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Ring-deactivated hydroxyalkylpyrrole-based inhibitors of α-chymotrypsin: synthesis and mechanism of action

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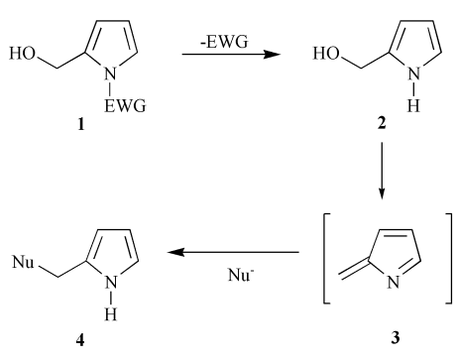
^{13}C NMR and mass spectrometry studies have been used to demonstrate that the inhibition of α-chymotrypsin by *N*-sulfonylhydroxymethylpyrrole inhibitors (**10**) is non-covalent. Hydroxyalkylpyrroles in which an electron-withdrawing group (acyl substituent) is introduced at the alternative C2 position have been synthesised and also shown to inactivate α-chymotrypsin. SAR studies on this class suggests that the incorporation of phenylalanine at C2 is favoured, however, there is little gain in introducing a hydrophobic substituent at C5.

Introduction

In previous work[1] we reported a series of hydroxymethylpyrroles substituted on nitrogen with an electron withdrawing group (EWG) (see **1** in Scheme 1) as a new class of inhibitor of α-chymotrypsin, a representative example of a class of enzyme known as serine proteases.[2] These compounds were postulated to act as mechanism-based inhibitors based upon model hydrolysis studies[3] and extensive chemical precedence.[4,5] In particular, hydrolytic removal of the EWG from **1**, in a process that mimics normal protease action on a substrate peptide, allows for the liberation of a highly electrophilic azafulvene (**3**) that is free to react with an available nucleophile (Scheme 1).[3] We proposed that this process could result in enzyme inactivation if it occurred in the vicinity of a catalytic group in the enzyme active site.[1]



Scheme 1

Peptidomimetics **1** can be considered to be chemically stable analogues of a dipeptide substrate of α-chymotrypsin where the EWG mimics an amino acid on the *N*-terminus of the substrate scissile bond as depicted in **5**, Fig. 1. In support of this notion we found[1] that the best inhibitors of type **1** contained a hydrophobic EWG that mimics the aromatic P_1 group (see Fig. 1)† of a natural substrate of α-chymotrypsin, *i.e.* the point of natural cleavage of a peptide by α-chymotrypsin.[6] We now report results of ^{13}C NMR and mass spectrometric studies, using α-chymotrypsin and labelled inhibitor, that suggest that the mechanism of inhibition is in fact non-covalent in nature. We also report a new but related series of C2-acylated hydroxyalkylpyrroles extended in the C-direction with an amino acid or dipeptide that also inhibit α-chymotrypsin by a non-covalent mechanism. These compounds have the EWG at C2 and as such mimic an alternative conformation of a natural substrate (see Fig. 1).

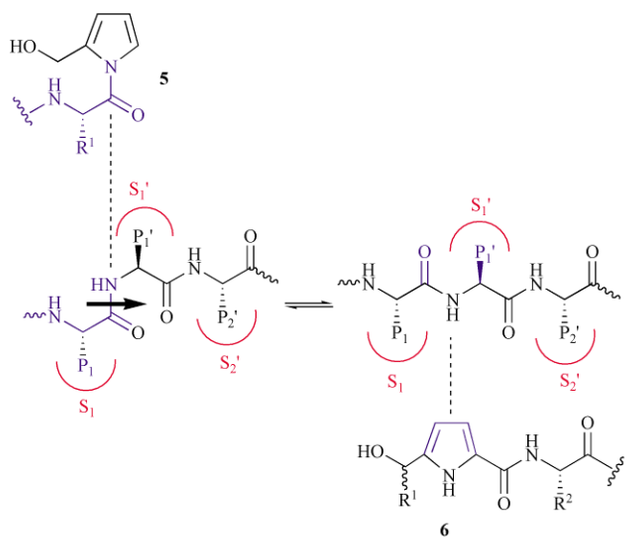


Fig. 1 Schematic representation of two conformations of a natural substrate (middle) bound to the active site of α-chymotrypsin, and how these might correlate with the proposed *N*-acylhydroxymethylpyrrole **5** (top) and the *C*2-acylhydroxyalkylpyrrole **6** (bottom) inhibitors. The point of cleavage of the natural substrate is indicated by the bold arrow. Note the use of Schechter–Berger nomenclature.†

Results and discussion

We chose to use *N*-phenylsulfonylpyrrole **10a** (see Scheme 2) for the mechanistic studies since it is the most potent inhibitor of this type identified to date and also because it is readily available.[1] Initial ^{1}H NMR studies on **10a**, whereby it was incubated in $CDCl_3$ at room temperature for 7 days without degradation, illustrated that this compound is relatively stable and hence suitable for mechanistic studies. In addition, we have demonstrated that an *N*-sulfonyl group, of the type found in **10a**, is the strongest ring-deactivating group of those studied, and as such it is the best at suppressing azafulvene formation.[5]

In the first series of experiments we decided to incorporate a ^{13}C-label into **10a** as a marker for monitoring changes to the electronic environment of the hydroxymethyl carbon on exposure to α-chymotrypsin. The target ^{13}C labelled compound

† Note the use of Schechter–Berger nomenclature[22] – the residues on the *N*-terminal side of the peptide bond that is cleaved are denoted (in order) P1–Pn, and those on the *C*-terminus are denoted $P_{1'}$–$P_{n'}$. In turn, the corresponding subsites on the enzyme are denoted S_n–$S_{n'}$

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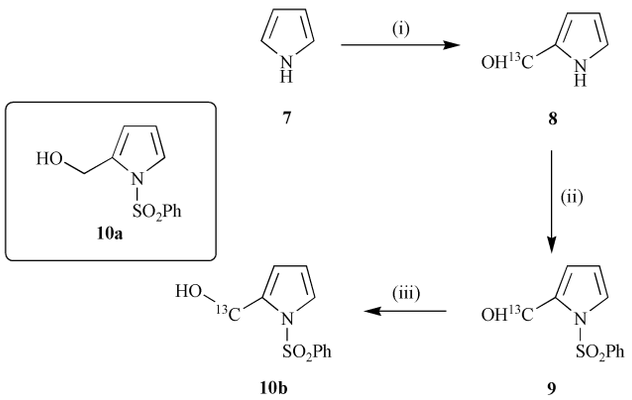


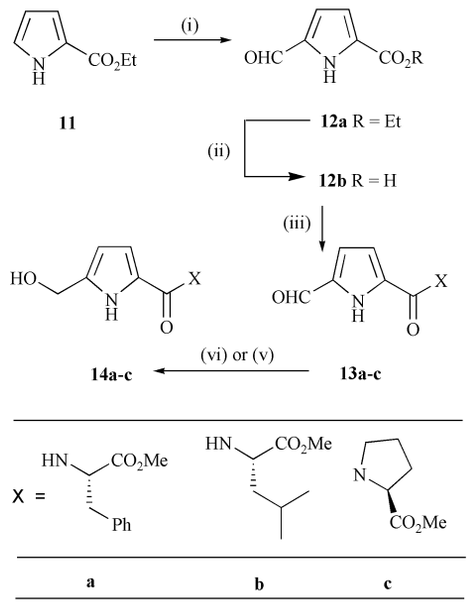
Scheme 2 *Reagents and conditions:* (i) $POCl_3$, ^{13}C-DMF, 1,2-DCE, reflux, 15 min, then $CH_3CO_2Na{\cdot}3H_2O_{(aq)}$, reflux, 15 min (59%); (ii) $PhSO_2Cl$, 30% $NaOH_{(aq)}$, Bu_4NI, CH_2Cl_2, rt, 30 h (91%); (iii) $Zn(BH_4)_2$, Et_2O, 0 °C, 30 min (90%).

10b was prepared using the method previously reported for **10a**[1] (Scheme 2) and a modification reported by Hinz *et al.*[7] In particular, pyrrole was formylated with ^{13}C–*N,N*-dimethylformamide under Vilismeier–Haack conditions[8] to give **8**. This was reacted with phenylsulfonyl chloride under phase transfer catalysis[7] to give **9**, which was subsequently reduced with zinc borohydride[5] to give the desired labelled derivative **10b**.

