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Reinforcing effect of bi- and tri-cyclopolyprenols on 'primitive' membranes made of polyprenyl phosphates

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This paper is dedicated with respect and affection to the memory of our mentor, Professor Guy Ourisson, who passed away on November 4th 2006

Abstract—Cholesterol plays the role of membrane reinforcer in eukaryotes, whereas hopanoids play the same role in bacteria. Which components could have reinforced 'primitive' membranes? We describe here an efficient biomimetic synthesis of bi- and tri-cyclopolyprenols and demonstrate that these compounds reinforce the membranes of polyprenyl phosphate, which we speculated as 'primitive' membranes.

1. Introduction

Biological membranes are protected from shear stress by polyprenoids that act as reinforcers. This function is assured by cholesterol in animals, phytosterols in plants and hopanoids and dihydroxylated carotenoids in bacteria. Our long term investigation in polyprenoids and membrane reinforcers has prompted us to propose a phylogenetic classification of membrane terpenoids, and to hypothesise that the membranes of primitive organisms were made of polyprenyl phosphates.[1] We have previously shown that acyclic and monocyclic polyprenols do stabilize these polyprenyl phosphate membranes.[2] As an extension of these studies, we now focus on the possible reinforcing effects of bi- and tri-cyclopolyprenols (Fig. 1). These tricyclopolyprenols may be the biogenetic precursors of tricyclopolyprenanes identified in organic fossils.[3]

Although we have previously shown that tricyclohexaprenol reinforces models of eukaryotic membranes less efficiently than cholesterol, its effects on other types of membranes remain to be explored.[4] To examine the reinforcing effects of bi- and tri-cyclopolyprenols on polyprenyl phosphate vesicles, which serve as a model of 'primitive' membranes, we have developed an efficient biomimetic synthesis of these compounds, which has permitted extensive biophysical studies.

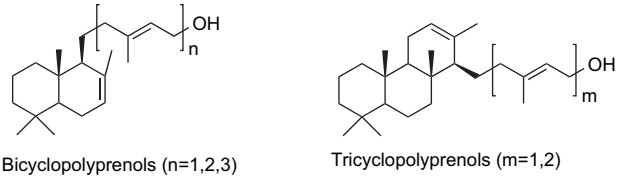

Figure 1. Polycyclopolyprenols.

2. Results and discussion

2.1. Synthesis of bi- and tri-cyclopolyprenyl alcohols

Tricyclohexaprenol has been synthesized by Corey and Burk as a mixture with its bicyclic homolog.[5] Heissler et al. have performed three hemisyntheses of this compound, starting from a non-commercial substance extracted from a Brazilian tree.[6–10] To overcome these limitations and to obtain enough material for extensive biophysical studies, we decided to use another approach based on a biomimetic cyclisation controlled by an allylsilane to synthesize bi- and tri-

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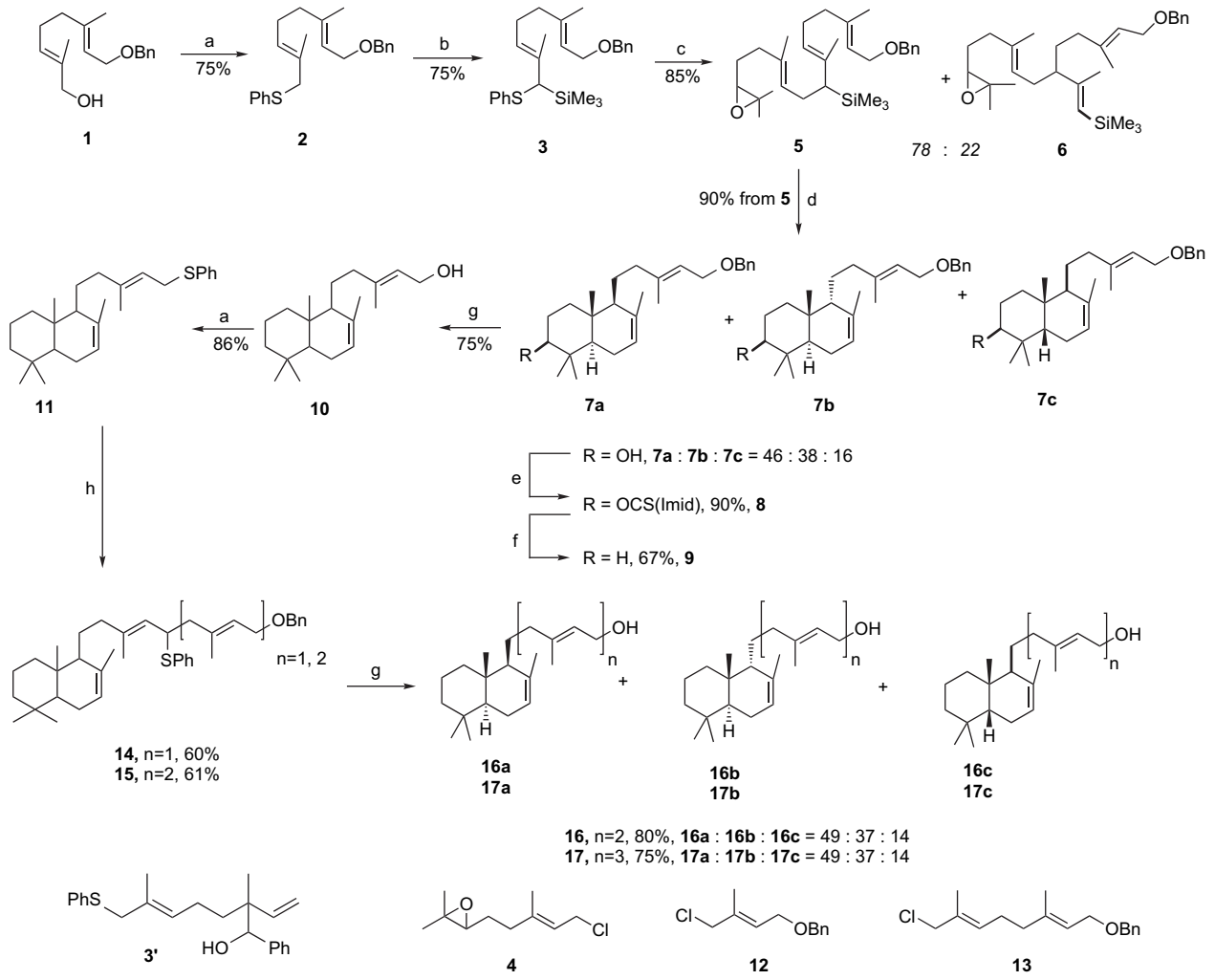
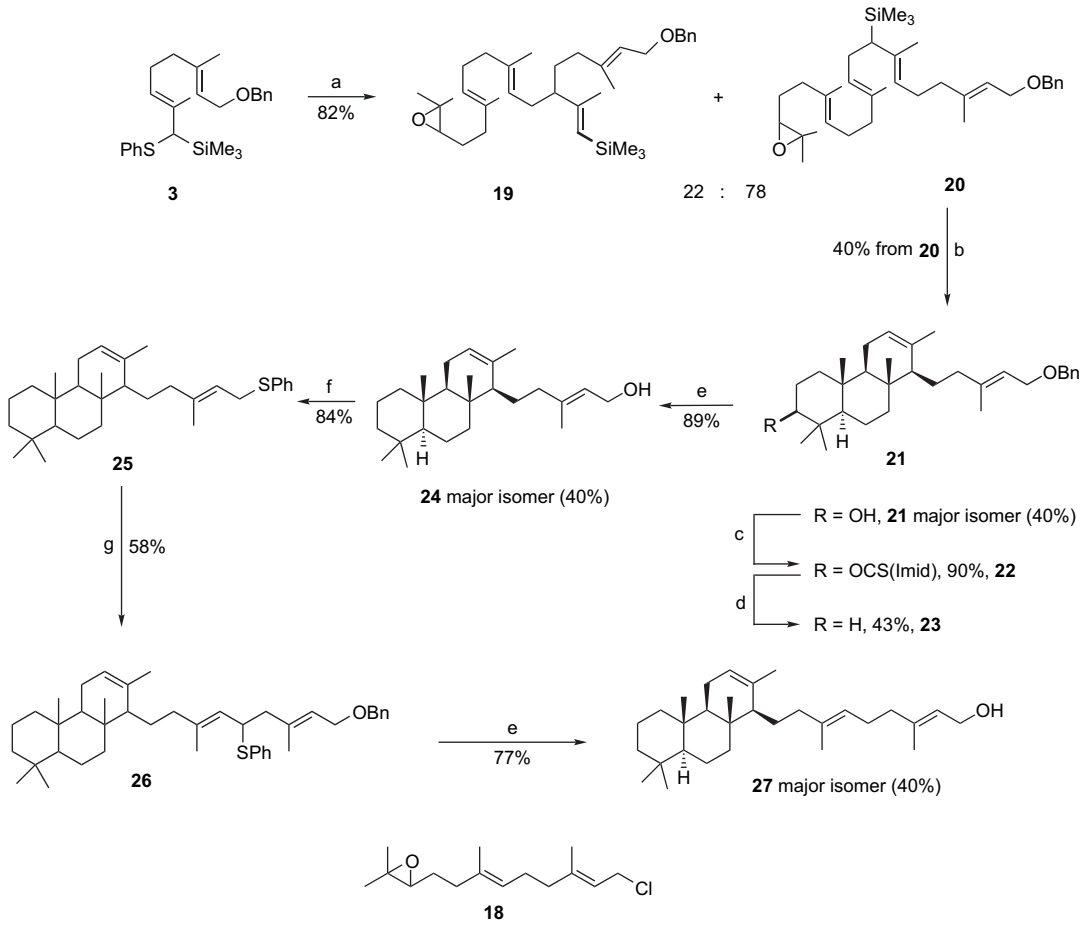


Scheme 1. Reagents and conditions: (a) PhSSPh, PBu_3, pyridine, RT, overnight; (b) LDA (1 M), TMSCl, THF, −78 °C, 2 h; (c) (i) lithium naphthalenide (2.2 eq), THF, −78 °C, 20 min, (ii) **4**, THF, −78 °C, 1 h; (d) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 °C, 2 h; (e) $(imid)_2CS$, DMAP, toluene, 65 °C, overnight; (f) Bu_3SnH, AIBN, toluene, reflux, overnight; (g) (i) $EtNH_2$, Li, THF, −78 °C, 4 h, (ii) MeOH; (h) *n*-BuLi, THF, −78 °C, then **12** or **13**, 2 h.

cyclopolyprenols. The biomimetic cyclisation of polyolefins is the most straightforward and elegant method to synthesize terpenes and steroids.[11] As far as we know, our approach is the first one to involve an allylsilane that is not located at the extremity of the polyenic chain. We recently reported our initial attempt to introduce regiospecifically a trimethylsilyl (TMS) group in the middle of a polyprenyl chain: our approach was based on the alkylation of a deprotonated allylic thioether to construct the polyprenyl chain and then transform this adduct into a silane by reductive lithiation.[12] Unfortunately, we were unable to achieve any regioselectivity in this second step. To overcome this problem, we modified our approach and introduced the TMS group prior to the alkylation in to the polyprenyl chain (Scheme 1). The rationale behind this strategy is that C–Si bonds are longer than C–C bonds,[13] hence the TMS moiety should display a smaller steric hindrance than the alkyl chain. We applied this approach first to the synthesis of bi-cyclopolyprenols and then to tri-cyclopolyprenols.

