Effect of *p*-Substitution of Aryl α-D-Mannosides on Inhibiting Mannose-Sensitive Adhesion of *Escherichia coli* – Syntheses and Testing

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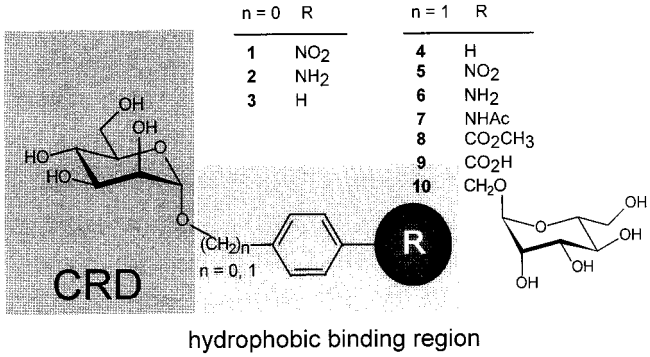
A series of *p*-substituted aryl α-D-mannosides was synthesized and tested with regard to their inhibitory capacity in the hemagglutination of guinea pig erythrocytes by type 1 fimbriated *Escherichia coli*. Synthesis of the bivalent, phenyl-linked mannoside cluster **10** was complicated by orthoester formation during the glycosylation reaction. Surprisingly, the inhibitory potency of **10** was lower than that of *p*-nitrophenyl α-D-mannoside (**1**) and this is an important finding for the further design of clustered ligands.

Microbial adhesion to host cells, occurring prior to infection is often dependent on the recognition of cell surface carbohydrates by microbial lectins[1]. In order to therapeutically manipulate these interactions, it is crucial to understand the detailed specificities of the carbohydrate recognition domains on microbial lectins.

Escherichia coli[2] is a commensal of the intestinal tract of mammals and has been recognized as an important pathogen involved in a variety of intestinal and extraintestinal diseases. *E. coli* are equipped with long filamentous appendages on their surfaces, called fimbriae or pili. Fimbriae carry lectin-like domains which enable them to bind to specific sugar epitopes on the surface of potential host cells[3]. Mannose-specific pili are called type 1 fimbriae and belong to the important virulence factors of *E. coli*[4]. Testing of their carbohydrate specificity has led to the following key results[5]. (1) The agglutinin on type 1 fimbriae is specific for α-D-mannosides, (2) several mannose-containing trisaccharides show enhanced binding potency and (3) aromatic α-mannosides are especially powerful inhibitors of hemagglutination by *E. coli*. These findings were integrated in a model about the carbohydrate recognition domain on type 1 fimbriae[6] in which the type 1 fimbrial lectin corresponds to the approximate size of a trisaccharide and an additional hydrophobic binding region is present, inside or close to the actual carbohydrate recognition domain. *p*-Nitrophenyl α-D-mannoside (**1**) became the prototype of potent aromatic mannoside ligands for type 1 fimbriae as its potency was usually not surpassed by the various oligosaccharides tested. Studies on the modification of the substituents at the phenyl moiety revealed different binding potencies in several cases[7]. This prompted us to further investigate the effect of different substituents in *p*-substituted aryl α-D-mannosides on the binding potencies of the respective mannosides towards type 1 fimbriae of *E. coli* HB 101 (pPKL4). To enable structural comparison to **1**, another *p*-R-benzyl α-D-mannosides became our target compounds (Figure 1).

Figure 1. Schematic presentation of α-mannoside binding to type 1 fimbriae: The mannose moiety is bound at the carbohydrate recognition domain (CRD); the hydrophobic part of the aromatic mannosides can improve its binding potencies by interacting with a hydrophobic binding region, present inside the CRD or close to it; the contribution of different *p*-substituents R was tested in inhibition hemagglutination tests



Our previous studies on the specificity of type 1 fimbriated *E. coli*, using multivalent mannosyl clusters[8] have shown that divalent and trivalent clusters possessed strongly enhanced binding potencies towards type 1 fimbriated *E. coli*. Thus, we were especially interested in the synthesis and testing of 1,4-bis(α-D-mannopyranosyloxymethyl)benzene (**10**), representing a divalent mannoside cluster. It could be expected to be an especially good inhibitor of *E. coli* adhesion, due to the combined effects of clustered mannose moieties and hydrophobicity of the aromatic

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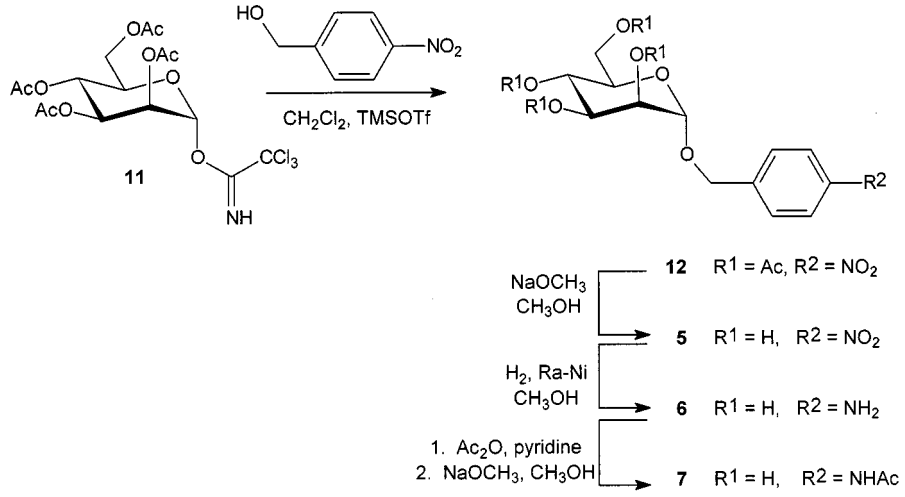
moiety. Syntheses and biological testing of the compounds listed in Figure 1 are presented in this paper.

p-Nitrobenzyl α-D-mannopyranoside (**5**) was obtained by mannosylation of *p*-nitrobenzyl alcohol under Lewis acid catalysis with the acetylated glycosyl trichloroacetimidate **11** as mannosyl donor and subsequent deacetylation of the resulting mannoside **12** in 86% overall yield (Scheme 1).

