

Novel chromogenic aminopeptidase substrates for the detection and identification of clinically important microorganisms

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Dedicated to the memory of Arthur L. James; 1936–2014.

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Abstract

A series of amino acid derivatives **8-10**, **42** and **43** have been prepared as chromogenic enzyme substrates in order to detect aminopeptidase activity in clinically important Gram-negative and Gram-positive bacteria. Enzymatic hydrolysis liberates the amino acid moiety and either a 4-aminophenol or a 4-dialkylaminoaniline derivative which undergoes oxidative coupling with 1-naphthol or a substituted 1-naphthol giving an indophenol dye. Substrates and 1-naphthols were incorporated into an agar-based culture medium and this allowed growth of intensely coloured bacterial colonies based on hydrolysis by specific enzymes. Red/pink coloured colonies were produced by the substrates **8-10** and blue coloured colonies were formed by the substrates **42** and **43**. The L-alanyl aminopeptidase substrates **8** targeted L-alanyl aminopeptidase activity and gave coloured colonies with a range of Gram-negative bacteria. Substrates **9** targeted β -alanyl aminopeptidase activity and generated coloured colonies with selected Gram-negative species including *Pseudomonas aeruginosa*. Three substrates for L-pyroglutamyl acid aminopeptidase (**10a**, **10c** and **43**) were hydrolysed by enterococci and *Streptococcus pyogenes* to generate coloured colonies. Two yeasts were also included in the study, but they did not produce coloured colonies with any of the substrates examined.

Keywords: aminopeptidase, bacteria detection, chromogenic substrates

1. Introduction

The detection and identification of pernicious microorganisms is of tremendous importance in the health-care sector (*e.g.* hospitals) and other broad areas such as food quality control and environmental monitoring (*e.g.* water contamination).¹⁻³ One important protocol that has emerged for the detection and identification of microorganisms is the application of synthetic enzyme substrates; microbial enzymes transform either weakly coloured (or weakly

fluorescent) substrates into strongly coloured (or highly fluorescent) products respectively. The ability of a microorganism to grow on a selective culture medium alongside the appearance of colour (or fluorescence) resulting from the activity of a specific enzyme (*e.g.* aminopeptidase, glycosidase, phosphatase *etc*) has great utility for establishing the presumptive identification of microbial species.⁴

The identification of specific types of aminopeptidase activity in microorganisms has proved useful in diagnostic microbiology. Of particular relevance to this paper are L-alanyl, β -alanyl and pyroglutamyl (PYRase) aminopeptidase activities. Thus, there has been longstanding interest in the detection of L-alanine aminopeptidase activity, which has enabled differentiation between Gram-positive and Gram-negative microorganisms.^{5,6} This enzyme is ubiquitous in Gram-negative microorganisms whereas, in contrast, it is generally absent from most Gram-positive microorganisms. β -Alanyl aminopeptidase has been detected in *Pseudomonas aeruginosa*, a common respiratory pathogen in cystic fibrosis patients.⁷ L-Pyroglutamyl aminopeptidase activity is useful for differentiation within the family Enterobacteriaceae^{3,8} and also for detection of enterococci⁹ and *Streptococcus pyogenes*.¹⁰

A diverse range of chromogenic aminopeptidase substrates have previously been described and some relevant examples (structures **1-5**, AA = amino acid) are shown in Figure 1. In these substrates, hydrolysis of the amide bond by an appropriate aminopeptidase enzyme liberates the corresponding coloured amine. L-Alanyl-*p*-nitroanilide **1** (AA = L-alanyl) liberates yellow *p*-nitroaniline in the presence of Gram-negative microorganisms.^{3,11} However, this substrate is not particularly suitable for use in agar media because of widespread diffusion of the *p*-nitroaniline. The phenoxazinone derivative **2** (AA = β -alanyl) has been evaluated for the detection of *Pseudomonas aeruginosa* in agar media and purple coloured colonies are produced.⁷ *N*-Methyllepidinium **3**¹² and *N*-methylacridinium **4**⁵ substrates bearing a range of pendent amino acids have been prepared and evaluated in agar media producing red or red-orange coloured colonies.

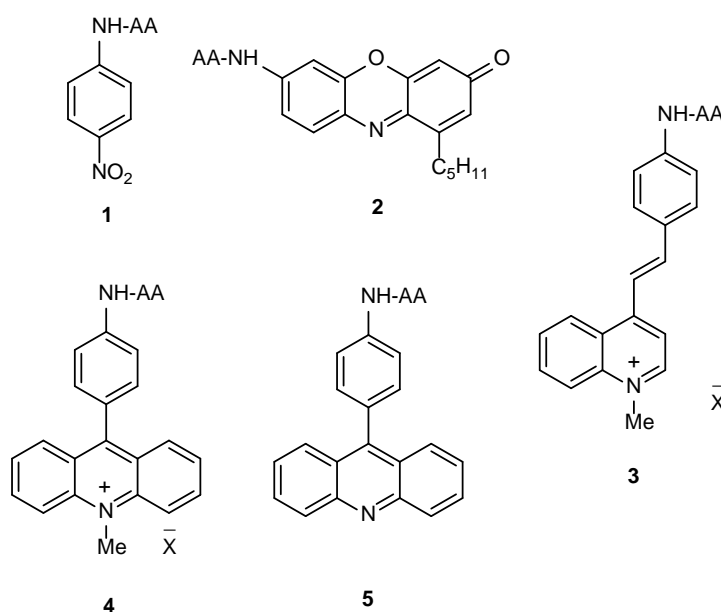
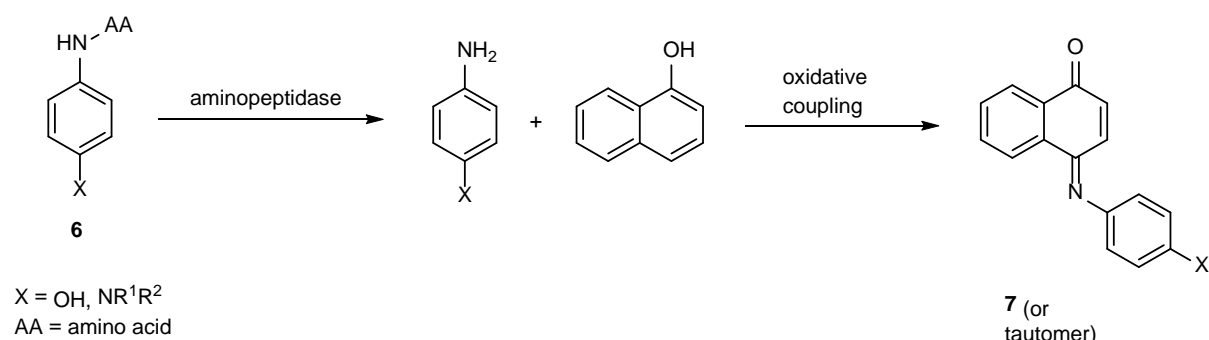


Figure 1. Chromogenic substrates for the detection of aminopeptidase activity

Amino acid derivatives of weakly coloured amines can also be used to detect aminopeptidase activity when the liberated amine is reacted with a secondary reagent in order to produce a coloured product. Thus, derivatives of the acridine substrates **5** (AA = L-alanyl, β -alanyl) produce various shades of red-coloured colonies in agar media after the addition of acetic acid.¹³ The function of the acetic acid is to protonate the acridine-nitrogen atom of the liberated amine because the free base is only weakly coloured. Amino acid derivatives of α - and β -naphthylamine can also be used to detect aminopeptidase activity when the liberated amine is reacted with a diazonium salt to produce a strongly coloured dye.^{3,8} Amino acid derivatives of 4-aminophenol and 4-dialkylaminoaniline and their analogues produce coloured indophenol products **7** when the liberated amine undergoes oxidative coupling with 1-naphthol in liquid media as illustrated in Scheme 1.¹⁴ This protocol has been extended to include glycoside derivatives of 1-naphthol in a 'dual' substrate procedure for microorganism identification; both glycosidase and aminopeptidase activity must be present in order for indophenol production.¹⁴

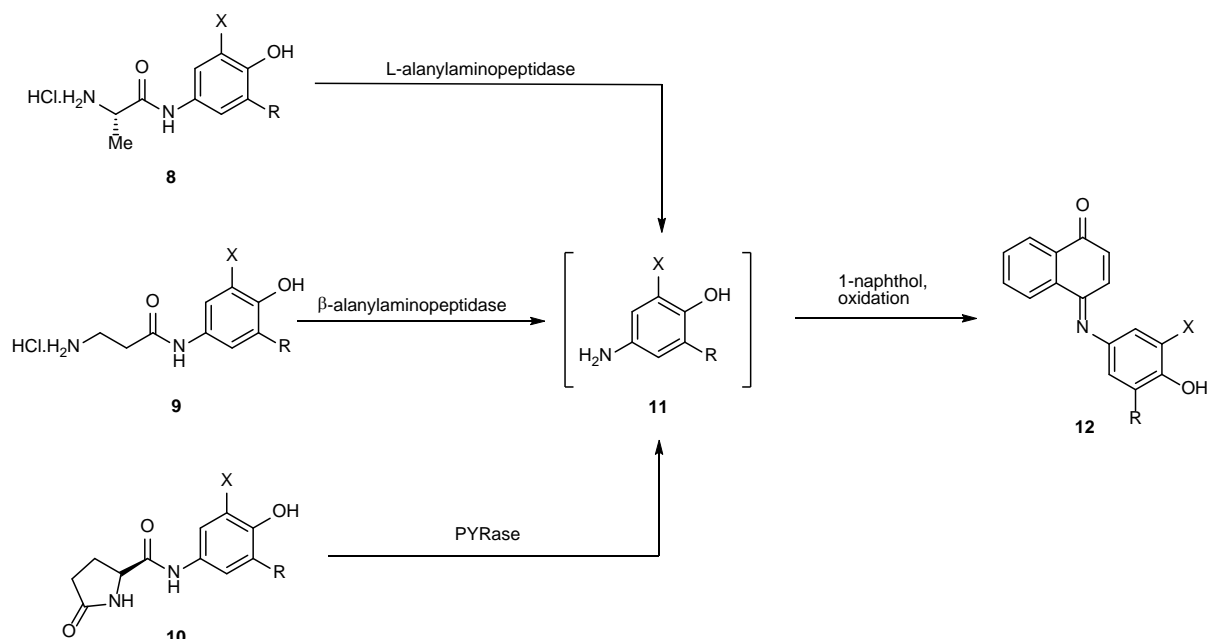
In this paper we describe the synthesis and application of the 4-aminophenol derivatives **8-10** and the 4-dialkylaminoaniline derivatives **42** and **43** as potential chromogenic substrates for use in agar media. Previous work on indophenol dye production has been confined to liquid media¹⁴ and an extension into agar media was thought to be highly desirable.



Scheme 1. Formation of indophenol dyes

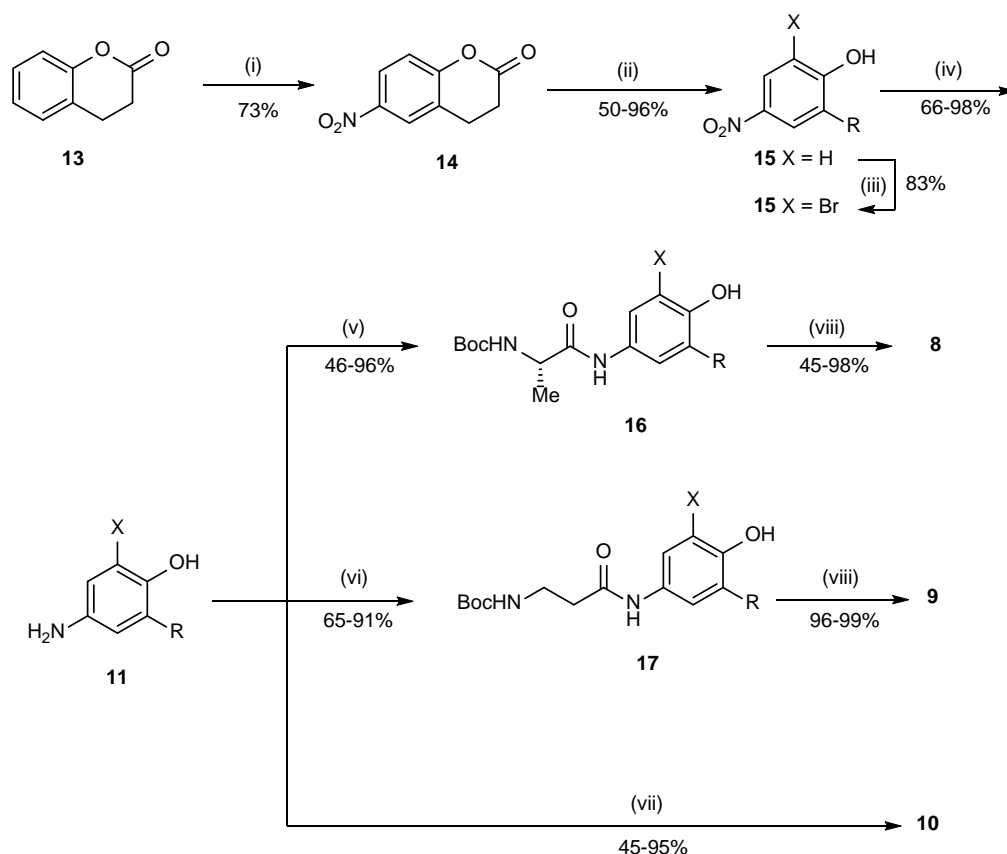
2. Synthesis of substrates **8-10**

We envisaged that hydrolysis of substrates of general structures **8-10** (Scheme 2, Table 1) would liberate the corresponding 4-aminophenol derivatives **11** which would subsequently undergo oxidative coupling with 1-naphthol (or a suitable analogue) producing the indophenol dyes **12**.



Scheme 2. Proposed formation of indophenol dyes **12** as a result of aminopeptidase activity on substrates **8-10**. See Table 1 for structures of X and R substituents.

The synthesis of the required substrates **8-10** is shown in Scheme 3. Commercially available and inexpensive 3,4-dihydrocoumarin (**13**) was selected as the starting material because, after nitration, treatment of the nitro-compound **14** with either amines or alcohols would be expected to result in ring-opening of the lactone moiety enabling access to a range nitrophenol derivatives **15** (X = H) from a common precursor. Halogenation of compounds **15** (X = H) could give additional structural diversity yielding halogenated products **15** (X = halogen). Thus, nitration of compound **13** following a literature procedure gave the nitro-derivative **14**. When compound was heated with ethanol and amines respectively, the ester **15a** (X = H) (69%) and the amides **15c-i** (X = H) (59-84%) were formed. Bromination of the ester **15a** with *N*-bromosuccinimide (NBS) in DMF solution gave compound **15b** (X = Br) (83%). Reduction of nitro-derivatives **15** using lithium formate in the presence of a palladium catalyst afforded the corresponding amines **11** [with the exception of compound **15b** which was reduced with tin(II) dichloride dihydrate in ethanol at reflux]. In the case of compound **15e**, the *O*-benzyl group was also removed under these conditions giving the product **11f**. A mixed anhydride coupling of the amines **11** to Boc-L-alanine and Boc-β-alanine gave the protected amino-acid derivatives **16** and **17** respectively and subsequent removal of the Boc-groups under acidic conditions yielded the required aminopeptidase substrates **8** and **9**. The mixed anhydride coupling of amines **11** with L-pyrroglutamic acid gave the substrates **10**.



Scheme 3. Synthesis of aminopeptidase substrates **8-10**. See Table 1 for structures of X and R groups. Reagents and conditions: (i) Ac_2O , HNO_3 , AcOH , 18-20 °C; (ii) EtOH, reflux, 1h (**15a**); appropriate aniline or amine, THF, reflux, 5 h (**15c-e**, **15g-i**); (iii) NBS, DMF, rt, 20 h (**15a** to **15b**); (iv) HC(O)OLi , 10% Pd/C, THF, reflux, 2-8 h or $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, EtOH, reflux (**15b** only); (v) (a) *N*-methylmorpholine, $^i\text{BuOC(O)Cl}$, Boc-L-alanine, THF, -5 °C, then add **11**, (b) rt overnight; (vi) (a) *N*-methylmorpholine, $^i\text{BuOC(O)Cl}$, Boc- β -alanine, THF, -5 °C, then add **11**, (b) rt overnight; (vii) (a) *N*-methylmorpholine, $^i\text{BuOC(O)Cl}$, L-pyroglutamic acid, THF/DMF 3:1, -5 °C, then add **11**, (b) rt overnight; (viii) EtOAc/HCl, rt, 3 h.

| | X | R | Yield of 15 (%) | Yield of 11 (%) | Yields of 16, 17 (%) | Yields of 8-10 (%) |
|----------|----|---|------------------------|------------------------|--------------------------------|---|
| a | H | | 69 | 66 | 16a 81 17a 79 | 8a 96 9a 96 10a 84 |
| b | Br | | 83 ^a | 89 | 16b 76 17b 65 | 8b 98 9b 99 10b 45 |

| | | | | | | |
|----------|---|--|-----------------|-----------------|--------------------------------|---|
| c | H | | 84 | 88 | 16c 96 17c 91 | 8c 92 9c 96 10c 95 |
| d | H | | 50 | 67 | 16d 81 | 8d 81 |
| e | H | | 59 ^b | -- | -- | -- |
| f | H | | -- | 80 ^b | 16f 46 | 8f 96 |
| g | H | | 86 | 91 | 16g 89 | 8g 97 |
| h | H | | 96 | 98 | 16h 68 | 8h 93 |
| i | H | | 65 | 87 | 16i 76 | 8i 81 |

^a Formed by bromination of **15a**.

^b Reduction of **15e** also resulted in de-benzylation giving compound **11f**.

Table 1. Structures and yields of compounds synthesised as shown in Scheme 3.

3. Evaluation of substrates 8-10

The substrates **8-10** have been evaluated in Columbia agar medium against a range of clinically important microorganisms including 10 Gram-negative bacteria, 8 Gram-positive bacteria and 2 yeasts. 1-Naphthol was incorporated into the growth media in order to react with the amine **11** to produce the indophenol dyes **12** as previously noted in Scheme 2.

Table 2 depicts the results of the evaluation of substrates **8a-c**. Plates were incubated at 37 °C in air for 24 h. The growth of the microorganisms was compared to control plates in which no substrate or 1-naphthol was present. The Gram-negative microorganisms all grew well on the control plates whereas the Gram-positive microorganisms and the yeasts showed only moderate growth. This extent of microorganism growth is generally observed when the substrate and 1-naphthol are both present in the plates with the exception of the yeasts which showed very little growth, suggesting that the substrates are inhibiting yeast growth (there is some growth of the yeasts in the presence of 1-naphthol and the substrates **9a-c** and **10a-c** indicating that the substrates **8a-c**, rather than 1-naphthol, are inhibitory). In the presence of 1-naphthol and substrates **8a** and **8c**, strongly red-coloured colonies were produced by most Gram-negative microorganisms as expected because these microorganisms generally exhibit L-alanyl aminopeptidase activity. Similarly, in the presence of 1-naphthol and the brominated substrate **8b** strongly coloured colonies were formed by the Gram-negative microorganisms but these colonies were pink, rather than red. The colourations produced by substrates **8a-c** are illustrated in Figure 2. The substrates **8d** and **8f-i** also produced red colonies (data not shown) but the colours formed were significantly less intense than those colours produced from substrates **8a-c**.

<Table 2>

Table 2. Evaluation of substrates **8a-c**. Substrate concentration = 300 mg L⁻¹; 1-naphthol concentration = 50 mg L⁻¹ (0.35 mM); inoculum = 100 000 colony-forming units (cfu)/spot.

<Figure 2>

Figure 2. Columbia agar plates depicting colour formation of substrates **8a-c** with various microorganisms.

There was some noticeable diffusion of colour around the microorganism colonies associated with the use of substrates **8a-c**. In agar media, it is preferable to have the colour restricted to the colonies as this allows clear differentiation of species that demonstrate enzyme activity from those that do not. When the coloured product diffuses through agar, there can be some uncertainty about which colonies are actually showing enzyme activity if colonies of several species are in close proximity to each other. Such polymicrobial cultures are frequently recovered from pathological specimens. We have therefore investigated whether diffusion of colour may be restricted by replacing 1-naphthol with analogues of this compound. A series of 2- and 8-substituted-1-naphthols **18-24** were prepared for this purpose (Figure 3). 2-Benzyl-1-naphthol (**18**) was prepared by a rhodium(III) chloride catalysed isomerisation of compound **25** in ethanol solution (90%). Treatment of commercially available phenyl 1-naphthol-2-carboxylate with 4-(aminomethyl)pyridine gave compound **19** (75%) and the reaction of phenylmagnesium bromide with 1,8-naphthosultone **26** afforded the known sulphone derivative **20**. The 1-naphthol derivatives **21-24** were all prepared by heating the lactone **27** with either ethanol [giving compound **21** (63%)] or an appropriate amine affording amides **22-24** (45-95%).

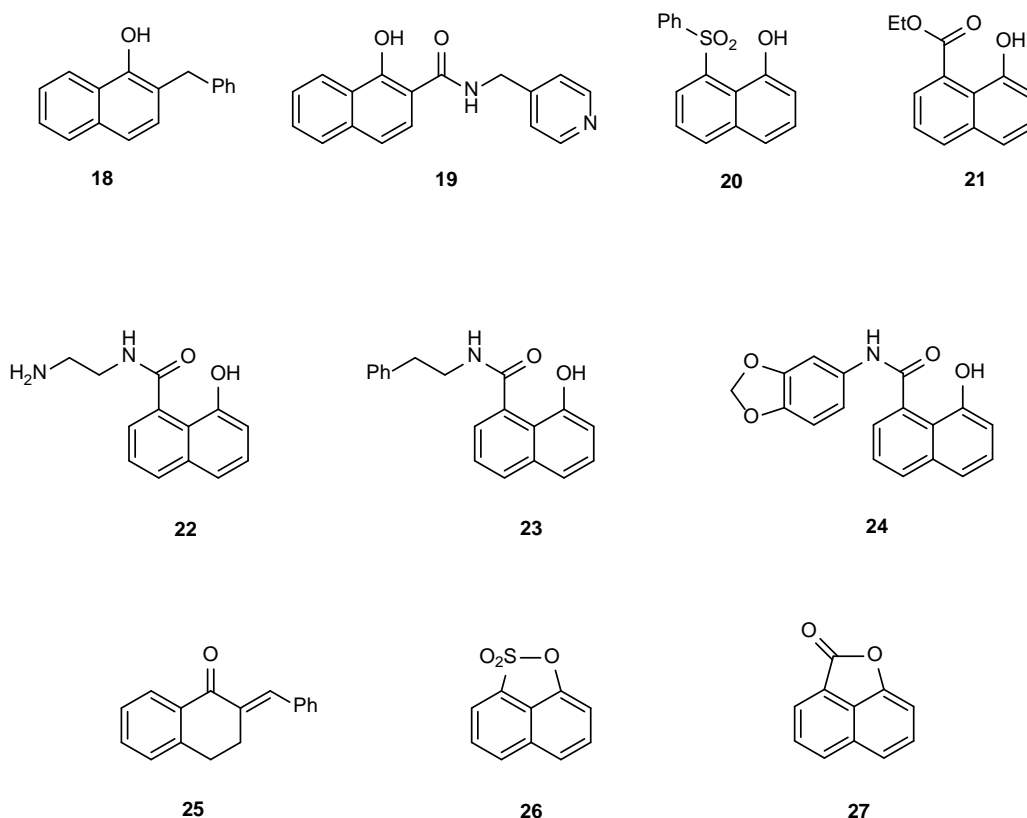


Figure 3. 1-Naphthol analogues **18-24** and their precursors **25-27**.

