Promoting helicity in carbohydrate-containing foldamers through long-range hydrogen bonds†

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A sensor system to probe the propensity of carbohydrates to induce helical structures through long-range hydrogen bonds, based on a C_2-symmetric xylylene bis(thiourea) arrangement, is reported; the formation of intermolecular complexes with benzoate anion promotes helix uncoiling, the free energy of the process being related to helix stability.

Emulating the close relationships between conformational and functional aspects typical of biological systems represents a challenging goal in supramolecular chemistry. Foldamers, oligomers that fold into a conformationally ordered state, offer a good opportunity to learn about such processes. Although the field has experienced significant progress during the last years, designability of definite folding patterns resulting in precise secondary structures is still limited to a few systems, among which β- and γ-peptides are paradigmatic examples.[1] Several carbohydrate–peptide foldamers have also been reported. The presence of the rigid sugar units usually exerts a drastic effect in the folding properties due to configurational and conformational biases. In all cases, however, long-range C=O···HN hydrogen bonds are the primary intramolecular interactions driving folding, the sugar oxygens playing only a minor role.[2]

Recently, we have reported the synthesis of pseudoamide (urea, thiourea, guanidine)-linked carbohydrate oligomers as phosphate anion binders.[3] Preliminary results suggested that such glycooligomers tend to take on 15-membered hydrogen-bond held helical conformations involving the sugar oxygens. We anticipated that the folding effect of preferred hydrogen bond patterns would be amplified in conjugates with a pseudo-C_2-symmetry axis, by virtue of concerted interactions, leading to helical arrangements. To prove this concept, the highly flexible *m*-xylylene unit has been chosen as helix handedness inversion centre, since it allows the formation of both left-handed (*M*) and right-handed (*P*) isomers.[4] Fixation of a secondary structure would anchor the conformation about the NH–C(=S) bonds. The rotameric *E*–*Z* conformational equilibrium in thioureas being slow in the chemical shift time scale, this fact makes it conceivable to structurally characterize the capability of a monosaccharide template to dictate a given sense upon hydrogen bonding by dynamic NMR spectroscopy (Fig. 1 A, B).[5] Interestingly, thiourea receptors can also establish intermolecular multipoint hydrogen bonds with complementary guests in a specific and predictable manner.[6] The influence of definite folding patterns on the complexing activity can be correlated, thus, to the stability of the corresponding secondary structure (Fig. 1 C).

In order to test the suitability of the above design criteria to assess 15-membered O(sugar)···HN hydrogen bond patterns, the conformational properties of the ditopic *m*-xylylene derivatives **2a–c** in chloroform-*d* solution and their complexing abilities towards benzoate anion were examined. The homologous monotopic benzylthioureas **1a–c** were also included in our study as reference compounds. In the absence of any definite secondary structure, the conformational behaviour of a bis-thiourea derivative should be the result of a statistic distribution per branch of the rotameric populations encountered in the monotopic counterpart. On the contrary, detection of a single conformer would necessary imply intramolecular interbranch interactions (Fig. 2).

In the D-xylopyranosyl derivative **2a**, the oxygen atom O-4 is located at 14 covalent bonds from the benzylic NH proton at the opposite branch. Notwithstanding, comparison of the low temperature NMR spectra for **1a** and **2a** discarded the existence

