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OXAZOLINE SYNTHESIS OF 1,2-*trans*-2-ACETAMIDO-2-DEOXYGLYCOSIDES. GLYCOSYLATION OF SECONDARY HYDROXYL GROUPS IN PARTIALLY PROTECTED SACCHARIDES

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ABSTRACT

2-Methyl-glyco[1′,2′:4,5]-2-oxazolines have been used for the glycosylation of partially protected saccharides having a free secondary hydroxyl group. By the oxazoline method, β-D-Glc*p*NAc-(1→2)-glycerol (**4**), α-D-Man*p*NAc-(1→2)-glycerol (**6**), β-D-Glc*p*NAc-(1→3)-D-Glc (**8**), and β-D-Glc*p*NAc-(1→3)-D-GlcNAc (**10**) have been synthesized in high yield. Disaccharide **10** was converted into 2-methyl-4,5-[3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4,6-di-*O*-acetyl-α-D-glucopyrano]-2-oxazoline (**11**) which was then used in the synthesis of the trisaccharide derivative β-D-Glc*p*NAc-(1→3)-β-D-Glc*p*NAc-(1→6)-β-D-Glc*p*NAc-(1→O)-$C_6H_4NO_2$-*p* (**13**).

INTRODUCTION

Synthesis of oligosaccharides containing 2-acetamido-2-deoxy sugar residues is of interest in connection with structural investigations of polysaccharides that contain residues of 2-amino-2-deoxy sugars, and also of enzyme substrates or inhibitors.

The methods known at present for the synthesis of such oligosaccharides are, however, not very effective. For example, the Koenigs–Knorr reaction gives low yields even in glycosylation of the primary hydroxyl group in partially protected saccharides[1–5].

Micheel[6] has shown that 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide is easily transformed into the corresponding amine hydrobromide by an N→O acyl migration *via* formation of an oxazoline ring. Structurally related glycosyl bromides, in which the amino function was protected[7–12] by, for example, benzylsulphonyl, benzylidene, 2,4-dinitrophenyl, or diphenoxyphosphinyl groups, were found to give satisfactory results only with reactive aglycons containing a primary hydroxyl group. These bromides were also used for glycosylation of secondary hydroxyl groups. Thus, 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-α-D-glucopyranosyl bromide was employed in the glycosylation of the secondary hydroxyl group in some glycerol and ribitol derivatives[8,9]. Also, α,β-D-

Glc*p*NAc-(1→3)-D-GlcNAc and α,β-D-Glc*p*NAc-(1→4)-D-GlcNAc were synthesized by using 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphinylamino-α-D-glucopyranosyl bromide as glycosylating agent[11,12]. However, the low stereoselectivity of the reaction and the multistep procedure essentially limit the preparative value of these methods.

The glycosylation of the primary and secondary hydroxyl groups in saccharides by means of 3,4,6-tri-*O*-benzoyl-2-deoxy-2-dichloroacetamido-α-D-glucopyranosyl bromide and its D-*galacto* analogue[13,14] is, to some extent, free of the above disadvantages. However, attempts to lengthen an oligosaccharide chain by two or more aminosaccharide units involve problems concerned with the synthesis of the desired glycosylating agents.

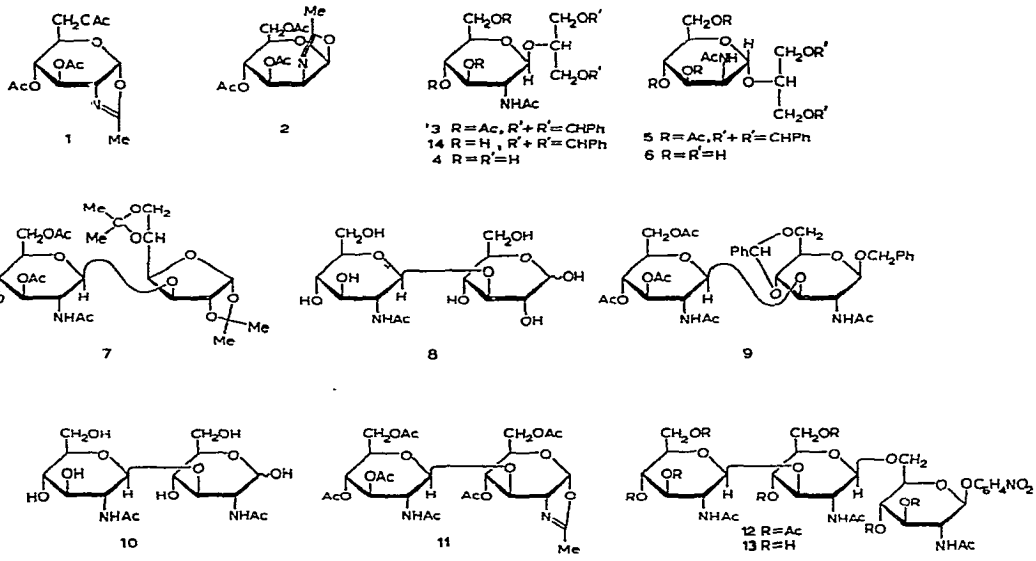
In our previous papers[15–18], 2-methyl-glyco[1′,2′:4,5]-2-oxazolines, including oxazoline derivatives of oligosaccharides, have been shown to be effective glycosylating agents. In an extension of the preparative scope of the oxazoline synthesis, the glycosylation of secondary hydroxyl groups in partially protected saccharides has been studied, and we now report on the synthesis of oligosaccharides having different combinations of 1,2-*trans*-2-acetamido-2-deoxyglycosidic bonds.

