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Preliminary communication

A new procedure for the preparation of oligosaccharide oxazolines†*

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Oligosaccharide "lipid intermediates" are required as exogenous glycosyl acceptors for studies of *N*-glycoprotein biosynthesis[1,2]. For the chemical synthesis of these intermediates, oligosaccharides having structures corresponding to those in the *N*-glycoprotein saccharide "core"[3] may be isolated from the urine of animals suffering from swainsonine-induced α-mannosidosis[4,5]. The synthetic sequence involves formation of a peracetylglycosyl phosphate, coupling with an "activated" derivative of dolichyl phosphate, and *O*-deacetylation of the resulting peracetylated diphosphoric diester[6]. When the "reducing" terminal is a 2-acetamido-2-deoxy-D-glucose residue, peracetyl oxazolines are appropriate precursors of oligosaccharide phosphates, because (a) they provide the α-D anomer in a reaction that involves net retention of configuration[6–8], and (b) phosphorylation occurs without any scission or modification of inter-residue glycosidic linkages. A key step, therefore, is the preparation, in high yield, of an oligosaccharide oxazoline from a peracetylated oligosaccharide. The synthetic challenge results from the occurrence, in the oligosaccharides, of α-D-(1→6) linkages that are very labile to the acidic conditions normally employed for formation of glycosyl halides, the usual precursors of glycooxazolines. Also, owing to the presence of a di-*N*-acetylchitobiose unit, any reagents employed must not adversely affect the acetamido groups, or cause significant hydrolysis of the β-D-(1→4) linkage between the two acetamidodeoxy sugar residues. Thus, "chloroacetolysis" (treatment with conc. hydrochloric acid in acetyl chloride), which we previously employed for the preparation of glycosyl chlorides from oligosaccharides [6–8], could not be used. Therefore a different reagent, namely hydrogen chloride, which has often served for the preparation of glycosyl chlorides, was tried, with either peracetylated tetrasaccharide **2** or heptasaccharide **3** (both preponderantly in the α anomeric form) as the starting compounds. Unfortunately, this method was also unsatisfactory because of

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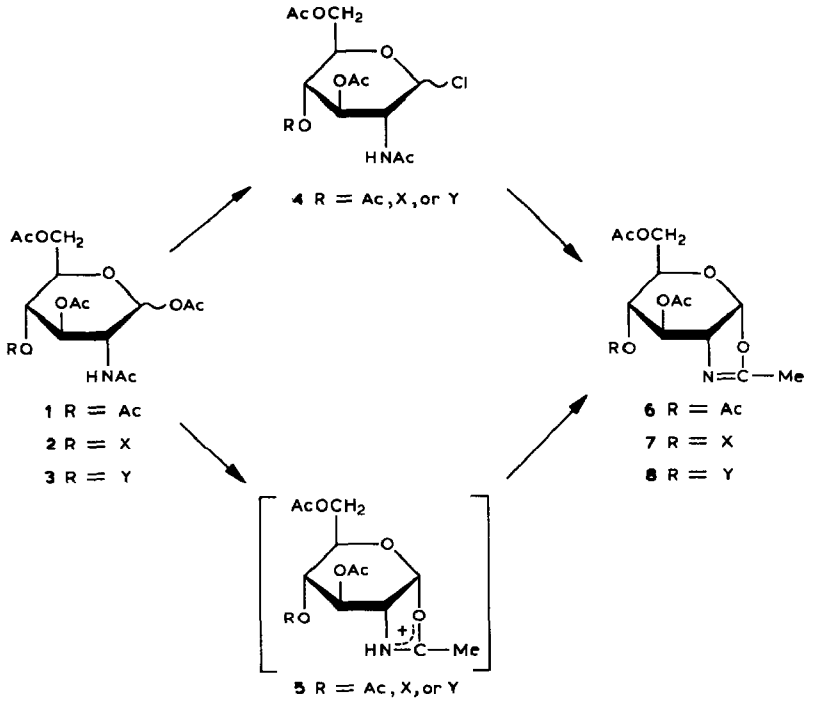

X = per-*O*-acetyl-α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→

Y = per-*O*-acetyl-α-D-Man*p*-(1→6)-α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→

3 3

1 1

α-D-Man*p* α-D-Man*p*

inter-residue bond cleavage (see Table I), and so a study was initiated using the α anomer of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose (**1**) as a model compound to try to identify a more satisfactory procedure. As can be seen from the results (Table I), none of the reagents that were used produced a high yield of glycosyl halide from the α-D anomer of the starting compound. Because of these problems, a new procedure was developed that involved treatment of the peracetylated oligosaccharide with trifluoromethanesulfonic acid (triflic acid) to form directly the oxazoline **6**, presumably *via* the ion **5**. Triflic acid, which is difficult to handle could be replaced by trimethylsilyl trifluoromethanesulfonate (Me_3Si-triflate), without any loss of yield (Table II). Indeed, preliminary ^{1}H-n.m.r. evidence indicated that triflic acid is detected when the latter reagent is employed. Trimethylsilyl triflate was greatly superior to stannic chloride[12], which we found unsatisfactory for the efficient synthesis of oligosaccharide oxazolines.

