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Conformational Effects of the Non-natural α-Methylserine on Small Peptides and Glycopeptides

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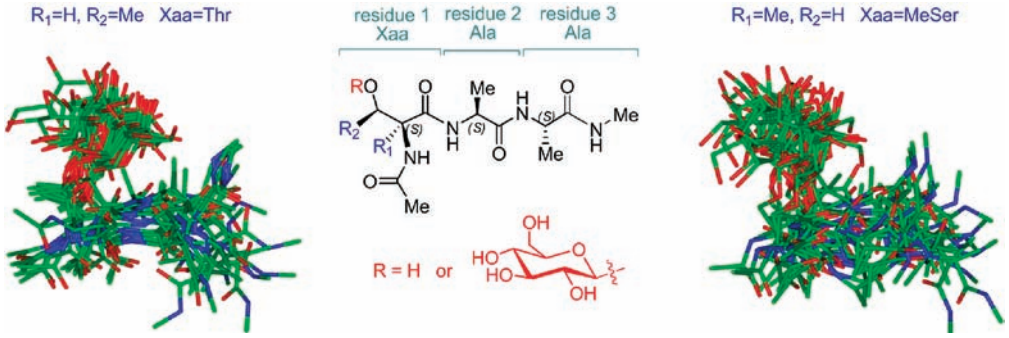

The synthesis and the conformational analysis in aqueous solution of a peptide and a glycopeptide containing the sequence threonine-alanine-alanine (Thr-Ala-Ala) are reported. Furthermore, the threonine residue has been replaced by the quaternary amino acid α-methylserine (MeSer) and their corresponding non-natural peptide and glycopeptide are also studied. The conformational analysis in aqueous solution combines NOEs and coupling constants data with Molecular Dynamics (MD) simulations with time-averaged restraints. The study reveals that the *β*-*O*-glycosylation produces a remarkable and completely different effect on the backbone of the peptide derived from Thr and MeSer. In the former, the *β*-*O*-glycosylation is responsible for the experimentally observed shift from extended conformations (peptide) to folded ones (glycopeptide). In contrast, the *β*-*O*-glycosylation of the MeSer-containing peptide, which clearly shows two main conformations in aqueous solution [extended ones (70%) and *β*-turn (30%)], causes a high degree of flexibility for the backbone.

Introduction

The synthesis of nonproteinogenic α-amino acids has received enormous attention because it represents a great advance in the design of modified peptides with altered biological activity. Particular attention has been paid to α, α-disubstituted α-amino acids;[1] for example, α-methylserine (α-MeSer) can be regarded as a potential α-helix-stabilizing building block.[2] By contrast, the field of modified glycopeptides remains relatively unexplored, and most of the modifications studied are centered around the carbohydrate moiety.[3] On this basis, and considering that various biologically active *O*-glycoproteins, such as epidermal growth factor (EGF) domains of different serum proteins[4] and Notch receptor,[5] incorporate the *β*-*O*-glucosylserine (*β*Glc-Ser) substructure, we have recently synthesized and studied the simplest non-natural *β*-*O*-glycosylated amino acids as well as a small peptide and glycopeptide containing a non-natural residue.[6] The studies showed that these non-natural molecules can stabilize conformations present in naturally occurring molecules or exhibit some atypical conformations.

On the other hand, the recent improvements in the synthetic methodology make it possible to gain access to novel synthetic glycopeptides that can be useful to study the structural influence of protein glycosylation. One of the main influences that glycosylation exerts is the stabilization of protein conformations, which is of pivotal importance for the biological activity of glycopeptides. On this basis, we have selected a glycosylated tripeptide (Thr*-Xaa-Xaa) and its analogue containing the noncoded α-amino acid MeSer (MeSer*-Xaa-Xaa) to unravel the role of carbohydrate

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attachment in the stabilization of peptide conformations. In previous works,[6] commented on above, we have studied this feature in the simplest model glycopeptides: amino acid diamides Ac-Thr-NHMe and Ac-MeSer-NHMe glycosylated with β-*O*-Glc. In the present paper, we want to demonstrate if the characteristics observed in these short models are kept when the peptide is bigger. To this aim, we have selected natural amino acids to add to the β-hydroxy-α-amino acid. Taking into account that some amino acids (Pro, Phe, etc.) can exert a structural role in the conformations of the backbone, we have decided to use an amino acid that does not cause these conformational effects. Moreover, the selected amino acid must be present around glycosylation sites in proteins.[7] In this context, alanine (Ala) appears as a good candidate for this purpose, therefore it was selected as the amino acid to increase the size of the peptidic chain (Xaa = Ala).

Herein, we have synthesized and studied the peptide and the corresponding β-*O*-glycopeptide derived from the sequence MeSer-Ala-Ala, where MeSer is the non-natural amino acid α-methylserine (Figure 1). In addition, the analogues molecules derived from the natural sequence Thr-Ala-Ala are also studied for a comparative purpose. Interestingly, this small peptide is the repeating tandem sequence found in antifreeze glycoproteins, which allow polar fish to survive in seas where the temperature is subzero.[8] In addition, this substructure

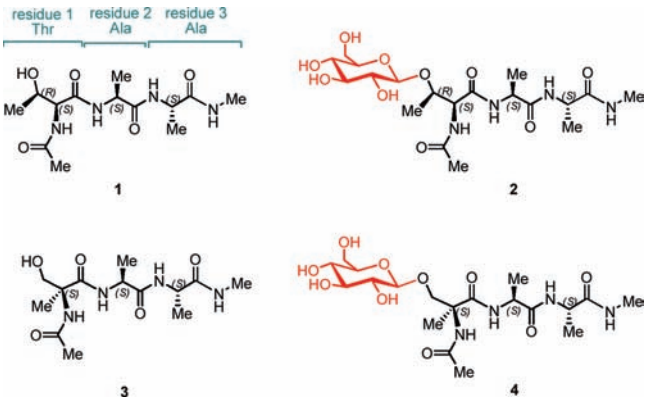

FIGURE 1. Compounds studied in this work.

appears to be important for the activity of a family of antimicrobial peptides called *Alyteserin*.[9] In fact, the derivatives that incorporate the Thr-Ala-Ala sequence display more activity against gram-positive bacteria than their analogue without this sequence.

Results and Discussion

Synthesis. The synthesis of peptide **1** was carried out as described in the Supporting Information. The corresponding glycopeptide **2** was obtained following the modified conditions of the Koenigs−Knorr glycosylation.[10] The treatment of peptide **1** with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide in the presence of AgTfO led to derivative **5** with a moderate yield. The hydroxyl groups of this compound were deprotected with MeONa to give glycopeptide **2** (Scheme 1). It is important to notice that these basic conditions did not produce racemization in the final compound **2**, since no other product was observed in the NMR spectra.

