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Synthesis of glycopeptides from the carbohydrate–protein linkage region of proteoglycans

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ABSTRACT

2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate was condensed with benzyl 2,3-*O*-isopropylidene-β-D-xylopyranoside to give the corresponding β-(1→4)-linked disaccharide derivative, which was transformed into 2,3-di-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-α-D-xylopyranosyl trichloroacetimidate. This glycosyl donor was condensed with a set of selectively *C*,*N*-protected L-seryl-glycine dipeptide units. Selective deblocking at the *C*- or *N*-termini of the glycosylated or non-glycosylated dipeptide segments, and coupling using the mixed-anhydride procedure allowed the construction in high yield of partially or fully glycosylated oligopeptides from the carbohydrate–protein linkage region of proteoglycans.

INTRODUCTION

Proteoglycans are macromolecular glycoproteins that contain a protein core to which side chains of glycosaminoglycans[1] are covalently attached. A polysaccharide–protein linkage region common to several proteoglycan species, namely heparin, heparan sulfate, chondroitin, and dermatan sulfate, has been identified as a β-D-GlcA-(1→3)-β-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Xyl tetrasaccharide sequence that joins each polysaccharide chain to an L-serine residue of the protein[2]. Recent studies of this region showed modifications by phosphorylation at O-2 of the xylose residue[3,4] and sulfation at O-4 or O-6 of the two galactose residues[5,6]. The cross-link, or core (Fig. 1), consists of a polypeptide that is composed of L-serine and glycine residues, occurring in alternating sequences. However, there are uncertainties about the proportion of L-serine residues that are substituted with polysaccharide chains. At least 2 out of 3 could be substituted in heparin, but many less in the case of chondroitin sulfate[7].

Glycopeptide linkages involving L-serine are alkali-sensitive (β-elimination or racemisation), and the xylose–serine bond is more or less acid-sensitive (hydrolysis or anomerisation). These drawbacks complicate greatly the isolation of glycopeptides from this region by chemical means. For these reasons, little is known about the exact

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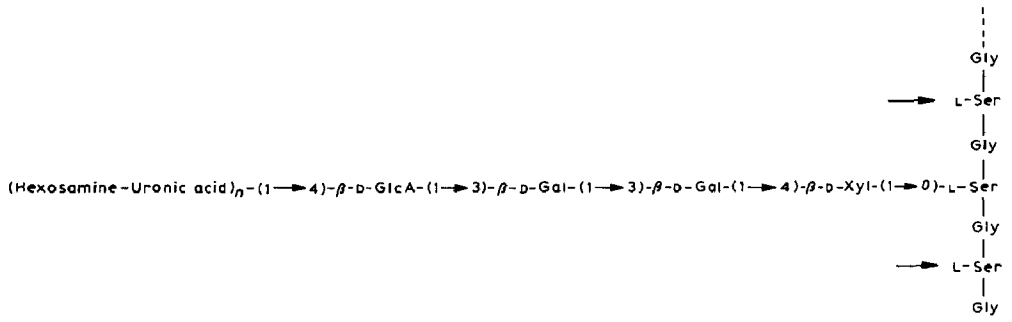


Fig. 1. The carbohydrate–protein linkage region of proteoglycans. The arrows indicate possible substitution with polysaccharides.

structures and biological activities of these molecules. However, it has been demonstrated[8] that a glycopeptide isolated from chondroitin sulfate proteoglycan accelerates the reactions of thrombin-AT III and factor Xa-AT III. In this case, both sugar and peptide moieties were required for the activity.

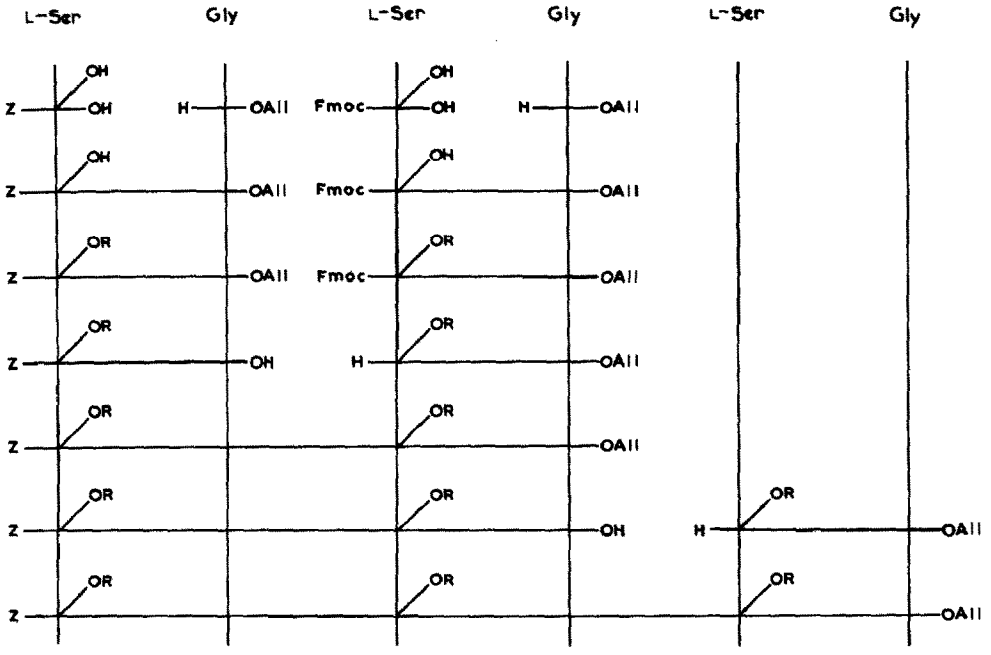
Thus, chemical synthesis remains the only alternative for solving such problems. Several fragments from the carbohydrate–protein linkage region of proteoglycans have been synthesised, either as oligosaccharides linked to a single L-serine residue[9,10], or as a peptide substitued by a single D-xylose residue[11]. As part of a program devoted to the synthesis and conformational studies of proteoglycan fragments[12], the synthesis of glycosylated oligopeptides from this region is now reported. A synthetic galactosyl-xylose disaccharide was used as the carbohydrate moiety.

RESULTS AND DISCUSSION

The strategy of the syntheses is based on the preparation of an activated disaccharide block, which is used as a glycosyl donor and condensed on preformed, selectively protected L-seryl-glycine dipeptide segments. These basic units were selectively deblocked at the *C*- or *N*-terminal part of the dipeptide moiety, then coupled by means of peptide chemistry techniques (Scheme 1). This procedure allowed construction of complex glycopeptides containing both glycosylated and non-glycosylated dipeptide sequences.

This approach required the glycosylation to be stereoselective and high-yielding. Deprotection at the *C*- or *N*-terminal part of the peptide has to be compatible with protecting groups and stability of the sugar moiety, and final deblocking must not be destructive. In summary, none of these reaction conditions should be acidic or basic.

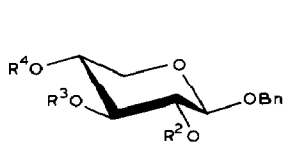
The disaccharide moiety. — For the preparation and activation of the galactosyl-xylose disaccharide, the trichloroacetimidate procedure[13] was selected. Treatment of benzyl β-D-xylopyranoside[14] (**1**) with 2-methoxypropene under kinetic control gave the crystalline 2,3-*O*-isopropylidene derivative **3** as the major product (78%), the structure of which was evident from its ^{1}H-n.m.r. spectrum. The 3,4-*O*-isopropylidene isomer **2** was also isolated (14%).



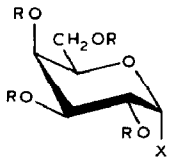
Scheme 1. Z = benzyloxycarbonyl, All = allyl, Fmoc = 9-(fluorenylmethoxycarbonyl), R = protected oligosaccharide or temporary protecting group.

β-D-Galactosylation of acceptor **3** was then studied. When **3** was condensed with acetobromogalactose under the catalysis of silver triflate with a limited amount of base (*sym*-collidine, 0.6 equiv.), extensive degradation was observed, due to the lability of the *O*-isopropylidene group under acidic conditions. With excess of base (2 equiv.), the major product was shown by ^{1}H-n.m.r. spectroscopy to be an orthodisaccharide [δ 5.87 (d, 1 H, $J_{1',2'}$ 5.0 Hz, H-1'), 1.70 (s, 3 H, CMe)]. Treatment of **3** with the *O*-acetylated trichloroacetimidate **5**[15] under the catalysis of trimethylsilyl triflate in toluene at $-20°$ gave the crystalline β-linked disaccharide **7** in 60% yield, along with ~30% of the above-described orthodisaccharide. These frustrating results prompted us to use a benzoyl group as a stereocontrolling auxiliary. Thus, 2,3,4,6-tetra-*O*-benzoyl-D-galactopyranose[16] (**6**) was treated with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene to give the amorphous imidate **7** (93%). That **7** was α was indicated by the resonance for H-1 at δ 6.92 (d, 1 H, $J_{1,2}$ 3.5 Hz). Condensation of **7** (1.33 equiv.) with **3** (1 equiv.) in dry toluene at $-20°$, in the presence of trimethylsilyl triflate (12% based on **7**), afforded 85% of the crystalline β-linked disaccharide derivative **9** (δ 5.02, d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1').

