Synthesis of the building blocks N^{α}-Fmoc-*O*-[α-D-Ac_3GalN_3p-(1→3)-α-D-Ac_2GalN_3p]-Thr-OPfp and N^{α}-Fmoc-*O*-[α-D-Ac_3GalN_3p-(1→6)-α-D-Ac_2GalN_3p]-Thr-OPfp and their application in the solid phase glycopeptide synthesis of core 5 and core 7 mucin *O*-glycopeptides

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The structures α-D-GalNAc-(1→3)-α-D-GalNAc-(1→O)-L-Thr and α-D-GalNAc-(1→6)-α-D-GalNAc-(1→O)-L-Thr are present as cores 5 and 7 of the mucin-type glycoproteins. The preparation of the corresponding building blocks **10** and **15** is described. Compounds **10** and **15** can be used directly in a multiple-column solid-phase glycopeptide peptide synthesis. In the resulting peptides the azido groups are reduced and acetylated on the solid support. The products are cleaved from the resin and the carbohydrate part is deprotected. A series of 20 *O*-glycopeptides has been prepared.

The N-linked and O-linked glycans of the glycoproteins have been found to play essential roles in the development and maintenance of the living cell.[1,2] They are involved in, *e.g.*, protein sorting and targeting,[3] cell–cell recognition,[4] control of growth and development,[5] and immunoregulatory functions.

The heterogeneity and often low abundance of the glycoproteins lead to difficulties in the isolation and characterization of the homogeneous glycans. Thus, synthesis of well defined glycopeptide fragments remains a valuable alternative in the study of the structure and function of the glycosylation in glycoproteins.

The mucin glycoproteins are widely distributed in the living organism and a high degree of structural heterogeneity has been detected.[6] This is due to the stepwise assembly of the structures by a range of glycosyl transferases which are present at various concentrations.[7] Therefore a number of different core structures have been identified. Among them, two disaccharides, α-D-GalNAc-(1→3)-α-D-GalNAc and α-D-GalNAc-(1→6)-α-D-GalNAc, have been detected in human meconium,[8,9] and adenocarcinoma[10] glycoproteins, and in the bovine submaxillary gland mucin,[11] respectively. The presence of these structures suggests the existence of two different *N*-acetylgalactosaminyl transferases which facilitates the formation of an α-(1→3) or α-(1→6) linkage, respectively, of GalNAc to the α-GalNAc unit linked at a threonine of the protein chain **1** (Fig. 1). Until now none of these enzymes responsible for the biosynthesis of the core 5 and core 7 structures has been characterized. No specific recognition sequence has been found for the general biosynthetic assembly of the *O*-glycoproteins.[12,13] Once an *N*-acetylgalactosamine moiety has been attached to the peptide backbone by the known and characterized *N*-acetylgalactosaminyl polypeptide transferase, the different mucin-type O-glycan core structures are assembled by stepwise glycosylation catalysed by specific glycosyl transferases.[7] Different glycopeptide derivatives **2** and **3** correspond to core structures 5 and 7 as well as common substrates **1** have been synthesized in order to isolate the two enzymes and study their enzymic activities.

It has previously been demonstrated for other glycosyl transferases that N-terminal acetylation and C-terminal amidation afforded superior substrates[14] and in the present work this derivatization has been preferred.

For a more detailed enzymic investigation a number of synthetic glycopeptides with a variation of the peptide sequence and the glycosylation site on the peptide were required. The currently most efficient strategy to build up a number of glycopeptides is the stepwise multiple-column solid-phase synthesis (MCPS)[15,16] using glycosylated amino acids as building blocks.

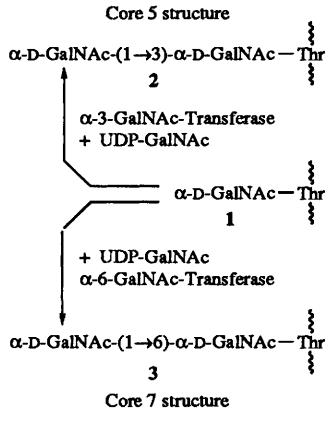
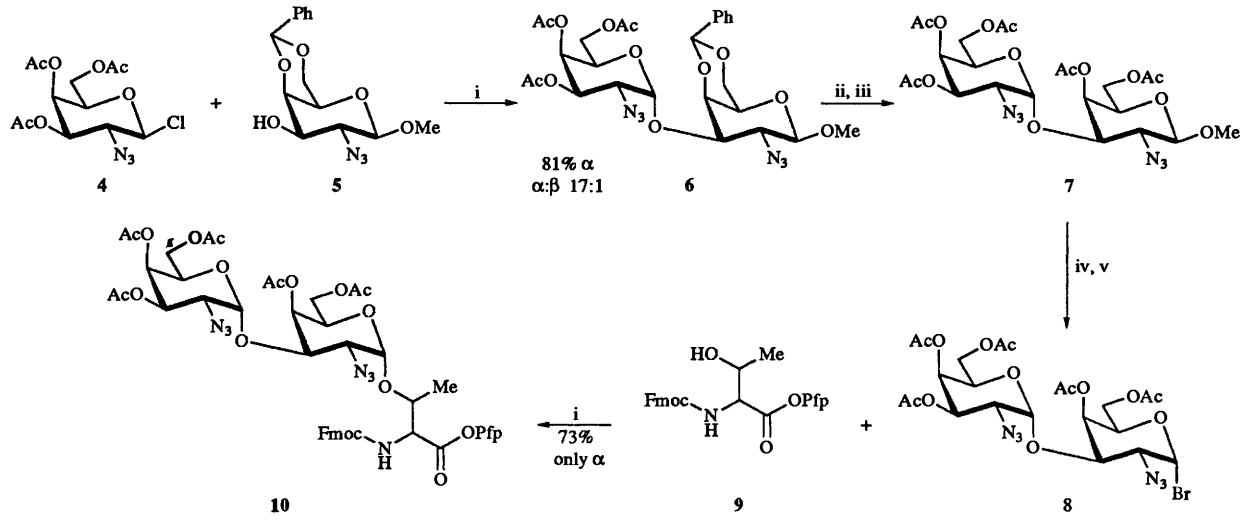


Fig. 1 Biosynthesis of core 5 and core 7 structures of mucin *O*-glycopeptides

Results and discussion

The strategy of MCPS of our mucin glycopeptides involves the preparation of the two building blocks **10** and **15**. The fluoren-9-ylmethoxycarbonyl (Fmoc) was selected as an amino-protecting group which can be selectively removed under mild conditions with the weak base morpholine[17] (after each peptide-coupling reaction) without cleavage or degradation of the carbohydrate part. The carboxy groups in intermediates **10** and **15** are activated as their pentafluorophenyl (Pfp) esters.[18] The acetyl groups used for protection of the carbohydrate part could easily be removed as the final step of the synthesis with a catalytic amount of sodium methoxide in methanol.

This approach[19,20] required the preparation of an activated disaccharide glycosyl donor and the condensation with the preformed Pfp ester of N^{α}-Fmoc-Thr.[21,22] The glycosylation of the amino acid acceptor must be stereoselective with a high yield. Thus the suitable building block is obtained in one step and can be directly used for peptide synthesis on the solid phase.



Scheme 1 *Reagents:* i, $AgClO_4$, Ag_2CO_3; ii, AcOH; iii, Ac_2O; iv, H_2SO_4–Ac_2O; v, $TiBr_4$

Synthesis of building block 10

Condensation between the halide **4**[18,23] (1.2 mol equiv.) and the acceptor **5**[24] (1 mol equiv.) in the presence of Ag_2CO_3/$AgClO_4$ afforded the α-linked disaccharide derivative **6** (δ 5.23, d, $J_{1',2'}$ 3.6 Hz, 1'-H) as a major product (81%) with a stereoselectivity α:β 17:1. Treatment of compound **6** with aq. 75% acetic acid followed by conventional acetylation gave compound **7** (86% overall yield). The ^{1}H NMR spectrum of compound **7** showed signals of two protons at δ 5.43 and 4.15 attributed to 4-H and 6-H_2 respectively. These deshielded signals are additional confirmation that glycosylation took place at 3-OH of compound **5**, and indicated no migration of the *O*-benzylidene group during the condensation.

Acetolysis of compound **7** in acetic anhydride gave the corresponding 1-*O*-acetate, which was directly treated with titanium tetrabromide to give the α-bromide **8** (80%). The amino acid derivative **9** (1 mol equiv.) was subsequently glycosylated with the halide **8** (1.3 mol equiv.) in the presence of Ag_2CO_3/$AgClO_4$ to give the desired building block **10** (73%). The glycosylation reaction gives stereoselectively the α-glycoside form **10** (Scheme 1).

Synthesis of building block 15

Glycosylation of compound **11**[25] (1 mol equiv.) with compound **4**[18,23] (1.2 mol equiv.), as described for the preparation of compound **6**, gave the α-linked disaccharide **12** (69%). The ^{1}H NMR spectrum of product **12** showed a doublet at δ 5.07 ($J_{1',2'}$ 3.0 Hz, 1'-H) characteristic of an α-linkage. About 20% of the related (1→3) α-linked derivative was also isolated as a by-product of the glycosylation reaction. Acetylation of this compound afforded the product **7**, demonstrating the partial migration of the isopropylidene group during the glycosylation reaction.

