A Novel and Efficient Synthesis of Neolacto Series Gangliosides 3′-nLM$_1$ and 6′-nLM$_1$

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Received September 14, 1993

Neolacto series gangliosides[1] are a subclass of glycosphingolipids found on the surface of human hematopoietic cells[2] which may play a role in the terminal granulocytic differentiation of these cells.[3] In order to facilitate analysis of neolacto gangliosides in the differentiation of hematopoietic cells, efficient syntheses of this family of compounds are needed. This report describes a combined chemical and enzymatic approach to the two simplest members of this family, gangliosides 3′-nLM$_1$ and 6′-nLM$_1$, whose structures are shown below:

NeuAcα(2,3)Galβ(1,4)GlcNAcβ(1,3)Galβ(1,4)Glcβ(1,1)Ceramide

3′-nLM$_1$ (**1a**)

NeuAcα(2,6)Galβ(1,4)GlcNAcβ(1,3)Galβ(1,4)Glcβ(1,1)Ceramide

6′-nLM$_1$ (**1b**)

The chemical synthesis of 3′-nLM$_1$ and 6′-nLM$_1$ is difficult because it involves construction of a glycosidic linkage to sialic acid (NeuAc)[4] and chemical glycosylation of a sphingosine derivative.[5,13,14,18]

Sialyltransferase-catalyzed[6] synthesis is a viable alternative to chemical synthesis of sialosides,[7] providing products in high overall yields. Enzymatic sialylation of a neutral glycosphingolipid in the late stages of a ganglioside synthesis not only would address the problem of constructing a sialic acid glycosidic linkage but also would avoid using valuable sialyloligosaccharides in a low-efficiency chemical glycosylation of a sphingosine derivative.[8]

Preliminary investigations carried out in these laboratories[9] indicated that purified sialyltransferases would not convert the neutral glycosphingolipid paragloboside (**2**) to ganglioside **1b** efficiently, even in the presence of non-ionic detergents. This is

Galβ(1,4)GlcNAcβ(1,3)Galβ(1,4)Glcβ(1,1)Ceramide

paragloboside (**2**)

NeuAcα(2,6)Galβ(1,4)GlcNAcβ(1,3)Galβ(1,4)Glcβ (1,1)Ceramide

6′-nLM$_1$ (**1b**)

surprising since **2** bears an oligosaccharide sequence identical to that of lacto-*N*-neotetraose (LNnT), known to be an exceilent sialyltransferase substrate.[7a] We reasoned that altering the ceramide portion of **2** might make it a better sialyltransferase substrate and allow for development of efficient ganglioside syntheses based on glycosphingolipid sialylation.

Lysoparagloboside (**3**) and derivatives **4** and **5** were then prepared via standard carbohydrate and glycosphingolipid chemistry methodology[10–14] (see supplementary material) and tested along with **2** as acceptor substrates in a reaction catalyzed by α(2,6) sialyltransferase under standardized conditions (see Scheme 1).[15]

The results are summarized in Figure 1. Three trends are noted in the Figure 1 graph. First, as previously observed, paragloboside (**2**) is a poor acceptor, yielding about 10% product **1b** after extended incubation. Second, the *N*-acetyl and *N*-monochloroacetyl derivatives of lysoparagloboside (**4** and **5**) are excellent substrates, providing sialylated products **8b** and **6b**, respectively. Third, lysoparagloboside (**3**), which lacks an acyl group, is an adequate substrate, providing >60% conversion to **7b**.

For preparative purposes, compound **5** or **3** can be converted into 3′-nLM$_1$ (**1a**) and 6′-nLM$_1$ (**1b**) employing either Galβ1,3-(4)GlcNAc α(2,3) sialyltransferase or Galβ(1,4)GlcNAc α(2,6) sialyltransferase, respectively. For example, according to Scheme

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(1) Gangliosides whose neutral core oligosaccharide structures are [Galβ-(1,4)GlcNAcβ(1,3)]$_n$Galβ(1,4)Glc where n = 1–4.

