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Synthesis of a photoactivatable phospholipidic probe containing tetrafluorophenylazide

Qing Peng,[a] Yi Xia,[a] Fanqi Qu,[a] Xiaojun Wu,[a] Daniel Campese[b] and Ling Peng[a,b,*]

[a]*College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, PR China*
[b]*Département de chimie, CNRS UMR 6114, 163, avenue de Luminy, 13288 Marseille, France*

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Abstract—In order to study lipid–lipid and lipid–protein interactions using a photolabeling approach, we synthesized and characterized a phospholipidic probe in which the photoactivatable tetrafluorophenylazido group is incorporated into the fatty acid chain. This probe is stable in the dark and becomes highly reactive upon being exposed to photoirradiation. It shows fast, clear-cut photochemical reactions and holds promise for further use to study lipid–lipid and protein–lipid interactions.

Biological membranes containing lipids and proteins serve as a barrier protecting the cells they surround, as well as being involved in some essential biological processes.[1] The lipids present in biomembranes are mainly phospholipids, which play an important role in various cellular processes.[2] Proteins associated with biomembrane lipid bilayers also mediate many biological functions, such as signal transduction, energy conversion, and the transport of ions and molecules across the membrane. In addition, membrane proteins are important targets for drug development, since more than 50% of the drugs on the market are targeted to membrane proteins.[3] Studies on lipid–lipid and lipid–protein interactions throw useful light on both the functional and structural properties of biological membranes as well as identifying novel therapeutic targets and contributing to drug development programs. However, structural information about membrane proteins and biomembranes is scarce because both X-ray crystallography and NMR spectroscopy are of limited applicability in this context.

Photoaffinity labeling[4–6] provides a useful chemical approach for obtaining the structural and functional data on biomembranes. This approach involves the use of photoactivatable lipidic probes. When exposed to light, these probes generate highly reactive species such as nitrene or carbene, and this leads to a process of covalent cross-linkage with the protein or lipid present at the interaction site. This process can be used to identify the proteins and lipids that interact with the lipid probes and to map the lipid-binding sites on proteins and biomembranes. This information can therefore be used to investigate specific lipid–protein interactions and the topology and patterns of distribution of membrane proteins in biomembranes.

The preparation of photoactivatable phospholipids was pioneered by Chakrabarti and Khorana,[7] and a number of additional examples have subsequently been synthesized, usually containing azide, or diazirine,[8] or benzophenone,[9] or a diazo or diazonium group,[10] for example. Among these photophores, aryl azides are those most frequently used as photoaffinity probes because they are small and can easily be synthesized, as well as being chemically stable in the dark and highly reactive upon being exposed to photoirradiation. Among the aryl azides, fluorinated aryl azides are particularly promising candidates because upon being photoactivated, they give much more efficient photolabeling than non-fluorinated aryl azides.[11] In addition, the presence of fluorine atoms in phospholipids can be used to probe specific lipid–lipid and lipid–protein interactions in biomembranes using ^{19}F NMR methods, which are highly suitable for investigating structural and dynamic features because of the wide range of

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* Corresponding author. Tel.: +33 4 91 82 91 54; fax: +33 4 91 82 93 01; e-mail: ling.peng@univmed.fr

