NOVEL REDOX DERIVATIVES OF TRYPTOPHAN

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Abstract - The design, synthesis and study of some physicochemical properties of several potential brain targeting chemical delivery systems (CDS) of D,L-tryptophan (Trp) are described. CDS's are based on a 1,4-dihydropyridine ↔ pyridinium salt-type redox system. The dihydropyridine moiety was chemically attached to the amino group of Trp by either substituted amide or substituted carbamate linkages. While the amide bond-containing derivatives are known to be rather stable toward *in vivo* hydrolysis, the parent Trp can be readily released from the substituted carbamate-type combinations. Lipophilicity and chemical oxidation studies performed on the new derivatives indicated that some of the new CDSs possess the properties required for an improved and specific brain delivery of Trp.

Although many drugs are available for the treatment of hypertension, a condition which affects tens of millions of people in the US alone, research aimed at finding safer and more potent compounds continues. L-Tryptophan (L-Trp), an essential amino acid commonly used as a nutrient, recently was investigated as a potential antihypertensive agent.[1-6] The mechanism of Trp action is not completely understood, but most evidence [4-6] indicates that its primary site of action is in the central nervous

system (CNS). Although Trp is transported to the CNS through the blood-brain-barrier (BBB) by the large neutral amino acid carrier (LNAA),[7] Trp's unfavorable polar and low lipophilicity properties and competition with other amino acids make its CNS uptake limited. In fact, the elimination from the CNS of Trp by active transport is significant, resulting in an unfavorable equilibrium between blood and brain. All these factors keep the brain concentration of L-Trp low after i.v. administration. Administration of high doses of Trp might have unwanted side-effects, including of bladder cancer.[8] Therefore, methods of enhancing CNS concentration of Trp, without of increasing the dose, could be of interest. Because the redox targetor-based chemical delivery system was successful in selectively and site-specifically transporting various drugs to the brain,[9,10] this method, which employs a dihydropyridine ↔ pyridinium salt-type targetor, covalently bound to the drug, was also applied to tryptophan.[11,12] In the CDSs investigated so far, the dihydropyridine moiety was chemically attached to the amino group of D,L-Trp by an amide-type bonding, while the carboxylic acid functionality was esterified to various alcohols.[11] Hypotensive activity studies in a deoxycorticosterone acetate-induced hypertensive rat models demonstrated that the CDSs reduced blood pressure more efficiently and for a longer time than did Trp itself. This paper describes the synthesis of some novel type CDSs of D,L-Trp (**1**) as well as the study of their physicochemical properties. The redox carrier in the CDSs is attached reversibly to Trp in a variety of ways.

RESULTS AND DISCUSSION

CDSs of Trp were designed to have CNS specificity. Two properties contributed by the dihydropyridine moiety are required by this purpose: 1) sufficient lipophilicity for BBB penetration and CNS uptake; 2) fairly rapidly oxidation for accumulation in the CNS and to accelerate peripheral elimination. The parent drug should be released by hydrolysis from the biologically inactive combination and also be active. In all cases, the carboxylic functionality of Trp is esterified to provide lipophilic character. Importantly, hydrolysis of the ester should occur *in vivo* prior to pharmacological action.

CDSs in which the redox targetor is attached to Trp by simple amide linkages were described before.[11] Here different combinations are investigated with the aim of synthesizing CDSs which can be readily

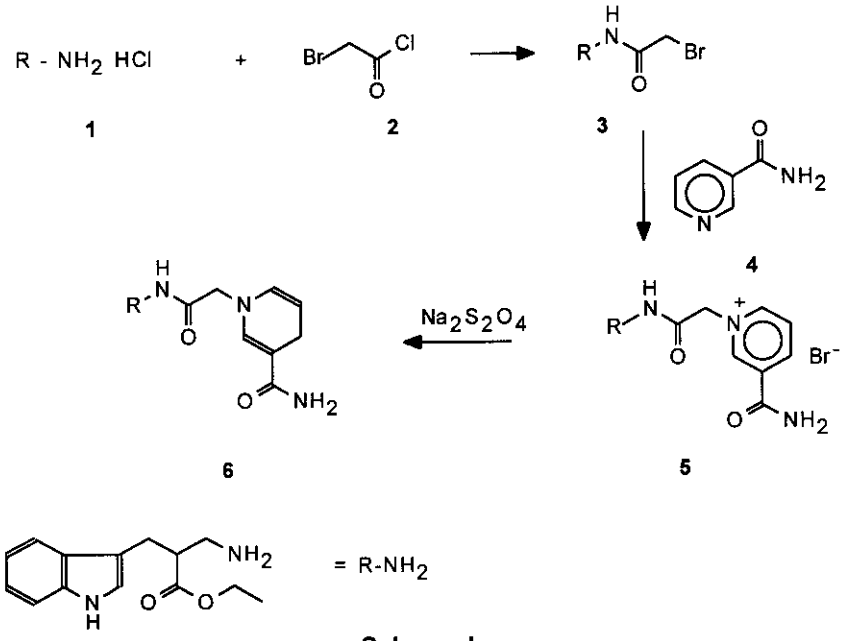
cleaved enzymatically. The poor hydrolytic potential of amidases prompted the preparation of some less stable substituted amide type, as well as substituted carbamate-type linkages Substituted carbamates are particularly suitable for the release of the drug since they are hydrolyzed by the active esterases present in a large concentration in the CNS.[13,14]

