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Benzophenone-containing fatty acids and their related photosensitive fluorescent new probes: Design, physico-chemical properties and preliminary functional investigations

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ABSTRACT

Hydrophobic photoaffinity labeling is a powerful strategy to identify hydrophobic segments within molecules, in particular membrane proteins. Here we report the design and synthesis of a novel family of fluorescent and photosensitive lipid tools, which have a common amino acid scaffold functionalized by three groups: (i) a first fatty acid chain grafted with a photoactivatable benzophenone moiety (Fatty Acid BenzoPhenone, FABP), (ii) a second fatty acid chain to ensure anchoring into a half-bilayer or hydrophobic environment, and (iii) a fluorescent carboxytetramethylrhodamine headgroup (CTMR) to detect the photolabeled compound. We present data of the synthesis and characterization of three lipid tools whose benzophenone ring is situated at various distances from the central scaffold. We could therefore establish structure/properties relationships dependent upon the depth of insertion of benzophenone into the membrane. Our lipid tools were extensively characterized both physico- and bio-chemically, and we assessed their functionality in vitro using bacterioRhodopsin (bR). We thus provide the scientific community with novel and reliable tools for the identification and study of hydrophobic regions in proteins.

1. Introduction

Among all photoreactive chemical functions,[1] benzophenone (BP) is one of the most popular, especially in the field of protein/protein and protein/lipid interaction studies.[2–4] BP activation wavelength is usually around 365 nm, which limits protein degradation and enables studies on cell cultures or other living systems. Moreover, the BP double ring has an unambiguous photoreactivity. Corresponding activated radicals react in a covalent manner and high yields with C–H groups[5,6] present in their hydrophobic surroundings. This allows for a precise identification of the chemical environment. Finally BP is chemically stable in moderate ambient light and compatible with particular synthesis conditions, like those encountered in solid-phase chemistry.[7–9] As a consequence, BP groups are widely used in numerous applications such as for example, photoaffinity probes,[10] photolithography[11] or photocrosslinking.[12]

En route toward a study of protein/lipid interaction, a novel family of fluorescent lipid tools aimed at photoaffinity labeling of hydrophobic peptides within transmembrane proteins was designed and their functionality studied. These tools have a common amino acid scaffold derivatized by three groups: (i) a first fatty acid chain containing a photoactivatable benzophenone group (FABP), (ii) a second fatty chain to ensure a stable anchoring into half-bilayers or micelles, and (iii) a fluorescent carboxytetramethylrhodamine headgroup (CTMR).

We designed a family of benzophenone-containing fatty acids (FABPs) as building blocks displaying two features essential for our study: (i) they are long enough to allow the probe insertion into a lipid half-bilayer; (ii) they are photoactivatable, thereby reacting with amino-acids present in their immediate vicinity. In this context, the position of the BP double ring within the fatty chain is of crucial importance.

Three lipid tools could be obtained in only few steps, within overall yields considered as good for this kind of lipid compounds (6% for 12 steps to 37% for four steps). Their fluorescent properties were fully characterized and their photoreactivity was precisely tuned. A relationship between photolabeling efficiency and depth of membrane insertion of the lipid tools could then be established using bacterioRhodopsin (bR) as a model membrane protein. More generally, the reactivity and the good recovery of the photolabeled protein indicate that this method can be transposed to other relevant targets for the identification and study of ill-defined hydrophobic regions of proteins.

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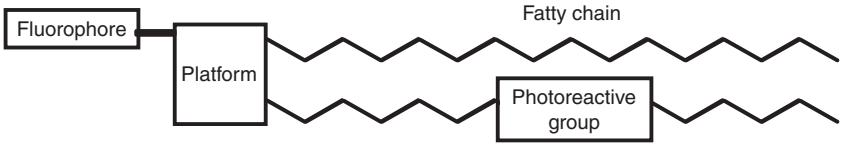


Figure 1. General structure of the lipid tools.

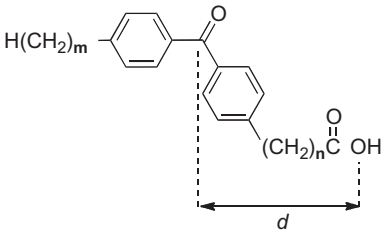


Figure 2. General structure of the benzophenone-containing fatty acids (FABPs) building blocks. m and n indicate the number of methylene. *d* is the distance (Å) between the cross-linking site (–CO) and the oxygen atoms of the hydroxyl group in the carboxylic acid (–COOH).

2. Bioorganic chemistry part

2.1. General conception of lipid tools with three functionalities

2.1.1. The central platform and the chemical bonds

In the present case, it was necessary to design lipid tools able to insert into lipid membranes where hydrophobic proteins are usually embedded. The partition of biomolecules modified by long alkyl chains between membranes and solutions has been extensively studied. Epand[13] showed that a stable insertion requires at least two alkyl chains spatially close to each other. Moreover, the lipid tools had to be photoreactive and detectable. Thus, at least three chemical functionalities were required on a common platform (Fig. 1). As we previously designed it,[14] we chose stable chemical bonds leading to efficient coupling, such as amides to assemble the expected tools. For convenience, we chose a commercially available alpha amino-acid platform, that is the *N*α-Boc-L-2,3-diaminopropionic acid (Boc-Dpr-OH). It has the advantage of displaying three different functions capable of reacting selectively and successively via a peptidic bond with three different partners. In the past, we had already chosen an amino-acid as a platform for such compounds, and demonstrated that functionality was preserved, without inducing any perturbation to the lipid bilayers.[15,16] This choice is in accordance with the chemical partners involved and the following of the synthesis.

2.1.2. Benzophenone

Benzophenone was chosen for the above-exposed reasons. Benzophenone-bearing carboxylic acids were first synthesized by Breslow et al. to study steroids[17] and membrane structure.[18] Hydrogen abstraction from lipids by triplet states of derivatized BP photosensitizers was performed by Patterson and co-workers,[19] notably in linoleate micelles.[20] Lala and coworkers were among the first to perform depth-dependent analysis of membranes[21] by designing suitable fluorescent and photoactivatable lipid tools containing a FABP.[22] More recently, some chemical routes to FABPs were also reported, especially by Spencer and coworkers,[23] to study cellular cholesterol efflux and HDL formation by the mean of analogs of cholesterol[24] or phospholipids.[25]

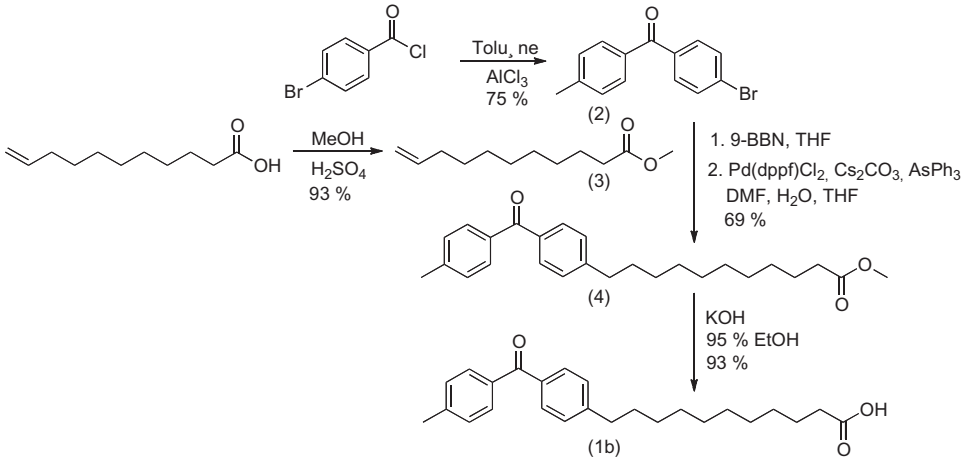
2.1.3. Rhodamine

Detection of the photoaffinity labeling by fluorescence was preferred to detection by radioactivity as a safer and more ubiquitous strategy. In order to follow the homogeneity of the bilayer insertion of the lipid tool and to track the adducts formed after photoactivation in a fine and sensitive way, we chose a fluorescent ring[26] such as carboxytetramethylrhodamine. This residue has a good stability in the envisaged conditions of synthesis; moreover rhodol derivatives have a very good photostability and are relatively insensitive to pH changes.

