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Design and Synthesis of Mannose Analogues as Inhibitors of α-Mannosidase

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Abstract : A series of N-, C- and S- mannopyranosyl derivatives (**4,9-16**) have been synthesised and their inhibitory activity tested towards jackbean α-mannosidase (EC 3.2.1.24). These compounds are of mechanistic and synthetic interest in the design of new α-mannosidase inhibitors.

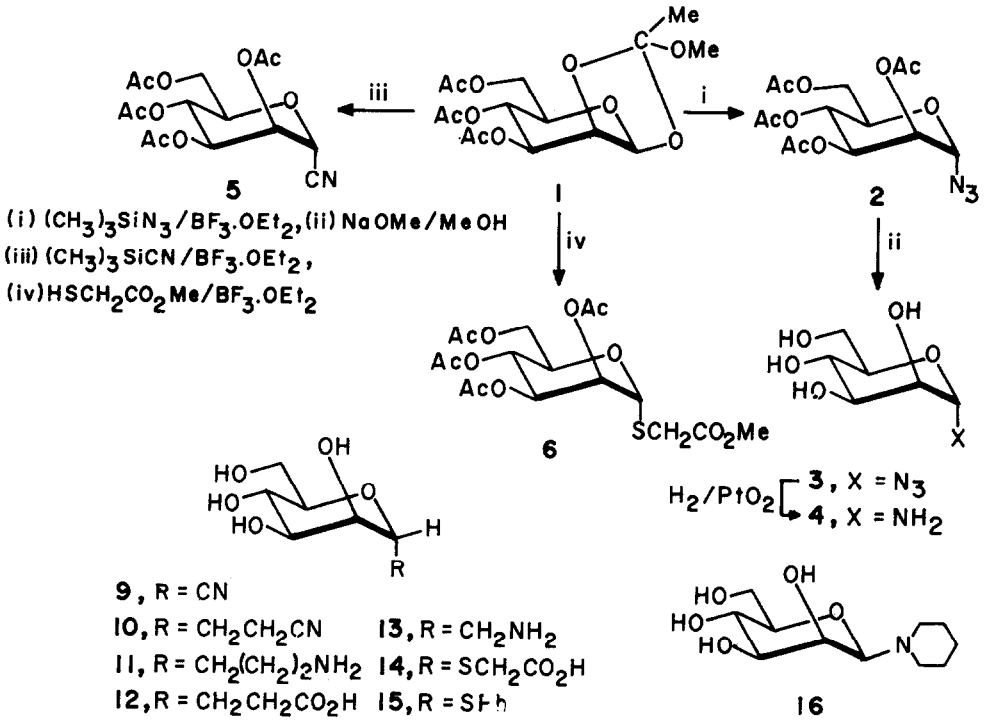
Synthesis of glycosidase inhibitors has been attempted many times[1-4] both for realising the mechanism of action of glycosidases, and for understanding the physiological role of such inhibitors found in plants[5] as well as fungi[6]. For the purpose of developing affinity chromatographic purification systems also, a ligand designed from rigorous chemical, stereochemical and biochemical considerations proves useful. Regarding the mechanism of action of glycosidases, it has been suggested that a reactive oxo-carbonium intermediate is involved in the reaction pathway[7]. The protonated inhibitors apparently mimic such carbocation intermediates in structures. However, such studies have mostly been concerned with glucosidase and galactosidase inhibitors, not much work having been done on the synthesis and kinetic studies of potential mannosidase inhibitors. We therefore designed several N-, C- and S- derivatives of mannose as possible inhibitors for α-mannosidase. The present report deals with the synthesis of such analogues and some preliminary studies in support of their role as mannosidase inhibitors.

