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Novel Sulfated Gangliosides, High-Affinity Ligands for Neural Siglecs, Inhibit NADase Activity of Leukocyte Cell Surface Antigen CD38☆

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Abstract—Three kinds of novel sulfated gangliosides structurally related to the Chol-1 (α-series) ganglioside GQ1bα were synthesized. These sulfated gangliosides were potent inhibitors of NADase activity of leukocyte cell surface antigen CD38. Among the synthetic gangliosides, GSC-338 (II^3III^6-disulfate of *iso*-GM1b) was surprisingly found to be the most potent structure in both the NADase inhibition and MAG-binding activity. The present study indicates that the sulfated gangliosides are useful to study the recognition of the internal tandem sialic acid residues α2-3-linked to Gal(II^3) as well as the siglec-dependent recognition including a terminal sialic acid residue.

It has been widely recognized that gangliosides, sialic acid-containing glycosphingolipids, are involved in various biological processes such as cell differentiation, cell growth, cell adhesion, immune response, oncogenesis, and many other receptor-mediated reactions.[2] We have succeeded[3] in the systematic total syntheses of a variety of gangliosides including their analogues and derivatives, and have revealed their biological functions at the molecular level.[4] Among them, the cholinergic neuron-specific Chol-1 (α-series) gangliosides[5] (Fig. 1) have been found[6] to be high affinity ligands for neural siglecs[7] (sialic acid-binding Ig-like lectins). The major determinant of neural siglec is α2,3-linked sialic acid and the internal sialic acids α2,3-linked to Gal (II^3) and α2,6-linked to GalNAc (III^6) greatly enhance the siglec-mediated cell adhesion. Especially, GQ1bα was a remarkably high affinity ligand for myelin-associated glycoprotein (MAG, siglec-4a).[6] Recently, we reported that the internal sialic acids could be substituted by other anionic groups based on the structure-siglec binding study.[8,9]

The leukocyte cell surface antigen CD38[10] is a predominant NAD^+ glycohydrolase in mammalian cells.[11] In consistent with its ubiquitous expression, widespread functional aspects of CD38 have been reported such as

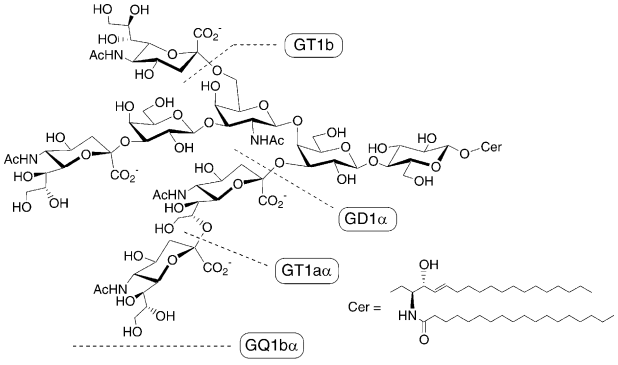

Figure 1. Structure of Chol-1 (α-series) gangliosides and GT1b.

☆Synthetic Studies on Sialoglycoconjugayes, Part 133. For part 132, see ref 1.

