First asymmetric synthesis of the Kelatorphan-like enkephalinase inhibitor (1*S*,2*R*,2′*S*)-2-[2′-(*N*-hydroxycarbamoylmethyl)-3′-phenylpropionylamino]cyclohexane-1-carboxylic acid

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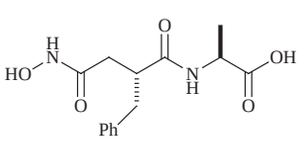
The first asymmetric synthesis of Kelatorphan-like enkephalinase inhibitor (1*S*,2*R*,2′*S*)-2-[2′-(*N*-hydroxycarbamoylmethyl)-3′-phenylpropionylamino]cyclohexane-1-carboxylic acid is achieved using lithium amide (*R*)-**5** and pyrrolidinone auxiliary (*R*)-**6**; the pyrrolidinone auxiliary is used to create (2*S*)-benzylsuccinic acid derivative (*S*)-**4** while lithium amide (*R*)-**5** is used to synthesize *tert*-butyl (1*S*,2*R*)-2-aminocyclohexanecarboxylate **3**.

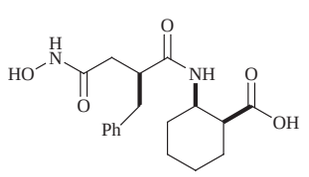
Introduction

The enkephalins[1] are just two of a series of peptides that make up the endogeneous morphine-like class of compounds in the brain called the endorphins. The enkephalins are believed to regulate perception and integration of pain impulses and behavioural response to pain. It has been suggested that they increase tolerance to pain by suppressing awareness of pain that would otherwise become incapacitating in life threatening situations.

The enkephalins (Tyr-Gly-Gly-Phe-Met and Tyr-Gly-Gly-Phe-Leu) have a short biological half life resulting from the action of three zinc proteinases; neutral endopeptidase 24.11 (NEP), dipeptidylaminopeptidase (DAP) and aminopeptidase N (EC 3.4.11.2) (APN). Compounds that could inhibit these enkephalin degrading enzymes should necessarily elicit desirable analgesic properties and to this end much research effort has been devoted. Extensive studies have revealed that the pseudopeptide Kelatorphan **1**[2] potently inhibits the action of



Kelatorphan **1**



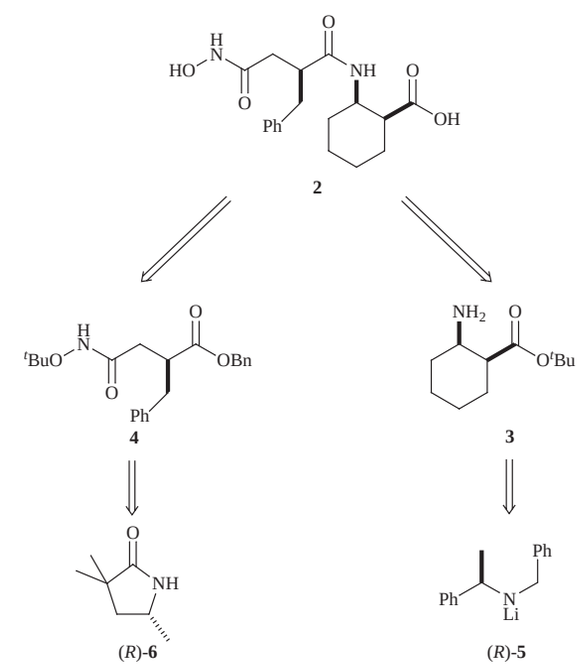
(±)-**2**

these enzymes. Further work showed that the related compound **2**[3] elicits comparable inhibitory action but to date has only been synthesised in racemic form.

The interesting combination of β-amino acid and succinic acid structural motifs contained within **2** combined with the potential to exploit chiral auxiliaries and methodologies developed within our laboratory made this compound an interesting synthetic target.

Herein we would like to report the full experimental details of the first asymmetric synthesis of (1*S*,2*R*,2′*S*)-2-[2′-(*N*-hydroxycarbamoylmethyl)-3-phenylpropionylamino]cyclohexane-1-carboxylic acid **2**.

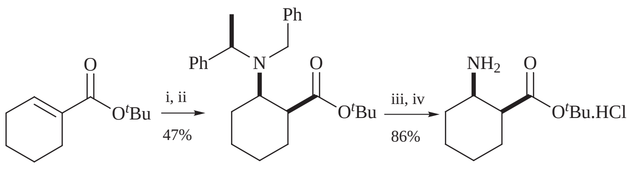
Our retrosynthetic analysis of **2** revealed a *syn*-cyclohexyl β-amino *tert*-butyl ester **3** and a (2*S*)-benzylsuccinic acid derivative (*S*)-**4**. It was envisaged that these fragments could be readily synthesised using the lithium amide (*R*)-**5** and the 'Quats' pyrrolidinone auxiliary (*R*)-**6** respectively. The *tert*-butyl group was chosen as the protecting group for both carboxylic acid and hydroxamate terminae. It was envisioned that



after debenzylation of (*S*)-**4** and successful coupling to **3**, both *tert*-butyl groups could be removed in one step by the mediation of a suitably acidic reagent.

Results and discussion

The highly diastereoselective Michael addition of lithium (*R*)-*N*-benzyl-*N*-α-methylbenzylamide (*R*)-**5** to cyclohex-1-enecarboxylic esters leading to the production of both *cis*- and *trans*-hexacin derivatives has been described.[4] Following this protocol, Michael acceptor **7** was treated with (*R*)-**5** at −78 °C for 21 h and the reaction mixture then quenched with 2,6-di-*tert*-butylphenol. After work-up, ^{1}H NMR spectroscopic analysis of the crude reaction product indicated that the reaction had occurred with high (>96% de) diastereoselectivity and to approximately 60% conversion. Chromatographic purification afforded the *syn*-Michael adduct **8** in moderate yield (47%) and as a mixture of diastereoisomers (>96% de). Hydrogenolysis of this material using Pearlman's catalyst and hydrogen (6 atm) occurred smoothly to afford, after treatment with gaseous hydrogen chloride, *tert*-butyl aminocyclohexanecarboxylate hydrochloride **3**·HCl in good overall yield (86%) (Scheme 1).



