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N,N'-Disuccinimidyl Carbonate as a Coupling Agent in the Synthesis of Thiophospholipids Used for Anchoring Biomembranes to Gold Surfaces

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Abstract: N,N'-Disuccinimidyl carbonate has been used to couple diacylglycerophosphatidylethanolamine with bis- ω -hydroxypolyethylenoxydisulfide. The resultant thiophospholipids can be used for anchoring biomembranes to gold surfaces. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

The attachment of biomembranes to solid supports is important in a variety of areas from fundamental studies of bilayer structure and function to the creation of biocompatible surfaces and biosensors.¹ Solid supported lipid bilayers can be constructed using Langmuir-Blodgett methodology² or by covalently attaching bilayers on gold coated surfaces using thiol chemistry.^{3,4} The latter method is particular attractive as it allows the construction of mechanically stable and biologically active supported bilayers. However, the thiol compounds required are usually complex and not commercially available. Recently we reported a simple approach to the attachment of lipid bilayers to surfaces which employs self-assembled monolayers (SAMs) comprised of a mixture in which the anchoring component is an ethylenoxy oligomer which is terminated with a cholesteryl group at one end and a thiol group at the other. The cholesteryl moiety penetrates into the hydrophobic region of the inner leaflet of the bilayer and acts as a 'hook', the ethylenoxy part provides a hydrophilic space between the gold and the bilayer and the thiol group covalently binds to the gold surface.^{5,6}

We now report the creation of equivalent 'phospholipid anchors' comprised of a thiol or disulfide at one end to bind to the gold, a polyethylenoxy chain of defined length (three or six ethylenoxy units) in the middle and a phospholipid residue at the other end to insert into the biomembrane.

To make such 'thiophospholipids', the main difficulty we encountered was to couple the diacylglycerophosphatidyl group with $bis-\omega$ -hydroxypolyethylenoxydisulfide. Such a coupling had been reported, in which dipalmitoylphosphatidic acid disodium salt was coupled with $bis-\omega$ -hydroxypolyethylenoxydisulfide by using 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl) as the coupling

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agent⁴ but, in our experience, this method suffers from purification difficulties and low irreproducible yields. In seeking an alternative approach it should be noted that the incompatibility of disulfide with oxidation reagents and hydrogenation catalysts make it difficult to exploit the sort of P^{III} chemistry usually employed in phospholipid synthesis.⁷ For instance, chloro-(*N*,*N*-diisopropylamino)-methoxyphosphine is an effective general reagent for the synthesis of glycerophospholipids,⁸ but such syntheses involve using *tert*-butyl hydroperoxide at some stage to oxidise P^{III} to P^v. These problems prompted us to search for an alternative P^v-based method to synthesise our target molecules.

N,N'-Disuccinimidyl carbonate (DSC) has been recognised as a versatile reagent for active ester synthesis⁹ and the reactions of N,N'-disuccinimidyl carbonate with alcohols to form mixed active carbonates and their subsequent utility in the synthesis of functionalized carbamates has been investigated.¹⁰ These mixed active carbonates have been used successfully both as chemical affinity reagents for probing enzyme activesites¹¹ and as coupling agents for making polyethylene glycol-lipid conjugates.¹² We envisaged that the reaction of N,N'-disuccinimidyl carbonate with ω -thioether or disulfide polyethylenoxy alcohols and the subsequent coupling with diacylglycerophosphatidylethanolamine would serve to produce our 'phospholipid anchors'. The carbamate linkage displays excellent stability¹³ and can maintain or enhance the hydrophilicity of the polyethylenoxy spacers, a feature which is crucial for incorporating protein into the bilayers formed.^{4,5}

