Stereoselective Synthesis of Stable Isotope-Labeled L-α-Amino Acids: Chemomicrobiological Synthesis of L-[β-^{13}C]-, L-[2'-^{13}C]-, and L-[1'-^{15}N]Tryptophan

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SUMMARY

We have developed a stereospecific chemomicrobiological synthesis of labeled tryptophan. L-[3-^{13}C]Serine, [1-^{15}N]- and [2-^{13}C]indole were used as precursors for the synthesis of L-[β-^{13}C]-, L-[1'-^{15}N]-, and L-[2'-^{13}C]tryptophan, respectively. The labeled precursors were incorporated quantitatively into tryptophan using a strain of *E. coli* engineered to overproduce tryptophan synthase. Labeled indoles were prepared by the base-promoted cyclization of appropriately labeled N-formyl-*o*-toluide; serine was prepared biosynthetically as described previously.

Keywords: L-[β-^{13}C]tryptophan, L-[3-^{13}C]tryptophan, L-[2'-^{13}C]tryptophan, L-[1'-^{15}N]tryptophan, L-[3-^{13}C]serine, [1-^{15}N]indole, and [2-^{13}C]indole.

INTRODUCTION

Stable isotope-labeled amino acids are required for studies of amino acid metabolism and for studies of peptide and protein structure and dynamics. For many of these applications, the naturally occurring L-configuration of the labeled amino acid is required. In general, specific labels have been introduced into racemic mixtures of α-amino acids which have been resolved using hog kidney acylase. We are developing strategies for the stereoselective synthesis of specifically labeled L-α-amino acids in which labeled L-serine, produced biosynthetically[1], serves as a template for the synthesis of more complex amino acids. The stereochemistry at the α-carbon produced during the biosynthesis of serine is retained in the product amino acid. In this manuscript, we

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describe the tryptophan synthase-catalyzed conversion of L-[3-^{13}C]serine to L-[β-^{13}C]tryptophan. In addition, we describe reproducible methods for the synthesis of [1-^{15}N]- and [2-^{13}C]indole and for their incorporation into L-tryptophan.

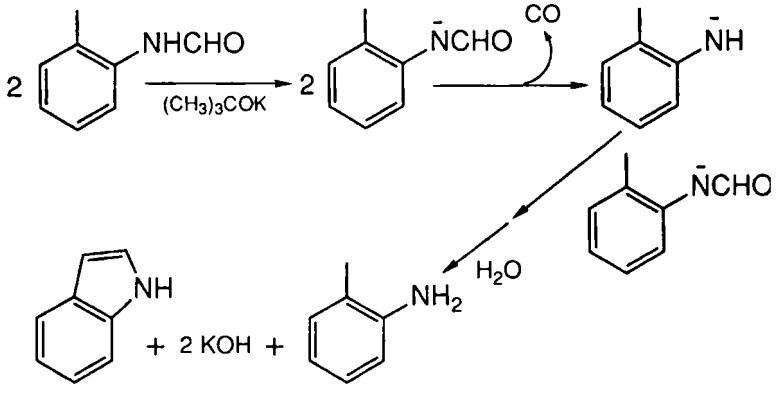
RESULTS AND DISCUSSION

The potential for the tryptophan synthase-catalyzed synthesis of L-tryptophan[2] and stable isotope-labeled L-tryptophan[3,4] has been recognized by others. In the cell, tryptophan synthase, a pyridoxyl phosphate-requiring enzyme, catalyzes the condensation of indoleglycerol phosphate (InGP) and L-serine to yield L-tryptophan and glyceraldehyde 3-phosphate. However, indole can substitute for InGP in the enzymatic reaction; therefore, labeled L-tryptophan can be produced from the appropriate isotopomer of L-serine or indole. Recently we reported the biosynthesis of specifically ^{13}C-labeled L-serine which can serve as a precursor for the synthesis of tryptophan labeled in the carbonyl, α-, and/or β-carbons[1].

