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The synthesis of chemically modified disaccharide derivatives of the *Shigella flexneri* Y polysaccharide antigen *

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ABSTRACT

Disaccharide analogs related to the 2-acetamido-2-deoxy-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranose element of the *Shigella flexneri* Y polysaccharide antigen have been synthesized and used to map the binding site of murine monoclonal antibodies GC-4 and SYA/J6 by solid-phase inhibition assays. *N*-Acetyl, *N*-trifluoroacetyl and *N*-benzyloxycarbonyl derivatives of methyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside **1**, **3**, and **4** were glycosylated by rhamnopyranosyl bromide and thioglycoside donors **5** and **6**. These in turn provided access to a series of α-L-Rha*p*-(1 → 3)-β-D-GlcN*p*-(1 → O)-Me disaccharide glycosides with amino **13**, *N*-acetyl **10**, *N*-propionyl **14**, *N*-pivaloyl **15**, and *N*-trifluoroacetyl **11** functionalities. Congeners of the disaccharide **10** were synthesized with monodeoxy groups introduced at the C-4 and C-6 positions of the GlcNAc residues and at the C-4′ position of the rhamnose unit. Chlorosulfation of the selectively protected disaccharide **24**, followed by reduction of the 4-chloro-4-deoxy compound **25**, was used to prepare the 4-deoxy congener **27**, while the C-6 and C-4′ deoxy derivatives **31** and **23** were assembled from their respective pre-functionalized monosaccharide building blocks **29** and **19**.

INTRODUCTION

The strategy of mapping carbohydrate binding sites of enzymes[1,2], lectins[3] and antibodies[3–5] by a series of functional group replacements employing the conservative substitution $>CH-OH \rightarrow >CH_2$ has provided an effective method to identify the oligosaccharide hydroxyl groups that form the most important polar contacts with protein. When this approach is combined with computer assisted modelling, an overall picture emerges that identifies the antigen surface exposed toward the antibody binding site as well as that pointing into solution[3]. By varying the size of the synthetic epitope, the extent of the complementary interacting surface may also be established[4,5].

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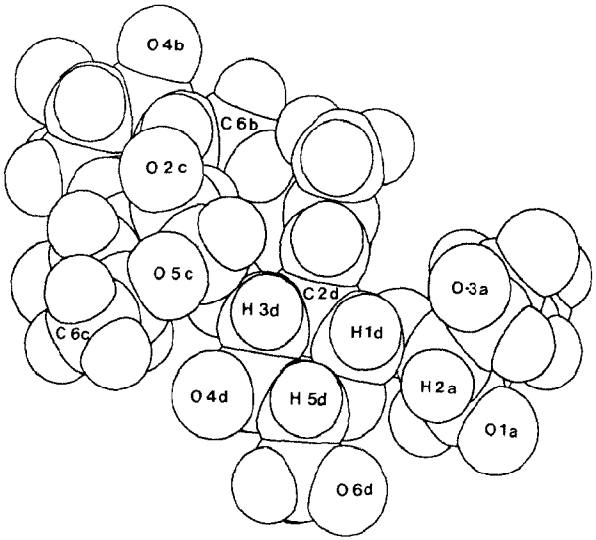


Fig. 1. The disaccharide element, CD, α-L-Rha-(1 → 3)-β-D-GlcNAc is viewed as part of the tetrasaccharide BCDA, and from a direction approximately perpendicular to the plane of the H-1d, H-3d and H-5d atoms. The acetamido group protrudes from the GlcN ring by a distance that is comparable to the dimension across the pyranose ring. This steric bulk is anticipated to significantly influence the formation of the oligosaccharide epitope.

O-Antigens are one of the principal antigens of Gram-negative bacteria, and their interaction with antibody is a vital part of the body's defense against infection. As part of a detailed investigation of the structure of two antibody–*Shigella flexneri* antigen complexes, extensive mapping of the binding site by oligosaccharides of various sizes and by monodeoxy congeners has been completed. For one antibody SYA/J6, this data will complement high resolution crystal structure studies[6] of the native Fab and Fab complexes with trisaccharide and pentasaccharide ligands.

At the outset of the modelling studies, it was evident that the presence of an acetamido moiety within the repeating unit of the *Shigella flexneri* O-antigen:

[→ 2)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-β-D-GlcNAc*p*-(1 →]

would significantly extend the surface topology of the glucosamine residue (Fig. 1). Therefore, it was considered essential to prepare analogs that would probe this molecular feature of the antibody–antigen interaction. However, previous efforts to obtain oligosaccharides with either 2-amino-2-deoxy or 2-acylamido-2-deoxy moieties encountered difficulties due to the known resistance toward alkaline amide hydrolysis of 2-acetamido-2-deoxy-3-*O*-(glycosyl)-glucosides[7,8]. This property of disaccharide **10** necessitated the use of selectively protected derivatives of glucosamine that would allow facile introduction of various *N*-acyl groups following elaboration of the oligosaccharide. The development of this strategy is described at the disaccharide level together with the synthesis of a number of

monodeoxy congeners of the disaccharide glycoside α-L-Rha*p*-(1 → 3)-β-D-GlcNAc*p*-(1 → O)-Me.

RESULTS AND DISCUSSION

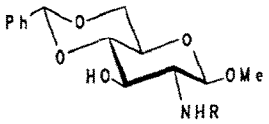
Alkaline hydrolysis of methyl 2-acetamido-2-deoxy-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside **10** was initially considered as a route to the corresponding amine **13** and, subsequently, the acylamido compounds **11**, **14**, and **15**. However, the well-documented resistance of 3-*O*-substituted 2-acetamido-2-deoxy-glucose to *N*-deacetylation reactions using aqueous base[7,8] resulted in only a 50–60% conversion of **10** to **13** even after prolonged treatment (2 M NaOH, 4 h). In order to provide access to a series of disaccharides with acylamido groups of increasing size or polarity, the monosaccharide **1** was hydrolyzed to the amine **2**, then used to prepare the trifluoroacetamide **3** and the *N*-benzyloxycarbonyl derivative **4** (benzyloxycarbonyl chloride, CH_2Cl_2–aq $KHCO_3$)[9].

Disaccharide **7** was synthesized by two methods: Helferich glycosylation of **1** by tri-*O*-acetyl-α-L-rhamnopyranosyl bromide **5** or by activation of the thioglycoside **6** by iodonium ions generated in situ from *N*-iodosuccinimide and triflic acid[10]. The latter was the method of choice, since it provided crystalline disaccharide **7** (71%) after simple workup. Disaccharide **8** was prepared from **3** and **6** in analogous fashion. Glycosylation of methyl 2-amino-4,6-*O*-benzylidene-2-deoxy-2-*N*-benzyloxycarbonyl-β-D-glucopyranoside **4** to yield **9** was accomplished by two procedures; in situ generation of the glycosyl bromide **5** by addition of bromine[11] to a solution of **4** and **6** containing mercuric bromide, or iodonium activation of **6** as described for the synthesis of **8**. A shorter reaction time (1 h vs. 30 h), as well as a higher yield (84 vs. 76%), accompanied the latter reaction.

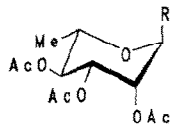
Disaccharide **7** was deprotected by hydrogenolysis of the benzylidene acetal, followed by treatment with methanolic ammonia to give crystalline **10** (97%). Transesterification of the trifluoroacetamido derivative **8** in sodium methoxide solution, followed by acid hydrolysis of the acetal protecting group, gave the disaccharide **11**. Residual silica gel was removed from **11** by gel-permeation chromatography using a Sephadex G-15 column.

