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Bioorganic & Medicinal Chemistry Letters 16 (2006) 5827–5831
Bioorganic & Medicinal Chemistry Letters

Fluorinated and hemifluorinated surfactants derived from maltose: Synthesis and application to handling membrane proteins in aqueous solution

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Received 13 July 2006; revised 13 August 2006; accepted 14 August 2006
Available online 8 September 2006

Abstract—Two novel fluorinated surfactants have been obtained by grafting by radical reaction either a fluorocarbon or an ethyl end-capped fluorocarbon chain onto the double bond of β-D-allyl maltose. The two compounds thus obtained form polydisperse aggregates in water. They can keep membrane proteins water-soluble, but the protein/surfactant complexes are polydisperse, which affects neither the native state nor the stability of the proteins.

Obtaining aqueous solutions of membrane proteins (MPs) so as to study their function and structure is of major scientific and biomedical importance. MPs represent 20–40% of open-reading frames in fully sequenced genomes.[1] They fulfil a wide variety of functions ranging from signal reception and transduction to solute translocation and are the target of more than half of pharmaceutical drugs. However, studying them is difficult, as reflected in the fact that <1% of current PDB entries describe membrane protein structures. Several bottlenecks explain this situation, among which the inactivating effect of the detergents that are used to extract MPs from their native membrane environment for the purpose of in vitro biochemical, functional and structural characterisation.[2] Detergents substitute for lipids around the hydrophobic domain of membrane proteins, forming water-soluble complexes. However, the dissociating effect of detergents, which allows them to solubilise biological membranes, can be difficult to control, resulting in the destabilisation and irreversible inactivation of solubilised MPs.[2] Two likely mechanisms leading to inactivation are the intrusion of the detergent into the transmembrane region of the protein and the dissociation of stabilising lipids, cofactors or subunits.[2,3]

To alleviate this problem, less 'aggressive' surfactants are being developed.[4,5] Non-ionic fluorinated surfactants[6,7] are lipophobic and, as such, do not solubilise biological membranes.[7,8] They are, however, able to keep water-soluble MPs that have been extracted using classical hydrogenated surfactants.[7] In order to improve their affinity for the hydrophobic, hydrocarbon-like transmembrane surface of MPs, we have introduced the so-called 'hemifluorinated' surfactants, which bear an ethyl hydrogenated tip at the extremity of the fluorinated chain (Fig. 1).[9–12]

The first hemifluorinated surfactant to be synthesised,[9] HF-TAC (Fig. 1), proved able to keep soluble a set of test MPs in their native and functional state, while stabilising them as compared to classical detergents used at comparable concentrations.[11] The large and highly soluble polar head of HF-TAC was chosen in order to counterbalance its highly insoluble hemifluorinated tail.

Keywords: Fluorinated surfactants; Maltoside; Membrane proteins; Solubilization; Aggregates.
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doi:10.1016/j.bmcl.2006.08.070

