Carbohydrate Research, 244 (1993) 295–313
Elsevier Science Publishers B.V., Amsterdam

Total synthesis of the carbohydrate–protein linkage region common to several mammalian 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)

(Received September 7th, 1992; accepted December 30th, 1992)

ABSTRACT

A stereocontrolled synthesis of β-D-Glc*p*A-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Xyl*p*-(1 → *O*)-L-Ser-Gly, the common glycopeptide sequence of the carbohydrate–protein linkage region of most mammalian proteoglycans, was achieved by use of *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)-2,3-di-*O*-benzoyl-α,β-D-xylopyranosyl trichloroacetimidate as the key intermediate. Condensation of this glycosyl donor with suitably protected L-seryl-glycine dipeptide segments, and peptide chain elongation, allowed the construction in high yield of complex structures of this linkage region.

INTRODUCTION

Although glycosaminoglycans have different repetitive disaccharide units, they are, with the exception of keratan sulfate, assumed to be bound to the protein cores through a common specific linkage region identified as a β-D-Glc*p*A-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Xyl*p*-(1 → *O*)-L-Ser sequence[1]. The cross-link is a polypeptide composed mainly of L-serine and glycine residues, occurring in an alternating sequence, in which certain L-serine units are substituted with polysaccharide chains[2].

Modifications by phosphorylation at O-2 of the xylose residue[3,4] or sulfations at O-4 or O-6 of the two galactose residues[5,6] were recently described.

Because of the known or suspected involvement of proteoglycans in important biological phenomena[7], their further characterisation is important. However, glycopeptide linkages involving L-serine are base-sensitive, and the xylose–serine bond is unstable under acidic conditions. This complicates greatly the isolation of

* Presented at the XVIth International Carbohydrate Symposium. Paris, 5–10 July, 1992.

** Corresponding author.

0008-6215/93/$06.00 © 1993 – Elsevier Science Publishers B.V. All rights reserved

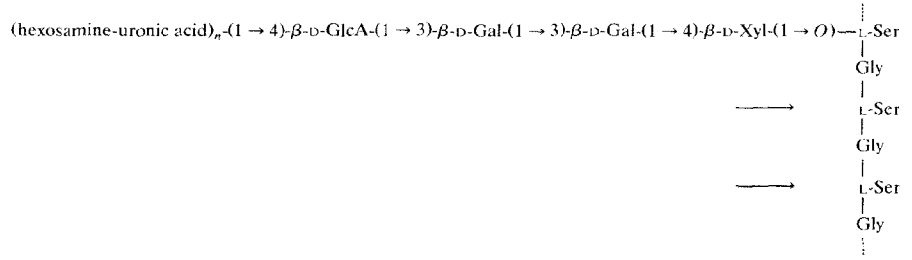


Fig. 1. The carbohydrate–protein linkage region of proteoglycans. The arrows indicate substitution with polysaccharides.

glycopeptides from this region by chemical means. Thus, synthesis remains the alternative of choice for studies of such structures.

As a part of a program devoted to the synthesis of proteoglycan fragments[8], we now report on the total synthesis of the common tetrasaccharide–dipeptide, as well as the preparation of more complex structures of this linkage region. A synthesis of a methyl glycoside of this tetrasaccharide was recently published[9].

RESULTS AND DISCUSSION

The strategy of these syntheses involves the preparation of an activated oligosaccharide block, which is used as a glycosyl donor and condensed with suitably protected L-seryl-glycine dipeptide acceptors, as we reported[10] previously. The trichloroacetimidate procedure[11] was selected for the activation of the oligoside moiety.

In order to study the glycosylating ability of such complex oligosaccharide blocks, we first undertook the synthesis of the β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Xyl trisaccharide. Several syntheses of this structure linked to a L-serine residue have been described[12,13], but involved the cumbersome preparation of a selectively protected xylosyl–serine fragment. Since all the linkages in these structures are 1,2-*trans*, and following previous observations[10], the benzoyl group was selected as a stereocontrolling auxiliary at O-2 of each sugar unit.

The stepwise construction of such structures requires a selectively protected D-galactopyranosyl precursor. Thus, benzyl 3-*O*-allyl-β-D-galactopyranoside[14] (**1**) was benzoylated, and directly *O*-deallylated[15] (Pd–C, *p*-TsOH, aqueous methanol) to give crystalline **2** (81% from **1**), the structure of which was evident from its ^{1}H NMR spectrum.

β-D-Galactosylation of **2** (1 equiv) with the *O*-benzoylated trichloroacetimidate[10] **3** (1.4 equiv), in 1,2-dichloroethane at 0°C, in the presence of trimethylsilyl triflate (15% based on **3**) afforded 78% of the crystalline β-linked disaccharide derivative **4** (δ 4.95, d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′). Catalytic hydrogenation (Pd–C) of **4** in ethyl

acetate gave the corresponding free hemiacetal, which upon treatment with trichloroacetonitrile and 1,8-diazabicyclo[5,4,0]undec-7-ene gave the crystalline imidate **5** (88%). The α configuration of **5** was indicated by the resonance for H-1 at δ 6.75 (d, 1 H, $J_{1,2}$ 4.0 Hz).

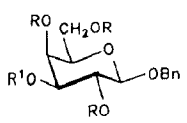
As in previous studies, benzyl 2,3-*O*-isopropylidene-β-D-xylopyranoside[10] (**6**) was selected as the xylosyl acceptor. Condensation of **5** (1 equiv) with **6** (1.3 equiv), in toluene at −20°C, in the presence of trimethylsilyl triflate (10% based on **5**) afforded the expected trisaccharide derivative which was more easily isolated after mild hydrolysis (aqueous 60% acetic acid) of the *O*-isopropylidene group, to give **7** (86% from **5**). The ^{1}H NMR spectrum of **7** showed a signal at δ 4.65 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), characteristic of a β linkage. Benzoylation of **7** gave crystalline **8** (96%). The ^{1}H NMR spectrum of **8** showed signals at δ 5.53 and 5.22 attributed, respectively, to H-3 and H-2 of a 2,3-di-*O*-benzoylated xylose residue, proving that glycosylation had occurred at O-4. Compound **7** was hydrogenated (Pd–C) and treated directly, as described for the preparation of **5**, to give 72% of the α-imidate **10** (δ 6.52, d, 1 H, $J_{1,2}$ 3.0 Hz, H-1α) and 16% of the β isomer **9** (δ 6.18, d, 1 H, $J_{1,2}$ 3.0 Hz, H-1β). The J values for **9** strongly suggested a significant departure from the $^4C_1$ conformation in solution, and are close to those reported[16] for *O*-benzoylated derivatives of D-xylose which adopt the $^1C_4$ conformation. This is probably due to the strong anomeric effect of the trichloroacetimidoyl group[11].

Glycosylation of a mixture of imidates **9** and **10** (1 equiv) with the dipeptide acceptor *N*-(benzyloxycarbonyl)-L-seryl-glycine benzyl ester[10] (**11a**, 1.5 equiv) was performed at −20°C in dichloromethane, with trimethylsilyl triflate as catalyst, to give crystalline **12** (91%). The ^{1}H NMR spectrum of **12** showed, *inter alia*, a signal at δ 4.50 (d, 1 H, $J_{1,2}$ 6.5 Hz, H-1) indicating the β configuration of the newly established linkage.

Final deprotection was then achieved through catalytic hydrogenation (Pd–C), followed by treatment with methanolic hydrazine, to give the expected trisaccharide–dipeptide **13** (90%). The ^{1}H and ^{13}C NMR data for **13** are in complete agreement with the postulated structure, and accord with those reported[12,17] for synthetic galactosyl-galactosyl-xylosyl-L-serine.

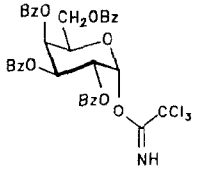
For the construction of the tetrasaccharide and related glycopeptides, an activated, suitably protected, D-glucopyranuronyl derivative was required. The temporary protecting ester group at C-6 must be stable during all the synthesis, with properties different from, and compatible with, those of the *C*-terminal part of the peptide moiety (thus allowing selective peptide-chain extension), and has to be cleaved under neutral conditions (to prevent β-elimination). On this basis, the 2-(trimethylsilyl)ethyl group was selected.

