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Synthesis and Analysis of Urea and Carbamate Prodrugs as Candidates for Melanocyte-Directed Enzyme Prodrug Therapy (MDEPT)

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Abstract—The suitability of 4-di(2-chloroethyl)aminoanilino-4-hydroxyphenethylaminomethanone **2** to act as a prodrug for melanocyte-directed enzyme prodrug therapy (MDEPT) is assessed. Thus its synthesis, ability to generate a cytotoxic agent upon exposure to tyrosinase, and stability within different sera are reported. A comparison is made to illustrate that the new urea prodrug **2** is a more suitable candidate for MDEPT than the corresponding carbamate prodrug **1**.

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 methods of treatment for this particular cancer.

A number of delivery systems have been developed for the localised delivery of prodrugs to certain cancers,[2] and these offer the advantage of minimal non-localised drug toxicity. For example, the antibody-directed enzyme prodrug therapy (ADEPT)[3] utilises antibodies to selectively deliver non-mammalian enzymes to tumour cells. Prodrugs, which are substrates for the introduced enzyme, are then administered with the knowledge that they will only release the toxic drug at the tumour site, where the antibody–enzyme conjugate resides. If an enzyme is expressed in high levels within a tumour cell, and if this enzyme is virtually absent from other cells, it is theoretically possible to consider localised drug delivery strategies based on prodrugs for this enzyme. Fortuitously, melanoma cells contain high levels of the tyrosinase enzyme, and since this enzyme is virtually absent from other cells, it is a good target for *in-situ* activation of prodrugs.[4] A number of tyrosinase dependent prodrug strategies have been investigated for the treatment of melanoma. For example, non-toxic phenol and catechol prodrugs have been oxidised by tyrosinase to afford toxic quinones within the vicinity of melanoma tumours.[5] In addition, we have recently reported a novel tyrosinase meditated drug delivery system which utilises tyrosinase to mediate the release of cytotoxic drugs, from prodrugs, via a cyclisation/delivery system outlined in Scheme 1.[6]

Although taxol, daunomycin, gemcitabine and nitrogen mustards have previously been incorporated within our prodrugs, previous results have illustrated that nitrogen mustards are the preferred cytotoxic agents for our system.[6,7] This is a consequence of steric hinderance affecting the ability of prodrugs derived from larger cytotoxic agents to act as substrates for tyrosinase.[6] Together with the fact that nitrogen mustards are often used in prodrug strategies such as ADEPT,[8] and are indeed employed in the clinic for the treatment of melanoma,[9] nitrogen mustards can be considered as suitable cytotoxic agents for incorporation within our MDEPT programme.

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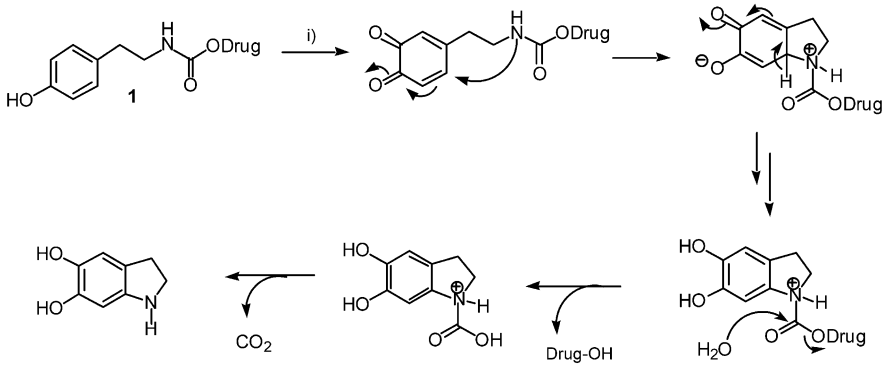
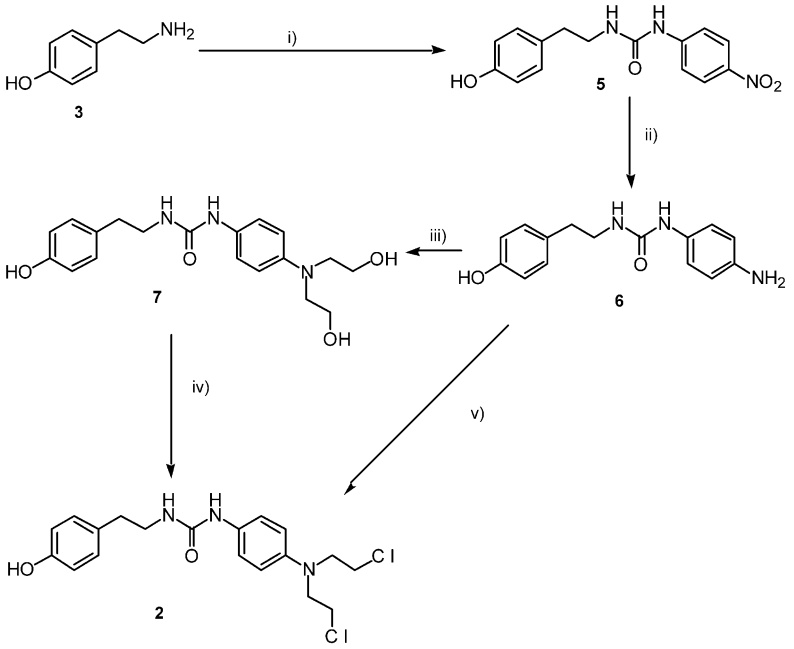


Scheme 1. Proposed drug release mechanism mediated by tyrosinase. Reagents: (i) Tyrosinase



Scheme 2. Synthesis of urea-linked aniline mustard **2**. Reagents: (i) *p*-Nitrophenyl isocyanate **4**, pyridine, 100%; (ii) 10% Pd on carbon (10 mol.%), abs ethanol, H_2, 1 atm, 100%; (iii) ethylene oxide, 54%; (iv) thionyl chloride, 52% (see Table 1); (v) chloroacetaldehyde, sodium cyanoborohydride, HCl/MeOH, pH 6, 69%.

