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ENANTIOSELECTIVE SYNTHESIS OF ^{11}C-LABELED PHENYLETHANOLAMINE

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SUMMARY

No-carrier-added [1-^{11}C]phenylethanolamine was prepared by the oxynitrilase-catalyzed addition of hydrogen [^{11}C]cyanide to benzaldehyde followed by reduction of the [^{11}C]cyanohydrin. Radiochemical yields of 2-4% at end-of-synthesis were obtained in a preparation time of 50-60 min from the end of hydrogen [^{11}C]cyanide production. The enantiomeric composition was determined through its conversion to the diastereomeric Mosher amide-esters; the enantiomeric purity of the [^{11}C]phenylethanolamine produced via $NaBH_4$-$CoCl_2$ reduction was 60% e.e. (R/S = 80/20) while that prepared through boran-THF complex reduction was 80% e.e. (R/S = 90/10).

Key Words: ^{11}C-labeling, [^{11}C]phenylethanolamine, enzymatic method, enantioselective labeling

INTRODUCTION

The 2-aminoalcohol functionality is present in a large number of biologically important compounds. Among them, phenylethanolamine (2-amino-1-phenylethanol) and p-octopamine (α-aminomethyl-4-hydroxybenzyl alcohol) have been the objects of growing interest as biogenic trace amines (1). We have been faced with the need of such ethanolamines and related compounds labeled with positron-emitting nuclides for in vivo biochemical studies in conjunction with positron emission tomography. These compounds have an asymmetric center and biological activity resides mainly in the R-configuration at the benzylic carbon (2, 3). Therefore, our attention has been focused on the development of advantageous technologies for preparing ^{11}C-labeled phenylethanolamine in enantiomeric pure or enriched form.

The phenylethanolamine derivative, [1-^{11}C]norepinephrine, has previously been prepared by the addition of [^{11}C]cyanide to the bisulfite addition product with

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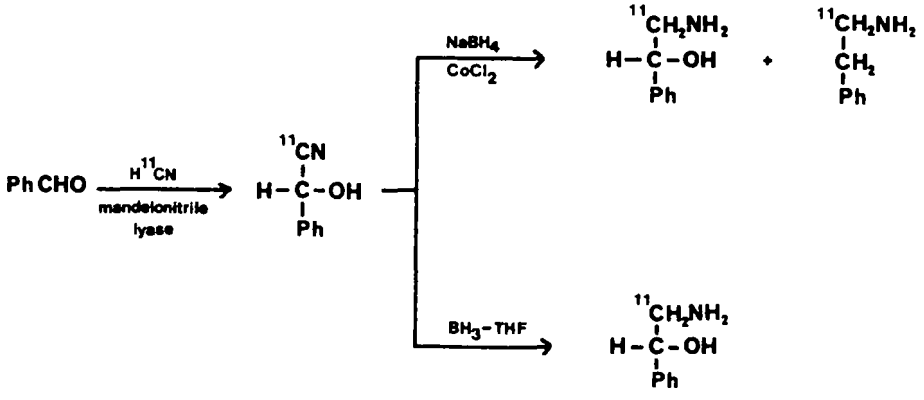
3,4-dihydroxybenzaldehyde, and the intermediate cyanohydrin reduced to the desired aminoalcohol (4). This approach represents an useful and general route to racemic mixtures of the labeled 2-aminoalcohols, using [^{11}C]cyanide as the labeled precursor. Soussain et al. (5) have reported the synthesis of ^{11}C-labeled (-)-epinephrine which consisted in methylating (-)-norepinephrine by using L-[methyl-^{11}C]methionine and two enzymes. It is known that almond oxynitrilase-mediated addition of hydrogen cyanide to a broad structural range of aldehydes occurs with stereospecificity and the (R)-cyanohydrins formed can be then converted into optically active substituted ethanolamines; it has been reported that the enzymatic reaction with benzaldehyde afforded a 95% of mandelonitrile consisting of 97% of the R-(+)-isomer and 3% of the S-(-)-enantiomer (6). We therefore have examined the possible use of the enzyme-catalyzed cyanohydrin synthesis as an efficient synthetic route leading to optically active phenylethanolamine labeled with ^{11}C.