The D-glucuronic acid unit was prepared as follows. Oxidation of benzyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside[18] (**14**) with chromic anhydride–sulfuric acid afforded the corresponding uronic acid derivative, which was converted into its chloride (oxalyl chloride) and then esterified [2-(trimethylsilyl)ethanol, pyridine] to give crystalline **15** (67% from **14**), whose structure was evident from its ^{1}H NMR

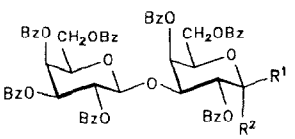


1 R = H, R^1 = $CH_2CH{=}CH_2$

2 R = Bz, R^1 = H

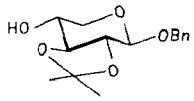


3

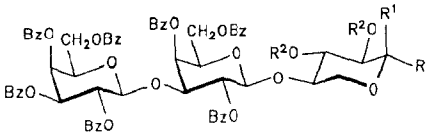


4 R^1 = OBn, R^2 = H

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



6



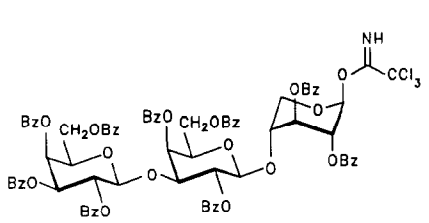
7 R = OBn, R^1 = R^2 = H

8 R = OBn, R^1 = H, R^2 = Bz

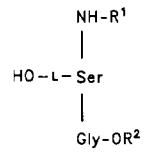
10 R = H, R^1 = $OC(NH)CCl_3$, R^2 = Bz

spectrum. Attempted direct esterification of the crude acid, using 2-(trimethylsilyl)ethanol, dicyclohexylcarbodiimide, and 4-dimethylaminopyridine, gave unsatisfactory results. Treatment of **15**, as described for the preparation of **5**, afforded the uronyl imidate **16** (90%), whose α configuration was indicated by the resonance for H-1 at δ 6.92 (d, 1 H, $J_{1,2}$ 3.8 Hz).

Preparation of the β-D-Glc*p*A-(1 → 3)-D-Gal block was then studied. When **16** or its corresponding unstable α-bromide (not described in the Experimental section) was condensed with the acceptor **2** (1.3 equiv) under several conditions (changes in catalyst, solvent, temperature), low yields (30–40%) of disaccharide were obtained. Since the nature of the donor was imposed by the strategy, a more reactive acceptor was selected. Thus, condensation of **16** (1 equiv) with benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside[19] (**17**, 1.3 equiv), as described for the preparation of **4**, afforded nearly insoluble, crystalline **18** (75%), the structure of which was evident from its ^{1}H NMR spectrum (δ 5.14, d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'). Treatment of **18** with aqueous 60% acetic acid followed by benzoylation gave crystalline **19** (88%). Catalytic hydrogenation (Pd–C) of **19** and treatment as described for the preparation of **5** afforded 86% of the crystalline α-imidate **20** (δ 6.77, d, 1 H, $J_{1,2}$ 3.8 Hz, H-1).



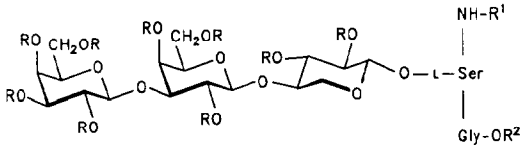
9



11a R^1 = COOBn, R^2 = Bn

11b R^1 = COOBn, R^2 = $CH_2CH{=}CH_2$

11c R^1 = Fmoc, R^2 = $CH_2CH{=}CH_2$



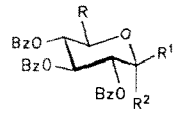
12 R = Bz, R^1 = COOBn, R^2 = Bn

13 R = R^1 = R^2 = H

Condensation of **20** (1 equiv) and **2** (1.5 equiv) at −20°C, as described for the preparation of **4**, gave smoothly the trisaccharide derivative **21** (79%). The ^{1}H NMR spectrum of **21** showed a doublet at δ 4.79 ($J_{1',2'}$ 8.0 Hz), characteristic of a β linkage. Transformation of **21**, as described for the preparation of **5**, afforded the α-imidate **22** (80%), showing the resonance for H-1 at δ 6.66 (d, 1 H, $J_{1,2}$ 4.0 Hz).

Glycosylation of **22** (1 equiv) with **6** (1.8 equiv), as described for the preparation of **4**, followed by mild acid hydrolysis (aqueous 60% acetic acid) and conventional benzoylation, gave crystalline **23** (85% from **22**). The ^{1}H NMR spectrum of **23** showed signals at δ 4.62 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1') and at δ 5.49 and 5.19 attributed, respectively, to H-3 and H-2. These deshielded signals are characteristic of a 2,3-di-*O*-benzoylated derivative of the xylose residue, proving that glycosylation occurred at O-4. Treatment of **23** as described for the preparation of **5** afforded an α,β-mixture (~5:1) of **24** (85%). The ^{1}H NMR spectrum of this mixture showed, *inter alia*, a signal at δ 6.13 (d, $J_{1,2}$ 3.0 Hz), characteristic of a β-xylosyl trichloroacetimidate which adopts a $^1C_4$ conformation in solution, as previously reported in the case of **9**.

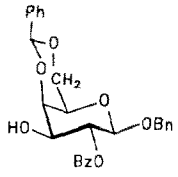
Crucial coupling between **24** (1 equiv) and the selectively *C*,*N*-protected dipeptides[10] **11a–c** (2 equiv) was performed at −20°C in dichloromethane, with



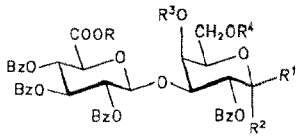
14 R = CH_2OH, R^1 = OBn, R^2 = H

15 R = $COOCH_2CH_2SiMe_3$, R^1 = OBn, R^2 = H

16 R = $COOCH_2CH_2SiMe_3$, R^1 = H, R^2 = $OC(NH)CCl_3$



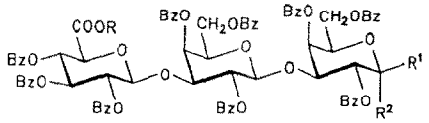
17



18 R = $CH_2CH_2SiMe_3$, R^1 = OBn, R^2 = H, R^3, R^4 = PhCH

19 R = $CH_2CH_2SiMe_3$, R^1 = OBn, R^2 = H, R^3 = R^4 = Bz

20 R = $CH_2CH_2SiMe_3$, R^1 = H, R^2 = $OC(NH)CCl_3$, R^3 = R^4 = Bz

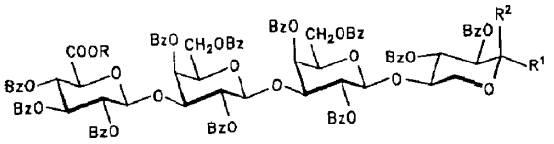


21 R = $CH_2CH_2SiMe_3$, R^1 = OBn, R^2 = H

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

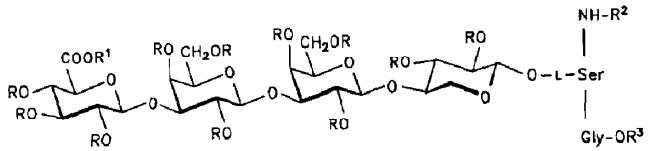
trimethylsilyl triflate (10% based on **24**) as catalyst. The glycopeptides **25a–c** were obtained in high yield (**25a**, 90%; **25b**, 92%; **25c**, 82%), and with a high stereoselectivity. The ^{1}H NMR spectrum of each glycopeptide derivative exhibited a signal at δ 5.15–5.12 ($J_{1,2}$ 6.0–6.5, $J_{2,3}$ 8.0–8.5 Hz) characteristic of H-2 in a β-linked xyloside in the $^4C_1$(D) conformation. The H-1 signals, in a crowded region of the spectra, were not firmly attributed.

Deprotection of **25a** was achieved through removal of the 2-(trimethylsilyl)ethyl ester (Bu_4NF in tetrahydrofuran), catalytic hydrogenation (Pd–C), and treatment with methanolic hydrazine, to give the target molecule **26** (50% from **25a**). No undesired side-reactions were observed in these transformations. The ^{1}H NMR



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

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



25a R = Bz, R^1 = $CH_2CH_2SiMe_3$, R^2 = COOBn, R^3 = Bn

25b R = Bz, R^1 = $CH_2CH_2SiMe_3$, R^2 = COOBn, R^3 = $CH_2CH{=}CH_2$

25c R = Bz, R^1 = $CH_2CH_2SiMe_3$, R^2 = Fmoc, R^3 = $CH_2CH{=}CH_2$

26 R = R^1 = R^2 = R^3 = H

spectrum of **26** shows the presence of the four anomeric protons [δ 4.71 (d, 1 H, $J_{1,2}$ 8.0 Hz, GlcA H-1), 4.69 (d, 1 H, $J_{1,2}$ 7.5 Hz, Gal H-1), 4.55 (d, 1 H, $J_{1,2}$ 8.0 Hz, Gal H-1), 4.52 (d, 1 H, $J_{1,2}$ 7.5 Hz, Xyl H-1)], and accords, at least for the tetrasaccharide–L-serine moiety, with those reported[5] for glycopeptide fragments isolated from proteoglycans of swarm rat chondrosarcoma. The ^{13}C NMR spectrum also accords with the postulated structure, and shows that no racemisation took place during final deprotections.