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(6) The sialyltransferase enzymes utilized in this work are the cloned enzymes CMP-*N*-acetylneuraminate:β-D-galactosyl-1,4-*N*-acetyl-β-D-glucosamine α-2,6-*N*-acetylneuraminyltransferase (EC 2.4.99.1) and CMP-*N*-acetylneuraminate:β-D-galactosyl-1,4-*N*-acetyl-β-D-glucosamine α-2,3-*N*-acetylneuraminyltransferase (EC 2.4.99.6). The rat liver counterpart of the cloned α(2,6) enzyme is available from Boehringer Mannheim Biochemicals, Indianapolis, IN. For cloning of this enzyme, see: Weinstein, J.; Lee, E. U.; McEntee, K.; Lai, P.; Paulson, J. C. *J. Biol. Chem.* **1987**, *262* (36), 17735–17743. For cloning of the α(2,3) enzyme, see: Wen, D. X.; Livingston, B. D.; Medzihradszky, K. F.; Kelm, S.; Burlingame, A. L.; Paulson, J. C. *J. Biol. Chem.* **1992**, *267* (29), 21011–21019.

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(8) The glycosylation of sphingosine derivatives with large oligosaccharides (four or more carbohydrate monomers) is known to be particularly difficult, typically providing product glycosphingolipids in yields of 20–60%. See refs 5, 13, and 14.

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(15) Standardized conditions are as follows: 10 mM [glycolipid substrate] in 100 mM sodium cacodylate, pH 6.5, 0.1% BSA, 0.6% Triton CF-54, 26 × 10^{-3} unit of sialyltransferase/μmol of substrate, 5 units of calf alkaline phosphatase/26 × 10^{-3} unit of sialyltransferase, 1.5 equiv of CMP-sialic acid, 37 °C.

(16) Yield calculated using thiobarbituric acid colorimetric assay for total sialic acid content. See: Warren, L. *J. Biol. Chem.* **1959**, *234* (8), 1971–1975.

(17) When lysoparagloboside (**3**) is used in preparative-scale reactions, twice as much enzyme is needed (52 × 10^{-3} unit/μmol) as in the reaction utilizing *N*-monochloroacetyl lysoparagloboside (**5**) (26 × 10^{-3} unit/μmol).

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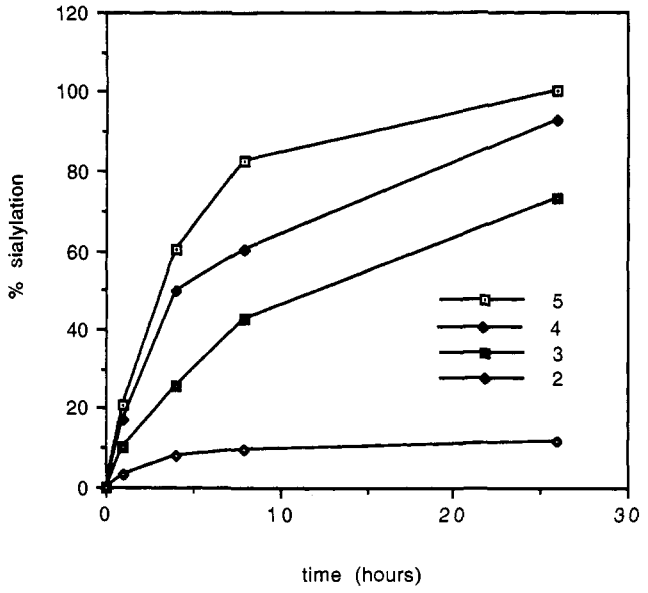
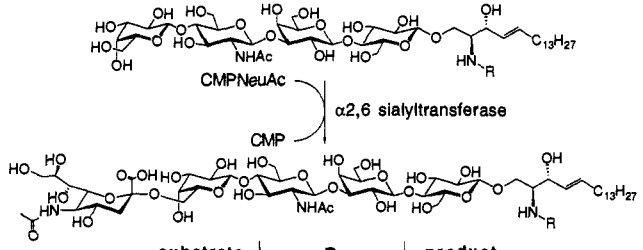


Figure 1. Plot illustrating the time course of $\alpha(2,6)$ sialyltransferase catalyzed conversion of lysoparagloboside (**3**) and derivatives **2**, **4**, and **5** to lyso-6′-nLM$_1$ (**7b**) and derivatives **1b**, **8b**, and **6b** under standardized conditions.

