Synthesis of glycopeptide sequences of repeating units of the mucins MUC 2 and MUC 3 containing oligosaccharide side-chains with core 1, core 2, core 3, core 4 and core 6 structure

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An efficient synthesis of glycosylamino acid building blocks containing core 1, core 2, core 3, core 4 or core 6 mucin core oligosaccharide structures linked O-glycosidically to threonine has been developed. These building blocks 6, 10, 16, 24 and 30 can be used directly for coupling reactions in a glycopeptide synthesis. In a multiple-column solid-phase synthesis, they have been used to prepare different series of glycopeptides. Decapeptide sequences have been synthesized from repeating units of the mucins MUC 2 and MUC 3 in which different threonine residues are each systematically glycosylated with an oligosaccharide of core 1, core 2, core 3, core 4 or core 6 structure. Glycopeptides are substrates for the study of the biosynthesis of the saccharide side-chains of mucins.

Introduction

Mucins are high relative-molecular-mass glycoproteins that are heavily glycosylated.[1] They may be membrane-associated on the surface of epithelial cells or secreted in gel form, and play an important role in the respiratory and intestinal tracts. The protein backbone has a high content not only of proline but also of threonine and serine residues, which have hydroxy groups linked O-glycosidically to saccharide side-chains.[1,2] The protein backbone of mucins of known structure consists of repeating units of approximately 10 to 20 amino acids which are joined to form larger molecules of up to about 20 tandem units. Even larger aggregates (400–1000 kDa) can be formed *via* disulfide bridges. The saccharide chains linked to the threonine and serine residues can vary in length, leading to a significant heterogeneity of the molecules. The carbohydrate content is high, constituting 50–85% of the total relative molecular mass.[1,3]

A characteristic feature of all mucins is, however, the linking region of the protein backbone with the saccharide side-chain. The first saccharide unit is always α-glycosidic linked 2-acetamido-2-deoxy-D-galactose connected to the hydroxy group of threonine or serine. This saccharide forms the inner part of the characteristic core oligosaccharides (core 1–7) which have the following structures:[1,3]

Core 1: Galβ(1→3)-GalNAcα-Thr(Ser)

Core 2: Galβ(1→3)(GlcNAcβ(1→6))-GalNAcα-Thr(Ser)

Core 3: GlcNAcβ(1→3)(GlcNAcβ(1→6))-GalNAcα-Thr(Ser)

Core 4: GlcNAcβ(1→3)-GalNAcα-Thr(Ser)

Core 5: GalNAcα(1→3)-GalNAcα-Thr(Ser)

Core 6: GlcNAcβ(1→6)-GalNAcα-Thr(Ser)

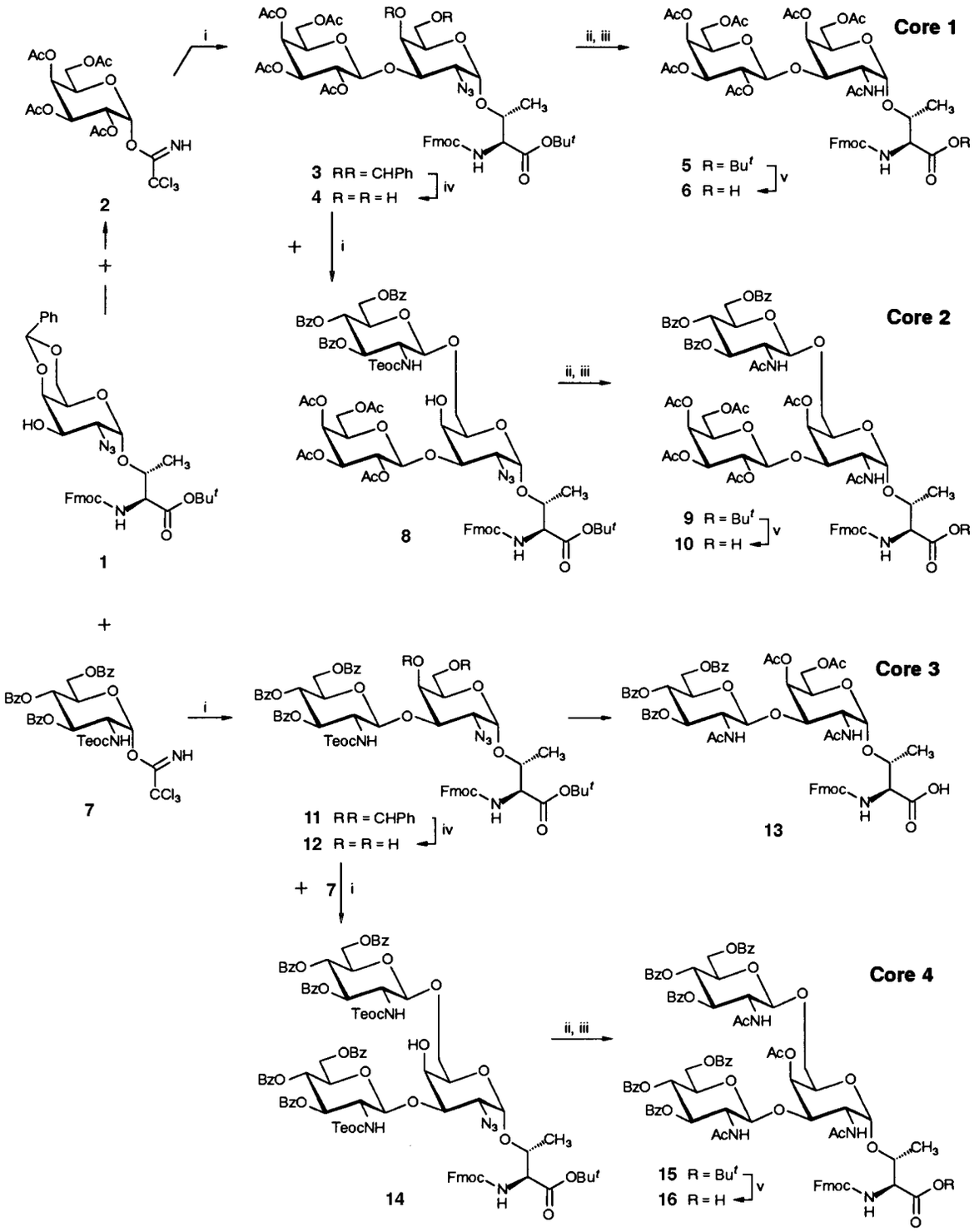
Core 7: GalNAcα(1→6)-GalNAcα-Thr(Ser)

Attached to the core structures are other saccharide chains of varying structure and length. In cancer cells, the carbohydrate side-chains are incomplete, and the core 1 structure or just the GalNAc unit may be exposed.[3,4] The biosynthesis of these glycopeptides proceeds as follows: first the 2-acetamido-2-deoxy-D-galactose is transferred from uridine 5′-diphosphate (UDP)-GalNAc to the hydroxy group of threonine or serine by the polypeptide-GalNAc-transferase and then further chain elongation takes place stepwise with catalysis of the corresponding stereoselective glycosyl transferases.[5] For isolation and precise characterisation of these enzymes the appropriate synthetic glycopeptides with defined core structures are needed as substrates and reference substances. A number of glycopeptides with core 5 and core 7 structures containing a further α-glycosidically linked GalNAc to GalNAc have already been synthesized.[6] In the present publication, the synthesis of a series of glycopeptides of varying amino acid sequence that contain the core 1, core 2, core 3, core 4 and core 6 structure is described. The compounds are substrates for a large number of enzymes.

To synthesize glycopeptides, glycosylamino acid building blocks are required which already contain the oligosaccharide chain and threonine or serine in a form that enables direct insertion of the building block in a multiple-column solid-phase glycopeptide synthesis.[7,8] Syntheses of some of this type of building block have already been described.[9,10] In the present publication, an improved, more efficient method is described which permits preparation of all five building blocks from a single starting material. This method also provides larger amounts, making it possible to synthesize series of glycopeptides with different types of linkage.

Results and discussion

The starting product for all syntheses was the glycosylamino acid derivative **1** easily available from compound **25**. The saccharide residue in the glycoside **1** contained a free OH group which could be coupled to afford disaccharide **3** by reaction with the trichloroacetimidate group of galactose **2**[11] in the presence of catalytic amounts of trimethylsilyl trifluoromethanesulfonate (TMSOTf). The benzylidene group was cleaved from compound **3** with acetic acid (80%) to give the diol **4**. Conversion of the azido group of the saccharide **4** into an acetamido group was most easily accomplished with activated zinc in acetic anhydride, acetic acid and tetrahydrofuran (THF)



Scheme 1 *Reagents:* i, TMSOTf; ii, Zn, Ac_2O, AcOH, THF; iii, Ac_2O, pyridine (Pyr); iv, AcOH (80%); v, TFA (95%)

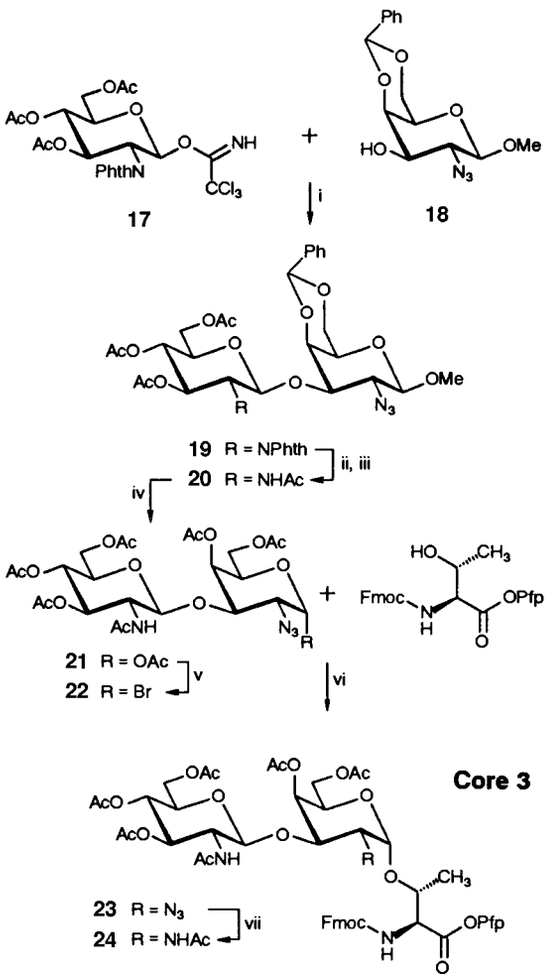
by reduction and acetylation.[12] Subsequent O-acetylation of the 4-OH and 6-OH groups afforded compound **5**, from which the *tert*-butyl group was cleaved with trifluoroacetic acid (TFA) to give the acid **6**. With compound **6** the desired core 1 building block was available for glycopeptide synthesis (Scheme 1).

The diol **4** obtained as the intermediate product was also the starting product for the synthesis of the core 2 building block **10**. It was important to protect the 2-amino group for coupling with glucosamine donors. It was also necessary to ensure the reaction to the β-glycoside and the possibility of removing the protecting group under mild conditions, thus avoiding degradation reactions. The best results were obtained with donor **7**, where the 2-amino group was protected with the trichloroethoxycarbonyl (Teoc) residue.[13] Reaction of the diol **3** proceeded regioselectively with β-glycosylation to give the trisaccharide **8**. Treatment of compound **8** with activated zinc in acetic anhydride, acetic acid and THF then resulted in a simultaneous reduction of the azido group and cleavage of the Teoc group, with *in situ* N-acetylation of both amino groups formed. Subsequent O-acetylation afforded compound **9**, providing the desired core 2 building block **10** ready for peptide synthesis after cleavage of the *tert*-butyl ester, making this building block available for a glycopeptide synthesis.

Synthesis of the core 3 and core 4 building block was possible in a similar reaction sequence. The benzylidene compound **1** could be treated with the glycosyl donor **7** in the presence of the TMSOTf promoter to give the disaccharide **11** in 82% yield. Selective hydrolysis of the benzylidene group in compound **11**

afforded the diol **12** which could, in turn, be glycosylated regioselectively with the donor **7** at the 6-OH group to give the trisaccharide **14** in 88% yield. This could be converted in one step by reduction with activated zinc and subsequent N-acetylation into the N-acetylated compound **15**. Hydrolysis of the ester group of the amino acid in compound **15** afforded the free carboxylic acid **16**, thus providing the core 4 building block for glycopeptide syntheses.

