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An optimized method for the synthesis of amino-functionalized phosphatidylcholine

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ABSTRACT

Phosphatidylcholine analogues were synthesised as affinity ligands for the capture of membrane proteins. Several protecting group strategies were investigated to synthesize the amino-functionalized phosphatidylcholine: 11-aminoundecyl 2-(trimethylammonio)ethyl phosphate (**4**). The acid-mediated deprotection of the Boc group generated a mixture of the target products which could only be purified by HPLC. However, an alternative strategy, using the hydrazine-labile phthalimide group route, followed by a gel filtration step proved straightforward to afford the desired amino-functionalized phosphatidylcholine product in high yield and purity.

Cytochrome P450s (CYPs) are a superfamily of mixed-function oxidases, members of which are present in virtually all living organisms. CYPs are characterized into families and sub-families by their sequence similarities. There are 103 isoforms identified in mouse and 57 in humans, predominantly expressed in the liver, but also specific forms occurring in other tissues, including tumours. Each member can also be characterized by their substrate (endogenous and foreign metabolites) specificity. CYPs are thought to have evolved, in part, as a protective adaptive response against the toxic effects of environmental chemicals.[1] They are the most important drug metabolizing enzymes in mammals, and, in humans, are responsible for the phase I metabolism of 70–80% of all clinically used drugs.[2] Essentially, by monitoring the changes in the protein expression profile of CYPs, the effect of drugs on target tissues can be determined, and hypotheses proposed as to the likely mechanisms of drug action. In addition to their detoxification role, CYPs are also responsible for the conversion of chemical toxins and procarcinogens into their toxic or carcinogenic forms.[3] The ability of CYPs to activate chemical toxins has been exploited in cancer chemotherapy where several anticancer drugs, including cyclophosphamide, tamoxifen and banoxantrone, are known to require metabolic CYP activation in order to exert their cytotoxic effects.[4] Because of their ability to metabolize drugs, CYPs have huge significance in pharmaceutical research.[5] The action of CYPs is one of the major causes of adverse drug reactions to many marketed drugs and drug-combination therapies, and many failures of novel drugs during their development have been attributed to their interactions with this class of enzyme. For example, drugs may be metabolized too rapidly by CYPs before they have had time to be effective, or they may be metabolized into smaller molecules which are toxic beyond their site of proposed action. Certain drugs may also inhibit the activity of a CYP enzyme which is involved in the metabolism of another drug that, given at the same time, can become elevated in the patient to levels which can cause side effects or become dangerous. There is now the potential to use the CYP profile of a subject to develop personalized medicines. A subject's genotype may impact on the pharmacodynamics (drug concentration vs time vs pharmacological effect), pharmacokinetics (absorption, distribution, metabolism and excretion) and/or the incidence of adverse events. The use of pharmacogenetic tests to determine this genetic variation can facilitate correct drug selection for treatment efficacy and minimize adverse side effects.

In the course of our research into the affinity purification of membrane proteins such as the CYP450s, we required a route to synthesize the amino-functionalized phosphatidylcholine: 11-aminoundecyl 2-(trimethylammonio)ethyl phosphate (**4**), which would be immobilized onto epoxy-activated softgel agarose to facilitate affinity enrichment-based proteomics experiments. There have been many papers and patents published outlining the synthesis of phosphatidylcholine derivatives,[6] but each of these methods has drawbacks. Some processes are lengthy and overly complex,[7] others use expensive and toxic reagents,[8] some require heavy metals during purification, or result in poor yields.[9] To address these issues, we report two new and simple methods for the synthesis and purification of phosphatidylcholine derivatives in excellent yields, utilizing relatively cheap, commercially available starting materials.

