Structure−Activity Relationships for 4-Nitrobenzyl Carbamates of 5-Aminobenz[*e*]indoline Minor Groove Alkylating Agents as Prodrugs for GDEPT in Conjunction with *E. coli* Nitroreductase

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Twelve substituted 4-nitrobenzyl carbamate prodrugs of the 5-aminobenz[*e*]indoline class of DNA minor groove alkylating agents were prepared and tested as prodrugs for gene-directed enzyme prodrug therapy (GDEPT) using a two-electron nitroreductase (NTR) from *E. coli* B. The prodrugs and effectors were tested in a cell line panel comprising parental and transfected human (SKOV/Skov-NTR^{neo}, WiDr/WiDr-NTR^{neo}), Chinese hamster (V79^{puro}/V79-NTR^{puro}), and murine (EMT6/EMT6-NTR^{puro}) cell line pairs. In the human cell line pairs, several analogues bearing neutral methoxyethoxy-, 2-hydroxyethoxy-, or 3-hydroxypropoxy-substituted side chains were good substrates for NTR as measured by cytotoxicity ratios, with NTR-ve/NTR+ve ratios similar to the established NTR substrates CB1954 (an aziridinyl dinitrobenzamide) and the analogous bromomustard. Selectivity for NTR decreased with increasing side-chain size or the presence of a basic amine group. Low to modest selectivity was observed in the Chinese hamster-derived cell line pair; however, in the murine EMT6/EMT6-NTR^{puro} cell line pair, the above hydroxyalkoxy analogues again showed significant selectivity for NTR. The activity of the 2-hydroxyethoxy analogue was evaluated against NTR-expressing EMT6 tumors comprising ca. 10% NTR+ve cells at the time of tumor treatment. A small decrease in NTR+ve cells was observed after treatment, with a lesser effect against NTR-ve target cells, but these effects were not statistically significant and were much less than for the dinitrobenzamides. These results suggest that useful GDEPT prodrugs based on the 4-nitrobenzyl carbamate and 5-aminobenz[*e*]indoline motifs may be developed if optimization of pharmacokinetics can be addressed.

Introduction

Gene-directed enzyme prodrug therapy (GDEPT) for cancer uses various vector systems to deliver genes for prodrug-activating enzymes (usually nonhuman) selectively to tumor tissue.[1,2] Subsequent systemic administration of a nontoxic prodrug that is a good substrate for the introduced enzyme then results in selective generation of active drug ("effector")[3] in the tumor cells. Provided this compound is able to diffuse to kill neighboring tumor cells (bystander effect),[4] the approach can compensate for the low efficiencies of gene transduction for most gene vector delivery systems. Nitroreductases are suitable enzymes for prodrug activation, since the very large electronic change generated in a prodrug by reduction of an aromatic nitro group (Hammett σ_p = 0.78) to the corresponding hydroxylamine ($\sigma_p = -0.34$) provides an efficient "switch" mechanism.[5] The most widely studied nitroreductase for GDEPT is the oxygen-insensitive enzyme from *E. coli* (the *nfsB* gene product NTR).[6,7]

The substrates reported for prodrug approaches with NTR fall into two classes. The first, dinitrobenzamide alkylating agents, is exemplified by the aziridine CB 1954 (**1**)[8−11] which is now in clinical trial as a prodrug for GDEPT using NTR.[12,13] More recently, related nitrogen mustards have been developed as prodrugs for NTR[14−16] and the bromomustard SN 24927 (**2**) has shown superior in vivo activity and a larger bystander effect compared to CB1954 in preclinical models.[17] The second class of substrates for NTR features a 4-nitrobenzyl carbamate motif (4-$NO_2PhCH_2OCONHR$). The reduction of such 4-nitrobenzyl carbamates to the corresponding hydroxylamines has been shown to induce spontaneous fragmentation, releasing amines RNH_2.[18−20] Examples of 4-nitrobenzyl carbamate prodrugs of DNA-reactive agents include aniline mustards,[21] mitomycin C,[21] enediynes,[22] and pyrrolobenzodiazepines.[23] Examples involving DNA-binding species include prodrugs of tallimustine derivatives,[24] doxorubicin, and actinomycin D.[21] Notably, all of these examples have used the unsubstituted 4-nitrobenzyl carbamate as a trigger.

We recently studied[19,20] the factors affecting the kinetics of spontaneous fragmentation of 4-hydroxylaminobenzyl carbamates ($HOHNPhCH_2OCONR_1R_2$). We showed that the unsubstituted 4-hydroxylaminobenzyl carbamate fragmented relatively slowly ($t^1/_2$: 16 min at 20 °C). Consistent with the proposed mechanism of fragmentation, this process could be accelerated by electron-donating substituents on the benzyl ring (stabilizing the developing positive charge on the benzylic carbon), according to the equation: $\log(t_{1/2}) = 0.57\sigma + 1.30$. This study suggested that appropriately

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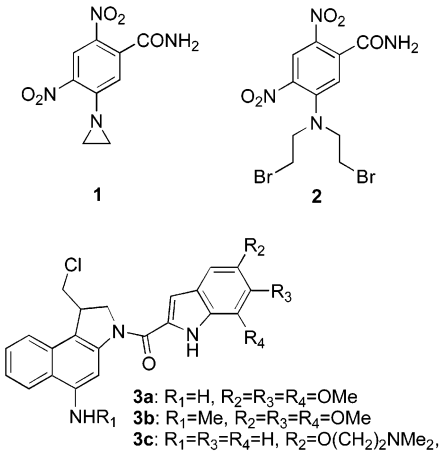


substituted 4-nitrobenzyl carbamates, particularly 2′-alkoxy analogues, could be superior triggers because of faster fragmentaion kinetics.

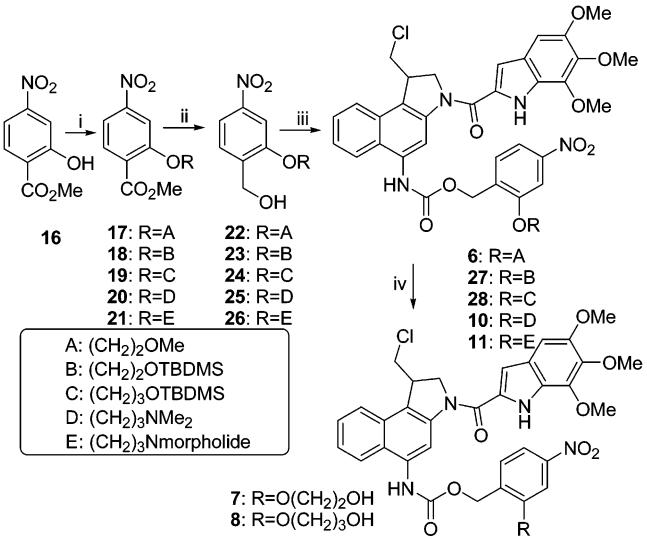
In this paper we build on these kinetic studies by linking modified 4-nitrobenzyl triggers (including 2′-alkoxy derivatives) to very potent 5-aminobenz[*e*]indoline cytotoxins. We have reported recently[25,26] on 5-aminobenz[*e*]indolines (e.g., **3a**−**c**), which are compounds related to the duocarmycins[27] that alkylate DNA in the minor groove at the N3 of adenines, preferentially in 5′-polyA sequences.[28] This process is deactivated by acylation of the amine, which makes these compounds of interest as effectors for 4-nitrobenzyl carbamate prodrugs. We report our in vitro and in vivo studies on the effectiveness of modified 4-nitrobenzylcarbamates **4**−**15** as prodrugs for a NTR-mediated GDEPT approach.

Chemistry

The benzyl alcohols **22**−**26**, required for carbamate formation, were prepared by alkylation of methyl 2-hydroxy-4-nitrobenzoate[19] (**16**), with the appropriate halides to give ethers **17**−**21**, followed by reduction with DIBAL-H (Scheme 1). The preparation of alcohol **32** involved alkylation of **16** with epichlorohydrin under basic conditions to give epoxide **29**, which was hydrolyzed with perchloric acid to the diol **30**. This was protected as the acetonide **31** and then reduced with DIBAL-H to the required benzyl alcohol **32** (Scheme 2). Alcohol **37** was prepared by activation of alcohol **34** as the 4-nitrophenyl carbonate **35** and subsequent reaction with 4-aminobenzyl-TBDMS ether to give **36** which was deprotected to provide **37** (Scheme 3).

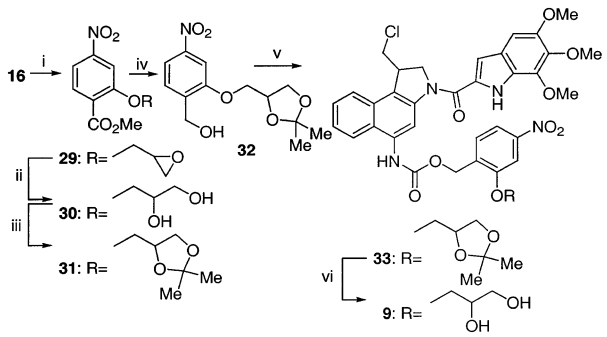
Two methods were used to couple amines **3a**[25] and **3b**[25] with the various alcohols. Reaction of the chloroformates of **34** and **38** wth **3a**, formed in situ, gave carbamates **5** and **12** in moderate yields (Scheme 4). Reaction of 4-nitrobenzyl chloroformate with **3a** and **3b** gave carbamates **4** and **14**, in modest yield (Scheme 5). Similarly, reaction of 3-NBoc derivative **39** with 4-nitrobenzyl chloroformate gave carbamate **40**, which was deprotected and coupled with 5-[2-(dimethylamino)-ethoxy]-1*H*-indole-2-carboxylic acid[26] to give carbamate **15**. Significantly higher yields (47−96%) were obtained by in situ formation of the isocyanate of **3a**, followed by reaction with alcohols and catalytic dibutyltindiacetate,

Scheme 1[a]



[a] Reagents: (i) K_2CO_3, DMF, then $Br(CH_2)_2OMe$ (**17**), $Br(CH_2)_2OTBDMS$ (**18**), $Br(CH_2)_3OTBDMS$ (**19**), $Cl(CH_2)_3NMe_2$ (**20**) or $Cl(CH_2)_3N$morpholide (**21**); (ii) DIBALH, THF; (iii) **3a**, triphosgene, Et_3N, DCM, then **22**−**26**, $^nBu_2Sn(OAc)_2$; (iv) for **27** and **28** HCl/MeOH.

Scheme 2[a]



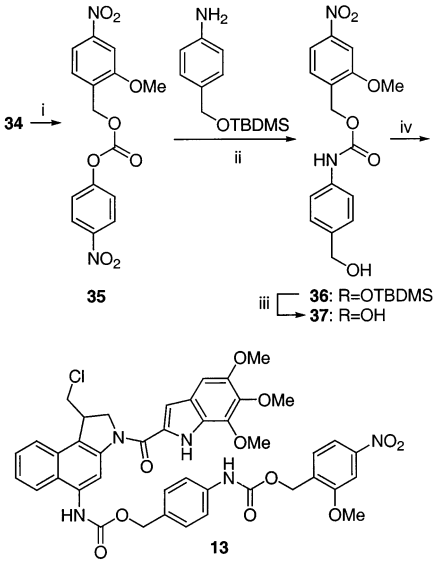
[a] Reagents: (i) epichlorohydrin, K_2CO_3, DMF; (ii) $HClO_4$, THF; (iii) dimethoxypropane, PPTS, DMF; (iv) DIBAL-H, THF; (v) **3a**, triphosgene, Et_3N, DCM, then **32**, $^nBu_2Sn(OAc)_2$; (vi) 1 M HCl, THF.

to give carbamates **6**-**11** and **13** (Schemes 1−3). This method is the preferred option for carbamate formation with deactivated amines such as **3**.

