Bioorganic & Medicinal Chemistry 19 (2011) 4803–4811

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, gp120 binding and anti-HIV activity of fatty acid esters of 1,1-linked disaccharides

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ARTICLE INFO

Article history:
Received 22 April 2011
Revised 22 June 2011
Accepted 27 June 2011
Available online 1 July 2011

Keywords:
Galactosylceramide
Glycolipid
Trehalose
Fatty acid
Antiviral
HIV

ABSTRACT

Inspired by the anti-human immunodeficiency virus (HIV) activity of analogues of β-galactosylceramide (GalCer), a set of mono- and di-saccharide fatty acid esters were designed as GalCer mimetics and their binding to the V3 loop peptide of HIV-1 and anti-HIV activity evaluated. 1,1-linked Gal-Man and Glu-Man disaccharides with an ester on the Man subunit bound the V3 loop peptide and inhibited HIV infectivity in single round infection assays with the TZM-bl cell line. IC_{50}'s were in the 50 μM range with no toxicity to the cells at concentrations up to 200 μM. These compounds appear to inhibit virus entry at early steps in viral infection since they were inactive if added post viral entry. Although these compounds were found to bind to the V3 loop peptide of gp120, it is not clear that this interaction is responsible for their anti-HIV activity because the relative binding affinity of closely related analogues did not correlate with their antiviral behavior. The low cytotoxicity of these 1,1-linked disaccharide fatty acid esters, combined with the easy accessibility to structurally diverse analogues make these molecules attractive leads for new topical anti-viral agents.

1. Introduction

While the fusion process mediated by gp41 per se remains well studied and targeted, the events preceding gp41 mediated fusion including gp120 binding to receptor and coreceptor present potential new targets for drug development.[1,2] Besides the well characterized receptor and coreceptor for HIV several cofactors are involved in facilitating gp120 binding to cells.[3,4] Membrane glycosphingolipids (GSL) including GalCer **1** constitute one well-known group of cofactors[5,6] (Fig. 1). In this vein, GSL analogues have attracted interest as potential inhibitors of HIV infectivity.[2,7–10] We have previously synthesized and tested the gp120 binding and antiviral activity of GalCer analogues with simple ceramide substitutes, against both X4 and R5 tropic virus.[11] Some of the lead compounds, represented by **2**, bound gp120 similar to GalCer and showed significant activity against HIV envelope glycoprotein (Env)-mediated fusion. The compounds act, presumably as GalCer mimetics, at an early step in HIV Env binding that precedes CD4 binding as determined by kinetic and temperature arrested state experiments. These GalCer analogues also showed inhibitory activity against vesicular stomatitis virus (VSV) pseudotype virus that was similar to other antiviral compounds like cyanovirin and suramin.[12,13] The further development of these compounds holds promise towards new therapies that can be used both as prophylaxis and post exposure treatment as the compounds act at an early step in the HIV cycle. Towards this end, with the aim of identifying new GalCer mimetics with anti-HIV activity, we designed a wider set of mono- and di-saccharide-derived lipids and evaluated their binding to the V3 loop peptide of gp120 and their HIV inhibitory activity. These results are discussed herein.

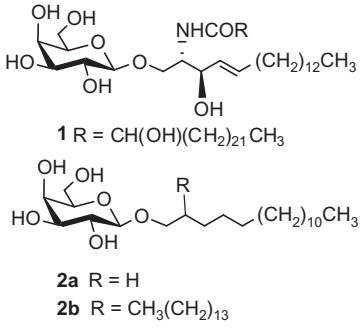

Figure 1. GalCer and analogues.

Our analog design is based on existing SAR data that suggests the Gal and hydrophobic residues of GalCer account for a significant part

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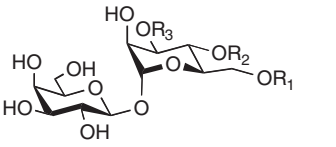
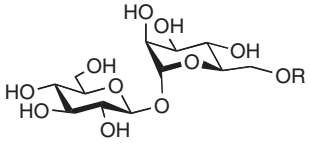
doi:10.1016/j.bmc.2011.06.078

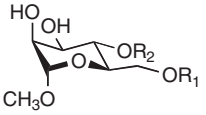
of the overall binding to gp120,[14] and the central polar segment that connects the two regions acts as a scaffold that controls the spatial positioning of the sugar and hydrophobic regions.[15,16] That our first generation analogues (e.g., **2**), in which the ceramide moiety in GalCer was replaced with simple hydrocarbon residues, showed binding properties comparable to GalCer is consistent with this model. To get insight into the conformational and spatial requirements of the ceramide residue in GalCer, for the purpose of developing more effective GalCer antagonists, we argued that a monosaccharide could be used as a replacement for the polar lipid head of GalCer. Indeed, it has been suggested that the GlcNHAc subunit in Galβ1-4GlcNAcβ1 is structurally analogous to this segment of GalCer and may mimic its binding to gp120.[17] In a similar vein, we argued that β-Gal disaccharides such as **3**, **4** and **5**, with one or two fatty acid esters in the mannose segment, may also act as GalCer mimetics (Fig. 2). While the gp120 binding of disaccharide glycolipids have been previously examined, these structures were conceived as mimetics of disaccharide GSL's, as opposed to the monosaccharide GalCer.[10,16] We opted for a 1,1-linked disaccharide template because of the well-defined conformational bias in the intersaccharide torsions of such frameworks,[18] and a mannose subunit, because of synthetic considerations relating to stereoselective glycoside bond construction and alcohol group differentiation. The β-Glu-disaccharide **6**, the α-Gal-disaccharide **9**, and the α-Man monosaccharides **7** and **8** were designed to probe the recognition specificity for the β-Gal motif. The Man-monosaccharide **7** was also of interest because the relative orientation of the 6-*O*-palmitoyl ester and the 2-OH group was similar to the positioning of the 4-OH and the anomeric lipid chain in Gal-monosaccharide **2a**, the reference compound from the earlier study.



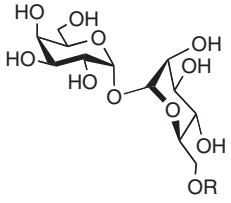
3 $R_1 = C(O)(CH_2)_{14}CH_3$; $R_2 = R_3 = H$
4 $R_3 = C(O)(CH_2)_{14}CH_3$; $R_1 = R_2 = H$
5 $R_1 = R_2 = C(O)(CH_2)_{14}CH_3$; $R_3 = H$



6 $R = C(O)(CH_2)_{14}CH_3$



7 $R_1 = C(O)(CH_2)_{14}CH_3$; $R_2 = H$
8 $R_1 = R_2 = C(O)(CH_2)_{14}CH_3$



9 $R = C(O)(CH_2)_{14}CH_3$

Figure 2. Proposed GalCer mimetics.

2. Results and discussion

2.1. Binding to V3 loop peptide of gp120

The binding of **3**–**8** to a synthetic V3 loop peptide, RIQRGPGRAFVTIGK, corresponding to the R15K peptide, from gp120 of HIV-1 IIIb isolate was evaluated. We and others have previously used the R15K peptide as a model for the glycolipid binding domain of gp120.[11,19] The change in surface pressure ($\Delta\pi$) at the air-water interface of individual glycolipid monolayers of the test compounds, on exposure to an aqueous solution of the R15K peptide (10 μM) was measured. Increase in surface pressure is associated with integration of the peptide into the glycolipid monolayer. The critical pressure of insertion (CPI), calculated by extrapolation for a null increase in surface pressure is often used as a measure of binding affinity. All the compounds tested interacted with the R15K V3 peptide with CPIs in the range of 18–49 mN/m (Fig. 3). These values were comparable to that measured for GalCer (CPI = 22–25 mN/m).[11] The likeness of **3**–**6** to, and the similarity of their CPIs to GalCer, are consistent with our hypothesis that these disaccharides mimic GalCer in their binding to the V3 peptide, but in the absence of additional data this conclusion is very speculative. It is also noteworthy that the monosaccharides **7** and **8** appear to bind appreciably more strongly to the V3 peptide than the disaccharides. However, give the structural disparity between the mono- and di-saccharide frameworks, the relative activity of the two groups of analogues does not provide any definitive SAR insight because the two sets of analogues may be binding to different domains on the peptide. Nevertheless given the possibility that **3**–**8** interact with the GalCer domain on the V3 peptide, and earlier findings that such compounds may inhibit the attachment of HIV to host cells, the next step was to examine their anti-HIV.

