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Synthesis and X-ray single crystal structure of a bivalent glycocluster

Manuela Tosin, Helge Müller-Bunz and Paul V. Murphy*

Centre for Synthesis and Chemical Biology, Department of Chemistry, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland. E-mail: paul.v.murphy@ucd.ie; Fax: +353-1-7162105; Tel: +353-1-7162504

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The crystal structure of a bivalent glycocluster containing aromatic amides reveals that alkylation of secondary amides alters amide configuration and thus carbohydrate presentation. This also facilitates non covalent interactions (azide–azide, carbonyl–pyranose and aromatic–pyranose) and thus carbohydrate–carbohydrate stacking.

There is little published work concerning the 3D structures of bivalent or higher order multivalent carbohydrate ligands. Such ligands[1,2] are relevant for the development of inhibitors or promoters of carbohydrate mediated biological processes.[3–11] Interest is not confined to the glycobiology area as applications for bivalent ligands include modulation of signal transduction[12] and as leads for development of new antibiotics.[13] There are recent indications that multivalent ligands with increased rigidity can have enhanced affinity and selectivities,[14] suggesting that 3D structure–activity relationships will be interesting. Earlier work[15] has led us to investigate the synthesis of bivalent carbohydrates such as **5–8** whose structure would depend on the conformational and configurational preferences displayed by their amides. It was expected that secondary amides (*e.g.* **1** where R^1 = H) (Fig. 1) would adopt the *Z*-configuration whereas it was unclear whether a steric interaction between the pyranose and the alkyl group would lead to tertiary amides (R^1 = alkyl) preferring to adopt the *E*-configuration.[16] If so then alkylation of the amide would alter the structural space occupied by the carbohydrates. We are interested in such bivalent compounds as scaffolding onto which ligands can be grafted for presentation to receptors. Alterations in the scaffold structure would also alter presentation of the grafted ligands. The concept of using carbohydrates as biologically relevant scaffolds has been introduced and validated previously.[17] The synthesis of bivalent compounds **5–8** is described herein and the structure of tertiary amide **7** has been determined in the solid state.

The acid **2**, prepared from D-glucuronic acid,[18] was the starting point for the synthetic work (Scheme 1, **2**). This was first converted to the β-azide **3** in three steps: preparation of the acid chloride was followed by synthesis of the allyl ester and subsequent introduction of the azide. The protection of the carboxylic acid as an ester was necessary to preclude formation of the α-azide for reasons that we have previously reported.[18] Removal of the allyl ester protecting group from **3**,[19] followed by preparation of the acid chloride **4** and its reaction with phenylene-1,4-diamine gave **5**. Deacetylation of **5** gave **6** whereas alkylation of **5** and deprotection gave the bivalent tertiary amide **8**.

Crystals suitable for X-ray diffraction were obtained for **7** and the solid state structure was determined.† One of the amides of **7** was found to be *E-anti* whereas the other amide was *E-syn* (Fig. 2).

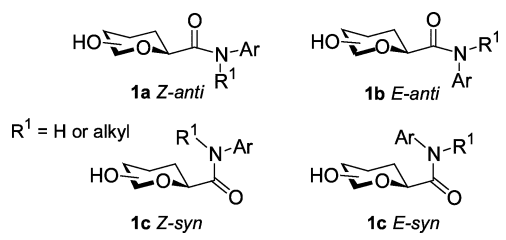
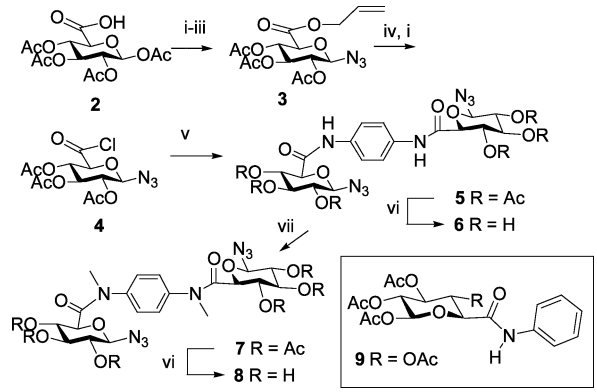


Fig. 1 Amide structure and nomenclature.

