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Synthesis of sulfated and phosphorylated glycopeptides from the carbohydrate–protein linkage region of proteoglycans

Sandrine Rio, Jean-Marie Beau and Jean-Claude Jacquinet *

Laboratoire de Biochimie Structurale, U.R.A. 499, U.F.R. Faculté des Sciences, Université d'Orléans, B.P. 6759, F-45067 Orléans (France)

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ABSTRACT

The synthesis of the tetrasaccharide dipeptide β-D-Glc*p*A-(1 → 3)-β-D-Gal*p*4SO_3Na-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Xyl*p*-(1 → O)-L-Ser-Gly was achieved by coupling a suitably protected tetrasaccharide trichloroacetimidate, built up from the nonreducing end by the stepwise addition of monosaccharide units, to the protected dipeptide Z-L-Ser-Gly-*O*Bn. Sulfation at O-4 of the second D-Gal unit and complete deprotection afforded the target molecule in high yield. Its phosphorylated analogue β-D-Glc*p*A-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Xyl*p*2PO_3Na_2-(1 → O)-L-Ser-Gly was synthesized by coupling a protected trisaccharide trichloroacetimidate to the 2,3-*O*-isopropylidene derivative of Z-(D-Xyl-)L-Ser-Gly-OBn. Hydrolysis of the *O*-isopropylidene group, regioselective acetylation at O-3 of the *O*-Xyl unit, and phosphorylation at O-2 followed by complete deprotection gave the phosphorylated tetrasaccharide dipeptide in high yield. These structures are found in the carbohydrate–protein linkage region of several proteoglycans.

INTRODUCTION

Proteoglycans are complex macromolecules that consist of a core protein to which a variable number of glycosaminoglycan chains is covalently attached[1]. In most mammalian proteoglycans, namely those carrying chondroitin sulfate, dermatan sulfate, heparin, and heparan sulfate, the glycosaminoglycan is assumed to be linked to an L-serine residue of the core through a specific tetrasaccharide sequence[2] (Fig. 1). Available information on the structure of the core protein suggests that the substituted L-serine is followed[3] by a glycine residue.

Recently, a sulfated structure was isolated[4] from the linkage region of chondroitin 4-sulfate proteoglycan from swarm rat chondrosarcoma. The location of the sulfate group at O-4 of the second D-galactose unit was established by high-field

* Corresponding author.

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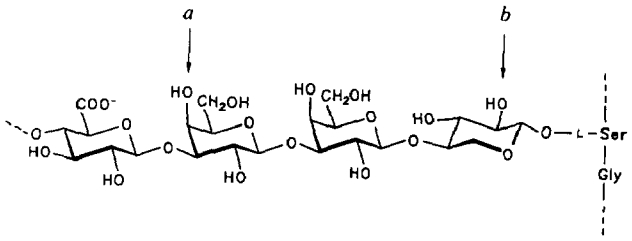
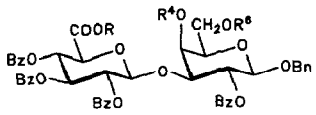


Fig. 1. The carbohydrate–protein linkage region of proteoglycans. The arrows indicate possible substitution with a sulfate (*a*) or a phosphate (*b*) group.

NMR. In addition, phosphate was detected at O-2 of the D-xylose unit in heparan sulfate[5] and heparin[6] from bovine lung, and in chondroitin sulfate from swarm rat chondrosarcoma[7], but the structure was not fully characterized by spectroscopic techniques.

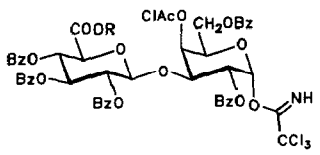
Isolation of glycopeptides from this linkage region by chemical means is complicated by the sensitivity of the xylose–peptide bond to acidic or basic conditions. Thus, chemical synthesis is the alternative of choice for confirming the postulated structures, and providing substrates for relevant biological studies. A synthesis of the sulfated glycotetraosyl-L-serine was recently reported[8], as well as that of a phosphorylated methyl glycoside[9] of the common tetrasaccharide.



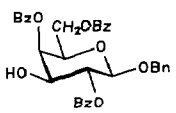
1 R = $CH_2CH_2SiMe_3$, R^4, R^6 = PhCH

2 R = $CH_2CH_2SiMe_3$, $R^4 = R^6$ = H

3 R = $CH_2CH_2SiMe_3$, R^4 = $ClCH_2CO$, R^6 = Bz



4 R = $CH_2CH_2SiMe_3$



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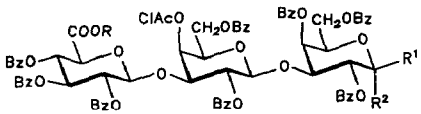
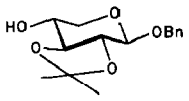


6 R = $CH_2CH_2SiMe_3$, R^1 = OBn, R^2 = H

7 R = $CH_2CH_2SiMe_3$, R^1 = H, R^2 = $OC(NH)CCl_3$



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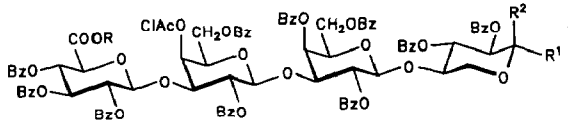
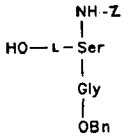
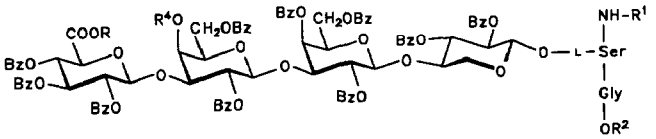


9 R = $CH_2CH_2SiMe_3$, R^1 = OBn, R^2 = H

10 R = $CH_2CH_2SiMe_3$, R^1, R^2 = H, OC(NH)CCl_3



11 Z = COOBn



12 R = $CH_2CH_2SiMe_3$, R^1 = COOBn, R^2 = Bn, R^4 = $ClCH_2CO$

13 R = $CH_2CH_2SiMe_3$, R^1 = COOBn, R^2 = Bn, R^4 = H

14 R = R^2 = Na, R^1 = Ac, R^4 = H

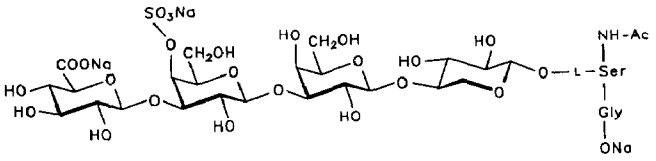
15 R = R^2 = Na, R^1 = Ac, R^4 = SO_3Na

As a part of a program devoted to synthetic and conformational studies of glycopeptides from the carbohydrate–protein linkage region of proteoglycans[10,11], we now report on a versatile approach to the synthesis of both sulfated and phosphorylated tetrasaccharide dipeptides from this region.

RESULTS AND DISCUSSION

The strategy for the synthesis of the sulfated structure involves the preparation of a suitably protected and activated tetrasaccharide block, which is used as a glycosyl donor in a condensation with the L-seryl-glycine dipeptide acceptor, as previously reported[11]. The oligosaccharide moiety, containing exclusively 1,2-*trans* linkages, was constructed stepwise from the nonreducing end by means of 2-*O*-benzoylated glycosyl units activated as their trichloroacetimidates[12]. Temporary protection was required at O-4 of the second D-galactose unit to allow introduction of the sulfate group after total synthesis of the backbone[13].

Benzyl *O*-[2-(trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate]-(1 → 3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside **1**, the synthesis of which was described elsewhere[11], was treated at 100°C in aqueous acetic acid, with 1,2-dimethoxyethane as a co-solvent, to give the crystalline diol **2** (90%). Selective benzoylation at O-6 was readily achieved by treatment of the diol with benzoyl cyanide[14] (1.4 equiv) in 1,2-dichloroethane–pyridine. The mixture, without isolation, was further treated with chloroacetic anhydride to give crystalline **3**



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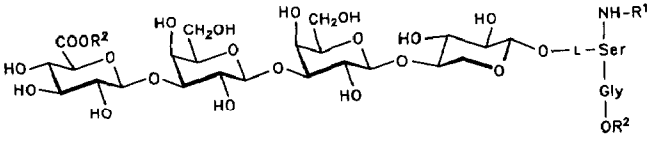
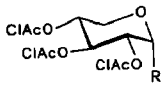


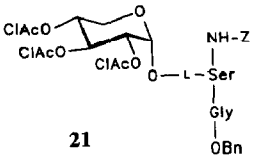
17 $R^1 = R^2 = H$

18 $R^1 = Ac$, $R^2 = Na$



19 $R = ClCH_2COO$

20 $R = Cl$



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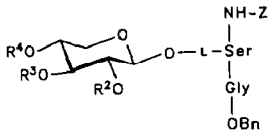


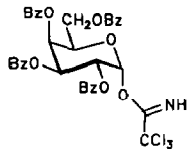
22 $R^2 = R^3 = R^4 = ClCH_2CO$

23 $R^2 = R^3 = R^4 = H$

24 $R^2 = H$, $R^3, R^4 = Me_2C$

25 $R^2, R^3 = Me_2C$, $R^4 = H$

(88%), the structure of which was evident from its ^1H NMR spectrum. Catalytic hydrogenation (Pd–C) of **3** in ethyl acetate gave the corresponding free hemiacetal, which was directly treated with trichloroacetonitrile and 1,8-diazabicyclo-[5.4.0]-undec-7-ene to give the imidate **4** (79%). The α configuration of **4** was indicated by the resonance for H-1 at δ 6.35 (J 3.5 Hz). Condensation of **4** (1 equiv) with benzyl 2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside[11] (**5**, 1.5 equiv) in dry 1,2-dichloroethane at −20°C, in the presence of trimethylsilyl triflate (15 mol % with respect to **4**), afforded the crystalline β-linked trisaccharide **6** (81% from **4**).



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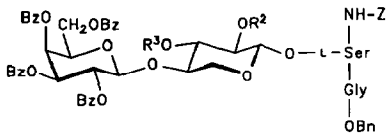


27 $R^2 = R^3 = Me_2C$

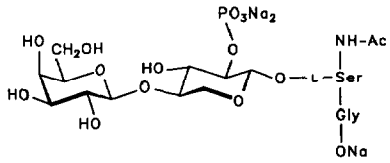
28 $R^2 = R^3 = H$

29 $R^2 = ClCH_2CO$, $R^3 = H$

30 $R^2 = H$, $R^3 = ClCH_2CO$

31 $R^2 = H$, $R^3 = Ac$

32 $R^2 = PO(OBn)_2$, $R^3 = Ac$



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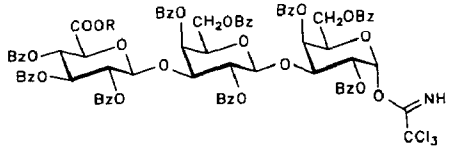
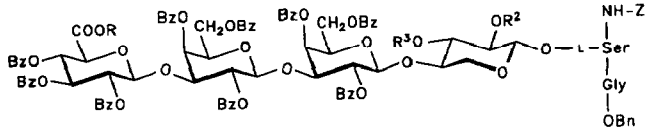
Catalytic hydrogenation of **6** and treatment of the hemiacetal as described for the preparation of **4**, gave 85% of the α-imidate **7**.

