N-1 and C-2 Substituted Tryptophans as Potential Inhibitors of Sickle Cell Hemoglobin Gelation

Noojaree Prasitpan [1a], Jayeshkumar N. Patel,
Philomen Z. De Croos [1b], Brenda L. Stockwell [1c],
Parthasarathy Manavalan [1d], Leela Kar,
Michael E. Johnson and Bruce L. Currie*

Department of Medicinal Chemistry and Pharmacognosy, m/c 781,
College of Pharmacy, University of Illinois at Chicago,
P. O. Box 6998, Chicago, IL 60680

Received July 24, 1991

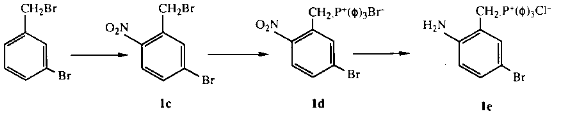
A series of N-1 or C-2 phenylalkyl substituted tryptophans and C-1 phenylalkyl substituted 3,4-dihydro-β-carbolines were prepared from the apropriate aniline, **1b** or **1e**, or tryptophan, **8** or **11**, by ring closure methods. None of the compounds prepared were more potent than 5-bromotryptophan (**11**) as inhibitors of sickle cell hemoglobin gelation.

J. Heterocyclic Chem., **29**, 335 (1992).

Until recently, 5-bromotryptophan (**11**) was the most potent non-covalent inhibitor of sickle cell hemoglobin gelation as reported by Poillon [2]. A series of dipeptides containing **11** was recently reported from our laboratory which demonstrated that substantial increases in potency were possible for larger, more hydrophobic compounds [3].

A recent computational study revealed several potential binding sites for aromatic compounds with known activity as inhibitors of sickle cell hemoglobin gelation [4]. One of these potential binding sites corresponded to the lateral contact region on the hemoglobin protein β-chain that has been designated as the so called "acceptor site" because it participates in a critical contact with the "mutation site" on the sickle cell hemoglobin protein during the gelation or aggregation process [5]. Binding studies with several aromatic ring containing compounds including the amino acids phenylalanine and tryptophan (**8**) and using various nmr techniques [6-8] also suggested that the lateral contact "acceptor" region was probably one of the binding sites for aromatic gelation inhibitors. Further exploration of this potential binding region using molecular graphics, indicated the addition of hydrophobic moieties of the appropriate length as substituents on the indole nucleus could lead to additional binding interactions and thus increased potency.

Scheme 1

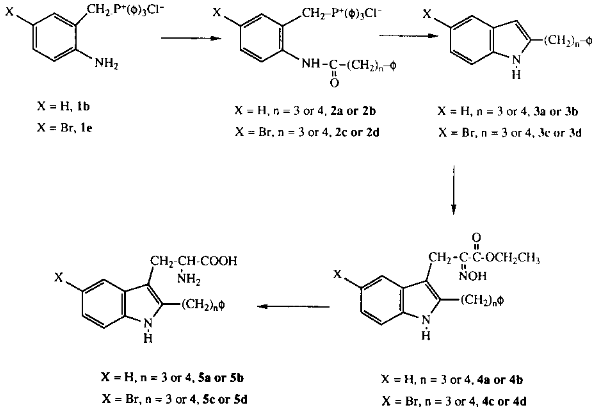


These results prompted us to synthesize compounds **5a-d**, **10**, **12**, and **21-24** as amino acid analogues with increased hydrophobicity that could be more potent inhibitors of sickle cell hemoglobin gelation.

Recently, Li *et al.* [9] reported a method whereby a substituent can be introduced at the C-2 position of an indole ring by the cyclization of an appropriately acylated aniline, and then, conversion of the resulting indole moiety into a substituted tryptophan. Electrophilic substitution of indole proceeds preferentially at C-3 even when a substituent is already present, as in the case of tryptophan. The α-amino group also presents further complications for direct substitution on a tryptophan at the C-2 position.

In order to obtain the corresponding 5-bromoindole moiety, it was necessary to prepare 5-bromo-2-nitrobenzyl bromide (**1c**). Nitration of 3-bromobenzyl bromide produced a mixture of products from which the desired **1c** was obtained in 40% yield. The bromide **1c** was treated with triphenylphosphine to provide the corresponding phosphonium bromide **1d** in quantitative yield. The nitro group was then reduced with zinc and acetic acid to the required aniline derivative **1e**. Subsequent acylation of anilines **1b** or **1e** with phenylbutanoyl chloride or phenylpentanoyl chloride provided amides **2a-d** which were then cyclized with potassium *t*-butoxide to the corresponding 2-(3-phenylpropyl)- and 2-(4-phenylbutyl)indoles **3a,b** or 5-

Scheme 2

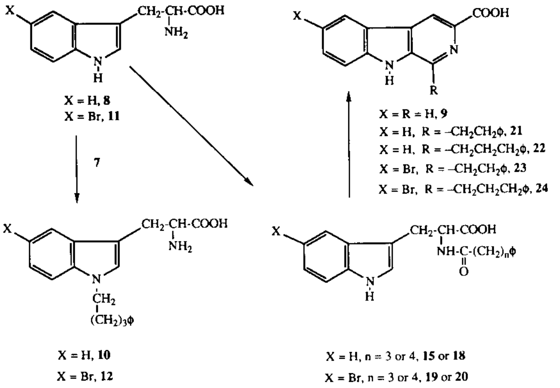


bromoindoles **3c,d** in good yield (62-88%). The method of Li *et al.* [9] was then used to add the amino acid side chain to the C-2 substituted indoles in two steps to provide tryptophans **5a-d** as shown in Scheme 2. The substituted indoles **3a-d** were synthesized in moderate yields in two steps from the requisite anilines **1b** or **1e** as shown in Scheme 2. This approach seems to be quite flexible for the introduction of an alkyl or arylalkyl moiety at the C-2 position of an indole and then subsequent conversion to a tryptophan.

The indole nitrogen of tryptophan can be alkylated directly by using sodium and liquid ammonia with an appropriate alkyl bromide by an adaptation of the method of Yamada *et al.* [10]. Either **8** or **11** could be substituted in this manner with 1-bromo-4-phenylbutane to provide the corresponding N-1 derivatives in 52-60% yield.