Hydrolysis of the ketal **12** with 70% aq. acetic acid followed by conventional acetylation gave compound **13** (96% overall yield). The ^{1}H NMR spectrum of product **13** showed signals of two protons at δ 5.37 and 4.82 attributed to 4-H and 3-H, respectively. These deshielded signals are characteristic for a 3,4-di-*O*-acetylated derivative, confirming that glycosylation occurred at the 6-OH position of substrate **11**.

Acetolysis of compound **13** followed by treatment with titanium tetrabromide afforded the α-bromide **14** (77% overall yield). Coupling between the threonine derivative **9** (1 mol equiv.) and the halide **14** (1.3 mol equiv.) in the presence of Ag_2CO_3/$AgClO_4$ gave the desired building block **15** (74%). Only the α-linked compound was formed (Scheme 2).

The multi-column solid-phase synthesis

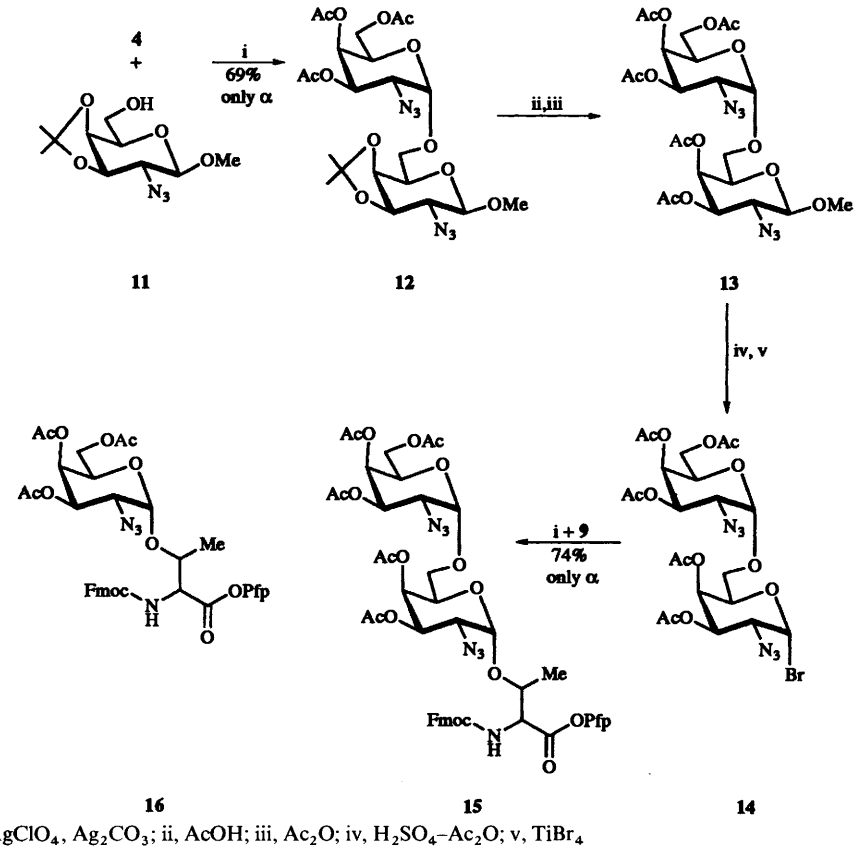
The glycosyl amino acids **10**, **15** and **16**[18] were introduced at different positions in a peptide sequence using an earlier described manual multiple-column peptide synthesizer.[18] A series of 20 *O*-glycosylated *N*-acetyl peptide carboxamides **20**–**39** were synthesized by this method.

The solid-phase glycopeptide synthesis was performed on a Kieselguhr-supported poly(dimethylacrylamide) resin[26] which was derivatized with norleucine as internal reference amino acid, and *p*-[α-amino-(2,4-dimethoxyphenyl)methyl]phenoxyacetic acid (Rink linker)[27] as an acid-labile amide linker. All Fmoc cleavages were carried out under mild conditions by treatment of the resin with 50% morpholine in dimethylformamide (DMF). The Fmoc amino acids were introduced into the peptide chains as Pfp esters. In all cases including the glycosylated amino acids **10**, **15** and **16**, 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (Dhbt-OH) was added as auxiliary nucleophile, in order to increase the rate of the coupling reaction and to allow us to follow the progress of the reaction visually by the disappearance of the strong yellow colour of the Dhbt-OH ammonium salt.

The side-chains of the non-glycosylated residue of Ser residues and the carboxylic group of Glu residues were protected as their *tert*-butyl ether and ester, respectively. After attachment of the last amino acid and removal of Fmoc the terminal amino groups were acetylated with Ac_2O in DMF to give the resin-bound glycopeptide **17** (Fig. 2). All azido groups on peptide **17** were reduced and transformed into *N*-acetyl groups on the resin in one step with thioacetic acid to yield product **18** (Fig. 2).

According to previously reported[18] methodology the reaction was followed by IR spectroscopy by observation of the complete disappearance of the azide absorption band at ν 2117 cm^{-1}. All 20 glycopeptides were cleaved off the resin by treatment with 95% aq. trifluoroacetic acid (TFA) with concurrent removal of the *tert*-butyl groups. Finally the *O*-acetyl groups of the carbohydrates were removed with sodium methoxide in methanol at pH 8.0, to afford the deprotected glycopeptides **19**.

The glycopeptides were purified by preparative reversed-phase HPLC. The pure *O*-glycopeptides **20**–**39** were obtained in yields of 60–80% after lyophilization based on the degree of the loading of the resin. 10–15% of the corresponding 2-deoxy-2-thioacetamido-α-D-galactopyranose derivative was isolated for each glycopeptide. This by-product was formed during the reduction of the azido group which lasted up to 8 days. The



Scheme 2 *Reagents:* i, $AgClO_4$, Ag_2CO_3; ii, AcOH; iii, Ac_2O; iv, H_2SO_4–Ac_2O; v, $TiBr_4$

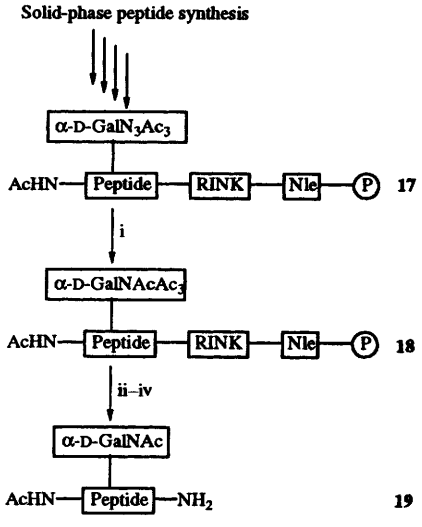


Fig. 2 Glycopeptide synthesis. RINK = Rink-linker,[27] Nle = norleucine, P = polymer. *Reagents and conditions:* i, AcSH; ii, TFA, scavenger; iii, NaOMe, MeOH; iv, HPLC

separation of the compounds by reversed-phase HPLC was in some cases difficult. The lower yields observed for the glycopeptides **20**, **24**, **27**, **33**, **35** and **36** may be explained by the fact that one of the coupling reactions was not complete. All *O*-glycopeptides were characterized by amino acid analyses (Table 1), FAB-MS and 1-D and 2-D NMR spectroscopy (Table 2).

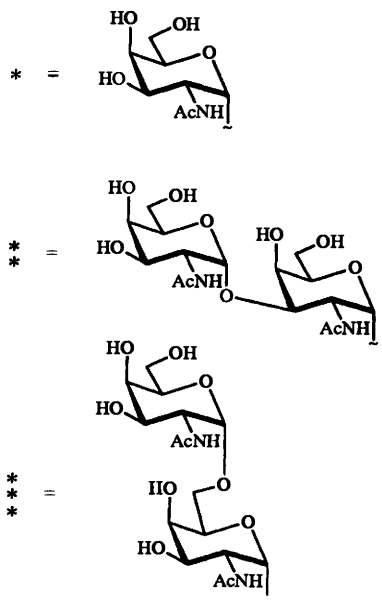
Conclusions

In conclusion the use of the present methodology involving the combined use of glycosylated Fmoc-amino acid pentafluorophenyl esters, azides as amino group precursors, and MCPS has allowed the simple preparation of 20 different glycopeptides **20**–**39** related to mucin core structures 5 and 7. All the glycopeptides will be tested for the specificity of the related glycosyl transferases and the results presented in a forthcoming publication.

Experimental

Materials and methods

All solvents were distilled at the appropriate pressure. DMF was analysed for free amines by addition of Dhbt-OH prior to use. Light petroleum refers to the fraction distilled in the range 60–70 °C. Reagents for peptide synthesis were purchased as follows: dicyclohexylcarbodiimide (DCCI), Dhbt-OH and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) from Fluka; Macrosorb SPR 250 from Sterling Organics; Fmoc amino acid Pfp esters and Fmoc-protected Rink linker from NovaBiochem. ^1H NMR spectra were recorded on a Bruker AMX 400 MHz spectrometer; δ-values are in ppm and J-values are in Hz (±0.3 Hz). Flash-column chromatography was performed on Silica Gel (ICN Biomedical, 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 for preparative separations) with buffer A (0.1% TFA in water) and buffer B (0.1% TFA in acetonitrile). Amino acid analyses were performed on a Pharmacia LKB Alpha Plus amino acid analyser after hydrolysis of the glycopeptides with 6 mol dm^{-3} HCl at 110 °C for 24 h. 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}.