An analysis of the ^{13}C NMR spectrum of a sample of **10b** incubated with α-chymotrypsin, using conditions previously reported for a different class of inhibitor,[9] revealed an enriched resonance at 56 ppm, the same chemical shift as was observed for **10b** in the absence of enzyme.‡ In addition, an electrospray mass spectrum of the product from the incubation of **10a** with α-chymotrypsin revealed signals at 25,236 g mol^{-1} (corresponding to the native enzyme), and 25,473 g mol^{-1}, a difference of 237 g mol^{-1} which corresponds to the molecular mass of intact **10a**. These results strongly suggest that inhibitors **10** are not covalently attached to α-chymotrypsin as previously postulated.[1]

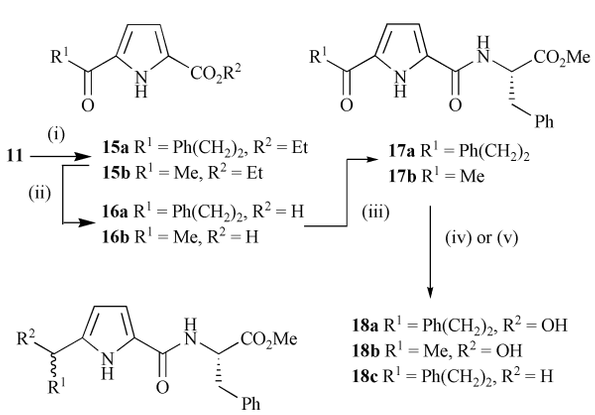
We next decided to investigate the effect of introducing an EWG at the alternative C2-position of a hydroxymethylpyrrole (see general structure **6** in Fig. 1). Unlike structures of type **1** and **5**, these derivatives do not have the EWG directly attached to the pyrrole nitrogen and as such they mimic an extended conformation of a peptide backbone as depicted in Fig. 1. We reasoned that, while a C2-acyl group as in **6** would still deactivate the pyrrole, it would do so to a lesser extent. As such azafulvene formation would be more favoured, which should in turn promote inactivation of α-chymotrypsin by a covalent mechanism. It is also important to note that compounds of type **6** allow for chain extension in the C-direction and also the incorporation of a range of groups in the P_1 position. A series of such compounds was prepared and assayed against α-chymotrypsin.

Scheme 3 details the synthesis of compounds of type **6** that bear a hydrogen at R^1 (P_1 group as defined for a substrate in Fig. 1). Three derivatives (**14a–c**) were prepared to investigate the effect of the nature of the appended amino acid on inhibitory potency. Phenylalanine was used (**14a**) as it is known that the S_2' subsite of α–chymotrypsin preferentially accommodates large, hydrophobic moieties of this type (see Fig. 1).[10,11] Leucine and proline were chosen in order to test this hypothesis in our series and to gain some possible insight into the mode of enzyme binding. The synthesis of compounds **14** began with the formylation of the pyrrole ester **11**[12] under Vilismeier–Haack conditions[13] to give **12a**, which would eventually give rise to the hydroxymethyl side chain of **14a–c**. Hydrolysis of the ester of **12a** with potassium hydroxide gave the key intermediate **12b** in 60% yield.[14] The free acid of **12b** was then separately coupled with L-phenylalanine methyl ester hydrochloride, L-leucine methyl ester hydrochloride and L-proline methyl ester hydrochloride, using standard EDCI conditions,[15] to give **13a**,**b** and **c** respectively. Reduction of the 5-formyl moiety of **13a–c** with zinc borohydride gave the desired dipeptide analogues **14a–c** in reasonable yields. The use of lithium borohydride, instead of zinc borohydride, for the reduction of **13b** gave an improvement in yield from 42% to 66%.



Scheme 3 *Reagents and conditions:* (i) $POCl_3$, DMF, 1,2-DCE, reflux, 15 min, then $CH_3CO_2Na{\cdot}3H_2O$, reflux, 15 min (61%); (ii) KOH, H_2O, 40–50 °C, 2 h (60%); (iii) EDCI, HOBT, DIEA, CH_2Cl_2, rt, 16 h and either L–PheOMe·HCl (**13a**, 71%), L–LeuOMe·HCl (**13b**, 61%), or L–ProOMe·HCl (**13c**, 74%); (vi) $Zn(BH_4)_2$, ether, 0 °C, 1 h (**14a**, 57%), (**14b**, 42%), (**14c**, 24%); (v) $LiBH_4$, ether, −78 °C, 1 h (**14b**, 66%).

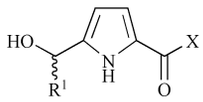
Next we prepared compounds **18a** (Scheme 4) in which R^1 (see general structure **6**, Fig. 1) more closely correlates to the known specificity of the P_1 group of a substrate of α–chymotrypsin (see Fig. 1). We also prepared compounds **18b** in which R^1 (Me) is of intermediary size, *cf.* H and $(CH_2)_2Ph$ of **14a–c** and **18a**, respectively. To this end, the pyrrole **11** was acylated with either hydrocinnamoyl chloride or acetyl chloride[16] to give **15a** and **15b**, respectively. Hydrolysis of the ethyl esters of **15a** and **15b** with potassium hydroxide gave the acids **16a** and **16b** which were then separately coupled with L-phenylalanine methyl ester hydrochloride (as for **12b**) to give **17a** and **17b**. A final reduction of the 5-keto group of **17a** and **17b** with zinc



Scheme 4 *Reagents and conditions:* (i) $ZnCl_2$, 1,2-DCE, 50 °C, 1 h and $Ph(CH_2)_2COCl$ (**15a**, 35%) or AcCl (**15b**, 37%); (ii) KOH, 2 : 1 H_2O–THF, 40–50 °C, 2 h (**16a**, 86%), (**16b**, 93%); (iii) L–PheOMe·HCl, EDCI, HOBT, DIEA, CH_2Cl_2, rt, 16 h (**17a**, 70%), (**17b**, 91%); (iv) $Zn(BH_4)_2$, ether, 0 °C, 1 h (**18a**, 21%), (**18c**); (v) $LiBH_4$, ether, −78 °C, 1 h, then rt, 1 h (**18b**, 46%).

‡ A difference spectrum was determined by subtracting the ^{13}C NMR spectrum of **10a** (unlabelled) with α-chymotrypsin from that of **10b** (labelled) with α-chymotrypsin according to the published method.[9]

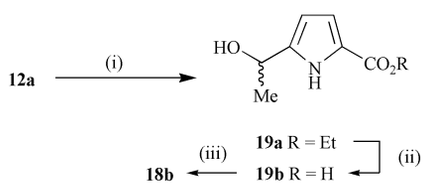
Table 1 Inhibition of α-chymotrypsin by **14a–c**, **18a** and **22**



Compound	R^1	X	% Inhibition (μg mL^{-1}) 1250	125	12.5
14a	H	NH–L–PheOMe	75	35	45
14b	H	NH–L–LeuOMe	10	5	20[a]
14c	H	N–L–ProOMe	45	25	5
18a	$Ph(CH_2)_2$	NH–L–PheOMe	75	35	15
22	H	NH–L–Phe–L–LeuOMe	100	20	15

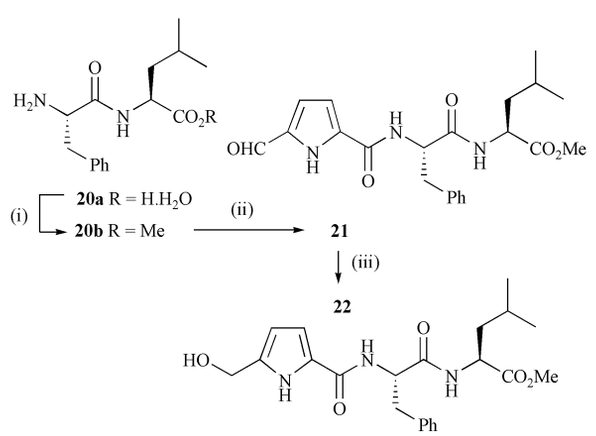
[a] This apparent anomalous result where greater inhibition is observed at lower concentration is under further investigation.

borohydride gave the extended peptide analogues **18a** and **18b** as 1 : 1 mixtures of epimers by ^{1}H NMR. An over reduction was observed in the case of **17a**, with the production of an almost equal amount of by-product **18c**. Compound **18b** proved to be somewhat unstable and its purification problematic. An alternative preparation of **18b** was also investigated as outlined in Scheme 5. Here, the formyl group of pyrrole **12a** was reacted with methyllithium to give **19a**. Subsequent ethyl ester hydrolysis with sodium hydroxide gave **19b** in low yield, which was then coupled to L-phenylalanine methyl ester hydrochloride to give **18b**, which again proved difficult to purify and was ultimately unstable.



Scheme 5 *Reagents and conditions:* (i) MeLi, THF, −23 °C, 5 h (95%); (ii) NaOH, 1 : 1 THF–H_2O, 40–50 °C, 24 h (17%); (iii) L–PheOMe·HCl, EDCI, HOBT, DIEA, CH_2Cl_2, rt, 16 h (54%).

