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Hybrid Double-Chain Maltose-Based Detergents: Synthesis, Colloidal and Biochemical Evaluation

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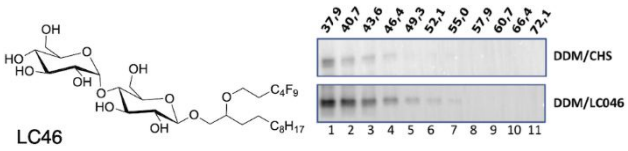


Abstract. Four hybrid double-chain surfactants with a maltose polar head were synthesized. The apolar domain consists of a hydrogenated chain, and a partially fluorinated chain made of a propyl hydrogenated spacer terminated by a perfluorinated core of various length. Their water solubility was found to be lower than 1g/L irrespective of the length of both chains. The self-assembling properties of pure hybrids in water was studied by dynamic light scattering and transmission electron microscopy, which revealed the formation of two populations of aggregates with diameters of 8–50 nm diameter and 80–300 nm. When mixed with the classical detergent *n*-dodecylmaltoside (DDM), the four hybrids were well soluble and formed small mixed micelles. DDM/hybrid mixtures were further evaluated for the extraction of the full-length, wild-type human GPCR adenosine receptor ($A_{2A}R$) and the bacterial transporter AcrB. The solubilization of $A_{2A}R$ showed extraction efficiencies ranging from 40% to 70%, while that of AcrB reached 60% to 90%. Finally, three of the hybrids exhibited significant thermostabilization when present as additives. The derivative with a C_{12} hydrogenated chain and a C_4F_9 fluorinated chain emerged as the most potent additive exhibiting both good extraction yields of $A_{2A}R$ and AcrB and thermostabilization of $A_{2A}R$ by ~7°C.

Introduction.

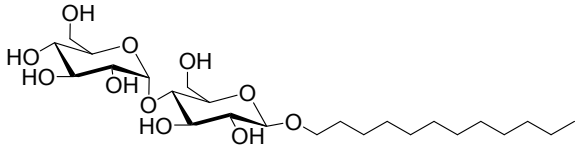
Sugar-based amphiphiles have been extensively developed over the past decades.[1] The growing interest arises from several reasons. The growing interest arises from several reasons. They can be prepared from abundant renewable raw vegetable materials; convenient organic chemistry allows structural diversity, which, in turn, gives access to different colloidal systems such as micelles, liposomes, foams, and emulsions; they exhibit low toxicity and high biodegradability. The diversity in chemical geometry goes from the rather simple "head-and-tail" alkyl glucosides derivatives that have been used successfully for membrane-protein solubilization and crystallization[2-4] or as hydrogelators,[5,6] to more sophisticated gemini surfactants[7] for gene delivery or highly fluorinated[8] derivatives for drug delivery, to name but a few.

Fluorinated chains present the peculiar property of being both hydrophobic and lipophobic, which provides to fluorinated surfactants (FSs) numerous industrial and medical applications.[9,10] FSs have also shown promising properties as milder alternatives to classical detergents for the study of membrane proteins (MPs).[11,12] Most of FSs used for the study of MPs have the same "head-and-tail" architecture as classical hydrogenated detergents, but they differ from the latter by the presence of several perfluorinated fluorinated carbons within the tail. More recently, their ability to solubilize artificial membranes[13] and to extract MPs has also been reported,[14] making them promising agents for both extraction and stabilization of membrane target proteins.

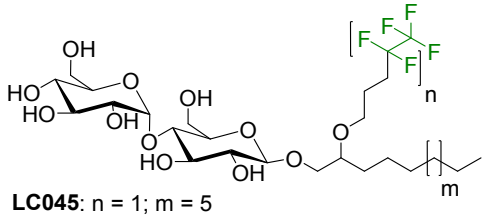
Fluorocarbon-hydrocarbon hybrid surfactants have an hydrophobic part made of a fluorinated and a hydrogenated chain and are referred to as hybrid double-chain surfactants.[15] Among the properties of such hybrid surfactants are the high viscosity of aqueous solutions, intramicellar phase separation between the two chains, and long micelle lifetimes.[15,16] We

have recently reported the synthesis of two hybrid double-chain surfactants with a di-glucose polar head whose apolar domain consists of a perfluorohexyl main chain and a butyl hydrogenated branch as a side chain.[17] They were both able to keep the membrane protein bacteriorhodopsin stable demonstrating the interest of hybrid surfactants for MPs stabilization.

We report herein the synthesis of a new series of hybrid double-chain detergents presenting structural similarity with conventional and widely used *n*-alkyl maltosides detergents. These new compounds have a maltose polar headgroup and two alkyl chains, one being fully hydrogenated while the other one is partially fluorinated. The *n*-dodecyl (C_{12}) hydrogenated derivatives **LC045-LC047** have a fluorinated chain of various lengths *i.e.* C_2F_5, C_4F_9 or C_6F_{13}, respectively while **LC057** has an *n*-octyl (C_8) hydrogenated chain and a C_4F_9 chain (Figure 1).



n-dodecyl β-D-maltoside



LC045: n = 1; m = 5
LC046: n = 3; m = 5
LC047: n = 5; m = 5
LC057: n = 3; m = 1

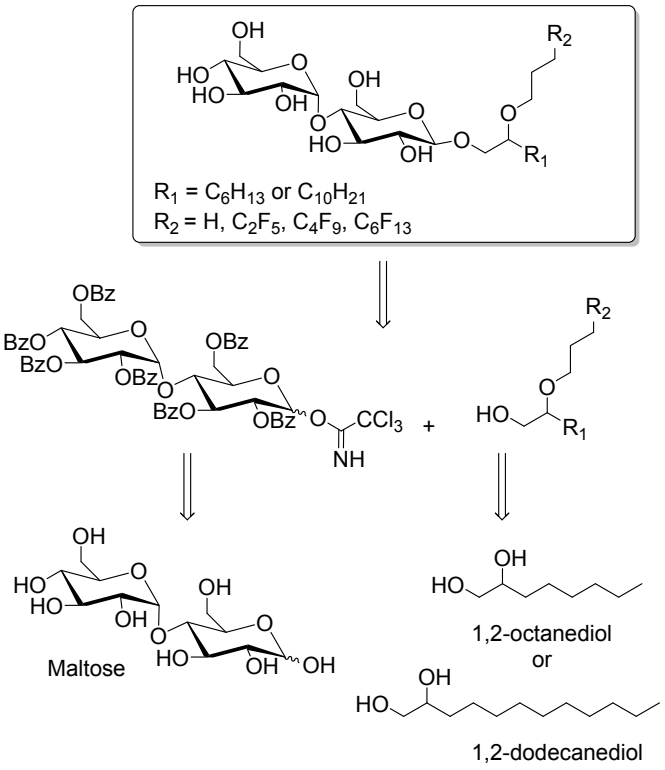
Figure 1. Chemical structures of *n*-dodecyl β-D-maltoside (DDM) a widely used detergent for MPs study and of the four hybrid double-chain surfactants **LC045**, **LC046**, **LC047** and **LC057**.

The self-aggregation of these four derivatives was further investigated by means of dynamic light scattering (DLS) and transmission electron microscopy (TEM). Because of the poor solubility of these derivatives, the self-aggregation of their mixtures with the classical DDM

detergent was also investigated. Finally, the ability of such mixtures to solubilize and stabilize membrane proteins was evaluated.

Results and Discussion

Synthesis. The synthesis follows a convergent synthetic route, based on three key steps: (i) synthesis of the hydrophobic parts; (ii) synthesis of the maltose polar head; and (iii) coupling of the hydrophobic and hydrophilic parts (**Scheme 1**). This convergent strategy allows versatility in chemical structure and would allow the synthesis of a large library of analogs by varying the nature of both polar head group and fluorinated chain.



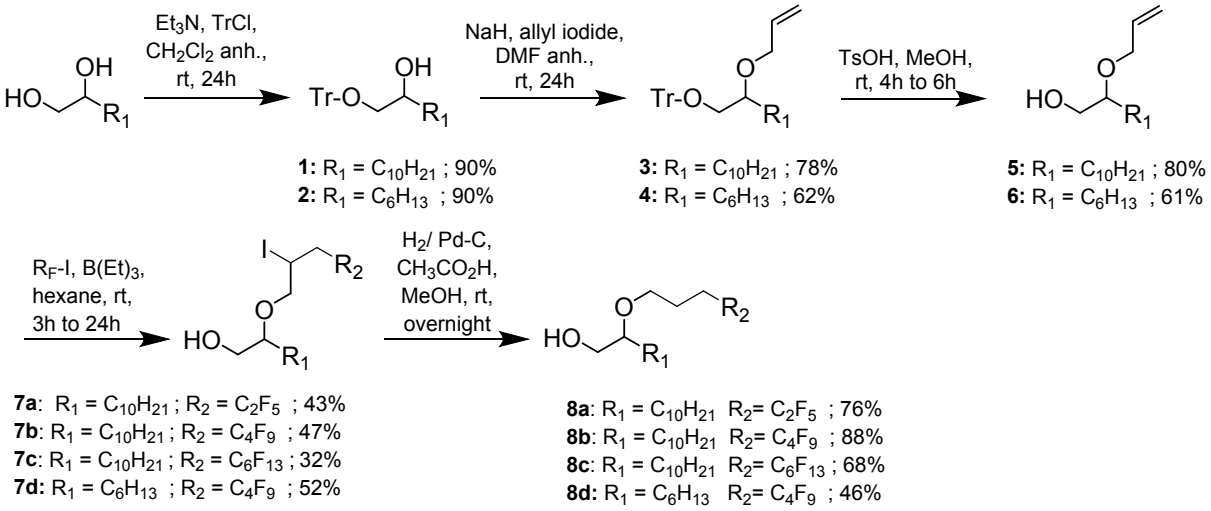
Scheme 1. Retrosynthetic pathway for compounds **LC045**, **LC046, LC047** and **LC057** from 1,2-dodecanediol or 1,2-octanediol.