1-Substituted 3,4-dihydro-β-carbolines were chosen as an alternative C-2 substituted indole system. The 3,4-dihydro-β-carbolines were prepared by acylation of the α-amino group of **8** or **11** with a phenylalkanoyl chloride in 60-80% yields. The resulting amides **15, 18-20** were cyclized by treatment with dicyclohexylcarbodiimide followed by anhydrous trifluoroacetic acid using a modification of the method of Letellier and Fleury [11] to provide the desired 3,4-dihydro-β-carbolines **21-24** in 21-65% yields as shown in Scheme 3. Similar N^{α}-acylindoles have been prepared previously by Takeuchi *et al.* [12-15] for a different purpose, including the phenylacyltryptophans reported herein, but without nmr spectral data or other corroborating evidence for the structures assigned.

Scheme 3



The method developed by Poillon *et al.* [16] as modified in our laboratory, and described by De Croos *et al.* [5] for the determination of the inhibition of gelation of purified human sickle cell hemoglobin, was used to measure the potency of the ten target compounds **5a-d, 10, 12, 21-24**. None of the ten were more potent than **11** as an inhibitor of sickle cell hemoglobin gelation. Further investigations of analogues and derivatives of **11** are currently underway in our laboratories in order to design and synthesize compounds with substantially increased potency as inhibitors of sickle cell hemoglobin gelation as potential anti-sickling agents.

EXPERIMENTAL

Melting points were taken on a Thomas Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (nmr) spectra were obtained on a Varian XL 300 spectrometer and are reported as δ (ppm) values downfield from tetramethylsilane as an internal standard in the solvent specified. Mass spectra were determined on a MAT-90 spectrometer (Finnigan MAT, San Jose, CA). The methods of ionization employed were chemical ionization (methane or ammonia) and fast atom bombardment (glycerol matrix). L-Tryptophan and all other starting materials for synthesis were purchased from Aldrich Chemical Company. Silica gel (E. Merck, 230-400 mesh) was used for flash column chromatography. Analytical thin layer chromatography (tlc) was conducted using E. Merck silica gel 60F-254 precoated (250 micron) glass plates. Compounds were detected on tlc plates by uv light (254 nm) and/or iodine vapor. Evaporations were carried out under aspirator reduced pressure or vacuum pump with a rotary evaporator using a bath temperature ranging from 30 to 50°. Elemental analyses were performed by Midwest Microlab, Indianapolis, IN.

5-Bromo-2-nitrobenzyl Bromide (**1c**).

To concentrated nitric acid (4 ml) was cautiously added concentrated sulfuric acid at 0°, then while maintaining the reaction mixture at room temperature, 3-bromobenzyl bromide (1.0 g, 4.0 mmoles) was added in portions with vigorous mixing. The mixture was stirred at room temperature for 2 hours and then poured into ice-water (200 ml). The oily liquid was collected, taken up into methylene chloride (20 ml), washed with water (3 x 20 ml), and dried (sodium sulfate). The solvent was removed *in vacuo* to give a yellowish oil (1.0 g, 85%). Three close running spots were observed on tlc for the three possible isomers. Column chromatography on silica gel (1% ethyl acetate in hexanes) as eluant gave the desired product as yellow crystals from methylene chloride or ether (0.4 g, 34%), mp 77-78° (lit [17] 62-66°); ^{1}H nmr (deuteriochloroform): δ 4.8, (s, 2H), 7.64 (dd, 1H), 7.76 (d, 1H), 7.96 (d, 1H); ms: (chemical ionization, ammonia) m/z 215 (molecular ion - Br).

Anal. Calcd. for $C_7H_5Br_2NO_2$ (294.9): C, 28.51; H, 1.71; Br, 54.19; N, 4.75. Found: C, 28.51; H, 1.57; Br, 54.01; N, 4.70.

The other major isomer, 3-bromo-4-nitrobenzyl bromide, was obtained as a yellowish oil (0.4 g, 34%); ^{1}H nmr (deuteriochloroform): δ 4.45 (s, 2H), 7.48 (dd, 1H), 7.77 (d, 1H), 7.84 (d, 1H).

2-Nitro-5-bromobenzyltriphenylphosphonium Bromide (**1d**).

A solution of **1c** (4.8 g, 16.3 mmoles) and triphenylphosphine (4.25 g, 16.2 mmoles) in chloroform (30 ml) was stirred at room temperature for 3 days. Ether (30 ml) was added and the resulting mixture kept at room temperature for an additional 24 hours. The deposited crystals were collected by filtration, washed with ether (20 ml) and air dried, 9.05 g (100%), mp 256-258° dec; ^{1}H nmr (deuteriochloroform): δ 6.24 (d, 2H, J = 15 Hz), 7.9-8.9 (m, 18H).

Anal. Calcd. for $C_{25}H_{20}Br_2NO_2P$ (557.2): C, 53.89; H, 3.62; Br, 28.68; N, 2.51; P, 5.56. Found: C, 54.08; H, 3.68; Br, 28.76; N, 2.55; P, 5.87.

2-Amino-5-bromobenzyltriphenylphosphonium Chloride (**1e**).

Zinc powder (10 g) was added in portions to a stirred solution of **1d** (10 g, 18 mmoles) in glacial acetic acid (200 ml) over 30 minutes with stirring under nitrogen without cooling. The mixture was stirred at room temperature overnight and was then filtered through Celite. The filtrate was concentrated *in vacuo* to a viscous oil which was dissolved in 1 *M* hydrochloric acid and evaporated to give a solid. The solid was triturated with a small amount of chloroform and collected by filtration to yield **1e** as the hydrochloride salt (6.2 g, 67%).

2-(4-Phenylbutanamido)benzyltriphenylphosphonium Chloride (**2a**).

4-Phenylbutanoyl chloride (0.8 g, 4.9 mmoles) was added dropwise to a stirred solution of *o*-aminobenzyltriphenylphosphonium chloride hydrochloride (1.6 g, 3.6 mmoles) in DMF (3 ml) and pyridine (1 ml) at 0°. The mixture was stirred at room temperature overnight, and then the solvents were removed *in vacuo.* The residue was taken up in chloroform, washed with 1 *M* hydrochloric acid (2 x 10 ml), brine (10 ml), dried (sodium sulfate), and filtered. Removal of the solvent *in vacuo* gave the desired product as a solid which was triturated with ether (20 ml), collected, and dried *in vacuo* to yield 1.8 g (90%); ^{1}H nmr (deuteriochloroform): δ 1.67 (m, 2H), 2.32 (t, 2H), 2.56 (t, 2H), 5.85 (d, 2H), 6.7-7.8 (m, 25H).

