Synthesis and anti-HIV activity of catanionic analogs of galactosylceramide

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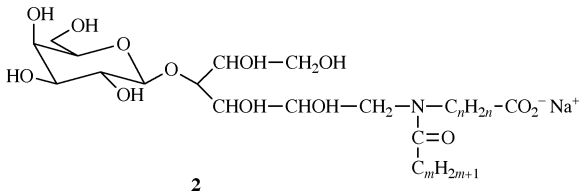
Two-chain and gemini catanionic analogs of galactosylceramide were easily prepared in quantitative yields, affording high anti-HIV activities with low cytotoxicities on human cells (*e.g.* for gemini 4b an EC_{50} of 0.5 μM with a CC_{50} of >100 μM were obtained).

Glycolipids play a vital role in molecular recognition mechanisms, as they are receptors for various pathogenic agents such as bacteria and viruses.[1,2] Recently, galactosylceramide has been identified as a new receptor of HIV,[3] because of its affinity with the viral glycoprotein gp 120. To avoid binding between the virus and the cells, the strategy was to synthesize galactosylceramide analogs as chimera for the virus.

Various analogs have already been synthesized, such as compound **2**, which possesses good antiviral activity.[4] However, this product requires multi-step synthesis followed by purification to obtain the desired compound with 50% yield.[5] To overcome this drawback, we synthesized catanionic analogs of galactosylceramide.

Catanionic compounds are easily obtained, according to the literature,[6–8] by mixing two oppositely charged surfactants or using an acido-basic reaction. To synthesize two-chain **3** or gemini **4** catanionic analogs of galactosylceramide, it was desirable to avoid the formation of residual salts by using an acido-basic reaction. In fact, this is a non-negligible advantage for products that are to be biologically tested.

As shown in Scheme 1, the amino sugar **1**[5,9] was mixed with 1 equiv. of carboxylic monoacid or 0.5 equiv. of dicarboxylic acid, yielding quantitatively two-chain **3** and gemini **4** catanionic analogs,[9] respectively.



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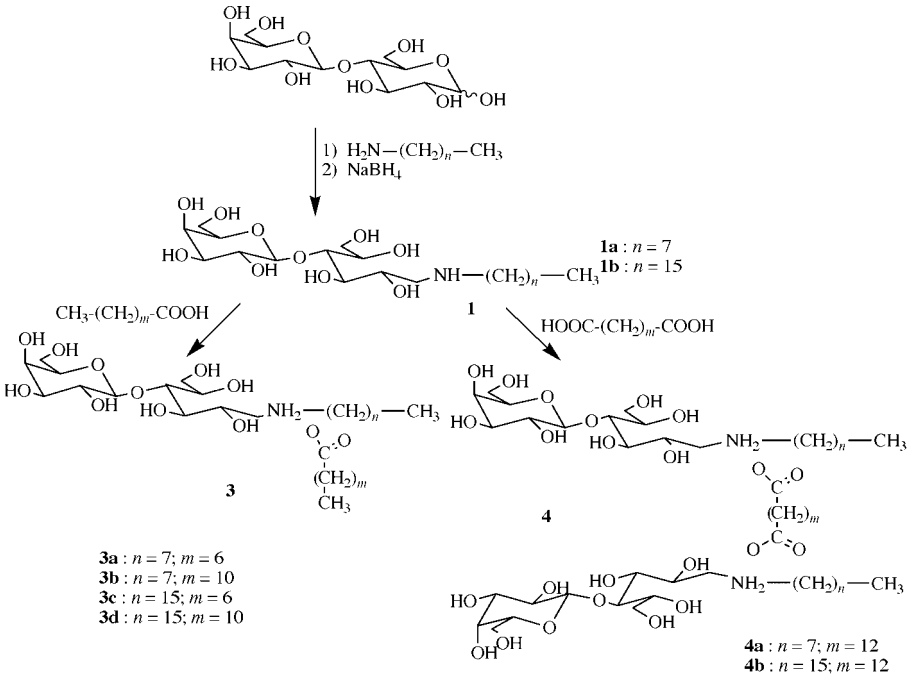
These new analogs of galactosylceramide were then tested on human cells to check their cytotoxicity[10] and anti-HIV activities.[11] The results were compared with compound **2**, which is the most active analog of galactosylceramide.[4] As shown in Table 1, products **3a** and **4a** are non-toxic and do not present an anti-HIV activity, while compound **3b** demonstrates a better anti-HIV activity (EC_{50} = 100 μM) but with a higher toxicity. Therefore, these three products do not seem of any biological interest. However, two-chain **3c** (EC_{50} = 16 μM) and **3d** (EC_{50} = 0.9 μM) are much more active than the reference compound **2** (EC_{50} = 50 μM), but they display a high toxicity that makes them unsuitable. One compound, gemini **4b**, was discovered that possessed both a low toxicity



