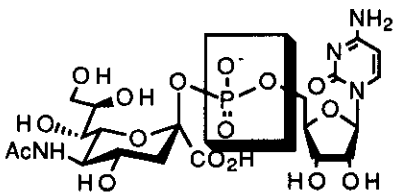
SYNTHESIS OF A CARBON-LINKED CMP-NANA ANALOG AND ITS INHIBITORY EFFECTS ON GM3 AND GD3 SYNTHASES

Yasumaru Hatanaka[a]*, Makoto Hashimoto[a], Kazuya I.-P. Jwa Hidari[b], Yutaka Sanai[b], Yoshitaka Nagai[c], and Yuichi Kanaoka[d]

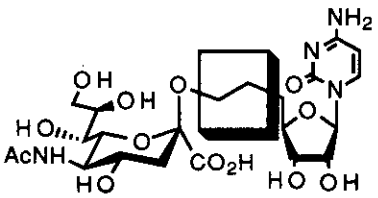
[a]Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama, 930-01 Japan, [b]The Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo, 113, [c]Mitsubishi Kasei Institute of Life Science, 11 Minami-ooya, Machida, Tokyo, 194, [d]Toyama Womens College, 444 Gankaiji, Toyama, 930-01

Abstract- A carbon-linked analog of cytidine monophospho-*N*-acetylneuraminic acid (CMP-NANA) was synthesized as the degradation resistant inhibitor for sialyltransferases. The compound is the first example of synthetic CMP-NANA analog that exhibited inhibitory effects on the activity of GM3 and GD3 synthases.

Gangliosides are the family of sialylated glycosphingolipids that are especially abundant on neuronal cell surfaces, and their biological roles have become increasingly appreciated, particularly in regard to cell growth, intercellular adhesion, and transmembrane signaling (reviewed in ref. 1). Sialyltransferases catalyze the transfer of sialic acids which are the characteristic component of gangliosides. The cDNA cloning of sialyltransferases has been recognized to be a major subject of glycobiology, and an important member of these enzymes in the ganglioside biosynthesis, GD3 synthase, has been cloned very recently.[2] The study of site-specific mutagenesis suggested that the conserved region between sialyltransferases, the sialyl-motif, appears to participate in binding of the common donor substrate CMP-sialic acid.[3] Therefore, the analogs mimicking the structure of donor substrate cytidine 5'-monophospho-*N*-acetylneuraminic acid (CMP-NANA) (**1**) is obviously useful for the development of bioactive compounds which potentially regulate the biological pathway of ganglioside synthesis. In this paper, we describe the synthesis and characterization of a novel carbon-linked CMP-NANA analog (**2**).