The synthesis of the various hydrophobic parts was achieved following a five-step route starting either from racemic 1,2-dodecanediol or 1,2-octanediol. The two hydroxyl groups allow the connection of both fluorinated chain and maltose polar head. We decided to

introduce the fluorinated chain on the secondary free hydroxyl group of the starting diol in order to keep the most reactive primary alcohol vacant for the glycosylation step.

In the first step, the primary alcohol group was selectively protected in the presence of trityl chloride and Et_3N to afford compound **1**.[18] Then, allylation of the secondary alcohol was achieved using allyl iodide in the presence of NaH. The insertion of the allyl moiety was confirmed by ^1H NMR with the presence of signals at 5.2 and 5.9 ppm, corresponding to the CH_2 and CH of the double bond, respectively. Deprotection of the trityl group with *p*-TsOH in methanol led to compound **3**. The addition of the fluorinated chain on the allyl moiety was achieved by radical addition of perfluoroalkyl iodide. In previous work, direct insertion of perfluoroalkyl chains was done under inert atmosphere using AIBN as initiator in a sealed tube.[17,19] With the aim to explore milder conditions we used the methodology described by Takeyama and co-workers, consisting in the use of triethylborane in the presence of oxygen.[20] Under these conditions insertion of the fluorinated chain was achieved in moderate to good yields ranging from 32% for compound **7c** to 43% for compound **7a**. The insertion of the fluorinated chain was further confirmed by ^1H NMR with the absence of unsaturated protons at 5.2 and 5.9 ppm and the presence of signals at 1.8 and 2.2 ppm related to the CH_2 in α and β position of the fluorinated segment. Reduction of the C-I bond was also improved compared to our previous work where we employed stoichiometric quantities of Bu_3SnH at 70°C in a sealed tube under inert condition.[17,19,21] Catalytic hydrogenation in the presence of Pd-C was chosen. This method was performed at room temperature in a hydrogenation reactor and led to the reduced compounds **8a-c** in good yields. This new synthetic route therefore provides a convenient method for both radical addition of fluorinated chains and reduction of the iodine group. A similar synthetic route was used for the synthesis of compound **8d** with 1,2-

octanediol as starting material (**Scheme 2**). Hydrophobic double-chain precursors were therefore prepared in 5 steps in 11–16% overall yields.

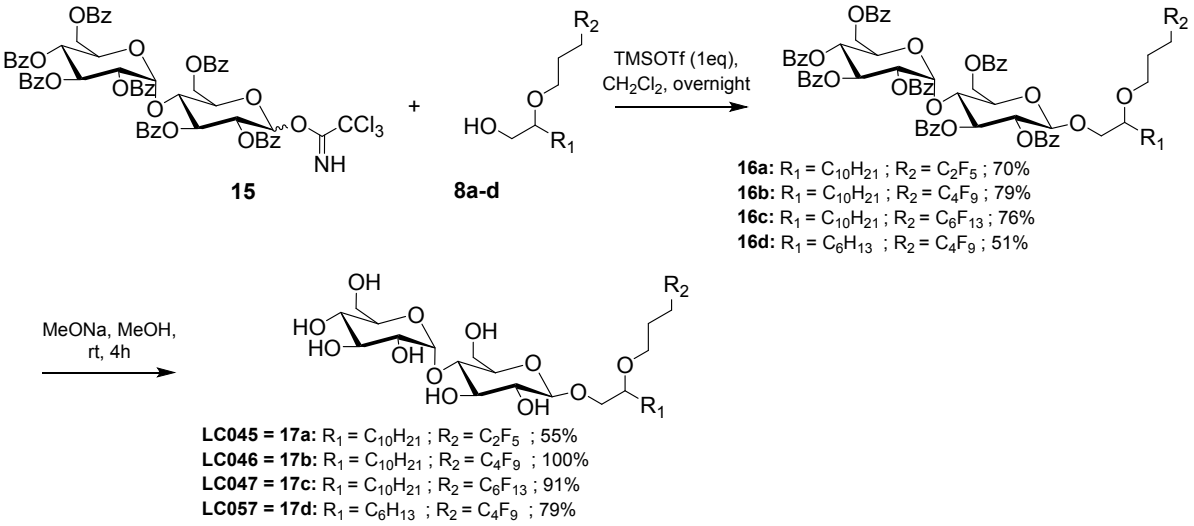


Scheme 2. Synthesis of the hydrophobic alcohols **8a-d**.

In order to obtain high yield and stereoselective *O*-glycosylation leading to the *β* anomer, activation of the anomeric position and protection of the other free hydroxy groups is preferable. Here, we decided to activate the maltose polar head into trichloroacetimidate and to use esters protecting groups.[22] Hepta-O-benzoyl-maltose-1-O-trichloroacetimidate **15** was readily prepared on large scale from commercially available maltose following a four steps synthetic route.[23]

The coupling between the hydrophilic and the lipophilic parts was next achieved following Schmidt reaction in the presence of TMSOTf as Lewis acid promoter.[24] Preliminary work showed that TMSOTf compared to $BF_3.OEt_2$ led to higher yields of reaction (data not shown). Under these conditions, hydrophobic alcohol **8a-d** were condensed onto the benzoylated maltose polar head **15**[23] in good yield in a few hours. Then, benzoyl groups were removed under Zemplén conditions to afford final hybrid derivatives **17a-d** (**Scheme 3**). Analysis of the proton NMR spectra showed the formation *β* anomer witnessed by a doublet between 4.0

and 4.5 ppm with a coupling constant of ~8 Hz (J_{trans}) corresponding to the anomeric proton H_1. The second anomeric proton between the two glucose units appeared at 5.2 ppm with a coupling constant of ~4 Hz (J_{cis}).



Scheme 3. Glycosylation and deprotection step leading to the formation of compounds **LC045**, **LC046**, **LC047** and **LC057**.

Colloidal characterization. We first evaluated the solubility of the hybrid series in water. At 1g/L and despite sonication and heating at 40°C, aqueous solutions of hybrid derivatives were opalescent for all derivatives. Centrifugation of the opalescent solutions led to the formation of a clear supernatant solution and a precipitate. For comparison DDM exhibits a solubility in water of 200 g/L. This shows that the additional fluorinated segment, regardless of its length, significantly affects the overall hydrophobicity and significantly reduces the solubility in water. We next conducted DLS experiments to determine whether the water-soluble fraction of these hybrid surfactants could form aggregates. The composition of the supernatant solution of a centrifuged suspension prepared at 1g/L was analyzed. Whatever the hybrid tested, volume-weighted particle size distributions indicated the presence of two populations of aggregates within the range of 20 to 300 nm (**Figure 2**). Number-weighted particle size distributions showed one main population of aggregates of ~15 to 50 nm hydrodynamic diameter, while intensity-weighted particle size distributions were dominated by a population

of larger aggregates with diameters between 80 and 300 nm (**Figure 2**). These results suggest the coexistence of small and large aggregates species in solution, smaller aggregates accounting for the major part.

