Synthesis of triphenylphosphonium vitamin E derivatives as mitochondria-targeted antioxidants

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Abstract

A series of mitochondria-targeted antioxidants comprising a lipophilic triphenylphosphonium cation attached to the antioxidant chroman moiety of vitamin E by an alkyl linker have been prepared. The synthesis of a series of mitochondria-targeted vitamin E derivatives with a range of alkyl linkers gave compounds of different hydrophobicities. This work will enable the dependence of antioxidant defence on hydrophobicity to be determined in vivo.

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1. Introduction

Mitochondria are essential to the functioning of most eukaryotic cells because they provide the energy necessary for cell activities in the form of an elevated adenosine triphosphate/diphosphate ratio by oxidative phosphorylation.1 Because free radicals are produced as a side product of this respiration, mitochondrial localised oxidative damage accumulates faster than in the rest of the cell. Mitochondrial dysfunction due to oxidative damage has been implicated in a wide range of conditions from ageing,2–4 ischaemia-reperfusion injury,5–7 cancer,8–10 epilepsy11 and to neurodegenerative diseases12 such as amyotrophic lateral sclerosis13 and Alzheimer’s14,15 and Parkinson’s16 diseases. Because of the roles mitochondria play in a wide range of pathologies, the engineering of molecules to prevent mitochondrial oxidative damage has therapeutic potential.20

Attaching a bioactive moiety to a lipophilic cation, such as triphenylphosphonium (TPP), enables non-mediated, membrane potential driven, accumulation of the active group within the mitochondrial matrix.21–23 These molecules have been shown to actively accumulate within mitochondria in tissues following oral delivery.24 Numerous examples in the literature, including in vitro,25,23,25–27 ex vivo,26 in vivo28 as well as human trials29 have demonstrated that mitochondria-targeted antioxidants are significantly more effective than non-targeted analogues at preventing mitochondrial oxidative damage in a range of pathologies.

To date, mitochondria-targeted antioxidants based on the natural antioxidants Coenzyme Q,22 lipoic acid,31 and vitamin E23 have been synthesised. A series of compounds based on coenzyme Q (viz. MitoQ) have been synthesised with 3-,21 5-,21 10-21,22 and 15-carbon21 alkyl chains linking the TPP and quinone functional groups (MitoQn3–15). This set of compounds displayed a wide range of lipophilicities21 and there was significant dependence of the protective behaviour in vitro on the linking chain length.32 The maximum antioxidant efficacy for the MitoQn series against mitochondrial oxidative damage was obtained with a 10-carbon alkyl chain (viz. MitoQ10). This intriguing chain-length dependence was found to be due to increased hydrophobicity which enhanced the extent of uptake into mitochondria by favouring adsorption to the mitochondrial inner membrane; longer linker chain-length which allowed the antioxidant quinol moiety to penetrate deeper into the core of the mitochondrial inner membrane relative to the TPP moiety which was localized close to the membrane surface; and longer chain length which allowed access of the ubiquinone moiety to the active site of mitochondrial complex II thereby facilitating its rapid reduction to the active ubiquinol antioxidant.32 Only one targeted vitamin E molecule (MitoE) has been reported to date and this contains a 2-carbon chain linking the functional, antioxidant chroman moiety of vitamin E to the targeting TPP cation and is thus
termed MitoE2 (1). As the antioxidant efficacy of the MitoQn compound depended on alkyl chain length, we have carried out a synthetic study to obtain mitochondria-targeted compounds based on vitamin E with varying alkyl chain lengths, and consequently varying lipophilicities. Previously some of the compounds obtained from this study have been used to create a series of vitamin E succinate derivatives to assay for anticancer activity and this report also provides full experimental support for the precursors involved in that work.

The previous synthesis of MitoE2 involved a large number of steps, was not amenable to the creation of analogues, and was not adaptable to scale-up. Retrosynthetic analysis of a generic MitoEn with chain length \( n \) (Fig. 1) shows it can be formed from B by displacement of a leaving group Y. The structure B is the key intermediate in the synthetic scheme as this establishes the basic carbon framework of the target molecule. The substituted heterocyclic ring in B can be derived from 2,3,5-trimethyl-p-hydroquinone (C) and a tertiary allylic alcohol (D) which in turn can be derived from a methyl ketone (E) and a vinyl organometallic species (F). The appropriate methyl ketone (E) can be formed from the corresponding \( \omega \)-hydroxy alkyne (G).

As a variety of \( \omega \)-hydroxy alkynes are available, especially by utilising acetylene zipper chemistry, this provides the required flexibility to form any MitoEn. Using this general approach we report the synthesis of MitoEn as the mesylate salts with \( n = 2, 4, 6 (1, 2, 3) \) and also MitoE10 (4) and MitoE11 (5) using related chemistry.

2. Results and discussion

The synthesis of MitoE6 (3) (Scheme 2), will be described in detail as an exemplar of the methodology. For this the required \( \omega \)-
hydroxy alkyne, 6-octyn-1-ol, (6) was treated with Hg(OTf)₂·(TMU)₂ in aqueous CH₂CN to afford 8-hydroxy-2-octanone (7) in 95% yield (Scheme 1). The primary hydroxyl group was then converted into a THP ether (8) in 86% yield followed by reaction with vinylmagnesium chloride to readily afford the tertiary allylic alcohol 9, following chromatography with 0.1% Et₃N in the eluant, in 96% yield. Reaction of 9 with 2,3,5-trimethyl-p-hydroquinone (10) in acid—preferably formic acid—afforded diol 11 in 53% yield.