1

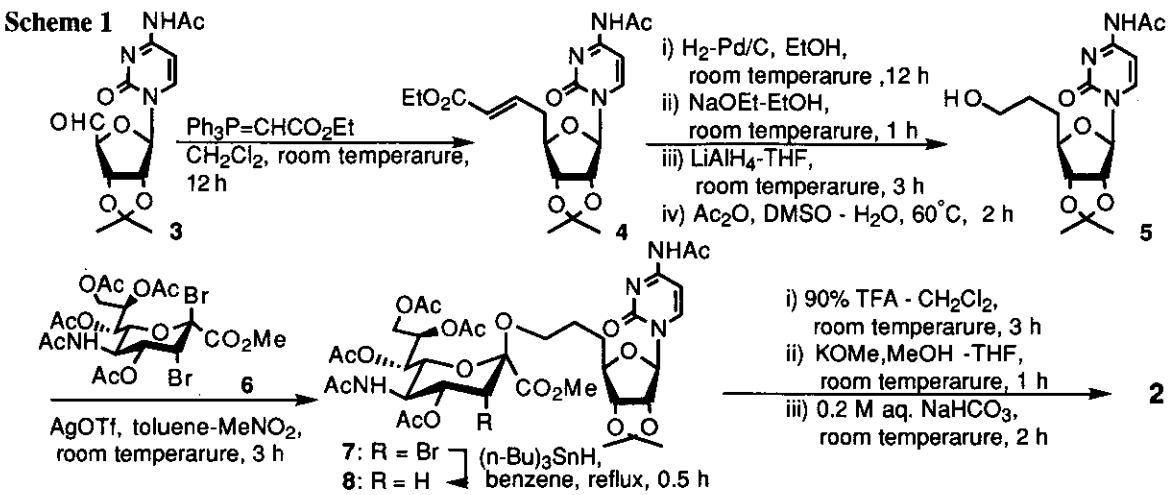


2

RESULTS AND DISCUSSION

Synthesis. By deleting the biologically labile phosphate bond, the truncated analogs of CMP-NANA were already reported as synthetic inhibitors for sialyltransferases.[4, 5] We designed a cytidine derivative modified at 5'-position where a two carbon unit is constructed in place of the phosphate linkage of cytidine monophosphate. This compound (**5**) was synthesized from the 5'-aldehyde (**3**)[6] as shown in Scheme 1. The Wittig reaction of **3** with carboethoxymethlenephosphorane gave an olefinic ester (**4**) in 94 % yield. After hydrogenation of **4** with Pd/C (93 %), the deprotection of *N*-acetyl group (79 %) followed by reduction of the ester with $LiAlH_4$ (88 %) and the selective reprotection of amino group[7] afforded the desired nucleoside (**5**) in 63 % yield.[8] This compound was subjected to the selective β-glycosylation with a dibromide (**6**)[9] to give a cytidine-NANA conjugate (**7**) (43 %). From an empirical rule based on the *J*-value between H"-7 proton and H"-8 proton (J = 2.0 Hz) and the difference of chemical shifts between two H"-9 protons (0.91 ppm) of the sialic acid moiety,[10] the introduced glycosidic linkage of **7** was determined as β-configuration. The reductive debromination of **7** gave **8** (93 %) which was sequentially deprotected to result the carbon-linked CMP-NANA analog (**2**) in 42 % yield.[8]

Scheme 1



Sialyltransferase assay. GM_3 synthase and GD_3 synthase catalyze different type of sialyltransfer, 2,3- and 2,8-sialylation, respectively, and are recognized to be potential candidate as a key regulatory

enzyme in the step of ganglioside biosynthesis.[11] Thus, the inhibitory activity of **2** for these typical sialyltransferases was examined with rat liver Golgi.[12] Lactosyl ceramide and GM_3 were used as acceptor substrates (0.3 mM) for GM_3 and GD_3 synthases, respectively. The competition between CMP-[^{3}H]NANA (0.35 mM) and **2** (21 mM or 35 mM) was determined and the results are listed in Table 1. The inhibition of GM_3 and GD_3 synthases with **2** was evident at near 10 mM concentrations and these values are higher in magnitude than well known inhibitors CMP, CDP, or CTP.[13] The phosphate bond deleted CMP-NANA analog[4] also reported to show low inhibitory activity for sialyltransferases,[14] but its resistivity against biological degradation is obviously useful.[15]

Table 1. Competitive inhibition of sialyltransfer to lactosyl ceramide and GM_3

Acceptor glycolipid	Inhibition of sialylation (%)	
	[CMP-NANA] : [**2**] = 1 : 60	[CMP-NANA] : [**2**] = 1 : 100
Lactosyl ceramide	22 ± 6 %	35 ± 2 %
GM_3	30 ± 5 %	35 ± 2 %

In conclusion, the compound (**2**) is the first example of synthetic CMP-NANA analog that exhibited inhibitory activities for GM_3 and GD_3 synthases, and the inhibition was more effective to GD_3 synthase than to GM_3 synthase. Because of this selective nature of inhibition and the potential resistivity of carbon-linkage to degradation, **2** should be a useful tool to investigate the biological functions of gangliosides.

