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Glycosylated foldamers: synthesis of carbohydrate-modified β^3hSer and incorporation into β-peptides‡

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Abstract: Fmoc-protected β^3hserine (β^3hSer) was prepared and *O*-linked to suitably protected *N*-acetylgalactosamine (GalNAc) and *N*-acetylglucosamine (GlcNAc) derivatives. Glycosylation of β^3hSer was made by two independent routes: either by direct glycosyl linkage to the β^3hSer, or linkage to natural L-Ser and then utilizing the carbohydrate moiety as a protecting group in an Arndt–Eistert homologation. Both procedures gave the novel glycosylated β^3-amino acids Fmoc-β^3hSer(α-D-GalNAc(Ac)$_3$)-OH (**1a**), its β-anomer (**1b**), and Fmoc-β^3hSer(β-D-GlcNAc(Ac)$_3$)-OH (**2**), which were utilized in the solid-phase peptide synthesis of four glycosylated dipeptides (**3a–d**) and two heptapeptides (**4a–b**). The preparation of β-amino acids bearing common post-translational modifiers represents an important step towards functionalized foldamers with broad applications in biomedical research. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: glycosylation; β-amino acids; glycopeptides; foldamers; conformational analysis

INTRODUCTION

Nature relies on post-translational modifications, such as lipidation, phosphorylation, and glycosylation, to help fine-tune properties and the activity of biomolecules. Among the various post-translational modifications found, glycosylation is the predominant form in higher organisms, e.g. more than 50% of the human proteins are glycoproteins [1–3]. The carbohydrate residues are often essential for the function of the biomolecule, and the ability to modify and control the composition and attachment of the sugars make possible the tailoring of biomolecular structure, folding, stability, immunogenicity, uptake, distribution, target recognition, and other properties and functions of these compounds [4]. For example, the activity and longevity of the approved glycoprotein drug erythropoietin (EPO) have been improved by modifying its glycosylation through genetic engineering [5–7]. Thus, the large current interest in glycosylation engineering [8] both from a fundamental and an applied point of view, is readily explained by this and other findings.

Over the last couple of years, our group has been interested in fundamental studies on how post-translational modification, especially glycosylation, may be used to affect the structure and function of artificial biomimetic oligomers. Our main interest has been related to oligomers that are capable of adopting well-defined secondary structures, the so-called foldamers [9]. Such molecular entities can be composed of completely abiotic building blocks, i.e. metals and ligands, or incorporate monomers more closely related to naturally occurring substances, e.g. amino acids. Oligomers of β-amino acids, i.e. β-peptides, are probably the most thoroughly investigated peptidomimetic foldamer to date [10,11]. β-Peptides with as few as six residues have a high propensity to fold into defined secondary structures similar to those found in nature [12]. This resemblance has sparked an interest in the search for biologically active β-peptides. Indeed, β-peptides with, e.g., antibacterial [13,14], antiproliferative [15], and somatostatin-mimicking [16] properties have been described and proven to be stable towards proteolytic [17] and metabolic [18] degradation.

The promising biomedical potential of β-peptides, e.g. folding of a reasonable molecular weight substance into predictable structures, proven receptor interaction potential, more favourable pharmacokinetic and pharmacodynamic properties than α-peptides, suggests that even better properties may be obtained by modifying the artificial backbone through glycosylation engineering. The resulting hybrid conjugate, in which a natural post-translational modification, e.g. a carbohydrate residue, is attached onto an artificial, but biomimetic, backbone is expected to be of interest not only for fundamental studies on the glycosylation's effect on the backbone structure itself, but

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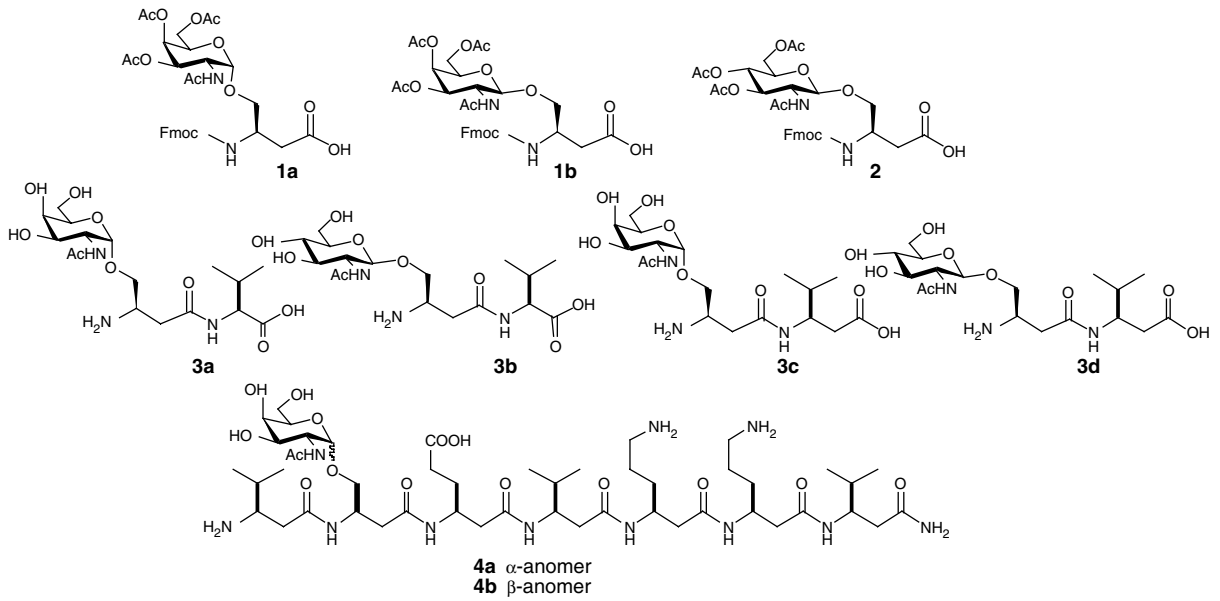
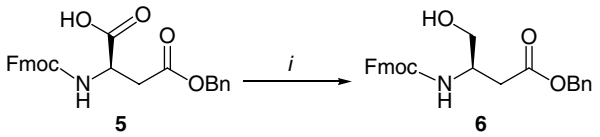


also for investigations on how the artificial backbone affects the properties and the biomolecular recognition of the natural sugars attached. Application of such hybrid conjugates in the area of vaccine development and other biomedical applications may also be envisioned.

In a first demonstration of this concept, we recently communicated the synthesis and solution-state structure of one glycosylated β-peptide [19] that was designed to fold into a 3_{14}-helical [20,21] conformation. It was demonstrated that the peptide was capable of folding into a 3_{14}-helical conformation in methanol despite incorporation of one GalNAc-modified β^3hSer residue. Here, we wish to present a full account on the synthesis of three novel β^3-amino acids, i.e. Fmoc-β^3hSer(α-D-GalNAc(Ac)$_3$)-OH (**1a**), its β-anomer (**1b**), and Fmoc-β^3hSer(β-D-GlcNAc(Ac)$_3$)-OH (**2**), and their incorporation into mixed α/β-peptides (**3a–b**) and β-dipeptides (**3c–d**). These carbohydrate-containing amino acids often represent the core sugar in more advanced glycosylation motifs, and are therefore an essential starting point for further synthesis through enzymatic methods and further studies in this field. In order to demonstrate the synthesis of longer, fully functionalized glycosylated β-peptides, we also present the synthesis and conformational study of glycosylated β^3-heptapeptides (**4a** and **4b**).

RESULTS AND DISCUSSION

β^3-Amino acids can efficiently and enantiospecifically be prepared over two steps using the Arndt–Eistert homologation of α-amino acid derivatives [22], a method which proceeds stereospecifically and most often in high yields. However, β^3hSer can also be synthesized



Scheme 1 Reagents and conditions: (i) *i*-Bu-OCO-Cl, NMM, −15 °C, 1 min, then $NaBH_4$ in H_2O, 82%.

using a less dangerous pathway, not including the use of the explosive and mutagenic diazomethane. This procedure (Scheme 1) takes advantage of the naturally occurring β-amino acid, aspartic acid. By reducing the free carboxyl group of Fmoc- and side-chain protected D-Asp derivative (**5**) to an alcohol according to a procedure reported by Martinez *et al.* [23], the suitably protected β^3hSer derivative (**6**) was obtained. This building block was then used as the glycosyl acceptor in the synthesis of glycosylated β-amino acids.

The synthesis of the Tn-antigen β^3-amino acid analogue (**1a**) and its β-anomer (**1b**) was performed (Scheme 2) using the monosaccharide building block 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide (**7**) as the glycosyl donor, which was prepared over four steps according to a procedure developed by Lemieux and Ratcliffe [24] and later improved by Kihlberg *et al.* [25].

