A Striking Example of the Interfacing of Glycal Chemistry with Enzymatically Mediated Sialylation: A Concise Synthesis of GM_3

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Gangliosides are sialic acid-containing glycolipids present in high concentrations on the cell surface of central nervous system cells where they may play a role in the process of signal transduction through the cell membrane.[1,2] Recent studies have tended to implicate gangliosides in other types of important biological settings such as cell–cell adhesion,[3,4] malignancy,[3,4] and cell growth regulation.[5] They have also been identified as tumor-associated antigens[6] and cell differentiation markers.[7] Ganglioside GM_3 was first isolated from equine erythrocytes by Yamakawa's group in 1952.[8] It has been shown to serve as the precursor for many complex gangliosides in the biosynthetic pathway[9] and is known to modulate the epidermal growth factor (EGF) and the platelet-derived growth factor (PDGF) receptors.[10] GM_3 was also found to be expressed in abnormally high concentration in tumor cells.[11] Given the extreme difficulty of isolating homogeneous gangliosides from natural sources,[12] effective syntheses are much to be desired.

Below we describe a straightforward synthesis of GM_3[13] starting with lactal (**1a**).[14] Our pathway exploits recent novel findings from enzymology and chemistry. *Remarkable for the field of ganglioside synthesis is the complete avoidance by this route of any selective protection–deprotection maneuvers.* All of the blocking groups are uniformly and trivially introduced and removed. A key discovery which makes the synthesis possible is that the anhydrosugar **2** reacts with the stannyl alkoxide **3a** (derived in situ from **3**)[15] to produce selectively the "pre-ceramide" β-glycoside (see **2** + **3a** → **4**, Scheme I). This type of stereospecific transformation is an important advance in the field of glycolipid synthesis. Another crucial discovery was that compound **4** is a competent substrate for enzymatically mediated sialylation at C3 (see **4** + **6** → **7**, Scheme I).[16] The synthesis is described below.

Pertriethylsilylation of lactal (**1a**) affords **1b** (81%) which, upon reaction with 2,2-dimethyldioxirane,[17] gives rise to 1,2-anhydrolactal derivative **2**. Attempts to use **2** as a glycosyl donor with **3** by application of our earlier methodology (with anhydrous zinc chloride as the promoter) gave disappointing results. Perhaps the presence of a diol linkage in the ceramide precursor **3** interferes with the ability of zinc chloride to orchestrate glycosidation in this case. Fortunately a new procedure, which avoided the need for differential protection of the diol, was developed. Treatment of the product (cf. **3a**) generated in situ from the reaction of **3** with bis(tributyltin) oxide with **2** in the presence of zinc triflate followed by exhaustive desilylation produced stereospecifically a β-glycoside (crude **4**). This purification was conducted into octaacetate **5** (44% yield from **1b**). A minor product (ca. 5%) derived from glycosidation of the C3 hydroxyl of **3** was identified. Per-deacylation of **5** provides homogeneous **4** in 95% yield.

The plan now contemplated introduction of the sialic acid residue by enzymatically mediated transfer from CMP–Nu5Ac (**6**)[18] using α-2,3-sialyltransferase.[13c,19] We noted that Paulson had reported that lactosyl ceramide is not a competent substrate toward this enzyme.[20] *However, we hoped that the less hydrophobic 4 would be tolerated by the transferase.* In the event, this hope was realized. Compound **4** indeed did accept sialic acid from CMP–Nu5Ac (**6**) under the conditions shown to produce **7** (75%). GM_3 was isolated in 40% yield from **7** via (i) reduction of the azido linkage and (ii) stearoylation.[21]

The utilization of the glycal linkage in the readily available lactal (**1a**) renders it a most attractive starting material for the construction of biologically important glycoconjugates (including adhesion molecules[22] and glycopeptides[23]). The use of epoxide **2** as a glycosyl donor avoids the need for a multistep installation of a unique directing group at C_2. The interfacing of emerging glycal chemistry with enzymatically driven processes elsewhere in the molecule holds considerable promise for massive simpli-

