Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1531–1545

BIOORGANIC & MEDICINAL CHEMISTRY

Effects of Glycosylation on Fragments of Tumour Associated Human Epithelial Mucin MUC1

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Received 26 October 1997; accepted 12 March 1998

Abstract—The glycodecapeptide AcPAPGS(αGalNAc)T(αGalNAc)APPA and the C-terminal glycohexapeptide AcS(αGalNAc)T(αGalNAc)APPA have been synthesized by applying the N-terminal Fmoc group in combination with the heptyl ester cleavable by lipase-catalyzed hydrolysis at pH 7. The solution conformation of these MUC1-related synthetic glycopeptides and the control, non-glycosylated decapeptide AcPAPGSTAPPA have been investigated using NMR spectroscopy. The structural studies indicate that the glycohexapeptide has a folded structure in solution. For this molecule, unrestrained molecular dynamics has been used to confirm the presence of the observed solution through-space connections. The results indicate that the non-globular nature of MUC1 is due to both protein core sequence and the effect of carbohydrate.

Introduction

MUC1 is a high molecular weight (>400kDa), *O*-glycosylated molecule located on the lumenal surface of epithelial cells such as those of the breast milk ducts, and the bladder.[1] The molecule is largely composed of an expressed variable number of tandem repeats (VNTR) of the sequence PDTRPAPGSTAPPA HGVTSA, which is extensively *O*-glycosylated through serine and threonine residues. The putative role of this molecule is one of lubrication and protection of epithelial cells. This reflects the high viscosity of MUC1, which has been shown to be largely an effect of carbohydrate groups which induce extended, rod-shaped conformations in mucin molecules, and which is a feature of the MUC1 macrostructure. MUC1 has received much attention due to the changes in expression which occur on malignant transformation of MUC1-positive cells. The MUC1 malignant phenotype features an up-regulation of expression, an altered cell distribution and changes in the nature and extent of glycosylation.[2a] Short saccharide side chains, in particular the monosaccharide GalNAc (Tn antigen), have been described to be tumour associated antigens on epithelial cells.[2b] Most anti-MUC1 antibodies bind to minimum epitopes in the protein core of the MUC1 VNTR within the sequence PDTRPAP.[3] NMR studies of synthetic non-glycosylated MUC1 core peptides show that this immunodominant region exists in a β-turn conformation.[4] Anti-MUC1 antibodies have been found to preferentially bind to MUC1 of malignant origin, which is thought to be due to altered glycosylation. Many such antibodies have therefore been exploited clinically to measure and/or visualize MUC1 of malignant origin.[5]

Structural studies of native mucins are complicated by the high molecular weight and heterogeneity of the molecule. Low resolution data from STM,[6] hydrodynamic,[7] and NMR studies[8] of native mucins show that these molecules form rod-like structures, which are critically affected by the carbohydrate moieties. In this study (glyco)peptides corresponding to the P^5-A^{14} region of the peptide core have been synthesized and analyzed using NMR spectroscopy. This region is of particular interest both structurally and functionally; glycosylation of the serine and threonine residues affects

Key words: *O*-Glycopeptides; enzymatic deprotection; conformation in solution; NMR spectroscopy; molecular dynamics simulations.
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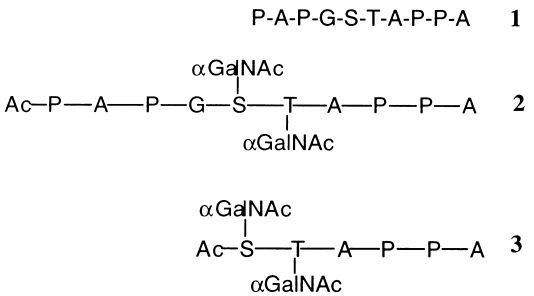
0968-0896/98/$—see front matter
PII: S0968-0896(98)00092-3

the binding reaction of antibodies to the distant MUC1 epitope, indicating that the carbohydrate groups in this position incur either steric or structural effects at the site of antibody binding. The presence of four proline residues in this sequence would be expected to destabilize secondary structure. Therefore the glycodecapeptide AcPAPGS(αGalNAc)T(αGalNAc)APPA (**2**), the truncated glycohexapeptide AcS(αGalNAc)T(αGalNAc)APPA (**3**), and the control decapeptide AcPAPGSTAPPA (**1**) were synthesized and subjected to NMR spectroscopic analysis.



Results and Discussion

Synthesis of glycopeptides

Glycopeptides with *O*-glycosidic bonds to serine and threonine are not only sensitive to strong acids but also prone to an easy base-catalyzed β-elimination of the carbohydrates.[9] A number of selective protecting group techniques have been developed during the past decade which enable the efficient chemical synthesis of glycopeptides of remarkable complexity.[10] Interesting reagents in this field are enzymatic methods.[11] For the syntheses of the glycopeptides **2** and **3** we applied a fragment condensation strategy. The fluorenylmethoxycarbonyl (Fmoc) group was used as the amino protecting group and selectively removed with morpholine.[12] The heptyl ester served as the carboxy protection which can be selectively hydrolyzed under catalysis by lipases.[13,14]

As has already been described,[15] Fmoc serine heptyl ester and Fmoc threonyl alanine heptyl ester were *O*-glycosylated by reaction with ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-β-D-galactopyranoside **4**[16] under activation with dimethyl(methylthio)sulfonium trifluoromethanesulfonate[17] (DMTST). Enzymatic hydrolysis of the *O*-glycosyl serine heptyl ester **5** catalyzed by lipase M (from *Mucor javanicus*) proceeded with complete selectivity.[15] The *O*-glycosylated threonine heptyl ester as well as its benzyloxycarbonyl protected analogue did not react under identical conditions. On the other hand, the Fmoc group was selectively cleaved off from **6**. Condensation of the selectively deblocked *O*-glycosyl amino acid derivatives **7** and **8** produced the clustered *O*-glycopeptide **9**. Transformation of the 2-azido to the 2-acetamidogalactosides furnished the twofold glycosylated glycopeptide **10** carrying the tumor associated Tn antigen structural element (Scheme 1).

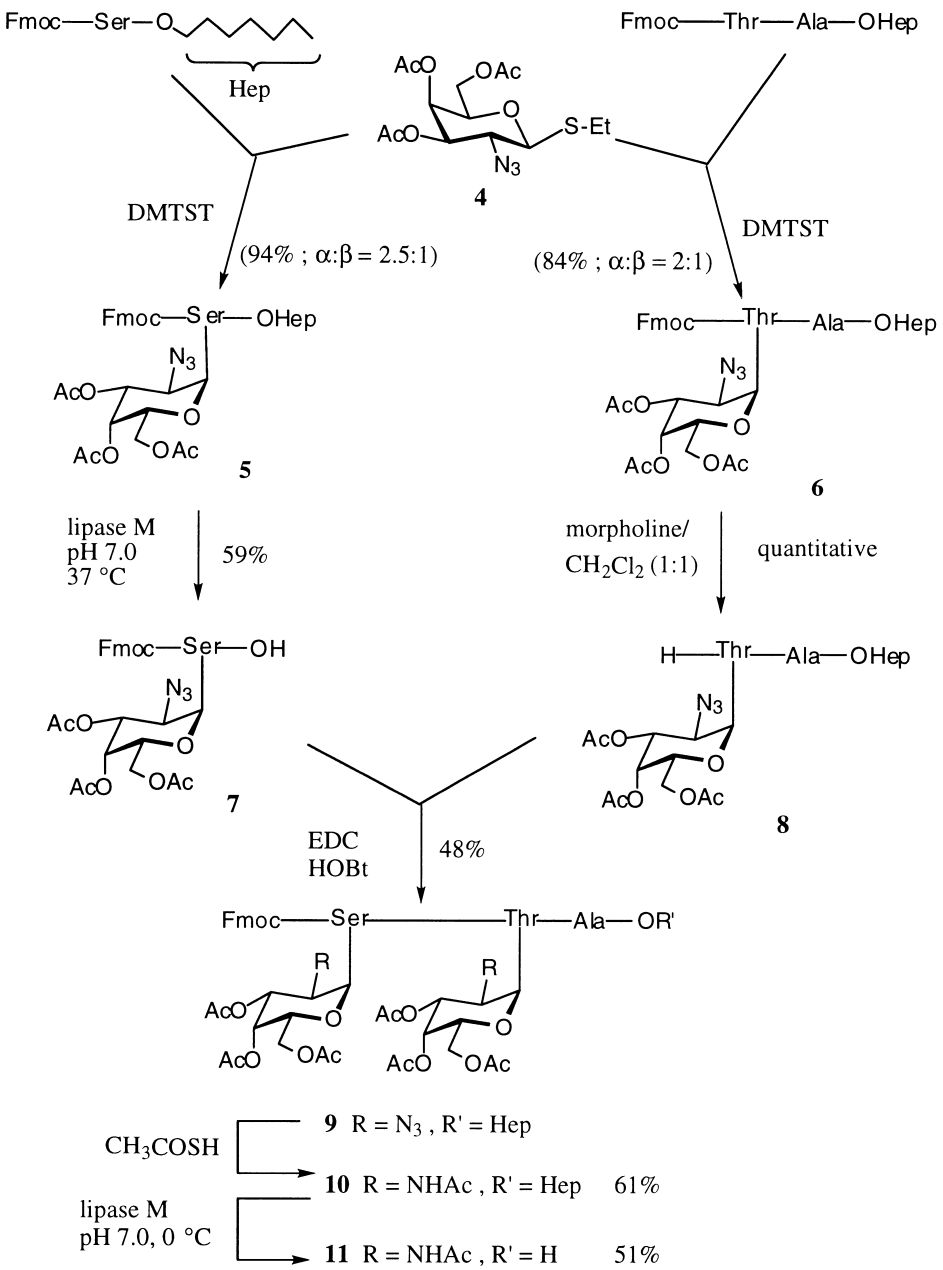
Hydrolysis of the heptyl ester catalyzed by lipase M gave the selectively carboxy-deblocked glycopeptide building block **11** required for the construction of the mucin tandem repeat fragments. In this context, it is noteworthy that *O*-glycosylated serine (**12**) as well as the corresponding threonine heptyl ester **13** carrying the tri-*O*-acetyl 2-acetamido-2-deoxy-α-D-galactosyl (Tn antigen) sidechain and *Z*-protection at the amino function were not accepted as substrates by any out of 15 lipases tested. In contrast, the recently developed analogous 2-(2-methoxy-ethoxy)-ethyl esters (MEE) **14** and **15**[18] were hydrolyzed by lipase M (Scheme 2). The reactions were slow but gave the selectively deblocked *O*-galactosaminyl amino acids **16** and **17** in pure form.

For fragment condensations the N- and C-terminal oligo-peptides were synthesized starting from glycine-(**18**) or alanine-heptyl ester,[13] **26**, respectively (Scheme 3). While the removal of the Fmoc group from peptide esters **19**, **21**, **23** or **27**, **29** proceeded quantitatively, the coupling reactions of and to proline units were slow and have not been optimized.

The hydrolysis of the tetrapeptide heptyl ester **24**, which according to its ^{13}C NMR spectrum exists in solution in four rotamers, was efficiently catalyzed by lipase N from *Rhizopus niveus* (Amano) to give **25** in a yield of 76%. For the protected tripeptide ester **29** synthesized in analogy to **21** three rotamers were detected by ^{1}H and ^{13}C NMR spectroscopy.

To couple the fragments, the Fmoc group was removed from **29**. Component **30** quantitatively obtained was condensed with glycopeptide **11** to produce the glycohexapeptide **31** in a yield of 50% after purification by flash-chromatography. It is interesting to note that the NMR spectra of **31** did not show rotamers, thus suggesting that the glycohexapeptide in contrast to the peptides **25** and **29** adopts one preferred conformation in solution (vide infra).