When the Me_3Si-triflate procedure was applied to **2**, R_F 0.27 (20 : 1 v/v chloroform–methanol), the tetrasaccharide oxazoline **7**, R_F 0.31, was obtained in 74% yield. The identity of the product was confirmed by examination of the ^{1}H-n.m.r. spectrum

TABLE I

FORMATION OF PERACETYLGLYCOSYL HALIDES FROM DERIVATIVES OF 2-ACETAMIDO-2-DEOXY-D-GLUCOSE

Starting compound	*Reagent*[a]	*Results*
2	HCl	40% yield of glycosyl chloride[b]
3	HCl	5–15% yield of glycosyl chloride[b]
1	Me_3SiCl	No reaction
1	Me_3SiBr (ref. 9)	Low yield of glycosyl bromide, decomposition
1	$TiCl_4$ (ref. 10)	Mixture of compounds[c], decomposition
1	$TiBr_4$ (ref. 11)	Mixture of compounds[c], decomposition

[a]All reactions were conducted at room temperature in 1,2-dichloroethane and the products identified by t.l.c. [b]Evidence of major side-reactions involving cleavage of glycosidic bonds. [c]Products included glycosyl halide, oxazoline, and starting material.

TABLE II

FORMATION OF 2-METHYL-(3,4,6-TRI-*O*-ACETYL-1,2-DIDEOXY-α-D-GLUCOPYRANO)-[2,1-*d*]-2-OXAZOLINE FROM COMPOUND 1

Anomer of 1	*Reagent*[a]	*Time* (*h*)	*Yield* (%)
α^b	Me_3Si-triflate	16	95
α^b	Triflic acid	12	97
β^c	Me_3Si-triflate	0.5	100

[a]A solution of the starting compound (0.1 mmol) in 1,2-dichloroethane was stirred at 50° with 1.1 equiv. of reagent. When t.l.c. (20 : 1 v/v chloroform–methanol) showed complete reaction, the mixture was made slightly alkaline with triethylamine, applied to a column of silica gel (Merck Kieselgel 60; 230–400 mesh), and eluted with 100 : 200 : 1 toluene–ethyl acetate–triethylamine. The product had R_F 0.43, $[\alpha]_D^{20}$ + 11° (*c* 1.35, chloroform), and was pure according to t.l.c. and the ^{1}H-n.m.r. spectrum. [b]R_F 0.37, $[\alpha]_D^{20}$ + 91° (*c* 1.4, chloroform). [c]R_F 0.34, $[\alpha]_D^{20}$ + 3° (*c* 1.75, chloroform).

(δ 5.89, $J_{1,2}$ 7.3 Hz, H-1), and by hydrolysis at room temperature with a dilute solution of *p*-toluenesulfonic acid in acetonitrile, followed by *O*-deacetylation with methanolic sodium methoxide, reduction with sodium borohydride, and comparison of the product by liquid chromatography (elevated pressure, 5-μm Amino-Spherisorb column, 7 : 3 acetonitrile–water) with an authentic specimen of the alditol derived from α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc. Similarly, when the Me_3Si-triflate procedure was applied to **3**, R_F 0.56 (10 : 1 v/v chloroform–methanol), the heptasaccharide oxazoline **8**, R_F 0.60, was obtained in 90% yield. In neither case was there any t.l.c. evidence for the formation of low-molecular-weight oxazolines indicative of glycosidic-bond cleavage[8]. T.l.c. clearly differentiated the required compounds **7** and **8** from the starting materials **2** and **3** respectively, and from the 1-hydroxy compounds that could have resulted from hydrolysis of the oxazolines, and also from any "glucal"-type

compounds that may have been formed as byproducts. Both oligosaccharide oxazolines gave satisfactory elemental analyses.

An important advantage of this new procedure for the synthesis of oligosaccharide oxazolines is that it can be applied equally well to the α or β anomer of the starting peracetylated compound, unlike the ferric chloride method[13], which can only utilize the relatively inaccessible β anomer. In recent work, the oxazoline 7 was converted into a tetrasaccharide phosphate and employed for the synthesis of a "lipid intermediately"[14].

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