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Development of chemical probes: Toward the mode of action of a methylene-linked di(aryl acetate) E1

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ABSTRACT

Analogues of the novel inhibitor of the PI3-K/PKB pathway, 2-[5-(2-chloroethyl)-2-acetoxy-benzyl]-4-(2-chloroethyl)-phenyl acetate (E1), have been prepared and preliminary SAR performed. This established that at least one of the chloroethyl *para*-substituents could be removed or modified and the ability to inhibit PKB/Akt activation retained. Synthetic methodologies were then developed to methylene-linked aryl acetates for use as molecular probes to identify the target of compound E1.

1. Introduction

The phosphoinositide 3-kinase (PI3-K) family of enzymes has a key function in cellular signalling pathways and operates predominantly via the down stream effector protein kinase B (PKB/Akt).[1] The PI3-K/PKB pathway regulates a range of cellular processes including cell growth, survival and proliferation.[1,2] Deregulation of the pathway is implicated in a number of disease states, notably cancer and there is therefore significant interest in the identification of targets for therapeutic intervention.

We have recently described a novel inhibitor of the PI3-K/PKB pathway, 2-[5-(2-chloroethyl)-2-acetoxy-benzyl]-4-(2-chloroethyl)-phenyl acetate (E1) (**1**).[3] This was identified when screening a library of analogues of compound 48/80 (**2**) (Fig. 1), an activator of protein kinase C (PKC) and calcium mediated processes which is produced as a mixture of oligomers.[4] The effect of **1** on the PI3-K/PKB/mammalian target of rapamycin (mTOR) pathway was studied, and was found to have a dose and time-dependent repressive effect on PKB and mTOR activity in PC-3 and MCF-7 cell lines.[3] Inhibition of PKB/mTOR activity also correlated with increased c-Jun NH_2-terminal kinase (JNK) phosphorylation, with a different mode of action to that of the mTOR inhibitor rapamycin. Further experiments indicated that repression of PKB/mTOR activity by compound E1 was mediated via JNK activation.

Compound E1 (**1**) was also found to function synergistically with suboptimal concentrations of paclitaxel to cause cell death in PC-3 and MCF-7 cells, suggesting that **1** and paxlitaxel operate through synergistic signalling mechanisms. It was concluded that the novel potent cytotoxic agent E1 causes cell death in both prostate and breast cancer cells through activation of JNK and supression of PKB/mTOR activity in a manner independent of PI3-K.[3] Other dimeric compounds structurally related to compound E1

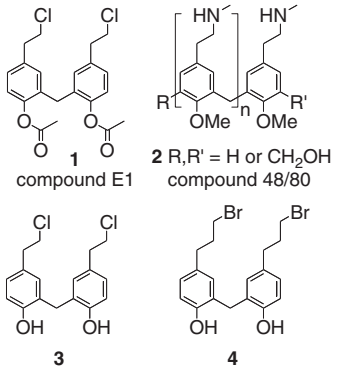

Figure 1. Structure of compounds **1**–**4**.

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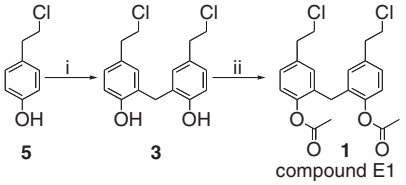
that also had inhibitory properties in the PKB immunoblotting assay included compounds **3** and **4**. With the aim of both developing syntheses to analogues of **1** for preliminary SAR and molecular probes based on E1, analogues were prepared, assessed and then selected compounds conjugated for future applications.

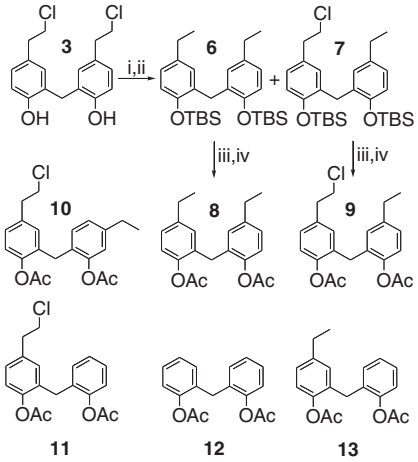
2. Results and discussion

There are several methods described in the literature for the preparation of dimeric methylene–linked phenols, although there are fewer examples for compounds possessing *para*-substituted functionalised groups. One route is via reaction of the monomeric phenol with formaldehyde under acidic conditions, but this will normally lead to the generation of oligomeric species from which the dimer must be purified.[5] Despite this, to ensure a direct rapid synthesis, the bis-phenol **3** and diacetate E1 (**1**) was readily prepared from the corresponding monomeric phenol **5** (Scheme 1). Other routes such as, *ortho*-hydroxymethylation or bromomethylation and subsequent coupling with the phenol have been used to compounds of this type, particularly when preparing hetero-coupled building blocks for convergent calixarene syntheses.[6] In addition, more recent strategies include the use of *ortho*-bromomethylated protected phenols, and cross-coupling under Negishi palladium catalysis conditions however in both of these cases preparation of the benzylic species is required.[7,8] Alternatively, Suzuki–Miyaura cross-coupling could be used, although the boronate substrates for coupling require synthesis and in all these approaches presence of the *ortho*-phenolic moiety may impede the reaction.[9] Treatment of 2-(4-hydroxyphenyl)-ethanol with thionyl chloride in toluene gave **5** in 89% yield.[5] Then, reaction of **5** with *para*-formaldehyde in sulfuric acid gave a complex mixture of oligomers from which the bis-phenol **3** was obtained using silica column chromatography and subsequent recrystallisation in 36% yield. An alternative more scalable purification procedure to **3** was developed by forming a slurry of the crude reaction product in chloroform, followed by filtration. Although lower yielding (26% isolated yield) it was a rapid method giving high purity product. Acetylation under standard conditions gave **1**.

With a view to preparing analogues of **1** to assess the importance of the two chloroethyl groups for inhibition of the PI3-K/PKB pathway, and to establish functionalisations tolerated for the preparation of active compounds for conjugation to biotin and fluorescent moieties, several analogues were prepared. Initially, compounds were prepared with ethyl groups rather than the chloroethyl functionality and also with the chloroethyl groups removed. Analogues possessing ethyl groups were readily prepared via a reduction strategy (Scheme 2). Compound **3** was diprotected using *t*-butyldimethyl silyl triflate (TBSOTf) in good yield, then reduced using an excess of lithium aluminium hydride which proceeded slowly over 24 h to give a mixture of **6** (21%) and **7** (31%) which could be separated by silica flash chromatography. Although not high yielding, in one step this gave access to both the diethylated analogues **8** and monochloroethyl–monoethyl analogue **9**, after deprotection and acetylation. The unsymmetrical *meta*-ethyl



Scheme 1. Preparation of **1** and **3**. Reagents and conditions: (i) $(CH_2O)_n$, H_2SO_4, 70 °C, 26–36%; (ii) Ac_2O, DMAP, pyridine, 80%.



Scheme 2. Preparation of **8** and **9**, structures **10**–**13**. Reagents and conditions: (i) TBSOTf, Et_3N, 86%; (ii) $LiAlH_4$, **6** 21%, **7** 31%; (iii) concd HCl, MeOH; (iv) Ac_2O, DMAP, pyridine, **8** 70% (two steps), **9** 89% (two steps).

analogue of **9**, compound **10**, was prepared via *ortho*-hydroxymethylation of one of the phenolic units. 3-Ethylphenol was *ortho*-formylated to give 4-ethyl-1-hydroxybenzaldehyde in 98% yield using a magnesium chloride-mediated addition of formaldehyde.[10] This was then reduced to the corresponding benzylalcohol with borane–dimethyl sulfide complex,[11] which due to poor stability was reacted directly with **1** under acidic conditions, and diacetylated to give **10**. The mono- and di-analogues lacking one or both chloroethyl groups, **11** and **12**, together with **13** possessing only one ethyl *para* group, were synthesised using the same method as for **1**, but using phenol with **5**, or phenol alone, or phenol with 4-ethyl phenol to form the dimers with subsequent acetylation. Though not high yielding these procedures generated compounds for screening the signalling pathway.

Compounds **8**–**13** were all tested with respect to their ability to inhibit PKB/Akt activation, which is monitored by detecting the phosphorylation at Ser473 by western blotting as described previously.[12] Compounds **8** (data in SI) and **9**–**11** (Fig. 2) inhibited Akt phosphorylation, whereas compounds **12** and **13** were inactive (data in SI). The inactivity of **12** and **13** highlighted the preference for at least one chloroethyl *para*-substituted ring. The data also revealed that **11** was more potent than compound E1, whereas compounds **9** and **10** appeared marginally less potent than E1. A dose response curve obtained for **9** (Fig. 2E and F) implies that the active compounds have effective IC_{50} values of 25 μM or lower. These modest affinities would explain the reversible activity observed for these compounds, since treated cells recovered quickly following elution of the compounds (data not shown). This observation would also suggest a reversible mode of action for these compounds, and that therefore no alkylation of the target protein by these compounds is occurring. In addition, the efficacy of compounds such as E1 and **9** was similar to that of LY294002, a well-established phosphoinositide 3-kinase inhibitor that is used in the 50 μM range.[13]

Initially compounds based on **14** were envisaged (Scheme 3) for probing the cellular target of compound E1, by retaining the bis-chloroethyl phenylacetate motif but incorporating an *ortho*-side chain linker for conjugation to biotin or a fluorophore. Initial linker coupling procedures were explored using the monomeric analogue **15** (readily synthesised via the iodination of phenol **5**) and *N*-diBoc protected alkene **16** under Heck ligandless coupling conditions.[14] Although some of the coupled product **17** could be detected by MS analysis, other side products were also formed and **17** could not be purified from the reaction mixture. The *ortho*-iodinated di-

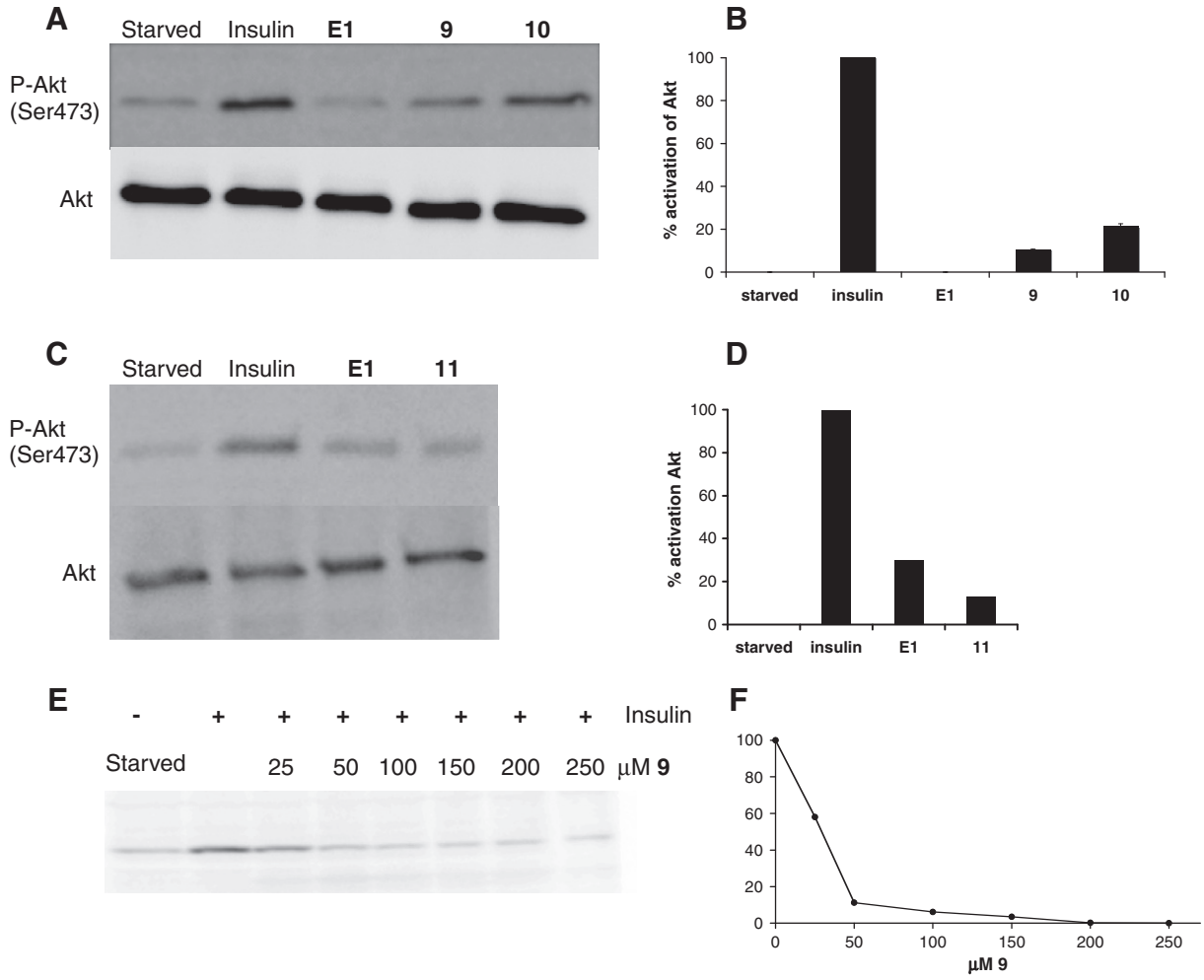
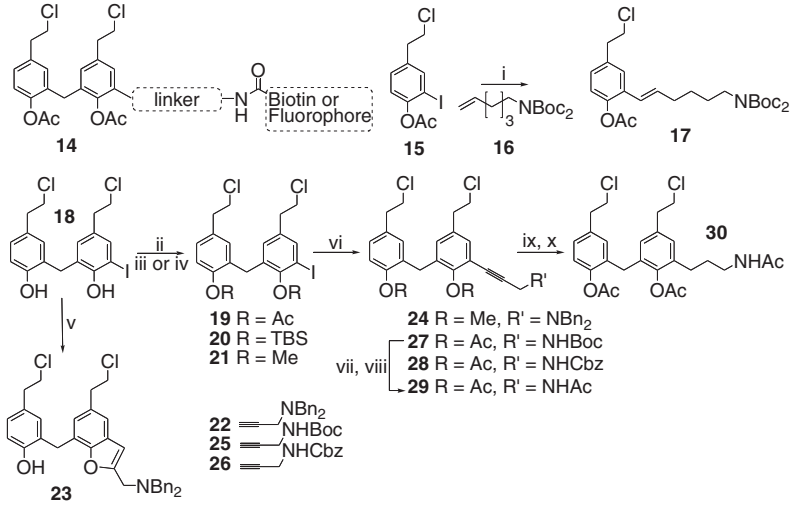