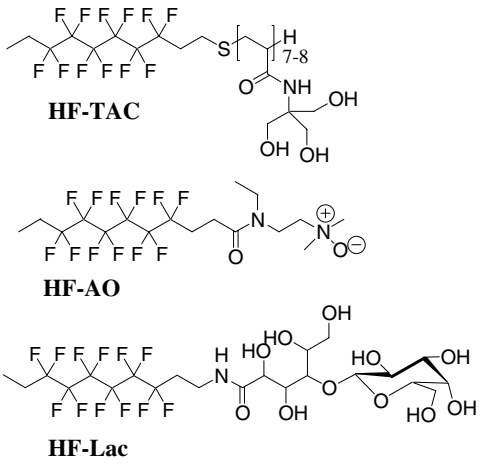
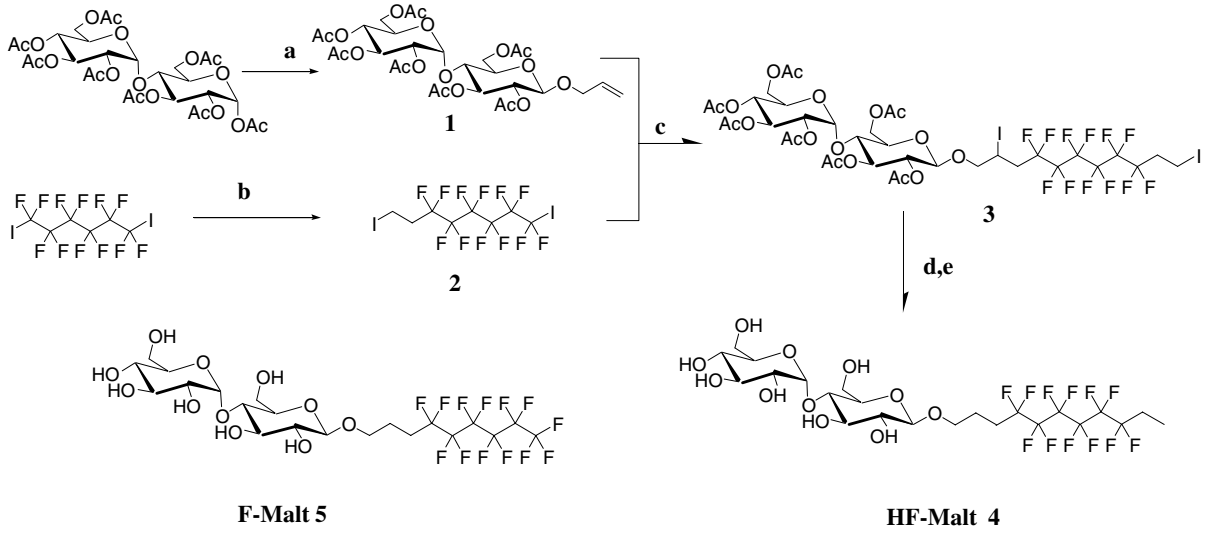


Figure 1. First-generation hemifluorinated non-ionic surfactants.[9–12]

While convenient to test the concept of hemifluorinated surfactants, HF-TAC suffers from a serious drawback from the point of view of biochemical and structural applications: because the synthesis of its polar head involves radical polymerisation, it is inevitably polydisperse, which can lead to batch-to-batch variations. To try to circumvent this problem, we have undertaken to prepare hemifluorocarbon surfactants bearing monodisperse polar heads (Fig. 1). The zwitterionic aminoxyde moiety of HF-AO,[10] however, was found to decrease MP stability. Since several very useful detergents in MP biochemistry, such as β-D-octylglucoside or β-D-dodecylmaltoside,[2] bear a glycosidic head, we focused on the synthesis of glycoside-derived fluorinated surfactants. In a first step, we synthesised surfactants with a lactobionamide head group (HF-Lac, Fig. 1).[12] HF-Lac was found to be efficient in keeping several test MPs water-soluble, active and monodisperse.[12] The lactobionamide polar head, however, proved destabilising as compared to the maltoside polar head of DDM, as evidenced by the inactivating character of the hydrogenated homologue of HF-Lac.[12] The work presented herein reports on the synthesis, physical–chemical and biochemical characterisation of fluorinated (F-Malt) and hemifluorinated (HF-Malt) homologues of the widely used detergent β-D-dodecylmaltoside (DDM).

The most efficient method to introduce an alkyl chain in the β anomeric position of maltose consists in (i) increasing the reactivity of the anomeric carbon 1 of a peracetylated maltose by grafting it with trichloroacetimidate, and (ii) substituting this group with an alcohol.[13] This reaction leads exclusively to the β-alkoxymaltoside isomer, generally in good yield. However, fluorocarbon alcohols exhibit a lower nucleophilic power than their hydrocarbon analogues, due to the electron-withdrawing effects of fluorine atoms. The coupling assays we performed with 3,3,4,4,5,5,6,6,7,7,8,8-dodecafluorooctanol and trichloroacetimidate maltoside proved negative.[14] To graft a fluorinated chain on the carbon anomer of different glycosides, Bonnet-Delpon et al.[15] used the Mitsunobu reaction. Another approach developed by Mietchen et al. consists in grafting a perfluoroalkyl iodide onto an allyl glycoside in the presence of a radical initiator.[16] We chose this chemical pathway. First, the allyl glycoside **1** (Scheme 1) was obtained by condensation of allyl alcohol on peracetylated maltoside in the presence of a Lewis acid.[17] Amongst the different Lewis acids assayed as catalyst, BF_3–etherate gave the highest coupling yield (89%). This reaction is accompanied by a partial deacetylation of the maltose derivative **1**, due to a transesterification reaction with the allyl alcohol used in excess. To minimise the effect of this side reaction, one can either use only 1.5 equiv of the allyl alcohol or, preferably, perform a peracetylation of crude products of the reaction in the presence of acetic anhydride/pyridine, followed by purification by silica gel chromatography. As regards the hemifluorocarbon diiodide **2**, it was synthesised by radical addition of diiodoperfluorohexane on ethylene following the method already reported by Manseri et al.[18] Condensation of compound **2** on allyl