2-(5-Phenylpentanamido)benzyltriphenylphosphonium Chloride (**2b**).

In a similar manner to that described above for **2a**, 5-phenylpentanoyl chloride (2.35 g, 11.95 mmoles), *o*-aminobenzyltriphenylphosphonium chloride hydrochloride (4.25 g, 9.56 mmoles), DMF (50 ml), and pyridine (20 ml) were used to obtain 4.5 g (83%) of **2b**; ^{1}H nmr (deuteriochloroform): δ 1.45 (m, 2H), 1.57 (m, 2H), 2.29 (t, 2H), 2.59 (t, 2H), 5.83 (d, 2H), 6.7-7.8 (m, 25H).

5-Bromo-2-(4-phenylbutanamido)benzyltriphenylphosphonium Chloride (**2c**).

As described above for **2a**, **1e** (4.6 g, 8.9 mmoles) and 4-phenylbutanoyl chloride (2.4 g, 14.6 mmoles) in DMF (40 ml) and pyridine (8 ml) were used for the preparation of **2c**, yield 4.9 g (88%); ^{1}H nmr (deuteriochloroform): δ 1.69 (m, 2H), 2.31 (t, 2H), 2.57 (t, 2H), 5.77 (d, 2H, J = 15 Hz), 6.88-7.78 (m, 23H), 10.38 (br s, 1H).

5-Bromo-2-(5-phenylpentanamido)benzyltriphenylphosphonium Chloride (**2d**).

As previously described for **2a**, **1e** (3.96 g, 7.6 mmoles) and 5-phenylpentanoyl chloride (2.2 g, 12.3 mmoles) in DMF (35 ml) and pyridine (8 ml) were used to prepare **2d**, yield 4.2 g (86%) after trituration in ether; ^{1}H nmr (deuteriochloroform): δ 1.47 (m, 2H), 1.60 (m, 2H), 2.28 (t, 2H), 2.60 (t, 2H), 5.92 (d, 2H, 15 Hz), 6.97-7.82 (m, 23H), 10.59 (br s, 1H).

2-(3-Phenylpropyl)indole (**3a**).

A mixture of **2a** (1.64 g, 2.98 mmole) and potassium *t*-butoxide (0.40 g, 3.54 mmoles) in toluene (20 ml) was stirred and heated at reflux for 30 minutes. Filtration followed by concentration of the filtrate *in vacuo* and purification by column chromatography (silica gel) using methylene chloride as eluant provided the desired product (0.6 g, 88%), mp 61-62°; ^{1}H nmr (deuteriochloroform): δ 2.05 (t, 2H), 2.72 (m, 4H), 6.26 (s, 1H), 7.05-7.75 (m, 10H).

Anal. Calcd. for $C_{17}H_{17}N$ (235.3): C, 86.77; H, 7.28; N, 5.95. Found: C, 86.71; H, 7.32; N, 5.91.

2-(4-Phenylbutyl)indole (**3b**).

As previously described for **3a**, a mixture of **2b** (4.5 g, 7.98 mmoles) and potassium *t*-butoxide (1.0 g, 8.9 mmoles) in toluene (50 ml) gave **3b** as a pale yellow solid (1.55 g, 78%), mp 79-80°; ^{1}H nmr (deuteriochloroform): δ 1.69 (m, 4H), 2.62 (t, 2H), 2.70 (t, 2H), 6.77 (s, 1H), 6.99-7.3 (m, 8H), 7.45-7.5 (m, 1H), 7.67 (br s, 1H).

Anal. Calcd. for $C_{18}H_{19}N$ (249.4): C, 86.70; H, 7.68; N, 5.62. Found: C, 86.39; H, 7.45; N, 5.65.

5-Bromo-2-(3-phenylpropyl)indole (**3c**).

As described above for **3a**, **2c** (4.55 g, 7.23 mmoles), potassium *t*-butoxide (1.04 g, 9.04 mmoles) and toluene (100 ml) were used for the preparation of **3c**, yield 1.89 g (83%). After column chromatography (silica gel) using methylene chloride as eluant and recrystallization from hexanes, **3c** was obtained as off-white plates, mp 85-86°; ^{1}H nmr (deuteriochloroform): δ 2.04 (m, 2H), 2.73 (m, 4H), 6.19 (s, 1H), 7.06-7.66 (m, 8H), 7.82 (br s, 1H).

Anal. Calcd. for $C_{17}H_{16}BrN$ (314.2): C, 64.98; H, 5.13; Br, 25.43; N, 4.43. Found: C, 64.56; H, 5.08; Br, 25.43; N, 4.43.

5-Bromo-2-(4-phenylbutyl)indole (**3d**).

In a similar manner to the preparation of **3a**, **2d** (3.8 g, 5.9 mmoles) and potassium *t*-butoxide (0.8 g, 7.4 mmoles) in toluene (80 ml) were used to prepare **3d**. After column chromatography (silica gel) with methylene chloride as eluant and recrystallization from hexanes, the pure product was obtained as pale yellow needles (1.2 g, 62%), mp 102-103°; ^{1}H nmr (deuteriochloroform): δ 1.72 (m, 4H), 2.65 (t, 2H), 2.75 (t, 2H), 6.16 (s, 1H), 7.05-7.34 (m, 7H), 7.63 (d, 1H), 7.82 (br s, 1H).

Anal. Calcd. for $C_{18}H_{18}BrN$ (328.2): C, 65.86; H, 5.53; Br, 24.34; N, 4.27. Found: C, 65.79; H, 5.53; Br, 24.14; N, 4.25.

Ethyl 2-Hydroximino-3-[2-(3-phenylpropyl)indol-3-yl]propanoate (**4a**).

A solution of ethyl 3-bromo-2-hydroximinopropanoate (**6**) (0.16 g, 0.76 mmole) in methylene chloride (2 ml) was added dropwise to a stirred mixture of **3a** (0.51 g, 1.7 mmoles) and sodium carbonate (0.16 g, 1.5 mmoles) in methylene chloride (5 ml) under a nitrogen atmosphere. The suspension was stirred overnight at room temperature. The mixture was filtered through Celite, the filtrate was concentrated to dryness *in vacuo,* and the residue purified by column chromatography (silica gel) with methylene chloride as eluant. The desired product was obtained as a white solid (0.2 g, 60%), mp 136-138°; ^{1}H nmr (deuteriochloroform): δ 1.23 (t, 3H), 1.97 (m, 2H), 2.68 (t, 2H), 2.89 (t, 2H), 4.01 (s, 2H), 4.18 (q, 2H), 7.03-7.75 (m, 10H), 9.6 (br s, 1H).

