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Synthesis of Single- and Double-chain Fluorocarbon and Hydrocarbon β-linked Galactose Amphiphiles derived from Serine

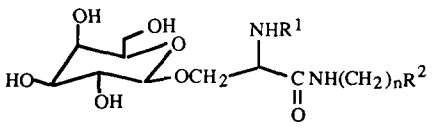
Laurence Clary, Jacques Greiner, Catherine Santaella and Pierre Vierling*

Laboratoire de Chimie Moléculaire, associé au CNRS, Université de Nice Sophia-Antipolis,
Faculté des Sciences, 06108 Nice, Cédex 2, France.

Abstract: Single- and double-chain β-linked galactose amphiphiles derived from serine were synthesized. Both types of compounds have potential as material for the formulation of liposomal drug carrier and targeting systems and as HIV inhibitors.

The achievement of a drug carrier and delivery system, such as liposomes, for specific targeting to cells and organs has become a major objective in biomedical research.[1,2] Specific cell targeting requires liposomes exhibiting extended in vivo blood circulation times and bearing, at their surface, ligands which are specifically recognized by receptors present on the cell.[1,2] Recently, long-circulating liposomes have been obtained by the use of highly fluorinated phospholipids.[3] Moreover hepatocytes and macrophages possess membrane lectins which are specific galactose receptors.[2,4] In order to enlarge the potential of the fluorinated liposomes as drug targeting devices to these cells, we designed and synthesized new galactosyl-labelled fluorinated amphiphiles based on serine.[5] Our interest in such serine-galactosyl compounds stemmed also from the ability of structurally closely related galactosphingolipid analogs to inhibit HIV uptake and infection of CD4-negative cells, as recently reported.[6]

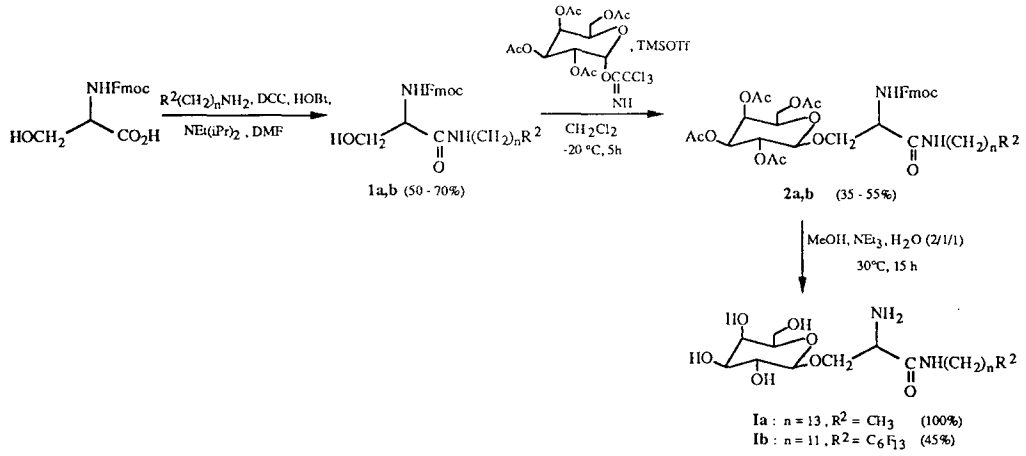
We report here the synthesis of various substituted fluorocarbon/hydrocarbon single- and double-chain galactose amphiphiles (Scheme 1). Their hydrophobic chains are connected via amide bonds to serine which is β-linked, via its remaining hydroxyl group, to the galactose polar head.



Ia : $R^1 = H$, $n = 13$, $R^2 = CH_3$
Ib : $R^1 = H$, $n = 11$, $R^2 = C_6F_{13}$
IIa : $R^1 = C_4F_9(CH_2)_{10}C(O)$- $n = 13$, $R^2 = CH_3$
IIb : $R^1 = C_6F_{13}(CH_2)_{10}C(O)$- $n = 15$, $R^2 = CH_3$
IIc : $R^1 = C_6F_{13}(CH_2)_{10}C(O)$- $n = 11$, $R^2 = C_4F_9$

Scheme 1 : Molecular structure of the single- and double-chain serine-galactosyl amphiphiles **Ia,b** and **IIa-c.**