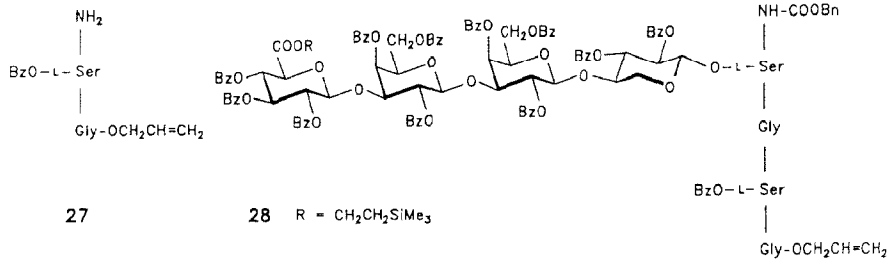
Elongation of the peptide chain was achieved as follows. Treatment[20] of **25b** with tetrakis(triphenylphosphine)palladium(0) in the presence of morpholine gave the corresponding *O*-deallylated glycopeptide, which was directly activated through the mixed-anhydride[21] procedure (isobutyl chloroformate, *N*-methylmorpholine), and condensed in situ with *O*-(benzoyl)-L-seryl-glycine allyl ester[10] (**27**, 1.1 equiv) to give **28** (83% from **25b**), the structure of which was evident from its ^1H NMR spectrum.

Removal of the allyl ester of **28** with palladium(0) afforded the corresponding acid, which was activated as described above, and coupled in situ with the amine (1.1 equiv) derived from **25c** by treatment[22] with morpholine, to give the crystalline glycopeptide **29** (73% from **28**). The ^1H NMR spectrum of **29** accords with the proposed structure, and shows the presence of the two oligosaccharide chains

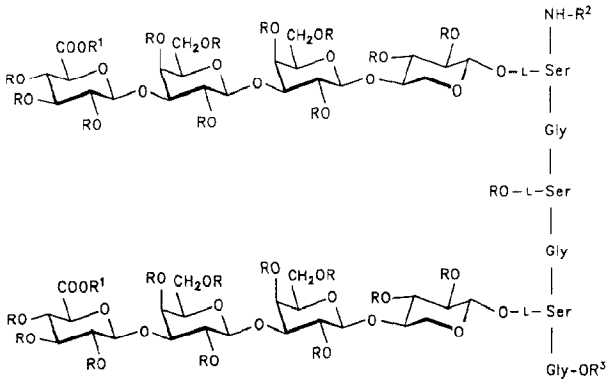
[δ 4.85, 4.84 (2 d, 2 H, $J_{1,2}$ 7.0 Hz, 2 GlcA H-1), 4.77, 4.76, 4.62, 4.53 (4 d, 4 H, $J_{1,2}$ 8.0 Hz, 4 Gal H-1)].

Complete deprotection of **29** was then achieved through removal of the 2-(trimethylsilyl)ethyl esters (Bu_4NF, tetrahydrofuran), *O*-deallylation (Pd(0), morpholine), catalytic hydrogenation[23] (Pd–C, 1,4-cyclohexadiene, *N*,*N*-dimethylformamide–ethanol), and treatment with methanolic hydrazine to give the octasaccharide–hexapeptide **30** (61% from **29**). The ^{1}H NMR data for **30** are in complete agreement with the postulated structure, and show clearly the presence of the two tetrasaccharide chains [δ 4.69 (d, 2 H, $J_{1,2}$ 7.5 Hz, 2 GlcA H-1), 4.68 (d, 2 H, $J_{1,2}$ 7.5 Hz, 2 Gal H-1), 4.55 (d, 2 H, $J_{1,2}$ 8.0 Hz, 2 Gal H-1), 4.49, 4.44 (2 d, 2 H, $J_{1,2}$ 7.5 Hz, 2 Xyl H-1)], as well as the three L-seryl residues [δ 4.74, 4.56, 4.43 (3 t, 3 H, J 5.0 Hz, 3 Ser α-CH)]. The ^{13}C NMR spectrum also accords with the expected structure, and shows the presence of two substitued L-serine residues (δ 69.20 and 67.70, Ser β-CH_2), as well as an unsubstitued one (δ 61.40, Ser β-CH_2).

The syntheses of **26** and **30** now reported open the way to the efficient preparation of more complex glycopeptides from the carbohydrate–protein linkage region common to most mammalian proteoglycans.



27 28 R = $CH_2CH_2SiMe_3$



29 R = Bz, R^1 = $CH_2CH_2SiMe_3$, R^2 = COOBn, R^3 = $CH_2CH{=}CH_2$

30 R = R^1 = R^2 = R^3 = H

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 ^1H (300 MHz) and ^{13}C NMR (75.4 MHz) spectra were recorded with a Bruker AM-300 WB spectrometer. Chemical shifts (δ) are given from the signal of internal Me_4Si unless otherwise stated. Unprimed numbers refer to the "reducing" unit and primed numbers to the "non-reducing" sugar unit. CI (ammonia)-mass spectra were recorded with a Ribermag R 10-10 spectrometer. The purity of the products was determined by TLC on Silica Gel F_{154} (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 Micro-Analyses du Centre National de la Recherche Scientifique (Vernaison, France).

*Benzyl 2,4,6-tri-*O*-benzoyl-β-*D*-galactopyranoside* (**2**).—Benzoyl chloride (2.9 mL) was added dropwise at 0°C to a solution of benzyl 3-*O*-allyl-β-D-galactopyranoside[14] (**1**, 1.86 g) and 4-dimethylaminopyridine (50 mg) in pyridine (20 mL), and the mixture was stirred at room temperature for 1 h. Methanol (2 mL) was then added, and the mixture was concentrated. A solution of the residue in CH_2Cl_2 (100 mL) was washed with aq 10% $KHSO_4$, satd aq $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. A mixture of the residue, *p*-toluenesulfonic acid monohydrate (200 mg), and 10% Pd–C (500 mg) in MeOH–H_2O (9 : 1, 50 mL) was stirred for 6 h under reflux, then filtered, and concentrated. A solution of the residue in CH_2Cl_2 (100 mL) was washed with satd aq $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. Crystallisation of the residue from EtOAc–hexane gave **2** (2.83 g, 81%); mp 150–151°C; $[\alpha]_D$ −28° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.72 (m, 20 H, 4 Ph), 5.75 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, H-4), 5.43 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2) 4.72 (d, 1 H, H-1), 4.12 (m, 1 H, $J_{3,OH}$ 6.0 Hz, H-3), 2.72 (d, 1 H, HO-3). Anal. Calcd for $C_{34}H_{30}O_9$: C, 70.09; H, 5.19. Found: C, 70.30; H, 5.17.

*Benzyl 2,4,6-tri-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**4**).—A mixture of **2** (500 mg), 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate[10] (**3**, 890 mg), and activated, powdered 4A molecular sieves (1 g) in dry 1,2-dichloroethane (14 mL) was stirred at room temperature under dry Ar, then cooled to 0°C. 0.5 M Trimethylsilyl triflate in toluene (0.36 mL) was added and the mixture was stirred for 45 min at 0°C. *N*,*N*-Diisopropylethylamine (0.5 mL) was added, and the mixture was filtered, then concentrated. The residue was chromatographed on silica gel (140 g), with toluene–EtOAc (15 : 1) as eluant, and crystallised from EtOH to give **4** (778 mg, 78%); mp 118–119°C; $[\alpha]_D$ +71° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.64 (m, 40 H, 8 Ph), 5.98 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, H-4), 5.86 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.0 Hz, H-4′), 5.68 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2), 5.57 (dd, 1 H, $J_{1',2'}$ 7.5, $J_{2',3'}$ 10.5 Hz, H-2′), 5.36 (dd, 1 H, H-3′), 4.97 (d, 1 H, H-1′), 4.57 (d, 1 H, H-1), 4.22 (dd, 1 H, H-3). Mass spectrum: *m/z* 1178 $(M + NH_4)^+$. Anal. Calcd for $C_{68}H_{56}O_{18}$: C, 70.33; H, 4.86. Found: C, 70.52; H, 4.62.

2,4,6-Tri-O-benzoyl-3-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-α-D-galactopyranosyl trichloroacetimidate (**5**).—A solution of **4** (3.23 g) in EtOAc (100 mL) was hydrogenated in the presence of 10% Pd–C (1 g) for 24 h, then filtered, and concentrated. A mixture of the residue, trichloroacetonitrile (2.78 mL), and 1,8-diazabicyclo[5,4,0]undec-7-ene (0.17 mL) in CH_2Cl_2 (50 mL) was stirred for 30 min at room temperature, then concentrated. The residue was chromatographed on silica gel (100 g), using toluene–EtOAc (15:1, containing 0.2% of triethylamine), and crystallised from EtOAc–hexane to give **5** (2.986 g, 88%); mp 194–195°C; $[\alpha]_D$ +120° (*c* 1, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 8.50 (s, 1 H, C=NH), 5.57 (m, 35 H, 7 Ph), 6.75 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 5.77 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 5.16 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.63 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3). Mass spectrum: *m/z* 1231 $(M + NH_4)^+$. Anal. Calcd for $C_{63}H_{50}Cl_3NO_{13}$: C, 62.26; H, 4.15; N, 1.15. Found: C, 62.34; H, 4.13; N, 1.06.