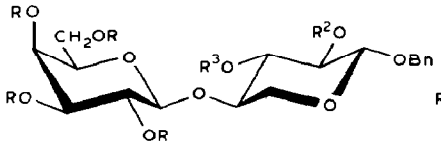
The use of the 2,3-*O*-isopropylidene group for protection of the xylose residue allowed flexibility. After mild hydrolysis of this acetal, any desired stereocontrolling auxiliary could be introduced. According to previous observations, the benzoyl group was selected. Treatment of **9** with aqueous 60% acetic acid followed by conventional benzoylation gave **10** (91% overall yield). The ^{1}H-n.m.r. spectrum of **10** showed signals



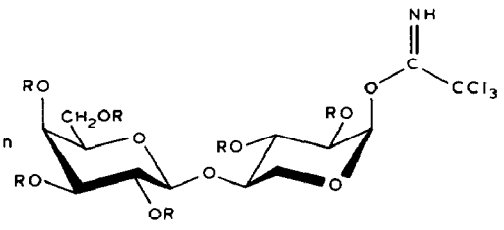
1 $R^2 = R^3 = R^4 = H$
2 $R^2 = H, R^3, R^4 = Me_2C$
3 $R^2, R^3 = Me_2C, R^4 = H$
4 $R^2 = R^3 = Bz, R^4 = H$



5 $X = OC(NH)CCl_3, R = Ac$
6 $X = H, OH, R = Bz$
7 $X = OC(NH)CCl_3, R = Bz$



8 $R = Ac, R^2, R^3 = Me_2C$
9 $R = Bz, R^2, R^3 = Me_2C$
10 $R = R^2 = R^3 = Bz$



11 R = Bz

at δ 5.64 and 5.34 attributed, respectively, to H-3 and H-2. These deshielded signals were an additional proof that galactosylation took place at O-4, and indicated that no migration of the *O*-isopropylidene group had occurred under the rather acidic conditions of the glycosylation.

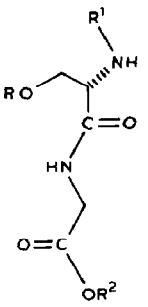
Another route to **10** was then examined. Selective chloroacetylation at HO-4 of **1** was readily achieved through the tin procedure[17]. The resulting crude product was then *O*-benzoylated and directly *O*-dechloroacetylated (thiourea in refluxing ethanol) to give crystalline **4** (81% from **1**), the structure of which was evident from its ^{1}H-n.m.r. spectrum. Condensation of **7** and **4**, as described for the preparation of **9**, afforded **10** (61%). Thus, the sequence **3** + **7** → **9** → **10** appeared to be the best route.

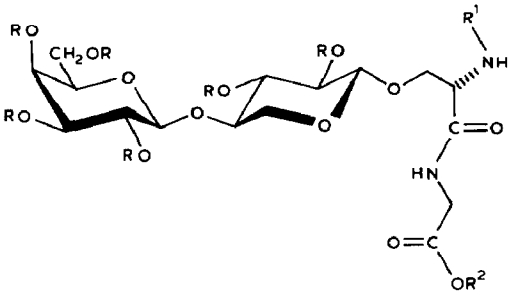
Catalytic hydrogenation (Pd–C) of **10** in ethyl acetate gave the corresponding free hemiacetal that was directly treated, as described for the preparation of **7**, to give crystalline 2,3-di-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-α-D-xylopyranosyl trichloroacetimidate (**11**, 78% from **10**). That **11** was α was indicated by the resonance for H-1 at δ 6.60 (d, 1 H, $J_{1,2}$ 3.5 Hz).

Protected dipeptides. — The choice of protecting groups for the dipeptide units was dictated by the requirement that these groups could be selectively removed, after condensation of the glycosyl donor and dipeptide acceptors, without cleavage or degradation of the synthetic glycopeptides. On this basis, benzyloxycarbonyl (Z), easily removed by catalytic hydrogenation, was selected as the permanent amino-protecting group. 9-(Fluorenylmethoxycarbonyl)[18] (Fmoc), which could be selectively eliminated with the weak base morpholine[19] (pK_a 8.2), was retained as the temporary amino-

protecting group. The allyl group, which could be removed under neutral conditions through palladium(0)-catalysed allyl transfer to morpholine[20], was selected for temporary carboxyl-protection (Scheme 1).

Dipeptides **12–15** were prepared by standard peptide-synthesis procedures, using carbodi-imide–hydroxybenzotriazole[21] activation. Dipeptide **12** was synthesised to prepare **25**, the basic repeating unit of the complex glycopeptides **32** and **35**. In order to obtain a non-glycosylated dipeptide segment, **14** was *O*-benzoylated (benzoyl chloride in pyridine) to give **18**. Attempted removal of the *N*-(9-fluorenylmethoxycarbonyl) group in **18** with morpholine[19] gave, in addition to the expected free amine **16** (50%), a major by-product identified by ^{1}H-n.m.r. and mass spectra as the *N*-benzoylated dipeptide **17**. Such *O*→*N* acyl migrations have been reported on L-serine derivatives[22], but were rather unexpected under these weakly basic conditions. In order to avoid this drawback, dipeptide **19** was prepared through *O*-benzoylation of **15**. Selective *N*-deprotection was then achieved by acid hydrolysis (trifluoroacetic acid in dichloromethane) to give, quantitatively, the stable trifluoroacetate of amine **16**. These acidic conditions had to be avoided with the acid-sensitive glycopeptides.

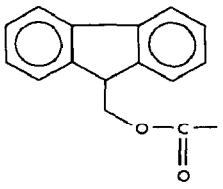




12 R = H, R^1 = Z, R^2 = Bn
13 R = H, R^1 = Z, R^2 = $CH_2CH{=}CH_2$
14 R = H, R^1 = Fmoc, R^2 = $CH_2CH{=}CH_2$
15 R = H, R^1 = Boc, R^2 = $CH_2CH{=}CH_2$
16 R = Bz, R^1 = H, R^2 = $CH_2CH{=}CH_2$
17 R = H, R^1 = Bz, R^2 = $CH_2CH{=}CH_2$
18 R = Bz, R^1 = Fmoc, R^2 = $CH_2CH{=}CH_2$
19 R = Bz, R^1 = Boc, R^2 = $CH_2CH{=}CH_2$

20 R = Bz, R^1 = R^2 = Bn
21 R = Bz, R^1 = Z, R^2 = $CH_2CH{=}CH_2$
22 R = Bz, R^1 = Fmoc, R^2 = $CH_2CH{=}CH_2$
23 R = Bz, R^1 = H, R^2 = $CH_2CH{=}CH_2$
24 R = Bz, R^1 = Z, R^2 = H
25 R = R^1 = R^2 = H

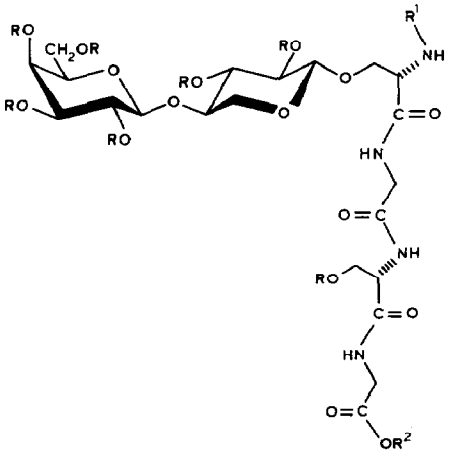
Fmoc



Protected glycopeptides. — Glycosylation reactions that involved donor **11** and the peptide acceptors **12–14** were performed at −20° in purified chloroform with trimethylsilyl triflate as a catalyst. Excess of dipeptide acceptor (1.5 equiv.) was routinely used. The crystalline glycopeptides **20–22** were obtained in high yields (>90%), and with a high stereoselectivity. The ^{1}H-n.m.r. spectrum of each β-linked glycopeptide derivative exhibited a doublet at δ 4.60 ($J_{1,2}$ 6.50–6.80 Hz) for H-1. Each c.i.(ammonia)-mass spectrum showed, in addition to the expected $(M + NH_4)^+$, a fragment (*m/z* 953) corresponding to the common sugar moiety $(M - \text{peptide} + NH_4)^+$.

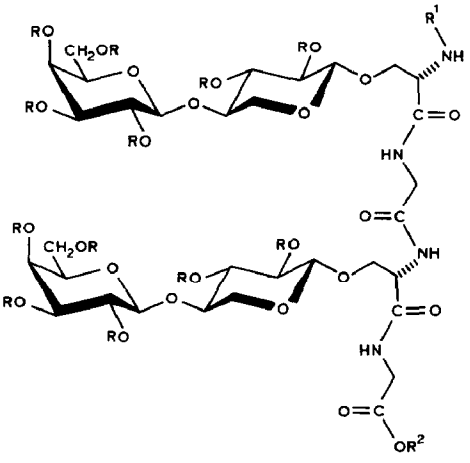
Compound **20** was then fully deprotected in order to obtain spectroscopic data for the basic glycopeptide unit. Catalytic hydrogenation (Pd–C) of **20**, followed by treatment[19] with methanolic hydrazine, which smoothly removed all of the *O*-benzoyl groups, afforded the free disaccharide–dipeptide **25** (90% from **20**). In these transformations, neither base-catalysed β-elimination nor racemisation were observed. The ^{1}H- and ^{13}C-n.m.r. data are in complete agreement with the proposed structures, and accord with those reported for synthetic galactosyl–xylosyl–serine[9,10].

C-*Terminal elongation of the peptide chain.* — Selective removal of the allyl ester of **21** was achieved by treatment[20] with tetrakis(triphenylphosphine)palladium(0) (10 mol%), in the presence of morpholine as accepting nucleophile, to give the crystalline acid **24** (95%). The ^{1}H-n.m.r. spectrum of **24** was in agreement with the expected structure and was devoid of signals for the allyl group. Acid **24** (1 equiv.) was condensed with the freshly prepared amine **16** (1 equiv.) in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline[23] (EEDQ) to give crystalline **26** (76%). The ^{1}H-n.m.r. spectrum of **26** contained, *inter alia*, a multiplet at δ 4.84 attributed to α-C*H* of the *O*-benzoyl-L-serine residue. Removal of the allyl ester of **26**, as described previously, gave the nearly insoluble acid **27** (91%).