Complete saponification of the acetylated methyl ester **13** gave the free acid **9**. Diagnostic ^{1}H-NMR chemical shifts of the *m*-aryl protons in the *p*-substituted aryl mannosides **5**−**10** are listed in Table 1.

Mannosylation of the diol 4-(hydroxymethyl)benzyl alcohol to obtain the bisglycoside **10**, unexpectedly did not occur under conditions, which were effective for the synthesis of **12** and **13**. Glycosylation using the Koenigs-Knorr donor 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide

Scheme 1

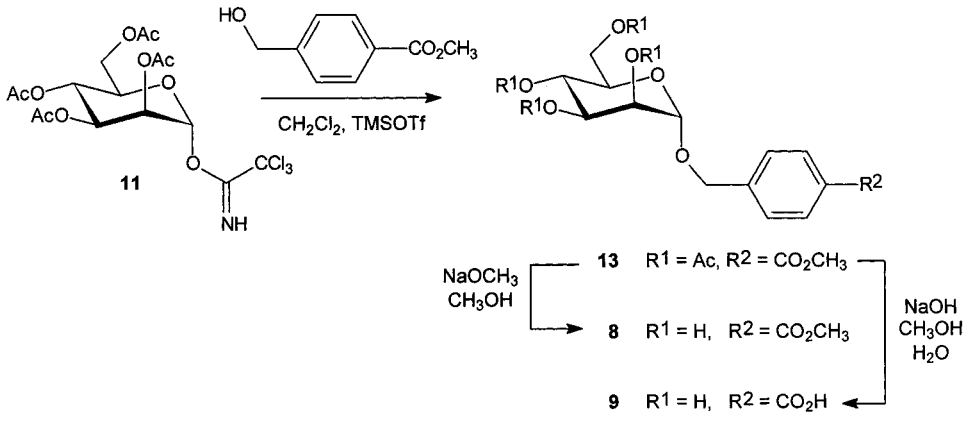


Chemoselective reduction of the nitro group in **5** to obtain **6** had to be performed without cleavage of the benzyl aglycon. This was achieved by Raney nickel catalyzed hydrogenation at short reaction times in yields around 70%. Complete acetylation of **6**, followed by Zemplen deprotection of the hydroxy groups led to the *p*-acetamidobenzyl mannoside (**7**) in 94% overall yield. In analogy to the synthesis of mannoside **12**, glycosylation of methyl 4-(hydroxymethyl)benzoate with the acetylated mannosyl trichloroacetimidate **11** gave access to the methoxycarbonyl-substituted benzyl mannoside **13**, which was carefully deacetylated by methanolic $NaOCH_3$ solution to give the target mannoside **8** while leaving the *p*-methoxycarbonyl substituent unchanged (Scheme 2).

Table 1. ^{1}H-NMR chemical shifts of the *m*-aryl protons in the *p*-substituted aryl mannosides **5**−**10**

p-Substituents	^{1}H-NMR chemical shifts of the *m*-aryl protons
NO_2 (**5**)	8.25
NH_2 (**6**)	7.15
NHAc (**7**)	7.31
CO_2CH_3 (**8**)	8.04
CO_2H (**9**)	7.83
α-mannosyloxymethyl (**10**)	7.28