The 1-naphthol analogues **18-24** were all evaluated with substrate **8b** and the results were compared to 1-naphthol (see Figure 4 for four illustrative plates). The range of microorganisms that produced coloured colonies with these additional naphthols was broadly similar to the range that produced coloured colonies with 1-naphthol. However, some diffusion of colour from the colonies into the surrounding agar was still apparent with these additional naphthol derivatives. Naphthols **20**, **21**, **23** and **24** produced red coloured colonies and the naphthol **18** gave orange coloured colonies. In contrast, the amides **19** and **22** bearing the basic pyridine and primary amine groups respectively, both afforded blue/purple coloured colonies.

<Figure 4>

Figure 4. Colours produced from substrate **8b** (concentration 300 mg L^{-1}) and microorganisms in the presence of four 1-naphthol analogues (concentration 0.35 mM). Top left, compound **23**; top right, compound **24**; bottom left, compound **22**; bottom right, compound **18**. See Figure 2 for the arrangement of the microorganisms on the plates.

In view of the most intense coloured colonies being produced with the L-alanyl aminopeptidase substrates **8a-c**, the preparation of β -alanyl aminopeptidase and PYRase substrates were therefore based on these three core structures. Table 3 shows the results obtained for the β -alanyl substrates **9a-c**. Gram-negative microorganisms grew well on the

media and the Gram-positive microorganisms and the yeasts generally exhibited moderate growth. Coloured colonies were not formed by any of the Gram-positive microorganisms or by the yeasts. Of the Gram-negative microorganisms, only *Pseudomonas aeruginosa* produced colonies with significant colouration; the colour produced with substrate **9b** was particularly strong. There were some weakly coloured colonies produced by *Serratia marcescens* with substrates **9a** and **9b**. As noted in the introduction, *Pseudomonas aeruginosa* exhibits β -alanyl aminopeptidase activity and this microorganism is being effectively detected by substrate **9b** although some diffusion of colour into the surrounding media was still apparent. Other than *P. aeruginosa*, a limited number of species have been reported to produce β -alanyl aminopeptidase including some strains of *Burkholderia cepacia* complex and *Serratia marcescens*.⁵ The specificity of substrate **9b** was therefore entirely consistent with previous reports.

<Table 3>

Table 3. Evaluation of substrates **9a-c**. Substrate concentration = 300 mg L⁻¹; 1-naphthol concentration = 50 mg L⁻¹ (0.35 mM); inoculum = 100 000 cfu/spot.

<Table 4>

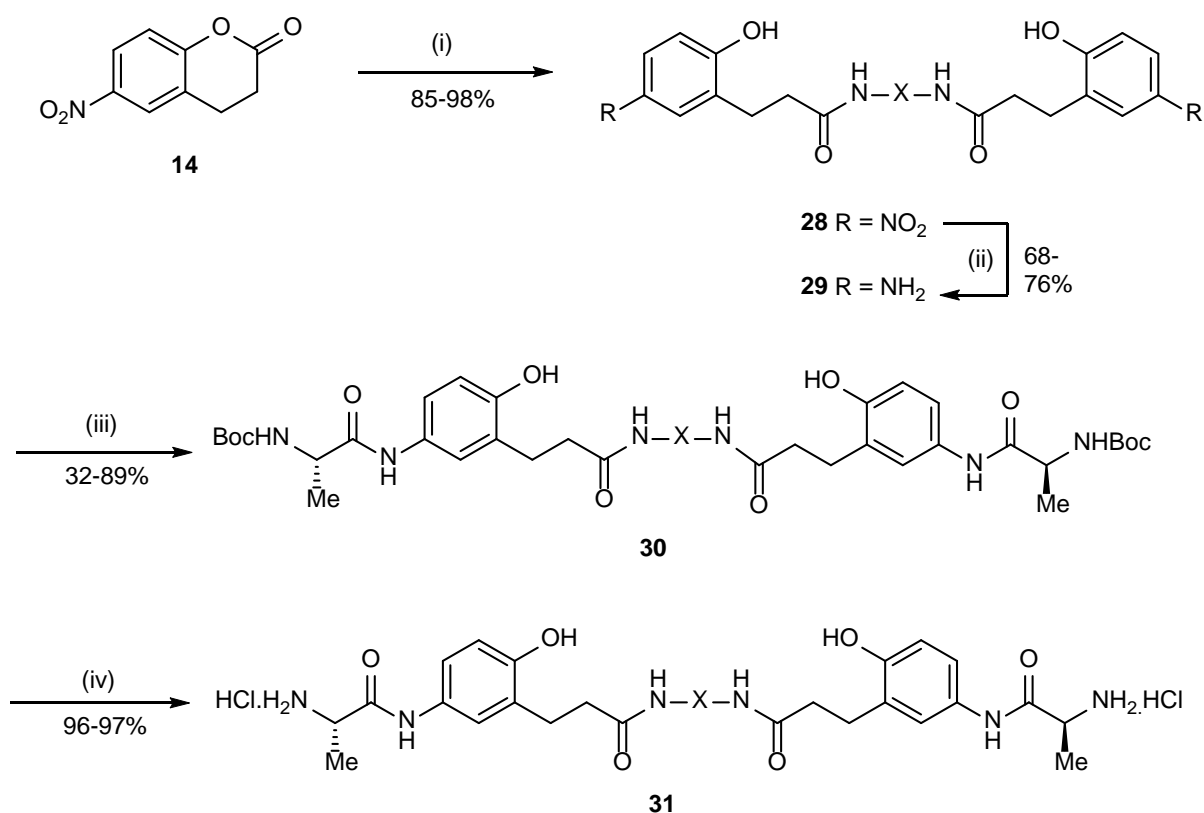
Table 4. Evaluation of substrates **10a-c**. Substrate concentration = 300 mg L⁻¹; 1-naphthol concentration = 50 mg L⁻¹ (0.35 mM); inoculum = 100 000 cfu/spot.

Substrates **10a** and **10c** were hydrolysed by enterococci and *Streptococcus pyogenes* to generate a pink coloration. The principal value of PYRase as a diagnostic marker is in the differentiation of *S. pyogenes* and enterococci from most other Gram-positive cocci.^{9, 15} A range of selective culture media have been designed for detection of enterococci and these have traditionally relied upon chromogenic substrates for detection of β -glucosidase activity, which is a less specific marker than PYRase. One reason for this is likely to be the lack of available chromogenic substrates for PYRase that are suitable for use in culture media. *Streptococcus pyogenes* is a significant human pathogen and the principal cause of bacterial pharyngitis and such substrates are potentially very useful for differentiation of this species from commensal bacteria.

4. Synthesis and evaluation of additional L-alanyl substrates

In order to try and restrict the diffusion of the chromophore within the media, the higher molecular mass *bis*-L-alanyl substrates **31** have been prepared from compound **14** (Scheme 4). Thus, reaction of compound **14** with either *para*-phenylene diamine hydrochloride under basic conditions or with ethylene diamine gave the nitro-compounds **28**. Reduction of compounds **28** afforded the corresponding amines **29** from which the Boc-protected amino acid derivatives **30** were synthesised using a mixed anhydride coupling procedure. Treatment

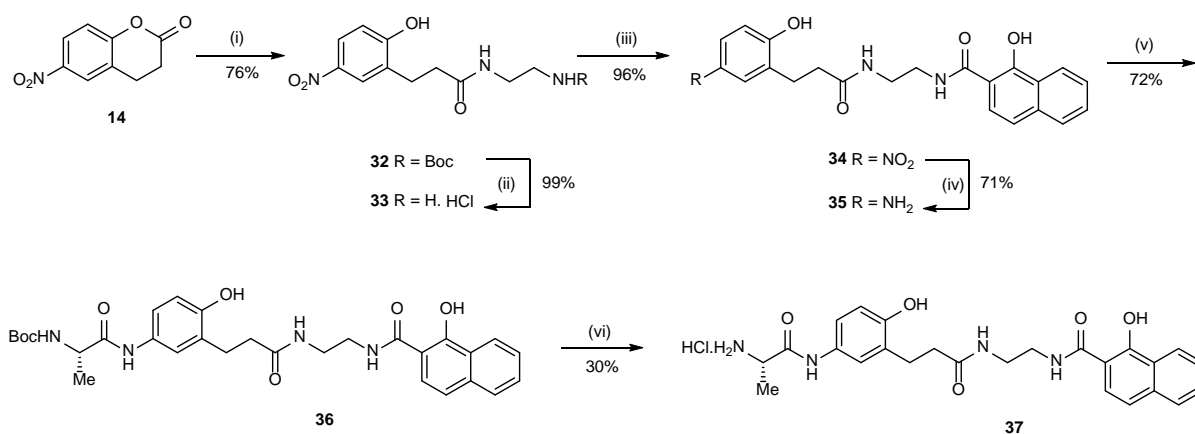
of compounds **30** with hydrogen chloride resulted in removal of the Boc-groups giving the required substrates **31**.



In formulae **28-31**; **a** X = 1,4-C₆H₄; **b** X = CH₂CH₂

Scheme 4. Synthesis of substrates **31**. Reagents and conditions: (i) 1,4-H₂NC₆H₄NH₂·HCl, NaHCO₃, THF, reflux (**28a**); H₂NCH₂CH₂NH₂, THF, reflux (**28b**); (ii) HC(O)OLi, 10% Pd/C, THF/DMF 2:1, 80 °C; (iii) (a) *N*-methylmorpholine, ^tBuOC(O)Cl, Boc-L-alanine, THF/DMF 2:1 (**29a**) or THF (**29b**), -5 °C, then add **29**, (b) rt overnight; (iv) EtOAc/HCl, rt, 3 h.

Additionally, the substrate **37** which bears both an L-alanyl moiety and a naphthol fragment within the same molecule was prepared (Scheme 5). It was anticipated that this substrate would undergo intermolecular oxidative coupling after hydrolysis of the L-alanyl group. Thus, reaction of the nitrocoumarin **14** with Boc-ethylenediamine gave the Boc-protected amine **32** from which the Boc-group was removed by treatment with hydrogen chloride in ethyl acetate affording compound **33**. Compound **33** was reacted under basic conditions with phenyl 1-hydroxy-2-naphthoate giving the nitro-derivative **34** which was then reduced yielding the amine **35**. A mixed anhydride coupling of this amine with Boc-L-alanine furnished compound **36** which, on treatment with hydrogen chloride in ethyl acetate afforded the required substrate **37**.



Scheme 5. Synthesis of substrate **37**. Reagents and conditions: (i) BocNHCH₂CH₂NH₂, THF, reflux; (ii) HCl/EtOAc, rt; (iii) phenyl 1-hydroxy-2-naphthoate, NaHCO₃, DMF/THF, reflux; (iv) HC(O)OLi, 10% Pd/C, THF/DMF 2:1, 80 °C; (v) (a) *N*-methylmorpholine, ⁱBuOC(O)Cl, Boc-L-alanine, THF/DMF 2:1, -5 °C, then add **35**, (b) rt overnight; (vi) HCl/EtOAc, rt, 3h.

Disappointingly, substrate **31a** gave only very weakly, pink coloured colonies with some Gram-negative microorganisms (data not shown) in the presence of 1-naphthol. Neither substrate **31b** (in the presence of 1-naphthol) nor substrate **37** (with no added 1-naphthol) produced any coloured colonies. This may be a consequence of the substrates being unable to penetrate into the bacterial cell. In support of this hypothesis, substrates **31b** and **37** were added to a cell-free *E. coli* extract (containing 1-naphthol in the case of substrate **31b**) and this resulted in the formation of pale orange solutions with both substrates, indicative of oxidative coupling and hence aminopeptidase activity (Figure 5). We have therefore speculated that these larger substrates may not pass efficiently through the bacterial cell membrane(s). When sodium periodate was added to the mixture (in order to assist the oxidative coupling to 1-naphthol), a slightly more intense colouration was produced. Also shown in Figure 5 is substrate **8b** which was selected as a comparator because this compound is known to give coloured colonies in agar media and hence was expected to produce a coloured solution with the cell-free extract in the absence of any additional oxidising agent.

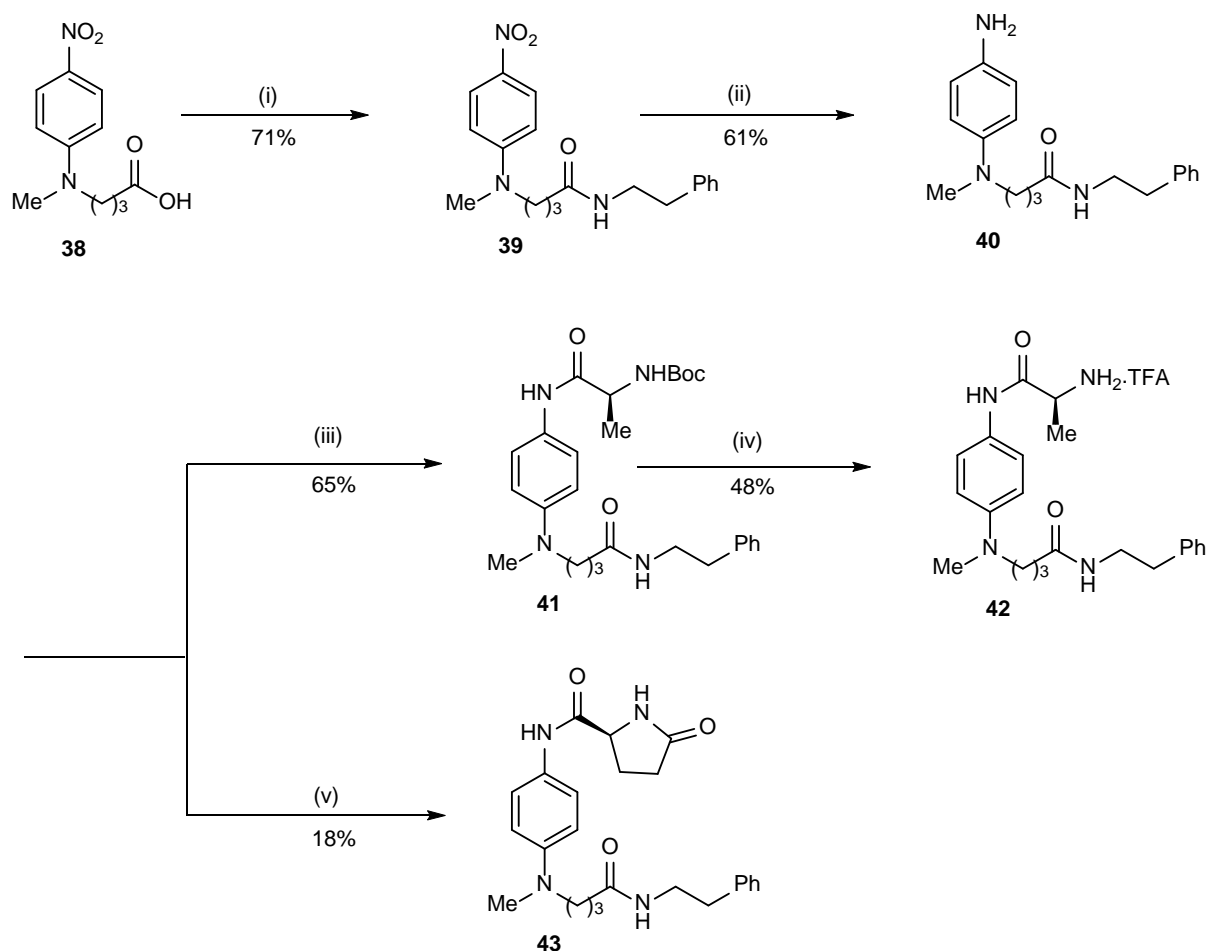
<Figure 5>

Figure 5. Performance of substrates **31b** (top left), **37** (top right) and **8b** (bottom) in the presence of an *E. coli* cell free extract (CFE). Left tube: buffer (0.1 M Tris pH 7.4) + substrate (150 mg L⁻¹); middle tube: buffer + *E. coli* CFE + substrate (150 mg L⁻¹); right tube: buffer + *E. coli* CFE + substrate (150 mg L⁻¹) + sodium periodate (150 mg L⁻¹). 1-Naphthol (50 mg L⁻¹) was added to all three tubes associated with substrates **31b** and **8b**.

5. Synthesis and evaluation of substrates **42** and **43**.

In view of the successful colour formation from substrates **8a** and **10**, we turned our attention to the preparation and evaluation of the corresponding *para*-phenylenediamine-derived substrates **42** and **43** (Scheme 6). 4-Fluoronitrobenzene was reacted with γ -aminobutyric acid under basic conditions yielding the carboxylic acid derivative **38** (62%) which was then

coupled to 2-phenylethylamine giving the amide **39**. This amide-containing chain was chosen in order to restrict diffusion in agar media of the dye that would be formed from the oxidative coupling of the hydrolysed substrates (*i.e.* amine **40**) and a 1-naphthol derivative. Reduction of compound **39** gave the amine **40** which was coupled with either Boc-L-alanine giving compound **41** or L-pyroglutamic acid affording the substrate **43**. Removal of the Boc-group from compound **41** yielded the substrate **42**.



Scheme 6. Synthesis of substrates **42** and **43**. Reagents and conditions: (i) *N*-methylmorpholine, ${}^i\text{BuOC}(\text{O})\text{Cl}$, THF, $-5\text{ }^\circ\text{C}$, then add $\text{PhCH}_2\text{CH}_2\text{NH}_2$; (ii) $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, EtOH, reflux; (iii), (a) *N*-methylmorpholine, ${}^i\text{BuOC}(\text{O})\text{Cl}$, Boc-L-alanine, THF, $-15\text{ }^\circ\text{C}$, then add **40**, (b) rt overnight (iv) TFA, CH_2Cl_2 , rt; (v) (a) *N*-methylmorpholine, ${}^i\text{BuOC}(\text{O})\text{Cl}$, L-pyroglutamic acid, THF/DMF 10:1, $-15\text{ }^\circ\text{C}$, then add **40**, (b) rt overnight.

The substrates **42** and **43** were evaluated in a similar manner to that described to that described above for substrates **8-10** (Table 5). In the presence of the naphthol derivative **20**, the L-alanyl substrate **42** produced intensely blue coloured colonies with the Gram-negative microorganisms as expected (Figure 6). However, blue coloured colonies were also produced with a number of the Gram-positive microorganisms which could limit the application of this substrate for the differentiation of Gram-negative and Gram-positive microorganisms. As noted earlier, PYRase substrates are useful for the identification of *S. pyogenes*. Substrate **43**

produced intensely blue coloured colonies with this microorganism in the presence of 1-naphthol.

<Table 5>

Table 5. Evaluation of substrates **42** and **43**. Substrate concentration = 300 mg L⁻¹; naphthol **20** concentration = 80 mg L⁻¹ or 1-naphthol = 50 mg L⁻¹; inoculum = 100 000 cfu/spot.

<Figure 6>

Figure 6. Evaluation of substrate **42** in the presence of naphthol derivative **20** against various microorganisms. Top plate: 20 microorganisms arranged as indicated in Figure 2; bottom left plate: *Pseudomonas aeruginosa*; bottom right plate: *E. coli*.

6. Conclusions

Compounds **8-10** are effective chromogenic substrates producing red/pink coloured colonies with selected Gram-negative and Gram-positive bacteria in agar media containing 1-naphthol. There is some diffusion of colour from the bacterial colonies which could limit their applications in agar media. The production of the coloured bacterial colonies is linked to the type of aminopeptidase activity associated with specific bacteria. Analogues of 1-naphthol that possess pendent basic functionalities can give rise to blue, rather than red coloured colonies. Higher molecular mass L-alanyl substrates are not effective chromogenic substrates for use in agar media but some colour development is apparent with a cell free *E. coli* extract in liquid media. Substrate **43** was an effective PYRase substrate producing blue coloured colonies. Of the two yeasts studied, neither produced any significant colour with any of the substrates evaluated, which is expected as neither is known to demonstrate the targeted enzymatic activities.

7. Experimental

7.1. Synthetic work

¹H-NMR spectra (270 or 400 MHz) and ¹³C-NMR spectra (68 or 101 MHz) were recorded on a Jeol EX270 or Jeol ECS400 instrument. Low resolution mass spectra (LRMS) were recorded *via* direct injection of dilute methanolic solutions (containing 0.1% formic acid) into a Thermo Finnigan LCQ Advantage MS detector using electrospray ionisation (ESI). High resolution mass spectrometry (HRMS) was performed by the EPSRC mass spectrometry service. Infrared spectra were obtained *via* a diamond anvil sample cell using a Perkin Elmer 1000 spectrometer. Melting points are reported uncorrected as determined on a Stuart SMP 1 melting point apparatus. Thin layer chromatography was performed on Merck plastic foil plates pre-coated with silica gel 60 F₂₅₄. Merck silica gel 60 was used for column chromatography.

7.1.1. 6-Nitro-3,4-dihydrocoumarin 14. To a stirred solution of 3,4-dihydrocoumarin **13** (18.00 g, 121.5 mmol) in acetic anhydride (90 mL) was added, dropwise, a mixture of

concentrated nitric acid (15 mL) and glacial acetic acid (30 mL) keeping the temperature between 18-20 °C . The mixture was stirred (1 h) and then poured into a mixture of ice and water (500 mL). The resulting precipitate was collected, washed well with water and dried in a desiccator under vacuum. The crude product was recrystallized from ethanol (100 mL) giving compound **14** (17.00 g, 73%) as yellow crystals, m.p. 128-130 °C, lit. m.p. 130 °C.¹⁶ LRMS (ES) for C₉H₇NO₄. Calculated mass of molecular ion: 216.16 [M+Na]⁺. Measured mass: 216.15; IR ν_{\max} cm⁻¹ 1778, 1514, 1327, 1246, 1089; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 8.28 (1H, d, *J* = 2.8 Hz, Ar-*H*), 8.15 (1H, dd, *J* = 8.7 and 2.8 Hz, Ar-*H*), 7.30 (1H, d, *J* = 8.7 Hz, Ar-*H*), 3.15 (2H, t, *J* = 7.3 Hz, CH₂), 2.88 (2H, t, *J* = 7.3 Hz, CH₂); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 167.7 (C=O), 156.9 (Ar-C), 143.9 (Ar-C), 125.7 (Ar-C), 124.6 (Ar-C), 124.3 (Ar-C), 118.0 (Ar-C), 28.2 (CH₂), 23.0 (CH₂).

