Synthesis and Evaluation of Nitroheterocyclic Carbamate Prodrugs for Use with Nitroreductase-Mediated Gene-Directed Enzyme Prodrug Therapy

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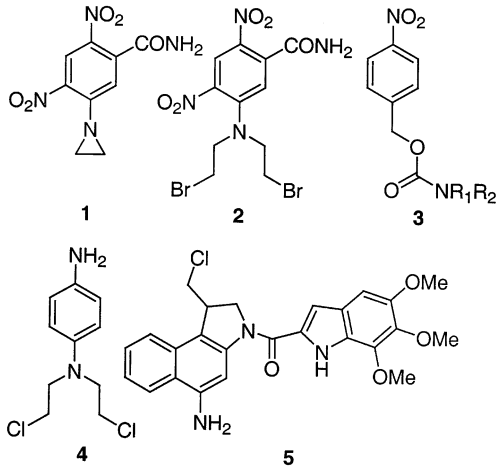
A variety of nitroheterocyclic carbamate prodrugs of phenylenediamine mustard and 5-amino-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indoline (amino-*seco*-CBI-TMI), covering a wide range of reduction potential, were prepared and evaluated for use in gene-directed enzyme prodrug therapy (GDEPT) using a two-electron nitroreductase (NTR) from *Escherichia coli* B. The carbamate prodrugs and corresponding amine effectors were tested in a cell line panel comprising parental and NTR-transfected human (SKOV3/SKOV3-NTR^{neo}, WiDr/WiDr-NTR^{neo}), Chinese hamster (V79^{puro}/V79-NTR^{puro}), and murine (EMT6/EMT6-NTR^{puro}) cell line pairs and were compared with the established NTR substrates CB1954 (an aziridinyl dinitrobenzamide) and the analogous dibromomustard. The 1-methyl-2-nitroimidazol-5-ylmethyl carbamate of phenylenediamine mustard was metabolized rapidly by EMT6-NTR^{neo} but not EMT6 cells, demonstrating that it is an efficient substrates for NTR. Despite this, the carbamates of phenylenediamine mustards show relatively low differential cytotoxicity for NTR+ve cells in IC_{50} assays, apparently because they retain sufficient alkylating reactivity that most of the prodrug reacts with nucleophiles during the drug exposure period. In contrast, the corresponding amino-*seco*-CBI-TMI prodrugs were less efficient NTR substrates but had greater chemical stability, were more potent, and showed substantial NTR−ve/NTR+ve ratios in the cell line panel, with ratios of 15−100-fold for the 1-methyl-2-nitro-1*H*-imidazol-5-ylmethyl and 1-methyl-5-nitro-1*H*-imidazol-2-ylmethyl carbamates of amino-*seco*-CBI-TMI. The activity of these two prodrugs was evaluated against NTR-expressing EMT6 tumors comprising ca. 10% NTR+ve cells. Small but not statistically significant killing of NTR+ve cells was observed, with no effect against NTR−ve target cells. The lack of activity against NTR+ve cells in tumors, despite potent and selective activity in culture, indicates that pharmacokinetic optimization will be required if in vivo efficacy against solid tumors is to be achieved with this new class of NTR prodrugs.

Introduction

Gene-directed enzyme prodrug therapy (GDEPT) is a novel approach to the treatment of cancer.[1−3] This approach uses various vector systems to selectively deliver a gene encoding a nonhuman prodrug-activating enzyme to tumor tissue. Administration of a suitable prodrug for the enzyme results in generation of the drug selectively in transfected tumor cells. Given the expectation that only a small proportion of tumor cells will express the activating enzyme, the released drug must diffuse to kill neighboring tumor cells (the bystander effect).

The minor aerobic nitroreductase (NTR) from *Escherichia coli* B[4−7] has been widely used in GDEPT, activating nitroaromatic prodrugs by reducing the nitro group (Hammet $\sigma_p = 0.78$) to the corresponding hydroxylamine ($\sigma_p = -0.34$). This electronic release activates a prepositioned functional group within the molecule to provide an active form of the drug.

Two main classes of prodrugs for NTR have been evaluated. The prototypical example of the dinitrobenzamide class of prodrug, CB1954 (**1**),[4,8,9] has advanced



1 **2** **3** **4** **5**

to clinical trial,[10,11] and the structure−activity relationships (SAR) of related nitrogen mustards have been determined empirically.[12−14] The bromomustard **2** has shown increased in vivo activity and a larger bystander effect compared to CB1954 in preclinical models.[15]

The second class comprises 4-nitrobenzylcarbamates of cytotoxic amines, exemplified by **3**. Stabilization by the carbamate occurs either because of the steric influence of the attached carbamate and/or because of the

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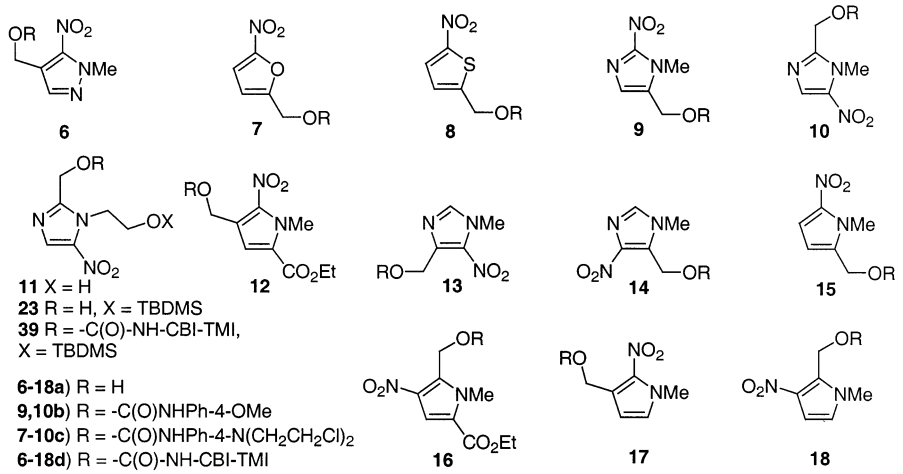
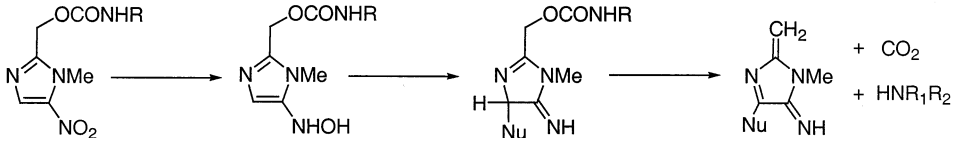
Chart 1. Compounds of Tables 1 and 2



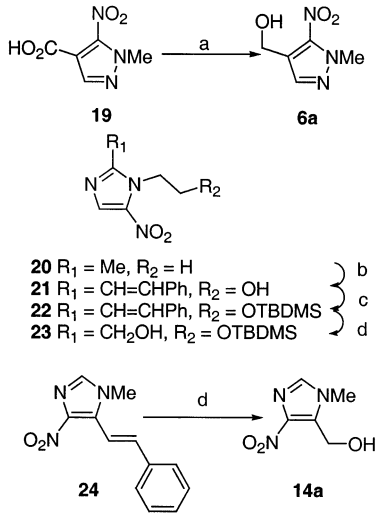
Scheme 1



electronic difference between the amine ($\sigma_p = -0.66$) and the carbamate ($\sigma_p = -0.17$). Reduction of the 4-NO_2 group to the hydroxylamine results in the fragmentation of the carbamate linkage, releasing the active amine. A wide range of amine-bearing DNA-reactive agents, including aniline mustard,[16] mitomycin,[16] enediynes,[17,18] *seco*-cyclopropylindoline derivatives,[19] pyrrolobenzodiazepines,[20] and DNA-binding agents including anthracyclines[16] and tallimustine analogues[21] have been protected as 4-nitrobenzyl carbamates. However, efficacy studies have been either rudimentary or failed to identify a lead compound with in vivo activity in a GDEPT protocol. We have recently shown that 2-alkoxy-4-nitrobenzylcarbamates of 5-aminobenz[*e*]indolines are good substrates for NTR in human cell lines in vitro, being more selective than the unsubstituted 4-nitrobenzyl carbamate.[22] However, these analogues were sparingly soluble, had reduction potentials considerably lower than those of the established substrates **1** and **2**, and placed considerable steric demand on the NTR binding site.

In an effort to improve all of these factors, we considered using a range of nitroheterocyclic carbamates **6c**,**d**−**18c**,**d** (Chart 1) as potential prodrugs for NTR. Fragmentation of 5-nitroimidazol-2-ylmethyl carbamates after nitro group reduction has been documented for ronidazole[23] and for aniline mustard derivatives.[24,25] Reactions of these 5-nitroimidazol-2-ylmethyl carbamates have been suggested[23−25] to proceed via reduction to the hydroxylamine and nucleophilic attack by thiols or water at the 4-position, with subsequent release of the amine (Scheme 1). The chemical reduction of 5-nitrofuran-2-ylmethyl carbamates (**7**; R = phenethyl) to the corresponding amines has been explored as a model for prodrug activation.[26] We chose two amine cytotoxin effectors for this study: the moderately potent phenylenediamine mustard[27] (**4**) and the very potent 5-amino-1-(chloromethyl)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indoline (amino-*seco*-CBI-TMI) (**5**).[28,29] These are both DNA alkylators[30,31] capable of killing both cycling and noncycling cells in a variety of hypoxic and acidic microenvironments.[32]

Scheme 2[a]

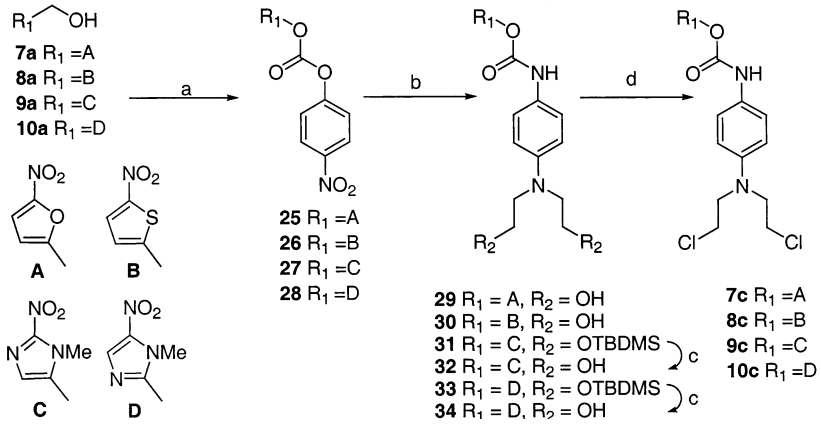


[a] Reagents: (a) BH_3·DMS; (b) NaOMe, PhCHO, DMSO; (c) TBDMSTf, pyridine, DCM; (d) O_3, DCM, MeOH, then $NaBH_4$, EtOH.

In a preliminary study[33] we showed that chemical, radiolytic, or enzymic reduction of the 2-nitroimidazol-5-ylmethyl carbamate model compound **9b** induced fragmentation of the carbamate and release of anisidine. Furthermore, the 2-nitroimidazol-5-ylmethyl carbamate **9d** of amino-*seco*-CBI-TMI (**5**) was 21-fold more toxic in cell culture against a cell line (SKOV3-NTR^{neo}) transfected with the NTR gene than the parent human ovarian carcinoma line SKOV3.

With these precedents in mind, we sought to expand the range of nitroheterocyclic carbamate-based prodrugs for use with NTR. We report here the preparation and in vitro and in vivo studies on a small set of 2- and 5-nitroimidazole, nitrothiophene, and nitrofuran pro-

Scheme 3[a]



[a] Reagents: (a) $NO_2PhOCOCl$, pyridine, THF; (b) **35**, pyridine; (c) TBAF, THF; (d) MsCl, pyridine; then LiCl, DMF.

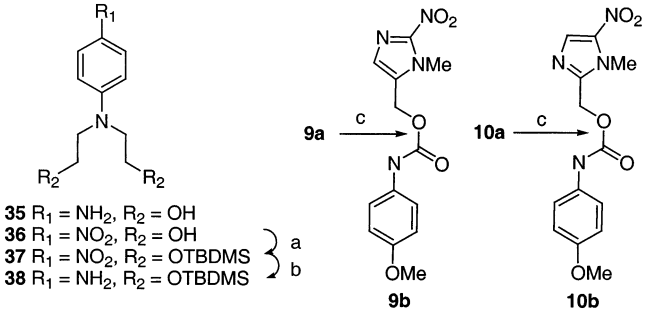
drugs (**7c**−**10c**) of phenylenediamine mustard **4** and a larger range of prodrugs (spanning a wider range of reduction potential and substitution pattern) of amino-*seco*-CBI-TMI (**5**) (**6d**−**18d**).

Chemistry

The alcohols **7a**,[26] **8a**,[34] **9a**,[35] **10a**,[35] **12a**,[36] **13a**,[37] and **15a**−**18a**[36] required for carbamate formation were synthesized as previously described. (1-Methyl-5-nitro-1*H*-pyrazol-4-yl)methanol (**6a**) was prepared by BH_3·DMS reduction of the corresponding acid[38] **19** (Scheme 2). Introduction of the hydroxymethyl substituent in the 2-position of the imidazole derivatives **23** was conveniently achieved by condensation of the corresponding methyl analogue **20** with benzaldehyde to give the styrene **21**.[39] This approach allowed selective protection of the side chain alcohol to give **22**, thus allowing regiospecific carbamate formation with alcohol **23**. Ozonolysis of the styrenes **22** and **24**, followed by a reductive workup, gave alcohols **23** and **14a** in good yields (88% and 78%, respectively).