ACKNOWLEDGMENTS

This work was supported by Grant-in-Aid for Scientific Research on Priority Areas (05274102) and Grant-in-Aid (06453193) from the Ministry of Education, Science and Culture, Japan. Research Fellow of the Japan Society for the Promotion of Science (M. H.)

REFERENCES AND NOTES

1 S. Hakomori and Y. Igarashi, *Adv. Lipid Res.*, 1993, **25,** 147; G. Tettamanti and L. Riboni, *ibid.*, 1993, **25,** 235.

2 K. Nara, Y. Watanabe, K. Maruyama, K. Kasahara, Y. Nagai, and Y. Sanai, *Proc. Natl. Acad. Sci. USA*, 1994, **91,** 7952; K. Sasaki, K. Kurata, N. Kojima, N. Kurosawa, S. Ohta, N. Hanai, S. Tsuji, and T. Nishi, *J. Biol. Chem.*, 1994, **269,** 15950; M. Haraguchi, S. Yamashiro, A. Yamamoto, K. Furukawa, K. Takamiya, K. O. Lloyd, H. Shiku, and K. Furukawa, *Proc. Natl. Acad. Sci. USA*, 1994, **91,** 10455.

3 A. K. Datta and J. C. Paulson, *J. Biol. Chem.*, 1995, **270,** 1497.

4 H. Ogura, K. Furuhata, M. Itoh, and Y. Shitori, *Carbohydr. Res.*, 1986, **158,** 37.

5 O. Kanie, J. Nakamura, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 1987, **6**, 105; S. Sato, K. Furuhata, M. Itoh, Y. Shitori, and H. Ogura, *Chem. Pharm. Bull.*, 1988, **36**, 914.

