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A stereoselective synthesis of pyruvic 4,6-acetals of D-hexopyranose residues*

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ABSTRACT

A stereoselective synthesis of pyruvic 4,6-acetals in their naturally occurring configurations on α-D-glucopyranose, α-D-mannopyranose, and β-D-galactopyranose residues is based on the preferential formation of 4,6-[1-(3,4-dimethoxyphenyl)ethylidene] acetals bearing equatorial methyl groups. 2,3-Di-*O*-acyl derivatives of these acetals are oxidized with ruthenium tetraoxide to yield the corresponding 4,6-(1-carboxyethylidene) acetals. Synthesis of allyl 4,6-*O*[1(*S*)-1-methoxycarbonylethylidene]-3-*O*-methyl-β-D-glucopyranoside has been achieved with protection of the allyl glycoside by epoxidation and subsequent regenerative deoxygenation with 3-methylbenzothiazole-2-selone. The allyl glycoside has been subjected in sequence to saponification, ozonolysis, and reductive amination in the presence of bovine serum albumin to furnish a neoantigen.

INTRODUCTION

Pyruvic acid residues, as cyclic acetals, first found in agar[1], are of widespread occurence in bacterial polyaccharides[2] and are found in some glycolipids[3,4]. Most commonly, these residues are present as 4,6-acetals of hexose residues, but they also occur as *cis* and *trans* 1,3-dioxolanes spanning secondary hydroxyl groups. Each of the 4,6-acetals encountered to date has the methyl substituent at the acetalic carbon of the 1-carboxyethylidene group in an equatorial orientation with the (*S*) configuration in D-glucose and D-mannose residues and the (*R*) configuration in D-galactose residues[5–7]. In these experiments, which led to unambiguous assignments of configuration, the pyruvate acetals were prepared by catalytic oxidation of 4,6-acetals of hydroxyacetone generated as mixtures of diastereomers. The first reported direct synthesis of pyruvate acetals by reaction of methyl β-D-glucopyranoside with ethyl pyruvate[8] gave a low yield of a mixture of diastereomers. A later synthesis of a *galacto* isomer from a protected glycoside proceeded in reasonable yield, but, although the 4,6-acetal with the desired (*R*) configuration was isolated, it was accompanied by an acyclic mixed acetal[9]. Recent syntheses have involved displacement of 4,6-disilyl ethers in the presence of trimethylsi-

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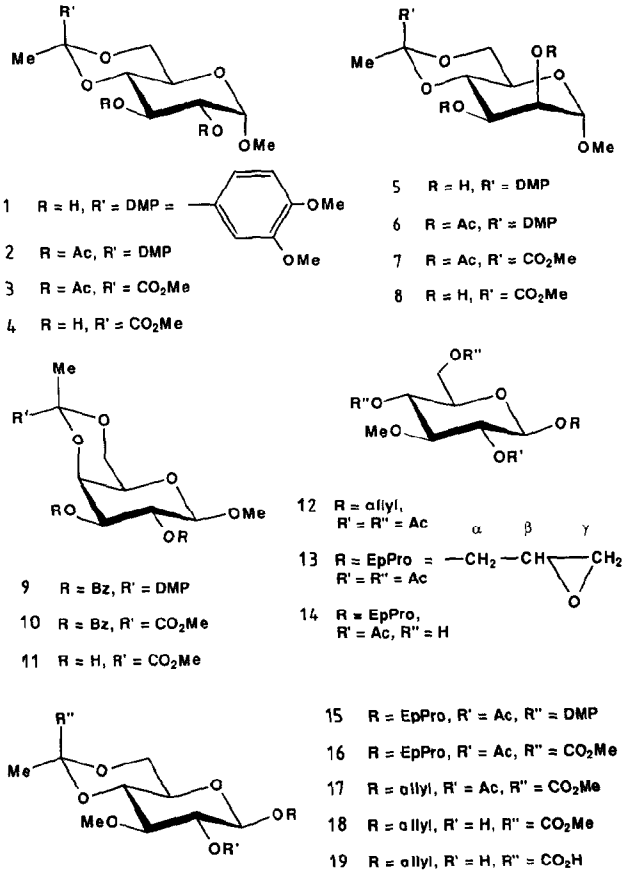
lyl triflate[10,11] or transacetalation from methyl pyruvate diphenyl dithioacetal[12] and have given higher yields. These syntheses include isolated examples of the stereoselective formation of acetals with the natural configuration, but mixtures of diastereomers are formed with other substrates.

