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SYNTHESIS OF STRUCTURAL VARIANTS OF PHOSPHOLIPIDS: INHIBITION OF PHOSPHOLIPASE A_2

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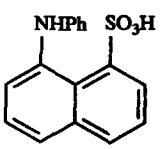
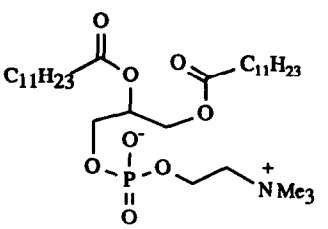
Abstract:

3-Arachidonyl-1,2,3,4-tetrahydro-4-[(O-phosphatidylethanamino)hydroxymethyl]furan-2-one has been synthesised as an inhibitor of extracellular PLA_2. This work has been facilitated by computer modelling of the active site of the enzyme.

Phospholipases A_2 (PLA_2) function *in vivo* to hydrolyse acyl esters of phosphoglycerides, thereby releasing arachidonic acid and initiating the biosynthesis of prostaglandins, thromboxanes, leukotrienes, and related molecules through the so called arachidonic acid cascade[1]. Recently we have developed a model of bovine PLA_2[2] which defines the stereochemistry and charge distribution of the active site and which accomodates known inhibitors such as 1,8-anilinonaphalenesulphonic acid (1)[3] with only minor perturbations of active site residues. Although the active site domain of PLA_2 has been identified by experiment[4], there is no X-ray structure depicting a ligand complexed with the enzyme.[5] Our work is based upon a model derived from crystallographic data from an apoenzyme,[6] further refined by minimizing with a model phospholipid substrate (2) and taking into account surrounding water molecules (see fig. 1).



(1)



(2)

The natural enzyme substrates are *O*-phosphoethanolaminoglycerides (3) which bear an arachidonyl unit at C2 and a long chain saturated acid residue at C3. We considered that a close mimic of this substrate might be a lactone of the type (4), where an arachidonyl unit is bonded to a potential phoshoglyceride, through a ketone function rather *via* an ester group.

Inspection of a model of the furanone (4) superimposed upon the receptor site conformation of the

phosphoglyceride (2) shows that the first three methylene groups adjoining the carbonyl function of the arachidonyl unit of the first compound correlate closely with those of compound (2) (see fig. 2). Thus we speculated that the complete arachidonyl fragment might be unnecessary for inhibition of the enzyme, and in one phase of our work we considered the synthesis of analogues (5), where the furanone bears an alkanoyl substituent.

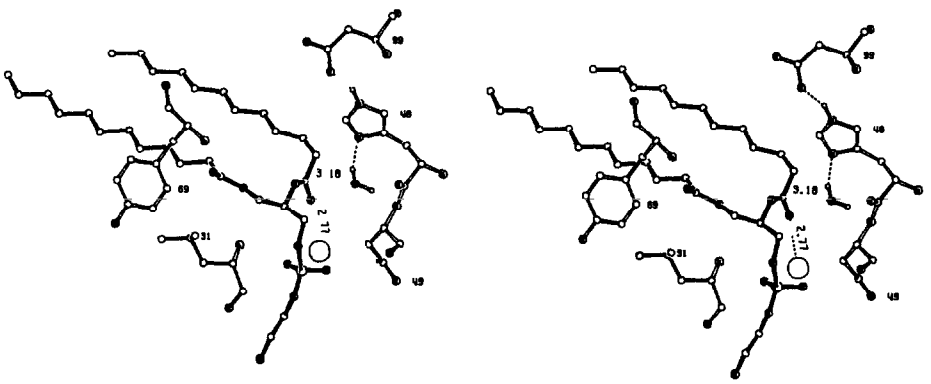
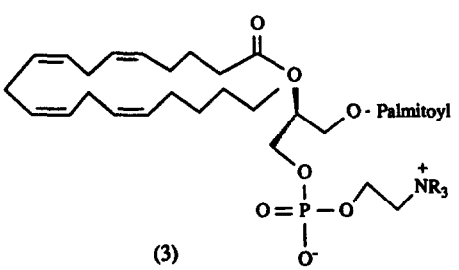
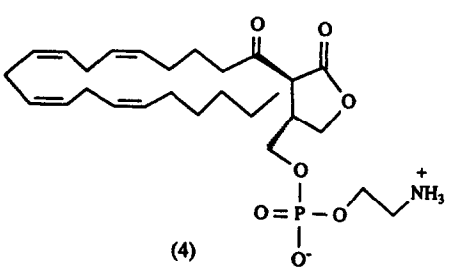


