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Synthesis of a novel dioxan sialic acid analog ☆

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Abstract—A preparative scale synthesis of a dioxan sialic acid analog was achieved from D-mannose. The conformation and the acidic character of this dioxan derivative, closely related to sialic acid, provides a scaffold for drug design.

Cell-surface glycoproteins are involved in a wide variety of biological cellular events[1] such as the specificity of cell function, cell–cell communication, cell–cell/cell-extracellular matrix recognition, or migration events, as well as transport mediation.

Glycoproteins comprising a terminal sialylated tetrasaccharide (sialyl lewis x or a sialylated epitope) are implicated in physiological and pathological situations[2] such as embryogenesis, cell differentiation, inflammation[3] (recruitment of leukocytes), cancer (metastasis),[4] *influenza* virus mediated infection, or immune-defense system detoxification.[5] Inhibition of adhesion events has been attempted via the design of selectin or sialyl Lewis x antagonists (tetrasaccharides),[6] sialyltransferase inhibitors (bisphosphates),[7] and half-chair oxonium transition state mimics.[8] Therefore, we proposed to construct compound **4**, a conformationally stable mimic of **2**, incorporating the key structural elements for sialyltransferase recognition[9] such as the strong acidic character of anomeric carboxylic acid ($pK_a \sim 3$), the glycerol side chain, the N-acetamide functionality[10] and removal of the nonessential hydroxyl at C_4 (Fig. 1).[2,7b]

In order to validate this hypothesis, various simplified monosubstituted cyclic ketals **5** and **6** were synthesized (Scheme 1).[11] Their probable pK_a's were calculated[12] and for the most interesting ones, pK_a's were determined experimentally[13] (Table 1). The various combinations, dioxane, dithiane, and hemithioacetal ring systems, appeared to have the lowest pK_a values, close to the pK_a of sialic acid. Moreover, the dioxane arrangement seemed the most appropriate since the cyclization proceeded in a stereospecific manner, leading solely to the requisite isomer. The stereoelectronic effect of the two oxygens, enabling electronic delocalization, favor an

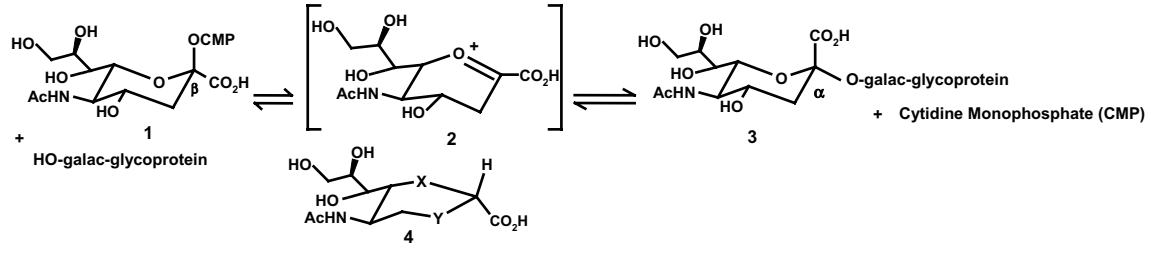

Figure 1. Sialyltransferase transition state.

Keywords: Sialic acid; Sialyltransferase; Sialidase.
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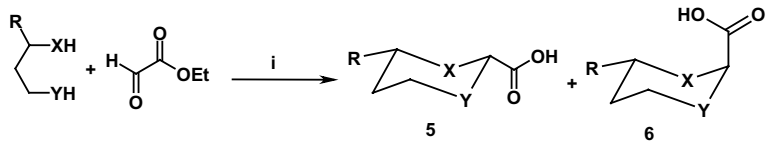


Scheme 1. Synthesis of various six membered-ring-[1,3]-heterocycles. Reagents and conditions: (i) (a) $BF_3{\cdot}OEt_2$, $HCOCO_2Et$, CH_2Cl_2; (b) LiOH, H_2O, MeOH.