The synthesis of MitoE₂ and MitoE₄ followed the same basic route although some specific modifications for producing the key intermediate allylic tertiary alcohol precursors (14,19) were required—requiring availability of suitable starting materials or undesired intramolecular reactions. Thus the synthesis of the MitoE₂ hydroxycroman intermediate (15, Scheme 1) was completed starting with 4-hydroxy-2-butaneone (12) and proceeding via the THP ether (13) and tertiary allylic alcohol (14). The synthesis of MitoE₄ required the use of 5-hexyn-1-ol (16) as the starting material. Reaction of 16 with aqueous Hg(OTf)₂ resulted in significant formation of the cyclic hemiketal, 2-methyl-tetrahydro-2H-pyran-2-ol. To overcome this undesired cyclization the primary alcohol was converted to an acetate (17) before transformation into the acetoxy methyl ketone (18). Treatment of 18 with excess vinylmagnesium chloride simultaneously formed the required tertiary allylic alcohol functionality and hydrolysed the acetate ester affording 19.

Preliminary studies on the formation of triphenylphosphonium salts from chromanols with an appropriate primary leaving group and triphenylphosphine showed substantial degradation of the heterocyclic system, particularly when the rate of phosphonium salt formation was relatively slow. While the nature of these undesired side reactions was not fully elucidated it was considered useful to protect the electron rich phenol function with an electron withdrawing group during phosphonium salt formation. There are several literature reports of the use of the methanesulfonyl (mesyl) group to protect phenols with subsequent removal using basic reagents. The mesyl group can therefore serve two functions, as an electronophilic activator of the primary alcohol by forming a mesyloxy group while also providing phenol protection during phosphonium salt formation.

Diol 11 was treated with two equivalents of MsCl in the presence of Et₃N and afforded the bis-mesylate 21 in 78% yield (Scheme 2). The bis-mesylate was then reacted with PPH₃ at 90 °C for 48 h to give 22 in 92% yield. Removal of the aryl mesyl ester was trialled with the mesyl derivative of vitamin E and, while reactions using NaBH₄, Cs₂CO₃ or MeONA/MeOH showed no change, reaction with three equivalents of LDA resulted in complete conversion to the phenol vitamin E (Supplementary data). To compensate for the likely ylide formation with the phosphonium substrates, the amount of LDA used in the reaction with 22 was increased to 6 equiv and this protocol afforded MitoE₆ (3) mesylate in 49% yield.

The synthesis of MitoE₄ (2) mesylate was also completed from 20 following the same sequence (viz. 20 → 23 → 24 → 2) (Scheme 2). For the synthesis of MitoE₂ (1) the bis-mesylate (25) was readily obtained from 15, but direct reaction of 25 with PPH₃ at 80–90 °C produced no phosphonium salt. However addition of 5 equiv of NaI to the melt gave 26, which was then treated with LDA to afford MitoE₂ (1) mesylate after anion exchange.

During the developmental phase the syntheses of MitoE₆ (4) and MitoE₉ (5) as the mesylate salts were also completed using less efficient earlier variations on the optimised route presented above (Supplementary data). MitoE₆ (4) was synthesised starting from 1-hydroxy-11-dodecylene, obtained by the addition of 1-bromononane to lithiated propargyl THP ether followed by triple bond migration using NaH and ethylenediamine. In both cases the final step involved reaction of triphenylphosphine with the mesyloxy phenol (57, S11, Supplementary data) and the adverse effects of extended exposure of the unprotected phenol moiety to these reaction conditions was evident.

The NMR spectra for the mesyles MitoE₂ (1), MitoE₄ (2), MitoE₆ (3), MitoE₁₀ (4) and MitoE₁₁ (5) displayed a number of common features which are summarized in Fig. 2 and Table 1. As an example the 1H NMR spectrum of MitoE₆ (3) contained a triplet at $\delta$ 2.55 ($J$=6.8 Hz), consistent with the signal from the methylene group at position 4. NOESY and gCOSY correlations from this resonance to a multiplet at $\delta$ 1.66–1.80 allowed assignment to the methylene protons at 3. Both of these signals had NOESY correlations to a 3-proton singlet at $\delta$ 1.17 therefore assigned to the methyl protons at 12. The resonances from the aryl methyl groups were identified from NOESY correlations as from the protons at 10 ($\delta$ 2.10) and 11 ($\delta$ 2.00) and a correlation between the signal from the remaining methyl group 9 ($\delta$ 2.07) and the methylene resonance of 4. The signal from the protons at position 6′ was clearly evident as a multiplet at $\delta$ 3.22–3.30.