Figure 1. Model of the substrate (2) (filled bonds) docked with the active site of energy minimised PLA_2 (open bonds, calcium atom hatched)

Structures of this type are available by the acylation of the known lactone (6, R=H),[7] protected as the TBDMS ether, and when the ether (6, R=BDMS) was reacted firstly with one molar equivalent of LDA, and the derived anion quenched with either acetyl chloride, or with butanoyl chloride the acetyl- and the butanoyl-compounds (7, R=Me) and (7, R=Pr) were formed respectively.



(3)



(4)

If excess butyl lithium was present in the LDA used for the first of these reactions the acetylated lactone (7, R=Me) was accompanied by a small amount of the diol (9, R=Me). The other possible regioisomer (10, R=Me) was not detected.

Both the acetyl- and butanoyl-lactones exist as mixtures of keto and enol isomers (8) as oils, but in chloroform solution the keto forms are preferred. Deuterium exchange of H-3 in these compounds is slow, but is quantitative if the corresponding anions are preformed and quenched with deuterium oxide. The remaining steps of the synthesis of the butanoyl model were achieved as follows: the lactone (7, R=Pr) was deprotected with hydrochloric acid and the product alcohol was treated with 2-chloro-2-oxa-1,2,3,4-dioxaphospholane (11), and then with ammonia to afford the phosphatidyl derivative (5, n=2).

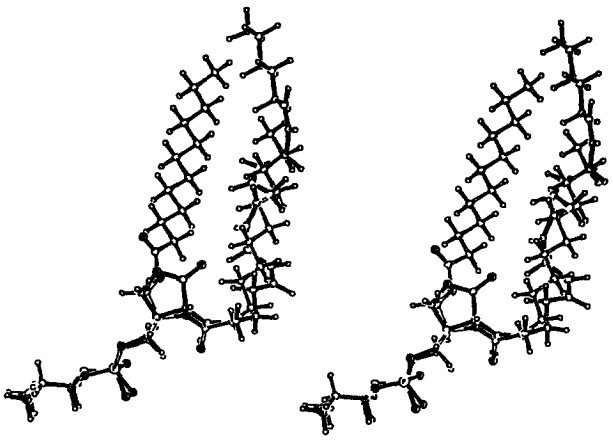
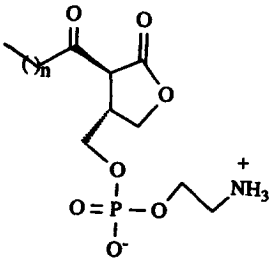
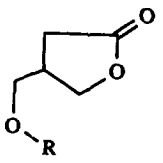


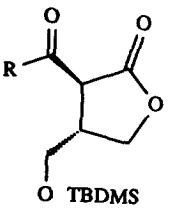
Figure 2. Furanone (4) (filled bonds) superimposed upon the receptor site conformation of the model substrate (2) (open bonds)



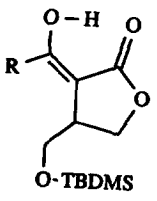
(5)



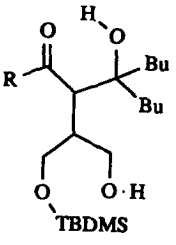
(6)



(7)



(8)



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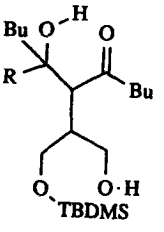
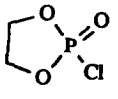


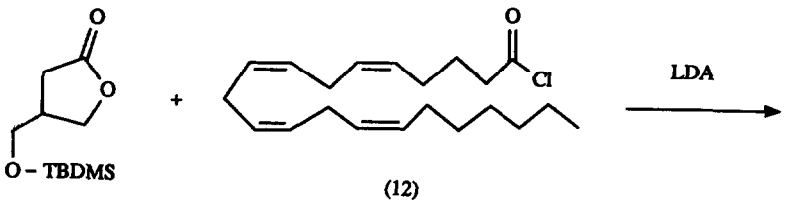
(10)



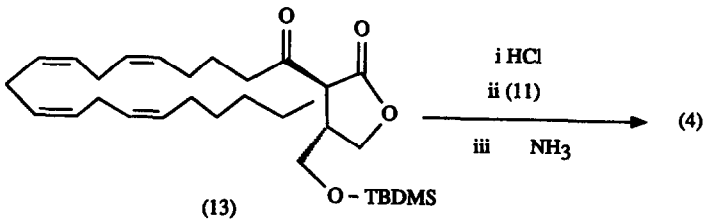
(11)

Unfortunately this compound exhibits no useful activity in PLA_2 inhibition assays, and our attention was directed to the synthesis of the arachidonyl derivative (4) which might better mimic the natural substrate.

