Substituent effects on the kinetics of reductively-initiated fragmentation of nitrobenzyl carbamates designed as triggers for bioreductive prodrugs

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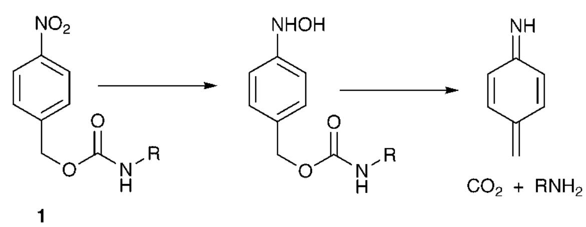
Received (in Cambridge, UK) 20th May 1999, Accepted 30th July 1999

4-Nitrobenzyl carbamates are of interest as triggers for bioreductive drugs, particularly in conjunction with the *E. coli* B nitroreductase, which efficiently reduces them to the corresponding hydroxylamines. These then fragment to release highly toxic amine-based toxins. While many 4-nitrobenzyl carbamate derivatives have been evaluated as bioreductive drugs, there has been no systematic study of substituent effects on the rate of this fragmentation (which should be as fast as possible following reduction). We therefore prepared a series of 2-, 3- and α-substituted 4-[*N*-methyl-*N*-(4-nitrobenzyloxycarbonyl)amino]phenylacetamides as model compounds to study these effects. The majority of the carbamates were prepared by *in situ* formation of the chloroformate of the appropriate 4-nitrobenzyl alcohol and reaction with methyl 4-(methylamino)phenylacetate, followed by ester hydrolysis and 1,1′-carbonyldiimidazole (CDI) mediated coupling with *N*,*N*-dimethylaminoethylamine. The hydroxylamines were generated by ^{60}Co γ-ray irradiation of the nitro compounds in aqueous phosphate-buffered-propan-2-ol. The reactions were analysed by reverse-phase HPLC to determine the maximum half-life ($Mt_{1/2}$) of the hydroxylamines generated, and the extent of release of amine from these after 10 half-lives (t_∞). The parent (unsubstituted) hydroxylaminobenzyl carbamate had a $Mt_{1/2}$ of 16 min under these conditions, while that of the corresponding α-methyl analogue was 9.5 min. Electron-donating substituents on the benzyl ring also accelerated fragmentation, with the data being fitted to the equation $\log(Mt_{1/2}) = 0.57\sigma + 1.30$, where σ represents σ_p for 2-substituents and σ_m for 3-substituents. The acceleration of fragmentation of the hydroxylamines with increasing substituent electron-donation is consistent with the proposed mechanism, and is presumably due to stabilisation of the developing positive charge on the benzylic carbon. The extent of release of amine (t_∞) also increased with increasing substituent electron-donation. These data suggest that the standard 4-nitrobenzyl carbamate trigger for nitroreductase enzyme (NTR) prodrugs can likely be improved on, by increasing the rate of fragmentation by the use of α-methyl and/or electron-donating benzyl substituents.

Introduction

Nitrobenzyl carbamates are of interest as potential bioreductive drugs, because of their ability to undergo fragmentation following reduction. In contrast to the reduction of nitrobenzyl halides[1] or quaternary salts,[2] which fragment at the radical anion stage following one-electron reduction,[3] nitrobenzyl carbamates undergo multi-electron reduction to electron-donating hydroxylamine or amine species, which then fragment to generate a quinomethane imine and an amine.[4] Initial interest centred around the reduction of compounds such as **1** (R = Me, Ph) by cellular nitroreductases, with the quinomethane or quinonimine species considered to be the potentially cytotoxic agent.[5] A subsequent study[6] considered the nitrobenzyl carbamates of 5-fluorouracil **1** (R = 5-FU) as bioreductive agents. However, the reduction potentials of compounds such as **1** are too low for efficient reduction by cellular nitroreductases, and the quinonimines generated were not particularly cytotoxic.

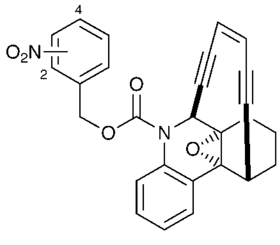
Renewed interest in nitrobenzyl carbamates as bioreductive drugs was generated by the discovery of an *E. coli* B nitroreductase enzyme (NTR)[7] which, in conjunction with NADH or NADPH, reduces certain aromatic nitro groups to the corresponding hydroxylamines.[8] Despite the low reduction potentials of typical 4-nitrobenzyl carbamates (*ca.* −490 mV),[9] they undergo reduction by the NTR enzyme (although with widely varying degrees of activation) to the corresponding unstable hydroxylamines. The latter, through increased electron release to the π-system stabilizing the developing positive charge on the benzylic carbon, readily fragment to release amines (Scheme 1). The 4-nitrobenzyl carbamate moiety has thus been proposed as a prodrug "trigger" that may deactivate highly cytotoxic amine "effectors".[10]



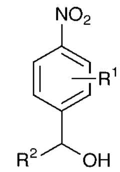
Scheme 1 Reduction and fragmentation of nitrobenzyl carbamates.

The *E. coli* enzyme has been used to activate such nitrobenzyl carbamate prodrugs in antibody-[11,12] and gene-directed enzyme prodrug therapy[13] (ADEPT and GDEPT, respectively) protocols. Several studies have shown that 4-nitrobenzyl carbamate derivatives of actinomycin,[11] mitomycin,[11] enediynes,[14] amino-*seco*-cyclopropylindoline derivatives,[15] and tallimustine derivatives[16] are substrates for *E. coli* NTR. The selectivity of the NTR enzyme for the 4-nitrobenzyl moiety is well-demonstrated in the enediyne analogues **2** and **3**, where only the 4-nitrobenzyl isomer **3** was a substrate for the enzyme.[14]

Factors of prime importance in the design of effective 4-nitrobenzyl carbamate prodrugs for nitroreductase-mediated prodrug therapy include the efficiency of the trigger unit as an



2 2-NO_2
3 4-NO_2



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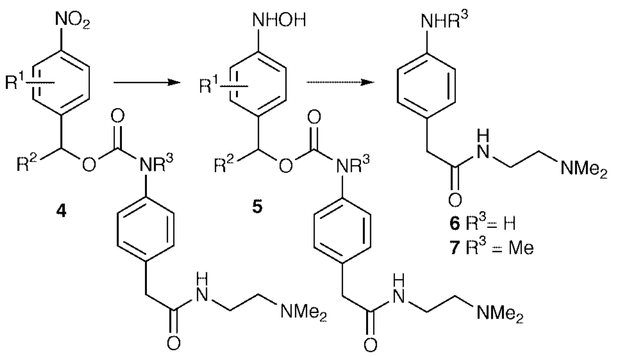
enzyme substrate and the rate of fragmentation following enzymic reduction. While kinetic structure–activity relationships (SAR) have been extensively studied for the one-electron reduction of nitrobenzyl halides[3] and quaternary salts,[2] the SAR involved in the reductive fragmentation of 4-nitrobenzyl carbamates has been neglected, with only the unsubstituted 4-nitrobenzyl derivatives being employed as prodrugs for NTR activation. The use of substituents on the nitrobenzyl moiety offers the prospect of modifying the rate of fragmentation of the reduced prodrug, and also providing a site of attachment for solubilising functionality.

In this paper we use a series of model compounds **4a**–**n** to study the effect of nitrobenzyl substituents on the rate of fragmentation of the intermediate hydroxylamines **5a**–**n** (generated by radiolysis) to release amines **6** or **7** (Scheme 2). Substituents



4,5	R^1	R^2	R^3	4,5	R^1	R^2	R^3
a	H	H	H	**h**	2-N(Me)CO_2Bu^t	H	Me
b	H	H	Me	**i**	3-N(Me)CO_2Bu^t	H	Me
c	H	Me	Me	**j**	2-NO_2	H	Me
d	2-Me	H	Me	**k**	3-NO_2	H	Me
e	3-Me	H	Me	**l**	3-CO_2Me	H	Me
f	2-OMe	H	Me	**m**	2-NHMe	H	Me
g	3-OMe	H	Me	**n**	3-NHMe	H	Me

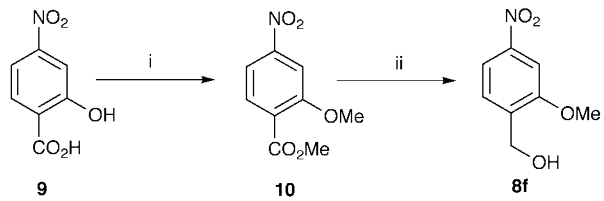
Scheme 2 Reduction and fragmentation of carbamates **4a**–**n**.

at both the 2- and 3-positions were selected to span a wide range of electronic properties, while also being able to serve as potential attachments for soluble side chains. The use of α-substituents was also expected to alter the rate of fragmentation and may be a useful site for the attachment of solubilising groups.[17] The amines **6** and **7**, released on fragmentation of **4a**–**n**, were designed as simple models of cyclopropylindoline and enediyne (*e.g.* **3**) prodrugs, while also being stable, synthetically accessible, water-soluble, and possessing a good UV signature for HPLC analysis.

Results and discussion

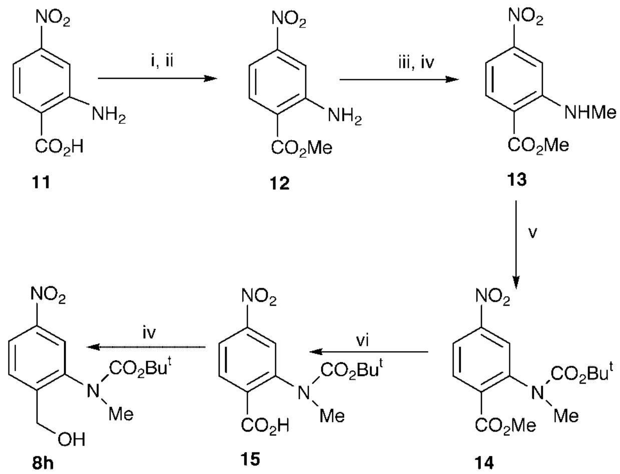
Synthesis of carbamates 4

Nitrobenzyl alcohols **8a** and **8k** for the preparation of the model carbamates were available commercially, and **8d**, **8e**, **8g**, **8l** were prepared directly by borane–dimethyl sulfide (BH_3·DMS) reduction of the corresponding acids. 1-(4-Nitrophenyl)-ethyl alcohol **8c**[18] and 2,4-dinitrobenzyl alcohol **8j**[19] were obtained as reported. 2-Methoxy-4-nitrobenzyl alcohol **8f** was conveniently prepared by methylation of 4-nitrosalicylic acid **9** and diisobutylaluminium hydride (DIBAL-H) reduction of the resulting ester **10** (Scheme 3).



Scheme 3 Synthesis of alcohol **8f**. *Reagents*: i, CH_2N_2 Et_2O; ii, DIBAL-H, THF.

2-(*N*-Methyl-*N*-*tert*-butyloxycarbonylamino)-4-nitrobenzoic acid **8h** was prepared from 4-nitroanthranilic acid **11** by esterification to give **12**, reductive methylation of the amine to give **13** and protection of **13** with the *tert*-butyloxycarbonyl (Boc) moiety to give the ester **14** (Scheme 4). Alkaline hydrolysis of **14**

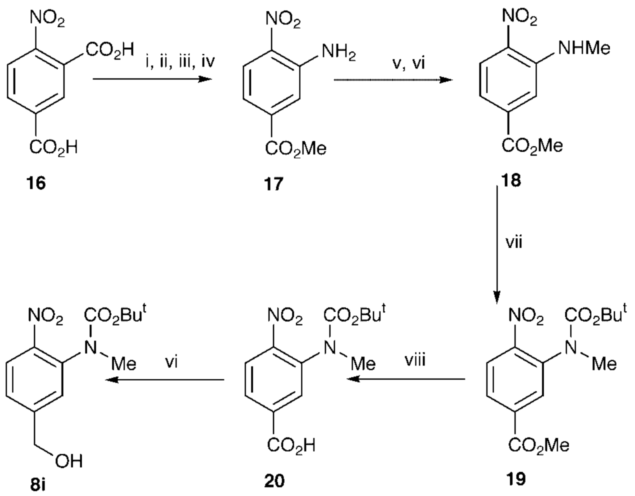


Scheme 4 Synthesis of alcohol **8h**. *Reagents*: i, $(COCl)_2$, DCM; ii, MeOH; iii, acetic formic anhydride; iv, BH_3·DMS, THF; v, di-*tert*-butyldicarbonate, DMAP, THF; vi, LiOH, MeOH.

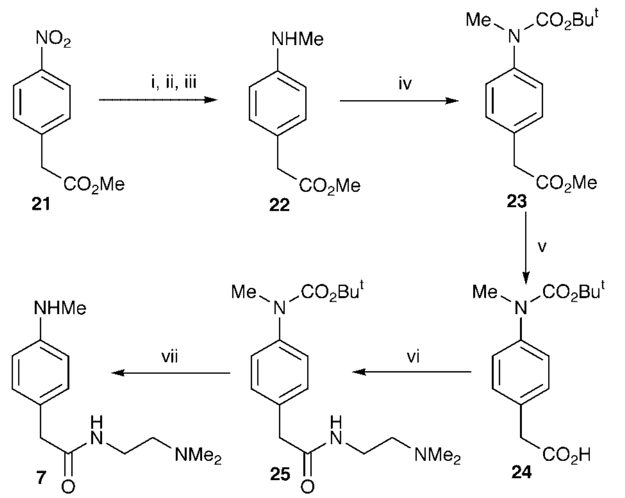
gave the acid **15** which was reduced to **8h**. Similarly, the isomer **8i** was synthesized from the corresponding amine **17**, prepared *via* a Curtius rearrangement from the 4-nitroisophthalic acid half ester **16**, through intermediates **18**–**20** (Scheme 5).

The amine **7** was readily available (Scheme 6) from methyl 4-nitrophenylacetate **21** by catalytic reduction and reductive amination to **22**. Protection of **22** as the Boc derivative **23**, hydrolysis of the ester and coupling of the resulting acid **24** with *N*,*N*-dimethylaminoethylamine provided the amide **25**. This was deprotected to give **7**, which was conveniently stored as the hydrochloride salt. The amine **6** was prepared by coupling 4-nitrophenylacetic acid **26** with *N*,*N*-dimethylaminoethylamine followed by catalytic reduction of the amide **27** and conversion to the hydrochloride salt (Scheme 7).