RESULTS AND DISCUSSION

Combined enzymatic and chemical synthesis

Hydrogen [^{11}C]cyanide, free of the ammonia added during processing, was prepared by minor modifications of the literature method (7-9) and collected by bubbling through a solution of 0.05 M 50% methanolic acetate buffer (pH = 5.4) held at -20°C. On the basis of the procedure described by Becker et al. (6), the enzyme-catalyzed cyanohydrin reaction was performed by adding benzaldehyde and salt-free mandelonitrile lyase to this trapping solution, in which reaction time, temperature and substrate concentration were randomly selected. From these results, the stirring for 10 min at room temperature (20-25°C) using 2-4 units of the enzyme and 2 µl of benzaldehyde allowed to transfer about 40% of the available activity into CH_2Cl_2 extract from the reaction mixture. Analysis of the CH_2Cl_2 extract containing any unreacted [^{11}C]cyanide by HPLC on a Partisil M9 PAC column showed a single radioactive peak with identical retention characteristics to authentic mandelonitrile. On the other hand, when the acetate buffer solution contaminated by ammonia was employed in the enzymatic reaction, at least four radiochemical peaks with a trace amount of the desired

mandelonitrile were observed on the same HPLC system. The [^{11}C]mandelonitrile intermediate obtained after evaporation of the CH_2Cl_2 ,in which 14-35% (average 23%, without decay correction) of the radioactivity remained in the reaction vessel, was used for the next step without further purification.



Scheme 1. Two steps synthesis of NCA [1-^{11}C]phenylethanolamine

Sodium borohydride-cobaltous chloride was initially chosen as the reducing agent for the conversion to an aminoalcohol because no anhydrous conditions are required (10). The reduction was carried out in methanol at room temperature for 10 min. The product fraction obtained after simple purification by a small cation-exchange column (H^+ form) was analyzed by analytical HPLC, indicating that the presence of two radioactive peaks, one corresponding to phenylethanolamine and another eluted behind the required ethanolamine on a reversed phase column. The ratio of the radioactivity in the two peaks was ~55:45. This radioactive byproduct was identified as [^{11}C]phenethylamine by its coelution with authentic material. Consequently, we examined the use of a preparative HPLC as an alternative method of purification. The reaction mixture was directly subjected to HPLC using a reversed phase column which had been eluted with a 4 : 1 mixture of 0.01 N HCl and methanol (Fig. 1a). It was found to be effective in removing all chemical and radiochemical impurities, and [^{11}C]phenylethanolamine hydrochloride with a radiochemical purity of >98% was isolated. The cobalt ion level in the purified solution was below the

sensitivity of the ammonium thiocyanate spot test (<20 ppm) (11).

In order to avoid the formation of the radioactive byproduct during the reduction, the second approach involved treatment of the cyanohydrin intermediate with boran-THF complex (4, 12). Reduction to the required aminoalcohol functionality without loss of the benzylic hydroxy-group was found to proceed at 50°C for 8 min as expected. The [^{11}C]phenylethanolamine hydrochloride with a radiochemical purity of better than 98% was isolated by extraction of the reduction mixture with CH_2Cl_2 followed by the same HPLC system as used in the $NaBH_4$-$CoCl_2$ reduction (Fig. 1b).

