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Synthesis of ^{14}C-labelled CGS 19755, a Selective NMDA Antagonist.

Alban J. Allentoff*, Mahesh Desai, Bohdan Markus and Timothy Duelfer

Radiochemistry Laboratory, Ciba Pharmaceuticals Division
556 Morris Avenue, Summit, New Jersey 07901-1398, USA

SUMMARY

CGS 19755 is a selective NMDA (N-methyl-D-aspartate) antagonist presently under development as a neuronal protecting agent after stroke and head trauma. It was synthesized with ^{14}C-labelling in the carboxylic acid side chain via the synthetic route displayed in Scheme 1. Key steps in the synthesis involved a Reissert-Henze addition of potassium [^{14}C]cyanide to a functionalized pyridine N-oxide, followed by hydrolysis and a selective hydrogenation affording the desired *cis*-substituted piperidine target. Using this procedure, [^{14}C]CGS 19755 was prepared with radiochemical purity of 99.6%. The synthesis demonstrates the utility of the Reissert-Henze reaction, in conjunction with palladium/rhodium-catalyzed hydrogenation, as a facile means of preparing ^{14}C-labelled *cis*-2,4-substituted cyclic amino acids.

KEYWORDS: [^{14}C]CGS 19755, NMDA antagonist, Reissert-Henze reaction, potassium [^{14}C]cyanide

INTRODUCTION

CGS 19755 is a selective NMDA (N-methyl-D-aspartate) antagonist currently under clinical development as a neuronal protecting agent for the treatment of stroke and head trauma (1). In order to support biodistribution and pharmacokinetic studies, a ^{14}C-labelled form of CGS 19755 was required. This paper describes the details of the radiosynthesis of [^{14}C]CGS 19755 (8, Scheme 1) and offers a method for purifying the final product by semi-preparative HPLC.

Examination of previous routes used to synthesize unlabelled 8 (2, 3) prompted us to place the ^{14}C-label in the carboxyl group shown. We felt that introduction of this group could be efficiently executed via a Reissert-Henze addition of ^{14}C-labelled potassium cyanide to a suitably substituted pyridine oxide (4, 5). Subsequent hydrogenation and hydrolysis would afford 8 with the desired *cis* relative stereochemistry. This known selectivity in catalytic hydrogenation of pyridines (2, 3, 6), the few steps involved, and the affordability of ^{14}C-labelled potassium cyanide made this an attractive approach for synthesizing 8.

**Author to whom correspondence should be addressed.*

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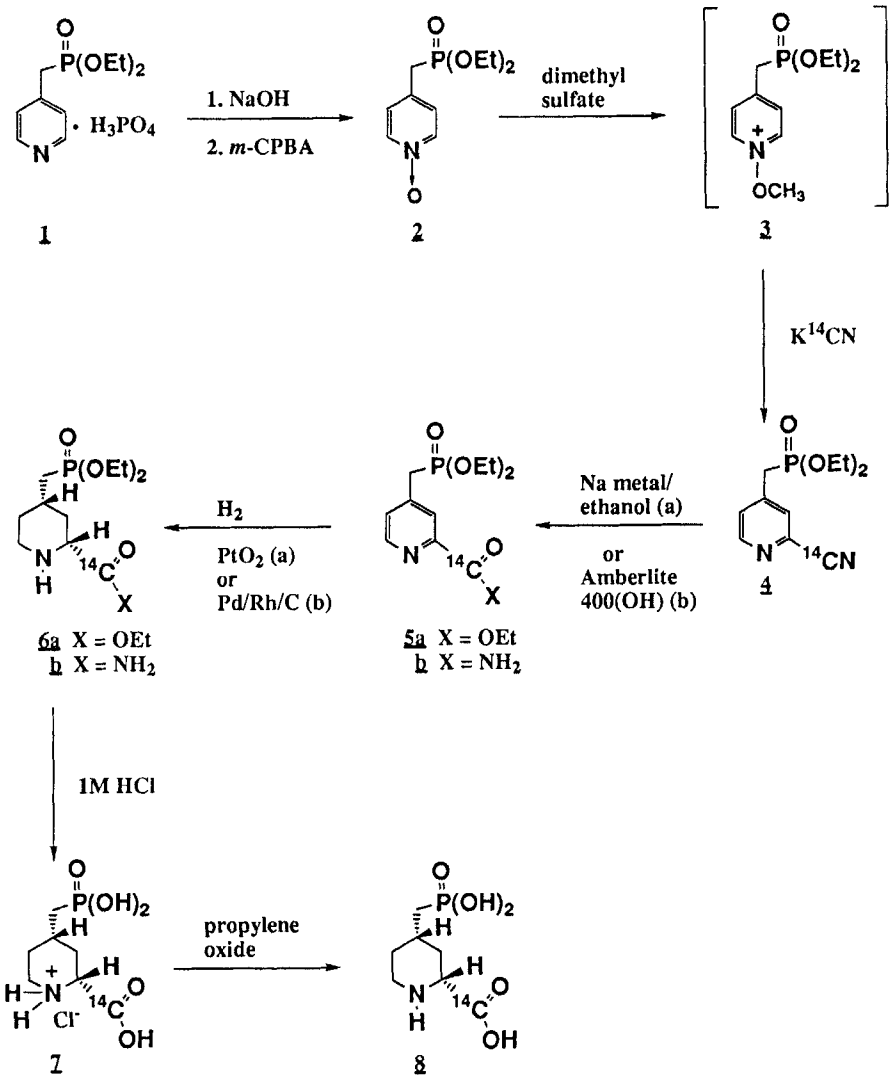
Scheme 1