For conformational studies the Fmoc group was cleaved off from **31** and exchanged with the *N*-acetyl group in **32**. The selective hydrolysis of the heptyl ester was efficiently catalyzed by lipase N in water/acetone (20:1) at 37 °C and pH 7. Finally, the *O*-acetyl groups were removed from **33** by Zemplén transesterification in methanol at an apparent pH of 8.5.[19,20] The pure glycohexapeptide **3** was obtained after neutralization with

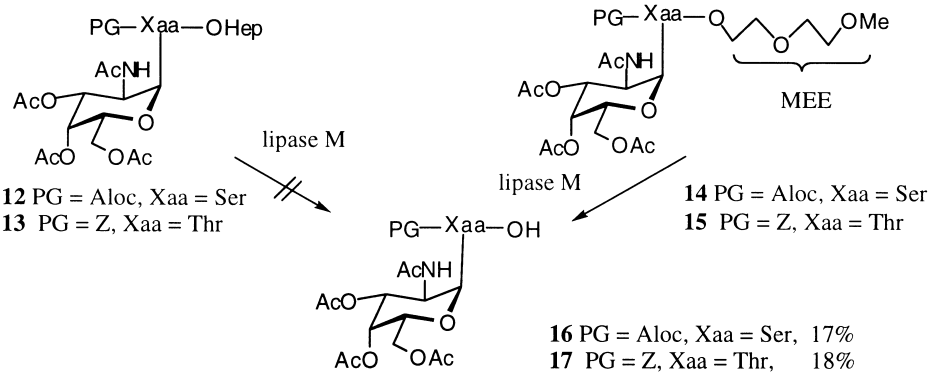


Scheme 1.

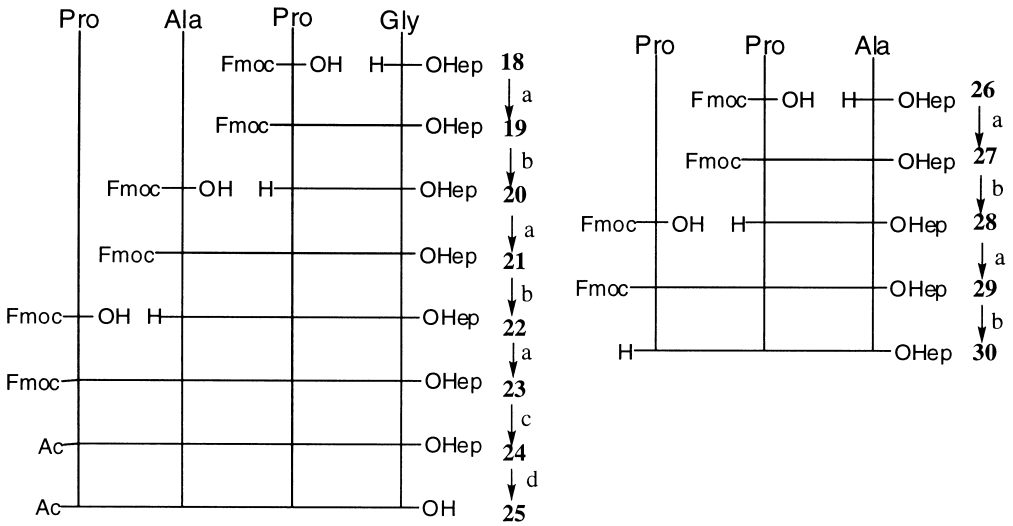
ion exchange resin (DOWEX 50WX-8) and preparative HPLC as an amorphous hygroscopic solid (Scheme 4).

To synthesize the glycodecapeptide **2**, the amino-deblocked product of **31** was condensed with the tetrapeptide **25** to give the protected glycodecapeptide **34**. During this coupling a byproduct was formed which could be isolated (25%) and identified as the *N*-acetyl glycohexapeptide **32**. Its formation is obviously due to an intermolecular acetyl group transfer from a carbohydrate ester[21] occurring during the long reaction time (Scheme 5).

As a general conclusion from the formation of **32** in this reaction one can learn that fragment condensation strategies should, if possible, avoid reactions at sterically demanding functions. However, in the described synthesis of **34** any other segmentation would be worse because of the proline units involved. The heptyl ester of **34** was subjected to hydrolysis catalyzed by lipase N.



Scheme 2.



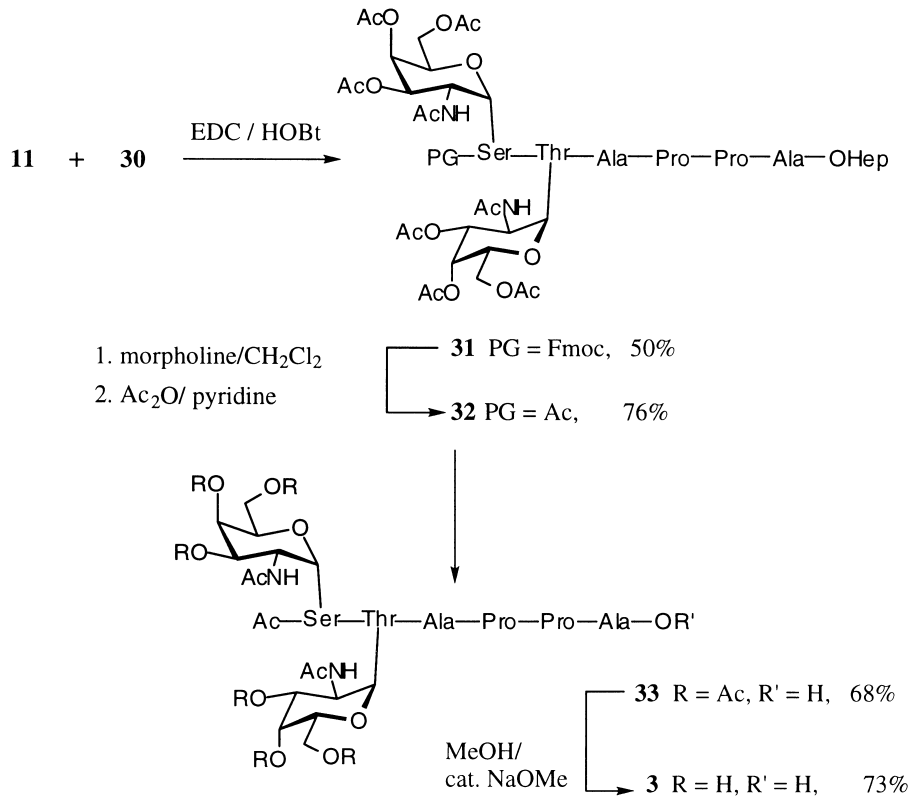
Scheme 3. (a)Ethyl-3-(3-dimethylamino-propyl carbodiimide (EDC)/1-hydroxy-benzotriazole (HOBt); (b) morpholine/CH_2Cl_2; (c) 1: morpholine/CH_2Cl_2, 2: Ac_2O/pyridine; (d) lipase N (Rhizopus niveus (Amano), pH 7, 37 °C.

From the selectively formed **35** the *O*-acetyl groups were removed by controlled base-catalyzed methanolysis to give the desired glycodecapeptide **2** required from the conformational studies.

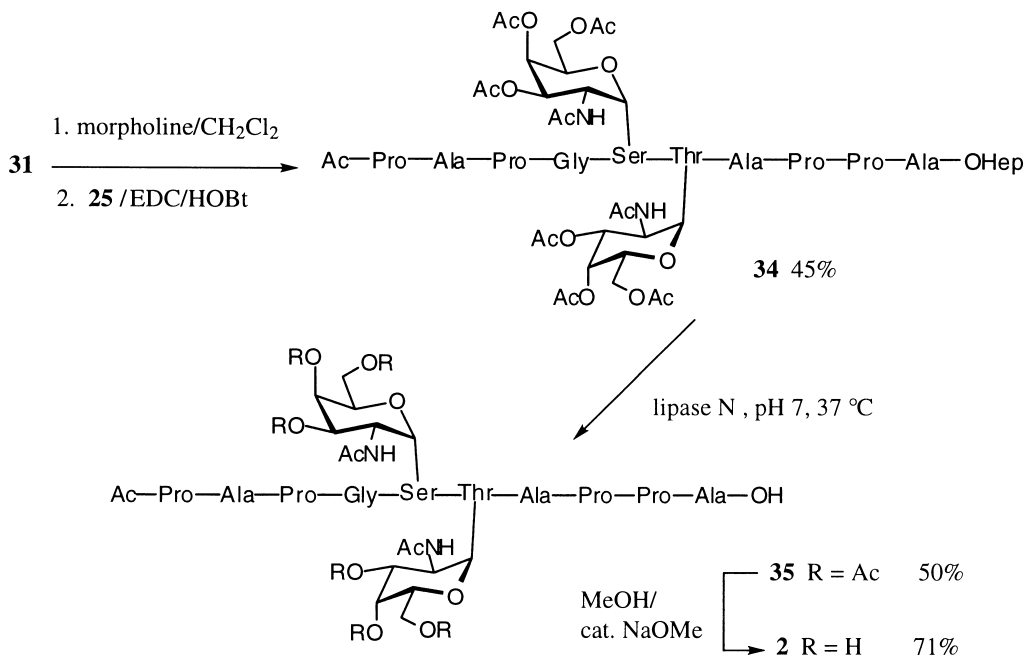
Structural studies of MUC1 related glycopeptides

Resonances were assigned on the basis of through bond and sequential through space connections utilizing two-dimensional COSY, HOHAHA, NOESY, and ROESY experiments. Amino acid attachment specificity for carbohydrate spin systems were deduced by examination of sugar resonance through space connections to attached amino acid αH and/or βH resonances. Coupling constants were measured from a DQF-COSY and 1-D spectra. Table 1 lists resonance assignments for the three (glyco)peptides. The coupling constant data for the three (glyco)peptides are displayed in Table 2, $^3J_{NH\text{-}\alpha H}$ data for the two deca(glyco)peptides **1** and **2** do not indicate the presence of any stable conformation. The $^3J_{NH\text{-}\alpha H}$ value for A^6 in glycohexapeptide **3** was found to be 3.67 Hz, suggesting some ordered structure in this region. The carbohydrate $^3J_{H1\text{-}H2}$ values are consistent with the sugar groups being in the α-anomeric conformation, and the $^3J_{H2\text{-}H3}$ values are consistent with the $^4C_1$ chair conformation with axial hydrogens as expected.

Distance restraints were derived from NOESY and ROESY spectra, and are indicated in Table 3. It is interesting to note that the NOESY spectrum of decapeptide **1** contained no crosspeaks, necessitating the use of the ROESY experiment, whereas the two glycopeptides (**2** and **3**) both produced adequate NOESY spectra. This reflects differences in solution mobility as a result of glycosylation. The glycodecapeptide **2** and



Scheme 4.



Scheme 5.

Table 1.