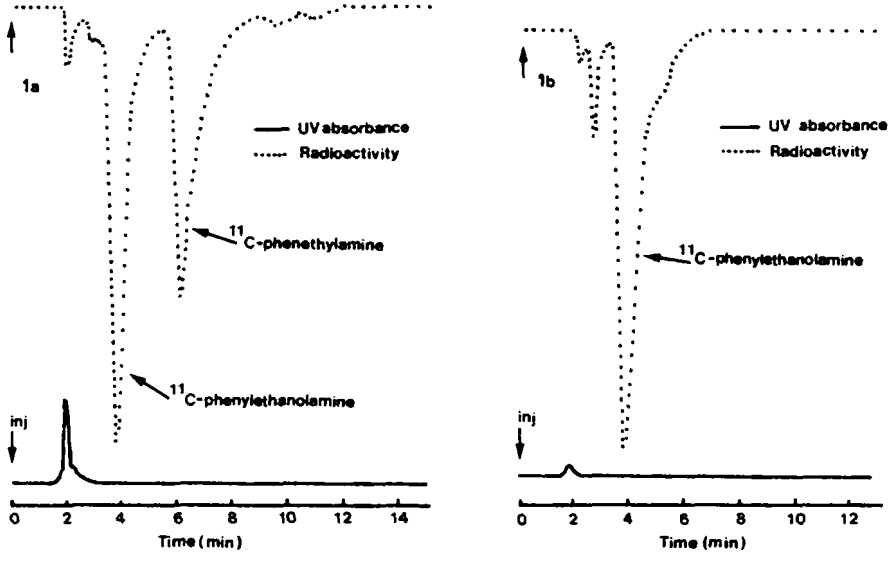


Figure 1. HPLC chromatograms of reaction mixtures followed reduction of the ^{11}C-cyanohydrin intermediate with (a) $NaBH_4$-$CoCl_2$ and (b) boran-THF complex (conditions described in the text)

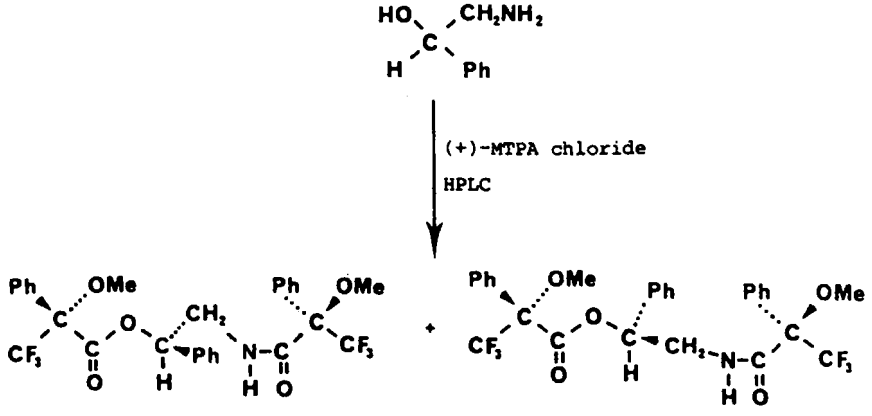
The two synthetic approaches to [^{11}C]phenylethanolamine afforded almost the same overall radiochemical yields (2-4%) at end-of-synthesis (EOS) in a synthesis time of 50-60 min. Although the procedure through reduction with boran-THF complex gave significantly higher yields (4-9%) of the [^{11}C]phenylethanolamine than that with $NaBH_4$-$CoCl_2$ (2-3%) as shown by direct HPLC analysis of the reaction mixture, the extraction step required before injection on the HPLC column to remove UV-absorbing materials in the former case led to losses of the [^{11}C]phenylethanolamine. It is still possible to improve

the technical handling in this preparation. The specific activity of the product obtained was ~95 Ci/mmol at the end of radiosynthesis as determined by UV spectroscopy and no protein was detected in the final product.

Enantiomeric composition

Racemic ^{11}C-labeled amino acids have been resolved into their enantiomers by either enzymatic amino-oxidation (13) or chromatographic techniques (14). Recently the transfer RNA method has also been developed for the determination of enantiomeric purity of [3-^{11}C]phenylalanine and [methyl-^{11}C]methionine (15).