Benzyl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-β-D-xylopyranoside (**7**).—A mixture of benzyl 2,3-*O*-isopropylidene-β-D-xylopyranoside[10] (**6**, 75 mg), **5** (250 mg), and activated, powdered 4A molecular sieves (500 mg) in dry toluene (3 mL) was stirred at room temperature under dry Ar, then cooled to −20°C. 0.5 M Trimethylsilyl triflate in toluene (42 μL) was added and the mixture was stirred for 15 min at −20°C. *N,N*-Diisopropylethylamine (0.2 mL) was added, and the mixture was filtered, then concentrated. A solution of the residue in aq 60% acetic acid (5 mL) was stirred at 100°C for 20 min, then cooled, and concentrated. The residue was chromatographed on silica gel (30 g), using toluene–acetone (5:1), to give amorphous **7** (230 mg, 86%), $[\alpha]_D$ +79° (*c* 1, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 7.63 (m, 40 H, 8 Ph), 4.99 (d, 1 H, $J_{1'',2''}$ 7.5 Hz, H-1″), 4.65 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.31 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 3.88 (d, 1 H, $J_{3,OH}$ 2.5 Hz, HO-3), 2.51 (d, 1 H, $J_{2,OH}$ 3.5 Hz, HO-2). Mass spectrum: *m/z* 1310 $(M + NH_4)^+$. Anal. Calcd for $C_{73}H_{64}O_{22} \cdot 2H_2O$: C, 65.96; H, 5.15. Found: C, 65.78; H, 4.99.

Benzyl O-(2,3,4,6-tetra-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-xylopyranoside (**8**).—Compound **7** (600 mg) was treated as described for the preparation of **2**. The product was chromatographed on silica gel (50 g), using toluene–EtOAc (7:1), and crystallised from EtOH to give **8** (670 mg, 96%); mp 118–119°C; $[\alpha]_D$ +43° (*c* 1, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 7.53 (m, 50 H, 10 Ph), 5.84 (dd, 1 H, $J_{3'',4''}$ 3.5, $J_{4'',5''}$ 1.0 Hz, H-4″), 5.83 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.0 Hz, H-4′); 5.54 (dd, 1 H, $J_{1'',2''}$ 8.0, $J_{2'',3''}$ 10.5 Hz, H-2″), 5.53 (t, 1 H, $J_{2,3} = J_{3,4}$ = 7.0 Hz, H-3), 5.51 (dd, 1 H, $J_{1',2'}$ 7.5, $J_{2',3'}$ 10.0 Hz, H-2′), 5.35 (dd, 1 H, H-3″), 5.22 (dd, 1 H, $J_{1,2}$ 5.5 Hz, H-2), 4.92 (d, 1 H, H-1″), 4.73 (d, 1 H, H-1′), 4.67 (d, 1 H, H-1). Anal. Calcd for $C_{87}H_{72}O_{24}$: C, 69.59; H, 4.83. Found: C, 69.58; H, 4.92.

O-(2,3,4,6-Tetra-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- (**9**) *and -α-D-xylopyranosyl trichloroacetimidate* (**10**).—A solution of **8** (252 mg) in EtOAc (10 mL) was hydrogenated in the presence of 10% Pd–C (100 mg) for 24 h, then filtered, and

concentrated. A mixture of the residue, trichloroacetonitrile (0.18 mL), and 1,8-diazabicyclo[5,4,0]undec-7-ene (10 μL) in CH_2Cl_2 (4 mL) was stirred for 30 min at room temperature, then concentrated. The residue was chromatographed on silica gel (20 g), using toluene–EtOAc (7:1, containing 0.2% of Et_3N), to give, first, the amorphous β-imidate **9** (43 mg, 16%); $[\alpha]_D$ +39° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 8.68 (s, 1 H, C=NH), 7.57 (m, 45 H, 9 Ph), 6.18 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 5.69 (t, 1 H, $J_{2,3} = J_{3,4} = 4.0$ Hz, H-3), 5.32 (d, 1 H, H-2), 4.95 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.85 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.11 (dd, 1 H, $J_{4,5eq}$ 2.5, $J_{5ax,5eq}$ 12.5 Hz, H-5*eq*), 3.53 (dd, 1 H, $J_{4,5ax}$ 4.0 Hz, H-5*ax*).

Further elution gave the amorphous α-imidate **10** (188 mg, 72%); $[\alpha]_D$ +65° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 8.48 (s, 1 H, C=NH), 7.56 (m, 45 H, 9 Ph), 6.52 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.89 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.5 Hz, H-3), 5.31 (dd, 1 H, H-2), 4.92 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.71 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.14 (m, 1 H, $J_{4,5ax}$ 11.5, $J_{4,5eq}$ 6.0 Hz, H-4). Anal. Calcd for $C_{82}H_{66}Cl_3NO_{24}$: C, 63.30; H, 4.28; N, 0.90. Found: C, 63.47; H, 4.56; N, 1.15.

N-(Benzyloxycarbonyl)-O-[O-(2,3,4,6-tetra-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-seryl-glycine benzyl ester (**12**).—A mixture of imidates **9** and **10** (78 mg), *N*-(benzyloxycarbonyl)-L-seryl-glycine benzyl ester[10] (**11a**, 29 mg), and activated, powdered 4A molecular sieves (50 mg) in CH_2Cl_2 (2 mL) was stirred at room temperature under dry argon, then cooled to −20°C. 0.5 M Trimethylsilyl triflate in toluene (12 μL) was added, and the mixture was stirred for 15 min at −20°C. *N*,*N*-Diisopropylethylamine (0.1 mL) was added, and the mixture was filtered, then concentrated. The residue was chromatographed on silica gel (10 g), using toluene–EtOAc (5:2), and crystallised from EtOAc–ether to give **12** (82 mg, 91%); mp 204–205°C; $[\alpha]_D$ +44° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.56 (m, 55 H, 11 Ph), 6.78 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 5.87 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.0 Hz, H-4′), 5.84 (dd, 1 H, $J_{3'',4''}$ 3.5, $J_{4'',5''}$ 1.0 Hz, H-4″), 5.66 (d, 1 H, *J* 8.0 Hz, Ser N*H*), 5.54 (dd, 1 H, $J_{1'',2''}$ 7.5, $J_{2'',3''}$ 10.5 Hz, H-2″), 5.42 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.0 Hz, H-2′), 5.36 (dd, 1 H, H-3″), 5.16 (dd, 1 H, $J_{1,2}$ 6.5, $J_{2,3}$ 8.5 Hz, H-2), 5.05 (ABq, 2 H, OCH_2Ph), 5.02 (s, 2 H, OCH_2Ph), 4.93 (d, 1 H, H-1″), 4.70 (d, 1 H, H-1′), 4.50 (d, 1 H, H-1), 4.21 (dd, 1 H, H-3′). Anal. Calcd for $C_{100}H_{86}N_2O_{29} \cdot H_2O$: C, 66.81; H, 4.93; N, 1.56. Found: C, 66.65; H, 5.08; N, 1.59.

O-[O-β-D-Galactopyranosyl-(1 → 3)-O-β-D-galactopyranosyl-(1 → 4)-β-D-xylopyranosyl]-L-seryl-glycine (**13**).—A solution of **12** (330 mg) in EtOAc (5 mL) and MeOH (10 mL) was hydrogenated in the presence of 10% Pd–C (200 mg) for 24 h at room temperature, then filtered, and concentrated. A mixture of the residue, MeOH (10 mL), and 98% hydrazine hydrate (3 mL) was stirred for 4 h at room temperature, then cooled to 0°C. Acetone (25 mL) was added cautiously, and the mixture was stirred for 30 min, then concentrated. The resulting syrup was triturated with EtOH (3 × 10 mL), and the residue was chromatographed on a column (2.2 × 120 cm) of Sephadex G-10 with water as eluant, to give amorphous, hygroscopic **13** (103 mg, 90%); $[\alpha]_D$ +7.4° (*c* 1, H_2O). NMR data: ^1H (D_2O,

internal TSP), δ 4.64 (d, 1 H, $J_{1'',2''}$ 7.5 Hz, H-1″), 4.56 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.51 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.28 (dd, 1 H, $J_{Ha,H\alpha}$ 6.0, $J_{Ha,Hb}$ 11.5 Hz, Ser β-C*Ha*), 4.22 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.0 Hz, H-4′), 4.15 (dd, 1 H, $J_{4,5eq}$ 5.0, $J_{5ax,5eq}$ 12.0 Hz, H-5*eq*), 4.12 (dd, 1 H, $J_{H\alpha,Hb}$ 4.0 Hz, Ser β-C*Hb*), 3.95 (dd, 1 H, $J_{3'',4''}$ 3.5, $J_{4'',5''}$ 1.0 Hz, H-4″), 3.93 (d, 1 H, *J* 17.0 Hz, Gly C*H*), 3.44 (dd, 1 H, $J_{4,5ax}$ 10.0 Hz, H-5*ax*), 3.41 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2); ^{13}C (D_2O, internal acetone), δ 104.44 (Gal C-1), 102.82 (Xyl C-1), 101.54 (Gal C-1), 82.21 (Gal C-3), 76.52 (Xyl C-4), 75.22, 75.10 (2 Gal C-5), 73.84 (Xyl C-3), 72.76, 72.69 (Xyl C-2, Gal C-3), 71.20 (Gal C-2), 68.73, 68.57 (2 Gal C-4), 67.65 (Ser β-CH_2), 63.17 (Xyl C-5), 61.21 61.11 (2 Gal C-6), 53.24 (Ser α-CH), 43.66 (Gly CH_2). Anal. Calcd for $C_{22}H_{38}N_2O_{18} \cdot 2H_2O$: C, 40.37; H, 6.47; N, 4.28. Found: C, 40.21; H, 6.55; N, 4.12.