The *N*-benzyloxycarbonyl derivative **12** (90%) was obtained as an analytically pure white powder after acetal hydrolysis and transesterification of the protected disaccharide **9**. Disaccharide amine **13** was precipitated as its hydrochloride salt after hydrogenolysis of the benzyloxycarbonyl group of **12**. Both the *N*-propionyl and *N*-pivaloyl disaccharide derivatives **14** and **15** were prepared by acylation in situ of the amine **13**.

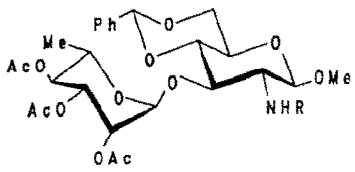
Methyl 4,6-dideoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranoside **16** was prepared by a published procedure based on a Barton–MacCombie reaction[12]. The isopropylidene acetal was hydrolyzed, and the resulting diol **17** was subjected to acetolysis. The triacetate **18** crystallized and was converted to a mixture of α and β thioethyl rhamnopyranosides **19** (67%) and **20** (16%). Ethyl 2,3-di-*O*-acetyl-4,6-di-



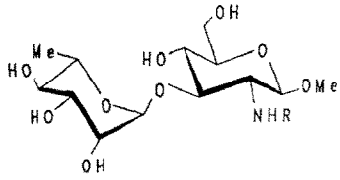
1 R=Ac
2 R=H
3 R=$COCF_3$
4 R=CO_2CH_2Ph



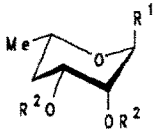
5 R=Br
6 R=SEt



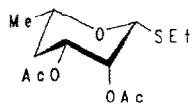
7 R=Ac
8 R=$COCF_3$
9 R=CO_2CH_2Ph



10 R=Ac
11 R=$COCF_3$
12 R=CO_2CH_2Ph
13 R=H
14 R=$COCH_2CH_3$
15 R=$COC(CH_3)_3$



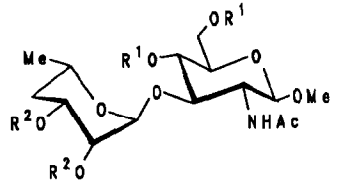
16 R^1=OMe, R^2=>CMe_2
17 R^1=OMe, R^2=H
18 R^1=OAc, R^2=Ac
19 R^1=SEt, R^2=Ac



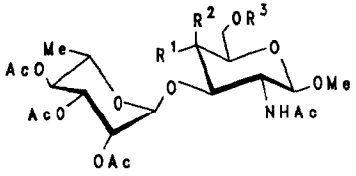
20

deoxy-1-thio-α-L-*lyxo*-hexopyranoside **19** was reacted with the alcohol **1** using iodonium ions as promoter to give the required disaccharide **21** (80%). Transesterification gave **22** and hydrogenolysis over palladium gave the 4′-deoxy congener **23**.

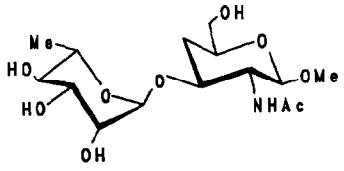
The introduction of a 4-deoxy functionality to oligosaccharides containing 2-acetamido-2-deoxy-glucose residues by radical deoxygenation type reactions has been shown to give variable yields[13]. In order to circumvent this problem, chlorosulfation[14] of a selectively protected disaccharide **24** was envisioned as a route to the 4-chloro-4-deoxy disaccharide **25**, which after reduction could provide the 4-deoxy disaccharide **27**. Reductive opening of the benzylidene acetal of disaccharide **7** gave the selectively protected disaccharide **24** (88%). Chlorosulfation and prolonged reaction at 0–25°C gave the 4-chloro-4-deoxy compound **25** (62%).



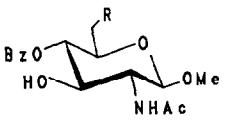
21 R^1=>CHPh, R^2=Ac
22 R^1=>CHPh, R^2=H
23 R^1=R^2=H



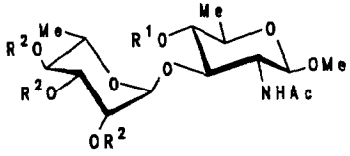
24 R^1=OH, R^2=H, R^3=Bn
25 R^1=H, R^2=Cl, R^3=Bn
26 R^1=R^2 H, R^3=Bn



27



28 R=Br
29 R=H



30 R^1=Bz, R^2=Ac
31 R^1=R^2=H

Tributyltin hydride efficiently reduced this compound to **26**, and removal of the acetyl and benzyl groups to give **27** was accomplished by standard reactions.

The 6-deoxy disaccharide congener **31** was synthesized from the selectively protected alcohol **29**, prepared by NBS opening of the benzylidene acetal[15] **1** and reduction of the 6-bromo-6-deoxy monosaccharide **28**. Helferich glycosylation of **29** by the rhamnopyranosyl bromide **5** gave the disaccharide **30** (64%) that was deprotected by transesterification.

The reaction products were characterized by fully assigned ^{1}H and ^{13}C NMR spectra, and anomeric configurations of newly formed glycosidic linkages were established by measurement of anomeric $^1J_{C,H}$ coupling constants[16]. The assignment and chemical shifts for the reported structures are recorded in Tables II–VI (Experimental section).

TABLE I

Direct competitive inhibition by disaccharide haptens of enzyme–polysaccharide antigen binding to the IgM monoclonal antibody GC-4 [17]

Inhibitor	Hapten concentration at 50% inhibition (μmol·L^{-1})	Relative potency (%)	$\Delta(\Delta G^{\circ})$ (kcal·mol^{-1})
BCDA			
α-L-Rha-(1 → 3)-α-L-Rha-(1 → 3)-β-D-GlcNAc-(1 → 2)-α-L-Rha-(1 → O)-Me [18]	53	100	0
CDA			
α-L-Rha-(1 → 3)-β-D-GlcNAc-(1 → 2)-α-L-Rha-(1 → O)-Me [19]	99	54	0.4
BCD			
α-L-Rha-(1 → 3)-α-L-Rha-(1 → 3)-β-D-GlcNAc-(1 → O)-Me	35	151	−0.3
CD			
α-L-Rha-(1 → 3)-β-D-GlcNAc-(1 → O)-Me	60	88	0.1
α-L-Rha-(1 → 3)-β-D-Glc-(1 → O)-Me [20]	1518	> 3.0	> 1.9
α-L-2-deoxy-Rha-(1 → 3)-β-D-GlcNAc-(1 → O)-Me [19]	49	108	−0.1
α-L-4-deoxy-Rha-(1 → 3)-β-D-GlcNAc-(1 → O)-Me	117	45	0.4
α-L-Rha-(1 → 3)-6-deoxy-β-D-GlcNAc-(1 → O)-Me	795	7	1.5
α-L-Rha-(1 → 3)-4-deoxy-β-D-GlcNAc-(1 → O)-Me	19400	0.3	3.4
α-L-Rha-(1 → 3)-β-D-GlcNHCOCF$_3$-(1 → O)-Me	86	62	0.3
α-L-Rha-(1 → 3)-β-D-GlcNHCOEt-(1 → O)-Me	17	300	−0.7
α-L-Rha-(1 → 3)-β-D-GlcNHCOtBu-(1 → O)-Me	115	46	0.4
α-L-Rha-(1 → 3)-β-D-GlcNH$_2$-(1 → O)-Me	inactive		