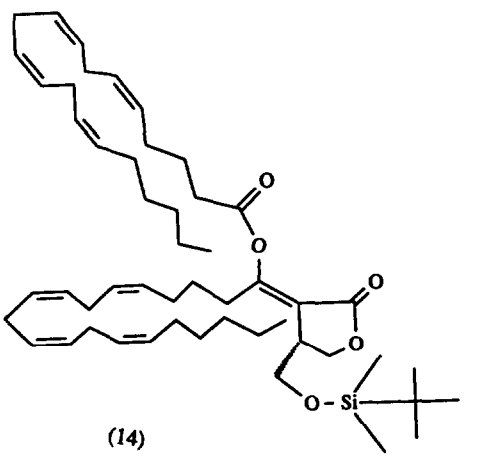
When arachidonyl chloride (12), was used to acylate the lactone (6, R=TBDMS) the ketolactone (13 R=TBDMS) was obtained, together with some of the enol ester (14). The ketolactone was then converted into the target molecule (4) by a similar series of reactions to those used for the butanoyl analogue.



(12)



(13)



(14)

This compound exhibits an IC_{50} value of 64 μM against PLA_2 in a cell free preparation and IC_{50} 44 μM in the macrophage test. These activities are of the same order as those shown by typical site-specific PLA_2 inhibitory drugs.

The ^1H NMR spectrum of the intermediate (13) recorded in deuteriochloroform shows it to exist as the keto isomer, although, the infrared spectrum of the neat compound exhibits a hydroxyl band at 3400-3325 cm^{-1}. A coupling constant of J=8Hz between the signals due to H-3 and H-4 in the ^1H NMR spectrum indicates the keto form to have the *trans* relationship as shown.

It should be noted that the best fit to our substrate model is obtained with the furanone (4) in the (*3S,4R*) configuration as depicted in figure 2, The two next best fits (*E,4R*) and (*3R,4R*), both have the plane of the lactone ring perpendicular to that defined by the lipid side chains. Isomers having (*4S*)-stereochemistry present the poorest fits. Work is now in progress to synthesise inhibitors related to the furanone (4) bearing the optimal absolute configuration.

EXPERIMENTAL

Infrared spectra were recorded as chloroform solutions, unless stated otherwise, on a Perkin Elmer 1310 infrared spectrometer.

^1H and ^{13}C NMR spectra were measured at 270MHz in deuteriochloroform solution with TMS or chloroform as internal standards, using a JEOL GMNGXFT instrument. Mass spectrometry was determined with a VG 7070E spectrometer with a VG database. CI measurements refer to the use of isobutane as the ionized medium, and FAB experiments utilised glycerol-hydrochloric acid. All column chromatography was conducted with simple pressure columns containing 60H Merck No. 7736 silica.

All solvents used were purified by distillation prior to use and, where necessary, dried by standard procedures. Petrol refers to petroleum ether boiling range 60-80°C.

4-^{t}Butyldimethylsilyloxymethyl-1,2,3,4-tetrahydrofuran-2-one (6, R=TBDMS)

