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Melanocyte-Directed Enzyme Prodrug Therapy (MDEPT): Development of Second Generation Prodrugs for Targeted Treatment of Malignant Melanoma

Allan M. Jordan[a,†], Tariq H. Khan,[b] Hugh Malkin,[a] Helen M.I. Osborn,[a,*] Andrew Photiou[c] and Patrick A. Riley[d]

[a]*Department of Chemistry, University of Reading, Whiteknights, Reading RG6 6AD, UK*
[b]*Department of Medical Oncology, Imperial College of Science, Technology and Medicine, Charing Cross Campus, London W6 8RP, UK*
[c]*Skin Tumour Unit, St. John's Institute of Dermatology, St. Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK*
[d]*Department of Molecular Pathology, Windeyer Institute, UCL Medical School, London W1P 6DB, UK*

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Abstract—Evaluation of second generation prodrugs for MDEPT, by oximetry, has highlighted structural properties that are advantageous and disadvantageous for efficient oxidation using mushroom tyrosinase. In particular, a sterically undemanding prodrug bis-(2-chloroethyl)amino-4-hydroxyphenylaminomethanone **28** was synthesised and found to be oxidised by mushroom tyrosinase at a superior rate to tyrosine methyl ester, the carboxylic acid of which is the natural substrate for tyrosinase. The more sterically demanding phenyl mustard prodrugs **9** and **10** were oxidised by mushroom tyrosinase at a similar rate to tyrosine methyl ester. In contrast, tyramine chain elongation via heteroatom insertion was detrimental and the rate of mushroom tyrosinase oxidation of phenyl mustard prodrugs **21** and **22** decreased by 10 nanomol/min.

Introduction

Malignant melanoma continues to be a serious clinical problem on account of its increasing incidence, the relatively young population that it affects and the poor prognosis of the disseminated disease.[1] The high mortality rate is a reflection of the failure of melanoma cells to respond to current cytotoxic treatment in the form of radiation and chemotherapy. Thus, there is an urgent need for improved treatment.

A selective strategy towards the treatment of malignant melanoma in the form of a melanocyte-directed enzyme prodrug therapy (MDEPT) has previously been reported by our group.[2] Based on the unique occurrence of tyrosinase expression in melanocytes,[3] MDEPT offers a highly selective drug delivery system. Therefore, with increased selectivity in the delivery of cytotoxic agents, intolerable side effects and the need for administration of large amounts of drugs should be minimised. Initial studies into MDEPT were carried out using prodrug **1** (Scheme 1). The proposed drug release mechanism, mediated by tyrosinase, is outlined in scheme 1.

Prodrug **1** can be viewed as a three component system, with a dopamine moiety acting as the tyrosinase substrate, a drug such as phenyl mustard, as the cytotoxic agent and a carbamate linkage coupling the two components together. In order to further develop this strategy, we have now prepared a more extensive range of compounds and examined their ability to be oxidised by mushroom tyrosinase. Since oxidation by tyrosinase is the first step in our proposed drug release mechanism, it is essential that good substrates for tyrosinase are identified at an early stage of our programme.

Three different cytotoxic agents were incorporated within the prodrugs, namely, phenyl mustard,[4] a bis-ethyl amine mustard[5] and daunomycin.[6] All three of these cytotoxic agents have been previously used as anticancer drugs in the clinic.

*Corresponding author. Tel.: +44-118-987-5123; fax: +44-118-931-6331; e-mail: h.m.i.osborn@reading.ac.uk
†Present address: Ribotargets Ltd, Granta Park, Abington, Cambridge CB1 6GB, UK.

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Herein, we describe the synthesis and analysis of the prodrugs, with particular emphasis on their ability to act as substrates for tyrosinase, as determined by oximetry.

Results and Discussion

Synthesis of phenyl mustard prodrugs

The general protocol for the synthesis of the various prodrugs was based on the premise of forming a reactive carbonate that was prone to nucleophilic attack by a primary or secondary amine. Prodrugs with a carbamate linkage were synthesised as previously reported, and required access to the reactive carbonate intermediate **6**. Facile preparation of *p*-hydroxyphenyl mustard hydrochloride **5** was achieved by treating benzyloxyaniline **2** with ethylene oxide to give diol **3**.[7] Conversion to the bis-chloroethyl amino compound **4** was facilitated using methane sulfonyl chloride in anhydrous pyridine.[8] Subsequent formation of the hydrochloride salt and benzyl cleavage by hydrogenation gave the desired *p*-hydroxyphenyl mustard hydrochloride **5**. Preparation of the carbonate **6**, ready for coupling with primary and secondary amines, was achieved by heating **5** at reflux in toluene in the presence of *p*-nitrophenyl chloroformate (Scheme 2).

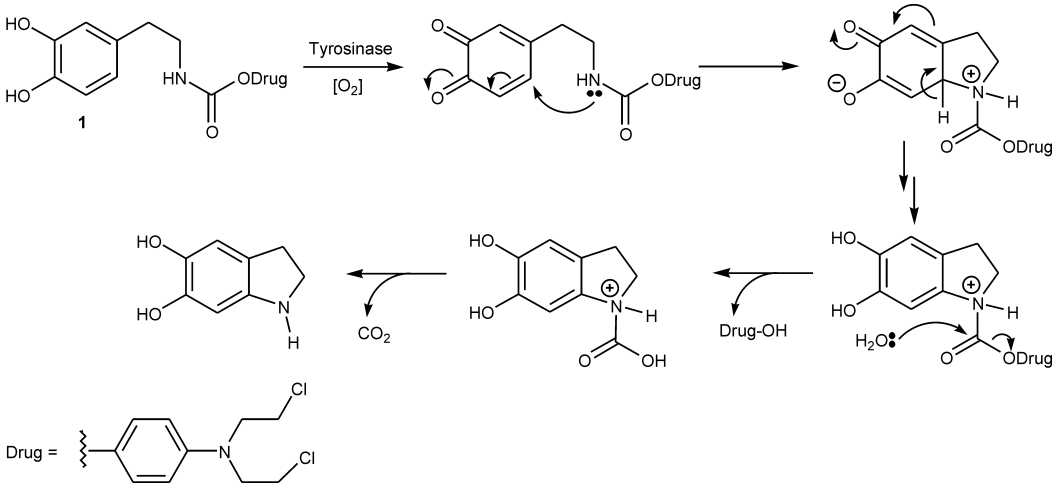
Coupling of carbonate **6** with various primary amines proceeded smoothly in anhydrous dimethylformamide to give the corresponding prodrugs in good to excellent yield (Table 1).

Since prodrug **12** was derived from an excellent tyrosinase substrate, tyrosine methyl ester, a thiocarbonate derivative was also prepared. Initial attempts at functional group interconversion, using Lawesson's reagent[9] were disappointing, and consequently an alternative synthesis was adopted. *p*-Hydroxyphenyl hydrochloride mustard **5** was treated with pentafluorophenylchlorothionoformate **23** to give thiocarbonate **24**, which was converted to prodrug **25** by treatment with tyrosine methyl ester (Scheme 3).

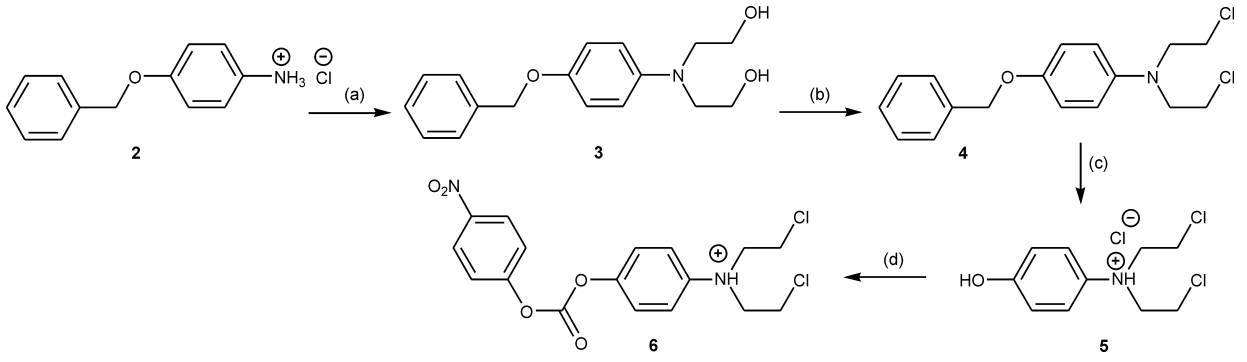
Synthesis of bis-(2-chloroethyl)amine urea mustards

For this series of prodrugs two synthetic approaches were examined. The initial protocol mirrored that used for the formation of the phenol mustard prodrugs and involved the synthesis of the *p*-nitrophenol carbamate **27** (Scheme 4).

