Acta Chemica Scandinavica B 29 (1975) 1085—1088

Synthesis of Methyl 2-*O*-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-α-D-glucopyranoside. A New Route to 2-Acetamido-2-deoxy-α-D-glucopyranosides

HANS B. BORÉN, GÖRAN EKBORG and JÖRGEN LÖNNGREN

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-104 05 Stockholm, Sweden

A new route to 2-acetamido-2-deoxy-α-D-glucopyranosides has been worked out and exemplified by the synthesis of methyl 2-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-α-D-glucopyranoside. Condensation of 3,4,6-tri-*O*-benzyl-1,2-*O*-methylorthoacetyl-β-D-mannose with methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside, followed by deacetylation, yielded a disaccharide derivative with a free hydroxyl at C-2 in the α-D-mannopyranosyl residue. Oxidation to the corresponding carbonyl derivative, oximation, stereoselective reduction with simultaneous removal of protecting groups, acetylation and *O*-deacetylation yielded the title compound.

We have recently developed a route for the synthesis of 1,2-*cis*-glycosides [1,2] which involves the preparation of the corresponding epimer with the reversed configuration at C-2, with all hydroxyls except that at C-2 suitably protected. The condensation was performed either by a Koenigs-Knorr reaction [1] or an orthoester synthesis, using a fully benzylated 1,2-*O*-methylorthoacetyl derivative.[2] Oxidation to the carbonyl compound followed by stereoselective reduction yielded the 1,2-*cis*-glycoside. The method has been used for the synthesis of β-D-mannopyranosides [1–3] and α-D-glucopyranosides.[2] Oximation of the carbonyl derivative followed by stereoselective reduction should yield a 1,2-*cis*-2-amino-2-deoxyglycoside.

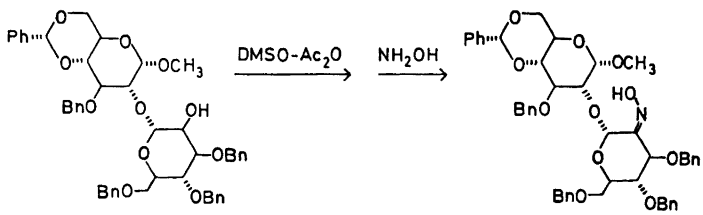
The disaccharide methyl 2-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-α-D-glucopyranoside (VII, title compound), contains the same sugars and linkages as a structural element [α-D-GlcNAc*p*-(1→2)-α-D-Glc*p*-(1→] of the common core oligosaccharide of *Salmonella* lipopolysaccharides.[4,5] The substance was needed in studies of the specificity of the bacteriophage ΦX 174 receptor [6] against this core. We therefore chose to demonstrate the new route to α-D-glucosaminides by the synthesis of this substance.

RESULTS and DISCUSSION

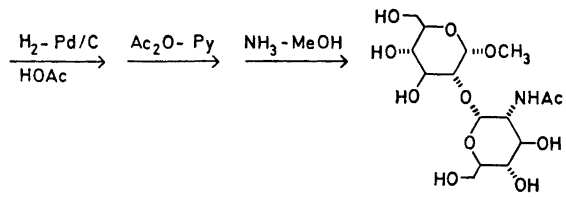
The aglycone, methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucoside (II) was prepared from methyl 4,6-*O*-benzylidene-2-*O*-*p*-toluenesulfonyl-α-D-glucoside [7] by benzylation, using benzyl bromide and silver oxide in dimethylformamide,[8,9] followed by treatment with lithium aluminium hydride.

The orthoester, 3,4,6-tri-*O*-benzyl-1,2-*O*-methylorthoacetyl-β-D-mannose was prepared as previously described,[2] and condensed with the aglycone (II) as devised by Kochetkov *et. al.*[10] Deacetylation of the product (III) to IV, and oxidation of IV to V, using dimethyl sulfoxide-acetic anhydride, was also performed as previously described.[1,2] The carbonyl derivative was not purified but transformed directly into the oxime (VI). The yield of crystalline oxime, starting from IV, was 82 %. A mixture of two stereoisomeric oximes should be formed, and the results indicate that one of these forms predominates.

