Multiple-column Solid-phase Glycopeptide Synthesis

Stefan Peters,[a] Tim Bielfeldt,[a] Morten Meldal,[b] Klaus Bock[b] and Hans Paulsen[a]
[a] *Institut für Organische Chemie, Martin-Luther-King-Platz 6, D-2000 Hamburg 13, Germany*
[b] *Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark*

The preparation of the two new building blocks N^{α}-Fmoc-Ser(Ac_3-α-D-Gal*p*NAc)-OPfp **6** and N^{α}-Fmoc-Thr(Ac_3-α-D-Gal*p*NAc)-OPfp **7** and their application in a simultaneous multiple-column solid-phase synthesis of 40 different *O*-glycopeptides from human intestinal mucin and porcine submaxillary gland mucin are described. All glycopeptides were obtained in excellent purity and were characterised by 1D- and 2D-^{1}H NMR spectroscopy and amino acid analyses.

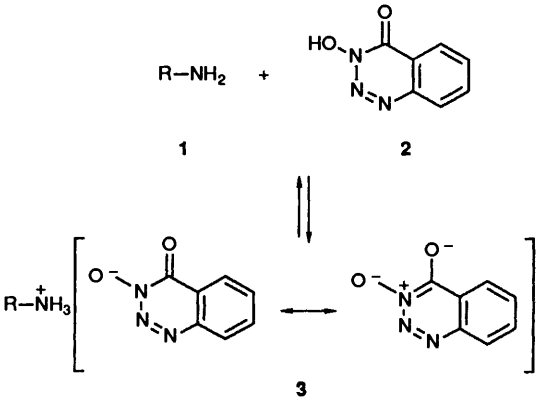
In recent years there has been an increasing interest in glycoproteins. The surface carbohydrates on glycoproteins and cell membranes are involved in important biological recognition phenomena and transport processes.[1] The isolation of glycoproteins from natural sources is difficult or often impossible due to the low concentration of homogeneous glycoconjugates in biosystems. Therefore, the chemical synthesis of glycoprotein fragments as model compounds for structure/activity studies is indispensable.

In the biosynthesis of *N* glycoproteins the peptide sequence Asn-Xxx-Thr/Ser (Xxx = any amino acid) initiates the enzymatic transfer of an oligosaccharide from an isoprenyl pyrophosphate precursor.[2] In contrast a specific recognition sequence has not been found for the biosynthetic assembly of the *O*-glycoproteins even though proline-rich regions have been suggested as being preferred.[3] Once a first *N*-acetylgalactosamine (GalNAc) moiety is attached to the peptide backbone the different mucin-type *O*-glycan core structures are constructed stepwise by specific enzymes.[4] In order to establish how the peptide sequence influences the activity of the enzymes that act on the GalNAc residue we synthesized several *O*-glycopeptides containing a GalNAc α-linked to the hydroxy group of serine and threonine.[5-7] These products were used as substrates for different glycosyltransferases. It was demonstrated that the activity of the β3-galactosyltransferase, which glycosylates the 3-OH position in GalNAc residues, is influenced by the structure of the peptide moiety.[8] The kinetic data of these experiments indicate that the transferase activity is dependent on the peptide sequence, the chain length, the attachment site of the GalNAc sugar, and the number of GalNAc residues in the substrates. The uncharged *N*-acetyl glycopeptide carboxamides resembling a natural protein were much better substrates than the corresponding molecules with charges at the *N*- and *C*-termini. For a more detailed enzymatic investigation a larger series of synthetic *O*-glycopeptides with a systematic variation of structure and glycosylation site on the peptide was required, thereby suggesting the application of multiple-column synthesis.

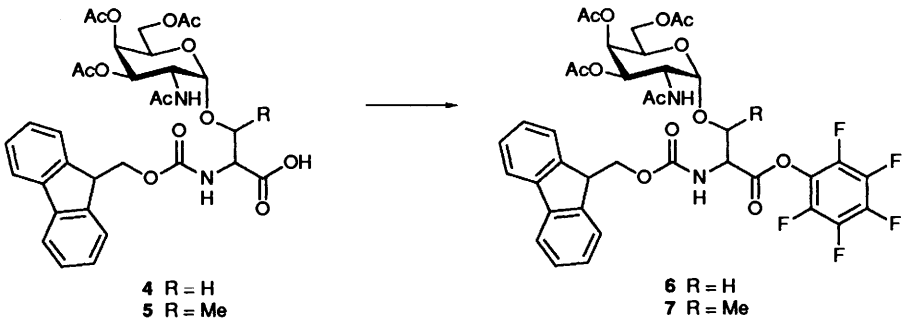
Several chemical methods for the synthesis of glycopeptides have been described.[9] However, the direct *O*-glycosylation of protected peptide fragments is in general not feasible due to the low solubility and reactivity of these peptides in the organic solvents required for glycosylation reactions.[10,11] The *in vitro* enzymatic glycosylation is frequently prohibited by the lack of isolated enzymes. The currently most efficient strategy for the assembly of glycopeptides is the stepwise, solid-phase synthesis using glycosylated amino acids as building blocks.[12-25] The *O*-glycosidic bond to serine and threonine is, however, sensitive to both strong acids and bases so that only a limited number of orthogonal protection groups may be utilised.

Results and Discussion

In this paper the application of the two new building blocks **6** and **7** is described.[26] The protecting-group pattern of these building blocks is suitable for solid-phase glycopeptide synthesis. The use of the fluoren-9-ylmethoxycarbonyl (Fmoc) group allows deprotection of the α-amino group under mild conditions with morpholine to occur without β-elimination of the carbohydrate.[27] *O*-Acetyl groups were employed for protection of the carbohydrate part due to their easy removal with sodium methoxide or hydrazine in methanol. The carboxy group is highly activated as the pentafluorophenyl ester for aminolysis and peptide-bond formation.[19,23,28] The reactivity of the pentafluorophenyl ester was further enhanced by the addition of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH). The use of Dhbt-OH as an auxillary nucleophile allows the progress of the peptide-bond formation to be followed visually.[29] A bright yellow colour of the ion pair **3**, formed between resin-bound amino groups **1** and Dhbt-OH **2**, fades away during the acylation.

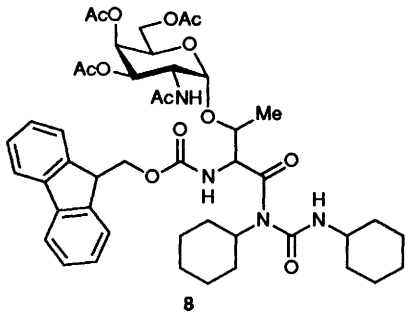


The key compounds **6** and **7** were synthesized by esterification of the acids **4** and **5**[5] using pentafluorophenol (Pfp-OH) and dicyclohexylcarbodiimide (DCCI). Initial experiments using 1.1 mol equiv. of both Pfp-OH and DCCI in tetrahydrofuran (THF) or ethyl acetate at 0 °C showed only 50% conversion of acids **4** and **5** into the desired products. The yield could not be improved by raising the excess of Pfp-OH and DCCI (1.5 mol equiv.) or by raising the temperature. At temperatures above 0 °C the *N*-acylurea **8** is formed as a by-product. The best results were obtained on addition of 1.1 mol equiv. of both Pfp-OH and DCCI at 0 °C in an as saturated as possible solution of substrate **4** or **5** in ethyl acetate. Under these conditions 75% of the activated ester **6** or **7** was isolated as a stable, solid material after



4 R = H
5 R = Me

6 R = H
7 R = Me



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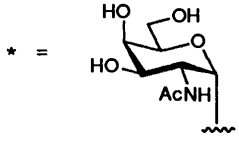
Reagents: Pfp-OH, DCCI.