Synthesis of the *p*-nitrophenyl carbamate **27** was easily achieved by reacting *p*-nitrophenyl chloroformate with bis-(2-chloroethyl)amine hydrochloride **26**. Mustard **27** was then coupled to primary and secondary amines as before. In these cases, the *p*-nitrophenol by-product had



Scheme 1. Proposed drug release mechanism mediated by tyrosinase.



Scheme 2. Synthesis of the reactive carbonate **6**. Reagents: (a) triethylamine, ethylene oxide, 88%; (b) mesyl chloride, pyridine, 59%; (c) $HCl_{(g)}$ then H_2, Pd/C, 51%; (d) *p*-nitrophenylchloroformate, triethylamine, 64%.

to be removed from the prodrugs using silica gel column chromatography.

In addition, with the aim of minimising purification procedures, two one-pot methodologies were developed. The one-pot strategies relied upon in situ formation of a reactive intermediate which, upon reaction with a primary or secondary amine, would afford the prodrug together with a *volatile* by-product. The one-pot rationale and formation of prodrugs **28**–**30** can be seen in Scheme 5.

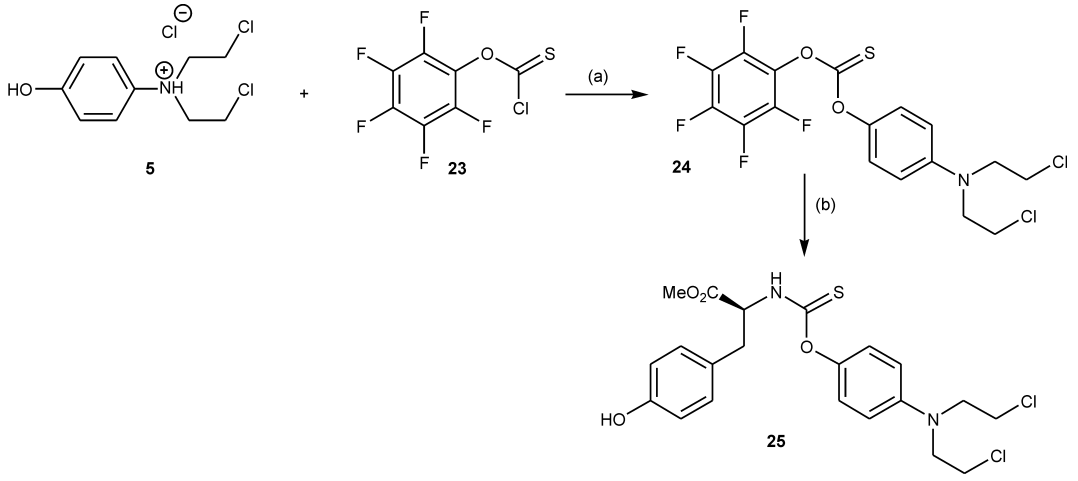
In all cases, the one-pot approach afforded higher yields than employing the *p*-nitrophenylchloroformate method. Therefore, when combined with easier purification, the one pot approach is, without doubt, a superior method for prodrug synthesis.

The final class of compounds synthesised and tested by oximetry were the daunomycin-based prodrugs. These were easily obtained using a similar two pot reaction scheme, via the reactive carbamate **31**. Addition of daunomycin then afforded the urea linked prodrug **32** (Scheme 6).

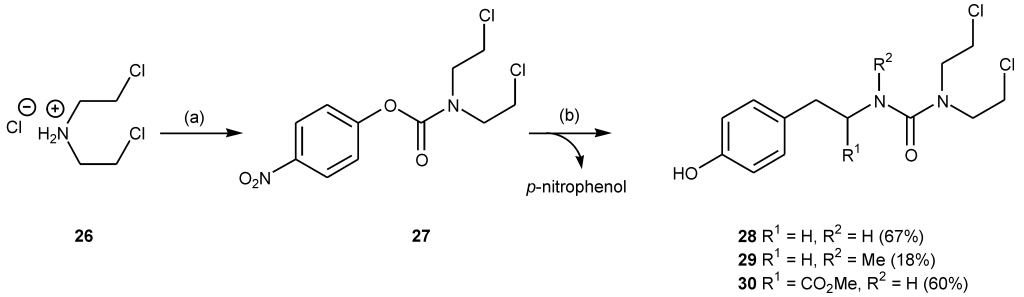
Oximetry

When tyrosinase substrates are oxidised according to the pathway in Scheme 1, molecular oxygen is absorbed from the surrounding solution. The resulting oxygen depletion can be measured using an oxygen sensor, thereby oxygen uptake is a measure of the rate of tyrosinase oxidation of the prodrugs. Using this technique we were able to examine the oxidation of the prodrugs by tyrosinase (Graph 1). The relative rates of oxygen uptake were used to estimate the efficiency of the prodrugs to act as tyrosinase substrates. In order to obtain a quantitative comparison of tyrosinase-catalysed oxidation of our prodrugs we also examined the rate of oxygen uptake in the presence of the methyl ester of the natural substrate tyrosine (entry TyMe, Graph 1).

Graph 1 shows that prodrug **28** bis-(2-chloroethylamino)-4-hydroxyphenylaminomethanone was an excellent tyrosinase substrate ($R_{max} = 20$ nanomol/min) compared with the model substrate tyrosine methyl ester ($R_{max} = 17.5$ nanomol/min). By comparing oxygen depletion in the oximetry cuvettes of prodrug **28** and tyrosine methyl ester over 400 s a brief lag period is evident for both substrates. Prodrugs **9, 10** and **30** were also good substrates, exhibiting similar oxidation rates to tyrosine methyl ester. Heteroatom incorporation (oxygen or sulfur) to afford prodrugs **21** and **22** resulted in slower oxidation. For example, the oxidation rate was only 7.5 nanomol/min for prodrug **21** and **22**.

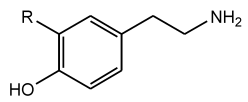
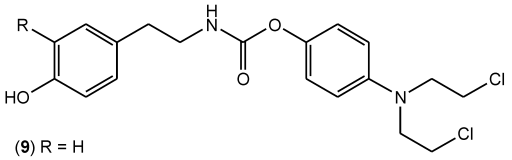
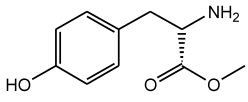
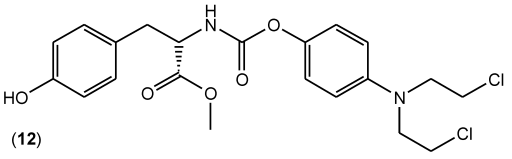
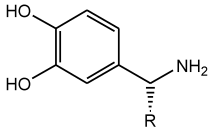
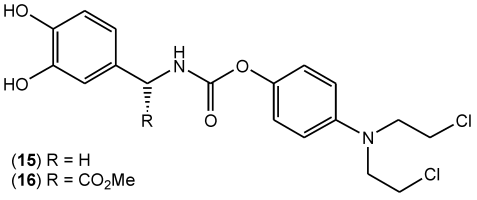
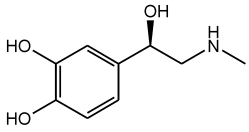
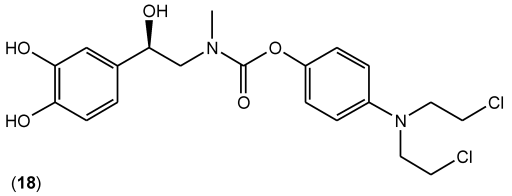
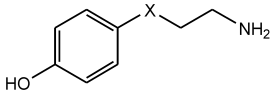
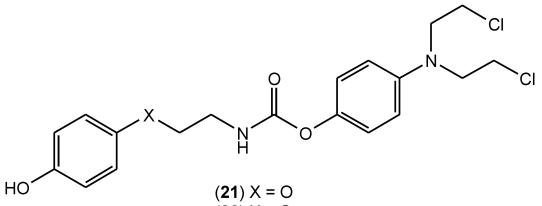


Scheme 3. Synthesis of thiocarbamate linked prodrug **25**. Reagents: (a) triethylamine, toluene; (b) tyrosine methyl ester, dimethylsulfoxide, N_2, 68% (overall yield).