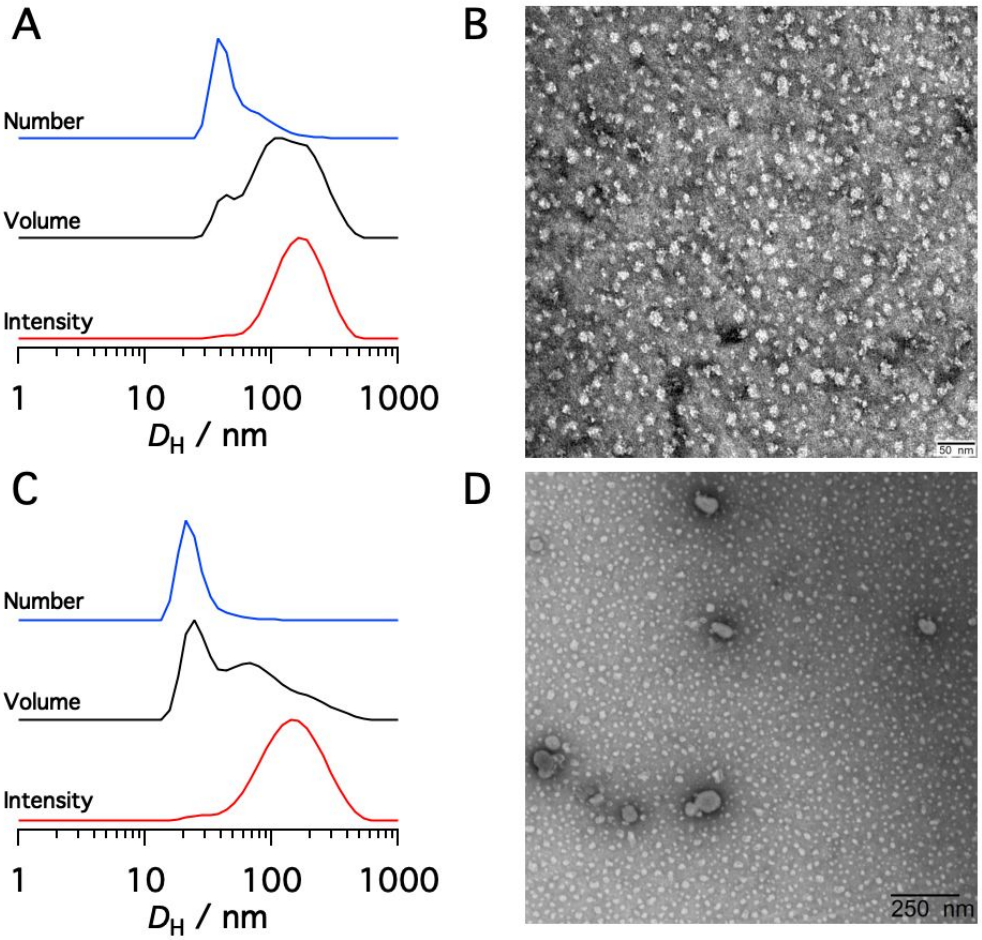


Figure 2. Hydrodynamic size distribution by Contin analysis for aqueous solutions of **LC045** (A) and **LC046** (C). TEM images of negatively stained samples **LC045** (B) and **LC046** (D). The supernatant of centrifuged suspensions at 1g/L were analyzed.

Then, we performed TEM experiments to further characterize the aggregates observed by DLS. The composition of the supernatant of a 1g/L saturated solution was analyzed using the negative staining method. As shown in Figure 2, **LC045** and **LC046** solutions allowed the observation of aggregates with an averaged diameter of ~15 ± 5 nm (**LC045**) and ~25 ± 10 nm (**LC046**). The presence of few aggregates with diameter of ~80 to 100 nm was also noted. This confirms the coexistence of small and large aggregates as observed by DLS. For the **LC047** and **LC057**, we also observed the coexistence of larger and smaller aggregates, the latter exhibiting

40 and 8.0 nm diameter, respectively, in agreement with the hydrodynamic diameters determined by number analysis (TEM images not shown).

To overcome the poor water-solubility of **LC045**, **LC046**, **LC047**, and **LC057** and to allow the evaluation of their potency as stabilizing agent for membrane proteins, we next prepared mixtures with DDM at different molar ratio. On the one hand, DDM is readily soluble in water and therefore is able to promote the solubilization of our hybrid molecules in water. On the other hand, the use of DDM/additive mixtures has already been successfully employed in several biochemical assays.[25,26] Below a DDM:**LC** molar ratio of 100:10, the four hybrids appeared well soluble in water, as witnessed by transparent solutions. Beyond this ratio, gradual addition of hybrids promoted the formation of a cloudy solution, the critical concentration of hybrid molecules at which the transfer from a clear to a cloudy solution occurred is reported in Table S1. Along the C_{12} series, **LC047** that has the longest fluorinated chain induced the lowest solubility of the mixture with the appearance of a cloudy solution at 100:20, while for compound **LC045** with the shortest fluorinated chain the mixture was still soluble at 100:50. We next performed hydrodynamic diameter measurements on DDM:**LC** mixtures.

We used the same DDM:**LC** molar ratios than those applied for membrane solubilization and stability assays (see below). DDM:**LC** molar ratios of 100:2 and 100:4 were studied for the C_{12} series while for **LC057** a 100:10 was studied. Contin distribution plots showed the presence of aggregates with hydrodynamic diameters of about 5 to 8 nm, for all hybrids and all mixing ratios tested (**Figure S59**). This indicates that DDM:hybrid mixtures self-assemble into small micellar aggregates. TEM experiments failed to show any of these aggregates. In general, aggregates smaller than 10 nm in diameter can only be visualized if the staining gives good

contrast, if a microscope with high acceleration voltage (300kV) is applied, or if cryo transmission electron microscopy is performed.

Solubilization and stabilization of membrane proteins. In order to determine the ability of the four hybrids compounds to act as solubilizing and stabilizing agents we conducted a biochemical evaluation on two membrane targets of biological interest: The full length and wild-type human GPCR adenosine receptor *(*$A_{2A}R$*)*, a eukaryotic protein that contains seven transmembrane domains and the bacterial transporter AcrB, a prokayriotic protein that contains twelves transmembrane domains. As above, DDM/hybrid mixtures ranging from 100:2 to 100:10 were studied. The solubilization of $A_{2A}R$ showed extraction yields varying from 40% to 70% of the total content of proteins (**Figure 3A**). These values are close to those obtained with SDS (not shown) and in DDM/cholesteryl hemisuccinate (CHS) mixture (**Figure 3A**), two very extracting conditions. The use of DDM/cholesteryl hemisuccinate (CHS) mixture to efficiently extract and stabilize $A_{2A}R$ has been reported in the literature including our previous work.[27]

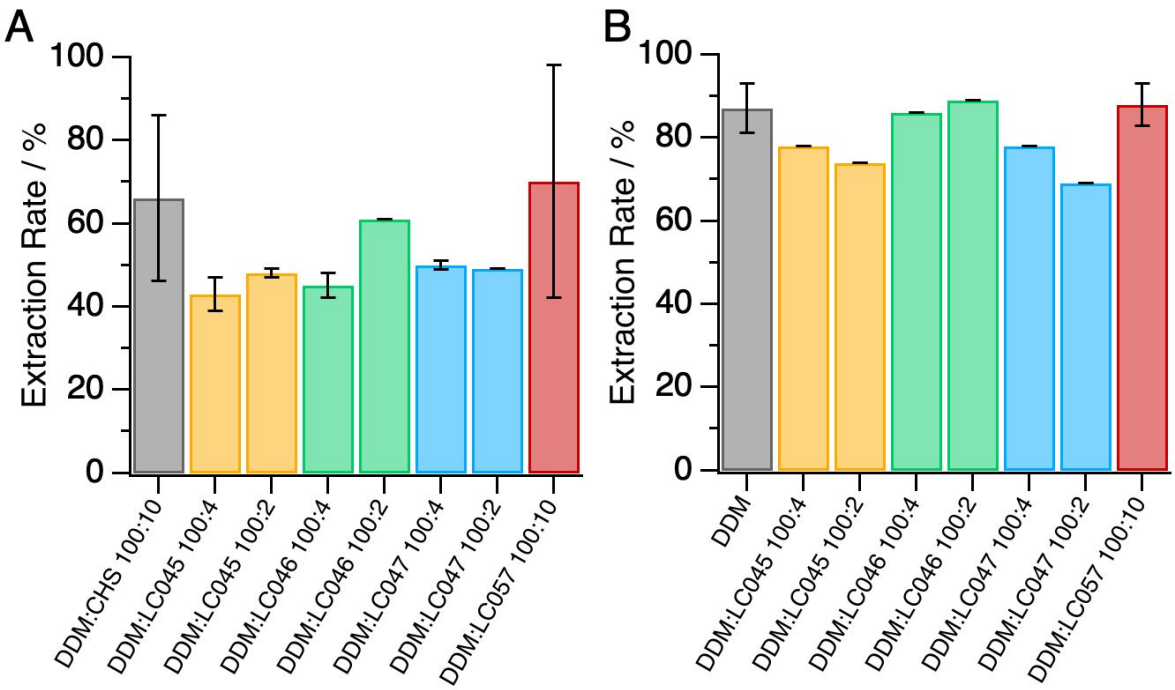
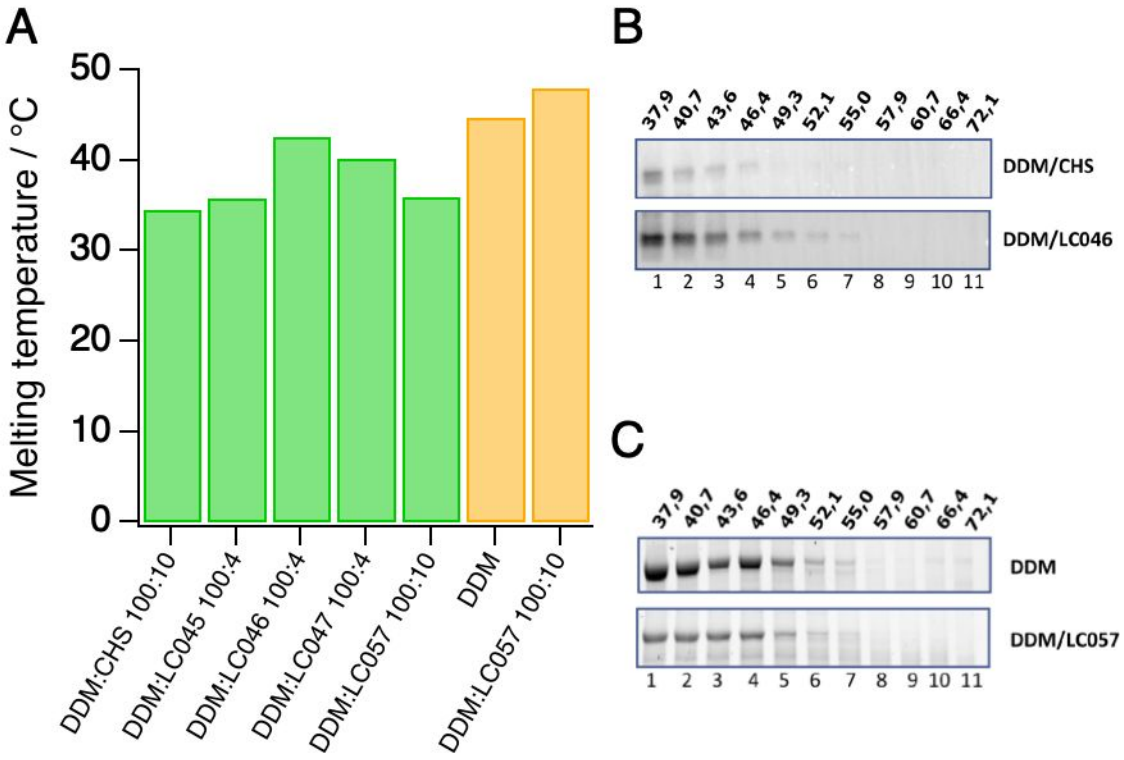