The success of a localised delivery strategy relies upon generation of the cytotoxic unit from the prodrug purely at the tumour site. This therefore necessitates stability of the prodrug within serum. Previous work within our group had illustrated that carbamate prodrugs of representative structure **1** were good substrates for tyrosinase, and their ability to release the cytotoxic phenol mustard moiety upon exposure to tyrosinase had been demonstrated.[6] We now wish to report our recent studies within this area, which have investigated the relative stabilities of carbamate and urea prodrugs within sera as well as the ability of urea prodrug **2** to release nitrogen mustard upon exposure to tyrosinase.

Results and Discussion

Synthesis of phenyl and aniline mustard prodrugs

Carbamate-linked prodrug **1** was synthesised as previously reported,[6] with an overall yield of 62%, over 5 steps. Synthesis of the urea prodrug **2** was accomplished using an isocyanate intermediate (Scheme 2). Thus formation of the urea prodrug skeleton was easily achieved by coupling tyramine **3** with *p*-nitrophenyl isocyanate **4** to afford urea **5**. Subsequent nitro reduction gave amine **6**, in quantitative yield over the two steps. Amine **6** was then either reacted with ethylene oxide to afford the corresponding bis-diol **7**, which was subsequently transformed to the bis-chloride **2** using several different chlorinating reagents (Table 1), or taken straight through to prodrug **2** by a reductive amination step, using chloroacetaldehyde. This reductive amination has previously been reported in the synthesis of mono-ethyl mustards,[10] but has not been reported for entry to bis-chloroethyl mustards. Although both synthetic routes successfully provided access to the urea prodrug **2**, reductive amination resulted in a superior overall yield (69%), compared to the traditional ethylene oxide approach (28%) and offered the added advantages of being consistently reproducible whilst avoiding the use of toxic ethylene oxide.

Table 1. Chlorinating reagents used in step (iv) of Scheme 2

Entry	Chlorinating reagent (step iv)	Yield of **2**
1	Thionyl chloride	52%
2	Mesyl chloride	37%
3	$CH_3N^+CHCl_2$	<5%
4	CCl_4, PPh_3	<5%

In order to achieve appreciable yields in the reductive amination the reaction was typically left to run over several days. Under standard Borch reaction conditions[11] an excess of amine is used to favour the otherwise slow formation of the iminium intermediate, thus increasing the overall rate of the reaction. To overcome the need for a large excess of amine, various modifications of the Borch conditions have been reported. For instance, Mattson et al.[12] reported the use of titanium (IV) isopropoxide to catalyse rapid equimolar reductive aminations between ketones/aldehydes and various primary and secondary amines and McCarthy et al.[13] employed titanium tetrachloride with diisopropylethylamine or triethylamine in the synthesis of hindered amines, with good effect. We therefore investigated a range of conditions that were considered to promote the rate of reductive amination between amine **6** and chloroacetaldehyde, including: Lewis acids and base catalysis, concentration and excess aldehyde (Table 2).

Table 2 shows that titanium (IV) isopropoxide (entry 4 & 5) increases the reaction rate, compared to the uncatalysed standard conditions (entry 1). However, more importantly, when the amount of aldehyde was increased from 4 equiv (entry 1) to 8 equiv (entry 6) it was observed that 50% of amine **6** was converted to the corresponding tertiary amine **2** in 3 h, compared to 48 h under the standard conditions. Furthermore, as expected, when more concentrated conditions were employed (entry 6 vs entry 7) further rate enhancement was observed. Therefore, in this instance, it can be concluded that instead of using an excess of amine to favour the slow formation of the iminium intermediate, it was possible to use an excess of the readily available aldehyde to achieve the same effect.

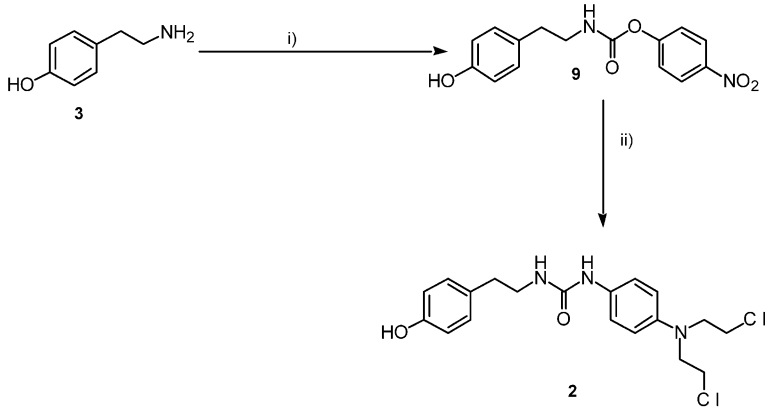
A convergent synthesis of urea prodrug **2** was also attempted as outlined in Scheme 3. In this case, tyramine **3** was coupled with *p*-nitrophenyl chloroformate **8** to give carbamate **9**. This was subsequently reacted with the aniline mustard **10**, which was prepared by two methods as outlined in Scheme 4.