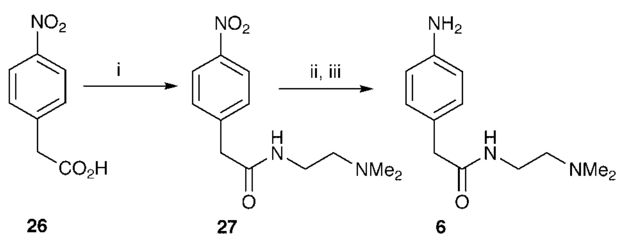
The majority of the model carbamates **4** were prepared by *in situ* formation of the chloroformate of the alcohols **8** and subsequent reaction with the amino ester **22** or methyl 4-aminophenylacetate to give the carbamate esters **28**. Basic



Scheme 5 Synthesis of alcohol **8i**. *Reagents*: i, $SOCl_2$; ii, NaN_3, acetone; iii, TMS–ethanol, toluene; iv, TBAF, THF; v, acetic formic anhydride; vi, BH_3·DMS, THF; vii, di-*tert*-butyldicarbonate, DMAP, THF; viii, LiOH, MeOH.

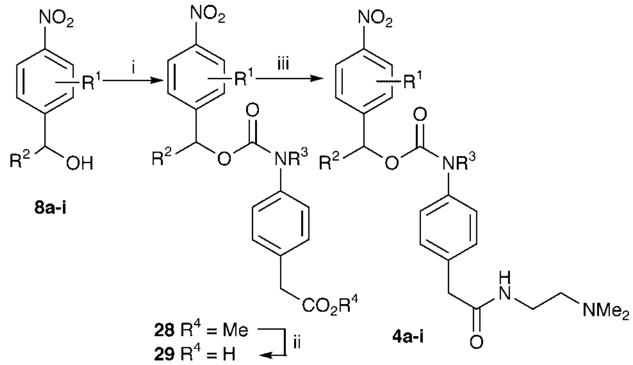


Scheme 6 Synthesis of amine **7**. *Reagents*; i, H_2, Pd/C, EtOH; ii, acetic formic anhydride; iii, BH_3·DMS, THF; iv, di-*tert*-butyldicarbonate, DMAP, THF; v, LiOH, MeOH; vi, CDI, $NH_2CH_2CH_2NMe_2$, DMF; vii, HCl, MeOH.

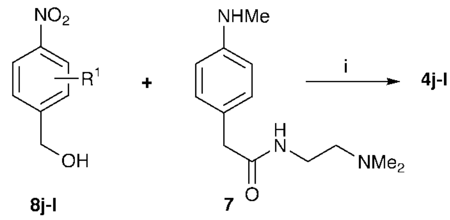


Scheme 7 Synthesis of amine **6**. *Reagents*: i, CDI, $NH_2CH_2CH_2NMe_2$, DMF; ii, H_2, Pd/C, EtOH; iii, HCl, MeOH.

hydrolysis of **28** to the acids **29**, followed by 1,1′-carbonyldiimidazole (CDI)-mediated coupling with *N*,*N*-dimethylaminoethylamine, gave carbamates **4** (Method A, Scheme 8). Carbamates **4j**, **4k**, and **4l** were not obtainable by Method A because of decomposition under the reaction conditions, and were made instead by direct coupling of the corresponding alcohols **8j**, **8k**, and **8l** to the amide **7**, with purification by semi-preparative HPLC (Method B, Scheme 9). Finally, carbamates **4m** and **4n** were made by direct HCl-mediated removal of the Boc group from **4f** and **4g**, respectively (Method C, Scheme 10).



Scheme 8 Preparation of carbamates **4a**–**i**. Method A. *Reagents*: i, $COCl_2$, then Cs_2CO_3, **22** or methyl 4-aminophenyl acetate, DMF; ii, LiOH, MeOH; iii, CDI, $NH_2CH_2CH_2NMe_2$, DMF.



Scheme 9 Preparation of carbamates **4j**–**l**. Method B. *Reagents*: i, $COCl_2$, THF.

4h, **i** R = N(Me)CO_2Bu^t —i→ **4m**, **n** R = NHMe

Scheme 10 Preparation of carbamates **4m**, **n**. Method C. *Reagents*: i, HCl, THF.

Radiolytic reduction of carbamates 4

Radiolytic reduction, rather than enzymic reduction by *E. coli* NTR enzyme, was used in order to investigate the substituent effects on carbamate fragmentation without the complicating influence of the kinetics of enzyme activation. The radiolysis of water is a useful method to study reductively-triggered reactions in aqueous media, because powerful, transient reductants can be generated in controlled quantities (*via* the exposure time of a given volume of solution at a known dose-rate) in solutions buffered over a wide pH range (2–11). It also provides information on the stoichiometry of reductive fragmentation, and hence the identity of the activated species that undergoes fragmentation. ^{60}Co γ-rays produce approximately equal amounts of reducing aquated electrons (e^-_{aq}) and oxidising hydroxyl radicals ($OH^•$) in water, and the presence of propan-2-ol produces a reducing environment by converting hydroxyl radicals to reducing 2-hydroxypropan-2-yl radicals, $(CH_3)_2C^•OH$.

A typical plot of the change in the composition of an aqueous solution (50 μM) of carbamate **4** with the extent of reductive activation by ^{60}Co γ-rays is shown in Fig. 1. Up to 6 stoichiometric equivalents of reducing radicals were added, and the compositions of the reduced solutions were examined by HPLC. Fig. 1 shows that **4b** is consumed with 4-fold stoichiometry, generating a transient species assumed to be the corresponding hydroxylamine **5b**. The instability of these compounds precluded formal identification, but reactions of **5b** with $Na_3[Fe(CN)_5NH_3]$ [sodium amminepentacyanoferrate(II)] are consistent with a hydroxyamino or nitroso compound.[20] There was also proportional release of the corresponding amine **6** or **7** with up to 4 reducing equivalents, but little further release if more than 4 reducing equivalents were used. The subsequent production of amine **7** after reduction of **4b** to the hydroxylamine **5b** may be attributed to the slow fragmentation kinetics of **5b** ($Mt_{1/2}$ = 16 min).

Table 1 Fragmentation of 4-nitrobenzyl carbamates

Compound	R^2	R^3	$Mt_{1/2}$/min [a]	t_0 [b] (%)	t_∞ [c] (%)
5a	H	H	10	52	54
5b	H	Me	16	40	49
5c	Me	Me	9.5	55	62

[a] Maximum half-life, see text for derivation. [b] % Amine released at earliest time measurable. [c] % Amine released after >10 half-lives. Values were determined in triplicate and were reproducible to within ±0.6%.

Table 2 Fragmentation of 2-substituted 4-nitrobenzyl carbamates

Compound	R^1	σ_p	$Mt_{1/2}$/min	t_o (%)	t_∞ (%)
5j	NO_2	0.78	88	18	33
5b	H	0.0	16	40	49
5j [a]	NHOH	−0.04	16	68	73
5h [b]	N(Me)CO_2tBu	−0.15	23	30	54
5d [c]	Me	−0.17	n.d.	n.d.	n.d.
5f	OMe	−0.27	12	48	55
5m	NHMe	−0.84	7.2	65	71

[a] Data collected for 8-electron reduction of **4j**. [b] σ_p ($NHCO_2Me$) used as best approximation. [c] Fragmentation rates not determined. Values were determined in triplicate and were reproducible to within ±0.6%.

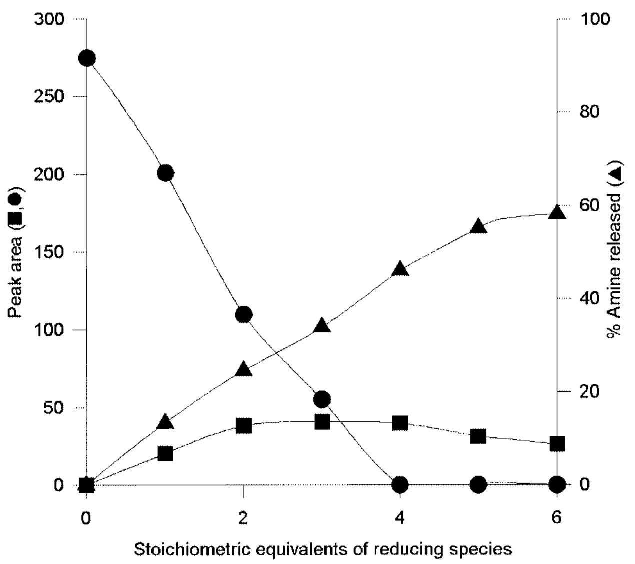


Fig. 1 Representative plot of changes in the composition of solutions of **4b** with changing extent of radiolytic reduction. Plot depicts (Left-hand y-axis) the peak areas of **4b** (●) and the major transient reduction product of **4b**, assumed to be the corresponding hydroxyaminobenzyl carbamate **5** (■) and (right-hand y-axis) the % release of amine **7** (▲). $[\mathbf{4b}]_0 = 50$ µM, pH 7.4 10 mM phosphate buffer, 4% (v/v) isopropyl alcohol, 20 °C.

These data were used to estimate the maximum half-life ($Mt_{1/2}$) of the hydroxyaminobenzyl carbamates **5**. Assuming first order conditions, half-life ($t_{1/2}$) is calculated from the equation $\ln([R]_0/[R]_t) = t(\ln 2/t_{1/2})$, where $[R]$ is the concentration of reactant at time t. Approximately 12 minutes were required to effect 4-electron reduction and assay the samples, thus only the fraction of the reduced carbamate which had not fragmented within 12 minutes could be calculated and substituted into the above equation. This gave an estimate of the maximum half-life of **5**, and these data are presented in Tables 1–3. Solutions of the nitrobenzyl carbamates **4** (50 µM, pH 7.4, 10 mM phosphate buffer, 4% (v/v) propan-2-ol, 20 °C) were reduced with 4-fold stoichiometry and assayed for **5** by HPLC either immediately (*ca.* 12 minutes after reduction was commenced) to give t_0, or after ten half-lives to give t_∞.

Radiolytic reduction of the 4-nitrobenzyl carbamates **4**

Table 3 Fragmentation of 3-substituted 4-nitrobenzyl carbamates

Compound	R^1	σ_m	$Mt_{1/2}$	t_0	t_∞
5k	NO_2	0.71	65	22	24
5l	CO_2Me	0.37	20	44	57
5g	OMe	0.12	17	37	58
5i [a]	N(Me)CO_2tBu	0.07	15	43	50
5b	H	0.00	16	40	49
5e [b]	Me	−0.07	n.d.	n.d.	n.d.
5n	NHMe	−0.30	14	42	44
5k [c]	NHOH	−0.34	22	49	52

[a] σ_p ($NHCO_2Me$) used as best approximation. [b] Fragmentation too fast to measure. [c] Data collected for 8-electron reduction of **4k**. Values were determined in triplicate and were reproducible to within ±0.6%.

occurred with 4-fold stoichiometry, which is consistent with reduction to the hydroxylamine **5**. While all of the initial carbamates **4** were consumed after the addition of 4 reducing equivalents, only about 50% (22–68%) of the expected amines were produced at the earliest analysis time (assigned t_0) (Tables 1–3). When the reduced solutions were maintained under anaerobic conditions at 20 °C for a further 10 half-lives (assigned t_∞), the presence of **5** was undetectable, yet little further amine was released. The unstable nature of **5** and the finite time required for its radiolytic generation, prevented quantification of chromatographic peaks and the direct measurement of the rate of fragmentation. Rather, quantification of the amine **6** or **7** released from **4** after reduction with 4 reducing equivalents was used to estimate the maximum half-life ($Mt_{1/2}$) for the fragmentation of hydroxylaminobenzyl-carbamates **5**. Assuming first order conditions, the half-life ($t_{1/2}$) of species R is calculated from the equation $\ln([R]_0/[R]_t) = t(\ln 2/t_{1/2})$. The ratio $[R]_0/[R]_t$ was taken as the fraction of nitrobenzyl carbamate **4** which had not released amine **6** or **7** after 4-fold reduction; *i.e.*, $[R]_0 = 50$ µM (the concentration of the nitrobenzyl carbamate **4** before radiolytic reduction), $[R]_t = 50$ µM − $[\text{amine}]_t$, and t = time between beginning 4-fold reduction and measuring the concentration of **6** or **7** by HPLC (approximately 12 minutes). This method yields a maximum value for the half-life of fragmentation. The rate of reduction and of fragmentation both contribute to the rate of amine release, but the two sequential processes are combined within the $Mt_{1/2}$ value. However, the rate of reduction is constant (dictated by the dose rate of the γ-rays), and $Mt_{1/2}$ indicates the relative rates of amine release *via* **5** which are presented in Tables 1–3.

Structure–activity relationships

The unsubstituted hydroxyaminobenzyl carbamate **5b** (generated from **4b**) had a $Mt_{1/2}$ of 16 minutes (Table 1). This is relatively long, and under biological conditions may permit substantial loss of material by side reactions not involving (activating) amine release. The α-methyl analogue **5c** had a $Mt_{1/2}$ of 9.5 min. This significant acceleration of fragmentation is presumably due to stabilisation of developing positive charge on the benzylic carbon. Compound **5a** which releases a primary amine **6** fragmented about twice as rapidly ($Mt_{1/2}$ = 10 minutes) as **5b**, indicating the nature of the leaving amine (**6** instead of **7**) also has some effect.

The remaining compounds (Tables 2 and 3) all released amine **7**, so that carbamate substituent effects alone could be discerned. In support of the proposed mechanism, electron-donating substituents at the 2-position on the benzyl ring also accelerated fragmentation, presumably by a similar effect. Thus the electron-donating 2-OMe (**5f**), 2-NHMe (**5m**) and 3-NHMe (**5n**) analogues were more unstable than the parent compound **5c** ($Mt_{1/2}$ values of 12, 7.2 and 14 min, compared with 16 min). Conversely the strongly electron-withdrawing 2-NO_2 and 3-NO_2 substituents significantly slowed fragmentation of **5j** and

5k (M$t_{1/2}$ values of 88 min and 65 min respectively). In the case of these compounds it is not known unequivocally which nitro group undergoes reduction.

The 2- and 3-Me derivatives **5d** and **5e** fragmented, with apparent 3-fold stoichiometry, too quickly to measure under our assay system. For the 12 compounds for which data was available, there was a quantitative relationship between M$t_{1/2}$ and substituent electronic properties, measured as σ_p for 2-substituents and σ_m for 3-substituents [eqn. (1)] (n is the sample number, r is the Pearson coefficient, s is the standard error estimate and F is the F statistic for Goodness of Fit.

$$\log(\mathrm{M}t_{1/2}) = 0.57(\pm 0.10)\sigma + 1.30(\pm 0.05) \qquad (1)$$
$$n = 12 \quad \mathrm{r} = 0.87 \quad \mathrm{s} = 0.15 \quad \mathrm{F} = 31.3$$

Note that, while the five 2-substituted analogues should ideally be represented by σ_o substituent constants, these are not readily available. Reliable σ_o values are reported [21] for only three of the five substituents, and these do not differ markedly (>25%) from the corresponding σ_p values. A recalculation of eqn, (1) using these values (not shown) did not change the conclusions to be drawn from it.