Finally, we prepared an extended tetrapeptide analogue **22** based upon the simple hydroxymethylpyrrole scaffold of compounds **14a–c**. We chose to extend the peptide in the C-direction using L-phenylalanyl-L-leucine since this sequence is known to favour binding to α-chymotrypsin.[17,18] This sequence also allows for a direct comparison with **14a**, which simply has L-phenylalanine attached. The required starting dipeptide ester **20b** was prepared from commercially available L-phenylalanyl-L-leucine hydrate **20a** on treatment with diazomethane (Scheme 6). This dipeptide methyl ester was then coupled to **12b** in the presence of BOP[19] to give **21** in 52% yield over two steps. Reduction with lithium borohydride then gave **22**.



Scheme 6 *Reagents and conditions:* (i) CH_2N_2, THF, rt, 15 h; (ii) **12b**, BOP, DIEA, CH_2Cl_2, rt, 16 h (52% yield over two steps); (iii) $LiBH_4$, THF, −78 °C, 1 h, then rt, 1 h (66%).

The peptidomimetics **14a–c**, **18a** (as a mixture of diastereomers) and **22** were assayed against α–chymotrypsin at three concentrations using a simple screening assay as described previously[1] and the results are presented in Table 1 (compound **18b** proved too unstable to assay). From these results it is apparent that there is not a significant difference in the inhibitor potency of compounds **14a** and **18a**, compounds that differ solely in the nature of R^1 (see **6** in Fig. 1). We had postulated that the hydrophobic group of **18a** might be favoured (see earlier for a discussion). However, it is important to remember that **18a** was assayed as a mixture of diastereomers and as such one isomer may be more active than the other. A comparison of the inhibitory assay results obtained for the four compounds (**14a–c** and **22**) that contain a hydroxymethylpyrrole moiety suggest a slight preference for a phenylalanine in the appended X group. The tetrapeptide analogue **22** proved the most potent and an IC_{50} value of 108 μM was determined for this compound. LCMS studies were also performed on α-chymotrypsin incubated with **14a** at 37 °C for 3 hours in 50 mM Tris–HCl. As for **10**, there was no evidence of covalent attachment of the inhibitor to the enzyme. Finally, it should be noted that 2-acylhydroxyalkylpyrroles of general structure **6** (see **14a–c**, **18a** and **22** in Table 1) are similarly active to the compounds in which the EWG is substituted on nitrogen (see **1** and **5**).[1]

Conclusion

N-Substituted hydroxymethylpyrroles of general type **1** are known to inhibit α-chymotrypsin. In this paper we have used a combination of mass spectrometry and ^{13}C NMR experiments to show that compounds of type **1** (specifically **10**) do not form a covalent attachment to the enzyme. A modified series of compounds was then prepared in which a peptidic side chain is attached to the pyrrole nucleus at C2. Assay results for this series reveal that, while compounds with a phenylalanine residue as part of the C2 side chain (X in Table 1) are the most potent inhibitors of α-chymotrypsin, there is little gain in introducing a more hydrophobic substituent at C5 (R^1 in Table 1). The IC_{50} value for the most potent compound (**22**) in this new series of inhibitor of α-chymotrypsin was determined to be 108 μM. Again it appears that these compounds inhibit α-chymotrypsin *via* a non-covalent mechanism.

Experimental

All melting points were obtained on an Electrothermal apparatus and are uncorrected. Proton NMR spectra were obtained on a Varian Inova spectrometer, operating at 500 MHz. Carbon NMR were obtained on a Varian Unity 300 spectrometer, operating at 75 MHz. IR spectra were obtained using a Shimadzu 8201PC series FTIR, either in $CHCl_3$ (solution phase), or in

solid KBr (diffuse reflectance). Molecular masses of products were detected on a Kratos MS80 RFA mass spectrometer. Inhibitor–enzyme samples were analysed initially by electrospray ionisation mass spectrometry (ESI) using a Micromass LCT mass spectrometer scanning at 100–2500 atomic mass units (scans every 0.9 s, 0.1 s delay). The source was at 80 °C (ESI probe = 150 °C, nebuliser = 190 L h^{-1}, desolvation = 350 L h^{-1}). Liquid chromatography mass spectrometry (LCMS) was carried out on a Waters 2790 LC coupled to the Micromass LCT spectrometer using a Waters 996 PDA Zorbax C3 column (150 mm × 2 mm × 5 μm, flow 0.2 cm^3 min^{-1}). The mobile phase consisted of a gradient of 100% [water–0.5% formic acid] to 40% [water–0.5% formic acid]–60% acetonitrile over 80 min, then to 100% acetonitrile over 5 min, held at 100% acetonitrile for 10 min, then back to 100% [water–0.5% formic acid] over 5 min. Data was processed with Maxent® software. α-Chymotrypsin assays were carried out in microtitre plates (twelve 1 × 8 well Titertek® microtitre strips) and read in a BMG Labtechnologies Fluostar Galaxy 96-well plate reader. Compounds **11**,[12] **12a**[13] and **12b**[14] were prepared by literature methods. Flash chromatography was performed using 230–400 mesh Merck Silica Gel 60 under positive pressure. All solvents were distilled prior to use using literature procedures.[20]

α-Chymotrypsin inhibition experiments

Assay[21]**.** Solutions of **14a**–**c**, **18a** and **22** were made to 1250 μg cm^3, 125 μg cm^3 and 12.5 μg cm^3 in methanol. Tris–HCl (50 μL of a 0.4 M solution in water, pH 7.6), distilled water (50 μL), inhibitor solution (50 μL) and α-chymotrypsin (50 μL, Sigma ex-bovine pancreas, 9 units cm^3 in 50 mM Tris–HCl, pH 7.6) were added to each well of the microtitre plate. Incubation at 37 °C for 30 min was followed by the addition of *N*-succinyl-L-phenylalanine-4-nitroanilide (100 μL, 1 mg per cm^3 solution in 50 mM Tris–HCl buffer, pH 7.6). The absorbance was read at 405 nm at hourly intervals until the maximum measured absorbance exceeded 1.00 absorbance units. Each inhibitor concentration was assayed in triplicate and average absorbances were used to calculate the percentage (%) inhibition. Sample blanks to determine the absorbance for each inhibitor concentration in the absence of enzyme (*i.e.* no substrate turnover) were run concurrently, in which Tris–HCl (50 μL of a 50 mM solution in water, pH 7.6), replaced α-chymotrypsin. Maximum substrate turnover was determined by averaging the absorbance readings from six wells in which methanol (50 μL) replaced the inhibitor solution. All percentage (%) inhibition figures reported are rounded to the nearest 5%. The IC_{50} for **22** was determined using the same general method except that solutions of **22** were made to 12.0 mM, 3.01 mM, 0.75 mM, 0.19 mM, 47.0 μM, 11.8 μM, 2.94 μM and 0.73 μM. Precipitation occurred in all wells where **22** was present at a concentration of 12.0 mM, so no inhibition value could be calculated at this concentration. Each sample was assayed in triplicate. The IC_{50} value was calculated by fitting the inhibitor concentration–percent inhibition data into Microsoft Excel.

^{13}C NMR and LCMS. Stock solution A was made up by dissolving **10b** (2 mg) in DMSO (225 μL), followed by addition of D_2O (2.775 cm^3) to give a final concentration of 2.8 mM. Stock solution B was made up by dissolving α-chymotrypsin (71 mg, Sigma ex-bovine pancreas) in DMSO (75 μL) followed by addition of D_2O (925 μL) to also give a concentration of 2.8 mM. Stock solution C was made up as for A using **10a** in place of **10b**. Solution A (300 μL) and separate mixtures of A and B (300 μL, 1 : 1) and B and C (300 μL, 1 : 1) were then left at room temperature for one hour, shaking occasionally. The samples were transferred to NMR tubes and the ^{13}C NMR spectra were determined and analysed as described.[9] LCMS experiments on enzyme inhibitor complexes were carried out on **10a**, **10b** and **14a** in solution in Tris–HCl buffer.

General procedure A: peptide couplings using EDCI

To a stirred solution (~0.1 M) of the pyrrole carboxylic acid (1 equiv.) and the L-amino acid ester hydrochloride (1.1 equiv.) in dry dichloromethane (~8 cm^3), at rt under an inert atmosphere, were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.3 equiv.) and 1-hydroxybenzotriazole hydrate (1.5 equiv.). *N*,*N*-Diisopropylethylamine (1.1 equiv.) was added, and the reaction mixture was stirred at rt for 16 h. The solution was then diluted with dichloromethane (10 cm^3), washed with 3 M aqueous hydrochloric acid (2 × 10 cm^3), water (2 × 10 cm^3), dried ($MgSO_4$) and the solvent was removed by evaporation under reduced pressure. The resultant material was purified by flash chromatography on silica. See individual experiments for details.

