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The preparation of side chain functionalized analogues of coenzyme Q for protein conjugation studies

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The synthesis of two analogues of CoQ (**10** and **13**) suitable for conjugation to a peptide or protein, and hence the development of an ELISA immunoassay, is presented. These analogues were synthesized from the protected quinone, 1-bromo-2-methyl-3,4,5,6-tetramethoxybenzene (**1**), itself prepared from commercially available CoQ-0 (**3**). Model coupling studies of one of the analogues (**10**) to *N*-acetyl-L-lysine methyl ester and a lysine containing dipeptide (*N*-acetyl-glycine-L-lysine methyl ester) were also undertaken as a first step to monoclonial antibody production.

Introduction

The coenzyme Q_n family, also known as ubiquinones (Fig. 1), have a number of important biofunctions that include acting as mobile mediators for electron transfer and protein translocation between redox enzymes in the electron transport chain of mitochondria and bacterial respiratory systems.[1–3] Coenzyme Q is also known to act as an antioxidant by reducing free radicals that can cause damage to structural lipids or proteins in the membrane, not only in mitochondria but in any cellular CoQ containing membranes.[3] In addition, a deficiency of CoQ has been observed in many diseased states, and supplementation with exogenous CoQ is thought to be beneficial to some disorders including cancer,[4–6] diabetes,[7,8] heart disease[9,10] and neurodegenerative disease.[11,12] For these reasons it is important to have efficient methods for the preparation and detection of these natural products and derivatives thereof.

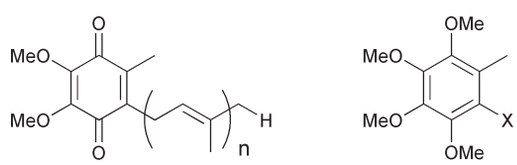


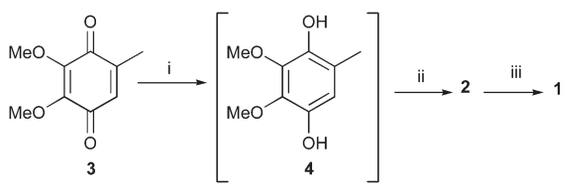
Fig. 1 Coenzyme Q and some important chemical intermediates.

The protected quinone 1-bromo-2-methyl-3,4,5,6-tetramethoxybenzene (**1**), a key starting material for the synthesis of coenzyme Q and its analogues, is conveniently derived from 1-methyl-2,3,4,5-tetramethoxybenzene (**2**).[13] However, published syntheses of **2** are time consuming, complex and low yielding. A review of the literature shows that a number of groups have synthesised **2** *via* complicated multistep procedures starting from highly functionalised starting materials such as glucose,[14] and pyrogallol.[13] Keinan and Eren[15] published a two step synthesis of **2** from *p*-cresol in 71% yield, but in our hands this procedure gave a complex, intractable mixture from which only a very low yield of the desired derivative **2** was isolated. There are also limited reports on the preparation of various analogues of coenzyme Q including fluorescently[16] and isotopically labelled analogues,[17] *O*-alkylated derivatives,[18,19] and analogues with side chain modifications.[20–23] These compounds were synthesised *via* a series of functional group interconversions of CoQ compounds with varying chain length.

In this paper we present an efficient and reproducible three step synthesis of **1** starting from commercially available 2,3-dimethoxy-5-methyl-1,4-benzoquinone (CoQ-0). We also report the preparation of analogues of coenzyme Q (**10** and **13**), from **1**, that bear a carboxyl group at the side-chain terminus suitable for conjugation to a peptide or protein. In addition, analogue **10** was coupled to *N*-acetyl-L-lysine methyl ester and a lysine containing dipeptide (*N*-acetyl-glycine-lysine methyl ester) to demonstrate the feasibility of its use in protein conjugation studies that would form a basis for monoclonal antibody production and hence the development of an ELISA based detection method.

Results and discussion

Our synthesis of **1** involved simple protection of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (CoQ-0) (**3**) followed by aryl bromination as detailed in Scheme 1. In particular, reaction of **3** with stannous chloride and concentrated hydrochloric acid afforded the hydroquinone **4**, which was not purified. This crude material was suspended in dimethylsulfate, potassium hydroxide was added in aliquots, and the mixture heated at 80 °C for one hour to give **2** in 85% over two steps. Finally, **2** was treated with bromine for 10 min to give 1-bromo-2-methyl-3,4,5,6-tetramethoxybenzene (**1**) in 84% yield. This simple three step protocol affords **1** in 71% overall yield from the commercially available starting material **3**. Importantly, the products do not require purification by chromatography and as such the methodology is suitable for large scale synthesis.



Scheme 1 i. $SnCl_2$, c.HCl, EtOH; ii. dimethylsulfate, KOH, 80 °C, 85% (two steps); iii. Br_2, CH_2Cl_2, 5 °C, 84%.

