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Synthesis of a potential enzyme-specific inhibitor of squalene synthase

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Abstract. A multi-step synthesis of phosphinylphosphonate analogue **12** of the proposed farnesyl-farnesyl-enzyme (FFE) intermediate in the enzymatic conversion of farnesyl pyrophosphate to squalene is described. In addition, evidence is presented of the inhibitory effect of **12** on squalene synthase.

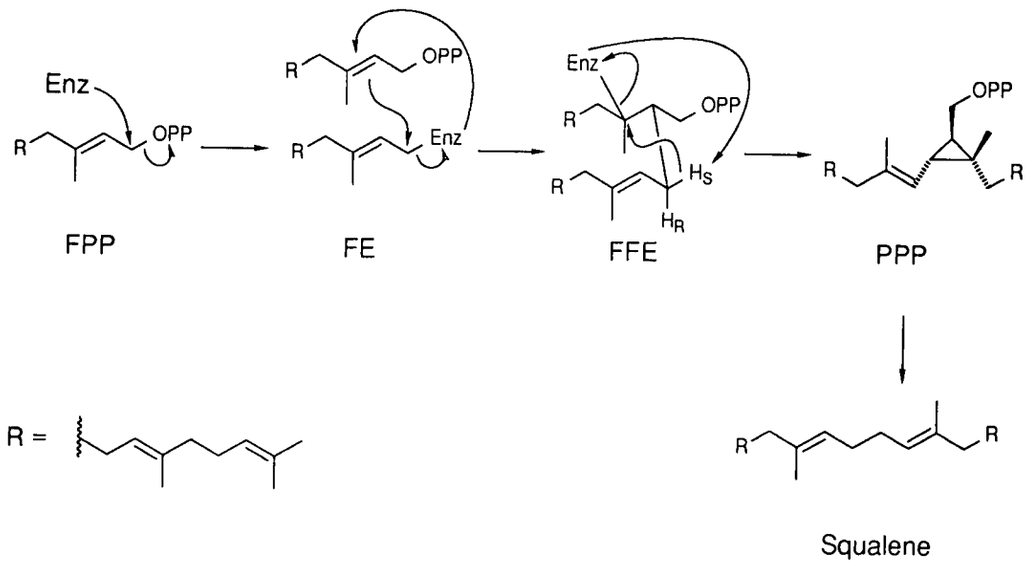
Introduction [a]

Squalene synthase (SS) is the first pathway-specific enzyme in the biosynthesis of cholesterol and catalyses, as depicted in Scheme 1, the head-to-head condensation of two molecules of farnesyl pyrophosphate (FPP) to form squalene. The precise mechanism of the enzymatic conversion is not fully understood, but it has been implied that a ping-pong reaction[1] is involved in the transformation of FPP into presqualene pyrophosphate (PPP). The first step comprises replacement of the pyrophosphate moiety of one FPP by a nucleophilic group of SS giving a farnesyl-enzyme intermediate (FE). The C2-C3 double bond of a second FPP is then activated by another nucleophilic group of SS, followed by attack on the C1′ of FE. The resulting compound (FFE) is cyclized to PPP by stereospecific abstraction of the pro-S proton at C1′, followed by nucleophilic attack on C3. Up to now, different classes of compounds have been proposed to inhibit the biosynthesis of cholesterol; for example, FPP analogues are a major class of inhibitors[3a-e]. However, this type of compounds lack specificity and are also potential inhibitors of other FPP-consuming enzymes (*e.g.*, geranyl-geranyl pyrophosphate synthase or protein-farnesyl transferase). In order to increase enzyme specificity, analogues of PPP have been synthesized[4]. Moreover, cationic mimics of FFE, based on the mechanism in Scheme 1, have recently been described by *Steiger*[5] and *Oehlschlager*[6].
As part of a programme[3d] directed towards the design and synthesis of inhibitors of the cholesterol biosynthesis, we here describe the preparation of a presumed enzyme-specific analogue of FFE, containing a phosphinylphosphonate function as a replacement for the pyrophosphate (*i.e.*, compound **12**).