Disaccharides were relatively weak inhibitors of the antibody SYA/J6 binding to polysaccharide antigen, but the apparently smaller binding site of the IgM antibody, GC-4, rendered it a suitable candidate to evaluate the synthetic inhibitors described here. The relative potency of disaccharide inhibitors is related to a tetrasaccharide, methyl glycoside BCDA[18] (Table I). The trisaccharide CDA[19] was only 0.4 kcal/mol less active than the tetrasaccharide BCDA, indicating that the relative free-energy of binding for the pyranose residue B was less than 0.5 kcal/mol. A conclusion that was confirmed by the activity of trisaccharide BCD relative to disaccharide CD. Since the disaccharide CD binds ~ 0.3 kcal/mol more tightly the trisaccharide CDA, it is concluded that the residue A may actually make some unfavourable contacts at the periphery of the binding site. The data therefore suggest that the disaccharide CD is a minimal epitope. It may be further concluded from the inactivity of the 4- and 6-monodeoxy GlcNAc disaccharides that both the 4-hydroxyl and 6-hydroxyl groups of the GlcNAc residue are essential for binding and are likely hydrogen bonded to protein. The inactivity of the Rha-(1 → 3)-GlcOMe[20] glycoside suggests that the acetamido function is also essential for binding, which is confirmed by the inactivity of the amino derivative of CD. Substitution of the *N*-acetyl residue by *N*-trifluoacetyl, *N*-propionyl, and

N-pivaloyl indicates that the polarity and size of this residue influences binding. The propionyl group appears to be accommodated within the binding site and makes productive contacts, whereas the bulky *tert*-butyl residue cannot be readily accommodated. Therefore, the epitope recognized by the GC4 antibody is estimated to correspond to a di- or tri-saccharide with principal polar contacts involving the GlcNAc D residue. In the rhamnose C residue only the 4-hydroxyl group appears to be important for complex formation, and its absence results in a small 0.4 kcal/mol loss of binding energy relative to the native disaccharide. Further evaluation of these compounds with the $IgG_3\kappa$ antibody, SYA/J6, as well as the activities of more elaborate structures built from them will be described in a subsequent paper.

EXPERIMENTAL

General methods.—Optical rotations were measured with a Perkin–Elmer 243 polarimeter, and melting points are uncorrected. TLC was performed on Silica Gel-60 F_{254} (E. Merck) and detected with UV light and charring with H_2SO_4. Silica Gel-60, 70–230 mesh or 230–400 mesh (E. Merck), was used for conventional column chromatography and medium-pressure column chromatography, respectively. Solvents were purified and dried according to standard procedures[21], and organic solutions were dried over Na_2SO_4 and concentrated at 40°C under reduced pressure. ^1H and ^{13}C NMR spectra were recorded at 300 K with Bruker AM 200 and AM 500 spectrometers for solutions in: $CDCl_3$ (internal standard, for ^1H: residual $CHCl_3$ δ 7.24; for ^{13}C: $CDCl_3$ δ 77.0), Me_2SO-d_6 [internal standard, for ^1H: residual Me_2SO δ 2.50, for ^{13}C: $(CD_3)_2SO$ δ 39.50], CD_3OD (internal standard, for ^1H: residual CH_3OD δ 3.31, for ^{13}C: CD_3OD δ 49.00) and D_2O (internal 1.0% acetone δ_H 2.225, δ_c 31.07). Carbon chemical shifts and first-order proton chemical shifts and coupling constants were obtained from one-dimensional ^{13}C and ^1H NMR spectra. Assignments of proton and carbon resonances were based on COSY and ^1H–^{13}C correlated HMQC experiments. The ^1H–^{13}C correlated HMQC experiments were carried out without carbon decoupling, permitting the measurement of the C–H coupling constants for the anomeric carbons in the F-2 dimension.

NMR data are given in Table II for glucopyranose monosaccharide derivatives, in Table III for rhamnose monosaccharide derivatives, and in Tables IV, V (^1H) and VI (^{13}C) for disaccharides.

Methyl 2-amino-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**2**).—A solution of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[22] (10 g, 30.9 mmol) in 4 M KOH in 95% EtOH (300 mL) was refluxed for 2 h and allowed to cool to room temperature. The solution was diluted with CH_2Cl_2 (500 mL) and washed with water until the pH of the aqueous layer became neutral (pH paper). The aqueous washings were re-extracted once with CH_2Cl_2 (200 mL), and the combined organic phases were dried and concentrated. Chromatography (10:1

TABLE II

^{1}H NMR data for the GlcNAc monosaccharide derivatives **2**, **3**, **4**, **28**, and **29**

Protons (J Hz)	**2** [a]	**3** [b]	**4** [b]	**28** [c]	**29** [c]
H-1	4.17	4.48	4.34	4.51	4.50
($J_{1,2}$)	(8.0)	(8.5)	(8.5)	(8.0)	(8.5)
H-2	2.75	3.63	3.31	3.3–3.5	3.57
($J_{2,3}$)	(9.0)	(~9.0)			(~9.0)
H-3	3.4–3.6	3.71	3.55	3.90	4.0
($J_{3,4}$)	(9.0)	(9.0)	(9.0)	(~9.0)	(~8.5)
H-4	3.4–3.6	3.49	3.43	4.95	4.90
($J_{4,5}$)		(9.0)	(9.0)	(9.0)	(9.0)
H-5	3.4–3.6	3.35	3.30	3.69	3.70
H-6	3.77	3.76	3.73	3.3–3.5	1.24
($J_{5,6}$)	(10.0)	(10.0)	(10.0)		(6.0)
H-6′	4.31	4.23	4.21	3.3–3.5	
($J_{5,6'}$; $J_{6,6'}$)	(4.5, 10.0)	(5.0, 10.0)	(5.0, 10.0)		
OCH_3	3.50	3.35	3.36	3.42	3.46
NHR		9.33			6.33
(2, NH)		(10.0)			(6.5)
$NHCOCH_3$				1.90	1.95
CHPh	5.42	5.62	5.60		
CH_2Ph			5.05		

[a] In $CDCl_3$. [b] In $(CD_3)_2SO$. [c] In 3:1 $CDCl_3$–CD_3OD.

$CHCl_3$–MeOH) of the residue gave pure **2** (7.4 g, 85%) as an amorphous white solid; $[\alpha]_D^{25}$ −73.9° (*c* 1.1, Me_2SO). Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.8; H, 6.8; N, 5.0. Found: C, 59.5; H, 6.8; N, 4.9.

*Methyl 4,6-O-benzylidene-2-deoxy-2-trifluoroacetamido-β-*D*-glucopyranoside* (**3**). —A solution of the amino glycoside **2** (100 mg, 0.35 mmol) in anhyd pyridine (9 mL) containing triethylamine (1 mL) and powdered 4A molecular sieves (700 mg) was stirred for 1 h under N_2 at 0°C. Trifluoroacetic anhydride (52 μL, 1 mol equiv) was then added to the mixture which was allowed to reach 10°C in 7 h. After cooling to 0°C more anhydride was added (10 μL, 0.2 mol equiv), and the mixture was allowed to warm to room temperature overnight. Solids were filtered off, rinsed 4:1 $CHCl_3$–MeOH (20 mL), and the combined filtrate and washings were concentrated to dryness. The residue was dissolved in EtOAc (30 mL), washed successively with M HCl (20 mL), satd aq $NaHCO_3$ (20 mL), satd aq NaCl (20 mL), and dried. Concentration gave **3** as a white amorphous powder (114 mg, 86%) that was isolated from diethyl ether; $[\alpha]_D^{25}$ −86.4° (*c* 0.6, MeOH). Anal. Calcd for $C_{16}H_{18}F_3NO_6$: C, 50.9; H, 4.8; N, 3.7. Found: C, 51.1; H, 4.8; N, 3.6.

*Methyl 2-amino-4,6,-O-benzylidene-2-*N*-benzyloxycarbonyl-2-deoxy-β-*D*-glucopyranoside* (**4**).—Benzyl chloroformate (1 mL, 1.2 mol equiv) was added to a solution of the amino compound **2** in $CHCl_3$–3% aq $KHCO_3$ (500 mL) stirred vigorously at 0°C. The mixture was allowed to reach room temperature overnight. The alcohol **4** (1.4 g, 59%) was filtered off from the crude mixture, washed successively with CH_2Cl_2 (20 mL), water (20 mL), acetone (20 mL), and dried.