1-Bromo-2-methyl-3,4,5,6-tetramethoxybenzene (**1**) was then used to prepare aldehyde **8**, a key precursor to side-chain functionalised analogues (**10** and **13**) of coenzyme Q, Schemes 2 and 3. The aryl bromide **1** was treated with nBuLi and copper bromide dimethylsulfide complex to give the corresponding diaryl cuprate which was coupled with geranyl bromide[13] to give **5** in an unoptimised yield of 48%. Treatment of **5** with *N*-bromosuccinimide then gave the bromohydrin (**6**), which upon reaction with potassium carbonate gave the corresponding epoxide (**7**). Cleavage of the epoxide (**7**) with periodic acid gave the desired aldehyde (**8**), which was used subsequently without purification.

With the key aldehyde **8** in hand we set about the preparation of side chain functionalised analogues of coenzyme Q, **10** and **13**, Scheme 3. Analogue **10** was synthesised in two steps; the aldehyde **8** was oxidized with silver nitrate and sodium hydroxide in ethanol to the carboxylic acid **9**, the quinone core of which was deprotected

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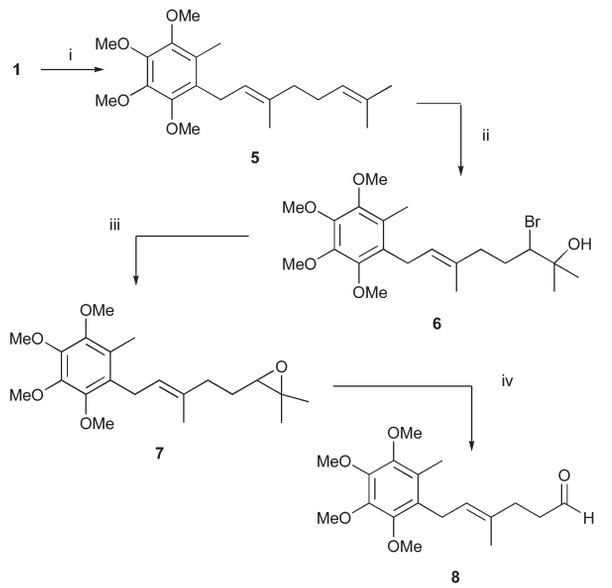
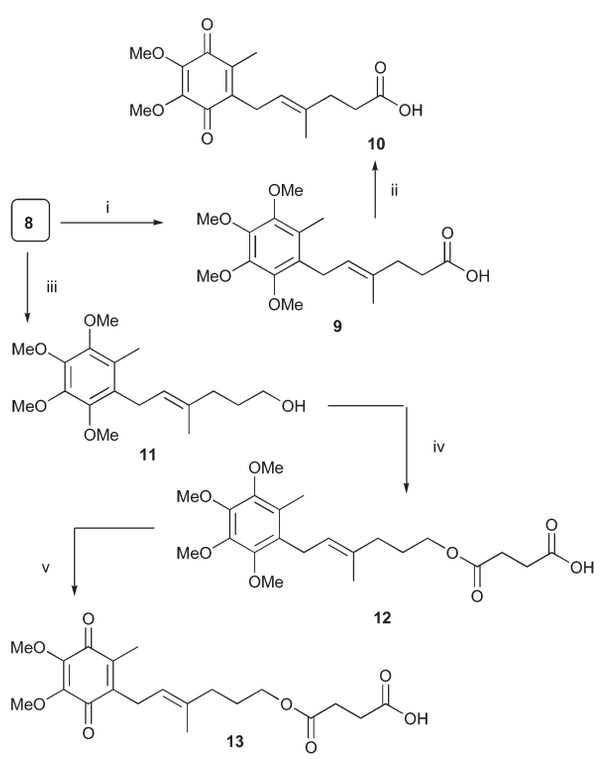


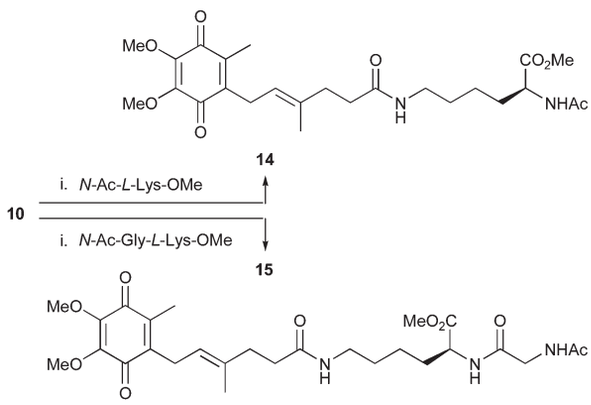
Scheme 2 i. nBuLi, CuBr·Me_2S, geranyl bromide, Et_2O, 0 °C–R.T., 48%; ii. NBS, THF/H_2O, 65%; iii. K_2CO_3, MeOH, 78%; iv. periodic acid, THF, Et_2O, 85%.

with cerric ammonium nitrate to give **10** (Scheme 3). Analogue **13** was prepared from aldehyde **8** as shown in Scheme 3; reduction of the aldehyde with sodium borohydride gave the corresponding alcohol (**11**), which was reacted with succinic anhydride in the presence of triethylamine and DMAP to give the corresponding succinic ester (**12**). Deprotection of the quinone with cerric ammonium nitrate afforded the target analogue **13**.