Concerning the non-natural peptide and glycopeptide, the synthesis of peptide **3** was started from dipeptide **6**, which was previously synthesized by our group.[6b] This compound was first deprotected with trifluoroacetic acid (TFA) to give the amine **7**. MeSer derivative **8**, also obtained in our group,[11] was then treated with **7**, and *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(benzotriazol-1-yl)uranium tetrafluoroborate (TBTU) as a coupling agent in the presence of diisopropylethylamine (DIEA) as a base to give the *N*-Boc protected peptide **9**, which was purified by column chromatography on silica gel. Deprotection of the terminal amino group and further acetylation with acetic anhydride (Ac_2O) and pyridine (Py) followed by subsequent hydrolysis of the acetyl group with MeONa gave the desired peptide **3** (Scheme 2).

Glycopeptide **4** was synthesized from non-natural amino acid **10**, previously obtained in our group, whose carboxylic acid group was protected as allyl ester (a selectively removable carboxy-protecting function), and the hydroxyl group as silyl ether (TBDMS) to give compound **11**. Further acetylation of the amino group (Ac_2O/Py) and subsequent deprotection of the hydroxyl group led to derivative **12** in a moderate yield. The key step in the synthesis of glycopeptide

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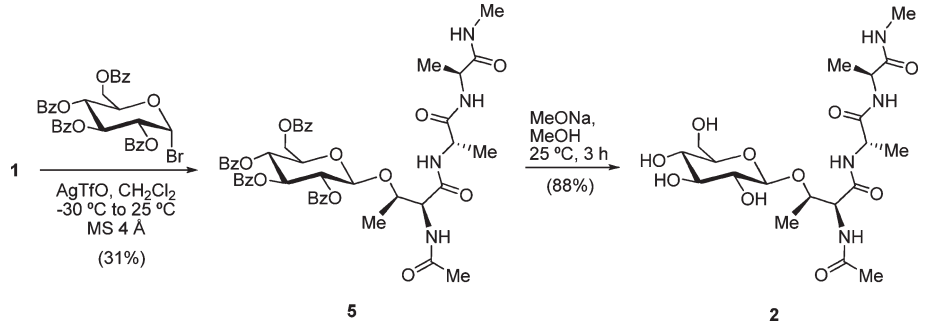
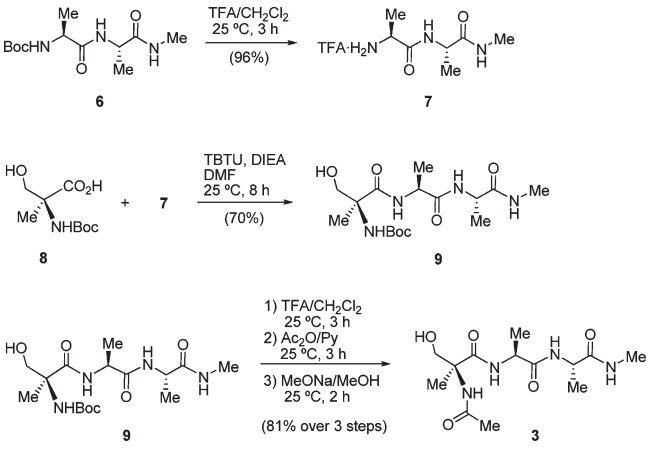
SCHEME 1. Synthesis of Glycopeptide Diamide 2



SCHEME 2. Synthesis of Peptide Diamide 3



4 was the glycosylation of MeSer derivative **12** following the modified conditions of the Koenigs−Knorr glycosylation[10] with the same glycosyl donor as commented on above, to give building block **13** in 50% yield. Selective removal of the allyl group in compound **13** was carried out with Pd^0-catalyzed allyl transfer to morpholine. Under these conditions, the deprotection proceeded without affecting other labile groups.[12] Further coupling of the acid group with the amine group of dipeptide **7** led to intermediate **14**. Finally, the hydrolysis of the benzoyl groups with sodium methoxide in methanol gave the desired glycopeptide **4** derived from MeSer (Scheme 3). In this case, racemization was not observed.

Conformational Analysis of the Desired Peptides and Glycopeptides. The conformational analysis in aqueous solution of all the compounds was carried out combining NMR data with molecular dynamics simulations with time-averaged restraints (MD-tar), following a protocol[13] identical with that recently applied by our group on different glycopeptides.

In the first step, full assignment of the protons of peptides **1** and **3**, as well as the corresponding glycopeptides **2** and **4**, was carried out with standard COSY and HSQC experiments (see the Supporting Information). Then, selective 1D-NOESY experiments in D_2O (25 °C, pH 4.8) and 2D-NOESY experiments in H_2O/D_2O (9/1) (25 °C, pH 4.8) were carried out for all the compounds.

The torsional angles and the atom labels used herein for the compounds are shown in Figure 2.

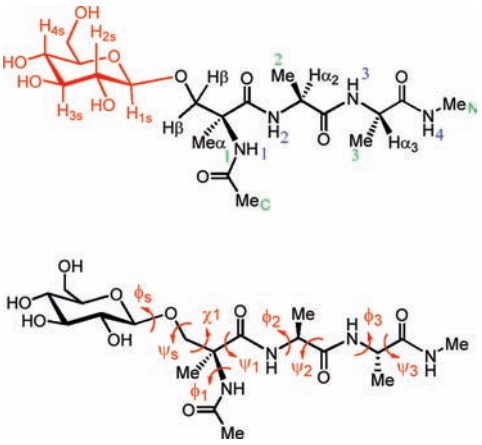


FIGURE 2. Molecular structure of glycopeptide **4**, showing the atom labels and the most relevant torsional angles. The letter "s" makes reference to the sugar moiety. The blue and green numbers refer to the numeration of the NH and methyl groups, respectively. The same atom labels were used for the rest of the molecules.

Conformational Analysis of Thr-Containing Derivatives. Figure 3a shows the amide region of the 2D NOESY spectra of glycopeptide **2**. The presence of consecutive NH−NH cross peaks suggests the existence of an important population of helix-like conformation. On the contrary, despite the overlapping of signals in the spectrum of the parent peptide, it seems clear that there is an absence of these NOEs, indicating that the backbone adopts almost exclusively extended conformations. This fact, along with the slight upfield shift of the NH protons of the glycosylated residues (CSD),[14] corroborates that β-*O*-glycosylation of peptides derived from natural amino acids promotes folded conformations for the peptide backbone within the glycopeptide. This finding confirms our previous work,[15] where β-*O*-glycosylation was found to be responsible for the experimental shift from extended conformations (model peptides derived from Ser and Thr) toward the folded ones (corresponding model glycopeptides).