A solution of ^{t}butyldimethylsilyl chloride (3.8g) in dry DMF (10cm^3) was added to a solution of 4-hydroxymethyl-1,2,3,4-tetrahydrofuran-2-one (1.4g) and imidazole (3.25g) in the same solvent (30cm^3), protected by a nitrogen atmosphere. After 24h, the solution was diluted with diethyl ether (40cm^3), washed with water (10cm^3) and then with 2M hydrochloric acid (10cm^3). The organic phase was dried and evaporated to give a pale yellow oil, which was purified by column chromatography eluting with 20% ethyl acetate in petrol. Yield 2.7g, 96%; υ_{max} 1760 cm^{-1}; δ_H 4.39 (1H, dd, J_1= 9H$_z$, J_2= 7H$_z$, 5-H$_\alpha$), 4.20 (1H, dd, J_1=9Hz, J_2= 5H$_z$, 5-H$_\beta$), 3.64 (2H, dd, J_1=6Hz, J_2= 4H$_z$, 6-H$_2$), 2.74 (1H, m, 4-H), 2.58 and 2.39 (2x1H, 2xdd, J_1=17H$_z$, J_2=8H$_z$, 3-H$_2$), 0.90 [9H, m, $(CH_3)_3CSi$], 0.08 [6H, s, $(CH_3)_2Si$]; δ_C 177.4 (s, C=O), 70.5 (t, 5-C), 63.2 (t, 6-C), 37.1 (d, 4-C), 30.6 (t, 3-C), 25.7 [q, $(CH_3)_3CSi$], -5.7[q, $(CH_3)_2Si$]; *m/z* (CI) 231 (M+1), 385.. [Found: C, 57.7; H, 10.7 $C_{11}H_{22}O_3Si$ requires: C, 57.7; H, 9.6%]

3-Acetyl-4-^{t}butyldimethylsilyloxymethyl-1,2,3,4-tetrahydrofuran-2-one (7, R=Me)

LDA [from the reaction of ^{n}butyllithium (7.6 cm^3 of a 1.6M solution in hexane) and diisopropylamine 1 cm^3] at -78°C was treated with 4-^{t}butyldimethylsilyloxymethyl-1,2,3,4-tetrahydrofuran-2-one (1g) in tetrahydrofuran (10cm^3), and the mixture was left stirring at -78°C for 1H. It was then added to a solution of acetyl chloride (0.6cm^3) in THF (10cm^3) at -78°C., and after 1.5h, the reaction mixture was allowed to warm to room temperature. Excess reagent was destroyed by the addition of saturated ammonium chloride solution (12cm^3), and the reaction mixture was extracted with ethyl acetate (3x25cm^3). The combined extracts were dried ($MgSO_4$) and evaporated to give a pale brown oil which was chromatographed, eluting with 4% ethyl acetate in petrol, to afford 4-butyl-3-(1-^{t}-butyldimethylsiloxy-3-hydroxy-prop-2-yl)-4-hydroxyoctan-2-one (9) as an almost colourless oil (150mg, 9%): υ_{max} 3600-3300, 1720 cm^{-1}; δ_H 3.97 (2H, m, CH_2OH), 3.69 (1H, dd, J_1=10H$_z$ J_2=5H$_z$, CH$_\alpha$OSi), 3.49 (1H, dd, J_1=10H$_z$, J_2=7H$_z$ CH$_\beta$OSi), 3.06 (1H, brs, OH), 2.09 (1H, m, 3-H), 2.03 (3H, s, CH_3CO), 1.47-1.38 (6H, m, $(CH_2)_2CO$ + CH_2CO), 1.3-1.2 (8H, m, 2x CH_2CH_2), 0.88 [15H, m, 2xCH_3, $(CH_3)_3CSi$], 0.05 [6H, s, $(CH_3)_2Si$]; δ_C 170.9 (s, C=O), 74.1 (s), 66.0 (t), 65.0 (t), 38.9 (t), 38.8 (t), 35.9 (d), 26.0 (q), 25.8(t), 25.7(t), 25.6(d), 23.3(t), 20.8 [q, $(CH_3)_3Si$], 14.0 (q, CH_3), -5.6 [q, $(CH_3)_2Si$]; *m/z* (FAB) 389 $(M+1)^+$.

Subsequent fractions from the column yielded the title compound (6, R=Me) as an almost colourless oil (450mg, 38%): υ_{max} 1765, 1710 cm^{-1}; δ_H 4.34 (1H, dd, J_1=9H$_z$, J_2=7H$_z$ 5-H$_\alpha$), 4.09 (1H, dd, J_1=9H$_z$, J_2=6H$_z$, 5-H$_\beta$), 3.61 (3H, m, 3-H + 6-H$_2$), 3.15 (1H, m, 4-H), 2.40 (3H, s CH_3), 0.83 [9H, m, $(CH_3)_3CSi$], 0.01 and 0 [2x3H, 2xs, $(CH_3)_2Si$]; δ_C 200.2 (s, C=O lactone), 172.2 (s, C=O), 69.0 (t, 5-C), 61.7 (t, 6-C), 55.5 (d, 3-C), 38.9 (d, 4-C), 29.5 (q, CH_3), 25.6 [q, $(CH_3)_3Si$], -5.7 [q, $(CH_3)_2Si$]; *m/z* (CI) 273 $(M+1)^+$