Scheme 3 i. $AgNO_3$, NaOH, EtOH, 78%; ii. CAN, MeCN/H_2O, 0 °C–R.T., 55%; iii. $NaBH_4$, MeOH, 0 °C–R.T., 74%; iv. succinic anhydride, DMAP, Et_3N, CH_2Cl_2, 37%; v. CAN, MeCN/H_2O, 0 °C–R.T., 90%.

Model studies were next carried out to investigate the feasibility of coupling a coenzyme Q analogue of this type to a protein as a first step to the development of an ELISA-based method for the detection of natural coenzyme Q, Scheme 4. We chose to use *N*-acetyl-L-lys methyl ester and a *N*-acetyl-gly-L-lys methyl ester for these studies, where the lysine side chain allows coupling to the acid of the analogue using standard peptide coupling conditions. It is important to note that lysine residues are commonly located on the surface of a protein so coupling to lysine is desirable for protein coupling experiments. The analogue **10** was used in these studies and its reaction with *N*-α-acteyl-lysine methyl ester and *N*-acetyl-glycine-lysine methyl ester proceeded cleanly in the presence of EDCI, HOBt and DIPEA in dichloromethane to give **14** and **15** in 63% and 34% yield, respectively (see Scheme 4).



Scheme 4 i. EDCI, HOBt, DIPEA, CH_2Cl_2.

The coupled products **14** and **15** were characterised by ^1H, ^{13}C, and two-dimensional NMR techniques, as well as by mass spectrometry. The ^1H NMR spectrum of **14** showed two amide resonances (5.77 and 6.40 ppm) and a multiplet at 4.55 ppm corresponding to the α-proton of the lysine residue. A series of multiplets at 1.35 (2H), 1.50 (2H), 1.69 (1H), 1.82 (1H), and 3.21 (2H) ppm were also observed for the side chain methylenes of lysine. Analysis of **15** by ^1H NMR spectroscopy revealed three amide resonances at 6.03, 6.74, and 7.08 ppm. The lysine α proton resonance was observed as a multiplet at 4.53 ppm, while the lysine side chain methylenes were observed as a series of multiplets at 1.34, 1.46, 1.71, 1.85, and 3.23 ppm. The α methylene of glycine was observed as two doublet of doublets at 3.91 and 4.01 ppm.

Conclusions

A new and efficient synthesis of 1-methyl-2,3,4,5-tetramethoxybenzene (**2**), and hence 1-bromo-2-methyl-3,4,5,6-tetramethoxybenzene (**1**), from the commercially available 2-methyl-5,6-dimethoxy-1,4-benzoquinone (**3**) has been developed. The reported procedure is quick and simple to carry out, the products are obtained in high yield and they do not require purification by chromatography. As such, this approach should be amenable to the large scale synthesis of these important precursors to coenzyme Q and analogues thereof. The synthesis of two side-chain functionalized analogues of CoQ from **1** is also described. We report an improved route to analogue **10**[22,24] and a preparation of the previously unknown analogue **13**. We have also have carried out studies on the coupling of **10** to lysine derivatives as a first step in the development of an immunoassay for the detection of coenzyme Q in biological tissues. This is an important goal since an assay of this type would allow the study and monitoring of diseases associated with coenzyme Q.

Experimental

General procedures

Proton detected NMR spectra were obtained on an Inova 500 spectrometer. Carbon detected NMR spectra were obtained on a Varian XL300 spectrometer or a Varian Unity 300 spectrometer both operating at 75 MHz. Chemical shifts are reported in parts per million (ppm), on the δ scale, and are referenced to the appropriate solvent peaks: $CDCl_3$ referenced to $(CH_3)_4Si$ at δ 0 ppm for ^1H spectra, and $CDCl_3$ at δ 77.0 ppm for ^{13}C spectra. All ^{13}C NMR spectra were obtained with a delay (D_1) of 1 s. ^1H and ^{13}C NMR spectra were assigned by comparison, with all assignments verified by 2-D NMR

experiments. Mass spectrometry was performed by Electron Impact (EI) at 4 kV using a Kratos MS80 Mass Spectrometer. All amino acid conjugates were analysed by electrospray mass spectrometry using a Micromass LCT spectrometer. IR spectra were obtained using a Shimadzu 8201PC series FTIR, oils were analysed neat on KBr disks. Unless otherwise stated all reactions were carried out in oven-dried, or flame-dried glassware under an N_2 atmosphere. Radial chromatography was carried out on 1 mm, 2 mm or 4 mm plates of Merck Silica Gel 60 PF_{254} containing gypsum, and visualised with short-wavelength ultraviolet light. Dichloromethane was distilled over calcium hydride, while diethyl ether and tetrahydrofuran were freshly distilled from sodium benzophenone ketal, under a nitrogen atmosphere before use. Coenzyme Q-0 (**3**) was obtained from Sigma Chemical Co®, and geranyl bromide was obtained from Aldrich Chemical Co®. Butyllithium was used fresh or titrated before use to determine exact concentration.

