

Sugar-Modified Foldamers as Conformationally Defined and Biologically Distinct Glycopeptide Mimics**

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It is predicted that over half of all eukaryotic proteins are glycosylated, and it is now well-established that co- and post-translational modification of proteins with glycans can have dramatic consequences on their folding, stability, and ultimately their function.^[1] Considerable effort has thus been invested in delineating the impact of appended carbohydrates on the conformational preferences of proteins and peptides in solution and vice versa,^[2] and also in understanding their interactions with their cognate receptors.^[3] These endeavours are not straightforward, and success in rationalizing such processes has been possible only in a handful of well-studied cases.^[4] Important insights into such questions have been gleaned from the study of glycoconjugate mimetics, whose interactions with cellular targets can impact a wide range of physiological phenomena, including fertilization, immune response, host-pathogen interactions, cell growth, and tumor metastasis.^[1] However, attempts to successfully correlate biological functions of structurally well-defined glycopeptides with their secondary structures have been relatively sparse,^[2-4] despite the importance of such targets in the quest for carbohydrate-based therapeutics.^[5]

Herein we examine the effects of appended sugar moieties on the conformational behavior of peptide foldamers derived from δ -sugar amino acids (δ -SAAs).^[6] The study of foldamers has in the past helped enlighten our understanding of the

origins of the preferred secondary structures and biological activities of biopolymers.^[7] Considering the endogenous and therapeutic importance of glycoproteins, we were struck by the dearth of reports describing the impact of glycosylation on the secondary structures of peptide foldamers.^[8] Appended sugars in the two families of newly synthesized δ -SAA-derived glycofoldamers indeed play a defining role on the preferred conformations of the peptide foldamer backbones and, far less commonly, are seen to do so even in water.^[9] Furthermore, the differences in conformation manifested by each glycofoldamer are shown to be mirrored in their distinct and contrasting interaction with selected targets including the lectin *Concanavalin A* (ConA)^[10a] and the bacterium *Escherichia coli* (*E. coli*).^[10b]

The families of δ -SAA-derived foldamers targeted for investigation herein, annotated *cis*- and *trans*- in the text, differ from one another in the configuration of the stereo-center at C2 of the furanoid rings of their constituent δ -SAA moieties: those with the “2S” configuration designated *cis*-foldamers, and those with “2R”, *trans*-foldamers (Scheme 1). Previous work has shown that in organic solvents, *cis*-foldamers adopt conformations reminiscent of a conventional β -turn, whereas the secondary structures of *trans*-foldamers are dependent on the substituent pattern of their constituent furanoid rings.^[11] The targeted families of δ -SAA-based

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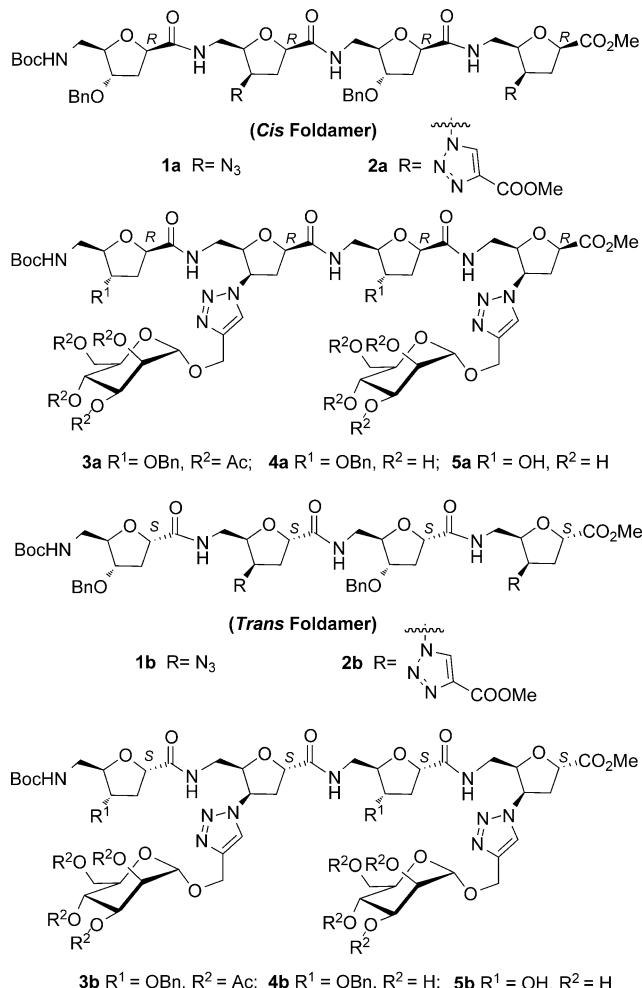
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Scheme 1. Representation of δ -SAA based hybrids.

