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Rapid Communication

Ganglioside GD1α analogues as high-affinity ligands for myelin-associated glycoprotein (MAG)☆

Naoki Sawada [a], Hideharu Ishida [a], Brian E. Collins [b], Ronald L. Schnaar [b], Makoto Kiso [a,*]

[a] *Department of Applied Bio-organic Chemistry, Gifu University, Gifu 501-1193, Japan*
[b] *Departments of Pharmacology and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA*

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Abstract

The first systematic synthesis of ganglioside GD1α analogues carrying *N*-acetyldeoxyneuraminic acids linked to C-6 of the GalNAc residue was accomplished. The suitably protected GM1b pentasaccharide derivative was regioselectively glycosylated with the phenyl 2-thioglycosides of 7-deoxy, 8-deoxy, and 9-deoxy-*N*-acetylneuraminic acid promoted by *N*-iodosuccinimide (NIS)–trifluoromethanesulfonic acid (TfOH) in acetonitrile, and the resulting hexasaccharides were converted to the target GD1α analogues. All of the analogues retained excellent efficiency in supporting the adhesion to myelin-associated glycoprotein (MAG), raising the possibility that the internal sialic acid linked to the GalNAc residue may be replaced by other anionic substituents, in contrast to the terminal sialic acid, which is essential for MAG binding.

Keywords: Ganglioside GD1α; Myelin-associated glycoprotein; Sialic acid; Sialoadhesins; Siglec

Gangliosides, sialic acid-containing glycosphingolipids, are commonly present in cell-surface membranes and are particularly abundant in tissues of the central nervous system. These molecules are considered to be involved in many biological processes such as cell growth, cell differentiation, cell adhesion, microbial and viral infections, immune responses, oncogenesis, and many other receptor-mediated reactions [2]. Ganglioside GD1α, an extremely minor disialoganglioside, was first isolated from rat ascites hepatoma cells [3] and later from adult bovine brains [4]. It has recently been reported [5] that GD1α functions as an adhesion molecule in the process of tumor metastasis.

Ganglioside GD1α has a unique structure that contains the internal sialic acid α-(2→6)-linked to the GalNAc residue in addition to the terminal sialic acid α-(2→3)-linked to the galactose residue. With our interest focussed on this unique structure and its biological functions, we have chemically synthesized ganglioside GD1α (**1**) [6] and found [7] that this molecule can efficiently support the adhesion to myelin-associated glycoprotein (MAG), a member of the sialic acid-depen-

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* Corresponding author. Fax: +81-58-293-2916/2840.
E-mail address: kiso@cc.gifu-u.ac.jp (M. Kiso)

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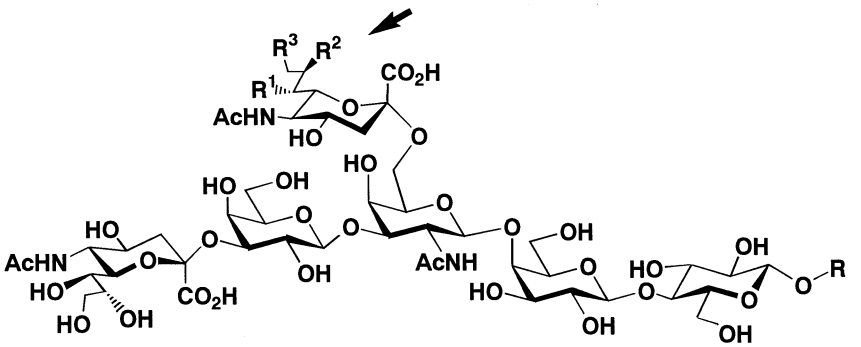
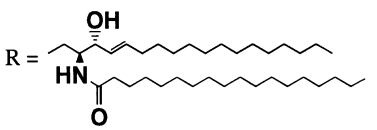
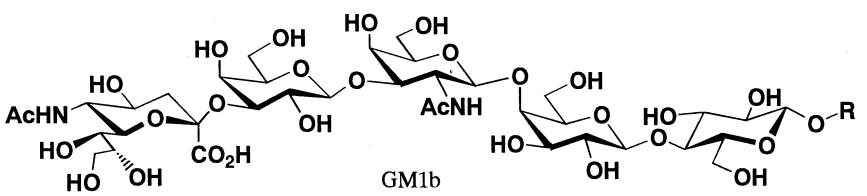