Reaction between the glycosyl donor (**7**) and the acceptor (**6**) was promoted by silver triflate (AgOTf) under dry and inert conditions, leading to the formation of the α- and β-anomers (**8a**) and (**8b**), respectively. By ^{1}H NMR of the crude reaction mixture, the α/β ratio could be estimated to 1.8 : 1. Chromatographic separation of the α- and the β-isomers [(**8a**) and (**8b**), respectively] and reductive acylation using Zn and $CuSO_4$ (sat.) in a mixed solvent of THF/Ac_2O/AcOH

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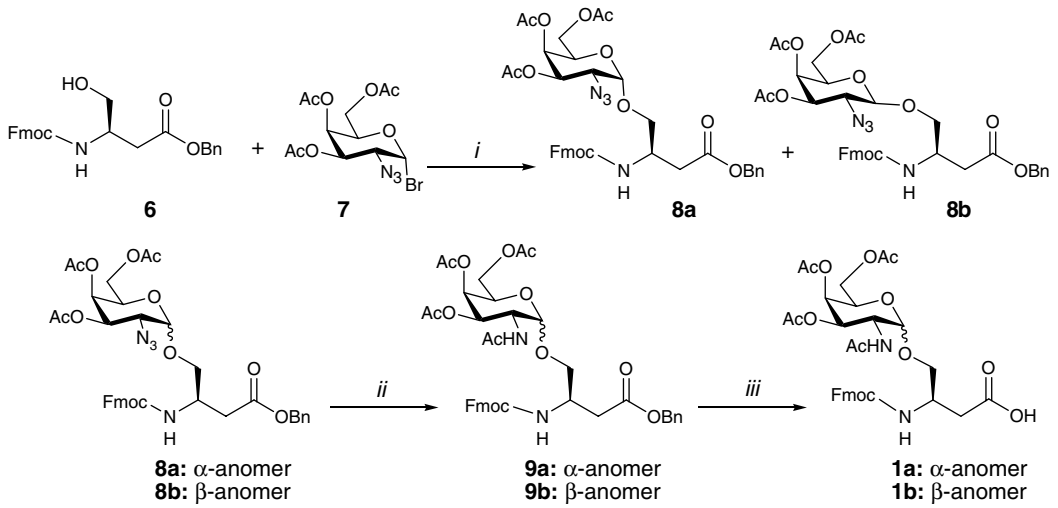
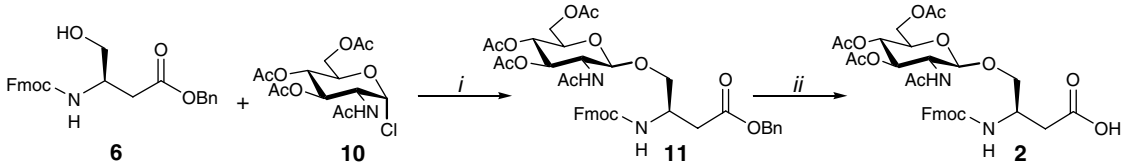
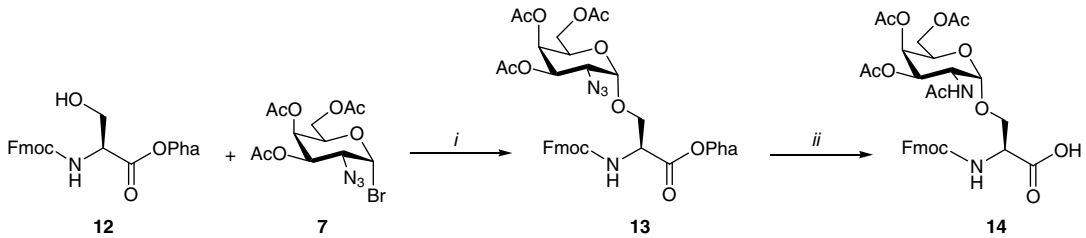


Scheme 2 Reagents and conditions: (i) DCM/THF (1 : 1), 4 Å MS, −40 °C, 1 h. Then AgOTf in toluene, −40 °C 6 h, 36% (α-anomer) and 29% (β-anomer); (ii) THF/Ac_2O/AcOH (3 : 2 : 1), activated Zn, $CuSO_4$ (sat.), r.t. 2 h, 84% (α-anomer) and 74% (β-anomer); (iii) 10% Pt/C, H_2, 1 atm., MeOH/H_2O (15 : 1), 92% (α-anomer) and 64% (β-anomer).



Scheme 3 Reagents and conditions: (i) AgOTf, DCM, 4 Å MS, reflux overnight, 35%; (ii) 10% Pt/C, H_2, 1 atm., MeOH/H_2O (15 : 1), 80%.



Scheme 4 Reagents and conditions: (i) DCM/THF (1 : 1), 4 Å MS, −40 °C, 1 h. Then AgOTf in toluene, −40 °C 6 h, 22%; (ii) activated Zn dust, $CuSO_4$ (sat.), Ac_2O/AcOH (2 : 1) r.t. 2 h, then H_2O and activated Zn dust, r.t., 13 h, 59%.

[26] followed by deprotection of the benzyl group by Pt/C [27] gave the final product (**1a**) in an overall yield of 28% with respect to the β^3hSer (**6**) residue [the overall yield of (**1b**) was 14%].

The synthesis (Scheme 3) of Fmoc-β^3hSer(β-D-GlcNAc(Ac)$_3$)-OH (**2**) was less cumbersome, as the β-linked anomer was formed exclusively because of the directing effect of the 2-*N*-acetyl group. Thus, commercially available chloride (**10**) could be directly coupled to the protected β^3hSer derivative (**6**) using AgOTf as activator [28], yielding Fmoc-β^3hSer(β-D-GlcNAc(Ac)$_3$)-OBn (**11**) in 35% yield. Debenzylation using Pt/C provided glycosylated β^3-amino acid (**2**) in 29% yield from the β^3hSer residue.

The two glycosylated β^3hSer derivatives (**1a**) and (**2**) could also be synthesized according to a strategy in which the saccharide moieties were linked to the α-amino acid and then used as a kind of protecting group during an Arndt–Eistert homologation.

Utilizing this method for the synthesis of glycosylated β^3hSer derivative (**1a**), the glycosyl donor (**7**) was linked to the Fmoc- and phenacyl ester [29] (Pha) protected L-Ser (**12**) according to the same coupling procedure used for β^3hSer, yielding the compound (**13**) in a diasteromeric mixture with the β-anomer (ratio 1.3 : 1, Scheme 4). Reductive acylation and removal of the phenacyl group of (**13**) were done in a one-pot reaction, producing (**14**) in 17% overall yield with respect to the Fmoc- and Pha-protected serine.

The synthesis of glycosylated β^3hSer derivative (**2**) was carried out starting from the Troc-protected amine

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(**15**). Troc-protection [30] of the C-2 amine of D-(+)-glucosamine followed by peracetylation [31] gave the glycosyl donor (**15**) in 68% yield over 2 steps. As an acyl group at the C-2 position of the carbohydrate moiety promotes the formation of the desired β-isomer, thus the Troc-group was not suitable only because of its quality as a protecting group of the free amine.

Compound (**15**) was linked to Fmoc-protected L-Ser according to a procedure described by Kihlberg *et al.* [32] forming β-glycosidic products (**16**) (Scheme 5). Troc-deprotection and acetylation was performed in the same step, producing (**17**) in 29% overall yield (from Fmoc-protected serine).

The suitably protected αGalNAc-L-Ser (**14**) and βGlcNAc-L-Ser (**17**) could be stereospecifically transformed into their β^3-amino acid analogues (**1a**) and (**2**) via Arndt–Eistert homologation, using a method developed by Seebach *et al.* [22] but with an ultrasound-promoted Wolff rearrangement [33]. This provided the glycosylated β^3-amino acids (**1a**) and (**2**) in 82% and 50%, respectively, over two steps, and in 14% and 15%, respectively, in overall yields with respect to the serine starting materials (Scheme 6).

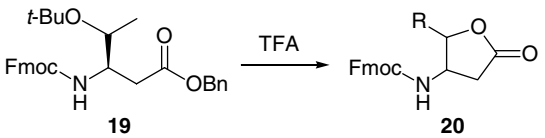
Since not only serine but also threonine is found to be glycosylated in biological systems, we set out to synthesize β^3hThr with the αGalNAc and βGlcNAc monosaccharide moieties *O*-linked to the side chain. The synthetic route for preparing the glycosyl acceptor β^3hThr was by Arndt–Eistert homologation, using Fmoc-L-Thr(O*t*-Bu)-OH as starting material. Suitable protecting groups for the backbone amino group and the side chains were required during these steps in order to minimize the number of side reactions. The homologation of Fmoc-L-Thr(O*t*-Bu)-OH gave Fmoc-β^3hThr(O*t*-Bu)-OBn (**19**) in 76% yield, according to a procedure developed by Seebach *et al.* [22]

However, to be able to link the amino acid to the carbohydrate moiety, deprotection of the *t*-Bu group of (**19**) was required. This should occur rapidly by the use of TFA and TIPS in CH_2Cl_2, leaving the amino acid residue with a free hydroxyl group. Unfortunately, as Fmoc-β^3hThr(O*t*-Bu)-OBn (**19**) was treated with the cleavage mixture, rapid lactonization occurred (Scheme 7). The same reaction occurred when Fmoc-β^3hSer-OBn (**6**) was treated with TFA.