Synthesis of the required epoxy silane **5** initiated with the conversion of the known alcohol **1** to thioether **2** resulted by action of diphenyl disulfide and tri-*n*-butylphosphine in the presence of pyridine in a 75% yield (Scheme 1).[14] Initial attempts to prepare silylated thioether **3** by deprotonation with *n*-BuLi followed by quenching with TMS chloride mainly produced the product of Wittig rearrangement **3′**.[15] To suppress this side reaction, the lithiation of **2** by LDA was performed in the presence of TMS chloride to trap the carbanion before it had time to undergo the Wittig rearrangement (75% yield). Reductive lithiation of **3** by a stoichiometric amount of lithium naphthalenide followed by quenching with the known epoxygeranylchloride **4**[16] resulted in an inseparable mixture of **5** and **6** in a 78:22 ratio (85% overall yield). Treatment of this mixture with $BF_3{\cdot}Et_2O$ in CH_2Cl_2 yielded bicyclic compounds **7a**, **7b** and **7c** in a 46:38:16 ratio (89% yield). These compounds could not be separated at this stage. The vinylsilane **6** did not react under these conditions and was easily removed by flash chromatography. Conversion of **7** to imidazoylthiocarbonate and exposure to Bu_3SnH and catalytic AIBN in toluene under reflux produced the deoxygenated compound **9** (60% for both steps).[17] Deprotection of the alcohol **9** was performed by reductive lithiation in ethylamine (75% yield). Conversion to thioether using the previous method and isoprenic homologation with chlorides **12** and **13** produced **14** and **15** in,



Scheme 2. Reagents and conditions: (a) (i) lithium naphthalenide (2.2 eq), THF, −78 °C, 20 min, (ii) **18**, THF, −78 °C, 1 h; (b) $BF_3 \cdot OEt_2$, CH_2Cl_2, −78 °C, 2 h; (c) $(imid)_2CS$, DMAP, toluene, 65 °C, overnight; (d) Bu_3SnH, AIBN, toluene, reflux, overnight; (e) (i) $EtNH_2$, Li, THF, −78 °C, 4 h, (ii) MeOH; (f) PhSSPh, PBu_3, pyridine, RT, overnight; (g) *n*-BuLi, THF, −78 °C, then **12**, 2 h.

respectively, 51% and 52% yields for both steps.[18] Reductive cleavage of phenylthioether and benzyl groups from the alkylated sulfides was conveniently accomplished by exposure to lithium in ethylamine to give the bicyclic polyprenols **16** and **17** (80% and 75%, respectively). Pure samples of **16a** and **17a**, with the chain in equatorial position, were obtained by preparative HPLC.

Scheme 2 outlines our final endeavour to synthesize tricyclohexaprenol **27**, following the method developed for bicyclic compounds **16** and **17** described above. An inseparable mixture of epoxy silanes **19** and **20** has been synthesized in a 22:78 ratio (82% overall yield). The critical cyclisation of **20** mediated by $BF_3 \cdot EtO_2$ resulted in a 40% yield and led to the desired stereomer **21** as the major product (40%) mixed with other diastereomers that could not be separated at this stage. Once again, pure samples of **24** and **27**, with the chain in equatorial position, were obtained by preparative HPLC.

2.2. Microscopic observation

Before measuring the water permeability of vesicles, we confirmed that a mixture of geranylgeranyl phosphate **28** and 10 mol % of polyprenyl alcohols forms vesicles at 20 °C by optical microscopy using Nile red as a probe. In the case of bi-cyclopolyprenols **16a** and **17a**, we observed that vesicle formation took place between pH 4.0 and 12.6 while vesicle formation of geranylgeranyl phosphate alone was observed in a pH range from 2.2 to 8.6. As for tri-cyclopolyprenols **24** and **27**, the vesicles were formed, respectively, in a pH range from 4.5 to 10.8 and pH 4.5 to 10.6. These results show that the addition of alcohols shifted vesicle formation of geranylgeranyl phosphate towards higher pHs. These data are in agreement with the model of Israelachvili et al.[19] that predict vesicle formation in function of the ratio of the hydrophilic and hydrophobic volume: the addition of alcohols in the membrane increases the hydrophobic volume and therefore stabilizes the vesicles at higher pHs. Moreover, the possible intermolecular hydrogen bonding between the head group area of polyprenyl phosphate and the polyprenyl alcohol could stabilize the vesicles formed.[20]

2.3. Water permeability

The water permeability of polyprenyl phosphate vesicles, with or without 10 mol % of free alcohol, was studied at 15 °C and at pH 5.81 by osmotic swelling of a suspension of unilamellar vesicles of homogeneous size using the stopped-flow/light scattering method.[21] The compounds tested and the results are presented in Figure 2 and Table 1.

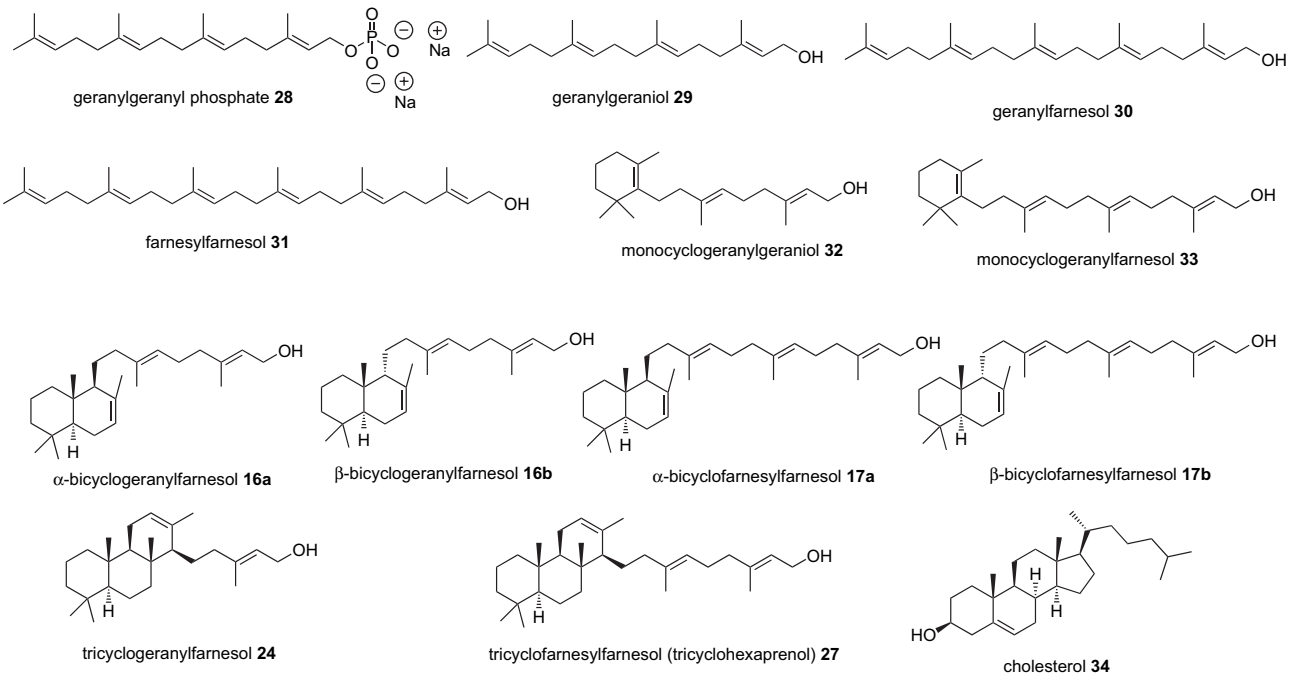


Figure 2. Terpenic compounds.

The presence of cyclohexene rings in the alcohol decreases the water permeability in the vesicles of geranylgeranyl phosphates **28**. For example, the addition of monocyclogeranylfarnesol **33**, bearing the same length of hydrophobic chain as geranylgeranyl phosphate **28**, decreases the water permeability of vesicles, whereas addition of geranylgeraniol **29** increases it (entries 2 and 6, Table 1 and see entries 1 and 3, Table 2). This shows that the incorporation of a rigid molecule improves the order and decreases the water permeability of the membrane. In contrast, the addition of a longer acyclic polyprenyl alcohol **30** or **31** decreases the water permeability, especially in the case of farnesylfarnesol **31**, which lowers the water permeability of the membrane as well as monocyclogeranylfarnesol **33** does (entries 3, 4 and 6, Table 1). These longer acyclic polyprenyl alcohols, probably, penetrate to the opposite leaflet in an interdigitated manner.[22] The additional van der Waals interactions may contribute to stabilize the membrane. The addition of bicyclogeranylfarnesol **16a** or tricyclofarnesylfarnesol **27**, with their hydrophobic chain length close to that of geranylgeranyl phosphate **28**, also decreases the water permeability of vesicles like the monocyclic homologues (entries 7 and 12, Table 1 and see entries 4 and 9, Table 2). Interestingly, the number of rings does not have any effect on the water permeability since the values measured for **33**, **16a** and **27** are similar (entries 6, 7 and 12, Table 1). It seems that chain length is the critical parameter for the water permeability of the system consisting of polyprenylphosphate/polycyclopolyprenol. In the case of monocyclogeranylgeraniol **32** or tricyclogeranylfarnesol **24**, which bears a chain much shorter than geranylgeranyl phosphate **28**, water permeability is not modified (entries 5 and 11, Table 1 and see entries 2 and 8, Table 2). On the other hand, with bicyclofarnesylfarnesol **17a** that bears a chain longer than the lipids **24** and **32**, water permeability decreases slightly (entry 9, Table 1 and see entry 6, Table 2). The suitable size of polycyclopolyprenol for an

Table 1. Water permeability of unilamellar vesicles obtained from a mixture of geranylgeranyl phosphate **1** and 10 mol % of alcohol at 15.0±0.1 °C measured by stopped-flow/light scattering

Entry	Polyprenyl alcohols added	Diameter[a] (nm)	$t_{1/2}$[b] (ms)
1	—[c]	180±16	20±1
2[e]	**29**	179±24	6.0±0.5
3[e]	**30**	185±23	53±1
4[e]	**31**	200±35	260±10
5[e]	**32**	187±26	17±2
6[e]	**33**	190±21	251±6
7	**16a**	160±56	240±20
8	**16b**	nd[d]	nd[d]
9	**17a**	154±55	100±10
10	**17b**	nd[d]	nd[d]
11	**24**	169±50	31±5
12	**27**	170±39	250±20
13[e]	**34**	162±35	190±10

[a] Average diameter±standard deviation.
[b] Average $t_{1/2}$±standard deviation.
[c] Without addition of polyprenyl alcohol.
[d] Not determined, vesicles were unstable.
[e] Values from Ref. 2.

Table 2. Predicted molecular length of polyprenols calculated after energy minimisation by the Gaussian03 method using PM3MM

Entry	Polycycloprenols	Length[c] (Å)
1	**29**	18.06
2	**32**	13.19
3	**33**	17.97
4	**16a**[a]	15.57
5	**16b**[b]	12.96
6	**17a**[a]	20.25
7	**17b**[b]	17.41
8	**24**	11.82
9	**27**	16.67

[a] Chain in equatorial position.
[b] Chain in axial position.
[c] Total length of polyprenyl alcohol.

optimal reinforcing effect against water permeability might be important for the enhancement of van der Waals interactions and the compactness of the membrane. In the same way, we were interested in comparing the reinforcing effect of cholesterol **34**, well known as the ubiquitous reinforcer of animal membranes.[23] In a previous paper, we have reported that cholesterol **34** reinforces membranes made of dimyristoylphosphatidylcholine (DMPC) better than tricyclohexaprenol **27**.[4] Here, we have demonstrated that tricyclohexaprenol **27** and some other terpenyl alcohols **16a**, **31** and **33** reinforce the membrane of geranylgeranyl phosphate better than cholesterol **34** (entries 4, 6, 7, 12 and 13, Table 1). These data show that the ability of a compound to reinforce membrane will depend on the structure of the lipids that constitute the membrane. To optimise this reinforcement, the structure of the reinforcer must match the lipidic structure of the membrane.

2.4. Molecular modelling

In our synthesis of bi- and tri-cyclopolyprenols, we have obtained the mixture of two pairs of diastereomers substituted by an equatorial or an axial chain. When we tried to prepare vesicles of geranylgeranyl phosphate **28** with the diastereomers **16b** and **17b** bearing an axial chain for the stopped-flow experiments, we could not obtain stable vesicles. In contrast, the diastereomers **16a** and **17a** carrying an equatorial chain formed stable vesicles. In order to explain these observations, we calculated the molecular length and modelled polyprenyl alcohols by the PM3MM method. The PM3MM Hamiltonian was used with the GAUSSIAN03 programme package (see Table 2 and Fig. 3).[24]

The diastereomers of bi- and tri-cyclopolyprenols, bearing the lateral chain in an equatorial position, form a plane structure. However, when the chain is in the axial position, we observed an important distortion of the chain with respect to the plane of bi- and tricycles. This could be the reason why we were unable to obtain stable vesicles of geranylgeranyl phosphate **28** with diastereomers **16b** and **17b** as they have an axial chain. Probably, this axial chain perturbs the cohesion of the membrane of the phosphate **28**.