7.1.2 Synthesis of Nitrophenols **15**, **28**, **32** and **34**

7.1.2.1. Ethyl 3-(2-Hydroxy-5-nitrophenyl)propanoate 15a. To a stirred solution of compound **13** (18.00 g, 121.5 mmol) in acetic anhydride (90 mL) was added, dropwise, a mixture of concentrated nitric acid (15 mL) and glacial acetic acid (30 mL), keeping the temperature between 18-20 °C . The mixture was stirred (1 h) and then poured into a mixture of ice and water (500 mL). The resulting precipitate was collected, and dried in a desiccator under vacuum. The dried product was then heated in ethanol (200 mL) at reflux for 1 h, allowed to cool to rt and then evaporated. Half of the crude product was kept to be used for synthesis of compound **15b**, the other half was washed with ice cold ethanol (30 mL) and dried to afford compound **15a** (10.02 g, 69%), m.p. 86-88 °C, lit. m.p. 89.5-90 °C.¹⁷ LRMS (ES) for C₁₁H₁₃NO₅. Calculated mass of molecular ion 262.22 [M+Na]⁺. Measured mass: 262.21; IR ν_{\max} cm⁻¹ 3320-3280, 1692 1493, 1332, 1231, 1081; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 11.18 (1H, s, broad, OH), 8.04-7.99 (2H, m, Ar-*H*), 6.97 (1H, d, *J* = 8.24 Hz, Ar-*H*), 4.05 (2H, q, *J* = 7.0 Hz, CH₂CH₃), 2.86 (2H, t, *J* = 7.3 Hz, CH₂CH₂CO), 2.62 (2H, t, *J* = 7.3 Hz, CH₂CO), 1.16 (3H, t, *J* = 7.1 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.7 (C=O), 162.5 (Ar-C), 139.8 (Ar-C), 128.3 (Ar-C), 126.2 (Ar-C), 124.6 (Ar-C), 115.5 (Ar-C), 60.4 (CH₂CH₃), 33.2 (CH₂), 25.6 (CH₂), 14.6 (CH₃).

7.1.2.2. Ethyl 3-(3-Bromo-2-hydroxy-5-nitrophenyl)propanoate 15b. Compound **15a** (1.00 g, 4.18 mmol) was dissolved in DMF (30 mL) and *N*-bromosuccinimide (0.82 g, 4.61 mmol) was added. The mixture was stirred for 20 h at rt after which time the solvent was evaporated. Water (20 mL) was added to the residue and the precipitate was collected and washed well with cold water (200 mL). The resulting orange granules were dried in a desiccator under vacuum giving compound **15b** (1.10 g, 83%), m.p. 99-100 °C. HRMS (APCI) for C₁₁H₁₂BrNO₅. Calculated mass of molecular ion 317.9972 [M+H]⁺. Measured mass: 317.9973; IR ν_{\max} cm⁻¹ 3500-2800, 1694, 1510, 1320, 1223, 1156, 702; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 11.01 (1H, s, broad, OH), 8.27 (1H, d, *J* = 2.8 Hz, Ar-*H*), 8.06 (1H, d, *J* = 2.8 Hz, Ar-*H*), 4.07 (2H, q, *J* = 7.2 Hz, CH₂O), 2.97 (2H, t, *J* = 7.3 Hz, CH₂CH₂CO), 2.64 (2H, t, *J* = 7.3 Hz, CH₂CO), 1.17 (3H, t, *J* = 7.1 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.5 (C=O), 158.7 (Ar-C), 140.3 (Ar-C), 130.4 (Ar-C), 127.2 (Ar-C), 125.4 (Ar-C), 111.0 (Ar-C), 60.5 (CH₂CH₃), 33.3 (CH₂), 26.3 (CH₂), 14.6 (CH₃).

7.1.2.3. *N*-(2*H*-1,3-Benzodioxol-5-yl)-3-(2-hydroxy-5-nitrophenyl)propanamide 15c. To a stirred solution of compound **14** (1.00 g, 5.18 mmol) in THF (30 mL) was added 3,4-(methylenedioxy)aniline (0.71 g, 5.18 mmol). The mixture was stirred at reflux for 5 h. The solution was allowed to cool and kept overnight. The resulting crystals were collected and dried affording compound **15c** (1.43 g, 84%) as shiny yellow crystals, m.p. 233-236 °C. HRMS (NSI) for C₁₆H₁₄N₂O₆. Calculated mass of molecular ion 331.0925 [M+H]⁺. Measured mass: 331.0924; IR ν_{\max} cm⁻¹ 3331, 3200, 1633, 1562, 1478, 1332, 1282, 1241, 1192, 1036, 786; ¹H-NMR (270 MHz; d₆-DMSO) δ_{H} 11.14 (1H, broad, s, OH), 9.85 (1H, s, NH), 8.07-7.98 (2H, m, Ar-H), 7.29 (1H, d, *J* = 2.0 Hz, Ar-H), 7.00-6.81 (3H, m, Ar-H), 5.97 (2H, s, CH₂O), 2.90 (2H, t, *J* = 7.6 Hz, CH₂CH₂CO), 2.60 (2H, t, *J* = 7.6 Hz, CH₂CO); ¹³C-NMR (68 MHz; d₆-DMSO) δ_{C} 170.5 (C=O), 162.5 (Ar-C), 147.5 (Ar-C), 143.3 (Ar-C), 139.9 (Ar-C), 134.2 (Ar-C), 129.2 (Ar-C), 126.1 (Ar-C), 124.5 (Ar-C), 115.6 (Ar-C), 112.4 (Ar-C), 108.6 (Ar-C), 101.9 (Ar-C), 101.5 (CH₂O), 35.9 (CH₂), 25.8 (CH₂).

7.1.2.4. *N*-(3,4-Dimethoxyphenyl)-3-(2-hydroxy-5-nitrophenyl)propanamide 15d. To a stirred solution of compound **14** (1.00 g, 5.18 mmol) in THF (20 mL) was added 3,4-dimethoxyaniline (0.79 g, 5.18 mmol). The resulting mixture was stirred at reflux for 2 h, filtered and evaporated. The residue was recrystallized from ethanol (20 mL) affording compound **15d** (0.90 g, 50%) as light brown crystals, m.p. 188-190 °C. HRMS (NSI) for C₁₇H₁₈N₂O₆. Calculated mass of molecular ion 347.1238 [M+H]⁺. Measured mass: 347.1242; IR ν_{\max} cm⁻¹ 3364, 3300-2700, 1635, 1509, 1327, 1290, 1230, 1023; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 11.16 (1H, s, broad, OH), 9.82 (1H, s, NH), 8.08 (1H, d, *J* = 2.8 Hz, Ar-H), 8.02 (1H, dd, *J* = 9.2 and 3.3 Hz, Ar-H), 7.29 (1H, d, *J* = 2.3 Hz, Ar-H), 7.08 (1H, dd, *J* = 8.7 and 2.3 Hz, Ar-H), 6.99 (1H, d, *J* = 9.2 Hz, Ar-H), 6.89 (1H, d, *J* = 8.7 Hz, Ar-H), 3.20 (2 x 3H, d, *J* = 4.1 Hz, 2 x CH₃), 2.93 (2H, t, *J* = 7.6 Hz, CH₂CH₂CO), 2.62 (2H, t, *J* = 7.6 Hz, CH₂CO); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 170.3 (C=O), 162.5 (Ar-4°C), 149.0 (Ar-4°C), 145.2 (Ar-C), 139.9 (Ar-C), 133.3 (Ar-C), 129.1 (Ar-C), 126.1 (Ar-C), 124.5 (Ar-C), 115.5 (Ar-C), 112.5 (Ar-C), 111.6 (Ar-C), 104.9 (Ar-C), 56.2 (CH₃), 55.8 (CH₃), 35.9 (CH₂), 25.9 (CH₂).

7.1.2.5. *N*-(4-Benzyloxyphenyl)-3-(2-hydroxy-5-nitrophenyl)propanamide 15e. To a stirred solution of compound **14** (1.00 g, 5.18 mmol) in THF (20 mL) was added NaHCO₃ (0.66 g, 7.77 mmol) and 4-benzyloxyaniline hydrochloride (1.22 g, 5.18 mmol). The resulting mixture was stirred at reflux for 12 h and then was evaporated. The residue was recrystallized from ethanol (50 mL) affording compound **15e** (1.20 g, 59%) as an orange powder, m.p. 239-241 °C. HRMS (NSI) for C₂₂H₂₀N₂O₅. Calculated mass of molecular ion 393.1445 [M+H]⁺. Measured mass: 393.1450; IR ν_{\max} cm⁻¹ 3364, 1612, 1539, 1326, 1286, 1241, 827; ¹H-NMR (270 MHz; d₆-DMSO) δ_{H} 11.14 (1H, broad, s, OH), 9.78 (1H, s, NH), 8.04 (1H, d, *J* = 3.0 Hz, Ar-H), 7.98 (1H, dd, *J* = 8.9 and 3.0 Hz, Ar-H), 7.49-7.28 (7H, m, Ar-H), 6.94 (3H, t, *J* = 8.5 Hz, Ar-H), 5.03 (2H, s, CH₂O), 2.88 (2H, t, *J* = 7.6 Hz, CH₂CH₂CO), 2.58 (2H, t, *J* = 7.6 Hz, CH₂CO); ¹³C-NMR (68 MHz; d₆-DMSO) δ_{C} 170.3 (C=O), 162.5 (Ar-C), 154.7 (Ar-C), 139.9 (Ar-C), 137.8 (Ar-C), 133.1 (Ar-C), 129.2 (Ar-C), 129.0 (2 x Ar-C), 128.3 (Ar-C), 128.2 (2 x Ar-C), 126.1 (Ar-C), 124.5 (Ar-C), 121.2 (2 x Ar-C), 115.6 (Ar-C), 115.4 (2 x Ar-C), 69.9 (CH₂O), 35.9 (CH₂), 25.8 (CH₂).

7.1.2.6. (S)-Methyl 2-[3-(2-Hydroxy-5-nitrophenyl)propanamido]-2-phenyl-2-carboxylate 15g. To a stirred solution of compound **14** (0.50 g, 2.59 mmol) in THF (20 mL) was added NaHCO₃ (0.23 g, 2.74 mmol) and (S)-(+)-2-phenylglycine methyl ester hydrochloride (0.52 g, 2.59 mmol). The resulting mixture was stirred at reflux for 3 h, filtered and was evaporated. The residue was recrystallized from ethanol/water affording compound **15g** (0.80 g, 86%) as a light yellow powder, m.p. 147-149 °C. HRMS (NSI) for C₁₈H₁₈N₂O₆. Calculated mass of molecular ion 359.1238 [M+H]⁺. Measured mass: 359.1242; IR ν_{\max} cm⁻¹ 3360, 3330-2750, 1729, 1622, 1538, 1336, 1287; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 8.79 (1H, d, *J* = 7.3 Hz, *NH*), 8.03-7.97 (2H, m, *Ar-H*), 7.40-7.30 (5H, m, *Ar-H*), 6.95 (1H, d, *J* = 8.7 Hz, *Ar-H*), 5.42 (1H, d, *J* = 6.9 Hz, *CHNH*), 3.61 (3H, s, *CH*₃), 2.83 (2H, t, *J* = 7.8 Hz, *CH*₂*CH*₂*CO*), 2.55-2.50 (2H, m, *CH*₂*CO*); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.0 (*C=O*), 171.6 (*C=O*), 162.6 (*Ar-C*), 139.8 (*Ar-C*), 136.8 (*Ar-C*), 129.2 (2 x *Ar-C*), 129.1 (*Ar-C*), 128.7 (2 x *Ar-C*), 128.2 (*Ar-C*), 126.1 (*Ar-C*), 124.4 (*Ar-C*), 115.5 (*Ar-C*), 56.7 (*CHNH*), 52.7 (*CH*₃), 34.4 (*CH*₂), 25.7 (*CH*₂).

7.1.2.7. 3-(2-Hydroxy-5-nitrophenyl)-*N*-(4-pyridylmethyl)propanamide 15h. To a stirred solution of compound **14** (1.00 g, 5.18 mmol) in THF (30 mL) was added 4-(aminomethyl)pyridine (0.56 g, 5.18 mmol). The resulting mixture was stirred at reflux for 2 h and then evaporated. The residue was recrystallized from ethanol (30 mL) affording compound **15h** (1.49 g, 96%) as light orange crystals, m.p. 230-233 °C. HRMS (NSI) for C₁₅H₁₅N₃O₄. Calculated mass of molecular ion 302.1135 [M+H]⁺. Measured mass: 302.1139; IR ν_{\max} cm⁻¹ 3318, 3250-2700, 1650, 1587, 1538, 1326, 1287, 1014; ¹H-NMR (270 MHz; d₆-DMSO) δ_{H} 11.15 (1H, broad, s, *OH*), 8.52 (1H, t, *J* = 5.9 Hz, *NH*), 8.42 (2H, d, *J* = 5.9 Hz, *Ar-H*), 8.05 (2H, m, *Ar-H*), 7.12 (2H, d, *J* = 5.9 Hz, *Ar-H*), 6.99 (1H, d, *J* = 9.2 Hz, *Ar-H*), 4.29 (2H, d, *J* = 5.9 Hz, *CH*₂*NH*), 2.89 (2H, t, *J* = 7.4 Hz, *CH*₂*CH*₂*CO*), 2.55 (2H, t, *J* = 7.4 Hz, *CH*₂*CO*); ¹³C-NMR (68 MHz; d₆-DMSO) δ_{C} 172.2 (*C=O*), 162.6 (*Ar-C*), 149.9 (2 x *Ar-C*), 149.1 (*Ar-C*), 139.9 (*Ar-C*), 129.1 (*Ar-C*), 126.2 (*Ar-C*), 124.5 (*Ar-C*), 122.5 (2 x *Ar-C*), 115.6 (*Ar-C*), 41.6 (*CH*₂*NH*), 34.8 (*CH*₂), 25.9 (*CH*₂).

7.1.2.8. 3-(2-Hydroxy-5-nitrophenyl)-*N*-(3-imidazol-1-ylpropyl)propanamide 15i. To a stirred solution of compound **14** (1.00 g, 5.18 mmol) in THF (30 mL) was added 1-(3-aminopropyl)imidazole (0.65 g, 5.18 mmol). The resulting mixture was stirred at reflux for 5 h and then was evaporated. The residue was recrystallized from methanol (15 mL) affording compound **15i** (1.07 g, 65%) as a yellow powder, m.p. 157-158 °C. HRMS (NSI) for C₁₅H₁₈N₄O₄. Calculated mass of molecular ion 319.1401 [M+H]⁺. Measured mass: 319.1404; IR ν_{\max} cm⁻¹ 3313, 3150-2400, 1640, 1492, 1330, 1288, 1228, 1082, 740; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 8.04-7.94 (3H, m, 2 x *Ar-H*, *NH*), 7.62 (1H, s, *Ar-H*), 7.15 (1H, s, *Ar-H*), 6.95 (1H, d, *J* = 8.7 Hz, *Ar-H*), 6.90 (1H, s, *Ar-H*), 3.90 (2H, t, *J* = 6.9 Hz, *CH*₂*CH*₂*CH*₂*NH*), 3.00 (2H, q, *J* = 6.4 Hz, *CH*₂*CH*₂*CH*₂*NH*), 2.84 (2H, t, *J* = 7.3 Hz, *CH*₂), 2.43 (2H, t, *J* = 7.3 Hz, *CH*₂), 1.79 (2H, p, *J* = 6.9 Hz, *CH*₂*CH*₂*CH*₂*NH*); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 171.9 (*C=O*), 162.9 (*Ar-C*), 139.6 (*Ar-C*), 137.8 (*Ar-C*), 129.2 (*Ar-C*), 128.7 (*Ar-C*), 126.0 (*Ar-C*), 124.4 (*Ar-C*), 119.8 (*Ar-C*), 115.4 (*Ar-C*), 44.0 (*CH*₂), 36.1 (*CH*₂), 34.9 (*CH*₂), 31.3 (*CH*₂), 25.9 (*CH*₂).

7.1.2.9. 3-(2-Hydroxy-5-nitrophenyl)-N-[4-[3-(2-hydroxy-5-nitrophenyl)propanamido]phenyl]propanamide 28a. To a stirred solution compound **14** (1.00 g, 5.18 mmol) in THF (40 mL), NaHCO₃ (0.44 g, 5.18 mmol) and *para*-phenylenediamine dihydrochloride (0.47 g, 2.59 mmol) were added. The mixture was stirred at reflux for 48 hours. The mixture was allowed to cool, filtered and the solid was washed well with water and then dried in a desiccator under vacuum giving compound **28a** (1.10 g, 85%) as a white powder, m.p. > 260 °C. LRMS (ES) for C₂₄H₂₂N₄O₈. Calculated mass of molecular ion 495.14 [M+H]⁺. Measured mass: 495.32; IR ν_{\max} cm⁻¹ 3368, 3134, 1634, 1562, 1331, 1276, 1084; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 11.46 (2H, broad, s, 2 x OH), 10.05 (2H, s, 2 x NH), 8.06 (2H, d, *J* = 3.2 Hz, Ar-H), 8.00 (2H, dd, *J* = 9.2 and 2.8 Hz, Ar-H), 7.51 (4H, s, Ar-H), 7.12 (2H, d, *J* = 8.7 Hz, Ar-H), 2.90 (4H, t, *J* = 7.6 Hz, 2 x CH₂CH₂CO), 2.63 (4H, t, *J* = 7.6 Hz, 2 x CH₂CO); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 170.5 (2 x C=O), 162.7 (2 x Ar-C), 139.7 (2 x Ar-C), 135.0 (2 x Ar-C), 129.1 (2 x Ar-C), 126.1 (2 x Ar-C), 124.5 (2 x Ar-C), 120.0 (4 x Ar-C), 115.5 (2 x Ar-C), 35.9 (2 x CH₂), 25.8 (2 x CH₂).

7.1.2.10. 3-(2-Hydroxy-5-nitrophenyl)-N-[2-[3-(2-hydroxy-5-nitrophenyl)propanamido]ethyl]propanamide 28b. To a stirred solution of compound **14** (1.50 g, 7.77 mmol) in THF (50 mL), ethylenediamine (0.24 g, 3.99 mmol) was added and the resulting mixture was stirred at reflux for 5 h. The volume was then reduced and the solution was left to cool overnight. The resulting solid was collected and dried affording compound **28b** (1.70 g, 98%) as a yellow powder, m.p. > 260 °C. HRMS (NSI) for C₂₀H₂₂N₄O₈. Calculated mass of molecular ion 445.1365 [M-H]⁻. Measured mass: 445.1360; IR ν_{\max} cm⁻¹ 3370, 3300-2500, 1622, 1583, 1540, 1326, 1286, 1240, 751; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 7.95-7.91 (4H, m, Ar-H), 7.89-7.85 (2H, m, 2 x NH), 6.87 (2H, d, *J* = 9.2 Hz, Ar-H), 3.30-2.97 (4H, m, 2 x CH₂NH), 2.76 (4H, t, *J* = 7.6 Hz, 2 x CH₂CH₂CO), 2.32 (4H, t, *J* = 7.6 Hz, 2 x CH₂CO); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.0 (2 x C=O), 163.4 (2 x Ar-C), 139.3 (2 x Ar-C), 129.3 (2 x Ar-C), 126.0 (2 x Ar-C), 124.5 (2 x Ar-C), 115.6 (2 x Ar-C), 38.8 (2 x CH₂NH), 35.0 (2 x CH₂), 25.9 (2 x CH₂).

7.1.2.11. *tert*-Butyl N-[2-[3-(2-hydroxy-5-nitrophenyl)propanamido]ethyl]carbamate 32. To a stirred solution of compound **14** (1.00 g, 5.18 mmol) in THF (50 mL), *N*-Boc-ethylenediamine (0.83 g, 5.18 mmol) was added. The resulting mixture was stirred at reflux for 12 h. The volume of the reaction mixture was reduced and the solution was then left to cool overnight. The solid that crystallized was collected and dried giving compound **32** (1.39 g, 76%) as light yellow crystals, m.p. 209-211 °C. HRMS (NSI) for C₁₆H₂₃N₃O₆. Calculated mass of molecular ion 376.1479 [M+Na]⁺. Measured mass: 376.1483; IR ν_{\max} cm⁻¹ 3372, 3340, 1684, 1584, 1540, 1488, 1326, 1278, 1252, 1159; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 11.15 (1H, broad, s, OH), 7.95-7.91 (2H, m, Ar-H), 7.88 (1H, t, *J* = 5.5 Hz, NH), 6.95 (1H, d, *J* = 9.6 Hz, Ar-H), 6.73 (1H, t, *J* = 5.5 Hz, NH), 3.00 (2H, q, *J* = 6.2 Hz, CH₂NH), 2.89 (2H, q, *J* = 6.2 Hz, CH₂NH), 2.76 (2H, t, *J* = 7.6 Hz, CH₂CH₂CO), 2.49 (2H, t, *J* = 7.6 Hz, CH₂CO), 1.32 (9H, s, Boc); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.0 (C=O), 162.5 (Ar-C), 156.1 (C=O), 139.8 (Ar-C), 129.2 (Ar-C), 126.0 (Ar-C), 124.3 (Ar-C), 115.5 (Ar-C), 78.1 (C(CH₃)₃), 40.3 (CH₂NH, signal obscured by d₆-DMSO signal, re-appears by DEPT), 39.2 (CH₂NH), 35.0 (CH₂), 28.7 (C(CH₃)₃), 25.9 (CH₂).

7.1.2.12. 1-Hydroxy-N-[2-[3-(2-hydroxy-5-nitrophenyl)propanamido]ethyl]naphthalene-2-carboxamide 34. A mixture of compound **33** (1.00 g, 3.46 mmol), phenyl-1-hydroxy-2-naphthoate (0.92 g, 3.46 mmol) and NaHCO₃ (0.29 g, 3.46 mmol) in THF/DMF (50 mL, 2:1) was heated at reflux for 72 h. The solvents were evaporated and the residue was recrystallized from ethanol/methanol (40 mL, 50:50) giving compound **34** (1.40 g, 96%) as a yellow powder, m.p. 232 °C. HRMS (NSI) for C₂₂H₂₁N₃O₆. Calculated mass of molecular ion 446.1323 [M+Na]⁺. Measured mass: 446.1321; IR ν_{max} cm⁻¹ 3368, 3300-2500, 1640, 1581, 1538, 1333, 1276, 1285, 1253, 756; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 9.22 (1H, m, NH), 8.30-8.22 (2H, m, NH, Ar-H), 8.01-7.90 (3H, m, Ar-H), 7.87 (1H, d, J = 8.2 Hz, Ar-H), 7.66-7.60 (1H, m, Ar-H), 7.57-7.52 (1H, m, Ar-H), 7.37 (1H, d, J = 8.7 Hz, Ar-H), 7.04 (1H, d, J = 9.2 Hz, Ar-H), 3.40 (2H, q, J = 6.4 Hz, CH₂NH), 3.30 (2H, q, J = 6.0 Hz, CH₂NH), 2.84 (2H, t, J = 7.8 Hz, CH₂), 2.43 (2H, t, J = 7.8 Hz, CH₂); ¹³C-NMR (101 MHz; d₆-DMSO) δ_C 172.3 (C=O), 171.1 (C=O), 163.3 (Ar-C), 160.2 (Ar-C), 139.3 (Ar-C), 136.3 (Ar-C), 129.2 (2 x Ar-C), 128.0 (Ar-C), 126.2 (Ar-C), 126.0 (Ar-C), 125.3 (Ar-C), 124.4 (Ar-C), 123.5 (Ar-C), 123.4 (Ar-C), 117.9 (Ar-C), 115.6 (Ar-C), 107.6 (Ar-C), 39.6 (CH₂NH, signal obscured by d₆-DMSO signal, re-appears by DEPT), 38.6 (CH₂), 35.1 (CH₂), 26.0 (CH₂).

