Design and synthesis of new amino-modified iminocyclitols: Selective inhibitors of α-galactosidase

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General considerations:

All solvents were purified by standard procedures. Anhydrous solvents were dried over sodium wire (benzene, hexane, THF) or molecular sieves (MeOH, DMF, CH₃CN). Thin-layer chromatography (TLC) was performed on Merck silica gel pre-coated on aluminium plates. Flash column chromatography was performed on 230–400 mesh silica gel. Optical rotations were recorded on an Autopol V (Rudolph Research Flanders, New Jersey) instrument. All the rotations were measured at 589 nm (sodium 'D line). Melting points of the compounds are uncorrected. IR spectra were taken over the 4000–400 cm⁻¹ range as KBr pellets on a Nicolet (Madison, USA) FTIR spectrophotometer (Model protégé 460). All the ¹H and ¹³C NMR spectra were recorded on a 300 MHz Bruker Spectrospin DPX FT-NMR spectrometer. Chemical shifts are reported as δ values (ppm) relative to Me₄Si as internal standard. Mass spectra were recorded on an Applied Biosystems Q-Star instrument. Freeze-drying of samples was done on a Freezone 2.5 (Labconco, USA) lyophilizer.

3,4,6-Tri-O-benzyl-1,2-dideoxy-1,2-(di-p-toluenesulfonamido)-D-glucitol 9:

![Chemical structure of 3,4,6-Tri-O-benzyl-1,2-dideoxy-1,2-(di-p-toluenesulfonamido)-D-glucitol 9](image)

Compound 8⁴ (5.0 g, 6.6 mmol) was taken in a flame dried 100 mL three-necked round bottom flask and 50 mL of THF was added to it. LiAlH₄ (0.377 g, 9.9 mmol) was then added and the reaction mixture was refluxed at 66 °C under argon atmosphere. The progress of the reaction was monitored by TLC. After 20 min., when TLC indicated the disappearance of the starting material, the reaction was then quenched with 10% aqueous HCl (100 mL) and the reaction mixture was extracted with CHCl₃ (3 x 100 mL). The combined organic layer was dried over sodium sulphate, filtered and then concentrated to give 9 in 95% (4.763 g) as colourless low melting solid. [α]D²⁸ +0.54 (c 1.5, CHCl₃). νmax (KBr)/cm⁻¹: 3496, 3280, 3032, 2920, 2866, 1451, 1330, 1159, 1088, 814, 742, 699, 666, 552; δH (300 MHz; CDCl₃): 7.66 (2H, d, J 7.8 Hz), 7.54 (2H, d, J 8.1 Hz), 7.35–7.13 (19H, m), 5.30 (1H, br s, exchangeable with D₂O), 4.71 (1H, br s, exchangeable with D₂O), 4.68 (2H, d, J 11.1 Hz), 4.57-4.44 (4H, m), 4.36 (1H, d, J 11.1 Hz), 3.82 (1H, m), 3.68 (1H, m), 3.55–3.52 (2H, m), 3.47-3.37 (2H, m), 2.88 (1H, m), 2.79 (1H, m), 2.38 (3H, s), 2.34 (3H, s); δC (300 MHz; CDCl₃) : 143.5 (C), 143.3 (C), 137.7 (C), 137.4 (C), 137.1 (C), 136.4 (C), 129.6 (CH), 128.5 (CH), 128.06 (CH), 127.9 (CH), 127.1 (CH), 126.9 (CH), 77.8 (2xCH), 74.7 (CH₂), 73.9 (CH₂), 73.3 (CH₂), 71.1 (CH), 70.7 (CH₂), 53.8 (CH), 44.4 (CH₂), 21.4 (2xCH₃); HRMS (ESI): [M+H]^+, Found: 759.2734, C₄₁H₄₇N₂O₈S₂ requires 759.2774.
(2S,3R,4R,5S)-2-(N-p-Toluenesulfonyl)aminomethyl-3,4-dibenzylxyloxy-5-benzylxymethyl-1N-(p-toluenesulfonyl)pyrrolidine 10:

Compound 9 (5.0 g, 6.59 mmol) was taken in a 100 mL flame dried three necked round bottom flask and 30 mL of dry THF was added to it followed by PPh₃ (2.246 g, 8.56 mmol). The reaction mixture was cooled to 0 °C. Diethyl azodicarboxylate, DEAD (1.517 mL, 9.23 mmol) was injected into the reaction mixture drop-wise under argon atmosphere. After the addition was over, the reaction mixture was brought to room temperature. The reaction was found to be completed in 30 min. (vide TLC). The reaction was then stopped and the solvent was evaporated. Flash chromatography (hexane-ethyl acetate 6:1) of resulting residue provided 10 in 90% (4.393 g) yield as a colorless viscous liquid; [α]²⁸D⁻1.1 (c 0.53, CHCl₃); νmax (KBr)/cm⁻¹; 3378, 3031, 2923, 2867, 2359, 1497, 1456, 1405, 1334, 1160, 1094, 741, 700, 554; δH (300MHz; CDCl₃) : 7.71 (2H,d, J 8.1 Hz), 7.67 (2H, d, J 8.1 Hz), 7.31–7.16 (17H, m), 6.91-6.89 (2H, m), 5.43 (1H, t, J = 7.2 Hz, exchangeable with D₂O), 4.63–4.44 (5H, m), 4.13–3.99 (4H, m), 3.88–3.82 (1H, m), 3.76 (1H, dd, J 9.9 Hz, 3.3 Hz), 3.48 (1H, d, J 9.9 Hz), 3.44–3.27 (2H, m), 2.39 (3H, s), 2.29 (3H, s); δC (300 MHz; CDCl₃) : 143.5 (C), 143.1 (C), 137.9 (C), 137.6 (C), 137.4 (C), 137.1 (C), 136.6 (C), 129.6 (CH), 129.5 (CH), 128.6 (CH), 128.5 (CH), 128.1 (CH), 128.1 (CH), 128.01 (CH), 127.9 (CH), 127.5 (CH), 127.4 (CH), 127.3 (CH), 127.2 (CH), 126.8 (CH), 80.1 (CH), 80.7 (CH), 73.5 (CH₂), 73.3 (CH₂), 72.6 (CH₂), 65.6 (CH₂), 58.4 (CH), 58.3 (CH), 43.8 (CH₂), 21.5 (CH₃), 21.5 (CH₃); HRMS (ESI): [M+H]⁺, Found: 741.2635, C₄₁H₄₅N₂O₇S₂ requires 741.2668.

(2S,3R,4R,5S)-2-Aminomethyl-3,4-dihydroxy-5-hydroxymethyl-pyrrolidine 12:

Liquid ammonia (40 mL) was collected in a flame dried 100 mL three-necked round bottom flask at -78 °C. Sodium metal (0.140 g, 6.073 mmol) was added to it. Deep blue colour appeared. Pyrrolidine 10 (1.0 g, 1.35 mmol) dissolved in THF (2 mL) was added to the reaction mixture and stirred at -78 °C for 3 h. The reaction was quenched by the addition of benzene until the blue colour disappeared followed by addition of water (10 mL). The reaction mixture was allowed to slowly warm to room temperature. The crude reaction mixture was dried in a lyophilizer. Column chromatography (CH₃CN-NH₄OH 4:1) of the residue over deactivated silica yielded
ADMDP 12 in 86% (0.188 g) as a pale yellow low melting solid. [α]^{28}_D +2.3 (c 0.30, D_2O); \nu_{\text{max}} (\text{KBr/\text{cm}^{-1}}): 3451, 2924, 2361, 1717, 1640, 1452, 1330, 1269, 1158, 1095, 812, 754, 706, 667, 553; \delta_H (300 MHz; D_2O): 4.29 (2H, d, J 10.5 Hz) 4.05 (1H, s), 3.90-3.79 (3H, m), 3.43-3.39 (2H, m); \delta_C (300 MHz; D_2O): 74.8 (CH), 74.4 (CH), 63.5 (CH), 58.2 (CH), 57.4 (CH_2), 36.2 (CH_2); HRMS (ESI): [M+H]^+, Found: 163.1080, C_6H_15N_2O_3 requires 163.1083.

(2S,3R,4R,5S)-2-(N-Acetyl-N-p-toluenesulfonyl)aminomethyl-3,4-dibenzyloxy-5-benzzyloxyethyl-1N-(p-toluenesulfonyl)pyrrolidine 13:

In a 50 mL single necked round bottom flask was taken compound 10 (3.0 g, 3.83 mmol) and dissolved in pyridine (30 mL). DMAP (0.467 g, 3.83 mmol) was then added and reaction mixture was cooled to 0°C. Ac_2O (0.724 mL, 7.66 mmol) was then added and the reaction mixture was allowed to come at room temperature and stirred for 24 h. The reaction was quenched with 10% aqueous HCl (100 mL) and the reaction mixture was extracted with EtOAc (3 x 150 mL). The organic layer then washed with brine solution and dried over sodium sulphate, filtered and then concentrated. Flash chromatography of crude reaction mixture was performed with hexane-ethyl acetate (6:1) to obtain 13 in 95% yield (3.012 g) as a colourless viscous liquid; [α]^{25}_D -1.9 (c 0.58, CHCl_3); \nu_{\text{max}} (\text{KBr/\text{cm}^{-1}}): 3026, 2924, 2861, 2360, 1700, 1645, 1455, 1354, 1162, 1096, 762, 663, 581, 547; \delta_H (300 MHz; CDCl_3); 7.79 (2H, d, J 7.8 Hz), 7.77 (2H, d, J 7.5 Hz), 7.29–7.10 (17H, m), 6.93-6.90 (2H, m), 4.66–4.48 (5H, m), 4.43–4.21 (4H, m), 4.14-4.07 (2H, m), 3.96 (1H, dd, J 12 Hz), 3.80 (1H, dd, J 9.9, 4.2 Hz), 3.57 (1H, d, J 9.9 Hz), 2.39 (3H, s), 2.26 (3H, s), 2.18 (3H, s); \delta_C (300 MHz; CDCl_3): 170.9 (C), 144.7 (C), 142.9 (C), 138.04 (C), 137.9 (C), 137.9 (C), 137.7 (C), 136.5 (C), 129.8 (CH), 129.6 (CH), 129.2 (CH), 129.02 (CH), 128.3 (CH), 128.2 (CH), 127.9 (CH), 127.8 (CH), 127.6 (CH), 127.4 (CH), 127.2 (CH), 127.1 (CH), 126.9 (CH), 81.3 (CH), 79.6 (CH), 73.3 (CH_2), 73.2 (CH_2), 72.5 (CH_2), 65.2 (CH_2), 58.8 (CH), 56.4 (CH), 46.2 (CH_2), 25.1 (CH_3), 21.6 (CH_3), 21.4 (CH_3); HRMS (ESI): [M+Na]^+, Found: 805.2584, C_{43}H_{46}N_2O_8S_2Na requires 805.2593.