hybrid peptides, **1a** and **1b**, were assembled following a convergent strategy from the corresponding monomeric δ -SAA precursors **6a** and **7a** (for **1a**) or **6b** and **7b** (for **1b**), respectively. The required monomeric δ -SAA building blocks were conveniently obtained from the same starting sugar, 2-deoxy-D-ribose.^[12]

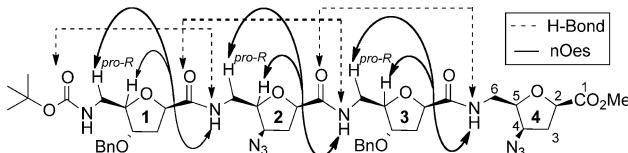
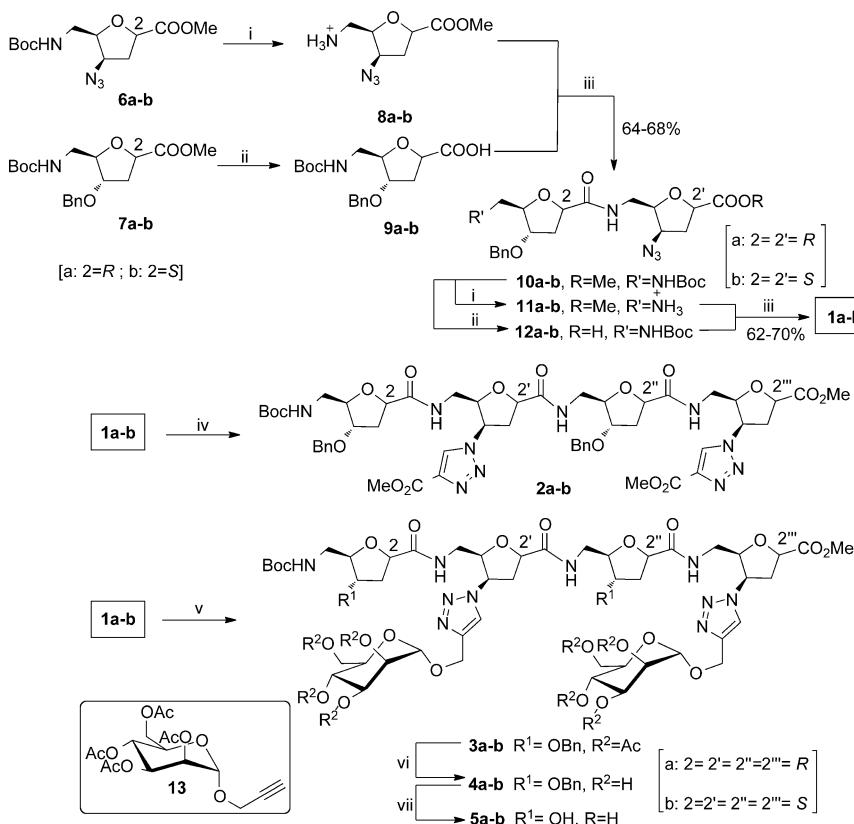
N-Boc deprotection of **6a** and saponification of the methyl ester function of **7a** gave precursors **8a** and **9a**, respectively, which when condensed under standard solution-phase peptide coupling conditions gave smoothly the corresponding *cis*- δ -SAA dimer **10a**. The dimer **10a** could be deprotected at either ends to give the precursors **11a** and **12a** which, when reacted together (standard peptide coupling conditions), gave the target *cis*- δ -SAA based tetramer **1a** (Scheme 2). An identical reaction sequence starting from the *trans*- δ -SAA monomers **6b** and **7b** allowed the straightforward assembly of the corresponding *trans*- δ -SAA derived tetramer **1b** (Scheme 2). It was envisaged that the azido functions, thus integrated, would subsequently allow ready conjugation of selected partners at precise positions along each δ -SAA-derived backbone post-assembly. As expected, reaction of either **1a** or **1b** with either non-sugar or sugar partners armed with propargyl functions proceeded smoothly

by a Cu^I-catalyzed Huisgen click cycloaddition^[13] to give the corresponding conjugates **2a,b** or **3a,b**, respectively (Scheme 2). This click conjugation method has found appeal previously in neoglycopeptide assembly, where the triazole moieties themselves can impact physiochemical properties or biological activities of the resulting glycoconjugates.^[14]