Modification to general procedure A

The reaction was performed in the manner as described in general procedure A, except that the combined aqueous washings were back-extracted with dichloromethane (2 × 10 cm^3), and the combined organic fractions were then dried ($MgSO_4$). The solvent was removed by evaporation under reduced pressure, and the resultant material was purified by flash chromatography on silica. See individual experiments for details.

General procedure B: zinc borohydride reductions

A stirred solution (~0.1 M) of the pyrrole (1 equiv.), dissolved in dry ether under an inert atmosphere, was cooled to 0 °C. Zinc borohydride (2.2 equiv. of a 0.14 M solution in ether) was added, and the resultant solution was stirred at 0 °C for 1 h. Water (1 cm^3), and then 10% aqueous acetic acid (1 cm^3), were carefully added to quench the reaction. The separated aqueous phase was extracted with dichloromethane (2 × 10 cm^3), and the combined organic phases were washed with water (2 × 10 cm^3), saturated aqueous brine (10 cm^3), dried ($MgSO_4$) and the solvent was removed by evaporation under reduced pressure. The resultant material was purified by flash chromatography on silica. See individual experiments for details.

[*Formyl*-^{13}*C*]-1*H*-pyrrole-2-carboxaldehyde (8). Prepared from **7** (210 mg, 3.13 mmol) using phosphorous oxychloride (526 mg, 3.43 mmol) and *N*,*N*-dimethylformamide-*carbonyl*-^{13}*C* (255 mg, 3.43 mmol) as described for unlabelled **8**[8] (177 mg, 59%); δ_H(500 MHz; $CDCl_3$; Me_4Si) 6.36 (1 H, m, pyrrole 4-H), 7.02 (1 H, m, pyrrole 3-H), 7.18 (1 H, m, pyrrole 5-H), 9.51 (1 H, m, *J* = 175.3 Hz, C*H*O), 10.39 (1 H, br s, N*H*); δ_C(75 MHz; $CDCl_3$; Me_4Si) 111.3, 121.8, 126.9, 132.7, 179.4 (*C*HO enriched peak).

[*Formyl*-^{13}*C*]-1-(phenylsulfonyl)pyrrole-2-carboxaldehyde (9). Prepared from **8** (110 mg, 1.14 mmol) as described for unlabelled **9**[7] (212 mg, 91%); δ_H(500 MHz; $CDCl_3$; Me_4Si) 6.42 (1 H, t, *J* = 3.4 Hz, pyrrole 4-H), 7.16 (1 H, m, pyrrole 3-H), 7.54–7.64 (4 H, m, pyrrole 5-H and Ar*H*), 7.92 (2 H, m, Ar*H*), 9.95 (1 H, d, *J* = 182.6 Hz, C*H*O); δ_C(75 MHz; $CDCl_3$; Me_4Si) 112.5, 124.8, 127.4, 129.5, 133.5, 134.5, 138.1, 178.7 (*C*HO enriched peak).

[*Methylene*-^{13}*C*]-2-hydroxymethyl-1-(phenylsulfonyl)pyrrole (10b). Prepared from **9** (62 mg mg, 0.30 mmol) as described for **10a**[1] (56 mg, 90%); δ_H(500 MHz; $CDCl_3$; Me_4Si) 2.78 (1 H, br s, O*H*), 4.60 (2 H, d, *J* = 145.0 Hz, CH_2OH), 6.24–6.27 (2 H, m, pyrrole 4-H and pyrrole 3-H), 7.27 (1 H, m, pyrrole 5-H), 7.50–7.62 (3 H, m, Ar*H*), 7.82 (2 H, m, Ar*H*); δ_C(75 MHz; $CDCl_3$; Me_4Si) 57.7 (*C*H_2OH enriched peak), 112.0, 115.4, 123.6, 126.6, 129.5, 134.0, 134.6, 139.0.

***N*-(5-Formyl-1*H*-pyrrole-2-carbonyl)-L-phenylalanine methyl ester (13a).** The pyrrole acid **12b**[14] (100 mg, 0.72 mmol, 1 equiv.) was coupled with L-phenylalanine methyl ester hydro-

chloride (171 mg, 0.79 mmol, 1.1 equiv.) according to general procedure A. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 2 : 1) to give **13a** (152 mg, 71%) as a cream solid; mp 48–51°; $\nu_{max}(CHCl_3)/cm^{-1}$ 1529.4, 1550.7, 1676.0, 1739.7, 3020.3, 3423.4; δ_H(500 MHz; $CDCl_3$; Me_4Si) 3.22 (2 H, m, CH_2Ph), 3.76 (3H, s, CO_2CH_3), 5.06 (1 H, m, NHCH), 6.51 (1 H, d, J = 7.3 Hz, NHCH), 6.61 (1 H, dd, J = 2.7, 4.2 Hz, pyrrole 3-H), 6.91 (1 H, dd, J = 2.7, 4.2 Hz, pyrrole 4-H), 7.10 (2 H, m, ArH), 7.27 (3 H, m, ArH), 9.61 (1 H, s, CHO), 10.11 (1 H, br s, pyrrole NH); ^{13}C NMR (75 MHz; $CDCl_3$; Me_4Si) δ 38.0, 52.6, 53.2, 111.2, 120.0, 127.2, 128.6, 129.3, 131.2, 134.0, 135.5, 159.3, 171.9, 180.3; *m*/*z* (ES) 301.1188 (M^+ + H) $C_{16}H_{17}N_2O_4$ requires 301.1188, 301 (100%).

***N*-(5-Formyl-1*H*-pyrrole-2-carbonyl)-L-leucine methyl ester (13b).** The pyrrole acid **12b**[14] (100 mg, 0.72 mmol, 1 equiv.) was coupled with L-leucine methyl ester hydrochloride (144 mg, 0.79 mmol, 1.1 equiv.) according to general procedure A. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 2 : 1) to give **13b** (116 mg, 61%) as a cream solid; mp 139–142 °C; $\nu_{max}(CHCl_3)/cm^{-1}$ 1529.4, 1550.7, 1676.0, 1739.7, 2960.5, 3018.4, 3425.3; δ_H(500 MHz; $CDCl_3$; Me_4Si) 0.96 (6 H, m, CH(CH_3)$_2$), 1.61–1.77 (3 H, m, CH_2CH), 3.77 (3 H, s, CO_2CH_3), 4.83 (1 H, m, NHCH), 6.62 (1 H, d, J = 8.3 Hz, NHCH), 6.68 (1 H, d, J = 2.4 Hz, pyrrole 3-H), 6.93 (1 H, d, J = 2.9 Hz, pyrrole 4-H), 9.62 (1 H, s, CHO), 10.18 (1 H, br s, pyrrole NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 21.9, 22.8, 24.9, 41.7, 50.9, 52.6, 111.0, 120.0, 131.2, 133.9, 159.6, 173.7, 180.2; *m*/*z* (EI) 266.1266 (M^+. $C_{13}H_{18}N_2O_4$ requires 266.1267), 210 (30%), 207 (38), 138 (12), 123 (26), 122 (75), 86 (100), 66 (20) and 44 (14).

***N*-(5-Formyl-1*H*-pyrrole-2-carbonyl)-L-proline methyl ester (13c).** The pyrrole acid **12b**[14] (100 mg, 0.72 mmol, 1 equiv.) was coupled with L-proline methyl ester hydrochloride (131 mg, 0.79 mmol, 1.1 equiv.) according to modified general procedure A. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 2 : 1 then 4 : 1) to give **13c** (133 mg, 74%) as a white solid; mp decomp. >40 °C (Found: C, 57.63; H, 5.73; N, 11.21. $C_{12}H_{14}N_2O_4$ requires C, 57.59; H, 5.64; N, 11.19%); $\nu_{max}(CHCl_3)/cm^{-1}$ 1544.9, 1616.2, 1672.2, 1745.5, 3419.6; δ_H(500 MHz; $CDCl_3$; Me_4Si) 2.01–2.27 (4 H, m, NCH$_2$CH_2CH_2CH), 3.73 (3 H, s, CO_2CH_3), 3.86 (1 H, m, NCH_{2a}CH$_2$CH$_2$CH), 3.97 (1 H, m, NCH_{2b}CH$_2$CH$_2$CH), 4.70 (1 H, m, NCH), 6.69 (1 H, m, pyrrole 3-H), 6.92 (1 H, m, pyrrole 4-H), 9.63 (1 H, s, CHO), 10.38 (1 H, br s, pyrrole NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 25.3, 28.6 (NCH$_2$CH_2CH_2CH), 48.5 (NCH$_2$CH$_2$CH$_2$CH), 52.3 (CO_2CH_3), 60.2 (NCH), 113.2 (pyrrole 3-C), 119.3 (pyrrole 4-C), 130.7 (pyrrole 2-C), 133.3 (pyrrole 5-C), 159.5 (*C*ONH), 172.3 (CO_2), 180.1 (*C*HO); *m*/*z* (EI) 250.0952 (M^+. $C_{12}H_{14}N_2O_4$ requires 250.0954), 218 (14%), 191 (47), 122 (26), 94 (12) and 70 (100).

