Reductive thioalkylation of a pyranonaphthoquinone

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Reduction of pyranonaphthoquinone **6** with sodium dithionite in the presence of the sulfur nucleophiles thiocresol, phenylmethanethiol and butanethiol afforded the thioalkylated products **7** and **8**, **9** and **10**, and **11** and **12** respectively. This observed reductive thioalkylation at C-4 supports the postulate that naturally occurring pyranonaphthoquinone antibiotics may act as bioreductive alkylating agents.

Successful treatment of cancerous tumours by chemotherapy is dependant upon exploiting the differences between host and target cells. Solid tumours, have regions of poor vascularity and therefore hypoxic (oxygen deficient) cells may exist in these areas. This difference in the population of hypoxic cells allows the possibility of designing drugs capable of bioactivation in a hypoxic environment. Quinones can be activated by a reductive mechanism and therefore offer potential for development as hypoxia selective cytotoxins.


kalafungin **1**

nanaomycin A **2**

nanaomycin D **3**
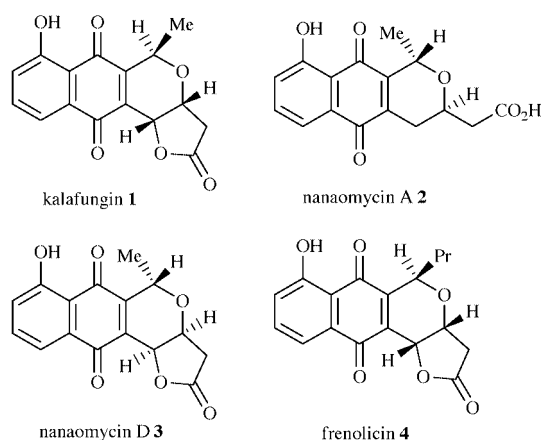
frenolicin **4**

Kalafungin **1**,[1] nanaomycin A **2**,[2] nanaomycin D **3**[3] and frenolicin **4**[4] belong to the pyranonaphthoquinone family of antibiotics, which are produced by various species of *Streptomyces* and have in common a benzoisochromenedione skeleton.[5] Moore and Czerniak[6,7] have proposed that *in vivo* reduction of pyranonaphthoquinones such as kalafungin **1** to a hydroquinone triggers a molecular rearrangement to give a quinone or a bis-quinomethane which may function as an alkylating agent or a bis-alkylating agent respectively (Scheme 1). These pyranonaphthoquinone antibiotics may well exhibit antitumour activity if the nucleophile involved were the nitrogenous base of a DNA molecule invoking a mechanism of action that resembles the anticancer agent mitomycin C **5**.[7] The work reported herein[8] describes our efforts to provide the first experimental evidence to support the Moore and Czerniak hypothesis that pyranonaphthoquinones can act as bioreductive alkylating agents.

The main thrust of research into hypoxia selective cytotoxins based on the bioreductive activation of a quinone functionality
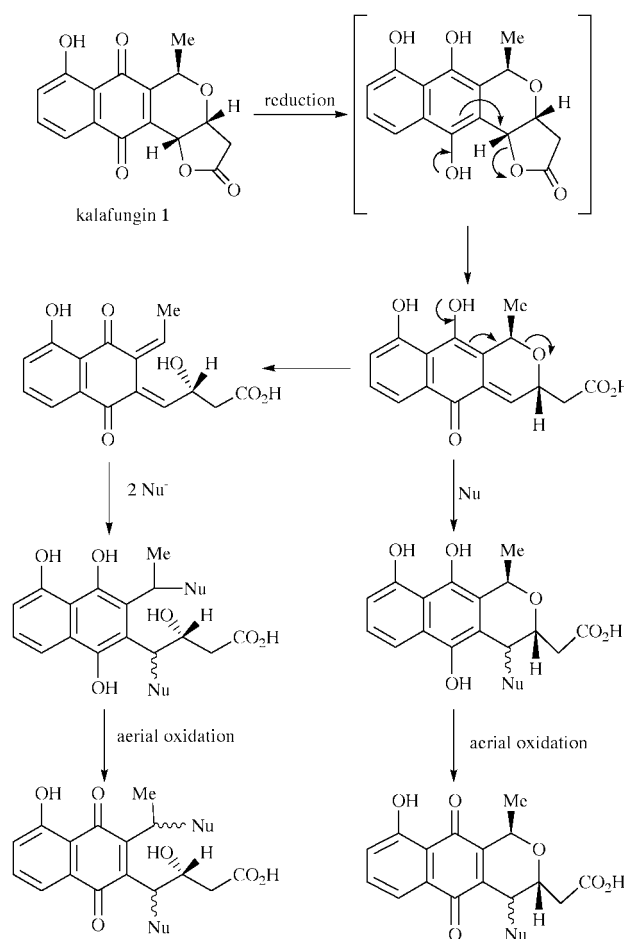
Scheme 1
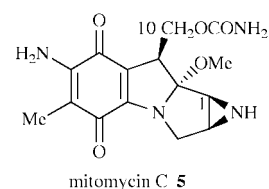
mitomycin C **5**

has focused on the use of mitomycin C **5** and analogues thereof,[9] anthracycline antitumour drugs[10,11] and model quinomethanes.[12] Previous experimentation under a variety of conditions—enzymatic,[13,14] catalytic,[15] chemical,[13,16] electrochemical[17] and pulse radiolysis[18]—led to the rapid activation of mitomycin C **5** at moderate pH (pH 7–8.5) to an unstable

quinomethane to give C-1 substituted products. These protocols were used as the basis for the present work using pyranonaphthoquinones.