*2-(Trimethylsilyl)ethyl (benzyl 2,3,4-tri-*O*-benzoyl-β-*D*-glucopyranosid)uronate* (**15**).—A solution of chromium trioxide (1.34 g) in concd H_2SO_4 (1.15 mL) and water (2.85 mL) was added dropwise at 0°C to a stirred solution of benzyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside[18] (**14**, 2.8 g) in acetone (50 mL). After 2 h at 0°C, 2-propanol (15 mL) was added, and the mixture was filtered and concentrated. A solution of the residue in EtOAc (100 mL) was washed with brine and water, dried (Na_2SO_4), and concentrated. Oxalyl chloride (0.64 mL) was added dropwise at 0°C to a solution of the residue in CH_2Cl_2 (25 mL) and pyridine (0.43 mL). The mixture was stirred for 30 min at 0°C. Pyridine (1.17 mL) and then 2-(trimethylsilyl)ethanol (1.39 mL) were added, and the mixture was stirred for 1 h at 0°C. The mixture was diluted with CH_2Cl_2 (250 mL), washed with aq satd $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (30 g), using toluene–EtOAc (8:1), and crystallised from hexane–EtOAc to give **15** (2.235 g, 67%); mp 155–156°C; $[\alpha]_D$ −22° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.47 (m, 20 H, 4 Ph), 5.84 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 5.74 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 5.61 (dd, 1 H, $J_{1,2}$ 7.5 Hz, H-2), 4.88 (d, 1 H, H-1), 4.84 (ABq, 2 H, OCH_2Ph), 4.29 (d, 1 H, H-5), 4.16 (m, 2 H, OCH_2), 0.79 (m, 2 H, CH_2Si), −0.04 (s, 9 H, 3 CH_3). Anal. Calcd for $C_{39}H_{40}O_{10}Si$: C, 67.22; H, 5.79. Found: C, 67.16; H, 5.64.

*2-(Trimethylsilyl)ethyl (2,3,4-tri-*O*-benzoyl-α-*D*-glucopyranosyl trichloroacetimidate)uronate* (**16**).—A solution of **15** (2.297 g) in EtOAc (50 mL) was hydrogenated in the presence of 10% Pd–C (350 mg) for 16 h, then filtered, and concentrated. A mixture of the residue, trichloroacetonitrile (3.3 mL), and 1,8-diazabicyclo[5,4,0]undec-7-ene (0.1 mL) in CH_2Cl_2 (8 mL) was stirred for 15 min at room temperature. The mixture was directly eluted from a column of silica gel (100 g) with hexane–EtOAc (7:2, containing 0.1% of Et_3N) to give amorphous **16** (2.243 g, 90%); $[\alpha]_D$ +56° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 8.67 (s, 1 H, C=NH), 7.64 (m, 15 H, 3 Ph), 6.92 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 6.28 (t, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.78 (t, 1 H, $J_{4,5}$ 10.0 Hz, H-4), 5.63 (dd, 1 H, H-2), 4.74 (d, 1 H, H-5), 4.16 (m, 2 H, OCH_2), 0.77 (m, 2 H, CH_2Si), −0.06 (s, 9 H, 3 CH_3). Anal. Calcd for $C_{34}H_{34}Cl_3NO_{10}Si$: C, 54.37; H, 4.56; N, 1.86. Found: C, 54.65; H, 4.43; N, 1.70.

Benzyl 2-O-benzoyl-4,6-O-benzylidene-3-O-[2-(trimethylsilyl)ethyl 2,3,4-tri-O-benzoyl-β-D-glucopyranosyluronate]-β-D-galactopyranoside (**18**).—A mixture of benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside[19] (**17**, 205 mg), **16** (256 mg), and activated, powdered 4A molecular sieves (500 mg) in dry 1,2-dichloroethane (7 mL) was stirred at room temperature under dry Ar, then cooled to 0°C. M Trimethylsilyl triflate in toluene (51 μL) was added, and the mixture was stirred for 15 min at 0°C. *N,N*-Diisopropylethylamine (14 μL) was added, and the mixture was filtered, then concentrated. The residue was chromatographed on silica gel (40 g), using toluene–acetone (15:1, containing 0.1% of Et_3N), and crystallised from toluene–acetone to give **18** (269 mg, 75%); mp 244–245°C; $[\alpha]_D$ +14° (*c* 1, $CHCl_3$). ^{1}H–NMR data ($CDCl_3$): δ 7.49 (m, 30 H, 6 Ph), 5.75 (t, 1 H, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, H-3'), 5.70 (t, 1 H, $J_{4',5'}$ 9.5 Hz, H-4'), 5.67 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 11.0 Hz, H-2), 5.60 (s, 1 H, PhC*H*), 5.47 (dd, 1 H, $J_{1',2'}$ 7.5 Hz, H-2'), 5.14 (d, 1 H, H-1'), 4.73 (ABq, 2 H, OCH_2Ph), 4.58 (d, 1 H, H-1), 0.68 (m, 2 H, CH_2Si), −0.08 (s, 9 H, 3 CH_3). Mass spectrum: *m/z* 1068 $(M + NH_4)^+$. Anal. Calcd for $C_{59}H_{58}O_{16}Si$: C, 67.41; H, 5.56. Found: C, 67.33; H, 5.59.

Benzyl 2,4,6-tri-O-benzoyl-3-O-[2-(trimethylsilyl)ethyl 2,3,4-tri-O-benzoyl-β-D-glucopyranosyluronate]-β-D-galactopyranoside (**19**).—A mixture of **18** (1.9 g), 1,2-dimethoxyethane (32 mL), acetic acid (98 mL), and water (42 mL) was stirred at 100°C for 30 min, then cooled, and concentrated. The residue was benzoylated as described for the preparation of **2**, and chromatographed on silica gel (100 g), using toluene–EtOAc (15:1), to give **19** (1.872 g, 88%); mp 125–126°C (from aq EtOH); $[\alpha]_D$ +22.5° (*c* 1, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 7.54 (m, 35 H, 7 Ph), 6.18 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2), 6.12 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, H-4), 6.05 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.5$ Hz, H-3'), 5.93 (t, 1 H, $J_{4',5'}$ 9.5 Hz, H-4'), 5.57 (dd, 1 H, $J_{1',2'}$ 7.5 Hz, H-2'), 5.03 (d, 1 H, H-1'), 4.66 (dd, 1 H, $J_{5,6a}$ 5.5, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.58 (dd, 1 H, $J_{5,6b}$ 7.0 Hz, H-6b), 4.48 (d, 1 H, H-1), 4.04 (dd, 1 H, H-3), 0.97 (m, 2 H, CH_2Si), −0.10 (s, 9 H, 3 CH_3). Mass spectrum: *m/z* 1188 $(M + NH_4)^+$. Anal. Calcd for $C_{66}H_{62}O_{18}Si \cdot 0.5H_2O$: C, 67.16; H, 5.38. Found: C, 67.19; H, 5.36.

2,4,6-Tri-O-benzoyl-3-O-[2-(trimethylsilyl)ethyl 2,3,4-tri-O-benzoyl-β-D-glucopyranosyluronate]-α-D-galactopyranosyl trichloroacetimidate (**20**).—Compound **19** (2.13 g) was treated as described for the preparation of **16**. The mixture was directly eluted from a column of silica gel (150 g) with toluene–EtOAc (15:1, containing 0.1% of Et_3N) to give **20** (1.926 g, 86%); mp 115°C (from ether–hexane); $[\alpha]_D$ +64° (*c* 1, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 8.50 (s, 1 H, C=NH), 7.62 (m, 30 H, 6 Ph), 6.77 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 5.21 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 0.84 (m, 2 H, CH_2Si), −0.03 (s, 9 H, 3 CH_3). Mass spectrum: *m/z* 1241 $(M + NH_4)^+$. Anal. Calcd for $C_{61}H_{56}Cl_3NO_{18}Si$: C, 59.78; H, 4.61; N, 1.14. Found: C, 60.03; H, 4.62; N, 0.93.

Benzyl 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)-2,4,6-tri-O-benzoyl-β-D-galactopyranoside (**21**).—A mixture of **20** (250 mg), **2** (179 mg), and activated,

powdered 4A molecular sieves (500 mg) in 1,2-dichloroethane (5 mL) was stirred at room temperature under dry Ar, then cooled to −20°C. M Trimethylsilyl triflate in toluene (31 μL) was added and the mixture was stirred for 15 min at −20°C. The mixture was treated as described for the preparation of **4** and chromatographed on silica gel (40 g), using toluene–EtOAc (7:1), to give amorphous **21** (267 mg, 79%); $[\alpha]_D$ +24° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.52 (m, 50 H, 10 Ph), 5.85 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, H-4), 5.83 (dd, 1 H, $J_{3',4'}$ 4.0, $J_{4',5'}$ 1.0 Hz, H-4′), 5.59 (t, 1 H, $J_{3'',4''} = J_{4'',5''} = 9.5$ Hz, H-4″), 4.83 (d, 1 H, $J_{1'',2''}$ 7.0 Hz, H-1″), 4.79 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.43 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.25 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, H-3′), 4.11 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-3), 4.03 (d, 1 H, H-5″), 0.75 (m, 2 H, CH_2Si), −0.06 (s, 9 H, 3 CH_3). Anal. Calcd for $C_{93}H_{84}O_{26}Si$: C, 67.87; H, 5.14. Found: C, 67.71; H, 5.00.