The syntheses of [2-^{13}C]- and [1-^{15}N]indole have been reported[3,4]. Lutenberg and coworkers prepared ^{13}C- and ^{15}N-labeled indole in good yield by reductive ring closure of *o*-nitrobenzyl cyanide[3]. This nitrile was prepared in by the treatment of *o*-nitrobenzyl bromide with potassium cyanide in aqueous ethanol. The ring closure reaction was carried out using dilute solutions of 2-nitrobenzyl cyanide containing a 10% palladium on carbon catalyst and using a Parr apparatus at relatively low hydrogen pressure ($3.9X10^5$ Pa). Although several of us attempted to reproduce these results, we obtained only very poor yields of indole by this route. Similar to the report of Dippy and Pratt[5], our many and varied attempts to produce *o*-nitrobenzyl cyanide by cyanide displacement of *o*-nitrobenzyl chloride resulted in complex mixtures that contained only a small amount of the desired product. In addition, variable yields for the preparation of indoles by the hydrogenation of *o*-nitrobenzyl cyanides have been reported[6,7] since this route was first described[8], which may reflect inconsistent preparations of the starting material.

A more reproducible method for the preparation of indole involves the base-promoted cyclization of N-formyl-*o*-toluide[7,9,10] (scheme 1). Using this general approach, Houck and coworkers prepared indole by treatment of N-[^{13}C]formyl-*o*-toluide with potassium formate[4]. However, the label in indole was diluted, presumably by exchange

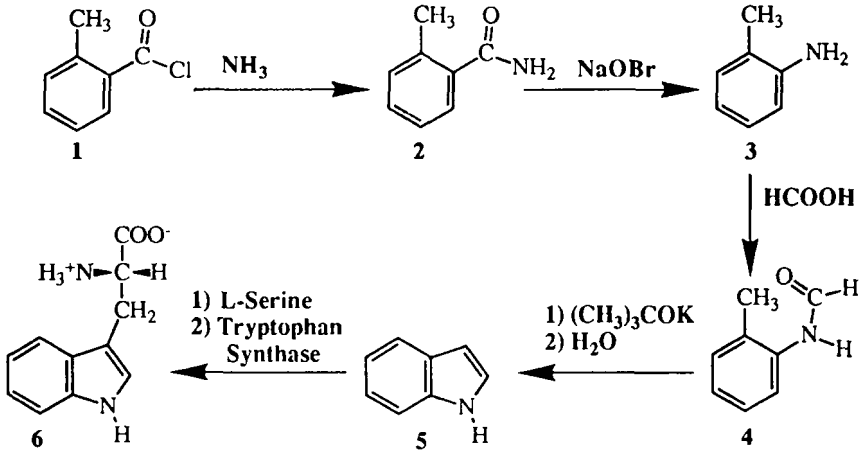
with potassium formate. By carrying out this reaction using potassium *tert*-butoxide, we did not dilute the label from N-[^{13}C]formyl-*o*-toluide. As diagrammed in scheme 1, the disadvantage of this reaction is that potassium o-toluide is thought to serve as the base in the cyclization reaction lowering the yield of indole from formyl-*o*-toluide to a maximum of 50%[7,10]. Another convenient route to indoles is the cyclization of *o*-(lithiomethyl)phenyl isocyanides[11,12]. We have not fully examined the utility of this route to labeled indole.



Scheme 1) Cyclization of *o*-Formotoluide

Our synthetic scheme to [1'-^{15}N]tryptophan is diagrammed in scheme 2. *o*-Toluoyl chloride (**1**) was converted to *o*-[^{15}N]toluamide (**2**) in 87% yield by treatment with [^{15}N]ammonia. Treatment of *o*-[^{15}N]toluamide (**2**) with sodium hypobromite afforded *o*-[^{15}N]toluidine (84% yield) (**3**) which was converted to N-formyl-*o*-[^{15}N]toluide in 89% yield (**4**) by treatment with formic acid. Similarly, N-[^{13}C]formyl-*o*-toluide was prepared by treatment of toluide with sodium [^{13}C]formate in the presence of acid. Cyclization of *o*-[^{15}N]- or N-[^{13}C]formyl-*o*-toluide by treatment with potassium *tert*-butoxide at 350°C afforded [^{15}N]indole (44%) or [2-^{13}C]indole (46%), respectively. As pointed out above, the theoretical yield achievable by this route is 50%; however, in the preparation of [^{15}N]indole, some of the label was recovered as [^{15}N]toluidine (27%), which could be recycled. No attempt was made to recover the [^{13}C]carbon monoxide lost during the cyclization of N-[^{13}C]formyl-*o*-toluide. Indole was converted to L-tryptophan using tryptophan synthase. The enzyme, provided by a genetically engineered strain of *E. coli* which constitutively overproduced tryptophan synthase[13], was used without purification. Whole cells were incubated in an ammonium acetate-buffered solution that contained