Figure 2. Screening of selected compounds. (A) MCF seven cells were starved overnight, incubated with 50 μg/mL of the compounds indicated (or vehicle) for 1 h, followed by 1 μg/mL insulin for 15 min. Cells were collected and analyzed by western blotting using phospho-Akt (serine 473) and Akt antibodies as described previously.[12] (B) The blots in (A) were quantified using ImageJ. The percentage of activation of Akt (P-Akt phosphorylation normalized for loading using the Akt blotting intensities) is shown. Starved cells were set at 0% and insulin treated cells at 100%, respectively. (C) MCF seven cells were incubated as described above with E1 and **11** (50 μg/mL for 45 min) and analysed for Akt activation by western blotting. (D) Quantification of the blot intensities shown in (C) (using ImageJ). The difference in activity for compound E1 in (B) and (D) was due to different incubation times (60 and 45 min, respectively). (E) MCF seven cells were incubated as described above with the concentrations of **9** indicated and analysed for P-Akt phosphorylation by western blotting. (F) Quantification of the blot intensities of dose response shown in (E) (using ImageJ).



Scheme 3. Structure of general probe **14** and preparation of **15**–**30**. Reagents and conditions: (i) range of Heck conditions; (ii) Ac_2O, pyridine, DMAP, 87%; (iii) TBSOTf, Et_3N, 83%; (iv) MeI, K_2CO_3, 60 °C, 87%; (v) $Pd(PPh_3)_4$, CuI, Et_3N, 80 °C, 49%; (vi) $Pd(PPh_3)_4$, CuI, Et_3N, 80 °C; **21** and **25**, 39%, **19** and **25**, 46%; **19** and **26**, 45%; (vii) TFA, 0 °C, then (viii) Ac_2O, pyridine, DMAP, 85% over two steps; (ix) H_2, Pd/C, then (x) Ac_2O, pyridine, DMAP, 87% over two steps.

mer **18** (synthesised via the iodination of phenol **3**) was diacetylated to give **19**, and when the Heck ligandless conditions were used with **18** and alkene **19**, no coupled product could be detected. Alternative Heck coupling conditions were then investigated with **15** and **16** including use of the electron rich bulky phosphine ligands SPhos and XPhos,[15] tri-*tert*-butyl phosphonium tetrafluoroborate[16] and triphenylphosphine with $Pd_2(dba)_3$. In most reactions inseparable mixtures were formed, although the use of SPhos did give some **17**, however again it could not readily be separated from the side products generated. An alternative strategy involving Friedel–Craft acylation at the *ortho*-position and reduction was also unsuccessful.

It was then decided to focus on alternative reactions with the *ortho*-iodinated dimer **18** and protected analogues. Accordingly, dimer **18** was di-TBS protected to give **20** and attempts were made to introduce a linker at the *ortho*-position with a model reaction utilising *n*-butyl zinc bromide under Negishi coupling conditions ($Cl_2Ni(PPh_3)_2$).[17] No reaction was observed, so the dimethoxy *ortho*-iodinated analogue, compound **21**, was prepared and reacted under both nickel-catalysed ($Cl_2Ni(PPh_3)_4$) and palladium catalysed ($Pd(PPh_3)_4$) Negishi coupling conditions with *n*-butyl zinc bromide.[18] Again the reaction did not proceed and the use of Kumada coupling conditions ($Cl_2Ni(PPh_3)_2$) were also unsuccessful.[19] The reason for the failure to undergo these coupling procedures is unclear but may be due to the functionalised electron rich bis-aryl systems and unfavourable steric interactions, together with the challenges encountered with such sp^2–sp^3 coupling reactions. Despite this, Sonogashira conditions (Scheme 3), involving coupling sp^2 and sp systems were then explored with **18** and dibenzyl protected propargylamine **22**. The reaction gave the coupled benzofuran **23** in 49% yield:[20] such reactions between terminal alkynes and *ortho*-iodophenols with subsequent cyclisation to benzofurans have been reported in the literature.[21] The successful coupling suggested that Sonagashira conditions might be the most productive method for attaching linkers but using a protected bis-phenol. Treatment of the dimethoxy analogue **21** in the Sonogashira reaction with **22** then gave **24** in 39% yield. Having identified suitable coupling conditions the diacetylated analogue **19** was reacted with both *N*-Boc and *N*-Cbz protected propargylamines, **25** and **26**, to give **27** and **28** in 46% and 45% yields, respectively. In order to establish that analogues possessing *ortho*-linkers were still biologically active, acetylene **27** was deprotected and directly acetylated to give compound **29** in 85% over two steps. The Cbz protected analogue **28** was deprotected and reduced, then directly acetylated to give **30** in 87% yield over two steps, yielding a saturated linker. However, both **29** and **30** were found to be inactive and did not inhibit PI3-K mediated signalling rendering the *ortho*-linker attachment strategy unviable. Nevertheless it did indicate the preferred coupling strategies to be used for the bis-phenolic compounds.

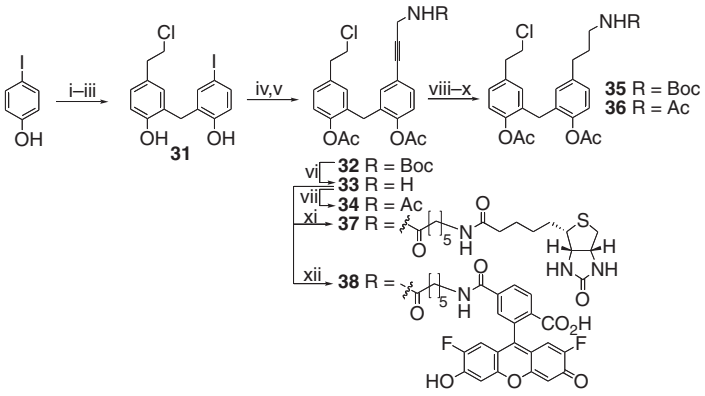
Since in the SAR study compounds **9** and **11** had been noted to have similar biological activity to E1 (**1**) the synthesis of *para*-labelled variants was then pursued (Scheme 4). *ortho*-Formylation as previously but using 4-iodophenol gave 2-hydroxy-5-iodobenzaldehyde which was reduced to the corresponding benzyl phenol using borane–dimethyl sulfide complex, and then condensed with phenol **5** to give **31** in 33% yield. Diacetylation and Sonogashira coupling as before to **25** gave **32** in good yield reflecting that the lower yields when performing the same reaction at the *ortho*-position are probably due to steric problems. Deprotection to the amine **33** and acetylation generated **34**, and hydrogenation to **35**, then deprotection and acetylation gave the variant **36** as before. Both acetylated analogues **34** and **36** were tested with respect to their ability to inhibit PKB/Akt activation, which revealed some retention of inhibitory activity. Biotin X-E and Oregon Green 488-X functionalised probes possessing the acetylene linker were therefore prepared via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) coupling or reaction with the corresponding *N*-hydroxysuccinimidyl esters to give **37** and **38**, respectively. A range of novel analogues and the methodology to access this series of probes has now been established. Experiments to identify the target of compound E1 (**1**) using these compounds will be described elsewhere.

In summary, preliminary SAR has been performed on compound E1, which established that at least one of the chloroethyl *para*-substituents could be removed or modified and the ability to inhibit PKB/Akt activation retained. Synthetic methodologies have also been developed to the methylene-linked aryl acetates for use as molecular probes to identify the target of compound E1.

3. Experimental

3.1. General

Unless otherwise noted, solvents and reagents were reagent grade from commercial suppliers and used without further purification. Dry CH_2Cl_2 and MeCN were obtained using anhydrous alumina columns.[22] All moisture-sensitive reactions were performed under a nitrogen or argon atmosphere using oven-dried glassware. Reactions were monitored by TLC on Kieselgel 60 F_{254} plates with detection by UV, potassium permanganate, and phosphomolybdic



Scheme 4. Preparation of **31**–**38**. Reagents and conditions: (i) $(CH_2O)_n$, $MgCl_2$, Et_3N, 70 °C, 52%; (ii) $BH_3{\cdot}Me_2S$, 98%; (iii) **5**, 25% H_2SO_4, 70 °C, 33%; (iv) Ac_2O, pyridine, DMAP, 81%; (v) **25**, $Pd(PPh_3)_4$, CuI, Et_3N, 70%; (vi) TFA, 0 °C, then (vii) Ac_2O, pyridine, DMAP, 57% over two steps; (viii) H_2, Pd/C, 100%; (ix) TFA, 0 °C, then (x) Ac_2O, pyridine, DMAP, 100% over two steps; (xi) **33** (from **32** as (vi)), then Biotin X-SE, EDC, Et_3N, 72% over steps (vi) and (xi); (xii) **33** (from **32** as (vi)), then Oregon Green 488-X (succinimydyl ester), Et_3N, 0 °C, 66% over steps (vi) and (xii).

acid stains. Flash column chromatography was carried out using silica gel (particle size 40–63 μm). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at the field indicated using Bruker AMX300 MHz, AMX400, Avance-500 MHz and Avance-600 MHz machines. Coupling constants are measure in hertz (Hz) and unless otherwise specified, spectra were acquired at 298 K. Mass spectra were recorded on Thermo Finnegan MAT 900XP and Micro Mass Quattro LC electrospray mass spectrometers VG ZAB 2SE. Infrared spectra were recorded on a Shimadzu FTIR-8700 spectrometer. HPLC analysis and purification was performed on a Varian Prostar system using a Discovery®BIO C18 column (Supelco). The purity of all compounds was checked prior to biological screening, and samples were further purified by HPLC if required. 4-(2-Chloroethyl)-phenol[5] **5**, *N,N*-dibenzylpropargylamine[23] **23**, prop-2-ynylcarbamic acid *tert*-butyl ester[24] **26**, and 2-hydroxy-5-iodobenzaldehyde[10,25] were prepared as previously described. Biotin X-SE and Oregon Green 488-X was purchased from Invitrogen Inc.