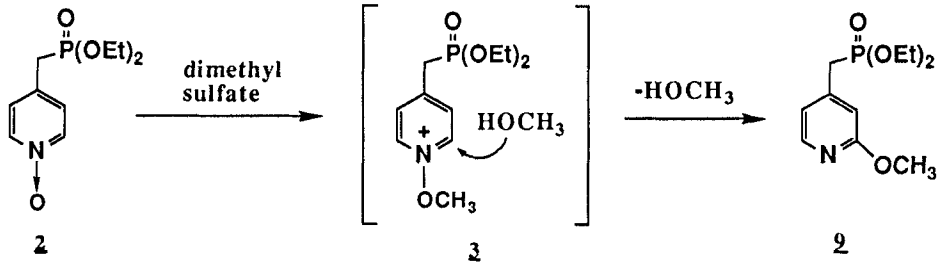
RESULTS AND DISCUSSION

The reaction sequence used to synthesize [^{14}C]CGS 19755 is displayed in Scheme 1. Commercially available phosphoric acid salt 1 was neutralized with aqueous sodium hydroxide and then oxidized by *m*-chloroperoxybenzoic acid in methylene chloride to give crude N-oxide 2. Compound 2 was methylated with dimethyl sulfate to yield the quaternary ammonium salt 3, which upon reaction *in situ* with ^{14}C-labelled potassium cyanide yielded nitrile 4 (4, 5, 6). ^{1}H NMR

analyses of developmental cold runs established that the crude product 4 was contaminated with approximately 25% of the 2-methoxy-substituted pyridine 9 (Scheme 2). Formation of this contaminant is consistent with the reaction displayed in Scheme 2; methanol formed as a side product competes with cyanide in adding to salt 3 yielding 9. The presence of 9 in the reaction mixture did not interfere with the subsequent step in the synthetic sequence.

Previously reported syntheses of unlabelled 4 (2, 3) have utilized a modification of the Reissert-Henze reaction which could potentially alleviate the formation of 9. This modification uses trimethylsilanecarbonitrile as the agent of cyanation, which is a disadvantage for the radiosynthesis, since extra steps are required to prepare the labelled form of this reagent from potassium cyanide. Therefore, despite the undesired formation of 9, the direct addition of labelled potassium cyanide to 3 is the perferred approach to the radiosynthesis of 4.

Scheme 2



2 3 9

In previous work on this radiosynthesis (7), nitrile 4 was converted to the ester (5a), via treatment with sodium metal in ethanol at ambient temperature. Ester 5a was then hydrogenated in glacial acetic acid with PtO_2 as catalyst to give the desired *cis*-substituted piperidine 6a with 90% stereoselectivity. The olefin configuration of the products was established by HPLC and ^{1}H-NMR analyses as previously described (2).