Resonance assignments for **1**: AcPAPGSTAPPA (chemical shifts measured in ppm)

Residue	NH	αH	βH	γH	δH
Pro^{1}		4.27	2.02	1.85	3.53
Ala^{2}	8.24	3.71	1.21		
Ala^{2}(min)	7.96	4.45	1.16		
Pro^{3}		4.30	2.03	1.88	3.55
Gly^{4}	8.21	3.71			
Ser^{5}	7.73	4.39	3.61		
Thr^{6}	7.67	4.19	1.03 (CH_3)		
Ala^{7}	7.81	4.46	1.15		
Pro^{8}		4.56	2.16	1.87	3.48, 3.49
Pro^{9}		4.35	2.18	1.76	3.38
Ala^{10}	8.02	4.12	1.24		
Pro(min)		4.44	2.11	1.61	3.42

Resonance assignments for **2**: AcPAPGS(αGalNAc)T(αGalNAc)APPA (chemical shifts measured in ppm)

Residue	NH	αH	βH	γH	δH	
Pro^{1}		4.34	2.16, 1.75	1.90	3.36	
Ala^{2}	8.24	4.55	1.23 (CH_3)			
Pro^{3}		4.30	2.05, 1.90	1.90	3.59	
Gly^{4}	8.15	3.75				
Ser^{5}	7.93	4.79	3.60, 3.75			
Thr^{6}	8.00	4.40	4.13	1.09 (CH_3)		
Ala^{7}	8.08	4.43	1.16 (CH_3)			
Pro^{8}		4.56	2.14, 1.89	1.93	3.61, 3.45	
Pro^{9}		4.28	2.00, 1.83	1.83	3.65, 3.49	
Ala^{10}	7.88	4.01	1.02 (CH_3)			
Ala min	7.95	4.44	1.18 (CH_3)			
Sugar group	NH	H1	H2	H3	H4/H5	H6
(Ser^{5})GalNAc	6.85	4.68	4.23	3.70	4.01	3.55
(Thr^{6})GalNAc	7.08	4.73	4.21	3.73	4.01	3.58

Resonance assignments for **3**: AcS(αGalNAc)T(αGalNAc)APPA (chemical shifts measured in ppm)

Residue	NH	αH	βH	γH	δH	
Ser^{1}	8.20	4.73	3.58, 3.73			
Thr^{2}	7.96	4.38	4.13	1.11 (CH_3)		
Ala^{3}	8.11	4.45	1.18 (CH_3)			
Pro^{4}		4.56	2.16	2.00, 1.87	3.60, 3.44	
Pro^{5}		4.22	1.95	1.87, 1.83	3.73, 3.55	
Ala^{6}	7.35	3.60	1.13 (CH_3)			
Sugar group	NH	H1	H2	H3	H4/H5	H6
(Ser^{1})GalNAc	6.96	4.69	4.30	3.70	4.00	3.55
(Thr^{2})GalNAc	7.12	4.71	4.29	3.72	4.00	3.57

control decapeptide **1** have no medium or long range peptide through-space connections, indicating that these molecules have no preferred secondary structure in solution. The glycohexapeptide however, has some long range through-space connections between the peptide termini (Thr^{2}NH-Ala^{6}NH, Ser^{1}NH-Pro^{5}βH/γH, Pro^{4}αH-Ser^{1}αH) suggesting gross folding of this glycopeptide.

Unrestrained molecular dynamics (MD) of the three peptide sequences was utilized to confirm the through-space

Table 2.

$^3J_{NH\text{-}\alpha H}$ (Hz) values for (glyco)peptides			
Residue	$^3J_{NH\text{-}\alpha H}$ (Hz) decapeptide **1**	$^3J_{NH\text{-}\alpha H}$ (Hz) glycodecapeptide **2**	$^3J_{NH\text{-}\alpha H}$ (Hz) glycohexapeptide **3**
Ala^2	N.D.	7.68	–
Gly^4	N.D.	9.29	–
Ser^5	9.69	8.49	8.26 (Ser^1)
Thr^6	10.29	9.30	10.1 (Thr^2)
Ala^7	8.48	9.30	6.43 (Ala^3)
Ala^{10}	8.48	7.27	3.67 (Ala^6)

Important saccharide proton coupling constants for the (glyco)peptides				
	Ser^5-linked GalNAc glycodecapeptide **2**	Thr^6-linked GalNAc glycodecapeptide **2**	Ser^1-linked GalNAc glycohexapeptide **3**	Thr^2-linked GalNAc glycohexapeptide **3**
$^3J_{H1\text{-}H2}$ (Hz)	3.69	4.00	3.47	3.76
$^3J_{H2\text{-}H3}$ (Hz)	10.80	10.80	N.D.[a]	N.D.
$^3J_{NH\text{-}H2}$ (Hz)	8.66	9.01	10.10	9.18

N.D. = not determined.

connections observed and to provide an indication of the nature of the turn in the glycohexapeptide. The simulations showed all of the through-space connections, the lowest energy conformations are displayed in Figure 1. From the MD data ambiguous through-space connections observed in the glycodecapeptide and the glycohexapeptide could be resolved, with the former showing connections between the resonance of the *N*-acetyl amide proton of the S^5 sugar to the γH resonance of P^9 and not to P^8, and the latter indicating that it is the γH resonances of P^5 which form the through-space connection to the *N*-acetyl amide proton of the S^1 sugar. The MD simulations indicated that long-range coupling could exist in all of the peptide sequences. The low energy conformations obtained for the decapeptide, for instance, showed an average inter-nuclei distance, of the low energy structures, between the amine protons of A^2 and S^5 of 4.5 Å. This analysis, however, neglects the dynamic flexibility of the molecule and is the subject of further work.

The glycohexapeptide exhibited H-bonding between the sugars and the backbone of the peptide. The *N*-acetyl amide proton of the S^1 sugar *H*-bonded to the terminal acetate carbonyl, the *N*-acetyl amide proton of the T^2 sugar to the carboxyl of the T^2 backbone, and the terminal carboxyl of A^6 to hydroxyl groups of the S^1 sugar. These H-bonds, and those seen between the P^4 and T^2, and A^3 and P^5, explain why the short peptide is folded into a tight turn. Figure 1 shows representations of the low energy structures found during the molecular dynamics analysis of (a) the decapeptide; (b) the glycodecapeptide; and (c) the glycohexapeptide.

Structural consequences of glycosylation

Peptide conformation and dynamics are known to be affected by the addition of carbohydrate groups.[22,23] Depending on the nature of glycosylation, and the peptide sequence, this effect may be an induction of secondary structure,[24] or simply a restriction of peptide mobility in solution.[8] The structural studies indicate that the glycohexapeptide **3** has a folded structure in dimethyl sulphoxide. The peptide chemical shifts and $^3J_{NH\text{-}H\alpha}$ for the control decapeptide, and glycodecapeptide show that glycosylation apparently has a significant effect on the electronic environment of the decapeptide. Comparison of chemical shifts indicates that the effect is particularly apparent around the site of glycosylation, and in the residues to the C-terminal side of the serine and threonine residues. In contrast to those systems studied by Hollosi et al.[24] through-space connection data for the deca(glyco)peptides does not indicate stabilization of secondary structure motifs by carbohydrate moieties. Mucin molecules are known from their hydrodynamic properties[7] and microscopic studies[6] to exist in their native form as extended, non-globular, possibly rod-like molecules, the dimensions of which reflect their viscoelastic properties. The manner in which the carbohydrate groups appear to incline along the peptide core, possibly stabilized through H-bonding, may restrict the conformational space available to the peptide, and result in a possible stiffening effect. Differences in molecular tumbling rates between the glycodecapeptide and control decapeptide (inferred from the lack of NOESY cross peaks in only the non-glycosylated form) may reflect differences in molecular dimensions

Table 3.

Pairs of PAPGS(αGalNAc)T(αGalNAc)-APPA **2** proton resonances that are connected by NOESY cross peaks

Pro^{1} αH	Ala^{2} NH
Gly^{4} NH	Pro^{3} αH
Gly^{4} NH	Ser^{5} β_2H
Ala^{7} NH	Thr^{6} αH
Ala^{7} NH	Thr^{6} βH
Thr^{6} NH	Ser^{5} αH
Thr^{6} NH	Ser^{5} β_1H
Thr^{6} NH	Ser^{5} β_2H
Ser^{5} NH	Ser^{5} αH
Ser^{5} NH	(Ser^{5})GalNAc H2
Thr^{6} βH	(Thr^{6})GalNAc H1
Thr^{6} αH	(Thr^{6})GalNAc H1
Ala^{7} NH	(Thr^{6})GalNAc H1
Thr^{6} ηH	$Pro^{8,9}$ αH
Thr^{6} γH	(Thr^{6})GalNAc H1
$Pro^{8,9}$ γH	Ala(min) βCH_3
Thr^{6} NH	(Thr^{6})GalNAc NH
Ser^{5} NH	(Ser^{5})GalNAc NH
Ser^{5} NH	Gly^{4} NH
Ala^{2} NH	Ala(min) NH

Pairs of PAPGSTAPPA **1** proton resonances which are connected by ROESY cross peaks

Ser^{5} NH	Gly^{4} αH
Thr^{6} NH	Ser^{5} αH
Ala^{2} NH	Pro^{1} αH
Ala^{7} NH	Thr^{6} αH
Ala^{2} NH (min)	Pro^{1} αH
Ala^{10} NH	Pro^{9} αH
Gly^{4} NH	Pro^{3} αH
Ala^{7} αH	Pro^{8} δ_1H, δ_2H
Thr^{6} αH	Ser^{5} αH
Ala^{2} αH	Pro^{3} δH

Pairs of S(αGalNAc)T(αGalNAc)APPA **3** proton resonances which are connected by NOESY cross peaks

Thr^{2} NH	Ala^{6} NH
Thr^{2} NH	(Thr^{2}) GalNAc NH
Ser^{1} NH	Pro^{5} bH/gH
Ala^{3} NH	Thr^{2} βH
Thr^{2} NH	Ser^{1} αH
Ala^{6} NH	Pro^{5} αH
Pro^{4} αH	Ser^{1} αH
Ser^{1} αH	Thr^{2} βH
Thr^{2} βH	(Thr^{2})GalNAc H1
Ala^{3} NH	Thr^{2} αH
Thr^{2} αH	(Thr^{2})GalNAc H1

between these molecules as a result of glycosylation. ^{13}C NMR studies of sequentially deglycosylated native ovine submaxilliary mucin (OSM) (*O*-glycosylated with saccharide chains which consist of only αGalNac, linked

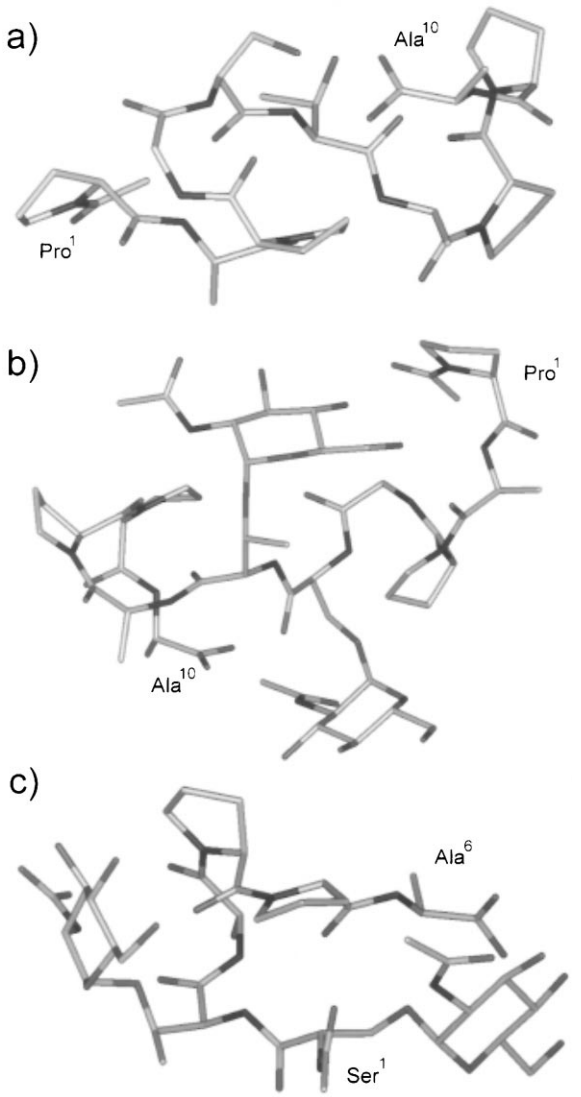


Figure 1. Stick representations of the lowest energy conformers determined for the (a) decapeptide, (b) glycodecapeptide, and (c) glycohexapeptide. The terminal amino acids for each peptide are labelled.

to sialic acid) demonstrated that glycosylation resulted in a stiffening effect at the site of glycosylation (reflected in reduced mobility of α-carbons (correlated to spin-lattice relaxation times), and which was transmitted to adjacent non-glycosylated residues.[8] Desialylated OSM was found to be much less flexible than totally deglycosylated OSM, and native OSM was found to be somewhat less flexible than desialylated OSM. These observations were complemented by light scattering studies[7] which indicated that sequential desialylation and total deglycosylation of OSM resulted in a progressively less extended, more globular molecular

structure. These observation indicate that αGalNac has a role in causing the extended, rod-shaped macrostructure of certain mucin glycoproteins. The studies of MUC1-related *O*-glycopeptides suggest that the carbohydrate moieties of these molecules appear to produce similar sorts of interactions as seen for other mucin-related systems. αGalNAc is therefore likely to affect the peptide core dynamics of MUC1, which may be reflected in some of the differences seen in antibody binding reactivity to MUC1 core related glycopeptides.[25,26]

^{1}H NMR studies of MUC1 core-related peptides identified secondary structure motifs only around the sequence PDTR.[27] The truncated glycohexapeptide studied here however, appears to be folded onto itself, the modeled low energy structure is displayed in Figure 1. This folding is not present in the longer glycodecapeptide, which contains the glycohexapeptide sequence, and confirms that the proline containing N-terminal sequence Ac-PAPG of the glycodecapeptide inhibits the formation of turn motifs around the glycohexapeptide sequence. This information implies that the non-globular structure of MUC1 may be due in part to the proline-rich sequence of the MUC1 peptide core, in addition to the effect of glycosylation.