It was not possible to directly separate the enantiomers of racemic phenylethanolamine on the HPLC columns packed with chiral stationary phases such as Chiralcel OC and Chiralpack WH (Daicel Chem. Ind. LTD., Japan). On the other hand, the most widely used method of determining enantiomeric ratio in organic synthetic work includes conversion of an enantiomeric mixture into a pair of diastereomers by reaction with an appropriate chiral compound and subsequent analysis by chromatography as well as NMR spectroscopy. Our successful strategy using (R)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA, the Mosher reagent) (16) as the chiral derivatizing agent, which is remarkably stable toward racemization, is illustrated in Scheme 2. To obtain a reference sample, unlabeled racemic phenylethanolamine reacted with (+)-MTPA chloride in pyridine to give a 1 : 1 mixture of the diastereomeric amide-esters, which were well



Scheme 2. Derivatization of phenylethanolamine with (+)-MTPA chloride

separated on a Partisil M9 PAC column using HPLC technique, allowing the isolation of the each diastereomerically pure MTPA derivative. The absolute configuration of the each diastereomer isolated was assigned on the basis of chemical shift differences for the diastereotopic methoxy groups in their NMR spectra and configurational correlation model described in the literature (16-18). The two OMe signals in the MTPA moiety for the (R, R, R)* diastereomer, eluting as the first peak on HPLC, appeared as two sets of quartets centered at δ 3.35 and 3.32 ppm due to long-range couplings with the CF_3 groups. The similar resonances of the two OMe groups from its (R, S, R)* counterpart, eluting as the second peak on HPLC, occurred at δ 3.45 and 3.27 ppm. This observed large chemical shift difference between the two OMe groups in the (R, S, R) diastereomer, in which the phenyl ring in the ethanolamine moiety is preferentially oriented toward one of the two OMe groups, can be reasonably interpreted by a shielding effect of the phenyl ring. The reaction mixtures obtained by treatment of the [^{11}C]phenylethanolamine (without HPLC purification)

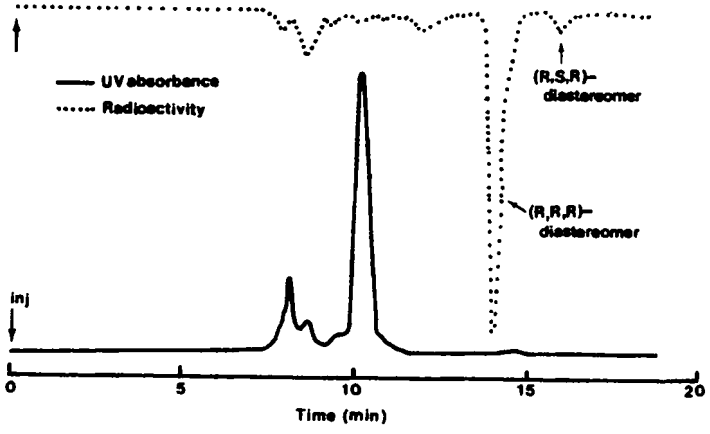


Figure 2. HPLC analysis of the MTPA derivatives of the [^{11}C]phenylethanolamine obtained through the boran-THF complex reduction (conditions described in the text)

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*The first and third assignments of configuration refer to the MTPA moiety; the second is that of the carbon atom which bears the methine group in the ethanolamine moiety.

with (+)-MTPA chloride were subjected to HPLC to assay the diastereomeric purity by ratio of radioactivity. Radio HPLC analysis of the MTPA derivatives of the [^{11}C]phenylethanolamine obtained through the $NaBH_4$-$CoCl_2$ reduction step showed a 80 : 20 ratio of (R, R, R) to (R, S, R) diastereomers while that via the boran-THF complex reduction gave a 90 : 10 ratio. When the CH_2Cl_2 extract of the derivatization reaction mixture was washed with 2N HCl, the assay of activity indicated about 10-15% of the activity to be in the acidic water phase. This suggested that the reaction to form the ^{11}C-labeled diastereomers was not completed with respect to the crude [^{11}C]phenylethanolamine, although the identity of the radioactive species in the water phase was not performed. The diastereomeric ratio obtained with the present procedure which can not exclude kinetic resolution, nevertheless, should be regarded as indicative of the starting [^{11}C]phenylethanolamine without a serious error.

