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Article

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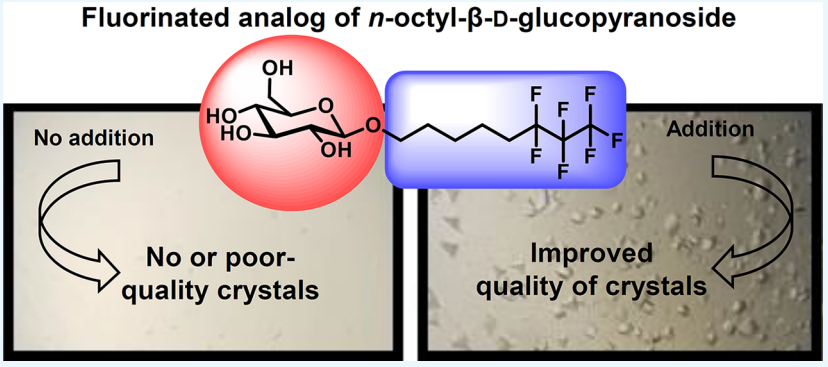

ABSTRACT: We report herein the synthesis of a series of fluorinated surfactants with a glucose moiety as a polar head group and whose alkyl chain was varied in length and in fluorine/hydrogen ratio. They were synthesized in two or four steps in 20 to 50% overall yields allowing gram-scale synthesis. Their solubility in water is between 0.2 and 13.8 g/L, which indicates low water solubility. Two derivatives of the series were found to form micelles in water at ∼11 mM. Their hydrophilic–lipophilic balance was determined both by Griffin's and Davies' methods; they may exhibit a "harsh" character toward membrane proteins. This, combined with their low water solubility, suggest that they could advantageously be used in detergent mixtures containing a "mild" detergent. Finally, the potency of one of the derivatives, F_3H_5-*β*-Glu, to act as an additive for the crystallization of AcrB was evaluated in detergent mixtures with *n*-dodecyl-*β*-D-maltopyranoside (DDM). Among the six crystallization conditions investigated, adding F_3H_5-*β*-Glu improved the crystallization for three of them, as compared to control drops without additives. Moreover, preliminary tests with other compounds of the series showed that none of them hampered crystallization and suggested improvement for three of them. These novel glucose-based fluorinated detergents should be regarded as potential additives that could be included in screening kits used in crystallization.

INTRODUCTION

Elucidating structures of membrane proteins (MPs), known to be among major drug targets, still remains a challenge. Progresses in various fields, diversification of hosts for protein expression, emergence of novel detergents,[1] new approaches in sample preparation like incorporation in nanodiscs,[2] and crystallization in lipidic cubic phases,[3] combined with functional and biophysical approaches, increased drastically the number of membrane proteins that are amenable to crystallography and cryoelectron microscopy (cryoEM).[4] However, a large number of membrane proteins are still unstable or not sufficiently soluble in artificial amphiphilic environments, leading to inhomogeneous solutions. Stability and homogeneity of the protein solution are also needed when forming nanodiscs used in cryoEM or when incorporating proteins into lipidic cubic phases for crystallization. In addition, crystallization of membrane proteins using the vapor diffusion method relies on the behavior of a ternary mixture of water, detergent, and precipitant (for review see ref 5 and references herein). Crystallization is known to be favored while approaching the consolution boundary above which a phase separation appears. Getting close to the boundary favors interactions between detergent micelles and thus between protein–detergent complexes.

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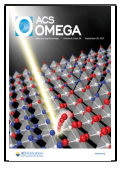

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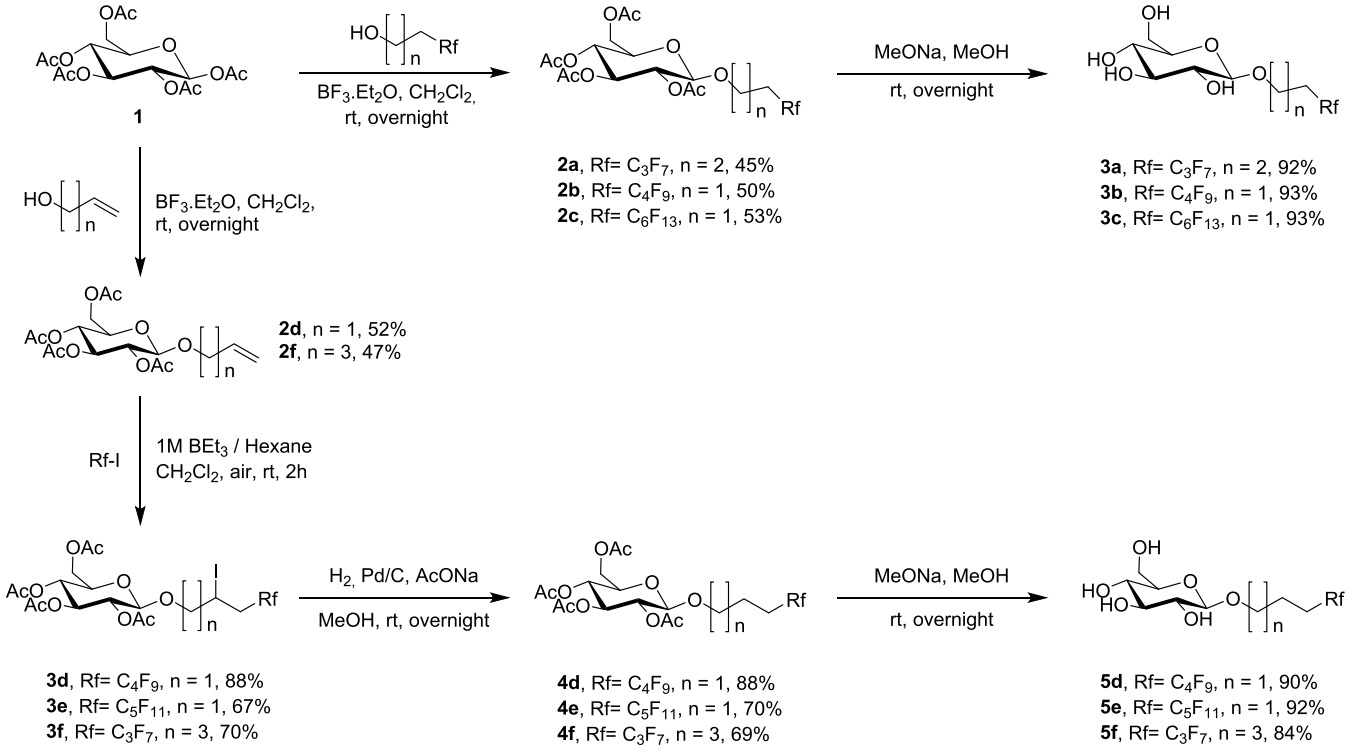
Scheme 1. Synthesis of Glucose-Based Fluorinated Derivatives



Sugar-based detergents have been the most widely used class of detergents for structural studies on MPs. The two maltose-based *n*-dodecyl-*β*-D-maltopyranoside (DDM) and *n*-decyl-*β*-D-maltopyranoside (DM) have been used for the determination of about half of the deposited structures of MPs in the Protein Data Bank (PDB). Including also the two glucose-based *n*-octyl-*β*-D-glucopyranoside (OG) and *n*-nonyl-*β*-D-glucopyranoside (NG) in the listing makes the number of deposited structures around two-thirds, demonstrating the vast use of sugar-based detergents.[6] Over the last decade, there has been a growing number of examples where chemical additives or secondary detergents were successfully used for the crystallization of MPs.[1,7] Additives are known to play an important role as they modulate the behavior of the protein−detergent complex. This was already observed in the crystallization of the first membrane protein that led to structure elucidation[8] and since then about one-third of integral MPs were crystallized in a mixture of detergents. As the effect of an additive will depend on the protein and the chemical nature of all of the compounds, in particular the detergent, and their concentrations, none of the additives is expected to be universal. Increasing the number of additives that showed crystallization improvement even in a limited number of cases is therefore of interest.