RESULTS AND DISCUSSION

The glycosylating capability of 2-methyl-4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyrano)-2-oxazoline (**1**) and 2-methyl-4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-β-D-mannopyrano)-2-oxazoline (**2**) was investigated by examination of their reactions with 1,3-*O*-benzylideneglycerol, 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, and benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside. 2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3-*O*-benzylideneglycerol(**3**) was obtained in 36% yield by heating the oxazoline **1** with 1,3-*O*-benzylideneglycerol in dry benzene in the presence of a catalytic amount of toluene-*p*-sulphonic acid (at pH 3–4). Deacetylation (Zemplén), followed by the removal of the benzylidene group by heating with aqueous acetic acid, gave 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)glycerol (**4**). By using oxazoline **2**, under similar conditions, 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranosyl)-1,3-*O*-benzylideneglycerol (**5**) was obtained in 31% yield, and was converted into 2-*O*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)-glycerol (**6**).

The high reactivity of the oxazoline **1** was shown in glycosylation of monosaccharides (pyranose and furanose rings) containing sterically hindered, unblocked hydroxyl groups. Condensation of the oxazoline **1** with 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose gave 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**7**) in 49% yield. From **7**, after deblocking reactions, 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-glucopyranose (**8**) was obtained.

Under the above conditions, benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside was treated with the oxazoline **1** to give benzyl 2-acetamido-3-*O*-



(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**9**) in 81% yield. The removal of the benzylidene group, deacetylation, and catalytic hydrogenolysis gave the known[11] 2-acetamido-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose (**10**).

The above data establish that the benzylidene and isopropylidene protecting groups can be used successfully in the oxazoline synthesis.

By the procedure described earlier[19], the disaccharide **10** has been converted into 2-methyl-4,5-[3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyrano]-2-oxazoline (**11**) in 53% yield. The reaction of the oxazoline **11** with *p*-nitrophenyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside led to *p*-nitrophenyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**12**) in 78% yield. Deacetylation of **12** gave *p*-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranoside (**13**).

The 1,2-*trans*-configuration of the glycosidic bonds of the synthetic 2-acetamido-2-deoxyglycosides was established by the enzymic hydrolysis of these compounds with 2-acetamido-2-deoxy-β-D-glucosidase from boar epididymis, and also by comparison of the observed and calculated values for molecular rotation.

The results obtained in this, as well as in previous papers[15–19], demonstrate that oxazoline derivatives of mono- and oligo-saccharides are effective glycosylating agents which offer a convenient route to 1,2-*trans*-2-acetamido-2-deoxyglycosides, including oligosaccharides.

EXPERIMENTAL

General. — Melting points were measured on a Kofler apparatus and are corrected. Optical rotations were determined with a SPU-M VNIIEKIProdmash polarimeter. Thin-layer chromatography (t.l.c.) was performed with Silicagel KSK-G with chloroform–methanol (20:1, 10:1, and 5:1). Paper chromatography (p.c.) was conducted on Filtrak Niederschlag FN3 paper with isopentyl alcohol–pyridine–water (5:5:4). The spots were revealed by treatment with ammonia vapour and or conc. sulphuric acid (t.l.c.), and aniline hydrogen phthalate, methanolic sodium methoxide, or chlorine–toluidine (p.c.). The compounds described subsequently were homogeneous on t.l.c. or p.c. Evaporations were performed at 35–40° *in vacuo*.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3-O-benzylideneglycerol (**3**). — A mixture of the oxazoline[19] **1** (1.06 g), *cis*-1,3-*O*-benzylideneglycerol[20] (0.62 g), and toluene-*p*-sulphonic acid (*ca.* 10 mg, pH 3–4) in dry benzene (4 ml) was heated for 30 min at 110–120°. The product was collected, washed with benzene, and recrystallised from propyl alcohol to give the title compound (0.585 g, 36%), m.p. 177–178° (decomp.), $[\alpha]_D^{20}$ −13° (*c* 0.29, methanol).