![Fig. 2.](image-url) (A) Numbering scheme for assignment of NMR spectra of MitoEn. (B) Key H–H correlations: $\rightarrow$ gCOSY, $\leftrightarrow$ NOESY. (C) Key H–C correlations: $\rightarrow$ HMBC, $\leftrightarrow$ mutual HMBC.

| Assignment | MitoE₂ | MitoE₄ | MitoE₆ | MitoE₁₀ |
|------------|--------|--------|--------|---------|
| $\delta^1$ | 2.44 $^a$, 2.58 $^a$ | 2.55 $^a$ | 2.55 $^a$ | 2.56 |
| 9          | 2.06   | 2.07   | 2.07   | 2.06   |
| 10         | 2.15   | 2.08   | 2.10   | 2.10   |
| 11         | 2.03   | 1.94   | 2.00   | 2.04   |
| 12         | 1.37   | 1.18   | 1.17   | 1.20   |
| $\delta^1$ | 1.74 $^b$, 1.98 $^b$ | 1.54 $^b$ | 1.50 $^b$ | 1.52 $^b$ |
| $\delta^2$ | 3.13 $^c$, 3.37 $^c$ | 3.19 $^b$ | 3.26 $^b$ | 3.28 $^b$ |
| $\delta^3$ | 2.60   | 2.66   | 2.50   | 2.53   |

$^a$ Chemical shift in CD₂Cl₂ referenced to solvent (5.31 ppm).
$^b$ Value of $\delta$ at centre of multiplet.
$^c$ Triplet ($J$=6.8 Hz).
$^d$ Methylene adjacent to P⁺.
Selected key $^1$H NMR resonances for the mesylates of MitoE$_2$, MitoE$_4$, MitoE$_6$ and MitoE$_{10}$ are summarised in Table 1. The close proximity of the functional groups in MitoE$_2$ was evident from the differences compared to the longer chain MitoE compounds.

Further structural confirmation for MitoE$_2$ (1) was provided by X-ray diffraction of the crystalline bromide (Fig. 3). In this structure, the six-membered heterocyclic ring of MitoE$_2$ adopted an envelope conformation with $\theta=54.5^\circ$ (lit.$^{40} 54.7^\circ$) and with C2 deviating from the O1–C3–C4–C4a–C8a plane by 0.699 Å, towards the phosphonium group.

To determine whether the biochemical properties and antioxidant efficacy of the chromanol moiety of MitoE were affected by changing the length of alkyl chain conjugating it to the TPP cation, the ability of the most (MitoE$_{10}$) and least (MitoE$_2$) lipophilic members of the MitoE$_n$ series to prevent lipid peroxidation was measured and compared. The ability of the compounds to act as chain breaking antioxidants in the rat brain homogenate system of lipid peroxidation$^{23}$ was assessed. This system was chosen as comparison of the effects of the antioxidants on preventing lipid peroxidation would not be confounded by differential uptake into mitochondria. Rat brain homogenates were allowed to undergo spontaneous lipid peroxidation, which was assessed by the production of thiobarbituric reactive species (TBARS)$^{22}$ The effect of MitoE$_2$, and MitoE$_{10}$ on preventing this peroxidation was then assessed (Fig. 4) and showed that both compounds were of comparable efficacy in preventing lipid peroxidation. Therefore it is concluded that conjugation to the TPP cation by differing alkyl chain lengths to the chromanol moiety does not significantly alter their intrinsic antioxidant efficacy. Consequently any changes in antioxidant efficacy seen in mitochondrial or cell studies can be assigned to differences in uptake, adsorption or recycling. The antioxidant efficacy of the MitoE compounds was far greater than that of Trolox, which contains the same antioxidant chromanol moiety as MitoE, connected to a short-chain carboxylic acid rather than a TPP function, making it far more hydrophilic. This suggests that the lipophilic nature of the TPP moiety enhances the interaction of the antioxidant moiety with the phospholipid bilayer.

This aspect was extended by measuring whether MitoE$_2$ and MitoE$_{10}$ compounds were accumulated by mitochondria in response to the mitochondrial membrane potential, as is expected for a compound linked to a TPP compound. To measure the uptake of the MitoE compounds an ion-selective electrode was used that responds to the concentration of the TPP cation in solution (Fig. 5).
When the MitoE compounds were added to a mitochondrial suspension the ion-selective electrode responded to the increase in concentration of the MitoE compound in the extracellular environment. When the mitochondria were energised with the respiratory substrate succinate a large membrane potential across the mitochondrial inner membrane was established. This led to the extensive accumulation of the compounds within mitochondria, thus lowering the extracellular concentration which is detected by the electrode. Addition of the uncoupler FCCP resulted in the dissipation of the membrane potential and consequent release of the compounds back in to the supernatant and this is evident from the electrode response.

Therefore these experiments showed that, as expected, both MitoE2 and MitoE10 were taken up by energised mitochondria in response to the membrane potential. To see if this accumulation within mitochondria enhanced the ability of the mitochondria-targeted compounds to decrease oxidative damage to isolated mitochondria the activity was assessed and compared with the antioxidant efficacy of Trolox, a chroman containing antioxidant molecule, which is not taken up by mitochondria (Fig. 6).

This showed that both MitoE2 and MitoE10 were comparably protective against mitochondrial lipid peroxidation and that both were more protective against oxidative damage to Trolox. Finally, the ability of the most effective version of MitoE, MitoE10, to protect against oxidative damage to mitochondrial DNA caused by the reductase cycling molecule menadione was determined (Fig. 7). This also showed that MitoE10 was able to protect against this form of mitochondrial oxidative damage more effectively than the control compound decylTPP. MitoE2 was not protective in this assay (data not shown) consistent with the greater protection of MitoE10 against lipid peroxidation.

