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C-Glycoside analogues of β-galactosylceramide with a simple ceramide substitute: Synthesis and binding to HIV-1 gp120

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Abstract—The synthesis and HIV-1 gp120 binding of *C*- and aza-*C*-glycoside analogues of β-galactosylceramide (GalCer) that contain a simple C-17 hydrocarbon chain as a ceramide substitute are described. Both compounds originate from stearic acid, and a carbohydrate-derived thioacetal-alcohol, and their syntheses are potentially general for β-*C*-galactosides and their aza-*C*-partners. They showed potent and specific affinity for gp120 in an assay based on the change of surface pressure when the glycolipid monolayers were exposed to solutions of gp120. Interestingly, the aza-*C*-glycoside exhibited a significantly higher affinity than GalCer, whereas the *C*-glycoside was as active as GalCer.

1. Introduction

It has been shown that interaction between the glycosphingolipid GalCer **1** and gp120 is important for HIV infectivity in CD4 negative cells (Fig. 1).[1,2] GalCer has also been proposed as a co-factor for gp120 attachment in CD4 presenting cells.[3] Consequently, GalCer analogues have attracted interest as mechanistic probes for the development of new HIV entry inhibitors. Structure–activity investigations have focused on variations in the sugar and the fatty acid chain. Trends vary considerably depending on the assay system used.[4] It is generally believed that specificity with respect to the galacto sugar subunit is high and that the presence or absence of the fatty acid residue or changes in its length or level of hydroxylation can be tolerated.[4–8] However, anomalies to these trends have been reported. Recent studies with water-soluble GalCer analogues suggest a much lower specificity for the sugar,[9] and investigations with analogues that were incorporated into monolayer, indicated high specificity for an α-hydroxylated fatty acid residue over the non-hydroxylated derivative.[8] Structure–activity investigations with analogues containing modifications in the sphingosine backbone have been less rigorous. Isolated examples of analogues with ceramide substitutes of varying polarities have been reported to show good binding affinity.[9–12] Studies have also shown a nonlinear concentration dependence of gp120 for GalCer, suggesting the involvement of GalCer-rich microdomains.[4,13] In view of potential therapeutic applications, we were interested in GalCer analogues that were hydrolytically stable and which contained simple replacements for the ceramide

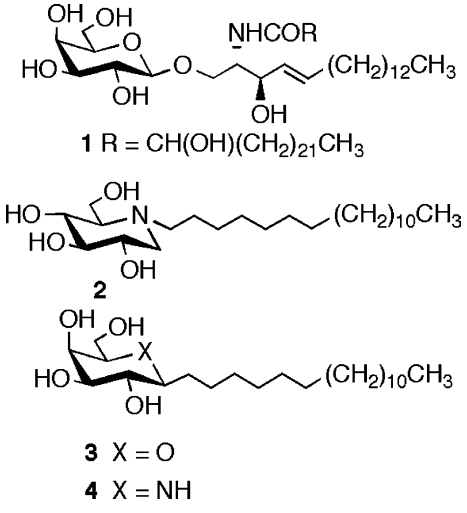

Figure 1.

Keywords: Galactosyl ceramide; *C*-Glycoside; Aza-*C*-glycoside gp120; Oxocarbenium ion.
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residue. Based on the observation that *N*-stearyl-1-deoxynojirimycin **2** and related derivatives with simple stearyl or stearoyl chains as ceramide substitutes exhibited gp120 affinity that was comparable to that of GalCer,[14] we set as initial goals, the synthesis of *C*-glycoside **3** and aza-*C*-glycoside **4**, and the determination of their binding to gp120.[15]

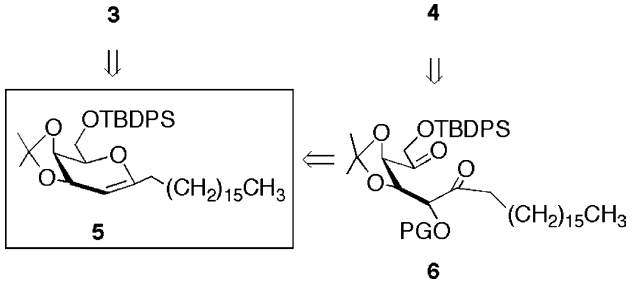
2. Synthesis

A synthetic strategy, in which **3** and **4** could be derived from a central C1-substituted galactal precursor **5**, was envisaged (Scheme 1). Thus, stereoselective hydroboration of **5** was expected to provide the β-*C*-glycoside framework, **3**.[16] Transformation of **5** to a diketone like **6** and stereoselective double reductive amination on **6** were plans for the β-aza-*C*-glycoside **4**.[17] Since C1-substituted galactals **5** with aglycone substituents of varying complexities are available through our oxocarbenium ion-enol ether cyclization methodology,[16] this protocol for **3** and **4** is a potentially general one for β-*C*-galactosides and their corresponding aza-*C*-derivatives.