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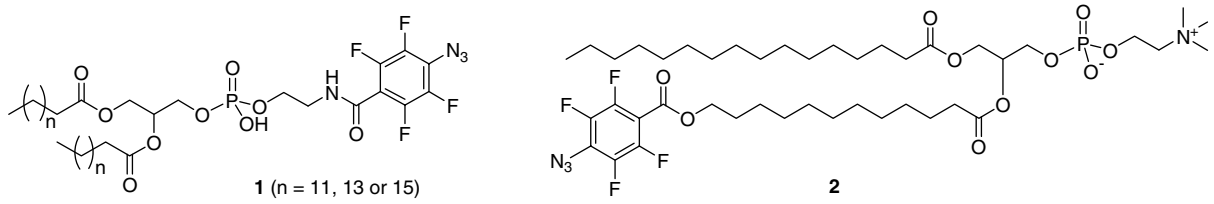
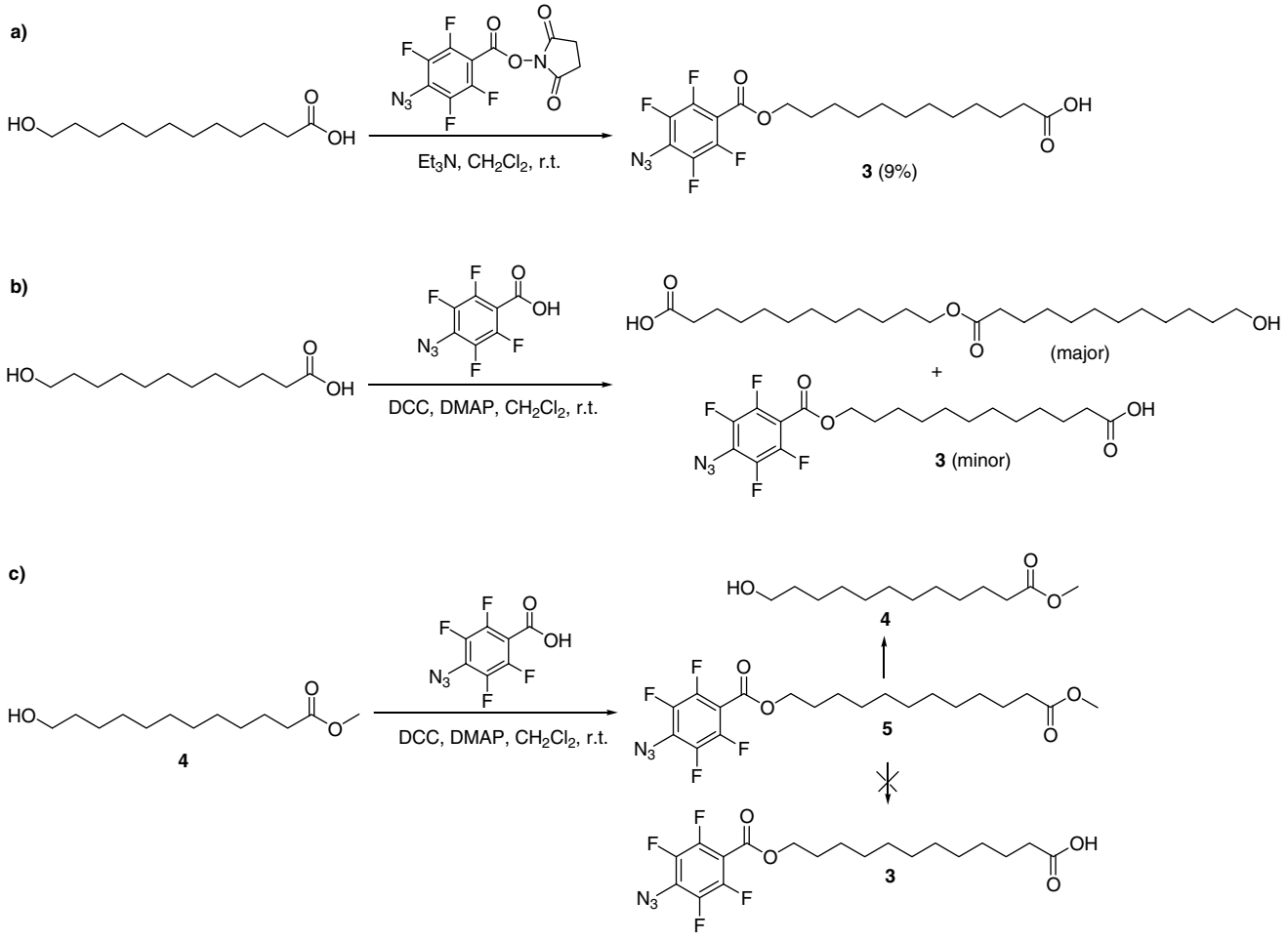


Scheme 1. Photoactivatable phospholipidic probes **1** and **2**.