Results and Discussion

Initially we chose 8-benzylthio-3,6-dioxaoctanol 2 to react with *N*,*N*'-disuccinimidyl carbonate to test this method, as the benzyl protected thiols are more robust than the disulfides and can be made easily. The synthetic route is shown in scheme 1. 8-Benzylthio-3,6-dioxaoctanol 2 was synthesised in excellent yield by reacting commercially available 2-(2-(2-chloroethoxy)ethoxy)ethanol 1 with BnSH in ethanol with EtONa as the base.⁵ The reaction of 2 with DSC was carried out at room temperature using two equivalent of DSC.¹⁰ The reaction is clean and no homocarbonate 5 was detected. Of particular note, is that 3 is quite stable and can be chromatographed on silica gel. This is the real advantage of using DSC as the coupling reagent compared with more conventional coupling reagents such as 1,1'-carbonyldiimidazole.¹⁴ Severe difficulties are experienced in the chromatography of the phospholipid anchors and the ability to chromatograph at this intermediate stage and the very clean nature of the final step provide the crucial advantage. The final coupling reaction of 3 with L- α -dimyristoylphosphatidylethanolamine (DMPE) in CHCl₃ was fast as monitored by TLC and marked by the resulting DMPE suspension in chloroform becoming completely clear. Even using this approach, the chromatography of the final product was time-consuming and gradient elution was required. Nevertheless the desired product 4 was obtained in good yield and satisfactory analytical and spectroscopic data were obtained.



Reagents and conditions,

i, BnSH, EtONa, EtOH, reflux, 96%; ii, DSC, Et₃N, MeCN, 79%; iii, DMPE, Et₃N, CHCl₃, 88%.



Scheme 1

Encouraged by this result, we next used this method to make the 'thiophospholipids' 9 and 10 (scheme 2 and scheme 3 respectively). Product 9 could also be obtained by deprotecting the benzyl group from 4 and then oxidising the thiol to give the disulfide by established methods.^{4,5} However, this involves two steps of manipulations of 4, and the purification difficulties make this route less attractive than the route shown in scheme 2. Thus 7 was made by following a literature procedure.⁴ The reaction of 7 with DSC was carried out using the conditions described for scheme 1. It should be noted that in this case it is essential to monitor the reaction closely as a longer reaction time was found to significantly reduce the yield. The coupling reaction of 8 with DMPE was quick and smooth and, followed by column chromatography on flash silica gel, it gave the desired product 9 in good yield.



Reagents and conditions: i, NaHS, EtOH, 80%; ii, I₂, K₂CO₃, 84%; iii, DSC, MeCN, Et₃N, 71%; iv, DMPE, Et₃N, CHCl₃, 85%.

Scheme 2

The synthesis of 10 (scheme 3) proved to be much more difficult. We first tried coupling bis(8-hydroxy-3,6-dioxaoctyl)disulfide 7 with tetrahydropyranyl 2-(2-(2-chloroethoxy)ethoxy)ethanol 11 in the presence of a phase transfer catalyst to avoid the two step reactions of protecting and deprotecting the thiol

group (scheme 4). Unfortunately the disulfide was incompatible with these harsh reaction conditions. Therefore, we have to use the benzyl group to protect the thiol. As shown in scheme 3, 8-benzylthio-3,6-dioxaoctanol 2 was used to couple with 11 under the same conditions described for scheme 4 to give 12 in good yield. The tetrahydropyranyl group of 12 was then cleaved using methanol and 10-camphorsulfonic acid. The aqueous work-up of deprotecting benzyl group of 13 using Na/NH₃ resulted in some losses of the product because of its solubility in water but yields were improved by using continuous extraction. Changing the work-up method by using powdered NH₄Cl instead of NH₄Cl aqueous solution gave satisfactory yields. Compound 16 was made in the same way as 8 except that the crude product was washed with NaHCO₃ aqueous solution and water successively and extracted with dichloromethane to remove the by-product *N*-hydroxysuccinimide before it was purified by column chromatography (the TLC behaviour of the by-product and of 16 are very similar). Finally 10 was made by the same procedure as 9 in good yield.



Reagents and conditions:

i, 2,3-dihydropyran, HCl, 90%; ii, NaOH, Bu₄NHSO₄, **2**, 82%; iii, camphor-10-sulfonic acid, MeOH, 80%; iv, Na/NH₃, 89%; v, I₂, K₂CO₃, MeOH, 78%; vi, DSC, Et₃N, MeCN, 75%; vii, DMPE, Et₃N, CHCl₃, 86%.

Scheme 3



Scheme 4









Fig. 2. Comparison of the formation Egg-PC bilayers on "cholesterol" 17 and "thiophospholipid" 9 SAMs as monitored by Impedance Spectroscopy.