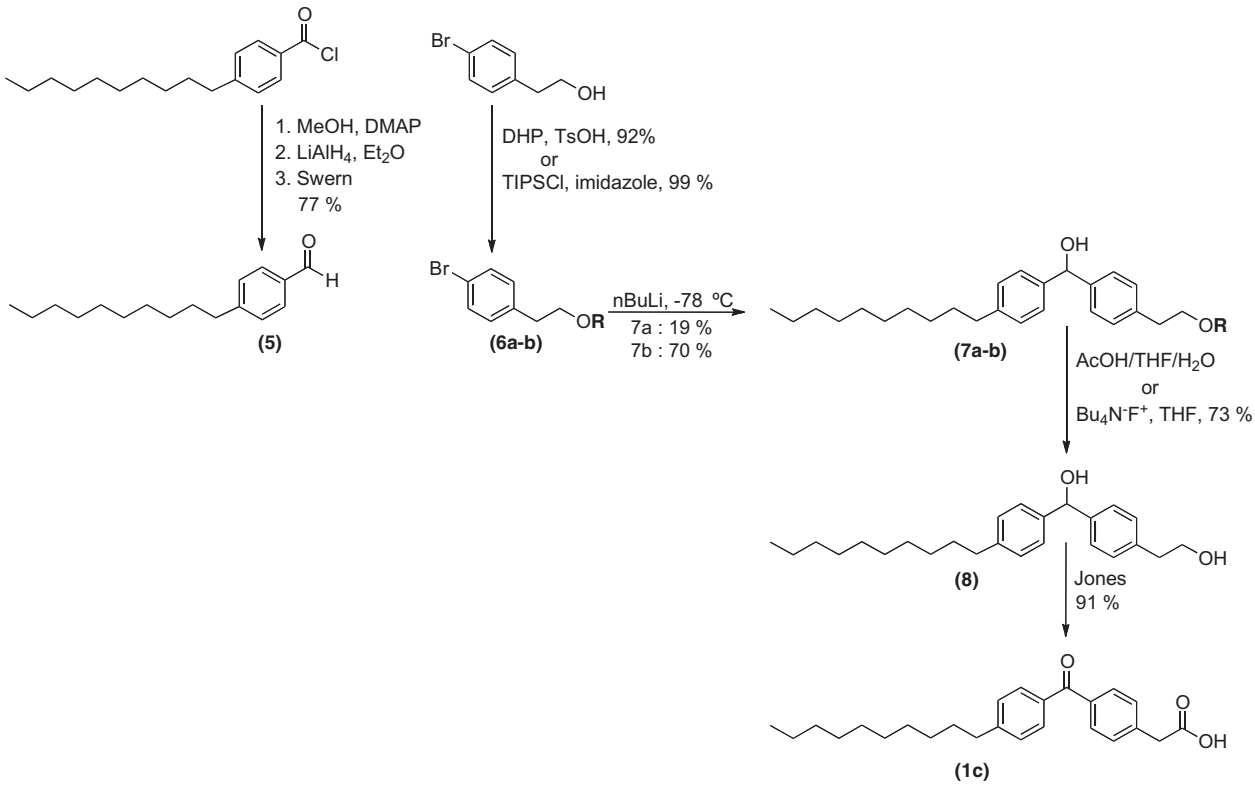
2.2. Preparation of the FABPs building blocks

2.2.1. Medium FABP, C_6-BP-C_5-COOH (1a)

En route toward a depth-dependent exploration, we aimed at the synthesis of a first FABP, where BP is situated in the middle of the fatty chain as starting material. We applied Lala's method[27] to obtain 6-[4-(4-*n*-hexylbenzoyl)phenyl]hexanoic acid ('C_6-BP-C_5-COOH') **1a** within a comparable overall yield. To implement the family, other analogs and isomers were required, particularly one where BP was



Scheme 1. Convergent synthesis of distal FABP **1b**.



Scheme 2. Convergent synthesis of proximal FABP **1c**.

far from the carboxylic acid function ('C_1-BP-C_{10}-COOH', **1b**, a distal FABP), and another one where BP was close to the carboxylic acid function ('C_{10}-BP-C_1-COOH', **1c**, a proximal FABP).

2.2.2. Distal FABP, C_1-BP-C_{10}-COOH (1b)

The preparation of distal FABP (where m = 1 and n = 10, Figure 2) followed the already reported protocol by Spencer[24] with slight variations. Briefly, 4-bromo-4′-methylbenzophenone was prepared according to Nakatani et al.,[28] by a Friedel–Crafts reaction of 4-bromobenzoyl chloride on toluene in the presence of aluminium chloride. This electrophilic aromatic substitution occurred preferentially in *para* position and gave the expected 4-bromo-4′-methylbenzophenone within good yields (75%). In parallel, undecanoic acid was first protected by esterification by methanol in the presence of a catalytic amount of sulfuric acid (93%), in order to avoid side-reactions in the further steps. A Suzuki coupling was then performed between the two reagents, with the help of 9-borabicyclo[3.3.1]nonane (9-BBN) in THF, in the presence of Pd^0 generated in situ to get the expected ketoester within 96% yield. The final saponification followed by a neutralization led to the free carboxylic acid within 96% yield. Finally, in our hands, two steps were necessary to get the expected 1-(4-(4′-methylbenzophenone))-10-undecanoic acid **1b** ('C_1-BP-C_{10}-COOH') within 92% yield (starting from 4-bromo-4′-methyl benzophenone), and four steps within 64% overall yield in a convergent route starting from the bromobenzoyl chloride (Scheme 1).

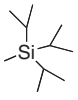
2.2.3. Proximal FABP, C_{10}-BP-C_1-COOH (1c)

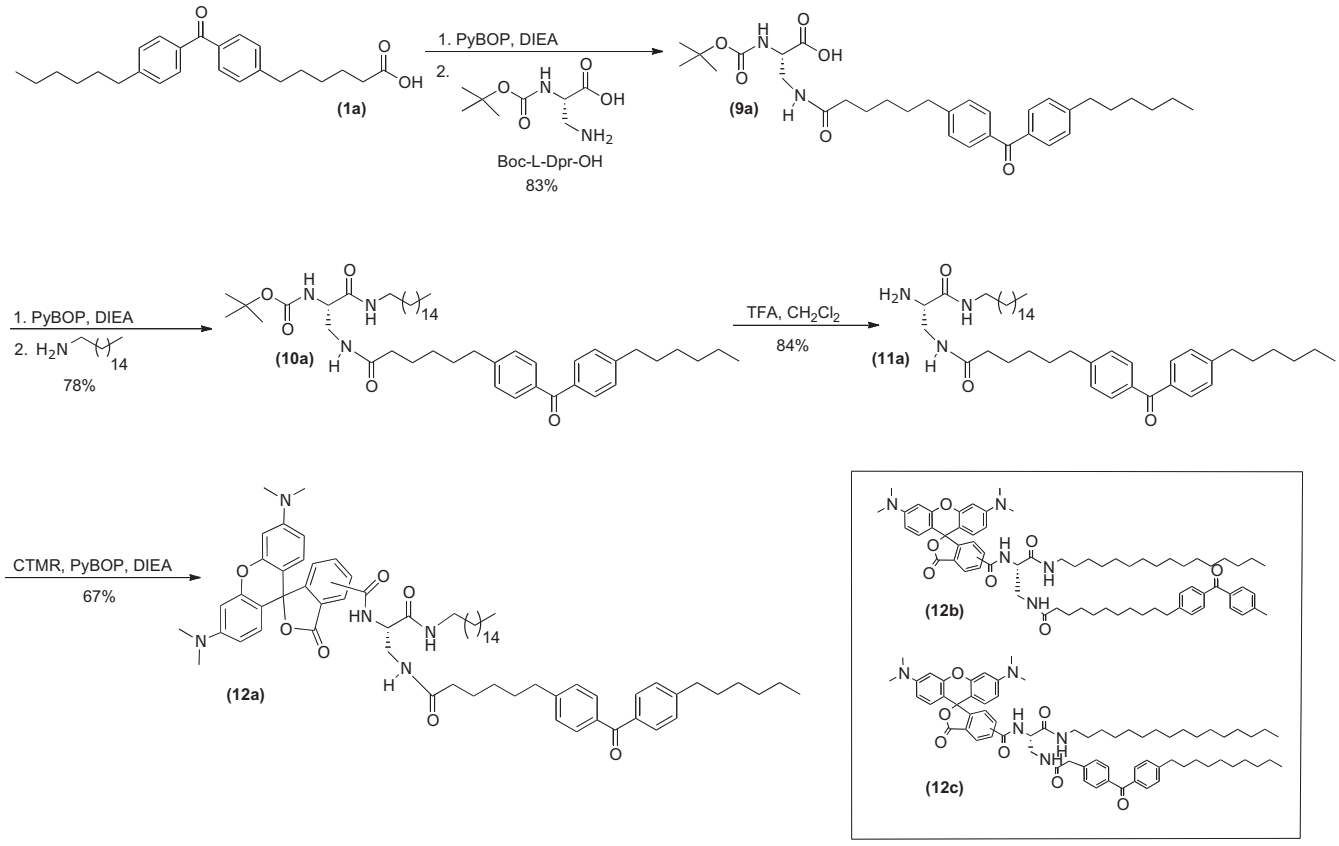
Conversely to what was obtained for the distal FABP, the synthesis of the expected proximal FABP (where m = 10 and n = 1,Fig. 2) was tricky. Lala's method remained unsuccessful. To circumvent the use of oxone already reported, we designed an alternate route to synthesize (4-(4-decylbenzoyl)phenyl)ethanoic acid ('C_{10}-BP-C_1-COOH', **1c**). Thus, the precursor 4-n-decylbenzaldehyde **5** was prepared in high yield (77%), as a colorless oil, in a three-step procedure starting from commercially available
4-decylbenzoyl chloride.

