Tetrahydro-pyrrolo-[2,3-b]indole-1,2,8-tricarboxylic Acid Ester in the Enantiospecific Preparation of α-Methyltryptophan: Application in the Preparation of Carbon-14 Labeled PD 145942 and PD 154075

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Summary

[2R-(2α, 3aβ, 8aβ)]-2,3,3a,8a-Tetrahydro-pyrrolo[2.3-b]indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester, its [2S-(2β, 3aα, 8aα)]-isomer, and the tribenzyl ester analogs were prepared. From these [2,3-b]indole-1,2,8-tricarboxylic acid esters we accomplished a simple, high yielding preparation of enantiopure α-methyltryptophan and methyl ester derivatives. Using this protocol, we inexpensively made (R)-α-[^{14}C]methyltryptophan methyl ester, and in subsequent reactions converted it into [1-(2-hydroxy-cyclohexylcarbamoyl)-2-(1H-indol-3-yl)-1-[^{14}C]methyl-ethyl]carbamic acid adamantan-2-yl ester (PD 145942) and [2-(1H-indole-3-yl)-1-[^{14}C]methyl-1(1-phenyl-ethylcarbamoyl)-ethyl]carbamic acid benzofuran-2-yl methyl ester (PD 154075). Both of these compounds are drug candidates in preclinical study for the treatment of anxiety and emesis respectively.

Keywords: *NK_1 receptor antagonist, CCK-B receptor antagonist, emesis, anxiety, enantioselective synthesis,(R)-α-[^{14}C]methyltryptophan methyl ester, [2,3-b]indole-1,2,8-tricarboxylic acid ester.*

Introduction

[1-(2-Hydroxy-cyclohexylcarbamoyl)-2-(1H-indol-3-yl)-1methyl-ethyl]carbamic acid adamantan-2-yl ester (PD 145942) is a CCK-B receptor antagonist, and it is being studied as an anti-anxiety drug candidate.[1] The structurally similar compound [R-(R*,S*)][2-(1H-indole-3-yl)-1-methyl-1(1-phenyl-ethylcarbamoyl)-ethyl]carbamic acid benzofuran-2-yl methyl ester (PD 154075) (Figure 1) is a selective, and specific high affinity NK_1 receptor antagonist.[2] PD 154075 is in preclinical evaluation for the treatment of emesis (nausea and vomiting) induced by chemotherapy and other emetogens. Radioisotope labeled forms of both compounds were requested for metabolic and pharmacokinetics investigations, and we carried out the synthetic studies described in this paper to provide these compounds. In earlier work both compounds were prepared from (R)-α-methyltryptophan methyl ester.[1,2] In our view, the radio-enantioselective preparation of (R)-α-[^{14}C]methyltryptophan methyl ester presented the most important added challenge to the synthesis of

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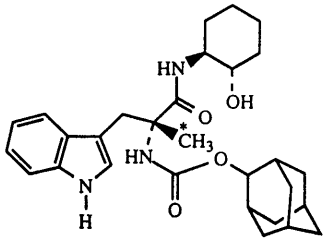
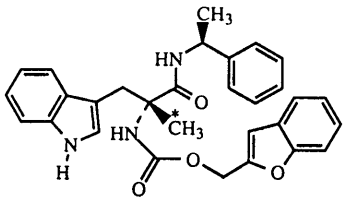
these compounds by the known sequence. As a consequence, we sought a preparation of (R)-α-[^{14}C]methyltryptophan methyl ester that would be sufficiently practical, high yielding and cost effective. In this paper we wish to describe a simple, enantiospecific and high yielding route to (S)- and (R)-α-methyltryptophan and the methyl ester derivatives.



PD 145942



PD 154075

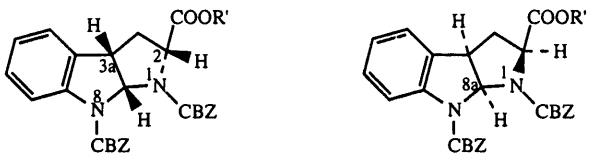
Figure 1

The present [3] sequence to (R)-α-methyl tryptophan methyl ester facilitated the synthesis of carbon-14 labelled PD 145942 and PD 154075 from a smaller quantity of ^{14}C-methyl iodide than could have been possible by reported methods .

Results and Discussion

We chose to first explore the synthesis of (R)-α-[^{14}C]methyltryptophan methyl ester (7) which was considered to be crucial to a cost managed preparation of PD 145942 and PD 154075. A number of preparative methods applicable to (R)-α-[^{14}C]methyltryptophan methyl ester have been reported over the years. [4] The more recent preparations are improvements on the earlier method involving the deprotonation of N-benzyliden-tryptophan methyl ester, alkylation with methyl iodide and deprotection. This method gives a mixture of enantiomers from which in our case, the desired (R)-enantiomer may be separated as the ester after the (S)-enantiomer has been selectively converted to the free acid by enzyme (chymotrypsin) action. [5] In order to avoid the loss of at least one-half the radioactivity in a single step as would be the result from these methods, we opted to examine the more recent reactions that are potentially enantiospecific. [6] [2R-(2α, 3aβ, 8aβ)]-2,3,3a,8a-Tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester (4), its (2S)-isomer (11) and their respective 1,2,8-tribenzyl ester analogs, (17) and (23), were selected for study as 'cold' precursors from which α-methyltryptophan methyl ester and α-methyltryptophan could be prepared.

The deprotonation of (4) was expected to be followed by a diastereofacially selective alkylation with methyl iodide. Subsequent transformations would result overall, in the enantiospecfic preparation of (R)-α-methyltryptophan methyl ester. There is precedence for these reactions in dimethyl 2(S), 3a(R),8a(S)-(+)-hexahydro-8-(phenylsulfonyl)pyrrolo[2,3-b]indole-1,2-dicarboxylate, [6] a compound that is structurally similar to (4). According to these reports, the alkylation of dimethyl 2(S), 3a(R),8a(S)-(+)-hexahydro-8-(phenylsulfonyl)pyrrolo[2,3-b]indole-1,2-dicarboxylate is followed by decyclization to the fully protected (S)-α-methyltryptophan methyl ester.



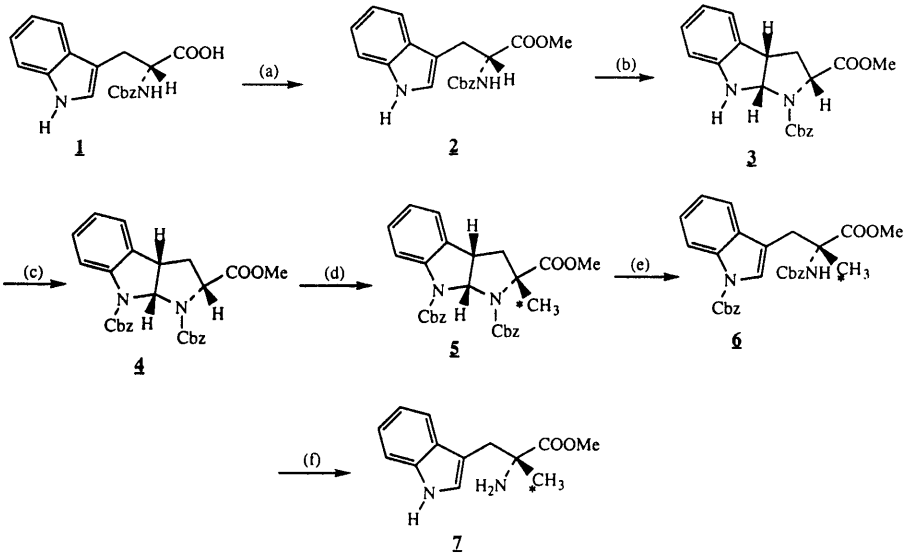
(2R) (4) R'= Me (17) R'= Bn (2S) (11) R'= Me (23) R'= Bn