Results and Discussion

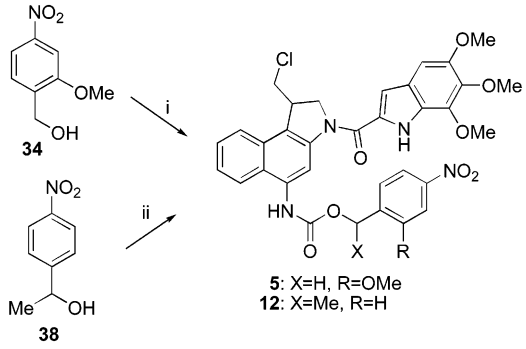
The prodrugs studied, together with the three amine effectors (**3a** for compounds **4**−**13**; **3b** for compound **14**, and **3c** for **15**), are listed in Table 1 along with CB1954 (**1**) and the dibromomustard **2**. The solubility of compounds **1**−**15** in α-MEM culture medium with 5% added fetal calf serum was determined by HPLC analysis of the supernatant of a saturated solution. The prodrugs **4**, **14**, and **15** were considerably less soluble than their corresponding amines **3a**−**c**. The most dramatic loss in solubility was seen for the unsubstituted nitrobenzyl prodrug **4** (ca. 246-fold). The addition of a 2-substituent to the nitrobenzyl group provided significant increases of solubility: 6-fold for the 2-OMe group **5**, up to 36-fold for the dihydroxypropyl side chain **9**. Addition of an α-methyl substituent **12** gave a 28-fold increase in solubility over **4**. The addition of a basic amino side chain positioned either on the nitrobenzyl unit, **10** and **11**, or on the indole unit **15** provided no increase in

Scheme 3[a]



[a] Reagents: (i) 4-nitrophenyl chloroformate, pyr; (ii) HOBT, Et_3N, 4A sieves, THF; (iii) 1 M HCl, MeOH; (iv) **3a**, triphosgene, Et_3N, then **37**, $^nBu_2Sn(OAc)_2$.

Scheme 4[a]

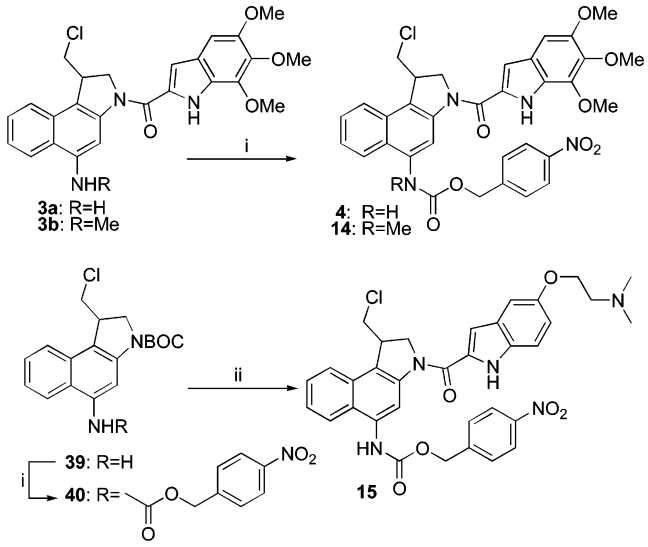


[a] Reagents: (i) $COCl_2$, THF, then **3a**, THF; (ii) triphosgene, pyr, DCM; then **3a**, THF.

solubility at pH 7 over neutral side chains despite their lower logD values. Remarkably, the addition of a second benzyl group in **13** reduced solubility by only a small degree. The low solubilities were not unexpected, as their calculated logP values [determined using ACDlogP software (Advanced Chemistry Development Inc., Toronto, Canada, v. 4.5)], were high, ranging from 3.55 for **9** to 6.04 for **13**, considerably greater than those of the amines themselves (1.87 to 2.87). The prodrugs **4**−**15** were considerably less soluble than **1**, but were similar to **2** despite having higher calculated logP values.

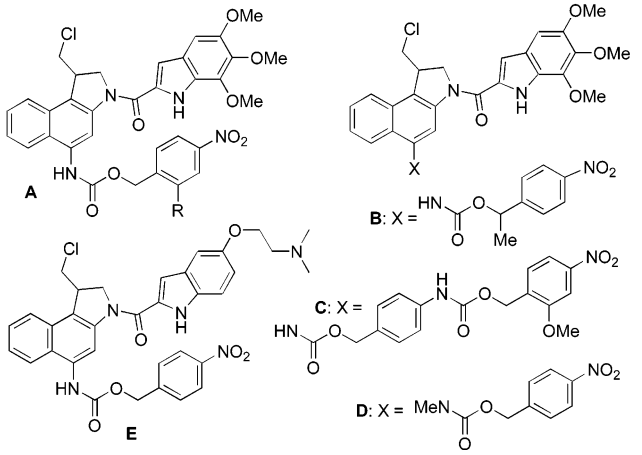
The effectors and prodrugs were evaluated for cytotoxicity in four pairs of cell lines, each comprising a non-NTR-expressing cell line: SKOV3 (human ovarian carcinoma), WiDr (human colon carcinoma), $V79^{puro}$ (Chinese hamster fibroblast) and EMT6 (mouse mammary carcinoma), and the corresponding transfectants stably expressing NTR. Cytotoxicity was measured as IC_{50} values (nM 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 (Table 2) as a measure of selectivity for NTR-expressing cells. The compounds were compared with CB1954 (**1**) and SN 24927 (**2**) in order to evaluate their potential value as GDEPT candidates.

Scheme 5[a]



[a] Reagents: (i) 4-nitrobenzyl chloroformate, THF; (ii) HCl, dioxan; then 5-[2-(dimethylamino)ethoxy]-1*H*-indole-2-carboxylic acid, EDCI·HCl, DMA.

Table 1. Structures and Physicochemical Properties of 4-Nitrobenzyl Carbamate Prodrugs



no.	formula	R	solubility[a]	LogP[b]
1			1600	1.28[c]
2			40	2.68[c]
3a			197	1.87
3b			310	2.87
3c			95	2.26 (0.53)[d]
4	A	H	0.8	4.55
5	A	2-OMe	5	4.77
6	A	2-O$(CH_2)_2$OMe	15	4.47
7	A	2-O$(CH_2)_2$OH	13	3.79
8	A	2-O$(CH_2)_3$OH	2	4.57
9	A	2-OCH_2CH(OH)CH_2OH	29	3.38
10	A	2-O$(CH_2)_3NMe_2$	12	4.95 (2.74)[d]
11	A	2O$(CH_2)_3$Nmorpholide	16	4.55 (4.06)[d]
12	B		23	4.90
13	C		3	6.09
14	D		27	3.98
15	E		17	4.94 (3.24)[d]

[a] Solubility (μM) in α-MEM culture medium, determined by HPLC. [b]logP calculated using the ACDLogP prediction software(v 4.5). [c]Measured values [**1**, 0.26; **2**, 1.97 (ref 16)]. [d]logD at pH 7 for compounds with ionizable groups (in brackets).

The effector **3a** had an average cytotoxicity across the four parental cell lines of 0.85 nM while the methylamino analogue **3b** was on average ca. 6-fold more

Table 2. IC_{50} Values and IC_{50} Ratios (NTR-ve/NTR+ve) for the Amino-CBI Cytotoxins **3a**−**c** and 4-Nitrobenzylcarbamate Prodrugs **4**−**15**

no.	SKOV (nM)[a]	SKOV/Skov-NTR^{neo} [a,b]	WiDr (nM)[a]	WiDr/WiDr-NTR^{neo} [a,b]	V79 (nM)[a]	V79^{puro}/V79-NTR^{puro} [a,b]	EMT6 (nM)[a]	EMT6/EMT6-NTR^{puro} [a,b]
1	174 000 ± 10 000	317 ± 21	54 000 ± 3000	51.2 ± 3	374 000 ± 14 000	2090 ± 210	710 000 ± 7 000	930 ± 140
2	58 000 ± 10 000	211 ± 16	40 000 ± 3000	174 ± 31	43 000 ± 5 000	302 ± 93	54 000	1380
3a	1.1 ± 0.1	0.6 ± 0.0	1.6 ± 0.1	0.9 ± 0.3	0.33 ± 0.07	0.9 ± 0.1	0.37 ± 0.06	2.0 ± 0.7
3b	0.2 ± 0.0	0.7 ± 0.2	0.2 ± 0.0	1.1 ± 0.1	0.08 ± 0.02	1.2 ± 0.2	0.11 ± 0.05	0.74 ± 0.18
3c	13 ± 1	1.6 ± 0.2	6 ± 2	1.9 ± 0.8	2.6 ± 0.3	1.2 ± 0.2	1.9 ± 0.8	0.6 ± 0.3
4	62 ± 8	12 ±1	391 ± 75	33 ± 6	89 ± 23	10 ± 3.3	193 ± 46	112 ± 44
5	59 ± 13	17 ± 4	141 ± 17	47 ± 13	39 ± 11	6 ± 3	174 ± 28	108 ± 29
6	106 ± 23	67 ± 8	110 ± 15	45 ±4	31 ± 1	17 ± 4	33 ± 6	78 ± 2
7	224 ± 37	84 ± 4	295 ± 127	148 ± 48	63 ± 22	11 ± 2	107 ± 22	104 ± 11
8	145 ± 12	58 ± 7	191 ± 15	91 ± 2	48 ± 5	11 ± 1	47 ± 9	91 ± 25
9	114 ± 26	40 ± 1	129 ± 41	25 ±1	42	1.75	44 ± 18	13 ± 3
10	189 ± 10	3.6 ± 0.4	269 ± 60	3.8 ± 1.1	45 ± 7	1.45 ± 0.03	74 ± 14	3.4 ± 0.3
11	88 ± 10	6.5 ± 1.0	98 ± 13	9.0 ± 0.1	20 ± 5	2.6 ± 1.1	24 ± 7	6 ± 3
12	158 ± 5	5.65	154 ± 3	6.60	62 ± 10	1.67	79 ± 12	11 ± 5
13	223 ± 48	0.7 ± 0.0	295 ± 61	1.8 ± 0.8	54 ± 8	1.03 ± 0.01	63 ± 1	1.8 ± 0.6
14	152 ± 27	10 ± 4	101 ± 24	15 ± 1	48 ± 15	8.4 ± 2.1	48 ± 13	27 ± 7
15	770 ± 107	41 ± 4	403 ± 24	20 ± 2	157 ± 20	4.3 ± 1.0	208 ± 54	31 ± 13

[a] Values are mean ± SEM for up to five independent experiments. [b] Intraexperiment ratios. Cell lines: SKOV3 (human ovarian carcinoma), WiDr (human colon carcinoma), V79^{puro} (Chinese hamster fibroblast), and EMT6 (mouse mammary carcinoma).

potent and the solubilized amine **3c** was ca. 7-fold less potent than **3a**. Prodrugs **4**−**13**, which share the same effector **3a**, were overall less cytotoxic in the human SKOV and WiDr wild-type lines (average IC_{50}s 127 and 207 nM, respectively), compared with the rodent lines T-78 and EMT6 (average IC_{50}s 49 and 83 nM, respectively). Across all cell lines, prodrugs **4**−**13** were, on average, deactivated relative to the effector **3a** by factors ranging from 66 to 273-fold. Prodrug **14** showed an even greater degree of deactivation (average of 575-fold) than **4**−**13**, primarily due to the enhanced cytotoxicity of **3b**. Prodrug **15** displayed modest deactivation (average 74-fold). However, the prodrugs **4**-**15** were still ca. 1000-fold more potent than the dinitrobenzamide prodrugs **1** and **2**.

