Carbohydrate Research, 204 (1990) 167–186
Elsevier Science Publishers B.V., Amsterdam

Synthesis of specifically deoxygenated ligands related to (1→6)-β-D-galacto-oligosaccharides, and studies on their binding to monoclonal antigalactan antibodies

Thomas Ziegler*, Viliam Pavliak†, Tsu-Hsing Lin‡, Pavol Kováč, and Cornelis P. J. Glaudemans
NIDDK, National Institutes of Health, Bethesda, Maryland 20892 (U.S.A.)

(Received November 1st, 1989; accepted for publication January 10th, 1990)

ABSTRACT

Synthetic deoxygenated derivatives of methyl β-glycosides of (1→6)-β-D-galacto-oligosaccharides were prepared, and their binding to antigalactan monoclonal antibodies X24 and J539 (Fab′) was studied. The results suggest the involvement of an additional, critical hydrogen bond in the highest affinity subsite (A), which now appears to require two hydrogen bonds *from* the 2- and 3-hydroxyls of the galactosyl residue to the protein, and one from the protein to O-4 of that residue. The data obtained with a series of oligosaccharides deoxygenated at position 3^1, 3^2, 3^3, 4^1, 4^2, or 4^3 support the binding patterns and subsite-arrangement inferred previously from studies with large numbers of deoxyfluoro-substituted ligands and this family of antibodies.

INTRODUCTION

This laboratory has reported on the subsite arrangements of monoclonal antigalactan[1a] and antidextran[2] antibodies. Hitherto, we have only evaluated (oligo)saccharides bearing deoxyfluoro groups at specific locations in order to elucidate the binding patterns. We here report the use of corresponding and related deoxygenated saccharides for a dual purpose: these experiments could verify the previous results, and also might reveal the presence of additional hydrogen bonds playing a role in the binding of ligands. Changes in binding resulting from the replacement of a hydroxyl group by fluorine or hydrogen in carbohydrate ligands that bind with proteins are interpreted in the following way: if a hydroxyl group is replaced by fluorine, and diminished (or no) binding is the result, it indicates H-bonding from the ligand to the protein (if no change in binding takes place there could still be H-bonding from the protein to the ligand, since fluorine can be a proton acceptor). If a hydroxyl group was replaced by fluorine and no change in binding occurred, and the hydroxyl group at the same position is now replaced by hydrogen, causing diminished (or no) binding, it indicates H-bonding from the protein to that hydroxyl of the carbohydrate ligand. Finally, if a hydroxyl group is

---

[*] On leave from the Institute of Organic Chemistry, University of Stuttgart, F. R. G.

[†] On leave from the Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Czechoslovakia.

[‡] Visiting Fellow, 1987–1988.

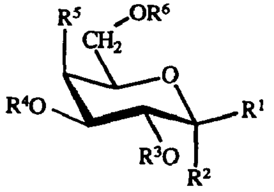
replaced sequentially by fluorine and hydrogen, and in either case no change of binding occurs, it indicates that that hydroxyl is not involved in H-bonding to the protein.

Based on the studies with deoxyfluoro sugars we have proposed[1a] that binding to antigalactan antibodies of the X24 genetic family[1b] is mediated by hydrogen bonds from the 2- and 3-hydroxyls of the galactosyl residue bound in the highest affinity subsite (A). Thus, that site should not bind a galactosyl residue deoxygenated at position 3. With a view to testing this inference, saccharides containing such a moiety were prepared (see Table IV for references). In the present work the methyl β-glycosides of (1→6)-linked di- and tri-D-galacto-oligosaccharides containing a 4-deoxy-β-D-*xylo*-hexopyranosyl residue at each of the possible positions were prepared, and these and the previously synthesized saccharides were evaluated for binding.

RESULTS AND DISCUSSION

Synthesis. — We have previously described[3] improved syntheses of the 2-, 3-, and 4-deoxy derivatives of methyl β-D-galactopyranoside (**1**) using tributyltin hydride reduction of the corresponding imidazolethiocarbonyl derivatives for the deoxygenation step. In order to prepare β-(1→6)-linked D-galacto-oligosaccharides having 4-deoxy-β-D-*xylo*-hexopyranosyl units as any desired residue in the chain, we have now extended this methodology to the deoxygenation of various methyl 6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranosides. Selective regeneration of OH-6 in the products then allowed further extension of the oligosaccharide chain at that position. The partial benzoylation of methyl 6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (**2**), which was prepared from methyl β-D-galactopyranoside (**1**) by a modification of the published procedures[4,5], resulted in a mixture of compounds **3**, **4**, **5**, and **6** (10%, 5%, 14%, and 70%, respectively), all of which were isolated by chromatography and fully characterized. Treatment of the main product **6** with thiocarbonyldiimidazole (TCDI) gave methyl 2,3-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-4-*O*-(imidazol-1-ylthiocarbonyl)-β-D-galactopyranoside (**7**, 94%), which reacted smoothly with tributyltin hydride to give the corresponding 4-deoxy glycoside **8** (95%).

This laboratory reported the use of the *tert*-butyldiphenylsilyl group in the synthesis of galacto-oligosaccharides[4], and the use of methanolic HCl for desilylations. The latter procedure requires much solvent, and so is not convenient for work on a large scale. Instead, we have found that the BF_3–methanol complex is a more efficient reagent for desilylation. For example, treatment of a relatively concentrated solution (*cf.* ref. 4) of methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (**3**) with this reagent furnished compound **17** in a virtually theoretical yield. Analogously, selective deprotection of compound **8** with the same reagent yielded methyl 2,3-di-*O*-benzoyl-4-deoxy-β-D-*xylo*-hexopyranoside (**9**, 91%), which on debenzoylation, gave methyl 4-deoxy-β-D-*xylo*-hexopyranoside (**10**). Alternatively the glycosyl acceptor **9**, which allows further extension of the chain at O-6, was synthesized from independently prepared[3] deoxyglycoside **10** *via* tritylation and benzoylation in a one-pot procedure, followed by iodotrimethylsilane-mediated detritylation of the formed intermediate **11**.



Cpd.	R^1	R^2	R^3	R^4	R^5	R^6
1	OMe	H	H	H	OH	H
2	OMe	H	H	H	OH	TBDPS
3	OMe	H	Bz	Bz	OBz	TBDPS
4	OMe	H	H	Bz	OBz	TBDPS
5	OMe	H	H	Bz	OH	TBDPS
6	OMe	H	Bz	Bz	OH	TBDPS
7	OMe	H	Bz	Bz	OTCI	TBDPS
8	OMe	H	Bz	Bz	H	TBDPS
9	OMe	H	Bz	Bz	H	H
10	OMe	H	H	H	H	H
11	OMe	H	Bz	Bz	H	Tr
12	OMe	H	Bz	Bz	H	Bz
13	H	Cl	Bz	Bz	H	Bz
14	H	OMe	Bz	Bz	H	Bz
15	H	Cl	Bz	Bz	H	TBDPS
16	H	Br	Bz	Bz	OBz	Bz
17	OMe	H	Bz	Bz	OBz	H
18	OMe	H	Bz	Bz	OH	H
19	H	Cl	Bz	Bz	OBz	TBDPS

TBDPS = *tert*-Butyldiphenylsilyl; OCTI = Imidazol-1-ylthiocarbonyloxy

The construction of methyl β-glycosides of β-(1→6)-linked D-galacto-oligosaccharides containing a terminal 4-deoxygenated D-galactosyl group requires a 4-deoxy-D-*xylo*-hexopyranosyl donor. This was readily obtained by treatment of methyl 2,3,6-tri-*O*-benzoyl-4-deoxy-β-D-*xylo*-hexopyranoside[3] (**12**) with dichloromethyl methyl ether (DCMME), which yielded the crystalline chloride **13**. As we have observed in other conversions[6,7] of methyl β-glycosides to glycosyl chlorides by DCMME, a small amount of compound **14**, formed by anomerization of the starting material, could also be isolated. Under these conditions, compound **8** was converted into the complex glycosyl donor **15** (85%), which permits extension of the saccharide chain at the primary position of the 4-deoxy-D-*xylo*-hexopyranosyl group after coupling to an acceptor. The facile, high yielding reduction of imidazolethiocarbonyl derivatives, previously used on simple galactosides[3], was also successfully applied to the deoxygenation of suitably protected disaccharides. Silver trifluoromethanesulfonate (silver triflate)-promoted, selective galactosylation of methyl 2,3-di-*O*-benzoyl-β-D-galactopyranoside[8] (**18**) with 2,3,4-tri-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranosyl chloride[4] (**19**), or with 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide[9] (**16**), under carefully controlled conditions, gave mainly the O-4^1 unprotected β-(1→6)-linked disaccharide glycosides **20** and **21**, respectively, isolated in 73% and 81% yields. The ^{13}C-n.m.r. spectra of the latter compounds (Table III) clearly showed that the 6^1 positions were