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)-2,4,6-tri-O-benzoyl-α-D-galactopyranosyl trichloroacetimidate (**22**).—Compound **21** (1.221 g) was treated as described for the preparation of **16**. The resulting mixture was directly chromatographed on silica gel (80 g), using toluene–EtOAc (9:1, containing 0.1% of Et_3N), to give amorphous **22** (1.01 g, 80%); $[\alpha]_D$ +62° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 8.42 (s, 1 H, C=NH), 7.52 (m, 45 H, 9 Ph), 6.66 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.10 (d, 1 H, $J_{4'',5''}$ 10.0 Hz, H-5″), 0.78 (m, 2 H, CH_2Si), −0.04 (s, 9 H, 3 CH_3). Anal. Calcd for $C_{88}H_{78}Cl_3NO_{26}Si$: C, 62.17; H, 4.62; N, 0.82. Found: C, 62.08; H, 4.60; N, 0.87.

Benzyl 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)-2,3-di-O-benzoyl-β-D-xylopyranoside (**23**).—A mixture of **22** (195 mg) and **6** (58 mg) was treated as described for the preparation of **21**. A solution of the residue in aq 70% acetic acid was stirred for 5 min at 100°C, then cooled, and concentrated. The residue was chromatographed on silica gel (30 g), using toluene–acetone (6:1), to give a fraction that was benzoylated as described for the preparation of **2**. The residue was eluted from a column of silica gel (8 g) with toluene–acetone (8:1), and crystallised from EtOH to give **23** (194 mg, 85%); mp 117–119°C; $[\alpha]_D$ +15° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.62 (m, 60 H, 12 Ph), 5.49 (t, 1 H, $J_{2,3} = J_{3,4} = 7.0$ Hz, Xyl H-3), 5.19 (dd, 1 H, $J_{1,2}$ 5.0 Hz, Xyl H-2), 4.82 (d, 1 H, $J_{1,2}$ 7.0 Hz, GlcA H-1), 4.78 (d, 1 H, $J_{1,2}$ 8.0 Hz, Gal H-1), 4.65 (d, 1 H, Xyl H-1), 4.62 (d, 1 H, $J_{1,2}$ 8.0 Hz, Gal H-1), 4.04 (d, 1 H, $J_{4,5}$ 10.0 Hz, GlcA H-5), 0.74 (m, 2 H, CH_2Si), −0.07 (s, 9 H, 3 CH_3). Anal. Calcd for $C_{112}H_{100}O_{32}Si$: C, 67.73; H, 5.07. Found: C, 67.94; H, 5.01.

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-(2,3,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-α,β-D-xylopyranosyl trichloroacetimidate (**24**).—Compound **23** (200 mg) was treated as described for the preparation of **16**. The resulting mixture was directly chromatographed on silica gel (15 g), using toluene–acetone (15:1, containing 0.1% of Et_3N), to give an α,β-mixture of **24**

(175 mg, 81%). ^{1}H NMR data ($CDCl_3$): δ 8.65 (s, C=NHβ), 8.47 (s, C=NHα), 6.48 (d, $J_{1,2}$ 3.8 Hz, Xyl H-1α), 6.13 (d, $J_{1,2}$ 3.0 Hz, Xyl H-1β), 4.03 (d, 1 H, $J_{4,5}$ 10.0 Hz, GlcA H-5), 0.74 (m, 2 H, CH_2Si), −0.07 (s, 9 H, 3 CH_3). Anal. Calcd for $C_{107}H_{94}Cl_3NO_{32}Si$: C, 62.99; H, 4.64; N, 0.69. Found: C, 62.71; H, 4.63; N, 0.60.

Rechromatography of a portion of the above described mixture allowed isolation of pure α-imidate; $[\alpha]_D$ +31° (*c* 1, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 8.47 (s, 1 H, C=NH), 6.48 (d, 1 H, $J_{1,2}$ 3.8 Hz, Xyl H-1).

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)*-*2,3-di*-O-*benzoyl*-β-D-*xylopyranosyl}*-L-*seryl-glycine benzyl ester* (**25a**).—A mixture of **24** (150 mg), *N*-(benzyloxycarbonyl)-L-seryl-glycine benzyl ester[10] (**11a**, 57 mg), and activated, powdered 4A molecular sieves (100 mg) in CH_2Cl_2 (4 mL) was stirred at room temperature under dry Ar, then cooled to −20°C. M Trimethylsilyl triflate in toluene (7.5 μL) was added, and the mixture was stirred for 15 min at −20°C. Triethylamine (2 μL) was added, and the mixture was filtered, then concentrated. The residue was chromatographed on silica gel (20 g), using toluene–acetone (5 : 1), and crystallised from EtOH to give **25a** (151 mg, 90%); mp 108–110°C; $[\alpha]_D$ +16.5° (*c* 1, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 7.56 (m, 65 H, 13 Ph), 6.78 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 5.83, 5.75 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, 2 Gal H-4), 5.59 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, GlcA H-4), 5.48 (t, 1 H, $J_{2,3}$ 9.5 Hz, GlcA H-3), 5.38, 5.35 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 5.17 (dd, 1 H, $J_{1,2}$ 7.0 Hz, GlcA H-2), 5.12 (dd, 1 H, $J_{1,2}$ 6.0, $J_{2,3}$ 8.0 Hz, Xyl H-2), 4.83 (d, 1 H, GlcA H-1), 4.78, 4.58 (2 d, 2 H, 2 Gal H-1), 4.06 (d, 1 H, GlcA H-5), 0.75 (m, 2 H, CH_2Si), −0.08 (s, 9 H, 3 CH_3). Anal. Calcd for $C_{125}H_{114}N_2O_{37}Si$: C, 66.30; H, 5.07; N, 1.24. Found: C, 66.31; H, 5.23; N, 1.27.

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)*-*2,3-di*-O-*benzoyl*-β-D-*xylopyranosyl}*-L-*seryl-glycine allyl ester* (**25b**).—A mixture of **24** (200 mg) and *N*-(benzyloxycarbonyl)-L-seryl-glycine allyl ester[10] (**11b**, 66 mg) was treated as described for the preparation of **25a**. The product was chromatographed on silica gel (20 g), using toluene–acetone (6 : 1), to give **25b** (200 mg, 92%); $[\alpha]_D$ +19.5° (*c* 1, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 7.58 (m, 60 H, 12 Ph), 6.78 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 5.82 (m, 1 H, $OCH_2CH{=}CH_2$), 5.13 (dd, 1 H, $J_{1,2}$ 6.0, $J_{2,3}$ 8.5 Hz, Xyl H-2), 4.04 (d, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-5), 0.75 (m, 2 H, CH_2Si), −0.07 (s, 9 H, 3 CH_3). Anal. Calcd for $C_{121}H_{112}N_2O_{37}Si$: C, 65.63; H, 5.10; N, 1.27. Found: C, 65.43; H, 4.93; N, 1.37.

N-*(9-Fluorenylmethoxycarbonyl)*-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)*-*2,3-di*-O-*benzoyl*-β-D-*xylopyranosyl}*-L-*seryl-glycine allyl ester* (**25c**).—A mixture of **24** (200 mg) and 9-(fluorenylmethoxycarbonyl)-L-seryl-glycine allyl ester[10] (**11c**, 84 mg) was treated as described for the preparation of **25a**. The product was chromatographed on

silica gel (20 g), using toluene–EtOAc (3:1), and crystallised from EtOH to give **25c** (186 mg, 82%); mp 118–120°C; $[\alpha]_D$ +18° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.57 (m, 55 H, 11 Ph), 6.80 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 5.83 (m, 1 H, $OCH_2CH{=}CH_2$), 5.15 (dd, 1 H, $J_{1,2}$ 6.5, $J_{2,3}$ 8.5 Hz, Xyl H-2), 4.04 (d, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-5), 0.74 (m, 2 H, CH_2Si), −0.08 (s, 9 H, 3 CH_3). Anal. Calcd for $C_{128}H_{116}N_2O_{37}Si$: C, 66.77; H, 5.08; N, 1.22. Found: C, 66.54; H, 5.21; N, 1.24.