3. Conclusion

The development of this generalised route to MitoE analogues has allowed a suite of targeted analogues to be prepared. We have assessed the biochemical properties of the most (MitoE10) and least (MitoE2) lipophilic members of the MitoE compounds and found that they are effective. By analogy with the MitoQ suite of compounds the series of MitoE compounds we have made will also have a range of lipophilicities and can be used to assess the effect of lipophilicity on the biological effects of MitoE. This strategy is both efficient and can be systematically varied to create a range of MitoE compounds. These compounds accumulate in mitochondria and preliminary biological data demonstrate that MitoE analogues show greater efficacy in preventing lipid peroxidation, mitochondrial oxidative damage and damage to mitochondrial DNA than non-targeted compounds. Further work needs to be conducted to fully understand the trend of biological behaviour of the MitoE series and determine the activity of the intermediate MitoE compounds and enable the dependence of the antioxidant efficacy of MitoE on chain length to be assessed in vivo.

4. Experimental section

4.1. General procedures

Thin Layer Chromatography (TLC) was performed with silica gel (Merck) 60F 254 coated on aluminium roll and were developed in solvent mixtures as indicated. Plates were visualised first with UV light (254 nm) then stained with vanillin or phosphomolybdic acid and heated. Column chromatography was performed using Merck 60 Silica (200–400 mesh, 40–63 μm) as the adsorbent. Columns were pre-equilibrated with the starting solvent before use and 50 g of adsorbent per g of crude product was used. Anion Exchange Chromatography was performed using Amberlite® IRA-400(Cl) ion exchange resin. The column was pre-equilibrated with 10% aqueous MeOH before use. Material was loaded in MeOH and the column was eluted with 1:1 MeOH:H2O.

Nuclear Magnetic Resonance (NMR) Spectroscopy ¹H and ¹³C NMR spectra were acquired on a Varian INOVA-300 spectrometer at 7.05 T and 298 K operating at 299.90 and 75.42 MHz, respectively, or
on a Varian INOVA-500 spectrometer at 11.74T and 298 K, operating at 499.74 MHz and 125.67 MHz, respectively as indicated. 

**H** and **C** NMR spectra were acquired on a Varian INOVA-300 spectrometer at 7.05 T and 298 K operating at 121.40 MHz. Spectra were acquired in 

**CDCl3**, **CD2Cl2** and **CD3OD** as indicated. The solvent peak was used as an internal reference for **H** and **C** NMR spectra. In **CDCl3** the **H** and **C** NMR spectra were referenced to 7.26 ppm and 77.08 ppm, respectively; in **CD2Cl2** **H** and **C** NMR spectra were referenced to 5.31 ppm and 53.8 ppm, respectively and **CD3OD** to 3.31 ppm and 49.0 ppm, respectively. Phosphoric acid (85%) was used as an external reference at 0 ppm for **31P** NMR spectra. Spectra were processed using standard Varian software. For each **H** NMR signal, chemical shifts (δ), relative integral, multiplicity, coupling constant (J), and assignment information are given unless otherwise stated. **13C** resonances were evident as singlets from a single carbon unless otherwise stated. The following standard abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet, dd=doublet of doublets, td=triplet of doublets, dt=doublet of triplets. Standard 2D experiments (gCOSY, NOESY, HSQC and HMBC) were employed for assignment of proton and carbon resonances.

Low resolution atmospheric pressure chemical ionisation (APCI) mass spectroscopy (MS) and low resolution electrospray ionisation (ESI) MS were performed using the Shimadzu LCMS WP8000x spectrometer operated in positive or negative ion mode as indicated. High resolution mass spectra (HRMS) were recorded on a Bruker microTOF-Q spectrometer operated in positive or negative ion mode as indicated. Data are presented as m/z values for the parent molecular ion. Combustion microanalyses were performed by M. Dick or R. McAllister (Campbell Microanalytical Laboratory, Department of Chemistry, University of Otago).

X-ray diffraction data were collected on a Bruker APEX II CCD diffractometer, with graphite monochromated Mo-Kα (λ=0.71073 Å) radiation. Intensities were corrected for Lorentz polarisation effects and a multiscan absorption correction was applied. The structure was solved by direct methods (SIR-97) and refined on R2 using all data by full-matrix least-squares procedures (SHELXL 97).

### 4.2. Syntheses

#### 4.2.1. General synthetic procedures

Argon was used for reactions requiring an inert atmosphere. Standard vacuum line Schleck techniques were employed; glassware was flame-fired before use and cannula were used for transferring liquids between reaction vessels. Removal of solvents was achieved by either rotary evaporation at temperatures of up to 50 °C, or by evaporation under a stream of argon. Some syntheses were carried out a 15 mL Kimax brand round bottomed 10 mm id borosilicate test-tubes fitted with a screw-top that enables a convenient sealed system under argon to be established.

Diisopropylamine was distilled from NaOH before use. Tri- fluoromethanesulfonic anhydride (Tf₂O) was distilled from P₂O₅ and used after distillation over CaH₂ and distilled directly before use. Diethyl ether (Et₂O) was distilled before use and stored over sodium wire. Tetrahydrofuran (THF) was distilled over KOH then distilled onto Na wire. The pre-dried solvent was then freshly distilled under an argon atmosphere over a benzophenone-K₂CO₃ amalgam (4:1) prior to use. All other solvents were used without purification.

