Table I

| Polypeptide | μg of protein N pptd at equiv pta | μg of protein N pptd by 1 after absorption[a] | % of protein N pptd by polypeptide[a] |
|---|---|---|---|
| (Tyr-Glu-Ala-Gly)$_n$Gly (1) | 106 | 0 | 100 |
| (Tyr-Asp-Ala-Gly)$_n$Gly (2) | 26 | 78 | 25 |

[a] Per milliliter of *anti*-poly(Tyr-Glu-Ala-Gly)Gly-1-$^{14}C$ Et ester-sera.

$Et_3N$ in 200 ml of $CH_2Cl_2$ was added 32.3 g (0.0565 mol) of Z-β-*tert*-Bu-Asp pentachlorophenyl ester. The mixture was stirred overnight at room temperature and concentrated, and the product was dissolved in EtOAc, washed with 1 *N* HCl and $H_2O$, then dried ($Na_2SO_4$), and concentrated under reduced pressure to give an oil. This material was chromatographed on a column of Silicar CC-7 using $CHCl_3$–EtOAc (1:1) as eluent to give the fully blocked tripeptide; crystallization from EtOAc–hexane yielded 16.3 g (62%): mp 122.5°; $[\alpha]^{24}D$ −7.7° (*c* 4.2, DMF). *Anal.* ($C_{22}H_{31}N_3O_8$) C, H, N.

Z-*O*-*tert*-Bu-Tyr-β-*tert*-Bu-Asp-Ala-Gly Me Ester (4). A suspension of 15.0 g (0.0322 mol) of 3 and 0.7 g of 10% Pd/C in 200 ml of MeOH was treated with 1.168 g (0.0322 mol) of dry HCl in MeOH, and the suspension was hydrogenated for 2 hr. The reaction mixture was filtered and the filtrate concentrated under reduced pressure. The residue was dissolved in 200 ml of $CH_2Cl_2$ and 3.58 g (0.0354 mol) of $Et_3N$ and 20.0 g (0.0324 mol) of Z-*O*-*tert*-Bu-Tyr pentachlorophenyl ester[‡] was then added. The reaction mixture was stirred overnight at room temperature and concentrated under reduced pressure, and the product was dissolved in EtOAc, washed with 1 *N* HCl and $H_2O$, then dried ($Na_2SO_4$), and concentrated under reduced pressure to give a solid. This material was chromatographed on a column of Silicar CC-7 using $CHCl_3$–EtOAc (1:1) as eluent to give the fully blocked tetrapeptide; crystallization from EtOAc–hexane yielded 13.0 g (58.7%): mp 131°; $[\alpha]^{24}D$ −20.2° (*c* 5.7, DMF). *Anal.* ($C_{35}H_{48}N_4O_{10}$) C, H, N.

Z-*O*-*tert*-Bu-Tyr-β-*tert*-Bu-Asp-Ala-Gly Pentachlorophenyl Ester (5). To a solution of 12.0 g (0.0175 mol) of 4 in 250 ml of MeOH was added 17.5 ml of 1 *N* KOH and the solution was stirred for 90 min at room temperature and then concentrated under reduced pressure. The residue was flooded with $H_2O$, acidified with 10% citric acid solution, and extracted into EtOAc. The EtOAc solution was dried ($Na_2SO_4$) and concentrated under reduced pressure to give 11.7 g (100%) of the tetrapeptide free acid. This material was dissolved in 250 ml of $CH_2Cl_2$, and 4.5 g (0.0167 mol) of pentachlorophenol and 7.1 g (0.0167 mol) of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methotoluene-*p*-sulfonate were added. The mixture was shaken for 2 days at room temperature and then concentrated under reduced pressure to give a solid. This material was washed with $H_2O$ and crystallized from MeOH to yield 1.1 g (8%): mp 188°; $[\alpha]^{24}D$ −19.8° (*c* 1.64, DMF). *Anal.* ($C_{40}H_{45}Cl_5N_4O_{10}$) C, H, N.

