Solid-Phase Synthesis and Characterization of *O*-Dimannosylated Heptadecapeptide Analogues of Human Insulin-like Growth Factor 1 (IGF-1)

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N^{α}-Fmoc-3-*O*-[Ac_4-α-D-Man*p*-(1→2)-Ac_3-α-D-Man*p*-1-]-Thr-OPfp, **7**, and N^{α}-Fmoc-3-*O*-[Ac_4-α-D-Man*p*-(1→2)-Ac_3-α-D-Man*p*-1-]-Ser-OPfp, **8**, were prepared and used as building blocks in automated continuous-flow solid-phase glycopeptide synthesis of two *O*-glycosylated heptadecapeptide analogues of human insulin-like growth factor **1**. The corresponding non-glycosylated fragments were also prepared, and comparative NMR studies regarding the influence of the sugar moiety on the peptide backbone showed only limited effects due to glycosylation.

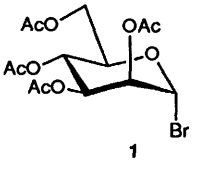
The oligosaccharides in *O*-glycosylated proteins play an important role in controlling the transport, stability, and recognition phenomena of the glycoproteins (for a review of *O*-glycoproteins, see ref. 1). In contrast to proteins the glycoproteins are not easily available by gene-technology, and isolated proteins exist as mixtures of many glyco-forms. Accordingly, there is a need for synthetic techniques to make well defined carbohydrate–peptide conjugates, in reasonable quantities (5–100 mg scale), in order to get a better understanding of the role of the carbohydrate part in glycopeptides and proteins. In this work we describe a sequential solid-phase synthesis of two glycosylated heptadecapeptides by using suitable protected glycosylated amino acids as building blocks.

Human insulin-like growth factor 1 (IGF-1, Somatomedin C) is a secretory polypeptide of 70 amino acid residues, involved in the regulation of cell growth. A glycosylated form of IGF-1, produced by the yeast *Saccharomyces cerevisiae*, has recently been isolated and characterized, in addition to the non-glycosylated form.[2] The glycosylation site was determined to be threonine-29 and it was mainly substituted with α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3). In order to study the influence of the sugar moiety on the peptide backbone, we have synthesized the heptadecapeptide fragment of IGF-1 corresponding to amino acid 22 (glycine) to 38 (alanine), both in the glycosylated, [Di-Man-Thr^{29}] IGF-1 (22–38), **10**, and non-glycosylated, IGF-1 (22–38), **13**, forms.[3] Furthermore the two corresponding serine analogues, [Di-Man-Ser^{29}] IGF-1 (22–38), **12**, and Ser^{29} IGF-1 (22–38), **14**, were prepared for comparative studies.

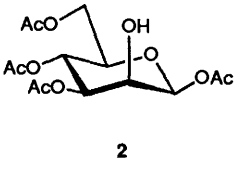
O-Glycosylated β-hydroxy α-amino acid derivatives are sensitive to both strong acid and strong base,[4] and therefore it is necessary to use protective groups that can be removed under mild conditions. Utilization of glycosylated amino acids as building blocks in solid-phase synthesis of *O*-glycopeptides has been reported by several groups.[5–12] We have recently described a fully automated solid-phase method for synthesis of *O*- and *N*-glycopeptides where the pentafluorophenyl (Pfp) esters of suitable protected glycosylated amino acids are used as building blocks.[3,13,14] The Pfp-ester serves the dual purpose of protecting the carboxylic function during glycosylation, and activating the carboxylic group in the acylation step.[15] Hence Pfp-esters are stable enough to survive both glycosylation and purification on silica gel under dry condtions.[14] The α-amino group is preferably protected with the fluoren-9-ylmethoxycarbonyl (Fmoc) group, which is suitable for synthesis of *O*-glycopeptides due to its sensitivity to mild organic secondary bases, such as morpholine.[16] The hydroxy groups of the disaccharide are best protected with acetyl or benzoyl groups, which confer stability to the bond between the disaccharide and the peptide during the synthesis and easily can be removed with sodium methoxide in methanol.[17] Syntheses were carried out on a custom-made continuous-flow peptide synthesizer using N^{α}-Fmoc-amino acid 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH) esters.[18] Acylation times were measured with a solid-phase spectrophotometer[19] and the coupling reactions of glycosylated Pfp-esters were monitored and catalysed by addition of Dhbt-OH. Using this technique, pure heptadeca *O*-glycopeptides have been prepared in a 30 mg scale.

Results and Discussion

Condensation of tetra-*O*-acetyl-α-D-mannopyranosyl brom-



1



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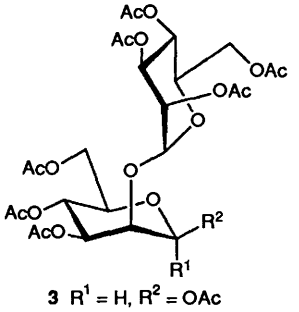
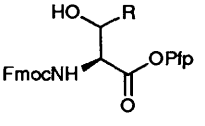
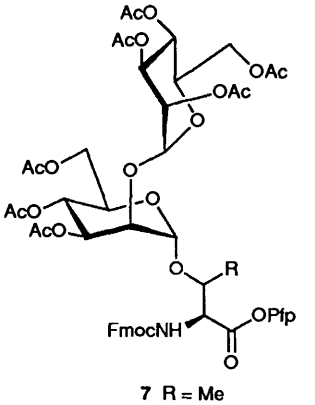


3 R^1 = H, R^2 = OAc
4 R^1 = Br, R^2 = H



5 R = Me
6 R = H



Fmoc = fluoren-9-ylmethoxycarbonyl
Pfp = pentafluorophenyl

7 R = Me
8 R = H

Table 1 ^{1}H NMR data (500 MHz) for compounds **3** and **5–8**

Proton[a]	Chemical shifts[b] (δ) for compound				
	3	**5**	**6**	**7**	**8**
1″-H	5.04			4.95	4.98
2″-H	5.34			5.29	5.32
3″-H	5.53			5.41	5.42
4″-H	5.41			5.33	5.32
5″-H	4.46			4.07	4.14
6″-H_2	4.36, 4.10			4.25, 4.22	4.27, 4.10
1′-H	5.83			5.15	5.06
2′-H	4.20			3.99	4.08
3′-H	5.15			5.30	5.33
4′-H	5.37			5.32	5.32
5′-H	3.82			4.09	4.00
6′-H_2	4.30, 4.22			4.22, 4.01	4.22, 4.22
OAc	2.20, 2.19, 2.17, 2.14, 2.13, 2.08, 2.06			2.18, 2.16, 2.13, 2.09, 2.08, 2.06, 2.04	2.19, 2.14, 2.13, 2.08, 2.07, 2.06, 2.05
α-H		4.55	4.56	4.87	5.04
β-H		4.30	3.86	4.57	4.22, 4.16
γ-H_3		1.24		1.45	
NH		7.81	8.00	5.75	6.08
OH		5.10	5.23		
Fmoc-CH		4.25	4.25	4.32	4.31
Fmoc-CH_2		4.37	4.37	4.54	4.52, 4.51
ArH		7.87, 7.75, 7.40, 7.31	7.89, 7.73, 7.41, 7.32	7.80, 7.67, 7.43, 7.35	7.81, 7.66, 7.44, 7.35