Previous work by this research group has established an efficient synthesis of several members of the pyranonaphthoquinone family of antibiotics using a furofuran annulation–oxidative rearrangement strategy.[19–22] The reduction potentials and pulse radiolysis decay kinetics of several synthetic pyranonaphthoquinones have also been investigated.[23] In the present work attention is focused on the reductive alkylation of kalafungin analogue 6, which was readily prepared following our published procedure.[19]

In the present work the lack of a "chemical library" of reductive alkylation products to facilitate product identification by HPLC necessitated that the reductive alkylation products were formed in sufficient quantities to enable full spectroscopic characterisation of the products formed. After reviewing the literature on the reductive activation of mitomycin C 5, several reducing agents, sodium dithionite,[9,10] 4-methoxyphenylhydrazine,[24] sodium borohydride,[25] PtO$_2$/H$_2$,[26] and chromium(II) perchlorate[27] were selected using imidazole, thiocresol, dithiothreitol and aniline as nucleophiles.

In the case of mitomycin C 5 the use of buffered methanol (pH 7.4, Trizma) as solvent simplified the product profile.[9] In the present work buffered 1:1 methanol–THF was employed as the solvent in order to facilitate the solubility of pyranonaphthoquinone 6. Treatment of pyranonaphthoquinone 6 with aqueous sodium dithionite under argon followed by the addition of thiocresol afforded a more polar product which proved difficult to isolate by flash chromatography. Subsequent treatment of the crude product with ethereal diazomethane afforded a less polar product (53%) which was then purified by flash chromatography. Analysis of this product by ^{1}H NMR established that it was in fact a 3.1:1 mixture of thioadducts 7:8 (Scheme 2).
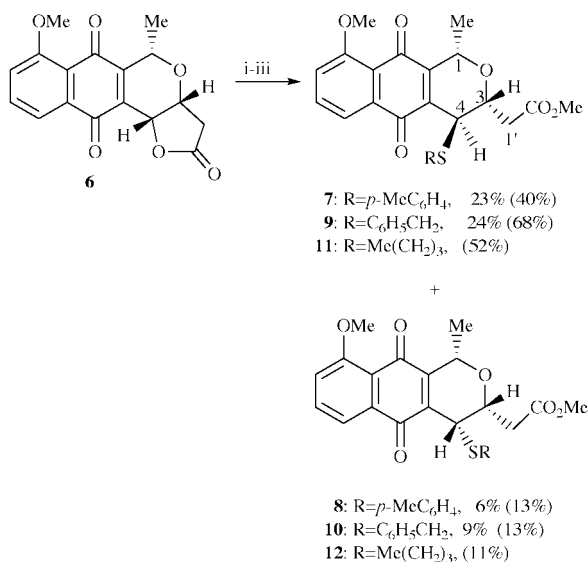
Scheme 2 *Reagents and conditions*: (i) sodium dithionite, 1:1 THF–MeOH, Trizma, Ar, then RSH (4.0 equiv.), rt, 4 h; (ii) oxidation (air); (iii) Et$_2$O, CH$_2$N$_2$. Yields in parentheses were determined by ^{1}H NMR analysis of the product mixture isolated after flash chromatography but prior to separation of the diastereomers by HPLC.

The products were separated by preparative HPLC and their ^{1}H NMR spectra exhibited differences in the chemical shifts for the resonances assigned to the methyl group at C-1 and the pyran ring proton, 4-H. The stereochemistry of the thiol adducts 7 and 8 was determined by the magnitude of the vicinal coupling between H-3 and H-4 (Fig. 1). Given that the methyl group at C-1 and the ester side chain at C-3 adopt pseudoequatorial positions on the flattened six-membered ring, the
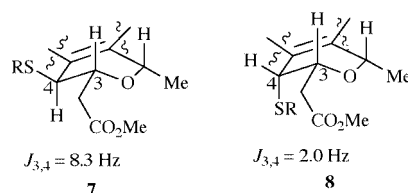

Fig. 1

large diaxial coupling constant, $J_{3,4} = 8.3$ Hz,‡ observed for the major isomer 7 established that the thiol group adopted a pseudoequatorial position. In the minor isomer 8, the coupling constant $J_{3,4} = 2.0$ Hz established that the thiol group adopted a pseudoaxial position. These assignments are consistent with preferential attack of the thiol from the top face of the molecule thereby avoiding unfavourable steric hindrance from the ester side chain at C-3. The minor isomer 8 converted to the major isomer 7 upon standing in deuteriochloroform overnight, presumably *via* formation of a quinomethane intermediate.

The assignment of stereochemistry to the phenylmethanethiol adducts 9 and 10 was made by analogy to the thiocresol adducts 7 and 8 with the *trans* isomer 9 exhibiting a large vicinal coupling constant, $J_{4,3} = 9.5$ Hz. In the case of the butanethiol adducts, however, the magnitude of this vicinal coupling constant in both the *trans* isomer 11 and the *cis* isomer 12 was the same, namely, $J_{4,3} = 2.0$ Hz. Presumably in this case the pyran ring is distorted to a skew boat conformation such that the (H)C-3–C-4(H) dihedral angle in both isomers is similar.

Thiocresol was the only nucleophile which reacted cleanly to afford one major product (albeit as a mixture of stereoisomers). Use of imidazole and aniline at both pH 7.4 (Trizma) and pH 5.5 (Bis-Tris)[10] afforded baseline material which proved to be intractable even after addition of diazomethane. A complex mixture was also afforded using dithiothreitol as a nucleophile.