*O*-*tert*-Bu-Tyr-β-*tert*-Bu-Asp-Ala-Gly Pentachlorophenyl Ester·HCl (6). A suspension of 3.3 g (0.0036 mol) of the tetrapeptide active ester 6 and 0.7 g of 10% Pd/C in 200 ml of MeOH was treated with 0.1314 g (0.0036 mol) of dry HCl in MeOH, and the suspension was hydrogenated for 2 hr. The reaction mixture was filtered and the filtrate concentrated. The residue was crystallized from MeOH–$Et_2O$ to give 2.3 g (78%): mp 152°; $[\alpha]^{24}D$ −7.5 (*c* 2.7, DMF). *Anal.* ($C_{32}H_{40}Cl_6 \cdot N_4O_8 \cdot H_2O$) C, H, N.

Poly(Tyr-Asp-Ala-Gly)Gly Me Ester (2). To a solution of 0.9 g (0.00896 mol) of $Et_3N$ and 0.64 mg of Gly Me ester·HCl in 5 ml of DMSO was added a solution of 2.1 g (0.00256 mol) of the polymerization unit 6 in 20 ml of DMSO. The mixture was shaken for 1 week and then centrifuged to yield the fully protected polymer which was washed with three 35-ml portions of $H_2O$, three 35-ml portions of MeOH, and three 35-ml portions of $Et_2O$ and dried to give the blocked polymer. This material was treated with 30 ml of 90% $F_3C \cdot CO_2H$ and stirred for 50 min and then concentrated under reduced pressure to yield the crude polypeptide 2. This material was suspended in 20 ml of $H_2O$ and dissolved by the addition of 1 *N* NaOH to pH 7.5. The solution was dialyzed against distilled $H_2O$ overnight and acidified with 6 *N* HCl to pH 2.5 and dialyzed against distilled $H_2O$ for a day. The precipitated polypeptide 2 was collected by centrifugation and then lyophilized to yield 0.1 g (10%); amino acid ratios of an acid hydrolysate, $Tyr_{1.0}$ $Asp_{1.0}$ $Ala_{1.0}$ $Gly_{1.0}$.

Molecular Weight Determination. The polypeptide 1 (0.1 g) was dissolved in 50 ml of $H_2O$ by the addition of 1 *N* NaOH to pH 7.4. This solution was separated into four different molecular weight ranges by successive diafiltrations through Diaflo membranes XM 50, UM 20E, and UM 10.[#] Each fraction was acidified with 6 *N* HCl, dialyzed, and lyophilized to yield 0.05 g, mol wt >50,000; 0.01 g, mol wt 20–50,000; 0.01 g, mol wt 10–20,000; 0.014 g, mol wt <10,000.

Immunochemical Procedures. Eight rabbits were treated with poly(Tyr-Glu-Ala-Gly)Gly-1-$^{14}C$ Et ester (1) at weekly intervals, using the immunization schedule previously described;[3] 25 days after the last injection all rabbits were bled using the standard heart puncture technique. Serum from each rabbit was tested for the precipitin reaction with the homologous antigen 1; serum from each animal gave a positive precipitin reaction. The serum from each animal was pooled and this combined serum was used for the following experiments. It was assumed that the antibodies produced by each rabbit after the same time interval were directed against the same antigenic determinants of poly(Tyr-Glu-Ala-Gly)Gly-1-$^{14}C$ Et ester (1).

Quantitative Precipitin Reactions. To 1-ml aliquots of the pooled rabbit serum was added incremental amounts of the polypeptide 2. Each tube was made up to a total of 2 ml with buffer (0.1 *M* NaCl–0.05 *M* $NaHCO_3$) and incubated for 1 hr at 37° and then kept at 4° for 48 hr. The tubes were centrifuged in the cold and the precipitates were washed twice with 1 ml of buffer (0.05 *M* $K_2HPO_4$–NaOH), pH 7.0. The total amount of protein precipitate was estimated by analysis for N (Kjeldahl). An identical procedure was used for the homologous polymer 1 which was run simultaneously with that used for the polypeptide 2. The comparative precipitin curves are shown in Figure 1.

Absorption Studies. The pooled rabbit serum was treated with the equivalent point amount of the heterologous polypeptide 2 as described above. The precipitate was centrifuged out and the supernatant liquid was poured off into a separate tube. To this was added 30 μg of the homologous antigen, poly(Tyr-Glu-Ala-Gly)Gly-1-$^{14}C$ Et ester (1). The mixture was incubated at 37° for 1 hr and then stored at 4° for 48 hr. The precipitate was collected by centrifugation and washed twice with 1 ml of buffer solution (0.05 *M* $K_2HPO_4$–NaOH), pH 7.0. The amount of protein precipitate was estimated by analysis for N (Kjeldahl). The amount of precipitate obtained using this procedure is shown in Table I. A control in which the serum was first absorbed with the homologous antigen 1 ascertained that the homologous antigen precipitated all of the antibody, since the supernatant liquid gave no further precipitation reaction when 30 μg of 1 was added.