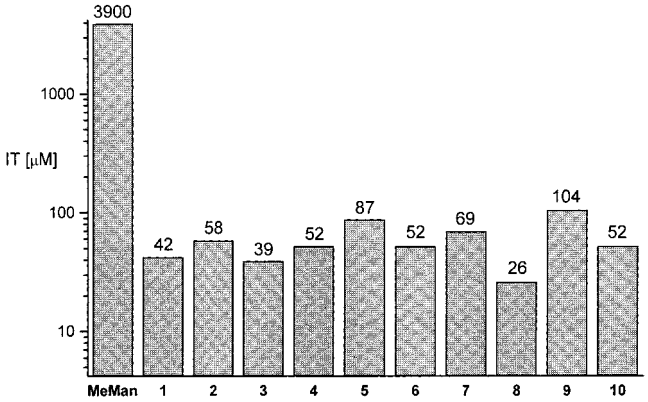
Scheme 2



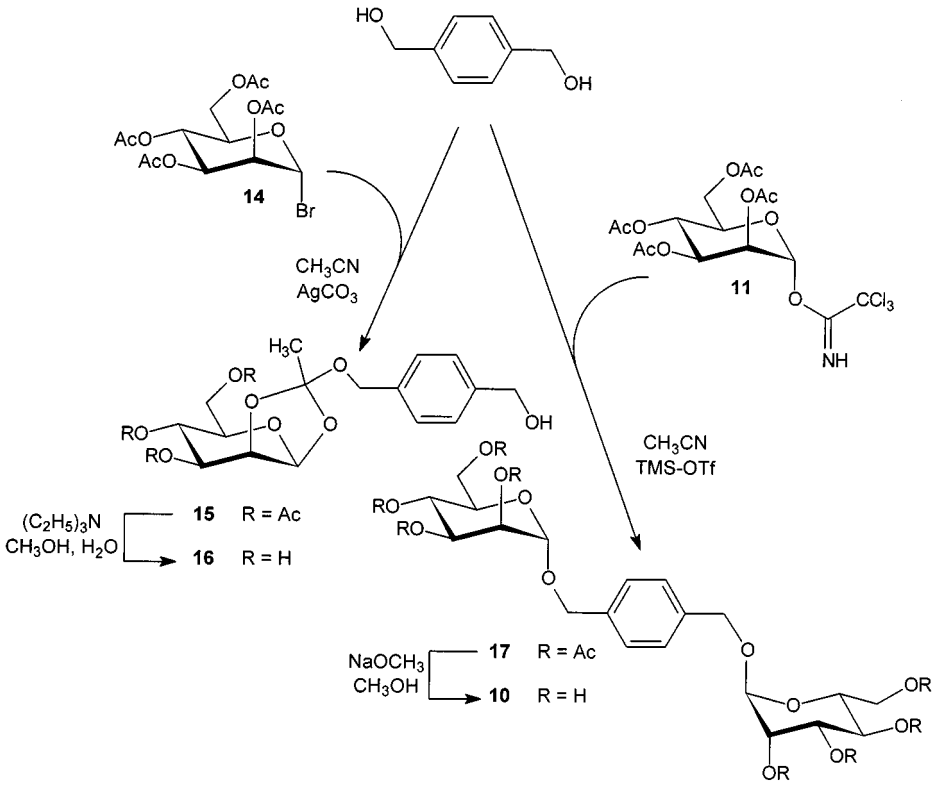
(**14**) and different silver salts as promotor led to complex mixtures of which only the 1,2-orthoacetate **15** could be isolated in its pure form. Typically, the signal of the anomeric proton of the glycosyl 1,2-orthoester **15** is shifted downfield (δ = 5.43) compared to the corresponding glycoside, whereas the signals of 2-H and 5-H are shiftet upfield (δ = 4.49 and 3.61, respectively). Monosubstitution was reflected by the unsymmetry of the ^{1}H-NMR spectrum regarding the signals for the acceptor diol and by the integration ratios of the sugar ring protons and those of the benzyl group. To further characterize the orthoester, it was deprotected with triethylamine to give **16**, which displayed the conserved orthoester methyl group at δ = 1.76. Unfortunately, all our attempts (using acidic conditions, TMSOTf catalysis or mercury salts) to isomerize the orthoacetate **15** to the corresponding glycoside failed. With benzyl-protected glycosyl donors the formation of glycosyl orthoesters naturally is excluded. However, this approach was not suitable for the synthesis of the "benzyl-coupled" bisglycoside **10** as the final debenzylating deprotection step would also cleave the glycosidic bonds. Finally, the divalent mannoside **17** was obtained in yields around 50% after employing the acetylated donor **11** in acetonitrile at 0°C or at lower temperature with the repeated addition of TMSOTf and long reaction times.. Deacetylation of **17** was easily performed to yield the unprotected target compound **10**, which could be unequivocally characterized by NMR spectroscopy.

In this assay, the inhibition titre denotes the lowest concentration of the inhibitor at which agglutination of guinea pig erythrocytes by *E. coli* is no longer visible by macroscopic inspection. As expected, the known high affinity inhibitor of type 1 fimbriae-mediated hemagglutination, *p*-nitrophenyl α-D-mannoside (**1**) had an IT of 42 μM, 80-fold lower when compared to methyl α-D-mannoside. The ITs of all other tested phenyl and benzyl mannosides are in a similar range and are shown in Figure 2.

Figure 2. Logarithmic presentation of the micromolar inhibition titres (IT) of all tested compounds, as determined by inhibition hemagglutination tests (MeMan: methyl α-D-mannopyranoside)



Scheme 3

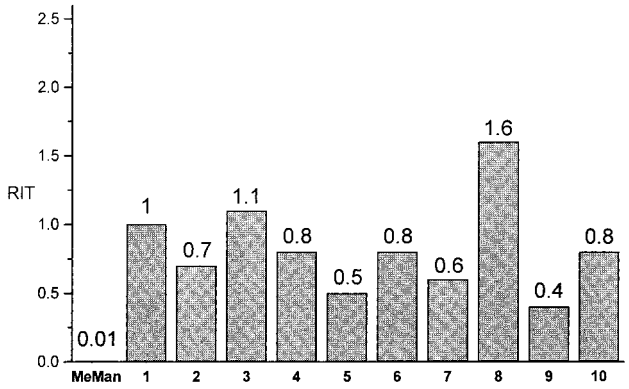


The *p*-substituted phenyl mannosides **1**–**3** and benzyl mannosides **4**–**10** were tested as inhibitors of mannose-specific adhesion in an inhibition hemagglutination test[9].

No substantial differences were observed. All tested compounds showed inhibitory concentrations 30- to 120-fold lower than methyl α-D-mannoside. The relative inhibition

titres (RIT) compared to **1** as the standard are shown in Figure 3.

Figure 3. Relative inhibition titres (RIT) of the tested compounds, based on *p*-nitrophenyl α-D-mannoside (**1**) as standard (≡ 1)



Considering the semi-quantitative character of the inhibition hemagglutination test[10], four derivatives could be identified to display relative inhibitory potencies different from that of **1** (Figure 3). The *p*-methoxycarbonyl-substituted derivative **8** was 1.6-fold more effective as inhibitor than **1** whereas *p*-nitrobenzyl mannoside **5**, *p*-acetamidobenzyl mannoside **7** and the acid **9** were found to be approximately half as potent as **1**. The aromatic bismannoside **10** showed an inhibitory binding similar to that of **1**. This was surprising as the combined effects of clustering and hydrophobicity were expected to add to better overall binding. This finding indicated that only one of the two mannose moieties was bound to the CRD of the fimbrial lectin.