Figure 3. Solubilization experiments of human $A_{2A}R$ (**A**) and of bacterial AcrB (**B**) in the presence of DDM:**LC045**, **LC046, LC047,** and **LC057** mixtures. Biological membranes were incubated for 2 h in the presence of detergents at 4°C and the solution was subjected to ultracentrifugation. The soluble fraction was analyzed by Western blotting to quantify the amount of solubilized protein.

The solubilization of *AcrB* indicates good extraction properties for DDM:**LC** mixtures with high yields from 60% to 90% close to that of DDM, the standard generally used to extract that protein (**Figure 3B**). It is also interesting to note that better extraction yields were obtained for *AcrB* compared to $A_{2A}R$. The solubilizing assays on the two membrane targets $A_{2A}R$ and *AcrB* showed that DDM:**LC057** and DDM:**LC046** mixtures exhibited good extraction potencies comparable to or even better than that of SDS and DDM/CHS. As for DDM:**LC045** and DDM:**LC047** mixtures, the potency remained similar to that of DDM:CHS, thus demonstrating that their addition does not affect the solubilization efficiency of DDM.

In order to evaluate the stabilizing properties of the DDM/hybrid mixtures, thermal shift (T_m) measurements were undertaken. The value of Tm corresponds to the temperature at which half of the proteins in solution are found in a denatured state. Thermostability assays on $A_{2A}R$ showed that DDM:**LC046** and DDM:**LC047** mixtures induced a thermal shift of +6.8 and +5.7°C, respectively, compared to the reference condition (**Figure 4**), while DDM:**LC045,** DDM:**LC057**, and DDM:CHS mixtures led to similar Tm values (**Figure 4**). Regarding wild-type, full-length $A_{2A}R$ receptor, no classical detergent was reported to stabilize the protein. Therefore, previously reported work describes the removal the C-terminal domain (96 amino acids) and heavy mutagenesis of the protein to improve its stability (8 mutations).[28] Having an improvement of +6.8 and +5.7°C when using DDM:**LC046** and DDM:**LC047** mixtures, in comparison with DDM/CHS, therefore constitutes a significant achievement.

Thermostability assays in the presence of **LC057** were also conducted on AcrB using DDM:**LC057** mixture, indicating a less pronounced thermal shift of +3.2°C in comparison with the reference (**Figure 4**).



*Ref = DDM 0.5% + CHS 1/10.

Figure 4. (**A**) Thermostability results on the membrane targets $A_{2A}R$ (green) and AcrB (yellow) in the presence of **LC045**, **LC046**, **LC047 and LC057**. For both proteins DDM is used at 0.5%. Proteins were submitted to heat treatment (30–80°C), followed by ultracentrifugation before loading of the supernatant onto SDS-PAGE and Western blotting. (**B**) $A_{2A}R$ and (**C**) AcrB representative gel bands.

Conclusion.

We have synthesized a new series of four fluorocarbon/hydrocarbon hybrid double-chain surfactants with a maltose polar head. One chain is made of either a *n*-dodecyl or a *n*-octyl hydrogenated chain, while the second chain is made of a propyl hydrogenated spacer terminated by a perfluorinated core of various length, that is, C_2F_5, C_4F_9 and C_6F_{13}. Whatever the length of the chains, these four compounds were found to be poorly soluble in water. The

supernatant of a 1 g/L saturated solution contains two populations of aggregates of ~8 to 50 nm for the smaller one and of ~80 to 300 nm for the larger one as observed by DLS and TEM. When mixed with DDM detergent at ratios varying from 2 to 10%, the four hybrids were well soluble and formed small mixed micelles. The potency of these DDM/hybrid mixtures to extract two membrane proteins, namely, the full-length, wild-type human GPCR adenosine receptor ($A_{2A}R$) and the bacterial transporter AcrB, was further demonstrated without affecting the potency of DDM itself, thereby indicating that they can be used as additives for membrane-protein studies. Finally, their ability to thermostabilize the two proteins was also investigated, and three hybrids exhibited thermostabilization. The derivative with a *n*-dodecyl hydrogenated chain and a C_4F_9 fluorinated chain appeared as the most potent additive exhibiting both good extraction yields of $A_{2A}R$ and AcrB and thermostabilization of $A_{2A}R$ by ~7°C. The main limitation of the new compounds lies in their low water solubility. With a convenient synthetic route in hand that allows versatility in the chemical structures, current work is focused on the synthesis of more water-soluble derivatives.

Experimental section.

Material & Methods. All starting materials were commercially available and were used without further purification. All solvents were of reagent grade and used as received unless otherwise indicated. Anhydrous solvents were dried by simple storage over activated 4-Å molecular sieves for at least 24h. Molecular sieves were activated by heating *in vacuo* for 24h. The progress of the reactions was monitored by thin layer chromatography. The compounds were detected either by exposure to ultraviolet light (254 nm) or by spraying with sulfuric acid (5% ethanol) and/or ninhydrin (5% ethanol), followed by heating at ~150°C. ^{1}H, ^{13}C, and ^{19}F NMR experiments were performed at 400, 100, and 375 MHz, respectively. Chemical shifts are given in ppm relative to the solvent residual peak as a heteronuclear reference for ^{1}H and

^{13}C. The coupling constants J are given in hertz and the chemical shifts in parts-per-million (ppm). Abbreviations used for signal patterns are: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublet; dt, doublet of triplet. High-resolution mass spectra were determined on a Synapt G2-S (Waters) mass spectrometer with a TOF mass analyzer in a positive ionization mode.

1-(trityloxy)dodecan-2-ol (**1**).[29] To a solution of 1,2-dodecanediol (12 g, 59.3 mmol, 1 eq) in anhydrous CH_2Cl_2, was added Et_3N (16.5 mL, 119.0 mmol, 2 eq). The solution was stirred for 20 min and trityl chloride (19.8 g, 71.2 mmol, 1.2 eq) was added portion-wise. The reaction mixture was stirred for 24 h, then concentrated *in vacuo.* The residue was diluted with cyclohexane and the precipitate was filtered off. The solution was concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 90:10 v/v) to yield **1** as a colorless oil (23.7 g, 90 %). **Rf** = 0.64 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): *δ* 7.45-7.43 (6H, m); 7.28-7.18 (9H, m); 3.70-3.65 (1H, m); 3.04 (1H, dd, *J* = 6 Hz, J = 12 Hz); 3.01 (1H, dd, *J* = 4 Hz, *J* = 12 Hz); 1.53 (2H, m); 1.25 (16H, bs); 0.88 (3H, t, *J* = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): *δ* 145.6; 129.9-128.3; 87.7; 71.8; 68.8; 34.9; 33.0; 30.4-30.7; 26.3; 23.7; 14.4. **HRMS** (ESI-TOF) m/z: $[M + Na]^+$ calcd for $C_{31}H_{40}O_2Na$ 467.2926; Found 467.2920.