On the basis of our previous studies benzyl 2,3-*O*-isopropylidene-β-D-xylopyranoside[10] (**8**) was selected as the xylosyl acceptor. Thus, condensation of **7** (1 equiv) with **8** (1.8 equiv), as described for the preparation of **6**, gave the expected tetrasaccharide derivative, which on mild acid hydrolysis (aqueous acetic acid) of the *O*-isopropylidene group and conventionnal benzoylation of the resulting diol furnished **9** (77% from **7**). The ^1H NMR spectrum of **9** showed signals at δ 4.68 and 4.62 (2 Gal H-1), as well as signals at δ 5.49 and 5.18 attributed by spin decoupling experiments to H-3 and H-2, respectively, of a 2,3-di-*O*-benzoylated D-xylopyranose unit. This proved that glycosylation took place at O-4, and indicated that no migration of the *O*-isopropylidene group had occurred during the glycosylation reaction. The *J* values ($J_{1,2}$ 5.0, $J_{2,3} = J_{3,4} = 6.5$ Hz) observed for the xylosyl residue in **9** suggested a significant departure from the $^4C_1$ conformation in solution. Catalytic hydrogenation of **9**, and treatment of the hemiacetal as described above gave 88% of the imidate **10** as an α,β mixture which could be separated on silica gel. The structure of the major, α anomer was evident from its ^1H NMR spectrum. Examination of the *J* values for the β anomer ($J_{1,2}$ 3.0,



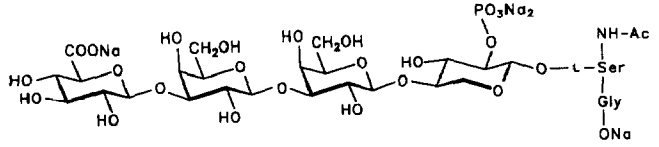
34 R = $CH_2CH_2SiMe_3$



35 R = $CH_2CH_2SiMe_3$, $R^2 = R^3 = H$

36 R = $CH_2CH_2SiMe_3$, $R^2 = H$, $R^3 = Ac$

37 R = $CH_2CH_2SiMe_3$, $R^2 = PO(OBn)_2$, $R^3 = Ac$



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$J_{2,3} = J_{3,4} = 4.0$ Hz) strongly suggested a significant change of the conformation in solution. These values accord with those previously reported[11] for similar structures, and are closed to those listed[15] for *O*-benzoylated derivatives of D-xylose that adopt the $^1C_4$ conformation.

Glycosylation of the dipeptide *N*-benzyloxycarbonyl-L-serylglycine benzyl ester[10] (**11**, 2 equiv) with the imidate **10** (1 equiv) in dry dichloromethane at −20°C, with trimethylsilyl triflate (15 mol % with respect to **10**) as a catalyst gave **12** (86% from **10**). The ^1H NMR spectrum of **12** accorded with the expected structure, and showed a signal at δ 5.11 characteristic for H-2 of a β-linked D-xylose. The H-1 signal, in the overcrowded region of the spectrum, was not identified.

Treatment of **12** with thiourea in ethanol–pyridine gave the alcohol **13** (84%). The ^1H NMR spectrum * of **13** contained, inter alia, a multiplet at δ 4.22

* When two galactose residues are present and need to be distinguished, unprimed locants are assigned to the reducing unit, or the unit nearer the reducing end, and primed locants to the unit nearer the nonreducing end.

attributed by spin-decoupling experiments and exchange with D_2O to Gal H-4′. Partial deprotection of **13** was achieved through removal of the 2-(trimethylsilyl)ethyl group (Bu_4NF in dry tetrahydrofuran at 0°C), followed by catalytic hydrogenation (Pd–C) and selective *N*-acetylation[16] of the resulting free amine (acetic anhydride in *N*,*N*-dimethylformamide). Treatment with an ion-exchange resin (Na^+ form) gave the disodium salt **14** (63% overall yield), the structure of which was confirmed by its ^1H NMR spectrum. It should be noted that attempted selective *N*-acetylation of the free amino group of the L-serine residue with acetic anhydride in aqueous ethyl acetate–methanol led to extensive formation of methyl esters of the uronic acid and/or glycine moieties, probably through acid-catalyzed esterification with methanol. These methyl esters could not be cleaved by treatment with lithium iodide in refluxing *sym*-collidine[17], or with lithium hydroperoxide[18] at low temperature, without extensive degradation of the molecule.

Attempted *O*-sulfation (sulfur trioxide–trimethylamine complex in dry *N*,*N*-dimethylformamide at 50°C) of the disodium salt of **14** was unsuccessful. The use of a large excess of reagent or a prolonged reaction time led to intractable mixtures. However, *O*-sulfation of the corresponding free acid under the same conditions proceeded smoothly, and gave **15**, isolated as its trisodium salt, in 89% yield. The very poor solubility of the salt **14**, or a possible dramatic change in the spatial arrangment of the molecule due to the presence of an ionized carboxyl group on the uronic acid residue, could explain this absence of reaction. Nevertheless, *O*-sulfation of similar structures (uronyl salts) has been reported[13]. Comparison of the ^1H NMR spectra of **15** and **14** (CD_3OD) showed the expected[13] downfield shift (0.73 ppm) of the signal for Gal H-4′ in **15**.

Final deprotection of **15** was achieved through hydrazinolysis[19] in methanol to give the target molecule **16** (85%). No loss or migration of the sulfate group was observed in this transformation.

The unsubstituted tetrasaccharide dipeptide **18** (Na salt) was prepared by selective *N*-acetylation (acetic anhydride in water at pH ~ 8.5) of the amino group in **17** (ref 11) to give a model for NMR comparison. The ^1H NMR spectrum of **16** is in complete agreement with the postulated structure, and the NMR data agree, at least for the tetrasaccharide moiety, with those reported[4] for naturally occurring samples. Sulfation at Gal O-4′ caused the expected[13] downfield shift (0.6 ppm) of the signal for Gal H-4′ in **16**, compared with its nonsulfated analogue **18**. The ^{13}C NMR spectrum of **16** (Table I) confirms the structure, and shows the expected downfield shift (7.6 ppm) of the signal for Gal C-4′, as well as the upfield shift (4.8 ppm) of the signal for Gal C-3′.

In planning the synthesis of the phosphorylated analogue, we first intended to use a protected phosphate group as a stereocontrolling auxiliary at O-2 of the D-xylose unit of the activated tetrasaccharide intermediate, and to condense this latter with dipeptide **11**. However, preliminary experiments with several 2-*O*-phosphorylated derivatives of D-xylose, under various activation conditions, showed that

TABLE I

^{13}C NMR data for synthetic glycopeptides (Na salts)

Carbon atom		Chemical shifts (ppm) [a]			
		Unsubstituted **18**	4-Sulfate **16**	2-Phosphate **33**	2-Phosphate **38**
Ser	α-CH	53.75	53.77	53.86	53.86
	β-CH_2	68.30	68.64	68.94	68.31
	N-$COCH_3$	22.14	22.13	22.19	22.17
Gly	CH_2	43.67	43.24	43.71	43.33
Xyl	C-1	103.26	103.13	102.08	102.15
	C-2	72.94	72.95	76.68	76.87
	C-3	74.01	74.02	73.38	73.41
	C-4	76.75	76.91	75.95	75.95
	C-5	63.24	63.24	62.77	62.83
Gal	C-1	101.64	101.67	101.87	101.52
	C-2	70.42	71.28	70.87	70.43
	C-3	82.66	82.41	72.89	82.61
	C-4	69.33	69.28	69.27	69.28
	C-5	75.19	75.21	75.56	75.20
	C-6	61.32	61.33	61.34	61.31
Gal	C-1′	104.20	104.33		104.20
	C-2′	70.10	70.12		70.06
	C-3′	82.26	77.45		82.23
	C-4′	68.72	76.31		68.76
	C-5′	75.06	74.72		75.02
	C-6′	61.23	61.21		61.22
GlcA	C-1	103.86	103.23		103.82
	C-2	73.43	73.35		73.41
	C-3	75.58	75.61		75.57
	C-4	72.03	72.07		72.00
	C-5	76.42	76.70		76.30
	C-6	175.90	175.57		175.75

[a] In D_2O at 27°C; quoted in ppm from external Me_4Si, measured from internal acetone (30.50 ppm).

this approach was not realistic. We thus decided to take advantage of the versatility of 2,3-*O*-isopropylidene-D-xylosides. A retrosynthetic analysis led us to design a glucosyluronic-galactosyl-galactosyl donor, and a 2,3-*O*-isopropylidene derivative of D-Xyl-L-Ser-Gly as an acceptor. After stereocontrolled coupling and mild acid hydrolysis, regioselective substitution at O-2 of the corresponding diol could be studied. A similar synthon has already been reported for the preparation of a galactosyl-galactosyl-xylosyl-L-serine glycopeptide[20], but the preparation of the xylosyl-L-serine acceptor (13 steps from D-arabinose) was rather lengthy.

Our novel short synthesis of acceptor **25** started from D-xylose. Treatment of 1,2,3,4-tetra-*O*-chloroacetyl-α-D-xylopyranose[21] (**19**) with titanium(IV) chloride (1.2 equiv) in dichloromethane gave 95% of the unstable α-chloride **20**. Condensation

of **20** (1 equiv) with dipeptide **11** (1.2 equiv) in dry 1,2-dichloroethane at 0°C, with silver triflate (2 equiv) as a promoter, gave the crystalline β-linked glycopeptide **22** (68%), together with the α isomer **21** (19%), the structures of which were evident from their ^{1}H NMR spectra. This rather low stereocontrolling power of the chloroacetate ester group in glycosylation reactions with soluble silver salts was recently reported[21]. Attempted glycosylation with insoluble silver silicate[22] as a promoter led essentially to the formation of complex imidates[23], resulting from attack by the peptide amidic carbonyl oxygen at the anomeric center of the sugar. Treatment of **22** with an excess of thiourea gave crystalline **23** (85%), without any undesired side reaction in the peptide moiety. Protection of **23** was achieved through treatment with 2-methoxypropene in *N*,*N*-dimethylformamide, with camphorsulfonic acid as a catalyst. The subsequent cleavage of the mixed acetal[24] with methanol at 0°C yielded the crystalline 2,3-*O*-isopropylidene derivative **25** (65%), along with its 3,4-*O*-isopropylidene isomer **24** (20%).