* = HO, OH, O, HO, AcNH

** = HO, OH, O, HO, AcNH, O, HO, OH, O, AcNH

*** = HO, OH, O, HO, AcNH, O, HO, O, HO, AcNH

20 Ac-Glu-Leu-Ser-Thr-Thr-Gly-Pro-Gly-NH_2

21 Ac-Glu-Leu-Ala-Thr-Thr-Gly-Pro-Gly-NH_2

22 Ac-Glu-Leu-Ala-Thr-Thr-Gly-Pro-Gly-NH_2

23 Ac-Glu-Leu-Ala-Thr-Thr-Gly-Pro-Gly-NH_2

24 Ac-Glu-Leu-Ala-Thr-Thr-Gly-Pro-Gly-NH_2

25 Ac-Glu-Leu-Ala-Thr-Thr-Gly-Pro-Gly-NH_2

26 Ac-Glu-Leu-Ala-Thr-Thr-Gly-Pro-Gly-NH_2

27 Ac-Glu-Leu-Ala-Thr-Thr-Gly-Pro-Gly-NH_2

28 Ac-Glu-Leu-Ala-Thr-Val-Gly-Pro-Gly-NH_2

29 Ac-Glu-Leu-Ala-Thr-Val-Gly-Pro-Gly-NH_2

30 Ac-Glu-Leu-Ala-Thr-Val-Gly-Pro-Gly-NH_2

31 Ac-Glu-Leu-Ala-Pro-Thr-Gly-Pro-Gly-NH_2

32 Ac-Glu-Leu-Ala-Pro-Thr-Gly-Pro-Gly-NH_2

33 Ac-Glu-Leu-Ala-Pro-Thr-Gly-Pro-Gly-NH_2

34 Ac-Glu-Leu-Ala-Thr-Gln-Gly-Pro-Gly-NH_2

35 Ac-Glu-Leu-Ala-Thr-Gln-Gly-Pro-Gly-NH_2

36 Ac-Glu-Leu-Ala-Thr-Gln-Gly-Pro-Gly-NH_2

37 Ac-Glu-Leu-Ala-Gln-Thr-Gly-Pro-Gly-NH_2

38 Ac-Glu-Leu-Ala-Gln-Thr-Gly-Pro-Gly-NH_2

39 Ac-Glu-Leu-Ala-Gln-Thr-Gly-Pro-Gly-NH_2

Table 1 Amino acid analyses of the glycopeptides **20**–**39**. Relative values referring to Pro = 1.00

Substance	Glu	Leu	Ala	Ser	Thr	Val	Gly	Pro
20	1.16	1.00		0.98	1.97		1.90	0.98
21	1.05	1.04	0.99		1.85		2.06	1.00
22	1.09	0.96	0.99		1.95		2.02	1.00
23	1.03	1.00	1.01		1.89		2.05	1.01
24	1.08	1.02	1.00		1.86		2.03	1.02
25	1.03	1.00	1.04		1.81		2.17	1.10
26	0.94	1.00	0.98		1.95		2.00	0.98
27	1.06	1.03	1.02		1.78		2.08	1.04
28	0.84	1.01	1.02		0.86	1.00	2.02	0.99
29	1.00	0.94	0.99		0.85	0.96	2.28	0.98
30	1.09	1.00	0.99		0.92	0.98	2.00	1.02
31	0.99	0.99	1.03		0.94		2.12	1.93
32	1.06	1.01	1.01		0.92		2.04	1.98
33	1.01	1.00	1.01		0.95		2.05	1.99
34	1.98	1.00	1.01		0.96		2.08	0.98
35	2.07	0.99	1.04		0.91		2.01	0.99
36	2.04	1.01	1.02		0.92		2.00	1.01
37	2.04	1.01	1.02		0.93		2.01	1.00
38	2.07	0.99	1.02		0.88		2.04	1.01
39	2.04	1.01	1.01		0.93		2.02	0.99

Methyl *O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside 6

A mixture of compound **5**[24] (100 mg, 0.32 mmol), activated powdered 4 Å molecular sieves and silver carbonate (179 mg, 0.65 mmol) in dry dichloromethane–toluene (1 : 1, 10 cm^3) was stirred at room temperature under nitrogen. After 1 h, silver perchlorate (18 mg) and a solution of the chloride **4**[18] (137 mg, 0.39 mmol) in dry dichloromethane–toluene (1 : 1; 1 cm^3) were added. The mixture was stirred 4 h at room temperature, and then filtered. The solution was washed successively with aq. $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated. The residue (ratio of α : β forms 17 : 1) was chromatographed on a silica gel column (10 g) with toluene–acetone (6 : 1) as eluent to give *title compound* **6** (164 mg, 81%); $[\alpha]_D$ +106 (*c* 1.0, $CHCl_3$); δ_H($CDCl_3$; standard Me_4Si) 2.03, 2.06 and 2.14 (9 H, 3 s, 3 × Ac), 3.42 (1 H, m, 5-H), 3.60 (3 H, s, OMe), 3.61 (1 H, dd, $J_{2,3}$ 10.7, $J_{3,4}$ 3.5, 3-H), 3.65 (1 H, dd, $J_{1',2'}$ 3.6, $J_{2',3'}$ 11.0, 2′-H), 3.88 (1 H, dd, $J_{1,2}$ 8.0, 2-H), 4.06 (1 H, dd, $J_{5',6'a}$ 6.6, $J_{6'a,6'b}$ 11.2, 6′-H^a), 4.11 (1 H, dd, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 12.0, 6-H^a), 4.15 (1 H, dd, $J_{5',6'b}$ 6.6, 6′-H^b), 4.24 (1 H, d, 1-H), 4.28 (1 H, dd, $J_{4,5}$ 1.0, 4-H), 4.39 (1 H, dd, $J_{5,6b}$ 1.5, 6-H^b), 4.53 (1 H, m, 5′-H), 5.23 (1 H, d, 1′-H), 5.43 (1 H, dd, $J_{3',4'}$ 3.0, 3′-H), 5.52 (1 H, dd, $J_{4',5'}$ 1.0, 4′-H), 5.59 (1 H, s, PhC*H*) and 7.12–7.57 (5 H, m, Ph) (Found: C, 50.0; H, 5.1; N, 13.3. $C_{26}H_{32}N_6O_{12}$ requires C, 50.3; H, 5.2; N, 13.5%).

Methyl *O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranoside 7

A mixture of benzylidene acetal **6** (335 mg, 0.54 mmol), acetic acid (12 cm^3) and water (3 cm^3) was stirred at 100 °C for 20 min, then was cooled and concentrated. To a solution of the residue in dry pyridine (6 cm^3) was added acetic anhydride (3 cm^3). After being stirred at room temp. for 30 min the solution was concentrated, and co-evaporated with toluene. The residue was chromatographed on a silica gel column (25 g) with toluene–acetone (6 : 1) as eluent, to give *title compound* **7** (286 mg, 86%), $[\alpha]_D$ +92 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; Me_4Si) 2.05, 2.07, 2.15 and 2.18 (15 H, 4 s, 5 × Ac), 3.61 (3 H, s, OMe), 3.62 (1 H, dd, $J_{2,3}$ 10.7, $J_{3,4}$ 3.1, 3-H), 3.67 (1 H, dd, $J_{1,2}$ 7.1, 2-H), 3.73 (1 H, dd, $J_{1',2'}$ 3.6, $J_{2',3'}$ 11.2, 2′-H), 3.78 (1 H, m, 5-H), 4.02 (1 H, dd, $J_{5',6'a}$ 6.1,

Table 2 ^{1}H NMR data (400 MHz; D_2O; internal reference MeOH at 300 K) of the amino acid protons of the glycopeptides **20–39** (δ-values given in ppm, J-values given in ± 0.3 Hz)

Peptide	Glu$^{\alpha}$	Glu$^{\beta}$	Glu$^{\gamma}$	Leu$^{\alpha}$	Leu$^{\beta}$	Leu$^{\gamma}$	Leu$^{\delta}$	Ser$^{\alpha}$	Ser$^{\beta}$	Ala$^{\alpha}$	Ala$^{\beta}$
20	4.32 (5.6)(8.6)	1.92–2.15	2.49 (7.6)	4.43	1.58–1.71	1.58–1.71	0.87, 0.93	4.63 (6.1)(6.0)	3.89		
21	4.34 (6.1)(8.6)	1.94–2.11	2.49 (7.6)	4.43	1.55–1.69	1.55–1.69	0.88, 0.94			4.51 (7.1)	1.43
22	4.33 (6.1)(2.2)	1.93–2.14	2.49 (7.6)	4.38	1.57–1.68	1.57–1.68	0.88, 0.93			4.52 (7.1)	1.41
23	4.33	1.91–2.14	2.49 (7.6)	4.39	1.53–1.69	1.53–1.69	0.88, 0.93			4.52 (7.1)	1.41
24	4.33 (6.1)(8.6)	1.92–2.14	2.49 (7.6)	4.36	1.56–1.68	1.56–1.68	0.88, 0.94			4.52 (7.1)	1.40
25	4.32 (6.1)(9.1)	1.94–2.13	2.49 (7.6)	4.39	1.58–1.69	1.58–1.69	0.88, 0.93			4.51 (7.1)	1.42
26	4.34 (5.6)(8.6)	1.93–2.16	2.50 (7.6)	4.38	1.57–1.71	1.57–1.71	0.88, 0.94			4.52 (7.1)	1.42
27	4.32 (5.6)	1.93–2.14	2.49 (7.6)	4.40	1.58–1.70	1.58–1.70	0.89, 0.94			4.52 (7.1)	1.43
28	4.33 (6.1)(9.2)	1.93–2.14	2.48 (7.6)	4.38 (5.1)(9.1)	1.55–1.69	1.55–1.69	0.88, 0.95			4.50 (7.1)	1.42
29	4.33 (6.1)(8.6)	1.89–2.13	2.48 (7.6)	4.38	1.53–1.67	1.53–1.67	0.88, 0.92			4.51 (7.1)	1.39
30	4.34 (6.1)(8.6)	1.93–2.16	2.49 (7.6)	4.38 (5.1)(9.2)	1.57–1.70	1.57–1.70	0.88, 0.94			4.52 (7.1)	1.42