Synthesis of the aniline mustard **10** is outlined in Scheme 4. Diol **12** could either be formed by reaction of *p*-nitrophenyl aniline **11** with ethylene oxide, under acidic conditions, or by an S_N2_{Ar} fusion between *p*-fluoronitrobenzene **13** and diethanolamine. In either case, diol **12** was then converted into the corresponding bis-chloride **14**, using mesyl chloride or thionyl chloride. Iron mediated nitro reduction and treatment with $HCl_{(g)}$ then gave **10** (HCl salt). The S_N2_{Ar} fusion route was found to proceed with enhanced overall yield; 63% versus 22% for the ethylene oxide route.

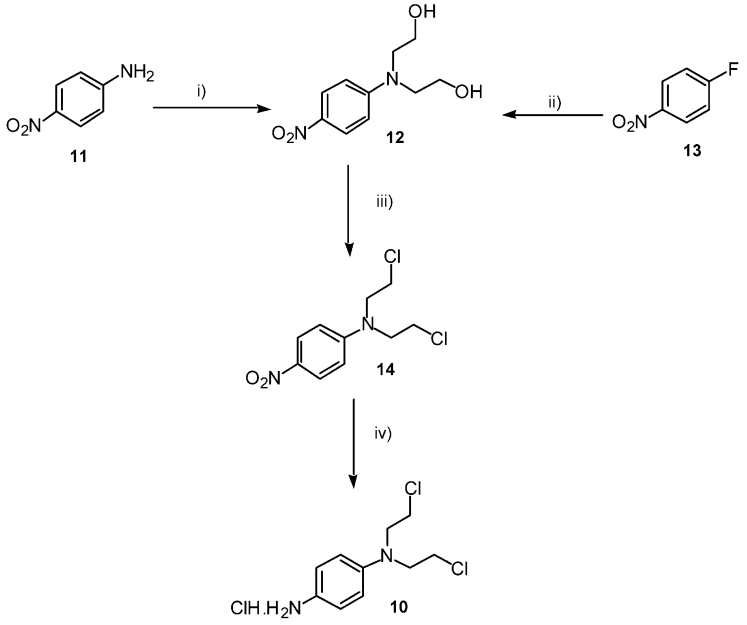
Table 2. Affect of reaction conditions on the reductive amination of **6** in Scheme 2

Entry	Conditions	Reaction time (h) required for 50% conversion of SM to product	Total% Yield of **2**
1	4 eq. aldehyde, 10 mL MeOH≡Standard conditions (SC)[a]	48	69
2	$TiCl_4$ under SC	No product	0
3	$TiCl_4 + Et_3N$ under SC	No product	0
4	$Ti(O^iPr)_4$ under SC	12	74
5	$Ti(O^iPr)_4 + Et_3N$ under SC	12	71
6	8 eq. aldehyde, 10 mL MeOH	3	77
7	8 eq. aldehyde, 5 mL MeOH	2	82

[a]SC≡Standard conditions: **6** (1 mmol), aldehyde (4 mmol), MeOH (10 mL), $NaBH_3CN$ (2.2 mmol), 6N HCl∼pH 6.



Scheme 3. Convergent synthesis of urea prodrug **3**. Reagents: (i) *p*-Nitrophenyl chloroformate **8**, CH_2Cl_2, 97%; (ii) **10**, dimethylformamide, triethylamine, 52%.



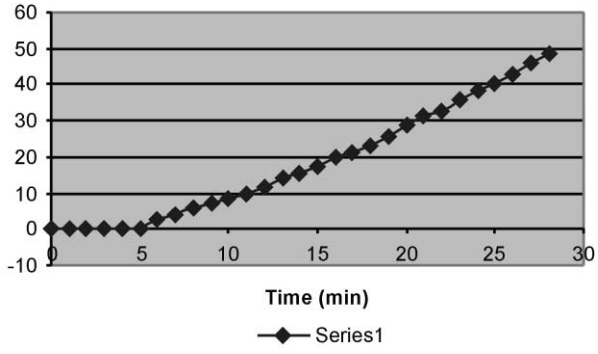
Scheme 4. Synthesis of aniline mustard hydrochloride **10**. Reagents: (i) ethylene oxide, acetic acid, 30%; (ii) *N,N*-diethanolamine, 130 °C, 84%; (iii) MsCl, pyridine, 83% or thionyl chloride, pyridine/CH_2Cl_2, 86%; (iv) Fe, HCl, EtOH and then $HCl_{(g)}$, diethyl ether, 87%.

When the nitrogen mustard **10** was used in the route outlined in Scheme 3 poor yields of urea prodrug **2** were obtained, due to instability of the aniline mustard **10**, in basic solution and attack of the liberated *p*-nitrophenol upon the newly formed urea prodrug **2**. Thus for further studies, urea prodrug **2** was synthesised according to the method outlined in Scheme 2.