3-Butanoyl-4-^{t}butyldimethylsilyloxymethyl-1,2,3,4-tetrahydrofuran-2-one (7, R=Pr)

LDA [from the reaction of ^{n}butyllithium (6 cm^3 of a 1.6M solution in hexane) and diisopropylamine 1.1 cm^3] at -78°C was treated with 4-^{t}butyldimethylsilyloxymethyl-1,2,3,4-tetrahydrofuran-2-one (1.8g) and the mixture was left stirring at -78°C for 1 h. It was then added to a solution of butanoyl chloride (0.85cm^3) in THF (15cm^3) at -78°C, and after 1.5h, the reaction mixture was allowed to warm to room temperature. Excess reagent was destroyed by the addition of saturated ammonium chloride solution (12cm^3), and the reaction mixture was extracted with ethyl acetate (3x25cm^3). The combined extracts were dried ($MgSO_4$) and evaporated to give a pale brown oil which was chromatographed , eluting with 4% ethyl acetate in petrol, to afford the title compound as an almost colourless oil (1.1g, 47%): υ_{max} 1755, 1720 cm^{-1}; δ_H 4.25 (2H, m, 5-H$_2$), 3.65 (2H, m, 6-H$_2$), 3.25 (1H, m, 3-H), 3.0 (1H, m, 4-H), 2.40 (2H, t, CH_2CH_2CO), 1.65 (2H, m, CH_2CH_2CO), 1.1-0.95 [12H, m, CH_3, $(CH_3)_3CSi$], 0.08 [6H, s, $(CH_3)_2Si$]; δ_C 202.4 (s, C=O lactone), 170.4 (s, C=O), 67.8 (t, 5-C), 62.9 (t, 6-C), 54.8 (d, 3-C), 41.2 (d, 4-C), 36.0 (t, CH_2CO), 31.3 (t, CH_2CH_3), 25.7 [q, $(CH_3)_3Si$], 13.8 (q, CH_3), -5.5 [q, $(CH_3)_2Si$]; *m/z* (CI) 301 $(M+1)^+$.

3-Butanoyl-4-hydroxymethyl-1,2,3,4-tetrahydrofuran-2-one

3-Butanoyl-4-^{t}butyldimethylsilyloxymethyl-1,2,3,4-tetrahydrofuran-2-one (7, R=Pr) (0.95g) in methanol (25cm^3) was treated with 5M hydrochloric acid (0.2cm^3) and after 36h, the solvent was removed to afford a pale yellow oil. This was chromatographed, eluting with 50% ethyl acetate in petrol,to give the title compound as a colourless oil (0.14g, 24%): υ_{max} 3500-3200, 1760, 1720 cm^{-1}; δ_H 4.3 (2H, m, 5-H$_2$), 3.75 (2H, m, 6-H$_2$), 3.50 (1H, brs, OH), 3.15 (1H, m, 3-H), 2.45 (3H, m, 4-H + CH_2CH_2CO), 1.55 (2H, m, CH_2CH_2CO), 0.95 (3H, t, *J*=8H$_z$, CH_3); m/z (CI) 187 $(M+1)^+$.

3-Butanoyl-1,2,3,4-tetrahydro-4-[(O-phosphatidylethananolamino)hydroxy methyl]furan-2-one (5, n=2)

2-Chloro-2-oxa-1,3,2-dioxaphospholane (250mg) in dry benzene (2 cm^3) was added to a solution of 3-butanoyl-4-hydroxymethyl-1,2,3,4-tetrahydrofuran-2-one (100mg) and triethylamine (0.25cm^3) in benzene (15 cm^3) maintained at 0°C. After 3h, the reaction mixture was filtered and the filtrate evaporated to give

3-butanoyl-4-(1,3,2-dioxaphospholanyloxymethyl)-1,2,3,4-tetrahydrofuran-2- one as a viscous oil (210mg). This material was used directly. It was dissolved in dry acetonitrile (15cm^3) and the solution then saturated with ammonia during 2h., after 2h, and a waxy solid which had formed was filtered off (45mg, 27% yield). This was shown to be the title compound: δ_H 4.5-4.1 (2H, 2xdd, J_1= 9H$_z$, J_2= 8H$_z$, 5-H$_\beta$), 3.90 (3H, m, POCH$_2$CH$_2$N$^+$H$_3$), 3.6 (4H, m, POCH$_2$CH$_2$N$^+$H$_3$), and 3.55 (2H, m, 6-H$_2$), 3.4 (1H, m, 3-H), 3.15 (1H, m, 4-H), 2.55 (2H, t, J=6H$_z$, CH$_2$CH$_2$CO), 1.50 (2H, tq, J_1= 7H$_z$, J_2= 6H$_z$, CH$_2$CH$_2$CO), 0.90 (3H, t, J=7H$_z$, CH$_3$); *m/z* (FAB HCl-Glycerol) 307(M-1)$^+$.