indole and serine; the unlabeled substrate was used in excess. Tryptophan was isolated from the culture filtrate. Incorporation of labeled indole into tryptophan was quantitative and proceeded without dilution.



Scheme 2) Synthesis of Labeled Tryptophan

Chemicals-- Carbon monoxide (99.2% ^{13}C) and anhydrous ammonia (98 % ^{15}N) were prepared at Los Alamos National Laboratory. Sodium [^{13}C]formate was prepared by treatment of [^{13}C]carbon monoxide with sodium hydroxide[14]. L-[3-^{13}C]Serine was prepared biosynthetically as described previously[1].

NMR Methods-- Proton and proton-decoupled ^{13}C FT-NMR spectra were obtained at 300.13 and 50.3 MHz, respectively, using Bruker (WM-300 WB and AM-200 WB) NMR spectrometers. ^{15}N FT-NMR spectra were obtained on the Bruker AM-200 operating at 28.283 MHz. Acquisition parameters were as follows: ^{1}H NMR --- 3 KHz sweep width, 32 K data points, 5.11 s acquisition time, 0.196 Hz/pt data point resolution, 256 scans, and 25°C; proton-decoupled ^{13}C NMR --- 10.869 KHz sweep width, 32 K data points, 1.51 s acquisition time, 5 s relaxation delay, 0.663 Hz/pt data point resolution, 1024 scans, and 25°C. L-Tryptophan samples (100 mg) were dissolved as zwitterions in D_2O for NMR analysis. Samples of intermediates were dissolved in $CDCl_3$ solutions. Signal intensities were determined by Lorentzian line shape analysis carried out on a MicroVax II using a modified Levenberg-Marquardt algorithm implemented by the NMR1 software package supplied by the National Institutes of Health Resource for NMR Data Analysis (Syracuse, NY). ^{1}H and ^{13}C chemical shifts are reported in ppm downfield from external TMS=0 ppm;

^{15}N chemical shifts are reported in ppm relative to a 2.5 M solution of potassium [^{15}N]nitrate.

Culture Conditions-- A strain of *E.coli* that constitutively overproduces tryptophan synthase was obtained from Professor Charles Yanofsky of Stanford University (Stanford, CA). This organism contains a plasmid, pWS1 that encodes for *E.coli trp C$^+$, B$^+$, A$^+$* and a chloramphenicol resistance marker; the parent strain, *E. coli* W3310 Δ[*tonB trpBA17*] *his*, produces tryptophan synthase constitutively[13]. The organism was maintained on nutrient agar plates with chloramphenicol (200 μg/L) and cultured in liquid medium that contained the following: NH_4OH (16.75 mM); K_2HPO_4 (10.0 g/L); $NaH_2PO_4 \cdot H_2O$ (2.32 g/L); $MgSO_4$ (98 mg/L); $FeCl_2$ (1.9 mg/L); citric acid (2 g/L); glucose (2 g/L); casamino acids (Difco) (5 g/L); and chloramphenicol (200 μg/L).

L-[β-^{13}C]-, L -[2'-^{13}C]- or L-[1'-^{15}N]Tryptophan (6)-- Using a sterile loop, the *E. coli* cells were transferred from an agar plate to a 50-mL fluted flask that contained 5 mL of sterile liquid culture medium. The organism was cultured at 37°C on a rotary shaker (200 rpm). After 24 h, the 5-mL culture was diluted into 1.5 L of sterile liquid culture medium in a fermentor at 37°C. The fermentor was oxygenated by bubbling air (1.5 L/m) and stirring (200 rpm). After 24 h, the cells were harvested from the culture broth by centrifugation. For the production of tryptophan, cells were resuspended in labeling medium prepared as follows. The labeling medium contained ammonium acetate (100 mM) and indole (20 mM) which were suspended to their respective final concentrations. The suspension was then heated gently on a hot plate until the indole dissolved. The solution was allowed to cool, then serine was added and the pH adjusted to 7.5 with ammonium hydroxide. When L-serine was the labeling precursor, it was used as a limiting reagent (10 mM); when indole was the labeled precursor, serine was added in excess (40 mM). The production medium was sterilized by filtration (0.22 μM filter).