Briefly, the acyl chloride was converted in the corresponding methyl ester, that was fully reduced into the corresponding 4-*n*-decylbenzylic alcohol (Scheme 2). The primary alcohol was oxidized into **5** according to the procedure first described by Omura and Swern.[29] Though an alternate route was described by Rosemund,[30] this route conducted successfully to the expected compound. In parallel, we developed two strategies to protect the commercially available 2-(4-bromophenyl)ethanol. First, dihydropyrane (DHP) was employed[31] and over 6 h, the corresponding acetal **6a** was obtained in 92% yield (Table 1). Nevertheless, it was tricky to condense **6a** with aldehyde **5** and only 19% of the expected secondary alcohol **7a** could be obtained.

An alternate strategy using triisopropylsilyl chloride (TIPSCl) in the presence of imidazole allowed the conversion of 2-(4-bromophenyl)ethanol into the corresponding ether **6b** in high yield (99%) over 2 h. Since our attempt to condense the protected bromo compound **6b** onto aldehyde **5** under Grignard conditions failed, compound **6b** was instead treated with *n*-butyl-lithium at −78 °C

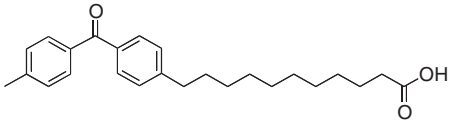
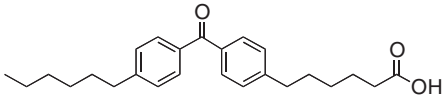
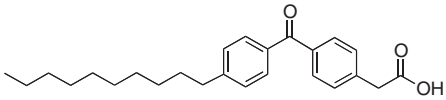
Table 1
Protection of the 2-(4-bromophenyl)ethanol by either DHP (a) or TIPSCl (b) and recovery of the protected secondary alcohol **7a** or **7b**.

	Protecting group (R) →	**6** (yield) (%) →	**7** (yield) (%)
a		92	19
b		99	70



Scheme 3. Total synthesis of lipid tool **12a**. In square: structures of **12b** and **12c** in comparison.

Table 2
Structures of benzophenone-containing fatty acids (FABPs) with predicted distances (*d*, Å) from the hydroxyl group oxygen atom to the keto carboxyl group atom (inspired by Spencer and coworkers[23])

Name	Formula	*d* (Å)
C_1-BP-C_{10}-COOH Distal FABP (**1b**)		18.7
C_6-BP-C_5-COOH Medium FABP (**1a**)		12.7
C_{10}-BP-C_1-COOH Proximal FABP (**1c**)		7.9

and coupled to **5** to give the secondary alcohol **7b** as a colorless oil in 70% yield.

After that, tetra-*n*-butylammonium fluoride (TBAF) was used to deprotect **7b** and primary alcohol **8** was obtained in 73% yield. Further, **8** was doubly oxidized by chromium trioxide in concentrated sulfuric acid aqueous solution to give **1c** in 91% yield, as a white solid. All compounds (final and intermediate) were characterized by MS and NMR. Starting form the 4-*n*-decylbenzaldehyde, the yield of the synthesis was as high as 46%, in comparison to the 22% previously reported. Finally, the overall yield of the total synthesis was 35%, seven steps were needed to get the final product from commercially available 4-decylbenzoyl chloride following a convergent strategy (Scheme 3). Thus, the fine tuning of the reaction allowed to obtain (4-(4-decylbenzoyl)phenyl)ethanoic acid ('C_{10}-BP-C_1-COOH', **1c**) in better overall yield than what was reported for a lower number of steps starting from the aldehyde. This improvement paves the way to the synthesis of new FABP analogs.

2.3. Distance prediction

In the FABPs described here, the cross-linking site (–CO) is situated at defined distances (*d* in Scheme 1) from the oxygen atoms of the hydroxyl group in the carboxylic acid (−COOH). These distances had previously been calculated by Spencer and coworkers using the Spartan program.[23] For our three compounds, these

Table 3
Yield of each step in the synthesis of the lipid tools. m and n are the number of methylene link in the fatty chain (see Fig. 1)

	m	n	**9** (%)	**10** (%)	**11** (%)	**12** (%)
a (medium BP)	6	5	83	78	84	67
b (distal BP)	1	10	90	64	86	74
c (proximal BP)	10	1	50	54	95	66

distances are now ranging from 7.9 Å ('C_{10}-BP-C_1-COOH') to 18.7 Å ('C_1-BP-C_{10}-COOH'), and the middle value for 'C_6-BP-C_5-COOH' can thus be estimated to 12.7 Å (Table 2).

2.4. Assembly of the probes: general procedure

Three FABPs (**1a–c**) were thus synthesized and engaged into the preparation of three novel lipid tools. Each experiment was performed away from light. In order to easily monitor the synthesis progress by UV, we chose to introduce the FABPs at the first step of the synthesis. Each FABP was activated in the presence of DIEA by PyBOP. Then Boc-Dpr-OH was added to the solution. After work-up, Boc-Dpr(FABP)-OH **9a–c** were isolated and activated by PyBOP in the presence of DIEA before the addition of hexadecylamine. Boc-Dpr(FABP)-NH-$C_{16}H_{33}$ **10a–c** were then isolated and

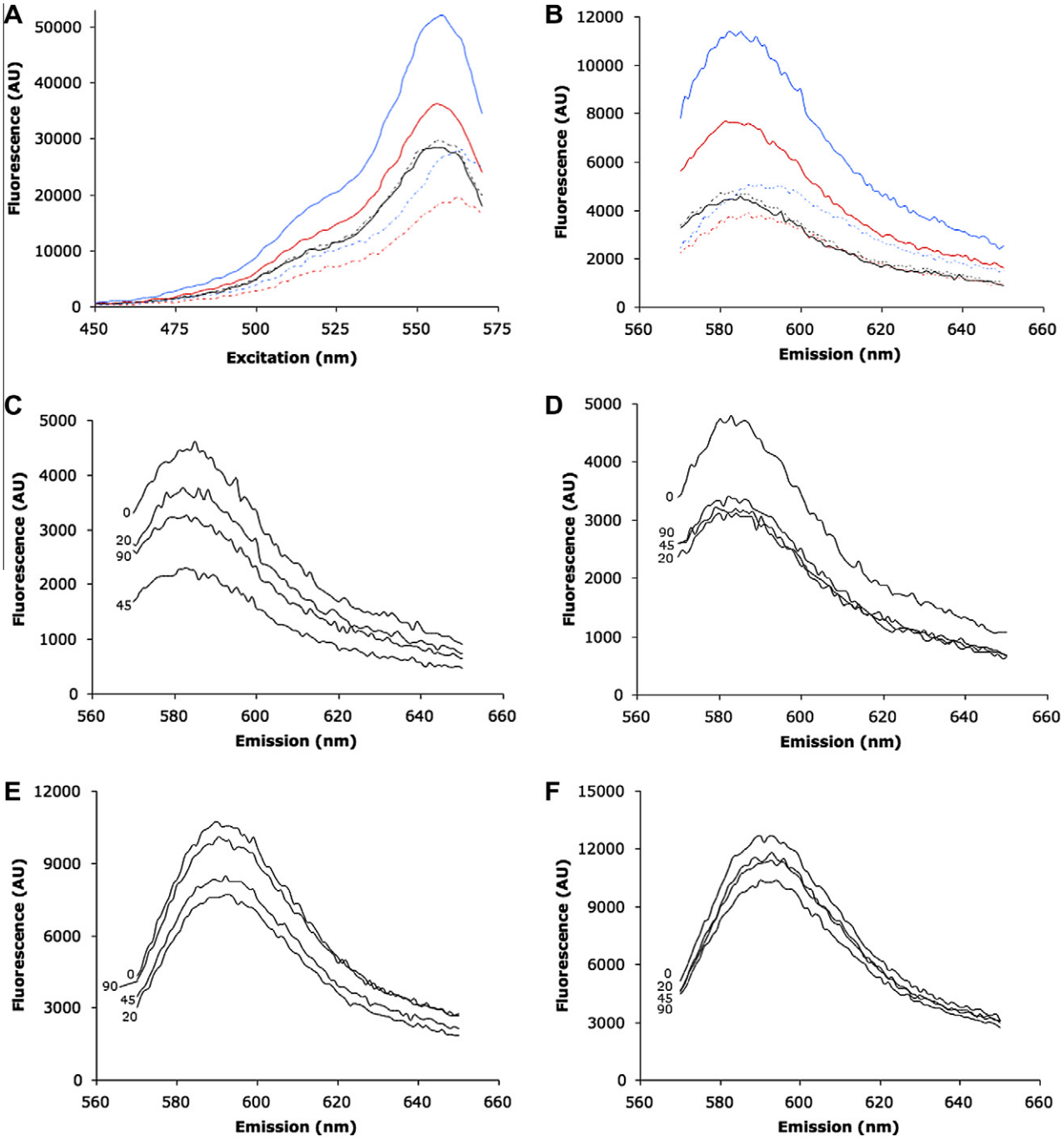


Figure 3. Fluorescence characteristics of lipid tools **12a**, **12b** and **12c**. Liposomes composed of PC/chol/lipid tool (65:30:5 molar ratio) were prepared in PBS buffer at pH 7.4 or pH 5.0. Excitation and emission spectra (50 μM final lipid composition) were recorded at 25 °C on an Infinite 1000 Tecan® spectrofluorimeter. (A) Excitation spectra (emission set at 590 nm) of **12a** (black), **12b** (red) and **12c** (blue), at pH 7.4 (solid lines) or 5.0 (dotted lines). (B) Emission spectra (excitation set at 560 nm) of **12a**, **12b** and **12c** (similar symbols and color codes as in A). (C and D) Emission spectra of **12a** at pH 7.4 (C) and 5.0 (D), after no. (0), 20, 45 or 90 s of UV irradiation. (E and F) Emission spectra of R18 at pH 7.4 (E) and 5.0 (F), after no. (0), 20, 45 or 90 s of UV irradiation.