The stereoselectivity on reduction of oximes analogous to VI has been investigated by Lemieux *et al.*[11] The low solubility of the oxime VI in methanol or ethanol precluded the use



IV VI



VII

of catalytic hydrogenation in these solvents and in the presence of hydrazine, a method which has shown good stereoselectivity. When tetrahydrofuran was added, in order to increase the solubility of VI, no reduction was obtained. However, hydrogenation of VI in acetic acid, using palladium as catalyst, was rapid and produced the α-D-glucosaminide in good yield. Acetylation of the reaction mixture and chromatography on silicic acid afforded the major component as a chromatographically pure syrup, identified by NMR as fully acetylated VII. *O*-Deacetylation of this product yielded VII, which was purified by gel filtration. The yield of crystalline VII, m.p. 240 – 245 °C (dec.), $[\alpha]_{578}^{23}$ + 169°, starting from the oxime (VI) was 38 %. Attempts to find a solvent from which it could be recrystallized were unsuccessful.

Acid hydrolysis of VII yielded equimolar amounts of D-glucose and D-glucosamine, analysed by GLC of their corresponding alditol acetates. No D-mannosamine was observed. That VII is an α-D-glucosaminide is evident from its high specific optical rotation and from chemical shifts, δ 5.04 and 4.94, and coupling constants, $J_{1,2}$ 3,0 Hz and 2.5 Hz, respectively, of its two anomeric protons.

Although the route described above for the synthesis of α-D-glucosaminides involves several steps, it provides a feasible alternative to the method developed by Lemieux and coworkers, starting from the dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride.[11] Our method could also be applicable for the synthesis of β-D-mannosaminides.

EXPERIMENTAL

General methods were the same as previously reported,[3] except that NMR spectra were also recorded with a Varian XL 100 instrument. For spectrum determination in D_2O, 3-trimethylsilyl-1,1,2,2,3,3-hexadeuteriopropane-sulfonic acid sodium salt was used as internal standard.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-p-toluenesulfonyl-α-D-glucoside (I). Methyl 4,6-*O*-benzylidene-2-*O*-*p*-toluenesulfonyl-α-D-glucoside [7] (65 g) was dissolved in dimethylformamide (130 ml) and benzylated with benzyl bromide (36 ml) and silver oxide (36 g) with stirring in the dark at room temperature for 20 h. Methanol (30 ml) was added and the mixture stirred for another 2 h. The mixture was filtered through kieselguhr. The filtrate was concentrated and the resulting syrup was crystallized from ethanol to give I (62.5 g; 80 %). Recrystallization from ethanol yielded the pure substance, m.p. 121.5 – 123.5 °C, $[\alpha]_{578}^{23}$ + 2° (*c* 1.1, chloroform). (Found: C 63.8; H 5.80; S 6.21. $C_{28}H_{30}O_6S$ requires: C 63.9; H 5.74; S 6.09). NMR ($CDCl_3$, 100 MHz): δ 7.70 (2 H, d, $J_{H,H}$ 9.0 Hz, *p*-$MeC_6H_4SO_2$-O, other 2 H in other aromatics), 7.05 – 7.55 (12 H, m, other aromatics), 5.45 (1 H, s, benzylidene proton), 4.91 (1 H, d, $J_{1,2}$ 4 Hz, H-1), 3.35 (3 H, s, OCH_3), 2.31 (3 H, s, aryl methyl).

Methyl 3-O-benzyl-4,6-O-benzylidene-α-D-glucoside (II). A solution of I (30 g) and lithium aluminium hydride in diethyl ether (750 ml) was refluxed overnight. Excess hydride was decomposed by addition of ethyl acetate, ethanol and water. The organic layer was separated and the aqueous layer extracted with chloroform (500 ml). The combined organic phases were washed with saturated aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$) and evaporated to yield crystalline II (16.3 g; 82 %). Crystallization from ethanol afforded the pure substance, m.p. 183–185 °C. $[\alpha]_{578}^{23}$ +87° (*c* 0.9, chloroform). (Found: C 67.9; H 6.58 $C_{21}H_{24}O_6$ requires: C 67.7; H 6.50). NMR ($CDCl_3$, 100 MHz): δ 7.10–7.55 (10 H, m, aromatics), 5.51 (1 H, s, benzylidene proton), 4.74 (1 H, d, $J_{1,2}$ 3 Hz, H-1), 3.39 (3 H, s, OCH_3).