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## Synthesis of 3,4-Dihydroxy-5-fluoro-DL-phenylalanine and 3,4-Dihydroxy-5-[$^{18}$F]fluoro-DL-phenylalanine

Gunter Firnau,* Claude Nahmias, and Stephen Garnett

*Nuclear Medicine, McMaster University, Hamilton, Ontario, Canada.*
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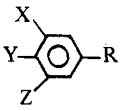
Radioactive agents ranging from simple ions to protein molecules have been used in an attempt to visualize malignant or vascular disease in the brain by means of external detectors. These agents are nonspecific and detect only

Table 1



| | Compound | R | X | Y | Z | Mp, °C | Yield, % |
|---|---|---|---|---|---|---|---|
| 1 | 3,4-Dimethoxy-5-nitrobenzaldehyde | CHO | MeO | MeO | $NO_2$ | 85 | 75 |
| 2 | 3,4-Dimethoxy-5-nitrobenzyl alcohol | $CH_2OH$ | MeO | MeO | $NO_2$ | 81 | 64 |
| 3 | 3,4-Dimethoxy-5-nitrobenzyl chloride | $CH_2Cl$ | MeO | MeO | $NO_2$ | 165 | 89 |
| 4 | Diethyl acetamido(3,4-dimethoxy-5-nitrobenzyl)-malonate | $C(COOC_2H_5)_2NHAc$ | MeO | MeO | $NO_2$ | 115 | 60 |
| 5 | Diethyl acetamido(3,4-dimethoxy-5-aminobenzyl)-malonate hydrochloride | $C(COOC_2H_5)_2NHAc$ | MeO | MeO | $NH_2HCl$ | 126 | *a* |
| 6 | 5-(2',2'-Dicarbethoxy-2'-acetamidoethyl)-2,3-dimethoxybenzyldiazonium fluoroborate | $C(COOC_2H_5)_2NHAc$ | MeO | MeO | $N_2BF_4$ | 132 dec | 76 |
| 7 | Diethyl acetamido(3,4-dimethoxy-5-fluorobenzyl)-malonate | $C(COOC_2H_5)_2NHAc$ | MeO | MeO | F | 105 | |
| 8 | 3,4-Dihydroxy-5-fluorophenylalanine hydrobromide | $CH_2CH(NH_3Br)COOH$ | OH | OH | F | 170 | 62[b] |

[a]Quantitative. [b]Calculated from 6.

about two-thirds of lesions. Dihydroxyphenylalanine (DOPA) enters healthy, mature brain capillaries where it is decarboxylated to dopamine. Dopamine can be concentrated in the capillaries by an amine oxidase inhibitor and demonstrated by fluoresence microscopy. Damaged or immature capillaries will not do this.[1] DOPA labeled with a suitable $\gamma$-emitting isotope would, therefore, seem to be a specific substance which might allow a distinction to be made between normal and abnormal brain capillaries by means of external $\gamma$-ray detection.

$^{18}F$ was chosen as the label since it has a high photon yield and a short half-life (110 min) and DOPA decarboxylase is unlikely to distinguish between DOPA and fluoro-DOPA owing to its broad specificity.[2] The purpose of this study was to develop a rapid synthesis for 3,4-dihydroxy-5-fluoro-DL-phenylalanine (5-fluoro-DOPA), which would be suitable for a $^{18}F$-labeling procedure.

The known syntheses of fluoro derivatives of aromatic amino acids use the following approach.[3-6] Fluorine is introduced by the Balz–Schiemann reaction sequence (arylamine, aryldiazonium fluoroborate, aryl fluoride) in a simple substituted aromatic compound. The amino acid side chain is then built up using either the malonic ester synthesis of Erlenmeyer's azlactone synthesis. This approach conforms to the generally accepted view that the Balz–Schiemann reaction yields best results when complicated side chains are absent.[7] Since the above procedures are time consuming, they cannot be used for labeling with $^{18}F$. This communication describes a new rapid synthesis for 5-fluoro-DOPA which is suitable as a $^{18}F$ labeling procedure.

