1,1′-Thionyldi-imidazole in the Synthesis of a Long-chain Phospholipidic Membrane Probe

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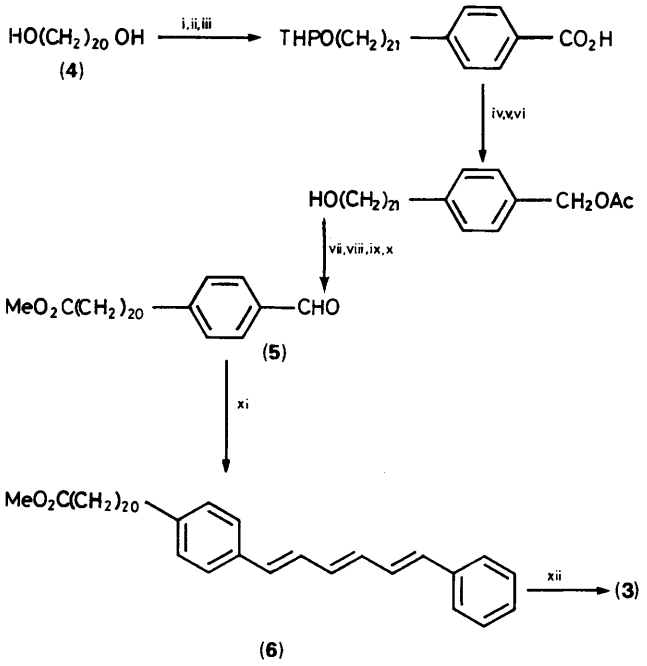
A long-chain fluorescent membrane probe could be synthesized from palmitoyl-lysophosphatidylcholine and 21-(diphenylhexatrienyl)henicosanoic acid only when the promoting agent was 1,1′-thionyldi-imidazole.

Fluorescence anisotropy is a very sensitive and convenient method to study membrane fluidity, and one of the most popular probes for such studies is 1,6-diphenyl-1,3,5-hexatriene (DPH).[1] However, it has been shown that the use of DPH with whole cells leads to its distribution in all the lipidic areas of the cells, thus preventing the measurement of the cell plasma-membrane fluidity.[2] In recent years, amphiphilic DPH-derivatives[2b,3] have been introduced that allow investigations on the cell plasma-membrane only, but they give an averaged fluidity of both the outer and the inner leaflets which have a different lipid composition and different physical properties.[4] In this communication, we report the synthesis of the phosphatidylcholine (**1**) bearing a DPH fluorophore at the end of a C_{21} spacer. This structure should allow the location of the DPH moiety in the inner leaflet of cell membranes, whereas the phosphorylcholine polar head would remain at the surface of the outer leaflet.

To synthesize the mixed-chain phosphatidylcholine (**1**), we planned to esterify commercial palmitoyl-lysophosphatidylcholine (**2**) (from Sigma Chemical Co.) with 21-DPH-henicosanoic acid (**3**), which had first to be prepared. This preparation was more difficult than expected since it soon turned out that some obvious intermediates were almost insoluble and thus of no use. After several unsuccessful approaches, we came to the conclusion that we had to design a synthetic route in which synthons larger than C_{20} did not bear two protic functional groups at a time (*e.g.*, OH and CO_2H) and in which the DPH moiety was introduced only at the end. With these guidelines in mind, we were able to synthesize the acid (**3**) according to Scheme 1.† As starting material, we selected icosane-1,20-diol (**4**), which was obtained either by saponification of its diacetate (KOH, MeOH, reflux) or by reduction of icosanedioic acid[5] [BH_3–Me_2S, tetrahydrofuran (THF)]. The diol (**4**) was transformed into the aldehyde (**5**) which was allowed to undergo a Wittig–Horner–Emmons reaction with a phosphonate[6] prepared in three steps from (2*E*,4*E*)-5-phenylpenta-2,4-dienal[7] [i, di-isobutylaluminium hydride, THF; ii, PCl_3, THF; iii, $P(OEt)_3$, 150 °C]. That the DPH moiety thus formed was all-*trans* could be shown by second order analysis of the ^1H NMR spectrum of the ester (**6**), using the Bruker routine PANIC (alkenic coupling constants *J* 15.6, 14.7, and 15.4 Hz).



