significant distortions along the three orthogonal axes defined by the three pairs of metal centers. It is, however, clear that the high-frequency modes at 1470 and 1570 cm^{-1} have been shifted to lower frequency by 30 cm^{-1} as opposed to less than 10 cm^{-1} for the monosubstituted systems. This is consistent with the increased π backbonding brought on by the six metal centers.

In conclusion, the Raman spectra of organometallic complexes involving C_{60} provide a clear probe into the symmetry reduction upon reaction of the C_{60} molecular system. This reduction in symmetry results in the observation of both "silent" modes and the splitting of degenerate modes in the Raman spectrum. As the degree of substitution is increased, the perturbations of the C_{60} vibrational modes become stronger and the correlations with the original vibrational spectrum of the parent become less clear. The spectra reported here should be useful benchmarks in characterizing structurally related C_{60} derivatives.

Acknowledgment. We acknowledge the assistance of B. Malone and E. Holler for the synthesis and purification of C_{60}.

Communications to the Editor

Synthesis and Conformational Analysis of GM_3 Lactam, a Hydrolytically Stable Analogue of GM_3 Ganglioside Lactone

Asim K. Ray, Ulf Nilsson, and Göran Magnusson*

Organic Chemistry 2, Chemical Center
Lund Institute of Technology, University of Lund
P.O. Box 124, 221 00 Lund, Sweden

Received November 12, 1991

Gangliosides have been found to occur in conjunction with the corresponding lactones (e.g., **1**) in brain tissue[1] and in the membranes of tumor cells.[2] The lactones are unstable at neutral pH. We now report the synthesis of the stable lactam **3** and derivatives corresponding to the natural GM_3 lactone. The conformations of **1** and **3** are similar, thus making the lactams potential substitutes for ganglioside lactones in biomedical research. The lactams might be natural products yet to be discovered.

The lactone of GM_3 ganglioside (**1**, "GM_3 lactone") has been suggested to be a tumor-associated antigen on cells of an experimental mouse melanoma.[2] In a comparative immunization with GM_3 ganglioside and **1**, it was shown that the latter was the stronger immunogen, and it was suggested that it could be the real immunogen despite being a minor membrane component.[2] The reactivity of a monoclonal anti-melanoma antibody (M2590) with various cells and liposomes was shown[2] to depend in a threshold, all-or-none fashion on the concentration of GM_3 ganglioside in the cell membrane or liposome. The antibody was found to cross-react with **1**.

Acidic conditions favor the lactone in the equilibrium GM_3 ganglioside $\rightleftarrows$ GM_3 lactone.[3] Since GM_3 ganglioside is an acidic glycolipid, it might induce its own lactonization when the concentration is high in a cell membrane or liposome. This can help to explain the threshold effect described above and may have implications for other sialic acid-containing saccharides.

The equilibrium concentration of GM_3 lactone is low at close-to-neutral pH, and it may therefore be a rather inefficient immunogen. In contrast, the corresponding lactam (cf. **3**, "GM_3 lactam") is perfectly stable at neutral pH and should be a good lactone substitute, provided that the overall shape of lactone and lactam is similar. We report the synthesis and conformational analysis of the 2-(trimethylsilyl)ethyl[4] (TMSEt) glycoside of GM_3 lactam **3** as well as the spacer glycoside **11** and the neoglycoprotein **12**.

