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Preliminary communication

A facile and regioselective synthesis of partially benzoylated 3′,4′-*O*-isopropylidene-β-lactosides as standardized key intermediates for sialyl Lewis X (sLe^X) analogues

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Cell-surface glycoconjugates may act as cell–cell recognition molecules via specific binding between carbohydrates on one cell and protein receptors on an opposing cell, or via specific interactions directly between carbohydrates on opposing cells[1]. Adhesion of cells in the immune system, therefore, has therapeutic potential when considering such indications as cancer, microbial infection, and inflammatory, allergic, or autoimmune diseases[2]. The adhesion of leukocytes to platelets or vascular endothelium is thought to be mediated by the selectin family of glycoproteins[3]. Adhesion is an early step in leukocyte extravasation which subsequent pathophysiology includes thrombosis, and inflammation[4,5]. Three protein receptors, E-, L-, and P-selectins are assigned to the selectin family based on their cDNA sequences. Defining the native carbohydrate ligands for each selectin receptor is currently the object of intense effort. Several laboratories have described the ligands for E-, L, and P-selectin as the sialyl Lewis X epitope **1** (sLe^X) (Scheme 1)[6–8].

Our strategy for the synthesis of sLe^X tetrasaccharide and its analogues as potential therapeutic agents was first to prepare partially protected lactoside building blocks having a free hydroxyl group at O-3 and O-3′. Then, the resulting derivatives would only require fucosylation at position-3 and sialylation at position-3′, or subsitution with the corresponding isosteres.

In the initial stage of the synthesis (see Scheme 1), catalytic *O*-deacetylation of allyl[9], benzyl[10], methoxyethyl[11], and 2-(trimethylsilyl)ethyl[12] hepta-*O*-acetyl-β-lactoside afforded the allyl[13], benzyl[10], methoxyethyl *, and 2-(trimethylsilyl)ethyl[14] 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosides (**2a–d**). Treatment of these β-lactosides with 2,2-dimethoxypropane in the presence of camphorsulfonic acid[15]

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* See footnote on the following page.

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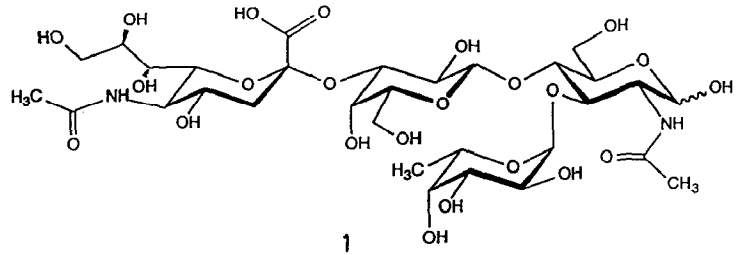

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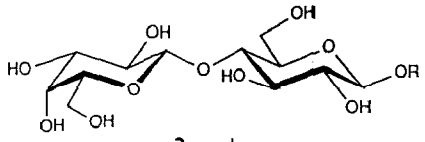
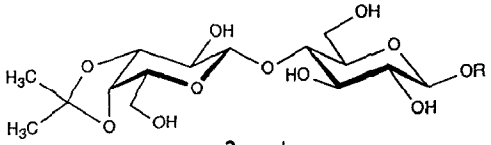


2a–d



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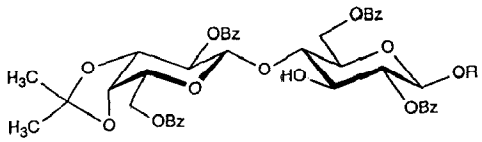

4a–d

Scheme 1. Series a, R = allyl; Series b, R = benzyl; Series c, R = $CH_2CH_2OCH_3$; Series d, R = $CH_2CH_2SiMe_3$; Bz = benzoyl.

gave allyl[9], benzyl[10], methoxyethyl *, and 2-(trimethylsilyl)ethyl * 4-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranosides (**3a–d**).

General procedure for regioselective benzoylation.—The 3′,4′-*O*-isopropylidene-β-lactoside derivative was dissolved in abs pyridine (20 mL per g), and the mixture was reacted with 4.2 mol equiv of benzoyl chloride[16] at −45°C for 3–4 h. It was then poured into $CHCl_3$, and the $CHCl_3$ solution was washed with 5% HCl, water, 5% $NaHCO_3$ and water. Evaporation of the dried (Na_2SO_4) $CHCl_3$ solution under diminished pressure gave the crude product. Crystallization from hot MeOH gave a single, crystalline tetrabenzoate (**4a–d**), together with some pentabenzoate with a yield of 68–78%. This product was used as a convenient starting material for the synthesis of sLe^X analogues. For identification purposes a portion of the product was chromatographed on a column of silica gel using 20:1 toluene–acetone as the eluent.

* An independent synthesis of these compounds will be described elsewhere.

TABLE I

^{1}H NMR data for key intermediates **4a–d** [a]

Compound	^{1}H Chemical shifts, δ (multiplicity, coupling constants, Hz) [b]							
	H-1′	H-2′	H-1	H-2	H-3	HO-3	H-4	R
4a	4.65 (d, 8.2)	5.36 (t, 7.8)	4.59 (d, 8.1)	5.25 (t, 8.2)	4.10 (dt, 8.1)	4.62 (d, 1.5)	3.75 (t, 8.1)	5.87–5.64 (m, 1 H, =CH–), 5.18–5.02 (m, 2 H, CH_2=), 4.49–3.96 (m, OCH_2–CH=)
4b	4.67 (d, 8.0)	5.35 (t, 7.8)	4.56 (d, 8.1)	5.30 (t, 8.0)	3.93 (dt, 9.6)	4.61 (d, 1.4)	3.75 (t, 9.5)	7.15 (m, 5 H, Ph–H), 4.60 (AB, 12.7 Hz, Ph–CH_2)
4c	4.67 (d, 8.3)	5.37 (t, 8.1)	4.66 (d, 8.1)	5.23 (t, 8.2)	4.01 (bt, 8.1)	4.64 (bs)	3.73 (t, 8.2)	3.88–3.35 (m, 4 H, OCH_2–CH_2), 3.11 (s, 3 H, –CH_3)
4d	4.82 (d, 8.2)	5.53 (t, 7.7)	4.71 (d, 8.1)	5.39 (t, 8.4)	4.16 (bt, 8.3)	4.78 (bs)	3.91 (t, 8.2)	3.71–3.58 (m, 2 H, OCH_2–CH_2), 1.10–0.85 (m, 2 H, –CH_2–Si), 0.19 [s, 9 H, –$Si(CH_3)_3$]

[a] Recorded on a Varian Gemini 300 MHz spectrometer at ambient temperature. [b] Determined in $CDCl_3$, with Me_4Si as the internal standard. Multiplicitier (d, doublet; s, singlet; t, triplet; b, broad) and spin–spin coupling constants appear in parentheses.

Structure elucidation. [†]—The tetrabenzoate derivatives (**4a–d**) were readily characterized as the desired lactoside building blocks by their ^{1}H NMR spectra, which showed two CH triplets downshifted to δ 5.53–5.35 and 5.39–5.23, respectively, deshielded by a geminal acyloxy group. Their resonances were identified by decoupling experiments as the signals for H-2′ and H-2, respectively. The signal for H-3 was found at δ 4.16–3.93, indicating that O-3 was not acylated. Therefore, the two benzoyl groups must be at positions-6 and -6′. We also found that the order of esterification of the five hydroxyl groups of 3′,4′-*O*-isopropylidene-β-lactoside derivatives is HO-6,6′ > HO-2,2′ > HO-3. All the key intermediates (**4a–d**) produced a common positive-ion, *m/z* 780.5, due to $(M - R)^+$.

*Allyl 2,6-di-*O*-benzoyl-4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**4a**).—Following the above general procedure, **3a** (ref. 13) yielded **4a** (78%) as fine needles: mp 191–193°C; $[\alpha]_D^{22}$ +21.6°, $[\alpha]_{436}^{22}$ +39.8° (*c* 1.3, $CHCl_3$); positive-ion LSIMS: *m/z* 861.0 $(M + Na)^+$; negative-ion LSIMS: *m/z* 991.4 $(M + m\text{-NBA})^-$ and 884.0 $(M + NO_2)^-$.

*Benzyl 2,6-di-benzoyl-4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**4b**).—The isopropylidene derivative (**3b**) was treated with benzoyl chloride according to the general procedure, affording the title compound **4b** (76%) as needles: mp 159–161°C; $[\alpha]_D^{22}$ −3.8°, $[\alpha]_{436}^{22}$ −11.3° (*c*, 0.71, $CHCl_3$); positive-ion LSIMS: *m/z* 889.7 $(M + H)^+$; negative-ion LSIMS: *m/z* 934.1 $(M + NO_2)^-$ and 1041.1 $(M + m\text{-NBA})^-$.

[†] All compounds described herein were purified to homogeneity by recrystallization or by chromatography on columns of silica gel when necessary. All compounds were characterized by ^{1}H NMR spectroscopy (see Table I). New compounds obtained in this study gave elemental analyses data in agreement with assigned structures.

Methoxyethyl 2,6-di-O-benzoyl-4-O-(2,6-di-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4c**).—The isopropylidene derivative (**3c**) was treated with benzoyl chloride according to the general procedure, giving **4c** (74%) as fine needles: mp 168–169°C; $[\alpha]_{436}^{22}$ + 43° (*c* 1.04, $CHCl_3$); negative-ion LSIMS: *m/z* 751.5 $(M - COPh)^-$ and 902.2 $(M - NO_2)^-$.

2-(Trimethylsilyl)ethyl 2,6-di-O-benzoyl-4-O-(2,6-di-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4d**).—Similarly **3d** afforded the title compound **4d** (68%) as needles: mp 173–174°C; $[\alpha]_D^{22}$ +18.8, $[\alpha]_{436}^{22}$ +34.5 *c* 1.6, $CHCl_3$); positive-ion LSIMS: *m/z* 921.5 $(M + Na)^+$ and 1031.4 $(M + Cs)^+$; negative-ion LSIMS: *m/z* 944.4 $(M + NO_2)^-$ and 1051.3 $(M + m\text{-NBA})^-$.

In conclusion, the key intermediates (**4a–d**) which are suitable for chain extension at positions O-3 and O-3′ (after removal of the isopropylidene group), were successfully synthesized. These intermediates are versatile as fucosylation at position-3 and sialylation at position-3′ produced the corresponding sLe^X analogues. Fucose and sialyic acid isosteres produced additional sLe^X analogues. Further versatility results from the use of a different aglycon. Thus, the allyl group (**4a**) is useful as an affinity ligand[17], and the methoxyethyl group (**4c**) can be derivatized to the bromide, which is useful as a donor to attach a lipid tail. The 2-(trimethylsilyl)ethyl group (**4d**) is useful for the ease of selective deprotection[18], and it can also serve as a lipid tail.

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