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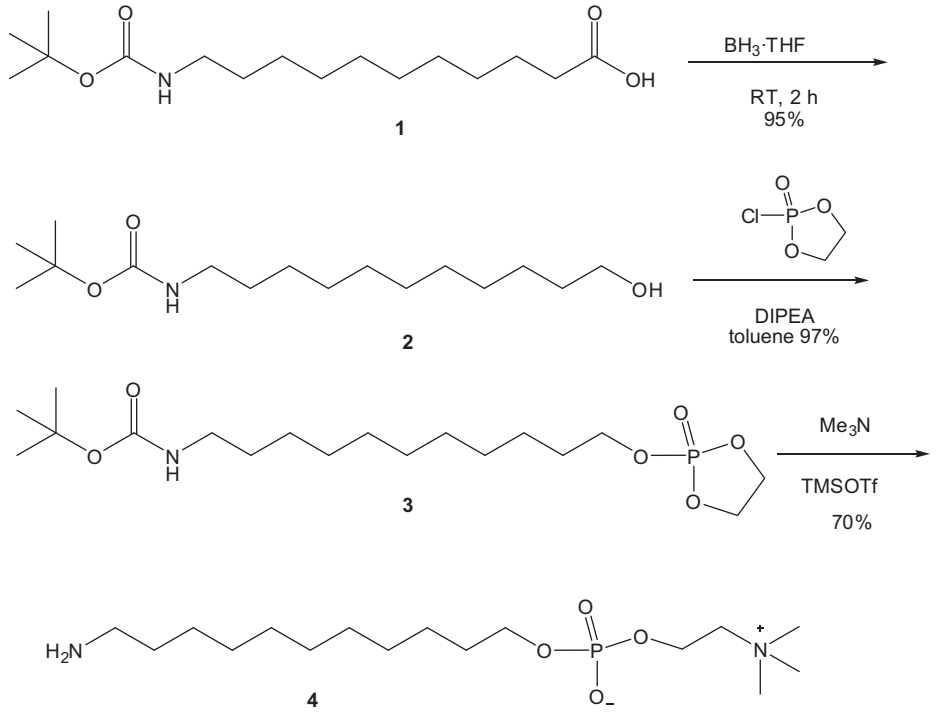
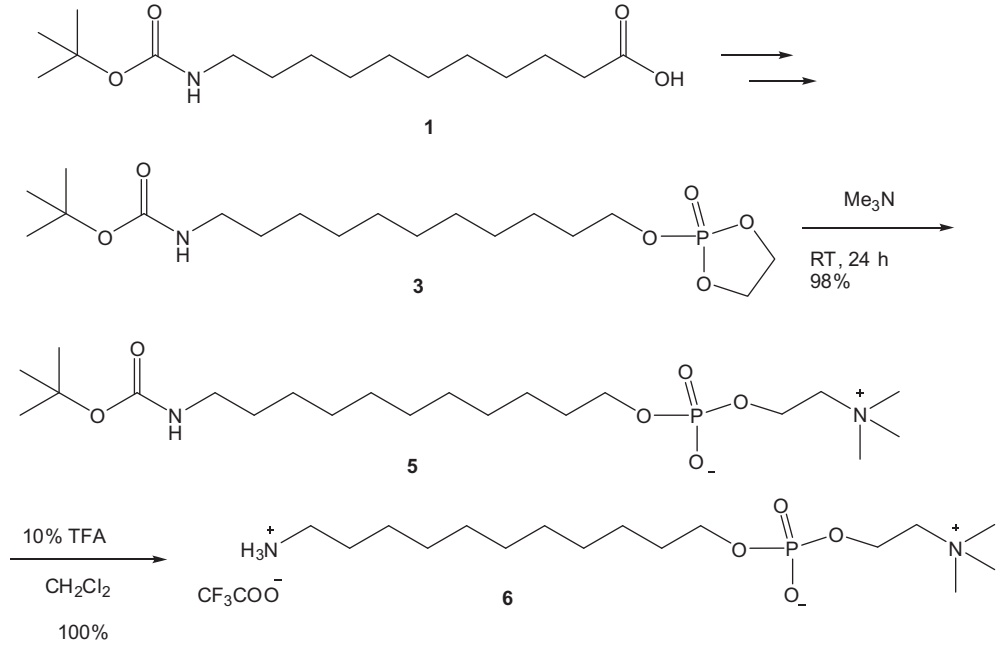
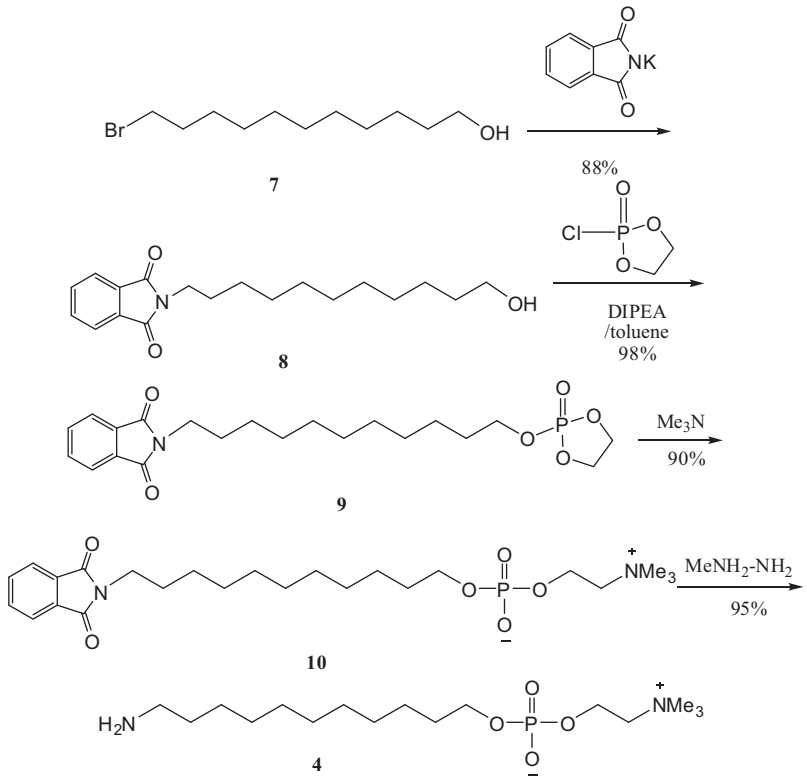
In our initial efforts (Scheme 1), we prepared the Boc protected long chain alcohol **2** through the selective reduction of commercially available acid **1** resulting in a 95% isolated yield of alcohol **2** as a white powder after flash silica gel chromatography[10] (methanol:dichloromethane = 5:95, R_f = 0.4). Alcohol **2** reacted with 2-chloro-2-oxo-1,3,2-dioxaphospholane to give phosphate **3** with ^{31}P NMR spectroscopy indicating the reaction yield was 97%. After removing the solvent in vacuo, the residue was used without further purification. In a glass Schlenk tube, phosphate **3** was dissolved in CH_3CN, cooled to −78 °C, and 1 mL of $(CH_3)_3N$ was added to the reaction mixture. The mixture was stirred at 60–65 °C for 5 h in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) under a condenser charged with dry ice/acetone. ^{31}P NMR spectroscopy[11] confirmed that 30%, 50% and 70% yields of the target compound **4** had been formed in the presence of 0.5, 1.0 and 2.0 equiv of TMSOTf, respectively. Further attempts to optimize the yield of this reaction proved unsuccessful. Hence, it was difficult to obtain pure phosphate **4** even after employing repeated gel filtration with a Sephadex LH-20 column.