Subsequent experimentation has demonstrated that higher selectivity towards formation of the *cis* product is achieved via hydrogenation of amide 5b using palladium/rhodium catalyst (5). Hence, nitrile 4 was converted to amide 5b by heating 4 with Amberlite IRA-400(OH) basic resin in aqueous solution for two hours (8). The mild reaction conditions allowed clean conversion of the nitrile to the amide without hydrolyzing the phosphonate esters. Crystallization from hexane:ethanol solution allowed facile removal of the contaminant 9 and gave pure 5b in 66% radiochemical yield in two steps from K^{14}CN.

Amide 5b was hydrogenated in water using palladium/rhodium catalyst to give *cis*-piperidine 6b with 97% selectivity of *cis* over *trans* as observed from HPLC analysis of hydrochloride salt 7. Hydrolysis of 6b was accomplished by treatment with dilute HCl at reflux to afford 7. The ammonium chloride produced as a side product was removed by binding crude 7 to Ambersep basic resin, washing with water, and then regenerating 7 (82% yield in two steps from 5b) through aqueous acid treatment of the resin-bound product.

Salt 7 was neutralized to target compound 8 ([^{14}C]CGS 19755) in 47% yield through treatment of 7 with excess propylene oxide in aqueous ethanol (2). The product possessed radiochemical purity of 90% and was purified to greater than 99.6% radiochemical purity via the preparation of the piperidine salt of 8, followed by semi-preparatory ion exchange HPLC. None of the *trans*-isomer was observed in the final product.

In conclusion, we have developed an efficient route to the synthesis of [^{14}C]CGS 19755 which provides very high purity material in five steps from potassium [^{14}C]cyanide. The synthesis demonstrates the utility of the Reissert-Henze reaction, in conjunction with palladium/rhodium-catalyzed hydrogenation, as a facile means of preparing ^{14}C-labelled *cis*-2,4-substituted cyclic amino acids.

EXPERIMENTAL

Potassium [^{14}C]cyanide was purchased from Amersham Corporation of Arlington Heights, IL or NEN DuPont of Boston, MA. Pyridine salt 1 was purchased from Zeneca Specialties of Wilmington, DE. Other unlabelled reagents were purchased from Aldrich Chemical Company of Milwaukee, WI. Thin layer chromatography was performed on Whatman LK6DF channeled plates of 250 mm thickness, which were analyzed by a Bioscan System 200 imaging scanner. HPLC analyses were performed on a system (Waters Associates) including a Model U6K injector, a Model 510 HPLC pump, a Model 486 tunable absorbance detector adjusted to 254 nm wavelength, and a Baseline 810 Chromatography Workstation. The HPLC system was also interfaced with an on–line radioactivity detector (Flo–One/Beta Series A–100 detector of Radiomatic Instrument Co.) for measuring radioactivity. The HPLC effluent was mixed with liquid scintillation solution (Flo–Scint III) at a ratio of 1:2 (v/v). Ion exchange HPLC analysis utilized a 10 cm x 4.6 mm i.d. Whatman Partisil 5 analytical column with a solvent system of 1:1 methanol:aqueous H_3PO_4 (15 mM at pH 4) (v/v). Semi-preparative HPLC utilized a Whatman

Magnum 9, Partisil 10 SAX, 25 cm x 9.4 mm i.d column. The column was eluted isocratically at a flow rate of 2 mL/min with a mobile phase consisting of 18 mM NH_4OAc adjusted to pH 4.0 with acetic acid. NMR spectra were recorded on a Varian XL-300 spectrometer in $CDCl_3$ with chemical shifts reported in δ and coupling constants in Hz. Mass spectra were obtained on a Perkin-Elmer Sciex API III triple quadrupole mass spectrometer with an ionspray interface using conditions described below. All melting points were taken in capillary tubes on an Electrothermal IA 9100 digital melting point apparatus and are uncorrected.