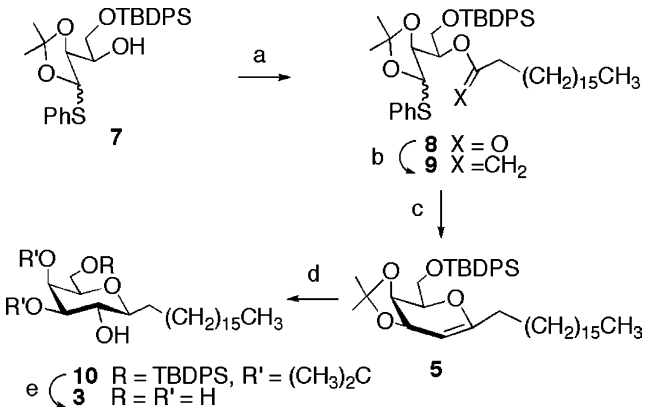
The synthesis of **5** started with the DCC mediated esterification of alcohol **7**[18] and stearic acid to give ester **8** (Scheme 2). Tebbe olefination of **8** gave enol ether **9** in 91% overall yield from **7**. Treatment of **9** with methyl triflate in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) provided galactal **5** in 87% yield as a single stereoisomer of the *O*-isopropylidene residue and a single regioisomer of the alkene. The reaction is presumed to proceed via attack of the enol ether on the cyclic oxocarbenium derived from thioacetal **9**. No evidence was observed for isomers of **5** that contained a *trans*-*O*-isopropylidene or an exocyclic alkene. These selectivities are a likely consequence of torsional factors associated with the 5,6 fused bicyclic framework.

Hydroboration of **5** provided the *C*-glycoside **10** as a single diastereomer.[19] The stereochemistry of **10** was confirmed by the 1H COSY analysis of the derived mono-acetate. The coupling constants ($J_{1,2}$ = 9.2, $J_{2,3}$ = 7.5, $J_{3,4}$ = 5.1, and $J_{4,5}$ = 2.2 Hz) are in agreement with those expected for the 3,4-*O*-isopropylidene 1β-galacto residue.[20] Removal of the silyl ether and acetonide protecting groups in **10** provided the desired *C*-glycoside **3** in 87% overall yield from **10**.

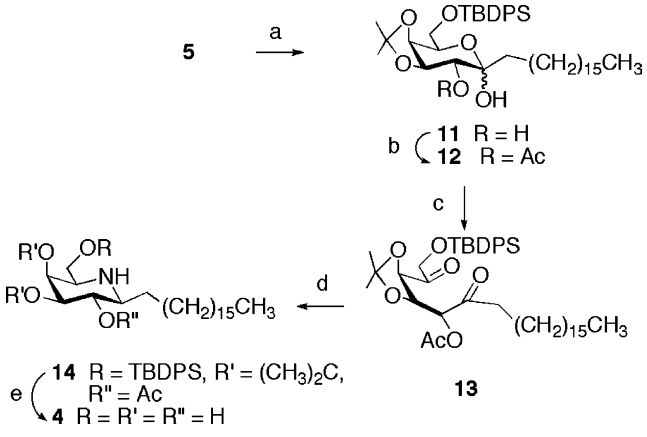
The first stage in the preparation of aza-*C*-glycoside **4** was the transformation of C1 galactal **5** to an appropriate diketone precursor. Dihydroxylation of **5** with osmium tetroxide/NMNO proceeded with complete α-selectivity to provide lactol **11** in 80% yield (Scheme 3). Selective acetylation of **11** and PCC oxidation of the monoacetate **12** led to diketone **13** in 78% overall yield from **11**. The key double reductive amination reaction was performed by treatment of **13** with ammonium formate and $NaCNBH_3$ in anhydrous methanol. The β-aza-*C*-galactoside **14** was obtained as a single stereoisomer in 72% yield. The stereochemistry of **14** was assigned on the basis of *J* values ($J_{1,2}$ = 9.9, $J_{2,3}$ = 7.7, $J_{3,4}$ = 5.1, and $J_{4,5}$ = 2.2 Hz) and observation of 1% NOE effects between H1, and H3 and H5, respectively. Removal of the alcohol protecting groups in **14** under standard conditions provided the desired β-aza-*C*-galactoside **4**. The high stereoselectivity of the double reductive amination is consistent with the Stevens model of a preferred axial attack by nucleophiles on six-membered half-chair-like, iminium ions.[21] That the galacto substrate **13** shows the same sense of stereochemical bias observed when related gluco-and manno-type diketones were subjected to similar conditions supports this stereochemistry model.[22]



Scheme 1.