Scheme 1. Synthesis of HF-Malt. Reagents and conditions: (a) allyl alcohol, BF_3/Et_2O, DCM, argon, 89% yield; (b) ethylene, CuI, 165 °C, 80% yield;[18] (c) Zn dust, DCM, argon, 61–88% yield; (d) Bu_3SnH, AIBN, acetonitrile, argon, 67% yield; (e) MeONa, MeOH, 99% yield.

maltoside derivative **1** was carried out in the presence of Zn powder in methylene choride to provide compound **3** in good yield (66%).[19] Iodide groups were then reduced using tributyltin hydride (Bu_3SnH) in the presence of α,α′ azo-bis-isobutyronitrile (AIBN), yielding hemifluorocarbon peracetylated maltoside. The observed partial deacetylation of the maltose moiety was again corrected by acetylation of crude products. After purification by silica gel chromatography, peracetylated hemifluoro-maltoside was isolated with 67% yield, and, finally, quantitatively deacetylated at room temperature in methanol in the presence of catalytic amount of sodium methylate following the Zemplen method. After final purification on a Sephadex LH20 column, HF-Malt **4** was isolated as a white powder and characterised by NMR.[20] The same procedure was applied to perfluorohexyl iodide to provide F-Malt (compound **5**) in 52% overall yield.

F- and HF-Malt are soluble in water. Physical–chemical parameters derived from tensiometric measurements were compared to those of their hydrogenated homologue DDM (Table 1).

As previously observed both for telomeric surfactants derived from Tris (HF-TAC and F-TAC)[9] and for lactobionamide derivatives (HF-Lac and F-Lac),[12] adding an ethyl group at the end of the fluorocarbon chain has little effect on the critical micellar concentration (CMC) (Table 1). Furthermore, whereas the limit surface tension of F-Malt is lower than that of DDM, as expected for fluorinated compounds,[21] that of HF-Malt has an intermediate value. The area per molecule of surfactant is higher for HF-Malt than for either DDM or F-Malt, a phenomenon already noted for HF-Lac.[12] These unusual features can be explained by a poorer packing between fluorinated and hydrogenated groups, by steric hindrance in the core of HF-Lac micelles and/or by the acidic character of the methylene group vicinal to the fluorocarbon chain.[12] Both the fluorinated chain and the presence of the ethyl tip at its extremity also affect the aggregation behaviour of F- and HF–Malt (Table 2): whereas DDM forms small, spherical micelles (~6 nm in diameter),[22] F-Malt, at 25 °C and at relatively low concentration (0.4 mM), forms larger aggregates (Table 2)—probably cylindrical assemblies, as do other fluorinated surfactants.[23] HF-Malt forms even larger and more polydisperse aggregates (Table 2). This behaviour is enhanced at higher concentration (5 mM) as well as at lower temperature (4 °C), conditions under which a population of micrometric particles is observed (Table 2).

Table 1. Physical–chemical properties of DDM, F-Malt and HF-Malt

Compound	CMC (mM)	γ_{cmc} (mN m^{-1})	Area per molecule (nm^2)
DDM	0.17[a]	35.4[a]	0.42[a]
F-Malt	0.20 ± 0.01	17.5 ± 0.5	0.38 ± 0.02
HF-Malt	0.22 ± 0.01	27 ± 0.5	0.51 ± 0.03

[a] Values from Ref. 22.