From the obtained results no definite conclusions can be drawn about the influence of the *p*-substituents in aromatic mannosides on their inhibitory properties in the examined adhesion system. Only smaller effects were detected, which can not clearly be correlated to the chemical nature of the substituents R. The conformation of the bismannoside **10** obviously did not allow complete binding to the CRD of the fimbrial lectin. This implies that the type 1 fimbrial carbohydrate binding site can not accomodate a structure "sugar-aryl-sugar" as represented in **10**. This is an important finding for further design of multivalent mannoside ligands for type 1 fimbriae.

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Experimental Section

General: TLC: Thin layer chromatography was carried out on Alugram® Sil G/UV_{254} plates (Machery & Nagel) or on Kieselgel 60 F_{254} plates (Merck). Detection was performed under UV light or by spraying with 20% ethanolic sulphuric acid or ninhydrin solution (2% in water/*i*PrOH, 1:1) with subsequent heating. − Flash chromatography: Merck silica gel 60 (0.040−0.063 mm, 230−400 mesh) was used. − Optical rotations: Optical rotation values were obtained with a Perkin-Elmer polarimeter 341 or 243 (Na-D line, 589 nm, cell length 10 cm). − Melting points: Melting points were determined with a Leitz heating stage microscope and are uncorrected. − NMR: NMR spectra were recorded with a Bruker AM 400 (400 MHz for ^{1}H NMR and 100.6 MHz for ^{13}C NMR) or a DRX 500 (500 MHz for ^{1}H NMR and 125.84 MHz for ^{13}C NMR); chemical shifts are in ppm; NMR spectra were calibrated relative to internal TMS (δ = 0.00 for ^{1}H and ^{13}C NMR) or solvent peaks {$CDCl_3$: δ = 7.26 for ^{1}H NMR and 77.00 for ^{13}C NMR; [D_4]MeOH: δ = 3.35 for ^{1}H NMR and 49.30 for ^{13}C NMR; [D_6]DMSO: δ = 2.49 for ^{1}H NMR and 39.70 for ^{13}C NMR; D_2O: δ = 4.65 for ^{1}H NMR and for ^{13}C NMR [D_3]acetonitile (δ = 1.30) or [D_6]acetone (δ = 30.60) was added}. − MS: Mass spectra were measured with a VG Analytical 70-250S (FAB MS). − Bacteria: A recombinant type 1 fimbriated *E. coli* strain, *E. coli* HB 101 (pPKl4)[11], was used and cultured as described elsewhere[8]. − Inhibition hemagglutination tests with guinea pig erythrocytes were performed as described[8][9]. − Carbohydrates: *p*-Nitrophenyl α-D-mannoside (**1**) was from SENN chemicals and benzyl α-D-mannoside (**4**) from Sigma. **2** was derived from **1** by catalytic hyrogenation. The mannosyl donor **11** was prepared from D-mannose in three steps as described in the literature[12]. TMSOTf was from Fluka and used without further purification.

p-Nitrobenzyl 2,3,4,6-Tetra-O-acetyl-α-D-mannopyranoside (**12**): A solution of 245 mg ($1.60\cdot10^{-3}$ mol) of 4-nitrobenzyl alcohol and 1.02 g ($2.08\cdot10^{-3}$ mol, 1.3 equiv.) of **11** in 20 ml of dry toluene was twice concentrated with 20 ml of dry toluene and then dissolved in 50 ml of dry acetonitrile. Molecular sieves (100 mg, 4 A) and 100 µl of a TMSOTf solution (0.02 M in dichloromethane) were added and the reaction mixture was stirred at 0°C for 45 min and at room temp. for 1 h. Additional 200 µl of the TMSOTf solution were added and it was stirred at room temp. overnight. To quench the reaction, 250 µl of Et_3N was added and the mixture was concentrated to dryness. Purification by flash chromatography (light petroleum ether/ethyl acetate, 2:1) afforded 0.7 g of **12** (90%), white amorphous solid, $[\alpha]_D^{20}$ = +44.5 (*c* = 1.07, $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.24 (d, *J* = 8.7 Hz, 2 H, *m*-aryl-H), 7.53 (d, 2 H, *o*-aryl-H), 5.39 (dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.7 Hz, 1 H, 3-H), 5.33 (dd, $J_{1,2}$ = 1.5 Hz, 1 H, 2-H), 5.33 (dd ≈ t, 1 H, 4-H), 4.92 (d, 1 H, 1-H), 4.84 (d, *J* = 13.2 Hz, 1 H, benzyl-C*H*H), 4.67 (d, 1 H, benzyl-CH*H*), 4.29 (dd, $J_{5,6}$ = 5.6 Hz, $J_{6,6'}$ = 12.7 Hz, 1 H, 6-H), 4.11 (dd, $J_{5,6'}$ = 2.6 Hz, 1 H, 6′-H), 4.00 (ddd, 1 H, 5-H), 2.16, 2.11, 2.05, 2.01 [each s, each 3 H, 4 C(O)CH_3]. − ^{13}C NMR (100.67 MHz, $CDCl_3$): δ = 170.52, 169.99, 169.90, 169.63 (each s, 4 C=O), 147.71 (s, *p*-aryl-C_q), 143.54 (s, *i*-aryl-C_q), 128.12, 123.83 (each s, aryl-CH), 97.10 (s, C-1), 69.32 (s, C-4), 68.99 (s, C-5), 68.87 (s, C-3), 68.35 (s, benzyl-CH_2), 65.98 (s, C-2), 62.37 (s, C-6), 20.80, 20.70, 20.64, 20.63 [each s, 4 C(O)*C*H_3]. − $C_{21}H_{25}NO_{12}$ (483.42): calcd. C 52.2, H 5.2, N 2.9; found C 52.3, H 5.1, N 2.8.