Table 1. Structures and yields of compounds **5** and **6** and pK_a for compounds **5**

R	X	Y	Yield (%)	**5**/**6**[22]	pK_a (**5**)		Ref.
					Calcd	Found	
CH_3	O	O	75	100/0	3.07	3.4	24
C_3H_7	S	O	70	85/15	3.1	3	25
H	S	S	50	**5** ⇌ **6**[23]	3.03	ND	25
C_2H_5	NH	NH	50	100/0	3.88	ND	26
CH_3	O	NH	30	100/0	3.95	ND	26
Sialic acid	O	CH_2	—	—	2.3	3.2	—

equatorial conformational of the carboxylic acid substituent.[14] Therefore, compound **4** (X, Y = O) fulfilled the required electronic elements to mimic the transition state of sialic acid.

To elaborate the five chiral centers on the dioxane backbone, natural sugars were envisioned as starting materials (Fig. 2). Starting from D-mannose, the anomeric position was first protected as an allyl ether followed by sequential 4,6-benzylidene formation,[15] masking the dioxane oxygens (Scheme 2). The 2,3-oxygen dibenzylation gave compound **7a**, which was selectively deallylated and reduced at the anomeric position[16] to form diol **8a**. The primary alcohol was selectively silylated as **8b**, the benzylidene precursor of dioxane ring **4**. The transformation of the remaining secondary α-hydroxy into the corresponding acetamide with retention of configuration was achieved in four steps, involving alcohol oxidation, and oxime formation of the resulting ketone.[17] The oxime reduction proceeded stereospecifically, from the α-face, presumably governed by steric and stereoelectronic effects and by a possible

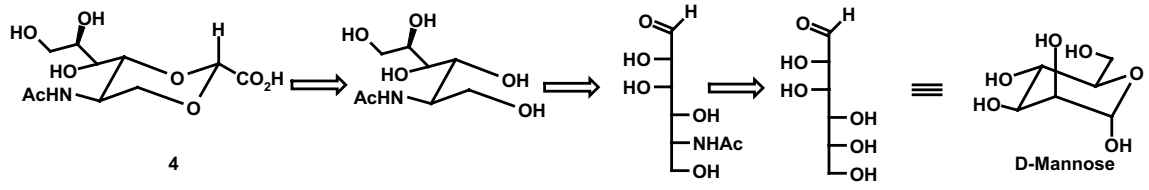
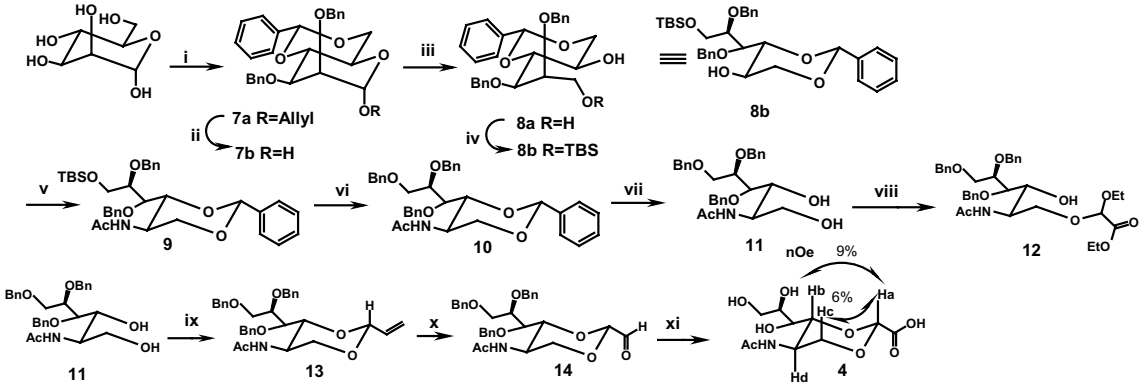


Figure 2. Retrosynthesis of compound **4**.



