ORGANIC LETTERS

2001
Vol. 3, No. 17
2629−2632

Application of Ugi Reactions in Synthesis of Divalent Neoglycoconjugates: Evidence That the Sugars Are Presented in Restricted Conformation

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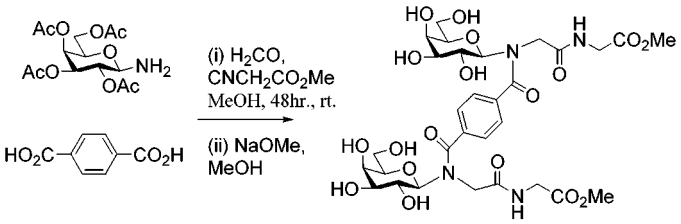
Received June 18, 2001

ABSTRACT



The Ugi reaction has been used to prepare divalent galactose derivatives. NMR analysis shows that a divalent neoglycoconjugate, where the glycopeptides are bridged by a terephthaloyl group, is an 83:17 mixture of two conformers; the amide groups of the major isomer have *E-anti* conformations. The spatial relationship and the relative orientation of the sugars are restricted, which may have consequences for the recognition of this and related structures in biological systems.

Oligosaccharides and proteins, heterogeneously coated on cell surfaces, provide a complex environment where a variety of functions take place. These include cell−cell interactions involved in the immune response, inflammation, cell signaling and infection by pathogens. The design, synthesis, and development of carbohydrate- and glycoconjugate-based drug molecules that interfere with the binding events at cell surfaces is an emerging therapeutic area.[1] Much recent work, for example, has focused on development of novel inhibitors of carbohydrate-selectin recognition.[2] Other areas of interest include the development of cancer vaccines,[3] influenza inhibitors,[4] and xenotransplantation.[5] Strategies that have been adopted for the purpose of discovery of therapeutics from carbohydrates include combinatorial synthesis,[6] the synthesis of carbohydrate mimetics[7] and of carbohydrates modified with hydrophobic groups that increase affinity for

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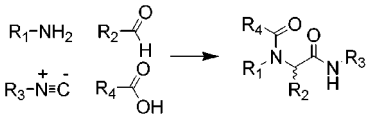
(1) Wong, C.-H. *Acc. Chem. Res.* **1999**, *32*, 376.
(2) Simanek, E. E.; McGarvey, G. J.; Jablonski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833 and references therein.
(3) (a) Liebe, B.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 618. (b) Keil, S.; Claus, C.; Dippold, W.; Kunz, H. *Angew. Chem., Int. Ed.* **2001**, *40*, 366 and references therein. (c) Deshpande, P.; Danishefsky, S. J. *Nature* **1997**, *387*, 167. (d) Hummel, G.; Schmidt, R. R. *Tetrahedron Lett.* **1997**, *38*, 1173.
(4) (a) von Itzstein, M.; Wu, W.-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M. *Nature* **1993**, *363*, 418. (b) Kamitakahara, H.; Suzuki, T.; Nishigori, N.; Suzuki, Y.; Kanie, O.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1998**, *37*, 1524.
(5) Liu, B.; Roy, R. *J. Chem. Soc., Perkin Trans. 1* **2001**, 773.
(6) Schweizer F.; Hindsgaul, O. *Curr. Opin. Chem. Biol.* **1999**, *3*, 291 and references therein.

10.1021/ol016294q CCC: $20.00 © 2001 American Chemical Society
Published on Web 08/02/2001

the receptor by subsite-assisted binding.[8] These approaches have met with some success, yet there is still considerable difficulty in discovery of high affinity ligands for carbohydrate receptors, as the monovalent epitopes tend to bind their receptors weakly (millimolar range). Carbohydrates on cell surfaces are displayed in clusters (or multivalent arrays), and it is most likely that cooperative effects are responsible for increasing the strength of binding of these ligands to their receptors and the resulting potency of these compounds.[9] Multivalent carbohydrates can also affect the nature of the biological response that results.[10] The development of strategies for synthesis of multivalent (di-, tri- and higher order) carbohydrate displays is required for the development of therapeutics from carbohydrates and for providing compounds, which are structurally well defined, for studies of the mechanisms of action of these ligands.