2.2. Anti HIV activity

The anti-HIV activity of the synthetic glycolipids was evaluated in a virus infection assay using the TZM-bl indicator cell line infection with CXCR4 tropic HIV (NL-Lai).[20] The β-Gal-disaccharide **4** with the *O*-3 ester on the mannose residue, the β-Glu-disaccharide

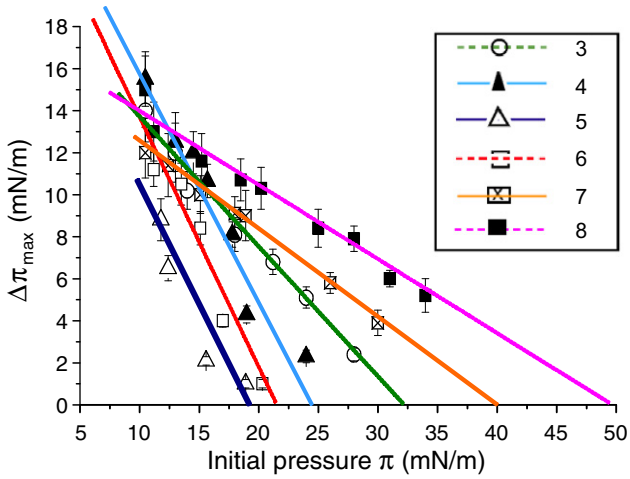


Figure 3. Binding of glycolipid analogues to the V3 loop peptide. Each analog was spread on the surface of a water subphase at various values of the initial surface pressure. A stable monomolecular film was formed after evaporation of the solvent. The synthetic V3 peptide was then added in the subphase at a final concentration of 10 μM. The maximal surface pressure increase ($\Delta\Pi_{max}$) was determined after 2–3 h of incubation, that is, when equilibrium was reached. The data were analyzed with the Filmware 2.5 program (Kibron, Inc.)

6 with the *O*-6 ester and the α-Gal-disaccharide **9** with the *O*-6 ester, showed appreciable activity with IC_{50}'s of 76, 53 and 48 μM, respectively, which was comparable to the activity of the previously assayed monosaccharide **2a** (44 μM, Fig. 4, Table 1). The β-Gal-disaccharide **3** with the ester at *O*-6 of the mannose ring, its di-*O*-ester **5**, and the monosaccharides **7** and **8** showed no significant activity up to 100 μM. Thus the data from the binding and anti-HIV assays indicate that V3 loop binding by itself is not a reliable predictor of anti-HIV activity. This situation may arise because the binding assay measures overall binding but anti-HIV activity requires binding to a specific domain of the peptide. Therefore, the binding data does not necessarily correlate with anti-HIV activity. Within the context of GalCer mimicry, it may be contended that the disaccharides interact with the GalCer binding domain on the V3 peptide but the monosaccharides bind other domains, so that the relative binding affinity of the mono- and di-saccharide analogues is not connected to their anti-HIV activity. However, this argument does not explain why the disaccharide **3** (which is very similar in structure to **4** and **6**), and to a lesser extent **5**, are active in the binding assay but not in the anti-HIV assay. One explanation for this apparent anomaly is that glycolipid presentation in the two assays are different,[7] so that structural factors that control the morphology of the active glycolipid species conflict with those that affect glycolipid-peptide recognition, thereby skewing the data. Of course, an alternative and arguably more obvious, explanation for the contrasting behavior of individual test compounds in the two assays is that the mechanism for anti-viral activity is not at all connected to V3 loop binding. Notwithstanding the mechanism for anti-viral activity, the data from the anti-HIV assay suggests that a disaccharide framework (vs monosaccharide) and one ester residue (vs two), are important for anti-viral activity. That the β-galactoside **4**, the β-glucoside **6** and the α-galactoside **9** were active but the β-galactoside **3** was not, also suggests that the location of the ester is important, and that the optimal position for the ester may vary with the structure of disaccharide framework.

We also examined the activity of glycerol monolaurate (GML, 1-*O*-lauryl-glycerol) in the TZM-bl/HIV assay. GML shows low micromolar activity against two other envelope viruses herpes-simplex virus 2 and bacteriophage ϕ6.[21] Since our active glycolipids and GML are both fatty acid esters, an obvious question was whether the glycolipids and GML inhibit HIV infectivity by a similar mechanism. However, GML was not active at concentrations of up to 100 μM. Parenthetically, it should be noted that GML has been shown to prevent simian immunodeficiency virus (SIV) in a rhesus macaque model.[22] The low activity observed for GML in our cellular assay suggests that the activity observed for GML in the animal model does not result from inhibition of HIV entry. Indeed the results in the animal studies have been linked to immunological effects. That the three disaccharide analogues **4**, **6** and **9** were active but the monosaccharide esters and GML were not, suggests a specificity for a disaccharide scaffold with a single mono fatty acid ester.

Table 1
IC_{50}'s of glycolipid analogues

Compound	IC_{50} (μM)
2a	43.51 ± 1.63
3	>100
4	75.99 ± 6.16
5	>100
6	52.51 ± 1.93
7	>100
8	>100
9	48.35 ± 1.74
GML	>100

IC_{50}'s were determined from the data in Figure 4 for compounds with IC_{50}'s of less than 100 mM.

2.3. Cytotoxicity assays

Cytotoxicity was determined for test compounds against TZM-bl cells over a 24 h incubation period using a MTS dye reduction assay.[23] None of the compounds were toxic up to a 200 μM dose.

2.4. Pre and post infection assays

To determine whether the compounds affected the viral entry process or events post viral entry, simple time of addition experiments were conducted.[11] TZM-bl cells were exposed to the virus in the presence of the compounds (100 μM) or the compounds were added 4 h after infection with the virus. We also used T20,[24] an inhibitor of gp41 mediated fusion and AMD 3100,[25] a CXCR4 antagonist that inhibits HIV Env mediated fusion and viral entry as controls in these experiments. All active compounds inhibited virus infection if added at the same time as the virus (Fig. 5). However, addition of the compounds 4 h post-infection failed to show significant inhibition. The same was true for the

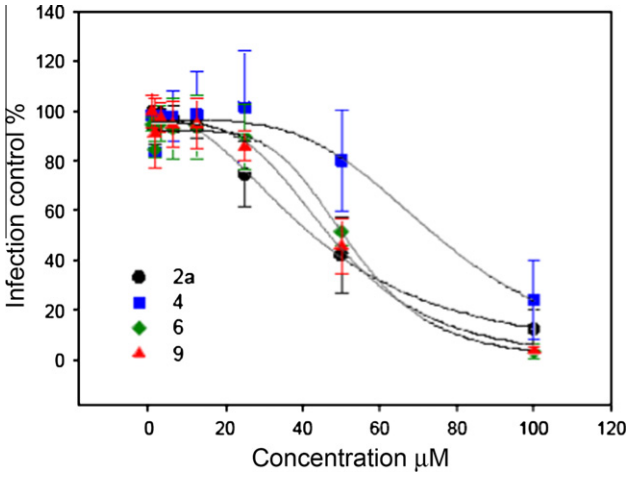


Figure 4. Anti HIV activity of glycolipid analogues. TZM-bl cells were infected with X4 HIV NL-Lai in the presence of serial dilutions of the compounds. Infectivity was determined 24 h later by measuring luciferase activity and normalized to control wells infected with virus in the absence of drug. Pooled data from three independent experiments was used to fit curves using Sigma Plot software.

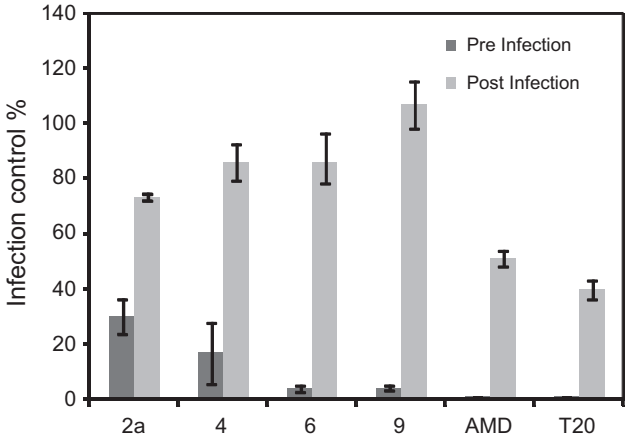


Figure 5. Glycolipid analogues show activity pre-infection only. TZM-bl cells were infected with NL-Lai virus. Compounds 100 μM were added either prior to infection (pre infection) or 4 h post infection. Infectivity was determined 24 h later by measuring luciferase activity. AMD and T20 controls are used at 2 mM. Data are mean ± SD of triplicate observations.