26 R = Bz, R^1 = Z, R^2 = $CH_2CH{=}CH_2$

27 R = Bz, R^1 = Z, R^2 = H



28 R = Bz, R^1 = Z, R^2 = $CH_2CH{=}CH_2$

29 R = Bz, R^1 = Z, R^2 = H

Treatment of **22** with morpholine afforded the free amine **23** (85%) which was immediately used in the next step. Condensation of acid **21** (1 equiv.) and amine **23** (1 equiv.) in the presence of EEDQ (2 equiv.) proceeded sluggishly within 12 days to give the crystalline tetrasaccharide–tetrapeptide derivative **28** (61%), the ^{1}H-n.m.r. spectrum of which clearly indicated the presence of two galactosyl–xylosyl residues [δ 4.93, 4.92 (2 d, 2 H, $J_{1',2'}$ 8.0 Hz, 2 H-1'), 4.64, 4.62 (2 d, 2 H, $J_{1,2}$ 6.5 Hz, 2 H-1)]. A major by-product (~30% from **23**) was also isolated, the ^{1}H-n.m.r. and mass spectra of which indicated it to be an *N*-ethoxycarbonyl derivative of **23**. Such transcarbamoylation reactions have been observed[24] in glycopeptide synthesis with EEDQ.

In order to avoid the important loss of amine **23** through formation of such undesired by-products, and to effect rapid and high-yielding coupling reactions, more powerful activation of the carboxyl-group was then examined. Attempted preparation of the chloride of acid **21** led to a complex mixture which was not further investigated. To the best of our knowledge, the mixed-anhydride[25] method of coupling had not been applied to the synthesis of complex glycopeptides. Thus, treatment of acid **21** (1 equiv.) with *N*-methylmorpholine (1 equiv.) and isobutyl chloroformate (1 equiv.) in anhydrous tetrahydrofuran at −20° gave the corresponding mixed anhydride which reacted readily with amine **23** (1 equiv.) to afford crystalline **28** in excellent yield (94%). In none of these reactions were transformations of amines **16** and **23** into the corresponding diketopiperazines or hydantoins observed. Removal of the allyl ester of **28** with palladium(0), as described before, gave the acid **29** (96%).

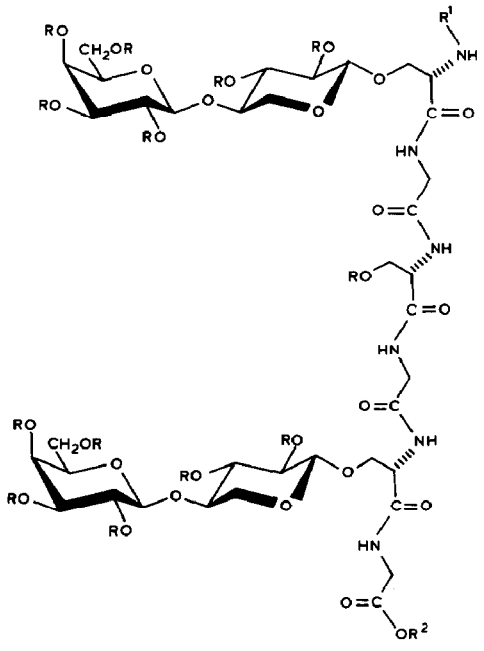
Acid **27** (1 equiv.) was condensed first with amine **23** (1 equiv.) in acetonitrile in the presence of benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent[26]) to give a complex mixture from which the tetrasaccharide–hexapeptide derivative **30** could be isolated (15%). The same coupling with EEDQ for 5 days gave 40% of crystalline **30**. However, coupling by the mixed-anhydride method, as described for the preparation of **28**, smoothly afforded **30** (61%). The ^{1}H-n.m.r. spectrum of **30** showed the presence of the two galactosyl–xylosyl residues [δ 4.99, 4.90 (2 d, 2 H, $J_{1',2'}$ 8.0 Hz, 2 H-1'), 4.66, 4.34 (2 d, 2 H, $J_{1,2}$ 6.0 and 7.5 Hz, 2 H-1)], as well as the *O*-benzoylated L-serine residue [δ 4.71 (m, 1 H, Ser α-C*H*)].

Surprisingly, condensation of acid **29** (1 equiv.) and amine **23** (1 equiv.) in the presence of EEDQ for 12 days afforded the crystalline hexasaccharide–hexapeptide **33** in 75% yield. The same coupling by the mixed-anhydride method quickly gave **33** in a slightly better yield (84%). The ^{1}H-n.m.r. spectrum of **33** indicated the presence of the three disaccharidic residues [δ 5.01, 4.94, and 4.93 (3 d, 3 H, $J_{1',2'}$ 8.0 Hz, 3 H-1'), 4.71, 4.58, and 4.47 (3 d, 3 H, $J_{1,2}$ 5.0, 6.5, and 7.0 Hz, respectively, 3 H-1)]. The *J* values ($J_{1,2}$ 5.0, $J_{1,3}$ 7.0 Hz) observed for one of the xylosyl residues strongly suggested a significant departure from the $^1C_4$ conformation in solution. Similar distortions have recently been reported[27] for O-benzoylated derivatives of D-xylose.

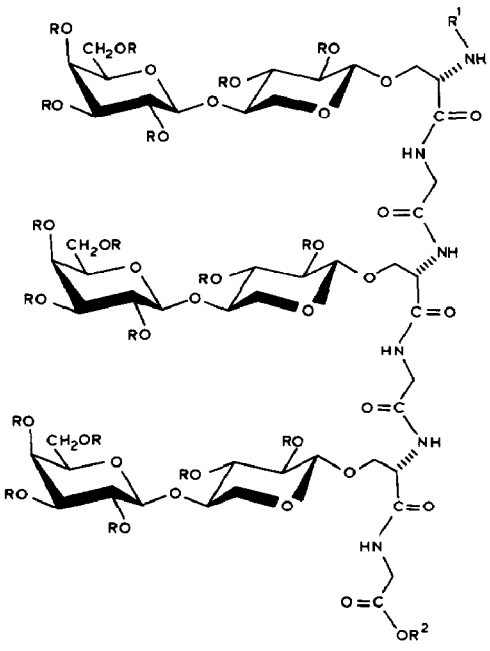
Removal of the allyl ester of **30** and **33** with palladium(0) afforded, respectively, **31** (93%) and **34** (91%), which are ready for a further *C*-terminal elongation. Final deprotection was achieved through catalytic hydrogenation (Pd–C) followed by treatment with methanolic hydrazine to give the target molecules **32** (81%) and **35** (80%), respectively. No undesired side-reactions were observed in these transformations.

The ^{1}H-n.m.r. data for **32** and **35** are in complete agreement with the postulated structures, and accord with those of the basic unit **25**. The ^{13}C-n.m.r. data (Table I) for **25**, **32**, and **35** also accord with expected structures, and are in close agreement with those reported for synthetic galactosyl-xylosyl-L-serine[9,10]. The presence of one unsubstituted L-serine residue in **32** was evident from the upfield shift (−6.50 p.p.m.) of the signal for β-CH_2, and the downfield shift (+2 p.p.m.) of the signal for α-CH, compared to those of *O*-glycosylated L-serine residues.

The synthesis of fragments of higher molecular weight is currently under investigation in our group.



30 R = Bz , R^1 = Z , R^2 = $CH_2CH{=}CH_2$
31 R = Bz , R^1 = Z , R^2 = H
32 R = R^1 = R^2 = H



33 R = Bz , R^1 = Z , R^2 = $CH_2CH{=}CH_2$
34 R = Bz , R^1 = Z , R^2 = H
35 R = R^1 = R^2 = 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° with a Perkin–Elmer Model 141 polarimeter. The ^{1}H- (300 MHz) and ^{13}C-n.m.r. (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. C.i. (ammonia)-mass spectra were recorded with a Ribermag R 10-10 spectrometer. The

TABLE I

^{13}C-N.m.r. parameters[a] (75.4 MHz) for the synthetic glycopeptides

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	L-*Ser*		*Gly* CH_2
												α.*CH*	β-CH_2	
25	102.95	72.79[b]	73.98	76.73	63.31	102.03	70.91	72.87[b]	68.87	75.58	61.36	53.10	67.93	43.72
32	102.95	72.87[b]	73.96	76.69	63.34	102.10	70.94	72.89[b]	68.90	75.58	61.44	53.19	67.75	42.88
	103.21	72.89[b]	(2)[c]	76.70	63.35	(2)	(2)	72.90[b]	(2)	(2)	(2)	55.70	61.44	42.89
												53.62	69.14	43.70
35	102.95	72.81[b]	74.05	76.78	63.35	102.06	70.88	72.81[b]	68.87	75.61	61.36	53.35	67.83	42.87
	103.23	72.82[b]	(2)	76.79	(2)	(3)	(3)	72.82[b]	(3)	(3)	(3)	53.61	69.16	42.89
	(2)	72.83[b]	74.06	(2)	63.36			72.83[b]				53.75	69.17	43.75

[a] For solutions in D_2O at 300K; chemical shifts in p.p.m. from internal acetone (30.50 p.p.m.). [b] Assignments for C-2 and C-3' may be reversed. [c] Values in brackets under a chemical shift indicated the corresponding number of carbons.

purity of products was determined by t.l.c. on Silica Gel 60 F_{154} (Merck) with detection by charring with sulfuric acid. Column chromatography was performed on Silica Gel 60 (Merck, 63–200 μm), and flash-column chromatography 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 3,4- (**2**) *and 2,3-*O*-isopropylidene-β-*D*-xylopyranoside* (**3**). — A mixture of benzyl β-D-xylopyranoside[14] (**1**, 480 mg) and camphorsulfonic acid (10 mg) in *N,N*-dimethylformamide (2.5 mL) was stirred at 60° with the exclusion of moisture. 2-Methoxypropene (0.4 mL) was added portionwise during 1 h. *N,N*-Di-isopropylethylamine (0.2 mL) was then added, and the mixture was cooled and concentrated. The residue was eluted from a column of silica gel (50 g) with hexane–ethyl acetate (4:3, containing 0.1% of triethylamine) to give, first, **2** (82 mg, 14%), m.p. 126° (from ether–hexane), $[\alpha]_D$ −87° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.38 (m, 5 H, Ph), 4.35 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 2.43 (d, 1 H, *J* 2.0 Hz, HO-2), 1.47 and 1.46 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 63.98; H, 7.19.