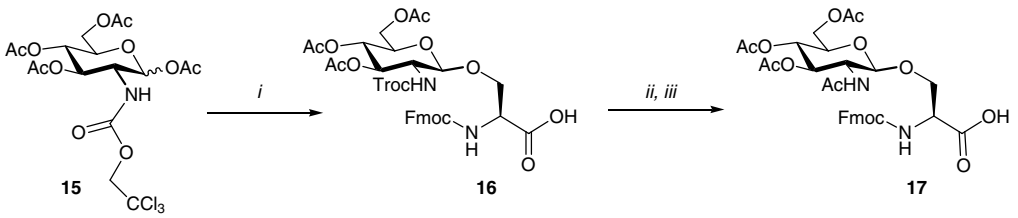
Even though many attempts were made, no satisfactory procedure to open the lactone was found. The possible strategies to recover the opened lactone were either by treating the lactone with water to form the free carboxylic acid or with an alcohol to form the ester-protected analogue under acidic, neutral or basic conditions. The opening of the lactone promoted by alcohol was further limited since an orthogonal protecting group strategy had to be considered.

The αGalNAcβ^3hSer derivative (**1a**), its β-linked analogue (**1b**) as well as the βGlcNAcβ^3hSer derivative (**2**) were used in solid-phase syntheses of four glycosylated dipeptides (**3a–d**) and two β^3-heptapeptides (**4a**) and (**4b**).

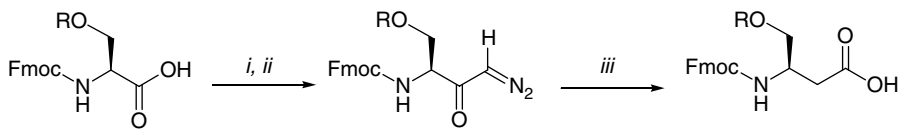
First, Fmoc-β^3hSer(α-D-GalNAc(Ac)$_3$)-OH (**1**) and Fmoc-β^3hSer(β-D-GlcNAc(Ac)$_3$)-OH (**2**) were used in the synthesis of the α/β^3-mixed dipeptides (**3a**) and (**3b**), respectively. By using a commercial Wang resin already anchored with the L-Val residue, the coupling of the glycosylated β^3hSer (22 h) using the standard



Scheme 7 Lactonization of Fmoc-β^3hThr(O*t*-Bu)-OBn when exposed to acid.



Scheme 5 Reagents and conditions: (i) $BF_3{\cdot}OEt_2$, Fmoc-Ser-OH, DCM, 5 days, 46% (ii) Zn, AcOH, 8 h, then Ac_2O, 13 h, 64%.



14 R=α-D-GalNAc(Ac)$_3$ **18a** R=α-D-GalNAc(Ac)$_3$ **1a** R=α-D-GalNAc(Ac)$_3$
17 R=β-D-GlcNAc(Ac)$_3$ **18b** R=β-D-GlcNAc(Ac)$_3$ **2** R=β-D-GlcNAc(Ac)$_3$

Scheme 6 Reagents and conditions: (i) NMM, *i*-Bu-OCO-Cl, THF, −15 °C, 20 min; (ii) diazomethane, Et_2O, 0 °C to r.t.; 5 h (iii) 15% H_2O in THF, silver benzoate, NMM, ultrasonication, 10 min.

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activation mixture HBTU/HOBt/DIPEA was the single step of this synthesis. One advantage of using β^3-amino acids in SPPS is that they do not racemize, an advantage that allows longer reaction times.

Fmoc deprotection followed by TFA-mediated cleavage (TFA/TIPS/H_2O, 95 : 2.5 : 2.5) from the resin and removal of the *O*-acetyl protecting groups using NH_3/MeOH gave the NH_2-β^3hSer(α-D-GalNAc)-Val-OH (**3a**) and NH_2-β^3hSer(β-D-GlcNAc)-Val-OH (**3b**) after reversed-phase HPLC purification in 65% and 98% yield, respectively.

Dipeptides (**3c**) and (**3d**) had the same peptide sequence as the former synthesized peptides (**3a**) and (**3b**), but with a β^3hVal residue included in the sequence instead of the normal L-Val. In the first step of this synthesis, the β^3hVal amino acid building block was attached to the Wang linker using DMAP and EDC in DMF. The loading levels were determined to be 85% and 47%, respectively. The glycosylated amino acids were linked to the β^3hVal over 19 h by standard activation procedures and a final Fmoc deprotection completed the SPPS. Cleavage from the resin and deacetylation followed by purification by reversed-phased preparative HPLC gave H_2N-β^3hSer(*O*-α-GalNAc)-β^3hVal-OH (**3c**) and H_2N-β^3hSer(*O*-β-GlcNAc)-β^3hVal-OH (**3d**) in 67% and 76% yield, respectively.

Since no immense problem arose upon synthesizing glycosylated β^3-peptides (**3a–d**), the two 7-residues long glycosylated β^3-peptides (**4a**) and (**4b**) were the next to be synthesized. The two peptides (**4a**) and (**4b**) differ from each other only by the anomeric configuration of the GalNAc moiety. Whereas (**4a**) has the monosaccharide introduced in a Tn-antigen type α-linkage, it contains the non-natural β-linkage in (**4b**). In the design of (**4a**) and (**4b**), a β^3hGlu and a β^3hOrn residue were positioned as residues 3 and 6, respectively, in order to form stabilizing salt-bridge interactions in a 3_{14}-helical conformation of the β-peptide, and β^3hVal residues were chosen as hydrophobic residues at position 1, 4, and 7. The glycosylated β^3hSer was positioned as residue 2, and a β^3hOrn at position 5 was incorporated for a possible hydrogen-bond formation with the carbohydrate C-2 acetamido group.

Unlike in the synthesis of the four dipeptides (**3a–d**), the peptide syntheses were performed this time using Rink amide resin. Linkage of the first amino acid (β^3hVal) to the resin was done by standard amino acid activation (HBTU/HOBt/DIPEA) giving a loading level of 96% for both peptides. Capping of unreacted amino groups was performed by acetylation [Ac_2O in CH_2Cl_2 (1 : 1 ratio), 3 h] after the first coupling to the resin and once again before coupling of the glycosylated amino acid residue.

Final removal of the Fmoc protecting group followed by cleavage from the resin and acetyl deprotection gave the crude products (**4a**) and (**4b**). The two glycosylated β^3-peptides were purified by reversed-phase HPLC (C18 column).

The pure peptides (**4a**) and (**4b**), obtained after purification by reversed-phased HPLC, were investigated by circular dichroism (CD) spectroscopy ($c = 0.1$ mM, 25 °C). The CD spectrum of a left-handed 3_{14}-helix of a β^3-peptide in methanol is well established and gives rise to a minimum, zero crossing, and maxima at 215, 208, and 198 nm, respectively. The CD spectrum of the glycosylated β^3-peptides (**4a**) and (**4b**) in methanol and PBC (1 mM sodium phosphate/borate/citrate, pH 7) are shown in Figure 1. In methanol, both peptides gave rise to spectra characteristic for a 3_{14}-helical conformation and no difference in intensity could be detected. However, in PBC buffer, a significant difference could be observed. From the intensities of the spectra from peptides (**4a**) and (**4b**), it could be concluded that the β-linked GalNAc moiety in (**4b**) has a far more destabilizing effect on the helical structure in aqueous solution compared to the α-linkage in (**4a**). Thus, peptide (**4a**) kept as much as 45% of its helical intensity on change of solvent as compared to 15% for (**4b**).

A full assignment of the peptide protons and a further study of the helical structures of (**4a**) and (**4b**) were performed by ^{1}H NMR and 2D NMR (TOCSY, PE COSY, ROESY) in CD_3OD/CH_3OH (9 : 1) using WET solvent suppression technique [34,35]. The ^{1}H NMR spectrum of both (**4a**) and (**4b**) showed good dispersion of the amide region signals indicating the presence of a secondary structure. The ^3J (NH–C(β)H) coupling constants were large in both cases (ranging between 8.9–9.2 Hz and 9.0–9.5 Hz, respectively), verifying the expected antiperiplanar orientation of these protons.

Hydrogen-bond patterns defining the helical structures of (**4a**) and (**4b**) were provided from the ROESY spectra. As expected, both glycosylated β^3-peptides showed seven out of seven possible multiple long-range NOE characteristics of a 3_{14}-helical conformation. In Figure 2 this is exemplified by the glycosylated β^3-peptide (**4a**), but it also applies to (**4b**).