TABLE III

^1H and ^{13}C NMR data [a] for 4-deoxy-α-L-*lyxo*-hexopyranoside derivatives **18–20**

	18	**19**	**20**
Proton			
(*J*, Hz)			
H-1	6.03	5.18	4.64
($J_{1,2}$)	(1.0)		
H-2	5.05	5.11	5.33
($J_{2,3}$)	(2.5)		(2.8)
H-3	5.24	5.12	4.98
H-4	1.77–1.80	1.69–1.75	1.73
H-4′	1.77–1.80	1.69–1.75	1.77
H-5	4.01	4.23	3.61
H-6	1.24	1.19	1.31
($J_{5,6}$)	(6.0)	(6.0)	(6.0)
OAc	1.99–2.12	1.94–2.07	1.99–2.15
SCH_2CH_3		2.55	2.72
SCH_2CH_3		1.22	1.26
Carbon [b]			
C-1	91.8	82.7	82.0
($J_{C,H}$)	(176)	(167)	(151)
C-2	66.5	69.5	68.8
C-3	66.5	67.3	69.7
C-4	33.0	33.7	32.9
C-5	66.8	64.8	72.6
C-6	21.0	21.0	21.2
OAc	20.8, 20.9	21.0, 20.9	20.8, 20.7
SCH_2CH_3		25.3	25.4
SCH_2CH_3		14.9	14.9

[a] In $CDCl_3$. [b] The numbers in parentheses denote the one-bond ^{13}C–^1H coupling constants (Hz) for the anomeric position of each pyranose ring.

Filtrate and washings were then transferred to a separating funnel, and the organic phase was decanted, washed with water (100 mL), dried and concentrated. More alcohol was then isolated as a white powder filtered off in $CHCl_3$ (527 mg, 22%); mp 246–247°C, $[\alpha]_D^{25}$ −40.4° (*c* 1.0, Me_2SO). Anal. Calcd for $C_{22}H_{25}NO_7$: C, 63.4; H, 6.1; N, 3.4. Found: C, 63.6; H, 6.0; N, 3.2.

Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**7**).—*Method A*. A mixture of the alcohol **1** (30 g, 93 mmol), the bromosugar **5** (58 g, 1.7 mol equiv), mercuric cyanide (35 g, 1.5 mol equiv), and activated powdered 4A molecular sieves (5 g) in anhyd CH_2Cl_2 (600 mL) was stirred at room temperature. After 4 h the solids were removed by filtration, and the filtrate was washed successively with KI solution (15%, 500 mL), satd aq $NaHCO_3$ (500 mL), and water (500 mL). The organic solution was dried and concentrated, and chromatography (10:1 EtOAc–hexanes) of the residue gave the disaccharide **7** (51.0 g, 92%), recrystallized from EtOAc–hexanes.

Method B. A mixture of the alcohol **1** (2 g, 6.3 mmol), the ethyl thiorhamnoside **6**[11,23,24] (2.54 g, 1.2 mol equiv), and powdered activated 4A molecular sieves (8 g) in

TABLE IV

^{1}H NMR data for disaccharides **7–15**

Proton (J, Hz)	**7** [a]	**8** [a]	**9** [b]	**10** [c]	**11** [c]	**12** [d]	**13** [c]	**14** [c]	**15** [c]
α-L-Rha Unit C									
H-1	4.80	4.72	4.88	4.84	4.81	4.99	4.99	4.84	4.81
($J_{1,2}$)	(1.5)	(1.5)			(1.5)	(1.0)	(2.0)	(2.0)	
H-2	5.09	5.14	5.07	3.70–3.82	3.75	3.91	4.12	3.77	3.75
($J_{2,3}$	(3.0)	(3.5)			(3.5)	(~ 2)	(3.0)	(3.0)	(2.5)
H-3	5.26	5.20	5.05	3.70–3.82	3.71	3.77	3.80	3.72	3.67
($J_{3,4}$)	(10.0)	(10.0)			(9.5)	(9.5)	(10.0)	(10.0)	(9.5)
H-4	4.92	4.90	4.74	3.42	3.43	3.49	3.49	3.43	3.40
($J_{4,5}$)	(10.0)	(10.0)	(10.0)	(10.0)	(9.5)	(9.5)	(10.0)	(10.0)	(9.5)
H-5	4.01	3.99	4.08	3.97	3.97	4.05	3.94	4.12	3.96
($J_{5,6}$)	(6.0)	(6.0)	(6.0)	(6.0)	(6.5)	(6.0)	(6.5)	(6.0)	(6.0)
H-6	0.60	0.60	0.53	1.23	1.23	1.34	1.28	1.25	1.22

β-D-GlcNAc Unit D									
H-1	4.96	4.86	4.38	4.49	4.58	4.40	4.67	4.50	4.49
($J_{1,2}$)	(8.0)	(8.0)	(8.0)	(8.5)	(8.5)	(8.0)	(9.0)	(8.5)	(8.0)
H-2	3.14	3.43	3.53	3.70–3.82	3.89	3.56	3.18	3.80	3.79
($J_{2,3}$)	(~ 9.5)	(~ 9.0)			(~ 10.0)	(~ 9.0)	(10.0)	(~ 10.0)	(~ 9.0)
H-3	4.50	4.41	3.80	3.45–3.56	3.71	3.64	3.85	3.61	3.70
($J_{3,4}$)	(~ 9.5)	(~ 9.0)			(~ 9.5)	(~ 9.5)	(9.0)	(~ 9.0)	(~ 9.0)
H-4	3.50	3.59	3.74	3.59	3.56	3.48	3.64	3.51	3.44
($J_{4,5}$)		(~ 9.0)		(10.0)	(~ 9.5)	(~ 9.5)	(~ 10.0)		
H-5	3.54	3.55	3.42	3.45–3.56	3.51	3.37	3.53	3.47	3.41
H-6	3.75	3.77	3.79	3.70–3.82	3.76	3.81	3.78	3.75	3.71
($J_{5,6}$)	(~ 10.0)	(~ 10.0)			(6.0)	(5.5)	(6.0)		
H-6′	4.34	4.36	4.26	3.92	3.95	3.99	3.96	3.93	3.90
($J_{5,6'}$; $J_{6,6'}$)	(4.5, 10.0)	(5.0, 11.0)	(5.0, 10.0)	(2.0, 12.0)	(1.5, 12.5)	(~ 1.0, 12.0)	(~ 2.0, 12.5)	(–, 12.5)	(–, 12.0)
OCH_3	3.48	3.47	3.37	3.51	3.52	3.57	3.61	3.50	3.47
NH	5.81	7.06							
($J_{2,NH}$)	(7.5)	(7.5)							
$NCOCH_3$	2.08			2.05					
NCOCHR								2.29	
$NCOCCH_3$								1.13	1.18
OCH_2Ph			4.91, 5.02						
CHPh	5.50	5.51	5.73						
OAc	1.45–1.47	1.95–2.05	1.92–2.05						

[a] In $CDCl_3$. [b] In $(CD_3)_2SO$. [c] In D_2O. [d] In CD_3O. [e] In 1:1 CD_3OD–D_2O.

