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Glycosylated Foldamers To Probe the Carbohydrate−Carbohydrate Interaction

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On the basis of the seminal studies of Hakamori[1a,b,2d] and Burger,[1c,2c] intercellular interactions involving complementary carbohydrate motifs are now recognized to be multivalent in nature, highly substrate specific, and associated with a number of key biological processes.[2] To date, study of this important recognition process has exploited the multivalent component,[3] with, for example, synthetic micelles, monolayers, and glyconanoparticles playing a key role.[4] Nevertheless, the molecular detail of these weak, sometimes Ca^{2+} dependent, interactions remains unclear, necessitating the need for models to probe the nature of this phenomenon in terms of individual carbohydrate (CHO) motifs.

Our proposal was to ligate carbohydrates onto a conformationally defined helical scaffold to allow carbohydrate−carbohydrate interactions to be studied within a controlled and *nonmultivalent* environment. We propose to evaluate these interactions directly by spectroscopic methods, which may also be associated within (and reportable via) the scaffold core. In this way, complementary (and sensitive) analytical methods could be employed to study this phenomenon. A suitable core was reported by Gellman[5] whose mixed cyclopentyl/pyrrolidine "foldamers" display a 12-helical secondary structure (i.e., 12-membered H-bonded ring C═O(i)−HN(i + 3)[5f]) in aqueous solution. Positioning the CHO moieties on a single face of the foldamer scaffold offers an efficient design that maximizes the opportunity for intrascaffold CHO-based interactions. To pursue this strategy we have targeted a hexapeptide **1**, which is selectively glycosylated at i + 1, i +3, and i +4 on the basis of a predicted 12-helical pattern (Figure 1(a) and (b)).

Peptide **1** is accessible via the requisite enantiomerically pure glycosylated pyrrolidine *trans*-β-amino acid, with peptide assembly exploiting cyclopentyl β-amino acids as spacers. In this paper we describe the synthesis and characterization of the first glycosylated foldamer which presents a carbohydrate surface which positions and maintains model CHO units in close proximity to one another.[6]

Our synthetic strategy centers on the enantiomerically pure pyrrolidine β-amino acid monomers (e.g., **7**) glycosylated via a sulfonamide-based[5d] linker (Scheme 1). Suitably protected *trans*-3-aminopyrrolidine-4-carboxylate **3** was prepared from β-keto ester **2**[7] utilizing a diastereoselective reductive amination/crystallization procedure as reported by Gellman.[5e] Protecting group manipulations and sulfonamide formation provided access to pyrrolidine **4** ready for carbohydrate attachment through cross metathesis (CM).

Optimal CM conditions required crotyl−crotyl containing components, and reactions involving **4** and four β-*O*-crotyl carbohydrates partners **5a**, **5b**, **5c**, and **5d** were employed. The latter were based on galactose, glucose, glucosamine, and lactose, respectively, and this led to the glycosylated monomers **6a**−**d** in moderate yield.[8]

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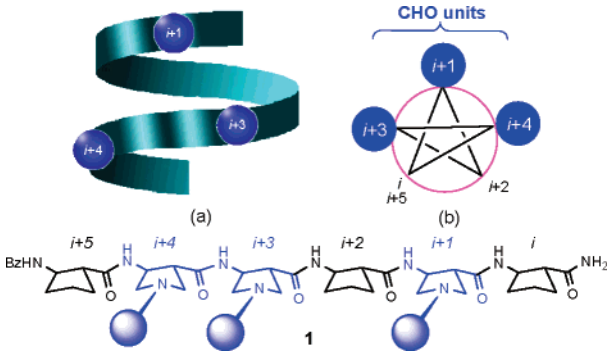
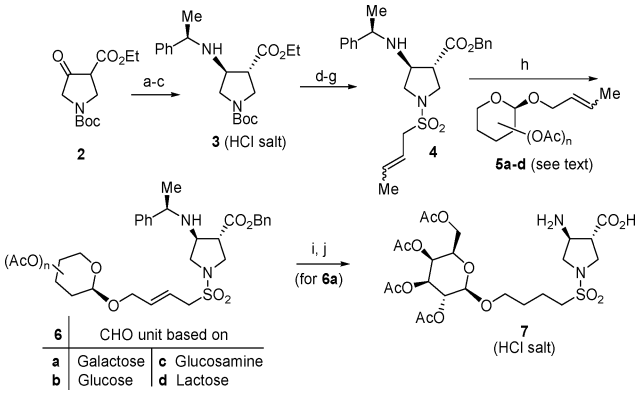