In conclusion, the simple two-step procedures involving enzymatic reaction described in this work allowed the preparation and isolation of (R)-[1-^{11}C]phenylethanolamine with a high enantiomeric excess, affording radiochemical yields of 2-4% at EOS. The extension of this reaction sequence to the preparation of optically active ^{11}C-labeled ring-hydroxylated phenylethanolamines of biological interest such as p-octopamine appears promising, which is currently under investigation.

EXPERIMENTAL

General

Mandelonitrile lyase (E.C. 4.1.2.10, activity = 120 units/mg) obtained from Sigma Chemical Co. (St. Louis, USA) was dialyzed against several changes of distilled water and, after freeze-drying, a solution of 4 units/20 µl in water was stored in the refrigerator. The benzaldehyde used was treated with $NaHCO_3$, purified by vacuum distillation under argon and stored in a sealed vial under argon pressure. The cation exchange resin (AG 50W-X12, H^+ form) was obtained from Bio Rad. All other reagents and chemicals were of analytical grade and used without further purifications. High pressure liquid chromatographic system equipped with a 2 ml sample loop was done using Waters model M-45 or Shimadzu

LC-6A liquid chromatographs fitted with one of the following columns: Whatman Partisil M9 10/50 PAC, M9 ODS-2, or Shim-pack CLC-ODS. Conditions for separation and purification are given in the following experimental sections. Column-effluent absorbance was monitored at 254 nm UV detector and effluent radioactivity was determined using a NaI(Tl) scintillation detector system. Radioactivity was also quantified with a Capintec radioisotope calibrator (CRC-30). The product peak was collected and their radiochemical purity was confirmed by the repeated injection of the isolated fractions into the HPLC. The identity of labeled products was supported by HPLC coinjection studies. The radiochemical yields were calculated by determining the amount of activity in the product fraction as a percentage of the initial activity of the $H^{11}CN$ trapped in the reaction vessel (without decay correction). ^{1}H-NMR spectra were obtained in $CDCl_3$ with a JEOL FX-100 spectrometer with tetramethylsilane as internal reference. Mass spectra were determined on a JEOL D-300 mass spectrometer and UV spectra were obtained on a Hitachi 220A spectrometer. IR spectra were taken on a JASCO IRA-1 spectrophotometer.

NCA Hydrogen [^{11}C]Cyanide

With slight modification no-carrier-added (NCA) hydrogen [^{11}C]cyanide was prepared according to the published methods (7-9). [^{11}C]Methane was produced by bombardment of a target gas (95% N_2/5% H_2) with 17 MeV protons from the Japan Steel Works 1710 cyclotron. The effluent from the target, carried by a stream of helium gas (100-130 ml/min) was mixed with anhydrous ammonia (flow rate: 20 ml/min) and passed over platinum wire heated at 920-930°C. The gases were passed through a U-tube containing powdered P_2O_5 and bubbled through 50% sulfuric acid (1 ml) heated at 60-70°C. Finally, the NCA hydrogen [^{11}C]cyanide, free of the ammonia added during processing, was collected by bubbling through a 300 µl solution of 0.05 M 50% methanolic acetate buffer (pH = 5.4) held at -20°C. The ^{11}C-activity of 17.6 mCi in this solution was obtained by a 15 min bombardment with a beam current of 20 µA. Activities greater than this were not required for our experiments.