3.2. 2-[5-(2-Chloroethyl)-2-hydroxybenzyl)-4-(2-chloroethyl] phenol[26] 3

para-Formaldehyde (679 mg, 22.6 mmol) was added to a vigorously stirred solution/suspension of phenol **5** (2.95 g, 18.8 mmol) in sulfuric acid (50 mL, 25% vol) and the mixture heated at 70 °C for 2 h. The mixture was filtered and the solid collected washed with water (3 × 50 mL) and dried under vacuum. The residue (~3.2 g) was suspended in chloroform (25 mL) and stirred at 50 °C for 20 min. The hot mixture was filtered, the solid collected washed with chloroform (50 mL) and then dried under vacuum to yield the desired compound as a white solid (795 mg, 26%). Mp 165–167 °C (petroleum ether/ethyl acetate); ν_{max}/cm^{-1} (KBr) 3258, 2928, 1501, 1431; δ_H (300 MHz; $(CD_3)_2CO$) 8.48 (2H, s, 2 × OH), 7.10 (2H, d, *J* 2.0, 3-ArH and 6-ArH(benzyl)), 6.95 (2H, dd, *J* 8.1 and 2.0, 5-ArH and 4-ArH(benzyl)), 6.79 (2H, d, *J* 8.1, 6-ArH and 3-ArH(benzyl)), 3.91 (2H, s, ArCH_2Ar), 3.66 (4H, t, *J* 7.5, 2 × CH_2Cl), 2.89 (4H, t, *J* 7.5, 2 × CH_2Ar); δ_C (75 MHz; acetone-d_6) 154.2, 132.0, 130.5, 128.5, 128.1, 116.1, 46.1, 39.1, 32.0; *m/z* (HRCI) found $[MH]^+$ 325.0753; $C_{17}H_{19}Cl_2O_2$ requires 325.0762.

3.3. 2-[5-(2-Chloroethyl)-2-acetoxybenzyl)-4-(2-chloroethyl] phenyl acetate (compound E1) 1

Diphenol **3** (300 mg, 0.924 mmol) was dissolved in pyridine (10 mL). Acetic anhydride (3 mL) and DMAP (cat.) were added and the solution stirred overnight at rt. The mixture was concentrated to dryness and the residue partitioned between ethyl acetate (50 mL) and 0.3 M potassium hydrogen sulfate (50 mL). The organic phase was washed with saturated sodium chloride solution (50 mL), dried ($MgSO_4$) and concentrated in vacuo to yield a residue which was purified by flash silica chromatography (ethyl acetate/petroleum ether 40:60, 1:4) to yield **1** as a white solid (300 mg, 80%). Mp 76–78 °C (acetone/toluene); ν_{max}/cm^{-1} (KBr) 2926, 1751, 1499; δ_H (300 MHz; $CDCl_3$) 7.13 (2H, d, *J* 8.2, 3-ArH and 6-ArH(benzyl)), 7.01 (2H, d, *J* 8.2, 6-ArH and 3-ArH(benzyl)), 6.90 (2H, d, *J* 1.9, 5-ArH and 4-ArH(benzyl)), 3.77 (2H, s, ArCH_2Ar), 3.65 (4H, t, *J* 7.4, 2 × CH_2Cl), 2.98 (4H, t, *J* 7.4, 2 × CH_2Ar), 2.19 (6H, s, 2 × COCH_3); δ_C (75 MHz; $CDCl_3$) 169.3 (C=O), 147.9, 136.1, 131.4, 131.1, 128.1, 122.5, 44.8, 38.5, 30.5, 20.7; *m/z* (HRFAB) found $[MNa]^+$ 431.0801; $C_{21}H_{22}Cl_2O_4$ requires 431.0793.

3.4. Bis[2-(*tert*-butyldimethylsilyloxy)-5-(2-chloroethyl)phenyl] methane

To diphenol **3** (161 mg, 0.495 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added triethylamine (152 μL, 1.09 mmol), then TBSOTf (239 μL, 1.04 mmol) was added dropwise and the mixture stirred for 18 h at rt. The reaction was diluted with dichloromethane (40 mL) and washed with 0.3 M potassium hydrogen sulfate (50 mL), satd sodium hydrogen carbonate (50 mL) and dried ($MgSO_4$). The organic phase was concentrated in vacuo to yield a residue that was purified by silica flash chromatography (ethyl acetate/hexane, 5:95) to give the titled compound as a colourless oil (237 mg, 86%). δ_H (300 MHz; $CDCl_3$) 6.93 (2H, dd, *J* 8.2 and 2.3, 2 × 4-ArH), 6.72–6.76 (4H, m, 2 × 3-ArH and 2 × 6-ArH), 3.89 (2H, s, ArCH_2Ar), 3.59 (4H, t, *J* 7.4, 2 × CH_2Cl), 2.91 (4H, t, *J* 7.4, 2 × CH_2Ar), 0.91 (18H, s, 2 × C(CH_3)$_3$), 0.19 (12H, s, 4 × CH_3Si); δ_C (75 MHz; $CDCl_3$) 152.6, 131.1, 130.8, 130.3, 127.2, 117.9, 45.3, 38.6, 30.8, 25.7, 18.1, −4.2; *m/z* (HRFAB) found $[MNa]^+$ 575.23123; $C_{29}H_{46}Cl_2O_2Si_2$ requires 575.23111.

3.5. Bis[2-(*tert*-butyldimethylsilyloxy)-5-ethylphenyl]methane 6 and *tert*-butyl[2-(2-(*tert*-butyldimethylsilyloxy)-5-(2-chloroethyl)benzyl)-4-ethylphenoxy]dimethylsilane 7

The reaction was carried out under anhydrous conditions. To bis[2-(*tert*-butyldimethylsilyloxy)-5-(2-chloroethyl)phenyl]methane (110 mg, 0.199 mmol) in THF (5 mL) at 0 °C was added lithium aluminium hydride (60 mg, 1.58 mmol) and the reaction was warmed to rt. The reaction was stirred for 48 h, then cooled to at 0 °C and 0.3 M potassium hydrogen sulfate (5 mL) added. The mixture was partitioned between ethyl acetate (50 mL) and water (50 mL) and the aqueous phase extracted with further ethyl acetate (50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to yield a residue which was purified by silica flash chromatography (hexane) to yield **6** (20 mg, 21%) and **7** (32 mg, 31%), both as colourless oils. Compound **6** δ_H (300 MHz; $CDCl_3$) 6.89 (2H, dd, *J* 8.1 and 2.2, 4-ArH), 6.74 (2H, d, *J* 2.2, 6-ArH), 6.71 (2H, d, *J* 8.1, 3-ArH), 3.89 (2H, s, ArCH_2Ar), 2.48 (4H, q, *J* 7.6, 2 × CH_2CH$_3$), 1.13 (6H, t, *J* 7.6, 2 × CH$_2$CH_3), 0.92 (18H, s, 2 × C(CH_3)$_3$)), 0.17 (12H, s, 4 × CH_3Si); δ_C (75 MHz; $CDCl_3$) 151.7, 136.6, 131.1, 130.1, 126.0, 117.8, 30.9, 28.2, 25.9, 18.3, 16.0, −4.1; *m/z* (HRFAB) found $[MH]^+$ 485.32599; $C_{29}H_{49}O_2Si_2$ requires $[MH]^+$ 485.32709. Compound **7** δ_H (300 MHz; $CDCl_3$) 6.93 (2H, apparent dd, *J* 8.2 and 2.3, 5-ArH and 4-ArH(benzyl)), 6.72–6.76 (4H, m, 3-ArH and 6-ArH, 3-ArH(benzyl) and 6-ArH(benzyl)), 3.90 (2H, s, ArCH_2Ar), 3.59 (2H, t, *J* 7.5, CH_2Cl), 2.91 (2H, t, *J* 7.5, CH_2Ar), 2.51 (2H, q, *J* 7.6, CH_2CH$_3$), 1.15 (3H, t, *J* 7.6, CH$_2$CH_3), 0.94 (9H, s, C(CH_3)$_3$), 0.91 (9H, s, C(CH_3)$_3$), 0.21 (6H, s, CH_3Si), 0.20 (6H, s, CH_3Si); δ_C (75 MHz; $CDCl_3$) 152.6, 151.6, 136.5, 131.6, 130.7, 130.5, 130.2, 130.0, 127.0, 126.1, 117.8, 117.7, 45.2, 38.7, 30.8, 28.1, 25.7, 18.2, 18.1, 15.9, −4.2; *m/z* (FAB) found $[MH]^+$ 519.28923; $C_{29}H_{48}ClO_2Si_2$ requires 519.28812.

3.6. 2,2′-Methylenebis(4-ethyl-2,1-phenylene) diacetate 8

To compound **6** (10 mg, 0.021 mmol) in methanol (5 mL) concd hydrochloric acid (two drops) was added and the reaction was stirred at rt for 3 d, then concentrated to dryness in vacuo. To the residual white solid was added pyridine (3 mL), acetic anhydride (1 mL) and DMAP (cat.). The reaction was stirred for 17 h, concentrated to dryness under vacuum and the residue partitioned between ethyl acetate (50 mL) and 0.3 M potassium hydrogen sulfate (50 mL). The organic phase was dried ($MgSO_4$), concentrated and purified using preparative HPLC (acetonitrile/water, 1:9 to 9:1 over 25 min, retention time 18.0 min) to give **8** as a colourless oil (5 mg, 70%). δ_H (300 MHz; $CDCl_3$) 7.06–7.09 (2H, m, 3-ArH), 6.90–6.96 (4H, m, 5-ArH and 6-ArH), 2.57 (4H, q, *J* 7.6, 2 × CH_2CH$_3$), 2.18 (6H, s, 2 × COCH_3), 1.18 (6H, t, *J* 7.6, 2 × CH$_2$CH_3); *m/z* (ES+) found $[MNa]^+$ 363.1577; $C_{21}H_{24}NaO_4$ requires 363.1572.

3.7. 2-(2-Acetoxy-5-(2-chloroethyl)benzyl)-4-ethylphenyl acetate 9

To compound **7** (20 mg, 0.039 mmol) in MeOH (5 mL) concd HCl (two drops) was added and the reaction was stirred at rt for 3 d, then concentrated to dryness in vacuo. To the residual white solid was added pyridine (3 mL), acetic anhydride (1 mL) and DMAP (cat.). The reaction was stirred for 17 h, concentrated to dryness under vacuum and the residue partitioned between ethyl acetate (50 mL) and 0.3 M potassium hydrogen sulfate (50 mL). The organic phase was dried ($MgSO_4$), concentrated and purified using preparative HPLC (CH_3CN/H_2O, 1:9 to 9:1 over 25 min, retention time 17.7 min) to give **9** as a colourless oil (13 mg, 89%). δ_H (300 MHz; $CDCl_3$) 6.88–7.12 (6H, m, Ar), 3.75 (2H, s, ArCH_2Ar), 3.64 (2H, t, *J* 7.4, CH_2Cl), 2.97 (2H, t, *J* 7.4, CH_2Ar), 2.57 (2H, q, *J* 7.6, CH_2CH$_3$), 2.21 (3H, s, COCH_3), 2.16 (3H, s, COCH_3), 1.18 (3H, t, *J* 7.6, CH$_2$CH_3); δ_C (75 MHz; $CDCl_3$) 169.5 (*C*=O), 169.3 (*C*=O), 147.8, 146.9, 142.2, 136.0, 131.8, 131.0, 130.8, 130.2, 127.9, 127.1, 122.4, 122.2, 44.8, 38.5, 30.7, 28.3, 20.8, 20.7, 15.5; *m*/*z* (HRFAB) found $[MH]^+$ 375.13528; $C_{21}H_{24}ClO_4$ requires 375.13630.