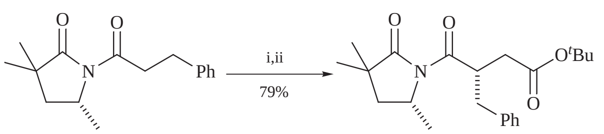
7 8, >96% de 3.HCl, 96% ee

Scheme 1 *Reagents and conditions*: i, (*R*)-**5**, THF, −78 °C, 21 h; ii, 2,6-di-*tert*-butylphenol, (1.05 equiv.), THF, −78 °C; iii, H_2 (6 atm), $Pd(OH)_2$, MeOH, RT, 16 h; iv, HCl (g).

The enantiomeric excess (96%) of **3** was determined by derivatisation as the Mosher's amide and was in accordance with the diastereomeric excess of **8**. With the β-amino ester fragment in hand our attention then focused on the asymmetric synthesis of (2*S*)-benzylsuccinic acid derivative (*S*)-**4**.

The highly diastereoselective synthesis of homochiral methylsuccinnic acid derivatives using the 'Quats' pyrrolidinone auxiliary (*R*)-**6** has been recently described.[5] It was our aim to apply that protocol directly in the asymmetric synthesis of (2*S*)-benzylsuccinic acid derivative (*S*)-**4**.

Accordingly, the readily prepared *N*-hydrocinnamoyl 'Quats' pyrrolidinone (*R*)-**9** was deprotonated with LDA in THF at −78 °C and subsequently treated with *tert*-butyl bromoacetate. After work-up, the desired succinate derivative **10** was obtained in good yield (79%) and with a high diastereomeric excess (>98% de) following purification by column chromatography and recrystallisation of the resulting solid from diethyl ether–pentane (Scheme 2).

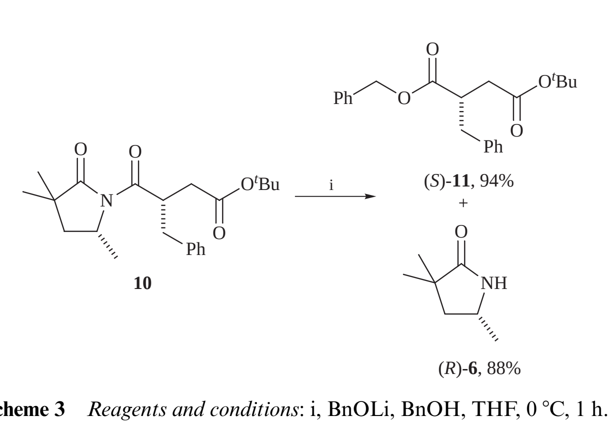


(*R*)-**9** **10**, >98% de

Scheme 2 *Reagents and conditions*: i, LDA (1.05 equiv.), THF, −78 °C, 1 h; ii, *tert*-butyl bromoacetate (1.5 equiv.), −78 °C to RT.

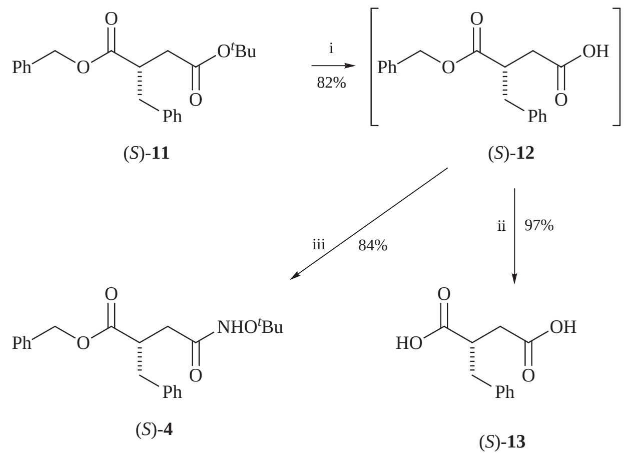
It is well documented that cleavage of chiral *N*-acyl side chains from oxazolidinone type auxiliaries with excess lithium phenylmethoxide at 0 °C in THF occurs rapidly, efficiently and without racemisation.[6] Preliminary studies in our laboratory have shown that cleavage of chiral *N*-acyl side chains from the pyrrolidinone auxiliary (*R*)-**6** occurs equally well.[7] We chose to adopt this non-destructive mode of auxiliary removal as it would necessarily lead to the formation of an orthogonally protected succinate derivative.

Accordingly, **10** was treated with lithium phenylmethoxide in THF at 0 °C for 1 h. Aqueous work-up and purification by chromatography on silica gel afforded benzyl *tert*-butyl (*S*)-benzylsuccinate (*S*)-**11** and pyrrolidinone auxiliary (*R*)-**6** both in good yield (Scheme 3).



Scheme 3 *Reagents and conditions*: i, BnOLi, BnOH, THF, 0 °C, 1 h.

With (*S*)-**11** in hand we were suitably poised to manipulate the *tert*-butyl ester into a suitably protected hydroxamic acid. Thus, (*S*)-**11** was treated with trifluoroacetic acid in dichloromethane for 1 h. After removal of volatiles *in vacuo* the residual material was purified by chromatography on silica gel. The resultant solid was further purified by recrystallisation from diethyl ether–pentane to afford the half acid (*S*)-**12**[8] in good yield. In order to confirm the absolute stereochemistry of **12** as (*S*) this material was subjected to standard hydrogenolysis conditions which afforded the parent (*S*)-benzylsuccinic acid (*S*)-**13**. The specific rotation, $[a]_D^{22}$ −27.0 (*c* 0.675, EtOAc) [lit.[9] $[a]_D^{21}$ −28.9 (*c* 0.665, EtOAc)] and melting point, 157–159 °C [lit.[9] 153–154 °C] of (*S*)-**13** were in good agreement with the reported data, hence confirming the (*S*)-configuration of the 2-position and hence the sense of the asymmetric induction in the enolate alkylation step. With the absolute stereochemistry established, the half acid (*S*)-**12** was then coupled to *O-tert*-butylhydroxylamine using standard procedures to afford the desired *O-tert*-butyl hydroxamate (*S*)-**4** in good yield (Scheme 4).