Figure 2

Sequential desulfonylation and removal of N_α-carbomethoxy protection gives the (S)-α-methyltryptophan methyl ester. The desulfonylation protocol uses sodium in liquid ammonia, and the yield is variable depending on the dryness of the reaction condition. We felt that such a testy step could severely limit the efficiency of the radiolabelled synthesis. Furthermore, the harsh reaction conditions such as refluxing in 6 N hydrochloric acid or 6 N potassium hydroxide overnight, or a reaction with conc. hydroiodic acid in a sealed tube at 210 °C that is required to remove the N_α-carbomethoxy protection would be an additional drawback with this synthon. In designing [2R-(2α, 3aβ, 8aβ)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester (4) we wanted to avoid these difficulties. We replaced the N_8-phenylsulfonyl and N_α-carbomethoxy protection in dimethyl 2(S),3a(R),8a(S)-(+)-hexahydro-8-(phenylsulfonyl)pyrrolo[2,3-b]indole-1,2-dicarboxylate with Cbz to give (4). It was planned that these changes would facilitate the preparation of (R)-α-methyltryptophan methyl ester (7) by exploiting the more practical and mild catalytic hydrogenolysis of Cbz groups in compound (6). The

workup of the hydrogenolysis reaction would be a mere filtration to remove spent catalyst, evaporation of solvent to isolate product , and a resultant improved yield of (7).

Scheme 1

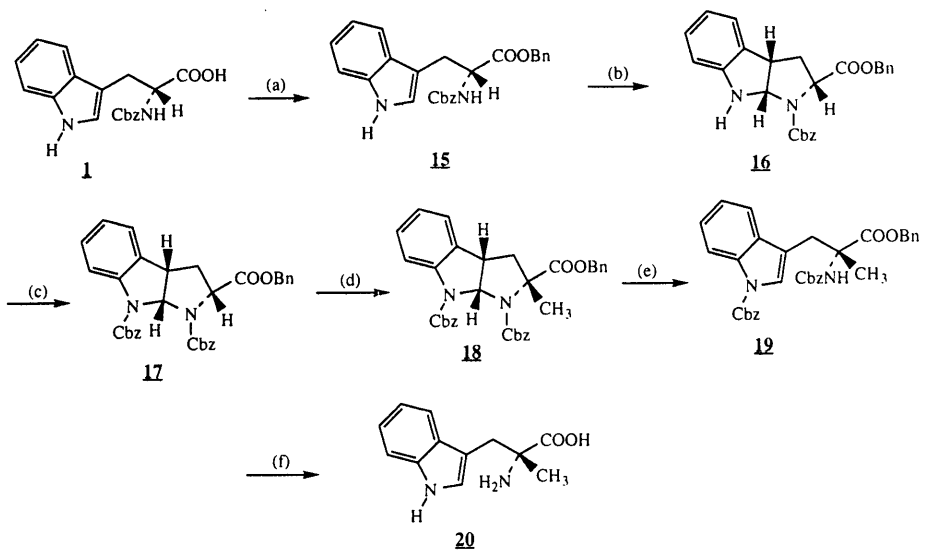


Reagents:- (a) CH_2N_2, Ether-CH_2Cl_2, RT (b) CF_3COOH, 4 days, RT
(c) Na_2CO_3, 10 % aq. Dioxane, $C_6H_5CH_2OCOCl$, 4 hr, 0 - 5 °C (d) LHMDS, THF, $*CH_3I$, -78 - RT
(e) CF_3COOH, 1hr, RT (f) 10 % Pd/C, H_2, EtOH

As shown in scheme 1, we prepared [2R-(2α, 3aβ, 8aβ)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester (4)[7] from the amino acid N_α-Cbz-D-tryptophan. The methyl ester derivative (2) of N_α-Cbz-D-tryptophan was made by a reaction with diazomethane. By stirring (2) in trifluoroacetic acid for four days it was cyclized to [2R-(2α, 3aβ, 8aβ,)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2-dicarboxylic acid-1-benzyl ester 2-methyl ester (3). Three products were indicated by tlc in this conversion, and the desired (3) was chromatographically least polar and major component by our analytical system. After the compound (3) was separated, it was immediately reacted with benzyl chloroformate to make the N_8 - pyrrolo-indole Cbz derivative (4). The reaction was best carried out in aqueous dioxane at 0 - 5 °C in the presence of sodium carbonate, and the resulting compound was satisfactorily purified on neutral

alumina column to give (4). From N_α-Cbz-L-tryptophan (8) by a reaction sequence comparable to Scheme 1 and a similar purification method the [2S-(2β, 3aα, 8aα)]-isomer (11) was synthesized. The tribenzyl ester analogs of these compounds were also made. Thus the reaction of N_α-Cbz-D-tryptophan (1) with benzyl bromide in the presence of cesium carbonate , illustrated in Scheme 2, furnished N_α-Cbz-D-tryptophan benzyl ester from which in turn the [2R-(2α, 3aβ, 8aβ)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,2,8-tribenzyl ester (17) was prepared. The [2S-(2β, 3aα, 8aα)]-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,2,8-tribenzyl ester (23) was synthesized from N_α-Cbz-L-tryptophan (8) in a sequence similar to the preparation of (17).

Scheme 2



1 15 16 17 18 19 20

Reagents:- (a) Cs_2CO_3, $C_6H_5CH_2Br$, DMF, RT (b) CF_3COOH, 4 days, RT
(c) Na_2CO_3, 10 % aq. Dioxane, $C_6H_5CH_2OCOCl$, 4 hr, 0 - 5 °C (d) LHMDS, THF, *CH_3I, -78 - RT
(e) CF_3COOH, 1hr, RT (f) 10 % Pd/C, H_2, EtOH

We examined the deprotonation of (4) with lithium diisopropylamide (LDA), and the enolate so prepared reacted with methyl iodide to give the desired product (5) along with a significant (5 - 10 %) amount of starting compound (4). On the other hand, Scheme 1, the deprotonation of (4) with lithium

bis(trimethylsilyl)amide consistently resulted in near quantitative yield of (5) upon reaction with methyl iodide. It was not neccessary to purify (5) before decyclization by stirring for 1 hr in trifluoroacetic acid to the fully protected (R)-α-methyltryptophan (6). After the solvent was removed by evaporation and azeotrope with toluene, the Cbz groups were hydrogenolyzed in absolute ethanol containing 10 % Pd/C to afford pure (R)-α-methyltryptophan methyl ester (7) in excellent yield (94 %) based on methyl iodide. In the preliminary experiments to establish the feasibility of this sequence the alkylation, decyclization and hydrogenolysis of pyrrolo[2,3-b]indole-1,2,8-tribenzyl ester (17) , Scheme 2, and its isomer (23) directly furnished the (2R)- (20) and (2S)-α-methyltryptophan (26).[9]

These initial explorations proved helpful in chiral hplc evaluation of the purity of (4), and the

