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Note

The crystal structure of a cyclic glycolipid reveals a carbohydrate–carbohydrate interaction interface

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Abstract—The crystal structure of an amphiphilic macrocycle [Velasco-Torrijos, T.; Murphy, P. V., *Tetrahedron: Asymmetry* **2005**, *16*, 261–272] derived from saccharides shows extensive hydrogen-bonding networks, including participation of water, that may have relevance for the modelling of carbohydrate–carbohydrate recognition at cell–cell interfaces. The structure may provide a basis for understanding the binding of the macrocycle to hydrophobic probes.

Keywords: Crystal structure; Glycolipid; Macrocycle; Hydrogen bonding; Hydrophobic interaction

We have recently synthesized the macrocycle **1** (Chart 1),[1] a water-soluble compound that contains a hydrophobic cavity and which bound to a hydrophobic probe, 8-anilino-1-naphthalenesulfonate (ANS). This property is also displayed by β-cyclodextrin.[2] The NMR analysis of **1** indicates that in solution there is a predominating C_2-symmetric isomer (85%) assigned to **1a** and/or **1b** as well as an unsymmetrical minor isomer (15%) corresponding to **1c** (Chart 1). Isomers **1a** and **1b** have two *E-anti*† amides whereas **1c** has one *E-anti* and one *Z-anti* amide. A description of the crystal structure which provides detailed coordinates for isomer **1c** is provided herein.

The macrocycle **1** crystallized in the monoclinic space group $P2_1$ and the crystal structure (Figs. 1 and 4) showed only one of the isomers, namely the L-shaped isomer **1c** (Chart 1). Each unit cell contained two identical macrocycles and four water molecules. The close-packed structure demonstrated carbohydrate–carbohydrate interactions, resulting from dense networks

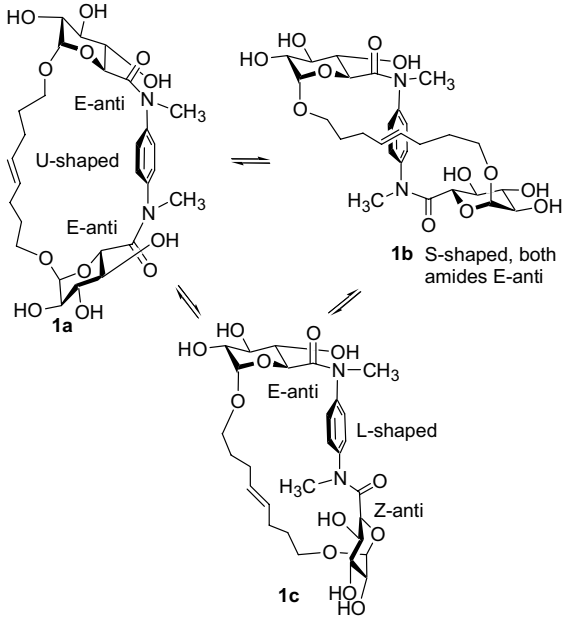

Chart 1. Structure of **1** in solution is 15:85 of **1c**:**1a/b**.

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† The *E* and *Z* nomenclature used refer to amide configuration. For the *Z*-isomer the two groups of highest priority according to Cahn Ingold Prelog rules (i.e. the aromatic group and oxygen atom) are on same side of the bond with double bond character (amide bond); for the *E*-isomer these same two groups are on opposite sides of the amide bond. *Anti* and *syn* nomenclature refers to torsion angle defined by H5-C5-C6-O6; for the *anti*-isomer this angle is 180 ± 90°.