Use of 4-methoxyphenylhydrazine[24] as the reducing agent gave comparable results to the dithionite case in that only use of thiocresol as the nucleophile afforded any reductive alkylation products. The yield of thioadducts 7 and 8 obtained, however, was much lower than when using dithionite as the reductant. Use of sodium borohydride[25] and chromium perchlorate[27] as reducing agents resulted in extensive and rapid loss of starting material to baseline material while hydrogenation of pyranonaphthoquinone 6 over platinum(IV) oxide resulted in rapid formation of the hydroquinone, however, subsequent addition of thiocresol afforded the thioadducts 7 and 8 in low yield (23%).

Given the success realised using thiocresol as a nucleophile with sodium dithionite as the reducing agent, phenylmethanethiol and butanethiol were also employed as nucleophiles under similar conditions and the analogous adducts were isolated (9 and 10, and 11 and 12 respectively). The phenylmethanethiol adducts 9 and 10 required separation by preparative HPLC, however, the thiobutyl adducts 11 and 12 were separable by flash chromatography. More complex thiols such as *N*-acetylcysteine[28] and potassium ethyl xanthate[29] were also used, however, the adducts proved to be extremely difficult to isolate. Carbon (sodium diethylmalonate)[12] and oxygen (ethanol and ethoxide)[12] based nucleophiles were also employed, however, no isolable material was recovered from these experiments.

The lack of success in reacting pyranonaphthoquinone 6 with nucleophiles other than thiols was reminiscent of recent work[12] which focused on anthracycline systems. In these systems sulfur based nucleophiles afforded stable adducts while oxygen and nitrogen based nucleophiles were thought to form unstable adducts due to the reversibility of the addition.

The intermediacy of the hydroquinone 13 of pyrano-

‡ This coupling constant was erroneously reported as 13.9 Hz in our earlier communication.[8]

naphthoquinone **6** was confirmed by reductive acetylation to diacetate **14** using sodium dithionite or hydrogenation over platinum(IV) oxide followed by treatment with acetic anhydride and triethylamine. Reaction of pyranonaphthoquinone **6** with thiols after reduction with dithionite therefore presumably occurs *via* intermediacy of the hydroquinone. Two pathways are then available for incorporation of a thiol at C-4 (Scheme 3).

Scheme 3
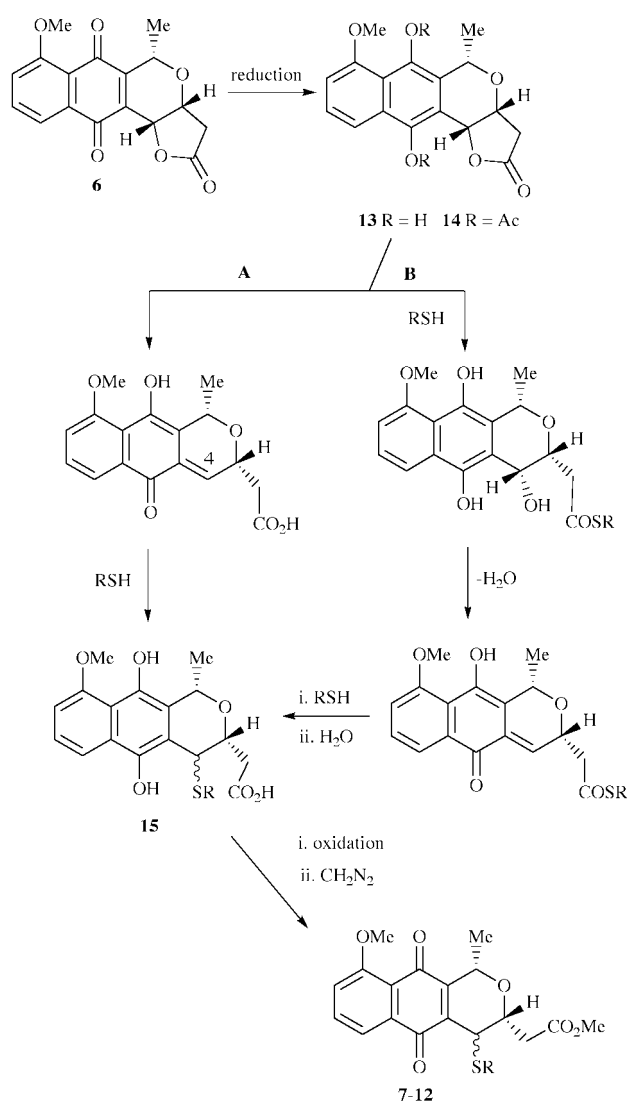
The first possibility (pathway **A**) is that the initial hydroquinone **13** undergoes rearrangement to a quinomethane intermediate which undergoes nucleophilic attack at C-4 by the thiol to afford a hydroquinone **15** that is readily oxidized to the final quinonoid product. Alternatively, the lactone of the initial hydroquinone **13** undergoes nucleophilic addition to afford a thioester (pathway **B**) which undergoes elimination of water to form a quinomethane. Subsequent addition of thiol and hydrolysis of the thioester affords the same intermediate **15** that is formed from pathway **A**.