p-Nitrobenzyl α-D-Mannopyranoside (**5**): To a solution of 424 mg ($0.88\cdot10^{-3}$ mol) of **12** in 50 ml of dry MeOH a catalytic amount of sodium was added and it was stirred at room temp. until the deprotection reaction was complete (TLC ethyl acetate/MeOH/water, 7:2:1). The solution was neutralized with ion exchange resin (DOWEX-H^+), filtered and the filtrate concentrated. Purification by flash chromatography (ethyl acetate/MeOH, 5:1) gave 263 mg of **5** (95%), white amorphous solid, $[\alpha]_D^{20}$ = +71.04 (*c* = 0.46, MeOH). − ^{1}H NMR (400 MHz, [D_4]MeOH): δ = 8.25 (d, *J* = 8.7 Hz, 2 H, *m*-aryl-H), 7.63 (d, 2 H, *o*-aryl-H), 4.93−4.88 (d, 2 H, 1-H, benzyl-C*H*H), 4.69 (d, *J* = 13.2, 1 H, benzyl-CH*H*), 3.90 (dd, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 3.6 Hz, 1 H, 2-H), 3.86 (dd, $J_{5,6}$ = 2.0 Hz, $J_{6,6'}$ = 11.7 Hz, 1 H, 6-H), 3.76 (dd, $J_{3,4}$ = 9.2 Hz, 1 H, 3-H), 3.73 (dd, $J_{5,6'}$ = 6.1 Hz, 1 H, 6′-H), 3.65 (dd ≈ t, $J_{4,5}$ = 9.7 Hz, 1 H, 4-H), 3.60 (ddd ≈ m, 1 H, 5-H). − ^{13}C NMR (100.67 MHz, [D_4]methanol): δ = 149.14, 147.21 (each s, aryl-C_q), 129.76, 124.91

(each s, aryl-CH), 101.66 (s, C-1), 75.59 (s, C-5), 73.03 (s, C-3), 72.46 (s, C-2), 69.09 (s, 4-H or benzyl-CH_2), 69.03 (s, benzyl-CH_2 or 4-H), 63.38 (s, C-6). − $C_{13}H_{17}NO_8$ (315.27): calcd. C 49.5, H 5.4, N 4.4; found C 49.4, H 5.5, N 4.3.

p-Aminobenzyl α-D-Mannopyranoside (**6**): To a solution of 170 mg ($0.54\cdot10^{-3}$ mol) of **5** in 10 ml of dry MeOH 200 mg of T1 Raney nickel (freshly prepared[13] and stored in dry ethanol) was added and the mixture was stirred under hydrogen for 30 min. The course of the reaction was carefully controlled by TLC (ethyl acetate/MeOH/water, 7:2:1) and the reduction reaction was stopped when the starting material was almost consumed and before cleavage of the glycosidic bond (mannose formation) occurred. After filtration, concentration and purification by flash chromtography (ethyl acetate/MeOH, 8:2) 118 mg of **6** (77%) was obtained, colourless syrup, R_f (ethylacetate/MeOH/water, 7:2:1) = 0.61 (**5**), 0.52 (**6**), 0.26 (mannose), $[\alpha]_D^{20}$ = +71.49 (c = 0.35, H_2O). − ^{1}H NMR (400 MHz, D_2O): δ = 7.15 (d, J = 8.14 Hz, 2 H, *m*-aryl-H), 6.75 (d, 2 H, *o*-aryl-H), 4.85 (d, $J_{1,2}$ = 1.5 Hz, 1 H, 1-H), 4.55 (d, J = 11.2 Hz, 1 H, benzyl-C*H*H), 4.36 (d, 1 H, benzyl-CH*H*) 3.80 (dd, $J_{2,3}$ = 3.6 Hz, 1 H, 2-H), 3.77 (dd, $J_{5,6}$ = 2.0 Hz, $J_{6,6'}$ = 12.2 Hz, 1 H, 6-H), 3.67 (dd, $J_{3,4}$ = 9.7 Hz, 1 H, 3-H), 3.64−3.53 (m, 3 H, 4-H, 5-H, 6′-H). − ^{13}C NMR (100.67 MHz, D_2O): δ = 149.43 (s, *i*-aryl-C_q), 146.78 (s, *p*-aryl-C_q), 130.65, 116.72 (each s, aryl-CH); 99.40 (s, C-1); 75.59 (s, C-5); 73.21, 70.97, 70.47, 67.12 (each s, C-2, C-3, C-4, C-5), 69.68 (benzyl-CH_2), 61.24 (C-6). − No elemental analysis was obtained.

