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The synthesis of 1,3-diamidophospholipids

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ABSTRACT

A straightforward synthesis of a small library of 1,3-diamidophospholipids is presented using readily available, cheap reagents and introducing a simple phosphoramidate protecting group strategy.

Lipids are at the very heart and beginning of life on Earth and owing to the tremendous efforts in lipidomics we are beginning to understand why hundreds of structurally different lipids can be found in a single cell.[1–3] This variety originates in the modular platform found by nature: Held together by glycerol, various fatty acids and phosphate head groups can be chosen to build ever different phospholipids.[4] Exchanging the natural glycerol for nonnatural molecules such as diaminopropanol could lead to phospholipids with new and interesting physical properties not found in natural systems.

Historically, the synthesis of nonnatural phospholipids containing amides at the interface has been motivated by increasing the stability of liposomes through additional hydrogen bonds and resistance to phospholipase A_2 cleavage.[5] As a simplified version of the natural sphingomyelin, 1,2-dimyristoylamido-l,2-deoxy-*sn*-glycero-3-phosphatidylcholine was found to significantly ease the reconstitution of membrane proteins into liposomes.[5] Such carriers showed a potential for antitumor and anti-HIV agents.[6,7] These studies were carried out using racemic mixtures, and only later was enantiopure material synthesized.[8] However, significant advances in liposomal stability were only achieved after moving to the symmetrical 1,3-diamido phospholipids carrying fluorinated alkyl chains.[9–11]

The synthesis of a 1,3-diamido phospholipid with hydrogenated alkyl chains had not been reported yet, therefore this symmetrical phospholipid seemed both an attractive chemical challenge and a versatile platform for biophysical and biomedical studies. The appearance of such scaffolds in the recent literature prompted us to communicate our own efforts in this field.[12,13]

Presently, there is a lack of a clear nomenclature for nonnatural phospholipids. The current IUPAC-recommended nomenclature for natural phospholipids is based on the Fischer projection of the glycerol placing the substituent on the secondary alcohol in an L-position.[14,15] Consecutive numbering of the glycerol carbon centers leads to names such as 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine abbreviated as POPC and 3-palmitoyl-2-oleoyl-*sn*-glycero-1-phosphocholine for its enantiomer. Recently, Szoka and Huang included the nature of the chemical linkage branching off the glycerol into a modified nomenclature.[16] Based on these two recommendations, we propose a two-letter code that unambiguously identifies the chemical linker. This code would follow the standard trivial abbreviation of fatty acids (P for palmitoyl, O for oleoyl, etc.) and would, for example, lead to Pes-Oes-PC being the natural ester phospholipid POPC and PC-Oes-Pes being its enantiomer. Pad-PE-Pad would be the 1,3-palmitoylamido-l,3-deoxy-*sn*-glycero-2-phosphatidyl-ethanolamine (**1c**) reported in this communication. Besides "ad" for amide, "an" might stand for amine, "et" for ether etc.

Our goal was to find an efficient synthesis based on readily available reagents. We opted for introducing the fatty acid chains late in the synthesis since we found 1,3-dipalmitoylamido-2-propanol to be virtually insoluble in any solvent.[13] Furthermore, such an approach would also allow us to rapidly access various lipids of different chain lengths.

Unfortunately, the general methods for phospholipid chemistry that we tried were not applicable to the less reactive secondary alcohol found in our scaffold, a problem that was also reported in earlier syntheses of diamidophospholipids.[9] Although being reactive, reagent cost and oxidation problems kept us away from using phosphoramidites.

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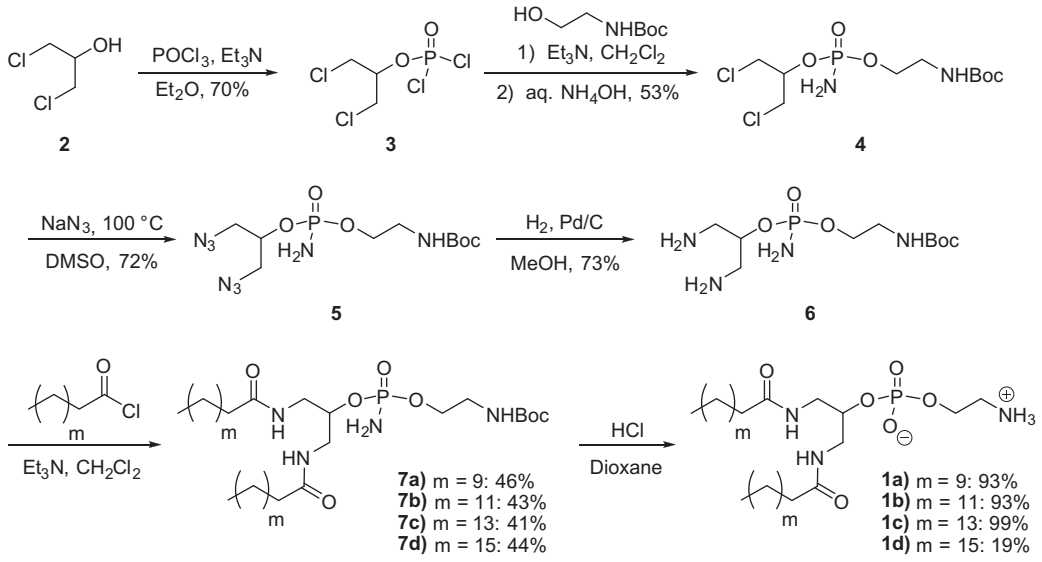

