Bioorganic & Medicinal Chemistry Vol. 1, No. 4, pp. 237-257, 1993
Printed in Great Britain

0968-0896/93 $6.00 + .00

Synthesis of Di- to Penta-Saccharides Related to the O-Specific Polysaccharide of *Shigella Dysenteriae* Type 1, and Their Nuclear Magnetic Resonance Study[1]

Vince Pozsgay,[a,b]* Bruce Coxon[c] and Herman Yeh[d]

[a]*Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, U.S.A.*

[b]*Laboratory of Medicinal Chemistry, National Institute of Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, U.S.A.*

[c]*Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, U.S.A.*

[d]*Laboratory of Analytical Chemistry, National Institute of Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, U.S.A.*

(*Received* 20 *May* 1993; *accepted* 15 *June* 1993)

Abstract—The syntheses of oligosaccharide fragments of the O-specific polysaccharide of the lipopolysaccharide of *Shigella dysenteriae* type 1 are described, including disaccharides methyl *O*-α-D-mannopyranosyl-(1→2)-α-D-galactopyranoside (**1**), and methyl *O*-(2-deoxy-2-propionamido-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**2**), trisaccharide methyl *O*-α-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**3**), tetrasaccharide methyl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamno-pyranoside (**4**), and pentasaccharide methyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**5**). The following monosaccharide building blocks were used as starting compounds: methyl 6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (**8**), methyl 3,4,6-tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (**11**), methyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α-D-glucopyranoside (**16**), methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside (**18**), methyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**21**), methyl 2,3,4-tri-*O*-benzoyl-1-thio-α-L-rhamnopyranoside (**22**), 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide (**23**), and methyl 4-*O*-benzyl-α-L-rhamnopyranoside (**24**). Nuclear magnetic resonance data indicate that oligosaccharides **4** and **5** partially mimic the conformation of the O-specific polysaccharide of *S. dys.* type 1.

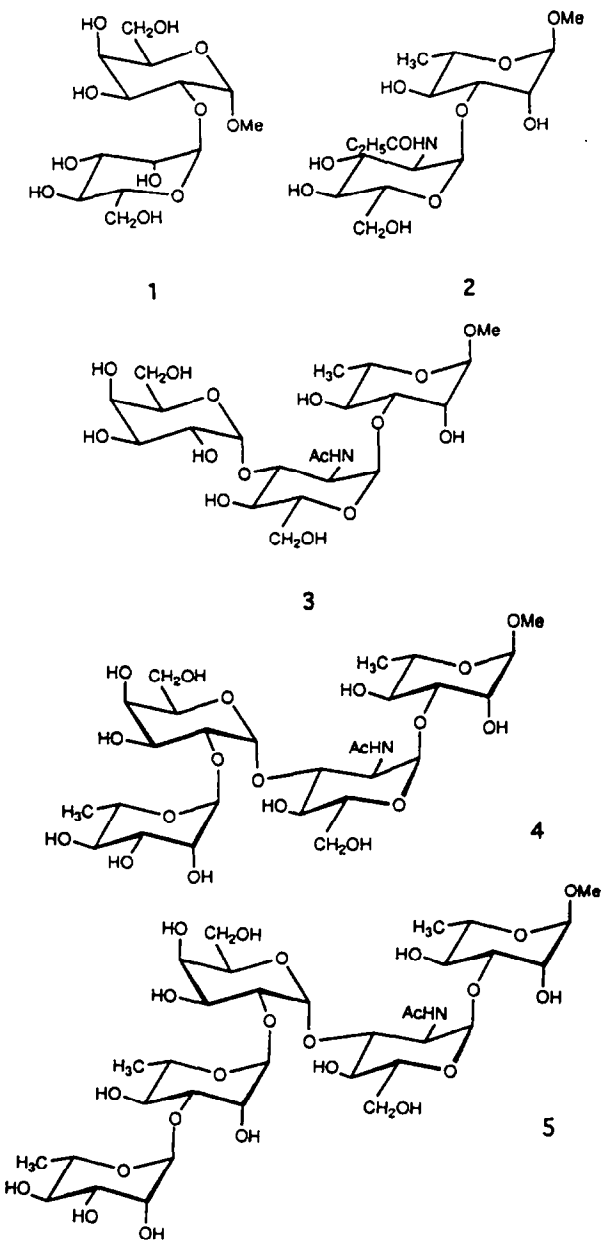
Shigella dysenteriae type 1 is a major cause of dysentery and has the potential for causing catastrophic public health problems in the developing countries.[2,3] The emergence of increasing resistance of *S. dys.* type 1 to antimicrobial drugs necessitates the exploration of other medical approaches for control of diseases caused by this pathogen.[4] An alternative option to prevent shigellosis could be vaccination, but there is as yet no licensed vaccine for *S. dys.* type 1. The hypothesis of Robbins *et al.*[5] that serum antibodies to the *O*-specific polysaccharide (*O*-SP) part of the lipopolysaccharide (LPS) of *S. dys.* type 1 can provide protective immunity against shigellae in humans has been the impetus for synthesizing oligosaccharide fragments of the O-specific polysaccharide of this pathogen.[6] The O-SP of *S. dys.* type 1 is a regular heteropolysaccharide, made up of the linear tetrasaccharide repeating unit **I** which contains α-linked L-rhamnose, *N*-acetyl-D-glucosamine and D-galactose residues.[7]

3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→

I

As a part of a program aimed at the development of a human vaccine against shigellosis, we describe the synthesis[8] of several oligosaccharides related to the O-SP.[9] These oligosaccharides (haptens[10]) can be used for the systematic characterization of the non-covalent interactions[11] between the O-specific polysaccharide epitope[10] and binding sites (paratopes[10]) of monoclonal antibodies raised against *S. dys.* type 1.[12] As an extension of this project, these oligosaccharides are being used in NMR spectroscopic studies to define fragments that express conformational features of the native O-SP. A synthetic fragment having modularity[10] that is judged to be similar to that represented by the native O-SP will be chosen for the preparation of an artificial, conjugate vaccine. Such a synthetic vaccine may be an alternative to vaccines based on the native, bacterial polysaccharides, and the exploration of this hypothesis is currently underway in our laboratories. In a preliminary communication,[1] we outlined the synthesis of fragments of the native O-SP. We now describe details of the syntheses of di- to penta-saccharide methyl glycosides **1–5** related to the O-SP of *S. dys.* type 1 and note some characteristics of the NMR

spectra of these compounds, for which we present complete assignments obtained through the use of a combination of one- and two-dimensional NMR techniques.



Results and Discussion

Synthesis

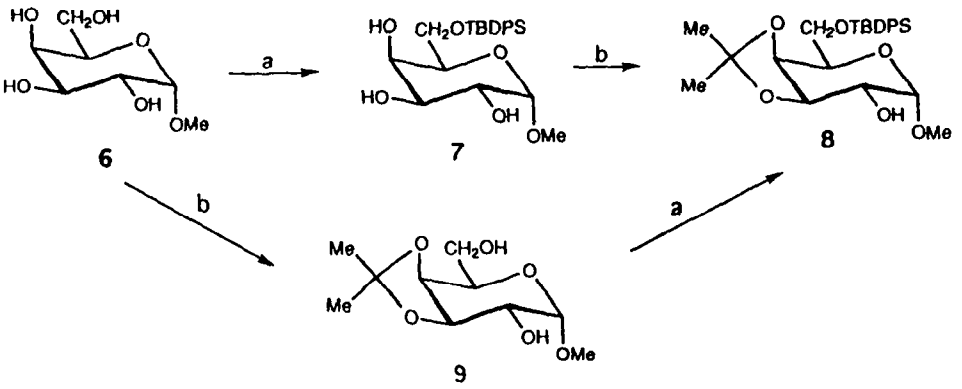
Compounds **1**–**4** were constructed in a stepwise manner from monosaccharide intermediates. In this approach, the sugar chain is extended gradually by one monosaccharide residue at a time, starting at the "reducing end". Pentasaccharide **5** was prepared in a block synthetic scheme using a rhamnobiose donor, and an α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p* trisaccharide acceptor.

Synthesis of the monosaccharide intermediates

D-Galactose units. The starting compound for the galactose acceptor **8** was methyl α-D-galactopyranoside **6** (Scheme I). Treatment of **6** with *tert*-butyldiphenylsilyl chloride[13] (TBDPSCl) afforded the silyl ether **7** (63 %) which upon reaction with 2,2-dimethoxypropane[14] gave compound **8**. An alternative sequence, involving the preparation of the isopropylidene derivative[15] **9** followed by silylation with TBDPSCl afforded **8** in 95 % yield. We have shown that the *O*-acetylated[9b] and *O*-benzoylated derivatives[9a] of the triol[1,9a,9b] **10** are excellent precursors for the construction of the α-galactosyl linkage, using methyl trifluoromethanesulfonate[16] (MeOTf) as the promoter.[17] In these donors, the 4-methoxybenzyl (MBn) group at O-2 permitted the formation of the 1,2-*cis* linkage in high yields and excellent stereoselectivity. After glycosylation, the MBn group could be selectively removed under mild oxidative conditions[18] without causing acyl migration or other untoward changes.[19] We caution that the MBn group is highly acid-sensitive. Therefore, all glycosylations involving *O*-4-methoxybenzylated donors were performed in the presence of the hindered base 2,6-di-*tert*-butyl-4-methylpyridine[20] (DTBMP). In the present study, we used the tri-*O*-benzylated derivative **11**, obtained by careful benzylation of compound **10** with benzyl bromide and sodium hydride. An excess of the reagent had to be avoided in this conversion to prevent side reactions. We chose **11** in the expectation that this donor would be more reactive than the *O*-acylated counterparts described previously.[9a,9b]

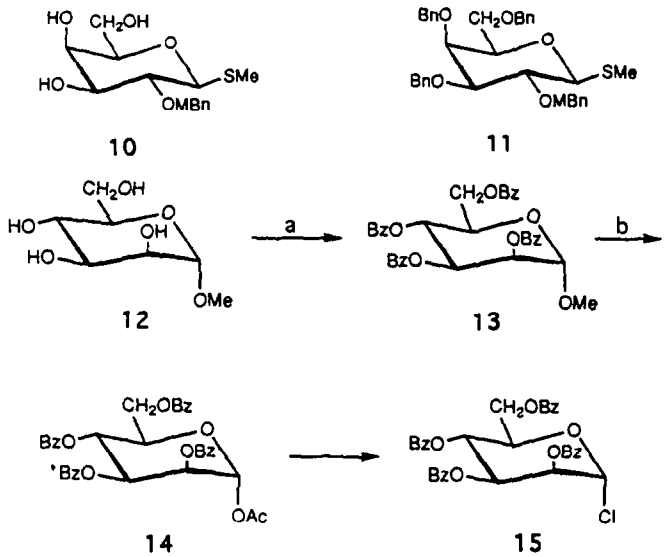
D-Mannose unit. The tetra-*O*-benzoylated mannosyl donor **15** was obtained from compound **12** (Scheme II). Routine conversions [(i) BzCl/Py →**13**, (ii) AC_2O/H_2SO_4] afforded the acetate **14** in admixture with a co-chromatographing side-product (5% or less, ^{1}H NMR), in a combined yield of 92%. Treatment of **14** with dichloromethyl methyl ether (DCMME)[21] gave the chloride **15** in 95% yield. As the catalyst in the chlorination reaction, we used the commercially available zinc chloride diethyl ether complex in methylene chloride. This catalyst avoids the complications associated with the conventional, "freshly fused $ZnCl_2$". The α anomeric configurations of compounds **14** and **15** were ascertained from the values of their $^1J_{C\text{-}1,H\text{-}1}$ coupling constants, 179 and 184 Hz, respectively.

D-Glucosamine units. We described the synthesis of the tri-*O*-acetylated azido-glucose derivatives **16** and **17** in which the non-participating azido group at C-2 allows the formation of *cis*-glycosides upon activation of the anomeric carbon atom by thiophilic reagents.[9a] We now prepared two multifunctional, 2-azido-2-deoxy-D-glucopyranosyl donors (**19** and **20**) that permit chemoselective deblocking of HO-3, i.e. the site of the chain elongation. Compound **19** was obtained from the known thioglycoside[9a] **18** by acetylation with acetic anhydride in pyridine in 88% yield. Subsequent chlorinolysis (Cl_2/CCl_4) yielded **20** in 85% yield (Scheme III). The β configuration of the anomeric carbon atom in **20** was indicated by the value $^3J_{H\text{-}1,H\text{-}2}$ 7.8 Hz.



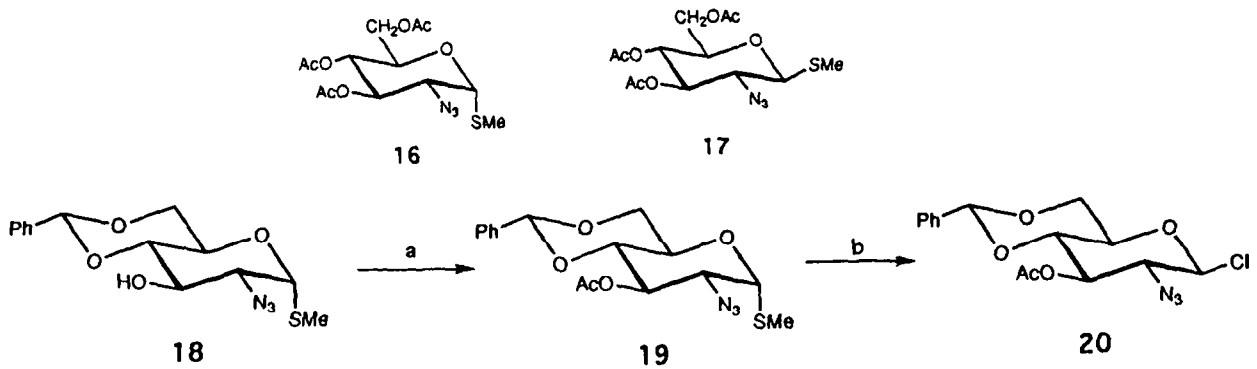
Key: (a) TBDPSCl, Py; (b) $(CH_3)_2C(OCH_3)_2$, CSA.

Scheme I.



Key: (a) BnCl, Py; (b) Ac_2O, H_2SO_4; (c) CH_3OCHCl_2, $ZnCl_2{\cdot}Et_2O$.

Scheme II.

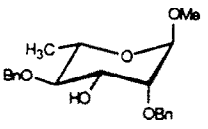


Key: (a) Ac_2O, Py; (b) Cl_2.

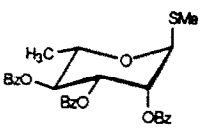
Scheme III.