p-Acetamidobenzyl α-D-Mannopyranoside (**7**): A solution of 100 mg ($0.35\cdot10^{-3}$ mol) of **6** in 5 ml of pyridine was treated with 2 ml of acetic anhydride and stirred at room temp. until the reaction was complete. The reaction mixture was repeatedly coconcentrated with toluene and the remaining syrup was dissolved in 10 ml of dry MeOH and treated with 100 µl of an NaOMe solution (1 M in MeOH) until the deprotection reation was complete (TLC: ethyl acetate/MeOH/water, 7:2:1). The solution was neutralized with ion exchange resin (DOWEX-H^+), filtered and the filtrate concentrated and purified by flash chromatography (ethyl acetate/MeOH, 8:2) to yield 105 mg of **7** (92%), colourless syrup, $[\alpha]_D^{20}$ = +89.44 (c = 0.59, H_2O). − ^{1}H NMR (400 MHz, D_2O) δ = 7.31 (d, 2 H, *m*-aryl-H), 6.75 (d, 2 H, *o*-aryl-H), 4.85 (d, $J_{1,2}$ = 1.5 Hz, 1 H, 1-H), 4.62 (d, J = 11.7 Hz, 1 H, benzyl-C*H*H), 4.43 (d, 1 H, benzyl-CH*H*) 3.84 (dd, $J_{2,3}$ = 3.6 Hz, 1 H, 2-H), 3.76 (dd ≈ d, $J_{6,6'}$ = 12.2 Hz, 1 H, 6-H), 3.69 (dd, $J_{3,4}$ = 9.7 Hz, 1 H, 3-H), 3.67−3.57 (m, 3 H, 4-H, 5-H, 6′-H). − ^{13}C NMR (100.67 MHz, D_2O): δ = 173.20 (s, NH*C*(O)CH_3), 137.31 (s, *i*-aryl-C_q), 133.96 (s, *p*-aryl-C_q), 129.71, 122.16 (each s, aryl-CH); 99.69 (s, C-1); 73.30, 70.97, 70.44, 67.09 (each s, C-2, C -3, C -4, C-5), 69.31 (s, benzyl-CH_2), 61.23 (s, C-6). − $C_{15}H_{21}NO_7$ (327.33): calcd. C 55.0, H 6.5, N 4.3; found C 55.0, H 6.5, N 4.1.

p-(Methoxycarbonyl)benzyl 2,3,4,6-Tetra-O-acetyl-α-D-mannopyranoside (**13**): A solution of 3.54 g ($7.2\cdot10^{-3}$ mol) of **11** and 929 mg ($5.52\cdot10^{-3}$ mol) of methyl 4-(hydroxymethyl)benzoate was twice concentrated with 20 ml of dry toluene. The remaining mixture was dissolved in 50 ml of dry dichloromethane, 2.5 ml of a TMSOTf solution (0.02 M in dry dichloromethane) was added and the reaction mixture was stirred at room temp. for 3 h. Then, 1 additional ml of TMSOTf (0.02 M in dry dichloromethane) was added and stirring was continued until the reaction was complete (TLC: light petroleum ether/ethyl acetate, 1:1). To stop the reaction 300 µl of Et_3N was added and the solution was concentrated to dryness and purified by flash chromatography (light petroleum ether/ethyl acetate, 1:1) to yield 2.44 g of **13** (89%), colourless syrup, $[\alpha]_D^{20}$ = +42.6 (c = 1.25, $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04 (d, J = 8.2 Hz, 2 H, *m*-aryl-H), 7.42 (d, 2 H, *o*-aryl-H), 5.39 (dd, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 10.1 Hz, 1 H, 3-H), 5.31 (dd ≈ t, $J_{4,5}$ = 10.1 Hz, 1 H, 4-H), 5.31 (dd, $J_{1,2}$ = 1.6 Hz, 1 H, 2-H), 4.90 (d, 1 H, 1-H), 4.77 (d, J = 12.6 Hz, 1 H, benzyl-C*H*H), 4.63 (d, 1 H, benzyl-CH*H*), 4.28 (dd, $J_{5,6}$ = 3.6 Hz, $J_{6,6'}$ = 12.3 Hz, 1 H, 6-H), 4.07 (dd, $J_{5,6'}$ = 2.5 Hz, 1 H, 6′-H), 4.00 (ddd, 1 H, 5-H), 3.93 (s, 3 H, CO_2CH_3), 2.15, 2.11, 2.04, 2.00 [each s, each 3 H, 4 C(O)CH_3]. − ^{13}C NMR (100.67 MHz, $CDCl_3$): δ = 170.54, 169.95, 169.85, 169.66 (each s, 4 C=O), 166.68 (s, *C*O_2CH_3), 146.04 (s, *p*-aryl-C_q), 141.30 (s, *i*-aryl-C_q), 129.85, 127.59 (each s, aryl-CH), 96.98 (s, C-1), 69.44 (s, C-2), 69.09 (benzyl-CH_2), 69.01 (s, C-5), 68.82 (s, C-3), 66.06 (s, C-4), 62.36 (s, C-6), 52.11 (s, CO_2CH$_3$), 20.78, 20.76, 20.62, 20.60 [each s, 4 C(O)CH_3]. − $C_{23}H_{28}O_{12}$ (496.46): calcd. C 55.6, H 5.7; found C 55.5, H 5.6.

p-(Methoxycarbonyl)benzyl α-D-Mannopyranoside (**8**): A solution of 490 mg ($0.987\cdot10^{-3}$ mol) of **13** in 20 ml of dry MeOH was treated with 2 ml of an NaOMe solution (1 M in MeOH) and stirred at room temp. for 20 min. Then the reaction mixture was neutralized with ion exchange resin (Levatit SP 1080 H^+), the resin was filtered off and the filtrate was concentrated. The resulting crude material was purified by flash chromatography (ethyl acetate/MeOH, 10:1) to yield 230 mg of **8** (71%), white amorphous solid, $[\alpha]_D^{20}$ = +80.0 (c = 0.45, MeOH). − ^{1}H NMR (400 MHz, [D$_4$]MeOH): δ = 8.04 (d, J = 8.14 Hz, 2 H, *m*-aryl-H), 7.52 (d, J = 8.65 Hz, 2 H, *o*-aryl-H), 4.90 (d, $J_{1,2}$ = 1.5 Hz, 1 H, 1-H), 4.87 (d, J = 12.71 Hz, 1 H, benzyl-C*H*H), 4.64 (d, 1 H, benzyl-CH*H*), 3.94 (s, 3 H, OCH_3), 3.91 (dd, $J_{2,3}$ = 3.6 Hz, 1 H, 2-H), 3.88 (dd, $J_{5,6}$ = 2.0 Hz, $J_{6,6'}$ = 11.7 Hz, 1 H, 6-H), 3.79 (dd, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.2 Hz, 1 H, 3-H), 3.75 (dd, $J_{5,6'}$ = 5.6 Hz, 1 H, 6′-H), 3.67 (dd ≈ t, $J_{4,5}$ = 9.7 Hz, 1 H, 4-H), 3.62 (ddd, 1 H, 5-H). − ^{13}C NMR (100.67 MHz, $CDCl_3$): δ = 168.67 (s, *C*O_2CH_3), 145.03 (s, aryl-C_q), 130.91 (s, *m*-aryl-C), 128.99 (s, *o*-aryl-C), 101.35 (s, C-1), 75.35 (s, C-5), 72.94 (s, C-3), 72.42 (s, C-2), 69.51 (s, benzyl-CH_2), 68.95 (s, C-4), 63.26 (s, C-6), 52.89 (s, OCH_3). − $C_{15}H_{20}O_8$ (328.31): calcd. C 54.9, H 6.1; found C 55.0, H 5.9.