The synthetic procedures are summarized in Schemes I-V. In the compounds (**6**)and (**9**) the brain targeting dihydropyridine moiety is attached to Trp *via* the pyridine nitrogen as a substituted acetamide and propionamide, respectively. By acylating the ethyl ester of Trp (**1**) with bromoacetyl chloride (**2**), the bromoacetamide (**3**) resulted. By reacting (**3**) with nicotinamide (**4**) in a polar aprotic solvent, the quaternary salt (**5**) was obtained. Reduction of (**5**) with aqueous basic sodium dithionite regioselectively produced the 1,4-dihydropyridine derivative (**6**) (Scheme I).

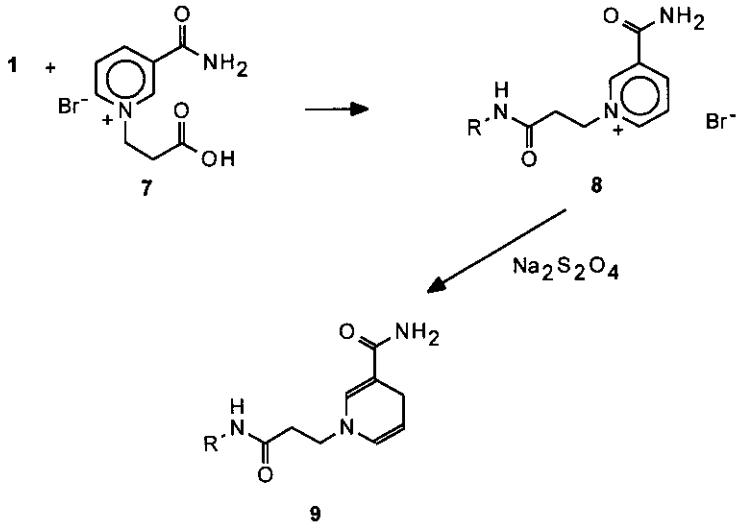
Compound (**9**) was obtained by reacting (**1**) with 3-carbamoyl-1-carboxyethylpyridinium bromide (**7**) (prepared by reacting nicotinamide with 3-bromopropionic acid) in the presence of dicyclohexylcarbodiimide, which was used as dehydrating agent, and by reducing the resulting quaternary salt (**8**) with sodium dithionite (Scheme II).

Another amide type of CDS (**13**) in which the pyridine moiety is attached to (**1**) as substituted acetamide containing an ester linkage, was obtained by reacting the bromoacetamide (**3**) with the triethylammonium salt of nicotinic acid (**10**). The resulting nicotinate (**11**) was then *N*-methylated and the quaternary salt (**12**) was reduced with sodium dithionite (Scheme III).

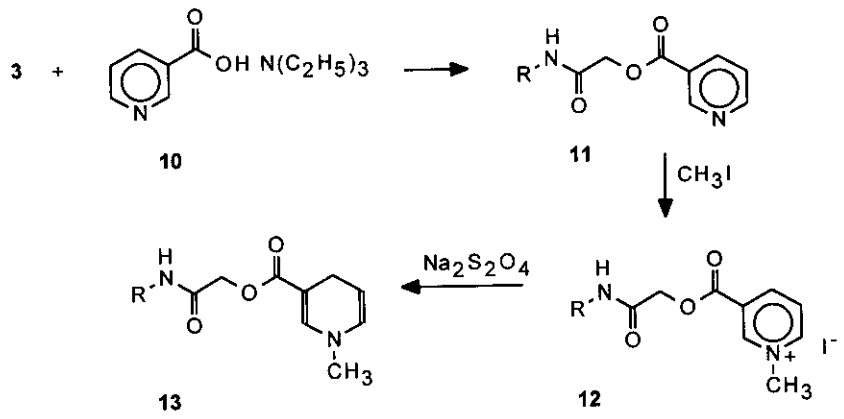
The synthesis of two CDSs in which the pyridine moiety is attached to the drug by a hydrolytically labile substituted carbamate linkage is shown in Scheme IV.



