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Synthesis and evaluation of bifunctional nitrocatechol inhibitors of pig liver catechol-*O*-methyltransferase

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Abstract—Bifunctional compounds were tested in vitro as potential inhibitors of pig liver catechol-*O*-methyltransferase (COMT) with respect to the catechol substrate 4-[(3,4-dihydroxyphenyl)azo]benzenesulfonate. The bifunctional compounds were a composite of either two nitrocatechols or one nitrocatechol and one phenol, linked by amide bonds to a spacer unit comprising two to five methylene groups. The unsymmetrical compounds *N*-[2-(4-hydroxybenzoylamine)ethyl]-3,4-dihydroxy-5-nitrobenzamide], *N*-[3-(4-hydroxybenzoyl-amine)propyl]-3,4-dihydroxy-5-nitrobenzamide] and *N*-[5-(4-hydroxybenzoylamine)pentyl]-3,4-dihydroxy-5-nitrobenzamide] demonstrated strong inhibitory action against COMT with K_i values in the 100 nM range. In comparison, the monofunctional nitrocatechol analogues of these compounds had K_i values that were significantly higher.

1. Introduction

Catechol-*O*-methyltransferase (COMT) catalyses the transfer of a methyl group from S-adenosylmethionine (AdoMet) to a catechol substrate. This ubiquitous enzyme plays an important role in the extraneuronal inactivation of catecholamine neurotransmitters.[1] Effective inhibitors for this enzyme have been shown to improve the effectiveness of current drug therapies for Parkinson's disease.[2]

The first series of inhibitors that were identified as having limited clinical use were the so-called 'first generation inhibitors'. Examples of these are pyrogallol,[3] tropolone,[4] catechin,[3] and gallic acid.[5] These inhibitors had K_i values in the 10^{-5} M range. The following so-called 'second-generation' inhibitors are characterized by having electron withdrawing groups, such as nitro groups, in the 3- and 5-position of the catechol ring.[6] These features have been incorporated into clinically useful inhibitors such as nitecapone,[7] tolcapone[8] and entacapone.[9]

Of the various COMT inhibitors that have been reported in the literature, a small group are of a 'bifunctional' nature, in that they have duplicated substructures for enzyme binding. Three such inhibitors are from the so-called first generation inhibitors and include D-catechin, desmethylpapaverine and nordihydroxyguaiaretic acid (Fig. 1).[10,11]

Recently, two new progressive series of bifunctional compounds were synthesized and examined for their inhibition characteristics.[12] The compounds were designed with dual substituted catechols for enzyme binding separated by linker sections of various lengths (Fig. 2). The catechol derivatives were either 3,4-dihydroxybenzamide or 3,4,5-trihydroxybenzamide groups, linked through the

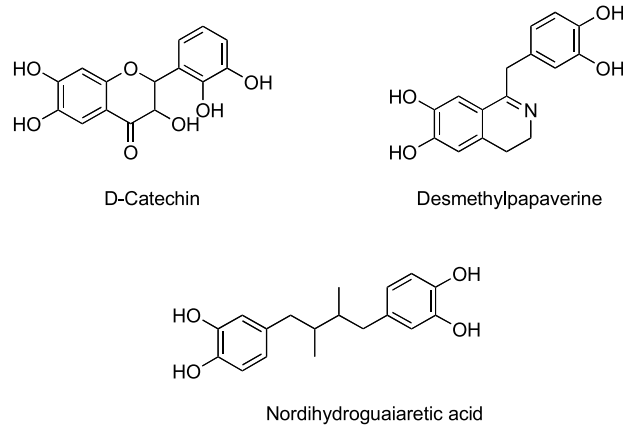

Figure 1. Bifunctional 'first generation' inhibitors.

Keywords: Catechol-*O*-methyltransferase; Enzyme inhibitors; Bifunctional.
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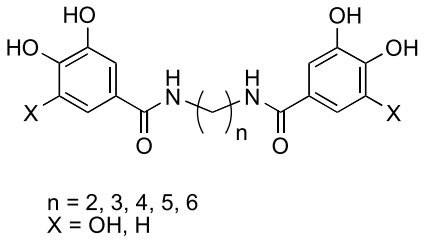


Figure 2. Previously evaluated polyhydroxybenzamide bifunctional inhibitors.

nitrogen atoms to a spacer section consisting of a varying number of methylene units. It was found that some of these bifunctional inhibitors were significantly more potent than their monofunctional counterparts.

In this paper, we report the preparation of a series of inhibitors that incorporate the extra binding substructure of a bifunctional inhibitor, with aspects of the 'second generation' inhibitors in an attempt to create even more potent inhibitors. In addition, we chose to investigate the effect of these inhibitors with pig liver COMT, as the structure and function of pig liver COMT has not been as well established as rat and human COMT,[13] both of which have very similar protein sequences.

In order to investigate the effect of having electron withdrawing groups on the catechol rings, we synthesized a series of bifunctional inhibitors of varying length that incorporated nitro group substitutions at the 5 and 5′ positions of the catechol rings (**1**–**4**) (Table 1). On the basis of the results from using these bis-nitrocatechols as COMT inhibitors, a second series of unsymmetrical bifunctional compounds were also synthesized (**5**–**8**). These inhibitors were composite of a nitrocatechol and phenol linked by amide bonds to a variable length spacer group.

2. Chemistry

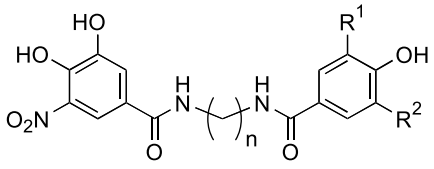
3,4-Dimethoxybenzoyl chloride was prepared from vanillin in a straightforward four-step process (Scheme 1). Vanillin was nitrated using a mixture of acetic acid and nitric acid to give 5-nitrovanillin (**9**).[6] Methylation of the hydroxy group of 5-nitrovanillin using a two-phase system containing the methylating reagent dimethylsulfate gave 3,4-dimethoxy-5-nitrobenzaldehyde (**10**).[14] The aldehyde was then oxidized to the corresponding carboxylic acid (**11**) using acidified chromium trioxide. Finally, the carboxylic acid was converted into the corresponding acid chloride (**12**) using phosphorus pentachloride.

To make the methylated precursors (**13**–**16**) of the diamides **1**–**4**, 3,4-dimethoxy-5-nitrobenzoyl chloride was coupled to a set of variable length diamines, ranging from 2 to 5 methylene units. The methyl groups were then removed by treatment with boron tribromide to give the bis-nitrocatechols (**1**–**4**).[15] To make the diamides **5** and **6**, a three step synthesis was used (Scheme 2). Anisoyl chloride was reacted with an excess of 1,2-diamino ethane and 1,3-diaminopropane to form the monoacylated diamines **17** and **18**, respectively.[16] The monoacylated diamines were then reacted with 3,4-dimethoxy-5-nitrobenzoylchloride to give compounds **19** and **20**. These diamides were demethylated using boron tribromide to give the first two compounds (**5** and **6**) that make up the phenol–catechol conjugate inhibitor series.

The formation of the butyl and pentyl phenol–catechol conjugate inhibitors (**7** and **8**) via the synthesis of mono-acylated diamines was impractical due to the high cost of the diamines and the low yields of the monoacylated diamines. Consequently, an alternative strategy was used to synthesize compounds **7** and **8** (Scheme 3).