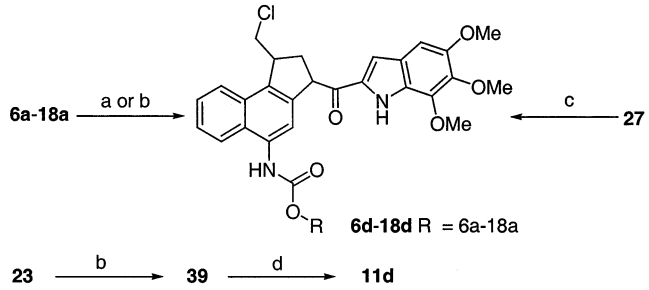
Two general methods were used for carbamate formation. For the more nucleophilic anilines, reaction with activated 4-nitrophenyl carbonates was used. Thus, alcohols **7a** and **8a** were activated as 4-nitrophenyl carbonates **25** and **26**, and displacement with **35** gave diols **29** and **30**, respectively, in moderate yields for the two steps (Scheme 3). Formation of mustards **7c** and **8c** was achieved in good yield via mesylation and displacement with LiCl in DMF. Similarly, activation of the nitroimidazoles **9a** and **10a** as carbonates **27** and **28** ensured facile reaction with aniline **38**, prepared from diol **34** (Scheme 4), to give the protected carbamates **31** and **33** in good yields (82 and 84%, respectively). The silyl protecting groups were readily removed using TBAF, and the resulting diols **32** and **34** elaborated to the mustards **9c** and **10c** using standard conditions. The use of the silyl protecting groups gave an improved yield in the coupling step compared to **25** and **26**, primarily due to improved isolation of the products. However, when combined with only good yields (88 and 82%) in the deprotection steps, this approach did not provide a significant benefit over the direct route. An alternate, more direct approach with activation of the alcohol **10a** using diphosgene and reaction of the resulting chloroformate with phenylenediamine mustard **4** gave a poor yield of carbamate **10c**. Although giving **10c** directly, this approach is less effective than the multistep approach used above.

Scheme 4[a]



[a] Reagents: (a) TBDMSCl, imidazole, DMF; (b) Pd/C, H_2, EtOH; (c) MeOPhNCO, $^nBu_2Sn(OAc)_2$, DCM.

Scheme 5[a]



[a] Reagents: (a) triphosgene, pyridine, DCM, then **5**; (b) **5**, triphosgene, Et_3N, DCM, then **6a**−**18a**, $^nBu_2Sn(OAc)_2$, DCM; (c) **5**, DIEA, HOBT, 4 Å molecular sieves, DMF; (d) HCl, aq MeOH.

For the more weakly nucleophilic amino-*seco*-CBI-TMI (**5**), attempts to form carbamates by in situ formation of the chloroformate of alcohol **9a** and reaction with **5** were plagued by low and variable yields (0−33%) (Scheme 5). Reaction of the carbonate **27** with amine **5** was extremely slow, and while addition of HOBT[40] provided an increase in rate, the reaction was still low-yielding (Scheme 5). Formation of the isocyanate of **5** in situ,[22] and subsequent reaction with alcohol **9a** in the presence of catalytic dibutyltin diacetate,[41] was found to be a superior method. The reaction did not proceed in the absence of dibutyltin diacetate. Thus, reaction of amine **5** with triphosgene and Et_3N in DCM followed by addition of alcohols **6a**−**18a** and dibutyltin diacetate gave variable yields (28−89%) of carbamates **6d**−**18d**.

Table 1. Physicochemical Data, IC_{50} Values for Parental Cells Lines, and IC_{50} Ratios (NTR−ve/NTR+ve) for Phenylenediamine Mustard (**4**) and Its Nitroheterocyclic Carbamate Prodrugs **8c**−**10c**; Comparison with the Reference Dinitrobenzamide Prodrugs CB 1954 (**1**) and **2**

no.	sol.[a]	*E*(1) pH 7.0 (mV)[b]	ref[c]	SKOV3 (μM)[d]	SKOV3/ SKOV3-NTR^{neo} [d,e]	WiDr (μM)[d]	WiDr/ WiDr-NTR^{neo} [d,e]	V79 (μM)[d]	V79/ V79-NTR^{puro} [d,e]	EMT6 (μM)[d]	EMT6/ EMT6-NTR^{puro} [d,e]
1	160 0	393 ± 7	BV	174 ± 10	317 ± 21	54 ± 3	51 ± 2	374 ± 14	2090 ± 210	71 ± 7	930 ± 140
2	40	nd[f]	nd	58 ± 10	211 ± 16	40 ± 3	174 ± 31	43 ± 5	302 ± 93	54	1380
4	nd	nd	nd	3.74 ± 0.06	0.97 ± 0.02	3.9 ± 1.0	1.3 ± 0.2	1.9 ± 0.2	0.57 ± 0.05	1.06 ± 0.05	0.51 ± 0.04
8c	10	−394 ± 7	MV	1.7 ± 0.2	2.9 ± 0.5	1.39 ± 0.07	2.8 ± 0.3	0.92 ± 0.02	2.3	1.16 ± 0.07	6.1 ± 1.3
9c	nd	−421 ± 8	MV	2.3 ± 0.2	28 ± 3	1.7 ± 0.2	6.7 ± 1.8	1.6 ± 0.2	5.3 ± 0.2	1.3 ± 0.3	22 ± 6
10c	11	−483 ± 8	MV	2.1 ± 0.2	3.7 ± 0.8	2.0 ± 0.1	2.01 ± 0.02	1.7 ± 0.2	1.3 ± 0.2	1.63 ± 0.03	6.1 ± 1.1

[a] Solubility (μM) in α-MEM culture medium, determined by HPLC. [b] Measured on **8a**−**10a**; one-electron potential in mV determined by pulse radiolysis. [c] BV, benzyl viologen (1,1′-dibenzyl-4,4′-bipyridinium) = −375 ± 10 mV;[55] MV, methyl viologen (1,1′-dimethyl-4,4′-bipyridinium) = −447 ± 7 mV.[56] [d] Values are mean ± SEM for up to five independent experiments. [e] Intraexperiment ratios. [f] Not determined.

Table 2. Physicochemical Data, IC_{50} Values for Parental Cells Lines, and IC_{50} Ratios (NTR−ve/NTR+ve) for Amino-*seco*-CBI-TMI (**5**) and Its Nitroheterocyclic Carbamate Prodrugs **6d**−**18d**

no.	sol.[a]	*E*(1)[b] pH 7.0 (mV)	ref[c]	SKOV3 (nM)[d]	SKOV3/ SKOV3-NTR^{neo} [d,e]	WiDr (nM)[d]	WiDR/ WiDr-NTR^{neo} [d,e]	V79 (nM)[d]	V79/ V79-NTR^{puro} [d,e]	EMT6 (nM)[d]	EMT6/ EMT6-NTR^{puro} [d,e]
5	197	nd[f]	nd	1.1 ± 0.08	0.55 ± 0.04	1.6 ± 0.1	0.9 ± 0.3	0.33 ± 0.07	0.9 ± 0.1	0.37 ± 0.06	2.0 ± 0.7
6d	8	−222 ± 6	MEN	32 ± 2	30.3 ± 0.7	11.3 ± 0.6	13.5 ± 1.8	41 ± 5	37 ± 4	39 ± 8	87 ± 21
7d	11	−381 ± 10	BV	76 ± 7	1.2 ± 0.2	89 ± 8	1.9 ± 0.2	20 ± 2	1.1 ± 0.2	18 ± 1	1.2 ± 0.2
8d	4	−394 ± 7	MV	10.6 ± 0.8	7 ± 0.8	18 ± 4	13.5 ± 1.9	3.5 ± 0.3	3.1 ± 0.9	4.3 ± 1.0	16 ± 3
9d	17	−421 ± 8	MV	75 ± 7	21 ± 2	75 ± 11	40 ± 6	24 ± 2	14.7 ± 1.7	19.6 ± 0.4	62± 4
10d	8	−483 ± 8	MV	146 ± 18	50 ± 1	230 ± 1.0	100 ± 19	68 ± 10	10 ± 4	78 ± 5	71 ± 5
11d	20	−482 ± 8	TQ	378 ± 79	26 ± 2	427 ± 41	12.4 ± 0.02	94 ± 11	1.2 ± 0.1	129 ± 32	11 ± 3
12d	nd	−496 ± 9	TQ	97 ± 8	9.6 ± 1.6	94 ± 13	13 ± 3	24 ± 2	2.0 ± 0.0	28 ± 3	12 ± 3
13d	5.5	−525 ± 8	MV	61 ± 3	5.6 ± 0.2	24 ± 1	3.4 ± 0.7	44 ± 9	1.4 ± 0.2	63 ± 5	6.5 ± 0.3
14d	13	−577 ± 8	MV	185 ± 13	4.8	201 ± 9	2.8 ± 0.8	55 ± 4	1.2 ± 0.0	73 ± 18	2.1
15d	9	−605 ± 11	TQ	265 ± 60	1.3 ± 0.5	182 ± 31	2.5 ± 0.1	53 ± 23	1.0 ± 0.1	49 ± 5	1.2 ± 0.6
16d	21	−611 ± 7	TQ	98 ± 22	0.8	223 ± 17	1.3	52 ± 5	1.8	nd	nd
17d	18	−627 ± 7	TQ	116 ± 4	0.5 ± 0.0	219 ± 27	1.8 ± 0.4	62 ± 5	1.5 ± 0.3	64 ± 3	1.3
18d	8.4	−671 ± 7	TQ	25 ± 6	0.7 ± 0.1	28 ± 4	1.4 ± 0.2	6 ± 1	0.9 ± 0.2	15 ± 6	1.5

[a] Solubility (μM) in α-MEM culture medium, determined by HPLC. [b] Measured on **6a**−**18a**; one-electron potential in mV determined by pulse radiolysis. [c] MEN, menadione = −203 ± 5 mV;[42] BV, benzyl viologen (1,1′-dibenzyl-4,4′-bipyridinium) = −375 ± 10 mV;[55] MV, methyl viologen (1,1′-dimethyl-4,4′-bipyridinium) = −447 ± 7 mV;[56] TQ, triquat (7,8-dihydrodipyrido-[1,2-*a*:2′,1′-*c*][1,4]diazepinediium) = −548 ± 7 mV.[56] [d] Values are mean ± SEM for up to five independent experiments. [e] Intraexperiment ratios. [f] Not determined.

Model carbamate **9b** (Scheme 4) was prepared as described previously[35] by reaction with 4-methoxyphenylisocyanate and di-*n*-butyltin diacetate in DCM, and **10b** was prepared similarly from **10a**.

Pulse radiolysis experiments were performed on a Dynaray 4 (4 MeV) linear accelerator (200 ns pulse length with a custom-built optical radical detection system). One-electron reduction potentials [*E*(1)] for the compounds were determined in anaerobic aqueous solutions containing 2-propanol (0.1 M) buffered at pH 7.0 (10 mM phosphate) by measuring the equilibrium constant[42] for the electron transfer between the radical anions of the compounds and the appropriate viologen or quinone reference standard. Data were obtained at three concentration ratios.

Results and Discussion

The solubility of compounds in α-MEM culture medium with 5% added fetal calf serum (FCS) was determined by HPLC analysis of the supernatant of a saturated solution. All the prodrugs (**8c**, **10c**, and **6d**−**18d**) were considerably less soluble than CB1954 (**1**) (4−21 μM, compared with 1600 μM) and slightly less soluble than the bromomustard **2** (Table 1). The prodrugs **6d**−**18d** were also less soluble than the corresponding amine **5** (197 μM) (Table 2). However, they were more soluble (5−40-fold) than the corresponding 4-nitrobenzyl carbamate of **5** (0.8 μM).[22]

The prodrugs **8c**−**10c** were unstable in α-MEM culture medium + 5% FCS over a 24 h period, whereas the prodrugs of **5** (with the exception of the 5-nitroimidazole **11d** and the pyrrole esters **12d** and **16d**) were stable under these conditions (as were the mustards **1** and **2**). 5-Nitroimidazole **11d** gave 30% of a more polar metabolite after 24 h, while the pyrrole esters **12d** and **16d** both underwent ca. 50% conversion to more polar metabolites, consistent with hydrolysis of the ester groups. Importantly, formation of the parent drug **5** was not observed for any of the amino-*seco*-CBI-TMI prodrugs **6d**−**18d**.