[a] Atoms belonging to the mannopyranosyl residue linked to the amino acid are primed, those to the second mannopyranosyl residue are doubly primed, and atoms belonging to the amino acid residues are designed α–γ. [b] For solutions in $CDCl_3$, except for **5** and **6**, which are in $(CD_3)_2SO$.

ide[20] **1** and 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose[21] **2**, using silver trifluoromethanesulfonate (silver triflate) in dichloromethane at low temperature (−40 °C), afforded 1,3,4,6-tetra-*O*-acetyl-2-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose **3**. The yield, after chromatography, was almost quantitative (97%). By using reversed addition in the reaction, acetyl migration to position two in compound **2** was avoided. This reaction has been reported previously, although with a moderate yield, using mercury(II) cyanide/mercury(II) bromide in acetonitrile.[22,23] Conversion of β-acetate **3** into the corresponding α-bromide **4**, followed by silver triflate-promoted reaction with N^{α}-Fmoc-Thr-OPfp,[3,24] **5**, or N^{α}-Fmoc-Ser-OPfp,[14,24] **6**, afforded the protected dimannosylated building blocks **7** and **8**, respectively. Long reaction times (7–8 h at −30 to −40 °C) were needed for the completion of the glycosylations, which might be due to the electronegative influence of the Pfp-esters. This has previously been observed in other glycosylation reactions.[14] The glycosylation products were purified by chromatography on dry silica gel using dry solvents, and the isolated yields were 80% of **7** and 65% of **8** NMR data of compounds **3**, **5**, **6**, **7** and **8** are given in Tables 1 and 2. Recently an alternative synthetic route to benzylated α-D-mannopyranosyl-(1→2)-α-D-mannopyranose linked to N^{α}-Fmoc-Thr-OBn and N^{α}-Fmoc-Ser-OBn, respectively, has been reported.[25]

The glycosylated amino acid derivatives **7** and **8** were then used in automated continuous-flow solid-phase synthesis of the heptadecapeptide fragments of IGF-1, [Ac_7-di-Man-Thr^{29}] IGF-1 (22–38), **9**, and [Ac_7-di-Man-Ser^{29}] IGF-1 (22–38), **11**, respectively. Kieselguhr-supported poly(dimethylacrylamide) resin was used as the solid phase[26] and *N*,*N*-dimethylformamide (DMF) as the solvent. The first amino acid (Ala) was coupled to the resin by using the symmetrical anhydride and 4-(dimethylamino)pyridine (DMAP) as a catalyst,[27] or by the recently described method where the N^{α}-Fmoc-protected amino acid is esterified, in high yield and without significant racemization, using 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT) in the presence of 1-methylimidazole[28] (MeIm). The synthesis was then continued with three mole equivalents of activated Dhbt-esters of N^{α}-Fmoc-protected amino acids[18] with *tert*-butyl (Bu^t)-based acid-labile protecting groups in the side-chains. Arginine was protected with the acid-labile 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) group.[29] In the coupling steps where the glycosylated amino acid derivatives were used (three mole equivalents of compound **7** and 2.7 mole equivalents of compound **8**, respectively), one mole equivalent of Dhbt-OH was added as an auxiliary nucleophile. The acylation steps were monitored at 440 nm by a solid-phase spectrophotometer recording the colouration of the resin due to formation of an ion-pair between Dhbt-OH and unchanged amino groups.[19] Minimum acylation time was programmed to 30 min. The reaction times varied from 30 min to 33 h, and 40% of the couplings were finished within one hour. The acylation times with compounds **7** and **8** were 115 min and 5 h, respectively. Deprotection of the α-amino groups was achieved by treatment with 50% morpholine in DMF for 20 min. The *O*-glycopeptides were cleaved from the resin by treatment twice with 95% trifluoroacetic acid (TFA) for 30 min, with simultaneous deprotection of the acid-labile side-chain-protecting groups, and solidified by trituration with diethyl ether. The crude yields of products **9** and **11** after solidification were quantitative, and, after preparative HPLC, 53% and 67%, respectively. *O*-Deacetylation was then successfully performed by treatment with sodium methoxide at pH 12 in room temperature for 30 min. According to HPLC the reaction was finished after 10 min, and no by-products could be detected by HPLC after 3 h. This observation is general and has since been well documented in our laboratory. The deprotected glycopeptides were then purified by preparative reversed-phase HPLC to give products **10** and **12** in overall yields of 37 and 33%, respectively. The corresponding non-glycosylated fragments, IGF-1 (22–38), **13**, and [Ser^{29}] IGF-1 (22–38), **14**, were synthesized by the same methodology.

Characterization of the glycopeptides and the peptides was performed by 1D- and 2D-NMR spectroscopy (see Tables 3–6), FAB-MS and amino acid analysis. Comparison of ^{1}H and ^{13}C chemical shifts of the glycosylated and non-glycosylated compounds shows that only the shifts of the glycosylated amino acid were affected by the glycosylation. The largest shift differences due to glycosylation were observed for the α-protons in Thr and Ser, which were shifted 0.15 and 0.16 ppm downfield, respectively; for the α-carbons, which were shifted 1.2 and 1.8 ppm upfield; and for the β-carbons, which were shifted 8.7 and 5.6 ppm downfield, respectively. These shift differences are

H-Gly-Phe-Tyr-Phe-Asn-Lys-Pro-NH-CH(R^1)OR^2-CO-Gly-Tyr-Gly-Ser-Ser-Ser-Arg-Arg-Ala-OH

9 R^1 = Me, R^2 = Ac_4-α-D-Man*p*-(1→2)-Ac_3-α-D-Man*p*-(1-
10 R^1 = Me, R^2 = α-D-Man*p*-(1→2)-α-D-Man*p*-(1-
11 R^1 = H, R^2 = Ac_4-α-D-Man*p*-(1→2)-Ac_3-α-D-Man*p*-(1-
12 R^1 = H, R^2 = α-D-Man*p*-(1→2)-α-D-Man*p*-(1-
13 R^1 = Me, R^2 = H
14 R^1 = R^2 = H