11 Ac-P-T-T-$\overset{*}{T}$-P-I-S-T-NH_2	**25** Ac-P-T-G-$\overset{*}{T}$-P-I-S-T-NH_2	**39** Ac-P-S-T-$\overset{*}{T}$-P-I-S-T-NH_2
12 Ac-P-T-$\overset{*}{T}$-T-P-I-S-T-NH_2	**26** Ac-P-$\overset{*}{T}$-G-T-P-I-S-T-NH_2	**40** Ac-P-S-S-$\overset{*}{T}$-P-I-S-T-NH_2
13 Ac-P-$\overset{*}{T}$-T-T-P-I-S-T-NH_2	**27** Ac-P-G-T-$\overset{*}{T}$-P-I-S-T-NH_2	**41** Ac-P-T-$\overset{*}{T}$-S-P-I-S-T-NH_2
14 Ac-P-T-T-$\overset{*}{T}$-G-I-S-T-NH_2	**28** Ac-P-G-$\overset{*}{T}$-T-P-I-S-T-NH_2	**42** Ac-P-S-$\overset{*}{T}$-T-P-I-S-T-NH_2
15 Ac-P-T-$\overset{*}{T}$-T-G-I-S-T-NH_2	**29** Ac-P-T-P-$\overset{*}{T}$-P-I-S-T-NH_2	**43** Ac-P-S-$\overset{*}{T}$-S-P-I-S-T-NH_2
16 Ac-P-$\overset{*}{T}$-T-T-G-I-S-T-NH_2	**30** Ac-P-$\overset{*}{T}$-P-T-P-I-S-T-NH_2	**44** Ac-P-$\overset{*}{T}$-T-S-P-I-S-T-NH_2
17 Ac-G-T-T-$\overset{*}{T}$-P-I-S-T-NH_2	**31** Ac-P-T-$\overset{*}{T}$-$\overset{*}{T}$-P-I-S-T-NH_2	**45** Ac-P-$\overset{*}{T}$-S-T-P-I-S-T-NH_2
18 Ac-G-T-$\overset{*}{T}$-T-P-I-S-T-NH_2	**32** Ac-P-$\overset{*}{T}$-T-$\overset{*}{T}$-P-I-S-T-NH_2	**46** Ac-P-$\overset{*}{T}$-S-S-P-I-S-T-NH_2
19 Ac-G-$\overset{*}{T}$-T-T-P-I-S-T-NH_2	**33** Ac-P-$\overset{*}{T}$-T-T-P-I-S-T-NH_2	**47** Ac-P-T-T-$\overset{*}{T}$-S-I-S-T-NH_2
20 Ac-G-T-T-$\overset{*}{T}$-G-I-S-T-NH_2	**34** Ac-P-$\overset{*}{T}$-$\overset{*}{T}$-$\overset{*}{T}$-P-I-S-T-NH_2	**48** Ac-G-S-S-$\overset{*}{S}$-G-S-P-G-NH_2
21 Ac-G-T-$\overset{*}{T}$-T-G-I-S-T-NH_2	**35** Ac-P-T-T-$\overset{*}{S}$-P-I-S-T-NH_2	**49** Ac-G-S-$\overset{*}{S}$-S-G-S-P-G-NH_2
22 Ac-G-$\overset{*}{T}$-T-T-G-I-S-T-NH_2	**36** Ac-P-T-$\overset{*}{S}$-T-P-I-S-T-NH_2	**50** Ac-G-$\overset{*}{S}$-S-S-G-S-P-G-NH_2
23 Ac-P-T-$\overset{*}{T}$-G-P-I-S-T-NH_2	**37** Ac-P-$\overset{*}{S}$-T-T-P-I-S-T-NH_2	
24 Ac-P-$\overset{*}{T}$-T-G-P-I-S-T-NH_2	**38** Ac-P-T-S-$\overset{*}{T}$-P-I-S-T-NH_2	

Fig. 1 *N*-Acetylglycopeptide carboxamides **11**–**50** synthesized by a multiple-column technique

flash chromatography on dry silica gel and precipitation from diethyl ether–pentane. A substantial portion (24%) of the unchanged acid **4** or **5** was recovered in each case.

The two glycosylamino acids **6** and **7** were used in a multiple-column solid-phase glycopeptide synthesis. As target molecules parts of the repeating units from human intestinal mucin octapeptide **9**, and porcine submaxillary gland mucin octapeptide **10**, were selected.[30,31]

Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr **9**

Gly-Ser-Ser-Ser-Gly-Ser-Pro-Gly **10**

Based on these two octapeptides, a series of 40 *O*-glycosylated *N*-acetyl peptide carboxamides were designed and synthesized as shown in Fig. 1. In the natural protein it is not known which hydroxy amino acid is glycosylated. However, the glycosylation sites of the designed glycopeptides were shifted between the three successive threonines (serines). The three *C*-terminal amino acids were left unchanged while the other amino acids near the glycosylation site were systematically substituted by glycines, prolines or serines.

The multiple-column peptide synthesizer[32,33] used for the simultaneous assembly of 40 different glycopeptides consists of a steel frame equipped with two parallel 96-channel washers,

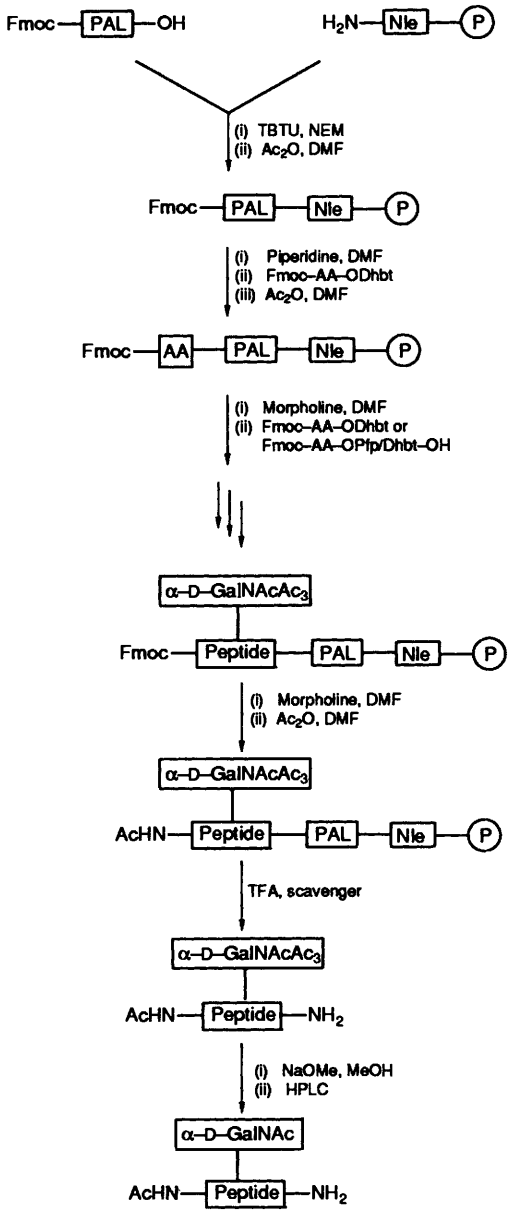
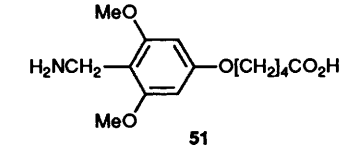


Fig. 2 Principle of multiple-column solid-phase glycopeptide synthesis. P, poly(dimethylacrylamide)resin; Nle, norleucine; AA, amino acid.

one connected to a dispensing bottle with *N,N*-dimethylformamide (DMF) and one to a dispensing bottle with deprotection reagent. The synthesis was carried out in a block of Teflon with 96 wells in an ELISA-type arrangement. Each well was equipped with a Teflon filter and a bottom outlet for either vacuum or nitrogen pressure. The columns (82 of the 96 wells were used for synthesis) were packed with 25 mg of resin each. All the glycopeptides were synthesized in two neighbouring wells. The addition of DMF or deprotection reagent was carried out from the dispensers while the Teflon block was mounted on an elevator table under the respective washers. The amino acid active esters were dissolved in a separate reagent tray with 96 compartments and were added to the resins by an 8-channel multipipette. During acylation steps the reactor was placed on a rocker table and a weak excess of pressure in the chamber below the wells ensured that the reagents remained in the columns. The resins were washed 4 times after acylation and 5 times after removal of Fmoc. The deprotection reagent contained a small amount of the deep red dye Azoruby to indicate a complete washing procedure.

Fig. 2 outlines the principle of the parallel solid-phase glycopeptide synthesis performed on a Kieselguhr-supported poly(dimethylacrylamide) resin[34] which was derivatised with norleucine as internal reference amino acid. Several linkers for a direct synthesis of peptide amides have been reported.[35] The acid lability, however, of the 5-(4-aminomethyl-3,5-dimethoxyphenoxy)valeric acid (PAL)[36] **51** or the *p*-(α-amino-2,4-dimethoxybenzyl)phenoxyacetic acid (Rink linker)[37] **52** is adequate considering the sensitivity of the glycosidic bond toward strong acids.[38] The PAL group was selected for the first assembly of glycopeptides.



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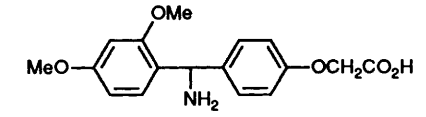

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The Fmoc-protected PAL group was coupled to the resin by activation with *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU)[39] and 4-ethylmorpholine (NEM). Non-reacted amino groups were capped with acetic anhydride in DMF. After removal of the Fmoc group with 20% piperidine in DMF the first amino acid was coupled to the linker as the Dhbt-ester. The incorporation of the first amino acid was 92% and 93% for threonine and for glycine, respectively, as estimated by amino acid analyses. The resin was again capped with Ac_2O in DMF. The resin was washed with diethyl ether, dried, and measured into 82 of the 96 wells of the Teflon block in which the parallel syntheses were continued. All the following Fmoc deprotections were effected under mild conditions with 50% morpholine in DMF to prevent β-elimination of the carbohydrate moiety. The non-glycosylated Fmoc-amino acids were introduced into the peptide chain as Dhbt-esters. The glycosyl amino acid building blocks **6** and **7** were used with the addition of Dhbt-OH as auxiliary nucleophile. The progress of the acylation reactions was indicated by the gradual disappearance of the yellow colour, and to assure complete peptide-bond formation the acylation reactions were in all cases continued overnight. The side-chains of non-glycosylated serine and threonine residues were protected as *tert*-butyl ethers. After coupling of the last amino acids the Fmoc groups were cleaved and the *N*-termini were acetylated with Ac_2O in DMF. The resins were simultaneously transferred into 82 small glass vials held in the 96 holes of a reagent tray. Cleavage of the glycopeptides was performed with 95% aq. trifluoroacetic acid (TFA) with concurrent removal of the Bu^t groups. The amino acid analyses (Table 1) of the crude products were all correct within 8%. Finally, the *O*-acetyl groups of the carbohydrates were removed with catalytic amounts of sodium methoxide in methanol at pH 8–9 (measured on dry pH paper). All deacetylations were complete after 3 h even in the case of polyglycosylated peptides and occurred without any β-elimination of the carbohydrate part as shown by TLC and HPLC, in accord with previous results.[23] The glycopeptides were purified by preparative reversed-phase HPLC. The purity of the final products was excellent as exemplified by analytical HPLC (Fig. 3).