Peptide	Thr$^{\alpha}$	Thr$^{\beta}$	Thr$^{\gamma}$	Thr$^{\alpha}$	Thr$^{\beta}$	Thr$^{\gamma}$	Val$^{\alpha}$	Val$^{\beta}$	Val$^{\gamma}$	Gly$^{\alpha}$	Pro$^{\alpha}$	Pro$^{\beta}$	Pro$^{\gamma}$	Pro$^{\delta}$	Gly$^{\alpha}$
20	4.74	4.35 (5.6)	1.31	4.61 (2.0)	4.35 (5.6)	1.28				4.26 3.88	4.42 (4.6)(9.1)	2.24–2.34	1.98–2.09	3.63	3.89
21	4.71 (2.0)	4.43	1.33	4.61 (2.0)	4.42	1.28				4.27 3.88	4.43 (4.6)(9.1)	2.26–2.33	2.03–2.08	3.65	3.90
22	4.73 (2.0)	4.41	1.33	4.59 (2.5)	4.36	1.29				4.24 3.87	4.43 (4.6)(8.6)	2.27–2.33	1.97–2.09	3.65	3.91
23	4.72 (2.0)	4.39	1.32	4.63 (1.5)	4.35	1.28				4.30 3.87	4.40	2.26–2.33	2.00–2.08	3.65	3.91
24	4.74	4.39	1.32	4.61 (2.0)	4.38	1.29				4.29 3.81	4.38	2.26–2.34	2.00–2.07	3.65	3.90
25	4.71 (2.5)	4.37	1.33	4.60 (2.5)	4.37	1.28				4.27 3.91	4.42 (5.1)(9.2)	2.24–2.34	2.00–2.03	3.65	3.90
26	4.72 (2.0)	4.41	1.33	4.62 (2.0)	4.31	1.30				4.28 3.95	4.44 (4.6)(9.1)	2.26–2.34	1.98–2.09	3.65	3.92
27	4.72	4.38	1.35	4.60 (2.0)	4.33	1.30				4.28 3.95	4.43 (4.6)(9.1)	2.27–2.34	1.99–2.08	3.65	3.91
28				4.59 (2.0)	4.31	1.28	4.21	2.03–2.16	0.98, 0.96	4.17 3.96	4.44 (5.1)(9.2)	2.25–2.34	1.94–2.07	3.68	3.91
29				4.59	4.32	1.28	4.13	2.01–2.12	0.97, 0.99	4.13 3.97	4.43 (4.6)(8.6)	2.24–2.34	1.98–2.09	3.67	3.91
30				4.59	4.29	1.31	4.21	2.02–2.16	0.97, 0.98	4.17 3.96	4.44 (4.6)(9.1)	2.25–2.35	1.97–2.08	3.68	3.92

Peptide	Glu$^{\alpha}$	Glu$^{\beta}$	Glu$^{\gamma}$	Leu$^{\alpha}$	Leu$^{\beta}$	Leu$^{\gamma}$	Leu$^{\delta}$	Ala$^{\alpha}$	Ala$^{\beta}$	Pro$^{\alpha}$	Pro$^{\beta}$	Pro$^{\gamma}$	Pro$^{\delta}$
31	4.34 (6.1)(8.6)	1.92–2.15	2.49 (7.6)	4.40 (5.6)(10.2)	1.55–1.70	1.55–1.70	0.88, 0.95	4.63 (7.1)	1.38	4.64	2.28–2.37	1.97–2.06	3.84
32	4.33 (6.1)(8.6)	1.92–2.13	2.49 (7.6)	4.38	1.53–1.69	1.53–1.69	0.88, 0.93	4.61 (7.1)	1.37	4.61	2.27–2.38	1.97–2.06	3.88
33	4.33 (6.1)(9.1)	1.91–2.14	2.49 (7.6)	4.41 (5.1)(10.2)	1.52–1.69	1.52–1.69	0.88, 0.93	4.61 (7.1)	1.37	4.62	2.29–2.37	1.98–2.07	3.82
34	4.32 (6.1)(9.1)	1.92–2.17	2.49 (7.6)	4.37 (3.6)(9.1)	1.58–1.69	1.58–1.69	0.88, 0.94	4.50 (7.1)	1.41				
35	4.33 (6.1)(9.1)	1.91–2.14	2.49 (7.6)	4.38 (5.1)(9.1)	1.55–1.69	1.55–1.69	0.88, 0.93	4.52 (7.1)	1.41				
36	4.33	1.94–2.14	2.50 (7.6)	4.35	1.55–1.70	1.55–1.70	0.89, 0.95	4.51 (7.1)	1.42				
37	4.33	1.87–2.12	2.49 (7.6)	4.38	1.54–1.71	1.54–1.71	0.89, 0.94	4.32	1.39				
38	4.33	1.87–2.14	2.49 (7.6)	4.37	1.54–1.69	1.54–1.69	0.88, 0.96	4.31	1.39				
39	4.33	1.94–2.13	2.49 (7.6)	4.37	1.53–1.69	1.53–1.69	0.88, 0.93	4.33	1.39				

Peptide	Thr$^{\alpha}$	Thr$^{\beta}$	Thr$^{\gamma}$	Glu$^{\alpha}$	Glu$^{\beta}$	Glu$^{\gamma}$	Gly$^{\alpha}$	Pro$^{\alpha}$	Pro$^{\beta}$	Pro$^{\gamma}$	Pro$^{\delta}$	Gly$^{\alpha}$
31	4.57 (2.5)	4.40	1.32				4.23, 4.02	4.44 (5.1)(9.2)	2.26–2.36	1.97–2.06	3.67	3.92
32	4.58 (1.5)	4.42	1.32				4.26, 3.99	4.42	2.25–2.34	1.97–2.06	3.65	3.91
33	4.57 (2.5)	4.38	1.32				4.21, 4.00	4.43 (5.1)(9.1)	2.26–2.33	1.98–2.07	3.67	3.90
34	4.54 (2.0)	4.31	1.28	4.39 (3.6)(9.1)	1.96–2.05 2.11–2.19	2.42 (7.6)	4.19, 3.97	4.43 (4.1)(9.1)	2.27–2.35	1.99–2.08	3.67	3.90
35	4.57 (2.0)	4.34	1.29	4.37 (5.6)(9.6)	1.97–2.18	2.43 (7.6)	4.15, 3.97	4.46 (5.1)(9.2)	2.27–2.36	1.98–2.09	3.67	3.92
36	4.55	4.31	1.30	4.41 (5.1)	1.95–2.16	2.42 (7.6)	4.17, 3.96	4.45 (4.6)(9.1)	2.26–2.35	1.96–2.06	3.66	3.91
37	4.60 (2.0)	4.41	1.27	4.52 (5.1)(8.6)	1.93–2.03 2.10–2.21	2.42 (7.6)	4.22, 4.01	4.42 (4.9)	2.26–2.33	2.00–2.09	3.66	3.90
38	4.61 (2.0)	4.43	1.27	4.53 (5.6)(9.1)	1.94–2.08 2.12–2.22	2.41 (7.6)	4.27, 3.87	4.41	2.24–2.33	1.97–2.07	3.66	3.90
39	4.59	4.40	1.28	4.52 (5.6)	1.98–2.07 2.13–2.23	2.42 (7.6)	4.21, 3.98	4.42	2.24–2.34	1.97–2.06	3.65	3.89

$J_{6'a,6'b}$ 11.2, 6'-H^a), 4.15 (2 H, m, 6-H_2), 4.17 (1 H, dd, 6'-H^b), 4.24 (1 H, d, 1-H), 4.51 (1 H, m, 5'-H), 5.21 (1 H, d, 1'-H), 5.33 (1 H, dd, $J_{3',4'}$ 3.0, 3'-H), 5.43 (1 H, dd, $J_{4,5}$ 1.0, 4-H) and 5.50 (1 H, dd, $J_{4',5'}$ 1.0, 4'-H) (Found: C, 44.4; H, 5.1; N, 13.5. $C_{23}H_{32}N_6O_{14}$ requires C, 44.8; H, 5.2; N, 13.6%).

