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Potent small molecule mouse CD22-inhibitors: Exploring the interaction of the residue at C-2 of sialic acid scaffold

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ABSTRACT

Our previous study revealed that compound **1** (9-(4′-hydroxy-4-biphenyl)acetamido-9-deoxy-Neu5Gcα2-6GalOMP) has the most promising affinity for mCD22. Replacing the subterminal galactose residue of **1** with benzyl or biphenylmethyl as aglycone led to 38- and 20-fold higher potency, respectively. This discovery represents a new direction in inhibitor design suitable for pharmaceutical development.

CD22 is a B cell-specific sialic acid binding immunoglobulin-like lectin (Siglec) whose function as a regulator of B cell signaling appears to be modulated by its interaction with glycan ligands bearing the sequence Neu5Ac/Gcα2-6Gal.[1] It exhibits low intrinsic affinity for its in vivo ligands (K_d = 0.2 mM), and their abundance provides cis and trans interactions that effectively mask the ligand binding site.[2] To overcome the threshold set by these interactions, high affinity ligand probes are required. Interestingly, high affinity ligand conjugated with toxin was shown to bind CD22 and eventually endocytosed, inducing B cell death. This finding represents a promising approach for B cell targeting.[3]

In addition to treatment of hematologic malignancies,[4] B cell-directed therapies have an important role in the treatment of autoimmune disorders.[5] It has been suggested that CD22-inhibitors could shorten the time required for antibody production, thereby augmenting the host defense against acute infectious diseases regardless of pathogens; as a 'universal vaccination'.[6]

A number of ligand specificity studies for CD22 have been carried out which have shown the importance of C-2, C-5 and C-9 substituents on sialic acids in modulating binding affinity and selectivity.[7–14] CD22 showed high specificity for Neu5Ac/Gcα2-6Gal. The axial position of the carboxyl group in α-sialosides is essential for the binding. In contrast to most members of siglec family, human (hCD22) and mouse (mCD22) show high affinity for Neu5Gc.[7] Moreover, 9-biphenylcarboxamido and 9-biphenylacetamido derivatives of Neu5Ac/Gc showed high affinity and selectivity for hCD22 and mCD22, respectively.[8,9] These key features of the CD22-sialic acid binding, provide the basis for sialoside design aimed at developing compounds with enhanced affinity and selectivity for CD22.

CD22 binding, similar to other siglecs, is almost entirely mediated through interactions with the Neu5Ac/Gc template, while the subterminal galactose residue contribute to a lesser degree and its hydroxyl groups are not involved in binding.[10]

Recently,[14] we have reported the dramatic improvement of binding affinity for CD22 achieved by the modifications at 9-position of Neu5Gcα2-6GalOMP core as exemplified by compound **1**, Figure 1, (9-(4′-hydroxy-4-biphenyl)acetamido-9-deoxy-Neu5Gcα2-6GalOMP) which exhibited the highest potency for mCD22. Our predicted binding model for compound **1**,[14] revealed that the interactions of GalOMP residue with its binding site is mainly hydrophobic in nature.

In this respect, Neu5AcαBn was 8–10-fold more potent than Nue5AcαMe for MAG (siglec-4) and sialoadhesin (Sn).[7] Since the

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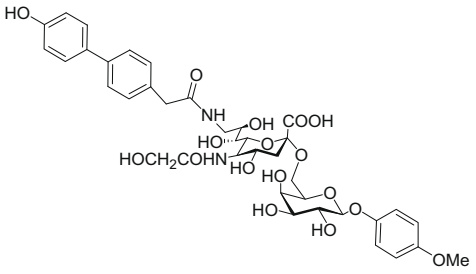


Figure 1. Compound **1**.[14]

binding sites of Sn and CD22 possess a high degree of sequence similarity;[12] we expected that hydrophobic substituent at the C-2 of sialic acid scaffold could improve the affinity for CD22 in analogy to Sn.

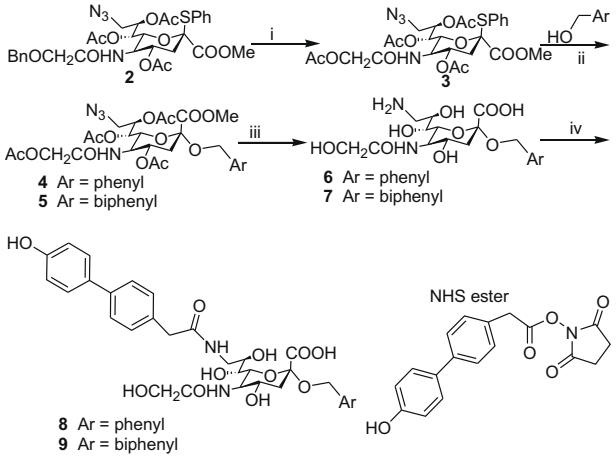
To explore the importance of α2-6 linkage, contribution of galactose residue to the binding and the nature of interaction at C-2 of sialic acid; mimetics **8** and **9** were synthesized by replacing GalOMP residue of **1** with benzyl or biphenylmethyl, respectively, as reducing end substituents.

