Synthetic Study on Neural Siglecs Ligands: Systematic Synthesis of α -Series Polysialogangliosides and Their Analogues

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Abstract: To elucidate the structure required as the ligands of neural Siglecs at the level of the sugar residue, α -series polysialogangliosides, GD1 α , GT1 α and GQ1b α , were successfully synthesized. In addition, a series of GD1 α analogues which contain the modified sialic acids, were synthesized to elucidate the structure-activity relationship of the MAG ligands at the level of the functional group.

1. Introduction

It has been widely recognized that sialoglycoconjugates, so-called gangliosides and sialoglycoconjugates, have important roles in biological processes such as cell growth, differentiation, adhesion, and oncogenesis.¹⁻⁴ In addition, the function of these molecules as ligands of animal lectins^{5,6} as well as receptors for viruses⁷ and bacteria⁸ has drawn much attention in connection with inflammatory or infectious diseases. Recently, a new family of carbohydrate-binding proteins, termed Siglecs (sialic acid-binding immunoglobulin superfamily lectins), has been identified.^{6,9,10} This family consists of sialoadhesin (Siglec-1), CD22 (Siglec-2), CD33 (Siglec-3), MAG (myelin-associated glycoprotein, Siglec-4a), SMP (Schwan cell myelin proteins, Siglec-4b) and Siglec 5-9. Sialoadhesin is found in bone marrow macrophages and may play a role in hematopsis. The nervous system Siglecs, MAG and SMP, are expressed on myelinating oligodendrocytes and Schwann cells. MAG is involved in myelin maintenance and in myelin-axon interactions. SMP may be the avian homologue of MAG. Although it is hypothesized that gangliosides are functional MAG ligands, the details of the structure required for the binding are still obscure. We describe herein the systematic syntheses of α -series polysialogangliosides, which are thought to be the ligands of MAG and SMP, to elucidate the structure required as Siglecs ligands. In addition, a series of analogues of the gangliosides were also designed and synthesized to get more detailed information.

2. Systematic synthesis of α -series polysialogangliosides

α -Series polysialogangliosides, such as GD1 α , GT1 α and GQ1b α (see Figure 1) were defined as a new series of gangliosides containing NeuAc linked to the C-6 of GalNAc of the gangliotetraosyl backbone.^{11,12} They have been thought to be only a minor component,¹³ and little is known about their physiological functions. Because the expression of these gangliosides was restricted to a particular region and a particular population in brain tissues, it is suggested that these gangliosides may serve as ligands for some neural proteins. In this section, a systematic synthesis of α -series polysialogangliosides is described. The activity of these gangliosides as the ligands of neural Siglecs is also described.

2.1 Total synthesis of ganglioside GD1 α ^{14,15}

Figure 2 shows a retrosynthetic analysis of GD1 α . This ganglioside was first synthesized by route A,¹⁴ in which the GM1b oligosaccharide was first constructed and then underwent sialylation on C-6 of the GalNAc residue. The coupling of GM1b and NeuAc, however, proceeded with rather poor stereoselectivity to give an anomeric mixture which could not be readily separated. Therefore, we employed another synthetic strategy, route B¹⁵, in which GD1 α was constructed by the coupling of a GM2 α acceptor and a sialyl α (2-3) galactose donor (NeuAcGal).

The detailed synthesis of GD1 α is shown in Scheme 1 and 2. Coupling of the suitably protected galactosamine donor **1** with the suitably protected lactose acceptor **2** was carried out

Figure 1. Structures of α -series polysialogangliosides.