RESULTS AND DISCUSSION

The reported synthesis of tetra-O-acetyl-α-mannopyranosyl azide[8] and cyanide[9] from tetra-O-acetyl-α-D-mannopyranosyl bromide suffers from low yield and contamination with other side products. In search of a better alternative, we decided to utilise the well known ortho-ester method[10] for 1,2-trans glycosidation. It occurred to us that the disadvantage of the method, i.e., formation of mixtures due to involvement of the alkoxy group of the ortho-ester as a nucleophile, could be avoided by using reactive nucleophiles[11] such as Me_3SiN_3 or Me_3SiCN. Also, a Lewis acid had to be used as catalyst. Thus, treatment of ortho-ester **1** with trimethylsilyl azide in the presence of $BF_3.OEt_2$

furnished (**Scheme 1**) 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl azide **2** as the sole product (95% yield). In an analogous fashion 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl cyanide **5** has been prepared by using trimethylsilyl cyanide.

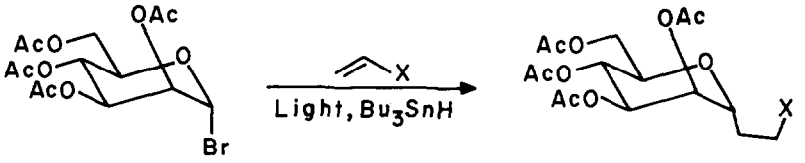
Scheme - I



Thioglycosidation has often proved unsatisfactory; in particular, excessive thioalkoxylation occasionally takes place yielding polysulphurised products. Ogawa *et al*[12] overcame this drawback by reducing the nucleophilicity of the thioalkoxylating reagent by employing thiostannanes in the presence of an equivalent amount of $SnCl_4$. However, similar attempts for thioglycosidation of either ortho-ester **1**[13] or pentaacetyl-α-D-mannopyranose using methoxycarbonylmethylthiotributyltin[14] in the presence of $SnCl_4$ failed. On the other hand, $BF_3.OEt_2$ catalysed reaction of ortho-ester **1** and methyl thioacetate afforded 2,3,4,6-tetra-O-acetyl-1-(methoxycarbonylmethyl)thio-α-D-mannopyranoside **6** in 70% yield (methyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside was also obtained in 7% yield as a side product[15]). Phenyl-α-D-thioglycoside was likewise prepared (95% yield) from pentaacetyl-α-D-mannose and thiophenol using $BF_3.OEt_2$ as a catalyst.

The C-glycosyl compound **7** was synthesised in 53% yield through radical condensation of 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide and acrylonitrile as described by Giese *et al*.[16] In an analogous fashion, the ester **8** could be prepared using methyl

acrylate and tetraacetate of α-D-mannopyranosyl bromide. Reduction of nitriles 5[17] and 7 with $LiAlH_4$ gave rise to amino alcohols 13 and 11[18] respectively.



7, X = CN
8, X = CO_2Me

The acetylated glycosides 2, 5, 6 and 7 were transesterified[20] with sodium methoxide in methanol in high yields (over 90%). Further alkaline hydrolysis (aq. NaOH) of 10 gave the acid 12.

α-D-Aminomannose 4 used in the present study was obtained as a crystalline solid from α-D-azidomannose 3 through hydrogenation in MeOH using Adams catalyst.[21]

The efficacy of the mannose analogues as inhibitors of jack-bean α-mannosidase was examined at pH 4.5 under standard assay conditions.[22] As shown in Fig.1, the enzyme loses

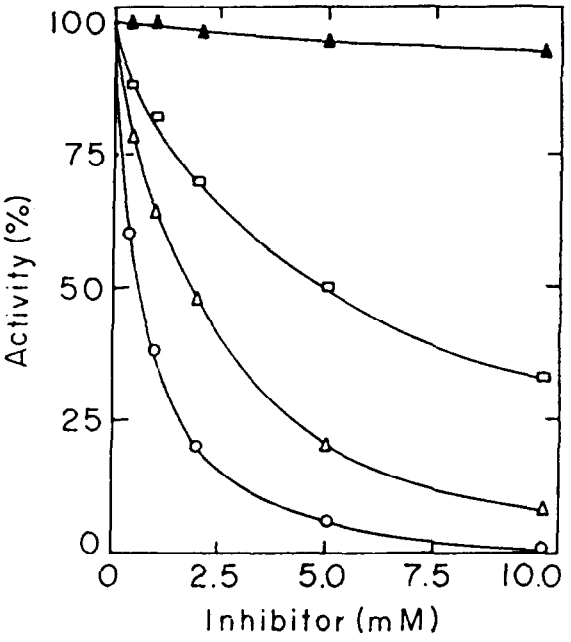


Fig.1. Effect of various mannose analogues on the activity of jack-bean α-mannosidase. (-○-) 4; (-△-) 13; (-□-) 16 and (-▲-) 15.