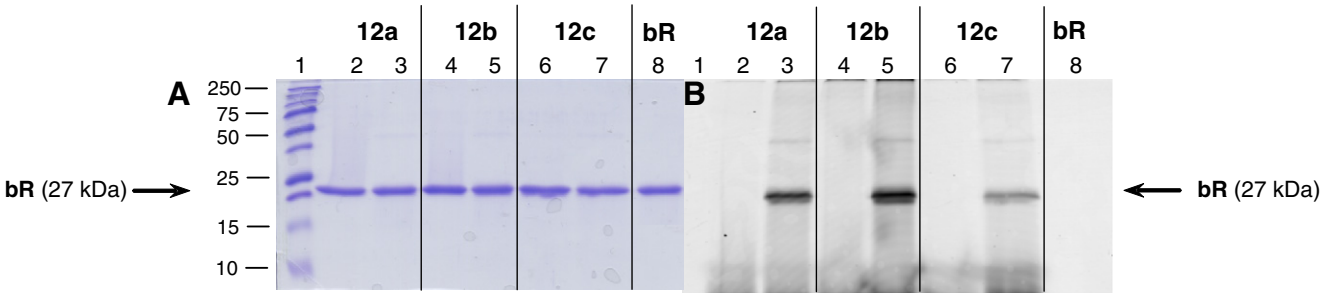


Figure 4. Photolabeling of bR with lipid tools (**12a**–**c**). (A) Coomassie coloration compared to all blue standard (left), (B) Fluorimager analysis. 10 μg of bR are used in each experiment (for each lane). Lane 1, molecular mass markers; protein standards in kDa are indicated on the left side. Lane 2, bR + **12a** not irradiated; lane 3, bR + **12a** irradiated; lane 4, bR + **12b** not irradiated; lane 5, bR + **12b** irradiated; lane 6, bR + **12c** not irradiated; lane 7, bR + **12c** irradiated; lane 8, bR alone.

deprotected by a mixture of TFA and dichloromethane (1/1, v/v). After work-up, H-Dpr(FABP)-NH-$C_{16}H_{33}$ **11a–c** were isolated. Finally CTMR was the last building block to be introduced. After its activation by PyBOP in the presence of DIEA, H-Dpr(FABP)-NH-$C_{16}H_{33}$ **11a–c** were added. The reaction progress was followed by TLC. After work-up, Rhod-Dpr(FABP)-NH-$C_{16}H_{33}$ **12a–c** were isolated as purple powders (Scheme 3 for **12a** synthesis).

Intermediate yields to each lipid tool were comparable (Table 3). Every compound (final and intermediate) was extensively characterized by MS, TLC and NMR whenever possible. Note that CTMR was used as the commercially available mixture of the 5- and 6-carboxytetramethylrhodamine isomers. The two regioisomers were sometimes detectable as two spots by TLC, but could not be further separated. The separation was not required for the biological use of the lipid tool.

3. Biochemistry part

3.1. Fluorescence and absorbance properties of the lipid tools

We characterized the spectral properties of lipid tools **12a**, **12b** and **12c** by fluorescence spectroscopy in liposomes. This was done using a chloroformic solution of lipid tool added to chloroformic solutions of phosphatidylcholine and cholesterol (PC/chol/lipid tool 65:30:5 molar ratio). Fluorescence spectra were then recorded at pH 7.4 and pH 5.0. Depending on the lipid tool considered, the influence of pH on its fluorescence remained low to negligible on both excitation (Fig. 3A) and emission spectra (Fig. 3B), in agreement with the well-documented stability of rhodol derivatives toward pH variations.[32] Excitation and emission maxima were found at 560 ± 2 nm and 585 ± 2 nm for both pH, respectively. These are therefore typical spectral properties of a rhodamine derivative. This indicates that CTMR grafted onto these novel lipid tools behaves as a regular rhodamine moiety.

Interestingly when comparing spectra obtained from liposomes containing our lipid tools, submitted or not to a 20 s, 45 s or 90 s—UV irradiation, we noticed a remarkable stability of the rhodamine moiety to UV light exposure for both pH tested (Fig. 3C and D for **12a**; data not shown for **12b** and **12c**). Not only the quantum yields of fluorescence were comparable, but also were the maxima of both excitation (data not shown) and emission spectra. This agrees well with the previously reported photostability of rhodamine present in other fluorophores.[33,34]

To check whether this stability under our specific experimental conditions was intrinsic to the rhodamine moiety or correlated to the chemical formula of lipid tools, we prepared PC/chol liposomes containing 5 mol % of octadecyl Rhodamine B chloride (R18), a well-documented lipid compound used in fluorescence spectroscopy, notably in viral fusion assays.[35,17] The behavior of R18 into PC/chol liposomes was comparable to that observed with **12a**, **12b** and **12c**, with respect to pH and irradiation conditions (Fig. 3E and F). Excitation (data not shown) and emission maxima remained unaffected by UV irradiation (Fig. 3E for pH 7.4 and Fig. 3F for pH 5.0). Taken together these data clearly demonstrate that the observed stability of rhodamine fluorescence to pH and UV irradiation is an intrinsic property of rhodamine, whatever the chemical lipid skeleton it is associated to.

3.2. Hydrophobic photoaffinity labeling of bacterioRhodopsin

To check the capability of lipid tools **12a**, **12b** and **12c** to label hydrophobic protein, we chose bacterioRhodopsin as a convenient membrane protein model. Indeed its three-dimensional structure is known,[36,37] revealing an arrangement with seven alpha-helicoidal transmembrane domains. Moreover it is commercially available, which renders its use very easy. BacterioRhodopsin resuspended in water was incubated with micelles containing lipid tools (**12a**, **12b** or **12c**). The micelles obtained were submitted to 45 s UV irradiation at 365 nm. The suspension was denaturated and submitted to SDS–PAGE protein gels (Fig. 4) followed by a Coomassie coloration (A), compared to all blue standard (left) and Fluorimager analysis (B). Interestingly when visualizing the gel under Fluorimager, we first noticed fluorescent labeling for a molecular weight corresponding to bacterioRhodopsin, which indicates that the covalent photolabeling procedure with our lipid tool was successful (Fig. 4B lanes 3, 5 and 7). Most importantly fluorescent labeling was not observed when the protein/lipid tool mixture was not irradiated (Fig. 4B lanes 2, 4 and 6). Also note that the protein was not damaged by the irradiation (Fig. 4A) and the protein alone did not give any fluorescent signal (Fig. 4 lane 8). When bR was heated to 95 °C before irradiation, we noticed the appearance of high-order oligomers that could be photolabeled (data not shown). This tends to indicate that bR treatment by heat leads to the formation of multimers hydrophobic by nature, and that photolabeling with our lipid tools is not dependent upon a specific protein conformation.

Figure 4B shows that the efficacy of the photo-crosslinking is dependent on the distance between the benzophenone double ring and the CO. In this particular case, the longer the distance (d), the more intense the crosslinking.

4. Conclusion

An alternate expedient chemical route is now available to reach functional FABPs. We therefore synthesized a novel generation of lipid tools for hydrophobic photoaffinity labeling, based upon a fluorescent detection via a rhodamine dye. This approach relies on the use of lipid tools containing a photoactivatable agent and a fluorescent probe for the detection of adducts of the reaction.

The corresponding lipid tools offer numerous advantages: their synthesis is relatively easy, they are stable in membranes due to their double fatty acid chain structure, photolabeling is performed at an ultraviolet wavelength which prevents degradation of the

proteins studied. After photoactivation, the benzophenone ring is expected to induce a covalent cross-linking reaction with its immediate environment in the membrane. The CTMR headgroup allows to detect and follow the cross-linking reaction products. Preliminary biochemical experiments efficiently proved the functionality of our new probes. We thus provide the scientific community with new, robust and efficient tools of study and identification of hydrophobic zones of proteins.[38]

5. Experimental procedures

5.1. Chemistry

5.1.1. Generals

The amino-acid Boc-L-Dpr-OH was purchased from Iris Biotech GmbH. The ninhydrin, PMA and TFA were from Sigma-Aldrich. The CTMR and PyBOP were purchased from Novabiochem. The hexadecylamine was from Aldrich and the DIEA from Alfa Aesar. 6-(4-(4-*n*-hexylbenzoyl) phenyl) hexanoic acid was synthesized following an already reported protocol.[39] Chemical reagents were used without further purification. CH_2Cl_2 and THF were distilled under argon atmosphere and dried over CaH_2 and sodium (in the presence of benzophenone) respectively. Chromatography was carried out on silica gel 60 (40–63 μm): powder and aluminium-supported sheets for TLC plates were purchased from Merck. All ^1H and ^{13}C NMR spectra were acquired on Brucker 300 MHz or 400 MHz spectrometers as mentioned. ^1H chemical shifts (δ) are reported in ppm as referenced to residual undeuterated solvent peak and coupling constants (J) are reported in Hz. Mass spectra (ES-MS) were recorded on an ESI/TOF Mariner mass spectrometer. When a compound is described for the first time, a high resolution mass result (HR-MS) acquired on a GC/TOF Agilent technology is provided. Melting points are measured on a Büchi B-540 apparatus. Analytical HPLC analyses were performed on an analytical Jupiter 5 μ C_4 300 Å column (150 × 4.6 mm) from Phenomenex with a flow rate of 1 mL min^{-1} using a 10 min linear gradient from 0% CH_3CN in water (0.1% TFA) to 100% CH_3CN (0.1% TFA) followed by 5 min at 100% CH_3CN (0.1% TFA). Retention times (Rt) from analytical RP-HPLC are reported in minutes.