A tentative rationalization in the selection and design of detergents for MP crystallization has been proposed by Breibeck and Rompel.[1] The concept of hydrophilic−lipophilic balance (HLB) allows to classify common detergents into "harsh" and "mild" groups. While harsh detergents are usually preferred to extract MPs because of their propensity to disrupt lipid bilayers, they are often exchanged with "milder" detergents in which the target proteins are more protected against denaturation. Harsh detergents with shorter alkyl chains such as OG are prone to favor crystal contacts and thus yield crystals diffracting to a higher resolution but often increase protein denaturation. By contrast, fluorinated surfactants exhibit rather limited miscibility with lipids and lipid cofactors and therefore can be considered as mild stabilizing detergents, particularly for fragile MPs (for a general review see ref 9). However, despite their well-recognized "mildness", recent findings demonstrated that fluorinated surfactants can also act as solubilizing agents and extract MPs.[10−13] Fluorinated surfactants have also been successfully used to reconstitute MPs in the artificial bilayer.[14,15] Mixtures of fluorinated and hydrogenated surfactants lead to various micellar organizations, depending on the molecule type and concentration.[16,17] It is thus expected that fluorinated surfactants affect membrane protein crystallization, even if benefits are hardly predictable.

Some original high-resolution structures of membrane proteins were obtained with the help of the commercially available fluorinated surfactant 1*H*,1*H*,2*H*,2*H*-perfluorooctyl)-*β*-D-maltopyranoside (F_6OM) or its phosphocholine analogue (F_6OPC). They were generally selected from a screen of secondary detergents as additives (at ∼0.1% while the hydrogenated detergent is in % amounts) to improve crystal quality. For example, addition of F_6OM improved the diffraction from 5 to 4 Å of crystals of RibU, the S component of the ECF-type riboflavin transporter of *Staphylococcus aureus*.[18] F_6OM was also used as an additive to crystallize the membrane domain of the electron transport chain complex I of *Escherichia coli*, leading to a structure at 3.9 Å resolution,[19] and F_6OPC as an additive for the crystallization of the entire complex I of *Tetraopes thermophilus*, leading to a structure at 3.3 Å resolution.[20] Crystals of mitochondrial cytochrome *c* oxidase from bovine heart, CcO, alone[21] or in complex with cytochrome *c*,[22] were crystallized using F_6OM as the main detergent, allowing structure determination at 1.77 and 2.0 Å, respectively. F_6OPC is also indicated as an additive improving the crystal quality of a couple of soluble proteins.[23,24]

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In this work, we focused our attention on the design and the synthesis of OG analogues bearing a partially fluorinated chain as additives for crystallization. We report herein the synthesis of a series of glucose-based fluorinated surfactants whose alkyl chain was varied in length and in fluorine/hydrogen ratio. The solubility in water of the series as well as their ability to assemble into micelles was further investigated and was correlated to their hydrophilic−lipophilic balance (HLB). Finally, their potency to act as additives for the crystallization of AcrB was evaluated in detergent mixtures with DDM.

RESULTS AND DISCUSSION

Synthesis. The fluorinated glucoside derivatives were synthesized, as illustrated in Scheme 1, using two different synthetic pathways depending on the availability of the fluorinated alcohols (Rf-OH). The first synthetic pathway involves the glycosylation of an excess of commercially available fluorinated alcohols (Rf-OH, 1.5 equiv) with β-D-glucose pentaacetate in the presence of $BF_3{\cdot}Et_2O$.[25] The crude compounds were purified by flash chromatography to afford pure compounds **2a−c** in 45−53% yields. The β-anomeric configuration of the three compounds was confirmed by ^1H and ^{13}C NMR spectra. The acetyl protective groups were then removed using a catalytic amount of MeONa in MeOH to afford the desired compounds **3a−c** in 92−93% yields after silica gel flash chromatography purification.

The other derivatives **5d−f** were prepared in four steps. This synthetic route relies on the insertion of an allyl group onto which fluorinated chains were further grafted. First, the allyl-based compounds **2d** and **2f** were obtained in ∼50% yield from β-D-glucose pentaacetate by glycosylation of allyl alcohol or 4-penten-1-ol. The double bond of the obtained compounds **2d** and **2f** was then subjected to a free radical reaction with perfluoroalkyl iodide in the presence of 1M BEt_3 in hexane and oxygen, using the methodology described by Takeyama and co-workers.[26] Under these conditions, the compounds **3d−f** were obtained in good yields, ranging from 67% for compound **3e** to 88% for compound **3d**. The addition of the fluoroalkyl chains on the double bond was confirmed by ^1H and ^{13}C NMR, which showed the disappearance of the signals corresponding to the CH_2 and CH of the double bond and the formation of new signals related to the CH−I group. The iodide group of compounds **3d−f** was then reduced under a H_2 atmosphere in the presence of Pd/C as the catalyst and led to compounds **4d−f** in 69−88% yields. Finally, the acetyl groups were removed using a catalytic amount of MeONa in MeOH to afford the desired compounds **5d−f** in 84−92% yields after silica gel flash chromatography purification. All of the fluorinated detergents were freeze-dried after purification.

Physical−Chemical Characterization. For the sake of clarity in the discussion, the fluorinated surfactants were denoted F_nH_m-β-Glu, where n and m indicate, respectively, the number of fluorinated and hydrogenated carbons within the hydrophobic chain and -β-Glu indicates the glucose polar head with a β configuration of the anomeric carbon.

Water Solubility. The water solubility of the F_nH_m-β-Glu series was determined by turbidity measurement of the aqueous solution using a CrystalEYES system. Figure 1 depicts the evolution of the turbidity of aqueous solutions of F_nH_m-β-Glu at varying concentrations. As we can see, at a low concentration, the turbidity remains constant over the first range of concentrations at about 0.5 to 1.0 Nephelometric Turbidity Units (NTU) and then a break is observed and the turbidity starts to increase steadily with the concentration. The linear fitting of the two sets of points led to a concentration value that was taken as the water solubility limit of the derivatives. The values are reported in Table 1 and they range from 5.2 to 36.0 mM, demonstrating the poor water solubility of the compounds. For F_6H_2-β-Glu and F_5H_3-β-Glu, the water solubility could not be determined by this technique as 1 mM solutions were already turbid. For these two compounds, a visual determination was conducted. A saturated solution was first prepared and then diluted until a clear limpid solution was observed.