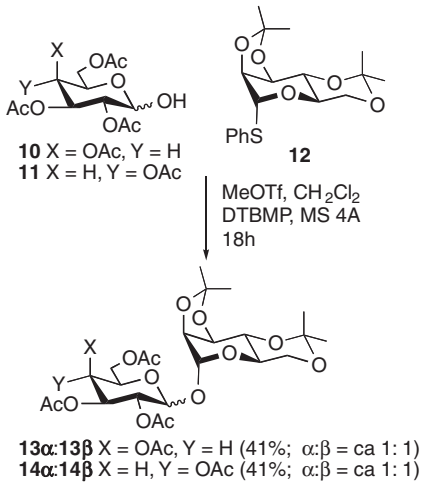
entry inhibitors T20 and AMD3100. The absence of activity when the compounds were added 4 h post-infection also suggest that they did not have a direct effect on cell viability or function and hence the inhibition is at the step of initial virus cell interaction. This mode of action, which appears to be similar to **2a**[11] contrasts with the one suggested for a 3-*O*-fatty acid ester of trehalose (α,α-1,1-Glu-Glu) that is structurally similar to **4**. The trehalose derivative is believed to prevent influenza proliferation by inhibiting viral replication.[26,27]

2.5. Critical micelle concentration (CMC)

The CMC's of selected analogues were measured in order to determine the physical state of the glycolipids at the concentrations used in the cellular assays. Values were obtained by tensiometer measurements of solutions of glycolipids in water, as previously described.[11] The CMC is taken as the concentration of glycolipid that does not induce any further decrease in surface tension. This data was obtained in triplicate: **3** (9.8, 10 and 10.5 μM); **4** (12, 12 and 12.5 μM); **6** (10, 10.5, and 12 μM); **7** (13, 13.5 and 13.7 μM). Thus at their active concentrations analogues **4** and **6**, exist as micelles, but this does not preclude the possibility that the active species is monomeric. The CMC's obtained for **3**, **4** and **6** were in the range observed for mono-fatty acid esters of related 1,1-linked disaccharides.[28,29] The diester **8** was difficult to study; a regular decrease in surface tension from 72.8 mN/m (pure water) to 0 mN/m (glycolipid film collapse) in the concentration range of 0.25–5 μM was observed. One interpretation of this result is that **8** forms precipitates rather than micelles in water above a concentration of 0.5 μM.

2.6. Synthesis

The synthesis of the disaccharide lipids **3**–**6** started with assembly of the 1,1-linked precursors **13**β and **14**β (Scheme 1). Initial glycosidation reactions using 2-O-acetylated Gal donors containing different alcohol protecting and glycosyl activating groups with Man acceptors gave low yields of disaccharide products. A solution to this problem, albeit not a stereoselective one, was found in the methyl triflate promoted glycosidation of thioglycoside Man donor **12** with Gal and Glu acceptors **10**[30] and **11**,[31] respectively. The phenylthio glycoside **12** was prepared from the tetra-*O*-acetate derivative following the procedure used for the corresponding ethylthio glycoside.[32,33] The reactions of **12** with **10** and **11** afforded in each case an approximately 40% yield of a 1:1 mixture of anomers, α:β disaccharides **13**α:β and **14** α:β. The stereochemistry at the anomeric position of the Gal and Glu rings was deduced from vicinal $J_{H,H}$ values (**13**α/β: $J_{1,2}$ (Gal) = ca. 3.5:8.0 Hz; **14**α/β: $J_{1,2}$(Glu) = 3.8:8.0 Hz). The α-configuration at C-1 for the Man subunit was assigned on the basis of $J_{C,H}$ values (**13**α/β: $J_{1,1}$ (Man) = 175:172; **14**α/β: $J_{1,1}$ (Man) = 175:172 Hz.[34]



Scheme 1. Glycoside synthesis.

The separated anomers from the glycosidation reactions and the known mannoside **21**[35] were next transformed to the target compounds **3**–**9** through established alcohol protecting group sequences (Scheme 2). These reactions were not optimized in all cases. Representative transformations are described. Acetate hydrolysis on **13**β followed by perbenzylation of the resulting tetraol provided the tetra-*O*-benzyl ether derivative. The 4,6-*O*-isopropylidene in this product was selectively cleaved under acidic conditions to provide diol **15**. DCC mediated esterification of **15** with 1 equiv of palmitic acid provided monoester **16** as the major product, and with excess acid, the diester **17**. Individual hydrogenolysis of **16** and **17** afforded the mono- and di-*O*-acylated disaccharide targets **3** and **5**. For the 3-*O*-ester **4**, **15** was first transformed to diol **18** via standard protecting group transformations. Selective acylation[36] of the equatorial alcohol in **18** followed by hydrogenolysis of the product provided **4**. The β,α-1,1-Glu-Man **6** and the α,α-1,1-Gal-Man disaccharide **9** were prepared from **14**β and **13**α, respectively, following the reaction sequence used for transformation of **13β** to **3**. For the monosaccharide esters **7** and **8**, **21** was first converted to the 2,3-*O*-isopropylidene-4-*O*-palmitoyl- or 2,3-*O*-isopropylidene-4,6-di-*O*-palmitoyl-derivatives following procedures used for the analogous disaccharides **16** and **17**. Acetal hydrolysis in these materials led to **7** and **8**, respectively.

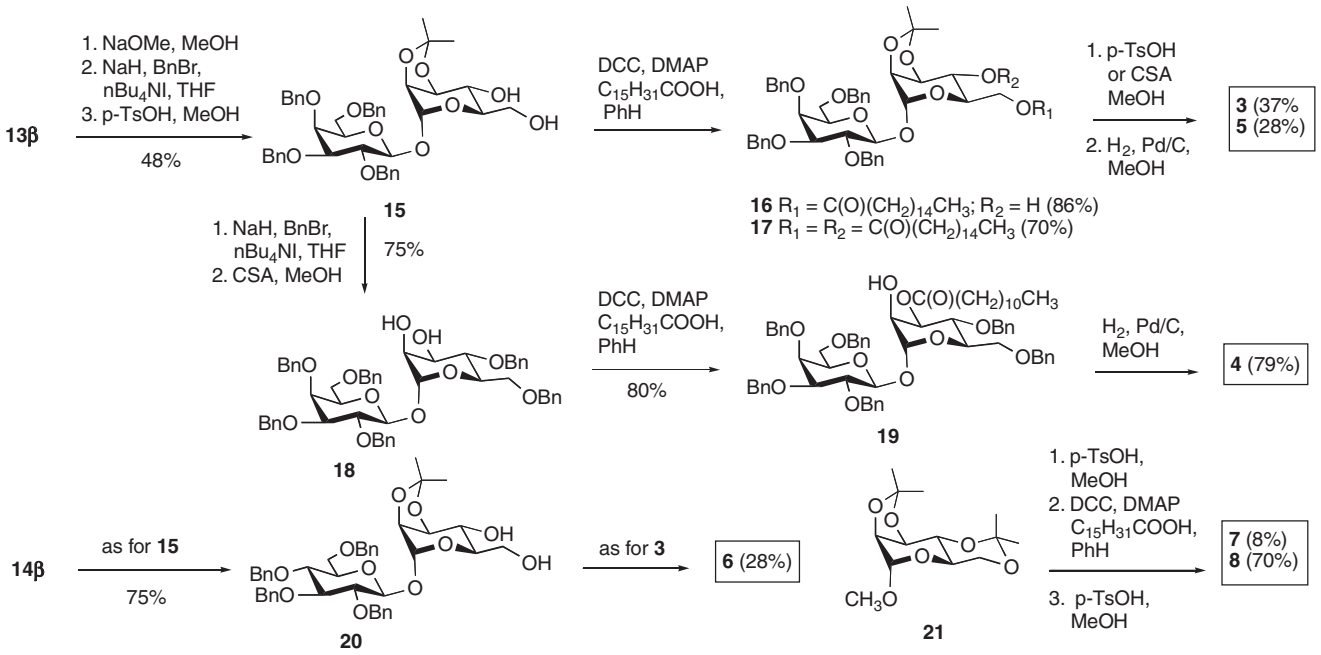
3. Summary

1,1-Linked Gal-Man and Glu-Man disaccharides with a long chain fatty acid ester on the mannose residue were found to inhibit HIV-infectivity in the 50 μM range, with no cytotoxicity at concentrations up to 200 μM. These compounds appear to inhibit virus entry at early steps in viral infection since they were inactive if added post viral entry. Their structural likeness to GalCer and the observation that they bind the V3 loop peptide of HIV-1 and inhibit HIV infectivity fits with the notion their anti-HIV activity results from their ability to bind the GalCer domain of the V3 peptide. However, in the absence of other data this conclusion is very tentative. That closely related disaccharide analogues showed similar binding to the V3 peptide but did not inhibit HIV infectivity suggests that factors other than V3 loop binding may contribute to anti-HIV activity, or may point to completely different mechanisms. In this context it is noteworthy that the viruscidal activity of fatty acid derivatives of related disaccharide structures and other small molecule amphiphilic lipids has been attributed to surfactant-like, membrane-perturbing properties.[21,37–41] Notwithstanding the mechanistic basis for their anti-HIV activity, the preliminary structure–activity data obtained from this investigation suggests that a disaccharide-type framework and the position of acylation are important for activity. Although the trehalose-type fatty acid esters reported here have similar anti-HIV potency to other small molecule lipids, their low cytotoxicity and other reports that trehalose-type disaccharide esters have broader viruscidal activity, combined with the easy accessibility to structurally diverse analogues, make them attractive leads for drug development.[42–44]

4. Experimental

4.1. Surface pressure measurements

The surface pressure of glycolipid monolayers was measured with a fully automated microtensiometer (μTROUGH SX; Kibron,



Scheme 2. Esterification and protecting group sequences.