Diethyl (4-pyridinylmethyl)phosphonate N-oxide (2)

An aqueous solution of 1 (20.0 g, 51.1 mmol) in 66 mL of water was treated dropwise with 21.2 mL of 33% sodium hydroxide solution at 0 °C over 10 min. Methylene chloride (30 mL) was added and the mixture was stirred for 20 min. The layers were separated and the aqueous layer extracted once with 30 mL of methylene chloride. The combined organic layers were washed with saturated brine and the solvent removed to yield 16.3 g of the free base of 1 as a yellow oil: ^1H NMR d 8.53 (m, 2 H), 7.22 (m, 2 H), 4.04 (dq, 4 H, J = 7, 7), 3.12 (d, 2 H, J = 30), 1.25 (t, 6 H, J = 7).

All of this material was immediately added dropwise over 20 min to a solution of *m*-chloroperbenzoic acid (24.6 g, 60% pure, 85.5 mmol) in 197 mL of methylene chloride at 0 °C. After the addition, the solution was stirred at room temperature for 48 hr. Powdered potassium carbonate (35.0 g, 253 mmol) was then added, and the mixture was stirred for an additional 3 h and then filtered through a fritted glass funnel. Removal of the solvent gave 14.2 g of the crude product as a yellow oil, which was used directly for the next step: ^1H NMR ($CDCl_3$) δ 8.13 (m, 2 H), 7.20 (m, 2H), 4.05 (dq, 4 H, J = 7, 7), 3.08 (d, 2 H, J = 30), 1.25 (t, 6 H, J = 7).

Diethyl (2-[^{14}C]-cyano-4-pyridinylmethyl)phosphonate (4) and diethyl (2-methoxy-4-pyridinylmethyl)phosphonate (9)

To a stirred solution of 7.1 g of N-oxide 2 in 71 mL of acetonitrile at room temperature was added dropwise dimethyl sulfate (3.6 g, 28.5 mmol) over 10 min. The resulting mixture was stirred at room temperature for 18 hr and was then cooled to 0 °C. A solution of labelled potassium cyanide (170 mg, 2.61 mmol, 145 mCi, 55 mCi/mmol) and unlabelled potassium cyanide (2.2 g, 33.8 mmol) in 3.3 mL of water was added dropwise over 3 min. The resulting red

solution rapidly formed a yellow precipitate and the mixture was stirred at 25 ^{0}C for 4 h. The solvent was then removed *in vacuo*, the residue partitioned between water and methylene chloride, the layers separated, and the aqueous layer extracted twice with methylene chloride. The combined organic layers were dried over $MgSO_4$ and the solvent removed to yield 6.5 g of a red oil. A second batch was run in identical scale and the products combined to give a total of 13.3 g of crude 4. TLC analysis in methanol:methylene chloride 20:80 (v/v) using radioscanning showed that the material was 98% radiochemically pure, but TLC analysis using UV detection, and ^{1}H NMR analysis of prior developmental cold runs showed that this material was approximately 75% pure and contained side products resulting from methoxylation rather than cyanation of 2. The material was used for the next steps without further purification.

The ^{1}H NMR data for unlabelled 4 and 9 were obtained from the mixture of products produced from a cold trial run.

The data for unlabelled 4:

^{1}H NMR ($CDCl_3$) δ = 8.59 (d, 1 H, J = 6.0), 7.61 (br s, 1 H), 7.43 (m, 1), 4.02 (dq, 4 H, J = 7,7), 3.13 (d, 2 H, J = 22.5) , 1.23 (t , 6 H, J = 7).

The data for 9:

^{1}H NMR ($CDCl_3$) δ = 8.55 (d, 1 H, J = 6.0), 7.61 (br s, 1 H), 7.32 (m, 1), 4.02 (dq, 4 H, J = 7,7), 3.40 (s, 3 H, OCH_3), 3.13 (d, 2 H, J = 22.5) , 1.21 (t , 6 H, J = 7).