Scheme 2. Synthesis of compound **4**. Reagents and conditions: (i) (a) AcCl, allylOH, pyridine (quant.); (b) $PhCH(OMe)_2$, PTSA (90%); (c) BnBr, NaH, (90%). (ii) SeO_2, AcOH (60%). (iii) (a) $NaBH_4$, MeOH (90%). (iv) TBDMSCl, imidazole (92%). (v) (a) PCC, (90%); (b) NH_2OH, (90%); (c) $LiAlH_4$, Et_2O (80%); (d) AcCl, NEt_3 (90%). (vi) (a) TBAF, (70%); (b) BnBr, NaH, (90%). (vii) PTSA, MeOH (64%). (viii) $BF_3{\cdot}OEt_2$, $HCOCO_2Et$, CH_2Cl_2 (70%). (ix) acrolein, nBuSnCl$_3$ (80%). (x) (a) OsO_4, NMO, tBuOH, H_2O (83%); (b) $NaIO_4$, CH_2Cl_2 (quant.). (xi) (a) AgO, CH_3CN (40%); (b) H_2, Pd/C (90%), (quant.).

aluminum complexation by the ketal oxygens, yielding the desired isomer **9** in 72% yield, after acetylation of the crude amine. Replacement of the *O*-silyl by a *O*-benzyl gave the orthogonally protected intermediate **10**, which was hydrolyzed to the key diol **11** in 20% overall yield from mannose. When compound **11** was subjected to diethoxyacetic acid ethyl ester as in the model study, no cyclization occurred in the various acidic reaction conditions attempted, compatible with the benzyl group stability. The transient α-carbethoxy oxonium was rapidly trapped by the ethoxy counterion, producing the corresponding mixed acetal **12**. To successfully perform the cyclization, it was necessary to stabilize the oxonium intermediate via the appropriate introduction of a substituent, precursor of a carbethoxy functionality and allowing charge delocalization, such as a vinyl group. When diol **11** was combined with an excess of acrolein in the presence of catalytic butyltintrichloride, the cyclization was performed in excellent yield, giving the desired isomer **13** as a single product.[18] Conventional functional group manipulation involving osmylation of the exocyclic olefin, sodium periodate-catalyzed bond breaking to aldehyde **14**, and subsequent silver nitrate oxidation afforded the desired carboxylic acid **4** (Scheme 2).

Its conformation was assessed by NMR,[19a] with nuclear Overhauser effects (NOE) between 1,3-diaxial hydrogens, and an order of magnitude of 9–10 Hz of the transdiaxial coupling constants.[18] Moreover NMR titration confirmed an experimental pK_a value of 2.8, close to that of sialic acid. Indeed, molecular modeling (MOPAC calculations),[20] comforting these data, showed superimposition of compound **4** with sialic acid (Fig. 3).

As a preliminary evaluation of the enzymatic activity, compound **4** was assayed on purified α-2,6-sialyltransferase.[21] However, as with sialic acid, no inhibition was found up to 1 mM. Indeed, the only active inhibitors described are the bisubstrate transition state mimics[6,7] containing either a bisacid functionality on the sialic acid backbone, reminiscent of CMP-sialic acid substrate, and/or a sugar-like substituent. Incorporation of the dioxan sialic acid moiety **4** at the terminus of sialyl-conjugates as new bisubstrate analogs is underway.

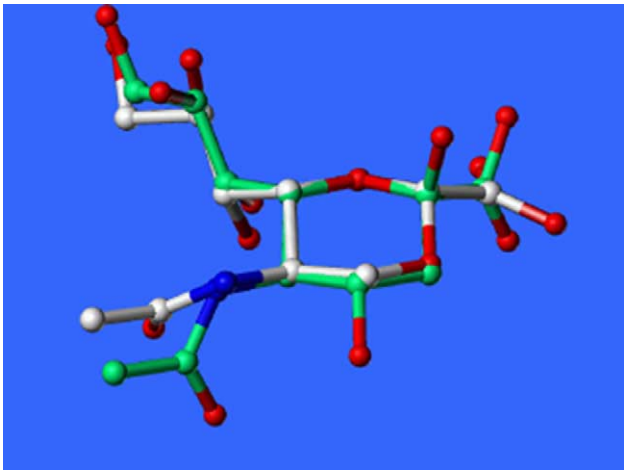


Figure 3. Comparison of sialic acid (*green*) and [1,3] dioxane analog **4** (*white*).

Supplementary material: spectroscopic and physical characterization data for compound **4** (^{1}H, ^{13}C NMR spectra, IR, CHN, MH, optical rotation).