***N*-(5-Hydroxymethyl-1*H*-pyrrole-2-carbonyl)-L-phenylalanine methyl ester (14a).** The pyrrole **13a** (106 mg, 0.35 mmol, 1 equiv.) was reduced with zinc borohydride according to general procedure B. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 4 : 1) to give **14a** (61 mg, 57%) as a tan solid; mp 105–107 °C (Found: C, 63.42; H, 5.90; N, 9.38. $C_{16}H_{18}N_2O_4$ requires C, 63.57; H, 6.00; N, 9.27%); $\nu_{max}(CHCl_3)/cm^{-1}$ 1525.6, 1637.5, 1739.7, 3035.7, 3435.0; δ_H(500 MHz; $CDCl_3$; Me_4Si) 2.97 (1 H, br s, CH$_2$OH), 3.20 (2 H, m, CH_2Ph), 3.74 (3 H, s, CO_2CH_3), 4.65 (2 H, s, CH_2OH), 5.00 (1 H, m, NHCH), 6.08 (1 H, t, J = 3.2 Hz, pyrrole 4-H), 6.37 (1 H, d, J = 7.8 Hz, NHCH), 6.47 (1 H, t, J = 3.2 Hz, pyrrole 3-H), 7.12 (2 H, d, J = 7.3 Hz, ArH), 7.27 (3 H, m, ArH), 10.31 (1 H, br s, pyrrole NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 37.9 (*C*H$_2$Ph), 52.4 (CO_2CH_3), 53.2 (NH*C*H), 57.2 (*C*H$_2$OH), 108.1 (pyrrole 4-C), 110.9 (pyrrole 3-C), 124.5 (pyrrole 2-C), 127.1, 128.5, 129.1, 135.7 (Ar*C*), 136.8 (pyrrole 5-H), 161.3 (*C*ONH), 172.4 (CO_2); *m*/*z* (EI) 302.1264 (M^+. $C_{16}H_{18}N_2O_4$ requires 302.1267), 284 (11%), 162 (20), 140 (77), 124 (100), 122 (21), 120 (21), 108 (30), 107 (24), 106 (88), 91 (70) and 78 (38).

***N*-(5-Hydroxymethyl-1*H*-pyrrole-2-carbonyl)-L-leucine methyl ester (14b).** *Method 1.* The pyrrole **13b** (116 mg, 0.44 mmol, 1 equiv.) was reduced with zinc borohydride according to general procedure B. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 4 : 1) to give **14b** (49 mg, 42%) as a cream solid; mp 92–96 °C (Found: C, 58.35; H, 7.37; N, 10.36. $C_{13}H_{20}N_2O_4$ requires C, 58.19; H, 7.51; N, 10.44%); $\nu_{max}(CHCl_3)/cm^{-1}$ 1525.6, 1641.3, 1739.7, 2958.6, 3438.8; δ_H(500 MHz; $CDCl_3$; Me_4Si) 0.94 (6 H, m, CH(CH_3)$_2$), 1.63–1.75 (3 H, m, CH_2CH), 3.76 (3 H, s, CO_2CH_3), 4.60 (2 H, d, J = 2.9 Hz, CH_2OH), 4.76 (1 H, m, NHCH), 6.04 (1 H, dd, J = 2.4, 3.4 Hz, pyrrole 4-H), 6.57 (1 H, dd, J = 2.4, 3.4 Hz, pyrrole 3-H), 6.62 (1 H, d, J = 8.8 Hz, NHCH), 10.51 (1 H, br s, pyrrole NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 21.6, 22.8 (CH(*C*H$_3$)$_2$), 24.8 (CH$_2$*C*H), 41.0 (*C*H$_2$CH), 50.7 (NH*C*H), 52.4 (CO_2CH_3), 57.3 (*C*H$_2$OH), 107.9 (pyrrole 4-C), 110.9 (pyrrole 3-C), 124.6 (pyrrole 2-C), 136.8 (pyrrole 5-C), 161.7 (*C*ONH), 174.5 (CO_2); *m*/*z* (EI) 268.1418 (M^+. $C_{13}H_{20}N_2O_4$ requires 268.1423), 212 (27%), 209 (12), 191 (19), 180 (11), 175 (13), 174 (96), 130 (15), 126 (18), 125 (12), 124 (100), 108 (21), 106 (70), 86 (48) and 78 (31).

Method 2. The pyrrole **13b** (35 mg, 0.13 mmol, 1 equiv.), dissolved in dry ether (5 cm^3) under an inert atmosphere, was cooled to −78 °C (dry ice–acetone). Lithium borohydride (6 mg, 0.26 mmol, 2 equiv.) was added, and the resultant solution was stirred at −78 °C for 1 h. Water (10 cm^3) was carefully added to quench the reaction, and the solution was extracted with ethyl acetate (3 × 10 cm^3). The combined organic phases were washed with saturated aqueous brine (10 cm^3), dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 4 : 1) to give **14b** (23 mg, 66%) as an off-white solid; mp 94–97 °C. Data as above.

***N*-(5-Hydroxymethyl-1*H*-pyrrole-2-carbonyl)-L-proline methyl ester (14c).** The pyrrole **13c** (120 mg, 0.48 mmol, 1 equiv.) was reduced with zinc borohydride according to general procedure B. The resultant material was purified by flash chromatography on silica (ethyl acetate–acetone, 10 : 1) to give **14c** (29 mg, 24%) as an orange oil; $\nu_{max}(CHCl_3)/cm^{-1}$ 1596.9, 1743.5, 3010.7, 3436.9, 3691.5; δ_H(500 MHz; $CDCl_3$; Me_4Si) 2.03–2.24 (4 H, m, NCH$_2$CH_2CH_2CH), 3.44 (1 H, br s, CH$_2$OH), 3.73 (3 H, s, CO_2CH_3), 3.85 (1 H, m, NCH_{2a}CH$_2$CH$_2$CH), 3.95 (1 H, m, NCH_{2b}CH$_2$CH$_2$CH), 4.61 (2 H, s, CH_2OH), 4.73 (1H, m, NCH), 6.13 (1 H, s, pyrrole 4-H), 6.60 (1 H, s, pyrrole 3-H), 10.75 (1 H, br s, pyrrole NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 25.2, 28.8 (NCH$_2$CH_2CH_2CH), 48.8 (NCH$_2$CH$_2$CH$_2$CH), 52.4 (CO_2CH_3), 57.4 (*C*H$_2$OH), 60.2 (NCH), 108.2 (pyrrole 4-C), 113.8 (pyrrole 3-C), 124.6 (pyrrole 2-C), 136.9 (pyrrole 5-C), 161.3 (*C*ON), 172.8 (CO_2); *m*/*z* (EI) 252.1119 (M^+. $C_{12}H_{16}N_2O_4$ requires 252.1110), 252 (20%), 193 (48), 191 (16), 176 (14), 175 (76), 147 (11), 147 (11), 128 (77), 124 (62), 122 (12), 108 (21), 107 (83), 106 (100), 96 (11), 94 (16), 79 (61), 78 (64), 70 (90) and 68 (26).

Ethyl 5-(3-phenylpropionyl)-1*H*-pyrrole-2-carboxylate (15a). A solution of pyrrole ester **11**[12] (2.91 g, 21 mmol, 1 equiv.) in dry 1,2-dichloroethane (35 cm^3) was added in a dropwise fashion over 5 min to a stirred suspension of hydrocinnamoyl chloride (7.04 g, 42 mmol, 2 equiv.) and zinc chloride (5.70 g, 42 mmol, 2 equiv.) in dry 1,2-dichloroethane (35 cm^3), cooled to 0 °C under an inert atmosphere. The suspension was heated to 50 °C for 1 h, after which the resultant solution was poured

onto ice–water and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (40 cm^3), and the combined organic fractions were washed with aqueous saturated sodium hydrogen carbonate (30 cm^3), aqueous saturated brine (30 cm^3), dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure. The crude material was dissolved in ethyl acetate (50 cm^3), washed with 1 M aqueous sodium hydroxide (4 × 20 cm^3) to remove excess hydrocinnamoyl chloride, dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 1 : 4) to give **15a** (2.0 g, 35%) as a cream residue; ν_{max}(KBr)/cm^{-1} 1548.7, 1604.7, 1662.5, 1710.7, 2987.5, 3006.8, 3429.2; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.36 (3 H, t, J = 7.1 Hz, CH_2CH_3), 3.04 (2 H, t, J = 7.3 Hz, $PhCH_2CH_2$), 3.13 (2 H, m, $PhCH_2CH_2$), 4.35 (2 H, q, J = 7.2 Hz, CH_2CH_3), 6.81 (1 H, dd, J = 2.9, 3.9 Hz, pyrrole H), 6.86 (1 H, dd, J = 2.4, 3.9 Hz, pyrrole H), 7.18–7.30 (5 H, m, Ar*H*), 9.89 (1 H, br s, pyrrole N*H*); δ_C(75 MHz; $CDCl_3$; Me_4Si) 14.2 (CH_2CH_3), 30.2 ($PhCH_2CH_2$), 40.0 ($PhCH_2CH_2$), 61.1 (CH_2CH_3), 115.4, 115.5 (pyrrole 3-C and 4-C), 126.2, 128.3, 128.5, 141.8 (Ar*C*), 127.2 (pyrrole 2-C), 133.7 (pyrrole 5-C), 160.3 (CO_2), 190.3 (CH_2CO); *m*/*z* (EI) 271.1220 (M^+. $C_{16}H_{17}NO_3$ requires 271.1208), 271 (85%), 226 (17), 198 (27), 166 (63), 139 (66), 120 (100), 104 (40) and 91 (48).