Prodrugs **4** and **5** generally showed a similar degree of activation by the NTR-expressing cell lines (mean IC_{50} ratio 42 and 45, respectively) but this was substantially less than the differential between effector **3a** and prodrugs **4** and **5**, suggesting partial metabolic conversion to **3a**. The methoxyethoxy prodrug **6** and alcohols **7** and **8** showed the largest degree of activation by NTR across the four cell lines (mean IC_{50} ratios 52, 87, and 63, respectively), while the larger diol **9** showed lesser activation (19-fold). Compounds **6**−**8** were clearly superior to **4** in the human cell lines, but showed only equivalent activation in the rodent cell lines. The amines **10** and **11** showed little activation, suggesting an unfavorable interaction between the side chain and the binding pocket of NTR. Compound **12** was examined to determine the effect of an α-methyl substituent on prodrug selectivity, since we had previously showed[19] that this accelerated the fragmentation of the corresponding hydroxylamines. This prodrug was somewhat less selective than the nonmethylated derivative **4** (mean activation 6-fold), with lower levels of activation in the NTR+ve lines. There have been several reports that placing large effector units close to the trigger domain of prodrugs results in a loss of enzyme binding, presumably due to steric hindrance. In those cases (e.g., prodrugs for carboxypeptidase[29] and *β*-glucuronidase[30]), interposition of a "spacer" group can restore activity. This was evaluated in the present case with compound **13**, where a 4-aminobenzyl carbamate unit was interposed. However, this showed no selectivity, with no increase in potency in the NR+ve lines, suggesting limited fragmentation of the more complex spacer or unfavorable binding interactions in the active site. To determine the effect of substitution on the carbamate amine, the NMe analogue **14** was evaluated. This prodrug achieved the highest degree of deactivation compared to its effector **3b** (430−760-fold), but showed only moderate differentials between NTR-expressing and parental lines (average of 15-fold) indicating relatively poor levels of activation by NTR. Limited studies[19] have suggested that the fragmentation of a similar NMe carbamate is a less efficient process than for the corresponding NH carbamate.

Overall, the IC_{50} ratios (NTR-ve/NTR+ve) of prodrugs **6**−**8** were similar to that of the dinitrobenzamides **1** and **2** for the human cell lines (SKOV3 and WiDr) but the ratios for the two rodent cell line pairs were much lower for the nitrobenzylcarbamate derivatives. Maximum in vitro selectivity for NTR was seen with compound **7** which has a 2-hydroxyethoxy side chain. Increasing the length of this by one carbon (**6** or **8**) was accompanied by a loss of selectivity which continued with diol **9**. Low selectivity was seen with **10** and **11** which bear basic side chains. The lack of tolerance for charged side chains has been observed for analogues of **2**.[14] Other modifications, e.g., **12**−**15** did not result in significant selectivity for NTR. Assuming comparable uptake and formation of a common cytotoxic metabolite, the ratios of the IC_{50}s in the NTR-ve and NTR+ve cells are a measure of the effectiveness of the prodrugs as substrates for the NTR enzyme. This will depend on a number of factors, the most important likely to be the reduction potential of the nitro group and the ability to fit the enzyme binding site.

Generally, 4-nitrobenzyl carbamates are likely to have considerably lower reduction potentials than the established NTR substrates such as CB1954 (E(1) −385 mV).[31] In contrast, the reduction potential of 4-nitrobenzyl alcohol is considerably lower (−494 ± 8 mV), which is likely to make the 4-nitrobenzyl carbamates harder to reduce. In agreement with this, various nitrobenzyl carbamates showed little activity when evaluated as bioreductive prodrugs (activation under hypoxic condi-

tions by endogenous enzymes).[32−34] Addition of a 2′-methoxy group (electron-withdrawing when meta to the NO_2) is likely to raise the $E(1)$ of compounds such as **6**−**8** slightly (2-methoxy-4-nitrobenzyl alcohol has an $E(1)$ of -470 ± 12 mV; R. F. Anderson, personal communication).

The ability of prodrugs to access the enzyme binding site is more difficult to evaluate, but X-ray studies of NTR reveal a V-shaped pocket narrowing toward the FMN cofactor which is only accessible from the *re* face.[7,35] The prodrugs were docked into the active site of the enzyme generated from the coordinates[35] for the crystal structure of NTR complexed with nicotinic acid as a mimic for the cofactor NADPH. Nicotinic acid was removed from the active site and energy-minimized prodrugs docked using GOLD (v1.2, Cambridge Crystallographic Centre) and Sybyl (v6.8, Tripos Inc., St Louis, MO). Docking of prodrug **4** into the active site showed a variety of binding modes, indicating that the cleft is large enough to accommodate it readily. Constraining the 4-nitro group to within 3.5 Å of N5 of FMN located the nitrobenzyl group above the FMN with the CBI-TMI unit adopting a range of configurations extending out into the binding cleft. Smaller side chains at the 2-position, e.g., methoxy **5** and 2-hydroxyethyl **7**, were also aligned into the cleft whereas longer side chains, e.g., **9**−**11** were aligned along the channel formed by Phe124 and Tyr68. The placement of the side chain along the channel resulted in displacement of the nitro group from above the N5 of FMN. This provides a possible explanation for the waning in selectivity for NTR seen from **7** down to **11**.

The maximum tolerated dose (MTD) of effector **3a** given ip in DMSO to C3H mice was 2.37 μmol kg^{-1}, whereas the MTD of **7** was 421 μmol kg^{-1} demonstrating considerable deactivation of the toxicity of the effector by the prodrug in vivo. The MTD of **1** was 240 μmol kg^{-1} given ip in DMSO whereas **2** was considerably less toxic (>1330 μmol kg^{-1}). The activity of **7** was evaluated against NTR-expressing EMT6 tumors comprising mixtures of NTR-ve and NTR+ve cells and compared with **1** and **2**. In this model nude mice are inoculated with 2:1 mixtures of EMT6-NTR^{puro} and EMT6 (NTR-ve) cells, respectively, providing ca. 5−10% NTR+ve cells at the time of tumor treatment.[36] This tumor model represents the likely situation in a GDEPT protocol where low rates of transfection of tumor tissue are expected. A small decrease in NTR+ve cells was observed after treatment with **7**, with a lesser effect against NTR-ve target cells, but neither of these effects were statistically significant (Figure 1). In contrast, dinitrobenzamides **1** and **2** given at 200 and 1330 μmol kg^{-1}, respectively, showed significant killing of activator (EMT6-NTR^{puro}) cells and target (EMT6) cells, indicating the operation of a bystander effect. Given that killing of NTR+ve cells in the tumors was at least as great for **1** as for **2**, but killing of NTR-ve cells was greater for **2**, the latter appears to have a more efficient bystander effect in this system as previously reported with WiDr tumor xenografts[17]

Conclusions

Several nitrobenzyl carbamate prodrugs, e.g., **6**−**8**, proved to be good substrates for NTR in the human cell lines in vitro and were significantly more selective for NTR in these cell lines than the unsubstituted nitrobenzyl carbamate **4**. These compounds, bearing neutral methoxyethoxy- (**6**), 2-hydroxyethoxy- (**7**), or 3-hydroxypropoxy-substituted (**8**) side chains, displayed NTR+ve/NTR-ve ratios similar to the dinitrobenzamides **1** and **2**, which are well established as useful substrates for NTR. Compounds **6**−**8** were active in vitro despite having lower reduction potentials and a large steric demand on the binding site relative to **1** and **2**. Compounds **6**−**8** showed modest selectivity (11−17-fold) for NTR in the V79^{puro}/T79-NTR^{puro} lines and good selectivity (78−104-fold) for NTR in the EMT6/EMT6-NTR^{puro} murine lines, matching the selectivity seen for **4** in these cell lines. Compound **7** did not display significant antitumor activity in the mixed EMT6/EMT6-NTR^{puro} tumor model. However, the dinitrobenzamides **1** and **2** which were considerably more selective for NTR in the rodent cell lines, displayed in vivo activity in the EMT6 model. These results suggest potentially useful GDEPT prodrugs of 5-aminobenz[*e*]indolines using the 4-nitrobenzyl carbamate motif may be developed if a number of factors can be addressed. In particular the optimization of pharmacokinetics, especially extravascular diffusion, may be required for the translation of the observed in vitro activity to in vivo efficacy. Improved activity might also be achieved through increasing the reduction potential of the nitro group and reducing the steric demand in the active site of the enzyme.

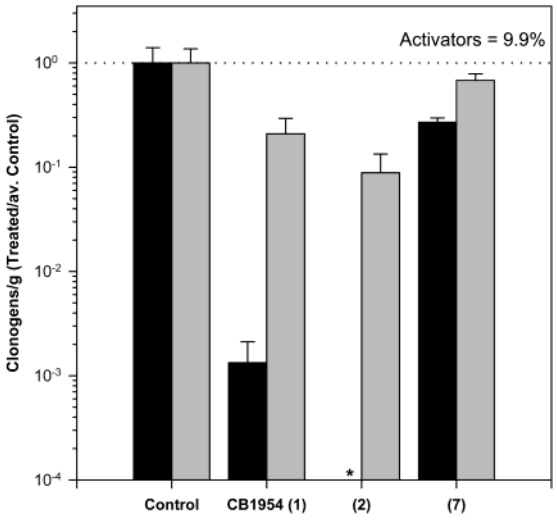


Figure 1. Comparison of in vivo activity and bystander effects for CB1954 (**1**), SN 24927 (**2**), and the nitrobenzylcarbamate **7**. Prodrugs given at 240, 1330, and 421 μmol kg^{-1} ip. In this experiment, tumors at excision comprised 9.9% EMT6-NTR^{puro} as assessed by the proportion of puromycin-resistant cells. * EMT6-NTR^{puro} < 10^{-4} clonogens/g.

Experimental Section

Chemistry. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 Melting Point Apparatus. 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. 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 10000 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 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 biological testing were analyzed at >99% purity by reverse phase HPLC using a Philips PU4100 liquid chromatograph, a Phenomenex BondClone 10-C18 stainless steel column (300 mm × 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 heptanesulfonic 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, DMF refers to dry dimethylformamide, ether refers to diethyl ether, EtOAc refers to ethyl acetate, EtOH refers to ethanol, $^iPr_2O$ refers to diisopropyl ether, MeOH refers to methanol, pet. ether refers to petroleum ether, boiling range 40−60 °C, and THF refers to tetrahydrofuran dried over sodium benzophenone ketyl. All solvents were freshly distilled.

Alkylation of Phenols. Methyl 2-(2-Methoxyethoxy)-4-nitrobenzoate (17). A mixture of methyl 2-hydroxy-4-nitrobenzoate (**16**)[19] (1.0 g, 5.07 mmol) and K_2CO_3 (1.05 g, 7.61 mmol) in DMF (25 mL) was stirred at 20 °C for 30 min. A solution of 2-bromoethyl methyl ether (0.72 mL, 7.61 mmol) in DMF (3 mL) was added and the mixture was stirred at 100 °C for 4 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), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 30% EtOAc/pet. ether, to give ether **17** (1.27 g, 98%) as a white solid, mp (EtOAc/pet. ether) 45−46 °C; ^{1}H NMR δ 7.90 (d, J = 8.3 Hz, 1 H, H-6), 7.82−7.86 (m, 2 H, H-3, H-5), 4.28−4.30 (m, 2 H, CH_2O), 3.93 (s, 3 H, OCH_3), 3.82−3.87 (m, 2 H, CH_2O), 3.48 (s, 3 H, OCH_3); ^{13}C NMR δ 165.1, 158.6, 150.6, 132.1, 126.4, 115.2, 108.4, 70.5, 69.4, 59.4, 52.5. Anal. ($C_{11}H_{13}NO_6$) C, H, N.

Methyl 2-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethoxy)-4-nitrobenzoate (18). Similarly, reaction of **16** and 2-bromoethyl *tert*-butyl(dimethyl)silyl ether gave ether **18** (77%) as a white solid, mp (EtOAc/pet. ether) 47−48 °C; ^{1}H NMR δ 7.89 (d, J = 1.3 Hz, 1 H, H-3), 7.81−7.85 (m, 2 H, H-5, H-6), 4.24 (t, J = 5.0 Hz, 2 H, CH_2O), 4.03 (t, J = 5.0 Hz, 2 H, CH_2O), 3.92 (s, 3 H, OCH_3), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.08 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR δ 165.5, 158.6, 150.4, 131.8, 126.7, 115.0, 108.3, 71.1, 61.7, 52.5, 25.7 (3), 18.3, −5.5 (2). Anal. ($C_{16}H_{25}NO_6Si$) C, H, N.