The carbohydrates stacked and adopted a *cis*‡ or U-shaped conformation. Carbohydrate stacking was mediated by non-covalent interactions (Table 1 and Fig. 3). Selected interatomic distances that provide evidence for intramolecular van der Waals interactions and other close contacts are given in Table 1: there were clear interactions between (i) the oxygen atom of both pyranoses with aromatic carbon and hydrogen atoms; (ii) the *E-syn* 2-acetate carbonyl oxygen atom with *E-anti* pyranose ring protons and (iii) the two azide groups.

Qualitative NOE data obtained for **6** in D_2O or for **9** in $CDCl_3$ do not provide evidence that the amides are *E-anti* or *E-syn*; *i.e.* there are no NOEs observed between H-5 and aromatic protons or between H-4 and aromatic protons as would be expected (the H-5 of the *E-anti* pyranose was 3.16 Å and the H-4 of the *E-syn* pyranose was 2.51 Å from the nearest aromatic protons in the crystal structure of **7**). The observation of both *E-syn* and *E-anti* conformations in the solid state structure of **7** would indicate that two signal sets of equal intensity should be observed if the structure was the same in solution. However this is not the case for **7** or **8**, where there is only one major set of signals in the NMR spectra of each compound. This could be due to both amides preferring to adopt *E-anti* conformers in solution (supported by NOE) or that the rate of *E-anti/E-syn* interconversion is too rapid to be detected by NMR. Qualitative NOE data obtained for **7** ($CDCl_3$) and **8** (D_2O) indicate that the *E-anti* amide is preferred in solution as an NOE



Scheme 1 *Reagents and conditions*: (i) oxalyl chloride, DMF, CH_2Cl_2; (ii) allyl alcohol, C_5H_5N; (iii) $TMSN_3$, $SnCl_4$, CH_2Cl_2; (iv) $Pd(PPh_3)_4$, pyrrolidine, MeCN; (v) 1,4-phenylenediamine, C_5H_5N, CH_2Cl_2; (vi) NaOMe, MeOH; (vii) NaH, MeI, DMF.

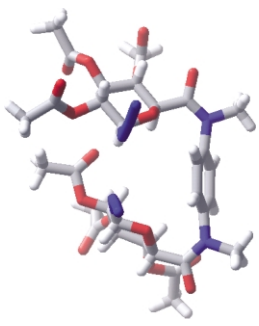


Fig. 2 X-ray single crystal structure of **7**.

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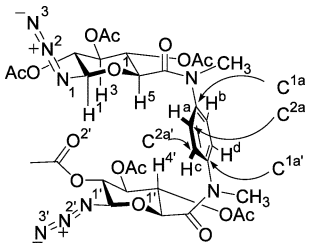
Table 1 Selected interatomic distances in X-ray single crystal structure of **7**

Atom X – Atom Y	Distance/Å
O2′–H1	2.57 (1)
O2′–H3	2.46 (1)
O2′–H5	3.01 (1)
O1′–Hc	2.78 (1)
O1–Ha	2.52 (1)
N1–N1′	3.22 (2)
N1–N2′	3.22 (2)
O1′–aromatic C2a′	2.97 (2)
O1′–aromatic C1a′	2.85 (2)
O1–aromatic C2a	3.04 (2)
O1–aromatic C1a	3.31 (2)



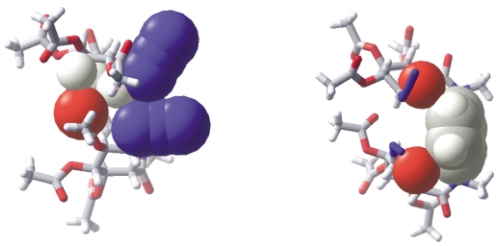


Fig. 3 Van der Waals surfaces were calculated using Macromodel 8.1 for **7**. Shown are the (a) azide–azide and carbonyl–pyranose proton interactions and (b) pyranose oxygen–aromatic interactions.

