SYNTHESIS OF 4-NITROPHENYL 2-ACETAMIDO-2-DEOXY-β-D-MANNOPYRANOSIDE AND 4-NITROPHENYL 2-ACETAMIDO-2-DEOXY-α-D-MANNOPYRANOSIDE

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The title compounds were synthesized by the selective reduction of the azido group in 4-nitrophenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside (**8**) and 4-nitrophenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-mannopyranoside (**11**), and by subsequent acetylation. Compound **8** was prepared by opening of the epoxide ring in methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-glucopyranoside (**1**) with sodium azide, followed by inversion of the configuration at C-3 in the resulting altropyranoside and glycosidation with 4-nitrophenol.
Keywords: *N*-Acetylmannosamine; β-Mannopyranosides; Aminosugars; Glycosylation; Glycosides; Glycosyl donors; Immunology; Immunostimulants.

2-Acetamido-2-deoxy-D-mannopyranose (ManNAc) is a frequently occurring glycosyl residue in a number of bacterial capsular polysaccharides and lipopolysaccharides (*e.g.*, *Haemophillus influenzae* and *Streptococcus pneumoniae*)[1–5]. In the Gram-positive bacteria, such as *Staphylococcus aureus* H, and *Bacillus subtilis*, the β-ManNAc residue is a component of the "linkage unit" attaching teichoic acids to the peptidoglycan[4]. Recently, ManNAc was identified as the strongest monosaccharidic ligand for the natural killer cell activating protein NKR-P1[6], and some ManNAc-containing saccharides were found to be strong immunostimulants[7]. The β-ManNAc units have potent implication in the virulence and pathogenity of some bacteria (*e.g.*, *S. pneumoniae* 19F and 19A)[4,5]. It was shown that the presence of mannos-

amine derivatives can cause modification of the cell wall synthesis and some changes in mucin production[8–10].

Despite its physiological importance, reports on the preparation of the structures containing β-ManNAc residues appeared quite recently – as reviewed by Gridley and Osborn[1] in 2000. It is universally accepted that preparation of the β-mannopyranosyl (β-Man), and particularly, of the β-ManNAc glycosidic linkages still remains one of the greatest challenges to carbohydrate chemists.

Our primary goal was to synthesize 4-nitrophenyl 2-acetamido-2-deoxy-β-D-mannopyranoside (**15**) as a chromogenic substrate for a screening of the hitherto unknown β-*N*-acetylmannosaminidase activity. Furthermore, our previous studies[6,7] showed that the β-ManNAc moiety, especially when attached to an aromatic system, should have strong affinity to activation receptor (NKR-P1) of the natural killer cells[6] and thus activate them against malignant tumours (*e.g.*, colon carcinoma, melanoma). This requirement should be perfectly met by compound **15**. Indeed, *in vitro* tests have shown that 4-nitrophenyl 2-acetamido-2-deoxy-β-D-mannopyranoside (**15**) is 10 times more potent than 4-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside, so far the strongest NK-cell monosaccharidic activator. Moreover, the nitro group is suitable for the construction of multivalent glycomimetics[11]. To our knowledge, compound **15** has not been prepared yet.

We also investigated some alternatives to the single described method[12] of preparation of the corresponding α-anomer **14** from rather expensive starting material 2-acetamido-2-deoxy-D-mannopyranose.

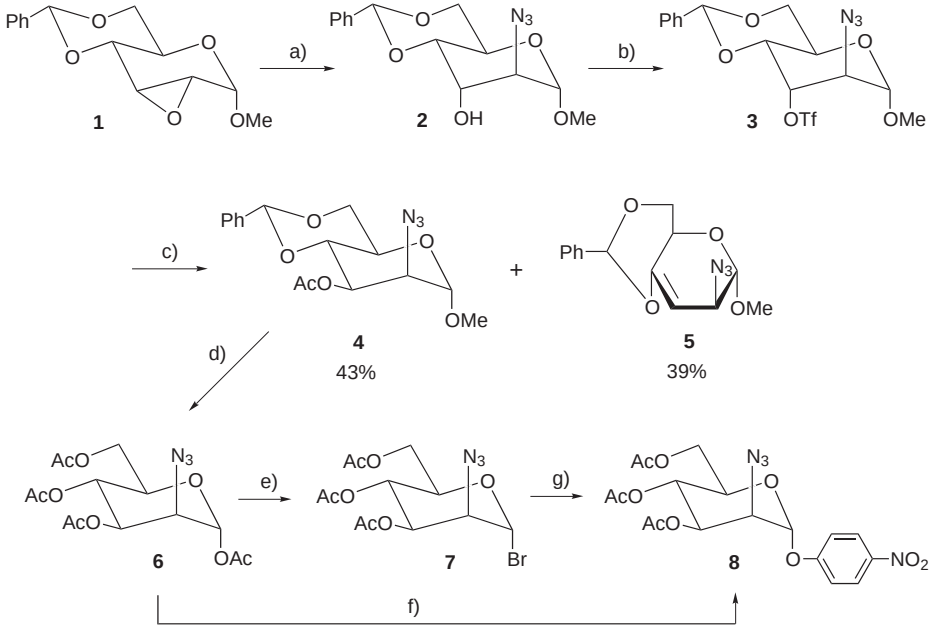
This paper describes new methods for the synthesis of both α and β 4-nitrophenyl 2-acetamido-2-deoxy-D-mannopyranosides (**14** and **15**).