If the correlations are performed separately for the 2- and 3-substituted analogues, the intercept is identical but the σ coefficient differs (0.67 for the 2- substituted, 0.46 for the 3- substituted). This greater dependence of the 2-substituted compounds on substituent electronic properties would be consistent with some resonance stabilisation of the putative developing benzylic cation in the transition state of the carbamate fragmentation.

The extent of release of amine **7** after 10 half-lives (t_∞) was clearly increased with increasing substituent electron-donation. Thus the 2-NHMe analogue **5m** produced a maximum of 71% of **7** (t_∞) whereas the 2-NO_2 derivative **5j** produced a maximum of only 33% of **7**. This property also correlated significantly with σ values, although the relationship was not as strong [eqn. (2)], and is more difficult to understand.

$$\log(t_\infty) = -0.21(\pm 0.07)\sigma + 1.70(\pm 0.03) \qquad (2)$$
$$n = 12 \quad \mathrm{r} = 0.71 \quad \mathrm{s} = 0.10 \quad \mathrm{F} = 10.3$$

The varying degree of release of the amine (as low as 25% in some cases) is of concern. Prodrugs of low pK_A effectors release to the least extent, with fragmentation of the hydroxylamine competing with another reaction that forms an unknown species. The nature of this species is currently under study.

These data suggest that the standard 4-nitrobenzyl carbamate trigger for NTR prodrugs may be improved upon. Electron-donating substituents in the 2-position and α-methyl substitution of the nitrobenzyl moiety will both favour accelerated fragmentation of the 4-hydroxylamines produced by enzymic reduction. Substituents such as 2-alkoxy or 2-aminoalkyl also provide potential linkage points for the attachment of side chains designed to improve prodrug solubility. Of course, the effect of such substitutions on the kinetics of enzymic reduction would have to be determined. Rates of hydroxylamine fragmentation are also likely to be influenced by the nature of the released amine, with prodrugs of primary amines fragmenting more rapidly than a comparable secondary amine. However, there may be less flexibility to alter the amine-based "effector".

Experimental

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 discs, unless otherwise stated. NMR spectra were obtained on a Bruker AM-400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C spectra. Spectra were obtained in deuteriochloroform unless otherwise specified, and are referenced to Me_4Si. Chemical shifts and coupling constants were recorded in units of ppm and Hz, respectively. Mass spectra were determined on a VG-70SE mass spectrometer using an 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 sodium sulfate, unless otherwise noted. Solvents were evaporated under reduced pressure on a rotary evaporator. Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck 60 F_{254}) with visualisation of components by UV light (254 nm) or exposure to I_2. Column chromatography was carried out on silica gel, (Merck 230–400 mesh). DCM refers to dichloromethane; THF refers to tetrahydrofuran dried over sodium benzophenone ketyl; DMF refers to dry dimethyl formamide; EtOAc refers to ethyl acetate; ether refers to diethyl ether; light petroleum refers to petroleum ether, boiling range 40–60 °C; MeOH refers to methanol; EtOH refers to ethanol. All solvents were freshly distilled. Compounds **28a**–**n**, **29a**–**n**, and **4a**–**n** are named as phenylacetic acid derivatives in order to provide a consistent numbering scheme throughout.

General preparation of nitrobenzyl alcohols 8d,e,g,l

BH_3·DMS (20 mmol) was added dropwise to a stirred solution of nitrobenzoic acid (10 mmol) and trimethyl borate (40 mmol) in THF (100 cm^3) at 20 °C. The solution was heated at reflux temperature for 6 h and cooled to 5 °C. The reaction was quenched carefully with MeOH (2 cm^3), the mixture stirred for 5 min, water (2 cm^3) was added, and the mixture stirred for 5 min. 5 M HCl (10 cm^3) was added and the mixture heated at 50 °C for 30 min. The solvent was removed under reduced pressure, the residue partitioned between EtOAc (100 cm^3) and water (100 cm^3). The organic fraction was dried, the solvent removed under reduced pressure, and the residue chromatographed, eluting with 50% EtOAc–light petroleum to give the nitrobenzyl alcohol **8**.

2-Methyl-4-nitrobenzyl alcohol 8d. Preparation from 2-methyl-4-nitrobenzoic acid as described above gave *alcohol* **8d** (93%) as colourless needles, mp (from EtOAc–light petroleum) 99–100 °C (Found: C, 57.7; H, 5.7; N, 8.5. $C_8H_9NO_3$ requires C, 57.5; H, 5.4; N, 8.4%); ν_{max}/cm^{-1} 3310, 1522, and 1343; δ_H 2.16 (1 H, t, J 5.3, OH), 2.40 (3 H, s, CH_3), 4.76 (2 H, d, J 5.3, CH_2O), 7.29 (1 H, d, J 8.3, 6-H), 8.03 (1 H, dd, J 8.3 and 2.4, 5-H), and 8.28 (1 H, d, J 2.4, 3-H); δ_C 18.8, 62.3, 121.6, 122.3, 130.9, 140.0, 143.4, and 146.5.

3-Methyl-4-nitrobenzyl alcohol 8e. Preparation from 3-methyl-4-nitrobenzoic acid as described above gave alcohol [22] **8e** (90%) as a white solid, mp (from EtOAc–light petroleum) 60–61 °C (Found: C, 57.2; H, 5.4; N, 8.4. $C_8H_9NO_3$ requires C, 57.5; H, 5.4; N, 8.4%).

3-Methoxy-4-nitrobenzyl alcohol 8g. Preparation from 3-methoxy-4-nitrobenzoic acid as described above gave *alcohol* **8g** (83%) as cream coloured needles, mp (from EtOAc–light petroleum) 96.5–97.0 °C (lit.[23] mp (from benzene–light petroleum) 96 °C); ν_{max}/cm^{-1} 3260, 1618, 1512, and 1283; δ_H 2.23 (1 H, br s, OH), 3.97 (3 H, s, OCH_3), 4.77 (2 H, d, J 4.3, CH_2O), 6.96 (1 H, dd, J 8.2 and 1.3, 6-H), 7.15 (1 H, d, J 1.3, 2-H), and 7.84 (1 H, d, J 8.2, 5-H); δ_C 56.5, 64.0, 111.1, 117.7, 126.0, 138.3, 148.3, and 153.4.

3-Methoxycarbonyl-4-nitrobenzyl alcohol 8l. Preparation from 3-methoxycarbonyl-4-nitrobenzoic acid as described above gave *alcohol* **8l** (89%) as a tan solid, mp (from EtOAc–light petroleum) 56–58 °C (Found: C, 51.4; H, 4.4; N, 6.7.

$C_9H_9NO_5$ requires C, 51.2; H, 4.3; N, 6.6%); ν_{max}/cm^{-1} 3287, 1724, 1532, and 1360; δ_H 2.30 (1 H, br s, OH), 3.92 (3 H, s, OCH_3), 4.82 (2 H, s, CH_2O), 7.60 (1 H, dd, *J* 8.4, and 1.4, 6-H), 7.68–7.70 (1 H, m, 2-H), and 7.91 (1 H, d, *J* 8.4, 5-H); δ_C 53.3, 63.4, 124.2, 127.3, 128.0, 129.0, 146.8, 147.0, and 166.2.

2-Methoxy-4-nitrobenzyl alcohol 8f. An etheral solution of diazomethane (**CAUTION**) was added to a solution of 4-nitrosalicylic acid **9** (1.0 g, 5.46 mmol) in ether (50 cm^3) until a yellow colour persisted and the solution stood at 20 °C for 4 h. The reaction was quenched with glacial acetic acid (2 cm^3), poured into sat. aq. $NaHCO_3$ solution and extracted with ether (2 × 50 cm^3). The combined organic fractions were dried and the solvent removed under reduced pressure to give ester **10** (1.11 g, 96%) as white needles, mp (from ether) 89–90 °C (lit.[24] mp (from MeOH) 86–88 °C) (Found: C, 51.5; H, 4.3; N, 6.7. $C_9H_9NO_5$ requires C, 51.2; H, 4.3; N, 6.6%).

A solution of **10** (0.9 g, 4.26 mmol) in THF (20 cm^3) was added dropwise to a stirred solution of DIBAL-H (1 M solution in toluene, 13.4 cm^3, 13.4 mmol) in THF (20 cm^3) at 2 °C and the solution stirred at 2 °C for 15 min. The solvent was removed under reduced pressure and the residue partitioned between EtOAc (100 cm^3) and water (100 cm^3). The aqueous fraction was extracted with EtOAc (2 × 50 cm^3) and the combined organic fraction dried and the solvent removed under reduced pressure. The residue was chromatographed, eluting with 50% EtOAc–light petroleum, to give *alcohol* **8f** (0.74 g, 93%) as cream needles, mp (EtOAc–light petroleum) 103–104 °C (Found: C, 52.4; H, 4.8; N, 7.4. $C_8H_9NO_4$ requires C, 52.5; H, 4.95; N, 7.65%); ν_{max}/cm^{-1} 3310, 1523, 1250, and 1036; δ_H 2.27 (1 H, br s, OH), 3.96 (3 H, s, OCH_3), 4.76 (2 H, d, *J* 5.5, CH_2O), 7.52 (1 H, d, *J* 8.3, 6-H), 7.71 (1 H, d, *J* 2.1, 3-H), and 7.86 (1 H, dd, *J* 8.3 and 2.1, 5-H); δ_C 55.9, 60.7, 105.0, 116.0, 127.9, 136.6, 148.3, and 157.1.

2-[*N*-Methyl-*N*-(*tert*-butyloxycarbonyl)amino]-4-nitrobenzyl alcohol 8h and 2-methylamino-4-nitrobenzyl alcohol 8m

Oxalyl chloride (4.3 cm^3, 49.4 mmol) was added dropwise to a mixture of 4-nitroanthranilic acid **11** (6.0 g, 32.9 mmol) and DMF (2 drops) in DCM (100 cm^3) at 2 °C and the mixture stirred at 20 °C for 16 h. The solvent was removed under reduced pressure and the residue dissolved in ice cold MeOH (100 cm^3) and the solution stirred for 16 h. The solvent was removed under reduced pressure and the residue partitioned between EtOAc (200 cm^3) and sat. aq. $NaHCO_3$ solution (200 cm^3). The organic fraction was washed with 0.1 M NaOH (100 cm^3), water (100 cm^3), brine (50 cm^3), dried and the solvent removed under reduced pressure. The residue was chromatographed, eluting with 20% EtOAc–light petroleum, to give methyl 4-nitroanthranilate **12** (4.48 g, 69%) as orange needles, mp (from EtOAc–light petroleum) 154–156 °C; ν_{max}/cm^{-1} 3493, 3381, 1701, 1587, 1518, 1348, and 1252; δ_H 3.94 (3 H, s, OCH_3), 6.05 (2 H, br s, NH_2), 7.40 (1 H, dd, *J* 8.8 and 2.3, 5-H), 7.50 (1 H, d, *J* 2.3, 3-H), and 8.00 (1 H, d, *J* 8.8, 6-H); δ_C 52.2, 110.6, 111.1, 114.9, 132.8, 150.7, 151.3, and 167.3.

Formic acid (1.35 cm^3, 35.9 mmol) was added dropwise to acetic anhydride (2.75 cm^3, 29.2 mmol) at 2 °C and then the mixture was heated at 50 °C for 30 min. The mixture was cooled to 2 °C and THF (20 cm^3) added. A solution of **12** (2.2 g, 11.2 mmol) in THF (20 ml) was added dropwise and the stirred solution allowed to warm to 20 °C over 1 h. The solvent was removed under reduced pressure and the residue dissolved in THF (100 cm^3). The solution was cooled to 2 °C and BH_3·DMS (2.8 cm^3, 28 mmol) added and the solution stirred at 2 °C for 3 h. The reaction was quenched carefully with MeOH (2 cm^3), stirred for 5 min, water (2 cm^3) added and the mixture stirred for 5 min. 1 M HCl (10 cm^3) was added and the mixture heated at 50 °C for 30 min. The solvent was removed under reduced pressure and the residue partitioned between EtOAc (150 cm^3) and sat. aq. $NaHCO_3$ (150 cm^3). The aqueous phase was extracted with EtOAc (2 × 50 cm^3), the combined organic fraction dried and the solvent removed under reduced pressure. The residue was chromatographed, eluting with a gradient (20–50%) of EtOAc–light petroleum to give (i) *methyl 2-methylamino-4-nitrobenzoate* **13** (0.63 g, 27%) as orange needles, mp (from EtOAc–light petroleum) 120–121 °C (Found C, 51.2; H, 4.65; N, 13.2. $C_9H_{10}N_2O_4$ requires C, 51.4; H, 4.8; N, 13.3%); ν_{max}/cm^{-1} 3376, 1689, 1547, 1345, and 1238; δ_H 2.98 (3 H, d, *J* 5.1, NCH_3), 3.90 (3 H, s, OCH_3), 7.34 (1 H, dd, *J* 8.7 and 2.2, 5-H), 7.46 (1 H, d, *J* 2.2, 3-H), 7.88 (1 H, br s, NH), and 8.02 (1 H, d, *J* 8.7, 6-H); δ_C 29.7, 52.1, 105.4, 108.2, 114.3, 132.9, 151.9, 152.3, and 167.8; (ii) starting material (0.25 g, 11%) spectroscopically identical to an authentic sample; and (iii) *2-methylamino-4-nitrobenzyl alcohol* **8m** (1.0 g, 49%) as orange prisms, mp (from EtOAc–light petroleum) 135–137 °C (Found: C, 52.5; H, 5.5; N, 15.4. $C_8H_{10}N_2O_3$ requires C, 52.7; H, 5.5; N, 15.4%); ν_{max}/cm^{-1} 3146, 1622, 1533, and 1367; δ_H 1.75 (1 H, br s, NH), 2.94 (3 H, s, NCH_3), 4.74 (2 H, s, CH_2O), 5.14 (1 H, br s, OH), 7.15 (1 H, d, *J* 8.1, 6-H), 7.40 (1 H, d, *J* 2.1, 3-H), and 7.48 (1 H, dd, *J* 8.1 and 2.1, 5-H); δ_C 30.2, 64.0, 104.0, 111.0, 128.8, 130.0, 149.3, and 149.4.