Scheme 1 Synthesis of new catanionic analogs of galactosylceramide.

Table 1 Anti-HIV activity of new catanionic analogs **3** and **4** of galactosylceramide compared to **1b** and **2**

Compound	EC_{50}/μM	CC_{50}/μM	SI	Log *P*
1b	50	70	1.4	2.6
3a	>1000	>1000	—	1.7
3b	100	>100	>1	3.3
3c	16	38	2.3	4.9
3d	0.9	2.5	2.7	6.5
4a	500	600	1.1	2.1
4b	0.5	>100	>200	8.4
2[a]	50	220	4.5	4.5

[a] $n = 10$ and $m = 15$.

($CC_{50} > 100$ μM) and a greater anti-HIV activity ($EC_{50} = 0.5$ μM) than reference compound **2**, with a selectivity index[12] 50 times higher (SI > 200) than that for compound **2** (SI = 4.5). Furthermore, the anti-HIV activity and the toxicity of compound **1b** ($EC_{50} = 50$ μM; $CC_{50} = 70$ μM), equivalent to illustrate that the sugar part of gemini **4b**, is less active and more toxic than compound **4b** itself. These results provide evidence for the ionic and hydrophobic associations of gemini **4b**, giving rise to a new and active anti-HIV compound.

Combining all the results previously obtained, we can note that greater hydrophobicity,[13] represented by a higher log *P* term, is necessary to allow an attractive anti-HIV activity.[14] In fact, only compounds with a log *P* value superior to 4.5 possess this activity, represented by an EC_{50} value lower than 50 μM. Other information can be extracted from Table 1, concerning the multiplicity of sugar moieties. Indeed, compounds with two sugar heads (**4**) seem to be more active overall than compounds with only one sugar moiety (**3**). These results confirm that gemini **4b**, which possesses both a high log *P* and two sugar heads, is the most active catanionic analog of galactosylceramide.

In conclusion, catanionic analogs of galactosylceramide were easily accessible in quantitative yields; gemini **4b** is especially interesting because of its high anti-HIV activity and low cytotoxicity. These results may open new horizons in anti-HIV chemotherapy.

Experimental

Preparation of two-chain catanionic glycolipids 3

To 0.66 mmol of **1** in 50 mL of distilled water was added 0.66 mmol of monocarboxylic acid. After 2 days of stirring at room temperature, we obtained a homogeneous solution that gave product **3** quantitatively after water removal.

Mixture of *N*-octylaminolactitol and octanoic acid 3a. ^{1}H NMR (200 MHz, D_2O): δ 4.39 (d, 1 H, anomeric H), 4.2–3.4 (m, 12 H, sugar part), 2.99 (m, 4 H, CH_2–NH_2–CH_2), 2.14 (t, 2 H, CH_2–COO), 1.64 (q, 2 H, NH_2–CH_2–CH_2), 1.48 (q, 2 H, CH_2–CH_2–COO), 1.23 (m, 18 H, CH_2), 0.80 (t, 6 H, CH_3). ^{13}C NMR (50 MHz, D_2O): δ 105.6 (anomeric C), 81.9–70.5 (CH sugar), 65.3 (CH_2–OH), 63.8 (CH_2–NH_2–CH_2), 53.7–24.7 (CH_2), 16.1 (CH_3).