L-Rhamnose units. Methyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside[22] (**21**), methyl 2,3,4-tri-*O*-benzoyl-1-thio-α-L-rhamnopyranoside[23] (**22**), and 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide[23] (**23**) were prepared as described. A starting compound for the rhamnobiose donor was the rhamnosyl acetate **29** which was prepared from the diol[24] **24**. A two-step conversion[25] (reactions a and b in Scheme IV) afforded the benzoate **26**. In the first step, a cyclic orthoester (**25**) is formed which is subsequently hydrolyzed by aqueous acetic acid. The reaction proceeded with complete regioselectivity, as predicted by the empirical rule of King and Allbutt.[26] According to this rule, hydrolysis of orthoesters formed on vicinal, *axial–equatorial* pairs of hydroxyl groups leads to products in which the *axial* hydroxyl group is acylated and the *equatorial* one is free. We found that no additional solvent is necessary for the orthoester formation, which proceeds rapidly at room temperature.[27] Removal of the alcohol formed during the orthoester formation step accelerates the conversion and suppresses side-reactions. The HO-3

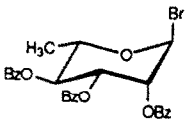
hydroxyl group in **26** was temporarily protected with a bromoacetyl group[28] (→**27**, 89%). Next, the anomeric methoxy group was replaced by an acetoxy group by controlled acetolysis using acetic anhydride and sulfuric acid. Compound **28** was obtained in anomerically pure form in 95% yield. The α configuration at C-1 was indicated by the large, $^1J_{C\text{-}1,H\text{-}1}$ coupling constant (177 Hz). The temporary, *O*-bromoacetyl group was removed by treatment with thiourea.[28] To our surprise, the reaction mixture contained both the expected compound (**29**) and the isomer **30**, as indicated by the ^1H NMR spectrum of the crude product. The extent of the *O*-benzoyl migration could be reduced by terminating the reaction *immediately* after consumption of **28** and avoiding aqueous work-up. Compound **29** could be isolated free of the isomeric impurity by chromatography, followed by crystallization in 84% yield.



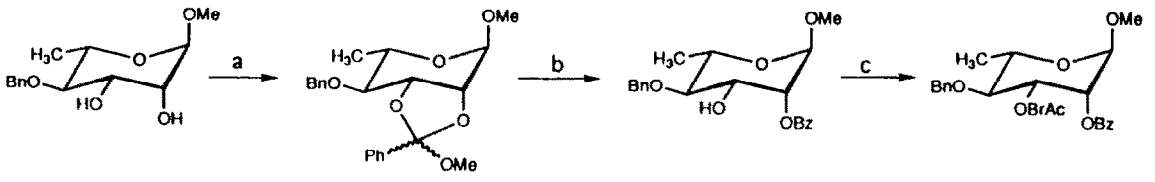
21



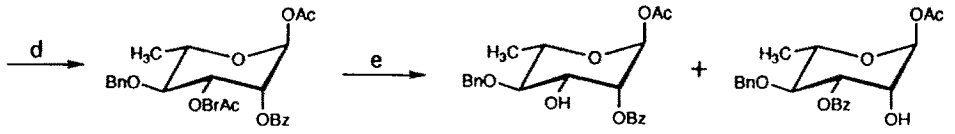
22



23



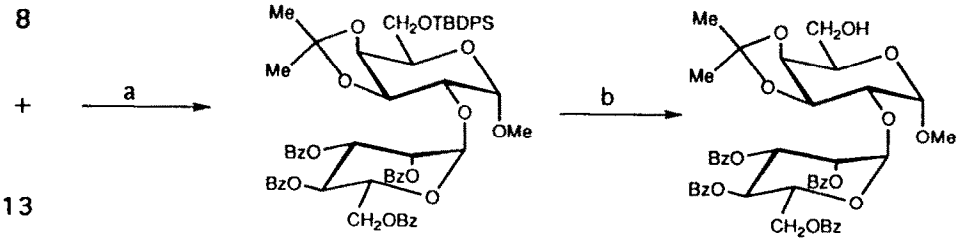
24 25 26 27



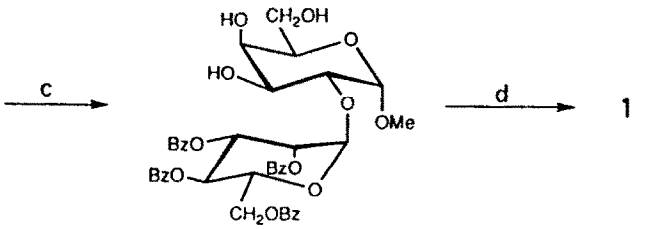
28 29 30

Key: (a) $PhC(OCH_3)_3$, CSA; (b) H_3O^+; (c) BrAcBr, Py; (d) Ac_2O, H_2SO_4; (e) $CS(NH_2)_2$.

Scheme IV.



31 32



33

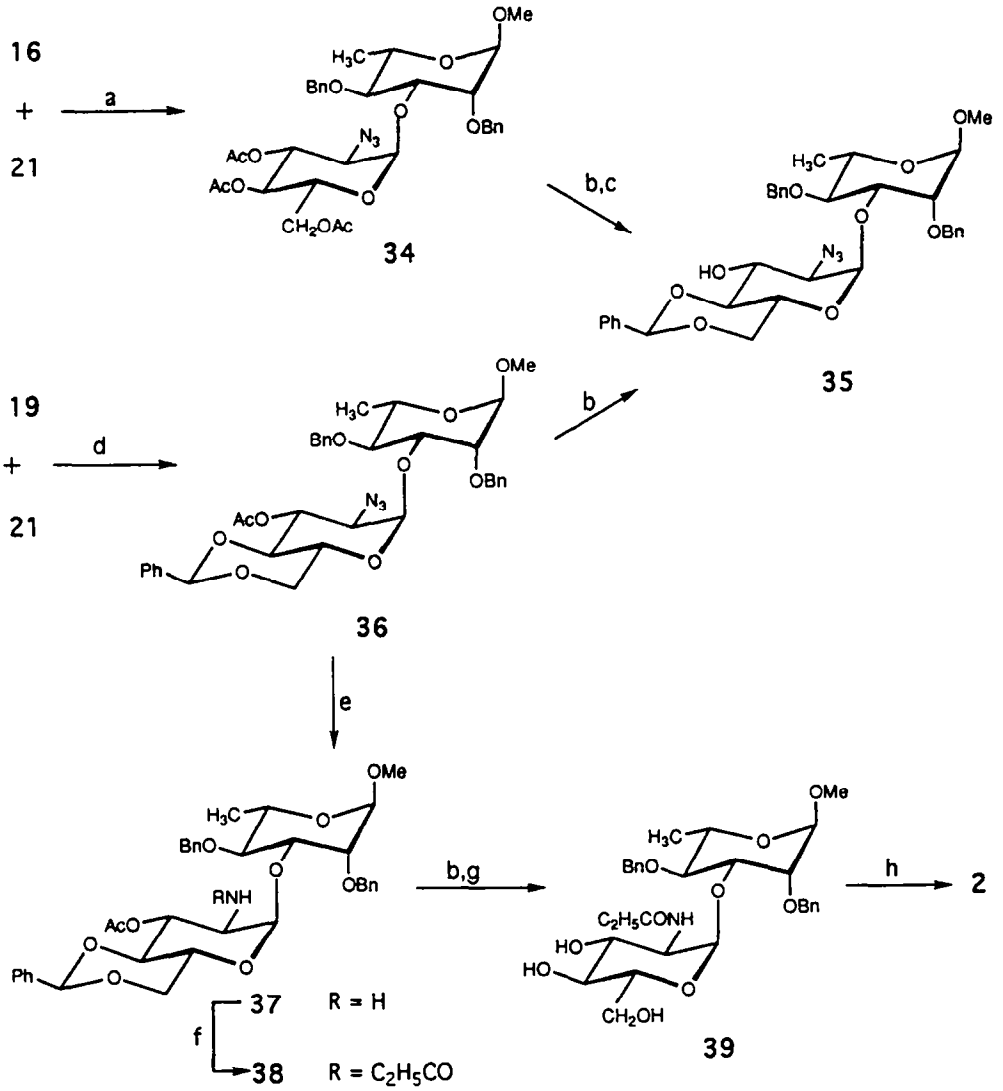
Key: (a) AgOTf, DTBMP, CH_2Cl_2; (b) Bu_4NF, THF; (c) AcOH, H_2O; (d) NaOMe, MeOH.

Scheme V.

Synthesis of the oligosaccharides

Mannosylation of the galactose acceptor **8** with chloride **15** (AgOTf, DTBMP) (Scheme V) afforded the disaccharide **31**. The 1,2-*trans* interglycosidic linkage in **31** is indicated by the $^{1}J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constant (see Experimental). Sequential removal of the protecting groups from the galactose residue [(i) Bu_4NF, →**32**, 76%, (ii) AcOH–H_2O, 85%] afforded the triol **33**. Transesterification (NaOMe/MeOH) gave the disaccharide methyl glycoside **1** (86%).

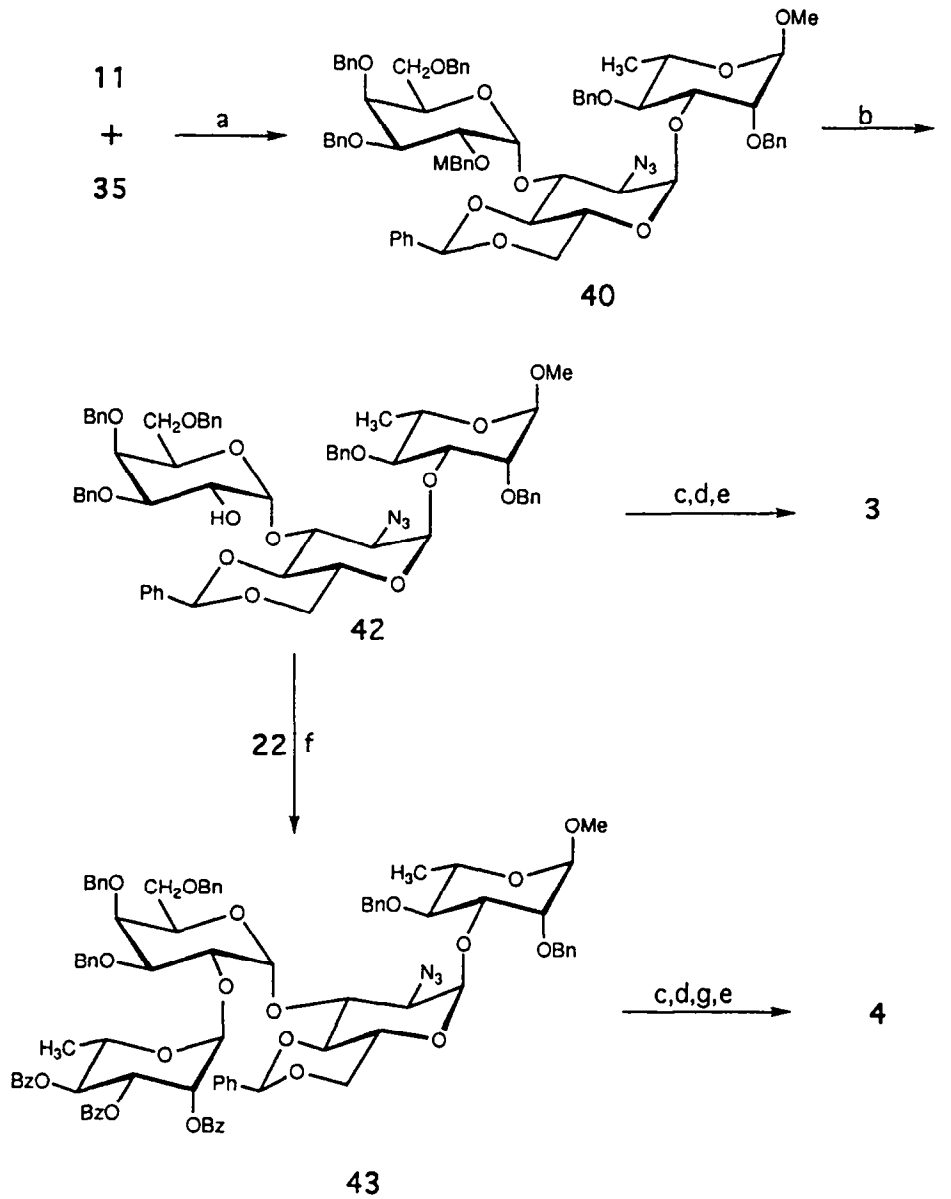
Reaction of the glucosamine donor[9a] **16** with the alcohol[22] **21** under MeOTf activation[16] afforded the disaccharide **34** in 75% yield (Scheme VI). The reaction proceeded with excellent stereoselectivity, but the glycosylation reaction was slower than desirable. Although the effect of the protecting groups on the reactivity of the HO-3 group of L-rhamnose residues in glycosylation reactions is not clear,[29] earlier successful experiments[9c] seem to give assurances on the sufficient nucleophilicity of **21**. In an effort to gain insight into the effect of the anomeric center of the glycosyl donor on the glycosylation process, reactions of **16** and its β anomer[9a] **17** with the alcohol **21** were compared. Under identical conditions (in diethyl ether, under MeOTf activation, at room temperature) reaction of the β anomer **17** with the acceptor **21** afforded the disaccharide **34** *ca.* three times faster than did the α anomer **16**.[30] Conventional transformations of **34** [(i) NaOMe, MeOH; (ii) $PhCH(OMe)_2$, H^+] afforded the benzylidene acetal **35** in which the free HO-3 group in the glucosyl residue is the site of the chain elongation. Compound **35** was also obtained by reaction of the rhamnose acceptor **21** with the thioglucoside donor **19** (→**36**, 54%) followed by deacetylation. Reduction of the intermediate **36** according to Paulsen[31] ($NiCl_2/H_3BO_3/NaBH_4$) (→**37**) followed by *N*-propionylation (propionic anhydride) afforded compound **38** which was conventionally deprotected [(i) NaOMe, MeOH; (ii) H_3O^+ →**39**, (iii) H_2/Pd-C] to give disaccharide **2**.



Key: MeOTf, DTBMP, CH_2Cl_2; (b) NaOMe, MeOH; (c) $PhCH(OMe)_2$, H^+; (d) MeOTf, CH_2Cl_2; (e) $NiCl_2/H_3BO_3/NaBH_4$; (f) $(C_2H_5CO)_2O$; (g) H_3O^+; (h) H_2/Pd-C.

Scheme VI.

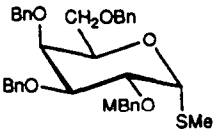
Condensation of the alcohol **35** with the galactosyl donor **11** under activation by MeOTf in diethyl ether gave trisaccharide derivative **40** in 76% yield (Scheme VII). Acid-induced decomposition was prevented by the use of DTBMP.[32] The 1,2-*cis* interglycosidic linkage in **40** is indicated by the $^{1}J_{C\text{-}1,H\text{-}1}$ one-bond, heteronuclear coupling constant for the galactose residue being 171 Hz. No *trans*-linked disaccharide could be detected in the reaction mixture. On the other hand, this glycosylation reaction was accompanied by anomerization of the glycosyl donor to the α anomer **41**. The accumulation of **41** in the reaction mixture indicates that it is less reactive than the β anomer **11**. The structure of compound **41** was established by the following: (1) NMR data confirmed the presence of the anomeric methylthio group in the α configuration ($^{3}J_{H\text{-}1,H\text{-}2}$: 5.5 Hz; $\delta_{C\text{-}1}$ 84.8 ppm; $^{1}J_{C\text{-}1,H\text{-}1}$: 166 Hz); (2) mass spectroscopic and elemental analytical data were consistent with the empirical formula of **41**. Reactivity differences between *O*-benzyl substituted anomers of thioglycosides as glycosyl donors have been noted earlier.[33] The unprecedented anomerization observed in this study may bear similarities to the acid-catalyzed anomerization of thioglycosides.[34] One-electron oxidation of compound **40** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone[18a] (DDQ) removed the 4-methoxybenzyl group to afford the trisaccharide alcohol **42** in 72% yield. A three-step conversion of compound **42** [(i) $NiCl_2/H_3BO_3/NaBH_4$; (ii) Ac_2O; (iii) H_2/Pd-C] afforded the trisaccharide methyl glycoside **3**.



Key: (a) MeOTf, DTBMP, $(C_2H_5)_2O$; (b) DDQ; (c) $NiCl_2/H_3BO_3/NaBH_4$; (d) Ac_2O; (e) H_2/Pd-C; (f) MeOTf, DTBMP, CH_2Cl_2; (g) NaOMe, MeOH.