The suitability of **25** as a glycosyl acceptor was first tested in a synthesis of a model disaccharide-peptide. Condensation of **25** (1 equiv) with 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate[10] (**26**, 1.2 equiv), as described for the preparation of **12**, gave **27** (75%), confirmed by its ^{1}H NMR spectrum. Mild hydrolysis (aqueous acetic acid) of **27** gave the diol **28** (94%). The ^{1}H NMR spectrum of **28**, recorded in Me_2SO-d_6, was in general agreement with the expected structure, and it showed multiplets at δ 3.18 and 2.92 that could be attributed by spin decoupling experiments and exchange with D_2O to Xyl H-3 and H-2, respectively. The attempted selective phosphorylation or acylation of the diol **28** led to complex mixtures. However, regioselective acylation could be readily achieved through the tin procedure[25]. Treatment of the 2,3-*O*-dibutylstannylene derived of **28** with chloroacetyl chloride (1.1 equiv) led to the formation of the 2-chloroacetate **29** as a major product, whereas reaction with the corresponding anhydride (1.1 equiv) gave essentially the 3-chloroacetate **30** (75%). Similar treatment with acetic anhydride gave the 3-acetate **31** (63%). Treatment of **31** with dibenzyl *N*,*N*-diisopropylphosphoramidite[26] and 1-*H*-tetrazole in dichloromethane gave the corresponding phosphite, which was oxidized[26] in situ with *m*-chloroperbenzoic acid to give the 2-phosphate **32** in 90% overall yield. Examination of the ^{1}H NMR spectrum of **32** showed, in the signal for Xyl H-2, an additional coupling, $^3J_{H,P} = 9.0$ Hz.

Deprotection of **32** was achieved through catalytic hydrogenation (Pd-C), followed by ion-exchange chromatography on Sephadex SP-C25 (Na^+), *N*-acetylation, and hydrazinolysis, as described before, to give the glycopeptide **33**, isolated as its trisodium salt (72% from **32**). The ^{1}H NMR spectrum of **33** is in full agreement with the expected structure, including the additional coupling $^3J_{H,P} = 9.0$ Hz in the signal for Xyl H-2. Phosphorylation at O-2 caused a downfield shift (0.5 ppm) of the signal for Xyl H-2, compared with that of the nonphosphorylated structure **18**. The ^{13}C NMR spectrum (Table I) also accords with the structure, and shows the additional couplings $^2J_{C,P} = 6.0$ and $^3J_{C,P} = 3.0$ Hz in the signals of Xyl

C-2 and C-3, respectively. The low value (3.0 Hz) observed for this $^3J_{C,P}$ indicates a *gauche* relationship between C-3 and the P atom. Phosphorylation at O-2 caused a downfield shift (3.7 ppm) of the signal for Xyl C-2, and upfield shifts (1.1 and 0.6 ppm) of the signals for Xyl C-1 and C-3, respectively, compared with those observed for **18**.

The synthesis of the phosphorylated tetrasaccharide dipeptide **38** was then accomplished as follows. The crucial coupling between the trisaccharide imidate[11] (**34**, 1 equiv) and **25** (1.25 equiv), as described for the preparation of **12**, followed by treatment of the crude mixture with aqueous acetic acid, gave the diol **35** (79%). No α isomer was detected. The ^{1}H NMR spectrum of **35**, recorded in Me_2SO-d_6, agreed with the expected structure, and the same argument as described above for the diol **28** confirmed that the glycosylation occurred at O-4. Regioselective acetylation of **35** at O-3 was achieved through the tin procedure, as described for **31**, to give **36** (81%), the structure of which was confirmed by ^{1}H NMR spectroscopy. Phosphorylation of **36**, as described for the preparation of **32**, gave **37** (91%), the ^{1}H NMR spectrum of which showed the additional coupling $^3J_{H,P} = 9.0$ Hz in the signal for Xyl H-2.

Final deprotection was achieved through cleavage of the 2-(trimethylsilyl) ethyl ester, catalytic hydrogenation followed by ion-exchange chromatography, *N*-acetylation, and hydrazinolysis, essentially as already described, to give the target molecule **38** (64% from **37**). The ^{1}H NMR spectrum of **38** is in complete agreement with the expected structure, including the additional coupling $^3J_{H,P} = 9.0$ Hz in the signal for Xyl H-2. Phosphorylation at O-2 caused a significant downfield shift (0.55 ppm) of the signal for Xyl H-2, compared with that of the unsubstituted analogue **18**. The ^{13}C NMR spectrum (Table I) also accords with the structure, and shows the characteristic coupling $^2J_{C,P} \sim 5$ Hz in the signal for Xyl C-2. Noticeable also is the downfield shift (3.93 ppm) of the signal for Xyl C-2, and the upfield shifts (1.1 and 0.6 ppm) of the signals for Xyl C-1 and C-3, respectively.

In conclusion, stereocontrolled and high-yielding syntheses of the substituted tetrasaccharide dipeptides **16** and **38** are reported. These compounds, obtained in reasonable amounts, are currently being evaluated in biological and conformational studies.

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 20–25°C with a Perkin–Elmer Model 141 polarimeter. The ^{1}H (300 MHz) and ^{13}C (75.4 MHz) NMR spectra were recorded at 300 K with a Bruker AM-300 WB spectrometer. Chemical shifts (δ) are given from the signal of internal Me_4Si unless otherwise stated. The purity of the products was checked by TLC on Silica Gel F_{254} Merck, with detection by charring with H_2SO_4. Flash-column chromatography was performed on Silica Gel Merck, 40–63 μm. Elemental analyses were performed by

the Service Central de Microanalyse du Centre National de la Recherche Scientifique (Vernaison, France).

Benzyl O-[*2-(trimethylsilyl)ethyl 2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranosyluronate*]-(*1 → 3*)-*2*-O-*benzoyl*-β-D-*galactopyranoside* (**2**).—A solution of benzyl *O*-[2-(trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate]-(1 → 3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside[11] (**1**, 2.36 g, 2.25 mmol) in 1,2-dimethoxyethane (40 mL), AcOH (120 mL), and water (50 mL) was stirred at 100°C for 40 min then concentrated, and the residue was crystallized from EtOAc to give **2** (1.96 g, 90%); mp 236–237°C; $[\alpha]_D$ −5° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.50 (m, 25 H, 5 Ph), 5.79 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, GlcA H-3) 5.68 (t, 1 H, $J_{4,5}$ 9.0 Hz, GlcA H-4), 5.58 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, Gal H-2), 5.47 (dd, 1 H, $J_{1,2}$ 7.0 Hz, GlcA H-2), 5.03 (d, 1 H, GlcA H-1), 4.72 (ABq, 2 H, OCH_2Ph), 4.54 (d, 1 H, Gal H-1), 4.31 (d, 1 H, GlcA H-5), 4.27 (m, 1 H, $J_{3,4}$ 3.5 Hz, Gal H-4), 3.97 (dd, 1 H, Gal H-3), 3.12 (br s, 1 H, Gal HO-4), 2.36 (br s, 1 H, Gal HO-6), 0.74 (m, 2 H, CH_2Si), and −0.07 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{52}H_{54}O_{16}Si$: C, 64.85; H, 5.65. Found: C, 64.91; H, 5.38.

Benzyl O-*[2-(trimethylsilyl)ethyl 2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranosyluronate*]-(*1 → 3*)-*2,6-di*-O-*benzoyl-4*-O-*chloroacetyl*-β-D-*galactopyranoside* (**3**).—Benzoyl cyanide in anhyd CH_2Cl_2 (1.0 M, 1.26 mL) was added to a solution of **2** (900 mg, 0.9 mmol) in 1,2-dichloroethane (5 mL) and pyridine (5 mL), and the mixture was stirred overnight at room temperature, then cooled to 0°C. 4-Dimethylaminopyridine (80 mg) and chloroacetic anhydride (480 mg, 2.8 mmol) were added and the mixture was stirred at 0°C for 30 min. Ice-cold water (1 mL) was added, and the mixture was stirred for 15 min at 0°C, then diluted with CH_2Cl_2 (100 mL), washed with water, satd aq $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (50 g) with 15:1 toluene–EtOAc to give **3** (940 mg, 88%); mp 203–204°C (from EtOAc–hexane); $[\alpha]_D$ −10° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.58 (m, 30 H, 6 Ph), 5.73 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, Gal H-4), 5.68 (m, 2 H, GlcA H-3 and H-4), 5.49 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, Gal H-2), 5.37 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.5 Hz, GlcA H-2), 4.91 (d, 1 H, GlcA H-1), 4.56 (dd, 1 H, $J_{5,6a}$ 7.5, $J_{6a,6b}$ 11.5 Hz, Gal H-6a), 4.41 (d, 1 H, Gal H-1), 4.42 (dd, 1 H, $J_{5,6b}$ 6.0 Hz Gal H-6b), 4.30 (ABq, 2 H, $COCH_2Cl$), 0.77 (m, 2 H, CH_2Si), and −0.06 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{61}H_{59}ClO_{18}Si$: C, 64.06; H, 5.20. Found: C, 63.83; H, 5.17.

O-*[2-(Trimethylsilyl)ethyl 2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranosyluronate*]-(*1 → 3*)-*2,6-di*-O-*benzoyl-4*-O-*chloroacetyl*-α-D-*galactopyranosyl trichloroacetimidate* (**4**).—A solution of **3** (1.28 g, 1.12 mmol) in EtOAc (20 mL) was hydrogenated in the presence of 10% Pd–C (500 mg) for 16 h, then filtered and concentrated. A mixture of the residue, trichloroacetonitrile (1.12 mL, 11.2 mmol), and 1,8-diazabicyclo[5.4.0]undec-7-ene (42 μL, 0.28 mmol) in CH_2Cl_2 (10 mL) was stirred for 15 min at room temperature, then directly eluted from a column of silica gel (80 g) with 15:1 toluene–EtOAc containing 0.1% of Et_3N, to give **4** (1.06 g, 79%); $[\alpha]_D$ +54° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.50 (s, 1 H, C=N*H*), 7.64 (m, 25 H,

5 Ph), 6.65 (d, 1 H, $J_{1,2}$ 3.5 Hz, Gal H-1), 5.92 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, Gal H-4), 5.77 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, GlcA H-3), 5.71 (t, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-4), 5.56 (dd, 1 H, $J_{2,3}$ 10.0 Hz, Gal H-2), 5.41 (dd, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-2), 5.11 (d, 1 H, Gal H-1), 4.32 (d, 1 H, GlcA H-5), 4.28 (ABq, 2 H, $COCH_2Cl$), 0.81 (m, 2 H, CH_2Si), and −0.04 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{56}H_{53}Cl_4NO_{18}$: C, 56.15; H, 4.46; N, 1.17. Found: C, 56.28; H, 4.35; N, 1.32.