The TMSEt 2′-azidodeoxylactoside **4** {$[\alpha]^{22}_D$ +19° (*c* 1, $CDCl_3$); ^1H NMR δ 4.40, 4.31 (H-1,1′)} was synthesized in 37% overall yield by glycosylation of 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[4] with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide[5] followed by deacetylation, formation of the 3′,4′-acetonide, benzylation of the 6′-position, and removal of the acetonide group. Glycosylation of **4** with the *N*-acetylneuraminic acid derivative **5**[6] according to the method of Lönn et al.[7] gave the trisaccharide derivative **6** in high yield {71%, $[\alpha]^{22}_D$ −13° (*c* 1, $CDCl_3$); ^1H NMR δ 4.50, 4.40 (H-1′,1)} together with the β-anomer (4%). The α-configuration of **6** was determined by NMR[8] spectroscopy ($J_{C1-H3ax}$ = 6.15 Hz). Nickel boride ($NaBH_4/NiCl_2{\cdot}6H_2O/H_3BO_3$) reduction of **6**, O-deacetylation of the resulting crude amine, treatment with pyridine to effect the lactam ring closure, and hydrogenolysis of the benzyl protecting groups gave **3** {54%, $[\alpha]^{22}_D$ −22.3° (*c* 0.7, MeOH); ^1H NMR δ 4.69, 4.47 (H-1′,1), 4.32 (H-4″), 3.23 (H-2), 2.59 (H-3″eq), 2.02 (NHAc), 1.67 (H-3″ax); *m/z* calcd for $C_{28}H_{51}O_{17}N_2Si$ (M + H) 715.2957, found 715.2958} after chromatographic purification. Compound **3** was acetylated to give **7** {98%, $[\alpha]^{29}_D$ −32° (*c* 0.8, $CDCl_3$); ^1H NMR δ 4.54, 4.40 (H-1,1′)}. The TMSEt glycoside **7** was transformed[9] into the α-chloro derivative **8** [100%; ^1H NMR δ 6.20 (H-1)]. Glycosylation of 2-bromoethanol with **8** gave the 2-bromoethyl glycoside **9** {54%, α/β 15:85; $[\alpha]^{25}_D$ −27° (*c* 1.2, $CDCl_3$); ^1H NMR δ 4.78 (H-1′)}. Glycoside **9** was treated[10] with methyl 3-mercaptopropionate to give **10** {82%, $[\alpha]^{22}_D$ −23° (*c* 1.1, $CDCl_3$); ^1H NMR δ 4.63 (H-1′), 3.69 (COOMe)}. Deacetylation of **10** gave the spacer glycoside **11** {86%, $[\alpha]^{25}_D$ −0.1° (*c* 0.5, MeOH); ^1H NMR δ 4.92, 4.69, 4.48 (H-1α, H-1′, H-1β)}, which was used for coupling[10] to bovine serum albumin to give the neoglycoprotein **12** (~21 mol of **11** per mol of BSA according to sulfur analysis[10]), useful as an antigen for immunization purposes (Scheme I).

A conformational analysis of GM_3 lactone (**1**), based on NMR data, was reported by Yu et al.[11] A highly rigid structure was proposed, where the lactone ring occupies a chairlike conformation. However, we suggest that a boatlike conformation is instead preferred: (i) both the lactones (**1** and **2**[12]) and the lactam (**3**) show a deshielding of H-4″ as compared to the open-form GM_3 ganglioside (δ 3.55 for GM_3 ganglioside and 4.12 for **1**, both in

(1) Gross, S. K.; Williams, M. A.; McCluer, R. H. *J. Neurochem.* **1980**, *34*, 1351-1361.
(2) Nores, G. A.; Dohi, T.; Taniguchi, M.; Hakomori, S.-I. *J. Immunol.* **1987**, *139*, 3171-3176.
(3) Wiegandt, H. *Ergeb. Physiol. Biol. Chem. Exp. Pharmacol.* **1966**, *57*, 190-222.
(4) Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G.; Dahmén, J.; Noori, G.; Stenvall, K. *J. Org. Chem.* **1988**, *53*, 5629-5647.
(5) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244-1251.
(6) Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1989**, *187*, 35-42.
(7) (a) Birberg, W.; Lönn, H. *Tetrahedron Lett.* **1991**, *32*, 7453-7456; 7457-7458. (b) Lönn, H.; Stenvall, K. *Tetrahedron Lett.* **1992**, *33*, 115-116.
(8) Hori, H.; Nakajima, T.; Nishida, Y.; Ohrui, H.; Maguro, H. *Tetrahedron Lett.* **1988**, *29*, 6317-6320.
(9) Jansson, K.; Noori, G.; Magnusson, G. *J. Org. Chem.* **1990**, *55*, 3181-3185.
(10) Dahmén, J.; Frejd, T.; Magnusson, G.; Noori, G.; Carlström, A.-S. *Carbohydr. Res.* **1984**, *127*, 15-25; *127*, 27-33; *129*, 63-71.
(11) Yu, R. K.; Koerner, T. A. W.; Ando, S.; Yohe, H. C.; Prestegard, J. H. *J. Biochem.* **1985**, *98*, 1367-1373.
(12) Gift from Dr. J. Dahmén, Symbicom AB, Ideon Research Park, Lund, Sweden.

