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Synthesis of (2*S*)-2-amino-3-(1*H*-4-indolyl)propanoic acid, a novel tryptophan analog for structural modification of bioactive peptides

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Abstract

A convenient, multigram synthesis of a novel α-amino acid (2*S*)-2-amino-3-(1*H*-4-indolyl)propanoic acid (**1a**), is reported. An Fmoc–*t*-Boc derivative of this novel regioisomer of the natural aromatic amino acid tryptophan could be readily incorporated into bioactive synthetic peptides using standard solid phase synthesis. The synthesis featured the use of Schöllkopf chiral auxiliary reagents for chirality induction during a key step.

1. Introduction

Tryptophan (Trp) is one of the essential amino acids and plays a key role in the structure and function of many bioactive proteins and peptides,[1] inter alia, serotonin,[2a] melatonin[2b] and dynorphin.[2c] Minor structural modifications of these and other naturally occurring macromolecules provide important probes for determining their function in biological systems. Other uses of the targeted structural modifications include the development of analogs and therapeutic drugs that modulate specific biological activities of receptors and enzymes. Certain analogs of tryptophan, for example, have been extensively used in time-resolved fluorescence spectroscopy, which is used to monitor molecular interactions and motions that occur in the picosecond–nanosecond time range.[3] During our work on the development of novel neurotensin receptor ligands,[4] we required neurotensin analogs with reduced sizes that would better fit the slightly smaller human neurotensin receptor relative to the rat receptor, as well as impart cell

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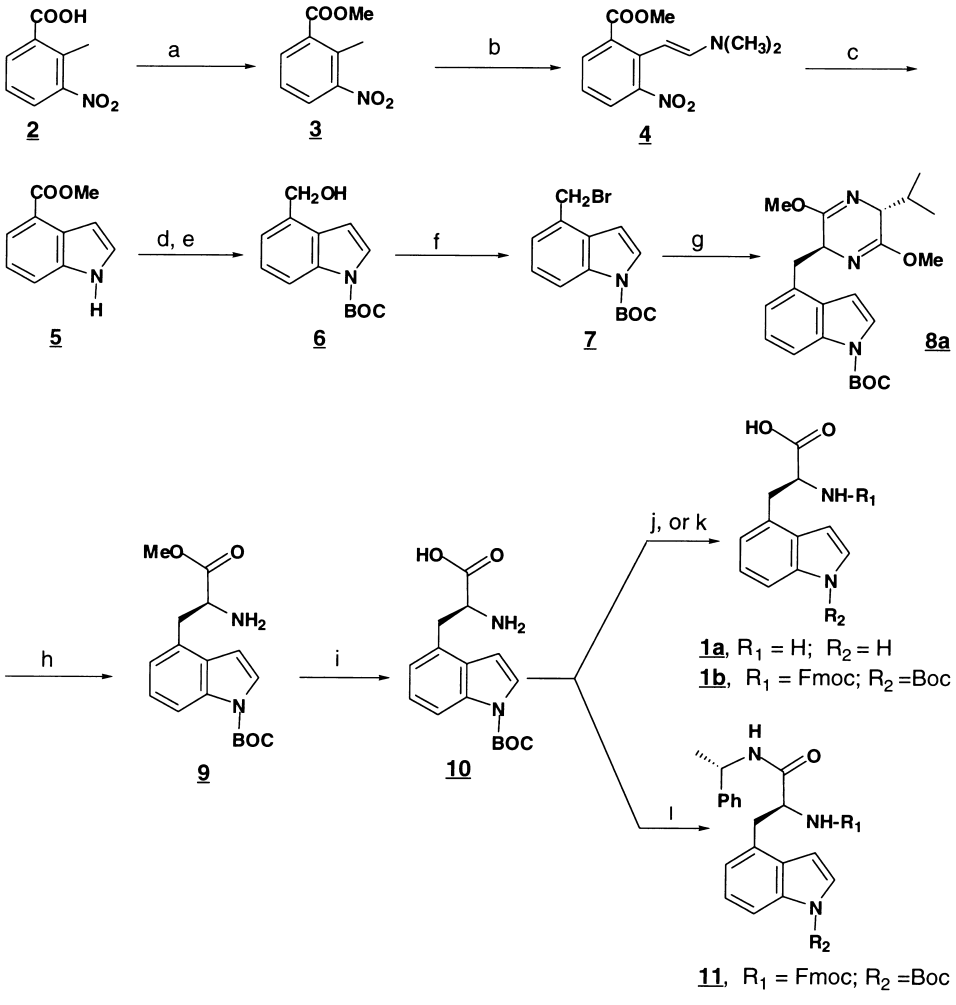
permeability and resistance to peptidases. Herein, we report the synthesis of a novel, enantiopure Trp isostere, (2*S*)-2-amino-3-(1*H*-4-indolyl)propanoic acid (**1a**) which was successfully incorporated into some brain-penetrating, hydrolysis resistant, and highly potent neurotensin analogs.[5]