We now describe a different approach based on the highly stereoselective transacetalation of methyl hexopyranosides with dimethyl acetals of acetophenone[13] followed by oxidation with ruthenium tetraoxide[14]. Yields of the order of 60–70% were obtained and no other products of oxidation were detected. Since the completion of this work, preliminary results have been reported[15] of an essentially similar approach. Our results are presented in the context of the synthesis of pyruvic acid acetals of allyl glycosides to be used for the preparation of neoglycoproteins[16] by ozonolysis followed by reductive-amination-conjugation to ε-amino groups of lysine residues in proteins[17]. Such a glycoside may be prepared by the ruthenium tetraoxide procedure if the allyl group is protected by epoxidation and subsequently regenerated by deoxygenation.

RESULTS AND DISCUSSION

Since oxidative degradations of aryl to carboxyl substituents take place more rapidly with 3,4-dimethoxyphenyl than for phenyl groups[18], oxidation with ruthenium tetraoxide[14] of acetals prepared by transacetalation from the dimethyl acetal of 3,4-dimethoxyacetophenone was explored. Transacetalation of methyl α-D-glucopyranoside in *N*,*N*-dimethylformamide afforded methyl 4,6-*O*-[(*S*)-1-(3,4-dimethoxyphenyl)-ethylidene]-α-D-glucopyranoside (**1**). The characteristic ^{13}C chemical shift of the resonance of the equatorial acetalic methyl substituent[13] supported the assigned configuration, and no trace of the (*R*)-diastereomer was detected. The 2,3-diacetate **2** was oxidized with ruthenium tetraoxide (continuously regenerated from ruthenium trichloride by sodium periodate in acetonitrile–carbon tetrachloride–water) to give the pyruvic acid acetal **3**, which was isolated after esterification with diazomethane. Catalytic *O*-deacetylation then afforded methyl 4,6-*O*-[(*S*)-1-methoxycarbonylethylidene]-α-D-glucopyranoside (**4**), the configuration of which was confirmed by the ^{13}C-chemical shift of the resonance of the acetal methyl carbon[7]. Similar syntheses were performed with derivatives of methyl α-D-mannopyranoside and methyl β-D-galactopyranoside, and yielded methyl pyruvate 4,6-acetals having the acetal methyl substituent equatorial. However, in each series, the initially formed acetephenone acetal was obtained in a relatively low yield due to lack of regioselectivity in direct acetalation.

Reaction of methyl α-D-mannopyranoside with 3,4-dimethoxyacetophenone dimethyl acetal gave the 4,6-acetal **5** as the main product together with two other compounds, the n.m.r. spectra of which, especially the ^{13}C resonances at ~108 and ~102 p.p.m. for acetal ring carbon atoms of 1,3-dioxolanes and 1,3-dioxanes[19], identified them as the 2,3-acetal and the 2,3:4,6-di-acetal. Acetal carbon resonances were distinguished from those of anomeric and aromatic carbons by using the DEPT sequence for peak multiplicities in C–H coupling. The 4,6-acetal **5** was converted into the 2,3-diacetate **6** which was oxidized with ruthenium tetraoxide. The product was



esterified to give the methyl pyruvate acetal **7**, which was *O*-deacetylated to give methyl 4,6-*O*-[(*S*)-1-methoxycarbonylethylidene]-*α*-D-mannopyranoside (**8**).

Preliminary experiments showed that direct acetalation of unprotected galactopyranosides gave the 3,4-acetal as the major product, so that synthesis of the 4,6-acetal could be performed only on 2,3-disubstituted derivatives. Acetalation of methyl 2,3-di-*O*-benzoyl-*β*-D-galactopyranoside[20] furnished methyl 2,3-di-*O*-benzoyl-4,6-*O*-[(*R*)-1-(3,4-dimethoxyphenyl)ethylidene]-*β*-D-galactopyranoside (**9**). Oxidation of **9** with ruthenium tetraoxide, followed by esterification and *O*-debenzoylation gave, successively, **10** and **11**, the latter having physical constants similar to those reported for methyl 4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-*β*-D-galactopyranoside[7].

The glycopeptidolipid antigen[3] from *Mycobacterium avium–M. intracellulare–M. scrofulaceum* (MAIS) serotype 8 carries a terminal 4,6-*O*-[(*S*)-1-carboxyethylidene]-3-*O*-methyl-*β*-D-glucopyranosyl unit 3-linked to the *α*-L-rhamnopyranosyl-(1→2)-6-deoxy-*α*-L-talopyranose residue which is common to the glycopeptidolipids of all the MAIS serotypes[17]. For the preparation of a neoglycoconjugate related to this serotype,

