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Synthesis of Glycolipid Analogues that Disrupt Binding of HIV-1 gp120 to Galactosylceramide

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Abstract—HIV-1 has been shown to infect CD4 negative cells by the binding of HIV gp120 to the glycolipid galactosylceramide (**1**) (GalCer). Several analogues of **1** were prepared to investigate the specific orientation of **1** in the membrane bilayer that is involved in gp120 binding. Interestingly, *N*-stearyl-1-deoxynojirimycin (**8**) displayed potent and specific affinity for gp120 equal to that of **1**, a finding that may shed light on the antiviral activity of *N*-butyl-1-deoxynojirimycin.

With the discovery of the role of chemokine-co-receptors, spectacular progress has been made in unravelling the pathway by which HIV infects cells expressing the CD4 antigen.[1] However, many cell lines, including hepatocytes,[2] brain-derived glial cells,[3] cervical epithelial cells[4] and natural killer cells,[5] do not express CD4, yet are nonetheless infected by HIV, suggesting a different infection mechanism.[6]

In 1991, Gonzalez-Scarano et al.[7] found that antibodies to galactosylceramide (GalCer, **1**, Fig. 1) blocked HIV infection in two neural cell lines that were CD4 negative, yet susceptible to HIV infection. By isolating lipids from those cell lines, it was shown that the viral glycoprotein gp120 bound selectively to **1**, with a K_d of 11.4 nM.

Several groups investigating structure–activity relationships in gp120/GalCer binding have noted the importance of the D-*galacto* configuration of the sugar. Neither the D-*gluco* analogue, GlcCer, nor lactosylceramide bound gp120, although substitution or sulfation of the 3-hydroxyl group in **1** was tolerated.[8–11] Studies have also shown a nonlinear concentration dependence of gp120 for GalCer, suggesting the involvement of GalCer-rich microdomains.[12,13] Although the effect of such microdomains on the conformation of the glycolipid is unknown, the fact that the saccharide rings of **1**[14] and GlcCer[15] adopt quite different conformations in the solid state suggest that minor structural changes in the saccharide might significantly influence the overall conformation of the glycolipid.

Here we report our efforts to probe the conformation of **1** involved in gp120 binding by synthesizing simple synthetic analogues that mimic specific orientations of the saccharide and lipid subunits. The analogues were tested for gp120 binding by analyzing the interaction of glycolipid monolayers with gp120 at the air–water interface.[16] The most active compound, *N*-stearyl-1-deoxynojirimycin **8**, displayed a potent and specific affinity for gp120 equal to **1** itself.

Topographic mapping of **1** suggested that three discrete orientations **2**–**4** (Fig. 2) of GalCer were possible in the membrane bilayer. In each orientation, glycolipid (**1**) presented different structural elements of the galactose ring to gp120. Synthetic analogues **5**–**8** of GalCer were designed to mimic these orientations of the lipid and saccharide subunits. In view of evidence that gp120 binding was independent of the lipid structure,[8] we decided to replace the ceramide group with a stearyl or stearoyl chain, resulting in a major synthetic simplification.

When oriented as in **2**, GalCer presented the 4- and 6-hydroxyl groups of GalCer to gp120, and could be mimicked by 2-stearoylamido-2-deoxy-D-galactopyranoside (**5**) having a 3-*O*-sulfate group to improve aqueous solubility and minimize any concentration-dependent hydrophobic interactions. Orientation **3**, which was

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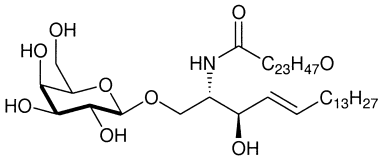


Figure 1. Galactosylceramide (GalCer) **1**.

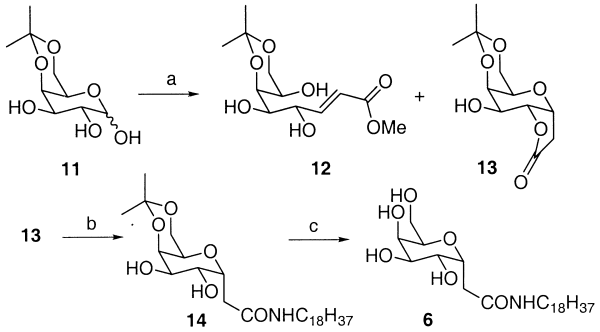
observed in the reported X-ray crystal structure of **1**,[14] presented the 3-, 4-, and 6-hydroxyl groups to gp120 and could be mimicked by the C-glycoside **6**. When oriented as in **4**, GalCer presented the 2-, 3-, and 4-hydroxyl groups to gp120, for which *N*-stearyl 5-amino-1,5-dideoxy-D-galactopyranoside (**7**) was designed as a conformational mimic. *N*-Stearyl-1-deoxynojirimycin **8** having the D-*gluco*-configuration was synthesized as an additional control to test the importance of the D-*galacto*-configuration in **1** noted in earlier work.