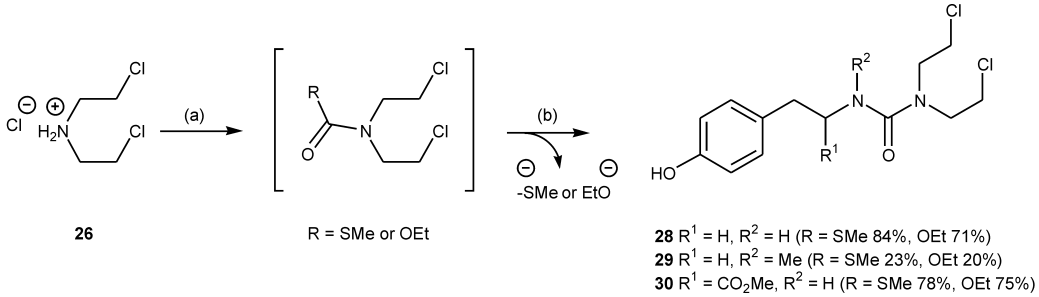


Scheme 4. Synthesis of bis-chloroethyl amine urea linked mustards. Reagents: (a) triethylamine, dimethylformamide, *p*-nitrophenylchloroformate, 70%; (b) amine **7**, **11** or *N*-methyl tyramine, reflux, N_2.

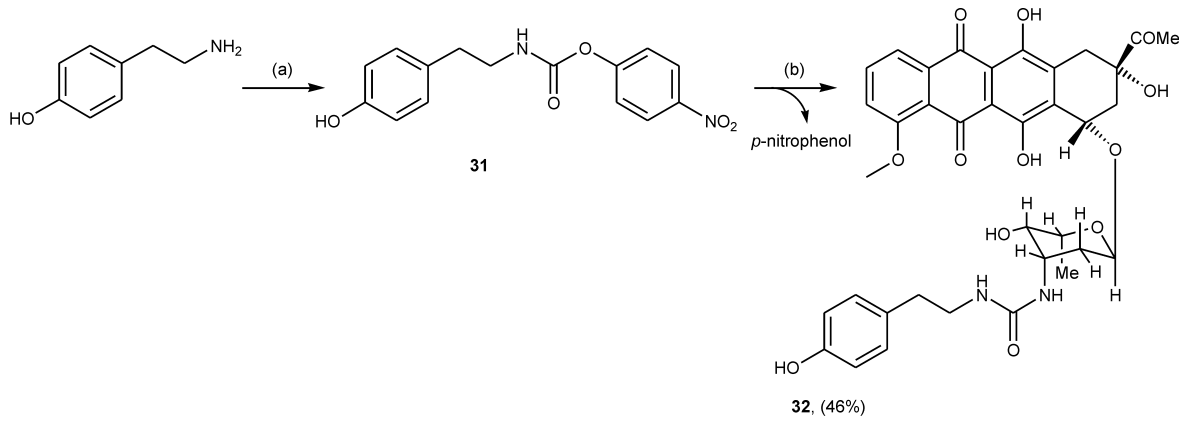
Table 1. Phenyl mustard prodrugs afforded from the reaction between various amines and carbonate **6**

Entry	Amine	Carbonate	Prodrug	Yield
1	 (**7**) R = H (**8**) R = OH	(**6**)	 (**9**) R = H (**10**) R = OH	(**9**) 47% (**10**) 73%
2	 (**11**)	(**6**)	 (**12**)	62%
3	 (**13**) R = H (**14**) R = CO_2Me	(**6**)	 (**15**) R = H (**16**) R = CO_2Me	(**15**) 52% (**16**) 54%
4	 (**17**)	(**6**)	 (**18**)	44%
5	 (**19**) X = O (**20**) X = S	(**6**)	 (**21**) X = O (**22**) X = S	(**21**) 27% (**22**) 39%

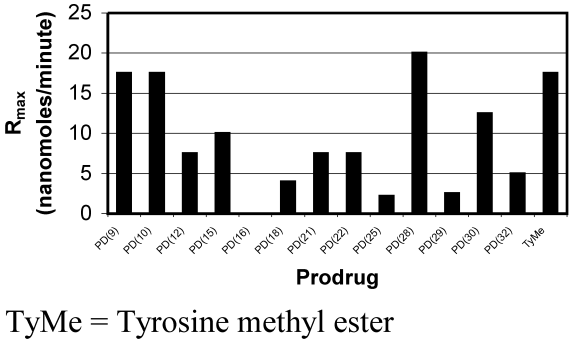
The results of the oximetry study highlight the structural properties that diminish oxidation rate. For example, prodrug **16** was not oxidised by tyrosinase within a 25 min incubation period. We conclude that, shortening the dopamine chain length from two carbons to one carbon resulted in reduced affinity to the enzyme. This may be due to increased steric hindrance close to the active site of the enzyme. Nitrogen methylation also resulted in a reduced rate of oxidation, e.g. (**29**), suggesting the importance of a primary or secondary amine function. Not surprisingly, the sterically hindered daunomycin-based, e.g. (**32**), was also a poor substrate with an R_{max} of only 5 nanomoles/min. From these results it was concluded that prodrugs **9**, **10**, and **28**



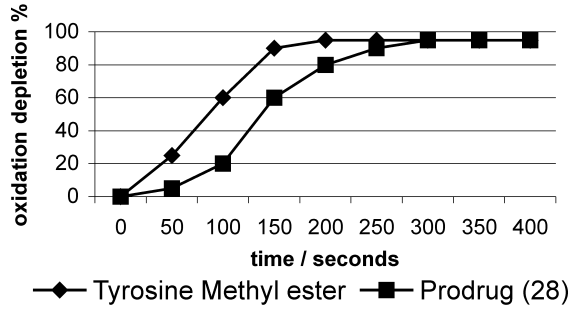
Scheme 5. One-pot synthesis of bis-chloroethyl amine urea linked mustards. Reagents: (a) triethylamine, dimethylformamide, ethyl chloroformate or methyl chlorothioformate; (b) amine **7**, **11** or *N*-methyl tyramine, reflux.



Scheme 6. Synthesis of daunomycin prodrug **32**. Reagents: (a) *p*-nitrophenylchloroformate, dichloromethane, reflux; (b) daunomycin, *N,N*-diisopropylethylamine, dimethylformamide.

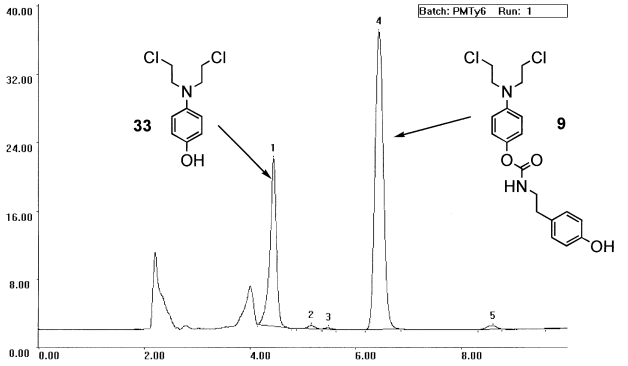


Graph 1. Oxidation rate of prodrugs by mushroom tyrosinase.



Graph 2. Tyrosinase oxidation of tyrosine methyl ester and prodrug **28**.

were the most efficient substrates for mushroom tyrosinase. In order to obtain further data on the suitability of these lead compounds individually for drug release under our MDEPT programme they were treated with tyrosinase in aqueous phosphate buffer and monitored by LCMS for drug release. Pleasingly, for compounds **9** and **10** the chromatograms illustrated liberation of the phenol mustard drug, suggesting that they be of utility in the MDEPT programme (see LCMS trace 1). Unfortunately, for compound **28** detection of liberated drug, upon exposure to tyrosinase, was not possible due to the instability of this compound in aqueous media.



