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Synthesis of Nickel-Chelating Fluorinated Lipids for Protein Monolayer Crystallizations

Waleed M. Hussein,[†] Benjamin P. Ross,[†] Michael J. Landsberg,[‡] Daniel Lévy,[§] Ben Hankamer,[‡] and Ross P. McGeary*,[†,||]

The University of Queensland, School of Molecular & Microbial Sciences, Institute for Molecular Bioscience, and School of Pharmacy QLD 4072, Australia, and Institut Curie, UMR CNRS 168, 11 rue P.M.Curie, F-75231 Paris, France

r.mcgeary@uq.edu.au

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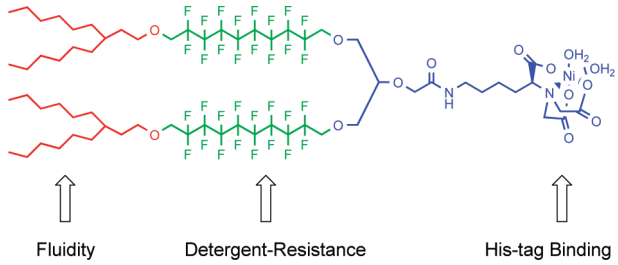

Nickel-chelating lipids have been synthesized for use as functionalized templates for 2-D crystallization of membrane proteins. These monolayer-forming lipids have been designed with three distinct components: (i) a branched hydrocarbon tail to confer fluidity of the monolayer, (ii) a perfluorinated central core for detergent resistance, and (iii) a nickel-chelating hydrophilic headgroup to facilitate binding of recombinant, polyhistidine-tagged fusion proteins. Alkylations of fluorinated alcohols used in these syntheses proceed in good yields only with the application of prolonged sonication and, in some cases, in the presence of phase-transfer catalysts. Formation of 2-D crystals of the His-tagged membrane protein BmrA from *Bacillus subtillis* is reported.

Introduction

Binding and adsorption of proteins on lipid monolayers is an elegant method to generate high concentrations of oriented proteins and protein complexes. Bound proteins can be either imaged directly or, under ideal conditions, induced to form 2-D crystalline arrays for subsequent structure determination by either single particle analysis[1] or 2-D electron crystallography,[2] respectively. 2-D crystals grown by this technique can also be used as templates for the growth of 3-D crystals for X-ray diffraction analysis.[3] Moreover, it has recently been demonstrated that purification and monolayer binding can be coupled in a single experiment by taking advantage of the high-affinity interaction between His-tagged proteins and the Ni^{2+}−NTA ligand.[4]

Monolayer-forming lipids for 2-D crystallization contain two domains with distinct properties: a long, branched hydrophobic tail with the necessary chemistry to impart fluidity of the lipid monolayer at the air−water interface,[5] and a hydrophilic headgroup which is responsible for orienting the lipid to the aqueous phase and often incorporates a functional moiety for specific interaction with target proteins and/or complexes.[6−8]

Many derivatized lipids have been reported, incorporating ligands such as ATP,[9] biotin,[10−14] and steroids,[15] and metal ions

[†] The University of Queensland, School of Molecular & Microbial Sciences.
[‡] The University of Queensland, Institute for Molecular Bioscience.
[§] Institut Curie.
[||] The University of Queensland, School of Pharmacy.

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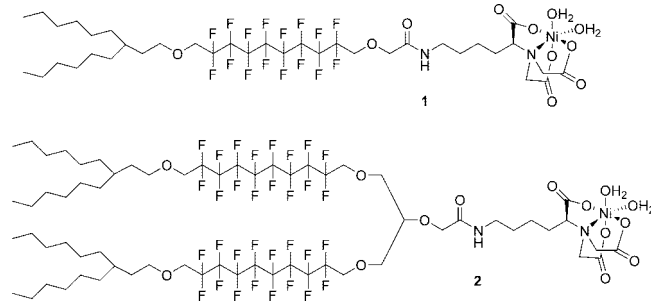
including Cu^{2+} [16] and Ni^{2+}.[17] These lipids bind proteins through either a natural or conferred affinity for the functionalized head groups. For example, Ni^{2+}-chelating lipids have been used to grow 2-D crystals of a number of proteins, including HIV-1 reverse transcriptase,[18] S-layer protein sbpA,[19] Moloney murine leukemia virus capsid protein (His−MoCa),[20] yeast RNA polymerase I,[21] murine MHC class I,[22] FhuA,[23,24] F_0F_1−ATP-synthase,[24] HC−Pro,[25] and a VE cadherin fragment.[26] The lipid headgroup is designed to take advantage of the strong binding interaction between the Ni^{2+} ion and a sequence of 4−8 consecutive histidine residues (His-tag), often incorporated at the N- or C-terminus of recombinantly expressed fusion proteins. Ni^{2+} is usually attached to the lipid headgroup via the quadridentate nitrilotriacetic acid (NTA, cf. compounds **1** and **2**) ligand, which presents an excellent coordination complex for the octahedral coordination sphere of Ni^{2+}.[17]