Scheme 2. Reagents: (a) stearic acid, DMAP, PhH; (b) Tebbe, py, THF/toluene, 91% two steps; (c) MeOTf, DTBMP, CH_2Cl_2, 87%; (d) $BH_3{\cdot}Me_2S$, THF, 76%; (e) (i)—Bu_4NF, THF; (ii)—HCl, MeOH, 87% two steps.



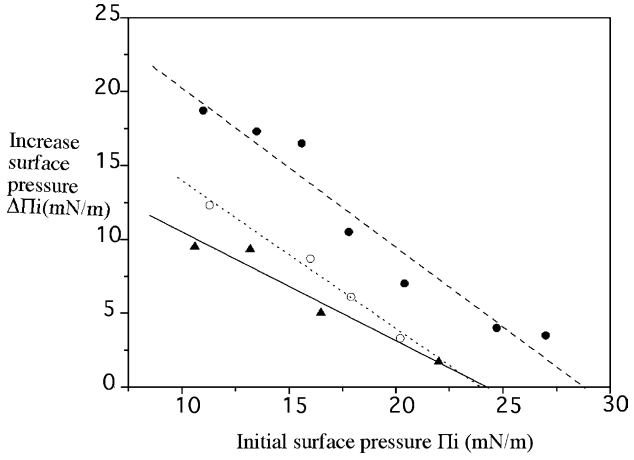
Scheme 3. Reagents: (a) OsO_4 (cat), NMNO, acetone, 80%; (b) Ac_2O, EtOAc, DMAP 95%; (c) PCC, CH_2Cl_2, Celite, NaOAc, MS 4A, 82%; (d) NH_4HCO_2, $NaCNBH_3$, MeOH, 72%; (e) (i)—K_2CO_3, MeOH; (ii)—Bu_4NF, THF; (iii)—HCl, MeOH, then NaOMe 57%.

3. HIV-1 gp120 binding

The binding of **3** and **4** to gp120 was evaluated by measuring the change in surface pressure ($\Delta\pi_i$) versus initial surface pressure (π_i) at the air–water interface of the glycolipid monolayer, on exposure to an aqueous solution of recombinant gp120 (10 nM).[23] Increase in surface pressure is associated with integration of gp120 into the glycolipid monolayer. The critical pressure of insertion, calculated by extrapolation for a null increase in surface pressure, represents the pressure above which lipid–lipid interactions are not disrupted by the protein, and is often taken as a measure of binding affinity. Together, a critical pressure greater than 10 mN/m and a linear variation of $\Delta\pi$ versus π_i is generally interpreted as an indication of specific binding.[24] The critical pressure of insertion was in the range of 24 mN/m for both GalCer **1** and the *C*-glycoside **3**, and 28.5 mN/m for the aza-*C*-glycoside **4**. These data suggest that gp120 exhibits similar binding to monolayers of **1** and **3**, and, appreciably higher affinity for the monolayer formed from **4** (Fig. 2).

4. Discussion

That **3** and **4** bind HIV-1 gp120 with comparable or even higher affinity than GalCer suggests that it may be possible to develop GalCer analogues with simple ceramide substitutes as potent inhibitors of GalCer-gp120 binding. This data is consistent with observations on related, stearyl, and stearoyl monosaccharide derivatives with single lipid chains,[14] but appear to disagree with other studies that point to the critical importance of the fatty acid residue in the ceramide moiety. Thus, in the identical monolayer assay, the GalCer analogue without the α-hydroxy group in the acyl chain was completely inactive[8] and in a solid-phase assay psychosine (without the acyl chain) is inactive.[6] The unusual behavior of the single chain analogues **3** and **4** also appears to be in conflict with the notion that the polar head region of the ceramide is preorganized for binding to gp120, because the simple C-linked hydrocarbon chains in **3** and **4** are not expected to favor a well-defined conformation. However, these results do not rule out the possibility that the lipid chains in GalCer might be important for clustering into microdomains, leading to multivalent binding to gp120.[4,13] In such a situation, substitution of the ceramide residue with simple hydrocarbon chains as in **3** and **4** might not have a pronounced effect on the topography of a multivalent assembly, and as a consequence, on binding affinity.[25]



1 (open circles, dotted line) **3** (full triangles, solid line)
4 (full circles, dashed line)

Figure 2. Binding of gp120 to glycolipid analogues.

That the interaction of gp120 with the aza analogue **4** is stronger than for GalCer is in of itself noteworthy and opens up new possibilities for mimetic design. This effect could be related to aggregation factors in microdomain formation, or to more intimate receptor contacts in the individual sugar residues.[26] In the absence of a wider set of analogues additional speculation is premature. Parenthetically, the related aza sugar *N*-butyl-1-deoxynorjimicin has been shown to reduce syncytia formation.[27] The mechanism of action is believed to be a result of biosynthetic alteration of the host cell receptor.[28] The binding behavior of **4** and **2** suggests that direct binding of Bu-DNM to gp120 may be an alternative explanation for the antiviral activity of Bu-DNM.