Ethyl 5-acetyl-1*H*-pyrrole-2-carboxylate (15b). The pyrrole **11**[12] (4.0 g, 29 mmol, 1 equiv.) was treated with acetyl chloride (4.51 g, 57 mmol, 2 equiv.) as described for **15a**. Washing with 1 M aqueous sodium hydroxide was not required in this case. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 1 : 2) to give **15b** (1.93 g, 37%) as a yellow solid; mp 54–57 °C (lit.,[16] 60–60.5 °C); δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.36 (3 H, t, J = 7.3 Hz, CH_2CH_3), 2.46 (3 H, s, $COCH_3$), 4.34 (2 H, q, J = 7.2 Hz, CH_2CH_3), 6.83 (1 H, t, J = 3.2 Hz, pyrrole H), 6.87 (1 H, t, J = 3.2 Hz, pyrrole H), 9.82 (1 H, br s, pyrrole N*H*).

5-(3-Phenylpropionyl)-1*H*-pyrrole-2-carboxylic acid (16a). A stirred solution of the pyrrole ester **15a** (801 mg, 2.95 mmol, 1 equiv.) and potassium hydroxide (663 mg, 11.8 mmol, 4 equiv.) in 2 : 1 water–tetrahydrofuran (20 cm^3) was heated to 40–50 °C for 2 h. After cooling to rt, the solution was washed with ether (20 cm^3), acidified to pH ~1 with concentrated aqueous hydrochloric acid, and extracted with ether (5 × 20 cm^3). The combined ethereal fractions were dried ($MgSO_4$) and the solvent was removed by evaporation under reduced pressure to give **16a** (616 mg, 86%) as a brown solid; mp 181–183 °C; ν_{max}(KBr)/cm^{-1} 1500.5, 1550.7, 1670.2, 1701.1, 2366.5, 2597.9, 3197.8; δ_H(500 MHz, acetone-d_6,) 3.11 (2 H, t, J = 7.6 Hz, $PhCH_2CH_2$), 3.32 (2 H, t, J = 7.6 Hz, $PhCH_2CH_2$), 6.97 (1 H, dd, J = 2.4, 3.9 Hz, pyrrole H), 7.15 (1 H, dd, J = 2.4, 3.9 Hz, pyrrole H), 7.29 (1 H, m, Ar*H*), 7.39 (4 H, m, Ar*H*), 11.23 (1 H, br s, pyrrole N*H*); δ_C(75 MHz, acetone-d_6) 30.1 ($PhCH_2CH_2$), 39.9 ($PhCH_2CH_2$), 115.5, 115.8, 126.1, 128.5, 128.6, 141.6, 127.9 (pyrrole 2-C), 134.8 (pyrrole 5-C), 161.2 (CO_2H), 190.0 (CH_2CO); *m*/*z* (EI) 243.0900 (M^+. $C_{14}H_{13}NO_3$ requires 243.0895), 243 (57%), 143 (60), 120 (100), 113 (63), 104 (72) and 94 (75).

5-Acetyl-1*H*-pyrrole-2-carboxylic acid (16b). The pyrrole **15b** (1.90 g, 10 mmol, 1 equiv.) was hydrolysed with potassium hydroxide (2.35 g, 42 mmol, 4 equiv.) as described for **15a** to give **16b** (1.50 g, 93%) as a yellow solid; mp 185–187 °C (Found: C, 54.93; H, 4.55; N, 9.07. $C_7H_7NO_3$ requires C, 54.90; H, 4.61; N, 9.15%); ν_{max}(KBr)/cm^{-1} 1546.8, 1670.2, 2360.7, 2511.1, 2584.4, 3124.5, 3436.9; δ_H(500 MHz, acetone-d_6,) 2.57 (3 H, s, CH_3CO), 6.98 (1 H, dd, J = 2.4, 3.9 Hz, pyrrole H), 7.11 (1 H, dd, J = 2.4, 3.9 Hz, pyrrole H), 11.20 (1 H, br s, pyrrole N*H*); δ_C(75 MHz, acetone-d_6) 25.5 (CH_3CO), 115.5, 116.2 (pyrrole 3-C and 4-C), 127.9 (pyrrole 2-C), 135.1 (pyrrole 5-C), 161.2 (CO_2H), 188.2 (CH_3CO); *m*/*z* (EI) 153.0426 (M^+. $C_7H_7NO_3$ requires 153.0426), 153 (45%), 138 (21) and 120 (100).

***N*-[5-(3-Phenylpropionyl)-1*H*-pyrrole-2-carbonyl]-L-phenylalanine methyl ester (17a).** The pyrrole acid **16a** (150 mg, 0.62 mmol, 1 equiv.) was coupled with L-phenylalanine methyl ester hydrochloride (146 mg, 0.68 mmol, 1.1 equiv.) according to modified general procedure A. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 2 : 3) to give **17a** (175 mg, 70%) as a brown residue (Found: C, 71.37; H, 6.15; N, 7.10. $C_{24}H_{24}N_2O_4$ requires C, 71.27; H, 5.98; N, 6.93%); ν_{max}(KBr)/cm^{-1} 1529.4, 1548.7, 1668.3, 1741.6, 3008.7, 3425.3; δ_H(500 MHz; $CDCl_3$; Me_4Si) 3.03 (2 H, m, $PhCH_2CH_2$), 3.14 (2 H, m, $PhCH_2CH_2$), 3.21 (2 H, m, CH_2Ph), 3.75 (3 H, s, CO_2CH_3), 5.05 (1 H, m, NHC*H*), 6.46 (2 H, m, pyrrole 3-H and N*H*CH), 6.78 (1 H, dd, J = 2.4, 3.9 Hz, pyrrole 4-H), 7.10 (2 H, m, Ar*H*), 7.17–7.30 (8 H, m, Ar*H*), 10.00 (1 H, br s, pyrrole N*H*); δ_C(75 MHz; $CDCl_3$; Me_4Si) 30.3 ($PhCH_2CH_2$), 37.9 ($CHCH_2Ph$), 40.0 ($PhCH_2CH_2$), 52.5 (CO_2CH_3), 53.1 (NH*C*H), 110.4 (pyrrole 3-C), 115.5 (pyrrole 4-C), 126.2, 127.2, 128.3, 128.5, 128.6, 129.3, 135.6, 140.9 (Ar*C*), 129.7 (pyrrole 2-C), 133.3 (pyrrole 5-C), 159.4 (*C*ONH), 171.9 (CO_2Me), 190.0 (CH_2CO); *m*/*z* (EI) 404.1745 (M^+. $C_{24}H_{24}N_2O_3$ requires 404.1736), 370 (33%), 265 (15), 242 (29), 238 (16), 226 (100), 214 (23), 208 (23), 120 (22) and 91 (81).