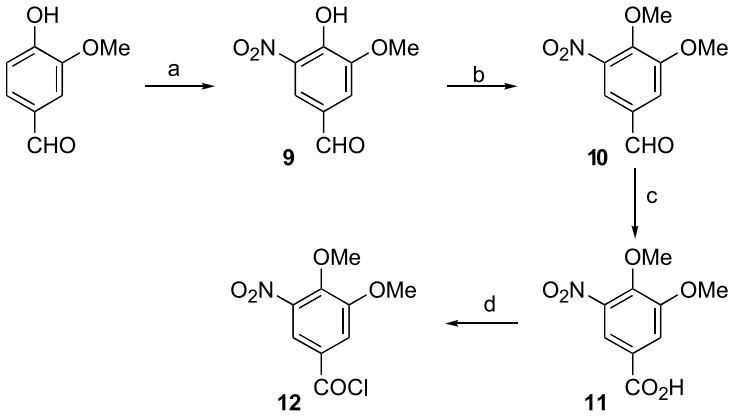
3,4-Dimethoxy-5-nitrobenzoylchloride was reacted with 4-aminobutanol and 5-aminopentanol to give compounds **21** and **22**, respectively. The hydroxy groups of these amide compounds were converted into amino groups via a three-step process. Compounds **21** and **22** were transformed into tosylate esters (**23** and **24**) which were then converted into the corresponding phthalamides **25** and **26**. Removal of the phthaloyl groups with hydrazine hydrate gave the monoacylated diamines,

Table 1. Structure, COMT inhibitory pattern, and kinetic inhibition constants of the compounds synthesized

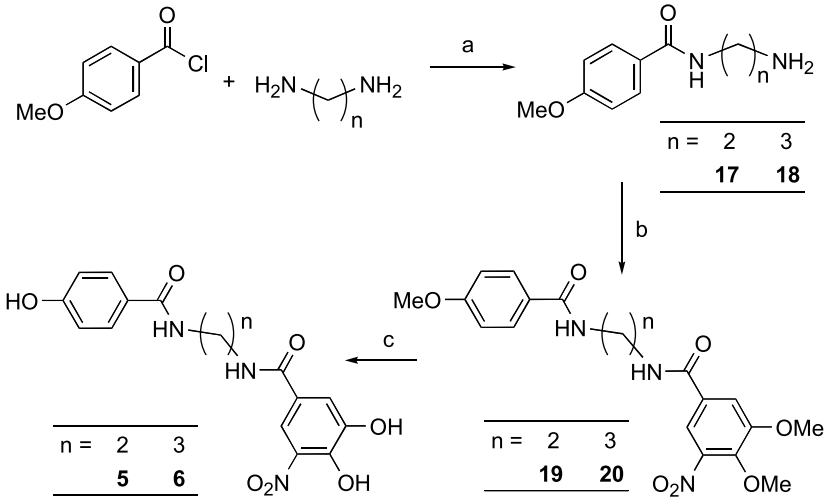


Compound	R^1	R^2	*n*	Inhibition pattern	Kinetic inhibition constant, K_i (μM)
1	NO_2	OH	2	C[a]	3.48 ± 0.30
2	NO_2	OH	3	C	5.47 ± 1.08
3	NO_2	OH	4	NC[b]	3.94 ± 0.60
4	NO_2	OH	5	NC	4.33 ± 0.76
5	H	H	2	C	0.64 ± 0.15
6	H	H	3	C	0.77 ± 0.03
7	H	H	4	C	11.8 ± 0.60
8	H	H	5	C	0.97 ± 0.06

[a] Competitive mode of inhibition.
[b] Noncompetitive mode of inhibition.



Scheme 1. Reagents: (a) HNO_3, AcOH; (b) Me_2SO_4, Bu_4NBr, NaOH, H_2O/CH_2Cl_2; (c) aqueous H_2SO_4, CrO_3; (d) PCl_5.



Scheme 2. Reagents: (a) CH_2Cl_2; (b) 12, K_2CO_3, H_2O/EtOAc; (c) BBr_3.

which were in turn reacted with anisoyl chloride to give the diamide products **27** and **28**. Removal of the methyl groups by treatment with boron tribromide gave the desired compounds **7** and **8**.

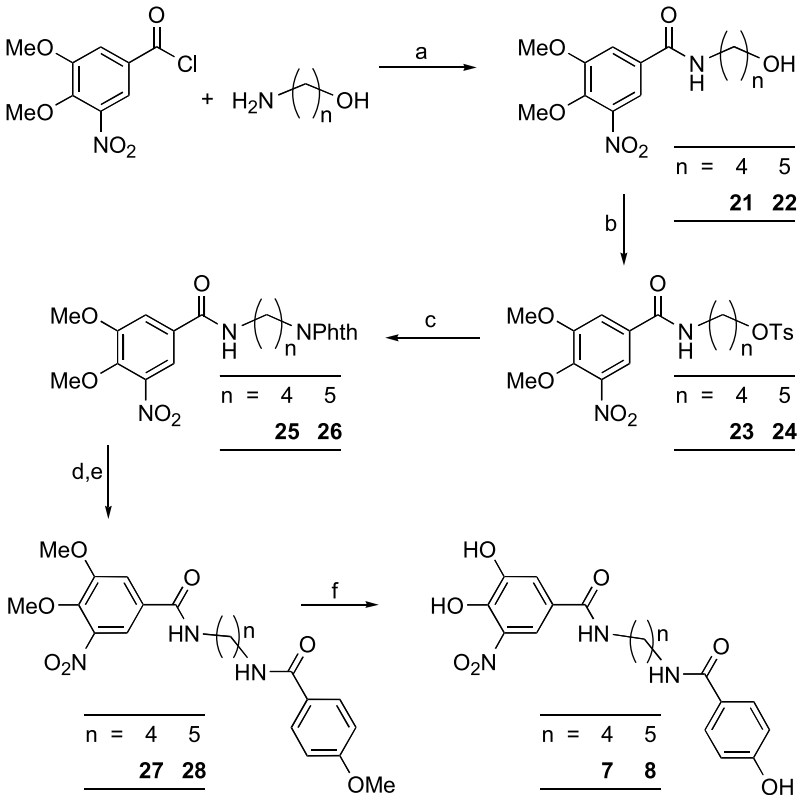
The monofunctional inhibitors (**31** and **32**) (Fig. 4) that were used for comparison were made by reacting 3,4-dimethoxy-5-nitrobenzoylchloride with methylamine and ethylamine to give the corresponding amides (**29** and **30**), which were then treated with boron tribromide to give the final products.

3. Biological results

Initial observations showed that the inhibitors tested in this study with pig liver COMT do not exhibit the tight binding behaviour usually associated with nitrocatechol compounds in human and rat COMT. A lower affinity of pig COMT relative to the human and rat enzyme of ~10- to 100-fold has been reported for nitrocatechol derivatives.[17] Accordingly, K_i values were derived using double reciprocal plots of $1/V$ versus 1/[S] at different inhibitor concentrations. Figure 3 shows a double reciprocal plot that is representative of the plots we obtained.

The K_i values for the bis-nitrocatechol inhibitors (**1**–**4**) range from 3.48 to 5.47 μM. The length of the variable spacer group does not contribute to any trend in the potency of inhibition. In comparison, the monofunctional analogues **31** and **32** gave K_i values of 2.56 and 3.41 μM, respectively (see Table 2), which are within the same order of magnitude as the K_i values obtained with the bis-nitrocatechol inhibitors. This would suggest that the additional functionality of the bis-nitrocatechol inhibitors **1**–**4** played no significant part in enhancing the potency of inhibition. However, compounds **3** and **4** showed noncompetitive modes of inhibition with respect to the catechol substrate, whereas the monofunctional analogues **31** and **32** and the bifunctional compounds **1** and **2** were competitive inhibitors. Presumably, the longer tethers in compounds **3** and **4** alter the interaction of the inhibitor with the enzyme. It was hoped that the bis-nitrocatechol inhibitors would show an increase in potency of at least one order of magnitude greater that the monofunctional inhibitors, because this increase in binding was observed for the bis-catechol compound **33** when compared to its monofunctional analogue **34**.

It was postulated that for inhibitor **33**, one end of the bifunctional inhibitor binds to the active site of COMT,



Scheme 3. Reagents: (a) triethylamine, MeCN; (b) tosyl chloride, pyridine; (c) potassium phthalamide, DMSO; (d) hydrazine hydrate, EtOH; (e) anisoyl chloride, K_2CO_3, H_2O/EtOAc; (f) BBr_3.