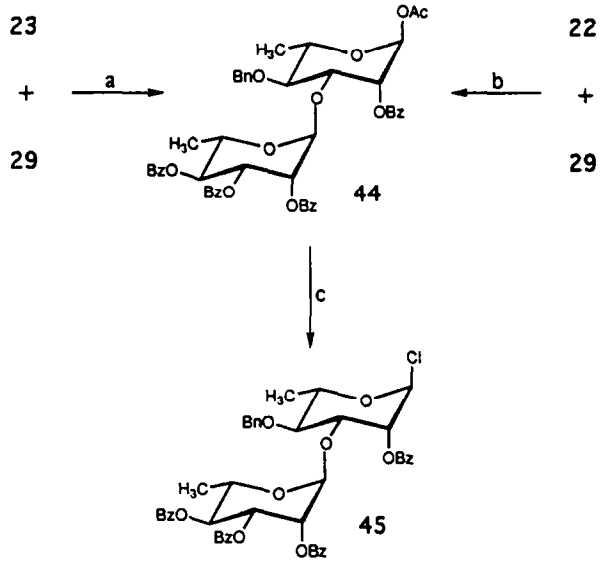
Scheme VII.

Condensation of **42** with the rhamnosyl donor **22** under promotion by MeOTf afforded the protected tetrasaccharide **45** in 94% yield. Conversion of the azido to an acetamido group[31] and removal of the *O*-protecting groups as described above afforded the tetrasaccharide methyl glycoside **4**.



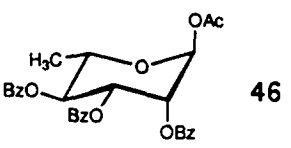
41

As noted earlier, pentasaccharide **5** was assembled in a convergent, [2+3] block scheme. The triose block (**42**) is already available. Next we describe the preparation of the rhamnobiose donor **45**. (Scheme VIII). Condensation of rhamnosyl bromide **23** with alcohol **29** (AgOTf, DTBMP) afforded the protected rhamnobiosyl acetate **44** in 93% yield. Compound **44** is a potential glycosyl donor itself, under activation by a powerful Lewis-acid, e.g. trimethylsilyl trifluoromethanesulfonate.[35] However, such glycosylations proceed under acidic conditions which may cause partial decomposition of the acetal **42**. Thus, the acetate **44** was converted to the chloride **45** by treatment with 1,1-dichloromethyl methyl ether in the presence of $ZnCl_2$–etherate, as described above. We note that extended treatment of **44** with DCMME in the presence of $ZnCl_2$ or $ZnCl_2$–etherate resulted in the cleavage of the *O*-benzyl group. As an alternative route to the disaccharide **44**, we examined the condensation of the alcohol **29** with the thiorhamnoside donor[23] **22**. Surprisingly, activation with MeOTf led to the formation of the rhamnosyl acetate **46** as a major side product, which was the major product when the donor **22** was activated with NOBF4.[36] A combination of MeOTf and DTBMP in dichloromethane afforded the disaccharide **44** in 72% yield.



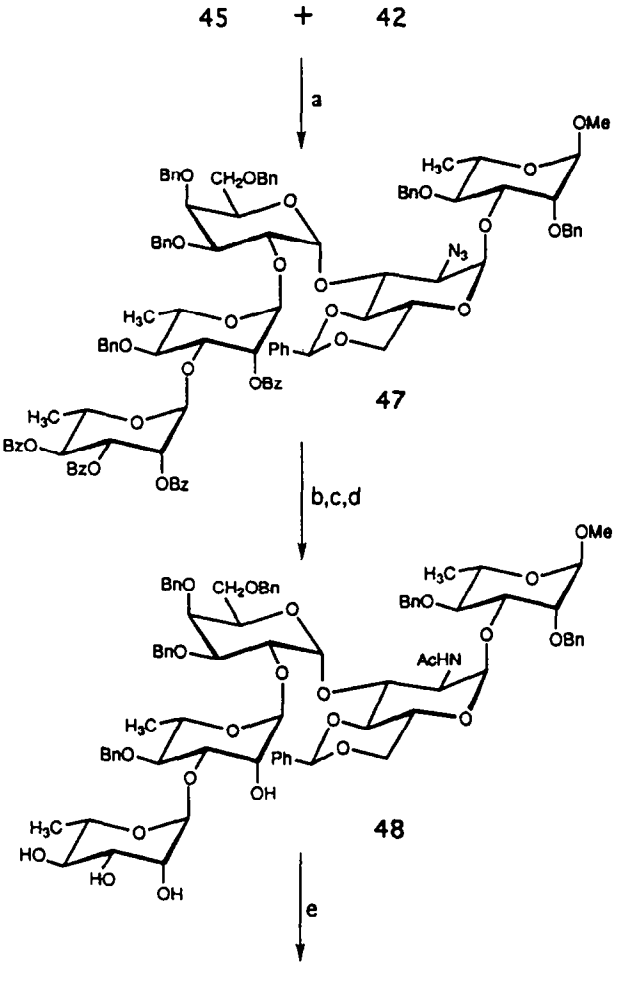
Key: (a) AgOTF, DTBMP, CH_2Cl_2; (b) MeOTf, DTBMP, CH_2Cl_2; (c) CH_3OCHCl_2, $ZnCl_2.Et_2O$

Scheme VIII.



46

Condensation of chloride **45** with the trisaccharide alcohol **42** (AgOTf, DTBMP) (Scheme IX) afforded the fully protected pentasaccharide methyl glycoside **47** in 79% yield. Reduction of the azido group, *N*-acetylation, and de-*O*-acetylation gave the intermediate **48**. Hydrogenolysis on palladium/charcoal afforded the pentasaccharide methyl glycoside **5**.



Key: (a) AgOTf, DTBMP, CH_2Cl_2; (b) $NiCl_2/H_3BO_3/NaBH_4$; (c) Ac_2O; (d) NaOMe, MeOH; (e) H_2/Pd-C

Scheme IX.

The structures of all intermediates and those of the final products **1–5** have been verified by elemental analyses, mass spectroscopy, and NMR spectroscopy.

NMR spectroscopy of oligosaccharides 2–5

A combination of one- and two-dimensional (1D and 2D) NMR spectroscopic techniques was used for complete assignment of the ^1H and ^{13}C NMR spectra of the free oligosaccharide methyl glycosides, including ^1H-^1H COSY (correlation spectroscopy),[37] TOCSY (total correlation spectroscopy),[38] HMQC (heteronuclear multiple quantum

correlation),[39] ^{13}C-detected ^{1}H–^{13}C shift correlation,[40] and DEPT (distortionless enhancement by polarization transfer).[41] The anomeric configurations were proved by the values of the one-bond, $^{1}J_{C,H}$ coupling constants.[42] These data (Tables 1–3) provided proof for the proposed structures and were used to estimate conformational similarity of the oligosaccharide methyl glycosides **2**–**5** to the O-SP.

Table 1. ^{1}H NMR chemical shifts for compounds **2**–**5**[a,b,c]

H-atom[d]	Compound			
	2[e]	**3**[e]	**4**[f]	**5**[f]
1_A	4.705	4.720	4.715	4.715
2_A	4.024	4.077	4.081	4.083
3_A	3.767	3.798	3.783	3.789
4_A	3.546	3.528	3.520	3.520
5_A	3.688	3.703	3.712	3.708
6_A	1.140	1.330	1.324	1.324
$6'_A$				
1_B	4.995	5.001	4.992	4.993
2_B	3.974	4.100	4.129	4.133
3_B	3.843	3.994	4.072	4.068
4_B	3.558	3.80	3.785	3.795
5_B	4.000	3.998	3.995	4.005
6_B	3.833	n.d.	3.805	3.810
$6'_B$	3.801	n.d.		
1_C		5.430	5.591	5.598
2_C		3.819	3.950	3.950
3_C		3.770	3.862	3.882
4_C		3.992	4.004	4.006
5_C		3.887	3.915	3.918
6_C		n.d.	3.755	3.755
$6'_C$		n.d.	3.785	3.786
1_D			5.074	5.056
2_D			4.064	4.162
3_D			3.789	3.874
4_D			3.472	3.559
5_D			3.853	3.883
6_D			1.296	1.306
1_E				5.074
2_E				4.065
3_E				3.848
4_E				3.462
5_E				3.846
6_E				1.313
CH_3O	3.390	3.395	3.402	3.395
CH_3CONH		2.059	2.051	2.051
CH_3CH_2	0.944			
CH_3CH_2	2.128			

[a] In ppm, using acetone (δ_H 2.225) as a secondary internal reference.
[b] At 300K, in D_2O.
[c] First-order data.
[d] For designations **A**–**E**, see Experimental Section, General.
[e] At 500 MHz.
[f] At 600 MHz.

Table 2. ^{13}C NMR chemical shifts for compounds 2–5[a,b]

C-atom[c]	Compound[d]			
	2[e]	**3**[e]	**4**[e]	**5**[f]
1_A	101.48	101.41	101.48	101.45
	(171)	(171)	(172)	(171)
2_A	67.68	67.26	67.22	67.19
3_A	76.54	75.90	75.76	75.73
4_A	71.08	70.95	71.02	71.03
5_A	69.25	69.28	69.24	69.23
6_A	17.65	17.52	17.45	17.45
1_B	95.39	95.05	94.86	94.85
	(171)	(171)	(171)	(173)
2_B	54.71	52.61	52.70	52.67
3_B	71.71	78.00	75.20	75.52
4_B	70.72	71.15	71.81	71.83
5_B	72.75	72.40	72.69	72.62
6_B	61.24	60.70	60.71	60.78
1_C		100.04	98.42	98.42
		(171)	(175)	(175)
2_C		69.24	74.39	74.53
3_C		70.04	69.78	70.09
4_C		69.69	70.24	70.21
5_C		71.52	71.63	71.62
6_C		61.22	61.51	61.47
1_D			102.30	102.21
			(172)	(173)
2_D			70.64	70.36
3_D			70.86	78.77
4_D			72.70	72.06
5_D			69.97	69.73
6_D			17.37	17.45
1_E				103.02
				(171)
2_D				70.45
3_E				70.82
4_E				72.85
5_E				69.88
6_E				17.45
CH_3O	55.50	55.53	55.58	55.58
CH_3CH_2	10.34			
CH_3CH_2	29.87			
CH_3CONH		22.81	22.81	22.80
CH_3CONH		174.67	174.89	

[a] In ppm from internal tetramethylsilane, via acetone (δ_C 31.00) as a secondary internal reference.
[b] At 300K, in D_2O.
[c] For designations A–E, see Experimental Section, General
[d] Data in parentheses are one-bond, ^{13}C–^1H coupling constants, in Hz.
[e] At 125 MHz.
[f] At 100 MHz.

Table 3. $^{3}J_{H-H}$ coupling constants for compounds 2–5[a]

$^{3}J_{H-H}$[b]	Compound			
	2	**3**	**4**	**5**
1_A-2_A	2.0	1.8	1.9	1.8
2_A-3_A	3.3	3.0	3.4	3.4
3_A-4_A	9.6	9.6	9.7	9.7
4_A-5_A	9.5	9.6	9.7	9.7
5_A-6_A	6.2	6.3	6.2	6.4
5_A-$6_{A'}$				
6_A-$6_{A'}$				
1_B-2_B	3.7	3.6	3.6	3.6
2_B-3_B	10.6	10.8	10.6	10.6
3_B-4_B	8.8	8.8	8.3	8.6
4_B-5_B	10.1	10.7	9.9	10[c]
5_B-6_B	2.6	3.4	3.4	2[c]
5_B-$6'_B$	4.2	6.6	8.3	5[c]
6_B-$6'_B$	-12.3	nd		
1_C-2_C		3.8	3.8	3.8
2_C-3_C		10.2	10.4	10.4
3_C-4_C		4.4	3.3	4[c]
4_C-5_C		1.1	1.2	<1
5_C-6_C		nd	7.1	8[c]
5_C-$6'_C$		nd	5.4	5.3
6_C-$6'_C$		nd	-11.5	-11.3
1_D-2_D			1.7	1.9
2_D-3_D			3.5	3.3
3_D-4_D			9.7	9.7
4_D-5_D			9.7	9.7
5_D-6_D			6.2	6.4
1_E-2_E				1.6
2_E-3_E				3.4
3_E-4_E				9.7
4_E-5_E				9.7
5_E-6_E				6.4

[a]In Hz.
[b]For designations **A**–**E**, see formulae.
[c]Estimated values. nd: not determined.

The assignment techniques for compounds **2** and **3** followed guidelines discussed earlier[19b] and need no particular comment. The ^{1}H NMR spectra of tetrasaccharide **4** and pentasaccharide **5** were assigned by the use of 1D and 2D TOCSY experiments. The successful application of the 1D TOCSY technique depends on the availability of at least one nucleus of a particular spin system that can be selectively excited. Following excitation, the magnetization is transferred sequentially through the spin system, provided that the coupling constants of vicinal protons are larger than *ca.* 1 Hz. Figure 1 shows the 1D TOCSY spectra of **5** for the individual sugar units **A**–**E** at 600 MHz. These spectra were obtained by selective excitation of the anomeric protons using a Gaussian-shaped pulse and an isotropic mixing time of 145 ms. Shorter mixing times were also used and resulted in subspectra exhibiting shorter propagation of magnetization. Spectra **a**, **d**, and **e** could immediately be assigned to residues **A**, **D**, and **E** based on the well-documented chemical shift and spin-coupling pattern of L-rhamnose residues.[9c,19a] Likewise, spectrum **b** was assigned to the glucosamine residue (**B**), and the remaining spectrum **c** assigned to the galactose residue (**C**). The assignment of subspectra **a**, **d**, and **e** to the individual rhamnose residues was based on the following arguments. The close similarity of the chemical shift for H-1 (4.715 ppm) in spectrum **a** to those of various, *O*-glycosylated derivatives of methyl α-L-rhamnopyranoside (Refs 9c, 23 and compound **2**) allowed assignment of spectrum **a** to residue **A**. The 2D ^{1}H–^{13}C heteronuclear correlation spectrum revealed correlation between H-3 of spectrum **d** with the carbon nucleus resonating at 78.77 ppm. This resonance must be due to C-3 of residue **D** based on its similarity to the chemical shift of C-3 i.e. the linkage carbon atom (78.82 ppm) in methyl *O*-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranoside[9c] (**49**). Spectrum **d** is thus assigned to residue **D**. The remaining spectrum (**e**) is then assigned to residue **E**.