1-(trityloxy)octan-2-ol (**2**). To a solution of 1,2-octanediol (20 g, 137 mmol, 1 eq) in anhydrous CH_2Cl_2, was added Et_3N (38 mL, 274 mmol, 2 eq). The solution was stirred for 20 min, and trityl chloride (45.7 g, 164 mmol, 1.2 eq) was added portion-wise. The reaction mixture was stirred for 24 h then concentrated *in vacuo*. The residue was diluted with cyclohexane and the precipitate was filtered off. The solution was concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 95:5 v/v) to yield **2** as a colorless oil (47.9 g, 90 %). **Rf** = 0.38 (cyclohexane/AcOEt 90:10 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): *δ* 7.45-7.30

(15H, m); 3.78 (1H, m); 3.20 (1H, dd, *J* = 4Hz, *J* = 12 Hz); 3.05 (1H, dd, *J* = 8 Hz, *J* = 12 Hz); 2.34 (1H, bs); 1.40 (2H, br); 1.26 (8H, bs); 0.89 (3H, t, *J* = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): *δ* 143.9; 128.7; 127.8; 127.1; 86.6; 71.0; 67.8; 33.3; 31.7; 29.2; 26.9; 26.9; 25.4; 22.6; 14.0. **HRMS** (ESI-TOF) m/z: $[M + Na]^+$ calcd for $C_{27}H_{32}O_2Na$ 411.2300; Found 411.2312.

(((2-(allyloxy)dodecyl)oxy)methanetriyl)tribenzene (**3**). To a suspension of NaH (0.52 g, 21.6 mmol, 2.4 eq) in anhydrous DMF, was added a solution of **1** (4 g, 9 mmol, 1 eq) in anhydrous DMF at 0 °C. The solution was stirred at 0 °C for 20 min, and allyl iodide (0.9 mL, 9.9 mmol, 1.2 eq) was added dropwise. The reaction mixture was stirred for 24 h, then water was added, and the solution was extracted twice with AcOEt. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 95:5 v/v) to yield **3** as a colorless oil (3.4 g, 78 %). **Rf** = 0.58 (cyclohexane/AcOEt 95:5 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): *δ* 7.50-7.20 (15H, m); 5.95 (1H, m); 5.27 (1H, d, *J* = 16 Hz); 5.15 (1H, d, *J* = 12 Hz); 4.18 (1H, dd, *J* = 4 Hz, J= 12 Hz); 4.00 (1H, dd, *J* = 8 Hz, *J* = 12 Hz); 3.45 (1H, m); 3.17 (1H, dd, *J* = 6 Hz, *J* = 10 Hz); 3.10 (1H, dd, *J* = 4 Hz, *J* = 10 Hz); 1.47 (2H, m); 1.26 (16H, bs); 0.89 (3H, t, *J* = 8 H). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): *δ* 144.4; 135.7; 128.9; 127.9; 127.0; 116.6; 86.7; 78.7; 71.4; 66.2; 32.4; 32.1; 29.8; 29.7; 29.5; 27.1; 25.6; 22.8; 14.3. **HRMS** (ESI-TOF) m/z: $[M + Na]^+$ calcd for $C_{34}H_{44}O_2Na$ 507.3239; Found 507.3238.

(((2-(allyloxy)octyl)oxy)methanetriyl)tribenzene (**4**). To a suspension of NaH (3.0 g, 123.5 mol, 2.4 eq) in anhydrous DMF, was added a solution of **2** (20 g, 51.5 mmol, 1 eq) in anhydrous DMF at 0 °C. The solution was stirred at 0 C for 20 min, and allyl iodide (5.8 mL, 61.8 mmol, 1.2 eq) was added dropwise. The reaction mixture was stirred for 24 h, then water was added, and the solution was extracted twice with AcOEt. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude compound **4** (13.8 g, 62 %)

was directly used without further purification. **Rf** = 0.65 (cyclohexane/AcOEt 95:5 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.55-7.20 (15H, m); 5.96 (1H, m); 5.30 (1H, d, *J* = 16 Hz); 5.15 (1H, d, *J* = 12 Hz); 4.20 (1H, dd, *J* = 4 Hz, *J* = 12 Hz); 4.02 (1H, dd, *J* = 8 Hz, *J* = 12 Hz); 3.48 (1H, qt, *J* = 8 Hz); 3.20 (1H, dd, *J* = 4 Hz, *J* = 12 Hz); 3.15 (1H, dd, *J* = 8 Hz, *J* = 12 Hz); 1.55 (2H, m); 1.27 (8H, bs); 0.88 (3H, t, *J* = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 144.1; 135.4; 128.6; 127.6; 126.8; 116.2; 86.4; 78.4; 71.1; 66.0; 32.1; 31.7; 29.2; 26.8; 25.3; 22.5; 14.0. **HRMS** (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{30}H_{36}O_2Na$ 451.2608; Found 451.2604.

2-(allyloxy)dodecan-1-ol (**5**). To a solution of **3** (3.4 g, 7.0 mmol, 1 eq) in MeOH was added portion-wise p-TsOH (85 mg, 0.7 mmol, 0.1 eq). The reaction mixture was stirred for 4 h then the solution was neutralized by addition of Et_3N and concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 90:10 v/v) to yield **5** as a colorless oil (1.37 g, 80 %). **Rf** = 0.21 (cyclohexane/AcOEt 90:10 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.90 (1H, m); 5.20 (2H, dd, *J* = 12 Hz, *J* = 16 Hz); 4.10 (2H, m); 3.65 (1H, m); 3.50 (1H, m); 3.40 (1H, m); 2.26 (1H, bs), 1.55 (1H, m); 1.45 (1H, m); 1.24 (16H, bs); 0.86 (3H, t, *J* = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 135.0; 116.8; 79.7; 70.4; 64.2; 31.8; 30.8; 29.8; 29.5; 29.3; 25.3; 22.6; 14.0. **HRMS** (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{15}H_{30}O_2Na$ 265.2143; Found 265.2142.

2-(allyloxy)octan-1-ol (**6**). To a solution of **4** (13.8 g, 32.2 mmol, 1 eq) in MeOH was added p-TsOH (0.38 g, 3.2 mmol, 0.1 eq), and the reaction mixture was stirred for 6 h. Then the solution was neutralized by addition of Et_3N, and concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 80:20 v/v) to yield **6** as colorless oil (3.66 g, 61 %). **Rf** = 0.35 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 5.90 (1H, m); 5.25 (1H, d, *J* = 16 Hz); 5.14 (1H, d, *J* = 12 Hz); 4.03 (2H, m); 3.63 (1H, m); 3.46 (1H, m); 3.38 (1H, m); 2.27 (1H, bs); 1.50 (2H, m); 1.26 (8H, bs); 0.85 (3H, t, *J* = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$,

100 MHz): δ 135.1; 116.8; 79.7; 70.5; 64.3; 31.7; 30.8; 29.4; 25.3; 22.6; 14.0. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{11}H_{23}O_2$ 187.1693; Found 187.1688.

2-((4,4,5,5,5-pentafluoro-2-iodopentyl)oxy)dodecan-1-ol (**7a**). To a solution of **5** (1.4 g, 5.8 mmol, 1 eq) in hexane, was added perfluoroethyl iodide (1.0 mL, 8.7 mmol, 1.5 eq) at -40 °C and in a sealed tube. The reaction mixture was stirred at -40 °C for 20 min, and triethylborane (0.5 mL, 3.5 mmol, 0.6 eq) was added dropwise. The reaction mixture was stirred for 24 h at room temperature then the solution was concentrated *in vacuo.* The crude compound was purified by flash chromatography (cyclohexane/AcOEt 90:10 v/v) to yield **7a** as a yellow oil (1.22 g, 43 %). **Rf** = 0.28 (cyclohexane/AcOEt 90:10 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.40 (1H, m); 3.80 (2H, m); 3.70 (1H, m); 3.60 (1H, m); 3.45 (1H, m); 3.10 (1H, m); 2.70 (1H, m); 2.40 (1H, m); 1.55 (1H, m); 1.46 (1H, m); 1.26 (16H, bs); 0.87 (3H, t, J = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 81.1; 73.9; 64.5; 37.5; 31.9; 30.8; 29.7; 29.6; 29.5; 29.4, 29.3; 25.4; 25.3; 22.7; 16.4; 15.8; 13.9. **^{19}F** ($CDCl_3$, 375 MHz): δ -85.8; -117.6.