Experimental

Glycopeptide synthesis

NMR spectra were usually run at 400 (^{1}H) and 100.6 MHz (^{13}C) if not indicated otherwise. Lipases were generous gifts by the Amano Pharmaceutical Company: Lipase M from Mucor javanicus and lipase N from Rhizopus niveus. Flash chromatography was performed on silica gel of 0.04–0.063 mm (E. Merck, Darmstadt, Germany). Preparative HPLC was carried out with a column of 20×250 mm filled with Spherisorb ODS II (5 μm) reversed-phase silica gel (Bischoff Comp., Leonberg, Germany).

***N*-(9-Fluorenylmethoxcycarbonyl)-L-prolyl-glycine heptyl ester (19).** To a solution of 3.4 g (0.01 mol) Fmoc proline, 2.0 g (0.01 mol) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) (2.7, 0.02 mol) in dry dichloromethane (20 mL) and dimethylformamide (20 mL) are added glycine heptyl ester hydro-*p*-toluenesulfonate[13] (3.5 g, 0.01 mol) and *N*-ethyl diisopropylamine (1.7 mL, 0.01 mmol). After 24 h stirring at room temperature and subsequent addition of dichloromethane (100 mL), the mixture was extracted three times with 0.1 N HCl (50 mL), satd $NaHCO_3$ solution (50 mL) and water. The organic layer was dried with $MgSO_4$ and evaporated to dryness in vacuo. Purification by flash chromatography on silica gel (300 g) in petroleum ether/ethyl acetate (3:1, v/v), gave **19**; yield 4.4 g (92%); oil; $[\alpha]_D$ −47.3 (*c* 1, $CHCl_3$); R_f 0.19 (petroleum ether/ethyl acetate, 1:1, v/v). ^{1}H NMR (200 MHz, $CDCl_3$, 2 rotamers): δ 7.75–7.24 (m, 8H, aromat. H), 7.10 (sb, 0.63 H, NH Gly), 6.44 (s_b, 0.37 H, NH Gly), 4.44–4.41 (m, 2H, O-CH_2 Fmoc), 4.21–3.84 (m, 6H, α-CH Pro, CH_2 Gly, 9-H Fmoc, OCH_2 Hep), 3.58–3.43 (m, 2H, δ-CH_2 Pro), 2.30–1.99 (m, 4H, β-CH_2 Pro, γ-CH_2 Pro), 1.60–1.54 (m, 2H, OCH_2-CH_2- Hep), 1.24 (m, 8H, -(CH_2)$_4$-CH_3), 0.86 (m, 3H, CH_3 Hep). Anal. calcd for $C_{29}H_{38}N_2O_5$: C, 69.67; H, 7.55; N, 5.83; found: C, 70.19; H, 7.65; N, 5.86.

***N*-(Fluorenylmethoxycarbonyl)-L-alanyl-L-prolyl-glycine heptyl ester (21).** A solution of **19** (4.0 g, 8.2 mmol) in dichloromethane (50 mL) and morpholine (50 mL) was stirred at room temperature for 2 h and concentrated in vacuo. Traces of morpholine were removed by codistillation with toluene (3 × 20 mL) in vacuo. The residue containing crude L-prolyl-L-glycine heptyl ester **20** was dissolved in dichloromethane (10 mL) and added to a solution of Fmoc alanine (2.84 g, 9.1 mmol), EDC (1.74 g, 9.1 mmol) and HOBt (2.46 g, 18.2 mmol) in dichloromethane/dimethylformamide (3:1, v/v, 20 mL). After stirring for 48 h at room temperature and addition of dichloromethane (60 mL), the mixture was extracted three times with each 0.1 N HCl (50 mL), satd $NaHCO_3$ solution (50 mL) and water. The organic layer was dried with $MgSO_4$ and concentrated in vacuo. Flash chromatography of the residue in petroleum ether/ethyl acetate (1:1 v/v) on silica gel (300 g) gave **21**, yield 2.57 g (55%); mp 52–55 °C; $[\alpha]_D^{22}$ −67.5 (*c* 1.0, $CHCl_3$); R_f 0.26 (petroleum ethyl/ethyl acetate, 1:2, v/v). Purification to analytical grade was carried of for the tetrapeptide. ^{1}H NMR (200 MHz $CDCl_3$): δ 7.76–7.25 (m, 8H, aromat. H), 7.18 (t, J = 4.9 Hz, 1H, NH Gly), 5.76 (d, J = 7.9 Hz, 1H, NH Ala), 4.60 (m, 1H, α-CH Pro), 4.55 (m, 1H, α-CH Ala), 4.34 (d, J = 7.0 Hz, 2H, O-CH_2 Fmoc), 4.21 (m, 1H, 9-H Fmoc), 4.11 (t, J = 6.7 Hz, 2H, OCH_2 Hep), 4.05–3.90 (m, 2H, CH_2 Gly), 3.68–3.51 (m, 2H, δ-CH_2 Pro), 2.34 (m, 1H, β-CH_{2a} Pro), 2.12–1.88 (m, 3H, β-CH_{2b} Pro, γ-CH_2 Pro), 1.64–1.58 (m, 2H,OCH_2-CH_2-Hep), 1.41 (d, J = 6.8 Hz, 3H, CH_3-Ala), 1.35–1.21 (m, 8H, -(CH_2)$_4$-CH_3), 0.85 (m, 3H, CH_3 Hep). ^{13}C NMR ($CDCl_3$, 2 rotamers): δ 172.52, 171.06, 169.63 (C=O), 155.55 (C=O, urethane), 143.67, 143.59, 141.04, 127.45, 126.85, 124.95, 119.75 (aromat. C), 66.76, 65.32 (O-CH_2 Fmoc, OCH_2 Hep), 60.58 (low intensity), 59.49 (α-C Pro), 48.88 (low intensity), 48.17 (α-C Ala), 47.01 (δ-C Pro), 46.91, 46.34 (low intensity) (C-9 Fmoc), 41.10 (α-C Gly), 31.45, 28.63, 28.29, 25.53, 22.34 (5 CH_2 Hep), 27.26 (β-C Pro), 24.80 (γ-C Pro), 18.25, 17.01 (low intensity) (CH_3 Ala), 13.86 (CH_3 Hep).

***N*-(9-Fluorenylmethoxycarbonyl)-L-prolyl-L-alanyl-L-prolyl-glycine heptyl ester (23).** The Fmoc-group was

removed from **21** (2.40 g, 4.3 mmol) in dichloromethane (30 mL) and morpholine (30 mL) and the crude **22** reacted with Fmoc proline (1.52 g, 4.5 mmol), and HOBt (1.22 g, 9.0 mmol) analogously to the synthesis of **21**. Purrification by flash chromatography in petroleum ether/ethyl acetate (1:2 v/v) gave **23**, yield 1.48 g (53%); mp 49–53 °C; $[\alpha]_D^{22}$ −90.6 (*c* 1.1, $CHCl_3$); R_f 0.12 (petroleum ether/ethyl acetate 1:10 v/v). ^{13}C NMR ($CDCl_3$, 2 rotamers): δ 171.35,171.05, 169.63 (C=O) 143.87, 141.26, 127.64, 127.03, 125.13, 125.05, 119.91 (aromat. C), 67.69, 66.42 (low intensity), 65.47, 65.32 (low intensity) (O-CH_2 Fmoc, OCH_2 Hep), 60.88, 59.58 (α-C Pro), 47.34 (α-C Ala, C-9 Fmoc), 47.06, 45.81 (low intensity) (δ-C Pro), 44.97 (low intensity) (C-9 Fmoc), 42.35 (low intensity), 41.44 (low intensity), 41.24 (α-C Gly), 31.61, 28.77, 28.48, 25.70, 22.47 (5 CH_2 Hep), 27.01 (β-C Pro), 24.92, 24.59 (γ-C Pro), 18.69, 18.09 (low intensity) (CH_3 Ala), 13.94 (CH_3 Hep). Anal. calcd for $C_{37}H_{48}N_4O_7$ (660.8): C, 67.25; H, 7.32; N, 8.48; found: C, 67.94; H, 7.98; N, 8.43.

***N*-Acetyl-L-prolyl-L-alanyl-L-prolyl-glycine heptyl ester (24).** A solution of **23** (1.3 g, 2.0 mmol) in dichloromethane (5 mL) and morpholine (5 mL) was stirred at room temperature for 1 h. After concentration and codistillation with toluene (3 × 10 mL) in high vacuum, the remainder was dissolved in dichloromethane/pyridine (5:1 v/v, 24 mL). At 0 °C acetic anhydride (0.41 g, 4.0 mmol) was added, and the mixture was stirred 16 h at room temperature. After concentration in vacuo the crude product was purified by flash-chromatography in petroleum ether/ethyl acetate (1:2, v/v)→ethyl acetate →ethanol on silica gel (200 g) to give **24**, yield 0.92 g (76%); amorphous solid; $[\alpha]_D^{22}$ −154.1 (*c* 0.8, $CHCl_3$); R_f 0.10 (ethyl acetate/ethanol 9:1). ^1H NMR ($CDCl_3$, 4 rotamers): δ 8.6 (d, (low intensity), NH Ala), 8.17 (t, J=5.9 Hz, 0.1H, NH Gly), 7.80 (t, low intensity), NH Gly), 7.58 (d, J=3.8 Hz, 0.1H, NH Ala), 7.41 (d, J=6.5 Hz, 0.8H, NH Ala), 7.27 (t, J=5.4 Hz, 0.1H, NH Gly), 7.19 (t, J=5.4 Hz, 0.8H, NH Gly), 7.06 (d, J=7.6 Hz, 0.1H, NH Ala), 4.65 (quint., J=7.2 Hz, 0.1H, α-CH Ala), 4.56 (m, 1.6H, α-CH Pro, α-CH Ala), 4.44 (m, 0.8H, α-CH Pro), 4.29–4.20 (m, 0.5H, α-CH Pro, α-CH Ala), 4.04–3.95 (m, 2H, OCH_2 Hep), 3.93–3.83 (m, 2H, CH_2 Gly), 3.68–3.33 (m, 4H, 2 δ-CH_2 Pro), 2.44 (dd, J_1=5.5 Hz, J_2=12.5 Hz, 0.2H, β-CH_{2a} Pro), 2.22–1.82 (m, 11H, 2 β-CH_2 Pro, 2 γ-CH_2 Pro, CH_3CO), 1.55–1.52 (m, 2H,OCH_2-CH_2- Hep), 1.32–1.19 (m, 11H, CH_3-Ala, -(CH_2)$_4$-CH_3), 0.80 (m, 3H, CH_3 Hep). ^{13}C NMR ($CDCl_3$, 3 rotamers): δ 172.76, 171.96, 171.82, 171.33, 171.17, 169.86, 169.49 (C=O), 77.20 (g.I.), 65.42, 65.20 (OCH_2 Hep), 61.84, 60.89, 59.89, 59.71 (α-C Pro), 48.88 (low intensity), 48.26 (α-C Ala), 47.17, 47.09, 46.95, 46.81, 46.64 (δ-C Pro), 41.34, 41.15 (α-C Gly), 31.94, 31.51, 30.72 (CH_2 Hep), 28.69, 28.57, 28.37, 28.07, 27.57 (β-C Pro, 2 CH_2 Hep), 25.60, 24.85, 24.73 (γ-C Pro, CH_2 Hep), 22.81, 22.43, 22.38, 21.87 (CH_2 Hep, CH_3CO), 17.70, 17.35, 16.25 (CH_3 Ala), 13.86 (CH_3 Hep). Anal. calcd for $C_{24}H_{40}N_4O_6$ * $0.5H_2O$ (489.6): C, 58.88; H, 8.44; N, 11.44; found: C, 58.60; H, 8.42; N, 10.53.