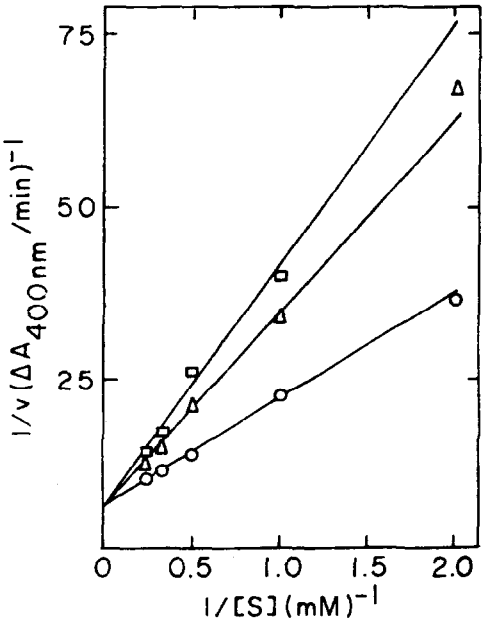


Fig.2(a). Determination of K_i for α-D-aminomannose 4. 0.5 mM (-□-); 0.3 mM 4 (-△-) and in absence of 4 (-○-).

almost 95% of its activity at about 5 mM concentration of 1-α-aminomannose **4**, whereas it retains 15% and 50% of its activity with similar concentrations of piperidinomannose **16** and 1-α-aminomethyl mannose **13** respectively. Other analogues tested, viz. **3**, **9**, **10**, **12** and **15**, have only negligible effect (data for **15** shown in **Fig.1**). Detailed kinetic analysis[23] of the three inhibitors **4**, **13** and **16** (**Figs** 2a-c) indicates that these

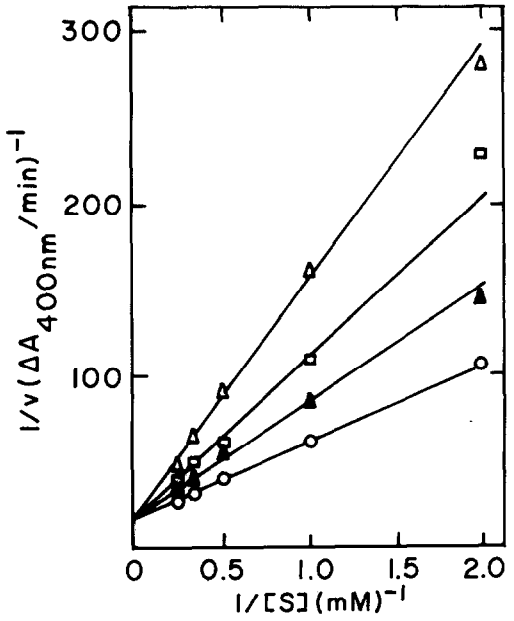


Fig.2(b). Determination of K_i for 1-α-aminomethylmannose **13**. 4.0 mM **13** (-Δ-); 2.0 mM **13** (-□-); 1.0 mM **13** (-▲-) and in absence of **13** (-○-).