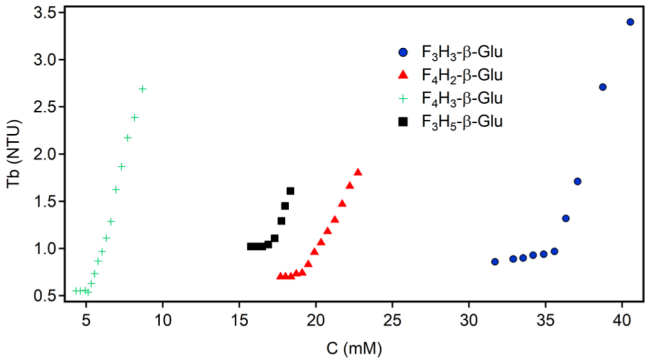

Figure 1. Turbidity measurement of an aqueous solution of F_3H_3-β-Glu (**3a**), F_4H_2-β-Glu (**3b**), F_4H_3-β-Glu (**5d**), and F_3H_5-β-Glu (**5f**) at 25 °C.

Surface Tension Measurement. The surface tension activity of aqueous solutions of F_nH_m-β-Glu was next investigated by the Wilhelmy plate method. Since surface tension requires large volumes of stock solution at ∼3−4 times the CMC and therefore needs large amounts of surfactants when the CMC is expected to be high, we did not test the most hydrophilic derivatives F_3H_3-β-Glu, F_3H_5-β-Glu, and F_4H_2-β-Glu. Stock solutions of F_4H_3-β-Glu, F_5H_3-β-Glu, and F_6H_2-β-Glu were prepared at 4.3, 0.73, and 0.32 mM, respectively. The surface tension of these stock solutions was measured, and as seen in Figure 2 for the three derivatives tested, a quite low surface tension was measured ranging from 20.8 and 27.2 mN/m. However, the constant addition of a fixed amount of water failed to show any plateau in the surface tension. Instead, a regular increase in the surface tension was observed with the dilution, demonstrating a surface activity of these components. This indicates that no micelles can be formed by these three compounds due to their limited water solubility. This suggests that precipitation of the derivatives occurs before reaching the "theoretical" CMC.

^{19}F Nuclear Magnetic Resonance. For the more water-soluble derivatives, F_4H_2-β-Glu and F_3H_5-β-Glu, the CMC could be measured by ^{19}F NMR, as shown in Figure 3. Indeed, ^{19}F NMR is an appropriate method for surfactants with high CMC as it requires low volumes of stock solution. We followed the signal of the CF_3 group and plotted the variation in chemical shift as a function of the surfactant concentration to derive the CMC value. The values of CMC are reported in Table 1 and show that the CMC values of F_4H_2-β-Glu and F_3H_5-β-Glu are very close to their water solubility, indicating that resorting to surface tension measurements to confirm the CMC would have been difficult. This clearly contrasts with the fully hydrogenated OG whose CMC ranges from 19 to 25 mM depending on the techniques used to evaluate it and whose water solubility is above 100 g/L.

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Table 1. Water Solubility, CMC, and Griffin (HLBG) and Davies (HLBD) Hydrophilic−Lipophilic Balance of the Synthesized Surfactants

			water solubility		CMC			
compound	F_nH_m-β-Glu	M (g/mol)	mM	g/L	mM	g/L	HLBG	HLBD
3a	F_3H_3-β-Glu	390.3	35.3	13.8	[c]		9.2	9.4
3b	F_4H_2-β-Glu	426.2	19.3	8.2	10.9[d]	4.6[d]	8.4	9.0
3c	F_6H_2-β-Glu	526.3	≈0.3[a]	≈0.2[a]	[e]		6.8	7.3
5d	F_4H_3-β-Glu	440.3	5.2	2.3	[e]		8.1	8.6
5e	F_5H_3-β-Glu	490.3	≈0.7[a]	≈0.3[a]	[e]		7.3	7.7
5f	F_3H_5-β-Glu	418.3	17.0	7.1	11.7[d]	4.9[d]	8.5	8.5
n-octyl-β-D-glucopyranoside		292.4	>342[b]	>100[b]	25[f]	7.3[f]	12.3 (11.2)[g]	9.7

[a]Visual determination (a limpid solution was observed). [b]Data from commercial sources. [c]Not determined. [d]Determined by ^{19}F NMR. [e]Measured by surface tension measurement; no micelle formation. [f]Measured by surface tension measurement; data from ref 27. [g]Data from ref 1.

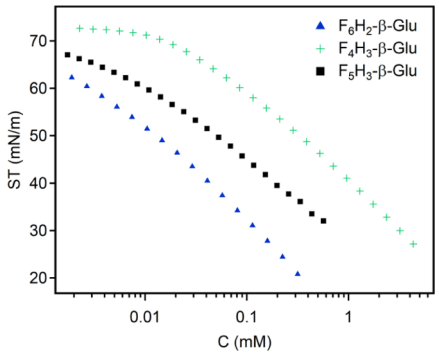

Figure 2. Surface tension measurement of F_6H_2-β-Glu (**3c**), F_4H_3-β-Glu (**5d**), and F_5H_3-β-Glu (**5e**) at 25 °C.

Hydrophilic−Lipophilic Balance (HLB). The hydrophilic−lipophilic balance (HLB) introduced by Griffin is a valuable reference to characterize a detergent. It is calculated from the ratio of the hydrophilic vs total molar mass of the surfactant.[28,29] Later, Davies suggested a method based on the nature of chemical groups of the molecule.[30] The advantage of this latter method is that it takes into account the effect of stronger and weaker hydrophilic groups as well as the contribution of the fluorinated carbons.[31,32] The higher accuracy of composition-calculated HLB over the Griffin method has been discussed in the literature.[33] The HLB of our derivatives was calculated according to both the Griffin (HLBG) and the Davies (HLBD) equations; the values are presented in Table 1. Since it is now admitted that the first methylene of the chain connected to the polar head group is part of the hydration sphere, it was excluded from the calculation of the HLBD.[1] The concept of hydrophilic−lipophilic balance allows to classify common detergents into harsh and mild groups and its use in the selection and design of detergents for MP crystallization has been reviewed and discussed by Breibeck and Rompel.[1] In the literature, the detergents that have been successfully used for the stabilization and/or crystallization of MPs have moderate HLBG values near 12, ensuring long-time compatibility with the protein.[1] However, the "harshness" of the detergent depends on other parameters. Indeed, detergents with a harsh character usually feature small and/or charged head groups and short alkyl chains. For example, the dodecyl maltoside DDM with an HLBG value of 13.4 (12.3 from ref 1) is classified as a mild detergent, while the octyl glucoside OG with an HLBG value of 12.2 (11.2 from ref 1) belongs to the harsh series. Resorting to the Davies method shows stronger differences in the HLB values of the fully hydrogenated detergents. Indeed, we calculated values of 13.3 and 9.7 for DDM and OG, respectively. With regard to our fluorinated series, a very good agreement was noted between the two methods, with HLB values being between 7 and 9. Thus, our fluorinated derivatives should be considered harsh detergents. The use of a detergent mixture containing a mild detergent in combination with our glucose-based detergents was therefore chosen so as to investigate their potency in MP crystallization.