After resuspension, cells were incubated in production medium at 20°C with stirring (200 rpm) but without aeration. The loss of serine and production of tryptophan were monitored quantitatively using a Beckman model 6300 automated amino acid analyzer. In indole limited reactions, its consumption was monitored qualitatively by thin layer chromatography as follows. Samples (1 mL) taken from the fementor were extracted

with ether (1 mL). The ether layer was separated and a small amount spotted onto silica gel plates (60f-254, MCB Manufacturing). The plates were developed using an ether/hexane (10:90) solvent system and monitored for indole (R_f = 0.5) with UV irradiation. When serine or indole disappeared from the production medium, the cells were removed by centrifugation and discarded. Labeled tryptophan was isolated from the culture broth as described below.

Purification of Labeled L-Tryptophan-- In serine limited reactions, the clarified culture broth was extracted with ether to remove excess indole. In general, incorporation of labeled indole into tryptophan was quantitative. However, in rare fermentations where the reaction was not complete, labeled indole could be extracted with ether and recovered by sublimation (50 °C) away from the nonvolatile solids left after evaporation of the ether layer. L-Tryptophan was recovered from the aqueous layer after concentration *in vacuo* using a rotary evaporator. The concentrated medium was dissolved in water (300 mL) and deionized using a column (5X75 cm) that contained Dowex AG 50-X8 (200-400 mesh) in the H^+ form. After loading the sample, the column was washed with water until chloride was no longer detected in the eluent with $AgNO_3$. L-Tryptophan and L-serine, if present, bound to the column and were eluted with 1.0 M NH_4OH (5.0 L). Column fractions (200 mL) were monitored for free amino groups by their colorimetric reaction with ninhydrin as follows. The sample (20 µL) was spotted on paper (Whatman 3 mm), dried with a heat gun and then sprayed with a ninhydrin solution (0.5% in 1-butanol). The colorimetric reaction was developed at 100 °C. Column fractions that gave a positive ninhydrin were monitored for amino acids by quantitative amino acid analysis. L-Serine eluted first (2 L) followed by tryptophan (4 L). L-Tryptophan was crystallized as its zwitterion from ethanol. The yield of tryptophan from labeled indole was essentially quantitative (98%). The yield from serine was lower (82%), although the overall recovery of label (96%) in tryptophan and serine was high.

Characterization of L-Tryptophan--The enantiomeric purity of labeled tryptophan isotopomers were determined by gas chromatography using a fused silica capillary column (25 meter) with a chiral stationary phase (Chirasil-Val III, Alltech Associates). L-Tryptophan was chromatographed as its N-pentafluoropropionyl amide isopropyl

ester[15] and monitored using a flame ionization detector. Isotopically labeled tryptophan eluted with a retention time (38.3 m) identical to L-tryptophan; no evidence was obtained for the D isomer (retention time 37.9 m). Based on this result, preparations of labeled tryptophan were determined to contain the L-isomer in enantiomeric excess of >99.5%. Isotopic purities were determined by proton and proton-decoupled ^{13}C NMR The intensities of ^{13}C satellites on the β- or 2'-proton resonances were used to estimate the enrichment of L-[β-^{13}C] and L-[2'-^{13}C]tryptophan, respectively. The intensity of the ^{15}N satellites on the natural abundance ^{13}C resonance of C-2' was used to estimate the enrichment of L-[1'-^{15}N]tryptophan. In all cases, the isotopic label from serine or indole was incorporated without dilution into tryptophan. Preparations of labeled tryptophan were characterized and shown to be pure by quantitative amino acid analysis. ^{13}C{^{1}H} NMR: (δ DMSO-D_6; C-3, 27.02; C-2, 54.65; 109.54; 111.18, 118.16, 118.27, 120.78; C2', 123.86; 127.10; C-1a', 136.20; C-1, 169.834; $^{1}J_{C2'-N1'}$ = 12.6 Hz, $^{1}J_{C1a'-N1'}$ = 14.6 Hz. Elem. Anal. Calcd. for L-[1'-^{15}N]Tryptophan: C, 64.38; H, 5.89; N, 14.13; Found: C, 63.76; H, 5.89; N, 14.23.