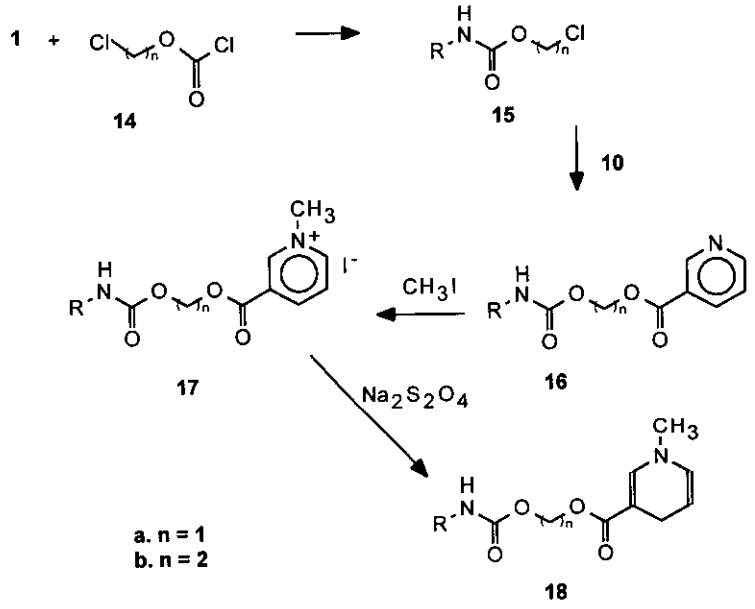
Scheme I



Scheme II



Scheme III



Scheme IV

The haloalkylcarbamate intermediates (**15a**) and (**15b**) were obtained by acylation of (**1**) with haloalkyl chloroformates (**14**). The haloalkylcarbamates were then reacted with the triethylammonium salt of nicotinic acid to give the carbonyloxyalkylpyridine carboxylates of **1** (**16**). By *N*-methylation of (**16**) and dithionite reduction of the resulting quaternary salts (**17a**) and (**17b**), CDSs (**18a**) and (**18b**) resulted. (Scheme IV).

The products were characterized by elemental analysis, ms, uv photospectrometry and ^{1}H-nmr spectroscopy. Thin layer chromatography indicated only one component in each case. The dihydropyridines were proven to be the 1,4-isomers by uv and ^{1}H-nmr methods.

All dihydropyridine derivatives were oxidized to the corresponding quaternary salts by means of *methanolic silver* nitrate solution or H_2O_2 in the presence of catalytic cupric ions. Ferricyanide-mediated oxidation studies performed on similar compounds indicated that the determined oxidation rates compared in an empirical sense with those of systems which readily oxidized *in vivo*. Based on these data it is presumed that *in vivo* oxidation of **6**, **9**, **13** and **18a, b** will take place.

Table 1. R_m Values[a] for 1,4-dihydropyridine type derivatives of tryptophan at various (acetone:water) concentrations.

Compound	Acetone %				
	30	50	60	70	80
6	0.98	0.15	-0.27	-0.57	-0.92
13	0.00	1.21	0.50	-0.04	-0.52
18a	0.00	0.85	0.34	-0.09	-0 52
18b	0.51	0.83	0.31	-0.12	-0.57

[a] $R_m = \log 1/(R_f-1)$

Table 2. Lipophilic indexes (extrapolated r_m values)[a] for 1,4-dihydropyridine-type derivatives of tryptophan as compared to the parent compound

Compound	R_m	r[b]
Tryptophan (acid)	0.00	0.998
4	2.08	0.997
9	4.30	0.996
13a	3.10	0.998
13b	2.83	0.997

[a] R_m values at 0% acetone were calculated by extrapolating from the linear part of the R_m value curve obtained from various mobile phases.