A solution of **13** (1.51 g, 7.18 mmol), di-*tert*-butyl dicarbonate (3.14 g, 14.37 mmol) and dimethylaminopyridine (DMAP) (80 mg, 0.72 mmol) in THF (100 cm^3) was heated at reflux temperature for 24 h. More di-*tert*-butyl dicarbonate (1.0 g, 4.58 mmol) was added and the solution heated at reflux temperature for a further 24 h. The solvent was removed under reduced pressure and the residue chromatographed, eluting with 10% EtOAc–light petroleum to give *methyl 2-[N-methyl-N-(tert-butyloxycarbonyl)amino]-4-nitrobenzoate* **14** (2.21 g, 97%) as a yellow oil, ν_{max}(thin film)/cm^{-1} 1734, 1713, 1531, 1350, and 1157; δ_H (2 rotamers) 1.31 and 1.52 (9 H, 2s, $C(CH_3)_3$), 3.29 and 3.33 (3 H, 2s, NCH_3), 3.91 and 3.93 (3 H, 2s, OCH_3), 7.95–8.03 (1 H, m, 6-H), and 8.08–8.18 (2 H, m, 5-H and 3-H); δ_C 28.0 and 28.2 (3), 37.4 and 37.8, 52.8, 81.2 and 81.5, 121.0, 123.2, 126.1, 131.8, 144.5, 150.3, 153.4, and 165.3; *m/z* 310.1162 (M^+. $C_{14}H_{18}N_2O_6$ requires 310.1165); *m/z* 310 (M^+, 2%), 237 (10), 210 (40), and 57 (100).

A solution of **14** (2.21 g, 7.12 mmol) in MeOH (50 cm^3) and 1 M LiOH solution (36 cm^3, 36 mmol) was stirred at 50 °C for 30 min. The solution was cooled to 2 °C, diluted with water (50 cm^3), extracted with ether (50 cm^3), and acidified to pH 4 with 1 M HCl. The mixture was extracted with EtOAc (3 × 50 cm^3), the combined extracts dried and the solvent removed under reduced pressure to give *2-[N-methyl-N-(tert-butyloxycarbonyl)amino]-4-nitrobenzoic acid* **15** (2.1 g, 99%) as a tan hygroscopic foam (Found: C, 52.5; H, 5.9; N, 9.1. $C_{13}H_{16}N_2O_6$ requires C, 52.7; H, 5.4; N, 9.5%); ν_{max}(thin film)/cm^{-1} 3457, 1710, 1533, 1350, and 1157; δ_H [$(CD_3)_2SO$] (2 rotamers) 1.32 and 1.40 (9 H, 2s, $C(CH_3)_3$), 3.20 (3 H, s, NCH_3), 7.98 (1 H, d, *J* 8.5, 6-H), 8.17 (1 H, dd, *J* 8.5 and 2.2, 5-H), 8.20 (1 H, br s, 3-H), and 13.53 (1 H, br s, CO_2H); δ_C 27.4 and 27.3 (3), 37.6 and 37.1, 82.4 and 80.1, 121.1, 122.6, 124.9, 131.8, 143.5, 149.7 and 149.4, 152.6, and 166.3.

BH_3·DMS reduction of **15** as described above gave *alcohol* **8h** (41%) as a pale orange oil, ν_{max}(thin film)/cm^{-1} 3447, 1705, 1682, 1528, 1350, and 1157 cm^{-1}; δ_H (2 rotamers) 1.27 and 1.35 (9 H, 2s, $C(CH_3)_3$), 3.23 (3 H, s, NCH_3), 4.60–4.70 (2 H, m, CH_2O), 7.74 (1 H, d, *J* 8.4, 6-H), 8.03 (1 H, br s, 3-H), and 8.22 (1 H, dd, *J* 8.4 and 2.2, 5-H); δ_C 28.2 (3), 38.0, 60.6, 82.0, 122.1, 122.4, 128.4, 130.9, 145.6, 147.8, and 155.7; *m/z* 282.1214 (M^+. $C_{13}H_{18}N_2O_5$ requires 282.1216); *m/z* 282 (M^+, 1%), 209 (10), 182 (20), and 57 (100).

3-[*N*-Methyl-*N*-(*tert*-butyloxycarbonyl)amino]-4-nitrobenzyl alcohol 8l

A mixture of 5-methoxycarbonyl-2-nitrobenzoic acid **16** (2.07 g, 9.19 mmol) (derived from 4-nitroisophthalic acid by Fischer esterification) and DMF (2 drops) in $SOCl_2$ (100 cm^3) was heated at reflux temperature for 3 h. The mixture was evaporated to dryness under reduced pressure, dissolved in acetone (50 cm^3) and a solution of NaN_3 (0.90 g, 13.8 mmol) in water (5 cm^3) added in one portion. The mixture was stirred at 20 °C for 15 min, partitioned between benzene (150 cm^3) and water (150 cm^3) and the aqueous fraction extracted with benzene (50 cm^3). The combined organic extracts were dried and the solvent removed under reduced pressure. The residue was dissolved in xylene (100 cm^3) and 2-(trimethylsilyl)ethanol (1.60 cm^3, 11.0 mmol) added. The solution was heated at reflux temperature for 16 h, the solvent removed under reduced pressure and the residue dissolved in THF (100 cm^3). A solution of tetrabutylammonium fluoride (TBAF) (1 M solution in THF, 14 cm^3, 14 mmol) in THF was added and the solution stirred at 20 °C for 30 min. The solvent was removed under reduced pressure and the residue chromatographed, eluting with a gradient (20–50%) of EtOAc–light petroleum, to give methyl 3-amino-4-nitrobenzoate **17** (1.60 g, 89%) as orange needles, mp (from EtOAc–light petroleum) 193–194.5 °C; ν_{max}/cm^{-1} 3493, 3370, 1720, 1633, 1580, 1320, and 1246; δ_H 3.90 (3 H, s, OCH_3), 7.15–7.18 (3 H, m, 6-H and NH_2), 7.71 (1 H, d, *J* 1.7, 2-H), 8.08 (1 H, d, *J* 8.9, 5-H); δ_C 51.7, 114.6, 120.5, 125.3, 132.2, 134.9, 144.7, and 164.7; which was used without further characterisation.

Reductive formylation of **17** (2.34 g, 11.9 mmol) as described above for **12** gave methyl 3-methylamino-4-nitrobenzoate **18** (1.07 g, 43%) as an orange powder, mp (from EtOAc–light petroleum) 129–130 °C; ν_{max}/cm^{-1} 3376, 1730, 1576, 1319, and 1226; δ_H 3.08 (3 H, d, *J* 5.1, NCH_3), 3.97 (3 H, s, OCH_3), 7.23 (1 H, dd, *J* 8.9 and 1.7, 6-H), 7.54 (1 H, d, *J* 1.7, 2-H), 8.00 (1 H, br s, NH), and 8.21 (1 H, d, *J* 8.9, 5-H); δ_C 29.9, 52.7, 115.1, 115.3, 127.0, 133.8, 136.5, 145.7, and 165.8; *m/z* 210.0633 (M^+, $C_9H_{10}N_2O_4$ requires 210.0641); *m/z* 210 (M^+, 100%), 179 (20), and 161 (80); which was used without further characterisation.

A solution of **18** (1.20 g, 5.7 mmol), di-*tert*-butyl dicarbonate (2.49 g, 11.4 mmol) and DMAP (64 mg, 0.6 mmol) in THF (100 cm^3) was heated at reflux temperature for 96 h. The solvent was removed under reduced pressure and the residue partitioned between EtOAc (150 cm^3) and water (150 cm^3). The organic fraction was dried, the solvent removed under reduced pressure and the residue chromatographed, eluting with 5% EtOAc–light petroleum, to give methyl 3-[*N*-methyl-*N*-(*tert*-butyloxycarbonyl)amino]-4-nitrobenzoate **19** (1.77 g, 99%) as a yellow oil; ν_{max}(thin film)/cm^{-1} 1730, 1717, 1533, 1352, and 1157; δ_H 1.27 (9 H, s, $C(CH_3)_3$), 3.33 (3 H, s, NCH_3), 3.99 (3 H, s, OCH_3), and 7.92–8.02 (3 H, m, 2-H, 5-H, and 6-H); δ_C 27.8 (3), 37.6, 52.9, 82.0, 124.8, 128.0, 130.0, 134.7, 137.5, 148.9, 152.7, and 164.7; *m/z* 310.1161 (M^+. $C_{14}H_{18}N_2O_6$ requires 310.1165); *m/z* 310 (M^+, 1%), 251 (2), 237 (5), 210 (10), and 57 (100); which was used without further characterisation.

1 M LiOH (25 cm^3, 25 mmol) was added to a stirred solution of **19** (1.75 g, 5.64 mmol) in MeOH (50 cm^3) and the mixture stirred at 50 °C for 30 min. The mixture was cooled to 5 °C, washed with ether (50 cm^3) and the pH adjusted to 4.0 with 1 M HCl. The suspension was extracted with EtOAc (3 × 50 cm^3), the combined organic fraction dried, and the solvent removed under reduced pressure to give *3-[N-methyl-N-(tert-butyloxycarbonyl)amino]-4-nitrobenzoic acid* **20** (1.48 g, 89%) as a pale yellow powder, mp (from EtOAc–light petroleum) 176–178 °C (Found: C, 52.95; H, 5.6; N, 9.5. $C_{13}H_{16}N_2O_6$ requires C, 52.7; H, 5.4; N, 9.5%); ν_{max}/cm^{-1} 3150, 1720, 1694, 1532, 1372, and 1155; δ_H 1.23 (9 H, s, $C(CH_3)_3$), 3.28 (3 H, s, NCH_3), 7.98 (1 H, dd, *J* 8.5 and 1.7, 6-H), 8.03 (1 H, d, *J* 1.6, 2-H), 8.10 (1 H, br d, *J* 8.5, 5-H), and 13.86 (1 H, br s, CO_2H); δ_C 27.2 (3), 36.9, 80.8, 125.2, 128.1, 129.3, 135.8, 136.4, 148.1, 151.9, and 165.3.

BH_3·DMS reduction of **20** as described above gave *alcohol* **8i** (92%) as yellow prisms, mp (from EtOAc–light petroleum) 106–107 °C (Found: C, 55.1; H, 6.6; N, 10.25. $C_{13}H_{18}N_2O_5$ requires C, 55.3; H, 6.4; N, 9.9%); ν_{max}/cm^{-1} 3510, 1707, 1512, 1358, and 1157; δ_H (2 rotamers) 1.29 and 1.49 (9 H, 2s, $C(CH_3)_3$), 3.27 and 3.28 (3 H, 2s, NCH_3), 4.74 and 4.78 (2 H, 2s, CH_2O), 7.34–7.36 (m, 2 H, 2-H and 5-H), and 7.88–7.93 (1 H, m, 6-H); δ_C 28.2 and 27.7 (3), 37.4 and 37.3, 63.5, 81.6, 124.7, 125.3, 126.9, 137.4, 145.0, 147.8, and 154.9 and 153.3.

Preparation of *N*-(*N′*,*N′*-dimethylaminoethyl) 4-(methylamino)phenylacetamide 7 (Scheme 6)

A mixture of methyl 4-nitrophenylacetate **21** (6.44 g, 33.0 mmol) and Pd/C (100 mg) in EtOH (100 cm^3) was stirred under H_2 (60 psi) for 1 h. The mixture was filtered through Celite washed with EtOH (2 × 20 cm^3) and the solvent removed under reduced pressure. The residue was dissolved in THF (50 cm^3) and added to a solution of acetic formic anhydride (prepared by adding formic acid (4.0 cm^3, 106 mmol) dropwise to acetic anhydride (8.1 cm^3, 86.0 mmol) as described above) in THF (50 cm^3) at −10 °C. The solution was stirred at −10 °C for 30 min, allowed to warm to 20 °C, the solvent removed under reduced pressure and the residue partitioned between EtOAc (150 cm^3) and sat. aq. $NaHCO_3$ (150 cm^3). The aqueous fraction was extracted with EtOAc (100 cm^3) and the combined organic fraction dried and the solvent removed under reduced pressure. The residue was dissolved in THF (100 cm^3) and BH_3·DMS (8.25 cm^3, 82.5 mmol) added slowly. The solution was stirred at 20 °C for 1 h, MeOH (10 cm^3) carefully added, the mixture stirred for 15 min. 1 M HCl (10 cm^3) was added and the mixture stirred at 40 °C for 30 min. The solvent was removed under reduced pressure and the residue partitioned between EtOAc (200 cm^3) and sat. aq. $NaHCO_3$ solution (100 cm^3). The aqueous fraction was extracted with EtOAc (100 cm^3), the combined organic fraction dried and the solvent removed under reduced pressure. The residue was chromatographed, eluting with a gradient (20–40%) EtOAc–light petroleum, to give methyl 4-(methylamino)phenylacetate **22** (4.48 g, 76%) as a colourless oil which was used directly.

A solution of **22** (4.48 g, 25.0 mmol), di-*tert*-butyl dicarbonate (8.18 g, 37.5 mmol) and DMAP (0.28 g, 2.5 mmol) in THF (100 cm^3) was heated at reflux temperature for 16 h. The solution was cooled to 20 °C and the solvent removed under reduced pressure. The residue was partitioned between EtOAc (100 cm^3) and sat. aq. $NaHCO_3$ solution (100 cm^3) and the aqueous fraction extracted with EtOAc (2 × 50 cm^3). The combined organic fraction was washed with water (2 × 50 cm^3), brine (50 cm^3), dried, and the solvent removed under reduced pressure. The residue was chromatographed, eluting with a gradient (10–30%) of EtOAc–light petroleum to give *methyl 4-[N-methyl-N-(tert-butyloxycarbonyl)amino]phenylacetate* **23** (4.30 g, 62%) as a pale yellow gum (Found: C, 64.6; H, 7.6; N, 5.2. $C_{15}H_{21}NO_4$ requires C, 64.5; H, 7.6; N, 5.0%); ν_{max}(thin film)/cm^{-1} 1738, 1699, 1366, and 1153; δ_H 1.45 (9 H, s, $C(CH_3)_3$), 3.24 (3 H, s, NCH_3), 3.66 (2 H, s, CH_2O), 3.69 (3 H, s, OCH_3), and 7.17–7.23 (4 H, m, 2-H, 3-H, 5-H, and 6-H); δ_C 28.3 (3), 37.2, 40.6, 52.0, 80.3, 125.5 (2), 129.4 (2), 130.9, 142.8, 154.7, and 171.9.