***o*-[^{15}N]Toluamide (2)**-- *o*-Toluoyl chloride (Aldrich Chemical Co., 60.6 mL, 71.8 g, 460 mMol) was added to 1.1 L of cold acetone, on ice, stirring under a nitrogen stream in a round bottomed flask. The flask was attached to a vacuum/pressure manifold and evacuated until the solvent refluxed. To this was slowly added [^{15}N]ammonia (18.4 g, 1.02 Mol) at 2-3 psi through a stainless steal regulator. Excess ammonia helped prevent formation of side-products. After all the [^{15}N]ammonia was added, the reaction was allowed to warm to room temperature and then was stirred overnight under a nitrogen atmosphere. The pH was adjusted to 2 with concentrated HCl. The solution was cooled on ice, and the acetone was removed by rotary evaporation. One liter of cold water was added, and the solution was stirred on ice for about 2 h. The *o*-[^{15}N]toluamide was then filtered off and placed in a desiccator. Excess [^{15}N]ammonia could be recovered from the [^{15}N]ammonium chloride in the water phase. The yield of *o*-[^{15}N]toluamide was 55.11 g (400 mMol), 87% from *o*-toluoyl chloride. Although a small amount of contaminant was detected by ^{13}C-NMR spectroscopy, the toluamide was used without further purification.

***o*-[^{15}N]Toluidine (3)**-- A magnetically stirred aqueous solution of NaOH (300 mL,

2.9 M, 875 mMol) in a 500-mL round bottom flask was chilled to -5 to 0 °C with an acetone/ice bath. To this solution was added 8.81 mL of bromine (170 mMol) dropwise. After stirring the amber solution for 15-30 m, 20 g of [^{15}N]toluamide (148 mMol) was added as a solid. The resulting suspension was stirred vigorously for 3 h, at which time the suspension had become homogeneous. TLC analysis (ether:hexane 20:80) indicated the reaction was depleted of starting material. Subsequently, the reaction was brought to 60-65 °C for 1.0 h to decompose the carbamate, giving the free amine. The reaction was extracted with ether until the aqueous layer was free of the *o*-toluidine, as indicated by TLC analysis. The ether extracts were dried with sodium sulfate and stirred with activated charcoal for 2-4 h. Filtration and evaporation of solvent afforded the crude amine. Kogelrohor distillation (60-70 °C, 0.1 mm Hg) afforded 13.3 g (84 %) of clear colorless oil. ^{1}H NMR d 2.37 (s, 3H), 3.74 (br s, 2H), 6.84 (d, J= 8.1 Hz, 1H), 6.94 (t, J= 6.6 Hz, 1H), 7.25 (m, 2H); ^{13}C d 17.02 (d, J^{13}_{C}-$^{15}_{N}$= 1.3 Hz), 114.6 (d, J^{13}_{C}-$^{15}_{N}$= 2.6 Hz), 118.3, 122 (d, J^{13}_{C}-$^{15}_{N}$= 2.3 Hz), 126.7 (d, J^{13}_{C}-$^{15}_{N}$= 1.3 Hz), 130.2 (d, J^{13}_{C}-$^{15}_{N}$= 0.7 Hz), 144.4 (d, J^{13}_{C}-$^{15}_{N}$= 10.7 Hz); ^{15}N] d 324 ppm.