1-Bromo-2-methyl-3,4,5,6-tetramethoxybenzene (1). To a stirred solution of **2** (490 mg, 2.30 mmol, 1 equiv) in dichloromethane (2.3 cm^3) at 3–5 °C was added slowly a solution of bromine (0.12 cm^3, 2.30 mmol, 1 equiv) in dichloromethane (0.46 cm^3). The resulting dark orange solution was stirred for a further 5 min. The reaction mixture was washed with water (3×), dilute aqueous NaOH (3×), water, dried ($MgSO_4$) and solvent removed to give **1** (566 mg, 84%) as a yellow oil; υ_{max}/cm^{-1} 2937, 2858; δ_H(500 MHz; $CDCl_3$; Me_4Si) 2.30 (3H, s, ArMe), 3.78 (3H, s, OMe), 3.85 (3H, s, OMe), 3.91 (6H, s, 2 × OMe); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 15.98, 60.86, 60.90, 61.15, 61.39, 114.32, 127.31, 145.50, 146.35, 147.23, 148.31; *m*/*z* (EI) 290.0163 ($C_{11}H_{15}O_4{}^{79}Br$ requires 290.0151).

1-Methyl-2,3,4,5-tetramethoxybenzene (2). 2,3-Dimethoxy-5-methyl-1,4-benzoquinone **3** (1 g, 5.49 mmol, 1 equiv) was dissolved in hot ethanol and cooled rapidly in ice. To this was added a solution of stannous chloride (4.95 g, 0.02 mol, 4 equiv) in conc. HCl (5 cm^3) during which time the red colour faded to pale yellow. This was extracted with ethyl acetate (3×), dried ($MgSO_4$) and solvent removed to give pale yellow crystals. This was suspended in dimethylsulfate (7.2 cm^3, 0.08 mmol, 14 equiv) and stirred in a two-necked flask under nitrogen. To this was added portionwise a solution of potassium hydroxide (12 g, 0.22 mol, 40 equiv) in water (26 cm^3) not allowing the solution to reflux. During this addition a thick white precipitate formed, which then redissolved leaving a yellow solution that faded to white. Upon completion of the addition of potassium hydroxide the reaction was heated to 80 °C under a nitrogen atmosphere for 1 h, then allowed to cool slowly. The reaction was diluted with water (180 cm^3) and extracted with ether (3×). The combined organic fractions were washed with 5% aqueous NaOH, water, dried ($MgSO_4$) and solvent removed to afford **2**[15] (990 mg, 85%) as a pale yellow oil; υ_{max}/cm^{-1} 2937, 2837, 1591; δ_H(500 MHz; $CDCl_3$; Me_4Si) δ 2.22 (3H, s, ArMe), 3.78 (3H, s, OMe), 3.81 (3H, s, OMe), 3.86 (3H, s, OMe), 3.93 (3H, s, OMe), 6.44 (1H, s, ArH); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 15.84, 56.10, 60.65, 61.08, 61.17, 108.31, 125.89, 140.79, 145.40, 147.00, 149.11; *m*/*z* (EI) 212.1057 ($C_{11}H_{16}O_4$ requires 212.1049).

2,6-Dimethyl-8-(2-methyl-3,4,5,6-tetramethoxyphenyl)octa-2,6-diene (5). A solution of **1** (735 mg, 2.50 mmol) in diethyl ether (4.2 cm^3) was cooled to 0 °C under an Ar atmosphere, and nBuLi (1.6 M solution in hexanes, 1.8 cm^3, 1.15 equiv) was added slowly. During this addition the reaction went green then dark red and after stirring at 0 °C for 30 min a pale precipitate formed. To this solution copper bromide dimethylsulfide complex (360 mg, 0.7 equiv) was added, and stirring continued for 2.5 h during which time the solution turned dark purple. To this geranyl bromide (0.55 cm^3, 2.78 mmol, 1.1 equiv) in ether (2.8 cm^3) was slowly added and the reaction stirred at 0 °C for 2 h and room temperature for 3 h. The reaction was quenched *via* the addition of 10% aqueous HCl and the ether layer separated. The aqueous phase was extracted with ether (×3), and the combined organic fractions were washed with saturated aqueous $NaHCO_3$, water, dried ($MgSO_4$) and solvent removed to give the crude product. Purification by radial chromatography eluting with 1 : 19 ethyl acetate : petroleum ether afforded **5**[15,23] (426 mg, 48%) as a yellow oil; υ_{max}/cm^{-1} 2932, 2860, 1468; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.57 (3H, s, $=CCH_3$), 1.64 (3H, s, $=CCH_3$), 1.77 (3H, s, $=CCH_3$), 2.00 (2H, m, CH_2), 2.07 (2H, m, CH_2), 2.14 (3H, s, ArMe), 3.32 (2H, d, *J* 6.8, $ArCH_2$), 3.78 (3H, s, OMe), 3.79 (3H, s, OMe), 3.90 (3H, s, OMe), 3.91 (3H, s, OMe), 5.04 (2H, m, 2 × CH=); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.61, 16.11, 17.61, 25.62, 25.73, 26.55, 39.64, 60.61, 61.01, 61.03, 107.01, 122.88, 124.23, 125.35, 129.23, 131.25, 134.93, 144.63, 144.87, 147.61, 147.80; *m*/*z* (EI) 348.2306 ($C_{21}H_{32}O_4$ requires 348.2301).