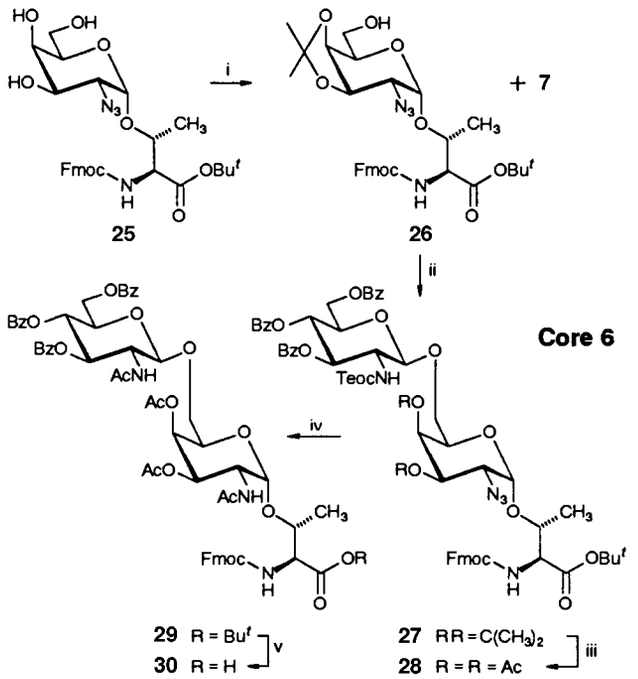
From the diol **11** the core 3 building block **13** was accessible *via* a reaction sequence similar to that of compounds **4**→**6**. We had, however, already synthesized the core 3 building block by another pathway. In this case the glycosyl donor **17**[11] was allowed to react with the methyl glycoside **18**[9,14] to give the disaccharide **19** (Scheme 2). After cleavage of the phthalimido



Scheme 2 *Reagents:* i, TMSOTf; ii, hydrazine; iii, Ac_2O, Pyr; iv, H_2SO_4, Ac_2O; v, $TiBr_4$; vi, AgOTf; vii, Zn, Ac_2O, AcOH, THF

group and N-acetylation to afford compound **20**, the methyl glycoside was cleaved by acetolysis to give the acetate **21**, which was subsequently converted with titanium tetrabromide into the glycosyl bromide **22**. The glycosylation of Fmoc-Thr-OPfp with the disaccharide donor **22** then afforded the α-glycoside **23**. Even in the labile pentafluorophenyl ester **23** reduction of the azido group to give compound **24** was possible with activated zinc in acetic anhydride. Since this pathway provided an adequate amount of the core 3 building block **24**, it was used for the other corresponding glycopeptide syntheses.

For synthesis of the core 6 building block the triol **25** was converted with 2,2-dimethoxypropane into the isopropylidene compound **26**. Treatment of the acceptor **26** with the glycosyl donor **7** then gave the disaccharide **27** (Scheme 3). After cleavage of the isopropylidene group and O-acetylation to afford the saccharide **28**, the azido group could be reduced with zinc in



Scheme 3 *Reagents:* i, $(CH_3)_2CH(OCH_3)_2$, PTSA; ii, TMSOTf; iii, AcOH, Ac_2O, Pyr; iv, Zn, Ac_2O, AcOH, THF; v, TFA (95%)

acetic anhydride and the Teoc group was cleaved to give, after N-acetylation, compound **29**. Cleavage of the ester then provided the core 6 building block **30** ready for glycopeptide synthesis.

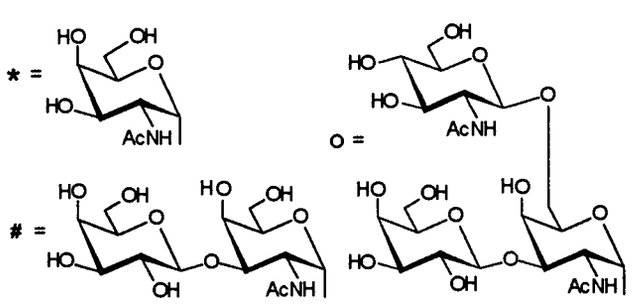
With the synthesized building blocks multiple-column solid-phase glycopeptide syntheses were performed. Two partial sequences of the repeating units of the two human intestinal mucins MUC 2 and MUC 3 which seemed promising for enzymic studies were selected as the target peptide sequence from MUC 2: Thr-Thr-Thr-Val-Thr-Pro-Thr-Pro-Thr-Gly and from MUC 3: Thr-Glu-Thr-Thr-Ser-His-Ser-Thr-Pro-Gly. These sequences have been synthesized with glycosylation on Thr at various positions.

The multiple-column solid-phase synthesis (MCPS) was carried out in a manual 20-column multiple synthesizer as previously described.[7] With this synthesizer 20 different glycopeptides can be prepared in a parallel fashion.[15] The Wang resin[16] was selected as support material. The building blocks of compounds **6** (core 1), **10** (core 2), **16** (core 4) and **30** (core 6) with free carboxy groups were activated for coupling with *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (TBTU). The non glycosylated amino acids were introduced as the *N*-fluoren-9-ylmethoxycarbonyl pentafluorophenyl (Fmoc-Pfp) esters and, as was the case with building block **24**, coupled, with addition of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH) in order to follow the progress of the coupling reaction visually by the disappearance of the yellow colour. Thr and Ser with free OH groups were introduced as the Bu^t ethers, the Glu as the Bu^t ester and His as the *tert*-butoxycarbonyl (Boc) compound. After removal of the synthesized glycopeptides from the resin with TFA, the compounds were treated with catalytic amounts of NaOMe in methanol at pH 8.5 to remove the acetyl groups of the saccharide part. After preparative reversed-phase HPLC separation, all compounds were isolated in their pure form. They were characterised by 1D- and 2D-^{1}H NMR experiments and by FAB mass spectra.

Thus it was possible to synthesize from the MUC 2 sequence the glycopeptides **31**–**36** with the monosaccharide (T^n-antigen structure), compounds **37**–**42** with the core 1 structure (T-antigen), compounds **43**–**48** with the core 2 structure (Table 1), as well as compounds **60**–**65** with the core 3 structure, com-

Table 1 Synthesized glycopeptides with T^n, Core 1 and Core 2 structure. FAB-MS data.

No.	Sequence	M + 1/Da	M_{calc}/Da
	MUC 2		
31	TTTVTPTPTG ★	1178.6	1177.6
32	TTTVTPTPTG ★	1178.9	1177.6
33	TTTVTPTPTG ★	1179.1	1177.6
34	TTTVTPTPTG ★	1179.0	1177.6
35	TTTVTPTPTG ★	1179.1	1177.6
36	TTTVTPTPTG ★ ★	1382.3	1380.7
37	TTTVTPTPTG #	1341.8	1340.4
38	TTTVTPTPTG #	1341.4	1340.4
39	TTTVTPTPTG #	1341.4	1340.4
40	TTTVTPTPTG #	1341.4	1340.4
41	TTTVTPTPTG # #	1341.7	1340.4
42	TTTVTPTPTG #	1706.6	1705.8
43	TTTVTPTPTG ○	1544.8	1543.6
44	TTTVTPTPTG ○	1544.7	1543.6
45	TTTVTPTPTG ○	1544.8	1543.6
46	TTTVTPTPTG ○	1544.8	1543.6
47	TTTVTPTPTG ○	1544.6	1543.6
48	TTTVTPTPTG ○ ○	2113.3	2112.2
49	TTTVTPTPTG ○ #	1910.0	1909.0
50	TTTVTPTPTG # ○	1910.1	1909.0
	MUC 3		
51	TETTSHSTPG ★	1220.8	1219.5
52	TETTSHSTPG ★	1221.3	1219.5
53	TETTSHSTPG ★	1221.1	1219.5
54	TETTSHSTPG #	1383.6	1382.4
55	TETTSHSTPG #	1383.8	1382.4
56	TETTSHSTPG #	1383.6	1382.4
57	TETTSHSTPG ○	1586.8	1585.6
58	TETTSHSTPG ○	1586.8	1585.6
59	TETTSHSTPG ○	1586.7	1585.6



pounds **68**–**73** with the core 4 structure and compounds **76**–**81** with the core 6 structure (Table 2). Compounds **49**, **50**, **66**, **67**, **74** and **75** are glycopeptides with two different saccharide side-chains. From the MUC 3 sequence the following compounds were obtained: **51**–**53** with a monosaccharide structure, compounds **54**–**56** with the core 1 structure, compounds **57**–**59** with the core 2 structure (Table 1), compounds **82**–**84** with the core 3 structure, compounds **85**–**87** with the core 4 structure and compounds **88**–**90** with the core 6 structure (Table 2). Thus a variety of glycopeptides with MUC 2 and MUC 3 sequence carrying all the different core structures are available by MCPS. Manual MCPS was found to be a very efficient method for the preparation of a large number of glycopeptides using only a small excess of the precious glycosylated amino acid building blocks.

Two types of carboxy-protection schemes were used and both were efficient; however, the stability of the Bu^t group under basic as well as weakly acidic conditions such as aq. acetic acid allows for a larger range of protecting-group manipulation when compared with the Pfp ester, leading to a large number of building blocks from a single precursor. On the other hand Fmoc-Thr/Ser-OPfp esters are sometimes more easily available.

This library of mucin-related compounds will be used for the characterisation of the specificity of the glycosyl transferases involved in the biosynthesis of the oligosaccharide side-chains of mucins. They can be used in the study of the following enzymes: polypeptide-GalNAc transferase, core 1-β3-Gal transferase, core 2-β6-GlcNAc transferase, core 3-β3-GlcNAc transferase, core 4-β4-GlcNAc transferase, core 6-β6-GlcNAc transferase, α3-sialyl transferase, α6-sialyl transferase, as well as transferases involved in elongation processes and also for sulfotransferases.

Experimental

Materials and methods

All solvents were distilled at the appropriate pressure. Light petroleum refers to the fraction distilled in the range 60–70 °C. Dimethylformamide (DMF) was analysed for free amines by addition of Dhbt-OH prior to use. Reagents for peptide synthesis were purchased as follows: Dhbt-OH and TBTU from Fluka; Wang resin from Bachem; Fmoc amino acid Pfp esters from NovaBiochem. ^{1}H NMR spectra were recorded on a Bruker AMX 400 spectrometer; δ-values are in ppm and J-values are in Hz (±0.3 Hz). Column chromatography was performed on Silica Gel (ICN Biochemical, 12–26 μm; 60 Å) with 1.5–6 bar pressure.† HPLC was performed on a Merck/Hitachi HPLC system with LiChrospher reversed-phase RP-18 columns (250 × 25 mm; 7 μm; flow rate 10 cm^3 min^{-1} for preparative separation) with buffer A (0.1% TFA in water) and buffer B (0.1% TFA in acetonitrile). FAB mass spectra were recorded on a double-focused VG-Analytical 70-250 S mass spectrometer with *m*-nitrobenzyl alcohol matrix. Optical rotations were recorded on a Perkin-Elmer Polarimeter 241, and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}.

