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Synthesis of Photoactivatable Phospholipidic Probes

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Abstract: We synthesized and characterized photoactivatable phospholipidic probes **1-3**. These probes have the perfluorinated aryl azide function at the polar head of phospholipid. They are stable in dark and become highly reactive upon photoirradiation. The preliminary results suggest that they are promising tools to study the topology of membrane proteins and protein-lipid interactions using photolabeling approach.

Keywords: Photoactivatable phospholipidic probes, photolabeling, photolabeling probe, protein-lipid interactions, membrane protein topology.

Membrane proteins associate with biomembrane lipid bilayers[1] and mediate important biological functions, such as signal transduction, energy conversion, transport of ions and molecules across the membrane[2]. They are important targets for drug development, since more than 70% of the drugs on the market are targeted to membrane proteins[3]. Understanding the structure and function of these membrane proteins is therefore necessary for the identification of novel therapeutic targets as well as to pursue drug development programs. The topological arrangement of the membrane proteins involved in lipid bilayers and protein-lipid interactions is crucial for understanding of their structural and functional properties. Although high-resolution structures of membrane proteins are now becoming available (~ 30), they are still far less available than the high-resolution structures of water-soluble proteins (> 3000)[4]. This is mainly associated with the difficulties involved in the crystallization of membrane proteins for the purpose of X-ray crystallographic purposes. The size of the protein-lipid complexes is relatively large for high-resolution NMR structural determination. It is therefore crucial to directly obtain the reliable evidence about the topology of membrane proteins and protein-lipid interactions. This communication reported that photo-affinity labeling with photoactiva- table phospholipidic probes seems to be a useful approach[5].

Photoaffinity labeling[6,7] is an efficient method of studying the interactions between biologically relevant ligands and their target proteins. This method involves the use of photoactivatable probes. These probes are exposed to light and can produce highly reactive species such as nitrene or carbene, leading to a process of covalent cross-linkage

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with the protein at the binding site. This process can be used to identify the proteins that interact with the probes and to map the binding sites on the proteins, and thus to study the ligand-protein interactions. Aryl azides are frequently used as photoaffinity probes. Because they can be easily synthesized, as well as being chemically stable in dark and highly reactive upon irradiation. Among the arylazides, fluorinated aryl azides are exceptionally promising because upon photoactivation, they lead to much more efficient photolabeling than non-fluorinated arylazides[8]. Phospholipidic probes with various photoactiovatable groups have been previously synthesized on the fatty acid chain of phospholipids for studying the membrane proteins using a photolabeling approach[9]. However, no photolabeling probes have been developed so far based on fluorinated aryl azide at the lipid polar head. These probes provide useful tools for studying protein- lipid interactions at water-membrane interfaces. Here we report the synthesis and characterization of photoactivatable phospholipidic probes **1-3,** which have a fluorinated aryl azide group at the lipid polar head**.**

The synthesis of probes **1-3** was performed by condensing the corresponding phosphatidylethanolamine with N-succinimidyl-4-azido-tetrafluororobenzoate (**Scheme 1**). It is worth to note that it was necessary to control the temperature in order to obtain a satisfactory yield. Higher temperatures led to the decomposition of the phosphatidyl-ethanolamine. While lower temperatures, the reaction rate considerably slowed down, resulting in the decomposition of the phospholipids. All three probes **1-3** were purified carefully on silica gel in dark and characterized by ^{1}H-NMR, ^{13}C-NMR, IR, UV and MS[10].

Probes **1-3** are stable in dark. They are soluble in MeOH/CH_2Cl_2, but not readily soluble in MeOH or phosphate buffer. The maximum UV absorption of these probes is around 261 nm, and the molar absorption coefficients are around 15000 $(mol/L)^{-1}cm^{-1}$, which is a value characteristic of fluorinated aryl azides[11].

A photochemical study[12] was carried out with **1-3** in CH_2Cl_2/MeOH and in phosphate buffer. All the probes underwent a fast, clean process of photodecomposi- tion upon irradiation at ≥ 300 nm. **Figures 1a** and **1b** show the photodecomposition of **3** in response to irradiation at 300 nm in MeOH/CH_2Cl_2 and in phosphate buffer, respectively.