Scheme 1. Synthesis of the symmetrical phospholipids Lad-PE-Lad (**1a**), Mad-PE-Mad (**1b**), Pad-PE-Pad (**1c**), and Sad-PE-Sad (**1d**).

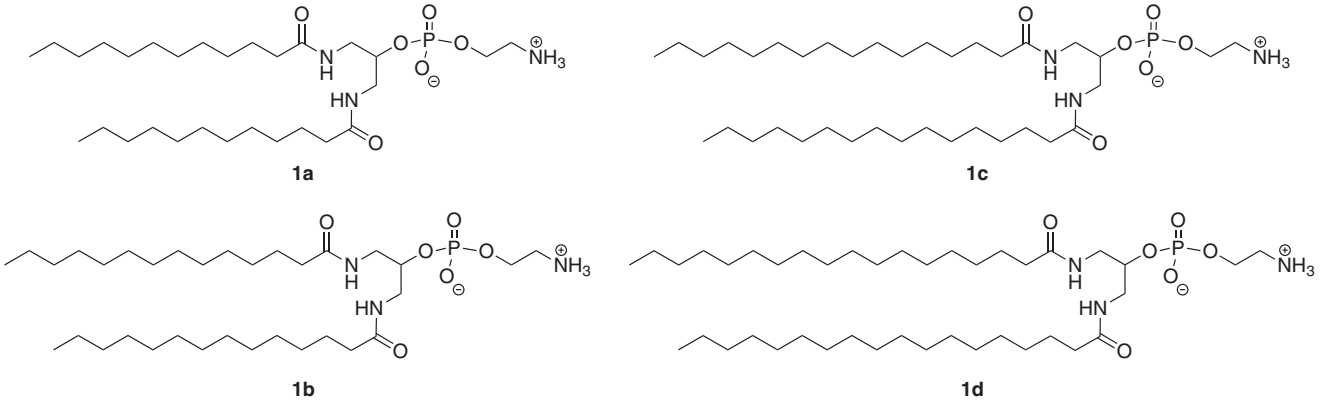

Figure 1. A small library of 1,3-diamidophospholipids containing various acyl chain lengths.

We finally identified phosphorodichloridate **3** as a versatile platform for our needs (see Scheme 1). The corresponding phosphate has been introduced as a flame retardant additive in polymer synthesis and low-yielding syntheses of isomeric mixtures of **3** have been reported.[17–19]

Starting from 1,3-dichloropropanol **2** a substitution of $POCl_3$ led to phosphorodichloridate **3** that could be readily purified by distillation (see Scheme 1). Any primary alcohol could then be used for a second substitution on the activated phosphorous compound. The obvious choice was a protected ethanolamine.[20] The resulting disubstituted phosphorochloridate could not be hydrolyzed directly into the phosphate. This problem could be circumvented by converting the phosphorochloridate to the corresponding phosphoramidate **4**. This simple protecting group was now readily removable under the same conditions used for BOC deprotection. To our knowledge, this was the first time that this strategy was applied to the synthesis of a phospholipid.

The dichloropropyl phosphoramidate **4** was transformed into the diazide **5** by substitution with sodium azide and further reduced to the diamine **6**.

Diamine **6** is an interesting intermediate, allowing the pursuit of several different synthetic directions. Here, the double substitution with acyl chains of different length led to a rapid access of a small library of 1,3-diamidophospholipids (see Fig. 1).

Amide bond formation with long chain acyl chlorides led to the protected diamido phospholipids **7a–d**: Reaction with lauryl chloride led to Lad-PE(Boc)-Lad (**7a**); reaction with myristoyl chloride led to Mad-PE(Boc)-Mad (**7b**); reaction with palmitoyl chloride led to Pad-PE(Boc)-Pad (**7c**); and reaction with stearoyl chloride led to Sad-PE(Boc)-Sad (**7d**). Treatment with concentrated hydrochloric acid both hydrolyzed the BOC protecting group as well as the amidophosphate yielding the title compounds **1a–d** in six linear steps.

In conclusion, we have reported a straightforward synthesis of several symmetrical 1,3-diamido phospholipids requiring no stringent exclusion of either air or water. The reported structures might serve as an interesting and flexible platform for further studies in biophysics and related fields. Work in this direction is currently ongoing.

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Supplementary data

Supplementary data (experimental procedures and full spectroscopic data for all compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.07.140.

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