O-(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→3)-4,6-di-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide 8

A solution of compound **6** (613 mg, 0.99 mmol) in acetic anhydride (5 cm^3) was stirred at −20 °C. A cold solution of acetic anhydride–sulfuric acid (50:1; 5 cm^3) was added. After 96 h at −20 °C, the solution was diluted in cold dichloromethane and washed successively with aq. $NaHCO_3$ and water, dried ($MgSO_4$), concentrated, and co-distilled with toluene to give the 1-*O*-acetate; δ_H ($CDCl_3$; Me_4Si) of the α-form 2.13–2.20 (18 H, 6 s, 6 × Ac), 3.75 (1 H, dd, $J_{1',2'}$ 3.6, $J_{2',3'}$ 11.2, 2'-H), 3.99 (1 H, dd, $J_{1,2}$ 4.07, $J_{2,3}$ 10.7, 2-H), 4.08 (1 H, dd, $J_{5,6a}$ 4.6, $J_{6a,6b}$ 11.2, 6-H^a), 4.12 (1 H, dd, $J_{5',6'a}$ 6.6, $J_{6'a,6'b}$ 11.7, 6'-H^a), 4.14 (1 H, dd, $J_{5,6b}$ 1.5, 6-H^b), 4.16 (1 H, dd, $J_{3,4}$ 3.5, 3-H), 4.21 (1 H, m, 5-H), 4.34 (1 H, m, 5'-H), 5.29 (1 H, d, 1'-H), 5.36 (1 H, dd, $J_{3',4'}$ 4.0, 3'-H), 5.51 (1 H, dd, $J_{4',5'}$ 1.0, 4'-H), 5.56 (1 H, dd, $J_{4,5}$ 1.0, 4-H) and 6.37 (1 H, d, 1-H).

A solution of the residue and titanium tetrabromide (539 mg, 1.46 mmol) in dry dichloromethane–ethyl acetate (10:1; 11 cm^3) was stirred for 16 h at room temp. The solution was diluted with dry toluene (10 cm^3), and dry sodium acetate was added until the mixture become colourless. The mixture was filtered and concentrated. The residue was chromatographed on a silica gel column (40 g) with light petroleum–ethyl acetate (2:1) as eluent, to give the *bromide* **8** (529 mg, 80%), $[\alpha]_D$ +212 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; Me_4Si) 2.05, 2.08, 2.09, 2.16 and 2.19 (15 H, 5 s, 5 × Ac), 3.73 (1 H, dd, $J_{1',2'}$ 3.1, $J_{2',3'}$ 10.7, 2'-H), 4.09 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 10.7, 2-H), 4.05–4.15 (2 H, m, 6- and 6'-H^a), 4.18 (1 H, dd, $J_{5',6'b}$ 4.6, $J_{6'a,6'b}$ 11.7, 6'-H^b), 4.21 (1 H, dd, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 12.2, 6-H^b), 4.26 (1 H, dd, $J_{3,4}$ 3.0, 3-H), 4.37 (1 H, m, 5'-H), 4.42 (1 H, m, 5-H), 5.29 (1 H, d, 1'-H), 5.37 (1 H, dd, $J_{3',4'}$ 3.05, 3'-H), 5.51 (1 H, dd, $J_{4',5'}$ 1.1, 4'-H), 5.60 (1 H, dd, $J_{4,5}$ 0.8, 4-H) and 6.53 (1 H, d, 1-H) (Found: C, 39.1; H, 4.5; N, 12.2. $C_{22}H_{29}BrN_6O_{13}$ requires C, 39.7; H, 4.4; N, 12.6%).

N^α-(Fluoren-9-ylmethoxycarbonyl)-O-[O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→3)-(4,6-di-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl]-L-threonine pentafluorophenyl ester 10

A mixture of compound **9** (168 mg, 0.33 mmol), activated powdered 4 Å molecular sieves and silver carbonate (110 mg, 0.4 mmol) in dry dichloromethane–toluene (1:1; 5 cm^3) was stirred at room temp. under nitrogen. After 1 h, silver perchlorate (11 mg) and a solution of the bromide **8** (265 mg, 0.4 mmol) in dry dichloromethane–toluene (1:1; 3 cm^3) were added. The mixture was stirred for 20 h at room temp. 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 (20 g) with toluene–ethyl acetate (3:1) as eluent, to give title compound **10** (265 mg, 73%), $[\alpha]_D$ +84 (*c* 1, $CHCl_3$); δ_H($CDCl_3$, standard Me_4Si) 1.42 (3 H, d, Thr γ-H_3), 1.95, 2.05, 2.06, 2.17 and 2.19 (15 H, 5 s, 5 × Ac), 3.68 (1 H, dd, $J_{1',2'}$ 4.1, $J_{2',3'}$ 11.2, 2'-H), 3.83 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 10.0, 2-H), 3.93 (1 H, dd, $J_{5,6a}$ 6.6, $J_{6a,6b}$ 10.2, 6-H^a), 4.05 (1 H, dd, $J_{5,6a}$ 7.1, $J_{6a,6b}$ 11.2, 6-H^a), 4.14 (1 H, dd, $J_{3,4}$ 3.6, 3-H), 4.16 (1 H, dd, $J_{5,6b}$ 7.1, $J_{6a,6b}$ 11.2, 6-H^b), 4.18–4.28 (4 H, m, Fmoc CH, 5-H, 5'-H, 6-H^b), 4.47 (1 H, dd, J_{CH,CH_2b} 7.1, J_{CH_2a,CH_2b} 10.7, Fmoc CH_2^{a}), 4.52 (1 H, dd, J_{CH,CH_2b} 7.2, Fmoc CH_2^{b}), 4.58 (1 H, dd, $J_{CHβ,CH}$ 6.1, $J_{CHα,CHβ}$ 2.0, Thr β-H), 4.79 (1 H, dd, Thr α-H), 5.19 (1 H, d, 1-H), 5.32 (1 H, d, 1'-H), 5.37 (1 H, dd, $J_{3',4'}$ 3.05, 3'-H), 5.48 (1 H, dd, $J_{4',5'}$ 1.0, 4'-H), 5.54 (1 H, dd, $J_{4,5}$ 1.0, 4-H), 6.66 (1 H, d, $J_{NH,CHα}$ 9.6, ThrNH) and 7.12–7.78 (8 H, m, ArH) [Found: MH$^+$ (FAB-MS), 1092.8. $C_{47}H_{46}F_5N_7O_{18}$ requires M, 1091.9].

Methyl O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→6)-2-azido-2-deoxy-3,4-O-isopropylidene-β-D-galactopyranoside 12

A mixture of the alcohol **11**[25] (1 g, 3.86 mmol), activated powdered 4 Å molecular sieves and silver carbonate (2.13 g, 7.72 mmol) in dry dichloromethane–toluene (1:1; 100 cm^3) was stirred at room temp. under nitrogen. After 1 h, silver perchlorate (213 mg) and a solution of the chloride **4**[18] (1.62 g, 4.63 mmol) were added. The mixture was stirred for 1 h at room temp. 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 (100 g) with toluene–ethyl acetate (4:1) as eluent, to give first the *title compound* **12** (1.53 g, 69%), $[\alpha]_D$ +112 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; Me_4Si), 1.36 and 1.54 (6 H, 2 s, Me_2C), 2.06 and 2.15 (9 H, 2 s, 3 × Ac), 3.37 (1 H, dd, $J_{1,2}$ 8.6, $J_{2,3}$ 8.1, 2-H), 3.57 (3 H, s, OMe), 3.68 (1 H, dd, $J_{1',2'}$ 3.0, $J_{2',3'}$ 11.2, 2'-H), 3.81 (1 H, dd, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 9.6, 6-H^a), 3.97 (1 H, dd, $J_{3,4}$ 5.6, 3-H), 3.99 (2 H, m, 5-H and 6-H^b), 4.06 (1 H, dd, $J_{5',6'a}$ 7.6, $J_{6'a,6'b}$ 6.6, 6'-H^a), 4.19 (1 H, dd, $J_{5',6'b}$ 9.7, 6'-H^b), 4.14 (1 H, d, 1-H), 4.17 (1 H, dd, $J_{4,5}$ 2.5, 4-H), 4.31 (1 H, m, 5'-H), 5.07 (1 H, d, 1'-H), 5.37 (1 H, $J_{3',4'}$ 3.5, 3'-H) and 5.45 (1 H, dd, $J_{4',5'}$ 1.0, 4'-H) (Found: C, 45.9; H, 5.7; N, 14.3. $C_{22}H_{32}N_6O_{12}$ requires C, 46.2; H, 5.6; N, 14.7%).