The synthesis of the target compounds is depicted in Scheme 1. Compound **2** was synthesized in seven steps starting from neuraminic acid.[14] Selective debenzylation of **2** with Hanessian reaction method[15] followed by acetylation afforded compound **3** in 87%.

Glycosylation of **3** with benzyl alcohol or biphenylmethanol under NIS/TfOH condition at −40 °C in acetonitrile gave the corresponding sialoside **4** or **5** in about 76% (α/β = 66/10). Saponification of the α-sialosides followed by chemoselective reduction of azide with Me_3P gave 9-amino derivative **6** and **7**. Acylation of the 9-amine with *N*-hydroxysuccinimide ester of 4′-hydroxybiphenyl-4-acetic acid[14] yielded the target compounds **8** and **9**.

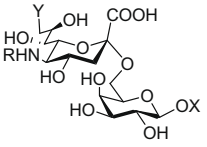
A competition enzyme-linked immunosorbent assay (ELISA) based on a biotinylated form of **1** has been developed.[16]Therefore the binding affinity of various synthetic sialosides was accurately and reliably determined. Inhibitory potencies of some previously reported compounds (Table 1), **1**, **8** and **9** (Table 2) were determined (see Supplementary data). The results are shown in Tables 1 and 2.

The results in Table 1 are included to show the higher affinity of mCD22 for *N*-Gc at C-5 of sialic acid and the dramatic improvement of the affinity of **GSC-633** upon amidation of 9-amino to give compound **1**.[14]



Scheme 1. Reagents and conditions: (i) (a) 10 equiv $PhSSiMe_3$, 5 equiv ZnI_2, 1.5 equiv Bu_4NI, DCE, 60 °C, 8 h; (b) Ac_2O/pyridine, two steps 87%; (ii) NIS, TfOH, MeCN, −40 °C; 36 h, 76%; (iii) (a) LiOH, EtOH/H_2O; (b) Me_3P, MeOH/H_2O, rt, 24 h, two steps 84%; (iv) NHS ester, $NaHCO_3$, MeCN/H_2O, 75%.

Table 1
Inhibitory potencies of representative Neu5Ac/Gcα2-6Gal derivatives for mCD22



Compd	Y	R	X	IC_{50}[a] (μM) ± SD
Neu5Acα2-GalβSE	OH	Ac	SE	N. D.
Neu5Gcα2-GalβSE	OH	Gc	SE	544.8 ± 23.5
GSC-715	OH	Gc	MP	305.2 ± 20.4
GSC-633	NH2	Gc	MP	82.9 ± 4.5

[a] Sialoside concentration which leads to 50% inhibition of binding. The values are the mean ± SD of triplicates. MP, *p*-methoxyphenyl; SE, 2-(trimethylsilyl)ethyl. N. D.; IC_{50} >1000 μM.

Table 2
Inhibitory Potencies of compounds **1**, **8** and **9** for mCD22

Compd	IC_{50}[a] (μM) ± SD	rIP[b]
1	3.84 ± 0.32	1
8	0.10 ± 0.00	38.4
9	0.19 ± 0.00	20.2

[a] Footnote of Table 1.
[b] The rIP of each sialoside was calculated by dividing the IC_{50} of the reference compound **1** by the IC_{50} of the compound of interest. This results in rIPs above 1.0 for derivatives binding better than **1** and rIPs below 1.0 for compounds with a lower affinity than **1.**

Interestingly, replacing the subterminal galactose residue of compound **1** with benzyl or biphenyl as aglycone enhanced the potency. Benzyl sialoside (**8**) exhibited an IC_{50} value 38-fold more potent than **1**. The sterically more demanding biphenyl of **9** led also to improved affinity but to a lower extent; 20-fold more potent than **1**. The differences in binding of these compounds must arise from the differences in the interactions made by the substituents at the C-2 of sialic acid moiety. These substituents are either benzyl or biphenylmethyl and the interaction may therefore be expected to be primarily hydrophobic in nature. However, dimerization of CD22 molecules, mediated through the phenyl (**8**) or biphenyl (**9**) of the ligand may be occurred as already observed in the crystal structure of Neu5AcαBn with Sn.[11] Based on these results it could be concluded that the α2-6 linkage and the subterminal galactose residue are not essential for CD22 binding and could be replaced with non-carbohydrate moiety with even improved affinity and drug like properties.

Further investigation is under way to clarify in more detail the role of subterminal glycan in CD22 binding affinity and selectivity. It is worth noting that benzyl and biphenyl groups were successfully employed to mimic Galβ(1-4)GlcNAc core in selectin antagonists and the Galβ(1-3)Gal*N*Ac core in MAG antagonists.[17a–d] Biphenyl moiety was identified as a feasible replacement for the core disaccharide Galβ(1-3)Gal*N*Ac according to saturation transfer difference (STD) NMR and molecular modeling investigations.[17c]

In summary, replacing the galactose residue of compound **1** with benzyl or biphenylmethyl resulted in compounds with simplified structure and improved potency. Benefits also include easier synthetic access and possibly enhanced bioavailability.

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Supplementary data

Supplementary data (the synthetic procedures, characterization of the compounds, and inhibition assay) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.044.

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