**General procedure for Na-Hg mediated detosylation reactions.**

In a flame dried 50 mL three necked round bottom flask were taken 3% Na/Hg and Na_2HPO_4 at room temperature under Ar atmosphere. Compound to be detosylated, dissolved in a solvent mixture (DMF-MeOH 8:1), was then added and the reaction mixture was heated at 60 °C for 3 h.
after which the reaction was stopped. The reaction mixture was then filtered through a celite pad. The filtrate was diluted with EtOAc and washed with brine solution, a few times, to remove DMF completely from the reaction mixture. The organic layer was dried over anhydrous sodium sulphate, filtered and then concentrated. Flash chromatography of crude reaction mixture afforded the corresponding detosylated compounds.

\((2S,3R,4R,5S)-2-(N-p-Toluenesulfonyl)aminomethyl-3,4-dibenzyloxy-5-benzylxoxymethyl-pyrrolidine 14:\)

![Diagram of compound 14]

Compound 14 (0.76 g, 96% yield) was obtained as a yellow gummy liquid from the reaction of compound 10 (1.0 g, 1.35 mmol) with 3% Na-Hg (52.0 g, 67.57 mmol) and Na₂HPO₄ (1.2 g, 6.76 mmol) following the general procedure described above. Product 14 was purified by column chromatography over silica using hexane-ethyl acetate (2:1). \([\alpha]_D^{28}-6.08 \ (c \ 0.44, \ CHCl₃); \nu_{\text{max}} \ (\text{KBr})/\text{cm}^{-1}: \ 3309, 3031, 2922, 2858, 1956, 1878, 1811, 1722, 1598, 1454, 1329, 1214, 1158, 1088, 910, 813, 741, 698, 664, 604, 552; \delta_H \ (300\text{MHz}; \text{CDCl}_3) \ 7.65 \ (2H, d, J \ 8.1 \ \text{Hz}), \ 7.36-7.22 \ (17H, m), \ 4.57-4.53 \ (5H, m), \ 4.35 \ (1H, d, J \ 11.7 \ \text{Hz}), \ 4.0 – 3.97 \ (2H, m), \ 3.64 – 3.52 \ (4H, m), \ 3.12(1H, dd, J \ 12.6, 6.3 \ \text{Hz}), \ 2.97(1H, dd, J \ 18.6, 6.3 \ \text{Hz}), \ 2.44 \ (3H, s); \delta_C \ (300 \ \text{MHz}; \text{CDCl}_3): \ 142.9 \ (C), \ 138.04 \ (C), \ 137.8 \ (C), \ 137.5 \ (C), \ 136.8 \ (C), \ 129.5 \ (CH) \ 128.5 \ (CH), \ 128.3 \ (CH), \ 128.3 \ (CH), \ 127.9 \ (CH), \ 127.7 \ (CH), \ 127.6 \ (CH), \ 127.6 \ (CH), \ 127.3 \ (CH), \ 126.9 \ (CH), \ 126.8 \ (CH), \ 82.8 \ (CH), \ 82.2 \ (CH), \ 73.2 \ (CH₂), \ 72.01 \ (CH₂), \ 71.9 \ (CH₂), \ 68.9 \ (CH₂), \ 64.8 \ (CH), \ 58.4 \ (CH), \ 57.6 \ (CH₂), \ 43.4 \ (CH₂), \ 21.3 \ (CH₃); \text{HRMS (ESI): [M+H]}^+, \text{Found: 587.2586, C}_{34}H_{39}N_{2}O_5S \text{requires 587.2580.}

\((2S,3R,4R,5S)-2-(p-Toluenesulfonyl)aminomethyl-3,4-dihydroxy-5-hydroxymethyl-pyrrolidine 15:\)

![Diagram of compound 15]