The experiments described above were carried out in such a way that reduction to a hydroquinone preceded the thioalkylation step, however, it was of note that when pyranonaphthoquinone **6** was treated with thiocresol in the absence of dithionite, thioalkylation products **7** and **8** were formed in addition to di-*p*-tolyl disulfide. This observation suggests that the thiol may effect initial reduction of the quinone to a semiquinone with thioalkylation proceeding *via* a semiquinone radical–thiyl radical coupling. Whilst the precise mechanism by which thioalkylation proceeds remains open for discussion, the

work reported herein supports the postulate that naturally occurring pyranonaphthoquinones such as kalafungin **1** may undergo similar reductive thioalkylations mediated by an enzyme *via* a mechanism similar to that proposed by Moore and Czerniak.[6,7]

Experimental

Mps were determined using a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded using a Perkin-Elmer 1600 Fourier Transform infra-red spectrophotometer as solutions in the solvent specified. Absorption maxima are expressed in wavenumbers (cm^{-1}) with the following abbreviations: vs = very strong, s = strong, m = medium, w = weak and br = broad. ^{1}H NMR spectra were recorded on a Bruker AC 200 (200 MHz) or a Bruker AM 400 (400 MHz) spectrometer at ambient temperature. All *J* values are given in Hz. Chemical shifts are expressed in parts per million downfield shift from tetramethylsilane as an internal standard, and reported as position (δ_H) (relative integral, multiplicity (s = singlet, br s = broad singlet, d = doublet, dd = double doublet, ddd = double double doublet, t = triplet, dt = doublet of triplets, q = quartet, qd = quartet doublet, m = multiplet), coupling constant (*J* Hz) and assignment). ^{13}C NMR spectra were recorded on a Bruker AC 200 (50.3 MHz) or a Bruker AM 400 (100.6 MHz) spectrometer at ambient temperature with complete proton decoupling. Data are expressed in parts per million downfield shift from tetramethylsilane as an internal standard and reported as position (δ_C), (multiplicity (aided by DEPT135, COSY and HETCOR experiments) and assignment). Some peaks may be coincidental. Low resolution mass spectra were recorded on either an AEI Kratos MS50 mass spectrometer operating with an ionization potential of 70 eV or a Kratos Concept ISQ with an ion source of 5.3 kV. LSIMS spectra were recorded between *m/z* 200–550. High resolution mass spectra (EI and LSIMS) were recorded at a nominal resolution of 7000. Major fragmentations are given as percentages relative to the base peak intensity and assigned where possible. Ionization methods employed were electron impact (EI) or liquid secondary ion mass spectrometry (LSIMS) using caesium(I) ions as the primary beam (10 kV) and *m*-nitrobenzyl alcohol as a matrix. Thin layer chromatography (TLC) was performed using 0.2 mm thick precoated silica gel plates (Merck Kieselgel 60 F$_{254}$ or Riedel-de Haen Kieselgel S F$_{254}$). Compounds were visualized by ultra-violet fluorescence or by staining with iodine or vanillin in methanolic sulfuric acid. Flash chromatography was performed using Merck Kieselgel 60 or Riedel-de Haen Kieselgel S silica gel (both 230–400 mesh) with the indicated solvents. Concentration "*in vacuo*" or "at reduced pressure" refers to concentration using a rotary evaporator connected to a water aspirator. Removal of residual solvent or volatile reagents where desired was achieved by evacuation (0.1–0.01 mmHg) with a high stage oil vacuum pump. Ether refers to diethyl ether, hexane refers to light petroleum (40/60), Trizma refers to tris(hydroxymethyl)aminomethane (pH 7.4). Preparative HPLC was carried out on a Whatman Partisil column (50 cm length, 22 mm id, 10 μm particle size) with 3:1 hexane–ethyl acetate as solvent, flow rate 13.5 mL min^{-1}, pressure 800 psi, UV detection at 254 nm. R_t refers to HPLC retention time (min).

General procedure for reductive thioalkylation of 6

A solution of **6** (6 mg, 0.020 mmol) in 1:1 THF–MeOH (2.0 mL) and Trizma buffer (pH 7.4, 0.7 mL) was degassed for 15 min with dry, oxygen-free argon. To this solution was added sodium dithionite (approx. 1 equiv.) to effect hydroquinone formation, followed by a solution of the appropriate nucleophile (4 equiv.) in degassed 1:1 THF–MeOH (0.3 mL). The reaction was stirred at room temperature under argon and

monitored periodically by TLC. Following extraction of the mixture by ethyl acetate (2×15 mL) the organic layer was washed with water (2×10 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue obtained dissolved in diethyl ether (2 mL). To this was added dry silica (230–400 mesh) and ethereal diazomethane, and the reaction stirred at room temperature for 15 min. A repeat of the previous work up procedure afforded an oil that was purified by flash chromatography using 1:2 hexane–ethyl acetate as eluant.

Using thiocresol as nucleophile

Treatment of **6** (25.6 mg, 0.082 mmol) with sodium dithionite and thiocresol following the general procedure described above afforded an orange solid (19.6 mg, 53%) which was established to be a 3.1:1 mixture of thioadducts **7**:**8** by ^{1}H NMR. Further purification of this mixture by HPLC on a Whatman Partisil column using 3:1 hexane–ethyl acetate as solvent afforded **7** and **8**.

