Second Generation Mimics of Ganglioside GM1 as Artificial Receptors for Cholera Toxin: Replacement of the Sialic Acid Moiety

Anna Bernardi,[a,*] Laura Carrettoni,[a] Antonio Grosso Ciponte,[a] Diego Monti[b] and Sandro Sonnino[c]

[a]Dipartimento di Chimica Organica e Industriale, Università di Milano, Via Venezian 21, 20133 Milano, Italy
[b]Centro CNR per lo Studio delle Sostanze Organiche Naturali, Milano, Italy
[c]Dipartimento di Chimica e Biochimica Medica, Università di Milano, LITA, Segrate (MI), Italy

Abstract—In a program directed towards the design and synthesis of mimics of ganglioside GM1, the NeuAc recognition domain was replaced by simple hydroxy acids, and the affinity of the new ligands to the cholera toxin was determined by fluorescence spectroscopy. The (R)-lactic acid derivative **4** was found to display the highest affinity of the series ($K_D = 190 \, \mu M$). © 2000 Elsevier Science Ltd. All rights reserved.

Carbohydrate–protein recognition events have emerged as a potential target for the cure of many human diseases, and the development of glycomimetic molecules, i.e., molecules that behave like structural and functional mimics of oligosaccharides, has attracted much attention.[1] One of the best characterized protein–carbohydrate complexes is formed by ganglioside GM1 (Galβ1-3 GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-1 Cer) and two AB$_5$ hexameric proteins, homologous by 80% and sharing a common mechanism of action: the cholera toxin from *Vibrio cholerae* (CT), or the heat-labile toxin of *Escherichia coli* (LT).[2] Both toxins recognize the oligosaccharide headgroup of ganglioside GM1 (o-GM1, **1**) on the host cell epitelial surface using the B5 pentamer. Biochemical[3] and structural[4] data have shown that five binding sites are present on the toxin and that the carbohydrate binds to them using the two terminal sugars at its non-reducing end, a galactose (Gal) unit and a sialic acid (NeuAc) residue, the latter interacting mainly through its carboxy group.[3a,b,4] Finding new receptors for bacterial enterotoxins may lead to drugs that block their docking to the target cells, thus preventing the onset of the disease.

In the course of a program aimed to identify viable computational tools for the study of protein–carbohydrate interactions and to develop approaches to the design of glycomimetic molecules,[5] the pseudo-oligosaccharide **2** was introduced as a functional and structural mimic of ganglioside GM1.[6] For the design of **2**, the Gal and NeuAc recognition determinants of GM1 were retained, and a new, conformationally restricted cyclohexanediol (DCCHD) was used as the scaffold element to replace the rest of the oligosaccharide. As a mimic of a 3,4-di-substituted galactose, DCCHD has many appealing features: it is easily available on a large scale and in enantiopure form;[7,8] it contains two carboxy groups that represent convenient handles for conjugation to various supports, and, as a carbocycle, it should enhance the metabolic stability of the structure. Mimic **2** was found to bind CT as efficiently as o-GM1 **1**, as determined by competitive inhibition assays.[6]

In order to simplify the molecular and synthetic complexity of **2**, replacement of the NeuAc moiety by simple α-hydroxyacids was examined in the series of second generation mimics **3**–**5** (Scheme 1).[9] Stereoselective sialylation is one of the unresolved problems in carbohydrate chemistry and, given the widespread occurrence of the sialic acid motif in protein-binding carbohydrates, the evaluation of functional substitutes for this residue in the context of the CT/LT model is worth pursuing. Indeed, sialylation of DCCHD represents the bottleneck of the entire reaction sequence leading to **2**, and replacement of the NeuAc residue with more treatable