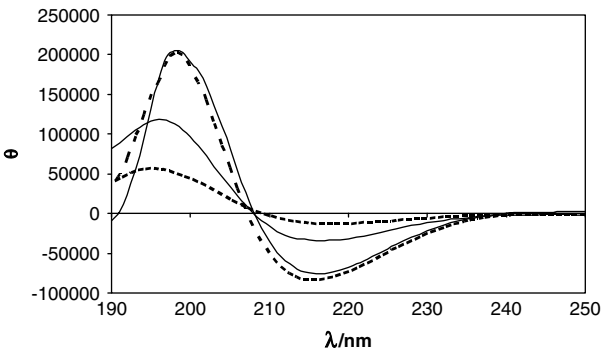

Figure 1 CD spectra of (**4a**) (———) and (**4b**) (- - - -) in methanol (strong bands) and pH 7 PBC buffer (weak bands).

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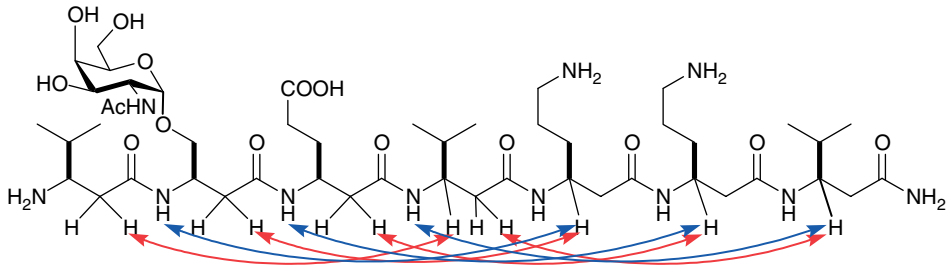

Figure 2 Backbone NOEs extracted from the ROESY spectrum of (**4a**); $C_\alpha H(i) \rightarrow C_\beta H(i+3)$ NOEs are in red and $C_N H(i) \rightarrow C_\beta H(i+3)$ NOEs are in blue. Similar NOEs, characteristic of the 3_{14} helix, were also observed for (**4b**).

EXPERIMENTAL SECTION

General Methods

Solvents and reagents. Solvents were dried and distilled according to standard methods. Diethyl ether and THF were distilled from sodium and benzophenone, respectively, and methylene chloride was distilled from sodium hydride. Melting points are uncorrected. Fmoc-protected α-amino acids, HOBt, HBTU, Rink amide resin and Wang resin were purchased from Senn Chemicals (Switzerland). DIPEA, NMM and piperidine were purchased from Acros Organics (Belgium), and DMF and TFA from Scharlau Chemie (Spain). All other reagents were purchased from Sigma-Aldrich.

Instruments. Solid-phase peptide synthesis was performed on a Quest 210 synthesizer (Argonaut Technologies, USA). RP-HPLC analyses were made using a Gilson system (Gilson 215 Liquid Handler, Gilson UV/VIS-152, Gilson 322 Pump), equipped with a Sedex 85 LT-ELSD, and a Finnigan AQA ESI mass spectrometer. Preparative RP-HPLC was performed using a Gilson system (Gilson 215 Liquid Handler, Gilson UV/VIS-156, Gilson 332 and 331 Pump, 819 Injection valve). Analytical runs were performed on a Phenomenex Gemini C18 column (100 × 3.0 mm, 5 μm), and preparative runs on a Grace Vydac C18 column (22 × 250 mm, 5 μm).

Upon analysis, products were eluted with 0.1% formic acid in acetonitrile/water using a linear gradient (A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile), and upon purification, 0.1% TFA in acetonitrile/water with a linear gradient (A: 0.1% TFA in water; B: 0.1 TFA in acetonitrile) was used. Optimization was made for every peptide at flow rates of 1 ml min^{-1} for analytical and 15 ml min^{-1} for the preparative column.

NMR spectra were recorded on a Varian Unity 500 (^1H at 500 MHz, ^{13}C at 125.8 MHz) or a Varian Unity 400 (^1H at 400 MHz, ^{13}C at 100.5 MHz) instrument. Additionally, ^{13}C spectra (75.4 MHz) were recorded on a Varian Mercury instrument plus spectrometer. Measurements were made at ambient temperature (unless otherwise stated) using the residual solvent signal as internal reference. Infrared spectra were obtained from a Perkin-Elmer 1760 IR FT spectrometer. CD spectra were measured with a Jasco J-810 spectropolarimeter (c = 0.1 mmol, 25 °C). UV spectra were recorded on a Varian Cary 3 Bio spectrophotometer. Flash Chromatography was carried out on silica gel (35–70 μm) from Matrex. Analytical TLC was performed on silica gel plates (Merck silica gel 60 F_{254}). The compounds containing a glycoside were visualized by dipping in a solution of 5% H_2SO_4 in ethanol followed by heating, and amine-containing compounds by dipping in ninhydrin in butanol solution followed by heating. Optical rotation was measured on a Perkin-Elmer 241 Polarimeter.

Solid-phase peptide synthesis of (3a–d) and (4). Capping of the unreacted amino groups by acetylation was performed after coupling the first amino acid to the resin. This was made by treatment with a mixture of Ac_2O in CH_2Cl_2 (1 : 1 ratio) for 3 h.

After loading of the first amino acid to the resin, one cycle is as follows: Fmoc deprotection with 2% piperidine and 2% DBU in DMF and then treatment for 5–24 h (β^3-amino acid do not racemize) with activated β^3-amino acid using the mixture of HBTU, HOBT, and DIPEA in DMF. Completeness was controlled by the TNBS test [36].

A milder Fmoc cleaving mixture composed of 20% DIPEA in DMF was, however, used after coupling of the glycosylated amino acid, owing to the base sensitivity of the glycoside *O*-acetyl groups.

The synthesis cycle was repeated until the desired length of the β-peptide was obtained. Between every step of the synthesis, the resin was washed at least five times with DMF to decrease the amount of by-products being formed.

After the final Fmoc deprotection, the peptide was cleaved off from the resin using a standard TFA/H_2O/TIPS mixture (95 : 2.5 : 2.5) followed by removal of the acetyl protecting groups of the carbohydrate moiety by overnight treatment with saturated methanolic ammonia.

Compounds **7**, **12**, and **15** were synthesized as described: **7** [24], **12** [28], **15** [30,31].

N$^\beta$-(Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-β^3-hserine (1a)

Procedure 1. The benzyl ester (**9a**) (0.360 g, 0.473 mmol) was dissolved in 12 ml MeOH/H_2O (15 : 1, v/v). In an atmosphere of argon, 10% Pt/C was added. The reaction mixture was left stirring under H_2 atmosphere at 1 atm. for 5 h and then filtered over Celite. Celite was thoroughly rinsed with MeOH. Flash chromatography (EtOAc/1% AcOH) gave (**1a**) (0.290 g, 92%) as a white solid. m.p. 52–55 °C.

Procedure 2. The glycosylated L-serine (**14**) (133 mg, 0.20 mmol) was dissolved in 3 ml dry THF under argon atmosphere, and cooled to −20 °C. After addition of isobutyl chloroformate (IBCF) (23 μl, 0.21 mmol) and NMM (28 μl, 0.21 mmol), the reaction mixture was kept stirred at −20 °C

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for 20 min. The resulting suspension was allowed to warm up to −10 °C, and a freshly distilled solution of CH_2N_2 in Et_2O was added (Warning. Diazomethane is explosive and a potent mutagen. Handle with care!). The reaction mixture was left to attain room temperature without stirring. After 5 h the excess CH_2N_2 was destroyed by the slow addition of a few drops of AcOH. The mixture was diluted with CH_2Cl_2 and washed with sat. $NaHCO_3$ and 1 M HCl. The pH of the $NaHCO_3$ phase was adjusted to 1–2 and extracted once again with CH_2Cl_2. The combined organic phase was dried with Na_2SO_4, and concentrated to give a yellow oil. Without any further purification, the crude diazoketone oil was dissolved in 10.0 ml THF/H_2O solvent mixture and silver benzoate (5 mg, ∼0.1 equiv.) dissolved in NMM (0.06 ml, 2.80 equiv.) was added. The reaction mixture was kept in an ultrasonic bath for 1 h. THF was removed under reduced pressure and the reaction mixture was then diluted with 1 M HCl and adjusted to pH1–2. Extraction was made with CH_2Cl_2 and the organic phase was then dried over Na_2SO_4. The product was concentrated and purified by RP-HPLC. Lyophilization gave (**1a**) as a white low-melting solid in 82% yield over two steps.

$[\alpha]_D^{30} = +49.0$ ($c = 0.5$, chloroform); TLC (EtOAc/1% AcOH): $R_f = 0.23$; IR: 3349 (m), 3065 (w), 3040 (w), 3020 (w), 2954 (w), 1750 (s), 1664 (m), 1536 (m).