TABLE I

^{1}H-N.m.r. data

Compound	*Chemical shifts (δ) and multiplicities*[a,b]									
	H-1	*H-2*	*H-3*	*H-4a*	*H-4b*	*H-5*	*H-6a*	*H-6b*	*OCH$_3$*	$(CH_3)_3C$
4	4.40d	4.01dd	5.39dd	5.96bd		3.98bt	3.79m	3.79m	3.69s	0.99s
5	4.29d	4.04dd	5.08dd	4.34bd		3.64bt	3.98dd	3.92dd	3.56s	1.05s
6	4.60d	5.79dd	5.32dd	4.46bd		3.75bt	4.04dd	3.98dd	3.51s	1.07s
7	4.96d	5.59dd	5.69dd	6.56bd		4.08bt	3.84dd	3.72dd	3.51s	1.02s
8[c]	4.78d	5.28dd	5.49ddd	2.44ddd	1.93–1.81m	4.06–4.00m	3.93dd	3.89dd	3.49s	1.08s
9[c]	4.75d	5.26dd	5.49ddd	2.39ddd	1.78m	3.89m	3.77–3.62m	3.77–3.62m	3.47s	
11[c]	4.80d	5.29dd	5.48ddd	2.39ddd	1.87m	4.08m	3.34dd	3.25dd	3.53s	
13	6.51d	5.48dd	5.87ddd	2.56ddd	2.01m	4.72m	4.68–4.46m	4.68–4.46m		
14	5.17d	5.32dd	5.79ddd	2.47ddd	1.90m	4.50–4.33m	4.50–4.33m	4.50–4.33	3.45s	
15	6.49d	5.41dd	5.84ddd	2.44ddd	1.98bq	4.41m	3.79d	3.79d		1.08s
20	4.43d	5.68–5.61m	5.68–5.61m	4.22bt		3.84–3.77m	4.14dd	3.84–3.77m	3.21s	
	4.89d	5.72dd	5.21dd	6.03bd		4.08bt	3.95dd	3.84–3.77m	1.00s	
21	4.47d	5.68dd	5.25dd	4.31bs		4.37bt	4.65dd	4.46dd	3.23s	
	4.99d	5.83dd	5.65dd	6.01bd		3.89bt	4.23dd	4.03dd		
22	4.56d	5.56–5.54m	5.56–5.54m	6.39bd		4.15bt	4.06dd	3.67–3.64m	3.24s	
	4.89d	4.68dd	5.59dd	6.02bd		4.00bt	3.78dd	3.67–3.64m		0.96s
23	4.62d	5.61–5.54m	5.61–5.54m	6.46bd		4.24bt	4.16dd	3.89dd	3.27s	
	4.89d	5.78dd	5.61–5.51m	5.96bd		4.33–4.29m	4.49dd	4.33–4.29m		
24	4.37d	5.27–5.24m	5.27–5.24m	2.24–2.19m	1.70–1.58m	3.85–3.78m	3.94dd	3.72dd	3.19s	
	4.88d	5.73dd	5.63dd	6.04bd		4.09bt	3.85–3.78m	3.85–3.78m		1.00s
25	4.47–4.34m	5.29–5.27m	5.29–5.27m	2.28bdd	1.69m	3.91–3.77m	4.04dd	3.91–3.77m	3.23s	
	4.97d	5.84dd	5.64dd	6.02bd		4.47–4.34m	4.71dd	4.47–4.34m		
26	4.39d	5.29–5.22m	5.29–5.22m	2.26bdd	1.65–1.59m	3.84–3.73m	3.96dd	3.84–3.73m	3.21s	
	4.87d	5.82dd	5.83dd	4.37bd		4.10bt	4.72dd	4.62dd		
27	4.56d	5.79dd[d]	5.58dd[d]	5.95bd[d]		4.46m	4.30–4.17m	3.87m	3.23s	
	4.92d	5.69dd[d]	5.52dd[d]	5.87bd[d]		4.30–4.17m	4.30–4.17m	4.30–4.17m		
28	6.42d	5.72dd	5.96dd	6.01bd[d]		4.77bt	4.38dd	3.90dd		
	4.90d	5.77dd	5.57dd	5.93bd[d]		4.24bt	4.22–4.13m	4.22–4.13m		
30	4.54d	5.67dd	4.50dd	5.85bd		4.15–4.07m	4.15–4.07m	3.83dd	3.24s	
	4.76d	5.43–5.38m	5.43–5.38m	2.43m	1.87m	4.01m	4.40–4.31m	4.40–4.31m		

31	4.50d	5.65dd	5.47dd	5.79bd		3.81–3.61m	4.09–4.00m	3.81–3.61m	3.17s	
	4.68d	5.38–5.35m	5.38–5.35m	2.40ddd	1.80–1.65m	3.81–3.61m	4.09–4.00m	3.81–3.61m		0.99s
32	4.58d	5.69dd	5.53dd	5.97bd		3.51–3.44m	4.09–4.02m	4.09–4.02m	3.36s	
	4.67d	5.36–5.32m	5.36–5.32m	2.23–2.16m	1.92–1.88m	3.87–3.82m	3.66–3.62m	3.51–3.44m		
35	4.70d	5.67dd[d]	5.51dd[d]	5.82bd[d]		4.03–3.97m[d]	3.80dd[d]	3.65–3.54m[d]	3.26s	
	4.56d	5.65dd[d]	5.43dd[d]	5.78bd[d]		4.03–3.97m[d]	3.76dd[d]	3.65–3.54m[d]		
	4.48d	5.38–5.23m	5.38–5.23m	2.41bdd	1.69–1.57m	4.12bdd	3.44dd	3.65–3.54m[d]		0.99s
36	4.78d	5.73–5.68m	5.52dd	5.97bd[d]		4.16–4.07m	3.84–3.76m	3.84–3.76m[d]	3.25s	
	4.56d	5.73–5.64m	5.52dd	5.87bd[d]		4.16–4.07m	3.84–3.76m	3.84–3.76m[d]		
	4.49d	5.28m	5.28m	2.13m	1.86m	4.01m	3.35m[d]	3.35m[d]		
37	4.74d	5.69dd	5.56–5.40m	5.86m		4.27–3.66m	4.27–3.66m	4.27–3.66m	3.29s	
	4.61d	5.69dd	5.56–5.40m	5.86m		4.27–3.66m	4.27–3.66m	4.27–3.66m		
	4.61d	5.56–5.40m	5.56–5.40m	2.40bdd	1.84m	3.96m	4.27–3.66m	4.27–3.66m		
39	4.81d	5.67dd	5.63dd[d]	6.00bd[d]		3.77–3.67m	3.99dd[d]	3.77–3.67m	3.24s	
	4.58d	5.33–5.19m	5.33–5.19m	2.29bdd	1.78–1.60m	4.08m	4.71dd	4.42dd		
	4.55d	5.77dd	5.49dd[d]	5.83bd[d]		4.33bt	3.92dd[d]	3.77–3.67m		
41	4.33d	5.23–5.19m	5.23–5.19m	2.20bd	1.48–1.36m	4.42m	3.76–3.65m	3.89dd	3.17s	
	4.90d[d]	5.78dd[d]	5.57dd[d]	5.94bd[d]		4.33–4.18m	4.33–4.18m	3.76–3.65m		
	4.81d[d]	5.74dd[d]	5.54dd[d]	5.90bd[d]		3.76–3.65m	3.76–3.65m	3.54dd		

---

[a] Data in the 1st row of each entry refer to sugar residue 1; data in the 2nd and 3rd row, if present, refer to sugar residues 2 and 3, respectively. Peak multiplicities: b, broad; d, doublet; m. multiplet; q, quartet; s, singlet; t, triplet. [b] Measured in chloroform-*d* unless otherwise indicated. [c] Measured in acetone-d_6. [d] Assignments in the same column may be reversed.