O-[O-(β-D-*Glucopyranosyluronic acid*)-(1 → 3)-O-β-D-*galactopyranosyl*-(1 → 3)-O-β-D-*galactopyranosyl*)-(1 → 4)-β-D-*xylopyranosyl*]-L-*seryl-glycine* (**26**).—A mixture of **25a** (198 mg) and tetrabutylammonium fluoride (100 mg) in dry tetrahydrofuran (2 mL) was stirred for 30 min at 0°C under dry Ar. The mixture was diluted with $CHCl_3$ (50 mL), washed with cold 0.5 M HCl, satd aq NH_4Cl, and water, dried ($MgSO_4$), and concentrated. A solution of the residue in EtOAc (12 mL), MeOH (2 mL) and water (1 mL) was hydrogenated in the presence of 10% Pd–C (100 mg) for 24 h, then filtered, and concentrated. A mixture of the residue, MeOH (10 mL), and 98% hydrazine hydrate (3 mL) was stirred for 3 h at room temperature, then worked up as described for the preparation of **13**. The product was chromatographed on a column (2.2 × 120 cm) of Sephadex G-10 with water as eluant, to give amorphous **26** (35 mg, 50% from **25a**); $[\alpha]_D$ −5° (*c* 1, H_2O). NMR data: ^1H (D_2O, internal TSP), δ 4.71 (d, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-1), 4.69 (d, 1 H, $J_{1,2}$ 7.5 Hz, Gal H-1), 4.55 (d, 1 H, $J_{1,2}$ 8.0 Hz, Gal H-1), 4.52 (d, 1 H, $J_{1,2}$ 7.5 Hz, Xyl H-1), 4.38 (dd, 1 H, $J_{H\alpha,Ha}$ 6.0, $J_{H\alpha,Hb}$ 4.0 Hz, Ser α-C*H*), 4.27 (dd, 1 H, $J_{Ha,Hb}$ 11.5 Hz, Ser β-C*Ha*), 4.22 (dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, 2 Gal H-4), 4.15 (dd, 1 H, $J_{4,5eq}$ 5.0, $J_{5ax,5eq}$ 12.0 Hz, Xyl H-5*eq*), 4.14 (dd, 1 H, Ser β-C*Hb*), 3.95 (d, 1 H, *J*, 17.0 Hz, Gly C*H*), 3.64 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, Xyl H-3), 3.55 (d, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-5), 3.47 (dd, 1 H, $J_{4,5ax}$ 9.5 Hz, Xyl H-5*ax*), 3.44 (dd, 1 H, $J_{2,3}$ 9.5 Hz, GlcA H-2), 3.41 (dd, 1 H, $J_{2,3}$ 9.0 Hz, Xyl H-2); ^{13}C (D_2O, internal acetone), δ 104.15 (Gal C-1), 103.78 (GlcA C-1), 102.85 (Xyl C-1), 101.58 (Gal C-1), 82.55, 82.21 (2 Gal C-3), 76.56 (Xyl C-4), 76.33 (GlcA C-5), 75.53 (GlcA C-3), 75.16, 75.01 (2 Gal C-5), 73.89 (Xyl C-3), 73.36 (GlcA C-2), 72.74 (Xyl C-2), 71.95 (GlcA C-4), 70.37, 70.03 (2 Gal C-2), 68.67, 68.26 (2 Gal C-4), 67.57 (Ser β-CH_2), 63.23 (Xyl C-5), 61.27, 61.18 (2 Gal C-6), 53.26 (Ser α-CH), 43.54 (Gly CH_2). Anal. Calcd for $C_{28}H_{46}N_2O_{24} \cdot H_2O$: C, 41.38; H, 5.95; N, 3.45. Found: C, 41.19; H, 6.05; N, 3.21.

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)-2,3-*di*-O-*benzoyl*-β-D-*xylopyranosyl*}-L-*seryl-glycyl*-O-(*benzoyl*)-L-*seryl-glycine allyl ester* (**28**).—A mixture of **25b** (271 mg), tetrakis(triphenylphosphine)palladium(0) (14 mg), and morpholine (108 μL) in dry tetrahydrofuran (7 mL) was stirred at room temperature under dry Ar for 30 min, then concentrated. The residue was chromatographed on silica gel (30 g), using EtOAc–MeOH (9:1 to 1:1), to give the corresponding carboxyl-deprotected glycopeptide (245 mg, 92%). ^1H NMR data [$(CD_3)_2SO$]: δ 8.32 (bs, 1 H, COOH), 7.44 (m, 60 H, 12 Ph), 4.82 (d, 1 H, $J_{1,2}$ 6.5 Hz, Xyl H-1), 4.67 (d, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-5), 0.59 (m, 2 H, CH_2Si), −0.10 (s, 9 H, 3 CH_3).

A solution of the above described acid (245 mg) in dry tetrahydrofuran (3.5 mL) and *N*,*N*-dimethylformamide (0.5 mL) was cooled to −20°C under dry Ar, and neutralised by stirring with *N*-methylmorpholine (12.5 μL). Isobutyl chloroformate (16 μL) was then added, followed 3 min later by a solution of *O*-(benzoyl)-L-seryl-glycine allyl ester[10] (**27**, 47 mg) in dry tetrahydrofuran (1.5 mL). The mixture was allowed to warm up slowly to room temperature, then concentrated. A solution of the residue in EtOAc (50 mL) was washed with cold M HCl, aq 5% NH_4Cl, and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on silica gel (30 g), using CH_2Cl_2–MeOH (19:1), to give amorphous **28** (250 mg, 90%); $[\alpha]_D = 12.5°$ (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 8.20–6.90 (m, 78 H, 15 Ph and 3 NH), 5.84, 5.76 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, 2 Gal H-4), 5.52 (m, 1 H, $OCH_2CH{=}CH_2$), 5.58 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, GlcA H-4), 5.47 (dd, $J_{2,3}$ 9.0 Hz, GlcA H-3), 5.43 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, Xyl H-3), 5.57, 5.54 (2 dd, 2 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 2 Gal H-2), 5.17 (dd, 1 H, $J_{1,2}$ 7.0 Hz, GlcA H-2), 5.07 (dd, 1 H, $J_{1,2}$ 7.0 Hz, Xyl H-2), 5.00 (s, 2 H, OCH_2Ph), 4.83 (d, 1 H, GlcA H-1), 4.78 (m, 1 H, Ser α-C*H*), 4.77, 4.58 (2 d, 2 H, 2 Gal H-1), 4.04 (d, 1 H, GlcA H-5), 0.74 (m, 2 H, CH_2Si), −0.07 (s, 9 H, 3 CH_3). Anal. Calcd for $C_{133}H_{124}N_4O_{41}Si \cdot H_2O$: C, 64.40; H, 5.12; N, 2.26. Found: C, 64.41; H, 5.03; N, 2.40.

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)*-*2,3-di*-O-*benzoyl*-β-D-*xylopyranosyl}*-L-*seryl-glycyl*-O-*(benzoyl)*-L-*seryl-glycyl*-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)*-*2,3-di*-O-*benzoyl*-β-D-*xylopyranosyl}*-L-*seryl-glycine allyl ester* (**29**).—A mixture of **25c** (76 mg) and morpholine (0.5 mL) was stirred at room temperature under dry Ar for 30 min, then concentrated. The residue was chromatographed on silica gel (8 g), using CH_2Cl_2–acetone (9:1 to 4:1), to give the corresponding amorphous amine (54 mg, 79%), which was immediately used in the next step. ^1H NMR data ($CDCl_3$): δ 7.54 (m, 55 H, 11 Ph), 5.87 (m, 1 H, $OCH_2CH{=}CH_2$), 4.82 (d, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-1), 4.77, 4.59 (2 d, 2 H, $J_{1,2}$ 8.0 Hz, 2 Gal H-1), 4.54 (d, 1 H, $J_{1,2}$ 6.0 Hz, Xyl H-1), 4.04 (d, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-5), 0.75 (m, 2 H, CH_2Si), −0.07 (s, 9 H, 3 CH_3).

Compound **28** (241 mg) was *O*-deallylated as described in its preparation from **25b**. The product was chromatographed on silica gel (20 g), using CH_2Cl_2–MeOH (12:1 to 6:1), to give the corresponding carboxyl-deprotected glycopeptide (202 mg, 85%). ^1H NMR data [$(CD_3)_2SO$]: δ 8.33 (bs, 1 H, COOH), 8.30 (d, 1 H, *J* 8.0 Hz, Ser N*H*), 8.15 (t, 1 H, *J* 5.5 Hz, Gly N*H*), 7.46 (m, 67 H, 13 Ph and 2 NH), 0.62 (m, 2 H, CH_2Si), −0.10 (s, 9 H, 3 CH_3).

A solution of the above described acid (70 mg) in dry tetrahydrofuran (1 mL) and *N*,*N*-dimethylformamide (0.25 mL) was cooled to −20°C under dry Ar, and neutralised by stirring with *N*-methylmorpholine (3.2 μL). Isobutyl chloroformate (4 μL) was then added, followed 5 min later by a solution of the above described

amine (54 mg) in dry tetrahydrofuran (0.5 mL) and *N,N*-dimethylformamide (0.1 mL). The mixture was allowed to warm up slowly to room temperature, and was treated as described for the preparation of **28**. The product was chromatographed on silica gel (10 g), using toluene–EtOH (19:1), and crystallised from EtOH to give **29** (100 mg, 73% from **28**); mp 139–140°C; $[\alpha]_D$ +4.5° (*c* 1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 8.10–6.90 (m, 124 H, 24 Ph and 4 NH), 5.83, 5.82, 5.78, 5.74 (4 dd, 4 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, 4 Gal H-4), 5.77 (m, 1 H, $OCH_2CH{=}CH_2$), 5.59 (dd, 2 H, $J_{3,4}$ 9.0, $J_{4,5}$ 9.5 Hz, 2 GlcA H-4), 5.47, 5.46 (2 t, 2 H, $J_{2,3}$ 9.0 Hz, 2 GlcA H-3), 5.37, 5.35, 5.34 (3 dd, 4 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, 4 Gal H-2), 5.18 (m, 3 H, 2 Gal H-2 and Xyl H-2), 4.97 (dd, 1 H, $J_{1,2}$ 7.0, $J_{2,3}$ 9.0 Hz, Xyl H-2), 4.87 (ABq, 2 H, OCH_2Ph), 4.85, 4.84 (2 d, 2 H, $J_{1,2}$ 7.0 Hz, 2 GlcA H-1), 4.77, 4.76, 4.62, 4.53 (4 d, 4 H, 4 Gal H-1), 4.51 (d, 1 H, $J_{1,2}$ 6.5 Hz, Xyl H-1), 4.05, 4.04 (2 d, 2 H, $J_{4,5}$ 9.5 Hz, 2 GlcA H-5), 0.75 (m, 4 H, 2 CH_2Si), −0.07 (s, 18 H, 6 CH_3). Anal. Calcd for $C_{243}H_{224}N_6O_{75}Si_2$: C, 65.08; H, 5.03; N, 1.87. Found: C, 65.01; H, 4.93; N, 1.60.