5.1.1.1. 4-Bromo-4′-methylbenzophenone (2). To a solution of 500 mg (2.28 mmol) of 4-bromobenzoyl chloride in 5 mL of toluene was added, in one portion, 456 mg (3.42 mmol) of $AlCl_3$. The resulting mixture was stirred at reflux for 2 h. The solution was cooled at room temperature and poured into an ice concentrated HCl solution. The aqueous layer was extracted with EtOAc. The combined organic extracts were washed with 1 N HCl aqueous solution, with water and brine and dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified on silica gel with 38:2–37:3 cyclohexane/ethyl acetate v/v to give, after recristallisation in *n*-hexane, 470 mg (75%) of **2** as a white solid. TLC: R_f = 0.4 (9:1 Cyclohexane/EtOAc); NMR ^1H (300 MHz; $CDCl_3$) δ 7.70–7.60 (m, 6H), 7.30–7.27 (m, 2H), 2.44 (s, 3H). NMR ^{13}C (75 MHz; $CDCl_3$) δ 195.9, 144.2, 137.3, 135.0, 132.1, 132.0, 130.9, 129.7, 127.8, 22.3. MS calcd for $C_{14}H_{11}BrO$ = 274.0, found 274.0.

5.1.1.2. Methyl 10-undecenoate (3). To a solution of 5.00 g (27.1 mmol) of 10-undecenoic acid in 100 mL of methanol, 1 mL of concentrated H_2SO_4 was added. The resulting mixture was stirred at reflux overnight. The solution was cooled to room temperature and 200 mL of petroleum ether was added. The combined organic extracts were washed with a saturated $NaHCO_3$ aqueous solution, with brine and dried over $MgSO_4$, filtered and concentrated under vacuum. The new product was dried under vacuum overnight to give 5.00 g (93%) of **3** as a yellow oil. TLC: R_f = 0.4 (9:1 Cyclohexane/EtOAc); NMR ^1H (400 MHz; $CDCl_3$) δ 5.83–5.77 (ddt, 1H, J = 17.1;10.1;6.8), 5.01–4.91 (m, 2H), 3.66 (s, 3H), 2.29 (t, 2H, J = 7.6), 2.04 (q, 2H, J = 7.0), 1.61 (quint, 2H, J = 7.3), 1.38–1.29 (m, 10H); NMR ^{13}C (100 MHz; $CDCl_3$) δ 174.9, 139.8, 114.8, 52.0, 34.8, 34.4, 29.8, 25.6. MS calcd for $C_{12}H_{23}O_2$ = 199.2, found 199.2.

5.1.1.3. Methyl 11-(4-(4-methylbenzoyl)phenyl)undecanoate (4). To a stirred solution of 181 mg (0.91 mmol) of **2** in 5 mL of THF at 0 °C under argon, 4 mL of a 0.5 M solution of 9-BBN in THF was added. The resulting mixture was stirred at room temperature for 4 h. To a solution of 890 mg (2.73 mmol) of cesium carbonate in 2 mL of DMF, 3 mL of THF and 0.6 mL of water, 55 mg (0.18 mmol) of triphenylarsine, 131.7 mg (0.18 mmol) of $Pd(dppf)Cl_2$ and 250 mg (0.91 mmol) of **3** were added. The first mixture mentioned was added, via a cannula, to the second one under argon. The resulting solution was stirred at room temperature for 22 h and poured into 15 mL of water and 15 mL of EtOAc. The organic layer was washed with water, with a saturated $NaHCO_3$ aqueous solution and with brine. The solution was filtrated through Celite and washed with EtOAc. The organic extract was dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified on silica gel with 100:0–100:10 cyclohexane/ethyl acetate v/v to give 247 mg (69%) of **4** as a yellow oil. TLC: R_f = 0.4 (9:1 Cyclohexane/EtOAc). NMR ^1H (300 MHz; $CDCl_3$) δ 7.73–7.71 (m, 4H), 7.37–7.29 (d, 4H, J = 7.4), 3.67 (s, 3H), 2.69 (t, 2H, J = 7.7), 2.45 (s, 3H), 2.31 (t, 2H, J = 7.5), 1.67–1.60 (m, 4H), 1.36–1.24 (m, 12H); NMR ^{13}C (75 MHz; $CDCl_3$) δ 196.3, 174.3, 147.9, 142.9, 135.4, 135.3, 130.2, 128.9, 128.3, 51.4, 36.0, 34.1, 31.2, 29.7, 29.5, 29.4, 29.3, 29.2, 29.2, 24.9, 21.6. MS calcd for $C_{26}H_{35}O_3$ = 395.2, found 395.2.

5.1.1.4. 11-(4-(4-methylbenzoyl)phenyl)undecanoic acid (1b). To a solution of 200 mg (0.51 mmol) of **4** in 10 mL of EtOH 95%, 85 mg (1.52 mmol) of sodium hydroxide was added. The resulting mixture was stirred overnight at room temperature. The solution was acidified to pH 1 with HCl 1 N aqueous solution. The aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine and dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified by recristallisation in petroleum ether to give 180 mg (93%) of **1b** as a white solid. NMR ^1H (300 MHz; $CDCl_3$) δ 7.74–7.71 (dd, 4H, J = 8.1, 2.7), 7.29–7.27 (d, 4H, J = 7.4), 2.69 (t, 2H, J = 7.7), 2.45 (s, 3H), 2.36 (t, 2H, J = 7.5), 1.65–1.61 (m, 4H), 1.41–1.22 (m, 12H); NMR ^{13}C (50 MHz; $CDCl_3$) δ 196.3, 179.1, 147.9, 142.9, 135.4, 135.2, 130.2, 128.9, 128.3, 36.0, 33.9, 31.2, 29.5, 29.4, 29.4, 29.3, 29.2, 29.0, 24.7, 21.6. MS calcd for $C_{25}H_{33}O_3$ = 381.2, found 381.2.

5.1.1.4.1. Methyl 4-decylbenzoate[40]*.* To a solution of 1.00 g (3.56 mmol) of 4-decylbenzoyl chloride in 10 mL of dry methanol, 43 mg (0.36 mmol) of dimethylaminopyridine were added. The resulting mixture was stirred at room temperature for 1 h and then concentrated to dryness. The crude solution was dissolved with 20 mL of CH_2Cl_2 and washed with water (2 × 20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were collected and dried over $MgSO_4$, filtered and concentrated under vacuum. The new product was dried under vacuum overnight to give 0.97 g (99%) of the expected compound as a colorless oil. NMR ^1H (400 MHz; $CDCl_3$): δ 7.96 (d, 2H, J = 8.2 Hz), 7.24 (d, 2H, J = 8.2 Hz), 3.90 (s, 3H), 2.66 (t, 2H, J = 7.8 Hz), 1.63 (m, 2H), 1.32–1.27 (m, 14H), 0.89 (t, 3H, J = 6.7 Hz). NMR ^{13}C (100 MHz; $CDCl_3$): δ 167.8, 149.1, 130.3, 129.0, 128.3, 52.5, 36.7, 32.6, 31.8, 30.2, 30.2,

30.1, 30.0, 29.9, 23.3, 14.7. MS calcd for $C_{18}H_{29}O_2$ = 277.2, found 277.2.

5.1.1.4.2. 4-Decylphenyl methanol[41]. A solution of 40 mg (1.03 mmol) of lithium aluminum hydride in 15 mL of ethylic ether was placed in a two-necked flask equipped with reflux condenser and a dropping funnel. Through the dropping funnel, 0.95 g (1.03 mmol) of methyl 4-decylbenzoate was introduced at a rate such as to produce a gentle reflux. The resulting mixture was stirred at room temperature for another 1 h. Then 20 mL of water were added dropwise and cautiously, the solution was poured onto ice and a solution of 10% of H_2SO_4 was added. The aqueous layer was extracted with ethylic ether. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified on silica gel with 95:5–80:20 cyclohexane/ethyl acetate v/v to give 0.77 g (90%) of 4-decylphenyl methanol as a white solid. NMR ^{1}H (300 MHz; $CDCl_3$): δ 7.29 (d, 2H, J = 7.9 Hz), 7.19 (d, 2H, J = 7.9 Hz), 4.65 (s, 2H), 2.63 (t, 2H, J = 7.8 Hz), 1.63 (m, 2H), 1.29 (m, 14H), 0.91 (t, 3H, J = 6.8 Hz). NMR ^{13}C (75 MHz; $CDCl_3$): δ 143.1, 138.8, 129.2, 127.8, 65.9, 36.3, 32.6, 32.2, 30.3, 30.2, 30.0, 23.3, 14.7. MS calcd for $C_{17}H_{28}O$ = 248.2, found 248.2.