LCMS Trace 1. This graph shows release of phenol mustard **33** after 30 min exposure of compound **9** to mushroom tyrosinase at 25 °C in H_2O/DMSO.

Conclusions

A second generation of prodrugs has been synthesised and the abilities of the individual prodrugs to act as substrates for tyrosinase have been examined. Prodrugs that closely resemble natural tyrosinase substrates were readily oxidised making them suitable MDEPT candidates. Functional group transformation, that is, carbamate to thiocarbamate resulted in a decrease in tyrosinase oxidation over 25 min incubation; Graph 1, PD(**12**) versus PD(**25**). Removal of steric bulk from the substrate's oxidative site via heteroatom insertion (sulfur or oxygen) resulted in a 10 nanomoles/min decrease in the rate of tyrosinase-catalysed oxidation. Steric bulk, *N*-methylation and removal of one carbon from the dopamine moiety, to give dihydroxyphenol-4-methylamine subunits **15** and **16**, all dramatically decreased the rate of tyrosinase-catalysed oxidation.

Experimental

All NMR spectra were recorded on a Bruker WM250, Bruker AC250, Bruker Avance DPX 250, Bruker AMX400 or Jeol AX400 spectrometer, using $CHCl_3$ as an internal standard unless stated otherwise (7.26 ppm for ^1H NMR, 77.0 ppm for ^{13}C NMR). ^{13}C spectra were

recorded using Distortionless Enhancement by Polarisation Transfer. Mass spectra were recorded on a Fisons VG Autospec. Infra-red spectra were recorded on a Perkin–Elmer Paragon 1000 FT-IR spectrometer. Optical activities were determined using a Perkin–Elmer 341 polarimeter. Melting points were determined using an Electrothemal digital melting point apparatus, and are uncorrected. Scanning oximetry was performed using a YSI model 5300 biological oxygen monitor. LCMS was performed using a Waters 600 system with a Micromass mass spectrometer. Stationary phase for LCMS was a Phenomenex Luna 5μ, C18(2), 250×4.6 mm.

Unless stated otherwise, all chemicals and materials were obtained from the Sigma-Aldrich Chemical Company (U.K.), the B.D.H. Merk Chemical Company (U.K.) or Lancaster Chemicals (U.K.) and were used as received. Silica gel for column chromatography was obtained from Merck (U.K.), with a pore diameter of 6 nm. Alumina column chromatography was performed using 150 mesh neutral aluminium oxide, obtained from the Aldrich Chemical Company. Silica and alumina thin layer chromatography was performed on pre-coated aluminium sheets, with a 0.2 mm thickness. Anhydrous solvents were purchased and used as received. Mushroom tyrosinase (3,520 units/mg) was used at a concentration of 300 units/mL in 0.1 M phosphate buffered saline (pH 7.4). LCMS samples were prepared by filtering through Waters Sep-Pak cartridges and run using a mobile phase of H_2O (0.1% TFA) 90%: acetonitrile 10% for 2 min to 100% acetonitrile over 10 min, at a flow rate of 1 mL/min.

Benzyl-*p*-(bis-2-hydroxyethylamino)phenyl ether (3). 4-Benzyloxyaniline hydrobromide (11.8 g, 0.05 mol) was suspended in glacial acetic acid (70 mL) and water (70 mL) and cooled to 0 °C. Ethylene oxide (17.64 g, 20 cm^3, 0.4 mol) was then added, in 1 mL portions, the solution allowed to warm to room temperature and stirred until no starting material remained by TLC. Additional ethylene oxide was added as required in order to drive the reaction to completion. After this time, the solution was concentrated in vacuo ($T<60$ °C) to give a red/brown syrup, which was re-dissolved in chloroform (100 mL). This solution was washed with water (50 mL) and saturated sodium bicarbonate solution (50 mL), dried (magnesium sulfate), filtered and concentrated in vacuo. Re-crystallisation (toluene/hexane) gave the diol as a pale-cream powder (12.55 g, 88%); mp 96–97 °C (lit.[10] 93–94 °C); R_f (silica, ethyl acetate) 0.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71–3.82 (8H, br m. 4×CH_2), 6.79 (2H, br d., $J=8.6$ Hz, PhCH_2), 7.39 (2H, br d., $J=8.6$ Hz, Ph), 7.41–7.55 (7H, m, Ar).

Benzyl-*p*-(bis-2-chloroethylamino)phenyl ether (4). The bis-(hydroxyethylamino)phenyl ether **3** (2.0 g, 7.2 mmol) was dissolved in anhydrous pyridine (11 mL) and cooled to 0 °C. Mesyl chloride (28.8 mmol, 2.23 mL) was added and the solution stirred at 2–4 °C for 20 min, followed by heating at 80 °C for 30 min. Ethyl acetate (30 mL) and water (30 mL) were then added, the organic fraction collected, dried (magnesium sulfate), filtered and concentrated in vacuo. Column chromatography (silica gel, dichloromethane) gave the dichloride (1.38 g, 59%) as a white powder; mp 108–109 °C (lit.[8] 105–106 °C); R_f 0.7 (silica, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.16–3.27 (8H, m, 4×CH_2), 5.00 (2H, s, PhCH_2), 6.73 (2H, d, $J=8.6$ Hz, Ph), 6.92 (2H, d, $J=8.6$ Hz, Ar), 7.29–7.44 (5H, m, Ar).

***p*-(Bis-2-chloroethylamino)phenol hydrochloride (5).** Hydrogen chloride gas was bubbled through a solution of the bis-(chloroethylamino)phenyl ether **4** (3.3 g, 0.01 mol) in methanol (35 mL), until complete dissolution occurred. Filtration and concentration in vacuo gave the hydrochloride salt as a white powder (mp 140–141 °C (lit.[11] 135–136 °C)), which was immediately re-suspended in ethanol (40 mL) containing 10% palladium on carbon (0.17 g). The suspension was stirred under an atmosphere of hydrogen until no starting material remained (TLC). The suspension was then filtered over Celite and concentrated in vacuo to give the phenol hydrochloride as a white solid (1.37 g, 51%); mp 176–178 °C (lit.[61] 170–173 °C); ^{1}H NMR (400 MHz, $CDCl_3$, free amine) δ 3.52–3.63 (8H, m, 4×CH_2), 6.57 (2H, d, $J=9.0$ Hz, Ar), 6.67 (2H, d, $J=9.0$ Hz, Ar).

Carbonic acid-*p*-(bis-2-chloroethylamino)phenyl ester-*p*-nitrophenyl ester (PNMC) (6). The bis-chloroethylamino hydrochloride salt **5** (1.35 g, 5.79 mmol) and triethylamine (1.17 g, 1.61 mL, 11.4 mmol) in toluene (15 mL) was slowly added, over 15 min, to a refluxing solution of *p*-nitrophenylchloroformate (1 g, 4.96 mmol) in toluene (15 mL) and the mixture was heated under reflux for 1 h. After this time, the reaction was cooled, concentrated in vacuo and purified by column chromatography (silica gel, dichloromethane) to give the diester as a yellow oil (1.27 g, 64%) which solidified upon standing; mp 97–99 °C; R_f 0.77 (silica, dichloromethane); ν_{max} (KBr disc) 1767, 1615, 1594, 1512, 1347, 1180, 814 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.61–3.68 (4H, m, 2×CH_2), 3.71–3.88 (4H, m, 2×CH_2), 6.70 (2H, d, $J=9.2$ Hz, Ar), 7.16 (2H, d, $J=9.2$ Hz, Ar), 7.47 (2H, d, $J=9.1$ Hz, Ar), 8.30 (2H, d, $J=9.1$ Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.2 (CH_2), 53.6 (CH_2), 112.4 (CH), 121.7 (CH), 121.8 (CH), 125.3 (CH), 140.5 (C), 141.3 (C), 142.7 (C), 143.1 (C), 153.6 (C); (CI: found 399.0525 [M+H]+. $C_{17}H_{16}Cl_2N_2O_5$ [M+H]+, requires 399.0514); *m*/*z* (CI) (399 [M+H]+, 75%), 348 (100), 120 (20), 63 (15).