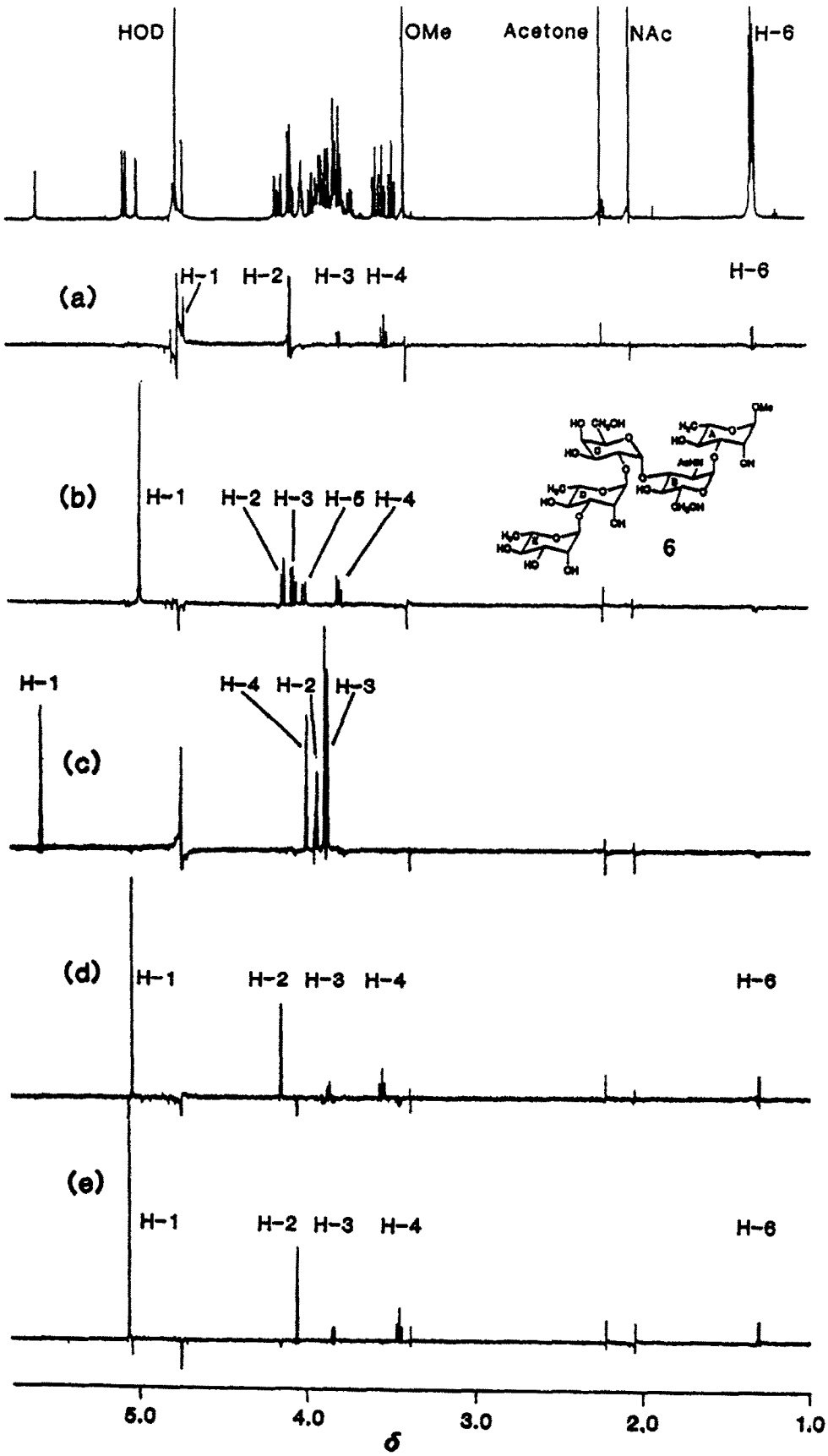
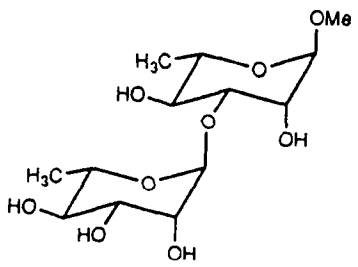


Figure 1. ^{1}H NMR spectra of pentasaccharide 5 in deuterium oxide at 600 HMz. Top trace: full spectrum. Spectra (a)–(e): 1D TOCSY subspectra for the individual glycose residues: (a) rhamnose residue A; (b) N-acetyl-glucosamine residue B; (c) galactose residue C, (d) rhamnose residue D; (e) rhamnose residue E. For experimental conditions see Experimental Section, General

We note that the resonances corresponding to H-6 and 6' for residue **B** and those corresponding to H-5, 6 and 6' for residue **C** are missing from spectra **b** and **c**, respectively. These resonances could easily be identified in the 2D TOCSY spectrum.

A noteworthy feature of the subspectra (Figure 1) is the periodic change in the ring proton signal intensities.[43] The starting point of the periodicity for the galactose residue is the anomeric proton and H-2 for the rhamnose residues. Surprisingly, the signal corresponding to H-5 is so weak that it could not be recognized in any of the spectra **a**, **d**, or **e**. On the other hand, the transfer of magnetization through this atom is indicated by the presence of the H-6 signals, the intensities of which were smaller than those corresponding to the H-4 protons. No such change is recognizable in the spectrum of the glucosamine unit.

The chemical shifts and coupling constants for the di- to pentasaccharide methyl glycosides **2**–**5** are within the expected range. Interestingly, the one-bond, heteronuclear $^1J_{C\text{-}1,H\text{-}1}$ coupling constants for the galactose residue in compounds **4** and **5** show an increase relative to compound **3** (175 Hz vs 171 Hz). The corresponding value for the native, O-SP is also 175 Hz (Ref. 44). The increase in the coupling constants for **4** and **5** relative to **3** is most likely due to an increase of steric crowding around the C-1, C-2 region for residues C in **4** and **5**, since the corresponding coupling constants for methyl *O*-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside[9f,g] and for methyl *O*-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranoside[9f,g] are only 170–172 Hz.[44] A conformational change brought about by the rhamnose residue (unit **D**) in the tetra- **4** and pentasaccharide **5** relative to trisaccharide **3** is also suggested by a comparison of the chemical shifts for C-3 of the *N*-acetylglucosamine residues of these compounds. This value is *ca.* 78 ppm for methyl *O*-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside[9f,g] (Ref. 44) and for the trisaccharide **3**, and 75.2–75.5 ppm for the tetra- **4** and pentasaccharide **5**. The corresponding chemical shift for the native polysaccharide is 75.5 ppm (Ref. 44). Based on these observations, we believe that the distribution of conformational states of the tetra- **4** and pentasaccharide **5** methyl glycosides more closely approaches that of the native O-SP than any of the disaccharides methyl *O*-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside, methyl *O*-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranoside, and **2**, and the trisaccharide **3**. On the other hand, a comparison of the chemical shifts of the anomeric protons of **2**–**5**, and **49** with those of the native O-SP (Table 4) shows that none of the chemical shifts of the anomeric protons in the di- and tri-saccharide sequences coincides with the corresponding shift for the O-SP. In the tetrasaccharide **4** the chemical shift of H-1 of the galactose residue is close to that of the corresponding proton in the O-SP, whereas in the penta-saccharide **5**, the chemical shifts of the anomeric protons of the galactose residue and a rhamnose residue (unit **D**) coincide with the corresponding shifts of the O-SP. Since conformational similarity assumes close similarity of the NMR parameters for the interchain residues, it appears, that the tetra- **4** and the pentasaccharide **5** only partially mimic the conformation of the native O-SP. We believe that a better similarity to the O-SP can be achieved by a relatively short increase in chain length over pentasaccharide **5**. These experiments are in progress in our laboratories and will be reported shortly.



49

Table 4. ^{1}H NMR chemical shifts[a] of the anomeric protons of the O-specific polysaccharide of *Shigella dysenteriae* type 1 (**I**), and oligosaccharides **2**–**5**,[b] and **49**

Compound	Chemical shift[c]						
	Residue						
	GlcN	Rha	Rha	Gal	GlcN	Rha	Rha
O-SP (**I**)	**5.045**	**5.110**	**5.056**	**5.604**	**5.045**	**5.110**	**5.056**
5		5.074	**5.056**	**5.598**	4.993	4.715	
4			5.074	5.591	4.992	4.715	
3				5.430	5.008	4.720	
2					4.995	4.705	
49[d]		5.027	4.666				

[a] In ppm.
[b] For experimental conditions, see Table 1.
[c] Chemical shifts for the anomeric protons of the O-SP and for those in the oligosaccharides which coincide with the corresponding resonances of the O-SP are shown in boldface.
[d] Data taken from Ref. 9c which reports full assignments of the NMR spectra for **49**.

Experimental Section

General

Melting points were taken on a Thomas Hoover* capillary melting point apparatus and are uncorrected. All chemicals were commercial grade and were used without purification. Anhydrous solvents were obtained from Aldrich. The O-specific polysaccharide of *S. dys.* was obtained as described in Ref. 2. Optical rotations were measured at 22 °C with a Perkin-Elmer Type 241MC polarimeter for $CHCl_3$ solutions, except where indicated otherwise. Column chromatography was performed on silica gel 60 (0.040–0.063 mm). The NMR spectra were measured at 300 K, by using Bruker WM-400 or AMX-600, and Varian VXR-500 S or Gemini 300 spectrometers. Internal references: TMS (0.000 ppm for ^1H for solutions in organic solvents), acetone (2.225 ppm for ^1H and 31.00 ppm for ^{13}C of solutions in D_2O), $CDCl_3$ (77.00 ppm for ^{13}C of solutions in $CDCl_3$), CD_3OD (49.90 ppm for ^{13}C of solutions in CD_3OD), $(CD_3)_2SO$ [39.5 ppm for ^{13}C of solutions in $(CD_3)_2SO$]. The NMR spectra of the intermediates were recorded at 300 MHz for ^1H, and 75.5 MHz for ^{13}C). Solutions of compounds **2–5** were prepared by lyophilization of the oligosaccharide (10–12 mg) with aliquots of deuterium oxide, followed by dissolution in deuterium oxide (0.5 mL). The solutions were contained in 5 mm sample tubes. One-dimensional ^1H NMR spectra of **4** and **5** were acquired at 600 MHz, by use of either 32,768 point data sets or 16,384 point data sets zero-filled to 32,768 points. Spectral widths of 2.5 and 3.0 kHz were employed, together with a 45° pulse (3.3–4.5 μs), a pulse recycle time of 6.0–6.6 s, and 64 or 128 scans. The spectra were resolution enhanced by Gaussian filtering of the free induction decay, using a line-broadening of -1.5 to -3.0 Hz, and a Gaussian truncation fraction of 0.3. One-dimensional TOCSY ^1H NMR spectra for **4** and **5** were acquired at 600 MHz with selective excitation of each anomeric proton in turn by a Gaussian shaped, low power pulse (150 ms) defined by 1,024 points of waveform memory. The observation frequency was set in the center of the spectrum, but the frequency offset for each anomeric proton was generated by application of a software-calculated, linear phase gradient to the observation frequency. Mixing times in the range of 17–215 ms were used, depending on the extent of coherence transfer desired. One-dimensional ^{13}C NMR spectra were recorded at 100.6 MHz by use of 32,768 point data sets, a spectral width of 10 kHz, a 40° pulse (4 μs), a pulse recycle time of 1 or 6 s, and continuous, WALTZ-16 composite pulse ^1H decoupling at 400 MHz. For tetrasaccharide **4** and pentasaccharide **5**, a DEPT-135 experiment was performed to identify the two methylene carbon resonances. For compound **5**, two-dimensional COSY-45 and 2D TOCSY ^1H NMR spectra were measured at 600 MHz in the phase-sensitive mode, by use of a spectral width of 3 kHz in both dimensions, a 90° pulse width of 8.9 μs, 1,024 (t_2) x 1,024 (t_1) point data sets, zero-filled to 2,048 points in both dimensions, and the States data acquisition protocol.[45] For 2D TOCSY, 72 dummy scans were used initially for temperature equilibration, followed by 24 scans per spectrum, with a mixing time of 70 ms. This data was subjected to a sine-bell window in both dimensions, with an offset of $\pi/3$ radians. The 2D COSY data were acquired by use of two dummy scans and 16 scans per spectrum, and were processed with a sine-bell window in both dimensions, using an offset of $\pi/2$ radians. For **4** and **5**, 2D heteronuclear, CH chemical shift correlated ^{13}C NMR spectra were recorded at 100.6 MHz, by using spectral widths of 10 kHz (^{13}C, t_2) and 1.87 kHz (^1H, t_1), 4,096 (t_2) x 256 (t_1) point data sets, zero-filled to 512 points in the t_1 dimension, two dummy scans and 512 scans per spectrum, a minimum, initial pulse recycle delay of 1 s, 90° ^1H and ^{13}C pulse widths of 26.8 and 9.5 μs, respectively, average delay periods $1/2J_{CH}$ = 3.52 and $1/4J_{CH}$ = 1.76 ms, and proton decoupling in both dimensions (WALTZ-16 in t_2 and BIRD in t_1). A sine-bell window was applied in both dimensions, with an offset of $\pi/2$ radians. The NMR spectra for compounds **2** and **3** were recorded on a Varian VXR-500S spectrometer, at 500 MHz for ^1H and 125 MHz for ^{13}C. The 1D data were acquired with a spectral width of 3 kHz (for ^1H) or 13 kHz (for ^{13}C), using 8,192 data points, zero-filled to 32,768 data points. The 2D COSY and HMQC data were acquired by the use of 512 or 1,024 (t_2) x 256 (t_1) point data sets, zero-filled to 1,024 points and were processed using a sine-bell window in both dimensions. For the HMQC experiments, the TPPI method was used with a ^{13}C spectral width of 26 kHz, i.e. twice the expected range. Subscripts A–E refer to the individual sugar residues, with A standing for the reducing-end unit. Low resolution mass spectra were obtained by the chemical ionization technique (CIMS), using NH_3 as the ionizing gas, and by the positive-ion fast atom-bombardment technique (FABMS) employing 3-nitrobenzyl alcohol or glycerol as the matrix.

Methyl 6-O-tert-butyldiphenylsilyl-α-D-galactopyranoside (7)

tert-Butyl-diphenylsilyl chloride[13] (23 mL) was added to a solution of methyl α-D-galactopyranoside (9.6 g) and imidazole (6.7 g) in pyridine (100 mL). The reaction mixture was stirred for 48 h at 25 °C, and then concentrated under reduced pressure. Column chromatography of the residue (2:1 ethyl acetate–hexane) afforded **7** as an amorphous solid (13.5 g, 63%): $[\alpha]_D$ +63° (c 1.0); NMR ($CDCl_3$): ^1H, δ 1.050 [s, 9H, $C(CH_3)_3$], 3.334 (s, 3H, OCH_3), 3.727 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-3), 3.75 (m, 1H, H-5), 3.8–3.92 (m, 3H, H-2,6,6'), 4.063 (dd, 1H, H-4), 4.765 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 7.33–7.71 (m, 10H, aromatic); ^{13}C, δ 19.1 [$C(CH_3)_3$], 26.8 [$C(CH_3)_3$], 55.2 (OCH_3), 63.4 (C-6), 69.5 (C-5), 69.6 (C-4), 70.0 (C-3), 71.2 (C-2), 99.5 (C-1), 127.7–135.6 (aromatic). CIMS: m/z 450 $(M + 18)^+$, 433 $(M + 1)^+$. Anal. calcd for $C_{23}H_{32}O_6Si$: C, 63.85; H, 7.45. Found: C, 63.65; H, 7.38.

*Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation by the National Institute of Standards and Technology, nor does it imply that the materials or equipment are necessarily the best available for the purpose.