(1) *New Comprehensive Biochemistry*; Neuberger, A.; van Deenen, L. L. M., Eds.; Elsevier: Amsterdam, 1985; Vol. 10, pp 199–260.
(2) Fishman, P. H. *J. Membr. Biol.* **1982**, *69*, 85.
(3) Taki, T.; Hirabayashi, Y.; Ishikawa, H.; Ando, S.; Kon, K.; Tanaka, Y.; Matsumoto, M. *J. Biol. Chem.* **1986**, *261*, 3075.
(4) Hirabayashi, Y.; Hyogo, A.; Nakao, T.; Tsuchiya, K.; Suzuki, Y.; Matsumoto, M.; Kon, K.; Ando, S. *J. Biol. Chem.* **1990**, *265*, 8144.
(5) (a) Matta, S. G.; Yorke, G.; Roisen, F. J. *Develop. Brain Res.* **1986**, *27*, 243. (b) Ledeen, R. W. *J. Neurosci. Res.* **1984**, *12*, 147.
(6) (a) Higashi, H.; Hirabayashi, Y.; Fukui, Y.; Kaiki, M.; Matsumoto, M.; Ueda, S.; Kato, S. *Cancer Res.* **1985**, *45*, 3796. (b) Hakomo, S. *Annu. Rev. Immunol.* **1984**, *2*, 103.
(7) (a) Ebel, F.; Schmitt, E.; Peter-Katalinic, J.; Kniep, B.; Muhlradt, P. F. *Biochemistry* **1992**, *31*, 12190. (b) Feizi, T. *Nature* **1985**, *314*, 53.
(8) Yamakawa, T.; Suzuki, S. *J. Biochem. (Tokyo)* **1952**, *39*, 383.
(9) Bouhours, D.; Bouhours, J.-F. *J. Biol. Chem.* **1991**, *266*, 12944.
(10) (a) Bremer, E.; Schlessinger, J.; Hakomori, S. *J. Biol. Chem.* **1986**, *261*, 2434. (b) Hanai, N.; Nores, G. A.; Torres-Mendez, C.-R.; Hakomori, S. *Biochem. Biophys. Res. Commun.* **1987**, *147*, 127.
(11) (a) Carubia, J. M.; Yu, R. K.; Macala, L. J.; Kirkwood, J. M.; Varga, J. M. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 500. (b) Portoukalian, J.; Zwingelstein, G.; Dore, J.-F.; Borgoin, J.-J. *Biochemie* **1976**, *58*, 1285.
(12) Ando, S.; Waki, H.; Kon, K.; Kishimoto, Y. In *Gangliosides and Modulation of Neuronal Functions*; Rahmann, H., Ed.; Springer-Verlag: Berlin-Heidelberg, 1987; pp 167–177.
(13) For previous total syntheses of GM_3: (a) Sugimoto, M.; Ogawa, T. *Glycocojugate J.* **1985**, *2*, 5. (b) Murase, T.; Ishida, H.; Kiso, M.; Hasewawa, A. *Carbohydr. Res.* **1989**, *188*, 71. (c) Ito, Y.; Paulson, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 1603.
(14) Haworth, W. N.; Hirst, E. L.; Plant, M. M. T.; Reynolds, R. J. W. *J. Chem. Soc.* **1930**, 2644.
(15) For other routes to ceramides, see: (a) Zimmermann, P.; Schmidt, R. R. *Liebigs. Ann. Chem.* **1988**, 663. (b) Nicolaou, K. C.; Caulfield, T.; Kataoka, H.; Kumazawa, T. *J. Am. Chem. Soc.* **1988**, *110*, 7910. (c) Kiso, M.; Nakamura, A.; Tomita, Y.; Hasegawa, A. *Carbohydr. Res.* **1986**, *158*, 101. (d) Koike, K.; Numata, M.; Sugimoto, M.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1986**, *158*, 113.
(16) For a recent application of this enzyme to a very different synthesis of GM_3 by Paulson and co-worker, see ref 13c. We thank Professor Paulson for providing us with a preprint of his contributions.
(17) (a) Halcomb, R. L.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6661. (b) Gordon, D. M.; Danishefsky, S. J. *Carbohydr. Res.* **1990**, *111*, 361. (c) Berkowitz, D. B.; Danishefsky, S. J.; Schulte, G. K. *J. Am. Chem. Soc.* **1992**, *114*, 4518.
(18) CMP-sialic acid is commercial available from Sigma and Genzyme Ltd.
(19) For sialyltransferases in organic syntheses: (a) Sabesan, S.; Paulson, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 2068. (b) Thiem, J.; Treder, W. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1096. (c) Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159. (d) Unverzag, C.; Kunz, H.; Paulson, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 9308. (e) Auge, C.; Fernandez-Fernandez, R.; Gautheron, C. *Carbohydr. Res.* **1990**, *200*, 257. (f) Ichikawa, Y.; Shen, G.-J.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 4698. (g) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceds, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283. (h) Lubineau, A.; Auge, C.; Francois, P. *Carbohydr. Res.* **1992**, *228*, 137. (i) Guilbert, B.; Khan, T. H.; Flitsch, S. L. *J. Chem. Soc., Chem. Commun.* **1992**, 1526. (k) Ito, Y.; Gaudino, J. J.; Paulson, J. C. *Pure Appl. Chem.* **1993**, *65*, 753.
(20) Rearick, J. I.; Sadler, J. E.; Paulson, J. C.; Hill, R. L. *J. Biol. Chem.* **1979**, *254*, 4444.
(21) (a) Zimmermann, P.; Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 725. (b) Bommer, R.; Schmidt, R. R. *Liebigs. Ann. Chem.* **1989**, 1107. (c) Zimmermann, P.; Bommer, R.; Bar, T.; Schmidt, R. R. *J. Carbohydr. Chem.* **1988**, *7*, 435.
(22) (a) Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Oriyama, T.; Griffith, D. A.; Wong, C.-H.; Dumas, D. P. *J. Am. Chem. Soc.* **1992**, *114*, 8329. (b) Danishefsky, S. J.; Koseki, K.; Griffith, D. A.; Gervat, J.; Peterson, J. M.; McDonald, F. E.; Oriyama, T. *J. Am. Chem. Soc.* **1992**, *114*, 8331.
(23) McDonald, F. E.; Danishefsky, S. J. *J. Org. Chem.* **1992**, *57*, 7001.