The data derived from NMR spectroscopy were used to establish the conformational preferences of the peptide foldamers, as this technique can provide discrete structural information at the atomic level.^[2-4] The ¹H NMR spectra of foldamers **1a,b** and their clicked analogues **2a,b** in CDCl₃ feature sharp resonances and well-resolved amide proton signals, suggesting the presence of predominantly well-defined folded structures in solution. The downfield chemical shifts observed for the amide protons in the spectra of **1a,b** and **2a,b** further suggest their participation in internal hydrogen bonding (H-bonding), a feature that is also supported by the minimal perturbation of these signals upon titration in 33% of [D₆]DMSO (v/v).^[12] For peptide foldamers **1a** and **2a**, the small changes in chemical shifts ($\Delta\delta$), observed for the NH₂, NH₃, and NH₄ in solvent titration studies, and the invariance of their chemical shifts in variable concentration studies, are also consistent with the participation of these protons in intramolecular H-bonding.^[15] The presence of sequential nOe cross-peaks between ²NH \leftrightarrow ¹C₆H_(pro-R), ¹C₃H_(pro-S), ³NH \leftrightarrow ²C₆H_(pro-R), ²C₃H_(pro-S), ⁴NH \leftrightarrow ³C₆H_(pro-R), and ³C₃H_(pro-S) in the ROESY spectrum of foldamer **1a** (Scheme 3) corroborates the participation of NH₂, NH₃, and NH₄ in H-bonding, consistent with a 10-membered turn network implicating NH(i)-CO(i-2).^[12] Furthermore, the medium-intensity nOes observed between ³NH \leftrightarrow ¹C₃H_(pro-R), ¹C₃H_(pro-S) and ⁴NH \leftrightarrow ²C₃H_(pro-S), ²C₃H_(pro-R) suggest that foldamer **1a** adopts a compact secondary structure. The clicked foldamer analogue **2a** afforded identical data to its precursor **1a**, suggesting that these two compounds share related secondary structures.

The NMR spectra of *cis*-glycofoldamer **3a**, featuring two acetylated mannose units, display similar nOes and H-bonding networks as seen for **1a** when recorded in CDCl₃. However a very weak medium-range nOe between ⁴NH \leftrightarrow ²C₆H_(pro-R) observable in the ROESY spectrum of *cis*-analogue **3a** (Figure 1) indicates the presence of an additional bend around the second H-bonding, which is also suggested by the absence of the nOe between ³NH \leftrightarrow ¹C₃H_(pro-S) present in **1a**. Titration studies carried out by sequential addition of 33% [D₆]DMSO (v/v) to **3a** gives a $\Delta\delta$ value for ³NH that is 0.26 ppm greater than those observed for the 2nd and 4th δ -SAA amide protons and further supports that in **3a** local distortions at ³NH occur. Taken together, these observations suggest that **3a** tends towards a higher-order structure than adopted by foldamer **1a**.

It was critical that the glycofoldamers would be soluble in water once deprotected, so that their biological evaluation might be undertaken. That these glycofoldamers would maintain distinct secondary structures in water was also imperative. Although short linear δ -SAA-based backbones (non-glycosylated) are predisposed towards defined secondary structures in organic solvents,^[9] these are invariably disrupted upon deprotection.^[16] We were nevertheless hope-



ful that the glycofoldamers synthesized herein, once deprotected, would behave similarly to short natural glycopeptides in water, in which attached carbohydrates have been demonstrated to stabilize secondary structures or compact conformations.^[17] Indeed, deacetylation or deacetylation/debenzylation of *cis*-glycofoldamer **3a** afforded the corresponding analogues **4a** or **5a**, respectively, which proved to be soluble in either methanol (**4a**) or water (**5a**).