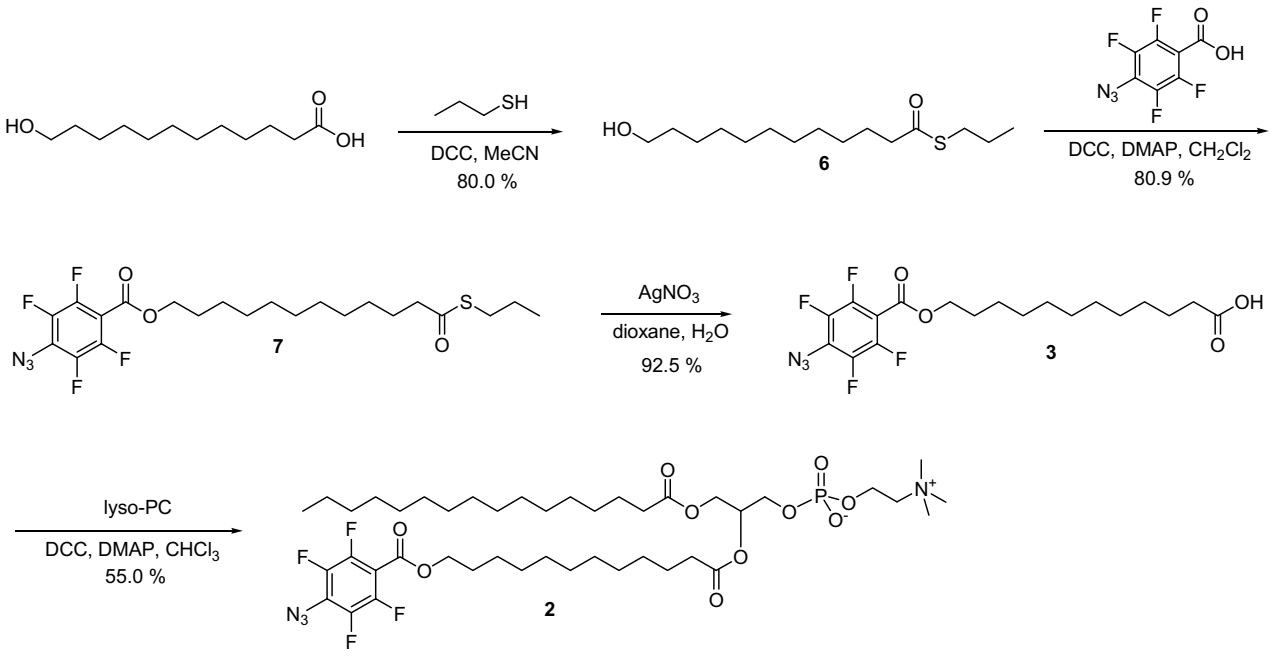
chemical shifts and the extreme sensitivity of these methods to environmental changes.[12] We therefore chose the tetrafluorophenylazido group as the starting point for preparing the photoactivatable phospholipidic probes.

In order to probe various areas of proteins and other lipids with which phospholipids interact, it was proposed to prepare two complementary types of photoactivatable phospholipidic probes **1** and **2** (Scheme 1), containing the tetrafluorophenylazido group at the polar head and in the fatty acid chain, respectively. Probe **1** has the photoactivatable moiety at the polar head and is designed for probing the lipid/water interface of biomembranes, while **2**, where the photoactivatable group is located in one fatty acid chain of the phospholipid, serves to probe the hydrophobic membrane core. We have previously reported on the synthesis and characterization of probe **1**.[13] Here we report on probe **2**.

To synthesize probe **2**, it was necessary to first prepare the tetrafluorophenylazido containing fatty acid surrogate carboxylic acid **3** (Scheme 2). Coupling 12-hydroxydodecanoic acid with *N*-succinimidyl-4-azidotetrafluorobenzoate[14] yielded only tiny amounts of **3** in our hands (Scheme 2a),[15] while condensing 12-hydroxydodecanoic acid with 4-azidotetrafluorobenzoic acid resulted in a mixture mainly containing the self-condensation product of 12-hydroxydodecanoic acid (Scheme 2b), which was difficult to remove. We therefore attempted to protect the acid group of 12-hydroxydodecanoic acid before introducing the tetrafluorophenylazido group.



Scheme 2. Attempted synthesis of **3**.



Scheme 3. Synthesis of **2**.

First we attempted to protect 12-hydroxydodecanoic acid by transforming it into a methyl ester (**4** in Scheme 2c), before coupling it with 4-azidotetrafluorobenzoic acid. However, the ester of 4-azidotetrafluorobenzoic acid is more reactive and more readily hydrolyzed than the methyl ester in **5**. Hydrolysis of **5** led to the hydrolysis of the ester of 4-azidotetrafluorobenzoic acid, giving **4** instead of **3**.

We then protected 12-hydroxydodecanoic acid by transforming it into thiolester **6** (Scheme 3),[16] which was further transformed into **7** by subsequently coupling the latter with 4-azidotetrafluorobenzoic acid.[17] The thiolester in **7** was selectively deprotected by $AgNO_3$[18] and gave **3** with a yield of more than 90%. Finally, coupling **3** with lyso-phosphocholine (lyso-PC) gave **2** with a yield of 55%.[19] It is worth noting that the coupling reactions with lyso-phosphocholine have to be performed under strictly anhydrous conditions in order to obtain satisfactory yields.[20]

The synthesized probe **2** is stable in the dark. It is soluble in MeOH–CH_2Cl_2, but not readily soluble in phosphate buffer. We attempted to solubilize it in phosphate buffer in the presence of the detergent sodium dodecylsulfate (SDS). The fact that the solubility of **2** in the buffer can be significantly increased by adding SDS (Fig. 1) suggests that **2** conserves the amphiphilic characteristics of phospholipids.