Anal. Calcd. for $C_{22}H_{24}N_2O_3$ (364.4): C, 72.51; H, 6.64; N, 7.69. Found: C, 72.41; H, 6.61; N, 7.68.

Ethyl 2-Hydroximino-3-[2-(4-phenylbutyl)indol-3-yl]propanoate (**4b**).

In a similar manner to that described for **4a**, **6** (0.3 g, 1.43 mmoles) in methylene chloride (3 ml) and 2-(4-phenylbutyl)indole

(1.2 g, 4.82 mmoles), sodium carbonate (0.32 g, 3.02 mmoles) in methylene chloride (10 ml) produced the desired product as a viscous oil (0.35 g, 65%) which was crystallized from ether/hexanes, mp 88-90°; ^{1}H nmr (deuteriochloroform): δ 1.24 (t, 3H), 1.7 (m, 4H), 2.65 (t, 2H), 2.89 (t, 2H), 4.03 (s, 2H), 4.17 (q, 2H), 7.03-7.3 (m, 7H), 7.68-7.76 (m, 3H), 9.72 (br s, 1H).

Anal. Calcd. for $C_{23}H_{26}N_2O_3$ (378.5): C, 72.99; H, 6.92; N, 7.40. Found: C, 72.79; H, 6.89; N, 7.34.

Ethyl 2-Hydroximino-3-[5-bromo-2-(3-phenylpropyl)indol-3-yl]propanoate (**4c**).

As described above for **4a**, **3c** (1.8 g, 5.73 mmoles), **6** (0.6 g, 2.86 mmoles), sodium carbonate (0.6 g, 5.66 mmoles), and methylene chloride (30 ml) were utilized for the preparation of **4c**. After workup and column chromatography (silica gel) using methylene chloride as eluant, the desired product was obtained as white crystals, from methylene chloride, 0.89 g (63%), mp 144-145°; ^{1}H nmr (deuteriochloroform): δ 1.3 (m, 3H), 1.97 (m, 2H), 2.70 (m, 2H), 2.8 (m, 2H), 3.97 (s, 2H), 4.21 (m, 2H), 6.9-7.9 (m, 8H), 9.24 (br s, 1H).

Anal. Calcd. for $C_{22}H_{23}BrN_2O_3$ (443.3): C, 59.60; H, 5.23; Br, 18.02; N, 6.32. Found: C, 59.42; H, 5.14; Br, 18.14; N, 6.28.

Ethyl 2-Hydroximino-3-[5-bromo-2-(4-phenylbutyl)indol-3-yl]propanoate (**4d**).

As described above for **4a**, **6** (0.38 g, 1.8 mmoles), **3d** (1.2 g, 3.66 mmoles), and sodium carbonate (0.4 g, 3.8 mmoles) in methylene chloride (30 ml) were used to prepare **4d**. After column chromatography (silica gel) with methylene chloride as eluant and recrystallization from methylene chloride, **4d** was obtained as white crystals (0.6 g, 75%), mp 118-119°; ^{1}H nmr (deuteriochloroform): δ 1.26 (t, 3H), 1.69 (m, 4H), 2.63 (t, 2H), 2.86 (t, 2H), 3.96 (s, 2H), 4.20 (q, 2H), 7.05-7.3 (m, 6H), 7.8 (m, 2H), 9.38 (br s, 1H).

Anal. Calcd. for $C_{23}H_{25}BrN_2O_3$ (457.4): C, 60.40; H, 5.51; Br, 17.47; N, 6.12. Found: C, 60.10; H, 5.48; Br, 17.74; N, 6.04.

2-(3-Phenylpropyl)tryptophan (**5a**).

Zinc powder (30 mg) was added to a solution of **4a** (165 mg, 0.38 mmole) in glacial acetic acid (5 ml). After stirring overnight, the mixture was filtered through Celite, and the filtrate was concentrated to dryness *in vacuo*. The oily residue was mixed with 1 *M* sodium hydroxide (2 ml) and methanol (5 ml) and stirred overnight at room temperature. The mixture was diluted with water and adjusted to *p*H 8 with 1 *M* hydrochloric acid. The precipitated solid was collected and recrystallized from methanol to give 100 mg (83%) of **5a**, mp 223-225° dec; ^{1}H nmr (DMSO-d$_6$): δ 1.95 (m, 2H), 2.62 (t, 2H), 2.79 (t, 2H), 2.86-2.89 (m, 1H), 3.17-3.44 (m, 2H), 6.89-7.54 (m, 10H), 10.88 (br s, 1H).

Anal. Calcd. for $C_{20}H_{22}N_2O_2+0.25H_2O$ (322.4+0.25H$_2$O): C, 73.48; H, 6.94; N, 8.57. Found: C, 73.25; H, 6.58; N, 8.45.

2-(4-Phenylbutyl)tryptophan (**5b**).

As previously described for **5a**, zinc powder (0.4 g) in glacial acetic acid (20 ml) was used to reduce **4b** (0.3 g, 0.79 mmole) to give a viscous oil. After saponification with 1 *M* sodium hydroxide (2 ml, 2 mmoles) in methanol (10 ml) followed by neutralization with 1 *M* hydrochloric acid, **5b** was obtained as an off-white solid 0.2 g (88%), mp 156-159°; ^{1}H nmr (DMSO-d$_6$): δ 1.63 (m, 4H), 2.5-2.9 (m, 5H), 3.2-3.4 (m, 2H), 6.85-7.6 (m, 10H), 10.8 (br s, 1H); ms: (positive fast atom bombardment) (% relative intensity) m/z 337 (molecular ion + H$^+$, 76).

Anal. Calcd. for $C_{21}H_{24}N_2O_2+H_2O$ (336.4+H$_2$O): C, 71.16; H, 7.39; N, 7.90. Found: C, 71.06; H, 7.02; N, 7.85.

5-Bromo-2-(3-phenylpropyl)tryptophan (**5c**).