***N*-[5-Acetyl-1*H*-pyrrole-2-carbonyl]-L-phenylalanine methyl ester (17b).** The pyrrole acid **16b** (200 mg, 1.31 mmol, 1 equiv.) was coupled with L-phenylalanine methyl ester hydrochloride (310 mg, 1.44 mmol, 1.1 equiv.) according to modified general procedure A. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 2 : 1) to give **17b** (375 mg, 91%) as a cream solid. An analytical sample was obtained by diffusion of petroleum ether into a solution of **17b** dissolved in ethyl acetate; mp 103–105 °C (Found: C, 65.13; H, 5.84; N, 8.77. $C_{17}H_{18}N_2O_4$ requires C, 64.96; H, 5.77; N, 8.91%); ν_{max}(KBr)/cm^{-1} 1546.8, 1654.8, 1741.6, 2956.7, 3031.9, 3112.9, 3352.1; δ_H(500 MHz; $CDCl_3$; Me_4Si) 2.42 (3 H, s, CH_3CO), 3.20 (2 H, m, CH_2Ph), 3.75 (3 H, s, CO_2CH_3), 5.06 (1 H, m, NHC*H*), 6.52 (1 H, dd, J = 2.9, 3.9 Hz, pyrrole 3-H), 6.67 (1 H, d, J = 7.8 Hz, N*H*CH), 6.88 (1 H, dd, J = 2.7, 4.2 Hz, pyrrole 4-H), 7.10 (2 H, m, Ar*H*), 7.25 (3 H, m, Ar*H*), 10.11 (1 H, br s, pyrrole N*H*); δ_C(75 MHz; $CDCl_3$; Me_4Si) 25.8 (CH_3CO), 37.9 (CH_2Ph), 52.5 (CO_2CH_3), 53.2 (NH*C*H), 110.9 (pyrrole 3-C), 116.3 (pyrrole 4-C), 127.2, 128.6, 129.2, 135.7 (Ar*C*), 130.0 (pyrrole 2-C), 133.6 (pyrrole 5-C), 159.4 (*C*ONH), 172.1 (CO_2), 188.6 (CH_3CO); *m*/*z* (EI) 314.1271 (M^+. $C_{17}H_{18}N_2O_4$ requires 314.1267), 280 (33%), 162 (24), 152 (73), 136 (100), 120 (32), 118 (15) and 91 (19).

(*R*,*S*)-*N*-[5-(1-Hydroxy-3-phenylpropyl)-1*H*-pyrrole-2-carbonyl]-L-phenylalanine methyl ester (18a). The pyrrole **17a** (112 mg, 0.28 mmol, 1 equiv.) was reduced with zinc borohydride according to general procedure B. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 1 : 1) to give a mixture of **17a** and **18c** (41 mg); δ_H(**18c** from mixture; 500 MHz; $CDCl_3$; Me_4Si) 1.95 (2 H, m, CH_2), 2.63 (4 H, m, 2 × CH_2), 3.18 (2 H, m, CH_2Ph), 3.71 (3 H, s, CO_2CH_3), 5.03 (1 H, m, NHC*H*), 5.93 (1 H, m, pyrrole 4-H), 6.23 (1 H, d, J = 7.8 Hz, N*H*CH), 6.45 (1 H, m, pyrrole 3-H), 7.11–7.29 (10 H, m, Ar*H*), 9.35 (1 H, br s, pyrrole N*H*); *m*/*z* (EI) 390.1953 (M^+. $C_{24}H_{26}N_2O_3$ requires 390.1943), 390 (10%), 356 (18), 228 (38), 212 (100), 208 (18), 124 (59), 120 (13), 107 (15), 91 (55) and 79 (31).

Further elution gave a 1 : 1 mixture of epimers **18a** (24 mg, 21%) as a light yellow oil; ν_{max}(KBr)/cm^{-1} 1521.7, 1602.7, 1641.3, 1741.6, 3012.6, 3030.0, 3436.9; δ_H(500 MHz; $CDCl_3$; Me_4Si) 2.13 (2 H, m, $PhCH_2CH_2$), 2.75 (2 H, m, $PhCH_2CH_2$),

3.20 (2 H, m, CHCH_2Ph), 3.71 and 3.72 (each 3 H, s, CO_2CH_3, isomers A and B), 4.75 (1 H, m, C*H*OH), 5.02 (1 H, m, NHC*H*), 6.02 (1 H, m, pyrrole 4-H), 6.42 (1 H, d, J = 7.3 Hz, N*H*CH), 6.48 (1 H, m, pyrrole 3-H), 7.11–7.29 (10 H, m, Ar*H*), 10.67 and 10.70 (each 1 H, br s, pyrrole N*H*, isomers A and B); δ_C(75 MHz; $CDCl_3$; Me_4Si) 31.8 (Ph*C*H_2CH_2), 38.2 (CH*C*H_2-Ph), 38.6 and 38.7 (PhCH_2*C*H_2, isomers A and B), 52.4 (CO_2*C*H_3), 53.1 (NH*C*H), 67.1 and 67.1 (*C*HOH, isomers A and B), 106.4 and 106.5 (pyrrole 4-C, isomers A and B), 110.7 (pyrrole 3-C), 124.0 (pyrrole 2-C), 125.9, 127.2, 128.4, 128.5, 128.6, 129.3, 135.6, 141.6 (Ar*C*), 140.1 (pyrrole 5-C), 161.1 (*C*ONH), 172.0 (CO_2Me); *m*/*z* (EI) 388.1800 ($M^+ - H_2O$. $C_{24}H_{24}N_2O_3$ requires 388.1787), 388 (17%), 226 (25), 210 (100), 208 (54), 180 (18) and 91 (23).

(*R*,*S*)-*N*-[5-(1-Hydroxyethyl)-1*H*-pyrrole-2-carbonyl]-L-phenylalanine methyl ester (18b). *Method 1.* A stirred solution of the pyrrole **17b** (150 mg, 0.48 mmol, 1 equiv.), dissolved in dry tetrahydrofuran (20 cm^3) under an inert atmosphere, was cooled to −78 °C (dry ice–acetone). Lithium borohydride (21 mg, 0.95 mmol, 2 equiv.) was added, and the resultant solution was stirred at −78 °C for 1 h, then warmed to rt and stirred for an additional 1 h. Water (10 cm^3) was carefully added to quench the reaction, and the solution was extracted with ethyl acetate (3 × 10 cm^3). The combined organic phases were washed with saturated aqueous brine (10 cm^3), dried ($MgSO_4$) and the solvent was removed by evaporation under reduced pressure. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 4 : 1) to give impure **18b** (70 mg, 46%) which could not be purified further; δ_H(from mixture; 500 MHz; $CDCl_3$; Me_4Si) 1.53 (3 H, m, CH_3CHOH), 3.20 (2 H, m, CH_2Ph), 3.74 (3 H, s, CO_2CH_3), 4.90 (1 H, m, C*H*OH), 5.03 (1 H, m, NHC*H*), 6.00 (1 H, s, pyrrole 4-H), 6.48 (1 H, t, J = 2.7 Hz, pyrrole 3-H), 6.68 (1 H, m, N*H*CH), 7.15 (2 H, d, J = 6.8 Hz, Ar*H*), 7.26 (m, 3 H, m, Ar*H*), 10.71 and 10.73 (each 1 H, br s, pyrrole N*H*, isomers A and B); *m*/*z* (EI) 316.1423 (M^+. $C_{17}H_{20}N_2O_4$ requires 316.1423), 298 (11%), 138 (12), 136 (61), 120 (100), 91 (18) and 65 (25).

Method 2. The pyrrole acid **19b** (20 mg, 0.13 mmol, 1 equiv.) was coupled with L-phenylalanine methyl ester hydrochloride (31 mg, 0.14 mmol, 1.1 equiv.) according to general procedure A, except in the workup whereby 0.1 M aqueous hydrochloric acid was used instead of the 3 M aqueous hydrochloric acid. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 4 : 1) to give **18b** (22 mg, 54%) as a colourless oil.

(*R*,*S*)-Ethyl 5-(1-hydroxyethyl)-1*H*-pyrrole-2-carboxylate (19a). A stirred solution of the pyrrole ester **12a**[13] (400 mg, 2.39 mmol, 1 equiv.) in dry tetrahydrofuran (40 cm^3) under an argon atmosphere was cooled to −23 °C (dry ice–carbon tetrachloride). Methyllithium (1.50 cm^3 of a 1.6 M solution in ether, 2.39 mmol, 1 equiv.) was added over 30 min, and the solution was stirred at −23 °C for 2.5 h. After this, a further 1.50 cm^3 of the methyllithium solution was added over 30 min, and the solution was again stirred at −23 °C for 2.5 h. The resultant solution was poured into ether–ice, and once the ice had melted the layers were separated. The organic layer was washed with aqueous saturated brine (25 cm^3), water (2 × 25 cm^3), then dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure to give **19a** (418 mg, 95%) as a brown oil (Found: C, 58.59; H, 7.22; N, 7.60. $C_9H_{13}NO_3$ requires C, 59.00; H, 7.15; N, 7.65%); ν_{max}(KBr)/cm^{-1} 1693.4, 2983.7, 3020.3, 3446.6; δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.34 (3 H, t, J = 7.1 Hz, CH_2CH_3), 1.55 (3 H, d, J = 6.8 Hz, CH_3CHOH), 3.08 (1 H, br s, CHO*H*), 4.29 (2 H, q, J = 7.2 Hz, CH_2CH$_3$), 4.96 (1 H, q, J = 6.5 Hz, C*H*OH), 6.05 (1 H, m, pyrrole 4-H), 6.83 (1 H, m, pyrrole 3-H), 9.77 (1 H, br s, pyrrole N*H*); δ_C(75 MHz; $CDCl_3$; Me_4Si) 14.4 (CH_2CH_3), 23.2 (*C*H$_3$CHOH), 60.4 (*C*H$_2$CH$_3$), 64.0 (*C*HOH), 106.3 (pyrrole 4-C), 115.9 (pyrrole 3-C), 121.8 (pyrrole 2-C), 141.5 (pyrrole 5-C), 161.9 (CO_2); *m*/*z* (EI) 183.0897 (M^+. $C_9H_{13}NO_3$ requires 183.0895), 183 (43%), 168 (56), 165 (25), 140 (24), 136 (14), 122 (100), 120 (54), 94 (12), 91 (17) and 65 (28).