We tested all of the compounds as additives for the crystallization of AcrB, an efflux pump located in the inner membrane of *E. coli*. AcrB is easy to purify and crystallize. Several structures are deposited in the PDB. Interestingly, large screening kits lead to hits with several crystallization conditions, only a few of them are amenable to high-resolution diffraction. This opens a large field of different conditions to explore. We used the 96 conditions of the MemGold screen

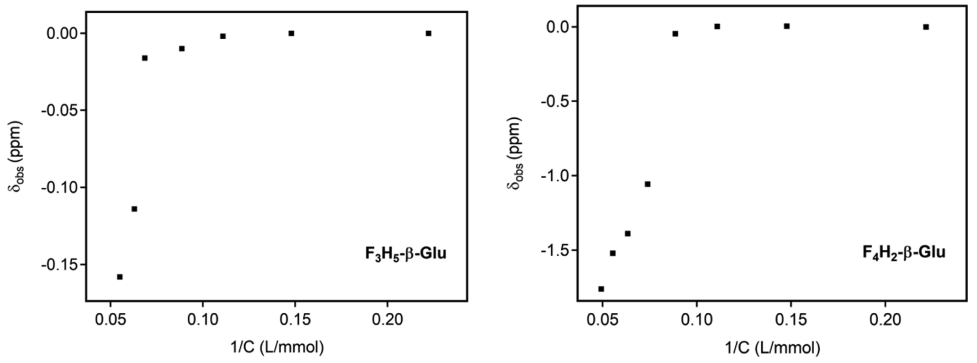

Figure 3. Determination of the CMC of F_3H_5-β-Glu (**5f**) and of F_4H_2-β-Glu (**3b**) by ^{19}F NMR (376 MHz) at 25 °C.

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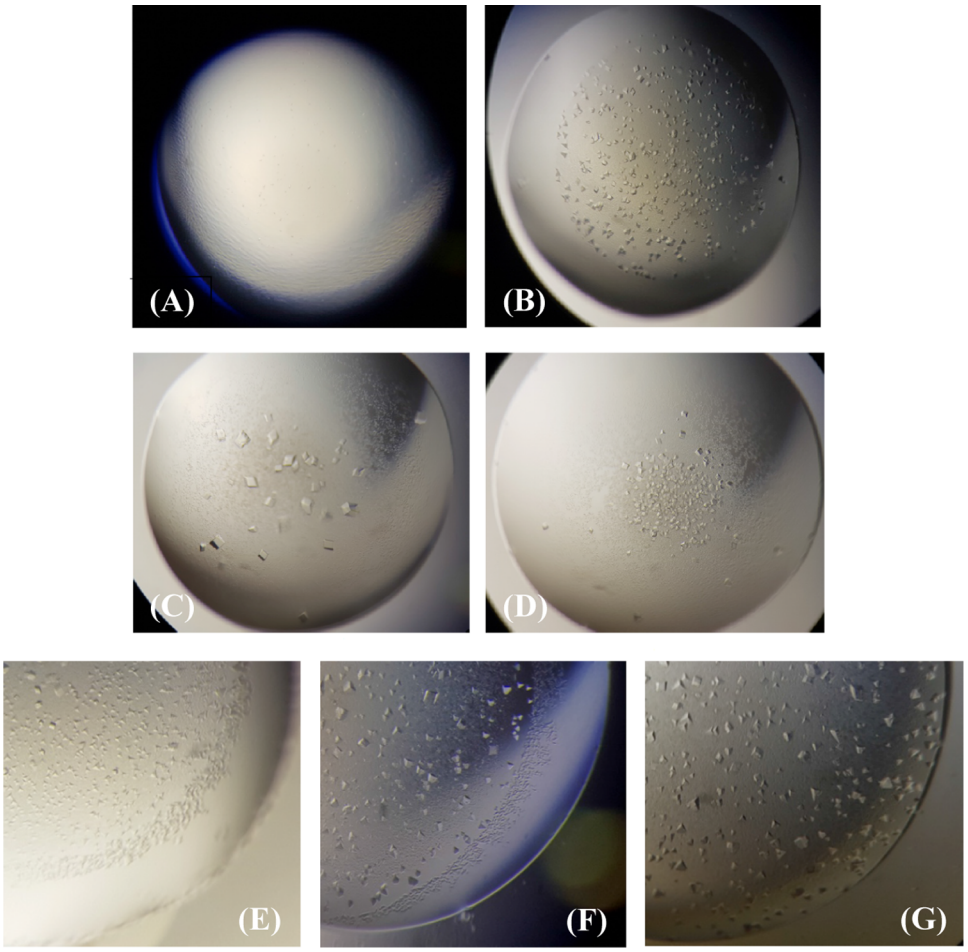

Figure 4. (A) Condition 1, control DDM (0.02%); (B) condition 1, DDM (0.02%)-F_3H_5-β-Glu (0.002%); (C) condition 4, DDM (0.02%)-F_3H_5-β-Glu (0.002%); (D) condition 4, DDM (0.02%)-F_3H_5-β-Glu (0.02%); (E) condition 5, control DDM (0.02%); (F) condition 5, DDM (0.02%)-F_3H_5-β-Glu (0.002%); and (G) condition 5, DDM (0.02%)-F_3H_5-β-Glu (0.02%).

diluted by two with water. From this screen, we selected up to six crystallization conditions that did not provide the best-looking crystals, that is, small crystals, poor or different crystal habits, multicrystalline particles, strong nucleation, and phase separation at the edge of crystallization to name but a few.

We reproduced these conditions manually in 24-well crystallization plates using the hanging drop method and added the fluorinated compounds as additives at different concentrations in the presence of a constant concentration of DDM (0.02%) and observed whether crystallization was affected. Among the additives tested, F_6H_2-, F_4H_2-, and F_3H_3-β-Glu showed no improvement (but also no negative effect), and F_5H_3-, F_4H_3-, and F_3H_5-β-Glu showed improved crystallization. As we performed more experiments with the latter, we focus herein on F_3H_5-β-Glu. AcrB was incubated with F_3H_5-β-Glu at different concentrations (0, 0.002, 0.02, and 0.04%) prior to crystallization. Three protein purifications coming from different membrane preparations were tested and gave converging results. Adding F_3H_5-β-Glu improved the crystallization for three crystallization conditions (1, 4, and 5) and did not affect crystallization for the three others as compared to control drops without additives.

For condition 1, the addition of 0.002% F_3H_5-β-Glu led to the formation of small nicely shaped crystals, whereas the control drop had a gel-like aspect with phase separation (Figure 4A,B). Crystallization with condition 4 was also drastically improved in the presence of 0.002% F_3H_5-β-Glu, going from a precipitate (not shown) to an ensemble of well-shaped crystals (Figure 4C). It has to be noted that a larger amount of additive, 0.02% (Figure 4D) and 0.04% (not shown), decreased the benefit of this additive. Although less spectacular, condition 5 is also improved in the presence of F_3H_5-β-Glu, the control drop exhibiting small crystalline particles that are mostly round-shaped with a few having nice crystal shapes (Figure 4E), while with increasing amounts of F_3H_5-β-Glu, 0.002 and 0.02%, the shapes of the crystals as well as their sizes are clearly improved (Figure 4F,G). Furthermore, preliminary tests with other compounds of the same family but having different chain lengths or fluorine contents were also tested. None of them hampered crystallization and F_6H_3-β-Glu, F_5H_3-β-Glu, and F_4H_3-β-Glu seemed to improve it (data not shown).