RESULTS AND DISCUSSION

*Synthesis of 4-Nitrophenyl 2-Azido-2-deoxy-α-D-mannopyranoside (**8**)*

For the synthesis of the target α-mannoside (**14**), commercial methyl α-D-glucopyranoside was used as the precursor. This compound was transformed into methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-glucopyranoside[13] (**1**). Opening of the epoxide ring in compound **1** with NaN_3 in aqueous methoxyethan-1-ol[14] gave methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside (**2**), which was converted into the 3-*O*-(trifluoromethanesulfonyl) derivative (**3**) (94%). Triflate displacement with tetrabutylammonium acetate in CH_2Cl_2 yielded 43% of methyl 3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside (**4**) *via* inversion

of the configuration at C-3, and 39% of the 2-azidohex-3-enopyranoside **5** as a result of competitive elimination facilitated by the *trans* arrangement of the 3-*O*-triflate leaving group and the adjacent *axial* hydrogen at C-4. The elimination process could be suppressed neither by changing the solvent (DMF, DMF–CH_2Cl_2, methyl ethyl ketone) nor by variation of the reaction temperature. The choice of 3-*O*-triflate as the leaving group compared to previously described 3-*O*-(*N*-imidazolylsulfonyl) group[15] gave comparable yield; however, it provided cleaner reaction mixture that enabled isolation of the hitherto unknown side product **5**, which was fully characterized. Acetolysis of **4** allowed simultaneous removal of the benzylidene acetal and the glycosidic aglycone to give 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranose (**6**) in high yield (92%). Van



a) NaN_3, NH_4Cl, 2-methoxyethanol–water, reflux[15]; b) Tf_2O, CH_2Cl_2–pyridine, 0 °C; c) tetrabutylammonium acetate, CH_2Cl_2, 0 °C; d) 5% H_2SO_4 in Ac_2O, 0 °C; e) $TiBr_4$, CH_2Cl_2–EtOAc, r.t.; f) $BF_3 \cdot Et_2O$, 4-nitrophenol, CH_2Cl_2, 0 °C; g) this reaction was done by three different methods: g-1) Ag-triflate, 4-nitrophenol, CH_2Cl_2–toluene, –20 °C; g-2) silver 4-nitrophenolate[20], acetonitrile, r.t. or g-3) Ag-silicate, 4-nitrophenol, acetonitrile, –20 °C

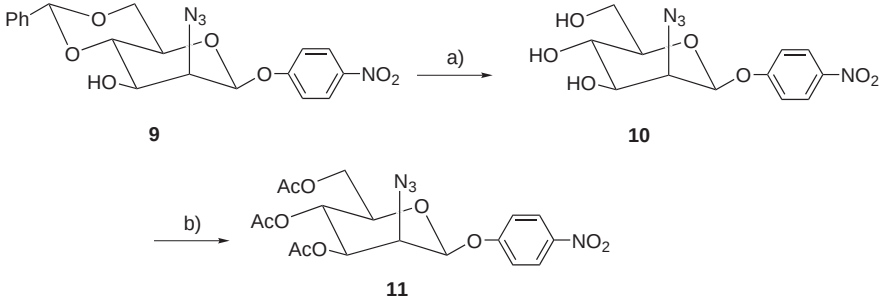
SCHEME 1

Boom *et al.*[16] described alternative synthesis of intermediate **6** in two steps from 2-deoxy-D-mannosamine hydrochloride in overall yield of 88%. However, our procedure starts from commercial (or easily preparable) methyl α-D-glucopyranoside that is more than 400 times cheaper (Sigma, Aldrich). The overall yield of our procedure is only 24%, but is compensated by a very low price of the starting material. Treatment of **6** with $TiBr_4$ in a mixture of CH_2Cl_2 and ethyl acetate[15] furnished the corresponding 1-α-bromosaccharide[17] **7** in 93% yield.

To obtain the desired glycoside **8**, the attachment of the 4-nitrophenyl glycosidic moiety to **7** was attempted using either silver 4-nitrophenolate (yield 40%); 4-nitrophenol in the presence of silver trifluoromethanesulfonate (yield 38%), or silver silicate (yield 61%)[18,19]. When 4-nitrophenol was reacted with **6** in the presence of boron trifluoride etherate, the yield of **8** was 30% [18,19] (Scheme 1). The yields were rather low and, in contrast to the literature data[17] reporting the formation of β-anomers or anomeric mixtures by the above processes, we were able to identify solely α-glycoside **8**.

*4-Nitrophenyl 2-Azido-2-deoxy-*β*-D-mannopyranoside (**11**)*

The introduction of the azido function and inversion of the configuration at C-2 in a suitably protected β-glucopyranoside is a feasible and inexpensive method for obtaining 4-nitrophenyl 2-azido-2-deoxy-β-D-mannopyranoside derivatives. Thus, using the Garegg's procedure[20], activation of the C-2 hydroxy group of 4-nitrophenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside with triflic anhydride, subsequent SN2 displacement of the resulting 2-*O*-triflate ester with NaN_3 and deprotection of the C-3 hydroxyl group afforded 4-nitrophenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranoside (**9**). Mild acid hydrolysis of the acetal protecting



a) 70% AcOH, 70 °C; b) Ac_2O, py, r.t.