Scheme 1. Synthesis of terminal amine phosphatidylcholine (PC) analogue **4** via the acid-mediated deprotection method.



Scheme 2. Synthesis of terminal amine phosphatidylcholine (PC) analogue **6** via the two step acid-mediated deprotection method.



Scheme 3. Synthesis of terminal amine phosphatidylcholine (PC) analogue **4** via the base-mediated deprotection method.

An alternative two-step preparation of phosphatidylcholine **6** from phosphate **3** (Scheme 2) was also performed. Phosphatidylcholine **6** is the trifluoroacetate salt form of the desired compound **4** and could easily be transformed into the amine **4** during the succeeding agarose bead coupling reaction. In a glass pressure tube, phosphate **3** was reacted with neat $(CH_3)_3N$ which was added to the reaction mixture from a cylinder with stirring at room temperature for 24 h. ^{31}P NMR spectroscopic data indicated that **5** had been formed in a 98% yield. After fully removing the excess $(CH_3)_3N$ by evaporation, crude phosphatidylcholine **5** was dissolved in 10% trifluoroacetic acid in dichloromethane and stirred at room temperature for 5 h, affording the phosphatidylcholine **6**, quantitatively. Following in vacuo removal of the solvent, phosphatidylcholine **6** was obtained in high purity (NMR acceptable product) after three successive gel filtration steps using a Sephadex LH-20 column. Unfortunately, this two-step procedure proved to be troublesome and non-reproducible: often four or five gel filtration steps were still insufficient to yield pure compound **6**. A further drawback of this method was that **6** still needed to be de-salted into **4** before coupling to agarose beads.