With these materials in hand, we have used them to anchor the bilayers to gold surfaces using the same methods as in our previous work on the cholesteryl derivatives⁶ (Fig. 1). Thus a gold coated glass slide was exposed to a dichloromethane solution of 9 and a self-assembled monolayer obtained. When this SAM was exposed to egg PC vesicles, a bilayer was formed and characterised by Impedance Spectroscopy. Preliminary results compared with the results obtained by using the 'cholesteryl anchor' 17^5 are shown in Fig. 2. As can be seen, as the bilayer forms the capacitance falls. For a perfect bilayer this should tend to a theoretical limit of ca. $0.5 \ \mu\text{F cm}^{-2.15}$ It is interesting to note that, under equivalent conditions, the bilayers generated using the 'cholesteryl anchors' are formed more slowly and are more imperfect than the bilayers formed using the 'cholesteryl anchors', despite the fact that phospholipids are more abundant than cholesterol in natural biomembranes, more bio-lookalike. It may be that the presence of the polar phosphate residue in the thiophospholipid (which itself will interact with the gold surface) gives a less ordered SAM. The relative size of the hydrophilic and hydrophobic residues of the thiolipids may also have an effect on the Impedance Spectroscopy results of the anchored bilayers.^{1b}

Conclusions

We have developed an efficient method for synthesising 'phospholipid anchors'. The key to this method is the use of DSC to couple diacylglycerophosphatidylethanolamine with bis- ω -hydroxypolyethylenoxydisulfides. Generally both lipids and polyethylenoxy derivatives are difficult to purify by column chromatography and the anchor molecules which contain both entities are particularly difficult. However, this strategy makes it possible to purify the intermediate mixed active carbonates and the subsequent coupling step is very clean.

In terms of a general strategy for anchoring biomembranes to a gold surface using natural membrane components, it might seem more obvious to bind through a phospholipid rather than a cholesterol lookalike. In our initial studies we chose to use the cholesteryl derivatives because they were simple to make and more robust in the physical, chemical and biochemical senses. This paper suggests that the cholesteryl derivatives may have a second unforeseen advantage. They actually gave better ordered self-assembled monolayers and more defect free anchored bilayers. The detailed physical characterisation of the anchored bilayers and the investigations of the influence of the lipid structure on the anchored bilayers will be reported in due course.

Experimental Section.

General procedures and instrumentation.

Nuclear magnetic resonance spectra were recorded on a General Electric QE 300 spectrometer or a Bruker AM 400 spectrometer. Chemical shifts are expressed in parts per million (ppm) downfield of tetramethylsilane for ¹H resonances. Mass spectra were recorded on a VG Autospec mass spectrometer. Microanalyses were carried out at Leeds University Microanalytical Laboratory. All C, H, N, S, and P analytical figures are percentage values. The lipid compounds were analysed as the corresponding hydrates.

Thin layer chromatography was carried out using precoated aluminium-backed silica plates which were visualised using ultraviolet light, permanganate stain and phosphomolybdic acid stain. Flash chromatography signifies column chromatography on Merck silica gel (230-400). Petroleum ether refers to petroleum ether (b.p. 40-60°C) unless otherwise stated. 8-Benzylthio-3,6-dioxaoctanol 2, tetrahydropyranyl 2-(2-(-2-chloroethoxy)ethoxy)ethanol 11, 17-Benzylthio-3,6,9,12,15-pentaoxaheptadecanol tetrahydro-pyranyl ether 12, 17-Benzylthio-3,6,9,12,15-pentaoxaheptadecanol 13 and 17-mercapto-3,6,9,12,15-pentaoxaheptadecanol 14 were prepared as reported before.⁵ 8-Mercapto-3,6-dioxaoctanol 6 and bis(8-hydroxy-3,6-dioxaoctyl)disulfide 7 were prepared using literature procedures.⁴

Experimental details.

N-(8-Benzylthio-3,6-dioxaoctoxycarbonyloxy)succinimide (3).

To a stirred solution of 8-benzylthio-3,6-dioxaoctanol 2^5 (0.52 g, 2.03 mmol) in dry MeCN (5 ml) at room temperature were added DSC (1.04 g, 4.06 mmol) and Et₃N (0.62 g, 0.85 ml, 6.09 mmol). The resulting mixture was stirred at room temperature for 4 hours and the solvent was removed under vacuum. The product was separated by column chromatography on silica gel (2:1 ethyl acetate : petroleum spirit) to give *N*-(8benzylthio-3,6-dioxaoctoxycarbonyloxy)succinimide **3** (0.64 g, 1.61 mmol, 79% yield) as a viscous pale yellow oil. $\delta_{H}(300 \text{ MHz}, \text{ CDCl}_3)$ 2.63 (2 H, t, J 6.6, SCH₂), 2.82 (4 H, s, succinimidyl), 3.58-3.67 (6 H, m, SCH₂CH₂OCH₂CH₂), 3.76-3.79 (4 H, m, PhCH₂, COOCH₂CH₂), 4.46 (2 H, t, J 4.5, COOCH₂), 7.26-7.34 (5 H, Ar). MS (EI) m/z 396 (M⁺-1, 11%), 248 (6), 186 (7), 151 (11), 122 (42), 91 (100). Calc. for C₁₈H₂₃NSO₇: C, 54.40; H, 5.83; N, 3.52; S, 8.07. Found: C, 54.65; H, 5.70; N, 3.25; S, 8.35%.