2. Results and discussion

The enantiomeric synthesis of **1a** and **1b** (see Scheme 1) began with 2-methyl-3-nitrobenzoic acid **2** which, after esterification, was followed by reaction with *N*,*N*-dimethylformamide dimethylacetal to furnish the enamine **4**.[6] Reductive cyclization using H_2/Pd–C gave the 4-substituted indole methyl ester **5**. Protection of the indole nitrogen of **5** with the *tert*-butoxycarbonyl (Boc) group[7] and reduction of the resulting ester with DIBAL proceeded uneventfully to give *N*-Boc-4-hydroxymethyl indole **6**. Next, conversion to the benzylic bromide **7** with phosphorus tribromide in ether was followed by the key S_N2 displacement of the bromide with the carbanion derived from the commercially available (*R*)-Schöllkopf reagent, (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine[8] to provide the diastereomeric bislactim products **8a** and **8b** in 10:1 diastereomeric ratio, in 67% yield of **8a**. The desired diastereomer **8a** could be easily isolated by flash silica gel chromatography and then subjected to mild acid (0.1 M TFA/CH_3CN) treatment which only hydrolyzed the bislactim, leaving the Boc group unaffected. The resulting amino ester **9**[9] was saponified to the N^{ind}-*t*-Boc amino acid **10**. Finally, the amino nitrogen was protected as its fluorenylmethoxycarbonyl (Fmoc) derivative **1b** for convenient incorporation of this novel amino acid into peptides by the commonly employed solid phase synthesis methods. For spectral, chemical, and optical characterization, the *t*-Boc group of compound **10** was removed to furnish the free amino acid **1a**. Following the above protocol, multigram synthesis of enantiopure **1b** was achieved in our laboratories in fairly short order. Since the *S*-enantiomer of the Schöllkopf reagent is commercially available, the (*R*)-2-amino-3-(1*H*-4-indolyl)propanoic acid can be similarly synthesized.

To determine the enantiomeric purity of the final Fmoc–Boc derivatives, **1b** and *ent*-**1b** were converted to the diastereomeric (*S*,*S*) amides **11** and **12** by EDC/HOBT-mediated coupling with (*S*)-α-methylbenzylamine. The (*R*,*S*) diastereomer **12** was obtained by carrying the minor (*R*,*R*) diastereomer of **8**, obtained during the Schöllkopf reaction, through the acidic and basic hydrolysis steps, followed by fluorenylmethoxycarbonylation, to the Fmoc–Boc derivative *ent*-**1b**. The diastereomers (*S*,*S*)-**11** and (*S*,*R*)-**12** exhibited important chemical shift differences in two regions of their ^{1}H NMR spectra, obtained for pure as well as for premixed samples of defined composition. Specifically, the ^{1}H NMR spectra of the pure samples of (*S*,*S*)-**11** and (*R*,*S*)-**12** gave sharp signals for the methyl protons of the (*S*)-α-methylbenzylamine portion at δ 1.31 (d, *J*=6.9 Hz, 3H) and 1.11 (d, *J*=6.9 Hz, 3H), respectively. Also, (*S*,*S*)-**11** showed a peak at δ 8.00 (d, *J*=8.3 Hz, 1H) for one of the aromatic protons of the (*S*)-amino acid portion, whereas the same peak was found at δ 8.11 (d, *J*=8.3 Hz, 1H) for the (*R*)-amino acid portion of the (*S*,*R*)-**12**. The analysis of the ^{1}H NMR spectra for both the pure diastereomers **11** and **12** showed only a trace diastereomeric impurity, and amounted to an estimated 98% ee for the final Fmoc–Boc-protected **1b** or *ent*-**1b**. Thus, the diastereomers **8** were obtained not only in almost pure form during the silica gel chromatography following the coupling reaction with the Schöllkopf reagent, but also the mild alkaline conditions employed during the hydrolysis of the methyl ester **9** and *ent*-**9** did not cause any significantly detectable racemization of the α-chiral center.