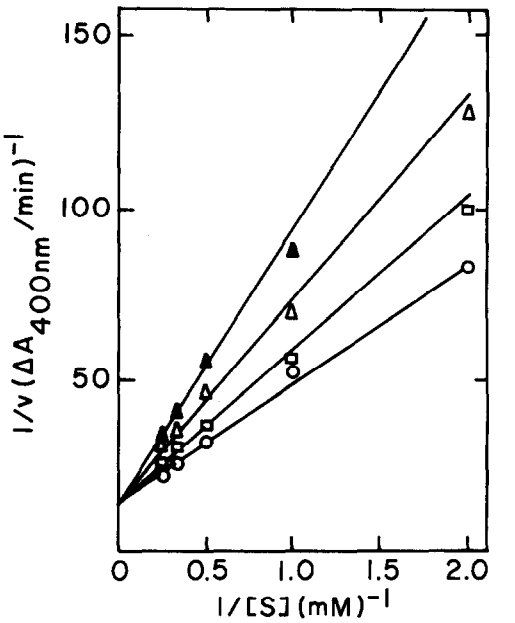


Fig.2(c). Determination of K_i for piperidinomannose **16**. 1.0 mM **16** (-▲-); 0.5 mM **16** (-Δ-); 0.25 mM **16** (-□-); and in absence of (-○-).

inhibitors are competitive in nature with K_i values of 0.3 mM, 0.65 mM and 0.7 mM respectively and this also identifies 1-α-aminomannose **4** as the most effective inhibitor.

The present findings indicate that among the various N-, S- and C- mannosides tested, only those carrying an amino group, which is capable of being protonated, show appreciable inhibitory activity. Shifting of the amino group away from the glycosidic bond decreases its inhibitory activity, perhaps due to reduction in its capability to function as a counter ion for the catalytically essential proton donating group of the enzyme.

EXPERIMENTAL

MPs were taken in open capillaries and are uncorrected. ^1H and ^{13}C NMR spectra were

taken in a JEOL FX-100 FT instrument with TMS as internal standard and chemical shifts are reported as δ values (ppm). Mass spectra were recorded on a JEOL AX-500 mass spectrometer. Optical rotations were measured in a Jasco DIP 360 polarimeter. Light petroleum refers to the fraction of b.p. 60-80°.

Jack-bean α-mannosidase (EC 3.2.1.24) was purchased from Sigma Chemical Co. U.S.A.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl azide 2 : To a stirred solution of ortho-ester 1 (3.62 g, 10 mmol) and azidotrimethylsilane (2.7 mL, 20 mmol) in CH_2Cl_2 (25 mL) was added dropwise $BF_3.OEt_2$ (1.2 mL, 10 mmol) under nitrogen during 5 minutes at 0°C. The reaction mixture was stirred for another 30 minutes at r.t., then aq. $NaHCO_3$ (10 mL) added and extracted with CH_2Cl_2 (3x25 mL). Evaporation of the solvent followed by chromatography on silica-gel (eluting with light petroleum - EtOAc; 3:1) of the resulting syrup gave the azide 2 (3.55 g, 95%) as a syrup. ^1H NMR ($CDCl_3$) 5.40 (d, 1H), 5.36-5.24 (m, 2H), 5.16 (d, 1H), 4.40-4.08 (m, 3H), 2.16 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H); ^{13}C NMR ($CDCl_3$) : 20.4 ($\underline{CH_3}COO$), 61.9 (C-6), 65.4 (C-4), 68.1 (C-2), 68.9 (C-3), 70.3 (C-5), 87.2 (C-1), 169.3, 169.5, 170.2 (CO); $[\alpha]_D^{27}$ +103° (c 1.0, $CHCl_3$) [Lit.[24] : $[\alpha]_D^{20}$+87° (c 1.0, $CHCl_3$)].

α-D-Mannopyranosyl azide 3 : Compound 2 (1.65 g, 4.4 mmol) was added to a stirred NaOMe solution prepared from 25 mg of sodium and 12 ml of dry MeOH. Complete dissolution occurred after 1 h when TLC [$CHCl_3$ -MeOH, 97:3 (v/v)] showed only the presence of nonmigrating material. The pale yellow solution was neutralised with Dowex-50 (H^+ form) ion-exchange resin and filtered; the filtrate was evaporated to yield a syrup (9.6 g). Crystallisation from ethanol afforded 3 (0.76 g, 83%), m.p. 123-124°; $[\alpha]_D^{27}$+221° (c 1.0, H_2O) [lit.[8] m.p. 120-121°, $[\alpha]_D$+223° (c 1.0, H_2O)].