A solution of **23** (4.00 g, 14.3 mmol) in MeOH (50 cm^3) and 1 M LiOH (73 cm^3, 73 mmol) was stirred at 50 °C for 30 min. The solution was cooled to 2 °C, washed with ether (50 cm^3), and the pH adjusted to 4.0 with 5 M HCl. The suspension was stirred at 2 °C for 30 min and filtered to give *4-[N-methyl-N-(tert-butyloxycarbonyl)amino]phenylacetic acid* **24** (3.67 g, 97%) as a white powder, mp (from MeOH–water) 114–116 °C (Found: C, 63.3; H, 7.1; N, 5.2. $C_{14}H_{19}NO_4$ requires C, 63.4; H, 7.2; N, 5.3%); ν_{max}/cm^{-1} 3101, 1732, 1659, 1377, and 1155; δ_H 1.45 (9 H, s, $C(CH_3)_3$), 3.24 (3 H, s, NCH_3), 3.61 (2 H, s, CH_2O), 7.18–7.24 (4 H, m, 2-H, 3-H, 5-H, and 6-H), and 10.55 (1 H, br s, CO_2H); δ_C 28.3 (3), 37.3, 40.4, 80.5, 125.6 (2), 129.5 (2), 130.3, 142.9, 154.8, and 177.0.

A solution of **24** (2.09 g, 7.88 mmol) and CDI (1.92 g, 15.8

mmol) in DMF (20 cm^3) was stirred at 50 °C for 10 min. *N′*,*N′*-Dimethylaminoethylamine (1.73 cm^3, 15.8 mmol) was added and the solution stirred at 20 °C for 2 h. The solution was poured into water (250 cm^3) and extracted with EtOAc (3 × 100 cm^3), the combined organic fractions washed with brine (50 cm^3), dried and the solvent removed under reduced pressure. The residue was chromatographed, eluting with 0.5% Et_3N–10% MeOH–EtOAc to give *N-(N′,N′-dimethylaminoethyl) 4-[N-methyl-N-(tert-butyloxycarbonyl)amino]phenylacetamide* **25** (2.33 g, 88%), as a colourless oil, ν_{max}/cm^{-1} 3324, 1701, 1655, 1512, 1365, and 1153; δ_H 1.45 (9 H, s, $C(CH_3)_3$), 2.23 (6 H, s, $N(CH_3)_2$), 2.44 (2 H, t, *J* 6.0, CH_2N), 3.24 (3 H, s, NCH_3), 3.31–3.36 (2 H, m, CH_2N), 3.49 (1 H, br s, CONH), 3.56 (2 H, s, CH_2CO), and 7.18–7.25 (4 H, m, 2-H, 3-H, 5-H, and 6-H); δ_C 28.3 (3), 36.7, 37.3, 43.0, 44.8 (2), 57.7, 80.3, 125.7 (2), 129.5 (2), 132.1, 142.7, 154.7, and 171.1; *m/z* 335.2200 (*M*$^+$. $C_{18}H_{29}N_3O_3$ requires 335.2209); *m/z* 335 (100%, M^+), 278 (5), 262 (50), 235 (30), and 219 (90).

A solution of **25** (1.0 g, 2.98 mmol) in HCl-saturated MeOH (50 cm^3) was stirred at 20 °C for 1 h. The solvent was removed under reduced pressure to give *acetamide* **7** as the dihydrochloride salt (0.87 g, 95%) as a hygroscopic foam, ν_{max}(thin film)/cm^{-1} 3435, 3230, 2672, and 1667; δ_H 2.75 (6 H, d, *J* 5.3, $N(CH_3)_2$), 2.89 (3 H, s, NCH_3), 3.12–3.17 (2 H, m, CH_2N), 3.41–3.47 (2 H, m, CH_2N), 3.62 (2 H, s, CH_2CO), 7.43 (2 H, br d, *J* 8.6, 3-H and 5-H), 7.49 (2 H, br d, *J* 8.6, 2-H and 6-H), 8.66 (1 H, t, *J* 5.6, CONH), 10.64 (1 H, br s, NHCl), and 11.35 (1 H, br s, NHCl); δ_C 34.3, 36.1, 41.9, 42.5 (2), 55.8, 121.8 (2), 130.5 (2), 136.4, 136.5, and 170.3; *m/z* 235.1684 (*M*$^+$. $C_{13}H_{21}N_3O$ requires 235.1685); *m/z* 235 (M^+, 20%), 120 (20), and 58 (100).

Preparation of *N*−(*N′*,*N′*-dimethylaminoethyl) 4-aminophenylacetamide 6 (Scheme 7)

A mixture of 4-nitrophenylacetic acid **26** (1.0 g, 5.52 mmol) and CDI (1.34 g, 8.28 mmol) in DMF (10 cm^3) was stirred at 50 °C for 10 min. The solution was cooled to 20 °C, *N′*,*N′*-dimethylaminoethylamine (1.21 cm^3, 11 mmol) was added dropwise and the solution stirred for 2 h. The solution was poured into water (150 cm^3) and extracted with EtOAc (3 × 75 cm^3). The combined organic extracts were washed with water (2 × 50 cm^3), brine (50 cm^3), dried, and the solvent removed under reduced pressure. The residue was chromatographed, eluting with a gradient (0–30%) of MeOH–EtOAc, to give *N-(N′,N′-dimethylaminoethyl) 4-nitrophenylacetamide* **27** (0.70 g, 58%) as a brown solid, mp (from EtOAc) 89–90 °C (Found: C, 57.2; H, 6.6; N, 16.6. $C_{12}H_{17}N_3O_3$ requires C, 57.35; H, 6.8; N, 16.7%); ν_{max}/cm^{-1} 3304, 3061, 1665, 1540, 1508, and 1350; δ_H 2.20 (6 H, s, $N(CH_3)_2$), 2.41 (2 H, t, *J* 5.9, CH_2N), 3.30–3.35 (2 H, m, CH_2N), 3.63 (2 H, s, CH_2CO), 6.28 (1 H, br s, CONH), 7.47 (2 H, dd, *J* 8.8 and 2.4, 2-H and 6-H), and 8.18 (2 H, dd, *J* 8.8 and 2.4, 3-H and 5-H); δ_C 36.9, 43.1, 45.0 (2), 57.5, 123.7 (2), 130.1 (2), 142.7, 147.0, and 169.1; *m/z* (CI) 252.1354 (*MH*$^+$. $C_{12}H_{18}N_3O_3$ requires 252.1348); *m/z* (CI) 252 (MH^+, 100%), 222 (95), and 151 (10).

A solution of **27** (0.68 g, 3.10 mmol) in EtOAc (30 cm^3) was stirred with Pd/C (50 mg) under H_2 (60 psi) for 1 h. The mixture was filtered through Celite, washed with EtOAc (50 cm^3) and the solvent removed under reduced pressure. The residue was dissolved in EtOAc (30 cm^3) and HCl-saturated EtOAc (30 cm^3) added. The solvent was removed under reduced pressure to give acetamide **6** (0.84 g, 92%) as a green foam, mp (from EtOAc) 70 °C (decomp.) (Found: C, 48.8; H, 7.2; N, 14.3; Cl, 24.1. $C_{12}H_{21}Cl_2N_3O$ requires C, 49.0; H, 7.2; N, 14.3; Cl, 24.1%); ν_{max}/cm^{-1} 3447, 3316, 2866, 1655, and 1530; δ_H 2.75 (6 H, s, $N(CH_3)_2$), 3.12 (2 H, t, *J* 5.9, CH_2N), 3.40–3.44 (2 H, m, CH_2N), 3.66 (2 H, s, CH_2CO), 7.30 (2 H, br d, *J* 8.3, 2-H and 6-H), 7.38 (2 H, br d, *J* 8.3, 3-H and 5-H), 8.58 (1 H, br s, CONH), 10.37 (1 H, br s, NHCl), and 10.54 (2 H, br s, NH_2); δ_C 33.9, 41.5, 42.1 (2), 55.5, 122.8 (2), 130.3 (2), 130.4, 135.7, and 170.3.

Preparation of methyl esters 28a–i. General method

Alcohol **8** was converted to the chloroformate by adding a solution of phosgene (**CAUTION**) in benzene (10 mmol) dropwise to a stirred solution of **8** (2 mmol) in THF (50 cm^3) at 0 °C under N_2. The solution was stirred at 20 °C for 16 h and the solvent removed under reduced pressure. The residue was dissolved in DMF (20 cm^3), Cs_2CO_3 (2.5 mmol) and a solution of amine **6** or **7** (2.2 mmol) in DMF (5 cm^3) added. The mixture was stirred at 20 °C for 16 h, poured into water (100 cm^3), and extracted with EtOAc (3 × 50 cm^3). The combined organic extract was washed with 1 M HCl (2 × 50 cm^3), water (50 cm^3), brine (40 cm^3), dried, and the solvent removed under reduced pressure. The residue was chromatographed, eluting with a gradient (20–50%) of EtOAc–light petroleum to give the ester **28**. Yields and characterisations of individual compounds are given below.

Methyl 4-[*N*-(4-nitrobenzyloxycarbonyl)amino]phenylacetate 28a. *Ester* **28a** was obtained (42%) as a pale yellow solid, mp (from EtOAc–light petroleum) 115.5–116 °C (Found: C, 59.2; H, 4.55; N, 8.0. $C_{17}H_{16}N_2O_6$ requires C, 59.3; H, 4.7; N, 8.1%); ν_{max}/cm^{-1} 3333, 1732, 1716, 1605, 1541, and 1324; δ_H 3.59 (2 H, s, CH_2CO), 3.69 (3 H, s, OCH_3), 5.29 (2 H, s, CH_2O), 6.82 (1 H, br s, $NHCO_2$), 7.23 (2 H, br d, *J* 8.1, 3-H and 5-H), 7.34 (2 H, br d, *J* 8.1, 2-H and 6-H), 7.54 (2 H, br d, *J* 8.7, 2′-H and 6′-H), 8.22 (2 H, ddd, *J* 8.7, 2.4, and 2.0, 3′-H and 5′-H); δ_C 40.4, 52.0, 65.4, 118.9 (2), 123.8 (2), 128.3 (2), 129.4, 130.0 (2), 136.4, 143.4, 147.7, 152.8, and 172.0.

Methyl 4-[*N*-methyl-*N*-(4-nitrobenzyloxycarbonyl)amino]phenylacetate 28b. *Ester* **28b** was obtained (76%) as a cream solid, mp (from EtOAc) 73–74 °C (Found: C, 60.1; H, 5.1; N, 7.5. $C_{18}H_{18}N_2O_6$ requires C, 60.3; H, 5.1; N, 7.8%); ν_{max}/cm^{-1} 1740, 1723, 1607, 1516, 1342, and 1157; δ_H 3.33 (3 H, s, NCH_3), 3.64 (2 H, s, CH_2CO), 3.71 (3 H, s, OCH_3), 5.23 (2 H, s, CH_2O), 7.21 (2 H, br d, *J* 8.1, 3-H and 5-H), 7.27 (2 H, d, *J* 8.4, 2-H and 6-H), 7.41 (2 H, br s, 2′-H and 6′-H), and 8.18 (2 H, d, *J* 8.4, 3′-H and 5′-H); δ_C 37.9, 40.5, 52.1, 65.8, 123.7 (2), 126.0 (2), 127.4 (2), 129.9 (2), 132.3, 141.8, 143.9, 147.5, 154.9, and 171.8.

Methyl 4-{*N*-methyl-*N*-[1-(4-nitrophenyl)ethyloxycarbonyl]-amino}phenylacetate 28c. *Ester* **28c** was obtained (61%) as a pale yellow gum, ν_{max}(thin film)/cm^{-1} 1736, 1707, 1518, 1346, and 1155; δ_H 1.52 (3 H, d, *J* 6.6, CH_3), 3.31 (3 H, s, NCH_3), 3.65 (2 H, s, CH_2CO), 3.72 (3 H, s, OCH_3), 5.88 (1 H, q, *J* 6.6, OCH), 7.19 (2 H, d, *J* 8.3, 3-H and 5-H), 7.29 (2 H, d, *J* 8.3, 2-H and 6-H), 7.38 (2 H, br s, 2′-H and 6′-H), 8.17 (2 H, d, *J* 8.6, 3′-H and 5′-H); δ_C ($(CD_3)_2SO$) 22.7, 37.7, 40.6, 52.1, 72.7, 123.8 (2), 126.0 (3), 126.5 (2), 129.8 (2), 141.9, 147.3, 149.6, 154.5, and 171.8; *m/z* (DEI) 372.1325 (*M*$^+$. $C_{19}H_{20}N_2O_6$ requires 372.1321); *m/z* (DEI) 372 (M^+, 50%), 328 (20), 313 (60), 223 (45), and 178 (100).

Methyl 4-[*N*-methyl-*N*-(2-methyl-4-nitrobenzyloxycarbonyl)-amino]phenylacetate 28d. *Ester* **28d** was obtained (53%) as a pale yellow oil, ν_{max}/cm^{-1} 1734, 1707, 1518, 1346, and 1153; δ_H 2.38 (3 H, s, CH_3), 3.33 (3 H, s, NCH_3), 3.66 (2 H, s, CH_2CO), 3.71 (3 H, s, OCH_3), 5.19 (2 H, s, CH_2O), 7.23–7.34 (5 H, m, 2-H, 3-H, 5-H, 6-H, and 6-H), and 8.19–8.21 (2 H, m, 3-H and 5-H); δ_C 19.0, 37.9, 40.6, 52.0, 64.2, 121.5, 122.6, 126.1 (2), 130.3 (2), 130.9, 131.0, 136.5, 141.7, 142.3, 146.4, 154.9, and 171.8; *m/z* (DEI) 372.1317 (*M*$^+$. $C_{19}H_{20}N_2O_6$ requires 372.1321); *m/z* (DEI) 372 (M^+, 60%), 312 (15), 269 (20), 178 (80), and 150 (100).

Methyl 4-[*N*-methyl-*N*-(3-methyl-4-nitrobenzyloxycarbonyl)-amino]phenylacetate 28e. *Ester* **28e** was obtained (52%) as a pale yellow oil, ν_{max}/cm^{-1} 1736, 1707, 1518, 1343, and 1153; δ_H 2.56 (3 H, s, CH_3), 3.32 (3 H, s, NCH_3), 3.64 (2 H, s, CH_2CO), 3.71 (3 H, s, OCH_3), 5.16 (2 H, s, CH_2O), 7.09–7.34 (6 H, m, 2-H, 3-H, 5-H, 6-H, 2′-H, and 6′-H), and 7.94 (1 H, d, *J* 8.8, 5′-H); δ_C 20.5, 37.9, 40.5, 52.1, 65.8, 125.0, 125.5 (2), 126.0, 129.9 (2), 130.4, 131.4, 133.9, 141.8, 142.1, 148.4, 155.0, and 171.8; *m*/*z* (DEI) 372.1317 (*M*⁺. $C_{19}H_{20}N_2O_6$ requires 372.1321); *m*/*z* (DEI) 372 (M^+, 100%), 328 (15), 313 (20) and 269 (100).