Results and discussion

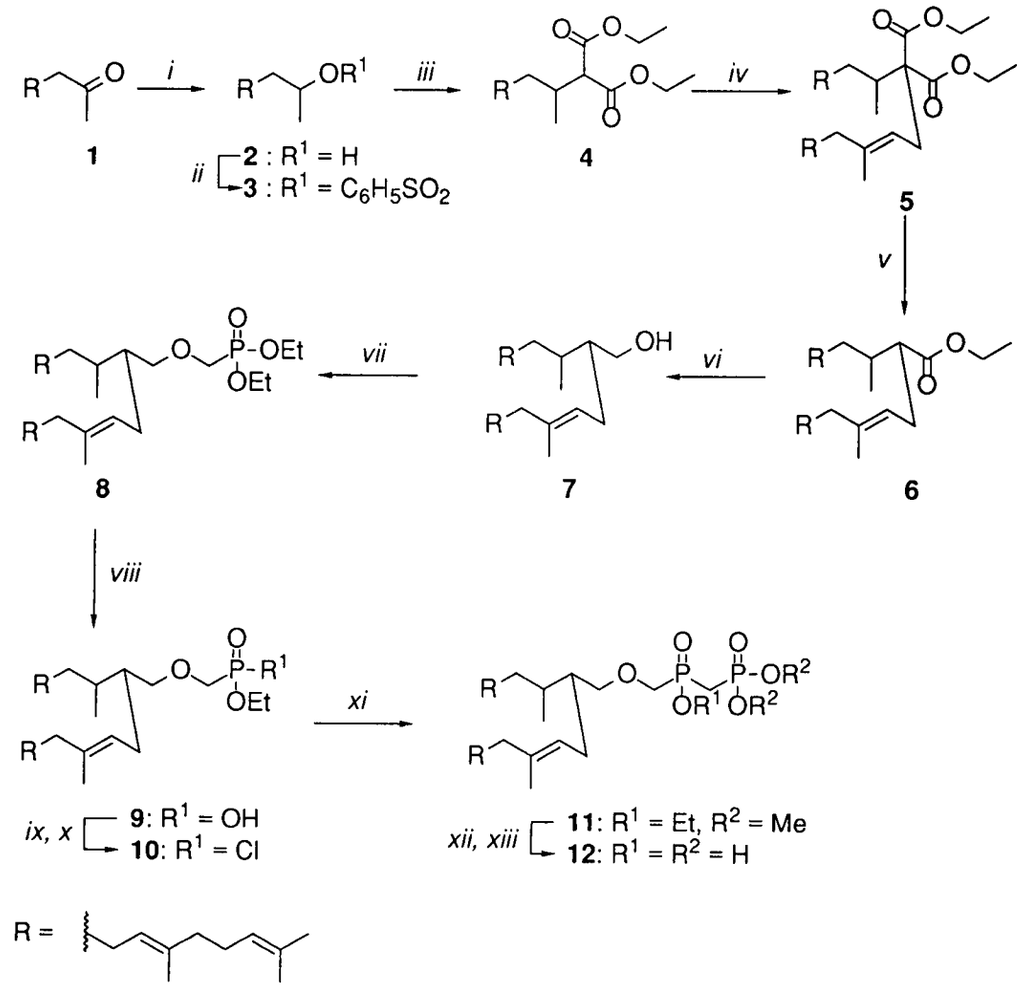
The synthetic route to the target compound **12** comprises, as outlined in Scheme 2, the following two distinct stages. The first stage deals with the build-up of the carbon skeleton and commences with reduction of geranylacetone (**1**) in ether to give, after purification by silica-gel chromatography, racemic (*E*)-6,10-dimethylundeca-5,9-dien-2-ol (**2**) in excellent yield. An attempt to separate the individual isomers of alcohol **2** by derivatization with the chiral auxiliaries menthoxyacetyl chloride or camphorsulfonyl chloride was abortive. Sulfonylation of **2** with benzenesulfonyl chloride in pyridine produced **3**, which was used for the alkylation of the sodium enolate of diethyl malonate in tetrahydrofuran at 68°C, to give **4**. In a similar way, alkylation of **4** in tetrahydrofuran with farnesyl bromide in the presence of sodium hydride afforded homogenous **5**. Decarboxylation[7] of **5** was effected by the action of excess sodium cyanide in refluxing DMSO, to yield the mono-ester derivative **6** as a mixture of diastereoisomers. ^{1}H- and ^{13}C-NMR spectroscopic analysis of **6** revealed that the decarboxylation process had proceeded without isomerisation of the double bonds[7,8]. Reduction of **6** resulted in the formation of target alcohol **7** which was isolated as an oil in 26% overall yield over the six steps.
The phosphinylphosphonate functionality was introduced by the following sequence of reactions. The alkoxide of **7**, generated *in situ* by the action of butyllithium in tetrahydrofuran at −78°C, was treated with (diethoxy phosphinyl)methyl triflate[9], to give the protected phosphonate **8** in satisfactory yield (δ_P 21.8 ppm). Hydrolysis of diester **8** to its corresponding mono-ester by refluxing for 16 h in a mixture of ethanol and 1N potassium hydroxide gave **9**,

[a] **Abbreviations and Chem. Abstr. names**: camphorsulfonyl chloride = (1*S*)-7,7-dimethyl-2-oxobicyclo[2.2.1]bicycloheptane-1-methanesulfonyl chloride; *sym*-collidine = 2,4,6-trimethylpyridine; DMF = *N,N*-dimethylformamide; DMSO = dimethylsulfoxide; farnesyl = (*E,E*)-3,7,11-trimethyldodeca-2,6,10-trienyl; FE = farnesyl-enzyme; FFE = farnesyl-farnesyl-enzyme; FPP = farnesyl pyrophosphate; geranyl = (*E*)-3,7-dimethylocta-2,6-dienyl; geranylacetone = (*E*)-6,10-dimethylundeca-5,9-dien-2-on (**1**); menthoxy = [2-methyl-5-(1-methylethyl)cyclohexyl]oxy; PPP = presqualene pyrophosphate; squalene = (all-*E*)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene; SS = squalene synthase; Tf = triflate = trifluormethanesulfonate; THF = tetrahydrofuran.