8-(L-α-Dimyristoylphosphatidylethanolaminocarbonyloxy)-3,6-dioxaoctylbenzyl sulfide (4).

DMPE (99 mg, 0.16 mmol, from Sigma) was added to a solution of N-(8-benzylthio-3,6dioxaoctoxycarbonyloxy)succinimide 3 (80 mg, 0.20 mmol) and Et₃N (47 mg, 0.46 mmol) in CHCl₃ (5 ml). The reaction mixture was stirred at 40°C for 1 hour, TLC showed that the DMPE had disappeared. The solvent was then removed under vacuum and the residue was subjected to column on flash silica gel (CHCl₃ \rightarrow 9:1 CHCl₃:MeOH \rightarrow 8:2 CHCl₃:MeOH) to give 8-(L- α -dimyristoylphosphatidylethanolaminocarbonyloxy)-3,6dioxaoctylbenzyl sulfide 4 (162 mg, 0.18 mmol, 88% yield) as white plates and dried over P₂O₅ under vacuum. $\delta_{H}(400 \text{ MHz}, \text{CDCl}_{3})$ 0.88 (6 H, t, J 6.8, CH₃ x 2), 1.25-1.29 (40 H, m, CH₃(CH₂)₁₀ x 2), 1.56 (4 H, s, br. CH₃(CH₂)₁₀CH₂ x 2), 2.24-2.28 (4 H, m, CH₃(CH₂)₁₁CH₂ x 2), 2.60 (2 H, t, J 6.8, SCH₂), 3.55-3.65 (10 H, m, NHCH₂, SCH₂CH₂O(CH₂)₂OCH₂), 3.73 (2 H, s, BnCH₂), 3.91 (4 H, s, br. NHCH₂CH₂, POCH₂), 4.20 (3 H, s, br. one of COOCH₂CH, COOCH₂CH₂), 4.36 (1 H, s, br., one of COOCH₂CH), 5.21 (1 H, br., COOCH₂CH), 7.227.30 (5 H, m, Ar). MS (FAB) m/z 941 (MNa⁺ 14%). Calc. for $C_{47}H_{84}NO_{12}PS \cdot H_2O$: C, 60.30; H, 9.26; N, 1.50; S, 3.42; P, 3.31. Found: C, 60.35; H, 9.20; N, 1.50; S, 3.40; P, 3.40%.

Bis(8-(N-succinimidyloxycarbonyloxy)-3,6-dioxaoctyl)disulfide (8).

To a stirred solution of bis(8-hydroxy-3,6-dioxaoctyl)disulfide 7⁴ (0.80 g, 2.42 mmol) in dry MeCN (5 ml) at room temperature were added DSC (2.48 g, 9.70 mmol) and Et₃N (1.47 g, 2.02 ml, 14.52 mmol). The resulting mixture was stirred at room temperature for 6 hours and then filtered and washed with ethyl acetate. The filtrate was collected and concentrated to give a viscous residue which was purified by column chromatography on flash silica gel (ethyl acetate) to give bis(8-(*N*-succinimidyloxycarbonyloxy)-3,6-dioxaoctyl)disulfide 8 (1.05 g, 1.72 mmol, 71% yield) as a viscous pale yellow oil. δ_{H} (400 MHz, CDCl₃) 2.83 (s, 8 H, succinimidyl), 2.90 (4 H, t, J 6.6, SCH₂), 3.64-3.68 (8 H, m, SCH₂CH₂OCH₂CH₂), 3.74 (4 H, t, J 6.6, SCH₂CH₂), 3.79 (4 H, t, J 4.6, COOCH₂CH₂), 4.46 (4 H, t, J 4.6, COOCH₂). MS (EI) m/z 612 (M⁺ 46%), 568 (10), 471 (36), 45 (100). Calc. for C₂₂H₃₂N₂O₁₄S₂: C, 43.13; H, 5.26; N, 4.57; S, 10.47. Found: C, 42.90; H, 5.30; N, 4.60; S, 10.20%.