α-D-Mannopyranosylamine 4 : A solution of 3 (205 mg) in MeOH (10 mL) was hydrogenated at atmospheric pressure in the presence of PtO_2 (200 mg) for 3 h. The catalyst was filtered off; crystallization of the residue from EtOAc afforded 4 (180 mg, 92%), m.p. 70-71°. Anal. calcd. for $C_6H_{13}NO_5$: C, 40.22; H, 7.32; N, 7.83. Found : C, 39.76; H, 6.70.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl cyanide 5 : To a stirred solution of ortho-ester 1 (1.8 g, 5 mmol) and trimethylsilyl cyanide (1.3 mL, 10 mmol) in nitromethane (10 mL) was added dropwise $BF_3.OEt_2$ (0.62 mL, 5 mmol) at r.t. during 10 min under nitrogen atmosphere. The mixture was stirred for 10 h at r.t., the solvent removed by evaporation and a solution of the residue in $CHCl_3$ (30 mL) was washed with chilled water (4x10 mL), dried (Na_2SO_4) and concentrated. Purification of the resulting syrup by repeated chromatography on silica gel (eluting solvent 2:1 ether : light petroleum) furnished the cyanide 5 as a colourless syrup (1.5 g). Crystallisation from EtOH afforded 5 (1.3 g, 72%), m.p. 56-57°, $[\alpha]_D^{27}$ +29.3° (c 3.4, $CHCl_3$) [lit.[9] m.p. 58-60°;

$[\alpha]_D$+28.6° (c 3.36, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$) : 5.44-5.24 (m, 3H), 4.90 (d, 1H), 4.44-4.00 (m, 3H), 2.18 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H).

Methoxycarbonylmethylthiotributyltin : A mixture of bis(tributyltin) oxide (5.96 g, 0.01 mol) and methyl thioglycolate (2.12 g, 0.02 mol) was shaken for 24 h; magnesium sulphate was added for drying and the product extracted with ether (100 mL). After removal of ether by evaporation, the product was purified by distillation; b.p. 160-165°/0.1 mm; ^{1}H NMR ($CDCl_3$) : 3.67 (s, 3H, $-COOCH_3$), 3.10 (s, 2H, $-SCH_2-$), 1.90-0.77 (m, 27H).

2,3,4,6-Tetra-O-acetyl-1-(methoxycarbonylmethyl)thio-α-mannopyranoside 6 : To a stirred solution of the ortho-ester **1** (1.8 g, 5 mmol) at 0°C, $BF_3.OEt_2$ (0.3 mL, 2.5 mmol) and methyl mercaptoacetate (0.8 g, 7.5 mmol) in CH_2Cl_2 (25 mL) were added successively under N_2 during 10 min. The mixture was stirred for another 30 min at r.t. and then diluted with CH_2Cl_2 (100 ml). The CH_2Cl_2 solution was washed with water (3x20 mL) and dried (Na_2SO_4). The crude product remaining upon evaporation of the solvent was chromatographed over silica-gel. Elution with light petroleum : ethyl acetate (4:1) afforded methyl2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside as a colourless solid (0.13 g, 7%) which after recrystallisation from ether-light petroleum had m.p. 63-64°, $[\alpha]_D^{27}$+47.6° (c 1.0, $CHCl_3$), [lit.[15] m.p. 63-64°, $[\alpha]_D$+49° (c 1.0, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$): 5.44-5.20 (m, 3H), 4.72 (d, 1H), 4.42-3.88 (m, 3H), 3.40 (s, 3H), 2.16 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H). Further elution with light petroleum : ethyl acetate (3:2) furnished **6** as a colourless syrup (1.53 g, 70%) which, after crystallisation from 3:1 (v/v) light petroleum : ether, had m.p. 59-60°, $[\alpha]_D^{27}$+116.9° (c 1.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$) : 5.54-5.14 (m, 4H), 4.46-4.0 (m, 3H), 3.74 (s, 3H, $-CO_2CH_3$), 3.36 (2xlHd, J= 16 Hz, SCH_2CO_2Me), 2.16 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 1.96 (s, 3H). Anal. Calcd. for $C_{17}H_{24}O_{11}S$: C, 46.79; H, 5.54. Found : C, 46.50; H, 5.05.