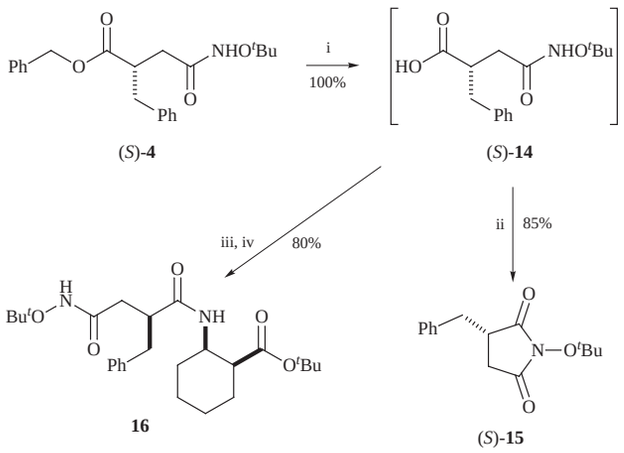
Scheme 4 *Reagents and conditions*: i, TFA–CH_2Cl_2 (1:1), RT, 1 h; ii, H_2 (1 atm), Pd/C, MeOH, RT; iii, DCC (1.1 equiv.), HOBt (1.5 equiv.), DMF, RT, 1 h, then ^{t}BuONH$_2$·HCl (1.2 equiv.), DMAP (2.0 equiv.), RT, overnight.

Subjection of (*S*)-**4** to standard hydrogenolysis conditions resulted in the quantitative production of the desired carboxylic acid (*S*)-**14**, which was used directly in the next step. In our initial attempt to couple acid (*S*)-**14** and β-amino ester **3**, standard peptide coupling procedures were adopted. These conditions were unsuccessful and led only to the efficient formation of the succinimide (*S*)-**15**. Accordingly, the crude carboxylic acid (*S*)-**14** was activated by formation of the mixed anhydride[10] with ethyl chloroformate and triethylamine in THF at −22 °C and then treated with **3**. Under these conditions the desired coupled product **16** was afforded in good yield (Scheme 5).

Interestingly, at room temperature in d_6-DMSO the ^{1}H NMR spectrum of **16** was generally broad and complicated by the presence of rotamers. However, at 90 °C in d_6-DMSO the ^{1}H NMR spectrum was sharpened and simplified.

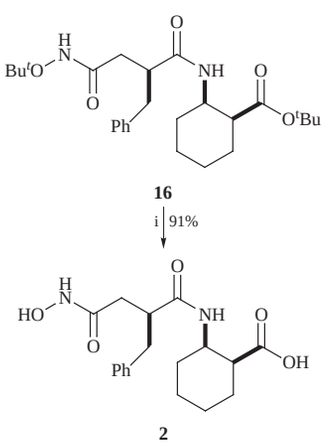
With the coupled product in hand, all that remained to complete the synthesis of enkephalinase inhibitor **2** was the removal of the two *tert*-butyl protecting groups. The deprotection of *tert*-butyl esters with TFA is well documented. However, to the best of our knowledge, the deprotection of *tert*-butyl hydroxamates has only been reported using the boron tris-trifluoroacetate reagent in TFA, at 0 °C.[11] The incredible cost of this reagent combined with the hazardous preparation encouraged us to look at the deprotection of **16** using TFA.

This reaction required much optimisation. At room temperature overnight, the hydroxamic acid grouping was still fully



Scheme 5 *Reagents and conditions*: i, H_2 (1 atm), Pd/C, MeOH, RT, 1 h; ii, HOBt (1.5 equiv.), **3**·HCl (1.2 equiv.), DMAP (1.1 equiv.), THF, −22 °C then DCC (1.05 equiv.), −22 °C, 1 h, then to RT over 5 h; iii, EtOCOCl (1.0 equiv.), Et_3N (1.1 equiv.), THF, −22 °C, 30 min; iv, **3**·HCl (1.1 equiv.), Et_3N (1.3 equiv.), THF, −22 °C, 1 h, then to RT overnight.

protected. Although labile in refluxing TFA, extensive product degradation was observed. Under the optimal conditions **16** was dissolved in neat TFA and the reaction flask was then immersed in a water bath at 39.5 °C for 4.5 h. On cooling, the volatiles were removed *in vacuo* affording a pink oil which was purified by HPLC using a reversed phase silica column, yielding the desired compound **2** in excellent yield (Scheme 6).



Scheme 6 *Reagents and conditions*: i, TFA, 39.5 °C, 4.5 h.

Although not identical, the ^{1}H NMR spectrum (500 MHz, d_6-DMSO) was comparable to the reported spectrum.[3] This slight difference could have been a result of the authors using hexamethyldisilazane as an internal standard (Table 1).

The R_f of synthesised **2**, R_f 0.45 [silica gel, eluent AcOEt–Py–AcOH–H_2O (160 : 20 : 6 : 11)] {lit.[3] R_f 0.47 [silica gel (Merck, 60F254), eluent AcOEt–Py–AcOH–H_2O (160 : 20 : 6 : 11)]} was in good agreement with the reported data and confirmed the relative stereochemistries of the substituents.

The ^{13}C NMR spectrum (125 MHz, d_6-DMSO), IR spectrum, low resolution mass spectrum and high resolution mass spectrum were all consistent with the proposed structure.

In summary, an efficient asymmetric route to Kelatorphan-like enkephalinase inhibitor (1*S*,2*R*,2′*S*)-2-[2′-(*N*-hydroxycarbamoylmethyl)-3′-phenylpropionoylamino]cyclohexane-1-carboxylic acid **2** has been established using lithium amide (*R*)-**5** and the 'Quats' pyrrolidine (*R*)-**6** chiral auxiliaries as sources of asymmetric induction. The route is convergent and could be applied in the future to the stereocontrolled production of many stereoisomers of **2**.

Table 1 Comparison of ^{1}H NMR data of **2** with literature.[3]

Assignment	δ_H (ppm)[a]	δ_H (lit.;[3] ppm)[b]
N*H*O	10.28	10.28
NO*H*	8.62	8.60
CHN*H*	7.63	7.88
Ph	7.27–7.16	
C*H*NH	4.08	3.88
C*H*CON	3.05–3.00	2.48
PhCH_2	2.85, 2.51–2.46	2.89, 2.77
CH_2CO$_2$	2.51–2.46	2.29
CH_2CO	2.21, 1.83	2.15, 1.81
CH_2	1.89–1.24	1.55–1.26

[a] ^{1}H NMR spectrum recorded in d_6-DMSO at 500 MHz. [b] ^{1}H NMR spectrum recorded in d_6-DMSO at 270 MHz using HMDS as internal standard.