the synthesis of the allyl glycoside of this presumed epitope was undertaken. Allyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranoside[21] (**12**), prepared by standard methods, was treated with 3-chloroperoxybenzoic acid to give the corresponding 2,3-epoxypropyl glycoside **13**. This and **14**–**16** were obtained as mixtures of diastereomers, the n.m.r. spectra of which contained two sets of signals for some protons and carbon atoms[22]. The extra chiral centre was removed later during the regeneration of the stereochemically homogeneous allyl glycoside **17**. Treatment of **13** with magnesium oxide[23] resulted in selective *O*-deacetylation[24] and the 2-acetate **14** formed was converted into the 4,6-acetal **15**, the ^{13}C-n.m.r. spectrum of which showed it to be the 1′(*S*) diastereomer. Oxidation of **15** with ruthenium tetraoxide followed by esterification with diazomethane gave the corresponding methyl pyruvate 4,6-acetal **16**. Deoxygenation of the epoxypropyl glycosidic substituent in **16**, to form the allyl glycoside **17**, proceeded smoothly on treatment[25] with 3-methylbenzothiazole-2-selone under anhydrous conditions in the presence of trifluoroacetic acid, and no loss of pyruvate acetal was detected. *O*-Deacetylation of **17** gave allyl 4,6-*O*-[(*S*)-1-methoxycarbonylethylidene]-3-*O*-methyl-β-D-glucopyranoside (**18**) as a convenient derivative from which the pyruvic acid 4,6-acetal **19** has been prepared by saponification for subsequent conversion, by ozonolysis and reductive amination in the presence of bovine serum albumin, into a neoglycoprotein. The immunological examination of this neoglycoprotein is reported elsewhere[17].

EXPERIMENTAL

General methods. — Evaporations were conducted under diminished pressure at ≤40°. Optical rotations were measured with a Perkin–Elmer 141 polarimeter at ~20°. N.m.r. spectra [^{1}H and ^{13}C (DEPT sequence)] were recorded with a Bruker AM 300 spectrometer for solutions in $CDCl_3$ (internal Me_4Si).

Methyl 4,6-O-[(S)-1-(3,4-dimethoxyphenyl)ethylidene]-α-D-glucopyranoside (**1**) *and the 2,3-diacetate* (**2**). — Methyl α-D-glucopyranoside (2.6 g, 13.4 mmol) was added to a solution of 3,4-dimethoxyacetophenone dimethyl acetal (5.25 g, 16.1 mmol) in *N*,*N*-dimethylformamide (25 mL) containing *p*-toluenesulfonic acid (10 mg), and the solution was stirred overnight. Sodium hydrogen carbonate was added, the solution was diluted with chloroform, and the organic layer was washed twice with water, dried, and concentrated. The residue was chromatographed on silica gel (chloroform–methanol, 97:3) to give **1** (3.82 g, 87%), m.p. 155°, $[\alpha]_D$ +138° (*c* 1, chloroform). N.m.r. data: ^{1}H, δ 6.89–7.02 (m, 3 H, aromatic), 4.86 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 3.89 (s, 6 H, 2 OMe), 3.42 (s, 3 H, OMe), 1.56 (s, 3 H, CH_3); ^{13}C, δ 149.4, 148.6, 132.4, 119.0, 111.4, 109.8 (aromatic), 101.9 (acetal C), 99.7 (C-1), 63.5 (C-6), 55.9, 55.8, 55.3 (3 OMe), 31.7 (acetal CH_3).

Anal. Calc. for $C_{17}H_{24}O_8$: C, 57.26; H, 6.78. Found: C, 57.35; H, 6.91.

Acetal **1** (2 g, 5.62 mmol) was acetylated in the usual way to give methyl 2,3-di-*O*-acetyl-4,6-*O*-[(*S*)-1-(3,4-dimethoxyphenyl)ethylidene]-α-D-glucopyranoside (**2**; 2.34 g, 95%) as a syrup, $[\alpha]_D$ +179° (*c* 1, chloroform). N.m.r. data: ^{1}H, δ 7.35, 6.89–6.92 (2 m, 3

H, aromatic), 5.51 (m, 1 H, H-3), 4.80 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.73 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2) 3.89, 3.90 (2 s, 3 H each, 2 OMe), 3.38 (s, 3 H, OMe), 2.06, 2.13 (2 s, 3 H each, 2 OAc), 1.52 (s, 3 H, CH_3).

Anal. Calc. for $C_{21}H_{28}O_{10}$: C, 57.26; H, 6.40. Found: C, 57.03; H, 6.41.