10% palladium on charcoal (1.5 g, 300% w/w) was taken in a 50 mL three-necked round bottom flask. Compound 14 (0.5 g, 0.85 mmol) dissolved in methanol (10 mL) was added to the reaction flask. H₂ gas was then bubbled slowly into reaction mixture for 5 h at 30 °C. The reaction mixture was then filtered through a celite pad. The filtrate was collected and the solvent
was evaporated to yield compound 15 as a white solid in 90% (0.243 g). mp 182 °C (recrystallized from MeOH/THF); $[\alpha]_{D}^{28} +7.4$ (c 0.35, H2O); $v_{\text{max}}$ (KBr)/cm$^{-1}$: 3150, 1745, 1632, 1406, 1325, 1158, 1089, 815, 666, 554; δ$H$ (300MHz; D$_2$O): 7.73 (2H, br s), 7.42 (2H, br s), 4.309 (1H, br s), 4.21 (1H, s), 3.94 (4H, m), 3.26 (2H, m), 2.37 (3H, s); δ$C$ (300 MHz; D$_2$O): 145.4 (C), 134.2 (C), 130.2 (CH), 126.8 (CH), 74.5 (CH), 74.4 (CH), 63.3 (CH), 61.05 (CH), 57.4 (CH$_2$), 39.5 (CH$_2$), 20.7 (CH$_3$); HRMS (ESI): [M+H]$^+$, Found: 317.1156, C$_{13}$H$_{21}$N$_2$O$_5$S requires 317.1171.

**General procedure for alkylation of compound 10:**

Compound 10 was taken in a flame dried three-necked round bottom flask and dissolved in dry DMF. Sodium hydride was added to it at 0 °C followed by alkyl halide and the reaction mixture was stirred at room temperature for the specified time after which the reaction was stopped. The reaction was then quenched with iced water. The reaction mixture was diluted with EtOAc and washed with brine solution, a few times, to remove DMF completely from the reaction mixture. The organic layer was dried over anhydrous sodium sulphate, filtered and then concentrated. Flash chromatography of crude reaction mixture afforded the corresponding alkylated compounds.

**(2S,3R,4R,5S)-2-(N-Methyl-N-p-toluenesulfon)aminomethyl-3,4-dibenzyloxy-5-benzyloxyethyl-1-N-(p-toluenesulfon)pyrrolidine 16:**

Compound 16 (1.814 g, 89% yield) was obtained in 3 h as a white crystalline solid from the reaction of compound 10 (2.0 g, 2.69 mmol) with NaH (0.432 g of 60% suspension in mineral oil) and methyl iodide (0.252 mL, 4.05 mmol) following the general procedure described above. Product 16 was purified by column chromatography over silica using hexane-ethyl acetate (8:1). mp 110 °C (recrystallized from benzene/hexane); $[\alpha]_{D}^{27} +28.4$ (c 0.69, CHCl$_3$); $v_{\text{max}}$ (KBr)/cm$^{-1}$: 3029, 2918, 2865, 1717, 1594, 1491, 1453, 1338, 1157, 1088, 1018, 810, 747, 699, 659, 601, 546; δ$H$ (300MHz; CDCl$_3$): 7.89 (2H, d, $J$ 7.8 Hz), 7.84 (2H, d, $J$ 8.1 Hz), 7.33 - 7.19 (17H, m), 6.89 (2H, m), 4.78 (1H, d, $J$ 11.7 Hz), 4.64 (1H, d, $J$ 11.7 Hz), 4.56 - 4.49 (4H, m), 4.11 - 3.88 (5H, m), 3.79-3.76 (1H, m), 3.60 (1H, d, $J$ 9.0 Hz), 2.88-2.80 (1H, m), 2.66 (3H, s), 2.43 (3H, s), 2.29 (3H, s); δ$C$ (300 MHz; CDCl$_3$): 143.5 (C), 142.9 (C), 137.9 (C), 137.8 (C), 137.5 (C), 132.2 (C), 129.6 (CH), 129.3 (CH), 128.3 (CH), 128.2 (CH), 127.9 (CH), 127.8 (CH), 127.6 (CH), 127.5 (CH), 127.3 (CH), 127.1 (CH), 126.8 (CH), 81.03
(CH), 79.1 (CH), 73.5 (CH2), 73.01 (CH2), 72.5 (CH2), 65.2 (CH2), 59.2 (CH), 58.8 (CH), 51.9 (CH2), 38.9 (CH3), 21.5 (CH3), 21.4 (CH3); HRMS (ESI): [M+H]+, Found: 755.2818, C42H47N2O7S2 requires 755.2825.

(2S,3R,4R,5S)-2-(N-Ethyl-N-p-toluenesulfonyl)aminomethyl-3,4-dibenzyloxy-5-benzyloxymethyl-1N-(p-toluenesulfonyl)pyrrolidine 17:

Compound 17 (2.413 g, 93% yield) was obtained in 5 h as colourless liquid from the reaction of compound 10 (2.5 g, 3.37 mmol) with NaH (0.404 g of 60% suspension in mineral oil) and ethyl iodide (0.329 mL, 4.05 mmol) following the general procedure described above. Product 17 was purified by column chromatography over silica using hexane-ethyl acetate (6:1). [α]28D +35.8 (c 0.48, CHCl3); v max(KBr)/cm⁻¹: 3409, 3062, 3031, 2929, 2871, 1710, 1598, 1494, 1453, 1453, 1340, 1155, 908, 814, 731, 659, 662, 598, 548; δH (300MHz; CDCl3): 7.86 (2H, d, J 8.4 Hz), 7.83 (2H,d, J 8.4 Hz), 7.30-7.13 (17H, m), 6.96-6.94 (2H, m), 4.71 (1H, d, J 11.7 Hz), 4.64-4.60 (2H, m), 4.53-4.47 (1H, dd, J 11.7, 4.5 Hz), 4.40 (1H, t, J 6.3Hz), 4.15 (1H, d, J 11.7Hz), 4.07-4.03 (2H, m), 3.95 (1H, d, J 14.4 Hz), 3.77-3.68 (4H ,m), 3.45-3.37 (1H, m), 3.25-3.14 (2H, m), 2.41 (3H, s), 2.29 (3H, s), 0.75 (3H, t, J 7.2 Hz ); δC (300 MHz; CDCl3): 143.3 (C), 143.1 (C), 138.0 (C), 137.9 (C), 137.9 (C), 136.4 (C), 129.7 (CH), 129.4 (CH), 128.4 (CH), 128.3 (CH), 128.1 (CH), 127.9 (CH) 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 127.4 (CH), 127.3 (CH), 127.04 (CH), 80.7 (CH), 78.9 (CH), 73.2 (CH2), 73.04 (CH2), 72.7 (CH2), 65.5 (CH2), 59.9 (CH), 59.7 (CH), 48.01 (CH2), 45.3 (CH2), 21.59 (CH3), 21.53 (CH3), 12.2 (CH3); HRMS (ESI): [M+Na]+, Found: 791.2810, C43H48N2O7S2Na requires 791.2801.

(2S,3R,4R,5S)-2-(N-Methyl)aminomethyl-3,4-dibenzyloxy-5-benzyloxymethyl-pyrrolidine 18:

Compound 18 (0.408 g, 69% yield) was obtained as a yellow gummy liquid from the reaction of compound 16 (1.0 g, 1.32 mmol) with 3% Na-Hg (126.939 gm, 165.57 mmol) and Na2HPO4 (2.948 gm, 16.56 mmol) following the general procedure described above. Product 18 was purified by column chromatography over silica using CHCl3:MeOH:NH4OH (100:2.5:2.5) as a solvent mixture. [α]27D 14.7 (c 0.43, CHCl3); v max(KBr)/cm⁻¹: 3835, 3020, 2924, 2861, 2963, 1709, 1552, 1459, 1407, 1218, 1099, 764, 694; δH (300MHz; CDCl3): 7.37-7.26
(15H, m), 4.58-4.48 (5H, m), 4.40 (1H, d, J = 12Hz), 4.03-3.93 (2H, m), 3.68-3.49 (4H, m), 2.73-2.67 (2H, m), 2.41 (3H, s), 1.96 (2H, br s, NH, exchangeable with D₂O), \( \delta_c \) (300 MHz; CDCl₃): 138.3 (C), 138.2 (C), 137.1 (C), 128.4 (CH), 127.3 (CH), 128.3 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 83.04 (CH), 82.4 (CH), 73.3 (CH₂), 72.2 (CH₂), 71.9 (CH₂), 69.6 (CH₂), 58.5 (2xCH), 51.7 (CH₂), 36.7 (CH₃); HRMS (ESI): [M+H]⁺, Found: 447.2648, C₂₈H₃₅N₂O₃ requires 447.2647.

(2S,3R,4R,5S)-2-(N-Ethyl)aminomethyl-3,4-dibenzyloxy-5-benzyloxyethyl-pyrrolidine 19:

\[
\begin{array}{c}
\text{BnO} \quad \text{OBn} \\
\text{EtN} \quad \text{N} \\
\text{CH} \quad \text{CH} \\
\text{OBn} \quad \text{BnO}
\end{array}
\]

Compound 19 (0.449 g, 75% yield) was obtained as a yellow gummy liquid from the reaction of compound 17 (1.0 g, 1.3 mmol) with 3% Na-Hg (124.62 g, 162.55 mmol) and Na₂HPO₄ (2.893 g, 16.26 mmol) following the general procedure described above. Product 19 was purified by column chromatography over silica using CHCl₃:MeOH:NH₄OH (10:0.1:0.1) as a solvent mixture. \( [\alpha]_{28}^{D} +8.9 \) (c 0.76, CHCl₃); \( \nu_{max} \) (KBr)/cm⁻¹: 3323, 3061, 3031, 2863, 1955, 1877, 1813, 1649, 1608, 1491, 1455, 1362, 1309, 1208, 1095, 1095, 911, 808, 697, 608, 465 and; \( \delta_H \) (300MHz; CDCl₃): 7.34-7.28 (15H, m), 4.60-4.50 (5H, m), 4.42 (d, 1H, J = 12 Hz), 4.06-3.97 (2H, m), 3.72-3.52 (4H, m), 2.84-2.73 (2H, m), 2.65 (2H, q, J = 7.2 Hz), 2.07 (2H, br s, NH exchangeable with D₂O), 1.08 (3H, t, J = 7.2 Hz); \( \delta_c \) (300 MHz; CDCl₃): 138.1 (C), 137.9 (C), 137.7 (C), 128.5 (CH), 128.4 (CH), 127.9 (CH), 127.8 (CH), 128.1 (CH), 127.8 (CH), 127.7 (CH), 127.3 (CH), 126.9 (CH), 82.7 (CH), 82.2 (CH), 73.4 (CH₂), 72.4 (2xCH₂), 69.1 (CH₂), 58.7 (CH), 57.1 (CH), 48.7 (CH₂), 43.9 (CH₂), 13.5 (CH₃); HRMS (ESI): [M+H]⁺, Found: 461.2806, C₂₉H₃₇N₂O₃ requires 461.2804.