The starting material, 3-methoxy-4-hydroxy-5-nitrobenzaldehyde, was methylated to 3,4-dimethoxy-5-nitrobenzaldehyde (**1**) which, in turn, was reduced to 3,4-dimethoxy-5-nitrobenzyl alcohol (**2**). The alcohol **2** was transformed into 3,4-dimethoxy-5-nitrobenzyl chloride (**3**). The chloride **3** was condensed with diethyl acetamidomalonate to give diethyl acetamido(3,4-dimethoxy-5-nitrobenzyl)malonate (**4**). The nitromalonic ester **4** was next reduced to the amino malonic ester **5**. This was then diazotized to give the diazonium fluoroborate **6**. The diazonium fluoroborate **6** was pyrolyzed to yield the fluoro malonic ester **7**. Finally, 3,4-dihydroxy-5-fluoro-DL-phenylalanine hydrobromide (**8**) was obtained by hydrolysis and ether cleavage of **7**. HBr (47%) was used instead of a 48% HI–$Ac_2O$ mixture since the latter damaged the molecule. It is essential that the reaction temperature during the HBr hydrolysis is maintained between 114 and 116° to eliminate by-reactions.

The diazonium fluoroborate **6** can be prepared in quantity and stored. A semimicro synthesis can be complete in 4 hr starting with **6**. 3,4-Dihydroxy-5-[$^{18}F$]fluorophenylalanine has been successfully synthesized using the procedure outlined above. **6** was allowed to exchange with the $^{18}F$ fluoride in aqueous solution, and the dried [$^{18}F$]diazonium fluoroborate ($^{18}F$-**6**) was then decomposed and hydrolyzed.

As far as is known, this is the first time that 5-fluoro-DOPA has been prepared. 2-Fluoro-DOPA has been synthesized in the traditional way[4] and 4-fluorophenylalanine has been prepared in a number of ways.[8-11] However, it should be pointed out that the approach described here can be used to introduce fluorine into other ring-substituted aromatic amino acids. The compounds are described in Table I.

## Experimental Section

**3,4-Dimethoxy-5-nitrobenzaldehyde (1).**[12] 5-Nitrovanillin (3-methoxy-4-hydroxy-5-nitrobenzaldehyde, Aldrich), 20 g (0.101 mol), was transformed to the K salt using concentrated KOH. The dried K salt was treated with an excess of dimethyl sulfate to yield **1** which was crystallized from 60% aqueous EtOH.

**3,4-Dimethoxy-5-nitrobenzyl Alcohol (2).**[13] A solution of 14 g (0.066 mol) of **1** in 60 ml of THF was dripped into a suspension of 5 g (0.132 mol) of $NaBH_4$ in 50 ml of THF and kept at 40°. The reaction was allowed to proceed for 1 hr under stirring. The excess $NaBH_4$ was destroyed by addition of water and a few crystals of $NaH_2PO_4$. THF and water were rotary evaporated, and the residue was extracted three times with ether. The ether layer was dried over $CaCl_2$ and evaporated yielding **2**: $\nu_{max}^{aryl\ NO_2}$ 1540 $cm^{-1}$ (KBr).

**3,4-Dimethoxy-5-nitrobenzyl Chloride (3).**[14] A suspension of 8 g (0.037 mol) of **2** in 50 ml of concentrated HCl (0.46 mol) was heated at 110° for 30 min. After cooling the solution was extracted three times with benzene. The benzene layer was dried over $CaCl_2$ and evaporated yielding **3**: $\nu_{max}^{aryl\ NO_2}$ 1540 $cm^{-1}$; $\nu_{max}^{CCl}$ 745 $cm^{-1}$ (KBr).

**Diethyl Acetamido(3,4-dimethoxy-5-nitrobenzyl)malonate (4).**[15] **3** (4 g, 0.017 mol) was condensed with 4 g (0.018 mol) of diethyl acetamidomalonate (Aldrich) in NaOEt yielding **4** which was recrystallized from 60% aqueous EtOH: $\nu_{max}^{aryl\ NO_2}$ 1540 $cm^{-1}$. *Anal.* ($C_{18}H_{24}O_9N_2$) C, H.