*Methyl 2,3-di-*O*-acetyl-4,6-*O*-[(S)-1-methoxycarbonylethylidene]-α-*D*-glucopyranoside* (**3**). — Ruthenium trichloride trihydrate (10 mg) was added to a suspension of sodium metaperiodate (7.3 g, 33.6 mmol) in water (40 mL) to which sodium hydrogen carbonate (2.7 g) had been added to bring the solution to pH 6. A solution of **2** (500 mg, 1.13 mmol) in carbon tetrachloride (20 mL) and acetonitrile (20 mL) containing tetrabutylammonium hydrogen sulfate (10 mg) was added with vigorous stirring at 0°. Stirring was continued at room temperature for 1 h when the colour of the mixture had faded. More ruthenium trichloride (10 mg) and sodium metaperiodate (720 mg, 3.36 mmol) were added, and stirring was continued for a further 6 h until starting material was no longer detected. The pH of the mixture was brought to 7 by the addition of sodium hydrogen carbonate, insoluble salts were removed, and the filtrate was extracted thrice with ethyl acetate. The aqueous solution at 0° was acidified to pH 1 and extracted thrice with ethyl acetate, and the combined extracts were washed with saturated brine then with water, dried, and concentrated. The residue was treated overnight with diazomethane in dichloromethane and then concentrated to a syrup which was chromatographed on silica gel (light petroleum–ethyl acetate, 7:3) to give **3** (243 mg, 59%), $[\alpha]_D$ +125° (*c* 4.3, chloroform). N.m.r. data: ^{1}H, δ 5.43 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 4.84 (m, 2 H, H-1,2), 3.83 (s, 3 H, ester OMe), 3.39 (s, 3 H, OMe), 2.07, 2.08 (2 s, each 3 H, 2 OAc), 1.51 (s, 3 H, CH_3).

Anal. Calc. for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.11. Found: C, 50.09; H, 5.98.

*Methyl 4,6-*O*-[(S)-1-methoxycarbonylethylidene]-α-*D*-glucopyranoside* (**4**). — Compound **3** (240 mg, 0.66 mmol) was treated conventionally with a catalytic amount of sodium methoxide in methanol, and the product was chromatographed on silica gel (chloroform–methanol, 97:3) to give **4** (117 mg, 63.5%), $[\alpha]_D$ +105° (*c* 1.3, chloroform); lit.[5] $[\alpha]_D$ +108° (chloroform). N.m.r. data: ^{1}H, δ 4.74 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 3.84 (s, 3 H, ester OMe), 3.43 (s, 3 H, OMe), 1.56 (s, 3 H, CH_3); ^{13}C, δ 170.2 (CO_2Me), 99.8 (C-1), 99.2 (acetal C), 65.3 (C-6), 61.9 (C-5), 55.5 (OMe), 52.8 (ester OMe), 25.3 (CH_3).

*Methyl 4,6-*O*-[(S)-1-(3,4-dimethoxyphenyl)ethylidene]-α-*D*-mannopyranoside (**5**) and the 2,3-diacetate (**6**).* — Methyl α-D-mannopyranoside (2.0 g, 10.3 mmol) was added to a stirred solution of 3,4-dimethoxyacetophenone dimethyl acetal (4.65 g, 14.2 mmol) and *p*-toluenesulfonic acid (20 mg) in *N,N*-dimethylformamide (40 mL) at room temperature. After 5 h, sodium hydrogen carbonate (1.5 g) was added, and the mixture was stirred for 30 min, diluted with dichloromethane (50 mL), washed with water (3 × 50 mL), dried, and concentrated. The residue was chromatographed on silica gel (chloroform–methanol, 97:3) to give 3 fractions.

Fraction 1 (1.77 g, 33%) had $[\alpha]_D$ +19° (*c* 1.1, water) and was assigned the structure methyl 2,3:4,6-di-*O*-[1-(3,4-dimethoxyphenyl)ethylidene]-α-D-mannopyranoside from the ^{13}C-n.m.r. data: δ 108.1–148.8 (12 aromatic carbons), 108.8 (acetal C of 1,3-dioxolane), 101.4 (acetal C of 1,3-dioxane), 98.4 (C-1), 62.2 (C-6), 54.9–55.6 (5 OMe), 31.5 (CH_3 of dioxane), 27.5 (CH_3 of dioxolane).

Fraction 2 (1.26 g, 34%), which crystallized from ether–hexane and had m.p. 167–168°, $[\alpha]_D$ +93.5° (*c* 1.1, chloroform), was characterized as **5** from the following n.m.r. data: ^{1}H, 6.86–6.99 (m, 3 H, aromatic), 4.63 (d, 1 H, $J_{1,2}$ 1.08 Hz, H-1), 3.88, 3.87, 3.36 (3 s, each 3 H, 3 OMe), 1.55 (CH_3); ^{13}C, δ 109.9 149.4 (6 aromatic carbons), 102.2 (acetal C), 101.2 (C-1), 63.3 (C-6), 54.9, 55.9, 56.0 (3 OMe), 31.9 (acetal CH_3).

Anal. Calc. for $C_{17}H_{24}O_8$: C, 57.26; H, 6.78. Found C: C, 57.35; H, 6.91.

