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Design, Synthesis and Evaluation of Imidazolylmethyl Carbamate Prodrugs of Alkylating Agents

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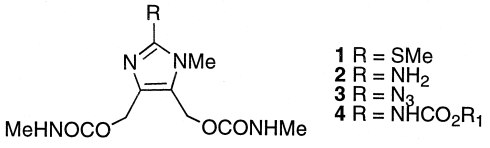
Abstract—Two approaches to prodrugs of alkylating agents based on an imidazolylmethyl carbamate nucleus were explored. A 2-azido analogue (**3**) of the bis-carbamate carmethizole (**1**) displayed similar aerobic cytotoxicity to **1** in a panel of human and murine cell lines. Approaches to the 2-amino and 2-carbamoyl analogues are described. In the second approach an imidazolylmethanol was used as a 'trigger' linked via a carbamate to the alkylating agent *N*,*N*-bis(2-chlorethyl)amine (BCEA). Nitroimidazole and methylsulphinylimidazole carbamate prodrugs **6**–**8** were 5–20-fold less toxic than BCEA. Despite this deactivation in the prodrug form, little increase in cytotoxicity was observed under hypoxia. The data suggest that BCEA released on bioreduction is not sufficiently potent to contribute significant additional cytotoxicity.

Most current cancer chemotherapeutic agents are cytotoxins with primary selectivity against dividing cells rather than cancer cells. In the search for new cancer therapeutics, there is an increasing interest in improving the tumour-selectivity of such cytotoxins by masking them as prodrugs, which may be activated in a tumour-specific manner.[1–3] Strategies to this end include antibody-directed enzyme prodrug therapy (ADEPT),[4–6] gene-directed enzyme prodrug therapy (GDEPT),[7,8] and the use of tumour hypoxia to facilitate bioreductive activation.[9,10] The presence of hypoxia within solid tumours[11] is a major factor contributing to their radioresistance.[12–14] It is also a significant physiological difference between solid tumours and normal tissue, providing an opportunity for tumour-selective activation of bioreductive prodrugs.[15] A requirement for such prodrugs is that they undergo initial reduction by endogenous enzymes to an oxygen-reversible one-electron adduct to provide a cytotoxic species.[9]

In the course of our studies into bioreductive prodrugs our attention was drawn to carmethizole (**1**), a novel bis-carbamate alkylating agent,[16] which forms DNA–protein and DNA–DNA cross-links in vitro,[17] and has antitumour activity against murine leukaemias, solid tumours and human tumour xenografts in vivo.[16,18] The antitumour activity of related bis-hydroxymethylimidazole carbamates was found to be enhanced by electron-donating 2-substituents, while electron-withdrawing 2-substituents led to compounds that were inactive.[16] This relationship was suggested to be a consequence of the ability of the 2-substituent to stabilise the transition state of the S_N1-type activation of the hydroxymethyl groups, which eventually leads to DNA alkylation.[19] These reports led us to consider the hydroxymethylimidazole nucleus as a potentially useful moiety around which to design prodrugs of alkylating agents for bioreductive activation in tumours. We explored two strategies in the development of such prodrugs.

In the first approach,[20] we aimed to use the bis-hydroxymethylimidazole nucleus derived from carmethizole as the alkylating unit, with 2-nitrogen substituents to deactivate the alkylating groups. While the 2-amino derivative (**2**) is expected to be more reactive than carmethizole (**1**) on the basis of its electron-donating properties [$\sigma_p(NH_2)=-0.66$, $\sigma_p(SMe)=0.00$][21] a 2-azido derivative **3** or a 2-carbamoyl derivative **4** contain considerably less electron-donating substituents [$\sigma_p(N_3)=0.15$, $\sigma_p(NHCO_2Me)=-0.15$],[22] and might therefore provide prodrug forms of **2**. The use of the azido group as a masked amine function is well known,[23] and metabolism of aryl azides to amines has been demonstrated.[24–26] Hence we targeted the 2-azido (**3**) and 2-carbamoyl analogues (**4**) of carmethizole (**1**) as examples of this class of prodrug.



1 R = SMe
2 R = NH_2
3 R = N_3
4 R = $NHCO_2R_1$

Keywords: antitumour compounds; carbamates; imidazoles/imidazolines.
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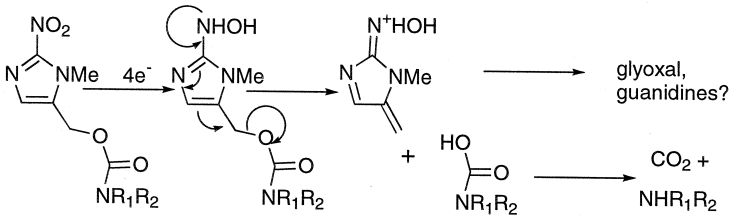
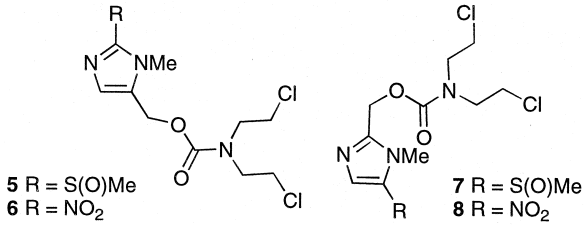


Scheme 1. Reductive activation of 2-nitroimidazole-5-methylcarbamates.

Our second strategy aimed to use a substituted hydroxymethylimidazole ring as the 'trigger' unit rather than as the alkylating unit. In this concept, reduction of the trigger unit leads to activation of a separate alkylating moiety, which is attached via a deactivating carbamoyl linker. Bioreduction of an electron-withdrawing substituent on the imidazole ring to a more electron donating moiety would activate the carbamate unit to nucleophilic attack, with subsequent fragmentation of the resulting carbamic acid, releasing a secondary amine as the cytotoxic 'effector' (Scheme 1). In the present study, the nitrogen mustard *N*,*N*-bis(2-chloroethyl)amine (BCEA), was selected as the alkylating agent. Nitrogen mustards are significantly deactivated by electron withdrawing substituents,[27] with mustard *N*-carbamates thus expected to be considerably less toxic than the free mustard.

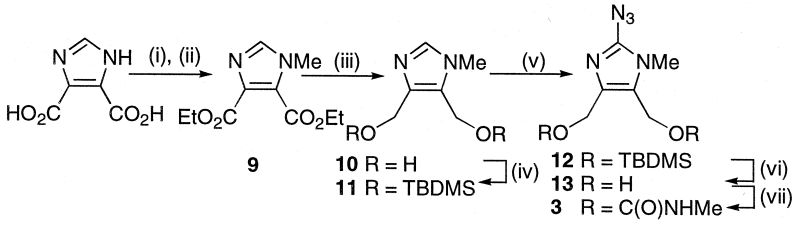
Nitro and sulphoxide substituents were selected as suitable electron-withdrawing substituents to place in conjugation to the methyl carbamate. Nitro groups are well documented as undergoing bioreduction under hypoxic conditions[15] and provide a large change in electron density [$\sigma_p(NO_2)=0.78$, $\sigma_p(NH_2)=-0.66$].[21] Precedent for the reductive elimination of 5-nitroimidazole carbamates exists in the mechanism of ronidazole[28] and examples of 5-nitroimidazole carbamates have been prepared.[3,29] A recent report demonstrated the fragmentation of analogous 2-nitroimidazolyl esters upon radiolytic reduction.[30] The fragmentation of a 4-nitro-5-methylimidazole quaternary salt of mechlorethamine by radiolytic reduction has also been recently reported.[31,32]

Analogous to the carmethizole analogues,[19] we expected a 2-sulphoxide group to deactivate the carbamate to displacement [$\sigma_p(S(O)Me)=0.49$, $\sigma_p(SMe)=0.00$].[21] Bioreduction of sulphoxides has been observed for a number of sulphoxide drugs,[33] most notably sulindac, a non-steroidal anti-inflammatory agent.[34,35]

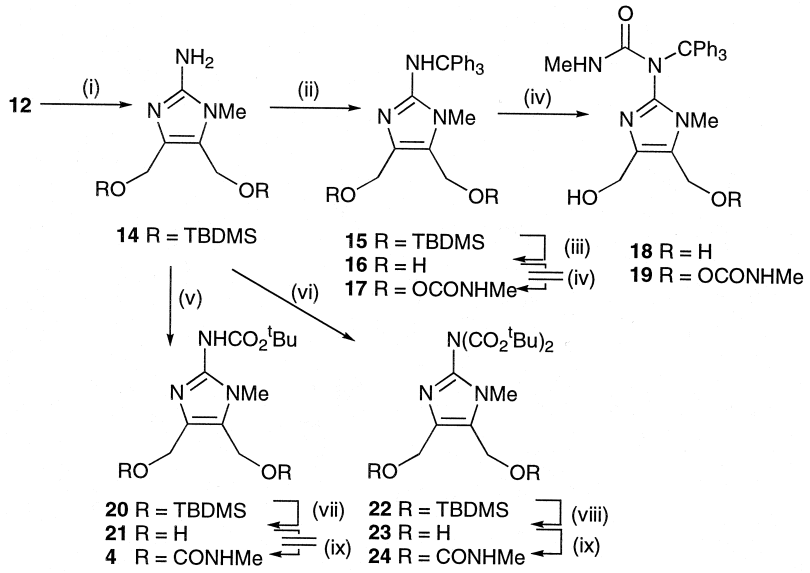


Synthesis of nitrogen analogues of carmethizole (**1**) was accomplished using a synthetic approach outlined previously in a preliminary account (Scheme 2).[20] Thus imidazole-4,5-dicarboxylic acid was esterified and *N*-alkylated to give the diester **9** (47% for 2 steps), which was reduced to the diol **10** with $LiAlH_4$(66%), and protected as the di-TBDMS ether **11** (74%). Lithiation and reaction with tosyl azide[36] (Caution) gave the azide **12** in 69% yield. Deprotection of the silyl ether groups with TBAF gave **13** (70%) and reaction with methyl isocyanate in the presence of catalytic dibutyltin diacetate gave the azide analogue **3** in 86% yield. Repeated attempts to reduce azide **3** to the corresponding amine **2** by catalytic hydrogenation using Pd/C, Pt/C, or Lindlar catalyst were unsuccessful, with rapid decomposition observed in all cases. Milder reduction conditions using Staudinger conditions[37] or PPh_3 were also unsuccessful, highlighting the inherent reactivity of imidazole 4,5-bis(methylcarbamates) with strongly electron-donating substituents at the 2-position.