5. Conclusion

In summary, novel analogues of GalCer with a simplified hydrocarbon chain as an aglycone substitute were prepared and shown, in a monolayer binding assay with gp120, to have similar properties as GalCer. A closer examination of the molecular basis of these effects using complementary binding assays is underway and will be reported in due course. From a synthetic standpoint, the preparation of **3** and **4** provides a potentially general protocol for accessing *C*- and aza-*C*-β-galactoside pairs from a common precursor.

6. Experimental

6.1. General synthesis

Solvents were purified by standard procedures or used from commercial sources as appropriate. Petroleum ether refers to the fraction of petroleum ether boiling between 40 and 60 °C. Ether refers to diethyl ether. Unless otherwise stated thin-layer chromatography (TLC) was done on 0.25 mm thick precoated Silica Gel 60 (HF-254, Whatman) aluminum sheets and flash chromatography (FCC) was performed using Silica Gel 60 (32–63 mesh, Scientific Adsorbents). Elution for FCC usually employed a stepwise solvent polarity gradient, correlated with TLC mobility. Chromatograms were observed under UV (short and long wavelength) light and/or were visualized by heating plates that were dipped in a solution of ammonium (VI) molybdate tetrahydrate (12.5 g) and cerium (IV) sulfate tetrahydrate (5.0 g) in 10% aqueous sulfuric acid (500 mL). Optical rotations ($[\alpha]_D$) were recorded using a Rudolph Autopol III polarimeter at 589 nm (sodium D-line). NMR spectra were

recorded using GE QE 300, JEOL 400 or Bruker Ultra Shield instruments. Unless otherwise stated spectra were recorded in $CDCl_3$ with residual $CHCl_3$ as internal standard (δ_H 7.26, δ_C 77.0). Chemical shifts are quoted in ppm relative to tetramethylsilane (δ_H 0.00) and coupling constants (J) are given in Hertz. First order approximations are employed throughout. High resolution mass spectrometry (HRMS) was performed on Micromass 70-SE-4F or Micromass Q-Tof Ultima instruments at the Mass Spectrometry Laboratory of the University of Illinois, Urbana-Champaign.

6.2. 1,2-*O*-Isopropylidene-3-*O*-octadecanoyl-4-*O*-*tert*-butyldiphenylsilyl-D-*threo*-*S*-phenyl-mono-thiohemiacetal 8

DCC (600 mg, mmol) was added at 0 °C to a solution of TIA–alcohol **7**[18] (1.00 g, 1.97 mmol), stearic acid (840 mg, 2.95 mmol), and DMAP (25 mg, 0.21 mmol) in anhydrous benzene (50 mL). The reaction was warmed to rt and stirred for 2 h. The mixture was diluted with ether and filtered through Celite. The filtrate was washed with 0.1 N aqueous HCl and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by FCC to give ester **8** (1.40 g, 92%): colorless oil; $R_f = 0.60$ (10% ethyl acetate: petroleum ether); $[\alpha]_D^{25} - 39.9$ (c 5.00, $CHCl_3$); ^{1}H NMR (500 MHz) δ 0.88 (t, $J = 6.9$ Hz, 3H), 1.03 (s, 9H), 1.25 (m, 28H), 1.44 (s, 3H), 1.48 (s, 3H), 1.58 (m, 2H), 2.29 (m, 2H), 4.34 (dd, $J = 3.6$, 6.6 Hz, 1H), 5.25 (m, 2H), 7.20–7.70 (m, 15H). ^{13}C NMR (125 MHz) δ 14.7, 23.3, 25.5, 26.8, 27.3, 27.8, 29.9, 30.0 (two peaks), 30.2, 30.3, 32.5, 34.9, 62.9, 72.0, 79.7, 85.9, 112.3, 128.2, 128.3, 129.6, 130.3, 130.3, 132.9, 133.6, 134.3, 136.2. HRMS (ESI) calcd for $C_{47}H_{70}O_5SSiNa$ (M+Na): 797.4572. Found: 797.4611.

6.3. 1,2-*O*-Isopropylidene-3-*O*-(2-nondecen-2-yl)-4-*O*-*tert*-butyldiphenylsilyl-D-*threo*-*S*-phenyl-mono-thiohemiacetal 9