crosspeak is observed between H-5 and aromatic protons but not between the methyl group and H-4 or H-5. An NOE enhancement between H-4 and aromatic proton would be expected if there was a significant population of *E-syn* in solution and it would be expected to be stronger than that observed between H-5 and the aromatic protons based on distances observed in the solid state structure (provided above); this NOE was not observed. The existence of both *E* and *Z* isomers would be expected to be detected by the presence of at least two signal sets in the ^{1}H-NMR as has been observed for diastereomeric tertiary amides previously.[18] The major set of signals observed for **7** and **8** is assigned to the *EE* isomer. It is not evident from the NMR spectra of **7** or **8** if a *cis* or U-shaped conformation where both amides are *E-anti* is the only structure that exists in solution. A *trans* or S-shaped conformation would be possible and calculations (Macromodel 8.1) indicate it is a low energy conformation.

In summary, amide modification in these bivalent structures alters amide configuration and thus carbohydrate presentation. A consequence is that non-covalent interactions are facilitated and observed in the solid state.

Notes and references

† **Crystal data and structure refinement for 7**. Crystals were obtained from CH_2Cl_2 and petroleum ether (1 : 3). $C_{32}H_{38}N_8O_{16}$. M = 790.70. Temperature 293(2) K. Wavelength 0.71073 Å. Crystal system, orthorhombic. Space group, $P2_12_12_1$ (#19). Unit cell dimensions a = 11.977(2), b = 15.047(3), c = 21.319(4) Å. Volume 3842.2(12) Å^3. Z = 4, calculated density = 1.367 Mg m^{-3}. Absorption coefficient, 0.111 mm^{-1}. F(000), 1656. Crystal size, 0.50 × 0.10 × 0.02 mm. Theta range for data collection, 1.66 to 19.00 deg. Limiting indices, $-10 \leqslant h \leqslant 10$, $-13 \leqslant k \leqslant 13$, $-13 \leqslant l \leqslant 19$. Reflections collected/unique 8199/3077 [R(int) = 0.1058]. Completeness to theta = 19.00, 99.8%. Absorption correction, numerical max. and min. transmission 0.9978 and 0.9465. Refinement method, full-matrix least-squares on F^2. Data/restraints/parameters 3077/0/225. Goodness-of-fit on F^2 0.997. Final R indices [$I > 2\sigma(I)$] $R1$ = 0.0947, $wR2$ = 0.2260. R indices (all data) $R1$ = 0.1456, $wR2$ = 0.2455. Absolute structure parameter fixed at 0.5 to avoid correlation with other parameters. Largest diff. peak and hole 0.570 and −0.410 e Å^{-3}. CCDC 223648. See http://www.rsc.org/suppdata/cc/b3/b313934d/ for crystallographic data in .cif or other electronic format.

^{1}H-NMR data for 5–8: **5** (300 MHz, $CDCl_3$): δ 8.30 (s, 2H, N*H*), 7.44 (s, 4H, aromatic H), 5.33 (apt t, 2H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0, H-3), 5.24 (apt t, 2H, H-4), 4.97 (apt t, 2H, $J_{1,2}$ 8.9, H-2), 4.87 (d, 2H, H-1), 4.15 (d, 2H, $J_{4,5}$ 9.5, H-5), 2.11, 2.08, 2.03 (each s, each 6H, each COC*H*$_3$); **6** (300 MHz, D_2O): δ 7.55 (s, 4H, Ar H), 4.92 (d, 2H, $J_{1,2}$ 8.8, H-1), 4.16 (d, 2H, $J_{4,5}$ 9.5, H-5), 3.73 (apt t, 2H, $J_{3,4}$ 9.3, H-4), 3.65 (apt t, 2H, $J_{2,3}$ 9.0, H-3), 3.40 (apt t, 2H, H-2); **7** (300 MHz, $CDCl_3$): δ 7.42 (s, 4H, aromatic H), 5.50 (apt t, 2H, $J_{3,4}$ 9.4, H-4), 5.15 (apt t, 2H, $J_{2,3}$ 9.2, H-3), 5.24 (apt t, 2H, H-4), 4.87 (apt t, 2H, H-2), 4.22 (d, 2H, $J_{1,2}$ 8.3, H-1), 4.11 (d, 2H, $J_{4,5}$ 9.2, H-5), 3.35 (s, 6H, NC*H*$_3$), 2.02 (2s, 12H, COC*H*$_3$), 2.00 (s, 12H, COC*H*$_3$); **8** (300 MHz, D_2O): δ 7.55 (s, 4H, Ar H), 4.53 (d, 2H, $J_{1,2}$ 8.3, H-1), 4.04 (d, 2H, $J_{4,5}$ 9.7, H-5), 3.81 (apt t, 2H, $J_{3,4}$ 9.3, H-4), 3.38 (s, 3H, NC*H*$_3$), 3.37 (overlapping signals, 8H, H-2 and NCH$_3$), 3.28 (apt t, 2H, $J_{2,3}$ 9.0, H-3).