In two columns the synthesis was carried out on Tenta Gel S® support[40] [polystyrene-based poly(ethylene glycol)-grafted gel-type support]. This matrix is supposed to have better

Table 1 Amino acid analyses of the crude glycopeptides **11–50**. Relative values referring to Ile = 1.00 (glycopeptides **11–47**) or Pro = 1.00 (glycopeptides **48–50**).

Peptide	Thr	Ser	Pro	Gly	Peptide	Thr	Ser	Pro	Gly	Peptide	Thr	Ser	Pro	Gly	Peptide	Thr	Ser	Pro	Gly
11	4.17	0.99	2.06		**21**	3.93	1.02		1.93	**31**	3.97	1.03	2.05		**41**	2.95	1.90	2.04	
12	4.00	1.05	2.06		**22**	3.76	1.06		2.0	**32**	4.04	1.03	2.04		**42**	2.71	2.08	2.04	
13	3.91	1.04	2.02		**23**	2.90	1.00	2.01	0.97	**33**	4.07	1.05	2.05		**43**	1.96	2.86	1.98	
14	3.88	0.98	1.00	1.01	**24**	2.91	0.97	2.03	0.98	**34**	4.06	1.04	2.05		**44**	3.05	1.98	2.04	
15	4.43	1.14	2.64	0.72	**25**	2.99	1.01	2.06	0.99	**35**	2.97	1.95	2.03		**45**	2.98	1.84	2.09	
16	3.77	1.05	0.91	1.01	**26**	3.06	1.05	2.12	1.01	**36**	3.01	1.92	2.01		**46**	1.95	2.74	2.02	
17	3.97	1.03	1.02	0.95	**27**	2.94	0.99	2.10	0.99	**37**	2.93	1.95	2.0		**47**	3.98	1.92	1.0	
18	4.00	1.04	1.02	0.99	**28**	3.03	1.03	2.11	1.04	**38**	2.99	1.94	2.02		**48**		3.4	1.0	2.97
19	3.95	1.04	1.03	1.01	**29**	2.99	1.03	3.0		**39**	2.87	1.80	1.99		**49**		3.37	1.0	2.87
20	3.93	1.01		1.96	**30**	3.0	1.04	3.03		**40**	2.08	2.96	2.09		**50**		3.60	1.0	3.05

Table 2 Optimised cleavage conditions for compound **13**

Entry	Linker	Reagent for cleavage[a]	Yield[b] (%)	Remaining peptide[c] on support (%)
1	**51**	TFA, water (95:5)	93	2.6
2	**51**	TFA CH_2Cl_2 Me_2S (14:5:1)	88	2.4
3	**51**	TFA CH_2Cl_2 (70:30)	78	3.5
4	**52**	TFA, water (95:5)	81	5.7
5	**52**	TFA CH_2Cl_2 (70:30)	81	9.7

[a] Conditions: 1 cm^3 reagent/30 mg resin; 2 h at 23 °C; 5 times wash with TFA (1 cm^3). [b] After deacetylation and purification (HPLC). [c] Determined by amino acid analysis.

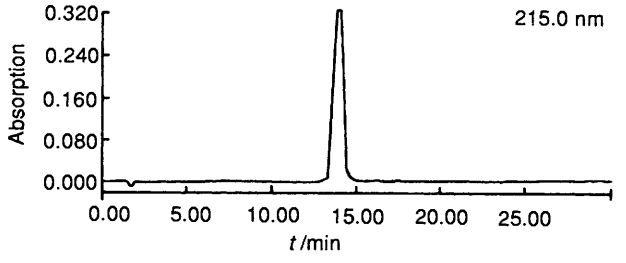

Fig. 3 Analytical HPLC chromatogram of the purified glycopeptide **33**. Conditions: buffer A–buffer B 99:1 ⟶ 75:25 (20 min) ⟶ 30:70 (10 min) (see the Experimental section).

properties for solid-phase synthesis due to its monodisperse character. The performance of this polymer and the purity of the product were equivalent to the results obtained with the polyacrylamide resin. The transfer, however, of the Tenta Gel resin into vials was hampered by the adherence of the dried beads to the Teflon and glass caused by static electricity.

The pure *O*-glycopeptides were obtained in yields of 30–40% after lyophilisation based on the substitution of the resin. An amino acid analysis of one resin sample after the cleavage with TFA indicated that 30% of the peptide product still remained in the support either covalently bound to the linker or not washed out during the TFA treatment. The latter is possible since the TFA solutions were recovered from the cleavage reactions by decantation of the TFA from the resin in order to facilitate the simultaneous handling of 40 samples. To determine the optimal cleavage procedure the synthesis of compound **13** was repeated with the same technique as described above in a multiple peptide synthesizer with 20 columns. Both the PAL and the Rink-linker were used to anchor the peptides to the support. Cleavage of the glycopeptides from the resins was performed by 2 h reactions with different mixtures of TFA and scavengers as summarised in Table 2. The resins were washed five times with TFA after the cleavage. The crude products were deacetylated as described, purified by reversed-phase HPLC, and weighed. The remaining supports were hydrolysed and analysed by amino acid analyses to determine the amount of glycopeptide retained. In case of the PAL-linker the glycopeptides from the 20 column synthesis were obtained in very high yields of 78–93%. Only about 3% of the peptide remained on the supports. It was demonstrated that the cleavage conditions did not influence the yield of the glycopeptide significantly. The amide bond to the Rink linker seems to be slightly more stable towards acid. About 6–10% of the peptides were not released after the 2 h TFA treatement. The purity of the glycopeptides synthesized on the Rink- and the PAL-linker was equally excellent. We therefore conclude that the reduced yields in the synthesis of the first 40 glycopeptides were mainly due to incomplete removal of peptide solutions from the resins.

In addition to the amino acid analyses the *O*-glycopeptides were characterised by 1D- and 2D-^{1}H NMR spectroscopy (Tables 3–5). The chemical shifts of the anomeric protons vary significantly in contrast to all other carbohydrate protons that have nearly constant chemical-shift values. The assignments of protons to specific amino acids may be interchanged when there are several identical amino acids in the sequence. The connectivities of α,β,γ and δ protons were, however, in all cases accessed by ^{1}H–^{1}H-cosy experiments. The glycosylated amino acids could be distinguished from non-glycosylated residues by a significant shift of the α-protons to lower field and, in the case of threonines, by smaller α–β coupling constants.

Preliminary results in the evaluation of the glycopeptides as substrates for the 1-3-β-galactosyltransferase indicate that the peptide substitution pattern has a large influence on the activity of the enzyme. The influence is complex and will be described 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. Fmoc amino acid Dhbt-esters[29] and the PAL-linker[36] were synthesized as previously described. Reagents for peptide synthesis were purchased as follows: DCCI, Dhbt-OH and TBTU from Fluka; Macrosorb SPR 500 from Sterling Organics; Tenta Gel S® from Rapp Polymere; Fmoc-protected Rink linker from Novabiochem. ^{1}H NMR spectra were recorded on a Bruker AMX 400 MHz spectrometer; δ-values are in ppm and *J*-values are in Hz. HPLC was performed on a Merck/Hitachi HPLC system with Li Chrospher C_{18} reversed-phase columns (250 × 4, 10 μm; flow rate 2 cm^3/min for analytical and 250 × 25, 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. Mass spectra were recorded on a double-focused VG-Analytical 70-250S mass spectrometer

Table 3 ^{1}H NMR data [400 MHz, internal reference DOH (δ 4.8) at 300 K] of the carbohydrate and acetate protons of the glycopeptides **11**–**50** (δ-values given in ppm, J-values given in Hz)