7.1.3. Synthesis of Aminophenols **11**, **29** and **35**.

7.1.3.1. General Procedure for Lithium Formate Reductions. To a stirred solution of the appropriate compound **15** (1 equiv.) in either THF or THF/ethanol, was added lithium formate (6 equiv. per nitro-group) and 10% palladium on activated carbon catalyst. The mixture was stirred at reflux for 1 h in air unless otherwise stated. The reaction was monitored by spotting the reaction mixture periodically onto filter paper and observing the disappearance (2–8 h) of the yellow colour associated with compound **15**. The reaction mixture was then filtered rapidly while hot and the filtrate was allowed to cool and kept overnight. If the product had crystallized, it was collected. Alternatively, the solvent volume was reduced or completely evaporated and the crude product was recrystallized from an appropriate solvent.

7.1.3.1.1 Ethyl 3-(5-Amino-2-hydroxyphenyl)propanoate 11a. Reduction of compound **15a** (1.00 g, 4.18 mmol) with lithium formate (1.76 g, 25.08 mmol) and 10% Pd/C (0.30 g) in THF (40 mL) gave compound **11a** (0.58 g, 66%) as light brown crystals after evaporation of the solvent and recrystallization of the residue from ethanol, m.p. 142-143 °C, lit. m.p. 144 °C.¹⁶ LRMS (ES) for C₁₁H₁₅NO₃. Calculated mass of molecular ion 210.25 [M+H]⁺. Measured mass: 209.96; IR ν_{max} cm⁻¹ 3350, 3300-2700, 1715, 1455, 1189, 931, 864; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 8.34 (1H, s, OH), 6.48 (1H, d, J = 8.24 Hz, Ar-H), 6.32 (1H, d, J = 2.3, Ar-H), 6.26 (1H, dd, J = 8.2 and 2.8 Hz, Ar-H), 4.35 (2H, s, broad, NH₂), 4.04 (2H, q, J = 7.2 Hz, CH₂CH₃), 2.65 (2H, t, J = 7.6 Hz, CH₂CH₂CO), 2.48 (2H, t, J = 7.6 Hz, CH₂CO), 1.17 (3H, t, J = 7.1 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_C 173.1 (C=O), 146.5 (Ar-C), 141.2 (Ar-C), 127.3 (Ar-C), 116.5 (Ar-C), 116.0 (Ar-C), 113.5 (Ar-C), 60.2 (CH₂CH₃), 34.3 (CH₂), 26.4 (CH₂), 14.7 (CH₃).

7.1.3.1.2. Ethyl 3-(5-Amino-3-bromo-2-hydroxyphenyl)propanoate 11b. A mixture of compound **15b** (2.00 g, 6.29 mmol) and tin(II) chloride dihydrate (7.15 g, 37.70 mmol) in

ethanol (80 mL) was heated at reflux for 3 h. The mixture was allowed to cool to rt and then neutralised by the addition concentrated aqueous sodium hydroxide solution. The mixture was filtered the filtrate was evaporated. The resulting oily residue was then stirred with diethyl ether (40 mL) overnight and then filtered. The filtrate was evaporated giving compound **11b** (1.62 g, 89%) as a light orange oil which crystallized on standing, m.p. 60-62 °C. HRMS (NSI) for C₁₁H₁₄BrNO₃. Calculated mass of molecular ion 288.0230 [M+H]⁺. Measured mass: 288.0237; IR ν_{\max} cm⁻¹ 3318, 3300-2600, 1698, 1475, 1445, 1175; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.67 (1H, s, broad, OH), 9.44 (2H, broad, s, NH₂), 7.33 (1H, d, *J* = 2.3 Hz, Ar-*H*), 7.05 (1H, d, *J* = 2.3 Hz, Ar-*H*), 4.05 (2H, q, *J* = 7.9 Hz, CH₂O), 2.88 (2H, t, *J* = 7.6 Hz, CH₂CH₂CO), 2.56 (2H, t, *J* = 7.6 Hz, CH₂CCO), 1.17 (3H, t, *J* = 7.1 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.6 (C=O), 150.9 (Ar-C), 131.6 (Ar-C), 126.7 (Ar-C), 124.7 (Ar-C), 123.5 (Ar-C), 112.0 (Ar-C), 60.5 (CH₂CH₃), 33.6 (CH₂), 26.5 (CH₂), 14.7 (CH₃).

7.1.3.1.3 3-(5-Amino-2-hydroxyphenyl)-*N*-(2*H*-1,3-benzodioxol-5-yl)propanamide **11c**.

Reduction of compound **15c** (1.00 g, 3.03 mmol) with lithium formate (1.27 g, 18.16 mmol) and 10% Pd/C (0.40 g) in THF (30 mL) gave compound **11c** (0.80 g, 88%) as a light brown powder, m.p. 153-155 °C. HRMS (NSI) for C₁₆H₁₆N₂O₄. Calculated mass of molecular ion 301.1183 [M+H]⁺. Measured mass: 301.1185; IR ν_{\max} cm⁻¹ 3420, 3300-2800, 1649, 1498, 1447, 1213, 1039, 794, 723; ¹H-NMR (270 MHz; d₆-DMSO) δ_{H} 9.85 (1H, s, NH), 8.38 (1H, broad, s, OH), 7.37 (1H, d, *J* = 2.0 Hz, Ar-*H*), 7.00 (1H, dd, *J* = 8.4 and 2.2 Hz, Ar-*H*), 6.88 (1H, d, *J* = 8.4 Hz, Ar-*H*), 6.55 (1H, d, *J* = 8.4 Hz, Ar-*H*), 6.42 (1H, d, *J* = 2.7 Hz, Ar-*H*), 6.31 (1H, dd, *J* = 8.4 and 2.7 Hz, Ar-*H*), 6.02 (2H, s, CH₂O), 4.39 (2H, broad, s, NH₂), 2.75 (2H, t, *J* = 7.8 Hz, CH₂CH₂CO), 2.54 (2H, m, CH₂CO); ¹³C-NMR (68 MHz; d₆-DMSO) δ_{C} 171.2 (C=O), 147.5 (Ar-C), 146.5 (Ar-C), 143.2 (Ar-C), 141.3 (Ar-C), 134.3 (Ar-C), 128.1 (Ar-C), 116.6 (Ar-C), 116.1 (Ar-C), 113.5 (Ar-C), 112.4 (Ar-C), 108.5 (Ar-C), 101.9 (Ar-C), 101.4 (CH₂O), 37.2 (CH₂), 26.5 (CH₂).

7.1.3.1.4 3-(5-Amino-2-hydroxyphenyl)-*N*-(3,4-dimethoxyphenyl)propanamide **11d**.

Reduction of the compound **15d** (1.00 g, 2.89 mmol) with lithium formate (1.22 g, 17.32 mmol) and 10% Pd/C (0.30 g) in THF (50 mL) gave compound **11d** (0.61 g, 67%) as brown crystals, m.p. 146-148 °C. HRMS (NSI) for C₁₇H₂₀N₂O₄. Calculated mass of molecular ion 317.1496 [M+H]⁺. Measured mass: 317.1500; IR ν_{\max} cm⁻¹ 3341, 3300-2700, 1652, 1513, 1461, 1402, 1214, 1133, 742; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.75 (1H, s, NH), 8.33 (1H, s, OH), 7.32 (1H, d, *J* = 2.8 Hz, Ar-*H*), 7.10 (1H, dd, *J* = 8.7 and 2.4 Hz, Ar-*H*), 6.87 (1H, d, *J* = 9.2 Hz, Ar-*H*), 6.51 (1H, d, *J* = 8.2 Hz, Ar-*H*), 6.38 (1H, d, *J* = 2.3 Hz, Ar-*H*), 6.27 (1H, dd, *J* = 8.2 and 2.8 Hz, Ar-*H*), 4.35 (2H, s, broad, NH₂), 3.72 (2 x 3H, d, *J* = 5.0 Hz, 2 x CH₃), 2.71 (2H, t, *J* = 7.6 Hz, CH₂CH₂CO), 2.51 (2H, m, CH₂CO); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 171.5 (C=O), 149.0 (Ar-C), 146.5 (Ar-C), 145.1 (Ar-C), 141.2 (Ar-C), 133.5 (Ar-C), 128.1 (Ar-C), 116.6 (Ar-C), 116.1 (Ar-C), 113.4 (Ar-C), 112.6 (Ar-C), 111.5 (Ar-C), 104.9 (Ar-C), 56.2 (CH₃), 55.8 (CH₃), 37.1 (CH₂), 26.6 (CH₂).

7.1.3.1.5 3-(5-Amino-2-hydroxyphenyl)-*N*-(4-hydroxyphenyl)propanamide **11f**.

Reduction of compound **15e** (0.45 g, 1.15 mmol) with lithium formate (0.48 g, 6.90 mmol) and 10% Pd/C (0.15 g) in THF (25 mL) gave compound **11f** (0.25 g, 80%) as a brown

powder, m.p. 112-114 °C. LRMS (ES) for C₁₅H₁₆N₂O₃. Calculated mass of molecular ion 273.30 [M+H]⁺. Measured mass: 273.31; IR ν_{\max} cm⁻¹ 3319, 3200-2900, 1738, 1640, 1586, 1370, 1209, 789; ¹H-NMR (270 MHz; d₆-DMSO) δ_{H} 9.61 (1H, s, NH), 9.14 (1H, s, OH), 8.31 (1H, s, OH), 7.35 (2H, m, Ar-H), 6.66 (2H, m, Ar-H), 6.48 (1H, d, *J* = 8.4 Hz, Ar-H), 6.35 (1H, d, *J* = 2.7 Hz, Ar-H), 6.24 (3H, dd, *J* = 8.4 and 2.7 Hz, Ar-H), 4.35 (2H, broad, s, NH₂), 2.68 (2H, t, *J* = 7.7 Hz, CH₂CH₂CO), 2.45 (2H, m, CH₂CO); ¹³C-NMR (68 MHz; d₆-DMSO) δ_{C} 170.9 (C=O), 153.7 (Ar-C), 146.5 (Ar-C), 141.3 (Ar-C), 131.6 (Ar-C), 128.3 (Ar-C), 121.5 (2 x Ar-C), 116.6 (Ar-C), 116.2 (Ar-C), 115.5 (2 x Ar-C), 113.4 (Ar-C), 37.1 (CH₂), 26.6 (CH₂).

7.1.3.1.6. (S)-Methyl 2-[3-(5-Amino-2-hydroxyphenyl)propanamido]-2-phenyl-2-carboxylate **11g**.

Reduction of compound **15g** (0.6 g, 1.67 mmol) with lithium formate (0.7 g, 10.00 mmol) and 10% Pd/C (0.15 g) in THF (30 mL) gave compound **11g** (0.50 g, 91%) as pink crystals, m.p. 175-177 °C. HRMS (NSI) for C₁₈H₂₀N₂O₄. Calculated mass of molecular ion 329.1496 [M+H]⁺. Measured mass: 329.1491; IR ν_{\max} cm⁻¹ 3318, 3300-2700, 1737, 1644, 1515, 1207, 696; ¹H-NMR (270 MHz; d₆-DMSO) δ_{H} 8.70 (1H, d, *J* = 7.2 Hz, NH), 8.26 (1H, s, OH), 7.36 (5H, m, Ar-H), 6.46 (1H, d, *J* = 8.4 Hz, Ar-H), 6.32 (1H, d, *J* = 2.7 Hz, Ar-H), 6.23 (1H, dd, *J* = 8.2 and 2.7 Hz, Ar-H), 5.41 (1H, d, *J* = 7.2 Hz, CH), 4.30 (2H, broad, s, NH₂), 3.61 (3H, s, CH₃), 2.79 (2H, m, CH₂CH₂CO), 2.51 (2H, t, *J* = 9.40 Hz, CH₂CO); ¹³C-NMR (68 MHz; d₆-DMSO) δ_{C} 172.7 (C=O), 171.8 (C=O), 146.5 (Ar-C), 141.3 (Ar-C), 136.9 (Ar-C), 129.2 (Ar-C), 128.8 (Ar-C), 128.4 (Ar-C), 128.2 (Ar-C), 116.5 (Ar-C), 116.1 (Ar-C), 113.4 (Ar-C), 56.8 (CHNH), 52.8 (CH₃), 35.6 (CH₂), 26.5 (CH₂).

7.1.3.1.7. 3-(5-Amino-2-hydroxyphenyl)-N-(4-pyridylmethyl)propanamide **11h**.

Reduction of compound **15h** (1.0 g, 3.32 mmol) with lithium formate (1.40 g, 19.90 mmol) and 10% Pd/C (0.45 g) in THF/EtOH (8:1, 45 mL) gave compound **11h** (0.88 g, 98%) as a brown oil after evaporation of the solvent. The oil gradually crystallized on standing giving a brown solid, m.p. 114-116 °C. HRMS (NSI) for C₁₅H₁₇N₃O₂. Calculated mass of molecular ion 272.1394 [M+H]⁺. Measured mass: 272.1393; IR ν_{\max} cm⁻¹ 3309, 3300-2700, 1638, 1542, 1505, 1421, 1216; ¹H-NMR (270 MHz; d₆-DMSO) δ_{H} 8.60-8.30 (4H, m, NH, OH, 2 x Ar-H), 7.13 (2H, d, *J* = 5.7 Hz, Ar-H), 6.49 (1H, d, *J* = 5.7 Hz, Ar-H), 6.34 (1H, d, *J* = 2.7 Hz, Ar-H), 6.27 (1H, dd, *J* = 8.2 and 2.6 Hz, Ar-H), 4.5-4.27 (2H, broad, s, NH₂), 4.26 (2H, d, *J* = 5.9 Hz, CH₂NH), 2.67 (2H, t, *J* = 7.6 Hz, CH₂CH₂CO), 2.40 (2H, t, *J* = 7.6 Hz, CH₂CO); ¹³C-NMR (68 MHz; d₆-DMSO) δ_{C} 172.9 (C=O), 150.0 (2 x Ar-C), 149.2 (Ar-C), 146.6 (Ar-C), 141.3 (Ar-C), 128.1 (Ar-C), 122.6 (Ar-C), 116.7 (Ar-C), 116.2 (Ar-C), 113.4 (Ar-C), 41.6 (CH₂NH), 36.2 (CH₂), 26.8 (CH₂).

7.1.3.1.8. 3-(5-Amino-2-hydroxyphenyl)-N-(3-imidazol-1-ylpropyl)propanamide **11i**.

Reduction of compound **15i** (1.6 g, 5.03 mmol) with lithium formate (2.1 g, 30.10 mmol) and 10% Pd/C (0.45 g) in THF/EtOH (8:1, 45 mL) gave compound **11i** (1.26 g, 87%) as a brown solid, m.p. 125-126 °C. LRMS (E.S) for C₁₅H₂₀N₄O₂. Calculated mass of molecular ion 295.28 [M+Li]⁺. Measured mass: 295.22; IR ν_{\max} cm⁻¹ 3401, 3280, 3100-2500, 1660, 1511, 1436, 1227, 1084, 827; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 8.32 (1H, broad, s, OH), 7.88 (1H, t, *J* = 5.5 Hz, NH), 7.60 (1H, s, Ar-H), 7.16 (1H, s, Ar-H), 6.88 (1H, s, Ar-H), 6.47 (1H, d, *J* = 8.2 Hz, Ar-H), 6.32 (1H, d, *J* = 2.8 Hz, Ar-H), 6.25 (1H, dd, *J* = 8.2 and 2.8 Hz, Ar-H),

4.33 (2H, s, broad, NH_2), 3.90 (2H, t, $J = 6.9$ Hz, $CH_2CH_2CH_2NH$), 2.99 (2H, q, $J = 6.9$ Hz, $CH_2CH_2CH_2NH$), 2.62 (2H, t, $J = 7$ Hz, CH_2), 2.30 (2H, t, $J = 7.6$ Hz, CH_2), 1.79 (2H, p, $J = 6.9$ Hz, $CH_2CH_2CH_2NH$); ^{13}C -NMR (101 MHz; d_6 -DMSO) δ_C 172.6 (C=O), 146.4 (Ar-C), 141.2 (Ar-C), 137.8 (Ar-C), 128.9 (Ar-C), 128.3 (Ar-C), 119.9 (Ar-C), 116.5 (Ar-C), 116.1 (Ar-C), 113.3 (Ar-C), 44.1 (CH_2), 36.3 (CH_2), 36.2 (CH_2), 31.3 (CH_2), 26.6 (CH_2).

7.1.3.1.9. 3-(5-Amino-2-hydroxyphenyl)-*N*-[4-[3-(5-amino-2-

hydroxyphenyl)propanamido]phenyl]propanamide **29a**. Reduction of compound **28a**

(1.00 g, 2.02 mmol) with lithium formate (0.85 g, 12.13 mmol) and 10% Pd/C (0.70 g) in THF/DMF (30 mL, 2:1) gave compound **29a** (0.60 g, 68%) as a brown powder, m.p. > 220 °C. HRMS (NSI) for $C_{24}H_{26}N_4O_4$. Calculated mass of molecular ion 435.2027 $[M+H]^+$.

Measured mass: 435.2028; IR ν_{max} cm^{-1} 3280, 3200-2500, 1657, 1544, 1512, 1401, 1217, 812, 704; 1H -NMR (400 MHz; d_6 -DMSO) δ_H 9.77 (2H, s, 2 x NH), 8.28 (2H, broad, s, 2 x OH), 7.46 (4H, s, Ar- H), 6.45 (2H, d, $J = 8.2$ Hz, Ar- H), 6.33 (2H, d, $J = 2.8$ Hz, Ar- H), 6.22 (2H, dd, $J = 8.2$ and 2.8 Hz, Ar- H), 4.33 (4H, broad, s, 2 x NH_2), 2.66 (4H, t, $J = 7.8$ Hz, 2 x CH_2CH_2CO), 2.46 (4H, m, 2 x CH_2CO); ^{13}C -NMR (101 MHz; d_6 -DMSO) δ_C 171.2 (2 x C=O), 146.5 (2 x Ar-C), 141.2 (2 x Ar-C), 135.1 (2 x Ar-C), 128.1 (2 x Ar-C), 120.0 (4 x Ar-C), 116.6 (2 x Ar-C), 116.1 (2 x Ar-C), 113.5 (2 x Ar-C), 37.1 (2 x CH_2), 26.5 (2 x CH_2).

7.1.3.1.10. 3-(5-Amino-2-hydroxyphenyl)-*N*-[2-[3-(5-amino-2-

hydroxyphenyl)propanamido]ethyl]propanamide **29b**. Reduction of compound **28b** (1.30

g, 2.91 mmol) with lithium formate (1.22 g, 17.44 mmol) and 10% Pd/C (0.80 g) in THF/DMF (50 mL, 2:1) gave compound **29b** (0.85 g, 75.5%) as a dark powder, m.p. 243-244 °C. LRMS (ES) for $C_{20}H_{26}N_4O_4$. Calculated mass of molecular ion 387.44 $[M+H]^+$.

Measured mass: 387.06; IR ν_{max} cm^{-1} 3317, 3200-2500, 1634, 1565, 1286, 1238; 1H -NMR (400 MHz; d_6 -DMSO) δ_H 8.08-8.01 (2H, m, 2 x NH), 7.81-7.77 (4H, m, Ar- H), 6.32 (2H, d, $J = 8.7$ Hz, Ar- H), 3.92 (4H, broad, s, 2 x NH_2), 3.09-3.03 (4H, m, 2 x CH_2NH), 2.67 (4H, t, $J = 7.6$ Hz, 2 x CH_2CH_2CO), 2.33 (4H, t, $J = 7.6$ Hz, 2 x CH_2CO); ^{13}C -NMR (101 MHz; d_6 -DMSO) δ_C 174.9 (2 x C=O), 172.8 (2 x Ar-C), 131.4 (2 x Ar-C), 130.2 (2 x Ar-C), 126.0 (2 x Ar-C), 125.9 (2 x Ar-C), 117.9 (2 x Ar-C), 38.9 (2 x CH_2NH), 35.9 (2 x CH_2), 26.6 (2 x CH_2).

7.1.3.1.11. 1-Hydroxy-*N*-[2-[3-(5-amino-2-

hydroxyphenyl)propanamido]ethyl]naphthalene-2-carboxamide **35**. Compound **34**

(0.90 g, 2.13 mmol) was reduced with lithium formate (0.90 g, 12.78 mmol) and 10% Pd/C (0.50 g) in THF/DMF (50 mL 2:1) under a nitrogen atmosphere. The reaction mixture was rapidly filtered and the solvent evaporated affording compound **35** (0.60 g, 72%) as a dark powder which was used directly in the synthesis of compound **36** without further characterisation.

7.1.4. Synthesis of Boc-protected amino acids **16**, **17**, **30** and **36**.

7.1.4.1. General procedure. The appropriate amine **11** (1 equiv. or 0.5 equiv. in the case of compounds **30**) was dissolved in dry THF or DMF and cooled to -5 °C in an ice/salt bath. In

a separate flask, to a stirred solution of Boc-L-alanine or Boc- β -alanine (1.05 equiv.) in dry THF and/or DMF was added *N*-methylmorpholine (1 equiv.) and the mixture was cooled to -5 °C. Isobutyl chloroformate (IBCF) (1 equiv.) was then added to this mixture and after stirring for 90 seconds at -5 °C, the previously prepared Boc-amino acid solution was added. The resulting mixture was stirred at -5 °C for 1 h and then at rt overnight. The solvent was evaporated and the residue was dissolved in either dichloromethane or ethyl acetate. The organic phase was washed sequentially with 0.1 M citric acid solution, 10% aqueous sodium hydrogen carbonate solution and water. The organic layer was dried (MgSO₄) and evaporated giving the product.

7.1.4.1.1. Ethyl 3-(5-Amino-2-hydroxyphenyl)propanoate; Boc L-alanine derivative 16a.

Compound **16a** was synthesized from compound **11a** (0.21 g, 1.00 mmol), Boc-L-alanine (0.20 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF (20 mL). No further purification of the product was required. Yield: (0.31 g, 81%) of an orange solid, m.p. 70-72 °C. HRMS (NSI) for C₁₉H₂₈N₂O₆. Calculated mass of molecular ion 381.2020 [M+H]⁺. Measured mass: 381.2020; IR ν_{\max} cm⁻¹ 3319, 3300-3100, 1745, 1657, 1507, 1438, 1232, 1161; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.55 (1H, s, NH), 9.17 (1H, s, OH), 7.24-7.18 (2H, m, Ar-H), 6.94 (1H, d, *J* = 7.3 Hz, NH), 6.66 (1H, d, *J* = 8.7 Hz, Ar-H), 4.05-3.96 (3H, m, CH₂O, CH), 2.69 (2H, t, *J* = 7.6 Hz, CH₂CH₂CO), 2.51-2.44 (2H, m, CH₂CO), 1.33 (9H, s, 3 x CH₃), 1.18 (3H, d, *J* = 7.3 Hz, CH₃), 1.12 (3H, t, *J* = 7.1 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.9 (C=O), 171.6 (C=O), 155.6 (C=O), 151.6 (Ar-C), 131.2 (Ar-C), 126.9 (Ar-C), 121.8 (Ar-C), 119.2 (Ar-C), 115.2 (Ar-C), 78.5 (C(CH₃)₃), 60.3 (CH₂CH₃), 50.7 (CHCH₃), 34.0 (CH₂), 28.7 (C(CH₃)₃), 26.3 (CH₂), 18.7 (CHCH₃), 14.6 (CH₂CH₃).

