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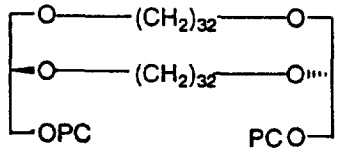
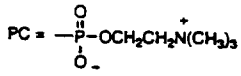
Synthesis of a Double-Phospholipid

F. M. Menger* and X. Y. Chen
Department of Chemistry, Emory University, Atlanta, GA 30322 USA

Abstract: A 72-membered macrocyclic phopholipid, which is a unimolecular version of the two-molecule unit spanning a membrane bilayer, was synthesized for the first time.

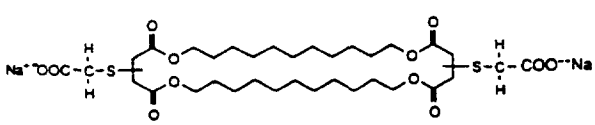
This communication describes the first synthesis of a "double-phospholipid", a unimolecular version of the bilayer assembly found in most biological membranes. The need to construct a giant 72-membered ring presented itself as a major challenge,



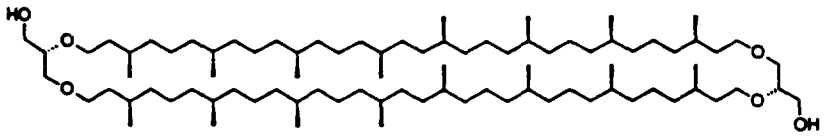


but the problem was solved using a strategy developed specifically by us to close such rings[1]. It should be stated at the outset that we could not predict the properties of the double-phospholipid owing to the fact that all four lipid chains, normally present in the two-molecule counterpart, become effectively immobilized within the giant ring. Since motional freedom of four independent chains is critical to many membrane properties, including their phase-transition[2], the interconnecting of the lipid pair by two new C-C bonds could have ramifications far exceeding the seemingly minor structural modification. As will be shown, this turned out to be the case.

"Bolaamphiphiles" are compounds that have two polar groups separated by a long hydrocarbon chain. Many different types are known.[3] Synthetic bolaamphiphiles whose hydrophobic region is comprised of a macrocyclic ring instead of a chain, as in the double-phospholipid, are rare. One of the few reported examples, prepared by the Fuhrhop group[4], is given below.



This 34-membered macrocycle spontaneously forms vesicles in water. Most examples of macrocyclic bolaamphiphile-type molecules are, however, not synthetic but are produced as membrane constituents by archaebacteria (ancient organisms that can live at low pH or high temperatures). One such membrane lipid is shown below.[5] Although the ring contains a 72-membered ring (as does the double-phospholipid), it possesses in addition numerous methyl substituents. As will be seen, the properties of our double-phospholipid analog revealed a likely function for the methyl groups.