Peptide	1-H ($J_{1,2}$)	2-H ($J_{2,3}$)	3-H ($J_{3,4}$)	4-H	5-H	6-H^a,6-H^b	Ac
11	5.10 (3.8)	4.16 (11.0)	3.93 (3.2)	4.03	4.09	3.83–3.78	2.18; 2.08
12	4.87 (3.8)	4.15 (11.0)	3.93 (3.2)	4.02	4.06	3.85–3.75	2.18; 2.11
13	4.99 (3.6)	4.15 (10.8)	3.95 (3.2)	4.04	4.10	3.86–3.77	2.17; 2.08
14	5.02 (3.8)	4.16 (11.2)	3.96 (3.0)	4.03	4.08	3.83–3.78	2.18; 2.07
15	4.98 (3.8)	4.17 (11.0)	3.95 (3.2)	4.03	4.08	3.83–3.77	2.18; 2.08
16	4.98 (3.8)	4.16 (11.0)	3.95 (3.2)	4.03	4.10	3.86–3.77	2.18; 2.10
17	5.04 (3.8)	4.16 (11.0)	3.93 (3.2)	4.03	4.09	3.84–3.78	2.12; 2.07
18	4.88 (3.8)	4.15 (11.0)	3.93 (3.2)	4.02	4.07	3.85–3.78	2.12; 2.11
19	5.00 (3.8)	4.15 (11.0)	3.91 (3.2)	4.04	4.07	3.83–3.77	2.11; 2.08
20	5.01 (3.8)	4.15 (11.0)	3.96 (3.0)	4.03	4.08	3.82–3.77	2.12; 2.07
21	4.98 (3.8)	4.16 (10.8)	3.93 (3.0)	4.03	4.08	3.82–3.77	2.12; 2.08
22	4.99 (3.8)	4.15 (11.2)	3.92 (3.2)	4.03	4.07	3.83–3.77	2.11; 2.10
23	5.04 (3.8)	4.15 (11.0)	3.95 (3.2)	4.02	4.08	3.83–3.78	2.18; 2.06
24	4.97 (3.8)	4.16 (11.0)	3.94 (3.2)	4.04	4.09	3.83–3.77	2.17; 2.07
25	5.08 (3.8)	4.16 (11.0)	3.91 (3.2)	4.03	4.08	3.83–3.78	2.18; 2.08
26	5.03 (3.8)	4.15 (11.0)	3.95 (3.2)	4.04	4.10	3.83–3.79	2.18; 2.06
27	5.10 (3.8)	4.16 (11.8)	3.92 (3.2)	4.01	4.08	3.83–3.77	2.18; 2.07
28	4.87 (3.8)	4.15 (11.0)	3.93 (3.2)	4.02	4.06	3.83–3.77	2.18; 2.10
29	5.08 (3.8)	4.17 (11.0)	3.94 (3.2)	4.03	4.12	3.86–3.76	2.17; 2.07
30	5.08 (3.8)	4.17 (11.0)	3.93 (3.2)	4.04	4.10	3.87–3.77	2.17; 2.09
31	5.07 (3.8),	4.15 (11.0),	3.93 (3.2),	4.02,	4.07,	3.85–3.77	2.18; 2.08;
	4.89 (3.8)	4.12 (11.0)	3.93 (3.2)	4.02	4.06		2.08
32	5.09 (3.8),	4.15 (11.0),	3.94 (2.8),	4.03,	4.09,	3.85–3.77	2.17; 2.09;
	5.00 (3.8)	4.15 (11.0)	3.91 (3.0)	4.03	4.09		2.08
33	4.93 (3.8),	4.14 (11.0),	3.94 (3.2),	4.01,	4.10,	3.85–3.77	2.17; 2.11;
	4.84 (3.8)	4.12 (11.2)	3.91 (3.2)	4.03	4.05		2.10
34	5.06 (3.8),	4.15 (11.0),	3.94 (3.2),	4.04,	4.09,	3.84–3.76	2.18; 2.11;
	4.90 (3.8),	4.13 (11.0),	3.92 (3.2),	4.03,	4.09,		2.10; 2.08
	4.88 (3.8)	4.09 (11.0)	3.91 (3.2)	4.01	4.04		
35	4.99 (3.6)	4.23 (11.0)	3.93 (3.2)	4.01	3.98	3.82–3.73	2.18; 2.09
36	4.93 (3.6)	4.22 (11.0)	3.89 (3.2)	3.99	3.89	3.82–3.75	2.17; 2.09
37	4.97 (3.6)	4.23 (11.0)	3.93 (3.2)	4.02	3.96	3.83–3.76	2.17; 2.09
38	5.08 (3.8)	4.16 (11.0)	3.92 (3.2)	4.02	4.09	3.94–3.77	2.18; 2.08
39	5.09 (3.8)	4.16 (11.0)	3.93 (3.2)	4.02	4.09	3.83–3.77	2.18; 2.07
40	5.09 (3.8)	4.16 (11.0)	3.93 (3.2)	4.02	4.09	3.83–3.77	2.18; 2.08
41	4.94 (3.8)	4.15 (11.0)	3.93 (3.2)	4.02	4.06	3.83–3.74	2.18; 2.09
42	4.88 (3.8)	4.16 (11.0)	3.94 (3.2)	4.02	4.07	3.83–3.74	2.18; 2.11
43	4.94 (3.8)	4.15 (11.0)	3.93 (3.2)	4.02	4.07	3.87–3.75	2.18; 2.09
44	4.99 (3.8)	4.16 (11.0)	3.94 (3.2)	4.04	4.10	3.86–3.73	2.18; 2.08
45	5.02 (3.8)	4.15 (11.0)	3.95 (3.2)	4.04	4.09	3.86–3.76	2.17; 2.09
46	5.02 (3.8)	4.16 (11.0)	3.94 (3.2)	4.04	4.09	3.84–3.75	2.17; 2.09
47	4.99 (3.8)	4.16 (11.0)	3.95 (3.2)	4.03	4.08	3.85–3.77	2.18; 2.10
48	4.97 (3.6)	4.22 (11.0)	3.93	4.04	4.08	3.84–3.75	2.12; 2.07
49	4.96 (3.6)	4.22 (11.0)	3.94	4.02	4.08	3.84–3.75	2.11; 2.08
50	4.96 (3.6)	4.22 (11.0)	3.92	4.02	4.03	3.83–3.75	2.11; 2.09

with *m*-nitrobenzyl alcohol matrix. Optical rotations were recorded on a Perkin-Elmer Polarimeter 241 and are given in units of 10^{-1} deg cm^2 g^{-1}; m.p.s were determined on a Leitz heating table instrument and are not corrected.

Preparation of O-(2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-α-D-*galactopyranosyl*)-N$^{\alpha}$-(*fluoren*-9-*ylmethoxycarbonyl*)-L-*serine Pentafluorophenyl Ester* **6**.—The glycosyl amino acid **4** (1.0 g, 1.52 mmol) was dissolved in dry ethyl acetate (6 cm^3). Pfp-OH (308 mg, 1.68 mmol) was added and the solution was cooled to 0 °C. After 5 min solid DCCI (355 mg, 1.68 mmol) was added and the mixture was stirred at 0 °C overnight. The dicyclohexylurea was removed by filtration and the solvent was evaporated off under reduced pressure. The residue was purified by flash chromatography on dry silica gel with dry solvents. The product was eluted with light petroleum (boiling range 60–70 °C)–ethyl acetate (1:2). The unchanged acid **4** (237 mg, 24%) was recovered by elution of the column with toluene–ethanol–acetic acid (5:2:0.01). The product fractions were concentrated, dissolved in diethyl ether–dichloromethane (10:1, 30 cm^3), and precipitated with pentane (50 cm^3) to yield the active ester **6** as a stable, solid material (938 mg, 75%), m.p. 96 °C [Found: MH$^+$ (FAB-mass spectrum), 823.0. $C_{38}H_{35}F_5N_2O_{13}$ requires *M*, 822.2]; $[\alpha]_D^{23}$ +43.8 (*c* 1.0, $CHCl_3$); δ_H(400 MHz; $CDCl_3$; standard Me_4Si) 1.90, 1.96, 2.01 and 2.18 (12 H, 4 s, 4 × Ac), 4.00–4.16 (5 H, m, ser β-H_2, 5-H and 6-H_2), 4.26 (1 H, t, Fmoc CH), 4.50 (2 H, d, J_{CH,CH_2} 6.8, Fmoc-CH_2), 4.59–4.69 (1 H, m, 2-H), 4.93 (1 H, d, $J_{1,2}$ 3.6, 1-H), 4.90–5.01 (1 H, m, ser α-H), 5.11 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.0, 3-H), 5.39 (1 H, dd, $J_{4,5}$ 0.6, 4-H), 5.56 (1 H, d, $J_{2,NH}$ 9.6, N*H*Ac), 5.97 (1 H, d, $J_{\alpha,NH}$ 8.4, ser NH) and 7.29–7.35, 7.38–7.44, 7.75–7.64 and 7.75–7.80 (8 H, 4 m, ArH).

