Synthesis and Biological Evaluation of Radiolabeled β-Ruthenocenylalanine

William H. Soine,† Clarence E. Guyer,‡ and Furn F. Knapp, Jr.*,‡

Department of Pharmaceutical Chemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298, and Nuclear Medicine Group, Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830. *Received February 8, 1983*

Carrier-free β-[^{103,106}Ru]ruthenocenylalanine was prepared from $^{103,106}RuCl_3$ by utilizing (ruthenocenylmethyl)trimethylammonium iodide as the key synthetic intermediate. The amino acid analogue was evaluated as a pancreatic imaging agent, but no selective uptake in the pancreas was observed in either rats or mice.

Ruthenium-97 has attractive properties for use in diagnostic nuclear medicine ($T_{1/2}$ = 2.9 days, 216-keV γ photon in 86% abundance), but only a few tissue-specific ruthenium radiolabeled agents have been evaluated. Initial studies on the biodistribution and excretion of some inorganic salts and complexes of ruthenium radionuclides were briefly evaluated in humans[1,2] and mice,[3] but no selective organ uptake was observed. A lack of specificity was also observed in rats and rabbits with the ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) chelates of ^{97}Ru.[4] The ruthenium–ammonia complex, [^{103}Ru]ruthenium red, showed some selectivity toward solid tumors in rats,[5] and recently the tumor uptake of a number of ^{97}Ru salts and chelates were evaluated and shown to have tumor specificity that compared favorably with gallium citrate.[6] Transferin labeled with ^{103}Ru and ^{97}Ru was evaluated in several mouse tumor models and shown to have almost twice the tumor uptake observed with gallium citrate.[7]

A different approach is the incorporation of ruthenium radionuclides into the ruthenocene moiety attached to tissue-specific carrier molecules. This approach has been studied by Wenzel and co-workers.[7-13] Through modification of the organic side chains attached to the ruthenocene ring system, organ selectivity has been observed for the adrenal gland with acetyl[^{103}Ru]ruthenocene and (hydroxyacetyl)[^{103}Ru]ruthenocene, for the thymus with cinnamoyl[^{103}Ru]ruthenocene, and for the kidney and liver with [^{103}Ru]ruthenocenecarboxylic acid and its 17β-estradiol and estrone esters. As a result of the limited chemical approaches reported for modification of radiolabeled ruthenocenes, we wished to prepare and evaluate the usefulness of the synthetically versatile quaternary ammonium salt, (ruthenocenylmethyl)trimethylammonium iodide.[18] We have prepared this salt from carrier-free $^{103,106}RuCl_3$ and synthesized β-ruthenocenylalanine, which has been evaluated as a potential pancreas-imaging agent.

Results and Discussion

The usual synthetic approach for incorporating the ruthenium radionuclide into the ruthenocene nucleus is through π ligand–π ligand exchange with the appropriately substituted ferrocene derivative.[8-17] This approach is very appealing due to the simplicity of the reaction conditions, the short reaction time, and the reasonable yields. There are some disadvantages to this approach, however, since the exchange reaction is occasionally unsuccussful, and frequently the separation of the ferrocene and ruthenocene derivatives is very difficult and requires administration of a mixture of the metallocenes. Although this is generally not perceived to interfere with the biological distribution, it would be desirable to administer the homogeneous ruthenocene agents. For these developmental studies, the longer-lived ^{103}Ru ($T_{1/2}$ = 39.4 days) and ^{106}Ru ($T_{1/2}$ = 368 days) were used, since this mixture of reactor-produced radionuclides is readily available. With this in mind, our approach was to prepare [^{103,106}Ru]ruthenocene (**1b**) from $^{103,106}RuCl_3$ and cyclopentadienylsodium. The literature reports describing the synthesis of ruthenocene from Ru^0 and Ru^{3+} indicated that long reaction times (80 h) were required and that the crude reaction mixtures were pyrophoric upon exposure to air.[19] A later report of Hofer and Schlogl[20] suggested that a much shorter reaction time (8 h) is possible and that the reaction mixtures are not pyrophoric. The initial attempts to synthesize **1b** with $^{103,106}RuCl_3$, Ru^0 and cyclopentadienylsodium gave **1b** in 8% yield. Modification of this procedure by pretreatment of $^{103,106}RuCl_3$ (Ru^0 omitted) with excess $TiCl_3 \cdot H_2$, followed by addition of cyclopentadienylsodium, and reaction of this mixture gave **1b** in 35% yield after 1 h. Longer reaction times up to 24 h did not improve the yield.