***N*-Acetyl-L-prolyl-L-alanyl-L-prolyl-glycine (25). Pretreatment of lipase N in order to inhibit serine proteases.** A mixture of lipase N (500 mg) and phenylmethylsulfonyl fluoride (PMSF, 10.5 mg) in 0.2 sodium phosphate buffer (pH 7.0, 5 mL) was stirred 1 h at 0 °C. After addition of sodium phosphate buffer (45 mL), the mixture was shaken at 37 °C for 1 h and can then be used for the enzymatic reactions.

Hydrolysis of the heptyl ester. To solution of lipase N (500 mg) in 0.2 M sodium phosphate buffer (pH 7.0, 50 mL) prepared according to a) was added dropwise a solution of **24** (0.5 g, 1.0 mmol) in acetone (2.5 mL) and vigorously shaken at 37 °C for 3 h. After lyophylization of the reaction mixture, the remainder was extracted five times with ethanol (20 mL), filtered and the ethanol evaporated in vacuo. The crude product was purified by flash-chromatography in ethyl acetate→ethyl acetate/ethanol→ethanol to give **25**, yield 280 mg (73%), amorphous solid, $[\alpha]_D^{22}$ −147.5 (*c* 1.3, CH_3OH), R_f 0.1 (ethyl acetate/methanol, 2:1, v/v) ^1H NMR (DMSO-d_6, 4 rotamers): δ 8.50, 8.40, 8.07, 8.01 (4d, 1H, NH Ala), 8.17 (t, J=5.9 Hz, 0.1H, NH Gly), 7.80 (t, NH Gly), 7.51 (s_b, 0.2H, NH Gly), 7.25 (s_b, 0.8H, NH Gly), 4.55–4.25 (m, 3H, 2 α-CH Pro, α-CH Ala), 3.65–3.25 (m, CH_2 Gly, 2 δ-CH_2 Pro, H_2O), 2.2–1.7 (m, 11H, 2 β-CH_2 Pro, 2 γ-CH_2 Pro, CH_3CO), 1.25, 1.20, 1.17, 1.13 (4d, 3H, CH_3-Ala). ^{13}C NMR (DMSO-d_6, 4 rotamers): δ 176.26, 174.17, 173.98, 173.85, 173.76, 173.51, 173.38, 172.41 (C=O), 62.16, 61.89, 61.83, 61.68, 61.08 (α-C Pro), 49.27, 48.51, 48.24, 47.95 (α-C Ala, δ-C Pro), 44.59 (α-C Gly), 33.09, 32.92, 30.83, 30.61, 30.38 (β-C Pro), 25.94, 25.64, 23.83, 23.06 (γ-C Pro), 24.16, 22.34, 22.29 (CH_3CO), 18.41, 17.57, 17.06, 16.97 (CH_3 Ala); FABMS, m/z 380.9 $(M-H)^-$.

***N*-(9-Fluorenylmethoxycarbonyl)-L-prolyl-L-alanine heptyl ester (27).** Starting from Fmoc proline (3.4 g, 0.01 mol), alanine heptyl ester hydro-*p*-toluenesulfonate (Braun et al.[13]) **26** (3.6 g, 0.01 mol), EDC (2 g, 0.01 mol) and HOBt (2.7 g, 0.02 mol) the dipeptide ester **27** was obtained in analogy the procedure described for **19**. Yield 3.56 g (72%); mp 121 °C; $[\alpha]_D^{22}$ −48.5 (*c* 1, $CHCl_3$); R_f 0.50 (petroleum ether/ethyl acetate, 1:1). ^1H NMR (200 MHz, $CDCl_3$, 2 rotamers): δ 7.77–7.29 (m, 8H, aromat. H), 7.06 (d, J=6.7 Hz, 0.6 H, NH Ala), 6.53 (s_b, 0.4 H, NH Ala), 4.71–4.00 (m, 7H, α-CH Pro, α-CH Ala, O-CH_2 Fmoc, 9-H Fmoc, OCH_2 Hep), 3.57–3.47 (m, 2H, δ-CH_2 Pro), 2.30–1.76 (m, 4H, β-CH_2 Pro, γ-CH_2 Pro), 1.57–1.45 (m, 2H, OCH_2 -CH_2-Hep), 1.38 (d, J=6.7 Hz, 3H, CH_3 Ala), 1.25 (mc, 8H,

-(CH_2)$_4$-CH_3), 0.86 (m, 3H, CH_3 Hep); ^{13}C NMR (50.3 MHz, $CDCl_3$, 2 rotamers): δ 172.8, 171.41 (C=O), 155.90, 155.16 (C=O, urethane), 143.93, 141.30, 127.73, 127.08, 125.11, 119.99 (aromat. C), 67.74, 65.57, (O-CH_2- Hep, O-CH_2- Fmoc), 60.50, 60.44 (α-C Pro), 48.32, 48.2 (α-C Ala, C-9 Fmoc), 48.0 (δ-C Pro), 31.68, 28.85, 28.49, 25.73, 22.57 (5 CH_2 Hep), 31.29 (β-C Pro), 24.63 (γ-C Pro), 18.3, 18.23 (CH_3 Ala), 14.09 (CH_3 Hep). Anal. calcd for $C_{29}H_{38}N_2O_5$ (494.6): C, 70.42; H, 7.74; N, 5.66; found: C, 70.47; H, 7.72; N, 5.40.

***N*-(9-Fluorenylmethoxycarbonyl)-L-prolyl-L-prolyl-L-alanine heptyl ester (29).** As described for **21**, the tripeptide ester **29** was obtained from **27** (2.5 g, 5 mmol), which was N-terminally deblocked by treatment with morpholine/dichloromethane (1:1, v/v, 30 mL), and Fmoc proline (1.69 g, 5 mmol), EDC (1.0 g, 5 mmol) and HOBt (1.4 g, 10 mmol). Purification was carried out by flash-chromatography in petroleum ether/ethyl acetate (2:1, v/v) on silica gel (300 g). Yield 1.88 g (62%); amorphous solid; $[\alpha]_D^{22}$ −59.8 (*c* 1, $CHCl_3$); R_f 0.15 (petroleum ether/ethyl acetate, 1:1, v/v). ^{1}H NMR [^{1}H-^{1}H-COSY] ($CDCl_3$, 3 rotamers): δ 8.26 (d, J=7.8 Hz, 0.33 H, NH Ala), 7.74–7.24 (m, 8H, aromat. H), 7.08 (d, J=7.3 Hz, 0.48 H, NH Ala) 7.02 (d, J=7.2 Hz, 0.18 H, NH Ala), 4.60–4.11 (m, 6H, α-CH Pro, α-CH Pro′ {4.58, 4.40, 4.32, 4.19ppm}, α-CH Ala {4.40ppm}, O-CH_2 Fmoc, 9-H Fmoc), 4.10–4.04 (m, 1.3H, OCH_2 Hep), 3.90–3.82 (m, 0.7H, OCH_2 Hep), 3.75–3.71 (m, 1.2H, δ-CH_2 Pro), 3.66–3.52 (m, 2.8H, δ-CH_2 Pro), 3.44 (m, 0.48H, δ-CH_{2a} Pro), 3.33 (m, 0.18H, δ-CH_{2a} Pro), 3.05 (m, 0.18H, δ-CH_{2b} Pro), 2.50 (dd, 0.33H, β-CH_{2a} Pro), 2.25–1.76 (m, 7.7H, β-CH_2 Pro, γ-CH_2 Pro, β-CH_2 Pro′, γ-CH_2 Pro), 1.61–1.56 (m, OCH_2-CH_2- Hep), 1.42 (d, J=7.4 Hz, CH_3 Ala), 1.39 (m, OCH_2-CH_2- Hep), 1.35 (d, J=7.4 Hz, CH_3 Ala), 1.31 (d, J=7.2 Hz, CH_3 Ala), 1.27–1.12 (m, 8H, -(CH_2)$_4$-CH_3), 0.85–0.81 (m, 3H, CH_3 Hep). ^{13}C NMR ($CDCl_3$ 3 rotamers): δ 172.95, 171.26, 172.10, 171.80, 171.65, 171.42, 170.93, 170.73 (C=O), 155.17, 154.91 (C=O, urethane), 144.47, 144.16, 144.01, 143.90, 141.24 (C-4$_{a/b}$, C-8$_a$, C-9$_a$ Fmoc), 127.57, 126.99, 126.88, 125.17, 125.05, 124.82, 124.65, 119.86, 119.70 (aromat. C), 67.73, 67.45, 66.27, 65.40, 65.10 (O-CH_2- Hep, O-CH_2- Fmoc), 60.93, 59.87, 59.63, 58.58, 58.15, 57.54 (α-C Pro), 48.93, 48.20, 47.64 (α-C Ala), 47.25 (C-9 Fmoc), 47.16, 47.11, 47.03, 46.92, 46.73, 46.53 (δ-C Pro), 31.83, 31.60, 31.54 (CH_2 Hep), 30.38, 29.47, 29.22 (β-C Pro), 28.75, 28.47, 28.42, 27.37, 27.22, 25.67 (β-C Pro, CH_2 Hep), 25.56, 25.16, 25.06 (γ-C Pro), 24.76, 24.32, 22.99, 22.20 (γ-C Pro, CH_2 Hep), 18.20, 16.29 (CH_3 Ala), 13.92 (CH_3 Hep). Anal. calcd for $C_{35}H_{45}N_3O_6$ (603.8): C, 69.63; H, 7.51; N, 6.96; found: C 69.62; H, 7.56; N, 7.13.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanyl-L-prolyl-L-prolyl-L-alanine heptyl ester (31).** A solution of **29** (160 mg, 0.26 mmol) in dichloromethane (2.5 mL) and morpholine (2.5 mL) was stirred at room temperature for 2 h. After concentration in vacuo, toluene (3 × 5 mL) was codistilled from the remainder in high vacuum. The residue containing crude **30** was dissolved in dichloromethane (2 mL) and added to solution of *N*-(9-fluorenylmethyloxycarbonyl)-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanine **11** (Braun et al.[15]) (300 mg, 0.26 mmol), EDC (100 mg, 0.52 mmol) and HOBt (70 mg, 0.52 mmol) in dichloromethane/dimethylformamide (3:1, v/v, 25 mL), which already had been stirred for 1 h. After stirring the mixture for 3 days at room temperature, dichloromethane (50 mL) was added and the solution extracted twice with each 0.1 N HCl (8 mL) and sat $NaHCO_3$ solution (10 mL). The organic layer was dried with $MgSO_4$ and evaporated to dryness in vacuo. The remainder was purified by flash-chromatography in ethyl acetate→ethyl acetate/ethanol (1:1, v/v) on silica gel (300 g) to give **31**, yield 199 mg (50%); mp 118 °C; $[\alpha]_D^{22}$ −23.4 (*c* 1.1, CH_3OH); R_f 0.64 (ethyl acetate/methanol, 7:3, v/v) ^{1}H NMR ($CDCl_3$): δ 7.73–7.26 (m, 10H, aromat. H, 2 NH), 7.22 (d, J=6.6 Hz, 1H, NH), 6.86 (d, J=7.1 Hz, 1H, NH), 6.56 (d, J=9.3 Hz, 1H, NH), 5.86 (d, J=7.3 Hz, 1H, NH Ser), 5.31 (d, 1H, 4-H), 5.29 (d, 1H, 4-H), 5.22 (d, $J_{1,2}$=3.4 Hz, 1H, 1-H), 5.10–5.14 (m, 2H, 2 3-H), 4.93 (d, $J_{1,2}$=3.2 Hz, 1H, 1-H), 4.71 (dd, J_1=3.5 Hz, J_2=7.5 Hz, 1H, α-CH Pro), 4.62 (m, 1H, α-CH Ala), 4.58–4.36 (m, 9H, 2 2-H, α-CH Ser, α-CH Pro, α-CH Ala, α-CH Thr, OCH_2 Fmoc, 9-H Fmoc), 4.21–4.16 (m, 3H, β-CH Thr, 2 5-H), 4.10–4.01 (m, 5H, 2 6-H$_a$, 6-H$_b$, OCH_2 Hep), 3.95 (dd, $J_{5,6b}$=6.1 Hz, $J_{6a,6b}$=9.9 Hz, 1H, 6-H$_b$), 3.80–3.55 (m, 6H, β-CH_2 Ser, 2 δ-CH_2 Pro), 2.39-1.86 (m+7s {2.12, 2.11, 1.98, 1.95, 1.93, 1.92, 1.91}, 32H, 8 CH_3CO, 2 β-CH_2 Pro, 2 γ-CH_2 Pro), 1.60–1.56 (m, 2H, OCH_2-CH_2- Hep), 1.39 (d, J=6.7 Hz, 3H, CH_3 Ala), 1.32 (d, J=7.2 Hz, 3H, CH_3 Ala), 1.26–1.23 (m, 8H, -(CH_2)$_4$-CH_3), 1.09 (d, J=6.5 Hz, 3H, CH_3 Thr), 0.84 (m, 3H, CH_3 Hep). ^{13}C NMR ($CDCl_3$): δ 172.81, 170.89, 170.73, 170.57, 170.42, 170.36, 170.26, 169.23, 167.57 (C=O), 155.80 (C=O, urethane), 143.64, 141.26, 127.75, 127.06, 124.92, 119.99 (aromat. C), 98.39, 97.94 (2 C-1), 73.56 (β-C Thr), 68.04, 67.98, 67.22, 67.12, 66.79 (2 C-3, 2 C-4, 2 C-5), 67.92, 65.52 (OCH_2 Fmoc, O-CH_2 Hep, β-C Ser), 62.04, 61.99 (2 C-6), 59.69, 58.02 (2 α-C Pro), 55.41 (α-C Thr), 53.72 (α-C Ser), 48.17 47.62, 47.56, 47.49 (2 α-C Ala, 2 C-2), 47.04 (C-9 Fmoc), 47.19, 48.87 (2 δ-C Pro), 31.59, 28.75, 28.44, 25.66, 22.46 (5 CH_2 Hep), 28.28, 27.89 (2 β-C Pro), 25.05, 24.64 (2 γ-C Pro), 22.98, 22.75 (*C*H_3CON), 20.71, 20.68, 20.61, 20.53 (*C*H_3CO), 18.09, 17.71 (2 CH_3 Ala), 16.09 (CH_3 Thr), 13.95 (CH_3 Hep). FABMS (3-NOBA) *m*/*z* 1522.2 $(M+H)^+$. FABMS (3-NOBA): 1522.2