Experimental

Optical rotations were recorded using a Perkin-Elmer 241 which has a thermally jacketed 10 cm cell and are given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analyses were obtained by the Dyson Perrins analytical department using a Carlo Erba 1106 analyser. Melting points were recorded using a Gallenkamp hot stage apparatus and are uncorrected. Infra-red spectra were obtained using a Perkin-Elmer 1750 spectrophotometer; solid samples as KBr discs and liquid samples as a thin film between sodium chloride plates. NMR spectra were recorded using either a Bruker AM500 (^{1}H; 500.13 MHz and ^{13}C; 125.8 MHz), WH 300 (^{1}H; 300.13 MHz), AM200 (^{1}H; 200 MHz and ^{13}C; 50.3 MHz) or Varian Gemini 200 (^{1}H; 200 MHz and ^{13}C; 50.32 MHz) spectrometer. All spectra were recorded using deuteriochloroform as solvent and internally referenced to residual protiochloroform (δ_H 7.27 and δ_C 77.0) unless otherwise stated. ^{1}H NMR spectra were run on a Bruker WH 300 spectrometer unless otherwise stated. ^{13}C NMR were obtained with DEPT editing or assigned by analogy with spectra so recorded. All chemical shifts are given in parts per million relative to tetramethylsilane (δ_H 0.00) and coupling constants (J) are given in Hz. Mass spectra were obtained in the Dyson Perrins analytical department using chemical ionisation (CI) or electronic ionisation (EI) on a VG MASSLAB VG 20-250 or on a Open Linx Micromass Platform 1 using APCI$^+$ or APCI$^-$. High resolution mass spectra were recorded using chemical ionisation (CI) on a VG-AutoSpec Instrument. Flash chromatography was carried out using silica gel (Kieselgel 60), Sephadex LH-20 or octadeca silane (ODS). High performance liquid chromatography (HPLC) was performed using a Waters 600E with a Waters 490E programmable multiwavelength detector set at 219 nm, using octadeca silane as the stationary phase with the mobile phase and flow rate as described. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Acetonitrile and dichloromethane were heated at reflux for 1 h over calcium hydride prior to distillation. Methanol was distilled from glass. *N*,*N*-Dimethylformamide was distilled under reduced pressure prior to use and stored over 4 Å molecular sieves. Petroleum ether refers to that fraction of petroleum ether boiling between 40 and 60 °C and was redistilled before use. All other solvents were used as received. Reactions were performed under an atmosphere of dry nitrogen unless otherwise stated.

tert-Butyl (1*S*,2*R*,α*R*)-2-(*N*-benzyl-*N*-α-methylbenzyl)amino-cyclohexanecarboxylate 8[4]

To a stirred solution of (*R*)-*N*-benzyl-*N*-α-methylbenzylamine (0.676 g; 3.20 mmol) in THF (3 mL) at −78 °C was added butyllithium (1.88 mL; 3.00 mmol) *via* syringe. The resultant pink solution was slowly warmed to 0 °C (10 min) and subsequently recooled to −78 °C. A solution of **7** (0.364 g, 2.00 mmol) in THF (1.5 mL) was then added dropwise *via* cannula to the

lithium amide solution. After stirring for 21 h at −78 °C the reaction was quenched by the dropwise addition of a solution of 2,6-di-*tert*-butylphenol (0.825 g, 4.00 mmol) in THF (1.5 mL) *via* cannula and allowed to warm to room temperature (15 min). The reaction mixture was partitioned between diethyl ether (30 mL) and brine (30 mL) and further extracted with diethyl ether (2 × 30 mL). The combined organic portions were dried (magnesium sulfate), filtered and concentrated *in vacuo* to yield a yellow oil. ^{1}H NMR (500 MHz) spectroscopic analysis of the crude product mixture indicated that the reaction had occurred with a diastereoselectivity of >96% and to ~60% completion. Purification by silica gel chromatography [petroleum ether–diethyl ether (50:1)] afforded the title compound **8** as a mixture of diastereoisomers (>96% de) and as a colourless oil (0.366 g, 47%); $[\alpha]_D^{23}$ +85.3 (*c* 1.00, $CHCl_3$); lit.[4] $[\alpha]_D^{25}$ +88.3 (*c* 1.00, $CHCl_3$); δ_H (lit.[4]) 7.47–7.18 (10H, m, Ph), 4.05 (1H, d, *J* 14.5, PhCH_2), 4.03 (1H, q, *J* 6.4, C*H*CH_3), 3.86 (1H, d, *J* 14.5, PhCH_2), 2.65 (1H, dt, *J* 12.6 and 4.2, C*H*N), 2.47 (1H, m, C*H*CO_2), 2.23 (1H, qd, *J* 12.6 and 3.6, C$H_{ax}H_{eq}$CHN), 1.83–1.11 [7H, m, $(CH_2)_3CH_{ax}CH_{eq}$] , 1.45 [9H, s, C(CH_3)$_3$], 1.31 (3H, d, *J* 6.7, CHCH_3).