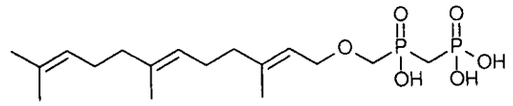


Scheme 1.

as gauged by ^{31}P-NMR spectroscopy (δ_P 17.4 ppm). Introduction of the second phosphonate was achieved by a three-step procedure[10]. Thus, silylation of crude **9** with *N,N*-diethyl(trimethylsilyl)amine in dichloromethane was followed by reaction with oxalyl chloride in dichloromethane/DMF. The resulting crude phosphonic



Scheme 2. Key: i: $LiAlH_4$, ether, reflux (97%); ii: benzenesulfonyl chloride (74%); iii: diethyl malonate, NaH (79%); iv: NaH, farnesyl bromide (82%); v: NaCN, DMSO, reflux, (73%); vi: $LiAlH_4$, ether (76%); vii: BuLi, $TfOCH_2P(O)(OEt)_2$ (84%); viii: KOH, ethanol, H_2O, reflux (96%); ix: Me_3SiNEt_2, CH_2Cl_2; x: oxalyl chloride, DMF, CH_2Cl_2, 0°C; xi: $LiCH_2P(O)(OMe)_2$, −78°C (59%, 3 steps); xii: Me_3SiBr, sym-collidine, CH_2Cl_2; xiii: NH_3, H_2O, dioxane (57%).



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chloride **10** was added to a solution of dimethyl (lithiomethyl)phosphonate[11] in tetrahydrofuran at −78°C to give **11** as a colourless oil. The appearance of two doublets at δ_P 23.08 and 41.36 ($J_{P\text{-}P}$ 3.66 Hz) in the ^{31}P-NMR spectrum firmly established the presence of the protected phosphinylphosphonate moiety. Two-step hydrolysis of triester **11** was accomplished by reaction with trimethylsilyl bromide in the presence of *sym*-collidine, followed by treatment with aqueous N ammonia in methanol, to give crude **12**. Purification was effected by CHP20P column chromatography, using a linear gradient of acetonitrile in water as the eluent, to give target compound **12** (NH_4^+ form) in 57% yield. The presence of two characteristic doublets at δ_P 17.7 and 32.2 ($J_{P\text{-}P}$ 17.1 Hz) was in complete accordance with the presence of the phosphinylphosphonate function in **12**. In addition, the mass spectrum of **12** (H^+ form) recorded in the positive or negative electrospray mode, showed peaks at m/z 599 $[M\text{-}H]^-$ and 618 $[M + NH_4]^+$, respectively, thus confirming the molecular weight of 600.

The inhibitory action of the thus obtained diastereoisomeric mixture of **12** on SS was tested in rat liver membrane preparations[12]. For the sake of comparison, the known[3c] FPP analogue **13**, having the same pyrophosphate modification as **12**, was tested under the same conditions.

The results presented in Figure 1 show that compounds **12** and **13** are both inhibitors of SS with IC_{50} values of 38.4 ± 19.9 μM and 2.4 ± 0.3 μM, respectively. It is evident that the diastereoisomeric mixture **12** is less active than **13**, however, it may not be excluded that only one of the individual stereoisomers of **12** is responsible for the inhibitory effect on SS.

In conclusion, we have presented a high-yielding synthesis of an inhibitor of SS, in which the individual steps are easy to perform. In order to attain better inhibition of SS and to get more insight in the stereochemical requirements of this kind of inhibitor, the separate isomers of **12** have to be synthesized and tested in different FPP-consuming enzyme assays (*i.e.*, SS and protein-farnesyl transferase).

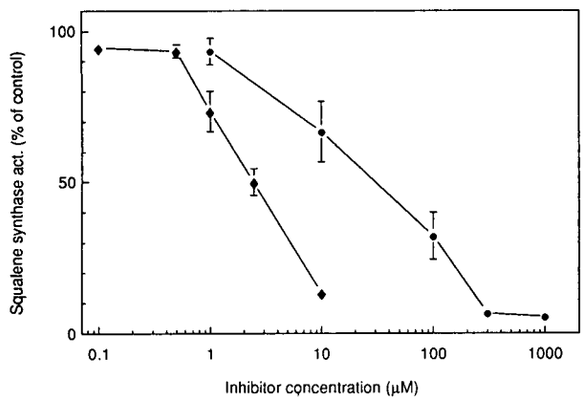

Figure 1. Inhibition of SS activity by compounds 12 and 13. SS assay was performed in the presence of the indicated concentrations of compound 12 (●) or compound 13 (◆). Values are the mean of three to four separately performed experiments (each experiment consisting of duplicate determinations at each concentration) and are expressed as a percentage of control values (2.15 ± 0.29 mmol squalene formed / min / mg of protein); bars depict standard error of mean.