Mixture of *N*-octylaminolactitol and lauric acid 3b. ^{1}H NMR (200 MHz, D_2O): δ 4.39 (d, 1 H, anomeric H), 4.2–3.4 (m, 12 H, sugar part), 2.99 (m, 4 H, CH_2–NH_2–CH_2), 2.14 (t, 2 H, CH_2–COO), 1.64 (q, 2 H, NH_2–CH_2–CH_2), 1.48 (q, 2 H, CH_2–CH_2–COO), 1.23 (m, 26 H, CH_2), 0.80 (t, 6 H, CH_3). ^{13}C NMR (50 MHz, D_2O): δ 105.6 (anomeric C), 81.9–70.5 (CH sugar), 65.3 (CH_2–OH), 63.8 (CH_2–NH_2–CH_2), 53.7–24.7 (CH_2), 16.1 (CH_3).

Mixture of *N*-hexadecylaminolactitol and octanoic acid 3c. ^{1}H NMR (200 MHz, D_2O): δ 4.39 (d, 1 H, anomeric H), 4.2–3.4 (m, 12 H, sugar part), 2.99 (m, 4 H, CH_2–NH_2–CH_2), 2.14 (t, 2 H, CH_2–COO), 1.64 (q, 2 H, NH_2–CH_2–CH_2), 1.48 (q, 2 H, CH_2–CH_2–COO), 1.23 (m, 34 H, CH_2), 0.80 (t, 6 H, CH_3). ^{13}C NMR (50 MHz, D_2O): δ 105.6 (anomeric C), 81.9–70.5 (CH sugar), 65.3 (CH_2–OH), 63.8 (CH_2–NH_2–CH_2), 53.7–24.7 (CH_2), 16.1 (CH_3).

Mixture of *N*-hexadecylaminolactitol and lauric acid 3d. ^{1}H NMR (200 MHz, D_2O): δ 4.39 (d, 1 H, anomeric H), 4.2–3.4 (m, 12 H, sugar part), 2.99 (m, 4 H, CH_2–NH_2–CH_2), 2.14 (t, 2 H, CH_2–COO), 1.64 (q, 2 H, NH_2–CH_2–CH_2), 1.48 (q, 2 H, CH_2–CH_2–COO), 1.23 (m, 42 H, CH_2), 0.80 (t, 6 H, CH_3). ^{13}C NMR (50 MHz, D_2O): δ 105.6 (anomeric C), 81.9–70.5 (CH sugar), 65.3 (CH_2–OH), 63.8 (CH_2–NH_2–CH_2), 53.7–24.7 (CH_2), 16.1 (CH_3).

Preparation of gemini catanionic glycolipids 4

To 0.78 mmol of **1** in 50 mL of distilled water was added 0.39 mmol of dodecyldicarboxylic acid. After 2 days of stirring at room temperature, we obtained a homogeneous solution that gave quantitatively product **4** after water removal.

Mixture of *N*-octylaminolactitol and dodecyldicarboxylic acid 4a. ^{1}H NMR (400 MHz, D_2O): δ 4.41 (d, 2 H, anomeric H), 4.2–3.4 (m, 24 H, sugar part), 3.05 (m, 8 H, CH_2–NH_2–CH_2), 2.12 (t, 4 H, CH_2–COO), 1.56 (q, 4 H, NH_2–CH_2–CH_2), 1.49 (q, 4 H, CH_2–CH_2–COO), 1.24 (m, 36 H, CH_2), 0.83 (t, 6 H, CH_3). ^{13}C NMR (100 MHz, D_2O): δ 105.6 (anomeric C), 81.8–70.3 (CH sugar), 65.4 (CH_2–OH), 63.6 (CH_2–NH_2–CH_2), 53.6–24.6 (CH_2), 16.0 (CH_3).

Mixture of *N*-hexadecylaminolactitol and dodecyldicarboxylic acid 4b. ^{1}H NMR (400 MHz, D_2O): δ 4.41 (d, 2 H, anomeric H), 4.2–3.4 (m, 24 H, sugar part), 3.05 (m, 8 H, CH_2–NH_2–CH_2), 2.12 (t, 4 H, CH_2–COO), 1.56 (q, 4 H, NH_2–CH_2–CH_2), 1.49 (q, 4 H, CH_2–CH_2–COO), 1.24 (m, 68 H, CH_2), 0.83 (t, 6 H, CH_3). ^{13}C NMR (100 MHz, D_2O): δ 105.6 (anomeric C), 81.8–70.3 (CH sugar), 65.4 (CH_2–OH), 63.6 (CH_2–NH_2–CH_2), 53.6–24.6 (CH_2), 16.0 (CH_3).