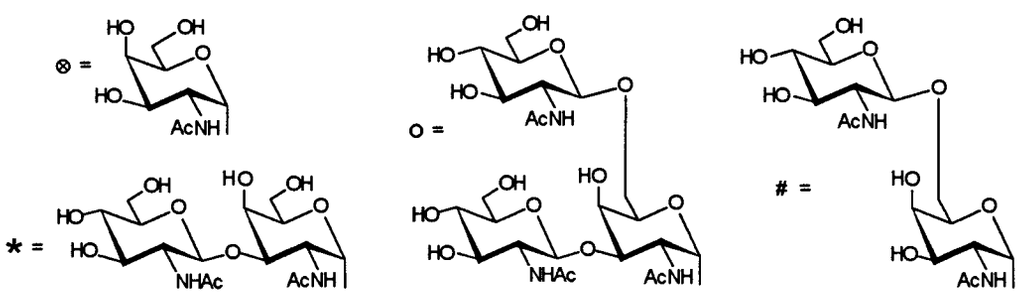
N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-L-threonine *tert*-butyl ester **1**

N-(Fluoren-9-ylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-threonine *tert*-butyl ester[17] (9.5 g, 13.3 mmol) was dissolved in methanol (200 cm^3). A solution of sodium methoxide (1 M in methanol; 1.5 cm^3) was carefully added in order to prevent cleavage of the Fmoc group. After 1 h (TLC chloroform–methanol, 10:1), the mixture was neutralised by addition of ion exchanger resin Amberlite CG-50I, filtered, and evaporated to give free triol **25**. A solution of compound **25** (7.75 g, 13.30 mmol), α,α-dimethoxytoluene (3.95 cm^3, 26.60 mmol) and toluene-4-sulfonic acid (PTSA) (630 mg) in nitromethane (130 cm^3) was stirred at room temperature. After 1 h (TLC toluene–acetone, 10:1), the mixture was neutralized with triethylamine and co-concentrated with toluene. The residue was chromatographed on a silica gel column with light petroleum–ethyl acetate (3:1) as eluent to give the *title compound* **1** (5.8 g, 65%), $[\alpha]_D$ +110 (*c* 1.07, $CHCl_3$);

† 1 bar = 10^5 Pa.

Table 2 Synthesized glycopeptides with Core 3, Core 4 and Core 6 structure. FAB-MS data.

No.	Sequence	M + 1/Da	M_{calc}/Da	No.	Sequence	M + 1/Da	M_{calc}/Da
	MUC 2						
60	TTTVTPTPTPG (★ on 1st P)	1381.8	1380.7	**76**	TTTVTPTPTPG (# on 3rd P)	1382.0	1380.7
61	TTTVTPTPTPG (★ on 2nd P)	1382.0	1380.7	**77**	TTTVTPTPTPG (# on 2nd P)	1382.0	1380.7
62	TTTVTPTPTPG (★ on 3rd P)	1382.0	1380.7	**78**	TTTVTPTPTPG (# on 3rd P)	1382.1	1380.7
63	TTTVTPTPTPG (★ on 1st P)	1382.2	1380.7	**79**	TTTVTPTPTPG (# on 1st P)	1382.0	1380.7
64	TTTVTPTPTPG (★ on 1st P)	1382.2	1380.7	**80**	TTTVTPTPTPG (# on 1st P)	1382.2	1380.7
65	TTTVTPTPTPG (★ on 1st P, ★ on 2nd P)	1788.0	1786.8	**81**	TTTVTPTPTPG (# on 1st P, # on 2nd P)	1787.8	1786.8
66	TTTVTPTPTPG (★ on 1st P, ⊗ on 2nd P)	1584.2	1583.7		**MUC 3**		
67	TTTVTPTPTPG (⊗ on 1st P, ★ on 2nd P)	1584.8	1583.7	**82**	TETTSHSTPG (★ on 9th P)	1423.3	1422.6
68	TTTVTPTPTPG (○ on 3rd P)	1584.8	1583.7	**83**	TETTSHSTPG (★ on 2nd P)	1423.8	1422.6
69	TTTVTPTPTPG (○ on 2nd P)	1585.0	1583.7	**84**	TETTSHSTPG (★ on 1st P)	1423.5	1422.6
70	TTTVTPTPTPG (○ on 3rd P)	1585.2	1583.7	**85**	TETTSHSTPG (○ on 9th P)	1627.1	1625.7
71	TTTVTPTPTPG (○ on 2nd P)	1585.2	1583.7	**86**	TETTSHSTPG (○ on 2nd P)	1626.8	1625.7
72	TTTVTPTPTPG (○ on 1st P)	1584.8	1583.7	**87**	TETTSHSTPG (○ on 1st P)	1627.0	1625.7
73	TTTVTPTPTPG (○ on 2nd P, ○ on 3rd P)	2194.2	2193.0	**88**	TETTSHSTPG (# on 9th P)	1424.1	1422.6
74	TTTVTPTPTPG (○ on 2nd P, # on 3rd P)	1991.3	1989.9	**89**	TETTSHSTPG (# on 2nd P)	1423.8	1422.6
75	TTTVTPTPTPG (# on 2nd P, ○ on 3rd P)	1991.4	1989.9	**90**	TETTSHSTPG (# on 1st P)	1424.1	1422.6



δ_H($CDCl_3$; $MeSi_4$) 7.82–7.25 (13 H, m, ArH), 5.75 (1 H, d, $J_{NH,CH\alpha}$ 9.1, NH), 5.57 (1 H, s, PhC*H*), 5.10 (1 H, d, $J_{1,2}$ 3.6, 1-H), 4.43 (2 H, m, Fmoc CH_2), 4.34 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.5, $J_{CH\beta,CH\gamma}$ 7.6, Thr CH^{β}), 4.30–4.22 (4 H, m, 4-H, 6-H_2 and Thr CH^{α}), 4.16 (1 H, ddd, $J_{4,5}$ 3.6, $J_{5,6a}$ 7.1, $J_{5,6b}$ 7.2, 5-H), 4.07 (1 H, dd, $J_{2,3}$ 10.7, 3-H), 3.78 (1 H, s, Fmoc CH), 3.57 (1 H, dd, 2-H), 2.51 (1 H, d, OH), 1.51 (9 H, s, Bu^t) and 1.31 (3 H, d, Thr C*H*$^{\gamma}$) (Found: C, 64.3; H, 6.1; N, 8.2. $C_{34}H_{40}N_4O_9$ requires C, 64.4; H, 6.0; N, 8.3%).

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-*O*-[*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-threonine *tert*-butyl ester 3

A mixture of compound **1** (2.77 g, 4.12 mmol), the imidate **2**[11] (2.76 g, 5.60 mmol) and activated powdered 4 Å molecular sieves in dry 1,2-dichloroethane (60 cm^3) was stirred at 0 °C under nitrogen. After 1 h, TMSOTf (80 mm^3, 0.44 mmol) was added. The mixture was stirred for 45 min at 0 °C (TLC toluene–acetone, 10:1), neutralized with triethylamine, filtered, and then concentrated. The residue was chromatographed on a silica gel column and eluted with light petroleum–ethyl acetate (2:1) to give *title compound* **3** (2.8 g, 68%), $[\alpha]_D$ +88.5 (*c* 1.03, $CHCl_3$); δ_H($CDCl_3$; $MeSi_4$) 7.80–7.28 (13 H, m, ArH), 5.74 (1 H, d, $J_{NH,CH\alpha}$ 9.2, NH), 5.56 (1 H, s, PhC*H*), 5.41 (1 H, d, $J_{3',4'}$ 3.1, 4′-H), 5.31 (1 H, dd, $J_{1',2'}$ 8.1, $J_{2',3'}$ 11.2, 2′-H), 5.13 (1 H, d, $J_{1,2}$ 3.6, 1-H), 5.04 (1 H, dd, 3′-H), 4.81 (1 H, d, 1′-H), 4.50 (1 H, ddd, $J_{4,5}$ 1.0, $J_{5,6a}$ 6.1, $J_{5,6b}$ 6.6, 5-H), 4.46 (1 H, dd, $J_{CH\alpha,CH\beta}$ 1.6, $J_{CH\beta,CH\gamma}$ 6.6, Thr CH^{β}), 4.41 (1 H, d, $J_{3,4}$ 2.5, 4-H), 4.35–4.23 (4 H, m, 6-H_2 and Fmoc CH_2), 4.21 (1 H, dd, 6′-H^a), 4.12 (1 H, dd, 6′-H^b), 4.08 (1 H, d, Thr CH^{α}), 4.05 (1 H, dd, $J_{2,3}$ 11.2, 3-H), 3.94 (1 H, m, 5′-H), 3.80 (1 H, dd, 2-H), 3.73 (1 H, s, Fmoc CH), 2.16, 2.06, 2.03 and 1.99 (12 H, 4 s, 4 × $COCH_3$), 1.50 (9 H, s, Bu^t) and 1.33 (3 H, d, Thr C*H*$^{\gamma}$) (Found: C, 60.0; H, 6.0; N, 5.3. $C_{50}H_{58}N_4O_{18}$ requires C, 59.9; H, 5.8; N, 5.6%).

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-*O*-[*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-azido-2-deoxy-α-D-galactopyranosyl]-L-threonine *tert*-butyl ester 4

A mixture of compound **3** (844 mg, 0.84 mmol), acetic acid (12 cm^3) and water (3 cm^3) was stirred at 80 °C for 2 h (TLC ethyl acetate–light petroleum, 3:1) then was cooled and co-concentrated with toluene. The residue was chromatographed on a silica gel column with ethyl acetate–light petroleum (3:1) as eluent to give *title compound* **4** (667 mg, 87%), $[\alpha]_D$ +66.0 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; $MeSi_4$) 7.81–7.28 (8 H, m, ArH), 5.70 (1 H, d, $J_{NH,CH\alpha}$ 9.7, NH), 5.41 (1 H, d, $J_{3',4'}$ 2.5, 4′-H), 5.30 (1 H, dd, $J_{1',2'}$ 7.6, $J_{2',3'}$ 10.2, 2′-H), 5.08 (1 H, d, $J_{1,2}$ 3.6, 1-H), 5.05 (1 H, dd, 3′-H), 4.75 (1 H, d, 1′-H), 4.47 (1 H, dd, $J_{CH\alpha,CH\beta}$ 1.5, $J_{CH\beta,CH\gamma}$ 6.1, Thr CH^{β}), 4.45 (1 H, s, Fmoc CH), 4.30 (1 H, d, Thr CH^{α}), 4.27 (2 H, ddd, $J_{CH,CH_2a} = J_{CH,CH_2b} = 7.1$, J_{CH_2a,CH_2b} 11.2, Fmoc CH_2), 4.21 (1 H, d, $J_{3,4}$ 2.5, 4-H), 4.18 (1 H, dd, $J_{5',6'a}$ 4.1, $J_{6'a,6'b}$ 11.7, 6′-H^a), 4.11 (1 H, dd, $J_{5',6'b}$ 5.2, 6′-H^b), 4.03 (1 H, dd, $J_{2,3}$ 10.7, 3-H), 3.98–3.89 (3 H, m, 5′-H and 6-H_2), 3.80 (1 H, m, 5-H), 3.58 (1 H, dd, 2-H), 2.21, 2.10, 2.05 and 2.00 (12 H, 4 s, 4 × $COCH_3$), 1.51 (9 H, s, Bu^t) and 1.26 (3 H, d, Thr C*H*$^{\gamma}$) (Found: C, 56.8; H, 6.1; N, 6.0. $C_{43}H_{54}N_4O_{18}$ requires C, 56.5; H, 6.0; N, 6.1%).

***N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-[*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl]-L-threonine *tert*-butyl ester 5**

Compound **4** (745 mg, 0.74 mmol) was dissolved in THF–acetic anhydride–acetic acid (3:2:1; 14 cm^3). Zinc powder, activated in 2% aq. copper sulfate, was added and the mixture was stirred at room temp. for 20 min (TLC ethyl acetate–light petroleum, 5:1). The solid was removed by filtration, and the solution was co-concentrated with toluene. To a solution of the residue in dry pyridine (10 cm^3) was added acetic anhydride (5 cm^3). After being stirred at room temp. for 2 h the solution was co-concentrated with toluene. The residue was chromatographed with ethyl acetate–light petroleum (3:1) as eluent to give *title compound* **5** (606 mg, 82%); $\delta_{\mathbf{H}}$($CDCl_3$; $MeSi_4$) 7.82–7.28 (8 H, m, ArH), 5.89 (1 H, d, $J_{\mathbf{2,NH}}$ 9.1, NH), 5.45 (1 H, d, $J_{\mathbf{NH,CH\alpha}}$ 9.7, NH Thr), 5.36 (2 H, d, $J_{\mathbf{3,4}}$ 3.0, 4- and 4′-H), 5.09 (1 H, dd, $J_{\mathbf{1',2'}}$ 7.6, $J_{\mathbf{2',3'}}$ 10.7, 2′-H), 4.93 (1 H, dd, 3′-H), 4.84 (1 H, d, $J_{\mathbf{1,2}}$ 3.6, 1-H), 4.54 (4 H, m, 1′-H and 2-H, Fmoc CH_2), 4.26 (2 H, m, 5-H and 6-H$^{\mathbf{a}}$), 4.19–4.10 (5 H, m, 6-H$^{\mathbf{b}}$, 6′-H_2 and Thr CH$^{\beta}$), 3.99 (1 H, dd, $J_{\mathbf{CH\alpha,CH\beta}}$ 2.1, Thr CH$^{\alpha}$), 3.87 (1 H, t, 5′-H), 3.78 (1 H, dd, $J_{\mathbf{2,3}}$ 10.2, 3-H), 2.16, 2.13, 2.06, 2.05, 2.04, 2.03 and 1.97 (21 H, 7 s, 7 × $COCH_3$), 1.46 (9 H, s, Bu^t) and 1.30 (3 H, d, $J_{\mathbf{CH\beta,CH\gamma}}$ 6.6, Thr CH$^{\gamma}$) (Found: C, 58.7; H, 6.4; N, 2.9. $C_{\mathbf{49}}H_{\mathbf{62}}N_2O_{\mathbf{20}}$ requires C, 58.9; H, 6.3; N, 2.8%).