Further elution gave **3** (437 mg, 78%), m.p. 78° (from ether–hexane), $[\alpha]_D$ −54° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.34 (m, 5 H, Ph), 4.73 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.03 (m, 1 H, $J_{3,4}$ 9.0, $J_{4,5ax}$ 7.0, $J_{4,5eq}$ 5.0, $J_{4,OH}$ 4.0 Hz, H-4), 2.33 (d, 1 H, HO-4), 1.46 and 1.45 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.39; H, 7.14.

*Benzyl 2,3-di-*O*-benzoyl-β-*D*-xylopyranoside* (**4**). — A mixture of **1**[14] (480 mg) and dibutyltin oxide (523 mg) in dry methanol (25 mL) was boiled under reflux for 2 h, then concentrated. A solution of purified chloroacetyl chloride (0.18 mL) in benzene (4 mL) was added dropwise to a solution of the residue in benzene (15 mL), and the mixture was stirred at room temperature for 30 min, then concentrated. Benzoyl chloride (0.7 mL) was added dropwise at 0° to a solution of the residue in pyridine (10 mL), and the mixture was stirred at 0° for 1 h. Methanol (2 mL) was then added, and the mixture was concentrated. A solution of the residue in dichloromethane (50 mL) was washed with aqueous 10% potassium hydrogensulfate, saturated aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), and concentrated. A solution of the residue in ethanol (15 mL) and pyridine (2 mL) was stirred for 16 h at 80° in the presence of thiourea (230 mg), then concentrated. A solution of the resulting solid in dichloromethane (50 mL) was washed with brine and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (50 g) with hexane–ethyl acetate (3:2) and crystallised from the same mixture of solvents to give **4** (724 mg, 81%), m.p. 120–121°, $[\alpha]_D$ +43° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.60 (m, 15 H, 3 Ph), 5.43 (dd, 1 H, $J_{1,2}$ 6.0, $J_{2,3}$ 8.0 Hz, H-2), 5.26 (t, 1 H, $J_{3,4}$ 8.0 Hz, H-3), 4.78 (d, 1 H, H-1), 4.02 (m, 1 H, $J_{4,5eq}$ 4.5, $J_{4,5ax}$ 8.0, $J_{4,OH}$ 6.0 Hz, H-4), 3.06 (d, 1 H, HO-4).

Anal. Calc. for $C_{26}H_{24}O_7$: C, 69.63; H, 5.39. Found: C, 69.54; H, 5.41.

*2,3,4,6-Tetra-*O*-benzoyl-α-*D*-galactopyranosyl trichloroacetimidate* (**7**). — A mixture of 2,3,4,6-tetra-*O*-benzoyl-D-galactopyranose[16] (**6**, 700 mg), trichloroacetonitrile (1.2 mL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (90 μL) in dry dichloromethane (10

mL) was stirred for 1 h at room temperature, then concentrated. The residue was eluted from a column of silica gel (50 g) with hexane–ethyl acetate (3:1, containing 0.5% of triethylamine) to give amorphous **7** (811 mg, 93%), $[\alpha]_D$ +113° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.64 (s, 1 H, C=NH), 7.68 (m, 20 H, 4 Ph), 6.92 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 6.18 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 1.0 Hz, H-4), 6.08 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-3), 5.96 (dd, 1 H, H-2), 4.86 (m, 1 H, H-5), 4.61 (dd, 1 H, $J_{5,6a}$ 7.0, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.44 (dd, 1 H, $J_{5,6b}$ 6.0 Hz, H-6b).

Anal. Calc. for $C_{36}H_{28}Cl_3NO_{10}$: C, 58.25; H, 3.81; N, 1.89. Found: C, 58.37; H, 3.80; N, 1.93.

Benzyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-xylopyranoside (**8**). — A mixture of **3** (42 mg), **5** (100 mg), and activated powdered 4A molecular sieves (100 mg) in dry toluene (2 mL) was stirred at room temperature under dry argon, then cooled to −20°. 0.5M Trimethylsilyl triflate in toluene (46 μL) was added, and the mixture was stirred for 30 min at −20°. *N,N*-Di-isopropylethylamine (0.2 mL) was added, and the mixture was filtered, then concentrated. The residue was eluted from a column of silica gel (15 g) with hexane–ethyl acetate (4:3, containing 0.2% of triethylamine), and crystallised from ether–hexane to give **8** (57 mg, 60%), m.p. 95°, $[\alpha]_D$ −28° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.32 (m, 5 H, Ph), 4.77 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.65 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 2.15, 2.07, 2.04, and 1.97 (4 s, 12 H, 4 Ac), 1.44 (s, 6 H, CMe_2).

Anal. Calc. for $C_{29}H_{38}O_{14}$: C, 57.04; H, 6.27. Found: C, 56.78; H, 6.44.

Benzyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-β-D-xylopyranoside (**9**). — A mixture of **3** (315 mg), **7** (1.1 g), and activated 4A molecular sieves (1 g) in dry toluene (15 mL) was stirred at room temperature under dry argon, then cooled to −20°. 0.5M Trimethylsilyl triflate in toluene (0.36 mL) was added, and the mixture was stirred for 30 min at −20°. *N,N*-Di-isopropylethylamine (0.5 mL) was added, and the mixture was filtered, then concentrated. The residue was eluted from a column of silica gel (100 g) with toluene–ethyl acetate (9:1, containing 0.2% of triethylamine), and crystallised from ether–hexane to give **9** (820 mg, 85%), m.p. 129–130°, $[\alpha]_D$ +51° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.68 (m, 25 H, 5 Ph), 6.00 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.0 Hz, H-4'), 5.79 (dd 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 5.61 (dd, 1 H, H-3'), 5.02 (d, 1 H, H-1'), 4.72 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.79 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 8.0 Hz, H-3), 3.42 (dd, 1 H, H-2), 1.42 (s, 6 H, CMe_2).

Anal. Calc. for $C_{49}H_{46}O_{14}$: C, 68.52; H, 5.39. Found: C, 68.60; H, 5.19.

Benzyl 2,3-di-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-β-D-xylopyranoside (**10**). — (*a*) *From* **9**. A solution of **9** (538 mg) in aqueous 60% acetic acid (20 mL) was stirred at 100° for 20 min, then cooled, and concentrated. Benzoyl chloride (0.3 mL) was added at 0° to a solution of the residue in pyridine (8 mL), and the mixture was stirred for 1 h at 0°. Methanol (1 mL) was added, and the mixture was concentrated. A solution of the residue in dichloromethane (50 mL) was washed with aqueous 10% potassium hydrogensulfate, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (60 g) with hexane–ethyl acetate (3:2), and crystallised from ethanol

to give **10** (600 mg, 91%), m.p. 103–104°, $[\alpha]_D$ +18° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.60 (m, 35 H, 7 Ph), 5.84 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.0 Hz, H-4'), 5.71 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 5.64 (t, 1 H, $J_{2,3} = J_{3,4}$ = 8.0 Hz, H-3), 5.52 (dd, 1 H, H-3'), 5.34 (dd, 1 H, $J_{1,2}$ 6.0 Hz, H-2), 4.96 (d, 1 H, H-1'), 4.72 (d, 1 H, H-1). Mass spectrum: *m/z* 1044 $(M + NH_4)^+$.

Anal. Calc. for $C_{60}H_{50}O_{16}$: C, 70.17; H, 4.91. Found: C, 70.30; H, 4.75.

(*b*) *From* **4**. A mixture of **4** (538 mg), **7** (1.0 g), and activated powdered 4A molecular sieves (1 g) in dry toluene (15 mL) was stirred at room temperature under dry argon, then cooled to −20°. M Trimethylsilyl triflate in toluene (0.25 mL) was added, and the mixture was stirred for 1 h at −20°. *N,N*-Di-isopropylethylamine (0.5 mL) was added, and the mixture was filtered, then concentrated. The residue was eluted from a column of silica gel (120 g) with toluene–ethyl acetate (14:1), and crystallised from ethanol to give **10** (751 mg, 61%), m.p. 103–104°.

2,3-Di-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-α-D-xylopyranosyl trichloroacetimidate (**11**). — A solution of **10** (1.26 g) in ethyl acetate (25 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 mL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (75 μL) in dichloromethane (20 mL) was stirred for 1 h at room temperature, then concentrated. The residue was eluted from a column of silica gel (100 g) with hexane–ethyl acetate (3:2, containing 0.2% of triethylamine), and crystallised from ether to give **11** (932 mg, 78%), m.p. 164°, $[\alpha]_D$ +65.5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.54 (s, 1 H, C=NH), 7.70 (m, 30 H, 6 Ph), 6.60 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 6.03 (t, 1 H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3), 5.85 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.0 Hz, H-4'), 5.67 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-4'), 5.51 (dd, 1 H, H-3'), 5.39 (dd, 1 H, H-2), 4.94 (d, 1 H, H-1'). Mass spectrum: *m/z* 1080 $(M + H)^+$.