One-electron reduction potentials [*E*(1)], determined by pulse radiolysis, have been used as a surrogate measurement of the potential for hydride transfer from FMN to the substrate in reductase enzymes.[12−14] For most two-electron reduction systems, the potential for addition of the second electron [*E*(2)] is more positive than for the first [*E*(1)][43] and the two-electron reduction potential is half the sum of *E*(1) and *E*(2).[44] Studies with a related oxygen-insensitive nitroreductase (NR; EC 1.6.99.7) from *Enterobacter cloacae* found a relationship between the initial rates of reduction and *E*(1) for a series of para-substituted nitrobenzenes.[45] Further studies with *E. cloacae* suggested a more complex parabolic relationship between *E*(1) and reduction rate.[46] Nonetheless, *E*(1) is expected to provide a guide to the rate of nitro group reduction for a substrate by NTR. The

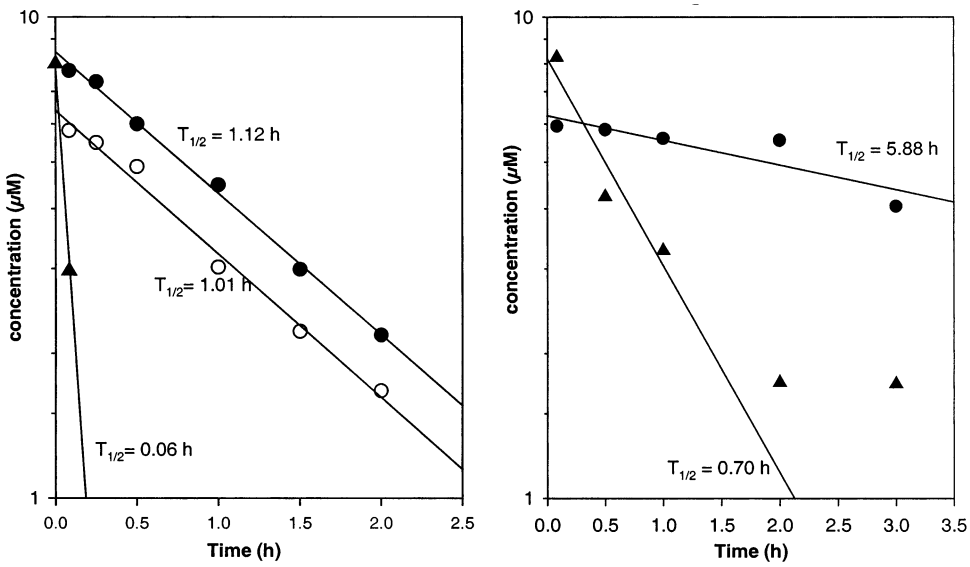


Figure 1. (A) Stability of **9c** (10 μM) in αMEM culture medium, ○; with EMT6 cells (10^6 mL^{-1}), ●; with EMT6-NTR^{puro} (10^6 mL^{-1}), ▲; under aerobic conditions. (B). Stability of **9d** (10 μM)) in αMEM culture medium with EMT6 cells (10^6 mL^{-1}), ●; with EMT6-NTR^{puro} (10^6 mL^{-1}), ▲; under aerobic conditions.

low solubility of the prodrugs **6d**−**18d** precluded $E(1)$ measurements on these compounds, but these were determined for the corresponding methanols **6a**−**18a** (Table 2) and two model carbamates **9b** and **10b**. The values for the latter (−419 ± 8 mV and −477 ± 9 mV, respectively) were indistinguishable from those of the parent alcohols, suggesting that the $E(1)$ values for **6a**−**18a** would reasonably approximate $E(1)$ values for **6d**−**18d**. The reduction potentials of these alcohols spanned a wide range [−222 ± 6 mV (**6a**) to −671 ± 7 mV (**18a**)] (Table 2), much larger than that previously covered. 4-Nitrobenzyl alcohol, which has been widely used previously to prepare carbamate substrates (**3**) for NTR,[16−22] has a reduction potential of −494 mV, while CB1954 (**1**) has a reduction potential of −385 mV[47] and the chloromustard analogue of **2** is intermediate at −421 ± 8 mV.[30]

The effectors and prodrugs were evaluated for cytotoxicity in four pairs of cell lines, each comprising a non-NTR-expressing cell line [SKOV3 (human ovarian carcinoma), WiDr (human colon carcinoma), V79^{puro} (Chinese hamster fibroblast), and EMT6 (mouse mammary carcinoma)], and the corresponding transfectants stably expressing NTR. Cytotoxicity was measured as IC_{50} values (following an 18 h drug exposure) in the NTR−ve lines, together with the ratios of the IC_{50} values between the NTR−ve and NTR+ve lines (Tables 1 and 2) as a measure of selectivity for NTR-expressing cells. The compounds were compared with CB1954 (**1**) and the related dibromomustard **2** in order to evaluate their potential as GDEPT candidates.

Phenylenediamine mustard (**4**) is an alkylating agent that has been used in a number of bioreductive prodrug approaches.[16,24,25] It has only moderate potency, with IC_{50} values of ca. 1−4 μM in the cell line panel (Table 1). The carbamate derivatives **8c**−**10c** were slightly more toxic (ca. 2-fold) than **4** in the wild-type human lines and equitoxic in the rodent cell lines. Given this lack of deactivation, the carbamates would not be expected to act as NTR prodrugs. However, surprisingly, all three showed significant increases in toxicity in NTR-expressing cells, with the largest NTR−ve/+ve ratios (5−28-fold) for the 2-nitroimidazole **9c**.

In an effort to explain the increase in toxicity displayed by **8c**−**10c** relative to **4**, the stability of **9c** and **9d** in culture medium was examined by LC/MS, following incubation in αMEM culture medium at 37 °C, with and without EMT6 or the transfected EMT6-NTR^{puro} cells at 10^6/mL, under aerobic conditions. Rapid loss of **9c** was observed in culture medium with a $T_{1/2}$ of ca. 1.0 h (Figure 1A), with no appreciable change in $T_{1/2}$ in the presence of EMT6 cells ($T_{1/2}$ = 1.1 h). The major products observed by mass spectrometry were consistent with the monohydroxy and dihydroxy hydrolysis products of **9c** (MH^+ = 398 and 380, respectively). In the presence of EMT6-NTR^{puro} cells the rate of loss of **9c** from the extracellular medium was increased 18-fold ($T_{1/2}$ = 0.06 h) and only very low levels of the mustard hydrolysis products were detected. In contrast, **9d** was stable in culture medium over 24 h and in the presence of EMT6 cells but was consumed ($T_{1/2}$ = 0.7 h) in the presence of EMT6-NTR^{puro} cells (Figure 1B). The relative rates of consumption indicate that **9c** is a better substrate than **9d**, but **9c** is hydrolytically unstable, despite the presumed deactivating effect of the carbamate linkage on mustard reactivity. Thus, the cytotoxicity of **4** (IC_{50} ca. 1−4 μM) observed in the cell line panel with an 18 h drug exposure appears underestimated due to rapid hydrolysis of the mustard group.[48] The mustard group in **9c** may be sufficiently stabilized by the increase in σ [σ_p(OCONH) = −0.17; σ_p(NH_2) = −0.66] to provide improved delivery of the mustard to the nuclear compartment and consequently increased cytotoxicity, but it is not sufficiently stabilized for it to be a useful prodrug.

This unfavorable balance between chemical reactivity and biological cytotoxicity for carbamates **7c**−**10c** prompted us to consider the more potent alkylating cytotoxin amino-*seco*-CBI-TMI (**5**), in which the chemical reactivity and cytotoxicity are less strongly linked. The effector **5** is ca. 1000-fold more potent than **4**,[28,29] with IC_{50} values of 0.3−1.6 nM across the panel of parental

(non-NTR-expressing) cell lines. Masking the amino group as a nitroheterocyclic carbamate (**6d**−**18d**) provided a variable (7−348-fold) decrease in cytotoxic potency in the NTR-negative cell lines, with the 5-nitroimidazole **11d** providing the greatest deactivation (66−348-fold).

For these compounds, the reduction potential significantly influenced selectivity. The nitropyrrole analogues (**15d**−**18d**), with $E(1)$ values below −600 mV, were not substrates for NTR, as shown by cell line ratios (NTR−ve/NTR+ve) of ca. 1. Analogues **12d**−**14d**, with $E(1)$ values between −500 and −600 mV, were poor substrates, inferred by cell line ratios of ca. 10 [conjugation of the ethyl carboxylate with the nitro group in **12d** raises the $E(1)$ sufficiently for reduction to occur, despite the nitro group being in a sterically hindered environment]. For compounds with $E(1)$ above ca. −500 mV, the data are more complex. As the $E(1)$ increases to ca. −394 mV (**10d**−**8d**) there is a corresponding increase in cytotoxic potency in the NTR−ve cell lines, yet the most electron-deficient compounds (the nitrofuran **7d** and the nitropyrazole **6d**) are relatively nontoxic. There is also a decrease in selectivity for NTR with increasing $E(1)$ for compounds **10d**−**7d** with the nitropyrazole **6d** displaying moderate selectivity. The 2-nitroimidazole **9d** had a selectivity of 15−62-fold while the 5-nitroimidazole **10d** was less potent (IC_{50} = 68−146 nM) but more selective (50−99-fold) for cells expressing NTR.

The addition of a larger *N*-1 side chain in compound **11d** resulted in lower cytotoxic potency (266−348 nM) and selectivity (1.2−26-fold). This suggests that steric bulk adjacent to the nitro group is not tolerated by the NTR enzyme in this series of prodrugs. Overall, the IC_{50} ratios (NTR−ve/NTR+ve) of prodrugs **6d**, **9d**, and **10d** were similar to that of the dinitrobenzamides **1** and **2** for the human cell lines (SKOV3 and WiDr), although their ratios in the two rodent cell line pairs were much lower. The reduction potentials, potencies, and cell line ratios for **9d** and **10d** are broadly similar to those previously seen for the related 2-alkoxy-4-nitrobenzyl carbamates of amino-*seco*-CBI-TMI.[22]

Prodrugs **9d** and **10d** were selected for in vivo evaluation because of their good selectivity in vitro. The maximum tolerated dose (MTD) in C3H mice of effector **5** given ip in DMSO was 2.37 μmol kg^{-1}, whereas the MTD of **9d** and **10d** was >1000 μmol kg^{-1}, demonstrating substantial masking of toxicity of the CBI effector in the prodrug form in vivo. In comparison, the MTD of dinitrobenzamide **1** was 240 μmol kg^{-1} given ip in 10% DMA/40% PEG400/50% water, whereas **2** (DMSO) was considerably less toxic (>1330 μmol kg^{-1}). The activities of **9d** and **10d** were evaluated against NTR-expressing EMT6 tumors containing mixtures of NTR−ve and NTR+ve cells. In this model nude mice are inoculated with 2:1 mixtures of EMT6-NTR^{puro} and EMT6 (NTR−ve) cells respectively, providing ca. 10% NTR+ve cells at the time of tumor treatment.[49] This tumor model represents the likely situation in a GDEPT protocol, where low rates of transfection of tumor tissue are expected. Although a small decrease in tumor cells was observed after treatment with **9d** or **10d**, these effects were not statistically significant (Figure 2). In contrast, as we have previously demonstrated,[22] dinitrobenzamides **1** and **2** given at 200 and 1330 μmol kg^{-1}, respectively, show significant killing of activator (EMT6-NTR^{puro}) cells and target (EMT6) cells, indicating the operation of a bystander effect. The lack of activity of **9d** and **10d** against solid tumors expressing only a fraction of NTR+ve cells may have several origins. The pharmacokinetics of these compounds have yet to be investigated and may not be optimal. Recent studies have also highlighted the requirement for high extravascular diffusion for bioreductive drugs targeted to the hypoxic subfraction of tumors.[50] Analogously, it is possible that the diffusion of these NTR prodrugs through the extravascular compartment to a limited number of NTR-transfected cells within the tumor may also be critical for in vivo activity. These possibilities require further investigation.

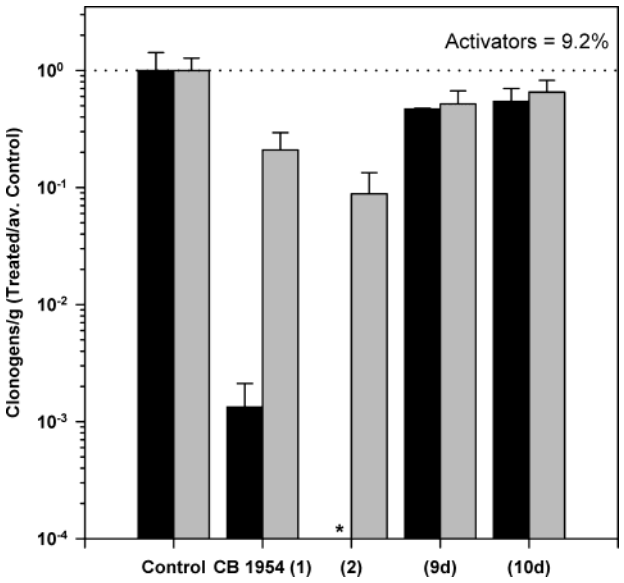


Figure 2. Comparison of in vivo activity and bystander effects for CB1954 (**1**), bromomustard **2**, 2-nitroimidazole carbamate **9d**, and 5-nitroimidazole carbamate **10d**. Prodrugs given at 200, 1330, 1000, and 1000 μmol kg^{-1} ip. In this experiment, tumors at excision comprised 9.2% EMT6-NTR^{puro} as assessed by the proportion of puromycin-resistant cells. Filled bars, EMT6-NTR^{puro}; shaded bars, EMT6. * EMT6-NTR^{puro} < 10^{-4} clonogens/g.

Conclusions

A range of nitroheterocyclic carbamates of the phenylenediamine mustard (**4**) showed low selectivity for NTR and were unsuitable as prodrugs because of their chemical reactivity and consequent instability in culture medium. A study of similar analogues of the amino-*seco*-CBI-TMI alkylating agent **5**, spanning a wider range of reduction potentials and structural variation, showed the important influence of reduction potential on both potency and selectivity for NTR-transduced cells. The selectivity of carbamates **6d**−**18d** for NTR across the cell line panel was greatest for those with $E(1)$ values between ca. −400 mV and ca. −500 mV, with the exception of the nitropyrazole carbamate **6d**. These prodrugs had solubilities broadly comparable to those of the "solubilized" nitrobenzyl carbamate prodrugs previously described.[22] This study identified analogues (**6d**, **9d**, and **10d**) with good selectivity for NTR in the human (SKOV3 and WiDr) and murine (EMT6) cell line pairs (although not as selective as the established dinitrobenzamide NTR prodrugs **1** and **2**). In contrast

to **1** and **2**, carbamates **9d** and **10d** did not show significant antitumor activity in the EMT6/EMT6-NTR^{puro} tumor model. The lack of activity against NTR+ve cells in tumors, despite potent and selective activity in culture, indicates that pharmacokinetic optimization will be required if in vivo efficacy against solid tumors is to be achieved with this new class of NTR prodrugs.

