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Synthesis of an α-CH_2CO_2H Functionalized Tryptophan and its Incorporation into an Analogue of Cholecystokinin.

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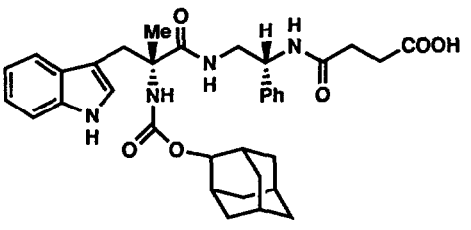
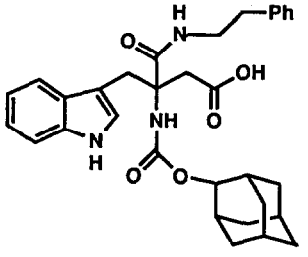
Abstract : *The synthesis of an α,α-disubstituted tryptophan derivative (2) predicted by computer assisted molecular modelling to have close structural and conformational analogy to the endogenous neuropeptide cholecystokinin (CCK) is described. Central to the synthesis of (2) is the alkylation of an isonitrile derivative of tryptophan.*

Introduction. We have previously reported[1] on the rational design of potent and selective 'dipeptoid' antagonists of the neuropeptide cholecystokinin (CCK). The representative compound (1) (PD134308) possesses potent anxiolytic and anti-gastrin properties. Using computer assisted molecular modelling selected examples of these dipeptoid antagonists have been compared with energy minimized conformations[2] of CCK 26-33 (the most abundant form of endogenous CCK[3]) and its C-terminal tetrapeptide CCK 30-33. Results from these studies indicated that the small molecule compound (2) would most closely overlay the Trp, Phe and Asp side chains of CCK 30-33.



(1)

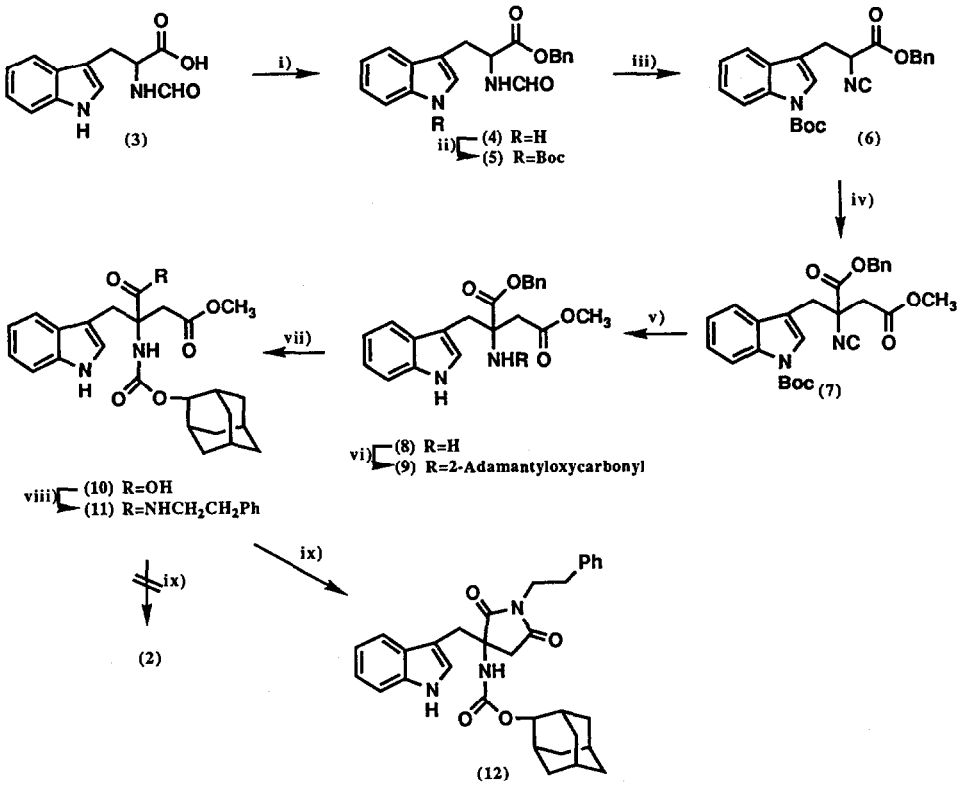


(2)

The most synthetically challenging feature of compound (2) is the incorporation of the α-functional group into the tryptophan moiety. An α-substituent would be expected to enhance the stability of a peptide bond[4] and modify biological activity.[5] Numerous methods for the preparation of α-CH_2-X functionalized amino acids have appeared in the literature.[6] However, many of these approaches are not suitable for the preparation of tryptophan

derivatives due to the reactive nature of the 2-position of the indole ring in the side chain. In this paper we describe the synthesis of an α-CH_2CO_2H derivatized tryptophan residue using the isonitrile method of Schöllkopf[7] developed by Pettig and Horwell[8] and its incorporation into the target molecule (2).