The susceptibility of lipid monolayers to detergent solubilization, however, limits their usefulness in the binding and crystallization of membrane proteins, which often require significant concentrations of detergent to maintain their solubility. Some success has been reported in the crystallization of detergent-solubilized membrane proteins via attachment to hydrogenated lipid monolayers; however, these successes have, so far, been limited to membrane proteins solubilized with low Critical Micelle Concentration detergents and require highly compressed monolayers. Lipids that are resistant to a broader range of detergents under more fluid conditions are, therefore, desirable.[27,28] In this respect, it has been demonstrated that partially fluorinated lipids have vastly improved stability in the presence of detergents.[29] In 2001, Lebeau et al. used partially fluorinated Ni^{2+}-chelating lipids to grow 2-D crystals of the plasma membrane proton-ATPase from *Arabidopsis thaliana* which yielded a projection map at 9 Å resolution.[30] Details of the syntheses of these partially fluorinated lipids, however, were not reported.

To extend the compatibility of the monolayer crystallization technique with membrane proteins, fluorinated lipids **1** and **2** with Ni^{2+}-chelating functional head groups have been designed to form a fluid yet detergent-resistant, monolayer template.[31] These compounds were designed to have fluorinated alkyl chains coupled to a Ni^{2+}-chelating headgroup for the binding of His-tagged proteins.[32−34] The two lipids contain one (compound **1**) or two (compound **2**) branched, partially fluorinated alkyl chains with the latter linked via a glycerol backbone. We also describe the synthesis of a "diluting" lipid (compound **18**) which, when mixed with compound **2** and spread at the air−water interface, is able to act as a template for 2-D membrane protein crystallization.

The synthesis of compounds containing perfluorinated moieties can be very problematic due to their unusual reactivities, hydrophobicity, and lipophobicity, which can lead to difficulties in handling, solubilization, and purification.[35,36] Here we report the synthesis of compounds **1** and **2**. One advantage of the procedures described is that they are modular, allowing for variation of the heads groups and lipid tails. We also report several improved techniques for preparing fluoroalkylated systems with good yields.



Results and Discussion

The branched, saturated methyl ester **4** was prepared by the conjugate addition of the Grignard reagent derived from 1-bromohexane with methyl non-2-enoate **3** in the presence of Cu(I) and in situ silylation[37] of the intermediate enolate (Scheme 1). The ester **4** was reduced to the corresponding alcohol **5**, either by using $NaBH_4$−LiCl in THF−ethanol (2:1) (64% yield) or by employing $LiAlH_4$ in THF, and quenching[38] with $Na_2SO_4 \cdot 10H_2O$ (83%). This quench was essential to avoid the formation of a gelatinous precipitate of $Al(OH)_3$ which hindered workup and led to low recovery of the desired product. No

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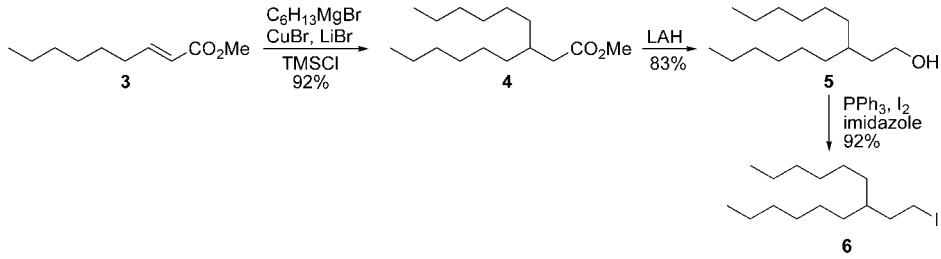
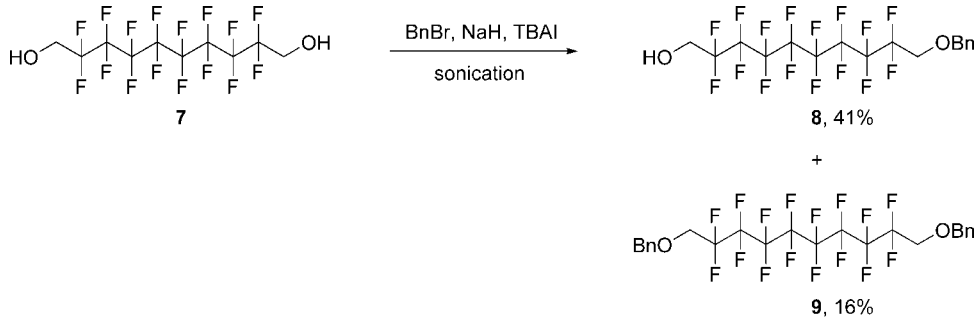
SCHEME 1