As described above for **5a**, zinc powder (0.6 g) in glacial acetic acid (10 ml) was used to reduce **4c** (0.6 g, 1.35 mmoles) to the corresponding amine obtained as a viscous oil. After saponification of the ester with 1 *N* sodium hydroxide in ethanol and then neutralization with 1 *M* hydrochloric acid, **5c** was obtained as an off-white solid, 0.3 g (55%), mp 151° dec; ^{1}H nmr (DMSO-d$_6$): δ 2.0 (m, 2H), 2.61 (t, 2H), 2.7-2.92 (m, 3H), 3.2-3.42 (m, 2H), 7.05-7.31 (m, 8H), 7.70 (m, 1H), 11.08 (br s, 1H); ms: (positive fast atom bombardment) (% relative intensity) m/z 403, 401 (molecular ion + H$^+$, 100, 99 bromine isotopes).

Anal. Calcd. for $C_{20}H_{21}BrN_2O_2+H_2O$ (401.3+H$_2$O): C, 57.29; H, 5.53; Br, 19.06; N, 6.68. Found: C, 57.39; H, 5.01; Br, 19.60 [18]; N, 6.58.

5-Bromo-2-(4-phenylbutyl)tryptophan (**5d**).

As previously described for **5a**, zinc powder (0.5 g) in glacial acetic acid (20 ml) was used to reduce **4d** (0.5 g, 1.1 mmoles). After the usual workup, the yellowish oil that was obtained was dissolved in ethanol (20 ml) and 1 *M* sodium hydroxide (10 ml) for saponification. After adjusting the *p*H to 8.5, the product was obtained as a yellowish solid which was recrystallized from ethanol/water, 0.2 g (44%), mp 160° dec; ^{1}H nmr (DMSO-d$_6$): δ 1.59 (m, 4H), 2.54 (m, 2H), 2.83 (m, 1H), 3.2-3.4 (m, 6H), 7.02-7.3 (m, 8H), 7.59 (br s, 1H), 11.00 (br s, 1H); ms: (positive fast atom bombardment) (% relative intensity) m/z 417, 415 (molecular ion + H$^+$, 8, 8 bromine isotopes), 185 (100).

Anal. Calcd. for $C_{21}H_{23}BrN_2O_2+2H_2O$ (415.3+2H$_2$O): C, 55.88; H, 6.03; Br, 17.70; N, 6.21. Found: C, 55.94; H, 4.96 [18]; Br, 17.53; N, 6.10.

1-Bromo-4-phenylbutane (**7**).

A mixture of 4-phenyl-1-butanol (5.0 g, 33.3 mmoles) and 48% hydrobromic acid (70 ml) was heated at reflux for 48 hours. The mixture was cooled and extracted with ether (4 x 50 ml). The ether layers were combined and neutralized over powdered sodium bicarbonate. After evolution of carbon dioxide had stopped, the ether extract was filtered, dried (magnesium sulfate), and evaporated to a dark brown oily residue. Column chromatography (silica gel) of this residue with petroleum ether eluant produced fractions (200 ml) containing the alkyl bromide (tlc, R$_f$ 0.5, petroleum ether) which were combined and the solvent evaporated to give **7** (6.4 g, 90%) as a colorless liquid; ^{1}H nmr (deuteriochloroform): δ 1.84 (m, 4H), 2.65 (t, J = 7.8 Hz, 2H), 3.42 (t, J = 6.6 Hz, 2H), 7.17-7.32 (br m, 5H).

3,4-Dihydro-β-carboline-3-carboxylic Acid (**9**).

To a mixture of **8** (1.02 g, 5 mmoles) and formic acid (2.0 ml) was added formic acetic anhydride (1.0 ml) with stirring and cooling in an ice-water bath for 2.5 hours under a nitrogen atmosphere. The mixture was evaporated *in vacuo*, water (3 x 2 ml) was added and evaporated to remove traces of formic acid. The product was recrystallized from ethanol/water and the white crystals were collected by filtration and air dried to provide 1.05 g (90%)

of N^{α}-formyltryptophan. The product was ninhydrin negative and the ^{1}H nmr spectrum matched a standard spectrum. The formyltryptophan was cyclized by the method of Letellier and Fleury [11] to obtain **9**. The nmr spectrum confirmed the assigned structure.

N^{i}-(4-Phenylbutyl)-L-tryptophan (**10**).

Metallic sodium (0.28 g, 0.012 g-atom) was added in small pieces with stirring to liquid ammonia (50 ml) containing ferric nitrate nonahydrate (0.015 g). After dissolution was complete, **8** (1.083 g, 5.3 mmoles) suspended in anhydrous ether (5 ml) was added to the stirred mixture. After 30 minutes, **7** (1.5 g, 7.0 mmoles) dissolved in anhydrous ether (2 ml) was added dropwise over 10 minutes. The mixture was allowed to warm to room temperature while stirring was continued in order to evaporate excess ammonia. The residue was dissolved in about 20 ml of water and the solution was extracted with ether (2 x 25 ml) to remove unreacted **7**. The aqueous phase was then adjusted to *p*H 6 with glacial acetic acid, and the precipitate obtained was collected and recrystallized from ethanol/ethyl acetate to give **10** (1.07 g, 60%), mp 186-187°; tlc, R_f = 0.44 (97:3, v:v, ethanol/30% ammonium hydroxide); ^{1}H nmr (perdeuteriomethanol + trifluoroacetic acid): δ 1.63 (m, 2H), 1.84 (m, 2H), 2.61 (t, J = 7.5 Hz, 2H), 3.33 (dd, J = 15 and 7.8 Hz, 1H), 3.48 (dd, J = 15 and 5 Hz, 1H), 4.15 (t, J = 7.1 Hz, 2H), 4.24 (dd, J = 7.8 and 5 Hz, 1H), 4.98 (s, all exchangeable protons), 7.06-7.25 (br m, 8H), 7.36 (d, J = 8.1 Hz, 1H), 7.61 (d, J = 7.8 Hz, 1H); ms: (chemical ionization) (% relative intensity) m/z 337 (molecular ion + H$^+$, 77), 320 (100), 262 (38).

Anal. Calcd. for $C_{21}H_{24}N_2O_2$ (336.4): C, 74.97; H, 7.19; N, 8.33. Found: C, 74.29 [18]; H, 7.18; N, 8.07.

5-Bromo-N^{i}-(4-phenylbutyl)tryptophan (**12**).