6 S. David, and G. de Senny, *J. Chem. Soc., Perkin Trans. I*, **1982**,1835

7 K. Takahashi, H. Hayatsu, and T. Ukita, *Biochim. Biophys. Acta*, 1969, **195**, 304.

8 **5**, colorless glass ; uv λmax 250 nm (ε=20,500), 300 nm (ε=8,300); $[\alpha]_D$ +48.6° (c=1.4, MeOH) ; ^{1}H-nmr ($CDCl_3$) δ 8.80 (1H, br, NH), 7.71 (1H, d, $J_{5,6}$=7.7Hz, H-6), 7.39 (1H, d, $J_{5,6}$=7.7Hz, H-5), 5.69 (1H, d, $J_{1',2'}$=1.7Hz, H-1'), 4.99 (1H, dd, $J_{1',2'}$=1.7Hz, $J_{2',3'}$=6.6Hz, H-2'), 4.64 (1H, dd, $J_{2',3'}$=6.6Hz, $J_{3',4'}$=4.4Hz, H-3'), 4.16 (1H, m, H-4'), 3.56 (2H, m, H-7'), 2.24 (3H, s, Ac), 1.85 (2H, m, H-5'), 1.71 (2H, m, H-6'), 1.57 and 1.34 (each 3H, s, Me_2C); FAB-ms m/z: 354 $(MH)^+$. HR FAB-ms m/z: Calcd for $C_{16}H_{24}N_3O_6$ $(MH)^+$: 354.1665. Found: 354.1661. **7**, colorless glass ; $[\alpha]_D$ -47.0° (c=0.5, $CHCl_3$); ^{1}H-nmr ($CDCl_3$) δ 9.13 (1H, br, 6-NH), 7.81 (1H, d, $J_{5'',NH}$=9.8Hz, 5''-NH), 7.50 (1H, d, $J_{5,6}$=7.8Hz, H-6), 7.33 (1H, d, $J_{5,6}$=7.8Hz, H-5), 5.78 (1H, d, $J_{1',2'}$=1.0Hz, H-1'), 5.53 (1H, dd, $J_{1',2'}$=1.0Hz, $J_{2',3'}$=6.3Hz, H-2'), 5.15 (1H, dd, $J_{3'',4''}$=3.4Hz, $J_{4'',5''}$=10.3Hz, H-4''), 5.02 (1H, m, $J_{7'',8''}$=2.0Hz, H-8''), 4.95 (1H, dd, $J_{8'',9''a}$=2.4Hz, $J_{9''a,9''b}$=-12.2Hz, H-9''a), 4.82 (1H, dd, $J_{2',3'}$=6.3Hz, $J_{3',4'}$=2.4Hz, H-3'), 4.62 (1H, d, $J_{3'',4''}$=3.4Hz, H-3''), 4.56 (1H, m, H-5''), 4.13 (1H, m, H-4'), 4.04 (1H, dd, $J_{8'',9''b}$=9.8Hz, $J_{9''a,9''b}$=-12.2Hz, H-9''b), 3.71 (3H, s, CO_2CH_3), 3.48 (2H, m, H-7'), 2.14, 2.13, 2.00, 1.84 and 1.79 (18H, 6s, OAc and NAc), 2.06 (2H, m, H-5'), 1.93 (2H, m, H-6'), 1.45 and 1.30 (each 3H, s, Me_2C); FAB-ms m/z: 905, 907 $(MH)^+$. **2**, white solid; $[\alpha]_D$= -26° (c= 0.1,H_2O) ; uv λmax 270 nm (ε=3,700); ^{1}H-nmr (D_2O) δ 7.64 (1H, d, $J_{5,6}$=7.3Hz, H-6), 6.06 (1H, d, $J_{5,6}$=7.3Hz, H-5), 5.85 (1H, d, $J_{1',2'}$=3.9Hz, H-1'), 4.29 (1H, dd, $J_{1',2'}$=3.9Hz, $J_{2',3'}$=4.9Hz, H-2'), 4.1- 4.0 (2H, m, H-4'' and H-6''), 3.9-3.8 (7H, m, H-3',4',7',5'',8'' and 9''), 4.95 (1H, dd, $J_{8'',9''a}$=2.0 Hz, $J_{9''a,9''b}$=-12.0Hz, H-9''a), 2.37 (1H, dd, $J_{3''eq,4''}$=4.9Hz, $J_{3''eq,3''ax}$=- 13.9Hz, H-3''*eq*), 2.04 (3H, s, NAc), 1.63 (1H, dd, $J_{3''eq,3''ax}$=- 13.9Hz, $J_{3''ax,4''}$=12.2Hz, H-3''*ax*); FAB-ms m/z: 563 $(MH)^+$. HR FAB-ms m/z: Calcd for $C_{22}H_{35}N_4O_{13}$ $(MH)^+$: 563.2201. Found: 563.2197.

9 K. Okamoto, T. Kondo, and T. Goto, *Tetrahrdron*, 1987, **43**, 5909.

10 H. Paulsen and H. Tietz, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 927.

11 G. van Echten and K. Sandhoff, *J. Biol. Chem.*, 1993, **268**, 5341.

12 K. I-P. Jwa Hidari, I. Kawashina, T. Tai, F. Inagaki, Y. Nagai, and Y. Sanai, *Eur. J. Biochem.*, 1994, **221**, 603.

13 W. D. Klohs, R. J. Bernacki, and W. Korytnyk, *Cancer Res.*, 1979, **39**, 1231.

14 I. Kijima-Suda, S. Toyoshima, M. Itoh, K. Furuhata, H. Ogura, and T. Osawa, *Chem. Pharm. Bull.*, 1985, **33**, 730.

15 I. Kijima-Suda, Y. Miyamoto, S. Toyoshima, M. Itoh, and T. Osawa, *Cancer Res.*, 1986, **46**, 858; I. Kijima-Suda, T. Miyazawa, M. Itoh, S. Toyoshima, and T. Osawa, *Cancer Res.*, 1988, **48**, 3728; B. E. Harvey and P. Thomas, *Biochem. Biophys. Res. Commun.*, 1993, **190**, 571.

Received, 22nd November, 1995