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 test compounds were spread on pure water subphases (volume of 800 μL) from hexane–chloroform–ethanol solution as described previously.[45] After spreading of the film, 5 min was allowed for solvent evaporation. To measure the interaction of the V3 peptide with the individual monolayers, the peptide was injected in the subphase with a 10 μL Hamilton syringe, and pressure increases ($\Delta\pi$) produced were recorded until reaching a stable value. The experiment was repeated at different values of the initial surface pressure (πi) 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 πi for the test compounds. The accuracy of the system under the experimental conditions was ±0.25 mN/m for surface pressure.

4.2. Antiviral assays

Testing of antiviral activity of test compounds was done as described previously.[11] Briefly, virus stocks were prepared by transfection of 293T cells with NL-Lai full length infectious clone of HIV using Ex gen 500 transfection reagent (Fermentas, Glen Burnie, MD. Virus supernatant was collected 48 h post transfection, cleared of cellular components by centrifugation, aliquoted, stored at −70 °C. Virus titers were determined in TZM-bl cells prior to use. For determination of antiviral activity TZM-bl cells were seeded in 96-well plates at 2×10^4 cells per well and allowed to adhere overnight. The subsequent day cells were infected with HIV virus in the presence of 20 μg/mL DEAE dextran and test compounds at various concentrations. Virus infection was determined 24 h post infection as luciferase activity using Brite Lite Luciferase substrate (Perkin Elmer). Percent infection was calculated based on control wells infected with virus in the absence of any compound. Normalized data was fit using Sigma Plot software and IC_{50} calculated based on the curve fit. Pooled data from at least three independent experiments was used for IC_{50} determination for each compound.

4.3. Cytotoxicity assays

Cytotoxicity of compounds was determined by a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) dye reduction assay. Briefly TZM-bl cells were seeded in 96-well plates and incubated overnight. Subsequent day serial dilutions of compounds starting at 200 μM were added to the cells. The cells were incubated for another 24 h before addition of the MTS reagent (Cell titer Aqueos one solution, Promega). Reduction of the MTS dye was determined by measuring OD at 490 nM and normalized to cells incubated with media only.

4.4. Pre and post infection assays

To determine the step at which the compounds showed activity a pre/post infection time of addition assay was conducted. TZM-bl cells were infected with NL-Lai virus. The compounds (100 μM) were either added prior to infection (pre infection) or 4 h post infection. For the post infection the uninfected virus was removed by washing with PBS followed by addition of fresh media containing the compounds. For controls T20 and AMD3100 were used at 2 μM concn.

4.5. CMC measurements

Stock solutions of glycolipids were prepared in hexane/chloroform/ethanol (11:5:4, v/v/v) and injected in water with a Hamilton microsyringe (dilution 1:1000). The surface tension was continuously recorded with the Kibron microtensiometer. Below the CMC, a drop in surface tension was recorded after each glycolipid injection. Increasing the concentration of the added glycolipid resulted in a linear decrease in surface tension. The CMC was determined as the concentration of glycolipid above which there was no further decrease in surface tension.

4.6. Synthesis—general

Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere in oven-dried glassware using standard

syringe and septa technique. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker 500 MHz spectrometer. Chemical shifts are relative to the deuterated solvent peak and are in parts per million (ppm). ^{13}C NMR peaks are listed to the nearest 0.1 ppm. When two or more signals occur as distinct paks within 0.1 ppm, the number of these signals are indicated in parenthesis. Assignments for selected nuclei were determined from ^{1}H COSY experiments. Thin layer chromatography (TLC) was done on 0.25 mm thick precoated silica gel HF_{254} aluminum sheets. Chromatograms were observed under UV (short and long wavelength) light, and 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). Flash column chromatography (FCC) was performed using Silica Gel 60 (230–400 mesh) and employed a stepwise solvent polarity gradient, correlated with TLC mobility. Solvents were purified by standard procedures or used from commercial sources as appropriate.

4.7. 2,3,4,6-Tetra-*O*-acetyl-α/β-D-galactopyranosyl-(1→1)-2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranoside 13α/β

A mixture of mannose donor **12**[32,33] (4.20 g, 11.9 mmol), galactose acceptor **10**[30] (4.10 g, 11.8 mmol), freshly activated, powdered, 4 Å molecular sieves, 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) (17.3 g, 83.3 mmol) and dry CH_2Cl_2 (50 mL) was stirred for 30 min, at rt. The mixture was then cooled to 0 °C and MeOTf (8.1 mL, 77.4 mmol) was slowly added. After stirring for an additional 20 h the reaction was quenched with triethylamine (3 mL). The mixture was diluted with CH_2Cl_2, filtered and concentrated in vacuo. FCC of the residue afforded **13**α (1.59 g, 23%) and **13**β (1.26 g, 18%) as white amorphous solids. For **13**α: R_f = 0.32 (30% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 3H), 1.42 (s, 3H), 1.52 (s, 3H), 1.57 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H), 2.14 (s, 3H), 2.17 (s, 3H), 3.50 (m, 1H), 3.74 (d, 1H, J = 8.1 Hz), 3.78 (m, 2H), 4.08 (m, 1H), 4.14 (m, 1H), 4.20 (d, 1H, J = 3.4 Hz), 4.29 (t, 1H, J = 6.7 Hz), 5.21 (dd, 1H, J = 3.7, 10.9 Hz), 5.30 (s, 1H), 5.36 (dd, 1H, J = 3.15, 10.9 Hz), 5.49 (apparent d, 2H, J = 3.5 Hz, H-1 (gal), H-4 (gal)); ^{13}C NMR ($CDCl_3$) δ 19.3, 21.2 (three signals), 26.7, 28.6, 29.6, 61.9, 62.2, 62.6, 67.6, 67.8, 67.9, 68.2, 73.0, 75.2, 76.1, 91.8 ($J_{H1,C1}$ (gal) = 172 Hz), 94.2 ($J_{H1,C1}$ (man) = 175 Hz), 100.3, 110.4, 170.6, 170.7, 170.8, 170.9; HRMS (ESI) calcd for $(M+Na)^+$ $C_{26}H_{38}NaO_{15}$ 613.2103, found 613.2110. For **13**β: R_f = 0.18 (30% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H), 1.43 (s, 3H), 1.53 (s, 3H), 1.57 (s, 3H), 2.01 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.20 (s, 3H), 3.71 (t, 1H, J = 9.81 Hz), 3.76 (d, 1H, J = 7.9 Hz), 3.82 (m, 1H), 3.89 (m, 1H), 3.97 (t, 1H, J = 6.9 Hz), 4.11 (m, 2H), 4.20 (m, 1H), 4.70 (d, 1H, J = 8.0 Hz, H-1 (gal)), 5.21 (s, 1H), 5.24 (dd, 1H, J = 8.1, 10.5 Hz), 5.41 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 18.9, 20.7, 20.8, 20.9, 21.0, 26.4, 28.3, 29.2, 61.7, 62.0, 62.3, 67.2, 69.2, 71.0, 71.4, 72.5, 75.0, 75.6, 99.8 ($J_{H1,C1}$ (man) = 172 Hz), 99.9 ($J_{H1,C1}$ (gal) = 154 Hz), 100.4, 109.9; HRMS (ESI) calcd for $(M+Na)^+$ $C_{26}H_{38}NaO_{15}$ 613.2103, found 613.2097.