*Corresponding author. Tel.: +39-02-2367593; fax: +39-02-2663079; e-mail: anna.bernardi@unimi.it
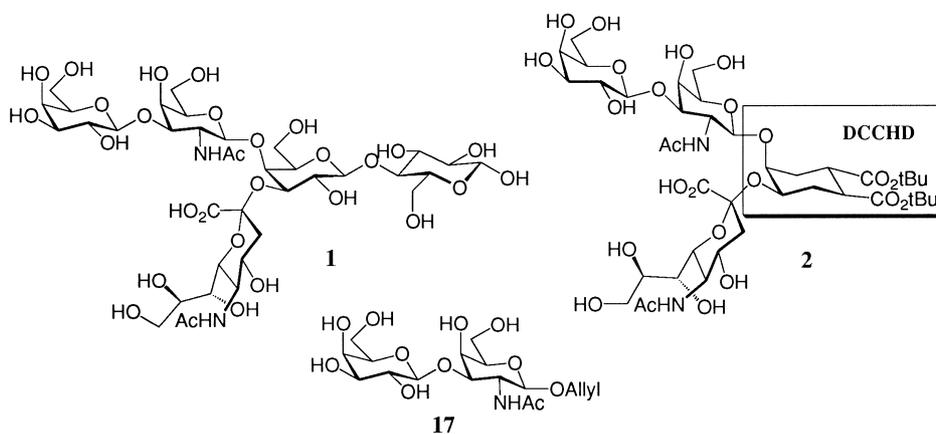
Chart 1. Ganglioside GM1 headgroup **1**, the artificial receptor **2**, and the Galβl-3GalNAc disaccharide **17**.
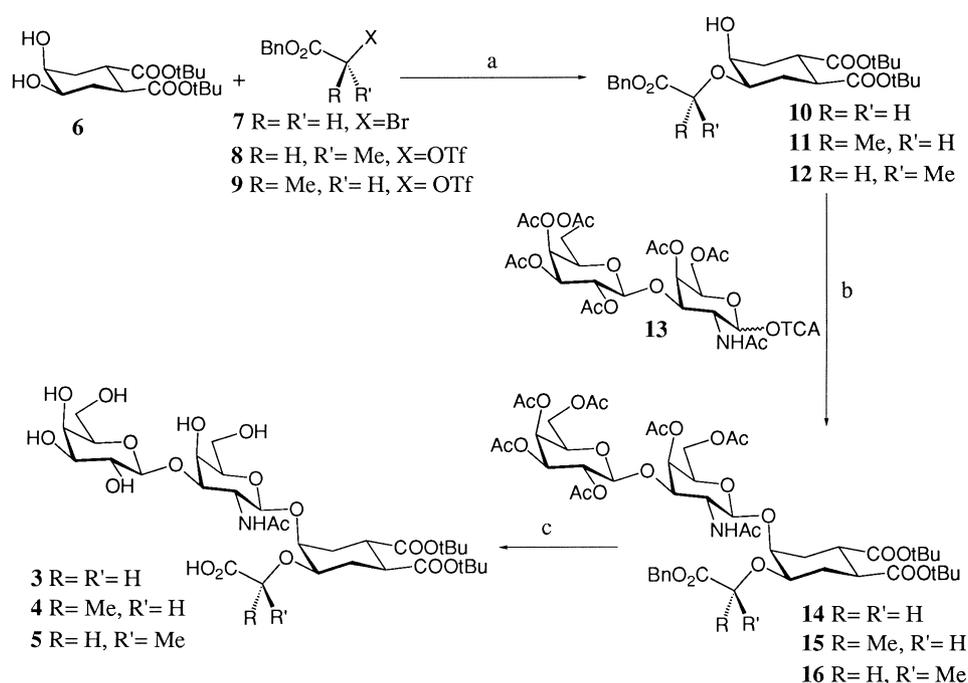
Scheme 1. General sequence for the synthesis of **3–5**. (a) Bu$_2$SnO, benzene, reflux, then Bu$_4$NI and **7**, or **8**, or **9** (50, 53, 76% yield). (b) **13** (0.5 equiv) and TMSOTf (0.3 equiv) in CH$_2$Cl$_2$, room temperature to reflux (48, 38, 38% yield). (c) H$_2$/Pd–C (90% yield); cat. MeONa in MeOH (80, 82, 85% yield).

chemical entities would considerably increase the accessibility of the artificial receptors. Conformational analysis of **3–5** showed that, although more flexible than **2**, the (R)-lactic acid derivative **4**, and to a minor extent, the glycolic acid derivative **3**, should be able to simultaneously fit the galactose and the carboxy binding sites of CT using low-energy conformations.[10]

The three substrates were synthesized following the sequence reported in Scheme 1. Thus, from the enantiomerically pure diol **6**,[7] the monoethers **10–12** were synthesized using Bu$_2$SnO and the appropriate nucleophile (**7–9**).[9,11] Glycosylation of the axial hydroxy group with the Galβl-3GalNAc donor **13**[7] was promoted with TMSOTf, to give the pseudotrisaccharides **14–16**, from which standard removal of the protecting groups yielded **3–5**.

The interaction of **1–5** with cholera toxin B5 pentamer (CTB) was studied using the intrinsic fluorescence of the Trp88 residue in the toxin binding site.[3e,12,13] Binding of **1–5** to CTB induces bathochromic shifts in the emission spectrum whose extent depends on the structure of the ligand.[14] The shift caused by GM1 binding is accompanied by a slight increase in fluorescence intensity, whereas a variable decrease in fluorescence is caused by **2–5**.[14] Thus, the resulting binding isotherms are more accurately compared by analyzing the intensity data, which requires a relatively high CTB concentration (0.5 μM, pentamer concentration). Wavelength titrations performed for **1** and **2** at lower toxin concentrations (0.1 μM) gave comparable results.

