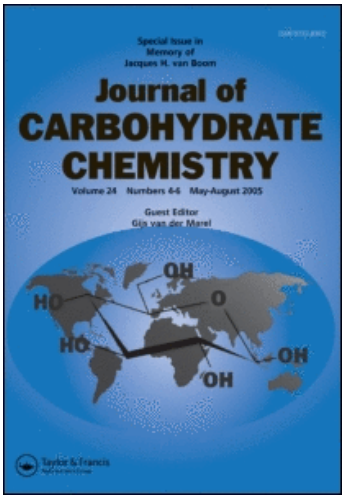

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A CONVENIENT MULTIGRAM PREPARATION OF FUNCTIONALIZED 2-AZIDO-2-DEOXY-D-MANNOSE AS A USEFUL ORTHOGONALLY PROTECTED BUILDING BLOCK FOR OLIGOSACCHARIDE SYNTHESIS

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ABSTRACT

Multigram preparation of strategically protected 2-azido-2-deoxy mannose **5** from pentaacetyl glucose **1** is described. This manno-derivative was obtained in a straightforward manner and in high overall yield and represents a flexible building-block for complex oligosaccharide synthesis.

INTRODUCTION

Mannosamine is a 2-deoxy-2-aminosugar playing different biological functions. It is an intermediate in the biosynthesis of *N*-acetyl D-neuraminic acid,[1] and it acts as inhibitor of GPI biosynthesis[2] and platelet aggregation factors in mammalian cells.[3] It is also, *inter alia*, a component of cell walls and of the capsular polysaccharides of different bacteria.[4] Due to its role in recognition phenomena, it represents a crucial building block in the preparation of natural and modified carbohydrate-based therapeutics. Complex carbohydrate synthesis often needs large amounts of strategically protected monosaccharides as intermediates. It is there-

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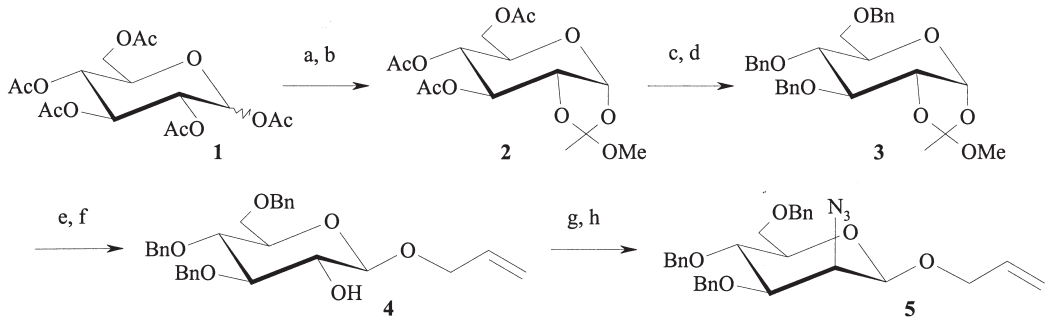
fore highly desirable to have a quick, simple and straightforward procedure to provide a properly protected mannosamine precursor in multi-gram quantities, and suitable for utilisation in the synthesis of *N*-acetyl mannosamine-containing oligosaccharides.

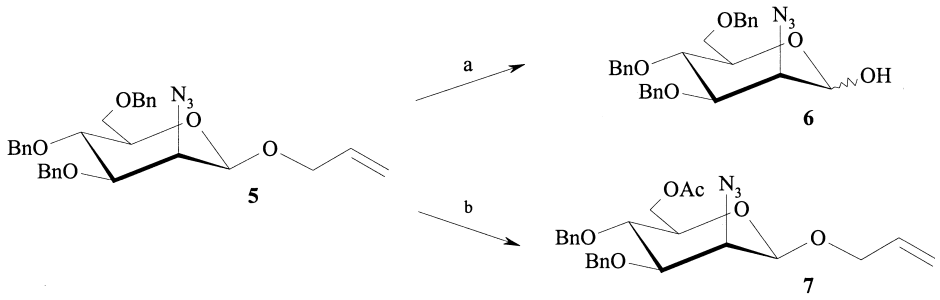
In a project aimed at synthesising analogues of bacterial capsular polysaccharides, we required a synthetic way of providing multi-gram amounts of protected mannosamine precursor **5** (Scheme 1), to be used as a building block in further steps of the synthesis. To date, only two methods have been applied to the preparation of a large amount of functionalised 2-azido-2-deoxy-D-mannosamine. Paulsen *et al.*[5b] adopted a poorly stereoselective azido nitration[5] on D-glucal, yielding a 2:1 manno:gluco mixture. Augè *et al.*[6] obtained 2-azido-2-deoxy-D-mannose in three steps from glucose, using *N,N′*-sulfuryl diimidazole as a key reagent. Nevertheless, this preparation furnishes very low overall yields due to the abundant formation of by-products in the early steps. Other procedures leading to mannosamine have also been reported, such as azido phenylselenylation[7] of D-glucal and electrophilic azidation of aldonolactones.[8] Unfortunately, they show some disadvantages such as low yields or poor stereoselectivities, use of undesired toxic reagents, or reaction conditions which make it difficult to scale-up for a multigram preparation. Finally, Ishido et al.[9] reported the nucleophilic displacement of a 2-*O*-triflate of D-glucose as a method to obtain 2-azido-2-deoxy-D-manno derivatives with high selectivity and yields. Therefore, we envisaged this reaction as the key step to prepare our target molecule on a large scale.