TABLE V

^{1}H NMR data for disaccharides **21**–**27** and **30**, **31**

Proton (J, H_2)	**21** [a]	**22** [b]	**23** [c]	**24** [a]	**25** [a]	**26** [a]	**27** [c]	**30** [a]	**31** [c]
α-L-Rha Unit C									
H-1	4.85	4.75	4.90	4.82	4.79	4.81	4.86	4.74	4.82
($J_{1,2}$)				(~ 2.0)		(1.5)	(~ 2.0)		
H-2	4.90	3.37	3.62	5.18	5.15	5.12	3.79	5.17	3.77
($J_{2,3}$)				(3.0)	(3.5)	(3.0)	(3.0)	(3.0)	(3.0)
H-3	5.16	3.60	3.99	5.24	5.22	5.22	3.72	5.12	3.72
($J_{3,4}$; $J_{3,4'}$)		(12.0, ~ 2.0)	(12.0, ~ 2.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.5)	(10.0)
H-4	1.55–1.62	1.37	1.57	5.07	5.03	5.03	3.42	4.85	3.42
($J_{4,5}$)		(12.0)	(12.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(9.5)
H-4′	1.55–1.62	1.25	1.72						
($J_{4',4}$; $J_{4',5}$)		(12.0, ~ 2.0)	(12.0, ~ 2.0)						
H-5	4.06	3.90	4.22	4.11	4.14	3.90	3.76	3.69	4.00
($J_{5,6}$)	(6.0)	(6.0)	(6.0)	(6.5)	(6.0)	(6.0)	(6.0)	(6.0)	(6.0)
H-6	0.66	0.64	1.17	1.19	1.15	1.15	1.27	0.6	1.23

β-D-GlcNAc Unit D									
H-1	4.86	4.40	4.49	4.85	4.93	4.80	4.44	5.06	4.46
($J_{1,2}$)	(8.0)		(8.5)	(8.5)	(8.5)	(7.5)	(8.5)	(8.0)	(8.5)
H-2	3.26	3.72	3.78	3.07	3.25	2.94	3.64	3.01	3.78
($J_{2,3}$)	(~8.0)		(9.5)	(10.0)	(11.0)	(11.0)	(9.5)	(9.5)	(9.5)
H-3	4.34	3.72	3.58	4.26	4.82	4.40	3.78	4.69	3.52
($J_{3,4}$; $J_{3,4'}$)	(~8.0)				(3.4)	(11.0, 5.0)	(11.5, 5.0)	(9.5)	(9.0)
H-4	3.49	3.54	3.48	3.50	4.45	1.50	1.58	4.98	3.26
($J_{4,5}$)	(9.0)	(9.0)			(~0.0)	(12.0)	(12.0)	(9.5)	(~9.0)
H-4′						2.07	2.07		
($J_{4',4}$; $J_{4',5}$)						(12.0, ~1.0)	(12.0, –)		
H-5	3.54	3.42	3.48	3.50	3.90	3.70	3.70–3.75	3.66	3.50
H-6	3.74	3.75	3.75	3.72	3.66	3.49	3.66	1.21	1.33
($J_{5,6}$)	(9.5)	(10.0)				(4.5)		(6.0)	(6.0)
H-6′	4.33	4.22	3.94	3.79	3.66	3.58	3.70–3.75		
($J_{5,6'}$; $J_{6,6'}$)	(4.5, 9.5)	(5.0, 10.0)	(–, 12.0)	(~1.0, 11.0)		(5.5, 11.0)			
OCH_3	3.43	3.34	3.52	3.47	3.46	3.47	3.52	3.49	3.49
NH	5.94			5.72	6.01	5.73		6.14	
($J_{2,NH}$)	(8.0)			(7.0)	(7.0)	(7.0)		(7.0)	
$NHCOCH_3$	2.07	1.84	2.06	2.03	2.02	2.02	2.05	1.98	2.05
$OCOCH_3$	1.98			1.97–2.11	1.94–2.08	1.96–2.10		1.90–2.07	
CHPh	5.47	5.63							
CH_2Ph				4.57, 4.62	4.52	4.55			

[a] In $CDCl_3$. [b] In $(CD_3)_2SO$. [c] In D_2O.

TABLE VI

^{13}C NMR data [a] for disaccharides **7–11**, **13–15**, **21**, **23**, **27**, **30**, and **31**

carbon	**7** [b]	**8** [b]	**9** [b]	**10** [c]	**11** [c]	**13** [c]	**14** [c]	**15** [d]	**21** [b]	**23** [c]	**27** [c]	**30** [b]	**31** [c]
α-L-Rha Unit C													
C-1	97.6	97.8	97.7	101.6	102.3	102.2	102.2	102.8	98.7	103.0	102.5	99.6	102.3
($J_{C,H}$)	(173)	(173)	(172)	(170)	(170)	(170)	(170)	(169)	(172)	(169)	(170)	(170)	(170)
C-2	70.4	69.7	70.0	81.8	71.4	71.0	71.5	71.9	68.4	69.2	71.3	69.8	71.6
C-3	68.8	68.8	68.9	70.9	71.1	70.8	71.1	71.7	66.8	65.9	70.9	69.2	71.0
C-4	71.2	70.8	71.1	72.1	72.7	72.7	72.7	73.1	33.1	35.2	72.8	70.5	72.7
C-5	66.4	66.7	66.3	69.1	69.9	70.5	69.7	70.0	64.4	66.5	69.8	67.3	69.7
C-6	16.5	16.4	16.5	16.7	17.3	17.4	17.2	17.6	20.2	20.7	17.3	16.8	17.2
β-D-GlcNAc Unit D													
C-1	100.7	100.2	101.5	101.5	101.5	100.4	102.2	102.3	101.0	102.1	102.6	99.4	102.0
($J_{C,H}$)	(166)	(164)	(162)	(162)	(163)	(163)	(161)	(161)	(165)	(162)	(162)	(162)	(162)
C-2	59.2	58.8	58.8	55.4	56.6	55.6	56.0	56.5	58.6	56.0	56.5	59.7	56.3
C-3	75.2	74.5	75.3	76.2	82.1	82.3	82.3	82.1	75.8	82.4	78.3	79.5	82.3
C-4	80.4	80.0	79.9	70.4	69.4	69.5	69.4	69.9	80.4	69.5	34.6	75.7	74.6
C-5	66.1	66.2	66.1	68.8	76.9	76.8	76.8	77.2	66.1	76.7	73.3	69.8	72.9
C-6	68.8	68.8	68.7	61.0	61.5	61.2	61.6	62.0	68.8	61.6	64.4	17.5	17.5

[a] Chemical shifts are given in ppm, and the numbers in parentheses denote the one-bond $^{13}C-^{1}H$ coupling constants for the anomeric carbons in Hz. [b] In $CDCl_3$. [c] In D_2O. [d] In 1:1 CD_3OD-D_2O.

anhyd CH_2Cl_2 (200 mL) was stirred under N_2 overnight at room temperature. *N*-Iodosuccinimide (1.9 g, 1.3 mol equiv) and a satd solution of triflic acid in CH_2Cl_2 (0.15 M, 5.2 mL, 0.12 mol equiv) were added to the stirred mixture in the dark. After 30 min at room temperature, more triflic acid (5.2 mL) was added, and stirring was continued for another 4 h. Triethylamine (1 mL) was then added, and the mixture was filtered and concentrated. The residue, dissolved in EtOAc (250 mL), was washed successively with a 5% solution of sodium thiosulfate in aq NaOH (0.5 M, 3 × 100 mL), N HCl (3 × 100 mL), satd aq $NaHCO_3$ (100 mL), satd aq NaCl (100 mL), and dried. After concentration the disaccharide was purified by crystallization in EtOAc–hexanes (2.7 g, 71%); mp 177–180°C; $[\alpha]_D^{25}$ −49.9° (*c* 0.9, $CHCl_3$). Anal. Calcd for $C_{28}H_{37}NO_{13}$: C, 56.5; H, 6.3; N, 2.3. Found: C, 56.2; H, 6.2; N, 2.6.