3.8. 4-Ethyl-2-hydroxybenzaldehyde

The reaction was carried out under anhydrous conditions. *para*-Formaldehyde (405 mg, 13.5 mmol) was added to a mixture of 3-ethylphenol (241 μL, 2 mmol), anhydrous magnesium chloride (285 mg, 3 mmol) and triethylamine (1.05 mL, 7.5 mmol) in acetonitrile (10 mL). The reaction was heated at 70 °C for 17 h, cooled to rt then partitioned between diethyl ether (100 mL) and 5% aqueous hydrochloric acid (100 mL). The organic phase was dried ($MgSO_4$) and concentrated to give a residue which was purified by silica flash chromatography (ethyl acetate/hexane, 1:9) to yield the titled compound as a colourless oil (294 mg, 98%).[27] δ_H (300 MHz; $CDCl_3$) 11.04 (1H, s, OH), 9.81 (1H, s, CHO), 7.43 (1H, d, *J* 7.9, 6-Ar*H*), 6.83 (1H, d, *J* 7.9, 5-Ar*H*), 6.80 (1H, s, 3-Ar*H*), 2.65 (2H, q, *J* 7.6, CH_2CH$_3$), 1.23 (3H, *J* 7.6, CH$_2$CH_3); δ_C (75 MHz; $CDCl_3$) 195.8 (*C*=O), 161.9, 155.0, 133.7, 120.0, 118.9, 116.5, 29.4, 14.7; *m*/*z* (HREI) found $[M]^+$ 150.06779; $C_9H_{10}O_2$ requires 150.06808.

3.9. 2-(2-Acetoxy-4-ethylbenzyl)-4-(2-chloroethyl)phenyl acetate 10

The reaction was carried out under anhydrous conditions. Borane–dimethyl sulfide complex (1.28 mL, 2.54 mmol; 2 M in THF) was added to a stirred solution of 4-ethyl-2-hydroxybenzaldehyde (193 mg, 1.27 mmol) in THF (10 mL). The reaction was stirred for 1 h and 5% aqueous hydrochloric acid (5 mL) added dropwise. The mixture was partitioned between ethyl acetate (50 mL) and water (50 mL) and the aqueous phase extracted with ethyl acetate (50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to dryness in vacuo at 30 °C for <10 min to yield a residue (190 mg) which was immediately added to a solution of **5** (196 mg, 1.25 mmol) in ethanol (5 mL) (196 mg, 1.25 mmol). Concd hydrochloric acid (0.5 mL) was added and the mixture heated at 80 °C for 17 h. The reaction was concentrated to dryness and partitioned between ethyl acetate (50 mL) and water (50 mL). The organic phase was dried ($MgSO_4$) and concentrated, then purified by silica flash chromatography (ethyl acetate/hexane, 1:9) to give an impure sample of 4-(2-chloroethyl)-2-(4-ethyl-2-hydroxybenzyl)phenol as a colourless oil (10 mg, 3%). The material was taken forward as isolated.

To the bis-phenol (10 mg, 0.034 mmol) in pyridine (3 mL) was added acetic anhydride (1 mL) and DMAP (cat.). The mixture was stirred for 17 h at rt, concentrated to dryness then purified by silica flash chromatography (ethyl acetate/hexane, 1:4) to give **10** as a colourless oil (5 mg, 39%). δ_H (600 MHz; $CDCl_3$) 7.10 (1H, dd, *J* 7.8 and 2.4, 5-Ar*H*), 6.97–7.00 (3H, m, 6-Ar*H* and 5-Ar*H*(benzyl) and 6-Ar*H*(benzyl)), 6.90 (1H, d, *J* 1.8, 3-Ar*H*(benzyl)), 6.88 (1H, d, *J* 1.2, 3-Ar*H*), 3.73 (2H, s, ArCH_2Ar), 3.64 (2H, t, *J* 7.8, CH_2Cl), 2.97 (2H, t, *J* 7.8, CH_2Ar), 2.64 (2H, q, *J* 7.2, CH_2CH$_3$), 2.21 (3H, s, COCH_3), 2.18 (3H, s, COCH_3), 1.23 (3H, t, *J* 7.2, CH$_2$CH_3); δ_C (150 MHz; $CDCl_3$) 169.5 (signals superimposed), 149.0, 147.9, 144.3, 136.1, 132.0, 131.1, 130.6, 128.4, 128.0, 125.9, 122.5, 121.8, 44.9, 38.7, 30.4, 28.4, 20.9, 15.3; *m*/*z* (HRCI) found $[MH]^+$ 375.13573; $C_{21}H_{24}ClO_4$ requires 375.13631.

3.10. 4-(2-Chloroethyl)-2-(2-hydroxybenzyl)phenol

To phenol **5** (500 mg, 3.19 mmol) and 2-hydroxybenzylalcohol (396 mg, 3.19 mmol) in ethanol (10 mL) was added concd hydrochloric acid (1 mL) and the reaction was heated at 80 °C for 17 h. The reaction was concentrated to dryness and the residue partitioned between ethyl acetate (50 mL) and water (50 mL). The organic phase was dried ($MgSO_4$) and concentrated, then purified by silica flash chromatography (ethyl acetate/hexane, 1:4) to give the titled compound as a yellow oil (58 mg, 7%). δ_H (300 MHz; $CDCl_3$) 7.31 (1H, d, *J* 7.5, 6-Ar*H*(benzyl)), 7.05–7.15 (2H, m, Ar), 6.89–6.95 (2H, m, Ar), 6.85 (1H, d, *J* 8.1, 5-Ar*H*), 6.79 (1H, d, *J* 8.1, 6-Ar*H*), 3.94 (2H, s, ArCH_2Ar), 3.66 (2H, t, *J* 7.5, CH_2Cl), 2.97 (2H, t, *J* 7.5, CH_2Ar); δ_C (75 MHz; $CDCl_3$) 152.4, 151.4, 131.3, 131.2, 130.8, 128.4, 128.2, 127.1, 126.9, 121.7, 116.1, 116.0, 45.2, 38.4, 31.0; *m*/*z* (HRCI) found $[MH]^+$ 263.08361; $C_{15}H_{16}ClO_2$ requires 263.08388.

3.11. 2-(2-Acetoxy-5-(2-chloroethyl)benzyl)phenyl acetate 11

To 4-(2-chloroethyl)-2-(2-hydroxybenzyl)phenol (50 mg, 0.190 mmol) in pyridine (3 mL), acetic anhydride (1 mL) and DMAP (cat.) were added. The mixture was stirred for 17 h at rt, concentrated to dryness and the residue partitioned between ethyl acetate (50 mL) and 0.3 M potassium hydrogen sulfate (50 mL). The organic phase was dried ($MgSO_4$), concentrated in vacuo and purified by silica flash chromatography (ethyl acetate/hexane, 1:4) to give **11** as a colourless oil (42 mg, 64%). δ_H (300 MHz; $CDCl_3$) 7.41–7.47 (1H, m, Ar), 7.16–7.35 (5H, m, Ar), 7.07 (1H, d, *J* 1.7, 6-Ar*H*(benzyl)), 3.95 (2H, s, ArCH_2Ar), 3.81 (2H, t, *J* 7.5, CH_2Cl), 3.14 (2H, t, *J* 7.5, CH_2Ar), 2.37 (3H, s, COCH_3), 2.35 (3H, s, COCH_3); δ_C (75 MHz; $CDCl_3$) 169.3 (*C*=O), 169.2 (*C*=O) 149.0, 147.9, 136.0, 131.7, 131.4, 131.0, 130.8, 128.0, 127.7, 126.2, 122.5, 122.4, 44.8, 38.5, 30.6, 20.8, 20.7; *m*/*z* (HRES) found $[MNa]^+$ 369.08740; $C_{19}H_{19}ClNaO_4$ requires 369.08700.

3.12. 2,2′-Methylenebis(2,1-phenylene) diacetate 12

Phenol (100 mg, 1.06 mmol) and 2-hydroxybenzylalcohol (132 mg, 1.06 mmol) were suspended in sulfuric acid (25% vol; 10 mL) and heated at 100 °C for 17 h. The mixture was diluted with water (50 mL) and extracted with diethyl ether (2 × 50 mL). The organic extracts were dried ($MgSO_4$) and concentrated in vacuo, then purified using silica flash chromatography (ethyl acetate/hexane, 1:4) to give a crude sample of the desired diphenol as a white solid (11 mg).[28] To the crude diphenol (5 mg, 0.025 mmol) in pyridine (3 mL) was added acetic anhydride (1 mL) and DMAP (cat.). The mixture was stirred for 17 h, concentrated to dryness and the residue partitioned between ethyl acetate (30 mL) and 0.3 M potassium hydrogen sulfate (30 mL). The organic phase was dried ($MgSO_4$), concentrated and purified by silica flash chromatography (ethyl acetate/hexane, 1:4) to give the desired compound as a colourless oil (7 mg, 5% yield over two steps). δ_H (500 MHz; $CDCl_3$) 7.24–7.28 (2H, m, 5-Ar*H*), 7.14–7.17 (2H, m, 4-Ar*H*), 7.08 (2H, dd, *J* 7.6 and 1.4, 6-Ar*H*), 7.05 (2H, dd, *J* 8.0 and 1.2, 3-Ar*H*), 3.81 (2H, s, ArCH_2Ar), 2.21 (6H, s, COCH_3); δ_C (125 MHz; $CDCl_3$) 169.3

(C=O), 149.0, 131.6, 130.8, 127.7, 126.3, 122.4, 30.7, 20.8;[29] *m*/*z* (HRCI) found $[MH]^+$ 285.11193; $C_{17}H_{17}O_4$ requires 285. 11268.

3.13. 2-(2-Acetoxy-5-ethylbenzyl)phenyl acetate 13

To 4-ethylphenol (100 mg, 0.82 mmol) and 2-hydroxybenzyl alcohol (102 mg, 0.82 mmol) in ethanol (10 mL), concd hydrochloric acid (1 mL) was added. The reaction was heated at 80 °C for 17 h, cooled to rt and concentrated to dryness in vacuo. The residue was purified by silica flash chromatography (ethyl acetate/hexane, 1:4) to give a crude sample of the desired diphenol. To the crude diphenol (25 mg) in pyridine (3 mL) acetic anhydride (1 mL) and DMAP (cat.) were added. The mixture was stirred for 17 h, concentrated to dryness and the residue partitioned between ethyl acetate (30 mL) and 0.3 M potassium hydrogen sulfate (30 mL). The organic phase was dried ($MgSO_4$) and concentrated, then purified by silica flash chromatography (ethyl acetate/hexane, 1:4) to give **13** as a colourless oil (15 mg, 6%). δ_H (500 MHz; $CDCl_3$) 7.23–7.26 (1H, m, Ar), 7.14–7.17 (1H, m, Ar), 7.04–7.09 (3H, m, Ar), 6.95 (1H, d, *J* 8.2, 6-Ar*H*), 6.91 (1H, d, *J* 1.9, 6-Ar*H*(benzyl)), 3.77 (2H, s, ArCH_2Ar), 2.57 (2H, q, *J* 7.6, CH_2CH$_3$), 2.21 (3H, s, COCH_3), 2.18 (3H, s, COCH_3), 1.18 (3H, t, *J* 7.6, CH$_2$CH_3); δ_C (125 MHz; $CDCl_3$) 169.6 (*C*=O), 169.3 (*C*=O), 149.0, 147.0, 131.8, 131.1, 130.7, 130.3, 127.6, 127.1, 126.2, 122.4, 122.2, 30.8, 28.3, 20.8 (signals superimposed), 15.5; *m*/*z* (HRCI) found $[MH]^+$ 313.14458; $C_{19}H_{21}O_4$ requires 313.14398.

3.14. 4-(2-Chloroethyl)-2-iodophenylacetate 15

Sodium hypochlorite (13% aqueous solution; 3.10 mL, 6.50 mmol) was added in small portions (until the red colour dissipated after each addition) over 45 min to a solution of phenol **5** (848 mg, 5.42 mmol), sodium iodide (812 mg, 5.42 mmol) and sodium hydroxide (217 mg, 5.42 mmol) in methanol (30 mL) at 0 °C. After a further 90 min 10% sodium thiosulfate solution (30 mL) was added. The solution was adjusted to pH 7 using 0.5 M hydrochloric acid and extracted with dichloromethane (60 mL). The organic layer was washed with brine (60 mL), dried ($MgSO_4$) and concentrated, then purified by silica flash chromatography (ethyl acetate/hexane, 1:9) to give 4-(2-chloroethyl)-2-iodophenol as a pale yellow oil (980 mg, 46%). R_F 0.52 (ethyl acetate/hexane, 1:4); ν_{max}/cm^{-1} (film) 3481, 2955, 2864, 1601, 1574, 1489; δ_H (300 MHz; $CDCl_3$) 7.53 (1H, s, 3-Ar*H*), 7.08 (1H, d, *J* 8.4, 4-Ar*H*), 6.97 (1H, d, *J* 8.4, 5-Ar*H*), 5.51 (1H, s, O*H*), 3.67 (2H, t, *J* 7.2, CH_2Cl), 2.95 (2H, t, *J* 7.2, CH_2Ar); δ_C (75 MHz; $CDCl_3$) 153.8, 138.5, 132.4, 130.4, 115.2, 85.7, 45.1, 37.7; *m*/*z* (HRCI) found $[MH]^+$ 282.9393; $C_8H_9Cl_2IO$ requires 282.9387.