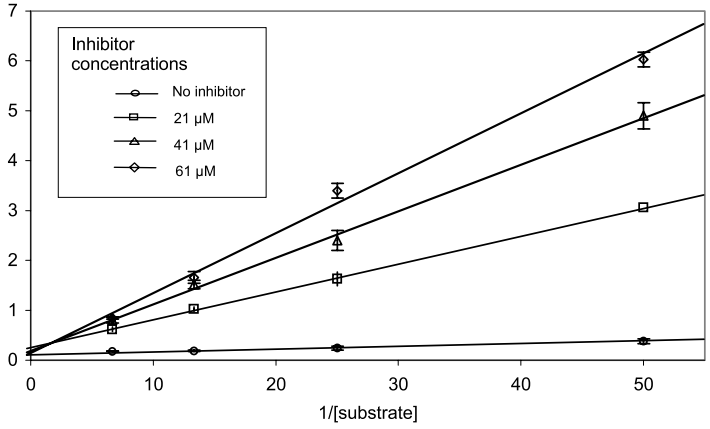


Figure 3. Burke–Lineweaver plot for inhibitor **2**.

Table 2. Structure, inhibition pattern, and kinetic inhibition constants of the monofunctional derivatives

Compound	R^1	Inhibition pattern	Kinetic inhibition constant, K_i (μM)
31	Me	C[a]	2.56 ± 0.67
32	Et	C	3.41 ± 0.68

whereas the other end interacts with some other part of the enzyme. Binding at the active site and the magnitude of the secondary interaction was optimal when the spacer group was three methylene units long. In the case of the bis-nitrocatechol inhibitors (**1**–**4**), there was no correlation between the spacer length and the degree of inhibition. This could suggest that while one end of the bifunctional inhibitor binds to the active site of COMT, the additional nitrocatechol functionality does not interact at all with the proposed secondary binding site. The large reported increase in binding observed for bifunctional inhibitor **33** when compared to the monofunctional inhibitor **34**[12] would suggest that a secondary binding is taking place, but this interaction is more favorable between the secondary binding site and an unsubstituted catechol. As this interaction is not evident with a nitrocatechol, the pK_a (and hence

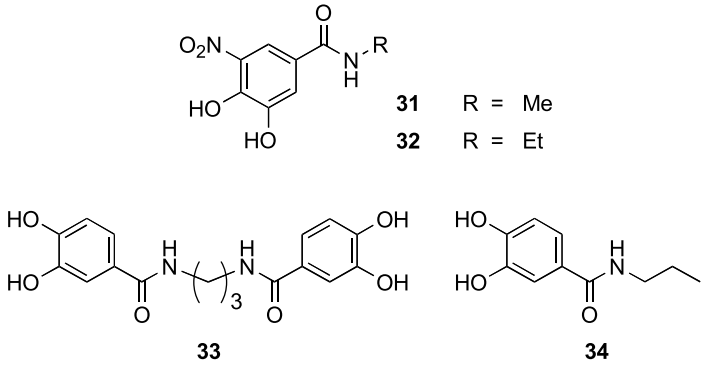


Figure 4.

ionization state) of the hydroxy groups may be the important factor that determines the magnitude of this secondary interaction. It is likely that an appropriate pK_a of the 4-hydroxy group of catechol is required for binding at the secondary site, as this hydroxy group would experience the largest change in pK_a with the incorporation of a nitro group at the 5-position. Hence, it was postulated that a nitrocatechol group at the end of a bis-nitrocatechol inhibitor could be replaced with a phenol to produce a better binding molecule. To test the validity of this theory, a new group of unsymmetrical inhibitors (**5**–**8**), as discussed earlier, were made and evaluated for inhibitory strength. Three of these phenol–catechol conjugate inhibitors (**5**, **6**, and **8**) had K_i values in the 100 nM range. As these three compounds gave values that were almost one order of magnitude lower than the bis-nitrocatechol series **1**–**4**, the proposed secondary binding interaction may be responsible for the increase in potency. Interestingly, the butyl linked inhibitor **7** has the highest K_i of all the inhibitors tested. This is perhaps due to unfavorable interactions of the butyl linker with the enzyme, which outweighs or disallows the secondary binding interaction.

Although this study has provided new insight to the enhanced binding observed with these bifunctional inhibitors, further work would be required to fully elucidate the nature of the secondary interaction.

4. Experimental

4.1. COMT assay

The activity of pig liver COMT was measured using a method described in a previous paper.[18] Accordingly, the activity of COMT was measured by determining the summed amount of the dyes 4-[(4-hydroxy-3-methoxyphenyl)azo]benzenesulfonate and 4-[(3-hydroxy-4-methoxyphenyl)azo]benzenesulfonate formed over time using HPLC with visible light detection. The buffer used in the COMT assays consisted of 0.2 M NaH_2PO_4 and 5 mM $MgCl_2{\cdot}6H_2O$ with the pH adjusted to 7.45. All incubation mixtures were made up to a total volume of 60 μL, each consisting of 6 μL of 20 mM AdoMet in buffer solution; 48 μL of COMT [125 U/mL, where one unit is defined as the methylation of 1 nmol of protocatechuic acid per hour at 37 °C using AdoMet as the methyl donor (Sigma^{TM})] in buffer solution; 3 μL of a stock solution of catechol dye (0.5–150 μM) and 3 μL of inhibitor (0–21 μM) in buffer solution. The mixture was incubated for 1 h at 37 °C, stopped by the addition of 20 μL of 4 M perchloric acid, and then diluted with 200 μL of buffer. An aliquot of 30 μL of the assay mixture was injected into a Microsorb-MV column (C18, 5 mm particle size, 4.6×250 mm^2), and the components of the mixture were eluted with a mobile phase which consisted of water/MeOH/trifluoroacetic acid (30:70:0.2, v/v/v), at a flow rate of 0.45 mL/min. Detection was at 370 nm, and the products, 4-[(4-hydroxy-3-methoxyphenyl)azo]benzenesulfonate and 4-[(3-hydroxy-4-methoxyphenyl)azo]benzenesulfonate, had retention times of 8.2 and 8.9 min, respectively. The enzyme activity was calculated as nanomole of product per minute per milligram of protein. Assays were performed in duplicates, and each assay had two 30 μL aliquots analysed. Conversion of substrate to product was less than 5%. The concentrations of AdoMet and Mg^{2+} used were saturating, and the product formation was linear with respect to protein concentration and reaction time. Bifunctional inhibitors were tested at concentrations up to 21 μM. Compounds which exhibited inhibitory activity less than the standard deviation, at this concentration, were classified as having no inhibitory action.

4.2. Analysis of kinetic data

All kinetic data were analyzed graphically using double reciprocal plots, where the reciprocal velocities versus the reciprocal substrate concentrations gave a linear relationship by which K_m and V_{max} could be calculated. Inhibition constants for competitive and noncompetitive inhibition were calculated from data fitting to equations $v = V_{max}[S]/(K_m(1 + [I]/K_i) + [S])$ and $v = V_{max}[S]/(([S] + K_m)(1 + [I]/K_i))$, respectively. Standard deviations were obtained from fitting all available data for a given compound. All calculations were performed with Microsoft Excel (Redmond, WA, USA).

4.3. Chemical methods

Elemental analyses were performed at the Campbell Microanalytical laboratory, University of Otago. The analytical results obtained were within ±0.4% of the

theoretical values. ^{1}H NMR spectra were measured on a Varian INOVA-300 MHz spectrometer. The signals are given in ppm relative to tetramethylsilane (TMS), HOD, CHD_2SOCD_3 or CHD_2COCD_3 as internal standards in $CDCl_3$, D_2O, DMSO-d_6 and acetone-d_6, respectively. The peak patterns are shown with the following abbreviations: br, broad; d, doublet; m, multiplet; t, triplet; q, quartet. Column chromatography was performed with silica gel (Sorbsil, particle size 32–63 mM). AdoMet as the *p*-toluenesulfonate salt, and pig liver COMT were purchased from Sigma. Boron tribromide, 1,2-diaminoethane, 1,3-diaminopropane, 1,4-diaminobutane and 1,5-diaminopentane, 4-aminobutanol and 5-aminopropanol were purchased from Aldrich. Solvent and inorganic bases were generally sourced.