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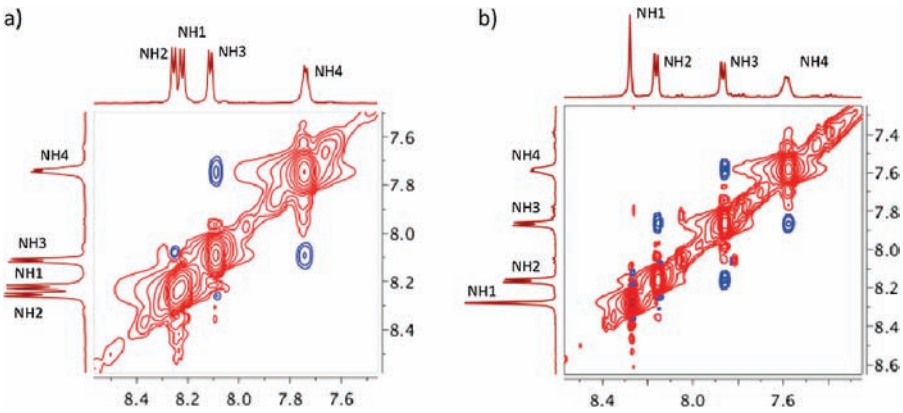
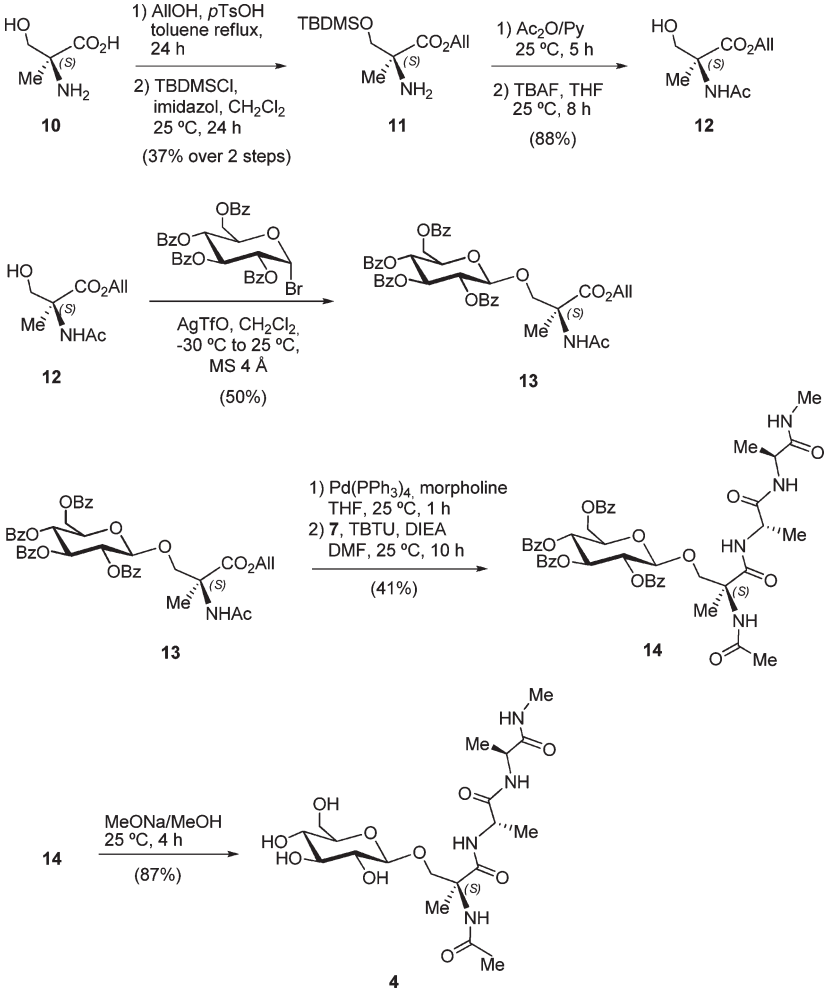


FIGURE 3. Amide region of the 800 ms 2D NOESY spectrum (400 MHz) in H_2O/D_2O (9:1) at 25 °C of glycopeptides **2** (a) and **4** (b).

SCHEME 3. Synthesis of Glycopeptide Diamide 4



The next step was to experimentally determine some proton−proton distances relevant to the conformational analysis. They were obtained from the corresponding NOE build-up curves[16] (see the Supporting Information). Additionally, distances involving NH protons were semiquantitatively determined by integrating the volume of the corresponding cross-peaks in the 2D NOESY spectrum. On the other hand, $^3J_{NH,H\alpha}$ coupling constants were measured from the splitting of the resonance signals in the 1D spectrum. All these experimentally determined distances, as well as the measured $^3J_{NH,H\alpha}$, were used as restraints in MD-tar simulations[17] with the aim of obtaining a distribution of

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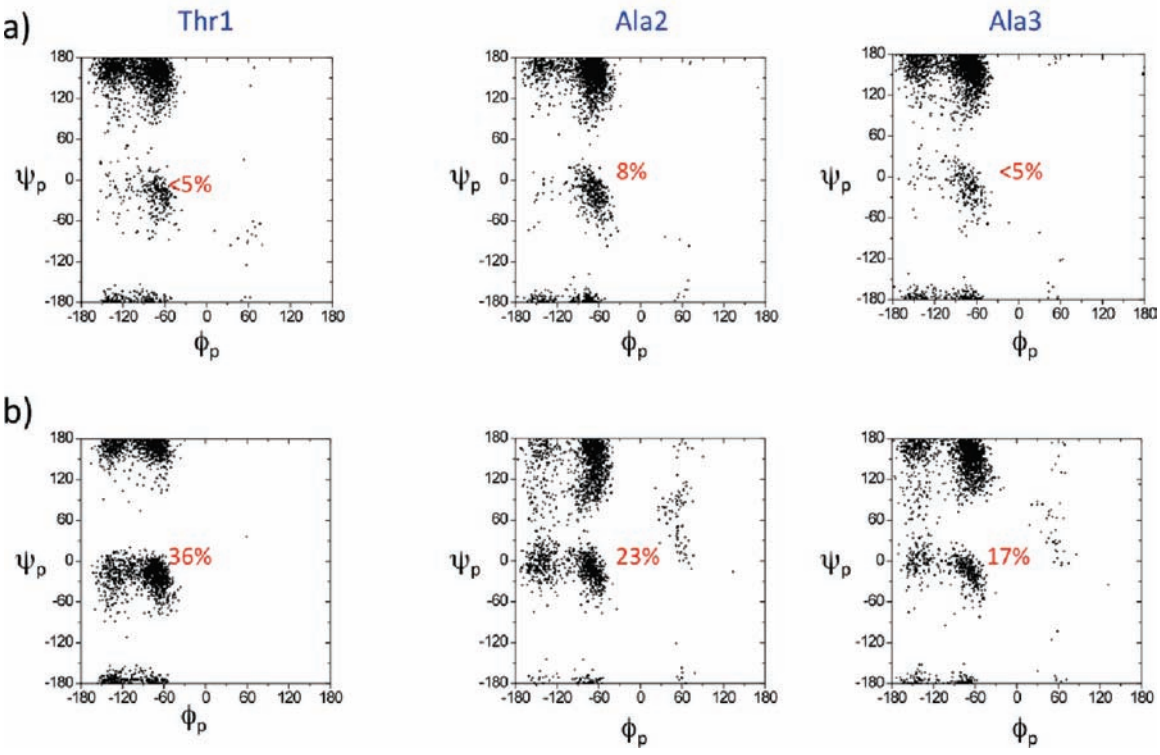

FIGURE 4. ϕ_p/ψ_p (p = peptide) distributions obtained from the MD-tar for compounds **1** (a) and **2** (b).