Acetic anhydride (0.22 mL, 2.33 mmol) was added to 4-(2-chloroethyl)-2-iodophenol (536 mg, 1.90 mmol) and 4-dimethylaminopyridine (cat.) in triethylamine (10 mL). The reaction was stirred for 17 h and concentrated in vacuo. The resulting residue was dissolved in ethyl acetate (20 mL) and water (20 mL) added. The aqueous layer was extracted with ethyl acetate (3 × 20 mL) and the combined organic extracts dried ($MgSO_4$) and concentrated to give the **15** as a pale yellow oil (560 mg, 91%). R_F 0.38 (ethyl acetate/hexane, 1:4); ν_{max}/cm^{-1} (film) 2960, 1767; δ_H (300 MHz; $CDCl_3$) 7.68 (1H, s, 3-Ar*H*), 7.21 (1H, d, *J* 8.2, 5-Ar*H*), 7.03 (1H, d, *J* 8.2, 6-Ar*H*), 3.66 (2H, t, *J* 7.2, CH_2Cl), 2.99 (2H, t, *J* 7.2, CH_2Ar), 2.35 (3H, s, COCH_3); δ_C (75 MHz; $CDCl_3$) 168.7 (*C*=O), 150.1, 139.6, 137.8, 122.9, 121.7, 90.7, 44.5, 37.9, 21.3 (COCH$_3$); *m*/*z* (HRCI) found $[MH]^+$ 324.9485; $C_{10}H_{10}ClIO_2$ requires 324.9492.

3.15. Di-*tert*-butyl-*N*-5-hexenyliminodicarboxylate 16

6-Bromohex-1-ene (0.80 mL, 6.00 mmol) was added to a stirring solution of di-*tert*-butyliminodicarboxylate (868 mg, 4.00 mmol) and cesium carbonate (2.61 g, 8.00 mmol) in 2-butanone (20 mL) and heated at reflux (90 °C) for 17 h. The solution was cooled to rt and brine (40 mL) added. The organic and aqueous layers were separated, and the aqueous layer extracted with diethyl ether (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to give the **16**[30] as a yellow oil (1.19 g, 100%). R_F 0.35 (ethyl acetate/hexane, 1:4); ν_{max}/cm^{-1} (film) 2980, 2937, 1716, 1630; δ_H (300 MHz; $CDCl_3$) 5.78–5.85 (1H, m, CH$_2$=C*H*CH$_2$), 4.90–5.00 (2H, m, CH_2=CHCH$_2$), 3.53 (2H, t, *J* 7.4, NCH_2CH$_2$), 2.04 (2H, q, *J* 6.7, CH$_2$=CHCH_2), 1.41–1.58 (20H, m, 2 × OC(CH_3)$_3$ and CH$_2$=CHCH$_2$CH_2); δ_C (75 MHz; $CDCl_3$) 152.7 (*C*=O), 138.5 (H$_2$C=*C*H), 114.6 (H$_2$*C*=CH), 82.0, 46.3, 33.4, 32.2, 28.5, 28.1, 26.0, 25.0; *m*/*z* (HRES) found $[2M+Na]^+$ 621.4072; $C_{32}H_{58}N_2NaO_8$ requires 621.4085.

3.16. 4-(2-Chloroethyl)-2-[5-2(chloroethyl)-2-hydroxybenzyl]-6-iodophenol 18

Sodium hypochlorite solution (13% aq solution; 630 μL, 1.323 mmol) was added in small portions (until the red colour dissipated after each addition) over 45 min to a solution of phenol **3** (430 mg, 1.32 mmol), sodium iodide (198 mg, 1.32 mmol) and sodium hydroxide (53 mg, 1.32 mmol) in methanol (30 mL) at 0 °C. After a further 95 min, 10% sodium thiosulfate solution (30 mL) was added and the solution was adjusted to pH 7 using 0.5 M hydrochloric acid. The mixture was extracted with dichloromethane (2 × 60 mL) and the combined organic extracts washed with brine (60 mL) and dried ($MgSO_4$). The organic extracts were concentrated under reduced pressure and the crude product purified twice by silica flash chromatography (petroleum ether 40:60/ethyl acetate, 4:1) to give **18** as a white solid (175 mg, 29%). Mp 134–136 °C (petroleum ether 40:60/ethyl acetate); ν_{max}/cm^{-1} (KBr) 3416, 2947, 1508, 1431; δ_H (300 MHz; $CDCl_3$) 7.40 (1H, s, 5-Ar*H*), 7.07 (2H, s, 3-Ar*H* and 6-Ar*H*(benzyl)), 6.97 (1H, d, *J* 8.2, 4-Ar*H*(benzyl)), 6.79 (1H, d, *J* 8.2, 3-Ar*H*(benzyl)), 6.31 (1H, s, O*H*), 6.11 (1H, s, O*H*), 3.94 (2H, s, ArCH_2-Ar), 3.60–3.70 (4H, m, 2 × CH_2Cl), 2.90–3.00 (4H, m, 2 × CH_2Ar); δ_C (75 MHz; $CDCl_3$) 152.0, 150.7, 136.9, 132.9, 131.7, 131.2, 130.9, 128.5, 126.9, 126.0, 116.4, 86.1, 45.3, 44.9, 38.3, 37.7 and 31.6; *m*/*z* (HRCI) found M^+ 449.9658; $C_{17}H_{17}Cl_2IO_2$ requires 449.9650.

3.17. 2-(2-Acetoxy-5-(2-chloroethyl)-3-iodobenzyl)-4-(2-chloroethyl)phenyl acetate 19

To diphenol **18** (370 mg, 0.820 mmol) in pyridine (6 mL) was added acetic anhydride (2 mL) and DMAP (cat.) and the reaction was stirred for 18 h. The mixture was concentrated to dryness in vacuo and the residue partitioned between ethyl acetate (50 mL) and 0.3 M potassium hydrogen sulfate (50 mL) and the organic phase dried ($MgSO_4$) and concentrated. The residue was purified using silica flash chromatography (ethyl acetate/hexane, 1:4) to give **19** as a yellow oil (383 mg, 87%). δ_H (300 MHz; $CDCl_3$) 7.56 (1H, d, *J* 2.0, 4-Ar*H*(benzyl)), 7.14 (1H, dd, *J* 8.2 and 2.1, 5-Ar*H*), 7.01 (1H, d, *J* 8.2, 6-Ar*H*), 6.94 (1H, d, *J* 2.1, 3-Ar*H*), 6.80 (1H, d, *J* 2.0, 6-Ar*H*(benzyl)), 3.76 (2H, s, ArCH_2Ar), 3.67 (2H, t, *J* 7.3, CH_2Cl), 3.62 (2H, t, *J* 7.3, CH_2Cl), 3.01 (2H, t, *J* 7.3, CH_2Ar), 2.92 (2H, t, *J* 7.2, CH_2Ar), 2.32 (3H, s, COCH_3), 2.04 (3H, s, COCH_3); δ_C (125 MHz; $CDCl_3$) 169.4, 168.3, 148.6, 148.0, 138.0, 137.9, 136.2, 133.6, 131.5, 131.1, 130.8, 128.5, 122.8, 91.7, 44.9, 44.5, 38.5, 37.9, 31.4, 21.1, 20.8; *m*/*z* (HRFAB) found $[MNa]^+$ 556.97648; $C_{21}H_{21}Cl_2INaO_4$ requires 556.97593.

3.18. *tert*-Butyl(2-(2-(*tert*-butyldimethylsilyloxy)-5-(2-chloroethyl)-3-iodobenzyl)-4-(2-chloroethyl)phenoxy) dimethylsilane 20

To diphenol **18** (175 mg, 0.388 mmol) in dichloromethane (10 mL) at 0 °C was added triethylamine (162 μL, 1.164 mmol)

and then dropwise, TBSOTf (267 μL, 1.164 mmol). The reaction was stirred at rt for 18 h, diluted with dichloromethane (20 mL) and the organic phase washed with 0.3 M potassium hydrogen sulfate (30 mL), satd sodium hydrogen carbonate (30 mL) and dried ($MgSO_4$). The organic phase was concentrated and the residue purified by silica flash chromatography (ethyl acetate/hexane, 95:5) to give **20** as a colourless oil (219 mg, 83%). δ_H (300 MHz; $CDCl_3$) 7.47 (1H, d, *J* 2.2, 4-Ar*H*(benzyl)), 6.96 (1H, dd, *J* 8.2 and 2.3, 5-Ar*H*), 6.80 (1H, d, *J* 2.2, 6-Ar*H*(benzyl)), 6.75 (1H, d, *J* 8.2, 6-Ar*H*), 6.62 (1H, d, *J* 2.3, 3-Ar*H*), 3.90 (2H, s, ArCH_2Ar), 3.63 (2H, t, *J* 7.4, CH_2Cl), 3.53 (2H, t, *J* 7.5, CH_2Cl), 2.94 (2H, t, *J* 7.5, CH_2Ar), 2.81 (2H, t, *J* 7.5, CH_2Ar), 1.04 (9H, s, (CH_3)$_3$C), 0.88 (9H, s, (CH_3)$_3$C), 0.32 (6H, s, CH_3Si), 0.17 (6H, s, CH_3Si); δ_C (75 MHz; $CDCl_3$) 152.6, 152.4, 137.8, 132.9, 132.5, 131.5, 130.8, 130.6, 130.5, 127.7, 118.1, 90.6, 45.3, 44.7, 38.4, 37.8, 32.0, 26.4, 25.6, 18.9, 18.1, −1.5, −4.2; *m*/*z* (HRFAB) found $[MH]^+$ 679.13895; $C_{29}H_{46}Cl_2IO_2Si_2$ requires 679.14580.

3.19. 5-(2-Chloroethyl)-1-(5-(2-chloroethyl)-2-methoxybenzyl)-3-iodo-2-methoxybenzene 21

Methyl iodide (211 μL, 3.39 mmol) was added to diphenol **18** (153 mg, 0.339 mmol) and potassium carbonate (234 mg, 1.70 mmol) in acetone (10 mL) in a pressure tube. The mixture was heated at 60 °C for 17 h and the contents cooled and filtered. The filtrate was concentrated under vacuum to give a residue which was purified using silica flash chromatography (ethyl acetate/hexane, 1:9) to yield **21** as a colourless oil (141 mg, 87%). δ_H (300 MHz; $CDCl_3$) 7.54 (1H, d, *J* 2.1, 4-Ar*H*), 7.07 (1H, dd, *J* 8.3 and 2.1, 4-Ar*H*(benzyl)), 6.82–6.87 (3H, m, 6-ArH, 3-Ar*H*(benzyl) and 6-Ar*H*(benzyl)), 4.00 (2H, s, ArCH_2Ar), 3.79 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.59–3.66 (4H, m, 2 × CH_2Cl), 2.95 (2H, t, *J* 7.7, CH_2Ar), 2.89 (2H, t, *J* 7.3, CH_2Ar); δ_C (75 MHz; $CDCl_3$) 156.9, 156.4, 137.5, 135.8, 135.1, 131.6, 131.0, 130.1, 128.5, 127.9, 110.5, 92.0, 60.9, 55.5, 45.4, 44.8, 38.3, 37.9, 30.5; *m*/*z* (HREI) found M^+ 477.99710; $C_{19}H_{21}Cl_2IO_2$ requires 477.99633.