***N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-[*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1′→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl]-L-threonine 6**

A solution of compound **5** (800 mg, 0.8 mmol) in TFA–water (95:5; 10 cm^3) was stirred at room temp. for 1 h (TLC ethyl acetate–light petroleum, 3:1). The solution was then co-concentrated with toluene to give *title compound* **6** (732 mg, 97%); $\delta_{\mathbf{H}}$($CDCl_3$, TFA 1%) 7.81–7.29 (8 H, m, ArH), 7.02 (1 H, d, $J_{\mathbf{2,NH}}$ 10.2, NH), 6.79 (1 H, d, $J_{\mathbf{NH,CH\alpha}}$ 9.7, NH Thr), 5.45 (1 H, d, $J_{\mathbf{1,2}}$ 3.1, 1-H), 5.39 (1 H, d, $J_{\mathbf{3',4'}}$ 3.0, 4′-H), 5.16 (1 H, d, $J_{\mathbf{3,4}}$ 3.0, 4-H), 5.09 (1 H, dd, $J_{\mathbf{1',2'}}$ 7.6, $J_{\mathbf{2',3'}}$ 10.1, 2′-H), 4.93 (1 H, dd, 3′-H), 4.65 (2 H, m, Fmoc CH_2), 4.54 (1 H, d, 1′-H), 4.36 (1 H, dd, $J_{\mathbf{CH\alpha,CH\beta}}$ 4.1, $J_{\mathbf{CH\beta,CH\gamma}}$ 6.6, Thr CH$^{\beta}$), 4.28–4.15 (4 H, m, 3-H, 6- and 6′-H$^{\mathbf{a}}$, Thr CH$^{\alpha}$), 4.16–4.01 (3 H, m, Fmoc CH, 6- and 6′-H$^{\mathbf{b}}$), 3.90 (1 H, dd, 2-H), 3.86 (1 H, t, 5-H), 3.82 (1 H, t, 5′-H), 2.17, 2.16, 2.10, 2.08, 2.05, 2.00 and 2.00 (21 H, 7 s, 7 × $COCH_3$) and 1.09 (3 H, d, Thr CH$^{\gamma}$) (Found: C, 57.14; H, 5.98; N, 3.03. $C_{\mathbf{45}}H_{\mathbf{54}}N_2O_{\mathbf{20}}$ requires C, 57.32; H, 5.77; N, 2.97%).

***N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-{*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1′→3)-*O*-[3″,4″,6″-tri-*O*-benzoyl-2″-deoxy-2″-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1″→6)]-2-azido-2-deoxy-α-D-galactopyranosyl}-L-threonine *tert*-butyl ester 8**

A mixture of disaccharide **4** (1.54 g, 1.68 mmol), imidate **7**[13] (1.77 g, 2.18 mmol) and activated powdered 4 Å molecular sieves in dry 1,2-dichloroethane (50 cm^3) was stirred at 0 °C under nitrogen. TMSOTf (40 mm^3, 0.22 mmol) was added. After 1 h (TLC ethyl acetate–light petroleum, 1:1) the mixture was neutralized by addition of triethylamine, filtered and concentrated. The residue was chromatographed with ethyl acetate–light petroleum (1:2) as eluent to give *title compound* **8** (2.0 g, 76%), $[\alpha]_{\mathbf{D}}$ +28.5 (*c* 1, $CHCl_3$); $\delta_{\mathbf{H}}$($CDCl_3$; $MeSi_4$) 8.04–7.29 (23 H, m, ArH), 5.78 (1 H, dd, $J_{\mathbf{2'',3''}}$ 10.2, $J_{\mathbf{3'',4''}}$ 9.1, 3″-H), 5.66 (1 H, d, $J_{\mathbf{NH,CH\alpha}}$ 9.7, N*H* Thr), 5.64 (1 H, dd, 4″-H), 5.56 (1 H, d, $J_{\mathbf{2'',NH}}$ 8.6, NH″), 5.40 (1 H, d, $J_{\mathbf{3',4'}}$ 3.0, 4′-H), 5.29 (1 H, dd, $J_{\mathbf{1',2'}}$ 7.1, $J_{\mathbf{2',3'}}$ 10.2, 2′-H), 5.04 (1 H, dd, 3′-H), 5.01 (1 H, d, $J_{\mathbf{1,2}}$ 3.6, 1-H), 4.90 (1 H, d, $J_{\mathbf{1'',2''}}$ 8.1, 1″-H), 4.77 (1 H, d, $J_{\mathbf{CH_2a,CH_2b}}$ 12.2, CH Teoc), 4.74 (1 H, d, 1′-H), 4.63 (1 H, dd, Fmoc CH), 4.51 (1 H, d, CH Teoc), 4.47 (2 H, m, Fmoc CH_2), 4.44 (1 H, dd, $J_{\mathbf{CH\alpha,CH\beta}}$ 2.8, $J_{\mathbf{CH\beta,CH\gamma}}$ 6.1, Thr CH$^{\beta}$), 4.28 (3 H, m, 6-H_2, Thr CH$^{\alpha}$), 4.19 (1 H, dd, 5″-H), 4.12 (1 H, d, $J_{\mathbf{3,4}}$ 3.0, 4-H), 4.10–4.02 (5 H, m, 5′-H, 6′- and 6″-H_2), 3.98 (1 H, dd, $J_{\mathbf{2,3}}$ 10.2, 3-H), 3.95 (1 H, dd, 2″-H), 3.87 (1 H, m, 5-H), 3.56 (1 H, dd, 2-H), 2.17, 2.11, 2.03 and 2.01 (12 H, 4 s, 4 × $COCH_3$), 1.51 (9 H, s, Bu^t) and 1.32 (3 H, d, Thr CH$^{\gamma}$) (Found: C, 55.9; H, 5.1; N, 4.7. $C_{\mathbf{73}}H_{\mathbf{78}}Cl_3N_5O_{\mathbf{27}}$ requires C, 56.1; H, 5.0; N, 4.5%).

***N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-{*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1′→3)-*O*-[2″-acetamido-3″,4″,6″-tri-*O*-benzoyl-2″-deoxy-β-D-glucopyranosyl-(1″→6)]-2-acetamido-4-*O*-acetyl-2-deoxy-α-D-galactopyranosyl}-L-threonine *tert*-butyl ester 9**

To a mixture of compound **8** (1.81 g, 1.15 mmol) in THF–acetic anhydride–acetic acid (3:2:1, 30 cm^3) was added zinc powder, activated in 2% aq. copper sulfate. The mixture was stirred at room temp. for 30 min (TLC toluene–acetone, 1:1), and was then filtered, and co-concentrated with toluene. To a solution of the residue in dry pyridine (20 cm^3) was added acetic anhydride (10 cm^3). After being stirred at room temp. for 10 h (TLC toluene–acetone, 1:1), the solution was co-concentrated with toluene. The residue was chromatographed with toluene–acetone (2:1) as eluent to give *title compound* **9** (1.24 g, 72%), $[\alpha]_{\mathbf{D}}$ +18.4 (*c* 1, $CHCl_3$); $\delta_{\mathbf{H}}$($CDCl_3$; $MeSi_4$) 8.04–7.17 (23 H, m, ArH), 5.97 (1 H, d, $J_{\mathbf{CH\alpha,NH}}$ 9.7, NH Thr), 5.77 (1 H, d, $J_{\mathbf{2'',NH}}$ 9.2, NH″), 5.72 (1 H, dd, $J_{\mathbf{2'',3''}}$ 10.2, $J_{\mathbf{3'',4''}}$ 9.7, 3″-H), 5.60 (1 H, d, $J_{\mathbf{2,NH}}$ 9.7, NH), 5.56 (1 H, dd, 4″-H), 5.34 (1 H, d, $J_{\mathbf{3',4'}}$ 2.5, 4′-H), 5.33 (1 H, d, $J_{\mathbf{3,4}}$ 3.0, 4-H), 5.09 (1 H, dd, $J_{\mathbf{1',2'}}$ 7.6, $J_{\mathbf{2',3'}}$ 10.2, 2′-H), 4.94 (1 H, dd, 3′-H), 4.85 (1 H, d, $J_{\mathbf{1,2}}$ 3.6, 1-H), 4.82 (1 H, d, $J_{\mathbf{1'',2''}}$ 7.6, 1″-H), 4.58 (1 H, m, Fmoc CH), 4.51 (1 H, d, 1′-H), 4.50 (1 H, dd, $J_{\mathbf{CH\alpha,CH\beta}}$ 2.4, Thr CH$^{\alpha}$), 4.43 (2 H, m, Fmoc CH_2), 4.24 (1 H, dd, $J_{\mathbf{CH\beta,CH\gamma}}$ 6.1, Thr CH$^{\beta}$), 4.20 (1 H, dd, 6′-H$^{\mathbf{a}}$), 4.14–4.05 (7 H, m, 2- and 2″-H, 6′-H$^{\mathbf{b}}$, and 6″- and 6-H_2), 3.95 (1 H, dd, 5-H), 3.84 (1 H, dd, $J_{\mathbf{2,3}}$ 10.7, 3-H), 3.80 (1 H, m, 5″-H), 3.56 (1 H, m, 5′-H), 2.14, 2.08, 2.07, 2.02, 1.99, 1.98 and 1.85 (21 H, 7 s, 7 × $COCH_3$), 1.47 (9 H, s, Bu^t) and 1.30 (3 H, d, Thr CH$^{\gamma}$) (Found: C, 61.6; H, 5.5; N, 2.8. $C_{\mathbf{76}}H_{\mathbf{85}}N_3O_{\mathbf{28}}$ requires C, 61.3; H, 5.8; N, 2.8%).

***N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-{*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1′→3)-*O*-[2″-acetamido-3″,4″,6″-tri-*O*-benzoyl-2″-deoxy-β-D-glucopyranosyl-(1″→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonine 10**

A solution of compound **9** (900 mg, 0.60 mmol) in TFA–water (95:5; 10 cm^3) was stirred at room temp. After 1 h (TLC chloroform–methanol, 20:1), the solution was co-concentrated with toluene to give *title compound* **10** (813 mg, 94%); $\delta_{\mathbf{H}}$($CDCl_3$; $MeSi_4$) 8.01–7.16 (23 H, m, ArH), 6.90 (1 H, d, $J_{\mathbf{CH\alpha,NH}}$ 9.7, NH Thr), 6.86 (1 H, d, $J_{\mathbf{2,NH}}$ 8.7, NH), 6.43 (1 H, d, $J_{\mathbf{2'',NH}}$ 8.6, NH″), 5.68 (1 H, dd, $J_{\mathbf{2'',3''}}$ 10.1, $J_{\mathbf{3'',4''}}$ 9.7, 3″-H), 5.66 (1 H, dd, 4″-H), 5.42 (1 H, d, $J_{\mathbf{3,4}}$ 3.0, 4-H), 5.38 (1 H, d, $J_{\mathbf{3',4'}}$ 2.6, 4′-H), 5.09 (1 H, dd, $J_{\mathbf{1',2'}}$ 7.6, $J_{\mathbf{2',3'}}$ 10.2, 2′-H), 4.93 (1 H, dd, 3′-H), 4.79 (1 H, d, $J_{\mathbf{1'',2''}}$ 8.1, 1″-H), 4.66 (1 H, dd, Fmoc CH), 4.56 (1 H, d, 1′-H), 4.55 (1 H, d, $J_{\mathbf{1,2}}$ 3.6, 1-H), 4.54 (1 H, dd, $J_{\mathbf{CH\alpha,CH\beta}}$ 2.6, Thr CH$^{\alpha}$), 4.44 (2 H, m, Fmoc CH_2), 4.35 (1 H, dd, $J_{\mathbf{CH\beta,CH\gamma}}$ 6.6, Thr CH$^{\beta}$), 4.25 (1 H, dd, 2″-H), 4.22 (2 H, m, 6″-H_2), 4.18–4.02 (5 H, m, 2-H, and 6′- and 6-H_2), 3.91 (1 H, dd, $J_{\mathbf{2,3}}$ 11.2, 3-H), 3.82 (2 H, m, 5- and 5′-H), 3.51 (1 H, m, 5″-H), 2.17, 2.13, 2.11, 2.06, 2.04, 2.03 and 1.98 (21 H, 7 s, 7 × $COCH_3$) and 1.19 (3 H, d, Thr CH$^{\gamma}$) (Found: C, 60.6; H, 5.7; N, 2.7. $C_{\mathbf{72}}H_{\mathbf{77}}N_3O_{\mathbf{28}}$ requires C, 60.4; H, 5.4; N, 2.9%).