{2′-(4″-Hydroxyphenyl)ethyl} carbamic acid *p*-(bis-2-chloroethylamino)phenyl ester (9). To a solution of the nitrophenyl carbonate **6** (0.20 g, 0.57 mmol) in chloroform (1 mL) was added tyramine hydrochloride (0.096 g, 0.50 mmol) and triethylamine (0.50 g, 0.08 mL, 0.50 mmol) and the mixture was heated under reflux for 4 h. After this time, the reaction was cooled, concentrated in vacuo and purified by column chromatography (silica gel, dichloromethane/ethyl acetate 95:5) to give the carbamate **9** as a colourless oil (0.046 mg, 47%); R_f 0.29 (silica, dichloromethane/ethyl acetate 95:5); ν_{max} (KBr disc) 3336, 1718, 1612, 1507, 1336, 1219 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.80 (2H, t, $J=6.9$ Hz, PhCH_2), 3.47 (2H, apparent dd, $J=6.9$,

6.0 Hz, CH_2NH), 3.61 (2H, d, J = 6.0 Hz, 2×NCH_2), 3.68 (4H, d, J = 6.0 Hz, 2×CH_2Cl), 6.67 (1H, br t, J = 6.0 Hz, NH), 6.67 (2H, d, J = 9.0 Hz, Ar), 6.77 (2H, d, J = 8.4 Hz, Ar), 6.99 (2H, d, J = 9.0 Hz, Ar), 7.03 (2H, d, J = 8.4 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.3 (CH_2), 40.5 (CH_2), 42.8 (CH_2), 54.3 (CH_2), 113.4 (CH), 115.8 (CH), 123.1 (CH), 130.2 (CH), 130.7 (C), 143.6 (C), 154.7(C), 155.8 (C and CO); (CI: found 397.1085 [M + H]+. $C_{19}H_{22}Cl_2N_2O_3$ requires 397.1085); m/z (CI) (397 [M + H]+, 45%), 234 (100), 184 (95), 107 (100).

{2′-(3″,4″-Dihydroxyphenyl)-ethyl} carbamic acid *p*-(bis-2-chloroethylamino)phenyl ester (10). A solution of the carbonate **6** (0.1 g, 0.26 mmol), 3-hydroxytyramine hydrochloride (0.1 g, 0.53 mmol) and triethylamine (0.05 g, 0.07 mL, 0.5 mmol) in anhydrous dimethylformamide (1.5 mL) was stirred for 3 days at ambient temperature. After this time, the mixture was concentrated to dryness in vacuo. Column chromatography (silica gel, dichloromethane/methanol 100:1 → 9:1 v/v) gave the carbamate **10** as a colourless viscous oil (0.08 g, 73%); R_f 0.45 (silica, dichloromethane/methanol, 9:1, v/v); ν_{max} (KBr disc) 3421, 1718, 1653, 1507, 1218 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.60 (2H, br t, J = 6.3 Hz, PhCH_2), 3.32 (2H, br q, J = 6.3 Hz, CH_2NH), 3.48 (4H, d, J = 6.2 Hz, 2×NCH_2), 3.55 (4H, d, J = 6.2 Hz, 2×CH_2Cl), 5.17 (1H, br t, J = 6.3 Hz, NH), 6.50 (2H, d, J = 7.9 Hz, Ar), 6.58 (2H, s, Ar), 6.64 (1H, d, J = 7.7 Hz, Ar), 6.88 (2H, d, J = 7.9 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.3 (CH_2), 40.7 (CH_2), 42.7 (CH_2), 53.9 (CH_2), 112.8 (CH), 115.6 (CH), 116.8 (CH), 120.9 (CH), 123.0 (CH), 130.9 (C), 142.5 (C), 143.0 (C), 144.1 (C), 144.4 (C), 156.1(C and CO); (CI: found [M + H]+, 413.1045. $C_{19}H_{22}Cl_2N_2O_4$ requires 413.1034); m/z (CI) 413 ([M + H]+, 10%), 233 (50), 184 (100), 123 (35).

(*R*)-[2′-Amino-3′-(4″-hydroxyphenyl)propionic acid methyl ester]-carbamic acid *p*-(bis-2-chloroethylamino) phenyl ester (12). To a solution of the nitrophenyl carbonate **6** (0.20 g, 0.57 mmol) in chloroform (1 mL) was added L-tyrosine methyl ester (0.127 g, 0.65 mmol) and the mixture was heated under reflux for 4 h. After this time, the reaction was cooled and concentrated in vacuo. Column chromatography (silica gel, dichloromethane/methanol 100:1) afforded carbamate **12** as a colourless oil (0.085 g, 62%); R_f 0.16 (silica, dichloromehane/ethyl acetate 95:5); $[\alpha]_D^{20}$ +29.0° (*c* 0.9, chloroform); ν_{max} (KBr disc) 3446, 1718, 1700, 1559, 1496, 1218 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.03 (2H, dq, J = 5.9 Hz, 14.2 Hz PhCH_2), 3.54 (2H, d, J = 6.2 Hz, 2×NCH_2), 3.68 (2H, d, J = 6.0 Hz, 2×CH_2Cl), 3.69 (3H, s, Me), 4.58 (2H, br d, J = 6.6 Hz, CH), 5.41 (1H, d, J = 8.0 Hz, NH), 6.61 (2H, d, J = 9.0 Hz, Ar), 6.68 (2H, d, J = 8.4 Hz, Ar), 6.92 (2H, d, J = 9.1 Hz, Ar), 6.94 (2H, d, J = 9.1 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.3 (CH_2), 40.3 (CH_2), 52.4 (CH_3), 53.7 (CH_2), 55.0 (CH), 112.6 (CH), 115.5 (CH), 122.7 (CH) 127.2 (C), 130.3 (CH), 142.3 (C), 143.8 (C), 154.7 (C), 155.0 (C), 172.0 (C); (CI: found 455.1148 [M + H]+. $C_{21}H_{24}Cl_2N_2O_5$ requires 455.1141); m/z (CI) (455 [M + H]+, 10%), 234 (15), 184 (35), 107 (100).

3′,4′-Dihydroxybenzylamino-carbamic acid *p*-(bis-2-chloroethylamino) phenyl ester (15). A solution of carbonate **6** (0.13 g, 0.32 mmol), 3,4-dihydroxybenzylamine hydrobromide (0.14 g, 0.65 mmol) and triethylamine (0.07 g, 0.09 mL, 0.65 mmol) in anhydrous dimethylformamide (2 cm^3) was stirred at room temperature for 72 h. After this time, the mixture was concentrated to dryness in vacuo. Column chromatography (silica gel, dichloromethane/methanol 100:1 → 9:1 v/v) gave carbamate **15** as a white powder (0.07 g, 52%); R_f 0.29 (silica, dichloromethane/methanol, 9:1, v/v); ν_{max} (KBr disc) 3322, 1689, 1616, 1507, 1427, 1281, 1215, 1037 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD) δ 3.65 (4H, d, J = 6.2 Hz, 2×NCH_2), 3.71 (4H, d, J = 6.2 Hz, 2×CH_2Cl), 4.17 (2H, br d, J = 7.3 Hz, CH_2), 6.64 (1H, br t, J = 7.3 Hz, NH), 6.74–6.81 (5H, m, Ar), 6.96 (2H, d, J = 7.4 Hz, Ar); ^{13}C NMR (100 MHz, CD_3OD) δ 41.6 (CH_2), 45.3 (CH_2), 54.6 (CH_2), 113.9 (CH), 115.7 (CH), 116.2 (CH), 119.9 (CH), 123.7 (CH), 144.0 (C), 145.4 (C), 145.6 (C), 146.3 (C), 158.0 (C and CO); (CI: found [M + H]+, 399.0870. $C_{18}H_{20}Cl_2N_2O_4$ requires 399.0878); m/z (CI) ([M + H]+, 20%), 363 (10), 233 (70), 184 (100).