4.4. General procedure for the preparation of *N*,*N*′-bis(3,4-dihydroxy-5- nitrobenzamide) derivatives

BBr_3 (15 mol equiv) was added to a cooled (0–5 °C) solution of the appropriate *N*,*N*′-bis(3,4-dimethoxy-5-nitrobenzamide) derivative suspended in CH_2Cl_2. The reaction was left to stir overnight and then water was carefully added to eliminate any unreacted BBr_3. The resulting yellow precipitate was removed by filtration and recrystallized from the appropriate solvent.

4.4.1. *N*,*N*′-1,2-Ethanediylbis(3,4-dihydroxy-5-nitrobenzamide) (1). The ethyl derivative was prepared from *N*,*N*′-1,2-ethanediylbis-(3,4-dimethoxy-5-nitro-benzamide) (**13**) (1 g, 2.1 mmol) and BBr_3 (2 mL) using the procedure described earlier. Recrystallization from methanol and water gave the product as small yellow crystals (0.84 g, 81%): mp 248–251 °C; ^{1}H NMR (acetone-d_6) δ 9.82 (4H, br s) 8.27 (2H, br t), 8.19 (2H, d, J = 2.1 Hz), 7.79 (2H, d, J = 2.1 Hz), 3.67 (4H, br s). Anal. Calcd for $C_{16}H_{14}N_4O_{10}\cdot 4H_2O$: C, 38.87; H, 4.48; N, 11.36. Found: C, 38.92; H, 4.50; N, 11.06.

4.4.2. *N*,*N*′-1,3-Propanediylbis(3,4-dihydroxy-5-nitrobenzamide) (2). The propyl derivative was prepared from *N*,*N*′-1,3-propanediylbis-(3,4-dimethoxy-5-nitro-benzamide) (**14**) (1 g, 2 mmol) and BBr_3 (2 mL) using the procedure described above. Recrystallization from ethanol and water gave the product as a bright yellow powder (0.48 g, 54%): mp 185–180 °C (dec); ^{1}H NMR (acetone-d_6) δ 8.23 (2H, d, J = 1.8 Hz), 8.19 (2H, br t), 7.85 (2H, d, J = 1.8 Hz), 3.59 (4H, dt, J = 3.6, 6.0 Hz), 1.92 (2H, quint, J = 6.0 Hz). Anal. Calcd for $C_{17}H_{16}N_4O_{10}$: C, 46.79; H, 3.70; N, 12.84. Found: C, 46.79; H, 3.78; N, 12.62.

4.4.3. *N*,*N*′-1,4-Butanediylbis(3,4-dihydroxy-5-nitrobenzamide) (3). The butyl derivative was prepared from *N*,*N*′-1,4-butanediylbis-(3,4-dimethoxy-5-nitro-benzamide) (**15**) (1 g, 2 mmol) and BBr_3 (2 mL) using the procedure described above. Recrystallization from ethanol and water gave the product as small orange crystals (0.62 g, 69%): mp 260–285 °C (dec); ^{1}H NMR (acetone-d_6) δ 8.18 (2H, d, J = 2.1 Hz), 8.06 (2H, br t), 7.81 (2H, d, J = 2.1 Hz), 3.67 (4H, m), 1.74 (4H, m). Anal. Calcd for $C_{18}H_{18}N_4O_{10}\cdot 0.25H_2O$: C, 47.52; H, 4.09; N, 12.32. Found C, 47.71; H, 3.95; N, 11.98.

4.4.4. *N*,*N*′-1,5-Pentanediylbis(3,4-dihydroxy-5-nitrobenzamide) (4). The pentyl derivative was prepared from *N*,*N*′-1,5-pentanediylbis-(3,4-dimethoxy-5-nitro-benzamide) (**16**) (1 g, 1.9 mmol) and BBr_3 (20 mL) using the procedure described above. Recrystallization from water and ethanol gave the product as flaky orange crystals (0.24 g, 26%): mp 226–228 °C; ^{1}H NMR (acetone-d_6) δ 8.17 (2H, d, J = 2.1 Hz), 7.96 (2H, br t), 1.51 (2H, m), 7.78 (2H, d, J = 2.1 Hz), 3.45 (4H, q, J = 6.9 Hz), 1.71 (4H, quint, J = 6.9 Hz). Anal. Calcd for $C_{19}H_{20}N_4O_{10}$: C, 49.14; H, 4.34; N, 12.07. Found: C, 49.44; H, 4.58; N, 11.76.

4.4.5. *N*-[2-(4-Hydroxybenzoylamino)ethyl]-3,4-dihydroxy-5-nitrobenzamide (5). An excess of BBr_3 (1.7 mL, 15 mol equiv) was added to a solution of *N*-[2-(4-methoxybenzoylamino)ethyl]-3,4-dimethoxy-5-nitrobenzamide (**19**) (0.5 g, 1.2 mmol) in dried CH_2Cl_2 (50 mL) under N_2. The mixture was left stirring for 24 h at room temperature. Water was carefully added to eliminate any unreacted BBr_3 and then removed along with the CH_2Cl_2 by rotary evaporation under reduced pressure to yield the crude product. The product was purified by recrystallization from acetone and water solution to give the final product as small yellow crystals (0.21 g, 48%): mp 268–270 °C (dec); ^{1}H NMR (acetone-d_6) δ 8.30 (1H, br s), 8.20 (1H, d, J = 2.1 Hz), 7.96 (1H, br s), 7.84 (2H, d, J = 8.7 Hz), 7.77 (1H, d, J = 2.1 Hz), 6.91 (2H, d, J = 8.7 Hz), 3.63 (4H, m). Anal. Calcd for $C_{16}H_{15}N_3O_7$: C, 53.18; H, 4.19; N, 11.63. Found: C, 53.23; H, 4.09; N, 11.55.

4.4.6. *N*-[3-(4-Hydroxybenzoylamino)propyl]-3,4-dihydroxy-5-nitrobenzamide (6). *N*-[3-(4-Methoxybenzoylamino)propyl]-3,4-dimethoxy-5-nitrobenzamide (**20**) (0.5 g, 1.2 mmol) was used in the BBr_3 procedure described above. The resulting crude material was recrystallized from water to give the final product as bright yellow crystals (0.27 g, 60%): mp 236–239 °C (dec); ^{1}H NMR (acetone-d_6) δ 8.31 (1H, br s), 8.25 (1H, d, J = 2.1 Hz), 7.92 (1H, br s), 7.87 (2H, d, J = 8.7 Hz), 7.83 (1H, d, J = 2.1 Hz), 6.92 (2H, d, J = 8.7 Hz), 3.52 (4H, m), 1.86 (2H, m). Anal. Calcd for $C_{17}H_{17}N_3O_7$: C, 54.25; H, 4.82; N, 11.17. Found: C, 54.52; H, 4.58; N, 11.14.

4.4.7. *N*-[4-(4-Hydroxybenzoylamino)butyl]-3,4-dihydroxy-5-nitrobenzamide (7). *N*-[4-(4-Methoxybenzoylamino)butyl]-3,4-dimethoxy-5-nitrobenzamide (**21**) (0.5 g, 1.2 mmol) was used in the BBr_3 procedure described above. The resulting crude material was recrystallized from methanol and water to give the final product as chunky orange/brown crystals (0.09 g, 20%): mp 251–255 °C (dec); ^{1}H NMR (acetone-d_6) δ 8.17 (1H, d, J = 2.1 Hz), 8.01 (1H, br s), 7.84 (2H, d, J = 9.0 Hz), 7.71 (1H, d, J = 2.1 Hz), 7.65 (1H, br s), 6.89 (2H, d, J = 9.0 Hz), 3.47 (4H, m), 1.71 (4H, m). Anal. Calcd for $C_{18}H_{19}N_3O_7$: C, 55.52; H, 4.92; N, 10.79. Found: C, 55.43; H, 4.77; N, 10.75.

