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

Razieh Eskandari, a Kyra Jones, b David R. Rose b and B. Mario Pinto*a

a Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada, V5A 1S6 Tel: (+1)7787824152
Fax: (+1)7787824860
E-mail: bpinto@sfu.ca

b Department of Biology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

Supporting Information

Table of Contents

Table of Contents Page #
1 General Experimental Section S2
2 Enzyme Inhibition Assays S2
2 Experimental Procedures and Physical Characterizations S2-4
3 NMR Spectra S5-9
4 Representative Lineweaver-Burk plot of ntSI S10
5 References S10
**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 $K_i = [I]/(K_m^{obs}/K_m)-1$. 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-toluensulphonate (11).** Benzyl 6-O-p-toluenesulfonyl-β-D-glucopyranoside $^9$ (310 mg, 0.73 mmol) and 1,4-dideoxy-2,3,5-tri-O-benzyl-1,4-anhydro-4-seleno-D-arabinitol $^10$ (409 mg, 0.88 mmol) were dissolved in HFIP (1.5 mL), containing anhydrous K$_2$CO$_3$ (10 mg). The mixture was stirred in a sealed reaction vessel in an oil bath at 65-70 °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 sulphonium salt 11 as a white amorphous solid (358 mg, 55%). $[\alpha]^{23}_D = + 22^\circ$. $^1$H NMR (MeOD) $\delta$ 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) $\delta$ 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$_6$Se (M+): 721.2278. Found: 721.2279.
1,4-Dideoxy-1,4-[[2S, 3S, 4R, 5S]-2,3,4,5,6-pentahydroxyhexyl]-(R/S)-epi-seleniumylidene-D-arabinitol chloride (7). Compound 11 (200 mg, 0.22 mmol) was dissolved in CH₂Cl₂ (20 mL), the mixture was cooled to -78 °C, and BCl₃ (1M solution in CH₂Cl₂, 3.6 mmol) was added under N₂. The reaction mixture was stirred at the same temperature for 30 min, and then allowed to warm to -5 °C for 6 h. The reaction was cooled to -78 °C 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 H₂O (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 NaBH₄ (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 the seleniumylidene-D-arabinitol chloride. The residue was purified by crystallization with minimum amount of MeOH to give 7 as a colorless solid (46 mg, 52%).

[α]²³ D + 12.8 °, (c = 0.5, H₂O). ^₁H NMR (D₂O) δ 4.76 (1H, dd, J₁,₂ = J₂,₃ = 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, J₄,₅ₐ = 12.2, J₅ₐ,₅ₐ = 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, J₆ₐ,₆ₐ = 10.7, J₅ₐ,₆ₐ = 3.7, J₅ₐ,₅ₐ = 5.9 Hz, H-6′b). ^₁³C NMR (D₂O) 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 C₁₁H₂₂O₆Se (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 K₂CO₃ (10 mg) at 65-70 °C for 4 days gave the selenonium salt 13 as a foam (473 mg, 45%) after purification by column chromatography (CHCl₃/MeOH (95:5)). Analysis by NMR showed that the product was a mixture of two isomers (~10:1) at the stereogenic selenium centre. [α]²³ D = +13.6 ° Data for the major diastereomer: ^₁H 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, 4CH₂-Ph), 4.58 (1H, m, H-3), 4.42 (1H, dd, J₃,₄ = 6.8, J₄,₅ = 9.4 Hz, H-4 ), 4.03 (1H, dd, J₂,₃ = 12.8 Hz, H-1a), 3.94 (2H, m, H-6′a, H-4′), 3.88 (1H, dd, J₁,₂ = 2.0, J₂,₃ = 2.7 Hz, H-2′) 3.83 (1H, dd, J₄,₅ = 6.7, J₅ₐ,₅ₐ = 10.3 Hz, H-5a), 3.78-3.73 (4H, m, H-1b, H-5b, H-3′, H-6b), 3.59 (1H, t, J₄,₅ = J₅ₐ,₅ₐ = 9.3 Hz, H-5′), 2.39 (3H, s, Me). ^₁³C 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
(4CH₂-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 C₃₉H₄₅O₈Se (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. [α][^D]₂₃ = +4°. ¹H NMR (D₂O) δ 4.85 (1H, d, J₁,₂ = 7.6, H-2), 4.54 (1H, t, J₃,₄ = J₂,₃ = 3.5 Hz, H-3), 4.30-4.23 (2H, m, H-2', H-4), 4.13 (1H, dd, J₄,₅ₐ = 12.4, J₅ₐ,₅ₜ = 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, J₆ₐ,₆ₕ = 11.7, J₅ₕ,₆ₕ = 5.6 Hz, H-6'b). ¹³C NMR (D₂O) 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 C₁₁H₂₃O₈Se (M+): 363.0553. Found: 363.0544.
Representative Lineweaver-Burk plot of ntSI inhibited by 5 at concentrations of 0 nM, 75 nM, 125 nM, and 200 nM.

References: