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SYNTHETIC, CONFORMATIONAL, AND IMMUNOCHEMICAL STUDIES OF MODIFIED LEWIS b AND Y HUMAN BLOOD-GROUP DETERMINANTS TO SERVE AS PROBES FOR THE COMBINING SITE OF THE LECTIN IV OF *Griffonia simplicifolia**†

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ABSTRACT

Syntheses of the methyl glycosides of the Lewis b {α-L-Fuc-(1→2)-β-D-Gal-(1→3)[α-L-Fuc-(1→4)]-β-D-GlcNAc-} and Y {α-L-Fuc-(1→2)-β-D-Gal-(1→4)[α-L-Fuc-(1→3)]-β-D-GlcNAc-} human blood-group determinants and both their 6a-deoxy and *N*-deacetylated derivatives are reported. In the case of the Lewis b structure (Le^b-OMe), the 6a-*O*-mesyl and 6a-deoxy-6a-iodo derivatives were also prepared. The conformational preferences predicted by HSEA calculation are shown to be in good agreement with expectations based on ^{1}H- and ^{13}C-n.m.r. spectroscopy. The immunochemical data based on inhibition and thermodynamic studies require that the binding of Le^b-OMe and Y-OMe by the lectin IV of *Griffonia simplicifolia* does not involve recognition of the OMe, NHAc, or 6a-OH group and, consequently, occurs at a cleft at the surface of the protein. The complex formed between the lectin and 6a-deoxy-6a-iodo-Le^b-OMe provided the heavy nuclei required for the solution of the X-ray crystal structure.

INTRODUCTION

The fourth lectin isolated from the seeds of *Griffonia simplicifolia* was found to recognize both the Lewis b (Le^b or α-L-Fuc-(1→2)-β-D-Gal-(1→3)[α-L-Fuc-(1→4)]-β-D-GlcNAc-} and Y human {α-L-Fuc-(1→2)-β-D-Gal-(1→4)[α-L-Fuc-(1→3)]-β-D-GlcNAc-} blood-group determinants[1]. ^{1}H-N.m.r. investigation of the synthetic tetrasaccharides[2,3] indicated similar topographies about the two fucose residues and the terminal end of the galactose residue. That this common surface is that recognized by the lectin was supported by a thorough probing of the combining site[4,5], using a wide range of systematically modified Le^b structures[4] in a radio-

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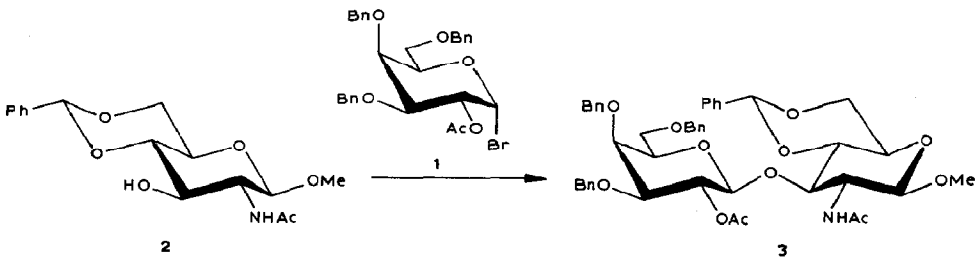
immunoassay. These results were subsequently confirmed by u.v.-difference-spectroscopy[5].

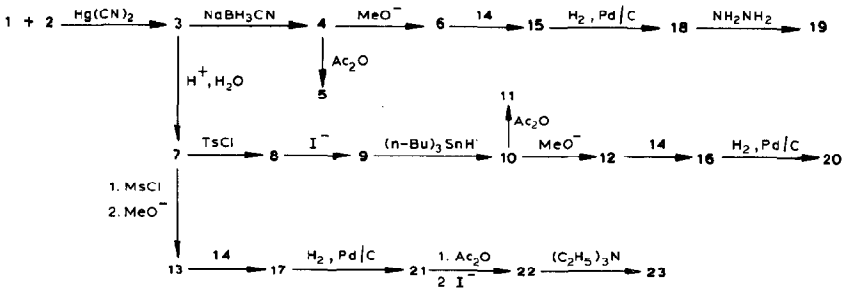
A cluster of three hydroxyl groups, namely, HO-3b and HO-4b of the β-D-Gal unit and HO-4c of the α-L-Fuc-(1→4) unit of the Le^b-hapten, provides the key polar interaction[6] between the protein and the Le^b tetrasaccharide. That the β-D-GlcNAc unit does not participate in the binding, but remains exposed to the aqueous phase, is now confirmed. Also, it is now established[5] that the CH_2OH-6b group is involved importantly in a non-polar interaction, probably with the hydroxyl group intramolecularly hydrogen-bonded to O-5b. The remaining hydroxyl groups appear to become involved in weak, polar interactions with either the protein or the aqueous environment. Firm conclusions in these and other regards are expected to arise from the X-ray analysis of crystals grown both from the lectin and several of its complexes with a variety of modified Le^b and Y structures[7].

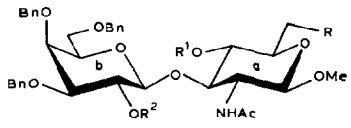
We now describe the chemical synthesis of the methyl glycosides of Le^b and Y tetrasaccharides (**18** and **38**) and derivatives to provide alterations of the β-D-GlcNAc unit. In addition to the binding studies, the conformational preferences of the Le^b and Y structures are discussed in terms of their n.m.r. characteristics.

DISCUSSION

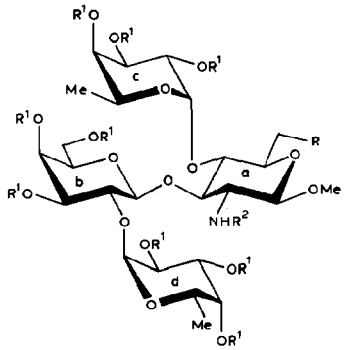
Synthesis. — The key intermediate for the synthesis of compounds **4–23**, following the route outlined in Scheme 1, was the crystalline, blocked disaccharide **3**. It was obtained in 89% yield by condensation of the glycosyl bromide **1**[8–10] with the well-known alcohol **2**[11,12] under Helferich conditions[13] at room temperature. The previously described bromide **1** was prepared from 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactopyranose[14,15] by treatment with acetyl bromide in the presence of tetraethylammonium bromide[3]. The ^{1}H-n.m.r. spectrum for **3** was in complete agreement with the structural assignment. The benzylidene ring in **3** was then reductively cleaved with sodium cyanoborohydride–hydrogen chloride as prescribed by Garegg *et al.*[16], to provide **4** which possesses a free hydroxyl at position 4a. The yield was 73% of a crystalline product which was also characterized as the diacetate **5**. The further elaboration of **3** to provide the methyl glycoside (**18**) of the Lewis b tetrasaccharide and the 6a-deoxy (**20**) and 6a-deoxy-6a-iodo (**23**) derivatives are outlined in Scheme 1.







	R	R^1	R^2
4	OBn	H	Ac
5	OBn	Ac	Ac
6	OBn	H	H
7	OH	H	H
8	OTs	H	Ac
9	I	H	Ac
10	H	H	Ac
11	H	Ac	Ac
12	H	H	H
13	OMs	H	H



	R	R^1	R^2
15	OBn	Bn	Ac
16	H	Bn	Ac
17	OMs	Bn	Ac
18	OH	H	Ac
19	OH	H	H
20	H	H	Ac
21	OMs	H	Ac
22	I	Ac	Ac
23	I	H	Ac

Scheme 1.

Zemplén deacetylation of **4** provided the diol **6** which was di-α-L-fucosylated using tri-*O*-benzyl-α-L-fucopyranosyl bromide[17] (**14**) under halide-ion catalyzed conditions[18]. The Vilsmeier bromide used in the preparation of **14** was generated *in situ* by reaction of *N,N*-dimethylformamide with oxalyl bromide in dichloromethane[19,20]. The blocked methyl Le^b-tetrasaccharide **15** was obtained in 75% yield. The benzyl groups were replaced by hydrogen in the usual way by hydrogenolysis over palladium-on-carbon. The identity and high purity of the product **18**, obtained in 81% yield, were established by ^1H- and ^{13}C-n.m.r. data (see Tables I–III). Treatment of **18** with hydrazine hydrate for 4 days at 145° allowed the isolation of the *N*-deacetylated methyl Le^b-tetrasaccharide (**19**) in 63% yield. This compound was required in order to test the involvment of the acetamido group in the binding of

TABLE I

COMPARISON OF ^{1}H-N.M.R. CHEMICAL SHIFTS (P.P.M.) AND COUPLING CONSTANTS (Hz) FOR THE Le^b AND Y BLOOD-GROUP DETERMINANTS (**18** AND **38**) AND RELATED STRUCTURES[a]

		Derivatives of Le^b-OMe (**18**)					*Derivatives of Y-OMe* (**38**)	
	Le^b-OMe (**18**)	*6a-Deoxy* (**20**)	*6a-OMs*[b] (**21**)	*6a-Deoxy-6a-iodo* (**23**)	N-*De-Ac* (**19**)	*Y-OMe* (**38**)	*6a-Deoxy* (**40**)	N-*De-Ac* (**39**)
β-D-GlcNAc a-unit								
H-1 ($J_{1,2}$)	4.32(8.3)	4.30(8.3)	4.39(8.3)	4.43(8.3)	4.33(7.8)	4.45(8.0)	4.43(8.3)	4.30(8.0)
H-2 ($J_{2,3}$)	3.81(10.3)	3.81(10.0)	3.81(10.0)	3.83(10.0)	2.72(9.5)	3.88	3.87	2.82(10.0)
H-3 ($J_{3,4}$)	4.12(9.3)	4.06(9.0)	4.17(9.0)	4.18(8.8)	3.85(9.5)	3.86¦		3.57
H-4 ($J_{4,5}$)	3.69(9.5)	3.35(9.2)	3.65(9.2)	3.58	3.65(9.5)	3.90	3.68	3.83
H-5 ($J_{5,6'}$)	3.53(1.8)	3.63(6.0)	3.81(1.8)	3.33	3.53(1.8)	3.45(1.5)	3.53(6.0)	3.44(1.8)
H-6′ ($J_{6',6''}$)	3.98(12.0)	1.42(Me)	4.74(11.5)	3.79	(12.0)	4.03(12.0)	1.44(Me)	4.03
H-6″ ($J_{5,6''}$)	3.85(4.0)	—	4.64(3.3)	3.50	3.83	3.82	—	3.80
CH_3O	3.48	3.47	3.47	3.50	3.56	3.49	3.47	3.56
$CH_3C{=}O$	2.07	2.06	2.05	2.06	—	2.02	2.00	—
					$\Delta(\delta-\delta_{18})$ *(p.p.m.)*			
β-D-Gal b-unit								
H-1 ($J_{1,2}$)	4.64(7.8)	−0.01	0.01	0.01	0.08	−0.14	0.01	−0.15
H-2 ($J_{2,3}$)	3.59(9.3)	−0.01	0.00	0.00	0.09	0.05	0.05	0.08
H-3	3.79	0.01	0.01	0.00	0.06	0.06	0.05	0.05
H-4	3.85	0.00	−0.02	0.01				
H-5	3.57	−0.01	0.01	0.01	0.02	0.02	0.02	0.02
H-6′¦	3.77	−0.04	−0.04	−0.03	−0.08	−0.02	−0.02	−0.05
H-6″¦	3.72	0.01	0.01	0.02	−0.03	−0.01	0.01	0.00
α-L-Fuc c-unit								
H-1 ($J_{1,2}$)	5.01(3.8)	0.08	−0.05	0.18	−0.03	0.07	0.07	0.21
H-2 ($J_{2,3}$)	3.80(10.0)	0.00	0.02	0.02	−0.04	−0.11	−0.13	−0.04
H-3 ($J_{3,4}$)	3.92(3.3)	0.01	0.00	0.00	−0.02	−0.01	−0.01	−0.02
H-4 ($J_{4,5}$)	3.81(< 1.0)	0.01	0.01	0.02	0.01	0.00	0.00	
H-5 ($J_{5,6}$)	4.84(6.5)	0.00	0.01	0.01	0.13	0.03	0.05	0.06
CH_3-6	1.27	−0.01	0.00	0.01	−0.04	−0.04	−0.04	−0.03

α-L-Fuc d-unit								
H-1 ($J_{1,2}$)	5.14(3.8)	−0.01	0.01	−0.01	0.19	0.14	0.13	0.15
H-2	3.73	0.00	0.01	0.03	0.05	0.07	0.05	0.08
H-3	3.73					0.06		
H-4 ($J_{4,5}$)	3.75(< 1.0)	0.01	0.00	0.02		0.08	0.04	0.05
H-5 ($J_{5,6}$)	4.34(6.5)	−0.01	−0.01	0.02	0.01	−0.08	−0.07	−0.07
CH_3-6	1.27	−0.01	−0.02	0.02	−0.01	0.00	−0.01	−0.01

[a]Measured at 360 MHz and 295 K, using 0.04M solutions in D_2O with acetone at 2.225 p.p.m. as internal reference. The assignments were based on homonuclear decoupling and homonuclear shift-correlated 2-D experiments (COSY) and are expected to be within ±0.01 p.p.m. except for those marked with ‡ which are within ±0.03 p.p.m. [b]CH_3SO_2, $\delta = 3.27$ p.p.m.

TABLE II

COMPARISON OF ^{13}C-N.M.R. CHEMICAL SHIFTS FOR THE Le^b AND Y BLOOD-GROUP DETERMINANTS (**18** AND **38**) AND RELATED STRUCTURES[a]

	δ(p.p.m.)	Δ(δ − δ$_{18}$) (p.p.m.)						
		Derivatives of Le^b-OMe (**18**)					*Derivatives of Y-OMe* (**38**)	
	Le^b-OMe (**18**)	*6a-Deoxy* (**20**)	*6a-OMs*[b] (**21**)	*6a-Deoxy-6a-iodo* (**23**)	N-*De-Ac* (**19**)	*Y-OMe* (**38**)	*6a-Deoxy* (**40**)	N-*De-Ac* (**39**)
β-D-GlcNAc a-unit								
C-1	103.52	−0.13	0.01	−0.39	0.65	−0.97	−1.07	0.98
C-2	56.35	0.18	−0.14	−0.05	2.55†[c]	0.30	0.44	2.43†
C-3	75.63	−0.17†	−0.40	−0.74†	4.74	0.82	−0.02†	4.54
C-4	73.12	5.42	0.06	1.17†	−0.63	1.13	6.02	0.81
C-5	76.33	−3.63†′	−2.75	−0.20†	0.14	−0.55	−3.69	0.41
C-6	60.60	−42.06	6.06	−52.23	−0.14	0.21	−42.73	0.24
CH_3(CO)	23.07	0.01	−0.02	−0.02	—	0.04	0.01	—
CO	174.45	−0.07	0.11	0.11	—	0.80	0.81	—
CH_3O	58.02	0.06	0.11	0.35	0.16†	−0.05	0.07	0.20†
β-D-Gal b-unit								
C-1	101.44	−0.02	−0.02	−0.13	0.82	−0.31	−0.15	−0.25
C-2	77.39	0.13	−0.10	0.0	−0.27	−0.10	−0.12	−0.27
C-3	74.55	−0.01	−0.11	−0.16	−0.17	−0.10	−0.12	−0.09
C-4	69.62	−0.03	−0.03	−0.05	0.07	−0.03	0.01	0.06
C-5	75.63	−0.02†	0.02	0.0	0.09	0.15	0.08†	0.07
C-6	62.38	−0.01	0.04	0.03	−0.04	−0.15	−0.07	−0.02
α-L-Fuc c-unit								
C-1	98.67	0.09	0.32	0.06	0.20	0.70	0.77	1.58†′
C-2	68.73	0.04	−0.15	−0.12	−0.01	0.42	0.40	0.38
C-3	70.01	−0.05	−0.04	−0.18	−0.03	0.07	0.01	0.14
C-4	72.87†	−0.04†′	−0.08†	−0.09	−0.02†′	−0.07	−0.08	−0.04
C-5	67.80	−0.06	0.31	0.25	0.02	−0.10†	−0.12	−0.05
C-6	16.21	0.0	−0.01	−0.03†′	0.09†″	0.05	0.03	0.23
α-L-Fuc d-unit								
C-1	100.42	0.06	0.01	0.08	−0.28	−0.12	−0.12	−0.10†′
C-2	69.18	0.0	−0.08	−0.07	−0.11	−0.59	−0.65	−0.34
C-3	70.35	−0.01	−0.08	−0.08	0.30	0.28	0.26	0.23
C-4	72.82†	0.01†′	−0.06†	−0.04	−0.09†′	−0.23	−0.24	−0.25
C-5	67.06	−0.04	0.01	0.04	0.60	0.54†	0.56	0.69
C-6	16.09	−0.01	−0.02	0.06†′	0.27†″	0.17	0.15	0.12

[a]For 0.05M solutions in D_2O with 1,4-dioxane as internal standard at 67.4 p.p.m., measured at 100 and 75 MHz at 295 K. [b]CH_3SO_2, δ = 37.65 p.p.m. [c]Assignments marked † are tentative and may be reversed.

the Le^b determinant by the lectin IV of *Griffonia simplicifolia*.

The 6a-deoxy derivative (**20**) of **18** was prepared in order to examine the possible involvment of HO-6a of **18** in the complexation. This was accomplished from

TABLE III

COMPARISON OF INTER-UNIT NUCLEAR OVERHAUSER ENHANCEMENTS WITH INTERNUCLEAR DISTANCES AS ESTIMATED BY HSEA CALCULATIONS[a]

Atom saturated	*Signal enhanced*	*% Enhancement*		*Internuclear distance (Å)*	
		Le^b-OMe (**18**)	*Y-OMe* (**38**)	*Le^b-OMe* (**18**)	*Y-OMe* (**38**)
H-1b	H-3b	7.3	[b]	2.57	2.57
	H-3a	4.4	—	2.63	—
	H-4a	—	[b]	—	2.52
H-1c	H-2c	10.5	13.5	2.46	2.46
	H-3a	—	[c]	—	2.57
	H-4a	6.0	—	2.67	—
H-5c	H-4c	5.9	8.5	2.36	2.35
	H-2b	7.6	5.2	2.40	2.30
H-1d	H-2d	7.6	11.6	2.46	2.46
	H-2b	6.5	13.1	2.39	2.41
H-5d	H-3d	7.2	5.5	2.35	2.35
	H-2a	[d]	—	2.60	—
	H-5a	—	3.7	—	2.28
		Le^a-OMe[e]	X-OMe[e]	Le^a-OMe[e]	X-OMe[e]
Me-6c	H-4c	5.5	6.5	2.51[f]	2.51[f]
	H-2b	8.0	11.6	2.60[f]	2.52[f]
		Le^d-OMe[e]	H type 2-OMe[e]	Le^d-OMe[e]	H type 2-OMe[e]
Me-6d	H-4c	4.9	4.7	2.51[f]	2.51[f]
	H-2a	5.9	—	2.83[f]	—
	H-4a	5.4	—	2.31[f]	—
	H-5a	—	2.8		2.45[f]

[a]Measured at 360 MHz and 300 K, using 0.04M solutions in D_2O. [b]The small chemical shift difference for H-1a and H-1b did not allow a useful experiment. [c]Definitely strongly enhanced, but second-order effects did not allow the determination of the % enhancement. [d]Definitely strongly enhanced, but the % enhancement could not be determined because the saturation of H-5d also saturated H-1a. [e]Le^a-OMe, β-D-Gal-(1→3)[α-L-Fuc-(1→4)]-β-D-GlcNAc-OMe; X-OMe, β-D-Gal-(1→4)[α-L-Fuc-(1→3)]-β-D-GlcNAc-OMe; Le^d-OMe, α-L-Fuc-(1→2)-β-D-Gal-(1→3)-β-D-GlcNAc-OMe; H type 2-OMe, α-L-Fuc-(1→2)-β-D-Gal-(1→4)-β-D-GlcNAc-OMe; [f]Distance between the nucleus of the hydrogen of the methyl group nearest to the hydrogen whose signal was enhanced.

the diol **7** which was obtained by acid hydrolysis of **3**. The primary hydroxyl group of **7** was preferentially tosylated to form **8** in 85% yield. Replacement of the tosyloxy group of **8** provided the iodo-compound **9** in 89% yield. Tributyltin hydride was chosen to replace the iodine atom by hydrogen, to form **10**, since the benzyloxy groups resist this reducing agent. Compound **10** was characterized as the diacetate **11**. Deacetylation then provided the diol **12**, from which the protected methyl 6a-deoxy-Le^b-tetrasaccharide (**16**) could be prepared in 75% yield by difucosylation under halide-ion-catalyzed conditions, as described above for the preparation of **18**. Hydrogenolysis to remove the benzyl groups then provided the desired methyl 6a-deoxy-Le^b-tetrasaccharide (**20**) in 71% yield. The characterization of this key product is presented in the discussion of the n.m.r. parameters.

As will be seen below, unequivocal evidence is obtained that the 6a-hydroxyl

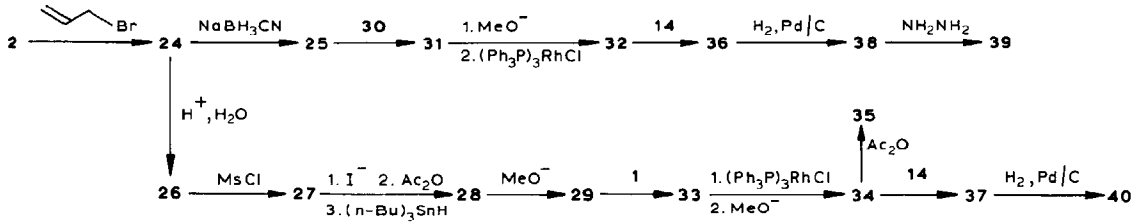
group of the Le^b determinant is not involved in its binding by the lectin IV of *Griffonia simplicifolia*. It was expected, therefore, that the lectin would also bind the 6a-deoxy-6a-iodo-derivative **23** (Scheme 1). Indeed, this proved to be the case, and the crystalline complex formed by the lectin and the iodide **23** proved instrumental in the solution of its crystal structure[7].

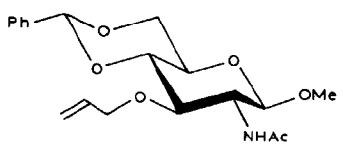
The synthesis of **23** started with the diol **7**. Preferential mesylation of the primary hydroxyl group and deacetylation (62% yield) followed by difucosylation of **13** (in 88% yield) provided the nona-*O*-benzyl derivative (**17**) of the methyl 6a-*O*-mesyl-Le^b-tetrasaccharide. This compound was then subjected to hydrogenolysis in the presence of palladium-on-carbon to remove the benzyl groups and form the 6a-mesylate **21**. Acetylation of **21** prior to treatment with sodium iodide in $HCONMe_2$ provided **22** in 74% yield. Deacetylation of **22** using triethylamine in aqueous methanol then afforded the desired iodide **23** in 80% yield. Compound **23** could also be obtained by reaction of **21** with iodide, but the purification proved to be more difficult. The ^{13}C-6a chemical shift for **23** was 8.37 p.p.m. as compared to 60.60 p.p.m. for this carbon in the methyl Le^b-tetrasaccharide (**18**).

The introduction of changes in the β-D-GlcNAc unit of the Y-OMe hapten (**38**) (Scheme 2) also started with the β-D-GlcNAc derivative **2**. Allylation furnished **24**, whose 4,6-*O*-benzylidene group was either reduced to form the benzyl ether **25** or hydrolyzed to provide the diol **26**. The yields were excellent.

Compound **25** was used to synthesize Y-OMe (**38**) by first preparing the blocked disaccharide **31** in 77% yield through a Helferich-type glycosylation employing the well-known[3] bromide **30**. Treatment of **31** with sodium methoxide in methanol followed by deallylation then provided **32**. This latter reaction first involved isomerization of the allyl group to a 1-propenyl group using tris(triphenylphosphine)rhodium(I) chloride as prescribed by Corey and Suggs[21] and appears more reliable than the use of palladium salts[22]. Hydrolysis of the 1-propenyl ether involved the use of mercuric chloride in the presence of mercuric oxide and provided the diol (**32**) in 86% yield. Conventional difucosylation then led to the blocked Y-tetrasaccharide (**36**) in 76% yield. Debenzylation under conditions for hydrogenolysis then provided Y-OMe (**38**), a key reference compound for the immunochemical studies. In order to examine possible involvement of its *N*-acetyl group in the binding reaction, **39** was prepared by treatment of **38** with hydrazine hydrate.

In order to synthesize 6a-deoxy-Y-OMe (**40**), the diol **34** was obtained by first preparing methyl 2-acetamido-3-*O*-allyl-2,6-dideoxy-β-D-glucopyranoside (**29**) by way of the 6-mesylate **27**, which was obtained in 90% yield. After replacement of the mesyloxy group by iodine and acetylation, the material was reduced, without further purification, using tributyltin hydride, to form **28**. Deacetylation then provided the alcohol **29** which was desired for glycosylation, using the glycosyl bromide **1**, to form **33**. The yield was only 34%, but no effort was made toward improvement, such as may have been the case using 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-galactosyl bromide (**30**) as reagent[3]. The blocked disaccharide **33** was then deallylated, as mentioned above for **31**, and the product was deacetylated without further





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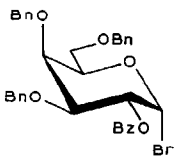
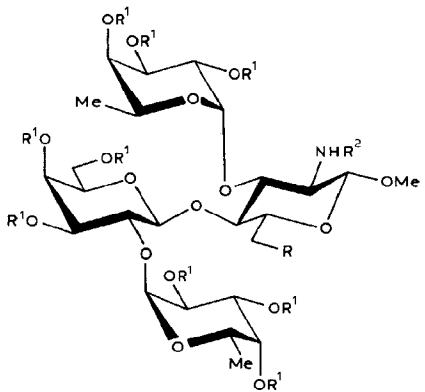
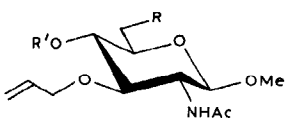
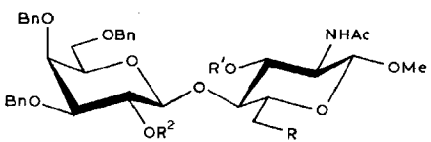


30





	R	R^1
25	OBn	H
26	OH	H
27	OMs	H
28	H	Ac
29	H	H



	R	R^1	R^2
31	OBn	Allyl	Bz
32	OBn	H	H
33	H	Allyl	Ac
34	H	H	H
35	H	Ac	Ac

	R	R^1	R^2
36	OBn	Bn	Ac
37	H	Bn	Ac
38	OH	H	Ac
39	OH	H	H
40	H	H	Ac

Scheme 2.

purification to provide the crystalline diol **34** in 76% overall yield. Difucosylation of **34**, under the conditions described for the preparation of **15**, provided **37** in 77% yield. Hydrogenolysis then gave 6a-deoxy-Y-OMe (**40**) in 66% yield. The characterization of this product will be discussed in the following section.

N.m.r. studies. — The structures of the various compounds which were synthesized for the first time are fully supported by the various n.m.r. parameters reported either in the Experimental or in Tables I and II, and do not require detailed consideration. However, although the conformational preferences for the Lewis b and Y human blood-group determinants have been considered[2,3], it seemed desirable to examine the conclusions then drawn, when the aglycon was the 8-methoxycarbonyloctyl linking-arm, in the light of data for the simple methyl glycosides which are presented in Tables I–III. Actually, it proved that the chemical shift data are in fine agreement and require little further consideration.

^{1}H-N.m.r. parameters for the Le^b-OMe (**18**) and Y-OMe (**38**) tetrasaccharides are reported in Table I together with those for structural modifications involving changes in the β-D-GlcNAc-OMe residues. The vicinal coupling constants require that these units remain in the $^4C_1$ conformation, and examination of the chemical shift data confirms the structural assignments. The lectin IV of *Griffonia simplicifolia* recognizes and non-covalently strongly binds both the Le^b-OMe and Y-OMe compounds[1] and, as previously mentioned[4], this probably happens because these two tetrasaccharides have very similar topographies about the terminal groups of the β-D-Gal and the two α-L-Fuc units. The ^{1}H-chemical shift data for these units support this contention. That appreciable chemical shift differences are found in the case of the ring-hydrogens of the α-L-Fuc d-units is not surprising since the conformational studies[2,23] require this unit in Le^b-OMe (**18**) to be in close proximity to the *N*-acetyl group but near the CH_2OH-6a group in the case of Y-OMe (**38**). That the 6a-deoxy derivative (**20**) has a chemical shift for H-1c different to that for H-1c in **18** is understandable since H-1c in **18** is known[2] to be in close proximity to the CH_2OH-6a group. This is not the case for Y-OMe (**38**) and, in fact, preparation of the 6a-deoxy derivative (**40**) had no influence on the chemical shift of H-1c. Inspection of the ^{13}C-chemical shift data in Table II further confirms that various derivatives of **18** and **38** have conformational preferences in water that are very similar to those of the parent compounds. Indeed, as seen in Table IV, all of these compounds, as expected, are strongly bound by the lectin.

Although it was not intended, in the context of this study, to perform detailed ^{1}H-n.m.r. examinations of the conformational preferences for Le^b-OMe (**18**) and Y-OMe (**38**), we wish to note that the n.O.e. data presented in Table IV are in good accord with the internuclear distances indicated by HSEA calculation[24]. Of particular significance are the enhancements of the signal for H-2b on saturating H-5c for both **18** and **38**. This result is in accord with the strong interunit deshielding of H-5c by O-4a and O-5b[2,3] (0.83 and 0.85 p.p.m. to lower field than for H-5 of α-L-Fuc-OMe) and supports the contention that the α-L-Fuc c-units of **18** and **38** are similarly oriented relative to the β-D-Gal b-unit. The very similar, strong enhancements for

TABLE IV

COMPARISON OF THE THERMODYNAMIC PARAMETERS FOR THE BINDING OF THE LEWIS b AND Y HUMAN BLOOD-GROUP DETERMINANTS AND DERIVATIVES BY THE LECTIN IV OF *Griffonia simplicifolia*

Idiotype	*Relative potency*[a]	$K_{Assoc.}$[b] ($x10^{-4}$)	$\Delta G^{\circ b}$	$\Delta H^{\circ b}$	$\Delta\Delta G^{\circ a}$	$\Delta S^{\circ b}$
			(kcal/mole)			*cal/mole/K*
Le^b-OMe (**18**)	100	4.4	−6.3	−13.3	0	−23.3
6a-Deoxy-Le^b-OMe (**20**)	95	3.9	−6.3	−13.1	0	−22.8
6a-*O*-Ms-Le^b-OMe (**21**)	110	3.8	−6.3	−12.3	−0.1	−20.3
6a-Deoxy-6a-iodo-Le^b-OMe (**23**)	83	—	—	—	0.1	—
N-Deacetylated Le^b-OMe (**19**)	67	—	—	—	0.2	—
Y-OMe (**38**)	53	2.0	−5.9	−10.0	0.4	−13.8
6a-Deoxy-Y-OMe (**40**)	50	—	—	—	0.4	—
N-Deacetylated Y-OMe (**39**)	23	—	—	—	0.9	—

[a]By radioimmunoassay under conditions where 50% inhibition was provided by 58 μmol/L of the reference Le^b-OMe inhibitor. [b]From changes in u.v. absorption in the temperature range 9–44°[4,5].

H-2b observed on saturating the Me-6c group of the Le^a-OMe and X-OMe trisaccharides confirm this orientation. As seen from Table III, the orientation of the α-L-Fuc d-unit is supported by the enhancement for H-2a in the case of **18** and H-5a in the case of **38** on saturation of their H-5d atoms. The orientation of the d-unit, as indicated by HSEA calculation, places the nucleus of H-1d of **18** at ~2.67 Å from that of O-3b. Therefore, H-1d should experience strong specific inter-unit deshielding by O-3b[23]. In fact, the chemical shift for H-1d of 3b-deoxy-Le^b-OMe is 0.33 p.p.m. to higher field than that (5.14 p.p.m.) of the parent compound. Lemieux and Bock[23] have commented on this type of evidence for the conformational preferences of inter-sugar glycosidic bonds. Thus, there can be little doubt that the HSEA conformer[24] represents a highly populated conformer within a narrow range of conformers in the deepest well of the potential energy surface for **18**. To date, we have not succeeded in obtaining crystals suitable for X-ray studies.

Immunochemical studies. — The main objective was to achieve unequivocal chemical evidence that the β-D-GlcNAc-OMe portions of both the Le^b-OMe (**18**) and Y-OMe (**38**) structures are not involved in the binding of these tetrasaccharides by the lectin IV of *Griffonia simplicifolia*. Although this information will become available through X-ray crystallographic studies[7], it was considered important to use this opportunity to test the value of conclusions based on the probing of receptor sites with chemically modified substrates, since an appreciation of the affinities exhibited by most combining sites of interest to biology will probably continue to depend on structure-activity studies based in chemical synthesis.

Because of the affinity displayed by the lectin for both **18** and **38**, it was expected that the 6a-deoxy-6a-iodo derivative (**23**) of **18** would be strongly bound and that the heavy iodine atoms of the resulting complex would serve as reference atoms for the solution of the crystal structures of the lectin and several of its com-

plexes[7]. In fact **23** provided a complex which crystallized well and examination of the crystals assisted in the solution of the diffraction pattern exhibited by the lectin[25]. It appears that, as was to be expected, the iodine atom is situated in a water channel at the surface of the lectin.

The immunochemical data presented in Table IV clearly require that, as previously suggested[4], the binding occurs essentially about the two fucose residues and the terminal atoms of the galactose residue. This situation was previously suggested on the basis of the relative potencies of Le^b and Y related compounds involving changes in the aglycon and *N*-deacetylation to the dipolar 8-carboxyoctylamino compounds. Since then, it has been recognized that a structural change that has little effect on the extent of binding does not necessarily mean that the particular structural feature is not directly involved in the complex formation[5]. Instead, involvement may occur with nearly the same decrease in free energy, because the substitution causes changes in the enthalpy and entropy terms that effectively cancel. This was not so for the structural changes reported in Table IV where it is seen that profound modifications at the 6a-position of Le^b-OMe (**18**) had, within experimental certainty, no effect on the thermodynamic parameters. Therefore, the chemical evidence that HO-6a is not involved in the binding is, in this case, in full agreement with the expectation that the iodine atom of **23** will remain in contact with water in complex formation with the lectin.

Although the lectin binds Y-OMe (**38**), the association is only about half as strong as that for Le^b-OMe (**18**). This results from decreases in both the changes in enthalpy and entropy. This observation is not necessarily surprising since it is to be expected that the hydration of **18** and **38** will differ, especially about the regions where the α-L-Fuc units are in contact with the β-D-GlcNAc-OMe residues. This proposal is supported by the inhibition data for the *N*-deacetylated derivatives **19** and **39**. It was previously established[4] that the α-L-Fuc c-unit is much more intimately involved in the binding than is the α-L-Fuc d-unit. Since the acetamido group is adjacent to the c-unit in the case of **38** but adjacent to the d-unit in the case of **18**, *N*-deacetylation should have a greater effect on the binding of **38**, as was found. As for **18**, deoxygenation at the 6a-portion of **38** to produce **40** had no measurable effect on the extent of binding. It is concluded, therefore, that the HO-6a, OMe, and NHAc groups of both **18** and **38** will be found, by X-ray crystallographic analysis, to remain in contact with the aqueous phase when these compounds are bound by the lectin IV of *Griffonia simplicifolia*.

EXPERIMENTAL

General methods. — ^{1}H-N.m.r. spectra were recorded at 360 and 400 MHz (Bruker WM 360 and WH 400 spectrometers) with tetramethylsilane as internal standard for $CDCl_3$ solutions. The reference standard for D_2O solutions was acetone (2.225 p.p.m.). ^{13}C-N.m.r. spectra were recorded at 100 and 75 MHz (Bruker WH 400 and AM 300 spectrometers), using D_2O as solvent and 1,4-dioxane (67.4

p.p.m.) as internal standard. Optical rotations were measured at room temperature (23 ±1°) in a 1-dm cell with a Perkin–Elmer 241 polarimeter. Thin-layer chromatography was performed on precoated plates of Silica Gel 60 F_{254} (Merck) with detection by spraying with 10% sulfuric acid in ethanol followed by heating. For column chromatography, Silica Gel 60 (230–400 mesh, Merck) and distilled solvents were used. Solvents and reagents were purified and dried according to standard procedures[26]. Melting points are uncorrected.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-galactopyranosyl bromide[9,10] (**1**). — A mixture of 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactopyranose[15] (21.0 g, 41.5 mmol), tetraethylammonium bromide (4.2 g, 20.0 mmol), 4 Å molecular sieves (33 g), and dichloromethane (160 mL) was stirred for 1 h under nitrogen. Then acetyl bromide (6 mL, 81.2 mmol) was added. After 1 h, the mixture was decanted from the sieves and poured into a stirred mixture of aqueous sodium hydrogencarbonate, ice, and dichloromethane. The organic solution was washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated to leave **1** as a syrup (22.3 g, 97%). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 15 H, 3 Ph), 6.75 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.18 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 4.93 and 4.55 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.71 (s, 2 H, CH_2Ph), 4.49 and 4.42 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.20 (t, 1 H, $J_{5,6A}$ = $J_{5,6B}$ = 6.5 Hz, H-5), 4.03 (s, 1 H, H-4), 4.00 (dd, 1 H, $J_{3,4}$ 2.5 Hz, H-3), 3.68–3.45 (m, 2 H, H-6A,6B), 2.08 (s, 3 H, Ac).

Methyl 2-acetamido-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**3**). — Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[11,12] (**2**; 10.0 g, 30.9 mmol), mercuric cyanide (16.0 g, 63.3 mmol), powdered calcium sulfate (10 g), and 4 Å molecular sieves (20 g) were stirred under nitrogen in a mixture of toluene and nitromethane (1:1, 1 L). A solution of bromide **1** (22.0 g, 39.6 mmol) in toluene–nitromethane (1:1, 120 mL) was added during 20 min at room temperature. After 15 min, the mixture was filtered and poured into a mixture of aqueous sodium hydrogencarbonate and dichloromethane. The organic solution was washed with water, dried, and evaporated to leave a solid residue. Recrystallization from chloroform–ethyl acetate provided **3** (19.2 g, 78%). More material (2.7 g, total yield 89%) was obtained by chromatography of the mother liquor on a column of silica gel.

Compound **3** had m.p. 212–213°, $[\alpha]_D$ −15° (*c* 0.8, dichloromethane). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.5–7.2 (m, 20 H, 4 Ph), 5.89 (d, 1 H, $J_{NH,2a}$ 6.5 Hz, NH), 5.45 (s, 1 H, C*H*Ph), 5.28 (dd, 1 H, $J_{1b,2b}$ 7.5, $J_{2b,3b}$ 9.5 Hz, H-2b), 5.15 (d, 1 H, $J_{1a,2a}$ 8.5 Hz, H-1a), 4.88 and 4.55 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.62 (d, 1 H, H-1b), 4.61 and 4.43 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.59 (t, overlapped by CH_2Ph, 1 H, $J_{2a,3a}$ = $J_{3a,4a}$ = 9.0 Hz, H-3a), 4.30 (dd, 1 H, $J_{6Aa,6Ba}$ 10.5, $J_{6Aa,5a}$ 4.5 Hz, H-6Aa), 4.26 (s, 2 H, CH_2Ph), 3.86 (d, 1 H, $J_{3b,4b}$ 2.5 Hz, H-4b), 3.72 (t, 1 H, $J_{5a,6Ba}$ 10.0 Hz, H-6Ba), 3.67 (t, 1 H, $J_{4a,5a}$ 9.0 Hz, H-4a), 3.50 (s, 3 H, MeO), 3.41 (dd, 1 H, H-3b), 3.29 (bt, 1 H, $J_{5b,6Ab}$ 5.5, $J_{5b,6Bb}$ 7.0 Hz, H-5b), 2.98 (m, 1 H, H-2a), 2.00 (s, 6 H, AcO, AcNH).

Anal. Calc. for $C_{45}H_{51}NO_{12}$: C, 67.74; H, 6.44; N, 1.76. Found: C, 67.61; H,

6.57; N, 1.99.

Methyl 2-acetamido-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**4**). — A mixture of compound **3** (14.0 g, 17.5 mmol), sodium cyanoborohydride (23 g), 3 Å molecular sieves (15 g), and a few crystals of Methyl Orange in tetrahydrofuran (400 mL) was stirred at 0° under nitrogen. Diethyl ether saturated with hydrogen chloride was added until the colour of the indicator turned red and gas evolution occurred. When t.l.c. indicated the reaction to be complete, the mixture was diluted with dichloromethane and poured into aqueous sodium hydrogencarbonate. The organic layer was washed with water, dried, and evaporated. The crude product was dissolved in methanol–dichloromethane (1:1) and deionized with mixed-bed ion-exchanger Amberlite MB-1. The resin was washed thoroughly with methanol–dichloromethane (1:1) and the solution evaporated to leave a white solid. Recrystallization from hot methanol provided **4** (8.9 g, 63%). More **4** (1.4 g, total yield 73%) was obtained by chromatography of the mother liquor on a column of silica gel (ethyl acetate). The analytical sample, recrystallized from dichloromethane–ethyl acetate, had m.p. 242–243°, $[\alpha]_D$ +12° (*c* 0.6, dichloromethane). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 20 H, 4 Ph), 5.77 (d, 1 H, $J_{NH,2a}$ 6.5 Hz, NH), 5.34 (dd, 1 H, $J_{1b,2b}$ 8.0, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.91 (d, 1 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.87 (d, 1 H, $J_{1a,2a}$ 8.0 Hz, H-1a), 4.68–4.35 (m, 7 H, CH_2Ph), 4.43 (d, 1 H, H-1b), 4.33 (dd, 1 H, $J_{2a,3a}$ 10.0, $J_{3a,4a}$ 8.0 Hz, H-3a), 4.26 (bs, 1 H, OH), 3.86 (d, overlapped, 1 H, $J_{3b,4b}$ 2.5 Hz, H-4b), 3.5 (dd, overlapped, H-3b), 3.46 (t, overlapped, $J_{4a,5a}$ 9.5 Hz, H-4a), 2.96 (m, 1 H, H-2a), 2.03, 1.96 (2 s, each 3 H, AcO, AcNH).

Anal. Calc. for $C_{45}H_{53}NO_{12}$: C, 67.52; H, 6.68; N, 1.75. Found: C, 67.33; H, 6.58; N, 1.71.

Methyl 2-acetamido-4-O-acetyl-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**5**). — A solution of compound **4** (250 mg, 0.31 mmol) in pyridine (4 mL) and acetic anhydride (1 mL) was kept for 12 h at room temperature, then poured into ice–water, extracted with dichloromethane, and evaporated. Recrystallization from chloroform–ethyl acetate provided the diacetate **5** (220 mg, 84%), m.p. 183.5–184.5°, $[\alpha]_D$ +12° (*c* 0.7, dichloromethane). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 20 H, 4 Ph), 5.78 (d, 1 H, $J_{NH,2a}$ 7.5 Hz, NH), 5.18 (dd, 1 H, $J_{1b,2b}$ 7.5, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.92 and 4.54 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.88 (t, overlapped by CH_2Ph, 1 H, $J_{3a,4a}$ 9.0, $J_{4a,5a}$ 8.5 Hz, H-4a), 4.79 (d, 1 H, $J_{1a,2a}$ 7.5 Hz, H-1a), 4.64 and 4.48 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.53 (s, 2 H, CH_2Ph), 4.46 (d, overlapped by CH_2Ph, 1 H, H-1b), 4.31 (t, 1 H, $J_{2a,3a}$ 8.0 Hz, H-3a), 3.90 (d, 1 H, $J_{3b,4b}$ 2.5 Hz, H-4b), 3.68 (m, 1 H, $J_{5a,6Aa}$ 4.0, $J_{5a,6Ba}$ 6.0 Hz, H-5a), 3.63–3.45 (m, 5 H, H-5b,6Ab,6Bb,6Aa,6Ba), 3.48 (dd, overlapped, H-3b), 3.47 (s, 3 H, MeO), 3.34 (m, 1 H, H-2a), 2.02, 1.90, 1.89 (3 s, each 3 H, 2 AcO, AcNH).

Anal. Calc. for $C_{47}H_{55}NO_{13}$: C, 67.05; H, 6.58; N, 1.66. Found: C, 67.03; H, 6.67; N, 1.85.

Methyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(3,4,6-tri-O-benzyl-β-D-galacto-

pyranosyl)-β-D-glucopyranoside (**6**). — A mixture of compound **4** (2.5 g, 3.13 mmol), methanolic 0.045M sodium methoxide (55 mL), and dichloromethane (25 mL) was kept for 24 h at room temperature. Neutralization with Amberlite IRC-50 (H^+) resin and evaporation left a white residue that was recrystallized from dichloromethane–ethyl acetate to give **6** (2.0 g, 84%), m.p. 234–235°, $[\alpha]_D$ −8° (*c* 0.6, dichloromethane). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 20 H, 4 Ph), 6.20 (bs, 1 H, NH), 4.83 and 4.51 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.77 (d, 1 H, $J_{1a,2a}$ 8.5 Hz, H-1a), 4.67 and 4.64 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.60 (s, 2 H, CH_2Ph), 4.46 and 4.37 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.29 (bs, 1 H, OH), 4.24 (d, 1 H, $J_{1b,2b}$ 7.5 Hz, H-1b), 4.17 (dd, 1 H, $J_{2a,3a}$ 10.0, $J_{3a,4a}$ 8.0 Hz, H-3a), 3.95 (dd, 1 H, $J_{2b,3b}$ 9.5 Hz, H-2b), 3.80 (d, 1 H, $J_{3b,4b}$ 2.0 Hz, H-4b), 3.50 (s, 3 H, MeO), 3.37 (dd, 1 H, H-3b), 3.26 (m, 1 H, $J_{2a,NH}$ 7.0 Hz, H-2a), 1.96 (s, 3 H, AcNH).

Anal. Calc. for $C_{43}H_{51}NO_{11}$: C, 68.15; H, 6.78; N, 1.85. Found: C, 68.15; H, 6.79; N, 1.80.

Methyl 2-acetamido-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (**7**). — The disaccharide **3** (1.5 g, 1.88 mmol) in 80% acetic acid (50 mL) was heated for 15 min at 80°. T.l.c. then showed the complete disappearance of the starting material. The solution was evaporated and co-evaporated a few times with water and finally with ethanol to leave a white solid. Chromatography on a column of silica gel (dichloromethane–5% methanol) provided **7** (1.24 g, 93%). Recrystallization from methanol gave the analytical sample, m.p. 218–220°, $[\alpha]_D$ +17.5° (*c* 0.6, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 15 H, 3 Ph), 5.65 (d, 1 H, $J_{NH,2a}$ 6.5 Hz, NH), 5.34 (dd, 1 H, $J_{1b,2b}$ 7.5, $J_{2b,3b}$ 9.5 Hz, H-2b), 3.50 (s, 3 H, MeO), 2.92 (m, 1 H, $J_{1a,2a}$ 8.5, $J_{2a,3a}$ 10.0 Hz, H-2a), 2.02, 1.97 (2 s, each 3 H, AcO, AcNH).

Anal. Calc. for $C_{38}H_{47}NO_{12}$: C, 64.30; H, 6.67; N, 1.97. Found: C, 64.14; H, 6.68; N, 2.12.

Methyl 2-acetamido-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2-deoxy-6-O-p-toluenesulfonyl-β-D-glucopyranoside (**8**). — A solution of *p*-toluenesulfonyl chloride (292 mg, 1.53 mmol) in dichloromethane (15 mL) was added dropwise to a solution of the diol **7** (840 mg, 1.18 mmol) in pyridine (20 mL) at ice-bath temperature. The mixture was allowed to warm up to room temperature, kept for 2 h, then poured into ice–water, and extracted with dichloromethane. The organic layer was dried and evaporated, and the residual material was applied to a column of silica gel. Elution with dichloromethane–5% methanol provided **8** (870 mg, 85%). The analytical sample, recrystallized from methanol, had no sharp m.p., but gradually decomposed at >170°; $[\alpha]_D$ +19° (*c* 0.4, chloroform). ^{1}H-N.m.r. data ($CDCl_3$: δ 7.85–7.2 (m, 19 H, 3 Ph, Ts aromatic), 5.85 (d, 1 H, $J_{NH,2a}$ 6.5 Hz, NH), 5.31 (dd, 1 H, $J_{1b,2b}$ 7.5, $J_{2b,3b}$ 9.5 Hz, H-2b), 4.78 (d, 1 H, $J_{1a,2a}$ 8.5 Hz, H-1a), 3.82 (d, 1 H, $J_{3b,4b}$ 2.0 Hz, H-4b), 3.41 (s, 3 H, MeO), 2.88 (m, 1 H, $J_{2a,3a}$ 10.0 Hz, H-2a), 2.21 (s, 3 H, Ts Me), 2.01, 1.95 (2 s, each 3 H, AcO, AcNH).

Anal. Calc. for $C_{45}H_{53}NO_{14}S$: C, 62.56; H, 6.18; N, 1.62; S, 3.71. Found: C, 62.60; H, 6.29; N, 1.71; S, 3.95.

Methyl 2-acetamido-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,6-dideoxy-6-iodo-β-D-glucopyranoside (**9**). — The tosylate **8** (1.3 g, 1.51 mmol) and potassium iodide (2.6 g, 15.66 mmol) in *N,N*-dimethylformamide (30 mL) were heated for 6 h at 90°. The mixture was diluted with dichloromethane and washed successively with water, aqueous sodium thiosulfate, and water. The residue obtained on drying and evaporation was chromatographed on a column of silica gel (dichloromethane–7% methanol) to give **9** (1.1 g, 89%). Recrystallization from dichloromethane–ethyl acetate provided the analytical sample, m.p. 225–226° (dec.), $[\alpha]_D$ +23° (*c* 0.5, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 15 H, 3 Ph), 5.78 (d, 1 H, $J_{NH,2a}$ 6.5 Hz, NH), 5.33 (dd, 1 H, $J_{1b,2b}$ 7.5, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.93 (d, 1 H, overlapped by CH_2Ph, $J_{1a,2a}$ 8.5 Hz, H-1a), 4.35 (dd, 1 H, $J_{2a,3a}$ 10.0, $J_{3a,4a}$ 8.0 Hz, H-3a), 3.82 (d, 1 H, $J_{3b,4b}$ 2.0 Hz, H-4b), 3.51 (s, 3 H, OMe), 2.91 (m, 1 H, H-2a), 2.03, 1.97 (2 s, each 3 H, AcO, AcNH).

Anal. Calc. for $C_{38}H_{46}INO_{11}$: C, 55.68; H, 5.66; I, 15.48; N, 1.71. Found: C, 56.05; H, 5.74; I, 15.15; N, 1.59.

Methyl 2-acetamido-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,6-dideoxy-β-D-glucopyranoside (**10**). — The iodo-compound **9** (1.2 g, 1.46 mmol), tributyltin hydride (1.8 mL, 6.7 mmol), and 2,2′-azobis(isobutyronitrile) (70 mg) in toluene (50 mL) were heated for 4 h at 80°. Although t.l.c. still revealed the presence of **9**, no further reaction occurred on prolonged heating. Therefore, the solvent was evaporated and the resulting material was applied to a column of silica gel and eluted with hexane–ethyl acetate (3:1) and dichloromethane–ethyl acetate (1:2). Evaporation of the first main fraction gave **9** (590 mg, 49%). Further elution provided impure **10** (542 mg) that was re-chromatographed (dichloromethane–ethyl acetate, 1:2), to give pure **10** (433 mg, 43%). On recrystallization from methanol, a gel-like precipitate formed that was filtered to give the analytical sample, m.p. 225–226° (dec.), $[\alpha]_D$ +18° (*c* 0.4, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 15 H, 3 Ph), 5.71 (d, 1 H, $J_{NH,2a}$ 7.0 Hz, NH), 5.34 (dd, 1 H, $J_{1b,2b}$ 8.0, $J_{2b,3b}$ 9.5 Hz, H-2b), 4.91 and 4.54 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.84 (d, 1 H, $J_{1a,2a}$ 8.5 Hz, H-1a), 4.66 and 4.50 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.46 and 4.41 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.42 (d, 1 H, H-1b), 4.29 (t, overlapped by OH, 1 H, $J_{3a,4a}$ 9.0, $J_{2a,3a}$ 10.0 Hz, H-3a), 4.29 (d, 1 H, $J_{4a,OH}$ 1.0 Hz, OH), 3.87 (d, 1 H, $J_{3b,4b}$ 2.5 Hz, H-4b), 3.50 (dd, overlapped, 1 H, H-3b), 3.16 (dt, 1 H, $J_{4a,5a}$ 9.0 Hz, H-4a), 2.93 (m, 1 H, H-2a), 2.03, 1.96 (2 s, each 3 H, AcO, AcNH), 1.31 (d, 3 H, $J_{5a,6a}$ 6.0 Hz, 3 H-6a).

Anal. Calc. for $C_{38}H_{47}NO_{11}$: C, 65.79; H, 6.83; N, 2.02. Found: C, 65.49; H, 6.86; N, 1.98.

Methyl 2-acetamido-4-O-acetyl-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,6-dideoxy-β-D-glucopyranoside (**11**). — (*a*) A solution of compound **9** (450 mg, 0.55 mmol) in pyridine (10 mL) and acetic anhydride (3 mL) was kept overnight at room temperature. It was then poured into ice–water and extracted with dichloromethane, and the extract was dried and evaporated to leave a white solid (470 mg). This material, tributyltin hydride (600 μL, 2.23 mmol), and 2,2′-azobis(isobutyronitrile) (20 mg) in toluene (15 mL) were heated at 80°. T.l.c. indicated

the reaction to be complete after 10 min. Solvent removal, followed by column chromatography on silica gel (hexane–ethyl acetate, 3:1; dichloromethane–ethyl acetate, 1:2), gave **11** (272 mg, 67%) as a white solid. Recrystallization from dichloromethane–ethyl acetate–hexane gave the analytical sample, m.p. 167–168°, $[\alpha]_D$ +20° (*c* 0.8, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 15 H, 3 Ph), 5.80 (d, 1 H, $J_{2a,NH}$ 7.0 Hz, NH), 5.18 (dd, 1 H, $J_{1b,2b}$ 7.5, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.94 and 4.55 (Abq, 2 H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.79 (d, 1 H, $J_{1a,2a}$ 8.0 Hz, H-1a), 4.69 (t, 1 H, $J_{3a,4a} = J_{4a,5a}$ = 9.0 Hz, H-4a), 4.66 and 4.49 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.45 (d, overlapped by CH_2Ph, H-1b), 4.45 (s, 2 H, CH_2Ph), 4.31 (t, 1 H, $J_{2a,3a}$ 10.0 Hz, H-3a), 3.91 (d, 1 H, $J_{3b,4b}$ 2.5 Hz, H-4b), 3.48 (dd, overlapped by MeO, H-3b), 3.46 (s, 3 H, MeO), 3.25 (m, 1 H, H-2a), 2.03, 1.97, 1.91 (3 s, each 3 H, 2 AcO, AcNH), 1.21 (d, 3 H, $J_{5a,6a}$ 6.5 Hz, 3 H-6a).

Anal. Calc. for $C_{40}H_{49}NO_{12}$: C, 65.29; H, 6.71; N, 1.90. Found: C, 64.92; H, 6.70; N, 1.76.

(*b*) A solution of compound **10** (50 mg, 0.07 mmol) in pyridine (1 mL) and acetic anhydride (0.5 mL) was kept for 5 h at room temperature. The usual work-up, followed by recrystallization (dichloromethane–ethyl acetate–hexane) afforded **11** (40.2 mg, 76%).

*Methyl 2-acetamido-2,6-dideoxy-3-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**12**). — A solution of acetate **10** (235 mg, 0.34 mmol) in methanolic 0.21M sodium methoxide (10 mL) was kept overnight at room temperature. It was neutralized with Amberlite IRC-50 (H^+) resin and evaporated to leave **12** (210 mg, 95%) as a solid. The analytical sample, recrystallized from methanol, had no sharp m.p. and decomposed at >220°, $[\alpha]_D$ +0.6°, $[\alpha]_{365}$ +2.4° (*c* 0.6, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 15 H, 3 Ph), 6.30 (d, 1 H, $J_{2a,NH}$ 7.0 Hz, NH), 4.84 and 4.51 (ABq, 2 H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.77 (d, 1 H, $J_{1a,2a}$ 8.5 Hz, H-1a), 4.68 and 4.64 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.46 and 4.37 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.30 (bs, 1 H, OH), 4.23 (d, 1 H, $J_{1b,2b}$ 8.0 Hz, H-1b), 4.14 (dd, 1 H, $J_{3a,4a}$ 9.0, $J_{2a,3a}$ 10.0 Hz, H-3a), 3.95 (dd, 1 H, $J_{2b,3b}$ 10.0 Hz, H-2b), 3.81 (d, 1 H, $J_{3b,4b}$ 2.5 Hz, H-4b), 3.49 (s, overlapped, MeO), 3.37 (dd, overlapped, H-3b), 3.23 (m, 1 H, H-2a), 3.18 (t, 1 H, $J_{4a,5a}$ 9.0 Hz, H-4a), 3.06 (bs, 1 H, OH), 1.97 (s, 3 H, AcNH), 1.35 (d, 3 H, $J_{5a,6a}$ 6.0 Hz, 3 H-6a).

Anal. Calc. for $C_{36}H_{45}NO_{10}$: C, 66.34; H, 6.96; N, 2.15. Found: C, 66.20; H, 6.97; N, 2.00.

*Methyl 2-acetamido-2-deoxy-6-O-methanesulfonyl-3-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**13**). — Methanesulfonyl chloride (260 μL, 3.36 mmol) was added dropwise to a solution of diol **7** (2.0 g, 2.82 mmol) in pyridine (20 mL) at −20°. After 2 h, the mixture was poured into ice–water and extracted with dichloromethane, and the organic solution was dried and evaporated. A solution of the residue in a mixture of dichloromethane (10 mL) and methanolic 0.14M sodium methoxide (55 mL) was kept for 2 h at room temperature. Deionization with Amberlite IRC-50 (H^+) resin, evaporation, and column chromatography (dichloromethane–5% methanol) afforded **13** as a white glass (1.3 g, 62%), $[\alpha]_D$

+4° (*c* 1.8, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 15 H, 3 Ph), 6.51 (d, 1 H, $J_{NH,2a}$ 7.5 Hz, NH), 4.85 (d, 1 H, overlapped by CH_2Ph, $J_{1a,2a}$ 8.5 Hz, H-1a), 4.67 and 4.60 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.26 (dd, 1 H, $J_{2a,3a}$ 10.0, $J_{3a,4a}$ 9.0 Hz, H-3a), 4.22 (d, 1 H, $J_{1b,2b}$ 7.5 Hz, H-1b), 3.94 (dt, 1 H, $J_{2b,3b}$ 10.0, $J_{2b,OH}$ 1.5 Hz, H-2b), 3.50 (s, 3 H, OMe), 3.13 (m, 1 H, H-2a), 3.0 (s, 3 H, MsO), 1.98 (s, 3 H, AcNH).

Anal. Calc. for $C_{37}H_{47}NO_{13}S$: C, 59.58; H, 6.35; N, 1.88; S, 4.30. Found: C, 59.29; H, 6.18; N, 1.99; S, 4.24.

2,3,4-Tri-O-benzyl-α-D-fucopyranosyl bromide[17] (**14**). — A solution of oxalyl bromide (900 μL, 9 mmol) in dichloromethane (5 mL) was added to a stirred solution of 2,3,4-tri-*O*-benzyl-L-fucopyranose[17] (2.28 g, 5.25 mmol) in dichloromethane (5 mL) and *N,N*-dimethylformamide (0.5 mL). The mixture was stirred for 20 min and then poured into ice–water. The organic solution was washed twice with cold water, dried with sodium sulfate and 3 Å molecular sieves, and concentrated before it was used in the following glycosidation reaction.

Methyl 2-acetamido-6-O-benzyl-2-deoxy-4-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-3-O-[3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**15**). — A solution of freshly prepared bromide **14** (5.25 mmol) in dichloromethane (4 mL) was added to a stirred mixture of diol **6** (800 mg, 1.06 mmol), tetraethylammonium bromide (230 mg, 1.1 mmol), dichloromethane (3 mL), *N,N*-dimethylformamide (1.2 mL), and 4 Å molecular sieves (3 g, powdered). Stirring under nitrogen was continued for 2 days, methanol (0.5 mL) was added, and the mixture was stirred for a further 2 h, then diluted with dichloromethane, filtered through Celite, washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. Chromatography of the crude product on a column of silica gel (hexane–ethyl acetate, 1:1; dichloromethane–ethyl acetate, 2:1, 1.5:1) gave **15** (1.27 g, 75%) as a foam, $[\alpha]_D$ −98° (*c* 0.9 dichloromethane). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.05 (m, 50 H, 10 Ph), 5.76 (d, 1 H, $J_{NH,2a}$ 7.5 Hz, NH), 5.70 (d, 1 H, $J_{1d,2d}$ 3.5 Hz, H-1d), 4.98 (d, 1 H, $J_{1c,2c}$ 3.5 Hz, H-1c), 3.33 (s, 3 H, MeO), 1.85 (s, 3 H, AcNH), 1.20, 1.12 (2 d, each 3 H, $J_{5c,6c} = J_{5d,6d}$ = 6.5 Hz, 3 H-6c, 3 H-6d).

Anal. Calc. for $C_{97}H_{107}NO_{19}$: C, 73.23; H, 6.78; N, 0.88. Found: C, 72.70; H, 6.73; N, 0.85.

Methyl 2-acetamido-2,6-dideoxy-4-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-3-O-[3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**16**): — A solution of bromide **14** (1.73 mmol) in dichloromethane (2 mL) was added to a mixture of diol **12** (200 mg, 0.31 mmol), tetraethylammonium bromide (150 mg, 0.71 mmol), and 4 Å molecular sieves (3 g) in dichloromethane (2 mL) and *N,N*-dimethylformamide (0.6 mL). This mixture was stirred for 40 h and then processed as described for **15**. Chromatography of the product on a column of silica gel (hexane–ethyl acetate, 3:1; dichloromethane–ethyl acetate, 2:1) gave **16** (343 mg, 75%). $[\alpha]_D$ −76° (*c* 0.4 chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.40–7.0 (m, 45 H, 9 Ph), 5.69 (d, 1 H, $J_{1d,2d}$ 3.5 Hz, H-1d), 5.61 (d,

1 H, $J_{NH,2a}$ 8.5 Hz, NH), 4.89 (d, 1 H, $J_{1c,2c}$ 3.5 Hz, H-1c), 3.41 (s, 3 H, OMe), 1.88 (s, 3 H, AcNH), 1.35, 1.18, 1.11 (3 d, each 3 H, $J_{5a,6a} = J_{5c,6c} = J_{5d,6d} = 6.5$ Hz, 3 H-6a, 3-H-6c, 3 H-6d).

Anal. Calc. for $C_{90}H_{101}NO_{18}$: C, 72.80; H, 6.86; N, 0.94. Found: C, 72.40; H, 6.86; N, 0.95.

Methyl 2-acetamido-2-deoxy-6-O-methanesulfonyl-4-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-3-O-[3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**17**). — To a stirred mixture of diol **13** (1.0 g, 1.34 mmol), tetraethylammonium bromide (600 mg, 2.86 mmol), and 4 Å molecular sieves (4 g) in dichloromethane (5 mL) and *N,N*-dimethylformamide (3 mL) was added a solution of bromide **14** (8.0 mmol) in dichloromethane (4 mL). After stirring for 24 h, the mixture was worked-up as described for the preparation of **15**, followed by column chromatography on silica gel (hexane–ethyl acetate, 3:1; dichloromethane–ethyl acetate, 3:1, 2:1, 1:1), to provide **17** (1.87 g, 88%) as a white foam, $[\alpha]_D$ −67.5° (*c* 0.8, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.46–7.08 (m, 45 H, 9 Ph), 5.96 (d, 1 H, $J_{2a,NH}$ 8.5 Hz, NH), 5.74 (d, 1 H, $J_{1d,2d}$ 3.5 Hz, H-1d), 4.94 (d, 1 H, $J_{1c,2c}$ 3.5 Hz, H-1c), 4.42 and 4.36 (m, overlapped, H-5c,5d), 4.09 (dd, overlapped, 2 H, $J_{2c,3c} = J_{2d,3d} = 10.0$ Hz, H-2c, 2d), 3.39 (s, 3 H, MeO), 2.85 (s, 3 H, MsO), 1.87 (s, 3 H, AcNH), 1.23 and 1.15 (2 d, each 3 H, $J_{5c,6c} = J_{5d,6d} = 6.5$ Hz, 3 H-6c, 3 H-6d).

Anal. Calc. for $C_{91}H_{103}NO_{21}S$: C, 69.23; H, 6.58; N, 0.89; S, 2.03. Found: C, 69.33; H, 6.42; N, 1.03; S, 2.11.

Methyl 2-acetamido-2-deoxy-4-O-(α-L-fucopyranosyl)-3-O-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**18**). — A mixture of compound **15** (234 mg, 0.15 mmol) and 5% palladium-on-carbon (234 mg) in 95% ethanol (9 mL) was hydrogenated for 5 days at 100 p.s.i. The catalyst was removed by filtration, the solvent evaporated, and the resulting residue passed through a column of Sephadex LH 20, using ethanol-water (1:1). Freeze-drying an aqueous solution provided **18** (82 mg, 81%) as a light powder, $[\alpha]_D$ −124° (*c* 0.7, water). The ^{1}H- and ^{13}C-n.m.r. data are reported in Tables I and II.

Methyl 2-amino-2-deoxy-4-O-(α-L-fucopyranosyl)-3-O-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**19**). — A solution of tetrasaccharide **18** (68 mg, 0.10 mmol) in 85% hydrazine hydrate (10 mL) was heated in a metal bomb for 4 days at 145°. After evaporation and co-distillation with water and methanol, the crude product was applied to a column of Sephadex LH 20 and eluted with ethanol-water (1:1). A second purification on the same column afforded, after freeze-drying an aqueous solution, **19** (40 mg, 63%) as a white powder, $[\alpha]_D$ −127° (*c* 0.8, water). The ^{1}H- and ^{13}C-n.m.r. data are reported in Tables I and II.

Methyl 2-acetamido-2,6-dideoxy-4-O-(α-L-fucopyranosyl)-3-O-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**20**). — Compound **16** (220 mg, 0.15 mmol) was hydrogenated for 3 days over 5% palladium-on-carbon, as described for the preparation of **18**. The product was precipitated twice from methanol–ethyl acetate to provide **20** (71.9 mg, 72%), $[\alpha]_D$ −125° (*c* 0.6, water). The ^{1}H- and ^{13}C-n.m.r. data are reported in Tables I and II.

Methyl 2-acetamido-2-deoxy-4-O-(α-L-fucopyranosyl)-3-O-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-6-O-methanesulfonyl-β-D-glucopyranoside (**21**). — Compound **17** (1.05 g, 0.67 mmol) was hydrogenated over 5% palladium-on-carbon for 24 h, as described for the preparation of **18**, to afford **21** (500 mg, 98%) as a white solid. A sample (60 mg) was passed through a column of Sephadex LH 20 (ethanol–water, 1:1) and was obtained as a light, white powder (47 mg) after freeze-drying an aqueous solution; $[\alpha]_D$ −125° (*c* 1.2, water). The ^{1}H- and ^{13}C-n.m.r. data are reported in Tables I and II.

Methyl 2-acetamido-2,6-dideoxy-6-iodo-4-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-3-O-[3,4,6-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**22**). — A solution of mesylate **21** (440 mg, 0.57 mmol) and 4-dimethylaminopyridine (5 mg, 0.04 mmol) in pyridine (10 mL) and acetic anhydride (5 mL) was left for 24 h at room temperature. Ice–water was added, the mixture extracted with dichloromethane, and the organic solution evaporated. The resulting solid (638 mg) and potassium iodide (1.4 g) were heated in *N,N*-dimethylformamide (15 mL) for 1.5 h at 100°. The mixture was diluted with dichloromethane, washed with water, saturated aqueous sodium thiosulfate, and water, and evaporated. Chromatography on a column of silica gel (ethyl acetate) afforded **22** (500 mg, 74%) as a white solid, $[\alpha]_D$ −111° (*c* 0.6, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 6.26 (d, 1 H, $J_{NH,2a}$ 7.5 Hz, NH), 4.76 (d, 1 H, $J_{1a,2a}$ 6.0 Hz, H-1a), 4.56 (t, 1 H, $J_{2a,3a} = J_{3a,4a}$ = 9.0 Hz, H-3a), 3.70 (t, 1 H, $J_{4a,5a}$ 9.0 Hz, H-4a), 3.59 (s, 3 H, MeO), 3.52 (dd, 1 H, $J_{5a,6Aa}$ 2.0, $J_{6Aa,6Ba}$ 10.5 Hz, H-6Aa), 3.45 (m, 1 H, H-5a), 3.40 (m, 1 H, H-2a), 2.20, 2.19, 2.17, 2.15, 2.08, 2.07, 2.05, 2.02, 2.01, 2.00 (10 s, each 3 H, AcNH, 9 AcO), 1.25, 1.21 (2 d, each 3 H, $J_{5c,6c} = J_{5d,6d}$ = 6.5 Hz, 3 H-6c, 3 H-6d).

Anal. Calc. for $C_{45}H_{64}INO_{27}$: C, 45.89; H, 5.48; I, 10.77; N, 1.19. Found: C, 45.91; H, 5.44; I, 10.82; N, 1.11.

Methyl 2-acetamido-2,6-dideoxy-4-O-(α-L-fucopyranosyl)-3-O-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-6-iodo-β-D-glucopyranoside (**23**). — (*a*) A solution of compound **22** (170 mg, 0.14 mmol) in methanol (3.4 mL), water (1.13 mL), and triethylamine (1.7 mL) was kept for 17 h at room temperature. After solvent removal, the residue was applied to a column of Sephadex LH 20, using ethanol–water (1:1) as eluant. The resulting material was precipitated twice from methanol–ethyl acetate to provide **23** (91.9 mg, 80%) as a white solid, $[\alpha]_D$ −87° (*c* 0.7, water). The ^{1}H- and ^{13}C-n.m.r. data are reported in Tables I and II.

(*b*) Tetrasaccharide **21** (10 mg, 0.013 mmol) and potassium iodide (10 mg) in *N,N*-dimethylformamide (600 μL) were kept for 1.5 h at 100°. The solution was taken to dryness, and the crude product was applied to a column of Sephadex LH 20 and eluted with ethanol–water (1:1) to provide a product identical (^{1}H-n.m.r. spectrum) to **23** (5.6 mg, 54%).

Methyl 2-acetamido-3-O-allyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**24**). — A mixture of compound **2** (7.0 g, 21.6 mmol), barium oxide (10 g, 65.2 mmol), barium hydroxide octahydrate (2.9 g, 9.2 mmol), and allyl bromide (3 mL,

34.7 mmol) in *N,N*-dimethylformamide (100 mL) was stirred for 2 h at room temperature. The mixture was diluted with dichloromethane, and filtered through a pad of Celite followed by thorough washing of the solids with dichloromethane. The combined filtrate was washed successively with aqueous 2% hydrochloric acid, aqueous saturated sodium hydrogencarbonate, and water, and concentrated to a smaller volume to which ether was added, to give white crystalline **24** (7.4 g, 90%). The analytical sample, obtained by recrystallization from ethanol, had m.p. 295–296° (dec.), $[\alpha]_D$ −62° (*c* 0.6, methyl sulfoxide). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.53–7.30 (m, 5 H, Ph), 5.88 (m, 1 H, $CH_2=CH$), 5.69 (d, 1 H, $J_{NH,2}$ 7.5 Hz, NH), 5.55 (s, 1 H, CHPh), 5.24 and 5.15 (2 m, 2 H, $CH_2=CH$), 4.99 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.36 and 4.13 (2 m, 2 H, $CH_2=CH$), 4.36 (dd, overlapped by $CH_2=CH$, $J_{6A,6B}$ 10.5, $J_{5,6A}$ 4.5 Hz, H-6A), 4.26 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 8.5 Hz, H-3), 3.78 (t, 1 H, $J_{5,6B}$ 9.5 Hz, H-6B), 3.59 (t, 1 H, $J_{4,5}$ 8.5 Hz, H-4), 3.53 (m, 1 H, overlapped by MeO, H-5), 3.52 (s, 3 H, MeO), 3.16 (m, 1 H, H-2), 2.02 (s, 3 H, AcNH).

Anal. Calc. for $C_{19}H_{25}NO_6$: C, 62.80; H, 6.93; N, 3.85. Found: C, 62.48; H, 6.71; N, 3.89.

*Methyl 2-acetamido-3-O-allyl-6-O-benzyl-2-deoxy-β-D-glucopyranoside** (**25**). — Compound **24** (1.0 g, 2.75 mmol) in dry tetrahydrofuran was treated with sodium cyanoborohydride (1.6 g, 25.7 mmol) as described for the preparation of **4**. The deionized, white solid was recrystallized from hot ethyl acetate to provide **25** (850 mg, 89%) as fine needles, m.p. 179–181°, $[\alpha]_D$ −20° (*c* 0.8, methanol). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.24 (m, 5 H, Ph), 5.89 (m, 1 H, $CH_2=CH$), 5.82 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.26 and 5.17 (2 m, 2 H, $CH_2=CH$), 4.80 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.61 and 4.57 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.27 and 4.17 (2 m, 2 H, $CH_2=CHCH_2$), 3.90 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 8.5 Hz, H-3), 3.76 (d, 2 H, $J_{5,6}$ 4.5 Hz, 2 H-6), 3.60 (t, 1 H, $J_{4,5}$ 8.5 Hz, H-4), 3.53 (m, 1 H, H-5), 3.47 (s, 3 H, MeO), 3.23 (m, 1 H, H-2), 2.97 (bs, 1 H, OH), 2.01 (s, 3 H, AcNH).

Anal. Calc. for $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83. Found: C, 62.40; H, 7.47; N, 3.75.

Methyl 2-acetamido-3-O-allyl-2-deoxy-β-D-glucopyranoside (**26**). — Compound **24** (4.0 g, 11.0 mmol) in aqueous 75% acetic acid (100 mL) was heated for 1 h at 70°. Solvent removal and co-evaporation with water and ethanol left a white solid, column chromatography (silica gel; dichloromethane–10% methanol) of which gave **26** (2.8 g, 92%). The analytical sample, recrystallized from ethanol–ethyl acetate, had m.p. 210–211°, $[\alpha]_D$ −37.5° (*c* 0.6, methanol). ^{1}H-N.m.r. (D_2O): 5.88 (m, 1 H, $CH_2=CH$), 5.28 and 5.24 (2 m, 2 H, $CH_2=CH$), 4.46 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.30 and 4.15 (2 m, 2 H, ABq, $J_{A,B}$ 12.5 Hz, $CH_2=CHCH_2$), 3.93 (dd, 1 H, $J_{6A,6B}$ 12.5, $J_{5,6A}$ 1.75 Hz, H-6A), 3.74 (dd, 1 H, overlapped by H-2, $J_{5,6B}$ 5.0 Hz, H-6B), 3.73 (dd, 1 H, overlapped, $J_{2,3}$ 9.0 Hz, H-2), 3.55–3.42 (m, 6 H, H-3,4,5, and MeO at 3.50), 2.03 (s, 3 H, AcNH).

* Compound **25** was prepared by S. Narasimhan.

Anal. Calc. for $C_{12}H_{21}NO_6$: C, 52.35; H, 7.69; N, 5.09. Found: C, 51.99; H, 7.78; N, 5.13.

Methyl 2-acetamido-3-O-allyl-2-deoxy-6-O-methanesulfonyl-β-D-glucopyranoside (**27**). — Methanesulfonyl chloride (900 μL, 11.6 mmol) was added to a solution of diol **26** (2.6 g, 9.44 mmol) in pyridine (20 mL) at −20°. After 1 h, the excess of methanesulfonyl chloride was reacted with methanol (1 mL), and the mixture was allowed to reach room temperature and then evaporated. Chromatography of the residue on a column of silica gel (dichloromethane–5% methanol) gave **27** (3.0 g, 90%) as a white solid. Recrystallization from ethanol provided the analytical sample, m.p. 160–162° (dec.), $[\alpha]_D$ −20° (*c* 0.7, methanol). ^{1}H-N.m.r. data (CD_3OD, CH_3 at 3.3 p.p.m.): δ 5.89 (m, 1 H, $CH_2=CH$), 5.23 and 5.10 (2 m, 2 H, $CH_2=CH$), 4.53 (dd, 1 H, $J_{6A,6B}$ 11.5, $J_{5,6A}$ 1.75 Hz, H-6A), 4.40 (dd, 1 H, $J_{5,6B}$ 5.5 Hz, H-6B), 4.38 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.33 and 4.13 (2 m, 2 H, $CH_2=CHCH_2$), 3.67 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 3.52 (ddd, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 3.44 (s, overlapped, MeO), ~3.4 (overlapped, H-4), 3.38 (t, 1 H, $J_{3,4}$ 9.0 Hz, H-3), 3.10 (s, 3 H, MsO), 1.97 (s, 3 H, AcNH).

Anal. Calc. for $C_{13}H_{23}NO_8S$: C, 44.18; H, 6.56; N, 3.96; S, 9.07. Found: C, 44.08; H, 6.67; N, 3.94; S, 9.07.

Methyl 2-acetamido-4-O-acetyl-3-O-allyl-2,6-dideoxy-β-D-glucopyranoside (**28**). — Mesylate **27** (1.2 g, 3.40 mmol) and potassium iodide (2.4 g, 14.46 mmol) in *N,N*-dimethylformamide (40 mL) were heated for 5.5 h at 95°. The mixture was processed, as described for the preparation of **9**, to provide a crystalline white residue (1.16 g, 3.01 mmol). This material was *O*-acetylated in pyridine–acetic anhydride (2:1; 45 mL) for 1 h and worked-up in the usual way. The resulting product (1.2 g), tributyltin hydride (2 mL, 10.12 mmol), and 2,2′-azobis(isobutyronitrile) (50 mg) in toluene (60 mL) were heated for 45 min at 80°. Evaporation followed by column chromatography on silica gel (dichloromethane–ethyl acetate, 1:1 and 1:2) provided **28** (750 mg, 73%). The analytical sample, recrystallized from ethanol, had m.p. 259–260°, $[\alpha]_D$ +42° (*c* 0.5, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.81 (m, overlapped by NH, $CH_2=CH$), 5.79 (d, overlapped, NH), 5.21 and 5.13 (2 m, 2 H, $CH_2=CH$), 4.92 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.72 (t, 1 H, $J_{3,4} \approx J_{4,5} \approx$ 9.5 Hz, H-4), 4.20 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-3), 4.07 (m, 2 H, $CH_2=CHCH_2$), 3.53 (m, 1 H, H-5), 3.49 (s, 3 H, MeO), 3.08 (m, 1 H, H-2), 2.06, 1.97 (2 s, each 3 H, AcO, AcNH), 1.22 (d, 3 H, $J_{5,6}$ 6.0 Hz, 3 H-6).

Anal. Calc. for $C_{14}H_{23}NO_6$: C, 55.80; H, 7.69; N, 4.65. Found: C, 55.43; H, 7.64; N, 4.66.

Methyl 2-acetamido-3-O-allyl-2,6-dideoxy-β-D-glucopyranoside (**29**). — (*a*) Mesylate **27** (1.2 g, 3.40 mmol) was treated with potassium iodide as described for **28**, and the resulting methyl 2-acetamido-3-*O*-allyl-2,6-dideoxy-6-iodo-β-D-glucopyranoside (1.2 g, 3.12 mmol) was reduced with tributyltin hydride in toluene (120 mL) as before. Evaporation with subsequent column chromatography of the product on silica gel (dichloromethane–5% and 7% methanol) provided **29** (660 mg, 75%) as a white solid. The analytical sample, recrystallized from ethanol, had m.p.

245–247° (dec.), $[\alpha]_D$ −15° (*c* 0.6, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.94 (m, 1 H, $CH_2=CH$), 5.64 (d, 1 H, $J_{NH,2}$ 7.5 Hz, NH), 5.29 and 5.20 (2 m, 2 H, $CH_2=CH$), 4.78 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.26–4.16 (m, 2 H, $CH_2=CHCH_2$), 3.92 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx$ 9.5 Hz, H-3), 3.47 (s, 3 H, MeO), 3.45 (m, 1 H, H-5), 3.23 (t, overlapped, H-4), 3.20 (m, overlapped, H-2), 2.01 (s, 3 H, AcNH), 1.32 (d, 3 H, $J_{5,6}$ 6.0 Hz, 3 H-6).

Anal. Calc. for $C_{12}H_{21}NO_5$: C, 55.59; H, 8.16; N, 5.40. Found: C, 55.27; H, 8.15; N, 5.58.

(*b*) Acetate **28** (680 mg, 2.26 mmol) was treated with methanolic 0.1M sodium methoxide (25 mL) for 24 h. Deionization with Amberlite IRC-50 (H^+), evaporation, and chromatography of the product on a column of silica gel (dichloromethane–ethyl acetate, 1:1; and ethyl acetate) gave **29** (543 mg 93%).

Methyl 2-acetamido-3-O-allyl-4-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**31**). — Alcohol **25** (800 mg, 2.19 mmol), mercuric cyanide (1.1 g, 4.37 mmol), powdered calcium sulfate (1 g), and 4 Å molecular sieves (3 g) were stirred under nitrogen in a mixture of nitromethane (10 mL) and toluene (10 mL) for 1.5 h. Then a solution of 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl bromide[3] (**30**) (3.69 mmol) in nitromethane–toluene (1:1, 10 mL) was added and stirring continued for 3 h. Further processing as described for **3** and chromatography of the syrupy residue on a column of silica gel (dichloromethane–ethyl acetate, 3:1, 2:1, 1:1) gave **31** (1.51 g, 77%) as a foam, $[\alpha]_D$ −13° (*c* 2.1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.0–7.2 (m, 25 H, 5 Ph), 6.24 (d, 1 H, $J_{NH,2a}$ 9.0 Hz, NH), 5.83 (m, 1 H, $CH_2=CH$), 5.63 (dd, 1 H, $J_{1b,2b}$ 8.0, $J_{2b,3b}$ 10.0 Hz, H-2b), 5.20 and 5.07 (2 m, 2 H, $CH_2=CH$), 4.51 (d, overlapped, H-1b), 4.10 (d, overlapped, H-1a), 3.93 (m, 1 H, H-2a), 3.02 (s, 3 H, MeO), 2.05 (s, 3 H, AcNH).

Anal. Calc. for $C_{53}H_{59}NO_{12}$: C, 70.57; H, 6.59; N, 1.55. Found: C, 70.28; H, 6.45; N, 1.51.

Methyl 2-acetamido-6-O-benzyl-2-deoxy-4-O-(3,4,6-tri-O-benzyl-β-D-galacto-pyranosyl)-β-D-glucopyranoside (**32**). — Compound **31** (645 mg, 0.72 mmol) was treated with methanolic 0.21M sodium methoxide (25 mL) for 2 days. A mixture of the material obtained on deionization with Amberlite IRC-50 (H^+) resin and evaporation, tris(triphenylphosphine)rhodium(I) chloride (50 mg, 0.054 mmol), 1,8-diazabicyclo[2.2.2.]octane (22 mg, 0.20 mmol), and 95% ethanol–toluene–water (7:3:1, 60 mL) was boiled under reflux for 20 h. The solvent was removed and the residue was dissolved in acetone (50 mL) containing mercuric oxide (10 mg). A solution of mercuric chloride (1.5 g) in acetone–water (9:1, 25 mL) was added and the mixture stirred for 30 min. After solvent removal, the residue was taken up in dichloromethane, the solution washed with aqueous saturated potassium bromide and water, and the organic solution concentrated. Chromatography on a column of silica gel (ethyl acetate) gave diol **32** (466 mg, 86%). The analytical sample, recrystallized from ethyl acetate–hexane, had m.p. 119–121°, $[\alpha]_D$ −4° (*c* 0.9, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.7–7.2 (m, 20 H, 4 Ph), 5.57 (d, 1 H, $J_{NH,2a}$ 7.5 Hz, NH),

4.86 and 4.56 (ABq, 2 H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.73 and 4.65 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.67 (d, 1 H, $J_{1a,2a}$ 8.0 Hz, H-1a), 4.61 and 4.57 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.26 (d, 1 H, $J_{1b,2b}$ 7.5 Hz, H-1b), 3.95 (dd, 1 H, $J_{2a,3a}$ 10.0, $J_{3a,4a}$ 8.5 Hz, H-3a), 3.90 (t, 1 H, $J_{2b,3b}$ 9.5 Hz, H-2b), 3.84 (d, 1 H, $J_{3b,4b}$ 2.5 Hz, H-4b), 3.55 (t, overlapped, 1 H, H-4a), 3.48 (s, 3 H, MeO), 3.42 (m, 1 H, H-2a), 3.34 (dd, 1 H, H-3b), 2.00 (s, 3 H, AcNH).

Anal. Calc. for $C_{43}H_{51}NO_{11}$: C, 68.15; H, 6.78; N, 1.85. Found: C, 67.60; H, 6.70; N, 1.97.

Methyl 2-acetamido-4-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-3-O-allyl-2,6-dideoxy-β-D-glucopyranoside (**33**). — A solution of bromide **1** (2.9 mmol) in toluene (10 mL) was added to a stirred mixture of **29** (480 mg, 1.85 mmol), mercuric cyanide (850 mg, 3.37 mmol), 4 Å molecular sieves (2 g), and powdered calcium sulfate (1 g) in nitromethane–toluene (1:1, 20 mL). Stirring was continued for 3 h and further processing was performed as described for **3**. Column chromatography (silica gel; dichloromethane–ethyl acetate, 3:1, 2:1, 1:1, 1:2) furnished **33** (460 mg, 34%) as a white solid. The analytical sample, obtained by recrystallization from dichloromethane–ethyl acetate, had m.p. 189–191°, $[\alpha]_D$ −8° (*c* 0.5, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.2 (m, 15 H, 3 Ph), 5,.83 (d, overlapped, 1 H, $J_{NH,2a}$ 8.0 Hz, NH), 5.81 (m, 1 H, $CH_2=CH$), 5.32 (dd, 1 H, $J_{1b,2b}$ 7.5, $J_{2b,3b}$ 10.0 Hz, H-2b), 5.17 and 5.04 (2 m, each 1 H, $CH_2=CH$), 4.93 and 4.56 (ABq, 2 H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.67 and 4.49 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.57 (d, overlapped, 1 H, $J_{1a,2a}$ 7.5 Hz, H-1a), 4.46 (d, 1 H, $J_{1b,2b}$ 7.5 Hz, H-1b), 4.43 and 4.49 (ABq, 2 H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.24 and 4.02 (2 m, 2 H, $CH_2=CHCH_2$), 3.97 (d, 1 H, $J_{3b,4b}$ 3.0 Hz, H-4b), 3.42 (s, 3 H, MeO), 2.02, 1.95 (2 s, each 3 H, AcO, AcNH), 1.33 (d, 3 H, $J_{5a,6a}$ 6.5 Hz, 3 H-6a).

Anal. Calc. for $C_{41}H_{51}NO_{11}$: C, 67.11; H, 7.01; N, 1.91. Found: C, 67.22; H, 6.92; N, 1.88.

Methyl 2-acetamido-2,6-dideoxy-4-O-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**34**). — Disaccharide **33** (280 mg, 0.38 mmol) was deallylated with subsequent deacetylation as described for the preparation of **32**. Column chromatography (silica gel; dichloromethane–3% methanol) of the product gave **34** (188 mg, 76%). The analytical sample, recrystallized from ethyl acetate–hexane, had m.p. 167–168°, $[\alpha]_D$ +1° (*c* 0.7, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.43–7.20 (m, 15 H, 3 Ph), 5.76 (d, 1 H, $J_{NH,2a}$ 7.5 Hz, NH), 4.60 (d, 1 H, $J_{1a,2a}$ 8.0 Hz, H-1a), 4.33 (d, 1 H, $J_{1b,2b}$ 7.5 Hz, H-1b), 3.98 (bdd, 1 H, $J_{2b,3b}$ 9.5 Hz, H-2b), 3.48 (s, overlapped, MeO), 2.00 (s, 3 H, AcNH), 1.37 (d, 3 H, $J_{5a,6a}$ 6.5 Hz, 3 H-6a).

Anal. Calc. for $C_{36}H_{45}NO_{10}$: C, 66.34; H, 6.96; N, 2.15. Found: C, 65.97; H, 7.02; N, 2.18.

Methyl 2-acetamido-3-O-acetyl-4-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,6-dideoxy-β-D-glucopyranoside (**35**). — Diol **34** (100 mg, 0.15 mmol) was acetylated in pyridine–acetic anhydride (2:1, 3.8 mL) overnight at room temperature. The mixture was processed in the usual way and the product was recrystallized from dichloromethane–ethyl acetate to give **35** (86 mg, 78%), m.p.

222–225°, $[\alpha]_D$ −17° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.4–7.15 (m, 15 H, 3 Ph), 5.63 (d, 1 H, $J_{NH,2a}$ 9.5 Hz, NH), 5.26 (dd, 1 H, $J_{1b,2b}$ 8.0, $J_{2b,3b}$ 10.0 Hz, H-2b), 4.92 (dd, 1 H, $J_{2a,3a}$ 10.0, $J_{3a,4a}$ 8.0 Hz, H-3a), 4.92 and 4.53 (ABq, 2 H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.65 and 4.47 (ABq, 2 H, $J_{A,B}$ 12.0 Hz, CH_2Ph), 4.56 (d, 1 H, $J_{1a,2a}$ 7.0 Hz, H-1a), 4.41 (s, 2 H, CH_2Ph), 4.21 (d, 1 H, H-1b), 4.03 (m, 1 H, H-2a), 3.96 (d, 1 H, $J_{3b,4b}$ 2.5 Hz, H-4b), 3.46 (overlapped, H-3b), 3.43 (s, overlapped, MeO), 1.99, 1.92, 1.90 (3 s, each 3 H, AcNH, 2 AcO), 1.30 (d, 3 H, $J_{5a,6a}$ 6.0 Hz, 3 H-6a).

Anal. Calc. for $C_{39}H_{49}NO_{12}$: C, 64.72; H, 6.82; N, 1.94. Found: C, 65.06; H, 6.74; N, 1.92.

*Methyl 2-acetamido-6-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-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* (**36**). — Diol **32** (290 mg, 0.38 mmol) was reacted with bromide **14** (2.30 mmol) for 40 h as described for the preparation of **15**. Chromatographic purification of the product on a column of silica gel (hexane–ethyl acetate, 3:1, dichloromethane–ethyl acetate, 3:1) provided **36** (464 mg, 76%) as a white foam, $[\alpha]_D$ −60° (*c* 1.1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.6–7.05 (m, 50 H, 10 Ph), 5.63 (d, 1 H, $J_{NH,2a}$ 6.5 Hz, NH), 5.10 (d, 1 H, $J_{1a,2a}$ 8.0 Hz, H-1a), 3.47 (s, 3 H, MeO), 2.98 (m, 1 H, H-2a), 1.58 (s, 3 H, AcNH), 1.26, 1.10 (2 d, each 3 H, $J_{5c,6c} \approx J_{5d,6d} \approx$ 6.5 Hz, 3 H-6c, 3 H-6d).

Anal. Calc. for $C_{97}H_{107}NO_{19}$: C, 73.23; H, 6.78; N, 0.88. Found: C, 73.49; H, 6.74; N, 0.95.

*Methyl 2-acetamido-2,6-dideoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-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* (**37**). — Compound **37** was prepared from diol **34** (215 mg, 0.33 mmol) and bromide **14** (1.97 mmol) as described for the synthesis of **15**. Purification of the product on a column of silica gel (hexane–ethyl acetate, 3:1; dichloromethane–ethyl acetate, 3:1, 2:1) afforded **37** (379 mg, 77%) as a foamy solid, $[\alpha]_D$ −66° (*c* 1.2, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.45–6.95 (m, 45 H, 9 Ph), 5.58 (d, 1 H, $J_{NH,2a}$ 6.5 Hz, NH), 5.13 (d, 1 H, $J_{1a,2a}$ 7.5 Hz, H-1a), 3.45 (s, 3 H, MeO), 2.88 (m, 1 H, H-2a), 1.55 (s, 3 H, AcNH), 1.34, 1.26, 1.12 (3 d, each 3 H, $J_{5a,6a} \approx J_{5c,6c} \approx J_{5d,6d} \approx$ 6.5 Hz, 3 H-6a, 3 H-6c, 3 H-6d).

Anal. Calc. for $C_{90}H_{101}NO_{18}$: C, 72.80; H, 6.86; N, 0.94. Found: C, 72.53; H, 6.78; N, 0.98.

*Methyl 2-acetamido-2-deoxy-3-*O*-(α-*L*-fucopyranosyl)-4-*O*-[2-*O*-(α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**38**). — Compound **36** (129 mg, 0.08 mmol) was hydrogenated for 24 h over 5% palladium-on-carbon and the product was precipitated three times from methanol–ethyl acetate to provide **38** (40 mg, 72%) as a white powder, $[\alpha]_D$ −126° (*c* 0.7, water). The ^{1}H- and ^{13}C-n.m.r. data are reported in Tables I and II.

*Methyl 2-amino-2-deoxy-3-*O*-(α-*L*-fucopyranosyl)-4-*O*-[2-*O*-(α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**39**). — Tetrasaccharide **38** (9.5 mg, 0.014 mmol) was *N*-deacetylated at 140° as described for the preparation of **19**.

Two-fold chromatographic purification provided **39** (5.5 mg, 62%) as a white solid, $[\alpha]_D$ −113° (*c* 0.3, water). The ^{1}H- and ^{13}C-n.m.r. data are reported in Tables I and II.

Methyl 2-acetamido-2,6-dideoxy-3-O-(α-L-fucopyranosyl)-4-O-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**40**). — Tetrasaccharide **37** (150 mg, 0.10 mmol) was hydrogenated over 5% palladium-on-carbon as described for compound **18**. The product was precipitated from methanol–ethyl acetate and column chromatography on Sephadex LH 20 (ethanol-water, 1:1) then provided **40** (45 mg, 66%), $[\alpha]_D$ −133° (*c* 0.8, water). The ^{1}H- and ^{13}C-n.m.r. data are reported in Tables I and II.

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