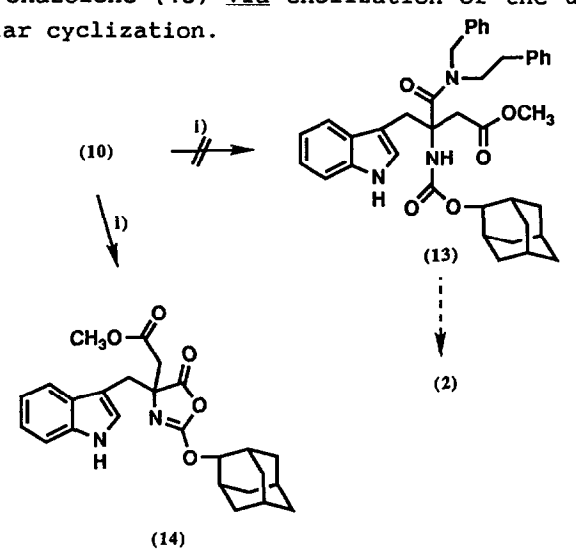
Results and Discussion. The initial approach to the synthesis of α,α-disubstituted tryptophan derivative (2) is described in Scheme I. Formation of the benzyl ester (4) was achieved in quantitative yield by reaction of the caesium salt of N-formyl-D,L-tryptophan (3) with benzyl bromide.[9] Protection



SCHEME 1

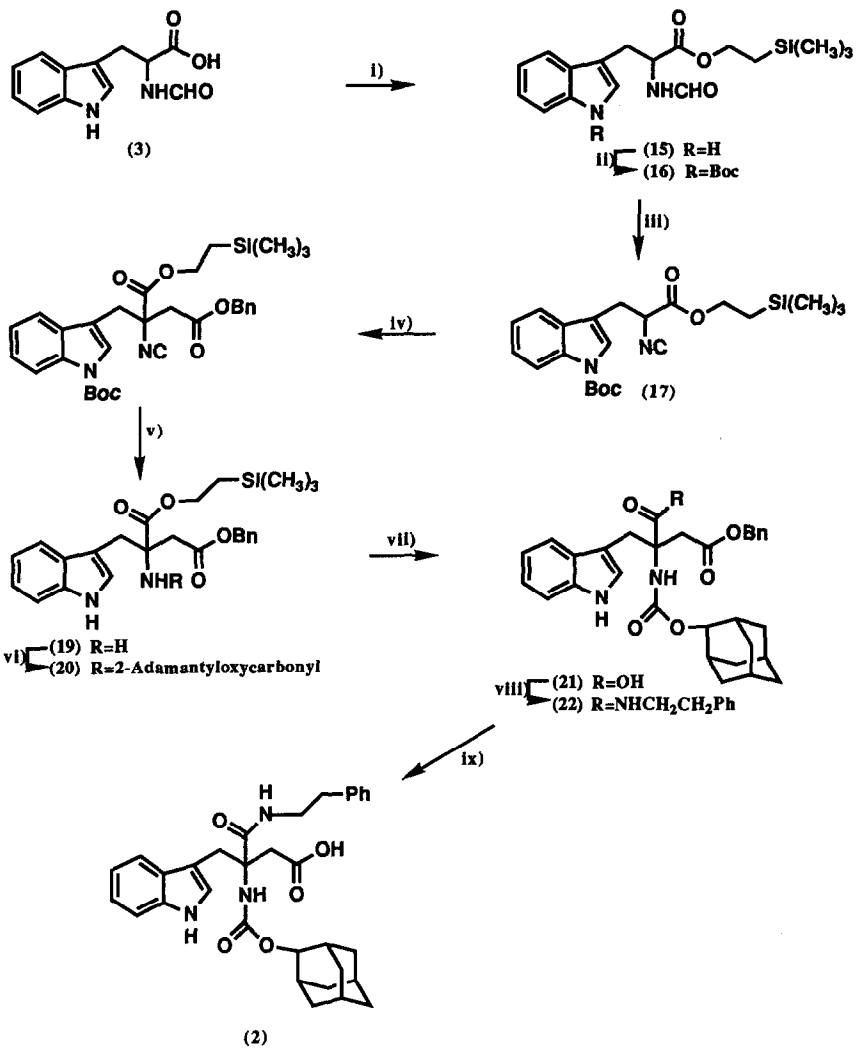
Reagents and conditions : i, Cs_2CO_3, H_2O, $PhCH_2Br$, DMF; ii, di-t-Butyldicarbonate, DMAP, DMF, 0°C; iii, Triphosgene, NEt_3, CH_2Cl_2, 0°C; iv, $BrCH_2CO_2CH_3$, HMPA, LHMDS, THF, -78°C; v, EtOH.HCl, -5°C; vi, 2-Adamantylchloroformate, NEt_3, THF, 0°C; vii, 10% Pd/C, H_2, 45psi, EtOH; viii, Pentafluorophenol, DCCI, DMAP, Phenethylamine, EtOAc; ix, 0.01M LiOH, THF/H_2O.