RESULTS

In this endeavour, we developed a very simple and efficient procedure leading in eight steps to protected mannosamine precursor **5** in 39% overall yield from 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose **1** (Scheme 1). In compound **5** the



Scheme 1. a) 33% HBr in AcOH, CH_2Cl_2, 0°C to rt, 2 h; b) *sym-collidine,*5 eq MeOH dry, 0.5 eq TBAB, 20 h, (92%o.y.); c) 0.2 eq MeONa 1M, MeOH, 14 min.; d) 9 eq BnBr, 4.5 eq NaH, DMF dry, 24 h (77%) from **2**); e) Allylic alcohol, 0.22 eq TMSOTf, 0°C , 15′; f) 0.2 eq MeONa, MeOH, 2 h (85% from **3**); g) 4 eq Py, 3 eq Tf_2O, CH_2Cl_2 dry, 0°C, 2 h; h) 3 eq $Bu_4N^+N_3^-$, dry toluene, 55–60°C, 24 h (65% from **4**).



Scheme 2. a) $HOAc/H_2O$ = 20:1 v/v, 2.4 eq NaOAc, 1.1 eq $PdCl_2$, 76%; b) Ac_2O/HOAc 2:1 v/v, 3 eq ZnCl2, **84**%.

amino group is masked by an azido group, widely used in the precursors of 2-deoxy-2-aminosugars for its poor steric hindrance and easy reconversion to an amino group. Our procedure utilises inexpensive reagents, requires little chromatographic purification and leads to the strategically protected azidomannose **5** on a multigram scale. In addition, compound **5** is a versatile building block, as it can be elongated from either its reducing or nonreducing end (Scheme 2).

1,2,3,4,6-Penta-*O*-acetyl-α-D-glucopyranose **1** was obtained by conventional *O*-acetylation of D-glucose. Compound **1** was transformed into the known orthoester **2** (10) (33% HBr in acetic acid, then *sym*-collidine and MeOH in dichloromethane) in 92% overall yield. After Zemplèn[11] deacetylation, reaction with benzyl bromide and sodium hydride yielded tribenzyl intermediate **3** (77% from **2**). The orthoester was then opened in allyl alcohol with trimethylsilyl triflate as a catalyst; subsequent 2-*O* deprotection with sodium methylate in methanol afforded allyl glycoside **4**[12] in 85% over two steps. The 2-OH was then activated as a triflate with triflic anhydride in dichloromethane, followed by nucleophilic displacement with tetrabutylammonium azide, providing target azidomannose **5** in 65% yield from **4** (39% o.y. from **1**). Compound **5** is a flexible monosaccharidic building block for oligosaccharide synthesis. In fact, it was easily deprotected at the anomeric position with $PdCl_2$ affording 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-mannopyranose (**6**, 76%), which can be employed as a glycosyl donor upon proper activation of the anomeric hydroxy group. On the other hand, chemoselective acetolysis of **5** with acetic acid and $ZnCl_2$ provided 6-*O*-acetyl azidomannose **7** (84%), allowing the elongation from the nonreducing end of the sugar.

CONCLUSIONS

In conclusion, we reported herein an easy, quick and straightforward synthetic route giving access to a strategically protected azidomannose in a multigram scale and in high overall yield. This compound is a versatile, useful tool for oligosaccharide synthesis, as demonstrated by selective deblocking of either the anomeric or the 6-*O*-position.

EXPERIMENTAL SECTION

General Methods. ^{1}H NMR spectra were recorded on Bruker AC 300 and Varian Gemini 200 spectrometers. Solvents were dried by standard procedures; allyl alcohol was dried over 4 Å m.s. Melting points were determined with a Büchi apparatus and are not corrected. Optical rotations were measured at room temperature (23°C) with a Perkin-Elmer 241 polarimeter. TLC was carried out on Merck Silica-gel 60 F_{254} plates (0.25 mm thickness), and spots were visualized by spraying with a solution containing H_2SO_4 (31 mL), ammonium molybdate (21 g) and $Ce(SO_4)_2$ (1 g) in 500 mL water, followed by heating at 110°C for 5 min Column chromatography was performed by the flash procedure using Merck Silica-gel 60 (230–400 mesh). Elemental analyses were performed using the Carlo Erba elemental analyzer 1108. Allyl alcohol was dried over m.s. 4 Å.