3-Bromo-2,6-dimethyl-8-(2-methyl-3,4,5,6-tetramethoxyphenyl)oct-6-en-2-ol (6). A solution of 5 (420 mg, 1.20 mmol) in THF (40 cm^3) was cooled in an ice bath and water was added slowly to the reaction until it remained turbid. THF was then added dropwise until the reaction just cleared. *N*-Bromosuccinimide (236 mg, 1.33 mmol, 1.1 equiv) was added and the reaction stirred at room temperature for 3 h. The reaction was extracted with ether (×3), dried ($MgSO_4$) and solvent removed to give the crude product. Purification by radial chromatography, eluting with 1 : 9 ethyl acetate : petroleum ether to 1 : 4 ethyl acetate : petroleum ether gave the bromohydrin 6 (347 mg, 65%) as a yellow oil; υ_{max}/cm^{-1} 3477, 2936, 1740; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.32 (3H, s, C(OH)CH_3) 1.33 (3H, s, C(OH)CH_3), 1.77 (3H, s, $=CCH_3$), 1.79 (1H, m, CH_{2a}CBr), 1.98 (1H, m, CH_{2b}CBr), 2.09 (1H, m, $=CCH_{2a}$), 2.35 (1H, m, $=CCH_{2b}$), 2.14 (3H, s, ArMe), 3.33 (2H, m, $ArCH_2$), 3.79 (3H, s, OMe), 3.78 (3H, s, OMe), 3.90 (3H, s, OMe), 3.91 (3H, s, OMe), 3.95 (1H, dd, *J* 1.5 and 11.7, CHBr), 5.13 (1H, t, *J* 6.4, CH=); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.78, 16.14, 25.81, 25.86, 26.62, 32.10, 38.23, 60.70 (×2), 61.10 (×2), 70.85, 72.44, 124.42, 125.28, 128.85, 133.24, 144.70, 145.00, 147.62, 147.87; *m*/*z* (EI) 444.1516 ($C_{21}H_{33}O_5{}^{79}Br$ requires 444.1509).

3-[3-Methyl-5-(2-methyl-3,4,5,6-tetramethoxyphenyl)pent-3-enyl]-2,2-dimethyloxirane (7). To a stirred solution of **6** (100 mg, 0.22 mmol) in methanol (0.25 cm^3) was added potassium carbonate (2.17 g, 0.02 mol, 70 equiv) and the reaction was stirred at room temperature for 16 h. The reaction was filtered and solvent removed. Water was added and the resulting solution was extracted with dichloromethane, dried ($MgSO_4$) and solvent removed under reduced pressure to give **7**[22] (64 mg, 78%) as a pale yellow oil; υ_{max}/cm^{-1} 2961, 2934, 2862, 2829; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.24 (3H, s, CCH_3), 1.25 (3H, s, CCH_3), 1.64 (2H, m, CH_2CO) 1.80 (3H, s, $=CCH_3$), 2.10 (2H, m, $=CCH_2$), 2.17 (3H, s, ArMe), 2.67 (1H, t, *J* 6.4, CHO), 3.32 (2H, d, *J* 2.9, $ArCH_2$), 3.78 (3H, s, OMe), 3.79 (3H, s, OMe), 3.90 (3H, s, OMe), 3.91 (3H, s, OMe), 5.10 (1H, t, *J* 5.4, CH=); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.62, 16.07, 18.59 (2×), 24.67, 25.67, 27.26, 36.19, 58.17, 60.51, 60.90, 60.93, 63.99, 123.37, 125.12, 128.79, 133.94, 144.57, 144.88, 147.50, 147.73; *m*/*z* (EI) 364.2254 ($C_{21}H_{32}O_5$ requires 364.2250).

4-Methyl-6-(2-methyl-3,4,5,6-tetramethoxyphenyl)hex-4-enal (8). To a stirred solution of **7** (120 mg, 0.33 mmol) in diethyl ether (16 cm^3) was added a solution of periodic acid (75 mg, 0.33 mmol, 1 equiv) in THF (8 cm^3). This was stirred at room temperature for 1.5 h during which time a fine precipitate formed. The reaction was quenched *via* the addition of water, the ether layer was separated and the aqueous phase extracted with ether (×2). The combined organic fractions were dried ($MgSO_4$) and solvent removed under reduced pressure to afford **8** (90 mg, 85%) as a pale yellow oil (Found: C, 66.9; H, 7.9. Calc. for $C_{18}H_{26}O_5$: C, 67.1; H, 8.1%); υ_{max}/cm^{-1} 2936, 1724; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.79 (3H, s, $=CCH_3$), 2.12 (3H, s, ArMe), 2.33 (2H, t, *J* 7.8, C$H_2$$CH_2$CHO), 2.52 (2H, dt, *J* 2.0 and 7.3, CH_2CHO), 3.32 (2H, d, *J* 5.9, $ArCH_2$), 3.78 (3H, s, OMe), 3.79 (3H, s, OMe), 3.90 (3H, s, OMe), 3.91 (3H, s, OMe), 5.09 (1H, t, *J* 6.4, CH=), 9.74 (1H, t, *J* 1.9, CHO); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.72, 16.27, 25.76, 31.79, 42.13, 60.68 (×2), 61.07 (×2), 123.99, 125.27, 128.62, 133.00, 144.68, 145.07, 147.62, 147.85, 202.47; *m*/*z* (EI) 322.1771 ($C_{18}H_{26}O_5$ requires 322.1780).