Methyl 6-O-tert-butyldiphenylsilyl-3,4-O-isopropylidene-α-D-galactopyranoside (8)

(a) A solution of **7** (10 g) in 2,2-dimethoxypropane[14] (100 mL) was treated with a catalytic amount of 10-camphorsulfonic acid at 25 °C. After 1 h the solution was treated with triethylamine (1 mL). The mixture was concentrated and the residue chromatographed (1:1 ethyl acetate–hexane) to give **8** as a syrup (10.4 g, 95%): $[\alpha]_D$ +65° (c 1.3); NMR ($CDCl_3$): ^1H, δ 1.063 [s, 9H, $C(CH_3)_3$], 1.336 and 1.479 [2 s, 6H, $C(CH_3)_2$], 3.414 (s, 3H, OCH_3), 3.777 (dd, 1H, $J_{2,3}$ 6.5, H-2), 3.860 (dd, 1H, $J_{5,6}$ 6.8 Hz, $J_{6,6}$ 10.1 Hz, H-6), 3.930 (dd, 1H, H-6'), 4.057 (dt, 1H, H-5), 4.192 (t 1H, H-3), 4.266 (dd, 1H, $J_{3,4}$ 6.0 Hz, $J_{4,5}$ 3.7 Hz, H-4), 4.725 (d, 1H, $J_{1,2}$ 3.9 Hz, H-1), 7.33-7.5 and 7.68–7.73 (aromatic); ^{13}C, δ 19.1 [$C(CH_3)_3$], 25.9 and 27.8 [$C(CH_3)_2$], 26.7 [$C(CH_3)_3$], 55.3 (OCH_3), 62.9 (C-6), 68.4, 69.8, 72.8, 76.2 (C-2,3,4,5), 98.5 (C-1), 109.4 [$C(CH_3)_2$], 127.6–135.8 (aromatic). CIMS: m/z 490 $(M+18)^+$, 473 $(M + 1)^+$. Anal. calcd for $C_{26}H_{36}O_6Si$: C, 66.06; H, 7.62. Found: C, 65.53; H, 7.57.

(b) A solution of methyl α-D-galactopyranoside was treated with 2,2-dimethoxypropane as described in Ref. 14 to give compound[15] **9**. A solution of **9** (6 g), *tert*-butyldiphenylsilyl chloride (11 g), imidazole (3 g) in pyridine (60 mL) was stirred for 24 h. Chromatographic work-up as described above afforded **9** (11.5 g, 95%) which was identical to the product obtained in (a).

Methyl 3,4,6-tri-O-benzyl-2-O-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (11)

A solution of compound[9a,9b] **10** (9.2 g) in *N,N*-dimethylformamide (60 mL) at 0 °C was treated under stirring with sodium hydride (4.5 g of a 60% suspension in oil). Benzyl bromide (12 mL) was added and stirring was continued until the temperature of the reaction mixture reached 25 °C. The mixture was cooled to 0 °C and the excess of sodium hydride was decomposed by careful addition of methanol. Extractive work-up followed by chromatography (6:1 hexane–ethyl acetate) afforded crystalline **11** (15.5 g, 93%): m.p. 74–75 °C; $[\alpha]_D$ +22° (c 1.2); NMR ($CDCl_3$): ^1H, δ 2.193 (s, 3H SCH_3), 3.54–3.60 (m, 4H, H-3,5,6,6'), 3.781 (OCH_3), 3.828 (t, 1H, H-2), 3.956 (dd, 1H, $J_{3,4}$ 3.5, $J_{4,5}$ <1 Hz, H-4), 4.316 (d, 1H, $J_{1,2}$ 9.6 Hz, H-1), 4.40, 4.45, 4.61, 4.73, 4.79, 4.95 (6 d, 6H, *J* 12 Hz), and 4.73 (s, 2H) [4 CH_2 (Bn and MBn)], 6.8–7.4 (aromatic); ^{13}C, δ 12.7 (SCH_3), 55.3 (OCH_3), 68.7 (C-6), 72.7, 73.5, 74.4, 75.3 [CH_2 (Bn and MBn)], 73.8 (C-4), 77.2 (C-5), 77.6 (C-2), 84.1 (C-3), 85.6 ($^1J_{C-1,H-1}$ 155 Hz, C-1), 113.7, 127.4–130.5, 137.9, 138.4, 138.8, and 159.3 (aromatic). Anal. calcd for $C_{36}H_{40}O_6S$: C, 71.97; H, 6.71; S, 5.43. Found: C, 72.05; H, 6.75; S, 5.29.

Methyl 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranoside (13)

To a stirred solution of methyl α-D-mannopyranoside (7 g) in pyridine (60 mL) at 0 °C was added benzoyl chloride (20 mL). After 1 h the volatiles were removed under vacuum. Extractive work-up afforded crystalline **13** (21 g, 96%): m.p. 138–140 °C, lit.[46] m.p. 136-137 °C; $[\alpha]_D$ -67° (c 0.8), lit.[46] [α] -67.5° ($CHCl_3$); NMR ($CDCl_3$): ^1H, 3.549 (s, 3H, CH_3O), 4.423 (ddd, 1H, H-5), 4.505 (dd, 1H, $J_{5,6'}$ 4.5 Hz, $J_{6,6'}$ 12.1 Hz, H-6'), 4.719 (dd, 1H, $J_{5,6}$ 1.5 Hz, H-6), 5.008 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.706 (dd, 1H, H-2), 5.918 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.1 Hz, H-3), 6.118 (t, 1H, $J_{3,4} = J_{4,5}$ = 10.1 Hz, H-4), 7.22–8.12 (aromatic); ^{13}C, δ 55.5 (CH_3O), 62.9 (C-6), 66.9 (C-4), 68.7 (C-5), 70.0 (C-3), 70.4 (C-2), 98.9 (C-1), 128.3–133.5 (aromatic), 165.55, 165.6 (2C), 166.3 (*C*=O). Anal. calcd for $C_{35}H_{30}O_{10}$: C, 68.85; H, 4.95. Found: C, 68.80; H, 4.98.

1-O-Acetyl-2,3,4,6-tetra-O-benzoyl-α-D-mannopyranose (14)

To a stirred solution of **13** (20 g) in acetic anhydride (100 mL) at 25 °C was added concentrated sulfuric acid (0.5 mL). After 2 days the reaction was terminated by the addition of an excess of $NaHCO_3$. Extractive work-up followed by chromatography (3:1 hexane–ethyl acetate) afforded an amorphous substance (20 g, 96%) consisting of **14** and an unidentified compound (5% or less): NMR data for **14** ($CDCl_3$): ^1H, δ 2.28 (s, 3H, CH_3CO), 4.43–4.74 (m, 3H, H-5,6,6'), 5.744 (dd, 1H, H-2), 5.927 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.1 Hz, H-3), 6.205 (t, 1H, $J_{3,4}$=$J_{4,5}$=9.3 Hz, H-4), 6.391 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1), 7.22–8.12 (aromatic); ^{13}C, δ 20.8 (CH_3CO), 62.3 (C-6), 66.2, 69.2, 69.7, 70.1 (C-2,3,4,5), 90.8 ($^1J_{C-1,H-1}$ 179 Hz, C-1), 128.5–133.6 (aromatic), 165.2, 165.4, 165.8, 166.2 [*C*=O (Bz)], 168.3 [*C*=O (Ac)]. Anal. calcd for $C_{36}H_{30}O_{11}$: C, 67.71; H, 4.73. Found: C, 67.70; H, 4.77.

2,3,4,6-Tetra-O-benzoyl-α-D-mannopyranosyl chloride (15)

To a solution of **14** (18 g) in dichloromethane (100 mL) at 25 °C was added dichloromethyl methyl ether (10 mL), followed by $ZnCl_2$·Et_2O (54%) in CH_2Cl_2 (2 mL). After 4 h an excess of solid $NaHCO_3$ was added. Extractive work-up followed by chromatography (6:1 hexane–ethyl acetate) afforded amorphous **15** (16.5 g, 95%): $[\alpha]_D$ -32° (c 0.8), lit.[46] [α] -30.5° ($CHCl_3$); NMR ($CDCl_3$): ^1H, δ 4.48–4.78 (m, 3H, H-5,6,6'), 5.861 (dd, 1H, $J_{2,3}$ 2.7 Hz, H-2), 6.16–6.27 (m, 2H, H-3,4), 6.326 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 7.22–8.14 (aromatic); ^{13}C, δ 61.9 (C-6), 65.9, 68.8, 71.6, 72.4 (C-2,3,4,5), 88.9 ($^1J_{C-1,H-1}$ 184 Hz, C-1), 128.5–133.9 (aromatic), 165.2, 165.4, 165.5 [*C*=O (Bz)]. CIMS: m/z 450 $(M + 18)^+$, 433 $(M + 1)^+$. Anal. calcd for $C_{34}H_{27}ClO_9$: C, 66.40; H, 4.42; Cl, 5.76. Found: C, 66.49; H, 4.45; Cl, 5.85.

Methyl 3-O-acetyl-2-azido-4,6-O-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside (19)

Acetic anhydride (5 mL) was added to a solution of compound[9a] **18** (2.1 g) in pyridine (10 mL) at 25 °C. After 2 h the volatiles were removed under vacuum. The residue was crystallized from ether–hexane to give **19** (2.1

g, 88%): m.p. 154–155 °C, $[\alpha]_D$ +119°(c 0.5); NMR ($CDCl_3$): ^1H, δ 2.122, 2.130 (CH_3CO and CH_3S), 3.622 (m, 1H, H-4), 3.75–3.84 (m, 1H, H-6), 3.992 (dd, 1H, $J_{1,2}$ 5.6 Hz, $J_{2,3}$ 10.2 Hz, H-2), 4.24–4.36 (m, 2H, H-5,6'), 5.330 (d, 1H, H-1), 5.439 (t, 1H, H-3), 5.497 (s, 1H, CHPh), 7.26–7.46 (aromatic); ^{13}C, δ 13.7 (CH_3S), 21.3 (CH_3CO), 63.0 (C-5), 63.7 (C-2), 69.2 (C-6), 71.0 (C-3), 80.2 (C-4), 85.7 (C-1), 102.2 (CHPh). Anal. calcd for $C_{16}H_{19}N_3O_5S$: C, 52.60; H, 5.24; N, 11.50; S, 8.77. Found: C, 52.46; H, 5.30; N, 11.40; S, 8.74.

3-O-Acetyl-2-azido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl chloride (20)

To a solution of **19** (1.0 g) in dichloromethane (20 mL) at 0 °C was added chlorine in carbon tetrachloride (3.2 mL of a 0.92 M solution). After 10 min, cyclohexene (2 mL) was added, and the solution was concentrated. Chromatography (8:1 hexane–ethyl acetate) of the residue afforded amorphous **20** (818 mg, 85%): $[\alpha]_D$ +74° (c 0.4); NMR ($CDCl_3$): ^1H, δ 2.15 (s, 3H, CH_3CO), 3.575 (ddd, 1H, H-5), 3.679 (dd, 1H, $J_{2,3}$ 9.3 Hz, H-2), 3.717 (t, 1H, $J_{3,4}$ 9.5 Hz, H-4), 3.799 (t, 1H, $J_{5,6} = J_{6,6'}$ = 10.2 Hz, H-6), 4.377 (dd, 1H, $J_{5,6'}$ 5.0 Hz, H-6'), 5.183 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 5.203 (t, 1H, H-3), 5.496 (s, 1H, CHPh), 7.3–7.44 (aromatic); ^{13}C, δ 20.7 (CH_3CO), 68.03 (C-6), 68.07 (C-2), 70.0 (C-5), 71.7 (C-3), 77.9 (C-4), 89.5 (C-1), 101.6 (CHPh), 126.0, 128.3, 129.2, 136.4 (aromatic), 169.4 (C=O). Anal. calcd for $C_{15}H_{16}ClN_3O_5$: C, 50.92; H, 4.56; N, 11.88. Found: C, 50.18; H, 4.50; N, 12.23.

Methyl 2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (26)

A mixture of compound[24] **24** (45 g), trimethyl orthobenzoate (50 g) and a catalytic amount of 10-camphorsulfonic acid was shaken until dissolution of **24** was complete. The volatiles were removed under the vacuum of a water aspirator. TLC (4:1 hexane–ethyl acetate) of the residue indicated the presence of the two isomers of the intermediate **25**. The syrupy intermediate was dissolved in chloroform (300 mL). To the stirred solution was added 50% aq. trifluoroacetic acid (5 mL) at 0 °C. After 15 min, water (100 mL) was added. The organic phase was concentrated. Column chromatography (6:1 hexane–ethyl acetate) of the residue afforded syrupy **26** (53 g, 85%): $[\alpha]_D$ +27° (c 1.1), lit.[25b] $[\alpha]_D$ +24.5° ($CHCl_3$); NMR ($CDCl_3$): ^1H, δ 1.394 (d, 3H, $J_{5,6}$ 6.3 Hz, H-6), 3.369 (s, 3H, CH_3O), 3.467 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.4 Hz, H-4), 3.791 (dq, 1H, H-5), 4.205 (dd, 1H, H-3), 4.745 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 4.742, 4.860 (2 d, 2H, CH_2), 5.336 (dd, 1H, H-2), 7.26–8.06 (aromatic); ^{13}C, δ 18.1 (C-6), 54.9 (CH_3O), 67.3 (C-5), 70.5 (C-3), 73.3 (C-2), 75.0 (CH_2), 81.7 (C-4), 98.5 (C-1), 127.7–138.3 (aromatic), 166.2 (C=O). Anal. calcd for $C_{21}H_{24}O_6$: C, 67.72; H, 6.49. Found: C, 67.71; H, 6.53.

Methyl 2-O-benzoyl-4-O-benzyl-3-O-bromoacetyl-α-L-rhamnopyranoside (27)

Bromoacetyl bromide (2.2 mL) was added to a solution of compound **26** (5.1 g) and 2,4,6-trimethylpyridine (10 mL) in dichloromethane (50 mL) at -15 °C. After 15 min ice–water was added. Extractive work-up followed by column chromatography using 6:1 hexane–ethyl acetate as eluant gave syrupy **27** (6.0 g, 89%): $[\alpha]_D$ +46° (c 0.8); NMR ($CDCl_3$): ^1H, δ 1.405 (d, 3H, $J_{5,6}$ 6.3 Hz, H-6), 3.352 (s, 3H, CH_3O), 3.639 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 3.644 and 3.699 (2 d, 2H, *J* 12.4 Hz, CH_2Br), 3.890 (dq, 1H, H-5), 4.465 and 4.770 [2 d, 2H, *J* 11 Hz, CH_2 (Bn)], 4.750 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.413 (dd, 1H, $J_{2,3}$ 3.4 Hz, H-3), 5.506 (dd, 1H, H-2), 7.25–7.66 and 8.04–8.09 (aromatic); ^{13}C, δ 18.0 (C-6), 25.4 (CH_2Br), 55.0 (CH_3O), 67.6 (C-5), 70.5 (C-3), 74.1 (C-2), 75.1 (CH_2), 78.6 (C-4), 98.5 (C-1), 127.8–133.3 and 137.9 (aromatic), 165.6, 166.1 (C=O). CIMS: m/z 512 $(M + 18)^+$. Anal. calcd for $C_{23}H_{25}BrO_7$: C, 55.99; H, 5.11; Br, 16.20. Found: C, 56.08; H, 5.12; Br, 16.26.

1-O-Acetyl-2-O-benzoyl-4-O-benzyl-3-O-bromoacetyl-α-L-rhamnopyranose (28)

To a solution of **27** (5.4 g) in acetic anhydride (20 mL) at 0 °C was added concentrated sulfuric acid (8 drops). After 45 min, solid $NaHCO_3$ was added, and the mixture was stirred for 5 min. Extractive work-up followed by column chromatography using 6:1 hexane–ethyl acetate as eluant gave crystalline **28** (5.4 g, 95%), m.p. 71–72 °C; $[\alpha]_D$ +21° (c 1.2); NMR ($CDCl_3$): ^1H, δ 1.410 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6), 2.156 (s, 3H, CH_3CO), 3.681 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 3.670 and 3.729 (2 d, 2H, *J* 12.4 Hz, CH_2Br), 4.101 (dq, 1H, H-5), 4.665 and 4.788 [2 d, 2H, *J* 11 Hz, CH_2 (Bn)], 5.431 (dd, 1H, $J_{2,3}$ 3.4 Hz, H-3), 5.523 (dd, 1H, H-2), 6.142 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1), 7.18–7.66 and 8.04–8.08 (aromatic); ^{13}C, δ 18.1 (C-6), 20.9 (CH_3CO), 25.3 (CH_2Br), 69.3 (C-5), 70.5 (C-3), 73.7 (C-2), 75.3 (CH_2), 78.0 (C-4), 90.8 ($^1J_{C\text{-}1,H\text{-}1}$ 177 Hz, C-1), 127.9–133.6, and 137.6 (aromatic), 165.4, 166.3, and 168.4 (C=O). CIMS: m/z 538 $(M + 18)^+$. Anal. calcd for $C_{24}H_{25}BrO_8$: C, 55.29; H, 4.83; Br, 15.33. Found: C, 55.16; H, 4.85; Br, 15.27.