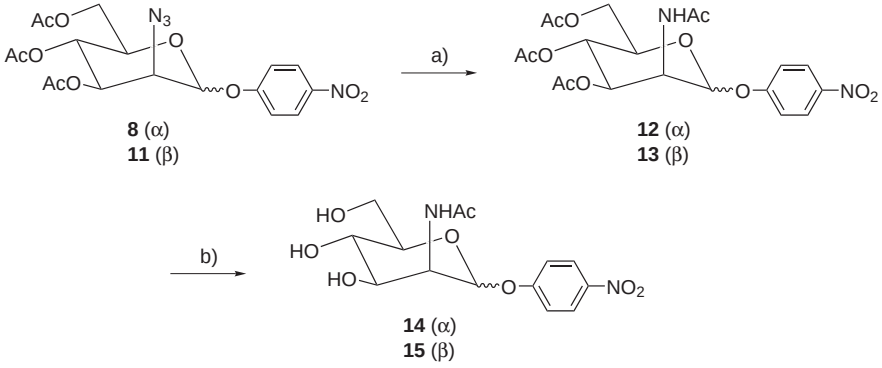
SCHEME 1

group in **9** gave **10** (82%), which was subsequently peracetylated yielding 4-nitrophenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-mannopyranoside (**11**) with a 79% overall yield (Scheme 2).

*4-Nitrophenyl 2-Acetamido-2-deoxy-α- and β-D-mannopyranoside (**14** and **15**)*

The C-2 azido group in the α- and β-glycosides **8** and **11** was chemoselectively reduced in the presence of the nitrophenyl group using triphenylphospine although alternatives (such as reduction with H_2S)[21] exist. Subsequent peracetylation (in 85% overall yield) followed by *O*-deacetylation (96%) (Scheme 3) afforded the title compounds **14** and **15**.

The couplings $J_{H\text{-}1,H\text{-}2}$ in α- and β-mannose derivatives are often very close[22] and unreliable; therefore, direct coupling $J_{C\text{-}1,H\text{-}1}$ was used to determine the anomeric configurations[23]. The respective $J_{C,H}$ extracted from coupled HMQC experiments made with **14** (178 Hz) and **15** (166 Hz) allowed unambiguous anomeric configuration assignments.



8 (α)
11 (β)

12 (α)
13 (β)

14 (α)
15 (β)

a) triphenylphosphine, CH_2Cl_2, 40 °C, H_2O, r.t. overnight; AcCl, CH_2Cl_2–pyridine, 0 °C;
b) NaOMe, MeOH, r.t.

SCHEME 3

CONCLUSIONS

Different glycosylation reactions of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-mannopyranose or 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl bromide yielded only 4-nitrophenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside. This anomer was obtained even when using silver silicate as a promoter that is known to catalyse formation of β-Man configuration[17–19]. Respective β-anomer can be obtained by indirect

route using 4-nitrophenyl β-glucopyranoside as the starting material *via* inversion of the configuration at C-2. The overall yields were not outstanding but the low price of the starting material substantiates the synthesis of the, hitherto unknown compound (**15**). Alternative, more effective syntheses were developed for the respective α-anomer (**14**). Both nitrophenyl glycosides can be used for the screening for hypothetical α- and β-*N*-acetylmannosaminidase and also for further construction of multivalent glycomimetic structures for immunological studies.

EXPERIMENTAL

General

NMR spectra were recorded on a Unity-Inova 400 MHz spectrometer (399.90 MHz for ^{1}H, 100.55 MHz for ^{13}C) in C_6D_6, $CDCl_3$, CD_3OD or D_2O at 30 °C. Chemical shifts are given in ppm (δ-scale). The assignments were based on COSY, HMQC, HMBC and differential NOE experiments performed using the manufacturer's software. Carbon chemical shifts were derived from ^{13}C NMR or HMQC and HMBC experiments. Acetone and residual solvent signals (HDO δ_H 4.508, acetone δ_C 30.5, $CDCl_3$ δ_H 7.265, δ_C 77.00, C_6D_6 δ_H 7.150, δ_C 128.00, CD_3OD δ_H 3.330, δ_C 49.30 ppm) were used as internal standards. Coupling constants (*J*) are given in Hz. Digital resolution was 0.0007 and 0.01 ppm for ^{1}H and ^{13}C, respectively. MALDI-TOF mass spectrometry: a saturated solution of α-cyano-4-hydroxycinnamic acid (Sigma) in aqueous 50% acetonitrile/0.2% TFA was used as the ionization matrix. Sample (2 μl) and matrix solution (2 μl) were premixed in a tube, 0.5 μl of the mixture was placed on the sample target and allowed to dry at ambient temperature. Positive ion MALDI mass spectra were measured on a Bruker BIFLEX reflectron time-of-flight mass spectrometer (Bruker–Franzen, Bremen, Germany) equipped with a SCOUT 26 sample inlet, a gridless delayed extraction ion source and a nitrogen laser (337 nm; Laser Science, Cambridge (MA), U.S.A.). Ion acceleration voltage was 19 kV and the reflectron voltage was set to 20 kV. Spectra were calibrated externally using the monoisotopic $[M + H]^+$ ion of α-cyano-4-hydroxycinnamic acid and a peptide standard (angiotensin II, Aldrich). Elemental analyses were performed using the Perkin Elmer 2400 II instrument. Column chromatography was performed on Silica Gel 60 (40–63 μm, 230–400 mesh, Merck). TLC was run on precoated Silica Gel 60 F_{254} aluminium sheets, detection with UV light (254 nm) and charring with 5% H_2SO_4 in EtOH.