7.1.4.1.2. Ethyl 3-(5-Amino-3-bromo-2-hydroxyphenyl)propanoate; Boc L-alanine derivative 16b.

Compound **16b** was synthesized from compound **11b** (0.58 g, 2.00 mmol), Boc-L-alanine (0.40 g, 2.10 mmol), *N*-methylmorpholine (0.20 g, 2.00 mmol) and IBCF (0.28 g, 2.00 mmol) in dry THF (30 mL). No further purification was required. Yield: (0.70 g, 76%) as an orange solid, m.p. 47 °C. HRMS (NSI) for C₁₉H₂₇BrN₂O₆. Calculated mass of molecular ion 459.1125 [M+H]⁺. Measured mass: 459.1133; IR ν_{\max} cm⁻¹ 3360, 3306, 2978, 1673, 1479, 1249, 1159; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.81 (1H, s, NH), 8.87 (1H, s, OH), 7.78 (1H, d, *J* = 2.3 Hz, Ar-H), 7.23 (1H, d, *J* = 2.1 Hz, Ar-H), 7.07 (1H, d, *J* = 7.3 Hz, NH), 4.07-4.01 (3H, m, CH₂O, CH), 2.83 (2H, t, *J* = 7.6 Hz, CH₂), 2.54 (2H, t, *J* = 7.6 Hz, CH₂), 1.38 (9H, s, 3 x CH₃), 1.22 (3H, d, *J* = 6.9 Hz, CH₃), 1.16 (3H, t, *J* = 7.3 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.7 (C=O), 172.1 (C=O), 155.7 (C=O), 147.8 (Ar-C), 132.9 (Ar-C), 130.6 (Ar-C), 121.7 (Ar-C), 120.8 (Ar-C), 111.6 (Ar-C), 78.6 (C(CH₃)₃), 60.4 (CH₂CH₃), 50.9 (CHCH₃), 33.9 (CH₂), 28.5 (C(CH₃)₃), 26.8 (CH₂), 18.5 (CHCH₃), 14.6 (CH₂CH₃).

7.1.4.1.3. 3-(5-Amino-2-hydroxyphenyl)-*N*-(2*H*-1,3-benzodioxol-5-yl)propanamide; Boc L-alanine derivative 16c.

Compound **16c** was synthesized from compound **11c** (0.30 g, 1.00 mmol), Boc-L-alanine (0.20 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF (20 mL). No further purification was required. Yield: (0.45 g, 95.5%) of white flakes, m.p. 178-179 °C. HRMS (NSI) for C₂₄H₂₉N₃O₇. Calculated

mass of molecular ion 472.2078 $[M+H]^+$. Measured mass: 472.2076; IR ν_{\max} cm^{-1} 3316, 3300-2750, 1678, 1504, 1234, 1183, 866, 798; $^1\text{H-NMR}$ (400 MHz; $\text{d}_6\text{-DMSO}$) δ_{H} 9.86 (1H, s, NH), 9.63 (1H, s, NH), 9.23 (1H, broad, s, OH), 7.32 (2H, d, $J = 1.3$ Hz, Ar-H), 7.25 (1H, dd, $J = 8.7$ and 2.8 Hz, Ar-H), 7.00 (1H, d, $J = 7.8$ Hz, NHCH), 6.95 (1H, dd, $J = 8.2$ and 1.8 Hz, Ar-H), 6.83 (1H, d, $J = 8.2$ Hz, Ar-H), 6.73 (1H, d, $J = 8.7$ Hz, Ar-H), 5.97 (2H, s, CH_2O), 4.07 (1H, p, $J = 7.3$ Hz, CH) 2.78 (2H, t, $J = 8.0$ Hz, $\text{CH}_2\text{CH}_2\text{CO}$), 2.53 (2H, m, CH_2CO), 1.38 (9H, s, 3 x CH_3), 1.23 (3H, d, $J = 7.3$ Hz, CH_3); $^{13}\text{C-NMR}$ (68 MHz; $\text{d}_6\text{-DMSO}$) δ_{C} 171.6 (C=O), 170.9 (C=O), 155.7 (C=O), 151.9 (Ar-C), 147.5 (Ar-C), 143.2 (Ar-C), 134.3 (Ar-C), 131.1 (Ar-C), 127.8 (Ar-C), 121.9 (Ar-C), 119.1 (Ar-C), 115.3 (Ar-C), 112.5 (Ar-C), 108.5 (Ar-C), 102.0 (Ar-C), 101.4 (CH_2O), 78.5 ($\text{C}(\text{CH}_3)_3$), 50.8 (CHCH_3), 36.9 (CH_2), 28.8 ($\text{C}(\text{CH}_3)_3$), 26.5 (CH_2), 18.8 (CHCH_3).

7.1.4.1.4. 3-(5-Amino-2-hydroxyphenyl)-N-(3,4-dimethoxyphenyl)propanamide; Boc L-alanine derivative 16d. Compound **16d** was synthesized from compound **11d** (0.32 g, 1.00 mmol), Boc-L-alanine (0.20 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF (20 mL). No further purification was required. Yield: (0.40 g, 81%) of light brown crystals, m.p. 86-88 °C. HRMS (NSI) for $\text{C}_{25}\text{H}_{33}\text{N}_3\text{O}_7$. Calculated mass of molecular ion 488.2391 $[M+H]^+$. Measured mass: 488.2391; IR ν_{\max} cm^{-1} 3281, 3250-2800, 1660, 1510, 1229, 1161, 1023; $^1\text{H-NMR}$ (400 MHz; $\text{d}_6\text{-DMSO}$) δ_{H} 9.77 (1H, s, NH), 9.61 (1H, s, broad, OH), 9.21 (1H, s, NH), 7.36-7.23 (3H, m, Ar-H, NH), 7.08 (1H, dd, $J = 8.7$ and 1.8 Hz, Ar-H), 6.98 (1H, d, $J = 7.3$ Hz, Ar-H), 6.86 (1H, d, $J = 8.7$ Hz, Ar-H), 6.72 (1H, d, $J = 8.7$ Hz, Ar-H), 4.03 (1H, p, $J = 6.6$ Hz, CH), 3.68 (2 x 3H, d, $J = 4.6$ Hz, 2 x CH_3), 2.79 (2H, t, $J = 7.8$ Hz, CH_2), 2.51 (2H, m, CH_2), 1.38 (9H, s, 3 x CH_3), 1.22 (3H, t, $J = 6.9$ Hz, CH_3); $^{13}\text{C-NMR}$ (101 MHz; $\text{d}_6\text{-DMSO}$) δ_{C} 171.6 (C=O), 170.8 (C=O), 155.6 (C=O), 151.6 (Ar-C), 149.0 (Ar-C), 145.1 (Ar-C), 133.5 (Ar-C), 131.2 (Ar-C), 127.8 (Ar-C), 121.9 (Ar-C), 119.1 (Ar-C), 115.2 (Ar-C), 112.5 (Ar-C), 111.5 (Ar-C), 104.9 (Ar-C), 78.5 ($\text{C}(\text{CH}_3)_3$), 56.2 (CH_3O), 55.8 (CH_3O), 50.7 (CHCH_3), 36.9 (CH_2), 28.7 ($\text{C}(\text{CH}_3)_3$), 26.5 (CH_2), 18.8 (CHCH_3).

7.1.4.1.5. 3-(5-Amino-2-hydroxyphenyl)-N-(4-hydroxyphenyl)propanamide; Boc L-alanine derivative 16f. Compound **16f** was synthesized from compound **11f** (0.60 g, 2.20 mmol), Boc-L-alanine (0.44 g, 2.30 mmol), *N*-methylmorpholine (0.22 g, 2.20 mmol) and IBCF (0.30 g, 2.20 mmol) in dry THF/DMF (25 mL, 4:1). No further purification was required. Yield: (0.45 g, 46%) of a white powder, m.p. 102-104 °C. LRMS (ES) for $\text{C}_{23}\text{H}_{29}\text{N}_3\text{O}_6$. Calculated mass of molecular ion 466.48 $[M+\text{Na}]^+$. Measured mass: 466.19; IR ν_{\max} cm^{-1} 3220, 3200-2470, 1651, 1510, 1227, 1112, 834; $^1\text{H-NMR}$ (400 MHz; $\text{d}_6\text{-DMSO}$) δ_{H} 9.65 (1H, s, NH), 9.60 (1H, s, NH), 9.19 (1H, s, broad, OH), 9.15 (1H, broad s, OH), 7.39-7.30 (3H, m, Ar-H), 7.25 (1H, dd, $J = 8.5$ and 2.5 Hz, Ar-H), 6.99 (1H, d, $J = 7.3$ Hz, NHCH), 6.72-6.65 (3H, m, Ar-H), 4.07 (1H, p, $J = 6.9$ Hz, CHNH), 2.78 (2H, t, $J = 7.8$ Hz, CH_2), 2.50 (2H, m, CH_2), 1.38 (9H, s, 3 x CH_3), 1.23 (3H, d, $J = 6.9$ Hz, CH_3); $^{13}\text{C-NMR}$ (101 MHz; $\text{d}_6\text{-DMSO}$) δ_{C} 171.6 (C=O), 170.5 (C=O), 155.6 (C=O), 153.6 (Ar-C), 151.6 (Ar-C), 131.5 (Ar-C), 131.2 (Ar-C), 127.9 (Ar-C), 121.9 (Ar-C), 121.5 (2 x Ar-C), 119.0 (Ar-C), 115.5 (2 x Ar-C), 115.2 (Ar-C), 78.5 ($\text{C}(\text{CH}_3)_3$), 50.74 (CHCH_3), 36.8 (CH_2), 28.7 ($\text{C}(\text{CH}_3)_3$), 26.6 (CH_2), 18.8 (CHCH_3).

7.1.4.1.6. (S)-Methyl 2-[3-(5-Amino-2-hydroxyphenyl)propanamido]-2-phenyl-2-carboxylate; Boc L-alanine derivative 16g. Compound **16g** was synthesized from compound **11g** (0.33 g, 1.00 mmol), Boc-L-alanine (0.20 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF (20 mL). No further purification was required. Yield: (0.45 g, 89%) of pale pink crystals, m.p. 82-84 °C. HRMS (NSI) for C₂₆H₃₃N₃O₇. Calculated mass of molecular ion 500.2391 [M+H]⁺. Measured mass: 500.2394; IR ν_{\max} cm⁻¹ 3304, 3300-2800, 1651, 1498, 1366, 1230, 1160, 1022, 697; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.59 (1H, s, NH), 9.16 (1H, s, OH), 8.73 (1H, d, *J* = 6.9 Hz, NH), 7.42-7.32 (5H, m, Ar-*H*), 7.25 (2H, m, Ar-*H*), 6.98 (1H, d, *J* = 7.3 Hz, NH), 6.69 (1H, d, *J* = 8.7 Hz, Ar-*H*), 5.42 (1H, d, *J* = 7.3 Hz, CH), 4.08 (1H, p, *J* = 7.1 Hz, CH), 3.62 (3H, s, CH₃), 2.70 (2H, m, CH₂), 2.44 (2H, t, *J* = 7.6 Hz, CH₂), 1.38 (9H, s, 3 x CH₃), 1.23 (3H, d, *J* = 7.3 Hz, CH₃); ¹³C-NMR (68 MHz; d₆-DMSO) δ_{C} 172.4 (C=O), 171.7 (C=O), 171.5 (C=O), 155.6 (C=O), 151.5 (Ar-C), 136.8 (Ar-C), 131.2 (Ar-C), 129.2 (2 x Ar-C), 128.7 (Ar-C), 128.3 (2 x Ar-C), 127.8 (Ar-C), 121.9 (Ar-C), 119.0 (Ar-C), 115.2 (Ar-C), 78.5 (C(CH₃)₃), 56.8 (CHNH), 52.7 (CH₃), 50.7 (CHCH₃), 35.4 (CH₂), 28.6 (C(CH₃)₃), 26.5 (CH₂), 18.8 (CHCH₃).

7.1.4.1.7. 3-(5-Amino-2-hydroxyphenyl)-*N*-(4-pyridylmethyl)propanamide; Boc L-alanine derivative 16h. Compound **16h** was synthesized from compound **11h** (0.27 g, 1.00 mmol), Boc-L-alanine (0.20 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF/DMF (20 mL, 3:1). No further purification was required. Yield: (0.30 g, 68%) as a pale pink powder, m.p. 115-117 °C. LRMS (ES) for C₂₃H₃₀N₄O₅. Calculated mass of molecular ion 465.50 [M+Na]⁺. Measured mass: 465.16; IR ν_{\max} cm⁻¹ 3294, 3200-2550, 1644, 1505, 1232, 1193, 1111; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.61(1H, s, NH), 9.18 (1H, broad, s, OH), 8.46-8.41 (3H, m, NHCH₂, 2 x Ar-*H*), 7.31-7.26 (2H, m, Ar-*H*), 7.12 (2H, d, *J* = 6.0 Hz, Ar-*H*), 6.99 (1H, d, *J* = 7.3 Hz, NHCH), 6.71 (1H, d, *J* = 9.2 Hz, Ar-*H*), 4.27 (2H, d, *J* = 6.0 Hz, CH₂NH), 4.07 (1H, p, *J* = 6.9 Hz, CH), 2.75 (2H, t, *J* = 7.6 Hz, CH₂), 2.44 (2H, t, *J* = 7.8 Hz, CH₂), 1.37 (9H, s, 3 x CH₃), 1.22 (3H, d, *J* = 7.3 Hz, CHCH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.5 (C=O), 171.5 (C=O), 155.6 (C=O), 151.6 (Ar-C), 150.0 (2 x Ar-C), 149.1 (Ar-C), 131.3 (Ar-C), 127.7 (Ar-C), 122.5 (2 x Ar-C), 121.9 (Ar-C), 119.0 (Ar-C), 115.3 (Ar-C) 78.5 (C(CH₃)₃), 50.7 (CHCH₃), 41.5 (CH₂NH), 35.8 (CH₂), 28.7 (C(CH₃)₃) 26.7 (CH₂), 18.8 (CH₃).

7.1.4.1.8. 3-(5-Amino-2-hydroxyphenyl)-*N*-(3-imidazol-1-ylpropyl)propanamide; Boc L-alanine derivative 16i. Compound **16i** was synthesized from compound **11i** (0.29 g, 1.00 mmol), Boc-L-alanine (0.20 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF/DMF (20 mL, 1:1). No further purification was required. Yield: (0.35 g, 76%) of an orange wax. LRMS (ES) for C₂₃H₃₃N₅O₅. Calculated mass of molecular ion 460.54 [M+H]⁺. Measured mass: 460.24; IR ν_{\max} cm⁻¹ 3241, 3200-2600, 1650, 1547, 1505, 1232, 1162, 745; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.60 (1H, broad, s, OH), 7.91 (1H, t, *J* = 5.7 Hz, NHCH₂), 7.60 (1H, s, Ar-*H*), 7.28 (1H, s, Ar-*H*), 7.23 (1H, dd, *J* = 8.7 and 2.8 Hz, Ar-*H*), 7.15 (1H, s, Ar-*H*), 7.08 (1H, d, *J* = 7.8 Hz, NH), 6.99 (1H, d, *J* = 7.3 Hz, NH), 6.88 (1H, s, Ar-*H*), 6.70 (1H, d, *J* = 8.7 Hz, Ar-*H*), 4.06 (1H, p, *J* = 7.1 Hz, CH), 3.89 (2H, t, *J* = 6.9 Hz, CH₂CH₂CH₂NH), 2.98 (2H, q, *J* = 6.4 Hz, CH₂CH₂CH₂NH), 2.70 (2H, t, *J* = 7.8 Hz, CH₂), 2.33 (2H, t, *J* = 7.8 Hz, CH₂), 1.79 (2H, p, *J*

= 6.9 Hz, CH₂CH₂CH₂NH) 1.38 (9H, s, 3 x CH₃), 1.22 (3H, d, *J* = 7.3 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_C 175.2 (C=O), 172.4 (C=O), 155.8 (C=O), 151.5 (Ar-C), 137.8 (Ar-C), 131.2 (Ar-C), 128.8 (Ar-C), 127.9 (Ar-C), 121.8 (Ar-C), 119.9 (Ar-C), 119.0 (Ar-C), 115.3 (Ar-C), 78.4 (C(CH₃)₃), 50.7 (CHCH₃), 44.1 (CH₂), 36.1 (CH₂), 36.0 (CH₂), 31.2 (CH₂), 28.7 (C(CH₃)₃), 26.6 (CH₂), 18.8 (CH₃).

7.1.4.1.9. Ethyl 3-(5-Amino-2-hydroxyphenyl)propanoate; Boc β-alanine derivative 17a.

Compound **17a** was synthesized from compound **11a** (0.21 g, 1.00 mmol), Boc-β-alanine (0.20 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF (20 mL). No further purification was required. Yield: (0.30 g, 79%) as a brown solid, m.p. 91-93 °C. HRMS (NSI) for C₁₉H₂₈N₂O₆. Calculated mass of molecular ion 381.2020 [M+H]⁺. Measured mass: 381.2023; IR ν_{max} cm⁻¹ 3296, 3163, 2982, 1734, 1687, 1549, 1440, 1364, 1284, 1247, 1161; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 9.64 (1H, s, NH), 9.21 (1H, s, broad, OH), 7.27 (1H, d, *J* = 2.3 Hz, Ar-*H*), 7.29 (1H, dd, *J* = 8.7 and 2.52 Hz, Ar-*H*), 6.86 (1H, t, *J* = 5.7 Hz, NHCH₂), 6.68 (1H, d, *J* = 8.7 Hz, Ar-*H*), 4.04 (2H, q, *J* = 7.0 Hz, CH₂CH₃), 3.18 (2H, q, *J* = 7.0 Hz, CH₂NH), 2.72 (2H, m, CH₂CH₂CO), 2.52 (2H, m, CH₂CO), 2.39 (2H, t, *J* = 7.1 Hz, CH₂CH₂NH), 1.38 (9H, s, 3 x CH₃), 1.16 (3H, t, *J* = 7.1 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_C 172.9 (C=O), 169.1 (C=O), 156.0 (C=O), 151.5 (Ar-C), 131.4 (Ar-C), 126.8 (Ar-C), 121.8 (Ar-C), 119.2 (Ar-C), 115.1 (Ar-C), 78.1 (C(CH₃)₃), 60.3 (CH₂CH₃), 37.2 (CH₂), 37.1 (CH₂), 34.1 (CH₂), 28.8 (C(CH₃)₃), 26.2 (CH₂), 14.6 (CH₃).

7.1.4.1.10. Ethyl 3-(5-Amino-3-bromo-2-hydroxyphenyl)propanoate; Boc β-alanine derivative 17b.

Compound **17b** was synthesized from compound **11b** (0.58 g, 2.00 mmol), Boc-β-alanine (0.40 g, 2.10 mmol), *N*-methylmorpholine (0.20 g, 2.00 mmol) and IBCF (0.28 g, 2.00 mmol) in dry THF (30 mL). No further purification was required. Yield: (0.60 g, 65%) as an orange wax. HRMS (NSI) for C₁₉H₂₇BrN₂O₆. Calculated mass of molecular ion 459.1125 [M+H]⁺. Measured mass: 459.1125; IR ν_{max} cm⁻¹ 3382, 3300-2500, 2979, 1682, 1478, 1247, 1160; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 9.77 (1H, s, NH), 8.80 (1H, s, broad, OH), 7.72 (1H, d, *J* = 2.8 Hz, Ar-*H*), 7.15 (1H, d, *J* = 2.3 Hz, Ar-*H*), 6.81 (1H, m, NHCH₂), 4.00 (2H, q, *J* = 6.9 Hz, CH₂CH₃), 3.15 (2H, q, *J* = 6.9 Hz, CH₂NH), 2.78-2.73 (2H, m, CH₂), 2.50-2.46 (2H, m, CH₂), 2.36 (2H, t, *J* = 7.1 Hz, CH₂CH₂NH), 1.33 (9H, s, 3 x CH₃), 1.15 (3H, t, *J* = 7.1 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_C 172.7 (C=O), 169.6 (C=O), 156.1 (C=O), 147.7 (Ar-C), 133.1 (Ar-C), 130.5 (Ar-C), 121.9 (Ar-C), 120.8 (Ar-C), 111.6 (Ar-C), 78.1 (C(CH₃)₃), 60.4 (CH₂CH₃), 37.3 (CH₂), 37.0 (CH₂), 33.9 (CH₂), 28.8 (C(CH₃)₃), 26.8 (CH₂), 14.5 (CH₃).

7.1.4.1.11. 3-(5-Amino-2-hydroxyphenyl)-*N*-(2*H*-1,3-benzodioxol-5-yl)propanamide; Boc β-alanine derivative 17c.

Compound **17c** was synthesized from compound **11c** (0.30 g, 1.00 mmol), Boc-β-alanine (0.20 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF (20 mL). No further purification was required. Yield: (0.43 g, 91.3%) as a light pink powder, m.p. 179-180 °C. HRMS (NSI) for C₂₄H₂₉N₃O₇. Calculated mass of molecular ion 472.2078 [M+H]⁺. Measured mass: 472.2068; IR ν_{max} cm⁻¹ 3322, 3300-2600, 2962, 1651, 1538, 1493, 1453, 1226, 1163, 1033, 797; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 9.85 (1H, s, NH), 9.66 (1H, s, NH), 9.21 (1H, broad, s, OH), 7.32 (2H,

d, $J = 2.3$ Hz, Ar-*H*), 7.22 (1H, dd, $J = 7.1$ and 2.3 Hz, Ar-*H*), 6.94 (1H, dd, $J = 8.2$ and 1.8 Hz, Ar-*H*), 6.87-6.81 (2H, m, NHCH₂, Ar-*H*), 6.70 (1H, d, $J = 8.2$ Hz, Ar-*H*), 5.97 (2H, s, CH₂O), 3.20 (2H, q, $J = 6.7$ Hz, CH₂NH), 2.76 (2H, t, $J = 7.8$ Hz, CH₂CH₂CO), 2.52 (2H, m, CH₂CO), 2.39 (2H, t, $J = 7.3$ Hz, CH₂CH₂NH), 1.37 (9H, s, 3 x CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_C 170.9 (C=O), 160.1 (C=O), 156.0 (C=O), 151.5 (Ar-C), 147.5 (Ar-C), 143.2 (Ar-C), 134.3 (Ar-C), 131.4 (Ar-C), 127.6 (Ar-C), 121.9 (Ar-C), 119.0 (Ar-C), 115.2 (Ar-C), 112.4 (Ar-C), 108.5 (Ar-C), 101.9 (Ar-C), 101.4 (CH₂O), 78.1 (C(CH₃)₃), 37.2 (CH₂), 37.1 (CH₂), 36.8 (CH₂), 28.8 (C(CH₃)₃), 26.5 (CH₂).