The ability of F- and HF-Malt to keep MPs water-soluble was investigated using three model proteins representative of different types of structures: (i) bacteriorhodopsin (BR), an archaebacterial protein folded into a bundle of seven transmembrane α-helices with small extramembrane loops.[24] A molecule of retinal is covalently but loosely bound to the protein, whose visible absorption spectrum is a sensitive and convenient reporter of whether it is in its native state or not; (ii) tOmpA, an eight-strand β-barrel that forms the transmembrane region of outer membrane protein A from the Gram-negative eubacterium *Escherichia coli*;[25] and (iii) cytochrome b_6f, a photosynthetic complex extracted from the chloroplast of *Chlamydomonas reinhardtii*. Cytochrome b_6f is a superdimer, each monomer being comprised of eight transmembrane subunits and numerous cofactors, including stabilising lipids.[26,27] Its stability can be monitored enzymatically.[26] All three proteins were purified using classical detergents.[27–29]

Figure 2 shows the migration of BR in 10–30% sucrose gradients (as described in Ref. 11). This procedure enables surfactant exchange, while the position of the protein/surfactant band yields an estimate of the sedimentation coefficient of the particles (which is related to their mass, size, shape and density) and its width an indication of their mono- or polydispersity. In DDM, BR migrates as a sharp band, in the upper part of the gradient. When transferred to either F- or HF-Malt, it migrates deeper. A similar behaviour was previously observed with other fluorinated surfactants,[11,12] and can be explained by the high density conferred to the surfactants by the fluorine atoms. The bands are also much broader in F- or HF-Malt than in detergent, which reveals a high polydispersity of the MP/surfactant

Table 2. Size of the aggregates formed by DDM, F-Malt and HF-Malt

Compound	25 °C, 0.4 mM[a]			4 °C, 0.4 mM[a]			4 °C, 5 mM[a]		
	Diameter (nm)	HHW[b] (nm)	% (volume)	Diameter (nm)	HHW[b] (nm)	% (volume)	Diameter (nm)	HHW[b] (nm)	% (volume)
DDM	6.3 ± 0.3	1.6 ± 0.1	100	7.2 ± 0.3	1.6 ± 0.1	100	8.2 ± 0.5	3.1 ± 0.5	100
F-Malt	11 ± 2	1.5 ± 0.3	99	20 ± 2	7 ± 1	97	25 ± 5	20 ± 10	99
HF-Malt	16 ± 2	4 ± 3	99	19 ± 5	12 ± 3	94	32 ± 20	21 ± 7	88
				650 ± 330	200 ± 100	6	1000 ± 300	480 ± 200	12

[a] Micelle sizes measured by dynamic light scattering using a Zetasizer Nano-S model 1600 (Malvern Co). The sample was prepared 24 h before the measurement and filtered through a 0.45-μm filter just before measurements.
[b] HHW, the width of the peak at half-height, an indication of the degree of polydispersity of the aggregates.

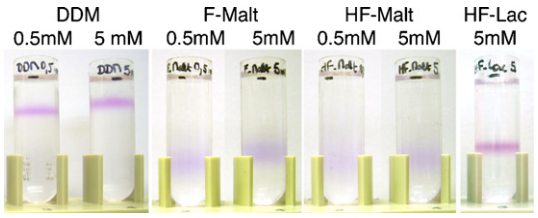


Figure 2. Migration of bacteriorhodopsin in 10–30% sucrose gradients in the presence of either DDM, F- or HF-Malt (0.5 or 5 mM) or HF-Lac (5 mM). Gradients were centrifuged 4 h at 200,000×*g*.

complexes. Polydispersity can stem from either component of the complex: the protein, which can form small, heterogeneous aggregates, or the surfactant, different amount of which can bind to the protein. BR migrates at the same position in F- or HF-Malt gradients as in an HF-Lac gradient (Fig. 2), suggesting that the protein has not aggregated and is present under the same oligomeric state in all conditions. On the other hand, dynamic light scattering measurements show that F- and HF-Malt form large and heterogeneous aggregates (Table 2). Thus, the high dispersity of BR/surfactant complexes seems more likely to stem from heterogeneous surfactant binding, presumably as a consequence of the tendency of the latter to form aggregates of variable size. The BR band in F-Malt gradients was observed to be slightly but reproducibly thinner than that in HF-Malt gradients, consistent with the fact that F-Malt forms by itself slightly less heterogeneous aggregates than does HF-Malt (Table 2).

