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Note

Efficient preparation of allyl 2,3,6,2′,3′,6′-hexa-*O*-benzyl-β-lactoside and its use as a glycosyl acceptor for chain extension at O-4′

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A lactose unit extended at position 3′ or 4′ is frequently found in the oligosaccharide moieties of glycoconjugates, particularly the glycosphingolipids [1], which include the gangliosides [2]. Knowledge of the importance of these glycoconjugates as antigens and recognition structures on cell surfaces [1–3] has stimulated interest in the synthesis of partial structures and analogues, and thus a demand for protected lactosides as starting materials. Protection, which must be complete except for OH-4′ when that position is to be substituted, has been accomplished with acyl (acetyl, benzoyl) groups, which can give satisfactory results [4], or with benzyl ether groups. The perception that *O*-benzyl groups provide a more ''friendly environment'' for glycosyl coupling reactions [5–9] has focussed attention on the benzyl ethers. Thus, preparations of 2,3,6,2′,3′,6′-hexa-*O*-benzyl-β-lactosides have been accomplished: (*a*) from 3′,4′- or 4′,6′-*O*-alkylidene lactosides (or mixtures of the two) by succesive benzylation, dealkylidenation, and selective benzylation of the resulting diol (methyl [5], benzyl [10,11], and allyl [12] as aglycons); or (*b*) from the 4′,6′-*O*-benzylidene lactosides by successive benzylation and reduction with cyanoborohydride-hydrogen chloride [13] (methyl [14] and trimethylsilylethyl [15] as aglycons). We have now used the latter approach for the preparation of allyl 2,3,6,2′,3′,6′-hexa-*O*-benzyl-β-lactoside (**7**), and record the details here. The synthesis is one step shorter than that via the 3′,4′-*O*-isopropylidene derivative reported by Youssef et al. [12], and it gave a crystalline product. We also record the use of the benzylated allyl lactoside as an acceptor for glycosylation with the differentially protected glucosamine **8**, which we described in a

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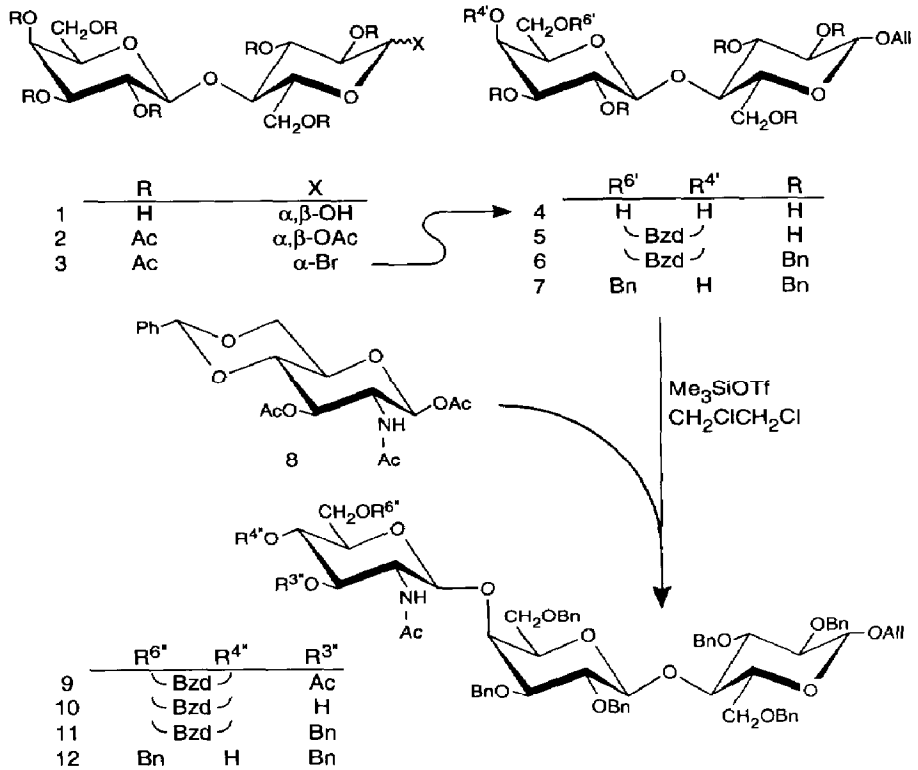
previous paper [16]. For the synthesis of **7**, lactose was acetylated using ferric chloride in acetic anhydride [17], and the acetobromolactose (**3**) obtained from the octaacetate (**2**) was converted into allyl β-lactoside (**4**) by reaction with allyl alcohol in the presence of mercuric oxide and mercuric bromide [18], followed by *O*-deacetylation. The overall yield from lactose was 84%. Benzylidenation with benzaldehyde and fused zinc chloride gave allyl 4′,6′-*O*-benzylidene-β-lactoside (**5**), and benzylation of this to give **6** followed by reductive debenzylidenation afforded the desired allyl 2,3,6,2′,3′,6′-hexa-*O*-benzyl-β-lactoside (**7**). The yields in these three steps were 89, 68, and 65%, respectively; probably the last two could be improved by careful attention to detail.



A test of the reactivity of **7** with an excess of the glucosamine donor 2-acetamido-1,3-di-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranose (**8**) [16] was conducted in refluxing 1,2-dichloroethane with trimethylsilyl trifluoromethanesulfonate as the catalyst. Addition of a second portion of donor midway in the 24-h reaction period partially compensated for the exhaustion of that component through side reactions, and enabled the formation of the protected trisaccharide **9** in ~55% yield, based on acceptor charged. The unreacted acceptor was recovered quantitatively. *O*-Deacetylation of **9** followed by benzylation afforded **11** (>95%), and this on reductive opening of the acetal ring (Garegg) gave a hydroxy compound (**12**).

Acetylation of **12** caused the appearance in the ^{1}H NMR spectrum of a triplet at δ 5.1 (J ~9 Hz), confirming the 4″ position of the free OH group. The ^{13}C NMR spectrum of the acetylated derivative contained signals characteristic of both the donor and acceptor moieties. Thus, **12** is a glucosamine analogue, protected, of the important glycosphingolipid

building unit gangliotriose [2]. Transformation of the *N*-acetylglucosamine unit of **12** into the *N*-acetylgalactosamine unit of the natural structure could be easily accomplished at this stage, and the product would be amenable to further synthetic manipulation.

1. Experimental

General methods.—The instrumental and chromatographic procedures employed were as previously described [16]. The following solvents were used for chromatography: *A*, 3:1, and *B*, 20:1 toluene–acetone; *C*, 8:1:1, *D*, 40:10:1, *E*, 200:8:1, and *F*, 300:8:1 toluene–acetone–MeOH; *G*, 1:1, and *H*, 2:1 hexane–EtOAc.

Allyl O-*β*-D-*galactopyranosyl-(1 → 4)-β*-D-*glucopyranoside (allyl β-lactoside)* (**4**).—Lactose monohydrate (**1**, 10 g) was added portionwise to a stirred solution of ferric chloride (600 mg) in Ac_2O (10 mL), maintained at 23–30°C. Extractive workup as previously described [17] afforded the octaacetate (**2**) as a syrup (18.5 g, 98%, R_f 0.34 in solvent *A*). This product was dissolved in CH_2Cl_2 (50 mL) and treated with 30% HBr in AcOH (15 mL) to give hepta-*O*-acetyllactosyl bromide (**3**, 19 g, R_f 0.42 in solvent *A*), readily isolated as a solid after the usual washing and evaporation of the organic layer. The bromide **3** (10 g) was treated with allyl alcohol (30 mL) in 1:1 toluene–nitromethane (50 mL) in the presence of HgO (4 g), $HgBr_2$ (0.5 g), and 3A molecular sieves (10 g). After 1 h, TLC showed complete conversion into a single product (R_f 0.5, solvent *A*). The mixture was filtered and the filtrate was thoroughly washed with satd aq KI, dried ($MgSO_4$), and concentrated. The solid residue was dissolved in dry MeOH (50 mL), and a small piece of sodium metal was added. After completion of the deacetylation the solution was neutralized [Amberlite IR 120 (H^+) resin], filtered, and concentrated. The solid that separated was recovered by filtration, washed with cold MeOH, and dried to give **4** (4.7 g, 86%); mp 173–174°C; $[\alpha]_D^{25}$ −1.0°, $[\alpha]_{436}^{25}$ −4.8° (*c* 2.5, H_2O); lit. mp 171–172°C, $[\alpha]_D^{20}$ +16° [19]; mp 168–170°C, $[\alpha]_{24}^{D}$ +2.1° [20]. Anal. Calcd for $C_{15}H_{26}O_{11}$ (382.36): C, 47.12; H, 6.85. Found: C, 46.49; H, 6.79. The slightly low carbon value suggests the retention of a little MeOH in the sample.

Allyl O-*(4,6-*O*-benzylidene-β*-D-*galactopyranosyl)-(1 → 4)-β*-D-*glucopyranoside (allyl 4′,6′-*O*-benzylidene-β-lactoside)* (**5**).—Compound **4** (4.4 g) was stirred with benzaldehyde (45 mL) and fused zinc chloride (4 g) at room temperature for 8 h, then the mixture was poured into hexane (350 mL) with vigorous stirring. The hexane layer was decanted from the gummy product, which was triturated with additional hexane (2 × 300 mL). The sticky mass was then dissolved in dry acetone (~10 mL, minimum volume), and the solution was left overnight at 0°C, whereupon finely divided compound **5** precipitated. Recovery by filtration, washing with cold acetone, and drying under vacuum gave **5** (4.8 g); mp 230–233°C; $[\alpha]_D^{25}$ −29°, $[\alpha]_{436}^{25}$ −58.7° (*c* 2.5, Me_2NCHO). A further 200 mg of **5** was isolated from the mother liquor, making the total yield 5.0 g (92%). Anal. Calcd for $C_{22}H_{30}O_{11}$ (470.47): C, 56.16; H, 6.43. Found: C, 55.7; H, 6.77.

Allyl O-*(2,3,6-tri-*O*-benzyl-β*-D-*galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β*-D-*glucopyranoside (allyl 2,3,6,2′,3′,6′-hexa-*O*-benzyl-β-lactoside)* (**7**).—The benzylation of **5** (3 g) was carried out by the usual method, using NaH and benzyl bromide in DMF [21]. Purification on a column of silica gel (packed dry, eluted with solvent *B*) gave a syrupy

material. On the addition of ether compound **6** (4 g) separated as a solid; mp 138–140°C; $[\alpha]_D^{25}$ +14° (*c* 1.1, $CHCl_3$).

A portion of the **6** (2 g) was dissolved in oxolane (25 mL) containing 3A molecular sieves (5 g), and the solution was stirred 45 min, then treated with $NaCNBH_3$ (1.6 g) and HCl according to the reported procedure [13]. TLC (solvent *A*) after 45 min indicated only partial conversion (~50%) into a product. The mixture was worked up and the reaction was repeated with fresh $NaCNBH_3$ (1.5 g), whereupon complete conversion took place. The crude residue (~3.0 g) from the subsequent workup was charged to a dry column of silica gel (100 g) and eluted with solvent *G* to afford pure **7** (1.3 g, 65%). Trituration of the syrupy product with hexane resulted in crystalline material; mp 108–109°C; $[\alpha]_D^{25}$ +19° (*c* 2.6, $CHCl_3$); lit. $[\alpha]_D$ +14° (CH_2Cl_2) [12]; ^{1}H NMR: δ 4.03 (br d, 1 H, *J* 3.0 Hz, H-4′); ^{13}C NMR (HETCOR): δ 139.1, 138.6 (2 C), 138.3, 138.2, 137.9 (6 C-1 of Ph), 134.1 (*C*H=CH_2), 117.2 (CH=*C*H_2), 102.7, 102.6 (C-1,1′), 75.4, 75.3, 75.0, 73.5, 73.1, 72.0 (6 C, Ph*C*H_2), 70.2 (O*C*H_2CH=), 68.4, and 68.3 (C-6,6′); additional signals at 82.9, 81.8, 81.1, 79.4, 76.6, 75.1, 72.7, and 66.1. Anal. Calcd for $C_{57}H_{62}O_{11}$ (923.11): C, 74.16; H, 6.77. Found: C, 74.47; H, 6.74.

A small sample of **7** was treated with Ac_2O–pyridine, and the ^{1}H NMR spectrum of the product was examined. Acetylation shifted the signal for H-4′ downfield to δ 5.58.