Methyl 4-[*N*-methyl-*N*-(2-methoxy-4-nitrobenzyloxycarbonyl)amino]phenylacetate 28f. *Ester* **28f** was obtained (66%) as a pale yellow oil, ν_{max}/cm^{-1} 1736, 1707, 1518, 1348, 1250, and 1155; δ_H 3.34 (3 H, s, NCH_3), 3.64 (2 H, s, CH_2CO), 3.71 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3), 5.23 (2 H, s, CH_2O), 7.22–7.31 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 7.53 (1 H, d, *J* 8.3, 6′-H), 7.71 (1 H, d, *J* 2.1, 3′-H), and 7.88 (1 H, dd, *J* 8.3 and 2.1, 5′-H); δ_C 37.8, 40.6, 52.1, 55.9, 62.1, 105.0, 115.7, 126.0 (2), 127.8, 129.9 (2), 132.9, 136.6, 141.9, 148.3, 155.0, 157.1, and 171.8; *m*/*z* (DEI) 388.1264 (M^+. $C_{19}H_{20}N_2O_7$ requires 388.1271); *m*/*z* (DEI) 388 (M^+, 20%), 344 (35), 285 (20), 178 (30), and 166 (100).

Methyl 4-[*N*-methyl-*N*-(3-methoxy-4-nitrobenzyloxycarbonyl)amino]phenylacetate 28g. *Ester* **28g** was obtained (67%) as a white powder, mp (from EtOAc–light petroleum) 80–83 °C (Found: C, 58.9; H, 5.2; N, 7.3. $C_{19}H_{20}N_2O_7$ requires C, 58.8; H, 5.2; N, 7.2%); ν_{max}/cm^{-1} 1736, 1707, 1611, 1518, 1344, and 1157; δ_H 3.33 (3 H, s, NCH_3), 3.63 (2 H, s, CH_2CO), 3.71 (3 H, s, OCH_3), 3.86 (3 H, br s, OCH_3), 5.17 (2 H, s, CH_2O), 6.89–6.93 (2 H, m, 2-H and 6-H), 7.21–7.23 (2 H, m, 2′-H and 6′-H), 7.30 (1 H, br d, *J* 8.4, 3-H and 5-H), and 7.81 (1 H, d, *J* 8.3, 5-H); δ_C 37.9, 40.4, 52.1, 56.4, 65.9, 111.9, 118.5, 125.8, 126.1 (2), 129.9 (2), 132.4, 138.7, 141.8, 143.7, 153.1, 154.9, and 171.7.

Methyl 4-(*N*-methyl-*N*-{2-[*N*-methyl-*N*-(*tert*-butyloxycarbonyl)amino]-4-nitrobenzyloxycarbonyl}amino)phenylacetate 28h. *Ester* **28h** was obtained (94%) as a pale yellow oil, ν_{max}(thin film)/cm^{-1} 1736, 1707, 1527, 1348, and 1153; δ_H (two rotamers) 1.33 and 1.51 (9 H, 2s, $C(CH_3)_3$), 3.18 (3 H, s, NCH_3), 3.32 (3 H, s, NCH_3), 3.64 (2 H, s, CH_2CO), 3.71 (3 H, s, OCH_3), 5.16 (2 H, s, CH_2O), 7.21 (2 H, br d, *J* 8.3, 2-H and 6-H), 7.30 (2 H, br d, *J* 8.3, 3-H and 5-H), 7.40 (1 H, br s, 6′-H), 8.00 (1 H, br s, 3′-H), and 8.09 (1 H, br d, *J* 8.0, 5′-H); δ_C 28.2 (3), 37.0, 38.0, 40.5, 52.1, 62.6, 81.3, 122.0, 122.3, 126.1 (2), 128.4, 130.0 (2), 132.6, 141.8, 142.1, 147.8, 153.8, 154.9, and 171.7; 2′-C not observed; *m*/*z* (DEI) 487.1969 (M^+. $C_{24}H_{29}N_3O_8$ requires 487.1956); *m*/*z* (DEI) 487 (M^+, 5%), 431 (21), 209 (90), and 57 (100).

Methyl 4-(*N*-methyl-*N*-{3-[*N*-methyl-*N*-(*tert*-butyloxycarbonyl)amino]-4-nitrobenzyloxycarbonyl}amino)phenylacetate 28i. *Ester* **28i** was obtained (89%) as a pale yellow oil, ν_{max}(thin film)/cm^{-1} 1736, 1711, 1528, 1354, and 1155; δ_H 1.27 and 1.47 (9 H, 2s, $C(CH_3)_3$), 2.93 (3 H, s, NCH_3), 3.25 (3 H, s, NCH_3), 3.64 (2 H, s, CH_2CO), 3.71 (3 H, s, OCH_3), 5.20 (2 H, s, CH_2O), 7.20–7.30 (6 H, m, 2-H, 3-H, 5-H, 6-H, 2′-H, and 6′-H), and 7.80 (1 H, br d, *J* 8.5, 5′-H); δ_C 28.2 (3), 37.3, 37.9, 40.5, 52.1, 65.4, 81.7, 125.0, 125.3, 126.0 (2), 127.0, 129.9 (2), 132.4, 137.5, 141.7, 143.1, 145.3, 153.0, 154.8, and 171.7; *m*/*z* (DEI) 487.1959 (M^+. $C_{24}H_{29}N_3O_8$ requires 487.1956); *m*/*z* (DEI) 487 (M^+, 10%), 399 (20), 387 (40), 178 (50), 165 (70), and 57 (100).

Hydrolysis of esters 28a–i. General method

A solution of ester **28** (2 mmol) in MeOH (40 cm^3) and 1 M NaOH solution (10 mmol) was stirred at 20 °C for 1 h. The solution was washed with ether (20 cm^3), the pH of the aqueous fraction adjusted to 2 with 5 M HCl, and extracted with EtOAc (3 × 30 cm^3). The combined organic extract was dried and the solvent removed under reduced pressure. The solid was recrystallised from EtOAc to give acid **29**. Yields and characterisations of individual compounds are given below.

4-[*N*-(4-Nitrobenzyloxycarbonyl)amino]phenylacetic acid 29a. *Acid* **29a** was obtained (80%) as a tan powder, mp (from MeOH–water) 182–183 °C (Found: C, 58.4; H, 4.3; N, 8.4. $C_{16}H_{14}N_2O_6$ requires C, 58.2; H, 4.3; N, 8.5%); ν_{max}/cm^{-1} 3393, 1736, 1690, 1610, 1541, 1344, and 1223; δ_H $[(CD_3)_2SO]$ 3.49 (2 H, s, CH_2CO), 5.30 (2 H, s, CH_2O), 7.17 (2 H, d, *J* 8.4, 3-H, 5-H), 7.40 (2 H, d, *J* 8.4, 2-H, 6-H), 7.69 (2 H, d, *J* 8.6, 2′-H, 6′-H), 8.26 (2 H, d, *J* 8.7, 3′-H, 5′-H), 9.87 (1 H, s, $NHCO_2$), and 12.26 (1 H, br s, CO_2H); δ_C $[(CD_3)_2SO]$ 39.9, 64.4, 118.1 (2), 123.5 (2), 128.4 (2), 129.1, 129.6 (2), 137.3, 144.6, 147.0, 153.0, and 172.7.

4-[*N*-Methyl-*N*-(4-nitrobenzyloxycarbonyl)amino]phenylacetic acid 29b. *Acid* **29b** was obtained (89%) as a white solid, mp (from EtOAc) 125–126 °C (Found: C, 59.2; H, 4.6; N, 8.1. $C_{17}H_{16}N_2O_6$ requires C, 59.3; H, 4.7; N, 8.1%); ν_{max}/cm^{-1} 3443, 3109, 1703, 1692, 1610, 1541, 1346, and 1167; δ_H $[(CD_3)_2SO]$ 3.26 (3 H, s, NCH_3), 3.58 (2 H, s, CH_2CO), 5.24 (2 H, s, CH_2O), 7.26–7.30 (4 H, m, 2-H, 3-H, 5-H, 6-H), 7.56 (2 H, br d, *J* 8.7, 2′-H, 6′-H), 8.21 (2 H, d, *J* 8.7, 3′-H, 5′-H), and 12.36 (1 H, br s, CO_2H); δ_C $[(CD_3)_2SO]$ 37.5, 40.0, 65.3, 123.5 (2), 125.6 (2), 127.9 (2), 129.8 (2), 132.9, 141.3, 144.6, 146.9, 154.3, and 172.6.

4-{*N*-Methyl-*N*-[1-(4-nitrophenyl)ethyloxycarbonyl]amino}phenylacetic acid 29c. *Acid* **29c** was obtained (75%) as a white powder, mp (from MeOH) 117–118.5 °C (Found: C, 60.3; H, 5.0; N, 7.7. $C_{18}H_{18}N_2O_6$ requires C, 60.3; H, 5.1; N, 7.8%); ν_{max}/cm^{-1} 3414, 2980, 1705, 1607, 1516, and 1344; δ_H $[(CD_3)_2SO]$ 1.45 (3 H, d, *J* 6.3, CH_3), 3.15 (3 H, s, NCH_3), 3.59 (2 H, s, CH_2CO), 5.85 (1 H, q, *J* 6.3, OCH), 7.28 (4 H, br s, 2-H, 3-H, 5-H, 6-H), 7.53–7.57 (2 H, m, 2′-H, 6′-H), 8.20 (2 H, d, *J* 8.7, 3′-H, 5′-H), and 12.37 (1 H, br s, CO_2H); δ_C $[(CD_3)_2SO]$ 22.3, 37.3, 40.0, 72.1, 123.6 (2), 125.4 (2), 126.6 (2), 129.7 (2), 132.7, 141.3, 146.7, 149.8, 153.7, and 172.6.

4-[*N*-Methyl-*N*-(2-methyl-4-nitrobenzyloxycarbonyl)amino]phenylacetic acid 29d. *Acid* **29d** was obtained (80%) as a pale yellow powder, mp (from EtOAc) 107–111 °C (Found: C, 60.4; H, 5.0; N, 7.8. $C_{18}H_{18}N_2O_6$ requires C, 60.3; H, 5.0; N, 7.8%); ν_{max}/cm^{-1} 3086, 2944, 1720, 1703, 1528, 1344, and 1157; δ_H $[(CD_3)_2SO]$ 2.36 (3 H, s, CH_3), 3.24 (3 H, s, NCH_3), 3.58 (2 H, s, CH_2CO), 5.20 (2 H, s, CH_2O), 7.28 (4 H, br s, 2-H, 3-H, 5-H, 6-H), 7.47 (1 H, d, *J* 8.3, 6′-H), 8.06–8.09 (2 H, m, 3′-H and 5′-H), and 12.40 (1 H, br s, CO_2H); δ_C $[(CD_3)_2SO]$ 18.5, 37.5, 40.0, 64.0, 122.2, 122.6 (2), 125.6 (2), 129.8 (2), 136.7, 131.3, 141.3, 144.6, 145.6, 154.2, and 172.6.

4-[*N*-Methyl-*N*-(3-methyl-4-nitrobenzyloxycarbonyl)amino]phenylacetic acid 29e. *Acid* **29e** was obtained (59%) as a cream powder, mp (from EtOAc) 118–119 °C (Found: C, 60.5; H, 5.1; N, 7.8. $C_{18}H_{18}N_2O_6$ requires C, 60.3; H, 5.0; N, 7.8%); ν_{max}/cm^{-1} 3034, 1707, 1613, 1516, 1339, and 1169; δ_H $[(CD_3)_2SO]$ 2.51 (3 H, s, CH_3), 3.25 (3 H, s, NCH_3), 3.58 (2 H, s, CH_2CO), 5.16 (2 H, s, CH_2O), 7.26–7.34 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 7.34–7.37 (2 H, m, 2′-H, 6′-H), 7.97 (1 H, d, *J* 8.2, 5′-H), and 12.36 (1 H, br s, CO_2H); δ_C $[(CD_3)_2SO]$ 19.6, 37.5, 40.0, 65.2, 124.6 (2), 125.5, 129.8 (2), 130.0, 130.9, 132.9, 141.3, 142.5 (2), 148.0, 154.2, and 172.5.

4-[*N*-Methyl-*N*-(2-methoxy-4-nitrobenzyloxycarbonyl)amino]phenylacetic acid 29f. *Acid* **29f** was obtained (85%) as a pale yellow powder, mp (from MeOH–water) 173.5–175.5 °C (Found: C, 57.8; H, 5.0; N, 7.4. $C_{18}H_{18}N_2O_7$ requires C, 57.8; H, 4.85; N, 7.5%); ν_{max}/cm^{-1} 3094, 2945, 1721, 1699, 1518, 1345, and 1155; δ_H $[(CD_3)_2SO]$ 3.26 (3 H, s, NCH_3), 3.58 (2 H, s,

CH_2CO), 3.95 (3 H, s, OCH_3), 5.15 (2 H, s, CH_2O), 7.26–7.31 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 7.39–7.41 (1 H, m, 6′-H), 7.77 (1 H, d, *J* 2.0, 3′-H), 7.83 (1 H, dd, *J* 8.3 and 2.0, 5′-H), and 12.36 (1 H, br s, CO_2H); δ_C [$(CD_3)_2SO$] 37.5, 40.0, 56.2, 61.5, 105.3, 115.5, 125.5 (2), 127.9, 129.8 (2), 132.5, 132.9, 141.3, 148.0, 154.2, 156.6, and 172.6.

4-[*N*-Methyl-*N*-(3-methoxy-4-nitrobenzyloxycarbonyl)-amino]phenylacetic acid 29g. *Acid* **29g** was obtained (83%) as a cream powder, mp (from MeOH–water) 158–160 °C (Found: C, 57.85; H, 4.85; N, 7.3. $C_{18}H_8N_2O_7$ requires C, 57.8; H, 4.85; N, 7.5%); ν_{max}/cm^{-1} 3119, 3055, 1701, 1614, 1516, 1340, and 1165; δ_H [$(CD_3)_2SO$] 3.26 (3 H, s, NCH_3), 3.58 (2 H, s, CH_2CO), 3.86 (3 H, s, OCH_3), 5.17 (2 H, s, CH_2O), 7.01 (1 H, br d, *J* 8.3, 6′-H), 7.23 (1 H, br s, 2′-H), 7.27–7.33 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 7.86 (1 H, d, *J* 8.3, 5′-H), and 12.37 (1 H, br s, CO_2H); δ_C [$(CD_3)_2SO$] 37.5, 40.0, 56.5, 65.3, 112.1, 118.4, 125.2 (2), 125.7, 129.8 (2), 133.0, 138.2, 141.3, 144.0, 152.0, 154.2, and 172.6.