Table 2 ^{13}C NMR data (125 MHz) for compounds **3** and **5–8**

Carbon[a]	Chemical shifts[b] (δ) for compound				
	3	**5**	**6**	**7**	**8**
C-1″	98.3			99.0	99.1
C-2″	69.9			69.7	69.6
C-3″	68.3			68.3	68.3
C-4″	66.1			66.2	66.4
C-5″	68.9			69.4	69.3
C-6″	62.2			62.2	62.1
C-1′	91.0			99.9	99.3
C-2′	74.6			76.8	76.3
C-3′	72.1			69.6	69.7
C-4′	65.7			66.3	66.2
C-5′	73.2			69.2	69.2
C-6′	61.8			62.2	62.5
*C*OMe	170.9, 170.5, 170.2, 169.8, 169.6, 169.3, 168.3			170.7, 170.4, 170.3, 169.8, 169.6, 169.4, 169.3	170.7, 170.5, 170.2, 169.8, 169.7, 169.5, 169.4
CO*Me*	20.9, 20.7, 20.6, 20.5			20.7, 20.6 20.5, 20.4,	20.8, 20.6
*C*O-OPfp		167.7	167.6	166.7	166.3
C-α		60.3	56.7	58.5	54.3
C-β		66.5	61.0	76.7	69.1
C-γ		19.8		18.1	
Fmoc-*C*H		46.6	46.6	47.1	47.0
Fmoc-*C*H_2		66.1	66.0	67.5	67.6
OCONH		156.5	156.1	156.4	155.8
Fmoc arom.-C		143.8, 143.7, 140.8, 127.7, 127.1, 125.3, 120.1	143.8, 143.7, 140.7, 127.6, 127.1, 125.3, 125.2, 120.1	143.6, 143.5, 141.3, 127.7, 127.1, 125.0, 120.0	143.6, 141.3, 127.8, 127.1, 125.0, 120.0
Pfp-C		141.5, 140.0, 139.6, 138.5, 138.1, 136.5, 124.9	141.4, 140.1, 139.5, 138.5, 138.1, 136.5, 124.4	141.9, 141.8, 140.8, 139.9, 138.9, 138.8, 136.9	141.8, 140.8, 139.9, 138.9, 136.9

[a] Atoms belonging to the mannopyranosyl residue linked to the amino acid are primed, those to the second mannopyranosyl residue are doubly primed, and atoms belonging to the amino acid residues are designed α–γ. [b] For solutions in $CDCl_3$, except for **5** and **6**, which are in $(CD_3)_2SO$.

believed to be due to substitution effects.[25] Comparison of qualitative rotating-frame nuclear Overhauser enhancements (ROE), measured from ROESY experiments for the glycosylated and non-glycosylated compounds, gave no indication of conformational effects due to glycosylation.

^{13}C Spin-lattice relaxation times (T_1), a useful tool for studies of macromolecular dynamics,[30] were measured for compounds **10**, **12**, **13** and **14** (see Table 7). A small T_1 value correlates with reduced flexibility in that region of the molecule. T_1 Values for the glycosylated and non-glycosylated analogues were measured at the same concentration and under the same experimental conditions in order to be comparable. The T_1 measurements indicated a rigid region of the peptide backbone extending for four amino acids around the glycosylated amino acid; this rigidity was more evident with glycosylated threonine than with glycosylated serine. Aside from an overall slower tumbling rate reducing T_1 by 5%, the other parts of the molecules seems to be unaffected by glycosylation. As expected, the mannosyl residue bound to the peptide backbone is less flexible than is the outer residue.

Comparison of the ^1H NMR shifts of α-D-mannopyranosyl-(1→2)-α-D-mannopyranose when it is linked to Thr in compound **10** with the shifts when it is linked to Thr itself[25] shows small upfield shifts (< 1 ppm) of the anomeric protons and of 2-H of the inner mannopyranosyl moiety. This may be a result of small changes in the glycosidic dihedral angles when the disaccharide is linked to Thr in the heptadecapeptide, these changes being due to steric interaction with the methyl group. This effect is not observed in the corresponding Ser analogue.

Recently the structure of IGF-1 in solution has been investigated, with a combination of NMR and restrained molecular dynamics methods.[31,32] These investigations clearly demonstrate that the 22–38 domain of IGF-1 has an undefined structure. This is in agreement with the lack of strong correlations in the ROESY experiments on the synthetic peptides **10**, and **12–14**. The amide protons have not been assigned for compound **13** but the size of the coupling constants $^3J_{H\alpha\text{-}N\alpha}$ are in the range 6–8 Hz, also indicating a random structure of the peptide backbone.[33]

In conclusion, the influence of the sugar moiety on the peptide backbone in compounds **10** and **12** has been observed to be limited.

Experimental

TLC was performed on Merck Silica Gel 60 F_{254} aluminium sheets with detection by charring with sulfuric acid, and by UV light when applicable. Flash column chromatography was performed on Merck Silica Gel (0.040–0.060 mm) and chromatography under dry conditions was performed on dried Silica Gel (120 °C; > 24 h) with distilled solvents kept over molecular sieves. DMF was freshly distilled by fractional distillation at reduced pressure and kept over 3 Å molecular sieves. Dichloromethane was distilled from phosphorus pentoxide and kept over 3 Å molecular sieves. Tetrahydrofuran (THF) was passed through a column of basic alumina prior to use. Light petroleum was the 60–80 °C fraction. Concentrations were performed under reduced pressure at a temperature < 40 °C (bath). The organic layers were dried over anhydrous $MgSO_4$. Suitable protected N^{α}-Fmoc amino acids were purchased from

Table 3 1H NMR assignments for dimannosylated peptide **10** and non-glycosylated peptide **13** (in parentheses)