TABLE II

^{1}H-N.m.r. coupling constants

Compound	Coupling constants (Hz)									
	$J_{1,2}$	$J_{2,3}$	$J_{3,4a}$	$J_{3,4b}$	$J_{4a,4b}$	$J_{4a,5}$	$J_{4b,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
4	7.7	10.1	3,4			<1		[a]	[a]	[a]
5	7.6	10.0	3.1			<1		6.1	4.9	10.7
6	7.9	10.2	3.1			<1		5.7	4.7	10.6
7	7.6	10.5	3.2			<1		5.8	8.2	10.3
8	8.0	9.7	5.4	11.5	12.6	1.8	[a]	4.7	4.5	10.0
9	7.8	9.7	5.4	11.6	11.6	1.9	11.6	[a]	[a]	[a]
11	7.8	9.4	5.4	11.4	11.4	1.9	11.4	5.4	4.5	9.6
13	3.9	9.9	5.2	12.9	12.9	2.2	12.9	[a]	[a]	[a]
15	3.8	10.0	5.1	11.4	12.9	2.2	[a]	[a]	[a]	[a]
20	7.9	[a]	[a]			<1		4.6	[a]	10.7
	7.7	10.4	3.1			<1		7.0	[a]	10.6
21	7.9	10.2	3.1			<1		6.5	6.3	11.3
	7.8	10.4	3.4			<1		4.9	6.9	10.6
22	7.5	[a]	[a]			<1		5.7	[a]	10.0
	7.6	10.4	3.3			<1		7.0	[a]	10.0
23	7.4	[a]	[a]			<1		5.1	7.1	10.0
	7.8	10.4	3.3			<1		8.7	[a]	13.6
24	7.6	[a]	[a]	[a]	[a]	[a]	[a]	2.9	7.0	10.4
	7.8	10.5	3.3			<1		6.9	[a]	[a]
25	[a]	[a]	[a]	12.3	12.3	2.0	12.3	2.6	[a]	10.2
	7.9	10.4	3.3			<1		6.3	[a]	11.0
26	7.7	[a]	3.1	[a]	12.9	[a]	[a]	2.6	[a]	9.9
	7.9	10.3	3.1			<1		6.4	6.4	11.4
27	7.9	10.4	3.4[b]			<1		[a]	[a]	[a]
	7.8	10.4	3.3[b]			<1		[a]	[a]	[a]
28	3.8	10.4	3.3			<1		5.3	7.2	10.7
	7.9	10.4	3.3			<1		[a]	[a]	[a]
30	7.9	10.4	3.2			<1		[a]	[a]	[a]
	7.3	[a]	1.7	11.5	11.5	1.7	11.5	[a]	[a]	[a]
31	7.9	10.4	3.4			<1		[a]	[a]	[a]
	7.5	[a]	[a]	[a]	~13	3.5	[a]	[a]	[a]	[a]
32	7.9	10.4	3.3			<1		[a]	[a]	[a]
	7.4	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
35	7.9	10.3[b]	3.2[b]			<1		6.2[b]	[a]	[a]
	7.8	10.4[b]	3.4[b]			<1		5.8[b]	[a]	[a]
	7.3	[a]	4.0	[a]	12.0	[a]	[a]	6.5[b]	[a]	[a]
36	7.8	10.4	3.3			<1		[a]	[a]	[a]
	7.8	10.4	3.3			<1		[a]	[a]	[a]
	7.3	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
37	7.7	10.2	3.3	[a]				[a]	[a]	[a]
	7.7	10.2	3.3					[a]	[a]	[a]
	7.9	[a]	[a]	11.6	11.6	2.8	11.6	[a]	[a]	[a]
39	7.8	10.4	3.3[b]			<1		4.1[b]	[a]	10.5[b]
	7.4	[a]	~4	~12	~12	[a]	[a]	6.3	6.8	11.0
	7.9	10.4	3.4[b]			<1		4.2[b]	[a]	9.8[b]
41	7.5	[a]	[a]	[a]	[a]	[a]	[a]	[a]	8.5	11.2
	7.8[b]	10.7[b]	3.3[b]			<1		[a]	[a]	[a]
	7.9[b]	11.0[b]	3.4[b]			<1		[a]	6.5	10.3

[a] Undetermined owing to overlap of signals. [b] Assignments in the same column may be reversed.

TABLE III

^{13}C-N.m.r. chemical shifts

Compound	*Chemical shifts (δ)*[a]							
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*	*$(CH_3)_3C$*
4	104.3	70.2	73.7[b]	68.0	73.8[b]	61.4	57.4	19.0
5	104.3	69.6	74.1	68.0	76.0	63.3	57.0	19.2
6	102.3	69.7	74.2	68.1	74.5	63.4	56.5	19.2
7	102.3	69.9	71.7	76.2	73.7	61.3	57.0	19.0
8	102.1	73.0[b]	72.0[b]	33.1	72.4[b]	66.0	56.6	19.3
9	102.3	72.8[b]	71.8[b]	32.3	72.4[b]	64.9	57.0	
10[c]	103.7	75.1	70.6	35.1	72.7	63.7	57.2	
11	102.1	72.9[b]	71.2[b]	33.5	72.0[b]	65.8	56.5	
13	92.0	72.0[b]	67.8[b]	32.5	69.0[b]	65.3		
14	97.9	72.7[b]	66.1[b]	33.2	68.4[b]	65.4	55.4	
15	92.6	72.4[b]	68.2[b]	32.3	71.4[b]	65.4		19.3
20	102.1	70.0[d]	73.7	67.6	74.1	68.0	56.5	
	101.5	69.6[d]	71.9	67.9	74.1	61.4		19.0
21	102.1	69.7[d]	71.6	67.5	74.1[d]	68.5	56.5	
	101.8	69.6[d]	71.6	68.3	73.7[d]	62.2		
22	102.1	70.0[d]	72.7	76.6	71.3	66.7	56.8	
	101.0	69.7[d]	71.8	67.7	73.8	60.9		
23	102.1	69.8[d]	71.6	76.8	71.4	67.0	56.8	
	101.1	69.7[d]	71.6[b]	68.0	72.7[b]	61.8		
24	102.0	72.7[b]	71.0[b]	32.9	71.6[b]	71.1	56.5	
	101.6	70.1	71.9	68.0	74.0	61.5		19.1
25	102.0	72.7[b]	71.6[b]	32.9	71.7[b,e]	71.0	56.5	
	101.7	69.8	71.6[e]	68.1	71.2	62.1		
26	102.0	72.7[b]	71.0[b]	32.9	71.6[b]	70.9	56.5	
	101.5	69.6	74.0	67.4	72.6	62.8		
28	91.4	68.0	69.0	68.5	71.7	67.1		
	101.4	69.7	71.7	68.0	71.4	61.7		
29[c]	103.7	75.2[e]	70.8[b]	34.4	70.4[b]	71.5	57.4	
	103.4	71.5	72.8	68.7	74.9[e]	61.1		
30	102.2	69.9	71.8	69.8	73.3	68.8	56.8	
	101.3	76.6[b]	71.5[b]	33.0	72.6[b]	68.2		
31	102.1	69.9	71.8	68.9	73.5	68.0	56.7	
	101.2	73.5[b]	71.8[b]	33.1	72.3[b]	65.7		19.2
32	102.4	69.8	71.9[d]	68.5	72.9[e]	67.4	57.0	
	101.1	72.8[b,e]	71.6[b,d]	31.9	72.4[b]	64.4		
33[c]	103.9	70.7[e]	72.7[f]	68.8	74.0	69.1	57.4	
	103.2	75.0	70.4[e]	34.3	72.8[f]	63.7		
35	102.2	70.0	71.8	68.7	73.0	68.1	56.8	
	101.2	70.0	71.8	67.9	72.8	66.4		
	100.9	72.8[b]	71.7[b]	33.2	72.2[b]	65.5		19.2
36	102.2	70.0[d]	71.9	68.7	73.1	68.0	56.8	
	101.3	69.9[d]	71.9	68.0	72.7	66.3		
	100.8	72.3[b]	71.9[b]	31.7	72.3[b]	64.2		
37	102.2	69.9	69.9	68.8[d]	72.8[d]	67.2	56.8	
	101.4[d]	69.9	71.9[d]	68.2[d]	72.6[d,e]	68.3		
	101.3[d]	73.0[b,e]	71.8[b,d]	32.8	71.5[b]	65.6		
38[c]	103.9	70.8[e]	72.7[f]	68.8	73.9[g]	69.3	57.4	
	103.4[d]	70.8[e]	72.7[f]	74.0[g]	73.9[g]	69.3		
	103.3[d]	75.0	70.4[e]	34.3	72.8[f]	63.7		

TABLE III *continued*

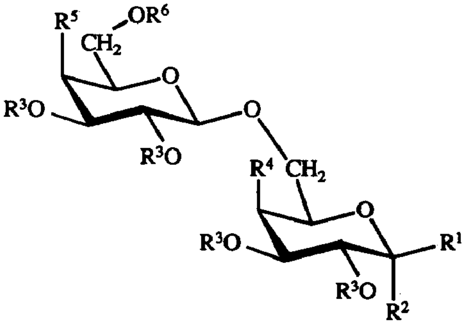
^{13}C-N.m.r. chemical shifts

Compound	*Chemical shifts* (*δ*)[a]							
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH₃*	*(CH₃)₃C*
39	102.2	69.9[d]	70.7	68.9	72.8	68.2	56.8	
	101.8	73.2[b]	71.7[b,d]	33.0	71.9[b,d]	71.1		
	101.3	69.8[d]	71.5[b,d]	68.2	71.4[b,d]	62.0		
40[c]	103.9	70.7	72.8[d]	68.8[d]	73.9	69.2	57.4	
	103.4	75.2[e]	70.3	34.3	71.6[f]	70.8		
	103.2	71.5[f]	72.7[d]	68.7[d]	74.9[e]	61.1		
41	101.9	73.2[b]	70.9[b]	32.7	72.7[b]	71.1	56.5	
	101.6	69.9	71.7	68.7	71.6	68.1		
	101.2	69.9	71.7	68.1	71.4	61.8		
42[c]	103.8[d]	75.3[e]	70.4[b,f]	34.4	70.8[b]	71.7	57.4	
	103.5[d]	70.8[f]	73.9	68.7	72.7[g]	69.2		
	103.4[d]	71.5	72.9[g]	68.7	75.0[e]	61.1		

[a] Measured in chloroform-*d* unless otherwise indicated. [b] Assignments in the same line may be reversed. [c] Measured in D_2O. [d] Assignments in the same column may be reversed. [e,f,g] Assignments of the corresponding signals may be reversed.

glycosylated ($\delta_{C-6'}$ 68.0 in **20**, and 68.5 in **21**). Proton spectra (Tables I and II) showed the positions 4^1 in **20** and **21** to be unsubstituted, by the presence of signals for H-4^1 at δ 4.22 and 4.31, respectively. Subsequent benzoylation of **21** afforded compound **27**. The free OH groups in **20** and **21** reacted smoothly with TCDI to give the intermediates **22** and **23**, which were treated with tributyltin hydride to furnish the corresponding 4^1-deoxygenated disaccharides **24** and **25** in 50% and 97% yield, respectively. The somewhat lower yield in the case of the conversion **22**→**24** was due to the formation of side-products during the reduction step.