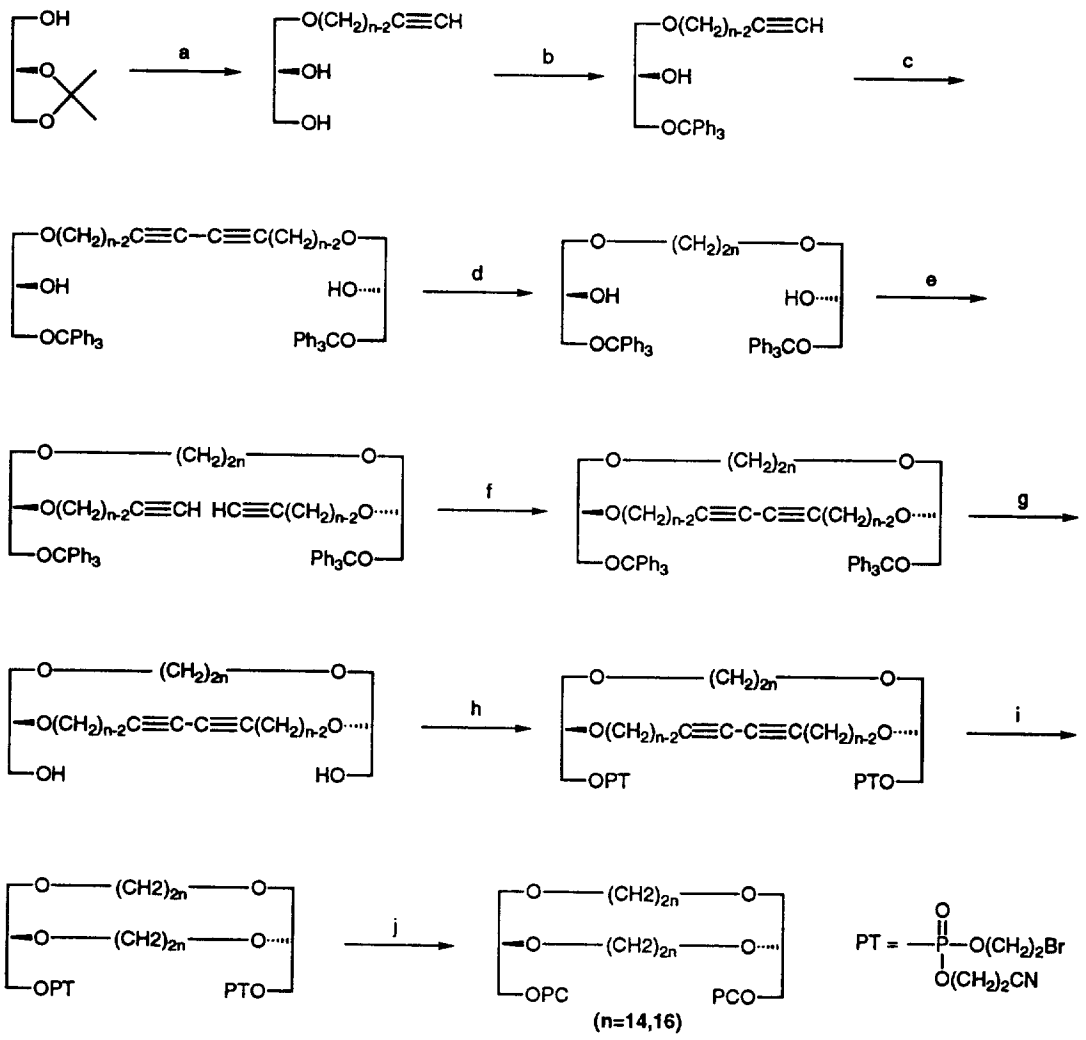


The 12-step synthesis of the double-phospholipid (formally named 2,2',3,3'-di-O-(dotriacontane-1,32-diyl)-sn-diglycero-1,1'-diphosphatidylcholine) is given in Scheme 1. Two particularly difficult steps were critical to the success of the venture: macrocylization via Glaser coupling (reaction f) and reduction of the resulting diyne to the saturated system (reaction i). These will now be described in detail using the "recipe format" that was introduced previously to abbreviate synthetic procedures.[6]