4.4.8. *N*-[5-(4-Hydroxybenzoylamino)pentyl]-3,4-dihydroxy-5-nitrobenzamide (8). *N*-[5-(4-Methoxybenzoylamino)pentyl]-3,4-dimethoxy-5-nitrobenzamide (**22**) (0.5 g, 1.1 mmol) was used in the BBr_3 procedure

described above. The resulting crude material was recrystallized from methanol and water to give the final product as bunched orange crystals (0.15 g, 31%): mp 107–110 °C; ^{1}H NMR (acetone-d_6) δ 10.57 (1H, s), 9.16 (1H, s), 8.94 (1H, s), 8.18 (1H, d, J = 1.8 Hz), 8.02 (1H, t, J = 5.9 Hz), 7.82 (1H, d, J = 1.8 Hz), 7.80 (2H, d, J = 8.7 Hz), 7.63 (1H, t, J = 5.9 Hz), 6.88 (2H, d, J = 8.7 Hz), 3.43 (4H, m), 1.68 (4H, quint, J = 7.2 Hz), 1.51 (2H, quint, J = 7.2 Hz). Anal. Calcd for $C_{19}H_{21}N_3O_7 \cdot 1 \cdot 5H_2O$: C, 53.02; H, 5.62; N, 9.76. Found: C, 53.36; H, 5.74; N, 9.63.

4.4.9. 4-Hydroxy-3-methoxy-5-nitrobenzaldehyde (9). Fuming HNO_3 (2 mL) was carefully added to a cooled (5 °C) solution of vanillin (5 g, 33 mmol) and acetic acid (50 mL) over a period of 30 min. The gold colored precipitate that formed was filtered, washed with water, and allowed to dry (5.21 g, 80%): mp 171 °C; ^{1}H NMR ($CDCl_3$) δ 9.88 (1H, s), 8.17 (1H, d, J = 1.8 Hz), 7.61 (1H, d, J = 1.8 Hz), 4.01 (3H, s). Anal. Calcd for $C_8H_7NO_5$: C, 48.73; H, 3.58; N, 7.11. Found: C, 48.64; H, 3.31; N, 7.32.

4.4.10. 3,4-Dimethoxy-5-nitrobenzaldehyde dihydrate (10). A mixture of CH_2Cl_2 (67.5 mL), water (67.5 mL), 4-hydroxy-3-methoxy-5-nitrobenzaldehyde (5.0 g, 25 mmol), sodium hydroxide (2 g, 50 mmol), tetrabutylammonium bromide (0.81 g, 2.5 mmol), and dimethylsulfate (12.5 mL, 132 mmol) were mixed together and stirred vigorously for 24 h under a nitrogen atmosphere. The aqueous layer was separated and washed with CH_2Cl_2. Rotary evaporation under reduced pressure of the pooled CH_2Cl_2 fractions gave a yellow oil which was redissolved in diethyl ether. The ether was washed with water, 2 M ammonia solution, and 2 M NaOH solution to remove unreacted phenol and dimethylsulfate. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) ether solution gave a brown oil that solidified on cooling. Purification by recrystallization from acetone and water gave the product as long needle-like cream colored crystals (5.21 g, 84%): mp 62–65 °C; ^{1}H NMR ($CDCl_3$) δ 9.92 (1H, s), 7.84 (1H, d, J = 1.9 Hz), 7.63 (1H, d, J = 1.9 Hz), 4.09 (3H, s), 4.01 (3H, s). Anal. Calcd for $C_9H_9NO_5 \cdot 2H_2O$: C, 43.72; H, 5.30; N, 5.67. Found: C, 43.47; H, 4.97; N, 5.56.

4.4.11. 3,4-Dimethoxy-5-nitrobenzoic acid (11). A solution of CrO_3 (6 g, 60 mmol), concentrated H_2SO_4 (5 mL), and water (100 mL) was added drop-wise to a stirring solution of 3,4-dimethoxy-5-nitrobenzaldehyde (10 g, 47 mmol), acetone (100 mL), and water (100 mL). The solution was stirred for a further 24 h and then isopropanol was added to eliminate any unreacted Cr(VI) species. Rotary evaporation, under reduced pressure, of the acetone and water mixture gave the crude product as a green sludge. The crude product was extracted into ethyl acetate and washed with 1 M HCl to remove any remaining Cr(III) species. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) ethyl acetate solution gave the crude product. Purification by recrystallization from water and ethanol gave the final product as long needle-like white crystals (7.4 g, 69%): mp 194–195 °C; ^{1}H NMR ($CDCl_3$) δ 8.10 (1H, d, J = 2.0), 7.81 (1H, d, J = 2.0), 4.08 (3H, s), 4.01 (3H, s). Anal. Calcd for $C_9H_9NO_6$: C, 47.58; H, 3.99; N, 6.17. Found: C, 47.58; H, 3.96; N, 6.20.

4.4.12. 3,4-Dimethoxy-5-nitrobenzoylchloride (12). 3,4-Dimethoxy-5-nitrobenzoic acid (5.2 g, 23 mmol), PCl_5 (9.5 g, 45 mmol) and CH_2Cl_2 (60 mL) were carefully mixed together and stirred overnight. The CH_2Cl_2 was quickly washed with cold water and then dried with $MgSO_4$. Rotary evaporation under reduced pressure of the CH_2Cl_2 solution gave the product as a cream colored solid (4.95 g, 88%): mp 68–70 °C; ^{1}H NMR ($CDCl_3$) δ 8.14 (1H, d, J = 2.2 Hz), 7.74 (1H, d, J = 2.2 Hz), 4.09 (3H, s), 4.01 (3H, s).

4.5. General procedure for the synthesis of *N*,*N*′-bis(3,4-dimethoxy-5-nitrobenzamide) derivatives

Freshly prepared 3,4-dimethoxy-5-nitrobenzoyl chloride (10 g scale) and potassium carbonate (1.2 mol equiv) was dissolved in H_2O and ethyl acetate (1:1, 350 mL total). The appropriate amount of diamine (0.45 mol equiv) was introduced into the acid chloride mixture and stirred overnight. The product precipitated out during the course of the reaction.

4.5.1. *N*,*N*′-1,2-Ethanediylbis(3,4-dimethoxy-5-nitrobenzamide) (13). The ethyl derivative was prepared from 3,4-dimethoxy-5-nitrobenzoyl chloride (10 g, 40 mmol), K_2CO_3 (6.8 g, 1.2 mol equiv) and 1,2-diaminoethane (1.2 g, 20 mmol) using the procedure described above. Recrystallization from methanol and DMSO gave the product as small white crystals (5.41 g, 57%): mp 235 °C; ^{1}H NMR ($CDCl_3$) δ 8.33 (2H, br t), 7.61 (2H, d, J = 1.9 Hz), 7.47 (2H, d, J = 1.9 Hz), 3.68 (6H, s), 3.66 (6H, s), 3.30 (4H, m). Anal. Calcd for $C_{20}H_{22}N_4O_{10}$: C, 50.21; H, 4.64; N, 11.71. Found: C, 50.16; H, 4.52; N, 11.60.

4.5.2. *N*,*N*′-1,3-Propanediylbis(3,4-dimethoxy-5-nitrobenzamide) (14). The propyl derivative was prepared from 3,4-dimethoxy-5-nitrobenzoyl chloride (10 g, 40 mmol), K_2CO_3 (6.8 g, 1.2 mol equiv) and 1,3-diaminopropane (1.48 g, 20 mmol) using the procedure described above. Recrystallization from methanol gave the product as long needle-like crystals (2.36 g, 24%): mp 200–201 °C; ^{1}H NMR ($CDCl_3$) δ 7.77 (2H, d, J = 2.0 Hz), 7.74 (2H, d, J = 2.0 Hz), 7.28 (2H, t, J = 5.9 Hz), 4.01 (6H, s), 3.99 (6H, s), 3.53 (4H, quint, J = 5.9 Hz), 1.87 (2H, quint, J = 5.9 Hz). Anal. Calcd for $C_{21}H_{24}N_4O_{10}$: C, 51.22; H, 4.91; N. 11.38. Found: C, 51.35; H, 4.89; N, 11.44.