Methyl 2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(trifluoromethanesulfonyl)-α-D-altropyranoside (**3**)

Triflic anhydride (165 μl, 0.97 mmol) in dry CH_2Cl_2 (2 ml) was added at 0 °C to a stirred solution of **2** (250 mg, 0.81 mmol)[14] in a dry CH_2Cl_2–pyridine mixture (2:1, 6 ml). The resulting mixture was kept at 0 °C for 30 min and then left to reach room temperature. After another 30 min, the reaction was quenched with water (5 ml). The organic phase was separated and the aqueous phase was extracted twice with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, evaporated to dryness, coevaporated with toluene, and purified (for spectral identification) by flash column chromatography (hexane–EtOAc, 9:1) to

yield **3** (335 mg, 94%) as white solid. ^{1}H NMR (C_6D_6): 2.784 (3 H, s, CH_3O); 3.326 (1 H, dd, *J* = 10.3, 10.4, H-6a); 3.560 (1 H, dd, *J* = 3.0, 9.8, H-4); 3.563 (1 H, dd, *J* = 0.9, 2.9, H-2); 3.951 (1 H, dd, *J* = 5.2, 10.4, H-6b); 4.079 (1 H, ddd, *J* = 5.2, 9.8, 10.3, H-5); 4.165 (1 H, m, H-1); 4.906 (1 H, dd, *J* = 2.9, 3.0, H-3); 5.169 (1 H, s, CH(O)O); 7.095 (1 H, m, H-*para*); 7.169 (2 H, m, H-*meta*); 7.544 (2 H, m, H-*ortho*). ^{13}C NMR (C_6D_6): 55.09 (q, CH_3O), 58.62 (d, C-5), 60.95 (d, C-2), 68.73 (t, C-6), 72.71 (d, C-4), 80.16 (d, C-3), 98.32 (d, C-1), 102.50 (d, CH(O)O), 119.11 (q, $J_{C,F}$ = 319.4, CF_3SO_2), 126.53 (d, 2 × C-*ortho*), 128.38 (d, 2 × C-*meta*), 129.36 (d, C-*para*). MALDI MS, *m/z*: 462.2 $[M + Na]^+$

Methyl 3-*O*-Acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside (**4**) and Methyl 2-Azido-4,6-*O*-benzylidene-2,3-dideoxy-β-D-*arabino*-hex-3-enopyranoside (**5**)

Compound **3** (without purification) was dissolved in CH_2Cl_2 (5 ml) and tetrabutylammonium acetate (370 mg, 1.2 mmol) was added at 0 °C under stirring. After 30 min the reaction mixture was diluted with CH_2Cl_2 (15 ml) and washed with aqueous 10% NaCl (3 × 5 ml), the organic phase was separated, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed (hexane–EtOAc, 85:15) to yield **4** (120 mg, 43%) as a colorless sirup and **5** (90 mg, 39%) as an amber sirup.

Compound **4**. ^{1}H NMR (C_6D_6): 1.656 (3 H, s, CH_3CO-3); 2.820 (3 H, s, CH_3O-1); 3.518 (1 H, dd, *J* = 10.3, 10.3, H-6a); 3.810 (1 H, dddd, *J* = 0.6, 4.8, 9.4, 10.3, H-5); 3.854 (1 H, dd, *J* = 1.5, 3.9, H-2); 4.024 (1 H, dd, *J* = 4.8, 10.3, H-6b); 4.092 (1 H, dd, *J* = 9.4, 10.3, H-4); 4.246 (1 H, dd, *J* = 0.6, 1.5, H-1); 5.300 (1 H, s, CH(O)O); 5.666 (1 H, dd, *J* = 3.9, 10.3, H-3); 7.070 (1 H, m, H-*para*); 7.131 (2 H, m, H-*meta*); 7.542 (2 H, m, H-*ortho*). ^{13}C NMR (C_6D_6): 19.9 (q, CH_3CO-3), 54.5 (q, CH_3O-1), 62.5 (d, C-2), 64.2 (d, C-5), 68.7 (t, C-6), 70.7 (d, C-3), 76.4 (d, C-4), 99.9 (d, C-1), 102.3 (d, CH(O)O), 126.8 (d, 2 × C-*ortho*), 128.3 (d, 2 × C-*meta*), 129.1 (d, C-*para*), 137.9 (s, C-*ipso*), 169.5 (s, CH_3CO-3). MALDI MS, *m/z*: 372.4 $[M + Na]^+$.