The doubly protected amino acid **1b** was readily incorporated into some novel biologically active neurotensin analogs by conventional Fmoc-related automated solid phase chemistry.[5] Interestingly, these analogs have not only provided more potent analogs for the cloned human neurotensin receptor, but they



Scheme 1. Reagents and conditions: (a) K_2CO_3, MeI, DMF, RT (100%); (b) *N*,*N*-dimethylformamide dimethylacetal, DMF, 120°C; (c) H_2, 10% Pd–C (cat), MeOH, RT, 50–55 psi, benzene (67% over 2 steps); (d) $(BOC)_2O$, CH_3CN, DMAP (cat), RT (100%); (e) DIBAL-H, CH_2Cl_2:ether, −78°C (88%); (f) PBr_3, ether:CH_2Cl_2 (95%); (g) (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine:BuLi, THF, −78°C, then **7** (67%); (h) 0.1 M aq. TFA, CH_3CN, RT (100% overall); (i) $LiOH \cdot H_2O$, THF:H_2O, RT (62%); (j) TFA:CH_2Cl_2, RT; (k) Fmoc–Suc, 10% $NaHCO_3$, acetone, 0°C–RT (72%); (l) α-methylbenzylamine, EDC, HOBT, DMF, RT (88%)

also appear to cross the blood–brain barrier and cause considerable resistance to the peptidases known to cleave neurotensin.[5]

Many potential applications of the apparently hitherto unknown amino acid **1a** can be envisioned. This novel amino acid could, in principle, be incorporated into molecules of biological and therapeutic interest in which tryptophan, or possibly tyrosine, appears to play a key role. These modified molecular species may then be re-evaluated for better potency and reduced toxicity. Our research in some of these sharply defined areas is continuing and will be reported in due course.

3. Experimental

3.1. General methods

Nuclear magnetic spectra (^{1}H, ^{13}C) were measured with a Bruker WH-300 instrument (^{1}H frequency 300 MHz, ^{13} C frequency 75 MHz) in the solvent noted. ^{1}H chemical shifts are expressed in parts per million, downfield from Me_4Si used as internal standard. Melting points were taken with a GallenKamp instrument and are uncorrected. The column chromatographic separations were performed with 'J. T. Baker' Silica gel (40 μm). Anhydrous DMF was obtained from Aldrich Chemicals. Tetrahydrofuran (THF) and diethyl ether were distilled over sodium benzophenone ketyl before use. Methylene chloride was distilled over calcium hydride or P_2O_5. Acetonitrile was reagent grade from E. M. Science and was used without further drying. Ethyl acetate and hexane were reagent grade and were used as received. The purity of all compounds was shown to be >95% by TLC and high field ^{1}H NMR and ^{13}C NMR (300 and 75 MHz Brucker instrument). Optical rotations were taken with a 241 Perkin–Elmer polarimeter (Na lamp). IR spectra were measured with a 2020 Galaxy Series FT-IR (Mattson Instruments). Mass spectra analyses were performed at the Mayo Clinic Rochester Mass Spectroscopy Facility, Rochester, Minnesota.