SCHEME 2

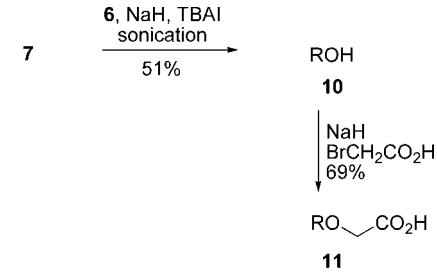


reduction of **4** occurred with $NaBH_4$−LiCl in refluxing THF alone. Conversion of the alcohol **5** to the alkyl iodide **6** was performed in two steps, via the tosylate intermediate (83%), and then onto the iodide **6** (68%). A higher yielding, direct transformation of alcohol **5** to the iodide **6** was achieved using PPh_3, iodine, and imidazole. For this reaction the choice of solvent was important: in benzene[39] the yield of iodide **6** was 50%, in dichloromethane[40] 75%, and in 1:3 acetonitrile:diethyl ether 92%.

The next step in the synthesis required the monobenzyl derivative of the fluorinated diol **7**. Performing chemical transformations on perfluorocarbon derivatives presents a number of challenges because of their poor solubility in many common organic solvents, unusual chemical properties, and reactivities.[41] In contrast to a recent report,[42] benzylation of diol **7** under usual conditions proved remarkably refractory. After many unsuccessful alkylation attempts, we showed that treatment of the diol **7** with NaH in THF led to rapid and extensive decomposition of the reactant. We eventually found that a moderate yield (41%) of the monobenzyl derivative **8** was achieved using benzyl bromide and NaH in DMF in the presence of the phase-transfer agent tetrabutylammonium iodide, and with prolonged sonication (Scheme 2). A small amount of the dibenzyl ether **9** (16%) was also obtained and recycled to the monobenzyl ether **8** by partial hydrogenolysis over palladium on carbon. Pasquato recently reported that attempted benzylation of **7**, under different conditions, led mainly to the formation of the dibenzyl ether **9** with low conversion and the formation of only a small amount of **8**.[41] Lebeau's group have also reported that alkylation of **7** with the very reactive *tert*-butyl bromoacetate only occurs in the presence of a THF−HMPA solvent mixture.[43]

Alkylation of the monobenzyl ether **8** with the iodide **6** under the usual conditions, again, proved problematic. Alkylation using

SCHEME 3



R = $(C_6H_{13})_2CHCH_2CH_2OCH_2(CF_2)_8CH_2$-

NaH in THF gave the corresponding benzyl ether in only 34% yield. The yield of this reaction was improved, to 47%, by sonicating the reaction mixture. Hydrogenolysis of the benzyl ether then yielded the fluorinated alcohol **10** in 83% yield (see Supporting Information). Alternatively, alcohol **10** was prepared directly from the diol **7**, in 51% yield, using sonication and a phase-transfer catalyst (Scheme 3). Reaction of the fluorinated alcohol **10** with bromoacetic acid and NaH in anhydrous THF gave the carboxylic acid **11** in 69% yield.

The next step involved coupling of the carboxylic acid **11** with the lysine-NTA derivative **15**. As shown in Scheme 4, the triester **15** was prepared by treating ε-Cbz-protected L-lysine **12** with bromoacetic acid under alkaline conditions to give the triacid[44,45] **13** in 92% yield. Esterification of **13** with acidic methanol yielded the triester[46] **14** in 76% yield, and hydrogenolysis of **14** in methanolic formic acid yielded the formate salt[46] **15** in 92% yield. The NMR spectra of **14** and **15** differed somewhat from those described by Roy[46] but were in agreement with those reported by Zhou.[47] It was necessary to store the amino triester **15** as its formate salt to prevent self-condensation.