‡ *Cis* is defined as the carbohydrate groups being on the same side of the plane defined by the aromatic ring. One referee referred to it as U-shaped.

1 Y. V. Lee, R. R. Townsend, M. R. Hardy, J. Lönngren, J. Arnarp, M. Haraldsson and H. Lönn, *J. Biol. Chem.*, 1983, **258**, 199.
2 M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754.
3 C. H. Wong, *Acc. Chem. Res.*, 1999, **32**, 376.
4 H. Kamitakahara, T. Suzuki, N. Nishigori, Y. Suzuki, O. Kanie and C.-H. Wong, *Angew. Chem., Int. Ed.*, 1998, **37**, 1524.
5 P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read and D. R. Bundle, *Nature*, 2000, **403**, 669.
6 B. Liu and R. Roy, *J. Chem. Soc., Perkin Trans. 1*, 2001, 773.
7 S. Andre, R. J. Pieters, I. Vrasidas, H. Kaltner, L. Kuwabara, F. T. Liu and R. M. J. Liskamp, *Chem. Bio. Chem.*, 2001, **2**, 822.
8 L. L. Kiessling and N. L. Pohl, *Chem. Biol.*, 1996, **3**, 71.
9 G. Kretzschmar, U. Sprengard, H. Kunz, E. Bartnik, W. Schmidt, A. Toepfer, B. Horsch, M. Krause and D. Seiffge, *Tetrahedron*, 1995, **51**, 13015.
10 J. C. Sacchettini, L. G. Baum and C. F. Brewer, *Biochemistry*, 2001, **40**, 3009.
11 J. B. Corbell, J. L. Lundquist and E. J. Toone, *Tetrahedron: Asymmetry*, 2000, **11**, 95.
12 J. D. Klemm, S. L. Schreiber and G. R. Crabtree, *Annu. Rev. Immunol.*, 1998, **21**, 418–422.
13 J. H. Rao and G. M. Whitesides, *J. Am. Chem. Soc.*, 1997, **119**, 10286–10290.
14 I. Vrasidas, S. Andre, P. Valentini, C. Bock, M. Lensch, H. Kaltner, R. M. J. Liskamp, H. J. Gabius and R. J. Pieters, *Org. Biomol. Chem.*, 2003, **1**, 803–810.
15 P. V. Murphy, H. Bradley, M. Tosin, N. Pitt, G. M. Fitzpatrick and W. K. Glass, *J. Org. Chem.*, 2003, **68**, 5693 and cited references.
16 R. Yamasaki, A. Tanatani, I. Azumaya, S. Saito, K. Yamaguchi and H. Kagechika, *Org. Lett.*, 2003, **5**, 1265 and cited references.
17 R. Hirschmann, K. C. Nicolaou, S. Pietranico, J. Salvino, E. Leahy, P. A. Sprengeler, G. Furst, A. B. Smith, C. D. Strader, M. A. Cascieri, M. R Candelore, C. Donaldson, W. Vale and L. Maechler, *J. Am. Chem. Soc.*, 1992, **114**, 9217.
18 M. Tosin and P. V. Murphy, *Org. Lett.*, 2002, **4**, 3675.
19 H. Kunz and H. Waldmann, *Angew. Chem., Int. Ed.*, 1984, **23**, 71.