CONCLUSIONS

In summary, six fluorinated surfactants with a glucose polar head group and whose alkyl chain was varied in length, from six to eight carbon atoms were synthesized. The presence of three to six perfluorinated carbon atoms within the chain significantly hampered the water solubility of the surfactants. Among the series, the two derivatives F_3H_5-β-Glu and F_4H_3-β-Glu exhibited a water solubility superior to 7−8 g/L and were found to form micelles at ∼11 mM. F_3H_5-β-Glu, which can be

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seen as a fluorinated analogue of *n*-octyl-β-D-glucopyranoside (OG), improved the crystallization of AcrB in detergent mixtures with DDM. Among the six investigated crystallization conditions, improvement for three of them was observed, while preliminary tests with other surfactants suggest also potential as additives for crystallization. This warrants further investigation of the whole series and may result in the near future in the development of screening kits for crystallization of membrane proteins.

EXPERIMENTAL SECTION

Synthesis. 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 24 h. Molecular sieves were activated by heating in vacuum. The progress of the reactions was monitored by thin-layer chromatography (60 F_{254} Merck plates). The compounds were detected either by exposure to ultraviolet light (254 nm) or by spraying with sulfuric acid (5% ethanol), followed by heating at ∼150 °C. Flash column chromatography was carried out on a silica gel (40−63 μm) with a CombiFlash system. ^{1}H, ^{13}C, and ^{19}F NMR analyses were performed on a Bruker AC400 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 constant *J* is given in hertz. Abbreviations used for signal patterns are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; and dt, doublet of triplets. High-resolution mass spectrometry (HRMS) was performed on a SYNAPT G2-S (Waters) mass spectrometer equipped with a time-of-flight (TOF) analyzer for ESI^{+} experiments. Milli-Q water (resistivity, 18.2 MΩ cm; surface tension, 71.45 mN/m at 25 °C) was employed for all physical−chemical experiments.

General Procedure for the Glycosylation Reaction (Compounds 2a−f). Under an argon atmosphere, β-D-glucose pentaacetate (1.0 equiv) was dissolved in dry CH_2Cl_2. At 0 °C, the corresponding alcohol (1.5 equiv) was added, followed by the dropwise addition of boron trifluoride diethyl ether complex (1.5 equiv). The reaction mixture was stirred at 0 °C for 2 h and then at room temperature overnight. After completion of the reaction, CH_2Cl_2 was added and the mixture was washed with saturated $NaHCO_3$ (2×) and brine (2×). The organic layers were collected, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The crude compound was purified by flash chromatography (cyclohexane/AcOEt, 7:3 v/v) to yield the desired compounds **2a−f**.

*4′,4′,5′,5′,6′,6′,6′-Heptafluorohexyl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**2a**).* **2a** was synthesized from β-D-glucose pentaacetate (1.50 g, 3.85 mmol), 4,4,5,5,6,6,6-heptafluorohexan-1-ol (1.62 g, 5.77 mmol), and boron trifluoride diethyl ether complex (0.73 mL, 5.77 mmol). It was obtained as a white powder (0.95 g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.19 (m, 1H), 5.07 (m, 1H), 4.98 (dd, *J* = 9.6 and 8.0 Hz, 1H), 4.52 (d, *J* = 8.0 Hz, 1H), 4.23 (m, 1H), 4.12 (m, 1H), 3.92 (m, 1H), 3.68 (m, 1H), 3.58 (m, 1H), 2.27−1.97 (m, 14H), 1.89 (m, 2H). ^{19}F NMR (375 MHz, $CDCl_3$): δ −80.6, −115.4, −127.8. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.4, 169.5, 169.4, 100.8, 72.9, 72.0, 71.3, 68.5, 68.4, 62.0, 27.4, 20.8, 20.8, 20.7, 20.6.

*3′,3′,4′,4′,5′,5′,6′,6′,6′-Nonafluorohexyl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**2b**).* **2b** was synthesized from β-D-glucose pentaacetate (1.20 g, 3.07 mmol), 3,3,4,4,5,5,6,6,6-nonafluorohexan-1-ol (1.22 g, 4.62 mmol), and boron trifluoride diethyl ether complex (0.65 mL, 4.61 mmol). It was obtained as a white powder (0.93 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.19 (m, 1H), 5.06 (m, 1H), 4.98 (dd, *J* = 9.5 and 8.0 Hz, 1H), 4.53 (d, *J* = 8.0 Hz, 1H), 4.24 (m, 1H), 4.12 (m, 2H), 3.83 (m, 1H), 3.72 (m, 1H), 2.41 (m, 2H), 2.09−1.96 (m, 12H). ^{19}F NMR (375 MHz, $CDCl_3$): δ −81.1, −113.6, −124.6, −126.0. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.3, 169.5, 169.4, 101.0, 72.7, 72.1, 71.1, 68.5, 62.0, 31.5, 20.7, 20.7, 20.5.

*3′,3′,4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,8′-Tridecafluorooctyl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**2c**).* **2c** was synthesized from β-D-glucose pentaacetate (1.25 g, 3.20 mmol), 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol (1.45 g, 4.0 mmol), and boron trifluoride diethyl ether complex (0.60 mL, 4.80 mmol). It was obtained as a white powder (1.50 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.19 (m, 1H), 5.07 (m, 1H), 4.98 (m, 1H), 4.53 (m, 1H), 4.25 (m, 1H), 4.12 (m, 2H), 3.82 (m, 1H), 3.70 (m, 1H), 2.41 (m, 2H), 2.14−1.91 (m, 12H). ^{19}F NMR (375 MHz, $CDCl_3$): δ −81.0, −113.4, −122.0, −123.0, −123.7, −126.3. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.4, 169.5, 101.0, 72.8, 72.1, 71.1, 68.5, 62.0, 31.6, 20.7, 20.6, 20.6, 20.4.

*Allyl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**2d**).* **2d** was synthesized from β-D-glucose pentaacetate (3.00 g, 7.68 mmol), allyl alcohol (0.67 g, 11.53 mmol), and boron trifluoride diethyl ether complex (1.5 mL, 11.53 mmol). It was obtained as a white powder (1.54 g, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.84 (m, 1H), 5.30−5.15 (m, 3H), 5.12−4.97 (m, 2H), 4.54 (d, *J* = 8.0 Hz, 1H), 4.32 (m, 1H), 4.24 (m, 1H), 4.16−4.04 (m, 2H), 3.67 (m, 1H), 2.12−1.95 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.4, 169.5, 169.4, 133.4, 117.7, 99.7, 73.0, 71.9, 71.4, 70.1, 68.6, 62.1, 20.8, 20.8, 20.7.