Absolute alcohol was dried over 4 A molecular sieves. Acetonitrile (CH₃CN) was refluxed over CaH₂ and distilled directly before use. Dichloromethane (CH₂Cl₂) was refluxed over P₂O₅ and distilled onto 4 A molecular sieves. Diethyl ether (Et₂O) was distilled before use and stored over sodium wire. Tetrahydrofuran (THF) was refluxed over KOH then distilled onto Na wire. The pre-dried solvent was then freshly distilled under an argon atmosphere over a benzophenone-K₂CO₃ amalgam (4:1) prior to use. All other solvents were used without purification.

**4.2.2. 9-(Tetrahydro-2H-pyran-2-yl oxy)-3-hydroxy-3-methyl-non-1-ene (9)**

Vinylmagnesium chloride (1.4 M in THF, 6.3 mL, 8.82 mmol) was added to a solution of 8 (Supplementary data) (1.006 g, 4.41 mmol) in anhydrous THF (60 mL) stirring at −78 °C. This was stirred for 2 h and then allowed to warm to room temperature over 30 min. Saturated aqueous NH₄Cl (50 mL) and then this was extracted with Et₂O (3×50 mL). The combined organic phase was washed with saturated aqueous NaCl (50 mL), dried over anhydrous MgSO₄, filtered and concentrated to give 9 as a pale yellow oil (1.086 g, 4.24 mmol, 96%) which was used without further purification. Analysis calcld for C₇H₁₄O₂: C 70.3, H 10.9; TLC: Rf 0.37 (1.9:Et₂O:CH₂Cl₂); HRMS (+ve ESI) m/z calcld for [M+Na]+: 279.1931, found: 279.1933; **1H** NMR (500 MHz, CD₂Cl₂): δ (ppm) 1.22 (3H, s, H5), 1.26–1.38 (6H, m, H₆–H₉), 1.49–1.60 (8H, m, H₄, H₆, H₈, H₁₁, H₁₂, H₁₃), 1.62–1.88 (1H, m, H₁₁b), 1.74–1.82 (1H, m, H₁₃b), 3.33 (1H, td, J=9.6, J=6.7 Hz, H₉a), 3.42–3.46 (1H, m, H₁₄a), 3.66 (1H, td, J=9.6, J=6.7 Hz, H₉b), 3.81 (1H, dd, J=11.3, J=3.0, 3.0 Hz, H₁₄b), 4.52 (1H, t, J=3.7 Hz, H₁₀), 5.00 (ABX system, 1H, dd, J=10.7 Hz, J=1.4 Hz, H₁₁a), 5.16 (ABX system, 1H, dd, J=10.7 Hz, J=1.4 Hz, H₁₁b), 5.50 (ABX system, 1H, dd, J=10.7 Hz, J=10.7 Hz, H₂). **13C** NMR (125 MHz, CD₂Cl₂): δ (ppm) 21.7 (C₂), 24.2 (C₆), 26.0 (C₁₃), 26.6 (C₇), 27.9 (C₅), 30.1 (C₈), 30.3 (C₆), 312 (C₁₄), 42.8 (C₄), 64.2 (C₄), 67.4 (C₉), 73.4 (C₃), 99.1 (C₁₀), 111.3 (C₁), 146.0 (C₂).
chromatography on silica gel and elution with 1:3 EtO\(_2\)CH\(_2\)Cl\(_2\) containing 0.1% Et\(_3\)N afforded pure 14 as a colourless liquid (1.091 g, 5.45 mmol, 93%). Analysis calc. for C\(_{11}\)H\(_{20}\)O\(_2\): C 66.0, H 10.1, found: C 65.9, H 10.2; TLC: R\(_f\) 0.71 (1:1 EtO\(_2\)CH\(_2\)Cl\(_2\); HPMS (+ve ES) m/z calc. for \([\text{M}+\text{Na}]^+: 223.1305, \text{found: 223.1305; } ^{13}\text{C}\) NMR (500 MHz, CDCl\(_3\)): δ (ppm) 125.3 (CH), 81.9 (2H, s), 59.5 (4H, s), 55.7 (2H, s), 54.7 (2H, s), 47.6 (2H, s), 45.8 (2H, s), 41.2 (CH), 40.0 (CH). TLC: R\(_f\) 0.19 (1:2 CH\(_2\)Cl\(_2\) : H\(_2\)O, 1.34, 3.74 (1H, s, H\(_9\)), 2.54 (2H, t, J = 6.8 Hz, H\(_7\)), 5.01 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_6\)), 5.17 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_B\)) NMR (125 MHz, CDCl\(_3\))): δ (ppm) 125.3 (CH), 81.9 (2H, s), 59.5 (4H, s), 55.7 (2H, s), 54.7 (2H, s), 47.6 (2H, s), 45.8 (2H, s), 41.2 (CH), 40.0 (CH).