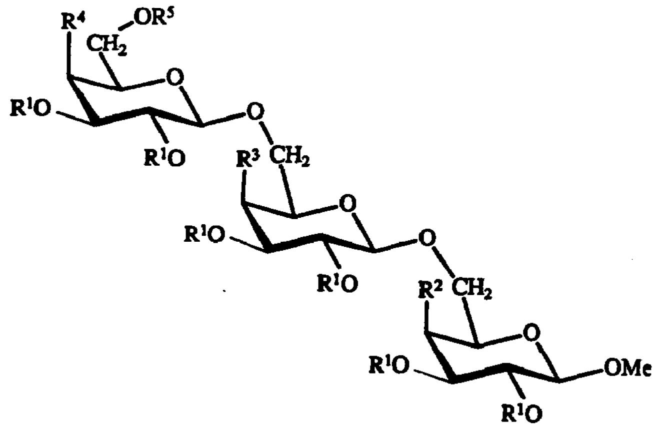
Treatment of compound **24** with boron trifluoride–methanol reagent, essentially under the same conditions as those applied for the high yielding conversion **3**→**17**, gave a single product (91%). Its proton-n.m.r. spectrum showed signals for H-4^2, H-6^2a and H-6^2b at 4.37, 4.72, and 4.62 p.p.m. In the spectrum of related compound **17** the corresponding signals appear[4] at 5.88, 3.86, and 3.70 p.p.m., respectively. This upfield shift of the signal of H-4 ($\Delta\delta$ -1.51 p.p.m.), and the downfield shift of the signals of H-6a and H-6b ($\Delta\delta$ $+0.86$ and $+0.92$ p.p.m.), in the spectrum of the product of desilylation of **24**, was evidence of O-4^2→O-6^2 benzoyl group migration during desilylation. The carbon-n.m.r. spectrum of the material also supported the presence of a benzoyl group at position O-6^2: the signals of C-4^2 and C-6^2 (δ 67.4 and 62.7, respectively) appeared at essentially the same position as those for the corresponding carbon nuclei of methyl 2,3,6-tri-*O*-benzoyl-*β*-D-galactopyranoside[10] (δ 67.2 and 62.9, respectively). Therefore, we assigned the product of the desilylation of **24** the structure **26**, and by benzoylation of the remaining OH group obtained the disaccharide **25**. An alternative synthesis of the fully benzoylated disaccharide **25** comprised condensation of methyl 2,3-di-*O*-benzoyl-4-deoxy-β-D-*xylo*-hexopyranoside (**9**) and the galactosyl bromide **16**, which furnished the compound in 63% yield. Debenzoylation afforded methyl *O*-β-D-galactopyranosyl-(1→6)-4-deoxy-β-D-*xylo*-hexopyranoside (**29**). The corresponding disaccharide **33**, having the 4-deoxy-β-D-*xylo*-hexopyranosyl reducing end group, was prepared similarly: first, the fully benzoylated deoxyglycosyl donor **13** was condensed with methyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (**17**) to give the benzoylated disaccharide **30** (85%), and this was subsequently deblocked.



Cpd.	R^1	R^2	R^3	R^4	R^5	R^6
20	OMe	H	Bz	OH	OBz	TBDPS
21	OMe	H	Bz	OH	OBz	Bz
22	OMe	H	Bz	OTCI	OBz	TBDPS
23	OMe	H	Bz	OTCI	OBz	Bz
24	OMe	H	Bz	H	OBz	TBDPS
25	OMe	H	Bz	H	OBz	Bz
26	OMe	H	Bz	H	OH	Bz
27	OMe	H	Bz	OBz	OBz	Bz
28	H	Cl	Bz	OBz	OBz	Bz
29	OMe	H	H	H	OH	H
30	OMe	H	Bz	OBz	H	Bz
31	OMe	H	Bz	OBz	H	TBDPS
32	OMe	H	Bz	OBz	H	H
33	OMe	H	H	OH	H	H
34	OMe	H	Bz	OBz	OBz	H

OTCI = Imidazol-1-ylthiocarbonyloxy

All three possible trisaccharide sequences having one 4-deoxygalactosyl moiety were synthesized. For the construction of the trisaccharide **38**, deoxygenated at position 4^3, we chose two approaches. Condensation of each of the glycosyl chlorides **13** and **15** with methyl *O*-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside[11,12] (**34**), resulted in intermediates **35** (93%) and **37** (86%), respectively. Compound **35** was further converted, in virtually theoretical yield, to the trisaccharide **36** by selective deprotection with boron trifluoride–methanol reagent. Trisaccharides **36** and **37** were then conventionally deprotected (Zemplén), in 85% and 96% yields, to give compound **38**. The trisaccharide **40**, having a 4-deoxy-β-D-*xylo*-hexopyranosyl internal residue, was prepared as follows: Condensation of the glycosyl donor **15** with the glycosyl acceptor **17** gave the disaccharide **31**. Subsequent desilylation of **31** gave compound **32** (98%), and this nucleophile was galactosylated at position O-6^2, using **16** as the glycosyl donor. Deprotection of the resulting fully benzoylated trisaccharide **39** gave the target compound **40**. For the synthesis of the trisaccharide deoxygenated at position 4^1, we chose the disaccharide chloride **28** as the glycosyl donor. It was prepared by coupling bromide **16** to the galactosyl acceptor **17**, to give the fully benzoylated methyl digalactoside **27**, and this was treated with DCMME to give the corresponding, crystalline glycosyl chloride **28**. Silver triflate promoted condensation of compounds **28** and **9** gave the blocked trisaccharide **41** (70%), and this on debenzoylation afforded the trisaccharide methyl β-glycoside **42** in 76% yield.



Cpd.	R^1	R^2	R^3	R^4	R^5
35	Bz	OBz	OBz	H	TBDPS
36	Bz	OBz	OBz	H	H
37	Bz	OBz	OBz	H	Bz
38	H	OH	OH	H	H
39	Bz	OBz	H	OBz	Bz
40	H	OH	H	OH	H
41	Bz	H	OBz	OBz	Bz
42	H	H	OH	OH	H

Binding studies. — Table IV shows the affinity constants and the maximal ligand-induced fluorescence changes[13] ($LIFC_{max}$) for the saccharides studied, some of them previously prepared[14]. To facilitate interpretation of the data a schematic illustration of the binding mode proposed for these saccharide epitopes and this class of antibodies, based on earlier findings[1], is shown in Fig. 1. It should be recalled that subsite **A** is the one that was shown to have critical hydrogen bonds from the 2- and 3-OHs of the ligand to the antibody. The approximate location in the combining area[1a,15] of the two solvent-exposed tryptophanyl residues (TRPs) is indicated as well.

We reported[16] that methyl 4-deoxy-4-fluroro-β-D-galactopyranoside exhibited binding equal to that of the the parent glycoside **1**, showing there is no hydrogen bond

Compound	C	A	B	D
1		Gal→Me		
45		Gal →	Gal→Me	
48	Gal →	Gal →	Gal→Me	
46		Gal →	3dGal→Me	
47	3dGal →	Gal→Me		
29		Gal →	4dGal→Me	
33	4dGal →	Gal→Me		
49	3dGal →	Gal →	Gal→Me	
50		Gal →	3dGal →	Gal→Me
51	Gal →	Gal →	3dGal→Me	
38	4dGal →	Gal →	Gal→Me	
40		Gal →	4dGal →	Gal→Me
42	Gal →	Gal →	4dGal→Me	
		TRP (33H)	**TRP** (91L)	

ANTIBODY COMBINING AREA

Fig. 1. Schematic depiction of the binding of various ligands to immunoglobulins X24 and J539. The letters **C**, **A**, **B**, and **D** represent the four galactosyl-binding subsites, and the location of the two solvent-exposed tryptophan residues in subsites **A** and **B** is indicated. For the meaning of the abbreviations see footnote *a*, Table IV. Note that subsite **A** always accomodates an unaltered (except for possible 6-substitution) galactopyranosyl residue.

donation from the 4 position of the galactosyl residue *to* the protein in the highest affinity subsite (**A**). Now, it can be seen that methyl 4-deoxy-β-D-galactopyranoside fails to bind, suggesting a critical requirement for a hydrogen bond *from* the antibody to O-4 of the residue in subsite **A**. And if that is so, di- and tri-saccharides containing a 4-deoxygalactosyl residue in various parts of their sequence should possess predictable binding patterns. For example disaccharide **29** should bind in subsites **A-B**, so that the 4-deoxygalactosyl residue would not occupy the "forbidden" subsite **A**, and the LIFC$_{\max}$ indicates this to be the binding mode. There is a somewhat reduced binding affinity, however, compared with that of **45**, showing that the 4-deoxygalactosyl residue has a K_a for subsite **B** of 13 in the case of J539, and 15 in the case of X24, compared to the affinities of 50 (J539) and 33 (X24) that a galactosyl residue has in that subsite ($\Delta G_{A\text{-}B} = \Delta G_A + \Delta G_B$, where ΔG is the free energy of binding). By contrast, ligand **33** must shift to subsites **C-A** in order to avoid having its 4-deoxygalactosyl moiety in subsite **A**. Table IV shows that this is so, as the LIFC$_{\max}$ has reverted to the value indicating perturbation of only one TRP residue. The affinity is exactly as expected for **C-A** (for **A**, X24 = 600, J539 = 10^3; for **C**, X24 = 16, J539 = 50). Thus, subsite **C** shows no involvement with the 4-OH of the residue it binds, while **B** shows some (noncritical) interaction with the 4-OH of its galactosyl residue. Trisaccharides **38**, **40**, and **42** can all utilize subsites **A-B**, so they all show the high values of LIFC$_{\max}$ indicative of perturbation of both solvent-exposed TRP residues. However, both **38** and **42** can occupy **C-A-B**, while **40** must shift