tert-Butyl (1*S*,2*R*)-2-aminocyclohexanecarboxylate hydrochloride 3·HCl

To a solution of **8** (0.363 g, 0.924 mmol) in methanol (5 mL) in a Fischer–Porter bottle was cautiously added Pearlman's catalyst (0.072 g, 20%). A pressure head was fitted and the flask was subsequently pressurised to 6 atm with hydrogen. The heterogeneous solution was stirred vigorously for 16 h at room temperature before the pressure was released and the reaction mixture filtered through a short plug of Celite®. The Celite® pad was washed with ethyl acetate and the filtrate concentrated *in vacuo* to yield a yellow oil which was subsequently dissolved in diethyl ether (5 mL). The ethereal solution was saturated with HCl (g) and concentrated *in vacuo* to afford a pale yellow oil which crystallised on standing. Trituration of the residue with diethyl ether afforded the title compound **3**·HCl as colourless crystals (0.187 g, 86%); mp 169–171 °C; $[\alpha]_D^{23}$ +7.1 (*c* 0.54, $CHCl_3$) (Found: C, 55.8; H, 9.6; N, 5.9. $C_{11}H_{22}NO_2Cl$ requires C, 56.0; H, 9.4; N, 5.9%); ν_{max}(KBr)/cm^{-1} 3140br s (N–H), 1712s (C=O); δ_H 8.54 (3H, br s, NH_3), 3.49–3.46 (1H, m, C*H*N), 3.08–3.06 (1H, m, C*H*CO_2), 2.23–2.09 and 1.88–1.18 [8H, m, $(CH_2)_4$], 1.47 [9H, s, C(CH_3)$_3$]; δ_C (50 MHz) 173.5 (*C*=O), 82.6 [*C*(CH$_3$)$_3$], 50.7 (*C*HN), 42.0 (*C*HCO_2), 27.9 [C(*C*H$_3$)$_3$], 27.0, 26.8, 23.1 and 22.1 (CH_2); *m*/*z* (APCI$^+$) 255 (17%), 200 (30%, MH$^+$), 144 (100%, MH$^+$ − C_4H_8).

(2′*S*,5*R*)-1-{2′-[(*tert*-Butoxycarbonyl)methyl]-3′-phenylpropionyl}-3,3,5-trimethylpyrrolidin-2-one 10

To a stirred solution of diisopropylamine (1.31 mL, 9.35 mmol) in THF (10 mL) at −78 °C was added butyllithium (5.36 mL; 8.57 mmol) *via* syringe. The resultant colourless solution was stirred at −78 °C (5 min), slowly warmed to 0 °C (10 min) and subsequently recooled to −78 °C. A solution of (*R*)-**9** (2.019 g, 7.795 mmol) in THF (5 mL) was then added dropwise *via* cannula to the lithium amide solution. Stirring was maintained at this temperature for 1 h before *tert*-butyl bromoacetate (1.89 mL, 11.69 mmol) was added neat *via* syringe. The reaction mixture was slowly warmed to room temperature overnight, quenched with saturated aqueous ammonium chloride solution (5 mL) and concentrated *in vacuo*. The residual material was partitioned between dichloromethane (50 mL) and distilled water (50 mL), and further extracted with dichloromethane (2 × 50 mL). The combined organic portions were washed with brine, dried (magnesium sulfate), filtered and concentrated *in vacuo* to yield a crude yellow oil which solidified on standing. Inspection of the crude ^{1}H NMR spectrum (500 MHz, $CDCl_3$) indicated that the major reaction product had formed with a diastereomeric excess of >95%. An accurate assignment of the reaction diastereoselectivity was not possible due to the lack of an authentic sample of minor diastereoisomeric product. Purification by silica gel chromatography [petroleum ether–diethyl ether (9:1)] gave a colourless solid which was further purified by a single recrystallisation from diethyl ether–pentane affording **10** as colourless crystals (2.199 g, 79%, >98% de); mp 87–88 °C; $[\alpha]_D^{21}$ −76.07 (*c* 0.89, $CHCl_3$) (Found: C, 70.7; H, 8.6; N, 3.7. $C_{22}H_{31}NO_4$ requires C, 70.75; H, 8.4; N, 3.75%); ν_{max}(KBr)/cm^{-1} 1735s (OC=O), 1718s and 1692s (NC=O); δ_H 7.29–7.16 (5H, m, Ph), 4.40–4.30 (1H, m, C*H*CO), 4.27–4.16 (1H, m, C*H*N), 3.06 (1H, dd, *J* 13.1 and 5.3, PhCH_2), 2.76 (1H, dd, *J* 16.8 and 4.2, CH_2CO_2), 2.46 (1H, dd, *J* 13.1 and 9.7, PhCH_2), 2.26 (1H, dd, *J* 16.8 and 10.8, CH_2CO_2), 2.03 (1H, dd, *J* 12.9 and 8.5, CH_2CHN), 1.57 (1H, dd, *J* 12.9 and 4.8, CH_2CHN), 1.37 [9H, s, C(CH_3)$_3$], 1.36 (3H, d, *J* 4.6, CHCH_3), 1.30 (3H, s, CH_3), 1.11 (3H, s, CH_3); δ_C (50 MHz) 180.6 (N*C*=O), 176.8 (CH*C*=O), 171.3 (O*C*=O), 138.6 (Ph:C_{ipso}), 129.3 and 128.3 (Ph:C_{ortho} and Ph:C_{meta}), 126.4 (Ph:C_{para}), 80.3 [*C*(CH$_3$)$_3$], 50.2 (*C*HN), 42.9 (*C*HCO), 42.0 [(CH$_3$)$_2$*C*CO], 40.3, 37.6 and 36.7 (CH_2), 27.9 [C(*C*H$_3$)$_3$], 26.3 [C(*C*H$_3$)$_2$], 26.0 [C(*C*H$_3$)$_2$], 21.0 (CH*C*H$_3$); *m*/*z* (APCI$^+$) 396 (7%, MNa$^+$), 318 (15%, MH$^+$ − C_4H_8), 300 (10%, $C_{18}H_{22}NO_3$), 128 (100%, $C_7H_{14}NO$).

Benzyl (2*S*)-2-[(*tert*-butoxycarbonyl)methyl]-3-phenylpropionate (*S*)-11

To a stirred solution of benzyl alcohol (0.908 g, 8.404 mmol) in THF (15 mL) at −78 °C was added butyllithium (3.94 mL, 6.30 mmol) dropwise *via* syringe. The resultant colourless solution was stirred at −78 °C (5 min) and slowly warmed to 0 °C (10 min). A chilled (0 °C) solution of **10** (1.500 g, 4.202 mmol) in THF (5 mL) was then added dropwise *via* cannula to the lithium phenylmethoxide solution. After stirring for 1 h at 0 °C the reaction was quenched by the addition of saturated ammonium chloride solution (5 mL) and allowed to warm to room temperature. The reaction mixture was partitioned between diethyl ether (50 mL) and brine (50 mL) and further extracted with diethyl ether (2 × 50 mL). The combined organic portions were dried (sodium sulfate), filtered and concentrated *in vacuo* to yield a colourless oil. This material was purified by silica gel chromatography [petroleum ether–diethyl ether (8:1), then ethyl acetate (100%)] eluting first the title compound (*S*)-**11** (1.397 g, 94%) as a colourless oil followed by the more polar pyrrolidinone (*R*)-**6** (0.471 g, 88%) as colourless crystals.