Further elution gave the methyl *O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→3)-2-azido-2-deoxy-4,6-*O*-isopropylidene-β-D-galactopyranoside (441 mg, 20%); δ_H($CDCl_3$; Me_4Si) 1.47 and 1.48 (6 H, 2 s, Me_2C), 2.05, 2.06 and 2.14 (9 H, 3 s, 3 × Ac), 3.29 (1 H, m, 5-H), 3.51 (1 H, dd, $J_{2,3}$ 10.6, $J_{3,4}$ 3.6, 3-H), 3.59 (3 H, s, OMe), 3.61 (1 H, dd, $J_{1',2'}$ 3.5, $J_{2',3'}$ 11.1, 2'-H), 3.84 (1 H, dd, $J_{1,2}$ 8.1, 2-H), 4.00 (1 H, dd, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 11.5, 6-H^a), 4.06 (1 H, dd, $J_{5',6'a}$ 6.6, $J_{6'a,6'b}$ 11.0, 6'-H^a), 4.09 (1 H, dd, $J_{5,6b}$ 2.5, 6-H^b), 4.15 (1 H, dd, $J_{5',6'b}$ 4.1, 6'-H^b), 4.17 (1 H, d, 1-H), 4.23 (1 H, dd, $J_{4,5}$ 1.0, 4-H), 4.53 (1 H, m, 5'-H), 5.12 (1 H, d, 1'-H), 5.46 (1 H, dd, $J_{3',4'}$ 3.5, 3'-H) and 5.53 (1 H, dd, $J_{4',5'}$ 1.0, 4'-H).

Methyl O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→6)-3,4-di-O-acetyl-2-azido-2-deoxy-β-D-galactopyranoside 13

A mixture of compound **12** (164 mg, 0.286 mmol), acetic acid (3.5 cm^3) and water (1.5 cm^3) was stirred at 100 °C for 20 min, then was cooled and concentrated. To a solution of the residue in dry pyridine (3 cm^3) was added acetic anhydride (1.5 cm^3). After being stirred at room temp. for 2 h, the solution was concentrated, and co-distilled with toluene. The residue was chromatographed on a silica gel column (20 g) and eluted with toluene–ethyl acetate (3:1) to give *title compound* **13** (171 mg, 96%), $[\alpha]_D$ +77 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; Me_4Si) 2.05, 2.14 and 2.16 (15 H, 3 s, 5 × Ac), 3.56 (1 H, dd, $J_{5,6a}$ 4.1, $J_{6a,6b}$ 10.2, 6-H^a), 3.62 (3 H, s, OMe), 3.65 (1 H, dd, $J_{1',2'}$ 3.6, $J_{2',3'}$ 11.0, 2'-H), 3.66 (1 H, dd, $J_{1,2}$ 8.1, $J_{2,3}$ 10.6, 2-H), 3.82 (1 H, dd, $J_{5,6b}$ 7.1, 6-H^b), 3.87 (1 H, m, 5-H), 4.06 (1 H, dd, $J_{5',6'a}$ 6.6, $J_{6'a,6'b}$ 11.1, 6'-H^a), 4.13 (1 H, dd, $J_{5',6'b}$ 6.6, 6'-H^b), 4.25 (1 H, m, 5-H), 4.30 (1 H, d, 1-H), 4.82 (1 H, dd, $J_{3,4}$ 3.0, 3-H), 4.94 (1 H, d, 1'-H), 5.33 (1 H, dd, $J_{3',4'}$ 3.0, 3'-H), 5.37 (1 H, dd, $J_{4,5}$ 1.0, 4-H) and 5.45 (1 H, dd, $J_{4',5'}$ 1.0, 4'-H) (Found: C, 44.7; H, 5.0; N, 13.3. $C_{23}H_{32}N_6O_{14}$ requires C, 44.8; H, 5.2; N, 13.6%).

O-(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→6)-3,4-di-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide 14

A solution of compound **13** (860 mg, 1.36 mmol) in acetic anhydride (7 cm^3) was stirred at −20 °C. A cold solution of acetic anhydride–sulfuric acid (50:1; 7 cm^3) was added. After

5 d at −20 °C, the reaction mixture was diluted in cold dichloromethane and washed successively with aq. $NaHCO_3$ and water, dried (Na_2SO_4), concentrated, and co-distilled with toluene. The residue was chromatographed on a silica gel column (80 g) and eluted with toluene–ethyl acetate (2:1), to give the corresponding 1-*O*-acetate (717 mg, 80%), $[\alpha]_D$ +176 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; Me_4Si) of the α-form 2.05–2.20 (18 H, 6 s, 6 × Ac), 3.54 (1 H, dd, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 10.7, 6-H^a), 3.60 (1 H, dd, $J_{1',2'}$ 3.0, $J_{2',3'}$ 11.2, 2'-H), 3.72 (1 H, dd, $J_{5,6b}$ 7.1, 6-H^b), 3.91 (1 H, dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.5, 2-H), 4.09 (2 H, m, 6'-H_2), 4.26 (2 H, m, 5- and 5'-H), 4.90 (1 H, d, 1'-H), 5.30 (1 H, dd, $J_{3',4'}$ 3.6, 3'-H), 5.33 (1 H, dd, $J_{3,4}$ 3.6, 3-H), 5.44 (1 H, dd, 4'-H), 5.53 (1 H, dd, 4-H) and 6.28 (1 H, d, 1-H).

A mixture of the residue (717 mg, 1.11 mmol) and titanium tetrabromide (612 mg, 1.66 mmol) in dry dichloromethane–ethyl acetate (10:1; 16.5 cm^3) was stirred overnight at room temp. The solution was diluted with dry toluene (15 cm^3), and dry sodium acetate was added until the reaction mixture became colourless. The mixture was filtered and concentrated to give the title compound **14** (713 mg, 96%), δ_H($CDCl_3$; Me_4Si) 2.05–2.17 (15 H, 5 s, 5 × Ac), 3.63 (1 H, dd, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 10.7, 6-H^a), 3.68 (1 H, dd, $J_{1',2'}$ 3.6, $J_{2',3'}$ 11.2, 2'-H), 3.78 (1 H, dd, $J_{5,6b}$ 7.1, 6-H^b), 3.99 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 10.7, 2-H), 4.09 (2 H, m, 6'-H_2), 4.21 (1 H, m, 5'-H), 4.50 (1 H, m, 5-H), 4.91 (1 H, d, 1'-H), 5.29 (1 H, dd, $J_{3',4'}$ 3.1, 3'-H), 5.36 (1 H, dd, $J_{3,4}$ 3.0, 3-H), 5.46 (1 H, dd, $J_{4',5'}$ 1.0, 4'-H), 5.55 (1 H, dd, $J_{4,5}$ 1.0, 4-H and 6.49 (1 H, d, 1-H). This labile compound was directly used for the next step.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-*O*-[*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→6)-3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl]-L-threonine pentafluorophenyl ester 15

A mixture of compound **9** (50 mg, 0.1 mmol), activated powdered 4 Å molecular sieves and silver carbonate (34 mg, 0.12 mmol) in dry dichloromethane–toluene (1:1; 2 cm^3) was stirred at room temp. under nitrogen. After 1 h, silver perchlorate (3 mg) and a solution of the bromide **14** (82 mg, 0.12 mmol) in dry dichloromethane–toluene (1:1; 1 cm^3) were added. The mixture was stirred for 36 h at room temp. 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 (10 g) with toluene–ethyl acetate (5:1) as eluent to give *compound* **15** (80 mg, 74%), $[\alpha]_D$ +77 (*c* 1, $CHCl_3$); δ_H($CDCl_3$; Me_4Si) 1.52 (3 H, d, $J_{CH\beta,CH\gamma}$ 6.1, Thr γ-H_3), 2.04–2.16 (15 H, 4 s, 5 × Ac), 3.54 (1 H, dd, $J_{5,6a}$ 3.0, $J_{6a,6b}$ 10.2, 6†-H^a), 3.70 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2-H), 3.74 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2†-H), 3.77 (1 H, dd, $J_{5,6b}$ 7.6, 6†-H^b), 4.05 (1 H, dd, $J_{5,6a}$ 7.1, $J_{6a,6b}$ 11.2, 6-H^a), 4.15 (1 H, dd, $J_{5,6b}$ 6.1, 6-H^b), 4.27–4.37 (3 H, m, 5-H_2 and Fmoc CH), 4.40 (1 H, dd, J_{CH,CH_2a} 7.1, J_{CH_2a,CH_2b} 10.2 Hz, Fmoc CH_2^{a}), 4.48 (1 H, dd, J_{CH,CH_2b} 7.6, Fmoc CH_2^{b}), 4.63 (1 H, dd, $J_{CH\alpha,CH\beta}$ 1.5, Thr β-H), 4.81 (1 H, dd, $J_{CH\alpha,NH}$ 9.1, Thr α-H), 4.94 (1 H, d, 1-H), 5.22 (1 H, d, 1†-H), 5.30 (1 H, dd, $J_{3,4}$ 3.0, 3-H), 5.33 (1 H, dd, $J_{3,4}$ 3.0, 3†-H), 5.47 (1 H, dd, 4-H), 5.50 (1 H, dd, 4†-H), 5.88 (1 H, d, Thr NH) and 7.14–7.78 (8 H, m, ArH) [Found: MH^+ (FAB-MS, 1092.9. $C_{47}H_{46}F_5N_7O_{18}$ requires M, 1091.9].