*Penten-1-yl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**2f**).* **2f** was synthesized from β-D-glucose pentaacetate (3.00 g, 7.68 mmol), penten-1-yl alcohol (0.99 g, 11.53 mmol), and boron trifluoride diethyl ether complex (1.5 mL, 11.53 mmol). It was obtained as a white powder (1.50 g, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.73 (m, 1H), 5.16 (m, 1H), 5.04 (m, 1H), 5.00−4.84 (m, 3H), 4.45 (d, *J* = 8.1 Hz, 1H), 4.22 (m, 1H), 4.08 (m, 1H), 3.83 (m, 1H), 3.64 (m, 1H), 3.45 (m, 1H), 2.20−1.83 (m, 14H), 1.63 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.3, 169.4, 169.3, 137.8, 115.1, 100.9, 72.9, 71.8, 71.4, 69.3, 68.5, 62.0, 29.9, 28.6, 20.8, 20.7, 20.6, 20.6.

General Procedure for the Free Radical Addition of the Fluorinated Chains (Compounds 3d−f). To a solution of the corresponding alkyl-β-D-2,3,4,6-tetra-*O*-acetyl-glucopyranoside (**2d−f**) (1.0 equiv) in CH_2Cl_2, perfluoroallyliodide (1.5 equiv) and 1M triethyl borane in hexane (0.2 equiv) were added. The reaction mixture was purged with air and stirred at room temperature for about 1 h. After completion of the reaction, a diluted solution of $Na_2S_2O_3$ was added and the aqueous layer was extracted with CH_2Cl_2 (3×). The organic layers were collected, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The crude product was purified by flash chromatography (cyclohexane/AcOEt, 7:3 v/v) to yield the desired compounds **3d−f**.

*4′,4′,5′,5′,6′,6′,7′,7′,7′-Octafluoro-2′-iodo-heptyl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**3d**).* **3d** was synthe-

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sized from compound **2d** (1.13 g, 2.90 mmol), perfluorobutyl iodide (1.51 g, 4.36 mmol), and 1M triethyl borane in hexane (0.6 mL, 0.60 mmol). It was obtained as a white powder (2.05 g, 88%) and as a mixture of two diastereoisomers (* indicates peaks from diastereotopic atoms). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.21 (m, 2H), 5.06 (m, 4H), 4.58 (d, J = 7.7 Hz, 1H), 4.57 (d, J = 8.0 Hz, 1H), 4.39 (m, 1H), 4.34−4.17 (m, 3H), 4.12 (m, 3H), 4.02 (m, 1H), 3.80 (m, 2H), 3.70 (m, 2H), 3.02 (m, 2H), 2.64 (m, 2H), 2.15−1.94 (m, 24H). ^{19}F NMR (375 MHz, $CDCl_3$): δ −81.0, −113.9, −124.6, −125.9. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.2, 169.4, 169.2, 101.1* and 100.2* ($\underline{C}H_{anom.}$), 74.8* and 73.6* (O$\underline{C}H_2$CHI), 72.6, 72.0, 71.0, 68.3, 61.8, 37.5, 20.7, 20.6, 20.6, 13.6* and 13.1* ($\underline{C}$HI).

*4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,8′-Undecafluoro-2′-iodo-pentyl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**3e**).* **3e** was synthesized from compound **2e** (0.78 g, 2.00 mmol), perfluoropentyl iodide (1.07 g, 2.71 mmol), and 1M triethyl borane in hexane (0.4 mL, 0.40 mmol). It was obtained as a white powder (1.00 g, 67%) and as a mixture of two diastereoisomers (* indicates peaks from diastereotopic atoms). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.21 (m, 2H). 5.06 (m, 4H), 4.58 (d, J = 8.0 Hz, 1H), 4.58 (d, J = 7.9 Hz, 1H), 4.39 (m, 1H), 4.34−4.18 (m, 3H), 4.13 (m, 3H), 4.03 (m, 1H), 3.80 (m, 2H), 3.70 (m, 2H), 3.02 (m, 2H), 2.64 (m, 2H), 2.17−1.92 (m, 24H). ^{19}F NMR (375 MHz, $CDCl_3$): δ −80.8, −113.7, −122.6, −123.8, −126.2. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.3, 169.5, 169.4, 101.2* and 100.3* ($\underline{C}H_{anom.}$), 74.9* and 73.8* (O$\underline{C}H_2$CHI), 72.8, 72.2, 71.2, 68.4, 62.0* and 61.9* ($\underline{C}H_2$OAc), 37.2, 20.8, 20.8, 20.7, 13.7* and 13.3*($\underline{C}$HI).

*4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,8′-Undecafluoro-4′-iodo-pentyl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**3f**).* **3f** was synthesized from compound **2f** (1.50 g, 3.60 mmol), perfluoropropyliodide (1.60 g, 5.4 mmol), and 1M triethyl borane in hexane (0.7 mL, 0.72 mmol). It was obtained as a white powder (1.80 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.20 (m, 1H), 5.08 (m, 1H), 4.98 (m, 1H), 4.50 (d, J = 8.1 Hz, 1H), 4.32 (m, 1H), 4.25 (m, 1H), 4.14 (m, 1H), 3.91 (m, 1H), 3.69 (m, 1H), 3.54 (m, 1H), 2.83 (m, 2H), 2.14−1.95 (m, 12H), 1.86 (m, 3H), 1.70 (m, 1H). ^{19}F NMR (375 MHz, $CDCl_3$): δ −80.3, −114.1, −127.9. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.3, 169.4, 169.2, 100.7, 72.8, 71.9, 71.2, 68.4, 61.9, 41.4, 36.9, 36.8, 29.8, 20.7, 20.6, 20.0.

General Procedure for the Elimination of the Iodine Group (Compounds 4d−f). The corresponding compounds **3d−f** (1.0 equiv) were dissolved in MeOH. To the resulting solution, Pd/C (catalytic amount) and sodium acetate (3.2 equiv) were added. The reaction mixture was stirred overnight at room temperature and under a hydrogen atmosphere (6.5 bar). After completion of the reaction, the resulting mixture was filtered through a pad of Celite and concentrated under vacuum. The crude compound was dissolved in CH_2Cl_2 and washed with a diluted solution of $Na_2S_2O_3$. The aqueous layer was then extracted with CH_2Cl_2 (2×). The organic layers were collected, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to yield the desired compounds **4d−f**, which were used for the next step without any further purification.

*4′,4′,5′,5′,6′,6′,7′,7′,7′-Octafluoro-heptyl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**4d**).* **4d** was synthesized from compound **3d** (2.05 g, 2.79 mmol), Pd/C (53 mg), and sodium acetate (0.70 g, 8.93 mmol). It was obtained as a white powder (1.50 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.19 (m, 1H). 5.07 (m, 1H), 4.98 (m, 1H), 4.50 (d, J = 8.0 Hz, 1H), 4.24 (m, 1H), 4.13 (m, 1H), 3.93 (m, 1H), 3.69 (m, 1H), 3.59 (m, 1H), 2.26−1.95 (m, 14H), 1.89 (m, 2H). ^{19}F NMR (375 MHz, $CDCl_3$): δ −81.1, −114.6, −124.5, −126.1. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.3, 169.4, 169.2, 100.7, 72.7, 71.9, 71.2, 68.4, 68.2, 61.9, 27.5, 20.7, 20.6, 20.6, 20.4.