3.2. Methyl 2-methyl-3-nitrobenzoate (3)

To well mixed 2-methyl-3-nitrobenzoic acid **2** (50 g, 0.28 mol) and $KHCO_3$ (84 g, 0.84 mol) was added DMF (130 ml). Since the mixture became highly viscous, it was heated to 40°C with manual shaking. Iodomethane (79 g, 0.56 mol) was added via syringe after the gas evolution had ceased. The resulting orange coloured solution was stirred for 12 h at room temperature. The reaction mixture was poured into water (800 ml) and the resulting precipitate was collected by filtration and dried over P_2O_5 to give pure product **3** (56 g, 100%) as a white solid: mp 64.2–65.5°C. ^{1}H NMR ($CDCl_3$) δ 8.00 (d, J=7.8 Hz, 1H), 7.85 (d, J=8.0 Hz, 1H), 7.39 (t, J=8.0 Hz, 1H), 3.95 (s, 3H), 2.63 (s, 3H); IR (KBr, cm^{-1}) 1724, 1548, 1279; MS (EI): 195 (M^+).

3.3. Methyl 1H-4-indolecarboxylate (5)

A solution of **3** (20 g, 0.1 mol), *N*,*N*-dimethylformamide dimethylacetal (40 ml, 0.3 mol) in DMF (50 ml) was stirred at 120°C under nitrogen for 12 h. The solution became deep red. The excess amount of *N*,*N*-dimethylformamide dimethylacetal and DMF was distilled under reduced pressure to give crude enamine **4** that was directly used in the next step without purification.

The crude enamine **4** was dissolved in anhydrous benzene (250 ml). To this solution was added Pd–C (10%, 2.8 g). The resulting mixture was hydrogenated at 55 psi. Warming was observed at the start of the reaction. The deep red mixture became dark gray after 12 h at room temperature. Pd–C was filtered off over Celite and the filtrate was concentrated under reduced pressure. Chomatography on silica gel (ethyl acetate:hexanes: 30:70 v/v, R_f=0.55) afforded **5** (11.8 g, 67%) as a light yellow solid: mp 67.5–69.0°C. ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 1H), 7.93 (d, J=8.3 Hz, 1H), 7.60 (d, J=8.9 Hz, 1H), 7.36 (t, J=3.0 Hz, 1H), 7.26 (t, J=3.7 Hz, 1H), 7.26–7.18 (m, 1H), 4.0 (s, 3H): IR (KBr, cm^{-1}) 3322, 1705, 1279; MS (ESI): 176 (M^++1).

3.4. 1-(tert-Butyl) 4-methyl 1H-1,4-indoledicarboxylate

To a solution of **5** (11.8 g, 67.4 mmol) in acetonitrile (50 ml) was added di-*tert*-butyl dicarbonate (14.7 g, 67.4 mmol) and DMAP (0.2 g). The mixture was stirred at room temperature for 12 h. Some bubbling was observed. The solvent was removed under reduced pressure to give a residue that was redissolved in ethyl acetate (200 ml). The solution was washed sequentially with cold 1 N HCl (80 ml), water (50 ml), and brine (50 ml), and then dried ($MgSO_4$). The solvent was removed under reduced pressure to give the pure product (18.5 g, 100%) as a light yellow oil. ^{1}H NMR ($CDCl_3$) δ 8.41 (d, *J*=8.2 Hz 1H), 7.98 (d, *J*=7.7 Hz, 1H), 7.71 (d, *J*=3.7 Hz, 1H), 7.36 (t, *J*=7.9 Hz, 1H), 7.28 (d, *J*=3.8 Hz, 1H), 3.98 (s, 3H), 1.68 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 187.3, 149.4, 135.9, 130.5, 127.8, 125.4, 123.5, 121.9, 119.7, 107.8, 84.1, 51.8, 28.1; IR (KBr, cm^{-1}) 1703, 1603, 1283, 1146; MS (ESI): 276 (M^++1).