3.20. 4-(2-Chloroethyl)-2-((5-(2-chloroethyl)-2-((dibenzylamino)-methyl)benzofuran-7-yl)methyl)phenol 23

The reaction was carried out under anhydrous conditions. Diphenol **18** (200 mg, 0.443 mmol), acetylene **22** (156 mg, 0.665 mmol), CuI (8 mg, 0.044 mmol) and $Pd(PPh_3)_4$ (26 mg, 0.022 mmol) in freshly distiled and degassed triethylamine (10 mL) and dimethylacetamide (3 mL) in a pressure tube were heated at 80 °C for 17 h. The reaction was cooled, concentrated to dryness in vacuo, partitioned between ethyl acetate (50 mL) and water (50 mL) and dried ($MgSO_4$). The organic phase was concentrated and the residue purified using silica flash chromatography (ethyl acetate/hexane, 1:4) to yield **23** as a brown oil (120 mg, 49%). δ_H (300 MHz; $CDCl_3$) 7.23–7.45 (11H, m, NBn and 4-Ar*H*(benzofuran)), 7.07 (1H, d, *J* 1.8, 3-Ar*H*), 6.97 (1H, dd, *J* 8.1 and 1.8, 5-Ar*H*), 6.94 (1H, s, 6-Ar*H*(benzofuran)), 6.79 (1H, d, *J* 8.1, 6-Ar*H*), 6.57 (1H, s, 3-Ar*H*(benzofuran)), 4.22 (2H, s, CH_2NBn$_2$), 3.77 (2H, s, ArCH_2Ar), 3.68–3.73 (6H, m, 2 × NCH_2Ar and CH_2Cl), 3.61 (2H, t, *J* 7.5, CH_2Cl), 3.09 (2H, t, *J* 7.5, CH_2Ar), 2.93 (2H, t, *J* 7.5, CH_2Ar); δ_C (75 MHz; $CDCl_3$) 156.1, 152.9, 152.5, 139.2, 133.0, 131.3, 130.5, 128.9, 128.6, 128.4, 128.2, 127.1, 126.2, 125.0, 123.3, 119.1, 116.3, 105.7, 57.8, 49.6, 45.6, 45.3, 39.2, 38.4, 30.2, 29.8; *m*/*z* (HRCI) found $[MH]^+$ 558.1972; $C_{34}H_{33}Cl_2NO_2$ requires 558.1967.

3.21. *N*,*N*-Dibenzyl-3-(5-(2-chloroethyl)-3-(5-(2-chloroethyl)-2-methoxybenzyl)-2-methoxyphenyl)prop-2-yn-1-amine 24

The reaction was carried out under anhydrous conditions. Aryl iodide **21** (153 mg, 0.319 mmol), acetylene **22** (112 mg, 0.479 mmol), CuI (6 mg, 0.032 mmol) and $Pd(PPh_3)_4$ (18 mg, 0.016 mmol) in freshly distiled and degassed triethylamine (15 mL) in a pressure tube were heated at 80 °C for 17 h. The reaction was cooled, concentrated to dryness in vacuo, partitioned between ethyl acetate (50 mL) and water (50 mL) and dried ($MgSO_4$). The organic phase was concentrated and the residue was purified using silica flash chromatography (ethyl acetate/hexane, 1:4) to yield **24** as an orange oil (73 mg, 39%). δ_H (300 MHz; $CDCl_3$) 7.44–7.46 (4H, m, NBn), 7.32–7.36 (4H, m, NBn), 7.24–7.29 (2H, m, NBn), 7.20 (1H, d, *J* 2.2, 6-Ar*H*), 7.06 (1H, dd, *J* 8.3 and 2.2, 4-Ar*H*(benzyl)), 6.89–6.91 (2H, m, 4-Ar*H* and 6-Ar*H*(benzyl)), 6.83 (1H, d, *J* 8.3, 3-Ar*H*(benzyl)), 3.98 (2H, s, ArCH_2Ar), 3.92 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.79 (4H, s, 2 × NCH_2Ar), 3.66 (2H, t, *J* 7.5, CH_2Cl), 3.65 (2H, t, *J* 7.3, CH_2Cl), 3.53 (2H, s, NCH_2), 2.96 (2H, t, *J* 7.4, CH_2Ar), 2.95 (2H, t, *J* 7.4, CH_2Ar); δ_C (75 MHz; $CDCl_3$) 158.5, 156.5, 139.0, 134.2, 133.3, 132.3, 131.5, 131.0, 130.0, 129.1, 129.0, 128.4, 127.7, 127.2, 117.0, 110.5, 89.0, 82.3, 61.1, 57.8, 55.5, 45.4, 44.9, 42.3, 38.4, 38.3, 29.9; *m*/*z* (HREI) found $[M]^+$ 585.22123; $C_{36}H_{37}Cl_2NO_2$ requires 585.22013.

3.22. *N*-(Benzyloxycarbonyl)propargylamine 26

To propargylamine (1 mL, 14.9 mmol) in dichloromethane (50 mL) and triethylamine (2.23 mL, 15.93 mmol) at 0 °C was added benzyl chloroformate (2.29 mL, 15.9 mmol) dropwise. The reaction was stirred for 17 h at rt, then washed with water (2 × 50 mL) and dried ($MgSO_4$). The organic phase was concentrated and the residue purified using silica flash chromatography (ethyl acetate/hexanes, 2:3) to yield **26**[31] as a colourless oil (1.10 g, 40%). δ_H (300 MHz; $CDCl_3$) 7.29–7.37 (5H, m, Ar), 5.13 (2H, s, CH_2Ar), 4.99 (1H, s, N*H*), 3.96–4.00 (2H, m, CH_2N), 2.25 (1H, t, *J* 4.1, CC*H*); δ_C (75 MHz; $CDCl_3$) 155.1, 136.2, 128.6, 128.3, 128.2, 79.6, 71.7, 67.1, 30.9.

3.23. 2-(2-Acetoxy-3-(3-(*tert*-butoxycarbonylamino)prop-1-ynyl)-5-(2-chloroethyl)benzyl)-4-(2-chloroethyl)phenyl acetate 27

The reaction was carried out under anhydrous conditions. Aryl iodide **19** (160 mg, 0.299 mmol), acetylene **2** (153 mg, 0.598 mmol), CuI (6 mg, 0.030 mmol) and $Pd(PPh_3)_4$ (17 mg, 0.015 mmol) in freshly distiled and degassed triethylamine (10 mL) were stirred for 17 h. The reaction was concentrated to dryness in vacuo, the residue partitioned between ethyl acetate (50 mL) and water (50 mL) and the organic phase dried ($MgSO_4$) then evaporated. The residue was purified using silica flash chromatography (ethyl acetate/hexane, 1:2) to yield **27** as a yellow oil (78 mg, 46%). δ_H (400 MHz; $CDCl_3$ at 55 °C) 7.21 (1H, d, *J* 2.2, 4-Ar*H*(benzyl)), 7.13 (1H, dd, *J* 8.2 and 2.2, 5-Ar*H*), 7.03 (1H, d, *J* 8.2, 6-Ar*H*), 6.93 (1H, d, *J* 2.2, 3-Ar*H*), 6.85 (1H, d, *J* 2.2, 6-Ar*H*(benzyl)), 4.14 (2H, d, *J* 5.7, CH_2N), 3.77 (2H, s, ArCH_2Ar), 3.67 (2H, t, *J* 7.3, CH_2Cl), 3.64 (2H, t, *J* 7.2, CH_2Cl), 3.01 (2H, t, *J* 7.3, CH_2Ar), 2.95 (2H, t, *J* 7.3, CH_2Ar), 2.27 (3H, s, COCH_3), 2.18 (3H, s, COCH_3), 1.49 (9H, s, C(CH_3)$_3$); δ_C (100 MHz; $CDCl_3$) 169.4, 168.7, 155.3, 149.2, 147.9, 136.2, 136.0, 132.5, 131.8, 131.3, 131.2, 131.0, 128.3, 122.6, 117.3, 90.5, 80.2, 78.2, 44.9, 44.6, 38.5, 38.3, 31.2, 30.5, 29.8, 28.4, 20.8, 20.6; *m*/*z* (HRFAB) found $[MNa]^+$ 584.15771; $C_{29}H_{33}Cl_2NNaO_6$ requires 584.15825.

3.24. 2-(2-Acetoxy-3-(3-(benzyloxycarbonylamino)prop-1-ynyl)-5-(2-chloroethyl)benzyl)-4-(2-chloroethyl)phenyl acetate 28

The reaction was carried out under anhydrous conditions. Aryl iodide **19** (161 mg, 0.301 mmol), acetylene **26** (114 mg, 0.602 mmol), CuI (6 mg, 0.030 mmol) and $Pd(PPh_3)_4$ (18 mg, 0.015 mmol) in freshly distiled and degassed triethylamine

(10 mL) were stirred for 17 h. The reaction was concentrated to dryness in vacuo, the residue partitioned between ethyl acetate (50 mL) and water (50 mL) and the organic phase dried ($MgSO_4$) then evaporated. The residue was purified using silica flash chromatography (acetone/toluene, 5:95) to yield **28** as an orange oil (80 mg, 45%). δ_H (300 MHz; $CDCl_3$) 7.29–7.41 (5H, m, Ar(carbamate)), 7.17–7.21 (1H, m, 4-Ar*H*(benzyl)), 7.13 (1H, d, *J* 8.3, 6-Ar*H*), 7.01 (1H, d, *J* 8.3, 5-Ar*H*), 6.91 (1H, s, 3-Ar*H*), 6.84 (1H, s, 6-Ar*H*(benzyl)), 5.05–5.20 (3H, m, OCH_2Ar and N*H*), 4.19 (2H, d, *J* 5.4, CH_2N), 3.75 (2H, s, ArCH_2-Ar), 3.69 (2H, t, *J* 7.3, CH_2Cl), 3.62 (2H, t, *J* 7.2, CH_2Cl), 2.99 (2H, t, *J* 7.3, CH_2Ar), 2.93 (2H, t, *J* 7.2, CH_2Ar), 2.22 (3H, s, COCH_3), 2.18 (3H, s, COCH_3); δ_C (75 MHz; $CDCl_3$) 169.4, 168.6, 155.9, 149.1, 147.9, 136.2, 136.1, 136.0, 132.5, 131.7, 131.3, 130.9, 129.1, 128.6, 128.3, 128.2, 122.6, 117.2, 90.0, 78.5, 67.1, 44.8, 44.5, 38.5, 38.2, 31.7, 30.4, 20.8, 20.5; *m*/*z* (HRFAB) found $[MNa]^+$ 618.14190; $C_{32}H_{31}Cl_2NNaO_6$ requires 618.14260.

3.25. 2-(3-(3-Acetamidoprop-1-ynyl)-2-acetoxy-5-(2-chloroethyl)benzyl)-4-(2-chloroethyl)phenyl acetate 29

TFA (1 mL) was added to a stirred solution of acetate **27** (78 mg, 0.154 mmol) in dichloromethane (4 mL) at 0 °C. The reaction was stirred at 0 °C for 1 h and the mixture then concentrated to dryness in vacuo. The residue was redissolved in pyridine (3 mL) and acetic anhydride (1 mL) and the reaction stirred for 17 h. The mixture was concentrated to dryness under vacuum and the residue partitioned between ethyl acetate (50 mL) and 0.3 M potassium hydrogen sulfate (50 mL) and the organic phase dried ($MgSO_4$) and evaporated. The residue was purified by silica flash chromatography to give **29** as a colourless oil (59 mg, 85%). δ_H (300 MHz; $CDCl_3$) 7.20 (1H, d, *J* 1.2, 4-Ar*H*(benzyl)), 7.11 (1H, dd, *J* 8.2 and 1.2, 5-Ar*H*), 6.99 (1H, d, *J* 8.2, 6-Ar*H*), 6.90 (1H, d, *J* 1.2, 3-Ar*H*), 6.84 (1H, d, *J* 1.2, 6-Ar*H*(benzyl)), 4.22 (2H, d, *J* 5.3, CH_2N), 3.74 (2H, s, ArCH_2Ar), 3.63 (4H, apparent quartet, *J* 7.5, 2 × CH_2Cl), 2.95 (4H, apparent quintet, *J* 7.3 and 7.2, 2 × CH_2Ar), 2.25 (3H, s, OCOCH_3), 2.17 (3H, s, OCOCH_3), 1.99 (3H, s, NHCOCH_3); δ_C (75 MHz; $CDCl_3$) 169.8, 169.4, 168.8, 149.2, 147.9, 136.2, 136.0, 132.5, 131.8, 131.4, 131.3, 131.0, 128.4, 122.6, 117.2, 89.9, 78.4, 44.9, 44.6, 38.5, 38.2, 30.5, 30.0, 23.1, 20.8, 20.6; *m*/*z* (HRFAB) found $[MNa]^+$ 526.11544; $C_{26}H_{27}Cl_2NNaO_5$ requires 526.11639.