Compound **5**. ^{1}H NMR (C_6D_6): 2.934 (3 H, s, CH_3O-1); 3.334 (1 H, ddd, *J* = 0.9, 1.0, 5.5, H-2); 3.672 (1 H, dd, *J* = 10.1, 10.6, H-6a); 4.174 (1 H, ddd, *J* = 0.5, 6.3, 10.1, H-6b); 4.255 (1 H, dddd, *J* = 1.0, 1.1, 6.3, 10.6, H-5); 4.543 (1 H, dd, *J* = 0.9, 1.1, H-1); 5.140 (1 H, dddd, *J* = 0.5, 1.1, 1.1, 5.5, H-3); 5.453 (1 H, s, CHO(O)); 7.084–7.169 (3 H, m, H-*meta*, H-*para*); 7.488 (2 H, m, H-*ortho*). ^{13}C NMR (C_6D_6): 55.44 (q, CH_3O-1), 56.97 (d, C-2), 61.04 (d, C-5), 70.29 (t, C-6), 97.87 (d, C-3), 100.30 (d, C-1), 104.37 (d, CHO(O)), 126.76 (d, 2 × C-*ortho*), 128.38 (d, 2 × C-*meta*), 129.49 (d, C-*para*), 137.12 (s, C-*ipso*), 156.87 (s, C-4). MALDI MS, *m/z*: 311.1 $[M + Na]^+$. For $C_{14}H_{15}N_3O_4$ (289.3) calculated: 58.13% C, 5.23% H, 14.53% N; found: 58.39% C, 5.44% H, 14.41% N.

1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranose (**6**)

To a solution of **4** (600 mg, 1.7 mmol) in acetic anhydride (30 ml), 4% sulfuric acid in acetic anhydride (1 ml) was added. The reaction mixture was stirred at 0 °C for 1 h and then it was diluted with EtOAc (30 ml). The organic phase was washed with water (3 × 30 ml), saturated aqueous $NaHCO_3$ (3 × 30 ml) and saturated aqueous NaCl (20 ml), dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography (hexane–EtOAc, 7:3) to give **6** (590 mg, 92%) in form of yellowish solid. ^{1}H NMR ($CDCl_3$): 2.048 (3 H, s, CH_3CO-4); 2.087 (3 H, s, CH_3CO-6); 2.108 (3 H, s, CH_3CO-3); 2.156 (3 H, s, CH_3CO-1); 4.000 (1 H, m, H-5); 4.026 (1 H, dd, *J* = 2.0, 3.4, H-2); 4.090 (1 H, dd, *J* = 2.4, 12.5, H-6a); 4.238 (1 H, dd, *J* = 4.5, 12.5, H-6b); 5.366–5.414 (2 H, m, H-3, H-4); 6.108 (1 H, d, *J* = 2.0,

H-1). ^{13}C NMR ($CDCl_3$) 20.4 (q, CH_3CO-3), 20.5 (q, CH_3CO-4), 20.6 (q, CH_3CO-6), 20.7 (q, CH_3CO-1), 60.5 (d, C-2), 61.8 (t, C-6), 65.3 (d, C-4), 70.5 (d, C-3), 70.7 (d, C-5), 91.4 (d, C-1), 168.2 (s, CH_3CO-1), 169.4 (s, CH_3CO-4), 170.0 (s, CH_3CO-3), 170.7 (s, CH_3CO-6). MALDI MS, *m/z*: 396.2 $[M + Na]^+$.

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl Bromide (**7**)

A solution of **6** (240 mg, 0.64 mmol) and $TiBr_4$ (285 mg, 0.77 mmol) in a mixture of CH_2Cl_2 (10 ml) and EtOAc (2 ml) was stirred at room temperature for 48 h. The reaction mixture was diluted with acetonitrile (5 ml). MeONa (200 mg) was added and stirring was continued until the red colour disappeared. The mixture was filtered, evaporated and the residue was chromatographed with hexane–EtOAc (7:3) to afford **7** (235 mg, 93%) in a form of yellowish solid. ^{1}H NMR ($CDCl_3$): 2.079 (3 H, s, CH_3CO-4); 2.106 (3 H, s, CH_3CO-6); 2.120 (3 H, s, CH_3CO-3); 4.136 (1 H, dd, *J* = 2.2, 12.5, H-6a); 4.178 (1 H, dddd, *J* = 0.8, 2.2, 4.4, 10.2, H-5); 4.297 (1 H, dd, *J* = 4.4, 12.5, H-6b); 4.325 (1 H, dd, *J* = 1.5, 3.9, H-2); 5.405 (1 H, dd, *J* = 9.7, 10.2, H-4); 5.708 (1 H, dd, *J* = 3.9, 9.7, H-3); 6.369 (1 H, dd, *J* = 0.8, 1.5, H-1). ^{13}C NMR ($CDCl_3$): 20.45 (q, CH_3CO-3), 20.56 (q, CH_3CO-4), 20.62 (q, CH_3CO-6), 61.19 (t, C-6), 64.97 (d, C-2), 65.05 (d, C-4), 69.99 (d, C-3), 73.05 (d, C-5), 83.80 (d, C-1), 169.33 (s, CH_3CO-4), 169.82 (s, CH_3CO-3), 170.52 (s, CH_3CO-6). MALDI MS, *m/z*: 416.1 $[M + Na]^+$.

4-Nitrophenyl 3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside (**8**)

Method A. Compound **7** (28 mg, 0.07 mmol) was slowly added under stirring to a mixture of 4-nitrophenol (10 mg, 0.07 mmol) and silver trifluoromethanesulfonate (19 mg, 0.07 mmol) in CH_2Cl_2–toluene (1:1, 4 ml) at –30 °C. After 10 min the reaction mixture was allowed to reach the room temperature, and after another 20 min it was evaporated *in vacuo*, dissolved in CH_2Cl_2 (10 ml), washed with water (3 × 10 ml), dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed (CH_2Cl_2–EtOAc, 9:1) to yield **8** (12 mg, 38%) in form of yellowish solid.