Benzyl O-[2-(trimethylsilyl)ethyl 2,3,4-tri-O-benzoyl-β-D-glucopyranosyluronate]-(1 → 3)-O-(2,6-di-O-benzoyl-4-O-chloroacetyl-β-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-O-benzoyl-β-D-galactopyranoside (**6**).—A mixture of **4** (0.68 g, 0.57 mmol), benzyl 2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside[11] (**5**, 0.5 g, 0.85 mmol), and activated powdered 4A molecular sieves (500 mg) in anhyd 1,2-dichloroethane (14 mL) was stirred for 1 h at room temperature under dry Ar, then cooled to −20°C. Trimethylsilyl triflate in toluene (1.0 M, 86 μL, 0.086 mmol) was added, and the mixture was stirred for 20 min at −20°C. Pyridine (0.28 mL) and trimethylsilyl chloride (0.32 mL, 2.5 mmol) were added and the mixture was stirred for 30 min at 0°c, then filtered, diluted with CH_2Cl_2 (50 mL), washed successively with brine and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (80 g) with 10:1 toluene–EtOAc containing 0.1% of Et_3N to give **6** (746 mg, 81%); mp 109–110°C (from hexane–EtOAc); $[\alpha]_D$ 0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.73 (m, 45 H, 9 Ph), 5.78, 5.61 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, 2 Gal H-4), 5.58 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, GlcA H-4), 5.54 (t, 1 H, $J_{2,3}$ 9.5 Hz, GlcA H-3), 5.53, 5.18 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 5.23 (dd, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-2), 4.72 (d, 1 H, GlcA H-1), 4.71, 4.42 (2 d, 2 H, 2 Gal H-1), 4.05 (d, 1 H, GlcA H-5), 4.04 (ABq, 2 H, $COCH_2Cl$), 0.74 (m, 2 H, CH_2Si), and −0.08 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{88}H_{81}ClO_{26}Si$: C, 65.32; H, 5.04. Found: C, 65.29; H, 5.03.

O-[2-(Trimethylsilyl)ethyl 2,3,4-tri-O-benzoyl-β-D-glucopyranosyluronate]-(1 → 3)-O-(2,6-di-O-benzoyl-4-O-chloroacetyl-β-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-O-benzoyl-α-D-galactopyranosyl trichloroacetimidate (**7**).—Compound **6** (726 mg, 0.45 mmol) was treated as described for the preparation of **4**. The mixture was directly eluted from a column of silica gel with 10:1 toluene–acetone containing 0.1% of Et_3N to give **7** (638 mg, 85%); $[\alpha]_D$ +39° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.42 (s, 1 H, C=N*H*), 7.55 (m, 40 H, 8 Ph), 6.63 (d, 1 H, $J_{1,2}$ 3.5 Hz, Gal H-1), 5.98 (dd, 1 H, $J_{3,4}$ 4.0, $J_{4,5}$ 1.0 Hz, Gal H-4), 5.68 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, Gal H-4′), 5.62 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, GlcA H-4), 5.61 (dd, 1 H, $J_{2,3}$ 10.0 Hz, Gal H-2), 5.56 (t, 1 H, $J_{2,3}$ 9.5 Hz, GlcA H-3), 5.24 (dd, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-2), 5.23 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, Gal H-2′), 4.89 (d, 1 H, Gal H-1′), 4.77 (d, 1 H, GlcA H-1), 4.09 (d, 1 H, GlcA H-5), 4.07 (ABq, 2 H, $COCH_2Cl$), 0.77 (m, 2 H, CH_2Si), and −0.06 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{83}H_{75}Cl_4NO_{26}Si$: C, 59.61; H, 4.52; N, 0.84. Found: C, 59.43; H, 4.55; N. 0.98.

Benzyl O-[2-(trimethylsilyl)ethyl 2,3,4-tri-O-benzoyl-β-D-glucopyranosyluronate]-(1 → 3)-O-(2,6-di-O-benzoyl-4-O-chloroacetyl-β-D-galactopyranosyl-(1 → 3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2,3,-di-O-benzoyl-β-D-xylopyranoside

(**9**).—A mixture of **7** (0.96 g, 0.57 mmol), benzyl 2,3-*O*-isopropylidene-β-D-xylopyranoside[10] (**8**, 290 mg, 1.03 mmol), and activated powdered 4A molecular sieves (500 mg) in anhyd 1,2-dichloroethane (14 mL) was stirred for 1 h at room temperature under dry Ar, then cooled to −20°C. Trimethylsilyl triflate in toluene (1.0 M, 86 μL) was added, and the mixture was stirred for 30 min at −20°C. Triethylamine (24 μL) was added, and the mixture was filtered, then concentrated. A solution of the residue in aq AcOH (70%, 30 mL) was stirred at 100°C for 5 min, then cooled, and concentrated. Benzoyl chloride (0.5 mL) and 4-dimethylaminopyridine (50 mg) were added at 0°C to a solution of the residue in anhyd pyridine (12 mL), and the mixture was stirred for 2 h at this temperature. MeOH (0.5 mL) was added, the mixture was diluted with CH_2Cl_2 (100 mL), washed with satd aq $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel with 12:1 toluene–acetone to give **9** (866 mg, 77%); $[\alpha]_D$ −3.5° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.56 (m, 55 H, 11 Ph), 5.64, 5.59 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, 2 Gal H-4), 5.58 (t, 1 H, $J_{3,4} = J_{4,5} = 9.0$ Hz, GlcA H-4), 5.54 (t, 1 H, $J_{2,3}$ 9.0 Hz, GlcA H-3), 5.49 (t, 1 H, $J_{2,3} = J_{3,4} = 6.5$ Hz, Xyl H-3), 5.38 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, Gal H-2), 5.22 (dd, 1 H, $J_{1,2}$ 7.0 Hz, GlcA H-2), 5.18 (dd, 1 H, $J_{1,2}$ 5.0 Hz, Xyl H-2), 5.16 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.5 Hz, Gal H-2), 4.72 (d, 1 H, GlcA H-1), 4.68, 4.62 (2 d, 2 H, 2 Gal H-1), 4.65 (d, 1 H, Xyl H-1), 4.60 (ABq, 2 H, OCH_2Ph), 3.99 (ABq, 2 H, $COCH_2Cl$), 0.73 (m, 2 H, CH_2Si), and −0.08 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{107}H_{97}ClO_{32}Si$: C, 65.62; H, 4.99. Found: C, 65.60; H, 4.91.

O-[2-(Trimethylsilyl)ethyl 2,3,4-tri-O-benzoyl-β-D-glucopyranosyluronate]-(1 → 3)-O-(2,6-di-O-benzoyl-4-O-chloroacetyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-α,β-D-xylopyranosyl trichloroacetimidate (**10**).—Compound **9** (455 mg, 0.23 mmol) was treated as described for the preparation of **4**. The mixture was directly eluted from a column of silica gel (35 g) with 7:1 toluene–EtOAc containing 0.1% of Et_3N to give first the β-imidate (113 mg, 28%); $[\alpha]_D$ −0.6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.67 (s, 1 H, C=N*H*), 7.58 (m, 50 H, 10 Ph), 6.17 (d, 1 H, $J_{1,2}$ 3.0 Hz, Xyl H-1), 5.68, 5.65 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, 2 Gal H-4), 5.63 (t, 1 H, $J_{2,3} = J_{3,4} = 4.0$ Hz, Xyl H-3), 5.47, 5.42 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 5.28 (dd, 1 H, Xyl H-2), 5.26 (dd, 1 H, $J_{1,2}$ 7.0, $J_{2,3}$ 10.0 Hz, GlcA H-2), 4.74, 4.71 (2 d, 2 H, 2 Gal H-1), 4.73 (d, 1 H, GlcA H-1), 3.94 (ABq, 2 H, $COCH_2Cl$), 0.72 (m, 2 H, CH_2Si), and −0.08 (s, 9 H, $SiMe_3$).

Next eluted was the α-imidate (263 mg, 60%); mp 137–138°C (from ethyl ether); $[\alpha]_D$ +15° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.48 (s, 1 H, C=N*H*), 7.57 (m, 50 H 10 Ph), 6.48 (d, 1 H, $J_{1,2}$ 3.5 Hz, Xyl H-1), 5.83 (t, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, GlcA H-4), 5.68, 5.60 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, 2 Gal H-4), 5.53 (dd, 1 H, $J_{2,3}$ 9.5 Hz, GlcA H-3), 5.32, 5.15 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 5.27 (dd, 1 H, $J_{2,3}$ 10.5 Hz, Xyl H-2), 5.22 (dd, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-2), 4.72 (d, 1 H, GlcA H-1), 4.68, 4.55 (2 d, 2 H, 2 Gal H-1), 4.07 (ABq, 2 H, $COCH_2Cl$), 4.03 (d, 1 H, GlcA H-5), 0.72 (m, 2 H, CH_2Si), and −0.08 (s, 9 H, $SiMe_3$). Anal. Calcd for

$C_{102}H_{91}Cl_4NO_{32}Si \cdot H_2O$: C, 60.33; H, 4.61; N, 0.69. Found: C, 60.15; H, 4.71; N, 0.72.

N-Benzyloxycarbonyl-O-{O-[2-(trimethylsilyl)ethyl 2,3,4-tri-O-benzoyl-β-D-glucopyranosyluronate]-(1 → 3)-O-(2,6-di-O-benzoyl-4-O-chloroacetyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(2,3-di-O-benzoyl-β-D-xylopyranosyl)}-L-serylglycine benzyl ester (**12**).—A mixture of **10** (0.75 g, 0.37 mmol), *N*-benzyloxycarbonyl-L-serylglycine benzyl ester[10] (**11**, 287 mg, 0.74 mmol), and activated powdered 4A molecular sieves (500 mg) in anhyd CH_2Cl_2 (15 mL) was stirred for 1 h at room temperature under dry Ar, then cooled to −20°C. Trimethylsilyl triflate in toluene (0.5 M, 112 μL) was gradually added, and the mixture was stirred for 45 min at −20°C. Triethylamine (16 μL) was added, and the mixture was filtered, then concentrated. The residue was eluted from a column of silica gel (50 g) with 3:1 toluene–EtOAc to give **12** (717 mg, 86%); $[\alpha]_D$ +3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.58 (m, 60 H, 12 Ph), 6.77 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 5.67, 5.59 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, 2 Gal H-4), 5.58 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, GlcA H-4), 5.53 (dd, 1 H, $J_{2,3}$ 9.0 Hz, GlcA H-3), 5.35, 5.17 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 5.22 (dd, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-2), 5.11 (dd, 1 H, $J_{1,2}$ 6.0, $J_{2,3}$ 8.0 Hz, Xyl H-2), 5.04 (ABq, 2 H, OCH_2Ph), 5.00 (s, 2 H, OCH_2Ph), 4.72 (d, 1 H, GlcA H-1), 4.68, 4.57 (2 d, 2 H, 2 Gal H-1), 4.02 (ABq, 2 H, COCH_2Cl), 0.72 (m, 2 H, CH_2Si), and −0.08 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{120}H_{111}ClN_2O_{37}Si \cdot H_2O$: C, 63.92; H, 5.05; N, 1.24. Found: C, 63.71; H, 5.10; N, 1.33.