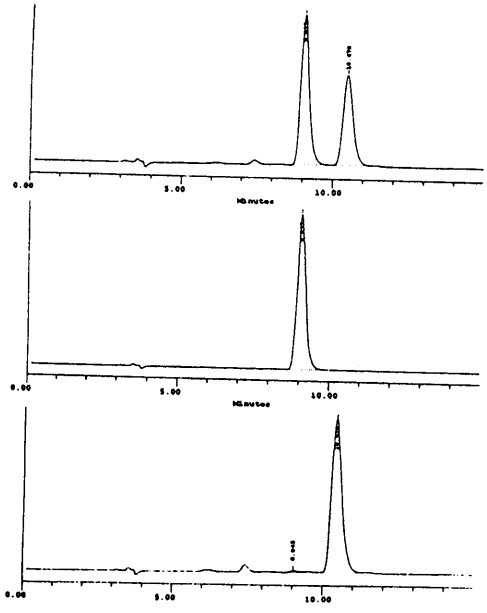


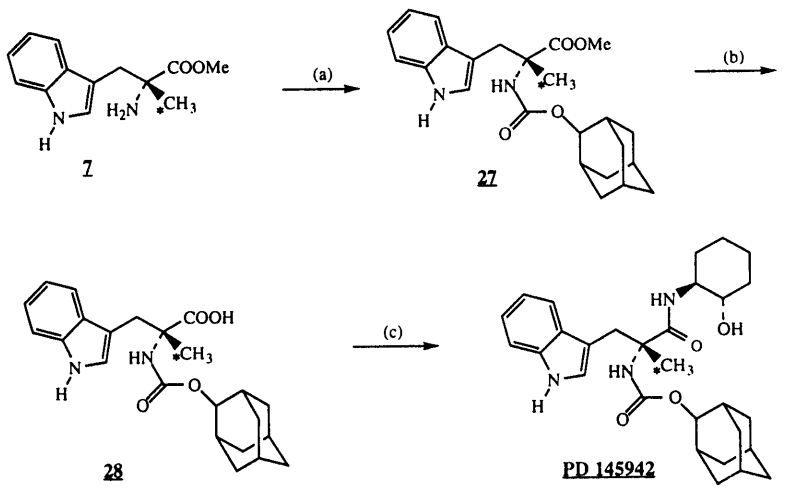
figure 3. Chiral purity determination by chiral HPLC: top: mixture of compound (4) and its (2S)-compound (11); mid: compound (4), 100% chiral purity; bottom: compound (11), 99.50% chiral purity. Chiral HPLC Conditions:Diacel Chiralpak AS, 4.6x250mm at 40 °C; Hexanes:IPA:DEA 82:18:0.1; flow rate: 1.0 mL/min; UV detection: 240 nm

Figure 3

product derived therefrom by alkylation with methyl iodide. By hplc we confirmed homogeneity of our starting compounds (4), (11), (17) and (23), and characterized the reaction products from the

alkylation reaction step. Reverse phase hplc was used to establish the absence of chemical contaminants, and by chiral hplc as shown with the chromatograms of (4) and its (2S)-compound (11), Figure 3, we determined the chiral purity for each compound to be greater than 99%. It is instructive to note that the requirement to protect the carboxylic acid group of (R)-α-methyltryptophan (20) prior to the carbamoylation reaction step dictated that compound (4) rather than the [2R-(2α, 3aβ, 8aβ)]-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,2,8-tribenzyl ester (17) be used as starting compound in the labeled synthesis. The excellent yields (>94 %) of α-methyltryptophan (7), (14),

Scheme 3



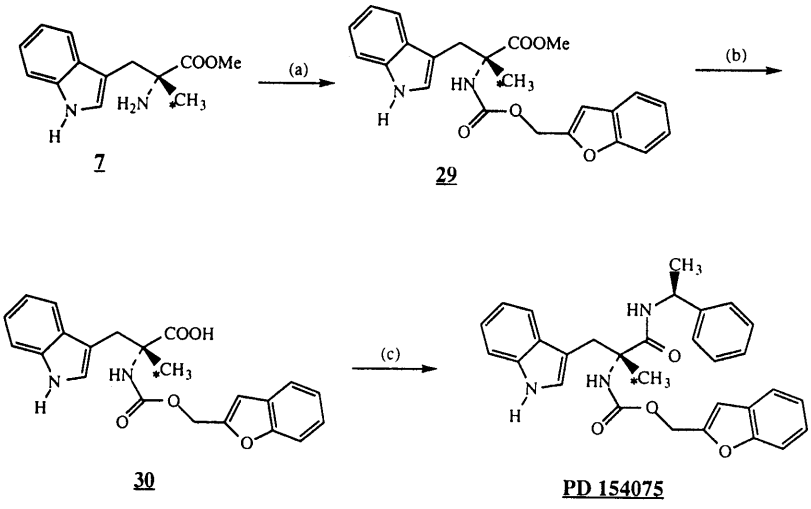
Reagents:- (a) Na_2CO_3, Adamantanyl chloroformate, aq. Dioxane, O°C, 3 hr.(b) LiOH, aq. THF, reflux, 3 days (c) HOBt, N-Methylmorpholin, EDC.HCl, 1S,2S-(+)-trans-Aminocyclohexanol, DMAP, overnight, RT.

(20) and (26) from these indole-tricarboxylic esters, and the minimum purification required to isolate products in the present sequence are noteworthy, and represent important improvements on published procedures. We were now prepared to make PD 145942 and PD 154075.

We made some minor modifications to the sequence developed by earlier workers and achieved the synthesis of PD 145942 as shown in scheme 3.[1] (R)-α-[^{14}C]Methyltryptophan methyl ester (7) was reacted with 2-adamantanyl chloroformate in 10 % aqueous dioxane containing Na_2CO_3 to give compound (27) in near quantitative yield. Hydrolysis of the methyl ester was expectedly slow, but

afforded the compound (28) which reacted with (1S,2S)-(+)-2-trans-aminocyclohexanol [8] to make target ^{14}C-labelled PD 145942 . As in peptide synthesis, either dicyclohexylcarbodiimide (DCC) or 1-(3-dimethylaminopropyl)-3-ethylcarbodiiimide hydrochloride (EDC.HCl) may be employed to couple these synthons and make PD 145942. We obtained a product contaminated with dicylohexylurea (DCU) when we employed DCC in the coupling reaction , and the removal of this contaminant proved tedious and costly. A repeat of this step using to 1-(3-dimethylaminopropyl)-3-ethylcarbodiiimide hydrochloride (EDC.HCl) gave a superior quality compound and in a vastly improved yield of 67 %.

Scheme 4



Reagents:- (a) Benzo[b]furan-2-yl methyl-4-nitrophenyl carbonate, DMAP, DMF, overnight, RT
(b) LiOH, aq. MeOH, RT, 6 days (c) HOBt, N-MM EDC.HCl, (S)-(-)-methylbenzylamine, DMF

By the similar reaction sequence of carbamoylation, hydrolysis of ester to acid, and amidation we also achieved the preparation of PD 154075. The subtle differences in the reaction steps are highlighted in the scheme 4. For example, in this sequence compound (29) was prepared in dimethylformamide at room temperature by the reaction of (R)-α-methyltryptophan methyl ester with benzo[b]furan-2-yl-methyl-4-nitrophenyl carbonate[8] in the presence of 22 mol. % of 4-DMAP. After a column purification to remove the 4-nitrophenol by-product, compound (29) was carefully hydrolyzed in six days at room temperature to afford compound (30) in 82 % yield. It is to be noted that the

benzo[b]furan-2-ylmethoxy moiety was found to be relatively more labile than the 2-adamantoxy-group in the preparation of PD 145942. Amidation of compound (30) with (S)-(-)-α-methylbenzylamine was accomplished by a coupling reaction in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride to give (13) in 68 % purified yield.