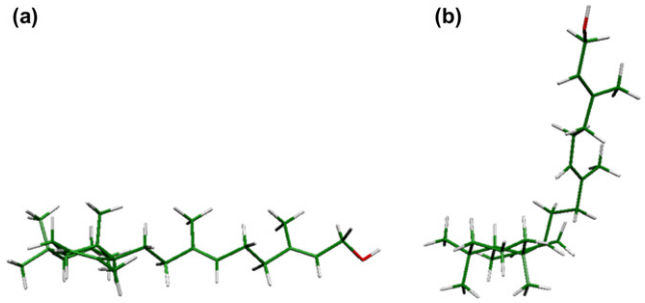


Figure 3. Example of modelling of bicyclogeranylfarnesol diastereomers: (a) chain in equatorial position; (b) chain in axial position.

3. Conclusion

We have described a biomimetic synthesis of bi- and tri-cyclopolyprenols and have provided evidence that these compounds reinforce the membranes of polyprenyl phosphate, when the chain length of the alcohols fits that of membrane constituents. We have also demonstrated that the reinforcing effect of tricyclohexaprenol and some other polyprenols is more important than that of cholesterol in the membranes made of polyprenyl phosphate. With eukaryotic membrane models made of DMPC, the result is opposite: cholesterol reinforces much better than tricyclohexaprenol. This indicates a possible matching relationship between the membrane constituents and the hydrophobic molecules to be inserted. Finally, these results provide a favourable argument for the hypothesis that polycyclopolyprenyl alcohols might have reinforced 'primitive' membranes made of polyprenyl phosphates.

4. Experimental

4.1. General

All reactions were carried out under an argon atmosphere. THF was distilled from Na/benzophenone. CH_2Cl_2 and pyridine were dried and distilled over CaH_2. Anhydrous ethylamine was purchased from Fluka and *n*-butyllithium in hexane (nominally 1.6 M) was purchased from Aldrich and titrated before each use. Thin layer chromatography (TLC) was performed on glass plates coated with silica gel 60 F254 (Merck). Column chromatography was carried out on silica gel 60 (Merck, 70–230 mesh). IR: Nicolet 380, characteristic absorption bands in cm^{-1} along with relative intensity (w=weak, m=medium, s=strong absorption). NMR: Bruker Advance (300 MHz) spectrometers; chemical shifts (δ) in ppm with respect to $CDCl_3$ (δ=7.26) as internal standard for ^1H NMR, to $CDCl_3$ (δ=77.0) as internal standard for ^{13}C NMR; significant ^1H NMR data are tabulated in the following order: chemical shift (δ), number of protons, multiplicity (s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet), values of coupling constants *J* in Hz. Column of preparative HPLC: DuPont Zorbax ODS (250×21.2 m; 8 μm; 80 Å), 20 mL/min^{-1}, eluents: MeOH and H_2O.

4.2. Synthesis

4.2.1. ((2*E*,6*E*)-8-(Benzyloxy)-2,6-dimethylocta-2,6-dienyl)(phenyl)sulfane (2). To a stirred solution of alcohol **1** (5 g, 19.2 mmol) in dry pyridine (7.8 mL, 96 mmol) under an argon atmosphere was added diphenyl disulfide (8.28 g, 38.4 mmol), the mixture was cooled at 0 °C in an ice bath and tri-*n*-butylphosphine (9.51 mL, 38.4 mmol) was added dropwise. The ice bath was removed and the solution was stirred overnight at room temperature. The reaction was quenched with distilled water and extracted three times with diethylether. The combined organic layer was washed with 2 M NaOH, 5% HCl, water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography on silica gel with hexane and hexane/CH_2Cl_2, yielded 5.08 g (75%) of sulfide **2** as a colourless oil: R_f=0.3 (CH_2Cl_2/Et_2O, 95:5); (Found: C, 78.47; H, 7.96. $C_{23}H_{28}OS$ requires C, 78.36; H, 8.01%); ^1H NMR (300 MHz, $CDCl_3$): δ=1.62 (3H, s, $CH_3(6')$), 1.75 (3H, s, $CH_3(2')$), 1.84–2.14 (4H, m, 2×CH_2 allylics(4,5)), 3.49 (2H, s, CH_2–SPh), 4.03 (2H, d, *J* 6.75 Hz, CH_2OBn), 4.51 (2H, s, OCH_2Ph), 5.19–5.26 (1H, t, *J* 6.75 Hz, CH vinylic(3)), 5.33–5.38 (1H, t, *J*

6.75 Hz, CH vinylic(7)), 7.19–7.36 (10H, m, H aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): δ=15.2 (C-6′), 16.4 (C-2′), 26.3 (C-4), 39.1 (C-1), 44.2 (C-5), 66.5 (C-8), 72.1 (O*C*H$_2$Ph), 121.0 (C-7), 126.2 (C-3), 127.5 (C-Ph), 128.4 (C-Ph), 128.4 (C-Ph), 128.6 (C-Ph), 128.6 (C-Ph), 130.8 (C-Ph), 136.5 (C-2), 138.5 (C-Ph), 139.9 (C-6).

4.2.2. ((2*E*,6*E*)-8-(Benzyloxy)-2,6-dimethyl-1-(phenylthio)octa-2,6-dienyl)trimethylsilane (3). To a stirred solution of sulfide **2** (3 g, 8.51 mmol) and TMSCl (1.6 mL, 12.76 mmol) in dry THF under an argon atmosphere at −78 °C was added a molar solution of lithium di-isobutylamine (9.4 mL, 9.36 mmol). After 1 h 30 min at −78 °C, the reaction was diluted with Et_2O and quenched with a saturated solution of NH_4Cl. The mixture was extracted with Et_2O. The organic layer was washed with 1 M HCl, distilled water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography on silica gel with heptane/Et_2O, 95:5, yielded 2.78 g (75%) of silane **3** as a colourless oil: R_f=0.4 (heptane/Et_2O, 9:1); (Found: C, 73.70; H, 8.40. $C_{26}H_{36}OSSi$ requires C, 73.53; H, 8.52%); IR (neat): 3060 (w), 3029 (w), 2952 (m), 2853 (m), 2358 (w), 1669 (w), 1583 (m), 1479 (m), 1453 (m), 1438 (m), 1380 (m), 1362 (m), 1248 (s), 1205 (m), 1087 (s), 1067 (s), 1026 (m), 941 (s), 837 (s), 734 (s), 690 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.14 (9H, s, Si(C*H*$_3$)$_3$), 1.60 (3H, s, CH$_3$(6′)), 1.67 (3H, s, CH$_3$(2′)), 1.94–2.11 (4H, m, 2×CH$_2$ allylics(4,5)), 3.23 (1H, s, SPhC*H*SiMe$_3$), 4.00 (2H, d, *J* 6.9 Hz, C*H*$_2$–OBn), 4.50 (2H, s, C*H*$_2$Ph), 5.17 (1H, t, *J* 6.9 Hz, CH vinylics(3)), 5.36 (1H, t, *J* 6.9 Hz, CH vinylics (7)), 7.10–7.35 (10H, m, CH aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): δ=−1.9 (Si(*C*H$_3$)$_3$), 15.0 (C-6′), 16.4 (C-2′), 26.4 (C-4), 39.4 (C-5), 45.5 (C-1), 66.6 (C-8), 72.1 (O–*C*–Ph), 120.9 (C-7), 125.4 (C-3), 125.9 (C-Ph), 127.5 (C-Ph), 127.8 (C-Ph), 128.3 (C-Ph), 128.4 (C-Ph), 129.1 (C-Ph), 133.2 (C-2), 138.4 (C-Ph), 138.6 (C-Ph), 140.1 (C-6); GC–HRMS (EI): calcd for $C_{26}H_{36}OSSi$: 424.2256; found: 424.2239.

4.2.3. ((3*E*,7*E*,11*E*)-13-(Benzyloxy)-3,7,11-trimethyl-1-(3,3-dimethyloxiran-2-yl)trideca-3,7,11-trien-6-yl)trimethylsilane (5) and ((5*E*)-3-((*E*)-5-(benzyloxy)-3-methylpent-3-enyl)-2,6-dimethyl-8-(3,3-dimethyloxiran-2-yl)-octa-1,5-dienyl)trimethylsilane (6). To a stirred solution of sulfide **3** (1 g, 2.35 mmol) in dry THF under an argon atmosphere at −78 °C was added dropwise a solution of lithium naphthalenide (0.6 M, 8.6 mL, 5.18 mmol) freshly prepared. After 20 min of reaction at −78 °C, a solution of chloride **4** (0.489 g, 2.593 mmol) in 3 mL of dry THF was added dropwise and the resulting solution was stirred for another hour at −78 °C. The reaction was quenched with a saturated solution of NH_4Cl and the mixture was extracted with Et_2O. The organic layer was washed with distilled water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography on silica gel with pentane/Et_2O, 9:1, yielded 0.94 g (85%) of the mixture of silane **5** and **6** with, respectively, 78% and 22% as a colourless oil: R_f=0.34 (pentane/Et_2O, 9:1); (Found: C, 76.86; H, 10.20. $C_{30}H_{48}O_2Si$ requires C, 76.86; H, 10.32%); IR (neat): 2955 (s), 2925 (s), 2852 (s), 2360 (m), 2342 (m), 1741 (w), 1667 (w), 1608 (w), 1453 (m), 1377 (m), 1246 (s), 1114 (m), 1087 (m), 1069 (m), 1028 (w), 834 (s), 735 (s), 696 (s) cm^{-1}; silane **5**: ^{1}H NMR (300 MHz, $CDCl_3$): δ=−0.03 (9H, s, Si(C*H*$_3$)$_3$), 1.25 (3H, s, CH$_3$), 1.29 (3H, s, CH$_3$), 1.42 (1H, t, *J* 7.9 Hz, C*H*SiMe$_3$), 1.51 (3H, s, CH$_3$), 1.56–1.64 (2H, m, (CH$_3$)$_2$COCHC*H*$_2$), 1.60 (3H, s, C*H*$_3$CCHCH$_2$OH), 1.64 (3H, s, (SiMe$_3$)CHCC*H*$_3$), 2.01–2.17 (8H, m, 4×CH$_2$), 2.69 (1H, t, *J* 6.3 Hz, (CH$_3$)$_2$COC*H*), 4.02 (2H, d, *J* 6.6 Hz, C*H*$_2$OBn), 4.50 (2H, s, CH$_2$(O–C*H*$_2$–Ph)), 4.95 (1H, t, *J* 6.6 Hz, CH vinylic), 5.09–5.14 (1H, m, CH vinylic), 5.40 (1H, tq, *J* 6.6 and 1.2 Hz, C*H*CH$_2$OBn), 7.25–7.36 (5H, m, H aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): δ=−2.2 (Si(*C*H$_3$)$_3$), 16.1 (CH$_3$), 16.4 (CH$_3$), 18.7 (CH$_3$), 24.9 (2×(*C*H$_3$)$_2$COCH), 26.4 (*C*H$_2$–CHSiMe$_3$), 26.7 (CH$_2$), 27.5 (CH$_2$), 36.3 (CH$_2$), 39.6 (*C*HSiMe$_3$), 39.9 (CH$_2$), 58.3 ((CH$_3$)$_2$*C*OCH), 64.2 ((CH$_3$)$_2$CO*C*H), 66.6 (*C*H$_2$–OBn), 72.0 (O–*C*H$_2$–Ph), 120.8 (CH vinylic), 122.7 (CH vinylic), 125.8 (CH vinylic), 127.5 (CH aromatic), 127.8 (CH aromatic), 128.3 (CH aromatic), 133.3 (C), 136.0 (C), 138.6 (C), 140.5 (C); GC–HRMS (EI): calcd for $C_{30}H_{48}O_2Si$: 468.3424; found: 468.3410.

