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Synthesis of lipid-based unsymmetrical *O,O*-dialkylphosphites

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ABSTRACT

An efficient method for the synthesis of unsymmetrical lipid-based *O,O*-dialkylphosphites is reported. The reaction implies the use of *H*-phosphonates monoester ammonium salt which is coupled with a fatty alcohol in the presence of an optimized quantity of pivaloyl chloride (Piv-Cl) as coupling reagent. The reaction conditions offer access to a wide panel of unsymmetrical *O,O*-dialkylphosphites including either non-functionalized lipid derivatives (lauryl, dodecyl tetradecyl, hexadecyl, octadecyl), ramified lipid (phytanyl), unsaturated lipids (oleyl, linoleyl) or ω-functionalized alkyl alcohols (azide, propargyl, alkenyl).

Lipophosphoramidates constitute a class of synthetic phospholipids that are used as non-viral gene vectors for both in vitro and in vivo transfection assay.[1–4] While these amphiphiles were originally intended for nucleic acids transfer, some of them exhibited outstanding antibacterial properties mainly against Gram positive bacteria (*Staphylococcus aureus*) and to a lesser extent against Gram negative strains (*Pseudomonas aeruginosa*),[5] thus increasing further the interest for these compounds. From a synthetic point of view, these bio-inspired vectors are prepared by the Atherton–Todd reaction, that is, by mixing an *O,O*-dialkylphosphite, an amine and $CBrCl_3$ in the presence of a base (DIPEA).[6] Following this strategy, a wide variety of lipophosphoramidates with symmetrical hydrophobic domains (two identical alkyl chains) were prepared.[7–9]

In a continuous effort to develop more efficient gene delivery vehicles, we were interested in the synthesis of unsymmetrical lipophosphoramidates that were characterized by the presence of two different lipid chains. Indeed, we and others have reported that some combinations of lipid chains included in the structure of cationic amphiphiles were beneficial for improving gene transfection efficacies.[10,11] On our side, we have recently published the synthesis of unsymmetrical lipophosphoramidates by an efficient 'one-pot' procedure starting from phosphorus oxychloride, a fatty alcohol, a primary amine and triethylamine as base.[12] Although this strategy was very efficient, we do not have isolated the unsymmetric lipophosphites which would be, by themselves, a very interesting lipid building block for the synthesis of other classes of amphiphilic compounds including lipophosphonates[13] or lipophosphates.[14] With respect to the synthesis of unsymmetrical *O,O*-dialkylphosphites, to the best of our knowledge, there is only one paper by Dal-Maso et al. which reports the synthesis of unsymmetrical *O,O*-dialkylphosphites that include two lipid chains.[15] The reaction was performed in two steps by reacting diphenylphosphite with the addition of two different fatty alcohols in pyridine. Unfortunately, in all our attempts, we found that the produced lipid-based dialkylphosphite contained some amount (around 10%) of symmetrical derivatives (two identical alkyl chains).

In the present Letter, we report a methodology to unsymmetrical lipid-based *O,O*-dialkylphosphites by a coupling reaction between *H*-phosphonate monoester ammonium salts and fatty alcohols by means of a coupling reagent (Scheme 1). Four coupling agents were evaluated; the best of all was retained to synthesize a panel of compounds that includes various saturated, unsaturated and ramified fatty alcohols of different lengths. Synthesis of a cholesteryl derivative with an aminoalkyl spacer and some ω-functionalized alcohols is also described.

The synthetic route for the synthesis of unsymmetrical *O,O*-dialkylphosphites is depicted in Scheme 1. The method used is inspired from the work by Lindh and Stawinski[16] who have reported the synthesis of some analogues of glycerophospholipids by the coupling of *H*-phosphonates and amino alcohols (serine or ethanolamine) in the presence of a coupling reagent. This methodology is herein adapted to the synthesis of *O,O*-dialkylphosphites possessing two different lipid chains. In the first step, the *H*-phosphonate monoester salts **2a–e** were synthesized by adapting the method of Kers et al.[17] These salts were prepared by ammonolysis of the symmetrical dialkylphosphites **1a–e** in THF with only a slight excess of aqueous NH_4OH 25% (5 equiv). The slight excess

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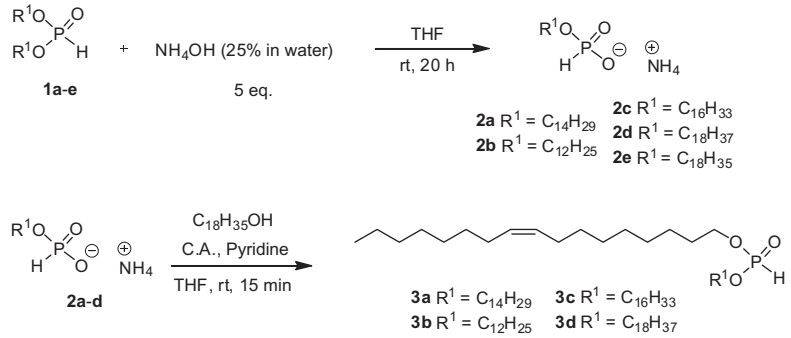