Amino acid[a]	Chemical shifts (δ) α-H	β-H	γ-H	δ-H	ε-H	ζ-H
H-Gly	3.57/3.53 (3.48/3.44)					
Phe	4.52 (4.53)	2.89/2.84 (2.93/2.86)		(7.11 (7.10)	7.27 (7.27)	7.25 (7.24)
Tyr	4.54 (4.52)	3.05/2.93 (3.05/2.95)		7.10 (7.09)	6.82 (6.81)	
Phe	4.50 (4.49)	3.04/2.94 (3.06/2.93)		7.20 (7.20)	7.32 (7.32)	7.25 (7.24)
Asn	4.59 (4.58)	2.71/2.61 (2.73/2.62)				
Lys	4.49 (4.50)	1.72/1.61 (1.72/1.62)	1.38 (1.37)	1.62 (1.61)	2.93 (2.92)	
Pro	4.52 (4.48)	2.26/1.92 (2.27/1.87)	1.99 (1.99)	3.75/3.59 (3.77/3.59)		
Thr	4.42 (4.27)	4.23 (4.16)	1.24 (1.19)			
Gly	3.93 (3.92)					
Tyr	4.47 (4.48)	2.91/2.80 (2.92/2.77)		7.01 (7.02)	6.78 (6.75)	
Gly	3.93 (3.95)					
Ser	4.43 (4.44)	3.85 (3.84)				
Ser	4.45 (4.47)	3.89/3.84 (3.90/3.84)				
Ser	4.49 (4.50)	3.89/3.86 (3.92/3.87)				
Arg	4.32 (4.31)	1.83/1.72 (1.83/1.72)	1.60 (1.61)	3.15 (3.16)		
Arg	4.32 (4.33)	1.83/1.72 (1.83/1.72)	1.60 (1.59)	3.15 (3.16)		
Ala	4.10 (4.12)	1.30 (1.30)				
Dimannosyl data of compound **10**:						
	1-H	2-H	3-H	4-H	5-H	6-H_2
Man(1→2)	4.94	4.02	3.79	3.63	3.71	3.70–3.75, 3.82–3.90
Man(1→Thr)	5.10	3.83	3.89	3.63	3.71	3.70–3.75, 3.82–3.90

[a] Assignments of protons to those amino acids of which there are more than one in the molecular entity 22–38 may be interchanged.

MilliGen (Taastrup, Denmark) or Bachem (Bubendorf, Switzerland), and the Dhbt-esters were prepared according to the method of Atherton *et al.*[18] Kieselguhr-supported poly(dimethylacrylamide) resin, Macrosorb SPR 100, purchased from Sterling Organics (Newcastle, England), was functionalized into the amine form with ethylenediamine[26] and derivatized with 4-(hydroxymethyl)phenoxyacetic acid Dhbt-ester. The peptides and the glycopeptides were hydrolysed with 6 mol dm^{-3} HCl at 110 °C for 24 h and the amino acid composition was determined on a Pharmacia LKB Alpa Plus amino acid analyser. Asn was determined as Asp. Nomenclature is according to IUPAC recommendations. Positive fast-atom-bombardment mass spectra were recorded on a JEOL SX 102 MS instrument. Ions were produced by a beam of xenon atoms, 6 keV, from a matrix of 3-nitrobenzyl alcohol. Optical rotations were measured with a Perkin-Elmer 241 polarimeter, and are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM 500 MHz spectrometer. Chemical shifts are given in ppm and referenced to internal $SiMe_4$ (δ_H, δ_C 0.00) for solutions in $CDCl_3$ at 300 K, and to external dioxane (δ_H 3.76, δ_C 67.40) for solutions in D_2O at 310 K. pD in the D_2O solutions was adjusted to 7.3 by addition of 0.2 mol dm^{-3} Na_2CO_3 in D_2O. The coupling constants $^3J_{H\alpha\text{-}N\alpha}$ were measured in 10% CD_3CO_2D in water at pH 3. For the assignment of signals, proton–proton and carbon–proton shift-correlation spectroscopy were used. ^1H NMR chemical shifts of overlapping signals were obtained from the centre of the cross-peaks in the proton–proton 2D homomuclear chemical-shift correlation (COSY) spectra. A mixing time of 200 ms was used for the 2D ROESY experiments. ^{13}C relaxation times, T_1, were determined by the inversion-recovery method with 4 delay times (t). The T_1-values for the glycosylated and non-glycosylated threonine analogues, **10** and **13** were measured in D_2O at a concentration of 4.5 mmol dm^{-3}, and for the serine analogues, **12** and **14**, at 7.7 mmol dm^{-3}. Preparative HPLC separations were performed on a Waters HPLC system using a DELTA PAK C-18 column (300 Å; 19 mm × 3 cm) with a flow rate of 10 cm^3 min^{-1} and detection at 215 nm and 280 nm with a photodiode array detector (Waters M 991). Solvent system A: 0.1% TFA. B: 0.1% TFA in 90% acetonitrile–10% water. Elemental analyses were carried out at LEO Pharmaceutical Products, Denmark.

Solid-phase Synthesis. General Procedure.—Synthesis of the glycopeptides was performed in DMF with a custom-made, fully automatic, continuous-flow peptide synthesizer using Kieselguhr supported poly(dimethylacrylamide) resin with 4-(hydroxymethyl)phenoxyacetic acid as a linker (0.5 g; 0.11 mequiv. g^{-1}; 0.055 mmol). The first amino acid (Ala) was coupled to the linker *via* the symmetrical anhydride, prepared

Table 4 ^{13}C NMR assignments for dimannosylated peptide **10** and non-glycosylated peptide **13** (in parentheses)

	Chemical shifts (δ)					
Amino acid[a]	C-α	C-β	C-γ	C-δ	C-ε	C-ζ
H-Gly	42.5 (42.5)					
Phe	55.7 (55.7)	37.9 (37.8)	137.1 (137.1)	130.0 (130.0)	129.7 (129.7)	128.1 (128.1)
Tyr	56.3 (56.3)	37.2 (37.2)	128.9 (128.9)	131.5 (131.5)	116.4 (116.4)	155.5 (155.6)
Phe	55.7 (55.7)	37.2 (37.1)	137.1 (137.1)	130.1 (130.1)	129.7 (129.7)	128.1 (128.1)
Asn	51.1 (51.1)	37.2 (37.1)				
Lys	55.6 (55.6)	31.0 (30.9)	22.7 (22.7)	27.3 (27.3)	40.2 (40.1)	
Pro	61.2 (61.4)	30.2 (30.3)	25.6 (25.6)	48.9 (48.9)		
Thr	58.9 (60.2)	76.7 (68.0)	18.6 (19.7)			
Gly	43.2 (43.3)					
Tyr	52.3 (52.4)	38.2 (38.2)	129.0 (128.9)	131.4 (131.4)	116.4 (116.4)	155.4 (155.5)
Gly	43.5 (43.5)					
Ser	56.6 (56.6)	62.0 (62.0)				
Ser	56.6 (56.6)	61.9 (61.9)				
Ser	56.6 (56.6)	61.9 (61.9)				
Arg	54.2 (54.2)	29.1 (29.1)	25.1 (25.1)	41.5 (41.5)	157.8 (157.8)	
Arg	54.2 (54.2)	29.1 (29.1)	25.2 (25.2)	41.5 (41.5)	157.8 (157.8)	
Ala	52.0 (52.0)	18.4 (18.5)				
Carbonyl carbons	185.0, 175.3, 174.7, 174.0, 173.3, 173.1, 172.7, 172.5, 172.4, 171.8 (180.9, 174.6, 173.9, 173.2, 173.0, 172.7, 172.5, 172.4, 172.3, 172.0)					
Dimannosyl data of compound **10**:						
	C-1	C-2	C-3	C-4	C-5	C-6
Man(1→2)	103.3	71.0	71.3	67.8	74.1	61.9
Man(1→Thr)	100.5	80.1	70.9	68.0	74.1	62.0