4.2.4. 5-((*E*)-5-(Benzyloxy)-3-methylpent-3-enyl)-1,2,3,4,4a,5,8,8a-octahydro-1,1,4a,6-tetramethylnaphthalen-2-ol (7). To a stirred solution of the mixture of epoxides **5** and **6** (164 mg, 0.35 mmol) in dry CH_2Cl_2 under an argon atmosphere at −78 °C was added a solution of $BF_3\cdot Et_2O$ (0.18 mL, 1.40 mmol). After 2 h at −78 °C, the reaction was quenched with 5% $NaHCO_3$ and the temperature was allowed to warm up to room temperature. The mixture was extracted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography on silica gel with heptane/Et_2O, 6:4, yielded 97 mg of the desired cyclic compound **7** (90% from epoxide **5**) as a colourless oil: R_f=0.4 (heptane/Et_2O, 6:4); (Found: C, 81.71; H, 10.23. $C_{27}H_{40}O_2$ requires C, 81.77; H, 10.17%); IR (neat): 3438 (broad signal), 3028 (w), 2960 (s), 2930 (s), 2851 (s), 1667 (w), 1496 (w), 1453 (s), 1379 (m), 1362 (m), 1329 (w), 1248 (w), 1202 (w), 1182 (w), 1159 (w), 1087 (s), 1061 (s), 1027 (s), 1004 (s), 937 (w), 907 (w), 844 (w), 809 (w), 734 (s), 696 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.71 (0.15H, s, CH$_3$), 0.75 (1.2H, s, CH$_3$), 0.79, 0.80 (0.5H, s, CH$_3$), 0.84, 0.85 (2.6H, s, CH$_3$), 0.88 (1H, s, CH$_3$), 0.90, 0.91 (0.5H, s, CH$_3$), 0.96 (1.5H, s, CH$_3$), 0.98 (1.1H, s, CH$_3$), 1.00 (0.5H, s, CH$_3$), 1.08–1.35 (4H, m, 2×CH$_2$), 1.48–1.59 (3H, m, CH$_2$ and CH), 1.65, 1.69 (6H, s, CH$_3$), 1.77–2.28 (5H, m, 2×CH$_2$ and CH), 3.14–3.28 (1H, m, C*H*–OH), 4.02 (2H, d, *J* 6.6 Hz, C*H*$_2$–OBn), 4.51 (2H, s, O–C*H*$_2$–Ph), 5.04 (0.08H, m, CH vinylic), 5.12 (0.08H, m, CH vinylic), 5.25 (0.38H, m, CH vinylic), 5.40 (1.46H, m, CH vinylic), 7.26–7.35 (m, 5H, CH aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.6 (CH$_3$), 15.1 (CH$_3$), 16.6 (CH$_3$), 22.0 (CH$_3$), 23.5 (CH$_2$), 25.5 (CH$_2$), 27.4 (CH$_2$), 27.9 (CH$_3$), 36.6 (C), 37.2 (CH$_2$), 38.6 (C), 42.0 (CH$_2$), 49.5 (CH), 54.0 (CH), 66.7 (*C*H$_2$–OBn), 72.1 (O–*C*H$_2$–Ph), 79.1 (*C*H–OH), 121.1 (CH vinylic), 122.1 (CH vinylic), 127.5 (CH aromatic), 127.8 (CH aromatic), 128.4 (CH aromatic), 135.2 (C), 138.5 (C), 140.7 (C); GC–HRMS: calcd for $C_{27}H_{40}O_2$: 396.3028; found: 396.3052.

4.2.5. *O*-5-((*E*)-5-(Benzyloxy)-3-methylpent-3-enyl)-1,2,3,4,4a,5,8,8a-octahydro-1,1,4a,6-tetramethylnaphthalen-2-yl 1H-imidazole-1-carbothioate (8). A solution

of alcohol **7** (310 mg, 0.78 mmol), thiocarbonyldiimidazole (278 mg, 1.56 mmol) and dimethylaminopyridine (286 mg, 2.34 mmol) in dry toluene (12 mL) was stirred in a pressure tube at 65 °C. After overnight reaction at 65 °C, the solvent was removed and the crude product was purified by chromatography on silica gel with heptane/Et_2O, 7:3, yielded 357 mg (90%) of the desired product **8** as a colourless oil: R_f=0.25 (heptane/Et_2O, 6:4); (Found: C, 73.54; H, 8.39; N, 5.17. $C_{31}H_{42}N_2O_2S$ requires C, 73.48; H, 8.35; N, 5.53%); IR (neat): 3122 (w), 3028 (w), 2961 (m), 2929 (m), 2852 (m), 2334 (w), 1667 (w), 1529 (w), 1460 (m), 1382 (s), 1347 (m), 1325 (m), 1280 (s), 1229 (s), 1093 (s), 1068 (m), 1039 (w), 975 (s), 920 (w), 892 (m), 831 (m), 734 (s), 697 (s), 656 (m), 642 (m); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.82 (1.30H, s, CH_3), 0.94, 0.96 (4.70H, s, CH_3), 1.02, 1.03 (0.5H, s, CH_3), 1.08, 1.09 (2.50H, s, CH_3), 1.27–1.67 (6H, m, 3×CH_2), 1.66, 1.67 and 1.71 (6H, s, CH_3), 1.76–1.83 (2H, m, CH_2), 1.93–2.07 (4H, m, CH_2 and 2×CH), 4.05, 4.04, 4.03, 4.02 (2H, d, *J* 6.6 Hz, CH_2–OBn), 4.50, 4.51, 4.52 (2H, s, O–CH_2–Ph), 5.19–5.29 (1.5H, m, C*H*OCS(imid) and CH vinylic), 5.41–5.45 (1.5H, m, CH vinylics), 7.03–7.04 (1H, m, CH imidazole), 7.29–7.36 (5H, m, CH aromatics), 7.62–7.64 (1H, m, CH imidazole), 8.33–8.35 (1H, m, CH imidazole); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.6 (CH_3), 16.8 (CH_3), 17.2 (CH_3), 22.0 (CH_3), 22.7 (CH_2), 23.1 (CH_2), 25.6 (CH_2), 27.9 (CH_3), 33.5 (CH_2), 36.5 (C), 36.6 (CH_2), 38.4 (C), 49.8 (CH), 54.0 (CH), 66.7 (*C*H_2OBn), 72.3 (O–*C*H_2–Ph), 91.8 (*C*–OCS(imid)), 117.8 (CH vinylic), 118.9 (CH vinylic), 121.2 (CH–imid), 121.6 (CH–imid), 127.6 (CH aromatic), 127.8 (CH aromatic), 128.4 (CH aromatic), 130.7 (CH–imid), 135.3 (C), 138.5 (C), 140.4 (C), 184.0 (C=S); GC–FABHRMS: calcd for $C_{31}H_{42}N_2O_2S$: 506.2967; found [M+H$^+$]=507.3061.

4.2.6. 5-((*E*)-5-(Benzyloxy)-3-methylpent-3-enyl)-1,2,3,4,4a,5,8,8a-octahydro-1,1,4a,6-tetramethylnaphthalene (9). To a stirred and degassed solution of the mixture of xanthate **8** (357 mg, 0.70 mmol) and azobisisobutyronitrile (35 mg, 0.21 mmol) in dry toluene (30 mL) under an argon atmosphere was added Bu_3SnH (0.60 mL, 2.11 mmol). After overnight reaction under reflux (111 °C), the solvent was removed and the crude product was purified by chromatography on silica gel with heptane/Et_2O, 95:5, yielded 181 mg (67%) of the reduced compound **9** as a colourless oil: R_f=0.55 (heptane/Et_2O, 9:1); (Found: C, 84.48; H, 10.46. $C_{27}H_{40}O$ requires C, 85.20; H, 10.59%); IR (neat): 3063 (w), 3028 (w), 2921 (s), 2845 (s), 1667 (s), 1496 (s), 1454 (s), 1377 (m), 1364 (m), 1307 (w), 1249 (w), 1203 (w), 1110 (m), 1085 (m), 1069 (s), 1028 (m), 982 (w), 942 (w), 921 (w), 843 (w), 819 (w), 732 (s), 696 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.72 (0.22H, s, CH_3), 0.75 (1.28H, s, CH_3), 0.85, 0.86, 0.88, 0.89, 0.90 (7.50H, s, CH_3), 1.09–1.59 (9H, m, 4×CH_2 and CH), 1.66, 1.69 (6H, s, 2×CH_3), 1.77–2.27 (5H, m, 2×CH_2 and CH), 4.03 (2H, d, *J* 6.6 Hz, CH_2OBn), 4.51 (2H, s, O–CH_2–Ph), 5.06 (0.06H, m, CH vinylic), 5.14 (0.09H, m, CH vinylic), 5.26 (0.35H, m, CH vinylic), 5.41 (1.51H, m, CH vinylic), 7.26–7.35 (5H, m, CH aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.6 (CH_3), 16.6 (CH_3), 18.8 (CH_2), 22.0 (CH_3), 22.2 (CH_3), 23.8 (CH_2), 25.5 (CH_2), 30.0 (CH_2), 33.2 (CH_3), 36.8 (C), 42.2 (C), 39.1 (CH_2), 42.3 (CH_2), 50.2 (CH), 54.4 (CH), 66.7 (*C*H_2OBn), 72.2 (O–*C*H_2–Ph), 120.9 (CH vinylic), 122.3 (CH vinylic), 127.5 (CH aromatic), 127.8 (CH aromatic), 128.4 (CH aromatic), 135.4 (C), 138.6 (C), 140.9 (C); GC–HRMS (EI): calcd for $C_{27}H_{40}O$: 380.3079; found: 380.3075.

4.2.7. (2*E*)-5-(1,2,3,4,4a,5,8,8a-Octahydro-1,1,4a,6-tetramethylnaphthalen-5-yl)-3-methylpent-2-en-1-ol (10). To a dry ethylamine (5 mL) at −78 °C, lithium wire was added (174 mg, 25.22 mmol) in small pieces and the solution was coloured in blue. The benzylether **9** (480 mg, 1.26 mmol), in dry THF (3 mL), was added slowly and the blue colour disappeared. The resulting solution was allowed to stir 4 h and the reaction colour became blue again. Maintaining the temperature at −78 °C, sodium benzoate was added until the blue colour was totally dissipated and the resulting yellow solution was then quenched with MeOH until colourless. After attaining room temperature, water was added until all the solids dissolved and the volatiles were carefully evaporated under reduced pressure. The resulting cloudy solution was extracted four times with ether. The combined organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography with pentane/Et_2O, 8:2, yielded 274 mg (75%) of alcohol **10** as colourless oil: R_f=0.26 (heptane/Et_2O, 6:4); (Found: C, 82.32; H, 11.83. $C_{20}H_{34}O$ requires C, 82.69; H, 11.80%); IR (neat): 3309 (m), 2920 (s), 2863 (s), 2845 (s), 2359 (w), 2342 (w), 1667 (w), 1456 (m), 1441 (m), 1381 (m), 1365 (m), 1095 (w), 1045 (w), 998 (m), 964 (w), 844 (w), 819 (w); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.73, 0.74 (1.58H, s, CH_3), 0.84, 0.85, 0.86, 0.88, 0.89 (7.42H, s, CH_3), 1.06–1.50 (9H, m, 4×CH_2 and CH), 1.60, 1.63, 1.64, 1.68 (6H, s, CH_3), 1.68–2.25 (5H, m, 2×CH_2 and CH), 4.14 (2H, d, *J* 6.9 Hz, CH_2OH), 5.05 (0.07H, t, *J* 1.2 Hz, CH vinylic), 5.13 (0.07 H, t, *J* 1.5 Hz, CH vinylic), 5.24 (0.34H, m, CH vinylic), 5.41 (1.52H, m, CH vinylic); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.6 (CH_3), 16.4 (CH_3), 18.8 (CH_2), 22.0 (CH_3), 22.2 (CH_3), 23.8 (CH_2), 25.6 (CH_2), 30.0 (CH_2), 33.2 (CH_3), 36.8 (C), 39.2 (CH_2), 42.2 (C), 42.3 (CH_2), 50.2 (CH), 54.5 (CH), 59.4 (CH_2OH), 122.3 (CH vinylic), 123.4 (CH vinylic), 135.3 (C), 140.8 (C); GC–HRMS (EI): calcd for $C_{20}H_{34}O$: 290.2610; found: 290.2603.