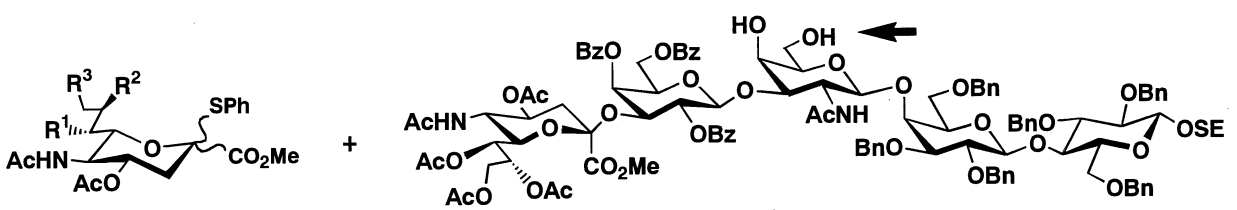
	R^1	R^2	R^3	
1	OH	OH	OH	(GD1α)
2	H	OH	OH	(7-deoxy)
3	OH	H	OH	(8-deoxy)
4	OH	OH	H	(9-deoxy)





GM1b

Scheme 1.

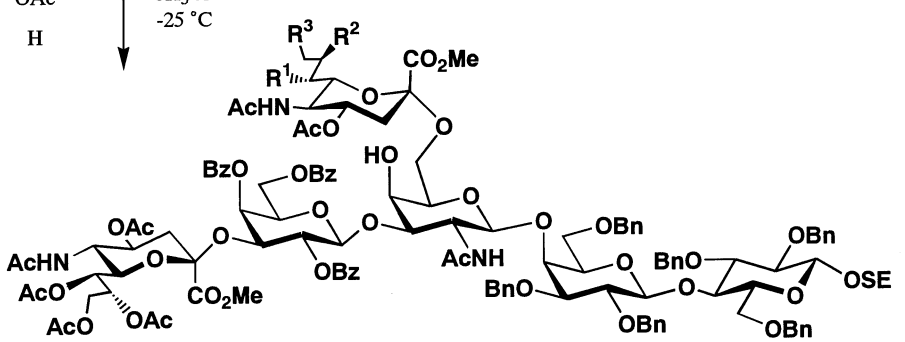


	R^1	R^2	R^3
5	H	OAc	OAc
6	OAc	H	OAc
7	OAc	OAc	H

NIS / TfOH
CH_3CN
-25 °C

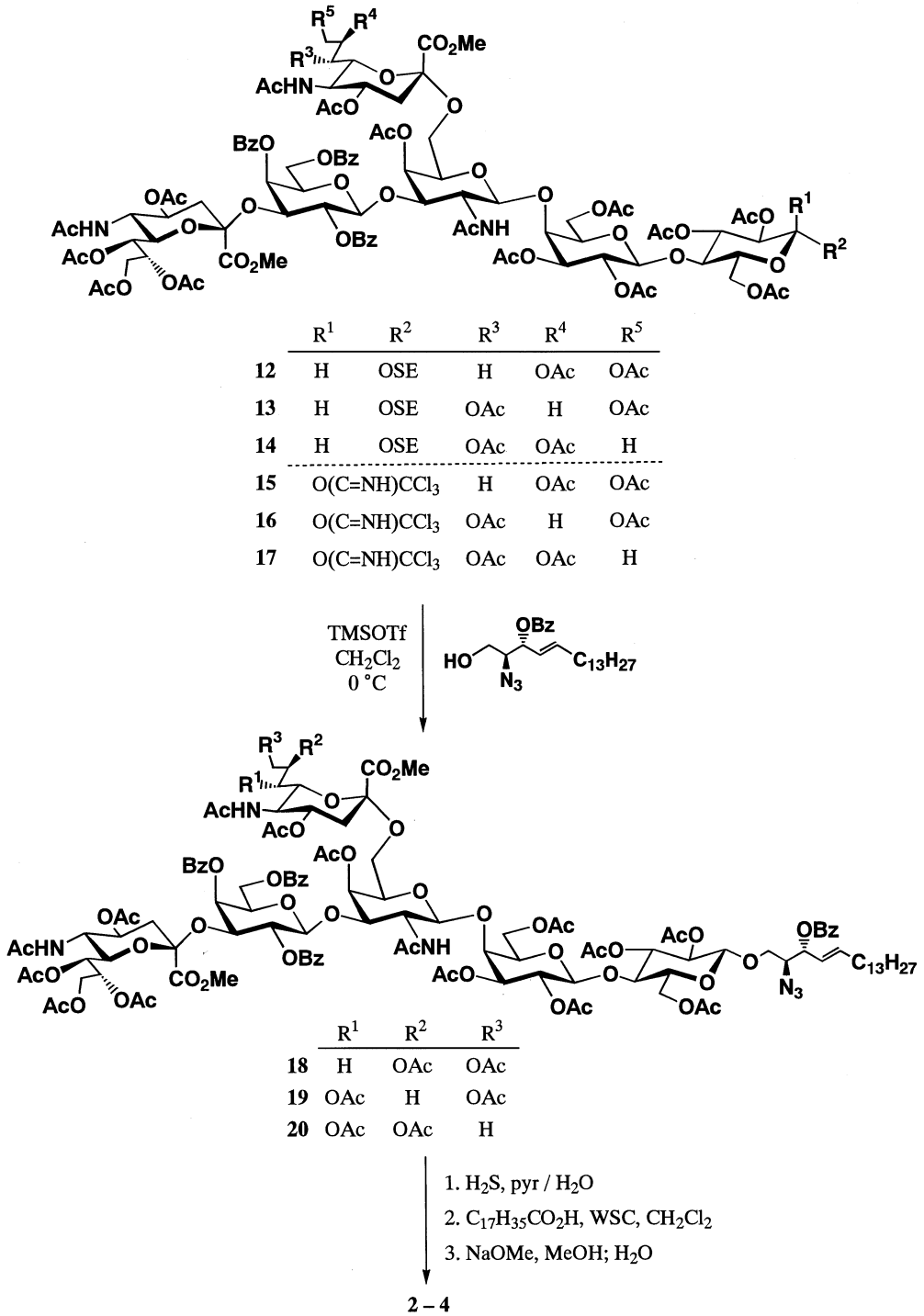
8

SE =



	R^1	R^2	R^3
9	H	OAc	OAc
10	OAc	H	OAc
11	OAc	OAc	H

Scheme 2.



	R^1	R^2	R^3	R^4	R^5
12	H	OSE	H	OAc	OAc
13	H	OSE	OAc	H	OAc
14	H	OSE	OAc	OAc	H
15	$O(C=NH)CCl_3$		H	OAc	OAc
16	$O(C=NH)CCl_3$		OAc	H	OAc
17	$O(C=NH)CCl_3$		OAc	OAc	H

	R^1	R^2	R^3
18	H	OAc	OAc
19	OAc	H	OAc
20	OAc	OAc	H

Scheme 3.

dent immunoglobulin lectin (siglec) family, previously termed sialoadhesins or I-type lectins [8]. The binding activity of GD1α to MAG was much higher than that of the monosialo ganglioside GM1b [9], indicating that the internal sialic acid linked to the GalNAc residue plays an important role in MAG binding.

In the course of study on sialic acid specificity of MAG binding with a series of gangliosides, we have demonstrated [10] that any chemical modification of the terminal sialic acid abrogated MAG binding, indicating the whole structure of the terminal sialic acid is required for the binding. However, the role of

the internal sialic acid at C-6 of the GalNAc residue is not clear. To elucidate the structural requirement of this sialic acid, we undertook, for the first time, the systematic synthesis of ganglioside GD1α analogues (**2**–**4**) carrying *N*-acetyl-deoxyneuraminic acids at C-6 of the GalNAc residue and examined their binding by MAG (Scheme 1).