† Virginia Commonwealth University.
‡ Oak Ridge National Laboratory.

(1) Yamagora, N.; Iwashima, K.; Iinuma, T. A.; Watari, K.; Nagai, T. *Health Phys.* **1969** *16*, 159–166.
(2) Webber, C. E.; Harvey, J. W. *Health Phys.* **1976**, *30*, 355–356.
(3) Wenzel, M.; Nipper, E.; Klose, W. *J. Nucl. Med.* **1977**, *18*, 367–372.
(4) Subramanian, G.; McAfee, J. G. *J. Nucl. Med.* **1970**, *11*, 365.
(5) Anghileri, L. J. *Strahlentherapic* **1975**, *149*, 173–175.
(6) Srivastava, S. C.; Richards, P.; Meinken, G. E.; Som, P.; Atkins, H. L.; Larson, S. M.; Grunbaum, Z.; Rasey, J. S.; Dowling, M.; Clarke, M. J. In "Radiopharmaceuticals II", Proceedings of the International Symposium on Radiopharmaceuticals, Seattle, 1979; Sorenson, J. A., Ed.; Society of Nuclear Medicine: New York, 1979; pp 207–223.
(7) Srivastava, S. C.; Richards, P.; Meinken, G. E.; Larson, S. M.; Grunbaum, Z.; In "Radiopharmaceuticals Structure–Activity Relationships"; Spencer, R. P., Ed.; Grune and Stratton: New York, 1981; pp 207–223.
(8) Langheim, D.; Wenzel, M.; Nipper, E. *Zentralatom. Chem. Ber.* **1975**, *108*, 146–154.
(9) Wenzel, M.; Subramanian, N.; Nipper, E. *Naturwissenchaften* **1976**, *63*, 341.
(10) Taylor, A. J.; Wenzel, M. *Naturwissenschaften* **1977**, *64*, 273–274.
(11) Stadlbauer, E. A.; Nipper, E.; Wenzel, M. *J. Labeled Compd. Radiopharm.* **1977**, *13*, 491–508.
(12) Riesselmann, B.; Wenzel, M. *Hoppe Seylers Z. Physiol. Chem.* **1977**, *358*, 1353–1357.
(13) Wenzel, M.; Herken, R.; Klose, W. *Z. Naturforsch.* **1977**, *32c*, 473–481.
(14) Taylor, A. J.; Wenzel, M. *Xenobiotica* **1978**, *8*, 107–112.
(15) Hoffman, K.; Riesselmann, B.; Wenzel, M. *J. Labeled Compd. Radiopharm.* **1980**, *17*, 421–430.
(16) Taylor, A. J.; Macha, J.; Wenzel, M. *J. Nucl. Med.* **1980**, *21*, 63–66.
(17) Schneider, M.; Wenzel, M. *J. Labeled Compd. Radiopharm.* **1981**, *18*, 293–301.
(18) Rosemblum, M. "Chemistry of the Iron Group Metallocenes: Ferrocene, Ruthenocene, Osmocene", Wiley: New York, 1965; p 137–142.
(19) Bublitz, D. E.; McEwen, W. E.; Kleinberg, J. *Org. Synth.* **1961**, *41*, 96–98.
(20) Hofer, O.; Schlogl, K. *J. Organomet. Chem.* **1968**, *13*, 443–456.