^{1}H NMR (400 MHz, CD_3OD): δ 7.79 (dd, $J = 7.7$ Hz, 2H), 7.65 (dd, $J = 5.6$, 7.7 Hz, 2H), 7.42 (tt, $J = 1.5$, 7.5 Hz, 2H), 7.32 (dt, $J = 1.5$, 7.5 Hz, 2H), 5.40 (d, $J = 1.3$, 3.5 Hz, 1H, H-1), 5.16 (dd, $J = 3.5$, 11.6 Hz, 1H, H-2), 4.88 (d, $J = 3.3$ Hz, 1H, H-4), 4.43 (dd, $J = 3.3$, 11.6 Hz, 1H) 4.40–4.37 (mult, 2H), 4.24–4.16 (mult, 3H), 4.04–3.98 (mult, 2H), 3.73 (dd, $J = 4.6$, 9.4 Hz, 1H, O-CH_2), 3.42 (dd, $J = 5.0$, 9.4 Hz, 1H, O-CH_2), 2.60 (dd, $J = 6.8$, 15.6 Hz, 1H, α-CH_2), 2.55 (dd, $J = 15.6$, 7.3 Hz, 1H, α-CH_2), 2.13 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.90 (s, 3H).

^{13}C NMR (100 MHz, CD_3OD): 173.6 (*C*O), 173.6 (*C*O), 172.1 (*C*O), 172.1 (*C*O), 171.2 (*C*O), 145.3 (*C*, Ar), 142.6 (*C*, Ar), 128.8 (*C*H, Ar), 128.2 (*C*H, Ar), 126.2 (*C*H, Ar), 126.1 (*C*H, Ar), 121.0, 99.3, 70.7, 69.7, 68.7, 68.0, 67.8, 63.1, 37.1, 22.6 (CH_3), 20.7 (CH_3), 20.5 (CH_3).

N^{β}-(Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-β^3-hserine (1b)

The benzyl ester (**9b**) (0.163 g, 0.214 mmol) was converted to (**1b**) (0.92 g, 64%, white foamy glass), analogously as for (**1a**).

^{1}H NMR (400 MHz, CD_3OD): δ 7.78 (dd, $J = 7.8$ Hz, 2H), 7.64 (dd, $J = 7.8$ Hz, 2H), 7.41–7.28 (mult, 4H), 5.33 (d, $J = 3.2$ Hz, 1H), 5.08 (dd, $J = 3.4$, 11.2 Hz, 1H), 4.59 (d, $J = 8.4$ Hz, 1H), 4.37 (dd, $J = 7.7$, 10.6 Hz, 1H), 4.28 (dd, $J = 7.3$, 10.6 Hz, 1H), 4.23–4.07 (mult, 5H), 4.01–3.97 (mult, 1H), 3.86 (dd, $J = 4.9$, 9.4 Hz, 1H), 3.62 (dd, $J = 5.7$, 9.4 Hz, 1H), 2.59 (dd, $J = 4.4$, 15.3 Hz, 1H), 2.49 (dd, $J = 6.9$, 15.3, Hz, 1H), 2.11 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H).

^{13}C NMR (100 MHz, CD_3OD): 175.2 (*C*O), 171.1 (*C*O), 171.0 (*C*O), 170.6 (*C*O), 144.2 (*C*, Ar), 141.4 (*C*, Ar), 128.8 (*C*H, Ar), 128.1 (*C*H, Ar), 127.6 (*C*H, Ar), 127.0 (*C*H, Ar), 125.1, 119.9 (*C*H), 101.6 (*C*H-1), 70.9 (Fmoc-CH_2), 70.7 (*C*H-3), 67.0 (*C*H-4), 66.6 (*C*H), 61.6 (CH_2), 50.4 (CH_2), 21.8 (CH_3), 20.3 (CH_3), 19.5 (CH_3), 19.4 (CH_3).

N^{β}-(Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-β^3-hserine (2)

Procedure 1. Glycoamino acid (**2**) was synthesized from (**11**) (0.122 g, 0.160 mmol) according to the same procedures as for (**1a**).

Flash chromatography [CH_2Cl_2 and 5 to 10% MeOH (gradient)] gave (**2**) (0.86 g, 80.0%) as a white solid. m.p. 194–199 °C.

Procedure 2. The glycosylated L-serine (**17**) (126 mg, 0.19 mmol) was dissolved in 3 ml dry THF under argon atmosphere, and cooled to −20 °C. After addition of IBCF (22 µl, 0.20 mmol) and NMM (26 µl, 0.20 mmol), the reaction mixture was stirred at −20 °C for 20 min. The resulting suspension was allowed to warm up to −10 °C, and a freshly distilled solution of CH_2N_2 in Et_2O was added (Warning. Diazomethane is explosive and a potent mutagen. Handle with care!). The reaction mixture was left to attain room temperature without stirring. After 5 h, the excess CH_2N_2 was destroyed by slow addition of a few drops of AcOH. The mixture was diluted with CH_2Cl_2 and washed with sat. $NaHCO_3$ and 1 M HCl. The pH of the $NaHCO_3$ phase was adjusted to 1–2 and extracted once again with CH_2Cl_2. The combined organic phase was dried with Na_2SO_4 and concentrated to give a yellow oil. Without any further purification, the crude diazoketone oil was dissolved in 15.0 ml THF/H_2O solvent mixture and silver benzoate (5 mg, ∼0.1 equiv.) dissolved in NMM (0.06 ml, 2.90 equiv.) was added. The reaction mixture was kept in an ultrasonic bath for 1 h. THF was removed under reduced pressure and the reaction mixture was then diluted with 1 M HCl and adjusted to pH1–2. Extraction was made with CH_2Cl_2 and the organic phase was then dried over Na_2SO_4. The product was concentrated and purified by RP-HPLC. Lyophilization gave compound (**2**) as a white, fluffy low-melting solid.

$[\alpha]_D^{31} - 15.0$ ($c = 1$, chloroform); TLC (EtOAc/1% AcOH): $R_f = 0.23$, IR: 3355 (m), 3068 (w), 3033 (w), 3019 (w), 2960 (w), 1748 (s), 1662 (m), 1539 (m).

^{1}H NMR (400 MHz, CD_3OD): δ 7.89 (d, $J = 7.8$ Hz, 2H), 7.67 (dd, $J = 7.8$ Hz, 2H), 7.42 (t, $J = 7.5$ Hz, 2H), 7.37–7.31 (t, $J = 7.5$ Hz, 2H), 5.06 (t, $J = 10.0$ Hz, 1H), 4.83 (t, $J = 10.0$ Hz, 1H), 4.62 (d, $J = 8.8$ Hz, 1H), 4.28–4.25 (mult, 2H), 4.22 (d, $J = 6.9$ Hz, 1H), 4.18 (dd, $J = 5.1$, 12.5 Hz, 1H), 4.00 (dd, $J = 2.3$, 12.5 Hz, 1H), 3.88–3.79 (mult, 2H), 3.75–3.68 (mult, 2H) 3.33 (br t, 2H), 2.28 (dd, $J = 5.7$, 2H), 2.00 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 1.77 (s, 3H).

^{13}C NMR (100 MHz, CD_3OD): 173.6 (*C*O), 172.4 (*C*O), 171.8 (*C*O), 171.3 (*C*O), 145.4 (C, Ar), 145.4 (C, Ar), 142.6 (C, Ar), 128.8 (CH, Ar), 128.2 (CH, Ar), 126.2 (CH, Ar), 126.2 (CH, Ar), 120.9 (CH, Ar), 102.2, 74.3, 73.0, 72.0, 70.2, 67.8, 63.3, 55.4, 38.0, 22.9 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 20.6 (CH_3).

NH_2-(*R*)-β^3hSer(*O*-α-GalNAc)-L-Val-OH (3a)

RP-HPLC purification gave dipeptide (**3a**) in 65% yield. Lyophilization provided the product as a white,, fluffy material. HPLC (C8-column; gradient MeCN 10 to 90%, 15 min) RT: 6.66 min. $[M]^+$(ESI) = 422.1.

^{1}H NMR (500 MHz, $CH_3OH:CD_3OD$ 8/2): δ 8.18 (d, $J =$ 7.6 Hz, 1H, αVal-N*H*), 7.82 (d, $J = 9.0$ Hz, 1H, GalN*H*Ac), 4.34 (mult, 1H, GalNAc-*H*), 4.29 (mult, 1H, αVal-CH), 3.94

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(mult, 2H, GalNAc-*H*, β^3hSer-*H*), 3.83 (mult, 5H, GalNAc-*H*, β^3hSer-*H*), 3.73 (dd, 1H, GalNAc-*H*), 3.53 (dd, $J = 7.3$, 10.3 Hz, 1H, β^3hSer-*H*), 2.74 (d, $J = 6.4$ Hz, 2 H, β^3hSer-α-CH_2), 2.20 (mult, 1H, αVal-C*H*), 2.04 (s, 3H, GalNAc-CH_3), 0.98 (dd, $J = 6.6$ Hz, 6H, αVal-CH_3).