5,6,7,9-Tetra-O-acetyl-4,8-anhydro-2,3-dideoxy-D-talo-nonanonitrile 7 : A boiling solution of tetra-O-acetyl-α-D-mannopyranosyl bromide (2.45 g, 6.0 mmol), acrylonitrile (3.2 g, 60 mmol) and tributyltin hydride (2.0 g, 6.85 mmol) in dry ether (100 mL) was irradiated with tungsten lamp. After 10 h, the mixture was filtered, additional acrylonitrile (1.5 g, 28.3 mmol) and tributyltin hydride (1.0 g, 3.45 mmol) were added to the filtrate which was again irradiated for 20 h. The solution was diluted with water, dried (Na_2SO_4) and solvent evaporated. Column chromatography on silica-gel (elution using 5:1 ether-hexane) of the residue gave 0.5 g of hydrocarbon. Further elution with ether furnished the nitrile **7** (1.2 g, 53%) as a syrup which after crystallisation from light petroleum : ether (1:3) had m.p. 67-68°; $[\alpha]_D^{27}$+15.8° (c 2.15, $CHCl_3$) [lit.[16] $[\alpha]_D$+10.3° (c 4.3, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$) : 5.22-5.36 (m, 1H), 5.02-5.16 (m, 2H), 4.60 (m, 1H), 4.24-3.88 (m, 3H), 2.50 (t, 2H, J=7 Hz), 2.13-1.90 (m, 14H). Anal. Calcd. for $C_{17}H_{23}O_9N$: C, 52.98; H, 6.02; N, 3.63. Found : C, 52.80; H, 6.20; N, 3.04.

Methyl 5,6,7,9-tetra-O-acetyl-4,8-anhydro-2,3-dideoxy-D-talo-nonanoate 8 : This compound was prepared from tetra-O-acetyl- α -D-mannopyranosyl bromide (1.23 g) and methyl acrylate according to the above mentioned procedure. Column chromatography, using ether as eluant, yielded **8** as a colourless syrup (0.56g, 45%); ^{1}H NMR ($CDCl_3$) : 5.44-4.84 (m, 3H), 4.64-3.36 (m, 4H), 3.72 (s, 3H, CO_2CH_3), 2.68-1.64 (m, 16H).

α-D-Mannopyranosyl cyanide 9 : To a solution of **5** (0.357g, 1 mmol) in dry MeOH (2 ml) was added 0.5M methanolic sodium methoxide (0.3 mL). After 1 h. at r.t. the mixture was deionized by passage through a small column of methanol-washed Amberlite IR-120(H^+) resin. Evaporation of the solvent gave **9** as a syrup (1.82 g, 96%); $[\alpha]_D^{27}$+18.7° (c 2.0, MeOH); ^{13}C NMR (CD_3OD):116.9 (CN), 80.1 (C-1), 72.9, 71.7, 69.4, 67.8, 62.3 (C-6).

1-Cyano-2-(α-D-mannopyranosyl)ethane 10 : The nitrile **7** (0.667g) was dissolved in dry MeOH (5 mL) and treated with sodium (60 mg) in 20 mL MeOH for 1 h at r.t. Then Dowex 50-Wx8 (H^+) was added to remove the sodium ions; the cation-exchange resin was filtered off, the solution was evaporated and the residue crystallised from methanol-ether; yield 0.350 g (80%); m.p. 133-134°; $[\alpha]_D^{27}$+49.6° (c 1.0, MeOH); ^{1}H NMR (D_2O) : 4.12-3.44 (m, 7H), 2.60 (t, J=7 Hz, 2H), 2.40-1.68 (m, 2H). Anal. Calcd. for $C_9H_{15}O_5N$: C, 49.76; H, 6.96; N, 6.45. Found : C, 50.07; H, 7.06; N, 6.23.