Ethyl 4-[(diethoxyphosphinyl)methyl]-2-pyridine-[^{14}C]-carboxylate (5a)

To a solution of 65 mg of sodium metal in ethanol (20 mL) was added a solution of 5.0 g of crude 4 in 10 mL of ethanol over 10 min. The reaction mixture was stirred for 24 h at ambient temperature and then cooled to 5 0C. A solution of 6N hydrochloric acid (10 mL) was added, and the mixture was stirred in an ice-bath for 2 h and then at ambient temperature for 36 h. The reaction mixture was evaporated to dryness *in vacuo* and the residue dissolved in water. The resulting solution was adjusted to pH 9 by addition of ammonium hydroxide solution, and was extracted twice with 50 mL of methylene chloride. The organic layer was washed with saturated brine and was dried with $MgSO_4$. Removal of the solvent gave 4.0 g of crude 5a as a brown gum. This material was used for conversion to 6a without further purification.

4-[(Diethoxyphosphinyl)methyl]-2-pyridine-[^{14}C]-carboxamide (5b)

A mixture of crude 4 (13.3 g), Amberlite-400(OH) basic resin (10.8 g) and water (131 mL) was heated at reflux for 7.5 h. The mixture was cooled, and the resin was filtered out. The

solvent was then removed *in vacuo*, and the residue partitioned between methylene chloride and water. After the layers were separated, the aqueous layer was extracted twice with methylene chloride. The combined organic layers were washed with saturated brine, dried over Na_2SO_4, and the solvent removed to yield 12.9 g of the crude product as an orange-red solid. This material was dissolved in 10 mL of boiling ethanol, 10 mL of hexane was added and the resulting solution was allowed to cool to -20 ^{0}C overnight. The resulting precipitate was filtered yielding 6.12 g (22.5 mmol) of a white crystalline solid; m.p. 141.1-141.6 ^{0}C. The specific activity was found to be 5.0 mCi/mmol (113 mCi total, 66% radiochemical yield in two steps from K^{14}CN). TLC analysis in methanol:methylene chloride 10:90 (v/v) using radioscanning and UV detection showed greater than 98% purity.

Ethyl *cis* 4-[(diethyloxyphosphinyl)methyl]-2-piperidine-[^{14}C]-carboxylate (6a)

To a solution of 4.0 g of 5a in 40 mL of glacial acetic acid was added 1.0 g of platinum oxide, and the mixture was hydrogenated in a Parr shaker at 40 psi for 24 h. The catalyst was filtered off and the filtrate evaporated *in vacuo* yielding a light-yellow oil, which was dissolved in methylene chloride and the resulting solution was neutralized by stirring with anhydrous sodium carbonate for 48 h. After filtering the mixture, the solid was washed with methylene chloride and the combined organic solutions were washed with water, and saturated brine and then dried over $MgSO_4$. Removal of the solvent yielded 5.2 g of 6a as a light-yellow oil.

***cis*-4-[(Diethoxyphosphinyl)methyl]-2-piperidine-[^{14}C]-carboxamide (6b)**

A mixture of amide 5b (6.1 g, 24.0 mmol), palladium/rhodium catalyst (1.2 g, 4.5% palladium, 0.5% rhodium on carbon, 50% water wet, Johnson Matthey) and 61.3 mL of water were shaken in a Parr hydrogenator at 50 psi hydrogen for 48 h. Another 200 mg of catalyst was added and shaking at 50 psi hydrogen was continued for an additional 62 h. The mixture was then concentrated to dryness, the residue dissolved in methylene chloride and then filtered through 20 g of celite. Removal of the solvent yielded 6b (6.5 g) as a grey, viscous oil. TLC analysis in methanol:methylene chloride 25:75 (v/v) using radioscanning showed greater than 96% radiochemical purity.