NH_2-(*R*)-β^3hSer(*O*-β-GlcNAc)-L-Val-OH (3b)

RP-HPLC purification gave dipeptide (**3b**) in 98% yield. Lyophilization provided the product as a white, fluffy material. HPLC (C8-column; gradient MeCN 10 to 90%, 15 min) RT: 6.61 min. $[M]^+$(ESI) = 422.1

^{1}H NMR (500 MHz, $CH_3OH : CD_3OD$ 9/1): δ 8.30 (d, $J = 8.6$ Hz, 1H, α-Val-NH), 8.16 (d, $J = 9.1$ Hz, 1H, GlcN*H*Ac), 4.40 (d, $J = 8.2$ Hz, 1H, GlcNHAc), 4.34 (dd, $J = 5.9$, 8.2 Hz, 1H, α-Val-C*H*), 3.95–3.88 (mult, 3H, β^3hSer-CH_2, GlcNAc-*H*), 3.78 (dd, $J = 3.6$, 10.9 Hz, 1H, β^3hSer-α-CH_2), 3.74–3.66 (mult, 5H, GlcNAc-*H*, β^3hSer-*H*), 2.71 (dd, $J = 5.2$, 16.0 Hz, 1 H, β^3hSer-α-CH_2), 2.64 (dd, $J = 7.8$, 16.0 Hz, 1 H, β^3hSer-α-CH_2), 2.23–2.15 (sextet, $J = 6.8$ Hz, 1H, α-Val-C*H*), 2.01 (s, 3H, GlcNAc-CH_3), 0.99 (d, $J = 4.2$ Hz, 3H, α-Val-CH_3), 0.97 (dd, $J = 6.8$ Hz, 3H, α-Val-CH_3).

NH_2-β^3hSer(*O*-α-GalNAc)-(*R*)-β^3-hVal-OH (3c)

RP-HPLC purification gave dipeptide (**3c**) in 67% yield. Lyophilization provided the product as a white, fluffy material. HPLC (C8-column; gradient MeCN 2 to 50%, 15 min) RT: 4.81 min. $[M]^+$(ESI) = 436.4.

^{1}H NMR (500 MHz, $CH_3OH : CD_3OD$ 9/1): δ 8.05 (d, $J = 9.4$ Hz, 1H, β^3hVal-N*H*), 7.68 (d, $J = 9.1$ Hz, 1H, GalN*H*Ac), 4.37–4.31 (mult, 1H, GalNAc-*H*), 4.16–4.10 (mult, 1H, β^3hVal-CH), 3.92 (d, $J = 3.9$, 1H, β^3hSer-*H*), 3.90 (mult, 1H, GalNAc-*H*), 3.82–3.76 (mult, 6H, GalNAc-*H*, β^3hSer-*H*), 3.71 (dd, $J = 3.3$, 10.0 Hz, 1H, GalNAc-*H*), 2.66 (dd, $J = 5.1$, 16.2 Hz, 1 H, β^3hSer-α-CH_2), 2.61–2.52 (mult, 2H, β^3hSer-α-CH_2, β^3hVal-CH_2), 2.37 (dd, $J = 9.8$, 15.5 Hz, 1H, β^3hVal-CH_2), 2.02 (s, 3H, GalNAc-CH_3), 1.85 (sextet, $J = 6.6$ Hz,1H, β^3hVal-C*H*), 0.94 (d, $J = 6.6$ Hz, 3H, β^3hVal-CH_3), 0.92 (d, $J = 4.4$ Hz, 3H, β^3hVal-CH_3).

NH_2-β^3hSer(*O*-β-GlcNAc)-(*R*)-β^3-hVal-OH (3d)

RP-HPLC purification gave dipeptide (**3d**) in 76% yield. Lyophilization provided the product as a white, fluffy material. HPLC (C8-column; gradient MeCN 2 to 50%, 15 min) RT: 4.94 min.$[M]^+$(ESI) = 436.6.

^{1}H NMR (500 MHz, $CH_3OH : CD_3OD$ 8/2): δ 8.14 (d, $J = 9.5$ Hz, 1H, β^3hVal-N*H*), 8.06 (d, $J = 8.7$ Hz, 1H, GlcN*H*Ac), 4.39 (d, $J = 8.5$ Hz, 1H, GlcNAc-*H*), 4.12 (mult, 1H, β^3hVal-CH), 3.94 (dd, $J = 1.6$,11.5 Hz, 1H, GlcNAc-*H*), 3.88 (dd, $J = 8.5$, 10.9 Hz, 2H, β^3hSer-*H*), 3.76 (dd, $J = 3.6$, 10.9 Hz, 1H, β^3hSer-*H*), 3.72–3.67 (mult, 5H, GlcNAc-*H*, β^3hSer-*H*), 2.61 (dd, $J = 5.2$, 16.1 Hz, 1 H, β^3hSer-α-CH_2), 2.57–2.50 (mult, 2H, β^3hSer-α-CH_2, β^3hVal-CH_2), 2.38 (dd, $J = 9.2$, 15.4 Hz, 1H, β^3hVal-CH_2), 2.01 (s, 3H, GlcNAc-CH_3), 1.82 (sextet, $J = 6.6$ Hz, 1H, β^3hVal-C*H*), 0.93 (d, $J = 4.4$ Hz, 3H, β^3hVal-CH_3), 0.92 (d, $J = 6.6$ Hz, 3H, β^3hVal-CH_3).

(*R*)-N^β-(Fluoren-9-ylmethoxycarbonyl)-butyric acid benzyl ester (6)

Compound (**6**) was synthesized according to the procedure described in Ref. 22 starting with 2.52 g (5.66 mmol) of starting material (**5**). Purification by flash chromatography (EtOAc/pentane, 4 : 6) followed by recrystallization in EtOAc/hexane gave (**6**) (1.96 g, 80%) as white, fluffy crystals.

m.p. 92–98 °C and $[\alpha]_D$ [30] −1° ($c = 0.5$, $CHCl_3$); TLC (EtOAc/Pentane 1 : 1): $R_f = 0.31$, IR: 3367 (m), 3065 (w), 3040 (w), 3009 (w), 2960 (w), 2940 (w), 2886 (w), 1735 (s), 1687 (s), 1534 (s).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (d, $J = 7.7$ Hz, 2H), 7.58 (d, $J = 7.7$ Hz, 2H), 7.40 (dd, $J = 7.7$ Hz, 2H), 7.37–7.28 (mult, 7H), 5.49 (d, $J = 7.7$ Hz, 1H, N*H*), 5.13 (s, 2H, Ph-CH_2), 4.39 (d, $J = 6.5$ Hz, 2H, Fmoc-CH_2), 4.20 (dd, $J = 6.5$ Hz, 1H, Fmoc-C*H*), 4.08 (br s, β-C*H*), 3.72 (mult, 1H, CH_2OH), 2.70 (d, $J = 4.6$,2H, α-CH_2), 2.41 (br s, O*H*).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6 (*C*O-OBn), 156.2 (Fmoc-*C*O), 143.8 (*C*, Ar), 141.3 (*C*, Ar), 135.5 (*C*, Ar), 128.6 (*C*H, Ar), 128.4 (*C*H, Ar), 128.3 (*C*H, Ar), 127.7 (*C*H, Ar), 127.0 (*C*H, Ar), 125.0 (*C*H, Ar), 120.0 (*C*H, Ar), 66.8, 66.7, 64.2, 49.7, 47.2, 35.9.

N^β-(Fluoren-9-ylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-β^3-hserine benzyl ester (8a)

With exclusion of moisture, the Fmoc-β^3hSer (**6**) (0.86 g, 1.99 mmol) and the azidobromide (**7**) (1.18 g, 2.99 mmol) were dissolved in 100 ml of CH_2Cl_2/THF (1 : 1) and mixed with 4 Å powdered molecular sieve (2.00 g). The reaction mixture was stirred at room temperature for 1 h and then cooled to −40 °C. AgOTf (0.917 g, 3.57 mmol) was diluted in toluene and added to the reaction mixture. After 5 h the reaction stopped, and a small amount of pyridine was added to neutralize the reaction. The mixture was allowed to attain r.t. and the THF was evaporated off. The reaction mixture was diluted with 100 ml Et_2O and filtered through Celite. The organic phase was washed with H_2O (1 × 100 ml) and brine (1 × 100 ml), and dried over Na_2SO_4. Concentration gave a crude mixture of (**8a**) and (**8b**). Purification by flash chromatography (EtOAc/pentane 3 : 7 to 4 : 6 to 1 : 1 (gradient)) gave the pure α-form (**8a**) (0.494 g, 36%) as a white solid.

m.p. 52–55 °C and $[\alpha]_D^{30} = +89.6$ ($c = 0.5$, chloroform); TLC (EtOAc/pentane 4 : 6): $R_f = 0.36$, IR: 3352 (m), 3066 (w), 3031 (w), 2953 (m), 2110 (s), 1733 (s).