Experimental

General procedures

(*E,E*)-Farnesol and (*E*)-geranylacetone were purchased from Aldrich and distilled. Toluene, dichloromethane and ether were dried by refluxing with P_2O_5 for 2 h and then distilled. Toluene and ether were stored over sodium wire. Dichloromethane was stored over molecular sieves (0.4 nm). THF and acetonitrile were dried by refluxing with CaH_2 for 16 h, distilled and stored over molecular sieves (0.4 nm). THF and ether were redistilled from $LiAlH_4$ directly before use. All reactions were carried out under a blanket of argon, unless stated otherwise. TLC analysis was performed on silica-gel (Schleicher & Schull, F 1500 LS 254). Compounds were visualised by spraying the TLC plates with $KMnO_4$ (1%) in aqueous Na_2CO_3 (2%). Column chromatography was performed on Merck Kieselgel (230–400 mesh ASTM). Evaporations were carried out below 40°C under reduced pressure (15 mmHg). ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were measured at 199.99, 50.1 and 80.7 MHz, respectively, using a JEOL JNM-FX 200 spectrometer on line with a JEC 980 B computer. ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded using a Bruker WM-300 spectrometer operating at 300, 75 and 121 MHz, respectively. ^{1}H and ^{13}C chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard and ^{31}P chemical shifts are given in ppm (δ) relative to 85% H_3PO_4 as external standard.

(E)-6,10-Dimethylundeca-5,9-dien-2-ol (**2**)

To a suspension of $LiAlH_4$ (133 mg, 3.5 mmol) in ether (5 ml) was added a solution of geranylacetone (**1**) (388 mg, 2 mmol) in ether (5 ml). After refluxing for 10 min TLC analysis (light-petroleum/ether, 95/5, v/v) showed complete disappearance of the starting compound. The reaction mixture was cooled to 0°C and dry ethyl acetate (4 ml) was added dropwise, followed by water (4 ml) and 15% NaOH (4 ml). Stirring was continued at 0°C for 1 h. The organic layer was separated and the remaining salts were washed with ether. The combined organic layers were washed with water, dried over $MgSO_4$ and concentrated. The product was purified by silica-gel column chromatography (elution: light-petroleum/ether, 1/0 → 9/1, v/v) to afford 97% of **2** as a colourless oil. ^{13}C{^{1}H}-NMR ($CDCl_3$) δ: 15.7, 17.4 (C12, C13); 23.2 (C1); 24.2, 26.4 (C4, C8); 25.5 (C11); 38.9, 39.5 (C3, C7); 67.4 (C2); 123.8, 124.1 (C5, C9); 131.0, 135.1 (C6, C10). ^{1}H-NMR ($CDCl_3$) δ: 1.19 (d, 3 H, H1, $J_{1,2}$ 6.17 Hz); 1.40–1.55 (m, 2 H, H2); 1.60, 1.63, 1.68 (3 s, 9 H, H11, H12, H13); 1.85–2.09 (m, 6 H, H4, H7, H8); 3.71 (sex, 1 H, H2); 5.01–5.15 (m, 2 H, H5, H9). Anal. calcd. for $C_{13}H_{24}O$ (196.34): C 79.53, H 12.32; found: C 79.50, H 12.25%.

(E)-1,5,9-Trimethylundeca-4,8-dienyl benzenesulfonate (**3**)

To a cooled (0°C) solution of compound **2** (196 mg, 1 mmol) in dry pyridine (2 ml) was added benzenesulfonyl chloride (160 μl, 1.25 mmol) in dry pyridine (2 ml). After stirring for 16 h at room temperature the reaction mixture was diluted with water and concentrated under reduced pressure. The remainder was dissolved in dichloromethane and washed with water, 10% $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, concentrated and the residue was applied to a silica-gel column, which was eluted with a gradient of light-petroleum/ether (1/0 → 1/1, v/v). Concentration of the appropriate fractions gave **3** (yield 74%) as a colourless oil. ^{13}C{^{1}H}-NMR ($CDCl_3$) δ: 15.8, 17.5 (5-CH_3, 9-CH_3); 20.7 (1-CH_3); 23.3, 26.5 (C3, C7); 25.5 (C10); 36.5, 39.5 (C2, C6); 80.5 (C1); 122.4, 124.0 (C4, C8); 127.5, 129.0, 133.3 (3 C_{arom}); 131.2, 136.1 (C5, C9); 137.5 ($C_{q\text{-}arom}$). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (d, 3 H, 1-CH_3, $J_{1,2}$ 6.19 Hz); 1.51, 1.59, 1.68 (3 s, 9 H, H10, 5-CH_3, 9-CH_3); 1.54–1.64 (m, 2 H, H2); 1.71–2.01 (m, 6 H, H3, H6, H7); 4.65 (sex, 1 H, H1); 4.90–4.96 and 5.01–5.06 (2 m, 2 H, H4, H8); 7.26–7.68 and 7.90–7.96 (2 m, 5 H, H_{arom}). Anal. calcd. for $C_{19}H_{28}O_3S$ (336.50): C 67.82, H 8.39; found C 67.76, H 8.35%.