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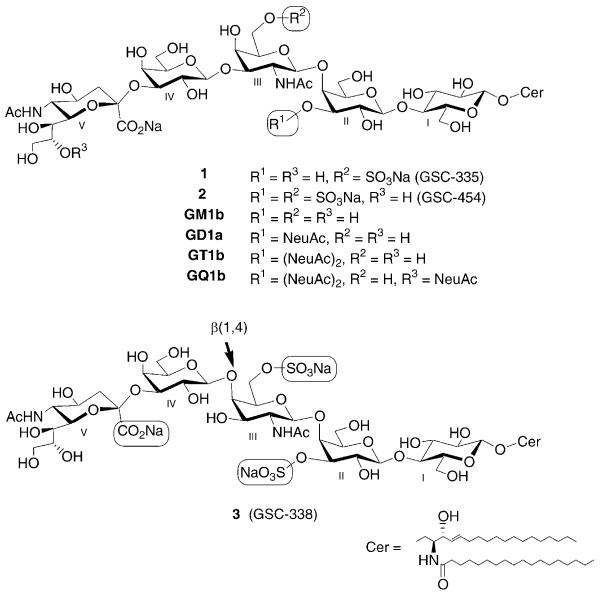
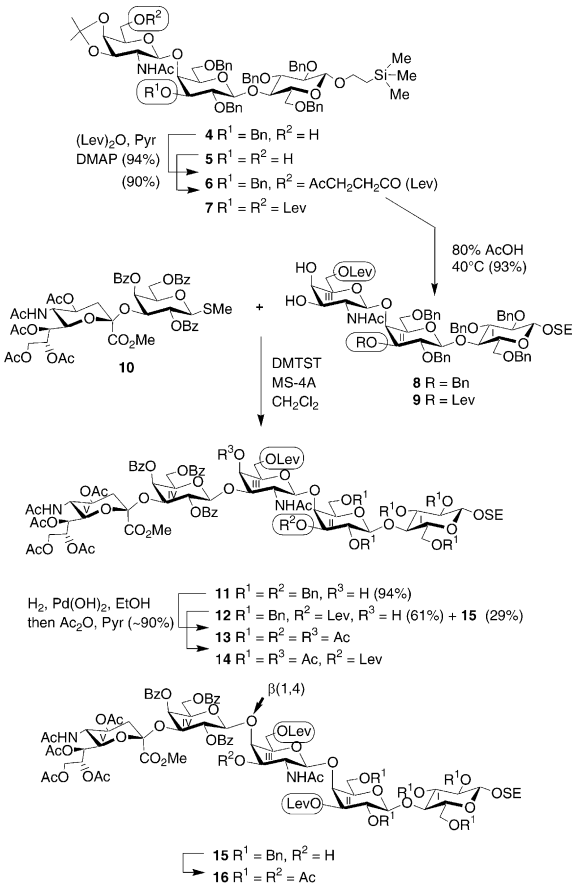


Figure 2. Structures of novel sulfated gangliosides **1**–**3** and ganglioside GM1b, GD1a, GT1b and GQ1b.

immunoregulation,[12] cell proliferation,[13] chemotaxis,[14] insulin release[15] or bone resorption.[16] The NADase activity is associated with the extracellular domain of CD38. It has been found[17,18] that these catalytic reactions of CD38 are efficiently inhibited by gangliosides such as GQ1bα, GQ1b and GT1b (Figs. 1 and 2). Thus, the internal tandem sialic acid residue α2,3-linked to Gal (II^3) is important for the inhibition. In the present study, we examined whether the crucial internal tandem sialic acid residues can be substituted by other anionic groups or not. We report here an efficient synthesis of novel sulfated gangliosides **1**–**3** (Fig. 2) in which the internal sialic acids of GQ1bα are substituted by the sulfate groups, and their inhibitory activity for NADase of CD38.

For the selective protection of the II^3 and III^6 hydroxyl groups, we selected the suitably protected ganglio-triose derivatives **4** and **5**[19] as the starting materials (Scheme 1), which had served as the key intermediate for the first total synthesis of ganglioside GQ1bα.[19] Treatment of **4** or **5** with levulinic anhydride in pyridine in the presence of 4-dimethylaminopyridine (DMAP) gave the III^6-levulinoyl (**6**) and II^3III^6-*di*-levulinoyl (**7**) derivatives in 94 and 90% yields, respectively. The 3,4-*O*-isopropylidene group in **6** or **7** was then cleaved by treatment with 80% acetic acid at 40 °C, to afford the desired glycosyl acceptors **8** and **9** in high yields. Dimethyl(methylthio)sulfonium triflate (DMTST)-promoted glycosylation[20] of **8** or **9** with a sialyl-α(2→3)-galactose donor **10**[21] was achieved β-stereoselectively to afford the protected pentasaccharide **11** (94%), and **12** (61%) accompanied by **15** (29%), respectively (Scheme 1). It is of interest to note that the glycosylation of **8** (R = Bn) with **10** gave **11** with high regioselectivity, while **9** (R = Lev) afforded a mixture of regioisomers (**12** and **15**). This result suggests that the steric hindrance of the Lev group in the adjacent Gal residue reduced the