***N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-{*O*-[3′,4′,6′-tri-*O*-benzoyl-2′-deoxy-2′-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-(1′→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl}-L-threonine *tert*-butyl ester 11**

A mixture of compound **1** (1.84 g, 2.74 mmol), imidate **7**[13] (2.60 g, 3.28 mmol) and activated powdered 4 Å molecular sieves in dry 1,2-dichloroethane (40 cm^3) was stirred at 0 °C under nitrogen. After 1 h, a solution of TMSOTf in dry dichloroethane (1 cm^3; 0.50 mmol) was added. The mixture was stirred for 45 min at 0 °C (TLC light petroleum–ethyl acetate, 1:1) then was neutralized with triethylamine, filtered and

concentrated. The residue was chromatographed on a silica gel column with light petroleum–ethyl acetate (5:2) as eluent to give *title compound* **11** (2.97 g, 82%), $[\alpha]_D$ +62.6 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; $MeSi_4$) 8.07–7.24 (28 H, m, ArH), 5.94 (1 H, t, $J_{2',3'}$ 10.2, 3′-H), 5.74 (1 H, d, $J_{NH,CH\alpha}$ 9.7, NH Thr), 5.62 (1 H, t, $J_{3',4'}$ 9.7, 4′-H), 5.43 (1 H, s, PhC*H*), 5.26 (1 H, d, $J_{NH,2'}$ 9.7, N*H* Teoc), 5.19 (1 H, d, $J_{1',2'}$ 7.6, 1′-H), 5.10 (1 H, d, $J_{1,2}$ 3.6, 1-H), 4.73 (1 H, dd, Fmoc CH), 4.65 (1 H, d, $J_{CHa,CHb}$ 12.2, CH Teoc), 4.48 (2 H, dd, Fmoc CH_2), 4.46 (1 H, m, 6-H^a), 4.45 (1 H, m, 5-H), 4.38 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.1, $J_{CH\beta,CH\gamma}$ 6.6, Thr CH$^\beta$), 4.35 (1 H, m, 6-H^b), 4.32 (1 H, dd, Thr CH$^\alpha$), 4.27 (1 H, d, $J_{3,4}$ 3.1, 4-H), 4.10 (1 H, m, 5′-H), 4.06 (1 H, dd, $J_{2,3}$ 11.7, 3-H), 3.91 (1 H, dd, 2′-H), 3.85 (1 H, dd, 2-H), 3.77 (2 H, m, 6′-H_2), 1.49 (9 H, s, Bu^t) and 1.27 (3 H, d, Thr CH$^\gamma$) (Found: C, 60.2; H, 5.0; N, 5.1. $C_{66}H_{64}Cl_3N_5O_{18}$ requires C, 60.0; H, 4.9; N, 5.3%).

N$^\alpha$-(Fluoren-9-ylmethoxycarbonyl)-*O*-{*O*-[3′,4′,6′-tri-*O*-benzoyl-2′-deoxy-2′-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-(1′→3)-2-azido-2-deoxy-α-D-galactopyranosyl}-L-threonine *tert*-butyl ester 12

A solution of compound **11** (2.97 g, 2.25 mmol) in a mixture of acetic acid (16 cm^3) and water (4 cm^3) was stirred at 80 °C. After 1 h (TLC ethyl acetate–light petroleum, 1:1), the solution was cooled, and co-concentrated with toluene. The residue was chromatographed on a silica gel column eluted with ethyl acetate–light petroleum (1:1) to give *title compound* **12** (2.28 g, 82%), $[\alpha]_D$ +39.4 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; $MeSi_4$) 8.07–7.31 (23 H, m, ArH), 5.89 (1 H, dd, $J_{2',3'}$ 10.2, $J_{3',4'}$ 9.7, 3′-H), 5.69 (1 H, d, $J_{NH,CH\alpha}$ 9.1, NH Thr), 5.58 (1 H, dd, $J_{4',5'}$ 10.2, 4′-H), 5.41 (1 H, d, $J_{NH,2'}$ 9.6, N*H* Teoc), 5.17 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 5.05 (1 H, d, $J_{1,2}$ 3.6, 1-H), 4.74 (1 H, dd, Fmoc CH), 4.69 (1 H, d, $J_{CHa,CHb}$ 12.2, CH Teoc), 4.54 (1 H, d, CH Teoc), 4.46–4.33 (3 H, m, Fmoc CH_2, Thr CH$^\beta$), 4.28 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.6, Thr CH$^\alpha$), 4.24 (1 H, d, $J_{3,4}$ 3.1, 4-H), 4.15–4.11 (3 H, m, 5′-H and 6′-H_2), 4.06 (1 H, dd, $J_{2,3}$ 10.7, 3-H), 3.89 (1 H, dd, 2′-H), 3.81 (1 H, m, 5-H), 3.74 (1 H, m, 6-H^a), 3.67 (1 H, dd, 2-H), 3.59 (1 H, m, 6-H^b), 2.85 (1 H, s, 4-OH), 2.39 (1 H, s, 6-OH), 1.50 (9 H, s, Bu^t) and 1.28 (3 H, d, $J_{CH\beta,CH\gamma}$ 6.6, Thr CH$^\gamma$) (Found: C, 57.3; H, 5.1; N, 5.6. $C_{59}H_{60}Cl_3N_5O_{18}$ requires C, 57.5; H, 4.9; N, 5.7%).

N$^\alpha$-(Fluoren-9-ylmethoxycarbonyl)-*O*-{*O*-[3′,4′,6′-tri-*O*-benzoyl-2′-deoxy-2′-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-(1′→3)-*O*-[3″,4″,6″-tri-*O*-benzoyl-2″-deoxy-2″-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-(1″→6)-2-azido-2-deoxy-α-D-galactopyranosyl}-L-threonine *tert*-butyl ester 14

A mixture of disaccharide **12** (2.20 g, 1.78 mmol), imidate **7**[13] (1.74 g, 2.14 mmol) and activated powdered 4 Å molecular sieves in dry 1,2-dichloroethane (40 cm^3) was stirred at 0 °C under nitrogen. After 1 h, TMSOTf (58 mm^3, 0.32 mmol) was added. After being stirred at 0 °C for 30 min (TLC light petroleum–ethyl acetate, 1:1), the solution was neutralized by addition of triethylamine, filtered, and concentrated. The residue was chromatographed on a silica gel column with light petroleum–ethyl acetate (2:1) as eluent to give *title compound* **14** (2.95 g, 88%), $[\alpha]_D$ +15.3 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; $MeSi_4$) 8.04–7.28 (38 H, m, ArH), 5.83 (1 H, dd, $J_{2',3'}$ 10.1, $J_{3',4'}$ 9.7, 3′-H), 5.78 (1 H, dd, $J_{2'',3''}$ 10.2, $J_{3'',4''}$ 9.7, 3″-H), 5.69 (1 H, d, $J_{2',NH}$ 9.6, NH′), 5.66 (1 H, dd, 4′-H), 5.59 (1 H, dd, 4″-H), 5.37 (1 H, d, $J_{2'',NH}$ 8.1, NH″), 5.14 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.98 (1 H, d, $J_{1,2}$ 3.5, 1-H), 4.87 (1 H, d, $J_{1'',2''}$ 8.1, 1″-H), 4.82 (1 H, d, $J_{CH\alpha,NH}$ 9.7, NH Thr), 4.70 (2 H, d, $J_{CHa,CHb}$ 12.2, CH Teoc), 4.64 (1 H, dd, Fmoc CH), 4.53 (2 H, d, CH Teoc), 4.48 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.5, Thr CH$^\alpha$), 4.42 (2 H, dd, Fmoc CH_2), 4.31–4.24 (3 H, m, 5″-H, 6′- and 6″-H^a and Thr CH$^\beta$), 4.16 (1 H, d, $J_{3,4}$ 3.0, 4-H), 4.11 (2 H, m, 6′- and 6″-H^b), 4.05 (1 H, m, 5′-H), 4.03 (1 H, dd, $J_{2,3}$ 10.7, 3-H), 3.94 (1 H, m, 5-H), 3.92–3.84 (4 H, m, 2′-, 2″-H and 6-H_2), 3.89 (1 H, dd, 2-H), 1.50 (9 H, s, Bu^t) and 1.26 (3 H, d, $J_{CH\beta,CH\gamma}$ 7.1, Thr CH$^\gamma$) (Found: C, 56.5; H, 4.3; N, 4.6. $C_{89}H_{84}Cl_6N_6O_{27}$ requires C, 56.8; H, 4.5; N, 4.5%).

N$^\alpha$-(Fluoren-9-ylmethoxycarbonyl)-*O*-{*O*-(2′-acetamido-3′,4′,6′-tri-*O*-benzoyl-2′-deoxy-β-D-glucopyranosyl)-(1′→3)-*O*-[(2″-acetamido-3″,4″,6″-tri-*O*-benzoyl-2″-deoxy-β-D-glucopyranosyl)-(1″→6)]-2-acetamido-4-*O*-acetyl-2-deoxy-α-D-galactopyranosyl}-L-threonine *tert*-butyl ester 15

Compound **14** (1.0 g, 0.53 mmol) was dissolved in THF–acetic anhydride–acetic acid (3:2:1; 10 cm^3). Zinc powder, activated in 2% aq. copper sulfate, was added and the mixture was stirred for 1 h at room temp. (TLC toluene–acetone, 1:2). The residue was acetylated as described for compound **9** and chromatographed on a silica gel column with elution with toluene–acetone (3:2) to give *title compound* **15** (530 mg, 60%), $[\alpha]_D$ +13.8 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; $MeSi_4$) 8.03–7.22 (38 H, m, ArH), 6.48 (1 H, d, $J_{CH\alpha,NH}$ 8.6, NH Thr), 6.03 (1 H, d, $J_{2',NH}$ 7.6, NH′), 5.93 (1 H, d, $J_{2'',NH}$ 8.6, NH″), 5.79 (2 H, dd, $J_{2',3'}$ 10.2, $J_{3',4'}$ 9.3, 3′- and 3″-H), 5.74 (1 H, d, $J_{2,NH}$ 9.1, NH), 5.59 (1 H, dd, 4″-H), 5.54 (1 H, dd, 4′-H), 5.45 (1 H, d, $J_{1,2}$ 3.1, 1-H), 5.12 (1 H, d, $J_{1',2'}$ 7.6, 1′-H), 4.88 (1 H, d, $J_{1'',2''}$ 8.1, 1″-H), 4.62 (1 H, dd, Fmoc CH), 4.52 (2 H, m, 6′- and 6″-H^a), 4.48 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.1, Thr CH$^\alpha$), 4.43 (2 H, m, 6′- and 6″-H^b), 4.24 (3 H, m, Fmoc CH_2, 5-H), 4.18 (1 H, dd, $J_{CH\beta,CH\gamma}$ 6.1, Thr CH$^\beta$), 4.10–3.98 (5 H, m, 4-H, 6-H^a, 5′-, 2″- and 5″-H), 3.92 (1 H, dd, $J_{2,3}$ 10.7, 2-H), 3.89 (1 H, dd, 6-H^b), 3.82 (1 H, dd, 2′-H), 3.55 (1 H, dd, 3-H), 2.06, 2.04, 1.90 and 1.84 (12 H, 4 s, 4 × $COCH_3$), 1.47 (9 H, s, Bu^t) and 1.22 (3 H, d, Thr CH$^\gamma$) (Found: C, 65.1; H, 5.8; N, 3.6. $C_{91}H_{92}N_4O_{27}$ requires C, 65.3; H, 5.5; N, 3.4%).