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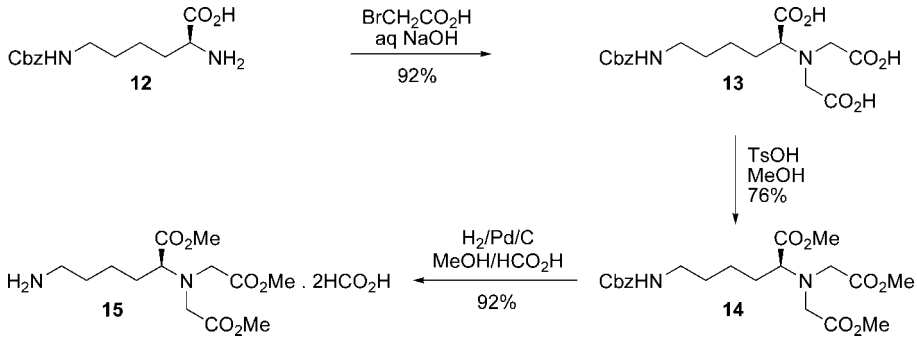
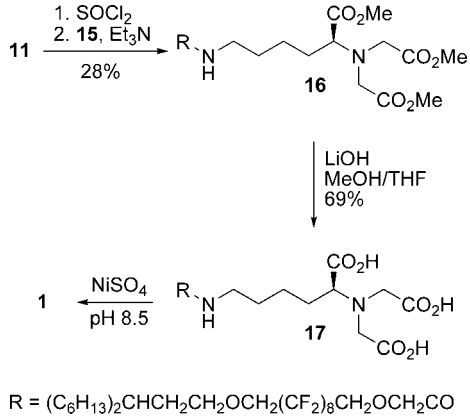
SCHEME 4

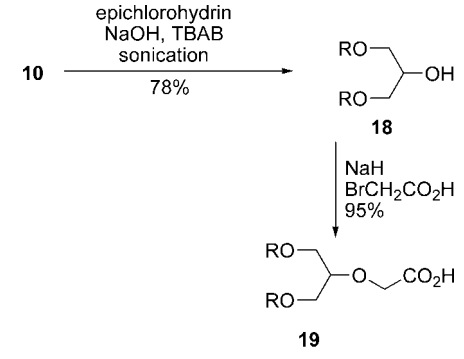


SCHEME 5



R = $(C_6H_{13})_2CHCH_2CH_2OCH_2(CF_2)_8CH_2OCH_2CO$

SCHEME 6

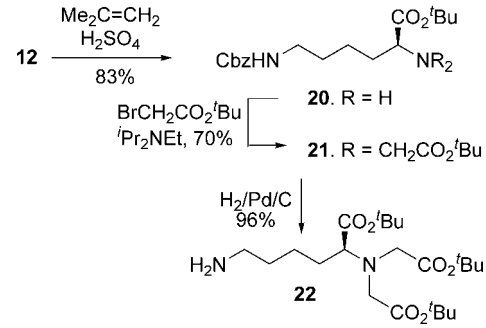


R = $(C_6H_{13})_2CHCH_2CH_2OCH_2(CF_2)_8CH_2$-

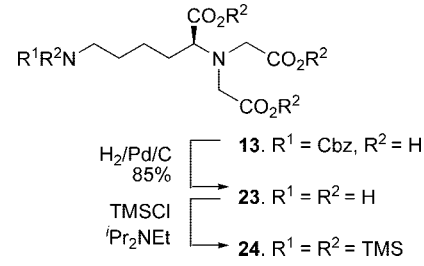
Coupling of the fluorinated acid **11** with the triester **15** to give the amide **16** was first attempted, without success, using peptide coupling agents, such as diisopropylcarbodiimide. A complex mixture was invariably obtained. After many attempts we found that the carboxylic acid **11** was converted using thionyl chloride into its corresponding acid chloride, and that this would react with **15** in the presence of triethylamine to yield the amide **16** in modest yield (Scheme 5). Hydrolysis of **16** with LiOH in THF−MeOH for 4 days gave the triacid **17** in 69% yield. Finally, the Ni−NTA complex **1** was prepared by treatment of **17** with $NiSO_4$ in Tris buffer.

The synthesis of the double-tailed carboxylic acid **19** is shown in Scheme 6. Conversion of fluorinated alcohol **10** to the tetraether **18** was accomplished in two steps by reaction with epichlorohydrin and powdered NaOH to give the corresponding oxirane (60% yield), followed by further reaction with additional **10** to yield **18** (43% yield). This same compound **18** was also made directly in 78% yield by heating a mixture of the alcohol **10**, epichlorohydrin, NaOH, and a phase-transfer catalyst at 30 °C followed by sonication. Compound **19** was prepared in 95% yield by alkylation of the alcohol **18** with bromoacetic acid and sodium hydride in THF. It could also be prepared in two steps by alkylation of **18** with *tert*-butyl bromoacetate and sodium hydride in DMF (45% yield), followed by deprotection of the ester with TFA (75% yield).