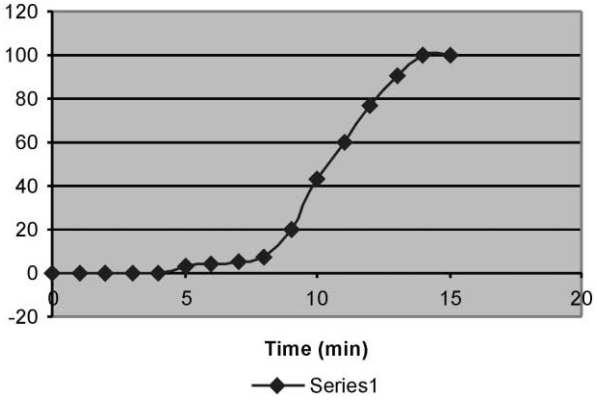
Ability of urea prodrug 2 to act as a substrate for tyrosinase

Upon establishing reliable synthetic entry to prodrug **2** we assessed its ability to act as a substrate for tyrosinase and, as before, this was achieved via oximetry studies.[6] If prodrug **2** is oxidised according to the pathway illustrated in Scheme 1, molecular oxygen will be absorbed from the surrounding solution. The resulting oxygen depletion can be measured using an oxygen sensor, and the rate of oxidation uptake provides an indication of the rate of tyrosinase oxidation of prodrug **2**. We have already illustrated that the carbamate prodrug **1** is a good substrate for tyrosinase ($R_{max} = 17.5$ nanomol/min compared with $R_{max} = 17.5$ nanomol/min for tyrosine methyl ester, the methyl ester of the natural substrate for tyrosinase).[6] Oximetry data illustrated that prodrug **2** was also a good substrate for tyrosinase ($R_{max} = 16.6$ nanomol/min, which again compares favourably with that for tyrosine methyl ester) but in this case a longer lag phase was evident at the begininning of the oxidation process (Graph 1).

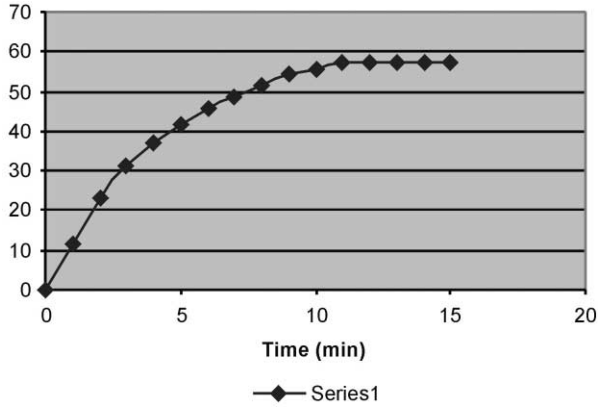
An initial lag phase is common during tyrosinase-catalysed oxidation of monohydric phenols and is a consequence of tyrosinase requiring activation by a catechol before oxidation of the phenol can commence.[14] To diminish this lag period, further oximetry studies were modified to allow addition of catalytic



Graph 1. Oximetry data for urea prodrug **2** (100 μL of a 10 mM solution).



Graph 2. Oximetry data for urea prodrug **2** (100 μL of 10 mM solution) with *N*-methyl dopamine (5 μL of 10 mM solution) being added at t = 8 min.

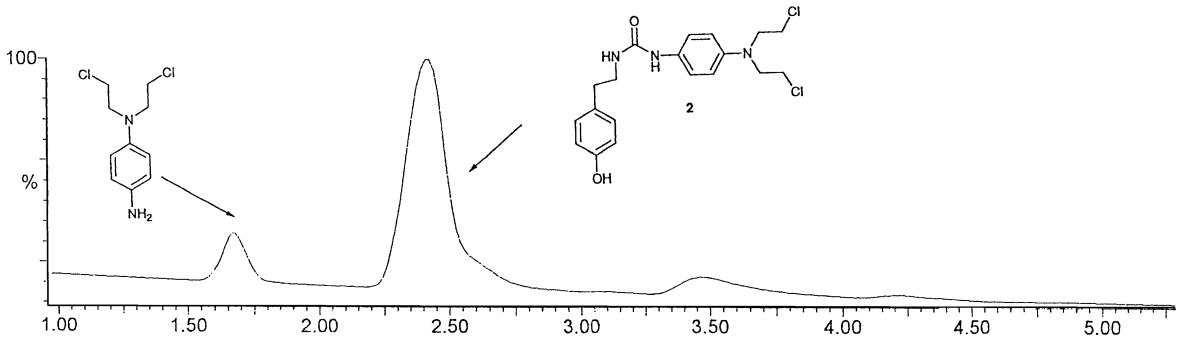


Graph 3. Oximetry data for *N*-methyl dopamine (100 μL of a 10 mM solution).