of the indole nitrogen using di-tert-butyldicarbonate gave (5) in 68% yield. The isonitrile (6) was readily prepared via the dehydration of the formamide moiety of (5) with triphosgene.[7,10] Subsequent alkylation[8] of (6) with methyl-2-bromoacetate gave the α-CH_2CO_2H substituted tryptophan protected derivative (7) in good yield (79%). The isonitrile moiety of (7) was hydrolysed by stirring with ethanolic HCl at room temperature under which conditions the indole N-Boc protecting group was also removed. Deprotection of the benzyl ester group by palladium catalysed reduction and subsequent coupling[11] of the pentafluorophenol active ester of (10) with phenethylamine, provided the desired amide (11). Hydrolysis of the methyl ester group of (11) with lithium hydroxide however did not give the acid (2) but resulted in the exclusive formation of a five membered cyclic imide (12). The mechanism probably involves base promoted deprotonation of the amide NH followed by intra-molecular cyclization. In order to stabilize the molecule towards basic conditions we considered the preparation of a tertiary amide (13) which could be reduced at the final step under neutral conditions to give (2) (see Scheme II). Unfortunately, reaction of the pentafluorophenol active ester of (10) with N-benzylphenethylamine gave the oxazolone (14) with no trace of the desired coupled amide (13). Presumably N-benzylphenethylamine is too sterically hindered to react with the active ester and instead promotes the formation of oxazolone (13) via enolization of the urethane and subsequent intra-molecular cyclization.



SCHEME 2

Reagents and conditions : i, Pentafluorophenol, DCCI, DMAP, N-Benzylphenethylamine, EtOAc, reflux.



SCHEME 3

Reagents and conditions : i, Trimethylsilylethanol, DCCI, DMAP, EtOAc; ii, di-t-Butyl-dicarbonate, DMAP, DMF, 0°C; iii, Triphosgene, NEt_3, CH_2Cl_2, 0°C; iv, $BrCH_2CO_2Bn$, HMPA, LHMDS, THF, -78°C; v, EtOH.HCl, -5°C; vi) 2-Adamantylchloroformate, NEt_3, THF; vii, TBAF, EtOAc, 0°C; viii, Pentafluorophenol, DCCI, DMAP, Phenethylamine, EtOAc; ix, 10% Pd/C, H_2, 45psi, EtOH.

In order to avoid the base promoted cyclic imide formation outlined in Scheme I, we followed the synthetic pathway described in Scheme III which has an ester deprotection under neutral conditions as the final step. The synthetic chemistry involved is similar to that described in Scheme I differing only in the nature of the protecting groups of the carboxyl moieties. The carboxyl group of N-formyl-D,L-tryptophan is protected as a 2-trimethylsilyl ester (15) and the alkylation step uses 2-bromobenzylacetate as the electrophile (18). Deprotection of the 2-trimethylsilyl ester is achieved with tetrabutylammonium fluoride to give (21). The final step involves a palladium catalysed reduction of the benzyl ester group (22) to give the target molecule (2) in 86% yield.

Conclusion. This synthesis further extends the scope of the isonitrile method of amino acid alkylation and provides the first example of the incorporation of an α-functionalized substituted tryptophan into analogues of neuropeptide active ligands.

Acknowledgements. We thank Dr G R Ratcliffe and Mrs L Terry for their expert technical assistance.

Experimental. Tlc (thin layer chromatography) was performed on silica gel 60_{254} plates (Merck Arb 5719); the chromatograms were viewed under u.v. light and/or developed with iodine vapour. Column chromatography was effected under pressure, using for normal phase Merck Kieselgel 60 (Merch Arb 9385 or Merck Arb 1115) and for reverse phase LiChromprep® RP-18 (Merck Art 13900).

Solvent evaporations were carried out using a Buchi rotary evaporator. Melting points were determined on either a Reichert Thermometer hot stage microscope, or a Mettler FP 800 auto mp apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 1750 Fourier transform spectrometer, as a thin film. ^{1}H NMR spectra were recorded on a Buchi AM 300 spectrometer at 300 Hz in $CDCl_3$ unless otherwise stated and chemical shifts are reported in δ parts per million downfield from tetramethylsilane. Fast atom bombardment (FAB), electron ionization (EI) and chemical ionization (CI) mass spectra were determined on a Finnegan 4500 spectrometer.

(±)-Benzyl-N-formyltryptophan (4). The formamide (3) (10.0 g, 43.1 mmol) was suspended in H_2O (100 ml). Caesium carbonate (7.70 g, 23.5 mmol) was added portion-wise to the solution. The solution was stirred until all the formamide (3) had dissolved completely. The solvent was then evaporated *in vacuo*, the residue dissolved in dimethylformamide (dry) (50 ml) and benzylbromide (7.50 g, 43.8 mmol) was added. The solution was left stirring for 4 h, ether (200 ml) added, and the solution washed with H_2O (100 ml). The

etheral layer was dried ($MgSO_4$) and evaporated *in vacuo* to yield the title compound (4) (14.3 g, ~100%), m.p. 85-86°C; ν_{max} 3294 (NH) 1739 (CO ester) and 1673 cm^{-1} (CO amide); δ 3.28 (2 H, d, *J* 7 Hz), 5.02 (3 H, m), 6.66 (1 H, d, *J* 8 Hz), 6.77 (1 H, s), 7.03-7.33 (8 H, m), 7.50 (1 H, d, *J* 7 Hz), 7.98 (1 H, s), 8.94 (1 H, s); *m/z* (CI) 323 (M+1, 100), 130 (19.8) (Found : C, 70.1; H, 5.7; N, 8.4. $C_{19}H_{18}N_2O_3$. $0.1H_2O$ requires C, 70.4; H, 5.7; N, 8.6%).