4-(*N*-Methyl-*N*-{2-[*N*-methyl-*N*-(*tert*-butyloxycarbonyl)-amino]-4-nitrobenzyloxycarbonyl}amino)phenylacetic acid 29h. *Acid* **29h** was obtained (90%) as a colourless oil, ν_{max}(thin film)/cm^{-1} 3202, 1710, 1528, 1348, and 1153; δ_H [$(CD_3)_2SO$] 1.28 and 1.46 (9 H, 2s, $C(CH_3)_3$), 3.11 (3 H, s, NCH_3), 3.26 (3 H, s, NCH_3), 3.58 (2 H, s, CH_2CO), 5.10 (2 H, s, CH_2O), 7.26–7.31 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 7.53–7.56 (1 H, m, 6′-H), 8.14–8.17 (2 H, m, 3′-H and 5′-H), and 12.60 (1 H, br s, CO_2H); δ_C [$(CD_3)_2SO$] 27.6 (3), 36.6, 37.6, 40.0, 59.7, 80.2, 122.0, 122.2, 125.6, 128.5 (2), 128.6, 129.8 (2), 133.0, 141.2, 142.1, 147.3, 153.0, 154.2, and 172.5; *m/z* (DEI) 473.1795. (M^+. $C_{23}H_{27}N_3O_8$ requires 473.1798); *m/z* (DEI) 473 (M^+, 5%), 417 (2), 373 (5), 209 (90), 165 (90), and 57 (100).

4-(*N*-Methyl-*N*-{3-[*N*-methyl-*N*-(*tert*-butoxycarbonyl)amino]-4-nitrobenzyloxycarbonyl}amino)phenylacetic acid 29i. *Acid* **29i** was obtained (91%) as a white foam (Found: C, 57.6; H, 6.0; N, 8.45. $C_{23}H_{27}N_3O_8 \cdot {}^1/_2MeOH$ requires C, 57.7; H, 6.0; N, 8.6%); ν_{max}/cm^{-1} 3360, 1711, 1528, 1356, and 1157; δ_H [$(CD_3)_2SO$] 1.22 and 1.42 (9 H, 2s, $C(CH_3)_3$), 3.18 (3 H, s, NCH_3), 3.10 (1.5 H, br s, residual CH_3OH), 3.26 (3 H, s, NCH_3), 3.58 (2 H, s, CH_2CO), 4.00 (0.5 H, m, residual CH_3OH), 5.19 (2 H, s, CH_2O), 7.26–7.31 (4 H, m, 2-H, 3-H, 5-H, 6-H), 7.37 (1 H, br d, *J* 8.3, 6′-H), 7.45 (1 H, br s, 2′-H), 7.95 (1 H, d, *J* 8.3, 5′-H), and 12.35 (1 H, br s, CO_2H); δ_C [$(CD_3)_2SO$] 27.8 (3), 37.0, 37.5, 39.9, 48.6, 65.0, 80.6, 124.9, 125.3, 125.6 (2), 126.7, 129.8 (2), 132.9, 136.3, 141.3, 143.7, 144.6, 152.1, 154.2, and 172.5.

Formation of carbamates 4a–i: example of general preparation by method A (Scheme 8)

A solution of acid **29** (1 mmol) and CDI (1.5 mmol) in DMF (10 cm^3) was heated at 50 °C for 10 min. *N′*,*N′*-Dimethylaminoethylamine (2 mmol) was added and the solution stirred at 20 °C for 2 h. The solution was poured into ice–water (100 cm^3) and extracted with EtOAc (3 × 50 cm^3). The combined organic fraction was washed with 1 M NaOH solution (10 cm^3), water (3 × 30 cm^3), brine (30 cm^3), dried, and the solvent removed under reduced pressure. The residue was chromatographed on neutral alumina, eluting with EtOAc to give the amide **4**. Amide **4** was dissolved in MeOH (10 cm^3) and HCl-saturated MeOH (10 cm^3) added and then the solvent was removed under reduced pressure to give **4** as the HCl salt. Yields and characterisations of individual compounds are given below.

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-(4-nitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4a.** *Acetamide hydrochloride* **4a** was obtained (66%) as a tan powder, mp (from MeOH–EtOAc) 161 °C (decomp.) (Found: C, 53.5; H, 5.7; N, 11.9. $C_{20}H_{25}ClN_4O_5$.MeOH requires C, 53.8; H, 6.2; N, 12.0%); ν_{max}/cm^{-1} 3385, 3283, 1738, 1649, 1607, 1539, 1346, and 1125; δ_H [$(CD_3)_2SO$] 2.75 (6 H, d, *J* 5.0, $N(CH_3)_2$), 3.34 (2 H, s, CH_2CO), 3.39–3.43 (4 H, m, 2 × CH_2N), 3.47 (3 H, br s, CH_3OH), 5.30 (2 H, s, CH_2O), 7.19 (2 H, d, *J* 8.4, 3-H, 5-H), 7.39 (2 H, d, *J* 8.4, 2-H, 6-H), 7.68 (2 H, d, *J* 8.6, 2′-H, 6′-H), 8.27 (2 H, d, *J* 8.7, 3′-H, 5′-H), 8.46 (1 H, t, *J* 5.5, CONH), 9.88 (1 H, s, $NHCO_2$), and 10.55 (1 H, s, NHCl); δ_C [$(CD_3)_2SO$] 33.9, 41.6, 42.1 (2), 49.0, 55.6, 64.4, 118.5 (2), 123.6 (2), 128.4 (2), 129.4 (2), 130.1, 137.2, 144.6, 147.0, 153.0, and 170.8.

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-methyl-*N*-(4-nitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4b.** *Acetamide hydrochloride* **4b** was obtained (58%) as a pale yellow gum (Found: C, 55.65; H, 6.3; N, 12.1; Cl, 8.1. $C_{21}H_{27}ClN_4O_5$ requires C, 55.9; H, 6.0; N, 12.4; Cl, 7.9%); ν_{max}/cm^{-1} 3289, 1701, 1651, 1610, 1541, 1346, and 1167; δ_H [$(CD_3)_2SO$] 2.75 (6 H, d, *J* 3.9, $N(CH_3)_2$), 3.12–3.15 (2 H, m, CH_2N), 3.25 (3 H, s, NCH_3), 3.40–3.44 (2 H, m, CH_2N), 3.48 (2 H, s, CH_2CO), 5.24 (2 H, s, CH_2O), 7.26–7.31 (4 H, m, 2-H, 3-H, 5-H, 6-H), 7.56 (2 H, br d, *J* 8.7, 2′-H, 6′-H), 8.22 (2 H, d, *J* 8.7, 3′-H, 5′-H), 8.56 (1 H, t, *J* 5.5, CONH), and 10.49 (1 H, br s, NHCl); δ_C [$(CD_3)_2SO$] 33.9, 37.6, 41.6, 42.1 (2), 55.6, 65.3, 123.5 (2), 125.5 (2), 127.9 (2), 129.6 (2), 133.9, 141.2, 144.6, 146.9, 154.3, and 170.6.

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-{*N*-methyl-*N*-[1-(4-nitrophenyl)ethyloxycarbonyl]amino}phenylacetamide hydrochloride 4c.** *Acetamide hydrochloride* **4c** was obtained (85%) as a yellow gum, ν_{max}(thin film)/cm^{-1} 3407, 1701, 1658, 1516, 1346, and 1159; δ_H [$(CD_3)_2SO$] 1.45 (3 H, d, *J* 6.6, CH_3), 2.81 (6 H, d, *J* 4.0, $N(CH_3)_2$), 3.14 (2 H, s, CH_2CO), 3.25 (3 H, s, NCH_3), 3.36–3.41 (4 H, m, 2 × CH_2N), 5.84 (1 H, q, *J* 6.6, OCH), 7.25–7.32 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 7.54–7.58 (2 H, m, 2′-H, 6′-H), 8.22 (2 H, d, *J* 8.7, 3′-H, 5′-H), 8.58 (1 H, t, *J* 5.5, CONH), and 10.56 (1 H, br s, HCl); δ_C [$(CD_3)_2SO$] 22.3, 33.9, 37.4, 41.6, 42.1 (2), 55.5, 72.1, 123.6 (2), 125.4 (2), 126.7 (2), 129.5 (2), 133.7, 141.2, 146.7, 149.8, 153.7, and 170.6; *m/z* (DEI) 428.2055 (M^+. $C_{22}H_{28}N_4O_5$ requires 428.2060); *m/z* (DEI) 428 (M^+, 60%), 409 (80), 385 (10), 235 (70), and 164 (100).

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-methyl-*N*-(2-methyl-4-nitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4d.** *Acetamide hydrochloride* **4d** was obtained (63%) as a brown hygroscopic foam, ν_{max}(thin film)/cm^{-1} 3396, 1705, 1672, 1516, 1346, and 1155; δ_H [$(CD_3)_2SO$] 2.75 (6 H, d, *J* 4.8, $N(CH_3)_2$), 2.50 (3 H, s, CH_3), 3.11–3.15 (2 H, m, CH_2N), 3.24 (3 H, s, NCH_3), 3.40–3.43 (2 H, m, CH_2N), 3.47 (2 H, s, CH_2CO), 5.20 (2 H, s, CH_2O), 7.22–7.31 (4 H, m, 2-H, 3-H, 5-H, 6-H), 7.49 (1 H, d, *J* 8.3, 6-H), 8.02–8.06 (1 H, m, 3′-H), 8.08 (1 H, dd, *J* 8.3 and 2.2, 5′-H), 8.52 (1 H, t, *J* 5.5, CONH), and 10.41 (1 H, s, NHCl); δ_C [$(CD_3)_2SO$] 18.5, 33.9, 37.5, 41.6, 42.1 (2), 55.7, 63.9, 122.1, 122.6, 125.5 (2), 129.6 (2), 131.3, 134.0, 136.7, 141.3, 144.6, 145.6, 154.2, and 170.6; *m/z* (DEI) 428.2049 (M^+. $C_{22}H_{28}N_4O_5$ requires 428.2060); *m/z* (DEI) 428 (M^+, 35%), 409 (60), 384 (60), 358 (80), and 327 (100).

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-methyl-*N*-(3-methyl-4-nitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4e.** *Acetamide hydrochloride* **4e** was obtained (85%) as a tan hygroscopic foam, ν_{max}(thin film)/cm^{-1} 3426, 1705, 1674, 1520, 1345, 1265, and 1161; δ_H [$(CD_3)_2SO$] 2.51 (3 H, s, CH_3), 2.75 (6 H, d, *J* 4.8, $N(CH_3)_2$), 3.10–3.15 (2 H, m, CH_2N), 3.24 (3 H, s, NCH_3), 3.40–3.45 (2 H, m, CH_2N), 3.47 (2 H, s, CH_2CO), 5.16 (2 H, s, CH_2O), 7.27–7.33 (4 H, m, 2-H, 3-H, 5-H, 6-H), 7.40–7.45 (2 H, m, 2′-H, 6′-H), 7.97 (1 H, d, *J* 8.3, 5′-H), 8.56 (1 H, t, *J* 5.5, CONH), and 10.50 (1 H, s, NHCl); δ_C [$(CD_3)_2SO$] 19.6, 33.9, 37.5, 41.6, 42.1 (2), 55.6, 65.2, 119.1, 124.7 (2), 125.6 (2), 129.6 (2), 130.9, 132.9, 141.2, 142.5, 148.0, 154.3, and 170.6; *m/z* (DEI) 428.2067 (M^+. $C_{22}H_{28}N_4O_5$ requires 428.2060); *m/z* (DEI) 428 (M^+, 30%), 409 (100), and 384 (60).

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-methyl-*N*-(2-methoxy-4-nitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4f.** *Acetamide hydrochloride* **4f** was obtained (76%) as a brown oil, ν_{max}(thin film)/cm^{-1} 3418, 1707, 1658, 1518, 1348, and 1155; δ_H [(CD$_3$)$_2$SO] 2.48 (6 H, d, *J* 4.5, N(CH$_3$)$_2$), 3.14 (2 H, dt, *J* 6.0 and 5.5, CH$_2$N), 3.25 (3 H, s, NCH$_3$), 3.38–3.48 (4 H, m, CH$_2$N and CH$_2$CO), 3.97 (3 H, s, OCH$_3$), 5.15 (2 H, s, CH$_2$O), 7.26–7.32 (4 H, m, 2-H, 3-H, 5-H, 6-H), 7.41–7.43 (1 H, m, 6′-H), 7.76 (1 H, d, *J* 1.8, 3′-H), 7.84 (1 H, dd, *J* 8.3 and 1.8, 5′-H), 8.64 (1 H, t, *J* 5.4, CONH), and 10.76 (1 H, s, NHCl); δ_C [(CD$_3$)$_2$SO] 33.9, 37.5, 41.6, 42.1 (2), 55.5, 56.2, 61.5, 105.4, 115.1, 125.5 (2), 128.0, 129.6 (2), 132.5, 134.0, 141.2, 148.0, 154.2, 156.7, and 170.5; *m*/*z* (DEI) 444.2013 (M^+. $C_{22}H_{28}N_4O_6$ requires 444.2009); *m*/*z* (DEI) 444 (M$^+$, 40%), 425 (100), 300 (15), 185 (40), and 235 (60).

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-methyl-*N*-(3-methoxy-4-nitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4g.** *Acetamide hydrochloride* **4g** was obtained (95%) as a brown gum (Found: C, 59.2; H, 6.2; N, 12.6. $C_{22}H_{28}N_4O_6$ requires C, 59.4; H, 6.35; N, 12.6%); ν_{max}/cm^{-1} 3464, 3299, 1707, 1643, 1612, 1518, 1332, and 1161; δ_H [(CD$_3$)$_2$SO] 2.75 (6 H, d, *J* 4.8, N(CH$_3$)$_2$), 3.11–3.14 (2 H, m, CH$_2$N), 3.25 (3 H, s, NCH$_3$), 3.42 (2 H, dt, *J* 6.3 and 5.9, CH$_2$N), 3.47 (2 H, s, CH$_2$CO), 3.87 (3 H, s, OCH$_3$), 5.17 (2 H, s, CH$_2$O), 7.00–7.04 (1 H, m, 6′-H), 7.20 (1 H, br s, 2′-H), 7.26–7.33 (4 H, m, 2-H, 3-H, 5-H, 6-H), 7.87 (1 H, d, *J* 8.3, 5′-H), 8.56 (1 H, t, *J* 5.5, CONH), and 10.53 (1 H, s, NHCl); δ_C [(CD$_3$)$_2$SO] 33.9, 37.6, 41.6, 42.1 (2), 56.5, 59.7, 65.3, 112.2, 118.4, 125.2 (2), 125.6, 129.6 (2), 134.0, 138.2, 141.2, 144.0, 152.2, 154.2, and 170.6.