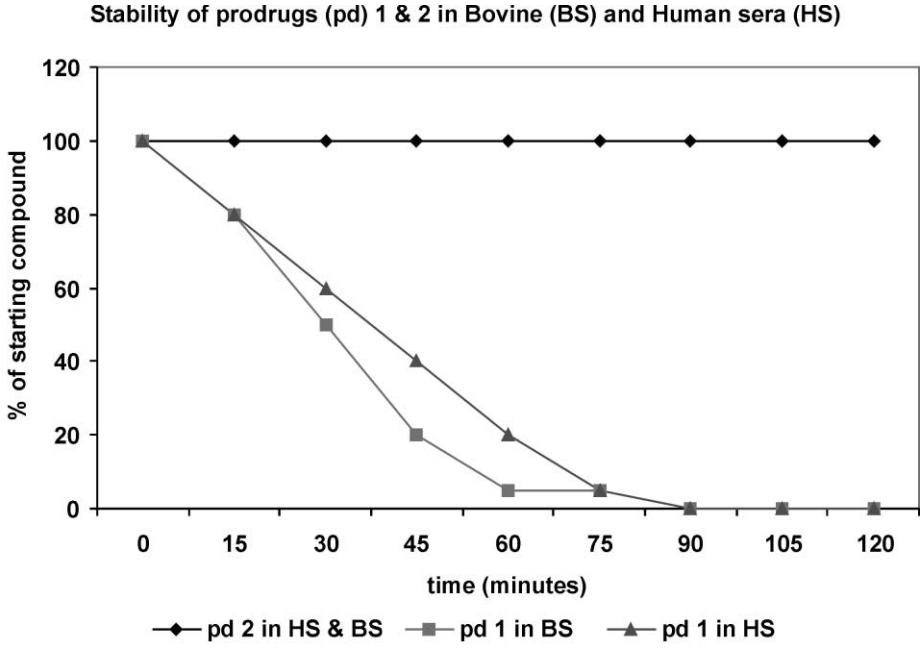
quantities of a catechol, *N*-methyl dopamine. As expected, catechol mediated activation of tyrosinase then allowed rapid oxidation of prodrug **2**, as illustrated in Graph 2. For comparison, oximetry data for *N*-methyl dopamine is presented in Graph 3.

Monitoring the ability of prodrug 2 to undergo cyclisation/drug release upon exposure to tyrosinase

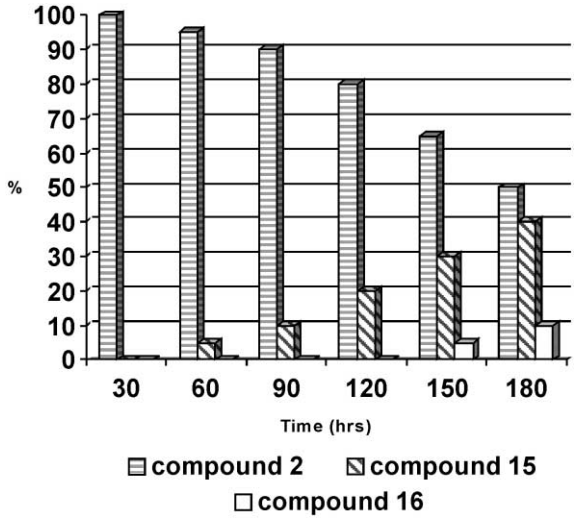
Having established that prodrug **2** was indeed a substrate for tyrosinase, our attention next focussed on determining whether tyrosinase mediated drug release would occur from prodrug **2**. Such a process had previously been proven by HPLC for prodrug **1**. Pleasingly, HPLC analysis indicated that drug release also occurred when prodrug **2** was exposed to mushroom tyrosinase at 25 °C in H_2O/DMSO (Trace 1).



Trace 1. This trace shows release of aniline mustard after 30 min exposure of urea prodrug **2** to mushroom tyrosinase at 25 °C in H_2O/DMSO (300 units of tyrosinase used per mL of solution).



Trace 2. Stability of carbamate prodrug **1** and urea prodrug **2** in human and bovine sera at 37 °C, as analysed by HPLC.



Graph 4. Investigation of halogen/DO exchange of compound **2** in D_2O/DMSO.

Stability of carbamate and urea prodrugs 1 and 2 in bovine and human sera

Efforts were next focussed on determining the stability of carbamate prodrug **1** and urea prodrug **2** in sera. To avoid systemic toxicity within prodrug strategies it is paramount that drug release from the prodrugs only occurs at the targeted site, thus localising alkylation within or around the malignant tumour. In order to achieve this, and generate prodrugs with useful half lives, in vivo stability of the urea/carbamate linker is imperative. HPLC was used to assess the stability of the prodrugs **1** and **2** in human and bovine sera at 37 °C for 2.5 h (Trace 2). Stability of the mustard moiety was also investigated by ^{1}H NMR in D_2O (Graph 4).

Pleasingly, it was evident that the urea prodrug **2** was stable for prolonged times under physiological conditions and hydrolytic conditions. This was in contrast to the results obtained for the carbamate prodrug **1**. Graph 4 demonstrates that the bis-chloro moiety of the urea mustard compound **2** was very stable to DO exchange in a solution of D_2O/d_6-DMSO. In fact, little exchange was observed over 48 h at room temperature and it was only after several days that appreciable exchange was observed (40% mono-chloro mono-hydroxy compound **15** and 10% bis-hydroxy compound **16**; see Scheme 5). Presumably exchange takes place via slow formation of an aziridine intermediate which is rapidly re-opened by D_2O, as shown in Scheme 5.