TABLE 1. Comparison of the Experimental and MD-tar Simulations Derived Distances and ^3J Coupling Constants for Compound 2

	exptl[a]	MD-tar (ε = 80)
$d_{NH1,NH2}$	2.9	3.0
$d_{NH2,NH3}$	2.9	3.0
$d_{NH3,NH4}$	2.9	2.9
$d_{NH4,H\alpha3}$	2.2	2.3
$d_{NH3,H\alpha3}$	2.5	2.6
$d_{NH3,H\alpha2}$	2.2	2.1
$d_{NH2,H\alpha2}$	2.5	2.4
$d_{NH1,H\alpha1}$	>2.7	2.8
$d_{H1s,H\beta}$	2.8	2.8
$^3J_{H\alpha1,H\beta1}$	4.3	3.8
$^3J_{NH1,H\alpha1}$	6.9	6.5
$^3J_{NH2,H\alpha2}$	5.9	5.4
$^3J_{NH3,H\alpha3}$	5.5	5.7

[a]Distances are given in Å and coupling constants in Hz.

conformers in aqueous solution able to reproduce quantitatively the experimental NMR data. All these data, and those theoretically obtained from the simulations, are shown in Table 1 for glycopeptide **2**.

The ϕ_i/ψ_i (i = 1, 2, or 3) distribution obtained from the MD-tar simulations for peptide **1** and glycopeptide **2** is shown in Figure 4. As can be seen, peptide **1** presents ϕ_i/ψ_i typical values of extended conformations for each residue, in accordance with the NOEs commented on above. On the other hand, this result is in agreement with those observed by other groups for small peptides, for which polyproline II (PPII) conformation seems to be the predominant one.[18] Additionally, this finding is similar to that previously found for the Cbz-Thr-Ala-Ala-CO_2Me derivative in DMSO.[19]

In contrast, for glycopeptide **2**, the three amino acids show a significant population corresponding to helix-like conformations (close to 36% for Thr and around 20% for the Ala residues). This result is in accordance with the NMR data and also with our previous results obtained for small peptides.

With regard to the glycosidic linkage (Figure 5a), the conformational preferences of the glycopeptide **2** are very similar to those observed for the Thr model glycopeptide previously studied by our group.[15] The ϕ_s dihedral angle was found to be rather rigid, exhibiting values close to −60°, in agreement with the exoanomeric effect.[20] The most significant feature is that ψ_s takes values around 120−140°, resulting in an eclipsed conformation for the Hβ−Cβ and O1−C1 bonds. This conformer avoids the steric repulsion between the methyl group at Cβ and the anomeric H1 proton, which would be present when this ψ_s angle is close to 180°. At this point, it has to be mentioned that the eclipsed conformation has been previously reported by our group for different model glycopeptides derived from Thr[6a,21] but this is the first time that we confirm this finding on a larger glycopeptide.

As far as the lateral chain is concerned, in glycopeptide **2**, the χ^1 torsional angle exhibits mainly the $g(+)$ conformation with only 10% of the *anti* conformer (Figure 5c). This result

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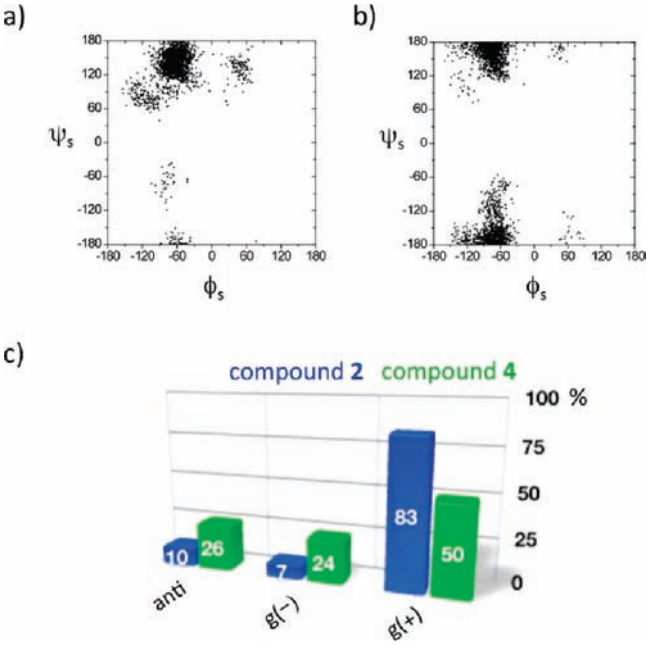

FIGURE 5. (a) ϕ_s/ψ_s (s = sugar) distribution obtained from the MD-tar simulations for compound **2**. (b) ϕ_s/ψ_s distribution obtained from the MD-tar simulations for compound **4**. (c) χ^1 distribution obtained from the MD-tar simulations for compounds **2** and **4**.

is in agreement with the small value experimentally observed for $^3J_{H\alpha,H\beta}$ (less than 4 Hz). Finally, Figure 6 shows the superimposition of 20 conformers randomly taken from the MD-tar simulations that quantitatively reproduce the experimental NMR data. As can be seen, peptide **1** (Figure 6a) presents a rather rigid structure with a rmsd value of 1.32 Å and adopts clearly an extended conformation. On the contrary, in the case of the corresponding glycopeptide, compound **2** (Figure 6b), a larger degree of flexibility was observed with a higher rmsd (close to 1.61 Å), showing mainly a folded conformation.

Conformational Analysis of MeSer-Containing Derivatives. The protocol described for peptide **1** and glycopeptide **2** was also used to quantitatively determine the conformers present in aqueous solution for their non-natural analogues derived from MeSer. Nevertheless, it is worth noting that not only was the synthesis of these non-natural target compounds difficult, but also the structural analysis was complicated. The absence of the Hα atom in the quaternary amino acid (MeSer) as well as the overlapping of the signals in the NMR spectrum corresponding to the Hα atoms in both alanine residues implicates the lack of some NOE interaction signals and coupling constants, which are important in eliciting the conformations.

Regarding peptide **3**, the medium NH2−NH3 and NH3−NH4 NOEs suggest the presence of a significant population of the helix-like conformations (Supporting Information). In this sense, the ϕ_p/ψ_p distribution obtained from the MD-tar simulations for this compound is shown in Figure 7a. The ϕ_1/ψ_1 dihedral angle values exhibited by the MeSer residue were typical for helix-like conformations, such as the right- and left-handed α-helix (α_D, 65%; α_L, 27%), in agreement with the NOE experiments. However,

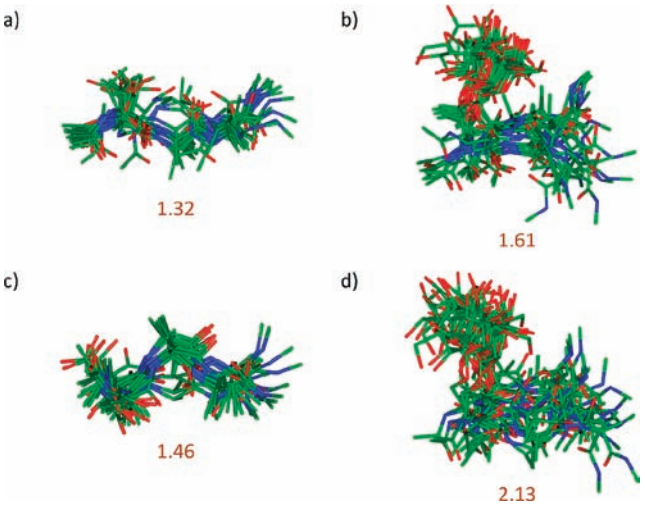

FIGURE 6. Calculated ensembles obtained from the MD-tar simulations for peptide **1** (a), glycopeptide **2** (b), peptide **3** (c), and glycopeptide **4** (d), showing the RMSDs for heavy atoms superimposition.

extended conformations (β-sheet and PPII) were the most populated ones for both Ala residues.

