Glycoconjugate Journal (1994) **11**:443–461

The recognition of three different epitopes for the H-type 2 human blood group determinant by lectins of *Ulex europaeus, Galactia tenuiflora* and *Psophocarpus tetragonolobus* (Winged Bean)*

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Received 6 May 1994, revised 8 July 1994

The chemical mapping of the regions of H-type 2 human blood group-related trisaccharide (Fucα(1-2)Galβ(1-4)GlcNAcβMe) that are recognized by three different lectins, the so-called epitopes, are reviewed together with an account of how and why oligosaccharides form specific complexes with proteins as presently viewed in this laboratory. The occasion is used to report the synthesis of the various mono-*O*-methyl derivatives of the above trisaccharide that were used in these investigations. Also, Fucα(1-2)Galβ(1-4)Xylβ Me was synthesized in order to examine whether or not the hydroxymethyl group of the GlcNAc residue participates in the binding reaction.

Keywords: Lectin specificities, H-type 2 blood group determinant, chemical mapping of epitopes, lectins of *Ulex europaeus, Galactia tenuiflora* and *Psophocarpus tetragonolobus.*

Abbreviations: Me, methyl; Bn, benzyl; Ac, acetyl; Bz, benzoyl; n-Bu, n-butyl; NMR, nuclear magnetic resonance; the GlcNAc, Gal and Fuc residues of the H-type 2 trisaccharide are designated as the a, b and c structural units, respectively.

Introduction

For the purposes of this communication, the definition of the epitope of an oligosaccharide is taken as that region of its surface that comes into intimate contact with the combining site of an acceptor protein. Studies of the effects on binding of replacing, one at a time, each of the hydroxyl groups of an oligosaccharide both by hydrogen and methoxy groups can provide a map of the epitope for a given association [1, 2]. By providing information as to which of the hydroxyl groups remain in the aqueous phase once the complex has formed, the method should prove particularly valuable to efforts aimed, for example, at modifying the oligosaccharide's structure to enhance the binding (stronger inhibitors) or to target therapeutics. This communication is to report the synthesis of mono-*O*-methyl derivatives of a trisaccharide and to review their use in mapping the epitopes of three different lectins. A brief review is presented of the contributions made from this laboratory to the recognition of oligosaccharides by lectins and antibodies.

Our appreciation of the chemical basis for the highly specific associations of complex molecular structures in aqueous biological fluids evolved from the proposal of the 'lock and key' concept for enzyme specificity made by Emil Fischer in 1894 [3, 4]. That complementarity is the basic requirement for specific molecular recognition is now a firmly established principle. Why the associations occur is a separate question.

We addressed the problem of how and why complex oliosaccharides are specifically recognized and bound by certain proteins once it became possible to synthesize oligosaccharides that were known to be specifically bound by well-defined monoclonal antibodies and lectins. The human blood group determinants at the tri- and tetrasaccharide levels were particularly useful [5]. The strategy was to systematically synthesize slightly altered forms of a given oligosaccharide for the probing, by way of binding studies, of the combining site presented by the protein.

Research designed to provide an appreciation of the many interactions within a complex assembly of molecules is necessarily an evolutionary process which requires adjustment as new, improved experimental insights are acquired. Indeed, we no longer hold notions on how and

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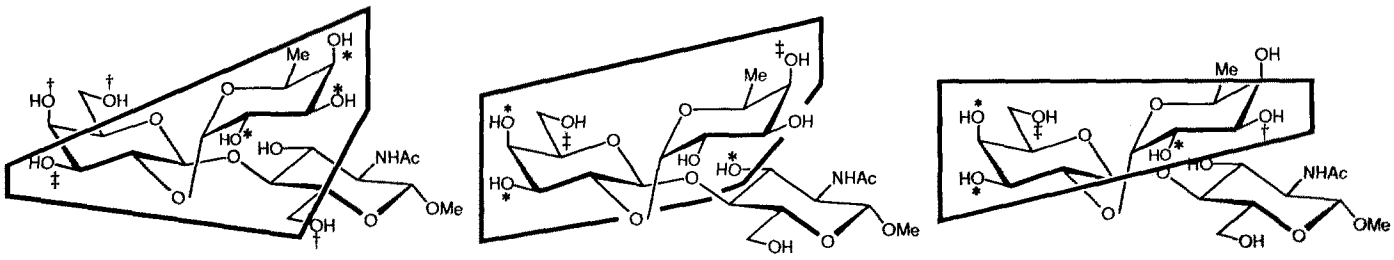


Ulex europaeus I ***Galactia tenuiflora*** ***Psophocarpus tetragonolobus II***

Figure 1. The involvements of the eight hydroxyl groups of H-type 2-OMe (**24**) in its complexes with the three different lectins. The unmarked hydroxyl groups remain in the aqueous phase; those marked † also remain hydrogen bonded to water but definitely near the periphery of the combining site; those marked ‡ are expected to be hydrogen bonded to the lectin at the periphery of the combining site; those marked * are considered to be involved in hydrogen bonds sufficiently deep within the combining site to not be in contact with the aqueous phase.

why certain biological associations occur that we held prior to the availability of the X-ray crystal structures of certain oligosaccharide-protein complexes. Since reference to only the earlier postulations could be misleading, it is important that citations include the most recent publications. In this regard, it is pertinent to this special issue on lectins to briefly review the evolution in thought that has occurred in this laboratory.

We have probed the combining sites of lectins of *Ulex europaeus* [6, 7], *Galactia tenuiflora* [8], and *Psophocarpus tetragonolobus* [9] using a wide variety of congeners of the methyl glycoside of the H-type 2 human blood group-related trisaccharide, Fucα(1-2)Galβ(1-4)GlcNAcβMe (H-type 2-OMe). The significance of the results in terms of the topographical features presented to the protein for binding have been discussed in detail. As schematically presented in Fig. 1, each lectin recognizes a different region of the surface of H-type 2-OMe. As previously discussed [7–9], all three epitopes comprise an assortment of polar groups adjacent to small nonpolar hydrophobic regions. Therefore, in each case, a unique hydrophilic mosaic of small amphiphilic structural units comes into direct interaction with the protein. The term 'polyamphiphilic' was coined to designate these surface areas which evidently can be immunogenic since structurally similar epitopes were detected for several human blood group-related monoclonal antibodies (anti-Lewis a [10], anti-Lewis b [11], anti-H-type 2 [7], and anti-B [12]). There can be no doubt that the affinities expressed by lectins and antibodies [13, 14] for oligosaccharides are similar in kind.

Following a preliminary study of the binding of the Galβ terminal units of an artificial antigen by a polyclonal preparation of anti-Galβ antibodies [15], the binding of the methyl glycoside of *N*-acetyl lactosamine by the myeloma monoclonal anti-I (Ma) antibody was examined [16]. Both these studies identified important involvement of hydrophobic regions for the epitopes. Therefore, the phenomenon that is associated with an increase in entropy caused by the release of water molecules to bulk – the 'hydrophobic effect' – was invoked as a dominant contribution to the driving force for these associations [15–17]. Since in several instances the deoxygenation of a hydroxyl group resulted in a stronger binding reaction, it was reasonable to expect that intramolecularly hydrogen bonded hydroxyl groups were accepted into hydrophobic combining sites [15, 18].

The foregoing considerations gave rise to the concept of the 'hydrated polar group effect' which suggested that the directional hydration of polar groups at the entrance to a hydrophobic region of the combining site added importantly to the specificity of the binding reaction by preventing nonspecific associations of the nonpolar cavities with nonpolar molecules of the surrounding medium [19]. The various probing studies had shown that the proposed intramolecular hydrogen bonded networks for acceptance into a hydrophobic site, if present, occurred near the periphery of the combining site and at least potentially in contact with the aqueous phase.

The turning point for the assessment of the role of water in the binding of oligosaccharides by proteins came [20] with the X-ray crystal structure [21, 22] for the complex formed by the lectin IV of *Griffonia simplicifolia* (GS-IV) [23] with the methyl glycoside of the Lewis-b human blood group determinant, Le^b-OMe (Fucα(1-2)Galβ(1-3)[Fucα(1-4)]GlcNAcβMe). It was found that the substitution by hydrogen of hydroxyl groups, that in the complex remained hydrogen bonded to the water present in the channels between lectin molecules, could strongly (up to 6 kcal mol^{-1}) influence the change in enthalpy for the binding reaction. That these hydroxyl groups were also bound to water when the complex is in free solution was confirmed by the fact that their *O*-methylation had only minor effects on the strength of the association [1]. Evidently, hydration forces have an important effect on the stability of the complex. The epitope revealed by the X-ray structure proved to be substantially smaller in area than that suggested by the probing data. Its hydrophilicity was

Table 1. A comparison of the involvements of the hydroxyl groups of H-type 2-OMe (**24**) in its complexes with lectins of *Ulex europaeus*, *Galactia tenuiflora* and *Psophocarpus tetragonolobus* (Winged bean) [7–9].

	Relative potencies of derivatives of ***24***[a]					
Lectin	Ulex		Galactia		Psophocarpus	
Derivatives	*Monodeoxy*	*Mono-O-Me*	*Monodeoxy*	*Mono-O-Me*	*Monodeoxy*	*Mono-O-Me*
Position altered						
3a	46	40	Inactive	Inactive	97	8
6b	60	45	30	12	10	38
4b	37	44	Inactive	Inactive	Inactive	Inactive
3b	3	70	Inactive	Inactive	4.5	Inactive
2c	0.6	Inactive	225	22	14	Inactive
3c	0.1	Inactive	110	85	10	7
4c	Inactive	Inactive	6	92	80	98
6a	250	150	138	96	88	112

[a] Calculated from 50% inhibition data and setting the potency of **24** at 100.

well exhibited by the presence in the X-ray crystal structure of seven (necessarily highly immobilized) water molecules within the combining site [22, 24]. Hydration of the complementary polyamphiphilic surfaces presented by the tetrasaccharide (Le^b-OMe) and the lectin (GS-IV) were expected to result in layers of water molecules that are perturbed because of the difficulty in adapting to the spacings and orientations of the polar groups [19] as compared to the water molecules in bulk solution where similar hindrances to association do not exist. Monte Carlo simulations both of Le^b-OMe [25] and the combining site of the lectin GS-IV [24] are in support of this contention. Evidently, the decrease in the exposure to water of the polyamphiphilic surfaces presented by the epitope and the combining site leads to a decrease in perturbed water which is an important contribution to the decrease in enthalpy that normally drives these associations.

Materials and methods

Enzyme linked immunosorbent assay (ELISA)

The relative potencies reported in Table 1 for the inhibition of the *Ulex* and *Galactia* lectins by the *O*-methyl derivatives of **24** were determined using the following conditions for an enzyme-linked immunosorbent assay (ELISA) [26]. The relative potencies determined in this manner agreed well within experimental error with the values obtained for several compounds using the radioimmunoassay developed in this laboratory [27] and which was used to establish the relative potencies reported in Table 1 for the deoxy compounds [6, 8, 9]. The relative potencies reported for the inhibition of the *Psophocarpus* lectin by the *O*-methyl derivatives were previously reported [9]. That for the 6a-*O*-methyl compound was determined using the radioimmunoassay.

The PBS buffer was 0.072 M Na_2HPO_4, 0.028 M NaH_2PO_4, 0.15M NaCl, 3 mM NaN_3, 0.1 mM $CaCl_2$, and 0.07 mM $MnCl_2$ pH 7.2 ± 0.1 and the DEA buffer was 1.0 M diethylamine-hydrochloride, 492 μM in $MgCl_2$ and 1% BSA pH 9.8 ± 0.1. The coating and inhibition procedure was essentially that reported by Spohr *et al.* [27]. The 96 wells of an ELISA plate were coated using 100 μl per well of the lectin solution in PBS buffer. In the case of the lectin I of *Ulex europaeus* the concentration was 30 μg ml^{-1}. The higher concentration of 100 μg ml^{-1} of the lectin of *Galactia tenuiflora* is that used previously in a radioimmunoassay [8]. The incubation was overnight at 4 °C. The coating of the wells was then completed by incubation at room temperature for 2 h with 200 μl of a 15% solution of BSA in PBS buffer. The wells were then washed by the addition of 200 μl per well of a 1% solution of BSA in PBS buffer, followed by aspiration, air dried for 30 min and kept at 4 °C.

The solutions of the inhibitors in PBS (50 μl) were then added to the wells in triplicate followed by the immediate addition of the solution of an artificial [Fucα(1-2)Galβ(1-4)[Fucα(1-3)]GlcNAc$(CH_2)_8CO]_{17}$ NH-BSA antigen (50 μl, 0.2 μg ml^{-1}) in PBS for the experiments with the *Ulex* lectin. In the case of the *Galactia* lectin, a solution of the [Fucα(1-2)Galβ(1-4)GlcNAcβ$(CH_2)_8CO]_8$ NH-BSA antigen (50 μl, 1.2 μg ml^{-1}) was used since these conditions were less demanding in inhibitor to achieve 50% inhibition. After incubation overnight at room temperature, it was aspirated and washed with PBS (4 × 200 μl). A solution of an anti-H antibody [7] in 1% BSA-PBS (100 μl, 0.5 μg ml^{-1}, or 4 μg ml^{-1} in the case of the *Galactia* lectin) was dispensed into each well. After incubation for 2.5 h, it was aspirated and washed with PBS (4 × 200 μl). Then a solution of alkaline phosphatase conjugated anti-mouse IgG antibody(Sigma, A-0162, 1:350) in 1% BSA-PBS (100 μl) was added to the wells, followed by incubation for

2.5 h at room temperature. After removal of the solution by aspiration and PBS washing (4 × 200 µl PBS), a solution of *p*-nitrophenyl phosphate (1 mg per 1 ml DEA buffer, 100 µl) was added and the absorbance at 405 nm was measured with an ELISA plate reader after 30 min. The 50% inhibitions were estimated from plots and the values obtained were rechecked in at least one separate similar experiment.

Methods

The ^{1}H-NMR spectra were measured at 300 and 360 MHz (Bruker AM300 and WM360) with tetramethylsilane as internal standard for $CDCl_3$ solutions. The reference standard for D_2O solutions was acetone (2.225 ppm). The ^{13}C-NMR spectra were recorded at 75 MHz using D_2O as solvent with 1,4-dioxane (67.4 ppm) as internal standard. Optical rotations were measured at room temperature (23 ± 1 °C) in a 1 dm cell on a Perkin-Elmer 241 polarimeter. Thin-layer chromatograms were performed on precoated plates of silica gel (60F254, E. Merck, Darmstadt) and visualized by spraying with 5% sulfuric acid in ethanol followed by heating. For column chromatography, silica gel 60 (230–400 mesh, E. Merck, Darmstadt) and Iatrobeads® (200–300 mesh, Iatron laboratories Inc.) were used. Solvents and reagents were purified and dried according to standard procedures. Melting points are uncorrected.

Syntheses

The carbohydrate nomenclature used in this section corresponds to internationally approved rules [28].

*Methyl 2-*O*-benzyl-3-*O*-methyl-α-*L*-fucopyranoside* (**2**)

A mixture of **1** (500 mg, 1.86 mmol) [29] and di-*n*-butyltin oxide (500 mg, 2.01 mmol) in benzene (16 ml) was boiled under reflux with azeotropic removal of water formed during stannylation. After 4 h, it was concentrated to ~4 ml, followed by the addition of tetrabutylammonium fluoride monohydrate (700 mg, 2.67 mmol), 4 Å molecular sieves (1.7 g) and methyl iodide (1 ml, 16 mmol). The mixture was stirred overnight, then the solids were removed by filtration and the solvent was evaporated. Column chromatography on silica gel (hexane-ethyl acetate, 3:1) provided syrupy **2** (437 mg, 83%). $[\alpha]_D$ −80.8° (*c* 0.7, chloroform). ^{1}H-NMR ($CDCl_3$) *δ*: 7.40–7.20 (m, 5H, Ph), 4.80 and 4.62 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.59 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 3.89 (m, 2H, H-4, H-5), 3.72 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.61 (dd, 1H, $J_{3,4}$ 3.0 Hz, H-3), 3.53 and 3.36 (2s, each 3H, 2CH_3O), 2.33 (bs, 1H, OH), 1.29 (d, 3H, $J_{5,6}$ 6.5 Hz, H_3-6). *Anal.* calcd. for $C_{15}H_{22}O_5$: C 63.81, H 7.85; found: C 63.66, H 7.89.

*Methyl 2,4-di-*O*-benzyl-3-*O*-methyl-α-*L*-fucopyranoside* (**3**)

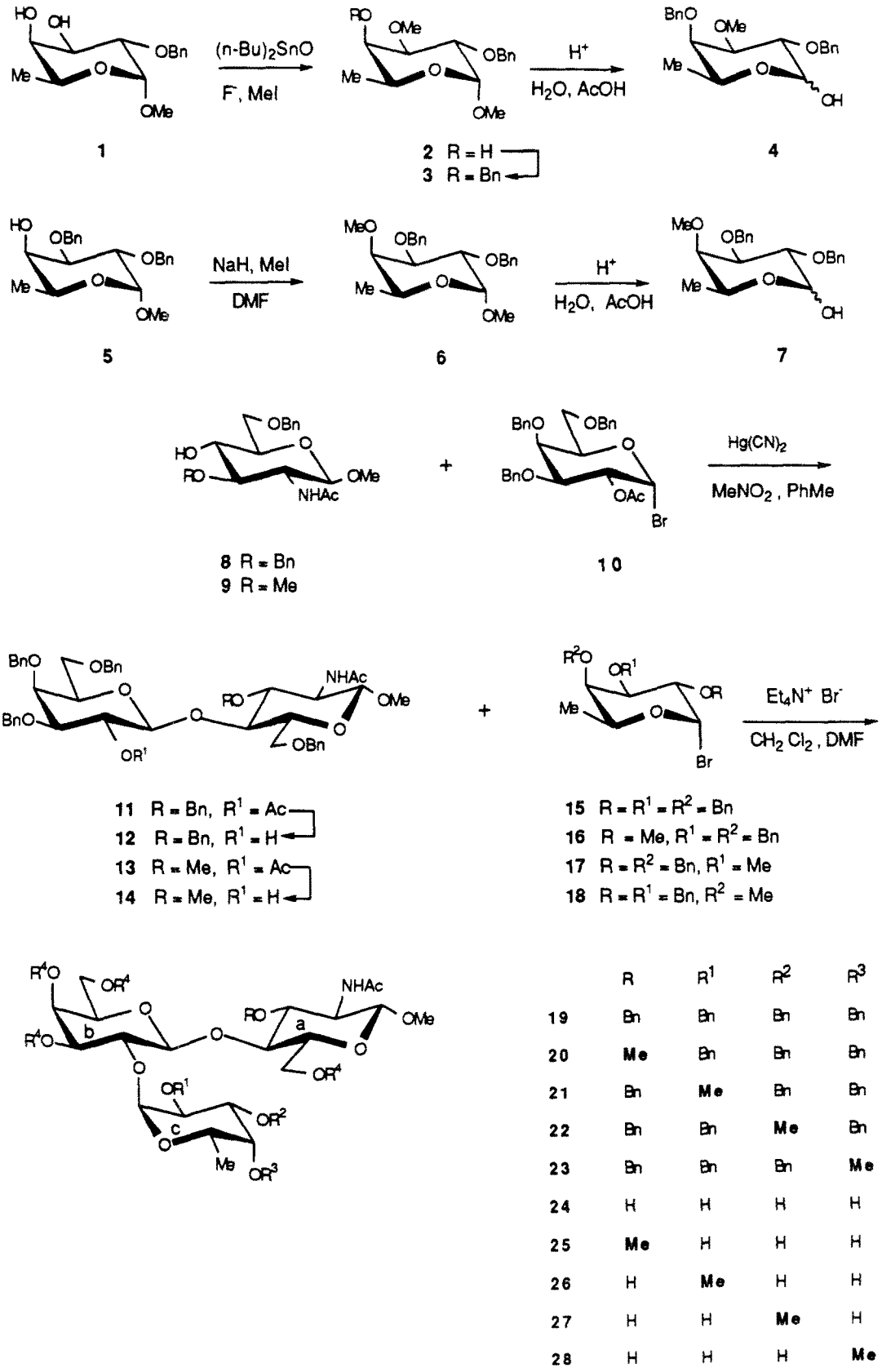
Sodium hydride (90 mg, 3.03 mmol, 80% oily suspension) was added to a stirred solution of **2** (427 mg, 1.51 mmol) in *N*,*N*-dimethylformamide (6 ml) at ice-bath temperature. After 30 min, benzyl bromide (0.33 ml, 2.8 mmol) was added and stirring continued for 3 h. Then methanol (200 µl) was carefully added to quench the reaction. The mixture was diluted with dichloromethane, washed with water and evaporated. Chromatographic purification of the resulting material on a column of silica gel (hexane-ethyl acetate, 5:1) afforded **3** (517 mg, 92%) as a syrup. $[\alpha]_D$ −28° (*c* 0.4, chloroform) {lit. [29], $[\alpha]_D$–25.5° (*c* 1.1, chloroform)}. ^{1}H-NMR ($CDCl_3$) *δ*: 7.50–7.20 (m, 10H, 2Ph), 4.95 and 4.63 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.84 and 4.65 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.61 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 3.93 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.83 (q, 1H, $J_{5,6}$ 6.5 Hz, $J_{4,5}$ < 1 Hz, H-5), 3.67 (m, 1H, H-4), 3.63 (dd, 1H, overlapped, $J_{3,4}$ 3.0 Hz, H-3), 3.55 and 3.33 (2s, each 3H, 2CH_3O), 1.13 (d, 3H, H_3-6). *Anal.* calcd. for $C_{22}H_{28}O_5$: C 70.95, H 7.58; found: C 70.74, H 7.64.

*2,4-Di-*O*-benzyl-3-*O*-methyl-*L*-fucopyranose* (**4**)

A solution of compound **3** (500 mg, 1.34 mmol) in glacial acetic acid (15 ml) containing 6 N hydrochloric acid (2 ml) was heated at 65 °C for 1 h. It was concentrated *in vacuo* and co-evaporated with toluene. The residue was chromatographed on a column of silica gel (hexane-ethyl acetate, 3:1) to provide syrupy **4** (342 mg, 71%). $[\alpha]_D$ −16.4° (*c* 0.4, chloroform). ^{1}H-NMR ($CDCl_3$, $\alpha:\beta = 2.3:1$) *δ*: 7.45–7.23 (m, 10H, 2Ph), 5.23 (bd, $J_{1,2}$ 3.5 Hz, H-1α), 4.59 (H-1β), 4.10 (q, $J_{5,6}$ 6.5 Hz, H-5α), 3.94 (dd, $J_{2,3}$ 10.0 Hz, H-2α), 3.69 (H-4α), 3.63 (H-2β), 3.63 (dd, $J_{3,4}$ 2.5 Hz, H-3α), 3.54 (s, CH_3O-α), 3.53 (s, CH_3O-β), 3.53 (overlapped, H-5β), 3.28 (dd, $J_{2,3}$ 9.7 Hz, $J_{3,4}$ 2.7 Hz, H-3β), 3.04 (bs, OH), 1.20 (d, $J_{5,6}$ 6.5 Hz, H_3-6β), 1.14 (d, H_3-6α). *Anal.* calcd. for $C_{21}H_{26}O_5$: C 70.37, H 7.31; found: C 70.40, H 7.38.

*Methyl 2,3-di-*O*-benzyl-4-*O*-methyl-α-*L*-fucopyranoside* (**6**)

Sodium hydride (200 mg, 4.17 mmol, approx. 50% dispersion in oil) was added to a solution of alcohol **5** (850 mg, 2.37 mmol) [29, 30] in *N*,*N*-dimethylformamide (10 ml) at ice-bath temperature. After 20 min, methyl iodide (270 µl, 4.33 mmol) was added and stirring continued for 1 h. The reaction mixture was diluted with dichloromethane, extracted with water and evaporated. Chromatographic purification of the resulting material on a column of silica gel (hexane-ethyl acetate, 3:1) provided methyl ether **6** (794 mg, 90%) as a syrup that slowly crystallized. The analytical sample was recrystallized from hexane-ether. M.p. 40–41 °C; $[\alpha]_D$ −38.5° (*c* 1.9, chloroform). ^{1}H-NMR ($CDCl_3$) *δ*: 7.50–7.20 (m, 10H, 2Ph), 4.85, 4.83, 4.72, 4.65 (2ABq, 4H, 2CH_2Ph), 4.61 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 3.93 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-2), 3.88 (d, 1H, $J_{3,4}$ 2.5 Hz, H-4), 3.86 (overlapped, H-3), 3.83 (q, 1H, $J_{5,6}$ 6.5 Hz, H-5), 3.61, 3.34 (2s, each 3H, 2CH_3O), 1.21 (d, 3H, H_3-6). *Anal.* calcd. for $C_{22}H_{28}O_5$: C 70.95, H 7.58; found: C 70.99, H 7.61.



	R	R^1	R^2	R^3	R^4
19	Bn	Bn	Bn	Bn	Bn
20	**Me**	Bn	Bn	Bn	Bn
21	Bn	**Me**	Bn	Bn	Bn
22	Bn	Bn	**Me**	Bn	Bn
23	Bn	Bn	Bn	**Me**	Bn
24	H	H	H	H	H
25	**Me**	H	H	H	H
26	H	**Me**	H	H	H
27	H	H	**Me**	H	H
28	H	H	H	**Me**	H

Scheme 1. The preparation of mono-*O*-methyl derivatives (**25**–**28**) of H-type 2-OMe (**24**).

2,3-Di-O-benzyl-4-O-methyl-L-fucopyranose (**7**)

A solution of compound **6** (750 mg, 2.01 mmol) in acetic acid (25 ml) containing 6 N hydrochloric acid (3.1 ml) was heated at 65 °C for 45 min. The mixture was diluted with dichloromethane, washed with water, aqueous saturated sodium hydrogen carbonate and water. After solvent removal, the crude product was applied to a column of silica gel and eluted with hexane-ethyl acetate (2:1, 3:2) to provide **7** (530 mg, 73%) as a crystallizing syrup. The analytical sample was obtained by recrystallization from ether/hexane. M.p. 83–84 °C; $[\alpha]_D$ −64.3° (after 5 min), −65.6° (after 20 h) (*c* 1.2, methanol). The ^{1}H-NMR spectrum of the recrystallized material required an $\alpha:\beta$ ratio of 4:1. ^{1}H-NMR ($CDCl_3$) δ: 7.50–7.25 (m, 10H, 2Ph), 5.23 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.83 and 4.66 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.80 and 4.77 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.10 (q, 1H, $J_{5,6}$ 6.5 Hz, H-5), 3.93 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-2), 3.83 (dd, 1H, $J_{3,4}$ 2.5 Hz, H-3), 3.62 (s, 3H, CH_3O), 3.39 (d, 1H, H-4), 3.00 (bs, 1H, OH-1), 1.23 (d, 3H, H_3-6). *Anal.* calcd. for $C_{21}H_{26}O_5$: C 70.37, H 7.31; found: C 70.19, H 7.35.

Methyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-methyl-β-D-glucopyranoside (**9**)

Ether saturated with hydrogen chloride was added at 0 °C to a stirred mixture of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-glucopyranoside [31, 32] (1.91 g, 5.66 mmol), sodium cyanoborohydride (3.9 g, 62.1 mmol) and a crystal of methyl orange in dry tetrahydrofuran (50 ml) until the colour of the indicator turned red and gas evolution occurred. When thin layer chromatography (TLC) indicated the reaction to be completed, it was diluted with dichloromethane and poured into aqueous saturated sodium hydrogen carbonate. The organic solution was washed with water, dried and concentrated. The crude product was purified by column chromatography on silica gel (dichloromethane-methanol, 19:1) to provide crystalline **9** (1.3 g, 68%). The analytical sample was recrystallized from ethyl acetate. M.p. 166–167 °C; $[\alpha]_D$ −26.4° (*c* 0.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.25 (m, 5H, Ph), 5.68 (d, 1H, $J_{NH,2}$ 7.5 Hz, NH), 4.78 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.63 and 4.57 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 3.78 (overlapped, H-3), 3.79–3.73 (m, H_2-6), 3.61 (dt, $J_{3,4} \sim J_{4,5} \sim$ 8.5 Hz, $J_{4,OH}$ 2.5 Hz, H-4), 3.54 (s, m, 4H, CH_3O, H-5), 3.48 (s, 3H, CH_3O), 3.26 (m, 1H, H-2). 2.89 (d, 1H, OH), 2.01 (s, 3H, CH_3CO). *Anal.* calcd. for $C_{17}H_{25}NO_6$: C 60.16, H 7.42, N 4.13; found: C 60.06, H 7.11, N 4.17.

Methyl 2-acetamido-4-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**11**)

A solution of bromide **10** [33] (2 g, 3.6 mmol) in nitromethane-toluene (1:1, 10 ml) was added to a mixture of **8** [8] (1.2 g, 2.9 mmol), mercuric cyanide (1.6 g, 6.33 mmol), powdered calcium sulfate (1 g) and 4 Å molecular sieves (2 g) in the same solvent (100 ml) under helium. After stirring for 1.5 h, more **10** (1.8 g, 3.24 mmol) was added and stirring continued for 6 h. The reaction mixture was diluted with dichloromethane and washed with aqueous saturated sodium hydrogen carbonate and water. The material obtained on evaporation was applied to a column of silica gel and eluted with hexane-acetone (1:1). **11** (2.25 g, 97%) was obtained as a foam. $[\alpha]_D$ −28.2° (*c* 0.4, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.15 (m, 25H, 5Ph), 6.18 (d, 1H, $J_{NH,2a}$ 9.0 Hz, NH), 5.29 (dd, 1H, $J_{1b,2b}$ 8.0 Hz, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.95–4.45 (m, 8H, 4CH_2Ph), 4.50 (d, 1H, overlapped by CH_2Ph, H-1a), 4.40 and 4.35 (ABq, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.33 (d, 1H, H-1b), 3.40 (s, 3H, CH_3O), 2.05, 1.95 (2s, each 3H, 2CH_3CO). *Anal.* calcd. for $C_{52}H_{59}NO_{12}$: C 70.17, H 6.68, N 1.57; found: C 69.92, H 6.87, N 1.65.

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**12**)

Compound **11** (98 mg, 0.11 mmol) was treated with methanolic 0.045N sodium methoxide (2.4 ml) for 25 h. Neutralization with Amberlite IRC 50 H^+, evaporation and column chromatography on silica gel (hexane-acetone, 1:1) provided **12** as a foam (90 mg, 95%). $[\alpha]_D$ + 10.7° (*c* 0.6, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.10 (m, 25H, 5Ph), 5.66 (d, 1H, $J_{NH,2a}$ 7.0 Hz, NH), 4.88, 4.71–4.51 (m, 8H, 4CH_2Ph), 4.70 and 4.47 (2d, each 1H, H-1a and H-1b), 4.33 and 4.26 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 3.41 (s, 3H, CH_3O), 1.85 (s, 3H, CH_3CO). *Anal.* calcd. for $C_{50}H_{57}NO_{11}$: C 70.82, H 6.78, N 1.65; found: C 70.42, H 7.03, N 1.78.

Methyl 2-acetamido-4-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-6-O-benzyl-2-deoxy-3-O-methyl-β-D-glucopyranoside (**13**)

Compound **9** (0.31 g, 0.92 mmol) was reacted with bromide **10** (1.15 g, 2.07 mmol) in the same way as in the preparation of **11**. White crystals of **13** (0.68 g, 90%) were obtained after recrystallization of the crude material from ethyl acetate. M.p. 164–166 °C. $[\alpha]_D$ −39.3° (*c* 0.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.25 (m, 20H, 4Ph), 6.45 (d, 1H, $J_{NH,2a}$ 9.0 Hz, NH), 5.29 (dd, 1H, $J_{1b,2b}$ 8.0 Hz, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.90 and 4.60 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.66 and 4.51 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.51 (s, 2H, CH_2Ph), 4.42 (s, 2H, CH_2Ph), 4.40 (d, $J_{1a,2a}$ 3.5 Hz, H-1a), 4.33 (d, 1H, $J_{1b,2b}$ 8.0 Hz, H-1b), 4.15 (m, 1H, $J_{2a,3a} \sim$ 3.5 Hz, H-2a), 3.51 (m, 2H, H-3a, H-3b), 3.39, 3.36 (2s, each 3H, 2CH_3O), 2.03 (s, 6H, 2CH_3CO). *Anal.* calcd. for $C_{46}H_{55}NO_{12}$: C 67.88, H 6.81, N 1.72; found: C 67.54, H 6.98, N 1.75.

Methyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-methyl-4-O-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**14**)

Compound **13** (0.11 g, 0.135 mmol) was treated with methanolic 0.045 N sodium methoxide (2.4 ml) for 26 h.

Neutralization with Amberlite IRC 50 H^+, evaporation and column chromatography on silica gel (hexane-acetone, 1:1) provided **14** as a white solid (87 mg, 83%). M.p. 140–142 °C (ethyl acetate-ether). $[\alpha]_D$ +4.2° (*c* 0.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.36–7.25 (m, 20H, 4Ph), 5.83 (d, 1H, $J_{NH,2a}$ 8.5 Hz, NH), 4.87 and 4.65 (ABq, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.70 and 4.55 (ABq, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.64 (d, 1H, overlapped by CH_2Ph, H-1a), 4.61 and 4.54 (ABq, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.47 (d, 1H, $J_{1b,2b}$ 8.0 Hz, H-1b), 4.42 and 4.38 (ABq, $J_{A,B}$ 11.5 Hz, CH_2Ph), 3.89 (overlapped, H-2b), 3.51 (m, 1H, H-2a), 3.45 (s, 6H, $2CH_3O$), 3.35 (dd, 1H, $J_{2b,3b}$ 10.0 Hz, $J_{3b,4b}$ 3.0 Hz, H-3b), 1.95 (s, 3H, CH_3CO). *Anal.* calcd. for $C_{44}H_{53}NO_{11}$: C 68.46, H 6.92, N 1.81; found: C 68.49, H 6.93, N 1.88.

General procedure for the preparation of substituted α-L-fucopyranosyl bromides (**15**) [34], (**16**) [1], (**17**) *and* (**18**)

A solution of oxalyl bromide (0.25 ml, 2.5 mmol) in dichloromethane (1.2 ml) was added to a stirred solution of 2,3,4-tri-*O*-alkyl-L-fucopyranose (0.55 g, *ca.* 1.54 mmol), in dichloromethane (1.2 ml) and *N,N*-dimethylformamide (0.12 ml) under helium. The solution was stirred for 0.5 h and poured into ice-water. The dichloromethane solution was washed with ice-water, dried over sodium sulfate, concentrated to a smaller volume and dried over 4 Å molecular sieves before use in the fucosylation reactions.

3,4-Di-O-benzyl-2-O-methyl-α-L-fucopyranosyl bromide (**16**). Prepared from 3,4-di-*O*-benzyl-2-*O*-methyl-L-fucopyranose [1]. ^{1}H-NMR ($CDCl_3$) δ: 7.50–7.20 (m, 10H, 2Ph), 6.65 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.04 and 4.66 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.94 and 4.72 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.12 (q, 1H, $J_{5,6}$ 6.5 Hz, H-5), 3.91 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 2.7 Hz, H-3), 3.77 (dd, 1H, H-2), 3.67 (dd, $J_{4,5}$ 1.0 Hz, H-4), 3.55 (s, 3H, CH_3O), 1.20 (d, 3H, $J_{5,6}$ 6.5 Hz, H_3-6).

2,4-Di-O-benzyl-3-O-methyl-α-L-fucopyranosyl bromide (**17**). Prepared from **4**. ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.20 (m, 10H, 2Ph), 6.49 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.95 and 4.63 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.76 and 4.69 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.13 (q, 1H, $J_{5,6}$ 6.5 Hz, H-5), 3.88 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-2), 3.73 (dd, 1H, $J_{3,4}$ 2.7 Hz, $J_{4,5}$ 0.8 Hz, H-4), 3.70 (dd, 1H, H-3), 3.56 (s, 3H, CH_3O), 1.19 (d, 3H, H_3-6).

2,3-Di-O-benzyl-4-O-methyl-α-L-fucopyranosyl bromide (**18**). Prepared from **7**. ^{1}H-NMR ($CDCl_3$) δ: 7.45–7.20 (m, 10H, 2Ph), 6.48 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.90 and 4.70 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.73 (s, 2H, CH_2Ph), 4.14 (q, 1H, $J_{5,6}$ 6.5 Hz, H-5), 3.93 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 2.5 Hz, H-3), 3.88 (dd, 1H, H-2), 3.64 (s, 3H, CH_3O), 3.42 (d, 1H, H-4), 1.26 (d, 3H, H_3-6).

General procedure for bromide-ion catalysed fucosylations

A solution of freshly prepared substituted fucopyranosyl bromide (**15**, **16**, **17** or **18**) (*ca.* 1.26 mmol) in dichloromethane (1.75 ml) was added to a mixture of the alcohol (0.53 mmol), tetraethylammonium bromide (0.53 mmol), powdered 4 Å molecular sieves (1.32 g) and *N,N*-dimethylformamide (0.53 ml) in dichloromethane (2 ml) with stirring under helium. The mixture was stirred for about 2 days. Then methanol (0.27 ml) was added and stirring continued for another 2 h. The reaction mixture was diluted with dichloromethane, filtered through a pad of Celite, washed with aqueous saturated sodium hydrogen carbonate and water. The crude product obtained on evaporation was applied to a silica gel column for purification.

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**19**)

Bromide **15** was reacted with alcohol **12** for 49 h following the general procedure for bromide-ion catalysed fucosylations. Column chromatography on silica gel (hexane-acetone, 3:2, dichloromethane-ethyl acetate, 3:1) provided **19** as a white foam (64%). $[\alpha]_D$ −27.5° (*c* 0.3, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.00 (m, 40H, 8Ph), 5.78 (m, 1H, NH), 5.70 (d, 1H, $J_{1c,2c}$ 4.0 Hz, H-1c), 4.93–4.30 (m, 16H, $8CH_2Ph$), 4.19 (dd, 1H, $J_{1b,2b}$ 7.5 Hz, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.02 (dd, 1H, $J_{2c,3c}$ 10.0 Hz, H-2c), 3.45 (s, 3H, CH_3O), 1.80 (s, 3H, CH_3CO), 1.20 (d, 3H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{77}H_{85}NO_{15}$: C 73.14, H 6.78, N 1.11; found: C 72.55, H 6.67, N 1.09.

Methyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-methyl-4-O-[3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**20**)

Bromide **15** was reacted with alcohol **14** for 49 h, following the general procedure for bromide-ion catalysed fucosylations. Column chromatography on silica gel (hexane-acetone, 3:2) provided **20** (70%). $[\alpha]_D$ −45° (*c* 0.3, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.35–7.00 (m, 35H, 7Ph), 6.15 (m, 1H, NH), 5.70 (d, 1H, $J_{1c,2c}$ 4.0 Hz, H-1c), 4.93–4.52 (m, 12H, $6CH_2Ph$), 4.49 and 4.42 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.34 (q, 1H, $J_{5c,6c}$ 6.5 Hz, H-5c), 4.12 (dd, 1H, $J_{1b,2b}$ 7.5 Hz, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.02 (dd, 1H, $J_{2c,3c}$ 10.0 Hz, H-2c), 3.43, 3.39 (2s, each 3H, $2CH_3O$), 1.80 (s, 3H, CH_3CO), 1.18 (d, 3H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{71}H_{81}NO_{15}$: C 71.76, H 6.87, N 1.18; found: C 71.54, H 6.88, N 1.21.

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[3,4,6-tri-O-benzyl-2-O-(3,4-di-O-benzyl-2-O-methyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**21**)

Bromide **16** was reacted with alcohol **12** for 25 h, following the general procedure for bromide-ion catalysed fucosylations. Column chromatography on silica gel (hexane-acetone, 3:2, dichloromethane-ethyl acetate, 4:1) provided **21** in 78.2% yield. $[\alpha]_D$ −40.2° (*c* 0.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.10 (m, 35H, 7Ph), 5.77 (d, 1H,

$J_{NH,2a}$ 7.0 Hz, NH), 5.64 (d, 1H, $J_{1c,2c}$ 3.3 Hz, H-1c), 4.95–4.30 (m, 14H, 7CH_2Ph), 4.31 (q, overlapped, H-5c), 4.15 (dd, 1H, $J_{1b,2b}$ 7.5 Hz, $J_{2b,3b}$ 10.0 Hz, H-2b), 3.45, 3.35 (2s, each 3H, 2CH_3O), 1.87 (s, 3H, CH_3CO), 1.22 (d, 3H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{71}H_{81}NO_{15}$: C 71.76, H 6.87, N 1.18; found: C 71.82, H 6.91, N 1.23.

*Methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,4-di-*O*-benzyl-3-*O*-methyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**22**)

Alcohol **12** was reacted with bromide **17** for 47 h, following the general procedure for bromide-ion catalysed fucosylations. Column chromatography on silica gel (hexane-ethyl acetate, 2:1, dichloromethane-ethyl acetate, 3:1, and hexane-acetone, 2:1) provided the title compound **22** (65.3%) as a white foam. $[\alpha]_D$ −34.8° (*c* 0.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.10 (m, 35H, 7Ph), 5.82 (d, 1H, $J_{NH,2a}$ 6.5 Hz, NH), 5.67 (d, 1H, $J_{1c,2c}$ 3.8 Hz, H-1c), 4.90–4.30 (m, 14H, 7CH_2Ph), 4.74 and 4.48 (2d, each 1H, H-1a and H-1b), 4.16 (dd, 1H, $J_{1b,2b}$ 7.5 Hz, $J_{2b,3b}$ 10.0 Hz, H-2b), 3.97 (q, 1H, $J_{5c,6c}$ 8.0 Hz, H-5c), 3.47, 3.43 (2s, each 3H, 2CH_3O), 1.82 (s, 3H, CH_3CO), 1.20 (d, 3H, $J_{5c,6c}$ 8.0 Hz, H_3-6c). *Anal.* calcd. for $C_{71}H_{81}NO_{15}$: C 71.76, H 6.87, N 1.18; found: C 71.58, H 6.85, N 1.19.

*Methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3-di-*O*-benzyl-4-*O*-methyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**23**)

Alcohol **12** was reacted with bromide **18** for 48 h, following the general procedure for bromide-ion catalysed fucosylations. Chromatography on a column of silica gel (hexane-acetone, 3:2) provided **23** in 61.2% yield. $[\alpha]_D$ −31.7° (*c* 0.4, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.00 (m, 35H, 7Ph), 5.85 (bd, 1H, NH), 5.70 (d, 1H, $J_{1c,2c}$ 3.5 Hz, H-1c), 4.85–4.28 (m, 14H, 7CH_2Ph), 4.17 (dd, 1H, $J_{1b,2b}$ 7.5 Hz, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.00 (q, 1H, $J_{5c,6c}$ 6.5 Hz, H-5c), 3.57, 3.45 (2s, each 3H, 2CH_3O), 1.85 (s, 3H, CH_3CO), 1.27 (d, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{71}H_{81}NO_{15}$: C 71.76, H 6.87, N 1.18; found: C 71.18, H 7.12, N 1.21.

General procedure for deblocking by hydrogenolysis to the final trisaccharides

The blocked trisaccharide (*ca* 0.4 g) and 5% palladium-on-carbon (0.4 g) in 95% ethanol (30 ml) were hydrogenated in the hydrogen stream for ~5 h. Removal of the catalyst by filtration, evaporation of the solvent and gel filtration of the remainder on a column of Sephadex LH 20 (ethanol-water, 1:1), provided the deblocked trisaccharide as a white solid after lyophilization of an aqueous solution. The ^{1}H- and ^{13}C-NMR data are reported in Tables 2 and 3.

*Methyl 2-acetamido-2-deoxy-4-*O*-[2-*O*-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**24**)

The hydrogenolysis of **19** produced **24** in 90% yield. $[\alpha]_D$–90.2° (*c* 0.5, water). {lit. [35], $[\alpha]_D$–94.8° (*c* 0.4, water)}.

*Methyl 2-acetamido-2-deoxy-4-*O*-[2-*O*-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-3-*O*-methyl-β-D-glucopyranoside* (**25**)

The hydrogenolysis of **20** produced **25** in 85.6% yield, $[\alpha]_D$ −96.3° (*c* 0.3, water).

*Methyl 2-acetamido-2-deoxy-4-*O*-[2-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**26**)

The hydrogenolysis of **21** produced **26** in 93.7% yield, $[\alpha]_D$ −103.1° (*c* 0.5, water).

*Methyl 2-acetamido-2-deoxy-4-*O*-[2-*O*-(3-*O*-methyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**27**)

The hydrogenolysis of **22** produced **27** in 88.3% yield, $[\alpha]_D$ −104.2° (*c* 0.5, water).

*Methyl 2-acetamido-2-deoxy-4-*O*-[2-*O*-(4-*O*-methyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**28**)

The hydrogenolysis of **23** produced **28** in 90% yield, $[\alpha]_D$ −118.9° (*c* 0.4, water).

*Methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside* (**30**) [8]

1. Glycosylation with mercuric cyanide as catalyst. This reaction was carried out as reported [8]. Compound **30** was obtained in 67% yield. As a minor product, *methyl 2-acetamido-4-O-acetyl-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside* (**32**) (24%) was also isolated. M.p. 164–166 °C (ethyl acetate). $[\alpha]_D$ +31° (*c* 0.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.37–7.20 (m, 10H, 2Ph), 5.72 (bs, 1H, NH), 5.00 (t, 1H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4), 4.91 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.62 and 4.57 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.54 (s, 2H, CH_2Ph), 4.31 (t, 1H, $J_{2,3} = J_{3,4}$ 9.0 Hz, H-3) 3.67 (m, 1H, H-5), 3.57 (m, 2H, H_2-6), 3.51 (s, 3H, CH_3O), 3.22 (m, 1H, H-2), 1.91, 1.89 (2s, each 3H, 2CH_3CO). *Anal.* calcd. for $C_{25}H_{31}NO_7$: C 65.63, H 6.83, N 3.06; found: C 65.17, H 6.74, N 3.06.

2. Glycosylation with silver trifluoromethanesulfonate-sym-collidine as catalyst. A solution of bromide **29** (0.74 g, 1.8 mmol) [36] in nitromethane (1 ml) was added to a mixture of compound **8** (0.47 g, 1.13 mmol), silver trifluoromethanesulfonate (0.49 g, 1.91 mmol), *sym*-collidine (0.13 ml, 0.95 mmol) and 4 Å molecular sieves (1 g) in nitromethane (2 ml) at –30°C under helium. After stirring for 1 h at –30°C,

Table 2. Some ^{1}H-NMR chemical shifts (ppm) and coupling constants (Hz) for the H-type 2 blood group determinant Fucα(1-2)Galβ(1-4)GlcNAcβMe (**24**) and related structures[a].

		Derivatives of H-type 2-OMe (24)							
	H-type 2-OMe (24)	*3a-OMe (25)*	*2c-OMe (26)*	*3c-OMe (27)*	*4c-OMe (28)*	*3b-OMe (39)*	*4b-OMe (49)*	*6b-OMe (56)*	*xylo- (63)*
β-D-GlcNAc a-unit									
H-1 ($J_{1,2}$)	4.43 (8.0)	4.44 (8.0)	4.42 (8.5)	4.44 (8.5)	4.42 (8.5)	4.44 (8.0)	4.43 (8.0)	4.43 (8.5)	4.32 (8.0)
H-6 ($J_{6,6'}$)	3.98 (12.0)	4.01 (12.0)	3.96 (12.0)	4.01 (12.0)	3.97 (12.0)	3.98 (12.0)	3.99 (12.0)	3.98 (12.0)	–
CH_3CO	2.05	2.03	2.00	2.05	2.00	2.03	2.03	2.04	–
CH_3O	3.50	3.49[b]	3.51	3.50	3.50	3.50	3.49	3.49	3.53
β-D-Gal b-unit									
H-1 ($J_{1,2}$)	4.52 (7.5)	4.52 (7.5)	4.52 (7.5)	4.53 (8.5)	4.50 (8.0)	4.54 (7.5)	4.50 (7.5)	4.53 (7.5)	4.60 (7.5)
H-3 ($J_{3,4}$)	3.86 (3.5)	3.87 (3.5)	3.83 (3.0)	3.84 (3.7)	3.83 (3.4)	3.54 (3.0)	3.92 (3.5)	3.84	3.87 (3.5)
H-4	3.88	3.89	3.85	3.88	3.86	4.18	3.61	3.86	3.88
CH_3O	–	–	–	–	–	3.44	3.50	3.39	–
α-L-Fuc c-unit									
H-1 ($J_{1,2}$)	5.30 (2.0)	5.30 (2.5)	5.53 (4.0)	5.30 (4.0)	5.26 (4.0)	5.24 (2.5)	5.30 (3.0)	5.31 (2.0)	5.25 (3.5)
H-4 ($J_{3,4}$)	3.78 (2.5)	3.79 (2.7)	3.79	4.09 (2.7)	3.53 (2.6)	3.77 (2.5)	3.79 (2.5)	3.79	*
H-5 ($J_{5,6}$)	4.21 (7.0)	4.27 (7.0)	4.20 (6.5)	4.20 (6.7)	4.20 (6.7)	4.21 (6.5)	4.20 (6.5)	4.21 (6.5)	4.27 (6.5)
H_3-6	1.23	1.24	1.21	1.23	1.25	1.22	1.22	1.20	1.24
CH_3O	–	–	3.49	3.43	3.55	–	–	–	–

[a] Measured at 360 or 300 MHz using 0.04 M solution in D_2O with acetone at 2.225 ppm as internal reference.
[b] CH_3O-3a 3.53 ppm.
* Was not assigned.

another portion of *sym*-collidine (0.13 ml, 0.95 mmol) was added and stirring continued for 0.5 h. The reaction mixture was allowed to warm to room temperature, diluted with dichloromethane, washed with water, aqueous 1 N hydrogen chloride and saturated sodium hydrogen carbonate. Evaporation and column chromatography on silica gel (ethyl acetate-dichloromethane, 1:2.5), provided orthoester **33** (0.51 g, 60%). M.p. 81–84 °C. $[\alpha]_D$ +53° (*c* 0.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.20 (m, 10H, 2Ph), 5.74 (d, 1H, $J_{1b,2b}$ 4.5 Hz, H-1b), 5.52 (d, 1H, $J_{NH,2a}$ 9.0 Hz, NH), 5.43 (dd, 1H, $J_{3b,4b}$ 3.5 Hz, $J_{4b,5b}$ 2.0 Hz, H-4b), 4.99 (dd, 1H, $J_{2b,3b}$ 7.0 Hz, H-3b), 4.80–4.50 (m, 4H, $2CH_2Ph$), 4.62 (d, 1H, $J_{1a,2a}$ 8.0 Hz, H-1a), 4.31 (dd, 1H, H-2b), 3.49 (s, 3H, CH_3O), 2.05, 1.97, 1.85, 1.71 (4s, each 3H, $4CH_3CO$), 1.71 (s, 3H, CH_3). *Anal.* calcd. for $C_{37}H_{47}NO_{15}$: C 59.59, H 6.35, N 1.88; found: C 60.02, H 6.36, N 1.97.

3. Glycosylation with silver trifluoromethanesulfonate as catalyst. Bromide **29** (1.85 g, 4.53 mmol) in nitromethane-toluene (1:1, 4 ml) was added to a mixture of **8** (1.18 g, 2.83 mmol), silver trifluoromethanesulfonate (1.23 g, 4.78 mmol) and 4 Å molecular sieves (2.5 g) in the same solvent (6 ml) at –30°C under helium. Stirring was continued for 0.5 h and *sym*-collidine (0.64 ml, 4.78 mmol) was added. The reaction mixture was worked up in the usual way and passed through a column of silica gel (hexane-acetone, 3:2) to provide **30** (1.7 g, 81%). The ^{1}H-NMR spectrum is identical to that previously reported [8].

*Methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[4,6-O-(4-methoxybenzylidene)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**34**)

A mixture of **31** [8] (1.14 g, 1.96 mmol), anisaldehyde dimethyl acetal (0.66 ml, 3.86 mmol) and *p*-toluenesulfonic acid monohydrate (45 mg) in dry acetonitrile (20 ml) was stirred for 1.5 h at room temperature. Neutralization with triethylamine and solvent evaporation gave a residue which was dissolved in methanol and precipitated from ether to provide **34** as a white solid (1.30 g, 94.9%). An analytical sample was recrystallized from ethyl acetate-methanol. M.p. 182–184 °C. $[\alpha]_D$ –54.5° (*c* 0.4, methanol). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.20 (m, 10H, 2Ph), 7.30 and 6.80 (4H, MeOPh), 5.63 (d, 1H, $J_{NH,2a}$ 7.5 Hz, NH), 5.44 (s, 1H, *CH*Ph), 5.04 and 4.73 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.75 (d, 1H, $J_{1a,2a}$ 7.5 Hz, H-1a), 4.68 and 4.55 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.50 (d, 1H, $J_{1b,2b}$ 8.0 Hz, H-1b), 3.77, 3.47 (2s, each 3H, $2CH_3O$), 1.89 (s, 3H, CH_3CO). *Anal.* calcd. for $C_{37}H_{45}NO_{12}$: C 63.87, H 6.52, N 2.01; found: C 63.61, H 6.66, N 2.07.

*Methyl 2-acetamido-4-*O*-[3-O-benzoyl-4,6-O-(4-methoxybenzylidene)-β-D-galactopyranosyl]-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside* (**35**)

34 (1.34 g, 1.93 mmol) and pyridine (1.0 ml, 12.4 mmol) were dissolved in dry dichloromethane (35 ml). Benzoyl chloride (0.34 ml, 2.89 mmol) was added slowly at –78 °C

Table 3. ^{13}C-NMR chemical shifts (ppm) for the H-type 2 blood group determinant Fucα(1-2)Glcβ(1-4)GlcNAcβMe (**24**) and related structures[a].

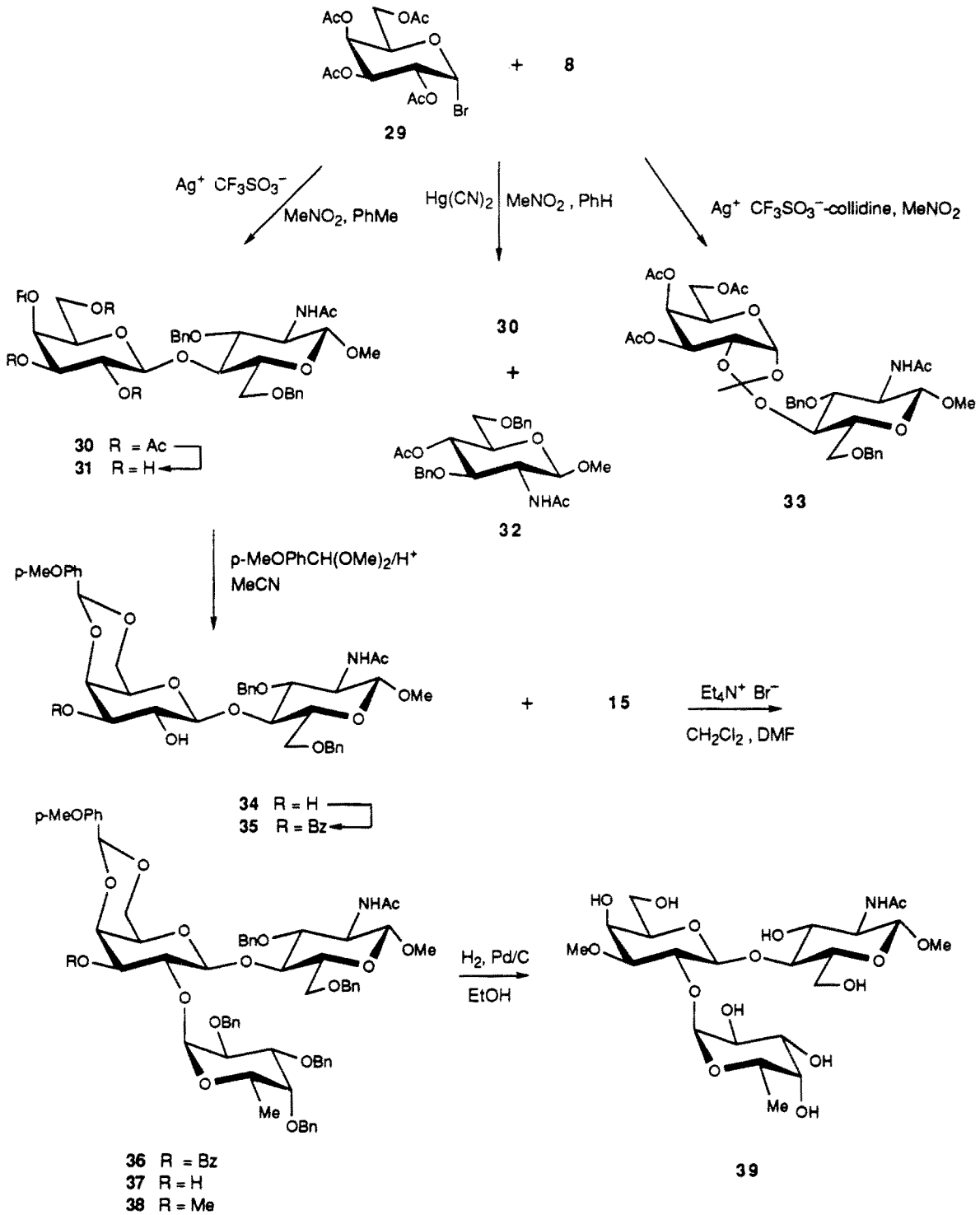
		Derivatives of H-type 2-OMe (*24*)							
	H-type 2-OMe (*24*)	*3a-OMe* (*25*)	*2c-OMe* (*26*)	*3c-OMe* (*27*)	*4c-OMe* (*28*)	*3b-OMe* (*39*)	*4b-OMe* (*49*)	*6b-OMe* (*56*)	*xylo-* (*63*)
β-D-GlcNAc a-unit									
C-1	102.73	102.88	102.77	102.77	102.78	102.79	102.80	102.72	104.78
C-2	56.03	54.43	55.99	55.98	55.98	55.97	55.93	55.99	73.68
C-3	73.23	81.76	73.31	73.26	73.26	73.30	73.28	73.24	75.01
C-4	77.26	74.77	77.06	77.14	77.00	77.01	77.09	77.67	76.82
C-5	76.14	76.37	76.17	76.18	76.16	76.17	76.15	76.09	63.73
C-6	61.10	60.82	61.06	61.07	61.05	61.04	61.05	61.11	–
CH_3O	57.90	57.96[b]	57.97	57.98	57.97	57.98	57.98	57.95	58.03
CH_3CO	23.05	22.97	23.02	23.02	23.01	23.03	23.04	23.02	–
CO	175.41	175.30	175.50	175.50	175.50	175.50	175.52	175.50	–
β-D-Gal b-unit									
C-1	101.19	101.26	100.99	101.13	101.07	101.04	101.01	101.22	100.60
C-2	77.32	77.22	78.08	77.14	77.25	75.98	77.20	76.89	77.57
C-3	74.43	74.48	74.58	74.42	74.36	84.05	74.80	74.31	74.46
C-4	69.97	70.05	69.53	69.96	69.95	65.33	80.42	70.11	69.72
C-5	76.09	76.04	76.01	76.10	76.09	75.26	76.27	73.92	75.97
C-6	61.87	61.94	61.93	61.93	61.92	62.02	61.48	72.29	61.90
CH_3O	–	–	–	–	–	57.10	62.25	59.27	–
α-L-Fuc c-unit									
C-1	100.26	100.21	97.17	100.02	100.20	100.06	100.18	100.17	100.28
C-2	69.13	69.03	76.01	67.99	69.35	68.82	68.99	69.01	69.16
C-3	70.54	70.52	70.10	79.72	70.83	70.44	70.49	70.44	70.41
C-4	72.56	72.52	72.39	68.11	83.20	72.54	72.53	72.51	72.60
C-5	67.74	67.70	67.32	67.57	67.81	67.58	67.68	67.74	67.70
C-6	16.14	16.17	16.05	16.21	16.22	16.08	16.13	16.12	16.18
CH_3O	–	–	55.99	56.77	55.98	–	–	–	–

[a] 0.05 M solutions in D_2O with dioxane as internal standard at 67.4 ppm were measured at 75 MHz at 295°. The assignments were made by inspection and are tentative.
[b] CH_3O-3a 59.05 ppm.

with stirring. When TLC examination showed the completion of the reaction, the excess of reagent was destroyed by the addition of methanol (0.7 ml). The mixture was allowed to reach room temperature and poured into ice-water. Washing of the organic layer with water, followed by drying and solvent evaporation left a white solid which was washed with ether (1.38 g, 89.3%). An analytical sample was recrystallized from ethyl acetate-hexane. M.p. 164–165 °C. $[\alpha]_D$ +66.8° (*c* 0.8, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 8.10, 7.60–7.20 and 6.75 (m, 19H, 3Ph and MeOPh), 5.68 (d, 1H, $J_{NH,2a}$ 7.5 Hz, NH), 5.39 (s, 1H, C*H*Ph), 5.05 and 4.71 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.97 (dd, 1H, $J_{2b,3b}$ 10.0 Hz, $J_{3b,4b}$ 3.5 Hz, H-3b), 4.78, 4.63 (2d, each 1H, *J* 7.5, 8.0 Hz, H-1a, H-1b), 4.71 and 4.58 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.31 (d, 1H, H-4b), 3.76, 3.48 (2s, each 3H, 2CH_3O), 1.90 (s, 3H, CH_3CO). *Anal.* calcd. for $C_{44}H_{49}NO_{13}$: C 66.07, H 6.17, N 1.75; found: C 65.93, H 6.36, N 1.76.

*Methyl 2-acetamido-4-*O*-[3-*O*-benzoyl-4,6-*O*-(4-methoxybenzylidene)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside* (**36**)

Bromide **15** was reacted with alcohol **35** for 20 h following the general procedure for bromide-ion catalysed fucosylations. *N*,*N*-diisopropylethylamine and methanol were added and stirring continued for 2 h. Usual work-up was followed by column chromatography on silica gel (hexane-acetone, 3:2) to provide **36** as a white foam (96%). $[\alpha]_D$ +7° (*c* 2.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 8.00–6.70 (m, 34H, 6Ph, MeOPh), 5.83 (bs, 1H, NH), 5.42 (d, 1H, $J_{1c,2c}$ 3.5 Hz, H-1c), 5.39 (s, 1H, C*H*Ph), 5.11 (dd, 1H, $J_{2b,3b}$ 10.0 Hz, $J_{3b,4b}$ 3.5 Hz, H-3b), 5.05 and 4.67 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.77 (d, 1H, $J_{1a,2a}$ 7.5 Hz, H-1a), 4.54 (d, 1H, $J_{1b,2b}$ 8.0 Hz, H-1b), 4.51 and 4.42 (ABq, 2H, $J_{A,B}$

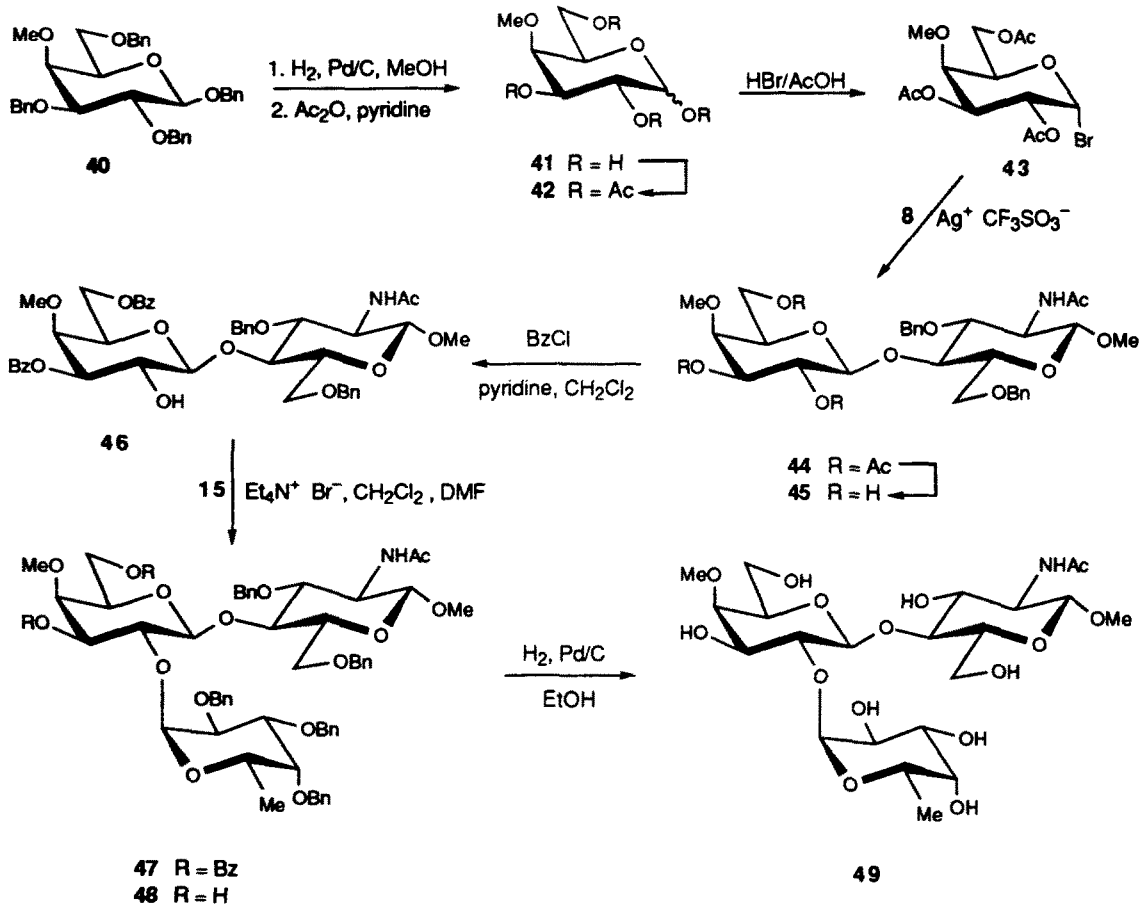


Scheme 2. The synthesis of 3b-*O*-methyl-H-type 2-OMe (**39**).

12.0 Hz, CH_2Ph), 4.38 (d, 1H, H-4b), 4.32 (dd, 1H, H-2b), 4.22 (m, 1H, H-5c), 3.95 (dd, 1H, $J_{2c,3c}$ 10.0 Hz, H-2c), 3.72, 3.48 (2s, each 3H, $2CH_3O$), 3.40 (m, 1H, H-2a), 1.88 (s, 3H, CH_3CO), 1.20 (d, 3H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{71}H_{77}NO_{17}$: C 70.11, H 6.38, N 1.15; found: C 69.93, H 6.81, N 1.18.

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[4,6-O-(4-methoxybenzylidene)-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**37**)

Compound **36** (0.4 g, 0.33 mmol) was treated with methanolic 0.07 N sodium methoxide (11 ml) for 20 h. Neutralization



Scheme 3. The synthesis of 4b-*O*-methyl-H-type 2-OMe (**49**).

with Amberlite IRC 50 H^+, filtration and evaporation provided **37** as a white solid (0.355 g, 97%). $[\alpha]_D$ −40° (c 0.6, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.10 and 6.80 (m, 29H, 5Ph, MeOPh), 5.68 (d, 1H, $J_{NH,2a}$ 7.5 Hz, NH), 5.50 (s, 1H, C*H*Ph) 5.04 (d, 1H, $J_{1c,2c}$ 3.5 Hz, H-1c), 3.78, 3.47 (2s, each 3H, $2CH_3O$), 1.88 (s, 3H, CH_3CO), 1.14 (d, 3H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{64}H_{73}NO_{16}$: C 69.11, H 6.62, N 1.26; found: C 68.71, H 6.94, N 1.31.

*Methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[4,6-*O*-(4-methoxybenzylidene)-3-*O*-methyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**38**)

A mixture of **37** (0.336 g, 0.30 mmol), barium oxide (0.139 g, 0.89 mmol), barium hydroxide octahydrate (40 mg, 0.13 mmol) and methyl iodide (0.34 ml, 5.43 mmol) in *N,N*-dimethylformamide (1.2 ml) was stirred under helium for 5 h. More methyl iodide (0.2 ml, 3.23 mmol) was added and stirring continued for 2 h. The reaction mixture was diluted with dichloromethane and filtered through a pad of Celite. The material obtained on evaporation was applied to a column of silica gel and eluted with hexane-acetone (3:2). **38** was obtained as a white foam (0.3 g, 88%). $[\alpha]_D$ −44.8° (c 0.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.45–7.20 (m, 25H, 5Ph), 7.15 and 6.70 (4H, MeOPh), 5.71 (d, 1H, $J_{NH,2a}$ 7.0 Hz, NH), 5.52 (d, 1H, $J_{1c,2c}$ 3.7 Hz, H-1c), 5.43 (s, 1H, C*H*Ph), 5.05–4.45 (m, 10H, $5CH_2$Ph), 4.74 (d, 1H, $J_{1a,2a}$ 7.0 Hz, H-1a), 4.48 (d, 1H, $J_{1b,2b}$ 8.0 Hz, H-1b), 4.27 (q, 1H, $J_{5c,6c}$ 6.5 Hz, H-5c), 4.08 (dd, $J_{2c,3c}$ 10.0 Hz, H-2c), 3.74, 3.48, 3.35 (3s, each 3H, $3CH_3O$), 1.88 (s, 3H, CH_3CO), 1.25 (d, 3H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{65}H_{75}NO_{16}$: C 69.31, H 6.71, N 1.24; found: C 69.01, H 6.46, N 1.30.

*Methyl 2-acetamido-2-deoxy-4-*O*-[2-O-(α-L-fucopyranosyl)-3-*O*-methyl-β-D-galactopyranosyl]-β-D-glucopyranoside* (**39**)

The hydrogenolysis of **38** produced **39** in 86.9% yield. $[\alpha]_D$ −74.7° (c 0.5, water). See Tables 2 and 3 for characterization by ^{1}H- and ^{13}C-NMR.

*4-*O*-Methyl-D-galactopyranose* (**41**) [38]

Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-methyl-β-D-galactopyranoside (**40**) [37] (0.95 g, 1.71 mmol) in methanol (50 ml) was hydrogenated in the hydrogen stream over 5% palladium-on-carbon (0.6 g) for 3 h. The catalyst was filtered off and the solvent evaporated to give a white solid of **42** (320 mg, 96.4%). M.p. 214–216 °C {lit. [38], m.p. 218–221 °C}.

2,3,6-Tri-O-acetyl-4-O-methyl-α-D-galactopyranosyl bromide (**43**)

41 (170 mg, 0.89 mmol) was acetylated with acetic anhydride (0.8 ml) in pyridine (1.2 ml) for 24 h. The reaction mixture was evaporated and co-evaporated with toluene to leave 1,2,3,6-tetra-*O*-acetyl-4-*O*-methyl-D-galactopyranose **42** [39] as a syrup which was treated with hydrogen bromide-acetic acid (25%, 0.8 ml) for 4 h. The solution was poured into ice-water and extracted with ether. The ether solution was washed with water, aqueous sodium hydrogen carbonate and water, followed by drying and evaporation, to provide **43** (275 mg, 81%) as a syrup. ^{1}H-NMR ($CDCl_3$) δ: 6.69 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.33 (dd, 1H, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 2.7 Hz, H-3), 5.15 (dd, 1H, H-2), 4.31–4.22 (m, 3H, H-5 and H-6, H-6'), 3.81 (d, 1H, H-4), 3.50 (s, 3H, CH_3O), 2.15, 2.10 (2s, 3H, 6H, $3CH_3CO$).

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(2,3,6-tri-O-acetyl-4-O-methyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**44**)

Bromide **43** was reacted with alcohol **8** in the same way as bromide **29** in the preparation of **30** using silver trifluoromethanesulfonate as a catalyst. After stirring for 2 h at −30° and 3 h at room temperature, *sym*-collidine was added and the reaction mixture was worked up in the usual way. After chromatography of the crude material on a column of silica gel (hexane-acetone, 3:2), **44** (60.6%) was obtained as a white solid. $[\alpha]_D$ −20° (*c* 0.3, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.20 (m, 10H, 2Ph), 6.05 (d, 1H, $J_{NH,2a}$ 8.0 Hz, NH), 5.23 (dd, 1H, $J_{1b,2b}$ 7.7 Hz, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.86 (dd, 1H, $J_{3b,4b}$ 3.2 Hz, H-3b), 4.66 (s, 2H, CH_2Ph), 4.61 and 4.50 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.55 (d, 1H, $J_{1a,2a}$ 4.5 Hz, H-1a), 4.41 (d, 1H, $J_{1b,2b}$ 7.7 Hz, H-1b), 4.19 (m, 2H, H_2-6b), 3.62 (dd, 1H, $J_{3b,4b}$ 3.2 Hz, $J_{4b,5b} < 1$ Hz, H-4b), 3.48, 3.42 (2s, each 3H, $2CH_3O$), 2.09, 2.05, 2.04 and 1.95 (4s, each 3H, $4CH_3CO$). *Anal.* calcd. for $C_{36}H_{47}NO_{14}$: C 60.24, H 6.60, N 1.95; found: C 60.06, H 6.92, N 1.90.

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(4-O-methyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**45**)

Compound **44** (50 mg, 0.07 mmol) was treated with methanolic 0.08N sodium methoxide (0.96 ml) for 22 h. Neutralization with Amberlite IRC 50 H^+, followed by evaporation, left **45** as a white solid (41 mg, 99%). $[\alpha]_D$ −12.7° (*c* 0.5, methanol). ^{1}H-NMR ($D_2O + CD_3OD$) δ: 7.40–7.20 (m, 10H, 2Ph), 4.99 and 4.56 (ABq, 2H, $J_{A,B}$ 10.5 Hz, CH_2Ph), 4.67 and 4.57 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.44, 4.33 (2d, each 1H, *J* 7.5, 7.7 Hz, H-1a, H-1b), 3.55, 3.40 (2s, each 3H, $2CH_3O$), 1.94 (s, 3H, CH_3CO). *Anal.* calcd. for $C_{30}H_{41}NO_{11}$: C 60.90, H 6.98, N 2.37; found: C 60.55, H 6.72, N 2.15.

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(3,6-di-O-benzoyl-4-O-methyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**46**)

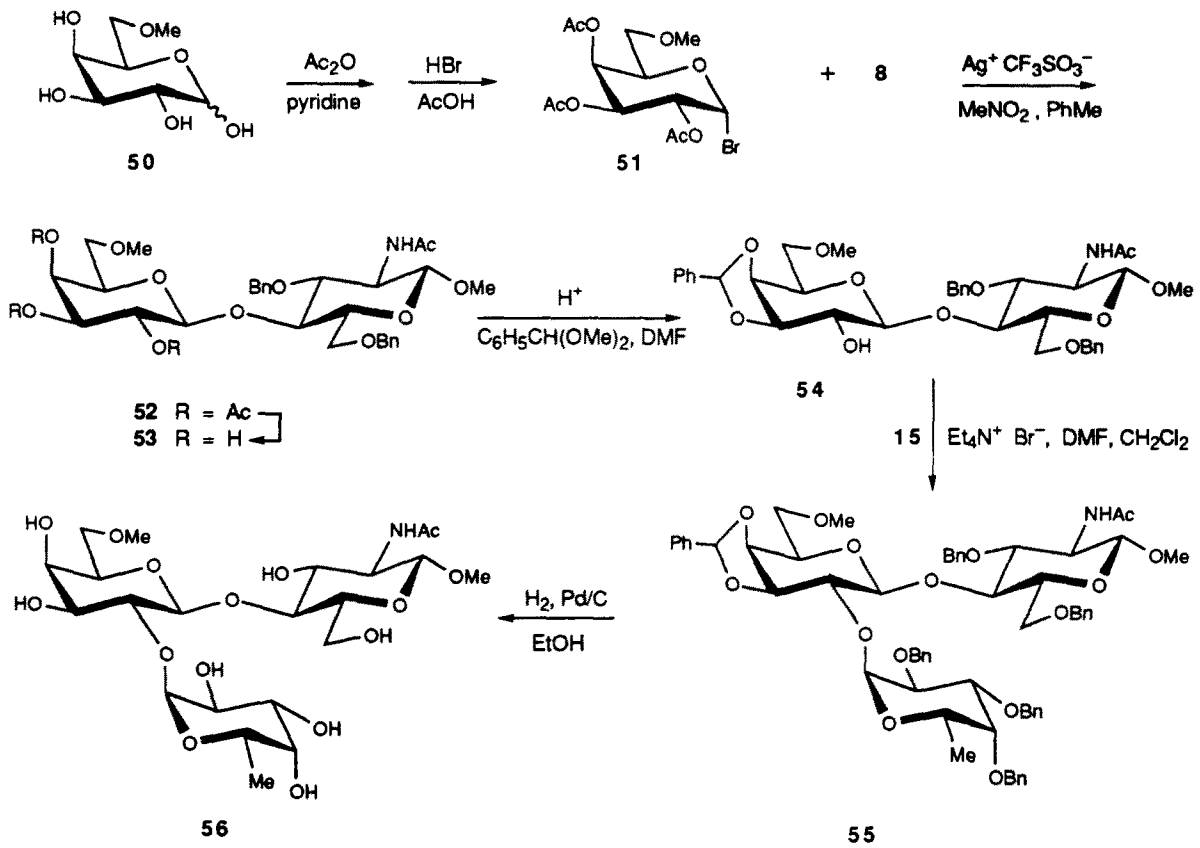
Compound **45** (100 mg, 0.17 mmol) was benzoylated in the same way as described for the preparation of **35**. **46** (90 mg, 66.7%) was obtained as a white solid after chromatography of the crude product on a column of silica gel (hexane-acetone, 3:2). $[\alpha]_D$ +0.7° (*c* 0.6, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 8.20–8.00, 7.65–7.20 (m, 20H, 4Ph), 5.65 (m, 1H, NH), 5.05 (dd, 1H, $J_{2b,3b}$ 10.0 Hz, $J_{3b,4b}$ 3.5 Hz, H-3b), 4.91 and 4.64 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.76, 4.61 (2d, each 1H, *J* 8.0, 8.5 Hz, H-1a, H-1b), 4.71 and 4.55 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.45 (dd, 1H, $J_{5b,6b}$ 6.5 Hz, $J_{6b,6b'}$ 11.5 Hz, H-6b), 4.30 (dd, 1H, $J_{5b,6b'}$ 6.5 Hz, H-6b'), 3.46, 3.45 (2s, each 3H, $2CH_3O$), 1.86 (s, 3H, CH_3CO). *Anal.* calcd. for $C_{44}H_{49}NO_{13}$: C 66.07, H 6.17, N 1.75; found: C 66.16, H 6.11, N 1.74.

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[3,6-di-O-benzoyl-4-O-methyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**47**)

Alcohol **46** was reacted with bromide **15** for 24 h, following the general procedure for the bromide-ion catalysed fucosylations. Column chromatography on silica gel (hexane-acetone, 3:2) provided a white foam of **47** (93.6%). $[\alpha]_D$ −43° (*c* 0.6, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 8.00–7.10 (m, 35H, 7Ph), 5.70 (d, 1H, $J_{NH,2a}$ 7.0 Hz, NH), 5.40 (d, 1H, $J_{1c,2c}$ 3.4 Hz, H-1c), 5.14 (dd, 1H, $J_{2b,3b}$ 9.5 Hz, $J_{3b,4b}$ 3.0 Hz, H-3b), 4.90–4.38 (m, 10H, $5CH_2$Ph), 4.77 (d, 1H, $J_{1a,2a}$ 7.0 Hz, H-1a), 4.22 (dd, 1H, $J_{1b,2b}$ 7.5 Hz, $J_{2b,3b}$ 9.5 Hz, H-2b), 4.21 (m, 1H, H-5c), 4.05 (m, 1H, H-3a), 3.94 (dd, 1H, $J_{1c,2c}$ 3.4 Hz, $J_{2c,3c}$ 10.0 Hz, H-2c), 3.73 (dd, 1H, $J_{3c,4c}$ 2.5 Hz, H-3c), 3.48, 3.40 (2s, each 3H, $2CH_3O$), 3.31 (m, 1H, H-2a), 1.90 (s, 3H, CH_3CO), 1.20 (d, 3H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{71}H_{77}NO_{17}$: C 70.10, H 6.38, N 1.15; found: C 70.06, H 6.48, N 1.18.

Methyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[4-O-methyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**48**)

Compound **47** was treated with methanolic 0.07 N sodium methoxide (8 ml) for 1 day. More methanolic 0.5 N sodium methoxide (1.5 ml) was added. After 4 days, it was neutralized with Amberlite IRC 50 H^+ and evaporated to leave a solid which was purified on a short column of silica gel (acetone-hexane, 3:2) to afford **48** (103 mg, 94.5%). $[\alpha]_D$ −46° (*c* 0.5, dichloromethane), ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.20 (m, 25H, 5Ph), 5.00–4.42 (m, 10H, $5CH_2$Ph), 4.95 (d, 1H, $J_{1c,2c}$ 3.5 Hz, H-1c), 4.62 and 4.29 (2d, each 1H, H-1a and H-1b), 3.50, 3.42 (2s, each 3H, $2CH_3O$), 1.90 (s, 3H, CH_3CO), 1.09 (d, 3H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{57}H_{69}NO_{15}$: C 67.91, H 6.90, N 1.39; found: C 67.84, H 7.05, N 1.40.



Scheme 4. The synthesis of 6b-*O*-methyl-H-type 2-OMe (**56**).

*Methyl 2-acetamido-2-deoxy-4-*O-[*2-*O-(*α-L-fucopyranosyl*)-*4-*O-*methyl-β-D-galactopyranosyl*]-*β-D-glucopyranoside* (**49**)

The hydrogenolysis of **48** produced **49** in 89% yield. $[\alpha]_D$ −94.4° (*c* 0.6, water). See Tables 2 and 3 for characterization by ^{1}H- and ^{13}C-NMR.

*2,3,4-Tri-*O-*acetyl-6-*O-*methyl-α-D-galactopyranosyl bromide* (**51**) [1]

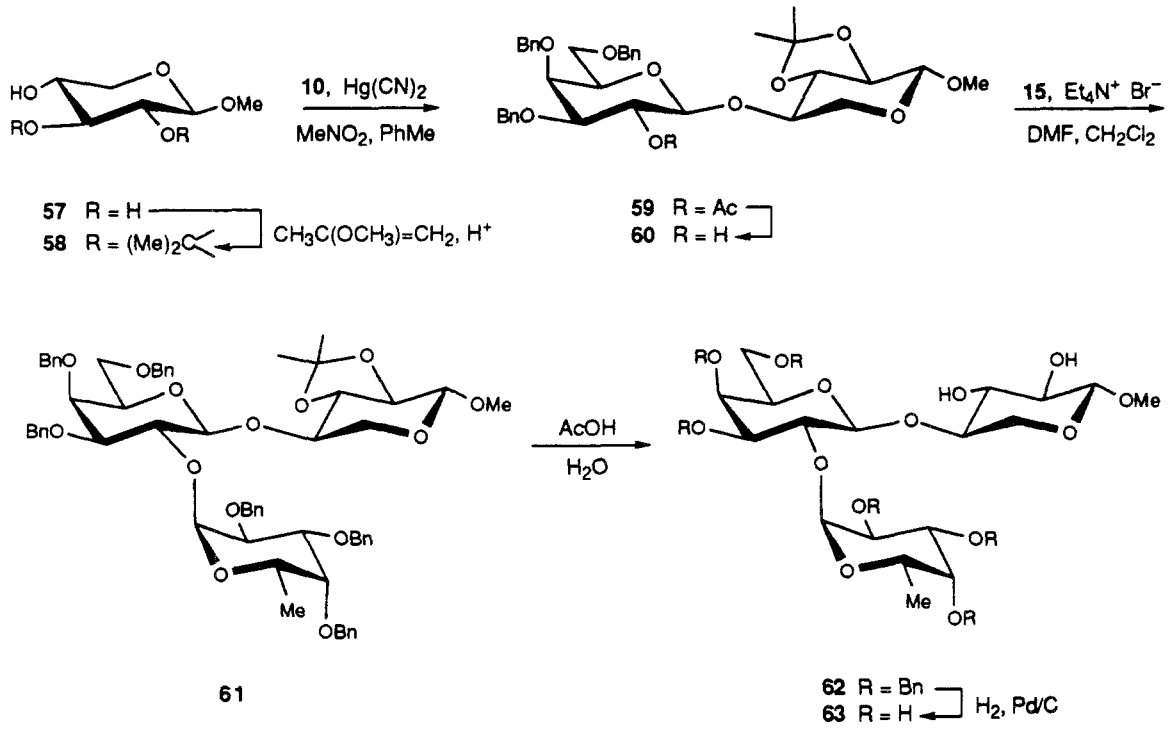
This compound was obtained by acetylation of **50** [40] (acetic anhydride/pyridine), followed by reaction with hydrogen bromide-acetic acid. ^{1}H-NMR ($CDCl_3$) δ: 6.71 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.52 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1.0 Hz, H-4), 5.40 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-3), 5.05 (dd, 1H, H-2), 4.42 (t, 1H, $J_{5,6}$ 5.5 Hz, H-5), 3.50 and 3.43 (2dd, 2H, H-6), 3.30 (s, 3H, CH_3O), 2.16, 2.10, 2.00 (3s, each 3H, $3CH_3CO$).

*Methyl 2-acetamido-3,6-di-*O-*benzyl-2-deoxy-4-*O-(*2,3,4-tri-*O-*acetyl-6-*O-*methyl-β-D-galactopyranosyl*)-*β-D-glucopyranoside* (**52**)

Bromide **51** was reacted with alcohol **8** in the same way as bromide **29** in the preparation of **30** using silver trifluoromethanesulfonate as a catalyst. After chromatography on a column of silica gel (hexane-acetone, 3:2), **52** was obtained as a white solid (85.8%). $[\alpha]_D$ −22.6° (*c* 0.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.20 (m, 10H, 2Ph), 5.93 (d, 1H, $J_{NH,2a}$ 7.5 Hz, NH), 5.37 (dd, 1H, $J_{2b,3b}$ 3.5 Hz, $J_{4b,5b} < 1$ Hz, H-4b), 5.11 (dd, 1H, $J_{1b,2b}$ 8.0 Hz, $J_{3b,4b}$ 10.0 Hz, H-2b), 4.91 (dd, 1H, $J_{3b,4b}$ 3.5 Hz, H-3b), 4.75 and 4.65 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.65 and 4.49 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.62 (d, 1H, $J_{1a,2a}$ 4.5 Hz, H-1a), 4.48 (d, 1H, $J_{1b,2b}$ 8.0 Hz, H-1b), 3.45, 3.26 (2s, each 3H, $2CH_3O$), 2.17–1.96 (4s, each 3H, $4CH_3CO$). *Anal.* calcd. for $C_{36}H_{47}NO_{14}$: C 60.24, H 6.60, N 1.95; found: C 59.38, H 6.52, N 1.93.

*Methyl 2-acetamido-3,6-di-*O-*benzyl-2-deoxy-4-*O-(*6-*O-*methyl-β-D-galactopyranosyl*)-*β-D-glucopyranoside* (**53**)

Compound **52** (210 mg, 0.29 mmol) was treated with methanolic 0.08 N sodium methoxide (4 ml) for 22 h. Neutralization with Amberlite IRC 50 H^+, followed by evaporation, left **53** as a white solid (0.17 g, 98%). $[\alpha]_D$ −5° (*c* 0.5, methanol). ^{1}H-NMR (D_2O + CD_3OD) δ: 7.50–7.30 (m, 10H, 2Ph), 4.85 and 4.63 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.72 and 4.53 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.42 (d, 1H, $J_{1a,2a}$ 8.0 Hz, H-1a), 4.20 (d, 1H, $J_{1b,2b}$ 7.7 Hz, H-1b), 3.45, 3.30 (2s, each 3H, $2CH_3O$), 1.85 (s, 3H, CH_3CO).



Scheme 5. The synthesis of Fucα(1-2)Galβ(1-4)XylβMe (**63**).

*Methyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-(3,4-*O*-benzylidene-6-*O*-methyl-β-*D*-galactopyranosyl)-2-deoxy-β-*D*-glucopyranoside* (**54**)

A mixture of **53** (240 mg, 0.35 mmol), α,α-dimethoxytoluene (0.32 ml, 2.79 mmol) and *p*-toluenesulfonic acid monohydrate (15 mg) in *N*,*N*-dimethylformamide (1 ml) was stirred at 50 °C under helium for 1 h. The residue obtained after neutralization with triethylamine and solvent evaporation was applied to a column of silica gel (hexane-acetone, 3:2) to provide **54** (190 mg, 78.5%) as a 1:1 mixture of two diastereoisomers. $[\alpha]_D$ +11.2° (*c* 0.7, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.50–7.20 (m, 15H, 3Ph), 6.13 and 5.96 (2s, each 0.5H, C*H*Ph), 5.64 (m, 1H, NH), 3.50, 3.49 (2s, each 1.5H, CH_3O), 3.31, 3.29 (2s, each 1.5H, CH_3O), 1.87 (s, 3H, CH_3CO). *Anal.* calcd. for $C_{37}H_{45}NO_{11}$: C 65.38, H 6.67, N 2.06; found: C 64.85, H 7.00, N 2.03. An analytical sample was acetylated conventionally (acetic anhydride/pyridine) to provide *methyl 2-acetamido-4-*O*-(2-*O*-acetyl-3,4 - O - benzylidene - 6 - O - methyl - β - D-galactopyranosyl) - 3,6 - di - O - benzyl - 2 - deoxy - β - D - glucopyranoside* which was characterized by ^{1}H-NMR spectroscopy. ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.20 (m, 15H, 3Ph), 6.16 and 5.91 (2s, each 0.5H, C*H*Ph), 6.04 (m, 1H, NH), 5.08, 4.98 (t,m, each 0.5H, $J_{1b,2b}$ 8.0 Hz, $J_{2b,3b}$ 8.5 Hz, H-2b), 3.44, 3.43 (2s, each 1.5H, CH_3O), 3.35, 3.34 (2s, each 1.5H, CH_3O), 2.10, 2.09 (2s, each 1.5H, CH_3CO), 1.95, 1.91 (2s, each 1.5H, CH_3CO).

*Methyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-[3,4-*O*-benzylidene-6-*O*-methyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-2-deoxy-β-*D*-glucopyranoside* (**55**)

Bromide **15** was reacted with alcohol **54** for 20 h following the general procedure for bromide-ion catalysed fucosylations. Chromatography on a silica gel column (hexane-acetone, 2:1, containing 0.5% triethylamine) provided **55** (86.7%) as white foam. $[\alpha]_D$ −41.5° (*c* 0.9, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.50–7.10 (m, 30H, 6Ph), 6.09 and 5.94 (2s, each 0.5H, C*H*Ph), 5.73 (d, 1H, $J_{NH,2a}$ 7.5 Hz, NH), 5.58, 5.48 (2d, each 0.5H, $J_{1c,2c}$ 3.7 Hz, H-1c), 3.51, 3.49 (2s, each 1.5H, CH_3O), 3.33, 3.31 (2s, each 1.5H, CH_3O), 1.90, 1.85 (2s, each 1.5H, CH_3CO), 1.23, 1.13 (2d, each 1.5H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{64}H_{73}NO_{15}$: C 70.12, H 6.71, N 1.28; found: C 69.69, H 6.72, N 1.31.

*Methyl 2-acetamido-2-deoxy-4-*O*-[2-*O*-(α-*L*-fucopyranosyl)-6-*O*-methyl-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**56**)

The hydrogenolysis of **55** produced **56** in 85% yield. $[\alpha]_D$ −80.4° (*c* 0.5, water). See Tables 2 and 3 for characterization by ^{1}H- and ^{13}C-NMR.

Methyl 2,3-O-*isopropylidene-β-D-xylopyranoside* (**58**) [41]

2-Methoxypropene (0.268 ml, 2.8 mmol) was added to a mixture of **57** (0.2 g, 1.2 mmol), *p*-toluenesulfonic acid

(20 mg) in *N,N*-dimethylformamide (0.6 ml) with stirring at room temperature. After 1 h, it was diluted with dichloromethane, washed with water, aqueous sodium hydrogen carbonate and water. The aqueous solution was reextracted with dichloromethane. Evaporation of the dried organic solution gave a syrup of **58** which turned into a solid in hexane (0.16 g, 65%). M.p. 71–73 °C (ether-hexane) {lit. [41], m.p. 73–75 °C}. An analytical sample was acetylated (acetic anhydride/pyridine) to give *methyl 4-O-acetyl-2,3-O-isopropylidene-β-D-xylopyranoside* which was characterized by ^{1}H-NMR spectroscopy. ^{1}H-NMR ($CDCl_3$) δ: 5.03 (td, 1H, $J_{4,5e}$ 5.0 Hz, $J_{4,5a}$ 5.7 Hz, $J_{3,4}$ 9.0 Hz, H-4), 4.64 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.20 (dd, 1H, $J_{5e,5a}$ 12.0 Hz, H-5e), 3.79 (dd, $J_{2,3}$ 10.0 Hz, H-3), 3.52 (s, 3H, CH_3O), 3.48 (dd, 1H, H-2), 3.37 (dd, 1H, H-5a), 2.11 (s, 3H, CH_3CO), 1.48 and 1.46 (2s, each 3H, $2CH_3$).

Methyl 4-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,3-O-isopropylidene-β-D-xylopyranoside (**59**)

This compound was prepared from **58** and bromide **10** under the conditions described for the preparation of **11**. The yield was 34% after column chromatography on silica gel (hexane-ethyl acetate, 2:1). $[\alpha]_D$ −8.3° (*c* 0.5, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.20 (m, 15H, 3Ph), 5.34 (dd, 1H, $J_{1b,2b}$ 8.0 Hz, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.93 and 4.58 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.65 and 4.48 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.49 (d, 1H, $J_{1b,2b}$ 8.0 Hz, H-1b), 4.46 (d, 1H, $J_{1a,2a}$ 7.0 Hz, H-1a), 4.43 and 4.39 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 3.98 (m, 1H, H-4b), 3.50 (s, 3H, CH_3O), 3.48 (dd, 1H, $J_{2b,3b}$ 10.0 Hz, $J_{3b,4b}$ 3.0 Hz, H-3b), 3.27 (m, 1H, H-2a), 2.03 (s, 3H, CH_3CO), 1.38 and 1.33 (2s, each 3H, $2CH_3$). *Anal.* calcd. for $C_{38}H_{46}O_{11}$: C 67.24, H 6.83; found: C 66.95, H 6.80.

Methyl 2,3-O-isopropylidene-4-O-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-β-D-xylopyranoside (**60**)

Compound **59** (0.37 g, 0.54 mmol) was treated with methanolic 0.045 N sodium methoxide (11.7 ml) for 20 h. Neutralization with Amberlite IRC 50 H^+, evaporation and column chromatography on silica gel (hexane-ethyl acetate, 1:1) provided **60** (0.328 g, 94.5%) as a white solid. $[\alpha]_D$ −12.5° (*c* 0.7, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.20 (m, 15H, 3Ph), 4.90 and 4.59 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.75 and 4.69 (ABq, 2H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.51, 4.40 (2d, 2H, *J* 8.0, 7.5 Hz, H-1b, H-1a), 4.46 (s, 2H, CH_2Ph), 3.93 (d, 1H, $J_{3b,4b}$ 3.0 Hz, H-4b), 3.52 (s, 3H, CH_3O), 3.43 (dd, 1H, $J_{2b,3b}$ 10.0 Hz, H-3b), 1.41 and 1.39 (2s, each 3H, $2CH_3$). *Anal.* calcd. for $C_{36}H_{44}O_{10}$: C 67.91, H 6.97; found: C 68.06, H 7.17.

Methyl 2,3-O-isopropylidene-4-O-[3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-xylopyranoside (**61**)

Alcohol **60** was reacted with bromide **15** for 30 h following the general procedure for the bromide-ion catalysed fucosylations. Column chromatography on silica gel (hexane-ethyl acetate, 2:1, containing 0.6% triethylamine) provided **61** (71%) as a foam. $[\alpha]_D$ −75° (*c* 1.3, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.00 (m, 30H, 6Ph), 5.65 (d, 1H, $J_{1c,2c}$ 3.7 Hz, H-1c), 4.90–4.48 (m, 10H, $5CH_2$Ph), 4.59, 4.50 (2d, each 1H, *J* 7.5, 8.0 Hz, H-1a, H-1b), 4.45 and 4.38 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.26 (q, 1H, $J_{5c,6c}$ 6.5 Hz, H-5c), 4.19 (dd, 1H, $J_{2b,3b}$ 10.0 Hz, $J_{1b,2b}$ 7.8 Hz, H-2b), 4.02 (dd, 1H, $J_{2c,3c}$ 10.0 Hz, H-2c), 3.50 (s, 3H, CH_3O), 3.30 (dd, 1H, $J_{1a,2a}$ 7.0 Hz, $J_{2a,3a}$ 10.0 Hz, H-2a), 1.40, 1.30 (2s, each 3H, $2CH_3$), 1.12 (d, 3H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{63}H_{72}O_{14}$: C 71.84, H 6.89; found: C 71.64, H 6.88.

Methyl 4-O-[3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-xylopyranoside (**62**)

61 (310 mg, 0.29 mmol) was treated with acetic acid (80%, 2.6 ml) at 70 °C for 2 h. The material obtained on evaporation was applied to a column of silica gel (hexane-ethyl acetate, 1:1) to provide **62** (280 mg, 94%) as a foam. $[\alpha]_D$ −59.9° (*c* 0.3, dichloromethane). ^{1}H-NMR ($CDCl_3$) δ: 7.40–7.00 (m, 30H, 6Ph), 5.65 (d, 1H, $J_{1c,2c}$ 3.7 Hz, H-1c), 4.95–4.50 (m, 10H, $5CH_2$Ph), 4.46 and 4.40 (ABq, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.17 (q, overlapped, H-5c), 4.04 (dd, 1H, $J_{2c,3c}$ 10.0 Hz, H-2c), 3.54 (s, 3H, CH_3O), 3.19 (dd, 1H, $J_{1a,2a}$ 7.0 Hz, $J_{2a,3a}$ 10.0 Hz, H-2a), 1.12 (d, 3H, $J_{5c,6c}$ 6.5 Hz, H_3-6c). *Anal.* calcd. for $C_{60}H_{68}O_{14}$: C 71.13, H 6.76; found: C 71.02, H 6.78.

Methyl 4-O-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-xylopyranoside (**63**)

The hydrogenolysis of **62** produced **63** in 91.3% yield. $[\alpha]_D$ −80.8° (*c* 0.4, water). See Tables 2 and 3 for characterization by ^{1}H- and ^{13}C-NMR.

Discussion of results

NMR spectroscopy

The ^{1}H-NMR parameters that characterize the various intermediates depicted in Schemes 1–5 are presented in the Material and methods section together with their mode of preparation. The structures assigned to the various derivatives of H-type 2-OMe (**24**) that were used in the binding studies are confirmed by the ^{1}H-NMR parameters presented in Table 2 since the differences from those for **24** correspond in each case to expectation based on the structural change. These structural assignments are thoroughly corroborated by the ^{13}C-NMR data presented in Table 3. The quality of the spectra all required a state of high purity.

It is of interest to note that the spacing which arises from $J_{1a,2a}$ coupling with $CDCl_3$ as solvent, for some of the blocked *N*-acetyllactosamine derivatives; for example, **13**, **44** and **52**, are substantially smaller (3.5–4.5 Hz) than are those, ~8 Hz, expected for H-1a and H-2a in an *anti*-periplanar relationship. As previously mentioned with regard to similar molecules [8], the small spacings can be ascribed to contributions to the time-averaged coupling by $^1C_4$ or $^{3,0}B$ conformations for the GlcNAc unit. These strained conformations are considered to become energetically acceptable because the opposing *syn*-axial relationship for the acetamido group and the O-4a atom allows the formation of an intramolecular hydrogen bond that is favoured by the aprotic solvent.

Synthesis

The reaction sequences for the syntheses of H-type 2-OMe (**24**) [8, 35] and its 3a-, 2c-, 3c- and 4c-mono-*O*-methyl congeners (**25**, **26**, **27** and **28**) are outlined in Scheme 1.

The protected *N*-acetyllactosamine intermediate (**11**) was prepared by galactosylation of **8** [8] with the bromide **10** [33] under Helferich conditions [42] and isolated in 97% yield. Zemplen deacetylation provided the alcohol **12** which served as precursor to the blocked trisaccharides **19**, **21**–**23**. The fucosyl bromides **15** [34], and **16** were prepared as previously reported [1, 43] from the appropriately blocked fucose using oxalyl bromide/*N*,*N*-dimethylformamide. For the preparation of **17**, the known diol **1** [29] was selectively methylated at the 3-position by treating the stannylidene intermediate [44, 45] with fluoride ion and methyl iodide (83% yield) [1]. Benzylation produced **3** [29] and acid hydrolysis provided **4**. Methylation of the known **5** [29, 30] gave **6** (90% yield) which was hydrolyzed to **7** (73% yield). The fucosylations of **12** under bromide-ion catalysis [46] produced the blocked trisaccharides **19**, **21**–**23** in yields ranging from 61 to 78%. Catalytic hydrogenolysis of the benzyl groups then afforded the desired trisaccharides **24** and **26**–**28**.

Reductive cleavage of the benzylidene ring of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-glucopyranoside [31, 32] with sodium cyanoborohydride-hydrogen chloride [47] provided **9** which was reacted with bromide **10** to produce **13** (90% yield) which was deacetylated to **14** (83% yield). Fucosylation of **14** provided the blocked trisaccharide **20** (70% yield). Hydrogenolysis then gave **25**.

The synthesis of the 3b- and 4b-*O*-methyl derivatives of **24** required the separate strategies outlined in Schemes 2 and 3.

The synthesis of the 3b-*O*-methyl congener **39** (Scheme 2) started from the known derivative **30** of *N*-acetyllactosamine [8] which was deacetylated to provide **31**. The yield of **30** on reacting acetobromogalactose (**29**) [36] (Scheme 1) with **8** using silver trifluoromethanesulfonate as promoter was 81%. The orthoester **33** was isolated in 60% yield when silver trifluoromethanesulfonate/*sym*-collidine was used. When the glycosidation was promoted with mercuric cyanide as reported [8], **30** was isolated in 67% yield along with the transesterification product **32** (24% yield).

p-Methoxybenzylidenation of **31** to diol **34** proceeded in 95% yield. Selective benzoylation of **34** at the 3b-position [8] provided **35** in 89% yield. α-L-Fucosylation of **35** using **15** as the reagent provided the blocked trisaccharide **36** (96% yield). Saponification of **36** and methylation of the product (**37**) with methyl iodide in the presence of barium oxide-barium hydroxide octahydrate gave **38** in 88% yield. The desired inhibitor (**39**) was then obtained by catalytic hydrogenolysis in the usual manner.

The synthesis of 4b-*O*-methyl-Le^{b}OMe (**49**) is displayed in Scheme 3. 4-*O*-Methyl-D-galactose (**41**) [38] was prepared from **40** [37] by catalytic hydrogenation and acetylated to the tetra-*O*-acetyl derivative **42** [39] from which the glycosyl bromide **43** was prepared. Reaction of **43** with **8** (Scheme 1) using silver trifluoromethanesulfonate provided **44** in 61% yield. The product of deacetylation (**45**) was selectively benzoylated at the 3b- and 6b-positions to form the alcohol **46** in 67% yield. Fucosylation using the bromide **15** provided **47** in 94% yield. Saponification to **48** and catalytic hydrogenolysis of the benzyl groups provided the 4b-*O*-methyl H-type 2 trisaccharide (**49**).

The synthesis of 6b-*O*-methyl-H-type 2-OMe (**56**) is outlined in Scheme 4. The galactosyl bromide (**51**) was prepared conventionally from 6-*O*-methyl-D-galactose (**50**) [40] and coupled with **8** (Scheme 1) using silver trifluoromethanesulfonate to provide the blocked disaccharide **52** in 86% yield. Deacetylation to **53** (98% yield) followed by benzylidenation gave a diastereoisomeric mixture of **54** (79% yield). The structural assignment was confirmed by the characteristic downfield shift of H-2b in the ^{1}H-NMR spectrum of 2b-*O*-acetyl-**54**. Fucosylation of **54** provided **55** (87% yield) and hydrogenolysis to remove the benzyl and benzylidene groups led to the target compound **56**.

The synthesis of **63** (Scheme 5) started from the known methyl 2,3-*O*-isopropylidene-β-D-xylopyranoside **58** [41]. Condensation of **58** with the galactosyl bromide (**10**, Scheme 1) under Helferich conditions provided the desired protected disaccharide (**59**) in poor yield (34%). Fucosylation of the deactylated compound (**60**) under standard conditions gave **61** in 71% yield. The isopropylidene group was then removed by acid hydrolysis to form the diol **62** which was subsequently hydrogenolysed to remove the benzyl group and form the desired **63**.

Binding studies

As seen in Table 1, for each lectin, three mono-*O*-methyl derivatives of H-type 2-OMe (**24**) are essentially inactive as inhibitors of the binding of an H-type 2 artificial antigen. In the case of the *Ulex* lectin, all three positions involve the Fucα c-unit in contrast to the *Galactia* lectin for which two positions in the Galβ b-unit and one in the GlcNAcβ a-unit are involved. The same two positions of the Galβ b-unit are

implicated in the case of the *Psophocarpus* lectin but the third position involves the Fucα c-unit. These *O*-methylations identify the so-called key hydroxyls of the epitope – a structural feature that is complementary to that portion of the combining site termed the polar gate [19]. Prior to complex formation, both the key hydroxyl and the gate are surely strongly hydrated. A prerequisite for binding is the provision of an energetically acceptable alternative to the binding by water. This condition can be met when the stereoelectronic fit of the complementary interacting surfaces allows as thorough an expulsion of the water molecules of hydration to bulk that maintains complementarity (the opening of the gate by the key polar groups). These associations are normally enthalpy driven associations and the force of attraction that is established between the complementary surfaces is not likely, on its own, to be sufficient to replace the attraction these surfaces had for water molecules prior to the association. As was discussed above, evidence has accumulated that the process is strongly assisted by the water molecules achieving an energetically more favourable environment [24].

As seen in Table 1, the deoxygenation of a key hydroxyl group may also cause a strong decrease in activity. The fact that some of the monodeoxy compounds are somewhat more active than the corresponding *O*-methyl derivatives is not surprising since the hydrogen atom is smaller than the hydroxyl group that it has replaced. In contrast, a methoxy group is much larger and more difficult to accommodate at the periphery of the combining site where it may interact with amino acid residues. This situation was encountered in the binding of Le^b-OMe by the lectin GS-IV where the results were shown to be in accordance with expectation based on molecular modelling of the *O*-methyl complexes [1].

The binding by the *Ulex* lectin of both the monodeoxy and mono-*O*-methyl derivatives of **24** at positions 3a, 6b and 4b was weaker than that for **24** (Table 1). It is considered, therefore, that the hydroxyl groups at these positions remain in the aqueous phase but close to the periphery of the combining site [1]. In the case of the substitutions at the 3b-position, the deoxygenation had an adverse effect on binding which was much greater than that of *O*-methylation. These results require that the 3b-hydroxyl of **24** becomes bound to the lectin at the periphery of its combining site. Furthermore, the high activity of the 3b-*O*-methyl compound required the 3b-hydroxyl to serve as a proton acceptor from an amino acid residue with the methyl group remaining in the aqueous phase. It is expected that the low activity of the 3b-deoxy compound is not due so much to the loss of a hydrogen bond with the lectin but to the fact that the proton donating amino acid of the combining site will necessarily remain hydrogen bonded to water and thereby cause a loss in complementarity [8, 48]. It is to be noted (Table 1) that similar results were obtained for the binding of **24** by both *Galactia* and *Psophocarpus* lectins. In the case of *Galactia* the 4c-deoxy derivative of **24** is much less active than is the 4c-*O*-methyl derivative. The 6b-position is similarly involved in the binding by the *Psophocarpus* lectin. It is to be noted in this regard that the inhibition data for these *O*-methyl derivatives, in the absence of the data for the corresponding deoxy derivatives, would suggest that the parent hydroxyl group is not bound to the protein. Therefore, the interpretations of binding data for *O*-methyl congeners only must always keep this possibility in mind.

The above-mentioned findings suggest important practical applications. Many oligosaccharide binding proteins are not available for structural analyses by way of X-ray crystallography. Whether these are enzymes, antibodies or lectins, it may be desired to develop strong inhibitors of an offensive *in vivo* binding reaction in order to alleviate the disease. Means that allow the detection of the part of an oligosaccharide which is not in the epitope and therefore available for chemical modification can be a major asset in this regard [2]. The structures displayed in Fig. 1 show that very different regions of the H-type 2-OMe ligand serve as epitopes for the three lectins. Therefore, it is possible that a short sequence of sugars in an oligosaccharide may be recognized in different ways by a number of different proteins for participation in a given biological process. The preparation of *O*-methyl derivatives may prove useful in the dissecting of such phenomena. For example, the 3b-*O*-methyl derivative of **24** is strongly bound by the *Ulex* lectin but inactive against the other two lectins. Although the *Galactia* lectin strongly binds the 3c-*O*-methyl derivative, this is not the case for *Ulex* and poorly for the *Psophocarpus* lectin. Insights on these matters could be gained by examining the effect of limited *O*-methylation of the natural oligosaccharides. Also, affinity chromatograms may be established using appropriately derivatized ligands at the surface of a solid support to separate proteins that recognize the same oligosaccharide by utilize different surfaces. The synthetic procedures reported herein for the preparation of mono-*O*-methyl derivatives of oligosaccharides should be useful in these regards.

In conclusion, we wish to take this opportunity to report on the participation of the 6a-hydroxymethyl group of GlcNAcβ residues in the binding of **24** by the lectins, as is depicted in Fig. 1. It is known that the 6a-hydroxyl group is nonessential for binding by the lectin I of *Ulex europaeus* [7]. Since the deoxygenation increased the activity, it was apparent that this hydroxyl group must come to reside close to the periphery of the combining site. It was of interest, therefore, to assess whether or not the 6a-methylene group was directly involved in the binding reaction. Replacement of the GlcNAcβ residue of **24** by XylNAcβ would provide an inhibitor that is appropriate for the examination of this possibility. However, it was decided to replace the GlcNAcβ residue by Xylβ (**63**) since this would involve a much more

facile synthesis and it was known that the acetamido group of **24** is not involved in the binding reaction [49].

Inhibition studies using compound **63** have allowed the following conclusions. Since the binding of **63** by the *Psophocarpus* lectin displayed a potency of 36 relative to that for **24** [9], it is concluded that the 6a-methylene group comes close to the periphery of the combining site but does not participate importantly in the binding reaction. The same conclusion applies to the binding of **63** by the *Galactia* lectin since its relative potency was found (ELISA) to be 30. The 6a-methylene group is, however, important to the binding of **24** by the *Ulex* lectin since compound **63** proved to be highly inactive.

Acknowledgements

This research was supported by the Natural Sciences and Engineering Research Council of Canada (Grant OPG-172 to R.U.L.) and a prize from the Pharmaceutical Manufacturers Association of Canada to R.U.L.

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