Method B. Freshly prepared well dried silver 4-nitrophenolate[19] (75 mg, 0.30 mmol) and **7** (40 mg, 0.10 mmol) were stirred with acetonitrile (5 ml). The reaction mixture was refluxed for 10 h. Then it was filtered, evaporated, dissolved in CH_2Cl_2, washed with water (3 × 10 ml), dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography (CH_2Cl_2–EtOAc, 9:1) to yield **8** (18 mg, 40%).

Method C. A mixture of 4-nitrophenol (7.8 mg, 0.05 mmol), freshly prepared well dried Ag-silicate[18] (60 mg), and 4 Å molecular sieves (100 mg) in dry MeCN (2 ml) was stirred at room temperature for 1 h. Then **7** (20 mg, 0.05 mmol) in MeCN (2 ml) was slowly added at –30 °C. The reaction mixture was allowed to warm up to room temperature, diluted with CH_2Cl_2 (10 ml), filtered, washed twice with water (10 ml), dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed with CH_2Cl_2–EtOAc (9:1) to yield **8** (14 mg, 61%).

Method D. A solution of 4-nitrophenol (13 mg, 0.09 mmol) and **6** (34 mg, 0.09 mmol) in CH_2Cl_2 (10 ml) was stirred with 4 Å molecular sieves (100 mg) for 1 h at 0 °C. Then $BF_3{\cdot}Et_2O$ (100 µl, 0.09 mmol) was added dropwise. The reaction mixture was stirred overnight at 0 °C, filtered, washed with water (10 ml), saturated $NaHCO_3$ (2 × 10 ml), dried over anhydrous Na_2SO_4, evaporated, and the residue was purified by column chromatography (CH_2Cl_2–EtOAc, 9:1) to yield **8** (12 mg, 30%). ^{1}H NMR ($CDCl_3$): 2.046 (3 H, s, CH_3CO-6);

2.069 (3 H, s, CH_3CO-4); 2.160 (3 H, s, CH_3CO-6); 3.954 (1 H, ddd, *J* = 2.3, 5.1, 10.0, H-5); 4.073 (1 H, dd, *J* = 2.3, 12.4, H-6a); 4.234 (1 H, dd, *J* = 5.1, 12.4, H-6b); 4.300 (1 H, dd, *J* = 1.9, 3.8, H-2); 5.434 (1 H, dd, *J* = 9.9, 10.0, H-4); 5.584 (1 H, dd, *J* = 3.8, 9.9, H-3); 5.640 (1 H, d, *J* = 1.9, H-1); 7.210 (2 H, m, H-*ortho*); 8.238 (2 H, m, H-*meta*). ^{13}C NMR ($CDCl_3$): 20.4 (q, CH_3CO-3), 20.5 (q, CH_3CO-4, CH_3CO-6), 61.1 (d, C-2), 61.8 (t, C-6), 65.5 (d, C-4), 69.8 (d, C-5), 70.6 (d, C-3), 96.3 (d, C-1), 116.5 (d, 2 × C-*ortho*), 126.0 (d, 2 × C-*meta*), 143.1 (s, C-*para*), 159.9 (s, C-*ipso*), 169.6 (s, CH_3CO-4), 170.1 (s, CH_3CO-3), 170.7 (s, CH_3CO-6). $J_{C\text{-}1,H\text{-}1}$ = 178. MALDI MS, *m/z*: 475.0 $[M + Na]^+$.

4-Nitrophenyl 2-Azido-2-deoxy-β-D-mannopyranoside (**10**)

Compound **9** (250 mg, 0.6 mmol)[20] was stirred with 70% aqueous acetic acid (25 ml) and the resulting mixture was warmed to 70 °C. The reaction was completed after complete dissolution of the starting material. The reaction mixture was diluted with EtOAc (10 ml), organic phase was washed with saturated $NaHCO_3$ (3 × 10 ml) and water (10 ml), and dried over anhydrous Na_2SO_4, concentrated and the residue was purified by column chromatography using CH_2Cl_2–MeOH (9:1) to yield **10** (160 mg, 82%) as a white solid. ^{1}H NMR (CD_3OD): 3.468 (1 H, ddd, *J* = 2.3, 6.0, 9.7, H-5); 3.585 (1 H, dd, *J* = 9.2, 9.7, H-4); 3.718 (1 H, dd, *J* = 6.0, 12.2, H-6a); 3.807 (1 H, dd, *J* = 3.7, 9.2, H-3); 3.927 (1 H, dd, *J* = 2.3, 12.2, H-6b); 4.180 (1 H, dd, *J* = 1.4, 3.7, H-2); 5.554 (d, *J* = 1.4, H-1); 7.232 (2 H, m, H-*ortho*); 8.236 (2 H, m, H-*meta*). ^{13}C NMR (CD_3OD): 62.82 (t, C-6), 66.58 (d, C-2), 68.62 (d, C-4), 74.38 (d, C-3), 79.35 (d, C-5), 98.69 (d, C-1), 117.88 (d, 2 × C-*ortho*), 126.95 (d, 2 × C-*meta*), 144.41 (s, C-*para*), 163.17 (s, C-*ipso*). MALDI MS, *m/z*: 349.2 $[M + Na]^+$.