Experimental Section

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 melting point apparatus. IR spectra were recorded on a Midac FT-IR as KBr disks, unless otherwise stated. NMR spectra were obtained on a Bruker Avance 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. Assignments were determined using COSY, HSQC, and HMBC two-dimensional experiments. Mass spectra were determined on a VG-70SE mass spectrometer using an ionizing 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 and solvents were evaporated under reduced pressure on a rotary evaporator. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F_{254}) with visualization 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 testing were analyzed at >99% purity by reverse phase HPLC using an Agilent 1100 liquid chromatograph, an Altima C18 (5 μM) stainless steel column (150 mm × 3.2 mm i.d.) and an Agilent 1100 diode array detector. Chromatograms were run using various gradients of aqueous (0.045 M ammonium formate and formic acid at pH 3.5) and organic (80% MeCN/MilliQ water) phases. DCM refers to dichloromethane; DMF refers to dry dimethyl formamide; ether refers to diethyl ether; EtOAc refers to ethyl acetate; EtOH refers to ethanol; MeOH refers to methanol; pet. ether refers to petroleum ether, boiling range 40−60 °C; THF refers to tetrahydrofuran dried over sodium benzophenone ketyl. All solvents were freshly distilled. Alcohols **7a**,[26] **8a**,[34] **9a**,[35] **10a**,[35] **12a**,[36] **13a**,[37] and **15a**−**18a**[36] were synthesized as previously described.

(1-Methyl-5-nitro-1*H*-pyrazol-4-yl)methanol (6a). Borane dimethyl sulfide (2 M solution in THF, 4.2 mL, 8.4 mmol) was added to a solution of 1-methyl-5-nitro-1*H*-pyrazole-4-carboxylic acid[38] (**19**) (1.11 g, 6.5 mmol) in THF (50 mL) under N_2, and the mixture stirred at reflux temperature for 80 min and then cooled. MeOH (5 mL) and then water (5 mL) and then 2 M HCl (5 mL) were added, the THF was evaporated, and the residue was diluted with water and extracted with EtOAc (3 × 50 mL). The combined organic extract was dried, the solvent evaporated, and the residue purified by chromatography, eluting with 50% EtOAc/pet. ether, to give **6a** (0.52 g, 51%) as a white solid: mp (benzene) 78−80 °C; ^{1}H NMR δ 7.58 (s, 1 H, H-3), 4.82 (d, J = 3.4 Hz, 2 H, CH_2O), 4.25 (s, 3 H, NCH_3), 2.39 (br s, 1 H, OH). Anal. ($C_5H_7N_3O_3$) C, H, N.

[1-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-5-nitro-1*H*-imidazol-2-yl]methanol (23). A solution of Na (2.0 g, 87.6 mmol) in dry MeOH (30 mL) was added in one portion to a stirred solution of metronidazole (**20**) (10.0 g, 58.4 mmol) and benzaldehyde (7.1 mL, 70 mmol) in DMSO (30 mL) at 20 °C. The mixture stood at 20 °C for 24 h and then water (80 mL) was added and the resulting solid filtered. The solid was dissolved in EtOAc (100 mL) and dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/pet. ether, to give 2-{5-nitro-2-[(*E*)-2-phenylethenyl]-1*H*-imidazol-1-yl}ethanol (**21**) (4.0 g, 26%) as a yellow powder: mp (EtOAc/pet. ether) 155 °C (lit.[39] mp 156−157 °C); ^{1}H NMR δ 8.06 (s, 1 H, H-4′), 7.83 (d, J = 15.8 Hz, 1 H, CH═), 7.52−7.58 (m, 2 H, H-2″, H-6″), 7.33−7.38 (m, 3 H, H-3″, H-4″, H-5″), 7.05 (d, J = 15.8 Hz, 1 H, CH═), 4.64 (dd, J = 5.1, 5.0 Hz, 2 H, H-1), 4.07 (dd, J = 5.1, 5.0 Hz, 2 H, H-2), 2.42 (br s, 1 H, OH); ^{13}C NMR δ 150.9, 140.0, 138.5, 135.3, 134.6, 129.7, 128.9 (2), 127.6 (2), 112.1, 61.8, 47.7.

TBDMS triflate (2.7 mL, 11.8 mmol) was added dropwise to a stirred solution of alcohol **21** (2.77 g, 10.7 mmol) and pyridine (1.3 mL, 16.0 mmol) in DCM (100 mL) at −5 °C and the solution stirred at 20 °C for 16 h. The reaction was quenched with MeOH (5 mL) and poured into saturated aqueous $KHCO_3$ (100 mL). The mixture was extracted with DCM (3 × 50 mL), the combined organic fraction dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/pet. ether, to give *tert*-butyl-(dimethyl)silyl 2-{5-nitro-2-[(*E*)-2-phenylethenyl]-1*H*-imidazol-1-yl}ethyl ether (**22**) (4.00 g, 100%) as a yellow solid: mp (EtOAc/pet. ether) 99−100.5 °C; ^{1}H NMR δ 8.13 (s, 1 H, H-4′), 7.87 (d, J = 15.8 Hz, 1 H, CH═), 7.57 (d, J = 6.8 Hz, 2 H, H-2″, H-6″), 7.34−7.41 (m, 3 H, H-3″, H-4″, H-5″), 7.06 (d, J = 15.8 Hz, 1 H, CH═), 4.62 (dd, J = 5.0, 4.8 Hz, 2 H, H-1), 4.00 (dd, J = 5.0, 4.8 Hz, 2 H, H-2), 0.77 [s, 9 H, $SiC(CH_3)_3$], 0.10 [s, 6 H, $Si(CH_3)_2$]; ^{13}C NMR δ 151.2, 139.3, 138.4, 135.5, 134.8, 129.6, 128.9 (2), 127.5 (2), 112.9, 62.3, 47.8, 25.7 (3), 18.1, −5.8 (2). Anal. ($C_{19}H_{27}N_3O_3Si$) C, H, N.

Ozone was bubbled into a solution of imidazole **22** (1.3 g, 3.5 mmol) in DCM/MeOH (1:1, 120 mL) at −78 °C until a blue color persisted. The solution was warmed to −40 °C with a N_2 purge to remove excess ozone. A solution of $NaBH_4$ (132 mg, 3.5 mmol) in EtOH (10 mL) was added dropwise over 15 min and the mixture stirred for 30 min. The mixture was treated with acetic acid (0.5 mL) and stirred for 10 min, and the solvent was evaporated. The residue was partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (50 mL) and brine (25 mL) and dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/pet. ether, to give **23** (0.92 g, 88%) as a white solid: mp 104−105 °C; ^{1}H NMR δ 7.97 (s, 1 H, H-4), 4.79 (s, 2 H, CH_2O), 4.62 (t, J = 4.8 Hz, 2 H, CH_2O), 3.97 (t, J = 4.8 Hz, 2 H, CH_2N), 3.80 (br s, 1 H, OH), 0.81 [s, 9 H, $SiC(CH_3)_3$], 0.10 [s, 6 H, $Si(CH_3)_2$]; ^{13}C NMR δ 157.2, 138.8, 132.3, 62.0, 57.2, 48.3, 25.7 (3), 18.2, −5.8 (2). Anal. ($C_{12}H_{23}N_3O_4Si$) C, H, N.

(1-Methyl-4-nitro-1*H*-imidazol-5-yl)methanol (14a). Ozone was bubbled through a solution of 1-methyl-4-nitro-5-[(*E*)-2-phenylethenyl]-1*H*-imidazole[37] (**24**) (1.0 g, 4.4 mmol) in DCM/MeOH (1:1, 120 mL) at −78 °C until a blue color persisted. The solution was warmed to −40 °C with a nitrogen purge to remove excess ozone. A solution of $NaBH_4$ (165 mg, 4.4 mmol) in EtOH (10 mL) was added dropwise over 15 min and the mixture stirred for 30 min. Acetic acid (0.5 mL) was added and the solvent evaporated. The residue was partitioned between water (50 mL) and pet. ether (50 mL). The aqueous fraction was evaporated and the residue triturated with hot acetone (60 mL). The mixture was filtered and the solution concentrated to give **14a** (523 mg, 78%) as a tan powder: mp (acetone) 135−137 °C [lit.[51] mp ($CHCl_3$) 239−240 °C]; ^{1}H NMR δ 7.78 (s, 1 H, H-2), 5.48 (t, J = 5.6 Hz, 1 H, OH), 4.85 (d, J = 5.6 Hz, 2 H, CH_2O), 3.75 (s, 3 H, NCH_3); ^{13}C NMR δ 143.5, 136.8, 133.2, 51.4, 32.5. Anal. ($C_5H_7N_3O_3$) C, H, N.

(5-Nitro-2-furyl)methyl 4-[Bis(2-chloroethyl)amino]-phenylcarbamate (7c). 4-Nitrophenyl chloroformate (4.17 g, 20.7 mmol) in dry THF (20 mL) was added slowly to a stirred solution of (5-nitro-2-furyl)methanol[26] (**7a**) (2.69 g, 18.7 mmol) and pyridine (1.67 mL, 20.7 mmol) in dry THF (100 mL) at 20 °C under N_2. The mixture was stirred at 20 °C for 4 h and then partitioned between EtOAc/water (200 mL). The organic layer was washed with water (100 mL) and brine (80 mL) and dried, and the solvent was evaporated to give (5-nitro-2-furyl)-methyl 4-nitrophenyl carbonate (**25**) (4.79 g, 83%) as a white solid: mp (EtOAc/pet. ether) 93−94 °C; IR ν 1775, 1501, 1352, 1215 cm^{-1}; ^{1}H NMR [$(CD_3)_2SO$] δ 8.34 (ddd, J = 9.2, 3.2, 2.1 Hz, 2 H, H-3, H-5), 7.72 (d, J = 3.8 Hz, 1 H, H-4′), 7.61 (ddd,

J = 9.2, 3.2, 2.1 Hz, 2 H, H-2, H-6), 7.07 (d, J = 3.8 Hz, 1 H, H-3′), 5.43 (s, 2 H, CH_2O); ^{13}C NMR [$(CD_3)_2SO$] δ 155.0, 151.8, 151.5, 146.0, 145.2, 125.4 (2), 122.5 (2), 115.1, 113.2, 61.5. Anal. ($C_{12}H_8N_2O_8$) C, H, N.

A solution of carbonate **25** (1.0 g, 3.2 mmol), *N*,*N*-bis(2-hydroxyethyl)-1,4-benzenediamine (**35**) (3.6 mmol), and pyridine (0.26 mL, 3.2 mmol) in THF (80 mL) was stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with EtOAc to give (5-nitro-2-furyl)methyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (**29**) (0.56 g, 64%) as an colorless oil: ^1H NMR [$(CD_3)_2SO$] δ 9.44 (s, 1 H, OCONH), 7.68 (d, J = 3.7 Hz, 1 H, H-4′), 7.20 (br d, J = 9.1 Hz, 2 H, H-2, H-6), 6.93 (d, J = 3.7 Hz, 1 H, H-3′), 6.62 (d, J = 9.1 Hz, 2 H, H-3, H-5), 5.19 (s, 2 H, CH_2O), 4.71 (t, J = 5.4 Hz, 2 H, 2 × OH), 3.48−3.54 (m, 4 H, 2 × CH_2O), 3.33−3.38 (m, 4 H, 2 × CH_2N); ^{13}C NMR [$(CD_3)_2SO$] δ 154.0, 152.7, 151.5, 144.2, 127.0, 120.4 (2), 113.8, 113.6, 111.4 (2), 58.1 (2), 57.1, 53.4 (2); MS (DEI) m/z 365 (M^+, 15%), 334 (70), 222 (20), 196 (40), 191 (100); HRMS (DEI) calcd for $C_{16}H_{19}N_3O_7$ (M^+) m/z 365.1223, found 365.1218.

Methanesulfonyl chloride (170 μL, 2.1 mmol) was added dropwise to a stirred solution of diol **29** (260 mg, 0.7 mmol) in pyridine (5 mL) at 5 °C and the solution stirred at 20 °C for 1 h. The solvent was evaporated and the residue partitioned between DCM (80 mL) and water (80 mL). The aqueous fraction was washed with DCM (2 × 50 mL), the combined organic extracts were dried, and the solvent was evaporated. The residue was dissolved in DMF (10 mL), LiCl (180 mg, 4.3 mmol) added, and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL), the combined extracts were dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/pet. ether, to give **7c** (237 mg, 83%) as an oil: ^1H NMR δ 9.57 (s, 1 H, OCONH), 7.69 (d, J = 3.7 Hz, 1 H, H-4′), 7.28 (br d, J = 9.1 Hz, 2 H, H-2, H-6), 6.95 (d, J = 3.7 Hz, 1 H, H-3′), 6.70 (d, J = 9.1 Hz, 2 H, H-3, H-5), 5.21 (s, 2 H, CH_2O), 3.63−3.72 (m, 8 H, 2 × CH_2N, 2 × CH_2Cl); ^{13}C NMR [$(CD_3)_2SO$] δ 153.9, 152.7, 151.5, 142.3, 128.7, 120.3 (2), 113.8, 113.6, 112.3 (2), 57.2, 52.3 (2), 41.1 (2); MS m/z 405 (M^+, 10%), 403 (M^+, 30), 401 (M^+, 50), 354 (40), 352 (100); HRMS (DEI) calcd for $C_{16}H_{17}{}^{35}Cl_2N_3O_5$ (M^+) m/z 401.0545, found 401.0546; calcd for $C_{16}H_{17}{}^{35}Cl^{37}ClN_3O_5$ (M^+) m/z 403.0516, found 403.0521; calcd for $C_{16}H_{17}{}^{37}Cl_2N_3O_5$ (M^+) m/z 405.0486, found 405.0498.