As a consequence, and according to the MD simulations, MeSer-containing peptide **3** is rather flexible in aqueous solution, showing the coexistence of extended conformations (70%) and folded ones (30%) (see the Supporting Information). However, it is important to note the exceptionally high population of this folded conformation, represented by a type I β-turn, for a small peptide (Figure 8).

With regard to the lateral chain (see the Supporting Information), peptide **3** exhibits a flexible behavior for the χ^1 angle, and the $g(+)$ conformation is the most populated one (χ^1 angle close to 60°).

Concerning glycopeptide **4**, the medium-strong Meα1−NH1 NOE (typical of folded conformations), along with the also observed Meα1−NH2 NOE (characteristic of extended ones), suggests the coexistence of both conformations in aqueous solution for the MeSer residue (see the Supporting Information). On the other hand, the presence of the NH2−NH3 and NH3−NH4 NOEs suggests the existence of a certain population of folded conformations for the two alanine residues, as already occurred for its parent peptide (Figure 3b). Moreover, the observation of the NH1−Hβ and NH2−Hβ NOE interactions seems to indicate some flexibility for the conformational preferences of the χ^1 torsional angle for glycopeptide **4**.

As shown in Figure 7b, the MeSer residue adopts mainly helix-like conformations (60%) with preference for the α_D helix, similar to that observed for the nonglycosylated compound. Regarding the two residues of alanine, they exhibited dispersed values of ϕ_i/ψ_i. While for Ala2 the helix-like conformations were the most populated ones (about 53% of the total trajectory time), extended conformations (β-sheet and PPII) were predominant for Ala3. Therefore, the conformational analysis of the backbone indicates that, in general, both folded conformations and extended ones coexist for the three residues, as suggested by the NOE experiments. As a consequence, the β-turn observed for peptide **3** is present only 18% of the total trajectory time for compound **4**. Moreover, an additional β-turn was also

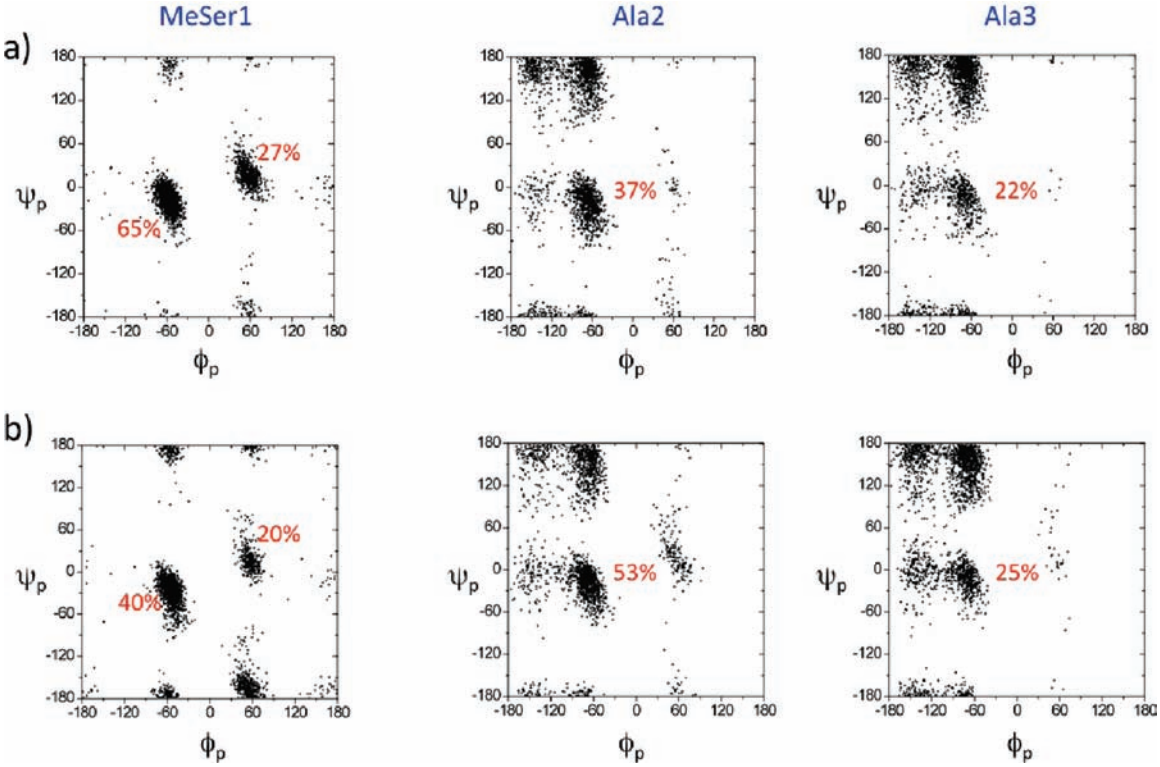

FIGURE 7. (a) ϕ_p/ψ_p distributions (backbone) obtained from the MD-tar simulations for peptide **3**. (b) ϕ_p/ψ_p distributions (backbone) obtained from the MD-tar simulations for glycopeptide **4**.

found (13% of the total time), in which the intramolecular hydrogen bond is formed between $i + 1$ and $i + 4$ residues (C2═O2···H4−N4, see the Supporting Information). These results are in agreement with the observed Me_C−NH3 and Meα1−NH4 NOEs, respectively, for each turn.

Concerning the glycosidic linkage (Figure 5b), the values of the ϕ_s dihedral angle are mainly determined by the exoanomeric effect. On the other hand, the ψ_s angle showed well-defined values around 180° (typical of alternate conformations). It is important to note that this is a completely different behavior to that observed for the Thr-containing glycopeptide **2**. This result demonstrates that the distinct substitution of the methyl group at the Cα or Cβ position (MeSer and Thr, respectively) drastically affects the geometry of the glycosidic linkage also in larger glycopeptides.

Finally, as shown in Figure 5c, it is possible to see that the lateral chain of the non-natural glycopeptide is rather flexible, showing a significant population of each staggered conformation for the χ^1 torsional angle. This fact is in accordance with the NH1−Hβ and NH2−Hβ NOE interactions commented on above.