[b] correlation coefficient

Chromatographic R_m values can be used as lipophilicity indexes [15] since they are related to the partition coefficient. The R_m values were determined from the R_f values by means of a reversed-phase tlc method with various concentrations of aqueous acetone as the mobile phase using the equation: $R_m = \log (1/R_f - 1)$. The calculated R_m values are shown in Table 1. The lipophilic indexes presented in Table 2 were then calculated by extrapolation of the linear part of the R_m curve to a totally aqueous mobile phase. All CDSs and analogs were more lipophilic than Trp ($R_m = 0$). The most lipophilic CDS was the substituted amide type (**13**). Followed by the substituted carbamates (**18a, b**). These data suggest that the CDSs will penetrate the BBB.

In the series of compounds produced, it is expected [13,14,16] that the amide type CDSs (**6**) and (**9**), as well as their quaternary salt-type synthetic precursors and metabolic successors, (**5**) and (**8**) are stable toward both chemical and enzymatic hydrolysis and will only slowly release the parent drug. Compounds (**12**) and (**13**) should be hydrolyzed faster at the ester function but the resulting hydroxyacetamide of **1** ($R\text{-}NHCOCH_2OH$) is probably stable toward further hydrolysis. However, the substituted carbamates (**18a, b**) should readily generate (**1**) by both chemical and enzymatic hydrolysis, because their labile functions are the acyloxyalkyl ester linkages. The hydroxyalkyloxycarbamates (hemiketals), which result as the first products of hydrolysis, are very unstable and should decompose spontaneously to the parent compound through an intermediate carbamic acid. It is known that brain amidases are by far less active than brain esterases. For this reason it is expected that CDSs based on substituted carbamate linkages (**18**) which are hydrolyzed by esterases, will release the native Trp in the CNS more quickly. However, because amide bond containing CDSs of Trp proved to have a greater antihypertensive effect than the native amino acid, it is possible that **6** and **9** are also effective. It is not certain if the activity of the amide-type CDSs can be attributed to the parent Trp released by hydrolysis or to the intrinsic activity of the derivatives.

In conclusion, the redox-type CDSs of Trp possess the required properties to penetrate the brain, across the BBB and generate by oxidation quaternary salt-type derivatives. While the release of **1** from

their polar species "locked-in" the CNS (but eliminated from periphery) by enzymatic hydrolysis is predicted to be faster in the case of (**17a**) and (**17b**), the other derivatives might be active themselves.

EXPERIMENTAL SECTION

Uncorrected melting points were determined on an Electrothermal® melting-point apparatus (Fischer Scientific). Elemental microcombustion analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. Ultraviolet spectra (uv) were obtained on a Hewlett-Packard 8451A diode array spectrophotometer. Proton nuclear magnetic resonance spectra (^{1}H-nmr) were recorded on a Varian XL 200 (200-MH2; FT mode) spectrometer. Samples were dissolved in an appropriate deuterated solvent and chemical shifts were reported as parts per million (δ) relative to the tetramethylsilane internal standard. Coupling constants (J) are reported in hertz (Hz). Mass spectra were recorded on a Kratos, MS 80-RFA double focusing instrument. Fast atom bombardment (FAB) ionization was performed using a xenon beam (6 KeV) and dissolving the samples in a glycerol matrix.[17] Thin layer chromatography (tlc) was performed on EM Reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with silica gel 60. A mixture of isopropanol:chloroform, 1:8 was used as solvent for development to determine R_f. All chemicals were reagent grade. Tryptophan esters were obtained from Sigma.

N-(Bromoacetyl)-D,L-tryptophan ethyl ester (3) To a suspension of 2.01 g (7.5 mmol) of D,L-tryptophan ethyl ester hydrochloride (**1**) and 2.52 g (30 mmol) of sodium bicarbonate in 60 ml of methylene chloride, 2.36 g (15 mmol) of bromoacetyl chloride (**3**) dissolved in 10 ml of methylene chloride was added dropwise while stirring over a 10 min period at 0-5° C. The mixture was stirred for 1 h at 20-25°C then poured onto ice. The organic layer was separated and washed successively with water, 0.1 N aqueous hydrochloric acid and water, then dried over anhydrous sodium sulfate and filtered. Removal of the solvent *in vacuo* afforded 2.56 g (97%) of the product as a brown oil, R_f:0.69, which was used in the next step of the synthesis.