SCHEME 7



SCHEME 8



With the acid **19** in hand, we now turned our attention to its coupling with a suitably protected NTA derivative. Several approaches were investigated: as well as using the previously synthesized trimethyl ester **15**, use of the tri-*tert*-butyl ester[48−50] **22** (Scheme 7) and the persilylated derivative[51,52] **24** (Scheme 8) was also examined.

The ε-Cbz-protected L-lysine **12** was converted to its *tert*-butyl ester **20** by treatment with isobutylene and sulfuric acid in a pressure bottle.[48] Bisalkylation of **20** with *tert*-butyl

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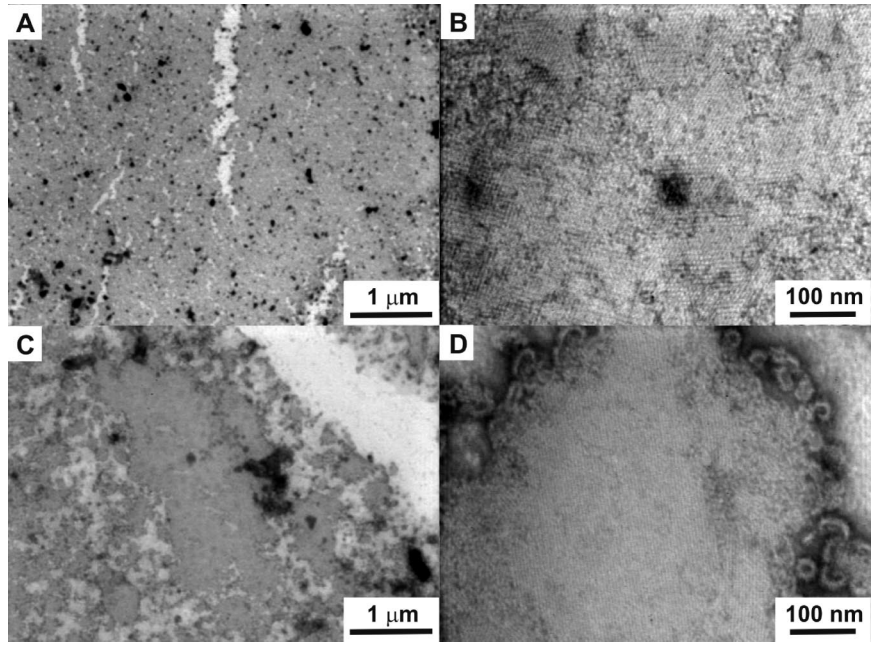
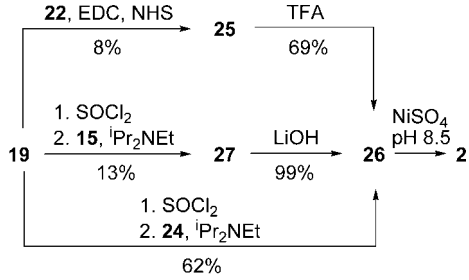
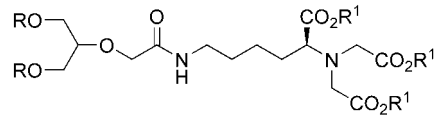


FIGURE 1. (A,C) At low magnification, large planar reconstituted membranes (dark gray) are seen. The membrane in A is broken as a result of transfer to the carbon grid. (B,D) Mosaic 2-D arrays of BmrA are clearly seen at higher magnification. Since structural analysis of BmrA is beyond the scope of this paper, we did not perform systematic trials to improve the crystallinity. However, the presence of 2-D arrays is only observed following binding of the protein to the functionalized fluorinated monolayer, thus demonstrating the effectiveness of compound **2**.

SCHEME 9





25.
R = $(C_6H_{13})_2CHCH_2CH_2OCH_2(CF_2)_8CH_2$- R^1 = ^{t}Bu
26.
R = $(C_6H_{13})_2CHCH_2CH_2OCH_2(CF_2)_8CH_2$- R^1 = H
27
R = $(C_6H_{13})_2CHCH_2CH_2OCH_2(CF_2)_8CH_2$- R^1 = Me

bromoacetate then gave the triester **21**, and hydrogenolysis yielded the deprotected aminotriester **22**.

The Cbz protecting group of the triacid **13** was removed by hydrogenolysis to give the amino-triacid[52] **23**, which was converted to the soluble silylated derivative[51] **24**, using chlorotrimethylsilane and diisopropylethylamine in toluene.

Coupling of the acid **19** with the tri-*tert*-butyl ester **22** using EDC and NHS in DMF gave a very poor yield of the desired amide **25** (Scheme 9). Similarly, coupling of the acid chloride derived from **19** with the trimethyl ester **15** in the presence of DIPEA gave only 13% of the desired amide **27**. Both of these products could be carried forward to the corresponding triacid **26**, either by treatment of **25** with TFA in chloroform (69%) or by saponification of **27** with LiOH in MeOH−THF (99%).