4.5.3. *N*,*N*′-1,4-Butanediylbis(3,4-dimethoxy-5-nitrobenzamide) (15). The butyl derivative was prepared from 3,4-dimethoxy-5-nitrobenzoyl chloride (10 g, 40 mmol), K_2CO_3 (6.8 g, 1.2 mol equiv) and 1,4-diaminobutane (1.76 g, 20 mmol) using the procedure described above. Recrystallization from methanol and DMSO gave the product as a white crystalline powder (5.15 g, 51%): mp 230–231 °C; ^{1}H NMR (DMSO-d_6) δ 8.14 (2H, t, J = 5.6 Hz), 7.83 (2H, d, J = 2.0 Hz), 7.66 (2H, d, J = 2.0 Hz), 3.86 (6H, s), 3.84 (6H, s), 3.33 (4H, quint,

J = 5.6 Hz), 1.57 (4H, m). Anal. Calcd for $C_{22}H_{26}N_4O_{10}$: C, 52.17; H, 5.17; N, 11.07. Found: C, 52.13; H, 5.26; N, 10.89.

4.5.4. *N*,*N*′-1,5-Pentanediylbis(3,4-dimethoxy-5-nitrobenzamide) (16). The pentyl derivative was prepared from 3,4-dimethoxy-5-nitrobenzoyl chloride (10 g, 40 mmol), K_2CO_3 (6.8 g, 1.2 mol equiv) and 1,5-diaminopentane (2.04 g, 20 mmol) using the procedure described above. Recrystallization from methanol gave the product as long needle-like crystals (4.79 g, 46%): mp 198–199 °C; ^{1}H NMR ($CDCl_3$) δ 7.63 (2H, d, J = 2.0 Hz), 7.61 (2H, d, J = 2.0 Hz), 6.34 (2H, t, J = 6.4 Hz), 3.99 (6H, s), 3.94 (6H, s), 3.45 (4H, quint, J = 6.4 Hz), 1.70 (4H, quint, J = 6.4 Hz), 1.49 (2H, quint, J = 6.4 Hz). Anal. Calcd for $C_{23}H_{28}N_4O_{10}$: C, 53.07; H, 5.42; N, 10.77. Found: C, 52.82; H, 5.38; N, 10.64.

4.5.5. 2-(4-Methoxybenzamide)ethylamine (17). A solution of 4-methoxybenzoylchloride (1 g, 5.7 mmol) and CH_2Cl_2 (100 mL) was added drop-wise into a vigorously stirred and cooled (−78 °C) solution of 1,2-diaminoethane (4 mL) and CH_2Cl_2 (200 mL). The CH_2Cl_2 mixture was washed with 1 M HCl solution to extract the monosubstituted amine and unreacted diamine. The pH of the combined aqueous extracts was adjusted to 14 by the addition of sodium hydroxide pellets and the amine product was extracted into ethyl acetate. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) ethyl acetate solution gave the crude product as an oil, which eventually solidified to a crystalline white solid (0.42 g, 38%): mp 81–85 °C; ^{1}H NMR ($CDCl_3$) δ 7.76 (2H, d, J = 8.8 Hz), 6.91 (2H, d, J = 8.8 Hz), 6.72 (1H, t, J = 5.9 Hz), 3.84 (3H, s), 3.48 (2H, q, J = 5.9 Hz), 2.93 (2H, t, J = 5.9 Hz).

4.5.6. 3-(4-Methoxybenzamide)propylamine (18). A solution of 4-methoxybenzoyl chloride (1 g, 5.7 mmol) and CH_2Cl_2 (100 mL) was added drop-wise into a vigorously stirred and cooled (−78 °C) solution of 1,3-diaminopropane (4 mL) and CH_2Cl_2 (200 mL). The CH_2Cl_2 mixture was washed with 1 M HCl solution to extract the monosubstituted amine and unreacted diamine. The pH of the combined aqueous extract was adjusted to 14 by the addition of sodium hydroxide pellets and the amine product was extracted into ethyl acetate. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) ethyl acetate solution gave the crude product as an oil. Further evaporation of the product under high vacuum gave the product as a crystalline white solid (0.84 g, 71%): mp 89–90 °C; ^{1}H NMR ($CDCl_3$) δ 7.76 (2H, d, J = 8.8 Hz), 7.56 (1H, t, J = 6.0 Hz), 6.91 (2H, d, J = 8.84 Hz), 3.85 (3H, s), 3.57 (2H, q, J = 6.0 Hz), 2.91 (2H, t, J = 6.0 Hz), 1.74 (2H, quint, J = 6.0 Hz).

4.5.7. *N*-[2-(4-Methoxybenzoylamino)ethyl]-3,4-dimethoxy-5-nitrobenzamide (19). Freshly prepared 3,4-dimethoxy-5-nitrobenzoyl chloride (1.5 g, 6.1 mmol) and potassium carbonate (0.84 g, 6.1 mmol) were dissolved in a two phase system consisting of H_2O (20 mL) and ethyl acetate (20 mL). 2-(4-Methoxybenzamide)ethylamine (1 g, 5.1 mmol) was added to the solution which was stirred overnight. The precipitate that formed was removed by filtration and recrystallized from water and ethanol to give the product as small white crystals (0.67 g, 27%): mp 177 °C; ^{1}H NMR (DMSO-d_6) δ 8.87 (1H, s), 8.52 (1H, s), 7.94 (1H, d, J = 2.1 Hz), 7.87 (2H, d, J = 8.8 Hz), 7.84 (1H, d, J = 2.1 Hz), 7.51 (2H, d, J = 8.8 Hz), 4.00 (3H, s), 3.95 (3H, s), 3.84 (3H, s), 3.48 (4H, br s). Anal. Calcd for $C_{19}H_{21}N_3O_7$: C, 56.57; H, 5.25; N, 10.42. Found: C, 56.53; H, 5.13; N, 10.19.

4.5.8. *N*-[3-(4-Methoxybenzoylamino)propyl]-3,4-dimethoxy-5-nitrobenzamide (20). Freshly prepared 3,4-dimethoxy-5-nitrobenzoyl chloride (1.42 g, 5.8 mmol) and potassium carbonate (0.80 g, 5.8 mmol) were dissolved into a two phase system consisting of H_2O (30 mL) and ethyl acetate (30 mL). 3-(4-Methoxybenzamide)propylamine (1 g, 4.8 mmol) was added to the solution which was then stirred overnight. The crude product was extracted with ethyl acetate and washed with 1 M NaOH to remove any carboxylate byproduct. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) ethyl acetate solution gave the crude product, which was purified by recrystallization from water and ethanol to give the final product as small white flaky crystals (0.83 g, 34%): mp 139 °C; ^{1}H NMR ($CDCl_3$) δ 7.89 (1H, d, J = 2.0 Hz), 7.83 (1H, t, J = 6.0 Hz), 7.78 (2H, d, J = 8.8 Hz), 7.77 (1H, d, J = 2.0 Hz), 6.94 (2H, d, J = 8.8 Hz), 6.70 (1H, t, J = 6.0 Hz), 4.01 (3H, s), 4.00 (3H, s), 3.86 (3H, s), 3.58 (2H, q, J = 6.0 Hz), 3.53 (2H, q, J = 6.0 Hz), 1.84 (2H, quint, J = 6.0 Hz). Anal. Calcd for $C_{20}H_{23}N_3O_7$: C, 57.55; H, 5.55; N, 10.07. Found: C, 57.32; H, 5.52; N, 10.03.