In another attempt to prepare carbamate derivative **4**, the azide **12** was reduced by catalytic hydrogenation to give amine **14** (91%) (Scheme 3). Reaction with trityl chloride gave the protected aminoimidazole **15** (93%), which was deprotected to give diol **16** in 77% yield. Reaction of diol



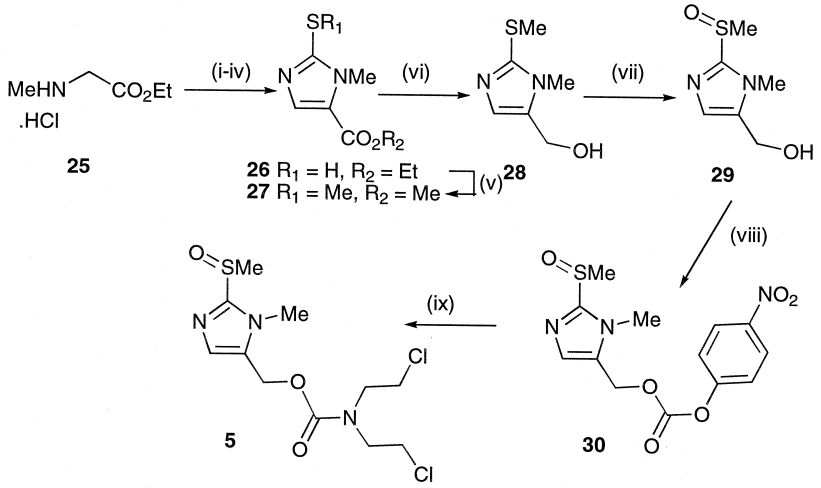
Scheme 2. Reagents: (i) $SOCl_2$, DMF, EtOH; (ii) K_2CO_3, MeI, DMF; (iii) $LiAlH_4$, THF; (iv) TBDMSCl, imidazole, DMF; (v) *n*-BuLi, tosylazide, THF; (vi) TBAF, THF; (vii) MeNCO, *n*-$Bu_2Sn(OAc)_2$, DCM.



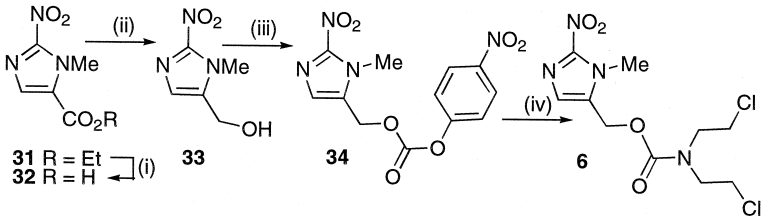
Scheme 3. Reagents: (i) H_2, Pd/C, EtOH; (ii) Ph_3CCl, Et_3N, DCM; TBAF, THF; (iv) MeNCO, *n*-$Bu_2Sn(OAc)_2$ DCM; (v) (*t*-$BuOCO)_2O$, DCM; (vi) (*t*-$BuOCO)_2O$, DMAP, DCM; (vii) TBAF, THF; (viii) HF pyridine, THF; (ix) MeNCO, *n*-$Bu_2Sn(OAc)_2$, DCM.

16 with methyl isocyanate and dibutyltin diacetate did not give the bis-carbamate **17**, but rather preferential reaction on nitrogen to give **18** (14%) and **19** (49%), as well as the starting material (17%). In an effort to deactivate the 2-amino group we elected to use the BOC protecting group. Reaction of amine **14** with di-*tert*-butyl dicarbonate in DCM gave mixtures of carbamate **20** (57%) and bis-carbamate **22** (22%), while addition of DMAP to the reaction gave clean conversion to bis-carbamate **22** (64%). Deprotection of **20** with TBAF gave the diol **21** (90%), which when reacted with MeNCO and dibutyltin diacetate gave a mixture of products rather than **4**. NMR analysis showed preferential acylation of the 2-carbamate over reaction at the 4-CH_2OH position. Attempts to deprotect the silyl groups of **22** with TBAF in THF, or KF in acetonitrile were unsuccessful. However, reaction of the bis-carbamate **22** with HF.pyridine gave the diol **23** in 81% yield, which was treated with MeNCO and dibutyltin diacetate to give imidodicarbamate **24** in 95% yield. Attempts to selectively remove the BOC groups using trifluoroacetic acid were not successful.

Synthesis of carbamate **5** required imidazole ring formation. Treatment of sarcosine ethyl ester hydrochloride[38] **25** with sodium formate in formic acid gave the *N*-formyl sarcosine derivative in 79% yield (Scheme 4). Formation of the C-formyl intermediate and ring formation with KSCN gave the 2-thioimidazole **26** (27%), which was methylated and transesterified to give **27** (96%). Reduction of ester **27** with Superhydride® (94%) and oxidation of sulphide **28** with MCPBA gave the 2-sulphinylimidazole methanol **29** in 98% yield. Formation of the carbonate **30** (48%) and displacement with bis-(2-chloroethyl)amine gave carbamate **5** in 52% yield.



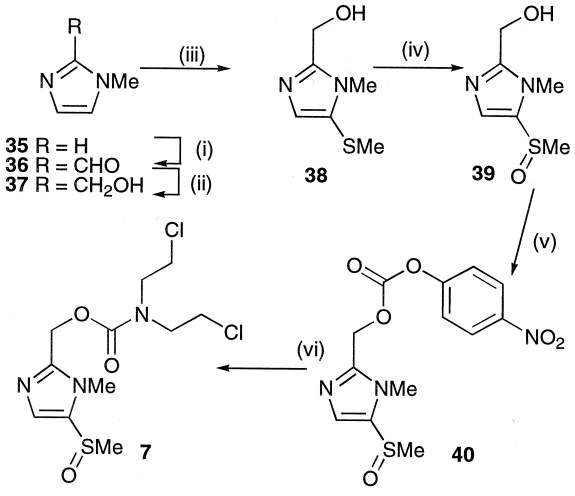
Scheme 4. Reagents: (i) HCO_2Na, HCO_2H; (ii) Ac_2O; (iii) NaOEt, HCO_2Et; (iv) KSCN; (v) K_2CO_3, MeI, DMF; (vi) Superhydride®, THF; (vii) MCPBA, DCM; (viii) $NO_2C_6H_4OCOCl$, pyridine, THF; (ix) $(ClCH_2CH_2)_2NH{\cdot}HCl$, pyridine.



Scheme 5. Reagents: (i) NaOH, H_2O; (ii) $NaBH_4$, EtOH; (iii) $NO_2C_6H_4OCOCl$, pyridine, THF; (iv) $(ClCH_2CH_2)NH.HCl$, pyridine.

The readily available ethyl 2-nitroimidazol-5-ylcarboxylate[39] (**31**) was hydrolysed under mild basic conditions to give the acid **32** quantitatively (Scheme 5). 2-Nitroimidazol-5-ylmethanol (**33**) was prepared by sodium borohydride reduction of the imidazolide[40] of the acid **32** in 68% yield. This represents a major improvement upon the literature procedure,[39] which used lithium borohydride reduction of the ester **31** to give **33** directly. In our hands, this procedure gave complex mixtures requiring tedious chromatography and gave poor yields (typically 5–10%) of the alcohol **33**. Activation of the alcohol **33** as the carbonate **34** (84%) and displacement with *N*,*N*-bis(2-chloroethyl)amine gave **6** in 74% yield.

Synthesis of 5-sulphoxide-2-carbamate **7** was achieved using sequential lithiation reactions.[41] Lithiation at the 2-position of *N*-methylimidazole **35** and reaction with DMF gave aldehyde **36** (80%), which was reduced to the alcohol **37** with $LiAlH_4$ in 90% yield (Scheme 6). Lithiation at the 5-position and reaction with methyldisulphide gave methyl sulphide **38** (51%), which was oxidised to the sulphoxide **39** with MCPBA in 90% yield. Activation of **39** as the carbonate **40** (81%) and displacement with *N*,*N*-bis(2-chloroethyl)amine gave the carbamate **7** in 80% yield.



Scheme 6. Reagents: (i) *n*-BuLi, DMF, THF; (ii) $LiAlH_4$, THF; (iii) *n*-BuLi, *t*-BuLi, Me_2S_2, THF; (iv) MCPBA, DCM; (v) $NO_2C_6H_4OCOCl$, pyridine, THF; (vi) $(ClCH_2CH_2)_2NH{\cdot}HCl$, pyridine.

Reaction of *N*-methyl 5-nitroimidazole[42] **41** with paraformaldehyde gave the alcohol **42** in 57% yield (Scheme 7). Activation of **42** as the carbonate **43** and displacement with *N*,*N*-bis(2-chloroethyl)amine gave the carbamate **8**[43] in 95% yield.

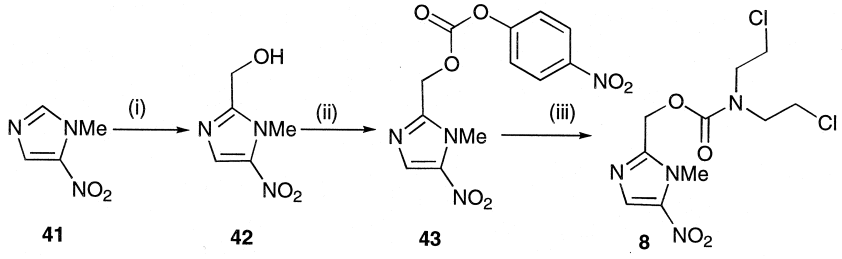
The cytotoxicities of the bis-hydroxymethylimidazoles **1** and **3** were determined under aerobic conditions against a panel of three cell lines (the Chinese hamster lines AA8 and UV4, and the murine mammary carcinoma EMT6) using a growth inhibition assay, which has been described in detail previously.[44] The UV4 cell line, a nucleotide excision repair (NER)-defective ERCC-1 mutant derived from AA8, is hypersensitive to agents whose cytotoxicity is due to bulky DNA adducts or cross-links,[45] and hence provides information on the mechanisms of cytotoxicity. Cells in log-phase growth were exposed to drug for 4 h in 96 well plates under aerobic conditions, and subsequent cell growth was measured after 72 h. IC_{50} values were calculated in each case (Table 1).

Carmethizole **1** showed an IC_{50} value of 178 μM against AA8 cells, and was 36-fold more toxic to UV4 cells in

Table 1. In vitro cytotoxicity of bis-hydroxylmethylimidazole prodrugs

Compound	Cytotoxicity (IC_{50})[a]			
	AA8	UV4	HF[b]	EMT6
1	178±34[c]	4.7±0.7	36±7	73±12
3	189±7	15.2±0.7	12±1	139±12

[a] Concentration (μM) for 50% inhibition of cell proliferation following 4 h exposure under aerobic conditions.
[b] Hypersensitivity Factor=IC_{50} AA8/IC_{50} UV4.
[c] Values are mean±SEM for replicate experiments.



Scheme 7. Reagents: (i) $(CHO)_n$, DMSO; (ii) $NO_2C_6H_4OCOCl$, pyridine, THF; $(ClCH_2CH_2)_2NH{\cdot}HCl$, pyridine.

Table 2. In vitro cytotoxicity of imidazolecarbamate mustard prodrugs

Compound	Cytotoxicity (IC_{50})[a]			
	AA8	UV4	HF[b]	EMT6
5	2290±70[c]	470±30	4.9±0.2	2170±170
6	590±70	153±12	4.4±0.9	500±8
7	2130±190	520±90	3.8±1.1	1070±170
8	1000±60	450±40	2.6±0.2	740±100
BCEA	110±30	6.1±2.0	36±6	46±7

[a] Concentration (μM) for 50% inhibition of cell proliferation following 18 h exposure under aerobic conditions.
[b] Hypersensitivity factor=IC_{50} AA8/IC_{50} UV4.
[c] Values are mean±SEM for replicate experiments.

vitro (HF of 36). This is in close agreement with previous results[17] ($HF_{(AA8/UV4)}$=37) and is consistent with formation of DNA cross-links being the major mechanism of cytotoxicity. Azide **3** shows similar potency against AA8 and EMT6 cells and an HF of 12, also consistent with DNA cross-linking and/or the formation of bulky DNA adducts. Compounds **1** and **3** were also evaluated against a panel of human tumour cell lines (MGH-U1 bladder carcinoma, SKOV3 ovarian carcinoma, BE and HT29 colon carcinoma; data not shown). The IC_{50} values for 4 h exposure to **1** ranged from 76–160 μM, compared to 190–280 μM for the 2-azido analogue **3**. The latter was no more than 2.9-fold less potent than **1** in any of the human (or rodent) cell lines. This differential was not considered sufficient to warrant further investigation of the azido derivative as a prodrug.