Methyl (1S*,3R*,4S*)-5,10-dioxo-9-methoxy-1-methyl-3,4,5, 10-tetrahydro-4-(p-tolylthio)-1H-naphtho[2,3-c]pyran-3-yl-acetate 7. A yellow solid (8.5 mg, 23%) (R_t 64.4 min); mp 156–158 °C (Found: MH$^+$, 453.13671. $C_{25}H_{25}O_6S$ requires MH$^+$, 453.13718); ν_{max} (CH$_2$Cl$_2$)/cm^{-1} 1740s (C=O, ester), 1662s, 1652s (C=O, quinone), 1586s (C=C) and 1266s (C–O); δ_H (400 MHz; CDCl$_3$) 1.44 (3H, d, J 6.7, CHMe), 2.28 (3H, s, C$_6$H$_4$$Me$), 2.62 (1H, dd, J_{gem} 15.6 and $J_{1'A,3}$ 8.3, CH^ACO$_2$Me), 3.09 (1H, dd, J_{gem} 15.6 and $J_{1'B,3}$ 3.0, CH^BCO$_2$Me), 3.72 (3H, s, CO$_2$Me), 3.99–4.07 (1H, m, 4-H), 4.00 (3H, s, OMe), 4.04 (1H, ddd, $J_{3,4}$ 8.3, $J_{3,1'B}$ 8.3 and $J_{3,1'B}$ 3.0, 3-H), 4.46 (1H, qd, $J_{1,Me}$ 6.7 and $J_{1,4}$ 2.2, CHMe), 7.03 (2H, d, J 7.9, C$_6$H$_4$Me), 7.26–7.31 (3H, m, C$_6$H$_4$Me and 6-H or 8-H), 7.67–7.71 (1H, m, 7-H) and 7.81 (1H, dd, J 7.6 and 1.0, 8-H or 6-H); δ_C (100.6 MHz; CDCl$_3$) 20.5 (CH$_3$, 1-Me), 21.2 (CH$_3$, C$_6$H$_4$$Me$), 38.4 (CH$_2$, C-1'), 44.7 (CH, C-4), 51.9 (CH$_3$, CO$_2$$Me$), 56.5(3) (CH$_3$, OMe), 70.2, 70.3 (CH, C-1), 74.2 (CH, C-3), 117.7(2) (CH, C-6 or C-8), 119.2, 119.5, 139.1, 140.4, 150.8 (C, C-4a, C-5a, C-9a and C-10a), 119.7 (CH, C-8 or C-6), 127.8 (C, C$_6$H$_4$Me), 129.8 (CH, C$_6$H$_4$), 134.4 (C, C$_6$H$_4$S), 135.0 (CH, C-7 and C$_6$H$_4$), 159.6 (C, C-9), 171.3 (C, C-2') and 183.0, 183.1 (C, C-5 and C-10); m/z (EI) 452 (M$^+$, 13%), 421 (M − OMe, 1), 379 (M − CH$_2$CO$_2$Me, 0.5), 350 (M − OCHCH$_2$CO$_2$Me, 2), 329 (M − SC$_6$H$_4$Me, 13), 313 (M − SC$_6$H$_4$Me-CH$_2$-2H, 9), 269 (M − SC$_6$H$_4$Me-HCO$_2$Me, 12), 255 ($C_{15}H_{11}O_4$, 18), 227 ($C_{14}H_{11}O_3$, 100), 213 ($C_{13}H_9O_3$, 6), 184 ($C_{11}H_4O_3$, 5), 128 (17), 124 (MeC$_6$H$_4$SH, 13), 123 (MeC$_6$H$_4$S, 19), 91 (MeC$_6$H$_4$, 39) and 43 (MeCO, 49); m/z (LSIMS) 453 (MH$^+$, 63%), 421 (MH − MeOH, 13), 379 (MH − MeOH − CH$_2$CO, 9), 330 (MH − MeC$_6$H$_4$S, 100), 292 (23), 273 (45), 242 (36) and 227 ($C_{14}H_{11}O_3$, 37).

Methyl (1S*,3R*,4R*)-5,10-dioxo-9-methoxy-1-methyl-3,4,5, 10-tetrahydro-4-(p-tolylthio)-1H-naphtho[2,3-c]pyran-3-yl-acetate 8. A yellow solid (2.2 mg, 6%) (R_t 67.8 min); mp 58–60 °C (Found: MH$^+$, 453.13703. $C_{25}H_{25}O_6S$ requires MH$^+$, 453.13718); ν_{max} (CH$_2$Cl$_2$)/cm^{-1} 1735s (C=O, ester), 1663s, 1654s (C=O, quinone), 1586s (C=C) and 1267s (C–O); δ_H (200 MHz; CDCl$_3$) 1.43 (3H, d, J 6.7, CHMe), 2.32 (3H, s, C$_6$H$_4$$Me$), 2.85 (1H, dd, J_{gem} 16.7 and $J_{1'A,3}$ 6.7, CH^ACO$_2$Me), 2.97 (1H, dd, J_{gem} 16.7 and $J_{1'B,3}$ 6.7, CH^BCO$_2$Me), 3.53 (3H, s, CO$_2$Me), 4.00 (3H, s, OMe), 4.14 (1H, ddd, $J_{3,1'A}$ 6.7, $J_{3,1'B}$ 6.7 and $J_{3,4}$ 2.0, 3-H), 4.39 (1H, dd, $J_{4,3}$ 2.0 and $J_{4,1}$ 2.0, 4-H), 4.87 (1H, qd, $J_{1,Me}$ 6.7 and $J_{1,4}$ 2.0, CHMe), 7.11 (2H, d, J 7.9, C$_6$H$_4$Me), 7.21–7.35 (2H, m, C$_6$H$_4$Me), 7.54–7.81 (3H, m, 6-H, 7-H and 8-H); δ_C (100.6 MHz; CDCl$_3$) 21.5 (CH$_3$, C$_6$H$_4$$Me$), 30.4 (CH$_3$, 1-Me), 38.1 (CH$_2$, C-1'), 46.1 (CH, C-4), 52.3 (CH$_3$, CO$_2$$Me$), 57.2 (CH$_3$, OMe), 71.2 (CH, C-1), 73.0 (CH, C-3), 118.5 (CH, C-6 or C-8), 120.0 (CH, C-8 or C-6), 121.0, 138.5, 140.5, 148.4 (C, C-4a, C-5a, C-9a and

C-10a), 130.5 (CH, C$_6$H$_4$), 132.4 (C, C$_6$H$_4$Me), 133.9 (CH, C$_6$H$_4$), 134.9 (C, C$_6$H$_4$S), 135.6 (CH, C-7), 160.1 (C, C-9), 171.7 (C, C-2') and 183.0, 184.0 (C, C-5 and C-10); m/z (LSIMS) 453 (MH$^+$, 84%), 379 (MH − MeOH − CH$_2$CO, 6), 330 (MH − MeC$_6$H$_4$S, 100), 273 (27), 255 ($C_{15}H_{11}O_4$, 25) and 227 ($C_{14}H_{11}O_3$, 32).