N-Benzyloxycarbonyl-O-{O-[2-(trimethylsilyl)ethyl 2,3,4-tri-O-benzoyl-β-D-glucopyranosyluronate]-(1 → 3)-O-(2,6-di-O-benzoyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(2,3-di-O-benzoyl-β-D-xylopyranosyl)}-L-serylglycine benzyl ester (**13**).—A mixture of **12** (260 mg, 0.11 mmol) and thiourea (44 mg, 0.6 mmol) in pyridine (1.5 mL) and EtOH (3.5 mL) was stirred at 80°C for 20 h, then cooled, and concentrated. A solution of the residue in $CHCl_3$ (50 mL) was washed with brine, and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (20 g) with 3:1 toluene–EtOAc to give **13** (211 mg, 84%); $[\alpha]_D$ +4° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.52 (m, 60 H, 12 Ph), 6.75 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 5.70 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.6 Hz, Gal H-4), 5.49 (t, 1 H, $J_{2,3} = J_{3,4} = 8.5$ Hz, Xyl H-3), 5.35, 5.23 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 5.31 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.0 Hz, GlcA H-2), 5.11 (dd, 1 H, $J_{1,2}$ 6.0 Hz, Xyl H-2), 4.88 (d, 1 H, GlcA H-1), 4.65, 4.54 (2 d, 2 H, 2 Gal H-1), 4.59 (d, 1 H, Xyl H-1), 4.22 (m, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.8, $J_{4,OH}$ 3.2 Hz, Gal H-4′), 4.19 (d, 1 H, $J_{4,5}$ 9.0 Hz, GlcA H-5), 3.00 (d, 1 H, Gal HO-4′), 0.71 (m, 2 H, CH_2Si), and −0.08 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{118}H_{110}N_2O_{36}Si$: C, 65.61; H, 5.13; N, 1.30. Found: C, 65.80; H, 4.85; N, 1.34.

N-Acetyl-O-[O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyluronic acid)-(1 → 3)-O-(2,6-di-O-benzoyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(2,3-di-O-benzoyl-β-D-xylopyranosyl)]-L-serylglycine, disodium salt (**14**).—A mixture of **13** (540 mg, 0.25 mmol), and Bu_4NF (261 mg, 1 mmol) in

anhyd THF (8 mL) was stirred for 1 h at 0°C under Ar. The mixture was diluted with $CHCl_3$ (100 mL), washed with cold 0.1 M HCl, satd aq NH_4Cl, and water, dried ($MgSO_4$), and concentrated. The residue was hydrogenated in 12:2:1 EtOAc–MeOH–water (20 mL) in the presence of 10% Pd–C (300 mg) for 48 h, then filtered, and concentrated. Acetic anhydride (0.5 mL) was added dropwise at 0°C to a solution of the residue in DMF (8 mL), and the mixture was stirred for 10 min at 0°C, then concentrated at < 30°C. The residue was eluted from a column of silica gel (20 g) with 8:2:1 EtOAc–MeOH–water, then from a column (1 × 20 cm) of Sephadex SP C-25 (Na^+ form) with the same mixture of solvents to give amorphous hygroscopic **14** (326 mg, 68%); $[\alpha]_D$ −5° (*c* 1, MeOH); ^1H NMR (CD_3OD): δ 7.62 (m, 50 H, 10 Ph), 5.68 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.5 Hz, Gal H-4), 5.63 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, GlcA H-3), 5.58 (t, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-4), 5.46 (t, 1 H, $J_{2,3} = J_{3,4} = 8.0$ Hz, Xyl H-3), 5.35 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.5 Hz, GlcA H-2), 5.32, 5.22 (2 dd, 2 H, J_{12} 8.0, $J_{2,3}$ 10.0 Hz, Gal H-2 and H-2′), 5.11 (d, 1 H, $J_{1,2}$ 6.5 Hz, Xyl H-2), 5.04 (d, 1 H, GlcA H-1), 4.79, 4.75 (2 d, 2 H, Gal H-1′ and H-1), 4.67 (d, 1 H, Xyl H-1), 4.34 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.5 Hz, Gal H-4′), 4.28, 4.01 (2 dd, 2 H, Gal H-3 and H-3′), 4.18 (d, 1 H, GlcA H-5), and 1.73 (s, 3 H, NAc). Anal. Calcd for $C_{100}H_{86}N_2Na_2O_{35} \cdot 2H_2O$: C, 61.35; H, 4.63; N, 1.43. Found: C, 61.21; H, 4.75; N, 1.32.

N-*Acetyl*-O-[O-(*2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranosyluronic acid*)-(1 → 3)-O-(*2,6-di*-O-*benzoyl*-*4*-O-*sulfo*-β-D-*galactopyranosyl*)-(1 → 3)-O-(*2,4,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl*)-(1 → 4)-(*2,3-di*-O-*benzoyl*-β-D-*xylopyranosyl*)]-L-*serylglycine, trisodium salt* (**15**). — A solution of **14** (97 mg, 50 μmol) in 5:2:1 EtOAc–MeOH–water (5 mL) was stirred at room temperature with Amberlite IR-120 (H^+) resin (1 mL) for 20 min, then filtered, concentrated, and dried in vacuo. A mixture of the residue and SO_3–Me_3N complex (70 mg, 0.5 mmol) in anhyd DMF (2 mL) was stirred for 48 h at 50°C under Ar, then cooled. MeOH (0.1 mL) was added, and the mixture was eluted from a column (2.5 × 60 cm) of Sephadex LH-20 with 1:1 CH_2Cl_2–MeOH. The residue was eluted from a column (1 × 20 cm) of Sephadex SP C-25 (Na^+) with 5:2:1 EtOAc–MeOH–water to give **15** (90 mg, 89%) $[\alpha]_D$ −8° (*c* 1, MeOH); ^1H NMR (CD_3OD): δ 7.50 (m, 50 H, 10 Ph), 5.69 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, GlcA H-4), 5.65 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.8 Hz, Gal H-4), 5.62 (t, 1 H, $J_{2,3}$ 9.5 Hz, GlcA H-3), 5.49 (dd, 1 H, $J_{1,2}$ 8.0 Hz, GlcA H-2), 5.45 (t, 1 H, $J_{2,3} = J_{3,4} = 8.0$ Hz, Xyl H-3), 5.31, 5.14 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, Gal H-2 and H-2′), 5.11 (dd, 1 H, $J_{1,2}$ 6.5 Hz, Xyl H-2), 5.07 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.5 Hz, Gal H-4′), 5.04 (d, 1 H, GlcA H-1), 4.77, 4.69 (2 d, 2 H, Gal H-1′ and H-1), 4.68 (d, 1 H, Xyl H-1), 4.25, 4.23 (2 dd, 2 H, Gal H-3′ and H-3), 4.17 (d, 1 H, GlcA H-5), and 1.74 (s, 3 H, NAc). Anal. Calcd for $C_{100}H_{85}N_2Na_2O_{38}S \cdot H_2O$: C, 58.82; H, 4.29; N, 1.37. Found: C, 58.61; H, 4.39; N, 1.32.

N-*Acetyl*-O-[O-(β-D-*glucopyranosyluronic acid*)-(1 → 3)-O-(*4*-O-*sulfo*-β-D-*galactopyranosyl*)-(1 → 3)-O-β-D-*galactopyranosyl*-(1 → 4)-β-D-*xylopyranosyl*]-L-*serylglycine, trisodium salt* (**16**).—A mixture of **15** (168 mg, 82 μmol) and 98% hydrazine hydrate (2 mL) in MeOH (5 mL) was stirred for 5 h at room tempera-

ture, then cooled to 0°C. Acetone (20 mL) was added cautiously, and the mixture was stirred for 30 min, then concentrated. The resulting syrup was triturated with abs EtOH (3 × 2 mL), and the residue was eluted from a column (2.3 × 120 cm) of Sephadex G-10 with water to give amorphous, hygroscopic **16** (70 mg, 85%); $[\alpha]_D$ −13° (*c* 1, H_2O); NMR data: ^1H (D_2O, internal H_2O, δ_H 4.754), δ 4.82 (dd, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ 0.6 Hz, Gal H-4′), 4.81 (d, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-1), 4.72 (d, 1 H, $J_{1,2}$ 8.0 Hz, Gal H-1′), 4.68 (dd, 1 H, $J_{H\alpha,Ha}$ 5.0, $J_{H\alpha,Hb}$ 5.5 Hz, Ser α-C*H*), 4.55 (d, 1 H, $J_{1,2}$ 8.0 Hz, Gal H-1), 4.47 (d, 1 H, $J_{1,2}$ 7.6 Hz, Xyl H-1), 4.22 (dd, H, $J_{Ha,Hb}$ 11.0 Hz, Ser β-C*H*a), 4.21 (dd, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ 0.8 Hz, Gal H-4), 4.13 (dd, 1 H, $J_{4,5eq}$ 5.0, $J_{5ax,5eq}$ 12.0 Hz, Xyl H-5*eq*), 4.08 (dd, 1 H, $J_{2,3}$ 10.0 Hz, Gal H-3′), 3.95 (dd, 1 H, Ser β-C*H*b), 3.86 (s, 2 H, Gly CH_2), 3.75 (d, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-5), 3.71 (dd, 1 H, $J_{2,3}$ 10.0 Hz, Gal H-2), 3.64 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, Xyl H-3), 3.58 (dd, 1 H, $J_{3,4}$ 9.0 Hz, GlcA H-4), 3.54 (t, 1 H, $J_{2,3}$ 9.0 Hz, GlcA H-3), 3.44 (dd, 1 H, GlcA H-2), 3.43 (dd, 1 H, $J_{4,5ax}$ 10.0 Hz, Xyl H-5*ax*), 3.37 (dd, 1 H, Xyl H-2), and 2.11 (s, 3 H, NAc); ^{13}C (D_2O, internal acetone), see Table I. Anal. Calcd for $C_{30}H_{45}N_2Na_3O_{28}S \cdot 3\ H_2O$: C, 34.75; H, 4.96; N, 2.70. Found: C, 34.61; H, 5.01; N, 2.60.

N-*Acetyl-O-[O-(β-D-glucopyranosyluronic acid)-(1 → 3)-O-β-D-galactopyranosyl-(1 → 3)-O-β-D-galactopyranosyl-(1 → 4)-β-D-xylopyranosyl]-L-serylglycine, disodium salt* (**18**).—Glycopeptide[11] **17** (16 mg, 20 μmol) was dissolved in water (2 mL), and the pH of the solution was adjusted to ~8.5 with satd aq $NaHCO_3$. Acetic anhydride (6 μL, 60 μmol) was added, and the pH was maintained at ~8 by additions of 5% aq $NaHCO_3$. The mixture was stirred for 1 h, then concentrated. The residue was eluted from a column (2.2 × 120 cm) of Sephadex G-10 with water to give amorphous, hygroscopic **18** (15 mg, 88%); $[\alpha]_D$ −8° (*c* 1, H_2O); NMR data: ^1H (D_2O, internal H_2O), δ 4.71 (d, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-1), 4.70, 4.57 (2 d, 2 H, $J_{1,2}$ 8.0 Hz, 2 Gal H-1), 4.69 (dd, 1 H, $J_{H\alpha,Ha}$ 5.0, $J_{H\alpha,Hb}$ 4.5 Hz, Ser α-C*H*), 4.47 (d, 1 H $J_{1,2}$ 7.5, Xyl H-1), 4.22 (dd, 1 H, $J_{Ha,Hb}$ 11.0, Ser β-C*H*a), 4.21 (dd, 2 H, $J_{3,4}$ 3.4, $J_{4,5}$ 0.6 Hz, 2 Gal H-4), 4.14 (dd, 1 H, $J_{4,5eq}$ 5.0, $J_{5ax,5eq}$ 12.0 Hz, Xyl H-5*eq*), 3.96 (dd, 1 H, Ser β-C*H*b), 3.88 (m, 1 H, $J_{3,4}$ 9.0, $J_{4,5ax}$ 10.0 Hz, Xyl H-4), 3.82 (s, 2 H, Gly CH_2), 3.74 (d, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-5), 3.72, 3.68 (2 dd, 2 H, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 3.63 (t, 1 H, $J_{2,3}$ 9.0 Hz, Xyl H-3), 3.55 (m, 2 H, GlcA H-3, H-4), 3.45 (dd, 1 H, $J_{2,3}$ 9.0 Hz, GlcA H-2), 3.43 (dd, 1 H, Xyl H-5*ax*), 3.37 (dd, 1 H, Xyl H-2), and 2.12 (s, 3 H, NAc); ^{13}C (D_2O, internal acetone), see Table I.