0002-7863/92/1514-2256$03.00/0

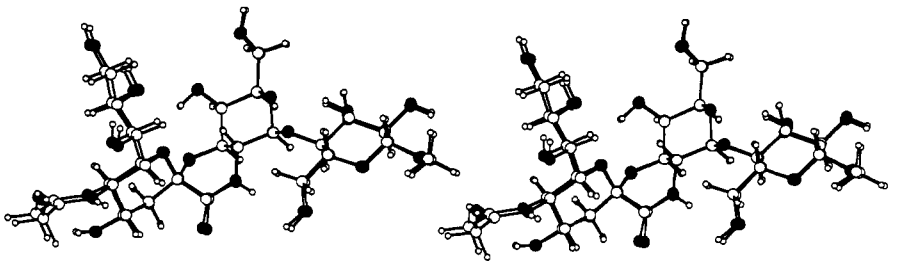
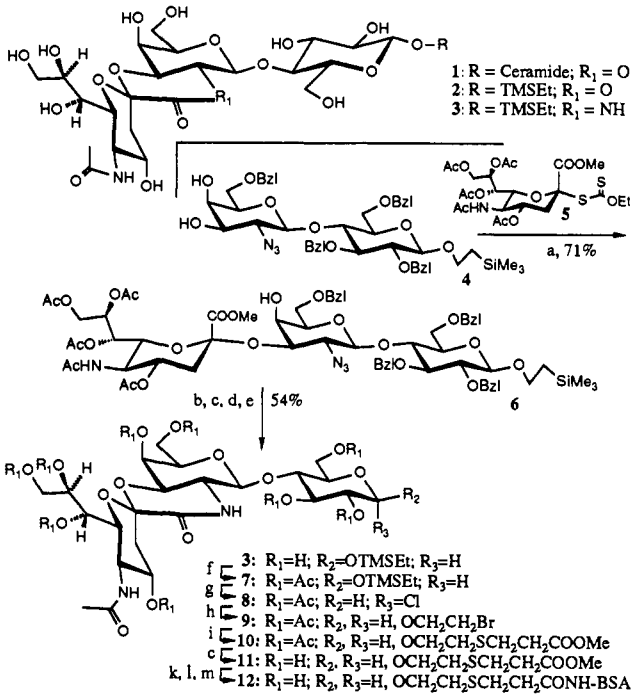


Figure 1. Stereoview of the superimposed low-energy [MM2(91)] boatlike conformers of the methyl glycosides that correspond to **2** and **3**.

Scheme I[a]



[a](a) $CF_3SO_3Ag/MeSBr/CH_3CN/CH_2Cl_2$/molecular sieves (3-Å)/2 h, then iPr_2NH/−78 °C/1 h. (b) $NiCl_2 \cdot 6H_2O/H_3BO_3/NaBH_4$/EtOH/0 °C/20 min. (c) MeONa/MeOH/22 °C/12 h. (d) Pyridine/22 °C/12 h. (e) H_2/Pd–C, 10%/1 atm. (f) Ac_2O/pyridine-/22 °C/24 h. (g) $Cl_2CHOMe/ZnCl_2/CHCl_3$/22 °C/12 h. (h) $BrCH_2CH_2OH/CF_3SO_3Ag$/molecular sieves (4 Å)/CH_2Cl_2/N_2/−28 → 22 °C/16 h. (i) $HSCH_2CH_2COOMe/Cs_2CO_3/N_2$/DMF/22 °C/2.5 h. (k) H_2NNH_2/EtOH/22 °C/12 h/freeze drying. (l) *t*-BuO-NO/HCl–dioxane/$HOSO_2NH_2$/DMSO. (m) BSA/pH ~9 buffer/22 °C/16 h/dialysis and freeze drying.

DMSO-d_6/D_2O 98:2[11]; 4.28 for **2**, 4.32 for **3**, and 4.32 for **11**, all in D_2O). Such deshielding has been observed[13,14] for ring protons that are in van der Waals contact (≤2.7 Å) with a hydroxyl, carbonyl, or ether oxygen atom. According to MM2 calculations, it is only the boatlike conformations of GM_3 lactone and lactam that place H-4″ in van der Waals contact with the carbonyl oxygen atom. (ii) In the chairlike conformation (Dreiding models), H-3″eq and H-3′ are closely situated (~2.2 Å); however, no strong NOE effect was observed, thus supporting the boatlike conformation (a ROESY experiment suggested a H-3″eq/H-3′ distance of 3.7–4.2 Å). (iii) A molecular mechanics [MM2-(91)[15,16]] calculation, where both a chair and a boat were used as starting conformations, resulted in boatlike conformations in both cases, with lactones as well as lactams (H-3″eq/H-3′ distance ~4 Å).