Figure 2. Retrosynthesis of GD1 α .

in the presence of *N*-iodosuccinimide-trifluoromethanesulfonic acid (NIS-TfOH) at $-30\text{ }^{\circ}\text{C}$ to give the desired trisaccharide, which was converted into the acceptor **4** by treatment with hydrazine monohydrate and chemoselective *N*-acetylation. The iodonium ion promoted glycosylation¹⁶ of **4** with sialyl donor **5**¹⁷ was performed at $-30\text{ }^{\circ}\text{C}$ in a solution of acetonitrile to give the desired tetrasaccharide in 80% (α ; 65%, β ; 15%) yield. Selective removal of the *p*-methoxyphenylmethyl (MPM) group by treatment with ceric ammonium nitrate (CAN) and H_2O afforded the key glycosyl acceptor **7** in 95% yield, which was glycosylated with sialyl α (2-3)galactose donor **8**¹⁸ by employing dimethyl(methylthio)sulfonium triflate (DMTST) as the glycosyl promoter in dichloromethane to construct the GD1 α in 80% yield. The oligosaccharide obtained was converted into the final compound, ganglioside GD1 α , by the following procedure as shown in Scheme 2: i) replacement of benzyl with acetyl groups,¹⁹ ii) removal of the 2-(trimethylsilyl)ethyl (SE) group by treatment with trifluoroacetic acid (TFA), iii) introduction of trichloroacetimidate¹⁸ as a leaving group, iv) coupling with the suitably protected azidosphingosine acceptor **13**,^{21,22} v) conversion of azido into a stearyl amino group, and vi) the complete deprotection of all protecting groups.

2.2 Total synthesis of ganglioside GT1a α ^{23,24}

Figure 3 shows a retrosynthetic analysis of GT1a α , in which the heptasaccharide of GT1a α was divided into the sialyl α (2-3) galactose donor and the GD2 α acceptor. We employed two synthetic strategies to construct the key intermediate GD2 α . In route A²³, we selected the suitably protected GM2 α derivative as a key glycosyl acceptor, which served as the intermediate also for GQ1b α (see 2.3). Strategy B employs the efficient coupling of the NeuAc-Gal donor

Scheme 1

Scheme 2

and the GM3 acceptor as a key reaction. Due to the low yield of the glycosylation of the GM2 α acceptor with the sialyl donor (NeuAc), a key reaction of route A, route B is recommended for the practical synthesis of GT1a α .

Figure 3. Retrosynthetic analysis of GT1a α .

Figure 3 shows the detailed synthesis by route B. The galactosamine acceptor **16** in which the amino group was protected with phthaloyl was glycosylated with sialyl donor **5** in the presence of NIS-TfOH at $-30\text{ }^{\circ}\text{C}$ to afford the desired sialyl α (2-6) galactose **17** in 80% yield; no β -glycoside was isolated. Removal of the *tert*-butyldimethylsilyl (TBDMS) group in **17** (83%) and trichloroacetimidate formation (99%) yielded the disaccharide donor **18**, which was coupled with sialyl α (2-3) lactose derivative **19**²⁵ to give the desired GM2 α pentasaccharide (76%). The phthalimido group was then converted to an acetamido group. The methyl ester had to be converted to the free carboxyl (**21**) before the removal of phthaloyl group and re-esterified (**22**) after the *N*-acetylation, to prevent the by-product formation. The physicochemical properties and spectral data of **22** were identical with those of **22** separately synthesized by route A.

The isopropylidene group in **22** was cleaved by treatment with 80% acetic acid to give **23** in 85% yield. Regioselective glycosylation of **23** with sialyl α (2-3) galactose donor **8** in the presence of DMTST gave the protected GT1a α heptasaccharide **24** in 95% yield. The oligosaccharide obtained was converted into the final compound, ganglioside GT1a α , by

Scheme 3

essentially the same procedure as described for ganglioside GD1 α (see 2.1).