A series of phenyl 2-thioglycosides of *N*-acetyldeoxyneuraminic acids (**5**–**7**), which were synthesized from the corresponding 2-(trimethylsilyl)ethyl glycoside of *N*-acetylneuraminic acid by the improved method of our previously reported procedure [11], were each coupled with the suitably protected GM1b pentasaccharide derivative **8** [6] in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) in acetonitrile at −20 °C [12]. The resulting products contained the desired sialyl-α-(2→6) hexasaccharides (**9**–**11**, 30–40%) and the sialyl-β-(2→6) isomers (20–25%), respectively (Scheme 2). The most significant signals in the ^{1}H NMR spectra of **9**–**11** were a one-proton doublet of doublets ($J_{gem} = 12.8$, $J_{3eq,4} = 4.8$ Hz) at δ 2.56 due to H-3*eq* of the newly introduced α-(2→6)-linked sialyl residue, and another one-proton doublet of doublets ($J_{gem} = 12.6$, $J_{3eq,4} = 4.6$ Hz) at δ 2.43 due to H-3*eq* of the α-(2→3)-linked terminal sialyl residue. The two three-proton singlets due to the methoxycarbonyl groups of the internal and terminal sialyl residues were observed at around δ 3.70 and 3.80, respectively. The benzyl groups in **9**–**11** were completely removed by hydrogenolysis, and then all hydroxyls were acetylated to give **12**–**14** in high yields. Selective removal of the 1-*O*-[2-(trimethylsilyl)ethyl] group [13] by treatment with trifluoroacetic acid in dichloromethane quantitatively gave the 1-OH derivatives, which were converted [14] to the trichloroacetimidates **15**–**17**.