Therefore, from the exhaustive conformational study of the MeSer-containing molecules, it can be concluded that in this case, the β-*O*-glycosylation is responsible for the conformational flexibility within the glycopeptide, which exhibits the highest root-mean-square deviation (rmsd) value (2.13 Å), regarding not only the peptide backbone but also the lateral chain (Figure 6d).

Conclusions

Herein we report the synthesis and the conformational analysis of a peptide and glycopeptide derived from the sequence Thr-Ala-Ala as well as of their non-natural analogues from α-MeSer. Some important conclusions can be drawn from this study. Comparing both peptides **1** and **3**, it is apparent that the incorporation of a methyl group at the Cα position of the amino acid induces folded conformations for the peptide backbone. This result confirms our recent work on small model peptides. However, in spite of the structural rigidity conferred by the quaternary amino acid (α-MeSer), neither peptide **3** nor glycopeptide **4** are conformationally rigid. Indeed, both molecules are rather flexible in aqueous solution. Concerning the glycosylated compounds, the study reveals that the β-*O*-glycosylation produces a remarkable and completely different effect on the peptide backbone of the diamide tripeptide derived from Thr and MeSer (compare panels b and d of Figure 6). In the former, the β-*O*-glycosylation is responsible for the experimentally observed shift from extended conformations (peptide) to folded ones (glycopeptide). This feature supports the tendency observed in our previous study on model peptides derived from Ser and Thr. However, the β-*O*-glycosylation of the non-natural peptide with MeSer does not seem to promote that shift toward helix-like conformations. This result is in good agreement with our recent study on non-natural model glycopeptides, indicating that the methyl group at the α-position, and not the β-*O*-glycosylation, is responsible for the backbone con formations in these non-natural compounds. In fact, for the diamide MeSer-Ala-Ala tripeptide, which clearly shows two main conformations in aqueous solution [extended ones (70%) and β-turn (30%)], the β-*O*-glycosylation causes a large degree of flexibility for the peptide backbone.

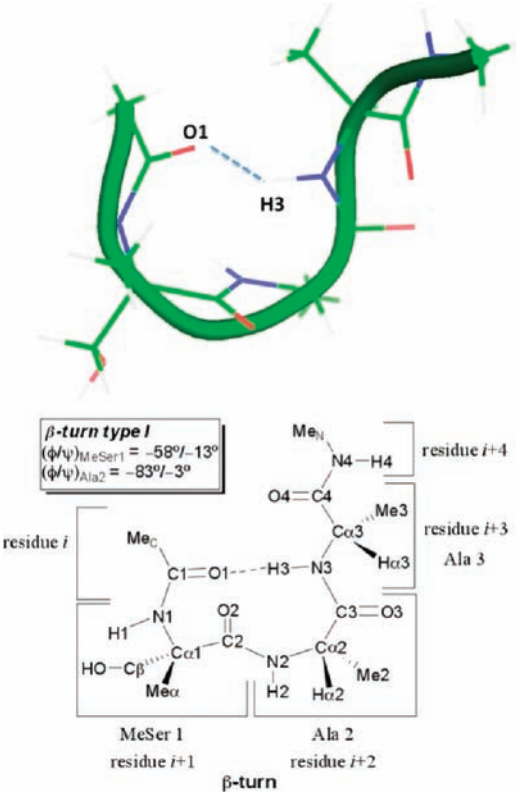

FIGURE 8. Scheme of the type I β-turn obtained from the MD-tar simulations in aqueous solution for peptide **3**. The β-turns are formed when the polypeptide chain adopts a folded structure involving four amino acid residues, with the carbonyl oxygen of the first residue (*i*) forming a hydrogen bond with the amino group hydrogen of the fourth ($i + 3$). Type I β-turn shows characteristic values for the dihedral angles ϕ/ψ of $-60°$, $-30°$ (ϕ_{i+1}/ψ_{i+1}) and $-90°$, $0°$ (ϕ_{i+2}/ψ_{i+2}).

Experimental Section

Synthesis of Compound 5. Silver triflate (60 mg, 0.25 mmol) was added to a suspension of **1** (35 mg, 0.15 mmol) and powdered molecular sieves (4 Å, 20 mg) in CH_2Cl_2 (3 mL), under an inert atmosphere. The mixture was stirred at -30 °C and then 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (570 mg, 0.86 mmol) in CH_2Cl_2 (3 mL) was added. The mixture was stirred at this temperature for 1 h and then was warmed to 25 °C and stirred for an additional 14 h. The crude was filtered, concentrated, and purified by silica gel column chromatography, eluting with CH_2Cl_2/MeOH (95:5), to give **5** as a colorless oil (31 mg, 31%), which was used without further characterization in the next step. Anal. Calcd for $C_{47}H_{50}N_4O_{14}$: C, 63.08; H, 5.63; N, 6.26. Found: C, 63.14; H, 5.60; N, 6.30.

Synthesis of Compound 2. A solution of **5** (30 mg, 0.03 mmol) in MeOH (5 mL) was treated with MeONa/MeOH (0.5M) to pH 9. After being stirred for 3 h at 25 °C, the mixture was neutralized with Dowex 50-X8, filtered, and concentrated. Purification of the residue with a C18 reverse-phase sep-pak cartridge gave 14 mg of **2**, as a colorless oil in 88% yield. ^{1}H NMR (400 MHz, D_2O): δ 1.27 (d, $J = 6.3$ Hz, 3H), 1.35−1.43 (m,6H), 2.10 (s, 3H), 2.74 (s, 3H), 3.22−3.30 (m, 1H), 3.35−3.42 (m, 1H), 3.43−3.53 (m, 2H), 3.73 (dd, $J_1 = 12.3$ Hz, $J_2 = 5.9$ Hz, 1H), 3.92 (dd, $J_1 = 12.2$ Hz, $J_2 = 1.8$ Hz, 1H), 4.24 (q, $J = 7.2$ Hz, 1H), 4.31−4.39 (m, 2H), 4.41 (d, $J = 4.6$ Hz, 1H), 4.55 (d, $J = 7.9$ Hz, 1H). ^{13}C NMR (100 MHz, D_2O): δ 15.6, 16.4, 16.5, 21.7, 25.8, 49.7, 49.8, 58.3, 60.8, 69.7, 73.0, 73.6, 75.6, 75.9, 99.7, 171.6, 174.4, 174.8, 175.2. Anal. Calcd for $C_{19}H_{34}N_4O_{10}$: C, 47.69; H, 7.16; N, 11.71. Found: C, 47.79; H, 7.20; N, 11.65.

Synthesis of Compound 9. TFA (2 mL) was added to a solution of **6** (66 mg, 0.24 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The reaction was maintained at 0 °C for 30 min then at 25 °C for 3 h, and then concentrated to give derivative **7** (0.23 mmol). This compound was then dissolved at 25 °C in DMF (3 mL) and treated with α-methylserine derivative **8** (43 mg, 0.20 mmol), DIEA (0.2 mL, 0.90 mmol), and TBTU (83 mg, 0.26 mmol). The reaction mixture was stirred at 25 °C for 8 h, the solvent was then removed, and the residue was purified by silica gel column chromatography, eluting with dichloromethane/MeOH (15:1) to give compound **9** (105 mg, 70%) as a colorless oil. This compound was used without further characterization in the next step. Anal. Calcd for $C_{16}H_{30}N_4O_6$: C, 51.32; H, 8.08; N, 14.96. Found: C, 51.40; H, 8.05; N, 14.92.