Aerobic cytotoxicity, measured as IC_{50} values, was also determined for the four imidazolecarbamate mustard prodrugs (the 2-methylsulphinylimidazole **5**, the 5-isomer **7**, and the 2- and 5-nitroimidazoles **6** and **8**) and for the expected alkylating product BCEA using 18 h drug exposures (Table 2). BCEA showed only modest cytotoxic potency, with IC_{50} values in the range 46–110 μM in the repair-competent rodent cells, and 104–214 μM in the above panel of human tumour cell lines (data not shown). The HF ratio for BCEA was 36. All the carbamate prodrugs were less cytotoxic than BCEA (20 fold for **5**, 19 fold for **7**, 5-fold for **6** and 9-fold for **8** against AA8 cells, with even higher differentials for the other cell lines), showing that formation of these carbamates does deactivate the mustard effectively. All of these compounds showed low HF values (2–5), suggesting that they are sufficiently deactivated to suppress DNA alkylation.

Given these large differentials between the imidazolecarbamates and BCEA, the possibility of activation of these potential prodrugs by endogenous mammalian reductases under anoxic conditions was assessed. Cytotoxic potency was evaluated against plateau-phase AA8 cells, using a clonogenic assay, with potency quantified as the concentration×time to reduce survival to 10% of controls (CT_{10}; Table 3). For BCEA, the aerobic CT_{10} was 0.88 mM-h for AA8 cells, with a 13-fold greater sensitivity for the ERCC1 mutant UV4, which is hypersensitive to bulky DNA monoadducts and crosslinks.[45,46] In contrast, the ERCC2 mutant UV5, which is defective in NER of bulky DNA monoadducts but not crosslinks,[45,46] showed no significant

Table 3. Cytotoxicity of imidazole carbamate mustard prodrugs against plateau phase cells under aerobic and anoxic conditions, determined by clonogenic assay of continuously stirred and gassed cell suspensions

Compound	Cell line	CT_{10}[a], air (mM-h)	CT_{10}, N_2 (mM-h)	HCR[b]
BCEA	AA8	0.88	0.66±0.01[c]	1.3
	UV4[d]	0.068±0.011	0.044±0.008	1.5±0.1
	UV5[e]	0.83±0.06	0.72±0.03	1.2±0.1
5	AA8	4.1±0.1	4.4	0.93
6	AA8	>2.5[f]	1.4±0.3	>1.8
	UV4	>2.5[f]	1.1±0.2	>2.3
7	AA8	7.2	6.3	1.1
8	AA8	17.6	10.4	1.7

[a] Concentration×time required to reduce cell survival to 10% of controls.
[b] Hypoxic cytotoxicity ratio=CT_{10}, air/CT_{10}, N_2.
[c] Errors are range or SEM for 2–4 separate experiments.
[d] ERCC1 mutant derived from AA8.
[e] ERCC2 mutant derived from AA8.
[f] Non-toxic at solubility limit (0.5 mM) for 5 h under aerobic conditions.

hypersensitivity relative to the parental AA8 line. The differential sensitivity of UV4 relative to both UV5 and AA8 confirms that cytotoxicity of BCEA is due to DNA crosslinks. As in the IC_{50} assay, the imidazolecarbamate mustards **5**–**8** were all considerably less toxic (4.7–20-fold) than BCEA towards aerobic AA8 cells in the clonogenic assay. However, little hypoxic selectivity was evident in these potential bioreductive prodrugs; with the exception of **6**, hypoxic cytotoxicity ratios (HCR) were in the range 0.93–1.7 and were little different than that for BCEA itself (HCR 1.3). In the case of the 2-nitroimidazole **6**, cell killing could be detected under anoxic but not aerobic conditions at the solubility limit (0.5 mM) for 5 h, with an HCR of >1.8. However, UV4 cells were not significantly more sensitive to **6** than were AA8 cells under anoxia. This clearly indicates that the hypoxic cytotoxicity of **6** is not due to bioreductive release of BCEA, or to any other DNA crosslinking metabolite. Given the modest cytotoxic potency of BCEA, it is probable that the hypoxic cytotoxicity of **6** is due to reactive intermediates derived from reduction of the nitro group itself, as for simple (non-alkylating) 2-nitroimidazoles such as misonidazole.[47,48]

In conclusion, synthetic difficulties precluded the use of 2-carbamates as prodrugs of amino analogues of carmethizole itself. However, the above data suggest that methylsulphinyl- and nitro-substituted imidazole carbamates might have potential as trigger units for release of secondary amine cytotoxins from prodrugs, although further development of this concept will require release of cytotoxic effectors more potent than the nitrogen mustard BCEA. In an extension of this approach we have recently reported that an analogue of **6** in which reduction of a 2-nitroimidazol-5-ylmethyl carbamate releases a potent amino-*seco*-CBI-TMI cytotoxic effector, and that this has promise both as a hypoxia-selective cytotoxin and as a substrate for nitroreductase-mediated GDEPT.[49]

Experimental

General procedures

Analyses were carried out in the Microchemical Laboratory,

University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal 2300 melting point apparatus. IR spectra were recorded on a Midac FT-IR as KBr discs, unless otherwise stated. NMR spectra were obtained on a Bruker AM-400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C spectra. Spectra were obtained in $CDCl_3$ unless otherwise specified, and are referenced to Me_4Si. Chemical shifts and coupling constants were recorded in units of ppm and Hz, respectively. Mass spectra were determined on a VG-70SE mass spectrometer using an ionising potential of 70 eV at a nominal resolution of 1000. High resolution spectra were obtained at nominal resolutions of 3000, 5000 or 10 000 as appropriate. All spectra were obtained as electron impact (EI) using PFK as the reference unless otherwise stated. Solutions in organic solvents were dried with anhydrous Na_2SO_4. Solvents were evaporated under reduced pressure on a rotary evaporator. Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck 60 F_{254}) with visualisation of components by UV light (254 nm) or exposure to I_2. Column chromatography was carried out on silica gel, (Merck 230–400 mesh). All compounds designated for biological testing were analysed at >99% purity by reverse phase HPLC using a Philips PU4100 liquid chromatograph, a Phenomenex BondClone 10-C18 stainless steel column (300×3.9 mm i.d.) and a Philips PU4120 diode array detector. Chromatograms were run using various gradients of aqueous (1 M NaH_2PO_4, 0.75 M heptanesulphonic acid, 0.5 M dibutylammonium phosphate, and MilliQ water in a 1:1:1:97 ratio) and organic (80% MeOH/MilliQ water) phases. DCM refers to dichloromethane; THF refers to tetrahydrofuran dried over sodium benzophenone ketyl; DMF refers to dry dimethylformamide; DMSO refers to dimethylsulphoxide; EtOAc refers to ethyl acetate; ether refers to diethyl ether; light petroleum refers to petroleum ether, boiling range 40–60°C; MeOH refers to methanol; EtOH refers to ethanol. All solvents were freshly distilled.

Synthesis of 3

Diethyl 1-methylimidazol-1*H*-yl-4,5-dicarboxylate (9). Thionyl chloride (23.4 mL, 320 mmol) was added dropwise to a stirred suspension of imidazole-4,5-dicarboxylic acid (10.0 g, 64 mmol) and DMF (3 drops) in EtOH (200 mL) and the mixture stirred at 80°C for 48 h. The solvent was removed and the residue dissolved in water (100 mL) and the pH adjusted to 9 with aq. KOH. The solution was extracted with EtOAc (4×100 mL), the combined extracts dried and the solvent removed to give crude diethyl imidazole-4,5-carboxylate (8.39 g, 62%), mp (EtOAc) 152.5–155.0°C (lit.[50] mp 151–152°C); ^{1}H NMR δ 9.20 (br s, 1H, NH), 7.90 (s, 1H, H-2), 4.38 (q, J=7.1 Hz, 4H, $2CH_2O$), 1.36 (t, J=7.1 Hz, 6H, $2CH_3$). A mixture of ester (8.0 g, 37.7 mmol), K_2CO_3 (5.5 g, 39.6 mmol) and MeI (2.82 mL, 45.2 mmol) in DMF (50 mL) was stirred at 40°C for 24 h. The solvent was removed and the residue partitioned between EtOAc (200 mL) and water (200 mL). The organic fraction was washed with water (3×50 mL), brine (50 mL), dried and the solvent removed to give **9** (6.48 g, 76%) as an oil, IR (thin film) ν 1717, 1506 1377 and 1277 cm^{-1}; ^{1}H NMR δ 7.52 (s, 1H, H-2), 4.39 (2q, J=7.1 Hz, 4H, $2CH_2O$), 3.85 (s, 3H, NCH_3) and 1.39 (t, J=7.1 Hz, 6H, $2CH_3$); ^{13}C NMR δ 162.5 (CO_2), 159.9 (CO_2), 139.9 (C-2), 137.4 (C-4), 125.1 (C-5), 61.6 (CH_2O), 61.1 (CH_2O), 34.1 (NCH_3), 14.1 (CH_3) and 13.9 (CH_3); MS m/z 226 (M^+, 30%), 181 (60), and 153 (100); HRMS calcd for $C_{10}H_{14}N_2O_4$ (M^+) m/z 226,0953, found 226.0960.

4,5-Bis(hydroxymethyl)-1-methyl-1*H*-imidazole (10). A solution of diester **9** (5.17 g, 22.8 mmol) in THF (20 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (2.60 g, 68.5 mmol) in THF (50 mL) at 0°C. The mixture was stirred for 5 h at 20°C and quenched with water (5 mL) (Caution), 2 M NaOH (2 mL) and water (5 mL). The suspension was filtered through a pad of Celite and the residue extracted in a Soxhlet apparatus with THF. The combined organic fraction was evaporated to give **10** (2.15 g, 66%) as white crystals, mp (THF) 124.5–126.5°C; IR ν 3329, 1518, 1356, 1221 cm^{-1}; ^{1}H NMR [$(CD_3)_2SO$] δ 7.46 (s, 1 H, H-2), 4.90 (br t, J=5.0 Hz, 1H, OH), 4.63 (br t, J=5.1 Hz, 1H, OH), 4.45 (d, J=5.0 Hz, 2H, CH_2O), 4.30 (d, J=5.0 Hz, 2H, CH_2O), and 3.59 (s, 3H, NCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 139.2 (C-4), 136.9 (C-2), 128.4 (C-5), 56.3 (CH_2O), 51.3 (CH_2O), and 31.1 (NCH_3); Anal. calcd for $C_6H_{10}N_2O_2$: C, 50.7; H, 7.1; N, 19.7; found C, 50.4; H, 7.2; N, 19.5%.