(*S*)-**11**; $[\alpha]_D^{24}$ −5.17 (*c* 0.87, $CHCl_3$) (Found: C, 74.3; H, 7.6. $C_{22}H_{26}O_4$ requires C, 74.55; H, 7.4%); ν_{max}(film)/cm^{-1} 1732s (C=O); δ_H 7.37–7.12 (10H, m, Ph), 5.13 (1H, d, *J* 12.4, PhCH_2O), 5.07 (1H, d, *J* 12.4, PhCH_2O), 3.19–3.09 (1H, m, C*H*CO), 3.03 (1H, dd, *J* 13.4 and 6.7, PhCH_2), 2.78 (1H, dd, *J* 13.4 and 7.9, PhCH_2), 2.62 (1H, dd, *J* 16.6 and 8.8, CH_2CO_2), 2.36 (1H, dd, *J* 16.6 and 5.3, CH_2CO_2), 1.40 [9H, s, C(CH_3)$_3$]; δ_C (50 MHz) 174.5 (CH*C*=O), 171.2 (CH$_2$*C*=O), 138.5 and 136.1 (Ph:C_{ipso}), 129.3, 128.7, 128.4, 128.3 and 126.9 (Ph:*C*), 80.8 [*C*(CH$_3$)$_3$], 66.5 (Ph*C*H$_2$O), 43.3 (*C*HCO), 37.7 and 36.4 (CH_2), 27.9 [C(*C*H$_3$)$_3$]; *m*/*z* (APCI$^+$) 377 (14%, MNa$^+$), 299 (100%, MH$^+$ − C_4H_8), 281 (85%, $C_{18}H_{17}O_3$), 263 (32%, $C_{15}H_{19}O_4$).

Benzyl (2*S*)-2-(carboxymethyl)-3-phenylpropionate (*S*)-12[8]

To a stirred solution of (*S*)-**11** (1.067 g, 3.014 mmol) in dichloromethane (6 mL) at room temperature was added trifluoroacetic acid (6 mL) *via* pipette. Stirring was maintained at this temperature for 1 h before the reaction mixture was concentrated *in vacuo* to afford a pale brown oil. Purification by silica gel chromatography [petroleum ether–diethyl ether (1:1)] afforded a colourless oil which crystallised on standing. This material was further purified by recrystallisation from diethyl ether–pentane affording the title compound (*S*)-**12** as colourless crystals (0.616 g). Concentration of the mother liquor and sub-

sequent recrystallisation of the residue afforded a further (0.121 g) of the title compound (*S*)-**12**. The combined yield of (*S*)-**12** was (0.737 g, 82%); mp 60–61 °C; $[\alpha]_D^{24}$ −16.02 (*c* 0.83, $CHCl_3$) (Found: C, 72.2; H, 6.0. $C_{18}H_{18}O_4$ requires C, 72.5; H, 6.1%); ν_{max}(film)/cm^{-1} 3031br s (O–H), 1734s (CHC=O), 1713s (CH_2-C=O); δ_H (lit.[8]) 7.37–7.12 (10H, m, Ph), 5.11 (2H, s, PhCH_2O), 3.22–3.04 (2H, m, C*H*CO and PhCH_2), 2.79 (1H, dd, *J* 14.0 and 8.2, PhCH_2), 2.74 (1H, dd, *J* 17.3 and 9.2, CH_2CO$_2$), 2.46 (1H, dd, *J* 17.3 and 4.7, CH_2CO$_2$); δ_C (50 MHz) 178.1 (CH_2C=O), 174.0 (CH*C*=O), 137.9 and 135.7 (Ph:*C*$_{ipso}$), 129.1, 128.7, 128.6, 128.2 and 126.8 (Ph:C), 66.7 (Ph*C*H_2O), 42.9 (*C*HCO), 37.6 (Ph*C*H_2) and 34.9 (*C*H_2CO); *m*/*z* (APCI$^-$) 297 (100%, M − H$^+$), 207 (50%, $C_{11}H_{11}O_4$), 189 (33%).

(*S*)-Benzylsuccinnic acid (*S*)-13[9]

To a solution of (*S*)-**12** (0.050 g, 0.168 mmol) in degassed methanol (3 mL) in a round-bottom flask was cautiously added palladium on carbon (0.010 g, 20%). The flask was fitted with a balloon filled with hydrogen. The heterogeneous solution was vigorously stirred for 2 h at room temperature before the balloon was removed and the reaction mixture filtered through a short plug of Celite®. The Celite® pad was washed with ethyl acetate and the filtrate concentrated *in vacuo* to yield a colourless oil which crystallised on standing. Recrystallisation of this material from distilled water afforded the title compound (*S*)-**13** as colourless crystals (0.034 g, 97%); mp 157–159 °C; lit.[9] mp 153–154 °C; $[\alpha]_D^{22}$ −26.96 (*c* 0.675, EtOAc); lit.[9] $[\alpha]_D^{21}$ −28.9 (*c* 0.665, EtOAc); δ_H (300 MHz, d_6-DMSO) 12.24 (2H, s, COO*H* and COO*H*), 7.31–7.17 (5H, m, Ph), 2.94–2.85 (2H, m, C*H*CO and PhCH_2), 2.73 (1H, dd, *J* 15.4 and 10.0, PhCH_2), 2.42 (1H, dd, *J* 16.6 and 8.1, CH_2CO$_2$), 2.24 (1H, dd, *J* 16.6 and 3.5, CH_2CO$_2$).

