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A facile method to enable a model phospholipid cell-permeable and photoactivatable

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ABSTRACT

Directly utilizing phospholipids in cellular studies is limited by their dynamic metabolism and lack of cell permeability. We have developed a facile method to enable a fluorescent model phospholipid cell-permeable and photoactivatable. This method features the reaction of a diazo-containing molecule with either phosphonic or phosphoric acids to form the corresponding photocaged esters under mild conditions, and should be generally applicable to endogenous phospholipids.

1. Introduction

Phospholipids play essential roles in all living organisms.[1,2] They are the major membrane constituents in mammalian, plant, and bacterial cells. In addition, many phospholipids function as signaling molecules through interactions with various proteins.[3] Consequently, phospholipids are involved in various cellular processes including cell proliferation and differentiation. Abnormal levels of phospholipids and aberrant regulation of their metabolizing enzymes have been implicated in onset and progression of various diseases.[4,5] For example, enhanced production of sphingosine 1-phosphate (S1P) is observed in patients with leukemia or multiple sclerosis.[6] Another phospholipid, phosphatidylinositol 3,4,5-trisphosphate (PIP_3), regulates multiple signaling events and the enzymes that are responsible for its biosynthesis and degradation are frequently mutated in human cancer.[7]

Directly manipulating the level of an individual phospholipid in cells is essential to understand its roles in various cellular processes. However, phospholipids are negatively charged making it challenging to deliver them into cells. Existing methods for delivering charged molecules such as DNAs include electroporation, microinjection, and transfection.[8,9] Analogous methods have been applied to phospholipids but often result in inconsistent efficiency and perturbed cellular signaling. Modifying phospholipids as the corresponding esters that can be hydrolyzed by esterases or are photoactivatable is one promising approach to increase cell permeability of phosphate-containing molecules. However, previously reported methods require multistep synthesis to obtain the desired products that are cell permeant.[10–16]

In this work, we report a general, one-step method to enable phospholipids cell permeable and photoactivatable. Trimethylsilyldiazomethane ($TMSCHN_2$) **1** (Fig. 1A) has been shown to react with PIP_3 in cell lysates to form the corresponding methyl ester under mild conditions.[17] However, the methyl esters are not easily hydrolyzable in cells. By replacing one hydrogen atom in CH_2N_2 with a nitrobenzyl group, we envision that reagent **2** (Fig. 1A) should have similar reactivity toward phosphonic or phosphoric acids **3** to generate the according photolabile esters **4** (Fig. 1B). Indeed, compound **2** has previously been used to photocage phosphate groups in nucleotides and inositol phosphates.[18] Because there are no net negative charges in phosphate esters **4**, they should be cell-permeable. Once delivered into cells, the esters **4** could be uncaged through light illumination to release the free phosphonic or phosphoric acids **3**.

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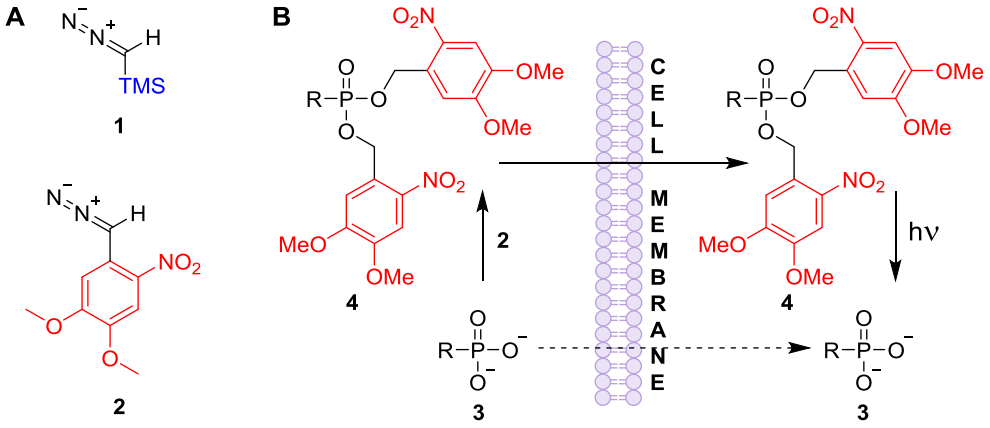

Fig. 1. A facile method to enable phospholipids cell-permeable and photoactivatable. **A.** Structures of two diazo-containing reagents. **B.** Schematic illustration of a general method to deliver phospholipids into cells.