Preparation of O-(2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-α-D-*galactopyranosyl*)-N$^{\alpha}$-(*fluoren*-9-*ylmethoxycarbonyl*)-L-*threonine Pentafluorophenyl Ester* **7**.—The glycosyl amio acid **5** (1.2 g, 1.79 mmol) was dissolved in dry ethyl acetate (4 cm^3) and treated with Pfp-OH (362 mg, 1.97 mmol) and DCCI (406 mg, 1.97 mmol) as described for compound **6**, to yield the active ester **7** (1.12 g, 75%), m.p. 110 °C [Found: MH$^+$ (FAB-mass spectroscopy), 837.3. $C_{39}H_{37}F_5N_2O_{13}$ requires *M*, 836.2]; $[\alpha]_D^{23}$ +34.0 (*c* 1.0, $CHCl_3$); δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.45

Table 4 ^{1}H NMR data [400 MHz, internal reference DOH (δ 4.8) at 300 K] of the amino acid protons of the glycopeptides **11**–**47** (δ-values given in ppm, J-values given in Hz)

Peptide	P_α	T_α ($J_{\alpha,\beta}$)	T_α ($J_{\alpha,\beta}$)	T_α ($J_{\alpha,\beta}$)	P_α ($J_{\alpha,\beta}$)	I_α ($J_{\alpha,\beta}$)	S_α ($J_{\alpha,\beta}$)	T_α ($J_{\alpha,\beta}$)	P_β ($J_{\alpha,\beta}$)	T_β ($J_{\alpha,\beta}$)	T_β ($J_{\beta,\gamma}$)	T_β ($J_{\beta,\gamma}$)	P_β ($J_{\beta,\gamma}$)	I_β	S_β ($J_{\beta a,\beta b}$)	T_β ($J_{\beta,\gamma}$)	P_γ	T_γ	T_γ	T_γ	P_γ	$I_{\gamma a}$ ($J_{\beta,\gamma a}$)	$I_{\gamma b}$	$I_{\gamma c}$	T_γ	P_δ	I_δ ($J_{\gamma,\delta}$)
11	4.55	4.58	4.50	4.77[a]	4.53	4.09	4.59	4.39	2.43–2.26,	4.28	4.35	4.33[a]	2.43–2.26,	1.92–1.80	3.97; 3.90	4.38	*e*	1.28	1.29	1.38[a]	*e*	1.0	1.69–1.57	1.34–1.20	1.27	3.80–3.65	0.97
	(3.6) (8.8)	(5.0)	(4.4)	(1.8)	(3.6) (8.0)	(7.4)	(5.6) (6.4)	(3.6)	2.13–1.91	(6.4)	(6.4)	(6.4)	2.13–1.91		(11.2)	(6.0)						(7.2)					(7.6)
12	4.54	4.65	4.71[a]	4.58	4.49	4.22	4.62	4.39	2.43–2.31,	4.20	4.40[a]	4.30	2.43–2.31,	1.98–1.87	3.96; 3.90	4.37	*e*	1.32	1.30[a]	1.31	*e*	1.0	1.64–1.52	1.38–1.21	1.27	3.80–3.65	0.94
	(4.6) (8.8)	(5.8)	(2.0)	(5.0)	(6.4) (8.4)	(7.4)	(5.8) (6.0)	(3.6)	2.16–1.87	(6.4)	(6.4)	(6.4)	2.16–1.87		(11.4)	(6.4)						(6.8)					(7.4)
13	4.64–4.59	4.70[a]	4.63	4.51	4.52	4.24	4.62	4.39	2.43–2.30,	4.39[a]	4.15	4.26	2.43–2.30,	2.00–1.88	3.96; 3.90	4.37	*e*	1.38[a]	1.33	1.25	*e*	1.0	1.64–1.51	1.33–1.19	1.27	3.80–3.52	0.94
		(2.2)	(5.6)	(4.4)	(6.4)(8.4)	(7.4)	(6.2) (6.2)	(3.6)	2.16–1.88	(6.4)	(6.4)	(6.4)	2.16–1.88		(11.2)	(6.2)						(6.8)					(7.4)
14	4.53	4.60	4.51	4.66[a]	4.11; 3.98[b]	4.27	4.61	4.39	2.43–2.32,	4.33	4.35	4.43[a]	*b*	1.98–1.85	3.96; 3.91	4.37	*e*	1.30	1.30	1.33[a]	*b*	0.98	1.59–1.46	1.30–1.17	1.27	3.77–3.52	0.93
	(5.0) (6.0)	(5.0)	(4.4)	(2.2)	(17.2)	(7.4)	(6.4) (6.0)	(3.6)	2.15–1.98	(6.4)	(6.4)	(6.4)			(11.4)	(6.4)						(6.8)					(7.2)
15	4.54	4.57	4.73[a]	4.43	4.10; 3.94[b]	4.30	4.62	4.39	2.43–2.32,	4.31	4.43[a]	4.26	*b*	1.99–1.88	3.97; 3.92	4.37	*e*	1.31	1.34[a]	1.29	*b*	0.98	1.59–1.47	1.33–1.18	1.27	3.80–3.53	0.93
	(4.0) (8.0)	(5.0)	(2.0)	(4.4)	(14.0)	(7.6)	(6.0) (6.0)	(3.6)	2.22–1.99	(6.6)	(6.6)	(6.8)			(11.4)	(6.2)						(6.8)					(7.4)
16	4.61	4.69[a]	4.56	4.42	4.08; 4.01[b]	4.30	4.62	4.39	2.45–2.31,	4.39[a]	4.31	4.27	*b*	1.99–1.88	3.97; 3.92	4.37	*e*	1.37[a]	1.26	1.28	*b*	0.98	1.59–1.45	1.29–1.18	1.27	3.75–3.52	0.93
	(4.0) (8.0)	(2.4)	(4.2)	(4.6)	(16.8)	(7.4)	(5.6) (6.0)	(3.6)	2.14–1.99	(6.4)	(6.4)	(6.8)			(11.4)	(6.2)						(6.8)					(7.2)
17	4.05[b]	4.59	4.51	4.77[a]	4.58	4.09	4.58	4.39	*b*	4.28	4.32	4.33[a]	2.40–2.27,	1.92–1.80	3.97; 3.90	4.37	*b*	1.29	1.27	1.37[a]	*e*	1.00	1.69–1.54	1.34–1.20	1.27	3.88–3.64	0.97
		(5.0)	(4.6)	(2.2)	(4.8) (8.2)	(7.4)	(5.8) (6.4)	(3.4)		(6.4)	(6.4)	(6.4)	2.10–1.92		(11.4)	(6.2)						(6.8)					(7.4)
18	4.07; 4.01[b]	4.65	4.71[a]	4.60	4.49	4.22	4.62	4.39	*b*	4.20	4.40[a]	4.29	2.42–2.31,	2.04–1.78	3.96; 3.90	4.37	*b*	1.32	1.31[a]	1.30	*e*	1.00	1.64–1.51	1.38–1.20	1.27	3.90–3.73	0.94
	(16.8)	(5.8)	(2.2)	(4.8)	(6.2) (8.2)	(7.4)	(5.8) (6.0)	(3.6)		(6.4)	(6.2)	(6.4)	2.18–1.97		(11.4)	(6.2)						(6.8)					(7.2)
19	4.11; 4.04[b]	4.71[a]	4.62	4.52	4.51	4.23	4.61	4.39	*b*	4.39[a]	4.15	4.26	2.42–2.30,	2.02–1.85	3.96; 3.90	4.36	*b*	1.35[a]	1.32	1.35	*e*	0.99	1.64–1.50	1.36–1.20	1.26	3.86–3.72	0.93
	(16.6)	(2.4)	(6.0)	(4.4)	(6.0) (8.4)	(7.8)	(6.0) (6.2)	(3.6)		(6.4)	(6.8)	(6.4)	2.18–1.99		(11.6)	(6.2)						(6.8)					(7.4)
20	4.11; 3.97[b]	4.60	4.51	4.66[a]	4.05[b]	4.27	4.61	4.38	*b*	4.32	4.32	4.43[a]	*b*	1.98–1.84	3.96; 3.91	4.37	*b*	1.30	1.27	1.32[a]	*b*	0.98	1.60–1.54	1.31–1.18	1.26		0.92
	(17.2)	(5.0)	(4.6)	(2.2)		(7.4)	(6.0) (6.0)	(3.6)		(6.6)	(6.4)	(6.4)			(11.4)	(6.2)						(6.8)					(7.4)
21	4.09; 3.95[b]	4.59	4.73[a]	4.42	4.03[b]	4.29	4.62	4.39	*b*	4.29	4.42[a]	4.26	*b*	1.99–1.87	3.95–3.92	4.37	*b*	1.29	1.34[a]	1.29	*b*	0.98	1.58–1.43	1.31–1.18	1.27		0.92
	(17.0)	(5.0)	(2.4)	(4.4)		(7.2)	(6.0) (6.0)	(3.4)		(6.4)	(6.4)	(6.6)			(11.6)	(6.4)						(6.8)					(7.4)
22	4.11; 4.00[b]	4.71[a]	4.57	4.41	4.08; 4.04[b]	4.30	4.62	4.39	*b*	4.40[a]	4.31	4.26	*b*	1.99–1.87	3.96; 3.91	4.36	*b*	1.34	1.24	1.28	*b*	0.98	1.58–1.45	1.32–1.18	1.24		0.92
	(16.8)	(2.6)	(4.4)	(4.6)	(16.6)	(7.6)	(6.0) (6.0)	(3.6)		(6.4)	(6.4)	(6.4)			(11.4)	(6.4)						(6.8)					(7.2)
23	4.54	4.58	4.69[a]	4.27; 4.09[b]	4.50	4.25	4.61	4.39	2.42–2.26,	4.33	4.46[a]	*b*	2.42–2.26,	2.02–1.88	3.95; 3.89	4.37	*e*	1.31	1.31[a]	*b*	*e*	0.99	1.64–1.50	1.37–1.21	1.26	3.78–3.53	0.93
	(4.8) (11.2)	(4.6)	(2.0)	(18.0)	(4.8) (8.6)	(7.6)	(6.2) (6.0)	(3.2)	2.12–1.88	(6.4)	(6.4)		2.12–1.88		(11.0)	(6.2)						(6.8)					(7.2)
24	4.61	4.71[a]	4.44	4.23; 4.02[b]	4.53	4.27	4.62	4.39	2.42–2.27,	4.41[a]	4.28	*b*	2.42–2.27,	2.00–1.88	3.96; 3.90	4.37	*e*	1.38[a]	1.29	*b*	*e*	0.99	1.62–1.49	1.33–1.20	1.27	3.77–3.55	0.93
	(4.4) (8.8)	(2.2)	(4.2)	(16.8)	(4.8) (8.4)	(8.0)	(5.8) (6.0)	(3.4)	2.13–1.88	(6.4)	(6.4)		2.13–1.88		(11.4)	(6.4)						(6.8)					(7.4)
25	4.59	4.46	4.16; 4.05[b]	4.78[a]	4.52	4.10	4.59	4.39	2.42–2.25,	4.35	*b*	4.30[a]	2.42–2.25,	2.00–1.80	3.97; 3.89	4.37	*e*	1.29	*b*	1.37[a]	*e*	1.00	1.68–1.54	1.34–1.20	1.27	3.86–3.53	0.96
	(4.8) (8.4)	(4.0)	(16.8)	(3.0)	(5.0) (9.2)	(7.4)	(5.6) (6.2)	(3.6)	2.12–1.91	(6.4)		(6.4)	2.12–1.91		(11.4)	(6.4)						(6.8)					(7.4)
26	4.64–4.59	4.65[a]	4.11; 4.01[b]	4.63	4.50	4.23	4.61	4.39	2.43–2.29,	4.42[a]	*b*	4.15	2.43–2.29,	2.04–1.88	3.96; 3.90	4.36	*e*	1.36[a]	*b*	1.32	*e*	0.99	1.64–1.50	1.35–1.18	1.27	3.80–3.55	0.93
		(2.2)	(17.2)	(6.2)	(3.0) (8.2)	(7.6)	(6.0) (6.0)	(3.6)	2.15–1.88	(6.4)		(6.4)	2.15–1.88		(11.4)	(6.2)						(6.8)					(7.4)
27	4.59–4.55	4.04[b]	4.58	4.80[a]	4.57	4.10	4.58	4.39	2.44–2.27,	*b*	4.27	4.33[a]	2.44–2.27,	1.93–1.80	3.97; 3.89	4.37	*e*	*b*	1.27	1.37[a]	*e*	0.99	1.68–1.55	1.38–1.19	1.27	3.87–3.60	0.96
			(4.6)	(2.2)	(5.0) (9.2)	(7.4)	(5.6) (6.2)	(3.4)	2.18–1.92		(6.2)	(6.4)	2.18–1.92		(11.4)	(6.2)						(6.8)					(7.4)
28	4.51–4.46	4.15; 4.03[b]	4.69[a]	4.64	4.51–4.46	4.22	4.61	4.39	2.46–2.29,	*b*	4.40[a]	4.19	2.46–2.29,	2.05–1.88	3.96; 3.90	4.36	*e*	*b*	1.30[a]	1.32	*e*	0.99	1.64–1.51	1.37–1.20	1.27	3.88–3.65	0.94
		(16.0)	(1.8)	(6.0)		(7.6)	(5.6) (6.0)	(3.4)	2.18–1.88		(6.4)	(6.4)	2.18–1.88		(11.4)	(6.2)						(6.8)					(7.4)