TABLE IV

Binding constants (K_a) and maximal ligand induced fluorescence changes (LIFC_{max}) for a series of ligands of IgAs X24 and J539 (Fab')

Ligand[a]		*Immunoglobulin*			
		X24		*J539 Fab'*	
		K_a	$LIFC_{max}$ (%)	K_a	$LIFC_{max}$ (%)
1	Gal→Me	0.6×10^3	18.2	1.0×10^3	18.8
43	2dGal→Me	0	0	0	0
44	3dGal→Me	0	0	0	0
10	4dGal→Me	0	0	0	0
45	Gal→Gal→Me	2.0×10^4	30	5.2×10^4	35
46	Gal→3dGal→Me	1.9×10^4	31	5.8×10^4	30
47	3dGal→Gal→Me	6.8×10^3	19	7.4×10^3	19
29	Gal→4dGal→Me	0.9×10^4	37	1.3×10^4 [b]	36
33	4dGal→Gal→Me	1.2×10^4	21	1.3×10^4 [b]	22
48	Gal→Gal→Gal→Me	3.2×10^5	41	4.8×10^5	43
49	3dGal→Gal→Gal→Me	1.9×10^5	34	4.8×10^5	37
50	Gal→3dGal→Gal→Me	2.6×10^4	31	5.2×10^4	36
51	Gal→Gal→3dGal→Me	3.5×10^5	37	4.4×10^5	38
38	4dGal→Gal→Gal→Me	2.3×10^5	41	4.8×10^5 [b]	44
40	Gal→4dGal→Gal→Me	2.2×10^4	37	4.7×10^4	35
42	Gal→Gal→4dGal→Me	1.6×10^5	30	1.8×10^5	38

[a] Names are abbreviated as follows: 2dGal→Me = methyl 2-deoxy-β-D-*lyxo*-hexopyranoside ("methyl 2-deoxy-β-D-galactopyranoside"), etc.; Gal→3dGal→Me = methyl *O*-β-D-galactopyranosyl-(1→6)-3-deoxy-β-D-*xylo*-hexopyranoside ("methyl *O*-β-D-galactopyranosyl-(1→6)-3-deoxy-β-D-galactopyranoside"), etc. All residues are in the pyranose form, and all linkages are β-(1→6). The synthesis of compounds **43**, **44**, and **10** (ref. 3), and **46**, **47**, **49**, **50**, and **51** (ref. 14) has been reported. [b] Measured on whole antibody.

to **A-B-D** in order to avoid having the 4-deoxy residue in subsite **A**. The K_as computed for these modes for **38**, **40**, and **42** (4.8×10^5, 1.6×10^4, and 1.4×10^5 respectively) are quite close to the values found (Table IV). The trisaccharides **49–51** show by their LIFC_{max} values that they too can all occupy subsites **A-B**, and the affinities reveal that **49** and **51** utilize the sequence **C-A-B**, while ligand **50** utilizes **A-B-D**, as would be expected knowing that in subsite **A** there is a critical hydrogen bond to the protein from OH-3 of the galactosyl residue. Thus, the data collected here on the 3- and 4-deoxygenated ligands related to (1→6)-β-D-galactopyranans completely confirm the binding modes proposed in earlier reports from this laboratory[1]. Also, an additional hydrogen bond from the protein to the ligand (position O-4) in subsite **A** is indicated by the data.

EXPERIMENTAL

General methods. — Optical rotations were measured at 25° with a Perkin–Elmer automatic polarimeter, model 241 MC. Thin-layer chromatography (t.l.c.) on precoated slides of Silica Gel G F254 (Analtech) was performed with solvent mixtures of appropriately adjusted polarity consisting of: *A*, carbon tetrachloride–acetone; *B*, toluene–acetone; *C*, dichloromethane–methanol; *D*, carbon tetrachloride–ethyl acetate; and *E*, toluene–ethyl acetate. Detection was effected by charring with 5% sulfuric acid in ethanol and, when applicable, with u.v. light. Preparative chromatography was performed by elution from columns of Silica Gel 60 (particle size 0.04–0.063 mm). N.m.r. data were extracted from spectra measured at 25° with a Varian XL 300 instrument, using tetramethylsilane as internal standard for solutions in $CDCl_3$. For the ^{13}C spectra taken in D_2O methanol was used as the internal standard ($\delta_{MeOH} vs. Me_4Si$ is 49.0 p.p.m.). Proton signal assignments were made by first-order analysis of the spectra, and were supported by homonuclear decoupling experiments. Of two magnetically nonequivalent geminal protons, the one resonating at lower field is denoted Ha and the one resonating at higher field, Hb. The superscripts used in reporting the n.m.r. data for oligosaccharides denote the sugar residues containing the designated proton or carbon atom. These are serially numbered, beginning with the residue bearing the aglycone. For example, H-1^2 refers to H-1 of the second sugar unit. Reactions requiring anhydrous conditions were performed under argon using common laboratory glassware, and reagents and solvents were handled with Hamilton series 1000 gas-tight syringes. Boron trifluoride–methanol complex (50% BF_3) was purchased from the Aldrich Chemical Co. Unless stated otherwise, solutions in organic solvents were dried with anhydrous sodium sulfate, and concentrated at 2 kPa and 40°.

Affinity constants were measured by the change in antibody tryptophanyl fluorescence as a function of incremental additions of putative ligands[13].

Immunoglobulins. — The purification of the monoclonal immunoglobulins has been described[1].

*Methyl 6-*O*-(*tert*-butyldiphenylsilyl)-β-*D*-galactopyranoside* (**2**). — A solution of methyl β-D-galactopyranoside (**1**; 9.68 g, 50 mmol), imidazole (7.51 g, 110 mmol) and *tert*-butylchlorodiphenylsilane (14.35 mL, 55 mmol) in *N*,*N*-dimethylformamide (150 mL) was stirred for 14 h at room temperature, and poured into water (~500 mL) with vigorous stirring. The precipitate was collected by filtration, dissolved in dichloromethane (100 mL), and combined with the dichloromethane extract of the filtrate. The organic phase was washed with aqueous sodium chloride solution (5%), and concentrated to give **2** in virtually theoretical yield, m.p. 91°; lit.[4] m.p. 92–93°.

*Methyl 2,3-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-*D*-galactopyranoside* (**6**). — A solution of benzoyl chloride (0.63 g, 4.5 mmol) in toluene (1 mL) was added at −20° to a solution of **2** (0.87 g, 2 mmol) in pyridine (2 mL), and the mixture was stirred at −20° until t.l.c. (solvent *A*, 5:1) showed complete conversion of compound **2** (~1 h). The mixture was diluted with dichloromethane and washed successively with dilute hydrochloric acid and aqueous sodium hydrogencarbonate solution. After evaporation of the solvent, the residue was chromatographed (gradient, carbon tetrachloride to solvent *A*, 10:1) to give first methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (**3**; 0.15 g, 10%), m.p. 192–193°; lit.[4] m.p. 193–194°.

Eluted next was compound **6** (0.90 g, 70%, white foam), $[\alpha]_D$ +56° (*c* 1.0, $CHCl_3$). *Anal.* Calc. for $C_{37}H_{40}O_8Si$: C, 69.35; H, 6.29. Found: C, 69.92; H, 6.32.

Eluted next was methyl 3,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (**4**; 67 mg, 5%, white foam), $[\alpha]_D$ +31° (*c* 0.8, $CHCl_3$).

Anal. Calc. for $C_{37}H_{40}O_8Si$: C, 69.35; H, 6.29. Found: C, 69.37; H, 6.25.

Eluted next was methyl 3-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (**5**; 0.15 g, 14%), m.p. 88–91° (from ethyl acetate–hexane), $[\alpha]_D$ +13.1° (*c* 1.2, $CHCl_3$).

Anal. Calc. for $C_{30}H_{36}O_7Si$: C, 67.14; H, 6.76. Found: C, 67.03; H, 6.81.

*Methyl 2,3-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-4-*O*-(imidazol-1-ylthiocarbonyl)-β-D-galactopyranoside* (**7**). — A solution of compound **6** (6.46 g, 10 mmol) and thiocarbonyldiimidazole (2.32 g, 13 mmol) in toluene (100 mL) was stirred at 100° until t.l.c. (solvent *A*, 5:1) showed complete conversion of **6** into a slower moving product (~15 h). After concentration, the residue was chromatographed (gradient, carbon tetrachloride to solvent *A*, 10:1) to give **7** (7.08 g, 94%), m.p. 79–81.5° (from acetone–hexane), $[\alpha]_D$ +100° (*c* 1.1, $CHCl_3$).

Anal. Calc. for $C_{41}H_{42}N_2O_8SSi$: C, 65.58; H, 5.64; N, 3.73; S, 4.27. Found: C, 65.36; H, 5.91; N, 3.60; S, 4.20.