(5-Nitro-2-thienyl)methyl 4-[Bis(2-chloroethyl)amino]-phenylcarbamate (8c). 4-Nitrophenyl chloroformate (2.58 g, 12.8 mmol) in dry THF (20 mL) was added slowly to a stirred solution of (5-nitrothien-2-yl)methanol[34] (**8a**) (1.85 g, 11.6 mmol) and pyridine (1.03 mL, 12.8 mmol) in dry THF (50 mL) at 20 °C under N_2. The mixture was stirred at 20 °C for 16 h and then partitioned between EtOAc and water. The organic layer was washed with saturated aqueous $NaHCO_3$ (50 mL) and water (50 mL) and dried and the solvent evaporated to give 4-nitrophenyl (5-nitro-2-thienyl)methyl carbonate (**26**) (1.86 g, 49%) as white solid: mp (EtOAc/pet. ether) 121−122 °C; IR ν 1763, 1522, 1345, 1231 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 8.33 (ddd, J = 9.2, 3.4, 2.2 Hz, 2 H, H-3, H-5), 8.08 (d, J = 4.2 Hz, 1 H, H-4′), 7.60 (ddd, J = 9.2, 3.4, 2.2 Hz, 2 H, H-2, H-6), 7.40 (d, J = 4.2 Hz, 1 H, H-3′), 5.56 (s, 2 H, CH_2O); ^{13}C NMR [$(CD_3)_2SO$] δ 155.0, 151.6, 151.5, 145.2, 144.8, 129.5, 129.0, 125.4 (2), 122.6 (2), 64.4. Anal. ($C_{12}H_8N_2O_7S$) C, H, N.

A solution of **26** (0.75 g, 2.3 mmol), *N*,*N*-bis(2-hydroxyethyl)-1,4-benzenediamine (**35**) (2.5 mmol), and pyridine (206 μL, 2.5 mmol) in THF (50 mL) was stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with EtOAc to give (5-nitro-2-thienyl)methyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (**30**) (0.56 g, 64%) as a cream solid: mp (EtOAc/pet. ether) 139−140.5 °C; IR ν 3360, 3208, 1730, 1530, 1337, 1215 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 9.46 (s, 1 H, OCONH), 8.02 (d, J = 4.2 Hz, 1 H, H-4′), 7.29 (d, J = 4.2 Hz, 1 H, H-3′), 7.21 (br d, J = 9.1 Hz, 2 H, H-2, H-6), 6.62 (d, J = 9.1 Hz, 2 H, H-3, H-5), 5.33 (s, 2 H, CH_2O), 4.72 (t, J = 5.5 Hz, 2 H, 2 × OH), 3.49−3.56 (m, 4 H, 2 × CH_2O), 3.36 (t, J = 6.2 Hz, 4 H, 2 × CH_2N); ^{13}C NMR [$(CD_3)_2SO$] δ 153.0, 150.8, 148.1, 144.3, 129.6, 127.5, 126.9, 120.5 (2), 111.4 (2), 60.1, 58.2 (2), 53.4 (2). Anal. ($C_{16}H_{19}N_3O_6S$) C, H; N, calcd 11.0, found 10.5%.

Methanesulfonyl chloride (260 μL, 3.4 mmol) was added dropwise to a stirred solution of diol **30** (0.43 g, 1.1 mmol) in pyridine (10 mL) at 5 °C and the solution stirred at 20 °C for 2 h. The solvent was evaporated and the residue partitioned between DCM (50 mL) and water (50 mL). The aqueous fraction was washed with DCM (2 × 50 mL), the combined organic extracts were dried, and the solvent was evaporated. The residue was dissolved in DMF (10 mL), LiCl (0.29 g, 6.8 mmol) added, and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL), the combined extracts were dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/pet. ether, to give **8c** (0.35 g, 69%) as pale green needles: mp (EtOAc/pet. ether) 99−100 °C; IR ν 3353, 1723, 1547, 1530, 1339, 1219 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 9.58 (br s, 1 H, OCONH), 8.04 (d, J = 4.2 Hz, 1 H, H-4′), 7.28−7.30 (m, 3 H, H-3′, H-2, H-6), 6.71 (d, J = 9.1 Hz, 2 H, H-3, H-5), 5.34 (s, 2 H, CH_2O), 3.65−3.72 (m, 8 H, 2 × CH_2N, 2 × CH_2Cl); ^{13}C NMR [$(CD_3)_2SO$] δ 153.0, 150.8, 148.2, 142.4, 129.5, 128.6, 127.5, 120.4 (2), 112.3 (2), 60.1, 52.2 (2), 41.1 (2). Anal. ($C_{16}H_{17}Cl_2N_3O_5S$) C, H, N, Cl.

***N*,*N*-Bis(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-4-nitroaniline (37).** A solution of TBDMSCl (4.20 g, 27.9 mmol) in DMF (15 mL) was added to a stirred solution of *N*,*N*-bis-(2-hydroxyethyl)-4-nitroaniline (**36**) (3.0 g, 13.3 mmol) and imidazole (3.79 g, 55.7 mmol) in DMF (50 mL), and the solution stirred at 20 °C for 48 h. The solvent was evaporated and the residue partitioned between EtOAc (150 mL) and water (150 mL). The organic fraction was washed with water (2 × 200 mL) and dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with 10% EtOAc/pet. ether, to give **37** (5.72 g, 95%) as a white solid: mp (pet. ether) 48−49 °C; IR ν 1597, 1520, 1300, 1202, 1107 cm^{-1}; ^1H NMR δ 8.07 (ddd, J = 9.5, 3.5, 2.1 Hz, 2 H, H-3, H-5), 6.67 (ddd, J = 9.5, 3.5, 2.1 Hz, 2 H, H-2, H-6), 3.80 (dd, J = 6.0, 5.7 Hz, 4 H, 2 × CH_2N), 3.63 (dd, J = 5.9, 5.7 Hz, 4 H, 2 × CH_2O), 0.86 [s, 18 H, 2 × $SiC(CH_3)_3$], 0.01 [s, 12 H, 2 × $Si(CH_3)_2$]; ^{13}C NMR δ 153.0, 138.6, 126.2 (2), 110.4 (2), 60.2 (2), 53.6 (2), 25.8 (6), 18.2 (2), −5.5 (4); MS (DEI) m/z 454 (M^+, 10%), 439 (5), 397 (10), 309 (100); HRMS (DEI) calcd for $C_{22}H_{42}N_2O_4Si_2$ (M^+) m/z 454.2683, found 454.2668. Anal. ($C_{22}H_{42}N_2O_4Si_2$) C, H, N.

***N*,*N*-Bis(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-1,4-benzenediamine (38).** A mixture of **37** (1.54 g, 3.4 mmol) and Pd/C (50 mg) in EtOAc/EtOH (1:1, 50 mL) was stirred under hydrogen (60 psi) for 30 min, filtered through Celite, and washed with EtOH (2 × 10 mL), and the solvent was evaporated to give crude benzenediamine **38** as an oil that was used directly without further purification or characterization.

(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-[Bis(2-chloroethyl)amino]phenylcarbamate (9c). (1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-nitrophenyl carbonate[35] (**27**) was prepared as previously described from alcohol **9a**. A solution of carbonate **27** (0.87 g, 2.7 mmol), aniline **38** (3.4 mmol), and pyridine (217 μL, 2.7 mmol) in THF (50 mL) was stirred at 20 °C for 48 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (2 × 50 mL) and brine (50 mL) and dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with a gradient (20−50%) of EtOAc/pet. ether, to give (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-[bis(2-{[*tert*-butyl(dimethyl)-silyl]oxy}ethyl)amino]phenylcarbamate (**31**) (1.37 g, 84%) as a white solid: mp 143−144 °C; IR ν 3258, 1721, 1539, 1257, 1103 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 7.23 (s, 1 H, H-4′), 7.15 (br d, J = 8.9 Hz, 2 H, H-2, H-6), 6.63 (d, J = 8.9 Hz, 2 H, H-3, H-5), 6.52 (br s, 1 H, OCONH), 5.20 (s, 2 H, CH_2O), 4.06 (s, 3 H, NCH_3), 3.73 (dd, J = 6.5, 6.3 Hz, 4 H, 2 × CH_2O), 3.47 (dd, J = 6.5, 6.3 Hz, 4 H, 2 × CH_2N), 0.88 [s, 18 H, 2 × $SiC(CH_3)_3$],

0.02 [s, 12 H, 2 × $Si(CH_3)_2$]; ^{13}C NMR [$(CD_3)_2SO$] δ 152.9, 146.1, 145.4, 132.5, 129.6, 125.4, 121.7 (2), 111.8 (2), 60.3 (2), 55.2, 53.6 (2), 34.3, 26.0 (6), 18.2 (2), −5.4 (4). Anal. ($C_{28}H_{49}N_5O_6Si_2$) C, H, N.

TBAF (1 M in THF, 3.9 mL, 3.9 mmol) was added dropwise to a stirred solution of **31** (1.07 g, 1.8 mmol) in THF (30 mL) at 5 °C. The solution was stirred for 30 min and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0−10%) of MeOH/EtOAc, to give (1-methyl-2-nitro-1*H*-imidazol-5-yl)methyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (**32**) (0.59 g, 88%) as a white solid: mp (MeOH) 171−174 °C; IR ν 3445, 3329, 3266, 1717, 1609, 1549, 1491, 1375, 1248 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 9.37 (s, 1 H, OCONH), 7.28 (s, 1 H, H-4′), 7.19 (br d, J = 9.1 Hz, 2 H, H-2, H-6), 6.61 (d, J = 9.1 Hz, 2 H, H-3, H-5), 5.23 (s, 2 H, CH_2O), 4.71 (t, J = 5.4 Hz, 2 H, 2 × OH), 3.96 (s, 3 H, NCH_3), 3.51 (dd, J = 6.4, 5.9 Hz, 4 H, 2 × CH_2O), 3.34 (dd, J = 6.2, 5.9 Hz, 4 H, 2 × CH_2N); ^{13}C NMR [$(CD_3)_2SO$] δ 152.8, 146.0, 144.2, 133.6, 128.6, 127.1, 120.3 (2), 111.4 (2), 59.7, 58.2 (2), 53.4 (2), 34.2; MS (FAB^+) m/z 380 (MH^+, 10%), 348 (5); HRMS (FAB^+) m/z calcd for $C_{16}H_{22}N_5O_6$ (MH^+) 380.1570, found 380.1579. Anal. ($C_{16}H_{21}N_5O_6$) C, H, N.

Methanesulfonyl chloride (190 μL, 2.5 mmol) was added dropwise to a stirred solution of diol **32** (312 mg, 0.8 mmol) in pyridine (10 mL) at 5 °C and the solution stirred at 20 °C for 1 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and water (100 mL). The aqueous fraction was washed with DCM (2 × 50 mL), the combined organic extracts were dried, and the solvent was evaporated. The residue was dissolved in DMF (10 mL), LiCl (210 mg, 4.9 mmol) added, and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL), the combined extracts were dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/pet. ether, to give **9c** (227 mg, 66%) as a white solid: mp (MeOH) 156−157 °C; IR ν 3408, 3246, 1725, 1531, 1354, 1221 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 9.49 (s, 1 H, OCONH), 7.26−7.29 (m, 3 H, H 4′, H-2, H-6), 6.70 (d, J = 9.1 Hz, 2 H, H-3, H-5), 5.24 (s, 2 H, CH_2O), 3.96 (s, 3 H, NCH_3), 3.64−3.70 (m, 8 H, 2 × CH_2N, 2 × CH_2Cl); ^{13}C NMR [$(CD_3)_2SO$] δ 152.8, 146.0, 142.3, 133.5, 128.7, 128.6, 120.3 (2), 112.3 (2), 54.9, 52.2 (2), 41.1 (2), 34.1. Anal. ($C_{16}H_{19}Cl_2N_5O_4$) C, H, N, Cl.

(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-[Bis(2-chloroethyl)amino]phenylcarbamate (10c). (1-Methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-nitrophenyl carbonate[35] (**28**) was prepared as previously described from alcohol **10a**. A solution of carbonate **28** (1.00 g, 3.1 mmol), aniline **38** (3.7 mmol), and pyridine (270 μL, 3.4 mmol) in THF (100 mL) was stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (2 × 50 mL) and brine (50 mL) and dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with a gradient (20−50%) of EtOAc/pet. ether, to give (1-methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-[bis(2-{[*tert*-butyl(dimethyl)-silyl]oxy}ethyl)amino]phenylcarbamate (**33**) (1.54 g, 82%) as a white solid: mp 117−118 °C; IR ν 3277, 1730, 1535, 1471, 1215, 1194 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 7.99 (s, 1 H, H-4′), 7.15 (br d, J = 8.9 Hz, 2 H, H-2, H-6), 6.63 (d, J = 8.9 Hz, 2 H, H-3, H-5), 6.59 (br s, 1 H, OCONH), 5.28 (s, 2 H, CH_2O), 4.05 (s, 3 H, NCH_3), 3.73 (dd, J = 6.5, 6.4 Hz, 4 H, 2 × CH_2O), 3.47 (dd, J = 6.5, 6.4 Hz, 4 H, 2 × CH_2N), 0.88 [s, 18 H, 2 × $SiC(CH_3)_3$], 0.02 [s, 12 H, 2 × $Si(CH_3)_2$]; ^{13}C NMR [$(CD_3)_2SO$] δ 152.9, 147.2, 145.4, 139.5, 132.1, 125.4, 121.7 (2), 111.8 (2), 60.3 (2), 57.8, 53.6 (2), 33.7, 25.9 (6), 18.3 (2), −5.4 (4). Anal. ($C_{28}H_{49}N_5O_6Si_2$) C, H, N.