1-[(1,1-Dimethylethoxy)carbonyl]-N-formyl-DL-tryptophan benzyl ester (5). The benzyl ester (4) (8.16 g, 24.8 mmol) was suspended in dry dimethylformamide (100 ml) under an atmosphere of nitrogen. 4-Dimethylaminopyridine (ca 0.1 g) dissolved in dimethylformamide (5 ml) was injected through a septum. Di-t-butyldicarbonate (5.43 g, 24.8 mmol) in dimethylformamide (30 ml) was added dropwise. The mixture was left stirring at room temperature for 24 h. The solution was evaporated to dryness and the residue was dissolved in ether (100 ml). The etheral solution was washed with 10% citric acid, dried, filtered and evaporated to dryness. The crude product was purified by column chromatography (ethyl acetate : hexane, 3:1) to give (5) as a yellow oil (7.16 g, 68%); ν_{max} 3257 (NH) 1734 (CO ester) and 1687 cm^{-1} (CO amide); δ 1.64 (9 H, s), 3.22 (1 H, d, *J* 17 Hz), 3.24 (1 H, d, *J* 17 Hz), 5.04 (3 H, m), 6.99 (1 H, d, *J* 8 Hz), 7.15-7.32 (7 H, m), 7.41 (1 H, s), 7.49 (1 H, d, *J* 8 Hz), 8.09 (1 H, d, *J* 8 Hz), 8.14 (1 H, s); *m/z* (CI) 422 (M+1, 5.1), 321 (4.5), 130 (100), 91 (54.0) (Found : C, 67.1; H, 6.2; N, 6.4. $C_{24}H_{26}N_2O_5$. $0.33H_2O$ requires C, 67.3; H, 6.3; N, 6.5%).

Benzyl-(±)-α-cyano-1-[(1,1-dimethylethoxy)carbonyl]-1H-indole-3-propanoate (6). The Boc protected indole benzyl ester (5) (3.04 g, 7.20 mmol) was dissolved in dichloromethane (10 ml) under an atmosphere of nitrogen. The solution was cooled to 0°C in an ice-salt bath. Triethylamine (2.21 g, 21.6 mmol) was added followed by triphosgene (0.800 g, 2.40 mmol) in dichloromethane (15 ml). The solution was allowed to warm to room temperature and was left to stir for 10 h. The solvent was then evaporated off *in vacuo*, and the residue was taken up in ether. Triethylamine hydrochloride was filtered off, the filtrate concentrated *in vacuo* and the product (6) isolated by flash chromatography (hexane : ethyl acetate, 3:1) as a yellow oil (2.54 g, 87%); ν_{max} 2149 (NC) and 1735 cm^{-1} (CO); δ 1.67 (9 H, s), 3.29 (1 H, dd, *J* 15 Hz, *J* 7 Hz), 3.41 (1 H, dd, *J* 15 Hz, *J* 7 Hz), 4.60 (1 H, dd, *J* 7 Hz, *J* 7 Hz,), 5.18 (2 H, s), 7.23-7.36 (7 H, m), 7.49 (1 H, d, *J* 8 Hz), 7.57 (1 H, s), 8.15 (1 H, d, *J* 8 Hz) (Found : C, 69.9; H, 6.0; N, 6.85; $C_{24}H_{24}N_2O_4.0.5H_2O$ requires C, 69.7; H, 6.1; N, 6.8%).

Methyl-(±)-β-cyano-1-[(1,1-dimethylethoxy)carbonyl]-β-[β-[(phenylmethoxy)carbonyl]-1H-indole-3-butanoate (7). The isonitrile (6) (2.05 g, 4.94 mmol) was dissolved in THF (15 ml) and the solution cooled to -78°C under an atmosphere of argon. Hexamethylphosphourous amide (HMPA)

(0.866 ml, 4.94 mmol) was added followed by a solution of lithium bistrimethylsilylamide (1.0M, 5.30 ml). After 30 min stirring at this temperature methylbromoacetate (0.81 g, 5.31 mmol) was added slowly. After a further 3 h, the mixture was allowed to warm to room temperature and was stirred for a further 1 h. The solvent was then evaporated in vacuo, the residue dissolved in 50% water : ether mixture, and the water layer was further extracted with ether (2 x 100 ml). The combined organic extracts were dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The product (6) was isolated by flash chromatography (hexane : ether, 9:1) as a white solid (7) (1.94 g, 79%), m.p. 29-30°C; ν_{max} 2138 (NC) and 1741 cm^{-1} (CO); δ 1.58 (9 H, s), 2.72 (1 H, d, J 17 Hz), 3.13 (1 H, d, J 17 Hz), 3.20 (1 H, d, J 15 Hz), 3.29 (1 H, d, J 15 Hz), 3.54 (3 H, s), 4.99 (1 H, d, J 12 Hz), 5.03 (1 H, d, J 12 Hz), 7.07-7.28 (7 H, m), 7.42 (1 H, d, J 8 Hz), 7.54 (1 H, s), 8.05 (1 H, d, J 8 Hz) (Found : C 67.8; H, 6.0; N, 5.8; $C_{27}H_{28}N_2O_6$ requires C, 68.05; H, 5.9; N, 5.9%).