We further characterized the photochemical properties of probe **2**. Its maximum UV absorption is around 265 nm, and the molar absorption coefficient is around 15,000 $(mol/L)^{-1}\,cm^{-1}$: this value is characteristic of fluorinated aryl azides.[11] A photochemical study was carried out with **2** in both organic solvent (CH_2Cl_2–MeOH, 1:1) and phosphate buffer. Compound **2** underwent a fast, clear-cut process of photodecomposition in response to irradiation at $\geqslant$300 nm (Fig. 2). Irradiation of the probe **2** quickly led to the disappearance of the absorption band. The isobestic points observed indicated that the photochemical reaction was a unique photodecomposition process. One important point is the fact that **2** can be activated at wavelength $\geqslant$300 nm, which ensures that the biological macromolecules will not be damaged by UV irradiation.

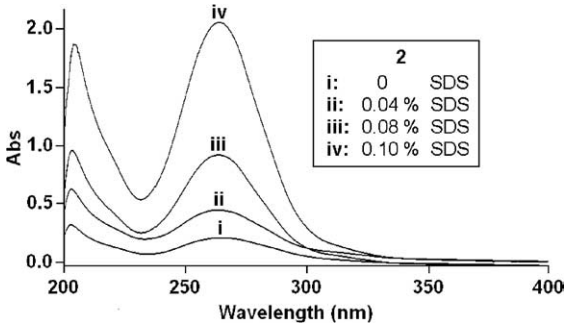


Figure 1. UV spectral recording of the increased solubility of **2** in buffer solution (100 mM phosphate, pH 7.4 at 20 °C) when adding the detergent, sodium dodecylsulfate (SDS).

In conclusion, a photoactivatable phospholipidic probe with fluorinated aryl azide introduced into the fatty acid chain was synthesized and characterized here as a potential tool for studying lipid–lipid and lipid–protein interactions using a photolabeling approach. The photochemical reactions occurring with this probe were investigated in both organic solvent and buffer solution, and it consistently showed a fast, clear-cut photochemical reaction, which suggests that it will provide promising tool for use in photolabeling studies. Furthermore, the

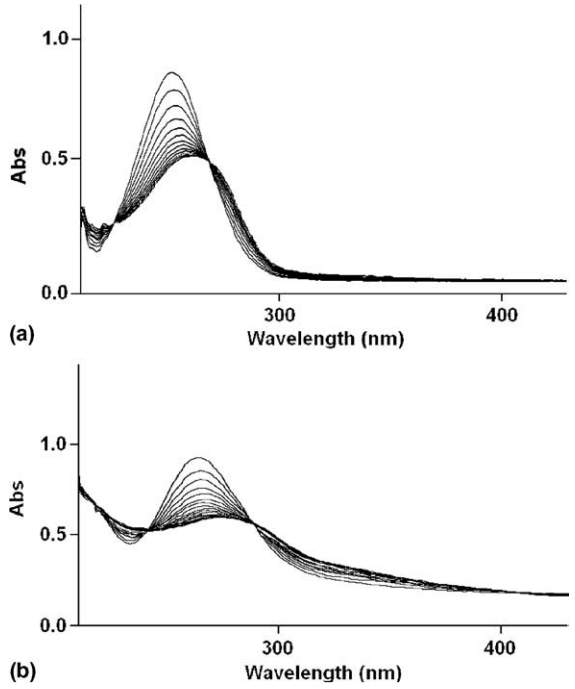


Figure 2. UV spectral recording of the photochemical reaction occurring with **2** in (a) MeOH–CH_2Cl_2 and (b) 100 mM phosphate buffer (pH 7.4) in response to irradiation at 300 nm at 20 °C.

presence of fluorine atoms in the probe makes it a useful means of studying specific lipid–lipid and lipid–protein interactions using ^{19}F NMR methods. Studies on the use of this probe for investigating biomembranes and protein–lipid interactions via a photolabeling approach and ^{19}F NMR are now underway at our laboratories.