*Methyl 2,3-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-4-deoxy-β-D-*xylo*-hexopyranoside* (**8**). — A solution of compound **7** (6.80 g, 9 mmol) in toluene (20 mL) was added to a solution of tributyltin hydride (5 mL, 15 mmol) in toluene (20 mL). The mixture was heated under reflux until t.l.c. (solvent *B*, 10:1) showed complete conversion into a single, faster moving product (~45 min). After concentration, the residue was chromatographed (gradient, carbon tetrachloride to solvent *A*, 30:1) to give **8** (5.32 g, 95%, white foam), $[\alpha]_D$ +49.6° (*c* 0.7, $CHCl_3$).

Anal. Calc. for $C_{37}H_{40}O_7Si$: C, 71.13; H, 6.45. Found: C, 70.89; H, 6.47.

*Methyl 2,3-di-*O*-benzoyl-4-deoxy-β-D-*xylo*-hexopyranoside* (**9**). — (*a*). A solution of compound **8** (0.63 g, 1 mmol) and boron trifluoride–methanol (0.2 mL) in 1:1 dichloromethane–methanol (10 mL) was kept at room temperature with occasional swirling until t.l.c. (solvent *A*, 5:1) showed complete conversion of the starting material into a slower moving product (~1.5 d). After concentration the residue was chromatographed (solvent *A*, 8:1) to give **9** (0.35 g, 91%, white foam), $[\alpha]_D$ +64° (*c* 1.0, $CHCl_3$).

Anal. Calc. for $C_{21}H_{22}O_7$: C, 64.47; H, 5.61. Found: C, 64.43; H, 5.65.

(*b*). Chlorotrimethylsilane (1.2 mL, 10 mmol) was added at 0°, with stirring, to a solution of **11** (2.1 g, 3.3 mmol) and sodium iodide (1.5 g, 10 mmol) in acetonitrile (50 mL). After 3 min, water (50 mL) was added, and the brown solution was decolorized by the addition of aqueous sodium thiosulfate. After concentration to ~50 mL the mixture was extracted with dichloromethane, and the combined organic phase was washed with water and concentrated. The residue was chromatographed to give compound **9** (0.96 g, 75%).

*Methyl 4-deoxy-β-D-*xylo*-hexopyranoside* (**10**). — A solution of compound **9** (386 mg, 1 mmol) and a catalytic amount of sodium methoxide in methanol (20 mL) was kept at room temperature for ~ 16 h. After neutralization of the mixture with Dowex (H^+) ion-exchange resin, and evaporation of the solvent, the residue was crystallized from methanol–ethyl acetate to give **10** (105 mg, 59%), m.p. 147°; lit.[3] m.p. 148–148.5°.

*Methyl 2,3-di-*O*-benzoyl-4-deoxy-6-*O*-trityl-β-D-*xylo*-hexopyranoside* (**11**). — To a solution of methyl 4-deoxy-β-D-*xylo*-hexopyranoside[1] (**10**; 0.89 g, 5 mmol), triethylamine (0.5 mL), and 4-dimethylaminopyridine (24 mg, 0.2 mmol) in *N,N*-dimethylformamide (10 mL) was added trityl chloride (1.54 g, 5.5 mmol), and the mixture was stirred at room temperature until t.l.c. (solvent *C*, 10:1) showed almost complete conversion of the starting material into a major, faster moving product (~12 h). Pyridine (5 mL) was added, followed by benzoyl chloride (1.7 mL, 15 mmol) at −30°, and the mixture was stirred at room temperature for additional 3 h. After conventional processing, the crude product was chromatographed (solvent *A*, 10:1) to give **11** (2.87 g, 91%, white foam), $[\alpha]_D$ +61° (*c* 0.6, $CHCl_3$).

Anal. Calc. for $C_{40}H_{36}O_7$: C, 76.40; H, 5.78. Found: C, 76.53; H, 5.78.

*2,3,6-Tri-O-benzoyl-4-deoxy-α-*D*-*xylo*-hexopyranosyl chloride* (**13**). — A solution of methyl 2,3,6-tri-*O*-benzoyl-4-deoxy-β-D-*xylo*-hexopyranoside[3] (**12**; 1.5 g, 3 mmol), dichloromethyl methyl ether (5.5 mL), and zinc chloride (~50 mg) in chloroform (5.5 mL) was heated and stirred under reflux until t.l.c. (solvent *A*, 35:1) showed complete conversion of the starting material into one major and one minor faster-moving product (~0.5 h). After concentration, and coevaporation with toluene, the residue was chromatographed (solvent *A*, 40:1) to give first compound **13** (1.02 g, 69%), m.p. 155.5–156° (from carbon tetrachloride), $[\alpha]_D$ +165.7° (*c* 0.9, $CHCl_3$).

Anal. Calc. for $C_{27}H_{23}ClO_7$: C, 65.56; H, 4.69; Cl, 7.17. Found: C, 65.29; H, 4.65; Cl, 7.30.

Eluted next was methyl 2,3,6-tri-*O*-benzoyl-4-deoxy-α-D-*xylo*-hexopyranoside (**14**; 0.17 g, 12%), m.p. 118–119.5° (from methanol), $[\alpha]_D$ +128° (*c* 0.8, $CHCl_3$).

Anal. Calc. for $C_{28}H_{26}O_8$: C, 68.54; H, 5.34. Found: C, 68.48; H, 5.36.

*2,3-Di-O-benzoyl-6-O-(*tert*-butyldiphenylsilyl)-4-deoxy-α-*D*-*xylo*-hexopyranosyl chloride* (**15**). — A solution of compound **8** (3.00 g, 4.8 mmol), dichloromethyl methyl ether (8 mL), and zinc chloride (~5 mg) in chloroform (10 mL) was stirred at 55° until t.l.c. (solvent *D*, 15:1) showed complete conversion of the starting material into a major, faster moving product (1.5 h). Workup as described for the preparation of compound **13**, and chromatography (gradient, carbon tetrachloride to solvent *E*, 10:1) gave **15** (2.56 g, 85%, white foam), $[\alpha]_D$ +138° (*c* 0.8, $CHCl_3$).

Anal. Calc. for $C_{36}H_{37}ClO_6Si$: C, 68.72; H, 5.93; Cl, 5.63. Found: C, 68.59; H, 5.96; Cl, 5.70.

*Methyl 2,3,4-tri-O-benzoyl-β-*D*-galactopyranoside* (**17**). — A solution of compound **3** (0.75 g, 1 mmol) in 1:1 dichloromethane–methanol (10 mL) was treated with boron trifluoride–methanol (0.2 mL) as described for the preparation of compound **9**. Chromatography (solvent *A*, 5:1) gave **17** (0.5 g, 99%), m.p. 90° (from diethyl ether–hexane); lit.[6] m.p. 90–91°.

*Methyl O-[2,3,4-tri-O-benzoyl-6-O-(*tert*-butyldiphenylsilyl)-β-*D*-galactopyranosyl]-(1→6)-2,3-di-O-benzoyl-β-*D*-galactopyranoside* (**20**). — A solution of methyl 2,3-di-*O*-benzoyl-β-D-galactopyranoside[8] (**18**; 402 mg, 1 mmol), methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-galactopyranosyl chloride[4] (**19**; 749 mg, 1 mmol), and 2,4,6-trimethylpyridine (109 mg, 0.9 mmol) in dichloromethane (5 mL) was added at −50° to a suspension of silver triflate (0.52 g, 1 mmol) and pulverized molecular sieves (4 Å, 1 g) in dichloromethane (5 mL), and the mixture was slowly warmed to 0°, when t.l.c. (solvent *A*, 5:1) showed complete conversion of the starting materials (~1 h). After neutralization with 2,4,6-trimethylpyridine, filtration, and washing of the filtrate with aqueous sodium thiosulfate, the solvent was evaporated. Chromatography of the residue gave **20** (0.81 g, 73%, white foam), $[\alpha]_D$ +87.6° (*c* 0.8, $CHCl_3$).

Anal. Calc. for $C_{64}H_{62}O_{16}Si$: C, 68.93; H, 5.60. Found: C, 68.68; H, 5.66.

Methyl O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→6)-2,3-di*-O-*benzoyl-β*-D-*galactopyranoside* (**21**). — A solution of compound **18** (1.21 g, 3 mmol), 2,3,4,6-tetra-*O*-benzoyl-*α*-D-galactopyranosyl bromide[9] (**16**; 1.98 g, 3 mmol), and 2,4,6-trimethylpyridine (0.35 g, 2.9 mmol) in dichloromethane (10 mL) was added at −70° to a suspension of silver triflate (1.03 g, 4 mmol) and pulverized molecular sieves (4 Å, 2 g) in dichloromethane (10 mL), and the mixture was slowly warmed to −10°, when t.l.c. (solvent *A*, 5:1) showed complete conversion of the starting materials (~0.5 h). Workup as described for the preparation of compound **20** gave **21** (2.38 g, 81%), m.p. 137–140° (from ethyl acetate–hexane), $[\alpha]_D$ +85° (*c* 0.8, $CHCl_3$).

Anal. Calc. for $C_{55}H_{48}O_{17}$: C, 67.34; H, 4.93. Found: C, 67.27; H, 4.97.