Scheme 1. Sialyltransferase-catalyzed conversion of lysoparagloboside (**3**) and derivatives **2**, **4**, and **5** to lyso-6′-nLM$_1$ (**7b**) and derivatives **1b**, **8b**, and **6b**, respectively

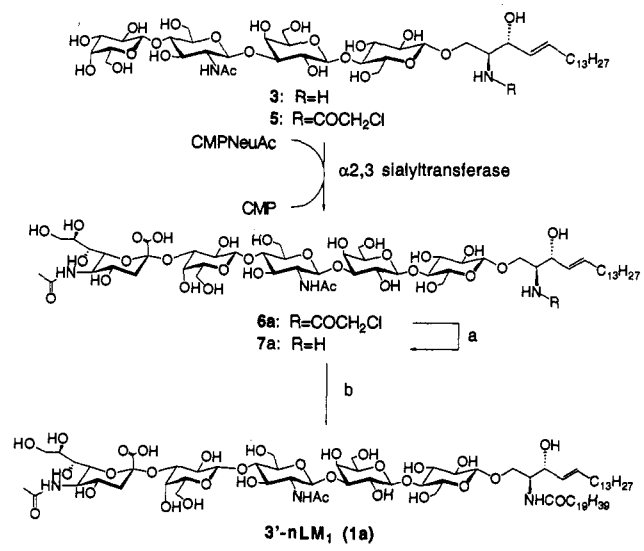


substrate	R	product
2	$CO(CH_2)_{18}CH_3$	1b
5	$COCH_2Cl$	6b
3	H	7b
4	$COCH_3$	8b

2, compounds **5** and **3** were converted to sialylated derivatives **6a** and **7a** on a 5-mg scale in 88% and 63% isolated yield[5,16,17] respectively, utilizing $\alpha(2,3)$ sialyltransferase. Compound **6a** was then converted to 3′-nLM$_1$ (**1a**) in 50% isolated yield[16] by removing the *N*-monochloroacetyl protecting group (thiourea, lutidine)[18] and then reacylating with steroyl chloride. Compound **7a** was converted directly to 3′-nLM$_1$ (**1a**) in 60% yield by acylating with steroyl chloride, obviating removal of the *N*-monochloroacetyl protecting group. When $\alpha(2,6)$ sialyltransferase was substituted for $\alpha(2,3)$ sialyltransferase, 6′-nLM$_1$ (**1b**) was obtained as the final product in similar yield.[16] 6′-nLM$_1$ and 3′-nLM$_1$ produced from **3** or **5** provided ^{1}H-NMR data which correlated well with data generated using materials from independent sources (see supplementary material).[19]

In conclusion, this report has demonstrated that altering the

Scheme 2[a]



[a] (a) Thiourea, 2,6-lutidine, 1:1 MeOH/CH_2Cl_2, 40 °C, 8 h. (b) Steroyl chloride, 0.1 M 1:1 $NaHCO_3/Et_2O$, 50% for two steps

lipid portion of neutral glycosphingolipids can alter the efficacy with which certain sialyltransferase enzymes utilize these compounds as substrates. Target gangliosides **1a** and **1b** were synthesized in 30–40% yield based on sialic acid, which is much higher than the yields achievable utilizing contemporary chemical glycosylation strategies.[20] Since cloned sialyltransferases[6] and CMP-sialic acid synthetase[21] are now available, and schemes for their utilization in multigram reactions have been developed,[7c] efficient use of valuable sialic acid in large-scale ganglioside synthesis is possible. Extension of this methodology to synthesis of rare neolacto series gangliosides containing hexa-, octa-, and decasaccharide core structures should provide useful amounts of these valuable compounds. In the future, ganglioside syntheses may be devised which utilize enzyme-catalyzed sialylation of glycosphingolipids, further extending the utility of sialyltransferases in combined chemical and enzymatic synthesis.

Acknowledgment. This research was supported by the National Institutes of Health Grants CA08823 and GM27904.

Supplementary Material Available: Experimental procedures for the preparation of compounds **1a,b**, **2–5**, **6a–8a**, and **6b–8b** and ^{1}H-NMR spectra of compounds **1a,b**, and **14** (20 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(19) ^{1}H-NMR data was generously provided by Professor Akira Hasegawa, Gifu University, Gifu, Japan, for 6′-nLM$_1$ synthesized via a chemical approach (ref 13c). Ganglioside 3′-nLM$_1$ produced from **3** or **5** was compared directly by ^{1}H-NMR with authentic 3′-nLM$_1$ isolated from natural sources by Edward Nudelman, University of Washington, Seattle, WA.

(20) The only other synthesis of a neolacto series ganglioside, the synthesis of 6′-nLM$_1$ by Hasegawa et al. (ref 13c), was completed in <5% yield based on sialic acid.

(21) CMP-sialic acid synthetase is available from Genzyme Corporation, Cambridge, MA. For preparation of CMP-sialic acid using this enzyme, see: Shames, S.; Simon, E. S.; Christopher, C. W.; Schmid, W.; Whitesides, G. M.; Yang, L.-L. *Glycobiology* **1991**, *1* (2), 187–191.