[a] Assignments of carbons to those amino acids of which there are more than one in the molecular entity 22–38 may be interchanged.

from N^{α}-Fmoc-Ala-OH (0.17 g, 0.55 mmol) and diisopropylcarbodiimide (43 mm^3, 0.27 mmol) in CH_2Cl_2–DMF (25:1) (5.2 cm^3) at 0 °C for 20 min, by addition of DMAP (7 mg, 0.055 mmol) in DMF (1.5 cm^3) (Procedure A), or by esterification of N^{α}-Fmoc-Ala-OH (68 mg, 0.22 mmol) promoted with MSNT (65 mg, 0.22 mmol) in the presence of MeIm[28] (13 mg, 0.16 mmol) (Procedure B). In both procedures capping of unchanged amino groups by addition of acetic anhydride (20 mm^3) was performed before coupling of the second amino acid. The incorporation of Ala, when using Procedure A, was not quantitatively determined but was estimated to be 65% [0.036 mmol $(0.5\ g)^{-1}$], as was determined for Procedure B by quantitative amino acid analysis. In coupling steps with glycosylated N^{α}-Fmoc-Thr-OPfp, **7** or N^{α}-Fmoc-Ser-OPfp, **8**, Dhbt-OH (1 mol equiv.) was added as an auxiliary nucleophile. All other amino acids were coupled as their N^{α}-Fmoc-protected Dhbt-esters (3 mol equiv.) with the side-chains protected with Pmc for arginine, Bu^t for serine and tyrosine, and *tert*-butyloxocarbonyl (Boc) for lysine. N^{α}-Fmoc deprotection was effected by treatment with 50% morpholine in DMF for 20 min and the acylation times were determined with a solid-phase spectrophotometer at 440 nm. After completion of the synthesis the resin was washed with diethyl ether (60 cm^3) and dried by nitrogen flow for 120 min. The resin was then removed from the column, and cleavage of the glycopeptide from the linker was performed by two successive treatments with 95% TFA (30 cm^3) for 30 min, followed by filtration. Acetic acid (3 cm^3) was added to the combined filtrates and, after concentration under reduced pressure, the residue was solidified by trituration with diethyl ether (2 × 25 cm^3). After decantation and evaporation the glycopeptide was purified by preparative HPLC.

The purified acetylated glycopeptide was dissolved in dry methanol (2 mg cm^{-3}) and 0.1 mol dm^{-3} sodium methoxide in methanol was added until a wetted pH-paper indicated pH 12. The mixture was stirred at this pH at room temperature for 20 min, neutralized with small pieces of solid CO_2, filtered, and lyophilized. The residue was dissolved in water (10 mg cm^{-3}) and purified by preparative HPLC.

1,3,4,6-*Tetra*-O-*acetyl*-2-O-(2,3,4,6-*tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-β-D-*mannopyranose* **3**.—Tetra-*O*-acetyl-α-D-mannopyranosyl bromide **1** (0.83 g, 2.0 mmol), silver triflate (0.52 g, 2.0 mmol) and powdered 3 Å molecular sieves was stirred in dry dichloromethane (8 cm^3) under argon at −40 °C.

Table 5 ^{1}H NMR assignments for dimannosylated peptide **12** and non-glycosylated peptide **14** (in parentheses)

	Chemical shifts (δ)					
Amino acid[a]	α-H	β-H	γ-H	δ-H	ε-H	ζ-H
H-Gly	3.47/3.42 (3.56/3.54)					
Phe	4.52 (4.52)	2.89/2.83 (2.87/2.81)		7.12 (7.09)	7.30 (7.26)	7.29 (7.25)
Tyr	4.49 (4.49)	3.04/2.95 (3.03/2.90)		7.11 (7.07)	6.84 (6.81)	
Phe	4.51 (4.49)	3.06/2.93 (3.03/2.90)		7.21 (7.18)	7.34 (7.31)	7.29 (7.25)
Asn	4.58 (4.58)	2.73/2.63 (2.70/2.61)				
Lys	4.47 (4.47)	1.72/1.63 (1.71/1.61)	1.34 (1.34)	1.59 (1.59)	2.91 (2.90)	
Pro	4.42 (4.39)	2.27/1.88 (2.25/1.89)	1.97 (1.96)	3.75/3.55 (3.73/3.55)		
Ser	4.53 (4.36)	3.93/3.77 (3.84/3.80)				
Gly	3.94 (3.91)					
Tyr	4.47 (4.47)	2.92/2.77 (2.89/2.77)		7.02 (7.00)	6.79 (6.77)	
Gly	3.94 (3.91)					
Ser	4.43 (4.43)	3.85/3.82 (3.84/3.80)				
Ser	4.46 (4.45)	3.90/3.84 (3.89/3.82)				
Ser	4.50 (4.49)	3.92/3.86 (3.90/3.84)				
Arg	4.32 (4.32)	1.81/1.71 (1.81/1.71)	1.57 (1.54)	3.15 (3.13)		
Arg	4.32 (4.30)	1.81/1.71 (1.83/1.71)	1.62 (1.61)	3.17 (3.14)		
Ala	4.11 (4.09)	1.30 (1.29)				
Dimannosyl data of compound **12**:						
	1-H	2-H	3-H	4-H	5-H	6-H_2
Man(1→2)	5.00	4.06	3.82	3.62	3.75	n.a.
Man(1→Ser)	5.10	3.93	3.88	3.67	3.55	3.85, 3.72

[a] Assignments of protons to those amino acids of which there are more than one in the molecular entity 22–38 may be interchanged. n.a. = Not assigned.

A solution of 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose **2** (0.47 g, 1.35 mmol) in dry dichloromethane (3 cm^3) was added and the mixture was stirred at −40 °C. After 40 min 2,4,6-trimethylpyridine (2,4,6-collidine) (0.21 cm^3, 1.6 mmol) was added and the temperature was slowly raised to ambient. After being stirred at this temperature for 45 min the mixture was filtered. The filter was washed dichloromethane and the combined washings and filtrate were poured onto ice. The organic layer was washed successively with cold 10% aq. sodium thiosulfate, water, 1 mol dm^{-3} sulfuric acid, 10% aq. sodium hydrogen carbonate and water, dried, and concentrated to a syrup. Purification on silica gel [ethyl acetate–light petroleum (55:45 followed by 60:40)] gave pure *disaccharide* **3** as a solid (0.88 g, 97%), $[\alpha]_D^{18}$ +0.7 (*c* 0.6, $CHCl_3$); ^{1}H and ^{13}C NMR data are presented in Table 1 and Table 2, respectively (Found: C, 49.6; H, 5.7. $C_{28}H_{38}O_{19}$ requires C, 49.56; H, 5.64%; *M*, 678.61).