The same overall behaviour was observed with the b_6f complex and with tOmpA: MP/surfactant complexes migrated deeper into F- and HF-Malt gradients than into DDM ones, and the bands were much broader (not shown). The behaviour of MP/HF-Malt complexes is noticeably different from that of complexes formed with either F- or HF-Lac.[12] F- and HF-Lac also form by themselves aggregates of variable size.[12] However, their complexes with MPs are monodisperse (Ref. 12 and Fig. 2). This likely results from F- and HF-Lac aggregates being smaller,[12] that is, featuring a stronger spontaneous interface curvature.

At low HF-Malt concentration (0.5 mM), tOmpA migrates near the bottom of the tube, indicating the onset of aggregation. When transferred into increasing surfactant concentrations (2–10 mM), the protein/surfactant band shifts towards lighter species, consistent with protein/surfactant interactions progressively overcoming protein/protein interactions. However, the position of the band remained beneath that observed in HF-Lac, suggesting that, even at 10 mM HF-Malt, tOmpA forms small aggregates (not shown).

The integrity of BR and of the b_6f complex in DDM, F- or HF-Malt was investigated by spectroscopic and/or enzymatic measurements. Notwithstanding the polydispersity of the complexes, BR and the b_6f complex were found to be in their native state, and the b_6f complex enzymatically as active as in DDM. Their stability over time was either comparable to (at low surfactant concentrations) or higher than (at higher surfactant concentrations) that in DDM (not shown), as previously observed both with HF-TAC[11] and HF-Lac.[12]

We have described the synthesis of novel fluorinated or hemifluorinated surfactants derived from maltose. In keeping with earlier observations,[9,10,12] addition of an ethyl tip to the fluorinated chain seems to destabilise the micelles, possibly because of unfavourable interactions between fluorinated and hydrogenated groups. Unlike surfactants of the TAC series,[30] however, F- and HF-Malt form large and polydisperse aggregates, as already observed for the lactobionamide series.[12] At variance with the latter case, however, the heterogeneity of (H)F-Malt aggregates seems to entail that of their complexes with MPs (Fig. 2). Future work will thus focus on optimising the chemical structure of fluorinated and hemifluorinated surfactants with the aim of obtaining compounds that form small, globular aggregates. Upon comparing the chemical structures and physical–chemical properties of compounds of the (H)F-TAC, (H)F-Lac and (H)F-Malt series, one is led to hypothesise that the introduction of a bulkier polar head should induce a decrease of the curvature radius of the aggregates and thus favour the formation of micelle-like aggregates and monodisperse MP/surfactant complexes, while preserving the useful biochemical properties exhibited thus far by hemifluorinated surfactants.

Acknowledgments

This work was supported by the C.N.R.S., the C.E.A., the Universities of 'Paris-7 Denis Diderot' and 'Avignon et Pays du Vaucluse', and by the E.C. Specific Targeted Research Project Innovative tools for membrane structural proteomics. F.L. and M.P. were the recipients of fellowships from the 'Ministére de l'Enseignement Supérieur et de la Recherche'.