Benzyl (2*S*)-2-(*N*-*tert*-butoxycarbamoylmethyl)-3-phenylpropionate (*S*)-4

To a stirred mixture of (*S*)-**12** (0.500 g, 1.678 mmol), dicyclohexylcarbodiimide (0.375 g, 1.846 mmol) and 1-hydroxybenzotriazole (0.340 g, 2.517 mmol) at room temperature was rapidly added DMF (3 mL) *via* syringe. The reaction mixture was stirred for 1 h before dimethylaminopyridine (0.410 g, 3.356 mmol) and *O*-*tert*-butylhydroxylamine hydrochloride (0.253 g, 2.01 mmol) were added solid and in one portion. Stirring was maintained for a further 16 h before the reaction mixture was diluted with ethyl acetate (50 mL), washed with aqueous hydrochloric acid (0.1 M, 2 × 50 mL) and then brine (50 mL), dried (magnesium sulfate), filtered and finally concentrated *in vacuo* to afford a solid yellow residue. Purification by silica gel chromatography [petroleum ether–diethyl ether (7:1)] afforded the title compound (*S*)-**4** as a colourless oil (0.518 g, 84%); $[\alpha]_D^{22}$ +2.26 (*c* 0.53, $CHCl_3$); ν_{max}(film)/cm^{-1} 3196br s (N–H), 1733s (CHC=O), 1663s (CH_2C=O); δ_H (300 MHz, d_6-DMSO) 10.36 (1H, s, N*H*), 7.37–7.11 (10H, m, Ph), 5.00 (2H, app s, PhCH_2O), 3.13–3.06 (1H, m, C*H*CO), 2.90–2.77 (2H, m, PhCH_2), 2.37 (1H, dd, *J* 15.2 and 8.8, CH_2CO$_2$), 2.21 (1H, dd, *J* 15.2 and 5.6, CH_2CO$_2$), 1.11 [9H, s, C(CH_3)$_3$]; δ_C (50 MHz) 174.9 (CH*C*=O), 170.5 (CH_2C=O), 138.2 and 135.8 (Ph:*C*$_{ipso}$), 129.3, 128.7, 128.4 and 126.9 (Ph:*C*), 66.7 (Ph*C*H_2O), 43.2 (*C*HCO), 37.9 (Ph*C*H_2) and 34.2 (*C*H_2CO), 26.1 [C(*C*H_3)$_3$]; *m*/*z* (APCI$^+$) 392 (28%, MNa$^+$), 370 (100%, MH$^+$), 314 (98%, MH$^+$ − C_4H_8) [Found (CI, NH_3): MH$^+$, 370.20280. $C_{22}H_{28}N_1O_4$ MH$^+$ requires, 370.20183].

(2*S*)-2-Benzyl-*N*-*tert*-butoxysuccinimide (*S*)-15

To a solution of (*S*)-**4** (0.100 g, 0.271 mmol) in degassed methanol (5 mL) in a round-bottom flask was cautiously added palladium on carbon (0.020 g, 20%). The flask was fitted with a balloon filled with hydrogen. The heterogeneous solution was vigorously stirred for 1 h at room temperature before the balloon was removed and the reaction mixture filtered through a short plug of Celite®. The Celite® pad was washed with ethyl acetate and the filtrate concentrated *in vacuo* to yield acid (*S*)-**14** as a colourless oil (0.076 g, 100%), *m*/*z* (APCI$^-$) 278 (100%, M − H$^+$), which was used in the next step without further purification or characterisation.

To (*S*)-**14** was added 1-hydroxybenzotriazole (0.055 g, 0.406 mmol), dimethylaminopyridine (0.036 g, 0.298 mmol) and **3**·HCl (0.077 g, 0.325 mmol) followed by THF (1 mL). The stirred reaction mixture was cooled to −22 °C and a solution of dicyclohexylcarbodiimide (0.059 g, 0.285 mmol) in THF (1 mL) was added dropwise *via* syringe. The stirred reaction mixture was maintained at −22 °C for 1 h and was then slowly warmed to room temperature over 5 h. The reaction mixture was diluted with ethyl acetate (100 mL), successively washed with aqueous hydrochloric acid (0.1 M, 2 × 50 mL), saturated sodium hydrogen carbonate (50 mL) and brine (50 mL), dried (magnesium sulfate), filtered and finally concentrated *in vacuo* to afford a solid yellow residue. Purification by silica gel chromatography [petroleum ether–diethyl ether (1:1)] afforded (*S*)-**15** as a colourless oil which crystallised on standing (0.060 g, 85%); mp 80–81 °C; $[\alpha]_D^{23}$ +75.3 (*c* 1.00, $CHCl_3$) (Found: C, 68.8; H, 7.4; N, 5.4. $C_{15}H_{19}NO_3$ requires C, 70.0; H, 7.3; N, 5.4%); ν_{max}(KBr)/cm^{-1} 1724s (C=O); δ_H (300 MHz) 7.34–7.17 (5H, m, Ph), 3.22–3.09 (2H, m, C*H*CO and PhCH_2), 2.92 (1H, dd, *J* 13.2 and 7.8, PhCH_2), 2.69 (1H, dd, *J* 18.0 and 8.6, CH_2CO$_2$), 2.46 (1H, dd, *J* 18.0 and 4.6, CH_2CO$_2$), 1.28 [9H, s, C(CH_3)$_3$]; δ_C (50 MHz) 175.6 and 172.8 (*C*=O), 136.7 (Ph:*C*$_{ipso}$), 129.4 and 129.1 (Ph:*C*$_{ortho}$ and *C*$_{meta}$), 127.4 (Ph:*C*$_{para}$), 87.2 [*C*(CH$_3$)$_3$], 38.4 (*C*HCO), 36.1 (Ph*C*H_2), 30.4 (*C*H_2CO), 27.2 [C(*C*H_3)$_3$]; *m*/*z* (CI, NH_3) 279 (68%, MNH$_4^+$), 262 (16%, MH$^+$), 223 (100%), 206 (20%), 91 (10%).

tert-Butyl (1*S*,2*R*,2′*S*)-2-[2′-(*N*-*tert*-butoxycarbamoylmethyl)-3′-phenylpropionylamino]cyclohexane-1-carboxylate 16

To a solution of (*S*)-**4** (0.100 g, 0.271 mmol) in degassed methanol (3 mL) in a round-bottom flask was cautiously added palladium on carbon (0.020 g, 20%). The flask was fitted with a balloon filled with hydrogen. The heterogeneous solution was vigorously stirred for 1 h at room temperature before the balloon was removed and the reaction mixture filtered through a short plug of Celite®. The Celite® pad was washed with ethyl acetate and the filtrate concentrated *in vacuo* to yield acid (*S*)-**14** as a colourless oil (0.076 g, 100%).