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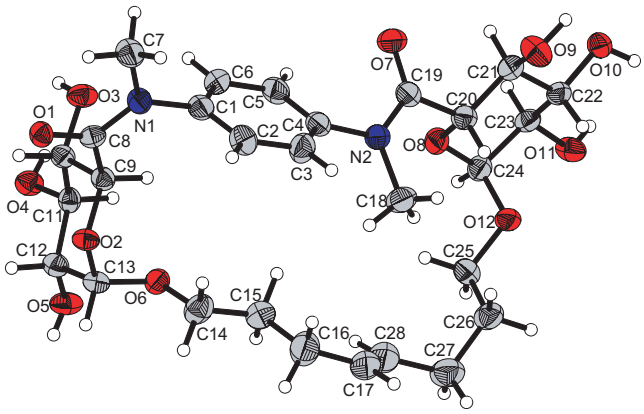

Figure 1. Structure of **1c** showing 80% probability displacement for ellipsoids.

of hydrogen bonds which occur through cooperation with water molecules and the association of the hydrophobic groups. The intermolecular hydrophobic interactions (Fig. 2) which are most significant are those which occur between a methyl with pyranose CH groups and between the octenyl and methyl groups with the aromatic ring. Intramolecular hydrophobic interactions

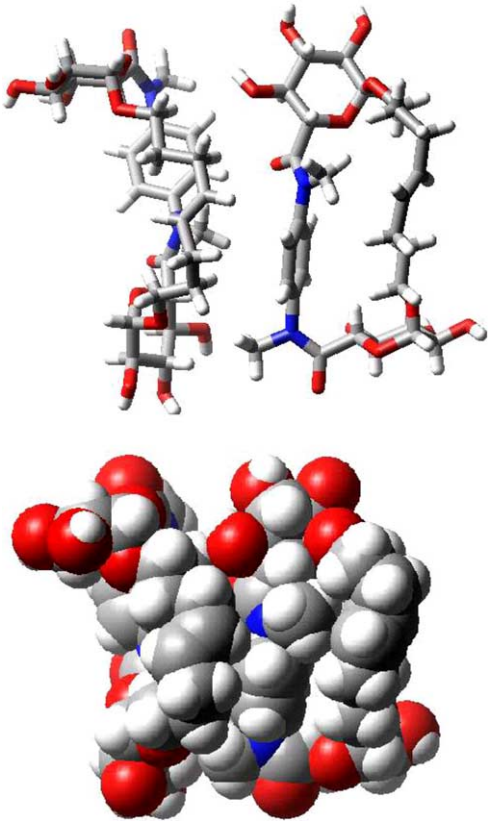

Figure 2. Two molecules of **1c** selected from the close packed structure are shown; van der Waals surfaces have been calculated (bottom). Inter- and intramolecular association of hydrophobic surfaces is evident. Oxygen atoms are red, nitrogen atoms are blue, carbon atoms grey and hydrogen atoms white.

are observed between one methyl group and the octenyl chain (Fig. 2). The interactions observed with the aromatic group may provide a basis for understanding the observed binding of **1** to ANS or for designing interactions with other amphiphilic or hydrophobic molecules.

Only hydrogen bonding interactions occur at the carbohydrate–carbohydrate interfaces leading to infinite stacking of macrocycle layers. Each sugar unit with the *E-anti* amide has three hydrogen bonds to water molecules and five to adjacent sugar units. Each *Z-anti* sugar has one hydrogen bond to water and five to the neighbouring sugar OH groups. The water molecules are arranged in dimeric clusters, hydrogen bonding to each other and to adjacent sugar residues. Jeffrey has identified patterns for hydrogen-bonding networking in crystals of carbohydrates and in this case there is a finite arrangement of hydrogen bonds within networks,[3] the networks being terminated by *O*-1 and *O*-6 of the glucuronic acid residues. For details on the hydrogen bonding see Table 1 and Figure 3. The glycolipid and macrocyclic nature of **1** seems to have facilitated crystallization, which is not straightforward in general for unprotected saccharides; the association of constrained hydrophobic surfaces is maximized as are the intermolecular contacts of the sugar hydroxyl groups. There are examples of biologically relevant carbohydrate–carbohydrate recognition.[4] It is believed that carbohydrate–carbohydrate interaction at a cell surface involves high ordered structures that result from multimerization of carbohydrates to generate sufficient affinity/avidity for their functioning. The gradual formation of two tightly linked carbohydrate chains on neighbouring cells has been likened to a 'zipper'.[4] There is little structural information available, although there are examples.[5] The strength of carbohydrate–carbohydrate interaction is similar to that which occurs between other biomolecule types[6] and calcium ions play an important role.[6,7] We provide a clear demonstration of the multimerization of a carbohydrate into an ordered structure in the absence of calcium; the carbohydrate–carbohydrate interactions observed are due to hydrogen bonding including participation of water. The structural information provided may be useful for modelling[8] carbohydrate–carbohydrate interactions at interfaces relevant to cell–cell adhesion and communication.