Fraction **3** (0.1 g, 3%), $[\alpha]_D$ +36.2° (*c* 0.9, chloroform), was assigned the structure methyl 2,3-*O*-[1-(3,4-dimethoxyphenyl)ethylidene]-α-D-mannopyranoside. N.m.r. data: ^{1}H, δ 6.80–7.10 (m, 3 H, aromatic), 5.03 (s, 1 H, H-1), 3.40, 3.87, 3.88 (3 s, each 3 H, 3 OMe), 1.62 (s, 3 H, CH_3); ^{13}C, δ 104.8–117.0 (3 aromatic C-H), 107.9 (acetal C of dioxolane), 62.5 (C-6), 55.1, 55.9, 56.0 (3 OMe), 28.4 (CH_3).

Treatment of **5** (1.26 g, 3.54 mmol) with acetic anhydride in pyridine afforded **6** (1.5 g, 96%), $[\alpha]_D$ +107° (*c* 1.1, chloroform). N.m.r. data: ^{1}H, δ 6.92–7.30 (m, 3 H, aromatic), 5.30 (m, 2 H, H-2,3), 4.55 (s, 1 H, H-1), 3.91, 3.90, 3.37 (3 s, each 3 H, 3 OMe), 2.07, 2.01 (2 s, each 3 H, 2 OAc), 1.53 (s, 3 H, CH_3); ^{13}C, δ 149.3–109.7 (6 aromatic C), 102.2 (acetal C), 99.5 (C-1), 63.4 (C-6), 55.9, 55.7, 55.0 (3 OMe), 31.8 (acetal CH_3), 20.8, 20.7 (2 OAc).

Anal. Calc. for $C_{21}H_{28}O_{10}$: C, 57.26; H, 6.40. Found: C, 57.03; H, 6.41.

Methyl 2,3-di-O-acetyl-4,6-O-[(S)-1-methoxycarbonylethylidene]-α-D-mannopyranoside (**7**). — Oxidation and esterification of **6** (500 mg, 1.13 mmol), as described in the synthesis of **3**, furnished **7** (243 mg, 59%), $[\alpha]_D$ +61° (*c* 1, chloroform). N.m.r. data: ^{1}H, δ 5.27 (m, 2 H, H-3), 4.60 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 3.85 (s, 3 H, ester OMe), 3.38 (s, 3 H, OMe), 2.12, 2.05 (2 s, each 3 H, 2 OAc), 1.51 (s, 3 H, CH_3); ^{13}C, δ 170.1, 170.0, 169.8 (CO_2Me, 2 CH_3CO), 99.6 (C-1), 99.4 (acetal C), 55.1 (OMe), 52.5 (ester OMe), 25.4 (acetal CH_3), 20.7 (2 OAc).

Anal. Calc. for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.11. Found: C, 50.25; H, 6.22.

Methyl 4,6-O-[(S)-1-methoxycarbonylethylidene]-α-D-mannopyranoside (**8**). — Catalytic deacetylation of **7** (129 mg, 0.29 mmol) conventionally with sodium methoxide in methanol afforded **8** (89 mg, 90%), which crystallized from ether and had m.p. 119–120°, $[\alpha]_D$ +79° (*c* 1, chloroform). N.m.r. data: ^{1}H, δ 4.71 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 3.83 (s, 3 H, ester OMe), 3.37 (s, 3 H, OMe), 2.77, 2.72 (2 s, 1 H each, 2 OH, exchangeable by D_2O), 1.54 (s, 3 H, CH_3); ^{13}C, δ 170.7 (CO_2Me), 101.4 (C-1), 99.5 (acetal C), 65.2 (C-6), 54.9 (OMe), 52.8 (CO_2CH_3), 25.4 (acetal CH_3).

Anal. Calc. for $C_{11}H_{18}O_8$: C, 47.48; H, 6.51. Found: C, 47.76; H, 6.61.

Methyl 2,3-di-O-benzoyl-4,6-O-[(R)-1-(3,4-dimethoxyphenyl)ethylidene]-β-D-galactopyranoside (**9**). — Methyl 2,3-di-*O*-benzoyl-β-D-galactopyranoside[20] (200 mg, 0.5 mmol) was treated with 3,4-dimethoxyacetophenone dimethyl acetal, as described in the synthesis of **1**. Chromatography of the product on silica gel (benzene–ethyl acetate, 94:6) furnished **9** (110 mg, 40%), $[\alpha]_D$ −10° (*c* 1, chloroform). N.m.r. data: ^{1}H, δ 8.10–7.33 (m, 10 H, 2 Ph), 6.90–6.70 (m, 3 H, aromatic), 5.93 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.3 Hz, H-2), 5.30 (dd, 1 H, $J_{3,4}$ 3.6 Hz, H-3), 4.65 (d, 1 H, H-1), 3.83, 3.57, 3.40 (3 s, 3 H each, 3 OMe), 1.63 (s, 3 H, CH_3); ^{13}C, δ 165.7, 165.2 (2 CO), 149.2–109.0 (aromatic), 101.8 (C-1), 101.1 (acetal C), 63.1 (C-6), 56.5, 55.7, 55.0 (3 OMe), 32.1 CH_3).