7.1.4.1.12. 3-(5-Amino-2-hydroxyphenyl)-*N*-[4-[3-(5-amino-2-hydroxyphenyl)propanamido]phenyl]propanamide; bis-Boc L-alanine derivative 30a.

Compound **30a** was synthesized from compound **29a** (0.43 g, 1.00 mmol), Boc-L-alanine (0.40 g, 2.10 mmol), *N*-methylmorpholine (0.20 g, 2.00 mmol) and IBCF (0.28 g, 2.00 mmol) in dry THF:DMF (20 mL, 2:1). No further purification was required. Yield: (0.69 g, 89%) as a brown powder, m.p. 215-217 °C; HRMS (NSI) for C₄₀H₅₂N₆O₁₀. Calculated mass of molecular ion 794.4083 [M+NH₄]⁺. Measured mass: 794.4085; IR ν_{max} cm⁻¹ 3311, 3300-2700, 1652m, 1498, 1241, 1165, 830; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 9.83 (2H, s, 2 x NH), 9.59 (2H, s, 2 x NH), 9.18 (2H, broad, s, 2 x OH), 7.46 (4H, s, Ar-*H*), 7.28 (2H, m, Ar-*H*), 7.20 (2H, dd, $J = 8.2$ and 2.5 Hz, Ar-*H*), 6.94 (2H, d, $J = 7.3$ Hz, 2 x NHCH), 6.68 (2H, d, $J = 8.7$ Hz, Ar-*H*), 4.03 (2H, p, $J = 7.2$ Hz, 2 x CH), 2.74 (4H, t, $J = 7.8$ Hz, 2 x CH₂CH₂CO), 2.50 (4H, t, $J = 7.8$ Hz, 2 x CH₂CO), 1.33 (18H, s, 6 x CH₃), 1.18 (6H, d, $J = 6.9$ Hz, 2 x CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_C 171.6 (2 x C=O), 170.9 (2 x C=O), 155.6 (2 x C=O), 151.6 (2 x Ar-C), 135.1 (2 x Ar-C), 131.2 (2 x Ar-C), 127.8 (2 x Ar-C), 121.9 (2 x Ar-C), 120.0 (4 x Ar-C), 119.1 (2 x Ar-C), 115.2 (2 x Ar-C), 78.5 (2 x C(CH₃)₃), 50.8 (2 x CHCH₃), 36.8 (2 x CH₂), 28.8 (2 x C(CH₃)₃), 26.5 (2 x CH₂), 18.8 (2 x CH₃).

7.1.4.1.13. 3-(5-Amino-2-hydroxyphenyl)-*N*-[2-[3-(5-amino-2-hydroxyphenyl)propanamido]ethyl]propanamide; bis-Boc L-alanine derivative 30b. Compound **30b** was synthesized from compound **29b** (0.77 g, 2.00 mmol), Boc-L-alanine (0.80 g, 4.20 mmol), *N*-methylmorpholine (0.40 g, 4.00 mmol) and IBCF (0.56 g, 4.00 mmol) in dry THF (30 mL). The crude product was purified by column chromatography (eluent; CH₂Cl₂/EtOH, 9:1) giving compound **30b** (0.46 g, 31.6%) as an orange solid, m.p. 65 °C. HRMS (NSI) for C₃₆H₅₂N₆O₁₀. Calculated mass of molecular ion 729.3818 [M+H]⁺. Measured mass: 729.3818; IR ν_{max} cm⁻¹ 3297, 3250-2750, 1655, 1508, 1438, 1365, 1230, 1162, 1018; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 9.59 (2H, broad, s, 2 x OH), 9.17 (2H, s, 2 x NH), 7.93-7.86 (2H, m, 2 x NHCH₂), 7.30-7.20 (4H, m, Ar-*H*), 6.98 (2H, d, $J = 7.3$ Hz, 2 x NHCH), 6.69 (2H, d, $J = 8.7$ Hz, Ar-*H*), 4.06 (2H, p, $J = 6.9$ Hz, 2 x CH), 3.12-3.04 (4H, m, 2 x CH₂NH), 2.70 (4H, t, $J = 7.8$ Hz, 2 x CH₂CH₂CO), 2.31 (4H, t, $J = 7.8$ Hz, 2 x CH₂CO), 1.38 (18H, s, 6 x CH₃), 1.18 (6H, d, $J = 7.3$ Hz, 2 x CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_C 172.4 (2 x C=O) 171.6 (2 x C=O), 155.6 (2 x C=O), 151.5 (2 x Ar-C), 131.2 (2 x Ar-C), 128.0 (2 x Ar-C), 121.8 (2 x Ar-C), 119.0 (2 x Ar-C), 115.3 (2 x Ar-C), 78.5 (2 x C(CH₃)₃), 50.8 (2 x CHCH₃), 38.9 (2 x CH₂NH), 36.0 (2 x CH₂), 28.7 (2 x C(CH₃)₃), 26.6 (2 x CH₂), 18.8 (2 x CH₃).

7.1.4.1.14. 1-Hydroxy-*N*-[2-[3-(5-amino-2-

hydroxyphenyl)propanamido]ethyl]naphthalene-2-carboxamide; Boc L-alanine

derivative 36. Compound **36** was synthesized from the crude compound **35** (0.39 g, 1.00 mmol), Boc-L-alanine (0.20 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF/DMF (20 mL, 2:1). The crude product was purified by column chromatography (eluent: CH₂Cl₂/EtOH 19:1) giving compound **36** (0.40 g, 72%) as a light pink powder, m.p. 114-115 °C. HRMS (NSI) for C₃₀H₃₆N₄O₇. Calculated mass of molecular ion 565.2657 [M+H]⁺. Measured mass: 565.2653; IR ν_{\max} cm⁻¹ 3350-3000, 2933, 1640, 1597, 1538, 1503, 1254, 1160, 764; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.59 (1H, s, NH), 9.18 (1H, broad, s, OH), 9.06-9.00 (1H, m, NHCH₂), 8.26 (1H, d, *J* = 8.2 Hz, Ar-*H*), 8.09 (1H, t, *J* = 5.5 Hz, NHCH₂), 7.88-7.83 (2H, m, Ar-*H*), 7.64 (1H, t, *J* = 8.2 Hz, Ar-*H*), 7.55 (1H, t, *J* = 8.2 Hz, Ar-*H*), 7.38 (1H, d, *J* = 9.2 Hz, Ar-*H*), 7.32-7.28 (1H, m, Ar-*H*), 7.22 (1H, dd, *J* = 8.7 and 2.3 Hz, Ar-*H*), 6.97 (1H, d, *J* = 7.3 Hz, NHCH), 6.69 (1H, d, *J* = 8.7 Hz, Ar-*H*), 4.06 (1H, p, *J* = 6.9 Hz, CH), 3.48-3.25 (4H, m, 2 x CH₂NH), 2.72 (2H, t, *J* = 7.3 Hz, CH₂), 2.34 (2H, t, *J* = 7.3 Hz, CH₂), 1.37 (9H, s, 3 x CH₃), 1.18 (3H, d, *J* = 7.3 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.7 (C=O), 171.6 (C=O), 171.2 (C=O), 160.2 (C=O), 155.6 (Ar-C), 151.5 (Ar-C), 136.3 (Ar-C), 131.2 (Ar-C), 129.3 (Ar-C), 128.0 (Ar-C), 126.3 (2 x Ar-C), 125.3 (Ar-C), 123.5 (Ar-C), 123.2 (Ar-C), 121.8 (Ar-C), 119.0 (Ar-C), 118.1 (Ar-C), 115.3 (Ar-C), 107.6 (Ar-C), 78.5 (C(CH₃)₃), 50.7 (CHCH₃), 39.6 (CH₂NH signal obscured by d₆-DMSO signal, can be seen by DEPT), 38.5 (CH₂NH), 36.1 (CH₂), 28.7 (C(CH₃)₃), 26.6 (CH₂), 18.8 (CH₃).

7.1.5. Synthesis of amino acid hydrochlorides **8**, **9**, **31** and **37** and of the amine hydrochloride **33**.

7.1.5.1. General Procedure. The Boc-protected aminophenol **16**, **17**, **30**, **32** or **36** was added to a saturated solution of dry HCl in ethyl acetate. The mixture was stirred at rt for 1-6 h.

7.1.5.1.1. Ethyl 3-(5-Amino-2-hydroxyphenyl)propanoate; L-alanine derivative HCl salt

8a. This substrate was prepared from compound **16a** (0.20 g) and anhydrous EtOAc/HCl (10 mL) for 4 hours. The solvent was evaporated yielding an oily residue which solidified overnight giving compound **8a** (0.16 g, 96%) as a red, waxy material. HRMS (NSI) for C₁₄H₂₀N₂O₄. Calculated mass of molecular ion 281.1496 [M+H]⁺. Measured mass: 281.1498; IR ν_{\max} cm⁻¹ 3500-2500, 1673, 1501, 1204, 1100, 816; ¹H-NMR (270 MHz; d₆-DMSO) δ_{H} 10.36 (1H, s, OH), 9.42 (1H, s, NH), 8.3 (3H, s, broad, NH₃⁺), 7.33-7.26 (2H, m, Ar-*H*), 6.77 (1H, d, *J* = 9.2 Hz, Ar-*H*), 4.09-3.94 (3H, m, CH₂O, CH), 2.74 (2H, t, *J* = 6.8 Hz, CH₂CH₂CO), 2.55-2.46 (2H, m, CH₂CO), 1.43 (3H, d, *J* = 7.2 Hz, CH₃), 1.16 (3H, t, *J* = 7.1 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.9 (C=O), 167.9 (C=O), 152.2 (Ar-C), 130.3 (Ar-C), 127.1 (Ar-C), 122.0 (Ar-C), 119.3 (Ar-C), 115.3 (Ar-C), 60.3 (CH₂CH₃), 49.3 (CHCH₃), 34.0 (CH₂), 26.2 (CH₂), 17.8 (CHCH₃), 14.6 (CH₂CH₃).

7.1.5.1.2. Ethyl 3-(5-Amino-3-bromo-2-hydroxyphenyl)propanoate; L-alanine derivative

HCl salt 8b. This substrate was prepared from compound **16b** (0.50 g) in anhydrous EtOAc/HCl (15 mL) for 3 h. The solvent was evaporated giving compound **8b** (0.42 g, 97.5%) as a brown wax. HRMS (NSI) for C₁₄H₁₉BrN₂O₄. Calculated mass of molecular ion

359.0601 [M+H]⁺. Measured mass: 359.0603; IR ν_{\max} cm⁻¹ 3600-2600, 1682, 1478, 1228, 1160; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 10.74 (1H, d, $J = 15.11$ Hz, *NH*), 8.93 (1H, s, broad, *OH*), 8.30 (3H, broad, s, NH_3^+), 7.72 (1H, t, $J = 2.3$ Hz, *Ar-H*), 7.24 (1H, t, $J = 2.3$ Hz, *Ar-H*), 4.02-3.92 (3H, m, CH_2O , *CH*), 2.78-2.70 (2H, m, $\text{CH}_2\text{CH}_2\text{CO}$), 2.48-2.35 (2H, m, CH_2CO), 1.36 (3H, d, $J = 6.9$ Hz, CH_3) 1.08 (3H, t, $J = 7.1$ Hz, CH_3). ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.6 (C=O), 168.4 (C=O), 148.4 (Ar-C) 132.1 (Ar-C), 130.8 (Ar-C), 122.1 (Ar-C), 121.0 (Ar-C), 111.7 (Ar-C), 60.4 (CH_2CH_3), 49.3 (CHNH_3), 33.9 (CH_2), 26.8 (CH_2), 17.7 (CH_3), 14.6 (CH_3).

7.1.5.1.3. 3-(5-Amino-2-hydroxyphenyl)-*N*-(2*H*-1,3-benzodioxol-5-yl)propanamide; L-alanine derivative HCl salt 8c. This substrate was prepared from compound **16c** (0.20 g) in anhydrous EtOAc/HCl (15 mL) for 3 h. The resulting precipitate was collected and dried giving compound **8c** (0.16 g, 92%) as an off white powder, m.p. 177-179 °C. HRMS (NSI) for C₁₉H₂₁N₃O₅. Calculated mass of molecular ion 372.1554 [M+H]⁺. Measured mass: 372.1547; IR ν_{\max} cm⁻¹ 3295, 3280-2500, 1657, 1564, 1489, 1237, 1043, 797; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 10.42 (1H, s, *NH*), 9.97 (1H, s, *NH*), 9.43 (1H, broad, s, *OH*), 8.33 (3H, d, $J = 3.7$ Hz, NH_3^+), 7.38-7.26 (3H, m, *Ar-H*), 6.97(1H, dd, $J = 8.7$ and 2.1 Hz, *Ar-H*), 6.85-6.76 (2H, m, *Ar-H*), 5.97 (2H, s, CH_2O), 4.00 (1H, m, *CH*) 2.79 (2H, t, $J = 7.6$ Hz, $\text{CH}_2\text{CH}_2\text{CO}$), 2.54 (2H, m, CH_2CO), 1.44 (3H, d, $J = 6.9$ Hz, CH_3); ¹³C-NMR (68 MHz; d₆-DMSO) δ_{C} 170.9 (C=O), 168.0 (C=O), 152.3 (Ar-C), 147.5 (Ar-C), 143.1 (Ar-C), 134.4 (Ar-C), 130.4 (Ar-C), 128.0 (Ar-C), 122.1 (Ar-C), 119.3 (Ar-C), 115.4 (Ar-C), 112.4 (Ar-C), 108.5 (Ar-C), 102.0 (Ar-C), 101.4 (CH_2O), 49.2 (CHCH_3), 36.8 (CH_2), 26.5 (CH_2), 17.9 (CHCH_3).

7.1.5.1.4. 3-(5-Amino-2-hydroxyphenyl)-*N*-(3,4-dimethoxyphenyl)propanamide; L-alanine derivative HCl salt 8d. This substrate was prepared from compound **16d** (0.30 g) in anhydrous EtOAc/HCl (10 mL) for 4 h. The resulting precipitate was collected giving compound **8d** (0.23 g, 81%) as a waxy, hygroscopic brown solid (0.23 g, 81%). HRMS (NSI) for C₂₀H₂₅N₃O₅. Calculated mass of molecular ion 388.1867 [M+H]⁺. Measured mass: 388.1860; IR ν_{\max} cm⁻¹ 3500-2750, 2750-2400, 1509, 1233, 1138, 1118; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 10.47 (1H, s, *NH*), 10.28 (1H, s, broad, *OH*), 9.44 (1H, s, *NH*), 8.37 (3H, s, broad, NH_3^+), 7.34-7.29 (2H, m, *Ar-H*), 7.05-6.99 (2H, m, *Ar-H*), 6.94 (1H, dd, $J = 8.7$ and 2.3 Hz, *Ar-H*), 6.88-6.77 (1H, m, *Ar-H*), 4.02 (1H, m, *CH*), 3.77 (2 x 3H, d, $J = 2.3$ Hz, 2 x CH_3), 2.72 (2H, t, $J = 7.6$ Hz, CH_2), 2.47 (2H, t, $J = 7.6$ Hz, CH_2) 1.45 (3H, t, $J = 6.9$ Hz, CH_3); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 174.5 (C=O), 167.9 (C=O), 152.2 (Ar-C), 149.6 (Ar-C), 148.7 (Ar-C), 130.3 (Ar-C), 127.5 (Ar-C), 125.0 (Ar-C), 121.9 (Ar-C), 119.3 (Ar-C), 115.6 (Ar-C), 115.4 (Ar-C), 112.6 (Ar-C), 107.6 (Ar-C), 56.3 (CH_3O), 56.2 (CH_3O), 59.3 (CHCH_3), 34.0 (CH_2), 26.2 (CH_2), 17.8 (CHCH_3).

7.1.5.1.5. 3-(5-Amino-2-hydroxyphenyl)-*N*-(4-hydroxyphenyl)propanamide; L-alanine derivative HCl salt 8f. This substrate was prepared from compound **16f** (0.40 g) in anhydrous EtOAc/HCl (10 mL) for 1 h. The resulting precipitate was collected and dried in a desiccator under vacuum giving compound **8f** (0.33 g, 96%) as an off white powder, m.p. 171-173 °C. HRMS (NSI) for C₁₈H₂₁N₃O₄. Calculated mass of molecular ion 344.1605 [M+H]⁺. Measured mass: 344.1605; IR ν_{\max} cm⁻¹ 3550-2450, 1656, 1508, 1217, 1105, 826;

¹H-NMR (270 MHz; d₆-DMSO) δ_H 10.38 (1H, s, NH), 9.70 (1H, s, NH), 8.28 (3H, m, NH₃⁺), 7.35-7.28 (3H, m, Ar-H), 7.24 (1H, dd, *J* = 8.7 and 2.3 Hz, Ar-H), 6.74 (1H, d, *J* = 8.7 Hz, Ar-H), 6.63 (2H, d, *J* = 8.7 Hz, Ar-H), 4.20 (1H, s, broad, OH), 3.95 (1H, m, CH), 2.73 (2H, t, *J* = 7.6 Hz, CH₂), 2.45 (2H, m, CH₂), 1.39 (3H, d, *J* = 6.9 Hz, CH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_C 170.5 (C=O), 167.7 (C=O), 153.7 (Ar-C), 152.3 (Ar-C), 131.5 (Ar-C), 130.3 (Ar-C), 128.1 (Ar-C), 122.1 (Ar-C), 121.4 (2 x Ar-C), 119.3 (Ar-C), 115.5 (2 x Ar-C), 115.4 (Ar-C), 49.3 (CHCH₃), 36.6 (CH₂), 26.5 (CH₂), 17.8 (CHCH₃).

7.1.5.1.6. (S)-Methyl 2-[3-(5-Amino-2-hydroxyphenyl)propanamido]-2-phenyl-2-carboxylate; L-alanine derivative HCl salt 8g. This substrate was prepared from compound **16g** (0.20 g) in anhydrous EtOAc/HCl (15 mL) for 4 h. The solvent was evaporated giving compound **8g** (0.17 g, 97%) as an oily product which solidified overnight affording red solid, m.p. 71-74 °C. HRMS (NSI) for C₂₁H₂₅N₃O₅. Calculated mass of molecular ion 400.1867 [M+H]⁺. Measured mass: 400.1860; IR ν_{max} cm⁻¹ 3550-2450, 1737, 1673, 1497, 1213, 1101, 697; ¹H-NMR (270 MHz; d₆-DMSO) δ_H 10.47 (1H, s, NH), 9.41 (1H, broad, s, OH), 8.77 (1H, d, *J* = 6.9 Hz, NH), 8.37 (3H, d, *J* = 3.2 Hz, NH₃⁺), 7.43-7.28 (7H, m, Ar-H), 6.78 (1H, d, *J* = 9.2 Hz, Ar-H), 5.42 (1H, d, *J* = 6.9 Hz, CH), 4.08 (1H, m, CH), 3.62 (3H, s, CH₃), 2.71 (2H, m, CH₂CH₂CO), 2.44 (2H, t, *J* = 7.2 Hz, CH₂CO), 1.45 (3H, d, *J* = 6.9 Hz, CH₃); ¹³C-NMR (68 MHz; d₆-DMSO) δ_C 172.5 (C=O), 171.8 (C=O), 167.9 (C=O), 152.3 (Ar-C), 136.8 (Ar-C), 130.4 (Ar-C), 129.2 (2 x Ar-C), 128.8 (Ar-C), 128.6 (2 x Ar-C), 128.0 (Ar-C), 122.1 (Ar-C), 119.3 (Ar-C), 115.4 (Ar-C), 56.8 (CHNH), 52.8 (CH₃), 49.3 (CHCH₃), 35.3 (CH₂), 26.6 (CH₂), 18.9 (CHCH₃).

7.1.5.1.7. 3-(5-Amino-2-hydroxyphenyl)-N-(4-pyridylmethyl)propanamide; L-alanine derivative HCl salt 8h. This substrate was prepared by dissolving compound **16h** (0.20 g) in EtOAc (3 mL) and then adding anhydrous EtOAc/HCl (5 mL) with stirring. The resulting precipitate was collected and dried in a desiccator under vacuum giving compound **8h** (0.16 g, 93%) as a grey powder, m.p. 183-184 °C. HRMS (NSI) for C₁₈H₂₂N₄O₃. Calculated mass of molecular ion 343.1765 [M+H]⁺. Measured mass: 343.1765; IR ν_{max} cm⁻¹ 3550-2500, 1639, 1503, 1232, 1105, 775; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 10.58 (1H, s, NH), 9.49 (1H, broad s, OH), 8.85 (1H, t, *J* = 5.6 Hz, NHCH₂), 8.81 (2H, d, *J* = 6.9 Hz, Ar-H), 8.41 (3H, d, *J* = 2.9 Hz, NH₃⁺), 7.76 (2H, d, *J* = 6.9 Hz, Ar-H), 7.36-7.30 (2H, m, Ar-H), 6.83 (1H, d, *J* = 8.7 Hz, Ar-H), 4.52 (2H, d, *J* = 6.0 Hz, CH₂NH), 4.07-4.00 (1H, m, CHNH₃⁺), 2.78 (2H, t, *J* = 7.6 Hz, CH₂), 2.54-2.48 (2H, m, CH₂), 1.45 (3H, d, *J* = 7.3 Hz, CHCH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_C 173.0 (C=O), 167.9 (C=O), 160.8 (Ar-C), 152.3 (Ar-C), 141.8 (2 x Ar-C), 130.3 (Ar-C), 127.7 (Ar-C), 125.1 (2 x Ar-C), 122.2 (Ar-C), 119.3 (Ar-C), 115.4 (Ar-C), 49.2 (CHCH₃), 42.2 (CH₂NH), 35.5 (CH₂), 26.7 (CH₂), 17.8 (CH₃).