4,5-Bis({*tert*-butyldimethylsilyloxy}methyl)-1-methyl-1*H*-imidazole (11). A solution of TBDMSCl (12.9 g, 85.5 mmol) in DMF (20 mL) was added dropwise to a stirred solution of diol **10** (3.04 g, 21.4 mmol) and imidazole (3.64 g, 53.5 mmol) in DMF (50 mL) at 5°C under N_2. The solution was stirred at 20°C for 96 h and the solvent removed. The residue was partitioned between EtOAC (200 mL) and water (200 mL). The organic fraction was washed with dil. HOAc (50 mL), water (3×50 mL), brine (50 mL), dried and the solvent removed. The residue was chromatographed, eluting with 10% MeOH/EtOAc, to give **11** as a clear oil (5.84 g, 74%), which solidified on standing, mp 134–136°C; IR ν 1516, 1467 and 1254 cm^{-1}; ^{1}H NMR δ 7.29 (s, 1H, H-2), 4.71 (s, 2H, CH_2O), 4.64 (s, 2H, CH_2O), 3.63 (s, 3H, NCH_3), 0.91 (s, 18H, $2OSiC(CH_3)_3$), and 0.11 (s, 12H, $2OSi(CH_3)_2$); ^{13}C NMR δ 139.8 (C-4), 136.6 (C-2), 128.5 (C-5), 60.3 (CH_2O), 53.4 (CH_2O), 31.5 (NCH_3), 25.9 ($2OSiC(\mathit{C}H_3)_3$), 18.3 ($2OSi\mathit{C}(CH_3)_3$), and −3.0 ($2OSi(CH_3)_2$); MS (CI, NH_3) m/z 371 (MH^+, 100%), 355 (5), and 313 (20); HRMS (CI, NH_3) calcd for $C_{18}H_{39}N_2O_2Si_2$ (MH^+) m/z 371.2550, found 371.2549.

2-Azido-4,5-bis(*tert*-butyldimethylsilyloxymethyl)-1-methyl-1*H*-imidazole (12). A solution of *n*-BuLi (2.5 M in hexanes) (4.5 mL, 11.3 mmol) was added dropwise to a stirred solution of imidazole (4.0 g, 10.8 mmol) in THF (100 mL) at −78°C. The solution was stirred for 10 min, a solution of tosyl azide[36] (Caution) (2.43 g, 11.9 mmol) in THF (15 mL) added dropwise and the solution stirred at −78°C for 30 min. The solution was allowed to warm to 20°C over 1 h and the reaction quenched with saturated aq. NH_4Cl solution (4 mL) (Caution). The mixture was poured into saturated aq. NH_4Cl solution (100 mL) and extracted with EtOAc (2×100 mL). The organic fraction was dried, the solvent removed, and the residue was chromatographed, eluting with 10% EtOAc/petroleum ether, to give the azide **12** (3.08 g, 69%) as a clear oil, IR ν (thin film) 2132, 1505 and 1256 cm^{-1}; ^{1}H NMR δ 4.67 (s, 2H, CH_2O), 4.61 (s, 2H,

CH_2O), 3.38 (s, 3H, NCH_3), 0.91 (s, 9H, $OSiC(CH_3)_3$), 0.88 (s, 9H, $OSiC(CH_3)_3$), 0.10 (s, 6H, $OSi(CH_3)_2$), and 0.06 (s, 6H, $OSi(CH_3)_2$); ^{13}C NMR δ 140.0 (C-2), 135.9 (C-4), 126.8 (C-5), 59.2 (CH_2O), 54.2 (CH_2O), 29.6 (NCH_3), 26.1 ($OSiC(\mathit{C}H_3)_3$), 25.8 ($OSiC(\mathit{C}H_3)_3$), 18.5 ($OSi\mathit{C}(CH_3)_3$, 18.1 ($OSi\mathit{C}(CH_3)_3$, −5.2 ($OSi(CH_3)_2$), and −5.4 ($OSi(CH_3)_2$); MS *m/z* 411 (M^+, 1%), 383 (4), 354 (10) and 326 (100); HRMS calcd for $C_{18}H_{37}N_5O_2Si_2$ (M^+) *m/z* 411.2486, found 411.2485.

2-Azido-4,5-bis(hydoxymethyl)-1-methyl-1*H*-imidazole (13). A solution of TBAF (1 M in THF) (10.5 mL, 10.5 mmol) was added dropwise to a stirred solution of bis(silyl ether) **12** (2.06 g, 5 mmol) in THF (80 mL) at −10°C and the solution stirred for 1 h. The solvent was removed and the residue chromatographed, eluting with a gradient (0–15%) MeOH/EtOAc, to give diol **13** (0.64 g, 70%) as a white solid, mp (EtOAc) 104°C (dec.); IR ν 3345, 3221, 2135, 1508 and 1259 cm^{-1}; ^1H NMR δ 4.93 (br s, 1H, OH), 4.75 (br s, 1H, OH), 4.41 (s, 2H, CH_2O), 4.27 (s, 2H, CH_2O), and 3.35 (s, 3H, NCH_3); ^{13}C NMR δ 138.3 (C-2), 136.7 (C-4), 128.6 (C-5), 55.6 (CH_2O), 51.3 (CH_2O), and 29.2 (NCH_3); MS *m/z* 183 (M^+, 15%), 155 (20), 124 (35) and 42 (100); HRMS calcd for $C_6H_9N_5O_2$ (M^+) *m/z* 183.0756, found 183.0757.

[2-Azido-1-methyl-4-({[(methylamino)carbonyl]oxy}-methyl)-1*H*-imidazol-5-yl]methyl methyl-carbamate (3). Dibutyltindiacetate (3 drops) was added to a stirred suspension of diol **13** (0.64 g, 3.5 mmol) and MeNCO (0.45 mL, 7.7 mmol) in DCM (30 mL) and the mixture stirred at 20°C for 16 h. The solvent was removed and the residue chromatographed, eluting with a gradient (0–10%) MeOH/EtOAc, to give the azido bis-carbamate **3** (0.89 g, 86%) as a pale yellow solid, mp (EtOAc) 126°C (dec.); IR ν 3424, 3368, 2172, 2120, 1707, 1560 and 1273 cm^{-1}; ^1H NMR δ 5.13 (s, 2H, CH_2O), 5.04 (s, 2H, CH_2O), 4.78 (br s, 1H, NH), 4.75 (br s, 1H, NH), 3.38 (s, 3H, NCH_3), and 2.77–2.80 (m 6H, $2CH_3$); ^{13}C NMR δ 156.8 (NCO_2), 156.4 (NCO_2), 141.6 (C-2), 134.9 (C-4), 124.9 (C-5), 59.1 (CH_2O), 54.8 (CH_2O), 29.6 (NCH_3), 27.6 (CH_3), and 27.5 (CH_3); MS (DEI) *m/z* 297 (M^+, 5%), 269 (5), 223 (20), 155 (30) and 42 (100); HRMS (DEI) calcd for $C_{10}H_{15}N_7O_4$ (M^+) *m/z* 297.1189, found 297.1188; Anal. calcd for $C_{10}H_{15}N_7O_4$: C, 40.4; H, 5.1; N, 33.0; found C, 40.1; H, 5.2; N, 32.4%.

4,5-Bis(*tert*-butyldimethylsilyloxymethyl)-1-methyl-1*H*-imidazol-2-ylamine (14). A solution of azide **12** (2.0 g, 4.86 mmol) and Pd/C (0.1 g) in EtOH (50 mL) was stirred at 20°C under H_2 (60 psi) for 1 h. The suspension was filtered through celite, washed with EtOH (2×10 mL) and the solvent removed to give amine **14** (1.71 g, 91%) as a colourless solid, mp 128–129.5°C; IR ν 3380, 3314, 1655, 1558 and 1256 cm^{-1}; ^1H NMR δ 4.63 (s, 2H, CH_2O), 4.52 (s, 2H, CH_2O), 3.87 (br s, 2H, NH_2), 3.38 (s, 3H, NCH_3), 0.91 (s, 9H, $OSiC(CH_3)_3$), 0.87 (s, 9H, $OSiC(CH_3)_3$), 0.09 (s, 6H, $OSi(CH_3)_2$), and 0.05 (s, 6H, $OSi(CH_3)_2$); ^{13}C NMR δ(147.4 (C-2), 133.3 (C-4), 123.6 (C-5), 59.0 (CH_2O), 54.3 (CH_2O), 29.3 (NCH_3), 26.1 ($OSiC(\mathit{C}H_3)_3$), 25.8 ($OSiC(\mathit{C}H_3)_3$), 18.5 ($2OSi\mathit{C}(CH_3)_3$), −5.2 ($OSi(CH_3)_2$), and −5.3 ($OSi(CH_3)_2$); MS *m/z* 385 (M^+, 5%), 370 (8) and 328 (100); HRMS calcd for $C_{18}H_{39}N_3O_2Si_2$ (M^+) *m/z* 385.2581, found 385.2577; Anal. calcd for $C_{18}H_{39}N_3O_2Si_2$ C, 56.05; H, 10.2; N, 10.9; found C, 55.7; H, 10.0; H, 10.7%.

***N*-[4,5-Bis(*tert*-butyldimethylsilyloxymethyl)-1-methyl-1*H*-imidazol-2-yl]-*N*-tritylamine (15).** A solution of trityl chloride (0.71 g, 2.53 mmol) in DCM (10 mL) was added dropwise to a stirred solution of amine **14** (0.93 g, 2.41 mmol) and Et_3N (0.37 mL, 2.65 mmol) in DCM (50 mL) at 20°C under N_2. The solution was stirred for 16 h, diluted with DCM (100 mL), washed with water (3×60 mL), brine (50 mL), dried and the solvent removed. The residue was chromatographed, eluting with 20% EtOAc/petroleum ether, to give tritylamine **15** (1.40 g, 93%) as a white solid, mp (EtOAc/petroleum ether) 113–116°C; IR ν 3254, 1493, 1471 and 1254 cm^{-1}; ^1H NMR δ 7.27–7.30 (m, 6H, H-3′, H-5′), 7.21–7.25 (m, 6H, H-2′, H-6′), 7.16–7.18 (m, 3H, H-4′), 4.51 (s, 2H, CH_2O), 4.49 (br s, 1H, NH), 4.39 (s, 2H, CH_2O), 2.92 (s, 3H, NCH_3), 0.84 (s, 9H, $OSiC(CH_3)_3$), 0.82 (s, 9H, $OSiC(CH_3)_3$), −0.01 (s, 6H, $OSi(CH_3)_2$), and −0.04 (s, 6H, $OSi(CH_3)_2$; ^{13}C NMR δ 145.5 (3, C-1′), 145.5 (C-2), 134.5 (C-4), 129.3 (6, C-2′ C-6′), 127.5 (6, C-3′, C-5′), 126.8 (3, C-4′), 124.3 (C-5), 72.6 (CPh_3), 59.0 (CH_2O), 54.4 (CH_2O), 28.9 (NCH_3), 26.0 ($OSiC(\mathit{C}H_3)_3$), 25.8 ($OSiC(\mathit{C}H_3)_3$), 18.3 ($OSi\mathit{C}(CH_3)_3$), 18.1 ($OSi\mathit{C}(CH_3)_3$), −5.1 ($OSi(CH_3)_2$), and −5.3 ($OSi(CH_3)_2$); MS (DEI) *m/z* 627 (M^+, 4%), 550 (1), 495 (15) and 243 (100); HRMS (DEI) calcd for $C_{37}H_{53}N_3O_2Si_2$ (M^+) *m/z* 627.3676, found 627.3646; Anal. calcd for $C_{37}H_{53}N_3O_2Si_2$: C, 70.8; H, 8.5; N, 6.7; found C, 70.9; H, 8.8; N, 6.7%.