3-Arachidonyl-4-^{t}butyldimethylsilyloxymethyl-1,2,3,4-tetrahydrofuran-2-one (13)

LDA [from the reaction of ^{n}butyllithium (2.5 cm^3 of a 1.6M solution in hexane) and diisopropylamine 0.54 cm^3] at -78°C was treated with 4-^{t}butyldimethylsilyloxymethyl-1,2,3,4-tetrahydrofuran-2-one (0.76g) and the mixture was left stirring at -78°C for 1h. It was then added to a solution containing 1.1 molar excess of arachidonyl chloride in THF (10cm^3) at -78°C, and after 15min, the reaction mixture was allowed to warm to room temperature. Excess reagent was destroyed by the addition of saturated ammonium chloride solution (10cm^3), and the reaction mixture was extracted with ethyl acetate (3x15cm^3). The combined extracts were dried ($MgSO_4$) and evaporated to give a pale brown oil which was chromatographed, eluting with 4% ethyl acetate in petrol, to afford the title compound as an almost colourless oil (0.7g, 41%): υ_{max} 1750, 1710, 1640 cm^{-1}; δ_H 5.42-5.35 (8H, m, 4x CH=CH), 4.35 (1H, dd, J_1= 8H$_z$, J_2= 6H$_z$, 5-H$_\alpha$), 4.15 (1H, dd, J_1=8Hz, J_2= 6H$_z$, 5-H$_\beta$), 3.63 (3H, m, 6-H$_2$+ 3-H), 3.13 (1H, m, 4-H), 2.95 and 2.59 (2x1H, 2xdt, J=19 and 9H$_z$, CH$_2$CH$_2$CO), 2.9-2.7 (6H, m, 3x =C-CH$_2$-C=), 2.06 (4H, m, 2x =C-CH$_2$), 1.67 (2H, q, J=6H$_z$, CH$_2$CH$_2$CO), 1.41-1.24 (6H, m, 3xCH$_2$), 0.87 [12H, m, CH$_3$, (CH$_3$)$_3$CSi], 0.05 and 0.04 (2x3H, 2xs, 2xCH$_3$Si); δ_C 202.4 (s, C=O lactone), 177.4 (s, C=O), 130.5 , 128.9, 128.8, 128.5, 128.2, 128.1, 127.8, 127.5 (8xd, 8x -CH=), 69.2 (t, 5-C), 61.9 (t, 6-C), 54.8 (d, 3-C), 41.9 (t, CH$_2$CO), 39.3 (d, 4-C), 31.5, 29.3, 27.2, 26.3 (4xt, 4xCH$_2$), 27.7 [q, (CH$_3$)$_3$Si], 25.6, 23.1, 22.6 (3xt, 5xCH$_2$), 14.1 (q, CH$_3$), -5.6 [q, (CH$_3$)$_2$Si]; *m/z* (CI) 517 (M+1)$^+$.

Later fractions from the column gave a second oil which was rechromatographed to give the enol-ether (14) as a yellow gum (0.21, 8%): λ_{max}236 nm; υ_{max}1760,1715,1610 cm^{-1}; δ_H 5.41-5.34 (16H, m, 8x CH=CH), 4.38, 4.12 (2x1H, 2xdd, J_1=9H$_z$, J_2=8H$_z$, 5-H$_\beta$), 3.63 (2H, dd, J_1 =5H$_z$, J_2=2H$_z$, 6-H$_2$), 3.16 (1H, m, 4-H) 2.99 and 2.58 (2x1H, m, CH$_2$COO), 2.66 (12H, m, 6x =C-CH$_2$-C=), 2.06 (8H, m, 4x C=CH$_2$), 1.68 (2H, m, COCH$_2$CH$_2$), 1.38 [2H, m, CH$_2$CH$_2$C(O)=)], 1.29 (12H, m, 6xCH$_2$), 1.15 [2H, m, CH$_2$CH$_2$C(O)=], 0.87 (15H, m, 2xCH$_3$, (CH$_3$)$_3$CSi), 0.04, 0.03 (2x3H, 2xs, 2xCH$_3$Si).