*4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,8′-Undecafluoro-pentyl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**4e**).* **4e** was synthesized from compound **3e** (1.00 g, 1.27 mmol), Pd/C (50 mg), and sodium acetate (0.33 g, 4.08 mmol). It was obtained as a white powder (0.80 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.20 (m, 1H), 5.08 (m, 1H), 4.99 (m, 1H), 4.50 (d, J = 8.0 Hz, 1H), 4.24 (m, 1H), 4.13 (m, 1H), 3.94 (m, 1H), 3.69 (m, 1H), 3.59 (m, 1H), 2.24−1.97 (m, 14H), 1.88 (m, 2H). ^{19}F NMR (375 MHz, $CDCl_3$): δ −81.8, −114.4, −122.7, −123.7, −126.3. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.4, 169.5, 169.4, 100.8, 72.9, 72.0, 71.3, 68.5, 68.4, 62.0, 27.6, 20.9, 20.8, 20.7, 20.6.

*6′,6′,7′,7′,8′,8′,8′-Heptafluoro-octyl-β-D-2,3,4,6-tetra-O-acetyl-glucopyranoside (**4f**).* **4f** was synthesized from compound **3f** (0.70 g, 0.98 mmol), Pd/C (50 mg), and sodium acetate (0.25 g, 3.14 mmol). It was obtained as a white powder (0.40 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.20 (m, 1H), 5.08 (m, 1H), 4.98 (m, 1H), 4.49 (d, J = 8.1 Hz, 1H), 4.26 (m, 1H), 4.13 (m, 1H), 3.88 (m, 1H), 3.69 (m, 1H), 3.49 (m, 1H), 2.11−1.97 (m, 14H), 1.61 (m, 4H), 1.43 (m, 2H). ^{19}F NMR (375 MHz, $CDCl_3$): δ −80.6, −115.4, −127.8. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.4, 169.5, 169.4, 100.9, 73.0, 71.9, 71.5, 69.6, 68.5, 62.1, 30.6, 29.2, 25.5, 20.8, 20.7, 19.9.

General Procedure for the Deprotection of Peracetylated Glucose (Compounds 3a−c and 5d−f). The corresponding compounds **2a−c** and **4d−f** were dissolved in MeOH and then a catalytic amount of sodium methoxide was added. The reaction mixture was stirred overnight at room temperature. The solution was neutralized by adding IRC-50, filtered, and concentrated under vacuum. The crude compound was purified by flash chromatography on a silica gel (DCM/MeOH, 85:15 v/v) to yield the desired compounds **2a−c** and **4d−f**.

*4′,4′,5′,5′,6′,6′,6′-Heptafluorohexyl-β-D-glucopyranoside (**3a**).* **3a** was synthesized from compound **2a** (0.90 g, 1.61 mmol) and sodium methoxide (43 mg, 0.80 mmol). It was obtained as a white powder (0.57 g, 92%). ^{1}H NMR (400 MHz, CD_3OD): δ 4.26 (d, J = 7.8 Hz, 1H), 3.99 (m, 1H), 3.87 (m, 1H), 3.67 (m, 2H), 3.38−3.25 (m, 3H), 3.18 (m, 1H), 2.32 (m, 2H), 1.91 (m, 2H). ^{19}F NMR (375 MHz, CD_3OD): δ −82.2, −116.5, −129.04. ^{13}C NMR (100 MHz, CD_3OD): δ 104.3, 78.0, 77.9, 75.0, 71.7, 69.1, 62.8, 28.6, 21.9. HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{12}H_{17}F_7O_6$, 391.0992; found, 391.0993.

*3′,3′,4′,4′,5′,5′,6′,6′,6′-Nonafluorohexyl-β-D-glucopyranoside (**3b**).* **3b** was synthesized from compound **2b** (0.90 g, 1.51 mmol) and sodium methoxide (40 mg, 0.75 mmol). It was obtained as a white powder (0.60 g, 93%). ^{1}H NMR (400 MHz, CD_3OD): δ 4.30 (d, J = 7.8 Hz, 1H), 4.19 (m, 1H), 3.88 (m, 2H), 3.67 (m, 1H), 3.39−3.26 (m, 3H), 3.18 (m, 1H), 2.57 (m, 2H). ^{19}F NMR (375 MHz, CD_3OD): δ −82.7, −114.7, −125.7, −127.2. ^{13}C NMR (100 MHz, CD_3OD): δ 104.6, 78.0, 78.0, 75.0, 71.6, 62.7, 62.6, 32.5. HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{12}H_{15}F_9O_6$, 427.0803; found, 427.0794.

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*3′,3′,4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,8′-Tridecafluorooctan-β-D-glucopyranoside (**3c**).* **3c** was synthesized from compound **2c** (1.40 g, 2.02 mmol) and sodium methoxide (55 mg, 0.50 mmol). It was obtained as a white powder (0.93 g, 93%). ^{1}H NMR (400 MHz, CD_3OD): δ 4.30 (d, J = 7.8 Hz, 1H), 4.19 (m, 1H), 3.88 (m, 2H), 3.67 (m, 1H), 3.39−3.27 (m, 3H), 3.18 (m, 1H), 2.58 (m, 2H). ^{19}F NMR (375 MHz, CD_3OD): δ −82.4, −114.5, −122.9, −123.9, −124.7, −127.3. ^{13}C NMR (100 MHz, CD_3OD): δ 104.6, 78.0, 78.0, 75.0, 71.6, 62.7, 62.6, 32.6. HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{14}H_{15}F_{13}O_6$, 527.0739; found, 527.0734.

*4′,4′,5′,5′,6′,6′,7′,7′,7′-Octafluoroheptyl-β-D-glucopyranoside (**5d**).* **5d** was synthesized from compound **4d** (1.50 g, 2.47 mmol) and sodium methoxide (50 mg, 0.98 mmol). It was obtained as a white powder (0.97 g, 90%). ^{1}H NMR (400 MHz, CD_3OD): δ 4.27 (d, J = 7.8 Hz, 1H), 3.99 (m, 1H), 3.87 (m, 1H), 3.66 (m, 2H), 3.40−3.25 (m, 3H), 3.18 (m, 1H), 2.34 (m, 2H), 1.91 (m, 2H). ^{19}F NMR (375 MHz, CD_3OD): δ −82.7, −115.7, −125.5, −127.3. ^{13}C NMR (100 MHz, CD_3OD): δ 104.3, 78.1, 78.0, 75.0, 71.7, 69.1, 62.8, 28.8, 21.9. HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{13}H_{17}F_9O_6$, 441.0960; found, 441.0946.

*4′,4′,5′,5′,6′,6′,7′,7′,8′,8′,8′-Undecafluorooctyl-β-D-glucopyranoside (**5e**).* **5e** was synthesized from compound **4e** (0.80 g, 1.21 mmol) and sodium methoxide (33 mg, 0.61 mmol). It was obtained as a white powder (0.57 g, 92%). ^{1}H NMR (400 MHz, CD_3OD): δ 4.28 (d, J = 7.8 Hz, 1H), 4.00 (m, 1H), 3.88 (m, 1H), 3.68 (m, 2H), 3.41−3.24 (m, 3H), 3.20 (m, 1H), 2.36 (m, 2H), 1.93 (m, 2H). ^{19}F NMR (375 MHz, CD_3OD): δ −82.5, −115.5, −123.8, −124.7, −127.5. ^{13}C NMR (100 MHz, CD_3OD): δ 104.3. 78.1, 78.0, 75.0, 71.7, 69.1, 62.8, 28.9, 21.9. HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{14}H_{17}F_{11}O_6$, 491.0928; found, 491.0919.