Figure 1. (a) Schematic β-peptide 12-helical scaffold; (b) helix periodicity wheel and glycosylation sites (blue spheres) for hexapeptide **1**.

Scheme 1. Synthesis of Glycosylated Pyrrolidine β-Amino Acid[a]



[a] Conditions: (a) (R)-(+)-α-methyl benzylamine, AcOH, EtOH, 4 h; (b) $NaCNBH_3$, 75 °C, 16 h; (c) 4 M HCl in dioxane, EtOAc, 25% (3 steps); (d) LiOH·H_2O, THF/MeOH/H_2O, 0 °C; (e) BnBr, Cs_2CO_3, DMF, 86% (2 steps); (f) TFA, CH_2Cl_2; (g) CH_3CH_2═$CHCH_2SO_2Cl$, DIPEA, CH_2Cl_2, 0 °C, 79% (2 steps); (h) Grubbs 2nd generation catalyst, CH_2Cl_2, 40 °C, 24 h, 38−60%; (i) 4 M HCl in dioxane; (j) H_2, 10% Pd/C, AcOH, room temp, 24 h, 86% (2 steps).

Completion of the glycosylated monomer synthesis was exemplified with β-galactose-based monomer **6a** via amino acid deprotection, to give the *O*-acetyl protected galactosyl β-amino acid **7**, which has been used as a model to illustrate the foldamer-based concept associated with a triglycosylated hexapeptide **1**.

The peptide coupling strategy employed the glycosylated and cyclopentyl derivatives **7** and **8** (and **10**), respectively (Scheme 2). We initially used a solid-phase synthesis strategy, but use of standard Fmoc protocols (with a HATU/HOBt/NMM coupling strategy) on Rink amide resin failed. This was because the hindered (i + 3)/(i + 4) coupling step involving two glycosylated units (**7** + **9** to generate **11**) did not go to completion. To overcome this problem, a convergent solution phase approach based on a more

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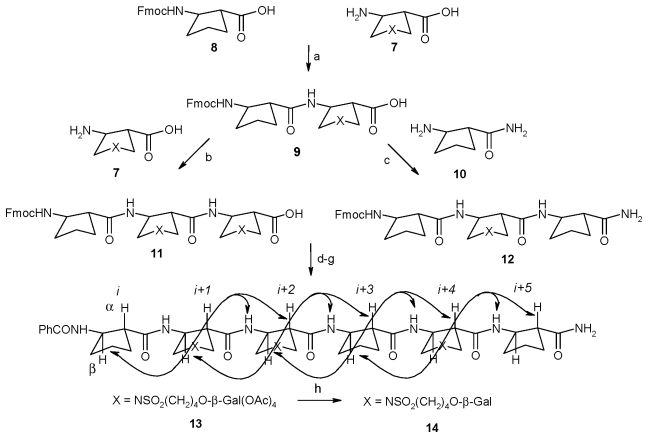
Scheme 2. Synthesis of Glycosylated Hexapeptides **13** and **14**[a]



[a] Conditions: (a) $ClCO_2{}^iBu$, NMM, −10 °C, 71%; (b) $ClCO_2{}^iBu$, NMM, −10 °C, 43%; (c) $ClCO_2{}^iBu$, NMM, −10 °C, 61%; (d) 20% piperidine in DMF; (e) $ClCO_2{}^iBu$, NMM, −10 °C, 74% (2 steps); (f) 20% piperidine in DMF; (g) BzCl, Et_3N, 0 °C, 80%; (h) MeOH/NH_3, 88%. Nonadjacent NOEs observed for peptides **13** and **14** in MeOH (see text) are shown with double headed arrows. NMM = *N*-methylmorpholine.