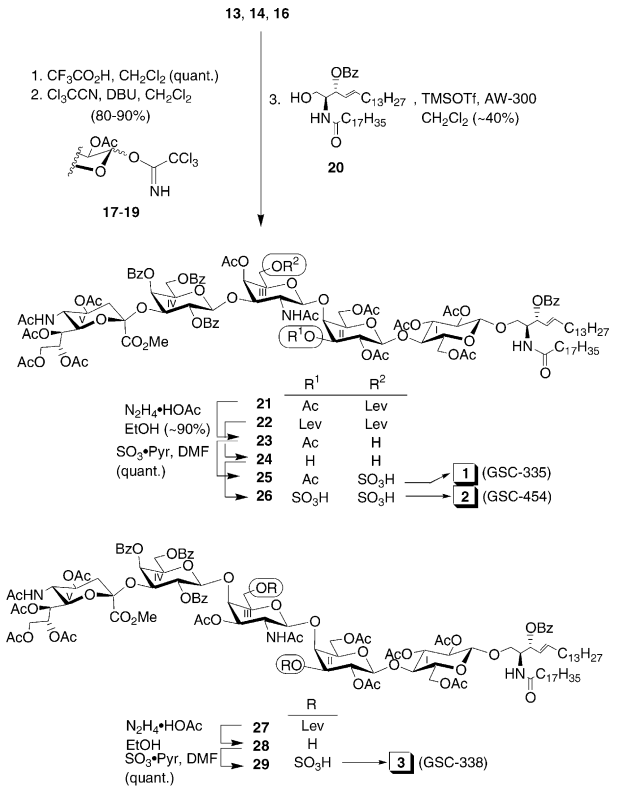


Scheme 1.

reactivity of the hydroxyl group at C-3 of the GalNAc residue.

Hydrogenolytic removal of the benzyl groups, in **11**, **12** and **15**, followed by acetylation, gave **13**, **14**, and **16**, respectively, which upon selective cleavage[22] of the 2-(trimethylsilyl)ethyl (SE) group, and treatment[23] with trichloroacetonitrile and DBU, afforded a series of imidate derivatives **17**–**19** (Scheme 2).

The direct glycosylation of the ceramide derivative **20**[24] with **17**–**19** was carried out in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and molecular sieves 4 Å (AW-300) in dichloromethane to yield the protected gangliosides **21**, **22**, and **27** in about 40% yields: most of the unreacted glycosyl donor (1-OH form) and acceptor **20** were recovered. The recovered hemiacetal was again transformed into the imidate donors. This result is comparable to that obtained by an efficient β-glycosidation procedure using the 2-*O*-pivaloyl group as an auxiliary.[25] Therefore, the yields of ~40% is satisfactory considering the additional steps in the azidosphingosine procedure, which has been employed for various ganglioside synthesis.[3] However, this incredibly high yield may be explained by the linear structure of the glycosyl donors which have no branched sugar residues. The levulinoyl groups in **21**, **22**, and **27** were selectively removed by treatment with hydrazine monoacetate in ethanol to give **23**, **24**, and **28** respectively, which were then sulfated quantitatively



Scheme 2.

with a sulfur trioxide–pyridine complex in DMF. Finally, all protective groups in **25**, **26** and **29** were removed by treatment with sodium methoxide in methanol, followed by addition of water, to afford the target compounds **1**–**3** in high yields (Scheme 2). The final products were purified by chromatography (5:4:0.7:0.07 $CHCl_3$–MeOH–H_2O–Et_3N) on a column of Sephadex LH-20. Selected physical data for synthetic compounds are provided in ref 26.