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20. Spectra of F-Malt and HF-Malt are summarised as follows: HF-Malt. Acetylated compound: R_f: 0.73 (ethyl acetate/Cy: 6/4), mp: 59 °C (dec), $[\alpha]_D^{20}$: +32.4 (*c*, 1, DCM), ^{1}H NMR (/$CDCl_3$): δ (ppm) 5.42 (1H, d, H'_1, $^3J_{H-H}$ = 4 Hz), 5.36(1H, t, H_3), 5.26 (1H, t, H'_3), 5,06(1H, m, H'_4), 4.86 (1H, m, H'_2), 4.79 (1H, m, H_2), 4.53 (1H, d, H_1, $^3J_{H-H}$ = 8 Hz), 4.45 (1H, m, H_6), 4.23 (2H, m, $2 \times H'_6$), 3.97 (5H, m, $H_4 + H_6 + H'_5 + CH_2O$), 3.61 (1H, m, H_5), 2.07 (27H, m, $7 \times CH_3CO + CH_2CH_2CF_2 + CH_2CH_3$), 1.14(3H, t, CH_2CH_3), ^{13}C NMR (/$CDCl_3$): δ (ppm) 170.6 (CH_3CO), 170.5 (CH_3CO), 170.3 (CH_3CO), 170 (CH_3CO), 169.9 (CH_3CO), 169.6 (CH_3CO), 169.5 (CH_3CO), 130.0 → 110.0 ($6 \times CF_2$), 100.2(C_1), 95.5(C'_1), 75.3(C_4), 72.6 (C_3), 72.1 (C_2), 72.0 (C_5), 70.0(C'_3), 69.3(C'_5), 68.5(C'_2), 68.4 (CH_2O), 68.0(C'_4), 62.7(C'_6), 61.4 (C_6), 27.9 ($OCH_2CH_2CH_2CF_2$), 27.6 ($CH_2CH_2CF_2$), 24.6 ($CF_2CH_2CH_3$), 21.0 (CH_3CO), 20.9 (CH_3CO), 20.8 (CH_3CO), 20.7 (CH_3CO), 20.6 (CH_3CO), 20.5 (CH_3CO), 20.4 (CH_3CO), 4.5 (CH_2CH_3), ^{19}F NMR (/$CDCl_3$): δ (ppm) −114.45 (2F, m, CF_2 $(CH_2)_3O$),−116.42 (2F, m, $CF_2CH_2CH_2CH_3$), −121.91 (4F, m, $CF_2CF_2(CF_2)_2CF_2CF_2$), −123.71 (4F, m, CF_2CF_2 $(CF_2)_2CF_2CF_2$), Deacetylated compound 4: R_f: 0.62 (ethyl acetate/MeOH/H_2O: 7/2/1), mp: 94 °C (dec), $[\alpha]_D^{20}$: +37.2 (C, 1, DCM), ^{1}H NMR (DMSO-d_6): δ (ppm) 5.52 (1H, m, O*H*), 5.46 (1H, m, O*H*), 5.18 (1H, d, H'_1, $^3J_{H-H}$ = 5 Hz), 5.02 (1H, m, O*H*), 4.92 (2H, m, $2 \times$ O*H*), 4.52 (2H, m, $2 \times$ O*H*), 4.20 (1H, d, H_1, $^3J_{H-H}$ = 8 Hz), 3.70 → 3.03 (12H, m, $H_2 + H_3 + H_4 + H_5 + 2 \times H_6 + H'_2 + H'_3 + H'_4 + H'_5 + 2 \times H'_6 + CH_2O$), 2.25 (4H, m, $2 \times CH_2CF_2$), 1.78 (2H, m, OCH_2-$CH_2CH_2CF_2$), 1.07 (3H, t, CH_2CH_3), ^{13}C NMR (DMSO-d_6): δ (ppm) 125.0 → 105.0 ($6 \times CF_2$), 103.1 (C_1), 101.3(C'_1), 80.4 (C_4), 76.8 (C_3), 75.6 (C_2), 73.9 (C_5), 73.8 (C'_3), 73.4 (C'_5), 72.9 (C'_2), 70.3(C'_4), 67.6 (CH_2O), 61.2(C'_6), 61.1 (C_6), 27.4 ($OCH_2CH_2CH_2CF_2$), 24.2 ($CF_2CH_2CH_3$), 20.9 ($OCH_2CH_2CH_2CF_2$), 4.8 (CH_2CH_3),^{19}F NMR (DMSO-d_6): δ (ppm) −113.35 (2F, m, $CF_2(CH_2)_3O$), −115.23 (2F, m, $CF_2CH_2CH_2CH_3$), −121.73 (4F, m, $CF_2CF_2(CF_2)_2CF_2CF_2$), −123.14 (4F, m, CF_2CF_2 $(CF_2)_2CF_2CF_2$). F-Malt. Acetylated compound: R_f : 0.67 (ethyl acetate/cyclohexane: 6/4), mp: 68 °C (dec), $[\alpha]_D^{20}$: +51.6 (*c*, 1, DCM), ^{1}H NMR (/$CDCl_3$): δ (ppm) 5.44 (1H, d, H'_1), 5.38 → 5.24(2H, m, $H_3 + H'_3$), 5.08 (1H, m, H'_4), 4.91 → 4.81 (2H, m, $H'_2 + H_2$), 4.55 (1H, d, H_1), 4.31 → 4.40 (9H, m, $H_4 + H_5 + 2 \times H_6 + H'_5 2 \times H'_6 + CH_2O$), 2.05 (25H, m, $7 \times CH_3CO + CH_2CF_2 + OCH_2$-$CH_2CH_2CF_2$), ^{13}C NMR (/$CDCl_3$): δ (ppm) 170.6 (CH_3CO), 170.5 (CH_3CO), 170.2 (CH_3CO), 170 (CH_3CO), 169.9 (CH_3CO), 169.6 (CH_3CO), 169.4 (CH_3CO), 130.0 → 110.0 ($5 \times CF_2 + CF_3$), 100.2 (C_1), 95.6(C'_1), 75.3 (C_4), 72.7 (C_3), 72.2 (C_2), 72.1 (C_5), 70.0(C'_3), 69.4(C'_5), 68.5(C'_2), 68.3 (CH_2O),68.1(C'_4), 67.0 ($OCH_2CH_2CH_2CF_2$), 62.7(C'_6), 61.5 (C_6), 27.5 (CH_2CF_2), 21.0 (CH_3CO), 20.9 (CH_3CO), 20.8 (CH_3CO), 20.7 (CH_3CO), 20.6 (CH_3CO), 20.5 (CH_3CO), 20.4 (CH_3CO), ^{19}F NMR (/$CDCl_3$): δ (ppm) −80.73 (3F, m, CF_3), −114.36 (2F, m, CF_2CH_2), −121.86 (2F, m, CF_2CF_3), −122.82 (2F, m, $CH_2CF_2CF_2CF_2$), −123.46 (2F, m, $CH_2CF_2CF_2$), −126.07 (2F, m, $CF_2CF_2CF_3$). Deacetylated compound 5: R_f : 0.61 (ethyl acetate/MeOH/H_2O: 7/2/1), $[\alpha]_D^{20}$: +40.7 (c, 1, DCM), ^{1}H NMR (/MeOH-d_4): δ (ppm) 5.18 (1H, m, O*H*), 4.96 (1H, d, H'_1), 4.90 (5H, m, $5 \times$ O*H*), 4.31 (1H, d, H_1), 4.10 → 3.20 (15H, m, $H_2 + H_3 + H_4 + H_5 + 2 \times H_6 + H'_2 + H'_3 + H'_4 + H'_5 + 2 \times H'_6 + CH_2O + OH$), 2.34 (2H, m, CH_2CF_2), 1.93 (2H, m, $OCH_2CH_2CH_2CF_2$), ^{13}C NMR (MeOH-d_4): δ (ppm) 130.0 → 110.0 ($5 \times CF_2 + CF_3$), 102.8 (C_1), 101.5(C'_1), 79.9 (C_4), 76,4(C$_3$), 75,2(C$_2$), 73.7 (C_5), 73.4(C'_3), 73.2(C'_5), 72.8(C'_2), 70.1(C'_4), 67.8 (CH_2O),61.3(C'_6), 60.8 (C_6), 27.4 ($OCH_2CH_2CH_2CF_2$), 20.5 ($OCH_2CH_2CH_2CF_2$), ^{19}F NMR (/MeOH-d_4): δ (ppm) −82.39 (3F, m, CF_3), −115.47 (2F, m, CF_2CH_2), −122.96 (2F, m, CF_2CF_3), −123.93 (2F, m, $CH_2CF_2CF_2CF_2$), −124.46 (2F, m, $CH_2CF_2CF_2$), −127.34 (2F, m, $CF_2CF_2CF_3$).
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