In conclusion, we achieved the synthesis of a number of [2,3-b]indole-1,2,8-tricarboxylic acid esters and have employed these compounds in a high yielding enantiospecific synthesis of α-methyltryptophan and the methyl ester derivatives. By this approach we accomplished a radioenantiospecific synthesis (R)-[^{14}C]methyltryptophan methyl ester, which in turn enabled us to prepare the radiolabelled versions of the drug candidates PD 145942 and PD 154075 in a cost effective manner.

Experimental

General Methods.

All reactions were carried out under an atmosphere of argon unless otherwise stated. Proton NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer. Proton chemical shifts (∂) are reported in ppm downfield from (TMS), and ^{13}C resonances were recorded using 77.0 ppm $CDCl_3$ resonanace of solvent as internal standard reference and are reported in ppm down field from TMS. MS and HRMS were performed on Finnigan MAT 900Q (Bremen, Germany), ESI , positive ion mode. Radiochemical purity of all the labeled compounds were determined by tlc radiochromatogram with Bioscan 200 imaging scanner. Carbon-14 labeled methyl iodide was purchased from Chemsyn Science Laboratories Lenexa, KS 66215, and diluted to required activity. Radiochemical counting was performed on a Packard 574 liquid scintillation counter using Beckman Readi-Solv MP cocktail. HPLC analyses of final products were performed on a Water Associates 600E solvent delivery system with on line PDA 996 photodiode array detector and an IN/US β-RAM radioactivity flow detector. Purifications were by column chromatography on a Merck Kieselgel 60 (230μ) or by flash column chromatography on Biotage Flash 40 System.

[2R-(2α, 3aβ, 8aβ,)]-2,3,3a,8a-Tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester (4)

An ethereal solution of diazomethane {generated from Diazald (23 g, 107.36 mmol), potassium hydroxide (10 g, 178.22 mmol) and 95 % ethanol (50 mL) water (15 mL) and ether (500 mL)} was

added slowly to a solution of N_α-Cbz-D-tryptophan (1) (20.0 g, 51.9 mmol) in ether-methylene chloride (600-60 mL, v/v). Upon the attainment of permanent yellowish coloration, addition was stopped and tlc (50 % ether in hexane, v/v) examination of the reaction mixture showed complete conversion. Trifluoroacetic acid was added dropwise to destroy the excess diazomethane as judged by the complete discharge of the color. It was concentrated to a viscous liquid product (2), azeotroped with toluene, dried on a vacuum pump, and stirred in trifluoroacetic acid (150 mL) at room temperature. After 4 days, the greenish trifluoroacetic acid solution was added slowly to a vigorously stirred mixture of 15 % aqueous Na_2CO_3 solution (2000 mL) and CH_2Cl_2 (600 mL) maintained at 0 °C temperature. The organic layer was separated and the aqueous portion was extracted with methylene chloride (2 X 300 mL). The combined extract was washed with 15 % Na_2CO_3 solution, brine and dried on sodium sulfate. Three components were indicated by tlc (ether : hexane 1:1 v/v), the desired (3) being the major and chromatographically fast moving spot. Using this tlc solvent system, the compound was isolated by column chromatography on silica gel to give [2R-(2α, 3aβ, 8aβ,)][2,3-b]indole-1,2-dicarboxylic acid-1-benzyl ester 2-methyl ester (3) as oil (11.65 g, 56 %).

To a stirred solution of (3) (11.65g, 33.09 mmol) in dioxane (116.2 mL) and water 11.62 (mL) maintained at 0 - 5 °C was added Na_2CO_3 (10.63 g, 100.29 mmol), followed by benzyl chloroformate (11.21 g, 13.4 mL, 65.7 mmol). The solvent was evaporated under reduced pressure after 4 hr , and the residue was suspended in ethyl acetate (300 mL). It was washed with 2 N HCl (100 mL), brine and dried. Purification by chromatography on neutral alumina (ll) column eluted with 15 % ethyl acetate in toluene gave (4) (10.5 g, 65 %). Chiral hplc at 40 °C on a Diacel Chiralpak AS 4.6 X 250 mm column, eluted with hexanes:IPA:DEA 82:18:0.1 at a flow rate of 1.0 mL/min and detected by UV at 240 nm showed the compound to be 100 % at a retention time of 9.03 min. IR ($CHCl_3$) cm^{-1} 2950, 1710, 1605. Proton NMR (200 MHz, $CDCl_3$) 2.55 (2H, m, 3-C$\underline{H}_2$); 3.11 (3H, s, 2-$CO_2C\underline{H}_3$); 4.00 (1H, t, 3a-H); 4.66 (1H, 2-H); 5.07 (3H, m, C$\underline{H}_2$Ph); 5.24 (1H, brd, C$\underline{H}_2$Ph); 6.50 (1H, d, 8a-H); 6.98 (1H, t, 6-H); 7.1 (1H, d, 5-H); 7.15 (1H, d, 7-H); 7.20 - 7.40 (10H, m, Ar-H); 7.68 (1H, d, 4-H). ^{13}C-NMR 172.15, 153.82, 143.08, 136.83, 136.67, 131.63, 129.16, 128.96, 128.78, 128.56, 124.49, 124.10, 117.30, 77.69, 68.05, 67.86, 59.95, 52.54, 45.53, and 34.44. MS (M^+) 486 (83), other ions at m/z 443(78), 410(8), 399(38), 382(79),

351(95), 335(16), 310(10), 292(15), 245(24), 220(38), 206(54), 185(24), 158(19), 130(75), 107(57), and 91(100). HRMS: Calcd. for $C_{28}H_{26}O_6N_2$: 487.1869 ($M + H^+$) . Found 487.1867

[2S-(2β,3aα,8aα,)]-2,3,3a,8a-Tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid -1,8-dibenzyl ester 2-methyl ester (11) .

$N_α$-Cbz-L-Tryptophan (8) (20.0 g, 59.10 mmoL) in CH_2Cl_2-ether (1:4 v/v) 200 mL was treated with diazomethane {generated from Diazald (23 .0 g), KOH (10 .0 g) and 95 % ethanol (50 mL), water (15 mL) and ether (350 mL)} as described in (4) . The solvent was removed, and the ester was dissolved in trifluoroacetic acid (150 mL) and stirred at room temperature for 4 days. A workup and purification as described in the preceeding experiment yielded (15), which was protected by a reaction with benzyl chloroformate (18.0 g) in the presence of sodium carbonate (16 g). The crude indole-1,2,8-tricarboxylic acid-1,8-dibenzyl 2-methyl ester was purified on neutral alumina ll-lll column eluted with toluene to give pure [S-(2β,3aα,8aα,)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester (11) (12.5 g, 43.5 %). Chiral hplc at 40 °C on a Diacel Chiralpak AS 4.6 X 250 mm column, eluted with hexanes:IPA:DEA 82:18:0.1 at a flow rate of 1.0 mL/min and detected by UV at 240 nm showed the compound to be 99.5 % with a retention time of 10.47 min. IR ($CHCl_3$) cm^{-1} 2950, 1710, 1605. Proton NMR (200 MHz, $CDCl_3$) 2.55 (2H, m, 3-C<u>H</u>$_2$); 3.12 (3H, s, 2-CO_2C<u>H</u>$_3$); 4.01 (1H, t, 3a-H); 4.68 (1H, d, 2-H); 5.07 (3H, m, C<u>H</u>$_2$Ph); 5.24 (1H, brd, C<u>H</u>$_2$Ph); 6.51 (1H, d, 8a-H); 6.98 (1H, t, 6-H); 7.1 (1H, d, 5-H); 7.15 (1H, d, 7-H); 7.20 - 7.40 (10H, m, Ar-H); 7.68 (1H, d, 4-H). ^{13}C-NMR 172.15, 153.83, 143.08, 136.84, 136.68, 131.63, 129.52, 129.16, 128.94, 128.78, 128.56, 124.50, 124.13, 117.30, 77.71, 68.05, 67.87, 59.95, 52.54, 45.58, and 34.43. HRMS: Calcd. for $C_{28}H_{26}O_6N_2$ 487.1869 ($M + H^+$). Found 487.1863