The NMR data for **4a** in CD_3OH features a sequence of $i \leftrightarrow i+2$ nOes along with those nOes observed in the spectra of **1a** and **3a**. The medium-range nOes between $(i)\text{NH} \leftrightarrow (i-2)\text{C}_6\text{H}_{(\text{pro-}R)}$ and $(i)\text{NH} \leftrightarrow (i-2)\text{C}_3\text{H}_{(\text{pro-}S)}$ (Scheme 4 and Figure 2) identified in the spectrum of **4a** for the 3rd and 4th residues, with the data of solvent exchange studies and minimal temperature dependencies, confirms participation of

these amide protons in H-bonding. The data taken together is consistent with the *cis*-glycofoldamer **4a** existing predominantly in a 16-membered H-bonded turn structure involving $^3\text{NH} \leftrightarrow \text{Boc-}CO$ and $^4\text{NH} \leftrightarrow ^1\text{CO}$.

The deacetylated/debenzylated *cis*-glycofoldamer **5a**, studied in 94% $\text{H}_2\text{O} + 6\%$ D_2O , showed an almost identical nOe pattern and H-bonding network as that observed for **4a**, suggesting similar secondary structures for both. As far as we are aware this is the first high-order 16-membered turn structure observed in water for any foldamer. The conformational switch evidenced for the *cis*-glycofoldamers **4a/5a** is driven uniquely by their appended mannosyl moieties and are not observed for the foldamer **2a**, which features an alternate aliphatic ester-appended backbone.

It is noteworthy that NMR data recorded for the members of the *trans*-foldamer family, **1b–3b**, are very similar to those of their *cis*-counterparts, suggesting that they share related conformational properties. For example, the *trans*-foldamer **1b** displayed sequential nOe peaks between $(i)\text{NH} \leftrightarrow (i-1)\text{C}_5\text{H}$, $(i)\text{NH} \leftrightarrow (i-1)\text{C}_6\text{H}_{(\text{pro-}S)}$ in its NMR spectrum together with those characteristic of a 10-membered H-bonded conformation for $(i)\text{NH} \leftrightarrow (i-2)\text{CO}$ similar to that observed for the *cis*-foldamer **1a**. Sol-

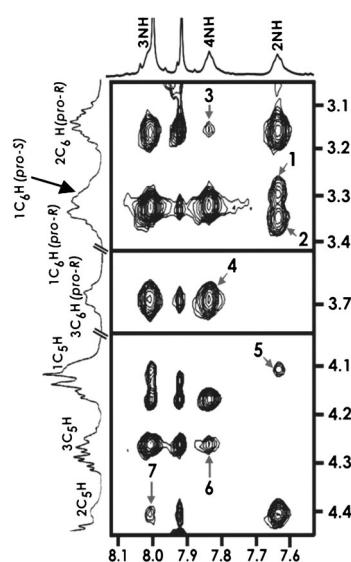
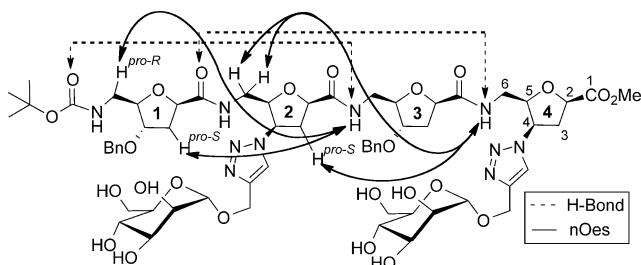


Figure 1. Expanded ROESY spectrum of **3a** in CDCl_3 (ca. 9 mM, 300 K). The nOes $^2\text{NH} \leftrightarrow ^1\text{C}_6\text{H}_{(\text{pro-}S)}$, $^2\text{NH} \leftrightarrow ^1\text{C}_6\text{H}_{(\text{pro-}R)}$, $^4\text{NH} \leftrightarrow ^2\text{C}_6\text{H}_{(\text{pro-}R)}$, $^4\text{NH} \leftrightarrow ^3\text{C}_6\text{H}_{(\text{pro-}R)}$, $^2\text{NH} \leftrightarrow ^1\text{C}_5\text{H}$, $^4\text{NH} \leftrightarrow ^3\text{C}_5\text{H}$, and $^3\text{NH} \leftrightarrow ^2\text{C}_5\text{H}$ are marked as 1–7.



Scheme 4. Characteristic nOEs and H-bonding pattern of the 16-membered helical turn structure of **4a** in CD_3OH .