Bis-(8-(L-α-dimyristoylphosphatidylethanolaminocarbonyloxy)-3,6-dioxaoctyl) disulfide (9).

DMPE (99.7 mg, 0.16 mmol) was added to a solution of bis(8-(*N*-succinimidyloxycarbonyloxy)-3,6dioxaoctyl)disulfide **8** (48 mg, 0.08 mmol) and Et₃N (48 mg, 0.47 mmol) in CHCl₃ (4 ml). The reaction mixture was stirred at 35°C for 2 hours and the solvent was then removed under vacuum and the residue was subjected to column on flash silica gel (CHCl₃ \rightarrow 9:1 CHCl₃:MeOH \rightarrow 8:2 CHCl₃:MeOH) to give the product bis-(8-(L- α dimyristoylphosphatidylethanolaminocarbonyloxy)-3,6-dioxaoctyl) disulfide **9** which was dissolved in chloroform and precipitated by acetone as a colourless solid, dried over P₂O₅ under vacuum (0.11 g, 0.07 mmol, 85% yield). $\delta_{H}(300 \text{ MHz}, \text{CDCl}_3) 0.88 (12 \text{ H}, \text{t}, \text{J} 6.3, \text{CH}_3 \text{ x} 4)$, 1.26 (80 H, s, br., $\text{CH}_3(\text{CH}_2)_{10} \text{ x} 4$), 1.57 (8 H, s, br. $\text{CH}_3(\text{CH}_2)_{10}\text{CH}_2 \text{ x} 4$), 2.27-2.30 (8 H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2 \text{ x} 4$), 2.93 (4 H, s, br., $\text{SCH}_2 \text{ x} 2$), 3.64-3.79 (20 H, m, NHCH₂ x 2, $\text{SCH}_2\text{CH}_2\text{O}(\text{CH}_2)_2\text{OCH}_2 \text{ x} 2$), 3.95 (8 H, s, br. NHCH₂CH₂ x 2, $\text{POCH}_2 \text{ x} 2$), 4.12-4.29 (6 H, m, one of $\text{COOCH}_2\text{CH} \text{ x} 2$, $\text{COOCH}_2\text{CH}_2 \text{ x} 2$), 4.37-4.42 (2 H, m, one of $\text{COOCH}_2\text{CH} \text{ x} 2$), 5.23 (2 H, s, br., $\text{COOCH}_2\text{CH} \text{ x} 2$). MS (FAB) m/z 1677 (MNa⁺ 14%). Calc. for $\text{C}_{80}\text{H}_{154}\text{N}_2\text{O}_{24}\text{S}_2\text{P}_2 \text{ *}2\text{H}_2\text{O}$: C, 56.85; H, 9.42; N, 1.66; S, 3.79; P, 3.67. Found: C, 56.65; H, 9.30; N, 1.50; S, 3.60; P, 3.45%.

Bis(17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)disulfide (15).

17-Mercapto-3,6,9,12,15-pentaoxaheptadecanol 14⁵ (0.30 g, 1.00 mmol) in methanol (4 ml) was mixed with a solution of potassium carbonate (0.07 g, 0.50 mmol) in water (2 ml). Iodine (0.13 g, 0.50 mmol) in methanol (4 ml) was added dropwise at room temperature. The potassium iodide was removed by filtration and the solvents were evaporated under vacuum. The residue was purified by column chromatography on flash silica (2:8 methanol : ethyl acetate) to give bis(17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)disulfide 15 (0.23 g, 0.39 mmol, 78% yield) as a colourless oil. $\delta_{H}(300 \text{ MHz}, \text{CDCl}_{3})$ 2.88 (4 H, t, J 6.7, SCH₂ x 2), 2.95 (2 H, s, br., OH x 2), 3.60-3.78 (44 H, m). MS (EI) m/z 594 (M⁺, 65%), 542 (19), 526 (22), 499 (27), 485 (34), 471 (37), 457 (42), 445 (49), 429 (56), 415 (61), 401 (62), 389 (65), 330 (25), 239 (25), 195 (87), 151 (85), 45 (100). Calc. for C₂₄H₅₀O₁₂S₂: C, 48.47, H, 8.57. Found: C, 48.25, H, 8.85%.