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References and notes

1. (a) Reutter, R.; Köttgen, E. K.; Bauer, C.; Gerok, W.; Biological, J. Significance of Sialic Acids. In *Sialic Acids Chemistry, metabolsm and Function, Cell Biology monographs*; Schauer, R., Ed.; Springer: Wien, 1982; Vol. 10, pp 263–305; (b) Varki, A. *Glycobiology* **1993**, *3*, 97–130; (c) Hodgson, J. *Biotechnology* **1991**, *9*, 609–613; (d) Neel, D.; Aubery, M.; Derappe, C. *Medicine/Sciences* **1992**, *8*, 233–238.
2. (a) Schauer, R. *Trends Biol. Sci.* **1985**, 357–360; (b) Kelm, S.; Schauer, R.; Croker, P. *Glycoconjugate J.* **1996**, *13*, 913–926; (c) Schauer, R. Sialic Acids Regulate Cellular and Molecular Recognition. In *Carbohydrates*; Ogura, H., Hasegawa, A., Suami, T., Eds.; VCH: Weinheim–New York, 1992; pp 340–401.
3. Musser, J. H.; Anderson, M. B.; Levy, D. E. *Curr. Pharm. Des.* **1995**, *1*, 221–232.
4. (a) Fukuda, M. *Cancer Res.* **1996**, *56*, 2237–2244; (b) Brooks, P. C. *Cancer Metastasis Rev.* **1996**, *15*, 187–194; (c) Brocke, C.; Kunz, H. *Bioorg. Med. Chem.* **2002**, *10*, 3085–3112.
5. (a) Burmeister, W.-P.; Daniels, R. S.; Dayan, S.; Gagnon, J.; Cusack, S.; Ruigrok, R. W. H. *Virology* **1991**, *180*, 266–272; (b) 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.; Penn, C. R. *Nature* **1993**, *363*, 418–423.
6. (a) Wong, C.-H.; Moris-Varas, F.; Hung, S.-C.; Marron, T. G.; Lin, C.-C.; Gong, K. W.; Weitz-Schmidt, G. *J. Am. Chem. Soc.* **1997**, *119*, 8152–8158; (b) Lin, C.-C.; Moris-Varas, F.; Weitz-Schmidt, G.; Wong, C.-H. *Bioorg. Med. Chem.* **1999**, 425–433; (c) Kaila, N.; Thomas, B. E. *Med. Res. Rev.* **2002**, *22*(6), 566–601.
7. (a) Amann, F.; Schaub, C.; Müller, B.; Schmidt, R. R. *Chem. Eur. J.* **1998**, *4*, 1106–1115; (b) Schröder, P. N.; Giannis, A. *Angew. Chem., Int. Ed.* **1999**, *38*, 1379–1380; (c) Whalen, L. J.; McEvoy, K. A.; Halcomb, R. L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 301–304; (d) Wang, X.; Niu, Y.; Cao, X.; Zhang, L.; Zhang, L.-H.; Ye, X.-S. *Bioorg. Med. Chem.* **2003**, *11*, 4217–4224; (e) Wang, X.; Zhang, L.-H.; Ye, X.-S. *Med. Res. Rev.* **2003**, *23*, 32–47.