(2S,3R,4R,5S)-2-(N-Methyl)aminomethyl-3,4-dihydroxy-5-hydroxymethyl-pyrrolidine 20:

\[
\begin{array}{c}
\text{HO} \\
\text{MeN} \\
\text{NH} \\
\text{HO}
\end{array}
\]

10% palladium on charcoal (0.4 g, 100% w/w) was taken in a 50 mL three-necked round bottom flask. Compound 18 (0.4 g, 0.89 mmol) dissolved in methanol (14 mL) was added to the reaction flask followed by the addition of 10% HCl in methanol (1.75 mL). H₂ gas was then bubbled slowly into reaction mixture for 12 h at 30 °C. The reaction mixture was then filtered through a celite pad and the filtrate was evaporated to get compound 20 as its hydrochloride salt which was then basified with triethylamine untill pH 12.0. Further purification of the product by
column chromatography with CH$_3$CN-NH$_4$OH (1:1) afforded the free base 20 in 94% (0.148 g) yield as a low melting white solid; \([\alpha]^{28}_D +3.8 \text{ (c 1.6, } \text{ H$_2$O)}; \nu_{\text{max}} \text{ (KBr)/cm}^{-1}: 3379, 1631, 1406, 1082, 571; \delta_H \text{ (300MHz; D$_2$O): } 4.31 \text{ (2H, m), 4.11-4.08 (1H, m), 3.90-3.79 (3H, m), 3.51 (1H, dd, } J \text{ 13.5, 6.0Hz), 3.41 (1H, dd, } J \text{ 13.5, 6.6 Hz), 2.75 (3H, s); \delta_C \text{ (300 MHz; D$_2$O): } 75.2 \text{ (CH), 74.7 (CH), 63.5 (CH), 57.8 (CH$_2$), 57.3 (CH), 45.9 (CH$_2$), 33.8 (CH$_3$); HRMS (ESI): } [\text{M+Na}]^+, \text{ Found: 199.1062, C$_7$H$_{16}$N$_2$O$_3$Na requires 199.1059.}

(2S,3R,4R,5S)-2-(N-ethyl)aminomethyl-3,4-dihydroxy-5-hydroxymethyl-pyrrolidine 21:

\[
\begin{align*}
\text{HO} & \\
\text{EtH} & \text{N} \\
\text{CH} & \text{CH} \\
\text{OH} & 
\end{align*}
\]

10% palladium on charcoal (0.4 g, 100% w/w) was taken in a 50 ml three necked round bottom flask. Compound 19 (0.4 g, 0.87 mmol) dissolved in methanol (14 mL) was added to the reaction flask followed by the addition of 10% HCl in methanol (1.75 mL). H$_2$ gas was then bubbled slowly into reaction mixture for 12 h at 30 °C. The reaction mixture was then filtered through a celite pad and the filtrate was evaporated to get compound 21 as its hydrochloride salt which was then basified with triethylamine until pH 12.0. Further purification of the product by column chromatography with CH$_3$CN-NH$_4$OH (1:1) afforded 21 in 90% (0.149 g) as a low melting white solid; \([\alpha]^{28}_D +5.6 \text{ (c 0.90, } \text{ H$_2$O)}; \nu_{\text{max}} \text{ (KBr)/cm}^{-1}: 3423, 2924, 2854, 2362, 1742, 1626, 1403, 1248, 1176, 1083, 555, 435, 403; \delta_H \text{ (300MHz; D$_2$O): } 4.22-4.18 (2H, m), 3.92-3.9 (1H, m), 3.79-3.68 (3H, m), 3.38 (1H, dd, } J \text{ 13.5, 6.0 Hz), 3.27 (1H, dd, } J \text{ 13.5, 6.6 Hz), 3.03 (2H, q, } J \text{ 7.5Hz), 1.15 (3H, t, } J \text{ 7.5Hz); \delta_C \text{ (300 MHz; D$_2$O): } 75.3 \text{ (CH), 74.9 (CH), 62.9 (CH), 57.9 (CH$_2$), 57.1 (CH), 44.2 (CH$_2$), 44.02 (CH$_2$), 10.6 (CH$_3$); HRMS (ESI): } [\text{M+H}]^+, \text{ Found: 191.1396, C$_8$H$_{19}$N$_2$O$_3$ requires 191.1396.}