2-((4,4,5,5,6,6,7,7,7-nonafluoro-2-iodoheptyl)oxy)dodecan-1-ol (**7b**). To a solution of **5** (1.5 g, 6.2 mmol, 1 eq) in hexane was added perfluorobutyl iodide (1.6 mL, 9.3 mmol, 1.5 eq) then the solution was cooled down to 0°C and triethylborane (0.55 mL, 3.8 mmol, 0.6 eq) was added dropwise. The mixture was stirred for 10 min at 0°C and for 24h at room temperature then the solution was concentrated *in vacuo.* The crude compound was purified by flash chromatography (cyclohexane/AcOEt 90:10 v/v) to yield **7b** as a yellow oil (1.7 g, 47 %). **Rf** = 0.49 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.40 (1H, m); 3.85-3.70 (2H, m); 3.65 (1H, m); 3.55 (1H, m); 3.42 (1H, m); 3.04 (1H, m); 2.70 (1H, m); 2.24-2.14 (1H, m); 1.55 (1H, m); 1.45 (1H, m); 1.24 (16H, bs); 0.86 (3H, t, J = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 81.2; 74.0; 64.4; 37.8; 37.6; 37.4; 31.9; 30.8; 29.7; 29.6; 29.5; 29.3; 26.9; 25.4; 25.3; 22.6; 16.3; 15.7; 14.0. **^{19}F** ($CDCl_3$, 375 MHz): δ -81.19; -114.01; -124.7; -126.0.

2-((4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluoro-2-iodononyl)oxy)dodecan-1-ol (**7c**). To a solution of **5** (0.5 g, 2.07 mmol, 1 eq) in hexane was added perfluorohexyl iodide (1.1g , 2.5 mmol, 1.2 eq) before the solution was cooled down to 0°C and triethylborane (0.4 mL, 0.4 mmol, 0.2 eq) was added dropwise. The mixture was stirred for 10 min at 0°C and for 3h at room temperature, then the solution was concentrated *in vacuo.* The crude compound was purified by flash chromatography (cyclohexane/AcOEt 90:10 v/v) to yield **7c** as a yellow oil (0.45 g, 32 %). **Rf** = 0.34 (cyclohexane/AcOEt 90:10 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.40 (1H, m); 3.90-3.70 (2H, m); 3.63 (1H, m); 3.51 (1H, m); 3.41 (1H, m); 3.10 (1H, m); 2.75 (1H, m); 2.45-2.36 (1H, m); 1.60 (1H, m). 1.50 (1H, m); 1.25 (16H, bs); 0.89 (3H, t, J = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 81.1; 74.3; 64.4; 37.7; 31.9; 31.0; 30.8; 29.7; 29.6; 29.6; 29.5; 29.5; 29.4; 29.3; 29.3; 25.4; 25.3; 22.6; 16.3; 15.7; 13.9. **^{19}F** ($CDCl_3$, 375 MHz): -81.2; -113.8; -122.0; -123.1; -123.9; -126.5. **MS** (ESI+) m/z = $[M + H]^+$ = 688.84.

2-((4,4,5,5,6,6,7,7,7-nonafluoro-2-iodoheptyl)oxy)octan-1-ol (**7d**). To a solution of **6** (1.0 g, 5.36 mmol, 1 eq) in hexane was added perfluorobutyl iodide (1.4 mL, 8.0 mmol, 1.5 eq), then the solution was cooled down to 0°C and triethylborane (0.47 mL, 3.22 mmol, 0.6 eq) was added dropwise. The mixture was stirred for 10 min at 0°C and for 24 h at room temperature then the solution was concentrated *in vacuo.* The crude compound was purified by flash chromatography (cyclohexane/AcOEt 90:10 v/v) to yield **7d** as a yellow oil (1.5 g, 52 %). **Rf** = 0.42 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 4.40 (1H, m); 3.82-3.70 (3H, m); 3.56 (1H, m); 3.46 (1H, m); 3.05 (1H, m); 2.70 (1H, m); 2.09-1.95 (1H, m); 1.58 (1H, m); 1.46 (1H, m); 1.28 (8H , bs); 0.88 (3H, t, J = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 81.2; 74.2; 64.5; 37.8; 37.6; 37.4; 31.7; 30.9; 30.8; 29.4; 25.3; 22.5; 16.3; 15.7; 14.0. **^{19}F** ($CDCl_3$, 375 MHz): -81.0; -113.8; -124.6; -125.9. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{15}H_{23}F_9IO_2$ 533.0599; Found 533.0605.

2-((4,4,5,5,5-pentafluoropentyl)oxy)dodecan-1-ol (**8a**). To a solution of **7a** (1.1 g, 2.3 mmol, 1 eq) in MeOH was added Pd-C (135 mg) and CH_3CO_2Na (0.60 g, 7.4 mmol, 3.2 eq). The reaction mixture was stirred overnight under a 6.5 bar pressure of H_2 in a hydrogenation reactor. Then, the solution was filtered through a pad of Celite and concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 80:20 v/v) to yield **8a** as a colorless oil (0.63 g, 76 %). **Rf** = 0.41 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.65 (1H, dd, J = 4 Hz, J = 12 Hz); 3.56 (2H, t, J = 8 Hz); 3.48 (1H, dd, J = 6 Hz, J = 12 Hz); 3.35 (1H, m); 2.15 (2H, m); 1.97 (1H, bs); 1.86 (2H, qt, J = 6 Hz); 1.59 (1H, m); 1.37 (1H, m); 1.25 (16H, bs); 0.87 (3H, t, J = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 80.4; 67.8; 64.3; 31.9; 30.7; 29.8; 29.6; 29.6; 29.5; 29.3; 27.9; 27.7; 27.5; 25.4; 22.7; 21.4; 14.0. **^{19}F** ($CDCl_3$, 375 MHz): δ -85.5; -118.3. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{17}H_{32}F_5O_2$ 363.2322; Found 363.2321.

2-((4,4,5,5,6,6,7,7,7-nonafluoroheptyl)oxy)dodecan-1-ol (**8b**). To a solution of **7b** (1.6 g, 2.7 mmol, 1 eq) in MeOH was added Pd-C (136 mg) and CH_3CO_2Na (0.70 g, 8.7 mmol, 3.2 eq). The reaction mixture was stirred overnight under a 6.5 bar pressure of H_2 in a hydrogenation reactor. Then, the solution was filtered through a pad of Celite and concentrated *in vacuo.* The crude compound was purified by flash chromatography (cyclohexane/AcOEt 80:20 v/v) to yield **8b** as a colorless oil (1.1 g, 88 %). **Rf** = 0.41 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.65 (1H, m); 3.57 (2H, m); 3.48 (1H, m); 3.34 (1H, m); 2.20 (2H, m); 2.08 (1H, bs); 1.88 (2H, qt, J = 6 Hz); 1.59 (1H, m); 1.37 (1H, m); 1.25 (16H, bs); 0.86 (3H, t, J = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 80.5; 67.9; 64.3; 31.9; 30.8; 29.8; 29.6; 29.6; 29.6; 29.5; 29.5; 29.3; 28.1; 27.8; 27.6; 25.4; 22.6; 21.2; 14.0. **^{19}F** ($CDCl_3$, 375 MHz): δ -81.2; -114.7; -124.6; -126.2. **HRMS** (ESI-TOF) m/z: $[M + Na]^+$ calcd for $C_{19}H_{31}F_9O_2Na$ 485.2078; Found 485.2072.

2-((4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)oxy)dodecan-1-ol (**8c**). To a solution of **7c** (1.0 g, 1.5 mmol, 1 eq) in MeOH was added Pd-C (80 mg) and CH_3CO_2Na (0.39 g, 4.8 mmol, 3.2 eq). The reaction mixture was stirred overnight under a pressure of H_2. Then, the solution was filtered through a pad of Celite and concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 80:20 v/v) to yield **8c** as a colorless oil (0.57 g, 68 %). **Rf** = 0.33 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.70 (1H, m); 3.60 (2H, m) ; 3.45 (1H, m); 3.35 (1H, m); 2.20 (2H, m); 2.00-1.80 (3H, m, CH_2); 1.60 (1H, m); 1.37 (1H, m); 1.25 (16H, bs); 0.86 (3H, t, J = 8 Hz). **^{13}C {1H}** NMR ($CDCl_3$, 100 MHz): δ 80.5; 67.9; 64.3; 31.9; 30.8; 29.8; 29.7; 29.6; 29.6; 29.5; 29.5; 29.4; 29.3; 28.2; 28.0; 27.7; 26.9; 25.4; 22.7; 21.2; 13.9. **^{19}F** ($CDCl_3$, 375 MHz): δ -81.2; -114.6; -122.2; -123.1; -123.7; -126.4. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{21}H_{32}F_{13}O_2$ 563.2195; Found 563.2192.

2-((4,4,5,5,6,6,7,7,7-nonafluoroheptyl)oxy)octan-1-ol (**8d**). To a solution of **7d** (1.5 g, 2.82 mmol, 1 eq) in MeOH was added Pd-C (160 mg) and CH_3CO_2Na (0.74 g, 9.0 mmol, 3.2 eq). The reaction mixture was stirred overnight under a 6.5 bar pressure of H_2 in a hydrogenation reactor. Then, the solution was filtered through a pad of Celite and concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 80:20 v/v) to yield **8d** as a colorless oil (0.53 g, 46 %). **Rf** = 0.38 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.63-3.41 (4H, m); 3.29 (1H, m); 2.52 (1H, bs); 2.14 (2H, m); 1.83 (2H, qt, J = 8 Hz); 1.47 (1H, m); 1.40 (1H, m); 1.24 (8H, bs); 0.83 (3H, t, J = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 80.5; 67.9; 64.2; 31.8; 31.7; 30.8; 29.4; 27.8; 26.8; 25.3; 22.5; 21.2; 13.8. **^{19}F** ($CDCl_3$, 375 MHz): δ -81.6; -114.9; -124.8; -126.4. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{15}H_{24}F_9O_2$ 407.1627; Found 407.1625.