Multicomponent reactions such as the Ugi reaction (Scheme 1) have generated much interest because of their synthetic

Scheme 1

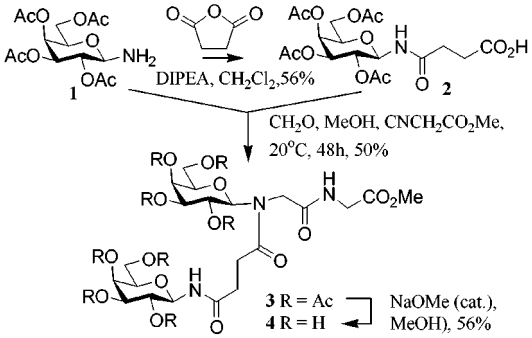


potential, their utility in combinatorial chemistry, and for the generation of molecular diversity.[11] The potential of the reaction in carbohydrate chemistry and biology has been recognized by other researchers.[12]

We have decided to investigate synthetic routes to divalent and higher order multivalent carbohydrates that will also facilitate introduction of diversity. Divalent carbohydrates can show improved biological activity,[13] and therefore we have initially investigated the suitability of the Ugi reaction for convergent one-pot synthesis of dimeric carbohydrates.

Thus galactose amine **1** can be used to prepare conjugate **2** by condensation with succinic anhydride in the presence of diisopropylethylamine in dichloromethane (56%, Scheme 2). The Ugi reaction of **1**, **2**, formaldehyde, and methyl

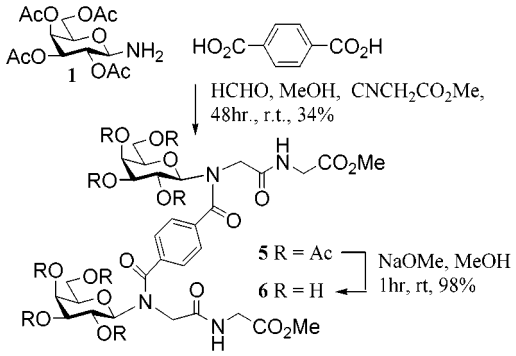
Scheme 2



isocyanoacetate gave the simple divalent galactose dimer **3**. The acetate groups can be removed using NaOMe/MeOH without degradation of the product to give the homodimeric glycoconjugate **4**.

The Ugi reaction of **1**, formaldehyde, methyl isocyanoacetate (2.0 equiv of each reagent), and terephthalic acid (1.0 equiv) gave the dimeric carbohydrate **5** (Scheme 3). The acetates can again be removed to give **6**.

Scheme 3



Having prepared **4** and **6**, we wanted to establish whether there are any preferred solution structures because of the potential relevance of conformation to the recognition of divalent and multivalent ligands in biological systems.[14] A number of carbohydrate derivatives **7**−**9** related to those found in the divalent compounds were also prepared (Scheme 4) to aid the structural studies.[15]

NMR analyses of β-glycosyl amido derivatives have been reported previously.[16,17] However, the conformation and configuration of the unprotected carbohydrates in D_2O has

(7) Murphy, P. V.; Hubbard, R. E.; Manallack, D. T.; Wills, R. E.; Montana, J. G.; Taylor, R. J. K. *Bioorg. Med. Chem.* **1998**, *6*, 2421.

(8) Arya, P.; Kutterer, K. M. K.; Qin, H.; Roby, J.; Barnes, M. L.; Kim, J. M.; Roy, R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1127.

(9) (a) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 696. (b) Mammen, M.; Choi, S. K. Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755.

(10) Gestwicki, J. E.; Strong, L. E.; Kiessling, L. L. *Chem. Biol.* **2000**, *7*, 583.