Synthesis of glycopeptides 20–39 on a manual 20-column peptide synthesizer

The derivatized Nle Macrosorb SPR 250 resin (2.0 g) was placed in a glass reactor and the resin was swelled in DMF (10 cm^3; 20 min). After removal of the Fmoc group by treatment of the resin with piperidine (20%) in DMF for 10 min, the Rink linker (350 mg, 0.65 mmol), TBTU (208 mg, 0.65 mmol), and 4-ethylmorpholine (94 mm^3, 0.75 mmol) in DMF (5 cm^3) were added. After being kept overnight in darkness, the resin was washed with DMF, acetylated with Ac_2O–DMF (1:7; 8 cm^3) for 20 min, and the Fmoc group was removed as described above. After washing of the resin with DMF, a mixture of Fmoc-Gly-OPfp (695 mg, 1.5 mmol) and Dhbt-OH (245 mg, 1.5 mmol) in DMF (5 cm^3) was added. After 16 h, the reagents were removed and the unreactive amino groups were acetylated. The resin was washed successively with DMF and diethyl ether (10 ×) and then dried. The incorporation of the glycine was 81.5%, and the loading of the resin was 0.21 mmol g^{-1} as estimated by quantitative amino acid analysis.

The derivatized resin was weighed out (100 mg/column) and packed in the 20 columns of the manual synthesizer. All reagents were removed by washing of the resin with DMF (10 ×). The Fmoc deprotections were performed by treatment with a 50% solution of morpholine in DMF, containing a small amount of the red dye Azorubin (0.01%) in order to indicate the end of the washing procedure. Each Fmoc amino acid Pfp ester and Dhbt-OH (3 mol equiv. for amino acid, 1.5 mol equiv. for building blocks **10**, **15**, **16**) were dissolved in DMF (0.5 cm^3) and the solutions were transferred to each well. After 24 h, the reaction mixtures were removed and the wells were washed with DMF. The uncoupled amino groups were capped by acetylation (Ac_2O–DMF, 1:7) after each coupling reaction. The synthesis cycle was repeated to complete the assembly of each glycopeptide (**20–39**) by using the respective activated Fmoc amino acids. After removal of the Fmoc groups the terminal amino groups were acetylated to give compound **17**.

The resins were washed successively with DMF (5 ×) and diethyl ether (5 ×) and dried. The reduction of the azido groups was performed by the addition of distilled thioacetic acid (0.75 cm^3; GLC > 99.5% purity) to each well. The progress of the reduction of the azido groups was followed by IR spectroscopy as previously described.[18] The acetamido compounds **18** were obtained after 2–8 days.

The resins were washed and dried as previously described, and carefully transferred to small Eppendorf tubes. The resins **18** were treated with 95% aq. TFA (2 cm^3) for 2 h at room temp. and were then filtered off, and washed with TFA (1 cm^3). The solutions were concentrated, and co-distilled first with toluene and then with MeOH–toluene (1:3). The residues were diluted in abs. methanol (2 cm^3). After addition of 1% methanolic sodium methoxide (40 mm^3), the reaction mixtures were stirred at room temp. for 3–5 h. The solutions were neutralized with acetic acid (30 mm^3), filtered, evaporated, and purified by preparative RP-HPLC [buffer A–buffer B 95:5 ⟶ 85:15 (20 min) ⟶ 50:50 (30 min)]. The pure glycopeptides **19** were eluted first. The tail of the eluting peak contained ~10% thioacetyl-containing by-product. In all NMR data below, * and ** correspond to protons belonging to the same respective sugar unit.

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-seryl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonylglycyl-L-prolylglycinamide 20. Pure compound **20** (4.1 mg, 17%), 4.6 mg (with 10% by-product) [Found: M + Na (FAB-MS), 1231.1. $C_{49}H_{81}N_{11}O_{24}$ requires M, 1207.5]; δ_H (D_2O; MeOH) carbohydrate protons: 1.92, 1.93 and 1.95 (9 H, 3 s, 3 × Ac), 3.75 (4 H, m, 4 × 6-H), 3.83–3.92 (2 H, m, 2 × 3-H), 3.95 and 3.97 (2 H, 2 dd, 2 × 4-H), 4.02 (2 H, m, 2 × 5-H), 4.07 (1 H, dd, $J_{1,2}$ 4.07 $J_{2,3}$ 11.1, 2-H), 4.08 (1 H, dd, $J_{1,2}$ 3.56, $J_{2,3}$ 11.1, 2*-H), 4.94 (1 H, d, 1-H) and 5.01 (1 H, d, 1*-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonylglycyl-L-prolylglycinamide 21. Compound **21** (11.6 mg pure, 51%), 8.0 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1193.2. C_{49}-

† Corresponds to protons belonging to the same sugar unit.

$H_{81}N_{11}O_{23}$ requires M, 1191.55]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.00, 2.01 and 2.03 (9 H, 3 s, 3 × Ac) 3.76 (4 H, m, 4 × 6-H), 3.91 (1 H, dd, $J_{2,3}$ 10.7, $J_{3,4}$ 3.0, 3-H), 3.92 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.0, 3*-H), 3.98 (2 H, m, 2 × 4-H), 4.03 (1 H, m, 5-H), 4.05 (1 H, m, 5-H), 4.10 (1 H, dd, $J_{1,2}$ 4.07, 2*-H), 4.11 (1 H, dd, $J_{1,2}$ 3.6, 2-H), 4.94 (1 H, d, 1-H) and 5.02 (1 H, d, 1*-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonylglycyl-L-prolylglycinamide 22. Compound **22** (11.0 mg pure, 40%), 8.0 mg (with 10% by-product) [Found: MH^+ (FAB MS), 1396.1. $C_{57}H_{94}N_{12}O_{28}$ requires M, 1394.63]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.01 and 2.04 (12 H, 2 s, 4 × Ac), 3.72 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.05, 3-H), 3.73–3.82 (6 H, m, 6 × 6-H), 3.90 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.6, 3*-H), 3.96–4.05 (6 H, m, 3-H, 2 × 4-H, 3 × 5-H), 4.11 (1 H, dd, $J_{1,2}$ 3.6, 2*-H), 4.15 (1 H, dd, 4-H), 4.21 (1 H, dd, $J_{1,2}$ 3.6, 2-H), 4.23 (1 H, dd, $J_{1,2}$ 4.1, 2-H), 5.02 (1 H, d, 1*-H), 5.04 (1 H, d, 1-H) and 5.06 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonyl-*O*-[*O*-2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonylglycyl-L-prolylglycinamide 23. Compound **23** (12.9 mg pure, 47%), 7.9 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1396.1. $C_{57}H_{94}N_{12}O_{28}$ requires M, 1394.63]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.02 (12 H, 3 s, 4 × Ac), 3.71–3.82 (7 H, m, 3-H and 6 × 6-H), 3.90 (1 H, dd, $J_{3,4}$ 3.6, 3*-H), 3.97–4.05 (6 H, m, 3**-H, 2 × 4-H and 3 × 5-H), 4.08 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 10.7, 2*-H), 4.14 (1 H, dd, 4-H), 4.21 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 10.7, 2-H), 4.27 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2**-H), 4.92 (1 H, d, 1*-H), 5.07 (1 H, d, 1-H) and 5.11 (1 H, d, 1**-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonylglycyl-L-prolylglycinamide 24. Compound **24** (3.2 mg pure, 11%), 4.3 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1599.2. $C_{65}H_{107}N_{13}O_{33}$ requires M, 1597.7]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.02 and 2.03 (15 H, 2 s, 5 × Ac), 3.72 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.05, 3-H), 3.72–3.82 (4 H, m, 4 × 6-H), 3.82 (1 H, dd, 3-H), 4.00 (8 H, m, 2 × 3-H, 2 × 4-H and 4 × 5-H), 4.15 (2 H, m, 2 × 4-H), 4.21 (2 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 10.7, 2 × 2-H), 4.25 (1 H, dd, 2-H), 4.27 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 11.2, 2-H), 5.01 (1 H, d, $J_{1,2}$ 3.6, 1-H), 5.07 (2 H, d, $J_{1,2}$ 4.1, 2 × 1-H) and 5.11 (1 H, d, $J_{1,2}$ 3.6, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-[*O*-2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonylglycyl-L-prolylglycinamide 25. Compound **25** (10.9 mg pure, 42%), 7.9 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1396.3. $C_{57}H_{94}N_{12}O_{28}$ requires M, 1394.63]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.00, 2.01, 2.03 and 2.05 (12 H, 4 s, 4 × Ac), 3.67–3.79 (5 H, m, 5 × 6-H), 3.83–3.92 (4 H, m, 3 × 3-H and 6-H), 3.95–4.05 (5 H, m, 3 × 4-H and 2 × 5-H), 4.10 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2-H), 4.11 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2-H), 4.17 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2*-H), 4.20 (1 H, m, 5-H), 4.95 (1 H, d, 1*-H), 4.96 (1 H, d, 1-H) and 5.02 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonylglycyl-L-prolylglycinamide 26. Compound **26** (10.5 mg pure, 38%), 9.3 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1396.5. $C_{57}H_{94}N_{12}O_{28}$ requires M, 1394.63]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.00, 2.02 and 2.06 (12 H, 3 s, 4 × Ac), 3.68–3.79 (5 H, m, 5 × 6-H), 3.83–3.94 (4 H, m, 3 × 3-H and 6-H), 3.97–4.02 (4 H, 3 × 4-H and 5-H), 4.05 (1 H, m, 5-H), 4.09 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 10.2, 2-H), 4.15 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2*-H), 4.18 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 10.7, 2**-H), 4.21 (1 H, m, 5-H), 4.94 (1 H, d, 1-H), 4.96 (1 H, d, 1**-H) and 5.04 (1 H, d, 1*-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-[*O*-2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonylglycyl-L-prolylglycinamide 27. Compound **27** (7.0 mg pure, 24%), 5.1 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1599.5. $C_{65}H_{107}N_{13}O_{33}$ requires M, 1597.71]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.01–2.06 (15 H, 4 s, 5 × Ac), 3.69–3.80 (10 H, m, 10 × 6-H), 3.83–4.05 (12 H, m, 4 × 3-H, 4 × 4-H, 2 × 5-H and 2 × 6-H), 4.12 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2-H), 4.13 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 11.2, 2*-H), 4.15–4.23 (4 H, m, 2 × 2-H and 2 × 5-H), 4.97 (3 H, d, $J_{1,2}$ 3.6, 3 × 1-H) and 5.03 (1 H, d, $J_{1,2}$ 4.1, 1*-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-valylglycyl-L-prolylglycinamide 28. Compound **28** (10.0 mg pure, 57%), 6.1 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 988.1. $C_{42}H_{70}N_{10}O_{17}$ requires M, 986.49]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.03 and 2.04 (6 H, 2 s, 2 × Ac), 3.76 (2 H, m, 2 × 6-H), 3.89 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.05, 3-H), 4.99 (1 H, dd, 4-H), 4.03 (1 H, m, 5-H), 4.12 (1 H, dd, $J_{1,2}$ 3.6, 2-H) and 4.86 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-valylglycyl-L-prolylglycinamide 29. Compound **29** (9.0 mg pure, 41%), 7.1 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1191.3. $C_{50}H_{83}N_{11}O_{22}$ requires M, 1189.57]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.02, 2.03 and 2.06 (9 H, 3 s, 3 × Ac), 3.72 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.05, 3-H), 3.73–3.82 (4 H, m, 2 × 6-H), 3.94–4.02 (5 H, m, 3*-H, 2 × 4-H and 2 × 5-H), 4.18 (1 H, dd, $J_{1,2}$ 4.1, 2-H), 4.23 (1 H, dd, $J_{1,2}$ 4.1, 2*-H), 4.89 (1 H, d, 1*-H) and 5.06 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-valylglycyl-L-prolylglycinamide 30. Compound **30** (9.2 mg pure, 42%), 8.7 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1190.8. $C_{50}H_{83}N_{11}O_{22}$ requires M, 1189.57]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.05 and 2.06 (9 H, 2 s, 3 × Ac), 3.71 (1 H, dd, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 10.7, 6-H^a), 3.77 (2 H, m, 2 × 6-H), 3.83–3.94 (3 H, m, 2 × 3-H and 6-H), 4.00 (3 H, m, 2 × 4-H and 5-H), 4.14 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2-H), 4.18 (1 H, dd, $J_{1,2}$ 4.6, $J_{2,3}$ 11.2, 2*-H), 4.21 (1 H, m, 5-H), 4.87 (1 H, d, 1-H) and 4.96 (1 H, d, 1*-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-L-prolyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonylglycyl-L-prolylglycinamide 31. Compound **31** (8.9 mg pure, 47%), 8.7 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 985.7. $C_{42}H_{68}N_{10}O_{17}$ requires M, 984.47]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.03 (6 H, s, 2 × Ac), 3.77 (2 H, m, 2 × 6-H), 3.93 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.6, 3-H), 3.98 (1 H, dd, 4-H), 4.04 (1 H, m, 5-H), 4.12 (1 H, dd, $J_{1,2}$ 3.6, 2-H) and 5.00 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-L-prolyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonylglycyl-L-prolylglycinamide 32. Compound **32** (10.7 mg pure, 47%), 9.4 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1189.1. $C_{50}H_{81}N_{11}O_{22}$ requires M, 1187.55]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.02 and 2.03 (9 H, 2 s, 3 × Ac), 3.72–