7.1.5.1.8. 3-(5-Amino-2-hydroxyphenyl)-N-(3-imidazol-1-ylpropyl)propanamide; L-alanine derivative HCl salt 8i. This substrate was prepared by dissolving compound **16i** (0.20 g) in methanol (4 mL) and then adding anhydrous EtOAc/HCl (6 mL) to the solution. The mixture was stirred for 1 h and then filtered. The filtrate was evaporated giving compound **8i** (0.14 g, 81%) as a red wax. LRMS (ES) for C₁₈H₂₅N₅O₃. Calculated mass of molecular ion 360.42 [M+H]⁺. Measured mass: 360.43; IR ν_{max} cm⁻¹ 3600-2550, 1608, 1440, 1260, 1085, 797; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 10.56 (1H, broad, s, OH), 9.22 (1H, s,

Ar-H), 8.45-8.30 (4H, m, NH_3^+ , NH), 8.19 (1H, t, $J = 5.73$ Hz, NHCH_2), 7.83-7.81 (1H, m, Ar-H), 7.73-7.70 (1H, m, Ar-H), 7.34-7.28 (2H, m, Ar-H), 6.79 (1H, d, $J = 8.7$ Hz, Ar-H), 4.16 (2H, t, $J = 6.7$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$), 4.07-3.99 (1H, m, CH), 3.02 (2H, q, $J = 6.0$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$), 2.72 (2H, t, $J = 7.8$ Hz, CH_2), 2.35 (2H, t, $J = 7.8$ Hz, CH_2), 1.92 (2H, p, $J = 6.4$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$) 1.44 (3H, d, $J = 6.9$ Hz, CH_3); ^{13}C -NMR (101 MHz; d_6 -DMSO) δ_{C} 172.5 (C=O), 167.9 (C=O), 152.2 (Ar-C), 135.9 (Ar-C), 130.4 (Ar-C), 128.0 (Ar-C), 122.5 (Ar-C), 122.0 (Ar-C), 120.3 (Ar-C), 119.2 (Ar-C), 115.4 (Ar-C), 49.2 (CHCH_3), 46.7 (CH_2), 35.8 (CH_2), 35.5 (CH_2), 30.2 (CH_2), 26.6 (CH_2), 17.8 (CH_3).

7.1.5.1.9. Ethyl 3-(5-Amino-2-hydroxyphenyl)propanoate; β -alanine derivative HCl salt

9a. This substrate was prepared from compound **17a** (0.20 g) in anhydrous EtOAc/HCl (15 mL) for 3 h. The solvent was evaporated to giving compound **9a** (0.16 g, 96%) as a brown wax. HRMS (NSI) for $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_4$. Calculated mass of molecular ion 281.1496 $[\text{M}+\text{H}]^+$. Measured mass: 281.1488; IR ν_{max} cm^{-1} 3600-2500, 1719, 1660, 1556, 1504s, 1223, 1184, 823; ^1H -NMR (400 MHz; d_6 -DMSO) δ_{H} 9.97 (1H, s, NH), 9.31 (1H, s, broad, OH), 7.98 (3H, s, broad, NH_3^+), 7.32-7.20 (2H, m, Ar-H), 6.73 (1H, d, $J = 8.7$ Hz, Ar-H), 4.03 (2H, q, $J = 7.1$ Hz, CH_2CH_3) 3.04 (2H, m, CH_2NH_3^+), 2.69 (4H, m, $\text{CH}_2\text{CH}_2\text{CO}$, $\text{CH}_2\text{CH}_2\text{NH}_3^+$), 2.44 (2H, t, $J = 7.3$ Hz, CH_2CO), 1.16 (3H, t, $J = 7.3$ Hz, CH_3); ^{13}C -NMR (101 MHz; d_6 -DMSO) δ_{C} 174.5 (C=O), 168.0 (C=O), 151.7 (Ar-C), 131.0 (Ar-C), 127.2 (Ar-C), 121.8 (Ar-C), 119.2 (Ar-C), 115.2 (Ar-C), 60.3 (CH_2CH_3), 35.6 (CH_2), 34.1 (CH_2), 33.4 (CH_2), 26.2 (CH_2), 14.6 (CH_3).

7.1.5.1.10. Ethyl 3-(5-Amino-3-bromo-2-hydroxyphenyl)propanoate; β -alanine derivative HCl salt

9b. This substrate was prepared from compound **17b** (0.50 g) in anhydrous EtOAc/HCl (15 mL) for 3 h. The solvent was evaporated giving compound **9b** (0.43 g, 99%) as a brown solid, m.p. 63-64 °C. HRMS (NSI) for $\text{C}_{14}\text{H}_{19}\text{BrN}_2\text{O}_4$. Calculated mass of molecular ion 359.0601 $[\text{M}+\text{H}]^+$. Measured mass: 359.0606; IR ν_{max} cm^{-1} 3350, 3314, 1707, 1640, 1585, 1470, 1440, 1245, 1171, 1096, 850; ^1H -NMR (400 MHz; d_6 -DMSO) δ_{H} 10.2 (1H, s, NH), 8.84 (1H, s, broad, OH), 7.97 (3H, s, broad, NH_3^+), 7.73 (1H, d, $J = 2.3$ Hz, Ar-H), 7.20 (1H, d, $J = 2.3$ Hz, Ar-H), 3.98 (2H, q, $J = 7.1$ Hz, CH_2CH_3), 2.96 (2H, m, CH_2NH_3^+), 2.76 (2H, t, $J = 7.6$ Hz, $\text{CH}_2\text{CH}_2\text{CO}$), 2.65 (2H, t, $J = 6.6$ Hz, $\text{CH}_2\text{CH}_2\text{NH}_3^+$) 2.49 (2H, m, CH_2CO), 1.10 (3H, t, $J = 7.1$ Hz, CH_3); ^{13}C -NMR (101 MHz; d_6 -DMSO) δ_{C} 172.7 (C=O), 168.5 (C=O), 147.9 (Ar-C), 132.8 (Ar-C), 130.6 (Ar-C), 122.0 (Ar-C), 120.8 (Ar-C), 111.7 (Ar-C), 60.4 (CH_2CH_3), 35.4 (CH_2), 33.9 (CH_2), 33.6 (CH_2), 26.8 (CH_2), 14.6 (CH_3).

7.1.5.1.11. 3-(5-Amino-2-hydroxyphenyl)-*N*-(2*H*-1,3-benzodioxol-5-yl)propanamide; β -alanine derivative HCl salt

9c. This substrate was prepared from compound **17c** (0.30 g) in anhydrous EtOAc/HCl (15 mL) for 3 h. The solvent was evaporated giving compound **9c** (0.25 g, 96%) as a brown powder, m.p. 155 °C. HRMS (NSI) for $\text{C}_{19}\text{H}_{21}\text{N}_3\text{O}_5$. Calculated mass of molecular ion 372.1554 $[\text{M}+\text{H}]^+$. Measured mass: 372.1556; IR ν_{max} cm^{-1} 3600-2500, 1653, 1557, 1490, 1229, 1034, 797; ^1H -NMR (400 MHz; d_6 -DMSO) δ_{H} 9.98 (1H, s, NH), 9.94 (1H, s, NH), 9.30 (1H, broad, s, OH), 8.01 (3H, broad, s, NH_3^+), 7.34 (2H, t, $J = 1.8$ Hz, Ar-H), 7.25 (1H, dd, $J = 8.7$ and 2.8 Hz, Ar-H), 6.97 (1H, dd, $J = 8.2$ and 1.8 Hz, Ar-H), 6.83 (1H, d, $J = 8.2$ Hz, Ar-H), 6.75 (1H, d, $J = 8.7$ Hz, Ar-H), 5.97 (2H, s, CH_2O), 3.04 (2H, sextet, $J = 6.4$ Hz, CH_2NH_3^+), 2.76 (2H, m, $\text{CH}_2\text{CH}_2\text{CO}$), 2.68 (2H, t, $J = 6.9$ Hz,

$\text{CH}_2\text{CH}_2\text{NH}_3^+$), 2.52 (2H, m, CH_2CO); ^{13}C -NMR (101 MHz; d_6 -DMSO) δ_{C} 170.9 (C=O), 168.0 (C=O), 151.8 (Ar-C), 147.4 (Ar-C), 143.1 (Ar-C), 134.3 (Ar-C), 131.0 (Ar-C), 127.7 (Ar-C), 122.0 (Ar-C), 119.1 (Ar-C), 115.2 (Ar-C), 112.4 (Ar-C), 108.5 (Ar-C), 101.9 (Ar-C), 101.4 (CH_2O), 36.7 (CH_2), 35.6 (CH_2), 33.4 (CH_2), 26.5 (CH_2).

7.1.5.1.12. 3-(5-Amino-2-hydroxyphenyl)-N-[4-[3-(5-amino-2-hydroxyphenyl)propanamido]phenyl]propanamide; bis-L-alanine derivative bis-HCl salt 31a. This substrate was prepared from compound **30a** (0.50 g) in anhydrous EtOAc/HCl (15 mL) for 3 h. The resulting precipitate was collected and dried giving compound **31a** (0.40 g, 96%) as a brick-red powder, m.p. 206-207 °C. HRMS (NSI) for $\text{C}_{30}\text{H}_{36}\text{N}_6\text{O}_6$. Calculated mass of molecular ion 577.2769 $[\text{M}+\text{H}]^+$. Measured mass: 577.2766; IR ν_{max} cm^{-1} 3600-2600, 1658, 1565, 1495, 1240, 830; ^1H -NMR (400 MHz; d_6 -DMSO) δ_{H} 10.42 (2H, s, 2 x NH), 9.95 (2H, s, 2 x NH), 9.40 (2H, broad, s, 2 x OH), 8.31 (6H, broad, s, 2 x NH_3^+), 7.46 (4H, s, Ar-H), 7.31 (2H, d, $J = 2.3$ Hz, Ar-H), 7.26 (2H, dd, $J = 8.7$ and 2.8 Hz, Ar-H), 6.75 (2H, d, $J = 8.7$ Hz, Ar-H), 4.03 (2H, m, 2 x CH), 2.75 (4H, t, $J = 7.3$ Hz, 2 x $\text{CH}_2\text{CH}_2\text{CO}$), 2.51 (4H, t, $J = 7.6$ Hz, 2 x CH_2CO), 1.40 (6H, d, $J = 6.9$ Hz, 2 x CH_3); ^{13}C -NMR (101 MHz; d_6 -DMSO) δ_{C} 170.9 (2 x C=O), 167.9 (2 x C=O), 152.3 (2 x Ar-C), 135.1 (2 x Ar-C), 130.3 (2 x Ar-C), 128.0 (2 x Ar-C), 122.1 (2 x Ar-C), 120.0 (4 x Ar-C), 119.3 (2 x Ar-C), 115.4 (2 x Ar-C), 49.3 (2 x CHCH₃), 36.7 (2 x CH₂), 26.5 (2 x CH₂), 17.7 (2 x CH₃).

7.1.5.1.13. 3-(5-Amino-2-hydroxyphenyl)-N-[2-[3-(5-amino-2-hydroxyphenyl)propanamido]ethyl]propanamide; bis-L-alanine derivative bis-HCl salt 31b. This substrate was prepared from compound **30b** (0.30 g) in anhydrous EtOAc/HCl (15 mL) for 3 h. The resulting precipitate was collected and dried giving compound **31b** (0.24 g, 97%) as a pink powder, m.p. 70-71 °C; HRMS (NSI) for $\text{C}_{26}\text{H}_{36}\text{N}_6\text{O}_6$. Calculated mass of molecular ion 529.2769 $[\text{M}+\text{H}]^+$. Measured mass: 529.2762; IR ν_{max} cm^{-1} 3600-2400, 1673, 1601, 1557, 1501, 1237, 1103, 819; ^1H -NMR (400 MHz; d_6 -DMSO) δ_{H} 10.44 (2H, s, 2 x NH), 9.35 (2H, broad, s, 2 x OH), 8.31 (6H, s, 2 x NH_3^+), 7.98-7.93 (2H, m, 2 x NHCH_2), 7.29-7.24 (4H, m, Ar-H), 6.73 (2H, d, $J = 8.7$ Hz, Ar-H), 4.03-3.97 (2H, m, 2 x CH), 3.06-3.01 (4H, m, 2 x CH_2NH), 2.66 (4H, t, $J = 7.6$ Hz, 2 x $\text{CH}_2\text{CH}_2\text{CO}$), 2.27 (4H, t, $J = 7.8$ Hz, 2 x CH_2CO), 1.40 (6H, d, $J = 6.9$ Hz, 2 x CH_3); ^{13}C -NMR (101 MHz; d_6 -DMSO) δ_{C} 172.4 (2 x C=O) 167.9 (2 x C=O), 152.2 (2 x Ar-C), 130.4 (2 x Ar-C), 128.1 (2 x Ar-C), 121.9 (2 x Ar-C), 119.2 (2 x Ar-C), 115.4 (2 x Ar-C), 49.3 (2 x CHCH₃), 38.9 (2 x CH_2NH), 35.9 (2 x CH₂), 26.5 (2 x CH₂), 17.8 (2 x CH₃).

7.1.5.1.14. N-(2-Aminoethyl)-3-(2-hydroxy-5-nitrophenyl)propanamide hydrochloride 33. Compound **32** (1.00 g, 2.83 mmol) was stirred in anhydrous EtOAc/HCl (20 mL) for 3 h. The resulting precipitate was collected and dried giving compound **33** (0.81 g, 99%) as a pale yellow solid, m.p. >260 °C. HRMS (NSI) for $\text{C}_{11}\text{H}_{15}\text{N}_3\text{O}_4$. Calculated mass of molecular ion 254.1135 $[\text{M}+\text{H}]^+$. Measured mass: 254.1142; IR ν_{max} cm^{-1} 3390, 3300-2500, 1644, 1582, 1547, 1488, 1325, 1276, 1264, 1082; ^1H -NMR (400 MHz; d_6 -DMSO) δ_{H} 11.29 (1H, broad, s, OH), 8.20 (1H, t, $J = 5.5$ Hz, NH), 8.02 (3H, broad, s, NH_3^+), 7.95-7.91 (2H, m, Ar-H), 7.01 (1H, d, $J = 9.6$ Hz, Ar-H), 3.24 (2H, q, $J = 6.2$ Hz, CH_2NH), 2.82-2.71 (4H, m, CH_2NH_3^+ , $\text{CH}_2\text{CH}_2\text{CO}$), 2.38 (2H, t, $J = 7.8$ Hz, CH_2CO); ^{13}C -NMR (101 MHz; d_6 -DMSO) δ_{C} 172.6

(C=O), 162.6 (Ar-C), 139.8 (Ar-C), 129.2 (Ar-C), 126.0 (Ar-C), 124.4 (Ar-C), 115.6 (Ar-C), 39.0 (CH₂), 36.9 (CH₂), 34.9 (CH₂), 25.8 (CH₂).

7.1.5.1.15. 1-Hydroxy-N-[2-[3-(5-amino-2-

hydroxyphenyl)propanamido]ethyl]naphthalene-2-carboxamide; L-alanine derivative HCl salt **37.**

Compound **36** (0.15 g) was stirred in anhydrous EtOAc/HCl (10 mL) for 3 h. The solvent was then evaporated and the crude product was purified by column chromatography (eluent: CH₂Cl₂ changing to MeOH). The methanol fraction was evaporated giving compound **37** (0.04 g, 30%) as a brown, hygroscopic solid. HRMS (NSI) for C₂₅H₂₈N₄O₅. Calculated mass of molecular ion 465.2132 [M+H]⁺. Measured mass: 465.2129; IR ν_{\max} cm⁻¹ 3224, 3310, 3100-2500, 1637, 1597, 1540, 1501, 1275, 1258, 1104, 764; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 10.42 (1H, s, NH), 9.40 (1H, broad, s, OH), 9.17 (1H, t, *J* = 5.3 Hz, NHCH₂), 8.36-8.30 (3H, s, broad, NH₃⁺), 8.27 (1H, d, *J* = 8.3 Hz, Ar-*H*), 8.19 (1H, t, *J* = 5.7 Hz, NHCH₂), 7.93 (1H, d, *J* = 8.7 Hz, Ar-*H*), 8.24 (1H, d, *J* = 8.2 Hz, Ar-*H*), 7.74-7.62 (2H, m, Ar-*H*), 7.55 (1H, t, *J* = 7.3 Hz, Ar-*H*), 7.38 (1H, d, *J* = 9.2 Hz, Ar-*H*), 7.31-7.28 (1H, m, Ar-*H*), 6.78 (1H, d, *J* = 8.7 Hz, Ar-*H*), 4.05-3.94 (1H, m, CHCH₃), 3.51-3.38 (2H, m, CH₂NH), 3.30 (2H, q, *J* = 6.0 Hz, CH₂NH) 2.74 (2H, t, *J* = 7.8 Hz, CH₂), 2.36 (2H, t, *J* = 7.8 Hz, CH₂), 1.44 (3H, d, *J* = 6.9 Hz, CHCH₃); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.6 (C=O), 171.2 (C=O), 160.1 (C=O), 152.2 (Ar-C), 136.3 (Ar-C), 132.1 (Ar-C), 130.3 (Ar-C), 129.3 (Ar-C), 129.2 (Ar-C), 128.0 (Ar-C), 126.3 (Ar-C), 125.2 (Ar-C), 123.5 (Ar-C), 123.3 (Ar-C), 122.0 (Ar-C), 119.3 (Ar-C), 118.1 (Ar-C), 115.4 (Ar-C), 107.6 (Ar-C), 49.3 (CHCH₃), 39.6 (CH₂NH, signal obscured by d₆-DMSO signal, can be seen by DEPT), 38.6 (CH₂NH), 35.9 (CH₂), 26.6 (CH₂), 17.8 (CH₃).

7.1.6. Synthesis of pyroglutamic acid derivatives **10**.

7.1.6.1. General procedure. Compounds **10** were prepared following the general procedure described in Section 6.1.4.1. using L-pyroglutamic acid as the amino acid.

7.1.6.1.1. Ethyl 3-(5-Amino-2-hydroxyphenyl)propanoate; L-pyroglutamic acid

derivative **10a.** This substrate was synthesized from compound **11a** (0.21 g, 1.00 mmol), L-pyroglutamic acid (0.13 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF/DMF (14 mL, 3:1). Yield: (0.27 g, 84%) as a brown powder, m.p. 91-92 °C. HRMS (NSI) for C₁₆H₂₀N₂O₅. Calculated mass of molecular ion 321.1445 [M+H]⁺. Measured mass: 321.1444; IR ν_{\max} cm⁻¹ 3283, 3216, 2962, 1722, 1659, 1549, 1436, 1223, 1175, 813, 710; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.80 (1H, s, NH), 9.31 (1H, s, broad, OH), 7.89 (1H, s, NH), 7.31-7.26 (2H, m, Ar-*H*), 6.72 (1H, d, *J* = 8.7 Hz, Ar-*H*), 4.14 (1H, m, CH) 4.04 (2H, q, *J* = 7.0 Hz, CH₂CH₃), 2.73 (2H, t, *J* = 7.8 Hz, CH₂CH₂CO), 2.52 (2H, m, CH₂CO), 2.34-2.24 (1H, m, γ -CH), 2.22-2.07 (2H, m, β -CH₂), 1.99-1.91 (1H, m, γ -CH), 1.16 (3H, t, *J* = 7.1 Hz, CH₃); ¹³C-NMR (101MHz; d₆-DMSO) δ_{C} 178.0 (C=O), 172.9 (C=O), 171.0 (C=O), 151.8 (Ar-C), 130.9 (Ar-C), 127.0 (Ar-C), 121.1 (Ar-C), 119.4 (Ar-C), 115.2 (Ar-C), 60.3 (CH₂CH₃), 56.8 (CHNH), 34.0 (CH₂), 29.8 (CH₂), 26.2 (CH₂), 25.9 (CH₂), 14.6 (CH₃).

7.1.6.1.2. Ethyl 3-(5-Amino-3-bromo-2-hydroxyphenyl)propanoate; L-pyroglutamic acid derivative 10b. Compound **10b** was synthesized from compound **11b** (0.58 g, 2.00 mmol), L-pyroglutamic acid (0.27 g, 2.10 mmol), *N*-methylmorpholine (0.20 g, 2.00 mmol) and IBCF (0.28 g, 2.00 mmol) in dry THF/DMF (20 mL, 3:1). Yield: (0.36 g, 45%) as a brown powder, m.p. 128 °C; HRMS (NSI) for C₁₆H₁₉BrN₂O₅. Calculated mass of molecular ion 399.0550 [M+H]⁺. Measured mass: 399.0558; IR ν_{\max} cm⁻¹ 3413, 3281, 2944, 1671, 1588, 1478, 1265, 1154, 1036; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 10.08 (1H, s, NH), 8.91 (1H, s, OH), 7.93 (1H, m, NH), 7.74 (1H, s, Ar-H), 7.24 (1H, s, Ar-H), 4.23 (1H, q, *J* = 4.6 Hz, CH), 4.05 (2H, q, *J* = 6.9 Hz, CH₂CH₃), 2.86 (2H, t, *J* = 7.3 Hz, CH₂), 2.57 (2H, m, CH₂), 2.48-2.39 (1H, m, γ -CH), 2.34-2.22 (2H, m, β -CH₂), 2.03-1.96 (1H, m, γ -CH), 1.15 (3H, t, *J* = 7.33 Hz, CH₃); ¹³C-NMR (101MHz; d₆-DMSO) δ_{C} 178.0 (C=O), 172.7 (C=O), 171.5 (C=O), 148.1 (Ar-C), 132.6 (Ar-C), 130.6 (Ar-C), 122.1 (Ar-C), 121.1 (Ar-C), 111.6 (Ar-C), 60.4 (CH₂CH₃), 56.8 (CHNH), 33.9 (CH₂), 29.7 (CH₂), 26.8 (CH₂), 25.8 (CH₂), 14.6 (CH₃).

7.1.6.1.3. 3-(5-Amino-2-hydroxyphenyl)-*N*-(2*H*-1,3-benzodioxol-5-yl)propanamide; L-pyroglutamic acid derivative 10c. This substrate was synthesized from compound **11c** (0.30 g, 1.00 mmol), L-pyroglutamic acid (0.13 g, 1.05 mmol), *N*-methylmorpholine (0.10 g, 1.00 mmol) and IBCF (0.14 g, 1.00 mmol) in dry THF/DMF (14 mL, 3:1). Yield: (0.39 g, 95%) as a white powder, m.p. 229-231 °C. HRMS (NSI) for C₂₁H₂₁N₃O₆. Calculated mass of molecular ion 412.1503 [M+H]⁺. Measured mass: 412.1500; IR ν_{\max} cm⁻¹ 3280, 3200-2600, 1652, 1548, 1493, 1227, 1042, 798, 743, 694; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.84 (1H, s, NH), 9.78 (1H, s, NH), 9.28 (1H, broad, s, OH), 7.88 (1H, s, NH), 7.32 (2H, dd, *J* = 10.5 and 2.3 Hz, Ar-H), 7.27 (1H, dd, *J* = 8.7 and 2.8 Hz, Ar-H), 6.94 (1H, dd, *J* = 8.7 and 1.8 Hz, Ar-H), 6.83 (1H, d, *J* = 8.7 Hz, Ar-H), 6.73 (1H, d, *J* = 8.7 Hz, Ar-H), 5.97 (2H, s, CH₂O), 4.13 (1H, m, CH), 2.78 (2H, t, *J* = 7.8 Hz, CH₂CH₂CO), 2.52 (2H, m, CH₂CO), 2.34-2.25 (1H, m, γ -CH), 2.22-2.07 (2H, m, β -CH₂), 1.99-1.90 (1H, m, γ -CH); ¹³C-NMR (101MHz; d₆-DMSO) δ_{C} 178.0 (C=O), 171.0 (C=O), 170.8 (C=O), 151.8 (Ar-C), 147.5 (Ar-C), 143.2 (Ar-C), 134.2 (Ar-C), 130.9 (Ar-C), 122.1 (Ar-C), 119.3 (Ar-C), 115.2 (Ar-C), 112.4 (Ar-C), 112.4 (Ar-C), 108.5 (Ar-C), 101.9 (Ar-C), 101.4 (CH₂O), 56.8 (CHNH), 36.7 (CH₂), 29.8 (CH₂), 26.4 (CH₂), 25.9 (CH₂).