4.8. 2,3,4,6-Tetra-*O*-acetyl-α/β-D-glucopyranosyl-(1→1)-2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranoside 14α/β

Disaccharides **14**α/β were prepared from mannose donor **12** (415 mg, 1.18 mmol), and glucose acceptor **11**[31] (373 mg, 1.07 mmol) via the procedure described for **13**α/β. For **14**α: (130 mg, 21%); R_f = 0.45 (40% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 3H), 1.43 (s, 3H), 1.58 (s, 6H), 2.05 (s, 3H), 2.06 (s, 3H), 3.52 (m, 1H), 3.76 (m, 3H), 4.08 (m, 1H), 4.13 (m, 2H), 4.24 (m, 2H), 4.30 (dd, 1H, J = 4.2, 4.3 Hz), 4.97 (dd, 1H, J = 3.9, 10.3 Hz), 5.13 (t, 1H, J = 9 Hz), 5.30 (s, 1H), 5.47 (d, 1H, J = 3.8 Hz, H1 (glu)), 5.50 (t, 1H, J = 9.9 Hz); ^{13}C NMR ($CDCl_3$) δ 18.9, 20.7, 20.8, 20.9, 26.4, 28.3, 29.3, 61.8, 61.9, 62.3, 68.3, 68.5, 70.0, 70.3, 72.6, 74.9, 75.7, 91.0 ($J_{H1,C1}$ (glu) = 172 Hz), 93.9 ($J_{H1,C1}$ (man) = 175 Hz), 100.0, 110.1, 169.7, 170.2, 170.4, 170.8; HRMS (ESI) calcd for $(M+Na)^+$ $C_{26}H_{38}NaO_{15}$ 613.2103, found 613.2095. For **14**β: (124 mg, 20%); R_f = 0.39 (40% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H), 1.43 (s, 3H), 1.52 (s, 3H), 1.57 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 2.08 (s, 3H), 2.12 (s, 3H), 3.74 (m, 4H), 3.89 (dd, 1H, J = 5.0, 10.1 Hz), 4.12 (d, 1H, J = 5.7 Hz), 4.19 (m, 3H), 4.74 (d, 1H, J = 8.0 Hz, H-1 (glu)), 5.01 (d, 1H, J = 9.6 Hz), 5.05 (t, 1H, J = 9.7 Hz), 5.23 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 18.9, 20.8 (two signals), 26.4, 28.4, 29.1, 61.6, 62.4, 62.5, 68.4, 71.6, 72.4, 72.6, 72.9, 75.0, 75.6, 99.7 ($J_{H1,C1}$ (man) = 172 Hz), 99.8 ($J_{H1,C1}$ (glu) = 152 Hz), 99.9, 109.9, 169.5, 169.6, 170.4, 170.9; HRMS (ESI) calcd for $(M+Na)^+$ $C_{26}H_{38}NaO_{15}$ 613.2103, found 613.2101.

4.9. 2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1→1)-2,3-*O*-isopropylidene-α-D-mannopyranoside 15

Tetra-*O*-acetate **13**β (0.514 g, 0.87 mmol) was treated with NaOMe (100 mg) in dry MeOH (10 mL) under N_2 for 15 min. The mixture was then neutralized with methanolic HCl and concentrated under reduced pressure. The crude product was dried in vacuo and taken up in dry THF (15 mL). TBAI (0.032 g, 0.087 mmol) and NaH (0.348 g, 60% dispersion in mineral oil, 8.7 mmol) were added to the solution at 0 °C, under nitrogen. After 45 min at this temperature, BnBr was added dropwise to the mixture and stirring continued at rt for 15 h. The reaction was then cooled to 0 °C, quenched with MeOH, diluted with water and extracted with ether. The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. FCC of the residue provided the tetra-*O*-benzylated derivative as a clear oil (0.498 g, 73%): R_f = 0.53 (30% EtOAc/petroleum ether). To a portion of this material (100 mg, 0.129 mmol), in dry MeOH (5 mL) was added *p*-TsOH (12 mg, 0.064 mmol) at rt. The mixture was stirred for 15 min, then neutralized with Et_3N and concentrated in vacuo. FCC of the residue afforded diol **15** (60 mg, 63%) as a white solid. R_f = 0.65 (70% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H), 1.53 (s, 3H), 3.39 (m, 1H), 3.56 (dd, 1H, J = 2.9, 6.9 Hz), 3.61 (m, 3H), 3.79 (dd, 1H, J = 3.1, 8.7 Hz), 3.83 (d, 1H, J = 2.8 Hz), 3.87 (t, 1H, J = 7.9 Hz), 4.01 (m, 1H), 4.06 (d, 1H, J = 5.7 Hz), 4.14 (m, 2H), 4.39 (d, 1H, J = 11.8 Hz), 4.48 (d, 1H, J = 11.7 Hz), 4.61 (d, 1H, J = 6.5 Hz), 4.64 (d, 1H, J = 2.6 Hz), 4.75 (d, 2H, J = 2.9 Hz), 4.80 (d, 1H, J = 11.2 Hz), 4.87 (d, 1H, J = 11.2 Hz), 4.94 (d, 1H, J = 11.7 Hz), 5.29 (s, 1H), 7.33 (m, 20 H); ^{13}C NMR ($CDCl_3$) δ 26.2, 28.0, 62.4, 69.3, 70.1, 70.6, 73.0, 73.3, 73.5, 73.8, 74.4, 75.3, 75.4, 78.5, 79.4, 82.5, 99.0, 102.2, 109.6, 127.6 (two signals), 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4 (three signals), 137.4, 138.2, 138.3, 138.5; HRMS (ESI) calcd for $(M+Na)^+$ $C_{43}H_{50}NaO_{11}$ 765.3245, found 765.3243.

4.10. 2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1→1)-2,3-*O*-isopropylidene-6-*O*-palmitate-α-D-mannopyranoside 16

To a solution of **15** (118 mg, 0.160 mmol) and palmitic acid (45 mg, 0.176 mmol), in dry benzene (4 mL) was added DCC (36 mg, 0.176 mmol) and DMAP (5 mg, 0.04 mmol) at 0 °C. The reaction was stirred for 18 h at rt, then diluted with ether and filtered through a bed of Celite. The filtrate was concentrated in vacuo and the residue purified by FCC to give **16** (100 mg, 86% brsm): R_f = 0.4 (30% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3H, J = 6.8, 7.0 Hz), 1.27 (br s, 25H), 1.36 (s, 3H), 1.51 (s, 3H), 1.62 (m, 2H), 2.35 (t, 2H, J = 7.5 Hz), 3.15 (br s, 1H), 3.46 (m, 2H), 3.55 (m, 2H), 3.60 (t, 1H, J = 6.2 Hz), 3.85 (t, 1H, J = 7.9 Hz), 3.89 (d, 1H, J = 2.4 Hz), 4.00 (m, 1H), 4.06 (m, 1H), 4.15 (t, 1H, J = 5.9 Hz), 4.38 (d, 1H, J = 11.6 Hz), 4.43 (d, 1H, J = 11.6 Hz), 4.57 (m, 2H), 4.63 (d, 1H, J = 11.7 Hz), 4.74 (s, 1H), 4.79 (d, 1H, J = 11.2 Hz), 4.86 (d, 1H, J = 11.2 Hz), 4.95 (d, 1H, J = 11.7 Hz), 5.30 (s, 1H), 7.34 (m, 20H); ^{13}C NMR ($CDCl_3$) δ 14.3, 22.9, 25.3, 26.4,

28.3, 29.4, 29.5 (two signals), 29.6, 29.8 (two signals), 29.9, 32.1, 34.4, 62.6, 68.5, 69.3, 69.4, 73.2, 73.4, 73.7, 74.0, 74.7, 75.4, 75.7, 77.8, 79.7, 82.8, 99.6, 102.7, 109.7, 127.7 (three signals), 128.0, 128.1, 128.2, 128.4, 128.6 (three signals), 137.8, 138.4, 138.6, 138.7, 175.4.