2.4.2. 3,7-Dihydroxy-3-methyl-hept-1-ene (19). Vinylmagnesium chloride (1.4 M in THF, 40.0 mL, 55.0 mmol) was added to a solution of 18 (Supplementary data) (1.615 g, 10.21 mmol) in anhydrous THF (100 mL) stirring at -78 °C. This was stirred for 2 h and then allowed to warm to room temperature over 30 min. To the reaction mixture was added dropwise aqueous saturated aqueous NH\(_4\)Cl (30 mL), dried over anhydrous MgSO\(_4\), filtered and concentrated to give a yellow oil. Chromatography on silica gel with elution with 1:1 EtO\(_2\)CH\(_2\)Cl\(_2\) containing 0.1% Et\(_3\)N gave 19 as a pale yellow oil (1.312 g, 9.10 mmol, 89%). Analysis calc. for C\(_{11}\)H\(_{20}\)O\(_2\); C 66.8, H 11.2, found: C 66.8, H 11.2; TLC: R\(_f\) 0.14 (1:1 EtO\(_2\)CH\(_2\)Cl\(_2\); HPMS (+ve ES) m/z calc. for [M+Na\(^+\): 167.1053, found: 167.1060; ^{13}\text{C}\) NMR (500 MHz, CDCl\(_3\)): δ (ppm) 123.3 (3H, s, H\(_8\)), 13.2 −14.0 (2H, m, H\(_4\)), 1.46−1.55 (4H, m, H\(_4\), H\(_6\)), 1.89 (2H, br, H\(_9\)), H\(_9\)), 3.57 (2H, t, J = 6.3 Hz, H\(_7\)), 5.01 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_6\)), 5.17 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_B\)) NMR (125 MHz, CDCl\(_3\))): δ (ppm) 123.3 (3H, s, H\(_8\)), 13.2 −14.0 (2H, m, H\(_4\)), 1.46−1.55 (4H, m, H\(_4\), H\(_6\)), 1.89 (2H, br, H\(_9\)), H\(_9\)), 3.57 (2H, t, J = 6.3 Hz, H\(_7\)), 5.01 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_6\)), 5.17 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_B\)) NMR (125 MHz, CDCl\(_3\))): δ (ppm) 123.3 (3H, s, H\(_8\)), 13.2 −14.0 (2H, m, H\(_4\)), 1.46−1.55 (4H, m, H\(_4\), H\(_6\)), 1.89 (2H, br, H\(_9\)), H\(_9\)), 3.57 (2H, t, J = 6.3 Hz, H\(_7\)), 5.01 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_6\)), 5.17 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_B\)) NMR (125 MHz, CDCl\(_3\))): δ (ppm) 123.3 (3H, s, H\(_8\)), 13.2 −14.0 (2H, m, H\(_4\)), 1.46−1.55 (4H, m, H\(_4\), H\(_6\)), 1.89 (2H, br, H\(_9\)), H\(_9\)), 3.57 (2H, t, J = 6.3 Hz, H\(_7\)), 5.01 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_6\)), 5.17 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_B\)) NMR (125 MHz, CDCl\(_3\))): δ (ppm) 123.3 (3H, s, H\(_8\)), 13.2 −14.0 (2H, m, H\(_4\)), 1.46−1.55 (4H, m, H\(_4\), H\(_6\)), 1.89 (2H, br, H\(_9\)), H\(_9\)), 3.57 (2H, t, J = 6.3 Hz, H\(_7\)), 5.01 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_6\)), 5.17 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_B\)) NMR (125 MHz, CDCl\(_3\))): δ (ppm) 123.3 (3H, s, H\(_8\)), 13.2 −14.0 (2H, m, H\(_4\)), 1.46−1.55 (4H, m, H\(_4\), H\(_6\)), 1.89 (2H, br, H\(_9\)), H\(_9\)), 3.57 (2H, t, J = 6.3 Hz, H\(_7\)), 5.01 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_6\)), 5.17 (ABX system, 1H, dd, J\(_{AB}\) = 10.8 Hz, J\(_{AX}\) = 1.3 Hz, H\(_B\)).
after recrystallisation from EtOH to give and precipitated from petroleum ether 40

C726C3PC11, 0

C8000eJ

C7000eJ

C10

C10

C9

C9

C8

C8

C7

C7

C6

C6

C5

C5

C4

C4

C3

C3

C2

C2

C1

C1

C0

C0

0.54 mmol). A solution of diisopropylium was prepared by adding diisopropylamine (0.30 mL, 0.216 g, 2.13 mmol) to anhydrous THF (4 mL) at -78 °C followed by n-Buti (1.8 M in hexane, 1.0 mL, 1.8 mmol). The solution was stirred at -78 °C for 30 min and then allowed to warm to 0 °C. The diisopropylethylamine solution was then added to a solution of 26 (0.195 g, 0.29 mmol) in anhydrous THF (4 mL) with stirring at 0 °C. After 30 min the solution was allowed to warm to room temperature and then aqueous saturated NH4Oms (10 mL) was added. The aqueous layer was extracted with CH2Cl2 (3 × 10 mL) and the combined organic phases dried over anhydrous MgSO4, filtered and concentrated in vacuo to give a pale yellow oil (1.003 g).