Methyl 2-(3-{[*tert*-Butyl(dimethyl)silyl]oxy}propoxy)-4-nitrobenzoate (19). Similarly, reaction of **16** and 3-bromopropyl *tert*-butyl(dimethyl)silyl ether gave ether **19** (93%) as a waxy solid, mp (EtOAc) 36.5−37 °C; ^{1}H NMR δ 7.88 (d, J = 8.9 Hz, 1 H, H-6), 7.80−7.84 (m, 2 H, H-3, H-5), 4.24 (t, J = 6.0 Hz, 2 H, CH_2O), 3.92 (s, 3 H, OCH_3), 3.85 (t, J = 5.9 Hz, 2 H, CH_2O), 2.04−2.09 (m, 2 H, CH_2), 0.88 (s, 9 H, $OSi(CH_3)_3$), 0.04 (s, 6 H, $OSi(CH_3)_2$); ^{13}C NMR δ 165.4, 158.7, 150.7, 132.0, 126.1, 114.8, 107.7, 64.0, 59.0, 52.5, 32.0, 25.9 (3), 18.3, −5.5 (2). Anal. ($C_{17}H_{27}NO_6Si$) C, H, N.

Methyl 2-[3-(Dimethylamino)propyloxy]-4-nitrobenzoate (20). Similarly, reaction of **16** and *N*-(3-chloropropyl)-*N*,*N*-dimethylamine gave ether **20** (71%) as a pale yellow oil which converted to the HCl salt, mp (EtOAc) 175−177 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.90 (br s, 1 H, NH^+Cl^-), 7.87−7.93 (m, 3 H, H-3, H-5, H-6), 4.32 (t, J = 6.0 Hz, 2 H, CH_2O), 3.89 (s, 3 H, OCH_3), 3.18−3.23 (m, 2 H, CH_2N), 2.77 (d, J = 4.8 Hz, 6 H, $N(CH_3)_2$), 2.18−2.24 (m, 2 H, CH_2); ^{13}C NMR [$(CD_3)_2SO$] δ 165.0, 157.3, 150.2, 131.6, 126.0, 115.3, 108.4, 66.5, 53.7, 52.6, 42.0 (2), 23.3. Anal. ($C_{13}H_{18}N_2O_5$.HCl) C, H, N, Cl.

Methyl 2-[3-(4-Morpholinyl)propoxy]-4-nitrobenzoate (21). Similarly, reaction of **16** and 4-(3-chloropropyl)morpholine gave ether **21** (80%) as an oil, ^{1}H NMR δ 7.88 (d, J = 9.1 Hz, 1 H, H-6), 7.81 (m, 2 H, H-3, H-5), 4.21 (t, J = 6.3 Hz, 2 H, CH_2O), 3.93 (s, 3 H, OCH_3), 3.69−3.74 (m, 4 H, 2 × CH_2O), 2.57 (t, J = 7.0 Hz, 2 H, CH_2N), 2.47−2.51 (m, 4 H, 2 × CH_2N), 2.02−2.07 (m, 2 H, CH_2); ^{13}C NMR δ 165.3, 158.6, 150.6, 132.0, 126.2, 114.8, 107.8, 67.6, 66.9 (2), 55.0, 53.7 (2), 52.5, 25.9. The hydrochloride salt had mp (EtOAc) 160−163 °C. Anal. ($C_{15}H_{20}N_2O_6$.HCl) C, H, Cl.

Methyl 4-Nitro-2-(2-oxiranylmethoxy)benzoate (29). Similarly, reaction of **16** and epichlorohydrin followed by chromatography successively gave starting material (18%), followed by ether **29** (59%) as a white solid, mp (EtOAc/pet. ether) 62−63 °C; ^{1}H NMR δ 7.91 (dd, J = 7.7, 1.0 Hz, 1 H, H-5), 7.84−7.86 (m, 2 H, H-3, H-6), 4.49 (dd, J = 11.2, 2.4 Hz, 1 H, H-3′), 4.14 (dd, J = 11.2, 5.2 Hz, 1 H, H-3′), 3.94 (s, 3 H, OCH_3), 3.40−3.44 (m, 1 H, H-2′), 2.91−2.97 (m, 2 H, H-1′); ^{13}C NMR δ 165.0, 158.1, 150.6, 132.3, 126.1, 115.6, 108.4, 69.6, 52.6, 49.7, 44.3; MS (CI, NH_3) m/z 295 (M + CH_3CN^+, 70%), 259 (MH^+, 100%). Anal. ($C_{11}H_{11}NO_6$) C, H, N.

Methyl 2-(2,3-Dihydroxypropoxy)-4-nitrobenzoate (30). Perchloric acid (1 mL) and water (3 mL) were added to a stirred solution of epoxide **29** (205 mg, 0.81 mmol) in THF (20 mL), and the solution was stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (50 mL) and water (50 mL). The organic fraction was washed with water (50 mL) and brine (25 mL) and dried and the solvent evaporated. The residue was purified by chromatography, eluting with 70% EtOAc/pet. ether, to give diol **30** (172 mg, 78%) as an oil which solidified on standing, mp 60−65 °C; ^{1}H NMR δ 8.02 (d, J = 8.5 Hz, 1 H, H-6), 7.87 (dd, J = 8.5, 2.0 Hz, 1 H, H-5), 7.84 (d, J = 2.0 Hz, 1 H, H-3), 4.38 (dd, J = 9.3, 3.1 Hz, 1 H, H-3′), 4.23 (dd, J = 9.3, 5.4 Hz, 1 H, H-3′), 4.10−4.14 (m, 1 H, H-2′), 3.95 (s, 3 H, OCH_3), 3.88 (br d, J = 4.1 Hz, 2 H, H-1′), 3.05 (br s, 1 H, OH), 1.95 (br s, 1 H, OH); ^{13}C NMR δ 164.8, 159.1, 151.0, 132.8, 124.9, 115.6, 108.8, 73.0, 69.2, 63.2, 52.8; MS (CI, NH_3) m/z 272 (MH^+, 1%), 240 (50%), 165 (100); HRMS (CI, NH_3) calcd for $C_{11}H_{14}NO_7$ (MH^+) m/z 272.0770, found 272.0766. Anal. ($C_{11}H_{13}NO_7$) C, H, N.

Methyl 2-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]-4-nitrobenzoate (31). 2,2-Dimethoxypropane (0.91 mL, 7.37 mmol) was added dropwise to a stirred solution of diol **30** (400 mg, 1.47 mmol) and PPTS (37 mg, 0.15 mmol) in DMF (20 mL) under N_2 and stirred at 20 °C for 24 h. The solvent was evaporated and the residue 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 evaporated. The residue was purified by chromatography, eluting with 30% EtOAc/pet. ether, to give acetonide **31** (458 mg, 100%) as a yellow oil, ^{1}H NMR δ 7.90 (d, J = 8.2 Hz, 1 H, H-6), 7.84−7.88 (m, 2 H, H-3, H-5), 4.49−4.54 (m, 1 H, H-4″), 4.25 (dd, J = 9.6, 4.6 Hz, 1 H, H-5″), 4.19 (dd, J = 8.5, 6.4 Hz, 1 H, H-2′), 4.15 (dd, J = 9.6, 4.6 Hz, 1 H, H-5″), 4.03 (dd, J = 8.5, 5.8 Hz, 1 H, H-2′), 3.94 (s, 3 H, OCH_3), 1.46 (s, 3 H, CH_3), 1.41 (s, 3 H, CH_3); ^{13}C NMR δ 165.1, 158.2, 150.6, 132.2, 126.4, 115.5, 109.9, 108.4, 73.6, 69.8, 66.5, 52.6, 26.6, 25.3; MS (CI, NH_3) m/z 312 (MH^+, 15%), 296 (95), 101 (95), 71 (100); HRMS (CI, NH_3) calcd for $C_{14}H_{18}NO_7$ (MH^+) m/z 312.1083, found 312.1092.

DIBAL-H Reduction of Esters. 2-[2-(Methoxy)ethoxy]-4-nitrobenzyl Alcohol (22). DIBAL-H (1 M in DCM, 16.4 mL, 16.4 mmol) was added dropwise to a stirred solution of ester **17** (1.27 g, 4.97 mmol) in THF (100 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of potassium sodium tartrate (1 M, 100 mL) and stirred vigorously for 30 min. The mixture was extracted with EtOAc (2 × 100 mL), the combined organic fraction washed with water (50 mL) and brine (50 mL) and dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with a gradient (30−50%) of EtOAc/pet. ether, to give alcohol **22** (1.03 g, 91%) as a white solid, mp (EtOAc/pet. ether) 89−90.5 °C; ^{1}H NMR δ 7.86 (dd, J = 8.2, 2.1 Hz, 1 H, H-5), 7.72 (d, J = 2.1 Hz, 1 H, H-3), 7.47 (d, J =

8.2 Hz, 1 H, H-6), 4.74 (br s, 2 H, CH_2O), 4.26−4.29 (m, 2 H, CH_2O), 3.78−3.80 (m, 2 H, CH_2O), 3.45 (s, 3 H, OCH_3), 3.10 (br s, 1 H, OH); ^{13}C NMR δ 156.8, 148.2, 137.5, 128.6, 116.5, 107.0, 70.5, 68.4, 61.1, 59.1. Anal. ($C_{10}H_{13}NO_5$) C, H, N.

[2-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethoxy)-4-nitrophenyl]methanol (23). Similarly, reduction of ester **18** gave alcohol **23** (97%) as a white solid, mp (EtOAc/pet. ether) 89−90 °C; ^{1}H NMR δ 7.85 (dd, J = 8.2, 2.1 Hz, 1 H, H-5), 7.74 (d, J = 2.1 Hz, 1 H, H-3), 7.46 (d, J = 8.2 Hz, 1 H, H-6), 4.75 (s, 2 H, CH_2O), 4.21 (dd, J = 4.9, 4.4 Hz, 2 H, CH_2O), 4.01 dd, J = 4.9, 4.4 Hz, 2 H, CH_2O), 2.84 (br s, 1 H, OH), 0.90 (s, 9 H, $SiC(CH_3)_3$), 0.06 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR δ 157.0, 148.3, 137.1, 128.4, 116.3, 106.8, 70.5, 61.6, 61.3, 25.8 (3), 18.3, −5.4 (2); Anal. ($C_{15}H_{25}NO_5Si$) C, H, N.

[2-(3-{[*tert*-Butyl(dimethyl)silyl]oxy}propoxy)-4-nitrophenyl]methanol (24). Similarly, reduction of ester **19** gave alcohol **24** (94%) as a white solid, mp (EtOAc/pet. ether) 48−49 °C; ^{1}H NMR δ 7.84 (dd, J = 8.3, 2.1 Hz, 1 H, H-5), 7.71 (d, J = 2.1 Hz, 1 H, H-3), 7.51 (d, J = 8.3 Hz, 1 H, H-6), 4.76 (d, J = 6.3 Hz, 2 H, CH_2O), 4.21 (t, J = 6.1 Hz, 2 H, CH_2O), 3.82 (t, J = 5.9 Hz, 2 H, CH_2OSi), 2.40 (t, J = 6.3 Hz, 1 H, OH), 2.02−2.08 (m, 2 H, CH_2), 0.89 (s, 9 H, $OSiC(CH_3)_3$), 0.06 (s, 6 H, $OSi(CH_3)_2$); ^{13}C NMR δ 156.5, 148.2, 136.7, 127.8, 115.9, 105.8, 65.5, 60.8, 59.3, 32.0, 25.9 (3), 18.3, −5.4 (2). Anal. ($C_{16}H_{27}NO_5Si$) C, H, N.

{**2-[3-(Dimethylamino)propoxy]-4-nitrophenyl**}**methanol (25).** Similarly, reduction of ester **20** gave alcohol **25** (86%) as a tan solid, mp (EtOAc) 104−105 °C; ^{1}H NMR δ 7.87 (dd, J = 8.3, 2.1 Hz, 1 H, H-5), 7.69 (d, J = 2.1 Hz, 1 H, H-3), 7.64 (d, J = 8.3 Hz, 1 H, H-6), 5.43 (br s, 1 H, OH), 4.58 (s, 2 H, CH_2O), 4.14 (t, J = 6.5 Hz, 2 H, CH_2O), 2.36 (t, J = 7.0 Hz, 2 H, CH_2N), 2.15 (s, 6 H, $N(CH_3)_2$), 1.85−1.91 (m, 2 H, CH_2); ^{13}C NMR δ 155.2, 147.0, 138.9, 126.7, 115.3, 105.2, 66.5, 57.6, 55.5, 45.1 (2), 26.5. Anal. ($C_{12}H_{18}N_2O_4$) C, H, N.