Anal. Calc. for $C_{55}H_{44}Cl_3NO_{16}$: C, 61.09; H, 4.10; N, 1.29. Found: C, 61.27; H, 3.89; N, 1.28.

General procedure for the preparation of protected dipeptides (mmol scale). — A solution of the *C*-terminal unit (1 mmol as its tosylate salt) in dry dichloromethane (10 mL) was treated at 0° with triethylamine (1 mmol). The *N*-protected amino acid (1 mmol) was then added, followed by di-cyclohexylcarbodi-imide (1 mmol) and 1-hydroxybenzotriazole[21] (1.2 mmol), and the mixture was stirred for 24 h at room temperature. The precipited dicyclohexylurea was removed, and the filtrate was washed with cold 0.1M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The following compounds were prepared by this procedure.

N-*(Benzyloxycarbonyl)-L-seryl-glycine benzyl ester* (**12**). — Prepared from *N*-(benzyloxycarbonyl)-L-serine (commercial, 239 mg) and glycine benzyl ester hydrotosylate[28] (377 mg), **12** (309 mg, 80%) had m.p. 94–95° (from ethyl acetate–hexane), $[\alpha]_D$ −8° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.35 (m, 10 H, 2 Ph), 6.98 (t, 1 H, *J* 5.5 Hz, Gly NH), 5.80 (d, 1 H, *J* 7.5 Hz, Ser NH), 2.96 (bs, 1 H, OH).

Anal. Calc. for $C_{20}H_{22}N_2O_6$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.23; N, 5.81; N, 7.12.

N-*(Benzyloxycarbonyl)*-L-*seryl-glycine allyl ester* (**13**). — Prepared from *N*-(benzyloxycarbonyl)-L-serine (commercial, 239 mg) and glycine allyl ester hydrotosylate[29] (287 mg), **13** (289 mg, 86%) had m.p. 85–86° (from ethyl acetate–hexane), $[\alpha]_D$ −9° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.35 (m, 5 H, Ph), 7.02 (t, 1 H, *J* 5.5 Hz, Gly NH), 5.90 (m, 1 H, $OCH_2CH{=}CH_2$), 5.86 (d, 1 H, *J* 8.0 Hz, Ser NH), 5.12 (s, 2 H, OCH_2Ph), 4.54 (m, 2 H, $OCH_2CH{=}CH_2$), 3.10 (bs, 1 H, OH).

Anal. Calc. for $C_{16}H_{20}N_2O_6$: C, 57.13; H, 5.99; N, 8.33. Found: C, 57.06; H, 5.89; N, 8.41.

N-*(9-Fluorenylmethoxycarbonyl)*-L-*seryl-glycine allyl ester* (**14**) *and its* O-*benzoylated derivative* (**18**). — Prepared from *N*-(9-fluorenylmethoxycarbonyl)-L-serine (commercial, 327 mg) and glycine allyl ester hydrotosylate[29] (287 mg), **14** (355 mg, 83%) had m.p. 133–134° (from methanol), $[\alpha]_D$ −13.5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.50 (m, 8 H, aromatic H), 7.00 (t, 1 H, *J* 5.5 Hz, Gly NH), 5.88 (m, 1 H, $OCH_2CH{=}CH_2$), 5.84 (d, 1 H, *J* 7.5 Hz, Ser NH), 3.10 (bs, 1 H, OH).

Anal. Calc. for $C_{23}H_{24}N_2O_6$: C, 65.08; H, 5.70; N, 6.60. Found: C, 65.10; H, 5.72; N, 6.42.

Benzoyl chloride (0.17 mL) was added dropwise at 0° to a solution of **14** (424 mg) in dry pyridine (5 mL). After 30 min, methanol (1 mL) was added, and the mixture was concentrated. A solution of the residue in dichloromethane (25 mL) was washed with brine and water, dried (Na_2SO_4), concentrated, and crystallised from ether to give **18** (470 mg, 89%), m.p. 157–158°, $[\alpha]_D$ +8.5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.60 (m, 13 H, aromatic H), 6.77 (t, 1 H, *J* 5.5 Hz, Gly NH), 5.88 (m, 1 H, $OCH_2CH{=}CH_2$), 5.73 (d, 1 H, *J* 7.5 Hz, Ser NH).

Anal. Calc. for $C_{30}H_{28}N_2O_7$: C, 68.13; H, 5.34; N, 5.30. Found: C, 68.32; H, 5.28; N, 5.11.

N-*(*tert-*Butoxycarbonyl)*-L-*seryl-glycine allyl ester* (**15**) *and its* O-*benzoylated derivative* (**19**). — Prepared from *N*-(*tert*-butoxycarbonyl)-L-serine (commercial, 205 mg) and glycine allyl ester hydrotosylate[29] (287 mg), followed by chromatography on a column of silica gel (20 g). Elution with dichloromethane–methanol (12:1) afforded amorphous **15** (237 mg, 78%), $[\alpha]_D$ −20° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.10 (t, 1 H, *J* 5.5 Hz, Gly NH), 5.82 (m, 1 H, $OCH_2CH{=}CH_2$), 5.56 (d, 1 H, *J* 8.0 Hz, Ser NH), 3.09 (dd, 1 H, *J* 5.0 and 8.0 Hz, OH), 1.45 (s, 9 H, ^{t}Bu).

Anal. Calc. for $C_{13}H_{22}N_2O_6$: C, 51.65; H, 7.33; N, 9.27. Found: C, 51.46; H, 7.21; N, 9.09.

Benzoylation of **15** (302 mg), as described for the preparation of **18**, and crystallisation of the residue from ethyl acetate–hexane gave **19** (370 mg, 91%), m.p. 71–72°, $[\alpha]_D$ +18° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.70 (m, 5 H, Ph), 6.88 (t, 1 H, *J* 5.5 Hz, Gly NH), 5.88 (m, 1 H, $OCH_2CH{=}CH_2$), 5.39 (d, 1 H, *J* 8.0 Hz, Ser NH), 1.43 (s, 9 H, ^{t}Bu).

Anal. Calc. for $C_{20}H_{26}N_2O_7$: C, 59.10; H, 6.45; N, 6.89. Found: C, 59.14; H, 6.48; N, 6.72.

N-*(Benzyloxycarbonyl)*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycine benzyl ester* (**20**). — A mixture of

11 (200 mg), **12** (107 mg), and activated 4A molecular sieves (200 mg) in dry chloroform (6 mL) was stirred at room temperature under dry argon, then cooled to −20°. 0.5M Trimethylsilyl triflate in toluene (45 μL) was added, and the mixture was stirred for 30 min at −20°. *N,N*-di-isopropylethylamine (0.2 mL) was added, and the mixture was filtered, then concentrated. The residue was eluted from a column of silica gel (20 g) with ethyl acetate–hexane (1:1) to give **20** (230 mg, 95%), m.p. 96–97° (from ethanol), $[\alpha]_D$ +26° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.60 (m, 40 H, 8 Ph), 6.82 (t, 1 H, *J* 5.5 Hz, Gly NH), 5.85 (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, $J_{2',3'}$ 10.5 Hz, H-2'), 5.66 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 5.52 (dd, 1 H, H-3'), 5.25 (dd, 1 H, $J_{1,2}$ 6.8 Hz, H-2), 5.07 (ABq, 2 H, OCH_2Ph), 5.04 (s, 2 H, OCH_2Ph), 4.92 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.58 (d, 1 H, H-1), 3.50 (dd, 1 H, $J_{Ha,Hb}$ 10.0, $J_{H\alpha,Ha}$ 8.0 Hz, Ser β-CHa). Mass spectrum: *m/z* 1322 $(M + NH_4)^+$.

Anal. Calc. for $C_{73}H_{64}N_2O_{21}$: C, 67.17; H, 4.94; N, 2.15. Found: C, 66.93; H, 5.00; N, 1.90.

N-*(Benzyloxycarbonyl)*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycine allyl ester* (**21**). — A mixture of **11** (600 mg) and **13** (280 mg) was treated as described for the preparation of **20**. The product was eluted from a column of silica gel (80 g) with ethyl acetate–hexane (4:3) to give **21** (660 mg, 94%), m.p. 97–98° (from ethanol), $[\alpha]_D$ +28.5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.62 (m, 35 H, 7 Ph), 6.84 (t, 1 H, *J* 5.5 Hz, Gly NH), 5.86 (m, 1 H, $OCH_2CH{=}CH_2$), 5.84 (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, $J_{2',3'}$ 10.5 Hz, H-2'), 5.52 (dd, 1 H, H-3'), 5.27 (dd, 1 H, $J_{1,2}$ 6.5, $J_{2,3}$ 9.0 Hz, H-2), 5.07 (ABq, 2 H, OCH_2Ph), 4.93 (d, 1 H, H-1'), 4.66 (d, 1 H, H-1), 3.57 (dd, 1 H, $J_{Ha,H\alpha}$ 8.0, $J_{Ha,Hb}$10.0 Hz, Ser β-CHa), Mass spectrum: *m/z* 1272 $(M + NH4)^+$.

Anal. Calc. for $C_{69}H_{62}N_2O_{21}$: C, 66.02; H, 4.98; N, 2.23. Found: C, 66.03; H, 5.10; N, 2.19.