3.26. 2-(3-(3-Acetamidopropyl)-2-acetoxy-5-(2-chloroethyl)-benzyl)-4-(2-chloroethyl)phenyl acetate 30

Palladium on carbon (50 mg; 10% wt/wt, dry form) was added to a stirred solution of carbamate **28** (75 mg, 0.126 mmol) in methanol (5 mL). The mixture was subjected to three cycles of pump/purge with nitrogen, then three cycles of pump/purge with hydrogen and the reaction stirred under hydrogen for 17 h. The reaction was subjected to three cycles of pump/purge with nitrogen and the catalyst removed by filtration. The filtrate was concentrated to dryness in vacuo to yield a colourless oil, to which was added pyridine (3 mL) and acetic anhydride (1 mL) and the mixture was stirred for a further 17 h. The reaction was concentrated to dryness under vacuum and the residue partitioned between ethyl acetate (10 mL) and 0.3 M potassium hydrogen sulfate (10 mL), then the organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by silica flash chromatography (ethyl acetate) to yield **30** as a colourless oil (56 mg, 87%). δ_H (300 MHz; $CDCl_3$) 7.11 (1H, dd, *J* 8.2 and 1.8, 5-Ar*H*), 6.95–7.03 (2H, m, 3-Ar*H* and 6-Ar*H*), 6.90 (1H, d, *J* 1.6, 4-Ar*H*(benzyl)) 6.71 (1H, d, *J* 1.6, 6-Ar*H* (benzyl)), 5.69 (1H, s, N*H*), 3.70 (2H, s, ArCH_2Ar), 3.65 (2H, t, *J* 7.4, CH_2Cl), 3.63 (2H, t, *J* 7.2, CH_2Cl), 3.22 (2H, t, *J* 6.8, CH_2N), 2.98 (2H, t, *J* 7.4, CH_2Ar), 2.94 (2H, t, *J* 7.2, CH_2Ar), 2.49 (2H, t, *J* 7.5, ArCH_2CH_2), 2.23 (3H, s, OCOCH_3), 2.16 (3H, s, OCOCH_3), 1.89 (3H, s, NHCOCH_3), 1.75–1.84 (2H, m, $CH_2CH_2CH_2$); δ_C (75 MHz; $CDCl_3$) 170.1, 169.4, 169.3, 147.9, 146.3, 136.1, 136.0, 133.7, 132.0, 131.3, 131.2, 128.9, 128.8, 128.1, 122.5, 44.9, 44.8, 39.2, 38.5, 30.8, 29.2, 27.6, 23.2, 20.7, 20.5. *m*/*z* (HRFAB) found $[MNa]^+$ 530.14820; $C_{26}H_{31}Cl_2NNaO_5$ requires 530.14769.

3.27. 2-(Hydroxymethyl)-4-iodophenol

The reaction was carried out under anhydrous conditions. Borane–dimethyl sulfide complex (2.02 mL, 4.04 mmol; 2 M solution in THF) was added to 2-hydroxy-5-iodobenzaldehyde (500 mg, 2.02 mmol) in THF (15 mL) and the reaction was stirred for 1 h, then quenched by the cautious addition of 5% hydrochloric acid solution (5 mL). The mixture was partitioned between ethyl acetate (100 mL) and water (100 mL) and extracted with further ethyl acetate (100 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo, then purified using silica flash chromatography (ethyl acetate/hexane, 1:1) to yield the titled compound as a white solid (498 mg, 98%).[32] δ_H (500 MHz; acetone-d_6) 7.62–7.64 (1H, m, 3-Ar*H*), 7.42 (1H, dd, *J* 8.4 and 2.3, 5-Ar*H*), 6.68 (1H, d, *J* 8.4, 6-Ar*H*), 4.71 (2H, s, CH_2); δ_C 125 MHz; acetone-d_6) 155.2, 136.8, 136.3, 131.4, 117.9, 81.0, 60.0; ; *m*/*z* (HREI) found M^+ 249.94856; $C_7H_7IO_2$ requires $[M]^+$ 249.94852.

3.28. 4-(2-Chloroethyl)-2-(2-hydroxy-5-iodobenzyl)phenol 31

Chlorophenol **5** (83 mg, 0.532 mmol) and 2-(hydroxymethyl)-4-iodophenol (133 mg, 0.532 mmol) were suspended in 25% aqueous sulfuric acid (25 mL) and heated at 100 °C for 4 h. The reaction was cooled to rt and the mixture diluted with water (25 mL), then extracted with diethyl ether (2 × 50 mL). The combined organics were dried ($MgSO_4$), concentrated in vacuo, and purified using silica flash chromatography (ethyl acetate/hexane, 1:4) to give **31** as a yellow oil (69 mg, 33%). δ_H (500 MHz; $CDCl_3$) 7.55 (1H, d, *J* 2.2, 6-Ar*H*(benzyl)), 7.37 (1H, dd, *J* 8.5 and 2.2, 4-Ar*H*(benzyl)), 7.09 (1H, d, *J* 2.2, 3-Ar*H*), 6.96 (1H, dd, *J* 8.2 and 2.2, 5-Ar*H*), 6.75 (1H, d, *J* 8.2, 6-Ar*H*), 6.59 (1H, d, *J* 8.5, 3-Ar*H*(benzyl)), 3.83 (2H, s, ArCH_2Ar), 3.66 (2H, t, *J* 7.4, CH_2Cl), 2.98 (2H, t, *J* 7.4, CH_2Ar); δ_C (125 MHz; $CDCl_3$) 153.1, 151.2, 139.2, 136.9, 131.5, 131.4, 129.5, 128.6, 126.2, 118.6, 116.0, 83.3, 45.3, 38.3, 30.5; *m*/*z* (-HRES) found $[M-H]^+$ 386.9669; $C_{15}H_{13}ClIO_2$ requires 386.9649.

3.29. 2-(2-Acetoxy-5-(2-chloroethyl)benzyl)-4-iodophenyl acetate

To diphenol **31** (300 mg, 0.772 mmol) in pyridine (3 mL) was added acetic anhydride (1 mL) and DMAP (cat.). The reaction was stirred for 17 h, concentrated to dryness in vacuo and the residue partitioned between ethyl acetate (50 mL) and 0.3 M potassium hydrogen sulfate (50 mL). The organic phase was dried ($MgSO_4$), concentrated under vacuum and purified by silica flash chromatography (ethyl acetate/hexane, 1:2) to give the titled compound as a colourless oil (295 mg, 81%). δ_H (500 MHz; $CDCl_3$) 7.58 (1H, dd, *J* 8.5 and 2.2, 5-Ar*H*), 7.43 (1H, d, *J* 2.2, 3-Ar*H*), 7.12 (1H, dd, *J* 8.2 and 2.1, 4-Ar*H*(benzyl)), 7.01 (1H, d, *J* 8.2, 3-Ar*H*(benzyl)), 6.88 (1H, d, *J* 2.1, 6-Ar*H*(benzyl)), 6.82 (1H, d, *J* 8.5, 6-Ar*H*), 3.73 (2H, s, ArCH_2Ar), 3.66 (2H, t, *J* 7.4, CH_2Cl), 2.99 (2H, t, *J* 7.4, CH_2Ar), 2.23 (3H, s, COCH_3), 2.16 (3H, s, COCH_3); δ_C (125 MHz; $CDCl_3$) 169.3, 168.9, 149.0, 147.8, 139.7, 136.9, 136.2, 134.0, 131.0, 130.9, 128.4, 124.7, 122.6, 90.5, 44.8, 38.6, 30.7, 20.9, 20.7; *m*/*z* (HRCI) found $[MH]^+$ 473.00232; $C_{19}H_{19}ClIO_4$ requires 473.00165.

3.30. 2-(2-Acetoxy-5-(2-chloroethyl)benzyl)-4-(3-(*tert*-butoxycarbonylamino)prop-1-ynyl)phenyl acetate 32

The reaction was carried out under anhydrous conditions. To 2-(2-acetoxy-5-(2-chloroethyl)benzyl)-4-iodophenyl acetate

(138 mg, 0.292 mmol) and acetylene **25** (149 mg, 0.584 mmol) in a mixture of degassed triethylamine (10 mL) and DMF (1 mL), $Pd(PPh_3)_4$ (17 mg, 0.015 mmol) and copper(I) iodide (6 mg, 0.029 mmol) were added and the reaction stirred at for 17 h. The mixture was concentrated to dryness in vacuo and the residue partitioned between ethyl acetate (50 mL) and water (50 mL). The organic phase was dried ($MgSO_4$), concentrated under vacuum and purified by silica flash chromatography (ethyl acetate/hexane, 1:2) to give **32** as a yellow oil (102 mg, 70%). δ_H (500 MHz; $CDCl_3$) 7.31 (1H, dd, *J* 8.3 and 1.5, 5-Ar*H*), 7.15 (1H, d, *J* 1.5, 3-Ar*H*), 7.12 (1H, dd, *J* 8.2 and 2.0, 4-Ar*H*(benzyl)), 6.99–7.01 (2H, m, 6-Ar*H* and 3-Ar*H*(benzyl)), 6.87 (1H, d, *J* 1.7, 6-Ar*H*(benzyl)), 4.74 (1H, br s, N*H*), 4.09–4.12 (2H, m, CH_2NH), 3.73 (2H, s, ArCH_2Ar), 3.65 (2H, t, *J* 7.4, CH_2Cl), 2.98 (2H, t, *J* 7.4, CH_2Cl), 2.22 (3H, s, $COCH_3$), 2.17 (3H, s, $COCH_3$), 1.45 (9H, s, $C(CH_3)_3$); δ_C (125 MHz; $CDCl_3$) 169.3, 169.0, 155.3, 149.0, 147.9, 136.2, 134.1, 131.8, 131.23, 131.20, 131.0, 128.3, 122.7, 122.6, 120.8, 85.6, 82.2, 80.1, 44.8, 38.6, 31.2, 30.6, 28.4, 20.8, 20.7; *m*/*z* (HRES) found $[MNa]^+$ 522.1670; $C_{27}H_{30}ClNNaO_6$ requires 522.1659.

3.31. 4-(3-Acetamidoprop-1-ynyl)-2-(2-acetoxy-5-(2-chloroethyl)-benzyl)phenyl acetate 34

To diacetate **32** (20 mg, 0.040 mmol) in dichloromethane (4 mL) at 0 °C was added TFA (1 mL) and the reaction was stirred at 0 °C for 2 h. The mixture was evaporated to dryness in vacuo and to the residue **33** in pyridine (3 mL), acetic anhydride (1 mL) and DMAP (cat.) were added. The reaction was stirred for 17 h, concentrated to dryness in vacuo and the residue partitioned between ethyl acetate (50 mL) and 0.3 M potassium hydrogen sulfate (50 mL). The organic phase was dried ($MgSO_4$), evaporated, and purified by silica flash chromatography (ethyl acetate/hexane, 1:1) to give **34** as a colourless oil (10 mg, 57%). δ_H (500 MHz; $CDCl_3$) 7.29 (1H, dd, *J* 8.3 and 2.0, 5-Ar*H*), 7.09–7.12 (2H, m, 3-Ar*H* and 4-Ar*H*(benzyl)), 6.98–7.00 (2H, m, 6-Ar*H* and 3-Ar*H*(benzyl)), 6.86 (1H, d, *J* 1.9, 6-Ar*H*(benzyl)), 5.77 (1H, br s, NH), 4.20 (2H, d, *J* 4.7, CH_2NH), 3.72 (2H, s, ArCH_2Ar), 3.63 (2H, t, *J* 7.4, CH_2Cl), 2.97 (2H, t, *J* 7.4, CH_2Ar), 2.20 (3H, s, $OCOCH_3$), 2.16 (3H, s, $OCOCH_3$), 1.99 (3H, s, $NHCOCH_3$); δ_C (125 MHz; $CDCl_3$) 170.0, 169.4, 169.0, 149.1, 147.9, 136.2, 134.1, 131.9, 131.2, 131.14, 131.11, 128.3, 122.8, 122.6, 120.6, 84.9, 82.7, 44.8, 38.5, 30.5, 30.2, 23.0, 20.83, 20.76; *m*/*z* (HRES) found $[MNa]^+$ 464.1242; $C_{24}H_{24}ClNNaO_5$ requires 464.1241.