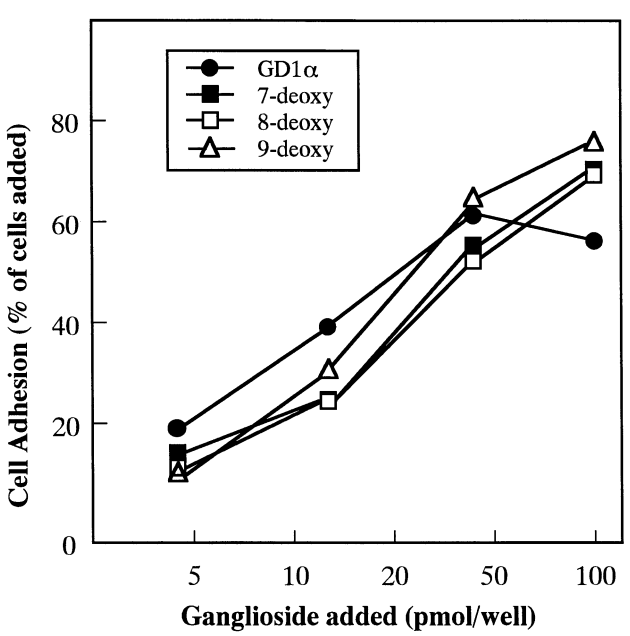


Fig. 1. MAG-mediated cell adhesion to GD1α (**1**) and the deoxy Neu5Ac analogues (**2**–**4**). Microwells were adsorbed with various amounts of GD1α (●), and 7-deoxy (■), 8-deoxy (□) or 9-deoxy (△) Neu5Ac analogues.

Glycosylation of (2*S*, 3*R*, 4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol [15,16] with **15**–**17** was performed in the presence of trimethylsilyl trifluoromethanesulfonate (Me_3-Si-OTf) in dichloromethane at 0 °C to afford the desired β-glycosides **18**–**20** in 70–80% yields (Scheme 3). The IR spectra of **18**–**20** showed the significant absorption peak at ν 2100 cm^{-1} due to the azido group. In the ^{1}H NMR spectra, a characteristic one-proton multiplet ($J_{4,5} = 14.2$, $J_{5,6} = J_{5,6'} = 7.7$ Hz) at around δ 5.90, due to H-5 of the sphingosine moiety, was clearly observed. Reduction of the azido group and subsequent *N*-acylation were carried out by the established method [17,18]. Finally, removal of all protective groups under basic conditions furnished the target GD1α analogues **2**–**4**. All new compounds were characterized by specific rotation, elemental analysis, IR and ^{1}H NMR spectroscopy, and FABMS.

In binding experiments with the MAG-transfected COS cells for the immobilized synthetic ganglioside GD1α (**1**) and its deoxyNeu5Ac analogues (**2**–**4**), we demonstrated that all of the analogues can efficiently support the adhesion as strongly as the parent GD1α (Fig. 1). From this result, we have raised the possibility that the internal sialic acid α-(2→6)-linked to the GalNAc residue may be replaced by other anionic substituents, as demonstrated in the study on selectin ligands [20], in contrast to the terminal sialic acid α-(2→3)-linked to the Gal residue, which is essential for MAG binding [9,10]. MAG is a

quantitatively minor protein constituent of central and peripheral nervous system myelin [19], and is thought to be implicated in myelin–neuron interactions. In particular, MAG has been shown to be a major neurite outgrowth inhibitor [21]. Therefore, detailed knowledge of the carbohydrate ligands that support MAG binding may provide opportunities for intervention in the control of neurite outgrowth and myelination [22].