[2R-(2α, 3aβ, 8aβ,)]-2,3,3a,8a-Tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,2, 8-tribenzyl ester (17)

$N_α$-Cbz-D-Tryptophan (1) (25.0 g,. 73.87 mmol) and Cs_2CO_3 (12.05 g, 36.93 mmoL) were stirred in anhydrous DMF (75 mL) while benzyl bromide (13.26 g, 9.22 mL, d 1.438, 77.57 mmol) was added. After the reaction was shown by tlc (pet ether:EtOAc 70/30 v/v) to be completed (2.5 hr), it was diluted with ethyl acetate, and filtered through a pad of celite. It was concentrated and passed

through a column of silica gel eluted with pet ether-ethyl acetate (75:25 v/v) to remove trace impurities. Crystallization from ethyl acetate-hexane gave (15) (28.5 g).

A solution of (15) (18.0g) in trifluoroacetic acid (100 mL) was stirred at room temperature for 4 days. It was poured slowly in 45 min into a vigorously stirred mixture of methylene chloride-15 % aqueous sodium carbonate maintained at 0 °C in an ice water bath. The organic phase was separated, and the aqueous portion was extracted with methylene chloride (2 X 300 mL). The combined organic extract was washed with 15 % aqueous sodium carbonate, water and brine. It was evaporated at reduced pressure, and the residue was chromatographed on silica gel eluted with hexane:ether (1:1 v/v) to give [2R-(2α, 3aβ, 8aβ,)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2-dicarboxylic acid-1,2-dibenzyl ester (16) (10.69 g). Compound (16) (10.69 g, 25 mmol) was dissolved in dioxane (75 mL) containing water (7.5 mL), and cooled to 0 °C in an ice-water bath. Sodium carbonate (5.28 g, 50 mmol) was added followed by benzyl chloroformate (8.26 mL). It was stirred at 0 °C while the reaction was followed by tlc (hexane:ether 1:1 v/v). After 2.5 hr the solvent was removed and the residue was taken up in methylene chloride (400 mL), washed with 1 N HCl , brine and dried. The compound was purified on neutral alumina (ll-lll) eluted with 10 - 20 % ethyl acetate in toluene to give (17) (MW 562, 8.93 g). Chiral hplc at 40 °C on a Diacel Chiralpak AS 4.6 X 250 mm column, eluted with hexanes:IPA:DEA (92: 8: 0.1) at a flow rate of 1.0 mL/min and detected by UV at 240 nm showed the compound at to be 99.91 % with a retention time of 17.44 min. IR ($CHCl_3$) cm^{-1} 2950, 1710, 1605. Proton NMR (200 MHz, $CDCl_3$) 2.63 (2H, m, 3-C$\underline{H}_2$); 4.02 (1H, t, 3a-H); 4.36 (1H, 2-H); 4.71 (2H, m, C$\underline{H}_2$Ph); 5.20 (4H, m, C$\underline{H}_2$Ph); 6.52 (1H, d, 8a-H); 6.98 (1H, t, 6-H); 7.1 (1H, d, 5-H); 7.15 (1H, d, 7-H); 7.20 - 7.40 (10H, m, Ar-H); 7.68 (1H, d, 4-H). ^{13}C-NMR 171.32, 153.80, 142.98, 136.76, 136.66, 135.75, 131.59, 129.17, 129.04, 128.94, 128.88, 128.73, 128.53, 128.37, 128.11, 127.46, 124.42, 124.07, 117.42, 77.73, 68.02, 67.86, 67.27, 60. 05, 45.51 and 34.26. MS (M^+) 562(35) and other ions at m/z 519(38), 475(10), 458(18), 427(39), 411(6), 383(7), 321(9), 293(7), 249(6), 220(16), 206(25), 181(10), 158(13), 130(43), 107(55), and 91(100). HRMS; Calcd. for $C_{34}H_{30}O_6N_2$: 563.2182 ($M + H^+$) . Found 563.2200.

[2S-(2β, 3aα, 8aα,)]-2,3,3a,8a-Tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,2, 8-tribenzyl ester (23)

N_α-Cbz-L-Tryptophan (8) (20.0 g, 59.1 mmol) was similarly converted as described in the preceeding reaction to [2S-(2β, 3aα, 8aα,)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,2, 8-tribenzyl ester (10.82 g, 19.25 mmol, 32 %). Chiral hplc at 40 °C on a Diacel Chiralpak AS 4.6 X 250 mm column, eluted with hexanes:IPA:DEA (92: 8: 0.1) at a flow rate of 1.0 mL/min and detected by UV at 240 nm showed the compound at to be 99.65 % with a retention time of 19.95 min. IR ($CHCl_3$) cm^{-1} 2950, 1710, 1605. Proton NMR (200 MHz, $CDCl_3$) 2.63 (2H, m, 3-C$\underline{H}_2$); 4.02 (1H, t, 3a-H); 4.36 (1H, 2-H); 4.71 (2H, m, C$\underline{H}_2$Ph); 5.20 (4H, m, C$\underline{H}_2$Ph); 6.52 (1H, d, 8a-H); 6.98 (1H, t, 6-H); 7.1 (1H, d, 5-H); 7.15 (1H, d, 7-H); 7.20 - 7.40 (10H, m, Ar-H); 7.68 (1H, d, 4-H). ^{13}C-NMR 171.32, 153.80, 142.98, 136.76, 136.66, 135.75, 131.59, 129.17, 129.04, 128.94, 128.88, 128.73, 128.53, 128.37, 128.11, 127.46, 124.42, 124.07, 117.42, 77.73, 68.02, 67.86, 67.27, 60. 05, 45.51 and 34.26. MS (M^+) 562(41) and other ions at m/z 519(38), 475(10), 458(18), 427(45), 411(6), 383(9), 321(7), 293(7), 249(6), 220(16), 206(27), 181(10), 158(13), 13042), 107(47), and 91(100). HRMS: Calcd. for $C_{34}H_{30}O_6N_2$: 563.2182 ($M + H^+$). Found 563.2178.