3.32. 2-(2-Acetoxy-5-(2-chloroethyl)benzyl)-4-(3-(*tert*-butoxy-carbonylamino)propyl)phenyl acetate 35

To diacetate **32** (89 mg, 0.178 mmol) in methanol (5 mL), palladium on carbon (20 mg; 10% wt, dry form) was added. The mixture was stirred under a hydrogen atmosphere for 3 d. The catalyst was removed by filtration and the solution concentrated to dryness to give the desired compound as a colourless oil (89 mg, 100%). δ_H (500 MHz; $CDCl_3$) 7.10 (1H, dd, *J* 8.2 and 2.2, 4-Ar*H*(benzyl)), 7.06 (1H, dd, *J* 8.2 and 2.1, 5-Ar*H*), 6.99 (1H, d, *J* 8.2, 3-Ar*H*(benzyl)), 6.95 (1H, d, *J* 8.2, 6-Ar*H*), 6.89 (1H, d, *J* 2.0, 6-Ar*H*(benzyl)), 6.84 (1H, d, *J* 2.0, 3-Ar*H*), 4.55 (1H, br s, NH), 3.74 (2H, s, ArCH_2Ar), 3.65 (2H, t, *J* 7.4, CH_2Cl), 3.06–3.12 (2H, m, CH_2NH), 2.98 (2H, t, *J* 7.4, $ClCH_2CH_2$Ar), 2.55 (2H, t, *J* 7.6, CH_2Ar), 2.19 (3H, s, $COCH_3$), 2.17 (3H, s, $COCH_3$), 1.71–1.77 (2H, m, $CH_2CH_2CH_2$), 1.43 (9H, s, $C(CH_3)_3$); δ_C (125 MHz; $CDCl_3$) 169.5, 169.4, 156.0, 147.9, 147.2, 139.6, 136.1, 131.7, 131.1, 130.6, 128.7, 128.1, 127.6, 122.5, 122.3, 79.2, 44.9, 38.6, 32.5, 31.6, 30.6, 30.1, 28.5, 20.8; *m*/*z* (HRES) found $[MNa]^+$ 526.1976; $C_{27}H_{34}ClNNaO_6$ requires 526.1972.

3.33. 4-(3-Acetamidopropyl)-2-(2-acetoxy-5-(2-chloroethyl) benzyl)phenyl acetate 36

To diacetate **35** (20 mg, 0.040 mmol) in dichloromethane (4 mL) at 0 °C TFA (1 mL) was added and the reaction was stirred at 0 °C for 2 h. The mixture was evaporated to dryness in vacuo and the residue in pyridine (3 mL) added to acetic anhydride (1 mL) and DMAP (cat.). The reaction was stirred for 17 h, concentrated to dryness and the residue partitioned between ethyl acetate (50 mL) and 0.3 M potassium hydrogen sulfate (50 mL). The organic phase was dried, concentrated under vacuum and purified by silica flash chromatography (ethyl acetate) to give **36** as a colourless oil (18 mg, 100%). δ_H (500 MHz; $CDCl_3$) 7.12 (1H, dd, *J* 8.2 and 2.1, 4-Ar*H*(benzyl)), 7.05 (1H, dd, *J* 8.2 and 2.0, 5-Ar*H*), 7.00 (1H, d, *J* 8.2, 3-Ar*H*(benzyl)), 6.95 (1H, d, *J* 8.2, 6-Ar*H*), 6.93 (1H, d, *J* 2.0, 6-Ar*H*(benzyl)), 6.81 (1H, d, *J* 2.0, 3-Ar*H*), 5.49 (1H, br s, N*H*), 3.75 (2H, s, ArCH_2Ar), 3.66 (2H, t, *J* 7.3, CH_2Cl), 3.19 (2H, apparent q, *J* 6.8, CH_2NH), 2.99 (2H, t, *J* 7.3, $ClCH_2CH_2$Ar), 2.55 (2H, t, *J* 7.5, CH_2Ar), 2.20 (3H, s, $OCOCH_3$), 2.17 (3H, s, $OCOCH_3$), 1.91 (3H, s, $NHCOCH_3$), 1.72–1.78 (2H, m, $CH_2CH_2CH_2$); δ_C (125 MHz; $CDCl_3$) 170.4, 169.6, 169.5, 147.9, 147.2, 139.4, 136.1, 131.6, 131.34, 131.29, 130.5, 128.1, 127.5, 122.6, 122.3, 44.9, 39.1, 38.5, 32.6, 30.8, 30.5, 23.3, 20.82, 20.80; *m*/*z* (HRES) found $[MNa]^+$ 468.1563; $C_{24}H_{28}ClNO_5$ requires 468.1554.

3.34. 2-(2-Acetoxy-5-(2-chloroethyl)benzyl)-4-(3-(6-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl) pentanamido)hexanamido)prop-1-ynyl)phenyl acetate 37

To diacetate **32** (50 mg, 0.100 mmol) in dichloromethane (4 mL) at 0 °C, TFA (1 mL) was added and the reaction was stirred at 0 °C for 2 h. The mixture was concentrated to dryness and the residue **33** added to dichloromethane (5 mL) and DMF (1 mL). The reaction was cooled to 0 °C and triethylamine (100 μL), Biotin X-SE (50 mg, 0.110 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (22 mg, 0.110 mmol) added. The reaction was stirred for 17 h at rt, then diluted with dichloromethane (50 mL), washed with 0.3 M potassium hydrogen sulfate (50 mL) and the aqueous phase extracted with further dichloromethane (50 mL). The combined organic extracts were dried ($MgSO_4$), concentrated under vacuum and purified using silica flash chromatography (ethyl acetate/methanol, 9:1 then neat methanol) to give **37** as a white solid (53 mg, 72%); δ_H (600 MHz; methanol-d_4) 7.34 (1H, dd, *J* 8.3 and 2.0, 5-Ar*H*), 7.21 (1H, dd, *J* 8.2 and 2.2, 4-Ar*H*(benzyl)), 7.16 (1H, d, *J* 2.0, 3-Ar*H*), 7.07 (1H, d, *J* 8.3, 6Ar*H*), 7.02–7.04 (2H, m, 3-Ar*H*(-benzyl) and 6-Ar*H*(benzyl)), 4.48–4.50 (1H, m, biotin CH_2CHNH), 4.31 (1H, dd, *J* 7.9 and 4.5, biotin CHCHNH), 4.14 (2H, s, CH_2NH), 3.79 (2H, s, ArCH_2Ar), 3.73 (2H, t, *J* 7.2, CH_2Cl), 3.15–3.23 (3H, m, CH_2NHCO and biotin CHS), 3.02 (2H, t, *J* 7.1, CH_2Ar), 2.93 (1H, dd, *J* 12.8 and 5.0, biotin CH_2S), 2.71 (1H, d, *J* 12.8, biotin CH_2S), 2.17–2.25 (10H, m, $2 \times CH_2$ and $2 \times COCH_3$), 1.51–1.77 (8H, m, $4 \times CH_2$), 1.34–1.47 (4H, m, $2 \times CH_2$); δ_C (150 MHz; $CDCl_3$) 174.6, 174.3, 169.6, 169.2, 164.7, 149.1, 147.9, 136.4, 133.5, 132.2, 131.1, 130.9, 130.5, 128.0, 122.6, 122.4, 85.0, 81.1, 62.0, 60.2, 55.6, 44.4, 39.7, 38.8, 38.0, 35.4, 35.3, 30.2, 28.73, 28.70, 28.4, 28.1, 26.1, 25.5, 25.1, 19.31, 19.25; *m*/*z* (HRES) found $[MNa]^+$ 761.2745; $C_{38}H_{47}ClN_4NaO_7S$ requires 761.2752.

3.35. 4-(6-(3-(4-Acetoxy-3-(2-acetoxy-5-(2-chloroethyl)benzyl) phenyl)prop-2-ynylamino)-6-oxohexylcarbamoyl)-2-(2,7-difluoro-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid 38

To diacetate **32** (8.0 mg, 0.016 mmol) in dichloromethane (4 mL) at 0 °C, TFA (1 mL) was added and the mixture stirred at 0 °C for 2 h. The mixture was concentrated to dryness and the residue **33** added to anhydrous dichloromethane (5 mL). Triethylamine (100 μL) and

Oregon Green 488-X succinimidyl ester (5.0 mg, 0.008 mmol) were then added and the reaction stirred at 0 °C for 1 h. The mixture was concentrated to dryness under vacuum and the residue purified by silica flash chromatography (gradient: ethyl acetate/methanol, 9:1, then neat methanol) to give the desired compound as an orange oil (4.8 mg, 66%). δ_H (600 MHz; methanol-d_4) 8.09 (1H, d, *J* 8.1, $ArCO_2H$ ring 6-H), 8.03 (1H, dd, *J* 8.1 and 1.7, $ArCO_2H$ ring 5-H), 7.65 (1H, d, *J* 1.7, $ArCO_2H$ ring 3-H), 7.27 (1H, dd, *J* 8.3 and 2.0, propynyl Ar ring 6-H), 7.14 (1H, dd, *J* 8.3 and 2.0, chloroethyl Ar ring 4-H), 7.11 (1H, d, *J* 2.0, propynyl Ar ring 2-H), 7.01 (1H, d, *J* 8.3, propynyl Ar ring 5-H), 6.96–6.97 (2H, m, chloroethyl Ar ring 3-H and 6-H), 6.71 (1H, s, Ar 8-H), 6.69 (1H, s, Ar 1-H), 6.64 (1H, s, Ar 4-H), 6.63 (1H, s, Ar 5-H), 4.08 (2H, s, $C{\equiv}CCH_2$), 3.74 (2H, s, $ArCH_2Ar$), 3.67 (2H, t, *J* 7.3, CH_2Cl), 3.35 (2H, t, *J* 7.1, CH_2CH_2NH), 2.96 (2H, t, *J* 7.3, CH_2Ar), 2.20 (2H, t, *J* 7.4, CH_2CONH), 2.16 (3H, s, $COCH_3$), 2.15 (3H, s, $COCH_3$), 1.59–1.66 (4H, m, $2 \times CH_2$), 1.37–1.41 (2H, m, CH_2); δ_C (150 MHz; $CDCl_3$) 177.3, 172.8, 169.0, 168.9, 168.0, 167.6, 165.9, 156.5, 154.5, 153.9, 152.2, 147.4, 146.3, 140.7, 134.8, 133.6, 131.9, 131.4, 130.6, 129.5, 129.3, 128.9, 128.1, 126.39, 126.36, 126.3, 121.0, 120.8, 119.0, 109.7, 109.5, 108.92, 108.86, 102.9, 83.3, 79.6, 42.8, 38.0, 36.5, 33.7, 28.6, 27.1, 27.0, 24.5, 23.5, 17.72, 17.66; *m*/*z* (HRES) found $[M-H]^+$ 905.2243; $C_{49}H_{40}ClF_2N_2O_{11}$ requires 905.2289.

3.36. Cell biology testing

MCF-7 Cells were deprived of serum overnight, and then incubated with the vehicle or compound for either 45 or 60 min (as indicated in the figure legends). The cells were stimulated with 1 μg/mL insulin for 15 min at 37 °C. After washing twice with ice-cold PBS, cells were harvested with 200 μL of lysis buffer (1.6 mL Tris 6.8 (2 M), 6 mL glycerol (80%), 2.5 mL β-mercaptoethanol, 10 mL 10% SDS, 5 mg bromophenol blue, distiled water to 50 mL). The cell lysate was boiled at 100 °C for 10 min and given a pulse spin. Then 20 μL aliquots of the samples were loaded on to a freshly prepared 10% SDS gel and the proteins separated by electrophoresis. The relevant part of the gel (around 100–40 kDa, assessed according to Bio-Rad protein ladder) was cut out and transferred to a (Whatman PROTRAN) nitrocellulose (NC) membrane at 400 mA at 4 °C (45 min). The NC membrane was blocked in 5% milk powder TBSt for 30 min and kept on a rocking platform at 4 °C in a suitable dilution of New England Biolabs P-Akt primary antibody (1:1000) in TBSt milk powder O/N. After three washes in TBSt (shaking for 5 min at rt), the membrane was incubated with a 1:1000 dilution of secondary antibody (Anti Rabbit, Bio-Rad) for 1 h and then again washed three times in TBSt, and TBS. The membrane was soaked in chemo-luminescence developer solution (ECL) from GE healthcare for 1 min and imaged (exposed for 1–15 min) using LAS-reader 3000 software and a Hamamatsu Orca camera. Blots were quantified using ImageJ (free software).

All blots shown were repeated at least once on different days with different batches of MCF cells.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.017.

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