1-Amino-3-(α-mannopyranosyl)propane 11 : A solution of nitrile **7** (0.562 g, 1.46 mmol) in dry THF (25 mL) was added dropwise at a rapid rate to a stirred suspension of $LiAlH_4$ (0.208 g, 5.48 mmol) in 15 mL of dry THF. The reaction mixture was boiled for 5 h under reflux, cooled and then ethanol was cautiously added to decompose the excess of hydride. Water (5 mL) was added, followed by conc. NH_4OH (15 mL) and filtered through celite (previously washed successively with water, 5N NH_4OH, and EtOH); the mixture was then stirred and filtered through a thin layer of filter celite. The solid was washed with 30 mL of 5N NH_4OH and the combined filtrate and washings were concentrated to a volume of 10 mL. The concentrated solution was passed through a column of Amberlite IR-120(H^+). The resin was washed with 100 mL of water and then rapidly eluted with 200 mL of 0.5N NH_4OH. Concentration of the eluate afforded **11** as an amorphous solid (hygroscopic) in 83% yield (0.271 g); m/z (f.a.b$^+$) : 222 (MH^+). Acetylation of **11** (100 mg) with acetic anhydride (0.6 mL) in 2 mL of pyridine furnished the pentaacetate as syrup; ^{1}H NMR ($CDCl_3$) : 6.12-5.88 (m, 1H), 5.40-5.04 (m, 3H), 4.52-3.80 (m, 4H), 3.44-3.20 (m, 2H), 2.14 (s, 3H , 2.10 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99-1.60 (m, 2H). Anal. Calcd. for $C_{19}H_{29}O_{10}N$: C, 52.89; H, 6.78; N, 3.25. Found : C, 52.72; H, 7.21; N, 3.10.

3-(α-D-Mannopyranosyl) propanoic acid 12 : A suspension of nitrile **7** (0.386 g, 1.0 mmol) in dry MeOH (5 mL) was mixed with 4M methanolic sodium methoxide (2.0 mL) and stirred for 4 h at r.t. in a stoppered r.b. flask. The resulting solution was then evaporated to to a solvent free foam which was dissolved in water (1.0 mL) and treated with 6M aq.

NaOH (0.2 mL). The solution was gently refluxed for 4 h., cooled to r.t., brought to pH 6 with Dowex 50W-x8(H^+) cation exchange resin. The mixture was filtered through celite, which was then washed extensively with water (25 mL). Evaporation of the filtrate gave a crystalline solid which on crystallisation from dry MeOH furnished **12** as solid (hygroscopic, homogeneous by TLC on silica gel G plates in the solvent system EtOAc : AcOH : Water 8:2:1), m.p. 114-116° (lit.[19] m.p. 111-115°); m/z (f.a.b$^+$) : 259 $(M+Na)^+$.

1-Amino-1-(α-mannopyranosyl)methane 13 : A solution of 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl cyanide **9** (0.714 g, 2 mmol) in THF (30 mL) was added to a stirred suspension of $LiAlH_4$ (0.475 g, 12.5 mmol) in THF (15 mL). The subsequent steps were as described for **11**. 1-Amino-1-(α-D-mannopyranosyl)methane **13** was obtained as a colourless syrup (0.3 g, 78%); m/z (f.a.b$^+$) : 194 $(MH)^+$.

1-Carboxymethyl-1-thio-α-D-mannopyranoside 14 : Deacetylation of **6** (0.436 g) as described for **12** afforded **14** (0.233 g, 92%) as a syrup, $[\alpha]_D^{27}$+393.6° (c 1.0, MeOH); m/z (f.a.b$^+$) : 277 $(MH)^+$; ^{13}C NMR (CD_3OD) : 86.0, 75.1, 77.0 (2XC), 68.6, 62.5, 32.6 (COOH signal too weak for detection).