3,4,6-Tri-*O*-acetyl-[1,2-*O*-(1-methoxyethylidene)]-α-D-glucopyranose (2). A solution of 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose **1** (50.5 g, 129.4 mmol) in 50 mL of dichloromethane was cooled to 0°C and hydrobromic acid 33% in acetic acid (50 mL, 252 mmol) was added dropwise. The reaction was monitored by TLC (eluent hexane/EtOAc = 7:3 v/v), and after 1.5 h the mixture was neutralised with ice-cold satd $NaHCO_3$, the organic layer was washed with water (2 × 200 mL) and dried (Na_2SO_4). Removal of the solvent afforded the bromide intermediate as a white glassy solid (53 g). Crude bromide (53 g, 129 mmol) was dissolved under a nitrogen atmosphere in 70 mL of *sym*-collidine and warmed to 45°C. TBAB (20.7 g, 64.5 mmol) and dry methanol (26 mL, 645 mmol) were added. The reaction was monitored by TLC (eluent hexane/EtOAc = 6:4 v/v). After 16 h the solution was diluted with dichloromethane, the organic layer was washed with water (2 × 200 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was suspended in diethyl ether (60 mL), the excess of TBAB was filtered off, and the ethereal solution was concentrated. Chromatographic purification (eluent hexane/EtOAc = 8:2 v/v containing 1% of TEA) afforded pure compound **2** as a white syrup (43.15 g, 92%), whose spectroscopic data were in agreement with those reported in literature.[10]

3,4,6-Tri-*O*-benzyl-[1,2-*O*-(1-methoxyethylidene)]-α-D-glucopyranose (3). Compound **2** (43.15 g, 119 mmol) was dissolved under a nitrogen atmosphere in dry methanol (60 mL) and a 1M solution of CH_3ONa in dry methanol (23.8 mL) was added dropwise. The reaction was monitored by TLC (eluent hexane/EtOAc = 7:3 v/v and CH_2Cl_2/MeOH = 85:15 v/v), and after 1.5 h the mixture was concentrated affording 28 g of the orthoester intermediate as a yellow oil. The crude compound (28 g, 119 mmol) was dissolved in dry DMF (100 mL) under a nitrogen atmosphere. After the dropwise addition of BnBr (63.6 mL, 535 mmol), NaH (25.70 g, 1.071 mol) was added portionwise. The reaction was stirred at room temperature (TLC: hexane/EtOAc 7:3 v/v), and after 20 h the excess of NaH was quenched by slow addition of methanol under vigorous stirring. The reaction mixture was diluted with diethyl ether, the organic layer was washed with water

(2×100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. A quick filtration on silica gel (eluent hexane/EtOAc = 9:1 v/v containing 1% of TEA) afforded compound **3** as a yellow syrup (46.53 g, 77%), whose spectroscopic data were in agreement with those reported in literature.[11]

Allyl (3,4,6-Tri-*O*-benzyl)-β-D-glucopyranoside (4). Compound **3** (46.53 g, 92 mmol) was dissolved in 100 mL of dry allyl alcohol under a nitrogen atmosphere. The solution was cooled to 0°C, and TMSOTf (3.2 mL, 20.2 mmol) was added dropwise. The reaction was monitored by TLC (eluent hexane/EtOAc = 7:3 v/v). After 20 min the reaction gave two products, the 2-*O*-acetyl and the 2-hydroxy derivatives respectively, in a ratio of about 1:1. The solution was neutralised with TEA and diluted with dichloromethane, the organic layer was washed with water (2×100 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was dissolved in 80 mL of dry methanol and a 1M solution of CH_3ONa in methanol (18.4 mL, 18.4 mmol) was added. The reaction was stirred at room temperature (TLC: hexane/EtOAc 7:3 v/v). After 20 h the mixture was neutralised with Amberlite IR-120 (H^+ form), filtered and concentrated. The residue was purified on silica gel (eluent hexane/EtOAc = 85:15 v/v) providing compound **4** (38.3 g, 85%), whose spectroscopic data were in agreement with those reported in literature.[12]