*6′,6′,7′,7′,8′,8′,8′-Heptafluorooctyl-β-D-glucopyranoside (**5f**).* **5f** was synthesized from compound **4f** (0.40 g, 0.68 mmol) and sodium methoxide (20 mg, 0.35 mmol). It was obtained as a white powder (0.24 g, 84%). ^{1}H NMR (400 MHz, CD_3OD): δ 4.25 (d, J = 7.8 Hz, 1H), 3.92 (m, 1H), 3.86 (m, 1H), 3.67 (m, 1H), 3.57 (m, 1H), 3.38−3.22 (m, 3H), 3.17 (m, 1H), 2.15 (m, 2H), 1.64 (m, 4H), 1.52 (m, 2H).^{19}F NMR (375 MHz, CD_3OD): δ −82.2, −116.4, −129.1. ^{13}C NMR (100 MHz, CD_3OD): δ 104.4, 78.1, 77.9, 75.1, 71.7, 70.4, 62.8, 31.5, 30.4, 26.6, 21.1. HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{14}H_{21}F_7O_6$, 419.1305; found, 419.1301.

Determination of Water Solubility. The turbidity of the aqueous solution of compounds was measured by a CrystalEYES system (manufactured by HEL Limited). This system includes a turbidity probe, a temperature probe, and a recorder. For each compound, a turbid solution, at a concentration around three times its limit of solubility, was prepared. The stock solution was maintained at 25 °C using a thermostat (Ministat 125 series, Huber) under stirring at 350 rpm. The turbidity and temperature probes were immersed in the stock solution and the turbidity was measured after 10 min of stirring. The stock solution was diluted by successive addition of water. After each addition of water, the stirring was continued for 10 min and the turbidity was recorded.

Surface Tension Measurements. The surface tension of the aqueous solution of compounds was determined using a K100 tensiometer (KRUSS, Hamburg, Germany). The Wilhelmy plate technique was employed. Surfactant solutions around the limit of solubility were prepared 24 h prior to measurements, and 20 mL of the solution was transferred to a 50 mL vessel supplied with a stirring bar. Surface tensions were determined by the automatic dilution of the stock solutions using a Metrohm 700 Dosino. In a typical experiment, 20−30 concentration steps were used with ∼5−10 min between each concentration step. All measurements were performed at 25.0 ± 0.5 °C. Sets of measurements to obtain equilibrium surface tension were taken until the change in surface tension was less than 0.05 mN/m.

^{19}F NMR for CMC Determination. Seven samples at different concentrations were prepared from a stock solution of each surfactant (8.7 g/L for F_4H_2-β-Glu and 7.6 g/L for F_3H_5-β-Glu) and were dissolved in 500 μL of a D_2O/H_2O (1:9, v/v) mixture. CF_3COONa (−73.53 ppm) was used as the internal reference and 20 μL of a solution at 1 g/L was added to each of the samples. ^{19}F NMR spectra were recorded on a Bruker AC400 at 376.50 MHz at 25 °C. The observed chemical shifts (δ_{obs}) of the terminal CF_3 group of the derivative were plotted as a function of the concentration below and above the CMC. Below the CMC, δ_{obs} corresponds to the chemical shift of the monomer (δ_{mon}), whereas above the CMC, δ_{obs} is the weighted average of the chemical shifts of the monomer and the formed micelle, assuming that the exchange between the bulk solution and the micelle is fast on the NMR time scale. If the monomer concentration is constant above the CMC, the observed chemical shift can be determined using eq 1

$$\delta_{obs} = \delta_{mic} - \left(\frac{CMC}{C}\right)(\delta_{mic} - \delta_{mon}) \quad (1)$$

Hydrophilic−Lipophilic Balance Determination. HLB values according to Griffin's method[29] were determined using eq 2

$$HLBG = 20 \times \frac{M_h}{M} \quad (2)$$

where M_h is the molecular weight of the hydrophilic part (here 179.1 g/mol) and M is the molecular weight of the whole molecule. HLB values according to Davies' method[30] were determined using eq 3

$$HLBD = 7 + \sum \text{hydrophilic group numbers} + \sum \text{hydrophobic group numbers} \quad (3)$$

For the glucose polar head group, we assigned a value of 1.9 for the primary OH group (1 × 1.9) and a value of 0.5 for the three secondary OH groups (3 × 0.5). A value of 1.3 was assigned for each ether group (2 × 1.3). For the alkyl chains, the group numbers were −0.475 per each CH_2 or CH_3 group and −0.870 per each CF_2 or CF_3 group.[32] The first methylene group of the chain connected to the polar head group was excluded from the calculation.

Crystallization of AcrB. AcrB was expressed and purified as described in ref 34. Purified AcrB was concentrated to more than 15 mg/mL (Amicon, 50kDa cutoff). The final buffer contains 10 mM Na-HEPES at pH 7 and 0.02% DDM (Anatrace, Anagrade). First crystallization was done on the PSB crystallization platform (High Throughput Crystallization Laboratory (HTX Lab) of the EMBL Grenoble,[35]) using the MemGold kit (Molecular Dimensions) diluted 50% (v/v) with water to identify initial crystallization conditions. F_3H_5-β-Glu was dissolved in the same buffer as AcrB at a concentration of up to 0.5%. Prior to crystallization, various amounts of F_3H_5-β-Glu were added to the protein solution to obtain final

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Table 2. Crystallization Conditions Selected from a 96-Conditions Screen[a]

conditions	buffer	precipitant	salt	effect of F_3H_5-β-Glu
1	25 mM bicine, pH 9.0	16.5% (v/v) PEG 300	50 mM NaCl	strong improvement
2	25 mM Na citrate, pH 4.5	11% (v/v) PEG400	35 mM NaCl	no effect
3	50 mM glycine, pH 9.3	15% (v/v) PEG400	50 mM $LiSO_4$	no effect
4	50 mM tris, pH 8.0	17.5% (v/v) PEG400	110 mM Na citrate	strong improvement
5	40 mM $NaPO_4$, pH 6.2	9% (w/v) PEG2000	10 mM Na citrate	moderate improvement
6	no buffer	11% (w/v) PEG6000	25 mM Li citrate, 50 mM $NaHPO_4$, 75 mM K citrate	no effect

[a]The improvement of crystallization in the presence of F_3H_5-β-Glu was observed in conditions 1, 4, and 5.

concentrations of 0, 0.002, 0.02, and 0.04% of F_3H_5-β-Glu, while keeping the same AcrB and DDM concentrations, respectively, 11.6 mg/mL and 0.02%, in all samples. Hand-made drops made of 1.2 μL of protein solution and 1.2 μL of the reservoir were suspended over 800 μL of reservoirs in 24-well plates. Crystals appear within a few days to 1 week. Selected crystallization conditions are shown in Table 2.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c02581.

^{1}H, ^{19}F, and ^{13}C NMR spectra and mass spectrometry data of compounds **3a−c** and **5d−f** (PDF)

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Notes

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