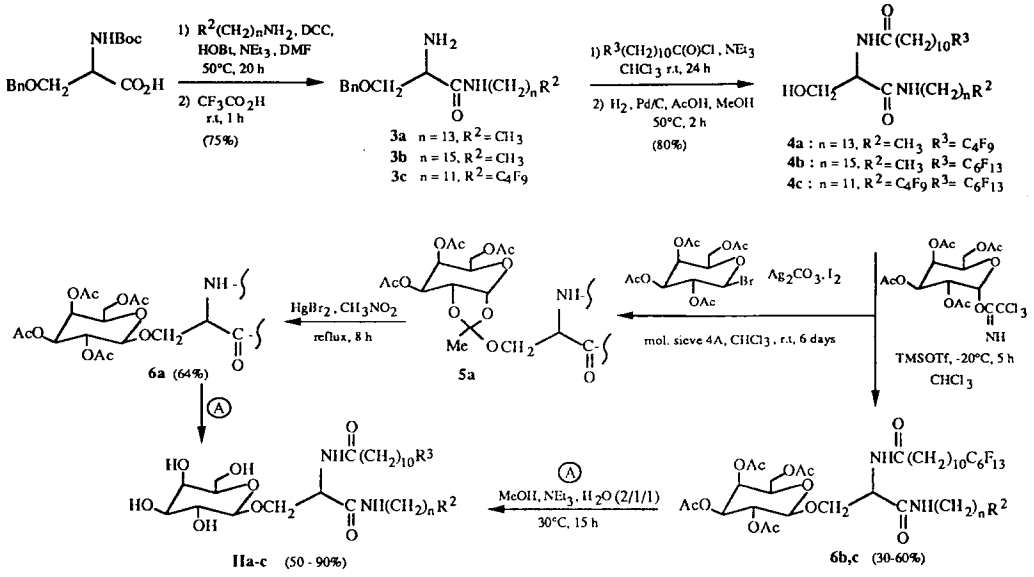
The synthesis of the single-chain amphiphiles **Ia-b** was performed in three steps starting from Fmoc-DL-serine (Scheme 2). The condensation of Fmoc-DL-serine with tetradecyl- or 11-(*F*-hexyl)-undecyl-amine[7] in the presence of DCC, HOBt and $NEt(iPr)_2$ (in order to avoid Fmoc-deprotection)[8] afforded **1a** (50% yield) and its fluorinated analog **1b** (70% yield), respectively. Glycosylation of these latter compounds with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate catalyzed by trimethylsilyltrifluoromethanesulfonate, TMSOTf (Schmidt method)[9], gave the β-galactosides **2a** and **2b** in 35 and 55% yields, respectively. After Fmoc and acetyl deprotection in a $MeOH/NEt_3/H_2O$ (2/1/1) mixture[10], the galactosides **Ia** and **Ib**[11] were respectively obtained in 100 and 45% yields.



Scheme 2 : Synthetic route to the hydrocarbon and fluorocarbon single-chain serine-galactosyl amphiphiles **Ia** and **Ib.**

The synthetic route to the mixed hydrocarbon/fluorocarbon and fluorocarbon/fluorocarbon double-chain amphiphiles **IIa,b** and **IIc** starting from Boc-*O*-benzyl-L(or DL)-serine is presented in Scheme 3. Condensation of Boc-*O*-benzyl-L(or DL)-serine with tetradecyl-, hexadecyl- or 11-(*F*-butyl)undecyl-amine[7] in the presence of DCC and HOBt, then Boc-deprotection and acylation of **3a-c** with the appropriate perfluoroalkylated acid chloride[7] and further hydrogenolysis of the benzyl group[12] afforded the diamidoalcohols **4a-c** in almost 60 % overall yields. Glycosylation of **4a** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in the usual conditions of the Koenigs-Knorr reaction[13] gave mainly the 1,2 orthoester **5a** together with the expected galactoside **6a.** **5a** was almost quantitatively converted into **6a** by refluxing **5a** in nitromethane with a catalytic amount of $HgBr_2$[14] (64% overall yield for the glycosylation). In view of the issue of the Koenigs-Knorr reaction, the glycosylation of **4b,c** was performed using the Schmidt reaction[9] which gave **6b,c** in 30 to 60% yields. Deacetylation of the galactosides **6a-c** in a $MeOH/NEt_3/H_2O$ (2/1/1) mixture[10] afforded **IIa-c**[11] in 50 to 90% yield.

Further investigations are underway to explore the potential of these new glycolipids (i) in the formulation of liposomes for drug targeting and (ii) in HIV uptake inhibition.