4.2.8. ((2*E*)-5-(1,2,3,4,4a,5,8,8a-Octahydro-1,1,4a,6-tetramethylnaphthalen-5-yl)-3-methylpent-2-enyl)(phenyl)-sulfane (11). To a stirred solution of bicyclogeranylgeraniol **10** (329 mg, 1.13 mmol) in dry pyridine (1 mL, 5.66 mmol) under an argon atmosphere was added diphenyl disulfide (494 mg, 2.26 mmol), the mixture was cooled at 0 °C in an ice bath and tri-*n*-butylphosphine (0.6 mL, 2.26 mmol) was added dropwise. The ice bath was removed and the solution was stirred overnight at room temperature. The reaction was quenched with Et_2O, washed with 1 M HCl and the mixture was extracted with Et_2O. The combined organic layer was washed with 1 M HCl, distilled water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography on silica gel with heptane/CH_2Cl_2, 8:2, yielded 377 mg (86%) of the desired sulfide **11** as a colourless oil: R_f=0.54 (heptane/CH_2Cl_2, 8:2); (Found: C, 81.61; H, 10.10. $C_{26}H_{38}S$ requires C, 81.61; H, 10.01%); IR (neat): 3058 (w), 2920 (s), 2863 (m), 2845 (m), 1661 (w),

1585 (w), 1479 (m), 1438 (m), 1455 (m), 1386 (m), 1378 (m), 1364 (m), 1303 (w), 1222 (w), 1157 (w), 1089 (w), 1067 (w), 1049 (w), 1025 (m), 982 (w), 961 (w), 894 (w), 851 (m), 810 (w), 735 (s), 689 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.74 (1.40H, s, CH_3), 0.86, 0.87, 0.88, 0.89, 0.91 (7.60H, s, CH_3), 1.10–1.30 (5H, m, 2×CH_2 and CH), 1.38–1.52 (4H, m, 2×CH_2), 1.58 (3H, br s, CH_3), 1.64, 1.67 (3H, br s, CH_3), 1.72–2.24 (5H, m, 2×CH_2 and CH), 3.55 (2H, d, *J* 7.8 Hz, C*H*$_2$SPh), 5.05 (0.08H, m, CH vinylic), 5.13 (0.08H, m, CH vinylic), 5.24 (0.38H, m, CH vinylic), 5.31 (1H, t, *J* 7.5 Hz, CH vinylic), 5.39 (0.48H, m, CH vinylic), 7.15–7.36 (m, 5H, CH aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.5 (CH_3), 16.2 (CH_3), 18.8 (CH_2), 22.0 (CH_3), 22.2 (CH_3), 23.8 (CH_2), 25.7 (CH_2), 30.1 (CH_2), 32.3 (*C*H$_2$SPh), 33.0 (C), 33.2 (CH_3), 36.8 (C), 39.1 (CH_2), 42.3 (CH_2), 50.1 (CH), 54.4 (CH), 119.3 (CH vinylic), 122.3 (CH vinylic), 126.1 (CH aromatic), 128.7 (CH aromatic), 130.1 (CH aromatic), 135.3 (C), 136.7 (C), 140.6 (C); GC–HRMS (EI): calcd for $C_{26}H_{38}S$: 382.2694; found: 382.2682.

4.2.9. ((2*E*,6*E*)-1-(Benzyloxy)-9-(1,2,3,4,4a,5,8,8a-octahydro-1,1,4a,6-tetramethylnaphthalen-5-yl)-3,7-dimethylnona-2,6-dien-5-yl)(phenyl)sulfane (14). To a stirred solution of sulfide **11** (150 mg, 0.39 mmol) in dry THF (2 mL) under argon atmosphere at −78 °C was added dropwise *n*-butyllithium (1.4 M in hexane solution, 0.35 mL; 0.43 mmol). The resulting yellow solution was stirred for 1 h at −78 °C. A solution of chloride **12** freshly prepared (91 mg, 0.43 mmol) in dry THF (1 mL) was added at −78 °C. After 1 h 30 min of reaction at −78 °C, the mixture was diluted in Et_2O, saturated aqueous NH_4Cl solution was carefully added and the mixture was extracted three times with Et_2O. The combined organic layer was washed with distilled water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography with pentane/Et_2O, 95:5, yielded 132 mg (60%) of the desired product **14** as a colourless oil: R_f=0.40 (pentane/Et_2O, 95:5); (Found: C, 81.48; H, 9.45. $C_{38}H_{52}OS$ requires C, 81.96; H, 9.41%); IR (neat): 3060 (w), 3028 (w), 2920 (m), 2846 (m), 1664 (w), 1584 (w), 1454 (m), 1438 (m), 1379 (m), 1364 (m), 1202 (w), 1089 (m), 1066 (m), 1026 (m), 860 (w), 735 (s), 693 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.72, 0.85, 0.87, 0.89 (9H, s, 3×CH_3), 1.14 (4H, m, 2×CH_2), 1.34 (3H, s, CH_3), 1.42 (2H, m, CH_2), 1.43 (1H, m, CH), 1.53 (2H, m, CH_2), 1.63 (6H, s, 2×CH_3), 1.76 (1H, s, CH), 1.71–2.12 (4H, m, 2×CH_2), 2.27 (1H, dd, *J* 13.5 and 9 Hz, C*H*$_2$–CHSPh), 2.45 (1H, dd, *J* 13.5 and 5.7 Hz, C*H*$_2$–CHSPh), 4.01 (2H, d, *J* 6.6 Hz, C*H*$_2$OH), 4.04–4.08 (1H, m, CH), 4.48 (2H, s, O–C*H*$_2$–Ph), 5.00 (1H, d, *J* 10.2 Hz, CH vinylic), 5.12–5.78 (2H, m, 2×CH vinylic), 7.23–7.42 (10H, m, CH aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.5 (CH_3), 16.2 (CH_3), 16.6 (CH_3), 18.8 (CH_2), 21.8 (CH_3), 22.0 (CH_3), 23.8 (CH_2), 24.1 (CH_2), 32.8 (C), 33.2 (CH_3), 36.3 (CH_2), 36.8 (C), 39.0 (CH_2), 42.9 (CH_2), 45.7 (CH_2), 45.9 (*C*HSPh), 50.1 (CH), 54.2 (CH), 66.3 (*C*H$_2$OBn), 71.7 (O–*C*H$_2$–Ph), 119.7 (CH vinylic), 122.2 (CH vinylic), 123.8 (C-Ph), 125.5 (CH vinylic), 127.4, 127.5, 127.8, 128.4, 128.5 (C-Ph), 135.3, 136.6, 137.3, 138.5, 138.8 (C-Ph); HRMS (MALDI-TOF): calcd for $C_{38}H_{52}OS$: 556.374; found [M+Na$^+$]=579.342; MS (70 eV, EI): *m*/*z* (%): 447 (1), 381 (2) [$C_{26}H_{37}S$], 339 (35), 271 (6), 245 (5), 243 (4), 205 (3), 201 (10), 191 (10), 189 (11), 177 (9), 163 (12), 147 (22), 135 (11), 123 (11) [CH_2SPh], 121 (16), 119 (16), 109 (36) [SPh], 91 (100), 81 (37), 69 (25) [isopentenyl], 55 (25) [isobutene].

4.2.10. (2*E*,6*E*)-9-(1,2,3,4,4a,5,8,8a-Octahydro-1,1,4a,6-tetramethylnaphthalen-5-yl)-3,7-dimethylnona-2,6-dien-1-ol (16). To dry ethylamine (3 mL) at −78 °C, lithium wire was added (28 mg, 4.09 mmol) in small pieces and the solution was coloured in blue. The coupled product **14** (114 mg, 0.20 mmol) in dry THF (1 mL) was added slowly, the blue colour disappeared and the resulting solution was allowed to stir 20 min until the reaction colour became blue again. Maintaining the temperature at −78 °C, sodium benzoate was added until the blue colour was totally dissipated and the resulting yellow solution was then quenched with MeOH until colourless. After attaining room temperature, water was added until all the solids dissolved and the volatiles were carefully evaporated under reduced pressure. The resulting cloudy solution was extracted four times with ether. The combined organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography with pentane/Et_2O, 8:2, yielded 59 mg (80%) of alcohol **16** as colourless oil: R_f=0.32 (pentane/Et_2O, 8:2); **16a** has been isolated by preparative HPLC: (Found: C, 83.56; H, 11.78. $C_{25}H_{42}O$ requires C, 83.73; H, 11.81%); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.75 (3H, s, CH_3), 0.85 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.88–0.97 (2H, m, CH_2), 1.17 (2H, dd, *J* 11.9 and 5.1 Hz, CH_2), 1.36–1.64 (5H, m, 2×CH_2 and CH), 1.61 (3H, s, CH_3), 1.68 (6H, br s, 2×CH_3), 1.80–2.16 (9H, m, 4×CH_2 and CH), 4.15 (2H, dd, *J* 6.9 and 0.5 Hz, C*H*$_2$OH), 5.11 (1H, m, CH vinylic), 5.38 (1H, m, CH vinylic), 5.42 (1H, tq, *J* 7.0 and 1.3 Hz, CH vinylic); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.6 (CH_3), 16.1 (CH_3), 16.3 (CH_3), 18.8 (CH_2), 21.8 (CH_3), 22.3 (CH_3), 23.8 (CH_2), 25.8 (CH_2), 26.3 (CH_2), 33.0 (C), 33.2 (CH_3), 36.7 (C), 39.1 (CH_2), 39.6 (CH_2), 42.2 (CH_2), 42.3 (CH_2), 50.2 (CH), 54.2 (CH), 59.4 (*C*H$_2$OH), 122.1 (CH vinylic), 123.4 (CH vinylic), 124.0 (CH vinylic), 135.6 (C), 136.0 (C), 139.8 (C); GC–MS (70 eV, EI): *m*/*z* 259 (1%), 205 (17), 204 (100), 191 (3), 189 (16), 161 (24), 148 (11), 135 (12), 121 (14), 109 (26), 95 (13), 81 (22), 69 (14) [isoprenyl], 55 (16).

4.2.11. ((3*E*,7*E*,11*E*)-13-(Benzyloxy)-1-(1,2,3,4,4a,5,8,8a-octahydro-1,1,4a,6-tetramethylnaphthalen-5-yl)-3,7,11-trimethyltrideca-3,7,11-trien-5-yl)(phenyl)sulfane (15). To a stirred solution of sulfide **11** (150 mg, 0.39 mmol) in dry THF (2 mL) under argon atmosphere at −78 °C was added dropwise *n*-butyllithium (1.4 M in hexane solution, 0.35 mL; 0.43 mmol). The resulting yellow solution was stirred for 1 h at −78 °C. A solution of chloride **13** freshly prepared (120 mg; 0.43 mmol) in dry THF (1 mL) was added at −78 °C. After 1 h 30 min of reaction at −78 °C, the mixture was diluted in Et_2O, saturated aqueous NH_4Cl solution was carefully added and the mixture was extracted three times with Et_2O. The combined organic layer was washed with distilled water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography with pentane/Et_2O, 95:5, yielded 150 mg (61%) of the desired product **15** as a colourless oil: R_f=0.45 (pentane/Et_2O, 95:5);

(Found: C, 82.59; H, 9.86. $C_{43}H_{60}OS$ requires C, 82.63; H, 9.68%); IR (neat): 3028 (w), 2920 (m), 2846 (m), 1665 (w), 1583 (w), 1453 (m), 1438 (m), 1378 (m), 1364 (m), 1203 (w), 1089 (m), 1066 (m), 1026 (m), 859 (w), 736 (s), 693 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.73 (1H, s, CH_3), 0.85, 0.88, 0.89 (8H, s, CH_3), 1.05–1.24 (4H, m, 2×CH_2(13,18)), 1.34, 1.36 (3H, s, CH_3), 1.40–1.45 (3H, m, CH_2 and CH), 1.52–1.55 (2H, m, CH_2), 1.59 (3H, s, CH_3), 1.64 (6H, s, 2×CH_3), 1.74–1.84 (2H, m, CH_2), 1.88–1.95 (2H, m, CH_2), 2.01–2.11 (5H, m, 2×CH_2 and CH), 2.19 (1H, dd, *J* 13.5 and 9 Hz, C*H*$_2$–CHSPh), 2.39 (1H, dd, *J* 13.5 and 5.7 Hz, C*H*$_2$–CHSPh), 4.02 (2H, d, *J* 6.6 Hz, C*H*$_2$OH), 3.96–4.04 (1H, m, C*H*SPh), 4.50 (2H, s, C*H*$_2$–Ph), 4.96–5.03 (1H, m, CH vinylic), 5.14–5.18 (1H, m, CH vinylic), 5.24 (0.41H, m, CH vinylic), 5.37–5.42 (1.49H, m, CH vinylic), 7.22–7.42 (10H, m, CH aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.6 (CH_3), 16.1 (CH_3), 16.2 (CH_3), 16.6 (CH_3), 18.8 (CH_2), 21.9 (CH_3), 22.1 (CH_3), 23.8 (CH_2), 24.2 (CH_2), 26.4 (CH_2), 33.0 (C), 33.2 (CH_3), 36.8 (C), 39.1 (CH_2), 39.4 (CH_2), 41.6 (CH_2), 42.3 (CH_2), 45.6 (CH_2), 45.9 (*C*H–SPh), 50.1 (CH), 54.6 (CH), 66.6 (*C*H$_2$OBn), 72.0 (O–*C*H$_2$–Ph), 119.7 (CH vinylic), 120.8 (CH vinylic), 126.9 (CH vinylic), 127.1 (CH vinylic), 127.2 (CH aromatic), 127.5 (CH aromatic), 127.8 (CH aromatic), 128.3 (CH aromatic), 128.5 (CH aromatic), 132.3 (C), 134.0 (CH aromatic), 134.9 (C-Ph), 135.3 (C), 136.7 (C), 138.4 (C-Ph), 140.3 (C); HRMS (MALDI-TOF): calcd for $C_{43}H_{60}OS$: 624.436; found [M+Na$^+$]=647.376; MS (70 eV, EI): *m*/*z* (%): 515 (0.92) (1) [M−SPh], 407 (18), 381 (10), 339 (9), 271 (9), 215 (8), 191 (24) [$C_{14}H_{23}$], 177 (13), 163 (14), 147 (21), 135 (15), 123 (14) [CH_2–SPh], 109 (46) [SPh], 91 (100), 81 (46), 69 (26) [isopentenyl], 55 (26) [isobutene].