reactive mixed anhydride was employed. The coupling of **7** and **8** to give **9** was successful, as was the reaction of **9** with each of **7** and **10** to give tripeptides **11** and **12**, respectively. A 3 + 3 coupling of tripeptides **11** and **12** gave the triglycosylated hexapeptide **13**, capped at the *N*- and *C*-termini with benzoyl and primary amide, respectively, and the carbohydrate moieties were deacetylated to give the target triglycosylated hexapeptide **14**. Both **13** and **14** were purified by reverse phase chromatography, and assignments were made by NMR (600 MHz) and ESI mass spectrometry.

2D NMR (NOESY and ROESY) analysis of *O*-protected peptide **13** in CD_3OH showed multiple *i* and *i* + *2* NOEs between backbone protons. Specifically $C_\beta H(i)-C_\alpha H(i+2)$ and $C_\beta H(i)-NH(i+2)$ NOEs are consistent with the expected 12-helical pattern and correlated well with Gellman's sulfonylated pyrrolidine/cyclopentyl foldamers, the structures of which have been assessed in detail.[5d] Peptide **14** shows a very similar set of nonadjacent NOEs in CD_3-OH, and analysis of **14** in H_2O also demonstrated a predominant 12-helical conformation.[9]

These results indicate that glycosylated peptide **14** is a 12-helical foldamer under aqueous solution and that incorporation of carbohydrate units (protected or unprotected) into this array does not alter the secondary structure. These findings were corroborated by circular dichroism (CD) studies (Figure 2). In MeOH, both peptides **13** and **14** showed a characteristic maximum at 204 nm and a weaker minimum at 230 nm. In water the λ_{max} of peptide **14** is shifted to 202 nm and λ_{min} to 225 nm.[10]

In conclusion, we have demonstrated a model system amenable to the study of carbohydrate−carbohydrate interactions, which complements multivalency-based approaches. Glycosylation of the foldamer scaffold does not perturb the helical structure necessary to maintain the carbohydrate units in close proximity, and the model triglycosylated peptide **14** also serves as an important control for future studies. Our next objective is to incorporate carbohydrate moieties that are associated with an established and biologically significant carbohydrate−carbohydrate interaction to study this process in comparative isolation and to detail the nature of the mechanisms involved.

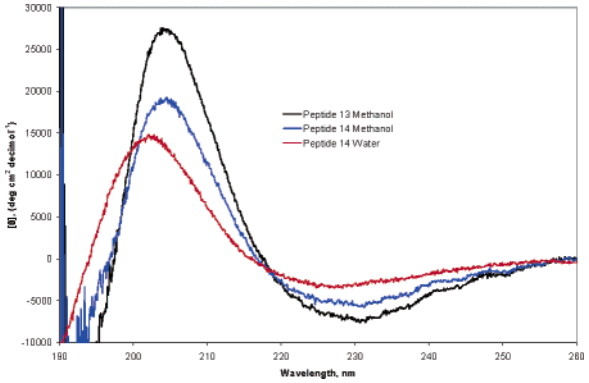


Figure 2. CD data for hexapeptides **13** and **14** in methanol and water. Data have been normalized for concentration and number of residues.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Of a range of substrates tested, crotyl−crotyl partners led to less alkene isomerization and limited the formation of homometathesis products.

(9) In total, three $C_\beta H(i)-C_\alpha H(i+2)$ and four $C_\beta H(i)-NH(i+2)$ NOEs were observed in the central portion of **14** in water (see Supporting Information). All possible $C_\beta H(i)-NH(i+1)$ NOEs were observed, consistent with the 12-helix.[5d,f]

(10) Titration of **14** with $CaCl_2$ (from 0.1 to 100 mM of Ca^{2+}) showed no effect (by CD) on the secondary structure of peptide **14**, and we infer that no interactions are associated with this simple Gal-based model.

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