TBAF (3.8 mL, 3.8 mmol) was added to dropwise to a stirred solution of carbamate **33** (1.06 g, 1.7 mmol) in THF (50 mL) at 5 °C. The solution was stirred for 30 min and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0−20%) of MeOH/EtOAc, to give (1-methyl-5-nitro-1*H*-imidazol-2-yl)methyl 4-[bis(2-hydroxyethyl)-amino]phenylcarbamate (**34**) (0.54 g, 82%) as a tan solid: mp (EtOAc/pet. ether) 157−158 °C; IR ν 3446, 3371, 1728, 1547, 1471, 1375, 1267 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 10.50 (br s, 1 H, OCONH), 8.02 (s, 1 H, H-4′), 7.20 (br d, J = 9.0 Hz, 2 H, H-2, H-6), 6.61 (br d, J = 9.0 Hz, 2 H, H-3, H-5), 5.25 (s, 2 H, CH_2O), 4.71 (br s, 2 H, 2 × OH), 3.95 (s, 3 H, NCH_3), 3.51 (dd, J = 6.1, 6.0 Hz, 4 H, 2 × CH_2O), 3.36 (dd, J = 6.2, 6.1 Hz, 4 H, 2 × CH_2N); ^{13}C NMR [$(CD_3)_2SO$] δ 152.7, 148.1, 144.2, 139.3, 131.9, 127.0, 120.3 (2), 111.4 (2), 58.2 (2), 57.3, 53.4 (2), 33.4; MS (DEI) m/z 379 (M^+, 35%), 348 (100), 318 (60); HRMS (DEI) calcd for $C_{16}H_{21}N_5O_6$ (M^+) m/z 379.1492, found 379.1482. Anal. ($C_{16}H_{21}N_5O_6$) C, H, N.

Methanesulfonyl chloride (240 μL, 3.1 mmol) was added dropwise to a stirred solution of diol **34** (390 mg, 1.0 mmol) in pyridine (10 mL) at 5 °C and the solution stirred at 20 °C for 1 h. The solvent was evaporated and the residue partitioned between DCM (100 mL) and water (100 mL). The aqueous fraction was washed with DCM (2 × 50 mL), the combined organic extracts were dried and the solvent evaporated. The residue was dissolved in DMF (10 mL), LiCl (260 mg, 6.2 mmol) added, and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL), the combined extracts were dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/pet. ether, to give **10c** (0.31 g, 72%) as a cream solid: mp ($CHCl_3$/pet. ether) 164−164.5 °C; IR ν 3250, 3185, 3127, 1723, 1603, 1547, 1516, 1381 cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 9.57 (br s, 1 H, OCONH), 8.08 (s, 1 H, H-4′), 7.27 (br d, J = 8.0 Hz, 2 H, H-2, H-6), 6.70 (d, J = 9.1 Hz, 2 H, H-3, H-5), 5.26 (s, 2 H, CH_2O), 3.95 (s, 3 H, NCH_3), 3.65−3.72 (m, 8 H, 2 × CH_2N, 2 × CH_2Cl); ^{13}C NMR [$(CD_3)_2SO$] δ 152.7, 148.0, 142.3, 139.3, 131.7, 128.7, 120.3 (2), 112.3 (2), 57.4, 52.2 (2), 41.1 (2), 33.4; MS (DEI) m/z 415 (M^+, 1%), 366 (2), 316 (2), 258 (20), 211 (30), 209 (100); HRMS (DEI) calcd for $C_{16}H_{19}{}^{35}Cl_2N_5O_4$ (M^+) m/z 415.0814, found 415.0808. Anal. ($C_{16}H_{19}Cl_2N_5O_4$) C, H, N.

Alternative Preparation of 10c. Diphosgene (85 μL, 0.7 mmol) was added dropwise to a stirred solution of alcohol **10a** (0.2 g, 1.3 mmol) and Et_3N (98 μL, 0.7 mmol) in THF (10 mL) at 5 °C. The suspension was stirred at 5 °C for 30 min and a mixture of *N*,*N*-bis(2-chloroethyl)-1,4-benzenediamine hydrochloride[27] (**4**) (0.38 g, 1.4 mmol) and Et_3N (195 μL, 1.4 mmol) in THF (4 mL) was added dropwise to the above suspension. The mixture was stirred at 20 °C for 4 h, the solvent evaporated, and the residue purified by chromatography, eluting with 50% EtOAc/pet. ether, to give carbamate **10c** (0.19 g, 36%); spectroscopically identical to the sample prepared above.

(1-Methyl-5-nitro-1*H*-pyrazol-4-yl)methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (6d). A solution of triphosgene (14.3 mg, 48 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine **5** (57 mg, 122 μmol) and Et_3N (38 μL, 275 μmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of alcohol **6a** (26 mg, 165 μmol) in DCM (2 mL) was added, followed by $^nBu_2Sn(OAc)_2$ (2 drops), and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20% EtOAc/DCM, to give **6d** (41 mg, 52%) as a white solid: mp (EtOAc/pet. ether) 201−202 °C; ^1H NMR [$(CD_3)_2SO$] δ 11.47 (br s, 1 H, indole-NH), 9.80 (br s, 1 H, OCONH), 8.56 (br s, 1 H, H-4), 8.06 (d, J = 8.5 Hz, 1 H, H-6), 7.98 (d, J = 8.3 Hz, 1 H, H-9), 7.74 (br s, 1 H, H-3″), 7.58 (dd, J = 8.3, 7.4 Hz, 1 H, H-8), 7.47 (dd, J = 8.5, 7.4 Hz, 1 H, H-7), 7.45 (d, J = 1.6 Hz, 1 H, H-3′), 6.98 (s, 1 H, H-4′), 5.33 (s, 2 H, CH_2O), 4.80 (dd, J = 11.0, 9.4 Hz, 1 H, H-2), 4.53 (dd, J = 11.0, 1.8 Hz, 1 H, H-2), 4.32−4.38 (m, 1 H, H-1), 4.17 (s, 3 H, NCH_3), 4.07 (dd, J = 11.0, 3.1 Hz, 1 H, CH_2Cl), 3.91−3.96 (m, 4 H, OCH_3, CH_2Cl), 3.82 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 160.2, 154.3, 149.1, 142.5, 141.5, 139.9, 139.0, 137.5, 134.2, 130.7, 129.4, 127.1, 125.4 (2), 124.3, 123.7, 123.3, 123.1, 122.0, 117.4, 113.0, 106.2, 98.0, 61.0, 60.9, 57.2, 55.9, 54.8, 47.5, 41.1, 40.8; MS (FAB^+) m/z 650 (MH^+, 2%),

648 (MH^+, 5); HRMS (FAB^+) calcd for $C_{31}H_{30}{}^{35}ClN_6O_8$ (MH^+) *m/z* 649.1814, found 649.1803; calcd for $C_{31}H_{30}{}^{37}ClN_6O_8$ (MH^+) *m/z* 651.1784, found 651.1796. Anal. ($C_{31}H_{29}ClN_6O_8$) C, H, N.

(5-Nitro-2-furyl)methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (7d). Similarly, reaction of amine **5** and alcohol **7a** gave **7d** (84%) as a white solid: mp (EtOAc/pet. ether) 185−187 °C; ^{1}H NMR δ 11.46 (s, 1 H, indole-NH), 9.92 (s, 1 H, OCONH), 8.54 (s, 1 H, H-4), 8.04 (d, J = 8.5 Hz, 1 H, H-6), 7.98 (d, J = 8.3 Hz, 1 H, H-9), 7.72 (d, J = 3.8 Hz, 1 H, H-4″), 7.58 (ddd, J = 8.3, 7.2, 0.7 Hz, 1 H, H-8), 7.45 (ddd, J = 8.5, 7.2, 0.7 Hz, 1 H, H-7), 7.09 (d, J = 2.1 Hz, 1 H, H-3′), 6.98−7.00 (m, 2 H, H-4′, H-3″), 5.30 (s, 2 H, CH_2O), 4.80 (dd, J = 10.8, 9.5 Hz, 1 H, H-2), 4.53 (dd, J = 10.8, 1.9 Hz, 1 H, H-2), 4.32−4.37 (m, 1 H, H-1), 4.07 (dd, J = 11.1, 3.0 Hz, 1 H, CH_2Cl), 3.91−3.96 (m, 4 H, OCH_3, CH_2Cl), 3.83 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3); ^{13}C NMR δ 160.1, 153.9, 153.8, 151.1, 149.2, 141.4, 139.8, 139.0, 134.0, 130.7, 129.4, 127.1, 125.4, 125.3, 124.3, 123.7, 123.3, 123.1, 122.2, 113.9, 113.6, 113.1, 106.2, 98.0, 61.0, 60.9, 57.7, 55.9, 54.8, 47.5, 41.1; MS (FAB^+) *m/z* 635 (MH^+, 6%), 637 (MH^+, 3); HRMS (FAB^+) calcd for $C_{31}H_{28}{}^{35}ClN_4O_9$ (MH^+) *m/z* 635.1545, found 635.1552; calcd for $C_{31}H_{28}{}^{37}ClN_4O_9$ (MH^+) *m/z* 637.1515, found 637.1514. Anal. ($C_{31}H_{27}ClN_4O_9$) C, H, N.

(5-Nitro-2-thienyl)methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (8d). Similarly, reaction of amine **5** and alcohol **8a** gave **8d** (89%) as a white solid: mp (EtOAc/pet. ether) 218−219 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.48 (br s, 1 H, indole-NH), 9.34 (br s, 1 H, OCONH), 8.55 (br s, 1 H, H-4), 8.08 (d, J = 4.1 Hz, 1 H, H-4″), 8.04 (d, J = 8.5 Hz, 1 H, H-6), 7.99 (d, J = 8.3 Hz, 1 H, H-9), 7.58 (dd, J = 8.3, 7.4 Hz, 1 H, H-8), 7.47 (dd, J = 8.5, 7.4 Hz, 1 H, H-7), 7.33 (d, J = 4.1 Hz, 1 H, H-3″), 7.10 (d, J = 1.9 Hz, 1 H, H-3′), 6.97 (s, 1 H, H-4′), 5.43 (s, 2 H, CH_2O), 4.80 (dd, J = 11.0, 9.5 Hz, 1 H, H-2), 4.54 (dd, J = 11.0, 1.8 Hz, 1 H, H-2), 4.32−4.36 (m, 1 H, H-1), 4.07 (dd, J = 11.0, 3.0 Hz, 1 H, CH_2Cl), 3.92−3.96 (m, 4 H, CH_2Cl, OCH_3), 3.92 (m, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 160.3, 154.4, 151.1, 149.3, 148.2, 141.6, 140.0, 139.1, 134.1, 130.8, 129.7, 129.6, 127.8, 127.4, 125.6, 125.5, 124.6, 123.8, 123.3, 123.2, 122.5, 113.5, 106.4, 98.1, 61.2, 61.0, 60.8, 56.0, 55.0, 47.7, 41.2; MS (FAB^+) *m/z* 653 (MH^+, 4%), 651 (MH^+, 8); HRMS (FAB^+) calcd for $C_{31}H_{28}{}^{35}ClN_4O_8S$ (MH^+) *m/z* 651.1316, found 651.1311; calcd for $C_{31}H_{28}{}^{37}ClN_4O_8S$ (MH^+) *m/z* 653.1287, found 653.1307. Anal. ($C_{31}H_{27}ClN_4O_8S$) C, H, N.

(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (9d). Similarly, reaction of amine **5** and alcohol **9a** gave **9d** (68%) as a tan powder: mp (EtOAc) 202−204 °C; ^{1}H NMR δ 9.49 (br s, 1 H, indole-NH), 8.81 (br s, 1 H, H-4), 7.86 (d, J = 8.5 Hz, 1 H, H-6), 7.78 (d, J = 8.3 Hz, 1 H, H-9), 7.57 (m, 1 H, H-8), 7.43 (m, 1 H, H-7), 7.25 (s, 1 H, H-4″), 7.21 (br s, 1 H, OCONH), 7.00 (d, J = 1.6 Hz, 1 H, H-3′), 6.87 (s, 1 H, H-4′), 5.31 (d, J = 13.6 Hz, 1 H, CH_2O), 5.25 (d, J = 13.6 Hz, 1 H, CH_2O), 4.80 (dd, J = 10.5, 1.6 Hz, 1 H, H-2), 4.65 (dd, J = 10.5, 8.7 Hz, 1 H, H-2), 4.13−4.20 (m, 1 H, H-1), 4.11 (s, 3 H, OCH_3), 4.01 (br s, 3 H, NCH_3), 3.94−3.98 (m, 4 H, CH_2Cl, OCH_3), 3.92 (s, 3 H, OCH_3), 3.47 (dd, J = 10.8, 10.8 Hz, 1 H, CH_2Cl); ^{13}C NMR δ 160.4, 153.5, 150.2, 146.4, 141.6, 140.7, 138.9, 133.3, 132.1, 129.8, 129.7, 129.5, 127.6, 125.7 (2), 125.1 (2), 123.6, 123.3, 122.3 (2), 106.6, 97.6, 61.5, 61.2, 56.3, 55.8, 54.9, 45.8, 43.4, 34.3; MS (FAB^+) *m/z* 649 (MH^+, 2%); HRMS (FAB^+) calcd for $C_{31}H_{30}{}^{35}ClN_6O_8$ (MH^+) *m/z* 649.1814, found 649.1767; calcd for $C_{31}H_{30}{}^{37}ClN_6O_8$ (MH^+) *m/z* 651.1784, found 651.1819. Anal. ($C_{31}H_{29}ClN_6O_8 \cdot {}^1/_2H_2O$) C, H, N.