The normalized changes in fluorescence emission intensity in CTB upon titration with **1–5** are collected in
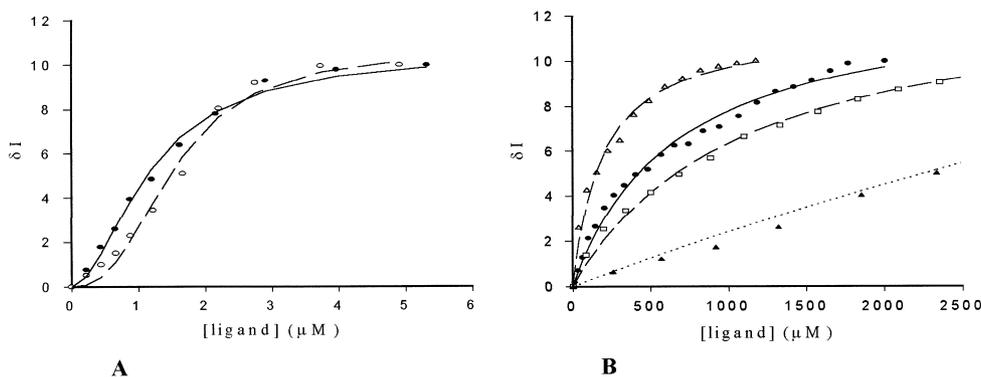
Figure 1. The effects of ligand binding on the fluorescence intensity of Trp88 in CTB. In **A** CTB (0.5 μM) was titrated with micromolar amounts of o-GM1 **1** (open circles, dashed line) or of mimic **2** (closed circle, solid line), which are shown plotted against the normalized, absolute value of the relative fluorescence intensity (|δI|) measured at the emission maximum of Trp88. In **B** the same experiment was performed using 0.5 μM CTB and micromolar concentrations of **4** (open triangles, dashed line), **3** (closed circles, solid line) and **5** (open squares, dashed line) or 2 μM CTB and micromolar concentrations of Galβ1-3GalNAc-OAllyl **17** (closed triangles, dotted line).

Figure 1. The effects measured for **1** and the first generation mimic **2** (Fig. 1(A)) confirm the comparable affinity of the two ligands previously established by ELISA inhibition assay.[6] Furthermore, both substrates appear to bind cooperatively to CTB, albeit with different cooperativity factors. Indeed, binding of **1** to CTB is known to occur cooperatively.[3e] Using calorimetric titrations, Freire and Schön[3e] have shown that the intrinsic association constant of **1** for the B_5 pentamer at 37 °C is 1.05×10^6 M^{-1}, which increases by a factor of 4 if an adjacent binding site on CTB is already occupied. In contrast, binding of **3–5** (Fig. 1(B)) does not display cooperative behavior. This suggests that the communications between CTB monomers elicited by **1** and **2** may be mediated by the NeuAc residue, which is present in **1** and **2** and missing in **3–5**. In the series **3–5**, the (R)-lactic acid derivative **4** (Figure 1(B), open triangles) displays the strongest affinity for CTB. The dissociation constants determined by non-linear regression analysis are 667 μM for **3**, 190 μM for **4**, and 1.1 mM for **5**. For comparison, CTB has a K_D of about 40 mM and 81 mM for galactose and lactose,[12] respectively, and the titration curve of 2 μM CTB with Galβ1-3GalNAc-OAllyl **17** (Chart 1) is also reported in Figure 1(B) (closed triangles). Asialo GM1, devoid of the NeuAc moiety, showed no detectable binding to CT at concentrations that were 320 times higher than the IC$_{50}$ of GM1.[3d] Thus the carboxy group of **4**, and to a lesser extent of **3**, appears to have a sizeable effect on the affinity of the artificial receptors for CT.

Further studies are in progress to determine the mode of binding of **4**, as well as to improve the affinity by varying the nature of the hydroxy acid side-chain. The hydroxy acid-containing mimics reported in this paper are easily accessible on a large scale and can be readily conjugated to aglycons. Thus they may be used to build multivalent ligands, which, ideally, should contain 5 pseudo-GM1 units capable of simultaneously interacting with the 5 B units of the toxin.[15]

Acknowledgements

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14. The observed emission maximum shifts ($\delta\lambda$) under saturation conditions were as follows: **1** 12.3 nm, **2** 12 nm, **3** 14.2 nm, **4** 12 nm, **5** 7.3 nm. The observed δI under saturation conditions at CTB 0.5 μM were: **1** +6.5, **2** −3.6, **3** −18.3, **1** −13.5, **5** −23.4.

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