Anal. Calc. for $C_{31}H_{32}H_{10}$: C, 65.95; H, 5.70. Found: C, 65.92; H, 5.52.

*Methyl 2,3-di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonylethylidene]-β-D-galactopyranoside (**10**).* — Oxidation and esterification of **9** (205 mg, 0.36 mmol), as described for the synthesis of **3**, gave **10** (117 mg, 66%), $[\alpha]_D$ +90° (*c*, 1.1, chloroform). N.m.r. data: ^1H, δ 8.01–7.35 (3 m, 10 H, 2 Ph), 5.81 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.3 Hz, H-2), 5.21 (dd, 1 H, $J_{3,4}$ 3.6 Hz, H-3), 4.62 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.65 (s, 3 H, ester OMe), 3.55 (s, 3 H, OMe), 1.60 (s, 3 H, CH_3); ^{13}C, δ 133.2–128.3 (aromatic), 102.0 (C-1), 98.8 (acetal C), 65.0 (C-6), 56.9 (OMe), 52.3 (ester OMe), 25.6 (CH_3).

Anal. Calc. for $C_{25}H_{26}O_{10}$: C, 61.72; H, 5.38. Found: 61.85; H, 5.16.

*Methyl 4,6-O-[(R)-1-methoxycarbonylethylidene]-β-D-galactopyranoside (**11**).* — Catalytic *O*-debenzoylation of **10** (90 mg, 0.18 mmol) with methanolic sodium methoxide followed by chromatography on silica gel (chloroform–methanol, 98.5:1.5) afforded **11** (27 mg, 59%), $[\alpha]_D$ −44° (*c* 0.8, chloroform); lit.[7] $[\alpha]_D$ −36° (chloroform). N.m.r. data: ^1H, δ 4.15 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.81 (s, 3 H ester OMe), 3.56 (s. 3 H, OMe), 1.59 (s, 3 H, CH_3); ^{13}C, δ 170.2 (CO), 103.7 (C-1), 98.7 (acetal C), 65.2 (C-6), 57.3 (OMe), 52.7 (ester OMe), 25.7 (CH_3).

2,3-Epoxypropyl 2,4,6-tri-O-acetyl-3-O-methyl-β-D-glucopyranoside (**13**). — 3-Chloroperoxybenzoic acid (2.9 g, 16.8 mmol) was added to a solution of allyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranoside[20] (**12**; 4.04 g, 11.2 mmol) in dichloromethane (80 mL), and the mixture was stirred at room temperature overnight. More oxidant (0.5 g, 2.9 mmol) was added and the mixture was kept for 24 h until no starting material remained. The solution was diluted with dichloromethane, washed successively with aqueous sodium hydrogen sulfite, aqueous sodium hydrogen carbonate, and water, dried, and concentrated. The residue (4.33 g) was separated by flash chromatography on silica gel (light petroleum–acetone, 7:3) to give syrupy **13** (3.7 g, 88%) as a mixture of diastereomers. N.m.r. data: ^1H, δ 4.88 (m, 2 H, H-2,4), 4.51 (4.56) [2 d, 1 H, $J_{1,2}$ 7.98 (8.00)* Hz, H-1], 3.38 (s, 3 H, OMe), 3.08 (m, 1 H, H′β), 2.50, 2.56, 2.71 (3 m, 2 H, H′γ), 2.00–2.11 (overlapping singlets, 9 H, 3 OAc); ^{13}C, δ 100.2 (100.0)* (C-1), 80.5 (C-3), 68.6 (69.8) (C′α), 61.5 (C-6), 58.5 (OCH_3), 49.2 (49.6) (C′β), 42.9 (43.0) (C′γ), 19.5, 19.6, 19.7 ($COCH_3$).

Anal. Calc. for $C_{16}H_{24}O_{10}$: C, 51.06; H, 6.38. Found: C, 51.08; H, 6.78.

One component of the mixture later crystallized from di-isopropyl ether and had m.p. 68–71°, $[\alpha]_D$ −126° (*c* 1.8, chloroform).

2,3-Epoxypropyl 2-O-acetyl-3-O-methyl-β-D-glucopyranoside (**14**). — A solution of the mixture of epoxides **13** (5.0 g, 13.3 mmol) in methanol (150 mL) was stirred with magnesium oxide (8.32 g) at room temperature for 72 h. The major component was purified by flash chromatography on silica gel (hexane–acetone, 7:3) to give **14** as a syrup (3.25 g, 84%), $[\alpha]_D$ −109° (*c* 1.5, chloroform). N.m.r. data: ^1H, δ 4.90 (dd, 1 H, $J_{2,3}$ 9.42 Hz, H-2), 4.43 (4.55) [2 d, 1 H, $J_{1,2}$ 7.95 (7.98) Hz, H-1], 4.08, 4.04, 4.02 (3 m, 2 H, H′γ of two isomers), 3.60 (m, 1 H, H-3), 3.53 (3.52) (2 s, 3 H, OMe), 3.15 (m 1 H, H′β), 2.80,

*Figures in parentheses denote resonances of the less abundant diastereomer.