(2-((4,4,5,5,5-pentafluoropentyl)oxy)dodecanyl tri-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**16a**). To a solution of **8a** (0.6 g, 1.66 mmol, 1 eq)

and **15** (2.43 g, 2.0 mmol, 1.2 eq) in anhydrous CH_2Cl_2, was added dropwise 1M TMSOTf in CH_2Cl_2 (1.6 mL, 1.66 mmol, 1 eq) at 0°C. The reaction mixture was stirred 2h at 0°C then overnight at room temperature. The solution was neutralized by addition of Et_3N and concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 80:20 v/v) to yield **16a** as a white solid (1.64 g, 70 %). **Rf** = 0.37 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 8.10-7.15 (35H, m); 6.30 (1H, t, *J* = 10 Hz); 6.01 (1H, m); 5. 80 (1H, m); 5.52 (1H, m); 5.08-4.45 (6H, m); 4.01-3.21 (9H, m); 2.13-1.80 (2H, m); 1.40-1.00 (20H, m); 0.90 (3H, t, *J* = 8 Hz). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 166.0-165.0; 133.0-128.0; 100.1; 96.9; 78.3; 75.5; 74.8; 73.4; 72.5; 71.4; 70.9; 69.9; 69.1; 68.8; 68.1; 63.4; 62.7; 31.7; 29.4; 29.3; 29.2; 29.1; 27.5; 27.4; 26.8; 25.2; 22.5; 21.3; 13.9. **^{19}F** ($CDCl_3$, 375 MHz): δ -85.4; -118.2. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{78}H_{80}F_5O_{19}$ 1415.5214; Found 1415.5193.

2-((4,4,5,5,6,6,7,7,7-nonafluoroheptyl)oxy)dodecanyl tri-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**16b**). To a solution of **8b** (1.1 g, 2.33 mmol, 1 eq) and **15** (2.92 g, 2.4 mmol, 1 eq) in anhydrous CH_2Cl_2, was added dropwise 1 M TMSOTf in CH_2Cl_2 (2.4 mL, 2.4 mmol, 1 eq) at 0°C. The reaction mixture was stirred for a few minutes at 0°C then 2h at room temperature. The solution was neutralized by addition of Et_3N and concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 80:20 v/v) to yield **16b** as a white solid (2.87 g, 79 %). **Rf** = 0.3 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 8.16-7.16 (35H, m); 6.30 (1H, t, *J* = 10 Hz); 6.10 (1H, m); 5.90-5.70 (2H, m); 5.52-5.28 (2H, m); 5.10-4.25 (7H, m); 4.20 (1H, m); 3.60-3.15 (4H, m); 2.0 (2H, m); 1.50-1.00 (20H, m); 0.89 (3H, m). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 166.0-165.0; 133.0-128.0; 100.0; 96.5; 78.4; 78.3; 74.9; 73.9; 73.3; 72.2; 71.7; 70.9; 69.9; 69.1; 68.4; 63.5; 62.5; 31.8; 29.5; 25.4; 22.7; 14.1. **^{19}F** ($CDCl_3$, 375 MHz): δ -81.1; -

114.6; -124.6; -126.6. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{80}H_{80}F_9O_{19}$ 1515.5150; Found 1515.5170.

2-((4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)oxy)dodecanyl tri-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**16c**). To a solution of **8c** (1.0 g, 1.78 mmol, 1 eq) and **15** (3.0 g, 2.5 mmol, 1.4 eq) in anhydrous CH_2Cl_2, was added dropwise 1 M TMSOTf in CH_2Cl_2 (1.8 mL, 1.78 mmol, 1 eq) at 0°C. The reaction mixture was stirred 2h at 0°C then overnight at room temperature. The solution was neutralized by addition of Et_3N and concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 80:20 v/v) to yield **16c** as a white solid (2.2 g, 76 %). **Rf** = 0.34 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 8.21-7.16 (35H, m); 6.34-6.16 (1H, m); 6.04 (1H, m); 5.82 (1H, m); 5.50 (1H, m); 5.10-4.40 (6H, m); 4.13-3.22 (9H, m); 2.21-1.84 (2H, m); 1.50-1.00 (20H, m); 0.92 (3H, m). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): δ 166.0-165.0; 133.4-128.3; 100.7; 97.0; 79.0; 78.4; 75.6; 74.7; 73.4; 72.5; 72.2; 71.4; 70.0; 69.1; 68.9; 63.4; 62.8; 31.9; 31.8; 31.6; 31.6; 29.5; 29.4; 29.3; 25.3; 25.2; 22.6; 14.1. **^{19}F** ($CDCl_3$, 375 MHz): δ –80.9; -114.3; -121.9; -122.8; -123.5; -126.1. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{82}H_{80}F_{13}O_{19}$ 1615.5086; Found 1615.5267.

2-((4,4,5,5,6,6,7,7,7-nonafluoroheptyl)oxy)octanyl tri-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**16d**). To a solution of **8d** (0.53 g, 1.3 mmol, 1 eq) and **15** (1.58 g, 1.3 mmol, 1 eq) in anhydrous CH_2Cl_2, was added dropwise TMSOTf (1.3 mL, 1.3 mmol, 1 eq) at 0°C. The reaction mixture was stirred for a few minutes at 0°C then overnight at room temperature. The solution was neutralized by addition of Et_3N and concentrated *in vacuo*. The crude compound was purified by flash chromatography (cyclohexane/AcOEt 80:20 v/v) to yield **16d** as a white solid (0.97 g, 51 %). **Rf** = 0.38 (cyclohexane/AcOEt 80:20 v/v). **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 8.16-7.16 (35H, m); 6.30 (1H, t, *J*

= 10 Hz); 6.10 (1H, m); 5.90-5.70 (2H, m); 5.52-5.28 (2H, m); 5.10-4.25 (7H, m); 4.20 (1H, m); 3.60-3.15 (5H, m); 2.0 (2H, m); 1.50-1.00 (12H, m); 0.89 (3H, m). **^{13}C {1H} NMR** ($CDCl_3$, 100 MHz): *δ* 166.0-165.0; 133.0-128.0; 100.0; 96.5; 78.4; 78.3; 74.9; 73.9; 73.3; 72.2; 71.7; 70.9; 69.9; 69.1; 68.4; 63.5; 62.5; 33.1; 31.8; 29.5; 25.4; 23.7; 22.7; 14.1.**^{19}F NMR** ($CDCl_3$, 375 MHz): *δ* -81.0; -114.5; -124.4; -126.0.

2-((4,4,5,5,5-pentafluoropentyl)oxy)dodecanyl 4-O-(α-D-glucopyranosyl)-β-D-glucopyranoside (**17a**). To a solution of **16a** (1.8 g, 1.27 mmol, 1 eq) in MeOH was added MeONa (0.027 g, 0.5 mmol, 0.4 eq) and the reaction mixture was stirred for 4 h. The solution was neutralized by addition of IRC-50, filtered and concentrated *in vacuo*. The crude compound was purified by flash chromatography ($CHCl_3$/MeOH 90:10 v/v) to yield **17a** as a white solid (480 mg, 55 %). **Rf** = 0.32 ($CHCl_3$/MeOH 80:20 v/v). **^{1}H NMR** (CD_3OD, 400 MHz): *δ* 5.12 (1H, m); 4.25 (1H, m); 3.92-3.16 (17H, m); 2.29-2.11 (2H, m); 1.77 (2H, qt, *J* = 8 Hz); 1.48 (2H, m); 1.26 (16H, bs); 0.86 (3H, t, *J* = 8 Hz). **^{13}C {1H} NMR** (CD_3OD, 100 MHz): *δ* 105.0; 103.1; 81.4; 80.2; 77.9; 76.7; 75.1; 74.8; 74.2; 73.2; 72.5; 71.5; 69.4; 62.8; 62.2; 33.0; 32.8; 30.7; 30.5; 28.7; 26.6; 23.7; 22.4; 14.4. **^{19}F** (CD_3OD, 375 MHz): *δ* - 85.4; -118.1. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{29}H_{52}F_5O_{12}$ 687.3379; Found 687.3373. **Anal.** Calcd for $C_{29}H_{51}F_5O_{12}$: C, 50.72; H, 7.49; Found: C, 49.44; H, 7.52.