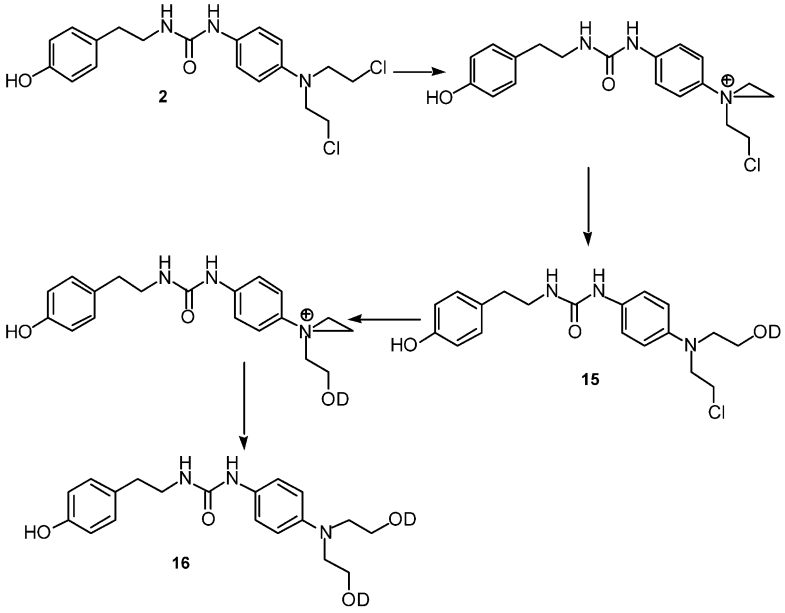
The enhanced stability and prolonged half life of the urea prodrug, relative to the carbamate prodrug, within sera, is a particularly interesting result. Since a number of prodrugs employed within conventional ADEPT strategies contain the carbamate functional group this work highlights the potential of increasing the stability and half lives of such prodrugs by the introduction of urea, rather than carbamate, linkages.

Conclusions

In conclusion, we have demonstrated that the urea prodrug **2** offers enhanced stability in sera compared with our previous lead prodrug **1**. Since prodrug **2** is a good substrate for tyrosinase, and is able to release a cytotoxic moiety upon exposure to tyrosinase, it remains a good candidate for the treatment of melanoma.

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



Scheme 5. Possible pathway of halogen/DO exchange, via an aziridine intermediate.

for ^{1}H 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 FTIR spectrometer. Melting points were determined using an Electrothemal digital melting point apparatus, and are uncorrected. 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. Oximetry was performed using a YSI model 5300 biological oxygen monitor.

Unless stated otherwise, all chemicals and materials were obtained from the Sigma–Aldrich Chemical Company, the B.D.H. Merk Chemical Company or Lancaster Chemicals and were used as received. Silica gel for column chromatography was obtained from Merck, with a pore diameter of 6 nm. 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. Mushroom tyrosinase (3520 units/mg) was used at a concentration of 300 units/mL in 0.1 M phosphate buffered saline (pH 7.4).

4 - Di(2 - chloroethyl)aminoanilino - 4 - hydroxyphenethyl - aminomethanone (2)

[Step e Scheme 3, thionyl chloride method]. Compound **7** (1 g, 2.7 mmol) was suspended in a solution of dichloromethane (20 mL) and pyridine (3 mL) and cooled to 0 °C. Thionyl chloride (0.42 mL, 5.8 mmol) was added and the mixture was heated at reflux for 1 h, allowed to cool and diluted with dichloromethane (100 mL). The mixture was washed with water (2×50 mL) and the organics were dried ($MgSO_4$) and concentrated in vacuo to give an off white solid, which was purified by column chromatography (silica gel, EtOAc) to yield mustard **2** as a white solid (550 mg, 52%).

[Step c Scheme 3, reductive amination method]. Compound **6** (500 mg, 1.8 mmol) was dissolved in methanol (5 mL). Chloroacetaldehyde (1.76 mL, 14.4 mmol) as a 45% aq solution and sodium cyanoborohydride (260 mg, 4.25 mmol) were added slowly. The mixture was acidified (pH 6) using acetic acid, stirred at room temperature for 5 h, acidified with concd HCl (pH 2) and concentrated in vacuo. The resulting oily residue was dissolved in dichloromethane (150 mL) and washed with a 10% sodium bicarbonate solution. The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give an off white solid. Purification by column chromatography (silica gel, EtOAc) yielded mustard **2** as a white solid (583 mg, 82%); mp 95 °C; ν_{max} (KBr disc) 3350, 1680, 1670, 1615, 1560, 1512, 1370, 1110 cm^{-1}; δ ^{1}H NMR (400 MHz, CD_3OD) 2.69 (2H, t, J = 7.0 Hz, Ph$\underline{CH_2}$), 3.34 (2H, t, J = 7.0 Hz, CH_2), 3.29–3.31 (8H, m, 2×Cl$\underline{CH_2CH_2}$N), 6.69–6.76 (4H, m, Ar), 7.04 (2H, d, J = 11.3 Hz, Ar), 7.14 (2H, d, J = 11.3 Hz, Ar); δ ^{13}C NMR (100 MHz, CD_3OD) 36.9, 2×(CH_2), 43.1, 2×(CH_2), 46.4 (CH_2), 55.1, (CH_2), 114.6, (C), 116.6, 4×(CH), 124.2, (C), 131.2, 4×(CH), 131.7, 2×(C), 157.3 (CO); (HRMS (CI), found: $[M+H]^+$ 396.1121. $C_{19}H_{23}Cl_2N_3O_2$ $[M+H]^+$, requires 395.1167); m/z (CI) (396 $[M+H]^+$, 15%), 259 (45), 209 (100), 146 (20), 107 (40).