(*S*)-**14** was dissolved in THF (0.5 mL) and cooled to −22 °C with stirring. A solution of ethyl chloroformate (0.0294 g, 0.271 mmol) in THF (0.5 mL) was then added to the crude acid *via* syringe, followed by a solution of triethylamine (0.0300 g, 0.297 mmol) in THF (0.5 mL) dropwise *via* pipette. The reaction mixture was stirred at −22 °C for 30 min before a mixture of **3**·HCl (0.072 g, 0.306 mmol) and triethylamine (0.035 g, 0.347 mmol) in THF (0.5 mL) were added *via* pipette. The reaction was maintained at −22 °C for 1 h and then was slowly warmed to room temperature overnight. The reaction mixture was diluted with ethyl acetate (100 mL), successively washed with aqueous hydrochloric acid (0.1 M, 2 × 50 mL), saturated sodium hydrogen carbonate (50 mL) and brine (50 mL), dried (magnesium sulfate), filtered and finally concentrated *in vacuo* to afford a yellow oil. Purification by silica gel chromatography [petroleum ether–diethyl ether (1:1)] afforded the title compound **16** as colourless oil which crystallised on standing (0.100 g, 80%); mp 64–65 °C; $[\alpha]_D^{22}$ +3.52 (*c* 0.625, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3266br (N–H), 1729s, 1676s and 1648s (C=O); δ_H (500 MHz, d_6-DMSO, 90 °C) 9.83 (1 H, br s, N*H*O), 7.28–7.13 (6H, m, Ph and N*H*), 4.07 (1H, tt, *J* 7.9 and 4.0, C*H*N), 3.02–2.95 (1H, m, C*H*CON), 2.91 (1H, dd, *J* 13.6 and 7.0, PhCH_2), 2.59 (1H, dd, *J* 13.6 and 7.2, PhCH_2), 2.48 (1H, dt, *J* 7.8 and 4.0, C*H*CO$_2$), 2.39 (1H, dd, *J* 15.2 and 8.4, CH_2CON), 2.06 (1H, dd, *J* 15.2 and 5.2, CH_2CON), 1.87–1.80, 1.74–1.68 and 1.62–1.31 [8H, m, (CH_2)$_4$], 1.37 [9H, s, COOC(CH_3)$_3$], 1.15 [9H, s, CON-

$HOC(CH_3)_3$]; δ_C (50 MHz) 173.1, 173.1 and 170.2 (*C*=O), 139.0 (Ph: C_{ipso}), 129.2 and 128.6 (Ph: C_{ortho} and C_{meta}), 126.6 (Ph: C_{para}), 81.6 and 80.9 [$C(CH_3)_3$], 47.9, 45.8 and 44.5 (*C*HCON, *C*HCO_2 and *C*HN), 38.8 and 35.6 (Ph*C*H_2 and *C*H_2CO), 29.1, 27.5, 24.2 and 22.0 (*C*H_2), 27.9 [$COOC(CH_3)_3$], 26.2 [$CONHOC(CH_3)_3$]; *m*/*z* ($APCI^+$) 483 (6%, MNa^+), 461 (100%, MH^+), 405 (7%, $MH^+ - C_4H_8$) [Found (CI, NH_3): MH^+, 461.30200. $C_{26}H_{41}N_2O_5$ MH^+ requires, 461.30155].

(1*S*,2*R*,2′*S*)-2-[2′-(*N*-Hydroxycarbamoylmethylene)-3′-phenylpropionylamino]cyclohexane-1-carboxylate acid 2

To **16** (0.0070 g, 0.0152 mmol) in a 5 mL round-bottomed flask was added trifluoroacetic acid (3 mL) *via* pipette. The flask was then flushed with nitrogen, immediately stoppered and lowered into a 39.5 °C water bath. After 4.5 h at this temperature, the reaction mixture was cooled to room temperature and the volatiles were removed *in vacuo* to afford a yellow oil. This material was purified by HPLC using an analytical column (4.6 mm × 250 mm) with ODS as the stationary phase and TFA (0.07%)–CH_3CN (80:20) as the mobile phase at a flow rate of 1.0 mL min^{-1} (retention time, 12 min 10 s) to afford the title compound **2** as a colourless oil (0.0048 g, 91%); R_F 0.45 [silica gel, eluent EtOAc–Py–AcOH–H_2O (160:20:6:11)]; lit.[3] R_f 0.47 [silica gel (Merck, 60F254), eluent EtOAc–Py–AcOH–H_2O (160:20:6:11)]; $[a]_D^{21}$ −5.8 (*c* 0.48, MeOH); ν_{max}(film)/cm^{-1} 3230br (N–H and O–H), 1714s (OC=O), 1644s (NC=O) and 1537s; δ_H (lit.;[3] 500 MHz, d_6-DMSO) 10.28 (1H, s, N*H*O), 8.62 (1H, br s, NO*H*), 7.63 (1H, d, *J* 8.6, CHN*H*), 7.27–7.16 (5H, m, Ph), 4.08 (1H, m, C*H*NH), 3.05–3.00 (1H, m, C*H*CON), 2.85 (1H, dd, *J* 13.7 and 6.6, PhCH_2), 2.51–2.46 (2H, m, PhCH_2 and C*H*CO_2), 2.21 (1H, dd, *J* 15.0 and 8.8, CH_2CO), 1.83 (1H, dd, *J* 15.0 and 5.5, CH_2CO), 1.89–1.81, 1.73–1.67 and 1.56–1.24 (8H, m, CH_2); δ_C (125 MHz, d_6-DMSO), 174.6, 172.9 and 167.9 (*C*=O), 139.6 (Ph: C_{ipso}), 129.0 and 128.3 (Ph), 126.1 (Ph: C_{para}), 46.6, 44.0 and 42.7 (*C*HCON, *C*HCO_2 and *C*HN), 38.0 and 33.6 (Ph*C*H_2 and *C*H_2CO), 29.2, 24.9, 23.1 and 22.1 (*C*H_2); *m*/*z* ($APCI^+$) 371 (13%, MNa^+), 349 (75%, MH^+), 333 (7%), 316 (36%), 305 (100%) [Found (CI, NH_3): MH^+, 349.17650. $C_{26}H_{41}N_2O_5$ MH^+ requires, 349.17635].

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