^{1}H NMR (500 MHz, DMSO): δ 7.78 (d, $J = 7.5$ Hz, 2H), 7.7 (mult, 3H), 7.41 (dd, $J = 7.5$, 12.9 Hz, 2H), 7.37–7.28 (mult, 6H), 7.19 (d, $J = 7.5$ Hz, 1H, N*H*), 5.42 (dd, $J = 3.1$, 11.4 Hz, 1H, H-2), 5.31 (d, $J = 3.1$ Hz, 1H, H-1), 5.10 (s, 2H, Ph- CH_2), 5.07 (d, $J = 3.5$ Hz, 1H, H-4), 4.24 (mult, 4H, H-5, H-6, Fmoc-CH), 4.13 (mult, 1H, β-H), 3.96 (dd, $J = 7.5$, 11.2 Hz, 1H, Fmoc-CH_2), 3.81 (dd, $J = 5.0$, 11.2 Hz, 1H, Fmoc-CH_2), 3.67 (dd, $J = 3.5$, 11.4 Hz, 1H, H-3), 3.63 (dd, $J = 4.9$, 9.6 Hz, 1H, *O*-CH_2), 3.51 (dd, $J = 4.9$, 9.6 Hz, 1H, *O*-CH_2), 2.71 (dd, $J = 5.4$, 15.4 Hz, 1H, α-CH_2), 2.65 (dd, $J = 9.0$, 15.4 Hz, 1H, α-CH_2), 2.10 (s, 3H), 2.00 (s, 3H), 1.86 (s, 3H).

^{13}C NMR (75 MHz, DMSO): 170.6 (*C*O), 170.0 (*C*O), 169.7 (*C*O), 169.4 (*C*O), 155.5 (*C*, Ar), 143.8 (*C*, Ar), 140.7 (*C*, Ar), 136.0 (*C*, Ar), 128.4 (*C*H, Ar), 128.0 (*C*H, Ar), 127.8 (*C*H, Ar), 127.6 (*C*H, Ar), 127.0 (*C*H, Ar), 125.2 (*C*H, Ar), 125.1 (*C*H, Ar),

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120.1 (*C*H, Ar), 97.2, 68.9, 67.6, 67.4, 66.2, 65.6, 65.5, 61.5, 57.0 (*C*H), 46.6 , 35.9, 20.4 (*C*H_3), 20.4 (*C*H_3), 20.3 (*C*H_3).

N$^{\beta}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl)-β^3-hserine benzyl ester (8b)

The β-anomer (**8b**) was formed as a by-product (29%) in the synthesis of (**8a**).

m.p. 52–60 °C, TLC (EtOAc/pentane 4 : 6): $R_f = 0.33$, IR: 3354 (m), 3060 (w), 3035 (w), 2948 (m), 2105 (s), 1738 (s).

^{1}H NMR (400 MHz, CD_3OD): δ 7.79 (d, $J = 7.5$ Hz, 2H), *7.63 (*dd, $J = 3.2$, 7.5 Hz, 2H), 7.39–7.25 (mult, 9H), 5.28 (d, $J = 4.3$ Hz, 1H), 5.11 (s, 2H, Ph-CH_2), 4.86 (dd, $J = 4.0$, 11.0 Hz, 1H), 4.48 (d, $J = 8.7$, 1H, H-1) 4.34–3.93 (mult, 8H), 3.89 (dd, $J = 5.2$, 10.3 Hz, 1H), 3.70–3.64 (mult, 1H), 2.77 (dd, $J = 5.4$, 15.9 Hz, 1H), 2.61 (dd, $J = 8.4$, 15.9 Hz, 1H), 2.09 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H).

^{13}C NMR (100 MHz, CD_3OD): 172.5 (CO), 172.1 (CO), 171.9 (CO), 171.3 (CO), 158.1 (C, Ar), 145.4 (C, Ar), 142.7 (C, Ar), 137.5 (C, Ar), 129.6 (CH, Ar), 129.3 (CH, Ar), 129.2 (CH, Ar), 128.8 (CH, Ar), 128.2 (CH, Ar), 126.3 (CH, Ar), 120.9 (CH, Ar), 103.3 (*C*H-1), 72.6, 72.0, 71.9, 68.0, 67.5, 62.5, 62.5, 37.2, 20.6 (CH_3), 20.6 (CH_3), 20.5 (CH_3).

N$^{\beta}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-β^3-hserine benzyl ester (9a)

Glycoamino acid (**9a**) was synthesized from (**8a**) (0.522 g, 0.701 mmol) according to the procedures in Ref. 26. Flash chromatography (EtOAc/pentane 1 : 1) gave the pure product (**9a**) (0.447 g, 84%) as a white solid.

m.p. 58–60 °C and $[\alpha]_D^{30} = +53.4$ ($c = 0.5$, chloroform); TLC (EtOAc): $R_f = 0.28$; IR: 3346 (m), 3070 (w), 3035 (w), 2953 (w), 1738 (s), 1666 (m), 1528 (m).

^{1}H NMR (500 MHz, DMSO): δ 7.89 (dd, $J = 8.4$, 7.7 Hz, 3H), 7.66 (d, $J = 7.5$ Hz, 2H), 7.45 (d, $J = 8.4$ Hz, 1H), 7.41 (dd, $J = 6.7$ Hz, 2H), 7.37–7.28 (mult, 6H), 5.31 (d, $J = 3.0$ Hz, 1H, H-1), 5.11–5.06 (mult, 3H), 4.80 (d, $J = 3.3$ Hz, 1H), 4.35–4.30 (mult, 2H), 4.24–4.17 (mult, 3H), 4.08 (mult, 1H) 3.96 (d, $J = 7.2$ Hz, 2H), 3.52 (dd, $J = 5.9$, 10.0 Hz, 1H), 3.41 (dd, $J = 4.3$, 10.0 Hz, 1H), 2.75 (dd, $J = 4.9$, 16.0 Hz, 1H, H-6), 2.59 (dd, $J = 9.4$, 16.0 Hz, 1H), 2.10 (s, 3H), 1.91 (s, 3H), 1.90 (s, 3H), 1.78 (s, 3H).

^{13}C NMR (75 MHz, DMSO): 170.6 (*C*O), 170.0 (*C*O), 169.8 (*C*O), 169.8 (*C*O), 169.6 (*C*O), 155.5 (*C*O), 143.8 (*C*, Ar), 143.8 (*C*, Ar), 140.7 (*C*, Ar), 136.0 (*C*, Ar), 128.9 (*C*H, Ar), 128.4 (*C*H, Ar), 128.0 (*C*H, Ar), 127.9 (*C*H, Ar), 127.6 (*C*H, Ar), 127.3 (*C*H, Ar), 127.1 (*C*H, Ar), 125.0 (*C*H, Ar), 120.1 (*C*H, Ar), 120.0 (*C*H, Ar), 97.0, 68.9, 67.5, 67.1, 66.1, 65.6, 65.4, 61.6, 47.1, 46.8, 35.9, 22.4 (CH_3), 20.6 (CH_3), 20.4 (CH_3), 20.4 (CH_3).

N$^{\beta}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-β^3-hserine benzyl ester (9b)

Compound (**9b**) was synthesized according to the same procedure as for (**9a**), starting from (**8b**) (0.284 g, 0.381 mmol). Flash chromatography (EtOAc/pentane 1 : 1) afforded (**9b**) (0.210 g, 0.276 mmol) in 74% yield as a white foamy glass.

^{1}H NMR (500 MHz, acetone-d_6): δ 7.83 (d, $J = 7.5$ Hz, 2H), 7.69–7.65 (mult, 2H), 7.41–7.26 (mult, 9H), 5.33 (dd, $J = 0.7$, 3.4 Hz, 1H), 5.11 (s, 2H), 5.10 (dd, $J = 11.4$, 3.4 Hz, 1H), 4.68 (d, $J = 8.4$ Hz, 1H), 4.36 (dd, $J = 7.1$, 10.4 Hz, 1H), 4.28 (dd, $J = 7.0$, 10.4 Hz, 1H), 4.25–4.18 (mult, 2H), 4.15–4.02 (mult, 4H), 3.91 (dd, $J = 5.2$, 10.4 Hz, 1H), 3.64 (dd, $J = 6.7$, 10.4 Hz, 1H), 2.76 (dd, $J = 5.3$, 15.5 Hz, 1H), 2.66 (dd, $J = 8.5$, 15.5 Hz, 1H, H-6), 2.10 (s, 3H), 1.97 (s, 3H), 1.92 (s, 3H), 1.86 (s, 3H).