29	4.66 (6.2) (8.4)	4.66 (6.2)	4.55[c] (5.2) (8.6)	4.74[a] (3.0)	4.51 (4.6) (8.6)	4.11 (7.4)	4.59 (5.6) (6.0)	4.39 (3.4)	2.57–2.28, 2.21–1.91	4.19 (6.4)	2.57–2.28[c] 2.21–1.91	4.29[a] (6.4)	2.57–2.28, 2.21–1.91	1.98–1.81	3.97; 3.90 (11.4)	4.37 (6.2)	*e*	1.33	*c*	1.40[a]	*e*	1.00 (6.8)	1.68–1.53	1.50–1.20	1.27	3.92–3.45	0.96 (7.4)
30	4.62 (4.8) (8.2)	4.77[a] (2.2)	4.58[c] (4.8) (8.6)	4.46 (6.4)	4.49 (6.4) (8.0)	4.23 (7.6)	4.61 (5.6) (6.0)	4.39 (3.6)	2.52–2.28, 2.20–2.00	4.32[a] (6.4)	2.52–2.28[c], 2.20–2.00	4.12 (6.4)	2.52–2.28, 2.20–2.00	2.05–1.87	3.96; 3.90 (11.4)	4.36 (6.2)	*e*	1.40[a]	*c*	1.36	*e*	0.99 (6.8)	1.62–1.50	1.39–1.18	1.26	3.95–3.53	0.93 (7.4)
31	4.54 (4.8) (8.4)	4.57 (5.2)	4.81[a] (1.8)	4.75[a] (2.4)	4.54 (4.8) (8.4)	4.05 (7.2)	4.58 (5.6) (6.2)	4.39 (3.4)	2.43–2.27, 2.20–1.92	4.29 (6.4)	4.41[a] (6.4)	4.33[a] (6.4)	2.43–2.27, 2.20–1.92	1.92–1.79	3.97; 3.89 (10.4)	4.37 (6.2)	*e*	1.31	1.33[a]	1.36[a]	*e*	1.00 (6.8)	1.71–1.58	1.36–1.18	1.26	3.86–3.54	0.97 (7.4)
32	4.61 (5.2) (8.8)	4.73[a] (1.6)	4.64 (4.2)	4.68[a] (2.6)	4.58 (4.8) (8.4)	4.06 (7.2)	4.58 (5.6) (6.2)	4.39 (3.6)	2.43–2.28, 2.13–1.92	4.35[a] (6.4)	4.28 (6.4)	4.40[a] (6.4)	2.43–2.28, 2.13–1.92	1.92–1.78	3.97; 3.89 (11.4)	4.37 (6.2)	*e*	1.39[a]	1.29	1.37[a]	*e*	0.99 (6.8)	1.69–1.58	1.36–1.19	1.27	3.86–3.67	0.97 (7.6)
33	4.62	4.80[a] (2.4)	4.71[a] (1.6)	4.61 (6.0)	4.48 (6.2) (8.4)	4.21 (7.4)	4.61 (6.0) (6.0)	4.39 (3.4)	2.45–2.30, 2.19–1.90	4.42[a] (6.2)	4.38[a] (6.4)	4.17 (6.4)	2.45–2.30, 2.19–1.90	2.02–1.85	3.96; 3.90 (11.2)	4.37 (6.4)	*e*	1.40[a]	1.32[a]	1.29	*e*	1.00 (6.8)	1.65–1.51	1.40–1.19	1.27	3.87–3.66	0.94 (7.4)
34	4.63 (5.0) (8.6)	4.85[a] (1.6)	4.80[a] (2.8)	4.72[a] (1.0)	4.55 (4.8) (8.4)	4.03 (7.2)	4.58 (5.6) (6.4)	4.39 (3.2)	2.44–2.29, 2.15–1.91	4.42[a] (6.4)	4.44[a] (6.4)	4.38[a] (6.4)	2.44–2.29, 2.15–1.91	1.91–1.78	3.97; 3.90 (11.4)	4.36 (6.0)	*e*	1.32[a]	1.40[a]	1.34[a]	*e*	1.00 (6.8)	1.72–1.59	1.37–1.19	1.27	3.86–3.65	0.98 (7.4)
35	4.53 (5.2) (8.2)	4.51 (4.4)	4.43 (4.6)	4.98[a,d] (5.0) (7.2)	4.53 (5.2) (8.2)	4.22 (7.2)	4.61 (5.8) (6.2)	4.39 (3.2)	2.48–2.28, 2.17–1.86	4.34 (6.4)	4.27 (6.4)	4.06; 3.81[a,d] (11.6)	2.48–2.28, 2.17–1.86	2.00–1.85	3.95; 3.89 (11.4)	4.38 (6.2)	*e*	1.29	1.27	*d*	*e*	0.99 (6.8)	1.65–1.52	1.35–1.20	1.27	3.90–3.51	0.94 (7.4)
36	4.53 (4.8) (8.2)	4.69 (6.0)	4.78[a,d] (5.6) (5.6)	4.45 (4.6)	4.50 (6.0) (8.0)	4.23 (7.2)	4.62 (5.8) (6.0)	4.39 (3.6)	2.45–2.29, 2.18–2.00	4.21 (6.4)	3.98; 3.85[a,d] (11.4)	4.29 (6.4)	2.45–2.29, 2.18–2.00	2.03–1.85	3.96; 3.90 (11.4)	4.37 (6.4)	*e*	1.32	*d*	1.29	*e*	0.99 (6.8)	1.64–1.50	1.33–1.20	1.27	3.87–3.52	0.93 (7.4)
37	4.59–4.48	4.79[a,d] (5.4) (6.2)	4.66 (5.8)	4.51 (4.4)	4.59–4.48	4.23 (7.6)	4.62 (5.8) (6.0)	4.39 (3.4)	2.43–2.30, 2.18–1.87	4.05; 3.86[a,d] (11.4)	4.18 (6.4)	4.25 (6.4)	2.43–2.30, 2.18–1.87	2.04–1.87	3.96; 3.90 (11.4)	4.36 (6.4)	*e*	*d*	1.32	1.25	*e*	0.99 (6.8)	1.62–1.50	1.36–1.20	1.27	3.82–3.54	0.93 (7.4)
38	4.56 (3.6) (8.4)	4.47 (4.4)	4.68[d] (5.8) (5.6)	4.78[a] (2.4)	4.53 (4.0) (8.0)	4.09 (7.4)	4.59 (5.6) (6.2)	4.39 (3.6)	2.45–2.27, 2.20–1.92	4.32 (6.4)	3.98–3.91[d]	4.32[a] (6.4)	2.45–2.27, 2.20–1.92	1.92–1.80	3.97; 3.89 (11.4)	4.37 (6.2)	*e*	1.29	*d*	1.37[a]	*e*	1.00 (6.8)	1.69–1.56	1.35–1.20	1.27	3.86–3.52	0.96 (7.4)
39	4.61–4.55	4.58[d] (5.8) (5.8)	4.57 (4.4)	4.77[a] (2.2)	4.50 (4.6) (8.6)	4.09 (7.4)	4.58 (6.4) (6.4)	4.39 (3.4)	2.45–2.27, 2.18–1.92	4.00–3.93[d]	4.31 (6.4)	4.33[a] (6.4)	2.45–2.27, 2.18–1.92	1.92–1.80	3.97; 3.89 (11.4)	4.36 (6.2)	*e*	*d*	1.28	1.38[a]	*e*	1.00 (6.8)	1.69–1.55	1.36–1.20	1.27	3.87–3.55	0.97 (7.6)
40	4.56 (3.6) (8.4)	4.67[d]	4.59[d]	4.78[a] (2.2)	4.50 (4.6) (8.4)	4.09 (7.4)	4.58	4.39 (3.4)	2.45–2.27, 2.13–1.92	3.98–3.92[d]	4.02–3.86[d]	4.32[a] (6.4)	2.45–2.27, 2.13–1.92	1.92–1.80	4.02; 3.86	4.36 (6.2)	*e*	*d*	*d*	1.37[a]	*e*	1.00 (6.8)	1.68–1.56	1.35–1.20	1.27	3.89–3.54	0.97 (7.4)
41	4.53 (5.0) (8.4)	4.67[a] (2.2)	4.58 (5.0)	4.80[d] (6.0) (7.2)	4.51 (5.4) (8.4)	4.22 (7.6)	4.61 (5.8) (6.0)	4.39 (3.6)	2.42–2.31, 2.17–1.96	4.31 (6.4)	4.40[a] (6.4)	3.94; 3.84[d] (11.6)	2.42–2.31, 2.17–1.96	1.99–1.85	3.96; 3.90 (11.6)	4.37 (6.2)	*e*	1.31	1.30[a]	*d*	*e*	0.99 (6.8)	1.62–1.50	1.35–1.19	1.27	3.89–3.52	0.94 (7.4)
42	4.52–4.46	4.67[d] (5.8) (5.8)	4.71 (2.0)	4.63 (6.0)	4.52–4.46	4.22 (7.6)	4.62 (6.0) (6.0)	4.39 (3.6)	2.42–2.31, 2.18–1.93	3.98–3.85[d]	4.42[a] (6.4)	4.18 (6.4)	2.42–2.31, 2.18–1.93	1.98–1.85	3.96; 3.90 (11.4)	4.37 (6.2)	*e*	*d*	1.31[a]	1.33	*e*	1.00 (6.8)	1.64–1.51	1.36–1.20	1.27	3.88–3.52	0.94 (7.4)
43	4.52 (5.4) (8.0)	4.80[d] (5.6) (7.0)	4.68[a] (1.8)	4.67[d]	4.50 (4.8) (8.0)	4.23 (7.6)	4.61 (6.2) (6.2)	4.39 (3.4)	2.44–2.30, 2.18–1.97	3.94; 3.85[d] (11.4)	4.44[a] (6.4)	4.00–3.92[d]	2.44–2.30, 2.18–1.97	2.00–1.85	3.96; 3.90 (11.4)	4.37 (6.2)	*e*	*d*	1.31[a]	*d*	*e*	0.99 (6.8)	1.62–1.50	1.33–1.19	1.27	3.91–3.51	0.94 (7.4)
44	4.62 (5.0 (8.6)	4.70[a] (2.2)	4.46 (4.0)	4.78[d] (6.0 (6.0)	4.55 (5.4) (8.4)	4.25 (7.6)	4.61 (5.8) (6.0)	4.39 (3.4)	2.46–2.31, 2.17–1.98	4.40[a] (6.4)	4.25 (6.4)	3.94–3.89[d]	2.46–2.31, 2.17–1.98	2.01–1.88	3.97; 3.91 (11.2)	4.37 (6.2)	*e*	1.38[a]	1.26	*d*	*e*	0.99 (6.8)	1.61–1.47	1.35–1.19	1.27	3.87–3.52	0.94 (7.4)
45	4.64–4.59	4.65[a] (2.2)	4.62[d]	4.62 (6.0)	4.51 (6.4) (8.2)	4.27 (7.8)	4.62	4.39 (3.4)	2.47–2.30, 2.26–1.95	4.40[a] (6.4)	3.99–3.84[d]	4.15 (6.4)	2.47–2.30, 2.26–1.95	2.03–1.86	3.99; 3.84	4.37 (6.4)	*e*	1.37[a]	*d*	1.33	*e*	0.99 (6.8)	1.64–1.50	1.39-1.21	1.27	3.87–3.52	0.94 (7.4)
46	4.62 (5.2) (8.2)	4.66[a] (2.0)	4.77[d]	4.62[d] (5.8) (6.0)	4.54 (5.6) (8.2)	4.25 (7.6)	4.59	4.39 (3.6)	2.42–2.30, 2.16–2.00	4.39[a] (6.4)	3.96–3.87[d]	4.00–3.85[d]	2.42–2.30, 2.16–2.00	2.05–1.88	4.00–3.85	4.37 (6.2)	*e*	1.38[a]	*d*	*d*	*e*	0.99 (6.8)	1.61–1.49	1.35–1.19	1.27	3.90–3.55	0.93 (7.4)
47	4.53 (5.4) (8.8)	4.59 (4.8)	4.50 (4.4)	4.66[a] (2.2)	4.62[d] (5.8) (5.2)	4.60 (5.6) (6.0)	4.25 (7.6)	4.39 (3.6)	2.42–2.32, 2.13–1.99	4.31 (6.4)	4.35 (6.4)	4.39[a] (6.4)	4.00–3.87[d]	2.00–1.88	4.00–3.87	4.38 (6.4)	*e*	1.30	1.30	1.34[a]	*d*	0.99 (6.8)	1.60–1.48	1.33–1.19	1.27	3.78–3.55	0.95 (7.4)