Crude 2,3,4-tri-O-chloroacetyl-α-D-xylopyranosyl chloride (**20**).—Titanium(IV) chloride (0.32 mL, 2.9 mmol) was added to a solution of 1,2,3,4-tetra-*O*-chloroacetyl-α-D-xylopyranose[21] (**19**, 1.1 g, 2.4 mmol) in anhyd CH_2Cl_2 (8 mL), and the mixture was stirred for 4 h at room temperature, then diluted with cold CH_2Cl_2 (100 mL), washed with ice-cold water, brine, and water, dried ($MgSO_4$), and concentrated. The residue was quickly eluted from a column of silica gel (40 g) with 3:1 hexane–EtOAc to give unstable syrupy **20** (912 mg, 95%); $[\alpha]_D$ +120° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.28 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1), 5.66 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 10.0 Hz, H-3), 5.15 (m, 1 H $J_{4,5eq}$ 6.5, $J_{4,5ax}$ 11.0 Hz, H-4), 4.09 (dd, 1 H,

$J_{5ax,5eq}$ 12.0 Hz, H-5*eq*), 4.08, 4.05, 4.03 (3 s, 6 H, 3 COCH_2Cl), and 4.00 (dd, 1 H, H-5*ax*).

N-*Benzyloxycarbonyl*-O-*(2,3,4-tri*-O-*chloroacetyl-α-* (**21**) *and -β-*D-*xylopyranosyl)-*L-*serylglycine benzyl ester* (**22**).—A mixture of freshly prepared **20** (1.03 g, 2.59 mmol), peptide[10] **11** (1.2 g, 3.11 mmol), and activated powdered 4A molecular sieves (1 g) in anhyd 1,2-dichloroethane (22 mL) was stirred for 30 min at room temperature under Ar, then cooled to 0°C. Silver triflate (1.34 g, 5.18 mmol) was added, and the mixture was stirred for 1 h 30 at 0°C. *sym*-Collidine (0.68 mL, 5.17 mmol) was added, and the mixture was diluted with CH_2Cl_2 (100 mL), filtered through a pad of Celite 545, washed with 5% aq $Na_2S_2O_3$, satd aq $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (120 g) with 1:1 hexane–EtOAc to give first **21** (372 mg, 19%); mp 138–139°C (from hexane–EtOAc); $[\alpha]_D$ +69° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36 (m, 10 H, 2 Ph), 6.78 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 5.54 (t, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.18 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.08, 4.06, and 4.00 (3 s, 6 H, 3 COCH_2Cl). Anal. Calcd for $C_{31}H_{33}Cl_3N_2O_{13}$: C, 49.78; H, 4.45; N, 3.74. Found: C, 49.99; H, 4.27; N, 3.74.

Next eluted was **22** (1.32 g, 68%); mp 102–103° (from hexane–EtOAc); $[\alpha]_D$ −10.5° (*c* 1, $CHCl_3$); ^{1}H NMR (Me_2SO-d_6): δ 8.42 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 7.50 (d, 1 H, *J* 8.5 Hz, Ser N*H*), 7.46 (m, 10 H, 2 Ph), 5.23 (t, 1 H, $J_{2,3} = J_{3,4} = 8.0$ Hz, H-3), 5.13, 5.05 (2 s, 4 H, 2 OCH_2Ph), 4.92 (m, 1 H, $J_{4,5eq}$ 5.0, $J_{4,5ax}$ 8.0 Hz, H-4), 4.88 (dd, 1 H, $J_{1,2}$ 6.0 Hz, H-2), 4.78 (d, 1 H, H-1), 4.38, 4.34, 4.32 (3 s, 6 H, 3 COCH_2Cl), 4.03 (dd, 1 H, $J_{5ax,5eq}$ 12.0 Hz, H-5*eq*), and 3.54 (dd, 1 H, H-5*ax*). Anal. Calcd for $C_{31}H_{33}Cl_3N_2O_{13}$: C, 49.78; H, 4.45; N, 3.74. Found: C, 50.03; H, 4.53; N, 3.99.

N-*Benzyloxycarbonyl*-O-*β-*D-*xylopyranosyl-*L-*serylglycine benzyl ester* (**23**).—A mixture of **22** (1.32 g, 1.77 mmol) and thiourea (810 mg, 10.6 mmol) in pyridine (4.5 mL) and EtOH (10.5 mL) was stirred at 80°C for 20 h, then cooled, and concentrated. A solution of the residue in EtOAc (100 mL) was washed with water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (30 g) with 9:1 CH_2Cl_2–MeOH to give **23** (784 mg, 85%); mp 135–136°C (from 2-propanol); $[\alpha]_D$ −19° (*c* 1, MeOH); ^{1}H NMR (Me_2SO-d_6): δ 8.32 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 7.47 (d, 1 H, *J* 8.5 Hz, Ser N*H*), 7.38 (m, 10 H, 2 Ph), 4.10 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1). Anal. Calcd for $C_{25}H_{30}N_2O_{10} \cdot 0.5H_2O$: C, 56.92; H, 5.92; N, 5.31. Found: C, 56.99; H, 5.95; N, 5.39.

N-*Benzyloxycarbonyl*-O-*(3,4-* (**24**) *and 2,3-*O-*isopropylidene-β-*D-*xylopyranosyl)-*L-*serylglycine benzyl ester* (**25**).—A mixture of **23** (0.74 g, 1.42 mmol) and camphorsulfonic acid (60 mg) in anhyd DMF (6 mL) was stirred at 40°C with the exclusion of moisture. 2-Methoxypropene (0.35 mL, 3.5 mmol) was added portionwise during 1 h. The mixture was then cooled to 0°C, and MeOH (0.5 mL) was added. After 30 min at this temperature, Et_3N (0.5 mL) was added, and the mixture was concentrated. The residue was eluted from a column of silica gel (50 g) with 3:1 EtOAc–hexane containing 0.2% of Et_3N to give first **24** (159 mg, 20%); $[\alpha]_D$ +5°

(*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36 (m, 10 H, 2 Ph), 7.07 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 6.18 (d, 1 H, *J* 8.0 Hz, Ser N*H*), 4.24 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 3.69 (m, 1 H, $J_{2,3}$ 10.0, $J_{2,OH}$ 3.5 Hz, H-2), and 1.43 (s, 6 H, CMe_2). Anal. Calcd for $C_{28}H_{34}N_2O_{10} \cdot 0.5H_2O$: C, 59.25; H, 6.22; N, 4.94. Found: C, 59.23; H, 6.06; N, 5.04.

Next eluted was **25** (517 mg, 65%); mp 87–88°C (from hexane–EtOAc) $[\alpha]_D$ +14.5° (*c* 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6): δ 8.42 (t, 1 H, *J* 6.0 Hz, Gly N*H*), 7.39 (d, 1 H, *J* 9.0 Hz, Ser N*H*), 7.37 (m, 10 H, 2 Ph), 5.38 (d, 1 H, $J_{4,OH}$ 4.5 Hz, HO-4), 5.13 (s, 2 H, OCH_2Ph), 5.04 (ABq, 2 H, OCH_2Ph), 4.66 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.82 (dd, 1 H, $J_{4,5eq}$ 5.0, $J_{5ax,5eq}$ 11.5 Hz, H-5*eq*), 3.73 (m, 1 H, $J_{3,4}$ 9.5, $J_{4,5ax}$ 8.0 Hz, H-4), 3.42 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-3), 3.18 (dd, 1 H, H-2), 3.10 (dd, 1 H, H-5*ax*), and 1.35 (s, 6 H, CMe_2). Anal. Calcd for $C_{28}H_{34}N_2O_{10}$: C, 60.21; H, 6.14; N, 5.01. Found: C, 60.33; H, 6.38; N, 4.99.

N-*Benzyloxycarbonyl*-O-[O-(*2,3,4,6-tetra*-O-*benzoyl*-β-D-*galactopyranosyl*)-(*1* → *4*)-(*2,3*-O-*isopropylidene*-β-D-*xylopyranosyl*)]-L-*serylglycine benzyl ester* (**27**).—A mixture of **25** (378 mg, 0.68 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate[10] (**26**, 0.6 g, 0.81 mmol) was treated as described for the preparation of **12**. The residue was eluted from a column of silica gel (80 g) with 1:1 EtOAc–hexane containing 0.2% of Et_3N to give **27** (577 mg, 75%); $[\alpha]_D$ +58° (*c* 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6): δ 8.42 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 7.62 (m, 30 H, 6 Ph), 7.38 (d, 1 H, *J* 8.0 Hz, Ser N*H*), 5.79 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, Gal H-4), 5.78 (dd, 1 H, $J_{1,2}$ 8.0 Hz, Gal H-2), 5.31 (d, 1 H, Gal H-1), 5.11, 5.03 (2 s, 4 H, 2 OCH_2Ph), 4.67 (d, 1 H, $J_{1,2}$ 7.8 Hz, Xyl H-1), 3.62 (t, 1 H, $J_{2,3}$ 9.0 Hz, Xyl H-3), 3.37 (dd, 1 H, Xyl H-2), 1.32, and 1.30 (2 s, 6 H, CMe_2). Anal. Calcd for $C_{62}H_{60}N_2O_{19}$: C, 65.49; H, 5.32; N, 2.46. Found: C, 65.62; H, 5.42; N, 2.49.