4-Nitrophenyl 3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-β-D-mannopyranoside (**11**)

Compound **10** (150 mg, 0.46 mmol) was dissolved in acetic anhydride–pyridine (1:1, 10 ml) and stirred at room temperature overnight. The reaction mixture was dissolved in CH_2Cl_2 (10 ml), washed with water (2 × 10 ml), saturated $NaHCO_3$ (3 × 15 ml) evaporated, and co-evaporated with toluene to yield **11** (200 mg, 96%) as a white solid. ^{1}H NMR ($CDCl_3$): 2.080 (3 H, s, CH_3CO-6); 2.081 (3 H, s, CH_3CO-4); 2.155 (3 H, s, CH_3CO-3); 3.844 (1 H, ddd, *J* = 2.8, 5.9, 9.5, H-5); 4.199 (1 H, dd, *J* = 2.8, 12.3, H-6a); 4.282 (1 H, dd, *J* = 5.9, 12.3, H-6b); 4.335 (1 H, dd, *J* = 1.5, 3.7, H-2); 5.123 (1 H, dd, *J* = 3.7, 9.6, H-3); 5.320 (1 H, dd, *J* = 9.5, 9.6, H-4); 5.389 (1 H, d, *J* = 1.5, H-1); 7.119 (2 H, m, H-*ortho*); 8.226 (2 H, m, H-*meta*). ^{13}C NMR ($CDCl_3$): 20.55, 20.56, 20.62 (q, CH_3CO-3, CH_3CO-4, CH_3CO-6), 61.21 (d, C-2), 62.13 (t, C-6), 65.41 (d, C-4), 71.42 (d, C-3), 73.02 (d, C-5), 96.84 (d, C-1), 116.49 (d, 2 × C-*ortho*), 125.77 (d, 2 × C-*meta*), 143.40 (s, C-*para*), 160.65 (s, C-*ipso*), 169.24 (s, CH_3CO-4), 170.04 (s, CH_3CO-3), 170.39 (s, CH_3CO-6). MALDI MS, *m/z*: 475.1 $[M + Na]^+$.

4-Nitrophenyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranoside (**12**) and 4-Nitrophenyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-mannopyranoside (**13**)

Triphenylphosphine (165 mg, 0.63 mmol) was added at a room temperature to a stirred solution of compound **8** or **11** (190 mg, 0.42 mmol) in CH_2Cl_2 (10 ml) and the reaction mixture was kept at 40 °C. After 4 h water (10 ml) was added and the stirring was continued overnight. The organic phase was separated, concentrated, and co-evaporated twice with toluene. The residue was dissolved in CH_2Cl_2–pyridine (1:1, 10 ml) and acetyl chloride (45 µl, 0.63 mmol) was added at 0 °C. After 1 h the mixture was concentrated and co-evaporated

twice with toluene. The residue obtained after evaporation was chromatographed (CH_2Cl_2–EtOAc, 2:3) to afford **12** or **13** (167 mg, 85%), both in form of yellowish solid.

Compound **12**. ^{1}H NMR ($CDCl_3$): 2.020 (3 H, s, CH_3CO-6); 2.045 (3 H, s, CH_3CO-3); 2.062 (3 H, s, CH_3CO-4); 2.101 (3 H, s, C**H**$_3$CONH-2); 4.009 (1 H, ddd, *J* = 2.8, 5.6, 10.2, H-5); 4.028 (1 H, dd, *J* = 2.8, 12.4, H-6a); 4.260 (1 H, dd, *J* = 5.6, 12.4, H-6b); 4.821 (1 H, ddd, *J* = 1.7, 4.8, 8.4, H-2); 5.201 (1 H, dd, *J* = 10.2, 10.2, H-4); 5.554 (1 H, dd, *J* = 4.8, 10.2, H-3); 5.626 (1 H, d, *J* = 1.7, H-1); 5.942 (1 H, d, *J* = 8.4, CH_3CONH-2); 7.202 (2 H, m, H-*ortho*); 8.222 (2 H, m, H-*meta*). ^{13}C NMR ($CDCl_3$): 20.57 (q, CH_3CO-4), 20.60 (q, CH_3CO-6), 20.66 (q, CH_3CO-3), 23.26 (q, CH_3CONH-2), 50.42 (d, C-2), 61.97 (t, C-6), 65.63 (d, C-4), 68.38 (d, C-3), 69.35 (d, C-5), 96.94 (d, C-1), 116.24 (d, 2 × C-*ortho*), 125.80 (d, 2 × C-*meta*), 143.21 (s, C-*para*), 160.24 (s, C-*ipso*), 169.66 (s, CH_3CO-3), 169.81 (s, CH_3CO-4), 170.22 (s, CH_3CO-6), 170.33 (s, CH_3CONH-2).