4.2.12. (2*E*,6*E*,10*E*)-13-(1,2,3,4,4a,5,8,8a-Octahydro-1,1,4a,6-tetramethylnaphthalen-5-yl)-3,7,11-trimethyltrideca-2,6,10-trien-1-ol (17). To dry ethylamine (3 mL) at −78 °C, lithium wire was added (32 mg, 4.54 mmol) in small pieces and the solution was coloured in blue. The coupled product **15** (142 mg, 0.23 mmol) in dry THF (1 mL) was added slowly, the blue colour disappeared and the resulting solution was allowed to stir 20 min until the reaction colour became blue again. Maintaining the temperature at −78 °C, sodium benzoate was added until the blue colour was totally dissipated and the resulting yellow solution was then quenched with MeOH until colourless. After attaining room temperature, water was added until all the solids dissolved and the volatiles were carefully evaporated under reduced pressure. The resulting cloudy solution was extracted four times with ether. The combined organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography with pentane/Et_2O, 7:3, yielded 84 mg (75%) of alcohol **17** as colourless oil: R_f=0.45 (pentane/Et_2O, 7:3); **17a** has been isolated by preparative HPLC: (Found: C, 84.03; H, 11.85. $C_{30}H_{50}O$ requires C, 84.44; H, 11.81%); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.74 (1.5H, s, CH_3), 0.85, 0.86, 0.87, 0.88 (7.5H, s, CH_3), 1.14–1.31 (6H, m, 3×CH_2), 1.37–1.50 (3H, m, CH_2 and CH), 1.60 (6H, s, 2×CH_3), 1.64, 1.68 (6H, s, 2×CH_3), 1.82–2.15 (13H, m, 6×CH_2 and CH), 4.15 (2H, d, *J* 6.9 Hz, C*H*$_2$OH), 5.11 (2H, m, CH vinylic), 5.23 (0.4H, m, CH vinylic), 5.41 (1.6H, m, CH vinylic); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.6 (CH_3), 16.1 (CH_3), 16.2 (CH_3), 16.3 (CH_3), 18.9 (CH_2), 21.9 (CH_3), 22.1 (CH_3), 23.8 (CH_2), 24.2 (CH_2), 26.4 (CH_2), 26.7 (CH_2), 33.0 (C), 33.2 (CH_3), 36.8 (C), 39.1 (CH_2), 39.6 (CH_2), 39.7 (CH_2), 41.9 (CH_2), 42.4 (CH_2), 50.2 (CH), 55.3 (CH), 59.4 (*C*H$_2$OH), 122.3 (CH vinylic), 123.3 (CH vinylic), 123.8 (CH vinylic), 124.4 (CH vinylic), 135.4 (C), 135.6 (2×C), 139.9 (C); GC–MS (70 eV, EI): *m*/*z* (%): 205 (23), 204 (100), 191 (4), 189 (15), 161 (21), 148 (9), 135 (11), 121 (14), 109 (24), 95 (15), 81 (24), 69 (14) [isoprenyl], 55 (15).

4.2.13. ((5*E*,9*E*)-3-((*E*)-5-(Benzyloxy)-3-methylpent-3-enyl)-2,6,10-trimethyl-12-(3,3-dimethyloxiran-2-yl)dodeca-1,5,9-trienyl)trimethylsilane (19) and ((2*E*,6*E*,10*E*,14*E*)-1-(benzyloxy)-3,7,11,15-tetramethyl-17-(3,3-dimethyloxiran-2-yl)heptadeca-2,6,10,14-tetraen-8-yl)trimethylsilane (20). To a stirred solution of sulfide **3** (2 g, 4.71 mmol) in dry THF (30 mL) under an argon atmosphere at −78 °C was added dropwise a solution of lithium naphthalenide (0.6 M, 17.2 mL, 10.37 mmol) freshly prepared. After 20 min of reaction at −78 °C, a solution of chloride **18** (1.33 g, 5.18 mmol) in 3 mL of dry THF was added dropwise and the resulting solution was stirred for another hour at −78 °C. The reaction was quenched with a saturated solution of NH_4Cl and the mixture was extracted with Et_2O. The organic layer was washed with distilled water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography on silica gel with pentane/Et_2O, 9:1, yielded 2.078 g (82%) of the mixture of silanes **19** and **20** with, respectively, 22% and 78% as a colourless oil: R_f=0.31 (pentane/Et_2O, 9:1); (Found: C, 78.66; H, 10.79. $C_{35}H_{56}O_2Si$ requires C, 78.30; H, 10.51%); IR (neat): 2956 (m), 2923 (m), 2851 (m), 1666 (w), 1609 (w), 1453 (m), 1377 (m), 1246 (s), 1114 (m), 1086 (m), 1069 (m), 834 (s), 734 (s), 696 (s); silane **20**: ^{1}H NMR (300 MHz, $CDCl_3$): δ=−0.02 (9H, s, Si(C*H*$_3$)$_3$), 1.25 (3H, s, CH_3), 1.29 (3H, s, CH_3), 1.43 (1H, t, *J* 7.9 Hz, C*H*–SiMe$_3$), 1.53 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.65 (3H, s, CH_3), 1.66–1.76 (2H, m, (CH$_3$)$_2$COCHC*H*$_2$), 1.94–2.17 (12H, m, 6×CH_2 allylics), 2.69 (1H, t, *J* 6.2 Hz, (CH$_3$)$_2$COC*H*), 4.03 (2H, d, *J* 6.7 Hz, C*H*$_2$OH), 4.50 (2H, s, O–C*H*$_2$–Ph), 4.96 (1H, t, *J* 6.6 Hz, CH vinylic), 5.09 (1H, t, *J* 6.6 Hz, CH vinylic), 5.14–5.18 (1H, m, CH vinylic), 5.41 (1H, tq, *J* 6.8 and 1.3 Hz, CH vinylic), 7.25–7.35 (5H, m, H aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): δ=−2.1 (Si(*C*H$_3$)$_3$), 16.0 (CH_3), 16.5 (CH_3), 16.8 (CH_3), 18.8 (CH_3), 24.9 (2×CH_3), 26.4 (CH_2), 26.8 (2×CH_2), 27.5 (CH_2), 36.3 (CH_2), 39.6 (*C*H–SiMe$_3$), 39.7 (CH_2), 40.0 (CH_2), 58.3 ((CH$_3$)$_2$*C*OCH), 64.2 ((CH$_3$)$_2$CO*C*H), 66.6 (*C*H$_2$OBn), 72.0 (O–*C*H$_2$–Ph), 120.7 (CH vinylic), 122.7 (CH vinylic), 125.0 (CH vinylic), 125.2 (CH vinylic), 127.5 (CH aromatic), 127.8 (CH aromatic), 128.3 (CH aromatic), 133.9 (C), 134.0 (C), 136.1 (C-Ph), 138.6 (C), 140.5 (C); GC–MS (70 eV, EI): *m*/*z* (%): 275 (4), 225 (3), 207 (5), 201 (6), 190 (10), 175 (7), 157 (17), 147 (16), 135 (32), 119 (31), 107 (36) [O–CH$_2$Ph], 91 (93) [CH_2–Ph], 81 (58), 73 (100) [Si(CH$_3$)$_3$], 69 (12) [isopentenyl], 55 (15).

4.2.14. 8-((*E*)-5-(Benzyloxy)-3-methylpent-3-enyl)-1,2,3,4,4a,4b,5,8,8a,9,10,10a-dodecahydro-1,1,4a,7,8a-pentamethylphenanthren-2-ol (21). To a stirred solution of the mixture of epoxides **19** and **20** (1.9 g, 3.54 mmol) in dry

CH_2Cl_2 under an argon atmosphere at −78 °C was added a solution of $BF_3 \cdot Et_2O$ (2 mL, 14.16 mmol). After 2 h at −78 °C, the reaction was quenched with 5% $NaHCO_3$ and the temperature was allowed to warm up to room temperature. The mixture was extracted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography on silica gel with pentane/Et_2O, 7:3, yielded 515 mg of the desired cyclic compound **21** (40% from epoxide **20**) as a colourless oil: R_f=0.51 (pentane/Et_2O, 6:4); (Found: C, 81.89; H, 10.45. $C_{32}H_{48}O_2$ requires C, 82.70; H, 10.41%); IR (neat): 3443 (m), 2930 (s), 2851 (s), 2360 (m), 2341 (m), 1668 (w), 1453 (s), 1382 (m), 1363 (m), 1247 (w), 1202 (w), 1067 (s), 1028 (s), 997 (s), 938 (m), 836 (m), 734 (s), 696 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.72, 0.78, 0.80, 0.87, 0.89, 0.97, 1.01 (12H, s, CH_3), 0.82–1.58 (13H, m), 1.66, 1.69 (6H, s, CH_3), 1.86–2.27 (5H, m), 3.15–3.25 (1H, m, C*H*–OH), 4.03 (2H, d, *J* 6.8 Hz, CH_2–OBn), 4.50, 4.51, 4.52 (2H, s, O–CH_2–Ph), 5.03 (0.1H, m, CH vinylic), 5.11 (0.1H, m, CH vinylic), 5.15 (0.1H, m, CH vinylic), 5.20 (0.3H, m, CH vinylic), 5.35–5.43 (1.4H, m, CH vinylic), 7.25–7.35 (5H, m, CH aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.3 (CH_3), 15.5 (CH_3), 16.6 (CH_3), 18.3 (CH_2), 18.5 (CH_2), 22.0 (CH_3), 22.9 (CH_2), 23.5 (CH_3), 27.3 (CH_2), 28.1 (CH_3), 36.7 (C), 36.9 (C), 38.3 (CH_2), 38.9 (C), 40.6 (CH_2), 42.1 (CH_2), 54.2 (CH), 54.9 (CH), 55.7 (CH), 66.7 (*C*H_2OBn), 72.3 (O–*C*H_2Ph), 78.9 (*C*H–OH), 119.1 (CH vinylic), 121.9 (CH vinylic), 127.6 (CH aromatic), 127.8 (CH aromatic), 128.4 (CH aromatic), 135.1 (C), 138.5 (C), 140.8 (C); GC–MS (70 eV, EI): *m*/*z* (%): 464 (1), 356 (55), 323 (4), 288 (12), 257 (10), 243 (8), 227 (19), 206 (27), 187 (22), 175 (16), 159 (18), 147 (16), 135 (38), 133 (39), 121 (33), 119 (37), 107 (51), 91 (100), 81 (38), 69 (24), 55 (29).