Good coupling yields were obtained, however, using the persilylated triester **24** with the acid chloride derived from **19** under microwave irradiation. Using this method, a yield of 62% of the triacid **26** was isolated after chromatography. Treatment of the triacid **26** with $NiSO_4$ in Tris buffer then gave the nickel complex **2**.

2-D Protein Crystallization

Using our previously published method,[24] we investigated the ability of the Ni^{2+}-chelating lipid **2** to induce 2-D protein crystallization of the His-tagged membrane protein BmrA,[53] an ABC transporter from *Bacillus subtillis*. A lipid monolayer composed of a 1:3 mixture of **2** (the functionalized lipid) and **18** (the diluent lipid) was spread at the air−water interface, following which detergent solubilized BmrA was injected below the lipid layer. After removal of the detergent and subsequent incubation for 4 days at 2 °C, the surface was transferred to a carbon-coated grid, negatively stained with 2% uranyl acetate, and examined by electron microscopy (see Supporting Information for more details). Figure 1 shows electron micrographs of the resulting 2-D crystalline arrays, in which oriented binding and concentration of the protein at the air−water interface can be seen.

Conclusions

Two partially fluorinated lipids with nickel-chelating head groups have been synthesized, for use in 2-D crystallization of His-tagged proteins and for structure determination by electron crystallography. The core fluorinated alcohol moieties of these lipids proved to be very difficult to alkylate under standard

(53) Steinfels, E.; Orelle, C.; Fantino, J.-R.; Dalmas, O.; Rigaud, J.-L.; Denizot, F.; Di Pietro, A.; Jault, J.-M. *Biochemistry* **2004**, *43*, 7491–7502.

conditions, leading to little expected product and extensive degradation of the reactants. These alcohols can, however, be alkylated in good yields using phase-transfer catalysts and prolonged sonication. The utility of fluorolipid **2** to act as a template for binding and orientation of His-tagged proteins has been demonstrated for the membrane protein BmrA.

Experimental Section

10-(Benzyloxy)-2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorodecan-1-ol (8) and 1,10-Bis(benzyloxy)-2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorodecane (9). Method A. Sodium hydride (60% dispersion in oil, 1.13 g, 28.2 mmol) was added portionwise to a solution of the fluorinated diol (**7**) (10.0 g, 21.8 mmol) in DMF (100 mL). The reaction mixture was sonicated under argon for 10 min. Benzyl bromide (4.80 g, 28.2 mmol) and TBAI (4.00 g, 10.8 mmol) were added, and the mixture was sonicated for 3 h. The solvent was evaporated in vacuo, and the residue was taken up in 5% HCl (100 mL). This solution was extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with 0.2 M $Na_2S_2O_3$ (2 × 50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo to afford a yellow oil. The crude product was purified by silica flash column chromatography (a gradient elution of 10−20% EtOAc in LP) to afford the monobenzyl fluorinated alcohol[42] (**8**) as a white solid (4.86 g, 41%), mp 48 °C. R_f: 0.37 (30% EtOAc in LP, $KMnO_4$ dip). ESI-MS, *m/z*: 551 $[M - H]^-$. HRMS calculated for $C_{17}H_{11}F_{16}O_2^-$ 551.0504, found 551.0518. HRMS calculated for $C_{17}H_{12}F_{16}NaO_2^+$ 575.0480, found 575.0482. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.52 (1H, br s), 3.93 (2H, t, *J* 13.9 Hz), 4.04 (2H, t, *J* 14.0 Hz), 4.67 (2H, s), 7.29 − 7.41 (5H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 60.5 (t, *J* 25.5 Hz), 66.7 (t, *J* 25.5 Hz), 74.5, 127.9, 128.3, 128.6, 136.4. Anal. calcd for $C_{17}H_{12}F_{16}O_2$: C, 36.97; H, 2.19. Found: C, 36.87; H, 2.14.

The fluorinated dibenzyl ether[41] (**9**) also was obtained as a colorless solid (2.17 g, 16%), mp 30−32 °C. R_f: 0.81 (30% EtOAc in LP, $KMnO_4$ dip). ESI-MS, *m/z*: 665 $[M + Na]^+$. HRMS calculated for $C_{24}H_{18}F_{16}NaO_2^+$ 665.0944, found 665.0956. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93 (4H, t, *J* 9.2 Hz), 4.67 (4H, s), 7.28−7.40 (10H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 66.7 (t, *J* 25.6 Hz), 74.4, 127.8, 128.3, 128.6, 136.4. Anal. calcd for $C_{24}H_{18}F_{16}O_2$: C, 44.87; H, 2.82. Found: C, 44.53; H, 2.81.