1-O-Acetyl-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranose (29)

Thiourea (0.9 g) was added to a solution of **28** (3.4 g) in methanol (50 mL) at 25 °C. After 20 min the solution was concentrated. The residue was treated with chloroform (50 mL), the mixture filtered, and the insoluble part discarded. Concentration followed by chromatographic purification (4:1 hexane–ethyl acetate) gave a crude product from which **29** (2.2 g, 84%) was obtained by crystallization, m.p. 104–106 °C; $[\alpha]_D$ +2.7° (c 1.3); NMR ($CDCl_3$): ^1H, δ 1.400 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6), 2.106 (s, 3H, CH_3CO), 3.507 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.870 (dq, 1H, H-5), 4.242 (ddd, 1H, H-3), 4.759 and 4.865 [2 d, 2H, *J* 11 Hz, CH_2 (Bn)], 5.523 (dd, 1H, H-2), 6.133 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1); ^{13}C, δ 18.3 (C-6), 20.9 (CH_3CO), 69.8 (C-5), 70.4 (C-3), 72.0 (C-2), 75.4 (CH_2), 81.1 (C-4), 90.9 (C-1), 128.0–133.4, and 137.8 (aromatic), 166.0, 168.5 (C=O). CIMS: m/z 818 $(2M + 18)^+$, 758 $(2M + 18 - AcOH)^+$, 681 $(2M + 1 - 2AcOH)^+$, 418 $(M + 18)^+$, 341

$(M + 1 - AcOH)^+$. Anal. calcd for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04. Found: C, 66.10; H, 6.09.

Methyl O-(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→2)-6-O-tert-butyldiphenylsilyl-3,4-O-isopropylidene-α-D-galactopyranoside (31)

Silver trifluoromethanesulfonate (2.4 g) was added to a stirred mixture of **8** (1.95 g), compound **15** (5.1 g), 2,6-di-*tert*-butyl-4-methylpyridine (1.6 g), 4A molecular sieves (1 g) and dichloromethane (50 mL) at 0 °C. After 2 h the reaction mixture was treated with ice-cold, aq. $NaHCO_3$ then filtered. Extractive work-up followed by column chromatography using 3:1 →2:1 hexane–ethyl acetate as eluant, afforded amorphous **31** (2.5 g, 58%): $[\alpha]_D$ +25° (c 1.2); NMR ($CDCl_3$): ^1H, δ 1.083 [s, 9H, $(CH_3)_3C$], 1.38 and 1.55 [2 s 6H, $(CH_3)_2C$], 3.429 (CH_3O), 3.82–4.48 (m, 8H, H-2_A,3_A,4_A,6_A,$6'_A$,5_B, 6_B,$6'_B$), 4.736 (m, 1H, H-5_A), 4.812 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1_A), 5.207 (d, 1H, H-1_B), 5.747 (dd, 1H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.2 Hz, H-2_B), 6.008 (dd, 1H, H-3_B), 6.245 (t, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4_B), 7.21–8.18 (aromatic); ^{13}C, δ 26.3 and 28.5 [$(CH_3)_2C$], 26.8 [$(CH_3)_3C$], 55.6 (CH_3O), 62.3, 62.9 (C-6_A,6_B), 66.7, 67.7, 68.8, 69.9, 70.7, 73.3, 74.6, 75.6 (C-2_A,3_A,4_A,5_A,2_B,3_B,4_B,5_B), 98.3 ($^1J_{C\text{-}1,H\text{-}1}$ 171 Hz, C-1_B), 98.8 ($^1J_{C\text{-}1,H\text{-}1}$ 168 Hz, C-1_A), 109.1 [$C(CH_3)_2$], 127–135.5 (aromatic), 165.3, 165.4, 166.1 (C=O). FABMS: m/z 1019 $(M + 1 - MeOH)^+$, 579 $(C_{37}H_{27}O_9)^+$. Anal. calcd for $C_{60}H_{52}O_{15}Si$: C, 68.55; H, 5.94. Found: C, 68.83; H, 6.10.

Methyl O-(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→2)-3,4-O-isopropylidene-α-D-galactopyranoside (32)

Tetrabutylammonium fluoride (2 mL of a 1.4 M solution in tetrahydrofuran) was added to a solution of **31** (2.2 g) in tetrahydrofuran (15 mL) at 25 °C. After 4 h the solution was concentrated. Column chromatography of the residue using 3:1 hexane–ethyl acetate as eluant gave amorphous **32** (1.3 g, 76%): $[\alpha]_D$ +35° (c 1.3); NMR ($CDCl_3$): ^1H, δ 1.393 and 1.569 [2 s, 6H, $(CH_3)_2C$)], 3.478 (s, 3H, CH_3O), 4.306 (dd, 1H, $J_{2,3}$ 5.6 Hz, $J_{3,4}$ 2.5 Hz, H-3_A), 4.883 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1_A), 5.220 (d, 1H, $J_{1,2}$ 2.6 Hz, H-1_B), 5.743 (dd, 1H, $J_{2,3}$ 5.0 Hz, H-2_B), 5.990 (dd, 1H, H-3_B), 6.244 (t, 1H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4_B), 7.2–8.14 (aromatic); ^{13}C, δ 26.3 and 28.2 [$(CH_3)_2C$], 55.7 (CH_3O), 62.2, 62.6 (C-6_A,6_B), 96.1, 97.1 (C-1_A,1_B), 109.7 [$(CH_3)_2C$], 128.3–133.5 (aromatic), 165.6, 166.3 (C=O). FABMS: m/z 811 $(M + 1 - H_2)$, 781 $(M + 1 - MeOH)^+$, 579 $(C_{37}H_{27}O_9)^+$. Anal. calcd for $C_{44}H_{44}O_{15}$: C, 65.02; H, 5.46. Found: C, 65.30; H, 5.71.

Methyl O-(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→2)-α-D-galactopyranoside (33)

A solution of **32** (1.1 g) in 80% aq. acetic acid was stirred at 70 °C for 1 h. The solution was concentrated. Column chromatography of the residue in 2:1 ethyl acetate–hexane afforded amorphous **33** (650 mg, 62%): $[\alpha]_D$ -4° (c 0.5); NMR ($CDCl_3$): ^1H, δ 3.458 (s, 3H, CH_3O), 3.84–4.14 (m, H-2_A,3_A,4_A,5_A,6_A,$6'_A$), 4.476 (dd, 1H, $J_{5,6}$ 5.0 Hz, $J_{6,6}$ 11.1 Hz, H-6_B), 4.730 (dd, 1H, $J_{5,6}$ 2.6 Hz, H-$6'_B$), 4.784 (ddd, 1H, H-5_B), 4.982 (d, 1H, $J_{1,2}$ 2.4 Hz, H-1_A), 5.232 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1_B), 5.732 (dd, 1H, H-2_B), 5.975 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.9 Hz, H-3_B), 6.072 (t, 1H, H-4_B), 7.23–8.12 (aromatic); ^{13}C, δ 55.6 (CH_3O), 62.9, 63.3 (C-6_A,6_B), 66.9, 68.5, 68.8, 69.4, 69.8, 70.6, 71.1 (C-3_A,4_A,5_A,2_B,3_B,4_B,5_B), 76.1 (C-2_A), 96.6, 97.7 (C-1_A,1_B), 128.4–133.7 (aromatic), 165.7 (C=O). FABMS: m/z 795 $(M + 23)^+$. Anal. calcd for $C_{41}H_{40}O_{15}$: C, 63.73; H, 5.22. Found: C, 63.66; H, 5.27.

Methyl O-α-D-mannopyranosyl-(1→2)-α-D-galactopyranoside (1)

A catalytic amount of sodium methoxide was added to a solution of **33** (560 mg) in methanol (10 mL) at 25 °C. After 48 h, the reaction mixture was neutralized (Dowex 50x2, H^+), filtered, and concentrated. The product was equilibrated between $CHCl_3$ and H_2O. Freeze-drying of the aqueous phase gave amorphous **1** (223 mg, 86%). A portion was purified by gel filtration (Biogel P-2) using 0.02 M pyridinium acetate as eluant: $[\alpha]_D$ +173° (c 0.3, H_2O); NMR (D_2O) ^1H, δ 3.431 (s, 3H, CH_3O), 3.672 (t, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4_B), 4.001 (dd, 1H, H-4_A), 5.014 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1_B), 5.103 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1_A); ^{13}C, δ 55.5 (CH_3O), 61.7, 62.0 (C-6_A,6_B), 67.5, 68.8, 70.1, 71.07, 71.15, 71.6, 72.8, 73.7 (C-2_A,3_A, 4_A,5_A,2_B,3_B,4_B,5_B), 97.2, 98.1 (C-1_A,1_B). CIMS: m/z 374 $(M + 18)^+$, 342 $(M + 18 - MeOH)^+$, 212 $(C_7H_{14}O_6 + 18)^+$, 180 $(C_6H_{11}O_5 + 18)^+$.

Methyl O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (34)

A stirred mixture of **16** (1.0 g), **21** (1.5 g), 4A molecular sieves (3 g) in dichloromethane (25 mL) was treated at 25 °C with methyl trifluoromethanesulfonate (660 μL) for one week. The usual work-up as described for **31**, followed by column chromatography using 7:1 hexane–ethyl acetate as the eluant gave amorphous **34** (1.4 g, 75%): $[\alpha]_D$ +118° (c 0.8); NMR ($CDCl_3$): ^1H, δ 1.372 (d, 1H, $J_{5,6}$ 6.4 Hz, H-6_A), 1.891, 2.019, 2.083 (3 s, 9H, CH_3CO), 3.305 (s, 3H, CH_3O), 3.363 (dd, 1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 10.6 Hz, H-2_B), 4.7–4.9 (2H, H-1_A,1_B), 5.567 (t, 1H, $J_{3,4}$ 10.5 Hz, H-3_A), 7.22–7.44 (aromatic); ^{13}C, δ 17.8 (C-6_A), 20.4, 20.5 (CH_3CO), 54.6 (CH_3O), 60.7 (C-6_B), 61.3 (C-2_B), 79.1 (C-4_A), 92.3 ($^1J_{C\text{-}1,H\text{-}1}$ 171 Hz, C-1_B), 98.1 (C-1_A), 169.4, 169.6, 170.3 (C=O). FABMS: m/z 670 $(M + 1 - H_2)^+$, 640 $(M + 1 - MeOH)^+$, 612 (M + 1 - AcOH). Anal. calcd for $C_{33}H_{41}N_3O_{12}$: C, 59.00; H, 6.16; N, 6.25. Found: C, 58.93; H, 6.17; N, 6.29.

Methyl O-(2-azido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (35)

(a) A catalytic amount of sodium methoxide was added at 25 °C to a solution of **34** (1.3 g) in methanol (40 mL).

After 4 h, the solution was neutralized with Dowex 50 x 2 (H^+), filtered, and concentrated. A solution of the residual syrup in acetonitrile (20 mL) and α,α-dimethoxytoluene (2 mL) was treated with 10-camphorsulfonic acid. After 1 h the solution was treated with triethylamine (1 mL). Concentration followed by column chromatography using 3:1 hexane–ethyl acetate afforded amorphous **35** (1.11 g, 91%): $[\alpha]_D$ +58° (c 0.5); NMR ($CDCl_3$): ^1H, δ 1.335 (d, 1H, $J_{5,6}$ 6.7 Hz, H-6_A), 3.290 (dd, 1H, H-2_B), 3.307 (s, 3H, CH_3O), 3.520 (t, 1H, H-4_A), 3.670 (dq, 1H, H-5_A), 3.838 (dd, 1H, H-2_A), 4.096 (dd, 1H, H-3_A), 4.280 (t, 1H, H-3_B), 4.587 and 4.898 (2 d, 2H, CH_2), 4.716 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1_A), 4.743 (s, 2H, CH_2), 5.013 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1_B), 5.500 (s, 1H, C*H*Ph), 7.2–7.44 (aromatic); ^{13}C, δ 17.9 (C-6_A), 54.8 (*C*H_3O), 62.9 (C-2_B),79.6 (C-4_A), 81.8 (C-4_B),94.1 (C-1_B),98.3 (C-1_A), 102.0 (*C*HPh), 126.4–137.8 (aromatic). FABMS: m/z 643 $(M + 1)^+$. Anal. calcd for $C_{34}H_{39}N_3O_9$: C, 64.44; H, 6.24; N, 6.63. Found: C, 64.50; H, 6.25; N, 6.60.

(b) A catalytic amount of sodium methoxide was added to a solution of **36** (1.9 g) in methanol (20 mL) at 25 °C. After 12 h the solution was neutralized [Dowex 50 x 2, (H^+)]. Concentration afforded **35** (1.72 g, 97%) which was identical to the preparation obtained in (a).

Methyl O-*(3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (36)*

Methyl trifluoromethanesulfonate (1 mL) was added to a stirred mixture of **19** (2.0 g), **21** (4.8 g), and 4A molecular sieves (2 g) in dichloromethane (50 mL) at 25 °C. After 4 days, the reaction mixture was processed as described for **34** to afford amorphous **36** (2.0 g, 54%): $[\alpha]_D$ +86° (c 1.1); NMR ($CDCl_3$): ^1H, δ 1.370 (d, 1H, $J_{5,6}$ 6.6 Hz, H-6_A), 2.135 (s, 3H, CH_3CO), 3.226 (dd, 1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 10.3 Hz, H-2_B), 3.309 (s, 3H, CH_3O), 3.847 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 3.2 Hz, H-2_A), 4.63 and 4.93 (2 d, 2H, CH_2), 5.043 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1_B), 5.451 (s, 1H, C*H*Ph), 5.727 (t, 1H, $J_{2,3} = J_{3,4}$ = 10.3 Hz, H-3_B). 7.08-7.45 (aromatic); ^{13}C, δ 18.5 (C-6_A), 21.4 (*C*H_3CO), 55.0 (*C*H_3O), 61.8 (C-2_B), 68.9 (C-6_B), 72.7, 76.5 (*C*H_2), 79.6, 79.7 (C 4_A,4_B), 94.4 ($J_{C\text{-}1,H\text{-}1}$ 171 Hz, C-1_B), 98.3 (C-1_A), 101.9 (*C*HPh), 126.4–137.7 (aromatic). CIMS: m/z 693 $(M + 18)^+$. Anal. calcd for $C_{36}H_{41}N_3O_{10}$: C, 63.98; H, 6.11; N, 6.22. Found: C, 64.46; H, 6.45; N, 6.10.

Methyl O-*(3-*O*-acetyl-2-amino-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (37)*

A solution of $NiCl_2 \cdot 6H_2O$ (2.5 g) and H_3BO_3 (1.2 g) in ethanol (30 mL) was added to a stirred solution of **36** (500 mg) in 1,2-dimethoxyethane (2 mL). To this solution was added under stirring at 25 °C a solution of sodium borohydride (300 mg) in methanol (15 mL) during 1 h. The mixture was concentrated. Extractive work-up followed by column chromatography using 1:1 hexane–ethyl acetate as eluant afforded amorphous **37** (390 mg, 81%); $[\alpha]_D$ +74° (c 1.1). FABMS: m/z 650 $(M + 1)^+$. Anal. calcd for $C_{36}H_{43}NO_{10}$: C, 66.55; H, 6.67; N, 2.15. Found: C, 66.42; H, 6.69; N, 2.00.