{**2-[3-(4-Morpholinyl)propoxy]-4-nitrophenyl**}**methanol (26).** Similarly, reduction of ester **21** gave alcohol **26** (88%) as a tan solid, mp (EtOAc) 105−106 °C; ^{1}H NMR δ 7.83 (dd, J = 8.2, 2.1 Hz, 1 H, H-5), 7.70 (d, J = 2.1 Hz, 1 H, H-3), 7.46 (d, J = 8.2 Hz, 1 H, H-6), 4.71 (s, 2 H, CH_2O), 4.18 (t, J = 6.0 Hz, 2 H, CH_2O), 3.74−3.77 (m, 4 H, 2 × CH_2O), 3.60 (br s, 1 H, OH), 2.56 (dd, J = 6.7, 6.5 Hz, 2 H, CH_2N), 2.45−2.49 (m, 4 H, 2 × CH_2N), 2.01−2.06 (m, 2 H, CH_2); ^{13}C NMR δ 156.7, 148.2, 137.2, 128.2, 116.1, 106.2, 67.7, 66.5 (2), 60.7, 56.3, 53.9 (2), 25.5. Anal. ($C_{14}H_{20}N_2O_5$) C, H, N.

{**2-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]-4-nitrophenyl**}**methanol (32).** Similarly, reduction of ester **31** gave alcohol **32** (92%) as a white solid, mp (EtOAc/pet. ether) 90−92 °C; ^{1}H NMR δ 7.87 (dd, J = 8.2, 2.1 Hz, 1 H, H-5), 7.72 (d, J = 2.1 Hz, 1 H, H-3), 7.50 (d, J = 8.2 Hz, 1 H, H-6), 4.82 (d, J = 14.1 Hz, 1 H, CH_2O), 4.70 (d, J = 14.1 Hz, 1 H, CH_2O), 4.51−4.57 (m, 1 H, H-4″), 4.23 (dd, J = 9.8, 4.0 Hz, 1 H, H-5″), 4.19 (dd, J = 9.8, 5.4 Hz, 1 H, H-2′), 4.11 (dd, J = 9.8, 5.4 Hz, 1 H, H-5″), 3.95 (dd, J = 8.7, 5.4 Hz, 1 H, H-2′), 3.25 (br s, 1 H, OH), 1.48 (s, 3 H, CH_3), 1.41 (s, 3 H, CH_3); ^{13}C NMR δ 156.5, 148.2, 137.2, 128.6, 116.6, 110.0, 106.4, 73.7, 69.7, 66.0, 61.0, 26.6, 25.0; MS m/z 283 (M^+, 3%), 268 (20), 225 (30), 101 (100); HRMS calcd for $C_{13}H_{17}NO_6$ (M^+) m/z 283.1056, found 283.1055. Anal. ($C_{13}H_{17}NO_6$) C, H, N.

Synthesis of Alcohol 37. 2-Methoxy-4-nitrobenzyl 4-Nitrophenyl Carbonate (35). A solution of 4-nitrophenyl chloroformate (1.00 g, 4.97 mmol) in pyridine (4 mL) was added dropwise to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol (**34**) (617 mg, 3.31 mmol) in pyridine (15 mL) at 20 °C, and the solution was stirred for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (20−50%) EtOAc/light petroleum, to give carbonate **35** (928 mg, 80%) as a white solid, mp (EtOAc/pet. ether) 105−106 °C; ^{1}H NMR δ 8.28 (ddd, J = 9.2, 3.1, 2.1 Hz, 2 H, H-3′), 7.89 (dd, J = 8.3, 2.1 Hz, 1 H, H-5), 7.77 (d, J = 2.1 Hz, 1 H, H-3), 7.58 (d, J = 8.3 Hz, 1 H, H-6), 7.40 (ddd, J = 8.3, 3.1, 2.1 Hz, 2 H, H-2′), 5.41 (s, 2 H, CH_2O), 4.00 (s, 3 H, OCH_3); ^{13}C NMR δ 157.6, 155.4, 152.3, 149.2, 145.5, 129.8, 129.3, 125.3 (2), 121.7 (2), 115.8, 105.5, 65.3, 56.2. Anal. ($C_{15}H_{12}N_2O_8$) C, H, N.

2-Methoxy-4-nitrobenzyl 4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)phenylcarbamate (36). Et_3N (0.40 mL, 2.84 mmol) was added to a stirred suspension of carbonate **35** (0.90 g, 2.58 mmol), 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)aniline (0.64 g, 2.71 mmol), HOBT (0.35 g, 2.58 mmol), and 4 Å molecular sieves (900 mg) in THF (80 mL) and the mixture 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 1 M HCl (2 × 40 mL), water (100 mL), and brine (50 mL) and dried and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/pet. ether, to give silyl ether **36** (0.89 g, 77%), mp (EtOAc/pet. ether) 120−122 °C; ^{1}H NMR δ 7.84 (dd, J = 8.3, 2.1 Hz, 1 H, H-5′), 7.72 (d, J = 2.1 Hz, 1 H, H-3′), 7.51 (d, J = 8.3 Hz, 1 H, H-6′), 7.35 (d, J = 8.3 Hz, 2 H, H-2, H-6), 7.26 (d, J = 8.3 Hz, 2 H, H-3, H-5), 6.76 (br s, 1 H, OCONH), 5.30 (s, 2 H, CH_2O), 4.69 (s, 2 H, CH_2OSi), 3.93 (s, 3 H, OCH_3), 0.92 (s, 9 H, $SiC(CH_3)_3$), 0.09 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR δ 157.3, 153.0, 148.7, 137.0, 136.4, 132.1, 128.7, 126.9 (2), 118.6 (2), 115.7, 105.2, 64.6, 61.4, 56.0, 26.9 (3), 18.4, −5.2 (2). Anal. ($C_{22}H_{30}N_2O_6Si$) C, H, N.

2-Methoxy-4-nitrobenzyl 4-(Hydroxymethyl)phenylcarbamate (37). A stirred solution of silyl ether **36** (0.89 g, 0.2 mmol) in MeOH (10 mL) was treated with 1 N HCl (4 mL, 4 mmol) and stirred at 20 °C for 1 h. The solution was poured into brine (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fraction was washed with water (50 mL) and dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20−50%) of EtOAc/pet. ether, to give alcohol **37** (628 mg, 95%) as a white solid, mp (EtOAc/pet. ether) 164−165 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.83 (br s, 1 H, OCONH), 7.90 (dd, J = 8.3, 2.1 Hz, 1 H, H-5′), 7.80 (d, J = 2.1 Hz, 1 H, H-3′), 7.63 (d, J = 8.3 Hz, 1 H, H-6′), 7.41 (d, J = 8.4 Hz, 2 H, H-2, H-6), 7.22 (d, J = 8.4 Hz, 2 H, H-3, H-5), 5.21 (s, 2 H, CH_2O), 5.07 (t, J = 5.6 Hz, 1 H, OH), 4.41 (t, J = 5.6 Hz, 2 H, CH_2O), 3.97 (s, 3 H, OCH_3); ^{13}C NMR [$(CD_3)_2SO$] δ 157.0, 153.0, 148.2, 137.4, 136.7, 132.3, 128.8, 127.0 (2), 117.9 (2), 115.5, 105.4, 62.5, 60.4, 56.0. Anal. ($C_{16}H_{16}N_2O_6$) C, H, N.

Coupling of Alcohols to 3a−c. 4-Nitrobenzyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (4). A solution of 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)-carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylamine (**3a**)[25] (430 mg, 0.92 mmol) in THF (50 mL) was treated at −5 °C with 4-nitrobenzyl chloroformate (298 mg, 1.38 mmol). The mixture was stirred at 10 °C for 30 min and then at 20 °C for 2 h, before being diluted with ^{i}Pr_2O/pet. ether. The precipitate was collected and extracted with DCM. The combined extracts were evaporated, and the residue was purified by chromatography, eluting with 10% EtOAc/DCM, to give **4** (290 mg, 49%) as a tan solid, mp (^{i}Pr_2O/DCM) 191−192 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.48 (d, J = 1.7 Hz, 1 H, indole-NH), 9.91 (s, 1 H, $NHCO_2$), 8.57 (br s, 1 H, H-4), 8.29 (d, J = 8.7 Hz, 2 H, H-3″, H-5″), 8.09 (d, J = 8.5 Hz, 1 H, H-6), 7.99 (d, J = 8.3 Hz, 1 H, H-9), 7.73 (d, J = 8.7 Hz, 2 H, H-2″, H-6″), 7.58 (t, J = 7.6 Hz, 1 H, H-8), 7.48 (t, J = 7.6 Hz, 1 H, H-7), 7.10 (d, J = 2.2 Hz, 1 H, H-3′), 6.98 (s, 1 H, H-4′), 5.36 (s, 2 H, CH_2O), 4.80 (dd, J = 10.8, 9.4 Hz, 1 H, H 2), 4.53 (dd, J = 11.1, 1.9 Hz, 1 H, H-2), 4.31−4.39 (m, 1 H, H-1), 4.07 (dd, J = 11.1, 3.1 Hz, 1 H, CH_2-Cl), 3.91−3.97 (m, 4 H, OCH_3, CH_2Cl), 3.82 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3). Anal. ($C_{33}H_{29}ClN_4O_8$) C, H, N.

2-Methoxy-4-nitrobenzyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (5). Phosgene (300 μL, 0.3 mmol, 1 M in toluene) was added to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol (**34**)[19] (20 mg, 0.11 mmol) in THF (10 mL) and stirred at 20 °C for 16 h. The solvent was evaporated, the residue dissolved in THF (10 mL), a solution of **3a** (50 mg, 0.11 mmol) in THF (10 mL) added, and the solution stirred at 20 °C for 4 days. The solvent was evaporated and the residue purified by chromatography, eluting with 50% EtOAc/pet. ether, to give **5** (31 mg, 43%) as a tan solid, mp (EtOAc/pet. ether) 162−165 °C; ^{1}H NMR δ 9.52 (s, 1 H, indole-NH), 8.90

(s, 1 H, OCONH), 7.90 (d, J = 8.7 Hz, 1 H, H-6), 7.80 (d, J = 8.7 Hz, 1 H, H-5″), 7.77 (d, J = 8.4 Hz, 1 H, H-9), 7.70 (br s, 1 H, H-3″), 7.50−7.57 (m, 2 H, H 8, H-6″), 7.42−7.47 (m, 1 H, H-7), 7.25 (br s, 1 H, H-4), 6.99 (d, J = 2.2 Hz, 1 H, H-3′), 6.87 (s, 1 H, H-4′), 5.34 (d, J = 1.9 Hz, 2 H, CH_2O), 4.78 (dd, J = 10.7, 1.6 Hz, 1 H, H-2), 4.64 (dd, J = 10.7, 8.8 Hz, 1 H, H-2), 4.07−4.17 (m, 5 H, H-1, CH_2Cl, OCH_3), 3.95 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.45 (t, J = 10.9 Hz, 1 H, CH_2Cl); ^{13}C NMR δ 160.3, 157.2, 154.0, 150.2, 148.6, 141.6, 140.6, 138.9, 133.9, 132.0, 129.7, 129.6, 128.8, 127.4, 127.2, 125.6, 125.0, 123.6, 123.1, 123.0, 122.4, 121.8, 115.7, 106.5, 105.1, 97.6, 61.8, 61.5, 61.1, 56.2, 56.0, 54.9, 45.8, 43.4; MS (FAB$^+$) m/z 675 (MH$^+$, 10%), 677 (4), 659 (1), 639 (1), 517 (5), 234 (25); HRMS (FAB$^+$) calcd for $C_{34}H_{32}{}^{35}ClN_4O_9$ (MH$^+$) m/z 675.1858, found 675.1832; calcd for $C_{34}H_{32}{}^{37}ClN_4O_9$ (MH$^+$) m/z 677.1828, found 677.1834; Anal. ($C_{34}H_{31}ClN_4O_9\cdot H_2O$) C, H, N.