Method B. A mixture of the fluorinated dibenzyl ether (**9**) (3.44 g, 5.35 mmol) and 5% Pd/C (1.00 g) in THF (50 mL) was stirred under an atmosphere of hydrogen for 2 days at room temperature. The reaction mixture was filtered, and the filtrate was evaporated in vacuo to afford a colorless oil. The crude product was purified by silica flash column chromatography (10% EtOAc in LP) to afford the monobenzyl fluorinated alcohol (**8**) as a white solid (0.47 g, 16%) with spectra in agreement with those described above.

2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(3-hexylnonyloxy)decan-1-ol (10). Sodium hydride (60% dispersion in oil, 346 mg, 8.66 mmol) was added to a solution of the fluorinated diol (**7**) (4.00 g, 8.66 mmol) in DMF (30 mL). The mixture was sonicated for 10 min. The alkyl iodide (**6**) (1.47 g, 4.33 mmol) and TBAI (80.0 mg, 0.22 mmol) were added, and the mixture was sonicated under argon for 2 h. The solvent was evaporated in vacuo, and the residue was taken up with 5% HCl (100 mL). The solution was extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with 0.2 M $Na_2S_2O_3$ (2 × 50 mL) and brine (50 mL), dried over Na_2SO_4, filtered, and evaporated in vacuo to afford a colorless oil. The crude product was purified by silica flash column chromatography (a gradient elution of 0 − 30% EtOAc in LP) to afford the fluorinated alcohol (**10**) as a colorless oil (1.50 g, 51%), R_f: 0.22 (10% EtOAc in LP, $KMnO_4$ dip). ESI-MS, *m/z*: 671 $[M - H]^-$, 707 $[M + {}^{35}Cl]^-$, 709 $[M + {}^{37}Cl]^-$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (6H, t, *J* 6.9 Hz), 1.20−1.28 (20H, m), 1.39−1.44 (1H, m), 1.53 (2H, q, *J* 6.9 Hz), 1.95−2.00 (1H, br s), 3.59 (2H, t, *J* 6.9 Hz), 3.89 (2H, t, *J* 14.0 Hz), 4.08 (2H, t, *J* 13.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.7, 26.5, 29.7, 31.9, 33.4, 33.7, 34.3, 60.6 (*J* 25.5 Hz), 67.8 (*J* 24.9 Hz), 71.8. Anal. calcd for $C_{25}H_{36}F_{16}O_2$: C, 44.65; H, 5.40. Found: C, 44.64; H, 5.41.

2-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(3-hexylnonyloxy)decyloxy)acetic Acid (11). This fluorinated acid was prepared according to the general method described by Elshani.[54] A solution of the fluorinated alcohol (**10**) (336 mg, 0.50 mmol) in dry THF (2.5 mL) was added dropwise over a period of 30 min to a mixture of NaH (60% dispersion in oil, 120 mg, 3.00 mmol) in dry THF (2.5 mL) under argon. The mixture was stirred for 1 h at room temperature, and a solution of bromoacetic acid (138 mg, 1.00 mmol) in dry THF (2.5 mL) was added dropwise over a period of 1 h. The mixture was stirred for 24 h, and then the excess NaH was destroyed by dropwise addition of water (5 mL). The THF was evaporated in vacuo, and 6 M HCl (20 mL) was added to the remaining mixture prior to extraction with CH_2Cl_2 (2 × 100 mL). The organic layer was washed with water (50 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo. The crude product was purified by silica flash column chromatography (a gradient elution of 10-70% EtOAc in LP) to afford the fluorinated acid (**11**) as a pale yellow oil (250 mg, 69%) R_f: 0.30 (70% EtOAc in LP, $KMnO_4$ dip). ESI-MS, *m/z*: 753 $[M + Na]^+$. HRMS calculated for $C_{27}H_{38}F_{16}NaO_4^+$ 753.2412, found 753.2437. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (6H, t, *J* 6.9 Hz), 1.20−1.30 (20H, m), 1.36−1.41 (1H, m), 1.52 (2H, q, *J* 6.8 Hz), 3.60 (2H, t, *J* 6.9 Hz), 3.90 (2H, t, *J* 13.9 Hz), 4.11 (2H, t, *J* 13.7 Hz), 4.30 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.7, 26.5, 29.7, 31.9, 33.4, 33.6, 34.3, 68.7, 71.7, 172.4.