[2R-Methyl-(2α, 3aβ, 8aβ,)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,2, 8-tribenzyl ester (18)

Lithium bis(trimethylsilyl)amide (1.0 M solution in THF, 2.93 mL, 2.93 mmol) was added slowly to a stirred solution of (17)1.50 g, 2.66 mmol) in dry THF (20 mL) at -78 °C under argon atmosphere. After 1 hr, methyl iodide (383.2 mg, 2.70 mmol) was added. It was stirred for 1hr, and allowed to warm to room temperature (cal. 2 hr). A mixture of chloroform and water (10 mL, 1:1 v/v) was added and the solvent was evaporated to dryness. Methylene chloride (40 mL) was added, followed by the addition of brine, and the organic phase was separated. The aqueous phase was acidified with 1 N HCl, and extracted with methylene chloride (2 X 20 mL). The combined organic extract was washed with cold 1 N HCl (20 mL), then brine and dried on magnesium sulfate. The crude product obtained by evaporation at reduced pressure was flashed on a Biotage Flash 40 system silica gel cartridge eluted with 25 % ethyl acetate in petroleum ether to give (18) (1.4 g, 92 %). Proton NMR (200 MHz, $CDCl_3$) 1.72 (3H, s, 2-C$\underline{H}_3$); 2.33 (1H, dd, 3-C$\underline{H}_2$); 2.86 (1H, d, 3-C$\underline{H}_2$); 3.90 (1H, t, 3a-H); 4.18 (1H, d, C$\underline{H}_2$Ph); 4.70 (2H, d, C$\underline{H}_2$Ph); 4.90 (1H, brd, C$\underline{H}_2$Ph);

5.10 (2H, d, C$\underline{H}_2$Ph); 6.50 (1H, d, 8a-H); 6.98- 7.50 (m). ^{13}C-NMR 173.58, 154.38, 154.07, 143.19, 136.80, 136.60, 136.00, 132.48, 128.96, 128.90, 128.78, 128.71, 128.61, 128.44, 128.33, 128.08, 124.10, 117.93, 79.08, 77.79, 68.08, 67.47, 67.18, 67.01, 43.73, 43.01 and 26.26. HRMS: Calcd. for $C_{35}H_{32}O_6N_2$: 577.2338 (M + H$^+$). Found 577.2330.

[2S-Methyl-(2β, 3aα, 8aα,)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,2, 8-tribenzyl ester (24)

[2S-(2β, 3aα, 8aα,)]-2,3,3a,8a-Tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,2, 8-tribenzyl ester (23) (2.00 g, 3.55 mmoL) was converted as described in the preceeding experiment to (24) (1.94 g, 95 %). Proton NMR (200 MHz, $CDCl_3$) 1.70 (3H, s, 2-C$\underline{H}_3$); 2.28 (1H, dd, 3-C$\underline{H}_2$); 2.84 (1H, d, 3-C$\underline{H}_2$); 3.88 (1H, t, 3a-H); 4.15 (1H, d, C$\underline{H}_2$Ph); 4.70 (2H, d, C$\underline{H}_2$Ph); 4.90 (2H, brd, C$\underline{H}_2$Ph); 5.10 (2H, d, C$\underline{H}_2$Ph); 6.50 (1H, d, 8a-H); 6.98- 7.50 (m). ^{13}C-NMR 173.58, 154.35, 154.06, 143.18, 136.79, 136.58, 135.98, 132.47, 128.95, 128.89, 128.77, 128.70, 128.60, 128.43, 128.32, 128.07, 124.09, 117.93, 79.06, 77.75, 68.06, 67.47, 67.17, 67.01, 43.72, 42.99 and 26.25. HRMS: Calcd. for $C_{35}H_{32}O_6N_2$: 577.2338 (M + H$^+$). Found 577.2328.

[2S-Methyl-(2β, 3aα, 8aα,)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester (12)

Lithium bis(trimethylsilyl)amide (1.0M solution in THF, 8.6 mL, 8.60 mmol) was added slowly to a stirred solution of (11) (3.61 g, 7.40 mmol) in dry THF (40 mL) at -78 °C under argon. After 1 hr methyl iodide (1.05 g, 460 μL, 7.40 mmol,) was added. It was stirred for another 1hr, and allowed to warm to room temperature. A mixture of chloroform and water (1:1 v/v) (10 mL) was added, and solvent was removed in vaccuo. Methylene chloride (80 mL) and brine (20 mL) were added to the residue, and the organic extract was separated. The aqueous layer was acidified with 1 N HCl (15 mL), and extracted again with methylene chloride (2 X 30 mL). The combined organic extract was washed with 1 N HCl, brine and dried. Solvent was removed to give an essentially clean oil, which however was Flash purified by a Biotage Flash system to give (12) (3.5 g, 94 %). Chiral hplc at 40 °C on a Diacel Chiraipak AS 4.6 X 250 mm column, eluted with hexanes:IPA:DEA 82:18:0.1 at a flow rate of 1.0 mL/min and UV 240 nm showed the compound to be 100 % with a

retention time of 8.99 min. Proton NMR (200 MHz, $CDCl_3$) 1.67 (3H, s, 2-$C\underline{H}_3$); 2.30 (1H, dd, 3-$C\underline{H}_2$); 2.80 (1H, d, 3-$C\underline{H}_2$); 3.00 (3H, s, 2-$CO_2C\underline{H}_3$); 3.87 (1H, t, 3a-H); 4.90 (1H, brd, $C\underline{H}_2Ph$); 5.10 (3H, q, $C\underline{H}_2Ph$); 6.50 (1H, d, 8a-H); 6.98 (1H, t, 6-H); 7.1 (1H, d, 5-H); 7.15 (1H, d, 7-H); 7.20 - 7.40 (10H, m, Ar-H); 7.68 (1H, d, 4-H). ^{13}C-NMR 174.44, 154.29, 154.10, 143.31, 137.01, 136.55, 132.57, 128.97, 128.86, 128.78, 128.64, 128.50, 128.41, 124.12, 117.84, 79.08, 77.74, 68.14, 67.34, 66.90, 52.52, 43.74, 43.06 and 26.16. HRMS: Calcd. for $C_{29}H_{28}O_6N_2$: 501.2025 (M + H^+). Found 501.2031.

[2R-[^{14}C]Methyl-(2α, 3aβ, 8aβ,)]-2,3,3a,8a-tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester (5)

Lithium bis(trimethylsilyl)amide (1.0M solution in THF, 4.8 mL, 4.8 mmol) was added slowly to a stirred solution of indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester ester (4) (1.944 g, 4.0 mmol) in dry THF (25 mL) at -78 °C under argon. After 1 hr [^{14}C]methyl iodide (567.76 mg, 4.0 mmol, Specific Activity ≈ 12 mCi/mmol) was added. It was stirred for another 1hr, and allowed to warm to room temperature. A mixture of chloroform and water (1:1 v/v) (10 mL) was added, and solvent was removed in vaccuo. Methylene chloride (40 mL) and brine (20 mL) were added to the residue, and the organic extract was separated. The aqueous layer was acidified with 1 N HCl (15 mL), and extracted again with methylene chloride (2 X 30 mL). The combined organic extract was washed with 1 N HCl, brine and dried. Solvent was removed to give an essentially clean (5) 1.87 g, 93.6 %). Proton NMR (200 MHz, $CDCl_3$) 1.67 (3H, s, 2-$C\underline{H}_3$); 2.30 (1H, dd, 3-$C\underline{H}_2$); 2.80 (1H, d, 3-$C\underline{H}_2$); 3.00 (3H, s, 2-$CO_2C\underline{H}_3$); 3.87 (1H, t, 3a-H); 4.90 (1H, brd, $C\underline{H}_2Ph$); 5.10 (3H, q, $C\underline{H}_2Ph$); 6.50 (1H, d, 8a-H); 6.98 (1H, t, 6-H); 7.1 (1H, d, 5-H); 7.15 (1H, d, 7-H); 7.20 - 7.40 (10H, m, Ar-H); 7.68 (1H, d, 4-H). ^{13}C-NMR ('cold prep. & ref')174.44, 154.29, 154.10, 143.31, 137.00, 136.54, 132.56, 128.96, 128.86, 128.78, 128.63, 128.50, 128.40, 128.31, 128.06, 124.11, 117.83, 79.06, 77.72, 68.13, 67.33, 66.89, 52.51, 43.70, 43.05 and 26.155