(*R*,*S*)-5-(1-Hydroxyethyl)-1*H*-pyrrole-2-carboxylic acid (19b). A stirred solution of the pyrrole ester **19a** (575 mg, 3.13 mmol, 1 equiv.), in 1 : 1 tetrahydrofuran–water (10 cm^3) under an inert atmosphere, was warmed to 40–50 °C. Sodium hydroxide (377 mg, 9.42 mmol, 3 equiv.) was added, and the progress of the reaction was monitored by thin layer chromatography (ethyl acetate–petroleum ether, 1 : 1). After 24 h, the solution was cooled to rt, washed with ether (10 cm^3), cooled to 0 °C and acidified to pH ~4 by the dropwise addition of 1 M aqueous hydrochloric acid. The resulting solution was extracted with ether (3 × 15 cm^3), the combined ethereal extracts were washed with aqueous saturated brine (15 cm^3), dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure to give **19b** (85 mg, 17%) as a purple solid. This product was used without further purification; mp 110–115 °C; ν_{max}(KBr)/cm^{-1} 1651.0, 3230.5, 3419.6; δ_H(300 MHz, acetone-d_6) 1.59 (3 H, d, J = 6.3 Hz, CH_3CHOH), 4.43 (1 H, br s, CHO*H*), 5.02 (1 H, m, CH_3C*H*OH), 6.20 (1 H, t, J = 3.2 Hz, pyrrole 4-H), 6.87 (1 H, t, J = 3.2 Hz, pyrrole 3-H), 10.47 (1 H, br s, pyrrole N*H*); δ_C(75 MHz, acetone-d_6) 23.2 (*C*H$_3$CHOH), 63.3 (CH_3*C*HOH), 105.9 (pyrrole 4-C), 115.6 (pyrrole 3-C), 121.8 (pyrrole 5-C), 143.2 (pyrrole 2-C), 161.8 (CO_2H); *m*/*z* (EI) 155.0579 (M^+. $C_7H_9NO_3$ requires 155.0582), 155 (40%), 140 (56), 137 (46), 122 (100), 119 (64), 112 (14), 91 (69), 69 (15) and 65 (50).

***N*-(5-Formyl-1*H*-pyrrole-2-carbonyl)-L-phenylalanyl-L-leucine methyl ester (21).** To a stirred suspension of L-phenylalanyl-L-leucine hydrate **20a** (200 mg, 0.67 mmol) in dry tetrahydrofuran (10 cm^3) at rt was added a 12-fold excess of an ethereal solution of diazomethane in a dropwise fashion over 10 min. After 15 h, the solvent was removed by evaporation under reduced pressure to give the methyl ester **20b** (202 mg, quantitative yield) as a light brown oil. This was immediately dissolved in dry dichloromethane (6 cm^3) at rt under an inert atmosphere, and to this was added the pyrrole acid **12b**[14] (87 mg, 0.63 mmol, 1 equiv.), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (306 mg, 0.69 mmol, 1.1 equiv.), and *N*,*N*-diisopropylethylamine (89 mg, 0.69 mmol, 1.1 equiv.). The reaction mixture was stirred at rt for 16 h, after which the solution was diluted with ethyl acetate (15 cm^3), washed with 1 M aqueous hydrochloric acid (10 cm^3), aqueous saturated sodium hydrogen carbonate (10 cm^3), aqueous saturated brine (10 cm^3), dried ($MgSO_4$) and the solvent was removed by evaporation under reduced pressure. The resultant material was purified by flash chromatography on silica (ethyl acetate–petroleum ether, 3 : 1), followed by diffusion of petroleum ether into a solution of the title product dissolved in ethyl acetate, to give **21** (135 mg, 52%) as a tan solid; mp 100–103 °C; ν_{max}(KBr)/cm^{-1} 1558.4, 1635.5, 1674.1, 1743.5, 2958.6, 3286.5; δ_H(500 MHz; $CDCl_3$; Me_4Si) 0.85 (6 H, m, CH(CH_3)$_2$), 1.43–1.61 (3 H, m, CH_2C*H*), 3.13 (2 H, m, CH_2Ph), 3.73 (3 H, s, CO_2CH_3), 4.56 (1 H, m, Leu α*H*), 5.06 (1 H, q, J = 7.3 Hz, Phe α*H*), 6.64 (1 H, dd, J = 2.4, 3.9 Hz, pyrrole 3-H), 6.90 (2 H, m, pyrrole 4-H and N*H*CH), 7.19 (6 H, m, Ar*H* and N*H*CH), 9.61 (1 H, s, C*H*O), 10.68 (1 H, br s, pyrrole N*H*); δ_C(75 MHz; $CDCl_3$; Me_4Si) 22.1, 22.6 (CH(*C*H$_3$)$_2$), 24.8, 41.6 (*C*H$_2$*C*H), 38.8 (*C*H$_2$Ph), 51.0 (Leu α*C*H), 52.5 (CO_2*C*H$_3$), 54.7 (Phe α*C*H), 111.3 (pyrrole 3-C), 119.6 (pyrrole 4-C), 126.9, 128.4, 129.4, 136.4 (Ar*C*), 131.3 (pyrrole 2-C), 134.1 (pyrrole 5-C), 159.7 (Pyr*C*ONH), 171.3 (CH*C*ONH), 173.1 (CO_2), 180.3 (*C*HO); *m*/*z* (ES) 414.2031 (M^+ + H. $C_{22}H_{28}N_3O_5$ requires 414.2029), 414 (100%), 293 (62), 288 (31), 268 (20), 247 (20), 225 (20) and 146 (29).

***N*-(5-Hydroxymethyl-1*H*-pyrrole-2-carbonyl)-L-phenylalanyl-L-leucine methyl ester (22).** The pyrrole **21** (82 mg, 0.20 mmol, 1

equiv.) was reduced with lithium borohydride (9 mg, 0.40 mmol, 2 equiv.) as described for **18b** (method 1). The resultant material was purified by flash chromatography on silica (ethyl acetate), followed by diffusion of petroleum ether into a solution of the title product in ethyl acetate, to give **22** (56 mg, 68%) as a cream solid; mp decomp. >85 °C; ν_{max}(KBr)/cm^{-1} 1541.0, 1624.0, 1664.5, 1735.8, 2958.6, 3267.2; δ_H(500 MHz; $CDCl_3$; Me_4Si) 0.84 (6 H, m, CH(CH_3)$_2$), 1.44–1.59 (3 H, m, CH_2CH), 3.11 (2 H, m, CH_2Ph), 3.69 (3 H, s, CO_2CH_3), 4.59 (3 H, m, Leu α*H* and CH_2OH), 5.05 (1 H, q, *J* = 7.7 Hz, Phe αH), 6.04 (1 H, t, *J* = 2.9 Hz, pyrrole 4-H), 6.53 (1 H, m, pyrrole 3-H), 7.03 (1 H, br s, N*H*CH), 7.16 (5 H, m, Ar*H*), 7.25 (1 H, br s, N*H*CH), 10.41 (1 H, br s, pyrrole N*H*); δ_C(75 MHz; $CDCl_3$; Me_4Si) 22.0, 22.6, 24.7, 41.5 (CH_2CH), 38.7 (CH_2Ph), 50.8 (Leu α*C*H), 52.3 (CO_2CH_3), 54.5 (Phe α*C*H), 57.5 (CH_2OH), 108.1 (pyrrole 4-C), 111.0 (pyrrole 3-C), 124.9 (pyrrole 2-C), 126.7, 128.4, 129.4, 136.3 (Ar*C*), 136.7 (pyrrole 5-C), 161.2 (Pyr*C*ONH), 172.0 (CH*C*ONH), 173.4 (CO_2); *m/z* (ES) 438.2023 (M^+ + Na. $C_{22}H_{29}N_3NaO_5$ requires 438.2005), 438 (100%), 416 (66) and 271 (63).

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