A suspension of **11** (prepared by the method of Prasitpan *et al.* [19]) (1.18 g, 4.17 mmoles) in liquid ammonia (30 ml) was added carefully to a stirred reaction mixture of sodium (0.40 g, 0.017 g-atom) in liquid ammonia (50 ml). After 30 minutes, a solution of **7** (1.19 g, 5.57 mmoles) in anhydrous ether (2 ml) was added dropwise to the reaction mixture over 10 minutes. The mixture was allowed to warm to room temperature while stirring overnight to evaporate excess ammonia. Compound **12** (0.9 g, 52%) was isolated from the residue in the same manner as described for **10** to give a white solid, mp 192-194°; tlc, R_f = 0.47 (97:3, v:v, ethanol/30% ammonium hydroxide); ^{1}H nmr (perdeuteriomethanol + trifluoroacetic acid): δ 1.62 (m, 2H), 1.83 (m, 2H), 2.61 (t, J = 7.5 Hz, 2H), 3.31 (dd, J = 15 and 7.5 Hz, 1H), 3.40 (dd, J = 15 and 5.4 Hz, 1H), 4.18 (t, J = 6.6 Hz, 2H), 4.22 (dd, J = 7.5 and 5.4 Hz, 1H), 4.95 (s, all exchangeable protons), 7.11-7.31 (br m, 8H), 7.76 (d, J = 1.5 Hz, 1H); ms: (positive fast atom bombardment) (% relative intensity) m/z 417, 415 (molecular ion + H$^+$, 77, 78 bromine isotopes), 400, 398 (100, 100).

Anal. Calcd. for $C_{21}H_{23}BrN_2O_2$ (415.3): C, 60.73; H, 5.58; Br, 19.24; N, 6.75. Found: C, 60.81; H, 5.55; Br, 18.95; N, 6.81.

N^{α}-(3-Phenylpropionyl)tryptophan (**15**).

A solution of hydrocinnamic acid, **13**, (3.0 g, 20 mmoles), benzene (10 ml, dried over sodium) and thionyl chloride (2.86 g, 24 mmoles) was refluxed for 3 hours under a nitrogen atmosphere. The mixture was evaporated to give the acid chloride **14** as a colorless liquid which was taken into anhydrous THF (5 ml) and added dropwise to a solultion of **8** (4.08 g, 20 mmoles) in 10% sodium hydroxide (10 ml) which had been cooled in an ice-water bath. The mixture was stirred for 30 minutes, and then acidified with 1 *N* hydrochloric acid to *p*H 3-4. The precipitated solid was collected by filtration, washed with water and air dried to give **15** (5.0 g, 75%). Recrystallization from methanol/chloroform gave the analytical sample, mp 161-162°; tlc, R_f = 0.43 (180:19:1, v:v:v, chloroform/methanol/acetic acid); ^{1}H nmr (perdeuteriomethanol): δ 2.43 (t, J = 7.7 Hz, 2H), 2.78 (t, J = 7.9 Hz, 2H), 3.12 (dd, J = 15 and 8.1 Hz, 1H), 3.30 (dd, J = 15 and 5 Hz, 1H), 4.72 (dd, J = 8.1 and 5 Hz, 1H), 4.95 (s, 3H), 6.96-7.22 (br m, 8H), 7.32 (d, J = 8.1 Hz, 1H), 7.54 (d, J = 6.9 Hz, 1H); ms: (chemical ionization) (% relative intensity) m/z 337 (molecular ion + H$^+$, 100).

Anal. Calcd. for $C_{20}H_{20}N_2O_3$ (336.4): C, 71.41; H, 5.99; N, 8.33. Found: C, 71.31; H, 5.89; N, 8.36.

N^{α}-(4-Phenylbutanoyl)tryptophan (**18**).

A solution of 4-phenylbutanoic acid, **16** (1.64 g, 10 mmoles), benzene (10 ml, dried over sodium), and thionyl chloride (2.86 g, 24 mmoles) was refluxed for 3 hours under a nitrogen atmosphere. Acid chloride **17** was obtained as a colorless liquid after evaporation of benzene which was then used to acylate **8**, (2.04 g, 10 mmoles) in a manner anlogous to that described for **15**, to give **18** (2.8 g, 80%). The amide was recrystallized from methanol/ethyl acetate to give the analytical sample, mp 158-159°; tlc, R_f = 0.42 (180:19:1, v:v:v, chloroform/methanol/acetic acid); ^{1}H nmr (perdeuteriomethanol): δ 1.75 (m, 2H), 2.03 (t, J = 7.5 Hz, 2H), 2.44 (t, J = 7.5 Hz, 2H), 3.16 (dd, J = 15 and 6 Hz, 1H), 3.27 (dd, J = 15 Hz, and 5.4 Hz, 1H), 4.11 (br s, 3H), 4.76 (dd, J = 6 and 5.4 Hz, 1H), 6.92-7.16 (br m, 9H), 7.25 (d, J = 8.1 Hz, 1H), 7.45 (d, J = 8.1 Hz, 1H); ms: (chemical ionization) (% relative intensity) m/z 351 (molecular ion + H$^+$, 100).

Anal. Calcd. for $C_{21}H_{22}N_2O_3$ (350.4): C, 71.98; H, 6.33; N, 7.99. Found: C, 72.21; H, 6.40; N, 8.02.

5-Bromo-N^{α}-(3-phenylpropionyl)tryptophan (**19**).

In a manner analogous to that described for **15**, acid chloride **14** was prepared from **13** (1.5 g, 10 mmoles) and mixed with **11** (2.83 g, 10 mmoles). The reaction mixture was acidified to *p*H 4 with 1 *N* hydrochloric acid and extracted with dichloromethane (4 x 50 ml). The organic layer was dried (magnesium sulfate) and evaporated to a small volume. Petroleum ether (50 ml) was added and a solid was obtained by triturating the oily residue. The solid was collected by filtration and dried to give **19** (3.0 g, 72%. Recrystallization from dichloromethane/petroleum ether provided the analytical sample, mp 151-152°; tlc, R_f = 0.54 (180:19:1, v:v:v, chloroform/methanol/acetic acid); ^{1}H nmr (deuteriochloroform + perdeuteriomethanol): δ 2.44 (t, J = 8.0 Hz, 2H), 2.89 (t, J = 7.8 Hz, 2H), 3.24 (m, 2H, indole-CH_2 overlapped with perdeuteriomethanol solvent peak), 4.84 (m, 1H), 6.30 (d, J = 7.8 Hz, < 1H due to partial deuterium exchange), 6.79 (s, 1H); 7.13-7.27 (br m, 7H), 7.67 (s, 1H), 8.85 (s, < 1H due to partial exchange); ms: (chemical ionization) (% relative intensity) m/z 417, 415 (molecular ion + H$^+$, 98, 100 bromine isotopes).