(*R*)-3,4-Hydroxyphenylglycine methyl ester (14). To a stirred solution of 3,4-hydroxyphenylglycine (0.5 g, 3.0 mmol) in 2,2-dimethoxypropane (30 mL) was added concentrated hydrochloric acid (3 mL). After stirring overnight at 20 °C, the mixture was concentrated to dryness in vacuo, and minimal methanol added to redissolve the residues. Diethyl ether (75 mL) was added and the resultant solid obtained by filtration. Re-dissolution in methanol (30 mL) and addition of triethylamine (0.3 g, 0.4 mL, 3 mmol), followed by concentration in vacuo and column chromatography (silica gel, dichloromethane/methanol 20:1 v/v) gave the free amine **14** as a white powder in quantitative yield; mp 172–173 °C (lit.[12] 178–180 °C); $[\alpha]_D^{20}$ −114.4° (*c* 0.25, 10% aq hydrochloric acid) (lit.[13] $[\alpha]_D^{20}$ −121.1° (*c* 1, aq hydrochloric acid)); R_f 0.55 (silica, dichloromethane/methanol, 10:1, v/v); ν_{max} (KBr disc) 3447, 1734, 1559, 1517, 1465, 1281, 1255, 1220, 1167 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.24 (2H, br s, NH_2), 3.60 (3H, s, Me), 4.46 (1H, br s., CH), 6.70 (2H, d, J = 8.6 Hz, Ar), 7.07 (2H, d, J = 8.6 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.1 (CH), 61.3 (CH_3), 118.9 (CH), 131.7 (CH), 134.0 (C), 161.0 (C), 178.2 (C); (CI: found [M + H]+, 182.0822. $C_9H_{11}NO_3$ requires 182.0818); m/z (CI) 182 ([M + H]+, 15%), 165 (50), 122 (100), 107 (5).

(*R*)-[1′-Amino-2′-(3,4″-hydroxyphenyl)ethanonic acid methyl ester]-carbamic acid *p*-(bis-2-chloroethylamino) phenyl ester (16). A solution of the carbonate **6** (0.13 g, 0.32 mmol) and the amino acid methyl ester **14** (0.14 g, 0.65 mmol) in anhydrous dimethylformamide (2 mL) was stirred at room temperature for 72 h. After this time, the mixture was concentrated to dryness in vacuo. Column chromatography (silica gel, dichloromethane/ethyl acetate, 95:5, v/v) gave carbamate **16** as a colourless viscous oil (0.07 g, 54%); $[\alpha]_D^{20}$ −115.8° (*c* 0.85, chloroform); R_f 0.14 (silica, dichloromethane/ethyl acetate, 95:5, v/v); ν_{max} (KBr disc) 3384, 1718, 1612, 1506, 1437, 1350, 1218, 1174, 1030 cm^{-1}. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 3.17 (3H, s, OMe), 3.48 (4H, d, $J=6.2$ Hz, $2\times NCH_2$), 3.57 (4H, d, $J=6.2$ Hz, $2\times CH_2Cl$), 5.23 (1H, d, $J=7.0$ Hz, NH), 6.08 (1H, d, $J=7.0$ Hz, CH), 6.52 (2H, d, $J=8.0$ Hz, Ar), 6.61 (2H, d, $J=7.7$ Hz, Ar), 6.90 (2H, d, $J=7.7$ Hz, Ar), 7.10 (2H, d, $J=8.0$ Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.3 (CH_2), 52.9 (CH_3), 53.6 (CH_2), 57.4 (CH), 112.5 (CH), 115.8 (CH), 122.6 (CH), 127.6 (C), 128.4 (CH), 142.1 (C), 143.8 (C), 154.5 (C), 156.4 (C), 171.5 (C); (CI: found [M + H]+, 441.0968. $C_{20}H_{22}Cl_2N_2O_5$ requires [M + H]+, 441.0984); *m/z* (CI) 441 ([M + H]+, 10%), 277 (10), 233 (35), 184 (70), 147 (100).

(*R*)-[1′-Amino-2′-hydroxy-2′-(4″-hydroxyphenyl)propionic acid methyl ester]-carbamic acid *p*-(bis-2-chloroethylamino) phenyl ester (18). A solution of the carbonate **6** (0.13 g, 0.32 mmol) and L-adrenaline (0.12 g, 0.65 mmol) in anhydrous dimethylformamide (2 mL) was stirred at room temperature for 72 h. After this time, the mixture was concentrated to dryness in vacuo. Column chromatography (silica gel, dichloromethane/methanol 100:1 → 9:1 v/v) gave carbamate **18** as a viscous colourless oil (0.06 g, 44%); $[\alpha]_D^{20}$ −21.9° (*c* 0.95, chloroform); R_f 0.29 (silica, dichloromethane/methanol, 9:1, v/v); ν_{max} (KBr disc) 3368, 1700, 1611, 1516, 1448, 1357, 121, 1219 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.41 (2H, d, $J=7.4$ Hz, CH_2), 3.52 (3H, s, Me), 3.53 (4H, d, $J=6.2$ Hz, $2\times NCH_2$), 3.64 (4H, d, $J=6.2$ Hz, $2\times CH_2Cl$), 4.81 (1H, d, $J=7.4$ Hz, CH), 6.57–6.92 (7H, m, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.7 (CH_3), 36.3 (CH_2), 40.4 (CH_2), 53.6 (CH_2), 72.5 (CH), 112.5 (CH), 113.1 (CH), 115.2 (CH), 118.2 (CH), 122.7 (CH), 133.5 (C), 142.5 (CH), 143.9 (C), 144.0 (C), 157.2 (C and CO); (CI: found [M + H]+, 443.1079. $C_{20}H_{24}Cl_2N_2O_5$ requires 443.1140); *m/z* (CI) 443 ([M + H]+, 20%), 425 (100), 234 (70), 184 (100), 184 (85), 121 (20).

2-(4-Hydroxyphenoxy)-ethylamine hydrochloride (19).[14] A solution of 2-(4-hydroxyphenoxy)acetamide (1.45 g, 8.8 mmol) in anhydrous tetrahydrofuran (63 mL) was slowly added under an inert atmosphere to a refluxing suspension of lithium aluminium hydride (1 M soln in anhydrous tetrahydrofuran, 32 mL) and the suspension refluxed for a further 12 h. After cooling, water was slowly added until hydrogen evolution ceased and the mixture concentrated in vacuo. After re-suspension in methanol, the mixture was eluted through a short pad of silica with methanol and the organic extracts concentrated in vacuo, re-dissolved in concentrated hydrochloric acid and re-concentrated in vacuo to give the amine hydrochloride as a white powder (0.2 g, 12%); mp 170–171 °C (lit.[14] 172–174 °C dec.). ^{1}H NMR (250 MHz, D_2O) δ 3.25 (2H, t, $J=5.2$ Hz, CH_2), 4.08 (2H, t, $J=5.2$ Hz, CH_2), 6.73 (2H, d, $J=9.1$ Hz, Ar), 6.82 (2H, d, $J=9.1$ Hz, Ar).

2-[(4-Hydroxyphenyl)thio]ethylamine hydrochloride (20). A mixture of 4-hydroxythiophenol (2.48 g, 19.7 mmol) and 2-methyl-2-oxazoline (1.67 g, 1.69 mL, 19.7 mmol) were refluxed (neat) under argon for 2 h. Upon cooling, the crude sticky solid was re-suspended in concentrated hydrochloric acid (aq) and refluxed for 12 h. The mixture was then poured into water (20 mL) and extracted with diethyl ether (2×20 mL). The aqueous liquors were concentrated to dryness and twice re-dissolved in water and re-concentrated. Re-crystallisation of the resultant solid (ethanol/diethyl ether) gave the product as a cream solid (0.45 g, 11%); mp 130–131 °C (lit.[15] 128–129 °C). ^{1}H NMR (400 MHz, D_2O) δ 3.07 (4H, br s, $2\times CH_2$), 6.87 (2H, d, $J=11.9$ Hz, Ar), 7.41 (2H, d, $J=11.9$ Hz, Ar).