b-Series gangliosides GQ1bα, GQ1b, and GT1b (Figs. 1 and 2) have a potent inhibitory activity on the NADase of the extracellular domain of CD38 expressed as a fusion protein with maltose-binding protein (MBP-CD38).[18] As GQ1bα and GT1b were more effective than GT1aα and GD1a, respectively, the tandem sialic acid [NeuAc-α(2,8)-NeuAc] residue α2,3-linked to the internal Gal (II) residue seems to be crucial for the inhibition. Furthermore, the lactonization of GT1b greatly diminished the inhibitory potency, indicating that the negative charges in the carboxyl group of NeuAc are involved in the inhibition. We have proposed that the negative charge cluster formed by the two carboxyl groups of the tandem sialic acid residues can mimic the diphosphate structure of NAD^+, which explains the inhibitory effect of b-series gangliosides.[17]

The effect of GSC-335, GSC-338, or GSC-454 on the NADase activity of MBP-CD38 was examined as described in ref 27. As shown in Figure 3, all the three sulfated gangliosides inhibited the NADase activity. However, GSC335 was less potent than the others. The presence of sulfate group on C-3 of the internal Gal (II) potentiated the inhibitory effect (GSC-454). GSC-338 differs from GSC-454 in that the linkage between terminal Gal (IV) and GalNAc (III) is unnatural β1-4 form, and inhibited the NADase activity more effectively than GSC-454. GSC-338 was as potent as GT1b with and without the preincubation step.

In GSC-454 and GSC-338, it is possible to locate the two sulfate groups on GalNAc (III) and Gal (II) close to each other, which can mimic the diphosphate structure of NAD just like the two carboxyl groups of the tandem sialic acid residues of GT1b. Proposed structural models constructed using CAChe program[28] are shown in Figure 4. When the Gal (IV) residue is linked to C-4 of GalNAc (GSC-338), the sugar residues I–IV align so that the terminal sialic acid may contribute to the recognition of the negative charge cluster of the sulfate groups by MBP-CD38 (see Fig. 4). On the other hand, when the Gal (IV) residue is linked to C-3 of GalNAc (GSC-454), the terminal sialic acid is protruded from the anion cluster. Such structural feature could explain why GSC-338 is more potent than GSC-454.

In conclusion, the novel sulfated gangliosides (GSC-335, GSC-454 and GSC-338) which have potent binding activity to neural siglecs[9] were systematically synthesized. They were found to be potent inhibitors for NADase activity of CD38. It is plausible that the two sulfate groups on GalNAc (III) and Gal (II) substitute the internal tandem sialic acid residues of b-series gangliosides. Among the synthetic gangliosides, GSC-338 (compound **3**) was the most potent structure. Since GSC-338 has also most potent MAG-binding activity,[9] the anion cluster including two sulfate groups and the terminal sialic acid must be important for expressing both activities. Recently, a novel ganglio-series ganglioside 3′-*O*-sulfo-GM1b [NeuAcα2-3Galβ1-3GalNAcβ1-4(HSO_3-3)Galβ1-4Glcβ1-1Cer] has been isolated from bovine cauda equina,[29] suggesting the possibility of occurrence of new sulfated gangliosides in nature. However, the biological function has not been elucidated because of insufficient availability of materials.

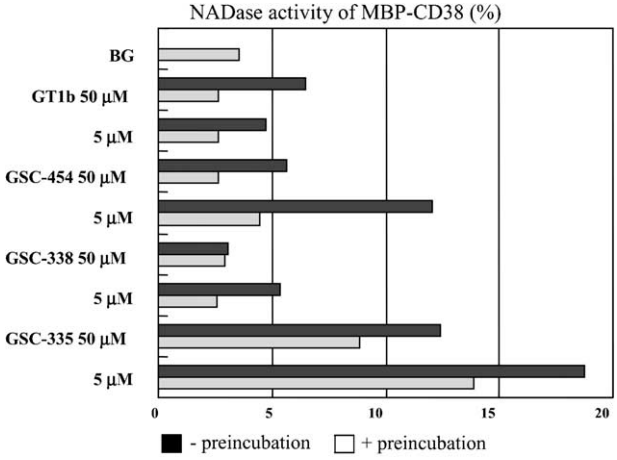


Figure 3. Inhibitory effect of GSC-335, 338, 454 on the NADase activity of MBP-CD38.