Methyl-(±)-β-[(phenylmethoxy)carbonyl]-β-[(tricyclo [3.3.1.$1^{3,7}$]dec-2-yloxy)carbonyl]amino]-1H-indole-3-butanoate (9). The isonitrile (7) (1.80 g, 3.78 mmol) was dissolved in ethanol (25 ml). The solution cooled to -5°C in an acetone-ice bath and ethanolic HCl (20 ml) was added dropwise. H_2O (1ml) was added and the reaction was warmed to room temperature. The solution was left to stir for 4 h and the solvent evaporated in vacuo. The oil was dissolved in ethylacetate (50 ml) and washed with 10% Na_2CO_3 solution (50 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated to dryness. The resulting oil was dissolved in dry THF (20 ml) under argon and triethylamine (0.53 ml, 3.80 mmol) was injected. The solution was cooled to 0°C in an ice-salt bath and 2-adamantylchloroformate[1] (0.816 g, 3.80 mmol) dissolved in THF (10 ml) was injected. The solution was stirred for 12 h at room temperature before triethylamine hydrochloride was filtered off. The filtrate was evaporated to dryness, the residue taken up in ether (25 ml) and the solution was washed with water (2 x 25 ml). The organic layer was dried ($MgSO_4$), filtered and evaporated to dryness. The crude product was purified by flash chromatography (ether : hexane, 1:1) to furnish the title compound (9) [(0.803 g, 39% from (7)], m.p. 61-62°C; ν_{max} 3412 NH and 1738 cm^{-1} (CO); δ 1.49-2.09 (14 H, m), 3.12 (1 H, d, J 15 Hz), 3.30 (1 H, d, J 15 Hz), 3.38 (3 H, s), 3.72 (1 H, d, J 15 Hz), 3.80 (1 H, d, J 15 Hz), 4.83 (1 H, br s), 4.98 (1 H, d, J 12 Hz), 5.11 (1 H, d, J 12 Hz), 6.88 (1 H, s, NH), 6.79 (1 H, s), 7.03 (1 H, t, J 7 Hz), 7.14 (1 H, t, J 7 Hz), 7.17-7.34 (6 H, m), 7.48 (1 H, d, J 8 Hz), 8.30 (1 H, s); m/z (FAB) 545 (M + 1, 5.3), 501 (4.6), 130 (100) (Found : C, 70.6; H, 6.8; N, 5.0; $C_{32}H_{36}N_2O_6$ requires C, 70.6; H, 6.7; N, 5.1%).

Methyl-(±)-β-carboxy-β-[[(tricyclo[3.3.1.$1^{3,7}$]dec-2-yloxy) carbonyl]amino]-1H-indole-3-butanoate (10). In a 250 ml glass vial the

urethane (9) (800 mg, 1.47 mmol), palladium on charcoal (10%, ca 20 mg) and ethanol (75 ml) were added. The vessel was sealed in a Parr Hydrogenation Apparatus and charged with H_2 gas (45 psi). Shaking was initiated after pressurization and continued for 12 h. Upon completion palladium on charcoal was filtered off and the filtrate evaporated to dryness. The crude product was purified by reverse phase flash chromatography (methanol : water, 2:1) to yield a white powder (10) (588 g, 88%), m.p. 108-9°C; ϑ_{max} 3413 (NH) and 1733 cm^{-1} (CO); δ 1.47-2.07 (14 H, m), 3.14 (1 H, d, J 16 Hz), 3.26 (1 H, d, J 16 Hz), 3.64 (3 H, s), 3.76 (1 H, d, J 15 Hz), 3.84 (1 H, d, J 15 Hz), 4.75 (1 H, br s), 4.83 (1 H, br s), 5.96 (1 H, s), 6.98-7.04 (2 H, m), 7.10 (1 H, t, J 7 Hz), 7.28 (1 H, d, J 8 Hz), 7.61 (1 H, d, J 8 Hz), 8.34 (1 H, s); m/z (FAB) 455 (M+1, 17.0), 411 (16.5), 217 (16.1), 135 (77.4), 130 (100) (Found : C, 65.7; H, 6.7; N, 6.0; $C_{25}H_{30}N_2O_6$ requires C, 66.1; H, 6.65; N, 6.2%).

Methyl-(±)-β-[[(2-phenylethyl)amino]carbonyl]-β-[[(tricyclo-[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]-1H-indole-3-butanoate (11). The acid (10) (0.20 mg, 0.44 mmol) was dissolved in dry THF (10 ml). Pentafluorophenol (88 mg, 0.48 mmol) was added followed by dicyclohexylcarbodiamide (100 mg, 0.48 mmol). The solution was left stirring for 2 h before phenylethylamine (60 mg, 0.50 mmol) was injected into the solution. The mixture was left stirring overnight. The solution was evaporated down to dryness, ethylacetate added and dicyclohexylurea filtered off. The filtrate was evaporated down to dryness once more and the product (11) was isolated by flash chromatography (hexane : ethyl acetate, 3:1) as a white solid (180 mg, 73%), m.p. 78-79°C; ϑ_{max} 3333 (NH), 1730 (CO) and 1659 cm^{-1} (CO amide); δ 1.51-2.04 (14 H, m), 2.61 (2 H, m), 2.94 (1 H, d, J 16 Hz), 3.21 (1 H, d, J 16 Hz), 3.37 (1 H, d, J 7 Hz), 3.41 (1 H, d, J 7 Hz), 3.46 (1 H, d, J 15 Hz), 3.57 (1 H, d, J 15 Hz), 3.62 (3 H, s), 4.78 (1 H, br s), 5.88 (1 H, br s), 6.58 (1 H, br s), 6.92 (1 H, d, J 2 Hz), 7.03-7.26 (7 H, m), 7.33 (1 H, d, J 8 Hz), 7.56 (1 H, d, J 8 Hz); m/z (FAB). 558 (M+1, 13.5), 362 (20.9), 331 (15.9), 231 (17.2), 135 (100), 130 (75.7), 105 (36.2) (Found : C, 69.0; H, 6.8; N, 7.2; $C_{33}H_{39}N_3O_5 . 0.75H_2O$ requires C, 69.4; H, 7.1; N, 7.4%).