Diethyl [(E)-1,5,9-trimethyldeca-4,8-dienyl]malonate (**4**)

To a stirred suspension of NaH (60 mg as 80% in oil, 2 mmol) in THF (4 ml) was added diethyl malonate (304 μl, 2 mmol) over a period of 5 min. After stirring for 30 min at room temperature,

compound **3** (168 mg, 0.5 mmol) in THF (2 ml) was added and stirring was continued for 4 h at reflux temperature. When TLC analysis (light-petroleum/ether, 4/1, v/v) showed complete disappearance of **3** the reaction mixture was diluted with ether and washed with saturated NH_4Cl and water. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (elution: light-petroleum/ether, 1/0 → 3/1, v/v) to give **4** as a colourless oil; yield 79%. $^{13}C\{^1H\}$-NMR ($CDCl_3$) δ: 13.8 (OCH_2CH_3); 15.6, 17.3 (C12, C13); 16.5 (C11); 25.3 (C10); 24.9, 26.4 (C3, C7); 32.7 (C1); 34.0 (C2); 39.4 (C6); 57.2 (CH (malonate)); 60.6 (OCH_2CH_3); 123.5, 124.0 (C4, C8); 130.8, 134.9 (C5, C9); 168.3, 168.5 (CO (malonate)). ^1H-NMR ($CDCl_3$) δ: 1.00 (d, 3 H, H3', $J_{3,3'}$ 6.7 Hz); 1.27 (t, 6 H, OCH_2CH_3); 1.34–1.55 (m, 2 H, H4); 1.60 (s, 6 H, H7', H11'); 1.68 (s, 3 H, H12); 1.98–2.05 (m, 6 H, H5, H8, H9); 2.19–2.33 (m, 1 H, H3); 3.25 (d, 1 H, H2, $J_{1,2}$ 7.96 Hz); 4.37 (q, 4 H, OCH_2CH_3); 5.05–5.09 (m, 2 H, H6, H10). Anal. calcd. for $C_{20}H_{34}O_4$ (338.49): C 70.97, H 10.13; found C 70.99, H 10.11%.

*Diethyl [(*E*)-1,5,9-trimethyldeca-4,8-dienyl][(*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl]malonate* (**5**)

To a stirred suspension of NaH (9 mg as 80% in oil, 0.3 mmol) in THF (1 ml) was added a solution of **4** (100 mg, 0.3 mmol) in THF (1 ml). After stirring for 30 min at room temperature the mixture was treated with a solution of farnesyl bromide (143 mg, 0.5 mmol) in THF (1 ml). After refluxing for for 4 h the reaction mixture was diluted with ether and washed with saturated NH_4Cl and water. The organic layer was dried ($MgSO_4$) and concentrated. Silica-gel column chromatography (elution: light-petroleum/ether, 1/0 → 9/1, v/v) of the crude mixture afforded **5** in a yield of 82%. $^{13}C\{^1H\}$-NMR ($CDCl_3$) δ: 13.9 (OCH_2CH_3); 14.5, 15.7, 15.9, 17.3 (C13, C14, C15, C11', C12', C13'); 25.4 (C12, C10'); 26.3, 26.5 (C5, C9, C3', C7'); 32.0 (C1); 32.9 (C2'); 36.3 (C1'); 39.5 (C8, C6'); 39.7 (C4); 60.3 (OCH_2CH_3); 61.8 (C_q (malonate)); 118.7 (C2); 123.8, 124.0, 124.2 (C6, C10, C4', C8'); 130.7 (C11, C9'); 134.6, 134.7 (C7, C5'); 137.4 (C3); 170.6, 170.8 (CO (malonate)); ^1H-NMR ($CDCl_3$) δ: 1.25 (t, 6 H, OCH_2CH_3); 1.60 (s, 15 H, H13, H14, H15, H12', H13'); 1.68 (s, 6 H, H12, H10'); 1.90–2.18 (m, 16 H, H4, H5, H8, H9, H2', H3', H6', H7'); 2.63 (d, 2 H, H1, $J_{1,2}$ 7.2 Hz); 4.16 (dq, 4 H, OCH_2CH_3); 5.01–5.08 (m, 5 H, H2, H6, H10, H4', H8'). Anal. calcd. for $C_{35}H_{58}O_4$ (542.85): C 77.44, H 10.77; found C 77.40, H 10.71%.