4.5.9. 4-(3,4-Dimethoxy-5-nitrobenzamide)butan-1-ol (21). 3,4-Methoxy-5-nitrobenzoyl chloride (3 g, 12.2 mmol), triethylamine (3 mL), 4-aminobutanol (1.25 g, 14 mmol) and acetonitrile (50 mL) were mixed together and heated under reflux for 5 h. Removal of the solvent by rotary evaporation under reduced pressure gave the crude product, which was then extracted into ethyl acetate. The ethyl acetate was washed with 10% aqueous HCl and 2 M NaOH to remove triethylamine and carboxylate byproducts. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) ethyl acetate solution gave the crude product, which was purified by column chromatography (eluting with chloroform/hexane 1:1) and then recrystallized from chloroform to give the final product as a white crystalline powder (2.84 g, 78%): mp 91–93 °C; ^{1}H NMR ($CDCl_3$) δ 7.73 (1H, d, J = 2.1 Hz), 7.68 (1H, d, J = 2.1 Hz), 6.88 (1H, t, J = 5.9 Hz), 4.80 (1H, br s), 4.04 (3H, s), 4.00 (3H, s), 3.78 (2H, t, J = 5.9 Hz), 3.52 (2H, q, J = 5.9 Hz), 1.74 (4H, m). Anal. Calcd for $C_{13}H_{18}N_2O_6$: C, 52.34; H, 6.08; N, 9.40. Found: C, 52.50; H, 5.89; N, 9.49.

4.5.10. 5-(3,4-Dimethoxy-5-nitrobenzamide)pentan-1-ol (22). 3,4-Methoxy-5-nitrobenzoyl chloride (3 g, 12.2 mmol), triethylamine (3 mL), 5-aminopropanol (1.44 g, 14 mmol) and acetonitrile (50 mL) were mixed together and heated under reflux for 5 h. Removal of the solvent by rotary evaporation under reduced pressure gave the crude product, which was then extracted into ethyl acetate. The ethyl acetate was washed with 10% aqueous HCl and 2 M NaOH to remove triethyl-

amine and carboxylate byproducts. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) ethyl acetate solution gave the crude product, which was purified by column chromatography (eluting with chloroform/hexane 1:1) followed by recrystallization from water and ethanol to give the final product as flaky white crystals (3.09 g, 81%): mp 86–88 °C; ^{1}H NMR ($CDCl_3$) δ 7.68 (1H, d, J = 1.8 Hz), 7.62 (1H, d, J = 1.8 Hz), 6.35 (1H, t, J = 6.6 Hz), 4.02 (3H, s), 3.98 (3H, s), 3.56 (2H, t, J = 6.6 Hz), 3.45 (2H, q, J = 6.6 Hz), 1.84 (2H, quint, J = 6.6 Hz), 1.66 (2H, quint, J = 6.6 Hz), 1.55 (2H, quint, J = 6.6 Hz). Anal. Calcd for $C_{14}H_{20}N_2O_6$: C, 53.84; H, 6.45; N, 8.97. Found: C, 53.90; H, 6.62; N, 8.95.

4.5.11. 4-(3,4-Dimethoxy-5-nitrobenzamide)butan-1-ol, *p*-toluenesulfonate ester (23). To a solution of 4-(3,4-dimethoxy-5-nitrobenzamide)butan-1-ol (**21**) (2.5 g, 8.4 mmol) and dry pyridine (30 mL) cooled to below 5 °C was added *p*-toluenesulfonyl chloride (1.93 g, 10.1 mmol). After stirring for 12 h, the mixture was extracted into CH_2Cl_2 and washed with ice-cold 1 M HCl solution to remove any dissolved pyridine. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) CH_2Cl_2 solution gave the crude product which was purified by column chromatography (eluting with chloroform/hexane 1:2) to give the final product as a white solid (2.04 g, 54%): mp 129 °C; ^{1}H NMR ($CDCl_3$) δ 7.79 (2H, d, J = 8.1 Hz), 7.65 (2H, m), 7.36 (2H, d, J = 8.1 Hz), 6.37 (1H, t, J = 5.8 Hz), 4.10 (2H, t, J = 5.8 Hz), 4.03 (3H, s), 3.99 (3H, s), 3.46 (2H, q, J = 5.8 Hz), 2.45 (3H, s), 1.75 (4H, m). Anal. Calcd for $C_{20}H_{24}N_2SO_8 \cdot 0.5H_2O$: C, 52.05; H, 5.46; N, 6.07; S, 6.95. Found: C, 51.94; H, 5.30; N, 6.10; S, 6.57.

4.5.12. 5-(3,4-Dimethoxy-5-nitrobenzamide)pentan-1-ol, *p*-toluenesulfonate ester (24). To a solution of 4-(3,4-methoxy-5-nitrobenzamide)pentan-1-ol (**22**) (2.5 g, 8.0 mmol) and dry pyridine (30 mL) cooled to below 5 °C was added *p*-toluenesulfonyl chloride (1.83 g, 9.6 mmol). After stirring for 12 h, the mixture was extracted into CH_2Cl_2 and washed with ice-cold 1 M HCl solution to remove any dissolved pyridine. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) CH_2Cl_2 solution gave the crude product, which was purified by column chromatography (eluting with chloroform/hexane 1:2) to give the final product as a clear oil (2.39 g, 64%): ^{1}H NMR ($CDCl_3$) δ 7.79 (2H, d, J = 8.4 Hz), 7.69 (1H, d, J = 2.1 Hz), 7.66 (1H, d, J = 2.1 Hz), 7.36 (2H, d, J = 8.4 Hz), 6.35 (1H, t, J = 6.6 Hz), 4.08 (2H, t, J = 6.6 Hz), 4.04 (3H, s), 4.00 (3H, s), 3.45 (2H, q, J = 6.6 Hz), 2.47 (3H, s), 1.75 (2H, quint, J = 6.6 Hz), 1.65 (2H, m), 1.50 (2H, m).

4.5.13. *N*-(4-Phthalimidobutyl)-3,4-dimethoxy-5-nitrobenzamide (25). 4-(3,4-Dihydroxy-5-nitro-benzamide)butan-1-ol, *p*-toluenesulfonate ester (**23**) (1.48 g, 3.27 mmol), potassium phthalamide (0.73 g, 3.93 mmol) and DMSO (30 mL) were mixed together and heated at 100 °C with stirring for 12 h. Water (150 mL) was added and the mixture stirred until a precipitate formed. The precipitate was extracted with chloroform and washed with saturated salt solution to remove any residual DMSO. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) chloroform solution gave the crude product which was purified by column chromatography (eluting with chloroform/hexane 3:1) to give the final product as a cream colored powder (0.74 g, 53%): mp 144 °C; ^{1}H NMR ($CDCl_3$) δ 7.85 (2H, m), 7.73 (2H, m), 7.72 (H, d, J = 2.0 Hz), 7.71 (H, d, J = 2.0 Hz), 6.63 (H, t, J = 6.8 Hz), 4.02 (3H, s), 3.98 (3H, s), 3.76 (2H, t, J = 6.8 Hz), 3.54 (2H, q, J = 6.8 Hz), 1.76 (4H, m). Anal. Calcd for $C_{21}H_{21}N_3O_7$: C, 59.01; H, 4.95; N, 9.83. Found: C, 58.87; H, 5.04; N, 9.71.

4.5.14. *N*-(5-Phthalimidopentyl)-3,4-dimethoxy-5-nitrobenzamide (26). 5-(3,4-Dimethoxy-5-nitro-benzamide)pentan-1-ol, *p*-toluenesulfonate ester (**24**) (1.8 g, 3.98 mmol), potassium phthalamide (0.88 g, 4.77 mmol) and DMSO (30 mL) were mixed together and heated at 100 °C with stirring for 12 h. Enough water was added to the mixture to allow the formation of a precipitate and then the mixture was reheated until the precipitate dissolved. The pure product crystallized as the mixture cooled (1.61 g, 96%): mp 118–119 °C; ^{1}H NMR ($CDCl_3$) δ 7.83 (2H, m), 7.73 (6H, m), 6.43 (H, t, J = 6.6 Hz), 4.04 (3H, s), 3.99 (3H, s), 3.75 (2H, t, J = 6.6 Hz), 3.49 (2H, q, J = 6.6 Hz), 1.75 (4H, m), 1.45 (2H, quint, J = 6.6 Hz). Anal. Calcd for $C_{22}H_{23}N_3O_7$: C, 59.86; H, 5.25; N, 9.52. Found: C, 59.81; H, 5.30; N, 9.42.