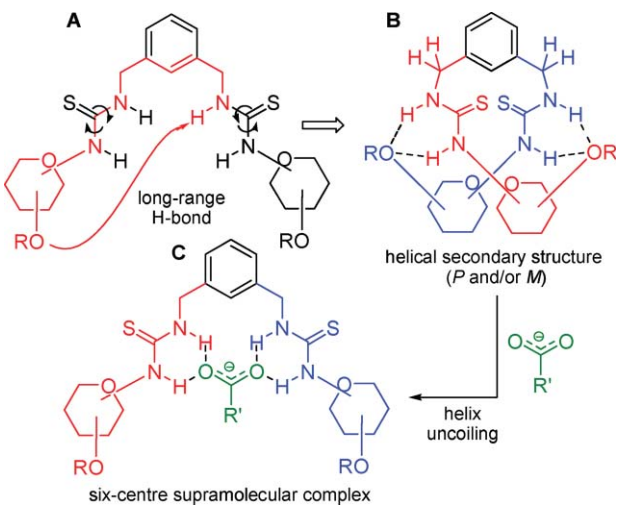

Fig. 1 Schematic representation of a sensor to probe the propensity of monosaccharides to induce long-range hydrogen bonds based on the xylylene bis(thiourea) core. (A) In the unfolded state, all rotameric configurations about the NH–(C=S) bonds are possible. (B) Cooperative long-range hydrogen bonds resulting in helical foldings would anchor the *Z*,*Z* configuration at both thiourea segments. (C) The formation of an intermolecular six-centre hydrogen-bonded complex with carboxylate guests should promote helix uncoiling, the free energy of the process being related to helix stability.

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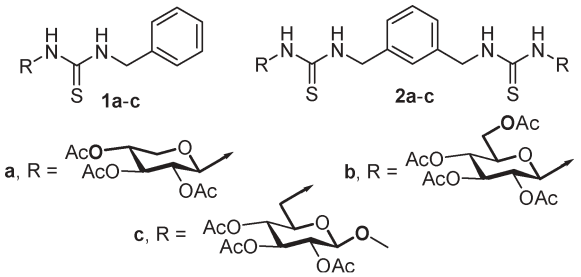

Fig. 2 Structures of the monotopic (**1a–c**) and ditopic thiourea derivatives (**2a–c**) considered in this study. Their synthesis was accomplished by coupling of the corresponding sugar isothiocyanate with benzylamine or *m*-(bis-aminomethyl)benzene, (see Supplementary Information†). The key oxygen centre located at 14 covalent bonds from the $PhCH_2NH$ proton at the opposite branch is shown in boldface.

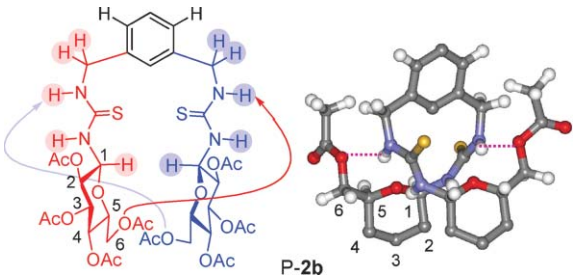

Fig. 3 Schematic representation and computer generated model of the *P*-helical conformation of compound **2b**.‡[1] The two key 15-membered H-bonds are indicated. Only the CH protons relevant for structural assignment (NOE) are depicted. The acetate groups at secondary carbons have been omitted for clarity.

of any defined folding (Table 1). In stark contrast, whilst the monotopic D-glucopyranosyl derivative **1b** existed as a mixture of the *Z*,*Z* and *Z*,*E* rotamers in chloroform-*d* solutions, a single conformer, having the *Z*,*Z* configuration at both thiourea segments, was detected in the case of **2b**, independently of the sample concentration. Moreover, the NH proton resonances in **2b** were significantly downfield shifted as compared to **1b**, with particularly low chemical shift temperature coefficients in the case of those located at benzylic positions, strongly supporting their participation in intramolecular hydrogen bonding, with the O-6 atoms as acceptors, in an overall helical architecture (Fig. 3).

It is noteworthy that only one of the two possible diastereomeric helices for **2b** was detected. In fact, the anomeric carbon (C-1)–NH–C(=S) segment is set in the anti-*Z* conformation in glycosylthioureas. According to molecular modelling, this rigid arrangement is only compatible with the *P*-handeness in the xylylene bis(thiourea) system. The existence of a fixed *gauche-trans* conformation at the C-5–C-6 bond ($J_{5,6a}$ and $J_{5,6b}$ 9.3 and <1.5 Hz, respectively) instead of the usual equilibrium between staggered rotamers, as well as diagnostic NOE contacts, additionally supported this assignment.