3-Carbamoyl-1-[[[(RS)-1-carboxy-2-indol-3-ylethyl]carbamoyl]methyl] pyridinium bromide ethyl ester (5) To a solution of 2.60 g (7 mmol) of **3** in 50 ml of nitromethane, 0.94 g (7.7 mmol) of nicotinamide was added. The mixture was stirred for six days at 40-50°C. The precipitate was filtered at the same temperature and washed with ether. After recrystallization from a mixture of methanol: ether 2.15 g (65%) of product was obtained, mp 168-170°; uv (MeOH) 218, 270 nm; ^{1}H-nmr (DMSO-d_6) δ:1.13 (*t*, 3H, J = 7.01), 3.17-3.21 (m, 2H), 4.01-4.08 (m, 2H), 4.60-4.63 (m, 1H), 5.61-5.65 (m, 1H), 5.70 (s, 2H), 6 99-7.51 (m, 4H), 7.78-7.80 (m, 1H), 8.11-8.42 (m, 3H), 8.61-9.61 (m, 5H); C+ (m/z): 395. Anal. Calcd for $C_{21}H_{23}N_4O_4Br$: C, 53.06; H, 4.87; N, 11.95; Br, 16.64. Found: C, 52.80; H, 4.52; N, 11.95; Br, 16.87.

N-[(3-Carbamoyl-1-(4H)-pyridyl)acetyl]-D,L-trytophan ethyl ester (6). To a solution of 2.55 g (5.36 mmol) of **5** in 250 ml of deaerated water and 250 ml of ethyl acetate, were added a mixture of 2.70 g (32.16 mmol) of sodium bicarbonate and 3.73 g (21.47 mmol) of sodium dithionite while stirring at 0-5° C. The system was maintained under an argon stream and was stirred for 3 h. The organic layer was separated and the aqueous layer extracted with 2 x 100 ml of ethyl acetate. The combined organic layers were washed with 2 x 100 ml of cold deaerated water, dried over sodium sulfate and evaporated under reduced pressure to give 1.8 g (85%) of **6** as a yellow solid: mp 135-7°C; R_f : 0.31; uv (MeOH):λ 218, 345 nm; ^{1}H-nmr (DMSO-d_6) δ: 1.14 (t, 3H, J=8.28), 2.95 (br s, 2H), 3.36 (s, 2H), 4.00-4.11 (m, 2H), 4.56-4.58 (m, 2H), 5.52 (d, 1H, J=6.11), 6.98 (br s, 1H), 6.83 (s, H), 6.92-7.21 (m, 5H), 8.10-8.26 (m, 1H). Anal. Calcd for $C_{21}H_{24}N_4O_4.0.5H_2O$: C, 62.20; H, 6.21; N, 13.81, Found: C, 62.35; H, 6.20; N, 13 78.

3-Carbamoyl-1-[[[(RS)-1-carboxy-2-indolylethyl]carbamoyl]ethyl]pyridinium bromide ethyl ester (8) A solution of 0.26 g (1 mmol) of **1**, 0.08 g (1 mmol) of sodium bicarbonate 0.3 g (1.1 mmol) of 3-carbamoyl-1-carboxyethylpyridinium bromide (**7**) and 0.22 g (1.1 mmol) of dicyclohexylcarbodiimide in 30 ml of dimethylformamide was stirred at 20-25°C for 4 days. The precipitated dicyclohexylurea was removed by filtration and the solvent removed *in vacuo*. The residue was crystallized from a

mixture of methanol:ether to give 0.34 g (70%) of **8** as off-white crystals, mp 165-169°C, uv (MeOH) λ 217, 272 nm; ^{1}H-nmr (DMSO-d6) δ: same as **5**, the singlet from 4.70 ppm replaced by 3.22 (t, 2H, J=6.4), 4.90 (t, 2H, J=6.16); C^+ (m/z): 409. Anal. Calcd for $C_{22}H_{25}N_4O_4Br$: C, 53.99; H. 51.5; N, 11.50; Br, 16.33, Found: C, 54.26; H, 5.30; N, 11.26; Br, 16.56.