4-Methyl-6-(2-methyl-3,4,5,6-tetramethoxyphenyl)hex-4-enoic acid (9). To a solution of silver nitrate (82 mg, 0.54 mmol, 1.5 equiv) in ethanol (3.9 cm^3) stirring at room temperature under a nitrogen atmosphere was added a solution of **8** (115 mg, 0.36 mmol) in ethanol (2.3 cm^3). To this was added dropwise a solution of NaOH (0.18 cm^3 of a 5 N aqueous solution) in ethanol (2.3 cm^3) over a period of 10 min. The resulting black solution was stirred at room temperature for 16 h. The reaction was filtered and ethanol removed under reduced pressure. Water was added, and the solution acidified and extracted with ethyl acetate (2×). Combined organic fractions were dried ($MgSO_4$) and solvent removed to afford **9**[24] (94 mg, 78%) as a yellow oil (Found: C, 64.1; H, 7.8. Calc. for $C_{18}H_{26}O_6$: C 63.9; H 7.7%); υ_{max}/cm^{-1} 2936, 2660, 1709; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.79 (3H, s, $=CCH_3$), 2.12 (3H, s, ArMe), 2.31 (2H, t, *J* 7.3, $=CCH_2$), 2.45 (2H, t, *J* 6.8, CH_2CO_2H), 3.32 (2H, d, *J* 6.4, $ArCH_2$), 3.77 (3H, s, OMe), 3.78 (3H, s, OMe), 3.89 (3H, s, OMe), 3.90 (3H, s, OMe), 5.09 (1H, t, *J* 6.8, CH=); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.69, 16.13, 25.75, 32.67, 34.27, 60.68 (×2), 61.08 (×2), 123.98, 125.32, 128.72, 133.03, 144.65, 145.02, 147.62, 147.83, 178.47; *m*/*z* (EI) 338.1729 ($C_{18}H_{26}O_6$ requires 338.1729).

4-Methyl-6-(3-methyl-5,6-dimethoxy-1,4-benzoquinon-2-yl)hex-4-enoic acid (10). To a solution of **9** (90 mg, 0.27 mmol) in a 2 : 1 acetonitrile : water mixture (1.1 cm^3) was added a cooled solution of cerric ammonium nitrate (CAN) (365 mg, 0.66 mmol, 2.5 equiv) in a 1 : 1 acetonitrile : water mixture (1.3 cm^3), over a 10 min period. The resulting solution was stirred at 0 °C for 20 min and room temperature for 10 min before quenching *via* the addition of water. The resulting reaction mixture was extracted with dichloromethane (×2) and the combined organic fractions washed with water, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by radial chromatography eluting with dichloromethane to 1 : 19 MeOH : dichloromethane gave **10**[24] (45 mg, 55%) as a bright orange oil; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.77 (3H, s, $=CCH_3$), 2.00 (3H, s, ArMe), 2.30 (2H, m, $=CCH_2$), 2.43 (2H, m, $=CCH_2CH_2$), 3.19 (2H, d, *J* 6.8, $ArCH_2$), 3.98 (3H, s, OMe), 3.99 (3H, s, OMe), 5.00 (1H, m, CH=); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.88, 16.13, 25.17, 32.61, 34.19, 61.09 (×2), 120.02, 135.50, 139.01, 141.17, 144.17, 144.27, 179.03, 183.79, 184.60; *m*/*z* (EI) 308.1263 ($C_{16}H_{20}O_6$ requires 308.1260).