(11) Domling, A.; Ugi, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 33168 and references therein.

(12) (a) Oertel, K.; Zech, G.; Kunz, H. *Angew. Chem., Int. Ed.* **2000**, *39*, 1431 and references therein. (b) Park, W. K. C.; Auer, M.; Jaksche, H. *J. Am. Chem. Soc.* **1996**, *118*, 10150. (c) Sutherlin, D. P.; Stark, T. M.; Armstrong, R. W. *J. Org. Chem.* **1996**, *61*, 8350. (d) Tsai, C.-Y.; Park, W. K. C.; Weitz-Schmidt, G.; Ernst, B.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2333. (e) Lockhoff, O. *Angew. Chem., Int. Ed.* **1998**, *37*, 3436. (f) Ziegler, T.; Gerling, S.; Lang M. *Angew. Chem., Int. Ed.* **2000**, *39*, 2109. Nunns, C. L.; Spence, L. A.; Slater, M. J.; Berrisford, D. J. *Tetrahedron Lett.* **1999**, *40*, 9341.

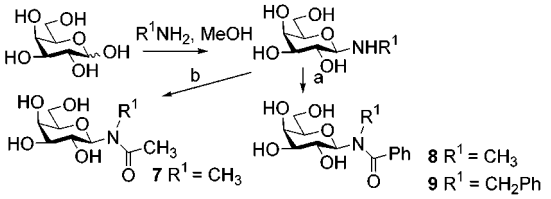
(13) Kogan, T. P.; Dupre, B.; Bui, H.; McAbee, K. L.; Kassir, J. M.; Scott, I. L.; Hu, X.; Vanderslice, P.; Beck, P. J.; Dixon, R. A. F. *J. Med. Chem.* **1998**, *41*, 1099.

(14) Roy, R. Das, S. K. Santoyo-Gonzalez, F. Hernandez-Mateo, F.; Dam, T. K.; Brewer, C. F. *Chem. Eur. J.* **2000**, *6*, 1757.

(15) Experimental protocols were as described previously; see: Retailleau, L.; Laplace, A.; Fensterbank, H.; Larpent, C. *J. Org. Chem.* **1998**, *63*, 608;

(16) The α-glycosides have not been reported. Conventions for describing the configurations and conformations of the β-derivatives have been described previously; see: Avalos, M.; Babiano, R.; Durān, C. J.; Jimēnez, J. L.; Palacios, J. C. *J. Chem. Soc., Perkin Trans. 2*, **1992**, 2205 and references therein.

Scheme 4[a]



[a] Reagents and conditions: (a) BzCl, Na_2CO_3, MeOH, 0 °C, 1 h; (b) Ac_2O, Na_2CO_3, MeOH, 0 °C, 1 h.

not been studied. The ^1H and ^{13}C NMR spectra for most of the known derivatives show two signal sets, and this has been attributed to observation of the *Z*- and *E*-isomers of the *β*-D-glycosylamide. Babiano and co-workers and others have found that an antiperiplanar (*anti*) rather than synperiplanar (*syn*) conformation is generally preferred (see Figure 1). They have established a set of rules based on

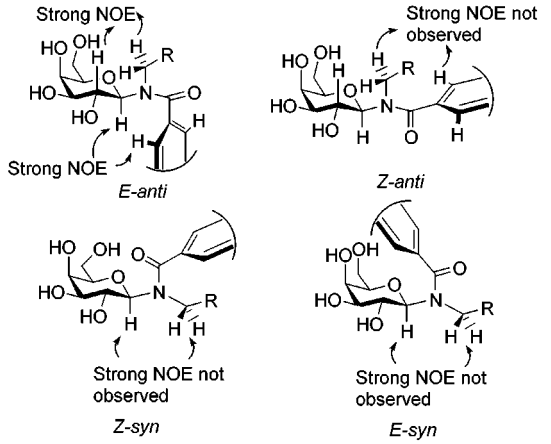


Figure 1. There are strong NOE enhancements for **6** between the aromatic protons and H-1 and also between H-2 and the methylene group adjacent to the anomeric center.