N-[(3-Carbamoyl-1-(4H)pyridyl)propionyl]-D,L-tryptophan ethyl ester (9) By the reduction of 0.045 g (0.09 mmol) of **8** in 10 ml of water and 10 m of ethyl acetate with 0.046 g (0.53 mmol) of sodium bicarbonate and 0.064 g (0.37 mmol) of sodium dithionite in 4 h, according to the procedure described for **6**, 0.026 g(70%) of **9** was obtained as an oil: R_f:0.40, uv (MeOH)λ 214, 352 nm; ^{1}H-nmr (DMSO-d_6) δ·1 09 (t, 3H, J=6.27), 2.33 (t, 2H, J=6 5), 3.09-3.38 (m, 7H), 3.99 (q, 2H, J=7.08), 4.50-4.52 (m; 1H), 5.81 (d, 1H, J=6.12), 6.53 (br s, 1H), 6.86-7.50 (m, 5H), 8.42 (d, 1H, J=7.18). Anal. Calcd for $C_{22}H_{26}N_4O_4$: C, 64.37; H, 6.39; N, 13.65, Found: C, 64.65; H, 6.52, N, 13.40.

N-[[(3-Pyridinylcarbonyl)oxy]acetyl-D,L-tryptophan ethyl ester (11) To a solution of 1.30 g (3.6 mmol) of **2** in 25 ml of nitromethane, 0.53 g (4 32 mmol) of nicotinic acid (**10**) and 0.44 g (4.32 mmol) of triethylamine in 20 ml nitromethane were added. The mixture was stirred at 20-25°C for 8 days. The solvent was removed *in vacuo* and the residue was dissolved in 250 ml of ethyl acetate. The resulting solution was washed with water (2 x 50 ml), dried over anhydrous sodium sulfate and evaporated *in vacuo* to give 0.50 g (35%) of **11** as a brown oil. This material was used for the next step.

(R,S)-3-[[2-[[2-Ethoxy-1-(1H-indol-3-ylmethyl)-2-oxoethyl]amino]-2-oxoethoxy]carbonyl-1-methyl-pyridinium iodide (12) To a solution of 0.50 g (1.26 mmol) of **11** in 50 ml of dry acetone was added 6 ml (96 mmol) of iodomethane, after which the reaction mixture was stirred at 20-25°C for 4 days. After removing the solvent *in vacuo*, the residue was slurried with ether, filtered and dried, giving 0.50 g (74%) of **12** as a hygroscopic solid C^+(m/z):426, uv (MeOH)λ 219, 272 nm; ^{1}H-nmr (DMSO-d_6) δ: 1.15 (t, 3H, J=7.12), 3.07-3.19 (m, 2H), 4.07-4.38 (m, 4H), 4.43 (s, 3H),4.52-4.54 (m, 1H), 6.97-7.51 (m, 4H), 8.29-8.31 (m, 1H), 8.61-8.64 (m, 1H), 9 20-9.32 (m, 2H), 9.57-9.58 (m, 1H), 10.89 (br s, 1H).

Anal. Calcd for $C_{22}H_{24}N_3O_5I.H_2O$: C, 45.57; H, 4.71; N, 7.56; I, 22.85. Found: C, 47.68; H, 4.70; N, 7.57; I, 22.78.

N-[[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]oxy]acetyl-D,L- tryptophan ethyl ester (13) By reducing 0.37 g (0 68 mmol) of **12** dissolved in a mixture of 45 ml of water and 35 ml of ethyl acetate with 0.34 g (4.1 mmol) of sodium bicarbonate and 0.47g (2.7 mmol) of sodium dithionite for 5 h, 0.50 g (54%) of **9** resulted as a hygroscopic solid. R_f: 0.68; uv (MeOH)λ 216, 281, 358 nm; ^{1}H-nmr (DMSO-d_6) δ: 1.15 (t, 3H, J=6.87), 2.98-3.07 (m, 5H), 3.27 - 3.31 (m, 2H), 3.97-4.11 (m, 4H), 4.47 - 4.49 (m, 1H), 4.77 - 4.89 (m, 1H), 5.60-5.62 (m, 1H,), 6.98 - 7.45 (m, 5H), 8.18 - 8.22 (m, 1H), 8.59 - 8.62 (m, 1H), 8.86 - 8.97 (m, 1H). Anal. Calcd for $C_{22}H_{25}N_3O_5$. 1.5 H_2O: C, 60.26; H, 6.43; N, 9.58. Found: C, 59.82; H, 6.11; N, 9.43.