N^{α}-(*Fluoren-9-ylmethoxycarbonyl*)-O-[3,4,6-*tri*-O-*acetyl*-2-O-(2,3,4,6-*tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-α-D-*mannopyranosyl*]-L-*threonine Pentafluorophenyl Ester* **7**.—To a solution of the octaacetate **3** (0.30 g, 0.44 mmol) in dry dichloromethane (1.5 cm^3) with molecular sieves 3 Å was added 4 mol dm^{-3} hydrogen bromide in acetic acid (1.1 cm^3, 4.4 mmol). The mixture was stirred at room temperature for 60 min, diluted with dichloromethane, and then poured onto ice. The organic layer was washed successively with cold water, saturated aq. sodium hydrogen carbonate and water. Drying and evaporation were followed by concentration under reduced pressure to constant weight (8 h; 0.5 mmHg).

The resulting syrup of the α-bromide **4** (0.30 g, 0.43 mmol) was dissolved in dry dichloromethane (2.5 cm^3) and added to a mixture of N^{α}-Fmoc-L-Thr-OPfp **5** (0.22 g, 0.43 mmol), silver triflate (0.11 g, 0.43 mmol) and powdered 3 Å molecular sieves in dry dichloromethane (4.5 cm^3) under argon at −40 °C. The mixture was stirred at between −30 and −40 °C for 8 h and the progress of reaction was followed by TLC [ethyl acetate–light petroleum (3:2)]. 2,4,6-Collidine (56 mm^3, 0.43 mmol) was then added by syringe, the temperature was slowly raised to room temperature, and the mixture was stirred overnight. Dilution with dry dichloromethane, filtration, and concentration of the filtrate gave a syrup, which was purified on dried silica gel with dry solvents [ethyl acetate–light petroleum (1:1)]. This afforded pure solid *title compound* **7** (0.39 g, 80%), $[\alpha]_D^{18}$ +13.4 (*c* 0.6, $CHCl_3$); ^{1}H and ^{13}C NMR data are presented in Table 1 and Table 2, respectively; *m/z* 1126 $(M + H)^+$ and 1148 $(M + Na)^+$ (Found: C, 54.1; H, 5.1; N, 1.6. $C_{51}H_{52}F_5NO_{22}$ requires C, 54.40;, 4.65; N, 1.24%; *M*, 1125.97).

Table 6 ^{13}C NMR assignments for dimannosylated peptide **12** and non-glycosylated peptide **14** (in parentheses)

Amino acid[a]	Chemical shifts (δ) C-α	C-β	C-γ	C-δ	C-ε	C-ζ
H-Gly	42.9 (42.6)					
Phe	55.7 (55.8)	38.1 (38.2)	137.1 (137.1)	130.1 (130.1)	129.7 (129.7)	128.1 (128.1)
Tyr	56.5 (56.4)	37.1 (37.0)	128.9 (128.9)	131.5 (131.5)	116.5 (116.4)	155.6 (155.5)
Phe	55.6 (55.6)	37.8 (37.9)	137.1 (137.1)	130.0 (130.0)	129.7 (129.7)	128.1 (128.1)
Asn	51.1 (51.1)	37.1 (37.1)				
Lys	55.6 (55.7)	30.9 (30.9)	22.7 (22.7)	27.3 (27.3)	40.2 (40.1)	
Pro	61.3 (61.5)	30.3 (30.3)	25.6 (25.6)	48.9 (48.9)		
Ser	54.8 (56.6)	67.5 (61.9)				
Gly	43.3 (43.4)					
Tyr	52.3 (52.4)	37.2 (37.2)	128.9 (128.9)	131.4 (131.4)	116.4 (116.4)	155.5 (155.4)
Gly	43.5 (43.5)					
Ser	56.6 (56.6)	61.9 (62.0)				
Ser	56.6 (56.6)	62.1 (62.0)				
Ser	56.6 (56.6)	61.9 (61.9)				
Arg	54.2 (54.2)	29.1 (29.1)	25.2 (25.3)	41.5 (41.5)	157.7 (157.7)	
Arg	54.2 (54.2)	29.1 (29.1)	25.1 (25.1)	41.5 (41.5)	157.7 (157.7)	
Ala	52.0 (52.0)	18.5 (18.5)				
Carbonyl carbons	180.5, 175.1, 174.7, 174.0, 173.3, 173.1, 172.5, 172.4, 171.9 (180.5, 175.1, 174.7, 174.0, 173.3, 173.1, 173.0, 172.7, 172.6, 172.5, 172.4, 172.1, 171.1)					

Dimannosyl data of compound **12**:

	C-1	C-2	C-3	C-4	C-5	C-6
Man(1→2)	103.2	70.9	71.3	67.9	74.2	62.0
Man(1→Ser)	99.8	79.5	71.0	67.9	74.0	61.9

[a] Assignment of carbons to those amino acids of which there are more than one in the molecular entity 22–38 may be interchanged.

N^{α}-(*Fluoren-9-ylmethoxycarbonyl*)-O-[3,4,6-*tri*-O-*acetyl*-2-O-(2,3,4,6-*tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-α-D-*mannopyranosyl*]-L-*serine Pentafluorophenyl Ester* **8**.—The preparation of the α-bromide **4** was performed as described above from compound **3** (0.10 g, 0.14 mmol). The resulting syrup (0.10 g, 0.14 mmol) was dissolved in dry dichloromethane (3 cm^3) and added to a mixture of N^{α}-Fmoc-L-Ser-OPfp **6** (69 mg, 0.14 mmol), silver triflate (36 mg, 0.14 mmol) and powdered 3 Å molecular sieves in dry dichloromethane (3 cm^3) under argon at −40 °C. The mixture was stirred at between −25 and −40 °C, and the reaction was followed by TLC [ethyl acetate–light petroleum (3:2)]. After 7 h 2,4,6-collidine (18 mm^3, 0.14 mmol) was added and the mixture was stirred at room temperature overnight. Dilution with dry dichloromethane, filtration, and concentration were followed by purification on dried silica gel with dry solvents [ethyl acetate–light petroleum (2:1.25)] which gave pure *title compound* **8** (103 mg, 65%), $[\alpha]_D^{18}$ +23.0 (*c* 0.4, $CHCl_3$); ^1H and ^{13}C NMR data are presented in Table 1 and Table 2, respectively; *m/z* 1112 $(M + H)^+$ and 1134 $(M + Na)^+$ (Found: C, 53.6; H, 4.8. $C_{50}H_{50}F_5NO_{22}$ requires C, 54.01; H, 4.53%; *M*, 1111.95).