(1-Methyl-5-nitro-1*H*-imidazol-2-yl)methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (10d). Similarly, reaction of amine **5** and alcohol **10a** gave **10d** (34%) as a tan powder: mp (EtOAc) 169−170 °C; ^{1}H NMR δ 9.47 (s, 1 H, indole-NH), 8.85 (s, 1 H, OCONH), 7.98 (s, 1 H, H-4″), 7.86 (d, J = 8.5 Hz, 1 H, H-6), 7.77 (d, J = 8.3 Hz, 1 H, H-9), 7.57 (br dd, J = 8.3, 7.4 Hz, 1 H, H-8), 7.44 (ddd, J = 8.5, 7.4, 0.7 Hz, 1 H, H-7), 7.37 (br s, 1 H, H-4), 6.99 (d, J = 2.3 Hz, 1 H, H-3′), 6.87 (s, 1 H, H-4′), 5.38 (d, J = 13.4 Hz, 1 H, CH_2O), 5.34 (d, J = 13.4 Hz, 1 H, CH_2O), 4.79 (dd, J = 10.7, 1.6 Hz, 1 H, H-2), 4.65 (dd, J = 10.7, 8.7 Hz, 1 H, H-2), 4.15−4.19 (m, 1 H, H-1), 4.09 (s, 3 H, OCH_3), 4.02 (br s, 3 H, NCH_3), 3.95 (s, 3 H, OCH_3), 3.92−3.94 (m, 4 H, OCH_3, CH_2Cl), 3.45 (dd, J = 10.9, 10.7 Hz, 1 H, CH_2Cl); ^{13}C NMR δ 160.4, 153.4, 150.2, 146.9, 141.6, 140.7, 139.6, 138.9, 133.3, 132.1, 129.7, 129.6, 127.6, 125.7, 125.2 (2), 123.6, 123.2, 122.3, 122.2, 112.8, 106.6, 97.7, 61.5, 61.2, 58.4, 56.3, 54.9, 45.8, 43.4, 33.8; MS (FAB^+) *m/z* 649 (MH^+, 3%), 651 (1.5); HRMS (FAB^+) calcd for $C_{31}H_{30}{}^{35}ClN_6O_8$ (MH^+) *m/z* 649.1814, found 649.1797; calcd for $C_{31}H_{30}{}^{37}ClN_6O_8$ (MH^+) *m/z* 651.1784, found 651.1802. Anal. ($C_{31}H_{29}ClN_6O_8$) C, H, N.

1-(2-Hydroxyethyl)-5-nitro-1*H*-imidazol-2-yl]methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (11d). Similarly, reaction of amine **5** and alcohol **23** gave [1-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-5-nitro-1*H*-imidazol-2-yl]methyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (**39**) (49%) as a colorless oil: ^{1}H NMR δ 9.41 (s, 1 H, indole-NH), 8.88 (s, 1 H, OCONH), 8.06 (s, 1 H, H-4″), 7.87 (d, J = 8.5 Hz, 1 H, H-6), 7.79 (d, J = 8.3 Hz, 1 H, H-9), 7.57 (dd, J = 8.3, 7.5 Hz, 1 H, H-8), 7.46 (dd, J = 8.5, 7.5 Hz, 1 H, H-7), 7.17 (br s, 1 H, H-4), 7.00 (d, J = 2.2 Hz, 1 H, H-3′), 6.88 (s, 1 H, H-4′), 5.44 (d, J = 13.5 Hz, 1 H, CH_2O), 5.39 (d, J = 13.5 Hz, 1 H, CH_2O), 4.81 (dd, J = 10.7, 1.5 Hz, 1 H, H-2), 4.65−4.74 (m, 3 H, H-2, CH_2N), 4.16−4.22 (m, 1 H, H-1), 4.10 (s, 3 H, OCH_3), 3.95−3.99 (m, 4 H, CH_2Cl, OCH_3), 3.89−3.93 (m, 5 H, OCH_3, CH_2N), 3.48 (t, J = 10.9 Hz, 1 H, CH_2Cl), 0.81 [s, 9 H, $SiC(CH_3)_3$], −0.08 [s, 6 H, $Si(CH_3)_2$]; MS (FAB^+) *m/z* 795 (MH^+, 12%), 793 (25); HRMS (FAB^+) calcd for $C_{38}H_{46}{}^{35}ClN_6O_9Si$ (MH^+) *m/z* 793.2784, found 793.2762; calcd for $C_{38}H_{46}{}^{37}ClN_6O_9Si$ (MH^+) *m/z* 795.2755, found 795.2751.

HCl (1 M, 0.23 mL, 230 μmol) was added to a stirred solution of silyl ether **39** (91 mg, 115 μmol) in MeOH (5 mL) and the solution stirred at 20 °C for 4 h. The solvent was evaporated and the residue partitioned between EtOAc (40 mL) and water (40 mL). The organic fraction was washed with water (25 mL) and brine (20 mL) and dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with a gradient (0−10%) of MeOH/40% EtOAc/DCM, to give **11d** as a white solid: mp (EtOAc/pet. ether) 148−150 °C (dec); ^{1}H NMR δ [$(CD_3)_2SO$] 11.45 (s, 1 H, indole-NH), 9.92 (s, 1 H, OCONH), 8.45 (s, 1 H, H-4), 8.16 (s, 1 H, H-4″), 8.06 (d, J = 8.5 Hz, 1 H, H-6), 7.97 (d, J = 8.3 Hz, 1 H, H-9), 7.58 (ddd, J = 8.3, 7.2, 0.8 Hz, 1 H, H-8), 7.46 (ddd, J = 8.5, 7.2, 0.8 Hz, 1 H, H-7), 7.09 (d, J = 2.0 Hz, 1 H, H-3′), 6.97 (s, 1 H, H-4′), 5.37 (s, 2 H, CH_2O), 5.12 (t, J = 5.4 Hz, 1 H, OH), 4.80 (dd, J = 10.7, 9.4 Hz, 1 H, H-2), 4.56−4.60 (m, 3 H, H-2, CH_2O), 4.32−4.38 (m, 1 H, H-1), 4.06 (dd, J = 11.1, 3.2 Hz, 1 H, CH_2Cl), 3.91−3.95 (m, 4 H, OCH_3, CH_2Cl), 3.83 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.70−3.75 (m, 2 H, CH_2N); ^{13}C NMR δ [$(CD_3)_2SO$] 160.1, 154.0, 149.1, 148.7, 141.4, 139.9, 139.0, 138.9, 134.1, 132.5, 130.7, 129.4, 127.1, 125.4, 125.3, 124.3, 123.8, 123.3, 123.1, 122.2, 113.2, 106.2, 98.0, 61.0, 60.9, 59.7, 58.1, 55.9, 54.9, 48.2, 47.8, 41.1; MS (FAB^+) *m/z* 681 (MH^+, 5%), 679 (MH^+, 12%); HRMS (FAB^+) calcd for $C_{32}H_{32}{}^{35}ClN_6O_9$ (MH^+) *m/z* 679.1919, found 679.1797; calcd for $C_{32}H_{32}{}^{37}ClN_6O_9$ (MH^+) *m/z* 681.1890, found 681.1892. Anal. ($C_{32}H_{31}ClN_6O_9$) C, H, N.

Ethyl 4-({[({1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-yl}-amino)carbonyl]oxy}methyl)-1-methyl-5-nitro-1*H*-pyrrole-2-carboxylate (12d). Similarly, reaction of amine **5** and alcohol **12a** gave **12d** (31%) as a white solid: mp (EtOAc/pet. ether) 248−250 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.45 (s, 1 H, indole-NH), 9.86 (s, 1 H, OCONH), 8.56 (br s, 1 H, H-4″), 8.09 (d, J = 8.5 Hz, 1 H, H-6″), 7.99 (d, J = 8.3 Hz, 1 H, H-9″), 7.59 (dd, J = 8.3, 7.7 Hz, 1 H, H-8″), 7.48 (dd, J = 8.5, 7.7 Hz, 1 H, H-7″), 7.10 (d, J = 2.0 Hz, 1 H, H-3″), 7.06 (br s, 1 H, H-3), 6.97 (s, 1 H, H-4″), 5.38 (s, 2 H, CH_2O), 4.80 (dd, J = 11.0, 9.6

Hz, 1 H, H-2″), 4.53 (dd, J = 11.0, 2.0 Hz, 1 H, H-2″), 4.35−4.40 (m, 1 H, H-1″), 4.29 (q, J = 7.1 Hz, 2 H, H-1′), 4.19 (s, 3 H, NCH_3), 4.07 (dd, J = 11.0, 3.0 Hz, 1 H, CH_2Cl), 3.92−3.96 (m, 4 H, CH_2Cl, OCH_3), 3.83 (m, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 1.31 (t, J = 7.1 Hz, 3 H, H-2′); ^{13}C NMR [$(CD_3)_2SO$] δ 160.2, 159.4, 154.3, 149.1, 141.5, 139.9, 139.0, 137.1, 134.2, 133.9, 130.7, 129.5, 127.1, 125.6, 125.5, 125.4, 124.3, 123.7, 123.3, 123.1, 122.1, 114.6, 113.0, 106.2, 98.0, 61.2, 61.0, 60.9, 59.6, 55.9, 54.9, 47.5, 41.1, 35.2, 14.0; MS (FAB$^+$) m/z 722 (MH^+, 0.3%), 720 (MH^+, 0.6); HRMS (FAB$^+$) calcd for $C_{35}H_{35}{}^{35}ClN_5O_{10}$ (MH^+) m/z 720.2073, found 720.2059; calcd for $C_{35}H_{35}{}^{37}ClN_5O_{10}$ (MH^+) m/z 722.2043, found 722.2031. Anal. ($C_{35}H_{34}ClN_5O_{10}$) C, H, N.

(1-Methyl-5-nitro-1*H*-imidazol-4-yl)methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (13d). Similarly, reaction of amine **5** and alcohol **13a** gave **13d** (79%) as a pale yellow powder: mp (EtOAc) 219−220 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.47 (s, 1 H, indole-NH), 9.80 (s, 1 H, OCONH), 8.54 (br s, 1 H, H-4), 8.08−8.11 (m, 2 H, H-6, H-2″), 7.97 (d, J = 8.4 Hz, 1 H, H-9), 7.57 (ddd, J = 8.4, 7.2, 0.7 Hz, 1 H, H-8), 7.45 (ddd, J = 8.5, 7.2, 0.7 Hz, 1 H, H-7), 7.09 (d, J = 2.0 Hz, 1 H, H-3′), 6.97 (s, 1 H, H-4′), 5.40 (s, 2 H, CH_2O), 4.79 (dd, J = 10.8, 1.4 Hz, 1 H, H-2), 4.52 (dd, J = 11.0, 1.9 Hz, 1 H, H-2), 4.31−4.36 (m, 1 H, H-1), 4.07 (dd, J = 11.1, 3.0 Hz, 1 H, CH_2Cl), 3.89−3.95 (m, 7 H, OCH_3, CH_2Cl, NCH_3), 3.83 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 160.1, 154.3, 154.2, 149.1, 141.4, 141.2, 139.9, 139.0, 135.1, 134.4, 130.8, 129.4, 127.0, 125.5, 125.4, 124.2, 123.9, 123.2, 123.1, 122.0, 113.1, 106.2, 98.0, 61.0, 60.9, 59.6, 55.9, 54.8, 47.5, 41.1, 35.1; MS (FAB$^+$) m/z 651 (MH^+, 1%), 649 (MH^+, 2); HRMS (FAB$^+$) calcd for $C_{31}H_{30}{}^{35}ClN_6O_8$ (MH^+) m/z 649.1814, found 649.1802; calcd for $C_{31}H_{30}{}^{37}ClN_6O_8$ (MH^+) m/z 651.1784, found 651.1761. Anal. ($C_{31}H_{29}ClN_6O_8$) C, H, N.

(1-Methyl-4-nitro-1*H*-imidazol-5-yl)methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (14d). Similarly, reaction of amine **5** and alcohol **14a** gave **14d** (56%) as a white powder: mp (EtOAc/pet. ether) 229−231 °C; ^{1}H NMR δ 9.48 (s, 1 H, indole-NH), 8.87 (s, 1 H, OCONH), 7.87 (d, J = 8.5 Hz, 1 H, H-6), 7.76 (d, J = 8.3 Hz, 1 H, H-9), 7.55 (ddd, J = 8.3, 7.4, 0.7 Hz, 1 H, H-8), 7.44 (ddd, J = 8.5, 7.4, 0.7 Hz, 1 H, H-7), 7.40 (s, 1 H, H-2″), 7.33 (br s, 1 H, H-4), 7.00 (d, J = 2.3 Hz, 1 H, H-3′), 6.87 (s, 1 H, H-4′), 5.61 (s, 2 H, CH_2O), 4.78 (dd, J = 10.7, 1.7 Hz, 1 H, H-2), 4.65 (dd, J = 10.7, 8.7 Hz, 1 H, H-2), 4.15−4.20 (m, 1 H, H-1), 4.05−4.10 (m, 4 H, OCH_3, CH_2Cl), 3.95 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.83 (br s, 3 H, NCH_3), 3.44 (dd, J = 10.9, 10.7 Hz, 1 H, CH_2Cl); ^{13}C NMR δ 160.4, 153.8, 150.2, 146.3, 141.5, 140.6, 138.8, 136.3, 133.4, 129.7, 129.5, 127.6, 126.9, 125.7, 125.2, 125.2, 123.6, 123.1, 122.3, 121.9, 112.5, 106.6, 97.7, 61.5, 61.1, 56.3, 54.9, 54.4, 45.8, 43.4, 33.2; MS (FAB$^+$) m/z 651 (MH^+, 1%), 649 (MH^+, 2); HRMS (FAB$^+$) calcd for $C_{31}H_{30}{}^{35}ClN_6O_8$ (MH^+) m/z 649.1814, found 649.1818; calcd for $C_{31}H_{30}{}^{37}ClN_6O_8$ (MH^+) m/z 651.1784, found 651.1805. Anal. ($C_{31}H_{29}ClN_6O_8$) C, H, N.