4-Hydroxyphenethylamino-4-nitroanilinomethanone (5). Tyramine **3** (5 g, 36.5 mmol) was dissolved in anhydrous pyridine (30 mL) and cooled to 0 °C. *p*-Nitrophenyl isocyanate **4** (5.98 g, 36.5 mmol) was added slowly and the mixture was stirred and allowed to warm to room temperature. The reaction was monitored by TLC (EtOAc) and upon completion (approx 3 h) the mixture was concentrated in vacuo to afford urea **5** as a yellow solid (10.98 g, 100%); mp 175–176 °C; λ_{max} (KBr disc) 3350, 1680, 1615, 1560, 1512, 1370, 1285, 1110, 825 cm^{-1}; δ ^{1}H NMR (400 MHz, CD_3OD) 2.72 (2H, t, J = 6.3 Hz, Ph$\underline{CH_2}$), 3.36 (2H, t, J = 6.3 Hz, CH_2), 6.71 (2H, d, J = 7.9 Hz, Ar), 7.04 (2H, d, J = 7.9 Hz, Ar), 7.54 (2H, d, J = 7.9 Hz, Ar), 8.11 (2H, d, J = 7.9 Hz, Ar); δ ^{13}C NMR (100 MHz, CD_3OD) 36.6 (CH_2), 42.9 (CH_2), 116.7, 2×(CH), 118.8, 2×(CH), 126.3, 2×(CH), 131.1, 2×(CH), 131.5 (C), 143.3 (C), 148.2 (C), 157.4 (C and CO); (HRMS (CI), found: $[M+H]^+$ 302.1055. $C_{15}H_{15}N_3O_4$ $[M+H]^+$, requires 301.1063; m/z (CI) (302 $[M+H]^+$, 10%), 163 (20), 139 (100), 123 (25) 107 (65).

4-Aminoanilino-4-hydroxyphenethylaminomethanone (6). Compound **5** (5 g, 16.6 mmol) was dissolved in ethanol$_{abs}$ (250 mL) and 10% palladium on carbon was added (1.5 g). The reaction mixture was stirred under a hydrogen atmosphere for 12 h, concentrated in vacuo and filtered through Celite® to afford **6** as an off white solid (4.5 g, 100%); mp 188–190 °C; ν_{max} (KBr disc) 3350, 1660, 1615, 1560, 1512, 1370, 1110, 825, 820 cm^{-1}; δ ^{1}H NMR (400 MHz, CD_3OD) 2.56 (2H, t, J = 7.0 Hz, Ph$\underline{CH_2}$), 3.22 (2H, t, J = 7.0 Hz, CH_2), 6.55–6.62 (4H, m, Ar), 6.88–6.98 (4H, m, Ar); δ ^{13}C NMR (100 MHz, CD_3OD) 36.9 (CH_2), 43.1 (CH_2), 116.6, 2×(CH), 117.5, 2×(CH), 124.1, 2×(CH), 131.2, 2×(CH), 131.7 (C), 133.7 (C), 145.0 (C), 156.1 (C), 159.7 (CO); (HRMS (CI), found: $[M+H]^+$ 272.1386. $C_{15}H_{17}N_3O_2$ $[M+H]^+$, requires 272.1399); m/z (CI) (272 $[M+H]^+$, 40%), 165 (80), 137 (85), 108 (35).

4-Di(2-hydroxyethyl)aminoanilino-4-hydroxybenzylaminomethanone (7). Compound **6** (2 g, 7.4 mmol) was dissolved in acetic acid (50 mL) and H_2O (50 mL) and cooled to 0 °C. To this mixture 8 equiv of ethylene oxide (59 mmol) was added slowly. The reaction mixture was stirred and allowed to warm to room temperature. After 12 h a further 8 equiv of ethylene oxide (59 mmol) was added slowly and the mixture was stirred for a further 12 h. After this time, the solution was concentrated in vacuo to give a black oil, which was purified by flash chromatography (silica gel EtOAc, then MeOH). The MeOH fraction was concentrated in vacuo and recrystalised (Acetone/EtOAc, 1.25:1) to give a brown solid, further recrystalisation (MeOH) afforded diol **7** as an off white solid (1.43 g, 54%); mp 186 °C; ν_{max} (KBr disc) 3350, 1680, 1615, 1560, 1400, 1285, 1110, 825 cm^{-1}; δ ^{1}H NMR (400 MHz, CD_3OD) 2.68 (2H, t, J = 7.0 Hz, Ph$\underline{CH_2}$), 3.32 (2H, t, J = 7.0 Hz, CH_2), 3.47 (4H, t,

$J = 6.1$ Hz, 2×$\underline{CH_2}$N), 3.68 (4H, t, $J = 6.1$ Hz, 2×$\underline{CH_2}$OH), 6.69–6.74 (4H, m, Ar), 7.03–7.11 (4H, m, Ar); δ ^{13}C NMR (100 MHz, CD_3OD) 36.7 (CH_2), 43.0 (CH_2), 56.8, 2×(CH_2), 61.8, 2×(CH_2), 116.7, 2×(CH), 120.8, 2×(CH), 124.5, 2×(CH), 131.2, 2×(CH), 131.6 (C), 143.8 (C), 157.3 (C and CO), 158.0 (C); (HRMS (CI), found: $[M+H]^+$ 360.1845. $C_{19}H_{25}N_3O_4$ $[M+H]^+$, requires 359.1845); *m*/*z* (CI) (360 $[M+H]^+$, 100%), 315 (20), 223 (25), 133 (15), 100 (35).

4-Hydroxyphenethylamino-4-nitrophenoxymethanone (9). Tyramine **3** (1 g, 7.3 mmol) and *p*-nitrophenylchloroformate **8** (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 **9** as a pale yellow solid (2.2 g, 97%); mp 157–159 °C; ν_{max} (KBr disc) 3400, 1658, 1440, 1380 cm^{-1}; δ ^{1}H NMR (400 MHz, $CDCl_3$) 2.74 (2H, t, $J = 7.0$ Hz, $\underline{CH_2}$Ar), 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); δ ^{13}C NMR (100 MHz, $CDCl_3$); 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).