[a] Amino acid is glycosylated. [b] Amino acid is substituted by glycine. [c] Amino acid is substituted by proline. [d] Amino acid is substituted by serine. [e] Assignment as for P_β.

Table 5 ^{1}H NMR data [400 MHz, internal reference DOH (δ 4.8 at 300 K] of the amino acid protons of the glycopeptides **48–50** (δ-values given in ppm, J-values given in Hz)

Peptide	G_α	S_α	S_α	S_α	G_α	S_α	P_α	G_α	S_β	S_β	S_β	S_β	$P_{\beta,\gamma}$	P_δ
48	4.12–3.84	4.74	4.60	4.85[a]	4.12–3.84	4.59	4.51 (6.0) (8.4)	4.12–3.84	4.03; 3.90	4.02–3.90	3.95; 3.87[a]	4.02–3.90	2.42–2.31 2.20–1.98	3.90–3.74
49	4.12–3.84	4.79	4.85[a] (5.4) (6.6)	4.58 (5.6) (5.6)	4.12–3.84	4.55 (5.4) (5.4)	4.51 (6.0) (8.4)	4.12–3.84	4.05; 3.90	3.94; 3.86[a]	3.99–3.88	3.99–3.88	2.42–1.31 2.19–1.98	3.90–3.73
50	4.12–3.83	4.85[a] (5.6) (6.8)	4.77 (5.4) (5.4)	4.63 (5.6) (5.6)	4.12–3.83	4.53 (5.2) (5.2)	4.51 (6.0) (8.4)	4.12–3.83	3.94; 3.86[a]	4.02; 3.89	3.98; 3.95	3.98; 3.94	2.42–2.31 2.19–1.98	3.88–3.72

[a] Amino acid is glycosylated.

(3 H, d, thr γ-H), 2.17, 2.05, 2.00 and 1.96 (12 H, 4 s, 4 × Ac), 4.08 (1 H, dd, $J_{5,6a}$ 7.4, $J_{6a,6b}$ 11.0, 6-H[a]), 4.13 (1 H, dd, $J_{5,6b}$ 5.6, 6-H[b]), 4.19–4.25 (1 H, m, 5-H), 4.25–4.31 (1 H, m, Fmoc CH), 4.43 (1 H, dq, $J_{\beta,\gamma}$ 6.2, thr β-H), 4.50–4.67 (3 H, m, 2-H and Fmoc-CH_2), 4.75 (1 H, dd, $J_{\alpha,\beta}$ 1.6, thr α-H), 5.03 (1 H, d, $J_{1,2}$ 3.2, 1-H), 5.08 (1 H, dd, $J_{2,3}$ 11.6, $J_{3,4}$ 3.0, 3-H), 5.40 (1 H, dd, $J_{4,5}$ 0.8, 4-H), 5.62 (1 H, d, $J_{2,NH}$ 9.6, N*H*Ac), 5.80 (d, 1 H, $J_{\alpha,NH}$ 9.6, thr NH) and 7.30–7.36, 7.37–7.44, 7.59–7.66 and 7.74–7.81 (8 H, 4 m, ArH). A portion (24%) of the acid **5** was recovered.