$(M+H)^+$. Anal. calcd for $C_{73}H_{100}N_8O_{27}$ (1521.6): C, 57.62; H, 6.62; N, 7.36; found: C, 56.51; H,6.14; N, 7.50.

***N*-Acetyl-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-αD-galactopyranosyl)-L-threonyl-L-alanyl-L-prolyl-L-prolyl-L-alanine heptyl ester (32).** A solution of **31** (135 mg, 89 μmol) in dichloromethane (5 mL) and morpholine (5 mL) was stirred at room temperature for 2 h. After evaporation of the solvents in vacuo and codistillation twice with 5 mL toluene in high vacuum, the remainder was dissolved in pyridine (2 mL). At 0 °C acetic anhydride (0.2 mL, 2 mmol) was added, and the mixture stirred for 4 h at room temperature. Dichloromethane (25 mL) and, subsequently, ice (2 g) were added, and the mixture was vigorously stirred for 30 min at room temperature. After phase separation the organic layer was extracted with saturated NaCl solution (15 mL), dried with $MgSO_4$ and concentrated in vacuo. The remainder was purified by flash-chromatography on silica gel (150 g) in ethyl acetate→ethyl acetate/ethanol (2:1, v/v). Yield 91 mg (76%); amorphous solid; $[\alpha]_D^{22}$ −19.8 (*c* 0.5, CH_3OH); R_f 0.82 (ethyl acetate/methanol, 2:1, v/v). ^1H NMR ($CDCl_3$): δ 7.43 (d, $J=6.4$ Hz, 1H, NH), 7.38 (d, $J=8.8$ Hz, 1H, NH), 7.25 (d, $J=8.5$ Hz, 1H, NH), 6.83 (d, $J=7.2$ Hz, 1H, NH), 6.60 (d, $J=9.2$ Hz, 2H, NH), 5.31 (m, 2H, 2 4-H), 5.21 (d, $J_{1,2}=3.7$ Hz, 1H, 1-H), 5.13 (dd, $J_{2,3}=11.2$ Hz, $J_{3,4}=3.2$ Hz, 1H, 3-H), 5.10 (dd, $J_{2,3}=11.0$ Hz, $J_{3,4}=3.2$ Hz, 1H, 3-H), 4.94 (d, $J_{1,2}=3.6$ Hz, 1H, 1-H), 4.72 (dd, $J_1=4.0$ Hz, $J_2=8.3$ Hz, 1H, α-CH Pro), 4.69–4.40 (m, 7H, 2 2-H, α-CH Ser, 2 α-CH Ala, α-CH Thr, α-CH Pro), 4.23–4.19 (m, 2H, β-CH Thr, 5-H), 4.12–4.02 (m, 6H, 5-H, 2 6-H_a, 6-H_b, OCH_2 Hep), 3.96 (dd, $J_{5,6b}=6.0$ Hz, $J_{6a,6b}=9.9$ Hz, 1H, 6-H_b), 3.78–3.53 (m, 6H, β-CH_2 Ser, 2 δ-CH_2 Pro), 2.28–1.88 (m + 9s {2.12, 2.11, 2.03, 2.01, 1.98, 1.96, 1.933, 1.931, 1.92}, 35H, 9 CH_3CO, 2 β-CH_2 Pro, 2 γ-CH_2 Pro), 1.60–1.57 (m, 2H, OCH_2-CH_2- Hep), 1.39 (d, $J=6.8$ Hz, 3H, CH_3 Ala), 1.32 (d, $J=7.1$ Hz, 3H, CH_3 Ala), 1.30–1.12 (m, 8H, -(CH_2)$_4$-CH_3), 1.09 (d, $J=6.4$ Hz, 3H, CH_3 Thr), 0.84 (m, 3H, CH_3 Hep). ^{13}C NMR ($CDCl_3$): δ 172.80, 170.95, 170.89, 170.68, 170.61, 170.46, 170.35, 170.27, 170.21, 169.39, 167.50 (C=O), 98.47, 97.84 (2 C-1), 73.29 (β-C Thr), 68.10, 67.90, 67.25, 67.16, 67.12, 66.81 (2 C-3, 2 C-4, 2 C-5), 67.65, 65.54 (O-CH_2 Hep, β-C Ser), 62.00 (2 C-6), 59.70, 58.03 (2 α-C Pro), 55.36 (α-C Thr), 52.17 (α-C Ser), 48.17, 47.57, 47.43 (2 α-C Ala, 2 C-2), 47.21, 46.86 (2 δ-C Pro), 31.60, 28.76, 28.44, 25.66 (4 CH_2 Hep), 28.31, 27.84 (2 β-C Pro), 25.08, 24.64 (2 γ-C Pro), 23.02, 22.98, 22.76 (*C*H_3CON), 22.46 (CH_2 Hep), 20.68, 20.62, 20.57 (*C*H_3CO), 18.13, 17.74 (2 CH_3 Ala), 16.16 (CH_3 Thr), 13.95 (CH_3 Hep). FABMS (3-NOBA): 1342.0 $(M+H)^+$; calcd: 1341.6. Anal. calcd for $C_{60}H_{92}N_8O_{26}$ * $1.5H_2O$ (1341.4): C, 52.66; H, 7.00; N, 8.19; found: C, 52.29; H, 6.96; N, 8.08.

***N*-Acetyl-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanyl-L-prolyl-L-prolyl-L-alanine (33).** To a solution of lipase N (120 mg) in 0.2 sodium phosphate buffer (pH 7.0, 12 mL), treated with PMSF as described for **25**, was added a solution of **32** (60 mg, 45 μmol) in acetone (0.6 mL). After vigorous shaking for 16 h, the mixture was lyophylized and the remainder extracted five times with 25 mL of ethanol. After filtration the ethanol was evaporated in vacuo and the crude product purified by preparative reversed-phase HPLC (flow rate 10 mL/min) in methanol/water (1:1, v/v). Yield 38 mg (68%); amorphous solid; $[\alpha]_D^{22}$ −26.5 (*c* 0.65, CH_3OH); R_f 0.22 (ethanol/methanol, 9:1). ^1H NMR [^1H-^1H-COSY] (CD_3OD): δ 5.42 (s_b, 2H, 4-H, 4′-H), 5.22 (d, $J_{1,2}=3.6$ Hz, 1H, 1-H), 5.20 (dd, $J_{2,3}=11.2$ Hz, $J_{3,4}=3.1$ Hz, 1H, 3-H), 5.17 (dd, $J_{2',3'}=10.3$ Hz, $J_{3',4'}=3.2$ Hz, 1H, 3′-H), 5.03 (d, $J_{1',2'}=3.5$ Hz, 1H, 1′-H), 4.83 (t, $J=4.4$ Hz, 1H, α-CH Ser), 4.72 (dd, $J_1=4.5$ Hz, $J_2=8.1$ Hz, 1H, α-CH Pro), 4.61 (d, $J=1.6$ Hz, 1H, α-CH Thr), 4.57 (quart, $J=7.1$ Hz, 1H, α-CH Ala), 4.49-4.34 (m, 5H, α-CH Ala′, α-CH Pro′, 2-H, 2′-H, β-CH Thr), 4.29–4.12 (m, 5H, 5-H, 5′-H, 6-$H_{a/b}$, 6′-H_a), 4.07 (dd, $J_{5',6'b}=6.9$ Hz, $J_{6'a,6'b}=11.0$ Hz, 1H, 6′-Hb), 4.03 (dd, $J_1=4.6$ Hz, $J_2=10.5$ Hz, 1H, β-CH_{2a} Ser), 3.93 (m, 1H, δ-CH_{2a} Pro), 3.89 (dd, $J_1=4.3$ Hz, $J_2=10.6$ Hz, 1H, β-CH_{2b} Ser), 3.78 (m, 2H, δ-CH_2 Pro′), 3.66 (m, 2H, δ-CH_{2b} Pro), 2.38–1.88 (m + 7s {2.18, 2.10, 2.08, 2.06, 2.03, 2.00, 1.98}, 35H, 9 CH_3CO, β-CH_2 Pro, γ-CH_2 Pro, β-CH_2 Pro′, γ-CH_2 Pro′), 1.40 (d, $J=7.1$ Hz, 3H, CH_3 Ala), 1.39 (d, $J=7.1$ Hz, 3H, CH_3 Ala′), 1.35 (d, $J=6.4$ Hz, 3H, CH_3 Thr). ^{13}C NMR (CD_3OD): δ 178.54, 173.40, 173.27, 173.22, 173.17, 172.89, 172.38, 172.21, 172.12, 172.01, 171.89, 171.36 (C=O), 100.04, 99.88 (2 C-1), 78.12 (β-C Thr), 70.49, 69.93, 69.02, 68.80, 68.21 (2 C-3, 2 C-4, 2 C-5), 69.67 (β-C Ser), 63.35, 63.11 (2 C-6), 61.77, 59.52 (2 α-C Pro), 57.76 (α-C Thr), 54.37 (α-C Ser), 51.27, 48.46 (2 α-C Ala, 2 C-2, 2 δ-C Pro, berlagert durch CD_3OD-Signal), 30.38, 29.55 (2 β-C Pro), 26.09, 25.92 (2 γ-C Pro), 23.39, 22.86, 22.45 (*C*H_3CON), 20.65, 20.55 (*C*H_3CO), 19.33, 19.01 (2 CH_3 Ala), 16.99 (CH_3 Thr). FABMS (3-NOBA): *m*/*z* 1241.7 $(M-H)^-$. Calcd: 1241.5.