4.11. 2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1→1)-2,3-*O*-isopropylidene-4,6-di-*O*-palmitate-α-D-mannopyranoside 17

Diol **15** (60 mg, 0.081 mmol) was treated as described in Section 4.10, with excess palmitic acid (52 mg, 0.2 mmol). The diester **17** (68 mg, 70%) was obtained as a white solid: R_f = 0.82 (25% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 6H), δ 1.29 (br s, 48H), δ 1.60 (s, 4H), 2.30 (s, 4H), 3.56 (m, 4H), 3.86 (dd, 1H, J = 7.8, 9.6 Hz), 3.93 (s, 1H), 4.02 (d, 2H, J = 13.7 Hz), 4.19 (d, 2H, J = 8.4 Hz), 4.28 (d, 1H, J = 12.4 Hz), 4.40 (d, 1H, J = 11.7 Hz), 4.48 (d, 1H, J = 11.7 Hz), 4.59 (d, 1H, J = 9.5 Hz), 4.64 (d, 1H, J = 13.4 Hz), 4.74 (s, 1H), 4.79 (d, 1H, J = 13.1 Hz), 4.88 (d, 1H, J = 13.1 Hz), 4.94 (d, 1H, J = 13.3 Hz), 5.18 (t, 1H, J = 10.2 Hz), 5.35 (s, 1H), 7.32 (m, 20H); ^{13}C NMR ($CDCl_3$) δ 14.3, 22.9, 25.0, 26.8, 27.9, 29.3, 29.4, 29.5 (two signals), 29.7 (two signals), 29.8, 29.9 (two signals), 32.1, 34.3, 34.4, 61.5, 67.0, 68.5, 68.8, 70.8, 73.2, 73.4, 73.7, 73.9, 74.9, 75.7 (two signals), 76.5, 79.7, 82.9, 99.3, 102.9, 110.3, 127.7, 127.9 (two signals), 128.1, 128.4, 128.6 (two signals), 137.9, 138.4, 138.6, 138.7, 172.5, 173.8; HRMS (ESI) calcd for $(M+Na)^+$ $C_{75}H1_{10}NaO_{13}$ 1241.7389, found 1241.7389.

4.12. 2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1→1)-4.6-*O*-benzyl-α-D-mannopyranoside 18

Diol **15** (61 mg, 0.082 mmol) was subjected to the standard benzylation procedure described in Section 4.9. FCC of the crude product yielded the di-*O*-benzylated derivative (71 mg, 0.08 mmol), which was taken up in dry MeOH (5 mL) and treated with CSA (100 mg, 0.43 mmol). The mixture was stirred for 30 min, quenched with Et_3N and concentrated in vacuo. FCC of the residue afforded **18** (55 mg, 75% two steps) as a clear oil: R_f = 0.55 (60% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 3.56 (m, 5H), 3.73 (dd, 1H, J = 3.15, 3.05 Hz), 3.81 (m, 2H), 3.88 (m, 2H), 3.97 (dd, 1H, J = 3.40, 3.50 Hz), 4.12 (m, 1H), 4.37 (s, 2H), 4.40 (s, 1H), 4.59 (m, 4H), 4.60 (d, 1H, J = 11.1 Hz), 4.73 (s, 2H), 4.78 (d, 1H, J = 11.1 Hz), 4.82 (d, 1H, J = 11.1 Hz), 4.94 (d, 1H, J = 11.7 Hz), 7.24–7.39 (m, 30H); ^{13}C NMR ($CDCl_3$) δ 68.7, 68.9, 71.1, 71.5, 71.7, 73.1, 73.6 (two signals), 73.8, 74.6, 74.8, 75.6, 75.8, 79.7, 82.7, 101.5, 103.5, 127.7, 127.8 (three signals), 127.9 (three signals), 128.0, 128.1, 128.49 (two signals), 128.5, 128.6 (three signals), 138.1, 138.4, 138.6, 138.7, 138.8.

4.13. 2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1→1)-4.6-*O*-benzyl-3-*O*-palmitate-α-D-mannopyranoside 19

To a solution of **18** (80 mg, 0.09 mmol) and palmitic acid (26 mg, 0.099 mmol) in dry benzene was added DCC (20 mg, 0.099 mmol) and DMAP (2 mg, 0.018 mmol) at 0 °C. The reaction was stirred for 18 h at rt, then diluted with ether, filtered through a bed of Celite and concentrated in vacuo. FCC of the residue yielded **19** (74 mg, 80% brsm) as a white solid. R_f = 0.30 (30% EtOAc/petroleum ether). ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, J = 6.8 Hz), 1.27 (m, 24H), 1.61 (m, 2H), 2.28 (m, 2H), 3.54 (m, 6H), 3.71 (dd, 1H, J = 3.5, 11.5 Hz), 3.89 (m, 2H), 4.03 (m, 2H), 4.23 (m, 1H), 4.35 (m, 3H), 4.47 (d, 1H, J = 11.3 Hz), 4.56 (m, 1H), 4.62 (m, 2H), 4.73 (m, 2H), 4.83 (s, 1H), 4.95 (d, 1H, J = 12.0 Hz), 5.11 (d, 1H, J = 1.5 Hz), 5.34 (dd, 1H, J = 3.2, 9.7 Hz), 7.34 (m, 35H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 24.8, 24.9, 25.5, 29.1, 29.2, 29.3, 29.4, 29.6 (three signals), 29.7, 31.9, 33.7, 34.4, 68.2, 68.7, 69.5, 71.7, 72.7, 72.9, 73.3, 73.6, 73.9, 74.5, 75.5, 79.3, 82.3, 101.4, 103.6, 127.4 (two signals), 127.5 9 (three signals), 127.6, 127.8 (two signals), 128.0, 128.2 (two signals), 128.3 (three signals), 128.4, 137.9, 138.3, 138.4, 138.5, 138.6, 172.6; HRMS (ESI) calcd for $(M+NH_4)^+$ $C_{70}H_{92}NO_{12}$ 1138.6614, found 1138.6606.

4.14. 2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl-(1→1)-2,3-*O*-isopropylidene-α-D-mannopyranoside 20

Protected disaccharide **14**β was transformed to **20** (58 mg, 75% brsm) following the three-step procedure described for the conversion of **13**β to **15** (Section 4.9) For **20**: white solid; R_f = 0.18 (40% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H), 1.54 (s, 3H), 2.22 (d, 1H, J = 4.4 Hz), 2.55 (dd, 1H, J = 5.7, 7.5 Hz), 3.48 (m, 2H), 3.56 (m, 2H), 3.61–3.70 (m, 4H), 3.78–3.82 (m, 1H), 3.95–3.99 (m, 1H), 4.04 (d, 1H, J = 5.8 Hz), 4.14 (m, 1H), 4.51 (m, 3H), 4.67 (d, 1H, J = 7.9 Hz), 4.83 (m, 4H), 4.92 (d, 1H, J = 10.9 Hz), 5.30 (s, 1H), 7.33 (m, 20H); ^{13}C NMR ($CDCl_3$) δ 26.4, 28.2, 62.6, 69.2, 70.3, 71.1, 73.6, 74.9, 75.2, 75.4, 75.6, 75.9, 78.0, 78.7, 82.4, 85.0, 99.4, 102.5, 109.9, 127.9, 128.0, 128.1 (two signals), 128.2, 128.3, 128.6 (two signals), 128 .7, 137.7, 138.0, 138.4, 138.5; HRMS (ESI) calcd for $(M+Na)^+$ $C_{43}H_{50}NaO_{11}$ 765.3245, found 765.3238.

4.15. β-D-Galactopyranosyl-(1→1)-6-*O*-palmitate-α-D-mannopyranoside 3

A solution of **16** (100 mg, 0.1 mmol) and *p*-TsOH (100 mg, 0.52 mmol) in MeOH (5 mL) was stirred for 1 h. The mixture was then quenched with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. FCC of the residue afforded the corresponding triol (70 mg, 73%). A portion of the triol (30 mg, 0.032 mmol), 10% Pd/C (40 mg) and HCOOH (0.1 mL) was stirred under H_2 atmosphere for 20 h. The mixture was then filtered through a bed of Celite and concentrated in vacuo. FCC of the residue gave **3** (9 mg, 50%): R_f = 0.36 (20% MeOH/EtOAc); ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, J = 6.8 Hz), 1.28 (m, 28H), 1.61 (m, 2H), 2.36 (t, 2H, J = 7.5 Hz), 3.46 (dd, 1H, J = 3.0, 9.7 Hz), 3.53 (m, 2H), 3.71 (m, 5H), 3.85 (d, 1H, J = 2.7 Hz), 3.95 (s, 1H), 4.05 (m, 1H), 4.25 (dd, 1H, J = 4.9, 11.95 Hz), 4.31 (d, 1H, J = 11.2 Hz), 4.41 (d, 1H, J = 7.7 Hz), 5.02 (s, 1H); ^{13}C NMR (CD_3OD) δ 14.6, 23.9, 26.2, 30.4, 30.6, 30.8, 30.9 (two signals), 33.2, 35.1, 62.2, 64.9, 68.5, 70.1, 71.6, 72.4, 72.7, 72.8, 75.1, 77.0, 103.2, 104.4, 175.9; HRMS (ESI) calcd for $(M+Na)^+$ $C_{28}H_{52}NaO_{12}$ 603.3351, found 603.3352.