2-((4,4,5,5,6,6,7,7,7-nonafluoroheptyl)oxy)dodecanyl 4-O-(α-D-glucopyranosyl)-β-D-glucopyranoside (**17b**). To a solution of **16b** (2.9 g, 1.91 mmol, 1 eq) in MeOH was added MeONa (0.041 g, 0.77 mmol, 0.4 eq) and the reaction mixture was stirred for 4 h. The solution was neutralized by addition of IRC-50, filtered, and concentrated *in vacuo*. The crude compound was purified by flash chromatography ($CHCl_3$/MeOH 90:10 v/v) to yield **17b** as a white solid (1.5 mg, 100 %). **Rf** = 0.38 ($CHCl_3$/MeOH 80:20 v/v). **^{1}H NMR** (CD_3OD, 400 MHz): *δ* 5.19 (1H, m); 4.31 (1H, m); 3.96-3.24 (17H, m); 2.38-2.20 (2H, m); 1.85 (2H, qt, *J* = 8 Hz); 1.54

(2H, m); 1.31 (16H, bs); 0.91 (3H, t, *J* = 8 Hz). **^{13}C {1H} NMR** (CD_3OD, 100 MHz): *δ* 104.8; 102.9; 81.4; 80.2; 77.8; 76.7; 75.1; 74.8; 74.2; 73.3; 72.6; 71.5; 69.4; 62.8; 62.3; 33.1; 32.9; 32.8; 30.8; 30.7; 30.5; 28.9; 26.7; 23.8; 22.2; 14.5. **^{19}F** (CD_3OD, 375 MHz): *δ* -82.58; -115.7; -125.5; -127.2. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{31}H_{52}F_9O_{12}$ 787.3315; Found 787.3314. **Anal.** Calcd for $C_{31}H_{51}F_9O_{12}$: C, 47.33; H, 6.53; Found: C, 48.16; H, 7.02.

2-((4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)oxy)dodecanyl 4-O-(α-D-glucopyranosyl)-β-D-glucopyranoside (**17c**). To a solution of **16c** (2.5 g, 1.55 mmol, 1 eq) in MeOH was added MeONa (0.033 g, 0.6 mmol, 0.4 eq), and the reaction mixture was stirred for 4 h. The solution was neutralized by addition of IRC-50, filtered, and concentrated *in vacuo*. The crude compound was purified by flash chromatography ($CHCl_3$/MeOH 90:10 v/v) to yield **17c** as a white solid (1.25 g, 91 %). **Rf** = 0.41 ($CHCl_3$/MeOH 80:20 v/v). **^{1}H NMR** (CD_3OD, 400 MHz): *δ* 5.18 (1H, m); 4.30 (1H, m); 3.96-3.23 (17H, m); 2.39-2.22 (2H, m); 1.86 (2H, qt, *J* = 8 Hz); 1.52 (2H, m); 1.23 (16H, bs); 0.91 (3H, t, *J* = 8 Hz). **^{13}C {1H} NMR** (CD_3OD, 100 MHz): *δ* 104.8; 102.9; 81.4; 80.1; 77.8; 76.6; 75.1; 74.7; 74.1; 73.2; 72.5; 71.5; 69.3; 62.7; 62.2; 33.0; 32.9; 32.7; 30.7; 30.4; 28.9; 26.5; 23.7; 22.2; 14.4. **^{19}F** (CD_3OD, 375 MHz): *δ* - 82.4; -115.5; -122.9; -123.9; -124.5; -127.3. **HRMS** (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{33}H_{52}F_{13}O_{12}$ 887.3251; Found 887.3273. **Anal.** Calcd for $C_{33}H_{51}F_{13}O_{12}$: C, 44.70; H, 5.80; Found: C, 44.01; H, 6.11.

2-((4,4,5,5,6,6,7,7,7-nonafluoroheptyl)oxy)octanyl 4-O-(α-D-glucopyranosyl)-β-D-glucopyranoside (**17d**). To a solution of **16d** (0.97 g, 0.66 mmol, 1 eq) in MeOH was added MeONa (0.014 g, 0.26 mmol, 0.4 eq), and the reaction mixture was stirred for 4 h. The solution was neutralized by addition of IRC-50, filtered, and concentrated *in vacuo*. The crude compound was purified by flash chromatography ($CHCl_3$/MeOH 90:10 v/v) to yield **17d** as a white solid (0.38 g, 79 %). **Rf** = 0.43 ($CHCl_3$/MeOH 80:20 v/v). **^{1}H NMR** (CD_3OD, 400 MHz): *δ* 5.12 (1H, m); 4.26 (1H, m); 3.91-3.17 (17H, m); 2.24 (2H, m); 1.80 (2H, qt, *J* = 8 Hz); 1.48 (2H,

m); 1.27 (8H, bs); 0.86 (3H, m). **^{13}C {1H} NMR** (CD_3OD, 100 MHz): δ 104.8; 102.8; 81.3; 80.1; 77.8; 76.6; 75.0; 74.7; 74.1; 73.2; 72.5; 71.4; 69.3; 62.7; 62.2; 33.0; 32.9; 30.4; 28.8; 26.6; 23.6; 22.2; 14.4. **^{19}F** (CD_3OD, 375 MHz): δ -82.6; -115.6; -125.5; -127.2. **HRMS** (ESI-TOF) m/z: $[M + Na]^+$ calcd for $C_{27}H_{43}F_9O_{12}Na$ 753.2509; Found 753.2515. **Anal.** Calcd for $C_{27}H_{43}F_9O_{12}$: C, 44.39; H, 5.83; Found: C, 43.49; H, 5.94.

Water solubility. *Pure compounds.* Stock solution of hybrid were prepared at 1g/L. The solution was sonicated in a sonic bath at 40°C for 15 min. Centrifugation for 10 min at 5000 rpm of the cloudy opalescent solutions obtained led to the formation of a clear supernatant solution and a precipitate. The supernatant solutions was further used for DLS and TEM experiments. *DDM:LC mixtures.* The mixtures were prepared using molar ratio DDM:LC 100:4 and 100:2 for **LC045-LC047** and 100:10 for **LC057**. The hybrid compounds were solubilized in a 1.7 mM solution of DDM (10 CMC). Mixtures were sonicated using a sonication bath for a few minutes then solutions were centrifuged at 5000 rpm for 10 min. The resulting solutions were further used for DLS and TEM experiments.

DLS measurements. Hydrodynamic particle size distributions were determined on a Zetasizer Nano-S model 1600 (Malvern Instruments, UK) equipped with a He−Ne laser (λ = 633 nm, 4.0 mW). All measurements were performed at (25 ± 0.5)°C. The time dependent correlation function of the scattered light intensity was measured at an angle of 173° (backscattering detection). The hydrodynamic diameter (D_H) of the particles was estimated from their diffusion coefficient (D) using the Stokes−Einstein equation, $D = k_BT/3\pi\eta D_H$, where k_B is Boltzmann's constant, T absolute temperature, and η the viscosity of the solvent. Contin analysis was used for evaluating autocorrelation functions. All measurements were performed at (25 ± 0.5)°C.

TEM. The negatively stained samples were prepared by spreading 5 µL of the surfactant solution (0.5 mM) onto a Cu grid coated with a Formvar-film (PLANO, Wetzlar, Germany). After 1 min excess liquid was blotted off with filter paper and 5 µL of 1% aqueous uranyl acetate solution were placed onto the grid and drained off after 1 min. The dried specimens were examined with an EM 900 transmission electron microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). Micrographs were taken with a SSCCD SM-1k-120 camera (TRS, Moorenweis, Germany).

Solubilization. The experiments were performed as previously described.[27] Briefly, membrane fractions were incubated for 2h at 4°C at a final concentration of 5mg/mL in 50mM Hepes buffer pH 7.4, 200 mM NaCl, 1X protease inhibitor cocktail, and with 10-fold the CMC of DDM in combination with CHS (100:10 ratio) or LC compounds (100:2, 100:4 or 100:10 ratios). After solubilization samples were centrifuged at 100000g for 45min at 4°C and an aliquot of the total extract, the pellet and the supernatant from each solubilization condition was analyzed by SDS-PAGE and western-blot using an antibody $A_{2A}R$ (7F6-G5-A2) and against the his-tag for $A_{2A}R$ and AcrB, respectively.

Thermostability assay. Membranes of $A_{2A}R$ or AcrB (4mg/mL total protein) were solubilized in different conditions (see solubilization method above) for 2 hours at 4°C. Solubilized fractions were obtained after 100,000g ultracentrifugation for 1h at 4°C. Solubilized fraction serves to make 50µL aliquots to be submitted to one temperature each as part of a gradient of temperature ranging from 25 to 72°C using PCR thermal cycler (PeqSTAR 2x gradient; Peqlab). Samples were then centrifuged 40min at 20000g and supernatants were analyzed by SDS-PAGE and western-blot using anti- $A_{2A}R$ antibody (7F6-G5-A2). The relative intensity of the target protein at each temperature was quantified on Western-blot using Image Lab software 4.1 from Bio-rad. Each condition was performed twice. Intensity was plotted as a

function of the temperature, normalized and fit to the Boltzmann equation with the least square method using Solver Adds-in of Excel software. The method is described by Ashok et al.[30]

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Supplementary material available. ^{1}H, ^{13}C {1H} and ^{19}F NMR spectra of compounds; Water-solubility of DDM:LC mixtures; Contin distribution plots for DDM:LC mixtures in water.

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