***N*-[4,5-Bis(hydroxymethyl)-1-methyl-1*H*-imidazol-2-yl]-*N*-tritylamine (16).** A solution of TBAF (1 M in THF) (4.6 mL, 4.6 mmol) was added dropwise to a stirred solution of tritylamine **15** (1.38 g, 2.2 mmol) in THF (50 mL) at 5°C and the solution was stirred for 2 h. The solvent was removed and the residue chromatographed, eluting with a gradient (0–10%) of MeOH/EtOAc, to give the diol **16** (0.68 g, 77%) as a white solid, mp (MeOH) 168°C (dec.); IR ν 3431, 3287, 2944, 1603, 1541 and 1448 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 7.33–7.38 (m, 6H, H-3′, H-5′), 7.20–7.25 (m, 6H, H-2′, H-6′), 7.14–7.18 (m, 3H, H-4′), 6.16 (s, 1H, NH), 4.57 (t, J=5.1 Hz, 1H, OH), 4.26 (d, J=5.1 Hz, 2H, CH_2O), 3.95–4.02 (m, 3H, CH_2O, OH), 3.25 (s, 3H, NCH_3), and 2.53 (s, 3H, $C\mathit{H}_3OH$); ^{13}C NMR [$(CD_3)_2SO$] δ 146.2 (3, C-1′), 145.5 (C 2), 134.0 (C-4), 129.0 (6, C-3′, C-5′), 127.1 (6, C-2′, C-6′), 124.0 (3, C-4′), 124.0 (C-5), 70.3 (CPh_3), 56.1 (CH_2O), 51.7 (CH_2O), 48.4 (CH_3OH) and 28.8 (NCH_3); MS (DEI) *m/z* 399 (M^+, 1%), 281 (15), 304 (10) and 243 (100); HRMS (DEI) calcd for $C_{25}H_{25}N_3O_2$ (M^+) *m/z* 399.1947, found 399.1943; Anal. calcd for $C_{25}H_{25}N_3O_2 \cdot 1/2CH_3OH$: C, 73.7; H, 6.6; N, 10.1; found C, 73.3; H, 6.7; N, 10.1%.

Reaction of diol 16 with methylisocyanate. Dibutyltindiacetate (3 drops) was added to a stirred solution of diol **16** (0.64 g, 1.6 mmol) and MeNCO (0.20 mL, 3.36 mmol) in DCM (30 mL) at 20°C and the solution stirred for 16 h. The solvent was removed and the residue chromatographed, eluting with a gradient (0–10%) of MeOH/EtOAc, to give: (i) *N*-[4,5-bis(hydroxymethyl)-1-methyl-1*H*-imidazol-2-yl]-*N*′-methyl-*N*-tritylurea (**18**) (0.10 g, 14%) ^1H NMR δ 7.15–7.28 (m, 15H, H_{arom}), 4.83 (br q, J=4.8 Hz, 1H, NH), 4.78 (s, 2H, CH_2O), 4.62 (br s, 1H, OH), 4.39 (s, 2H,

CH_2O), 4.12 (br s, 1H, OH), 2.92 s, 3H, NCH_3) and 2.67 (d, J=4.8 Hz, 3H, CH_3); ^{13}C NMR δ 157.7 (NCO_2), 146.0 (C-2), 145.3 (3, C-1′), 130.8 (C-4), 129.3 (6, C-3′, C-5′), 128.0 (C-5), 127.6 (6, C-2′, C-6′), 126.9 (3, C-4′), 72.9 (CPh_3), 59.4 (CH_2O), 52.9 (CH_2O), 28.8 (NCH_3) and 27.3 ($NHCH_3$); (ii) starting material **16** (0.11 g, 17%) spectroscopically identical to sample prepared above; and (iii) {4-(hydroxymethyl)-1-methyl-2-[[(methylamino)carbonyl]-(trityl)-amino]-1*H*-imidazol-5-yl}methyl methyl-carbamate (0.40 g, 49%) (**19**) as a white solid, mp (EtOAc) 186–187°C; IR ν 3326, 3069, 1738, 1638 ,1545 and 1377 cm^{-1}; ^1H NMR δ 7.30–7.33 (m, 6H, H_{arom}), 7.23–7.27 (m, 9H, H_{arom}), 4.61 (s, 1H, OH), 4.50 (q, J=4.6 Hz, 1H, NH), 4.39 (br s, 1H, NH), 4.33 (s, 2H, CH_2O), 4.26 (s, 2H, CH_2O), 3.00 (s, 3H, NCH_3), 2.78 (d, J=4.6 Hz, 3H, NCH_3), and 2.60 (s, 3H, NCH_3); ^{13}C NMR δ 158.7 (NCO_2), 154.3 (NCO_2), 146.3 (C-2), 145.3 (3, C-1′), 136.4 (C-4), 129.1 (6, C-3′, C-5′), 127.7 (6, C-2′, C-6′), 126.8 (3, C4′), 119.9 (C-5), 72.6 (CPh_3), 56.9 (CH_2O), 40.0 (CH_2O), 32.2 (NCH_3), 29.1 (NCH_3) and 27.6 (NCH_3); Anal. calcd for $C_{29}H_{31}N_5O_3$: C, 70.0, H, 6.3; N, 14.1; found C, 69.9; H, 6.8; N, 14.4%.

Reaction of amine 14 with di-*tert*-butyldicarbonate. A solution of di-*tert*-butyldicarbonate (1.06 g, 4.86 mmol) in DCM (10 mL) was added to a stirred solution of amine (1.25 g, 3.24 mmol) in DCM (50 mL) at 20°C and stirred for 2 h. The solution was diluted with DCM (150 mL), washed with water (200 mL), brine (50 mL), dried and the solvent removed. The residue was chromatographed, eluting with 30% EtOAc/petroleum ether, to give: (i) *tert*-butyl 4,5-bis(*tert*-butyldimethylsilyloxymethyl)-1-methyl-1*H*-imidazol-2-ylcarbamate (**20**) (0.90 g, 57%) as a white solid, mp 100–101.5°C; IR ν 2957, 2930, 1719, 1570 and 1283 cm^{-1}; ^1H NMR δ 10.96 (br s, 1H, NH), 4.56 (br s, 2H, CH_2O), 4.50 (s, 2H, CH_2O), 3.46 (s, 3H, NCH_3), 1.49 (s, 9H, $OC(CH_3)_3$), 0.88 (s, 9H, $OSiC(CH_3)_3$), 0.85 (s, 9 H, $OSiC(CH_3)_3$), 0.07 (s, 6H, $OSi(CH_3)_2$), and 0.04 (s, 6H, $OSi(CH_3)_2$); ^{13}C NMR δ 154.0 (NCO_2), 146.4 (C-2), 138.5 (C-4), 127.8 (C-5), 83.5 (O*C*(CH3)3), 55.4 (CH_2O), 53.7 (CH_2O), 29.4 (NCH_3), 28.4 ($OC(CH_3)_3$), 25.9 ($OSiC(CH_3)_3$), 25.7 ($OSiC(CH_3)_3$), 18.3 ($OSiC(CH_3)_3$), 18.1 ($OSiC(CH_3)_3$), −5.3 ($OSi(CH_3)_2$), and −5.4 ($OSi(CH_3)_2$); Anal calcd for $C_{23}H_{47}N_3O_4Si_2$: C, 56.9; H, 9.75; N, 8.65; found C, 56.9; H, 10.0; N, 8.69%; and (ii) di(*tert*-butyl) 4,5-bis(*tert*-butyldimethylsilyloxymethyl)-1-methyl-1*H*-imidazol-2-ylimidodicarbonate (**22**) (0.44 g, 23%) as a white solid, mp (EtOAc) 166–168°C; IR ν 1767, 1655, 1576, 1340 and 1248 cm^{-1}; ^1H NMR δ 4.68 (s, 2H, CH_2O), 4.51 (s, 2H, CH_2O), 3.38 (s, 3H, NCH_3), 1.60 (s, 9H, $OC(CH_3)_3$), 1.50 (s, 9H, $OC(CH_3)_3$), 0.88 (s, 9H, $OSiC(CH_3)_3$), 0.85 (s, 9H, $OSiC(CH_3)_3$), 0.09 (s, 6H, $OSi(CH_3)_2$) and 0.04 (s, 6H, $OSi(CH_3)_2$); ^{13}C NMR δ 160.4 (NCO_2), 149.4 (NCO_2), 147.3 (C-2), 122.8 (C-4), 121.4 (C-5), 85.2 ($OC(CH_3)_3$), 77.7 ($OC(CH_3)_3$), 54.3 (CH_2O), 53.4 (CH_2O), 30.0 (NCH_3), 28.7 ($OC(CH_3)_3$), 27.8 ($OC(CH_3)_3$), 25.9 ($OSiC(CH_3)_3$), 25.7 ($OSiC(CH_3)_3$), 18.4 ($OSiC(CH_3)_3$), 18.1 ($OSiC(CH_3)_3$), −5.2 ($OSi(CH_3)_2$), and −5.5 ($OSi(CH_3)_2$); Anal calcd for $C_{28}H_{55}N_3O_6Si_2$: C, 57.4; H, 9.5; N, 7.2; found C, 57.4; H, 9.5; N, 7.0%.

Di(*tert*-butyl) 4,5-bis(*tert*-butyldimethylsilyloxymethyl)-1-methyl-1*H*-imidazol-2-ylimidodicarbonate (22). Compound **22** was also prepared by adding a solution of di-*tert*-butyl dicarbonate (2.4 g, 11.0 mmol) to a stirred solution of amine **14** (1.7 g, 4.4 mmol) and DMAP (0.1 g, 0.9 mmol) in DCM (100 mL) at 20°C and the solution stirred for 16 h. Workup as above gave **22** (1.66 g, 64%) as a white solid, mp (EtOAc) 166–167°C, spectroscopically identical to the above sample.

***tert*-Butyl 4,5-bis(hydroxymethyl)-1-methyl-1*H*-imidazol-2-ylcarbamate (21).** A solution of TBAF (1 M in THF) (3.7 mL, 3.7 mmol) was added slowly to a stirred solution of carbamate **20** (0.82 g, 1.69 mmol) in THF (30 mL) at 0°C and stirred for 1 h. The solvent was removed and the residue chromatographed, eluting with a gradient (0–10%) of MeOH/EtOAc, to give carbamate **21** (0.39 g, 90%) as a white solid, mp (MeOH/EtOAc) 200°C (dec.); IR ν 3345, 3169, 1728, 1576, 1462 and 1248 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 9.10 (br s, 1H, OCONH), 4.92 (br s, 1H, OH), 4.64 (br s, 1H, OH), 4.41 (d, J=4.3 Hz, 2H, CH_2O), 4.27 (d, J=4.5 Hz, 2H, CH_2O), 3.34 (s, 3H, NCH_3), 3.30 (br s, 3H, CH_3OH), and 1.43 (s, 9 H, $OC(CH_3)_3$); ^{13}C NMR [$(CD_3)_2SO$] δ 154.0 ($NHCO_2$), 145.8 (C-2), 138.0 (C-4), 127.0 (C-5), 79.5 ($OC(CH_3)_3$, 55.8 (CH_2O), 51.4 (CH_2O), 48.5 (CH_3OH), 29.4 (NCH_3) and 28.0 ($OC(CH_3)_3$); Anal calcd for $C_{11}H_{19}N_3O_4 \cdot 0.5CH_3OH$: C, 50.5; H, 7.7; N, 15.4; found C, 50.6; H, 7.6; N, 15.5%.