N-*Benzyloxycarbonyl*-O-[O-(*2,3,4,6-tetra*-O-*benzoyl*-β-D-*galactopyranosyl*)-(*1* → *4*)-β-D-*xylopyranosyl*]-L-*serylglycine benzyl ester* (**28**).—A solution of **27** (450 mg) in aq AcOH (70%, 20 mL) was stirred at 100°C for 15 min, then cooled, and concentrated. The residue was eluted from a column of silica gel (20 g) with 2:1 EtOAc–hexane to give **28** (411 mg, 94%); $[\alpha]_D$ +69° (*c* 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6): δ 8.18 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 7.56 (m, 30 H, 6 Ph), 7.33 (d, 1 H, *J* 8.5 Hz, Ser N*H*), 5.78 (dd, 1 H, $J_{3,4}$ 4.0, $J_{4,5}$ 1.0 Hz, Gal H-4), 5.68 (dd, 1 H, $J_{2,3}$ 10.5 Hz, Gal H-3), 5.43 (dd, 1 H, $J_{1,2}$ 8.0 Hz, Gal H-2), 5.22 (d, 1 H, Gal H-1), 5.08 (d, 1 H, $J_{2,OH}$ 4.0 Hz, Xyl HO-2), 5.02, 4.94 (2 s, 4 H, 2 OCH_2Ph), 4.91 (d, 1 H, $J_{3,OH}$ 5.0 Hz, Xyl HO-3), 3.96 (d, 1 H, $J_{1,2}$ 7.5 Hz, Xyl H-1), 3.18 (m, 1 H, $J_{2,3}$ 10.0 Hz, Xyl H-3),, and 2.92 (m, 1 H, Xyl H-2). Anal. Calcd for $C_{59}H_{56}N_2O_{19}$: C, 64.59; H, 5.15; N, 2.55. Found: C, 64.68; H, 5.30; N, 2.62.

N-*Benzyloxycarbonyl*-O-[O-(*2,3,4,6-tetra*-O-*benzoyl*-β-D-*galactopyranosyl*)-(*1* → *4*)-(*2*-O- (**29**) *and 3*-O-*chloroacetyl*-β-D-*xylopyranosyl*)]-L-*serylglycine benzyl ester* (**30**).—A mixture of **28** (110 mg, 0.1 mmol) and dibutyltin oxide (26 mg, 105 μmol) was heated for 15 h in refluxing benzene (20 mL) with azeotropic removal of water. Solvent (15 mL) was then slowly distilled at atmospheric pressure, and the mixture was cooled. Chloroacetic anhydride (188 mg, 0.11 mmol) in anhyd benzene (2 mL)

was added at room temperature, and the mixture was stirred for 3 h, then concentrated. The residue was eluted from a column of silica gel (10 g), with 4:3 EtOAc–hexane to give first **29** (15 mg, 13%); $[\alpha]_D$ +67° (*c* 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6): δ 5.37 (d, 1 H, $J_{3,OH}$ 5.2 Hz, Xyl HO-3), 4.58 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.0 Hz, Xyl H-2), 4.40 (ABq, 2 H, $COCH_2Cl$), 3.57 (m, 1 H, $J_{3,4}$ 9.0 Hz, Xyl H-3). Anal. Calcd for $C_{61}H_{57}ClN_2O_{20}$: C, 62.43; H, 4.89; N, 2.39. Found: C, 62.51; H, 4.75; N, 2.21.

Next eluted was **30** (88 mg, 75%); $[\alpha]_D$ +36° (*c* 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6): δ 5.53 (d, 1 H, $J_{2,OH}$ 4.2 Hz, Xyl HO-2), 4.90 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, Xyl (H-3), 4.33 (ABq, 2 H, $COCH_2Cl$), 4.28 (d, 1 H, $J_{1,2}$ 7.5 Hz, Xyl H-1), and 3.18 (m, 1 H, Xyl H-2). Anal. Calcd for $C_{61}H_{57}ClN_2O_{20}$: C, 62.43; H, 4.89; N, 2.39. Found: C, 62.31; H, 4.91; N, 2.31.

N-Benzyloxycarbonyl-O-[O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(3-O-acetyl-β-D-xylopyranosyl)]-L-serylglycine benzyl ester (**31**).—Compound **28** (110 mg, 0.1 mmol) was treated as described for the preparation of **30**, except that Ac_2O (11 μL, 115 μmol) was the reagent added to the dibutylstannylene derivative. The residue was eluted from a column of silica gel (10 g) with 3:2 EtOAc–hexane to give **31** (72 mg, 63%); $[\alpha]_D$ +41° (*c* 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6): δ 5.42 (d, 1 H, $J_{2,OH}$ 4.0 Hz, Xyl HO-2), 4.84 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, Xyl H-3), 4.24 (d, 1 H, $J_{1,2}$ 7.5 Hz, Xyl H-1), 3.18 (m, 1 H, Xyl H-2), and 1.94 (s, 3 H, OAc). Anal. Calcd for $C_{61}H_{58}N_2O_{20}$: C, 64.32; H, 5.13; N, 2.46. Found: C, 64.30; H, 5.18; N, 2.39.

N-Benzyloxycarbonyl-O-[O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(3-O-acetyl-2-O-dibenzyloxyphosphinyl-β-D-xylopyranosyl)]-L-serylglycine benzyl ester (**32**).—A mixture of **31** (70 mg, 61 μmol) and 1-*H*-tetazole (9 mg, 0.12 mmol) in anhyd CH_2Cl_2 (2 mL) was stirred at room temperature under Ar. Dibenzyl *N,N*-diisopropylphosphoramidite[26] (35 mg, 0.1 mmol) in anhyd CH_2Cl_2 (0.25 mL) was added, and the mixture was stirred for 30 min, then cooled to 0°C. *m*-Chloroperbenzoic acid (85%, 40 mg, 0.2 mmol) was added, the mixture was stirred for 1 h at 0°C, then diluted with CH_2Cl_2 (20 mL), washed with aq 5% $Na_2S_2O_3$, satd aq $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (5 g) with 4:3 EtOAc–hexane to give **32** (77 mg, 90%); $[\alpha]_D$ +27° (*c* 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6): δ 8.33 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 7.65 (m, 41 H, 8 Ph, Ser N*H*), 5.87 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.8 Hz, Gal H-4), 5.73 (dd, 1 H, $J_{2,3}$ 10.0 Hz, Gal H-3), 5.43 (dd, 1 H, $J_{1,2}$ 8.0 Hz, Gal H-2), 5.17 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, Xyl H-3), 5.00 (m, 8 H, 4 OCH_2Ph), 4.66 (d, 1 H, $J_{1,2}$ 7.5 Hz, Xyl H-1), 4.09 (m, 1 H, $J_{2,P}$ 9.0 Hz, Xyl H-2), and 1.77 (s, 3 H, OAc). Anal. Calcd for $C_{75}H_{71}N_2O_{23}P$: C, 64.37; H, 5.11; N, 2.00. Found: C, 64.51; H, 5.06; N, 1.89.

N-Acetyl-O-[O-β-D-galactopyranosyl-(1 → 4)-O-(2-O-phosphono-β-D-xylopyranosyl)]-L-serylglycine, trisodium salt (**33**).—A solution of **32** (90 mg) in 12:2:1 EtOAc–MeOH–water (15 mL) was hydrogenated in the presence of 10% Pd–C (50 mg) for 15 h, then filtered and concentrated. The residue was eluted from a column (1 × 20 cm) of Sephadex SP-C25 (Na^+) with 5:2:1 EtOAc–MeOH–water

to give the partially deprotected amino compound as its trisodium salt (65 mg); ^{1}H NMR (CD_3OD): δ 4.07 (m, 1 H, $J_{1,2}$ 7.5, $J_{2,3} = J_{2,P} = 9.0$ Hz, Xyl H-2). Acetic anhydride (0.1 mL) was added at 0°C to a solution of the amine in DMF (2 mL), and the mixture was stirred for 10 min, then concentrated at < 30°C. A mixture of the residue and 98% hydrazine hydrate (2 mL) in MeOH (4 mL) was stirred for 4 h at room temperature, then treated as described for the preparation of **16** to give amorphous, hygroscopic **33** (30 mg, 72% from **32**); $[\alpha]_D$ −30° (*c* 1, H_2O); NMR data: ^{1}H (D_2O, internal H_2O), δ 4.63 (t, 1 H, *J* 4.0 Hz, Ser α-C*H*), 4.58 (d, 1 H, $J_{1,2}$ 7.0 Hz, Xyl H-1), 4.51 (d, 1 H, $J_{1,2}$ 8.0 Hz, Gal H-1), 4.29 (dd, 1 H, $J_{H\alpha,Ha}$ 4.0, $J_{Ha,Hb}$ 10.0 Hz, Ser β-C*H*a), 4.12 (dd, 1 H, $J_{4,5eq}$ 5.0, $J_{5ax,5eq}$ 12.0 Hz, Xyl H-5*eq*), 3.95 (dd, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ 0.6 Hz, Gal H-4), 3.94 (m, 1 H, $J_{3,4} = J_{4,5ax} = 9.0$ Hz, Xyl H-4), 3.87 (m, 1 H, $J_{2,3} = J_{2,P} = 9.0$ Hz, Xyl H-2), 3.82 (t, 1 H, Xyl H-3), 3.81 (s, 2 H, Gly CH_2), 3.67 (dd, 1 H, $J_{2,3}$ 10.0 Hz, Gal H-3), 3.54 (dd, 1 H, Gal H-2), 3.47 (dd, 1 H, Xyl H-5*ax*) and 2.15 (s, 3 H, NAc); ^{13}C (D_2O, internal acetone), see Table I. Anal. Calcd for $C_{18}H_{28}N_2Na_3O_{17}P \cdot 2H_2O$: C, 31.77; H, 4.74; N, 4.12. Found: C, 31.62; H, 4.89; N, 4.01.

N-*Benzyloxycarbonyl*-O-{O-*[2-(trimethylsilyl)ethyl 2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranosyluronate]*-(1 → 3)-O-*(2,4,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl)*-(1 → 3)-O-*(2,4,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl)*-(1 → 4)-β-D-*xylopyranosyl}*-L-*serylglycine benzyl ester* (**35**).—A mixture of **25** (268 mg, 0.48 mmol) and trisaccharide imidate[11] **34** (650 mg, 0.38 mmol) was treated as described for the preparation of **12**. A solution of the initial product in aq AcOH (70%, 20 mL) was stirred at 100°C for 10 min, then cooled, and concentrated. The residue was eluted from a column of silica gel (60 g) with 2:1 EtOAc–hexane to give syrupy **35** (620 mg, 79% from **34**); $[\alpha]_D$ +38° (*c* 1, $CHCl_3$); ^{1}H NMR (Me_2SO-d_6): δ 8.24 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 7.50 (m, 56 H, 11 Ph, Ser N*H*), 5.84, 5.76 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.8 Hz, 2 Gal H-4), 5.32 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, GlcA H-3), 5.32 (t, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-4), 5.27 (d, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-1), 5.24, 5.17 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 5.10, 5.01 (2 s, 4 H, 2 OCH_2Ph), 5.08 (d, 1 H, $J_{2,OH}$ 4.0 Hz, Xyl HO-2), 5.06, 4.91 (2 d, 2 H, 2 Gal H-1), 5.04 (dd, 1 H, GlcA H-2), 4.75 (d, 1 H, $J_{3,OH}$ 4.5 Hz, Xyl HO-3), 4.67 (d, 1 H, GlcA H-5), 4.43, 4.40 (2 dd, 2 H, 2 Gal H-3), 3.91 (d, 1 H, $J_{1,2}$ 7.0 Hz, Xyl H-1), 3.12 (m, 1 H, $J_{2,3} = J_{3,4} = 8.5$ Hz, Xyl H-3), 2.90 (m, 1 H, Xyl H-2), 0.61 (m, 2 H, CH_2Si), and −0.10 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{111}H_{106}N_2O_{35}Si$: C, 64.84; H, 5.16; N, 1.36. Found: C, 64.72; H, 5.21; N, 1.24.