4.16. β-D-Galactopyranosyl-(1→1)-3-*O*-palmitate-α-D-mannopyranoside 4

The hexa-*O*-benzylether **19** (54 mg, 0.048 mmol) was subjected to the hydrogenolysis procedure described for **3**. FCC of the crude product provided **4** (22 mg, 79%): R_f = 0.58 (20% MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.8 Hz), 1.16 (m, 5H), 1.29 (br s, 26H), 1.63 (m, 4H), 1.71 (m, 2H), 1.85 (m, 2H), 2.41 (t, 2H, J = 7.45 Hz), 3.46 (m, 2H), 3.56 (m, 1H), 3.65 (m, 3H), 3.78 (m, 3H), 3.88 (d, 1H, J = 10.2 Hz), 4.10 (m, 2H), 4.44 (d, 1H, J = 7.85 Hz), 5.04 (dd, 1H, J = 3.2, 9.8 Hz), 5.06 (s, 1H); ^{13}C NMR (CD_3OD) δ 14.6, 23.9, 26.1, 26.2, 26.9, 30.3, 30.6, 30.8, 30.9 (two signals), 33.2, 34.9, 35.2, 63.0, 63.1, 66.3, 69.7, 70.5, 72.5, 75.0, 75.3, 75.6, 77.4, 102.9, 104.7, 175.5; HRMS (ESI) calcd for $C_{12}H_{22}NaO_{11}$ $(M+Na)^+$ 603.3351, found 603.3351.

4.17. β-D-Galactopyranosyl-(1→1)-4,6-di-*O*-palmitate-α-D-mannopyranoside 5

The diester **17** (72 mg, 0.06 mmol) was dissolved in a mixture of dry MeOH (3 mL) and dry CH_2Cl_2 (1 mL). CSA (25 mg, 0.11 mmol) was then added. The reaction mixture was stirred for 1 h, then

neutralized with Et_3N and evaporated under reduced pressure. FCC of the residue gave the derived diol (32 mg, 46%): white solid. R_f = 0.54 (35% EtOAc/petroleum ether). This material (32 mg, 0.027 mmol) was subjected to the hydrogenolysis procedure described for **3**. FCC of the product afforded **5** (13 mg, 60%): white solid; R_f = 0.32 (10% MeOH/EtOAc); ^{1}H NMR (CD_3OD) δ 0.90 (t, 6H, J = 6.8 Hz), 1.31 (s, 48H), 1.61 (m, 4H), 2.35 (m, 4H), 3.48 (dd, 1H, J = 3.3, 9.7 Hz), 3.54 (m, 2H), 3.72 (d, 2H, J = 6.3 Hz), δ 3.85 (d, 1H, J = 3.3 Hz), 3.89 (dd, 1H, J = 3.3, 9.8 Hz), 4.01 (m, 1H), 4.05 (m, 1H), 4.21 (m, 1H), 4.43 (d, 1H, J = 7.7 Hz), 5.07 (s, 1H), 5.24 (t, 1H, J = 9.8 Hz); ^{13}C NMR δ 14.6, 23.9, 26.0, 26.1, 30.3, 30.4, 30.6, 30.7, 30.8 (two signals), 30.9, 31.0, 33.2, 35.0, 35.2, 62.4, 63.8, 70.1, 70.2, 70.6, 71.7, 72.7, 75.1, 77.1, 103.3, 104.7, 174.9, 175.5; HRMS (ESI) calcd for $(M+Na)^+$ $C_{44}H_{82}NaO_{13}$ 841.5641, found 841.5648.

4.18. β-D-Glucopyranosyl-(1→1)-6-*O*-palmitate-α-D-mannopyranoside 6

Diol **20** was transformed to **6** (9 mg, 28% brsm) following the three step procedure described for the conversion of **15** to **3** (Sections 4.9, 4.10, 4.15). For **6**: R_f = 0.37 (20% MeOH/EtOAc); ^{1}H NMR (CD_3OD) δ 0.92 (t, 3H, J = 6.8 Hz), 1.20 (t, 3H, J = 7.0 Hz), 1.31 (br s, 24H), 1.64 (m, 2H), 2.23 (t, 2H, J = 7.4 Hz), 3.23 (t, 1H, J = 8.0 Hz), 3.33 (m, 1H), 3.50 (q, 2H, J = 7.0 Hz), 3.70 (m, 3H), 3.87 (dd, 1H, J = 2.2, 11.9 Hz), 3.98 (m, 1H), 4.10 (m, 1H), 4.29 (dd, 1H, J = 5.2, 11.9 Hz), 4.36 (dd, 1H, J = 2.2, 12.0 Hz), 4.48 (d, 1H, J = 8.0 Hz), 5.03 (s, 1H); ^{13}C NMR (CD_3OD) δ 12.1, 13.6, 21.9, 24.2, 28.4, 28.6, 28.8, 28.9, 31.2, 33.1, 60.7, 62.9, 65.0, 66.5, 69.3, 60.6, 70.4, 70.8, 73.3, 76.2, 76.4, 101.1, 101.7, 173.9; HRMS (ESI) calcd for $C_{28}H_{52}NaO_{12}$ $(M+Na)^+$ 603.3351, found 603.3344.

4.19. Methyl-6-*O*-palmitate-α-D-mannopyranoside 7

Compound **7** was prepared from **21**[35] following procedures used in the conversion of **13**β to **3**. For **7**: R_f = 0.60 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.9 Hz), 1.26 (br s, 24H), 1.65 (m, 2H), 2.28 (br s, 1H), 2.41 (t, 2H, J = 7.4 Hz), 3.39 (s, 3H), 3.56 (t, 1H, J = 9.6 Hz), 3.69 (m, 1H), 3.83 (dd, 1H, J = 3.3, 9.3 Hz), 3.97 (s, 1H), 4.21 (dd, 1H, J = 2.0, 12.4 Hz), 4.66 (dd, 1H, J = 3.8, 12.4 Hz), 4.77 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.3, 22.9, 25.1, 29.3, 29.4, 29.5, 29.6, 29.8 (two signals), 29.9, 32.1, 34.4, 55.3, 63.3, 67.7, 70.4, 70.5, 71.4, 101.0, 175.5; HRMS (ESI) calcd for $(M+Na)^+$ $C_{23}H_{44}NaO_7$ 455.2979, found 455.2986.

4.20. Methyl-4,6-di-*O*-palmitate-α-D-mannopyranoside 8

Compound **8** was prepared from **21** following procedures used in the conversion of **13**β to **5**. For **8**: R_f = 0.6 (80% EtOAc/petroleum ether); ^{1}H NMR δ 0.90 (t, 6H, J = 6.8 Hz), 1.27 (br s, 48H), 1.64 (m, 4H), 2.36 (m, 4H), 2.72 (br s, 1H), 3.12 (br s, 1H), 3.41 (s, 3H), 3.90 (m, 2H), 3.96 (s, 1H), 4.19 (dd, 1H, J = 2.2, 12.1 Hz), 4.28 (dd, 1H, J = 5.5, 12.1 Hz), 4.81 (s, 1H), 5.02 (t, 1H, J = 9.8 Hz). ^{13}C NMR δ 14.3, 22.9, 25.0 (two signals), 29.2, 29.3, 29.4, 29.5 (two signals), 29.6, 29.7, 29.8 (three signals), 29.9, 32.1, 34.3, 34.5, 55.3, 62.7, 68.0, 70.1, 70.6, 70.7, 100.7, 173.8, 175.0; HRMS (ESI) calcd for $C_{39}H_{74}NaO_8$ $(M+Na)^+$ 693.5276, found 693.5278.

4.21. α-D-Galactopyranosyl-(1→1)-6-*O*-palmitate-α-D-mannopyranoside 9

Disaccharide **13**α was transformed to **9** (60 mg, 13.2% bsrm) following the procedures described for the conversion of **13**β to **3**. For **9**: R_f = 0.2 (20% MeOH/EtOAc); ^{1}H NMR (CD_3OD) δ 0.81 (t, 3H, J = 6.9 Hz), 1.21 (br s, 23H), 1.52 (m, 2H), 2.26 (t, 2H, J = 7.5 Hz), 3.21 (m, 1H), 3.61 (m, 4H), 3.74 (m, 4H), 3.82 (d, 1H, J = 2.5 Hz), 3.87 (m, 1H), 4.13 (dd, 1H, J = 5.9, 11.8 Hz), 4.29 (br d, 1H, J = 11.7 Hz), 4.99 (s, 1H), 5.03 (d, 1H, J = 3.7 Hz); ^{13}C NMR (CD_3OD) δ 14.5, 23.8, 26.1, 30.4, 30.6 (two signals), 30.7, 30.9 (two signals), 33.2, 35.1, 62.8, 64.8, 68.7, 69.7, 71.1, 71.3, 72.2, 72.5, 73.3, 95.5, 96.7, 175.7; HRMS (ESI) calcd for $C_{28}H_{56}NO_{12}$ $(M+NH_4)^+$, 589.3799 found 589.3797.