Synthesis of Compound 3. TFA (3 mL) was added to a solution of **9** (100 mg, 0.27 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The reaction was stirred at 0 °C for 30 min then at 25 °C for 2.5 h, and then concentrated. The residue was dissolved in pyridine/acetic anhydride (2:1, 6 mL) and the mixture was stirred at 25 °C for 3 h to give the corresponding *O*,*N*-diacetylated compound, which was used in the next step without purification. A solution of this compound in MeOH (5 mL) was treated with sodium methoxide/MeOH (0.5M) to pH 9. After being stirred at 25 °C for 2 h, the mixture was neutralized with Dowex 50-X8, filtered, and concentrated. Finally, the residue was purified with a C_{18} reverse-phase sep-pak cartridge to give **3** (69 mg), as a colorless oil, in 81% yield. $[\alpha]^{25}_D$ −15.0 (*c* 0.24, CH_3OH). ^{1}H NMR (400 MHz, D_2O): δ 1.36−1.47 (m, 6H), 1.45 (s, 3H), 2.05 (s, 3H), 2.75 (s, 3H), 3.74−3.87 (m, 2H), 4.22−4.34 (m, 2H). ^{1}H NMR (400 MHz, H_2O/D_2O): δ 1.31−1.37 (m, 6H), 1.40 (s, 3H), 2.00 (s, 3H), 2.71 (d, $J = 4.5$ Hz, 3H), 3.69−3.84 (m, 2H), 4.15−4.31 (m, 2H), 7.66 (br s, 1H), 7.98 (d, $J = 6.0$ Hz, 1H), 8.21 (d, $J = 5.6$ Hz, 1H), 8.24 (s, 1H). ^{13}C NMR (100 MHz, D_2O): δ 15.9, 16.4, 19.3, 22.2, 25.8, 49.8, 50.1, 60.3, 64.5, 174.3, 175.1, 175.3, 175.4. Anal. Calcd for $C_{13}H_{24}N_4O_5$: C, 49.36; H, 7.65; N, 17.71. Found: C, 49.44; H, 7.63; N, 17.74.

Synthesis of Compound 11. A mixture of allylic alcohol (1.7 mL, 25.0 mmol), (*S*)-α-methylserine **10** (430 mg, 2.76 mmol), *p*-TsOH (635 mg, 3.34 mmol), and toluene (20 mL) was refluxed for 24 h by using a Dean−Stark trap and then concentrated. The crude salt was suspended in CH_2Cl_2 (15 mL) and imidazol (946 mg, 13.9 mmol) and TBDMSCl (922 mg, 6.12 mmol) were added. The resulting suspension was stirred at 25 °C for 24 h. The solvent was then removed and the mixture was partitioned between NaOH (1 N, 10 mL) and ethyl acetate (10 mL). The organic layer was washed with NaOH (1 N, 3 × 10 mL) and brine (10 mL), dried, filtered, and evaporated. Finally, the residue was purified by silica gel column chromatography (ethyl acetate/hexane, 8:2) to give compound **11** (280 mg, 37%) as a yellow oil. $[\alpha]^{25}_D$ −5.9 (*c* 1.10, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.00−0.11 (m, 6H), 0.87 (s, 9H), 1.25 (s, 3H), 1.90 (s, 1H), 3.48 (d, $J = 9.1$ Hz, 1H) 3.90 (d, $J = 9.1$ Hz, 1H), 4.52−4.69 (m, 2H), 5.14−5.51 (m, 2H), 5.80−6.03 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.8,−5.7,18.0, 22.3, 25.6, 59.5, 65.5, 69.9, 118.0, 131.9, 176.2. Anal. Calcd for $C_{13}H_{27}NO_3Si$: C, 57.10; H, 9.95; N, 5.12. Found: C, 57.22; H, 9.90; N, 5.10.

Synthesis of Compound 12. A solution of **11** (260 mg, 0.95 mmol) in pyridine/acetic anhydride (2:1, 6 mL) was stirred at 25 °C for 5 h and then concentrated. The residue was dissolved in THF (7 mL) and a solution of TBAF 1 M in THF (1.25 mL, 1.25 mmol) was added. The reaction was stirred for 8 h, then the solvent was removed and the mixture partitioned between $CHCl_3$/^{i}PrOH (4:1, 15 mL) and saturated NH_4Cl solution (5 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to give a residue that was purified by silica gel column chromatography, eluting with ethyl acetate/MeOH

(95:5) to give **12** (170 mg, 88%), as a colorless oil. $[\alpha]^{25}_D$ −10.7 (*c* 1.10, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 3H), 1.97 (s, 3H), 3.59 (br s, 1H), 3.76 (d, J = 11.3 Hz, 1H), 4.01 (d, J = 11.5 Hz, 1H), 4.52−4.72 (m, 2H), 5.11−5.41 (m, 2H), 5.73−5.97 (m, 1H), 6.36 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.3, 23.8, 62.3, 66.5, 66.6, 118.9, 131.4, 170.8, 172.9. Anal. Calcd for $C_9H_{15}NO_4$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.61; H, 7.54; N, 7.01.

Synthesis of Compound 13. Silver triflate (149 mg, 0.58 mmol) was added to a suspension of **12** (70 mg, 0.35 mmol) and powdered molecular sieves (4 Å, 20 mg) in CH_2Cl_2 (4 mL), under an inert atmosphere. The mixture was stirred at −30 °C and then 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (323 mg, 0.49 mmol) in CH_2Cl_2 (4 mL) was added. The mixture was stirred at this temperature for 1 h and then was warmed to 25 °C and stirred for an additional 14 h. The crude was filtered, concentrated, and purified by silica gel column chromatography, eluting with ethyl acetate/hexane (1:1), to give **13** as a colorless oil (135 mg, 50%). $[\alpha]^{25}_D$ +3.7 (*c* 1.25, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 1.38 (s, 3H), 1.56 (s, 3H), 4.01−4.08 (m, 1H), 4.11 (d, J = 9.9 Hz, 1H), 4.18 (d, J = 9.9 Hz, 1H), 4.39 (dd, J_1 = 12.2 Hz, J_2 = 4.8 Hz, 1H), 4.48−4.61 (m, 3H), 4.74 (d, J = 7.9 Hz, 1H), 5.12 (d, J = 11.0 Hz, 1H), 5.17−5.28 (m, 1H), 5.40 (dd, J_1 = 9.7 Hz, J_2 = 8.0 Hz, 1H), 5.61 ("t", J = 9.7 Hz, 1H), 5.72−5.87 (m, 2H), 6.16 (br s, 1H), 7.16−7.23 (m, 2H), 7.24−7.30 (m, 2H), 7.31−7.38 (m, 5H), 7.39−7.53 (m, 3H), 7.73−7.79 (m, 2H), 7.80−7.86 (m, 2H), 7.87−7.92 (m, 2H), 7.93−8.01 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.6, 23.4, 60.3, 63.0, 66.5, 69.5, 71.5, 71.8, 72.3, 72.6, 101.5, 118.5, 128.3, 128.4, 128.5, 128.7, 128.8, 129.3, 129.6, 129.8, 129.8, 129.9, 131.5, 133.2, 133.3, 133.4, 133.5, 165.0, 165.2, 165.8, 166.2, 169.7, 171.9. Anal. Calcd for $C_{43}H_{41}NO_{13}$: C, 66.23; H, 5.30; N, 1.80. Found: C, 66.16; H, 5.35; N, 1.77.