N-[(Chloromethoxy)carbonyl]-D,L-tryptophan ethyl ester (15a) To a suspension of 5.37 g (20 mmol) of **1** in 60 ml of methylene chloride, 3.10g (24 mmol) of chloromethyl chloroformate (**14a**) (obtained from phosgene and formaldehyde monomer) in 10 ml of methylene chloride was added dropwise. To the resulting mixture, cooled to 0.5 °C, 4.45 g (44 mmol) of the triethylamine in 20 ml of methylene chloride was added dropwise over a 25 min period. The mixture was stirred at 20-25°C for 1.5 h, then the layers were separated; the organics were extracted successively with water, 3% aqueous hydrochloric acid and water, dried over anhydrous sodium sulfate and evaporated. The product (5.61 g, 87% yield) was obtained as a white solid. mp 127-30°C; Rf, 0.73; ^{1}H-nmr (DMSO-d_6) δ: 1.17 (t, 3H, J=7.01), 2.98 - 3.21 (m, 2H), 3.97 - 4.10 (m, 2H), 4 15 -4.20 (m, 1H), 5.92 (s, 2H), 6.97-7.61 (m, 5H), 8.11 (d, 1H, J=3.12), 10.98 (bs, 1H) Anal. Calcd for $C_{15}H_{17}N_2O_4Cl$: C, 55.74; H. 5.27; N, 8.62; Cl, 10.94. Found: C, 55.39; H, 5.33; N, 8.57; Cl, 10.98.

N-[(Chloroethoxy)carbonyl]-D,L-tryptophan ethyl ester (15b) By reacting 0.67 g (2.5 mmol) of **1** in 15 ml of methylene chloride with 0.43 g (3 mmol) of chloroethyl chloroformate (**14b**) in 5 ml of methylene chloride and 0.56 g (5.5 mmol) of triethylamine in 5 ml of methylene chloride, according to the procedure described for **15a**, 0.80 g (94%) of **15b** was obtained as a brown oil, Rf: 0.63; ^{1}H-nmr

(DMSO -d_6) δ: 1.10 (t, 3h, J=7.03), 3.12 - 3.14 (m, 2H), 3.59 (t, 2H, J-5.61), 3.62 - 3.66 (m, 2H), 4.03 - 4.11 (m, 3h), 4.20 -4.26 (m, 1H), 6.97 - 7.57 (m, 5H), 10.86 (br s, 1H). Anal. Calcd for $C_{16}H_{19}N_2O_4Cl$. 0.5 H_2O: C, 55.25; H, 5.79; N, 8.05; Cl, 10.19. Found: C, 54.85; H, 5.49; N, 7.96; Cl, 10.17.

N-[[[[(3-Pyridine)carbonyl]oxy]methoxy]carbonyl]-D,L-tryptophan ethyl ester (16a) To a solution of 5.00 g (15.4 mmol) of **15a** in 50 ml of dimethylformamide, a solution of 2.28 g (18.5 mmol) of nicotinic acid and 1.87 g (18.8 mmol) of triethylamine in 50 ml of dimethylformamide were added dropwise at O°C. After the mixture was stirred for 3 days at 20-25°C, 500 ml of ethyl acetate was added. The solution was washed twice with water and twice with brine, then it was dried over anhydrous sodium sulfate. After recovering the solvent *in vacuo*, 5.14 g (81.3%) of **16a** was obtained as a hygroscopic solid, mp 56-62°C; Rf: 0.65, ^{1}H-nmr (DMSO-d_6) δ: 1.13 (t, 3H, J=7.03), 3.07 - 3.18 (m, 2H), 3.91-4.20 (m, 2H), 4.12-4.17 (m, 1H), 5.89 (s, 2H), 6.98-7.45 (m, 5H), 8.18-8.22 (m, 1H), 8.61-8.59(m, 1H), 8.86-8.97(m, 1H), 9.07 (s, 1H), 10.95 (br s, 1H). The material was used in the next step.

N-[[[(3-Pyridinyl)carbonyl]oxy]ethoxy]carbonyl]-D,L-tryptophan ethyl ester (16b) Following the procedures used for the synthesis of **16a**, starting from 0.70 g (1.8 mmol) of **15b**, 0.27 g (2.2 mmol) of nicotinic acid, 0.22 g (2.2 mmol) of triethylamine and 13 ml of dimethylformamide (reaction time 7 days), 0.70 g (91.4%) of **16b** was obtained as an oil. Rf 0.70: ^{1}H-nmr (DMSO-d_6) δ: similar to **11a** (singlet at 5.89 ppm replaced by 2+2 proton multiplets at 3.50-3.52 and 3.61-3.62 ppm. Used for next step.

