the addition of enzyme at increasing maltose concentrations (from 0.5 to 30 mM) in the presence of increasing inhibitor concentration (0-200 nM) by a two-step glucose oxidase assay in a 96-well plate. The enzyme was allowed to act on the maltose substrate in the presence of inhibitor for 45 minutes at 37°C. The reactions were then quenched with Tris-HCl to a final concentration of 1M. Glucose oxidase reagent (Sigma-Aldrich) was then added to each well (125µl) and the reactions were allowed to develop for 30 minutes at 37°C. Reactions were performed in quadruplicate and absorbance measured at 405 nM by a SpectraMax 190 Plate Reader (Molecular Devices). Absorbance readings were averaged to give the final value, which was compared to a glucose standard curve to determine the amount of glucose released by the enzyme from the substrate. The program KaleidaGraph 4.1 was used to fit the data to the Michaelis-Menten equation and estimate K_m, K_m^{obs} (K_m in the presence of the inhibitor) and V_{max} of the catalytic subunits. K_i values for each inhibitor were determined using the equation $K_i = [I]/(K_{mobs}/K_m)-1)$. The K_i values reported for each inhibitor were determined by averaging the K_i values from three different inhibitor concentrations. The weight of compounds **8** was adjusted for the presence of the major isomer. The data was also plotted on Lineweaver-Burk plots to verify that the inhibitors were acting as competitive inhibitors. The methods used for kinetic assays were reported previously.[1]

Benzyl 6-deoxy-6-[2,3,5-tri-*O*-benzyl-1,4-dideoxy-(R)-epi-seleniumylidene-D-arabinitol]-β-D-glycopyranoside-*p*-toluenesulfonate (11). Benzyl 6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside **9**[2] (310 mg, 0.73 mmol) and 1,4-dideoxy-2,3,5-tri-*O*-benzyl-1,4-anhydro-4-seleno-D-arabinitol **10**[3] (409 mg, 0.88 mmol) were dissolved in HFIP (1.5 mL), containing anhydrous K_2CO_3 (10 mg). The mixture was stirred in a sealed reaction vessel in an oil bath at 65-70 ^{o}C for 4 days. The mixture was cooled, then diluted with EtOAc, and evaporated to give a syrupy residue. Purification by column chromatography ($CHCl_3$/MeOH 95:5) gave the sulfonium salt **11** as a white amorphous solid (358 mg, 55%). $[\alpha]_D^{23}$ = + 22^{o}. ^{1}H NMR (MeOD) δ 7.74-7.24 (24H, m, Ar), 4.78 (1H, dd, $J_{1,2}$ = 7.8, $J_{2,3}$ = 4.2 Hz, H-2), 4.68-4.49 (8H, m, 4CH_2-Ph), 4.55 (1H, m, H-3), 4.48 (1H, m, H-4), 4.41 (1H, d, $J_{1',2'}$ = 7.8 Hz, H-1′), 3.98 (1H, m, H-6′a), 3.91 (1H, m, H-1a), 3.84 (1H, dd, $J_{5a,4}$ = 6.3, $J_{5a,5b}$ = 10.3 Hz, H-5a), 3.75 (3H, m, H-6′b, H-3′, H-5b), 3.70 (1H, dd, $J_{1,2}$ = 2.8, $J_{1a,1b}$ = 12.5 Hz, H-1b), 3.40 (1H, t, $J_{4',5'}$ = $J_{6',5'}$ = 9.1 Hz, H-5′), 3.28 (2H, m, H-2′, H-4′), 2.38 (3H, s, Me). ^{13}C NMR (MeOD) δ 142.2-125.5 (m, Ar), 103.0 (C-1′), 83.8 (C-2), 83.5 (C-3), 75.8 (C-5′), 73.4 (C-4′), 73.3 (C-2′), 73.1, 72.0, 71.7, 71.3 (4CH_2-Ph), 71.8 (C-3′), 66.5 (C-5), 66.2 (C-4), 45.5 (C-1), 45.2 (C-6′), 19.8 (Me). HRMS Calcd for $C_{39}H_{45}O_8Se$ (M+.): 721.2278. Found: 721.2279.