chemical shifts in ^1H and ^{13}C spectra that can be used to assign the *Z* or *E* configuration; these have proven very useful herein.[16] Thus, for the glycosylamides, the chemical shift of the anomeric proton of the *Z*-isomer will be greater than that of the *E*-isomer as a result of deshielding caused by the carbonyl group; the chemical shift of the anomeric carbon of the *Z*-isomer will be less than that of the E-isomer. In our experience, the protected products such as **5** can exhibit fluxional behavior, presumably through the interconversion of rotamers, and this resulted in broad signals in the NMR spectra at room temperature. The signals in ^1H and ^{13}C NMR spectra of **5** recorded in C_5D_5N at 100 °C did coalesce and were consistent with the structure proposed. The signals in the ^1H- and ^{13}C NMR spectra of unprotected **4**, **6**, and **7**−**9** in D_2O at 10−40 °C were not broadened, and this simplified the determination of the preferred solution structures. The resonances for the anomeric proton were generally doublets and had coupling constants consistent with assignment of the *β*-configuration; the signals and NOE enhancements observed for the ring protons confirmed that the $^4C_1$ conformation is favored for the pyranose in each case. The NMR spectra, recorded in D_2O, of **4**, **7**, and **8** clearly show two signal sets (Table 1); NOE and chemical shift data

Table 1. Selected NMR Data[a] for Glycosylamides

		δ H-1 (J_{1-2}, Hz)		δ C-1	
entry	compd	*E-anti*	*Z-anti*	*E-anti*	*Z-anti*
1	**4**[b]	5.06 (8.0)	5.45 (9.0)	89.0	85.3
2	**6**[c]	4.90 (9.0)		88.5	
3	**7**	4.98 (9.0)	5.40 (9.0)	87.5	82.5
4	**8**	4.71 (9.0)	5.63 (9.5)	88.0	82.7
5	**9**	4.90 (9.0)		88.5	

[a] NMR were recorded in D_2O from 10 to 40 °C. [b] Data corresponds to the galactose residue adjacent to the tertiary amide. [c] Data given for the major conformational isomer.

indicate that the *anti*- rather than *syn*-conformation is preferred. Therefore the signal sets can be assigned to *Z*- and *E-anti* isomers, and their respective proportions at equilibrium can be determined by integration of anomeric proton signals in the ^1H NMR spectra (**4**, 30:70; **7**, 45:55; **8**, 15:85). The NMR spectra for **6** (and also **9**) indicate that there is one major isomer present in solution; the *E-anti* conformation can be assigned to this isomer on the basis of the chemical shift of the anomeric proton (^1H NMR, *δ* 4.98, d, 40 °C). Also, a series of 1D NOE, 2D NOESY, and 2D ROESY experiments for **6** showed strong NOE enhancements (Figure 1) for the major isomer consistent with the amides adopting *E-anti* conformations. The presence of a minor isomer, where one amide adopts the *E-anti* and the other adopts the *Z-anti* conformation (Figure 2), can be

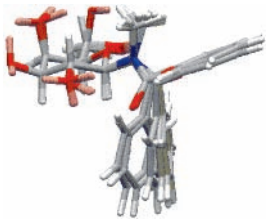


Figure 2. The 10 lowest energy conformations of **8** predicted by a Monte Carlo conformational search are shown; the pyranose ring atoms have been superimposed.

detected from the NMR data as there are two anomeric signals (^1H NMR, *δ* 5.90 and *δ* 4.90, 40 °C)[18] that show

(17) Related tertiary amides that have been prepared previously include *N*-alkylglucosyl(meth)acrylamides as surfactants and amphiphilic glycolipid analogues as stimulators of specific immune responses against antigens; see: Lockhoff, O.; Sadler, P. *Carbohydr. Res.* **1998**, *314*, 13 and ref 15.

