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SYNTHESIS AND PROPERTIES OF A PHOTOREACTIVE TRANSMEMBRANE PROBE

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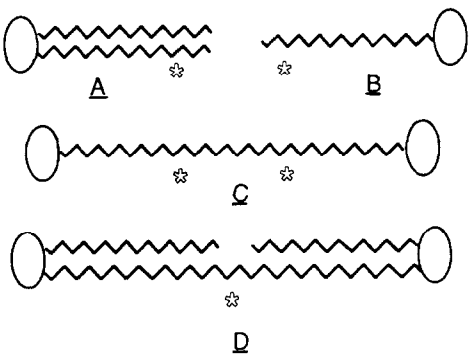
Phospholipid 1, bearing a benzophenone probe constrained in the middle of the double layer, has been synthesized by condensation of the bis (imidazolide) 6 with the $CdCl_2$ - complex of the lysophosphatidylcholine 5. It forms photosensitive liposomes and vesicles, alone and with dimyristoylphosphatidylcholine (DMPC).

The topology of the interior of phospholipid bilayers, containing or not intrinsic proteins, can be explored by physical methods (e.g. fluorescence, EPR or NMR), or with chemical probes, several types of which have been tested since the pionneering work of Khorana:[1] photoactivatable phospholipids of type A,[1] or amphiphilic molecules of types B and C.[2] In every case, the distribution of cross-linkings after irradiation was broad,indi-

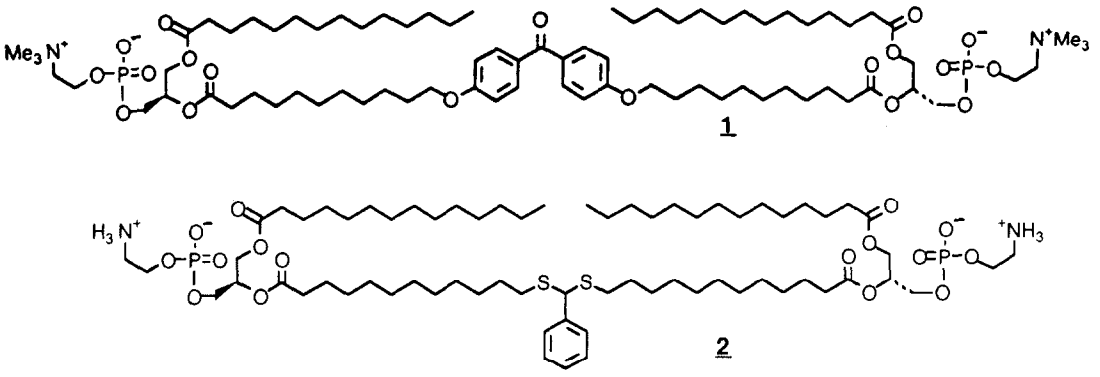


cating extensive dynamic disorder of the phospholipid matrix, of the probe, or of the inserted protein, or of all.

We have conceived, synthesized and begun to study a novel type of membrane probe D, encompassing the following desirable characteristics: it is a phospholipid, so as to remain as unobtrusive a probe as possible, it carries the photoreactive group on a transmembrane chain, so as to restrict its transverse movements, and its synthesis allows for structural variations in the nature of the head and photosensitive groups, and in the positioning of the probe at different depths; also, the photosensitive probe could be replaced by a spin label.

Our plan has been kindled by the structures of membrane-spanning archaebacterial phospholipids, either established,[4] or deduced from those of their molecular fossils.[5]. But we were also directly motivated by our general theory of the biochemical evolution of membrane reinforcers,[6] by the vindication of segments of this theory in a study of α,ω-dipolar carotenoids in vesicles,[7] and by the results obtained by an associated team on the structures of myelin proteolipids,[8] which should later provide interesting study goals.

We describe now the synthesis of the prototype probe 1 and some of its membrane properties, which appear to live up to our expectations. This preliminary report is prompted by a recent publication describing the synthesis of a probe model 2, of the same general type D, and announcing reports on the synthesis of photosensitive analogues of 2 and on their membrane properties.[3]



1

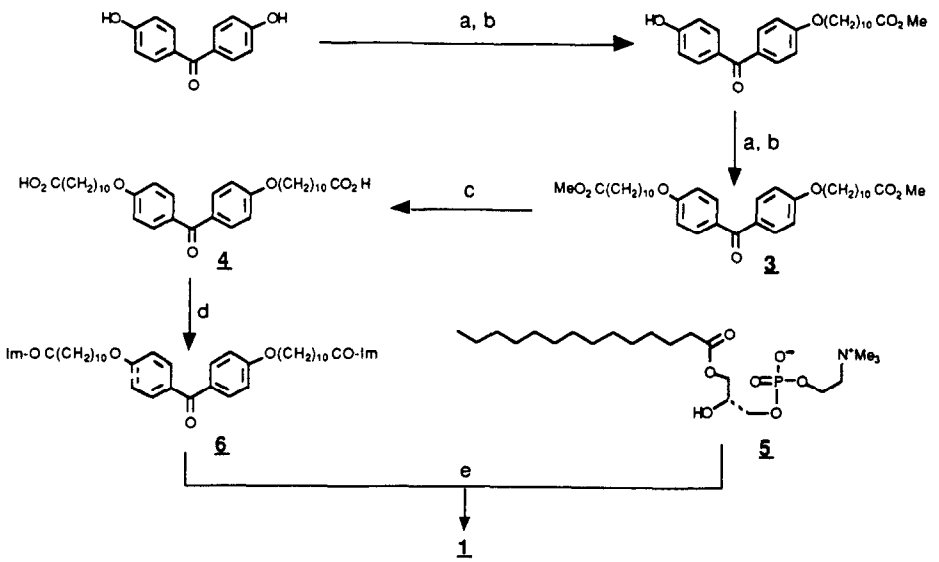
2

The core diacid 4 . The dicarboxylic acid 4 required was obtained by an obvious route: methyl 11-iodoundecanoate and p,p'-dihydroxybenzophenone gave, under basic phase-transfer catalysis, first the mono-ester and then the di-ester 3 . Such a two-step reaction, once properly optimized, will easily lead to unsymmetrical cores. Diester 3 shows λ_{max}=289nm in n-decanol when fully solubilized by warming; this will serve as a model for the absorption of the chromophore in a medium of low polarity. The solution, upon cooling, gives a suspension of fine solid particles of 3, serving as a model of chromophore aggregates, and showing λ_{max}=268nm. These values will serve later.