Allyl (2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy)-β-D-mannopyranoside (5). To a solution of compound **4** (38.3 g, 78.2 mmol) in dry dichloromethane (150 mL) under a nitrogen atmosphere, pyridine (25.3 mL, 313 mmol) was added. The mixture was cooled to 0°C, and after 15 min triflic anhydride (38.7 mL, 234.6 mmol) was added dropwise. After stirring 1.5 h the reaction was diluted with dichloromethane, the organic layer was washed with HCl 5% until acidic pH, then with satd $NaHCO_3$ until neutralisation, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude 2-*O*-triflate intermediate was dissolved under a nitrogen atmosphere in dry toluene (200 mL), $Bu_4N^+N_3^-$ (55.46 mL, 195 mmol) was added quickly, and the solution was stirred at 55°C (TLC: hexane/EtOAc 7:3 v/v). After 24 h the reaction mixture was concentrated and chromatographic purification (eluent hexane/EtOAc = 9:1 v/v) afforded compound **5** as a yellow syrup (26.17 g, 65%). $[\alpha]_D^{20} = -36.8°$ (*c* 1, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$) δ7.43−7.04 (m, 15H, H_{Ar}), 5.89−5.76 (m, 1H, C*H*═CH_2), 5.28−5.22 (m, 1H, CH═C*H*H), 5.04−5.01 (m, 1H, CH═CH*H*), 4.89 (d, 1H, J = 11.1 Hz, C*H*HPh), 4.56−4.40 (m, 4H, 3/2 CH_2Ph, H-1), 4.38−4.14 (m, 4H, CH_2Ph, CH_{all}, H-2), 4.01 (t, 1H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.93−3.85 (m, 1H, CH_{all}), 3.71 (d, 2H, H-6, H-6′), 3.48 (dd, 1H, $J_{3,2} = J_{3,4} = 8.8$ Hz, H-3), 3.32 (dt, 1H, $J_{5,4} = 9.6$ Hz, $J_{5,6} = J_{5,6'} = 3.3$ Hz, H-5).

Anal. Calcd for $C_{30}H_{33}O_5N_3$ (515.61): C, 69.88; H, 6.45; N, 8.15. Found: C, 69.73; H, 6.48; N, 8.10.

2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α,β-D-mannopyranose (6). Compound **5** (26.17 g, 50.83 mmol) was dissolved in a HOAc/H_2O = 20:1 v/v mixture

(100 mL), then NaOAc (16.6 g, 122 mmol) and $PdCl_2$ (6.56 g, 56 mmol) were added. The reaction was monitored by TLC (eluent hexane/EtOAc = 7:3 v/v). After 24 h the reaction mixture was diluted with EtOAc and filtered over celite, washed with satd $NaHCO_3$ until neutralisation, dried (Na_2SO_4), filtered and concentrated. Purification by flash chromatography (eluent hexane/EtOAc 9:1 v/v) afforded pure compound **6** as a yellow syrup (18.37g, 76%) as a mixture of α/β anomers. The deprotection of the anomeric hydroxyl was ascertained by the disappearance of the allyl signals detected in ^1H NMR spectrum (multiplets at 5.8, 5.2 and 5.0 ppm).

Anal. Calcd for $C_{27}H_{29}O_5N_3$ (475.54): C, 68.19; H, 6.14; N, 8.83. Found: C, 68.31; H, 6.12; N, 8.81.

Allyl 6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranoside (7). Compound **5** (768 mg, 1.49 mmol) was dissolved in a mixture of Ac_2O/HOAc 2:1 v/v containing freshly melted $ZnCl_2$ (610 mg, 4.47 mmol). After 6 h the reaction was diluted with water and EtOAc, washed with satd $NaHCO_3$ until neutralisation, dried (Na_2SO_4), filtered and concentrated. Flash chromatography of the crude compound (eluent hexane/EtOAc = 9:1 v/v) afforded mannopyranoside **7** as a yellow syrup (585 mg, 84%). $[\alpha]_D^{20} = -13.8°$ (*c* 1, chloroform); ^1H NMR (200 MHz, $CDCl_3$) δ7.50−7.10 (m, 10H, H_{Ar}), 6.01−5.81 (m, 1H, C*H*═CH_2), 5.34−5.20 (m, 2H, CH═CH_2), 4.91 (d, 1H, J = 10.8 Hz, C*H*HPh), 4.80−4.66 (2H, AB system, CH_2Ph), 4.59 (d, 1H, J = 10.8 Hz, CH*H*Ph), 4.52 (s, 1H, H-1), 4.43−4.32 (m, 2H, H-6, H-6′), 4.28−4.19 (m, 1H, CH_{all}), 4.12−4.02 (m, 1H, CH_{all}), 3.95 (d, 1H, $J_{2,3}$ = 3.2 Hz, H-2), 3.77 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 3.67 (dd, 1H, $J_{3,4}$ = 9.2 Hz, $J_{3,2}$ = 3.2 Hz, H-3), 3.41 (ddd, 1H, $J_{5,4}$ = 9.2 Hz, $J_{5,6}$ = 2.6 Hz, $J_{5,6'}$ = 5.2 Hz, H-5), 2.09 (s, 3H, OAc).

Anal. Calcd for $C_{25}H_{29}O_6N_3$ (467.52): C, 64.23; H, 6.25; N, 8.99. Found: C, 64.14; H, 6.26; N, 9.00.

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