3-Arachidonyl-4-hydroxymethyl-1,2,3,4-tetrahydrofuran-2-one

The silyloxy compound (7, R=arachidonyl)(48mg) in methanol (7.5cm^3) was treated with 5M hydrochloric acid (0.2cm^3) and left to stir overnight. The solvents were then removed under reduced pressure and the residue chromatographed, eluting with 20% ethyl acetate in petrol, to afford a pale yellow oil (30mg, 80%); υ_{max} 3500-3200, 1765, 1715, 1635 cm^{-1}; δ_H 5.45-5.29 (8H, m, 8x CH=), 4.46 (1H, dd, J_1= 8H$_z$, J_2= 6H$_z$, 5-H$_\alpha$), 4.15 (1H, dd, J_1=8Hz, J_2= 6H$_z$, 5-H$_\beta$), 3.70 (3H, m, 6-H$_2$+ 3-H), 3.43 (1H, brs, OH), 3.20 (1H, m, 4-H), 3.02 and 2.65 (2x1H, 2xdt, J=19 and 9H$_z$, CH$_2$CH$_2$CO), 2.83 (6H, m, 3x =C-CH$_2$-CH=), 2.18-2.02 (4H, m, 2x =C-CH$_2$), 1.72 (2H, m, CH$_2$CH$_2$CO), 1.41-1.25 (6H, m, 3xCH$_2$), 0.89 (3H, t, J=7H$_z$, CH$_3$); δ_C 202.4 (s, C=O lactone), 173.4 (s, C=O), 130.5 , 129.0, 128.6, 128.1, 127.9, 127.5 (6xd, 8x -CH=), 64.7 (t, 5-C), 61.7(t, 6-C), 54.9 (d, 3-C), 41.8 (t, CH$_2$CO), 39.0 (d, 4-C), 36.2, 31.5, 29.3, 27.2, 26.3, 25.6 (6xt, 8x CH$_2$), 14.1 (q, CH$_3$); *m/z* (CI) 403 (M+1)$^+$, 385.

3-Arachidonyl-1,2,3,4-tetrahydro-4-[(O-phosphatidylethananolamino)hydroxym hyl]furan-2-one (4)

2-Chloro-2-oxa-1,3,2-dioxaphospholane (114mg) in dry benzene (1 cm^3) was added to a solution of 3-arachidonyl-4-hydroxymethyl-1,2,3,4-tetrahydrofuran-2-one (320mg) and triethylamine (0.1) in benzene (15 cm^3) maintained at 0°C. After 2.5h, the reaction mixture was filtered and the filtrate evaporated to give 3-arachidonyl-4-(1,3,2-dioxaphospholanyloxymethyl)-1,2,3,4-tetrahydrofuran -2-one as a brown oil (450mg). This material was diluted with dry acetonitrile (15cm^3) and saturated with ammonia in the course of 2h. The reaction mixture was left for a further 2h, and then the waxy solid which had formed was filtered off (78mg, 19% yield). This corresponds to the title compound: υ_{max} 3250, 1760, 1700, 1615, 1200-1000 cm^{-1}; δ_H 5.45 -5.39(8H, m, 8x CH=), 4.50 (1H, dd, J_1= 9H$_z$, J_2= 8H$_z$, 5-H$_\alpha$), 4.18 (1H, m, 3-H), 4.13 (1H, dd, J_1=9Hz, J_2= 6H$_z$, 5-H$_\beta$), 4.00-3.06 (7H, m, 4-H + 6-H$_2$ + POCH$_2$CH$_2$N$^+$H$_3$), 3.04 and 2.61 (2x1H, 2xdt, J=19 and 9H$_z$, CH$_2$CH$_2$CO), 2.71 (6H, m, 3x=C-CH$_2$-C=), 2.10 (4H, m, 2x =C-CH$_2$-), 1.75 (2H, q, J=6H$_z$, CH$_2$CH$_2$CO), 1.29 (6H, m, 3xCH$_2$), 0.90 (3H, t, J=7H$_z$, CH$_3$); *m/z* (FAB HCl-Glycerol) 525 (M-1)$^+$, 125.

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