In the bis(thiourea) derivative **2c** the relative disposition of the key oxygen and nitrogen atoms has been exchanged as compared to **2b**. This subtle modification does not alter the distance between donor and aceptor centres. Yet, it has a dramatic influence on the chain flexibility, the C-5–C-6–NH–C(=S) segment exhibiting a broad conformational freedom. Thus, the three possible rotameric forms, *Z*,*Z*, *Z*,*E* and *E*,*Z* contribute to the conformational equilibrium of the monotopic thiourea **1c** in $CDCl_3$, meaning that up to six conformations are accessible for the ditopic homologue **2c** in the unfolded state. Instead, only two species, in relative proportions close to 1:1, were detected in the low temperature NMR spectra, both of them displaying the (*Z*,*Z*:*Z*,*Z*) configuration at the thiourea segments as confirmed by NOE, corresponding to the *P* and *M* helical foldings (Fig. 4).

In order to get information on the energy of the 15-membered hydrogen bonds responsible for helical folding, a comparative analysis of the complexing properties of the mono (**1a–c**) *versus* the ditopic (**2a–c**) receptors was carried out. Benzoate anion was used as an external probe for this purpose. In the absence of any intramolecular interaction, an above 10-fold increase in the association constant (K_{as}) on going from the four-centre to the six-centre 1:1 complex (Fig. 5) was expected.[7] Consistently, the Job's plots and binding isotherms for **1a** and **2a** were indicative of 1:1 stoichiometries, with K_{as} values of 1749 ± 34 and 18140 ± 180 M^{-1}, respectively (Table 1).[8]

Titration experiments for the **1b/2b** and **1c/2c** pairs against benzoate revealed a totally different scenario. Whereas the

Table 1 Thermodynamic data for **1a–c** and **2a–c** and their benzoate complexes

Receptor	Rotameric populations[a]	$K_{as(1:1)}$[b] /M^{-1}	$K_{as(2:1)}$[b] /M^{-1}
1a	*Z*,*E*/*Z*,*Z* 1.0:2.5	1749 ± 34	—
2a	n.d.[c]	18140 ± 180	—
1b	*Z*,*E*/*Z*,*Z* 1.0:3.6	929 ± 8	—
2b	(*Z*,*Z*:*Z*,*Z*) 100%	1106 ± 49	87 ± 6
1c	*Z*,*E*/*Z*,*Z*/*E*,*Z* 1.6:1.2:1.0	625 ± 14	—
2c	(*Z*,*Z*:*Z*,*Z*) 100%	1930 ± 80	30 ± 5

[a] Obtained by digital integration at 233 K. The first stereochemical sign refers to the N–C(=S) bond next to the monosaccharide residue. [b] At 298 K. Average values from at least three separate experiments. [c] Not determined due to extensive overlapping.

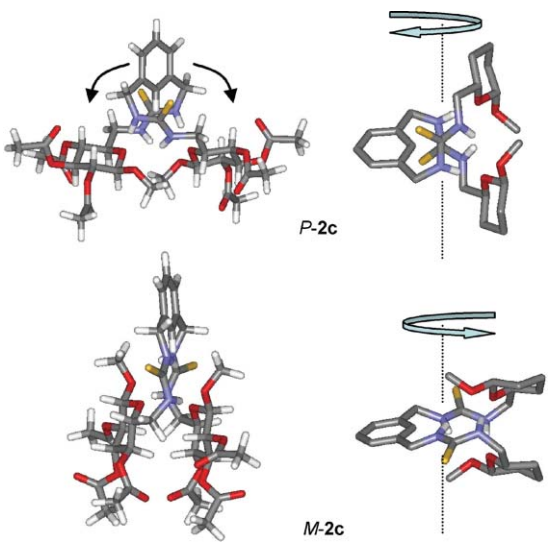

Fig. 4 Computer generated helical conformations for **2c**. The curve arrows in the *P*-isomer indicate the aromatic ring flipping, approaching the sugar protons at the β-face, which allows helix assignment (see Supplementary Information†). In the left-side structures, CH protons and acetate groups have been omitted for clarity.