1*N*,1*N*-Di(2-chloroethyl)-1,4-benzenediamine hydrochloride (10). Compound **14** (5 g, 19 mmol) was dissolved in ethanol (200 mL) and 10% palladium on carbon (500 mg) was added. The mixture was stirred under an atmosphere of hydrogen for 12 h, filtered through Celite® and concentrated in vacuo to give a grey solid. The solid was redissolved in ethanol (100 mL) and diethyl ether (100 mL) and hydrogen chloride gas was passed through it. The resulting precipitate was filtered off under a nitrogen atmosphere to yield aniline mustard hydrochloride 10 as a white solid (4.4 g, 87%); mp 213–215 °C (lit.[15] 210–214 °C); δ ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 3.75–3.85 (8H, m, 2×ArN$\underline{CH_2CH_2}$), 6.76–6.81 (2H, m, 2×Ar$\underline{H}$), 7.15–7.20 (2H, m, 2×Ar$\underline{H}$); δ ^{13}C NMR (100 MHz, $(CD_3)_2SO$); 44.3 (2×CH_2), 58.3 (2×CH_2), 113.9 (2×CH), 116.0 (2×CH), 134.5 (C), 136.2 (C); (HRMS (CI), found: $[M+H]^+$ 234.1367. $C_{10}H_{14}Cl_2N_2$ $[M+H]^+$, requires 233.1370); *m*/*z* (CI) (233 $[M+H]^+$, 63%), 183 (100), 120 (51), 66 (15).

2-(2-Hydroxyethyl-4-nitroanilino)-1-ethanol (12).

[Step i Scheme 4, *N*-alkylation of 4-nitroaniline]. 4-Nitroaniline **11** (13.8 g, 100 mmol) was suspended in glacial acetic acid (140 mL) and water (140 mL) and cooled to 0 °C. Ethylene oxide (240 mmol) was added slowly and the mixture was stirred and allowed to warm to room temperature. The reaction mixture was stirred (4 days) until no starting material remained by TLC (ethyl acetate/methanol 9:1) and concentrated in vacuo to give a black oil. The oil was dissolved in ethyl acetate (1 L), washed with water (300 mL), dried ($MgSO_4$) and concentrated in vacuo to give a brown oil. Purification by column chromatography (hexane/ethyl acetate 1:1) afforded diol **12** as a pale yellow solid (6.8 g, 30%).

[Step ii Scheme 4, fusion between 1-fluoro-4-nitro-benzene and diethanolamine]. 1-Fluoro-4-nitro-benzene **13** (11.75 g, 83.3 mmol) and *N*,*N*-diethanolamine (83.3 mmol) were mixed together and heated at 130 °C for 2 h. The mixture was allowed to cool to 60 °C before 0.6% sodium hydroxide solution (890 mL) was added to give a yellow precipitate. The precipitate was filtered and dried ($MgSO_4$) to afford diol **12** as a yellow solid (16 g, 84%). Mp 104–105 °C (lit.[16] 103–104 °C); δ ^{1}H NMR (400 MHz, (CD_3OD) 3.66–3.81 (8H, m, 2×ArN$\underline{CH_2CH_2}$), 6.80 (2H, d, $J = 13.1$ 2×Ar$\underline{H}$), 8.0 (2H, d, $J = 13.1$ 2×Ar$\underline{H}$); δ ^{13}C NMR (100 MHz, (CD_3OD); 55.2 (2×CH_2), 60.4 (2×CH_2), 112.3 (2×CH), 127.3 (2×CH), 138.2 (C), 155.1 (C); (HRMS (CI), found: $[M+H]^+$ 227.1002. $C_{10}H_{14}O_2N_2$ $[M+H]^+$, requires 226.0954); *m*/*z* (CI) (227 $[M+H]^+$, 100%), 195 (35).

1*N*-(2-Chloroethyl)-1*N*-(3-chloropropyl)-4-nitroaniline (14). Compound **12** (16 g, 71 mmol) was suspended in dichloromethane (160 mL) and pyridine (10 mL) and cooled to 0 °C. Thionyl chloride (160 mmol) was added slowly and the mixture was stirred and heated at reflux for 1 h. After cooling the mixture was diluted with dichloromethane (160 mL) and carefully washed with water (200 mL). The organics were dried ($MgSO_4$) and concentrated in vacuo to give mustard **14** as a dark yellow solid (16.1 g, 86%); mp 92–94 °C (lit.[17] 94–95 °C); δ ^{1}H NMR (400 MHz, (CD_3OD) 3.58—3.68 (4H, m, 2×$\underline{CH_2}$Cl), 3.75–3.84 (4H, m, 2×ArN$\underline{CH_2}$), 6.68 (2H, dd, $J = 7.5$ Hz, 2×Ar$\underline{H}$), 7.93 (2H, dd, $J = 7.5$ Hz, 2×Ar$\underline{H}$); δ ^{13}C NMR (100 MHz, (CD_3OD); 41.6 (2×$\underline{CH_2}$), 54.4 (2×CH_2), 112.3 (2×CH), 127.4 (2×CH), 138.8 (C), 153.7 (C).

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References and Notes

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