p-Carboxybenzyl α-D-Mannopyranoside (**9**): A solution of 559 mg ($1.13\cdot10^{-3}$ mol) of **13** in 25 ml of MeOH/water (3:1) and 5 ml of aqueous NaOH (10%) was strirred at room temp. for 3 h. Then the reaction mixture was neutralized with ion exchange resin (Levatit SP 1080 H^+), the resin was filtered off and the filtrate was concentrated. The resulting crude material was purified by size exclusion chromatography on Sephadex G-15 (2 × 90 cm column, distilled H_2O as eluent) to yield 333 mg of **9** (94%), white lyophilisate, $[\alpha]_D^{20}$ = +52.1 (c = 1.13, DMSO). − ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.83 (d, 2 H, J = 7.63 Hz, *m*-aryl-H), 7.23 (d, 2 H, J = 8.12 Hz, *o*-aryl-H), 4.68 (d, 1 H, $J_{1,2}$ = 1.5 Hz, 1-H), 4.65 (d, 1 H, J = 12.21 Hz, benzyl-C*H*H), 4.40 (d, 1 H, benzyl-CH*H*), 3.67 (dd, 1 H, $J_{6,6'}$ = 12.2 Hz, 6′-H),3.64 (dd, 1 H, $J_{2,3}$ = 3.1 Hz, 2-H), 3.54−3.41 (m, 3 H, 3-H, 5-H, 6-H), 3.48 (dd ≈ t, 1 H, $J_{4,5}$ = 9.2 Hz, 4-H). − ^{13}C NMR (100.67 MHz, [D$_6$]DMSO): δ = 169.81 (s, CO_2H), 138.47 (s, aryl-C_q), 129.18 (s, *m*-aryl-C), 126.74 (s, *o*-aryl-C), 99.20 (s, C-1), 74.46, 71.23, 67.29 (each s, C-3, C-4, C-5), 70.51 (s, C-2), 67.63 (s, benzyl-CH_2), 61.50 (s, C-6). − No elemental analysis was obtained.

3,4,6-Tri-O-acetyl-1,2-O-[p-(hydroxymethyl)benzyloxyethylidene]-β-D-mannopyranose (**15**): A mixture of 330 mg ($1.2\cdot10^{-3}$ mol) of silver carbonate, 1 g of molecular sieves (3 A) and 80 mg ($0.58\cdot10^{-3}$ mol) of 4-(hydroxymethyl)benzyl alcohol in 20 ml of dry acetonitrile (20 ml) was stirred at room temp. under nitrogen and exclusion of light. Then a solution of 576 mg ($1.40\cdot10^{-3}$ mol) of **14** in dry acetonitrile (20 ml) was added dropwise and the reaction mixture was stirred at room temp. for 20 h. TLC (toluene/ethyl

acetate, 1:1) showed at least 4 product spots. The mixture was filtered through Celite, concentrated and purified by flash chromatography (toluene/ethyl acetate, 4:1) to yield 120 mg of **15** (R_f = 0.23 in toluene/ethyl acetate, 1:1; 44%), colourless crystals, m.p. 135−142°C, $[\alpha]_D^{22}$ = +4.7 (*c* = 0.55, dichloromethane). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.28 (s, 4 H, aryl-H), 5.43 (d, $J_{1,2}$ = 2.5 Hz, 1 H, 1-H), 5.23 (dd ≈ t, $J_{3,4}$ = 9.7 Hz, $J_{4,5}$ = 9.4 Hz, 1 H, 4-H), 5.05 (dd, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.7 Hz, 1 H, 3-H), 4.59 (d, 2 H, benzyl-CH_2), 4.54−4.45 (m, 3 H, benzyl-CH_2, 2-H), 4.17 (dd, $J_{5,6}$ = 5.1 Hz, $J_{6,6'}$ = 12.2 Hz, 1 H, 6-H), 4.08 (dd, $J_{5,6'}$ = 2.5 Hz, 1 H, 6′-H), 3.61 (ddd, 1 H, 5-H), 2.02, 2.00, 1.98 [each s, each 3 H, 3 C(O)CH_3], 1.75 (s, 3 H, orthoester-C(O)CH_3).