Tricyclo[3.3.1.1.^{3,7}]dec-2-yl-(±)-[3-(1H-indol-3-ylmethyl)-2,5-dioxo-1-(2-phenylethyl)-3-pyrrolidinyl]carbamate (12). The ester (11) (110 mg, 0.20 mmol) was dissolved in THF (20 ml) and cooled to 0°C. Lithium hydroxide (21 ml, 0.01 M) was added dropwise to the solution over a 3 h period. The solution was kept stirring for a further 1 h and then allowed to warm to room temperature. Hydrochloric acid (2.1 ml, 0.1 M) was added and the aqueous solution extracted with ethylacetate (3x20 ml). The organic layers were combined, dried over $MgSO_4$ filtered and evaporated to dryness, to afford the crude product. The pure product (12) was isolated by reverse phase flash

chromatography (methanol : water, 4:1) as a white powder (84 mg, 78.5%), m.p. 157-9°C; ν_{max} 3347 (NH), 2912 (CH); 1781 (CO) and 1701 (CO); δ 1.55-1.97 (14 H, m), 2.38 (2 H, m), 3.00 (1 H, d, J 18 Hz), 3.05 (1 H, d, J 15 Hz), 3.47 (2 H, t, J 8 Hz), 4.80 (1 H, s), 5.49 (1 H, s), 7.04-7.35 (8 H, m), 7.56 (1 H, d, J 8 Hz), 8.68 (1 H, s); m/z (CI) 528 (M+1, (15.5), 527 (35.5), 526 (100), 374 (67.7), 331 (44.2), 130 (35.5) (Found : C, 72.6; H, 6.7; N, 7.9; $C_{32}H_{35}N_3O_4.0.25H_2O$ requires C, 72.5; H, 6.7; N, 7.9%).

Methyl-(±)-4,5-dihydro-4-(1H-indole-3-ylmethyl)-5-oxo-2-(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)-4-oxazolonacetate (14). The acid (10) (220mg, 0.44 mmol) was dissolved in dry THF (10 ml). Pentafluorophenol (88 mg, 0.48 mmol) was added followed by dicyclohexylcarbodiamide (100 mg, 0.48 mmol). The solution was left stirring for 2 h before N-benzyl-phenylethylamine (80 mg, 0.51 mmol) was injected into the solution. The solution was refluxed for 24 h, cooled and dicyclohexylurea filtered off. The filtrate was evaporated to dryness and the product (12) was isolated by flash chromatography (hexane : ethyl acetate, 3:1) to give a white solid (120 mg, 62.5%), m.p. 231-2°C; ν_{max} 3652 (NH); 3191 (Ar-H); 2909; 1832 (CO oxazolone); 1737 (CO ester) and 1667 cm^{-1} (CO urethane); δ 1.43-2.04 (14 H, m), 3.01 (1 H, d, J 16 Hz), 3.10 (1 H, d, J 16 Hz), 3.19 (1 H, d, J 14 Hz), 3.23 (1 H, d, J 14 Hz), 3.64 (3 H, s), 4.72 (1 H, m), 7.03-7.16 (3 H, m), 7.31 (1 H, d, J 8 Hz), 7.62 (1 H, d, J 8 Hz), 8.48 (1 H, s, NH); m/z (CI) 437 (M+1, (100)), 130 (81.1) (Found : C, 68,85; H, 6.5, N, 6.4; $C_{25}H_{28}N_2O_5$ requires C, 68.8; H, 6.5; N, 6.3%).

(±)-2-(Trimethylsilyl)ethyl-N-formyltryptophan (15). To a suspension of the formamide (3) (10.0 g, 43.1 mmol) in ethyl acetate (80 ml), 4-dimethyl-aminopyridine (ca ~10 mg) and 2-trimethylsilyl ethanol (5.10 g, 43.1 mmol) was added. Dicyclohexylcarbodiamide (8.90 g, 43.1 mmol) was added portion wise to the reaction mixture. The mixture was left stirring at room temperature for 12 h before dicyclohexylurea was filtered off. The solution was washed with HCl (2x50 ml) and then brine (2x50 ml). The organic phase was separated, dried ($MgSO_4$) and evaporated to dryness. The crude product was purified by flash chromatography (ether : hexane, 2:1) to give the title compound (15) as a clear oil (11.7 g, 82%); ν_{max} 3368 (NH), 2953 (CH), 1733 (CO ester) and 1674 cm^{-1} (CO amide); δ 0.03 (9 H, s), 0.90 (2 H, t, J 8 Hz), 3.32 (2 H, m), 4.13 (2 H, m), 4.94 (1 H, m), 6.12 (1 H, br d, J 8 Hz), 6.97 (1 H, d, J 2 Hz), 7.05 - 7.19 (2 H, m), 7.31 (1 H, d, J 8 Hz), 7.52 (1 H, d, J 8 Hz), 8.12 (1 H, s), 8.18 (1 H, s); m/z (FAB) 332 (49), 305 (87), 187 (82), 130 (100) (Found : C, 60.6; H, 7.2; N, 8.3; $C_{17}H_{24}N_2O_3Si.\ 0.25H_2O$ requires C, 60.6; H, 7.2; 8.3%).