(R)-α-Methyltryptophan (20)

[2R-Methyl-[2,3-b]indole-1,2,8-tricarboxylic acid-1,2, 8-tribenzyl ester (17) (2.30 g, 4.0 mmoL) was dissolved in trifluoroacetic acid (10 mL) and stirred for 1.5 hr at room temperature. The solvent was evaporated under reduced pressure, and the residual trifluoroacetic acid was removed by azeotrope with toluene. The residue was dissolved in absolute ethanol, and a suspension of 10 %

Pd/C (400 mg) in absolute ethanol was added. After the reaction was degassed, a balloon of hydrogen was connected, and it was stirred at room temperature for 3 hr. It was filtered through a pad of celite, the celite was washed thoroughly with methanol and the combined wash was evaporated to give (20) (850 mg, 96 %). It was dissolved in 2 N HCl (6 mL) and immediately frozen in liquid nitrogen bath and evaporated to dryness. It was passed through a C-18 column and eluted with 20 % methanol in water. It was evaporated, transferred with methanol into a flask and evaporated to give white solid (860 mg). NMR (200 MHz, D_2O) 1.45 (s, 3H), 3.0 (d, 1H), 3.23 (d, 1H), 7.01 - 7.29 (m, 3H), 7.41 (d, 1H), 7.53 (d, 1H). ^{13}C-NMR (D_2O) 179.28, 138.82, 130.20, 128.25, 124.78, 122.28, 121.24, 114.65, 109.63, 64.78, 35.34 and 24.96.

(S)-α-Methyltryptophan (26)[9]

[2S-Methyl-[2,3-b]indole-1,2,8-tricarboxylic acid-1,2, 8-tribenzyl ester (23) (1.6 g, 2.77 mmoL) was converted as in the preceeding experiment to (26) (560 mg, 93 %). NMR (200 MHz, D_2O) 1.11 (s, 3H), 2.70 (d, 1H), 2.98 (d, 1H), 6.90 - 7.02 (m, 3H), 7.24 - 7.28 (m, 1H), 7.48 - 7.52 (m, 1H). ^{13}C-NMR (D_2O) 183.52, 135.69, 127.78, 124.51, 121.42, 118.89, 118.87, 111.71, 110.23, 59.62, 35.71, and 25.72.

(S)-α-Methyltryptophan methyl ester (14)

[2S-Methyl-[2,3-b]indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester (11) (1.5 g,) was converted as described for (R)-α-[^{14}C]methyltryptophan methyl ester (7) to (14) (840 mg, 99.6 %). Proton NMR (200 MHz, $CDCl_3$) 1.55 (3H, s, 2-C$\underline{H}_3$); 3.20 (2H, q, 3-C$\underline{H}_2$); 3.56 (3H, s, -$CO_2C\underline{H}_3$); 5.51 (brs, N$\underline{H}_2$); 7.06 - 7.33 (4-H, m , indole-H); 7.51 (1H, d,Indole-H); and 8.52 (1-H, brs, -N$\underline{H}$). ^{13}C-NMR 175.53, 136.58, 128.14, 125.34, 122.46, 120.00, 119.21, 111.96, 108.69, 60.37, 53.24, 35.27, and 24.87.

(R)-α-[^{14}C]Methyltryptophan methyl ester (7)

Carbon-14 labeled tetrahydro-pyrrolo[2,3-b]indole-1,2,8-tricarboxylic acid-1,8-dibenzyl ester 2-methyl ester (4) (1.8 g, 3.6 mmol) was dissolved in trifluoroacetic acid (12 mL) and stirred for 1.5 hr at room temperature. The solvent was evaporated under reduced pressure, and the residual trifluoroacetic acid was removed by azeotrope with toluene. The residue was dissolved in absolute ethanol, and a suspension of 10 % Pd/C (400 mg) in absolute ethanol was added. The reaction was

degassed, a balloon of hydrogen was connected, and it was stirred at room temperature for 3 hr. It was filtered through a pad of celite, the celite was washed with methanol and the combined wash was evaporated to give (R)-α-[^{14}C]methyltryptophan methyl ester (7) (840 mg, 99.6 %). Proton NMR (200 MHz, $CDCl_3$) 1.46 (3H, s, 2-C$\underline{H}_3$); 1.75 (2H, s, 2-N$\underline{H}_2$); 3.20 (2H, q, 3-C$\underline{H}_2$); 3.68 (3H, s, -$CO_2C\underline{H}_3$); 7.00 - 7.36 (4-H, m , indole-H); 7.65 (1H, d,Indole-H); and 8.35 (1-H, brs, -N$\underline{H}$). ^{13}C-NMR ('cold prep & ref') 178.52, 136.47, 128.60, 123.75, 122.47, 120.00, 119.65, 111.58, 111.29, 59.66, 52.64, 36.83, and 27.24.

2-Adamantyloxycarbonyl-(R)-α-[^{14}C]methyltryptophan methyl ester (27)

(R)-α-[^{14}C]Methyltryptophan methyl ester (7) (469 mg, 2.00 mmol) in dioxane (10 mL), and water (1.0 mL) was stirred in an ice bath. Sodium carbonate (286 mg, 2.70 mmol) was added followed by 2-adamantanyl chloroformate (572 mg, 2.6 mmol). Reaction was complete in 3 hr as judged by tlc (5 % EtOH in CH_2Cl_2). Usual workup gave 2-adamanyloxycarbonyl-(R)-α-[^{14}C]methyltryptophan methyl ester (27) (830 mg). Proton NMR (200 MHz, $CDCl_3$) 8.12 (brs), 7.56 - 6.93 (m), 5. 46 (brs), 4.89 (brs), 3.68 (s, OMe), 3.66 - 3.34 (m), 2.0 - 1.56 (m)

[1-(2-Hydroxy-cyclohexylcarbamoyl)-2-(1H-indol-3-yl)-1-methyl-ethyl]arbamic acid adamantan-2-yl ester (PD 145942)

2-Adamantyloxycarbonyl-(R)-α-[^{14}C]methyltryptophan methyl ester (27) (830 mg, 2.01 mmol) and LiOH (1.67 g, 40 mmol) in THF (60 mL) and water (6.0 mL) was refluxed for 3 days and progress was monitored by tlc (5 % methanol in ethyl acetate). Reaction was shown to be 94 % complete. Workup gave 2-adamantyloxycarbonyl-(R)-α-[^{14}C]methyltryptophan (28) (820 mg).