O-*[O-(β-*D*-Glucopyranosyluronic acid)-(1 → 3)*-O-*β*-D*-galactopyranosyl-(1 → 3)*-O-*β*-D*-galactopyranosyl-(1 → 4)*-*β*-D*-xylopyranosyl]*-L*-seryl-glycyl-*L*-seryl-glycyl*-O-*[O-(β-*D*-glucopyranosyluronic acid)-(1 → 3)*-O-*β*-D*-galactopyranosyl-(1 → 3)*-O-*β*-D*-galactopyranosyl-(1 → 4)*-*β*-D*-xylopyranosyl]*-L*-seryl-glycine* (**30**).—A mixture of **29** (98 mg) and tetrabutylammonium fluoride (46 mg) in dry tetrahydrofuran (1 mL) was stirred for 1 h at 0°C under dry Ar. The mixture was diluted with $CHCl_3$ (20 mL), washed with cold 0.5 M HCl, brine, and water, dried ($MgSO_4$), and concentrated. A mixture of the residue, tetrakis(triphenylphosphine)palladium(0) (4 mg), and morpholine (20 μL) in dry tetrahydrofuran (1.5 mL) was stirred at room temperature under dry Ar for 30 min, then concentrated. The residue was chromatographed on silica gel (10 g), using EtOAc–MeOH–H_2O (4:1:0 to 15:2:1), to give the carboxyl-deprotected glycopeptide (84 mg). A mixture of the above described product, 1,4-cyclohexadiene (37 μL), and 10% Pd–C (50 mg) in *N,N*-dimethylformamide (1 mL) and EtOH (1 mL) was stirred at room temperature for 24 h, then filtered, and concentrated. A mixture of the residue, MeOH (10 mL), and 98% hydrazine hydrate (3 mL) was stirred for 5 h at room temperature, then worked up as described for the preparation of **13**. The product was eluted from a column (2.2 × 120 cm) of Sephadex G-10 with water to give amorphous **30** (23 mg, 61% from **29**); $[\alpha]_D$ −14° (*c* 1, H_2O). NMR data: ^1H (D_2O, internal TSP), δ 4.74 (t, 1 H, *J* 5.0 Hz, Ser α-C*H*), 4.69 (d, 2 H, $J_{1,2}$ 7.5 Hz, 2 GlcA H-1), 4.68 (d, 2 H, $J_{1,2}$ 7.5 Hz, 2 Gal H-1), 4.56 (t, 1 H, *J* 5.0 Hz, Ser α-C*H*), 4.55 (d, 2 H, $J_{1,2}$ 8.0 Hz, 2 Gal H-1), 4.49, 4.44 (2 d, 2 H, $J_{1,2}$ 7.5 Hz, 2 Xyl H-1), 4.43 (t, 1 H, *J* 5.5 Hz, Ser α-C*H*), 4.29 (dd, 1 H, $J_{Ha,H\alpha}$ 5.0, $J_{Ha,Hb}$ 11.5 Hz, Ser β-C*Ha*), 4.20 (dd, 4 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.8 Hz, 4 Gal H-4), 4.09, 4.08 (2 d, 2 H, *J* 17.0 Hz, 2 Gly C*H*), 3.54 (d, 2 H, $J_{4,5}$ 9.5 Hz, 2 GlcA H-5), 3.39 (dd, 2 H, $J_{2,3}$ 9.5 Hz, 2 GlcA H-2), 3.34 (dd, 2 H, $J_{2,3}$ 9.5 Hz, 2 Xyl H-2); ^{13}C (D_2O, internal acetone), δ 104.10 (2 Gal C-1), 103.73 (2 GlcA C-1), 103.10, 103.02 (2 Xyl C-1), 101.54 (2 Gal C-1), 82.52, 82.15 (4 Gal C-3), 76.56 (2 Xyl C-4), 76.31 (2 GlcA C-5), 75.48 (2 GlcA C-3), 75.11, 74.96 (4 Gal C-5), 73.86 (2 Xyl C-3), 73.31 (2 GlcA C-2), 72.83, 72.68 (2 Xyl C-2), 71.91 (2

GlcA C-4), 70.32, 69.98 (4 Gal C-2), 69.20 (Ser β-CH_2), 68.62, 68.20 (4 Gal C-4), 67.70 (Ser β-CH_2), 63.20, 63.10 (2 Xyl C-5), 61.40 (Ser β-CH_2), 61.23, 61.13 (4 Gal C-6), 55.61, 53.52, 53.17 (3 Ser α-CH), 43.30, 42.80, 42.70 (3 Gly CH_2). Anal. Calcd for $C_{61}H_{98}N_6O_{50} \cdot 4H_2O$: C, 40.99; H, 5.98; N, 4.70. Found: C, 40.72; H, 6.08; N, 4.48.

REFERENCES

1 U. Lindahl and L. Rodén, in A. Gottschalk (Ed.), *Glycoproteins*, Elsevier, New York, 1972, pp 491–517.
2 H.C. Robinson, A.A. Horner, M. Höök, S. Ögren, and U. Lindahl, *J. Biol. Chem.*, 253 (1978) 6687–6693.
3 T.R. Oegema, E.L. Kraft, E.W. Jourdian, and T.R. van Valen, *J. Biol. Chem.*, 250 (1984) 1720–1726.
4 L.Å. Fransson, I. Silverberg, and I. Carlstedt, *J. Biol. Chem.*, 260 (1985) 14722–14726.
5 K. Sugahara, I. Yamashina, P. de Waard, H. van Halbeek, and J.F.G. Vliegenthart, *J. Biol. Chem.*, 263 (1988) 10168–10174.
6 K. Sugahara, Y. Ohi, T. Harada, I. Yamashina, P. de Waard, and J.F.G. Vliegenthart, *Abstr. Pap. Int. Carbohydr. Symp., XVth, Yokohama, Japan, 1990*, B-023.
7 L. Kjellen and U. Lindahl, *Annu. Rev. Biochem.*, 60 (1991) 443–475.
8 J.-C. Jacquinet, *Carbohydr. Res.*, 199 (1990) 153–181, and references therein.
9 M. Nilsson, C.-M. Svahn, and J. Westman, *Abstr. Pap. Eurocarb VI, Edinburgh, Scotland, 1991*, B-69.
10 S. Rio, J.-M. Beau, and J.-C. Jacquinet, *Carbohydr. Res.*, 219 (1991) 71–90.
11 R.R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 212–235.
12 G. Ekborg, T. Curenton, N. Rama Krishna, and L. Rodén, *J. Carbohydr. Chem.*, 9 (1990) 15–37.
13 P.J. Garegg, B. Lindberg, and T. Norberg, *Acta Chem. Scand., Ser. B*, 33 (1979) 449–452.
14 S. David, A. Thiéffry, and A. Veyrières, *J. Chem. Soc., Perkin Trans. 1*, (1981) 1796–1801.
15 R. Boss and R. Scheffold, *Angew. Chem. Int. Ed. Engl.*, 15 (1976) 558–559.
16 F.W. Lichtenhaler and H.J. Lindner, *Carbohydr. Res.*, 200 (1990) 91–99.
17 H. van Halbeek, L. Dorland, G.A. Veldink, J.F.G. Vliegenthart, P.J. Garegg, T. Norberg, and B. Lindberg, *Eur. J. Biochem.*, 127 (1982) 1–6.
18 R. Neszmélyi, A. Lipták, P. Nánási, and J. Szejtli, *Acta Chim. Hung.*, 113 (1983) 431.
19 G.J.F. Chittenden and J.G. Buchanan, *Carbohydr. Res.*, 11 (1969) 379–385.
20 S. Friedrich-Bochnitschek, H. Waldmann, and H. Kunz, *J. Org. Chem.*, 54 (1989) 751–756.
21 J.R. Vaughan and R.L. Osato, *J. Am. Chem. Soc.*, 74 (1952) 676–678.
22 P. Schultheiss-Reimann and H. Kunz, *Angew. Chem. Int. Ed. Engl.*, 22 (1983) 62–63.
23 J.S. Bajwa, *Tetrahedron Lett.*, (1992) 2299–2302.