2.60, 2.57 (3 m, 2 H, H′α of two isomers), 2.13 (2.12) (2 s, 3 H, OAc); ^{13}C, δ 101.7 (100.8) (C-1), 84.1 (83.9) (C-3), 70.5 (70.1) (C′α), 62.4 (62.0) (C-6), 59.8 (59.9) (OCH_3), 51.0 (50.8) (C′β), 44.4 (44.1) (C′γ), 20.9 ($COCH_3$).

Anal. Calc. for $C_{12}H_{20}O_8$: C, 49.32; H, 6.85. Found: C, 48.96; H, 6.56.

2,3-Epoxypropyl 2-O-acetyl-4,6-O-[(S)-1-(3,4-dimethoxyphenyl)ethylidene]-3-O-methyl-β-D-glucopyranoside (**15**). — Epoxy glycoside **14** (3.47 g, 11.9 mmol) was treated with 3,4-dimethoxyacetophenone dimethyl acetal (4 mL) in benzene (130 mL) with *p*-toluenesulfonic acid (5 mg), and the solution was concentrated to ~60 mL under diminished pressure at 60°, cooled, neutralized by the addition of triethylamine, and concentrated. A solution of the residue in dichloromethane was washed with water, dried, and concentrated to a syrup which was purified by flash chromatography on silica gel (light petroleum–acetone, 4:1) to give **15** (5.16 g, 96%) as a syrup, $[\alpha]_D$ −219° (*c* 1.2, chloroform). N.m.r. data: ^{1}H, δ 6.88–6.99 (m, 3 H, aromatic), 4.82 (m, 1 H, H-2), 4.44 (4.53) [2 s, 1 H, $J_{1,2}$ 7.94 (7.89) Hz, H-1], 3.90, 3.88 (2 s, each 3 H, OMe), 3.63 (3.64) (2 s, 3 H, OMe), 3.05 (m, 1 H, H′β), 2.49, 2.60, 2.72 (3 m, 2 H, H′γ), 2.11 (s, 3 H, OAc), 1.55 (s, 3 H, CH_3); ^{13}C, δ 169.5 ($OCOCH_3$), 109.7, 111.5, 119.1, 132.2, 148.7, 149.5 (aromatic), 101.8 (acetal C), 101.5 (101.0) (C-1), 81.0 (81.1) (C-3), 68.4 (70.0) (C′α), 63.2 (C-6), 60.3 (OMe), 55.8, 55.9 (2 OMe), 50.1 (50.5) (C′β), 44.1 (C′γ), 31.7 (acetal CH_3), 20.8 ($OCOCH_3$).

Anal. Calc. for $C_{22}H_{30}O_{10}$: C, 58.15; H, 6.61. Found: C, 58.58; H, 6.83.

2,3-Epoxypropyl 2-O-acetyl-4,6-O-[(S)-1-methoxycarbonylethylidene]-3-O-methyl-β-D-glucopyranoside (**16**). — A solution of **15** (140 mg, 0.39 mmol) in carbon tetrachloride–acetonitrile (9.4 mL, 1:1) was added dropwise at 5° to a well-stirred solution of sodium metaperiodate (8 g, 37 mmol) and ruthenium trichloride (8 mg) to which sodium carbonate (0.4 g) had been added. The mixture was stirred vigorously for 3 h at room temperature, the inorganic salts were removed, solid sodium hydrogen carbonate was added to the filtrate until the pH was 7.9, salts were removed, and the filtrate was extracted with dichloromethane but no neutral starting material or product was obtained. Acidification of the aqueous solution to pH 1, extraction with dichloromethane, drying, and concentration gave a residual syrup which was treated immediately and kept overnight with diazomethane in ether. Removal of solvent without further purification afforded **16** as a syrup (78 mg, 67%), $[\alpha]_D$ −19° (*c* 0.8, chloroform). N.m.r. data: ^{1}H, δ 4.91 (m, 1 H, H-2), 4.48 (4.58) [2 s, 1 H, $J_{1,2}$ 7.88 (7.75) Hz, H-1], 3.83 (s, 3 H, OMe), 3.52 (3.53) (2 s, 3 H, OMe), 3.10 (m, 1 H, H′β), 2.53, 2.63, 2.77 (3 m, 2 H, H′γ), 2.12 (s, 3 H, OAc), 1.55 (s, 3 H, CH_3); ^{13}C, δ 169.5, 169.1 ($OCOCH_3$ and CO_2CH_3), 101.8 (101.3) (C-1), 99.6 (acetal C), 80.3 (C-3), 69.3 (70.8) (C′α), 65.4 (C-6), 59.3 (OMe), 53.1 (OMe), 50.6 (51.1) (C′β), 44.9 (C′γ), 26.0 (acetal CH_3), 21.8 ($OCOCH_3$).