Phenylthio-α-D-mannopyranoside 15 : $BF_3.Et_2O$ (1.9 mL, 15 mmol) was added dropwise to a stirred solution of peracetyl-α-D-mannose (3.9 g, 10 mmol) and thiophenol (1.65 g, 15 mmol) in CH_2Cl_2 (50 mL) under N_2 and the mixture was stirred at r.t. for 10 h. Usual work up followed by chromatography over silica gel (eluting with light petroleum : EtOAc, 3:2) gave 2,3,4,6-tetra-O-acetyl-α-phenylthio-D-mannopyranoside (4.27 g, 95%) as a colourless solid. Crystallisation of this material from ether : light petroleum (1:3) furnished white needles (3.6 g, 83%), m.p. 87°, $[\alpha]_D^{27}$+107.2° (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) : 7.60-7.24 (m, 5H), 5.66-5.28 (m, 4H), 4.68-4.00 (m, 3H), 2.14 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H). Deacetylation of tetraacetyl α-phenylthio D-mannopyranoside (1.32 g) with sodium methoxide in methanol as described for **9** afforded colourless crystals of **15** (0.79 g, 97%). An additional crystallization of **15** from ethanol-ether (3:1) furnished the analytical sample, m.p. 128-129°, $[\alpha]_D^{27}$+253.2° (c 3.0, EtOH) [lit.[25] $[\alpha]_D$+252.5° (c 3.0, EtOH)]; ^1H NMR (DMSO-d_6) : 7.60-7.28 (m, 5H), 5.36 (brs, 1H), 5.10 (d, J=4 Hz, 1H), 4.90-4.72 (m, 2H), 4.48 (t, J=6 Hz, 1H), 3.96-3.40 (m, 2H). Anal. Calcd. for $C_{12}H_{18}O_5S$: C, 52.94; H, 5.92. Found : C, 52.45; H, 6.12.

N-D-Mannopyranosylpiperidine 16 : Finely powdered anhydrous D-mannose (1.8 g, 10 mmol) and piperidine (1.7 g, 20 mmol) were stirred together for a few minutes until heat was evolved. The flask was then warmed on a steam-bath at 70-80° until a clear amber syrup was obtained (~ 20 min). Absolute MeOH (2.5 mL) and acetone (2.5 mL) were then added, and the solution was filtered. To the filtrate was added acetone (25 mL). After keeping for 5 days at 0°C, the separated crystals were filtered, washed with 1:4 MeOH-acetone, and dried in vacuo over $CaCl_2$. The product was recrystallised from 1:3 MeOH-acetone (40 mL), producing 1.6 g (65%) of pure white needles of **16**, m.p. 125-126°, $[\alpha]_D^{27}$ -15.0° (c 1.0, MeOH, 1 min), [lit.[26] m.p. 115-116°]; m/z (f.a.b+); 248 $(MH)^+$.

α-Mannosidase assay : The enzyme was assayed by the hydrolysis of p-nitrophenyl-α-D-mannopyranoside according to the procedure described by Li[22]. The reaction mixture contained in a total volume of 100 µl, 50 mM Na-acetate buffer, pH 4.50 and 2.0 mM substrate; the reaction was initiated by the addition of the enzyme and then incubated at 37°C for a specified period. The reaction was stopped by the addition of 0.9 mL of 0.2 M borate buffer and the liberated p-nitrophenol was measured at 400 nm. An enzyme unit is defined as the amount of enzyme required to liberate 1.00 µmole of p-nitrophenol per min under the conditions of assay.

Enzyme Inhibition Studies : α-Mannosidase (0.015 unit) was preincubated with different concentrations of the mannose analogues (**3, 4, 9, 10, 11, 12, 14** and **15**) and the enzyme activity was measured as described above. For kinetic studies, suitable amounts of the enzyme was assayed in the presence of fixed concentrations of the analogues with varying concentrations of the p-nitrophenyl-α-D-mannopyranoside. All rate measurements were done in the linear range.

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