The structure of the molecule was characterized by FAB MS (negative ion mode) and ^1H NMR spectrometry. The molecular ion species of the synthetic GT1a α were clearly detected at m/z 2172.04 [$\text{M}-\text{Na}^+$] ($\text{C}_{95}\text{H}_{162}\text{N}_5\text{Na}_2\text{O}_{47}$ MW, Exact 2171.0236, Ave. 2172.3161), 2150.06, 2149.07 [$\text{M}-2\text{Na}^+$], and 2127.06 [$\text{M}-3\text{Na}^+$], accompanied with significant fragment ions at m/z 1858.0, 1857.0 [$\text{M}-2\text{Na}-\text{NeuAc}^-$], 1696.0 [$\text{M}-2\text{Na}-\text{NeuAc}-\text{Gal}^-$], and 1545.0 [$\text{M}-3\text{Na}-2\text{NeuAc}^-$], providing unambiguous evidences for the structure assigned. The characteristic fragment ions at m/z 888.7 [lactosylceramide] $^-$, 726.6 [glucosylceramide] $^-$, and 564.6 [ceramide] $^-$, provide further evidence for the assigned structure including the ceramide moiety. In the ^1H NMR spectrum (500MHz) of the synthetic GT1a α in $\text{DMSO}-d_6$ - D_2O , the three characteristic H-3eq of NeuAc (δ 2.73–2.89), four anomeric protons due to the β glycosidic linkages in the GgOse4 core structure (δ 4.15, 4.30, 4.43 and 4.84), and two olefinic protons of ceramide (δ 5.32 and 5.75) were clearly observed. In addition, TLC-immunostaining of the synthetic GT1a α was performed for comparison with the native GT1a α , which showed the two compounds to be identical.

2.3 Total synthesis of ganglioside GQ1b α ²⁶

Ganglioside GQ1b α , which has the most complex struc-

ture amongst the α -series polysialogangliosides, was successfully synthesized by employing the GT2b α acceptor as a key intermediate, which was prepared by successive sialylation of the gangliotriaosyl acceptor (Figure 4 route A). When route B was employed as in the synthesis of GT1a α , the conversion of phthalimido to an acetamido group was rather tedious and difficult because of the presence of the sialyl α (2-8) sialyl residue.

Figure 4. Retrosynthetic analysis of GT2b α , a key intermediate of GQ1b α .

The condensation of the gangliotriaosyl acceptor **25** with the sialyl donor **5** in the acetonitrile in the presence of NIS-TfOH gave the α -glycoside **26** solely at the desired position in 45% yield. The regio-chemistry was deduced from the ^1H NMR spectrum of the acetylated compound; the observed chemical shifts of the galactose residue for H-3 (δ 4.94) indicate the glycosylated position to be HO-6 of the GalNAc residue. The glycosylation of the tetrasaccharide acceptor **26** with the sialyl α (2-8)sialyl donor **27** under the same conditions as described for **26** gave the expected hexasaccharide derivative **28** in 42% yield. The monomeric sialic acid was introduced to the same position in a poor yield in the same condition. Taking these results into the consideration, one can say that the sialyl α (2-8) sialyl donor **27**²⁷ served as a more reactive glycosyl donor than the monomeric sialyl donor **5**. The hexasaccharide acceptor **29** was formed by removal of the isopropylidene group, and glycosylated with the sialyl α (2-3) galactosyl donor **8** in the presence of DMTST to give the desired octasaccharide **30** in 85% yield. The octasaccharide was also transformed into the final com-

pound, ganglioside GQ1b α , by the same procedure as described for GD1 α as well as GT1a α .

Scheme 4

2.4 Binding of α -series polysialogangliosides to MAG, SMP and sialoadhesin²⁸

As shown in the Table, MAG and SMP displayed avidity for α -series polysialogangliosides. The most complex ganglioside GQ1b α was ~10-fold more potent in supporting MAG- and SMP-mediated adhesion than was the closely related major brain ganglioside lacking the α 2,6-NeuAc residue, GT1b (Figure 5). The other α -series polysialogangliosides tested, GD1 α and GT1a α , displayed more potency compared with GD1a and GT1b. In contrast to the neural Siglecs, MAG and SMP, α -series gangliosides and GD1a were equally potent in supporting sialoadhesin-mediated cell adhesion. These data indicate that MAG and SMP require a terminal "NeuAc α (2 $\rightarrow$ 3)" determinant as the primary structural requirement for binding but that additional (secondary) sialic acids on the same core greatly enhance binding. The relative placement of the secondary sialic acids appear to be a key factor, with the NeuAc α (2 $\rightarrow$ 6)GalNAc determinant preferred.

Figure 5. Structures of gangliosides tested.