N$^\alpha$-(Fluoren-9-ylmethoxycarbonyl)-*O*-{*O*-(2′-acetamido-3′,4′,6′-tri-*O*-benzoyl-2′-deoxy-β-D-glucopyranosyl)-(1′→3)-*O*-[(2″-acetamido-3″,4″,6″-tri-*O*-benzoyl-2″-deoxy-β-D-glucopyranosyl)-(1″→6)]-2-acetamido-4-*O*-acetyl-2-deoxy-α-D-galactopyranosyl}-L-threonine 16

A solution of compound **15** (450 mg, 0.27 mmol) in TFA–water (95:5; 6 cm^3) was stirred at room temp. for 1 h (TLC chloroform–methanol, 19:1). The solution was then co-concentrated with toluene to give *title compound* **16** (422 mg, 96%); δ_H($CDCl_3$; TFA 1%) 7.99–7.14 (38 H, m, ArH), 6.98 (1 H, d, $J_{CH\alpha,NH}$ 9.7, NH Thr), 6.94 (1 H, d, $J_{2',NH}$ 9.2, NH′), 6.72 (1 H, d, $J_{2,NH}$ 9.2, NH), 6.61 (1 H, d, $J_{2'',NH}$ 9.1, NH″), 5.72 (2 H, dd, $J_{2',3'} = J_{2'',3''} = 10.2$, $J_{3',4'} = J_{3'',4''} = 9.7$, 3′- and 3″-H), 5.68 (1 H, dd, 4″-H), 5.66 (1 H, dd, 4′-H), 5.50 (1 H, d, $J_{3,4}$ 3.1, 4-H), 5.03 (1 H, d, $J_{1,2}$ 3.6, 1-H), 4.85 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.82 (1 H, d, $J_{1'',2''}$ 7.6, 1″-H), 4.67 (1 H, dd, Fmoc CH), 4.65–4.54 (2 H, m, 6′-H_2), 4.52 (2 H, m, 5′- and 5″-H), 4.46 (2 H, dd, Fmoc CH_2), 4.29–4.21 (4 H, m, 2- and 2′-H, 6″-H^a and Thr CH$^\beta$), 4.15 (1 H, dd, 2″-H), 4.11 (1 H, dd, 6″-H^b), 4.03 (1 H, dd, 6-H^a), 3.99 (1 H, dd, 6-H^b), 3.96 (1 H, dd, $J_{2,3}$ 10.7, 3-H), 3.92 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.7, Thr CH$^\alpha$), 3.49 (1 H, m, 5-H), 2.06, 2.04, 2.01 and 1.91 (12 H, 4 s, 4 × $COCH_3$) and 1.12 (3 H, d, $J_{CH\beta,CH\gamma}$ 6.1, Thr CH$^\gamma$) (Found: C, 65.0; H, 5.0; N, 3.6. $C_{87}H_{84}N_4O_{27}$ requires C, 64.6; H, 5.2; N, 3.5%).

Methyl *O*-(3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-phthalimido-β-D-glucopyranosyl)-(1′→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside 19

A mixture of compound **18**[9,14] (310 mg, 1 mmol), imidate **17** (700 mg, 1.2 mmol) and activated powdered 4 Å molecular sieves in dry 1,2-dichlorethane (10 cm^3) was stirred at −20 °C under nitrogen. A solution of TMSOTf in dry toluene (0.5 M; 0.3 cm^3) was added. After 2 h (TLC toluene–ethyl acetate, 2:1), the solution was neutralized with triethylamine, filtered and concentrated. The residue was chromatographed on a silica gel column and eluted with toluene–ethyl acetate (2:1) to give *title compound* **19** (610 mg, 84%), $[\alpha]_D$ −2.2 (*c* 1, $CHCl_3$); δ_H($CDCl_3$;

$MeSi_4$) 7.88–7.32 (9 H, m, ArH), 5.80 (1 H, dd, $J_{2',3'}$ 10.5, $J_{3',4'}$ 9.0, 3′-H), 5.60 (1 H, d, $J_{1',2'}$ 8.2, 1′-H), 5.54 (1 H, s, PhC*H*), 5.19 (1 H, dd, $J_{4',5'}$ 9.5, 4′-H), 4.42 (1 H, dd, 2′-H), 4.38 (1 H, dd, $J_{5',6'a}$ 2.5, $J_{6'a,6'b}$ 12.0, 6′-H[a]), 4.30 (1 H, dd, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 12.5, 6-H[a]), 4.24 (1 H, dd, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0, 4-H), 4.17 (1 H, dd, $J_{5',6'b}$ 4.5, 6′-H[b]), 4.09 (1 H, d, $J_{1,2}$ 8.0, 1-H), 4.05 (1 H, dd, $J_{5,6b}$ 2.0, 6-H[b]), 3.86 (1 H, m, 5′-H), 3.59 (1 H, dd, 2-H), 3.47 (3 H, s, OMe), 3.35 (1 H, dd, $J_{2,3}$ 10.5, 3-H), 3.33 (1 H, m, 5-H) and 2.05, 2.03 and 1.88 (9 H, 3 s, 3 × $COCH_3$) (Found: C, 56.4; H, 5.2. $C_{34}H_{36}N_4O_{14}$ requires C, 56.4; H, 5.0%).

Methyl *O*-(2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-(1′→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside 20

A solution of disaccharide **19** (600 mg, 0.8 mmol) in a mixture of hydrazine hydrate (6 cm[3]) and ethanol (13 cm[3]) was stirred at 70 °C for 15 min (TLC toluene–ethyl acetate, 2:1). The solution was then co-concentrated with ethanol. To a solution of the residue in dry pyridine (18 cm[3]) was added acetic anhydride (9 cm[3]). After being stirred at 90 °C for 20 min (TLC toluene–acetone, 1:1), the solution was co-concentrated with toluene. The residue was chromatographed on a silica gel column with toluene–acetone (2:1) as eluent to give *title compound* **20** (471 mg, 86%), $[\alpha]_D$ +21.0 (*c* 1, $CHCl_3$); δ_H($CDCl_3$) 7.55–7.22 (5 H, m, Ph), 6.60 (1 H, s, NH), 5.54 (1 H, s, PhC*H*), 5.41 (1 H, dd, $J_{2',3'}$ 10.2, $J_{3',4'}$ 9.2, 3′-H), 5.06 (1 H, d, $J_{1',2'}$ 8.6, 1′-H), 5.00 (1 H, dd, $J_{4',5'}$ 9.6, 4′-H), 4.29 (1 H, dd, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0, 4-H), 4.25 (1 H, dd, $J_{5',6'a}$ 2.6, $J_{6'a,6'b}$ 12.2, 6′-H[a]), 4.23 (1 H, m, 6-H[a]), 4.20 (1 H, d, $J_{1,2}$ 8.1, 1-H), 4.15 (1 H, dd, $J_{5',6'b}$ 4.6, 6′-H[b]), 4.08 (1 H, dd, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 12.7, 6-H[b]), 3.75 (1 H, m, 5′-H), 3.69 (1 H, dd, $J_{2,3}$ 10.7, 2-H), 3.66 (1 H, dd, 2′-H), 3.56 (3 H, s, OMe), 3.52 (1 H, dd, 3-H), 3.43 (1 H, m, 5-H) and 2.05, 2.04, 2.01 and 1.93 (12 H, 4 s, 4 × $COCH_3$) (Found: C, 53.1; H, 5.6. $C_{28}H_{36}N_4O_{13}$ requires C, 52.8; H, 5.7%).

***O*-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-(1′→3)-1,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranose 21**

A solution of methyl glycoside **20** (1.25 g, 1.82 mmol) in acetic anhydride (12 cm[3]) was stirred at 0 °C. A cold solution of acetic anhydride–sulfuric acid (50:1; 12 cm[3]) was added. After 15 h at 0 °C (TLC ethyl acetate–light petroleum, 4:1), the solution was diluted with cold dichloromethane and washed successively with aq. $NaHCO_3$ and water, dried (Na_2SO_4), and co-concentrated with toluene to give the 1-*O*-acetate **21** (1.17 g, 97%); δ_H($CDCl_3$) α-anomer 6.25 (1 H, d, $J_{1,2}$ 3.6, 1-H), 5.62 (1 H, d, $J_{2',NH}$ 8.6, NH), 5.50 (1 H, dd, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0, 4-H), 5.34 (1 H, dd, $J_{2',3'}$ 10.6, $J_{3',4'}$ 9.1, 3′-H), 5.10 (1 H, dd, $J_{4',5'}$ 9.7, 4′-H), 4.99 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.32 (1 H, dd, $J_{5',6'a}$ 2.6, $J_{6'a,6'b}$ 12.2, 6′-H[a]), 4.19–3.97 (3 H, m, 6-H_2 and 6′-H[b]), 3.93 (1 H, dd, $J_{2,3}$ 10.2, 2-H), 3.78 (1 H, dd, 2′-H), 3.73 (1 H, m, 5′-H), 3.67 (1 H, dd, 3-H), 3.57 (1 H, m, 5-H) and 2.16, 2.12, 2.10, 2.07, 2.03, 2.02 and 1.95 (21 H, 7 s, 7 × $COCH_3$).

***O*-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-(1′→3)-4,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide 22**

A solution of compound **21** (140 mg, 0.21 mmol) and titanium tetrabromide (118 mg, 0.3 mmol) in dry dichloromethane–ethyl acetate (10:1; 2.2 cm[3]) was stirred at room temp. for 14 h. The solution was diluted with dry toluene (5 cm[3]), and dry sodium acetate was added until the mixture became colourless. The mixture was filtered and concentrated to give the bromide **22** (130 mg, 91%); δ_H($CDCl_3$) 6.46 (1 H, d, $J_{1,2}$ 4.1, 1-H), 5.56 (1 H, d, $J_{2',NH}$ 6.6, NH), 5.54 (1 H, d, $J_{3,4}$ 3.6, 4-H), 5.23 (1 H, dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 9.7, 3′-H), 5.10 (1 H, dd, $J_{4',5'}$ 10.2, 4′-H), 4.86 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.39 (1 H, m, 5-H), 4.31 (1 H, dd, $J_{5',6'a}$ 2.0, $J_{6'a,6'b}$ 12.2, 6′-H[a]), 4.20 (1 H, dd, $J_{5,6a}$ 4.6, $J_{6a,6b}$ 11.7, 6-H[a]), 4.14 (1 H, dd, $J_{5',6'b}$ 4.6, 6′-H[b]), 4.13 (1 H, dd, $J_{2,3}$ 10.2, 3-H), 4.03 (1 H, dd, $J_{5,6b}$ 7.6, 6-H[b]), 3.94 (1 H, dd, 2-H), 3.90 (1 H, dd, 2′-H), 3.71 (1 H, m, 5′-H) and 2.15, 2.12, 2.07, 2.04, 2.03 and 1.93 (18 H, 6 s, 6 × $COCH_3$).