1,2-O-[p-(Hydroxymethyl)benzyloxyethylidene]-β-D-mannopyranose (**16**): A mixture of 100 mg ($0.21\cdot10^{-3}$ mol) of **15** and 3 ml of Et_3N in 10 ml of MeOH and 0.7 ml of water was stirred for 20 h. Then the mixture was coconcentrated with toluene and purified by flash chromatography (ethyl acetate/MeOH, 7:3) to yield 45 mg of **16** (62%), colourless syrup, $[\alpha]_D^{22}$ = +27.5 (*c* = 0.38, dichloromethane). − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.28 (s, 4 H, aryl-H), 5.53 (d, 1 H, $J_{1,2}$ = 2.5 Hz, 1-H), 4.68−4.60 (m, 4 H, benzyl-CH_2), 4.58 (dd, 1 H, $J_{2,3}$ = 3.8 Hz, 2-H), 3.90 (dd, 1 H, $J_{5,6}$ = 2.5, $J_{6,6'}$ = 12.0 Hz, 6-H), 3.76 (dd, 1 H, $J_{3,4}$ = 9.5 Hz, 3-H), 3.72 (dd, 1 H, $J_{5,6'}$ = 6.3 Hz, 6′-H), 3.63 (dd ≈ t, 1 H, $J_{4,5}$ = 9.5 Hz, 4-H), 3.29 (ddd, 1 H, 5-H), 1.76 (s, 3 H, orthoester-CH_3).

1,4-Bis(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyloxymethyl)benzene (**17**): A mixture of 212 mg ($0.44\cdot10^{-3}$ mol) of **11** and 20 mg ($0.14\cdot10^{-3}$ mol) of 4-(hydroxymethyl)benzyl alcohol was coconcentrated with 10 ml of dry toluene three times and then dissolved in 10 ml of dry acetonitrile under nitrogen. The reaction was started by the dropwise addition of 100 µl of a TMSOTf solution (0.02 M in dry dichloromethane) at 0°C over 10 min. The reaction mixture was stirred at 0°C for 30 min followed by stirring at room temp. for 30 min. Then additional 100 µl of a TMSOTf solution (0.02 M in dry dichloromethane) was added and the mixture was stirred for 3 d at room temp. The reaction was stopped by adding 100 µl of Et_3N, it was coconcentrated with toluene and the residue was purified by flash chromatography (light petroleum ether/ethyl acetate, 1:1) yielding 61 mg of **17** (53%), colourless syrup, $[\alpha]_D^{22}$ = +68.6 (*c* = 0.48, $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.28 (s, 4 H, aryl-H), 5.38 (dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.7 Hz, 2 H, 2 3-H), 5.33−5.28 (m, 4 H, 2 2-H, 2 4-H), 4.89 (d, $J_{1,2}$ = 1.5 Hz, 2 H, 2 1-H), 4.73 (d, 2 H, benzyl-CH_2), 4.56 (d, 2 H, benzyl-CH_2), 4.29 (dd, $J_{5,6}$ = 5.1 Hz, $J_{6,6'}$ = 12.2 Hz, 2 H, 2 6-H), 4.08 (dd, $J_{5,6'}$ = 2.6 Hz, 2 H, 2 6′-H), 4.03 (ddd, $J_{4,5}$ = 10.2 Hz, 2 H, 2 5-H), 2.15, 2.12, 2.04, 1.99 [each s, each 6 H, 8 C(O)CH_3]. − ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 170.4, 169.8, 169.7, 169.5 (each s, C=O), 136.0 (s, aryl-C_q), 128.1 (s, aryl-CH), 96.5 (s, C-1), 69.3 (s, C-4), 69.0 (s, benzyl-CH_2), 68.9 (s, C-3), 68.5 (s, C-5), 65.9 (s, C-2), 62.2 (s, C-6), 20.7, 20.6, 20.49, 20.47 [each s, C(O)CH_3]. − FAB-MS; *m/z*: 799.5 [M + H]$^+$&csdilla; 821.5 [M + Na]$^+$.

1,4-Bis(α-D-mannopyranosyloxymethyl)benzene (**10**): A solution of 42 mg ($0.053\cdot10^{-3}$ mol) of **17** in 10 ml of dry MeOH (10 ml) was treated with 150 µl of an NaOMe solution (1 M in MeOH) and stirred at room temp. until the reaction was complete (TLC: ethyl acetate/MeOH/water, 7:2:1). The mixture was neutralized with ion exchange resin (Levatit SP 1080 H^+, Merck), filtered and concentrated in vacuo. The residue was purified by size exclusion chromatography on Sephadex LH-20 (1.5 × 90 cm column, MeOH as eluent) to yield 22 mg of **10** (90%), colourless syrup, $[\alpha]_D^{21}$ = +99.5 (*c* = 1.0, MeOH). − ^{1}H NMR (400 MHz, $[D_4]$MeOH): δ = 7.28 (s, 4 H, aryl-H), 4.87 (d, $J_{1,2}$ = 1.5 Hz, 2 H, 2 1-H), 4.79 (d, 2 H, benzyl-CH_2), 4.57 (d, 2 H, benzyl-CH_2), 3.89 (dd, $J_{5,6}$ = 2.0, $J_{6,6'}$ = 12.2 Hz, 2 H, 2 6-H), 3.86 (dd, $J_{2,3}$ = 3.6 Hz, 2 H, 2 2-H), 3.76 (dd, $J_{5,6'}$ = 5.1 Hz, 2 H, 2 6′-H), 3.77 (dd, $J_{2,3}$ = 3.6, $J_{3,4}$ = 8.1 Hz, 2 H, 2 3-H), 3.67 (dd ≈ t, $J_{4,5}$ = 8.1 Hz, 2 H, 2 4-H), 3.63 (m, 2 H, 2 5-H). − ^{13}C NMR (100.6 MHz, $[D_4]$ MeOH): δ = 138.9 (s, aryl-C_q), 129.5 (s, aryl-CH), 101.0 (s, C-1), 75.2 (s, C-5), 72.9 (s, C-3), 72.5 (s, C-2), 69.9 (s, benzyl-CH_2), 68.9 (s, C-4), 63.2 (s, C-6). − FAB-MS; *m/z*: 485.3 [M + Na]$^+$.

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