2-(2-Methoxyethoxy)-4-nitrobenzyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (6). A solution of triphosgene (12 mg, 40 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine **3a** (53 mg, 0.114 mmol) and Et_3N (32 μL, 0.228 mmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of alcohol **22** (28 mg, 0.125 mmol) in DCM (2 mL) was added, followed by ^{n}Bu$_2$Sn(OAc)$_2$ (2 drops), and the solution was stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20% EtOAc/DCM, to give **6** (75 mg, 91%) as a tan gum, ^{1}H NMR δ 9.49 (s, 1 H, indole-NH), 8.19 (s, 1 H, OCONH), 7.92 (d, J = 8.5 Hz, 1 H, H-6), 7.80−7.82 (m, 1 H, H-5″), 7.78 (d, J = 8.3 Hz, 1 H, H-9), 7.71 (d, J = 1.8 Hz, 1 H, H-3″), 7.56 (ddd, J = 8.3, 7.1, 0.8 Hz, 1 H, H-8), 7.49−7.54 (m, 1 H, H-6″), 7.45 (ddd, J = 8.5, 7.1, 0.8 Hz, 1 H, H-7), 7.27 (br s, 1 H, H-4), 7.00 (d, J = 2.3 Hz, 1 H, H-3′), 6.87 (s, 1 H, H-4′), 5.39 (s, 2 H, CH_2O), 4.79 (dd, J = 10.7, 1.7 Hz, 1 H, H-2), 4.66 (dd, J = 10.7, 8.7 Hz, 1 H, H-2), 4.23 (dd, J = 4.6, 4.4 Hz, 2 H, CH_2O), 4.15−4.20 (m, 1 H, H-1), 4.08 (s, 3 H, OCH_3), 3.94−3.98 (m, 4 H, OCH_3, CH_2Cl), 3.91 (s, 3 H, OCH_3), 3.80 (dd, J = 4.6, 4.4 Hz, 2 H, CH_2O), 3.47 (d, J = 10.9 Hz, 1 H, CH_2Cl), 3.44 (s, 3 H, OCH_3); ^{13}C NMR δ 160.3, 156.5, 154.0, 150.2, 148.5, 141.7, 140.6, 138.9, 133.9, 132.4, 129.7, 129.6, 128.7, 127.5, 125.6, 125.0, 123.6, 123.1, 122.5 (2), 121.8, 116.0, 112.7, 106.5, 106.3, 97.7, 70.7, 68.5, 61.9, 61.5, 61.1, 59.3, 56.3, 54.9, 45.9, 43.1; MS (FAB$^+$) m/z 721 (MH$^+$, 1.5%), 719 (MH$^+$, 3.5); HRMS (FAB$^+$) calcd for $C_{36}H_{36}{}^{37}ClN_4O_{10}$ (MH$^+$) m/z 721.2091, found 721.2131; calcd for $C_{36}H_{36}{}^{35}ClN_4O_{10}$ (MH$^+$) m/z 719.2120, found 719.2133. Anal. ($C_{36}H_{35}ClN_4O_{10}$) C, H, N.

2-(2-Hydroxyethoxy)-4-nitrobenzyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (7). Similarly, reaction of amine **3a** with alcohol **23** gave 2-(2-{[*tert*-butyl(dimethyl)silyl]-oxy}ethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (**27**) (94%) as a white solid, mp (EtOAc/pet. ether) 182−185 °C; ^{1}H NMR δ 9.45 (s, 1 H, indole-NH), 8.93 (s, 1 H, OCONH), 7.92 (d, J = 8.5 Hz, 1 H, H-6), 7.76−7.83 (m, 3 H, H-9, H-3″, H-5″), 7.53−7.60 (m, 2 H, H-8, H-6″), 7.47 (ddd, J = 8.5, 7.1, 0.8 Hz, 1 H, H-7), 7.13 (br s, 1 H, H-4), 7.01 (d, J = 2.2 Hz, 1 H, H-3′), 6.88 (s, 1 H, H-4′), 5.39 (s, 2 H, CH_2O), 4.81 (dd, J = 10.7, 1.8 Hz, 1 H, H-2), 4.67 (dd, J = 10.7, 8.7 Hz, 1 H, H-2), 4.21 (br dd, J = 5.0, 4.8 Hz, 2 H, CH_2O), 4.15−4.18 (m, 1 H, H-1), 4.09 (s, 3 H, OCH_3), 4.02 (br d, J = 5.0 Hz, 2 H, CH_2O), 3.97 (dd, J = 11.5, 3.1 Hz, 1 H, CH_2Cl), 3.95 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.48 (dd, J = 11.5, 10.9 Hz, 1 H, CH_2Cl), 0.90 (s, 9 H, $SiC(CH_3)_3$), 0.10 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR δ 160.3, 156.7, 154.0, 150.2, 148.5, 141.7, 140.6, 138.9, 133.8, 132.3, 129.7, 129.6, 128.7, 127.5, 125.6, 125.0, 123.6, 123.1, 122.4 (2), 121.8, 115.8, 112.8, 106.5 (2), 97.7, 70.7, 61.8, 61.7, 61.5, 61.1, 56.3, 54.9, 45.8, 43.5, 25.8 (3), 18.3, −5.4 (2); MS (FAB$^+$) m/z 819 (MH$^+$, 25%), 821 (MH$^+$, 12); HRMS (FAB$^+$) calcd for $C_{41}H_{48}{}^{35}ClN_4O_{10}Si$ (MH$^+$) m/z 819.2828, found 819.2804; calcd for $C_{41}H_{48}{}^{37}ClN_4O_{10}Si$ (MH$^+$) m/z 821.2799, found 821.2803; Anal. ($C_{41}H_{47}ClN_4O_{10}Si$) C, H, N.

HCl (1 M) (0.4 mL, 0.40 mmol) was added to a stirred solution of silyl ether **27** (157 mg, 0.19 mmol) in MeOH (5 mL) and the solution stirred at 20 °C for 1 h. The solvent was evaporated and the residue partitoned between EtOAc (50 mL) and water (50 mL). The organic fraction was washed with water (50 mL) and brine (25 mL) and dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20−50%) of EtOAc/pet. ether, to give carbamate **7** (119 mg, 88%) as a hygroscopic white solid, ^{1}H NMR δ 9.72 (s, 1 H, indole-NH), 8.80 (s, 1 H, OCONH), 7.86 (d, J = 8.5 Hz, 1 H, H-6), 7.79 (br d, J = 8.1 Hz, 1 H, H-5″), 7.67−7.73 (m, 2 H, H 9, H-3″), 7.47−7.53 (m, 3 H, H-4, H-8, H-6″), 7.37 (ddd, J = 8.5, 7.1, 0.8 Hz, 1 H, H-7), 6.97 (d, J = 2.2 Hz, 1 H, H-3′), 6.87 (s, 1 H, H-4′), 5.40 (s, 2 H, CH_2O), 4.73 (dd, J = 10.7, 1.6 Hz, 1 H, H-2), 4.59 (dd, J = 10.7, 8.7 Hz, 1 H, H-2), 4.21 (br dd, J = 4.6, 4.0 Hz, 2 H, CH_2O), 4.07−4.11 (m, 4 H, H-1, OCH_3), 4.00−4.04 (m, 2 H, CH_2O), 3.95 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.85 (d, J = 11.3, 3.0 Hz, 1 H, CH_2Cl), 3.39 (br s, 1 H, OH), 3.28 (dd, J = 11.3, 10.9 Hz, 1 H, CH_2Cl); ^{13}C NMR δ 160.5, 157.2, 154.4, 150.2, 148.9, 141.4, 140.6, 138.9, 133.8, 131.9, 130.3, 129.6 (2), 127.4, 125.8, 125.0, 123.6, 123.0, 122.5 (2), 121.9, 115.9, 112.8, 106.7, 106.6, 97.7, 70.7, 62.0, 61.5, 61.1, 60.9, 56.3, 55.1, 45.6, 43.3; MS (FAB$^+$) m/z 707 (MH$^+$, 5%), 705 (MH$^+$, 14); HRMS (FAB$^+$) calcd for $C_{35}H_{34}{}^{35}ClN_4O_{10}$ (MH$^+$) m/z 705.1964, found 705.1919; calcd for $C_{35}H_{34}{}^{37}ClN_4O_{10}$ (MH$^+$) m/z 707.1934, found 707.1931. Anal. ($C_{35}H_{33}ClN_4O_{10}$) C, H, N.

2-(3-Hydroxypropoxy)-4-nitrobenzyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (8). Similarly, reaction of amine **3a** and alcohol **24** gave 2-(3-{[*tert*-butyl(dimethyl)silyl]-oxy}propoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (**28**) (67%) as a white solid, mp (MeOH) 149−150 °C; ^{1}H NMR δ 9.42 (s, 1 H, indole-NH), 8.96 (s, 1 H, OCONH), 7.91 (d, J = 8.4 Hz, 1 H, H-6), 7.78−7.85 (m, 2 H, H 9, H-5″), 7.75 (d, J = 1.7 Hz, 1 H, H-3″), 7.53−7.59 (m, 2 H, H-8, H-6″), 7.47 (ddd, J = 8.4, 7.4, 0.8 Hz, 1 H, H-7), 7.08 (br s, 1 H, H-4), 7.02 (d, J = 2.2 Hz, 1 H, H-3′), 6.89 (s, 1 H, H-4′), 5.38 (s, 2 H, CH_2O), 4.82 (dd, J = 10.7, 1.7 Hz, 1 H, H-2), 4.69 (dd, J = 10.7, 8.7 Hz, 1 H, H-2), 4.21 (t, J = 6.0 Hz, 2 H, CH_2O), 4.17−4.20 (m, 1 H, CH_2Cl), 4.09 (s, 3 H, OCH_3), 3.99 (dd, J = 11.3, 2.9 Hz, 1 H, H-1), 3.95 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.83 (t, J = 5.9 Hz, 2 H, CH_2O), 3.49 (t, J = 11.0 Hz, 1 H, CH_2Cl), 2.02−2.08 (m, 2 H, CH_2), 0.88 (s, 9 H, $OSiC(CH_3)_3$), 0.04 (s, 6 H, $OSi(CH_3)_2$; MS (FAB$^+$) m/z 833 (MH$^+$, 25%), 835 (MH$^+$, 12), 775 (5), 599 (5); HRMS (FAB$^+$) calcd for $C_{42}H_{50}{}^{35}ClN_4O_{10}Si$ (MH$^+$) m/z 833.2985, found 833.3008; calcd for $C_{42}H_{50}{}^{37}ClN_4O_{10}Si$ (MH$^+$) m/z 835.2955, found 835.2982. Anal. ($C_{42}H_{49}ClN_4O_{10}Si$) C, H, N.