4-Methyl-6-(2-methyl-3,4,5,6-tetramethoxyphenyl)hex-4-en-1-ol (11). To a stirred solution of sodium borohydride (10 mg, 0.27 mmol, 1 equiv) in methanol (0.55 cm^3) at 0 °C was added a solution of **8** (88 mg, 0.27 mmol) in methanol (1.1 cm^3). The reaction was stirred and allowed to warm to room temperature. After 20 min saturated aqueous NH_4Cl was added to quench the reaction, and methanol was removed under reduced pressure. The resulting aqueous phase was extracted with dichloromethane (×2), and the combined organic fractions washed with saturated aqueous NaCl, water, dried ($MgSO_4$) and solvent removed under reduced pressure affording **11** (65 mg, 74%) as a yellow oil (Found: C, 66.7; H, 8.6. Calc. for $C_{18}H_{28}O_5$: C, 66.6; H, 8.7%); υ_{max}/cm^{-1} 3435, 2936, 1466, 1408; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.65 (2H, m, CH_2CH_2OH), 1.76 (3H, s, $=CCH_3$), 2.04 (2H, t, *J* 6.8, $=CCH_2$), 2.11 (3H, s, ArMe), 3.30 (2H, d, *J* 6.8, $ArCH_2$), 3.59 (2H, t, *J* 6.4, CH_2OH), 3.76 (3H, s, OMe), 3.77 (3H, s, OMe), 3.88 (3H, s, OMe), 3.89 (3H, s, OMe), 5.06 (1H, dt, *J* 1.5 and 6.8, CH=); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.88, 16.08, 25.90, 30.77, 36.26, 60.94, 61.99, 61.24 (×2), 62.76, 123.24, 125.22, 129.03, 134.82, 144.72, 145.03, 147.62, 147.92; *m*/*z* (EI) 324.1937 ($C_{18}H_{28}O_5$ requires 324.1937).

4-Methyl-6-(2-methyl-3,4,5,6-tetramethoxyphenyl)hex-4-enyl hydrogen succinate (12). A solution of succinic anhydride (34 mg, 0.34 mmol, 2.1 equiv), *N*,*N*-dimethylaminopyridine (1 mg, 0.01 mmol, 0.05 equiv) and triethylamine (0.03 cm^3, 0.24 mmol, 1.5 equiv) in dichloromethane (0.6 cm^3) was added slowly to a solution of **11** (52 mg, 0.16 mmol) in dichloromethane (1.6 cm^3). This was stirred at room temperature under an Ar atmosphere for 16 h. The reaction was diluted with dichloromethane and washed with 2 N aqueous HCl, water, saturated aqueous $NaHCO_3$, water, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by radial chromatography eluting with 1 : 9 ethyl acetate : petroleum ether afforded **12** (24 mg, 37%) as a pale yellow oil (Found: C 62.4; H 7.7. Calc. for $C_{22}H_{32}O_8$: C, 62.3; H, 7.6%); υ_{max}/cm^{-1} 2937, 1738; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.73 (2H, m, CH_2CH_2O), 1.76 (3H, s, $=CCH_3$), 2.03 (2H, t, *J* 6.8, $=CCH_2$), 2.12 (3H, s, ArMe), 2.59 and 2.65 (4H, m, $COCH_2CH_2CO_2H$), 3.30 (2H, d, *J* 6.4, $ArCH_2$), 3.78 (3H, s, OMe), 3.78 (3H, s, OMe), 3.89 (3H, s, OMe), 3.90 (3H, s, OMe), 4.05 (2H, dt, *J* 2.4 and 6.8, CH_2O), 5.05 (1H, t, *J* 6.4, CH=); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.66, 15.98, 25.71, 26.19, 26.69, 28.87, 35.65, 60.65, 61.05, 64.46, 64.66, 123.57, 125.32, 126.98, 128.92, 133.71, 144.59, 144.90, 147.48, 147.78, 172.22, 176.96; *m*/*z* (EI) 424.2129 ($C_{22}H_{32}O_8$ requires 424.2097).

4-Methyl-6-(3-methyl-5,6-dimethoxy-1,4-benzoquinon-2-yl)-hex-4-enyl hydrogen succinate (13). To a solution of **12** (24 mg, 0.06 mmol) in a 2 : 1 acetonitrile : water mixture (0.25 cm^3) was added a cooled solution of cerric ammonium nitrate (CAN) (77 mg, 0.14 mmol, 2.5 equiv) in a 1 : 1 acetonitrile : water mixture (0.3 cm^3), over a 10 min period. The resulting solution was stirred at 0 °C for 20 min and room temperature for 10 min before quenching *via* the addition of water. The resulting reaction mixture was extracted with dichloromethane (×2) and the combined organic fractions washed with water, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by radial chromatography eluting with dichloromethane to 1 : 19 MeOH : dichloromethane gave **13** (20 mg, 90%) as an orange oil; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.72 (2H, m, CH_2CH_2O), 1.74 (3H, s, $=CCH_3$), 2.02 (3H, s, ArMe), 2.03 (2H, m, $=CCH_2$), 2.60–2.66 (4H, m, $COCH_2CH_2CO_2H$), 3.18 (2H, d, *J* 6.8, $ArCH_2$), 3.98 (3H, s, OMe), 4.00 (3H, s, OMe), 4.03 (2H, t, *J* 6.3, CH_2O), 4.95 (1H, t, *J* 7.1, CH=); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.95, 16.12, 25.32, 26.58, 28.79, 28.82, 35.66, 61.14 (×2), 64.29, 119.73, 136.27, 138.94, 141.38, 144.21, 144.34, 172.13, 177.24, 183.93, 184.74; *m*/*z* (EI) 394.1621 ($C_{20}H_{26}O_8$ requires 394.1623).