1-[(1,1-Dimethylethoxy)carbonyl]-N-formyl-DL-tryptophan-2-(trimethylsilyl)ethyl ester (16). Prepared in a similar manner to (5) except using (15). A yellow oil (10.1 g, 85%); ν_{max} 3330 (NH), 1734 (CO ester) and

1668 cm^{-1} (CO amide); δ 0.03 (9 H, s), 0.95 (2 H, m), 1.64 (9 H, s), 3.26 (2 H, m), 4.15 (2 H, m), 4.95 (1 H, m), 6.15 (1 H, br d), 7.17-7.31 (2 H, m), 7.38 (1 H, s), 7.48 (1 H, d, J 8 Hz), 8.08 (1 H, d, J 8 Hz), 8.18 (1 H, s); m/z (EI) 432 (9.9), 349 (15.2), 305 (20.9), 215 (17.0), 130 (100) (Found : C, 60.3; H, 7.4; N, 6.4; $C_{22}H_{32}N_2O_5Si$. $0.25H_2O$ requires C, 60.45; H, 7.4; N, 6.4%).

2-(Trimethylsilyl)ethyl-(±)-α-cyano-1-[(1,1-dimethylethoxy) carbonyl]-1H-indole-3-propanoate (17). Prepared in an similar manner to (6) except using (16). A yellow oil (8.91 g, 93%); ν_{max} 2956 (CH), 2147 (NC), 1734 (CO ester) and 1668 cm^{-1} (CO amide); δ 0.03 (9 H, s), 0.96 (2 H, m), 1.68 (9 H, s), 3.28 (1 H, dd, J 15, 5 Hz), 3.39 (1 H, dd, J 15, 8 Hz), 4.29 (2 H, m), 4.53 (1 H, dd, J 8, 5 Hz), 7.26 (1 H, t, J 8 Hz), 7.34 (1 H, t, J 8 Hz), 7.53 (1 H, d, J 8 Hz), 7.59 (1 H, s), 8.16 (1 H, d, J 8 Hz); m/z (FAB) 414 (31.5), 358 (100), 331 (50.2), 304 (45.6), 260 (39.9) (Found : C, 63.2; H, 7.3; N, 6.7; $C_{22}H_{30}N_2O_4Si$. $0.25H_2O$ requires C, 63.05; H, 7.3; N, 6.7%).

Phenylmethyl-(±)-β-cyano-[(1,1-dimethylethoxy)carbonyl]-β-[β-2-(trimethylsilyl) ethoxycarbonyl]-1H-indole-3-butanoate (17). Prepared in a similar manner to (7) except using (18). A yellow oil (2.27 g, 89%); ν_{max} 2137 (NC) and 1731 cm^{-1} (CO amide); δ 0.09 (9 H, s), 0.78 (2 H, m), 1.71 (9 H, s), 2.95 (1 H, d, J 16 Hz), 3.29 (1 H, d, J 16 Hz), 3.30 (1 H, d, J 14 Hz), 3.40 (1 H, d, J 14 Hz), 4.09 (2 H, m), 5.18 (2 H, s), 7.39-7.22 *7 H, m), 7.52 (1 H, d, J 8 Hz), 7.67 (1 H, s), 8.17 (1 H, d, J 8 Hz); m/z (FAB) 562 (14), 507 (44), 220 (73), 174 (53), 130 (100) (Found : C, 66.0; H, 6.7; N, 4.9; $C_{31}H_{38}N_2O_6Si$ requires C, 66.2; H, 6.8; N, 5.0%).

Benzyl-(±)-β-[[2-(trimethylsilyl)ethoxy]carbonyl]-β-[(tricyclo [3.3.1.$1^{3,7}$]dec-2-yloxy)carbonyl]amino]-1H-3-butanoate (20). Prepared in a similar manner to (9) except using (18). A white solid [1.20 g, 51% from (17)], m.p. 40-2°C; ν_{max} 3410 (NH) and 1738 cm^{-1} (CO); δ 0.09 (9 H, s), 0.88 (2 H, m), 1.57-2.05 (14 H, m), 3.08 (1 H, d, J 15 Hz), 3.28 (1 H, d, J 15 Hz), 3.68 (1 H, d, J 15 Hz), 3.82 (1 H, d, J 15 Hz), 4.08 (2 H, m), 4.83 (1 H, s), 5.12 (2 H, d, J 13 Hz), 5.91 (1 H, s), 6.76 (1 H, s), 7.03 (1 H, t, J 7 Hz), 7.14 (1 H, t, J 7 Hz), 7.25-7.34 (6 H, m), 7.49 (1 H, d, J 7.5 Hz), 7.96 (1 H, s, NH); m/z (FAB) 632 (3.5), 560 (2.4), 435 (2.3), 130 (100) (Found : C, 68.7; H, 7.4; N, 4.4; $C_{36}H_{46}N_2O_6Si$ requires C, 68.5; H, 7.3; N, 4.4%).