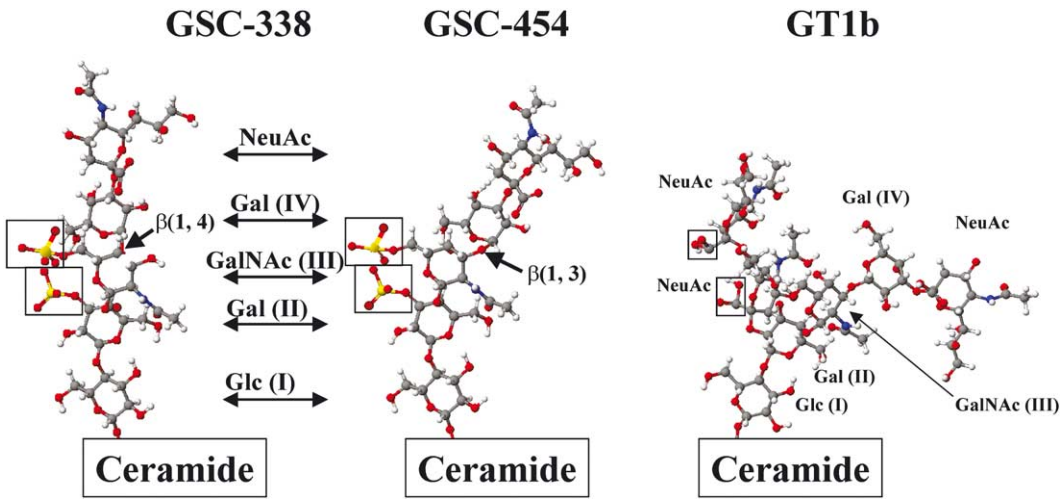


Figure 4. Proposed structural models of GSC-338, GSC-454 and GT1b. The *boxed outlines* enclose either the sulfate groups in GSC-338 and GSC-454, or the carboxyl groups of the internal tandem sialic acids $(NeuAc)_2$ in GT1b.

Therefore, the synthetic sulfated gangliosides may open new perspectives on their structure–function study and development of novel therapeutic agents.