Methyl O-*(3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-propionamido-α-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (38)*

Propionic anhydride (200 μL) was added to a solution of **37** (350 mg) in methanol at 25 °C. After 10 min the solution was concentrated. Column chromatography using 4:1 ethyl acetate–hexane as eluant afforded amorphous **38** (350 mg, 92%): $[\alpha]_D$ +44° (c 0.3). CIMS: m/z 723 $(M + 18)^+$, 706 $(M + 1)^+$. Anal. calcd for $C_{36}H_{43}NO_{10}$: C, 66.36; H, 6.71; N, 1.99. Found: C, 66.27; H, 6.76; N, 1.97.

Methyl O-*(2-deoxy-2-propionamido-α-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (39)*

Sodium methoxide was added to a solution of **38** (250 mg) in methanol (5 mL) at 25 °C. After 3 h the solution was neutralized with Dowex 50 x 2 (H^+), and the solution was concentrated. A solution of the residue in 80% aq. acetic acid was warmed at 60 °C for 3 h. Removal of the volatiles followed by chromatographic purification using 18:1 ethyl acetate–hexane as eluant gave amorphous **39** (165 mg, 81%): $[\alpha]_D$ +31° (c 0.4). CIMS: m/z 576 $(M + 1)^+$. Anal. calcd for $C_{30}H_{41}NO_{10}$: C, 62.59; H, 7.18; N, 2.43. Found: C, 62.15; H, 7.28; N, 2.35.

Methyl O-*(2-deoxy-2-propionamido-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (2)*

A mixture of **39** (130 mg), 10% palladium-on-charcoal (200 mg), ethanol (15 mL), and acetic acid (15 mL) was stirred under hydrogen at atmospheric pressure, at 25 °C for 24 h. The usual work-up afforded amorphous **2** (75 mg, 84%): $[\alpha]_D$ +99° (c 0.4, H_2O). For NMR data, see Tables 1–3. FABMS: m/z 396 $(M + 1)^+$.

Methyl O-*[3,4,6-tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-(1→3)-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (40)*

Methyl trifluoromethanesulfonate (100 μL) was added to a stirred mixture of **11** (2.5 g), **35** (1.54 g), 2,6-di-*tert*-butyl-4-methylpyridine (1 g), 4A molecular sieves (4 g), and diethyl ether (60 mL) at 25 °C. More **11** (1 g) and methyl trifluoromethanesulfonate (500 μL) were added over a period of 24 h. The reaction was terminated by addition of triethylamine (3 mL). Work-up in the usual manner followed by chromatography (3:1 hexane–ethyl acetate) afforded first **41** (0.7 g): $[\alpha]_D$ +98° (c 0.6); NMR ($CDCl_3$): ^1H, δ 2.022 (CH_3S), 3.52–3.54 (m, 2H, H-6,6'), 3.788 (s, 3H, OCH_3), 3.790 (dd, 1H, H-3), 3.905 (dd, 1H, H-4), 4.247 (m, 1H, H-5), 4.273 (dd, 1H, $J_{2,3}$ 8.9 Hz, H-2), 4.36–4.96 [8d, 8H, CH_2 (Bn and MBn)] 5.340 (d, 1H, $J_{1,2}$ 5.5 Hz, H-1), 6.82–6.86, 7.22–7.36 (aromatic); ^{13}C, δ

12.3 (CH_3S), 69.2 (C-6), 69.6 (C-5), 72.1, 73.4 (2C), 74.7 [CH_2 (Bn and MBn)], 75.2 (C-4), 76.0 (C-2), 79.5 (C-3), 84.8 ($^1J_{C\text{-}1,H\text{-}1}$ 166 Hz, C-1), 113.7, 127.3–130.3, 138.1, 138.7, 138.8, 159.2 (aromatic). FABMS: m/z 599 $(M + 1)^+$. Anal. calcd for $C_{36}H_{40}O_6S$: C, 71.97; H, 6.71; S, 5.43. Found: C, 72.03; H, 6.73; S, 5.42.

Subsequent elution yielded amorphous **40** (2.2 g, 76%): $[\alpha]_D$ +83° (c 0.6). NMR ($CDCl_3$): ^1H, δ 1.353 (d, 1H, $J_{5,6}$ 6.8 Hz, H-6_A), 3.315 (s, 3H, CH_3O), 3.357 (dd, 1H, $J_{1,2}$ 3.4 Hz, $J_{2,3}$ 9.8 Hz, H-2_B), 5.423 (s, 1H, CHPh), 6.56–7.5 (aromatic); ^{13}C, δ 18.0 (C-6_A), 54.8, 55.2 (2 CH_3O), 68.5, 68.8 (C-6_B,6_C), 71.7, 72.5, 73.0, 73.3, 75.0, 75.9 [CH_2 (Bn and MBn)], 79.6, 79.7 (C-3_B,4_A), 83.0 (C-4_B), 93.8 ($J_{C\text{-}1,H\text{-}1}$ 171 Hz, C-1_B), 97.2 ($J_{C\text{-}1,H\text{-}1}$ 173 Hz, C-1_C), 98.1 ($J_{C\text{-}1,H\text{-}1}$ 168 Hz, C-1_A), 101.8 (CHPh), 126.2–130.2, 136.9–138.8 (aromatic). FABMS: m/z 1278 $(M + C_8H_9O - N_2)^+$, 1248 $(M + C_7H_7 - N_2)^+$, 1184 $(M + 1 - N_2)$, 1158 $(M + 1 - N_2)^+$, 1126 $(M + 1 - N_2 - MeOH)^+$. Anal. calcd for $C_{67}H_{75}N_3O_{15}$: C, 69.85; H, 6.37; N, 3.54. Found: C, 69.93; H, 6.42; N, 3.49.

Methyl O-(3,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-O-(2-azido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (42)

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (170 mg) was added to a stirred mixture of **40** (800 mg) in dichloromethane (20 mL) and water (4 mL) at 25 °C. After 4 h, the organic phase was washed with aq. $NaHCO_3$ and water. Removal of the volatiles followed by chromatography (2:1 hexane–ethyl acetate) gave amorphous **42** (520 mg, 72%) $[\alpha]_D$ +90° (c 1.3). NMR ($CDCl_3$): ^1H, 1.355 (d, 1H, $J_{5,6}$ 6.8 Hz, H-6_A), 3.207 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.0 Hz, H-2_B), 3.307 (s, 3H, CH_3O), 3.834 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 3 Hz, H-2_A), 5.025, 5.469 (2d, 2H, H-1_B,1_C), 5.519 (s, 1H, CHPh), 7.02–7.46 (aromatic); ^{13}C, δ 18.0 (C-6_A), 54.8 (CH_3O), 68.4, 68.6 (C-6_B,6_C), 72.3, 72.5, 73.4, 74.9, 76.0 (CH_2), 79.3. 79.5 (C-3_B,4_A), 82.3 (C-4_B), 94.0 (C-1_B), 98.0, 99.9 (C-1_A,1_C), 101.2 (CHPh), 126–128, 136.8–138.5 (aromatic). FABMS: m/z 1128 $(M + C_7H_7 - N_2)^+$, 1064 $(M + 1 - H_2)^+$, 1038 $(M + 1 - N_2)^+$, 1036 $(M + 1 - H_2 - N_2)$, 1006 $(M + 1 - N_2 - MeOH)$ Anal. calcd for $C_{61}H_{67}N_3O_{14}$: C, 68.71; H, 6.33; N, 3.94. Found: C, 68.80; H, 6.43; N, 3.93.

Methyl O-α-D-galactopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (3)

A solution of $NiCl_2 \cdot 6H_2O$ (1.2 g) and H_3BO_3 (0.6 g) in ethanol (30 mL) was added to a solution of **42** (400 mg) in 1,2-dimethoxyethane (1 mL). To this solution, stirred at 25 °C, was added a solution of sodium borohydride (300 mg) in ethanol (20 mL) in small portions over a period of 1 h. The resulting mixture was cooled to 0 °C and acetic anhydride (4 mL) was added. After 10 min the volatiles were removed under vacuum. Column chromatographic purification of the residue, using 1:1 hexane–ethyl acetate as eluant, afforded an amorphous solid (290 mg, FABMS: m/z 1082 $(M + 1)^+$). A solution of this solid (250 mg) in ethanol–acetic acid was hydrogenolyzed as described for **2**. The crude product was purified by gel filtration through a column of Biogel P-2, using 0.02 M pyridinium acetate as eluant to give amorphous **3**: $[\alpha]_D$ +125° (c 0.7, H_2O). For NMR data, see Tables 1–3. FABMS: m/z 582 $(M + K)^+$, 566 $(M + Na)^+$, 544 $(M + 1)^+$.

Methyl O-(2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-O-(2-azido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (43)

Methyl trifluoromethanesulfonate (280 μL) was added to a stirred mixture of **42** (260 mg), **22** (220 mg), 4A molecular sieves (2 g), and dichloromethane (30 mL) at 25 °C, over a period of 60 h. The customary work-up and chromatography (3:1 hexane–ethyl acetate) afforded amorphous **43** (350 mg, 94%): $[\alpha]_D$ +91° (c 0.7). NMR ($CDCl_3$): ^1H, δ 0.617, 1.370 (2d, 2H, H-6_A,6_B), 3.326 (s, 3H, CH_3O), 5.028 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1_C), 5.416 (t, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4_D), 5.85 (dd, 1H, H-2_D),5.935 (dd, 1H, H-3_D); ^{13}C, δ 17.4, 18.0 (C-6_A,6_B), 54.8 (CH_3O), 62.3 (C-2_A), 68.5, 68.7 (C-6_B,6_C), 72.5, 72.6, 73.4, 75.1, 76.0 (CH_2), 79.6, 79.7 (C-3_B,4_A), 83.4 (C-4_B), 93.7 (C-1_B), 97.2, 98.2 (2C) (C-1_B,1_C,1_D), 100.8 (CHPh), 126–138.4 (aromatic), 165.1, 165.8 (2C) (C=O). FABMS: m/z 1586 $(M + C_7H_7 - N_2)^+$, 1522 $(M + 1 - H_2)^+$, 1496 $(M + 1 - N_2)^+$, 1494 $(M + 1 - H_2 - N_2)^+$, 1464 $(M + 1 - N_2 - MeOH)^+$. Anal. calcd for $C_{88}H_{89}N_3O_{21}$: C, 69.32; H, 5.88; N, 2.75. Found: C, 70.34; H, 6.44; N, 2.64.

Methyl O-α-L-rhamnopyranosyl-(1→2)-O-α-D-galactopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (4)

A solution of **43** (300 mg) in 1,2-dimethoxyethane (0.5 mL) was treated first with a solution of $NiCl_2 \cdot 6\ H_2O$ and H_3BO_3 in ethanol, then with sodium borohydride in methanol as described for the preparation of **37**. *In situ N*-acetylation (acetic anhydride, 0 °C), followed by chromatographic purification using 2:1 → 1:1 hexane–ethyl acetate as eluant afforded an amorphous solid (220 mg) which was dissolved in methanol (10 mL). To this solution was added a catalytic amount of sodium methoxide at 25 °C. After 6 h, the solution was neutralized with Dowex 50 x 2 (H^+), then concentrated. Chromatography of the residue using 20:1 ethyl acetate–methanol afforded an amorphous solid (180 mg) which was stirred in a 6:1 mixture of ethanol–acetic acid (50 mL) in the presence of 10% palladium-on-charcoal (370 mg) under hydrogen, at 25 °C, under atmospheric pressure for 5 days. The usual work-up afforded amorphous **4** (104 mg): $[\alpha]_D$ +84° (c 0.2, H_2O). For NMR data, see Tables 1–3. FABMS: m/z 728 $(M + K)^+$, 712 $(M + Na)^+$.

O-(2,3,4-Tri-O-benzoyl-α-L-rhamnopyranosyl)-(1→3)-1-O-acetyl-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranose (44)

(a) Silver trifluoromethanesulfonate (2.3 g) was added to a stirred mixture of **23** (4.5 g), **29** (3.0 g), 2,6-di-*tert*-butyl-4-methylpyridine (1.6 g), and 4A molecular sieves (0.5 g) in dichloromethane (50 mL) at -40 °C. The mixture was stirred at -10 °C for 2 h. Work-up as described for **31** followed by chromatography using 4:1 hexane–ethyl acetate as eluant afforded amorphous **44** (6.0 g, 93%): $[\alpha]_D$ +103° (c 0.8). NMR ($CDCl_3$): ^1H, δ 1.220, 1.418 (2d, 2H, H-6_A,6_B), 3.788 (t, 1H, H-4_A), 3.923, 4.180 (2dq, 2H, H-5_A,5_B), 4.369 (dd, 1H, H-3_A), 4.822, 5.110 (2d, 2H, CH_2), 5.378 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1_B), 5.456 (dd, 1H, H-2_A), 5.607 (t, 1H, H-4_B). 5.751 (dd, 1H, H-3_B). 5.76 (dd, 1H, H-2_B), 6.228 (s, 1H, $J_{1,2}$ 1.9 Hz, H-1_A); ^{13}C, δ 17.4, 18.2 (C-6_A,6_B), 20.9 (CH_3CO), 76.0 (CH_2), 78.7 (C-3_A), 79.7 (C-4_A). 90.5 (C-1_A), 99.8 (C-1_B). CIMS: m/z 876 $(M + 18)^+$, 799 $(M + 1 - AcOH)^+$. Anal. calcd for $C_{49}H_{46}O_{14}$: C, 68.52; H, 5.40. Found: C, 68.40; H, 5.37.

(b) Methyl trifluoromethanesulfonate (150 μL) was added to a stirred mixture of **22** (105 mg), **29** (60 mg), 2,6-di-*tert*-butyl-4-methylpyridine (42 mg), 4A molecular sieves (100 mg), and dichloromethane (5 mL) at 25 °C, over a period of 2 days. The usual work-up followed by chromatography as described above afforded amorphous **44** (92 mg, 72%) which was identical to the preparation obtained in (a).

O-(2,3,4-Tri-O-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl chloride (45)

To a solution of **44** (500 mg) in dichloromethane (20 mL) at 0 °C was added α,α-dichlorodimethyl ether (2 mL), followed by $ZnCl_2 \cdot Et_2O$ (54%) in CH_2Cl_2 (100 μL). After 10 min the solution was extracted with aq. $NaHCO_3$ at 0 °C. Chromatographic work-up using 4:1 hexane–ethyl acetate afforded amorphous **45** (420 mg, 88%): $[\alpha]_D$ +87° (c 0.5). NMR ($CDCl_3$): ^1H, δ 1.245, 1.440 (2d, 2H, H-6_A,6_B), 4.170 (dq, 1H, H-5_A), 4.660 (dd, 1H, H-3_A), 4.85, 5.13 (2d 2H, CH_2), 5.410 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1_B), 5.616 (dd, 1H, H-2_B), 5.638 (t, 1H, H-4_B). 5.773 (dd, 1H, H-3_B), 5.822 (dd, 1H, H-2_A), 6.224 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1_A), 7.17–8.25 (aromatic); ^{13}C, δ 17.3, 17.7 (C-6_A,6_B), 77.4 (C-3_A), 79.2 (C-4_A), 89.5 ($J_{C\text{-}1,H\text{-}1}$ 183 Hz, C-1_A), 99.8 ($J_{C\text{-}1,H\text{-}1}$ 173 Hz, C-1_B), 127.9–137.5 (aromatic), 165.2, 165.3 (2C), 165.7 (C=O). CIMS: m/z 852 $(M + 18)^+$, 799 $(M + 1 - HCl)^+$. Anal. calcd for $C_{47}H_{43}ClO_{12}$: C, 67.58; H, 5.16; Cl, 4.24. Found: C, 67.57; H, 5.21; Cl, 4.27.