Acknowledgments

This investigation was supported by grants R01 GM57865 and SCORE S06 GM60654 from the National Institute of General Medical Sciences of the National Institutes of Health (NIH). 'Research Centers in Minority Institutions' award RR-03037 from the National Center for Research Resources of the NIH, which supports the infrastructure and instrumentation of the Chemistry Department at Hunter College, is also acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.06.078.

References and notes

1. Meadows, D. C.; Gervay-Hague, J. *ChemMedChem* **2006**, *1*, 16.
2. McReynolds, K. D.; Gervay-Hague, J. *Chem. Rev.* **2007**, *107*, 1533.
3. Rawat, S. S.; Johnson, B. T.; Puri, A. *Biosci. Rep.* **2005**, *25*, 329.
4. Rawat, S. S.; Viard, M.; Gallo, S. A.; Blumenthal, R.; Puri, A. *Glycoconjugate J.* **2006**, *23*, 189.
5. Fantini, J.; Hammache, D.; Pieroni, G.; Yahi, N. *Glycoconjugate J.* **2000**, *17*, 199.
6. Hammache, D.; Pieroni, G.; Yahi, N.; Delezay, O.; Koch, N.; Lafont, H.; Tamalet, C.; Fantini, J. *J. Biol. Chem.* **1998**, *273*, 7967.
7. Conboy, J. C.; McReynolds, K. D.; Gervay-Hague, J.; Saavedra, S. S. *J. Am. Chem. Soc.* **2002**, *124*, 968.
8. Villard, R.; Hammache, D.; Delapierre, G.; Fotiadu, F.; Buono, G.; Fantini, J. *Chembiochem* **2002**, *3*, 517.
9. Kensinger, R. D.; Yowler, B. C.; Benesi, A. J.; Schengrund, C.-L. *Bioconjugate Chem.* **2004**, *15*, 349.
10. Lund, N.; Branch, D. R.; Mylvaganam, M.; Chark, D.; Ma, X. Z.; Sakac, D.; Binnington, B.; Fantini, J.; Puri, A.; Blumenthal, R.; Lingwood, C. A. *AIDS* **2006**, *20*, 333.
11. Garg, H.; Francella, N.; Tony, K. A.; Augustin, L. A.; Fantini, J.; Barchi, J. J., Jr.; Puri, A.; Mootoo, D. R.; Blumenthal, R. *Antiviral Res.* **2008**, *80*, 54.
12. Dey, B.; Lerner, D. L.; Lusso, P.; Boyd, M. R.; Elder, J. H.; Berger, E. A. *J. Virol.* **2000**, *74*, 4562.
13. Yahi, N.; Sabatier, J. M.; Nickel, P.; Mabrouk, K.; Gonzalez-Scarano, F.; Fantini, J. *J. Biol. Chem.* **1994**, *269*, 24349.
14. Bhat, S.; Spitalnik, S. L.; Gonzalez-Scarano, F.; Silberberg, D. H. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7131.
15. Faroux-Corlay, B.; Clary, L.; Gadras, C.; Hammache, D.; Greiner, J.; Santaella, C.; Aubertin, A.-M.; Vierling, P.; Fantini, J. *Carbohydr. Res.* **2000**, *327*, 223.
16. McReynolds, K. D.; Bhat, A.; Conboy, J. C.; Saavedra, S. S.; Gervay-Hague, J. *Bioorg. Med. Chem.* **2002**, *10*, 625.
17. Székeley, Z. *J. Mol. Struct.* **1995**, *334*, 93.
18. Asensio, J. L.; Cañada, F. J.; Cheng, X.; Khan, N.; Mootoo, D. R.; Jiménez-Barbero, J. *Chem. Eur. J.* **2000**, *6*, 1035.
19. Nehete, P. N.; Vela, E. M.; Hossain, M. M.; Sarkar, A. K.; Yahi, N.; Fantini, J.; Sastry, K. J. *Antiviral Res.* **2002**, *56*, 233.
20. Garg, H.; Joshi, A.; Freed, E. O.; Blumenthal, R. *J. Biol. Chem.* **2007**, *282*, 16899.
21. Sands, J.; Auperin, D.; Snipes, W. *Antimicrob. Agents Chemother.* **1979**, *15*, 67.
22. Li, Q.; Estes, J. D.; Schlievert, P. M.; Duan, L.; Brosnahan, A. J.; Southern, P. J.; Reilly, C. S.; Peterson, M. L.; Schultz-Darken, N.; Brunner, K. G.; Nephew, K. R.; Pambuccian, S.; Lifson, J. D.; Carlis, J. V.; Haase, A. T. *Nature* **2009**, *458*, 1034.
23. Cory, A. H.; Owen, T. C.; Barltrop, J. A.; Cory, J. G. *Cancer Commun.* **1991**, *3*, 207.
24. Wild, C. T.; Shugars, D. C.; Greenwell, T. K.; McDanal, C. B.; Matthews, T. J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9770.
25. De Clercq, E.; Yamamoto, N.; Pauwels, R.; Balzarini, J.; Witvrouw, M.; De Vreese, K.; Debyser, Z.; Rosenwirth, B.; Peichl, P.; Datema, R.; Thornton, D.; Skerlj, R.; Gaul, F.; Padmanabhan, S.; Bridger, G.; Henson, G.; Abrams, M. *Antimicrob. Agents Chemother.* **1994**, *38*, 668.
26. Boros, C.; Katz, B.; Mitchell, S.; Pearce, C.; Swinbank, K.; Taylor, D. J. *Nat. Prod.* **2002**, *65*, 108.
27. Kitamoto, D.; Isoda, H.; Nakahara, T. *J. Biosci. Bioeng.* **2002**, *94*, 187.
28. Vlahov, I. R.; Vlahova, P. I.; Linhardt, R. J. *J. Carbohydr. Chem.* **1997**, *16*, 1.
29. Lang, S.; Philp, J. C.; Van Leeuw, A. *J. Microbiol.* **1998**, *74*, 59.
30. Gum, A. G.; Kappes-Roth, T.; Waldmann, H. *Chem. Eur. J.* **2000**, *6*, 3714.
31. Tiwari, P.; Misra, A. K. *Tetrahedron Lett.* **2006**, *47*, 3573.

32. Crich, D.; Sun, S. *Tetrahedron* **1998**, *54*, 8321.
33. Gómez, A. M.; Casillas, M.; Barrio, A.; Gawel, A.; López, J.-C. *Eur. J. Org. Chem.* **2008**, 3933.
34. Bock, K.; Pedersen, C. *J. Chem. Soc., Perkins. Trans. II* **1974**, 293.
35. Liptak, A.; Imre, J.; Nanasi, P. *Carbohydr. Res.* **1981**, *92*, 154.
36. Haines, A. B. *Adv. Carbohydr. Chem. Biochem.* **1976**, *33*, 11.
37. Shah, V.; Doncel, G. F.; Seyoum, T.; Eaton, K. M.; Zalenskaya, I.; Hagyer, R.; Azin, A.; Gross, R. *Antimicrob. Agents Chemother.* **2005**, *49*, 4093.
38. Azim, A.; Shah, V.; Doncel, G. F.; Peterson, N.; Gao, W.; Gross, R. *Bioconjugate Chem.* **2006**, *17*, 1523.
39. Katz, D. H.; Marcelletti, J. F.; Pope, L. E.; Mohammed, H. K.; Katz, L. R.; McFadden, R. *Ann. N.Y. Acad. Sci.* **1994**, *724*, 472.
40. Wong, Y.-L.; Hubieki, M. P.; Curfman, C. L.; Doncel, G. F.; Dudding, T. C.; Savle, P. S.; Gandour, R. D. *Bioorg. Med. Chem.* **2002**, *10*, 3599.
41. Macri, R. V.; Karlovská, J.; Doncel, G. F.; Du, X.; Maisuria, B. B.; Williams, A. A.; Sugandhi, E. W.; Falkinham, J. O.; Esker, A. R.; Gandour, R. *Bioorg. Med. Chem.* **2009**, *17*, 3162.
42. Franzetti, A.; Gandolfi, I.; Bestetti, G.; Smyth, T. J. P.; Banat, I. M. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 617.
43. Rodrigues, L.; Banat, I. M.; Teixeira, J.; Oliveira, R. *J. Antimicrob. Chemother.* **2006**, *57*, 609.
44. Kitamoto, D.; Morita, T.; Fukuoka, T.; Konoshi, M.; Imura, T. *Curr. Opin. Colloid Interface Sci.* **2009**, *14*, 315.
45. Garmy, N.; Taieb, N.; Yahi, N.; Fantini, J. *J. Lipid Res.* **2005**, *46*, 36.