Methyl 2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannosyl)-3-O-benzyl-4,6-O-benzylidene-α-D-glucoside (III). *II* (6.8 g) and 3,4,6-tri-*O*-benzyl-1,2-*O*-methylorthoacetyl-β-D-mannose [2] (7.4 g) were dissolved in nitromethane (180 ml), mercuric bromide (0.62 g) was added and the mixture was distilled. The volume was kept constant by addition of nitromethane. After 3 h, when TLC indicated a complete reaction, the solvent was evaporated. Preparative TLC (silica gel, ethyl acetate-toluene, 1:4) yielded III (2.5 g; 20 %) as a homogeneous syrup (R_F = 0.44), having $[\alpha]_{578}^{22}$ +16° (*c* 2.2, chloroform). NMR ($CDCl_3$, 100 MHz): δ 7.00–7.60 (25 H, m, aromatics), 5.50 (1 H, s, benzylidene proton), 3.43 (3 H, s, OCH_3), 2.13 (3 H, s, $OCOCH_3$).

Methyl 3-O-benzyl-2-O-(3,4,6-tri-O-benzyl-α-D-mannosyl)-4,6-O-benzylidene-α-D-glucoside (IV). III (1.37 g) was dissolved in 7 % methanolic ammonia (30 ml) and was left at room temperature for 72 h. After concentration the product was purified by preparative TLC (silica gel, ethyl acetate-light petroleum, 2:3) to yield IV (0.98 g, 75 %) as a homogeneous syrup (R_F = 0.23), $[\alpha]_{578}^{23}$ +31° (*c* 1.4, chloroform). NMR ($CDCl_3$, 60 MHz): δ 7.00–7.50 (25 H, m, aromatics), 5.51 (1 H, s, benzylidene proton); 5.05 (1 H, s, $J_{1,2}$ < 1 Hz, D-mannosyl residue), 4.93 (1 H, d, $J_{1,2}$ 2 Hz, H-1 D-glucosyl residue), 3.42 (3 H, s, OCH_3).

Methyl 3-O-benzyl-(3,4,6-tri-O-benzyl-α-D-arabino-hexoside-2-ulose)-4,6-O-benzylidene-α-D-glucoside (V). IV (0.91 g) was dissolved in a mixture of dimethyl sulfoxide (90 ml) and acetic anhydride (4.5 ml). The solution was kept under nitrogen at room temperature for 2 days and the reagents were removed by lyophilization. The crude product was used without purification in the following step. IR (KBr) showed, *inter alia*, a strong absorption at 1740 cm^{-1} (C=O).

Methyl 3-O-benzyl-(3,4,6-tri-O-benzyl-2-oximino-α-D-arabino-hexosyl)-4,6-O-benzylidene-α-D-glucoside (VI). V (from 0.91 g of IV) was dissolved in tetrahydrofuran (11.5 ml) and methanol (3 ml). Hydroxylamine hydrochloride (0.36 g) in water (2.25 ml) and pyridine (2.25 ml) were added. After 7 h at room temperature, the solvents were evaporated and the residue suspended in toluene (125 ml). The organic phase was washed with water (100 ml), 10 % sulfuric acid (2 × 50 ml), saturated aqueous sodium hydrogen carbonate (2 × 50 ml) and water (50 ml), dried over magnesium sulfate and evaporated. The crude product was purified by preparative TLC (silica gel, ethyl acetate-light petroleum, 2:3) to yield VI (0.75 g; 82 %). Crystallization from methanol yielded the pure substance, m.p. 164.5–166 °C, $[\alpha]_{578}^{23}$ +44° (*c* 0.6, chloroform). (Found: C 70.3; H 6.22; N 1.80. Calc. for $C_{48}H_{51}O_{11}N$: C 70.5; H 6.29; N 1.71). NMR ($CDCl_3$, 100 MHz): δ 8.54 (1 H, s, oxime hydroxyl), 7.00–7.60 (25 H, m, aromatics), 6.10 (1 H, s, H-1 oximated residue), 5.51 (1 H, s, benzylidene proton), 3.35 (3 H, s, OCH_3).