3.5. tert-Butyl 4-(hydroxymethyl) 1H-1-indolecarboxylate (6)

To a solution of the Boc-protected indole (18.5 g, 67 mmol, obtained in the previous step) in ether (150 ml), was added DIBAL (1.0 M in CH_2Cl_2, 163 mmol) at −78°C in 30 min under nitrogen. Stirring was continued at this temperature for another 30 min at which point the reaction was quenched with saturated citric acid at −78°C. A precipitate immediately formed which, after warming to room temperature, was acidified to pH 1 with 1 N HCl and extracted with ethyl acetate (3×200 ml). The combined extracts were washed with water (100 ml), and brine (200 ml), and then dried ($MgSO_4$). The solvent was evaporated under vacuum to give a residue that was purified by chomatography on silica gel (ethyl acetate:hexanes 30:30 v/v, R_f=0.45) to yield **6** (14.5 g, 88%) as a light yellow solid: mp 62.9–64.1°C. ^{1}H NMR ($CDCl_3$) δ 8.09 (d, *J*=8.1 Hz 1H), 7.6 (d, *J*=3.7 Hz, 1H), 7.28 (t, *J*=7.5 Hz, 1H), 7.20 (d, *J*=7.3 Hz, 1H), 6.69 (d, *J*=3.7 Hz, 1H), 4.90 (s, 2H), 2.01 (s, 1H), 1.67 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 149.7, 135.3, 132.7, 128.8, 125.9, 124.2, 121.2, 114.8, 105.2, 83.7, 63.4, 28.1; IR (KBr, cm^{-1}) 3364, 1732, 1130; MS (ESI): 248 (M^++1).

3.6. tert-Butyl 4-{[(2S,5R)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-2-pyrazinyl]methyl}-1H-1-indolecarboxylate (8a)

To a stirred solution of the alcohol **6** (6.0 g, 24.3 mmol) in ether (80 ml) and CH_2Cl_2 (20 ml) under nitrogen was added dropwise PBr_3 (2.4 ml, 25.8 mmol) at 0°C under nitrogen. The reaction was complete 30 min after the addition. The mixture was poured into cold aq. $NaHCO_3$ solution (100 ml) and extracted with ethyl acetate (3×80 ml). The combined extract was washed with water (80 ml) and brine (80 ml), then dried ($MgSO_4$), filtered, and finally concentrated to provide *tert*-butyl 4-(bromomethyl)-1*H*-1-indolecarboxylate (**7**) (6.3 g, 84%) as an oil which was immediately taken to the next step.

To a solution of (2*R*)-2-isopropyl-3,6-dimethoxy-1,5-dihydropyrazine (3.8 g, 20.4 mmol) in THF (70 ml) under nitrogen at −78°C was added dropwise n-BuLi (2.5 M in hexane) via syringe. The carbanion was allowed to form for 10 min at the same temperature, at which point a solution of the bromide **7** in THF (40 ml) was added in a dropwise fashion. The reaction proceeded to completion in 1 h at −78°C. Saturated NH_4Cl aq (80 ml) was added at −78°C and the THF was evaporated under reduced pressure. The aqueous phase was extracted with ethyl acetate (3×80 ml). The combined extracts were washed with brine (100 ml), dried ($MgSO_4$), and concentrated. The residue was purified on a silica gel column (ethyl acetate:hexanes 5:95 then 10:90 v/v, R_f=0.60 for the major product) to afford the desired product **8a** (5.8 g, 67%) as a colourless oil: $[\alpha]_D^{25}$ +26.7 (*c* 14.8 mg/ml, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.98 (d, *J*=8.2 Hz 1H), 7.54 (s, 3H), 7.17 (t, *J*=7.8 Hz, 1H), 6.96 (d, *J*=7.3 Hz, 1H), 6.69 (d, *J*=4.0 Hz, 1H), 4.45–4.38 (m,

1H), 3.69 (s, 3H), 3.61 (s, 3H), 3.45–3.20 (m, 3H), 2.15–2.05 (m, 1H), 1.67 (s, 9H), 0.91 (d, *J*=6.8 Hz, 3H), 0.58 (d, *J*=6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 163.7, 162.2, 149.8, 134.9, 131.0, 130.0, 125.0, 124.0, 121.8, 113.3, 106.5, 83.4, 60.1, 56.7, 52.3, 52.1, 36.9, 31.1, 28.2, 18.9, 16.4; IR (KBr, cm^{-1}) 1734, 1696, 1346,1128; MS (ESI): 414 (M^++1).