HCl (1 M) (0.2 mL, 0.20 mmol) was added to a stirred solution of silyl ether **28** (64 mg, 0.08 mmol) in MeOH (5 mL) and the solution stirred at 20 °C for 30 min. The solvent was evaporated, the residue dissolved in EtOAc (50 mL), washed with water (2 × 50 mL) and brine (25 mL), and dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (50−100%) of EtOAc/pet. ether, to give carbamate **8** (52 mg, 94%) as a white solid, mp (EtOAc) 122−126 °C; ^{1}H NMR δ 9.51 (s, 1 H, indole-NH), 8.90 (s, 1 H, OCONH), 7.92 (d, J = 8.5 Hz, 1 H, H-6), 7.80 (d, J = 8.2 Hz, 1 H, H-5″), 7.77 (d, J = 8.3 Hz, 1 H, H-9), 7.73 (d, J = 1.8 Hz, 1 H, H-3″), 7.50−7.57 (m, 2 H, H-8, H-6″), 7.40−7.46 (m, 2 H, H-4, H-7), 6.99 (d, J = 2.2 Hz, 1 H, H-3′), 6.87 (s, 1 H, H-4′), 5.37 (d, J = 13.1 Hz, 1 H, CH_2O), 5.32 (d, J = 13.1 Hz, 1 H, CH_2O), 4.77 (dd, J = 10.8, 1.6 Hz, 1 H, H-2), 4.64 (dd, J = 10.8, 8.6 Hz, 1 H, H-2), 4.27 (t, J = 5.7 Hz, 2 H, CH_2O), 4.11−4.18 (m, 1 H, CH_2Cl), 4.09 (s, 3 H, OCH_3), 3.96 (s, 3 H, OCH_3), 3.91−3.95 (m, 3 H, H 1, CH_2O), 3.90 (s, 3 H, OCH_3), 3.44 (t, J = 10.9 Hz, 1 H, CH_2Cl), 2.75 (br s, 1 H, OH), 2.12−2.18 (m, 2 H, CH_2); ^{13}C NMR δ 160.4, 157.2, 153.8, 150.2, 148.9, 141.6, 140.6, 138.9, 134.0, 131.6, 130.1, 129.7, 129.6, 127.5, 125.7, 125.0, 123.6, 123.1, 122.4 (2), 121.6, 115.7, 112.2, 106.6, 106.1, 97.7, 66.8, 62.2, 61.5, 61.1, 60.1, 56.3, 55.0, 45.8, 43.4, 31.6; MS (FAB$^+$) m/z 721 (MH$^+$, 2%), 719 (MH$^+$, 4); HRMS (FAB$^+$)

calcd for $C_{36}H_{35}{}^{35}ClN_4O_{10}$ (MH^+) *m/z* 719.2120, found 719.2107; calcd for $C_{36}H_{35}{}^{37}ClN_4O_{10}$ (MH^+) *m/z* 721.2091, found 721.2093. Anal. ($C_{36}H_{35}ClN_4O_{10}$) C, H, N.

2-(2,3-Dihydroxypropoxy)-4-nitrobenzyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (9). Similarly, reaction of amine **3a** and alcohol **32** gave 2-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]-indol-5-ylcarbamate (**33**) (96%) as a gum, ^{1}H NMR δ 9.44 (s, 1 H, indole-NH), 8.94 (s, 1 H, OCONH), 7.92 (d, J = 8.5 Hz, 1 H, H-6), 7.87 (dd, J = 8.2, 2.1 Hz, 1 H, H-5″), 7.81 (d, J = 8.2 Hz, 1 H, H-9), 7.73 (d, J = 2.1 Hz, 1 H, H-3″), 7.58 (ddd, J = 8.2, 7.3, 0.7 Hz, 1 H, H-8), 7.45−7.51 (m, 2 H, H-7, H-6″), 7.13 (br s, 1 H, H-4), 7.02 (d, J = 2.2 Hz, 1 H, H-3′), 6.89 (s, 1 H, H-4′), 5.38 (s, 2 H, CH_2O), 4.83 (dd, J = 10.8, 1.7 Hz, 1 H, H-2), 4.69 (dd, J = 10.8, 8.7 Hz, 1 H, H-2), 4.50−4.56 (m, 1 H, H-4‴), 4.23 (dd, J = 9.8, 4.0 Hz, 1 H, H-5‴), 4.15−4.20 (m, 2 H, H-1, H-2‴), 4.09−4.14 (m, 4 H, OCH_3, H-5‴), 3.95−4.00 (m, 5 H, OCH_3, CH_2Cl, H-2‴), 3.92 (s, 3 H, OCH_3), 3.50 (dd, J = 10.9, 10.8 Hz, 1 H, CH_2Cl), 1.45 (s, 3 H, CH_3), 1.39 (s, 3 H, CH_3); MS (FAB^+) *m/z* 777 (MH^+, 10%), 775 (MH^+, 35); HRMS (FAB^+) calcd for $C_{39}H_{40}{}^{35}ClN_4O_{11}$ (MH^+) *m/z* 775.2381, found 777.2379; calcd for $C_{39}H_{40}{}^{37}ClN_4O_{11}$ (MH^+) *m/z* 777.2535, found 777.2354.

HCl (1 M) (1 mL) was added to a stirred suspension of acetonide **33** (160 mg, 0.06 mmol) in THF (20 mL) and the mixture stirred at 20 °C for 16 h. The mixture was evaporated and the residue partitioned between DCM (50 mL) and water (50 mL). The organic fraction was washed with water (30 mL) and brine (30 mL) and dried and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/DCM, to give carbamate **9** (87 mg, 56%), as a tan solid, mp (MeOH/^{i}Pr$_2$O) 147−149 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.46 (s, 1 H, indole-NH), 9.90 (s, 1 H, OCONH), 8.56 (s, 1 H, H-4), 8.12 (d, J = 8.5 Hz, 1 H, H-6), 7.98 (d, J = 8.3 Hz, 1 H, H-9), 7.92 (dd, J = 8.3, 1.9 Hz, 1 H, H-5″), 7.83 (d, J = 1.9 Hz, 1 H, H-3″), 7.69 (d, J = 8.3 Hz, 1 H, H-6″), 7.59 (dd, J = 8.2, 7.6 Hz, 1 H, H-8), 7.49 (dd, J = 8.5, 7.6 Hz, 1 H, H-7), 7.09 (d, J = 2.1 Hz, 1 H, H-3′), 6.98 (s, 1 H, H-4′), 5.33 (s, 2 H, CH_2O), 5.07 (d, J = 5.2 Hz, 1 H, OH), 4.81 (dd, J = 11.0, 9.7 Hz, 1 H, H-2), 4.73 (t, J = 5.7 Hz, 1 H, H-3‴), 4.53 (dd, J = 11.0, 3.5 Hz, 1 H, H-2), 4.32−4.37 (m, 1 H, H-1), 4.24 (dd, J = 10.0, 3.9 Hz, 1 H, CH_2Cl), 4.09−4.13 (m, 1 H, H-2‴), 4.02−4.06 (m, 1 H, H-3′), 3.93−3.96 (m, 4 H, OCH_3, CH_2Cl), 3.84−3.89 (m, 1 H, OH), 3.83 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.51 (t, J = 5.7 Hz, 2 H, H-1‴); ^{13}C NMR [$(CD_3)_2SO$] δ 160.2, 156.2, 154.3, 149.2, 148.0, 141.5, 139.9, 139.0, 134.3, 133.0, 130.7, 129.4, 128.0, 127.1, 125.4 (2), 124.3, 123.9, 123.3, 123.1, 122.0, 115.4, 113.0, 106.3, 106.2, 98.0, 70.7, 69.7, 62.4, 61.0, 60.9, 60.7, 55.9, 54.9, 47.5, 41.4; MS (FAB^+) *m/z* 737 (MH^+, 3%), 735 (MH^+, 8); HRMS (FAB^+) calcd for $C_{36}H_{36}{}^{35}ClN_4O_{11}$ (MH^+) *m/z* 735.2069, found 735.2050; calcd for $C_{36}H_{36}{}^{37}ClN_4O_{11}$ (MH^+) *m/z* 737.2040, found 737.2000. Anal. ($C_{36}H_{35}ClN_4O_{11}\cdot CH_3OH$) C, H, N.

2-[3-(Dimethylamino)propoxy]-4-nitrobenzyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (10). Similarly, reaction of amine **3a** and alcohol **25** gave carbamate **10** (55%) as a yellow solid which was converted to the hydrochloride salt, mp (MeOH) 176−180 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.43 (s, 1 H, indole-NH), 10.47 (br s, 1 H, NH^+Cl^-), 9.92 (s, 1 H, OCONH), 8.58 (s, 1 H, H 4), 8.10 (d, J = 8.5 Hz, 1 H, H-6), 7.97 (d, J = 8.3 Hz, 1 H, H-9), 7.93 (dd, J = 8.4, 2.0 Hz, 1 H, H-5″), 7.82 (d, J = 2.0 Hz, 1 H, H-3″), 7.71 (br d, J = 8.4 Hz, 1 H, H-6″), 7.56−7.61 (m, 1 H, H-8), 7.46−7.51 (m, 1 H, H-7), 7.10 (d, J = 2.1 Hz, 1 H, H-3′), 6.97 (s, 1 H, H-4′), 5.34 (s, 2 H, CH_2O), 4.81 (dd, J = 10.8, 9.5 Hz, 1 H, H-2), 4.53 (dd, J = 10.8, 1.7 Hz, 1 H, H-2), 4.33−4.38 (m, 1 H, H-1), 4.30 (t, J = 5.9 Hz, 2 H, CH_2O), 4.07 (dd, J = 11.1, 2.9 Hz, 1 H, CH_2Cl), 3.94−3.97 (m, 4 H, CH_2Cl, OCH_3), 3.83 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.23−3.27 (t, J = 7.5 Hz, 2 H, CH_2N), 2.75 (s, 6 H, $N(CH_3)_2$), 2.17−2.23 (m, 2 H, CH_2); ^{13}C NMR [$(CD_3)_2SO$] δ 160.2, 155.8, 154.3, 149.2, 148.0, 141.5, 139.9, 138.9, 134.3, 132.9, 130.7, 129.5, 128.5, 127.2, 125.4, 124.4, 123.7, 123.3, 123.1, 122.1, 121.2, 115.7, 113.0, 106.3 (2), 98.0, 66.8, 61.0, 60.9, 60.7, 55.9, 54.9, 53.7, 47.5, 42.0 (2), 41.1, 23.6; MS (FAB^+) *m/z* 746 (MH^+, 16%), 748 (MH^+, 7); HRMS (FAB^+) calcd for $C_{38}H_{41}{}^{35}ClN_5O_9$ (MH^+) *m/z* 746.2593, found 746.2582; calcd for $C_{38}H_{41}{}^{37}ClN_5O_9$ (MH^+) *m/z* 748.2563, found 748.2577. Anal. ($C_{38}H_{40}ClN_5O_9\cdot 2HCl$) C, H, N.

2-[3-(4-Morpholinyl)propoxy]-4-nitrobenzyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (11). Similarly, reaction of amine **3a** and alcohol **26** gave carbamate **11** (92%) as a tan solid, mp (EtOAc) 102−107 °C; ^{1}H NMR δ 9.48 (s, 1 H, indole-NH), 8.93 (s, 1 H, OCONH), 7.91 (d, J = 8.5 Hz, 1 H, H-6), 7.78−7.84 (m, 2 H, H 9, H-5″), 7.73 (d, J = 1.8 Hz, 1 H, H-3″), 7.57 (ddd, J = 8.2, 7.0, 1.0 Hz, 1 H, H-8), 7.50−7.53 (m, 1 H, H-6″), 7.45 (ddd, J = 8.5, 7.0, 1.0 Hz, 1 H, H-7), 7.28 (br s, 1 H, H-4), 7.00 (d, J = 2.2 Hz, 1 H, H-3′), 6.88 (s, 1 H, H-4′), 5.36 (s, 2 H, CH_2O), 4.81 (dd, J = 10.8, 1.8 Hz, 1 H, H-2), 4.68 (dd, J = 10.8, 8.7 Hz, 1 H, H-2), 4.16−4.22 (m, 3 H, CH_2O, CH_2Cl), 4.08 (s, 3 H, OCH_3), 3.93−3.99 (m, 4 H, H-1, OCH_3), 3.92 (s, 3 H, OCH_3), 3.67−3.69 (m, 4 H, 2 × CH_2O), 3.49 (t, J = 10.9 Hz, 1 H, CH_2Cl), 2.55 (t, J = 7.1 Hz, 2 H, CH_2N), 2.43−2.49 (m, 4 H, 2 × CH_2N), 2.00−2.08 (m, 2 H, CH_2); ^{13}C NMR δ 160.4, 156.7, 154.0, 150.2, 148.6, 141.7, 140.6, 138.9, 133.9, 132.2, 129.8, 129.6, 128.8, 127.5, 125.7, 125.0, 123.6, 123.2, 122.4 (2), 121.6, 115.7, 112.2, 106.5, 106.1, 97.7, 67.1, 66.8 (2), 61.9, 61.5, 61.1, 56.3, 55.2, 54.9, 53.6 (2), 45.8, 43.4, 26.1; MS (FAB^+) *m/z* 788 (MH^+, 6%), 790 (MH^+, 3); HRMS (FAB^+) calcd for $C_{40}H_{43}{}^{35}ClN_5O_{10}$ (MH^+) *m/z* 788.2699, found 788.2721; calcd for $C_{40}H_{43}{}^{37}ClN_5O_{10}$ (MH^+) *m/z* 790.2699, found 790.2728. Anal. ($C_{40}H_{42}ClN_5O_{10}\cdot{}^1/_2H_2O$) C, H, N.