***N*-Acetyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-seryl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanyl-L-prolyl-L-prolyl-L-alanine (3).** To a solution of **33** (30 mg, 24 μmol) in dry methanol (5 mL) was added a solution of 1% sodium methanolate in methanol until the pH is adjusted to 8.5. After stirring for 2 h at room temperature, neutralization was carried out by addition of acidic ion-exchange resin (DOWEX 50 WX-8, H^+ form). After filtration and concentration in vacuo, preparative HPLC was performed as described for **33**. Yield of **3**: 17 mg (73%); amorphous solid;

$[\alpha]_D^{22}$ −13.6 (*c* 1.75, CH_3OH). ^1H NMR [^1H-^1H-COSY and NOE] (D_2O): δ 4.93 (d, $J_{1,2}$ = 3.8 Hz, 1H, 1-HThr), 4.85 (d, $J_{1',2'}$ = 3.7 Hz, 1H, 1′-HSer), 4.72 (t, J = 4.7 Hz, 1H, α-CH Ser), 4.62 (dd, J_1 = 5.5 Hz, J_2 = 8.4 Hz, 1H, α-CH Pro), 4.57 (d, J = 1.8 Hz, 1H, α-CH Thr), 4.54 (quart, J = 7.1 Hz, 1H, α-CH Ala), 4.34 (dd, J_1 = 5.4 Hz, J_2 = 8.5 Hz, 1H, α-CH Pro′), 4.25 (dq, J_1 = 1.7 Hz, J_2 = 8.5 Hz, 1H, β-CH Thr), 4.10 (dd; $J_{1',2'}$ = 3.8 Hz, $J_{2',3'}$ = 10.8 Hz, 1H, 2′-H), 4.07 (m, 2H, 2-H, α-CH Ala′), 4.00 (dd, J_1 = 4.2 Hz, J_2 = 11.4 Hz, 1H, β-CH_{2a} Ser), 3.98 (t, J = 7.0 Hz, 1H, 5-H), 3.93 (d, J = 2.9 Hz, 2H, 4-H, 4′-H), 3.89–3.80 (m, 4H, 5′-H {3.87ppm}, 3′-H {3.83ppm} 3-H {3.82ppm}, β-CH_{2b} Ser {3.81pp}), 3.78–3.75 (m, 2H, δ-CH_{2a} Pro, δ-CH_{2a} Pro′), 2.40–2.20 (m, 2H, β-CH_{2a} Pro {2.34ppm}, β-CH_{2a} Pro′ {2.27ppm}), 2.10–1.87 (m, 15H, 3 CH_3CO, β-CH_{2b} Pro {1.95}, γ-CH_2 Pro {2.04ppm}, β-CH_{2b} Pro′ {1.91ppm}, γ-CH_2 Pro′ {2.04ppm}), 1.33 (d, J = 7.2 Hz, 3H, CH_3 Ala), 1.31 (d, J = 7.3 Hz, 3H, CH_3 Ala), 1.24 (d, J = 6.4 Hz, 3H, CH_3 Thr). ^{13}C NMR (D_2O): δ 174.18, 173.69, 172.97, 172.19,171.91, 171.70, 170.28 (C = O), 98.77, 98.15 (2 C-1), 76.56 (β-C Thr), 71.30, 68.60, 68.45, 68.40, 67.84 (2 C-3, 2 C-4, 2 C-5), 67.63 (β-C Ser), 61.32, 61.16 (2 C-6), 60.55, 58.46 (2 α-C Pro), 56.85 (α-C Thr), 53.60 (α-C Ser), 50.90, 49.74, 49.59, 47.31 (2 α-C Ala, 2 C-2), 47.86, 47.64 (2 δ-C Pro), 29.15, 28.07 (2 β-C Pro), 24.66, 24.57 (2 γ-C Pro), 22.37, 22.07, 21.65 (CH_3CON), 18.37, 17.60 (2 CH_3 Ala), 15.49 (CH_3 Thr). FABMS (glycerol): *m*/*z* 989.0 $(M-H)^-$; calcd: 989.4.

N-Acetyl-L-prolyl-L-alanyl-L-prolyl-glycyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanyl-L-prolyl-L-prolyl-L-alanine heptyl ester (34). A solution of **31** (345 mg, 227 mmol) in toluene (5 mL) and morpholine (5 mL) was stirred for 2 h at room temperature. The solvent was evaporated in vacuo. The residue was dried by codistillation with toluene (10 mL) in high vacuum and dissolved in dichloromethane (5 mL). This solution was added to a mixture of **25** (125 mg, 458 μmol), EDC (90 mg, 496μmol) and HOBt (125mg, 925 μmol) in dichloromethane/dimethylformamide (1:1, v/v, 10 mL), which already had been stirred for 1 h at room temperature. After 3 days the solvents were removed in high vacuum, and the remainder was purified by preparative HPLC as described for **33**, eluent methanol/water (65:35). As a by-product the *N*-acetyl glycohexapeptide **32** was isolated: 77 mg (25%). Yield of glycodecapeptide **34**: 170 mg (45%); amorphous solid; $[\alpha]_D^{22}$ −60.0 (*c* 0.5, CH_3OH). ^1H NMR ($CDCl_3$): δ 9.11 (d, 1H, NH), 8.22 (d, J = 11.4 Hz, 1H, NH), 7.94 (d, J = 9.1 Hz, 1H, NH), 7.81 (d, J = 6.0 Hz, 1H, NH), 7.21 (d, J = 10.4 Hz, 1H, NH), 7.02 (d, J = 8.5 Hz, 1H, NH), 6.94 (d, J = 7.0 Hz, 1H, NH), 6.43 (d, J = 9.9 Hz, 1H, NH), 5.33–5.28 (m, 2H), 5.11–5.06 (m, 2H), 4.99 (dd, J_1 = 3.3 Hz, J_2 = 11.9 Hz, 1H), 4.82–4.66 (m, 3H), 4.60–4.46 (m, 5H), 4.43 (t, J = 7.1 Hz, 1H), 4.39 (dd, J_1 = 2.3 Hz, J_1 = 8.5 Hz, 1H), 4.31–4.19 (m, 4H), 4.11–4.02 (m, 7H), 3.94 (dd, J_1 = 5.6 Hz, J_2 = 10.3 Hz, 1H), 3.88 (m, 1H), 3.81–3.46 (m, 9H), 3.38–3.33 (m, 1H), 2.29–1.86 (m, 43H, 9 CH_3CO, 4 β-CH_2 Pro, 4 γ-CH_2 Pro), 1.61–1.58 (m, 2H, OCH_2-CH_2- Hep), 1.42–1.17 (m, 17H, 3 CH_3 Ala, $(CH_2)_4$-CH_3), 1.14 (d, J = 7.3 Hz, 3H, CH_3 Thr), 0.85 (m, 3H, CH_3 Hep). ^{13}C NMR ($CDCl_3$): δ 174.42, 173.39, 173.23, 172.80, 171.75, 171.70, 171.06, 170.82, 170.55, 170.36, 170.20, 169.99, 169.44, 169.19 (C = O), 100.12, 98.05 (2 C-1), 73.94 (β-C Thr), 68.66, 68.44, 67.43, 67.37, 66.81 (2 C-3, 2 C-4, 2 C-5), 65.56, 65.44 (O-CH_2 Hep, β-C Ser), 62.29, 61.42 (2 C-6), 61.34, 60.61, 60.20, 59.90 (4 α-C Pro), 58.50 (α-C Thr), 56.10 (α-C Ser), 48.18, 48.05, 47.01, 46.86 (4 δ-C Pro), 47.96, 47.69, 47.33, 47.21 (3 α-C Ala, 2 C-2), 43.54 (α-C Gly), 31.56, 28.72, 28.42, 25.63, 22.43 (4 CH_2 Hep), 30.20, 27.70 (β-C Pro), 25.56, 25.35, 25.05, 23.94 (4 γ-C Pro), 23.16, 22.92, 22.67 (CH_3CON), 20.65, 20.57, 20.53 (CH_3CO), 18.58, 18.31, 18.09 (3 CH_3 Ala), 16.69 (CH_3 Thr), 13.91 (CH_3 Hep). FABMS (3-NOBA): 1664.7 $(M+H)^+$; calcd: 1663.8 (tetrahydrate). Anal. calcd for $C_{75}H_{114}N_{12}O_{30}$ * $4H_2O$ (1663.8): C, 51.90; H, 7.08; N, 9.68; found: C, 52.06; H, 6.92; N, 9.27.

N-Acetyl-L-prolyl-L-alanyl-L-prolyl-glycyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanyl-L-prolyl-L-prolyl-L-alanine (35). To solution of **34** lipase N (200 mg) in 20 mL of sodium phosphate buffer (pH 7.0), which had been treated with PMSF as described for **25**, was added a solution of **34** (100 mg, 60 mmol) in acetone (1 mL). The mixture was vigorously shaken for 16 h at 37 °C, then lyophylized and the residue was extracted five times with 20 mL of ethanol. After evaporation of the ethanol the crude product was purified by preparative HPLC in methanol/water (4:1, v/v) as described for **33**. Yield: 47 mg (50%), amorphous solid, $[\alpha]_D^{22}$ −49.5 (*c* 0.5, CH_3OH). ^1H NMR [^1H-^1H-COSY] (CD_3OD): δ 5.43 (m, 2H, 4-H, 4′-H), 5.18–5.22 (m, 3H, 3-H, 3′-H, 1-H), 5.03 (d, 1H, 1′-H), 4.80 (m, 1H, α-CH Ser), 4.75 (m, 1H, α-CH Pro), 4.67 (m, 1H, α-CH Ala), 4.65 (m, 1H, α-CH Thr), 4.57 (m, 1H, α-CH Ala′), 4.45 (m, 4H, 2′-H, 3 α-CH Pro), 4.42 (m, 3H, 2-H, β-CH Thr, 5-H), 4.30–4.20 (m, 2H, α-CH Ala, 5′-H), 4.20–4.04 (m, 5H, 6-$H_{a/b}$, 6′-$H_{a/b}$, β-CH_{2a} Ser), 3.45–4.00 (m, 12H, α-CH_2 Gly, β-CH_{2b} Ser, 4 δ-$CH_{2a/b}$ Pro), 2.45–1.90 (m, 43H, 9 CH_3CO, 4 β-CH_2 Pro, 4 γ-CH_2 Pro), 1.45–1.30 (m, 12H, 3 CH_3 Ala, CH_3 Thr). ^{13}C NMR (CD_3OD): δ 179.34, 175.03, 174.22, 174.03, 173.41, 173.28, 173.14, 173.01, 172.82, 172.36, 172.28, 172.11, 172.05, 171.94, 171.80, 171.20 (C = O), 100.45, 99.58 (2 C-1), 78.25 (β-C Thr), 70.48, 69.91, 69.01, 68.84, 68.28, 68.17 (2 C-3, 2 C-4, 2 C-5), 68.95 (β-C Ser), 63.37, 63.14 (2 C-6), 62.18, 61.91, 61.05, 59.53 (4

α-C Pro), 57.76 (α-C Thr), 54.61 (α-C Ser), 51.86, 48.44 (3 α-C Ala, 2 C-2), 48.02 (4 δ-C Pro), 43.79 (α-C Gly), 30.90, 30.68, 30.43, 29.56 (4 β-C Pro), 26.11, 25.89, 25.74 (4 γ-C Pro), 23.41, 22.89, 22.24 (CH_3CON), 20.71, 20.58, 20.52 (CH_3CO), 19.41, 19.31, 16.99 (3 CH_3 Ala), 16.43 (CH_3 Thr). FAB MS (3-NOBA): *m/z* 1566.8 $(M+H)^+$; calcd: 1565.7; 1589.8 $(M+Na)^+$; calcd: 1587.7.