[*Di-Man-Thr*^{29}]*IGF*-1 (22–38) **10**.—The solid-phase synthesis was carried out according to the general procedure as described above. The first amino acid (Ala) was coupled to the resin according to procedure A. The loading of Ala was estimated to be 65% (0.036 mmol). In the coupling step with the dimannosylated threonine derivative **7** three mole equivalents (130 mg, 0.11 mmol) were used. The acylation times varied from 30 min (Gly) to 17 h (Pro) and the acylation time for substrate for **7** was 115 min. After cleavage from the resin, IGF-1 [Ac_7-Di-Man-Thr^{29}] (22–38) **9** was purified by preparative HPLC using a linear gradient of 20–100% solvent B during 80 min (retention time 37 min). The yield of heptaacetate **9** was 48 mg, 53%.

Compound **9** was deacetylated with sodium methoxide as described above. Preparative HPLC using the same gradient as for compound **9** (retention time 20 min) afforded pure title compound **10** (29 mg, overall yield of 37%). ^1H and ^{13}C NMR data are presented in Table 3 and Table 4, respectively. Amino acid analyses (theoretical value in parenthesis): Ala 0.95(1), Arg 2.04(2), Ser 2.79(3), Gly 2.93(3), Tyr 1.87(2), Thr 1.02(1), Pro 1.24(1), Lys 0.99(1), Asn 1.17(1) and Phe 1.95(2); *m/z* 2220 $(M + H)^+$ ($C_{97}H_{143}N_{25}O_{35}$ requires *M*, 2219.37).

Table 7 ^{13}H Relaxation times (T_1/s) for dimannosylated peptide **10** and **12** and non-glycosylated peptide **13** and **14** (in parentheses)

Amino acid[a]	C-α	C-β	C-γ	C-δ	C-ε	C-ζ
[**10** and (**13**)]						
H-Gly	n.d.					
Phe	0.44 (0.42)	0.17 (0.19)	1.14 (1.10)	0.41 (0.49)	0.42 (0.49)	0.29 (0.30)
Tyr	0.28 (0.45)	0.14 (0.19)	0.87 (1.17)	0.34 (0.37)	0.36 (0.38)	n.d.
Phe	0.32 (0.39)	0.17 (0.21)	1.14 (1.01)	0.36 (0.46)	0.38 (0.45)	0.29 (0.33)
Asn	0.33 (0.41)	0.17 (0.21)				
Lys	0.32 (0.41)	0.14 (0.16)	0.28 (0.30)	0.47 (0.49)	0.55 (0.76)	
Pro	0.24 (0.36)	0.23 (0.37)	0.32 (0.48)	0.17 (0.20)		
Thr	0.23 (0.38)	0.22 (0.42)	0.43 (0.51)			
Gly	0.14 (0.15)					
Tyr	0.25 (0.43)	0.23 (0.18)	0.86 (1.17)	0.32 (0.34)	0.34 (0.37)	
Gly	0.20 (0.17)					
Ser	0.34 (0.38)	0.23 (0.23)				
Ser	0.36 (0.43)	0.24 (0.24)				
Ser	0.36 (0.43)	0.24 (0.24)				
Arg	0.36 (0.34)	0.23 (0.23)	0.33 (0.42)	0.37 (0.39)		
Arg	0.36 (0.34)	0.23 (0.23)	0.29 (0.32)	0.37 (0.39)		
Ala	0.70 (1.09)	0.79 (1.11)				
[**12** and (**14**)]						
H-Gly	0.45 (0.41)					
Phe	0.36 (0.36)	0.25 (0.23)	0.92 (0.90)	0.35 (0.37)	0.37 (0.37)	0.30 (0.28)
Tyr	0.31 (0.30)	0.14 (0.13)	1.09 (1.04)	0.32 (0.33)	0.33 (0.36)	0.80 (0.82)
Phe	0.31 (0.32)	0.13 (0.12)	0.81 (0.82)	0.46 (0.46)	0.44 (0.45)	0.30 (0.28)
Asn	0.32 (0.32)	0.14 (0.12)				
Lys	0.31 (0.32)	0.13 (0.13)	0.28 (0.30)	0.41 (0.46)	0.64 (0.69)	
Pro	0.28 (0.33)	0.28 (0.30)	0.28 (0.29)	0.12 (0.12)		
Ser	0.26 (0.35)	0.15 (0.22)				
Gly	0.12 (0.13)					
Tyr	0.27 (0.29)	0.13 (0.13)	1.09 (1.04)	0.32 (0.32)	0.33 (0.34)	0.80 (0.75)
Gly	0.13 (0.13)					
Ser	0.34 (0.34)	0.21 (0.22)				
Ser	0.34 (0.35)	0.22 (0.22)				
Ser	0.34 (0.36)	0.21 (0.22)				
Arg	0.35 (0.33)	0.22 (0.22)	0.29 (0.29)	0.36 (0.35)		
Arg	0.35 (0.36)	0.22 (0.22)	0.31 (0.32)	0.36 (0.35)		
Ala	0.67 (0.74)	1.01 (1.01)				
Dimannosyl data of **10** and **12**:						
	C-1	C-2	C-3	C-4	C-5	C-6
[**10**]						
Man(1→2)	0.35	0.36	0.36	0.33	0.31	0.24
Man(1→Thr)	0.31	0.30	0.27	0.28	0.31	0.23
[**12**]						
Man(1→2)	0.36	0.40	0.38	0.36	0.36	0.21
Man(1→Ser)	0.34	0.29	0.31	0.26	0.27	0.21

[a] Assignment of carbons to those amino acids of which there are more than one in the molecular entity 22–38 may be interchanged.

[*Di-Man-Ser*^{29}]*IGF*-1 (22–38) **12**.—The first amino acid was coupled to the resin according to procedure B (incorporation of Ala was determined to be 62%, 0.034 mmol), and the solid-phase synthesis was performed as described above. Dimannosylated serine derivative **8** (2.7 mol equiv.) was used (105 mg, 0.094 mmol), and the acylation times varied from 30 min (Ser, Gly, Tyr, Pro and Phe) to 5 h (**8**). Cleavage from the resin and purification with preparative HPLC with a linear gradient of 20–100% B during 40 min, retention time 24 min, afforded pure heptaacetate **11** (67 mg, 67%).

Deacetylation of compound **11** with sodium methoxide and purification by preparative HPLC with the same gradient (retention time 18 min) gave pure title compound **12** (27 mg, overall yield of 33%). ^1H and ^{13}C NMR data are presented in Table 5 and Table 6, respectively. Amino acid analyses (theoretical value in parenthesis): Ala 1.03(1), Arg 2.12(2), Ser 3.77(4), Gly 3.05(3), Tyr 1.93(2), Pro 1.10(1), Lys 0.98(1), Asn 1.02(1) and Phe 1.99(2); *m/z* 2206 $(M + H)^+$ ($C_{96}H_{141}N_{25}O_{35}$ requires *M*, 2205.34).