4-({[(2-Methoxy-4-nitrobenzyl)oxy]carbonyl}amino)-benzyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (13). Similarly, reaction of amine **3a** and alcohol **37** gave carbamate **13** (47%) as a white solid, mp 135−141 °C; ^{1}H NMR δ 11.47 (s, 1 H, indole-NH), 9.97 (s, 1 H, $NHCO_2$), 9.71 (s, 1 H, $NHCO_2$), 8.56 (s, 1 H, H-4), 8.05 (d, J = 8.6 Hz, 1 H, H-6), 7.97 (d, J = 8.3 Hz, 1 H, H-9), 7.91 (dd, J = 8.3, 2.1 Hz, 1 H, H-5‴), 7.81 (d, J = 2.1 Hz, 1 H, H-3‴), 7.64 (d, J = 8.3 Hz, 1 H, H-6‴), 7.57 (t, J = 7.6 Hz, 1 H, H-8), 7.51 (d, J = 8.4 Hz, 2 H, H-3″, H-5″), 7.44 (t, J = 7.7 Hz, 1 H, H-7), 7.40 (d, J = 8.5 Hz, 2 H, H-2″, H-6″), 7.09 (s, 1 H, H-3′), 6.98 (s, 1 H, H-4′), 5.23 (s, 2 H, CH_2O), 5.12 (s, 2 H, CH_2O), 4.80 (t, J = 10.1 Hz, 1 H, 1 H, H-2), 4.52 (dd, J = 11.1, 1.6 Hz, 1 H, H-2), 4.28−4.38 (m, 1 H, H-1), 4.06 (dd, J = 11.2, 3.1 Hz, 1 H, CH_2Cl), 3.97 (s, 3 H, OCH_3), 3.89−3.96 (m, 4 H, CH_2Cl, OCH_3), 3.82 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3). Anal. ($C_{42}H_{38}ClN_5O_{11}$) C, H, N, Cl.

1-(4-Nitrophenyl)ethyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo-[*e*]indol-5-ylcarbamate (12). A solution of 1-(4-nitrophenyl)-ethanol[37] (**38**) (18 mg, 0.11 mmol) in DCM (2 mL) was added dropwise to a stirred solution of triphosgene (16 mg, 0.054 mmol) and pyridine (9 μL, 0.11 mmol) in DCM (2 mL) at 20 °C. The mixture was stirred at 20 °C for 2 h, the solvent evaporated, and the residue dissolved in THF (5 mL). A solution of **3a** (50 mg, 0.11 mmol) 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 25% EtOAc/pet. ether, to give **12** (23 mg, 32%) as a tan solid, mp (EtOAc/pet. ether) 175−178 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.49 (s, 1 H, indole-NH), 8.88 (s, 1 H, OCONH), 8.18 (d, J = 7.6 Hz, 2 H, H-3″, H-5″), 7.88 (d, J = 8.3 Hz, 1 H, H-6), 7.78 (d, J = 8.3 Hz, 1 H, H-9), 7.52−7.58 (m, 3 H, H-8, H-2″, H-6″), 7.45 (dd, J = 7.8, 7.5 Hz, 1 H, H-7), 7.16 (br s, 1 H, H-4), 7.00 (d, J = 1.9 Hz, 1 H, H-3′), 6.87 (s, 1 H, H-4′), 6.00 (q, J = 6.6 Hz, 1 H, CHO), 4.80 (dd, J = 10.7, 1.2 Hz, 1 H, H-2), 4.65 (dd, J = 10.7, 8.8 Hz, 1 H, H-2), 4.11−4.17 (m, 1 H, CH_2Cl), 4.08 (s, 3 H, OCH_3), 3.93−3.97 (m, 4 H, OCH_3, CH_2Cl), 3.91 (s, 3 H, OCH_3), 3.45 (dt, J = 10.7, 3.3 Hz, 1 H, H-1), 1.65 (br d, J = 6.6 Hz, 3 H, CH_3); ^{13}C NMR δ 160.3,

153.4, 150.2, 149.0, 147.5, 141.6, 140.6, 138.9, 133.8, 130.4, 129.7, 129.6, 127.4, 126.8 (2), 125.6, 125.0, 123.9, 123.8 (2), 123.6, 123.1, 122.3, 121.7, 106.5, 97.6, 72.6, 61.5, 61.1, 56.3, 54.9, 45.8, 43.4, 22.6; MS (FAB$^+$) *m*/*z* 659 (MH$^+$, 6%), 658 (6), 510 (1), 234 (10); HRMS (FAB$^+$) calcd for $C_{34}H_{32}{}^{35}ClN_4O_8$ (MH$^+$) *m*/*z* 659.1909, found 659.1881; calcd for $C_{34}H_{32}{}^{37}ClN_4O_8$ (MH$^+$) *m*/*z* 661.1879, found 661.1882. Anal. ($C_{34}H_{31}ClN_4O_8$) C, H, N. Starting material **3a** (30 mg, 60%) was also recovered.

4-Nitrobenzyl 1-(Chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-yl(methyl)carbamate (14). A solution of 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-yl(methyl)amine (**3b**)[25] (77 mg, 0.16 mmol) in dry DCM containing pyridine (32 μL, 0.40 mmol) was treated portionwise at 0 °C with 4-nitrobenzyl chloroformate (69 mg, 0.32 mmol). The mixture was stirred at 10 °C for a further 45 min, then diluted with DCM (20 mL) and washed with water (2 × 10 mL) and dried. Solvent was evaporated and the residue was purified on alumina, eluting with 15% EtOAc/DCM, to give **14** (84 mg, 79%) as a tan solid, mp (EtOAc/^{i}Pr$_2$O) 125−128 °C; ^{1}H NMR δ (2 rotamers) 9.38 (br s, 1 H, indole-NH), 8.55 (br s, 1 H, H-4), 8.02 and 8.07 (2 × d, *J* = 8.5 Hz, 2 H, H-3″, H-5″), 7.86 (br d, *J* = 8.3 Hz, 1 H, H-6), 7.78−7.82 (m 1 H, H-9), 7.60−7.64 (m, 1 H, H-8), 7.47 (ddd, *J* = 8.3, 7.0, 0.9 Hz, 1 H, H-7), 7.11 and 7.19 (2 × d, *J* = 8.5 Hz, 2 H, H-2″, H-6″), 7.04 (d, *J* = 2.2 Hz, 1 H, H-3′), 6.89 (s, 1 H, H-4′), 5.12−5.20 (m, 2 H, CH_2O), 4.86 (br d, *J* = 10.7 Hz, 1 H, H-2), 4.73 (br dd, *J* = 10.7, 8.7 Hz, 1 H, H-2), 4.24−4.28 (m, 1 H, H-1), 4.11 (s, 3 H, OCH_3), 4.00−4.07 (m, 1 H, CH_2Cl), 3.96 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.50−3.57 (m, 1 H, CH_2Cl), 3.40 and 3.46 (2 × s, 3 H, NCH_3). Anal. ($C_{34}H_{31}ClN_4O_8$) C, H, N.

4-Nitrobenzyl 1-(Chloromethyl)-3-({5-[2-(dimethylamino)ethoxy]-1*H*-indol-2-yl}carbonyl)-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (15). A stirred solution of *tert*-butyl 5-amino-1-(chloromethyl)-1,2-dihydro-3*H*-benzo[*e*]indole-3-carboxylate (**39**)[26] [(400 mg, 1.20 mmol) in THF (25 mL) was treated at 0 °C with 4-nitrobenzyl chloroformate (388 mg, 1.80 mmol). The mixture was stirred at 0 °C for 45 min and stirred for a further 1 h at 20 °C. The mixture was concentrated to 10 mL, DCM (40 mL) was added, the solution was washed with dilute $KHCO_3$ (50 mL) and water (50 mL) and then dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give *tert*-butyl 1-(chloromethyl)-5-({[(4-nitrobenzyl)oxy]carbonyl}amino)-1,2-dihydro-3*H*-benzo[*e*]indole-3-carboxylate (**40**) (344 mg, 56%) as a white solid, mp (EtOAc/^{i}Pr$_2$O) 152 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 9.86 (s, 1 H, NH), 8.28 (d, *J* = 8.7 Hz, 2 H, H-3′, H-5′), 8.10 (br s, 1 H, H-4), 8.06 (d, *J* = 8.5 Hz, 1 H, H-6), 7.88 (d, *J* = 8.3 Hz, 1 H, H-9), 7.73 (d, *J* = 8.7 Hz, 2 H, H-2′, H-6′), 7.53 (t, *J* = 7.6 Hz, 1 H, H-8), 7.40 (t, *J* = 7.6 Hz, 1 H, H-7), 5.35 (s, 2 H, CH_2O), 4.27−4.11 (m, 2 H, H-2), 4.11−3.97 (m, 2 H, H-1, CH_2Cl), 3.88 (dd, *J* = 10.8, 6.7 Hz, 1 H, CH_2Cl), 1.52 (s, 9 H, $C(CH_3)_3$). Anal. ($C_{26}H_{26}ClN_3O_6$) C, H, N, Cl.

A solution of **40** (296 mg, 0.58 mmol) in HCl-saturated dioxane (10 mL) was stirred at 20 °C for 45 min, then the solvent evaporated below 30 °C. 5-[2-(Dimethylamino)ethoxy]-1*H*-indole-2-carboxylic acid[26] (174 mg, 0.61 mmol), EDCI·HCl (278 mg, 1.45 mmol), and DMA (2 mL) were then added, and the mixture was stirred at 20 °C for 6 h. The mixture was basified with dilute NH_3, and the precipitated solid was collected and purified by chromatography on alumina-90. Elution with 5% MeOH/EtOAc gave **15** (219 mg, 59%), mp (^{i}Pr$_2$O/EtOAc) 115−118 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 11.62 (s, 1 H, indole NH), 9.91 (s, 1 H, $NHCO_2$), 8.61 (s, 1 H, H-4), 8.29 (d, *J* = 8.6 Hz, 2 H, H-3″, H-5″), 8.08 (d, *J* = 8.5 Hz, 1 H, H-6), 8.00 (d, *J* = 8.3 Hz, 1 H, H-9), 7.74 (d, *J* = 8.6 Hz, 2 H, H-2″, H 6″), 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), 7.40 (d, *J* = 8.9 Hz, 1 H, H-7′), 7.18 (d, *J* = 2.2 Hz, 1 H, H-4′), 7.12 (s, 1 H, H-3′), 6.92 (dd, *J* = 8.9, 2.3 Hz, 1 H, H-6′), 5.36 (s, 2 H, CH_2O), 4.85 (t, *J* = 10.1 Hz, 1 H, H-2), 4.61 (dd, *J* = 10.1, 1.9 Hz, 1 H, H-2), 4.35−4.43 (m, 1 H, H-1), 4.04−4.12 (m, 3 H, CH_2O, CH_2Cl), 3.98 (dd, *J* = 11.1, 6.7 Hz, 1 H, CH_2Cl), 2.65 (t, *J* = 5.8 Hz, 2 H, CH_2N), 2.24 (s, 6 H, $N(CH_3)_2$). Anal. ($C_{34}H_{32}ClN_5O_6$) C, H, N, Cl.

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.[16] Skov-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.[11] 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.[36] 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}; SKOV-NTR^{neo}).

Growth Inhibition Assays. Growth inhibitory potencies were determined under aerobic conditions using log-phase cultures in 96-well plates, as described previously.[38,39] 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. Compounds 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 1 μL/g body weight, 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 against NTR-expressing and parental (NTR-ve) tumor cells 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.[36] 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 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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