(1-Methyl-5-nitro-1*H*-pyrrol-2-yl)methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (15d). Similarly, reaction of amine **5** and alcohol **15a** gave **15d** (63%) as a white solid: mp (EtOAc) 212−214 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.45 (br s, 1 H, indole-NH), 9.82 (br s, 1 H, OCONH), 8.55 (br s, 1 H, H-4), 8.04 (d, J = 8.5 Hz, 1 H, H-6), 7.89 (d, J = 8.3 Hz, 1 H, H-9), 7.57 (dd, J = 8.3, 7.2 Hz, 1 H, H-8), 7.45 (dd, J = 8.5, 7.2 Hz, 1 H, H-7), 7.24 (d, J = 4.4 Hz, 1 H, H-4″), 7.09 (d, J = 2.0 Hz, 1 H, H-3′), 6.98 (s, 1 H, H-4′), 6.45 (d, J = 4.4 Hz, 1 H, H-3″), 5.30 (s, 2 H, CH_2O), 4.80 (dd, J = 11.0, 9.4 Hz, 1 H, H-2), 4.53 (dd, J = 11.0, 1.8 Hz, 1 H, H-2), 4.32−4.37 (m, 1 H, H-1), 4.07 (dd, J = 11.1, 3.1 Hz, 1 H, CH_2Cl), 3.91−3.96 (m, 7 H, CH_2Cl, NCH_3, OCH_3), 3.83 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 160.2, 154.0, 149.1, 142.1, 141.4, 139.9, 138.2, 136.1, 134.2, 130.7, 129.4, 127.1, 125.3, 125.2, 124.3, 123.7, 123.3, 123.1, 122.1, 113.1, 113.0, 110.6, 106.2, 98.0, 61.0, 60.9, 57.6, 55.9, 54.8, 47.5, 41.1, 33.9; MS (FAB$^+$) m/z 650 (MH^+, 1%), 648 (MH^+, 2); HRMS (FAB$^+$) calcd for $C_{32}H_{31}{}^{35}ClN_5O_8$ (MH^+) m/z 648.1861, found 648.1852; calcd for $C_{32}H_{31}{}^{37}ClN_5O_8$ (MH^+) m/z 650.1832, found 650.1836. Anal. ($C_{32}H_{30}ClN_5O_8$) C, H, N.

Ethyl 5-({[({1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-yl}amino)carbonyl]oxy}methyl)-1-methyl-4-nitro-1*H*-pyrrole-2-carboxylate (16d). Similarly, reaction of amine **5** and alcohol **16a** gave **16d** (62%) as a white solid: mp (EtOAc/pet. ether) 227−229 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.46 (s, 1 H, indole-NH), 9.83 (s, 1 H, OCONH), 8.56 (br s, 1 H, H-4″), 8.02 (d, J = 8.5 Hz, 1 H, H-6″), 7.97 (d, J = 8.3 Hz, 1 H, H-9″), 7.57 (dd, J = 8.3, 7.4 Hz, 1 H, H-8″), 7.46 (dd, J = 8.5, 7.4 Hz, 1 H, H-7″), 7.43 (s, 1 H, H-3), 7.10 (d, J = 2.0 Hz, 1 H, H-3″), 6.98 (s, 1 H, H-4″), 5.63 (s, 2 H, CH_2O), 4.80 (dd, J = 11.0, 9.4 Hz, 1 H, H-2″), 4.53 (dd, J = 11.0, 1.9 Hz, 1 H, H-2″), 4.33−4.37 (m, 1 H, H-1″), 4.29 (q, J = 7.1 Hz, 2 H, H-1′), 4.00−4.08 (m, 4 H, CH_2Cl, NCH_3), 3.91−3.95 (m, 4 H, CH_2Cl, OCH_3), 3.83 (m, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 1.31 (t, J = 7.1 Hz, 3 H, H-2′); ^{13}C NMR [$(CD_3)_2SO$] δ 160.1, 159.3, 154.0, 149.1, 141.4, 139.9, 139.0, 134.1, 133.6, 133.4, 130.7, 129.4, 127.1, 125.4, 125.3, 124.3, 123.7, 123.3, 123.1, 122.4, 122.1, 113.0, 111.2, 106.2, 98.0, 61.0, 60.9, 60.8, 55.9, 54.8, 54.6, 47.5, 41.1, 33.6, 13.9; MS (FAB$^+$) m/z 722 (MH^+, 2.5%), 720 (MH^+, 6); HRMS (FAB$^+$) calcd for $C_{35}H_{35}{}^{35}ClN_5O_{10}$ (MH^+) m/z 720.2073, found 720.2045; calcd for $C_{35}H_{35}{}^{37}ClN_5O_{10}$ (MH^+) m/z 722.2043, found 722.2039. Anal. ($C_{35}H_{34}ClN_5O_{10}$) C, H, N.

(1-Methyl-2-nitro-1*H*-pyrrol-3-yl)methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (17d). Similarly, reaction of amine **5** and alcohol **17a** gave **17d** (28%) as a white solid: mp (EtOAc) 218−220 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.46 (br s, 1 H, indole-NH), 9.80 (br s, 1 H, OCONH), 8.55 (br s, 1 H, H-4), 8.09 (d, J = 8.5 Hz, 1 H, H-6), 7.98 (d, J = 8.3 Hz, 1 H, H-9), 7.58 (dd, J = 8.3, 7.3 Hz, 1 H, H-8), 7.48 (dd, J = 8.5, 7.3 Hz, 1 H, H-7), 7.33 (d, J = 2.6 Hz, 1 H, H-4″), 7.09 (d, J = 1.9 Hz, 1 H, H-3′), 6.98 (s, 1 H, H-4′), 6.37 (br s, 1 H, H-3″), 5.37 (s, 2 H, CH_2O), 4.80 (dd, J = 11.0, 9.3 Hz, 1 H, H-2), 4.52 (dd, J = 11.0, 1.9 Hz, 1 H, H-2), 4.31−4.37 (m, 1 H, H-1), 4.07 (dd, J = 11.1, 3.0 Hz, 1 H, CH_2Cl), 3.96 (s, 3 H, NCH_3), 3.91−3.94 (m, 4 H, CH_2Cl, OCH_3), 3.82 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 160.2, 154.4, 149.1, 141.5, 139.9, 139.0, 134.3, 133.2, 130.7, 130.4, 129.4, 127.1, 125.4, 125.3, 124.3, 123.8, 123.3, 123.1, 122.6, 122.0, 113.0, 108.1, 106.1, 98.0, 61.0, 60.9, 60.2, 55.9, 54.8, 47.5, 41.1, 37.9; MS (FAB$^+$) m/z 650 (MH^+, 1.5%), 648 (MH^+, 3.5); HRMS (FAB$^+$) calcd for $C_{32}H_{31}{}^{35}ClN_5O_8$ (MH^+) m/z 648.1861, found 648.1844; calcd for $C_{32}H_{31}{}^{37}ClN_5O_8$ (MH^+) m/z 650.1832, found 650.1826. Anal. ($C_{32}H_{30}ClN_5O_8$) C, H, N.

(1-Methyl-3-nitro-1*H*-pyrrol-2-yl)methyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-1-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (18d). Similarly, reaction of amine **5** and alcohol **18a** gave **18d** (28%) as a white solid: mp (EtOAc) 218−220 °C; ^{1}H NMR δ 9.42 (s, 1 H, indole-NH), 8.94 (s, 1 H, OCONH), 7.87 (d, J = 8.4 Hz, 1 H, H-6), 7.79 (d, J = 8.2 Hz, 1 H, H-9), 7.57 (ddd, J = 8.2, 7.4, 0.9 Hz, 1 H, H-8), 7.47 (ddd, J = 8.4, 7.4, 0.9 Hz, 1 H, H-7), 7.08 (br s, 1 H, H-4), 7.01 (d, J = 2.2 Hz, 1 H, H-3′), 6.89 (s, 1 H, H-4′), 6.80 (d, J = 3.3 Hz, 1 H, H-5″), 6.57 (d, J = 3.3 Hz, 1 H, H-4″), 5.65 (s, 2 H, CH_2O), 4.81 (dd, J = 10.7, 9.7 Hz, 1 H, H-2), 4.67 (dd, J = 10.7, 8.6 Hz, 1 H, H-2), 4.15−4.20 (m, 1 H, H-1), 4.10 (s, 3 H, OCH_3), 3.95−3.99 (m, 4 H, OCH_3, CH_2Cl), 3.92 (s, 3 H, OCH_3), 3.80 (br s, 3 H, NCH_3), 3.48 (dd, J = 11.0, 10.7 Hz, 1 H, CH_2Cl); ^{13}C NMR δ 160.4, 153.9, 150.2, 141.6, 140.6, 138.9, 133.7, 130.9, 129.7, 129.6, 128.8, 127.5, 125.6, 125.1 (2), 123.6, 123.1, 122.6, 122.2, 121.6, 113.0, 106.5, 106.1, 97.7, 61.5, 61.1, 56.3, 55.3, 54.9, 45.8, 43.4, 35.3; MS (FAB$^+$) m/z 650 (MH^+, 0.6%), 648 (MH^+, 1.5); HRMS (FAB$^+$) calcd for $C_{32}H_{31}{}^{35}ClN_5O_8$ (MH^+) m/z 648.1861, found 648.1850; calcd for $C_{32}H_{31}{}^{37}ClN_5O_8$ (MH^+) m/z 650.1832, found 650.1841. Anal. ($C_{32}H_{30}ClN_5O_8$) C, H, N.

Alternative Preparations of 9d. Method 1. A solution of **9a** (17 mg, 110 μmol) in DCM (2 mL) was added dropwise to a stirred solution of triphosgene (12 mg, 40 μmol) and pyridine (9 μL, 110 μmol) in DCM (2 mL) at 20 °C. The mixture

was stirred at 20 °C for 2 h, the solvent removed under reduced pressure, and the residue dissolved in anhydrous THF (5 mL). A solution of amine **5** (50 mg, 110 μmol) in THF (5 mL) was added and the solution stirred at 20 °C for 16 h. The mixture was partitioned between EtOAc (50 mL) and saturated aqueous $KHCO_3$ solution (50 mL), the organic fraction dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (50−100%) of EtOAc/pet. ether, to give **9d** (23 mg, 33%) as a tan solid: mp 200−205 °C (dec); spectroscopically identical with an authentic sample prepared above.

Method 2. Diethylisopropylamine (25 μL, 140 μmol) was added to a stirred mixture of carbonate **27** (42 mg, 130 μmol), amine **5** (57 mg, 140 μmol), HOBT (19 mg, 140 μmol), and 4 Å powdered molecular sieves (200 mg) in dry DMF (5 mL) under N_2. The mixture was stirred for 6 days, filtered, diluted with EtOAc (50 mL), washed with water (4 × 30 mL) and brine (25 mL), and dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/pet. ether, to give (i) starting amine **5** (40 mg, 70%) and (ii) carbamate **9d** (19 mg, 19%), spectroscopically identical with an authentic sample prepared above.

Cell Lines. Four pairs of cell lines, each comprising a tumor cell line and corresponding transfectant stably expressing NTR, were grown as monolayers in αMEM containing 5% fetal bovine serum. V79-NTR^{puro}, also known as T79-A3, is a Chinese hamster fibroblast which expresses NTR from an CMV promoter; the corresponding NTR−ve line, here referred to as V79^{puro}, has been transfected with the empty shuttle vector and is also known as T78-1.[14] SKOV3-NTR^{neo} and WiDr-NTR^{neo}, also known as SC3.2 and WC14, respectively, are human ovarian and colon carcinoma lines derived from SKOV3 and WiDr, which also express NTR from a CMV promoter.[52] EMT6-NTR^{puro}, also known as EN2A, is a murine breast carcinoma line derived from EMT6 and expresses NTR from a bicistronic cassette with an EF-1α promoter.[49] Selection for NTR expression was maintained during passage, but not during experiments, using 15 μM puromycin (V79-NTR^{puro}), 5 μM puromycin (EMT6-NTR^{puro}), or 300 μg/mL G418 (WiDr-NTR^{neo}; SKOV3-NTR^{neo}).

Growth Inhibition Assays. Growth inhibitory potencies were determined under aerobic conditions using log-phase cultures in 96-well plates, as described previously.[53,54] Cultures were initiated 24 h before an 18 h drug exposure, with cell densities determined 4−5 days later by staining with sulforhodamine B. IC_{50} values were calculated as the drug concentration providing 50% inhibition of growth relative to controls on the same plate.

Mouse Toxicity. Compound **1** was formulated in 10% DMA/40% PEG400/50% water, and compounds **2**, **9d**, and **10d** were formulated in DMSO immediately before use. Groups of six male C3H mice (ca. 25 g) were treated ip with single doses of compounds at 10 μL/g body weight for **1** and 1 μL/g for **2**, **9d**, and **10d** using $10^{1/8}$-fold dose increments and were observed daily for 60 days. Any animals losing >15% body weight or becoming moribund during the study were terminated.

In Vivo Excision Assay. Activity was assessed by treating mice with tumors containing mixtures of EMT6-NTR^{puro} cells and EMT6 cells. CD-1 nude mice were inoculated subcutaneously with 3×10^6 cells using a 2:1 mixture of EMT6-NTR^{puro} and EMT6 cells. When the tumors reached a mean diameter (length × width) of 9 ± 1 mm, the animals were randomized to treatment groups (five animals/group). Mice were treated ip with single doses of prodrugs, at the MTD as determined in C_3H mice, and tumors were removed 18 h later to determine cell killing by clonogenic assay as reported elsewhere.[49] Briefly, tumors were dissected, weighed, and dissociated in a Pronase/collagenase/DNAase cocktail. Cell numbers were determined with a particle counter (Coulter Electronics) and up to 10^5 cells were plated in medium containing 3 μM puromycin or nonselective medium to quantify survival of EMT6-NTR^{puro} and total tumor cells, respectively. Plates were incubated for 8 days, and colonies of >50 cells were counted. The plating efficiency of EMT6 cells was estimated from the difference between plating efficiency in puromycin and nonselective medium, and the number of clonogens of both types was calculated per gram of tumor tissue for control and treated tumors. Statistical significance of drug effects was determined by ANOVA using Dunnett's test to compare groups.

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