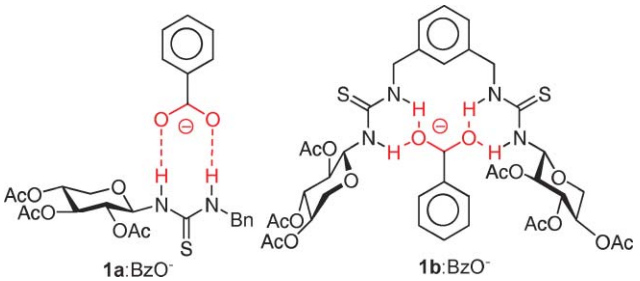

Fig. 5 Structures of the **1a**:benzoate (four-centre) and **1b**:benzoate (six-centre) complexes.

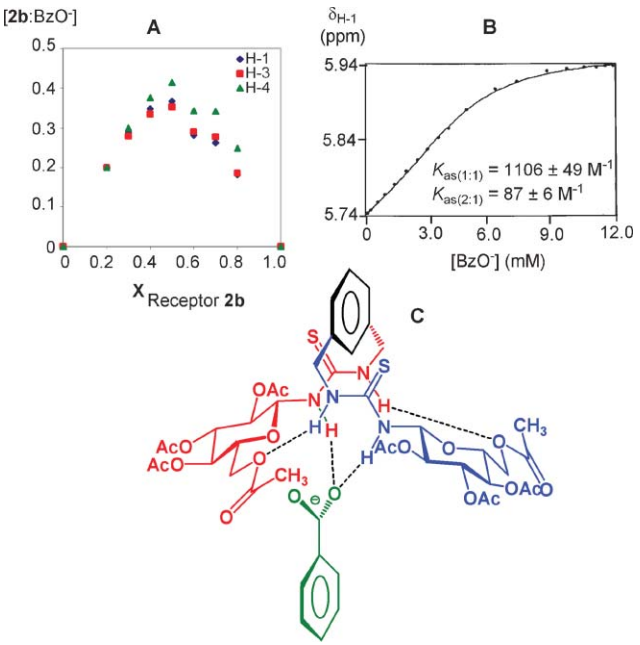

Fig. 6 Job's plot (A) and binding isotherm (B) for the complexation of benzoate by receptor **2b**. The proposed structure for the 2:1 complex is depicted (C; only one of the two molecules of **2b** in the complex is represented).

monotopic benzylthioureas **1b** and **1c** formed 1:1 complexes, the helix-forming derivatives **2b** and **2c** provided sigmoid binding isotherms and Job's plots consistent with 2:1/1:1 host:guest equilibria (Fig. 6 A, B).[8] Although probably both NH protons at each thiourea segment contribute to helix stability, just those located at 14-bonds from the oxygen acceptors are strictly necessary, the other two remaining accessible for intermolecular interactions in the 2:1 complex (Fig. 6 C). In the presence of excess benzoate, the six-centre hydrogen-bonded 1:1 complex is formed. Yet, this process has a significant penalty associated to helix disruption. The lower increment in the $K_{as(1:1)}$ values on going from **1b**:benzoate (929 $\pm$ 8 M^{-1}) or **1c**:benzoate (625 $\pm$ 14 M^{-1}) to **2b**:benzoate (1106 $\pm$ 49 M^{-1}) or **2c**:benzoate (1930 $\pm$ 80 M^{-1}), as compared with the corresponding data for **1a** and **2a**, reflects the extra-energy needed to destroy the two concerted 15-membered intramolecular hydrogen bonds. From these data, the stabilising free energy associated to the helical folding pattern can be estimated at 60 and 25 J mol^{-1} for **2b** and **2c**, respectively.

The above differences in the propensity of different orientations of equivalent structural motifs to induce a given secondary structure are remarkable and underline the potential of carbohydrates as conformational modulators. Particularly notable is the conformational uniqueness and stability associated to the *N*-glucopyranosyl derivative **2b**. Additional dynamic NMR studies confirmed that the 15-membered intramolecular hydrogen bonds holding the helix structure survived in the presence of up to 30% dimethyl sulfoxide, which is consistent with previous observations for natural *N*-glycopeptides in non aqueous solvents.[9] The conclusions of this study should make the rational design of this new family of carbohydrate-based foldamers feasible.

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Notes and references

‡ Calculations were performed with the MACROMODEL 6.0 package and the GB/SA continuous solvent model for chloroform.

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