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Preliminary communication

Synthesis of α-D-galactopyranosyl-linked oligosaccharides having an anomeric 4-nitrophenyl group by the use of a 2,6-di-*O*-(4-methoxybenzyl) derivative of D-galactose as a donor*

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Among the various reagents developed for α-glycosylation of D-glucose, D-galactose, and L-fucose[2], practically all of them possess a 2-*O*-benzyl group as a nonparticipating protecting group, which necessitates hydrogenolysis for its removal. As a result, the synthesis of oligosaccharides containing either 4-nitrophenyl or benzyl anomeric groups is not practical. There is a need for such 4-nitrophenyl or benzyl glycosides of oligosaccharides for rapid assays of glycosyltransferases and glycosidases. Moreover, these 4-nitrophenyl glycosides become unique chromogenic substrates for a search of endoglycosidases. Recently, we reported[3] the use of methyl 3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-1-thio-β-L-fucopyranoside as a glycosylating reagent for the synthesis of α-L-fucosylated oligosaccharides containing 4-nitrophenyl and benzyl aglycons. We report herein the use of a 2,6-di-*O*-(4-methoxybenzyl)derivative of D-galactose in similar synthesis.

Methyl 3,4-*O*-isopropylidene-2,6-di-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (**3**) was prepared in four steps from 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose. Treatment of this peracetate with (methylthio)trimethylsilane and trimethylsilyl triflate in dichloromethane produced a high yield (86%) of known methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside[4] (**1**). *O*-Deacetylation of **1** with methanolic sodium methoxide, followed with acetonation by the procedure of Catelani *et al.*[5], afforded known methyl 3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside[4] (**2**) in 70% yield. Alkylation of **2** with sodium hydride and 4-methoxybenzyl chloride in *N,N*-dimethylformamide gave methyl 3,4-*O*-isopropylidene-2,6-di-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (**3**) in 78.6% yield after silica gel column chromatography, $[\alpha]_D^{25}$

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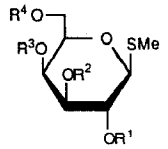
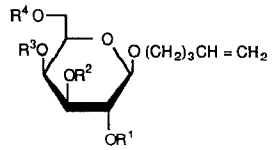
$-11.7°$ (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.15 (m, 4 H, arom.), 6.82 (d, $J \sim 9$ Hz, 4 H, arom.), 3.78 (s. 6 H, 2 OMe), 2.18 (s, 3 H, SMe), 1.43 and 1.33 (each s, 3 H, CMe). Similarly, pentenyl 3,4-*O*-isopropylidene-2,6-di-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (**6**) was prepared in four steps starting with 1,2,3,4-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[6]. Condensation of the bromide with 4-pentenol in the presence of active silver carbonate in dichloromethane gave a crude intermediate which, after *O*-deacetylation with sodium methoxide–methanol, provided pentenyl β-D-galactopyranoside (**4**) in 80% yield after silica gel column chromatography, $[\alpha]_D^{20}$ $-17.5°$ (methanol); ^{13}C-n.m.r. [$(CD_3)_2SO$]: δ 138.48 (CH=), 114.9 (=CH_2), and 103.56 (C-1). Isopropylidenation of **4**, to give **5**, followed by alkylation, as described for the preparation of **3**, afforded pentenyl 3,4-*O*-isopropylidene-2,6-di-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (**6**) in 76.5% yield, $[\alpha]_D^{25}$ $+18°$ (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.37–7.10 (m, 4 H, arom.), 7.13 (d, $J \sim 9$ Hz, 4 H, arom.), 6.07–5.54 (m, 1 H, =CH), 3.78 (s, 6 H, 2 OMe), 1.35 and 1.30 (each s, 3 H, CMe).

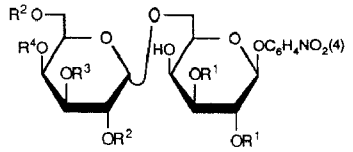
In an initial attempt to explore the utility of **3** as a glycosyl donor, a solution of **3** (0.65 g, 1.3 mmol) and 4-nitrophenyl 2,3-di-*O*-acetyl-β-D-galactopyranoside[7] (0.38 g, 1 mmol) in 5:1 (v/v) dichloroethane–*N*,*N*-dimethylformamide (36 mL) was stirred in the



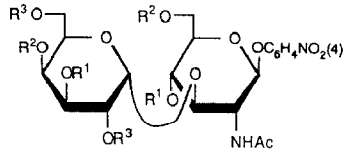
1 $R^1 = R^2 = R^3 = R^4 =$ Ac
2 $R^1 = R^4 =$ H; $R^2, R^3 = CMe_2$
3 $R^1 = R^4 =$ 4-MeOBn; $R^2, R^3 = CMe_2$



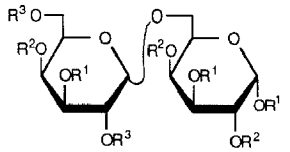
4 $R^1 = R^2 = R^3 = R^4 =$ H
5 $R^1 = R^4 =$ H; $R^2, R^3 = CMe_2$
6 $R^1 = R^4 =$ 4-MeOBn; $R^2, R^3 = CMe_2$



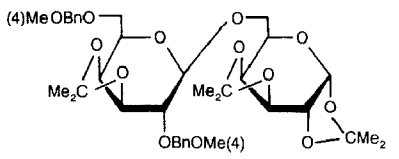
7 R^1 = Ac; R^2 = 4-MeOBn; $R^3, R^4 = CMe_2$
8 $R^1 = R^2 = R^3 = R^4 =$ H