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15. Compound **3**: white solid. Mp: 71 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 4.36 (t, 2H, J = 6.6 Hz), 2.35 (t, 2H, J = 7.5 Hz), 1.72–1.76 (m, 2H), 1.61–1.65 (m, 2H), 1.28 (m, 14H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 176.2, 155.5, 140.4–142.1 (ds, 2C, $^1J_{CF}$ = 255.9 Hz), 135.5–137.2 (dd, 2C, $^1J_{CF}$ = 249.6 Hz, $^2J_{CF}$ = 15.9 Hz), 119.1, 104.0–104.1 (d, 1C, $^2J_{CF}$ = 15.3 Hz), 62.8, 30.0, 25.4, 25.3, 25.2, 25.1, 25.0, 24.4, 21.7, 20.6; ^{19}F NMR (564.6 MHz, $CDCl_3$): δ −139.2 (m, 2F), −151.4 (m, 2F); IR (KBr): 2138.1 cm^{-1}; MS (ESI): 431.8 $(M-H)^-$, 864.6 $(2M-H)^-$; UV (CH_2Cl_2): λ_{max} = 265 nm, ε = 20,665 $(mol/L)^{-1}$ cm^{-1}.
16. Compound **6**: white solid. Mp: 35 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 3.64 (m, 2H), 2.84 (t, 2H, J = 6.9 Hz), 2.53 (t, 2H, J = 7.5 Hz), 1.54–1.67 (m, 6H), 1.27 (m, 14H), 0.96 (t, 3H, J = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 200.2, 63.2, 44.4, 33.0, 30.9, 29.8, 29.7, 29.6, 29.6, 29.4, 29.1, 25.9, 25.9, 23.2, 13.5; MS (ESI): 274.5 $(M)^+$.
17. Compound **7**: white solid. Mp: 34 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 4.35 (t, 2H, J = 6.6 Hz), 2.84 (t, 2 H, J = 7.2 Hz), 2.53 (t, 2H, J = 7.5 Hz), 1.57–1.73 (m, 6H), 1.27–1.39 (m, 14H), 0.95 (t, 3H, J = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 200.0, 159.6, 144.6–146.3 (ds, 2C, $^1J_{CF}$ = 256.3 Hz), 139.7–141.5 (dd, 2C, $^1J_{CF}$ = 258.5 Hz, $^2J_{CF}$ = 15.5 Hz), 123.3, 108.3, 67.0, 44.3, 30.9, 29.6, 29.6, 29.4, 29.3, 29.1, 28.6, 26.0, 25.9, 23.2, 13.6; ^{19}F NMR (564.6 MHz, $CDCl_3$): δ −139.3 (dd, 2F, J = 20.6, 11.0 Hz), −151.4 (dd, 2F, J = 21.5, 10.2 Hz); IR (CH_2Cl_2): 2127.5 cm^{-1}; MS (ESI): 515.0 $(M+Na)^+$; UV (CH_2Cl_2): λ_{max} = 265 nm, ε = 20,954 $(mol/L)^{-1}$ cm^{-1}.
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19. Compound **2**: waxy solid. ^1H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 5.21 (m, 1H), 4.34–4.42 (m, 3H), 4.10–4.17 (m, 1H), 3.99 (t, 2H, J = 6.0 Hz), 3.38 (m, 2H), 3.22 (s, 9H), 2.27–2.34 (m, 4H), 1.72–1.77 (m, 2H), 1.59 (m, 6H), 1.25–1.28 (m, 38H), 0.88 (t, 3H, J = 6.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$/CD_3OD): δ 174.0, 173.6, 159.7, 144.5–146.3 (ds, 2C, $^1J_{CF}$ = 261.6 Hz), 139.7–141.5 (dd, 2C, $^1J_{CF}$ = 250.5 Hz, $^2J_{CF}$ = 14.9 Hz), 123.4, 108.1, 70.5, 67.0, 66.6, 63.7, 62.9, 59.3, 54.5, 34.5, 34.3, 32.1, 31.1, 30.0, 29.8,

29.7, 29.6, 29.5, 29.4, 29.3, 28.6, 26.0, 25.0, 22.9, 14.3; ^{19}F NMR (564.6 MHz, $CDCl_3/CD_3OD$): δ −139.4 (m, 2F), −151.5 (m, 2F); ^{31}P NMR (242.9 MHz, $CDCl_3/CD_3OD$): δ 0.26; IR (CH_2Cl_2): 2127.4 cm^{-1}; MS (ESI): 933.9 $(M-H+Na)^+$, 1845.3 $(2M-H+Na)^+$; UV (CH_2Cl_2): λ_{max} = 265 nm, ε = 13,933 $(mol/L)^{-1}$ cm^{-1}.

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