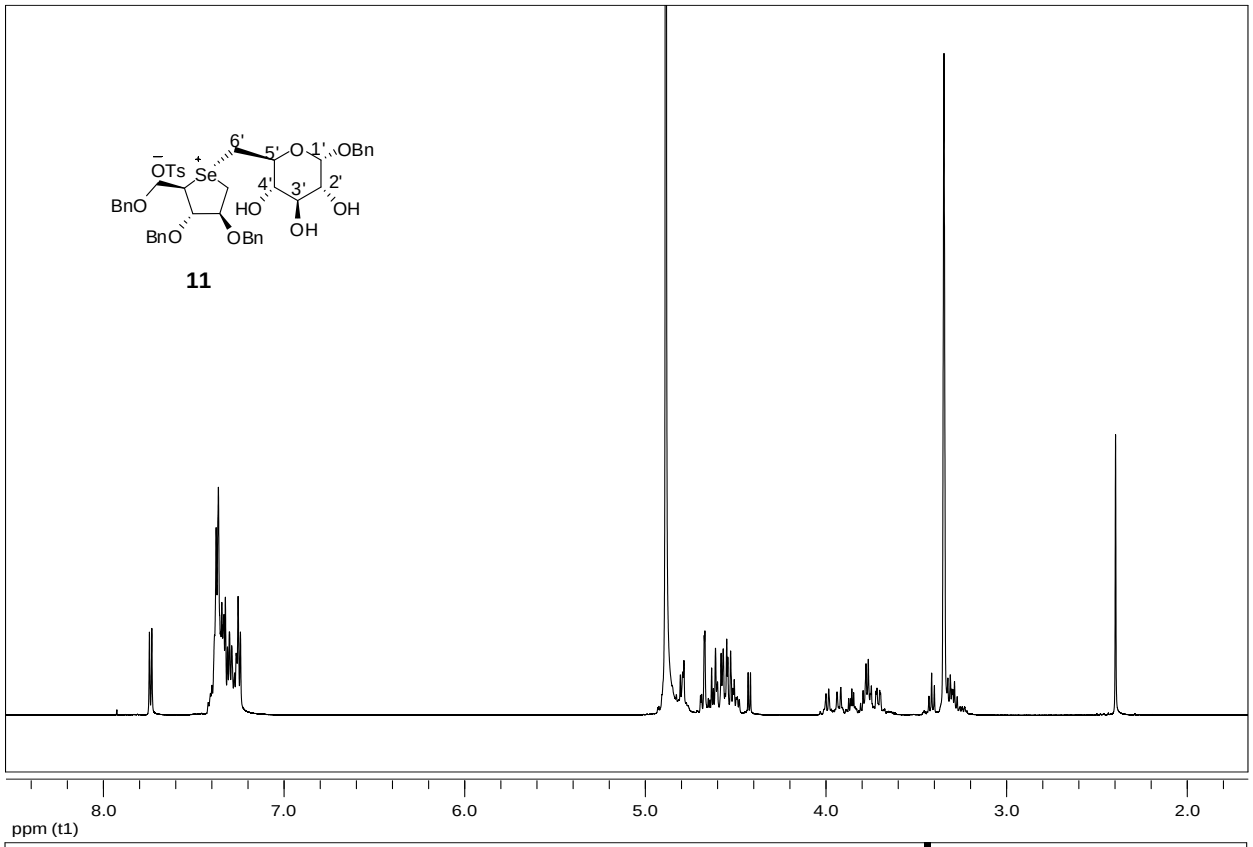
1,4-Dideoxy-1,4-[[2S, 3S, 4R, 5S]-2,3,4,5,6-pentahydroxyhexyl]-(R/S)-epi-seleniumylidine]-D-arabinitol chloride (7). Compound **11** (200 mg, 0.22 mmol) was dissolved in CH_2Cl_2 (20 mL), the mixture was cooled to -78 ^oC, and BCl_3 (1M solution in CH_2Cl_2, 3.6 mmol) was added under N_2. The reaction mixture was stirred at the same temperature for 30 min, and then allowed to warm to -5 ^oC for 6 h. The reaction was cooled to -78 ^oC and quenched by addition of MeOH (5 mL), the solvents were removed, and the residue was co-evaporated with MeOH (2 × 5 mL). The crude residue was dissolved in H_2O (10 mL), Amberlyst A-26 resin (200 mg) was added, and the reaction mixture was stirred at room temperature for 3 h. Filtration through cotton, followed by solvent removal gave the crude hemiacetal. The crude product was dissolved in water (8 mL), and the solution was stirred at room temperature while $NaBH_4$ (34 mg, 0.9 mmol) was added in small portions over 30 min. Stirring was continued for another 3 h and the mixture was acidified to pH < 4 by dropwise addition of 2M HCl. The mixture was evaporated to dryness and the residue was co-evaporated with anhydrous MeOH (3 × 30 mL). Treatment of the solid residue with 50% EtOAc:MeOH (5-10 mL) resulted in precipitation of most of the borate salt. Filtration through cotton, followed by solvent removal gave the crude compound. The residue was purified by crystallization with minimum amount of MeOH to give **7** as a colorless solid (46 mg, 52%).

$[\alpha]_D^{23}$ = + 12.8 ^{o}, (*c* = 0.5 , H_2O). ^{1}H NMR (D_2O) δ 4.76 (1H, dd, $J_{1,2}$ = $J_{2,3}$ = 3.7 Hz, H-2), 4.46 (1H, t, br, H-3), 4.20 (1H, m, H-2′), 4.15 (1H, m, H-4), 4.05 (1H, dd, $J_{4,5a}$ = 12.2, $J_{5a,5b}$ = 4.8 Hz, 5a) 3.92-3.87 (2H, m, H-5b, H-1′a), 3.78-3.74 (5H, m, H-5′, H-4′, H-1′b, H-1a, H-1b), 3.69-3.65 (2H, m, H-3′, H-6′a), 3.55 (1H, dd, $J_{6'a,6,b}$ = 10.7, $J_{5',6'b}$ = 5.9 Hz, H-6′b). ^{13}C NMR (D_2O) δ 77.8 (C-3), 77.2 (C-2), 73.2 (C-3′), 72.2 (C-5′), 69.1 (C-4), 69.0 (C-4′), 67.0 (C-2′), 61.8(C-6′), 558.9 (C-5), 47.0 (C-1′), 44.7 (C-1). HRMS Calcd for $C_{11}H_{23}O_8Se$ (M+.): 363.0551. Found: 363.0559.