**Diethyl Acetamido(3,4-dimethoxy-5-aminobenzyl)malonate Hydrochloride (5).**[10] **4** (10 g, 0.0237 mol) dissolved in AcOH was reduced in a hydrogenerator under a pressure of 2.5 atm at room temperature using Pd/C (5%). The catalyst was filtered off and the solution dripped into an excess of concentrated HCl. Evaporation of the AcOH yielded a syrup from which **5** was recrystallized using 60% aqueous EtOH: $\nu_{max}^{aryl\ NO_2}$ absent (KBr). *Anal.* ($C_{18}H_{27}O_9N_2$) C, H.

**5-(2',2'-Dicarbethoxy-2'-acetamidoethyl)-2,3-dimethoxybenzyldiazonium Fluoroborate (5).** A solution of 5 g (0.0119 mol) of **5** in

42 ml of HCl (0.0168 mol) was cooled to 0° and a solution of 0.82 g of $NaNO_2$ (0.0119 mol) in 5 ml of water was added over a period of 15 min under constant stirring. $HBF_4$ (48%) (7 ml, 0.04 mol) was then added at once. While stirring in an ice bath, little yellow crystals of 6 separated which were recrystallized from $Me_2CO$ by adding $Et_2O$. *Anal.* ($C_{18}H_{24}O_7N_3BF_4$) C, H, F.

**Diethyl Acetamido(3,4-dimethoxy-5-fluorobenzyl)malonate (7).** A suspension of 2 g ($4.17 \times 10^{-3}$ mol) of **6** in 200 ml of xylene decomposed smoothly at 132° for 1–2 hr. During the decomposition 7 went into solution, leaving a minimal amount of tar undissolved. Xylene was rotary evaporated yielding 7 which usually was processed further without purification. In order to identify the product, one batch was purified in the following way. The remainder, after the xylene evaporation, was dissolved in absolute EtOH and neutralized with aqueous NaOH. After evaporation of the EtOH, the product was dissolved in a small amount of hot benzene and passed through an alumina III column (1.6 × 8 cm). Approximately 100 ml of benzene was used to elute the product. After evaporation of the benzene 7 crystallized upon cooling. *Anal.* ($C_{18}H_{24}O_7NF$) C, H, N.

**3,4-Dihydroxy-5-fluorophenylalanine Hydrobromide (8).** The solution of the above residue from xylene evaporation of 7 in 20 ml of HBr (47%) was heated in an oil bath at 146–148° (*e.g.*, temperature of reaction mixture 114–116°) for 2 hr, while $H_2$ was passed through. HBr was removed by rotary evaporation with 10 ml of water. The dry, reddish crystalline residue was dissolved in 10 ml of water and boiled with activated charcoal. After filtration, the clear filtrate was evaporated to dryness. The yellowish residue was recrystallized from *i*-$Pr_2O$–*i*-PrOH (1:1) yielding white crystals. *Anal.* ($C_9H_{11}O_4NBrF$) C, H, N, F. The product appeared homogenous in the tlc (cellulose layer, *n*-BuOH–AcOH–$H_2O$, 10:1:1, detection with ninhydrin) and ion-exchange column chromatography (Dowex 50-X8, 200–400 mesh, $Na^+$ form; 1.4 × 24 cm, 0.1 *M* phosphate buffer, pH 6.8):[16] $\lambda_{max}^{0.1\ N\ HCl}$ 276 nm ($\epsilon$ 660); $\nu_{max}^{aryl\ F}$ 1190 $cm^{-1}$ (KBr); $^1H$ nmr ($D_2O$) δ ($H_2O$) +1.94 (2 H, m, aromatic), −0.2 (1 H, q, $J_{ab}$ = 5 Hz, $J_{bc}$ = 6 Hz, -C*H*$NH_2$), −1.6 ppm (2 H, m, $CH_2$); $^{19}F$ nmr ($D_2O$) δ ($CFCl_3$) +135 ppm (upfield) (1 F, d, separation 12.7 Hz, X portion of ABX system).

**3,4-Dihydroxy-5-[$^{18}$F]fluorophenylalanine.** Lithium carbonate (2 g, 95% enriched in $^6Li^+$) was irradiated for 4 hr in the McMaster 5-MW swimming pool nuclear reactor at a neutron flux of $2 \times 10^{13}$ neutrons $cm^{-2}$ $sec^{-1}$. Fluorine-18 is produced by the nuclear reaction sequence $^6Li(n,^4He)^3H$ and $^{16}O(^3H,n)^{18}F$. The lithium carbonate was dissolved with 10 ml of diluted sulfuric acid (4 ml of concentrated $H_2SO_4$ + 6 ml of water).