1. Experimental

1.1. Methods for X-ray crystal structure determination

Single crystals ($0.20 \times 0.15 \times 0.01$ mm) of **1**, were obtained from ethanol. Crystal data were collected using a Bruker SMART APEX CCD area detector diffractometer. A full sphere of the reciprocal space was scanned by phi-omega scans. Semi-empirical absorption correction

Table 1. Hydrogen bonds for **1c** [Å and °]

D–H ··· A	*d*(D–H)	*d*(H ··· A)	*d*(D ··· A)	<(DHA)
O(3)–H(3O) ··· O(11)[a]	0.77(3)	1.98(3)	2.739(2)	167(3)
O(4)–H(4O) ··· O(12)[a]	0.79(3)	2.15(3)	2.900(2)	161(3)
O(5)–H(5O) ··· O(10)[b]	0.75(3)	2.21(3)	2.771(2)	133(3)
O(9)–H(9O) ··· O(14)[c]	0.94(3)	1.87(3)	2.809(2)	175(2)
O(10)–H(10O) ··· O(13)[d]	0.83(3)	1.87(3)	2.703(2)	175(3)
O(11)–H(11O) ··· O(1)[e]	0.74(3)	2.01(3)	2.743(2)	174(3)
O(14)–H(1W) ··· O(4)[f]	0.80(3)	1.99(3)	2.791(2)	174(2)
O(14)–H(2W) ··· O(2)	0.84(3)	2.06(3)	2.895(2)	174(2)
O(13)–H(3W) ··· O(5)	0.77(3)	2.08(3)	2.838(2)	174(3)
O(13)–H(4W) ··· O(14)[g]	0.83(3)	2.03(3)	2.836(2)	165(3)

Symmetry transformations used to generate equivalent atoms:
[a] $-x+1, y-1/2, -z+2$.
[b] $-x+1, y+1/2, -z+2$.
[c] $-x+2, y-1/2, -z+2$.
[d] $x+1, y, z+1$.
[e] $x, y, z+1$.
[f] $-x+1, y+1/2, -z+1$.
[g] $x-1, y, z$. For atom numbering see Figures 1 and 3.

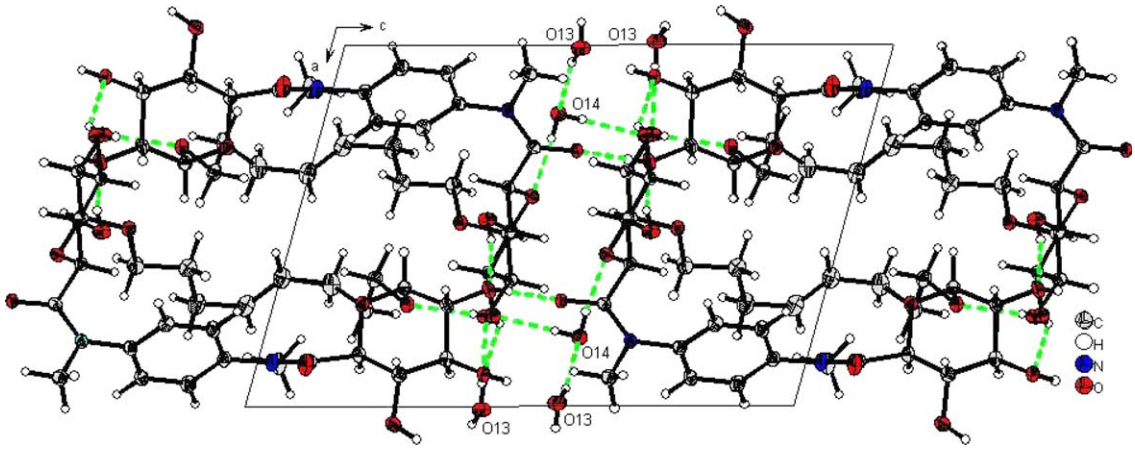

Figure 3. X-ray crystal structure of **1**. View of the close-packed structure along [0 1 0]; thermal ellipsoids are drawn on the 50% probability level. Hydrogen bonding is shown with dashed lines. Oxygen atoms of water molecules are labelled.