7.1.7. Synthesis of 1-Naphthol derivatives 18-24.

7.1.7.1. 2-Benzyl-naphthalen-1-ol 18. To a stirred solution of compound **25**¹⁸ (1.0 g, 4.27 mmol) in ethanol (50 mL) was added rhodium(III) chloride hydrate (100 mg, 0.47 mmol). The mixture was heated at reflux for 24 h and then evaporated. Ethyl acetate (40 mL) was added to the residue and the resulting mixture was washed with water (40 mL). The organic layer was separated, dried (MgSO₄) and evaporated. The crude product was purified by column chromatography (eluent: ether/petroleum ether, b.p. 60-80 °C 1:9) giving compound **18** (0.90 g, 90%) as grey crystals, m.p. 73 °C, lit. m.p. 73-74 °C.¹⁹ ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.25 (1H, s, OH), 8.18 (1H, d, *J* = 7.3 Hz, Ar-H), 7.74 (1H, dd, *J* = 7.2 and 2.1 Hz, Ar-H), 7.43-7.36 (2H, m, Ar-H), 7.31 (1H, d, *J* = 8.2 Hz, Ar-H), 7.24-7.18 (5H, m, Ar-H), 7.14-7.08 (1H, m, Ar-H), 4.10 (2H, s, CH₂); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 149.9 (Ar-C), 141.9 (Ar-C), 133.7 (Ar-C), 129.5 (Ar-C), 129.1 (2 x Ar-C), 128.8 (2 x Ar-C), 128.0

(Ar-C), 126.2 (Ar-C), 125.9 (Ar-C), 125.9 (Ar-C), 125.4 (Ar-C), 122.5 (2 x Ar-C), 119.9 (Ar-C), 35.8 (CH₂).

7.1.7.2. 8-Hydroxy-N-(pyridine-4-ylmethyl)naphthalene-1-carboxamide 19. To a stirred solution of phenyl 1-hydroxy-2-naphthoate (0.53 g, 2.0 mmol) in THF (25 mL) was added 4-picolylamine (0.22 g, 2.0 mmol). The mixture was stirred at reflux for 24 h and then evaporated. The crude product was purified by column chromatography (eluent: CH₂Cl₂ then CH₂Cl₂/MeOH 7:3) giving compound **19** (0.42 g, 75%) as an orange solid, m.p. 132-134 °C. HRMS (NSI) for C₁₇H₁₄N₂O₂. Calculated mass of molecular ion 279.1128 [M+H]⁺. Measured mass: 279.1132; IR ν_{\max} cm⁻¹ 3244; 3044; 1622, 1597; 1548, 1401, 1332, 1272, 999, 790, 764; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 9.82 (1H, s, broad, NH), 8.56 (2H, d, *J* = 6.0 Hz, Ar-H), 8.32 (1H, d, *J* = 8.2 Hz, Ar-H), 8.01 (1H, d, *J* = 8.7 Hz, Ar-H), 7.90 (1H, d, *J* = 7.8 Hz, Ar-H), 7.66 (1H, t, *J* = 7.6 Hz, Ar-H), 7.57 (1H, t, *J* = 7.1 Hz, Ar-H), 7.46-7.36 (3H, m, Ar-H), 4.64 (2H, d, *J* = 5.0 Hz, CH₂NH); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 171.3 (C=O), 160.6 (Ar-C), 150.2 (2 x Ar-C), 148.4 (Ar-C), 136.5 (Ar-C), 129.4 (Ar-C), 128.0 (Ar-C), 126.23 (Ar-C), 125.5 (Ar-C), 123.7 (Ar-C), 123.3 (Ar-C), 122.7 (2 x Ar-C), 118.0 (Ar-C), 107.5 (Ar-C), 42.1 (CH₂).

7.1.7.3. 8-Phenylsulphonyl-1-naphthol 20. The synthesis of this compound has been described in the literature.²⁰

7.1.7.4. Ethyl 8-Hydroxy-1-naphthoate 21. The synthesis of this compound has been described in the literature.²¹

7.1.7.5. N-(2-Aminoethyl)-8-hydroxynaphthalene-1-carboxamide 22. To a stirred solution of ethylenediamine (1.41 g, 23.52 mmol) in THF (30 mL) at reflux was added a solution of compound **27** (1.00 g, 5.88 mmol) in THF (3 mL) dropwise. The mixture was kept at that temperature for 1 h after which time it was allowed to cool and then filtered. The resulting solid was washed with cold THF (20 mL) and then dried giving compound **22** (1.29 g, 95%) as a cream powder, m.p. 200-201 °C. HRMS (NSI) for C₁₃H₁₄N₂O₂. Calculated mass of molecular ion 231.1128 [M+H]⁺. Measured mass: 231.1127; IR ν_{\max} cm⁻¹ 3338, 3271, 3200-2400, 1641, 1554, 1272, 1011, 825, 766; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 8.20 (1H, s, broad, OH), 7.82 (1H, dd, *J* = 8.2 and 1.4 Hz, Ar-H), 7.42-7.35 (1H, m, Ar-H), 7.32-7.26 (2H, m, Ar-H), 7.23 (1H, dd, *J* = 8.2 Hz, *J* = 1.4 Hz, Ar-H), 6.80 (1H, dd, *J* = 6.9 and 1.8 Hz, Ar-H) 4.90 (2H, s, broad, NH₂), 3.31 (2H, t, *J* = 6.0 Hz, CH₂), 2.76 (2H, t, *J* = 6.2 Hz, CH₂); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.5 (C=O), 155.7 (Ar-C), 135.8 (Ar-C), 135.1 (Ar-C), 129.2 (Ar-C), 127.6 (Ar-C), 125.3 (Ar-C), 124.6 (Ar-C), 122.0 (Ar-C), 117.9 (Ar-C), 110.8 (Ar-C), 42.5 (CH₂), 41.5 (CH₂).

7.1.7.6. 8-Hydroxy-N-(2-phenylethyl)naphthalene-1-carboxamide 23. To a stirred solution of compound **27**²² (0.50 g, 2.94 mmol) in THF (30 mL) was added 2-phenylethylamine (0.36 g, 2.94 mmol). The mixture was stirred at reflux for 3 h and then evaporated. The crude product was dissolved in CH₂Cl₂ (20 mL) and filtered through silica (5 g). The silica was washed with CH₂Cl₂ (10 mL) and the combined organic filtrates were evaporated giving compound **23** (0.81 g, 95%) as a light yellow powder, m.p. 113-114 °C. HRMS (NSI) for

C₁₉H₁₇NO₂. Calculated mass of molecular ion 292.1332 [M+H]⁺. Measured mass: 292.1332; IR ν_{\max} cm⁻¹ 3335, 3300-2700, 1621, 1538, 1261, 822, 764, 698; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 10.25 (1H, s, broad, OH), 8.41 (1H, t, *J* = 5.0 Hz, NH), 7.87 (1H, d, *J* = 7.3 Hz, Ar-H), 7.45-7.20 (9H, m, Ar-H), 6.89 (1H, dd, *J* = 7.3 and 1.4 Hz, Ar-H), 3.49 (2H, q, *J* = 7.3 Hz, CH₂NH) 2.89 (2H, t, *J* = 7.8 Hz, CH₂CH₂NH); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 172.0 (C=O), 153.9 (Ar-C), 140.3 (Ar-C), 135.7 (Ar-C), 134.7 (Ar-C), 129.5 (Ar-C), 129.2 (2 x Ar-C), 128.9 (2 x Ar-C), 127.4 (Ar-C), 126.6 (Ar-C), 125.5 (Ar-C), 125.4 (Ar-C), 121.3 (Ar-C), 119.5 (Ar-C), 110.9 (Ar-C), 41.6 (CH₂), 35.4 (CH₂).

7.1.7.7. *N*-(2*H*-1,3-Benzodioxol-5-yl)-8-hydroxynaphthalene-1-carboxamide 24. To a stirred solution of compound **27**²² (0.50 g, 2.94 mmol) in THF (30 mL) was added 3,4-(methylenedioxy)aniline (0.41 g, 2.94 mmol). The mixture was stirred at reflux for 5 h and then evaporated. The crude product was purified by column chromatography (eluent: CH₂Cl₂ changing to CH₂Cl₂/ MeOH 9.5:0.5) giving compound **24** (0.41 g, 45%) as a cream powder, m.p. 162 °C. HRMS (NSI) for C₁₈H₁₃NO₄. Calculated mass of molecular ion 308.0917 [M+H]⁺. Measured mass: 308.0919; IR ν_{\max} cm⁻¹ 3242, 3150-2400, 1612, 1557, 1214, 1038, 812, 755; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 10.22 (1H, s, broad, OH), 10.05 (1H, s, NH), 7.90 (1H, dd, *J* = 8.7 and 1.2 Hz, Ar-H), 7.51-7.33 (5H, m, Ar-H), 7.11 (1H, dd, *J* = 8.7 and 2.1 Hz, Ar-H), 6.86 (1H, d, *J* = 8.2 Hz, Ar-H), 6.84 (1H, dd, *J* = 7.3 and 1.4 Hz, Ar-H), 5.99 (2H, s, CH₂); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 169.7 (C=O), 153.8 (Ar-C), 147.5 (Ar-C), 143.1 (Ar-C), 135.5 (Ar-C), 135.1 (2 x Ar-C), 129.0 (Ar-C), 127.4 (Ar-C), 125.8 (Ar-C), 124.7 (Ar-C), 121.1 (Ar-C), 119.2 (Ar-C), 112.6 (Ar-C), 110.1 (Ar-C), 108.5 (Ar-C), 102.0 (Ar-C), 101.3 (CH₂).

7.1.7.8 4-[Methyl(4-nitrophenyl)amino]butanoic acid 38. A mixture of 1-fluoro-4-nitrobenzene (1.00 g, 7.09 mmol), *N*-methyl- γ -aminobutyric acid hydrochloride (1.20 g, 7.80 mmol) and NaHCO₃ (1.49 g, 17.73 mmol) in EtOH/H₂O (1:1, 100 mL) was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature and the ethanol was evaporated. The remaining solution was acidified to pH 1-2 by the addition of 2M aqueous HCl solution. The mixture was extracted twice with EtOAc, the combined organic extracts were washed with H₂O and then brine, dried (MgSO₄) and evaporated giving compound **38** (1.04 g, 62%) as a yellow solid, m.p. 139-141 °C. LRMS (ESI) for C₁₁H₁₅N₂O₄. Calculated mass of molecular ion [M+H]⁺ 239.25. Measured mass: 239.05; IR ν_{\max} cm⁻¹ 3000, 1686, 1577, 1477, 1226; ¹H-NMR (270 MHz; d₆-DMSO) δ_{H} 12.17 (1H, s, broad, OH), 8.04 (2H, d, *J* = 9.14 Hz, Ar-H), 6.80 (2H, d, *J* = 9.7 Hz, Ar-H), 3.49 (2H, t, *J* = 7.4 Hz, CH₂), 3.05 (3H, s, *N*-CH₃), 2.29 (2H, t, *J* = 7.4 Hz, CH₂), 1.74 (2H, quintet, *J* = 7.4 Hz, CH₂); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 174.7 (C=O), 154.0 (Ar-C), 135.9 (Ar-C), 126.4 (2 x Ar-C), 111.1 (2 x Ar-C), 51.4 (CH₂), 38.8 (CH₃), 31.1 (CH₂), 22.2 (CH₂).

7.1.7.9. 4-[Methyl(4-nitrophenyl)amino]-*N*-2-(phenylethyl)butanamide 39. Compound **38** (1.00 g, 4.20 mmol) and *N*-methylmorpholine (0.43 g, 4.20 mmol) were dissolved in dry THF (20 mL) and cooled to -5°C. IBCF (0.55 g, 4.00 mmol) was then added dropwise to the reaction mixture. After 90 seconds, 2-phenylethylamine (0.48 g, 4.00 mmol) was added dropwise and the reaction was stirred at -5 °C for 1 h before being allowed to warm to room temperature and stirred for 16 h. The THF was evaporated and the residue dissolved in

CH₂Cl₂. The solution was then washed with 0.1 M aqueous citric acid solution, saturated aqueous NaHCO₃ solution, water and brine. The organic layer was dried (MgSO₄) and evaporated giving compound **39** (0.97 g, 71%) as a yellow solid, m.p. 131-133 °C. LRMS (ESI) for C₁₉H₂₄N₃O₃. Calculated mass of molecular ion [M+H]⁺ 342.41. Measured mass: 342.04; IR ν_{\max} cm⁻¹ 3289, 1643, 1594, 1477, 1279; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 8.05 (2H, d, *J* = 9.2 Hz, Ar-*H*), 7.99 (1H, t, *J* = 6.0 Hz, NH), 7.30-7.17 (5H, m, Ar-*H*), 6.78 (2H, d, *J* = 8.7 Hz, Ar-*H*), 3.40 (2H, t, *J* = 6.9 Hz, CH₂), 3.29 (2H, q, *J* = 6.0 Hz, CH₂), 3.03 (3H, s, *N*-CH₃), 2.71 (2H, t, *J* = 7.3 Hz, CH₂), 2.11 (2H, t, *J* = 7.3 Hz, CH₂), 1.75 (2H, quintet, *J* = 7.3 Hz, CH₂); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 171.9 (C=O), 154.1 (Ar-C), 140.0 (Ar-C), 135.9 (Ar-C), 129.1 (2 x Ar-C), 128.8 (2 x Ar-C), 126.6 (Ar-C), 126.4 (2 x Ar-C), 111.1 (2 x Ar-C), 51.7 (CH₂), 40.7 (CH₂), 38.9 (CH₃), 35.7 (CH₂), 32.5 (CH₂), 22.8 (CH₂).

7.1.7.10. 4-[4-Aminophenyl](methylamino)-*N*-2-(phenylethyl)butanamide 40. A mixture of compound **39** (0.31g, 1.00 mmol) and SnCl₂·2H₂O (0.68 g, 3.00 mmol) in ethanol (30 mL) was heated at reflux for 16 h. The solution was allowed to cool to rt and the pH was adjusted to 8-10 by the addition of 2M aqueous NaOH solution. The solution was then filtered through celite and the ethanol evaporated. The residue was dissolved in CH₂Cl₂ (30 mL) and the solution was filtered. The organic layer was washed with water and then brine and dried (MgSO₄). The solvent was evaporated giving compound **40** (0.22 g, 78%) as a brown, oily solid. LRMS (ESI) for C₁₉H₂₃N₃O₃. Calculated mass of molecular ion M⁺ 312.44. Measured mass: 312.07; ¹H-NMR (400 MHz; d₆-DMSO) δ_{H} 7.80 (1H, t, *J* = 5.5 Hz, NH), 7.25-7.11 (5H, m, Ar-*H*), 6.44 (4H, q, *J* = 8.7 Hz, Ar-*H*), 4.40 (2H, broad s, NH₂), 3.55 (2H, s, CH₂), 3.27 (2H, q, *J* = 6.4 Hz, CH₂), 2.73 (3H, s, CH₃), 2.65 (2H, t, *J* = 7.3 Hz, CH₂); ¹³C-NMR (101 MHz; d₆-DMSO) δ_{C} 170.7 (C=O), 141.9 (Ar-C), 140.8 (Ar-C), 139.9 (Ar-C), 129.2 (2 x Ar-C), 128.8 (2 x Ar-C), 126.6 (Ar-C), 115.6 (2 x Ar-C), 115.2 (2 x Ar-C), 58.7 (CH₂), 40.7 (CH₃), 40.5 (CH₂), 35.6 (CH₂).

7.1.7.11. 4-[4-Aminophenyl](methylamino)-*N*-2-(phenylethyl)butanamide; Boc L-alanine derivative 41. Using a similar procedure to that described in Section 7.1.4, but at -15 °C, compound **41** was prepared from Boc-L-alanine (0.19 g, 0.42 mmol), *N*-methylmorpholine (0.04 g, 0.42 mmol), IBCF (0.06 g, 0.40 mmol) and compound **40** (0.13 g, 0.40 mmol) in dry THF (20 mL). Compound **41** (0.13 g, 65%) was obtained as a yellow oil. HRMS (NSI) for C₂₇H₃₉N₄O₄. Calculated mass of molecular ion [M+H]⁺ 483.2966. Measured mass: 483.2962; ¹H-NMR (400 MHz; CDCl₃) δ_{H} 9.58 (1H, s, NH), 7.91 (1H, t, *J* = 5.5 Hz, NH), 7.38 (2H, *J* = 9.2 Hz, Ar-*H*), 7.29-7.19 (6H, 5 x Ar-*H* NH), 6.64 (2H, d, *J* = 9.2 Hz, Ar-*H*), 4.11-4.04 (1H, m, CH), 3.31-3.19 (4H, m, CH₂), 2.81 (3H, s, CH₃), 2.70 (2H, t, *J* = 6.9 Hz, CH₂), 2.07 (2H, t, *J* = 7.8 Hz, CH₂), 1.67 (2H, quintet, *J* = 7.3 Hz, CH₂), 1.38 (9H, s, 3 x CH₃), 1.24 (3H, d, *J* = 7.3 Hz, CH₃); ¹³C-NMR (101 MHz; CDCl₃) δ_{C} 172.6 (C=O), 170.9 (C=O), 155.9 (C=O), 146.8 (Ar-C), 139.0 (Ar-C), 128.8 (2 x Ar-C), 128.7 (2 x Ar-C), 127.3 (Ar-C), 126.6 (Ar-C), 122.1 (2 x Ar-C), 112.7 (Ar-C), 80.3 (C(CH₃)₃), 52.2 (CH₂), 50.7 (CH), 40.7 (CH₂), 38.6 (CH₃), 35.7 (CH₂), 33.7 (CH₂), 28.4 (3 x CH₃), 22.8 (CH₂), 18.3 (CH₃).

7.1.7.12. 4-[4-Aminophenyl](methylamino)-*N*-2-(phenylethyl)butanamide; L-alanine derivative TFA salt 42. A solution of compound **41** (0.09 g, 0.19 mmol) in a mixture

CH₂Cl₂/TFA (2:1, 15 mL) was stirred at room temperature for 2 h. The solvent was evaporated giving compound **42** (0.05 g, 48%) as a brown, oily solid. HRMS (NSI) for C₂₂H₃₂N₄O₂. Calculated mass of molecular ion [M+H]⁺ 383.2442. Measured mass: 383.2441; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 10.37 (1H, s, NH), 8.24 (3H, s, NH₃⁺), 7.98 (1H, t, *J* = 5.5 Hz, NH), 7.52 (2H, d, *J* = 8.7 Hz, Ar-*H*), 7.29-7.19 (5H, m, Ar-*H*), 6.99 (2H, broad s, Ar-*H*), 4.00-3.98 (1H, m, CH), 3.33-3.24 (4H, m, 2 x CH₂), 2.94 (3H, s, CH₃), 2.70 (2H, t, *J* = 7.3 Hz, CH₂), 2.09 (2H, t, *J* = 7.3 Hz, CH₂), 1.64 (2H, m, CH₂), 1.45 (3H, d, *J* = 7.3 Hz, CH₃); ¹³C-NMR (101 MHz; CDCl₃) δ_C 171.7 (C=O), 168.6 (C=O), 140.0 (Ar-C), 129.1 (2 x Ar-C), 128.8 (2 x Ar-C), 126.6 (2 x Ar-C), 121.3 (2 x Ar-C), 117.4 (Ar-C), 114.5 (Ar-C), 111.1 (Ar-C), 51.7 (CH₂), 49.5 (CH), 40.6 (CH₂), 38.8 (CH₃), 35.6 (CH₂), 32.5 (CH₂), 21.8 (CH₂), 17.7 (CH₃).

7.1.7.13. 4-[4-Aminophenyl](methylamino)-*N*-2-(phenylethyl)butanamide; L-pyroglutamic acid derivative **43.** Using a similar procedure to that described in Section 7.1.6, but at -15 °C, compound **43** was prepared from L-pyroglutamic acid (0.72 g, 5.56 mmol), *N*-methylmorpholine (0.56 g, 5.56 mmol), IBCF (0.72 g, 5.30 mmol) and compound **40** (1.65 g, 5.30 mmol) in a mixture of dry THF/DMF (10:1, 10 mL). The crude dark coloured oil was purified by column chromatography on silica gel (eluent: CH₂Cl₂/EtOAc 1:1 changing to EtOAc/MeOH 99:1) giving compound **43** (0.40 g, 18%) as a dark coloured solid, m.p. 100-103 °C. LRMS (ESI) for C₂₄H₃₀N₄O₃. Calculated mass of molecular ion [M+H]⁺ 423.53. Measured mass: 423.10; IR ν_{max} cm⁻¹ 3287, 1658, 1519; ¹H-NMR (400 MHz; d₆-DMSO) δ_H 9.72 (1H, s, NH), 7.95-7.87 (2H, m, 2 x NH), 7.41 (2H, d, *J* = 8.7 Hz, Ar-*H*), 7.29-7.17 (5H, m, Ar-*H*), 6.65 (2H, d, *J* = 9.2 Hz, Ar-*H*), 4.14-4.11 (1H, m, CH), 3.31-3.19 (4H, m, 2 x CH₂), 2.82 (3H, s, CH₃), 2.73-2.68 (2H, m, CH₂), 2.32-1.91 (6H, m, 3 x CH₂), 1.68-1.65 (2H, m, CH₂); ¹³C-NMR (101 MHz; CDCl₃) δ_C 178.0 (C=O), 172.2 (C=O), 170.8 (C=O), 146.2 (Ar-C), 140.0 (Ar-C), 129.1 (2 x Ar-C), 128.8 (2 x Ar-C), 128.5 (Ar-C), 126.6 (2 x Ar-C), 121.4 (2 x Ar-C), 112.6 (Ar-C), 56.8 (CH), 52.1 (CH₂), 40.6 (CH₂), 38.5 (CH₃), 35.7 (CH₂), 33.1 (CH₂), 29.8 (CH₂), 25.9 (CH₂), 22.5 (CH₂).

7.2. Microbiological work

7.2.1. Agar plate preparation

Each substrate (30 mg) was dissolved in a minimal volume of 1-methyl-2-pyrrolidone (200-400 μL) and added to molten Columbia agar (99 mL) (Oxoid, Basingstoke) at 50 °C to a final concentration of 300 mg L⁻¹. 1-Naphthol (5 mg) was dissolved in deionised water (1 mL) and four drops of 10 M aqueous sodium hydroxide were added to aid dissolution before addition. Agar plates were then prepared and dried to remove excess moisture. Bacterial strains and yeasts obtained from various national culture collections (see Tables) were sub-cultured onto Columbia agar. Colonies of each strain were sampled using a loop and suspended in 0.85 % sterile physiological saline to generate a suspension equivalent to 10⁸ colony forming units (cfu) per mL using a densitometer. Each agar plate was then inoculated with 1 μL of this suspension using a multipoint inoculator that delivered suspensions of 20 strains per plate. Plates were incubated at 37 °C in air for 24 h. Columbia agar without substrate (but with 1-naphthol included) were prepared and inoculated concomitantly.

7.2.2. Cell free extract

A cell free extract was prepared as described previously.²³

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