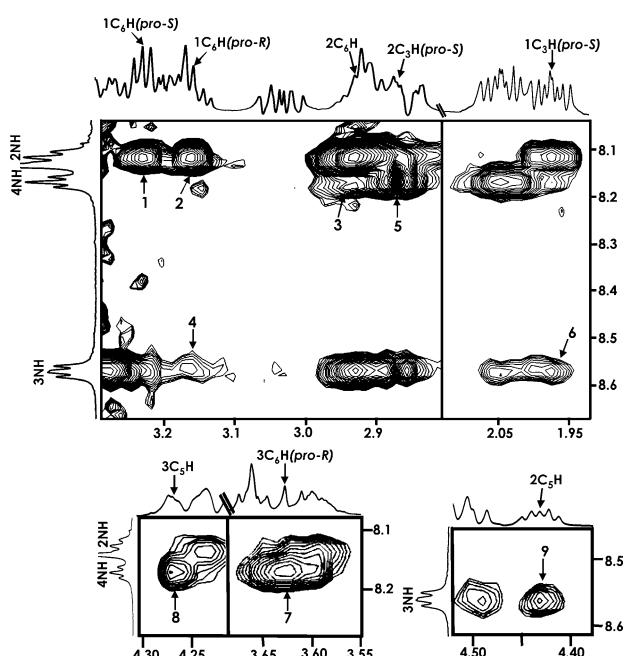


Figure 2. Expanded ROESY spectrum of **4a** in CD_3OH (ca. 8 mM, 300 K). The nOEs $^2\text{NH} \leftrightarrow ^1\text{C}_6\text{H}_{(\text{pro-S})}$, $^2\text{NH} \leftrightarrow ^1\text{C}_6\text{H}_{(\text{pro-R})}$, $^4\text{NH} \leftrightarrow ^2\text{C}_6\text{H}$, $^3\text{NH} \leftrightarrow ^1\text{C}_6\text{H}_{(\text{pro-R})}$, $^4\text{NH} \leftrightarrow ^2\text{C}_3\text{H}_{(\text{pro-S})}$, $^3\text{NH} \leftrightarrow ^1\text{C}_3\text{H}_{(\text{pro-S})}$, $^4\text{NH} \leftrightarrow ^3\text{C}_6\text{H}_{(\text{pro-R})}$, $^4\text{NH} \leftrightarrow ^3\text{C}_5\text{H}$, and $^3\text{NH} \leftrightarrow ^2\text{C}_5\text{H}$ are marked as **1-9**.

vent titration studies further supported the adoption of a 10-membered H-bonding pattern by **1b**.^[12] Likewise, the *trans*-glycofoldamers **2b** and **3b** also exist in a stable 10-membered H-bonded conformation similar to that observed for the corresponding *cis*-glycofoldamers **2a** and **3a**. However, careful observation of the deacetylated, or deacetylated/debenzylated *trans*-glycofoldamers, **4b** and **5b** respectively, showed that they do not form the higher-order structures observed in their *cis*-counterparts **4a** and **5a**, but rather maintain the conformational preferences adopted prior to their being appended with sugar moieties.

Restrained molecular dynamics (MD) studies were carried out using experimental distance and torsional constraints derived from nOe volume integrals and vicinal coupling constants (3J), respectively. The average structures of the 5 ns MD run of each *cis*-glycofoldamers are presented in Figure 3. The average distance between the anomeric carbons of mannose residues, as calculated from the structure ensembles, is 6.73 Å for the *cis*-analogue, **5a** (20 least-energy

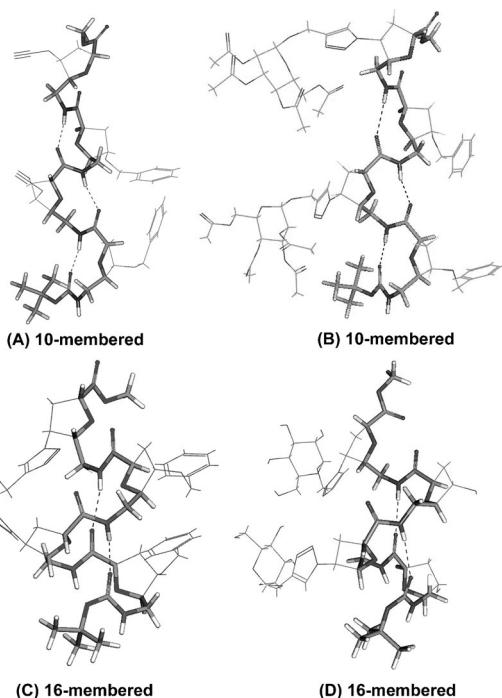


Figure 3. 10- and 16-membered *cis*- δ -foldamers and *cis*- δ -glycofoldamers. H-bonded average MD structure of A) **1a**, B) **3a**, C) **4a**, and D) **5a**.

conformations range from 5.45 Å to 9.40 Å), and 6.58 Å for the *trans*-compound, **5b** (20 least-energy conformations vary from 5.25 Å to 8.19 Å) respectively.

We were curious to establish whether or not the mannose residues in glycofoldamers **5a** and **5b** interacted with selected biological targets differently, despite the fact that the average distance between these residues differs by less than 0.2 Å (from MD calculations). Any difference in binding between **5a** and **5b** was anticipated to provide additional insights into the impact of foldamer conformational properties. The protein ConA, which is known to bind mannopyranosyl moieties preferentially, and the bacterium, *E. coli* in which the lectin FimH contributes specifically to bladder colonization through binding to terminal α -D-mannosyl units that are present on glycoproteins such as uroplakins, were selected for examination.^[18,10b]

The predisposition of glycofoldamers **5a** and **5b** to bind our targets was initially probed in a superimposition study in which the structures of the glycofoldamers (determined experimentally) and those of the proteins were kept rigid.^[19] Each of the two mannose residues in **5a** and **5b** were then superimposed onto the mannose residues of ligands^[12] complexed in the binding pocket of ConA or that of FimH (PDB codes: 1CVN, 2VCO), respectively. This resulted in two possible poses for the pair of glycofoldamers with each protein, all of which were evaluated for steric collisions. Significant steric clashes were observed between ConA and both **5a** and **5b** for all but one pose.^[12] The superposition of glycofoldamers **5a** and **5b** onto the $\text{Man}_3\text{GlcNAc}_2$ in FimH also resulted in unacceptable interactions.^[12] The preliminary study indicates that some level of induced fit would be

expected for binding of the glycofoldamers **5a** or **5b** with either of the selected targets.

An enzyme-linked lectin assay (ELLA)^[20] providing information on binding affinity between the sugar ligand and a single CRD in the lectin indeed confirms that both glycofoldamers are recognized by ConA, with the *cis*-compound, **5a** giving an IC_{50} (inhibitory concentration 50) some 1.3 fold lower ($IC_{50} = 347 \pm 10 \mu\text{M}$) than its *trans*-counterpart **5b**, ($IC_{50} = 440 \pm 10 \mu\text{M}$).^[12] However, nearly four- to sixfold affinity enhancement was found for Man₃ ($IC_{50} = 75 \pm 6 \mu\text{M}$), which supports the hypothesis that induced fit would be required for an increase in binding of either glycofoldamers with ConA, as suggested also by the findings of the superimposition studies. Peptide glycofoldamers **5a** and **5b** were also evaluated as inhibitors for FimH-expressing K-12 *E. coli* adhesion.^[21] In this study, the *cis*-analogue **5a** gave an inhibition titer (IT) fourfold lower (IT = 0.087 mm) than the *trans*-compound **5b** (IT = 0.35 mm) in the yeast agglutination assay.^[12] The IT was considered as the lowest compound concentration able to inhibit agglutination. Likewise, both glycofoldamers were seen to reduce the binding of *E. coli* to T24 bladder cells, the *cis*-glycofoldamer **5a** giving an IC_{50} of some 2.2-fold lower ($IC_{50} = 0.02986 \text{ mm}$) than its *trans*-counterpart **5b** ($IC_{50} = 0.06621 \text{ mm}$). Binding to an *E. coli* strain not expressing FimH was not seen.^[12] The differences observed between glycofoldamers **5a** and **5b**, in assays with both ConA as well as *E. coli*, are consistent with their contrasting conformational preferences. The origin of these differences may at present only be conjectured upon.

The data presented is the first demonstration that appended carbohydrates can have a defining influence on the structural preferences of δ -SAA-derived foldamer backbones in water. Of particular significance is the observation that the grafted mannose moieties provoke a conformational switch in the *cis*-foldamer backbone (and not in its *trans*-foldamer counterpart) from a 10-membered H-bonded turn structure, when not appended with sugars, to an unprecedented 16-membered helical one when mannose units are grafted. The variations are seen to be exquisitely dependent on the particular backbone to which sugars are appended and is consistent with a scenario in which the mannose units modulate the backbone torsional preferences of a given foldamer as defined by its backbone stereochemistry (*cis*- or *trans*-) and amplified by variations in its constituent δ -SAA furanoid (2R- or 2S-) conformations. Our findings mirror those of Imperiali et al.,^[22] who observed a chitobiose-driven conformational switch of a short peptide backbone and further showed by NMR that this was not due to any observable interaction between the peptide backbones and their constituent sugar moieties.^[2,22] Likewise, in neither glycofoldamer **5a** or **5b** are any interactions observable between the peptide backbone and the constituent mannose residues by NMR spectroscopy.