1-O-Acetyl-2,3,4-tri-O-benzoyl-α-L-rhamnopyranose (46)

Nitrosyl tetrafluoroborate (150 mg) was added to a stirred mixture of **22** (950 mg), **29** (500 mg), 4A molecular sieves (200 mg), and dichloromethane (15 mL) at 0 °C. After 20 min, the solution was extracted with ice-cold, aq. $NaHCO_3$ and concentrated. Column chromatography using 4:1 hexane–ethyl acetate as eluant afforded amorphous **46** (380 mg, 39%): $[\alpha]_D$ +135° (c 0.8). NMR ($CDCl_3$): ^1H, δ 1.391 (d, 1H, $J_{5,6}$ 6.2 Hz, H-6), 4.266 (dq, 1H, H-5), 5.70 (dd, 1H, H-2), 5.723 (t, 1H, H-4), 5.851 (dd, 1H, H-3), 6.307 (s, 1H, $J_{1,2}$ 1.7 Hz, H-1), 7.23–8.10 (aromatic); ^{13}C, δ 17.7 (C-6), 20.9 (CH_3CO), 69.1, 69.7 (2C), 71.2 (C-2,3,4,5), 90.8 (C-1), 128.8–133.6 (aromatic), 165.2, 165.6 (2C) [C=O (Bz)], 168.3 [C=O (Ac)]. CIMS: m/z 536 $(M + 18)^+$, 459 $(M + 1 - AcOH)^+$. Anal. calcd for $C_{29}H_{26}O_9$: C, 67.17; H, 5.05. Found: C, 67.38; H, 5.28.

Methyl O-(2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl)-(1→3)-O-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-O-(2-azido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (47)

Silver trifluoromethanesulfonate (310 mg) was added to a stirred mixture of **42** (700 mg), **45** (900 mg), 2,6-di-*tert*-butyl-4-methylpyridine (200 mg), 4A molecular sieves (1 g), and dichloromethane (25 mL) at -40 °C. The mixture was allowed to reach 10 °C in 2 h. Work-up as described for **31** followed by chromatography using 6:1 → 4:1 hexane–ethyl acetate as eluant afforded amorphous **47** (970 mg, 79%): $[\alpha]_D$ +88° (C 0.4). NMR ($CDCl_3$): ^1H, δ 0.762, 1.242, 1.333 (3d, 3H, H-6_A,6_D,6_E), 3.303 (s, 3H, CH_3O); ^{13}C, δ 17.6, 17.9, 18.1 (C-6_A,6_D,6_E), 54.7 (CH_3O), 68.4, 68.5 (C-6_B,6_C), 72.5, 72.8, 72.9, 74.9, 76.0 (CH_2), 79.0, 79.3, 79.5 (C-2_C,3_A,4_A), 80.4 (C-3_D), 83.1 (C-4_B), 93.6 (C-1_B), 96.9, 97.5, 98.2, 99.6 (C-1_A,1_C,1_D,1_E), 100.8 (CHPh), 126.4–138.5 (aromatic), 165.3, 165.4, 165.5 (2C) (C=O). FABMS: m/z 1927 $(M + C_7H_7 - N_2)^+$, 1864 $(M + 1)^+$, 1836 $(M + 1 - N_2)^+$, 799 $(C_{47}H_{43}O_{12})^+$, 459 $(C_{27}H_{23}O_7)^+$.

Methyl O-α-L-rhamnopyranosyl-(1→3)-O-(4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (48)

A solution of $NiCl_2 \cdot 6H_2O$ (1.0 g) and H_3BO_3 (0.6 g) in ethanol (30 mL) was added to a solution of **47** (850 mg) in 1,2-dimethoxyethane (1 mL). To this solution was added under stirring, at 25 °C, a solution of sodium borohydride (0.5 g) in ethanol (15 mL), in small portions over a period of 1 h. Extractive work-up, followed by column chromatography using 2:1 hexane–ethyl acetate as eluant gave a solid glass (610 mg) which was dissolved in a 5:1 mixture of methanol-1,2-dimethoxyethane (20 mL). To this solution was added acetic anhydride (1 mL) at 0 °C. After 10 min the volatiles were removed under vacuum. A solution of the residue in methanol (10 mL) was treated with a catalytic amount of sodium methoxide for 2 days. The solution was neutralized with Dowex 50 x 2 (H^+) and concentrated. Column chromatography of the residue using ethyl acetate as eluant gave amorphous **48** (250 mg, 37%): $[\alpha]_D$ +23° (c 0.2). FABMS: m/z 1502 $(M + K)^+$, 1486 $(M + Na)^+$, 1464 $(M + 1)^+$. Anal. calcd for $C_{82}H_{97}NO_{23}$: C, 67.24; H, 6.67; N, 0.96. Found: C, 66.93; H, 6.68; N, 0.95.

Methyl O-α-L-rhamnopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-O-α-D-galactopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (5)

A solution of **48** (320 mg) in a 1:1 mixture of ethanol–acetic acid (40 mL) was hydrogenolyzed as described for **2**. The usual work-up afforded amorphous **5** (163 mg, 92%): $[\alpha]_D$ +44° (c 1.1, H_2O). For NMR spectral data, see Tables 1–3. FABMS: m/z 836 $(M + 1)^+$.

Acknowledgements

We are grateful to Drs Cornelis P. J. Glaudemans, John B. Robbins, and Rachel Schneerson for their helpful comments during the preparation of this manuscript. We thank Dr Lewis Pannell, Mr Noel Whittaker, and Ms Towanda Carroll for the FAB and CI mass spectra.

References and Notes

1. For a preliminary publication see: Pozsgay, V.; Glaudemans, C. P. J.; Robbins, J. B.; Schneerson, R. *BioMed. Chem. Lett.* **1992**, *2*, 255.

2. Chu, C. Y.; Liu, B.; Watson, D.; Szu, S.; Bryla, D.; Shiloach, J.; Schneerson, R.; Robbins, J. B. *Infect. Immunol.* **1991**, *59*, 4450.

3. Levine, M. M. *Lancet* **1990**, *335*, 958.

4. Cohen, M. L. *Science* **1992**, *257*, 1050.

5. Robbins, J. B.; Chu, C.; Schneerson, R. *Clin. Inf. Dis.* **1992**, *15*, 346.

6. For recent developments on the synthesis of carbohydrate antigens of other human pathogenic bacteria, see: (a) *Carbohydrate Antigens, ACS Symp. Ser.* **1993**, 519; (b) Bundle, D. R. *Topics Curr. Chem.* **1990**, *154*, 1.

7. (a) Chemical repeating unit: Dmitriev, B. A.; Knirel, Y. A.; Kochetkov, N. K.; Hofman, I. L. *Eur. J. Biochem.* **1976**, *66*, 559; (b) Biological repeating unit: Sturm, S.; Jann, B.; Fortnagel, P.; Timmis, K. N. *Microbial Pathogenesis* **1986**, *1*, 307.

8. For a recent review on the synthesis of oligosaccharides, see: Kanie, O.; Hindsgaul, O. *Curr. Opin. Struct. Biol.* **1992**, *2*, 674; which contains a number of references to earlier reviews on this subject.

9. Syntheses of fully protected, frame-shifted tetrasaccharide repeating units that permit further chain elongation were described: (a) Pozsgay, V.; Glaudemans, C. P. J.; Robbins, J. B.; Schneerson, R. *Tetrahedron* **1992**, *48*, 10249; (b) Pozsgay, V.; Glaudemans, C. P. J.; Robbins, J. B.; Schneerson, R. *Carbohydr. Res.* **1993**, *244*, 259. For the synthesis of related oligosaccharides, see: (c) Pozsgay, V.; Brisson, J.-R.; Jennings, H. J. *Can. J. Chem.* **1987**, *65*, 2764; (d) Garegg, P. J.; Hallgren, C. *J. Carbohydr. Chem.* **1992**, *11*, 445; (e) Pavliak, V.; Kovac, P.; Glaudemans, C. P. J. *Carbohydr. Res.* **1992**, *229*, 103; (f) Kovac, P.; Edgar, K. J. *J. Org. Chem.* **1992**, *57*, 2455; (g) Pozsgay, V.; Coxon, B.; Kovac, P.; Glaudemans, C. P. J. Manuscript in preparation.

10. For a review of the development of this concept, see: Greenspan, N. S. *Bull. Inst. Pasteur* **1992**, *90*, 267.

11. For recent reviews on studies of interactions between proteins and carbohydrates at the atomic level see: (a) Vyas, N. K. *Curr. Opin. Struct. Biol.* **1991**, *1*, 732. (b). Bundle, D. R.; Young, N. M. *Curr. Opin. Struct. Biol.* **1992**, *2*, 666.

12. For a detailed, quantitative characterization of the interaction between a monoclonal, anti-*Shigella dysenteriae* type 1 antibody and the oligosaccharides described in this paper, see: Pavliak, V.; Nashed, E. M.; Pozsgay, V.; Kovac, P.; Karpas, A.; Chu, C.; Schneerson, R.; Robbins, J. B.; Glaudemans, C. P. J. *J. Biol. Chem.* **1993**, *268*, in press.

13. Hanessian, S.; Lavallee, P. *Can. J. Chem.* **1975**, *53*, 2975.

14. Liptak, A.; Imre, J.; Nanasi, P. *Carbohydr. Res.* **1981**, *92*, 154.

15. (a) Catelani, G.; Colonna, F.; Marra, A. *Carbohydr. Res.* **1988**, *182*, 297; (b) Buchanan, J. G.; Saunders, R. M. *J. Chem. Soc.* **1964**, 1796; (c) Wolfrom, M. L.; Shafizadeh, F.; Armstrong, R. K.; Shen Han, T. M. *J. Am. Chem. Soc.* **1959**, *81*, 3715.

16. (a) Lonn, H. *Carbohydr. Res.* **1985**, *139*, 105; (b). Lonn, H. *J. Carbohydr. Chem.* **1987**, *6*, 301.

17. For a comprehensive review of glycosyl donors and methods for their activation, see: Lockhoff, O. In *Methoden der organischen Chemie (Houben-Weyl)*, Vol. E14a/3, p. 621, Hagemann, H.; Klamann, D., Eds.; G. Thieme Verlag; Stuttgart, 1992.

18. (a) Oikawa, Y.; Yoshioko, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 885; (b) Johansson R.; Samuelsson, B. *J. C. S. Perkin I* **1984**, 2371.

19. For further examples of the use of the 4-methoxybenzyl protecting group in oligosaccharide syntheses, see: (a) Pozsgay, V.; Jennings, H. J. *J. Carbohydr. Chem.* **1987**, *6*, 41; (b) Pozsgay, V.; Brisson, J.-R.; Jennings, H. J.; Allen, S.; Paulson, J. C. *J. Org. Chem.* **1991**, *56*, 3377; (c) Jain, R. K.; Locke, R. D.; Matta, K. L. *Carbohydr. Res.* **1991**, *212*, c1; (d) Jain, R. K.; Sarkar, A. K.; Matta, K. L. *Carbohydr. Res.* **1991**, *220*, c1.

20. For earlier observations, leading to the introduction of the related, non-nucleophilic base 2,6-di-*tert*-butylpyridine in oligosaccharide synthesis, see: van Boeckel, C. A. A.; Beetz, T.; Vos, J. N.; de Jong, A. J. M.; van Aelst, S. F.; van den Bosch, R. H.; Mertens, J. M. R.; van der Vlugt, F. A. *J. Carbohydr. Chem.* **1985**, *4*, 293.

21. Gross, H.; Farkas, I. *Chem. Ber.* **1960**, *93*, 95.

22. Pozsgay, V. *Carbohydr. Res.* **1979**, *69*, 284.

23. Pozsgay, V.; Jennings, H. J. *J. Org. Chem.* **1988**, *53*, 4042.

24. Haines, A. H. *Carbohydr. Res.* **1969**, *10*, 466.

25. (a) Garegg, P. J.; Hultberg, H. *Carbohydr. Res.* **1979**, *72*, 276; (b) Wessel, H.-P.; Bundle, D. R. *J. Chem. Soc. Perkin Trans. 1* **1985**, 2251.

26. King, J. F.; Allbutt, A. D. *Can. J. Chem.* **1970**, *48*, 1754.

27. In earlier experimental protocols, similar conversions were performed in *N,N*-dimethylformamide at room temperature for 48 h (Ref. 25a) or at 50° for 1 h (Ref. 25b). Reported conditions for acetonitrile as the solvent: room temperature, 5 h (Wessel, H.-P.; Bundle, D. R. *Carbohydr. Res.* **1983**, *124*, 301); room temperature, 16 h (Tsvetkov, Y. E.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1989**, *193*, 75).

28. van Boeckel, C. A. A.; Beetz, T. *Tetrahedron Lett.* **1983**, *24*, 3775.

29. Paulsen, H.; Lorentzen, J. P. *Carbohydr. Res.* **1987**, *165*, 207.

30. Related findings include: (a) van der Klein, P. A. M.; Filemon, W.; Boons, G. J. P. H.; Veeneman, G. H.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1992**, *48*, 4649; (b) Paulsen, H. In *New Synthetic Methodology and Functionally Interesting Compounds*, Yoshida, Z-I., Ed.; Kodansha/Elsevier, 1986.

31. Paulsen, H.; Sinwell, V. *Chem. Ber.* **1978**, *111*, 869.

32. Although the non-participating properties of the MBn group at *O*-2 of thioglycoside-based glycosyl donors were exploited previously (Refs 19c and d) the reported yields were low even with highly reactive, primary hydroxyl group-containing acceptors and with the donors employed in excess. This is probably due to acid-induced decomposition of the 4-methoxybenzyl group-containing starting compounds and/or products.

33. Pozsgay, V. In *Carbohydrates, Synthetic Methods and Applications in Medicinal Chemistry*, p. 188, Suami, T.; Hasegawa, A.; Ogura, H., Eds.; Kodansha/VCH, 1992.

34. Erbing, B.; Lindberg, B. *Acta Chem. Scand., Ser. B* **1976**, *30*, 611.

35. For a number of examples, see Ref. 17, p 776.

36. (a) Pozsgay, V.; Jennings, H. J. *J. Org. Chem.* **1987**, *52*, 4635; (b) Pozsgay, V.; Jennings, H. J. *Carbohydr. Res.* **1988**, *178*, 61; (c) Ref. 23.

37. Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229.

38. Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355.

39. Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565.

40. Bax, A. *J. Magn. Reson.* **1983**, *53*, 517.

41. Doddrell, D. M.; Pegg, D. T.; Bendall, M. L. *J. Magn. Reson.* **1982**, *48*, 323.

42. Bock, K.; Pedersen, C. *Acta Chem. Scand., Ser B.* **1975**, *29*, 258.

43. A report of this phenomenon has been given by Byrd, R. A. *12th Blue Hen NMR Symp.*, University of Delaware, Newark, Delaware, June 8, 1992.

44. Pozsgay, V. unpublished results.

45. States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286.

46. Ness, R. K.; Fletcher, Jr, H. G.; Hudson, C. S. *J. Am. Chem. Soc.* **1950**, *72*, 2200.