Scheme 1. Synthesis of 21-DPH-henicosanoic acid (THP = tetrahydropyran-2-yl). i, 48% aqueous HBr, light petroleum (b.p. 110—130 °C), reflux, 24 h (40% yield); ii, dihydropyran, pyridinium toluene-*p*-sulphonate (PPTS), CH_2Cl_2, 20 h (87%); iii, *p*-$LiCH_2C_6H_4CO_2Li$, THF, 0 °C, 0.5 h (33%);[12] iv, BH_3–THF, THF, 0 °C then room temp., 20 h (67%); v, acetic anhydride, 4-(dimethylamino)pyridine, pyridine, CH_2Cl_2, 20 h (85%); vi, PPTS, EtOH, reflux, 1 h (95%); vii, Jones reagent, acetone (89%); viii, CH_2N_2, Et_2O (78%); ix, K_2CO_3, MeOH, THF, 45 min (69%); x, CrO_3–2-pyridine, CH_2Cl_2 (76%); xi, $PhCH{=}CHCH{=}CHCH_2P(O)(EtO)_2$, MeONa, THF, 0.5 h (66%); xii, KOH, MeOH, toluene, reflux, 20 h (78%).

Finally, the target compound (**1**) could be prepared by activating the acid (**3**) in THF with the little used 1,1′-thionyldi-imidazole[11] (TDI), and by allowing the so obtained imidazolide to react for 5 days at 50 °C with the lysoPC (**2**) in chloroform (8% yield).‡ The structure of the phosphatidylcholine (**1**) was affirmed by its UV, fluorescence, ^1H NMR, and FAB mass spectra.

With the acid (**3**) in hand, we could focus our efforts on its esterification with the little reactive lysophosphatidylcholine (lysoPC) (**2**). This kind of reaction is usually performed by acylating the lysoPC with an appropriate acid derivative, but in our case it was complicated by the size of the acid (**3**) and by the presence of the acid-sensitive DPH moiety. Thus, acylation attempts *via* anhydrides (mixed[3b] or symmetric[8]) or some other derivatives[9] of the acid (**3**) did not afford the expected lecithin (**1**). We decided then to explore the imidazolide route.[10] The activated derivative was first formed by treating the acid (**3**) in THF with commercial 1,1′-carbonyldi-imidazole (CDI; from Aldrich Chemical Co.). However, after addition of this imidazolide solution to either the lysoPC (**2**) or the lysoPC (**2**)–$CdCl_2$ complex in chloroform, no phosphatidylcholine (**1**) was obtained.

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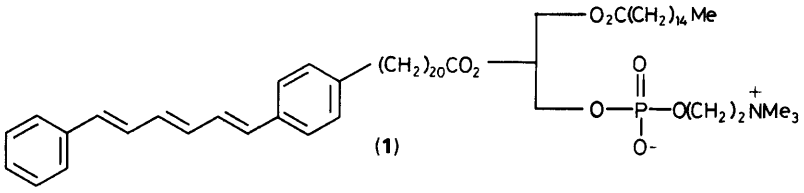
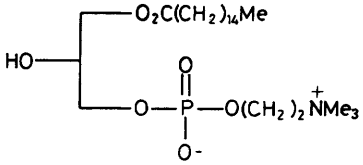
† All new compounds gave the expected IR, NMR, and mass spectra and satisfactory elemental analyses.

‡ We could also obtain other esters with significantly better yields when TDI was used as the activating agent instead of CDI.



(1)



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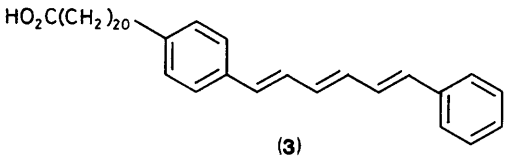

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