O-*[2,3-Di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-N-*(9-fluorenylmethoxycarbonyl)*-L-*seryl-glycine allyl ester* (**22**). — A mixture of **11** (270 mg) and **14** (159 mg) was treated as described for the preparation of **20**. The product was eluted from a column of silica gel (45 g) with ethyl acetate–hexane (4:3) to give **22** (319 mg, 95%), m.p. 109–110° (from aqueous ethanol), $[\alpha]_D$ +26.5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.63 (m, 38 H, aromatic H), 6.87 (t, 1 H, *J* 5.5 Hz, Gly NH), 5.87 (m, 1 H, $OCH_2CH{=}CH_2$), 5.85 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, H-4'), 5.70 (t, 1 H, $J_{2,3} = J_{3,4}$ = 8.5 Hz, H-3), 5.68 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 5.52 (dd, 1 H, H-3'), 5.29 (dd, 1 H, $J_{1,2}$ 6.5 Hz, H-2), 4.92 (d, 1 H, H-1'), 4.69 (d, 1 H, H-1). Mass spectrum: *m/z* 1360 $(M + NH_4)^+$.

Anal. Calc. for $C_{76}H_{66}N_2O_{21}{\cdot}2H_2O$: C, 66.17; H, 5.11; N, 2.03. Found: C, 66.19; H, 5.03; N, 1.83.

N-*(Benzyloxycarbonyl)*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycine* (**24**). — A mixture of **21** (660 mg), tetrakis(triphenylphosphine)palladium(0) (60 mg), and morpholine (0.46 mL) in dry tetrahydrofuran (6 mL) was stirred at room temperature under dry argon for 30 min, then concentrated. The residue was eluted from a column of silica gel (50 g) with

dichloromethane–methanol (9:1 to 1:1) to give **24** (611 mg, 95%), m.p. 173–174° (from aqueous ethanol), $[\alpha]_D$ −11.5° (*c* 1, chloroform). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 7.60 (m, 37 H, 7 Ph and 2 NH), 5.76 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1.0 Hz, H-4'), 5.72 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, H-3'), 5.54 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 5.38 (dd, 1 H, $J_{1',2'}$ 8.0 Hz, H-2'), 5.33 (d, 1 H, H-1'), 5.11 (dd, 1 H, $J_{1,2}$ 7.0 Hz, H-2), 4.93 (d, 1 H, H-1), 4.80 (ABq, 2 H, OCH_2Ph).

Anal. Calc. for $C_{66}H_{53}N_2O_{21}\cdot 1.5H_2O$: C, 63.81; H, 4.94; N, 2.25. Found: C, 63.76; H, 4.72; N, 2.30.

O-[4-O-(*β*-D-*Galactopyranosyl*)-*β*-D-*xylopyranosyl*]-L-*seryl-glycine* (**25**). — A solution of **20** (200 mg) in ethyl acetate (4 mL) and methanol (2 mL) was hydrogenated in the presence of 10% Pd–C (100 mg) for 2 h, then filtered, and concentrated. A mixture of the residue, methanol (10 mL), and 98% hydrazine hydrate (2 mL) was stirred for 3 h at room temperature, then cooled to 0°. Acetone (20 mL) was added cautiously, and the mixture was stirred for 30 min, then concentrated. The resulting syrup was triturated with ethanol (3 × 3 mL), and the residue was eluted from a column (2.2 × 120 cm) of Sephadex G-10 with water to give amorphous, hygroscopic **25** (63 mg, 90%), $[\alpha]_D$ −4° (*c* 1, water). N.m.r. data: ^{1}H (D_2O, internal TSP), δ 4.48 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.47 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.19 (dd, 1 H, $J_{Ha,H\alpha}$ 4.5, $J_{Ha,Hb}$ 11.5 Hz, Ser *β*-CHa), 4.12 (dd, 1 H, $J_{4,5eq}$ 5.5, $J_{5ax,5eq}$ 12.0 Hz, H-5*eq*), 4.04 (dd, 1 H, $J_{Hb,H\alpha}$ 5.5 Hz, Ser *β*-CHb), 3.93 (dd, 1 H, $J_{3',4'}$ 3.6, $J_{4',5'}$ 1.0 Hz, H-4'), 3.90 (d, 1 H, *J* 17.0 Hz, Gly CH), 3.75 (d, 1 H, Gly CH), 3.66 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, H-3'), 3.62 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.52 (dd, 1 H, $J_{1',2'}$ 8.0 Hz, H-2'), 3.42 (dd, 1 H, $J_{4,5ax}$ 10.0 Hz, H-5*ax*), 3.38 (dd, 1 H, H-2); ^{13}C (D_2O, internal acetone), δ 176.45 (C=O), 172.10 (C=O), 102.95 (C-1), 102.03 (C-1'), 76.73 (C-4), 75.58 (C-5'), 73.98 (C-3), 72.87 and 72.79 (C-2,3'), 70.91 (C-2'), 68.87 (C-4'), 67.93 (Ser *β*-CH_2), 63.31 (C-5), 61.36 (C-6'), 53.10 (Ser α-CH), 43.72 (Gly CH_2).

Anal. Calc. for $C_{16}H_{28}N_2O_{13}\cdot 2H_2O$: C, 39.02; H, 6.55; N, 5.69. Found: C, 39.23; H, 6.38; N, 5.46.

N-(*Benzyloxycarbonyl*)-O-[*2,3-di*-O-*benzoyl*-*4*-O-(*2,3,4,6-tetra*-O-*benzoyl*-*β*-D-*galactopyranosyl*)-*β*-D-*xylopyranosyl*]-L-*seryl-glycyl*-O-(*benzoyl*)-L-*seryl-glycine allyl ester* (**26**). — A solution of **19** (115 mg) in 3:7 trifluoroacetic acid–dichloromethane (5 mL) was stirred at room temperature for 3 h, then concentrated to give quantitatively the trifluoroacetate salt of **16**. This salt was dissolved immediately at 0° in chloroform (2 mL), and treated with triethylamine (40 μL). The resulting solution was added to a mixture of **24** (345 mg) and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline[22] (EEDQ, 140 mg) in chloroform (4 mL) and *N*,*N*-dimethylformamide (1 mL). The mixture was stirred at room temperature for 24 h, then concentrated. A solution of the residue in ethyl acetate (50 mL) was washed with cold M hydrochloric acid, brine, and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (40 g) with dichloromethane–methanol (19:1), and crystallised from ethyl acetate–hexane to give **26** (325 mg, 76%), m.p. 115–116°, $[\alpha]_D$ +7.5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.62 (m, 36 H, 7 Ph and 1 NH), 7.13 (t, 1 H, *J* 5.5 Hz, Gly NH), 6.98 (d, 1 H, *J* 8.0 Hz, Ser NH), 5.83 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, H-4'), 5.78 (m, 1 H, $OCH_2CH{=}CH_2$), 5.71 (d, 1 H, *J* 8.0 Hz, Ser NH), 5.65 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 5.60 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 5.50 (dd, 1 H, H-3'), 5.21 (dd, 1 H, $J_{1,2}$ 7.0

Hz, H-2), 5.02 (s, 2 H, OCH_2Ph), 4.92 (d, 1 H, H-1'), 4.84 (m, 1 H, $J_{H\alpha,Ha}$ 4.0, $J_{H\alpha,Hb}$ 6.0, $J_{H\alpha,NH}$ 8.0 Hz, Ser α-CH), 4.61 (dd, 1 H, $J_{Ha,Hb}$ 11.5 Hz, Ser β-CHb), 4.37 (d, 1 H, H-1), 4.33 (dd, 1 H, Ser β-CHa).

Anal. Calc. for $C_{81}H_{74}N_4O_{25}$: C, 64.71; H, 4.96; N, 3.73. Found: C, 64.82; H, 4.96; N, 3.64.

N-*(Benzyloxycarbonyl)*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycyl*-O-*(benzoyl)*-L-*seryl-glycine* (**27**). — Compound **26** (670 mg) was treated and purified, as described for the preparation of **24**, to give **27** as a white solid (594 mg, 91%), $[\alpha]_D$ +10.5° (*c* 1, *N,N*-dimethylformamide). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 8.37 (d, 1 H, *J* 8.0 Hz, Ser NH), 8.23 (t, 1 H, *J* 5.5 Hz, Gly NH), 7.58 (m, 42 H, 8 Ph and 2 NH), 5.75 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, H-4'), 5.71 (dd, 1 H, $J_{2',3'}$ 9.0 Hz, H-3'), 5.52 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 5.37 (dd, 1 H, $J_{1',2'}$ 8.0 Hz, H-2'), 5.32 (d, 1 H, H-1'), 5.09 (dd, 1 H, $J_{1,2}$ 7.0 Hz, H-2), 4.91 (d, 1 H, H-1), 4.78 (ABq, 2 H, OCH_2Ph), 4.77 (m, 1 H, $J_{H\alpha,Ha}$ 4.5, $J_{H\alpha,Hb}$ 7.0, $J_{H\alpha,NH}$ 8.0 Hz, Ser α-CH).

No satisfactory elemental analysis could be obtained for this nearly insoluble compound which strongly retained traces of solvents.

N-*(Benzyloxycarbonyl)*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycyl*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycine allyl ester* (**28**). — (*a*) A mixture of **22** (64 mg) and morpholine (0.5 mL) was stirred at room temperature under dry argon for 30 min, then concentrated. The residue was eluted from a column of silica gel (5 g) with toluene–ethanol (12:1) to give the amorphous amine **23** (47 mg, 85%), which was immediately used in the next step. ^{1}H-N.m.r. data ($CDCl_3$ + D_2O): δ 7.58 (m, 30 H, 6 Ph), 5.88 (m, 1 H, $OCH_2CH=CH_2$), 5.84 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, H-4'), 5.68 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 5.66 (t, 1 H, $J_{2,3} = J_{3,4}$ = 8.5 Hz, H-3), 5.51 (dd, 1 H, H-3'), 5.26 (dd, 1 H, $J_{1,2}$ 6.5 Hz, H-2), 4.93 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.64 (d, 1 H, H-1), 3.52 (dd, 1 H, $J_{H\alpha,Hb}$ 4.5, $J_{H\alpha,Ha}$ 7.0 Hz, Ser α-CH).