Anal. Calc. for $C_{24}H_{31}NO_{11}$: C, 56.4; H, 6.0; N, 2.7. Found: C, 56.4; H, 5.6; N, 2.9.

2-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-1,3-O-benzylideneglycerol (**14**). — Compound **3** (200 mg) was deacetylated at 20° for 16 h with 0.1M methanolic sodium methoxide (5 ml). The product was collected, washed with methanol, and recrystallised from propan-2-ol to give the title compound (148 mg, 99%), m.p. 166.5–167° (decomp.), $[\alpha]_D^{20}$ +2° (*c* 0.12, methanol).

Anal. Calc. for $C_{18}H_{25}NO_8$: C, 56.6; H, 6.6; N, 3.7. Found: C, 55.8; H, 6.7; N, 3.7.

2-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)glycerol (**4**). — The benzylidene derivative **14** (100 mg) was heated in 50% aqueous acetic acid (10 ml) for 1 h at 90°. The solution was then evaporated, traces of acetic acid were removed by codistillation with toluene, and the product was crystallised from aqueous methanol to give compound **4** (65 mg, 84%), m.p. 157–158°, $[\alpha]_D^{20}$ 0 ±2° (*c* 0.25, water), R_{Glc} 0.46 (p.c.).

Anal. Calc. for $C_{11}H_{21}NO_8$: C, 45.2; H, 6.9. Found: C, 44.9; H, 7.1.

Compound **4** was treated with 2-acetamido-2-deoxy-β-D-glucosidase from boar epididymis in citrate–phosphate buffer (pH 4.8) for 3 h at 37°, and 2-acetamido-2-deoxy-D-glucose (R_{Glc} 1.64, p.c.) was detected in the hydrolysate.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-mannopyranosyl)-1,3-O-benzylideneglycerol (**5**). — A solution of the oxazoline[19] **2** (1.00 g), *cis*-1,3-*O*-benzylideneglycerol (1.01 g), and toluene-*p*-sulphonic acid (10 mg) in dry toluene (10 ml) was heated for 1 h at 110–120°. The reaction mixture was treated with one drop of pyridine and evaporated. Compound **5** was isolated by elution of the residue from a column (30 × 2.5 cm) of neutral alumina (Brockmann IV) with ether (300 ml), chloroform (300 ml), and chloroform–methanol with an increase in methanol content to 20%. Yield, 0.48 g (31%) of chromatographically homogeneous **5**. After recrystallisation

from propyl alcohol, the product had m.p. 170° (decomp.), $[\alpha]_D^{20}$ +4° (*c* 0.25, chloroform).

Anal. Calc. for $C_{24}H_{31}NO_{11}$: C, 56.4; H, 6.0. Found: C, 56.4; H, 6.1.

2-*O*-(2-Acetamido-2-deoxy-α-D-mannopyranosyl)glycerol (**6**), $[\alpha]_D^{20}$ −28° (*c* 0.55, water), obtained from **5** by removal of blocking groups, was not affected by incubation for 24 h with 2-acetamido-2-deoxy-β-D-glucosidase.

3-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (**7**). — A solution of the oxazoline **1** (1.00 g), 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (0.80 g), and a catalytic amount of toluene-*p*-sulphonic acid in dry nitromethane–toluene (1:1, 10 ml) was heated for 50 min at 110°. The product (1.08 g) was collected, washed with toluene and ether, and recrystallised from ethanol to give compound **7** (0.87 g, 49%), m.p. 219–220°, $[\alpha]_D^{20}$ +68° (*c* 0.28, chloroform).

Anal. Calc. for $C_{26}H_{39}NO_{14}$: C, 52.9; H, 6.7. Found: C, 52.7; H, 6.7.

3-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D-glucopyranose (**8**). — Compound **7** (200 mg) was deacetylated in methanol with triethylamine (0.5 ml). The residue remaining after evaporation of the solution was heated with 50% aqueous acetic acid for 20 min at 100°. The solution was evaporated to give chromatographically pure product (120 mg, 90%), R_{Glc} 0.60 (p.c.). From 90% aqueous methanol, on cooling, the monohydrate of disaccharide **8** was precipitated as an amorphous powder, $[\alpha]_D^{20}$ +59 (10 min) → +39° (24 h, equilibrium, *c* 0.6, water).

Enzymic hydrolysis of **8**, as described above, yielded 2-acetamido-2-deoxy-D-glucose (R_{Glc} 1.64) and glucose (p.c.).