Bis(17-(N-succinimidyloxycarbonyloxy)-3,6,9,12,15-pentaoxaheptadecyl)disulfide (16).

DSC (0.35 g, 1.36 mmol) was added in bis(17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)disulfide **15** (0.20 g, 0.34 mmol) and Et₃N (0.21 g, 0.3 ml, 2.04 mmol) in MeCN (4 ml) at room temperature. The solvents were removed under vacuum after the mixture was stirred for 40 minutes. Dichloromethane (20 ml) was added and the residue was washed with NaHCO₃ aqueous solution and water successively. The organic layer was collected, dried (MgSO₄), filtered, and the solvent was removed under vacuum and the residue was purified by column chromatography on flash silica gel (1 : 9 methanol : ethyl acetate) to give bis(17-(N-succinimidyloxycarbonyloxy)-3,6,9,12,15-pentaoxaheptadecyl)disulfide **16** (0.22 g, 0.25 mmol, 75% yield) as a pale yellow oil. $\delta_{H}(300 \text{ MHz}, \text{CDCl}_3)$ 2.84 (8 H, s, succinimidyl), 2.88 (4 H, t, J 6.7, SCH₂), 3.64-3.81 (40 H, m, $CH_2(OCH_2CH_2)_4OCH_2$), 4.46 (4 H, t, J 4.5, COOCH₂). MS (EI) m/z 876 (M⁺, 44%), 832 (11), 793 (45), 620 (42), 443 (21), 274 (32), 186 (42), 84 (53), 55 (100). Calc. for C₃₄H₅₆N₂O₂₀S₂: C, 46.57; H, 6.44; N, 3.19. Found: C, 46.30; H, 6.55; N, 3.15%.

Bis-(17-(L-α-dimyristoylphosphatidylethanolaminocarbonyloxy)-3,6,9,12,15-pentaoxaheptadecyl) disulfide (10).

DMPE (204 mg, 0.32 mmol) was added to a solution of bis(17-(*N*-succinimidyloxycarbonyloxy)-3,6,9,12,15-pentaoxaheptadecyl)disulfide 16 (136 mg, 0.16 mmol) and Et₃N (94 mg, 0.93 mmol) in CHCl₃ (5 ml). The reaction mixture was stirred at 35°C for 2 hours and the solvent was then removed under vacuum and the residue was subjected to column on flash silica gel (CHCl₃ \rightarrow 9:1 CHCl₃:MeOH \rightarrow 8:2 CHCl₃:MeOH) to give bis-(17-(L- α -dimyristoylphosphatidylethanolaminocarbonyloxy)-3,6,9,12,15-pentaoxaheptadecyl) disulfide 10 which was dissolved in chloroform and precipitated by acetone as a colourless solid, dried over P₂O₅ under vacuum (257 mg, 0.13 mmol, 86% yield). $\delta_{H}(300 \text{ MHz}, \text{CDCl}_3) 0.87$ (12 H, t, J 6.8, CH₃ x 4), 1.25 (80 H, s, br., CH₃(CH₂)₁₀ x 4), 1.57 (8 H, s, br. CH₃(CH₂)₁₀CH₂ x 4), 2.26-2.28 (8 H, m, CH₃(CH₂)₁₁CH₂ x 4), 2.92 (4 H, t, J 7.1, SCH₂ x 2), 3.64-3.78 (44 H, m, NHCH₂ x 2, SCH₂CH₂(OCH₂CH₂)₄OCH₂ x 2), 3.89-3.96 (8 H, m, NHCH₂CH₂ x 2, POCH₂ x 2), 4.11-4.29 (6 H, m, one of COOCH₂CH x 2). MS (EI) m/z 495 (4%), (CH₂CH(OCOC₁₃H₂₇)CH₂OCOC₁₃H₂₇)⁺, 465 (6) (S(CH₂CH₂O)₆CONHCH₂CH₂)-OPO(OH)O + H⁺), 451 (18), 409 (5), 395 (26), 381 (11), 353 (7), (S(CH₂CH₂O)₆CONHCH₂), 339 (100) (S(CH₂CH₂O)₆CONH, 326 (6), 285 (23), 211 (58), 185 (9), 171 (18), 158 (10), 129 (13), 71 (38), 55 (83), 43 (75). Calc. for C₂₉H₁₇₈O₃₀N₂S₂P₂•2H₂O: C, 56.54; H, 9.39; N,1.43; S, 3.28; P, 3.17. Found: C, 56.30; H, 9.30; N,1.35; S, 3.30; P, 3.30%.