A mixture of **23** (47 mg), acid **21** (50 mg), and EEDQ (20 mg) in purified chloroform (1.5 mL) was stirred at room temperature for 12 days, then diluted with chloroform (20 mL), washed with cold M hydrochloric acid, brine, and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (10 g) with toluene–ethanol (9:1), and crystallised from ethanol to give **28** (60 mg, 61%), m.p. 129–130°, $[\alpha]_D$ +20.3° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.60 (m, 65 H, 13 Ph), 6.98 (t, 1 H, *J* 5.5 Hz, Gly NH), 6.95 (t, 1 H, Gly NH), 6.76 (d, 1 H, *J* 8.0 Hz, Ser NH), 5.84 (dd, 2 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, 2 H-4'), 5.78 (m, 1 H, $OCH_2CH=CH_2$), 5.68, 5.67 (2 dd, 2 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, 2 H-2'), 5.66 (t, 2 H, $J_{2,3} = J_{3,4}$ = 8.5 Hz, 2 H-3), 5.52, 5.51 (2 dd, 2 H, 2 H-3'), 5.25, 5.24 (2 dd, 1 H, $J_{1,2}$ 6.5 Hz, 2 H-2), 5.04 (ABq, 2 H, OCH_2Ph), 4.93, 4.92 (2 d, 2 H, 2 H-1'), 4.64, 4.62 (2 d, 2 H, 2 H-1), 4.55 (m, 2 H, $J_{4,5ax}$ 7.0, $J_{4,5eq}$ 5.0 Hz, 2 H-4); 4.34 (m, 1 H, $J_{H\alpha,Ha}$ 4.0, $J_{H\alpha,Hb}$ 7.0, $J_{H\alpha,NH}$ 8.0 Hz, Ser α-CH).

Anal. Calc. for $C_{127}H_{112}N_4O_{39}$: C, 65.80; H, 4.87; N, 2.41. Found: C, 65.64; H, 4.78; N, 2.50.

(*b*) A solution of **21** (54 mg) in dry tetrahydrofuran (1 mL) was cooled to −20° under dry argon, and neutralised by stirring with *N*-methylmorpholine (4.9 μL).

Isobutyl chloroformate (6.2 μL) was then added, followed 3 min later by a solution of **23** (50 mg) in dry tetrahydrofuran (1 mL). The mixture was allowed to warm up slowly to room temperature, then concentrated. A solution of the residue in ethyl acetate (25 mL) was washed with cold M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (10 g) with toluene–ethanol (10:1) to give **28** (97 mg, 94%), m.p. 130° (from ethanol), $[\alpha]_D$ +19.5° (*c* 1, chloroform).

N-*(Benzyloxycarbonyl)*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopryanosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycyl*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,-6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycine* (**29**). — Compound **28** (150 mg) was treated as described for the preparation of **24**. The product was eluted from a column of silica gel (15 g) with ethyl acetate–methanol (12:1 to 1:1) to give amorphous **29** (141 mg, 96%), $[\alpha]_D$ +13° (*c* 1, *N*,*N*-dimethylformamide). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 8.05 (d, 1 H, *J* 8.0 Hz, Ser NH), 7.56 (m, 68 H, 13 Ph and 3 NH), 5.74 (dd, 2 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, 2 H-4'), 5.71 (dd, 2 H, $J_{2',3'}$ 9.0 Hz, 2 H-3'), 5.53, 5.52 (2 t, 2 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, 2 H-3), 5.37 (dd, 2 H, $J_{1',2'}$ 8.0 Hz, 2 H-2'), 5.33, 5.32 (2 d, 2 H, 2 H-1'), 5.11, 5.09 (2 dd, 2 H, $J_{1,2}$ 7.0 Hz, 2 H-2), 4.95, 4.91 (2 d, 2 H, 2 H-1), 4.78 (ABq, 2 H, OCH_2Ph).

A satisfactory elemental analysis could not be obtained for this compound.

N-*(Benzyloxycarbonyl)*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycyl*-O-*(benzoyl)*-L-*seryl-glycyl*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycine allyl ester* (**30**). — (*a*) A mixture of **27** (26 mg), freshly prepared **23** (20 mg), benzotriazol-l-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate[26] (8 mg), and triethylamine (8 μL) in dry acetonitrile (1.5 mL) was stirred at room temperature for 2 days, then concentrated. A solution of the residue in ethyl acetate (20 mL) was washed with cold M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (5 g) with ethyl acetate–methanol (24:1), and crystallised from methanol to give **30** (7 mg, 15%), m.p. 149–150°, $[\alpha]_D$ +0.5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.61 (m, 74 H, 14 Ph and 4 NH), 7.09 (t, 1 H, *J* 5.5 Hz, Gly NH), 7.05 (d, 1 H, *J* 8.0 Hz, Ser NH), 5.96, 5.94 (2 dd, 2 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, 2 H-4'), 5.79 (m, 1 H, $OCH_2CH{=}CH_2$), 5.71, 5.65 (2 dd, 2 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, 2 H-2'), 5.62, 5.58 (2 t, 2 H, $J_{2,3} = J_{3,4}$ = 8.5 Hz, 2 H-3), 5.53, 5.51 (2 dd, 2 H, 2 H-3'), 5.33 (dd, 1 H, $J_{1,2}$ 6.0, $J_{2,3}$ 8.0 Hz, H-2), 5.12 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.5 Hz, H-2), 4.99 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.94 (ABq, 2 H, OCH_2Ph), 4.90 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.71 (m, 1 H, $J_{H\alpha,Ha}$ 4.5, $J_{H\alpha,Hb}$ 7.5, $J_{H\alpha,NH}$ 8.0 Hz, Ser α-CH), 4.66 (d, 1 H, $J_{1,2}$ 6.0 Hz, H-1), 4.34 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1).

Anal. Calc. for $C_{139}H_{124}N_6O_{43}$: C, 65.05; H, 4.87; N, 3.27. Found: C, 65.00; H, 4.99; N, 3.01.

(*b*) A mixture of **27** (128 mg), freshly prepared **23** (98 mg), and EEDQ (45 mg) in dichloromethane (2 mL) and *N*,*N*-dimethylformamide (1 mL) was stirred at room temperature for 5 days, then concentrated. The residue was worked-up and purified as

described in (*a*) to give **30** (90 mg, 40%), m.p. 149–150°, $[\alpha]_D$ +0.6° (*c* 1, chloroform).

(*c*) Compound **27** (46 mg) and amine **23** (35 mg) were treated as described for the preparation of **28** [method (*b*)]. The product was eluted from a column of silica gel (10 g) with toluene–ethanol (12:1) to give **30** (65 mg, 81%), m.p. 149–150°, $[\alpha]_D$ +0.8° (*c* 1, chloroform).

O-*[4*-O-*(β*-D-*Galactopyranosyl)*-*β*-D-*xylopyranosyl]*-L-*seryl-glycyl*-L-*seryl-glycyl*-O-*[4*-O-*(β*-D-*galactopyranosyl)*-*β*-D-*xylopyranosyl]*-L-*seryl-glycine* (**32**). — Compound **30** (156 mg) was treated as described for the preparation of **24**. The product was eluted from a column of silica gel (15 g) with ethyl acetate–methanol (12:1 to 1:1) to give **31** (145 mg, 93%). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 8.67 (bs, 1 H, COOH), 8.44 (d, 1 H, *J* 8.0 Hz, Ser NH), 8.29 (t, 1 H, *J* 5.5 Hz, Gly NH), 8.11 (d, 1 H, *J* 8.0 Hz, Ser NH), 7.57 (m, 73 H, 14 Ph and 3 NH), 5.75 (dd, 2 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, 2 H-4'), 5.71 (dd, 2 H, $J_{2',3'}$ 9.5 Hz, 2 H-3'), 5.53, 5.50 (2 t, 2 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, 2 H-3), 5.38 (dd, 2 H, $J_{1',2'}$ 8.0 Hz, 2 H-2'), 5.32, 5.31 (2 d, 2 H, 2 H-1'), 5.09, 5.07 (2 dd, 2 H, $J_{1,2}$ 7.0 Hz, 2 H-2), 4.89, 4.83 (2 d, 2 H, 2 H-1), 4.75 (ABq, 2 H, OCH_2Ph), 4.74 (m, 1 H, $J_{H\alpha,Ha}$ 5.0, $J_{H\alpha,Hb}$ 7.0, $J_{H\alpha,NH}$ 8.0 Hz, Ser α-CH).