Di(*tert*-butyl) 4,5-bis(hydroxymethyl)-1-methyl-1*H*-imidazol-2-ylimidodicarbonate (23). HF-pyridine (10 drops) was added to a stirred solution of bis(silylether) **22** (0.63 g, 1.1 mmol) in THF (50 mL) at 20°C and the solution stirred for 16 h. The solvent was removed and the residue chromatographed, eluting with 20% MeOH/EtOAc, to give the diol **23** (0.32 mg, 81%) as a white solid, mp (EtOAc) 177–178°C; IR ν 3246, 1769, 1732, 1516 and 1371 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 4.97 (t, J=5.4 Hz, 1H, OH), 4.70 (t, J=5.4 Hz, 1H, OH), 4.46 (d, J=5.4 Hz, 2H, CH_2O), 4.70 (d, J=5.4 Hz, 2H, CH_2O), 3.34 (s, 3H, NCH_3), and 1.41 (s, 18H, $2OC(CH_3)_3$); ^{13}C NMR [$(CD_3)_2SO$] δ 150.1 ($2CO_2$), 136.6 (C-2), 136.5 (C-4), 128.5 (C-5), 83.2 ($2C(CH_3)_3$), 56.0 (CH_2O), 51.6 (CH_2O), 29.3 (NCH_3) and 27.3 ($2OC(CH_3)_3$); Anal calcd for $C_{16}H_{27}N_3O_6$: C, 53.8; H, 7.6; N, 11.8; found C, 53.6; H, 7.55, N, 11.8%.

Di(*tert*-butyl) 1-methyl-4,5-bis({[(methylamino)carbonyl]-oxy}methyl)-1*H*-imidazol-2-ylimidodicarbon-ate (24). Dibutyltindiacetate (3 drops) was added to a stirred solution of diol **23** (0.31 g, 0.87 mmol) and methyl isocyanate (0.11 mL, 1.91 mmol) in DCM (20 mL) and the solution stirred for 16 h. The solvent was removed and the residue chromatographed, eluting with 10% EtOAc/petroleum ether, to give dicarbonate **24** (0.39 g, 95%) as a colourless oil, IR (thin film) ν 3380, 1800, 1767, 1721, 1514 and 1251 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 7.09 (q, J=4.3 Hz, 1H, NH), 6.97 (q, J=4.3 Hz, 1H, NH), 5.10 (s, 2H, CH_2O), 4.89 (s, 2H, CH_2O), 3.37 (s, 3H, NCH_3), 2.51 (br d, J=4.3 Hz, 6H, $2CH_3$) and 1.40 (s, 18 H, $2OC(CH_3)_3$); ^{13}C NMR [$(CD_3)_2SO$] δ 156.5 (NCO_2), 156.1 (NCO_2), 149.8 ($2CO_2$), 138.0 (C-2), 133.9 (C-4), 126.0 (C-5), 83.5 ($2OC(CH_3)_3$), 58.0 (CH_2O), 54.0 (CH_2O), 29.5 (NCH_3) and 27.3 ($2OC(CH_3)_3$); MS (DEI) m/z 471 (M^+,10%), 397 (10), 315 (30), 223 (40) and 165 (100); HRMS (DEI) calcd for $C_{20}H_{33}N_5O_8$ (M^+) m/z 471.2329, found 471.2337.

Synthesis of 5

Ethyl 1-methyl-2-sulphanyl-1*H*-imidazole-5-carboxylate (26). Sarcosine ethyl ester hydrochloride[38] (**25**) (168 g, 1.09 mol) was dissolved in warm formic acid (140 mL) and a hot solution of sodium formate (82 g, 1.21 mol) in formic acid (110 mL) was added. The solution was stirred at 20°C for 4 h. Ac_2O (490 mL) was added carefully and the solution heated at 100°C for 1 h. The mixture was cooled to 20°C, filtered and the solvent removed. The residue was suspended in acetone (550 mL), filtered, the solvent removed and the residue distilled. The fraction that distilled between 84 and 94°C at 0.3 mmHg was collected to give ethyl *N*-formylsarcosinate (125 g, 79%) as a yellow liquid, ^{1}H NMR δ (two rotamers) 8.12, 8.04 (2s, 1H, CHO), 4.22, 4.20 (2q, J=7.1 Hz, 2H, OCH_2), 4.18, 3.98 (2s, 2H, CH_2), 3.04, 2.93 (2s, 3H, NCH_3), and 1.30, 1.28 (2t, J=7.1 Hz, 3H, CH_3); ^{13}C NMR δ 168.2 (CO_2), 163.1, 162.9 (CHO), 61.7, 61.4 (OCH_2), 50.9, 45.6 (NCH_3), 35.2, 30.8 (CH_2) and 14.1 (CH_3).

A suspension of NaOEt (4.92 g, 72.3 mmol) in dry benzene (20 mL) was added slowly to a stirred solution of ethyl *N*-formylsarcosinate (10.0 g, 68.9 mmol) in ethyl formate (22.3 mL) at 0°C. The solution was stirred at 20°C for 2 h, water was (50 mL) added, and the mixture stirred until a solution formed. The benzene was separated and cHCl (8.3 mL, 83 mmol) added dropwise to the stirred aqueous solution at 0°C. The solution was stirred for 10 min and KSCN (8.03 g, 82.7 mL) added in four portions. The solution was stirred at 100°C for 3 h and then stood at 20°C for 16 h. The precipitate was filtered, washed with water and air dried to give ester **26** (3.52 g, 27%) as white needles, mp (H_2O) 178–180°C; ^{1}H NMR [$(CD_3)_2SO$] δ 12.86 (s, 1H, SH), 7.77 (s, 1H, H-4), 4.23 (q, J=7.1 Hz, 2H, CH_2), 3.69 (s, 3H, NCH_3), and 1.27 (t, J=7.1 Hz, 3H, CH_3); ^{13}C NMR δ 164.9 (CO_2), 158.3 (C-2), 123.4 (C-4), 120.4 (C-5), 60.4 (NCH_3), 32.4 (CH_2) and 14.0 (CH_3); Anal. calcd for $C_7H_{10}N_2O_2S$: C, 45.1; H, 5.4; N, 15.0; S, 17.2; found C, 45.4; H, 5.5; N, 12.2; S, 17.4%.

Methyl 1-methyl-2-(methylsulphanyl)-1*H*-imidazole-5-carboxylate (27). Iodomethane (1.53 mL, 24.6 mmol) was added to a stirred suspension of ester **26** (3.82 g, 20.5 mmol) and K_2CO_3 (2.84 g, 20.5 mmol) in MeOH (50 mL). The suspension was stirred at 20°C for 1 h, filtered and the solvent removed. The residue was suspended in EtOAc (50 mL), filtered and the solvent removed to give methyl sulphide **27** (3.67 g, 96%) as a white solid, mp (EtOAc) 56–58°C; (lit.[51] mp 43–45°C) ^{1}H NMR δ 7.72 (s, 1H, H-4), 3.83 (s, 3H, NCH_3), 3.80 (s, 3H, OCH_3) and 2.68 (SCH_3); ^{13}C NMR δ 160.6 (CO_2), 150.4 (C-2), 137.4 (C-4), 124.2 (C-5), 51.3 (OCH_3), 32.4 (NCH_3) and 14.8 (SCH_3); MS m/z (M^+, 100%), 153 (45) and 121 (30); HRMS calcd for $C_7H_{10}N_2O_2S$ (M^+) m/z 186.0463, found 186.0456.

[1-Methyl-2-(methylsulphanyl)-1*H*-imidazol-5-yl]methanol (28). A solution of Superhydride® in (1 M in THF) (41.2 mL, 41.2 mmol) was added slowly to a stirred solution of ester **27** (3.67 g, 19.6 mmol) in THF (50 mL) at 0°C. The solution was stirred at 20°C for 3 h, water (10 mL) added carefully and the solvent was removed. The residue was chromatographed, eluting with a gradient (0–10%) MeOH/EtOAc to give alcohol **28** (2.90 g, 94%) as a white solid, mp (EtOAc) 93–94.5°C; ^{1}H NMR [$(CD_3)_2SO$] δ 6.83 (s, 1H, H-4), 5.08 (br. s, 1H, OH), 4.40 (br. s, 2H, CH_2O), 3.51 (s, 3H, NCH_3), and 2.48 (s, 3H, SCH_3); ^{13}C NMR δ 141.9 (C-2), 133.9 (C-5), 127.0 (C-4), 52.8 (CH_2O), 30.3 (NCH_3) and 15.6 (SCH_3); MS m/z 158 (M^+, 100%), 141 (35) and 125 (85); HRMS calcd for $C_6H_{10}N_2O_6S$ (M^+), m/z 158.0514, found 158.0513; Anal calcd for $C_6H_{10}N_2OS$: C, 45.5; H, 6.4; N, 17.7; S, 20.3; found C, 45.5; H, 6.6; N, 17.8; S, 20.0%.

[1-Methyl-2-(methylsulphinyl)-1*H*-imidazol-5-yl]methanol (29). A solution of MCPBA (4.32 g, 20.0 mmol) in DCM (50 mL) was added slowly to a stirred solution alcohol **28** (3.02 g, 19.1 mmol) in DCM (100 mL) at −78°C. The solution was stirred at −78°C for 1 h, warmed to 20°C, and stirred for 1 h. The solvent was removed and the residue chromatographed, eluting with a gradient (0–20%) of MeOH/EtOAc to give sulphoxide **29** (3.28 g, 98%) as an oil, ^{1}H NMR [$(CD_3)_2SO$] δ 7.04 (s, 1H, H-4), 5.27 (t, J=5.3 Hz, 1H, OH), 4.50 (d, J=5.3 Hz, 2H, CH_2O), 3.82 (s, 3H, NCH_3) and 3.02 (s, 3H, $SOCH_3$); ^{13}C NMR δ 146.7 (C-2), 136.4 (C-5), 127.2 (C-4), 52.4 (CH_2O), 37.6 ($SOCH_3$) and 30.8 (NCH_3); MS m/z 174 (M^+, 30%), 159 (100), 142 (20) and 127 (60); HRMS calcd for $C_6H_{10}N_2O_2S$ (M^+) m/z 174.0463, found 174.0464.

[1-Methyl-2-(methylsulphinyl)-1*H*-imidazol-5-yl]methyl 4-nitrophenyl carbonate (30). 4-Nitrophenyl chloroformate (3.63 g, 17.5 mmol) in THF (20 mL) was added slowly to a stirred solution of alcohol **29** (2.90 g, 16.6 mmol) and pyridine (1.5 mL, 18.3 mmol) in THF (50 mL) at 20°C under N_2. The solution was stirred at 20°C for 16 h, diluted with DCM (200 mL), washed with 10% aq. $NaHCO_3$ (50 mL), brine (50 mL) and dried. The solvent was removed and the residue chromatographed, eluting with a gradient (0–10%) of MeOH/EtOAc, to give 4-nitrophenylcarbonate **30** (2.70 g, 48%) as an oil, ^{1}H NMR δ 8.28 (ddd, J=9.1, 3.2, 2.1 Hz, 2H, H-3′, H-5′), 7.38 (ddd, J=9.1, 3.2, 2.1 Hz, 2H, H-2′, H-6′), 7.34 (s, 1H, H-4), 5.33 (s, 2H, CH_2O), 4.02 (s, 3H, NCH_3) and 3.17 (s, 3H, $SOCH_3$); ^{13}C NMR δ 155.3 (CO_2), 152.1 (C-2), 148.2 (C-1′), 145.6 (C-4′), 131.8 (C-5), 129.6 (C-4), 125.4 (C-2′, C-6′), 121.7 (C-3′, C-5′), 59.1 (CH_2O), 38.1 ($SOCH_3$) and 31.5 (NCH_3); MS m/z (DEI) 339 (M^+, 80%), 324 (100), 292 (50) and 279 (70); HRMS (DEI) calcd for $C_{13}H_{13}N_3O_6S$ (M^+) m/z 339.0525, found 339.0520.