Tebbe reagent in toluene (7.84 mL of a 0.5 M solution, 3.91 mmol) was added at −78 °C, dropwise under an atmosphere of argon, to a solution of ester **8** (1.40 g, 1.80 mmol) in a mixture of pyridine (0.2 mL), anhydrous toluene (18 mL), and THF (6 mL). The reaction mixture was warmed to 0 °C, stirred at this temperature for 1 h, and then for an additional 15 min at rt. The mixture was then slowly poured into 1 N aqueous NaOH at 0 °C. The resulting suspension was extracted with ether, and the organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by FCC on basic alumina (Brockmann I, 150 mesh) to give the enol ether **9** (1.39 g, 99%): colorless oil; $R_f = 0.65$ (2% ethyl acetate: petroleum ether); $[\alpha]_D^{25} - 57.0$ (c 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 0.92 (t, $J = 7.0$ Hz, 3H), 1.18 (s, 9H), 1.33 (m, 30H), 1.52 (s, 3H), 1.53 (s, 3H), 2.16 (t, $J = 7.9$ Hz, 2H), 3.95 (m, 2H), 4.15 (m, 2H), 4.52 (m, 1H), 4.77 (dd, $J = 1.8$, 7.0 Hz, 1H), 5.81 (d, $J = 7.0$ Hz, 1H), 7.10–7.80 (m, 15H). ^{13}C NMR (125 MHz, C_6D_6) δ 14.6, 23.3, 27.2, 30.0, 30.3, 30.8, 32.0, 32.5, 33.3, 34.0, 37.6, 62.2, 74.7, 80.6, 82.2, 85.5, 111.9, 128.7, 129.4, 130.2, 132.4, 133.9, 135.2, 135.2, 163.3.

6.4. (1*S*)-1,5-Anhydro-2-deoxy-1-*C*-heptadecyl-3,4-*O*-isopropylidene-6-*O*-*tert*-butyldiphenylsilyl-D-*lyxo*-hex-2-enitol 6

A mixture of TIA–enol ether **9** (1.39 g, 1.80 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (4.40 g, 21.5 mmol), and freshly activated, powdered 4 Å molecular sieves (2.00 g) in anhydrous CH_2Cl_2 (200 mL) was stirred for 15 min at rt under an argon atmosphere. The mixture was cooled to 0 °C. Methyl triflate (2.05 mL, 18.1 mmol) was then introduced and the reaction mixture was warmed to rt, and maintained at this temperature for an additional 24 h, at which time triethylamine (2.60 mL) was added. The mixture was diluted with ether, washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by FCC over basic alumina (Brockmann I, 150 mesh) to give glycal **6** (1.19 mg, 87%): clear oil; $R_f = 0.55$ (2% ethyl acetate:petroleum ether); $[\alpha]_D^{25} + 18$ (c 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 0.92 (t, $J = 7.0$ Hz, 3H), 1.21 (s, 9H), 1.35 (m, 28H), 1.44 (s, 3H), 1.46 (s, 3H), 2.11 (m, 2H), 4.00 (t, $J = 6.7$ Hz, 1H), 4.17 (q, $J = 6.5$ Hz, 2H), 4.25 (m, 2H), 4.57 (dd, $J = 3.0$, 7.0 Hz, 1H), 4.72 (d, $J = 8.3$ Hz, 1H), 7.24–7.81 (m, 10H). ^{13}C NMR (125 MHz, C_6D_6): δ 14.9, 23.7, 27.6, 28.4, 29.2, 29.7, 30.7, 30.8, 31.2, 32.4, 32.9, 34.6, 38.1, 41.5, 64.7, 70.9, 72.7, 76.8, 98.7, 110.7, 127.8, 128.1, 128.9, 130.6, 136.6, 136.7, 157.3. HRMS (FAB) calcd for $C_{42}H_{65}O_4Si$ (M−H): 661.4652. Found: 661.4649.

6.5. (1*S*)-1,5-Anhydro-1-*C*-heptadecyl-3,4-*O*-isopropylidene-6-*O*-*tert*-butyldiphenylsilyl-D-galactitol 10

$BH_3{\cdot}Me_2S$ (1.09 mL of a 1 M solution in CH_2Cl_2, 1.09 mmol) was added at 0 °C to a solution of glycal **6** (180 mg, 0.27 mmol) in anhydrous THF, under an atmosphere of argon. The mixture was warmed to rt and stirred for an additional 1 h at this temperature. The solution was then re-cooled to 0 °C, and a mixture of 3 N NaOH (2 mL) and 30% aqueous H_2O_2 (2 mL) was carefully added. After stirring at this temperature for an additional 30 min, the mixture was diluted with ether and washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. FCC of the residue provided **10** (185 mg, 76%): clear oil; $R_f = 0.30$ (15% ethyl acetate: petroleum ether); $[\alpha]_D^{25} + 5.1$ (c 1.7, $CHCl_3$); ^{1}H NMR (400 MHz) δ 0.89 (t, $J = 7.0$ Hz, 3H), 1.07 (s, 9H), 1.25 (m, 30H), 1.36 (s, 3H), 1.51 (s, 3H), 1.54 (m, partly buried under singlet at 1.51, 1H), 1.83 (m, 1H), 2.81 (br s, 1H, D_2O ex.), 3.04 (br t, $J = 7.0$ Hz, 1H), 3.41 (dd, $J = 7.4$, 9.9 Hz, 1H), 3.80 (dt, $J = 2.2$, 8.1 Hz, 1H), 3.92 (m, 2H), 3.98 (dd, $J = 5.5$, 7.0 Hz, 1H), 4.30 (dd, $J = 2.2$, 5.5 Hz, 1H), 7.39–7.72 (m, 10H). ^{13}C NMR (75 MHz): 14.4, 19.5, 23.0, 25.6, 26.7, 27.1, 28.6, 29.6, 29.7, 30.0, 31.8, 32.2, 63.2, 74.0, 76.8, 78.4, 79.5, 109.8, 127.7, 127.8, 127.9, 129.7, 130.0, 133.6, 135.7, 135.8. HRMS (FAB) calcd for $C_{38}H_{59}O_4Si$ (M–C_4H_9): 623.4132. Found: 623.4133.