Benzyl 2-acetamido-3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**9**). — A solution of the oxazoline **1** (1.00 g), benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[21] (0.85 g), and toluene-*p*-sulphonic acid (10 mg) in dry nitromethane (50 ml) was heated for 30 min at 110°. The resulting solid was collected, washed with nitromethane and ether, and recrystallised from *p*-dioxane to give the title compound (1.25 g, 81%), m.p. 297–298°, $[\alpha]_D^{20}$ −43° (*c* 2.8, pyridine).

Anal. Calc. for $C_{36}H_{44}N_2O_{14}$: C, 59.3; H, 6.1. Found: C, 59.0; H, 6.0.

2-Acetamido-3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose (**10**). — Compound **9** (200 mg) was heated in 50% aqueous acetic acid (10 ml) for 20 min at 90°, the solution was evaporated, and the residue was crystallised from methanol to give benzyl 2-acetamido-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside (150 mg, 84%), m.p. 219–220°, $[\alpha]_D^{20}$ −8° (*c* 2.6, acetic acid). The latter compound (100 mg) was deacetylated with 0.05M methanolic sodium methoxide (10 ml) for 16 h at 20°. The resulting benzyl 2-acetamido-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside (75 mg), which decomposed at 194–195°, was hydrogenated in methanol (20 ml) over Adam's catalyst. The filtered solution was evaporated, and the residue was crystallised from aqueous methanol to give disaccharide **10** (59 mg, 79%), which

decomposed at 180°, $[\alpha]_D^{20}$ +40 (10 min) → +4° (24 h, equilibrium, *c* 0.17, water). R_{GlcNAc} 0.68 (p.c.).

Anal. Calc. for $C_{16}H_{28}N_2O_{11}$: C, 45.2; H, 6.6. Found: C, 45.2; H, 6.9.

For the monohydrate of **10**, Heyns *et al.* reported[11] m.p. 198–199° (decomp.), $[\alpha]_D^{20}$ +14.5 → +6.5° (water).

Enzymic hydrolysis of disaccharide **10**, as described above, yielded 2-acetamido-2-deoxy-D-glucose, R_{Glc} 1.64 (p.c.).

2-Methyl-4,5-[3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4,6-di-O-acetyl-2-deoxy-α-D-glucopyrano]-2-oxazoline (**11**). — The oxazoline **11** was prepared, as previously reported[19] from disaccharide **10**, in 53% yield, m.p. 181–182° (from chloroform–ether–light petroleum), $[\alpha]_D^{20}$ +8 ±2° (*c* 0.2, chloroform).

Anal. Calc. for $C_{26}H_{36}N_2O_{15}$: C, 50.6; H, 5.9. Found: C, 50.6; H, 5.8.

p-*Nitrophenyl* O-(*2-acetamido*-3,4,6-*tri*-O-*acetyl-2-deoxy-β-D-glucopyranosyl*)-(*1→3*)-O-(*2-acetamido-4,6-di*-O-*acetyl-2-deoxy-β-D-glucopyranosyl*)-(*1→6*)-*2-acetamido-3,4-di*-O-*acetyl-2-deoxy-β-D-glucopyranoside* (**12**). — A mixture of the oxazoline **11** (0.36 g), *p*-nitrophenyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside[4] (0.33 g), and toluene-*p*-sulphonic acid (10 mg) in dry nitromethane–toluene (1:1, 3.5 ml) was heated for 30 min at 100–110°. The product was collected, washed with nitromethane–toluene and ether, and recrystallised from methanol to give compound **12** (0.48 g, 78%), m.p. 280–281°, $[\alpha]_D^{20}$ +75° (*c* 0.13, chloroform–methanol).

Anal. Calc. for $C_{44}H_{58}N_4O_{25}$: C, 50.7; H, 5.6; N, 5.4. Found: C, 50.6; H, 5.5; N, 5.3.

p-*Nitrophenyl* O-(*2-acetamido-2-deoxy-β-D-glucopyranosyl*)-(*1→3*)-O-(*2-acetamido-2-deoxy-β-D-glucopyranosyl*)-(*1→6*)-*2-acetamido-2-deoxy-β-D-glucopyranoside* (**13**). — A suspension of compound **12** (480 mg) in dry methanol (60 ml) and M methanolic sodium methoxide (0.6 ml) was heated at 40–45° until dissolution was complete. The mixture was maintained at 5° for 16 h, and the product was collected and then washed with methanol to give the trisaccharide glycoside **13** (120 mg). From the mother liquor was isolated a further amount of **13** (210 mg, overall yield 95%); m.p. 209–210° (decomp.) (from aqueous ethanol), $[\alpha]_D^{20}$ +8° (*c* 0.18, aqueous methanol), R_{GlcNAc} 0.84 (p.c.).

Anal. Calc. for $C_{30}H_{44}N_4O_{18}$: C, 48.1; H, 5.9. Found: C, 48.2; H, 5.7.

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