[^{11}C]Mandelonitrile

The enzymatic reaction was started by addition of 2-4 µl of benzaldehyde and

20 µl of an aqueous solution containing 2-4 units of salt-free mandelonitrile lyase to a 0.05 M 50% methanolic acetate buffer (pH = 5.4) solution containing the hydrogen [^{11}C]cyanide. The vessel was stoppered and the mixture was kept at room temperature for 10 min with magnetic stirring. The reaction mixture was extracted three times with 500 µl of CH_2Cl_2. The combined extracts were then washed with 500 µl of water. The organic phase was filtered through anhydrous Na_2SO_4 powder. An aliquot of the filtrate was injected onto a Partisil M9 10/50 PAC HPLC column using a mixture of $CHCl_3$ and acetonitrile (7:3, V/V) as the mobile phase. [^{11}C]Mandelonitrile had a retention time of 7.6 min with a flow rate of 4 ml/min. The filtrate was rapidly blown to dryness using a stream of argon at 90-100°C. 14-35% (average 23%) of the radioactivity remained in the reaction vessel, which was employed for the next reduction step without further purification.

[1-^{11}C]Phenylethanolamine hydrochloride

(A) By $NaBH_4$-$CoCl_2$ reduction

To a vessel containing the [^{11}C]mandelonitrile prepared as described above was added 300 µl of methanol. 24 mg of $CoCl_2 \cdot 6H_2O$ was then dissolved in this solution and 19 mg of $NaBH_4$ was added in portions with stirring. Stirring at room temperature in an open system was continued for 10 min. The reaction was then quenched by addition of 300 µl of 3 N HCl. Most of the methanol was removed using a stream of argon at 90-100°C, and the resulting solution was diluted with 0.5-1 ml of water. This mixture was applied to a column (0.9 x 2.5 cm) of AG 50W-X12 that had been rinsed with water. Unreacted starting material and other nonbasic impurities were washed from the column with ~20 ml of 0.01 N HCl and discarded. The fractions eluted with ~40 ml of a mixture of methanol-3 N HCl (6 : 1, V/V) were collected. HPLC analysis [shim-pack CLC-ODS (0.6 X 15 cm), eluting with 10 mM phosphate buffer (pH = 2.6)-acetonitrile (20 : 1, V/V), 1.5 ml/min] of this sample showed two radioactive peaks in a ratio of 55:45, one of them corresponded to [^{11}C]phenylethanolamine (t_R = 3.7 min) and the other to [^{11}C]phenethylamine (t_R = 8.3 min). As an alternative purification, the reaction mixture diluted with water was directly injected onto a preparative HPLC column (Partisil ODS-2) using a mixture of 0.01 N HCl-MeOH (4 : 1, V/V) as the

mobile phase with a flow rate of 5 ml/min. A radioactive peak corresponding to the retention time (3.6 min) of phenylethanolamine was collected in a total volume of about 8 ml. The byproduct, [^{11}C]phenethylamine eluted at t_R = 6.1 min. By this procedure [^{11}C]phenylethanolamine hydrochloride was obtained with overall radiochemical yields of 2-3% (based on [^{11}C]HCN) at end-of-synthesis. Protein measurement in the purified solution was carried out using the Folin phenol reagent (19). The concentration of the cobalt ion present in the solution was also estimated by the ammonium thiocyanate spot test (11). The chemical and radiochemical purities was >98% as determined by HPLC. The entire procedures took 50-60 min after the end of the [^{11}C]HCN collection.

(B) By BH_3-THF reduction

To a vessel containing the [^{11}C]mandelonitrile was added 200 µl of boran-THF complex in a 1.0 M THF solution. The vial was sealed and heated at 55°C for 8 min. The mixture was blown to dryness using a stream of argon at 100°C leaving a white residue. The residue was dissolved in 300 µl of 1 N HCl and 400 µl of 1 N NaOH was then added. The mixture was extracted three times 500 µl of CH_2Cl_2 and the CH_2Cl_2 was removed using a stream of argon. The radioactive residue was then taken into a mixture of 0.01 N HCl and MeOH (4 : 1, V/V) and purified by HPLC using the same column and conditions as those described in method A. The overall yield of the product ranged from 3-4% at end-of-synthesis. The chemical and radiochemical purities was >98% as determined by HPLC. The entire procedures took 50-60 min after the end of the [^{11}C]HCN collection. Protein concentrations were estimated by the method of Lowry et al. (19).