***N*[α]-(Fluoren-9-ylmethoxycarbonyl)-*O*-[*O*-(2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-(1′→3)-4,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl]-L-threonine pentafluorophenyl ester 23**

A mixture of Fmoc-Thr-OPfp (188 mg, 0.37 mmol), activated powdered 4 Å molecular sieves and silver trifluoromethanesulfonate (226 mg, 0.88 mmol) in dry dichloromethane (10 cm[3]) was stirred at room temp. under nitrogen. After 1 h, the mixture was cooled at −20 °C and a solution of the bromide **22** (300 mg, 0.44 mmol) in dry dichloromethane was added. The mixture was stirred for 14 h at −20 °C (TLC toluene–acetone, 2:1) and was then filtered. The solution was washed successively with aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on a silica gel column with toluene–acetone (3:1) as eluent to give *title compound* **23** (197 mg, 48%), $[\alpha]_D$ +25.2 (*c* 1, $CHCl_3$); δ_H($CDCl_3$) 7.74–7.12 (8 H, m, ArH), 5.88 (1 H, d, $J_{CH\alpha,NH}$ 9.1, NH), 5.53 (1 H, d, $J_{2',NH}$ 8.7, NH′), 5.47 (1 H, d, $J_{3,4}$ 2.5, 4-H), 5.28 (1 H, dd, $J_{2',3'}$ 9.7, $J_{3',4'}$ 10.2, 3′-H), 5.10 (1 H, d, $J_{1,2}$ 4.6, 1-H), 5.09 (1 H, dd, $J_{4',5'}$ 9.2, 4′-H), 4.92 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.75 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.0, Thr CH[α]), 4.58 (1 H, dd, J_{CH,CH_2a} 7.1, J_{CH_2a,CH_2b} 10.7, Fmoc CH_2[a]), 4.54 (1 H, dd, $J_{CH\beta,CH\gamma}$ 6.1, Thr CH[β]), 4.45 (1 H, dd, J_{CH,CH_2b} 7.1, Fmoc CH_2[b]), 4.29 (2 H, m, Fmoc CH, 6′-H[a]), 4.17 (3 H, m, 6-H_2 and 6′-H[b]), 4.03 (1 H, dd, $J_{2,3}$ 10.1, 3-H), 3.99 (1 H, m, 5-H), 3.80 (1 H, dd, 2′-H), 3.76 (1 H, dd, 2-H), 3.69 (1 H, m, 5′-H), 2.13, 2.10, 2.06, 2.03, 2.02 and 1.90 (18 H, 6 s, 6 × $COCH_3$) and 1.42 (3 H, d, Thr CH[γ]) (Found: C, 53.0; H, 4.8. $C_{49}H_{50}F_5N_5O_{19}$ requires C, 53.1; H, 4.6%).

***N*[α]-(Fluoren-9-ylmethoxycarbonyl)-*O*-[*O*-(2′-acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-(1′→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl]-L-threonine pentafluorophenyl ester 24**

Compound **23** (292 mg, 0.26 mmol) was dissolved in THF–acetic anhydride–acetic acid (3:2:1; 10 cm[3]). Zinc powder, activated in 2% aq. copper sulfate, was added and the mixture was stirred at room temp. for 10 min (TLC toluene–acetone, 1:1). The mixture was filtered and co-concentrated with toluene. The residue was chromatographed on a silica gel column eluted with toluene–acetone (2:1) to give *title compound* **24** (213 mg, 73%), $[\alpha]_D$ +51.0 (*c* 1, $CHCl_3$); δ_H($CDCl_3$) 7.78–7.29 (8 H, m, ArH), 6.18 (1 H, d, $J_{CH\alpha,NH}$ 9.1, NH), 6.08 (1 H, d, $J_{2,NH}$ 8.6, NH), 5.81 (1 H, d, $J_{2',NH'}$ 8.1, NH′), 5.37 (1 H, dd, $J_{2',3'}$ 9.1, $J_{3',4'}$ 10.7, 3′-H), 5.34 (1 H, d, $J_{3,4}$ 2.6, 4-H), 5.05 (1 H, dd, $J_{4',5'}$ 9.7, 4′-H), 5.03 (1 H, d, $J_{1,2}$ 4.1, 1-H), 4.92 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.70 (1 H, dd, $J_{CH\alpha,CH\beta}$ 1.6, Thr CH[α]), 4.57 (2 H, ddd, J_{CH,CH_2a} = J_{CH,CH_2b} = 6.1, J_{CH_2a,CH_2b} 11.8, Fmoc CH_2), 4.46–4.34 (3 H, m, Thr CH[β], 2- and 5′-H), 4.27 (1 H, t, Fmoc CH), 4.13 (2 H, m, 6′-H_2), 4.07–3.99 (2 H, m, 6-H_2), 3.90 (1 H, dd, $J_{2,3}$ 9.7, 3-H), 3.70 (1 H, m, 5-H), 3.54 (1 H, dd, 2′-H), 2.11, 2.10, 2.08, 2.06, 1.92, 1.85 and 1.77 (21 H, 7 s, 7 × $COCH_3$) and 1.40 (3 H, d, $J_{CH\beta,CH\gamma}$ 6.1, Thr CH[γ]) (Found: C, 54.7; H, 5.0. $C_{51}H_{54}F_5N_3O_{20}$ requires C, 54.5; H, 4.8%).

***N*[α]-(Fluoren-9-ylmethoxycarbonyl)-*O*-(2-azido-2-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-L-threonine *tert*-butyl ester 26**

A solution of compound **25** (800 mg, 1.37 mmol) prepared as previously described[11] with compound **1** and PTSA (11 mg) in 2,2-dimethoxypropane (14 cm[3]) was stirred at room temp. for 12 h (TLC ethyl acetate–light petroleum, 1:1). The solution was then neutralized by addition of triethylamine, and co-concentrated with toluene. A solution of the residue in methanol–water (10:1; 11 cm[3]) was stirred at 80 °C. After 1 h (TLC ethyl acetate–light petroleum, 1:1) the solution was co-concentrated with toluene. The residue was chromatographed

on a silica gel column with ethyl acetate–light petroleum (1:2) as eluent to give *title compound* **26** (590 mg, 69%), $[\alpha]_D$ +99.5 (*c* 1, $CHCl_3$); δ_H($CDCl_3$) 7.79–7.13 (8 H, m, ArH), 5.63 (1 H, d, $J_{NH,CH\alpha}$ 9.1, NH), 5.02 (1 H, d, $J_{1,2}$ 3.6, 1-H), 4.44 (1 H, dd, J_{CH,CH_2a} 3.0, J_{CH,CH_2b} 10.7, Fmoc CH), 4.41 (1 H, dd, $J_{2,3}$ 8.1, $J_{3,4}$ 2.9, 3-H), 4.39 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.0, $J_{CH\beta,CH\gamma}$ 6.6, Thr CH^{β}), 4.33 (1 H, dd, J_{CH_2a,CH_2b} 7.6, Fmoc CH_2[a]), 4.29 (1 H, dd, Thr CH^{α}), 4.26 (1 H, dd, Fmoc CH_2[b]), 4.24 (1 H, dd, 4-H), 4.15 (1 H, m, 5-H), 3.93 (1 H, dd, $J_{5,6a}$ 6.6, $J_{6a,6b}$ 11.7, 6-H[a]), 3.84 (1 H, dd, $J_{5,6b}$ 4.1, 6-H[b]), 3.41 (1 H, dd, 2-H), 2.48 (1 H, d, OH), 1.52 (3 H, s, CH_3), 1.49 (9 H, s, Bu^t), 1.47 (3 H, s, CH_3) and 1.33 (3 H, d, Thr CH^{γ}) (Found: C, 61.7; H, 6.7; N, 9.1. $C_{32}H_{40}N_4O_9$ requires C, 61.5; H, 6.5; N, 9.0%).

N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-{*O*-[3′,4′,6′-tri-*O*-benzoyl-2′-deoxy-2′-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-(1′→6)-2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl}-L-threonine *tert*-butyl ester 27

A mixture of compound **26** (660 mg, 1.08 mmol), imidate **7** (1.03 g, 1.26 mmol) and activated powdered 4 Å molecular sieves in dry 1,2-dichloroethane (15 cm^3) was stirred at room temp. under nitrogen. After 1 h, TMSOTf (36 mm^3, 0.19 mmol) was added. After being stirred at room temp. for 25 min (TLC light petroleum–ethyl acetate, 2:1), the solution was neutralized by addition of triethylamine, filtered, and concentrated. The residue was chromatographed on a silica gel column eluted with light petroleum–ethyl acetate (3:1) to give *title compound* **27** (1.2 g, 81%), $[\alpha]_D$ +30.4 (*c* 1, $CHCl_3$); δ_H($CDCl_3$) 8.02–7.25 (23 H, m, ArH), 5.79 (1 H, dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 9.7, 3′-H), 5.62 (1 H, d, $J_{NH,2'}$ 9.1, NH Teoc), 5.60 (1 H, dd, 4′-H), 4.97 (1 H, d, $J_{1,2}$ 3.1, 1-H), 4.92 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.81 (1 H, d, $J_{NH,CH\alpha}$ 9.0, NH Thr), 4.59 (1 H, dd, J_{CH,CH_2a} 4.1, J_{CH,CH_2b} 12.2, Fmoc CH), 4.53 (1 H, d, $J_{CHa,CHb}$ 12.2, CH[a] Teoc), 4.51 (1 H, d, CH[b] Teoc), 4.46 (1 H, d, $J_{3,4}$ 3.0, 4-H), 4.39 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.0, $J_{CH\beta,CH\gamma}$ 6.6, Thr CH^{β}), 4.34–4.19 (4 H, m, 3-H, Thr CH^{α} and Fmoc CH_2), 4.10 (4 H, m, 6- and 6′-H_2), 3.92 (1 H, dd, 2′-H), 3.90 (1 H, m, 5′-H), 3.88 (1 H, m, 5-H), 3.36 (1 H, dd, $J_{2,3}$ 8.1, 2-H), 1.51 (9 H, s, Bu^t), 1.47 (6 H, s, 2 × CH_3) and 1.34 (3 H, d, Thr CH^{γ}) (Found: C, 58.2; H, 5.2; N, 5.6. $C_{62}H_{64}Cl_3N_5O_{18}$ requires C, 58.5; H, 5.1; N, 5.4%).

N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-{*O*-[3′,4′,6′-tri-*O*-benzoyl-2′-deoxy-2′-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-(1′→6)-3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl}-L-threonine *tert*-butyl ester 28

A solution of disaccharide **27** (680 mg, 0.52 mmol) in a mixture of acetic acid (16 cm^3) and water (4 cm^3) was stirred at 80 °C. After 30 min (TLC light petroleum–ethyl acetate, 1:1), the solution was co-concentrated with toluene. To a solution of the residue in dry pyridine (3 cm^3) was added anhydride acetic (1.5 cm^3). After 1 h (TLC light petroleum–ethyl acetate, 2:1), the solution was co-concentrated with toluene. The residue was chromatographed on a silica gel column and eluted with light petroleum–ethyl acetate (2:1) to give *title compound* **28** (612 mg, 87%), $[\alpha]_D$ +28.6 (*c* 1, $CHCl_3$); δ_H($CDCl_3$) 8.02–7.28 (23 H, m, ArH), 5.84 (1 H, dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 9.7, 3′-H), 5.65 (1 H, d, $J_{NH,CH\alpha}$ 9.7, NH Thr), 5.58 (1 H, dd, 4′-H), 5.47 (1 H, d, $J_{3,4}$ 3.0, 4-H), 5.43 (1 H, d, $J_{NH,2'}$ 9.1, NH Teoc), 5.35 (1 H, dd, $J_{2,3}$ 11.2, 3-H), 5.09 (1 H, d, $J_{1,2}$ 3.1, 1-H), 4.92 (1 H, d, $J_{1',2'}$ 7.6, 1′-H), 4.60 (1 H, dd, J_{CH,CH_2a} 3.0, J_{CH,CH_2b} 12.2, Fmoc CH), 4.52 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.0, Thr CH^{α}), 4.45 (1 H, d, $J_{CHa,CHb}$ 12.2, CH[a] Teoc), 4.43 (1 H, dd, $J_{CH\beta,CH\gamma}$ 6.6, Thr CH^{β}), 4.39 (2 H, m, Fmoc CH_2), 4.35 (1 H, d, CH[b] Teoc), 4.30–4.22 (3 H, m, 5-H and 6′-H_2), 4.07 (1 H, m, 5′-H), 3.89 (1 H, dd, 6-H[a]), 3.78 (1 H, dd, 2′-H), 3.66 (1 H, dd, 6-H[b]), 3.60 (1 H, dd, 2-H), 2.07 and 2.06 (6 H, 2 s, 2 × $COCH_3$), 1.50 (9 H, s, Bu^t), 1.33 (3 H, d, Thr CH^{γ}) (Found: C, 57.0; H, 5.0; N, 5.4. $C_{63}H_{64}Cl_3N_5O_{20}$ requires C, 57.4; H, 4.9; N, 5.3%).