Compound **13**. ^{1}H NMR ($CDCl_3$): 2.043 (3 H, s, CH_3CO-6); 2.070 (3 H, s, CH_3CO-3); 2.101 (3 H, s, CH_3CO-4); 2.116 (3 H, s, CH_3CO-2); 3.924 (1 H, ddd, *J* = 3.4, 6.5, 8.3, H-5); 4.189 (1 H, dd, *J* = 3.4, 12.2, H-6a); 4.289 (1 H, dd, *J* = 6.5, 12.2, H-6b); 4.990 (1 H, ddd, *J* = 2.1, 3.9, 9.0, H-2); 5.107 (1 H, dd, *J* = 3.9, 9.0, H-3); 5.162 (1 H, dd, *J* = 8.3, 9.0, H-4); 5.416 (1 H, d, *J* = 2.1, H-1); 5.945 (1 H, d, *J* = 9.0, CH_3CONH-2); 7.069 (2 H, m, H-*ortho*); 8.206 (2 H, m, H-*meta*). ^{13}C NMR ($CDCl_3$): 20.59 (q, CH_3CO-6), 20.65 (q, CH_3CO-4), 20.70 (q, CH_3CO-3), 23.34 (q, CH_3CO-2), 49.39 (d, C-2), 62.33 (t, C-6), 65.80 (d, C-4), 70.72 (d, C-3), 73.03 (d, C-5), 96.22 (d, C-1), 116.49 (d, 2 × C-*ortho*), 125.75 (d, 2 × C-*meta*), 143.24 (s, C-*para*), 160.90 (s, C-*ipso*), 169.58 (s, CH_3CO-4), 170.11 (s, CH_3CO-3), 170.23 (s, CH_3CO-6), 170.60 (s, CH_3CO-2). MALDI MS, *m/z*: 491.2 $[M + Na]^+$.

4-Nitrophenyl 2-Acetamido-2-deoxy-α-D-mannopyranoside (**14**)

To a solution of **12** (50 mg, 0.11 mmol) in methanol (5 ml), sodium methoxide (2 M in methanol) was added to reach pH 10, and the mixture was stirred for 2 h until the complete conversion of **12** (TLC; CH_2Cl_2–EtOAc, 1:4). The reaction mixture was neutralized with Dowex 50W-X2 (H form), filtered and concentrated to give **14** (35 mg, 96%) as a white solid. ^{1}H NMR (D_2O): 1.880 (3 H, s, C**H**$_3$CONH-2); 3.445 (1 H, dd, *J* = 2.4, 4.6, 10.0, H-5); 3.521 (1 H, dd, *J* = 9.6, 9.8, H-4); 3.533 (1 H, dd, *J* = 2.4, 12.4, H-6a); 3.587 (1 H, dd, *J* = 4.6, 12.4, H-6b); 4.053 (1 H, dd, *J* = 4.8, 9.6, H-3); 4.386 (1 H, dd, *J* = 1.6, 4.8, H-2); 5.487 (1 H, d, *J* = 1.6, H-1); 5.942 (1 H, d, *J* = 8.4, CH_3CONH-2); 7.034 (2 H, m, H-*ortho*); 8.013 (2 H, m, H-*meta*). ^{13}C NMR (D_2O): 22.07 (q, CH_3CONH-2), 52.40 (d, C-2), 60.25 (t, C-6), 66.55 (d, C-4), 68.93 (d, C-3), 73.52 (d, C-5), 96.92 (d, C-1), 116.76 (d, 2 × C-*ortho*), 126.16 (d, 2 × C-*meta*), 142.48 (s, C-*para*), 160.84 (s, C-*ipso*), 175.06 (s, CH_3CONH-2). $[\alpha]_D^{22}$ +53 (*c* 0.69 g/mol, water). For $C_{14}H_{18}N_2O_8$ (342.3) calculated: 49.12% C, 5.30% H, 8.18% N; found: 49.33% C, 5.45% H, 8.21% N.

4-Nitrophenyl 2-Acetamido-2-deoxy-β-D-mannopyranoside (**15**)

Compound **15** was prepared from **13** (50 mg, 0.11 mmol), using an analogous procedure for compound **14**, in the yield of 35 mg (96%) as a white solid. ^{1}H NMR (D_2O): 1.925 (3 H, s, C**H**$_3$CONH-2); 3.44 (1 H, m, H-5); 3.45 (1 H, m, H-4); 3.646 (1 H, dd, *J* = 4.6, 12.4, H-6a); 3.737 (1 H, dd, *J* = 1.8, 12.4, H-6b); 3.74 (1 H, m, H-3); 4.51 (1 H, m, H-2); 5.378 (d, *J* = 1.7, H-1); 6.939 (2 H, m, H-*ortho*); 8.006 (2 H, m, H-*meta*). ^{13}C NMR (D_2O): 22.32 (q, CH_3CO-2), 53.14 (d, C-2), 60.58 (t, C-6), 66.83 (d, C-4), 71.93 (d, C-3), 77.00 (d, C-5), 96.74 (d, C-1), 116.77 (d, 2 × C-*ortho*), 126.38 (d, 2 × C-*meta*), 142.86 (s, C-*para*), 161.53 (s, C-*ipso*), 175.96

(s, CH_3CO-2). MALDI MS, *m/z*: 365.1 $[M + Na]^+$. $[\alpha]_D^{22}$ –139 (*c* 0.69 g/mol, water). For $C_{14}H_{18}N_2O_8$ (342.3) calculated: 49.12% C, 5.30% H, 8.18% N; found: 49.20% C, 5.39% H, 8.11% N.

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