Anal. Calc. for $C_{16}H_{24}O_{10}$: 51.06; H, 6.28. Found: C, 51.22; H, 6.43.

Allyl 2-O-acetyl-4,6-O-[(S)-1-methoxycarbonylethylidene]-3-O-methyl-β-D-glucopyranoside (**17**). — Trifluoroacetic acid (8 μL) was added with stirring to **16** (130 mg, 0.35 mmol) and 3-methylbenzothiazole-2-selone (108 mg, 0.47 mmol) in dichloromethane at −10°. The solution was stirred for 2 h at room temperature with deposition of selenium as a red solid. The filtered solution was concentrated to a residue which was purified by flash chromatography on silica gel (light petroleum–acetone, 4:1) to give **17**

as a syrup (112 mg, 90%), $[\alpha]_D$ −82° (*c* 1, chloroform). N.m.r. data: ^{1}H, δ 5.82 (m, 1 H, –C*H*=CH$_2$), 5.21 (m, 2 H, –CH=C*H*$_2$), 4.94 (m, 1 H, H-2), 4.49 (d, 1 H, $J_{1,2}$ 7.85 Hz, H-1), 4.05 (m, 2 H, OCH$_2$–), 3.83 (s, 3 H, OMe), 3.52 (s, 3 H, OMe), 2.10 (s, 3 H, OAc), 1.55 (s, 3 H, CH$_3$); ^{13}C, δ 170.0, 169.4 (O*C*OCH$_3$, *C*O$_2$CH$_3$), 133.5 (*C*H$_2$=CH–), 117.3 (CH$_2$=*C*H–), 100.3 (C-1), 99.1 (acetal C), 80.0 (C-3), 69.8 (OCH$_2$–), 65.1 (C-6), 58.3 (OMe), 52.7 (OMe), 25.4 (acetal CH$_3$), 20.8 (OCO*C*H$_3$).

Anal. Calc. for $C_{16}H_{24}O_9$: C, 53.33; H, 6.67. Found: C, 53.09; H, 6.74.

*Allyl 4,6-O-[(S)-1-methoxycarbonylethylidene]-3-O-methyl-β-*D*-glucopyranoside* (**18**). — Sodium (0.2 mg) was added to a solution of **17** (198 mg, 0.55 mmol) in methanol (20 mL), and the solution was kept overnight, stirred with Dowex 50 (H$^+$) resin, filtered, and concentrated to give **18** (170 mg, 97%), $[\alpha]_D$ −71° (*c* 1.2, chloroform); the ^{1}H- and ^{13}C-n.m.r. data showed absence of resonances for OAc.

Anal. Calc. for $C_{14}H_{22}O_8$: C, 52.83; H, 6.92. Found: C, 53.02; H, 7.09.

Formation from bovine serum albumin of a neoglycoconjugate containing N-{*4,6-O-[(S)-1-carboxyethylidene]-3-O-methyl-β-*D*-glucopyranosyl-(1→2)-oxyethyl}lysine residues.* — A solution of **18** (102 mg) in methanol (25 mL) was treated overnight with 5M sodium hydroxide (0.5 mL), and the resulting solution was shaken with Dowex 50 (H$^+$) resin to remove sodium ions, and concentrated to give syrupy allyl 4,6-*O*-[(*S*)-1-carboxyethylidene]-3-*O*-methyl-β-D-glucopyranoside (**19**, 95 mg), $[\alpha]_D$ −93° (*c* 1.3, chloroform). The ^{1}H- and ^{13}C-n.m.r. data showed the presence of an equatorial acetal methyl substituent (δ_H 1.59, δ_C 25.3), and an absence of ester OMe.

A solution of **19** (10 mg) in methanol (30 mL) was ozonized for 10 min at −78°, then treated with dimethyl sulfide (15 μL) for 2 h, and concentrated to a syrup. The product (9 mg) and bovine serum albumin (15 mg) in 0.2M sodium phosphate buffer (2 mL, pH 7.8) were treated with sodium cyanoborohydride (15 mg) at 37° for 72 h. The resulting solution was passed through a column of Sephadex G-25 equilibrated in, and eluted with, 0.2M phosphate buffer at pH 7.8, and carbohydrate-rich fractions were dialyzed against distilled water and freeze-dried to give the neoglycoconjugate (18 mg), which contained 3.6% of carbohydrates as determined by the phenol–sulfuric acid reagent[26], using 3-*O*-methyl-D-glucose as reference.

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