{2′-(4″-Hydroxyphenyl)ethylamine} carbamic acid *p*-(bis-2-chloroethylamino)phenyl ester (21). A solution of **19** (0.2 g, 1 mmol), **6** (0.3 g, 0.75 mmol) and triethylamine (0.1 g, 0.14 mL, 1 mmol) in anhydrous chloroform (3 mL) was heated under reflux under an inert atmosphere for 4 h and concentrated in vacuo. Column chromatography (silica gel, ethyl acetate:dichloromethane 1:19 v/v) gave carbamate **21** as a colourless oil (0.11 g, 27%); R_f 0.26 (silica, ethyl acetate/dichloromethane 1:19 v/v); νmax (KBr disc) 3358, 1713, 1612, 1506, 1452, 1337, 1217, 1109, 1068, 826 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.47 (4H, d, $J=7.8$ Hz, $2\times CH_2$), 3.56 (2H, t, $J=5.0$ Hz, CH_2), 3.65 (4H, d, $J=7.8$ Hz, $2\times CH_2$), 3.91 (2H, t, $J=5.0$ Hz, CH_2), 5.55 (1H, t, $J=5.8$ Hz, NH), 6.53 (2H, d, $J=9.1$ Hz, Ar), 6.67 (4H, d, $J=1.4$ Hz, Ar), 6.90 (2H, d, $J=9.1$ Hz, Ar); ^{13}C NMR (62.8 MHz, $CDCl_3$) δ 40.7 (CH_2), 41.3 (CH_2), 54.1 (CH_2), 67.6 (CH_2), 112.9 (CH), 115.9 (CH), 116.5 (CH), 123.2 (CH), 142.7 (C), 144.2 (C), 150.7 (C), 152.6 (C), 156.2 (C and CO); (CI: found [M + H]+, 413.1049. $C_{19}H_{22}N_2O_4$ requires 413.1034); *m/z* (CI) 413 ([M + H]+, 15%), 234 (70), 184 (100), 135 (10), 110 (55), 65 (15).

{2′-[(4″-Hydroxyphenyl)thio]ethylamine} carbamic acid *p*-(bis-2-chloroethylamino)phenyl ester (22). A solution of **20** (0.2 g, 1 mmol) and triethylamine (0.1 g, 0.14 mL, 1 mmol) in anhydrous chloroform (3 mL) was brought to reflux and **6** (0.3 g, 0.75 mmol) added. After refluxing under an inert atmosphere for 4 h and concentration in vacuo, column chromatography (silica gel, ethyl acetate/dichloromethane 1:19 v/v) gave carbamate **22** as a colourless oil (0.16 g, 39%); R_f 0.34 (silica, ethyl acetate/dichloromethane 1:19 v/v); ν_{max} (KBr disc) 3333, 1700, 1651, 1612, 1556, 1495, 1455, 1397, 1335, 1266, 1218, 1182, 1110, 1048 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.88 (2H, d, $J=6.3$ Hz CH_2), 3.34 (2H, q, $J=6.3$ Hz, CH_2), 3.51 (4H, d, $J=$ Hz, $2\times CH_2$), 3.58 (4H, d, $J=$ Hz, $2\times CH_2$), 5.44 (1H, t, $J=6.0$ Hz, NH), 6.57 (2H, d, $J=9.1$ Hz, Ar), 6.64 (2H, d, $J=11.7$ Hz, Ar), 6.91 (2H, d, $J=9.1$ Hz, Ar), 7.20 (2H, d, $J=11.7$ Hz, Ar); ^{13}C NMR (62.8 MHz, $CDCl_3$) δ 36.1 (CH_2), 40.5 (CH_2), 40.7 (CH_2), 54.0 (CH_2), 112.9 (CH), 116.7 (CH), 123.1 (CH), 126.4 (CH), 134.5 (CH), 142.6 (C), 144.3 (C), 156.1 (C), 156.4 (C and CO).

{2′-(4″-Hydroxyphenyl)ethyl} thiocarbamic acid *p*-(bis-2-chloroethylamino)phenyl ester (25). A solution of the mustard **5** (0.2 g, 0.86 mmol) and triethylamine (0.174 g, 0.24 mL, 1.72 mmol) was slowly added to a refluxing solution of pentafluorophenylchloro-thionoformate in toluene (3 mL) and the mixture was heated under reflux for 2 h. After this time, the reaction was cooled and concentrated in vacuo. The resultant product and tyrosine methyl ester (0.336 g, 1.72 mmol) were then dissolved in anhydrous dimethylformamide (5 mL) and

stirred under an inert atmosphere at ambient temperature overnight. After this time the reaction was concentrated in vacuo and purified by column chromatography (silica gel, dichloromethane/ethyl acetate 95:5) to give thiocarbamate **25** as a colourless oil (0.30 g, 68%); $[\alpha]_D^{20}$ +53.4° (*c* 2.3, chloroform); R_f 0.42 (silica, dichloromethane/ethyl acetate 95:5); ν_{max} (KBr disc) 3400, 1737, 1614, 1507, 1444, 1378, 1147 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 3.16 (1H, dd, J = 13.2 Hz, 4.8 Hz, CH_2CH), 3.31 (1H, dd, J = 14.3 Hz, 5.5 Hz, CH_2CH), 3.62 (4H, t, J = 5.8 Hz, 2×NCH_2), 3.69 (4H, t, J = 5.8 Hz, 2×CH_2Cl), 3.77 (3H, s, OMe), 5.16 (1H, dd, J = 13.2 Hz, 5.5 Hz, CH), 5.56 (1H, br s, NH), 6.63 (2H, d, J = 8.8 Hz, Ar), 6.77 (2H, d, J = 7.9 Hz, Ar), 6.96 (2H, d, J = 8.8 Hz, Ar), 6.99 (2H, d, J = 7.9 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ 36.1 (CH_2), 40.3 (CH_2), 52.6 (CH_3), 52.7 (CH_2), 58.9 (CH), 112.0 (CH), 115.6 (CH), 123.5 (CH), 127.0 (C), 130.4 (CH), 144.2 (C), 144.4 (C), 154.9 (C), 171.4 (C), 189.8.

Di-(2-chloroethyl)amino-4-nitrophenoxymethanone (27). Bis-(2-chloroethylamine) hydrochloride (5.6 mmol, 1 g) and *p*-nitrophenylchloroformate (5.4 mmol, 1 g) were dissolved in dimethylformamide (15 mL) and triethylamine (11.5 mmol, 1.6 mL) was added slowly. The mixture was heated under reflux with stirring for 6 h before being concentrated in vacuo to give a brown oil. Purification by column chromatography (silica gel, dichloromethane) yielded carbamate **27** as a yellow oil (1.2 g, 70%). ν_{max} 1660, 1446, 1378, 1145 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.21–1.33 (4H, m, CH_2), 3.38–3.47 (4H, m, CH_2), 7.51 (2H, d, J = 5.7 Hz, 2×ArH), 8.32 (2H, d, J = 5.7 Hz, 2×ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.8 (CH_2), 42.1 (CH_2), 44.4 (CH_2), 44.7 (CH_2), 115.9 (2×CH), 131.2 (2×CH), 129.3 (C), 157.6 (C), 160.5 (C). *m*/*z* (CI) 273 (65%), 150 (50), 134 (100), 100 (10), 56 (25).

Bis-(2-chloroethyl)amino-4-hydroxyphenylaminomethanone (28). Compound **27** (860 mg, 2.8 mmol) was dissolved in dimethylformamide (20 mL) and triethylamine (0.9 mL, 5.6 mmol) was added. The mixture was stirred at reflux for 30 min and tyramine (0.76 mg, 5.6 mmol) was added. The mixture was heated under reflux for a further 6 h and then concentrated in vacuo. Purification by column chromatography (silica gel, dichloromethane then methanol) gave prodrug **28** as an orange/brown oil (571 mg, 67%). ν_{max} 3400, 1660, 1444, 1380, 1145 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.61 (2H, t, J = 3.72 Hz, 4, CH_2Ar), 3.17–3.22 (10H, m, $ArCH_2CH_2$, 2×CH_2CH_2Cl), 6.67 (2H, d, J = 5.7 Hz, 2×ArH), 6.9 (2H, d, J = 5.7 Hz, 2×ArH), 7.82 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.1 (CH_2), 43.1 (CH_2), 43.4 (CH_2), 44.2 (CH_2), 46.2 (CH_2), 46.7 (CH_2), 115.9 (2×CH), 130 (2×CH), 130.1 (C), 155.6 (C), 161.5 (C). *m*/*z* (CI) 269 (87%), 224 (50), 138 (100), 121 (45), 108 (35).

Bis-(2-chloroethyl)amino-4-hydroxyphenylaminomethanone (28). One-pot method. bis-(2-chloroethyl)amine hydrochloride **26** (200 mg, 1.1 mmol) was dissolved in dichloromethane (15 mL) and triethylamine (0.45 mL, 3.3 mmol) was added. The mixture was stirred for 5 min at ambient temperature and ethyl chloroformate or methyl chlorothioformate (1.32 mmol) was added. The mixture was stirred until no bis-(2-chloroethyl)amine remained by TLC (ethyl acetate). Tyramine (300 mg, 2.2 mmol) was added and the mixture was heated under reflux for 4 h. The mixture was allowed to cool, purified by dry flash column chromatography (silica gel, dichloromethane 200 cm^3) and concentrated in vacuo to yield prodrug **28** as an orange/brown oil (234 mg, 67% for methyl chlorothioformate and 229 mg, 64% for ethyl chloroformate).