Of particular significance is the demonstration that the interactions of the *cis*- and *trans*-glycofoldamers **5a** and **5b** with both the lectin ConA and the bacterium *E. coli* are distinct, suggesting that these differences may have their seeds in the underlying conformational preferences of this pair of neoglycopeptides. These data further confirm the

notion that subtle differences in solution conformation, and it is likely that dynamics preferences should indeed have consequences on the underlying biology of natural glycopeptides even when these might be structurally closely related.^[2-4]

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- [1] a) G. W. Hart, R. J. Copeland, *Cell* **2010**, *143*, 672–676; b) A. Helenius, M. Aebi, *Science* **2001**, *291*, 2364–2369; c) A. Varki, *Glycobiology* **1993**, *3*, 97–130; d) R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683–720.
- [2] a) A. Larkin, B. Imperiali, *Biochemistry* **2011**, *50*, 4411–4426; b) D. Shental-Bechor, Y. Levy, *Curr. Opin. Struct. Biol.* **2009**, *19*, 524–533; c) C. R. Ellis, B. Maiti, W. G. Noid, *J. Am. Chem. Soc.* **2012**, *134*, 8184–8193.
- [3] a) F. Corzana, J. H. Bustos, F. Marcelo, M. García de Luis, J. L. Asensio, S. Martín-Santamaría, J. Jiménez-Barbero, A. Avenoza, J. M. Peregrina, *Chem. Eur. J.* **2011**, *17*, 3105–3110; b) F. Corzana, J. H. Bustos, F. Marcelo, M. G. Luis, J. L. Asensio, S. Martín-Santamaría, Y. Sáenz, C. Torres, J. Jiménez-Barbero, A. Avenoza, J. M. Peregrina, *Chem. Commun.* **2011**, *47*, 5319–5321.
- [4] a) B. Meyer, H. Möller, *Top. Curr. Chem.* **2007**, *267*, 187–251; b) U. Westerlind, *Beilstein J. Org. Chem.* **2012**, *8*, 804–818.
- [5] a) S. M. Muthana, C. T. Campbell, J. C. Gildersleeve, *ACS Chem. Biol.* **2012**, *7*, 31–43; b) B. Ernst, J. L. Magnani, *Nat. Rev. Drug Discovery* **2009**, *8*, 661–677.
- [6] a) T. K. Chakraborty, P. Srinivasu, S. Tapadar, B. K. Mohan, *Glycoconjugate J.* **2005**, *22*, 83–93; b) J. L. Jiménez Blanco, F. Ortega-Caballero, C. Ortiz Mellet, J. M. García Fernández, *Beilstein J. Org. Chem.* **2010**, *6*, No. 20.
- [7] a) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180; b) C. A. Goodman, S. Choi, S. Shandler, W. F. De Grado, *Nat. Chem. Biol.* **2007**, *3*, 252–262; c) G. Guichard, I. Huc, *Chem. Commun.* **2011**, *47*, 5933–5941.
- [8] a) G. L. Simpson, A. H. Gordon, D. M. Lindsay, N. Promsawan, M. P. Crump, K. Mulholland, B. R. Hayter, T. Gallagher, *J. Am. Chem. Soc.* **2006**, *128*, 10638–10639; b) A. S. Norgren, M. Geitmann, U. H. Danielson, P. I. Arvidsson, *J. Mol. Recognit.* **2007**, *20*, 132–138; c) A. S. Norgren, P. I. Arvidsson, *J. Org. Chem.* **2008**, *73*, 5272–5278; d) A. S. Norgren, P. I. Arvidsson, *Org. Biomol. Chem.* **2005**, *3*, 1359–1361.
- [9] a) T. K. Chakraborty, S. Jayaprakash, P. V. Diwan, R. Nagaraj, S. R. B. Jampani, A. C. Kunwar, *J. Am. Chem. Soc.* **1998**, *120*, 12962–12963; b) T. K. Chakraborty, S. Ghosh, S. Jayaprakash, J. A. R. P. Sharma, V. Ravikanth, P. V. Diwan, R. Nagaraj, A. C. Kunwar, *J. Org. Chem.* **2000**, *65*, 6441–6457; c) D. D. Long, M. D. Smith, D. G. Marquess, T. D. W. Claridge, G. W. J. Fleet, *Tetrahedron Lett.* **1998**, *39*, 9293–9296; d) T. D. W. Claridge, D. D. Long, C. M. Baker, B. Odell, G. H. Grant, A. A. Edwards, G. E. Tranter, G. W. J. Fleet, M. D. Smith, *J. Org. Chem.* **2005**, *70*, 2082–2090.
- [10] a) T. A. Morris, A. W. Peterson, M. J. Tarlov, *Anal. Chem.* **2009**, *81*, 5413–5420; b) S. D. Knight, J. Bouckaert, *Top. Curr. Chem.* **2009**, *288*, 67–107.
- [11] R. Baron, D. Bakowies, W. F. Van Gunsteren, *Angew. Chem.* **2004**, *116*, 4147–4151; *Angew. Chem. Int. Ed.* **2004**, *43*, 4055–4059.
- [12] See the Supporting Information.