Benzyl 6-deoxy-6-[2,3,5-tri-*O*-benzyl-1,4-dideoxy-(R)-epi-seleniumylidene-D-arabinitol]-α-D-mannopyranoside-*p*-toluenesulfonate (13). Reaction of 1,4-dideoxy-2,3,5-tri-*O*-benzyl-1,4-anhydro-4-seleno-D-arabinitol **10** (660 mg, 1.4 mmol) with benzyl 6-*O*-*p*-toluenesulfonyl-β-D-mannopyranoside **13** (500 mg, 1.2 mmol) in HFIP (1.5 mL), containing anhydrous K_2CO_3 (10 mg) at 65-70 ^oC for 4 days gave the selenonium salt **13** as a foam (473 mg, 45%) after purification by column chromatography ($CHCl_3$/MeOH (95:5)). Analysis by NMR showed that the product was a mixture of two isomers (~10:1) at the stereogenic selenium centre. $[\alpha]_D^{23}$ = + 13.6 ^{o} Data for the major diastereomer: ^{1}H NMR (MeOD) δ 7.74-7.24 (24H, m, Ar), 4.86 (1H, m, H-1′), 4.81 (1H, m, H-2), 4.71-4.50 (8H, m, 4C*H*$_2$-Ph), 4.58 (1H, m, H-3), 4.42 (1H, dd, $J_{3,4}$ = 6.8, $J_{4,5}$ = 9.4 Hz, H-4), 4.03 (1H, d, $J_{1,2}$ = 12.8 Hz, H-1a), 3.94 (2H, m, H-6′a, H-4′), 3.88 (1H, dd, $J_{1',2'}$ =2.0 $J_{2',3'}$ = 2.7 Hz, H-2′) 3.83 (1H, dd, $J_{4,5}$ = 6.7, $J_{5a,5b}$ = 10.3 Hz, H-5a), 3.78-3.73 (4H, m, H-1b, H-5b, H-3′, H-6b), 3.59 (1H, t, $J_{4',5'}$ = $J_{5',6'}$ = 9.3 Hz, H-5′), 2.39 (3H, s, Me). ^{13}C NMR (MeOD) δ 141.7-125.1 (m, Ar), 100.0 (C-1′), 83.7 (C-2), 83.2 (C-3), 72.6, 71.5, 71.2, 69.5

($4CH_2$-Ph), 70.2 (C-2′), 70.0(C-5′), 69.9 (C-3′), 68.9 (C-4′), 66.1 (C-5),65.7 (C-4), 45.9 (C-1), 45.6 (C-6′), 19.4 (Me). HRMS Calcd for $C_{39}H_{45}O_8Se$ (M+.): 721.2278. Found: 721.2278.

1,4-Dideoxy-1,4-[[2S, 3S, 4R, 5R]-2,3,4,5,6-pentahydroxy-hexyl]-(R/S)-epi-seleniumylidene]-D-arabinitol chloride (8). Compound **8** was obtained as a colorless solid (40 mg, 45%) from **13** (200 mg, 0.22 mmol) using the same procedure that was used to obtain **7**. $[\alpha]_D^{23}$ = + 4°. ^{1}H NMR (D_2O) δ 4.85 (1H, dd, $J_{1,2}$ =7.6, $J_{2,3}$ = 3.85, H-2), 4.54 (1H, t, $J_{3,4}$ = $J_{2,3}$ = 3.5 Hz,, H-3), 4.30-4.23 (2H, m, H-2′, H-4), 4.13 (1H, dd, $J_{4,5a}$ = 12.4, $J_{5a,5b}$ = 5.2 Hz, 5a) 4.02-3.97 (2H, m, H-1′a,H-5b), 3.94-3.82 (5H, m, H-6′a, H-3′, H-1′b, H-1a, H-1b), 3.78-3.73 (2H, m, H-4′, H-5′b) 3.68 (1H, dd, $J_{6'a,6,b}$ = 11.7, $J_{5',6'b}$ = 5.6 Hz, H-6′b). ^{13}C NMR (D_2O) δ 78.3 (C-3), 77.9 (C-2), 72.1 (C-3′), 70.7(C-5′), 69.6 (C-4), 69.2 (C-4′), 67.5 (C-2′), 