4.5.15. *N*-[4-(4-Methoxybenzoylamino)butyl]-3,4-dimethoxy-5-nitrobenzamide (27). *N*-(4-Phthalimidobutyl)-3,4-dimethoxy-5-nitrobenzamide (**25**) (0.65 g, 1.5 mmol), hydrazine monohydrate (0.22 mL, 4.5 mmol) and ethanol (30 mL) were mixed together and stirred for 12 h. The precipitate that formed was removed by filtration and the ethanol and hydrazine were removed by high vacuum to yield the crude amine. The crude amine was resuspended in a mixture of 4-methoxybenzoyl chloride (0.31 g, 1.8 mmol), potassium carbonate (0.25 g, 1.8 mmol), H_2O (25 mL) and ethyl acetate (25 mL) and stirred overnight. The ethyl acetate mixture was separated and washed with 1 M NaOH to remove any carboxylate byproduct. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) ethyl acetate solution gave the crude product, which was purified by column chromatography (eluting with chloroform) to give the final product as a white powder (0.23 g, 36%): mp 128 °C; ^{1}H NMR ($CDCl_3$) δ 7.93 (1H, d, J = 2.1 Hz), 7.81 (1H, d, J = 2.1 Hz), 7.77 (2H, d, J = 9.0 Hz), 7.51 (1H, t, J = 6.0 Hz), 6.95 (2H, d, J = 9.0 Hz), 6.40 (1H, t, J = 6.0 Hz), 4.03 (3H, s), 4.02 (3H, s), 3.88 (3H, s), 3.59 (2H, q, J = 6.0 Hz), 3.54 (2H, q, J = 6.0 Hz), 1.75 (4H, m). Anal. Calcd for $C_{21}H_{25}N_3O_7$: C, 58.46; H, 5.84; N, 9.74. Found: C, 58.08; H, 5.86; N, 9.90.

4.5.16. *N*-[5-(4-Methoxybenzoylamino)pentyl]-3,4-dimethoxy-5-nitrobenzamide (28). *N*-(5-Phthalimidopentyl)-3,4-dimethoxy-5-nitrobenzamide (**26**) (1 g, 2.3 mmol), hydrazine hydrate (0.35 mL, 6.9 mmol) and ethanol (30 mL) were mixed together and stirred for 12 h. The precipitate that formed was removed by filtration and the ethanol and hydrazine were removed by high vacuum to yield the crude amine. The crude amine was resuspended in a mixture of 4-methoxybenzoyl

chloride (0.48 g, 2.8 mmol), potassium carbonate (0.39 g, 2.8 mmol), H_2O (25 mL) and ethyl acetate (25 mL) and stirred overnight. The ethyl acetate solution was separated and washed with 1 M NaOH to remove any carboxylate byproduct. Rotary evaporation under reduced pressure of the dried (Na_2SO_4) ethyl acetate solution gave the crude product, which was purified by column chromatography (eluting with chloroform) to give the final product as a clear oil (0.49 g, 47%): ^{1}H NMR ($CDCl_3$) δ 7.75 (1H, d, J = 2.1 Hz), 7.69 (1H, d, J = 2.1 Hz), 7.65 (2H, d, J = 9.0 Hz), 6.88 (2H, d, J = 9.0 Hz), 6.86 (1H, t, J = 6.9 Hz), 6.25 (1H, t, J = 6.9 Hz), 4.01 (3H, s), 3.94 (3H, s), 3.84 (3H, s), 3.47 (4H, q, J = 6.9 Hz), 1.68 (4H, quint, J = 6.9 Hz), 1.45 (2H, quint, J = 6.9 Hz).

4.5.17. *N*-Methyl-(3,4-dimethoxy-5-nitrobenzamide) (29). Methylamine in ethanol (33% w/w) (1.3 mL, 11 mmol) was added to a solution of 3,4-dimethoxy-5-nitrobenzoylchloride (2.5 g, 10.1 mmol), triethylamine (2 mL) and ethyl acetate (50 mL) and the mixture was stirred overnight. The ethyl acetate was washed with 10% aqueous HCl solution and 2 M NaOH solution. Rotary evaporation under reduced pressure of the dried ($MgSO_4$) ethyl acetate solution gave the crude product, which was purified by column chromatography (3:1 chloroform/hexane) to give the final product as a white powder (2.1 g, 86%): mp 158–162 °C (dec); ^{1}H NMR ($CDCl_3$) δ 7.66 (1H, d, J = 2.1 Hz), 7.60 (1H, d, J = 2.1 Hz), 6.20 (1H, br s), 4.01 (3H, s), 3.96 (3H, s), 3.01 (3H, d, J = 7.1 Hz). Anal. Calcd for $C_{10}H_{12}N_2O_5$: C, 50.00; H, 5.04; N, 11.66. Found: C, 50.24; H, 4.97; N, 11.34.

4.5.18. *N*-Ethyl-(3,4-dimethoxy-5-nitrobenzamide) (30). Ethylamine in ethanol (33% w/w) (1.95 mL, 11 mmol) was added to a solution of 3,4-dimethoxy-5-nitrobenzoylchloride (2.5 g, 10.1 mmol), triethylamine (2 mL) in ethyl acetate (50 mL) and the mixture was stirred overnight. The reaction mixture was washed with 10% aqueous HCl and 2 M NaOH solution. Rotary evaporation under reduced pressure of the dried ($MgSO_4$) ethyl acetate solution gave the crude product, which was recrystallized from water and ethanol to give the product as white flaky crystals (1.4 g, 54%): mp 111 °C; ^{1}H NMR ($CDCl_3$) δ 7.68 (1H, d, J = 2.1 Hz), 7.61 (1H, d, J = 2.1 Hz), 6.20 (1H, br s), 4.02 (3H, s), 3.98 (3H, s), 3.51 (2H, dq, J = 5.6, 7.1 Hz), 1.27 (3H, t, J = 7.1 Hz). Anal. Calcd for $C_{11}H_{14}N_2O_5$: C, 51.96; H, 5.55; N, 11.02. Found: C, 52.07; H, 5.46; N, 10.79.

4.5.19. *N*-Methyl-(3,4-dihydroxy-5-nitrobenzamide) (31). An excess of BBr_3 (1.2 mL, 15 mol equiv) was added to a solution of *N*-methyl (3,4-dihydroxy-5-nitrobenzamide) (**29**) (0.2 g, 0.8 mmol) in dried CH_2Cl_2 (20 mL) under N_2. The mixture was left stirring for 24 h at room temperature. Water was carefully added to eliminate any unreacted BBr_3. The mixture was evaporated to dryness by rotary evaporation under reduced pressure to yield the crude product. The product was purified by recrystallization from an acetone and water solution to give the final product as long yellow crystals (80 mg, 45%): mp 217–219 °C; ^{1}H NMR (acetone-d_6) δ 10.40 (1H, br s), 8.92 (1H, br s), 8.00 (1H, d, J = 2.1 Hz), 7.73 (1H, d, J = 4.8 Hz), 7.60 (1H, d, J = 2.1 Hz), 2.74 (3H, d, J = 4.8 Hz). Anal. Calcd for $C_8H_8N_2O_5$: C, 45.28; H, 3.80; N, 13.21. Found: C, 45.35; H, 3.82; N, 12.91.

4.5.20. *N*-Ethyl-(3,4-dihydroxy-5-nitrobenzamide) (32). The ethyl derivative was prepared using *N*-ethyl-(3,4-dimethoxy-5-nitro-benzamide) (**30**) (0.2 g, 0.8 mmol) and BBr_3 (1.2 mL, 15 mol equiv) using the procedure described above (96 mg, 54%): mp 176–177 °C; ^{1}H NMR (acetone-d_6) δ 10.60 (2H, br s), 8.00 (1H, d, J = 2.1 Hz), 7.78 (1H, br s), 7.62 (1H, d, J = 2.1 Hz), 3.28 (2H, dq, J = 5.6, 7.2 Hz), 1.05 (3H, t, J = 7.2 Hz). Anal. Calcd for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.45; N, 12.38. Found: C, 48.02; H, 4.43; N, 12.09.

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