Analogue **5** was prepared (Scheme 1)[17] by the *N*-acylation of methyl 2-amino-2-deoxygalactopyranoside **9** with stearic acid using benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate (BOP reagent)[18] to afford amide **10**. Regioselective sulfation of **10** was achieved by the method of Guilbert et al.[19] in which the intermediate dibutylstannylene acetal was treated with sulfur trioxide-trimethylamine complex.

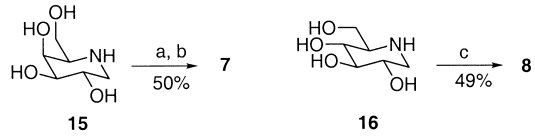
To synthesize GalCer analogue **6** (Scheme 2), Wittig olefination[20] of protected galactopyranose (**11**) afforded the *trans*-unsaturated methyl ester **12** and lactone **13**, which presumably arose by spontaneous cyclization of the *cis*-unsaturated ester and subsequent lactonization. The two products were easily separated, and upon treatment with $NaOCH_3$, **12** formed **13** in 91% yield. Reaction of **13** with stearylamine gave the amide **14**. Hydrolysis of the isopropylidene group in **14** afforded **6** in 75% yield.

GalCer analogue **7** was synthesized from 1-deoxygalactostatin **15**[21] by reductive amination with stearaldehyde (Scheme 3) using $NaBH_3CN$ according to the method of Fleet et al.[22] Analogue **8** was similarly prepared from 1-deoxynojirimycin **16** and stearaldehyde by hydrogenation.[23]

Analogues **5**, **6**, **7**, and **8** were screened for gp120 binding by monitoring the penetration of recombinant protein into glycolipid monolayers at the air–water interface, which could be measured by the increase in surface pressure $\Delta\Pi$, as described previously for GalCer.[16,24] Isotherms recording the variation of surface pressure



Scheme 2. (a) $Ph_3P{=}CH\text{-}CO_2CH_3$; (b) $C_{18}H_{37}NH_2$ (2 equiv), $CHCl_3$, reflux, 2 days 71%; (c) 0.5 M HCl, rt, 1 h.



Scheme 3. (a) $C_{17}H_{35}CHO$, 2:1:1 $THF:H_2O:CH_3OH$, rt, 3 days; (b) $NaBH_3CN$, $ZnCl_2$; (c) $C_{17}H_{35}CHO$, EtOH-HOAc, Pd/C, H_2, 4 h, rt.

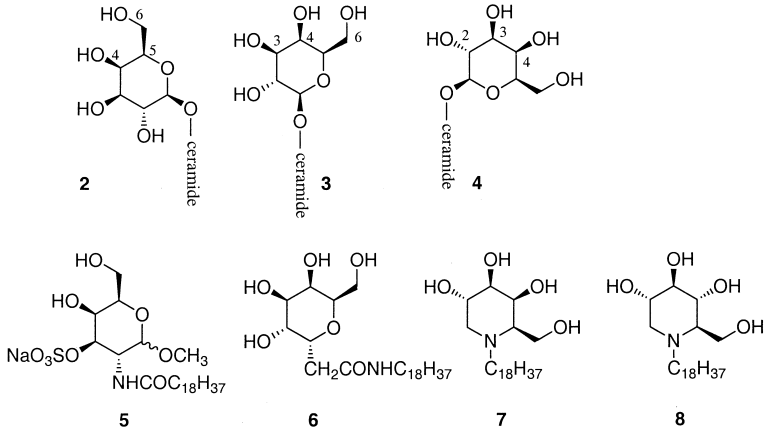
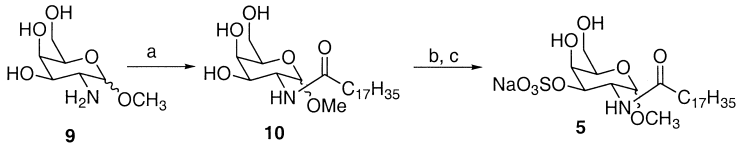


Figure 2. Synthetic GalCer analogues.



Scheme 1. (a) BOP, Et_3N, $C_{17}H_{35}CO_2H$, DMF, rt, 4 days, 48%; (b) $Bu_2Sn{=}O$, CH_3OH, reflux, 2 h; (c) $SO_3\text{-}N(CH_3)_3$, THF, rt, 2 days.

versus apparent molecular area at the air–water interface indicated that each of the synthetic GalCer analogues formed monomolecular films. The high compressibility noted at all film pressures and the absence of discontinuities in the isotherms indicated that all the analogues existed in the liquid expanded state up to film collapse (data not shown). Recombinant gp120 (HIV-1, IIIB isolate) was then added to monolayers prepared at an initial pressure of 8–10 mN/m, and $\Delta\Pi$ was measured until no further increases were noted (i.e., saturation; see Fig. 3a).