Reaction f. This high-temperature Glaser coupling is based on the methodology developed to prepare single-phospholipids with cyclized chains:[7] Add CuCl (0.12 g, 1.2 mmol), tetramethylethylenediamine (0.18 mL, 1.2 mmol) and 300 mL of xylene to a 500-mL 3-neck round-bottom flask equipped with a magnetic stirring bar, a condenser, a rubber stopper with a glass tube, and a septum. Heat to 140° with an oil bath while passing a gentle stream of O_2 through the glass tube. Add over a 4.5 h period, with the aid of a syringe pump (50-mL syringe), a solution of the acyclic diacetylenic precursor (0.73 g, 0.47 mmol in 4 OmL of xylene). Following the addition, turn off the O_2, cool, remove xylene under reduced pressure, add water to the residue, and extract the resulting mixture three times with ethyl acetate. Combine the ethyl acetate extracts, wash with water and brine, and dry over Na_2SO_4. Remove the solvent with a rotary evaporator, and purify the residue by silica gel chromatography (5:95 v/v ethyl acetate/hexane). Purify further by recrystallization from hexane to give pure product (0.33 g, 45%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ 1.29 (s, 96H), 1.57 (m, 12H), 2.25 (t, J = 6.8 Hz, 4H), 3.19 (d, J = 4.5 Hz, 4H), 3.44 (t, J = 6.4 Hz, 4H), 3.51-3.60 (m, 10H), 7.21-7.50 (m, 30H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 19.18, 26.11, 28.32, 28.80, 29.07, 29.44, 29.48, 29.61, 29.69, 30.10, 63.59, 65.31, 70.67, 71.43, 71.53, 78.37, 86.49, 126.84, 127.67, 128.71, 144.11. Anal. Calcd for $C_{108}H_{160}O_6$: C, 83.45; H, 10.37. Found: C, 83.54; H, 10.40. LRMS (electrospray ionization): m/z 1576 $(M + Na)^+$.

Reaction i. The diacetylene within the macrocycle resisted reduction, and rather heavy-handed conditions

Scheme I [a]



[a](a) (1) ROMs, KOH, DMSO; (2) p-TsOH, MeOH (77-86%); (b) TrCl, Et_3N, ^tBuOH (90-95%); (c) CuCl, TMEDA, O_2, 140 °C (66-76%); (d) H_2/Pt, EtOH (79-86%); (e) ROMs, NaH, 150 °C (82-87%); (f) CuCl, TMEDA, O_2, 140 °C (42-45%); (g) p-TsOH, CH_3OH-$CHCl_3$ (90%); (h) (1) N,N-diisopropylaminochlorophosphite, 2-bromoethanol, Et_3N; (2) tetrazole, H_2O_2 (40-43%); (i) H_2/Pd, THF-EtOH, 50 °C (32-35%); (j) $(CH_3)_3N$, CH_3CN, Δ.

were required: Hydrogenate at 1 atmosphere the macrocyclic diacetylene (66 mg) in 8 mL THF/EtOH (4:1 v/v) mixed with 10% Pd/C (33 mg, 50% H_2O). Filter through a layer of Celite and wash the pad with hot chloroform. Evaporate filtrate and purify crude product by recrystallization from ethyl acetate to afford a white solid product (21 mg, 32%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 96H), 1.55 (m, 8H), 2.78 (t, J = 6.0 Hz, 4H), 3.44-3.63 (m, 18H), 4.11-4.37 (m, 12H). ^{13}C NMR (75.1 MHz, $CDCl_3$): δ 19.57, 19.67, 26.01, 26.07, 29.25, 29.28, 29.40, 29.58, 29.66, 29.98, 61.99 ($^2J_{PC}$ = 4.5 Hz), 67.07 ($^2J_{PC}$ = 4.8 Hz), 67.84 ($^2J_{PC}$ = 6.3 Hz), 69.63, 70.68, 71.79, 116.20. LSIMS: m/z 1445 $(M+H)^+$.

The final product, i.e. the double-phospholipid produced in reaction j, was an intractable solid. Owing to its insolubility in all the common solvents, an NMR was impossible. Confidence that the desired compound was indeed in hand comes from the following: The direct precursor of the final product in Scheme 1 is a compound that could be fully purified and fully characterized. And this precursor was subjected to a reaction that normally goes in high (92%) yield.[8] Finally, a high-resolution mass spectrum gave a parent peak with the correct precise mass: Calcd for $C_{80}H_{164}O_{12}P_2$: m/z 1408.1837 $(M + H)^+$; Obsd: m/z 1408.1838.

Judging from the intractability of the double-phospholipid, the methyl groups in the archaebacterial lipids are needed to "fluidize" the membrane, i.e. to spread the chains apart and thereby reduce interlipid attraction. Mammalian membranes use unsaturation (particularly cis double-bonds) for the same purpose.

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