(R,S)-3-[[[[[[2-Ethoxy-1-(1H)indol-3-ylmethyl)-2-oxoethyl]amino]carbonyl]oxy]methoxy]carbonyl-1-methylpyridinium iodide (17a) By reacting 5.14 g (12.5 mmol) of **16a** in 60 ml of acetone with 15 ml (240 mmol) of methyl iodide for 3 days, 5.84 g (85%) of **17a** was obtained as a yellow solid, mp 80-88°C; uv (MeOH)λ 220-270 nm; ^{1}H-nmr (DMSO-d_6) δ: 1.07 (t, 3H, J=7.11), 2.97 - 3.20 (m, 2H), 3.96 - 4.11 (m, 2H), 4.11 - 4.18 (m, 1H), 4.43 (s, 3H), 5.98 (s, 2H), 6.97 - 7.61 (m, 5H), 8.11-8.21 (m, 1H), 8.84-8.86 (m, 1H), 9.10-9.17 (m, 1H), 9.67 (s, 1H), 10.67 (bs, 1H). Anal. Calcd for $C_{22}H_{24}N_3O_6I$: C, 47.75; H, 4.37; N, 7.59; I, 22.93. Found: C, 47.56; H, 4.42; N, 7.49; I, 22.76. C^+ (m/z) 426.

(R,S)-3-[[[[[[2-Ethoxy-1-(1H-indol-3-ylmethyl)-2-oxoethyl]amino]carbonyl]oxy]ethoxy]carbonyl-1-methylpyridinium iodide (17b) In the same way as in the case of **17a**, from 0.70 g (1.5 mmol) of **16b** and 10 ml (165 mmol) of methyl iodide in 100 ml of acetone, 0.80 g (90%) of **17b** was obtained as a very hygroscopic product. uv (MeOH)λ 219, 272 nm; ^{1}H-nmr (DMSO-d_6) δ: Similar to **17a** (singlet at 5.98 ppm replaced by t at 4.36 ppm (2H, J=6.11) and t at 4.50 ppm (2H, J=5.97). Anal. Calcd for $C_{23}H_{26}N_3O_6I$. $2H_2O$: C, 45.78; H, 5.01; N, 6.96; I, 21.03. Found: C, 46.10; H, 5.04; N, 6.56, I, 21.16. C^+ (m/z) 440.

N-[[[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]oxy]methoxy]carbonyl]-D,L-tryptophan ethyl ester (18a) By reducing 5.00 g (9 mmol) of **17a** in a mixture of 500 ml of water and 450 ml of ethyl acetate with 4.53 g (54 mmol) of sodium bicarbonate and 6.26 g (36 mmol) of sodium dithionite 2.52g (66%) of **18a** was obtained as a yellow solid, mp 117-121°C; Rf: 0.65; uv (MeOH): 220, 281, 360 nm. ^{1}H-nmr ($CDCl_3$) δ: 1.15 (t, 3H, J=7.12), 2.90 - 2.98 (m, 5H), 3.19 -3.21 (m, 2H), 3.97 - 4.11 (m, 2H), 4.50-4.61 (m, 1H), 5.51 (d, 1H, J=7.6), 5.71 (s, 2H), 6.97-7.41 (m, 7H), 8.71 (br s, 1H). Anal. Calcd for $C_{22}H_{25}N_3O_6$: C, 61.82; H, 5.90; N, 9.83. Found: C, 61,83; H, 5.91; N, 9.79.

N-[[2-[[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]oxy]ethoxy]carbonyl]-D,L-tryptophan ethyl ester (18b) By reduction of 0.20 g (0.38 mmol) of **17b** in 30 ml of water and 25 ml of ethyl acetate with 0.19 g (2.3 mmol) of sodium bicarbonate and 0.26 g (1.5 mmol) of sodium dithionite 0.13 g (77%) of **18b** was obtained as an oil. Rf: 0.70, uv (MeOH): 220, 362 nm; ^{1}H-nmr ($CDCl_3$) (similar to **18a**, except the 5.71 (s, 2H), replaced by 2+2 proton multiplets at 3.45 - 3.50 and 3 59-3.52 ppm. Anal. Calcd for $C_{23}H_{27}N_3O_6$, 2.5 H_2O: C, 56.77; H, 6.63; N, 8.63. Found: C, 56.64; H, 6.25; N, 8.80.

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