N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-[*O*-(2′-acetamido-3′,4′,6′-tri-*O*-benzoyl-2′-deoxy-β-D-glucopyranosyl)-(1′→6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl]-L-threonine *tert*-butyl ester 29

To a solution of compound **28** (450 mg, 0.34 mmol) in THF–acetic anhydride–acetic acid (3:2:1; 10 cm^3) was added zinc powder, activated in 2% aq. copper sulfate. The mixture was stirred at room temp. for 30 min (TLC toluene–acetone, 2:1) and was then filtered and co-concentrated with toluene. The residue was chromatographed on a silica gel column eluted with toluene–acetone (2:1) to give *title compound* **29** (305 mg, 73%), $[\alpha]_D$ +18.6 (*c* 1, $CHCl_3$); δ_H($CDCl_3$) 8.02–7.28 (23 H, m, ArH), 6.08 (1 H, d, $J_{NH,CH\alpha}$ 10.2, NHThr), 5.83 (1 H, d, $J_{NH,2}$ 9.6, NH), 5.81 (1 H, d, $J_{NH',2'}$ 10.2, NH′), 5.76 (1 H, dd, $J_{2',3'}$ 11.2, $J_{3',4'}$ 8.7, 3′-H), 5.56 (1 H, dd, 4′-H), 5.35 (1 H, d, $J_{3,4}$ 2.6, 4-H), 5.08 (1 H, dd, $J_{2,3}$ 10.7, 3-H), 5.01 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.88 (1 H, d, $J_{1,2}$ 2.5, 1-H), 4.54 (3 H, m, Thr-CH^{α}, 2- and 6′-H[a]), 4.46 (2 H, m, 6′-H[b] and Fmoc-CH), 4.25 (1 H, m, 5-H), 4.23 (2 H, m, Fmoc CH_2), 4.20 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.0, $J_{CH\beta,CH\gamma}$ 6.6, Thr CH^{β}), 4.08 (1 H, m, 5′-H), 3.90 (1 H, dd, 6-H[a]), 3.86 (1 H, dd, 2′-H), 3.65 (1 H, dd, 6-H[b]), 2.10, 1.99, 1.98 and 1.87 (12 H, 4 s, 4 × $COCH_3$), 1.47 (9 H, s, Bu^t) and 1.32 (3 H, d, Thr CH^{γ}) (Found: C, 64.2; H, 5.9; N, 3.5. $C_{64}H_{69}N_3O_{20}$ requires C, 64.0; H, 5.8; N, 3.5%).

N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-[*O*-(2′-acetamido-3′,4′,6′-tri-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl]-L-threonine 30

A solution of ester **29** (453 mg, 0.38 mmol) in TFA–water (95:5; 4 cm^3) was stirred at room temp. for 1 h (TLC dichloromethane–methanol, 20:1). The solution was then co-concentrated with toluene to give *title compound* **30** (414 mg, 96%); δ_H($CDCl_3$) 8.01–7.12 (23 H, m, ArH), 6.29 (1 H, d, $J_{NH,CH\alpha}$ 9.6, NH Thr), 6.12 (1 H, d, $J_{NH,2'}$ 8.7, NH′), 6.07 (1 H, d, $J_{NH,2}$ 9.1, NH), 5.91 (1 H, dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 9.8, 3′-H), 5.58 (1 H, dd, $J_{4',5'}$ 10.2, 4′-H), 5.35 (1 H, d, $J_{3,4}$ 2.6, 4-H), 5.12 (1 H, dd, $J_{2,3}$ 9.2, 3-H), 5.09 (1 H, d, $J_{1',2'}$ 7.6, 1′-H), 5.00 (1 H, d, $J_{1,2}$ 3.1, 1-H), 4.56 (1 H, dd, Fmoc CH), 4.49 (1 H, dd, 2-H), 4.48–4.36 (4 H, m, Thr CH^{α}, Thr CH^{β}, Fmoc CH_2), 4.22 (2 H, m, 5- and 5′-H), 4.15 (1 H, m, 6′-H[a]), 4.07 (1 H, m, 6′-H[b]), 3.90 (1 H, dd, 2′-H), 3.87 (1 H, dd, 6-H[a]), 3.62 (1 H, dd, 6-H[b]), 2.08, 1.99, 1.93 and 1.89 (12 H, 4 s, 4 × $COCH_3$) and 1.31 (3 H, d, $J_{CH\beta,CH\gamma}$ 6.6, Thr CH^{γ}) (Found: C, 63.1; H, 5.3; N, 3.5. $C_{60}H_{61}N_3O_{20}$ requires C, 63.0; H, 5.4; N, 3.7%).

Synthesis of glycopeptides 31–90 on a manual 20-column peptide synthesizer

The Wang resin (6.0 g) was placed in a glass reactor and swelled in dichloromethane (15 cm^3; 10 min). After washing of the resin with dichloromethane, a mixture of Fmoc-Gly-OH (2.4 g, 8.1 mmol), 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole (2.4 g, 8.1 mmol) and methylimidazole (504 mm^3, 5.4 mmol) in dichloromethane (15 cm^3) was added. After 2 h, the resin was washed successively with dichloromethane and DMF (3×) and the unchanged amino groups were acetylated with Ac_2O–DMF (1:7; 15 cm^3). The resin was washed successively with dichloromethane and DMF (6×) and then dried.

The derivatized resin was weighed out and packed in the 20 columns of the manual synthesizer.[15] All reagents were removed by washing of the resins with DMF (10×). The Fmoc deprotections were performed by treatment with piperidine (20%) in DMF (20 min). Each Fmoc amino acid Pfp ester and Dhbt-OH (3 mol equiv., 1.5 mol equiv. for building block **24**) and the building blocks **6**, **10**, **16** and **30**, TBTU and *N*-ethyldiisopropylamine (1.5 mol equiv.) were dissolved in DMF (0.75 cm^3) and the solutions were transferred to the respective wells. After 10–18 h, the reaction mixtures were removed and the wells were washed with DMF. The synthesis cycle was repeated to complete the assembly of each glycopeptide **31**–**90** by using the appropriate amino acids. After removal of the last Fmoc

Table 3 Synthesized glycopeptides

Substance	Formula	Yield [mg (%)]	Substance	Formula	Yield [mg (%)]
31	$C_{49}H_{83}N_{11}O_{22}$	4.8 (41)	**61**	$C_{57}H_{96}N_{12}O_{27}$	4.1 (16)
32	$C_{49}H_{83}N_{11}O_{22}$	3.8 (19)	**62**	$C_{57}H_{96}N_{12}O_{27}$	11.0 (36)
33	$C_{49}H_{83}N_{11}O_{22}$	2.6 (19)	**63**	$C_{57}H_{96}N_{12}O_{27}$	7.0 (28)
34	$C_{49}H_{83}N_{11}O_{22}$	3.8 (21)	**64**	$C_{57}H_{96}N_{12}O_{27}$	7.8 (33)
35	$C_{49}H_{83}N_{11}O_{22}$	4.3 (24)	**65**	$C_{73}H_{122}N_{14}O_{37}$	12.1 (44)
36	$C_{57}H_{96}N_{12}O_{27}$	5.6 (31)	**66**	$C_{65}H_{109}N_{13}O_{32}$	9.0 (25)
37	$C_{55}H_{93}N_{11}O_{27}$	6.6 (37)	**67**	$C_{65}H_{109}N_{13}O_{32}$	7.9 (24)
38	$C_{55}H_{93}N_{11}O_{27}$	8.4 (41)	**68**	$C_{65}H_{109}N_{13}O_{32}$	7.6 (30)
39	$C_{55}H_{93}N_{11}O_{27}$	9.3 (43)	**69**	$C_{65}H_{109}N_{13}O_{32}$	9.4 (37)
40	$C_{55}H_{93}N_{11}O_{27}$	6.6 (38)	**70**	$C_{65}H_{109}N_{13}O_{32}$	10.1 (39)
41	$C_{55}H_{93}N_{11}O_{27}$	7.5 (41)	**71**	$C_{65}H_{109}N_{13}O_{32}$	8.3 (30)
42	$C_{69}H_{116}N_{12}O_{37}$	10.0 (34)	**72**	$C_{65}H_{109}N_{13}O_{32}$	11.2 (43)
43	$C_{63}H_{106}N_{12}O_{32}$	8.2 (52)	**73**	$C_{89}H_{148}N_{16}O_{47}$	10.7 (26)
44	$C_{63}H_{106}N_{12}O_{32}$	7.8 (50)	**74**	$C_{81}H_{135}N_{15}O_{42}$	8.8 (31)
45	$C_{63}H_{106}N_{12}O_{32}$	6.5 (48)	**75**	$C_{81}H_{135}N_{15}O_{42}$	9.7 (37)
46	$C_{63}H_{106}N_{12}O_{32}$	6.8 (41)	**76**	$C_{57}H_{96}N_{12}O_{27}$	7.5 (41)
47	$C_{63}H_{106}N_{12}O_{32}$	9.8 (59)	**77**	$C_{57}H_{96}N_{12}O_{27}$	8.4 (45)
48	$C_{85}H_{142}N_{14}O_{47}$	14.2 (53)	**78**	$C_{57}H_{96}N_{12}O_{27}$	6.6 (37)
49	$C_{77}H_{129}N_{13}O_{42}$	12.1 (43)	**79**	$C_{57}H_{96}N_{12}O_{27}$	7.8 (43)
50	$C_{77}H_{129}N_{13}O_{42}$	8.6 (34)	**80**	$C_{57}H_{96}N_{12}O_{27}$	5.7 (31)
51	$C_{48}H_{77}N_{10}O_{24}$	4.6 (19)	**81**	$C_{73}H_{112}N_{14}O_{37}$	8.1 (41)
52	$C_{48}H_{77}N_{10}O_{24}$	7.2 (31)	**82**	$C_{56}H_{90}N_{14}O_{29}$	2.3 (9)
53	$C_{48}H_{77}N_{10}O_{24}$	4.2 (17)	**83**	$C_{56}H_{90}N_{14}O_{29}$	3.4 (14)
54	$C_{54}H_{87}N_{13}O_{29}$	5.6 (38)	**84**	$C_{56}H_{90}N_{14}O_{29}$	7.8 (29)
55	$C_{54}H_{87}N_{13}O_{29}$	6.1 (43)	**85**	$C_{64}H_{103}N_{15}O_{34}$	8.1 (52)
56	$C_{54}H_{87}N_{13}O_{29}$	7.8 (44)	**86**	$C_{64}H_{103}N_{5}O_{34}$	6.4 (40)
57	$C_{62}H_{100}N_{14}O_{34}$	6.8 (35)	**87**	$C_{64}H_{103}N_{5}O_{34}$	7.7 (48)
58	$C_{62}H_{100}N_{14}O_{34}$	4.3 (22)	**88**	$C_{56}H_{90}N_{14}O_{29}$	5.6 (43)
59	$C_{62}H_{100}N_{14}O_{34}$	4.8 (31)	**89**	$C_{56}H_{90}N_{14}O_{29}$	4.8 (40)
60	$C_{57}H_{96}N_{12}O_{27}$	9.4 (33)	**90**	$C_{56}H_{90}N_{14}O_{29}$	6.0 (43)

groups, the resins were washed successively with DMF (8×) and dichloromethane (5×), dried, and transferred to Eppendorf tubes.

The resins were treated with 95% aq. TFA (2 cm^3) for 2 h at room temp., were then filtered off and washed with TFA (1 cm^3, 3×). The solutions were concentrated, and co-distilled first with toluene and then with toluene–methanol (3:1). The residues were dissolved in methanol (1 cm^3) and a solution of 1% methanolic sodium methoxide was added until pH 7–8. The reaction mixtures were stirred at room temp. for 6–9 h. The solutions were neutralized with acetic acid, filtered, evaporated and purified by preparative RP-HPLC [buffer A–buffer B 95:5→85:5 (20 min)→50:50 (30 min)]. The yields of the substances are given in Table 3. All glycopeptides **31**–**90** were characterized by 1D and 2D ^{1}H NMR spectroscopy, and the ^{1}H NMR data are available as supplementary material on request.

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