Methyl O-*[2,3,4-tri*-O-*benzoyl-6*-O-*(*tert-*butyldiphenylsilyl)-β*-D-*galactopyranosyl]-(1→6)-2,3-di*-O-*benzoyl-4*-O-*(imidazol-1-ylthiocarbonyl)-β*-D-*galactopyranoside* (**22**). — A solution of compound **20** (669 mg, 0.6 mmol) and thiocarbonyldiimidazole (143 mg, 0.8 mmol) in toluene (20 mL) was heated under reflux until t.l.c. (solvent *E*, 4:1) showed complete conversion into a single, slower moving product (~2 h). Workup as described for compound **7**, and chromatography (solvent *A*, 15:1), gave **22** (622 mg, 85%, white foam), $[\alpha]_D$ +142° (*c* 0.3, $CHCl_3$).

Anal. Calc. for $C_{68}H_{64}N_2O_{16}SSi$: C, 66.65; H, 5.26; N, 2.29; S, 2.62. Found: C, 66.48; H, 5.33; N, 2.22; S, 2.57.

Methyl O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→6)-2,3-di*-O-*benzoyl-4*-O-*(imidazol-1-ylthiocarbonyl)-β*-D-*galactopyranoside* (**23**). — A solution of compound **21** (1.96 g, 2 mmol) and thiocarbonyldiimidazole (0.45 g, 2.5 mmol) in toluene (20 mL) was heated at 100° until t.l.c. (solvent *E*, 4:1) showed complete conversion into a single, slower moving product (~4 h). Workup, as described for compound **7**, and chromatography (solvent *E*, 6:1), gave **23** (2.08 g, 95%, white foam), $[\alpha]_D$ +120° (*c* 0.3, $CHCl_3$).

Anal. Calc. for $C_{59}H_{50}N_2O_{17}S$: C, 64.95; H, 4.62; N, 2.57; S, 2.94. Found: C, 64.86; H, 4.64; N, 2.56; S, 2.90.

Methyl O-*[2,3,4-tri*-O-*benzoyl-6*-O-*(*tert-*butyldiphenylsilyl)-β*-D-*galactopyranosyl]-(1→6)-2,3-di*-O-*benzoyl-4-deoxy-β*-D-xylo-*hexopyranoside* (**24**). — A solution of compound **22** (490 mg, 0.4 mmol) in toluene (5 mL) was added to a solution of tributyltin hydride (233 mg, 0.8 mmol) in toluene (10 mL), and the mixture was heated under reflux until t.l.c. (solvent *A*, 5:1) showed complete conversion into a major, faster moving product (~1 h). Workup, as described for compound **8**, and chromatography (gradient, carbon tetrachloride to solvent *A*, 5:1), gave **24** (218 mg, 50%, white foam), $[\alpha]_D$ +68° (*c* 0.7, $CHCl_3$).

Anal. Calc. for $C_{64}H_{62}O_{15}Si$: C, 69.93; H, 5.94. Found: C, 69.82; H, 5.67.

Methyl O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→6)-2,3-di*-O-*ben-*

*zoyl-4-deoxy-β-*D-xylo*-hexopyranoside* (**25**). — (*a*). Compound **23** (1.86 g, 1.7 mmol) was treated with tributyltin hydride (0.64 g, 2.2 mmol) as described for the preparation of compound **24**. T.l.c. (solvent *A*, 5:1) showed complete conversion into a single, faster moving product after ~1 h. Workup as described for compound **8**, and chromatography (solvent *A*, 10:1), gave **25** (1.55 g, 97%), m.p. 150–152° (from acetone–hexane), $[\alpha]_D$ +84° (*c* 1.1, $CHCl_3$).

Anal. Calc. for $C_{55}H_{48}O_{16}$: C, 68.46; H, 5.01. Found: C, 68.30; H, 5.06.

(*b*). A solution of **9** (0.63 g, 1.6 mmol), **16** (1.3 g, 1.9 mmol), and 2,4,6-trimethylpyridine (0.23 mL, 1.7 mmol) in dichloromethane (8.2 mL) was added at −25° to a suspension of silver triflate (0.6 g, 2.3 mmol) in dichloromethane (5 mL), and the mixture was stirred at room temperature until t.l.c. (solvent *B*, 15:1) showed complete conversion of the starting materials (~2 h). Workup as described for compound **20**, and chromatography (solvent *B*, 10:1), gave **25** (1.2 g, 63%).

(*c*). A portion of compound **26** was benzoylated in the usual way to give **25**.

Methyl O-*(2,3,6-tri-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→6)-2,3-di-*O*-benzoyl-4-deoxy-β-*D-xylo*-hexopyranoside* (**26**). — A solution of **24** (167 mg, 0.15 mmol) in 1:1 dichloromethane–methanol (2 mL) was treated with boron trifluoride–methanol (0.1 mL), as described for the preparation of compound **9**. Chromatography (solvent *A*, 10:1) gave **26** (117.5 mg, 91%, white foam), $[\alpha]_D$ +54.5° (*c* 0.3, $CHCl_3$).

Anal. Calc. for $C_{48}H_{44}O_{15}$: C, 66.97; H, 5.15. Found: C, 66.90; H, 5.17.

Methyl O-*(2,3,4,6-tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-*D*-galactopyranoside* (**27**). — (*a*). A solution of **17** (1.01 g, 2 mmol), **16** (1.45 g, 2.2 mmol), and 2,4,6-trimethylpyridine (0.24 g, 2 mmol) in dichloromethane (10 mL), was added at 0° to a suspension of silver triflate (0.77 g, 3.3 mmol) in dichloromethane (10 mL), and the mixture was stirred at room temperature until t.l.c.; solvent *A*, 5:1 showed complete conversion of the starting materials. Workup as described for compound **20** gave **27** (1.86 g, 86%), m.p. 248.5–250° (lit.[17] m.p. 251–252°).

(*b*). A portion of compound **21** was benzoylated in the usual way to give **27**, m.p. 250–251°.

O-*(2,3,4,6-Tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α-*D*-galactopyranosyl chloride* (**28**). — A stirred mixture of **27** (1 g, 0.92 mmol), dichloromethyl methyl ether (2.5 mL), and zinc chloride (~10 mg) in chloroform (5 mL) was heated at 65° until t.l.c. (solvent *A*, 5:1) showed complete conversion into a major, faster moving product (~2.5 h). Workup as described for compound **13** gave, after chromatography (gradient, carbon tetrachloride to solvent *A*, 10:1), **28** (0.62 g, 62%), m.p. 228–229° (from acetone–hexane), $[\alpha]_D$ +155° (*c* 0.2, $CHCl_3$).

Anal. Calc. for $C_{61}H_{49}ClO_{17}$: C, 67.25; H, 4.53; Cl, 3.25. Found: C, 66.75; H, 4.56; Cl, 3.05.

Methyl O-*β-*D*-galactopyranosyl-(1→6)-4-deoxy-β-*D-xylo*-hexopyranoside* (**29**). — A solution of **25** (1 g, 1 mmol) and a catalytic amount of sodium methoxide in methanol (50 mL) was heated at ~35° for 2 h. After neutralization of the mixture with Amberlite IR-120 (H^+) ion-exchange resin, the solvent was evaporated. Chromatography (solvent C, 2:1) gave compound **29** (0.3 g, 88%, hygroscopic, amorphous solid). A carefully dried sample showed $[\alpha]_D$ −22.4° (*c* 0.8, H_2O).

Methyl O-*(2,3,6-tri*-O-*benzoyl-4-deoxy-β*-D-xylo-*hexopyranosyl)-(1→6)-2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranoside* (**30**). — A solution of **17** (506 mg, 1 mmol), **13** (594 mg, 1.2 mmol), and 2,4,6-trimethylpyridine (0.14 mL, 1.1 mmol) in dichloromethane (5 mL) was added at room temperature to a suspension of silver triflate (0.36 g, 1.4 mmol) in dichloromethane (10 mL), and the mixture was stirred until t.l.c. (solvent *B*, 15:1) showed complete conversion of the starting materials (~30 min). Workup as described for compound **20**, and chromatography (solvent *B*, 25:1), gave **30** (0.82 g, 85%), m.p. 154–155° (from methanol), $[\alpha]_D$ +115° (*c* 0.8, $CHCl_3$).

Anal. Calc. for $C_{55}H_{48}O_{16}$: C, 68.44; H, 5.02. Found: C, 68.18; H, 5.11.

Methyl O-*[2,3-di*-O-*benzoyl-6*-O-*(*tert-*butyldiphenylsilyl)-4-deoxy-β*-D-xylo-*hexopyranosyl]-(1→6)-2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranoside* (**31**). — A solution of compound **17** (0.51 g, 1 mmol), compound **15** (0.69 g, 1.1 mmol), and 2,4,6-trimethylpyridine (0.11 g, 0.9 mmol) in dichloromethane (5 mL) was added at room temperature to a suspension of molecular sieves (4 Å, 1 g) and silver triflate (0.51 g, 2 mmol) in dichloromethane (5 mL), and the mixture was stirred until t.l.c. (solvent *A*, 5:1) showed complete conversion of the starting materials. Workup as described for compound **20**, and crystallization from methanol, gave **31** (0.8 g). Concentration of the mother liquor and chromatography (solvent *A*, 15:1) of the residue gave additional material (0.29 g, total yield of **31**, 1.09 g, 99%), m.p. 224–225°, $[\alpha]_D$ +120° (*c* 0.8, $CHCl_3$).

Anal. Calc. for $C_{64}H_{62}O_{15}Si$: C, 69.93; H, 5.69. Found: C, 69.77; H, 5.76.