3.7. tert-*Butyl 4-[(2*S*)-2-amino-3-methoxy-3-oxopropyl]-1*H*-1-indolecarboxylate (**9**)*

To a solution of **8** (3.5 g, 8.6 mmol) in acetonitrile (95 ml) was added TFA (0.15 N, 24 mmol). The mixture was purged with nitrogen and stirred for 12 h at room temperature. The acetonitrile was evaporated and the water phase was extracted with CH_2Cl_2 (5×60 ml). The combined extract was washed with water (3×100 ml) and brine (80 ml), then dried ($MgSO_4$). Filtration and evaporation of the solvent left the amino ester **9** as a colourless oil (2.7 g, 98%). $[\alpha]_D^{25}$ +17.6 (*c* 12.8 mg/ml, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.06 (d, *J*=8.3 Hz 1H), 7.61 (d, *J*=3.7 Hz, 1H), 7.26 (t, *J*=7.6 Hz, 1H), 7.06 (d, *J*=3.7 Hz, 1H), 3.84 (dd, *J*=5.1, 8.1 Hz, 1H), 3.70 (s, 3H), 3.36 (dd, *J*=5.1, 13.5 Hz, 1H), 3.06 (dd, *J*=8.2, 13.6 Hz, 1H), 1.67 (s, 9H), 1.46 (s 2H); ^{13}C NMR ($CDCl_3$) δ 175.4, 149.7, 135.2, 130.2, 129.5, 125.8, 124.3, 114.0, 105.3, 83.7, 55.5, 52.0, 38.5, 28.1; IR (neat, cm^{-1}) 3383, 1732, 1346, 1155; MS (ESI): 319 (M^++1).

*3.8. (2*S*)-2-Amino-3-[1-(*tert-*butoxycarbonyl)-1*H*-4-indolyl]propanoic acid (**10**)*

To a solution of **9** (2.7 g, 8.5 mmol) in THF (200 ml), was added $LiOH\cdot H_2O$ (980 mg, 26 mmol) dissolved in H_2O (100 ml) at room temperature. The reaction was judged complete after 10 min (close monitoring by TLC). After neutralizing with 1N HCl (30 ml), the THF and most of the water were evaporated in vacuo. The precipitated product was collected by filtration and dried over P_2O_5 under high vacuum (1.8 g, 62%). Mp 169.5–171.2°C (dec). $[\alpha]_D^{25}$ −9.8 (*c* 6.6 mg/ml, EtOH); ^{1}H NMR (DMSO-d_6) δ 7.89 (d, *J*=8.2 Hz 1H), 7.69 (d, *J*=3.8 Hz, 1H), 7.27 (t, *J*=7.7 Hz, 1H), 7.14 (d, *J*=7.5 Hz, 1H), 6.96 (d, *J*=3.8 Hz, 1H), 3.99 (t, *J*=6.6 Hz, 1H), 3.45–3.26 (m, 2H), 1.63 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 170.3, 149.1, 134.6, 130.0, 128.0, 126.0, 124.3, 123.8, 113.7, 105.8, 83.8, 53.3, 33.5, 27.6; IR (KBr, cm^{-1}) 3432, 3179, 1734, 1603, 1051; MS (ESI): 305 (M^++1).