Anti-viral assay

The cell cultures were maintained at 37 °C in 5% CO_2. The effects of the synthesized compounds on the replication of HIV-1 in CEM-SS cells were studied. The CEM-SS were obtained from Dr. Peter Nara through the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH). Cells were infected with the HIV-1 LAI isolate at 10 times the tissue culture infective dose 50, 10($TCID_{50}$), a virus dose that decreases the number of viable cells by 50% within 5 days, which was determined by evaluating the reverse transcriptase (RT) activity released from infected cells after 5 days at 37 °C.

The assay procedure for measuring the anti-HIV-1 activity of compounds in CEM-SS cells was based on a quantitative detection of reverse transcriptase activity in the culture supernatant. After adsorption for 30 min at 37 °C, the CEM-SS infected cells were washed twice to remove unadsorbed virus particles and cultured at a final concentration of 10^5 cells mL^{-1} in the presence of various dilutions of the test compounds. On day 5 following virus infection at 37 °C the RT activity in the supernatant was measured as below.

The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity: the toxic effect on non-infected CEM cells was assessed by a colorimetric reaction based on the ability of living cells to reduce MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl]tetrazolium bromide into formazan after 4 days incubation in the presence of different concentrations of the substances under test. The results are expressed as

the lowest concentration leading to 50% inhibition of the formation of formazan.

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References and notes

1 K. A. Karlsson, *Annu. Rev. Biochem*, 1989, **58**, 309.
2 M. Mammen, S. K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754.
3 N. Yahi, S. Baghdiguian, M. Moreau and J. Fantini, *J. Virol.*, 1992, **66**, 4848.
4 J. Fantini, D. Hammache, O. Delézay, N. Yahi, C. André–Barrès, I. Rico–Lattes and A. Lattes, *J. Biol. Chem.*, 1997, **272**, 7245.
5 I. Rico–Lattes, J. C. Garrigues, E. Perez, C. André–Barrès, C. Madelaine–Dupuich and A. Lattes, *New J. Chem.*, 1995, **19**, 341.
6 H. Fukuda, K. Kawata and H. Okuda, *J. Am. Chem. Soc.*, 1990, **112**, 1635.
7 (*a*) R. Zana and H. Lévy, *Langmuir*, 1997, **13**, 402. (*b*) S. Bhattacharya and S. De, *J. Chem. Soc., Chem. Commun.*, 1995, 651.
8 (*a*) S. Bhattacharya and S. De, *Langmuir*, 1999, **15**, 3400. (*b*) S. Bhattacharya, S. De and M. Subramanian, *J. Org. Chem.*, 1998, **63**, 7640.
9 M. Blanzat, E. Perez, I. Rico–Lattes, D. Promé, J. C. Promé and A. Lattes, A. *Langmuir*, 1999, **15**, 6163.
10 The cytotoxicity is represented by CC_{50}, which is the concentration leading to the death of 50% of the healthy cells. The higher the CC_{50}, the lower the cytotoxicity of the compound.
11 The anti-HIV activity is represented by EC_{50}, which is the concentration of compound that saves 50% of the infected cells. The lower the EC_{50}, the higher the product efficiency.
12 The selectivity index ($SI = CC_{50}/EC_{50}$) should be as high as possible.
13 The lipophilicity is represented by log *P* (partition coefficient of a compound between octanol and water), which was calculated using TSAR software (TSAR version 2.02, Oxford Molecular). The higher the log *P*, the higher the product solubility in octanol and the higher the product lipophilicity. For example, the value of the calculated log *P* of phospholipids such as dipalmitoylphosphatidylcholine is 10.43.
14 (*a*) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, 1964, **86**, 1616. (*b*) C. Hansch and E. J. Lien, *J. Med. Chem.*, 1971, **14**, 653.

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