63.1 (C-6′), 59.4 (C-5), 48.1 (C-1′), 45.3 (C-1). HRMS Calcd for $C_{11}H_{23}O_8Se$ (M+.): 363.0553. Found: 363.0544.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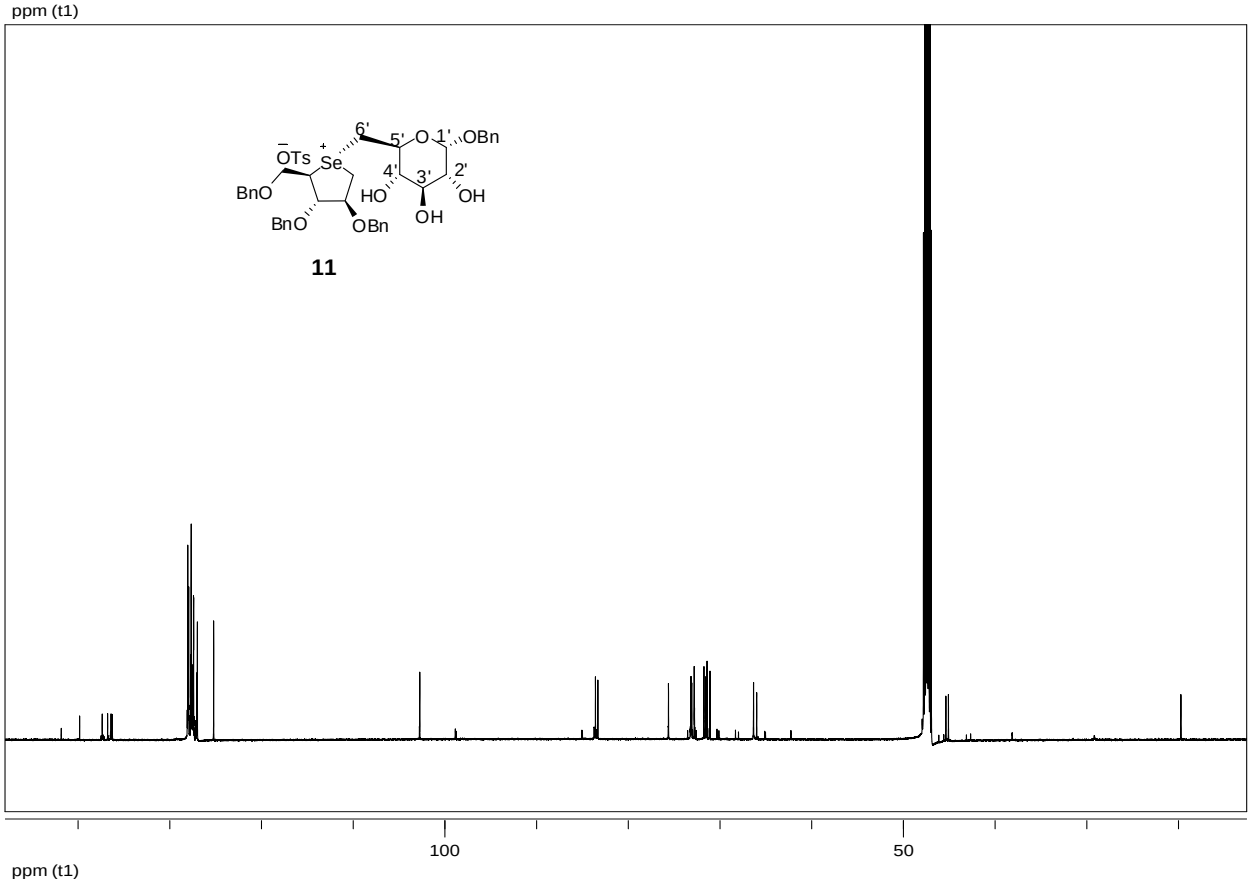
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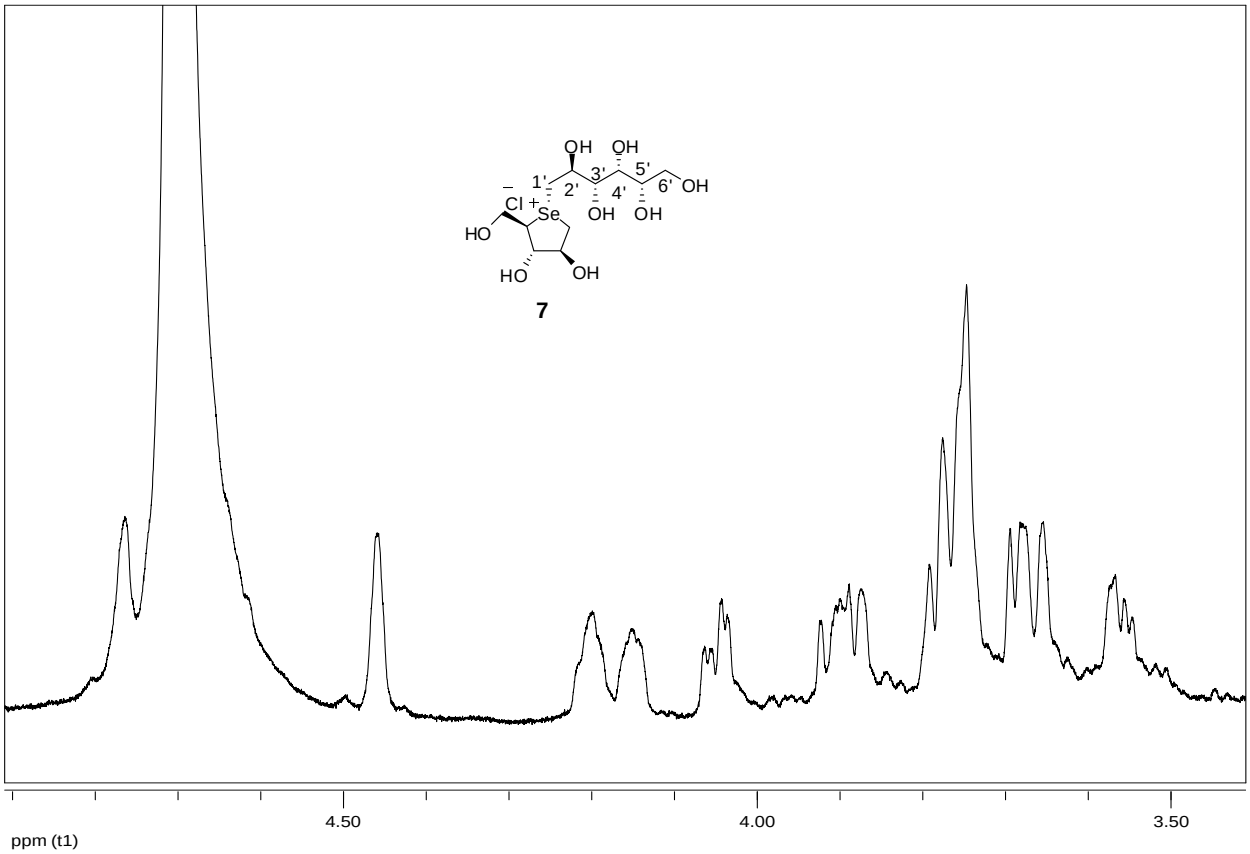
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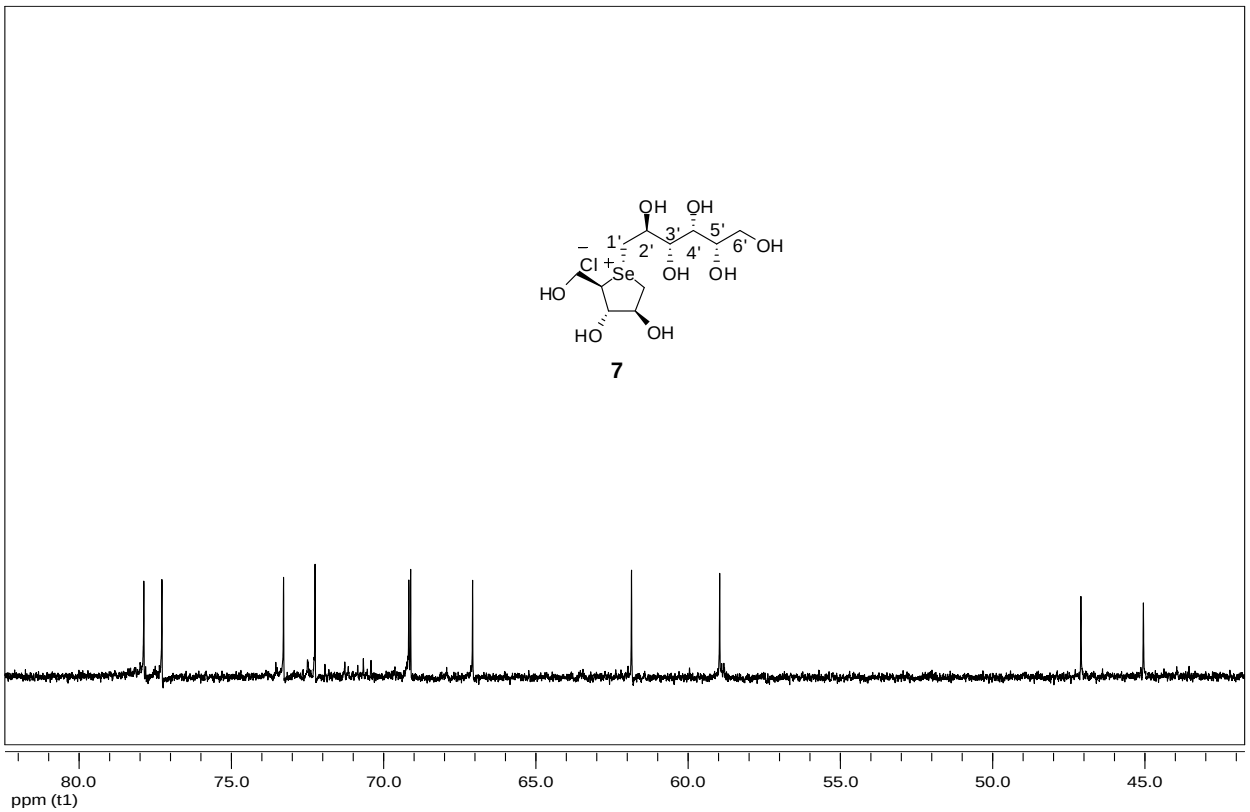
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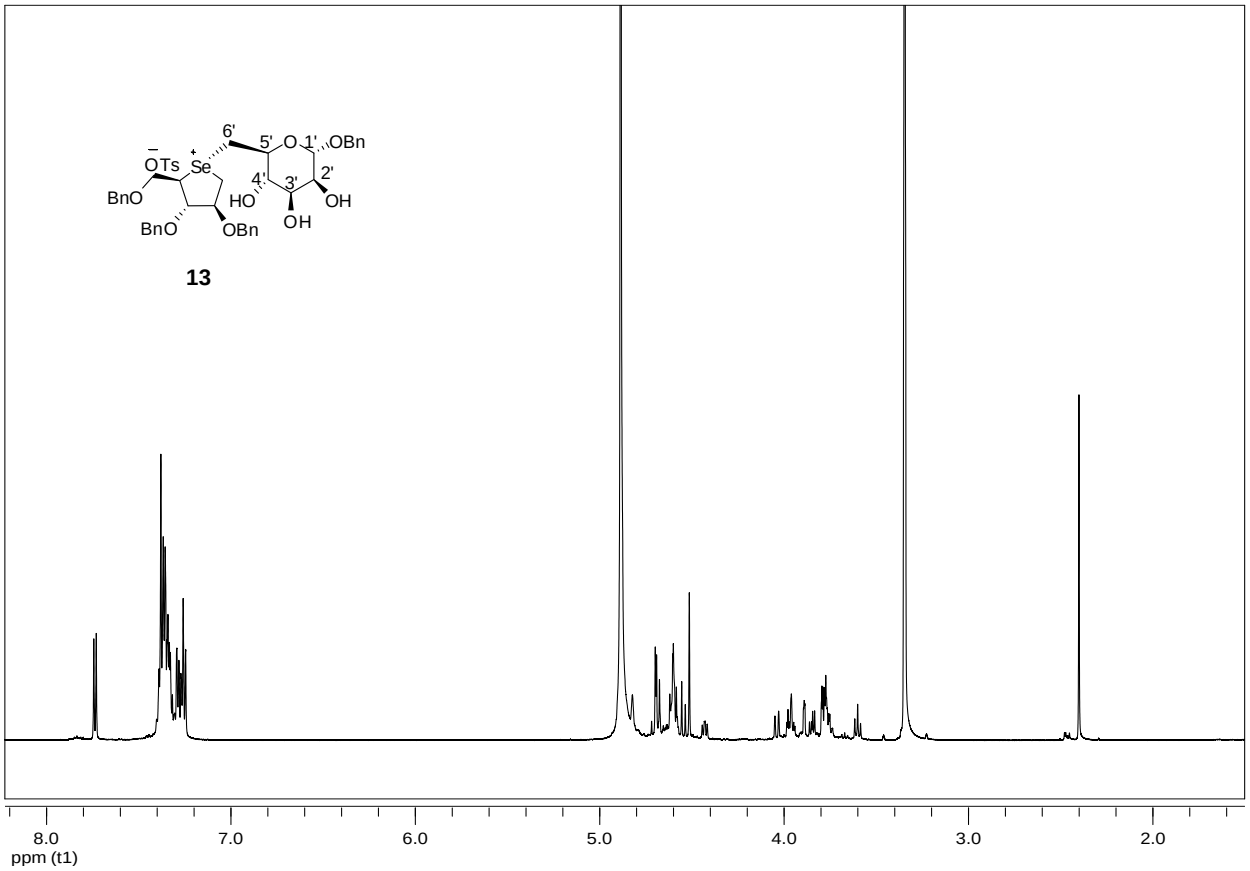
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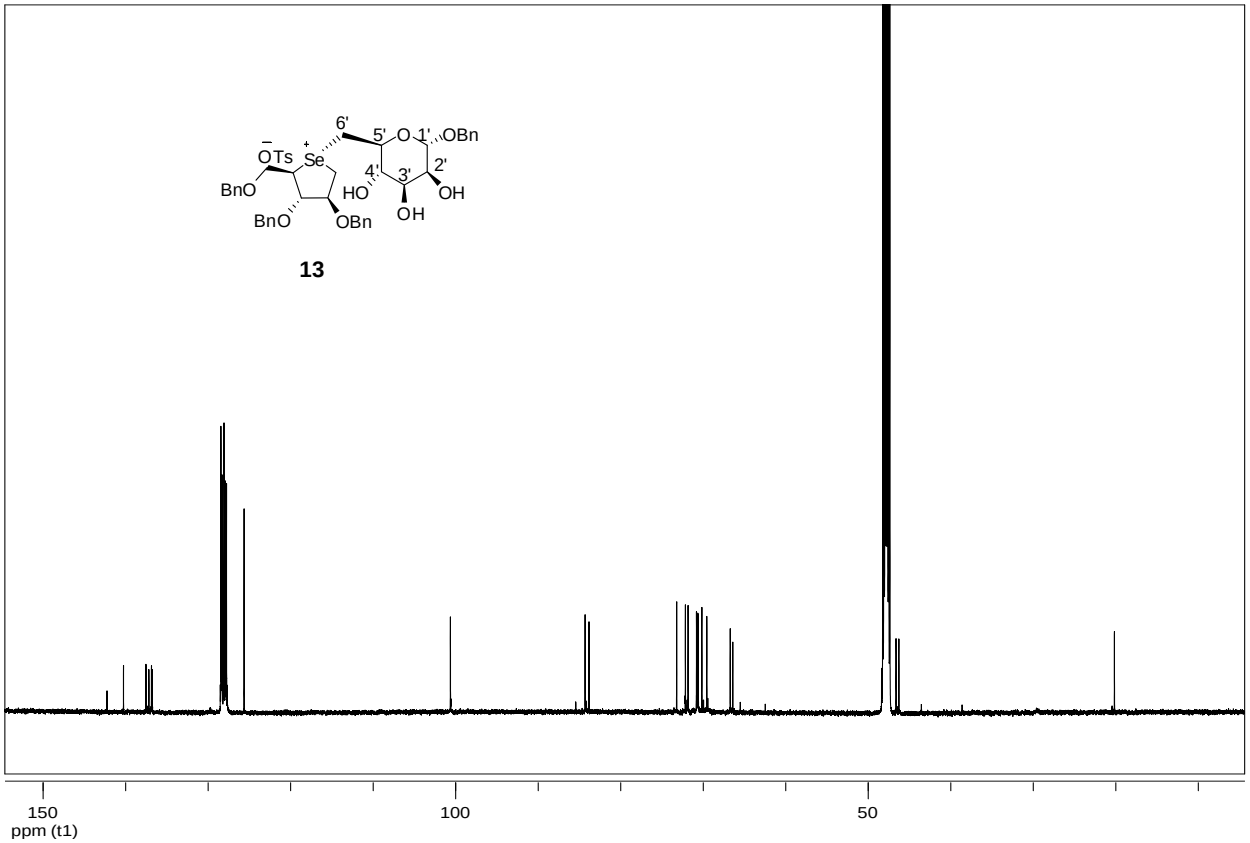