3.79 (5 H, m, 3-H and 4 × 6-H), 4.01 (4 H, m, 3*-H, 4-H and 2 × 5-H), 4.14 (1 H, dd, 4*-H), 4.21 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 11.2, 2-H), 4.27 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 11.2, 2*-H), 5.05 (1 H, d, 1*-H) and 5.07 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-L-prolyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonylglycyl-L-prolylglycinamide 33. Compound **33** (7.4 mg pure, 32%), 5.3 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1189.1. $C_{50}H_{81}N_{11}O_{22}$ requires M, 1189.56]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.01, 2.02 and 2.04 (9 H, 3, s, 3 × Ac), 3.71 (1 H, dd, 6-H), 3.87 (2 H, m, 2 × 6-H), 3.84–3.93 (3 H, m, 3-H, 3*-H and 6-H), 3.97–4.04 (3 H, m, 2 × 4-H and 5-H), 4.11 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 11.2, 2-H), 4.18 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 11.2, 2*-H), 4.23 (1 H, m, 5-H), 4.97 (1 H, d, 1*-H) and 4.98 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-glutaminylglycyl-L-prolylglycinamide 34. Compound **34** (9.8 mg pure, 54%), 8.7 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1016.7. $C_{42}H_{69}N_{11}O_{18}$ requires M, 1015.48]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.03 (6 H, s, 2 × Ac), 3.76 (2 H, m, 2 × 6-H), 3.88 (1 H, dd, $J_{2,3}$ 10.7, $J_{3,4}$ 3.05, 3-H), 3.99 (1 H, dd, 4-H), 4.02 (1 H, m, 5-H), 4.11 (1 H, dd, $J_{1,2}$ 3.6, 2-H) and 4.91 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-glutaminylglycyl-L-prolylglycinamide 35. Compound **35** (6.3 mg pure, 29%), 6.6 mg (with 10% by-product) [Found MH^+ (FAB-MS), 1219.9. $C_{50}H_{82}N_{12}O_{23}$ requires M, 1218.56]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.02, 2.03 and 2.05 (9 H, 3 s, 3 × Ac), 3.72 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.05, 3-H), 3.74–3.83 (4 H, m, 4 × 6-H), 3.91 (1 H, m, 5-H), 3.98 (1 H, dd, $J_{2,3}$ 10.7, $J_{3,4}$ 3.6, 3*-H), 4.00 (3 H, m, 2 × 4-H and 5-H), 4.21 (1 H, dd, $J_{1,2}$ 4.1, 2-H), 4.23 (1 H, dd, $J_{1,2}$ 4.1, 2*-H), 4.97 (1 H, d, 1*-H) and 5.08 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-glutaminylglycyl-L-prolylglycinamide 36. Compound **36** (4.5 mg pure, 19%), 2.9 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1221.2. $C_{50}H_{82}N_{12}O_{23}$ requires M, 1218.56]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.04 and 2.05 (9 H, 2 s, 3 × Ac),3.70 (1 H, dd, 6-H), 3.77 (2 H, m, 2 × 6-H), 3.74–3.94 (3 H, m, 2 × 3-H and 6-H), 4.00 (3 H, m, 2 × 4-H and 5-H), 4.14 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 10.7, 2-H), 4.19 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 11.2, 2*-H), 4.91 (1 H, d, 1-H) and 4.95 (1 H, d, 1*-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-L-glutaminyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonylglycyl-L-prolylglycinamide 37. Compound **37** (9.0 mg pure, 45%), 8.4 mg (with 10% by-product) [Found: M + Na^+ (FAB-MS), 1039.0. $C_{42}H_{69}N_{11}O_{18}$ requires M, 1015.48]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.02 and 2.03 (6 H, 2 s, 2 × Ac), 3.76 (2 H, m, 2 × 6-H), 3.82 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.05, 3-H), 3.99 (1 H, dd, 4-H), 4.04 (1 H, m, 5-H), 4.11 (1 H, dd, $J_{1,2}$ 3.6, 2-H) and 4.99 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-L-glutaminyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonylglycyl-L-prolylglycinamide 38. Compound **38** (7.7 mg pure, 33%), 9.3 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1219.9 $C_{50}H_{82}N_{12}O_{23}$ requires M, 1218.56]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.04 and 2.05 (9 H, 2 s, 3 × Ac), 3.73–3.87 (5 H, m, 3-H and 4 × 6-H), 3.95–4.03 (4 H, m, 3*-H, 4-H and 2 × 5-H), 4.13 (1 H, dd, 4*-H), 4.21 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2-H), 4.28 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 11.2, 2*-H), 5.06 (1 H, d, 1*-H) and 5.08 (1 H, d, 1-H).

N^{α}-Acetyl-L-glutamyl-L-leucyl-L-alanyl-L-glutaminyl-*O*-[*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonylglycyl-L-prolylglycinamide 39. Compound **39** (10.1 mg pure, 42%), 7.8 mg (with 10% by-product) [Found: MH^+ (FAB-MS), 1220.1. $C_{50}H_{82}N_{12}O_{23}$ requires M, 1218.56]; $\delta_H(D_2O$; MeOH) carbohydrate protons: 2.01, 2.02 and 2.03 (9 H, 3 s, 3 × Ac), 3.69 (1 H, dd, 6-H), 3.76 (2 H, m, 2 × 6-H), 3.81–3.95 (3 H, m, 2 × 3-H and 6-H), 4.00 (3 H, m, 2 × 4-H and 5-H), 4.11 (1 H, dd, $J_{1,2}$ 4.1, $J_{2,3}$ 11.2, 2-H), 4.18 (1 H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 11.2, 2*-H), 4.94 (1 H, d, 1*-H) and 4.99 (1 H, d, 1-H).

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