Acknowledgements

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26. Compound **1** (GSC-335): $[\alpha]_D$ +14.1° (*c* 0.2, 5:4:0.7 $CHCl_3$–MeOH–H_2O); ^{1}H NMR ($(CD_3)_2SO$–D_2O): δ 0.85 (t, 6H, J_{Me,CH_2} 7.0 Hz, 2 *Me*CH_2), 1.23 (s, 52H, 26 CH_2), 1.40 (m, 2H, $COCH_2CH_2$), 1.80 and 1.90 (2 s, 6H, 2 AcN), 2.74 (m, 1H, H-3^{V}eq), 4.17 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1^{I}), 4.19 (d, 1H, $J_{1,2}$ = 7.0 Hz, H-1^{II}), 4.25 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1^{IV}), 4.52 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1^{III}), 5.33 (m, 1H, ceramide-4), and 5.52 (m, 1H, ceramide-5). FAB-MS (negative ion mode, triethanolamine matrix): *m*/*z* 1646.9 [M−Na]$^-$, 1624.9 [M−2Na]$^-$, 1333.8 [M−Na–NeuAc]$^-$, 1171.8 [1333.8−Gal]$^-$, 888.7 [lactosyl ceramide]$^-$, 726.7 [glucosyl ceramide]$^-$, 564.6 [ceramide]$^-$; calcd for $C_{73}H_{129}N_3NaO_{34}S$ (M−Na): 1646.8076; found: 1646.86. Compound **2** (GSC-454): $[\alpha]_D$ +0.3° (*c* 0.6, 5:4:0.7 $CHCl_3$–MeOH–H_2O); ^{1}H NMR ($(CD_3)_2SO$–D_2O): δ 0.83 (t, 6H, J_{Me,CH_2} = 7.0 Hz, 2 *Me*CH_2), 1.22 (s, 52H, 26 CH_2), 1.41 (m, 2H, $COCH_2CH_2$), 1.85 and 1.92 (2 s, 6H, 2 AcN), 2.74 (m, 1H, H-3^{V}eq), 4.08 (m, 1H, H-1^{I}), 4.28 (d, 1H, $J_{1,2}$ = 7.1 Hz, H-1^{II}), 4.31 (m, 1H, H-1^{IV}), 4.50 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1^{III}), 5.27 (m, 1H, ceramide-4), and 5.54 (m, 1H, ceramide-5). FAB-MS (negative ion mode, triethanolamine matrix): *m*/*z* 1726.8 [M−2Na]$^-$, 1625.0 [M−2Na–SO_3Na + H]$^-$, 1435.8 [*M*−Na–NeuAc]$^-$, 1333.8 [1435.8−SO_3Na + H]$^-$, 1171.7 [1333.8−Gal]$^-$, 968.7 [3′-*O*-sulfo lactosyl ceramide]′, 726.6 [glucosyl ceramide]$^-$, 564.6 [ceramide]$^-$; calcd for $C_{73}H_{129}N_3NaO_{37}S_2$ (M−2Na): 1726.7644; found: 1726.80. Compound **3** (GSC-338): $[\alpha]_D$ −12.7° (*c* 0.2, 5:4:0.7 $CHCl_3$–MeOH–H_2O); ^{1}H NMR ($(CD_3)_2SO$–D_2O): δ 0.85 (t, 6H, JM_{e,CH_2} = 7.0 Hz, 2 *Me*CH_2), 1.23 (s, 52H, 26 CH_2), 1.44 (m, 2H, $COCH_2CH_2$), 1.87 and 1.90 (2 s, 6H, 2 AcN), 2.71 (m, 1H, H-3^{V}equiv), 4.17 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1^{I}), 4.23 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1^{II}), 4.29 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1^{IV}), 4.42 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1^{III}), 5.32 (m, 1H, ceramide-4), and 5.52 (m, 1H, ceramide-5). FAB-MS (negative ion mode, triethanolamine matrix): *m*/*z* 1726.9 [M−2Na]$^-$, 1625.0 [M−2Na–SO_3Na + H]$^-$, 1435.8 [M−Na–NeuAc]$^-$, 1333.7 [1435.8−SO_3Na + H]$^-$, 1171.8 [1333.7−Gal]$^-$, 968.7 [3′-*O*-sulfo lactosyl ceramide]$^-$, 726.5 [glucosyl ceramide]$^-$, 564.6 [ceramide]$^-$; calcd for $C_{73}H_{129}N_3NaO_{37}S_2$ (M−2Na): 1726.7644; found: 1726.88.
27. NADase assay: GSC-335, GSC-338, or GSC-454 dissolved in DMSO–H_2O (1:1, v/v) or GT1b in methanol were dried up in test tubes. To measure the NADase activity of MBP-CD38, the reaction mixture (10 μL) containing 5 nM MBP-CD38 in Buffer A (50 mM Tris–HCl, pH 8.0, 1 mM EDTA) was put into the test tubes. The reaction was started by the addition of 10 μL of 9.25 μM [*carbonyl*-^{14}C]NAD (5 nCi/assay) in Buffer A. After 10 min of incubation at 37 °C, the release of [^{14}C]nicotinamide was measured as described previously.[18]
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