based on redundant reflections was performed by the program SADABS.[9] The structure was solved by direct methods using SHELXS-97[10] and refined by full matrix least-squares on F^2 for all data using SHELXL-97.[11] Hydrogen atoms attached to oxygen were located in the difference Fourier map and allowed to refine freely with isotropic temperature factors. All other hydrogens were added at calculated positions and refined using a riding model. Their isotropic temperature factors were fixed to 1.2 (1.5 for methyl hydrogens) times the equivalent isotropic displacement parameters of the carbon atom the H-atom is attached to. Anisotropic temperature factors were used for all non-hydrogen atoms.

1.2. Crystal data

Molecular formula, $C_{28}H_{40}N_2O_{12}\cdot 2H_2O$ $M = 632.65$. Temperature, 100(2) K. Wavelength, 0.71073 Å. Crystal system, monoclinic. Space group, $P2_1$. Unit cell dimensions: $a = 10.5877(11)$ Å, $\alpha = 90°$; $b = 9.7464(10)$ Å, $\beta = 105.434(2)°$; $c = 15.4723(16)$ Å $\gamma = 90°$. Volume, 1539.0(3) Å^3. Z, 2. Density (calculated), 1.365 mg/m^3. Absorption coefficient, 0.110 mm^{-1}. $F(000)$, 676. Crystal size, $0.20 \times 0.15 \times 0.01$ mm^3. Theta range for data collection, 2.00–26.00°. Index ranges, $-13 \leqslant h \leqslant 13$, $-11 \leqslant k \leqslant 12$, $-19 \leqslant l \leqslant 19$. Reflections collected, 11842. Independent reflections, 5921 [R(int) = 0.0205]. Completeness to theta = 26.00°, 99.8%. Absorption correction, semi-empirical from equivalents. Max. and min. transmission, 0.9989 and 0.9784. Refinement method, full–matrix least–squares on F^2. Data/restraints/parameters, 5921/1/439. Goodness-of-fit on F^2, 1.022. Final R indices [$I > 2$sigma(I)]. $R1 = 0.0353$, $wR2 = 0.0799$. R indices (all data). $R1 = 0.0427$, $wR2 = 0.0830$. Absolute structure parameter, −0.2(6). Largest diff. peak and hole, 0.252 and −0.169 eÅ^{-3}.

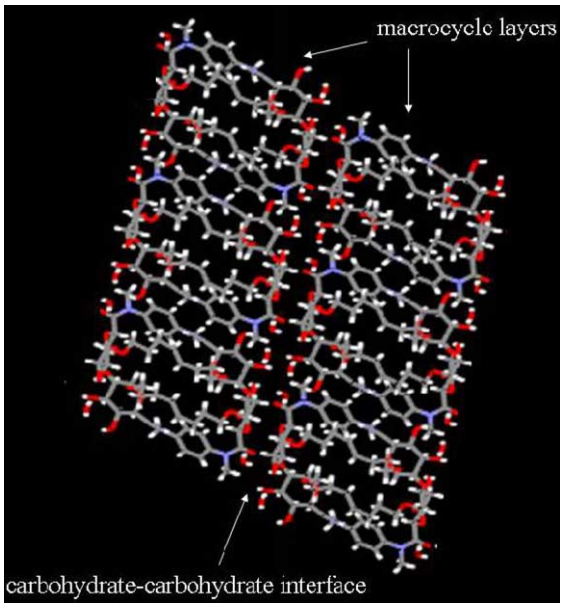

Figure 4. View of carbohydrate–carbohydrate interface between two macrocycle layers.

Full crystallographic details, excluding structure features, have been deposited (deposition no. CCDC 250845) with the Cambridge Crystallographic Data Centre. These data may be obtained, on request, from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (tel.: +44 1223 336408; fax: +44 1223 336033; email: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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Supplementary data

Extensive data (bond distances, angles etc.) and the crystallographic information file have been provided as supplementary data files with this paper. Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.carres.2005.02.026.

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