5.1.1.5. 4-Decylbenzaldehyde (5). To a solution of 390 μL (4.63 mmol) of oxalyl chloride at −78 °C, 460 μL (6.49 mmol) of dimethylsulfoxyde in 10 mL of CH_2Cl_2 were cautiously added. The resulting mixture was stirred for 10 min and a solution of 0.77 g (3.09 mmol) of 4-decylphenyl methanol solubilized in 10 mL of CH_2Cl_2 was added cautiously at −78 °C. The resulting solution was stirred for another 20 min and 1.73 mL (12.4 mmol) of triethylamine was added and the solution was allowed to reach room temperature over 1.5 h. 20 mL of a saturated $NaHCO_3$ aqueous solution were added. The aqueous layer was extracted with ethylic ether (1 × 50 mL). The combined organic extracts were washed with saturated $NaHCO_3$ aqueous solution, with brine and dried over $MgSO_4$, filtered and concentrated under vacuum. The new product was dried overnight to give 0.66 g (87%) of **5** as a colorless oil. NMR ^{1}H (300 MHz; $CDCl_3$): δ 9.98 (s, 1H), 7.80 (d, 2H, J = 8.1 Hz), 7.34 (d, 2H, J = 8.1 Hz), 2.69 (t, 2H, J = 7.8 Hz), 1.65 (m, 2H), 1.27 (m, 14H), 0.89 (t, 3H, J = 6.6 Hz). NMR ^{13}C (75 MHz; $CDCl_3$): δ 192.5, 151.1, 135.1, 130.5, 129.7, 36.9, 32.6, 31.7, 30.2, 30.1, 30.0, 23.3, 14.7. HRMS calcd for $C_{17}H_{26}O$ = 246.1984, found 246.2004.

5.1.1.6. 4-(Bromophenethoxy)triisopropylsilane (6b)[42]. To a mixture of 0.50 g (2.48 mmol) of 4-bromophenethyl alcohol in 10 mL of CH_2Cl_2 was added a solution of 0.72 g (3.73 mmol) of triisopropylsilyl chloride and 0.51 g (7.44 mmol) of imidazole. The resulting mixture was stirred at room temperature for 2 h. The organic layer was washed with water (10 mL) and brine (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried with $MgSO_4$, filtered and evaporated. The crude product was purified on silica gel with 100:0 to 100:10 cyclohexane/ethyl acetate v/v to give 0.88 g (99%) of **6b** as a colorless oil. NMR ^{1}H (300 MHz; $CDCl_3$): δ 7.40 (m, 2H), 7.11 (m, 2H), 3.87 (t, 1H, J = 6.9 Hz), 2.81 (t, 1H, J = 6.9 Hz), 1.05 (m, 21H). NMR ^{13}C (75 MHz; $CDCl_3$): δ 139.0, 131.9, 131.6, 120.6, 65.1, 39.8, 18.6, 12.7. HRMS calcd for $C_{14}H_{22}BrOSi$ = 313.0623 (with ^{79}Br), found 313.0638.

5.1.1.7. (4-Decylphenyl)(4-(2-5-triisopropylsilyloxy) ethyl)phenyl) methanol (7b). To a solution of 0.96 g (2.67 mmol) of **6b** in 10 mL of dry THF at −78 °C under argon, 1.8 mL (2.92 mmol) of *n*-butyllithium (1.6 M in hexane) was added dropwise with a syringe. After 1 h at −78 °C, a solution of 0.60 g (2.43 mmol) of **5** in dry THF was added drop by drop. The resulting mixture was allowed to reach room temperature over 1.5 h and kept stirring over night. It was quenched by a saturated $NaHCO_3$ aqueous solution (20 mL). The aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine and dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified on silica gel with 100:0–100:7 cyclohexane/ethyl acetate v/v to give 0.89 g (70%) of **7b** as a colorless oil. NMR ^{1}H (400 MHz; $CDCl_3$): δ 7.32–7.13 (m, 8H), 5.81 (s, 1H), 3.89 (dt, 2H, J = 10.6, 7.2 Hz), 2.86 (dt, 2H, J = 10.6, 7.2 Hz), 2.59 (t, 2H, J = 7.8 Hz), 1.59 (m, 2H), 1.30 (m, 14H), 1.05 (m, 21H), 0.89 (t, 3H, J = 6.7 Hz). NMR ^{13}C (100 MHz; $CDCl_3$): δ 142.9, 142.5, 141.9, 139.2, 129.9, 129.1, 127.1, 76.7, 65.5, 40.1, 36.3, 32.6, 32.2, 30.3, 30.2, 30.0, 23.4, 18.6, 14.8, 12.7. HRMS calcd for $C_{31}H_{49}O_2Si$ = 481.3502 (one isopropyl residue is lost), found 481.3496.

5.1.1.8. 2-(4-((4-decylphenyl)(hydroxy)methyl)phenyl)ethanol (8). To a solution of 0.84 g (1.60 mmol) of **7b** in 10 mL of THF, 3 mL (3 mmol) of *n*-tetrabutylammonium fluoride were added. The resulting mixture was stirred at room temperature for 1.5 h and was quenched by a saturated $NaHCO_3$ aqueous solution (20 mL). The aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine and dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified on silica gel with 100:0–98:2 cyclohexane/ethyl acetate v/v to give 0.43 g (73%) of **8** as a white solid. NMR ^{1}H (300 MHz; $CDCl_3$): δ 7.36–7.14 (m, 8H), 5.82 (s, 1H), 3.85 (m, 2H), 2.86 (t, 2H, J = 6.5 Hz), 2.58 (t, 2H, J = 7.8 Hz), 1.59 (m, 2H), 1.26 (m, 14H), 0.89 (t, 3H, J = 6.7 Hz). NMR ^{13}C (75 MHz; $CDCl_3$): δ 143.0, 142.9, 141.8, 138.3, 129.7, 129.2, 127.4, 127.1, 76.6, 64.2, 39.5, 36.3, 32.6, 32.1, 30.3, 30.2, 30.0, 23.4, 14.8. HRMS calcd for $C_{25}H_{36}O_2$ = 368.2715, found 368.2738.

5.1.1.9. 2-(4-((4-decylphenyl)(hydroxy)methyl)phenyl)acetic acid (1c). To a solution of 0.41 g (1.11 mmol) of **8** in 10 mL of acetone was added, drop by drop, 1 mL of Jones reagent (26.72 g of CrO_3 in 77 mL of H_2O and 23 mL of concentrated H_2SO_4). The resulting mixture was stirred at room temperature for 2 h and quenched with 2-propanol. Water (20 mL) was added to the solution and most of the solvent was removed under reduced pressure. The aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified on silica gel with 95:5–85:15 cyclohexane/ethyl acetate v/v to give 0.38 g (91%) of **1c** as a white solid. TLC: R_f = 0.4 (9:1 CH_2Cl_2/MeOH); mp 71.0–72.5 °C; NMR ^{1}H (300 MHz; $CDCl_3$): δ 7.77 (dd, 4H, J = 8.1, 12.8 Hz), 7.35 (dd, 4H, J = 8.1, 38.7 Hz), 3.76 (s, 2H), 2.69 (t, 2H, J = 7.7 Hz), 1.64 (m, 2H), 1.28 (m, 14H), 0.89 (t, 3H, J = 6.4 Hz). NMR ^{13}C (75 MHz; $CDCl_3$): δ 196.8, 177.4, 149.0, 138.3, 137.6, 135.6, 131.0, 130.0, 129.0, 41.6, 36.7, 32.6, 31.8, 30.2, 30.0, 23.3, 14.8. MS calcd for $C_{25}H_{33}O_3$ = 381.2, found 381.2.

5.1.2. General procedure to synthesize lipid tools

5.1.2.1. Synthesis of Boc-L-Dpr(FABP)-OH (9). To a solution of FABP (1 equiv) in anhydrous dichloromethane, DIEA (5 equiv) and PyBOP (1 equiv) were added. The resulting mixture was stirred for 10 min (acid activation). Then Boc-L-Dpr-OH (1 equiv) solubilized in a mixture of anhydrous dichloromethane and methanol (3:2 v/v), were added to the above mentioned activated solution. The resulting mixture was stirred at room temperature protected from the light. The organic layer was washed with saturated $NaHCO_3$ aqueous solution and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified on silica gel with 100:0–100:15 CH_2Cl_2/MeOH v/v eluent.

5.1.2.2. Synthesis of Boc-L-Dpr(FABP)-NH-$C_{16}H_{33}$ (10). To a solution of Boc-L-Dpr(FABP)-OH (1 equiv) in anhydrous dichloromethane, DIEA (5 equiv) and PyBOP (1.3 equiv) were added. The

resulting mixture was stirred for 10 min (acid activation). Then hexadecylamine (1 equiv) was added. The resulting mixture was stirred at room temperature protected from the light. The organic layer was washed with saturated $NaHCO_3$ aqueous solution and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified on silica gel with 100:0–100:4 CH_2Cl_2/MeOH v/v eluent.