Anal. Calcd. for $C_{20}H_{19}BrN_2O_3$ (415.3): C, 57.85; H, 4.61; Br, 19.24; N, 6.75. Found: C, 57.81; H, 4.72; Br, 19.23; N, 6.73.

5-Bromo-N^{α}-(4-phenylbutanoyl)tryptophan (**20**).

In a manner analogous to that described for **19**, **11** (2.83 g, 10 mmoles) was acylated with acid chloride **17** prepared as above from **16** (1.64 g, 10 mmoles). The amide **20** (2.5 g, 60%) obtained was recrystallized from chloroform/petroleum ether, mp 175-176°; tlc, R_f = 0.39 (180:19:1, v:v:v, chloroform/methanol/acetic acid); ^{1}H nmr (deuteriochloroform + perdeuteriomethanol): δ 1.83 (m, 2H), 2.11 (t, J = 7.5 Hz, 2H), 2.52 (t, J = 7.7 Hz, 2H), 3.17 (dd, J = 15 Hz and 5.7 Hz, 1H), 3.28 (dd, J = 15 and 5.1 Hz, 1H), 3.37 (br s, 2H), 4.80 (dd, J = 5.1 Hz and 5.7 Hz, 1H), 6.97 (s, 1H), 7.01-7.22 (br m, 7H), 7.63 (s, 1H); ms: (chemical ionization) (% relative intensity) m/z 431, 429 (molecular ion + H^+, 94, 100 bromine isotopes).

Anal. Calcd. for $C_{21}H_{21}BrN_2O_3$ (429.3): C, 58.75; H, 4.93; Br, 18.61; N, 6.53. Found: C, 59.06; H, 4.99; Br, 18.52; N, 6.54.

1-(2-Phenylethyl)-3,4-dihydro-β-carboline-3-carboxylic Acid (**21**).

To a suspension of **15** (500 mg, 1.49 mmoles) in dichloromethane (15 ml) was added dicyclohexylcarbodiimide (614 mg, 2.98 mmoles) and the mixture was stirred under a nitrogen atmosphere for 3 hours in a water bath maintained at 20-25°. The mixture was then filtered into a flask containing trifluoroacetic acid (20 ml). This mixture was heated at reflux (50-55°) in an oil bath overnight. Toluene (25 ml) was added to the cooled solution and the mixture was evaporated *in vacuo* to an oily residue. The residue was dissolved in ethyl acetate (2 ml) and allowed to stand. Dicyclohexylurea crystallized and was removed by filtration. This process was repeated until the greenish-yellow β-carboline was obtained during the second or third crystallization. Compound **21** was collected by filtration and obtained as the trifluoroacetate salt (325 mg, 68%). Recrystallization from methanol/chloroform gave the analytical sample, mp 168-169°; tlc, R_f = 0.14 (180:19:1, v:v:v, chloroform/methanol/acetic acid); ^{1}H nmr (DMSO-d_6): δ 3.05 (t, J = 8.1 Hz, 2H), 3.39 (t, J = 8.1 Hz, 2H), 3.49 (m, 1H), 3.64 (dd, J = 18 and 6.9 Hz, 1H), 5.03 (dd, J = 7.8 and 7.2 Hz, 1H), 7.19-7.33 (br m, 6H), 7.48 (t, J = 7.5 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 8.1 Hz, 1H), 8.32 (s, 1H), 12.67 (s, 1H); ms: (chemical ionization) (% relative intensity) m/z 303 (13), 276 (23), 275 (100).

Anal. Calcd. for $C_{20}H_{18}N_2O_2 \cdot CF_3COOH$ (318.4): C, 61.11; H, 4.43; N, 6.48. Found: C, 61.40; H, 4.52; N, 6.47.

1-(3-Phenylpropyl)-3,4-dihydro-β-carboline-3-carboxylic Acid (**22**).

In a manner similar to that described for the preparation of **21**, amide **18** (500 mg, 1.43 mmoles) was treated with dicyclohexylcarbodiimide (590 mg, 2.86 mmoles) in dichloromethane (15 ml) and then with trifluoroacetic acid (20 ml). The yellowish β-carboline derivative **22** (10 mg, 21%) was obtained by crystallization and dried *in vacuo*, mp 138-140°; tlc, R_f = 0.15 (180:19:1, v:v:v, chloroform/methanol/acetic acid); ^{1}H nmr (perdeuteriomethanol + trifluoroacetic acid): δ 2.20 (m, 2H), 2.84 (t, J = 7.5 Hz, 2H), 3.15 (m, less than 2H due to exchange), 3.50 (dd, J = 18.2 and 8.3 Hz, 1H), 3.72 (dd, J = 18.2 and 5.9 Hz, 1H), 4.90 (dd, J = 8.3 and 5.9 Hz, 1H), 5.1 (br s, all exchangeable protons), 7.13-7.23 (br m, 6H), 7.50 (m, 2H), 7.73 (d, J = 8.4 Hz, 1H); ms: (chemical ionization) (% relative intensity) m/z 317 (14), 290 (24), 289 (100).

Anal. Calcd. for $C_{21}H_{20}N_2O_2 \cdot 0.5H_2O$ (332.4 + $0.5H_2O$): C, 73.88; H, 6.20; N, 8.20. Found: C, 73.11 [18]; H, 6.02; N, 8.06.

6-Bromo-1-(2-phenylethyl)-3,4-dihydro-β-carboline-3-carboxylic Acid (**23**).