4.2.15. *O*-8-((*E*)-5-(Benzyloxy)-3-methylpent-3-enyl)-1,2,3,4,4a,4b,5,8,8a,9,10,10a-dodecahydro-1,1,4a,7,8a-pentamethylphenanthren-2-yl 1H-imidazole-1-carbothioate (22). A solution of alcohol **21** (564 mg, 1.21 mmol), thiocarbonyldiimidazole (433 mg, 2.43 mmol) and dimethylaminopyridine (445 mg, 3.64 mmol) in dry toluene (20 mL) was stirred in a pressure tube at 65 °C. After overnight reaction at 65 °C, the solvent was removed and the crude product was purified by chromatography on silica gel with pentane/Et_2O, 6:4, yielded 599 mg (90%) of the desired product **22** as a colourless oil: R_f=0.25 (pentane/Et_2O, 6:4); (Found: C, 75.14; H, 8.96; N, 4.19. $C_{36}H_{50}N_2O_2S$ requires C, 75.22; H, 8.77; N, 4.87%); IR (neat): 2928 (m), 2852 (m), 1734 (w), 1669 (w), 1529 (w), 1461 (m), 1382 (s), 1346 (m), 1325 (m), 1280 (s), 1229 (s), 1092 (m), 1068 (m), 1008 (w), 972 (s), 891 (m), 832 (m), 734 (m), 696 (m), 656 (m), 642 (m); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.75, 0.89, 0.94, 0.95, 0.97, 0.99, 1.02, 1.04 (12H, s, CH_3), 1.10–1.60 (10H, m), 1.64, 1.66, 1.70 (6H, s, CH_3), 1.88–2.26 (7H, m), 4.03 (2H, d, *J* 6.6 Hz, CH_2OBn), 4.50, 4.51, 4.52 (2H, s, O–CH_2–Ph), 5.04 (0.1H, m, CH vinylic), 5.11–5.27 (1.45H, m, CH vinylic and CH–OCS(imid)), 5.36–5.42 (1.45H, m, CH vinylic), 7.04 (1H, br s, CH imidazole), 7.25–7.36 (5H, m, CH aromatics), 7.63 (1H, br s, CH imidazole), 8.34 (1H, br s, CH imidazole); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.3 (CH_3), 15.6 (CH_3), 16.6 (CH_3), 17.7 (CH_3), 18.3 (CH_2), 22.3 (CH_2), 23.2 (CH_2), 23.5 (CH_3), 25.4 (CH_2), 28.2 (CH_3), 36.8 (C), 36.9 (C), 37.8 (CH_2), 38.6 (C), 40.4 (CH_2), 42.1 (CH_2), 54.1 (CH), 54.7 (CH), 55.8 (CH), 66.7 (*C*H_2–OBn), 72.3 (O–*C*H_2–Ph), 91.6 (*C*H–CS(imid)), 118.9 (CH vinylic), 120.8 (CH vinylic), 121.1 (CH imidazole), 121.6 (CH imidazole), 127.6 (CH aromatic), 127.8 (CH aromatic), 128.4 (CH aromatic), 130.6 (CH imidazole), 135.1 (C), 136.8 (C), 140.7 (C), 184.0 (*C*=S); GC–HRMS (ESI): calcd for $C_{36}H_{50}N_2O_2S$: 574.8594; found [M+H$^+$]=575.3666.

4.2.16. 8-((*E*)-5-(Benzyloxy)-3-methylpent-3-enyl)-1,2,3,4,4a,4b,5,8,8a,9,10,10a-dodecahydro-1,1,4a,7,8a-pentamethylphenanthrene (23). To a stirred and degassed solution of the mixture of xanthate **22** (572 mg, 0.99 mmol) and azobisisobutyronitrile (49 mg, 0.30 mmol) in dry toluene (20 mL) under an argon atmosphere was added Bu_3SnH (0.80 mL, 2.98 mmol). After overnight reaction under reflux (111 °C), the solvent was removed and the crude product was purified by chromatography on silica gel with pentane/Et_2O, 95:5, yielded 191 mg (43%) of the reduced compound **23** as a colourless oil: R_f=0.39 (pentane/Et_2O, 95:5); (Found: C, 84.92; H, 10.93. $C_{32}H_{48}O$ requires C, 85.65; H, 10.78%); IR (neat): 3028 (w), 2922 (s), 2848 (s), 2361 (w), 1739 (w), 1668 (w), 1454 (s), 1381 (m), 1364 (m), 1247 (w), 1206 (w), 1110 (m), 1088 (m), 1069 (s), 1028 (m), 1000 (w), 972 (w), 942 (w), 841 (w), 824 (w), 732 (s), 696 (s); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.73, 0.82, 0.83, 0.85, 0.87, 0.88 (12H, s, CH_3), 0.90–1.60 (14H, m), 1.66, 1.69 (6H, br s, CH_3), 1.77–2.27 (5H, m), 4.03 (2H, d, *J* 6.8 Hz, CH_2–OBn), 4.50, 4.51, 4.52 (2H, s, O–CH_2–Ph), 5.03 (0.06H, m, CH vinylic), 5.11 (0.1H, m, CH vinylic), 5.15 (0.07H, m, CH vinylic), 5.22 (0.31H, m, CH vinylic), 5.37–5.44 (1.45H, m, CH vinylic), 7.26–7.36 (5H, m, CH aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.4 (CH_3), 15.5 (CH_3), 16.6 (CH_3), 18.6 (CH_2), 18.8 (CH_2), 22.1 (CH_3), 22.8 (CH_2), 23.5 (CH_3), 25.4 (CH_2), 33.1 (C), 33.4 (CH_3), 36.9 (C), 37.2 (C), 39.8 (CH_2), 40.7 (CH_2), 41.9 (CH_2), 42.2 (CH_2), 55.0 (CH), 55.1 (CH), 56.3 (CH), 66.7 (*C*H_2OBn), 72.1 (O–*C*H_2Ph), 119.4 (CH), 120.9 (CH), 127.5 (CH), 127.8 (CH), 128.4 (CH), 135.1 (C), 136.7 (C), 141.0 (C); GC–MS (70 eV, EI): *m*/*z* (%): 272 (100), 257 (18), 243 (2), 190 (41), 177 (9), 175 (10), 147 (3), 149 (3), 135 (11), 121 (8), 119 (8), 109 (9), 107 (13) [OBn], 105 (10), 95 (17), 93 (21), 91 (92) [CH_2Ph], 81 (38), 79 (19), 77 (9) [Ph], 69 (23) [isoprenyl], 67 (12), 55 (29).

4.2.17. (2*E*)-5-(1,2,3,4,4a,4b,5,8,8a,9,10,10a-Dodecahydro-1,1,4a,7,8a-pentamethylphenanthren-8-yl)-3-methylpent-2-en-1-ol (24). To dry ethylamine (5 mL) at −78 °C, lithium wire was added (46 mg, 6.64 mmol) in small pieces and the solution was coloured in blue. The product **23** (149 mg, 0.33 mmol) in dry THF (2 mL) was added slowly, the blue colour disappeared and the resulting solution was allowed to stir 20 min until the reaction colour became blue again. Maintaining the temperature at −78 °C, sodium benzoate was added until the blue colour was totally dissipated and the resulting yellow solution was then quenched with MeOH until colourless. After attaining room temperature, water was added until all the solids dissolved and the volatiles were carefully evaporated under reduced pressure. The resulting cloudy solution was extracted four times with ether. The combined organic layer was washed with water

and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography with pentane/Et_2O, 7:3, yielded 106 mg (89%) of alcohol **24** as colourless oil: R_f=0.41 (pentane/Et_2O, 7:3); (Found: C, 85.38; H, 12.65. $C_{25}H_{42}O$ requires C, 83.73; H, 11.81%); IR (acetone): 3310 (w), 2921 (s), 2847 (s), 1734 (w), 1457 (m), 1382 (m), 1208 (w), 1000 (m), 669 (m), 649 (w); pure sample of **24** has been isolated by preparative HPLC: ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.72 (3H, s), 0.81 (3H, s), 0.85 (3H, s), 0.87 (3H, s), 0.78–1.63 (14H, m), 1.69 (6H, br s), 1.89–2.27 (5H, m), 4.15 (2H, d, *J* 9.6 Hz, C*H*$_2$OBn), 5.36 (1H, br s), 5.42 (1H, t, *J* 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.3 (CH_3), 15.5 (CH_3), 16.4 (CH_3), 18.6 (CH_2), 18.8 (CH_2), 21.7 (CH_3), 22.0 (CH_3), 22.8 (CH_2), 25.5 (CH_2), 33.1 (C), 33.4 (CH_3), 36.9 (C), 37.2 (C), 39.8 (CH_2), 40.7 (CH_2), 41.9 (CH_2), 42.2 (CH_2), 55.0 (CH), 55.1 (CH), 56.3 (CH), 59.4 (CH_2), 122.2 (CH), 123.3 (CH), 135.0 (C), 140.4 (C); GC–MS (70 eV, EI): *m*/*z* (%): 272 (88), 257 (23), 243 (4), 229 (3), 203 (5), 201 (4), 190 (100), 177 (37), 175 (11), 173 (10), 163 (6), 161 (6), 159 (6), 149 (12), 135 (36), 121 (29), 119 (37), 107 (50), 95 (46), 93 (54), 91 (31), 81 (75), 69 (47) [isoprenyl], 55 (44).

4.2.18. ((2*E*)-5-(1,2,3,4,4a,4b,5,8,8a,9,10,10a-Dodecahydro-1,1,4a,7,8a-pentamethylphenanthren-8-yl)-3-methylpent-2-enyl)(phenyl)sulfane (25). To a stirred solution of tricyclogeranylfarnesol **24** (154 mg, 0.43 mmol) in dry pyridine (1.5 mL, 8.49 mmol) under an argon atmosphere was added diphenyl disulfide (187 mg, 0.86 mmol), the mixture was cooled at 0 °C in an ice bath and tri-*n*-butylphosphine (0.22 mL, 0.86 mmol) was added dropwise. The ice bath was removed and the solution was stirred overnight at room temperature. The reaction was quenched with Et_2O, washed with 1 M HCl and the mixture was extracted with Et_2O. The combined organic layer was washed with 1 M HCl, distilled water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography on silica gel with heptane/CH_2Cl_2, 8:2, yielded 164 mg (84%) of the desired sulfide **25** as a colourless oil: R_f=0.31 (pentane); (Found: C, 82.70; H, 10.62. $C_{31}H_{46}S$ requires C, 82.60; H, 10.29%); IR (acetone): 2922 (s), 2847 (m), 1739 (s), 1716 (s), 1584 (w), 1507 (w), 1456 (s), 1438 (s), 1364 (s), 1217 (s), 1090 (w), 1025 (w), 854 (w), 736 (s), 689 (s), 669 (s), 650 (w); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.72 (1H, s, CH_3), 0.82, 0.84, 0.86, 0.87, 0.89 (11H, s, CH_3), 0.77–1.55 (14H, m), 1.58 (3H, s), 1.64 (1.5H, br s, CH_3), 1.67 (1.5H, br s, CH_3), 1.75–2.24 (5H, m), 3.55 (2H, d, *J* 7.7 Hz, C*H*$_2$SPh), 5.01 (0.1H, m, CH vinylic), 5.09 (0.1H, m, CH vinylic), 5.14 (0.1H, m, CH vinylic), 5.21 (0.3H, m, CH vinylic), 5.28–5.34 (1.5H, m, CH vinylic), 7.15–7.37 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.3 (CH_3), 15.5 (CH_3), 16.1 (CH_3), 18.6 (CH_2), 18.8 (CH_2), 21.7 (CH_3), 22.1 (CH_3), 22.8 (CH_2), 25.5 (CH_2), 32.3 (CH_2), 33.1 (C), 33.5 (CH_3), 36.9 (C), 37.2 (C), 39.8 (CH_2), 40.6 (CH_2), 42.0 (CH_2), 42.2 (CH_2), 54.3 (CH), 55.0 (CH), 56.3 (CH), 119.2 (CH), 122.1 (CH), 126.1 (CH), 128.7 (CH), 130.0 (CH), 135.0 (C), 136.6 (C), 140.7 (C); GC–MS (70 eV, EI): *m*/*z* (%): 450 (2) [M^+], 272 (7), 257 (3), 203 (2), 190 (13), 178 (100), 163 (4), 149 (10), 135 (16), 123 (12) [CH_2–SPh], 109 (14) [SPh], 93 (16), 81 (28), 69 (12) [isoprenyl], 55 (11).