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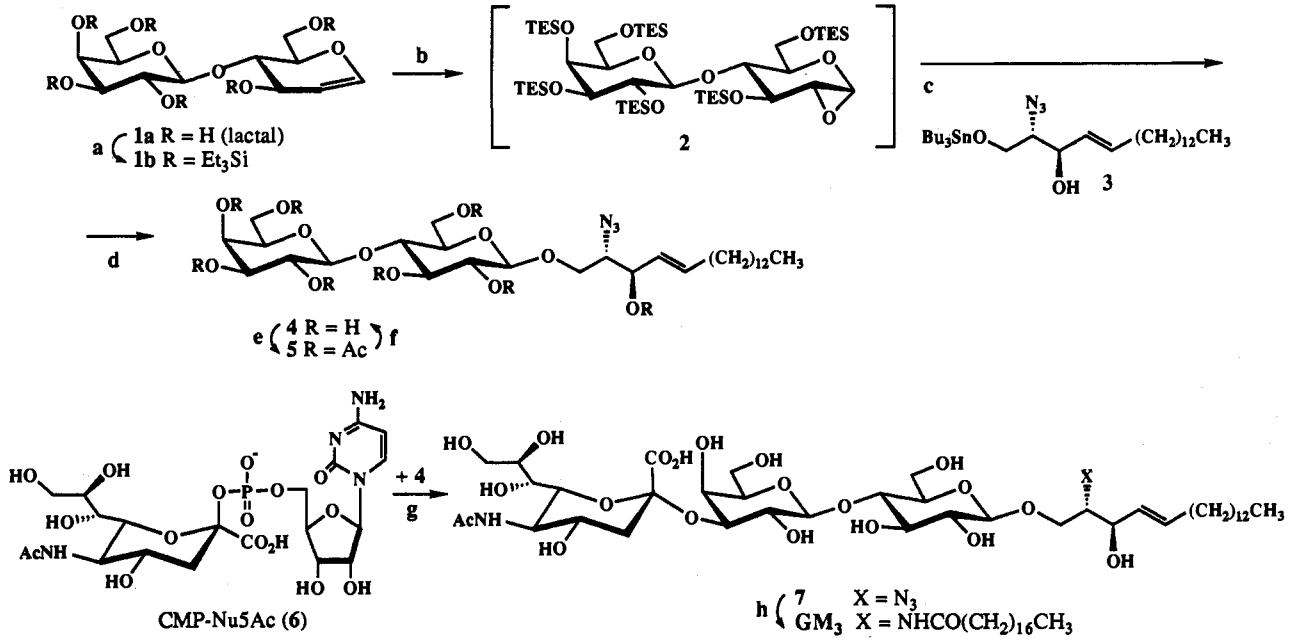
Scheme I[a]



[a] (a) Nine equiv of TESOTf, catalyst DMAP, pyridine (81%). (b) 2,2-Dimethyldioxirane, acetone, 0 °C. (c) Two equiv of **3**, 2 equiv of $Zn(OTf)_2$, THF, 0 °C → r.t. (d) TBAF, THF. (e) Acetic anhydride, catalyst DMAP, pyridine (44%, **5** → **1b**). (f) NaOMe, MeOH (95%). (g) α-2,3 Sialyltransferase ([EC 2.4.99.4]), Triton CF-54 (0.5%), bovine serium albumin, calf intestine alkaline phosphatase, HEPE buffer (pH 7.4), 5 days, r.t. (75%). (h) (i) H_2S, pyridine/water = 1:1; (ii) stearoyl chloride, 50% NaOAc/THF = 1:2 (40%, two steps).

fications in this area of glycolipid synthesis.[24] The solution to the problem of GM_3 synthesis is indicative of future directions. Other applications are under active consideration.

(24) For instance, the route described herein, where the ceramide precursor is introduced via a glycal is much shorter than was possible by classical glycosylation.[13] The latter required in each synthesis extensive manipulations to expose the glycosyl donor.

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