***N*-Acetyl-L-prolyl-L-alanyl-L-prolyl-glycyl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-seryl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanyl-L-prolyl-L-prolyl-L-alanine (2).** To a solution of **35** (40 mg, 25.5 mmol) in dry methanol (5 mL) was added a solution of 1% sodium methanolate in methanol until the pH was adjusted to 8.5. The mixture was stirred for 2 h and then neutralized by addition of ion-exchange resin (DOWEX WX 50-8, H^+ form). After filtration and concentration in vacuo, the remainder was purified by preparative HPLC in analogy to the procedure for **3**, eluent:methanol/water (1:1, v/v). Yield: 24 mg (71%); amorphous solid; $[\alpha]_D^{22}$ −38.8 (*c* 0.8, CH_3OH). ^1H NMR (CD_3OD): δ 5.04 (d, $J_{1,2}=3.8$ Hz, 1H, 1-H), 4.91 (d, $J_{1',2'}=3.7$ Hz, 1H, 1-H′), 4.85 (t, $J=5.7$ Hz, 1H, α-CH Ser), 4.72 (dd, $J_1=4.4$ Hz, $J_2=8.1$ Hz, 1H, α-CH Pro), 4.67 (m, 1H, α-CH Ala), 4.59 (m, 2H, α-CH Ala, α-CH Thr), 4.48–4.41 (m, 3H, 3 α-CH Pro), 4.31–4.23 (m, 4H, 2 α-CH Ala, 2-H, 2′-H), 4.08 (dd, $J_1=5.2$ Hz, $J_2=10.4$ Hz, β-CH_{2a} Ser), 4.00–3.60 (m, 21H, α-CH_2 Gly {4.0}, β-CH_{2b} Ser {3.84}, 3-H {3.78}, 3(-H {3.78}, 6-$H_{a/b}$, 6′-$H_{a/b}$, 5-H, 5′-H, 4-H, 4′-H, 4 δ-CH_2 Pro′, 2.37–1.96 (m + 3s {2.12, 2.08, 2.06}, 25H, 3 CH_3CO, 4 β-CH_2 Pro, 4 γ-CH_2 Pro), 1.41 (d, $J=7.0$ Hz, 3H, CH_3 Ala), 1.40 (d, $J=6.2$ Hz, 3H, CH_3 Ala), 1.39 (d, $J=6.9$ Hz, 3H, CH_3 Ala), 1.30 (d, $J=6.4$ Hz, 3H, CH_3 Thr). ^{13}C NMR ($CDCl_3$): δ 175.15, 174.28, 173.91, 173.64, 173.51, 172.99, 172.72, 172.31, 172.06, 171.73, 171.37 (C=O), 100.11, 99.66 (2 C-1), 76.99 (β-C Thr), 72.90, 70.64, 70.41, 70.33, 69.95 (2 C-3, 2 C-4, 2 C-5), 68.57 (β-C Ser), 62.89, 62.83 (2 C-6), 62.14, 61.53, 60.99, 59.61 (4 α-C Pro), 57.91 (α-C Thr), 54.68 (α-C Ser), 51.46, 51.32, 48.55, 48.43, 45.41 (3 α-C Ala, 2 C-2), 49.49, 48.75, 48.62, 48.39 (4 δ-C Pro), 43.82 (α-C Gly), 30.99, 30.48, 30.34, 29.43 (4 β-C Pro), 26.13, 26.02, 25.85, 25.74 (4 γ-C Pro), 23.43, 23.09, 22.24 (3 CH_3CON), 19.23, 18.31, 16.95 (3 CH_3 Ala), 16.62 (CH_3 Thr). FABMS (glycerol): *m/z* 1312.9 $(M-H)^-$; calcd: 1311.6.

Structual studies of 1, 2, and 3

The three (glyco)peptides, purified to homogeneity using HPLC, were dissolved in DMSO-d_6 containing 0.09 mM tetramethyl silane, to produce 4 mM solutions in 0.5 mL. DMSO-d_6 was used as solvent. ^1H Homonuclear 1- and 2-dimensional (1-D and 2-D) NMR spectroscopy was undertaken at 400 and 500 MHz on Brucker AX500, AMX500, and AX400 NMR spectrometers, using standard Bruker software for acquisition and processing of data. Chemical shifts were measured, accurate to 0.01 ppm relative to the TMS resonance. 1-D spectra were recorded over 16 k data points, zero filled to 32 k using a sweep width of 15 ppm. 2-D COSY, HOHAHA, NOESY, and ROESY experiments were undertaken typically over sweep widths of 15×15 ppm, acquiring 512×2 k spectra. The HOHAHA experiments were undertaken with a 100 ms mixing time, using an mLEV17 pulse sequence and time proportional phase incrementation (TPPI). The NOESY experiments used mixing times of between 200 and 600 ms. The ROESY experiment used a 150 ms mixing time, and a fileld strength of 1–2 kHz for spin locking, which was achieved with a continuous wave pulse.

Molecular simulation of 1, 2, and 3

The decapeptide, glycodecapeptide, and glycohexapeptide were constructed using the Builder module of InsightII (version 3.0.0; MSI, San Diego, CA). The structures were energy-minimized using 1000 iterations of molecular dynamics within the Discover (version 2.9.8; MSI, San Diego, CA) force-field followed by up to 5000 iterations of Fletcher–Reeves conjugate gradient minimization. The structures were exported to the Genesis II Graphics System[28] for simulation using the Dynamic/Monte-Carlo (DMC) method of Morley.[29] Partial atomic charges were re-assigned using the Charge method[30] and the structures re-minimized within the COSMIC(90) force-field[31] using up to 1000 iterations of Fletcher–Reeves conjugate gradients. The starting conformation for the simulation for each structure was chosen by performing 10 runs of simulated annealing (Monte-Carlo torsional rearrangements, initial Metropolis temperature of 1500 K, 25 temperature blocks, temperature increase factor of 0.93 per block, 250 Metropolis trials-per-block, five rearrangements per trial) and the conformer with the lowest energy after 1000 iterations of conjugate gradient minimization chosen.

Molecular dynamics was performed on each of the three peptides in conformations produced by the Monte-Carlo sampling above. From an initial Metropolis sampling temperature of 1000 K, low-energy conformers for each peptide were produced using 10 runs of COSMIC-style molecular dynamics[32] and a simulated annealing schedule within DMC (COSMIC-MD atomic rearrangements, initial Metropolis temperature of 1000 K, 25 temperature blocks, temperature increase factor of 0.93 per block, 250 trials-per-block, five MD iterations-per-trial, 1 femtosecond integration-per-MD step). The final 10 conformations of each peptide were energy

minimized and unique conformations (within 5 kcal/mol) extracted.

When used, inter-atomic restraints were applied by converting each distance violation to an energy penalty through a quadratic function. Inter-proton distances were recorded from a Boltzmann-average (taken at 298 K) of those in the final conformations.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie. GMD acknowledges funding from a BBSRC CASE Award with Unilever plc.

References

1. Gendler, S.; Spicer, A. P.; Lalani, E.-N.; Duhig, T.; Peat, N.; Burchell, J.; Pemperton, L.; Boshell, M.; Taylor-Papadimitriou, *J. Am. Rev. Respir. Dis*. **1991**, *144*, 42.

2. (a) Hull, S. R.; Carraway, K. L. *FASEB J*. **1988**, *2*, 2380. (b) Springer, G. F. *Science* **1984**, *224*, 1198.

3. Briggs, S.; Price, M. R.; Tendler, S. J. B. *Eur. J. Cancer* **1993**, *29A*, 230.

4. Scanlon, M. J.; Morley, S. D.; Jackson, D. E.; Price, M. R.; Tendler, S. J. B. *Biochem. J*. **1992**, *284*, 137.

5. Price, M. R.; Hudecz, F.; O'Sullivan, C.; Baldwin, R. W.; Edwards, P. M.; Tendler, S. J. B. *Mol. Immunol*. **1990**, *62*, 795.

6. Roberts, C. J.; Sekowski, M.; Davies, M. C.; Jackson, D. E.; Proce, M. R.; Tendler, S. J. B. *Biochem. J*. **1992**, *283*, 181.

7. Shogren, R.; Gerken, T. A.; Jentoft, N. *Biochemistry* **1989**, *28*, 5525.

8. Gerken, T. A.; Butenhof, K. J.; Shogren, R. *Biochemistry* **1989**, *28*, 5536.

9. Andersen, B.; Hoffmann, P.; Meyer, K. *Biochem. Biophys. Acta* **1963**, *74*, 309.

10. Garg, H. G.; Bruch, K. v. d.; Kunz, H. *Adv. Carbohydr. Chem. Biochem*. **1994**, *50*, 277.

11. Reidel, A.; Waldmann, H. *J. Prakt. Chem*. **1993**, *335*, 109.

12. Schultheiss-Reimann, P.; Kunz, H. *Angew. Chem. Int. Ed. Engl*. **1983**, *22*, 62; Schultheiss-Reimann, P.; Kunz, H. *Angew. Chem. Suppl*. **1983**, 39.

13. Braun, P.; Waldmann, H.; Vogt, W.; Kunz, H. *Liebigs. Ann. Chem*. **1991**, 165.

14. Braun, P.; Waldmann, H.; Kunz, H. *Synlett* **1992**, 39.

15. Braun, P.; Waldmann, H.; Kunz, H. *Bioorg. Med. Chem*. **1993**, *1*, 197.

16. Paulsen, H.; Rauwald, W.; Weichert, U. *Liebigs. Ann. Chem*. **1988**, 75.

17. Fügedi, P.; Garegg, P. J. *Carbohydr. Res*. **1986**, *149*, C9.

18. Kunz, H.; Kowalczyk, D.; Braun, P.; Braum, G. *Angew. Chem. Int. Ed. Engl*. **1994**, *33*, 336.

19. Zemplén, G.; Bascu, E. *Ber. Dtsch. Chem. Ges*. **1929**, *62*, 1613.

20. Live, D. H.; Kumar, R. A.; Beebe, X.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A*. **1996**, *93*, 12759.

21. Peters, S.; Bielefeldt, T.; Meldal, M.; Bock, K.; Paulsen, H. *J. Chem. Soc. Perkin Trans I* **1992**, *9*, 1163.

22. Kunz, H.; Kullmann, R. *Tetrahedron Lett*. **1992**, *33*, 6115.

23. Mimura, J, Yamamoto, Y, Inoue, Y, and Chujo, R. *Int. J. Biol. Macromol*. **1992**, *14*, 242.

24. Hollosi, M.; Perczel, A.; Fasman, G. D. *Biopolymers* **1990**, *29*, 1549.

25. Spencer, D. I. R.; Price, M. R.; Tendler, S. J. B.; DeMatteis, C. I.; Stadie, T.; Hanisch, F.-G. *Cancer Lett*. **1996**, *100*, 11.

26. Scanlon, M. J.; University of Nottingham, 1993.

27. Stadie, T. R. E.; Chai, W.; Lawson, A. M.; Byfield, P. G.; Hanisch, F.-G. *Eur. J. Biochem*. **1995**, *229*, 140.

28. Williams, P. M.; Davies, M. C.; Jackson, D. E.; Roberts, C. J.; Tendler, S. J. B.; Wilkins, M. J. *Nanotechnology* **1991**, *2*, 172.

29. Morley, S. D.; Jackson, D. E.; Saunders, M. R.; Vinter, J. G. *J. Comp. Chem*. **1992**, *13*, 693.

30. Abraham, R. J.; Grant, G. H.; Haworth, I. S.; Smith, P. E. *J. Computer-Aided Mol. Design* **1991**, *5*, 21.

31. Morley, S. D.; Abraham, R. J.; Haworth, I. S.; Jackson, D. E.; Saunders, M. R.; Vinter, J. G. *J. Computer-Aided Mol. Design* **1991**, *5*, 475.

32. Vinter, J. G.; Davis, A.; Saunders, M. R. *J. Computer-Aided Mol. Design* **1987**, *1*, 31.