The crude product was dissolved in CH2Cl2 (0.5 mL) and precipitated from EtOH (20 mL) twice then chromatographed on silica gel with elution with 1:9 EtOH:CH2Cl2 and finally passed through an anion exchange column loaded with OMs in methanol. The residual solvents were removed by freeze drying to give 1 as a white solid (52.8 mg, 89.3 μmol, 31%). HRMS (−ve ESI) m/z calculated for [M]+: 495.2459, found: 495.2462; 1H NMR (500 MHz, CDCl3): δ (ppm) 1.37 (3H, s, H12), 1.87 (2H, t, J=7.0 Hz, H3), 1.69–1.78 (2H, 2 × s, C7, C8), 115.9 (36C, 1C, J=28.5 Hz, C5), 135.8 (36C, 3C, J=31.3 Hz, C6), 144.2 (C8a), 146.6 (C6); 31P NMR (121 MHz, CDCl3): δ (ppm) 26.1.

4.2.14. (4-Hydroxy-2,5,7,8-tetramethyl-chromen-2-yl)ethyltriphenylphosphonium methanesulfonate, MitoE2 (2). A solution of lithium diisopropylethylamine in anhydrous THF (0.4 mL) was added for 2 above and added to a solution of 24 (0.192 g, 0.28 mmol) in anhydrous THF (4 mL) stirring at 0 °C. After 30 min the solution was allowed to warm up to room temperature and then aqueous saturated NH4Oms (10 mL) was added. The aqueous layer was extracted with CH2Cl2 (3 × 10 mL). The combined organic phases were dried over anhydrous MgSO4, filtered and concentrated in vacuo to give a pale yellow oil. The crude product was dissolved in CH2Cl2 (0.5 mL) and precipitated from EtOH (20 mL) twice then purified by column chromatography on silica gel and elution with 1:9 EtOH:CH2Cl2 to afford 2 as a white solid (0.091 g, 0.15 mmol, 53%). TLC: Rf 0.22 (1.9 EtOH:CH2Cl2); HRMS (+ve ESI) m/z calculated for [M]+: 523.2760, found: 523.2771; 1H NMR (500 MHz, CDCl3): δ (ppm) 1.18 (3H, s, H12), 1.42–1.66 (2H, 2 × s, C7, C8), 1.66–1.79 (6H, 6 × s, H2, H3, H2), 1.94 (3H, s, H11), 2.07 (3H, s, H9), 2.08 (3H, s, H10), 2.55 (2H, t, J=6.8 Hz, H4), 2.61 (3H, s, H11), 3.14–3.24 (2H, 2 × s, H4), 3.47 (1H, s, H5), 7.62–7.72 (12H, 2 × s, H6, H7, H8), 7.84 (3H, t, J=3.7 Hz, H8b), 12.7 (C6, CD3S); δ (ppm) 11.6 (C9), 11.9 (C11), 12.5 (C10), 21.0 (C4), 23.0 (1C, J=50.6 Hz, C8), 23.2 (C3); 24.1 (C12) 25.1 (1C, J=15.9 Hz, C2), 321 (C3), 32.8 (C2), 39.6 (C1), 74.3 (C12), 117.6 (C4a), 118.3 (1C, J=58.6 Hz, C5), 118.7 (C5), 122.0, 122.2 (2C, 2 × s, C7, C8), 130.9 (6C, J=125.8 Hz, C7), 133.8 (3C, J=98.8 Hz, C6), 135.7 (3C, J=26.8 Hz, C8), 145.2 (C6), 145.5 (C8a); 31P NMR (121 MHz, CDCl3): δ (ppm) 24.8.

4.2.15. (6-(6-Hydroxy-2,5,7,8-tetramethyl-chromen-2-yl)hexyltiphenylphosphonium methanesulfonate, MitoE3 (3). A solution of
lithium disopropylamide (1.49 mmol) in anhydrous THF (5.0 mL) was prepared as for 2 above and added to a solution of 22 (0.152 g, 0.21 mmol) in anhydrous THF (10 mL) with stirring at 0 °C. After 30 min the solution was allowed to warm to room temperature and then aqueous saturated NH₄OH solutions (10 mL) was added. The aqueous layer was extracted with CH₂Cl₂ (3 × 1 mL). The combined organic phases were dried over anhydrous MgSO₄, filtered and concentrated in vacuo to give a pale yellow oil which was dissolved in CH₂Cl₂ (0.5 mL) and precipitated from Et₂O (20 mL) twice. Purification by column chromatography on silica gel and elution with 1:9 EtOAc:CH₂Cl₂ followed by freeze drying gave 3 as a white solid (0.067 g, 0.10 mmol, 49%). TLC: Rf 0.19 (1:9 EtOAc:CH₂Cl₂); HRMS (+ve ESI) m/z calc for [M]+: 351.0376, found: 351.0377; 1H NMR (500 MHz, CDCl₃) δ (ppm) 1.17 (3H, s, H₁₂), 1.24–1.27 (4H, m, H₂, H₃), 1.42–1.57 (4H, m, H₄, H⁷), 1.57–1.66 (2H, m, H₆), 1.66–1.80 (2H, m, H₃), 2.00 (3H, s, H₁₁), 2.07 (3H, s, H₉), 2.10 (3H, s, H₁₀), 2.50 (3H, s, H₁₁°), 2.55 (2H, t, J=6.9 Hz, H₄), 3.22–3.30 (2H, m, H₆), 7.66–7.77 (12H, m, H₈, H⁹, H¹₀), 7.82–7.86 (3H, m, H₁₀), 118.3 (1C, d, JCP=16.8 Hz, C⁰), 121.5, 122.4 (2C, 2 × C⁶, C⁷, C⁸), 133.9 (6C, d, JCP=12.6 Hz, C⁹), 133.9 (6C, d, JCP=9.8 Hz, C⁸), 135.6 (3C, d, JCP=9.3 Hz, C¹⁰), 145.3 (C⁶), 145.6 (C⁸a); 13C NMR (125 MHz, CDCl₃) δ (ppm) 24.8.