(2S,3R,4R,5S)-2-(N-Acetyl)aminomethyl-3,4-dihydroxy-5-hydroxymethyl-pyrrolidine 22:

\[
\begin{align*}
\text{HO} & \\
\text{AeHN} & \text{H} \\
\text{CH} & \text{CH} \\
\text{OH} & 
\end{align*}
\]

In flame dried 50 mL three-necked round bottom flask was taken compound 10 (1.0 g, 6.17 mmol). Acetic anhydride (0.582mL, 6.17 mmol) was added to it at 0 °C under argon atmosphere. The reaction mixture was sonicated under solvent free condition for 15 minutes at room temperature, after which the reaction was quenched with liquor ammonia until the solution reaches a pH of 9. Column purification using CH$_3$CN-NH$_4$OH (1:1) afforded compound 22 in
85% (1.07 g) yield as low melting white solid. \([\alpha]^{28}_{D} -4.3 \text{ (c 0.73, H}_{2}\text{O); } \nu_{\text{max}} \text{ (KBr)/cm}^{-1}: 3183, 2304, 1649, 1406, 1099, 618; \delta_{\text{H}} \text{ (300MHz; D}_{2}\text{O): 4.27 (1H, br s), 4.21 (1H, br s), 3.94-3.79 (4H, m), 3.54-3.52 (2H, m), 1.94 (3H, s); } \delta_{\text{C}} \text{ (300 MHz; D}_{2}\text{O): 175.2 (C), 74.7 (CH), 74.3 (CH), 63.2 (CH), 60.9 (CH), 57.5 (CH}_{2}\text{), 36.3 (CH}_{2}\text{), 21.9 (CH}_{3}\text{); } \text{HRMS (ESI): } [\text{M+1}]^{+}, \text{ Found: 205.1190, } C_{8}H_{17}N_{2}O_{4} \text{ requires 205.1188.}

**General procedure for enzyme assay and calculation of IC\(_{50}\) values:**

Our experimental design was to measure enzyme velocity at constant substrate concentration with varying concentrations of an inhibitor\(^{2}\) spectrophotometrically,\(^{3}\) by carrying out the inhibition assay of the glycosidases in the presence of the iminosugars as inhibitors utilizing the corresponding \(p\)-nitrophenyl glycosides as the substrates.

\(\alpha\)-Glucosidase type I from Baker’s yeast, \(\alpha\)-galactosidase from green coffee beans, \(\beta\)-galactosidase from *Escherichia coli* and \(p\)-nitrophenyl-\(\alpha\)-D-galactopyranoside were purchased from Sigma Chemicals Co. USA.

\(\beta\)-glucosidase from almond, \(p\)NP-\(\alpha\)-D-glucopyranoside, \(p\)NP-\(\beta\)-D-glucopyranoside and \(p\)NP-\(\beta\)-D-galactopyranoside were purchased from SRL Chemicals Ltd., India.

Glycosidase was pre-incubated with various concentrations (0.5-12 mM) of inhibitor for 30 minutes at its optimum pH and temperature. 20\(\mu\)L of 25mM \(p\)-nitrophenyl glycopyranoside (\(p\)-NPG) in 0.1M phosphate buffer was added to the reaction mixture to initiate the reaction. In the case of \(\beta\)-glucosidase acetate buffer was used. The final volume of the reaction mixture was adjusted to 1.1 mL with buffer. Control was also run in parallel without inhibitor. The reaction was then incubated at the same pH and temperature for 10 minutes and quenched by adding 1mL of 1M Na\(_2\)CO\(_3\) solution. The glycosidase activity was determined by measuring the \(p\)-nitrophenol released from \(p\)-nitrophenyl glycopyranosides at 405 nm using Shimadzu UV-Visible Spectrophotometer (Model UV-1800). IC\(_{50}\) values were determined by plotting experimental data as \(V_{i}/V_{0}\) (fractional activity) versus the inhibitor concentration \([I]\) at a constant concentration of substrate, where \(V_{i}\) and \(V_{0}\) represent the enzyme velocity (activity) in the presence and absence of inhibitor, respectively. IC\(_{50}\) values were calculated from the inhibitor concentration \([I]\) corresponding to fractional activity of 0.5. IC\(_{50}\) value was defined as the concentration of the inhibitor to inhibit 50% of enzyme activity under the assay conditions.

Each experiment was repeated thrice to get a range of IC\(_{50}\) values and the mean IC\(_{50}\) values were reported in the manuscript.

**Preparations:**

Buffer solutions: 0.1M phosphate buffer for all enzymes.

For \(\beta\)-glucosidase 0.1M acetate buffer was used.