[1-Methyl-2-(methylsulphinyl)-1*H*-imidazol-5-yl]methyl bis(2-chloroethyl)carbamate (5). A solution of 4-nitrophenylcarbonate **30** (2.68 g, 7.90 mmol) in pyridine (20 mL) was added to a stirred solution of *N*,*N*-bis(2-chloroethyl)amine hydrochloride (1.69 g, 9.5 mmol) in pyridine (80 mL) at 0°C. The solution was stirred at 20°C for 16 h, the solvent removed and the residue partitioned between DCM and 5% aq. citric acid (100 mL). The organic phase was washed with brine (50 mL), dried and the solvent removed. The residue was chromatographed, eluting with a gradient (0–15%) of MeOH/EtOAc, to give carbamate **5** (1.39 g, 52%) as an oil, ^{1}H NMR [$(CD_3)_2SO$] δ 7.22 (s, 1H, H-4), 5.19 (s, 2H, CH_2O), 3.85 (s, 3H, NCH_3), 3.68–3.74 (m, 4H, $2NCH_2$), 3.59 (t, J=6.3 Hz, 4 H, $2CH_2Cl$) and 3.03

(s, 3H, $SOCH_3$); ^{13}C NMR δ 154.8 (NCO_2), 147.8 (C-2), 131.1 (C-5), 129.7 (C-4), 56.0 (CH_2O), 48.7 (NCH_2), 48.3 (NCH_2), 42.0 (CH_2Cl), 41.3 (CH_2Cl), 37.6 ($SOCH_3$) and 30.9 (NCH_3); MS *m/z* 345 (M^+, 3%), 343 (15), 341 (20), 330 (10), 328 (40) and 326 (100); HRMS calcd for $C_{11}H_{17}{}^{37}Cl_2N_3O_3S$ (M^+) *m/z* 345.0308, found 345.0297; calcd for $C_{11}H_{17}{}^{37}Cl^{35}ClN_3O_3S$ (M^+) *m/z* 343.0338, found 343.0325; calcd for $C_{11}H_{17}{}^{35}Cl_2N_3O_3S$ (M^+) *m/z* 341.0367, found 341.0344; Anal. calcd for $C_{11}H_{17}Cl_2N_3O_3S$: C, 38.6; H, 5.0; N, 12.3; Cl, 20.7; S, 9.4; found C, 38.1; H, 5.0; N, 12.1; Cl, 20.3; S, 9.6%.

Synthesis of 6

1-Methyl-2-nitro-1*H*-imidazole-5-carboxylate (32). Sodium hydroxide solution (1 M, 125 mL, 125 mmol) was added slowly to a stirred suspension of ethyl 1-methyl-2-nitro-1*H*-imidazole-5-carboxylate[39] (**31**) (5.0 g, 25.1 mmol) in water (50 mL) and the mixture stirred at 20°C until complete dissolution occurred. The solution was washed with ether (50 mL), the pH of the solution adjusted to 3 with HCl (5 N) and the mixture extracted with EtOAc (3×100 mL). The combined organic fractions were dried and the solvent evaporated to give carboxylic acid **31** (4.29 g, 100%) as a tan powder, mp 160–161°C (lit.[52] mp (EtOAc) 161–163°C); ^1H NMR [$(CD_3)_2SO$] δ 13.60 (br s, 1H, CO_2H), 7.37 (s, 1H, H-4), and 4.20 (s, 3H, NCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 160.3 (CO_2), 147.2 (C-2), 133.7 (C-5), 127.0 (C-4) and 35.0 (NCH_3).

(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methanol (33). A solution of CDI (7.0 g, 43.1 mmol) and carboxylic acid **32** was stirred at 20°C for 30 min and then added to a stirred solution of $NaBH_4$ (4.07 g, 108 mmol) in EtOH (10 mL) and the mixture stirred at 20°C for 1 h. HCl (5 M, 20 mL) was added carefully and the mixture stirred for 30 min. The solvent was evaporated and the residue chromatographed, eluting with EtOAc, to give alcohol **33** (2.23 g, 68%) as a cream solid, mp (EtOAc/petroleum ether) 138–140°C (lit.[39] 142–144°C); ^1H NMR [$(CD_3)_2SO$] δ 7.12 (s, 1H, H-4), 5.49 (br s, 1H, OH), 4.55 (s, 2 H, CH_2O) and 3.92 (s, 3H, NCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 145.6 (C-2), 138.6 (C-5), 126.5 (C-4), 52.9 (CH_2O) and 34.0 (NCH_3).

(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-nitrophenyl carbonate (34). A solution of 4-nitrophenyl-chloroformate (0.67 g, 3.34 mmol) in THF (5 mL) was added to a stirred solution of alcohol **33** (0.50 g, 3.18 mmol) and pyridine (283 μL, 3.50 mmol) in THF (50 mL) at 20°C under N_2. The solution was stirred at 20°C for 16 h, the solvent evaporated, and the residue dissolved in EtOAc (100 mL). The solution was washed with water (2×50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was chromatographed, eluting with 50% EtOAc/petroleum ether, to give 4-nitrophenylcarbonate **34** (0.87 g, 84%) as a tan solid, mp (EtOAc) 156.5–157.5°C; IR ν 1771, 1537 and 1359 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 8.33 (ddd, *J*=9.1, 3.2, 2.1 Hz, 2H, H-3′, H-5′), 7.59 (ddd, *J*=9.1, 3.2, 2.1 Hz, 2H, H-2′, H-6′), 7.37 (s, 1H, H-4), 5.48 (s, 2H, CH_2O) and 4.00 (s, 3H, NCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 155.1 (OCO_2), 151.4 (C-1′), 146.3 (C-4′), 145.2 (C-2), 131.5 (C-5), 129.6 (C-4), 125.4 (C-3′, C-5′), 122.5 (C-2′, C-6′), 59.4 (CH_2O) and 34.3 (NCH_3); Anal. calcd for $C_{12}H_{10}N_4O_7$: C, 44.7; H, 3.1; N, 17.4; found C, 45.0; H, 3.0; N, 16.7%.

(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl bis(2-chloroethyl)carbamate (6). A solution of 4-nitro-phenylcarbonate **34** (0.68 g, 2.11 mmol) in pyridine (3 mL) was added to a solution of *N*,*N*-bis(2-chloroethyl)amine hydrochloride (0.75 g, 4.22 mmol) in pyridine (30 mL) under N_2. The solution was stirred at 20°C for 16 h and the solvent evaporated. The residue was dissolved in DCM (100 mL) and washed with 2% citric acid solution (2×50 mL), water (50 mL), brine (50 mL), dried and the solvent evaporated. The residue was chromatographed, eluting with 50% EtOAc/petroleum ether, to give carbamate **6** (0.51 g, 74%) as a cream solid, mp (EtOAc) 100–101°C; IR ν 1703, 1489 and 1344 cm^{-1}; ^1H NMR δ 7.23 (s, 1H, H-4), 5.21 (s, 2H, CH_2O), 4.05 (s, 3H, NCH_3) and 3.58–3.70 (m, 8H, $2CH_2N$, $2CH_2Cl$); ^{13}C NMR δ 154.8 (NCO_2), 144.5 (C-2), 132.1 (C-5), 129.7 (C-4), 56.2 (CH_2O), 50.8 ($2CH_2N$), 41.6 ($2CH_2Cl$) and 34.3 (NCH_3); Anal. calcd for $C_{10}H_{14}Cl_2N_4O_4$ C, 36.9; H, 4.3; N, 17.2; Cl, 21.8; found C, 37.4; H, 4.1; N, 17.2; Cl, 21.8%.

Synthesis of 7

1-Methyl-1*H*-imidazole-2-carbaldehyde (36). *n*-Butyllithium (52.7 mL, 0.132 mol) was added dropwise to a solution of *N*-methylimidazole (**35**) (10 mL, 0.125 mol) in dry THF (150 mL) at −78°C. The solution was stirred at −78°C for 30 min. DMF (19 mL, 0.25 mol) was added and the mixture allowed to warm to 20°C and stirred for 2 h. Water (5 mL) was added carefully and the mixture partitioned between EtOAc and water (500 mL) and the organic fraction washed with water (2×50 mL), brine (50 mL), dried, and the solvent removed to give aldehyde **36** (11.2 g, 80%) as a tan solid, mp (EtOAc/petroleum ether) 36–38°C (lit.[53] mp 38–39°C); ^1H NMR δ 9.82 (s, 1H, CHO), 7.27 (s, 1H, H-4), 7.12 (s, 1H, H-5), and 4.03 (s, 3H, NCH_3); ^{13}C NMR δ 182.2 (CHO), 143.4 (C-2), 131.5 (C-5), 127.3 (C-4) and 34.9 (N-CH_3).

(1-Methyl-1*H*-imidazol-2-yl)methanol (37). A solution of aldehyde **36** (11.2 g, 0.102 mol) in dry THF (50 mL) was added to a stirred suspension of $LiAlH_4$ (4.25 g, 0.11 mol) in THF (100 mL) at 0°C. The suspension was stirred at 20°C for 1 h, water (20 mL) carefully added and the suspension filtered through Celite. The pad was washed with hot THF (200 mL) and the solvent removed from the filtrate to give alcohol **37** (10.3 g, 90%) as a white solid, mp (MeOH/*i*Pr_2O) 114–116°C (lit.[54]mp 116°C); ^1H NMR [$(CD_3)_2SO$] δ 7.05 (d, *J*=1.0 Hz, 1H, H-4), 6.75 (d, *J*=1.0 Hz, 1H, H-5), 5.30 (br. s, 1H, OH), 4.46 (s, 2H, CH_2O), and 3.63 (s, 3H, N–CH_3); ^{13}C NMR δ 147.3 (C-2), 126.0 (C-4), 121.8 (C-5), 55.5 (CH_2O) and 32.3 (NCH_3).

[1-Methyl-5-(methylsulphanyl)-1*H*-imidazol-2-yl]methanol (38). *n*-Butyllithium (18.4 mL, 29.4 mmol) was added dropwise to a stirred solution of alcohol **37** (3.0 g, 26.8 mmol) in THF (200 mL) at −78°C. The suspension was stirred at −78°C for 15 min. *tert*-Butyllithium (17.3 mL, 29.4 mmol) was added dropwise and the suspension stirred at −78°C for 15 min. Methyl disulphide (5.3 mL, 58.9 mmol) was added and the mixture allowed to warm to 20°C and stirred for 2 h. Water (5 mL) was

added carefully and the mixture partitioned between EtOAc and water (500 mL). The aqueous layer was extracted with EtOAc (2×50 mL), the combined organic extracts washed with brine (100 mL), dried and the solvent removed. The residue was chromatographed, eluting with a gradient (0–10%) of MeOH/EtOAc, to give alcohol **38** (2.16 g, 51%) as a cream coloured solid, mp (EtOAc) 92–94°C; ^{1}H NMR δ 6.99 (s, 1H, H-4), 5.46 (br. s, 1H, OH), 4.62 (s, 2H, CH_2O), 3.72 (s, 3H, NCH_3) and 2.24 (s, 3H, SCH_3); ^{13}C NMR δ 150.0 (C-2), 132.4 (C-4), 125.0 (C-5), 56.0 (CH_2O), 30.0 (NCH_3) and 20.5 (SCH_3); Anal. calcd for $C_6H_{10}N_2OS$: C, 45.5; H, 6.4; N, 17.7; S, 20.3; found C, 45.3; H, 6.1; N, 17.6; S, 20.3%.