Synthesis of Compound 14. A solution of derivative **13** (130 mg, 0.17 mmol) in THF (10 mL) was stirred under an argon atmosphere at 25 °C, then $Pd(PPh_3)_4$ (2 mg, 1.7×10^{-3} mmol) and morpholine (52 μL, 0.60 mmol) were subsequently added. After the mixture was stirred for 2 h, the solvent was evaporated and the residue was taken up in 10 mL of ethyl acetate. The organic layer was washed with 1 N HCl (5 mL), dried, and concentrated to give the desired acid, which was directly used for the next step. A solution of the acid in DMF (5 mL) was treated with DIEA (0.13 mL, 0.80 mmol), amine **7** (63 mg, 0.22 mmol), and TBTU (71 mg, 0.22 mmol). The reaction mixture was stirred at 25 °C for 10 h and then evaporated to give a residue that was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 15:1) to give **14** as a colorless oil (60 mg, 41%). $[\alpha]^{25}_D$ +1.0 (*c* 1.10, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 1.30−1.55 (m, 9H), 1.72 (s, 3H), 2.74 (d, J = 4.8 Hz, 3H), 3.74 (d, J = 9.9 Hz, 1H), 4.00 (d, J = 9.9 Hz, 1H), 4.15−4.31 (m, 2H), 4.39−4.56 (m, 2H), 4.72 (dd, J_1 = 12.0 Hz, J_2 = 2.8 Hz, 1H), 4.94 (d, J = 7.8 Hz, 1H), 5.42−5.55 (m, 1H), 5.72 ("t", J = 9.8 Hz,1H), 6.00 ("t", J = 9.9 Hz, 1H), 6.60 (s, 1H), 6.72 (d, J = 4.5 Hz, 1H), 6.85−6.98 (m, 1H), 7.22−7.62 (m, 13H), 7.78−8.07 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.1, 17.3, 19.2, 23.1, 26.3, 29.7, 49.4, 51.4, 59.8, 62.6, 69.3, 72.1, 72.2, 72.4, 72.5, 101.0, 128.4, 128.5, 128.5, 128.6, 128.7, 128.8, 129.4, 129.7, 129.8, 133.4, 133.5, 133.6, 134.0, 165.2, 165.6, 165.7, 166.2, 171.6, 172.2, 172.7, 173.4. Anal. Calcd for $C_{47}H_{50}N_4O_{14}$: C, 63.08; H, 5.63; N, 6.26. Found: C, 63.18; H, 5.57; N, 6.22.

Synthesis of Compound 4. A solution of **14** (30 mg, 0.03 mmol) in MeOH (5 mL) was treated with MeONa/MeOH (0.5M) to pH 9. After being stirred for 3 h at 25 °C, the mixture was neutralized with Dowex 50-X8, filtered, and concentrated. Purification of the residue with an C18 reverse-phase sep-pak cartridge gave 14 mg of **4**, as a colorless oil in 88% yield. $[\alpha]^{25}_D$ −26.0 (*c* 1.20, H_2O). ^1H NMR (400 MHz, D_2O): δ 1.26−1.33 (m, 6H), 1.40 (s, 3H), 1.95 (s, 3H), 2.65 (s, 3H), 3.18−3.32 (m, 2H), 3.33−3.44 (m, 2H), 3.63 (dd, J_1 = 12.3 Hz, J_2 = 6.1 Hz, 1H), 3.73−3.89 (m, 2H), 4.04 (d, J = 10.3 Hz, 1H), 4.12−4.25 (m, 2H), 4.39 (d, J = 7.9 Hz, 1H). ^{13}C NMR (100 MHz, D_2O): δ 15.9, 16.4, 19.4, 22.3, 25.8, 49.8, 50.2, 59.4, 60.8, 69.7, 71.8, 73.1, 75.6, 76.0, 102.4, 174.3, 174.8, 175.1, 175.3. Anal. Calcd for $C_{19}H_{34}N_4O_{10}$: C, 47.69; H, 7.16; N, 11.71. Found: C, 47.78; H, 7.13; N, 11.78.

2D NMR Experiments. NMR experiments were recorded on a 400 MHz spectrometer at 293 K. Magnitude-mode ge-2D COSY spectra were recorded with gradients and with the cosygpqf pulse program with 90 degree pulse width. Phase-sensitive ge-2D HSQC spectra were recorded with z-filter and selection before t1 removing the decoupling during acquisition by use of the invigpndph pulse program with CNST2 (JHC) = 145. 2D NOESY experiments were made with phase-sensitive ge-2D NOESY for $CDCl_3$ spectra and phase-sensitive ge-2D NOESY with WATERGATE for H_2O/D_2O (9:1) spectra. Selective ge-1D NOESY experiments were carried out with the 1D-DPFGE NOE pulse sequence. NOEs intensities were normalized with respect to the diagonal peak at zero mixing time. Experimental NOEs were fitted to a double exponential function, $f(t) = p_0(e^{-p_1}t)(1 - e^{-p_2}t)$ with p_0, p_1, and p_2 being adjustable parameters.[16] The initial slope was determined from the first derivative at time $t = 0$, $f'(0) = p_0p_2$. From the initial slopes, interproton distances were obtained by employing the isolated spin pair approximation.

MD Simulations: MD-tar Simulations. MD-tar simulations were performed with AMBER[22] 6.0 and AMBER94 force field,[23] which was implemented with GLYCAM 04[24] parameters to accurately simulate the conformational behavior of the carbohydrate moiety. NOE-derived distances were included as time-averaged distance constraints, and scalar coupling constants J as time-averaged coupling constraints. A $\langle r^{-6}\rangle^{-1/6}$ average was used for the distances and a linear average was used for the coupling constants. Final trajectories were run with an exponential decay constant of 8000 ps and a simulation length of 80 ns with a dielectric constant $\varepsilon = 80$.

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Supporting Information Available: ^1H and ^{13}C NMR spectra as well as COSY and HSQC correlations for compounds **1**−**4**, ^1H and ^{13}C NMR spectra for compounds **11**−**14**, 2D NOESY correlations for compounds **1**−**4**, scheme of the second β-turn obtained from the MD-tar simulations in aqueous solution for compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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