IGF-1 (22–38) **13**.—Solid-phase synthesis of peptide **13** was carried out according to the general procedure and the first amino acid was coupled to the resin according to procedure A. The loading of Ala was estimated to 65% (0.036 mmol). Acylation times varied from 30 to 90 min, except for lysine which was coupled for 7.5 h. After cleavage from the resin the peptide was purified by preparative HPLC using 10% solvent B for 10 min, a linear gradient of 10–25% B during 30 min, 25% B for 10 min and a linear gradient of 25–100% B for 20 min (retention time 40 min). This afforded pure title compound **13** (24 mg, 35%). ^1H and ^{13}C NMR data are presented in Table 3 and Table 4, respectively. Amino acid analyses (theoretical values in parenthesis): Ala 1.13(1), Arg 2.17(2), Ser 2.90(3), Gly 3.13(3), Tyr 1.68(2), Thr 0.94(1), Pro 1.00(1), Lys 1.01(1) Asn 1.06(1) and Phe 1.98(2); *m/z* 1896 $(M + H)^+$ ($C_{85}H_{123}N_{25}O_{25}$ requires *M*, 1895.08).

[*Ser*^{29}]*IGF*-1 (22–38) **14**.—The first amino acid was coupled to the resin according to procedure B (incorporation of Ala was

determined to 73%, 0.044 mmol) and preparation of peptide **14** was performed according to the general procedure. Acylation times varied from 30 min to 33 h (Lys) and purification, after cleavage from the resin, by preparative HPLC with a linear gradient of 10–100% solvent B during 40 min (retention time 24 min), afforded pure title compound **14** (36 mg, 43%). ^1H and ^{13}C NMR data are presented in Table 5 and Table 6, respectively. Amino acid analyses (theoretical values in parenthesis): Ala 1.14(1), Arg 2.27(2), Ser 3.74(4), Gly 3.03(3), Tyr 1.90(2), Pro 0.93(1), Lys 1.02(1), Asn 0.99(1) and Phe 2.00(2); *m/z* 1882 $(M + H)^+$ ($C_{84}H_{121}N_{25}O_{25}$ requires *M*, 1881.05).

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References

1 J. Montreuil, *Adv. Carbohydr. Chem. Biochem.*, 1980, **37**, 157.
2 P. Gellerfors, K. Axelsson, A. Helander, S. Johansson, L. Kenne, S. Lindqvist, B. Pavlu, A. Skottner and L. Fryklund, *J. Biol. Chem.*, 1989, **264**, 11444.
3 A. M. Jansson, M. Meldal and K. Bock, *Tetrahedron Lett.*, 1990, **31**, 6991.
4 K. Wakabayashi and W. Pigman, *Carbohydr. Res.*, 1974, **35**, 3.
5 S. Lavielle, N. C. Ling, R. Saltman and R. C. Guillemin, *Carbohydr. Res.*, 1981, **89**, 229.
6 H. Paulsen, G. Merz and U. Weichert, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 1365.
7 B. Lüning, T. Norberg and J. Tejbrant, *J. Chem. Soc., Chem. Commun.*, 1989, 1267.
8 F. Filira, L. Biondi, B. Scolaro, M. T. Foffani, S. Mammi, E. Peggion and R. Rocchi, *Int. J. Biol. Macromol.*, 1990, **12**, 41.
9 F. Filira, L. Biondi, F. Cavaggion, B. Scolaro and R. Rocchi, *Int. J. Pept. Protein. Res.*, 1990, **36**, 86.
10 H. Paulsen, G. Merz, S. Peters and U. Weichert, *Leibigs Ann. Chem.*, 1990, 1165.
11 E. Bardaji, J. L. Torres, P. Clapés, F. Albericio, G. Barany, R. E. Rodriguez, M. P. Sacristàn and G. Valencia, *J. Chem. Soc., Perkin Trans. 1*, 1991, 1755.
12 S. Peters, T. Bielfeldt, M. Meldal, K. Bock and H. Paulsen, *Tetrahedron Lett.*, 1991, **32**, 5067.
13 M. Meldal and K. Bock, *Tetrahedron Lett.*, 1990, **31**, 6987.
14 M. Meldal and K. J. Jensen, *J. Chem. Soc., Chem. Commun.*, 1990, 483.
15 E. Atherton, L. R. Cameron and R. C. Sheppard, *Tetrahedron*, 1988, **44**, 843.
16 P. Schultheiss-Reimann and H. Kunz, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 62.
17 H. Paulsen and J.-P. Hölck, *Carbohydr. Res.*, 1982, **109**, 89.
18 E. Atherton, J. L. Holder, M. Meldal, R. C. Sheppard and R. M. Valerio, *J. Chem. Soc., Perkin Trans. 1*, 1988, 2887.
19 L. R. Cameron, J. L. Holder, M. Meldal and R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, 1988, 2895.
20 P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, 1931, **90**, 89.
21 J. O. Deferrari, E. G. Gros and I. O. Mastronardi, *Carbohydr. Res.*, 1967, **4**, 432.
22 P. L. Durette and T. Y. Shen, *Carbohydr. Res.*, 1979, **69**, 316.
23 C. M. Reichert, *Carbohydr. Res.*, 1979, **77**, 141.
24 L. Kisfaludy and I. Schön, *Synthesis*, 1983, 325.
25 A. Helander and L. Kenne, presented at the XVth International Carbohydrate Symposium, Yokohama, 1990.
26 R. Arshady, E. Atherton, D. L. J. Clive and R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, 1981, 529.
27 A. Dryland and R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, 1986, 125.
28 B. Blankemeyer-Menge, M. Nimtz and R. Frank, *Tetrahedron Lett.*, 1990, **31**, 1701.
29 R. Ramage and J. Green, *Tetrahedron Lett.*, 1987, **28**, 2287.
30 D. Doddrell, V. Glushko and A. Allerhand, *J. Chem. Phys.*, 1972, **56**, 3683.
31 R. M. Cooke, T. S. Harvey and I. D. Campbell, *Biochemistry*, 1991, **30**, 5484.
32 Y. Kobayashi, S. Nishimura, T. Ohkubo, Y. Kyogoku, A. Sato, S. Koyama, M. Kobayashi and T. Yasuda, Proceedings of the European Peptide Symposium, 21st, 1990 (Publ. 1991, ESCOM Science Publishers, eds. E. Giralt and D. Andrew), p. 565.
33 A. Pardi, M. Billeter and K. Wüthrich, *J. Mol. Biol.*, 1984, **180**, 741.

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