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References.

- a). Mrksich, M. Current Opinion in Colloid & Interface Science, 1997, 2, 83-88; Sackmann, E. Science, 1996, 271, 43-48; b). Cornell, B. A.; Braach-Maksvytis, V. L. B.; King, L. G.; Osman, P. D. J.; Raguse, B.; Wleczorek, L.; Pace, R. J. Nature, 1997, 387(5), 580-583.
- Ulman, A. An Introduction to Ultrathin Organic Films. Academic Press, Inc.; San Diego, CA, 1991, 101-236.
- Naumann, R.; Jonczyk, A.; Kopp, R.; van Esch, J.; Ringsdorf, H.; Knoll, W.; Gräber, P. Angew. Chem. Int. Ed. Engl., 1995, 34, 2056-2058; Spinke, J; Yang, J.; Wolf, H.; Liley, M.; Ringsdorf, H.; Knoll, W. Biophys. J., 1992, 63, 1667-1671; Brink, G.; Schmitt, L.; Tampé, R.; Sackmann, E. Biochim. Biophys. Acta, 1994, 1196, 227-230; Duschl, C.; liley, M.; Vogel, H. Angew. Chem. Int. Ed. Engl., 1994, 33, 1274-1276; Duschl, C.; liley, M.; Corradin, G.; Vogel, H. Biophys. J., 1994, 67, 1229-1237.
- 4. Lang, H.; Duschl, C.; Vogel, H. Langmuir, 1994, 10, 197-210.
- 5. Boden, N.; Bushby, R. J.; Clarkson, S.; Evans, S. D.; Knowles, P. F.; Marsh, A. Tetrahedron, 1997, 53 (31), 10939-10952.
- a), Williams. L. M.; Evans, S. D.; Flynn, T. M.; Marsh, A.; Knowles, P. F.; Bushby, R. J.; Boden, N. Langmuir, 1997, 13, 751-757. b), Cheng Y.; Boden, N.; Bushby, R. J.; Clarkson, S.; Evans, S. D.; Knowles, P. F.; Marsh, A.; Miles, R. E. Langmuir, 1998, 14, 839-844. c), Jenkins, A. T. A.; Bushby, R. J.; Boden, N.; Evans, S. D.; Knowles, P. F.; Liu, Q.; Miles, R. E.; Ogier, S. D. submitted to Langmuir, 1998.
- 7. Cevc, G. (Editor), Phospholipids Handbook. New York, Marcel Dekker, Inc., 1993, 211-213.
- 8. Bruzik, K. S.; Salamonczyk, G.; Stec, G. W. J. Org. Chem., 1986, 51, 2368-2370.
- 9. Ogura, H.; Kobayashi, T.; Kawabe, K.; Takeda, K. Tetrahedron Lett., 1979, 4745-4746.
- 10. Ghosh, A. K.; Duong, T. T.; Mckee, S. P.; Thompson, W. J. Tetrahedron Lett., 1992, 2781-2784.
- Zarling, D. A.; Watson, A.; Bach, F. H. J. Immunol., 1980, 124, 913-920; Howard, A.; de La Baume, S.; Gioannini, T. L.; Hiller, J. M.; Simon, E. J. J. Biol. Chem., 1985, 260, 10833-10839; Bouizar, Z.; Fouchereau-Person, M.; Taboulet, J.; Moukhtar, M. S.; Milhaud, G. Eur. J. Biochem., 1986, 155, 141-147.
- 12. Lasic, D.; Martin, F. (Editors), Stealth Liposomes, Boca Raton: CRC Press, 1995, 94-102.

- 13. Hermanson, G. T., Bioconjugate Techniques, Academic press, INC. 1996, 202.
- 14. Allen, T. M.; Hansen, C.; Martin, F.; Redemann, C.; Yau-Young, A. Biochim. Biophys. Acta, 1991, 1066, 29-36;
- 15. Steinem, C.; Janshoff, A.; Ulrich, W. P.; Sieber, M.; Galla, H. J. Biochim. Biophys. Acta, 1996, 1279, 169-180.