Using phenylmethanethiol as nucleophile

Treatment of **6** (23.2 mg, 0.074 mmol) with sodium dithionite and phenylmethanethiol following the general procedure described above afforded an orange solid (27 mg, 81%) which was established to be a 5.2:1 mixture of thioadducts **9**:**10** by ^{1}H NMR. Further purification of this mixture by HPLC on a Whatman Partisil column using 3:1 hexane–ethyl acetate as solvent afforded **9** and **10**.

Methyl (1S*,3R*,4S*)-5,10-dioxo-9-methoxy-1-methyl-4-phenylmethylthio-3,4,5,10-tetrahydro-1H-naphtho[2,3-c]pyran-3-ylacetate 9. An orange solid (8.0 mg, 24%) (R_t 69.6 min); mp 48–55 °C (Found: MH$^+$, 453.13770. $C_{25}H_{25}O_6S$ requires MH$^+$, 453.13718); ν_{max} (CH$_2$Cl$_2$)/cm^{-1} 1733s (C=O, ester), 1653s (C=O, quinone), 1583s (C=C) and 1263s (C–O); δ_H (400 MHz; CDCl$_3$) 1.46 (3H, d, J 6.6, CHMe), 2.08 (1H, dd, J_{gem} 15.9 and $J_{1'A,3}$ 9.3, CH^ACO$_2$Me), 2.61 (1H, dd, J_{gem} 15.9 and $J_{1'B,3}$ 2.9, CH^B-CO$_2$Me), 3.54 (1H, dd, $J_{4,3}$ 9.5 and $J_{4,1}$ 2.5, 4-H), 3.67 (3H, s, CO$_2$Me), 3.80 (1H, ddd, $J_{3,4}$ 9.5, $J_{3,1'A}$ 9.3 and $J_{3,1'B}$ 2.9, 3-H), 3.88 (1H, d, J_{gem} 13.3, CH^APh), 4.00 (3H, s, OMe), 4.18 (1H, d, J_{gem} 13.3, CH^BPh), 4.69 (1H, qd, $J_{1,Me}$ 6.6 and $J_{1,4}$ 2.5, CHMe), 7.17 (1H, d, J $_{4'',3''}$ 7.3, 4''-H), 7.22–7.29 (3H, m, 6-H or 8-H and 3''-H), 7.36 (2H, d, $J_{2'',3''}$ 7.1, 2''-H), 7.64–7.68 (1H, m, 7-H) and 7.75 (1H, dd, J 7.6 and 1.0, 8-H or 6-H); δ_C (100.6 MHz; CDCl$_3$) 20.5 (CH$_3$, 1-Me), 37.3, 40.5 (CH$_2$, C-1' and SCH$_2$), 37.4 (CH, C-4), 51.7 (CH$_3$, CO$_2$$Me$), 56.5 (CH$_3$, OMe), 70.7, 74.3 (CH, C-1 and C-3), 117.8, 119.1 (CH, C-6 and C-8), 127.4, 128.6, 129.4 (CH, Ph), 134.5, 134.7, 137.9, 149.1 (C, C-4a, C-5a, C-9a and C-10a), 142.5 (C, Ph), 159.6 (C, C-9), 171.5 (C, C-2') and 183.4, 184.0 (C, C-5 and C-10); m/z (LSIMS) 453 (MH$^+$, 70%), 408 (8), 361 (12), 330 (MH − C$_6$H$_5$-CH$_2$S, 100), 287 (14), 255 ($C_{15}H_{11}O_4$, 22) and 227 ($C_{14}H_{11}O_3$, 21).

Methyl (1S*,3R*,4R*)-5,10-dioxo-9-methoxy-1-methyl-4-phenylmethylthio-3,4,5,10-tetrahydro-1H-naphtho[2,3-c]pyran-3-ylacetate 10. An orange solid (3.0 mg, 9%) (R_t 80.7 min); mp 58–72 °C (Found: MH$^+$, 453.13650. $C_{25}H_{25}O_6S$ requires MH$^+$, 453.13718); ν_{max} (CH$_2$Cl$_2$)/cm^{-1} 1735s (C=O, ester), 1653s (C=O, quinone), 1586s (C=C) and 1268s (C–O); δ_H (200 MHz; CDCl$_3$) 1.49 (3H, d, J 6.7, CHMe), 2.22 (1H, dd, J_{gem} 16.7 and $J_{1'A,3}$ 5.5, CH^ACO$_2$Me), 2.71 (1H, dd, J_{gem} 16.7 and $J_{1'B,3}$ 7.3, CH^B-CO$_2$Me), 3.61 (3H, s, CO$_2$Me), 3.72–4.05 (4H, m, 3-H, 4-H and CH_2Ph), 4.00 (3H, s, OMe), 4.87 (1H, qd, $J_{1,Me}$ 6.6 and $J_{1,4}$ 1.8, CHMe), 7.16–7.45 (6H, m, 6-H or 8-H and Ph) and 7.64–7.82 (2H, m, 7-H and 8-H or 6-H); δ_C (50.3 MHz; CDCl$_3$) 20.8 (CH$_3$, 1-Me), 37.2, 39.4 (CH$_2$, C-1' and SCH$_2$), 37.6 (CH, C-4), 51.7 (CH$_3$, CO$_2$$Me$), 56.4 (CH$_3$, OMe), 70.7, 71.9 (CH, C-1 and C-3), 117.9, 119.2 (CH, C-6 and C-8), 120.2, 134.0, 138.3, 145.6 (C, C-4a, C-5a, C-9a and C-10a), 127.1, 128.5, 129.4 (CH, Ph), 134.7 (CH, C-7), 141.8 (C, Ph), 159.4 (C, C-9), 171.1 (C, C-2') and 183.0, 183.4 (C, C-5 and C-10); m/z (LSIMS) 453 (MH$^+$, 61%), 421 (MH − MeOH, 26), 391 (27), 363 (6), 331 (92), 330 (MH − C$_6$H$_5$CH$_2$S, 100), 309 (31), 292 (32), 273 (43), 257 (33), 242 (33) and 229 (26).