***cis*-4-[(Dihydroxyphosphinyl)methyl]-2-piperidine-[^{14}C]-carboxylic acid hydrochloride (7) from 6a**

Compound 6a (5.0 g) was dissolved in 15 mL of 6N HCl and the mixture was heated at reflux for 48 h. The solvent was removed under reduced pressure, methanol was added to the

resulting residue and the mixture was filtered. The filtrate was then evaporated to give a gum, which was dried by repeated additions and removal of toluene *in vacuo,* affording hydrochloride salt 7 (1.2 g) as a light-yellow solid.

cis-4-[(Dihydroxyphosphinyl)methyl]-2-piperidine-[^{14}C]-carboxylic acid hydrochloride (7) from 6b

A solution of amide 6b (0.50 g) in 5.3 mL of 6N HCl was heated at reflux for 4.5 h. The solvent was removed *in vacuo* to yield 0.65 g of an off-white oily solid. Ambersep basic resin (Rohm and Haas Co., 6.5 mL) was placed in a fritted funnel and was washed three times with water, twice with methanol:water 95:5 (v/v), then three times with water and was then added in one portion to a solution of the crude product (0.65 g) in 9.0 mL of water. The resulting mixture was stirred for 40 min at ambient temperature. The resin was removed by filtration, washed twice with water, and stirred at 25 ^{0}C with a solution of 3N HCl (10.5 mL) for 2.5 h. The resin was then filtered out and the solvent evaporated *in vacuo*, yielding a white crystalline solid (0.42 g, 1.6 mmol, 82% yield in two steps from 5b). Ion exchange HPLC analysis showed the HCl salt (RT = 9.0 min) with approximately 86% radiochemical purity, containing 11% contamination with an unidentified polar impurity (RT = 6.5 min) and 3% contamination with the HCl salt of the *trans*-isomer (RT = 11.4 min). The product was used in the next step without further purification.

4-[(Dihydroxyphosphinyl)methyl]-2-piperidine-[^{14}C]-carboxylic acid ([^{14}C]CGS 19755; 8)

HCl salt 7 (0.42 g, 1.6 mmol) was dissolved in a mixture of water (1.6 mL) and ethanol (4.9 mL). To the slightly cloudy solution was added propylene oxide (0.2 mL) in 50 mL portions over 30 min. After 20 min of reaction, a white precipitate began to form. When the addition was complete, the mixture was allowed to stir for 18 h at 25 ^{0}C and then the precipitate was collected by filtration and dried under vacuum to yield 0.17 g (0.75 mmol, 3.4 mCi total activity, 47% yield) of a white, powdery solid, which was [^{14}C]CGS 19755. Ion exchange HPLC analysis of this product showed 90.1% radiochemical purity.

Purification of [^{14}C]CGS 19755 (8) via semi-preparative HPLC of the piperidine salt

To crude [^{14}C]CGS 19755 (1.0 g, 4.5 mmol, 5.5 mCi) was added water (2.5 mL) and piperidine (4.7 mmol, 0.4 g) and the resulting solution was then injected on a semi-preparative anion exchange HPLC column in 100 μL portions (40 mg compound/injection). The product (8)

eluted as the free acid at approximately 45 min retention time. The elution fractions containing the product were combined and were subjected to repeated evaporations on a rotary evaporator to remove the buffer from the mobile phase. Water was added between each evaporation. The product (0.315 g) was then placed under vacuum for 18 h at ambient temperature and the resulting white solid was dissolved in 15 mL of warm water (50 ^{0}C). This solution was then cooled to room temperature, adjusted to pH 2.4 and diluted with 15 mL of ethanol, causing a precipitate to form within 15 min. After standing at room temperature for 2 h, the solution was cooled to –20 ^{0}C overnight. The resulting precipitated [^{14}C]CGS 19755 was collected by vacuum filtration, washed with ethanol, and dried under vacuum at 45 ^{0}C yielding 157 mg (0.704 mmol) of pure product as a white powder (m.p. = 283.6-284.7 ^{0}C). The specific activity was found to be 2.7 mCi/mmol (1.9 mCi total).

Ion exchange HPLC analysis of the final product showed a single UV peak which had identical retention time as that of an authentic unlabelled standard. The radiochemical purity was 99.6%. None of the *trans*-isomer of 8 was observed in this analysis. MS/MS analysis using an ionspray interface with direct infusion as an aqueous solution via syringe pump (5 mL/min) showed that the mass spectrum of [^{14}C]CGS 19755 was identical to that of an authentic unlabelled standard. The spectrum contained a prominent fragment at m/z = 222 (M–H).

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