6.6. (1*S*)-1,5-Anhydro-1-*C*-heptadecyl-D-galactitol 3

TBAF (0.85 mL of 1 M solution) was added at rt to a solution of **11** (190 mg, 0.279 mmol) in anhydrous THF (2 mL) under nitrogen. The reaction mixture was stirred for 4 h and then concentrated under reduced pressure. FCC of the residue provided a homogeneous material (127 mg): $R_f = 0.13$ (20% ethyl acetate:petroleum ether). This material was dissolved in methanol (2 mL) and treated with an approximately 1 N solution of HCl in ether (0.1 mL) at rt for 10 min. The mixture was then neutralized with a solution of NaOMe in methanol, diluted with brine, and extracted with ether. The organic extract was dried (Na_2SO_4), filtered, and evaporated in vacuo. FCC of the residue provided **3** (100 mg, 87 %): clear oil; $R_f = 0.23$ (10% methanol: chloroform); ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (t, $J = 7.0$ Hz, 3H), 1.29 (m, 30H), 1.59 (m, 1H), 1.84 (m, 1H), 3.06 (t, $J = 7.9$ Hz, 1H), 3.38 (m, 3H), 3.69 (m, 2H), 3.87 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 14.6, 23.8, 26.8, 30.6, 30.8, 30.9, 33.0, 33.2, 62.9, 71.0, 72.9, 76.7, 80.2, 81.7. HRMS (FAB) calcd for $C_{23}H_{47}O_4$ (M+H): 403.3424. Found: 403.3423.

6.7. (3*R*,4*R*,5*S*,6*R*)-2-Heptadecyl-tetrahydro-4,5-*O*-isopropylidene-6-*tert*-butyldiphenylsilyloxy-methyl-2*H*-pyran-2,3-diol 11

N-Methylmorpholine-*N*-oxide (0.57 mL, 60 wt% in H_2O, 3.32 mmol) and osmium tetroxide (2.08 mL 2.5 wt% in *tert*-butanol, 0.17 mmol) were added to a solution of **6** (1.1 g, 1.66 mmol) in acetone (20 mL). The reaction mixture was stirred at rt for 0.5 h, at which time a solution of 1 N aqueous sodium bisulfite (0.57 mL, 1 N) was added and the mixture was stirred for additional 0.5 h. Most of the solvent was evaporated under reduced pressure, and the residue was diluted with water and extracted with ethyl acetate. The combined organic phase was dried (Na_2SO_4), filtered, and evaporated in vacuo. FCC of the residue gave mixture **11** (0.93 g, 80%): colorless oil; $R_f = 0.10$ (10% ethyl acetate:petroleum ether); ^{1}H NMR (500 MHz) δ 0.91 (t, $J = 7.0$ Hz, 3H), 1.08 (s, ca. 3H), 1.10 (s. 6H), 1.35 (s. ca 1H), 1.38 (s, 2H), 1.50 (s, ca. 2H), 1.52 (s, ca. 1H), 1.25–1.70 (m, 30H), 1.74 (m, ca. 1.33 H), 2.19 (d, $J = 6.0$ Hz, ca. 0.67 H, D_2O ex), 2.57 (dt, $J = 7.5$, 17.5 Hz, ca. 0.33H), 2.68 (dt, $J = 7.0$, 17.5 Hz, ca. 0.33H), 3.41 (br s, ca. 0.33H, D_2O ex), 3.59 (t, $J = 6.0$ Hz, ca. 0.67 H), 3.75–4.00 (m, ca. 2.33H), 4.18–4.40 (m, ca. 2.33 H), 4.35 (m, ca. 0.33H), 4.51 (m, ca. 0.33H), 7.40–7.80 (m, 10H). ^{13}C NMR (125 MHz) δ 14.4, 19.4, 22.39, 22.9, 25.2, 26.3, 27.7, 28.3, 29.6, 29.7, 32.1, 38.7, 63.2, 65.3, 69.0, 69.6, 72.7, 73.3, 76.3, 98.1, 109.4, 127.8, 128.0, 129.8, 130.1, 133.7, 135.7, 135.9. HRMS (FAB) calcd for $C_{42}H_{68}O_6Si$ (M+Na): 719.4684. Found: 719.4683.