N-*Benzyloxycarbonyl*-O-{O-*[2-(trimethylsilyl)ethyl 2,3,4,-tri*-O-*benzoyl*-β-D-*glucopyranosyluronate]*-(1 → 3)-O-*(2,4,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl)*-(1 → 3)-O-*(2,4,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl)*-(1 → 4)-3-O-*acetyl*-β-D-*xylopyranosyl)}*-L-*serylglycine benzyl ester* (**36**).—A mixture of **35** (0.48 g, 233 μmol) and dibutyltin oxide (63 mg, 252 μmol) was heated for 10 h in refluxing benzene (60 mL) with azeotropic removal of water. Solvent (40 mL) was then slowly distilled at atmospheric pressure, and the mixture was cooled to room temperature. Acetic anhydride (30 μL, 315 μmol) in anhyd benzene (0.5 mL) was added, and the mixture was stirred for 15 h, then concentrated. The residue was eluted from a column of

silica gel (40 g) with 7:4 EtOAc–hexane to give **36** (396 mg, 81%); $[\alpha]_D$ +22° (*c* 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6): δ 8.23 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 7.45 (m, 56 H, 11 Ph, Ser N*H*), 5.82, 5.77 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.8 Hz, 2 Gal H-4), 5.75 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, GlcA H-3), 5.32 (t, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-4), 5.31 (d, 1 H, $J_{2,OH}$ 4.0 Hz, Xyl HO-2), 5.26 (d, 1 H, $J_{1,2}$ 8.0 Hz, GlcA H-1), 5.17, 5.15 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 5.09, 5.01 (2 s, 4 H, 2 OCH_2Ph 5.04, 4.82 (2 d, 2 H, 2 Gal H-1), 4.67 (d, 1 H, GlcA H-5), 4.66 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, Xyl H-3), 4.11 (d, 1 H, $J_{1,2}$ 7.5 Hz, Xyl H-1), 3.04 (m, 1 H, Xyl H-2), and 1.72 (s, 3 H, OAc). Anal. Calcd for $C_{113}H_{108}N_2O_{36}Si$: C, 64.68; H, 5.19; N, 1.33. Found: C, 64.71; H, 5.08; N, 1.30.

N-*Benzyloxycarbonyl*-O{O-*[2-(trimethylsilyl)ethyl 2,3,4,-tri*-O-*benzoyl*-β-D-*glucopyranosyluronate]*-(1 → 3)-O-*(2,4,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl)*-(1 → 3)-O-*(2,4,6-tri*-O-*benzoyl*-β-D-*galactopyranosyl)*-(1 → 4)-*(3*-O-*acetyl*-2-O-*dibenzyloxyphosphinyl*-β-D-*xylopyranosyl)*}-L-*serylglycine benzyl ester* (**37**).—Compound **36** (453 mg, 216 μmol) was treated exactly as described for the preparation of **32**. The residue was eluted from a column of silica gel (40 g) with 4:3 EtOAc–hexane to give **37** (463 mg, 91%); $[\alpha]_D$ +17° (*c* 1, $CHCl_3$); ^1H NMR (Me_2SO-d_6): δ 8.30 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 7.45 (m, 66 H, 13 Ph, Ser N*H*), 5.82, 5.76 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.8 Hz, 2 Gal H-4), 5.74 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, GlcA H-3), 5.30 (t, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-4), 5.26 (d, 1 H, $J_{1,2}$ 8.0 Hz, GlcA H-1), 5.16, 5.15 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 5.08 (m, 4 H, 2 OCH_2Ph), 5.06, 4.88 (2 d, 2 H, 2 Gal H-1) 5.03 (dd, 1 H, GlcA H-2), 4.99 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, Xyl H-3), 4.87 (m, 4 H, 2 OCH_2Ph), 4.67 (d, 1 H, GlcA H-5), 4.54 (d, 1 H, $J_{1,2}$ 7.5 Hz, Xyl H-1), 3.98 (m, 1 H, $J_{2,P}$ 9.0 Hz, Xyl H-2), and 1.56 (s, 3 H, OAc). Anal. Calcd for $C_{127}H_{121}N_2O_{39}PSi$: C, 64.68; H, 5.17; N, 1.19. Found: C, 64.54; H, 5.09; N, 1.12.

N-*Acetyl*-O-*[*O-*(*β-D-*glucopyranosyluronic acid)*-(1 → 3)-O-β-D-*galactopyranosyl*-(1 → 3)-O-β-D-*galactopyranosyl*-(1 → 4)-*(2*-O-*phosphono*-β-D-*xylopyranosyl)]*-L-*serylglycine, tetrasodium salt* (**38**).—Compound **37** (0.33 g, 0.14 mmol) was treated with Bu_4NF (130 mg, 0.5 mmol) as described for the preparation of **14**. The residue was hydrogenated, treated with ion-exchange resin (Na^+), *N*-acetylated, and hydrazinolyzed as ascribed for the preparation of **33**. The residue was eluted from a column (2.2 × 120 cm) of Sephadex G-10 with water to give amorphous, hygroscopic **38** (90 mg, 64% from **37**); $[\alpha]_D$ −22° (*c* 1, H_2O); NMR data: ^1H (D_2O, internal H_2O), δ 4.71 (d, 1 H, $J_{1,2}$ 7.8 Hz, GlcA H-1), 4.69, 4.56 (2 d, 2 H, $J_{1,2}$ 7.5 Hz, 2 Gal H-1), 4.63 (t, 1 H, $J_{H\alpha,Ha} = J_{H\alpha,Hb} = 3.8$ Hz, Ser α-C*H*), 4.58 (d, 1 H, $J_{1,2}$ 6.5 Hz, Xyl H-1), 4.29 (dd, 1 H, $J_{Ha,Hb}$ 10.5 Hz, Ser β-C*H*a), 4.22, 4.21 (2 dd, 2 H, $J_{3,4}$ 3.2, $J_{4,5}$ 0.8 Hz, 2 Gal H-4), 4.12 (dd, 1 H, $J_{4,5eq}$ 5.0, $J_{5ax,5eq}$ 12.0 Hz, Xyl H-5*eq*), 3.95 (m, 1 H, $J_{3,4} = J_{4,5ax} = 9.0$ Hz, Xyl H-4), 3.92 (m, 1 H, $J_{2,3} = J_{2,P} = 9.0$ Hz, Xyl H-2), 3.89 (dd, 1 H, Ser β-C*H* b), 3.85 (s, 2 H, Gly CH_2), 3.75 (d, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-5), 3.72 (dd, 1 H, $J_{2,3}$ 10.0 Hz, Gal H-2), 3.55 (m, 2 H, GlcA H-3, H-4), 3.47 (dd, 1 H, Xyl H-5*ax*), 3.44 (dd, 1 H, $J_{2,3}$ 9.0 Hz, GlcA H-2), and 2.14 (s, 3 H, NAc); ^{13}C (D_2O, internal acetone), see Table I. Anal. Calcd for

$C_{30}H_{45}N_2Na_4O_{28}P \cdot 4H_2O$: C, 33.47; H, 4.96; N, 2.60. Found: C, 33.29; H, 5.01; N, 2.41.

REFERENCES

1 L. Kjellen and U. Lindahl, *Annu. Rev. Biochem.*, 60 (1991) 443–475.
2 U. Lindahl and L. Rodén, in A. Gottschalk (Ed.), *Glycoproteins*, Elsevier, New York, 1972, pp 491–517.
3 H.C. Robinson, A.A. Horner, M. Höök, S. Ögren, and U. Lindahl, *J. Biol. Chem.*, 253 (1978) 6687–6693.
4 K. Sugahara, I. Yamashina, P. de Waard, H. van Halbeek, and J.F.G. Vliegenthart, *J. Biol. Chem.*, 263 (1988) 10168–10174.
5 L.-Å. Fransson, I. Silverberg, and I. Carlstedt, *J. Biol. Chem.*, 260 (1985) 14722–14726.
6 L. Rosenfeldt and I. Danishefsky, *J. Biol. Chem.*, 263 (1988) 262–266.
7 T.R. Oegema, Jr., E.L. Kraft, G.W. Jourdian, and T.R. Van Valen, *J. Biol. Chem.*, 259 (1984) 1720–1726.
8 F. Goto and T. Ogawa, *Tetrahedron Lett.*, 33 (1992) 5099–5102.
9 M. Nilsson, J. Westman, and C.-M. Swahn, *J. Carbohydr. Chem.*, 12 (1993) 23–37.
10 S. Rio, J.-M. Beau, and J.-C. Jacquinet, *Carbohydr. Res.*, 219 (1991) 71–90.
11 S. Rio, J.-M. Beau, and J.-C. Jacquinet, *Carbohydr. Res.*, 244 (1993) 295–313.
12 R.R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 212–235.
13 J.-C. Jacquinet, *Carbohydr. Res.*, 199 (1990) 153–181, and references therein.
14 S.A. Abbas and A.H. Haines, *Carbohydr. Res.*, 39 (1975) 358–363.
15 F.W. Lichtenthaler and H.J. Lindner, *Carbohydr. Res.*, 200 (1990) 91–99.
16 S. Peters, T. Bielfeldt, M. Meldal, K. Bock, and H. Paulsen, *J. Chem. Soc., Perkin Trans. 1*, (1992) 1163–1171.
17 F. Helsinger, J. Schreiber, and A. Eschenmoser, *Helv. Chim. Acta*, 43 (1960) 113–116.
18 J.D. Evanseck, J.F. Blake, and J.C. Jorgensen, *J. Am. Chem. Soc.*, 109 (1987) 2349–2353.
19 P. Schultheiss-Reimann and H. Kunz, *Angew. Chem. Int. Ed. Engl.*, 22 (1983) 62–63.
20 P.J. Garegg, B. Lindberg, and T. Norberg, *Acta Chem. Scand., Ser. B*, 33 (1979) 449–452.
21 T. Ziegler, *Liebigs Ann. Chem.*, (1990) 1125–1131.
22 H. Paulsen and O. Lockhoff, *Chem. Ber.*, 114 (1981) 3102–3114.
23 J.-R. Pougny and P. Sinaÿ, *Carbohydr. Res.*, 47 (1976) 69–79.
24 R.F. Helm, J. Ralph, and L. Anderson, *J. Org. Chem.*, 56 (1991) 7015–7021.
25 S. David and S. Hanessian, *Tetrahedron*, 41 (1985) 643–663.
26 K.L. Yu and B. Fraser-Reid, *Tetrahedron Lett.*, 29 (1988) 979–982.