N-[O-(2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-α-D-*galacto*-*pyranosyl*)-N$^\alpha$-(*fluoren*-9-*ylmethoxycarbonyl*)-L-*threonyl*]*dicyclohexylurea* **8**.—The *N*-acylurea was formed as a by-product in the synthesis of compound **7** when the reaction temperature was above 0 °C [Found: MH^+ (FAB-mass spectroscopy), 877.6. $C_{46}H_{60}N_4O_{13}$ requires *M*, 876.4]; δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.36 (3 H, d, $J_{\beta,\gamma}$ 6.4 thr γ-H), 1.03–2.08 (20 H, m, cyclohexyl H), 2.00, 2.02 and 2.15 (12 H, 4 s, 4 × Ac), 3.60–3.73 (1 H, m, cyclohexyl H), 4.03–4.18 (4 H, m, thr β-H, 6-H_2 and cyclohexyl H), 4.23–4.31 (2 H, m, 5-H and Fmoc CH), 4.36–4.43 (2 H, m, Fmoc-CH_2), 4.47 (1 H, dq, thr α-H), 4.61 (1 H, ddd, $J_{2,3}$ 11.4, 2-H), 4.86 (1 H, d, $J_{1,2}$ 3.6, 1-H), 5.12 (1 H, dd, $J_{3,4}$ 3.2, 3-H), 5.42 (1 H, dd, $J_{4,5}$ 0.8, 4-H), 5.66 (1 H, d, $J_{\alpha,NH}$ 8.8, thr NH), 6.39 (1 H, d, $J_{CH,NH}$ 8.0, cyclohexyl NH), 6.79 (1 H, d, $J_{2,NH}$ 9.6, N*H*Ac) and 7.30–7.37, 7.38–7.45, 7.59–7.68 and 7.70–7.80 (8 H, 4 m, ArH).

Multiple-column synthesis of Glycopeptides **11–50** *on a 96-column synthesizer.*—(a) *Anchoring of the linker and the first amino acid.* Macrosorb SPR 500 was treated with ethylendiamine for 96 h, washed with DMF, and derivatised with a mixture of Fmoc-Nle-OPfp and Dhbt-OH (1.5 mol equiv.). Anchoring of the linker and the first amino acid (threonine or glycine) was carried out in a glass reactor with a filter in two separate batches. All reagents were removed by washing with DMF (10 ×). Fmoc deprotections were performed by a 1 min and a 10 min treatment of the resin with piperidine (20%) in DMF. Unchanged amino groups were capped by two 10 min treatments of the resin with Ac_2O–DMF (1:7). The derivatised Macrosorb SPR 500 (2.5 g) was placed in the reactor and the resin was swelled in DMF (10 cm^3; 20 min.) After removal of Fmoc the (4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy)-valeric acid (PAL) linker (822 mg, 1.63 mmol), TBTU (522 mg, 1.63 mmol), and 4-ethylmorpholine (237 mm^3, 1.88 mmol) were added in DMF (10 cm^3). The mixture was allowed to react for 1 h in the dark. The resin was washed with DMF, unchanged amino groups were capped, and the Fmoc group was removed. After washing of the resin with DMF, a mixture of Fmoc-Thr(OBu^t)-ODhbt (2.03 g, 3.75 mmol) in DMF (10 cm^3) was added and the mixture was allowed to react overnight. The reagents were removed and unchanged amino groups were capped. The resin was washed successively with DMF and diethyl ether (10 ×) and then dried. The same procedure was employed in a reactor using Macrosorb SPR 500 (150 mg), Fmoc-protected PAL-linker (49 mg, 97 μmol), TBTU (31 mg. 97 μmol), NEM (14 mm^3, 0.11 mmol), and Fmoc-Gly-ODhbt (100 mg, 0.23 mmol). The incorporation of threonine and glycine was 92% and 93%, respectively, as estimated by quantitative amino acid analyses, and the substitution of the resin was 0.24 mmol/g, Tenta Gel S® resin (100 mg, substitution 0.21 mmol/g) already containing the PAL-linker was derivatised with Fmoc-Thr(OBu^t)-ODhbt (34 mg, 63 μmol) as described above.

(b) *Performance of the multiple-column solid-phase synthesis.* Corresponding to the sequences in Fig. 1 the derivatised resins were weighed out (25 mg/well) and packed in 82 of the 96 wells in the multiple-column synthesizer. Each glycopeptide was synthesized in two neighbouring wells. DMF or deprotection reagents were added using two separate 96-channel washers connected to dispensing bottles. The deprotection reagent contained a small amount of the red dye Azoruby (0.01%) to indicate completion of the washing procedure. The solvents in the wells were removed through sintered Teflon filters by vacuum suction through a pressure chamber beneath. The Fmoc groups were removed and the following synthesis cycle was entered: Washing after deprotection: DMF, 5 ×, 2 min, 470 mm^3/well; Coupling: Fmoc amino acid Dhbt ester or Pfp ester/Dhbt-OH, (6.1 mol equiv.) in DMF (200 mm^3), 15–22 h; Washing after coupling: DMF, 4 ×, 2 min, 470 mm^3/well; Deprotection: 50% morpholine–DMF, 3 min and 2 × 20 min, 420 mm^3/well.

The Fmoc amino acids Dhbt esters (6.1 mol equiv.) corresponding to position 2 from the *C*-terminal end of glycopeptides **11–50** in Fig. 1 were dissolved in DMF (200 mm^3 each) in test tubes and transferred into a separate reagent tray with 96 compartments. During acylations with the glycosyl-amino acid pentafluorophenylester **6** or **7** an equivalent amount of Dhbt-OH was added as auxiliary nucleophile. The solutions were then transferred to the resins with an 8 channel multipipette while a slight overpressure of air below the wells was established to ensure that the reagents remained in the columns. During the acylation reactions the reactor was placed on a rocker table. The progress of formation of the peptide bonds was indicated in the decrease in the intensity of the colour in each column. The active esters were, however, allowed to react for 15–22 h, independently of the resin colour. None of the 574 coupling reactions had to be repeated. The reaction mixtures were removed and the wells were washed with DMF. The synthesis cycle was repeated to complete the assembly of each of the glycopeptides **11–50** by application of the respective activated Fmoc amino acids (6.1 mol equiv.). After removal of the Fmoc groups the terminal amino groups were acetylated with an Ac_2O–DMF mixture (1:7; 20 min; 400 mm^3 in each well). The resins were washed successively with DMF (5 ×,

~470 mm^3) and diethyl ether (5 ×, 600 mm^3) and dried by a flow of air through the resins (2 h). The resins were carefully loosened with a spatula and simultaneously transferred into 82 small glass vials. The resins were treated with 95% aq. TFA (0.8 cm^3) for 2 h at room temperature. The solutions were pipetted off and the resins were decanted four times with TFA (1.5 cm^3). The combined TFA fractions of each glycopeptide were evaporated using a stream of pressure air. The residues were dissolved in methanol (1 cm^3) and centrifuged. The clear solutions were again evaporated using pressure air, dissolved in water (1 cm^3), and evaporated under reduced pressure. The deacylations of the carbohydrate moieties were performed by dissolving the crude products in abs. methanol (200 mm^3/mg raw product) and addition of sodium methoxide solution (1%; 35 mm^3). The solutions were stirred at room temperature. All deacetylations were complete after 3 h according to TLC [chloroform–methanol–water (10:4:0.5) or butan-1-ol–pyridine–acetic acid–water (4:1:1:2)]. The solutions were acidified with acetic acid (23 mm^3), filtered, evaporated and purified by preparative RP-HPLC [for glycopeptides **11–47**: buffer A–buffer B 90:10 ⟶ 80:20 (10 min) ⟶ 30:70 (20 min); for glycopeptides **48–50**: buffer A–buffer B 99:1 ⟶ 90:10 (10 min) ⟶ 30:70 (20 min)]. All glycopeptides showed excellent purity according to analytical HPLC and ^{1}H NMR spectroscopy and were isolated in amounts of 4–6 mg.

Optimisation of Cleavage Conditions.—The synthesis of compound **13** was repeated in a manual 20 column peptide synthesizer. By employing the same technique and materials as described for the 96 column synthesizer two parallel syntheses were performed on a resin derivatised either with the PAL-linker or the Rink-linker. Samples of the resins according to the entries in Table 2 were treated with different TFA/scavenger mixtures (1 cm^3/30 mg resin) for 2 h at room temperature. The mixtures were filtered and the resins were carefully washed five times with TFA (1 cm^3). The combined TFA fractions of each experiment were evaporated under reduced pressure. The raw products were deacetylated as described above, filtered and purified by preparative reversed-phase HPLC [buffer A–buffer B 90:10 ⟶ 80:20 (10 min) ⟶ 30:70 (20 min)]. The yields of the pure lyophilysed products based on the substitution of the resin are summarised in Table 2. The remaining supports were washed successively with methanol and diethyl ether, dried, hydrolysed by 6 mol dm^{-3} HCl, and analysed by quantitative amino acid analysis. The amount of peptide that had not been cleaved by TFA treatment was calculated from the ratio of the corresponding amino acids and norleucine.

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