clear cross-peaks with the anomeric signal at δ 4.98 in the NOESY and ROESY spectra; these cross-peaks have opposite sign to the NOE enhancements supporting exchange between the isomers.[19]

Conformational searches using Macromodel 6.0 were carried out for **8** (Figure 2).[20] The low energy structures found comprised *Z*- and *E*-*anti* isomers.[21]

On the basis of our interpretation of the data obtained we can propose that the major conformational isomer adopted by **6** in D_2O is either **6a** or **6b** (Figure 3). Both of these isomers contain a C-2 axis of symmetry; for **6a** the carbohydrates are in a *cis* arrangement, and for **6b** they are *trans*. Calculations similar to those carried out for **8** indicate that **6b** may be more stable, but there is no experimental evidence to support this. Further work is necessary to establish the structure of the preferred conformer, if indeed there is one. However, the preliminary data indicate that for **6** there is a significant population of isomers where the topographical arrangement between the carbohydrates is restricted. The biological recognition of this and related amides should be of interest and is under investigation.

In summary, multicomponent Ugi reactions have been used for convergent synthesis of divalent carbohydrate derivatives. Further work will be required to improve the yields of the reactions and to simplify the purification procedures. These compounds exhibit interesting conformational preferences. An interesting goal will now be to design and synthesize conformationally diverse libraries of divalent and higher order multivalent ligands where each member will have a restricted, yet different, topographical arrangement between the recognition components. Such libraries will be useful for further studies related to the mechanism of action of multivalent ligands. They will also aid the development of potent carbohydrate-based therapeutics. This work is currently underway.

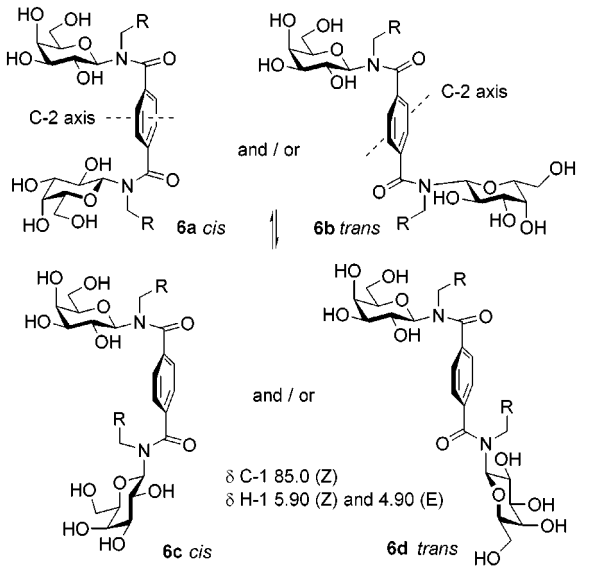


Figure 3. NMR indicates that an 83:17 mixture of *E*/*E* (**6a**/**6b**) and *Z*/*E* isomers (**6c**/**6d**) is present at 40 °C in D_2O.

Acknowledgment. The authors thank Enterprise Ireland, Pfizer Pharmaceuticals Inc., Co. Cork for an Irish American Partnership; DAAD; HRB/Wellcome Trust, Dr. Martin Walsh and Professor Richard J. K. Taylor.

Supporting Information Available: Experimental procedures for synthesis of **4** and **6**; 1D and 2D NMR spectra for **4** and **6**−**9**; and X-ray crystallography statistics for aromatic tertiary amides from CCDC. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) At 10 °C these signals are doublets at δ 5.58 and δ 4.60.

(19) Exchange between *Z*- and *E*-*anti* conformations has been observed also for **8** from NOE and variable temperature NMR experiments.

(20) Calculations were carried out on an O^2 silicon graphics workstation using Macromodel 6.0. Monte Carlo conformational searches were carried out using the SUMM method and the all-atom amber Force Field.

(21) It was noted that the aromatic ring was not planar to the carbonyl group in the calculated structures. X-ray crystallographic data indicate this is preferred. See Supplementary Information for statistics on data deposited at the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.