Scheme 3 : Synthetic route to the mixed fluorocarbon/hydrocarbon and fluorocarbon/fluorocarbon double-chain serine-galactosyl amphiphiles IIa,b and IIc.

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11. The following NMR data for **Ia,b** and **IIa-c** are consistent with their structures.

Ia and Ib consist in a mixture of diastereoisomers. **Ia** (in CD_3OD) : ^1H NMR (200 MHz) : δ 1.0 (t, J = 7 Hz, 3H, CH_3), 1.3-1.8 (m, 24H, $(CH_2)_{12}$), 3.2 (t, J = 7 Hz, 2H, CH_2N), 2.9-4.2 (m, 9H, GalH2 to GalH6 and OCH_2CH), 4.3 (d, J = 7 Hz, 1H, GalH1). ^{13}C NMR (50.3 MHz) : δ 14.3 (s, CH_3), 23.6 (s, $\underline{C}H_2CH_3$), 27.9 (s, $\underline{C}H_2CH_2N$), 30.0 to 30.6 $(CH_2)_8$, 32.9 (s, $\underline{C}H_2(CH_2)_2N$), 40.4 (s, $\underline{C}H_2N$), 55.5 and 55.8 (s, CHN), 62.4 (s, GalC6), 70.1 (s, GalC4), 71.7 and 71.9 (s, $\underline{C}H_2OGal$), 72.2 and 72.3 (s, GalC2), 74.6 and 74.7 (s, GalC3), 76.8 (s, GalC5), 104.9 and 105.3 (s, GalC1β), 172.3 and 172.6 (s, CO).

Ib (in $CDCl_3/CD_3OD$) : ^1H NMR (200 MHz) : δ 1.2-1.8 (m, 18H, $(CH_2)_9$), 2.1 (tt, J = 18 and 8 Hz, 2H, CH_2CF_2), 3.25 (t, J = 7 Hz, 2H, CH_2N), 3.4-4.2 (m, 9H, GalH2 to Gal H6 and OCH_2CH), 4.30 and 4.31 (d, J = 7 Hz, 1H, GalH1). ^{19}F NMR (188.3 MHz) : δ -80.5 (3F, CF_3), -113.8 (2F, CF_2CH_2), -121.2, -122.2, -122.8 (2F, 2F, 2F $(\underline{C}F_2)_3CF_2CH_2$), -125.5 (2F, $CF_3\underline{C}F_2$) . ^{13}C NMR (50.3 MHz) : δ 19.3 (t, J = 3.5 Hz, $\underline{C}H_2CH_2CF_2$), 26.1 (s, $\underline{C}H_2CH_2N$), 28.2 to 28.7 $(CH_2)_7$, 30.0 (t, J = 22.3 Hz, $\underline{C}H_2CF_2$), 38.7 (s, $\underline{C}H_2N$), 53.9 and 54.3 (s, CHN), 60.6 (s, GalC6), 68.3 (s, GalC4), 70.4 and 70.5 (s, GalC2), 70.7 and 71.1 (s, $\underline{C}H_2OGal$), 72.8 (s, GalC3), 74.7 (s, GalC5), 102.9 and 103.3 (s, GalC1β), 172.2 and 172.3 (s, CO).

IIa was obtained starting from Boc-*O*-benzyl-DL-serine. It consists in a mixture of diastereoisomers. ^1H NMR (200 MHz, $CDCl_3/CD_3OD$) : δ 0.8 (t, J = 7 Hz, 3H, CH_3), 1.1-1.7 (m, 40H, $(CH_2)_{12}$ and $(CH_2)_8$), 2.1 (tt, J = 18 and 8 Hz, 2H, CH_2CF_2), 2.2 (t, J = 7 Hz, 2H, CH_2CO), 3.2 (t, J = 7 Hz, 2H, CH_2N), 3.4-4.5 (m, 9H, GalH1 to GalH6 and OCH_2), 4.55 (t, J = 5 Hz, 1H, CHN). ^{19}F NMR (188.3 MHz) : δ -80.9 (3F, CF_3), -114.1 (2F, CF_2CH_2), -124.0 (2F, $\underline{C}F_2CF_2CH_2$), -125.6 (2F, CF_2CF_3). ^{13}C NMR (50.3 MHz) : δ 13.9 (s, CH_3), 19.9 (t, J = 3.5 Hz, $\underline{C}H_2CH_2CF_2$), 22.5 (s, $\underline{C}H_2CH_3$), 25.4 (s, $\underline{C}H_2CH_2CO$), 26.8 (s, $\underline{C}H_2CH_2N$), 28.9 to 30.1 $(CH_2)_6$ and $(CH_2)_9$, 30.6 (t, J = 22.3 Hz, $\underline{C}H_2CF_2$), 31.8 (s, $\underline{C}H_2(CH_2)_2N$), 36.0 and 36.1 (s, $\underline{C}H_2CO$), 39.6 (s, $\underline{C}H_2N$), 52.5 and 52.8 (s, CHN), 61.4 and 61.7 (s, GalC6), 68.8 and 69.3 (s, $\underline{C}H_2OGal$), 69.0 (s, GalC4), 71.0 (s, GalC2), 73.3 (s, GalC3), 74.7 and 75.0 (s, GalC5), 103.3 and 103.8 (s, GalC1β), 170.0 and 170.1 (s, $CH\underline{C}O$), 174.2 and174.4 (s,$CH_2\underline{C}O$).