6.8. (3*R*,4*S*,5*S*,6*R*)-2-Heptadecyl-tetrahydro-2-hydroxy-4,5-*O*-isopropylidene-6-*tert*-butyl-diphenylsilyloxymethyl-2*H*-pyran-3-yl acetate 12

To a solution of **11** (90 mg, 1.3 mmol) and DMAP (15.9 mg, 0.13 mmol) in ethyl acetate (10 mL) was added acetic anhydride (0.16 mL, 1.56 mmol) dropwise. The reaction mixture was stirred at rt for 5 min, then quenched with methanol (0.5 mL) and evaporated under reduced pressure. The residue was purified by FCC to give **12** (91 mg, 95%): clear oil; $R_f = 0.85$ (20% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz) δ 0.86 (t, $J = 7.0$ Hz, 3H), 1.10 (s, 9H), 1.25 (s, 30H), 1.32 (s, 3H), 1.53 (s, 3H), 2.10 (m, 2H), 2.12 (s, 3H), 3.85, 3.96 (both m, 1H ea), 4.32 (m, 3H), 4.95 (d, $J = 7.50$ Hz, 1H), 7.40–7.60 (m, 10H); ^{13}C NMR (75 MHz) δ 19.6, 21.4, 22.1, 23.0, 26.9, 27.0, 27.1, 27.2, 28.0, 29.7, 29.8, 30.0, 30.2, 32.2, 38.3, 63.2, 69.2, 73.4, 73.7, 75.3, 76.8, 97.9, 109.7, 127.7, 127.8, 127.9, 128.0, 128.1, 129.7, 129.8, 130.0, 133.1, 133.7, 133.8, 135.8, 170.3. HRMS(FAB) calcd for $C_{44}H_{71}O_5SiN$: 722.5180. Found: 722.5183.

6.9. (3*R*,4*S*,5*R*)-3,4-*O*-Isopropylidene-1-*tert*-butyldiphenylsilyloxy-2,6-dioxotricosan-5-yl acetate 13

To a mixture of PCC (147 mg, 0.136 mmol), Celite (147 mg), florisil (15 mg), sodium acetate (56 mg, 0.68 mmol), freshly activated 4 Å molecular sieves (200 mg), and CH_2Cl_2 (3 mL), under an argon atmosphere, was added a solution of **12** (100 mg, 0.136 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred at rt for 3 h and then filtered through a bed of Celite. The filtrate was concentrated under reduced pressure, and the residue was purified by FCC to give **13** (82 mg, 82%): colorless oil; $R_f = 0.80$ (10% ethyl acetate:petroleum ether); ^{1}H NMR (400 MHz) δ 0.86 (t, $J = 7.0$ Hz, 3H), 1.10 (s, 9H), 1.25 (br s, 30H), 1.27, 1.5 (both s, each 3H) 2.08 (s, 3H), 2.42 (m, 2H), 4.40 (ABq, $J = 17.0$ Hz, $\Delta\delta = 0.15$ ppm, 1H), 4.66 (d, $J = 2.2$ Hz, 1H), 4.95 (dd, $J = 2.2$, 8.4 Hz, 1H), 5.17 (d, $J = 8.4$ Hz, 1H), 7.40–7.60 (m, 10H); ^{13}C NMR (125 MHz) δ 14.7, 19.8, 20.9, 23.3, 23.4, 24.9, 26.7, 27.3, 29.6, 29.9, 30.0, 30.2, 30.3, 32.5, 39.4, 69.1, 77.1, 80.1, 111.2, 128.3, 128.5, 130.2, 130.6, 132.9, 133.3, 135.4, 136.0, 136.2, 171.2, 206.0, 207.2. HRMS(FAB) calcd for $C_{44}H_{68}O_7Si$ (M+Na): 759.4629. Found: 759.4632.

6.10. (1*S*)-2-*O*-Acetyl-1,5-dideoxy-1-*C*-heptadecyl-3,4-*O*-isopropylidene-1,5-imino-D-galactitol 4 14