8. (a) *Drug Future* **1996**, *21*, 375–382; (b) Florio, P.; Thomson, R. J.; Alafaci, A.; Abo, S.; von Itzstein, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2065–2068; (c) Smith, P. W.; Sollis, S. L.; Howes, P. D.; Cherry, P. C.; Starkey, I. D.; Cobley, K. N.; Weston, H.; Scicinski, J.; Merrit, A.; Whittington, A.; Wyatt, P.; Taylor, N.; Green, D.; Bethell, R.; Madar, S.; Fenton, R. J.; Morlay, P. J.; Pateman, T.; Beresford, A. *J. Med. Chem.* **1998**, *41*, 787–797.
9. (a) Horenstein, B. A.; Bruner, M. *J. Am. Chem. Soc.* **1996**, *118*, 10371–10379; (b) Brunner, M.; Horenstein, B. A. *Biochemistry* **1998**, *37*, 289–297.
10. Gross, H. J.; Brossmer, R. *Glycoconjugate J.* **1995**, *12*, 739–746.
11. All synthetic intermediates and final compounds gave satisfactory ^{1}H NMR, ^{13}C NMR, and/or mass spectral data, in full agreement with their assigned structures and stereochemistries.
12. pK_a were obtained via quantum calculations calibrated with known carboxylic acids; Gaussian 92/DFT, Revision F.3: Frisch, M. J.; Trucks, G. W.; Schlegel H. B.; Gill, P. M. W.; Johnson, B. G.; Wong, M. W.; Foresman, J. B.; Robb, M. A.; Head-Gordon, M.; Replogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R.L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A., Gaussian, Inc., Pittsburgh PA, 1993.
13. Experimental pK_a's were found by NMR titration (solvent D_2O): Wüthrich, K. In *NMR in Biological Research: Peptides and Proteins*; Elsevier: New York, 1976.
14. (a) Eliel, E. L.; Carmeline Knoeber, Sr.-M. *J. Am. Chem. Soc.* **1968**, *90*, 3444–3458; (b) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon: New York, 1983.
15. Winnik, F. M.; Carver, J. P.; Krepinsky, J. J. *J. Org. Chem.* **1982**, *47*, 2701–2707.
16. Gigg, R.; Warren, C. D. *J. Chem. Soc.* **1965**, 2205–2210.
17. Horton, D.; Weckerle, W. *Carbohydr. Res.* **1975**, *44*, 227–240.
18. Marton, D.; Slaviero, P.; Tagliavini, G. *Gazz. Chim. Ital.* **1989**, *119*, 359–361.
19. (a) Nuclear Overhauser effects (NOE) were measured using the DPFGSE-NOE sequence[a]. NMR experiments were carried out in D_2O, at a temperature of 37 °C and using a mixing time of 1 s. Integration of NOE signals was performed after an exponential multiplication (lb = 0.3 Hz) and a baseline correction, Stott, K.; Stonehouse, J.; Keeler, J.; Hwang, T.-L.; Shaka, A. J. *J. Am. Chem. Soc.* **1995**, *112*, 4199–4200; (b) H^1 NMR (500 MHz, DMSO d^6): $J_{bd} = 10$ Hz, $J_{cd} = 12$ Hz; $[\alpha]_D^{20}$ −44 (*c* 1 in H_2O).
20. Stewart James, J. P. MOPAC a Semiempirical Molecular Orbital Program (Special Issue). *J. Comput. Aided Mol. Des.* **1990**, *4*(1), 1–105.
21. Fluorometric assay for inhibition of α-2,6-sialyltransferase activity: (a) inhibition of α-2,6-sialyltransferase activity was performed based on the 96-well radioactive assay[a] and modified to use a fluorescent donor.[b] Inhibitors were added to a reaction mixture containing 2 mg/mL asialofetuin as acceptor, 0.5 mU/mL purified rat liver α-2,6-sialyltransferase (Boehringer M. 981 583) and 1 μM CMP-9-fluoresceinyl-Neu5Ac. After 1 h incubation at 37 °C, fluorescence of the reaction product was read at 530 nm. Laroy, W.; Maras, M.; Fiers, W.; Contreras, R. *Anal. Biochem.* **1997**, *249*, 108–111; (b) Gross, H. J.; Sticher, U.; Brossmer, R. *Anal. Biochem.* **1990**, *186*, 127–134.
22. The ratio of compounds **5** and **6** was determined on the amount of each compound isolated by flash chromatography (silica gel).
23. Coalescence was observed by ^{1}HNMR (CD_2Cl_2 400 MHz) at 27 °C. Moreover, ^{13}CNMR (CD_2Cl_2 100.6 MHz) at −90 °C showed, by integration of ^{13}CNMR signal areas, a 1/9 ratio of compounds **5/6** in favor of the axial conformation.
24. Suemune, H.; Tanaka, N.; Sakai, K. *Chem. Parm. Bull.* **1990**, *38*, 3155–3157.
25. (a) Tschierske, C.; Köhler, H.; Zaschke, H.; Kleinpeter, E. *Tetrahedron* **1989**, *45*, 6987–6998; (b) Eliel, E. L.; Rao, V. S.; Riddell, F. G. *J. Am. Chem. Soc.* **1976**, *98*, 3583–3590.
26. A solution of the amine (1 equiv) ethyl glycoxylate (1.2 equiv) and paradisulfonic acid (0.01 equiv) was refluxed in toluene until complexion of the reaction.