2-Adamantyloxycarbonyl-(R)-α-[^{14}C]methyltryptophan (28) (820 mg, 2.0 mmol), HOBt (324 mg, 2.4 mmol), N-methylmorpholin (NMM) (485.5 mg, 527 µL, 4.80 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl) (460.1 mg, 2.4 mmol) and (1S,2S)-(+)-trans-aminocyclohexanol (277.6 mg, 2.4 mmol) in anhydrous dimethylformamide (DMF) (5.0 mL) was stirred at room temperature overnight. The solvent was removed on a vaccum pump and the residue was taken up in ethyl acetate (60 mL). It was washed repeatedly with water and finally brine. It was dried on $MgSO_4$, concentrated, chromatographed on silica gel , and crystallized

from hexane-ethyl acetate to give [1-(2-Hydroxy-cyclohexylcarbamoyl)-2-(1H-indol-3-yl)-1-methyl-ethyl]carbamic acid adamantan-2-yl ester, PD 145942, (411 mg, 67 %). Hplc (on a Waters symmetry C-18 5μ, 3.9 X 150 mm, eluted with 0.05 M $NH_4H_2PO_4$ pH 3.5 w/H_3PO_4 : CH_3CN [45:55 v/v] at a flow rate of 1.0 mL/min and detection at 280 nm) gave retention time of 7.3 min which matched that of reference standard Lot W, as did the proton nmr spectrum in deuterochloroform solvent. Radiochemical purity was determined to be 99.87 %, chemical purity was found to be 99.88 %, and percent purity by weight as compared against reference standard Lot T was determined to be 99.1 %. Specific Activity was found to be 11.0 mCi/mmol based on MW 493.65. NMR 200 MHz ($CDCl_3$) 8.30 (s) , 7.62 - 7.03 (m), 6.05 (d), 5.15 (s), 4.82 (s), 3.69 (bs), 3.4 (q), 3.22 (m), 1.51 -1.99 (m), 1.25 (m). ^{13}C-NMR ('cold prep & ref') 175.28, 156.11, 136.48, 128.86, 124.59, 122.60, 120.25, 119.47, 111.72, 110.50, 78.86, 75.47, 61.20, 56.24, 37.80, 36.78, 34.02, 32.51, 31.73, 27.63, 27.38, 25.22, 24.58, and 24.48.

(R)-N-[(Benzo[b]furan-2-yl-methoxy)carbonyl]-(R)-[^{14}C]methyl-tryptophan methyl ester (29)

(R)-α-[^{14}C]Methyltryptophan methyl ester (7) (416.4 mg, 1.80 mmol), benzo[b]furan-2-yl methyl-4-nitrophenyl carbonate (819 mg, 2.61 mmol), 4-dimethylaminopyridine (73 mg, 0.6 mmol) in anhydrous dimethylformamide (DMF) (4.0 mL) was stirred at room temperature over a weekend. It was concentrated on a vacuum pump, diluted with ethyl acetate (50 mL), and washed repeatedly with 10 % K_2CO_3 until the organic phase became clear. It was dried , and the product was purified by column chromatography on silica gel eluted with 2 % ethyl acetate in methylene chloride to give after fast running impurities, (R)-N-[(benzo[b]furan-2-yl-methoxy)carbonyl]-(R)-[^{14}C]methyl-tryptophan methyl ester (29) (637.6 mg, 88.3 %). Proton NMR (200 MHz, $CDCl_3$) 1.69 (3H, s, $C\underline{H}_3$); 3.49 (2H, q, $C\underline{H}_2$); 3.69 (3H, s, $COOCH_3$); 5.23 (2H,q, CH_2); 5.59 (1H, m, N$\underline{H}$CO); 6.99 and 7.00 - 7.70 (m, Ar-H); 7.90 (1H, br, N$\underline{H}$).

[2-1H-Indol-3-yl)-1-[^{14}C]methyl-1-(1-phenyl-ethylcarbamoyl)-ethyl]-carbamic acid benzofuran-2-yl methyl,PD 154075

To a stirred solution of (R)-N-[(benzo[b]furan-2-yl-methoxy)carbonyl]-(R)-[^{14}C]methyl-tryptophan methyl ester (29) (637.6 mg, 1.568 mmol) in THF was added a solution of lithium hydroxide (78.37 mg, 1.568 mmol) in water (1.5 mL). Methanol was added till the precipitation that occurred clear and more THF (20 mL) was added. It was stirred for 6 days while being monitored by tlc (5 % methanol in ethyl acetate), evaporated. The residue was dissolved in ethyl acetate-methylene

chloride (5:1 v/v) (30 mL), and 10 % HCl (10 mL) was added. The organic phase was separated, and the aqueous was extracted several times with dichloromethane (5 X 20 mL) and dried. Solvent was removed to give (R)-N-[(benzo[b]furan-2-yl-methoxy)carbonyl-2(R)-[^{14}C]methyl tryptophan (30) (509 mg, 82.7 %)

A solution of (R)-N-[(benzo[b]furan-2-yl-methoxy)carbonyl-2-methyl tryptophan (30) (509 mg, 1.29 mmol), 1-hydroxybenzotriazole hydrate (HOBt) (210 mg, 1.556 mmol), N-methylmorpholin (NMM)(315 mg, 342 μL, 3.112 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl) (298.3 mg, 1.556 mmol) and (S)-(-)-α-methylbenzylamine (237 mg, 200 μL, 1.96 mmol) in dry dimethylformamide (DMF) was stirred under argon overnight and concentrated. Ethyl acetate (30 mL) was added, followed by water (20 mL). The aqueous portion was separated and the organic phase was washed with 10 % HCl (3 X 15 mL), followedby 10 % K_2CO_3 and finally brine (20 mL). It was dried and the solvent was evaporated to afford an impure creamy product. It was purified by column chromatography on silica gel eluted first with 35 % ethyl acetate in hexane. The pure fractions were combined and evaporated. Crystallization from hexane-CH_2Cl_2 gave 2-1H-Indol-3-yl)-1-methyl-1-(1-phenyl-ethylcarbamoyl)-ethyl]-carbamic acid benzofuran-2-yl methyl, PD 154075 as a white solid (437 mg, 68 %). Hplc (on a Waters symmetry C-18 5μ, 3.9 X 150 mm, eluted with 0.05 M $NH_4H_2PO_4$ pH 3.5 w/H_3PO_4 : CH_3CN [42:58 v/v] at a flow rate of 1.0 mL/min and detection at 220 nm) gave retention time of 7 min which matched that of reference standart Lot T, as did the proton nmr spectrum in deuterochloroform solvent. Radiochemical purity was determined to be 99.09 %, chemical purity was found to be 99.33 %, and percent purity by weight as compared against reference standard Lot W was determined to be 99.3 %. Specific Activity was found to be 11.1 mCi/mmol based on MW 495.58. Proton NMR (200 MHz, $CDCl_3$) 1.31 (3H, d); 1.63 (3H, s); 3.36 (2H, q); 5.05 (1H, m); 5.19 (4H, q); 5.44 (1H, s); 6.35 (1H, d); 6.78 (2H, superimposed s & d); 7.04 - 7.59 (14H, m, aromatic H); 7.97 (1H, brs, NH). ^{13}C-NMR ('cold prep & ref') 173.27, 155.62, 155.20, 152.77, 143.57, 136.35, 129.04, 128.67, 128.39, 127.69, 126.63, 125.34, 124.41, 123.48, 122.63, 121.90, 120.32, 119.30, 111.85, 111.70, 110.05, 107.42, 61.37, 59.43, 49.62, 33.38, 24.10, and 21.88

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