[13] a) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; b) C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064; c) P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Frechet, K. B. Sharpless, V. V. Fokin, *Angew. Chem.* **2004**, *116*, 4018–4022; *Angew. Chem. Int. Ed.* **2004**, *43*, 3928–3932.

[14] a) M. Kümin, L.-S. Sonntag, H. Wennemers, *J. Am. Chem. Soc.* **2007**, *129*, 466–467; b) R. S. Erdmann, H. Wennemers, *J. Am. Chem. Soc.* **2010**, *132*, 13957–13959; c) H. Lin, C. T. Walsh, *J. Am. Chem. Soc.* **2004**, *126*, 13998–14003; d) O. Schwardt, S. Rabbani, M. Hartmann, D. Abgottsporn, M. Wittwer, S. Kleeb, A. Zalewski, M. Smieško, B. Cutting, B. Ernst, *Bioorg. Med. Chem.* **2011**, *19*, 6454–6473.

[15] M. Ohnishi, D. W. Urry, *Biochem. Biophys. Res. Commun.* **1969**, *36*, 194–202.

[16] T. K. Chakraborty, S. Jayaprakash, P. Srinivasu, M. Govardhanan Chary, P. V. Diwan, R. Nagaraj, A. Ravi Sankar, A. C. Kunwar, *Tetrahedron Lett.* **2000**, *41*, 8167–8171.

[17] a) I. Laczko, M. Hollosi, L. Urge, K. E. Ugen, D. B. Weiner, H. H. Mantsch, J. Thurin, L., Jr. Ottos, *Biochemistry* **1992**, *31*, 4282–4288; b) A. H. Andreotti, D. Kahne, *J. Am. Chem. Soc.* **1993**, *115*, 3352–3353; c) D. H. Live, R. A. Kumar, X. Beebe, S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 12759–12761; d) S. E. O'Connor, B. Imperiali, *J. Am. Chem. Soc.* **1997**, *119*, 2295–2296; e) V. Slynko, M. Schubert, S. Numao, M. Kowarik, M. Aebi, F. H. T. Allain, *J. Am. Chem. Soc.* **2009**, *131*, 1274–1281.

[18] a) N. Sharon, H. Lis, *Glycobiology* **2004**, *14*, 53R–62R; b) R. J. Pieters, *Org. Biomol. Chem.* **2009**, *7*, 2013–2025.

[19] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, *25*, 1605–1612.

[20] M. Gómez-García, J. M. Benito, A. P. Butera, C. Ortiz Mellet, J. M. García Fernández, J. L. Jiménez Blanco, *J. Org. Chem.* **2012**, *77*, 1273–1288.

[21] a) C. G. Korea, R. Badouraly, M. C. Prevost, J. M. Ghigo, C. Beloin, *Environ. Microbiol.* **2010**, *12*, 1957–1977; b) A. Barras, F. A. Martin, O. Bande, J. S. Baumann, J. M. Ghigo, R. Boukherroub, C. Beloin, A. Siriwardena, S. Szunerits, *Nanoscale* **2013**, *5*, 2307–2316.

[22] C. J. Bosques, S. M. Tschampel, R. J. Woods, B. Imperiali, *J. Am. Chem. Soc.* **2004**, *126*, 8421–8425.