5.1.2.3. Synthesis of H-L-Dpr(FABP)-NH-C16H33 (11). Boc-L-Dpr(FABP)-NH-$C_{16}H_{33}$ was dissolved in a mixture of CH_2Cl_2/TFA 1:1 v/v. The resulting mixture was stirred for 1 h at room temperature protected from the light. The solution was diluted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$ aqueous solution and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under vacuum.

5.1.2.4. Synthesis of Rhod-Dpr(FABP)-NH-$C_{16}H_{33}$ (12). To a solution of CTMR (1.3 equiv) in anhydrous dichloromethane, DIEA (5 equiv) and PyBOP (1.3 equiv) were added. The resulting mixture was stirred for 10 min (acid activation). Then H-L-Dpr(FABP)-NH-$C_{16}H_{33}$ (1 equiv) solubilized in anhydrous dichloromethane was added to the above mentioned activated solution. The solution was concentrated under vacuum. The crude product was purified on silica gel with 100:0–100:15 CH_2Cl_2/MeOH v/v eluent.

5.1.3. Specific characterizations

5.1.3.1. (*S*)-2-(*tert*-Butoxycarbonylamino)-3-(6-(4-(4-hexylbenzoyl)phenyl)hexanamido) propanoic acid (9a). Reaction time: 12 h; TLC: R_f = 0.3 (9:1 CH_2Cl_2/MeOH); NMR ^{1}H (300 MHz; $CDCl_3$) δ 7.67 (m, 4H), 7.24 (m, 4H), 4.14 (m, 1H), 3.73 (q, 1H, J = 7.0), 3.14 (q, 1H, J = 7.0), 2.65 (m, 4H), 2.17 (m, 2H), 1.62–1.21 (m, 23H), 0.87 (m, 3H). MS calcd for $C_{33}H_{47}N_2O_6$ = 567.3, found 567.4.

5.1.3.2. (*S*)-*tert*-Butyl 1-(hexadecylamino)-3-(6-(4-(4-hexylbenzoyl)phenyl)hexanamido)-1-oxopropan-2-ylcarbamate (10a). Reaction time: 6 h; TLC: R_f = 0.4 (9:2 CH_2Cl_2/MeOH); NMR ^{1}H (300 MHz; $CDCl_3$) δ 7.73 (m, 4H), 7.27 (m, 4H), 6.86 (s, 1H), 6.35 (s, 1H), 5.98 (d, 1H, J = 6.0), 4.16 (m, 1H), 3.71 (m, 1H), 3.51 (m, 1H), 3.23 (m, 2H), 2.69 (t, 4H, J = 7.5), 2.20 (t, 2H, J = 7.5), 1.70–1.25 (m, 51H), 0.88 (m, 6H); NMR ^{13}C (75 MHz ; $CDCl_3$) δ 196.9, 175.5, 171.1, 148.6, 148.0, 136.3, 136.0, 130.9, 128.9, 81.0, 42.7, 40.2, 37.0, 36.7, 36.4, 32.6, 32.4, 31.8, 31.5, 30.4–29.5, 29.0, 27.5, 26.1, 23.3, 14.7. MS calcd for $C_{49}H_{80}N_3O_5$ = 790.7, found 790.6.

5.1.3.3. (*S*)-*N*-(2-Amino-3-(hexadecylamino)-3-oxopropyl)-6-(4-(4-hexylbenzoyl)phenyl) hexanamide (11a). Reaction time: 1 h; TLC: R_f = 0.4 (9:1 CH_2Cl_2/MeOH); NMR ^{1}H (300 MHz ; $CDCl_3$) δ 7.73 (m, 4H), 7.50 (m, 1H), 7.27 (m, 4H), 6.29 (m, 1H), 3.65 (m, 1H), 3.47–3.37 (m, 2H), 3.23 (m, 2H), 2.69 (t, 4H, J = 7.5), 2.20 (t, 2H, J = 7.5), 1.66-1.25 (m, 42H), 0.88 (m, 6H); NMR ^{13}C (75 MHz ; $CDCl_3$) δ 196.9, 174.8, 173.9, 148.6, 148.0, 136.2, 136.0, 130.8, 128.9, 55.9, 44.3, 39.8, 37.2, 36.6, 36.4, 32.6, 32.3, 31.8, 31.4, 30.3–29.5, 27.6, 26.1, 23.3, 14.7. MS calcd for $C_{44}H_{72}N_3O_3$ = 690.5, found 690.5.

5.1.3.4. Rhod-Dpr(CO-C_5-BP-C_6)-NH-$C_{16}H_{33}$ (12a). Reaction time: 24 h; TLC: R_f = [0.7 ; 0.5] (9:2 CH_2Cl_2/MeOH); HPLC: Rt = 13,6 min. RMN ^{1}H (500 MHz; $CDCl_3$) δ 11.05 (s, 1H), 8.90 (m, 1H), 8.15 (m, 2H), 7.69 (m, 4H), 7.23 (m, 4H), 6.86 (m, 2H), 6.57 (m, 4H), 3.75 (m, 1H), 4.72–4.62 (m, 2H), 3.18 (m, 2H), 3.10 (m,12H), 2.68 (m, 4H), 2.17 (m, 2H), 1.64–1.22 (m, 42H), 0.88 (m, 6H). RMN ^{13}C (125 MHz; $CDCl_3$) δ 196.9, 176.0, 175.9, 170.7, 170.5, 169.0, 167.5, 167.3, 156.0, 155.6, 148.6, 148.0, 136.2, 136.0, 130.8, 128.9, 126.2, 125.3, 118.5, 112.5, 111.8, 97.8, 56.3, 54.3, 51.5, 44.3, 39.8, 37.2, 36.6, 36.4, 32.6, 32.3, 31.8, 31.4, 30.3–29.5, 27.6, 26.1, 23.3, 14.7. HRMS calcd for $C_{69}H_{92}N_5O_7$ = 1102.6997, found 1102.6961; MS calcd for $C_{69}H_{92}N_5O_7$ = 1102.7 found 1102.9.

5.1.3.5. (*S*)-2-(*tert*-Butoxycarbonylamino)-3-(11-(4-(4-methylbenzoyl)phenyl)undecanamido) propanoic acid (9b). Reaction time: 2.5 h; TLC: R_f = 0.3 (9:1 CH_2Cl_2/MeOH); NMR ^{1}H (300 MHz; $CDCl_3$) δ 7.69 (m, 4H), 7.27 (m, 4H), 4.14 (m, 1H), 3.74 (q, 1H, J = 7.0), 2.65 (m, 2H), 2.42 (s, 3H), 2.17 (m, 2H), 1.62–1.21 (m, 25H). MS calcd for $C_{33}H_{47}N_2O_6$ = 567.3, found 567.3.

5.1.3.6. (*S*)-*tert*-Butyl 1-(hexadecylamino)-3-(11-(4-(4-methylbenzoyl)phenyl)undecanamido)-1-oxopropan-2-ylcarbamate (10b). Reaction time: 5 h; TLC: R_f = 0.8 (9:1 CH_2Cl_2/MeOH); NMR ^{1}H (300 MHz; $CDCl_3$) δ 7.73 (dd, 4H, J = 8.1;2.7), 7,29 (d, 4H, J = 7.4), 6.82 (s, 1H), 6.27 (s, 1H), 5.99 (s, 1H), 4.16 (m, 1H), 3.74 (m, 1H), 3.51 (m, 1H), 3.23 (m, 2H), 2.69 (t, 2H, J = 7.6), 2.45 (s, $3H_a$), 2.19 (t, 2H, J = 7.7), 1.67–1.25 (m, 53H), 0.89 (m, 3H); NMR ^{13}C (75 MHz; $CDCl_3$) δ 196.9, 148.5, 143.6, 136.1, 135.9, 130.8, 129.6, 128.9, 81.0, 55.9, 42.7, 40.2, 37.3, 36.7, 32.6, 31.8, 30.4, 30.2, 30.2, 30.1, 30.0, 29.9, 29.0, 27.5, 26.4, 23.4, 22.3, 14.8. MS calcd for $C_{49}H_{80}N_3O_5$ = 790.6, found 790.6.

5.1.3.7. (*S*)-*N*-(2-Amino-3-(hexadecylamino)-3-oxopropyl)-11-(4-(4-methylbenzoyl)phenyl) undecanamide (11b). Reaction time: 2 h; TLC: R_f = 0.4 (9:1 CH_2Cl_2/MeOH); NMR ^{1}H (400 MHz; $CDCl_3$) δ 7.72 (dd, 4H, J = 8.1, 2.7), 7.53 (t, 1H, J = 5.6), 7.29 (d, 4H, J = 7.4), 6.31 (m, 1H), 3.65 (m, 1H), 3.47 (m, 2H), 3.24 (q, 2H, J = 6.7), 2.68 (t, 2H, J = 7.7), 2.45 (s, 3H), 2.18 (t, 2H, J = 7.6), 1.63 (m, 4H), 1.51 (m, 2H), 1.28 (m, 40H), 0.89 (t, $3H_s$, J = 6.8); NMR ^{13}C (100 MHz; $CDCl_3$) δ 196.9, 175.0, 173.9, 148.5, 143.6, 136.1, 135.9, 130.9, 129.6, 128.9, 55.9, 44.3, 39.9, 37.5, 36.7, 32.6, 31.8, 30.4, 30.3, 30.2, 30.1, 30.0, 29.9, 27.7, 26.4, 23.4, 22.3, 14.8. MS calcd for $C_{44}H_{72}N_3O_3$ = 690.5, found 690.5.