^{13}C NMR (75 MHz, acetone-d_6): δ 171.4 (*C*O), 171.0 (*C*O), 170.4 (*C*O), 170.3 (*C*O), 170.1 (*C*O), 156.3 (*C*O), 144.4 (*C*, Ar), 144.4 (*C*, Ar), 141.4 (*C*, Ar), 136.6 (*C*, Ar), 128.6 (*C*H, Ar), 128.3 (*C*H, Ar), 128.2 (*C*H, Ar), 128.1 (*C*H, Ar), 127.8 (*C*H, Ar), 127.3 (*C*H, Ar), 125.4 (*C*H, Ar), 120.1 (*C*H, Ar), 101.8, 70.8, 70.6, 67.0, 66.4, 61.6, 50.4, 47.3, 36.1, 22.3 (CH_3), 19.8 (CH_3), 9.8 (CH_3).

N$^{\beta}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-β^3-hserine benzyl ester (11)

With exclusion of moisture, the Fmoc-β^3-homoserine (**6**) (0.307 g, 0.711 mmol) and 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl chloride (**10**) (0.325 g, 0.889 mmol, from Aldrich) were dissolved in 11 ml of CH_2Cl_2 and mixed with 4 Å molecular sieve (1.00 g). The reaction mixture was stirred at room temperature for 20 min before AgOTf (0.294 g, 0.114 mmol) was added, and the reaction mixture was refluxed at 40 °C overnight. The reaction was neutralized by a small amount of triethylamine, and then diluted with CH_2Cl_2. The organic phase was filtered, washed with ice-cold saturated $NaHCO_3$ and water, dried with Na_2SO_4, and concentrated to give a brown solid. Purification by flash column chromatography (EtOAc/pentane 7 : 3) gave (**11**) as a white foamy glass (0.138 g, 35.0%).

TLC (EtOAc): $R_f = 0.28$, IR: 3352 (m), 3068 (w), 3030 (w), 2952 (w), 1742 (s), 1665 (m), 1522 (m).

^{1}H NMR (500 MHz, DMSO): δ 7.91 (d, $J = 9.2$ Hz, 1H, N*H*), 7.88 (d, $J = 7.5$ Hz, 2H), 7.66 (mult, 2H), 7.40 (t, $J = 7.5$, 2H), 7.34–7.29 (mult, 7H), 7.27 (d, $J = 8.6$ Hz, 1H, N*H*), 5.11–5.02 (mult, 3H), 4.82 (t, $J = 9.9$ Hz, 1H), 4.61 (d, $J = 8.9$ Hz, 1H, H-1), 4.30–4.25 (mult, 2H), 4.22–4.13 (mult, 2H), 4.03–3.94 (mult, 2H), 3.81 (ddd, $J = 2.4$, 4.5, 10.1 Hz, 1H), 3.74–3.65 (mult, 2H), 3.35–3.30 (signal hidden behind solvent peak, 2H), 2.61 (dd, $J = 4.2$, 15.8 Hz, 1H), 1.98 (s, 3H), 1.96 (s, 3H), 1.90 (s, 3H), 1.72 (s, 3H).

^{13}C NMR (75 MHz, DMSO): 171.3 (*C*O-OBn), 170.8 (*C*O), 170.4 (*C*O), 170.0 (*C*=O), 169.9 (*C*O), 156.1 (Fmoc-*C*O), 144.5 (*C*, Ar), 141.5 (*C*, Ar), 136.8 (*C*, Ar), 129.1 (*C*, Ar), 128.6 (*C*H, Ar), 128.5 (*C*H, Ar), 128.3 (*C*H, Ar), 127.8 (*C*H, Ar), 125.8 (*C*H, Ar), 120.9 (*C*H, Ar), 101.3, 99.3, 73.4, 73.2, 71.5, 69.2, 66.3, 62.5, 53.8, 47.4, 23.3 (CH_3), 21.2 (CH_3), 21.1 (CH_3), 21.0 CH_3).

N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-L-serine (14)

Compound (**13**) was synthesized according to the same procedure as for (**8a**), starting from (**12**) (0.720 g, 1.62 mmol) and (**7**) (0.700 g, 1.78 mmol). Purification by flash chromatography (EtOAc/pentane 3 : 7 to 4 : 6 to 1 : 1 (gradient)) gave the pure α-form (**13**) (0.266 g, 22%) as a yellow oil.

A mixture of compound (**13**) (160 mg, 0.211 mmol), activated Zn (450 mg, 20 equiv.) and 2 ml sat. $CuSO_4$ in Ac_2O/HOAc 2 : 1 (20 ml) was stirred at room temperature. After 4 h, H_2O (10 ml) and Zn (450 mg, 20 equiv.) were added and stirred for another 13 h. The reaction mixture was filtered through Celite and co-evaporated with toluene. Purification of the crude by flash chromatography (dichloromethane–methanol–acetic acid 97.5 : 2.5 : 0.5, then 95 : 4:1) afforded (**14**) in 59% yield. Spectral data were in agreement with those reported earlier [37].

N^{β}-(Fluoren-9-ylmethoxycarbonyl)-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl-amino-β-D-glucopyranosyl)-L-serine (16)

Glycoamino acid (**16**) was synthesized from (**15**) (3.00 g, 5.74 mmol) and Fmoc-Ser-OH (2.25 g, 6.89 mmol) according to the procedure described in Ref. 32. Flash chromatography (dichloromethane–methanol–acetic acid 97.5 : 2.5 : 0.5) gave the pure product (**16**) (2.10 g, 46%) as a colorless oil. Spectral data were in agreement with those reported earlier [32].

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-2-β-glucopyranosyl)-L-threonine (17)

Compound (**17**) was synthesized from (**15**) ((2.10 g 2.66 mmol) according to the procedure described in Ref. 32. RP-HPLC purification gave (**17**) in 64% yield. Lyophilization provided the product as a white, fluffy material. Spectral data were in agreement with those reported earlier [38].

(2-Methyl-5-oxo-tetrahydro-furan-3-yl)-carbamic acid 9H-fluoren-9-yl methylester (20)

m.p. 169–173 °C; TLC : (EtOAc/pentane 4 : 6) : $R_f = 0.30$ IR: 3384 (m), 3043 (w), 3017 (w), 2985 (w), 2956 (w), 1774 (s), 1727 (s), 1529 (s) 1450 (m).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (d, $J = 7.7$ Hz, 2H), 7.58 (d, $J = 7.7$ Hz, 2H), 7.40 (m, 2H), 7.30 (ddd, $J = 0.9$, 7.7 Hz, 2H), 5.53 (d, $J = 8.4$ Hz, 1H, *NH*), 4.73–4.66 (mult, 1H, C*H*-CH_3), 4.56–4.48 (m, 2H, Fmoc-CH_2), 4.39 (dd, $J = 10.3$, 6.9 Hz, 1H, H-3), 4.19 (t, $J = 6.9$ Hz, 1H, Fmoc-*CH*), 2.91 (dd, $J = 17.7$, 7.6, 1H, H-4), 2.46 (d, $J = 17.7$,1H, H-4), 1.30 (d, $J = 6.6$ Hz CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 175.4 (lactone-*C*O), 156.2 (Fmoc-*C*O), 143.9 (*C*, Ar), 141.6 (*C*, Ar), 128.1 (*C*H, Ar), 127.3 (*C*H, Ar), 125.3 (*C*H, Ar), 120.3 (*C*H, Ar), 80.2, 67.1, 51.2, 47.5, 36.6, 14.7 (CH_3).

CONCLUSION

We have shown that the two novel glycosylated β^3-amino acids (**1a**) and (**2**) can be synthesized according to two different strategies: either by utilizing the carbohydrate moiety as 'protecting group' upon Arndt–Eistert homologation, or by linking the carbohydrate moieties to a suitably protected β^3hSer derivative, synthesized from D-aspartic acid. Considering both the hazard associated with diazomethane use and the low overall yield of the former strategy, the latter should be preferred.

Attempted syntheses of the glycosylated β^3hThr analogues of compounds (**1a**) and (**2**) by linkage of the carbohydrate moieties to β^3hThr were unfortunately not successful because of lactone formation during acidic deprotection of a β^3hThr derivative. This could, however, possibly be avoided by the use of the homologation strategy.

The glycosylated β^3-amino acid building blocks were then used in solid-phase synthesis of two mixed α/β-dipeptides, two β-dipeptides, and two β-heptapeptides. The two 7-residues long glycosylated β^3-peptides (**4a**) and (**4b**) were shown to fold into stable 3_{14}-helical secondary structure in methanol. In water (PBC buffer, pH 7), however, peptide (**4b**) kept only 15% of its overall mean 3_{14}-helical population, whereas glycosylated β^3-peptide (**4a**) retained as much as 45% of the helical intensity observed in methanol (based on CD spectral intensity at 215 nm). A dramatic effect on the stereochemistry of the carbohydrate linkage (i.e. α *vs* β) on the aqueous helical stability is thus observed for these β-peptides.

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