2. Result and discussion

2.1. Reactions of phosphonic and phosphoric acids with a diazo-containing reagent

The diazo-containing compound **2** was synthesized through first coupling of the 4,5-dimethoxy-2-nitrobenzaldehyde **5** with hydrazine (NH_2NH_2) and then oxidation of the resulting product with manganese oxide (MnO_2) (Fig. 2).[19,20] The reaction was clean so the product was used directly without further purification after the solids were filtered off and the solvent was removed. When phenylphosphonic acid **3a** in CH_3CN was mixed with reagent **2** at room temperature for only 5 min, the starting material was cleanly converted to the corresponding photocaged product as judged by ^{31}P NMR of the reaction mixture. Likewise, the reaction with an alkyl phosphonic acid **3b** proceeded cleanly to generate the caged product in 75% isolated yield. Similar reactivity was observed for phosphoric acid-containing molecules. Phosphorylated vitamin E, **3c**, and phosphatidic acid derivative, **3d**, were also efficiently converted into the corresponding triphosphate esters, respectively. Taken together, these results suggested that the diazo-containing reagent **2** is effective to transform phosphonic and phosphoric acids into the corresponding esters under mild conditions.

Derivatives of phosphonic or phosphoric acids are often available in the form of sodium or ammonium salts. When these salts were used in the reaction, it is important to acidify them with hydrogen chloride (HCl) before reagent **2** was added.

2.2. Design and synthesis of a fluorescent model phospholipid

To demonstrate that the phospholipids are cell-permeable, we designed and synthesized a fluorescent, model phospholipid **10** so that cell permeability can be assessed through confocal microscopy. Compound **10** (Scheme 1) is designed to contain a fluorophore 4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), a phosphate group, and an alkyl chain (C_6H_{13}). These are functional groups that are needed to mimic a fluorescent phospholipids while the synthesis is still relatively straightforward.

As shown in Scheme 1, the synthesis of compound **10** started with the amine **6**, which was generated via reduction of the corresponding azide over Raney nickel in the presence of hydrogen gas.[21] Coupling of **6** with N-Boc-protected caproic acid in the presence of dicyclohexycarbodiimide (DCC) and catalytic amount of 4-dimethylaminopyridine (DMAP) followed by hydrogenolysis over Pd/C to remove the carboxybenzyl (Cbz) protective group provided compound **7**. Another amide coupling between **7** and heptanoic

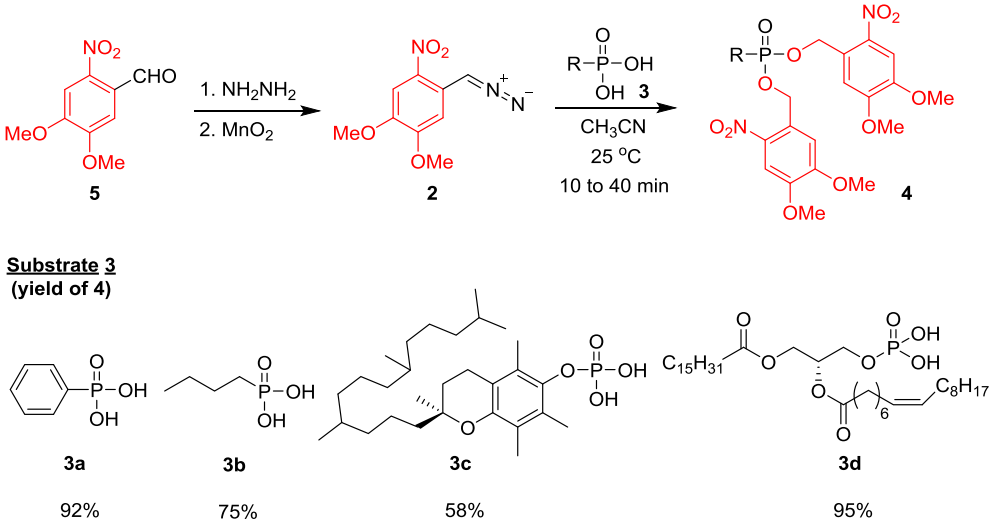

Fig. 2. Synthesis of reagent **2** and its reactions with phosphonic and phosphoric acids.