*Methyl 4,6-O-benzylidene-2-deoxy-2-trifluoroacetamido-3-O-(2,3,4-tri-O-acetyl-α-*L*-rhamnopyranosyl)-β-*D*-glucopyranoside* (**8**).—A mixture of the alcohol **3** (69.6 mg, 0.18 mmol), the ethyl thioglycoside **6** (76.5 mg, 1.26 mol equiv), and activated 4A powdered molecular sieves (580 mg), in anhyd CH_2Cl_2 (6 mL) was stirred overnight under N_2 at room temperature. *N*-Iodosuccinimide (59 mg, 1.4 mol equiv) and a satd solution of triflic acid in CH_2Cl_2 (170 μL, 0.14 mol equiv) were added to the mixture that was stirred for 1 h in the dark at room temperature. Triethylamine (50 μL) was added to quench the reaction, which was worked up as described for the preparation of **7** (method B). Disaccharide **8** was then purified (114 mg, 95%) by flash chromatography (8:2 toluene–EtOAc); $[\alpha]_D^{25}$ −48.7° (*c* 1.5, $CHCl_3$). Anal. Calcd for $C_{28}H_{33}F_3NO_{13}$: C, 51.8; H, 5.1; N, 2.2; Found: C, 51.4; H, 5.2; N, 2.3.

*Methyl 2-amino-4,6-O-benzylidene-2-N-benzyloxycarbonyl-2-deoxy-3-O-(2,3,4-tri-O-acetyl-α-*L*-rhamnopyranosyl)-β-*D*-glucopyranoside* (**9**).—*Method A.* A mixture of the acceptor **4** (100 mg, 0.24 mmol), the donor **6** (120 mg, 1.50 mol equiv), mercuric bromide (170 mg, 2 mol equiv), and activated 4A powdered molecular sieves (1 g) in anhyd 1,2-dichloroethane (10 mL) was stirred for 1.5 h at room temperature. Bromine (18 μL, 1.5 mol equiv) was then added to the mixture that was stirred for 30 h under N_2 at room temperature. After adding triethylamine (100 μL) the mixture was filtered, and the filtrate washed successively with N HCl (40 mL), satd aq $NaHCO_3$ (40 mL), water (40 mL), and dried. Chromatography (8:2 toluene–EtOAc, 60 mL; 7:3, 70 mL) of the residue obtained after concentration gave the pure disaccharide **9** as a colourless glass (127 mg, 76%).

Method B. The alcohol **4** (411 mg, 0.99 mmol) was glycosylated with the ethyl thioglycoside **6** (424 mg, 1.3 mol equiv) as described for the synthesis of disaccharide **8**. Chromatography (as described above in method A) gave the pure disaccharide **9** (577 mg, 84%) as a colourless glass; $[\alpha]_D^{25}$ −50.5° (*c* 0.9, $CHCl_3$). Anal. Calcd fo $C_{34}H_{41}NO_{14}$: C, 59.4; H, 6.0; N, 2.0. Found: C, 59.2; H, 6.1; N, 1.7.

*Methyl 2-acetamido-2-deoxy-3-O-(α-*L*-rhamnopyranosyl)-β-*D*-glucopyranoside* (**10**).—A solution of the protected disaccharide **7** (5 g, 8.4 mmol) in 80% aq acetic acid (90 mL) was hydrogenolyzed for 30 h at atmospheric pressure in the presence of 10% Pd–C. Filtration and repeated coevaporation with toluene gave a solid that

was dissolved in a satd solution of methanolic ammonia (300 mL), and kept overnight at room temperature in a tightly closed flask. The mixture was evaporated and chromatography on a silica gel column using 2:1 EtOAc–MeOH as eluant gave 3.1 g (97%) of **10**; mp 284–285°C (recrystallized from EtOH); $[\alpha]_D^{25}$ −82.3° (*c* 1.0, MeOH). Anal. Calcd for $C_{51}H_{27}NO_{10}$: C, 47.2; H, 7.1; N, 3.7. Found: C, 47.1; H, 7.1; N, 3.6.

Methyl 2-deoxy-3-O-(α-L-rhamnopyranosyl)-2-trifluoroacetamido-β-D-glucopyranoside (**11**).—A solution of the disaccharide **8** (103 mg, 0.16 mmol) in methanolic NaOMe (10 mL, 0.04 M) was stirred for 2.5 h at room temperature and deionized with Amberlite IR-120 resin (H^+). The resin was filtered off and rinsed with MeOH, and the combined filtrate and washings were concentrated to dryness. The residue was dissolved at 0°C in 90% trifluoroacetic acid (15 mL), stirred for 45 min and concentrated at 20°C under high vacuum. Residual traces of acid were coevaporated with MeOH (3 × 3 mL), and flash chromatography (9:1 $CHCl_3$–MeOH, 90 mL; 85:15, 100 mL) of the residue gave the disaccharide **11** that was obtained free of silica gel by chromatography on a column of Sephadex G-15 eluted with water (44 mg, 64%); $[\alpha]_D^{25}$ −72.4° (*c* 0.4, MeOH). Anal. Calcd for $C_{15}H_{24}F_3NO_{10}$: C, 41.4; H, 5.6; N, 3.2; Found C, 41.3; H, 5.6; N, 3.4.

Methyl 2-amino-2-N-benzyloxycarbonyl-2-deoxy-3-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (**12**).—A solution of the protected disaccharide **9** in 60% acetic acid (30 mL) was heated for 45 min at 100°C and concentrated to dryness. Residual traces of acid were coevaporated with 10:1 toluene–MeOH (2 × 10 mL), and the residue was dissolved in a methanolic solution of NaOMe (0.02 M, 30 mL) and stirred 1 h at room temperature. Sodium ions were removed with Amberlite IR-120 resin (H^+), and the resin was filtered off and rinsed with MeOH (20 mL). Concentration of the methanolic solutions and flash chromatography (9:1 $CHCl_3$–MeOH, 100 mL; 85:15, 150 mL) of the residue gave the pure disaccharide **12** as a white powder (224 mg, 90%); mp 228–230°C (dec); $[\alpha]_D^{25}$ −26.5° (*c* 0.9, H_2O). Anal. Calcd for $C_{21}H_{31}NO_{11}$: C, 53.3; H, 6.6; N, 3.0. Found: C, 53.4; H, 6.3; N, 2.6.

Methyl 2-amino-2-deoxy-3-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (**13**).—A solution of the disaccharide **12** (33 mg, 0.07 mmol) in MeOH (3 mL) was hydrogenolyzed for 2 h at atmospheric pressure in the presence of 10% Pd–C (20 mg). the catalyst was filtered off, rinsed with MeOH (2 mL), and the combined filtrate and washings were concentrated to dryness. The residue was dissolved in water (1.5 mL), and the pH lowered to pH 4.2 by addition of 0.1 M HCl. After concentration, the pure disaccharide **13** was precipitated (EtOH–acetone) as the hydrochloride salt, washed (acetone, 2 × 1 mL), and dried (20 mg, 76%): $[\alpha]_D^{25}$ −31.2° (*c* 0.3, H_2O). Anal. Calcd for $C_{13}H_{26}ClNO_9$: C, 41.5; H, 7.0; N, 3.7. Found: C, 41.6; H, 7.0; N, 3.6.