Water (7 ml) was then distilled from this solution. Air was passed through the apparatus to speed the distillation. More than 80% of the original $^{18}F$ activity was distilled.

Typically, two distillations were performed and the distillates were combined so that a final volume of 14 ml (pH 4) contained approximately 20 mCi of carrier free $^{18}F$. The diazonium fluoroborate **6** (90 mg, $1.84 \times 10^{-4}$ mol) was dissolved in this solution. The solution was maintained at 50° and was shaken occasionally.

After 30 min, the water was rotary evaporated and the yellow crystalline residue dried over $P_2O_5$ *in vacuo* for 15 min. The dried material was taken up in 5 ml of dioxane and filtered to remove any inorganic salts remaining. The filtrate was heated to 80° in a 50-ml flask and xylene (26 ml) was then added very slowly. Care was taken to ensure that the temperature in the flask did not fall below 80°. When all xylene had been added, the temperature was raised until the mixture refluxed. Decomposition of the diazonium fluoroborate ($^{18}F$-**6**) was complete when the deep yellow color of the mixture changed to pale yellow. (This usually took 30 min.) The dioxane–xylene mixture was cooled and then rotary evaporated until a yellowish-brown residue remained. This residue, fluoromalonic ester **7**, was dissolved in 10 ml of HBr (47%) and heated in an oil bath at 146–148°. $H_2$ was bubbled through this solution during the hydrolysis. After 45 min, HBr was rotary evaporated. In order to remove the excess of HBr, HF, and $HBF_4$, the dry residue was twice redissolved in water and evaporated. Water (15 ml) was used at each step. The residue was redissolved for a third time in water (7 ml) and was decolorized by boiling with activated charcoal. The final colorless solution contained 5-[$^{18}$F]fluoro-DOPA with a specific activity ranging from 0.2 to 2.0 μCi/mg of amino acid.

For the determination of the specific activity, an aliquot was chromatographed on an ion-exchange resin column (1.4 × 24 cm, AG50-X8, 200–400 mesh, $Na^+$ form; eluent 0.1 *M* Na phosphate buffer, pH 6.8).[16] The effluent was collected in fractions. Each fraction was measured for $^{18}F$ radioactivity using a 5 × 5 cm NaI (Tl) well-type crystal coupled to a single channel analyzer. The counts measured represent the intensity of the 511 keV γ peak (efficiency of the crystal 7%). The obtained elution pattern shows only a single $^{18}F$ peak after approximately three void volumes. This elution position corresponds to that of authentic 5-fluoro-DOPA. The $^{18}F$-containing fractions were combined and the amount of 5-fluoro-DOPA was estimated by uv absorption at 276 mm.

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# Alkaloids in Mammalian Tissues. 3. Condensation of L-Tryptophan and L-5-Hydroxytryptophan with Formaldehyde and Acetaldehyde[1]

Arnold Brossi,* Antonino Focella, and Sidney Teitel

*Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.* *Received September 25, 1972*

Biogenic primary amines and their amino acid precursors react readily *in vitro* with carbonyl compounds under so-called physiological conditions to afford 1,2,3,4-tetrahydroisoquinolines and related condensation products.[2] Since it has been speculated that similar reactions might also take place *in vivo* to generate alkaloidal-type substances (for leading references, see ref 3), a variety of substituted tetrahydroisoquinolines have recently been prepared from dopamine[1] and its biogenic precursor L-dopa.[4] As an extension of this study, we now report the *in vitro* condensation of L-tryptophan and L-5-hydroxytryptophan, two physiologically important amino acids found in mammalian tissues,[5] with $CH_2O$ and $CH_3CHO$ as well as the preliminary pharmacological evaluation of the resulting 3-carboxy-substituted 1,2,3,4-tetrahydro-β-carbolines **1a**,**c**, **2a**,**c**, and **3a**,**c**, and the related *N*-methyl derivatives **1b**,**d** and **2b**,**d**.

All of the secondary amino acids were obtained by a Pictet–Spengler condensation between the substrate and the

†Oak Ridge National Laboratory, Oak Ridge, Tenn.