*3.9. (2*S*)-3-[1-(*tert-*Butoxycarbonyl)-1*H*-4-indolyl]-2-{[(9*H*-fluorenylmethoxy)carbonyl]amino}-propanoic acid (**1b**)*

A mixture of **10** (1.8 g, 5.28 mmol) in 10% aq. $NaHCO_3$ (30 ml) was stirred for 1 h at room temperature. To this mixture was added a solution of Fmoc–Suc (1.9 g, 5.55 mmol) in acetone (30 ml). The resulting mixture was stirred for 12 h at room temperature. Acetone was evaporated under reduced pressure. The aqueous phase was acidified to pH 5 with 1N HCl, and extracted with ethyl acetate (3×60 ml). The combined extracts were washed with brine (80 ml), then dried (Na_2SO_4), and concentrated. The resulting residue was purified on silica gel (MeOH:CH_2Cl_2 5:95 v/v. R_f=0.3) as a white solid: mp 92.1–93.8°C. $[\alpha]_D^{25}$ +1.9 (*c* 3.6 mg/ml, $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 7.93 (d, *J*=8.2 Hz 1H), 7.87 (d, *J*=7.6 Hz, 2H), 7.78 (d, *J*=8.7 Hz, 1H), 7.67 (d, *J*=6.0 Hz, 1H), 7.59 (dd, *J*=7.5, 10.6 Hz, 2H), 7.45–7.36 (m, 2H), 7.36–7.19 (m, 3H), 7.15 (d, *J*=7.2 Hz, 1H), 6.85 (d, *J*=3.6 Hz, 1H), 4.34–4.23 (m, 1H), 4.23–4.10 (m, 2H), 3.41–3.32 (m, 2H), 3.20–3.08 (m, 1H), 1.62 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 173.4, 156.0, 149.2, 143.9, 140.8, 134.6, 130.7, 129.9, 127.8, 127.2 126.0, 125.4, 124.3, 123.6, 120.2, 113.3, 105.8, 84.0, 65.8, 55.2, 46.6, 34.2, 27.8; IR (KBr, cm^{-1}) 3308, 1703, 1346, 1128; MS (ESI): 563 (M+K^+), 549 (M+Na^+); HRMS (FAB) calcd for (M^++1) $C_{31}H_{31}N_2O_2$ 527.2182, found 527.2212. Anal.

calcd for $C_{31}H_{30}N_2O_2 \cdot 0.5H_2O$: C, 69.52; H, 5.83; N, 5.23. Found: C, 69.54; H, 6.40; N, 5.05. The *ent*-**1b** showed $[\alpha]_D^{25}$ −1.4 (*c* 10.0 mg/ml, $CHCl_3$).

*3.10. (2S)-2-Amino-3-(1H-4-indolyl)propanoic acid (**1a**)*

A solution of **10** (10 mg, 0.03 mmol) in TFA (1 ml) and CH_2Cl_2 (2 ml) was stirred for 90 min. The solvent was evaporated under reduced pressure. The residue was purified on reverse phase HPLC on a Vydak C_8 column (15–20 μm particle size, 250×22 mm i.d) using a gradient of 10% B to 90% B in 30 min (buffer A 0.1% TFA in H_2O, buffer B 80% CH_3CN in buffer A; UV detection at λ_{max} 220 nm; flow rate 8 ml/min) to give the desired product **12** as a trifluoroacetate salt: mp 110.0–111.8°C. $[\alpha]_D^{25}$ +31.8 (*c* 1.1 mg/ml, H_2O); ^{1}H NMR (DMSO-d_6) δ 11.22 (s, 1H), 8.27 (s, 3H), 7.42–7.34 (m, 2H), 7.04 (t, *J*=7.6 Hz, 1H), 6.87 (d, *J*=7.1 Hz, 1H), 6.53 (s, 1H), 4.17 (s, 1H), 3.41–3.26 (m, 2H); IR (KBr, cm^{-1}) 3399, 1736; MS (ESI): 205 (M^++1); HRMS (EI) calcd for $C_{31}H_{31}N_2O_2$ 204.0899, found 204.0976.