Using butanethiol as nucleophile

Treatment of **6** (8.5 mg, 0.027 mmol) with sodium dithionite and butanethiol following the general procedure described above afforded an orange oil that was purified by flash chromatography using 1:2 hexane–ethyl acetate as eluant to give **11** and **12**.

Methyl (1*S**,3*R**,4*S**)-4-butylthio-5,10-dioxo-9-methoxy-1-methyl-3,4,5,10-tetrahydro-1*H*-naphtho[2,3-*c*]pyran-3-ylacetate 11. An orange oil (5.9 mg, 52%) (Found: MH⁺, 419.15250. $C_{22}H_{26}O_6S$ requires MH⁺, 419.15284); ν_{max} (CH_2Cl_2)/cm⁻¹ 1732s (C=O, ester), 1652vs (C=O, quinone), 1585s (C=C) and 1264s (C–O); δ_H (200 MHz; CDCl₃); 0.90 (3H, t, *J* 7.1, CH_2Me), 1.31–1.67 (4H, m, CH_2CH_2Me), 1.51 (3H, d, *J* 6.6, CH*Me*), 2.70–2.80 (2H, m, SCH_2), 2.95 (2H, d, *J* 6.6, CH_2CO_2Me), 3.72 (3H, s, CO_2Me), 3.88 (1H, dd, $J_{4,3}$ 2.0 and $J_{4,1}$ 2.0, 4-H), 3.99 (3H, s, OMe), 4.09 (1H, ddd, $J_{3,1'A}$ 6.6, $J_{3,1'B}$ 6.6 and $J_{3,4}$ 2.0, 3-H), 4.89 (1H, qd, $J_{1,Me}$ 6.6 and $J_{1,4}$ 2.0, C*H*Me), 7.28 (1H, dd, *J* 8.7 and *J* 1.2, 6-H or 8-H) and 7.62–7.78 (2H, m, 7-H and 8-H or 6-H); δ_C (50.3 MHz; CDCl₃) 13.6 (CH₃, CH_2Me), 20.8 (CH₃, 1-Me), 22.0 (CH₂, CH_2Me), 31.7 (CH₂, SCH_2CH_2), 33.4 (CH₂, SCH_2), 37.5 (CH₂, C-1'), 38.5 (CH, C-4), 51.8 (CH₃, CO_2Me), 56.5 (CH₃, OMe), 70.6, 72.0 (CH, C-1 and C-3), 117.8, 119.2 (CH, C-6 and C-8), 120.2, 134.1, 141.2, 145.9 (C, C-4a, C-5a, C-9a and C-10a), 134.7 (CH, C-7), 159.4 (C, C-9), 171.3 (C, C-2') and 182.7, 183.5 (C, C-5 and C-10); *m/z* (EI) 418 (M⁺, 2%), 374 (M − CO₂, 4), 357 (8), 331 (36), 329 [M − S(CH₂)₃Me, 9], 313 [M − S(CH₂)₃Me − CH₂ − 2H, 27], 269 (19), 257 (24), 255 ($C_{15}H_{11}O_4$, 18), 227 ($C_{14}H_{11}O_3$, 34), 91 (14), 89 [Me(CH₂)₃S, 9], 57 [Me(CH₂)₃, 46] and 43 [Me(CH₂)₂, 100]; *m/z* (LSIMS) 419 (MH⁺, 68%), 387 (MH − MeOH, 45), 330 [MH − Me(CH₂)₃S, 100], 313 (23), 298 ($C_{17}H_{13}O_5$, 17), 273 (14), 256 ($C_{15}H_{12}O_4$, 24) and 227 ($C_{14}H_{11}O_3$, 34).