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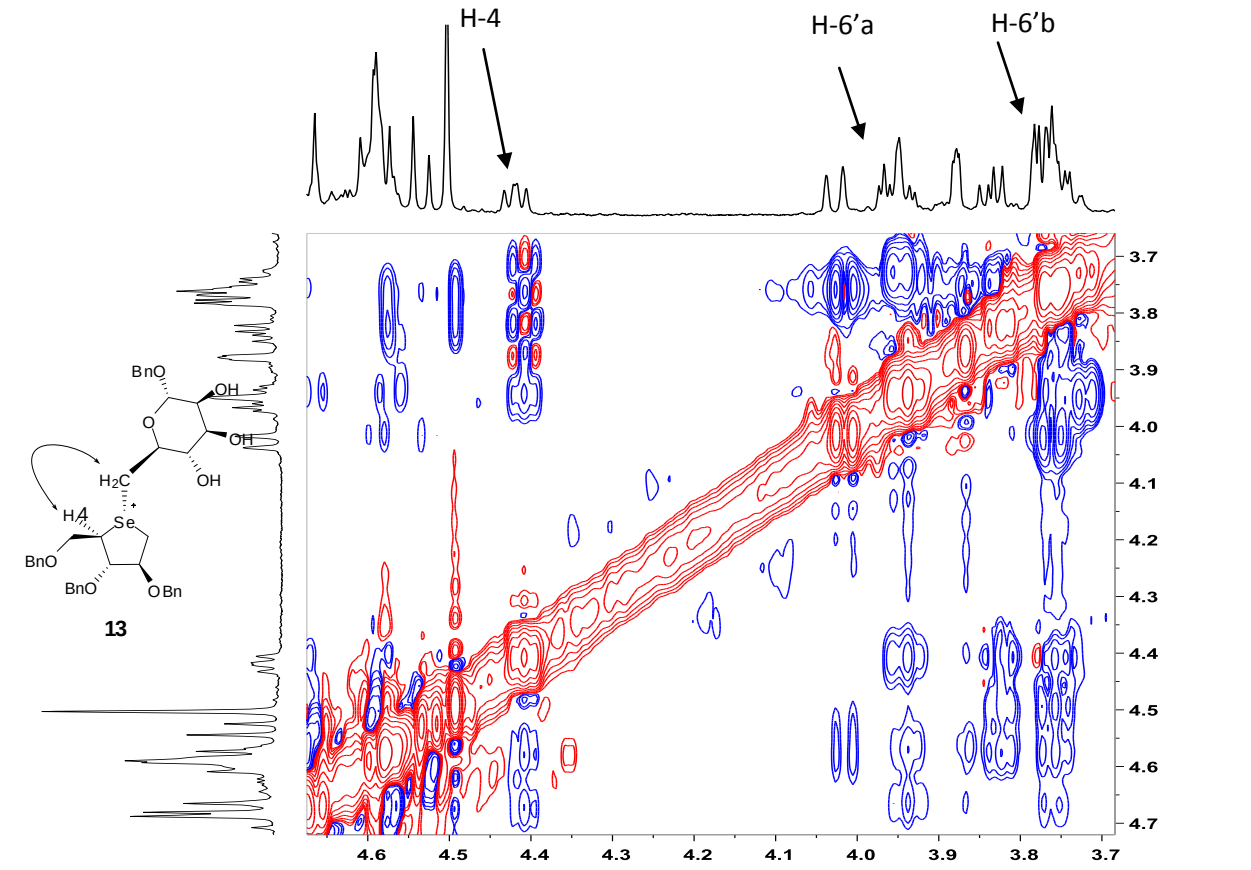
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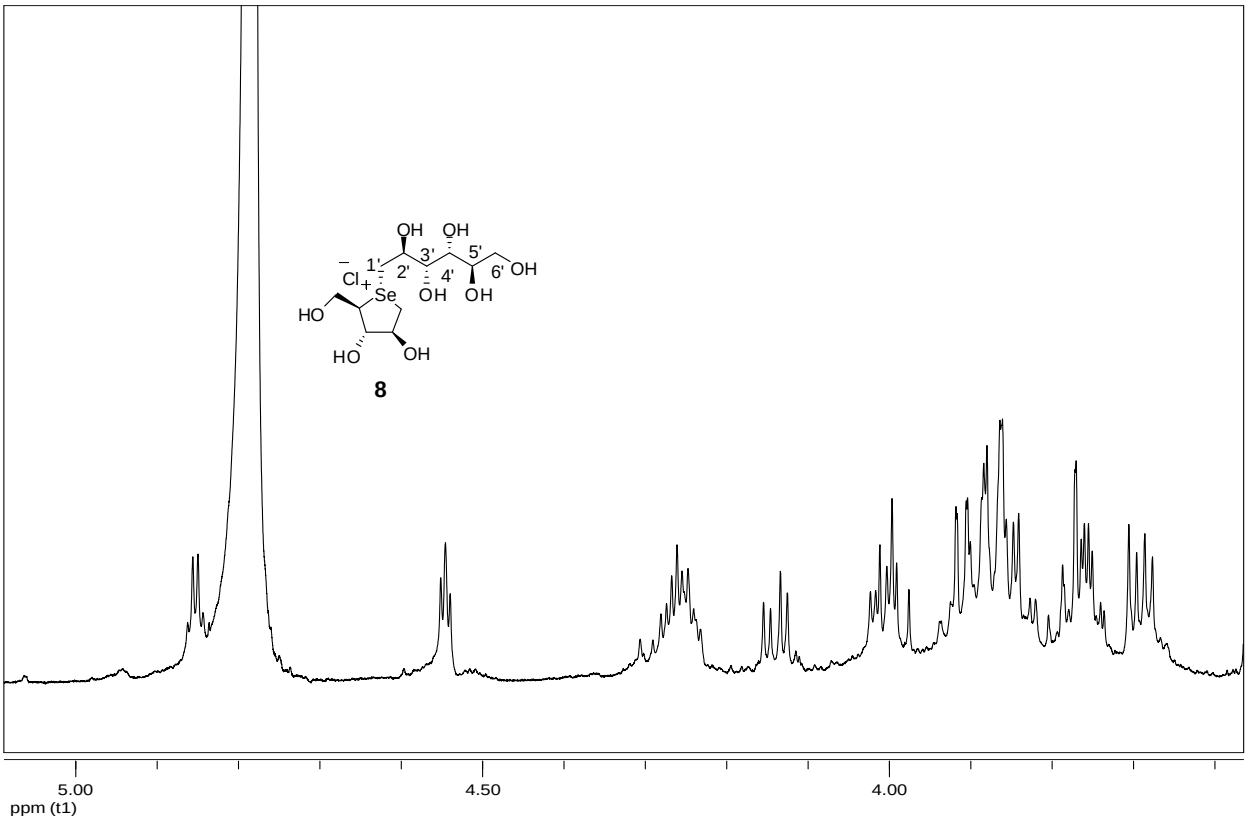
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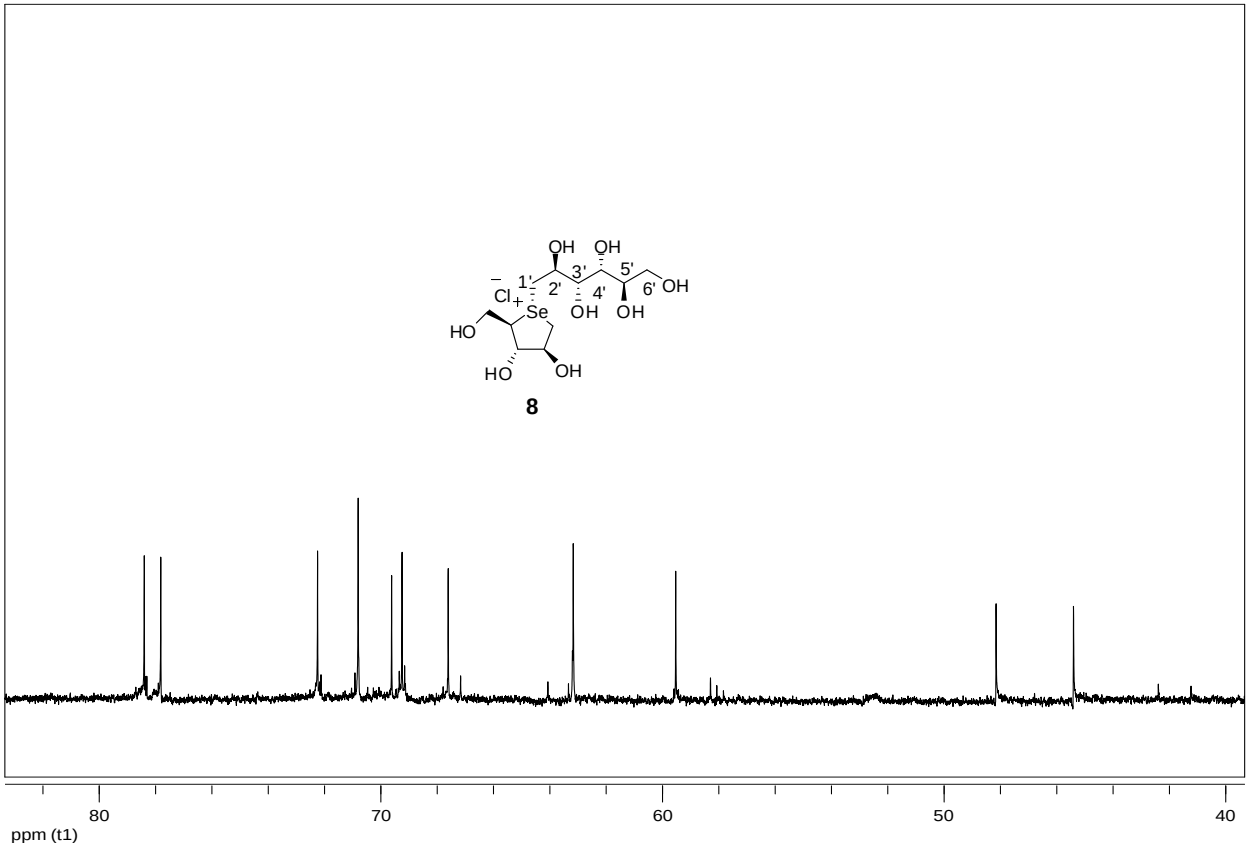
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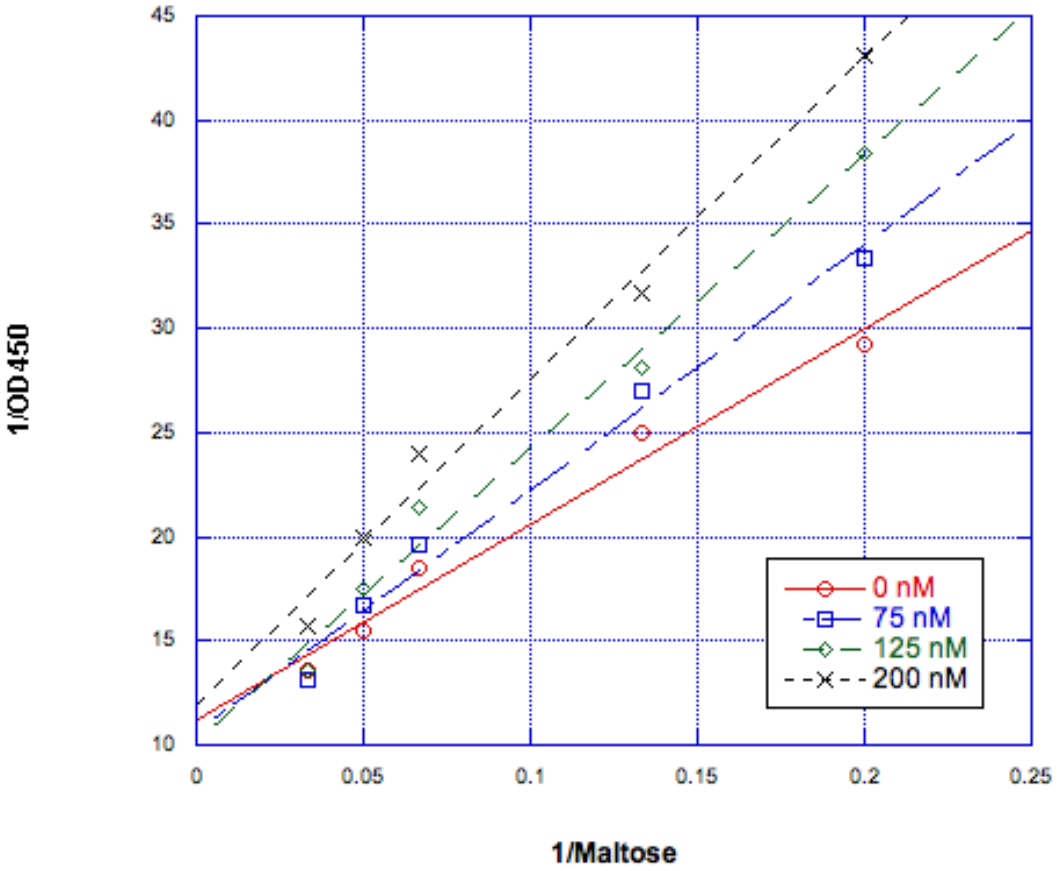

Representative Lineweaver-Burk plot of ntSI inhibited by **5** at concentrations of 0 nM, 75 nM, 125 nM, and 200 nM.

References:

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