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References

[1] H. Ando, H. Ishida, M. Kiso, *J. Carbohydr. Chem.*, 18 (1999) in press.
[2] (a) S. Hakomori, *J. Biol. Chem.*, 265 (1990) 18713–18716. (b) R.L. Schnaar, *Glycobiology*, 1 (1991) 477–485.
[3] T. Taki, Y. Hirabayashi, H. Ishikawa, S. Ando, K. Kon, Y. Tanaka, M. Matsumoto, *J. Biol. Chem.*, 261 (1986) 3075–3078.
[4] Y. Hirabayashi, A. Hyogo, T. Nakao, K. Tsuchiya, Y. Suzuki, M. Matsumoto, K. Kon, S. Ando, *J. Biol. Chem.*, 265 (1990) 8144–8151.
[5] T. Taki, D. Ishikawa, M. Ogura, M. Nakajima, S. Handa, *Cancer Res.*, 57 (1997) 1882–1888.
[6] (a) H. Prabhanjan, M. Kiso, A. Hasegawa, *Carbohydr. Res.*, 222 (1991) c1–c4. (b) H. Prabhanjan, K. Aoyama, M. Kiso, A. Hasegawa, *Carbohydr. Res.*, 233 (1992) 87–99.
[7] B.E. Collins, H. Ito, H. Ishida, M. Kiso, R.L. Schnaar, *19th Int. Carbohydr. Symp.*, 1998, Abstr. no. CK005.
[8] (a) S. Kelm, A. Pelz, R. Schauer, M.T. Filbin, S. Tong, M.-E. de Bellard, R.L. Schnaar, J.A. Mahoney, A. Hartnell, P. Bradfield, P.R. Crocker, *Curr. Biol.*, 4 (1994) 965–972. (b) L.D. Powell, A. Varki, *J. Biol. Chem.*, 270 (1995) 14243–14246. (c) P.R. Crocker, E.A. Clark, M. Filbin, J.H. Gordon, Y. Jones, J.H. Kehrl, S. Kelm, N. Le Douarin, L. Powell, J. Roder, R.L. Schnaar, D.C. Sgroi, I. Stamenkovic, R. Schauer, M. Schachner, T.K. van den Berg, P.A. van der Merwe, S.M. Watt, A. Varki, *Glycobiology*, 8 (2) (1998) v.
[9] L.J.-S. Yang, C.B. Zeller, N.L. Shaper, M. Kiso, A. Hasegawa, R.E. Shapiro, R.L. Schnaar, *Proc. Natl. Acad. Sci. USA*, 93 (1996) 814–818.
[10] (a) B.E. Collins, M. Kiso, A. Hasegawa, M.B. Tropak, J.C. Roder, P.R. Crocker, R.L. Schnaar, *J. Biol. Chem.*, 272 (1997) 16889–16895. (b) B.E. Collins, L.J.-S. Yang, G. Mukhopadhyay, M.T. Filbin, M. Kiso, A. Hasegawa, R.L. Schnaar, *J. Biol. Chem.*, 272 (1997) 1248–1255.
[11] A. Hasegawa, K. Adachi, M. Yoshida, M. Kiso, *Carbohydr. Res.*, 230 (1992) 257–272.
[12] (a) A. Hasegawa, T. Nagahama, H. Ohki, K. Hotta, H. Ishida, M. Kiso, *J. Carbohydr. Chem.*, 10 (1991) 493–498. (b) M. Kiso, A. Hasegawa, *Methods Enzymol.*, 242 (1994) 171–183. (c) K. Hotta, H. Ishida, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.*, 14 (1995) 491–506.
[13] K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori, K. Stenvall, *J. Org. Chem.*, 53 (1988) 5629–5647.
[14] R.R. Schmidt, G. Grundler, *Synthesis*, (1981) 885–887.
[15] R.R. Schmidt, P. Zimmermann, *Tetrahedron Lett.*, 27 (1986) 481–484.
[16] (a) M. Kiso, A. Nakamura, Y. Tomita, A. Hasegawa, *Carbohydr. Res.*, 158 (1986) 101–111. (b) Y. Ito, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.*, 8 (1989) 285–294.
[17] R.R. Schmidt, P. Zimmermann, *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 725–726.
[18] T. Murase, H. Ishida, M. Kiso, A. Hasegawa, *Carbohydr. Res.*, 188 (1989) 71–80.
[19] A. Hasegawa, M. Kiso, in Z.J. Witczak, K.A. Nieforth (Eds.), *Carbohydrates in Drug Design*, Marcel Dekker Inc., New York, 1997, pp. 137–155.
[20] B.D. Trapp, *Ann. NY Acad. Sci.*, 605 (1990) 29–43.
[21] (a) M.E. Schwab, J.P. Kapfhammer, C.E. Bandtlow, *Annu. Rev. Neurosci.*, 16 (1993) 565–595. (b) G. Mukhopadhyay, P. Doherty, F.S. Walsh, P.R. Crocker, M.T. Filbin, *Neuron*, 12 (1994) 757–767. (c) L. McKracher, S. David, D.L. Jackson, V. Kottis, R.J. Dunn, P.E. Braun, *Neuron*, 13 (1994) 805–811.
[22] R.L. Schnaar, B.E. Collins, L.P. Wright, M. Kiso, M.B. Tropak, J.C. Roder, P.R. Crocker, *Ann. NY Acad. Sci.*, 845 (1998) 92–105.