Methyl 2-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-α-D-glucopyranoside (VII). The oxime VI (0.65 g) was dissolved in glacial acetic acid (80 ml) and hydrogenated at atmospheric pressure over 10 % palladium on charcoal (1.30 g). After processing, a ninhydrine-positive product was obtained. Acetylation with acetic anhydride (5 ml) and pyridine (8 ml) yielded a crude product (0.52 g) which on TLC (silica gel, methanol-chloroform, 5:95) showed one major (R_F = 0.45) and several minor, faster moving components. The major component (0.31 g) was isolated by preparative TLC, and showed an NMR-spectrum compatible with peracetylated VII. This product was *O*-deacetylated by treatment with 5 % methanolic ammonia (8 ml) for 17 h, to yield crude VII (0.16 g). After gel filtration (Sephadex G-15, water), pure VII (0.12 g, 38 %) was obtained as a slowly crystallizing syrup, which could not be recrystallized. M.p. 240–245 °C (dec.), $[\alpha]_{578}^{23}$ +169° (*c* 0.7, water). NMR (D_2O, 100 MHz): δ 5.04 (1 H, d, $J_{1,2}$ 3 Hz, anomeric proton), 4.94 (1 H, d, $J_{1,2}$ 2.5 Hz, anomeric proton), 3.41 (3 H, s, OCH_3), 2.06 (3 H, s, $N\text{-}COCH_3$).

Sugar analysis of VII. The disaccharide (5 mg) was hydrolysed first with 90 % formic acid (100 °C, 2 h) and then with 0.25 M sulfuric acid (100 °C, 15 h), neutralized ($BaCO_3$) and the sugars were transformed into their alditol acetates by reduction (sodium borohydride) and acetylation (acetic anhydride-pyridine, 1:1). The mixture was analysed by GLC.[12]

Acknowledgements. The authors are indebted to Professor Bengt Lindberg and Dr. Per J. Garegg for their interest and to Miss Birthe Abrahamsson for her skilled technical assistance. This work was supported by the Swedish Natural Science Research Council.

REFERENCES

1. Ekborg, G., Lindberg, B. and Lönngren, J. *Acta Chem. Scand. 26* (1972) 3287.
2. Borén, H. B., Ekborg, G., Eklind, K., Garegg, P. J., Pilotti, Å. and Swahn, C. G. *Acta Chem. Scand. 27* (1973) 2639.
3. Ekborg, G., Lönngren, J. and Svensson, S. *Acta Chem. Scand. B 29* (1975) 1031.
4. Hellerqvist, C. G. and Lindberg, A. A. *Carbohyd. Res. 16* (1971) 39.
5. Nikaido, H. *J. Biol. Chem. 244* (1969) 2835.
6. Lindberg, A. A. *Annu. Rev. Microbiol. 27* (1973) 205.
7. Wiggins, L. F. *Methods Carbohyd. Chem. 2* (1962) 188.
8. Kuhn, R., Löw, I. and Trischmann, H. *Chem. Ber. 90* (1957) 203.
9. Croon, I. and Lindberg, B. *Acta Chem. Scand. 13* (1959) 593.
10. Kochetkov, N. K., Khorlin, A. J. and Bochkov, A. F. *Tetrahedron 23* (1967) 693.
11. Lemieux, R. U., James, K., Nagabhushan, T. L. and Ito, Y. *Can. J. Chem. 51* (1973) 33.
12. Sawardeker, J. S., Sloneker, J. H. and Jeanes, A. *Anal. Chem. 37* (1965) 1602.

Received April 17, 1975.