Methyl (1*S**,3*R**,4*R**)-4-butylthio-5,10-dioxo-9-methoxy-1-methyl-3,4,5,10-tetrahydro-1*H*-naphtho[2,3-*c*]pyran-3-ylacetate 12. An orange oil (1.2 mg, 11%) (Found: MH⁺, 419.15429. $C_{22}H_{26}O_6S$ requires MH⁺, 419.15284); ν_{max} (CH_2Cl_2)/cm⁻¹ 1738s (C=O, ester), 1696, 1659m (C=O, quinone), 1585s (C=C) and 1265s (C–O); δ_H (400 MHz; CDCl₃) 0.77 (3H, t, *J* 3.6, CH_2Me), 1.15–1.44 (4H, m, CH_2CH_2Me), 1.58 (3H, d, *J* 6.0, CH*Me*), 2.43–2.60 (2H, m, CH_2S), 2.62 (1H, dd, J_{gem} 16.0 and $J_{1'A,3}$ 7.3, C*H*^{A}CO_2Me), 2.74 (1H, dd, J_{gem} 16.0 and $J_{1'B,3}$ 7.3, C*H*^{B}CO_2Me), 3.73 (3H, s, CO_2Me), 3.94 (3H, s, OMe), 3.99 (1H, d, $J_{4,3}$ 2.0, 4-H), 4.32 (1H, q, $J_{1,Me}$ 6.0, C*H*Me), 4.74 (1H, ddd, $J_{3,1'A}$ 7.3, $J_{3,1'B}$ 7.3 and $J_{3,4}$ 2.0, 3-H), 7.28 (1H, dd, *J* 8.4 and 0.7, 6-H or 8-H), 7.62–7.68 (1H, m, 7-H) and 7.79 (1H, dd, *J* 7.7 and 0.9, 8-H or 6-H); δ_C (100.6 MHz; CDCl₃) 14.2 (CH₃, CH_2Me), 19.1 (CH₃, 1-Me), 22.6 (CH₂, CH_2Me), 30.4 (CH, C-4), 31.8, 32.6 (CH₂, SCH_2CH_2), 39.8 (CH₂, C-1'), 52.7 (CH₃, CO_2Me), 57.1 (CH₃, OMe), 71.3, 75.2 (CH, C-1 and C-3), 118.4, 119.9 (CH, C-6 and C-8), 134.4, 135.9, 140.4 (C, C-4a, C-5a, C-9a and C-10a), 135.1 (CH, C-7), 159.4 (C, C-9), 171.1 (C, C-2'), 184.0 (C, C-5 or C-10) and 192.3 (C, C-10 or C-5); *m/z* (LSIMS) 419 (MH⁺, 77%), 375 [M − Me(CH₂)₂, 26], 345 (M − CH₂CO₂Me, 21), 343 (23), 329 [MH − Me(CH₂)₃SH, 100], 292 (23), 273 (33), 255 ($C_{15}H_{11}O_4$, 36), 242 (27), 227 ($C_{14}H_{11}O_3$, 34) and 212 (14).

(3a*R**,5*S**,11b*R**)-6,11-Diacetoxy-7-methoxy-5-methyl-3,3a,5,11b-tetrahydro-2*H*-benzo[*g*]furo[3,2-*c*]isochromen-2-one 14

Using sodium dithionite as the reductant. A solution of **6** (8.2 mg, 0.026 mmol) in diethyl ether (4 mL) was degassed by passing an argon stream through it for 10 min. To this solution was added sodium dithionite (28 mg, 0.161 mmol—6 equiv.) in water (0.1 mL) and the resultant mixture stirred vigorously under argon at room temperature for 20 min. Excess sodium sulfate was added to the reaction along with dichloromethane (2 mL) and the dried liquid transferred by cannula to a new reaction vessel. To this was added acetic anhydride (0.1 mL, 1.06 mmol), triethylamine (0.1 mL, 0.72 mmol) and a catalytic amount of 4-dimethylaminopyridine. After approximately 40 min the reaction was poured into dichloromethane (15 mL), washed with water (2 × 5 mL) and dried over sodium sulfate. Removal of the solvent under reduced pressure and purification of the residue by flash chromatography using hexane–ethyl acetate (1:2) gave the *title compound* **14** (6.2 mg, 60%) as a colourless solid, mp 240–241.5 °C (Found: M⁺, 400.11576. $C_{21}H_{20}O_8$ requires *M*, 400.11582); ν_{max} (CH_2Cl_2)/cm⁻¹ 1767vs (C=O, γ-lactone and aromatic acetates), 1575m (C=C) and 1192s (C–O); δ_H (200 MHz; CDCl₃) 1.68 (3H, d, *J* 6.1, CH*Me*), 2.39 (3H, s, OAc), 2.49 (3H, s, OAc), 2.75 (1H, d, J_{gem} 17.6, 3-H^A), 2.91 (1H, dd, J_{gem} 17.6 and $J_{3,3a}$ 4.2, 3-H^B), 3.93 (3H, s, OMe), 4.40 (1H, dd, $J_{3a,3}$ 4.2 and $J_{3a,11b}$ 2.5, 3a-H), 4.87 (1H, q, $J_{5,Me}$ 6.1, 5-H), 5.30 (1H, d, $J_{11b,3a}$ 2.5, 11b-H), 6.93 (1H, dd, *J* 7.5 and 1.0, 8-H or 10–H) and 7.33–7.42 (2H, m, 9-H and 10-H or 8-H); δ_C (100.6 MHz; CDCl₃) 21.4, 21.8, 22.1 (CH₃, 3 × Me), 38.3 (CH₂, C-3), 57.0 (CH₃, OMe), 70.8, 72.0, 73.2 (CH, C-3a, C-5, C-11b), 108.5 (CH, C-10), 115.2 (CH, C-8), 119.8 (C, C-6a), 128.3 (CH, C-9), 129.5 (C, C-10a), 136.3, 136.4 (C, C-5a, C-11a), 141.2, 146.3 (C, C-6, C-11), 156.1 (C, C-7), 169.7, 169.9 (C, 2 × COMe) and 175.6 (C, C-2); *m/z* (EI) 400 (M⁺, 6%), 358 (MH − COMe, 77), 316 (MH − COMe − CH₂CO, 100), 301 (47), 272 (MH − COMe − CH₂CO − CO₂, 20), 256 ($C_{15}H_{12}O_4$, 77), 229 ($C_{13}H_9O_4$, 71) and 43 (MeCO, 75).

Using platinum(IV) oxide and hydrogen as the reductant. PtO₂ (3.6 mg, 0.016 mmol) was added to a solution of **6** (8.2 mg, 0.026 mmol) in THF (4.0 mL) under an atmosphere of hydrogen. Once the solution decolourised (*ca.* 5 min), the above acetylating agents were added to the suspension. Removal of the catalyst by filtration, evaporation of the solvent and purification by flash chromatography gave **14** (6.8 mg, 66%).

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