Di-(2-chloroethyl)amino-4-hydroxyphenethyl(methyl)aminomethanone (29). Bis-(2-chloroethyl)amine hydrochloride **26** (200 mg, 1.1 mmol) was dissolved in dichloromethane (15 mL) and triethylamine (0.45 mL, 3.3 mmol) was added. The mixture was stirred for 5 min at ambient temperature and methyl chlorothioformate (1.32 mmol) was added. The mixture was stirred until no bis-(2-chloroethyl)amine remained by TLC (ethyl acetate). *N*-Methyl tyramine (322 mg, 2.1 mmol) was added and the mixture was heated under reflux for 4 h. The mixture was allowed to cool, purified by dry flash column chromatography (silica gel, dichloromethane 200 mL) and concentrated in vacuo to yield prodrug **29** as an orange/brown oil (68 mg, 18%). ν_{max} 3400, 1660, 1444, 1380, 1145 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.32 (3H, s, CH_3) 2.60 (2H, t, J = 3.72 Hz, CH_2Ar), 3.17–3.22 (10H, m, $ArCH_2CH_2$, 2×CH_2CH_2Cl), 6.67 (2H, d, J = 5.7 Hz, 2×ArH), 6.9 (2H, d, J = 5.7 Hz, 2×ArH), 7.82 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.1 (CH_2), 43.3 (CH_2), 44.1 (CH_2), 44.2 (CH_2), 45.2 (CH_2), 46.5 (CH_3) 46.6 (CH_2), 115.9 (2×CH), 130 (2×CH), 130.1 (C), 155.6 (C), 161.5 (C). *m*/*z* (CI) 283 (87%), 238 (50), 152 (100), 121 (45), 108 (35).

Methyl-2-di(2-chloroethyl)aminocarbonylamino-3-(4-hydroxyphenyl)proponoate (30). Bis-(2-chloroethyl)amine hydrochloride **26** (200 mg, 1.1 mmol) was dissolved in dichloromethane (15 mL) and triethylamine (0.45 mL, 3.3 mmol) was added. The mixture was stirred for 5 min at ambient temperature and methyl chlorothioformate (1.32 mmol) was added. The mixture was stirred until no bis-(2-chloroethyl)amine remained by TLC (ethyl acetate). Tyrosine methyl ester (411 mg, 2.1 mmol) was added and the mixture was heated under reflux for 4 h. The mixture was allowed to cool purified by dry flash column chromatography (silica gel, dichloromethane 200 mL) and concentrated in vacuo to yield prodrug **30** as an orange/brown oil (278 mg, 60%). ν_{max} 3400, 1740, 1660, 1444, 1380, 1145 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.32 (3H, s, CH_3) 2.60 (2H, t, J = 3.72 Hz, CH_2Ar), 2.64 (3H, s, CH_3) 3.12–3.25 (9H, m, $ArCH_2CH$, 2×CH_2CH_2Cl), 6.67 (2H, d, J = 5.7 Hz, 2×ArH), 6.9 (2H, d, J = 5.7 Hz, 2×ArH), 7.82 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.8 (CH_2), 43.3 (CH_2), 44.1 (CH_2), 44.2 (CH_2), 45.2 (CH_2), 46.6 (CH_2), 47.1 (CH_3) 115.9 (2×CH), 130 (2×CH), 130.1 (C), 155.6 (C), 160.3 (C) 161.5 (C). *m*/*z* (CI) 242 (72%), 227 (45), 183 (75) 152 (100), 121 (45), 108 (35).

4-Hydroxyphenethylamino-4-nitrophenoxymethanone (31). Tyramine (1 g, 7.3 mmol) and *p*-nitrophenylchloro-

formate (1.4 g, 7.3 mmol) were dissolved in anhydrous dichloromethane and heated under reflux for 2 h. The reaction mixture was allowed to cool, concentrated in vacuo and purified by dry flash column chromatography (silica, dichloromethane and then ethylacetate) to afford carbamate **31** as a pale yellow solid (2.2 g, 97%); mp 157–159 °C; ν_{max} (KBr disc) 3400, 1658, 1440, 1380 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.74 (2H, t, $J=7.0$ Hz, CH_2Ar), 3.4 (2H, t, $J=7.0$ Hz, CH_2) 6.76 (2H, d, $J=8.5$ Hz, 2×ArH), 7.06 (2H, d, $J=8.5$ Hz, 2×ArH), 7.29 (2H, d, $J=9.2$ Hz, 2×ArH), 8.24 (2H, d, $J=9.2$ Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 36.4 (CH_2), 44.2 (CH_2), 116.6 (2×CH), 123.7 (2×CH), 126.4 (2×CH), 131.2 (2×CH), 131.3 (C) 146.9 (C), 156.0 (C), 157.4 (C), 158.1 (C). *m*/*z* (CI) 163 (25%), 139 (10), 107 (100) 65 (15).

3-Acetyl-3,5,12-trihydroxy-1-[5-hydroxy-4-(4-hydroxyphenylaminocarbonylamino)-6-methylperhydro-2-pyranoloxy]-10-methoxy-(1*S*,3*S*)-1,2,3,4,6,11-hexaydro-6,11-naphthacenedione (32). Daunomyocin[16] (20 mg, 0.038 mmol) and carbamate **31** (15 mg, 0.049 mmol) were dissolved in dimethylformamide (1 mL) and diisopropylethylamine (7.5 μL, 0.042 mmol) was added. The flask was wrapped in tinfoil to exclude light and the mixture was stirred for 3 h. Diethyl ether (5 mL) was added to give a red precipitate. The precipitate was collected by filtering across a cotton wool plug. The solid was then washed off the cotton wool using methanol (5 mL) and concentrated in vacuo to yield prodrug **32** as a red oily solid (12 mg, 46%). ν_{max} 3400, 2720, 1740, 1750, 1690, 1520, 1435, 1147 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 1.25 (2H, d, $J=4.5$ Hz, CH_2), 1.32–1.4 (5H, m, 5′ CH_3 and CH_2CCOCH_3), 1.72–1.89 (2H, m, $2CH_2$), 2.35 (3H, s, $COCH_3$), 2.55 (2H, t, $J=3.72$ Hz, $ArCH_2CH_2N$), 3.18 (2H, t, $J=3.72$ Hz, $ArCH_2CH_2N$), 3.54–3.55 (1H, m, 4′*CH*OH), 3.71 (1H, m, $C(CHO)CH_2$), 3.83 (3H, s, $ArOCH_3$), 3.92 (1H, brd, 1′CH), 4.25 (1H, q, $J=3.8$ Hz, 5′CH), 5.31–5.33 (1H, m, 3′CH), 6.58 (2H, d, $J=5.6$ Hz, 2×ArH), 6.91 (2H, d, $J=5.6$ Hz, 2×ArH), 7.27 (1H, t, $J=3.3$ Hz, ArH), 7.55–7.57 (2H, m, 2×ArH); ^{13}C NMR (100 MHz, CD_3OD) δ 25.1 (CH_3), 31.2 (CH), 32.0 (CH_2), 33.9 (CH_2), 37.0 (CH_2), 43.2 (CH_2), 44.2 (CH_2), 47.6 (CH) 56.2 (CH), 57.4 (CH), 69.1 (CH), 74.3 (CH_3) 77.9 (C), 80.0 (CH_3) 102.7 (CH), 131.1 (3×CH), 131.8 (CH), 135.9 (2×C), 136.2 (2×C), 136.3 (2×C), 137.4 (2×CH), 156.4 (C), 157.2 (C), 157.7 (2×C), 160.6 (2×C), 187.6 (C), 187.9 (C), 214.1 (C); *m*/*z* (CI) 383 (20%), 363 (100), 347 (15), 293 (urea linked tyramine to 4-OH-5-Me-hexose(15)), 174 (10), 138 (10), 107 (10), 74 (10).

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