Determination of enantiomeric purity of [1-^{11}C]phenylethanolamine following conversion to the MTPA diastereomers

(a) (+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride [(+)-MTPA chloride] was prepared by refluxing (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (99+%) [(+)- MTPA] in thionyl chloride for 50 hr according to the literature procedure (16). To a solution of 280 mg of racemic phenylethanolamine in 12 ml of dry pyridine and 4 ml of CCl_4 was added 1.45 g of (+)-MTPA chloride. The mixture was allowed to stand at room temperature for 1 hr and diluted with water, and extracted with ether. The

ether extract was washed with 2 N HCl, saturated aqueous Na_2CO_3, water, and dried (Na_2SO_4). The ether was evaporated to give a residual syrup. The syrup was purified by column chromatography on silica gel [Mallinchrodt Silica CC-4, benzene-petroleum ether (2 : 1, V/V) and benzene as eluant] to give a colorless syrup (710 mg) that showed a mixture of the desired MTPA diastereomers as its ^{1}H-NMR and mass spectra. IR (neat) 1700 and 1760 cm^{-1}; MS m/e 569 (M^+). A small sample was dissolved in the following HPLC solvent system. HPLC separation was carried out using a Whatman Partisil M9 10/50 PAC column with n-hexane-$CHCl_3$ (3 : 2, V/V) (mobile phase A) or CH_2Cl_2 (mobile phase B) at a flow rate of 3 ml/min as the eluent. The collected fractions obtained from several separations were combined respectively. A small amount of a mixed-cut was obtained and was utilized in subsequent separations. Evaporation of the combined less polar fractions gave 3 mg of the (R, R, R) diastereomer (t_R = 17.0 min in the mobile phase A, 13.7 min in the mobile phase B). ^{1}H-NMR ($CDCl_3$) δ 3.32 (q, 3H, OCH_3), 3.35 (q, 3H, OCH_3), 3.41-4.10 (m, 2H, CH_2), 6.02 (q, 1H, J = 4.0, 8.4 Hz, CH), 7.03 (broad t, 1H, NH) and 7.2-7.5 ppm (m, 15H, C_6H_5). Evaporation of the combined solution of the more polar fractions gave 3 mg of the (R, S, R) diastereomer (t_R = 18.2 min in the mobile phase A, 15.7 min in the mobile phase B). ^{1}H-NMR ($CDCl_3$) δ 3.27 (q, 3H, OCH_3), 3.45 (q, 3H, OCH_3), 3.51-4.06 (m, 2H, CH_2), 6.09 (q, 1H, J = 3.9, 8.1 Hz, CH), 7.02 (broad t, 1H, NH) and 7.2-7.4 ppm (m, 15H, C_6H_5). The superimposed ^{1}H-NMR spectrum of the each diastereomerically pure MTPA derivative was identical with that of a 1 : 1 mixture of the diastereomers.

(b) To a vessel containing the [^{11}C]phenylethanolamine (110-400 μCi) without HPLC purification was added 100 μl of dry pyridine and 20 μl of (+)-MTPA chloride. The mixture was sealed, heated for 3 min at 50°C, and allowed to stand at room temperature for an additional 4 min. The mixture was diluted with water and extracted with CH_2Cl_2 (or ether). The combined organic extracts were washed with 2N-HCl, saturated aqueous Na_2CO_3, water, and dried (Na_2SO_4). The radioactive residue, after removal of the solvent using a stream of argon, was dissolved in n-hexane-$CHCl_3$ (3 : 2, V/V) or CH_2Cl_2 and injected onto the same HPLC chromatographic system as described in part (a). The radioactive fractions

corresponding to the authentic diastereomers were directly collected from the column. Relative ratios of the diastereomers were calculated from both the peak areas and radioactivity of the isolated fractions. The MTPA derivatives obtained through the $NaBH_4$-$CoCl_2$ reduction step showed a 80 : 20 ratio of (R, R, R) to (R, S, R) diastereomer while that via the boran-THF complex reduction showed a 90 : 10 ratio.

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