Methyl O-*(2,3-di*-O-*benzoyl-4-deoxy-β*-D-xylo-*hexopyranosyl)-(1→6)-2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranoside* (**32**). — A solution of **31** (0.55 g, 0.5 mmol) in 1:1 dichloromethane–methanol (6 mL) was treated with boron trifluoride–methanol (0.15 mL) as described for the preparation of compound **9**. Chromatography (solvent *A*, 7:1) gave **32** in virtually theoretical yield, m.p. 235–236° (from acetone–hexane), $[\alpha]_D$ +134° (*c* 0.3, $CHCl_3$).

Anal. Calc. for $C_{48}H_{44}O_{15}$: C, 66.97; H, 5.15. Found: C, 66.84; H, 5.17.

Methyl O-*(4-deoxy-β*-D-xylo-*hexopyranosyl)-(1→6)-β*-D-*galactopyranoside* (**33**). — A solution of **30** (1 g, 1 mmol) and a catalytic amount of sodium methoxide in methanol (300 mL) was kept at ~30° with occasional swirling. Workup as described for the preparation of compound **9**, and chromatography (solvent *C*, 2:1), gave **33** (0.27 g, 80%) crystallysed from ethanol, m.p. 176°, $[\alpha]_D$ −33° (*c* 0.8, H_2O).

Methyl O-*[2,3-di*-O-*benzoyl-6*-O-*(*tert-*butyldiphenylsilyl)-4-deoxy-β*-D-xylo-*hexopyranosyl]-(1→6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→6)-2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranoside* (**35**). — A solution of **15** (0.5 g, 0.8 mmol) and 2,4,6-trimethylpyridine (85 mg, 0.7 mmol) in dichloromethane (4 mL) was added at room temperature to a suspension of methyl *O*-(2,3,4-tri-*O*-benzoyl-*β*-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-*β*-D-galactopyranoside[11,12] (**34**, 0.59 g, 0.6 mmol), silver triflate (0.89 g, 1.6 mmol), and molecular sieves (4 Å, 1 g) in dichloromethane (5 mL), and the mixture was stirred until t.l.c. (solvent *A*, 5:1) showed complete conversion of the starting materials (~15 min). Workup as described for compound **20** gave crude **35**, which on crystallization from methanol furnished 0.75 g of product. Concentration of the mother liquor and chromatography (solvent *A*, 10:1) of the residue gave additional material (0.13 g, total yield 0.88 g, 93%), m.p. 219°, $[\alpha]_D$ +104° (*c* 0.7 g, $CHCl_3$).

Anal. Calc. for $C_{91}H_{84}O_{23}Si$: C, 69.45; H, 5.38. Found: C, 69.35; H, 5.37.

*Methyl O-(2,3-di-O-benzoyl-4-deoxy-β-*D-xylo-*hexopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-*D-*galactopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-β-*D-*galactopyranoside* (**36**). — A solution of **35** (472 mg, 0.3 mmol) in 1:1 dichloromethane–methanol (5 mL) was treated with boron trifluoride–methanol (0.1 mL) as described for the preparation of compound **9**. Chromatography (solvent *A*, 7:1) gave **36** (389 mg, 99%), m.p. 234° (from acetone–hexane), $[\alpha]_D$ +134° (*c* 0.9, $CHCl_3$).

Anal. Calc. for $C_{75}H_{66}O_{23}$: C, 67.46; H, 4.98. Found: C, 67.20; H, 5.01.

*Methyl O-(2,3,6-tri-O-benzoyl-4-deoxy-β-*D-xylo-*hexopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-*D-*galactopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-β-*D-*galactopyranoside* (**37**). — This compound was prepared from **34** (0.53 g, 0.54 mmol) and **13** (0.35 g, 0.7 mmol) as described for the preparation of compound **30**. Chromatography (solvent *B*, 20:1) gave **37** (0.67 g, 86%), m.p. 152–154° (from dichloromethane–methanol), $[\alpha]_D$ +116° (*c* 0.8, $CHCl_3$).

Anal. Calc. for $C_{82}H_{70}O_{24}$: C, 68.42; H, 4.99. Found: C, 68.01; H, 5.00.

*Methyl O-(4-deoxy-β-*D-xylo-*hexopyranosyl)-(1→6)-O-β-*D-*galactopyranosyl-(1→6)-β-*D-*galactopyranoside* (**38**). — (*a*). A solution of **36** (133.5 mg, 0.1 mmol) and a catalytic amount of sodium methoxide in 1:1 toluene–methanol (10 mL) was kept at room temperature, with occasional swirling, for ~20 h. After neutralization with Dowex (H^+) ion-exchange resin the solvent was evaporated. Chromatography of the residue (solvent *C*, 1:1) gave **38** (42.9 mg, 85%). A carefully dried sample showed $[\alpha]_D$ −20.7° (*c* 0.3, H_2O).

(*b*). Deprotection of **37** (143.9 mg, 0.1 mmol), as described in (*a*), gave **38** (48.1 mg, 96%).

*Methyl O-β-*D-*galactopyranosyl)-(1→6)-O-(4-deoxy-β-*D-xylo-*hexopyranosyl)-(1→6)-β-*D-*galactopyranoside* (**40**). — Compound **39** was prepared from **32** (231.2 mg, 0.27 mmol) and **16** (264 mg, 0.4 mmol) as described for the synthesis of compound **35**. Chromatography (solvent *B*, 20:1) gave **39** (0.3 g, 78%, white hygroscopic foam), $[\alpha]_D$ +112° (*c* 0.2, $CHCl_3$). Deprotection of **39** (143.9 mg, 0.1 mmol), as described for the preparation of compound **38** (*a*), gave **40** (40.8 mg, 81%). A carefully dried sample showed $[\alpha]_D$ −19.8° (*c* 0.2, H_2O).

*Methyl O-(2,3,4,6-tetra-O-benzoyl-β-*D-*galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-*D-*galactopyranosyl)-(1→6)-2,3-di-O-benzoyl-4-deoxy-β-*D-xylo-*hexopyranoside* (**41**). — This compound was prepared from **9** (0.12 g, 0.3 mmol) and **28** (0.39 g, 0.36 mmol) as described for the synthesis of compound **35**. Chromatography (solvent *A*, 15:1) gave **41** (0.3 g, 70%, white foam), $[\alpha]_D$ +108° (*c* 0.4, $CHCl_3$).

Anal. Calc. for $C_{82}H_{70}O_{24}$: C, 68.42; H, 4.90. Found: C, 68.28; H, 4.92.

*Methyl O-β-*D-*galactopyranosyl-(1→6)-O-β-*D-*galactopyranosyl-(1→6)-4-deoxy-β-*D-xylo-*hexopyranoside* (**42**). — Deprotection of **41** (143.9 mg, 0.1 mmol), as described for the preparation of compound **38** (*a*), gave **42** (38.2 mg, 76%). A carefully dried sample showed $[\alpha]_D$ −18.2° (*c* 0.4, H_2O).

REFERENCES

1 (a) C. P. J. Glaudemans, *Mol. Immunol.*, 24 (1987) 371–377; (b) R. Ollo, C. Auffray, J. L. S. Sikorav, and F. Rougen, *Nucleic Acid Res.*, 9 (1981) 4099–4109.
2 C. P. J. Glaudemans, P. Kováč, and A. S. Rao, *Carbohydr. Res.*, 190 (1989) 267–277.
3 T. -H. Lin, P. Kováč, and C. P. J. Glaudemans, *Carbohydr. Res.*, 188 (1989) 228–238.
4 E. M. Nashed and C. P. J. Glaudemans, *J. Org. Chem.*, 52 (1987) 5255–5260.
5 R. Bubey, D. Reynolds, S. A. Abbas, and K. L. Matta, *Carbohydr. Res.*, 183 (1988) 155–162.
6 T. Ziegler, P. Kováč, and C. P. J. Glaudemans, *Carbohydr. Res.*, 194 (1989) 185–198.
7 P. Kováč, N. F. Whittaker, and C. P. J. Glaudemans, *J. Carbohydr. Chem.*, 4 (1985) 243–254.
8 J. Kihlberg, T. Frejd, K. Jansson, A. Sundin, and G. Magnusson, *Carbohydr. Res.*, 176 (1988) 271–286.
9 I. Lundt and C. Pedersen, *Acta Chem. Scand., Ser. B*, 30 (1976) 680–684.
10 P. Kováč and R. B. Taylor, *Carbohydr. Res.*, 167 (1987) 153–173.
11 T. Ziegler, P. Kováč, and C. P. J. Glaudemans, *Carbohydr. Res.*, 203, (1990) 224–239.
12 P. Kováč, *Carbohydr. Res.*, 153 (1986) 237–251.
13 M. E. Jolley and C. P. J. Glaudemans, *Carbohydr. Res.*, 33 (1974) 377–381.
14 P. Kováč and K. J. Edgar, *Carbohydr. Res.*, 201 (1990) 79–94.
15 S. W. Suh, T. N. Bhat, M. A. Navia, G. H. Cohen, D. N. Rao, S. Rudikoff, and D. R. Davies, *Proteins: Struct. Funct. Genet.*, 1 (1986) 74–80.
16 Y. Ittah and C. P. J. Glaudemans, *Carbohydr. Res.*, 95 (1981) 189–194.
17 P. Kováč, E. A. Sokoloski, and C. P. J. Glaudemans, *Carbohydr. Res.*, 128 (1984) 101–109.