*Ethyl (*E,E*)-2-[(*E*)-1,5,9-trimethyldeca-4,8-dienyl]-5,9,13-trimethyltetradeca-4,8,12-trienoate* (**6**)

To a solution of **5** (1 g, 1.9 mmol) in DMSO (10 ml) was added NaCN (360 mg, 7.4 mmol) and the mixture was heated for 5 h at 180°C. As TLC analysis (light-petroleum/ether, 95/5, v/v) showed complete conversion of the starting compound, the reaction mixture was cooled to room temperature and brine was added. This mixture was extracted three times with light-petroleum. The combined organic layers were washed with water, dried over $MgSO_4$ and concentrated. The crude compound was purified by silica-gel column chromatography (elution: light-petroleum/ether, 1/0 → 9/1, v/v) to give **6** (73% yield) as an oil. $^{13}C\{^1H\}$-NMR ($CDCl_3$) δ: 14.2 (OCH_2CH_3); 15.7, 15.8, 15.9, 17.3, (C13, C14, C15, C11', C12', C13'); 25.5 (C12, C10'); 26.3, 26.5 (C5, C9, C3', C7'); 32.0 (C1); 32.9 (C2'); 36.3 (C1'); 39.5 (C8, C6'); 39.7 (C4); 60.3 (OCH_2CH_3); 61.8 (C_q (malonate)); 118.7 (C2); 123.8, 124.0, 124.2 (C6, C10, C4', C8'); 130.7 (C11, C9'); 134.6, 134.7 (C7, C5'); 137.4 (C3); 174.8 (CO (malonate)); ^1H-NMR ($CDCl_3$) δ: 0.85–0.95 (m, 4 H, H1', H11'); 1.11–1.20 (m, 2 H, H2'); 1.24 (t, 3 H, OCH_2CH_3); 1.60 (s, 15 H, H13, H14, H15, H12', H13'); 1.68 (s, 6 H, H12, H10'); 1.91–2.11 (m, 14 H, H4, H5, H8, H9, H3', H6', H7'); 2.17–2.26 (m, 3 H, H2, H3); 4.10 (q, 2 H, OCH_2CH_3); 5.02–5.14 (m, 5 H, H4, H8, H12, H4', H8'). Anal. calcd. for $C_{32}H_{54}O_2$ (470.78): C 81.64, H 11.56; found C 81.58, H 11.50%.

*(*E,E*)-2-[(*E*)-1,5,9-Trimethyldeca-4,8-dienyl]-5,9,13-trimethyltetradeca-4,8,12-trien-1-ol* (**7**)

To a suspension of $LiAlH_4$ (133 mg, 3.5 mmol) in ether (5 ml) was added a solution of **6** (655 mg, 1.4 mmol) in ether (5 ml). After refluxing for 10 min TLC analysis (light-petroleum/ether, 95/5, v/v) showed complete disappearance of the starting compound. The reaction mixture was cooled to 0°C and dry ethyl acetate (4 ml) was added dropwise, followed by water (4 ml) and 15% NaOH (4 ml). Stirring was continued at 0°C for 1 h. The organic layer was separated and the remaining salts were washed with ether (2 × 5 ml). The combined organic layers were washed with water, dried over $MgSO_4$ and concentrated. The product was purified by silica-gel column chromatography (elution: light-petroleum/ether, 1/0 → 9/1, v/v) to afford 76% of **7** as a colourless oil. $^{13}C\{^1H\}$-NMR ($CDCl_3$) δ: 15.5, 15.8, 15.9, 16.1, 17.5 (C13, C14, C15, C11', C12', C13'); 25.5 (C14, C10'); 26.4 (C7, C11, C3', C7'); 31.8 (C3); 32.5, 32.9 (C1'); 34.3 (C2'); 39.6, 39.7 (C6, C10, C6'); 46.0 (C2); 63.5, 64.1 (C1); 123–125 (C4, C8, C12, C4', C8'); 131.0, 134.8, 135.8 (C5, C9, C13, C5', C9'); ^1H-NMR ($CDCl_3$ δ: 0.88 (2 d, 3 H, H11'); 1.16–1.26 (m, 2 H, H2, H1'); 1.60 (s, 15 H, H15, H16, H17, H12', H13'); 1.68 (s, 6 H, H14, H10'); 1.92–2.11 (m, 18 H, H3, H6, H7, H10, H11, H2', H3', H6', H7'); 3.56 (d(b), 2 H, H1); 5.01–5.16 (5 H, H4, H8, H12, H4', H8'). Anal. calcd. for $C_{30}H_{52}O$ (428.75): C 84.04, H 12.23; found C 84.09, H 12.19%.