9 $R^1, R^2 = CMe_2$; R^3 = 4-MeOBn
10 $R^1 = R^2 = R^3 =$ H



11 $R^1, R^2 = CMe_2$; R^3 = 4-MeOBn
12 $R^1 = R^2 = R^3 =$ H



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presence of $CuBr_2$–Bu_4NBr (2 mmol each) and 4A molecular sieves (4 g) for 16 h. at room temperature. Further amounts of **3** (0.32 g, 0.65 mmol) and $CuBr_2$–Bu_4NBr (1 mmol each) were added, and the stirring was continued for another 16 h. Examination of the mixture by t.l.c. with 2:3 (v/v) ethyl acetate–hexane revealed the presence of a major product migrating faster than the starting material. The mixture was filtered through Celite, the solids were thoroughly washed with chloroform, and the filtrate and washings were combined, successively washed with saturated aqueous $NaHCO_3$, water, dried, and concentrated. The residue was purified in a silica gel column to afford, in 44% yield, the protected, α-linked disaccharide **7**, $[\alpha]_D^{25}$ +25° (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.17–8.07 (d, $J \sim 9$ Hz, 2 H, arom.), 7.30–7.05 (m, 6 H, arom.), 6.80 (d, $J \sim 9$ Hz, 4 H, arom.), 5.05 (d, $J \sim 3$ Hz, 1 H, H-1′), 3.77 (s, 6 H, 2 OMe), 2.09 and 2.03 (each s, 3 H, OAc), 1.37 and 1.28 (each s, 3 H, CMe). *O*-Deacetylation of **7** (0.15 g) in methanolic sodium methoxide was followed by treatment of the intermediate in chloroform solution (30 mL) containing trifluoroacetic acid (1.5 mL) and water (0.3 mL) for 2 h at room temperature, and then evaporation to dryness. The residue was dissolved in methanol and addition of ether caused the precipitation of amorphous **8** (0.05 g, 60%), $[\alpha]_D^{25}$ +19° (water); ^{13}C-n.m.r., see Table I. A similar condensation of 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose with **3** afforded the α-linked D-galactosyl derivatives, **9** and **11**, respectively, further deprotected to provide **10** and **12**. The ^{13}C-n.m.r. spectra of the final compounds (**8**, **10**, and **12**) were in agreement with the structures assigned.

TABLE I

Compd.[a]	*Yield (%)*	$[\alpha]^{25}$,[b] *(degree)*	*Partial*	^{13}*C-n.m.r. data (δ)*
8	60	+19	98.36	(C-1), 96.86 (C-1′), and 65.15 (C-6)
10	39[c]	+67.5	98.41	(C-1), 97.67 (C-1′), and78.25 (C-3)
12	87	+131.5	97.73	(C-1′), 95.69 (C-1β), 91.57 (C-1α), 65.92 (C-6α), 65.69 (C-6β), and 60.25 (C-6′)

[a] All products listed gave satisfactory elemental analyses. [b] For a solution in water. [c] On the basis of 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside.

In the study of the glycosylating capability of donor **6** for synthesizing α-linked D-*galacto*-oligosaccharides, **6** (0.87 g, 1.6 mmol) and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (0.4 g, 1.5 mmol) were dissolved in dry dichloromethane (30 mL) under Ar. Powdered 4A molecular sieve and iodonium di(2,4,6-trimethylpyridine) perchlorate (1.7 g, 3.6 mmol) were added, and the mixture was stirred for 2 h at room temperature, and then filtered through Celite. The organic layer was washed with saturated $NaHCO_3$, dried, and concentrated under reduced pressure. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 20–25% ethyl acetate in hexane. The earlier fractions collected contained the faster migrating α-D anomer **11**

(0.15 g, 21.4%), $[\alpha]_D^{25}$ +19.5° (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.38–7.18 (m, 4 H, arom.), 6.80 (d, $J \sim 9$ Hz, 4 H, arom.), 5.50 (d, $J \sim 4$ Hz, 1 H, H-1), 4.88 (d, $J \sim 3.5$ Hz, 1 H, H-1'), 3.80 (s, 6 H, 2 OMe), and 1.45–1.21 (m, 18 H, 3 CMe). The latter fractions contained the pure β-D anomer **13** (0.45 g, 64%), $[\alpha]_D^{20}$ −8.5° (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.36–7.16 (m, 4 H, arom.), 6.95–6.73 (m, 4 H, arom.), 5.53 (d, $J \sim 4$ Hz, 1 H, H-1), 4.45 (d, $J \sim 9$ Hz, 1 H, H-1'), 3.78 (s, 6 H, 2 OMe), and 1.53–1.20 (m, 18 H, 3 CMe_2). When this reaction was carried out in 4:1 (v/v) ether–dichloromethane, the stereoselectivity was reversed and the α (**11**) and β anomer (**13**) were obtained in a 8:5 ratio. Condensation of 4-nitrophenyl 2,3-di-*O*-acetyl-β-D-galactopyranoside and 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside with glycosyl donor **6** again afforded a mixture of α- and β-linked derivatives.

In conclusion, the condensation of a thioglycoside having a nonparticipating group at O-2 in the presence of $CuBr_2$–Bu_4NBr complex as a promotor afforded, at room temperature, 1,2-*cis*-glycosylation products with complete stereoselectivity. In contrast to the case of the pentenyl glycosyl donor, as reported by Mootoo *et al.*[8], activation with iodonium di (2,4,6-trimethylpyridine) perchlorate yielded a mixture of the corresponding anomers. With the pentenyl donor **6**, the ratio of disaccharide anomers was found to depend on the solvent, dichloromethane favoring formation of the β anomer, and ether that of the α anomers[8].

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