Scheme 1 Synthesis of photoactivatable phospholipidicprobes **1-3**

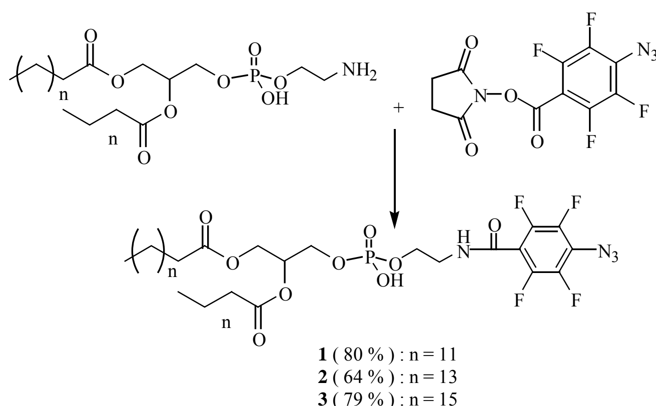
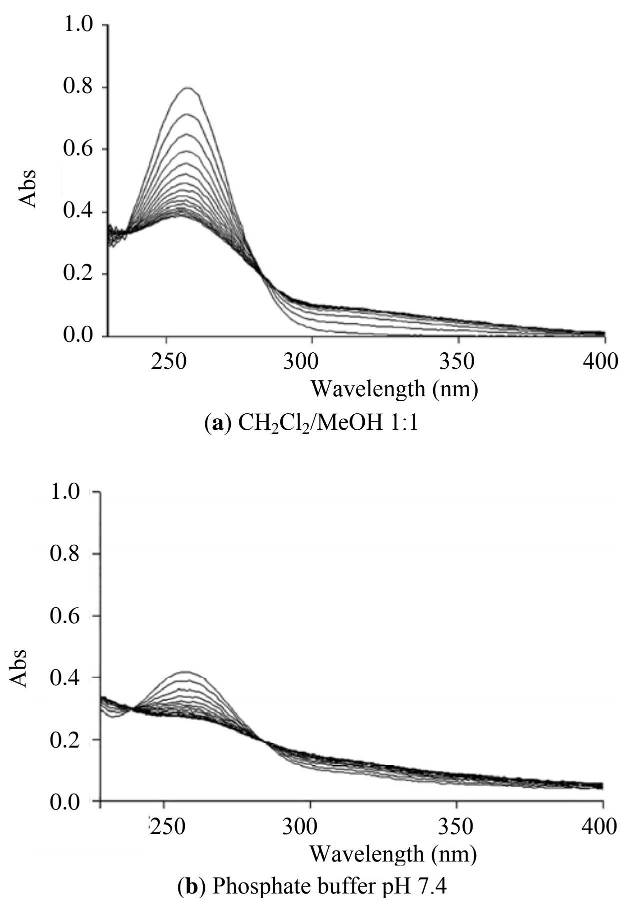


Figure 1 UV spectra taken from the photochemical reaction of **3** with irradiation at 300 nm at 20 °C with an interval of 1 min



Irradiation of **1-3** led to the disappearance of the absorption band quickly. The observed isobestic points indicated that the photochemical reaction was a single photo-decomposition process. It is important that all these probes can be activated at wavelength ≥ 300 nm, to ensure that the biological macromolecules will not be damaged by UV irradiation.

In conclusion, photoactivatable phospholipidic probes **1**-**3** with fluorinate aryl azide at the lipid polar head were synthesized and characterized with the aim to study the membrane proteins using a photolabeling approach. The photochemistry of **1**-**3** was studied in both organic solvent and buffer solution and consistently showed a fast, clear-cut photochemical reaction, which suggested that they are promising tools in photolabeling studies. Studies on the use of these probes for investigating the topology of membrane proteins and protein-lipid interactions *via* a photolabeling approach are now under way.

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10. Analytical data for compounds **1**-**3** :
    1: ^{1}H-NMR (300 MHz, $CDCl_3/CF_3COOH$, δ ppm): 5.31 (m, 1H), 4.42-4.39 (m, 2H), 4.25-4.15 (m, 4H), 3.80 (m, 2H), 2.40-2.36 (m, 4H), 1.59 (m, 4H), 1.26 (m, 40H), 0.91-0.86 (m, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 174.0, 158.2, 144.8, 143.1, 141.2, 139.5, 121.5, 111.9, 70.2, 64.0, 63.6 62.9, 40.8, 34.1, 33.8, 32.0, 29.8, 29.4, 29.3, 24.8, 22.7, 14.1. IR (KBr) 2123.9 cm^{-1}. MS (ESI) *m/z* 851.2 ($M-H^+$). UV (CH_2Cl_2) λ_{max} =261 nm, ε =13278 $M^{-1}cm^{-1}$.
    2: ^{1}H-NMR (300 MHz, $CDCl_3/CF_3COOH$, δ ppm): 5.27 (m, 1H), 4.37-4.34 (m, 2H), 4.18-4.10 (m, 4H), 3.73 (m, 2H), 2.34-2.31 (m, 4H), 1.53 (m, 4H), 1.19 (m, 48H), 0.81-0.79 (m, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 173.9, 158.7, 145.0, 143.39, 141.4, 139.7, 121.9, 111.8, 70.0, 65.4, 64.7, 62.6, 40.9, 34.3, 34.2, 32.2, 30.0, 29.7, 29.4, 25.0, 22.9, 14.3. IR (KBr) 2124.6 cm^{-1}. MS (ESI) *m/z* 907.2 ($M-H^+$). UV (CH_2Cl_2) λ_{max} =261 nm, ε = 14403 $M^{-1}cm^{-1}$
    3: ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$, δ ppm): 5.11 (m, 1H), 4.08-4.01 (m, 2H), 3.88-3.85 (m, 4H), 3.53 (m, 2H), 2.23-2.18 (m, 4H), 1.50 (m, 4H), 1.23 (m, 56H), 0.81-0.77 (m, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 173.7, 158.4, 145.5, 141.9, 141.7, 138.6, 121.6, 119.0, 70.9, 64.9, 64.2, 63.5, 41.6, 35.0, 34.8, 32.8, 30.7, 30.3, 30.1, 25.7, 23.6, 15.1. IR (KBr) 2124.2 cm^{-1}. MS (ESI) *m/z* 963.2 ($M-H^+$). UV (CH_2Cl_2) λ_{max} =261 nm, ε =17017 $M^{-1}cm^{-1}$.
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12. General procedure: The probe was dissolved in the corresponding solvent at concentrations of around $1.5x10^{-4}$ mol/L. The solutions were photolyzed with stirring using a 150 W USHIO Xenon Short Arc Lamp for 0- 30 minutes at 20 °C. The absorption spectra of the irradiated samples were recorded using a CARY UV spectrophotometer.

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