5.1.3.8. Rhod-Dpr(CO-C_{10}-BP-C_1)-NH-$C_{16}H_{33}$ (12b). Reaction time: 24 h; TLC: R_f = [0.8; 0.7] (8.5:1.5 CH_2Cl_2/MeOH); HPLC: Rt = 13.6 min. RMN ^{1}H (500 MHz; $CDCl_3$) δ 8.79 (m,1H), 8.65 (m,1H), 8.09 (m,1H), 7.75 (m, 2H), 7.72 (d, 4H, J = 8,1), 7.56 (m, 2H), 7.26 (m, 4H), 6.91 (m, 1H), 6.56 (m, 2H), 4.11 (m, 1H), 3.90 (m, 2H), 3.25–3.13 (m, 14H), 2.65 (m, 2H), 2.44 (s, 3H), 2.19 (m, 2H), 1.61 (m, 4H), 1.28 (m, 40H), 0.87 (m, 3H). RMN ^{13}C (125 MHz; CDCl3) δ 197.0, 176.4, 170.7, 167.8, 156.5, 156.0, 148.6, 143.6, 142.9, 136.0, 135.8, 130.9, 129.6, 128.9, 126.2, 125.3, 118.7, 111.6, 97.6, 64.7, 54.4, 44.2, 42.7, 41.3, 40.5, 37.5, 36.7, 32.6, 31.8, 30.4, 30.3, 30.2, 30.1, 30.0, 29.9, 27.7, 26.4, 23.4, 22.3, 14.8. HRMS calcd for $C_{69}H_{92}N_5O_7$ = 1102.6997 found 1102.6951.

5.1.3.9. (*S*)-2-(*tert*-Butoxycarbonylamino)-3-(2-(4-(4-decylbenzoyl)phenyl)acetamido)propanoic acid (9c). Reaction time: 12 h; Used for the next step as such.

5.1.3.10. (*S*)-*tert*-butyl 3-(2-(4-(4-Decylbenzoyl)phenyl)acetamido)-1-(hexadecylamino)-1-oxo propan-2-ylcarbamate (10c) . Reaction time: 12 h; TLC: R_f = 0.5 (9:1 CH_2Cl_2/MeOH); NMR ^{1}H (400 MHz ; $CDCl_3$) δ 7.77 (dd, 4H, J = 17.5, 8.1), 7.33 (m, 4H), 6.68 (s, 1H), 6.37 (s, 1H), 5.83 (d, 1H, J = 6.5), 4.17 (m, 1H), 3.72 (m, 1H), 3.64 (s, 2H), 3.50 (m, 1H), 3.21 (q, 2H, J = 6.5), 2.69 (t, 2H, J = 7.6), 1.67–1.25 (m, 53H), 0.89 (t, 6H, J = 6.6); NMR ^{13}C (100 MHz; $CDCl_3$) δ 196.5, 172.6, 170.9, 149.0, 139.5, 137.8, 135.6, 131.3, 130.9, 129.8, 129.1, 81.2, 44.2, 40.3, 36.7, 31.9, 30.4,

30.3, 30.2, 30.1, 30.0, 29.0, 27.5, 23.4, 14.8. MS calcd for $C_{44}H_{72}N_3O_3$ = 690.5, found 690.4.

5.1.3.11. (*S*)-2-Amino-3-(2-(4-(4-decylbenzoyl)phenyl)acetamido)-*N*-hexadecylpropanamide (11c). Reaction time: 5 h; TLC: R_f = 0.4 (9:1 CH_2Cl_2/MeOH); NMR ^{1}H (400 MHz; $CDCl_3$) δ 7.75 (dd, 4H, J = 17.5, 8.1), 7.44 (s, 1H), 7.34 (m, 4H), 6.52 (s, 1H), 3.63 (s, 2H), 3.45 (m, 2H), 3.21 (m, 2H), 2.69 (t, 2H, J = 7.6), 1.65 (m, 2H), 1.48 (m, 2H), 1.25 (m, 42H), 0.88 (t, 6H, J = 6.6); NMR ^{13}C (100 MHz; $CDCl_3$) δ 196.5, 172.6, 149.0, 139.5, 137.8, 135.6, 131.3, 130.9, 129.8, 129.1, 55.7, 44.2, 40.3, 36.7, 31.9, 30.4, 30.3, 30.2, 30.1, 30.0, 27.5, 23.4, 14.8. MS calcd for $C_{44}H_{72}N_3O_3$ = 690.5, found 690.5.

5.1.3.12. Rhod-Dpr(CO-C_1-BP-C_{10})-NH-$C_{16}H_{33}$ (12c). Reaction time: 24 h; TLC: R_{fs} = 0.5 and 0.6 (8.5:1.5 CH_2Cl_2/MeOH); HPLC: Rts = 14.1 and 14.3 min. RMN ^{1}H (500 MHz; $CDCl_3$) δ 11.01 (s, 1H), 8.78 (m, 1H), 8.12 (m, 1H), 7.78 (m, 2H), 7.61 (m, 4H), 7.27 (m, 4H), 7.10 (m, 2H), 6.66 (m, 1H), 6.52 (m, 2H), 3.63 (s, 2H), 4.75 (m, 2H), 3.18–3.07 (m, 14H), 2.69 (m, 2H), 1.60–1.18 (m, 44H), 0.87 (t, 6H, J = 6,5 Hz). RMN ^{13}C (125 MHz; $CDCl_3$) δ 196.8, 170.6, 157.1, 156.6, 149.3, 148.8, 142.7, 137.8, 135.6, 131.3, 130.9, 129.8, 129.1, 126.4, 125.4, 118.7, 111.6, 97.2, 54.3, 44.2, 42.6, 40.3, 36.7, 31.9, 30.4, 30.3, 30.2, 30.1, 30.0, 27.5, 23.4, 14.8. HRMS calcd for $C_{69}H_{92}N_5O_7$ = 1102.6997 found 1102.6946; MS calcd for $C_{69}H_{92}N_5O_7$ = 1102.7, found 1102.6.

5.2. Biochemistry

5.2.1. Generals

Dodecylphosphocholine (DPC) and lyophilized bacteriorhodopsin from *Halobacterium salinarum* were purchased from Sigma. Egg yolk phosphatidylcholine (PC) and cholesterol (chol, 99% pure) were purchased from Avanti Polar Lipids (Alabaster, USA). Octadecyl Rhodamine B chloride (R18) was purchased from Fluoprobes.

5.2.2. Liposome preparation

Phospholipid (PC), cholesterol and lipid tool (**12a–c**) (65:30:5 molar ratio) dissolved in chloroform were mixed in a test tube. After solvent evaporation by heat (45 min at 37 °C), the dried lipid film was hydrated by addition of 300 μL of PBS pH 7.4 to obtain a final concentration of 5 mM of lipids. The mixture was vortexed and the resulting suspension was heated (37 °C) and cooled (liquid nitrogen) five times and passed through an extruder equipped with a 100 nm polycarbonate filter (30–50 times).

5.2.3. Fluorescence spectroscopy

Excitation and emission spectra of lipid tools in liposomes were recorded at 32 °C on a TECAN Infinite M1000 spectrofluorimeter. The liposome composition, unless otherwise indicated, was PC/chol/lipid tools (65:30:5 molar ratio). Liposomes were 100 nm unilamellar vesicles and were prepared as described earlier.[43] Blank liposomes were composed of PC/chol (70:30), and their background fluorescence was substracted from the recorded spectra.

5.2.4. BacterioRhodopsin photolabeling

To a solution of lipid tool in chloroform/methanol 2/1 v/v, DPC (molar ratio lipid tool/detergent 1:50) was added. After evaporation under high vacuum (2 h), the dried lipid film was hydrated by addition of 50 μL of water and 50 μL of Tris–HCl pH 8.0 buffer solution. BacterioRhodopsin suspended in H_2O was incubated with lipid tools in DPC micelles at 1:5 molar protein-to-lipid ratios. The mixture was then left 1 h in the dark at 4 °C, and submitted to 45 s irradiation by UV light with a Flood Dymax lamp-house equipped with a 400 W short-arc high pressure mercury lamp (Equipements Scientifiques SA, Garches), at a 10 cm-distance from the UV source in a quartz cell. Laëmmli buffer was then added to the photolabeled mixture, followed by a 15 min-incubation at room temperature and analysis by SDS–PAGE on 15% acrylamide/20% glycerol gels. After migration, the gel was first analyzed for rhodamine fluorescence with a Fluorimager Typhoon 8600 equipped with a rhodamine filter set, and second stained with Coomassie blue to visualize all protein bands.

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