Benzyl-(±)-β-carboxyl-β-[[(tricyclo[3.3.1.$1^{3,7}$]dec-2-yloxy)carbonyl]amino]-1H-indole-3-butanoate (21). The TMS-ethyl ester (19) (1.10 g, 1.82 mmol) was dissolved in ethyl acetate (10 ml) and chilled in an ice-salt bath. Tetrabutylammonium fluoride (3.64 ml, 1.0M) was added dropwise to the stirred solution. The reaction mixture was allowed to warm to room temperature and was left to stir for 3 h. The solvent was partially evaporated down, ethyl acetate (20 ml) added and the solution was washed with

10% citric acid (2x20 ml) and then water (2x20 ml). The solution was dried ($MgSO_4$), filtered and evaporated to dryness to give a white foam. The acid (20) was purified by recrystallization from carbon tetrachloride to give white crystals (0.84 g, 89%), m.p. 72-4°C; ν_{max} 3412 (NH) and 1728 cm^{-1} (CO); δ (DMSO) 1.44-1.94 (14 H, m), 3.35-3.08 (4 H, m), 4.67 (1 H, s), 5.09 (2 H, m), 6.46 (1 H, s), 6.90-7.33 (9 H, m), 7.45 (1 H, d, J 7 Hz), 11.93 (1 H, s, NH), 13.12 (1 H, br s); m/z (FAB) 532 (0.4), 531 (1.2), 487 (0.5), 323 (5.0), 217 (94.8), 109 (100) (Found : C, 68.7; H, 6.4; N, 5.1; $C_{31}H_{34}N_2O_6$. $0.5H_2O$ requires C, 69.0; H, 6.5; N, 5.2%).

Benzyl-(±)-β-[[(2-pheneylethyl)amino]carbonyl]-β-[[(tricyclo [3.3.1.$1^{3,7}$]dec-2-yloxy)carbonyl]amino-1H-indole-3-butanoate (22). Prepared in a similar manner to (11) except using (21). A white solid (0.707 g, 74%), m.p. 56-8°C; ν_{max} 3389 (NH), 2912 (CH), 1704 (CO ester, urethane) and 1659 cm^{-1} (CO amide); δ 1.48-1.94 (14 H, m), 2.56 (2 H, m), 3.00 (1 H, d, J 16 Hz), 3.25 (1 H, d, J 16 Hz), 3.35 (2 H, m), 3.45 (1 H, d, J 14 Hz), 3.55 (1 H, d, J 14 Hz), 4.75 (1 H, s), 5.07 (2 H, s), 5.75 (1 H br s), 6.45 (1 H, br s), 6.90 (1 H, d, J 2 Hz),7.01-7.34 (14 H, m), 7.52 (1 H, d, J 8 Hz), 8.04 (1 H, s, NH); m/z (FAB) 634 (24.1), 217 (90), 109 (100) (Found : C, 73.8; H, 7.0; N, 6.6; $C_{39}H_{43}N_3O_5$ requires C, 73.9; H, 6.8; N, 6.6%).

(±)-β-[[2-Phenylethyl)amino]carbonyl]-β-[[tricyclo [3.3.1.$1^{3,7}$]dec-2-yloxy)carbonyl]amino]-1H-indole-3-butanoic acid (2). In a 250 ml glass vial, the benzyl ester (21) (500 mg, 0.789 mmol), palladium on charcoal (10%, ca 10 mg) and ethanol (75 ml) were added. The vessel was sealed in a Parr Hydrogenation Apparatus and charged with H_2 gas (45 psi). Shaking was initiated after pressurization and continued for 3 h. Upon completion, palladium on charcoal was filtered off and the filtrate was evaporated to dryness. The product was isolated by reverse phase flash chromatography (methanol : water, 2:1) and purified by recrystallization from acetonitrile to yield white crystals (2) (370 mg, 86%), m.p. 157-9°C; ν_{max} 3348 (NH), 2928 (CH), 1706 (CO acid + urethane) and 1659 cm^{-1} (CO amide); δ (DMSO) 1.50-2.03 (14 H, m), 2.53 (1 H, d, J 15 Hz), 2.61 (2 H, m), 2.77 (1 H, br d), 3.28 (2 H, m), 3.41 (1 H, d, J 14.5 Hz), 3.52 (1 H, d, J 14.5 Hz), 4.67 (1 H, s), 6.84-7.29 (12 H, m), 7.44 (1 H, d, J 8 Hz), 9.00 (1 H, br s), 10.82 (1 H, s); m/z (FAB) 544.2811 (M + 1, 34%; $C_{32}H_{38}N_3O_5$ requires M + 1, 544.28115), 130 (100) (Found : C, 69.8; H, 7.3; N, 7.0; $C_{32}H_{38}N_3O_5$. $0.5H_2O$ requires C, 69.5; H, 6.9; N, 7.6).

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