*Diethyl [({(*E,E*)-2-[(*E*)-1,5,9-trimethyldeca-4,8-dienyl]-5,9,13-trimethyltetradeca-4,8,12--trienyl}oxy)methyl]phosphonate* (**8**)

To a cooled (−78°C) and stirred solution of **7** (429 mg, 1 mmol) in THF (2 ml) under argon was added BuLi (625 μl as 1.6 M in hexane, 1 mmol) over 15 min. The reaction was stirred for 40 min at −78°C, when (diethoxy phosphinyl)methyl triflate (450 mg, 1.5 mmol) in THF (2 ml) was added. After 30 min at −78°C, the reaction was allowed to warm to 0°C in 2 h. The reaction was quenched with saturated NH_4Cl and partitioned between ether and water. The ether layer was washed with brine, dried ($MgSO_4$) and evaporated. The crude product was purified by silica-gel column chromatography (elution: light-petroleum/ether, 1/0 → 3/2, v/v) to give homogenous **8** in a yield of 84%. $^{13}C\{^1H\}$-NMR ($CDCl_3$) δ: 15.1 (OCH_2CH_3); 16.0, 16.1, 17.2 (C15, C16, C17, C11', C12', C13'); 25.2 (C14, C10'); 26.4 (C7, C11, C3', C7'); 31.7 (C3); 32.2, 32.6 (C1'); 34.0 (C2'); 39.4 (C6, C10, C6'); 43.1 (C2); 61.7, 61.8 (OCH_2CH_3); 64.8 (d, OCH_2P, $J_{C\text{-}P}$ 155.6 Hz); 73.8, 74.0 (2 d, C1); 123–125 (C4, C8, C12, C4', C8'); 130.5, 134.3, 135.3 (C5, C9, C13, C5', C9'); ^1H-NMR ($CDCl_3$) δ: 0.85–0.88 (m, 4 H, H1', H11'); 1.34 (t, 6 H, OCH_2CH_3); 1.60 (s, 15 H, H15, H16, H17, H12', H13'); 1.68 (s, 6 H, H14, H10'); 1.85–2.10 (m, 18 H, H3, H6, H7, H10, H11, H2', H3', H6', H7'); 3.39–3.48 (m, 2 H, H1); 3.73 (d, 2 H, OCH_2P, J 8.5 Hz); 4.16 (dq, 4 H, OCH_2CH_3); 5.01–5.12 (m, 5 H, H4, H8, H12, H4', H8'); $^{31}P\{^1H\}$-NMR ($CDCl_3$) δ: 21.8; Anal. calcd. for $C_{35}H_{63}O_4P$ (578.86): C 72.62, H 10.97; found C 72.68, H 10.95%.

*Ethyl [({(*E,E*)-2-[(*E*)-1,5,9-trimethyldeca-4,8-dienyl]-5,9,13-trimethyltetradeca-4,8,12--trienyl}oxy)methyl]phosphonate* (**9**)

To a solution of **8** (372 mg, 0.64 mmol) in ethanol (3.2 ml) was added 1N KOH (3.2 ml), and the reaction was refluxed for 16 h. After cooling to room temperature, the ethanol was evaporated, the aqueous residue was stirred with dichloromethane and acidified with 10% HCl. The organic layer was washed with water and brine, dried ($MgSO_4$) and concentrated to provide 96% of **9**. This was used without further purification in the next step. $^{31}P\{^1H\}$-NMR ($CDCl_3$) δ: 17.4.

*Dimethyl ({ethoxy [({(*E,E*)-2-[(*E*)-1,5,9-trimethyldeca-4,8-dienyl]-5,9,13-trimethyltetradeca-4,8,12-trienyl}oxy)methyl]phosphinyl}methyl)phosphonate* (**11**)

To a stirred solution of monoester **9** (339 mg, 0.62 mmol) in CH_2Cl_2 under argon was added *N,N*-diethyl(trimethylsilyl)amine (220 μl, 1.16 mmol). The reaction was stirred for $1\frac{1}{2}$ h at room temperature, the solvent was evaporated and the residue was dissolved in toluene, concentrated and then pumped at high vacuum. The remainder was dissolved in CH_2Cl_2 (2 ml) containing one drop of DMF under argon at 0°C, and oxalyl chloride (107 μl, 1.23 mmol) was added dropwise over a period of 10 min. After 45 min at 0°C and 45 min at room temperature the solution was concentrated and the residue was twice dissolved in toluene and concentrated to give crude **10**, which was used directly in the next step.