A solution of **31** (120 mg) in ethyl acetate–methanol–water (5:2:1, 8 mL) was hydrogenated in the presence of 10% Pd–C (100 mg) for 16 h, then filtered, and concentrated. A mixture of the residue, methanol (8 mL), and 98% hydrazine hydrate (3 mL) was stirred for 3 h at room temperature, then cooled to 0°. Acetone (25 mL) was added dropwise, and the mixture was stirred for 30 min, then concentrated. The resulting solid was triturated with ethanol (3 × 3 mL), and the residue was eluted from a column (2.2 × 120 cm) of Sephadex G-10 with water to give **32** as a colorless glass (42 mg, 81%), $[\alpha]_D$ −23° (*c* 1, water). N.m.r. data: ^{1}H (D_2O, internal TSP), δ 4.73 (t, 1 H, *J* 5.5 Hz, Ser α-CH), 4.55 (t, 1 H, *J* 5.0 Hz, Ser α-CH), 4.47 (d, 2 H, $J_{1',2'}$ 8.0 Hz, 2 H-1'), 4.46 (d, 2 H, $J_{1,2}$ 7.5 Hz, 2 H-1), 4.19 (dd, 1 H, $J_{H\alpha,Ha}$ 5.5, $J_{Ha,Hb}$ 11.0 Hz, Ser β-CHa), 4.11 (dd, 2 H, $J_{4,5eq}$ 5.5 $J_{5ax,5eq}$ 12.0 Hz, 2 H-5*eq*), 4.09 (d, 1 H, *J* 17.0 Hz, Gly CH), 4.02 (d, 1 H, *J* 17.0 Hz, Gly CH), 3.98 (dd, 2 H, $J_{3',4'}$ 3.4, $J_{4',5'}$ 0.8 Hz, 2 H-4'), 3.65 (dd, 2 H, $J_{2',3'}$ 10.0 Hz, 2 H-3'), 3.61 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, 2 H-3), 3.52 (dd, 2 H, 2 H-2'), 3.40 (dd, 2 H, $J_{4,5ax}$ 10.0 Hz, 2 H-5*ax*), 3.34 (dd, 2 H, 2 H-2); ^{13}C (D_2O, internal acetone), δ 176.80, 173.20, 172.90, 172.30, 171.30, and 169.80 (6 C=O), 103.21 (C-1), 102.95 (C-1), 102.10 (2 C-1'), 76.70, 76.69 (2 C-4), 75.58 (2 C-5'), 73.96 (2 C-3), 72.90, 72.89, and 72.87 (C-2,3'), 70.94 (2 C-2'), 69.14 (Ser β-CH_2), 68.90 (2 C-4'), 67.75 (Ser β-CH_2), 63.35, 63.34 (2 C-5), 61.44 (2 C-6' and Ser β-CH_2), 55.70, 53.62, and 53.19 (3 Ser α-CH), 43.70, 42.89, and 42.88 (3 Gly CH_2).

Anal. Calc. for $C_{37}H_{62}N_6O_{28}\cdot 3H_2O$: C, 40.66; H, 6.27; N, 7.69. Found: C, 40.58; N, 6.38; N, 7.42.

N-*(Benzyloxycarbonyl)*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)*-*β*-D-*xylopyranosyl]*-L-*seryl-glycyl*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)*-*β*-D-*xylopyranosyl]*-L-*seryl-glycyl*-O-*[2,3-di*-O-*benzoyl-4*-O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)*-*β*-D-*xylopyranosyl]*-L-*seryl-glycine allyl ester* (**33**). — (*a*) A mixture of **29** (139 mg), freshly prepared **23** (69 mg), and EEDQ (30 mg) in dry chloroform (3 mL) was stirred at room temperature for 12

days, then concentrated. A solution of the residue in ethyl acetate (30 mL) was washed with cold M hydrochloric acid, brine, and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (25 g) with toluene–ethanol (9:1), and crystallised from methanol to give **33** (158 mg, 75%), m.p. 153–154°, $[\alpha]_D$ +11.2° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.62 (m, 98 H, 19 Ph and 3 NH), 7.13 (t, 1 H, *J* 5.5 Hz, Gly NH), 7.07 (d, 1 H, *J* 8.0 Hz, Ser NH), 6.93 (d, 1 H, *J* 8.0 Hz, Ser NH), 5.88, 5.85 (2 dd, 3 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, 3 H-4'), 5.80 (m, 1 H, $OCH_2CH=CH_2$), 5.74, 5.68, and 5.65 (3 dd, 3 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, 3 H-2'), 5.54, 5.52 (2 dd, 3 H, $J_{3',4'}$ 3.5 Hz, 3 H-3'), 5.40 (dd, 1 H, $J_{1,2}$ 5.0, $J_{2,3}$ 7.0 Hz, H-2), 5.25 (dd, 1 H, $J_{1,2}$ 6.5, $J_{2,3}$ 9.0 Hz, H-2), 5.15 (dd, 1 H, $J_{1,2}$ 7.0, $J_{2,3}$ 9.0 Hz, H-2), 5.01, 4.94, and 4.93 (3 d, 3 H, 3 H-1'), 4.71 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1), 4.58 (d, 1 H, $J_{1,2}$ 6.5 Hz, H-1), 4.47 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1).

Anal. Calc. for $C_{185}H_{162}N_6O_{57}$: C, 65.72; H, 4.83; N, 2.48. Found: C, 65.71; H, 4.86; N, 2.55.

(*b*) Compounds **29** (144 mg) and **23** (71 mg) were treated as described for the preparation of **28** [method (*b*)]. The product was eluted from a column of silica gel (20 g) with toluene–ethanol (9:1) to give **33** (180 mg, 84%), m.p. 152–153°, $[\alpha]_D$ +11° (*c* 1, chloroform).

O-*[4*-O-(*β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycyl*-O-*[4*-O-*(β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycyl*-O-*[4*-O-(*β*-D-*galactopyranosyl)-β*-D-*xylopyranosyl]*-L-*seryl-glycine* (**35**). — Compound **33** (139 mg) was treated as described for the preparation of **24**. The product was eluted from a column of silica gel (15 g) with ethyl acetate–methanol (12:1 to 1:1) to give **34** (125 mg, 91%). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 8.33 (bs, 1 H, COOH), 8.10 (d, 1 H, *J* 8.0 Hz, Ser NH), 8.04 (d, 1 H, *J* 8.0 Hz, Ser NH), 7.98 (t, 1 H, *J* 5.5 Hz, Gly NH), 7.58 (m, 98 H, 19 Ph and 3 NH), 5.76 (dd, 3 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8 Hz, 3 H-4'), 5.73 (dd, 3 H, $J_{2',3'}$ 10.5 Hz, 3 H-3'), 5.54, 5.53, and 5.52 (3 t, 3 H, $J_{2,3} = J_{3,4}$ = 8.5 Hz, 3 H-3), 5.38 (dd, 3 H, $J_{1',2'}$ 8.0 Hz, 3 H-2'), 5.33 (d, 3 H, 3 H-1'), 5.10 (dd, 3 H, $J_{1,2}$ 7.0 Hz, 3 H-2), 4.94, 4.92, and 4.87 (3 d, 3 H, 3 H-1), 4.78 (ABq, 2 H, OCH_2Ph).

A solution of **34** (125 mg) in ethyl acetate–methanol–water (12:2:1, 10 mL) was hydrogenated in the presence of 10% Pd–C (100 mg) for 16 h, then filtered, and concentrated. A mixture of the residue, methanol (8 mL), and 98% hydrazine hydrate (2 mL) was stirred for 3 h at room temperature, then cooled to 0°. Acetone (25 mL) was added dropwise, and the mixture was stirred for 1 h, then concentrated. The solid residue was triturated with ethanol (3 × 3 mL), and the resulting precipitate was eluted from a column (2.2 × 120 cm) of Sephadex G-10 with water to give amorphous **35** (40 mg, 80%), $[\alpha]_D$ −20° (*c* 1, water). N.m.r. data: ^{1}H (D_2O, internal TSP), δ 4.72 (t, 1 H, *J* 5.0 Hz, Ser α-CH), 4.48 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.47 (d, 2 H, $J_{1',2'}$ 8.0 Hz, 2 H-1'), 4.46, 4.45, and 4.44 (3 d, 3 H, $J_{1,2}$ 7.5 Hz, 3 H-1), 4.22 (dd, 1 H, $J_{H\alpha,Ha}$ 5.5, $J_{Ha,Hb}$ 11.0 Hz, Ser *β*-CHa), 4.19 (dd, 1 H, Ser *β*-CHa), 4.14 (d, 1 H, *J* 17.0 Hz, Gly CH), 4.12, 4.11 (2 dd, 3 H, $J_{4,5eq}$ 5.5, $J_{5ax,5eq}$ 12.0 Hz, 3 H-5*eq*), 4.04 (d, 1 H, Gly CH), 3.93 (dd, 3 H, $J_{3',4'}$ 3.4, $J_{4',5'}$ 1.0 Hz, 3 H-4'), 3.65 (dd, 3 H, $J_{2',3'}$ 10.0 Hz, 3 H-3'), 3.62, 3.61 (2 t, 3 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, 3 H-3), 3.52 (dd, 3 H, 3 H-2'), 3.42, 3.41 (2 dd, 3 H, $J_{4,5ax}$ 10.0 Hz, 3 H-5*ax*), 3.35, 3.34 (2 dd, 3 H, 3 H-2); ^{13}C (D_2O, internal acetone), δ 176,90, 173.10, 172.90, 172.20, 171.30,

and 169.70 (6 C=O), 103.23 (2 C-1), 102.95 (C-1), 102.06 (2 C-1'), 76.79, 76.78 (3 C-4), 75.61 (3 C-5'), 74.06, 74.05 (3 C-3), 72.83, 72.82, and 72.81 (3 C-2,3'), 70.88 (3 C-2'), 69.17, 69.16 (2 Ser β-CH_2), 68.87 (3 C-4'), 67.83 (ser β-CH_2), 63.36, 63.35 (3 C-5), 61.36 (3 C-6'), 53.75, 53.61, and 53.35 (3 Ser α-CH), 43.75, 42.89, and 42.87 (3 Gly CH_2).

Anal. Calc. for $C_{48}H_{80}N_6O_{37}\cdot H_2O$: C, 42.67; H, 6.12; N, 6.22. Found: C, 42.51; H, 6.28; N.6.01.

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