[1-Methyl-5-(methylsulphinyl)-1*H*-imidazol-2-yl]methanol (39). A solution of MCPBA (3.05 g, 15.0 mmol) in DCM (20 mL) was added dropwise to a stirred solution of alcohol **38** (2.16 g, 13.7 mmol) in DCM (80 mL) at −78°C. The solution was stirred at −78°C for 1 h, allowed to warm to 20°C and stirred for 1 h. The solvent was removed and the residue chromatographed, eluting with a gradient (0–20%) of MeOH/EtOAc, to give sulphoxide **39** (2.16 g, 90%) as a white solid, mp (MeOH/EtOAc) 102–105°C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.39 (s, 1H, H-4), 5.49 (t, J=5.6 Hz, 1H, OH), 4.54 (t, J=5.6 Hz, 2H, CH_2O), 3.82 (s, 3H, NCH_3) and 3.01 (s, 3 H, $SOCH_3$); ^{13}C NMR δ 151.6 (C-2), 133.0 (C-4), 128.6 (C-5), 55.5 (CH_2O), 38.2 ($SOCH_3$) and 31.1 (NCH_3); MS m/z 174 (M^+, 25%), 159 (100) and 130 (65); HRMS calcd for $C_6H_{10}N_2O_2S$ (M^+) m/z 174.0463, found 174.0462; Anal. calcd for $C_6H_{10}N_2O_2S$: C, 41.4; H, 5.8; N, 16.1; S, 18.4; found C, 41.3; H, 5.8; N, 16.0; S, 18.1%.

[1-Methyl-5-(methylsulphinyl)-1*H*-imidazol-2-yl]methyl 4-nitrophenyl carbonate (40). A solution of 4-nitrophenyl-chloroformate (2.41 g, 11.9 mmol) in THF (10 mL) was added slowly to a stirred solution of sulphoxide **39** (1.98 g, 11.4 mmol) and pyridine (1.0 mL, 12.5 mmol) in THF (60 mL) at 20°C under N_2. The solution was stirred at 20°C for 16 h, diluted with DCM (150 mL) and washed with a saturated solution of $NaHCO_3$ (50 mL), dried and the solvent removed. The residue was chromatographed, eluting with a gradient (0–15%) of MeOH/EtOAc, to give 4-nitrophenyl carbonate **40** (3.13 g, 81%) as an oil, ^{1}H NMR [$(CD_3)_2SO$] δ 8.33 (ddd, J=9.1, 3.3, 2.1 Hz, 2H, H-3′, H-5′), 7.60 (ddd, J=9.1, 3.3, 2.1 Hz, 2H, H-2′, H-6′), 7.55 (s, 1H, H-4), 5.44 (s, 2H, CH_2O), 3.90 (s, 3H, NCH_3) and 3.07 (s, 3H, $SOCH_3$); ^{13}C NMR δ 155.1 (OCO_2), 151.5 (C-2), 145.4 (C-1′), 145.2 (C-4′), 134.4 (C-5), 129.4 (C-4), 125.3 (C-2′, C-6′), 122.5 (C-3′, C-5′), 61.5 (CH_2O), 38.3 ($SOCH_3$) and 31.4 (NCH_3); MS (DEI) m/z 339 (M^+, 20%), 324 (60) and 57 (100); HRMS (DEI) calcd for $C_{13}H_{13}N_3O_6S$ (M^+) m/z 339.0525, found 339.0524.

[1-Methyl-5-(methylsulphinyl)-1*H*-imidazol-2-yl]methyl bis(2-chloroethyl)carbamate (7). A solution of 4-nitrophenyl carbonate **40** (1.27 g, 3.74 mmol) in pyridine (10 mL) was added to a stirred solution of *N*,*N*-bis(2-chloroethyl)amine hydrochloride (0.80 g, 4.49 mmol) in pyridine (50 mL) at 0°C. The solution was stirred at 20°C for 16 h and the solvent removed. The residue was partitioned between DCM and 5% citric acid solution (100 mL) and the organic fraction washed with brine (50 mL), dried and the solvent removed. The residue was chromatographed, eluting with a gradient (0–15%) of MeOH/EtOAc, to give carbamate **7** (1.02 g, 80%) as a hygroscopic oil, ^{1}H NMR δ 7.40 (s, 1H, H-4), 5.25 (s, 2H, CH_2O), 3.96 (s, 3H, NCH_3), 3.59–3.69 (m, 8H, $2NCH_2$, $2CH_2Cl$), and 3.04 (s, 3H, $SOCH_3$); ^{13}C NMR δ 154.8 (OCON), 147.2 (C-2), 133.2 (C-5), 129.8 (C-4), 58.2 (CH_2O), 50.9 (NCH_2), 50.2 (NCH_2), 41.7 (CH_2Cl), 41.4 (CH_2Cl), 38.4 ($SOCH_3$) and 31.6 (NCH_3); MS (DEI) m/z 345 (M^+, 1%), 343 (7), 341 (9), 330 (1), 328 (7), 326 (10) and 173 (100); HRMS (DEI) calcd for $C_{11}H_{17}{}^{35}Cl_2N_3O_3S$ (M^+) m/z 341.0368, found 341.0376; calcd for $C_{11}H_{17}{}^{35}Cl^{37}ClN_3O_3S$ (M^+) m/z 343.0338, found 343.0348; calcd for $C_{11}H_{17}{}^{37}Cl_2N_3O_3S$ (M^+) m/z 345.0309, found 345.0311; Anal. calcd for $C_{11}H_{15}N_3O_3S.H_2O$: C, 36.7; H, 5.3; N, 11.7; found C, 36.9; H, 5.5; N, 11.6%.

Synthesis of 8

(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methanol (42). A mixture of *N*-methyl-5-nitroimidazole[42] (**41**) (1.0 g, 7.9 mmol) and paraformaldehyde (1.4 g, 15.7 mmol) in DMSO (10 mL) was heated in a sealed tube at 100°C for 24 h. The mixture was cooled to 20°C, EtOH (50 mL) was added, and the suspension was filtered. The filtrate was evaporated and the residue was chromatographed on alumina, eluting with a gradient (0–10%) of MeOH/$CHCl_3$, to give alcohol **42** (0.71 g, 57%) as a white powder, mp ($CHCl_3$) 116–118°C (lit.[55] mp 111°C); ^{1}H NMR [$(CD_3)_2SO$] δ 7.90 (s, 1H, H-4), 5.62 (t, J=5.8 Hz, 1H, OH), 4.62 (d, J=5.8 Hz, 2H, CH_2O) and 3.99 (s, 3H, NCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 151.8 (C-2), 138.8 (C-5), 130.9 (C-4), 56.1 (CH_2O) and 32.9 (NCH_3).

(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-nitrophenyl carbonate (43). 4-Nitrophenyl chloroformate (1.48 g, 7.4 mmol) in THF (8 mL) was added slowly to a stirred solution of alcohol **42** (1.10 g, 7.0 mmol) and pyridine (0.62 mL, 7.7 mmol) in THF (50 mL) at 20°C under N_2. The mixture was stirred at 20°C for 16 h, then partitioned between EtOAc and H_2O. The organic layer was washed with saturated aq. $NaHCO_3$ (50 mL), and the solvent evaporated to give 4-nitrophenylcarbonate **43** (2.04 g, 94%) as a white solid, mp (EtOAc/petroleum ether) 175–177°C; IR ν 1769, 1526, 1476, 1350 and 1261 cm^{-1}; ^{1}H NMR [$(CD_3)_2SO$] δ 8.33 (ddd, J=9.1, 3.2, 2.2 Hz, 2H, H-3′, H-5′), 8.11 (s, 1H, H-4), 7.59 (ddd, J=9.1, 3.2, 2.2 Hz, 2H, H-2′, H-6′), 5.48 (s, 2H, CH_2O) and 3.97 (s, 3H, NCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 155.0 (OCO_2), 151.3 (C-2), 145.9 (C-1′), 145.2 (C-4′), 139.5 (C-5), 131.6 (C-4), 125.3 (C-2′, C-6′), 122.4 (C-3′, C-5′), 61.7 (CH_2O) and 33.5 (NCH_3); Anal. calcd for $C_{12}H_{10}N_4O_7$ C, 44.7; H, 3.1; N, 17.4; found C, 44.9; H, 3.0; N, 17.3%.

(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methyl bis(2-chloroethyl)carbamate (8). A solution of 4-nitro-phenylcarbonate **43** (2.0 g, 6.5 mmol) in pyridine (5 mL) was added to a stirred solution of *N*,*N*-bis(2-chloroethyl)amine hydrochloride (1.5 g, 8.4 mmol) in pyridine (30 mL) at 0°C. The solution was stirred at 20°C for 16 h, then solvent was evaporated and the residue was partitioned between DCM and 10% aq. citric acid. The organic layer was dried and the solvent evaporated, and the residue was chromatographed, eluting with 50% EtOAc/light petroleum, to give carbamate

8 (2.0 g, 95%) as an oil, ^{1}H NMR δ 7.99 (s, 1H, H-4), 5.27 (s, 2H, CH_2O), 4.03 (s, 3H, NCH_3) and 3.60–3.71 (m, 8 H, $2CH_2N$, $2CH_2Cl$); ^{13}C NMR δ 154.8 (NCO_2), 146.9 (C-2), 139.6 (C-5), 132.2 (C-4), 58.7 (CH_2O), 51.1 ($2CH_2N$), 41.8 ($2CH_2Cl$), and 39.7 (NCH_3); MS *m/z* 328 (M^+, 10%), 326 (55), 324 (75), 289 (70), 245 (75) and 231 (100); HRMS calcd for $C_{10}H_{14}Cl_2N_4O_4$ (M^+) *m/z* 324.1392, found 324.1381.

Biological testing

Compounds were dissolved directly in culture medium (αMEM containing 5% foetal calf serum), filter sterilised, and the drug concentrations were checked by spectrophotometry. Solutions were used within 1–2 h. BCEA was dissolved in 0.01N HCl and diluted into the culture medium immediately before use. Growth inhibition under aerobic conditions was assessed using log-phase cultures in 96 well plates. Cells were exposed to drugs for 4 or 18 h, the exposure was terminated by washing 3 times with fresh culture medium, and cultures were stained with methylene blue 3 days later to determine cell density.[56] IC_{50} values were determined as the drug concentration providing 50% inhibition relative to controls on the same plate. Clonogenic assays were performed using early plateau phase spinner flask cultures (10^6 cells/mL for AA8, 5×10^5cells/mL for UV4 or UV5 cells). The cells were exposed to drugs under aerobic or anoxic conditions in fresh medium at 10^6 cells/mL, using continuously stirred and gassed (5% CO_2 in air or N_2, respectively) single cell suspensions as detailed previously.[57] Samples were withdrawn at intervals up to 5 h, cells were washed by centrifugation, and plating efficiency determined. A range of drug concentrations were examined for each compound and the concentration×time to reduce plating efficiency to 10% of controls (CT_{10}) was determined for a pair of curves showing similar kinetics of cell kill (where possible, giving 10% survival between 1 and 2 h).

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