While the two-step Boc deprotection sequence worked occasionally, a more robust and reproducible synthesis was required. We next examined the methyl hydrazine promoted deprotection of a phthalimide group (Scheme 3). We prepared the phthalimide protected long chain amino alcohol **8** from commercially available bromo alcohol **7** in an 88% isolated yield after recrystallization from methanol following the method of Terashima et al.[12] Phthalimide protected alcohol **8** was reacted with 2-chloro-2-oxo-1,3,2-dioxaphospholane to give phosphate **9** in a 98% yield (as calculated by ^{31}P NMR spectroscopy). After removing the solvent in vacuo, the residue was used in the amination without further purification. In a glass pressure tube, phosphate **9** was dissolved in CH_3CN, cooled to −78 °C, and $(CH_3)_3N$ was added to the mixture. The mixture was stirred at room temperature for 48 h. ^{31}P NMR spectroscopy showed that 90% of product **10** had been formed. The solvents were removed under reduced pressure, the mixture passed first through a Sephadex LH-20 size exclusion column ($CHCl_3$/MeOH; 50:50), followed by silica column chromatography (CH_2Cl_2:MeOH:H_2O; 65:25:4) to give the product phosphatidylcholine **10** as a white powder. Compound **10** underwent base-promoted deprotection to give the final product **4**. A simple gel filtration through Sephadex LH-20 yielded compound **4** in high yield and purity.

In conclusion, we have developed simple, robust and reproducible routes to 11-aminoundecyl 2-(trimethylammonio)ethyl phosphate (**4**) via methyl hydrazine promoted deprotection as the key step to obtain the phosphatidylcholine in high purity.[13]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.11.100.

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13. The optimized procedure for the synthesis of 11-aminoundecyl 2-(trimethylammonio)ethyl phosphate (**4**): Under an atmosphere of Ar, phthalimide protected alcohol **8** (500 mg, 1.742 mmol) was dissolved in toluene (25 mL). DIPEA (3.484 mmol) was added and the solution turned pale yellow. 2-Chloro-2-oxo-1,3,2-dioxaphospholane (2.09 mmol) was subsequently added and the reaction solution turned brown. The reaction was stirred for 24 h, then another aliquot of phosphorylation reagent was added and the mixture stirred for another 24 h. The solvent was removed under reduced pressure giving a yield (as determined by ^{31}P NMR spectroscopy) of 98%. The crude phosphate **9** was dissolved in CH_3CN (2 mL) and cooled to −78 °C. Pre-cooled (on ice) $(CH_3)_3N$ (1 mL) was then added to the reaction mixture. The mixture was stirred at room temperature for 48 h, then the solvents were removed under reduced pressure. The mixture was passed through a Sephadex LH-20 size exclusion chromatography column ($CHCl_3$/MeOH; 50:50), followed by silica column chromatography (CH_2Cl_2:MeOH:H_2O; 65:25:4) to give the final product **10** (210 mg) as a white powder. A small analytical sample was obtained via preparative inverse phase C18 HPLC purification or by preparative TLC (65:25:4; CH_2Cl_2:MeOH:H_2O). Phosphatidylcholine **10** (1.85 mmol) was dissolved in absolute EtOH (20 mL) and cooled to 0 °C. $MeNH_2NH_2$ (27.75 mmol) was added and the mixture stirred at 50 °C for 3 d. After removal of the solvent, the mixture was passed through a Sephadex LH-20 size exclusion chromatography column ($CHCl_3$/MeOH; 50:50), followed by silica column chromatography (65:25:4; CH_2Cl_2:MeOH:H_2O) to give the final product **4** (200 mg) as a white powder. ^{31}P NMR spectroscopy indicated the reaction yield to be approx. 95%. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.27–1.38 (m, 10H), 1.50–1.68 (m, 4H), 2.86–2.88 (m, 2H), 3.06–3.08 (m, 2H), 3.20 (s, 9H), 3.28–3.31 (m, 2H), 3.60–3.64 (m, 2H), 3.88–3.90 (m, 2H), 4.26–4.34 (m, 2H); ^{13}C NMR (62.9 MHz, CD_3OD): δ 21.8, 24.2, 25.2, 26.1, 28.8, 28.9, 29.1, 30.5, 39.5, 52.1, 53.2 (2C), 59.0, 59.2, 66.0, 66.2. MS (ES^+) $C_{16}H_{37}N_2O_4P$ (352) *m*/*z* (%) 353 $[M+H]^+$.