Allyl O-*(2-acetamido-3,6-di-*O-*benzyl-2-deoxy-β-*D-*glucopyranosyl)-(1→4)-*O-*(2,3,6-tri-*O-*benzyl-β-*D-*galactopyranosyl)-(1→4)-2,3,6-tri-*O-*benzyl-β-*D-*glucopyranoside* (**12**).—Compound **7** (170 mg, 184 mmol) and the donor [16] (**8**; 200 mg, 508 mmol) [1] were transferred into a small round-bottomed flask and dried [22], and 1,2-dichloroethane (8.0 mL), 3A molecular sieves (1 g), and 1,1,3,3-tetramethylurea (100 μL) were added. The suspension was stirred 1 h under N_2 at room temperature and cooled (0–5°C), and trimethylsilyl triflate (98 μL) was injected into it. After 10–15 min stirring at room temperature, the mixture was heated under reflux for a total of 24 h, with the addition of more donor (156 mg, 396 mmol), tetramethylurea (50 μL), and trimethylsilyl triflate (50 μL) at room temperature after 12 h. After cooling, pyridine (800 μL) was added, and the mixture was filtered. The residue was washed with CH_2Cl_2 (5 mL), and the total filtrate was washed successively with aq $NaHCO_3$ and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated to a syrup, which was charged to a dry column of silica gel (20 g) and eluted with solvent *E* (120 mL) and then solvent *F*. This gave first unreacted **7** (~80 mg, identified by its ^{1}H NMR spectrum), then the pure trisaccharide **9** (125 mg, 54% based on **7**), R_f 0.67 in solvent *G*; $[\alpha]_D^{25}$ −5.5° (*c* 1.8, $CHCl_3$); ^{13}C NMR: δ 170.5, 169.5 (2 *C*O), 134.1 (*C*H=CH_2), 116.8 (CH=*C*H_2), 103.3, 102.6 (2 C) (C-1, 1′,1″), 101.4 (Ph*C*H), 54.8 (C-2″), 22.9 (NHCO*C*H_3), 20.7 (OCO*C*H_3), and other signals at 82.5, 81.8, 80.4, 78.5, 77.6, 76.9, 75.3, 75.1, 74.9, 74.2, 73.1, 72.8, 70.1, 68.6, 68.2, and 66.8.

For deacetylation, compound **9** (122 mg) was dissolved in abs MeOH (4.0 mL) and treated with NaOMe (5.0 mg). TLC (solvent *D*) showed the conversion of the starting material, R_f 0.68, into a single product, R_f 0.58. The mixture was neutralized [IR 120 (H^+) resin], diluted with $CHCl_3$ (10 mL), and filtered. The organic layer was washed with water,

[1] For the successful preparation of **8** its precursor, 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranose, must be preponderantly in the β-anomeric form.

dried (Na_2SO_4), filtered, and concentrated to give the 3″-OH derivative **10**; $[\alpha]_D^{25}$ −9.2° (*c* 2.5, $CHCl_3$).

This product in DMF (4.0 mL) was immediately treated with benzyl bromide (60 mL) in the presence of BaO (0.45 g) and $Ba(OH)_2 \cdot 8H_2O$ (0.22 g). The mixture was stirred overnight at room temperature, then diluted with $CHCl_3$ (5 mL), filtered, and concentrated to an oil (0.3 g) containing a product having R_f 0.76 in solvent *D*. This oil was charged to a dry column of silica gel (60 g) and eluted with 100:10:1 toluene–acetone–MeOH to give allyl *O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**11**) as a syrup (120 mg, 95% from **9**); $[\alpha]_D^{25}$ +18° (*c* 2.5, $CHCl_3$); ^{13}C NMR: δ 169.6 (*C*O), 134.2 (*C*H=CH_2), 117.1 (CH=*C*H_2), 102.68, 102.6, 102.4 (C-1, 1′,1″), 101.2 (Ph*C*H), 56.5 (C-2″), and 23.4 (NHCO*C*H_3).

Compound **11** (120 mg) was dissolved in oxolane (2.5 mL) and treated with $NaCNBH_3$ (85 mg) and HCl in ether (see above). After 1 h TLC (solvent *H*) showed nearly complete conversion into a single product. The mixture was diluted with CH_2Cl_2 (10 mL) and filtered, and the filtrate was washed with aq $NaHCO_3$, then water, and dried ($MgSO_4$). Filtration and concentration afforded a syrup (130 mg) which on elution from a silica gel column (35 g, packed dry) with solvent *G* (40 mL) and solvent *H* afforded pure **12** (syrup, 120 mg, quant.) $[\alpha]_D^{25}$ +17° (*c* 4.7, $CHCl_3$). Trituration with hexane afforded a fine powder; mp 108–109°C; $[\alpha]_D^{25}$ +22° (*c* 4.0, $CHCl_3$); ^1H NMR: δ 7.5–7.2 (m, Ph-H), 5.95 (m, 1 H, C*H*=CH_2), 5.45 (d, 1 H, N*H*), 5.35–5.15 (m, CH=CH_2), 5.0–4.8 (3 d, $J_{1,2}$ ~8.0 Hz, H-1, 1′,1″), 2.63 (br s, 1 H, O*H*), and 1.7 (s, 3 H, NHCOCH_3); ^{13}C NMR: δ 169.7 (*C*O), 134.1 (*C*H=CH_2), 117.0 (CH=*C*H_2), 102.6, 102.45, 102.1 (C-1, 1′,1″), and 23.3 (NHCO*C*H_3). Anal. Calcd for $C_{79}H_{87}NO_{16}$ (1306.56): C, 72.62; H, 6.71. Found: C, 72.58, H, 6.77.

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