Sodium cyanoborohydride (75 mg, 1.1 mmol) was added to a mixture of **13** (250 mg, 0.34 mmol), ammonium formate (38.0 mg, 0.6 mmol), and 4 Å powdered molecular sieves (100 mg) in anhydrous methanol (10 mL), under an argon atmosphere. The reaction mixture was stirred for 30 min at rt and then filtered through a bed of Celite. The filter cake was washed with ether and the filtrate was concentrated under reduced pressure. The residue was dissolved in ether (20 mL) and washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. FCC of the residue to give **14** (176.3 mg, 72%): clear oil; $R_f = 0.45$ (15% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz) δ 0.86 (br t, $J = 7.0$ Hz, 3H), 1.10 (s, 9H), 1.25 (s, 30H), 1.33 (s, 3H), 1.62 (s, 3H), 1.20–1.40 (m, partially buried under singlets at 1.33 and 1.62 ppm, 2H), 2.45 (t, $J = 9.3$ Hz, 1H), 3.11 (m, 1H), 3.82 (m, 2H), 3.98 (m, 1H), 4.18 (m, 1H), 4.83 (dd, $J = 7.8$, 9.9 Hz, 1H), 7.40–7.60 (m, 10H); ^{13}C

NMR (75 MHz) δ 14.4, 19.5, 21.4, 23.0, 25.9, 26.8, 26.9, 27.1, 28.1, 29.6, 29.9, 30.0, 31.6, 32.2, 57.3, 57.8, 64.4, 74.1, 76.5, 78.7, 86.9, 109.9, 127.8, 129.8, 133.5, 133.6, 134.9, 135.7, 170.3. HRMS(FAB) calcd for $C_{44}H_{71}O_5S$-iN (M+H): 722.5183. Found: 722.5179.

6.11. (1*S*)-1,5-Dideoxy-1-*C*-heptadecyl-1,5-imino-D-galactitol 4

To a solution of **14** (150 mg, 0.21 mmol) in anhydrous methanol (5 mL) was added potassium carbonate (21 mg, 0.21 mmol). The reaction mixture was stirred at rt for 0.5 h and then evaporated in vacuo. The crude material was dissolved in THF (5 mL) and treated with *n*-Bu_4NF (0.4 mL, 1 M in THF) for 1 h at rt. The mixture was then diluted with water and extracted with ether. The organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. FCC of the residue afforded the derived diol (64 mg, 72%): clear oil; $R_f = 0.1$ (40% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (br t, $J = 7.0$ Hz, 3H), 1.25 (br s, 32H), 1.32 (s, 3H), 1.52 (s, 3H), 2.42 (m, 1H), 3.11 (m, 1H), 3.32 (dd, $J = 7.8$, 9.9 Hz, 1H), 3.82 (m, 2H), 3.98 (m, 1H), 4.18 (m, 1H).

An approximately 1 N solution of HCl in ether (0.1 mL) was added at rt to a solution of the material from the previous step (60 mg, 0.14 mmol) and in anhydrous methanol (5 mL). The reaction was maintained at this temperature and monitored by TLC. When the starting material had completely disappeared, solid NaOMe was carefully added to a pH of 7. The solution was then concentrated in vacuo and the residue as purified by FCC to give **4** (44 mg, 80%): clear oil; $R_f = 0.1$ (20% methanol:ethyl acetate). ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (br t, $J = 7.0$ Hz, 3H), 1.30 (m, 28H), 1.50 (m, 2H), 1.62 (m, 1H), 1.94 (m, 1H), 2.00 (s, 1H, –NH), 2.78 (m, 1H), 3.15 (t, $J = 6.5$ Hz, 1H), 3.42 (dd, $J = 3.0$, 8.5 Hz, 1H), 3.62 (t, $J = 10.0$ Hz, 1H), 3.78 (m, 2H), 4.00 (br s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 14.6, 23.9, 26.8, 30.6, 30.7, 30.9, 31.1, 32.0, 32.2, 60.9, 61.2, 61.5, 68.9, 71.7, 75.6. HRMS(FAB) calcd for $C_{23}H_{48}NO_4$ (M+H): 402.3583. Found: 402.3584.

6.12. Surface pressure measurements

The surface pressure was measured with a fully automated microtensiometer (μTROUGH SX; Kibron, Inc., Helsinki, Finland). The apparatus allowed the real-time recording of the kinetics of interaction of a soluble ligand with the monomolecular film, using a set of specially designed Teflon troughs. All experiments were carried out in a controlled atmosphere at 20 ± 1 °C. Monomolecular films of **1**, **3**, and **4** were spread on pure water subphases (volume of 800 μL) from hexane–chloroform–ethanol solution as described previously.[21] After spreading of the film, 5 min was allowed for solvent evaporation. To measure the interaction of gp120 with GalCer and its derivatives, recombinant gp120 (IIIB isolate, 10 nM) was injected in the subphase with a 10 μL Hamilton syringe, and pressure increases produced were recorded until reaching a stable value ($\Delta\pi$). The experiment was repeated at different values of the initial surface pressure (π_i) of the monolayer. The data were analyzed with the Filmware 2.5 program (Kibron, Inc.). The results are expressed as the variations of $\Delta\pi$ as a function of π_i for compounds **1**, **3**, and **4**. The accuracy of the system under our experimental conditions was ±0.25 mN/m for surface pressure.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmc.2005.09.044.

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