12,12,13,13,14,14,15,15,16,16,17,17,18,18,19,19,27,27,28,28,29,29,30,30,31,31,32,32, 33,33,34,34-Dotriacontafluoro-7,39-dihexyl-10,21,25,36-tetraoxapentatetracontan-23-ol (18). A mixture of the fluorinated alcohol (**10**) (482 mg, 0.72 mmol), TBABr (11.60 mg, 0.04 mmol), and powdered NaOH (31.60 mg, 0.79 mmol) was stirred for 15 min at 30 °C. Epichlorohydrin (16.59 mg, 14 μL, 0.18 mmol) was added to the reaction mixture. The mixture was stirred for 14 h at 30 °C and then sonicated for another 7 h. Saturated ammonium chloride (50 mL) was added, and the mixture was extracted with Et_2O (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated in vacuo to afford a colorless oil. The crude product was purified by silica flash column chromatography (a gradient elution of 5−10% EtOAc in LP) to afford the diglyceride (**18**) as a colorless oil (196 mg, 78%), R_f: 0.10 (10% EtOAc in LP, $KMnO_4$ dip). ESI-MS, *m/z*: 1423 $[M + Na]^+$. HRMS calculated for $C_{53}H_{76}F_{32}NaO_5^+$ 1423.508, found 1423.510. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (12H, t, *J* 6.8 Hz), 1.20−1.30 (40H, m), 1.38−1.43 (2H, m), 1.53 (4H, q, *J* 6.6 Hz), 2.30−2.35 (1H, br s), 3.60 (4H, t, *J* 6.9 Hz), 3.67−3.74 (4H, m), 3.91 (4H, t, *J* 9.3 Hz), 3.94−4.04 (5H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.7, 26.5, 29.7, 31.9, 33.4, 33.6, 34.3, 67.8, 68.4, 69.3, 71.7, 73.2.

2-(12,12,13,13,14,14,15,15,16,16,17,17,18,18,19,19,27,27,28,28,29,29,30,30,31,31,32,32,33,33,34,34-Dotriacontafluoro-7,39-dihexyl-10,21,25,36-tetraoxapentatetracontan-23-yloxy)acetic Acid (19). This fluorinated diglyceride acid was prepared according to the general method described by Elshani.[54] A solution of fluorinated diglyceride alcohol (**18**) (1.41 g, 1.00 mmol) in dry THF (5 mL) was added dropwise over a period of 15 min to a mixture of NaH (60% dispersion in oil, 240 mg, 6.00 mmol) in dry THF (5 mL) under argon at 0 °C. The mixture was stirred for 30 min at 0 °C, and a solution of bromoacetic acid (280 mg, 2.00 mmol) in dry THF (5 mL) was added dropwise over a period of 10 min. The mixture was stirred for 24 h at room temperature, and the excess NaH was destroyed by dropwise addition of water (5 mL). The THF was evaporated in vacuo, and to the remaining mixture 6 M HCl (40 mL) was added. The mixture was extracted with CH_2Cl_2 (3 × 100 mL), dried over Na_2SO_4, filtered, and evaporated in vacuo.

(54) Elshani, S.; Kobzar, E.; Bartsch, R. A. *Tetrahedron* **2000**, *56*, 3291–3301.

The crude product was purified by silica flash column chromatography (5−30% EtOAc in LP) to afford the fluorinated diglyceride acid (**19**) as a colorless oil (1.39 g, 95%), R_f: 0.53 (70% EtOAc in LP, $KMnO_4$ dip). ESI-MS, *m*/*z*: 1481 $[M + Na]^+$. HRMS calculated for $C_{55}H_{78}F_{32}NaO_7^+$ 1481.5134, found 1481.5173. ^1H NMR (300 MHz, $CDCl_3$) δ 0.86 (12H, t, *J* 6.9 Hz), 1.20−1.28 (40H, m), 1.35−1.41 (2H, m), 1.53 (4H, q, *J* 6.8 Hz), 3.58 (4H, t, *J* 6.9 Hz), 3.75−4.03 (13H, m), 4.29 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.7, 26.5, 29.7, 33.4, 33.7, 34.4, 67.9, 68.1, 68.5, 71.8, 72.5, 78.9, 173.8. Anal. calcd for $C_{55}H_{78}F_{32}O_7$: C, 45.27; H, 5.39. Found: C, 45.08; H, 5.46.

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Supporting Information Available: Experimental procedures for compounds **1**, **2**, **4**−**6**, **13**−**17**, **20**−**22**, and **24**−**27**. Alternate experimental procedures for compounds **10**, **18** and **19**. ^1H and ^{13}C NMR spectra of compounds **4**−**6**, **8**−**11**, **16**−**19**, **25**−**27**. Detailed description of formation of 2-D crystals of BmrA using **2** and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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