N-formyl-*o*-[^{15}N]toluidine (4)-- In a 25-mL round bottom flask containing a stir bar was placed 14.75 g of *o*-[^{15}N]toluidine (135 mMol). To this was added 6.01 mL of 95% formic acid (151 mMole). This mixture was heated at 130-140 °C (heating mantle temperature), under a nitrogen atmosphere, until (usually 2-4 h) consumption of the *o*-[^{15}N]toluidine was complete by TLC (ether:hexane 35:65). The crude reaction mixture was subjected directly to Kogelrohor distillation. The N-formyl-*o*-[^{15}N]toluidine was distilled at 110-120 °C at 0.1 mm Hg giving 16.54 g of a white solid (89%). NMR analysis indicated a 55:45 ratio of E and Z formamide geometric isomers. ^{1}H NMR d 2.37 (s, 3H), 2.23 (s. 3H), 6.99-7.28 (m, 8H), 7.4-8.65 (m, 4H); ^{13}C NMR d 17.64, 120.9, 123.3, 125.6, 126.2, 126.8, 127.2, 130.1, 131.3, 134.9,135.1,159.5 (d, J^{13}_{C}-$^{15}_{N}$= 13 Hz), 163.8 (d, J^{13}_{C}-$^{15}_{N}$= 14 Hz); ^{15}N NMR d 241.9 (dd, J^{15}_{N}-$^{1}_{H}$= 91, 13 Hz), 245.8 (dd, J^{15}_{N}-$^{1}_{H}$= 124, 17 Hz) ppm.

N-[^{13}C]formyl-*o*-toluide (4)-- A Teflon™-lined Parr Bomb (Series 4748, 125 mL) was charged with 2.41 g (35 mMol) of sodium [^{13}C]formate, 5.25 g (38.8 mMol) of *o*-toluidine (Aldrich Chemical Co.), 2.06 mL of conc. HCl, 1.10 mL of conc. phosphoric acid, 20 mL of di-n-propyl ether, and a stir bar. The sealed bomb was stirred magnetically

and heated at 135°C for 17 h. The deep purple product was filtered into an evaporating flask through a glass wool-plugged funnel. The residual solid was rinsed on the filter six times with approximately 10-mL portions of diethyl ether. After evaporation of all the volatiles, 5.61 g (34.4 mMol, 98% crude yield) of purple-stained N-[^{13}C]formyl-*o*-toluide crystals were obtained. This material was used without further purification in the preparation of indole. NMR analysis indicated a 55:45 ratio of E and Z formamide geometric isomers in slow exchange. ^{13}C NMR 163.8, 159.9; 134.9, 134.4; 130.8, 130.2; 130.0, 129.6; 126.6, 126.0; 125.7, 125.2; 123.3, 120.8; 117.3, 117.2.

[2-^{13}C]- and [^{15}N]Indole (5)-- A 500-ml three-necked round bottom flask containing 200 mL of dried *tert*-butyl alcohol (activated 3 A sieves) was fitted with a short condensor, a septum, and an inverted U-tube trap. While stirring rapidly and purging with nitrogen, 3.32 g (85 mMol) of chunked metallic potassium was added. The mixture was then heated to 70 °C until the potassium was consumed and gas evolution ceased. Solid formyl-*o*-toluide (7.40 g, 55 mMol) was added portion-wise, and allowed to dissolve. The reaction mixture was stirred at 70 °C for 1 h. A vacuum pump was then connected to the apparatus, and volatiles were removed at reduced pressure until the pressure stabilized. The resulting solid was placed under a stream of nitrogen and slowly heated with a heating mantle to 330-350 °C (heating mantle temperature). At 300 °C, the solid began melting with evolution of gas (CO and *o*-toluidine). Upon melting, the reaction mixture was stirred rapidly for 20-30 m. When the starting material was N-formyl-*o*-[^{15}N]toluidine, free *o*-[^{15}N]toluidine was recovered with the aid of a trap cooled with a dry ice/acetone bath. The resulting black anion was allowed to cool. At slightly above ambient temperature the anion was quenched with the addition of water. Then methylene chloride was added, and the mixture was stirred magnetically while the solid dissolved (10-15 m). The organic layer containing the indole was separated from the aqueous layer using a separatory funnel. The aqueous layer was then extracted 3x with 25 mL portions of methylene chloride. The methylene chloride washings were combined and then washed with 5% HCl to remove any traces of the amine. The aqueous layer from the acid wash was saved and for recovery of *o*-[^{15}N]toluidine as described below. The organic layer was dried over sodium sulfate, filtered, and volatiles evaporated to yielded the crude [^{15}N]Indole. Purification by Kogelrohor distillation (75-85 °C, 0.05 mm Hg) afforded 2.83