Quencher : 1M sodium carbonate for all experiments
Enzyme solution: 0.3 mg/mL for α- and β-glucosidase
0.045 mg/mL for α-galactosidase
0.1 mg/mL for β-galactosidase

Substrate concentration: 25 mM made from buffer in all cases

Inhibitor concentration: Made from buffer in varying concentrations ranging from 0.5 mM to 15 mM

**Standardisation:** OD values were standardized in terms of amount of p-nitrophenol under given buffer condition,

1 OD = X micromoles of p-nitrophenol (pNP)

From experimental values, OD in terms of amount of p-nitrophenol were obtained as follows:

1 OD = 0.093183 micromoles of p-nitrophenol for α-glucosidase
1 OD = 0.105579 micromoles of p-nitrophenol for β-glucosidase
1 OD = 0.092232 micromoles of p-nitrophenol for α-galactosidase
1 OD = 0.079403 micromoles of p-nitrophenol for β-galactosidase

**Activity:** Enzyme activities were calculated as follows:

20 μL of Z mg/mL of Enzyme gives OD value of Y in 10 minutes of incubation period with 20μL of 25 mM Substrate

\[ \text{Activity} = \frac{Y X}{20 Z X} \]

Y is the absorbance (@405 nm) of the p-nitrophenol released at the end of the reaction.

Z is the concentration of the enzyme solution (0.3 mg/mL for α- and β-glucosidase; 0.045 mg/mL for α-galactosidase, 0.1 mg/mL for β-galactosidase).

20 μL of enzyme solution and 20 μL of substrate solution were used in each experiment.

Activity at zero concentration of inhibitor (in the absence of inhibitor) = \( V_0 \)

Activity at various concentrations of inhibitor = \( V_i \)

Fractional activity = \( \frac{V_i}{V_0} \)

**Plot:** (Concentration-response plot)

Fractional activity (\( V_i/V_0 \)) (on the Y-axis) was plotted against inhibitor concentration [I] (on the X-axis)

IC\(_{50}\) values were calculated from the inhibitor concentration [I] corresponding to fractional activity of 0.5.
Result:
Compounds 12 and 22 did not inhibit any of the enzymes up to 12 mM concentration. The inhibition studies of compounds 15, 20 and 21 are given in Table 1 below.

Table 1: Inhibition activities of compounds 15, 20 and 21

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Enzyme</th>
<th>Source</th>
<th>Condition</th>
<th>IC50 (mM)</th>
<th>Range (mM)</th>
<th>Mean IC50 (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>α-glucosidase type 1</td>
<td>Baker’s yeast</td>
<td>37 °C, 10 min, pH = 6.8</td>
<td>3.6</td>
<td>3.3-3.6</td>
<td>3.5</td>
</tr>
<tr>
<td>15</td>
<td>β-glucosidase</td>
<td>Almond</td>
<td>37 °C, 10 min, pH = 5</td>
<td>5.6</td>
<td>5.6-6.9</td>
<td>6.3</td>
</tr>
<tr>
<td>15</td>
<td>β-galactosidase</td>
<td><em>Escherichia coli</em></td>
<td>37 °C, 10 min, pH = 7.3</td>
<td>6.1</td>
<td>4.8-6.1</td>
<td>5.4</td>
</tr>
<tr>
<td>20</td>
<td>α-galactosidase</td>
<td>Green coffee beans</td>
<td>25 °C, 10 min, pH = 6.5</td>
<td>6.3</td>
<td>6.3-7.8</td>
<td>6.9</td>
</tr>
<tr>
<td>21</td>
<td>α-galactosidase</td>
<td>Green coffee beans</td>
<td>25 °C, 10 min, pH = 6.5</td>
<td>8.8</td>
<td>7.3-8.8</td>
<td>8.1</td>
</tr>
</tbody>
</table>

References:


Exchangeable with D$_2$O
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Current Data Parameters
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EXPN0  5
PROCNO  1

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TD  665.36
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NS  331
DS  14
SWH  17985.611 Hz
FIDRES  0.274450 Hz
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BG  32768
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DE  6.000000 usec
TE  300.0 K
DI  2.00000000 sec
di1  0.03000000 sec
DELTA  1.69999998 sec
T00  1

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PL1  -2.00 dB
SP01  75.4752955 MHz

---------- CHANNEL f2 ----------
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PCN02  80.00 usec
PL2  -2.00 dB
PL12  16.00 dB
PL13  20.00 dB
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F2 - Processing parameters
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SP  75.4677557 MHz
WDW  EM
SSB  0
LB  2.00 Hz
GR  0
PC  1.40
Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Current Data Parameters
NAME Oct-26-2009
EXPNO 27
PROCNO 1

F2 - Acquisition Parameters
Date 20091026
Time 13.09
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PROBHD 5 mm DUL J3C-1
PULPROG zgpp30
TD 65336
SOLVENT CDCl3
NS 803
DS 4
SWH 17985.611 Hz
FIDRES 0.274439 Hz
A0 1.821908 sec
RG 32768
DW 27.800 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.00000000 sec
DELTA 1.89999998 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
F1 8.39 usec
PL1 -2.00 dB
SF01 75.4752553 MHz

======== CHANNEL f2 ========
COPPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -2.00 dB
PLL2 16.00 dB
PLL3 20.00 dB
SF02 300.1312095 MHz

F2 - Processing parameters
F1 SF 75.4677528 MHz
WDW EM
S2b 0
LB 1.00 Hz
GB 0
FC 1.40