3.11. tert-*Butyl 4-((2S)-2-{[(9H-9-fluorenylmethoxy)carbonyl]amino}-3-oxo-3-{[(1S)-1-phenylethyl]-amino}propyl)-1H-1-indolecarboxylate (**11**)*

To a solution of **1b** (300 mg, 0.57 mmol), HOBT (48 mg, 0.62 mmol) and EDCI (120 mg, 0.62 mmol) in DMF (10 ml), was added (*S*)-1-phenylethyl amine (65 mg, 0.54 mmol) at room temperature. After 12 h stirring, the mixture was poured into saturated $NaHCO_3$ (40 ml), and then extracted with ethyl acetate (3×50 ml). The combined extract was washed with water (2×40 ml) and brine (50 ml), then dried (Na_2SO_4), concentrated, and the residue was purified on silica gel column (ethyl acetate:hexanes 30:70 v/v. R_f=0.4) to afford **11** (315 mg, 88%) as a white solid: mp 170.5–171.9°C. ^{1}H NMR ($CDCl_3$) δ 8.00 (d, *J*=8.3 Hz, 1H), 7.76 (d, *J*=7.4 Hz, 2H), 7.60–7.48 (m, 3H), 7.40 (t, *J*=7.3 Hz, 2H), 7.30 (t, *J*=7.5 Hz, 2H), 7.25–7.16 (m, 3H), 7.10 (t, *J*=7.7 Hz, 1H), 6.95–6.85 (m, 3H), 6.82–6.73 (m, 1H), 5.66 (d, *J*=6.7 Hz, 1H), 5.52 (d, *J*=7.4 Hz, 1H), 4.97 (t, *J*=7.1 Hz, 1H), 4.52–4.32 (m, 3H), 4.20 (t, *J*=6.8 Hz, 1H), 3.45–3.36 (m, 1H), 3.15 (dd, *J*=9.3, 13.4 Hz, 1H), 1.68 (s, 9H), 1.31 (d, *J*=6.9 Hz, 3H); IR (KBr, cm^{-1}) 3297, 1732, 1651, 1537, 1032; MS (ESI): 630 ($M+H^+$).

3.12. tert-*Butyl 4-((2R)-2-{[(9H-9-fluorenylmethoxy)carbonyl]amino}-3-oxo-3-{[(1S)-1-phenylethyl]-amino}propyl)-1H-1-indolecarboxylate (**12**)*

This amide was prepared in 80% yield as a white solid by the same procedure as that used for amide **11**: mp 89.9–91.0°C. ^{1}H NMR ($CDCl_3$) δ 8.11 (d, *J*=8.3 Hz, 1H), 7.77 (d, *J*=7.5 Hz, 2H), 7.64–7.53 (m, 3H), 7.40 (t, *J*=7.3 Hz, 2H), 7.36–7.15 (m, 6H), 7.12–7.02 (m, 3H), 7.11–6.95 (m, 1H), 5.65 (d, *J*=7.1 Hz, 1H), 5.32 (d, *J*=7.7 Hz, 1H), 4.91 (t, *J*=7.1 Hz, 1H), 4.52–4.31 (m, 3H), 4.19 (t, *J*=7.0 Hz, 1H), 3.56–3.48 (m, 1H), 3.14 (dd, *J*=9.5, 13.3 Hz, 1H), 1.68 (s, 9H), 1.12 (d, *J*=6.8 Hz, 3H); IR (KBr, cm^{-1}) 33037, 1732, 1653, 1346, 1047; MS (ESI): 630 ($M+H^+$).

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7. The indole nitrogen was initially protected with the benzyl or carbobenzyloxy (Cbz) group but these groups turned out to be problematic later in the synthetic sequence. Thus, the indole nitrogen benzyl group could not be removed with catalytic hydrogenolysis, and the Cbz group gave lower yields due to its partial removal during the reduction of the methyl ester moiety with DIBAL.
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9. The TFA-salt of the amino ester **9** could be easily isolated in pure form by extraction with methylene chloride leaving the corresponding D-valine amino ester in the aqueous phase.