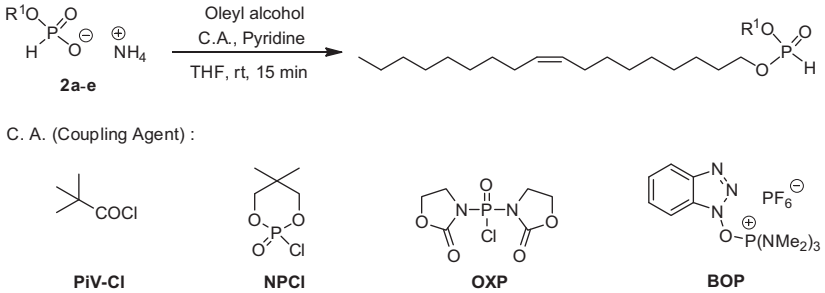
Scheme 1. General method for the synthesis of lipid-based unsymmetrical *O,O*-dialkylphosphites. (C.A.: Coupling Agent.)

used herein, in place of a large excess according to the method of Kers et al.[17] is essential to facilitate the purification step. After azeotropic distillation with toluene, the salts **2a**–**d** were precipitated by trituration in diethylether. The monoester ammonium salts with alkyl chains C12, C14, C16 and C18 were isolated as white solids in nearly quantitative yields (85–100%). The monoester salt with oleyl chain (**2e**) which does not crystallize was synthesized by the same procedure but its purification was achieved by flash column chromatography (eluent CH_2Cl_2/MeOH/NH_4OH: 25/5/1) to reach sufficient purity (90% yield).

In the second step, the salts **2a**–**e** were reacted with a lipid alcohol and a coupling agent. In order to find the best conditions, different coupling reagents were evaluated for the coupling of the monotetradecyl phosphonate ester ammonium salt with oleyl alcohol (Table 1). The choice of the coupling reagents was largely inspired by the work of Kraszewski dedicated to the chemistry of *H*-phosphonate.[18] Indeed, various reagents such as pivaloyl chloride (Piv-Cl), 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphirane (NPCl) and the bis[2-oxo-3-oxazolidinyl)phosphinic chloride (OXP) have been used as coupling reagents in *H*-phosphonate chemistry for the condensation of oligonucleotides. Furthermore, it was reported that many reagents initially designed for the synthesis of peptide have been successfully used for the formation of *H*-phosphonate internucleotidic linkages.[19,20] So, as a representative of these reagents we have selected (benzotriazol-1-yloxy)tris (dimethylamino)phosphonium hexafluorophosphate (BOP). With the aim to develop a rapid and facile route to the synthesis of unsymmetrical *O,O*-dialkylphosphites, we decided to explore the efficiency of these reagents.

Initially, we tried the conditions described by Lindh and Stawinski, (i.e., 2 equiv of alcohol and 3–5 equiv of the condensing agent in pyridine).[16] Under these conditions the product was isolated in satisfactory yields but further purifications were often required to eliminate pivalic acid (a by-product of this reaction) which is actually quite difficult to separate likely due to the amphiphilic character of the produced phosphites (Table 1, entry 1). Then, we reduced the quantities of reagents and found that similar yields could be reached by using only 1.1 equiv of alcohol and 1.25 equiv of the coupling reagent (Table 1, entry 2). Also, under our conditions, THF was used as a solvent and only a slight excess of pyridine (1.25 equiv), without decreasing the yields. We also observed that the use of reduced quantities of pyridine and Piv-Cl deeply simplified the purification step. The absence of free fatty alcohol and pivalic acid was assessed by ^1H and ^{13}C NMR analyses.

Table 1
Coupling reaction between oleyl alcohol (1.1 equiv) and various alkyl *H*-phosphonate monoester ammonium salts in the presence of Coupling Agent (C.A.; 1.25 equiv)



Entry	Ammonium salt	C.A.	Yield (%)
1	R^1 = C14:0	Piv-Cl	87[a]
2	R^1 = C14:0	Piv-Cl	85[b]
3	R^1 = C14:0	NPCl	50[b]
4	R^1 = C14:0	OXP	38[b]
5	R^1 = C14:0	BOP	45[b]
6	R^1 = C14:0	NPCl	45[b,c]
7	R^1 = C12:0	Piv-Cl	82[b]
8	R^1 = C16:0	Piv-Cl	85[b]
9	R^1 = C18:0	Piv-Cl	85[b]

[a] The coupling reaction was performed according to Lindh and Stawinski conditions.
[b] Conditions described in this Letter.
[c] The reaction was performed with butanol instead of oleyl alcohol.