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-(*N*-methyl-*N*-{2-[*N*-methyl-*N*-(*tert*-butyloxycarbonyl)amino]-4-nitrobenzyloxycarbonyl}amino)phenylacetamide 4h.** *Acetamide* **4h** was obtained (80%) as a pale yellow oil, ν_{max}(thin film)/cm^{-1} 3389, 1709, 1670, 1527, 1348, and 1158; δ_H [(CD$_3$)$_2$SO] 1.45 and 1.27 (9 H, 2s, C(CH$_3$)$_3$), 2.17 (6 H, d, *J* 4.5, N(CH$_3$)$_2$), 2.26 (2 H, t, *J* 6.7, CH$_2$N), 3.10–3.15 (5 H, m, NCH$_3$ and CH$_2$N), 3.48 (2 H, s, CH$_2$CO), 3.23 (3 H, s, NCH$_3$), 5.08 (2 H, s, CH$_2$O), 7.24–7.30 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 7.54 (1 H, br s, 3′-H), 8.00 (1 H, t, *J* 5.5, CONH), 8.15–8.19 (2 H, m, 5′-H and 6′-H); δ_C [(CD$_3$)$_2$SO] 27.6 (3), 35.7, 36.4, 36.6, 37.6, 41.7, 45.1 (2), 58.1, 80.2, 122.0, 122.2, 125.5, 127.5, 128.3 (2), 129.3 (2), 134.6, 141.2, 142.1, 147.3, 153.0, 154.2, and 169.7; *m*/*z* (DEI) 543.2686 (M^+. $C_{27}H_{37}N_5O_7$ requires 543.2693); *m*/*z* (DCI, NH$_3$) 544 (MH$^+$, 100%), 488 (10), 250 (10), and 236 (90).

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-(*N*-methyl-*N*-{3-[*N*-methyl-*N*-(*tert*-butoxycarbonyl)amino]-4-nitrobenzyloxycarbonyl}amino)phenylacetamide 4i.** *Acetamide* **4i** was obtained (66%) as a pale yellow oil, ν_{max}(thin film)/cm^{-1} 3359, 1709, 1659, 1528, 1345, and 1155; δ_H [(CD$_3$)$_2$SO] 1.42 and 1.22 (9 H, 2s, C(CH$_3$)$_3$), 2.13 (6 H, d, *J* 4.0, N(CH$_3$)$_2$), 2.27 (2 H, t, *J* 6.6, CH$_2$N), 3.13 (2 H, dt, *J* 6.6, 5.8, CH$_2$N), 3.19 (3 H, s, NCH$_3$), 3.26 (3 H, s, NCH$_3$), 3.40 (2 H, s, CH$_2$CO), 5.19 (2 H, s, CH$_2$O), 7.24–7.29 (4 H, m, 2-H, 3-H, 5-H, 6-H), 7.36–7.43 (2 H, m, 2′-H, 6′-H), and 7.95–8.03 (2 H, m, 5′-H and CONH); δ_C [(CD$_3$)$_2$SO] 27.7 (3), 36.7, 37.0, 37.6, 41.6, 45.1 (2), 58.1, 64.9, 80.6, 124.9, 125.3, 125.6 (2), 126.7, 129.3 (2), 134.5, 136.3, 141.0, 143.7, 144.6, 152.1, 154.2, and 169.8; *m*/*z* (DEI) 543.2687 (M^+. $C_{27}H_{37}N_5O_7$ requires 543.2693); *m*/*z* (CI, NH$_3$) 544 (MH$^+$, 80%), 367 (30), 236 (70), and 71 (100).

Formation of carbamates 4j–l: example of general preparation by method B (Scheme 9)

A solution of the chloroformate of the alcohol **8** (2 mmol) in THF (50 cm^3) was prepared *in situ* using phosgene and the alcohol **8** as described in Method A above. To this solution amine **7** in THF (5 cm^3) was added and the solution stirred at 20 °C for 16 h. The solution was poured into sat. aq. NaHCO$_3$ solution (100 cm^3), and extracted with EtOAc (3 × 50 cm^3). The combined organic extract was dried and the solvent removed under reduced pressure. The residue was chromatographed on neutral alumina, eluting with a gradient (0–10%) MeOH–EtOAc, to give the carbamate **4**. Final purification was achieved by semi-preparative reverse phase HPLC using a Philips PU4100 liquid chromatograph, a Phenomenex Bondclone 10 C18 column (300 × 21.2 mm id) monitoring at 254 nm. Chromatograms were run in 50% acetonitrile–ammonium formate at 10 cm^3 min^{-1}. Yields and characterisations of individual compounds are given below.

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-methyl-*N*-(2,4-dinitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4j.** *Acetamide hydrochloride* **4j** was obtained (5%) as a red–brown gum, ν_{max}(thin film)/cm^{-1} 3321, 1711, 1657, 1535, 1346, and 1154; δ_H [(CD$_3$)$_2$SO] 2.55 (6 H, d, *J* 5.0, N(CH$_3$)$_2$), 2.95 (2 H, t, *J* 6.5, CH$_2$N), 3.12–3.16 (2 H, m, CH$_2$N), 3.26 (3 H, s, NCH$_3$), 3.36 (2 H, s, CH$_2$CO), 5.53 (2 H, s, CH$_2$O), 7.26–7.30 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 7.50–7.54 (1 H, m, 6′-H), 8.56 (1 H, dd, *J* 8.6 and 2.2, 5′-H), 8.64 (1 H, t, *J* 5.4, CONH), 8.78 (1 H, d, *J* 2.2, 3′-H), and 10.76 (1 H, s, NHCl); δ_C [(CD$_3$)$_2$SO] 36.7, 37.6, 40.0, 45.0 (2), 58.1, 63.1, 120.3, 125.7 (2), 128.0, 129.5 (2), 129.8, 134.8, 139.3, 140.9, 146.7, 146.8, 153.9, and 169.8; *m*/*z* (DCI, NH$_3$) 460.1798 (MH^+. $C_{21}H_{26}N_5O_7$ requires 460.1832); *m*/*z* (DCI, NH$_3$) 460 (MH$^+$, 2%), 293 (3), and 236 (100).

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-methyl-*N*-(3,4-dinitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4k.** *Acetamide hydrochloride* **4k** was obtained (26%) as a red–brown gum, ν_{max}(thin film)/cm^{-1} 3342, 1707, 1653, 1543, 1350, and 1155; δ_H [(CD$_3$)$_2$SO] 2.32 (2 H, t, *J* 6.7, CH$_2$N), 2.76 (6 H, d, *J* 5.0, N(CH$_3$)$_2$), 3.15 (2 H, dt, *J* 6.6 and 5.7, CH$_2$N), 3.30–3.38 (5 H, m, NCH$_3$ and CH$_2$CO), 5.27 (2 H, s, CH$_2$O), 7.25–7.30 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 7.83–7.87 (1 H, m, 6′-H), 8.03 (1 H, t, *J* 5.4, CONH), 8.11 (1 H, br s, 2′-H), 8.22 (1 H, d, *J* 8.3, 5′-H), and 10.50 (1 H, s, NHCl); δ_C [(CD$_3$)$_2$SO] 36.6, 37.7, 41.7, 44.9 (2), 59.8, 64.5, 123.6, 125.6 (2), 125.9, 129.5 (2), 132.3, 134.5, 141.0, 142.1, 144.7 (2), 154.1, and 170.0; *m*/*z* (DCI, NH$_3$) 460.1811. (MH^+. $C_{21}H_{26}N_5O_7$ requires 460.1832); *m*/*z* (DCI, NH$_3$) 460 (MH$^+$, 5%), 430 (2), 412 (3), and 236 (100).

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-methyl-*N*-(3-methyloxycarbonyl-4-nitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4l.** *Acetamide hydrochloride* **4l** was obtained (12%) as a tan gum; ν_{max}(thin film)/cm^{-1} 3415, 1736, 1707, 1655, 1533, 1346, and 1155; δ_H [(CD$_3$)$_2$SO] 2.27 (2 H, t, *J* 6.7, CH$_2$N), 2.66 (6 H, d, *J* 4.5, N(CH$_3$)$_2$), 3.11–3.16 (2 H, m, CH$_2$N), 3.24 (3 H, s, NCH$_3$), 3.41 (2 H, s, CH$_2$CO), 3.87 (3 H, s, OCH$_3$), 5.23 (2 H, s, CH$_2$O), 7.25–7.30 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 7.69–7.75 (2 H, m, 2′-H and 6′-H), 8.03 (1 H, t, *J* 5.4, CONH), 8.06 (1 H, d, *J* 8.4, 5′-H), 10.35 (1 H, s, NHCl); δ_C [(CD$_3$)$_2$SO] 36.7, 37.6, 41.6, 45.1 (2), 53.2, 58.1, 64.8, 124.4, 125.5 (2), 126.4, 128.0, 129.3 (2), 130.8, 134.5, 141.0, 143.2, 146.8, 154.2, 164.9, and 169.8; *m*/*z* (DEI) 472.1956 (M^+. $C_{23}H_{28}N_4O_7$ requires 472.1958); *m*/*z* (DEI) 472 (M$^+$, 1%), 402 (2), 357 (3), 71 (20), and 58 (100).

Formation of carbamates 4m,n: example of general preparation by method C (Scheme 10)

HCl-saturated MeOH (10 cm^3) was added to a stirred solution of **4h** or **4i** (0.26 mmol) in MeOH (10 cm^3) and the solution stirred at 20 °C for 4 h. The solvent was removed under reduced pressure and the residue dissolved in water (10 cm^3), the pH adjusted to 10 with aq. Na$_2$CO$_3$ solution, and extracted with CHCl$_3$ (3 × 25 cm^3). The combined organic extracts were washed with brine (30 cm^3), dried, and the solvent removed under reduced pressure. The residue was chromatographed on neutral alumina, eluting with a gradient (0–10%) MeOH–EtOAc, to give the carbamate **4**. Yields and characterisations of individual compounds are given below.

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-methyl-*N*-(2-methylamino-4-nitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4m.** *Acetamide hydrochloride* **4m** was obtained (28%) as a pale yellow oil, ν_{max}(thin film)/cm^{-1} 3340, 1707, 1650, 1576, 1338, and 1145; δ_H [$(CD_3)_2SO$] 2.65 (6 H, d, *J* 4.9, $N(CH_3)_2$), 2.75 (2 H, t, *J* 6.7, CH_2N), 2.87 (3 H, s, NCH_3), 3.11–3.16 (2 H, m, NCH_2), 3.23 (3 H, s, $NHCH_3$), 3.39–3.43 (2 H, m, CH_2N), 3.47 (2 H, s, CH_2CO), 5.21 (2 H, s, CH_2O), 5.50 (1 H, s, $NHCH_3$), 7.24–7.35 (7 H, m, 2-H, 3-H, 5-H, 6-H, 3′-H, 5′-H, and 6′-H), 8.61 (1 H, t, *J* 5.5, CONH), and 10.71 (1 H, br s, NHCl); δ_C [$(CD_3)_2SO$] 33.9, 34.3, 37.5, 41.6, 42.1 (2), 55.5, 63.7, 113.1, 114.0, 125.4 (2), 128.9, 129.5 (2), 129.8, 133.9, 141.7, 146.9, 149.3, 154.2, and 170.5; *m*/*z* (DEI) 443.2160 (M^+. $C_{22}H_{29}N_5O_5$ requires 443.2169); *m*/*z* (DEI) 443 (M^+, 15%), 426 (10), 396 (30), and 235 (100).

***N*-[2-(*N*,*N*-Dimethylamino)ethyl] 4-[*N*-methyl-*N*-(3-methylamino-4-nitrobenzyloxycarbonyl)amino]phenylacetamide hydrochloride 4n.** *Acetamide hydrochloride* **4n** was obtained (28%) as a pale yellow oil, ν_{max}(thin film)/cm^{-1} 3387, 1707, 1655, 1626, 1580, 1336, and 1157; δ_H [$(CD_3)_2SO$] 2.78 (6 H, d, *J* 4.9, $N(CH_3)_2$), 2.88 (3 H, s, NCH_3), 3.12–3.16 (2 H, m, NCH_2), 3.25 (3 H, br s, $NHCH_3$), 3.40–3.43 (2 H, m, CH_2N), 3.47 (2 H, s, CH_2CO), 3.55 (1 H, s, $NHCH_3$), 5.11 (2 H, s, CH_2O), 6.55–6.59 (1 H, m, 6-H), 6.77 (1 H, br s, 2-H), 7.28–7.35 (4 H, m, 2-H, 3-H, 5-H, and 6-H), 8.03 (1 H, d, *J* 8.8, 5-H), 8.19 (1 H, s, NHCl), 8.51 (1 H, t, *J* 5.4, CONH), and 10.16 (1 H, br s, NHCl); δ_C [$(CD_3)_2SO$] 29.6, 34.1, 37.7, 41.6, 42.4 (2), 55.7, 65.5, 111.3, 113.1, 125.8 (2), 126.5, 129.7 (2), 130.0, 134.0, 141.3, 145.8, 146.0, 154.3, and 170.8; *m*/*z* (DEI) 443.2165 (M^+. $C_{22}H_{29}N_5O_5$ requires 443.2169); *m*/*z* (DEI) 443 (M^+, 25%), 426 (20), 408 (60), 396 (20), and 235 (100).

Radiolytic reduction of nitrobenzyl carbamates 4a–n

Solutions of the carbamates **4** (50 μM) in 10 mM phosphate buffer (5 cm^3, pH 7.4) and propan-2-ol (4%, v/v) were degassed by evacuation under reduced pressure, and radiolytically reduced at 20 °C by γ-irradiation, using a ^{60}Co source. The dose rate of the ^{60}Co source was measured as 0.721 J dm^{-3} s^{-1} using the NaCl modified Fricke dosimeter,[25] and corrected for decay of ^{60}Co in subsequent irradiations. The time, *t*, required to form 1 molar equivalent of reducing species in a solution of prodrug (50 μM, 5 cm^3) was calculated from $t = n/(Gdv)$, where n = mole of prodrug, G = radiation chemical yield (taken as 0.62 μmol J^{-1}, $G(e^-_{aq})$ + $G(^{\bullet}H)$ + $G(OH)$) d = dose rate and v = the volume of radiolysis solution. Samples were analysed by HPLC, injecting 0.05 cm^3 samples through a 0.025 cm^3 sample loop, on an Econosphere C-18 analytical column (reverse phase, 5 μm particle size, 250 × 4.6 mm id). Samples were eluted using both gradient and isocratic solvent systems, comprised of mixtures of MeOH, pH 6.5, 10 mM phosphate buffer and 10 mM heptane sulfonic acid, at a flow rate of 0.8 cm^3 min^{-1}. An HP1040M series II detector permitted collection of the spectra of chromatographic peaks. HPLC was used to quantify the release of amines **6** or **7**, using authentic samples to prepare calibration plots.

Acknowledgements

This work was supported by the US National Cancer Institute (Contract NO1-CM 47019), the Auckland Division of the Cancer Society of New Zealand, and a University of Auckland Post-Doctoral Fellowship (B. M. S).

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Paper 9/04067F