Alkaline hydrolysis of 3 gave the corresponding diacid 4 in a 66% overall yield based on dihydroxybenzophenone.

Synthesis of phospholipid 1 . Double condensation of the diacid 4 with lyso-sn-myristoylphosphatidylcholine 5 was achieved by activation of the diacid 4 as its bis-imidazolide 6 and protection of the phosphatidylcholine head-group as its cadmium chloride complex 5.[9] This required considerable experimentation (including the use of other activating substituents), and 1 was obtained only after careful drying of the diacid 4 (evaporation of a THF-toluene solution, followed by 24h at 50°C under vacuum) and elimination of methanol, used to crystallize the $CdCl_2$-complex 5, by evaporation of the chloroform-toluene suspension, followed by 24h at 50°C under vacuum (even then, a small proportion of diester 3 was formed after reaction with 6, and later improvements should include replacing methanol by a non-reactive solvent).

The bis-imidazolide 6 was prepared from the diacid 4 and carbonyl di-imidazole in THF with DMAP as catalyst. It was characterized by its very easy and complete conversion to the



a: 1 eq. NaH, BuN_4I cat.; b: $I(CH_2)_{10}CO_2Me$; c: KOH/EtOH, aq.
d: 2.4 eq. carbonyldiimidazole, 2 eq. DMAP, THF/43°C/3h ; e: 1 eq. 6 + 4 eq. 5.

diester 3 with methanol. The bis-imidazolide was added to a suspension of the $CdCl_2$-complex 5 in dry, alcohol-free, chloroform; this was sonicated at 20°C for 3h and then heated for 24h at 43°C with magnetic stirring. The resulting solution was passed over a 1:1 mixture of Amberlite IRC-50 and Amberlyst A-21 ion exchangers to retain the cadmium salts; the crude product was purified on silicic acid, and finally on a Sephadex LH-20 gel column. It gave the expected NMR, UV and MS-FAB spectra. The yield is only 29% from the diacid 4.

Membrane properties of phospholipid 1 . Phospholipid 1 forms, by vortex dispersion in deionized water, multilamellar vesicles (liposomes). These show, by differential microcalorimetry, a sharp transition at 51°C.

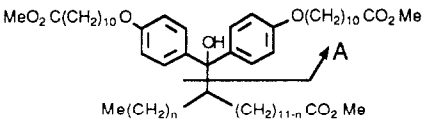
Mixtures of 1 and of DMPC can form, by sonication at about 50°C in water, mixed vesicles which have been purified from large aggregates by filtration through polycarbonate filters and a Sephadex gel.[7] Electron microscopy shows however that these vesicles are not homogeneous in size. Their probe content has been evaluated by UV spectrophotometry and phosphorus analysis.[7] Over the range 2-40% molar at least, the probe appears to be practically completely miscible with DMPC.

The mixed vesicles of 1 and DMPC show a remarkable differential microcalorimetry profile: in every case, the transition of DMPC remains sharp, its intensity decreasing progressively with increased content of the probe. Above this DMPC transition at 24°C, another one appears progressively and culminates in a medium-sharp peak, observed at temperatures lower than the T_m = 51°C of pure 1 : e.g. at 39°C for 20%, and 44°C for 40% molar 1 in DMPC. These observations can be interpreted as showing, in the range investigated, complete miscibility in the liquid crystal phase, and precipitation at lower

temperatures of pure 1, with virtually no miscibility of the gel phases.

This interpretation is nicely corroborated by the results of the UV study of these vesicles, which responds of course only to the probe. At low temperatures, the UV absorption observed (267-268 nm) is characteristic of aggregated 1 . As the temperature is raised, there is a sigmoid shift to a value (281-283 nm) compatible only with the localization of the chromophore in a non-polar solvent (i.e. inside the lipidic double layer). With increasing 1/DMPC ratios, the sigmoid middle-point shifts from about 24°C (for 2% 1) to about 39°C (for to38% 1).

Photochemistry of 1. So far, we have only investigated, above their higher T_m, the photochemical behaviour of DMPC liposomes containing 30% molar 1. Irradiation of a suspension in water, in Pyrex, with a medium-pressure mercury lamp, leads to the complete disappearance of the benzophenone chromophore in 5h. After evaporation to dryness in vacuum, methanol and hydrochloric acid were added to cleave the polar heads by ester interchange. The crude product shows the expected changes in the aromatic portion of its NMR spectrum, but mostly its MS (CI-NH_3) shows the expected molecular peak and fragments.



M = 852
MH^+-H_2O = 835
Base peak A = 611

No peak at 610 is observed; this would have been the molecular ion of the product resulting from the attack of the excited carbonyl on one of the chains of the probe itself, a process excluded if the probe is extended, transannular. The only photochemical reaction is therefore that of the excited carbonyl on one of the myristoyl chains, either that of the probe itself, or of one of the DMPC matrix. This point has not yet been clarified, and we have not yet established whether, as we hope, the attack on the myristoyl chain shows an enhanced site specificity.

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