Methyl 2-deoxy-2-propionamido-3-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (**14**).—Hydrogenolysis of the disaccharide **12** (51 mg, 0.10 mmol) was performed as described (compound **13**), and after filtration of the catalyst and concentration, the

intermediate amino disaccharide was dissolved in dry MeOH (3 mL), and treated 1 h at room temperature with propionic anhydride (100 μL). More anhydride (100 μL) was added, and, after an additional 15 min at room temperature, the excess reagent was destroyed by addition of water (200 μL). The solution was then passed through a column (15 × 15 mm) of Rexyn 201 resin (OH^-) that was eluted with MeOH (60 mL). The combined fractions containing the product were concentrated, and purification of the residue on a column of Bio Gel P-2 eluted with water gave **14** (35.3 mg, 82%) obtained as an amorphous white solid after freeze-drying. HRMS data: Calcd for $C_{16}H_{30}NO_{10}$: *m/z* 396.1870. Found: *m/z* 396.1877. Anal. Calcd for $C_{16}H_{29}NO_{10}$: C, 48.6; H, 7.41; N, 3.5. Found: C, 48.6; H, 7.3; N, 3.5.

*Methyl 2-amino-2-deoxy2-*N*-phthaloyl-3-*O*-(α-*L*-rhamnopyranosyl)-β-*D*-glucopyranoside* (**15**).–Hydrogenolysis of the disaccharide **12** (53 mg, 0.11 mmol) was performed as described (compound **13**) and after filtration of the catalyst and concentration, the intermediate amino disaccharide was dissolved in MeOH (2 mL). Triethylamine (500 mL) and pivaloyl chloride (200 mL) were added to the solution that was stirred at 17°C (water bath). After 1 h, more triethylamine (300 mL) and pivaloyl chloride (100 mL) were added, and the solution was stirred overnight at 18°C and concentrated. Residual triethylamine was coevaporated with MeOH (3 × 5 mL), and the residue, dissolved in MeOH (1 mL), was deionized on a column [(20 × 1.5 cm) top-half Rexyn resin 201 (OH^-) and bottom-half Amberlite IR-120 resin (H^+)]. Elution with MeOH and concentration of the effluent gave **15** that was purified on a Bio Gel P-2 column (water), and obtained as an amorphous powder (3.5 mg, 74%) upon freeze-drying: $[\alpha]_D^{25}$ −63.9° (*c* 0.4, MeOH). Anal. Calcd for $C_{18}H_{33}NO_{10}$: C, 51.0; H, 7.8; N, 3.3. Found: C, 50.5; H, 7.8; N, 3.1.

*1,2,3-Tri-*O*-acetyl-4,6-dideoxy-α-*L*-lyxo-hexopyranoside* (**18**).—A solution of the acetal **16**[12] (8.0 g; 39.5 mmol) in 80% acetic acid (250 mL) was heated for 30 min at 80°C and concentrated to dryness. The resulting diol **17** was dissolved in a 2:5 glacial acetic acid–acetic anhydride (700 mL), and a solution of H_2SO_4 in glacial acetic acid (0.47 M, 25 mL) was added dropwise to the mixture stirred at 20°C (water bath). After a total time of 1 h at 20°C, the mixture was poured slowly into ice-cold satd aq $NaHCO_3$ (1 L), and extracted with EtOAc (2 × 300 mL). The organic phase was washed with satd aq NaCl (300 mL), dried, and concentrated. Chromatography of the residue (4:1 hexanes–EtOAc) gave the pure peracetate **18** (8.1 g, 75%) that crystallized on standing; mp 70.5–72.5°C; $[\alpha]_D^{25}$ −58.1° (*c* 0.7, $CHCl_3$). Anal. Calcd for $C_{12}H_{18}O_7$: C, 52.5; H, 6.6. Found: C, 52.1; H, 6.6. [lit.[25] mp 73–74°C; $[\alpha]_D^{20}$ + 20° (*c* 3.4, chloroform)]

*Ethyl 2,3-di-*O*-acetyl-4,6-dideoxy-1-thio-α-*L*-lyxo-hexopyranoside* (**19**) *and ethyl 2,3-di-*O*-acetyl-4,6-dideoxy-1-thio-β-*L*-lyxo-hexopyranoside* (**20**).—The triacetate **18** (7.8 g, 28.4 mmol) was dissolved in dry CH_2Cl_2 (500 mL) and treated for 45 min at room temperature with ethanethiol (2.8 mL, 1.3 mol equiv) and $BF_3 \cdot Et_2O$ (4.2 mL, 1.2 mol equiv). Triethylamine (8.5 mL) was added slowly to the solution that was subsequently concentrated to dryness. The residue, dissolved in EtOAc (300

mL), was washed with satd aq $NaHCO_3$ (2 × 300 mL), dried, and concentrated. Chromatography (4:1 hexanes–EtOAc) of the resulting syrup gave the ethyl α-thioglycoside **19** (5.3 g, 67%) as a syrup; $[\alpha]_D^{25}$ − 139.3° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{12}H_{20}O_5S$: C, 52.2; H, 7.3. Found: C, 51.8; H, 7.4. Further elution gave the β anomer, **20** (1.2 g, 16%) as a pure product that crystallized on standing; mp 104°C; $[\alpha]_D^{25}$ + 99.9° (*c* 1.0, $CHCl_3$). Anal. Found: C, 52.5; H, 7.5.

*Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3-di-O-acetyl-4,6-dideoxy-α-*L*-lyxo-hexopyranosyl)-β-*D*-glucopyranoside* (**21**).—The acceptor **1** (1 g, 3.1 mmol) and the ethyl thioglycoside **19** (1 g, 1.2 mol equiv) were condensed as described for the preparation of **8**. Workup of the reaction, as described, gave the disaccharide **21** (1.35 g, 80%) isolated from EtOAc as a white powder; mp 190–194°C (dec); $[\alpha]_D^{25}$ −52.2° (*c* 0.7, $CHCl_3$). Anal. Calcd for: $C_{26}H_{35}NO_{11}$: C, 58.1; H, 6.6; N, 2.6. Found: C, 58.3; H, 6.8; N, 2.8.

*Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(4,6-dideoxy-α-*L*-lyxo-hexopyranosyl)-β-*D*-glucopyranoside* (**22**).—A solution of the disaccharide **21** (1.25 g, 2.3 mmol) in methanolic NaOMe (0.02 N, 120 mL) was stirred overnight at room temperature, deionized with Amberlite IR-120 resin (H^+) and concentrated to dryness. The diol **22** was isolated as a white powder from EtOAc–petroleum ether (950 mg, 90%); mp 256–260°C (dec); $[\alpha]_D^{25}$ −100.1° (*c* 0.8, Me_2SO). Anal. Calcd for $C_{22}H_{31}NO_9$: C, 58.2; H, 6.9; N, 3.1. Found: C, 58.1; H, 6.8; N, 3.2.

*Methyl 2-acetamido-2-deoxy-3-O-(4,6-dideoxy-α-*L*-lyxo-hexopyranosyl)-β-*D*-glucopyranoside* (**23**).—A solution of the diol **22** (102 mg, 0.22 mmol) in 90% acetic acid (10 mL) containing Pd–C (10%, 100 mg) was hydrogenolyzed (60 psi) overnight at room temperature. The catalyst as filtered off, washed with 90% acetic acid, and the combined filtrates were concentrated to dryness, residual traces of acid being coevaporated with water (2 × 2 mL). The disaccharide **23** was isolated (64 mg, 78%) as a pure white powder that was centrifuged from EtOH, washed with acetone (1 mL), and dried; $[\alpha]_D^{25}$ −91.4° (*c* 0.6, H_2O). Anal. Calcd for $C_{15}H_{27}NO_9$: C, 49.3; H, 7.4; N, 3.8. Found: C, 49.2; H, 7.5; N, 3.6.

*Methyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(2,3,4-tri-O-acetyl-α-*L*-rhamno-pyranosyl)-β-*D*-glucopyranoside* (**24**).—A satd solution of HCl in ether was added dropwise to a stirred mixture of disaccharide **7** (2.57 g, 4.3 mmol), sodium cyanoborohydride (2.59 g, 41 mmol), and 3A molecular sieves (8 g) in anhyd THF (70 mL) at 0°C. After the mixture became acidic (as determined with pH paper), the addition was continued very slowly during 15 min, and the reaction was allowed to proceed 1 h at 0°C and 1.5 h at room temperature. Solids were filtered off, rinsed with EtOAc (100 mL), and the combined organic solutions were washed with satd aq $NaHCO_3$ (3 × 300 mL) and water (200 mL), dried and concentrated. Chromatography (40:1 chloroform–$CHCl_3$–MeOH) of the residue gave the pure alcohol **24** (2.3 g, 88%) as a colourless glass; $[\alpha]_D^{25}$ −8.6° (*c* 0.8, $CHCl_3$). Anal. Calcd for $C_{28}H_{39}NO_{13}$: C, 56.3; H, 6.6; N, 2.3. Found: C, 55.9, H, 6.4; N, 2.8.

*Methyl 2-acetamido-6-O-benzyl-4-chloro-2,4-dideoxy-3-O-(2,3,4-tri-O-acetyl-α-*L*-rhamnopyranosyl)-β-*D*-galactopyranoside* (**25**).—Anhydrous pyridine (10 mL) and

sulfuryl chloride (520 mL, 2 mol equiv) were added to a solution of **24** in anhyd CH_2Cl_2 and stirred at 0°C. More sulfuryl chloride (1.04 mL, 4 mol equiv) was added every hour in four portions, and the mixture was stirred for 3.5 days at 0°C and 1 h at room temperature. The solution was diluted with CH_2Cl_2 (100 mL), washed successively with aq HCl (1 M, 3 × 100 mL), satd aq $NaHCO_3$ (2 × 100 mL), water (100 mL), and dried. Concentration and flash chromatography (4:1 EtOAc–hexanes) of the residue gave the pure disaccharide **25** (1.25 g, 62%) as a colourless glass; $[\alpha]_D^{25}$ + 15.7° (*c* 0.7, $CHCl_3$). Anal. Calcd for $C_{28}H_{38}ClNO_{12}$: C, 54.6; H, 6.2; N, 2.3. Found: C, 54.5; H, 6.2; N, 2.0.

Methyl 2-acetamido-6-O-benzyl-2,4-dideoxy-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-xylo-hexopyranoside (**26**).—Tributyltin hydride (680 mL, 1.3 mol equiv) and α,α′-azo-bisisobutyronitrile (70 mg) were added to a solution of the disaccharide **24** in toluene (90 mL) that was at reflux under N_2. After 1.5 h, more hydride (500 mL, 0.9 mol equiv) and catalyst (40 mg) were added to the mixture that was kept at reflux for 4.5 h, during which time more catalyst (20 mg) was added in two portions. The solution was allowed to cool to room temperature and was concentrated to an oily residue that was diluted in acetonitrile (200 mL), washed with hexanes (5 × 100 mL), and concentrated. Flash chromatography (4:1 EtOAc–hexanes) of the dry residue gave the pure disaccharide **26** (847 mg, 75%) as a colourless glass; $[\alpha]_D^{25}$ −20.0° (*c* 0.4, $CHCl_3$). Anal. Calcd for $C_{28}H_{39}NO_{12}$: C, 57.8; H, 6.8; N, 2.4. Found: C, 58.1; H, 6.6; N, 2.2.

Methyl 2-acetamido-2,4-dideoxy-3-O-(α-L-rhamnopyranosyl)-β-D-xylo-hexopyranoside (**27**).—The disaccharide **26** (50 mg, 0.09 mmol) was deacetylated under the same conditions used to prepare **22**. The isolated triol was dissolved in MeOH (3 mL) and hydrogenolyzed (60 psi) overnight in the presence of 10% Pd–C (17 mg). Filtration, concentration of the filtrate, and gel chromatography (Bio Gel P-2, eluted with water) of the residue gave the pure disaccharide **27** (26 mg, 82%) obtained as a white amorphous powder by freeze-drying; $[\alpha]_D^{25}$ −61.3° (*c* 0.4, MeOH). HRMS data: Calcd for $C_{15}H_{28}NO_9$: *m/z* 366.1764. Found: *m/z* 366.1740. Anal. Calcd for $C_{15}H_{27}NO_9$: C, 49.3; H, 7.4; N, 3.8. Found: C, 49.1; H, 7.6; N, 3.9.

Methyl 2-acetamido-4-O-benzoyl-2,6-dideoxy-6-bromo-β-D-glucopyranoside (**28**).—A mixture of the benzylidene acetal **1** (10 g, 31 mmol), *N*-bromosuccinimide (11 g, 2 mol equiv), and $BaCO_3$ (15 g, 2.5 mol equiv) in CCl_4 (800 mL) was refluxed for 2.5 h and then evaporated. The solid residue was dissolved in CH_2Cl_2 (500 mL) and filtered. The filtrate was washed with $KHCO_3$, followed by water, and dried. Filtration, concentration, and purification over silica gel (10:1 EtOAc–MeOH) gave the benzoate **28** (4.8 g, 39%) that crystallized on standing; mp 106–108°C; $[\alpha]_D^{25}$ −34.3° (*c* 0.9, $CHCl_3$). Anal. Calcd for $C_{16}H_{20}BrNO_6$: C, 47.8; H, 5.0; N, 3.5. Found: C, 47.5; H, 5.0; N, 3.7.

Methyl 2-acetamido-4-O-benzoyl-2,6-dideoxy-β-D-glucopyranoside (**29**).—A solution of compound **28** (4.6 g, 11 mmol) in EtOH (99%, 125 mL) containing triethylamine (2 mL) was hydrogenated for 7 days at atmospheric pressure in the presence of 10% Pd–C. The mixture was filtered, evaporated, and purified on

silica gel (1:1 EtOAc–hexanes, followed by EtOAc) to give **29** (2.6 g, 70%) that crystallized on standing; mp 102–105°C; $[\alpha]_D^{25}$ −45.0° (*c* 0.8, $CHCl_3$). Anal. Calcd for $C_{16}H_{21}NO_6$: C, 59.4; H, 6.5; N, 4.3. Found: C, 59.5; H, 6.7; N, 4.1.

Methyl 2-acetamido-4-O-benzoyl-2,6-dideoxy-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**30**).—A mixture of compound **29** (0.59 g, 1.83 mmol), rhamnosyl bromide **5** (0.95 g, 1.5 mol equiv), and mercuric cyanide (0.68 g, 1.5 mol equiv) in dry CH_2Cl_2 (25 mL) containing 3A molecular sieves (0.5 g) was stirred overnight at room temperature. The mixture was then filtered, and the solid was washed with CH_2Cl_2 (50 mL). The combined CH_2Cl_2 solutions were washed with a 15% solution of KI, followed by water, dried ($MgSO_4$), filtered, and evaporated. Chromatography (2:1 EtOAc–hexanes) of the residue gave the pure disaccharide **30** (700 mg, 64%) as a white powder; mp 211–213°C; $[\alpha]_D^{25}$ −2.27° (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{28}H_{37}NO_{13}$: C, 56.6; H, 6.3; N, 2.4. Found: C, 56.4 H, 6.2; N, 2.3.

Methyl 2-acetamido-3-O-(α-L-rhamnopyranosyl)-2,6-dideoxy-β-D-glucopyranoside (**31**).—Disaccharide **30** (280 mg, 0.44 mmol) was deacylated as described for the preparation of diol **22**. The deprotected disaccharide **31** was obtained as a white powder (138 mg, 80%); mp 250–253°C; $[\alpha]_D^{25}$ −86.0° (*c* 0.8, MeOH). *Anal.* Calcd for $C_{15}H_{27}NO_9$: C, 49.3; H, 7.4; N, 3.8. Found: C, 49.2; H, 7.3; N, 3.6.

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