Table 1. Siglec-mediated cell adhesion to gangliosides and ganglioside derivatives

Ganglioside	Ganglioside concentration supporting half-maximal cell adhesion by:		
	MAG	SMP	Sialoadhesin
	<i>pmol/well</i>		
GM1b	80	150	87
GD1a	50	>100	24
GT1b	50	>100	47
GQ1bα	6.0	8.5	21
GT1αα	17	30	22
GD1α	19	22	23
GD1α analogs			
GD1α, 7-deoxy-NeuAc	28	34	42
GD1α, 8-deoxy-NeuAc	31	34	44
GD1α, 9-deoxy-NeuAc	25	24	27

3. Structure-activity relationship of neural siglecs ligands at the level of functional group²⁹

Previously, we demonstrated³⁰ that any chemical modification of the terminal sialic acid, termed as the primary sialic acid, abrogated MAG binding, indicating the whole structure of the terminal sialic acid is required for the binding. However, the structural requirement on the internal sialic acid at C-6 of the GalNAc residue, termed as the secondary sialic acid, has not been elucidated. In this section, we undertook the systematic synthesis of ganglioside GD1α analogues carrying *N*-acetyl-deoxyneuraminic acids at C-6 of the GalNAc residue and examined their binding to MAG in a study of the structure-activity relationship of neural Siglecs ligand at the level of the functional group.

3.1 Systematic synthesis of ganglioside GD1α analogues carrying modified sialic acids

For the systematic synthesis of a series of GD1α analogues we employed the strategy in which GM1b oligosaccharide was first constructed and then sialylated on C-6 of GalNAc (discussed in 2.1).

A series of phenyl 2-thioglycosides of *N*-acetyldeoxyneuraminic acids (31–33)³¹, which were prepared from the corresponding 2-(trimethylsilyl)ethyl glycoside of *N*-acetylneuraminic acid by our previous procedure with some improvements, were each coupled with the suitably protected GM1b pentasaccharide 34¹⁴ in the presence of NIS-TfOH in acetonitrile at -20°C to give the desired NeuAcα(2-6) hexasaccharide (35–37, 30–40%) accompanied by NeuAcβ(2-6) isomers (20–25%). The most significant signals in the $^1\text{H-NMR}$ spectra of 35 were a one-proton doublet of doublets ($J_{\text{gem}} = 12.8$, $J_{3\text{eq},4} = 4.8\text{Hz}$) at δ 2.56 due to H-3eq of the newly introduced α(2-6)-linked sialyl residue, and another one-proton doublet of doublets ($J_{\text{gem}} = 12.6$, $J_{3\text{eq},4} = 4.6\text{Hz}$) at δ 2.43 due to H-3eq of the α(2-3)-linked terminal sialyl residue. The protected hexasaccharides 35–37 were transformed into the corresponding gangliosides, by the same procedure as described for the synthesis of GD1α (see 2.1).

Scheme 5

3.2 Binding of MAG and SMP to ganglioside GD1α analogues²⁸

We tested the role of α(2-6)NeuAc residue using synthetic GD1α analogues. As shown in the Table, GD1α analogues bearing a 7-, 8-, or 9-deoxysialic acid linked to GalNAc were comparable with the parent GD1α in supporting MAG and SMP binding and were much more potent than GM1b. This result led us to test the possibility that the secondary 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,³² in contrast to the primary sialic acid $\alpha(2-3)$ -linked to the Gal residue. In fact, structures bearing a sulfate group in place of the GD1 α secondary NeuAc residue displayed equivalent enhanced affinity (GM1b III⁶ sulfate) with its NeuAc-containing compound GD1 α .³³

4. Conclusion

In this paper, we have described the results of a study to elucidate the structure required as the ligands of neural Siglecs, mainly an organic synthetic approach, at the level of the sugar residue as well as the functional group. MAG is thought to be implicated in myelin-neuron interactions and, in particular, has been shown to be a neurite outgrowth inhibitor; therefore, detailed knowledge of the carbohydrate ligands that support MAG binding may provide opportunities for design of methodology in the control of neurite outgrowth and myelination.

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