Methyl 2-acetylamino-6-[4-methyl-6-(3-methyl-5,6-dimethoxy-1,4-benzoquinon-2-yl)hex-4-enoylamino]hexanoate (14). To a solution of **10** (10 mg, 0.032 mmol) and *N*-α-acetyl-L-lys methyl ester hydrochloride (8.5 mg, 0.036 mmol, 1.1 equiv) in dichloromethane (0.3 cm^3) stirring under an Ar atmosphere, was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl (EDCI) (8 mg, 0.042 mmol, 1.3 equiv), 1-hydroxybenzotriazole hydrate (6.5 mg, 0.048 mmol, 1.5 equiv) and diisopropylethylamine (6 μL, 0.035 mmol, 1.1 equiv). The resulting solution was stirred at room temperature for 16 h. The reaction was quenched *via* the addition of 3 M aqueous NaCl solution and the layers separated. The aqueous phase was extracted with dichloromethane (×2), and combined organic fractions were dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification by radial chromatography eluting with 1 : 19 MeOH : dichloromethane afforded **14** (10 mg, 63%) as an orange oil; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.35 (2H, m, $NHCH_2CH_2$), 1.50 (2H, m, $NHCH_2CH_2CH_2$), 1.68 (1H, m, $CH_{2a}CHCO_2Me$), 1.76 (3H, s, $=CCH_3$), 1.82 (1H, m, $CH_{2b}CHCO_2Me$), 2.01 (3H, s, ArMe), 2.04 (3H, s, COMe), 2.24 (2H, m, $=CCH_2$), 2.29 (2H, m, $=CCH_2CH_2$), 3.18 (2H, d, *J* 6.8, $ArCH_2$), 3.21 (2H, m, $NHCH_2$), 3.74 (3H, s, CO_2Me), 3.99 (3H, s, OMe), 4.00 (3H, s, OMe), 4.55 (1H, m, $CHCO_2Me$), 4.99 (1H, t, *J* 7.3, CH=), 5.77 (1H, brs, NH), 6.40 (1H, d, *J* 6.8, NH); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.95, 16.23, 22.25, 23.05, 25.33, 28.84, 31.79, 35.13, 35.27, 38.68, 51.82, 52.38, 61.13, 61.16, 119.91, 136.23, 139.06, 141.24, 144.20, 170.30, 172.85, 172.98, 182.30, 183.90; *m*/*z* (ES) 492.2465 ($C_{25}H_{36}N_2O_8$ requires 492.2472).

Methyl 2-(2-acetylamino-acetylamino)-6-[4-methyl-6-(3-methyl-5,6-dimethoxy-1,4-benzoquinon-2-yl)hex-4-enoylamino]hexanoate (15). To a solution of **10** (10 mg, 0.03 mmol) and *N*-acetyl-gly-L-lys methyl ester acetate (11 mg, 0.033 mmol, 1.1 equiv) in dichloromethane (0.3 cm^3) stirring under an Ar atmosphere, was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl (EDCI) (7.5 mg, 0.039 mmol, 1.3 equiv), 1-hydroxybenzotriazole hydrate (6 mg, 0.045 mmol, 1.5 equiv) and

diisopropylethylamine (6 μL, 0.033 mmol, 1.1 equiv). The resulting solution was stirred at room temperature for 16 h. The reaction was quenched *via* the addition of 3 M aqueous NaCl solution and the layers separated. The aqueous phase was extracted with dichloromethane (×2), and combined organic fractions were dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification by radial chromatography eluting with 1 : 19 MeOH : dichloromethane afforded **15** (6 mg, 34%) as an orange oil; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.34 (2H, m, $NHCH_2CH_2$), 1.46 (2H, m, $NHCH_2CH_2CH_2$), 1.71 (1H, m, $CH_{2a}CHCO_2Me$), 1.76 (3H, s, $=CCH_3$), 1.85 (1H, m, $CH_{2b}CHCO_2Me$), 2.01 (3H, s, ArMe), 2.05 (3H, s, COMe), 2.28 (2H, m, $=CCH_2$), 3.15 (1H, m, $NHCH_{2a}$), 3.17 (2H, d, *J* 7.3, $ArCH_2$), 3.23 (1H, m, $NHCH_{2b}$), 3.74 (3H, s, CO_2Me), 3.91 (1H, dd, *J* 5.4 and 16.1, $COCH_{2a}NH$), 3.99 (3H, s, OMe), 4.00 (3H, s, OMe), 4.01 (1H, dd, *J* 5.4 and 16.1, $COCH_{2b}NH$), 4.53 (1H, m, $CHCO_2Me$), 4.98 (1H, t, *J* 6.8, CH=), 6.03 (1H, brs, NH), 6.74 (1H, brs, NH), 7.08 (1H, d, *J* 7.3, NH); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 11.97, 16.28, 22.17, 22.89, 25.37, 28.86, 31.21, 35.04, 35.23, 38.55, 43.13, 52.01, 52.45, 61.15, 61.18, 119.85, 136.27, 139.10, 141.25, 143.01, 144.32, 169.08, 170.91, 172.51, 173.01, 183.96, 184.65; *m*/*z* (ES) 549.2677 ($C_{27}H_{39}O_9N_3$ requires 549.2686).

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