To a solution of amide **19** (500 mg, 1.2 mmoles) in anhydrous dichloroethane (15 ml) was added dicyclohexylcarbodiimide (500 mg, 2.4 mmoles), and the resulting mixture was stirred under a nitrogen atmosphere for 5 hours in a water bath maintained at 20-25°. The mixture was then filtered into a flask containing trifluoroacetic acid (15 ml), and the resulting solution was heated at reflux (80-85°) in an oil bath overnight. The mixture was evaporated to dryness on a rotary evaporator *in vacuo*, and dicyclohexylurea was removed by crystallization from ether. The yellowish β-carboline derivative **23** was obtained from the filtrate after purification by flash chromatography on silica gel. Initial elution with ethyl acetate/chloroform (1:1) was followed by chloroform/-methanol (49:1) and then stepwise to chloroform/methanol (17:3). The fractions containing **23** with R_f = 0.15 (180:19:1, v:v:v, chloroform/methanol/acetic acid) on tlc were pooled and evaporated to dryness. The residue was crystallized from methanol/ethyl acetate to provide 260 mg (65%) of **23**, mp 182-184°; ^{1}H nmr (DMSO-d_6): δ 3.06 (t, J = 8 Hz, 2H), 3.39-3.53 (m, 3H, 3.68 (dd, J = 18 and 6.6 Hz, 1H), 5.10 (dd, J = 7.5 and 6.6 Hz, 1H), 7.22-7.38 (br m, 7H), 7.57 (s, 1H), 8.18 (s, 1H), 12.99 (s, 1H); ms: (chemical ionization) (% relative intensity) m/z 355, 353 (81, 100 bromine isotopes).

Anal. Calcd. for $C_{20}H_{17}BrN_2O_2 \cdot 0.5H_2O$ (397.3 + $0.5H_2O$): C, 59.13; H, 4.47; Br, 19.67. Found: C, 59.16; H, 4.28; Br, 20.28.

6-Bromo-1-(3-phenylpropyl)-3,4-dihydro-β-carboline-3-carboxylic Acid (**24**).

In a manner analogous to that described for **23**, amide **20** (500 mg, 1.17 mmoles) was treated with dicyclohexylcarbodiimide (481 mg, 2.33 mmoles) in dichloroethane (15 ml). The filtrate obtained was heated at reflux (80-85°) with trifluoroacetic acid (20 ml) overnight. The light yellowish β-carboline derivative **24** (190 mg, 40%) was obtained by crystallization followed by flash chromatography (silica gel) with chloroform/methanol (49:1) to chloroform/methanol (17:3) eluants, mp 162-163°; tlc, R_f = 0.15 (180:19:1, v:v:v, chloroform/methanol/acetic acid); ^{1}H nmr (perdeuteriomethanol + trifluoroacetic acid): δ 2.24 (m, 2H), 2.87 (t, J = 7.5 Hz, 2H), 3.19 (m, less than 2 protons due to exchange, $-N=C-CH_2-$), 3.51 (dd, J = 18 and 8.3 Hz, 1H), 3.71 (dd, J = 18 and 6.3 Hz, 1H), 4.95 (m, 1H); 4.97 (br s, all exchangeable protons), 7.16-7.28 (m, 5H), 7.49 (dd, J = 9.7 and 1.5 Hz, 1H), 7.61 (dd, J = 9 Hz and 1.8 Hz, 1H), 7.97 (s, 1H); ms: (chemical ionization) (% relative intensity) m/z 413, 411 (molecular ion + H^+, 4, 5 bromine isotopes), 397, 396 (8, 11), 369, 367 (81, 100).

Anal. Calcd. for $C_{21}H_{19}BrN_2O_2$ (411.3): C, 61.33; H, 4.66; Br, 19.43; N, 6.81. Found: C, 60.93; H, 4.61; Br, 19.35; N, 6.73.

Acknowledgements.

The authors gratefully acknowledge NIH grants HL 23697, HL 33134 and HL 45977 for partial support of this work. We especially thank Mr. Richard Dvorak for obtaining the mass spectral data.

REFERENCES AND NOTES

[1a] On leave from the Department of Chemistry, Kasetsart University, Bangkok, Thailand; [b] Present address: Hoechst-Celanese, Providence, RI; [c] Present address: Abbott Laboratories, Abbott Park, IL; [d] Present address: Genzyme Corp., Framingham, MA.

[2] W. N. Poillon, *Biochemistry*, **21**, 1400 (1982).

[3] P. Z. De Croos, P. Sangdee, B. L. Stockwell, L. Kar, E. B. Thompson, M. E. Johnson and B. L. Currie, *J. Med. Chem.*, **33**, 3138 (1990).

[4] P. Manavalan, M. Prabhakaran and M. E. Johnson, *J. Mol. Biol.*, in press (1991).

[5] E. A. Padlan and W. E. Love, *J. Biol. Chem.*, **260**, 8280 (1985).

[6] H.-Z. Lu, B. L. Currie and M. E. Johnson, *FEBS Letters*, **173**, 259 (1984).

[7] Y.-H. Lee, B. L. Currie and M. E. Johnson, *Biochemistry*, **25**, 5647 (1986).

[8] I. M. Russu, A. K.-L. C. Lin, C. P. Yang and C. Ho, *Biochemistry*, **25**, 808 (1986).

[9] J. P. Li, K. A. Newlander and T. O. Kellin, *Synthesis*, 73 (1988).

[10] S. Yamada, T. Shiori, T. Itaya, T. Hara and R. Matsueda, *Chem. Pharm. Bull.*, **13**, 88 (1965).

[11] S. Letellier and B. Fleury, *J. Heterocyclic Chem.*, **25**, 1265 (1988).

[12] H. Takeuchi and H. Tamura, *Br. J. Pharmacol.*, **69**, 29 (1980).

[13] Y. Ariyoshi and H. Takeuchi, *Br. J. Pharmacol.*, **77**, 631 (1982).

[14] H. Takeuchi and Y. Ariyoshi, *Comp. Biochem. Physiol.*, **72C**, 225 (1982).

[15] H. Takeuchi, Y. Ariyoshi and M. Okutsu, *Comp. Biochem. Physiol.*, **75C**, 329 (1983).

[16] L. Mazhani, B. C. Kim and W. N. Poillon, *Hemoglobin*, **8**, 129 (1984).

[17] T. J. McCord, D. H. Kelley, J. A. Rabon, D. C. Foyt and A. L. Davis, *J. Heterocyclic Chem.*, **9**, 119 (1972).

[18] More satisfactory analytical values for the following were unattainable for a variety of reasons: Br for **5c**; H for **5d**; C for **10**; and C for **22**.

[19] N. Prasitpan, M. E. Johnson and B. L. Currie, *Synth. Commun.*, **20**, 3459 (1990).