4.2.16. (10-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-decyl)triphosphonium methanesulfonate, MitoE₂ (4). A mixture of 10-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl) decyl methanesulfonate (57, Supplementary data) (0.095 g, 0.21 mmol) and triphenylphosphate (0.282 g, 1.07 mmol) was mixed in a Kimax tube then the tube was flushed with argon, sealed and stirred at 80 °C for 30 min. The solution was allowed to warm to room temperature and aqueous saturated NH₄OMs (10 mL) was added. The aqueous phase was dried over anhydrous MgSO₄, filtered and concentrated in vacuo to give 4 as a pale yellow oil which was dissolved in CH₂Cl₂ (0.5 mL) and precipitated twice from Et₂O to give a pale yellow oil (0.027 g). The oil was dissolved in EtOH (0.5 mL) and then diluted with H₂O (5 mL) and any volatile solvents were removed in vacuo. The aqueous solution was freeze dried to give 4 as a fluffy white solid (270 mg, 37.4 µmol, 18%). HRMS (+ve ESI) m/z calc for [M]+: 607.3699, found: 607.3687; 1H NMR (500 MHz, CDCl₃) δ (ppm) 1.26–1.33 (8H, m, H₁–H₆), 1.34–1.46 (2H, m, H₂, H₃), 1.46–1.57 (4H, m, H₄, H⁷), 1.57–1.67 (4H, m, H₈, H⁹, H₁₀), 1.69–1.81 (2H, m, H₆), 2.04 (3H, s, H₁₁), 2.06 (3H, s, H₉), 2.10 (3H, s, H₁₀), 2.53 (3H, s, H₁₁°), 2.56 (2H, t, J=6.8 Hz, H₄), 3.25–3.31 (2H, m, H₁₀), 6.78–7.73 (12H, m, H₁, H₂, H₁₂), 7.18–7.85 (3H, m, H₁₄), 121.5, 122.4 (2C, 2 × C⁶, C⁷, C⁸), 127.8, 128.0 (6C, d, JCP=12.6 Hz, C⁹), 133.9 (6C, d, JCP=9.8 Hz, C⁸), 135.6 (3C, d, JCP=9.3 Hz, C¹⁰), 145.3 (C⁶), 145.6 (C⁸a); 13C NMR (121 MHz, CDCl₃) δ (ppm) 24.8.

4.3. Measurement of lipid peroxidation rat brain homogenates

Rat brain homogenates were prepared and incubated for 30 min at 37 °C as described with a range of concentration of MitoE compounds, Trolox or ethanol carrier. The extent of lipid peroxidation was assessed by measuring TBARS. Values were corrected for the background level at t=0 and were expressed as a % of the control with ETOH carrier. Data are the means±SD of 4–6 independent experiments.

4.4. Mitochondrial and cell experiments

Rat liver mitochondria were prepared as described. Mitochondrial uptake of MitoE₂, MitoE₁₀ was measured using an ion-selective electrode for TPP, as previously described. Briefly, isolated rat liver mitochondria (1 mg protein/mL) were incubated in 120 mM KCl, 10 mM HEPES, 1 mM EGTA, pH 7.2, KOH supplemented with 4 µg/mL rotenone at 37 °C with stirring. The TPP-electrode response was calibrated with five successive additions of 1 µM MitoE₂ or MitoE₁₀ (from a 1 mM stock in EtOH). The mitochondria were energised with 10 mM succinate as a respiratory substrate to measure the background level at 

To measure mitochondrial lipid peroxidation, isolated rat liver mitochondria (2 mg protein/mL) were incubated in KCl medium as above, supplemented with 10 mM succinate and 4 µg/mL rotenone at 37 °C and pretreated with MitoE for 2 min. Lipid peroxidation was then initiated with 1 mM cumene hydroperoxide, and the mitochondria were incubated for a further 15 min. Lipid peroxidation was assayed as TBARS above. Values were corrected for background level without cumene hydroperoxide and were expressed as a % of the control with ETOH carrier. Data are the means±SD of 3–4 independent experiments.

To measure mitochondrial DNA damage, C2C12 cells were seeded at 20,000 cells/cm² in six-well culture plates and grown overnight at 37 °C. Cells were then pre-incubated in 100 nM MitoE₁₀ or n-decyltriphenylphosphonium bromide (decylTPP) for 30 min, and then 25 µM menadione was added to generate oxidative damage within the cells and incubated for a further 1 h. Cells were washed and DNA was isolated using the DNeasy Blood and Tissue kit from Qiagen, quantitated using the Picogreen Assay.
experiments. Statistical significance was determined by Student’s t-test. **p<0.01 compared against menadione treated samples.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2015.09.014.

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