4.2.19. ((2*E*,6*E*)-1-(Benzyloxy)-9-(1,2,3,4,4a,4b,5,8,8a,9,10,10a-dodecahydro-1,1,4a,7,8a-pentamethylphenanthren-8-yl)-3,7-dimethylnona-2,6-dien-5-yl)(phenyl)sulfane (26). To a stirred solution of sulfide **25** (90 mg, 0.20 mmol) in dry THF (2 mL) under argon atmosphere at −78 °C was added dropwise *n*-butyllithium (1.6 M in hexane solution, 0.2 mL). The resulting yellow solution was stirred for 1 h at −78 °C. A solution of chloride **12** freshly prepared (51 mg, 0.24 mmol) in dry THF (1 mL) was added at −78 °C. After 1 h 30 min of reaction at −78 °C, the mixture was diluted in Et_2O, saturated aqueous NH_4Cl solution was carefully added and the mixture was extracted three times with Et_2O. The combined organic layer was washed with distilled water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography with heptane/Et_2O, 95:5, yielded 73 mg (58%) of the desired product **26** as a colourless oil: R_f=0.41 (pentane/Et_2O, 9:1); (Found: C, 82.18; H, 10.29. $C_{43}H_{60}OS$ requires C, 82.63; H, 9.68%); IR (acetone): 2927 (m), 1739 (s), 1456 (m), 1365 (s), 1229 (s), 1217 (s), 1066 (w), 735 (w), 692 (m), 669 (m); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.69, 0.82, 0.84, 0.85, 0.89 (12H, s, CH_3), 1.34, 1.35 (3H, s, CH_3), 1.63 (6H, br s, CH_3), 0.94–1.54 (14H, m), 1.73–2.14 (5H, m), 2.23–2.31 (1H, m, CH_2–CHSPh), 2.42–2.49 (1H, m, CH_2–CHSPh), 4.01 (2H, d, *J* 6.8 Hz, CH_2–OBn), 4.06 (1H, m, CH–SPh), 4.48 (2H, s, O–CH_2–Ph), 5.01 (1H, m), 5.08 (0.1H, m), 5.12 (0.1H, m), 5.21 (0.4H, br s), 5.34 (0.4H, br s), 5.44 (1H, m), 7.22–7.44 (10H, m, CH aromatics); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.3 (CH_3), 15.5 (CH_3), 16.2 (CH_3), 16.6 (CH_3), 18.6 (CH_2), 18.8 (CH_2), 21.7 (CH_3), 22.1 (CH_3), 22.8 (CH_2), 25.7 (CH_2), 33.1 (C), 33.6 (CH_3), 36.9 (C), 37.2 (C), 37.4 (CH_2), 39.9 (CH_2), 40.6 (CH_2), 41.9 (CH_2), 45.4 (CH_2), 45.7 (*C*H–SPh), 54.3 (CH), 55.1 (CH), 56.2 (CH), 66.2 (CH_2OBn), 71.7 (O–CH_2–Ph), 119.4 (CH vinylic), 122.1 (CH vinylic), 123.8 (CH aromatic), 125.2 (CH vinylic), 127.5 (CH aromatic), 128.3 (CH aromatic), 128.5 (CH aromatic), 134.2 (CH aromatic), 134.3 (CH aromatic), 135.0 (C), 137.3 (C), 137.4 (C), 138.5 (C), 138.9 (C); HRMS (MALDI-TOF): calcd for $C_{43}H_{60}OS$: 624.436; found [M+Na^+]=647.439; MS (70 eV, EI): *m*/*z* (%): 624 (1) [M^+], 449 (4), 407 (35), 339 (3), 272 (6), 269 (5), 259 (5), 257 (4), 189 (7), 177 (5), 175 (4), 163 (12), 147 (15), 135 (7), 119 (15), 110 (51), 109 (32) [SPh], 107 (28) [OCH_2Ph], 95 (24), 91 (100), 81 (42), 79 (33), 77 (35) [Ph], 69 (31) [isopentenyl], 55 (42), 51 (45).

4.2.20. (2*E*,6*E*)-9-(1,2,3,4,4a,4b,5,8,8a,9,10,10a-Dodecahydro-1,1,4a,7,8a-pentamethylphenanthren-8-yl)-3,7-dimethylnona-2,6-dien-1-ol (27). To dry ethylamine (3 mL) at −78 °C, lithium wire was added (26 mg, 3.65 mmol) in small pieces and the solution was coloured in blue. The coupled product **26** (114 mg, 0.18 mmol) in dry THF (1 mL) was added slowly, the blue colour disappeared and the resulting solution was allowed to stir 20 min until the reaction colour became blue again. Maintaining the temperature at −78 °C, sodium benzoate was added until the blue colour was totally dissipated and the resulting yellow solution was then quenched with MeOH until colourless. After attaining room temperature, water was added until all the solids dissolved and the volatiles were carefully evaporated under reduced pressure. The resulting cloudy solution was extracted four times with ether. The combined organic layer

was washed with water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the resulting yellow oily residue by chromatography with pentane/Et_2O, 7:3, yielded 60 mg (77%) of alcohol **27** as colourless oil; (Found: C, 85.89; H, 12.53. $C_{30}H_{50}O$ requires C, 84.44; H, 11.81%); IR (acetone): 3310 (w), 2922 (s), 2850 (m), 1739 (m), 1457 (m), 1376 (m), 1217 (m), 669 (m), 650 (m); ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.72 (3H, s, CH_3), 0.81 (3H, s, CH_3), 0.86 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.75–1.63 (15H, m), 1.61 (3H, s, CH_3), 1.68 (6H, br s, CH_3), 1.84–2.16 (9H, m), 4.15 (2H, d, J 6.9 Hz, CH_2OH), 5.11 (1H, m), 5.35 (1H, br s), 5.42 (1H, tq, J 6.9 and 1.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.4 (CH_3), 15.5 (CH_3), 16.1 (CH_3), 16.3 (CH_3), 18.6 (CH_2), 18.8 (CH_2), 21.7 (CH_3), 22.1 (CH_3), 22.8 (CH_2), 25.7 (CH_2), 26.3 (CH_2), 33.1 (C), 33.5 (CH_3), 36.8 (C), 37.2 (C), 39.6 (CH_2), 39.9 (CH_2), 40.6 (CH_2), 41.9 (CH_2), 42.3 (CH_2), 54.8 (CH), 55.1 (CH), 56.3 (CH), 59.4 (CH_2OH), 122.0 (CH), 123.4 (CH), 123.9 (CH), 135.3 (C), 136.0 (C), 139.8 (C); GC–MS (70 eV, EI): m/z (%): 327 (1), 272 (81), 257 (24), 243 (4), 229 (3), 216 (3), 203 (5), 190 (100), 177 (14), 175 (10), 173 (10), 163 (5), 161 (6), 159 (6), 148 (8), 135 (26), 121 (24), 119 (26), 108 (34), 107 (32), 95 (34), 93 (40), 81 (63), 69 (38) [isoprenyl], 55 (32).

4.3. Microscopic observation

4.3.1. Preparation of samples. A typical procedure was as follows: a mixture of lipid (3 mg) with or without 10 mol % of terpenic alcohol was dissolved in 3 mL of 1:1 (v/v) mixture of chloroform and methanol. An aliquot (5 μl) of the solution was dropped on a coverglass (0.17 mm thick). After 10 min of drying at room temperature, the lamellar solid remaining on the slide was brought into focus and 50 μL of a buffer at 25 °C was added. Aqueous buffers were prepared with MilliQ water (a citric acid–Na_2HPO_4 buffer: pH 2.6–7.6, a Na_2HPO_4–NaH_2PO_4 buffer: pH 7.8–8.0, a glycine–NaOH buffer: pH 8.6–10.6, a Na_2HPO_4–NaOH buffer: pH 11.0–11.9 or a NaCl–NaOH: pH 12.0–13.0). Samples were unsealed. Vesicles were observed to grow from the edges of the solid.

4.3.2. Differential interference contrast microscopy. The sample was observed by differential interference contrast microscopy: Axiovert 135, 63x/1.40 Plan Achromat Oil DIC objective, 2.5× insertion lens, light sources: Hg and halogen lamps, Carl Zeiss. Video system: CCD camera (C 2400-75H) and image processor (Argus 20), Hamamatsu Photonics.

4.3.3. Fluorescence microscopy. To a mixture of lipid with or without 10 mol % of terpenic alcohol (total: 3 mg) dissolved in 300 μL of methanol/chloroform (1:1, v/v) was added 5 mol % of Nile red (λ_{ex}=559 nm, λ_{em}=640 nm). The sample was handled as described above and was observed by differential interference contrast optical microscopy using the fluorescence mode.

4.4. Water permeability of vesicles measured by stopped-flow/light scattering method

4.4.1. Preparation of unilamellar vesicles of homogeneous size. A mixture of lipid with or without 10 mol % of terpenic alcohol (total weight is 30 mg) was dissolved in 3 mL of 1:1 (v/v) mixture of chloroform and methanol in a single round-bottomed flask. The solvents were removed by rotary evaporator to make a homogeneous dry film of lipid. Vesicles of the phospholipids were prepared by the freeze-thaw method using 10 mL of adjusted buffer (A): citric acid–Na_2HPO_4 buffer, 150 mM NaCl, pH 5.81. For the stopped-flow experiments, we used the buffer (B): citric acid–Na_2HPO_4 buffer, 0 mM NaCl, pH 5.81. The vesicle suspension was then extruded 10 times through two polycarbonate membranes (pore size 200 nm or 100 nm, Nucleopore, Corning Costar) under 5–15 atm N_2 pressure. Vesicle size distribution and its dispersity were evaluated by the photon correlation spectroscopy (PSC) on a Coulter-Counter N4MD instrument, using laser light scattering at 33 °C with a 90° scattering angle and the following parameters: viscosity: 0.006 poise, refraction index: 1.33, for supposed infinitely diluted vesicular solutions ($\sim 10^{-4}$ M) in low concentrated aqueous buffers (component concentrations $\sim 10^{-4}$ M). The average diameter measured by PSC is 180 nm.

4.4.2. Stopped-flow experiments. Variation of scattered light intensity (I) versus time (t) upon osmotic shock thermostated at T=(15.0±0.1) °C (MT/2 Lauda thermostat) was followed at the fixed wavelength of 400 nm (entrance and exit slit width=2 mm) on a Biosequential DX-17 MV stopped-flow ASVD spectrofluorimeter (2 mm path length cuvette, Applied Photophysics). Analysis of data: Bio-Kine Analysis V 3.14 software (Bio-Logic). The vesicle dispersions were subjected to osmotic shock for 60 min after their preparation. The stability of the samples was checked by comparison of the average size of the vesicles (PSC) just after their preparation and their average size 5 h later. A sample was deemed stable if it remained monodispersed and the average size of the vesicles was constant. An aliquot of vesicle dispersions prepared with the buffer A was rapidly mixed with the hypomolar buffer B in the stopped-flow instrument. To achieve thermal equilibrium, they were left at (15±0.1) °C in the drive syringes for at least 10 min before the beginning of the kinetic swelling experiments. Numerical fitting of the kinetic data was achieved by means of the exponential relationship 1 in agreement with the theoretical model.

$$I = I_\infty + A e^{-kt} \quad (1)$$

Each rate constant value k was calculated by averaging rate constant values k obtained in turn by computerised fitting of average curves (I) versus (t) derived from the superimposition of several experimental curves (typically 10–15 for each average curve). Each experimental curve (1000 points) was obtained by monitoring the change in scattered light intensity (I) following the rapid mixing ($t \leq 3$ ms) of equal volumes (100 μl) of sample and hypertonic buffer (Fig. 4). Typically 10–15 injections provided independent experimental kinetic curves, which were superimposed, averaged and numerically treated by the Bio-Kine software, which uses a factor analysis method and a Simplex algorithm. The results of 10–15 runs of experiments were then averaged: the corresponding k values and standard deviation are given in tables. The values of the first-order rate constants k determined for the theoretical exponential model measure the H_2O permeability of the vesicles.

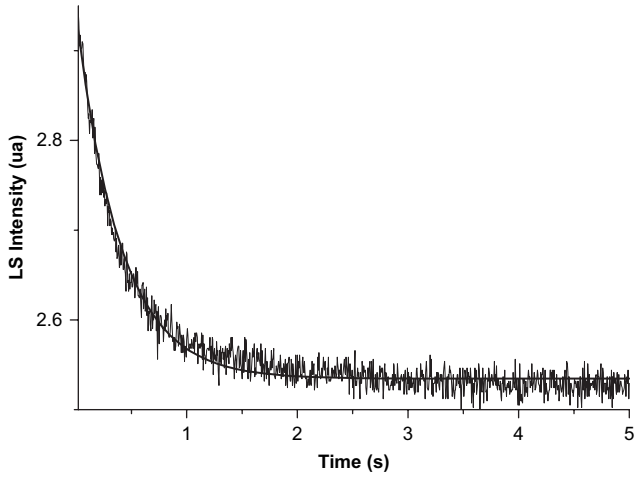


Figure 4. Variation of scattered light (LS) intensity as a function of time. Unilamellar vesicle obtained from a mixture of geranylgeranyl phosphate **28** and 10 mol % of tricyclohexaprenol **27**. pH 5.81; T=15.0±0.1 °C; size=170±39 nm; λ_{exc}=400 nm; cut-off filter 408 nm.

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