IIb and **IIc** were obtained starting from Boc-*O*-benzyl-L-serine. **IIb** (in $CDCl_3/CD_3OD$) : ^1H NMR (200 MHz) : δ 0.85 (t, 3H, CH_3), 1.1-1.7 (m, 44H, $(CH_2)_{14}$ and $(CH_2)_8$), 2.0 (tt, J = 18 and 8 Hz, 2H, CH_2CF_2), 2.2 (t, J = 8 Hz, 2H, CH_2CO), 3.2 (t, J = 7 Hz, 2H, CH_2N), 3.4-4.0 (m, 7H, GalH2, GalH6, two of GalH3-5, OCH_2), 4.05 (dd, J = 10 and 5 Hz, 1H, GalH3 or 4 or 5), 4.25 (d, J = 6.5 Hz, 1H, GalH1), 4.6 (t, J = 6 Hz, 1H, CHN). ^{19}F NMR (188.3 MHz) : identical to that of **Ib**. ^{13}C NMR (50.3 MHz) : δ 13.9 (s, CH_3), 20.0 (t, J = 3.5 Hz, $\underline{C}H_2CH_2CF_2$), 22.6 (s, $\underline{C}H_2CH_3$), 25.5 (s, $\underline{C}H_2CH_2CO$), 26.8 (s, $\underline{C}H_2CH_2N$), 29.0 to 29.6 $(CH_2)_6$ and $(CH_2)_{11}$, 30.8 (t, J = 22.3 Hz, $\underline{C}H_2CF_2$), 31.8 (s, $\underline{C}H_2(CH_2)_2N$), 36.2 (s, $\underline{C}H_2CO$), 39.7 (s, $\underline{C}H_2N$), 52.8 (s, $\underline{C}HN$), 62.0 (s, GalC6), 69.3 (s, GalC4), 69.6 (s, $\underline{C}H_2OGal$), 71.1 (s, GalC2), 73.3 (s, GalC3), 74.8 (s, GalC5), 103.8 (s, GalC1β), 170.0 (s, $CH\underline{C}O$), 174.2 (s, $CH_2\underline{C}O$).

IIc (in $CDCl_3/CD_3OD$) : ^1H NMR (200 MHz) : δ 1.1-1.7 (m, 34H, CH_2, $(CH_2)_9$ and $(CH_2)_8$), 2.0 (tt, J = 18 and 8 Hz, 4H, CH_2CF_2), then from 2.2 to 4.6 ppm identical to **IIb**. ^{19}F NMR (188.3 MHz) : δ -81.4, -81.6 (3F, 3F, CF_3), -115.1 (4F, CF_2CH_2), -122.5, -123.4, -124.1, -125.2 (2F, 2F, 2F, 2F, $(\underline{C}F_2)_3CF_2CH_2$ and $\underline{C}F_2CF_2CH_2$), -126.6 (4F, $CF_3\underline{C}F_2$). ^{13}C NMR (50.3 MHz) : δ 20.0 (t, J = 3.5 Hz, $\underline{C}H_2CH_2CF_2$), 25.5 (s, $\underline{C}H_2CH_2CO$), 26.8 (s, $\underline{C}H_2CH_2N$), 29.0 to 29.6 $(CH_2)_5$ and $(CH_2)_7$, 30.7 and 30.8 (t, J = 22.3 Hz, $\underline{C}H_2CF_2$), then from 36.2 to 174.2 ppm identical to **IIb**.

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