g of a white solid (44.1%). Using this method, we also prepared [2-^{13}C]indole (46%) by starting with N-[^{13}C]formyl-*o*-toluide. In addition, similar yields were obtained using commercial potassium *tert*-butoxide that had been purified by sublimation. ^{1}H NMR d 6.7 (m, 1H), 7.3-7.4 (m 3H), 7.57 (d, J= 8 Hz, 1H), 7.9 (d, J= 8 Hz, 1H), 9.2 (d, J= 97 Hz, 1H); ^{13}C d 102.3 (d, J^{13}_{C}-$^{15}_{N}$= 2.7 Hz), 111.0 (d, J^{13}_{C}-$^{15}_{N}$= 1.5 Hz), 119.7, 120.6 (d, J^{13}_{C}-$^{15}_{N}$= 1.0 Hz), 121.8 (d, J^{13}_{C}-$^{15}_{N}$= 2.0 Hz), 124.13 (d, J^{13}_{C}-$^{15}_{N}$= 13.2 Hz), 127.68 (d, J^{13}_{C}-$^{15}_{N}$= 5.0 Hz), 135.6 (d, J^{13}_{C}-$^{15}_{N}$= 15.5 Hz); ^{15}N d 251.4 (apparent dt, J^{15}_{N}-$^{1}_{H}$= 97, 4.7 Hz) ppm.

Recovery of *o*-[^{15}N]toluidine--The aqueous layer from the HCl wash was adjusted to pH 10 with NaOH and the resulting solution was extracted with diethyl ether. The ether layer was dried with sodium sulfate and filtered. The amine recovered in a dry ice cooled trap used during the cyclization reaction was combined with the ether washings. The ether washings were then filtered and condensed to give the crude recovered amine. Kogelrohor distillation afforded 1.59 g (27%) of a clear colorless oil.

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References

1. Hanners, J. L., Gibson, R., Velarde, K., Hammer, J., Alvarez, M., Griego, J. and Unkefer, C., - J. Labelled Comp. Radiopharm. 29: 781-790 (1991).
2. Miles, E. W. - Coenzymes and Cofactors, 1: 253-310 (1986).
3. van den Berg, E. M. M. , Baldew, A. U., de Goede, A. T. J. W., Raap, J. and Leutenberg, J. - Recl. Trav. Chim. Pays-Bas. 107: 73-81 (1988).
4. Houck, D. R., Chen, L. C., Keller, P. J., Beale, J. M., and Floss, H. G. - J. Am. Chem. Soc. 110: 5800-5806 (1988).
5. Dippy, F. J. and Pratt, T. H. - Chem. and Ind. 105-6 (1935).
6. Snyder, H. R., Merica, E. P., Force, C. G., and White, E. G. J. Am. Chem. Soc. 80: 4622-4625 (1958).
7. Tyson, F. T. - in *Organic Synthesis Collective Vol III* (Horning, E. C. ed) pp 479-482 J. Wiley & Sons New York (1955).
8. Stephen, H. - J. Chem. Soc. 127: 1874 (1925).
9. Tyson, F. T. - J. Am. Chem. Soc. 63: 2024-5 (1941).
10. Tyson, F. T. - J. Am. Chem. Soc. 72: 2801-3 (1950).
11. Ito, Y, Kobayashi, K., and Saegusa, T. - J. Am. Chem. Soc. 99: 3532-3534 (1977).
12. Ito, Y, Kobayashi, K., Seko, N. and Saegusa, T. - Bull. Chem. Soc. Jpn. 57: 73-84 (1984).
13. Schneider, W. P., Nichols, B. P., and Yanofsky, C. - Proc. Natl. Acad. Sci. USA 78: 2169-2173 (1981).
14. Ott, D. G. in *Synthesis with Stable Isotopes of Carbon, Nitrogen, and Oxygen*, pp. 18-19, John Wiley and Sons, New York (1981).
15. Knapp, D. R. in *Handbook of Analytical Derivatization Reactions*, pp 255, John Wiley and Sons, New York (1979).