A second series of experiments to investigate the specificity of penetration was conducted with each GalCer analogue. In these studies, the increase in surface pressure caused by penetration of gp120 (10 nM) into the monolayer was measured as a function of initial surface pressure of the monolayer (Fig. 3b). Since the lipid monolayers are expected to show decreased compressibility with increasing surface pressure, values of $\Delta\Pi$ should decrease as the initial surface pressure of the monolayer increases.[25]

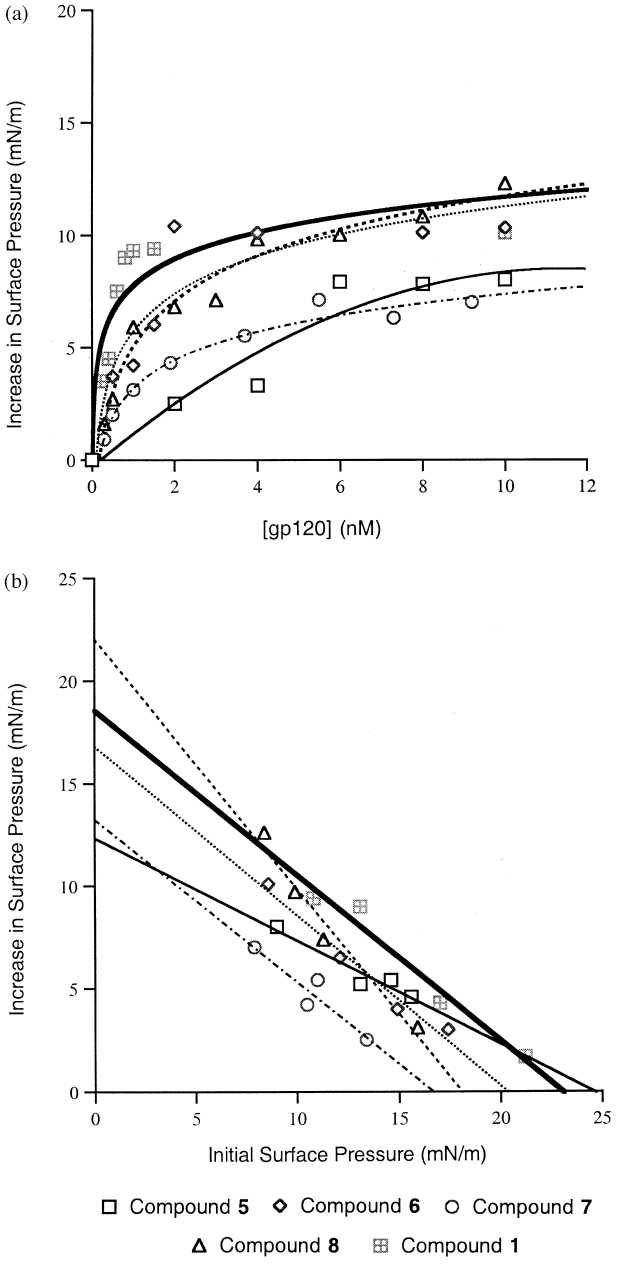


Figure 3. (a) Binding of gp120 to glycolipid analogues at the air–water interface. (b) Specificity of fp120 binding to glycolipid analogues.

As the data in Figure 3 indicate, monomolecular films of the analogues **5**–**8** interacted specifically with gp120 with a range of affinities. Acylated galactosamine **5** showed only a modest increase in surface pressure. Similar results were noted with *N*-stearyl-1-deoxygalactostatin **7**. By contrast, amide **6**, which was designed to mimic the orientation of GalCer in the solid-state,[14] showed significantly better affinity for gp120. The most potent binding to gp120 was noted with *N*-stearyl-1-deoxynojirimycin **8** (dashed line, Fig. 3a and b), whose activity and specificity matched that of authentic **1** (bold line, Fig. 3a and b).

Interestingly, *N*-butyl-1-deoxynojirimycin (Bu-DNM), a less lipophilic analogue of **8**, has been found to reduce the yield of infectious HIV particles by five orders of magnitude.[26] Further studies suggested that glucosidase inhibition was a candidate mechanism for the antiviral activity of this compound.[27] Pure Bu-DNM did not form a stable monolayer under the experimental conditions of this study. However, in preliminary experiments using a monolayer consisting of a 3:2 mixture of Bu-DNM with the inactive glycosphingolipid GlcCer, a maximal interaction was observed with gp120 (8 mM/m). These data suggest that direct binding of Bu-DNM to HIV-1 gp120 may also be a mechanism of antiviral activity.

The unexpectedly high affinity of **8** for gp120 suggests that the *N*-stearyl group, which in **8** is frame-shifted by one ring atom compared to GlcCer, significantly alters the orientation of the polyhydroxylated ring when the synthetic glycolipid is anchored in a membrane bilayer. In their solid state structures, the galactose ring of **1** adopts a conformation normal to the membrane bilayer, whereas the glucose ring in GlcCer is aligned tangential to the membrane bilayer. Crystallographic studies on **8**, which are underway, may shed light on conformational factors involved in gp120–glycolipid interactions and provide further insight into chemotherapeutic strategies against HIV.

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