To a solution of dimethyl methylphosphonate (152 μl, 1.4 mmol) in THF (4 ml) at −78°C under argon was added BuLi (0.85 ml as 1.6 M in hexane, 1.36 mmol) over 5 min. After 40 min, the acid chloride **10** prepared above was added in THF (2 ml) over 10 min. The reaction was stirred for 1 h at −78°C when it was quenched with saturated NH_4Cl and diluted with ether. The aqueous layer was made acidic with 10% HCl and the organic layer was separated and washed with brine. The aqueous layer was re-extracted with CH_2Cl_2, and the CH_2Cl_2 was washed with brine. The combined organic layers were dried ($MgSO_4$) and concentrated. The crude product was applied to a silica-gel column which was eluted with a gradient of

CH_2Cl_2/MeOH (1/0 → 9/1, v/v) to give **11** as a colourless oil; yield 59%. $^{13}C\{^1H\}$-NMR ($CDCl_3$) δ: 15.8 (OCH_2CH_3); 13.6, 16.3, 16.4, 17.5 (C15, C16, C17, C1′, C12′, C13′); 25.5 (C14, C10′); 25.9, 26.6 (C7, C11, C3′, C7′); 26.5 (q, PCH_2P, J_{C-P} 74.6 Hz); 34.0 (C3); 34.3 (C2′); 39.6, 39.7 (C6, C10, C6′); 43.2 (C2); 61.4 (OCH_2CH_3); 66.7 (d, OCH_2P, J_{C-P} 119.9 Hz); 75.0 (C1); 122.9, 123.0, 124.0, 124.4, 124.9 (C4, C8, C12, C4′, C8′); 131.0, 134.6, 134.7, 135.8 (C5, C9, C13, C5′, C9′). ^{1}H-NMR ($CDCl_3$) δ: 0.85–0.88 (m, 4 H, H1′, H11′); 1.34 (t, OCH_2CH_3); 1.60 (s, 15 H, H15, H16, H17, H12′, H13′); 1.68 (s, 6 H, H14, H10′); 1.85–2.06 (m, 18 H, H3, H6, H7, H10, H11, H2′, H3′, H6′, H7′); 2.36–2.58 (m, 2 H, PCH_2P); 3.40–3.50 (m, OCH_2P); 3.78–3.85 (m, 8 H, OCH_3, OCH_2CH_3); 4.13–4.21 (m, 2 H, H1); 4.99–5.12 (m, 5 H, H4, H8, H12, H4′, H8′). ^{31}P-NMR ($CDCl_3$) δ: 23.1, 41.4 (2 d, J_{P-P} 3.66 Hz); Anal. calcd. for $C_{36}H_{66}O_6P_2$ (656.87): C 65.83, H 10.13; found C 65.80, H 10.09%.

Triammonium ({hydroxy [({(E,E)-2-[(E)-1,5,9-trimethyldeca-4,8-dienyl]-5,9,13-trimethyltetradeca-4,8,12-trienyl}oxy)methyl]phosphinyl}methyl)phosphonate (**12**)

To a stirred solution of **11** (240 mg, 0.36 mmol) in CH_2Cl_2 (2 ml) at room temperature was added *sym*-collidine (199 μl, 0.9 mmol) followed by bromotrimethylsilane (237 μl, 1.8 mmol). The reaction was stirred for 23 h at room temperature, the solution was evaporated, the residue was dissolved in toluene, concentrated and pumped at high vacuum. The remainder was dissolved in 1M NH_4OH (5 ml) and stirred for 30 min at room temperature, diluted with water and lyophilized. The crude compound was purified on a CHP20P column that was eluted with a linear gradient of 80% acetonitrile/water in 5% methanol/water. Freeze drying of the appropriate fractions yielded the deprotected compound **12** as an amorphous and hygroscopic white solid. $^{13}C\{^1H\}$-NMR (D_2O) δ 15.7, 16.5, 16.6, 17.1, 18,0 (C15, C16, C17, C11′, C12′, C13′); 26.0 (C14, C10′); 27.5 (C7, C11, C7′); 33.5 (C3); 35.5 (C2′); 40.5 (C6, C10, C6′); 43.6 (C2); 71.5 (OCH_2P, J_{C-P} 117 Hz); 75.5 (C1); 123.4, 124.5, 125.3, 125.6, 125.7 (C4, C8, C12, C4′, C8′); 131.4, 135.3, 136.2, 136.9 (C5, C9, C13, C5′, C9′). ^{1}H-NMR (D_2O) δ: 0.83 and 0.91 (2 d, 3 H, H11′, $J_{11,1}$ 6.1 Hz); 1.55, 1.57, 1.36 (3 s, 24 H, H14, H15, H16, H17, H10′, H11′, H12′, H13′); 1.91–2.11 (m, 18 H, H3, H6, H7, H10, H11, H2′, H3′, H6′, H7′); 3.37–3.63 (m, 4 H, H1 and OCH_2P); 5.07–5.15 (m, 5 H, H4, H8, H12, H4′, H8′); $^{31}P\{^1H\}$-NMR (D_2O) δ: 17.7, 32.2 (2 d, J_{P-P} 17.1 Hz); Anal. calcd. for $C_{32}H_{67}N_3O_6P_2$ (651.85): C 58.96, H 10.36, P 9.50; found C 58.89, H 10.30, P 9.49%.

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