Superimposition and RMS fitting[16] of the low-energy boat conformations of the methyl glycosides corresponding to **2** and **3** (using all ring atoms) showed them to have very similar overall shapes (RMS = 0.097 Å), as depicted in Figure 1.

Acknowledgment. This work was supported by the Swedish Natural Science Research Council.

(13) Thögersen, H.; Lemieux, R. U.; Bock, K.; Meyer, B. *Can. J. Chem.* **1982**, *60*, 44–57.

(14) (a) Bock, K.; Frejd, T.; Kihlberg, J.; Magnusson, G. *Carbohydr. Res.* **1988**, *176*, 253–270. (b) Rehnberg, N.; Sundin, A.; Magnusson, G. *J. Org. Chem.* **1990**, *55*, 5477–5483.

(15) Burkert, U.; Allinger, N. L. *Molecular Mechanics*; American Chemical Society: Washington, DC, 1982.

(16) Molecular construction and energy minimization (dielectric constant was set at 80) were made with the MacMimic/MM2(91) package: InStar Software, Ideon Research Park, S-22370 Lund, Sweden.

Highly Enantioselective Protonation Catalyzed by an Antibody[1]

Jean-Louis Reymond,* Kim D. Janda,* and Richard A. Lerner*

The Scripps Research Institute
Departments of Molecular Biology and Chemistry
10666 North Torrey Pines Road
La Jolla, California 92037
Received December 4, 1991

One of the goals of antibody catalysis[2] is to facilitate unique chemical transformations. Herein we report an antibody which catalyzes the carbon protonation of a prochiral enol ether. This catalytic system allows highly enantioselective proton delivery, thereby accomplishing a reaction which to date has remained difficult for conventional organic chemistry.

The enantioface selective protonation of prochiral enol derivatives is a very simple and attractive route for the preparation of optically active carbonyl compounds. Examples have been reported where stoichiometric protonation of a metal enolate by a chiral proton source at low temperature leads to optical yields from 20 to 85% ee.[3] Enantioselectivities between 41 and 96% ee for enol protonation were reported for the yeast esterase catalyzed hydrolysis of 1-acetoxycycloalkenes.[4] Recently, an antibody from our laboratories was shown to catalyze a similar transformation with 42% ee.[5] All of these reactions involved enolates under basic conditions. The acid-promoted hydrolysis of enol ethers is an interesting alternative which has not been investigated for enantioselectivity.[6] Hydrolysis of enol ethers

(1) This research was supported in part by the Fonds National Suisse pour la Recherche Scientifique (J.-L.R.) and The National Institutes of Health (GM-43858, K.D.J.). We wish to thank Dee-Hua Huang for the ^1H NMR experiments.

(2) Review: Lerner, R. A.; Benkovic, S. J.; Schultz, P. G. *Science* **1991**, *252*, 659.

(3) (a) Review: Fehr, C. *Chimia* **1991**, *45*, 253. (b) Duhamel, L.; Plaquevent, J.-C. *J. Am. Chem. Soc.* **1978**, *100*, 7415. (c) Gerlach, U.; Hünig, S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1283. (d) Fehr, C.; Galindo, J. *J. Am. Chem. Soc.* **1988**, *110*, 6909. (e) Potin, D.; Williams, K.; Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1420. (f) Piva, O.; Pete, J.-P. *Tetrahedron Lett.* **1990**, *31*, 5157. (g) Matsumoto, K.; Ohta, H. *Tetrahedron* **1991**, *32*, 4729.

(4) Matsumoto, K.; Tsutsumi, S.; Ihori, T.; Ohta, H. *J. Am. Chem. Soc.* **1990**, *112*, 9614.

(5) Fujii, I.; Lerner, R. A.; Janda, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 9528.

(6) A related reaction is the diastereoselective hydrolysis of glycals catalyzed by glycosidases. (a) Lehmann, J.; Zieger, B. *Carbohydr. Res.* **1977**, *58*, 73. (b) Hehre, E. J.; Genghof, D. S.; Steinlicht, D. S.; Brewer, C. F. *Biochemistry* **1977**, *16*, 1780. (c) Chiba, S.; Brewer, C. F.; Okada, G.; Matsui, H.; Hehre, E. J. *Biochemistry* **1988**, *27*, 1564.

0002-7863/92/1514-2257$03.00/0