0022-2623/84/1827-0803$01.50/0

Table I. Distribution of Radioactivity in Tissues of Fischer 344 Rats following Intravenous Administration of β-[^{103,106}Ru]Ruthenocenylalanine (5b)[a]

tissue	mean % injected dose/g (range) at the following times after injection			
	5 min	1 h	4 h	1 day
blood	1.949 (1.81-2.12)	0.698 (0.65-0.78)	0.134 (0.13-0.14)	0.051 (0.05-0.05)
liver	2.844 (2.52-3.17)	1.270 (1.11-1.42)	0.278 (0.26-0.31)	0.150 (0.14-0.17)
kidneys	5.438 (4.82-6.24)	2.717 (2.56-2.83)	1.148 (1.07-1.21)	1.128 (0.98-1.23)
lungs	1.123 (1.03-1.21)	0.424 (0.39-0.50)	0.124 (0.12-0.13)	0.065 (0.06-0.07)
stomach	0.243 (0.20-0.34)	0.081 (0.06-0.10)	0.028 (0.02-0.04)	0.023 (0.01-0.04)
small intestine	0.620 (0.54-0.71)	1.421 (1.30-1.56)	0.218 (0.18-0.27)	0.044 (0.03-0.07)
spleen	0.355 (0.31-0.46)	0.140 (0.13-0.15)	0.062 (0.06-0.07)	0.065 (0.06-0.07)
pancreas	0.361 (0.30-0.40)	0.299 (0.14-0.75)	0.044 (0.04-0.05)	0.035 (0.03-0.04)
adrenals	0.275 (0.25-0.31)	0.141 (0.12-0.19)	0.047 (0.04-0.06)	0.044 (0.03-0.05)
thymus	0.335 (0.28-0.39)	0.130 (0.10-0.20)	0.035 (0.03-0.04)	0.024 (0.01-0.04)

[a] Four rats were used for each time period. Each rat received ~6.3 μCi of **5b** administered by injection in a lateral tail vein in saline solution (0.05 mL).

Table II. Distribution of Radioactivity in Tissues of CD-1 Mice following Intravenous Administration of β-[^{103,106}Ru]Ruthenocenylalanine[a]

tissue	mean % injected dose/g (range) at the following times after injection			
	5 min	1 h	4 h	1 day
blood	6.768 (4.71-8.69)	1.039 (0.78-1.59)	0.220 (0.17-0.27)	0.086 (0.06-0.10)
liver	7.749 (6.73-9.24)	1.784 (1.31-2.49)	1.010 (0.54-1.47)	0.508 (0.33-0.65)
kidneys	15.745 (9.01-19.69)	4.594 (3.47-5.66)	1.321 (0.98-1.73)	0.871 (0.51-1.06)
lungs	3.603 (2.23-4.43)	1.015 (0.77-1.42)	0.470 (0.28-0.74)	0.252 (0.12-0.44)
stomach	0.987 (0.40-1.52)	0.326 (0.17-0.52)	0.166 (0.05-0.41)	0.032 (0.02-0.05)
small intestine	1.739 (1.04-2.12)	2.641 (2.01-3.46)	0.367 (0.24-0.53)	0.059 (0.04-0.07)
spleen	1.348 (0.88-1.79)	0.384 (0.24-0.70)	0.206 (0.14-0.30)	0.189 (0.11-0.45)
pancreas	1.749 (1.00-2.44)	0.247 (0.09-0.35)	0.160 (0.11-0.27)	0.067 (0.05-0.08)
adrenals	6.850 (-2.13-31.92)	1.054 (0.45-1.46)	0.786 (-0.88-3.19)	0.556 (0.24-0.95)
thymus	2.579 (0.09-5.19)	0.350 (0.18-0.58)	0.195 (0.10-0.29)	0.138 (0.07-0.24)

[a] Four mice were used for each time period. Each mouse was administered ~1.48 μCi of **5b** by injection into a lateral tail vein in saline solution (0.05 mL).

The functionalization of the ruthenocene ring by the aminomethylation reaction and conversion of the amine to the quaternary ammonium salt proceeded in an overall 50% yield, and only monosubstitution was observed. Further homologation by alkylation with diethylsodioformamidomalonate, followed by hydrolysis to β-ruthenocenylalanine, is similar to the synthesis of β-ferrocenylalanine.[21-23] The final hydrolysis of the diethyl ([^{103,106}Ru]ruthenocenylmethyl)formamidomalonate under acid conditions[21,23] caused considerable decomposition of the ruthenocene nucleus. Very little decomposition was detected, however, under strongly basic conditions.[22] The overall yield of **5b** from $^{103,106}RuCl_3$ was 15%. The carrier-free β-ruthenocenylalanine appeared as a major radioactive component (>95%) upon thin-layer chromatographic analysis and cochromatographed with the unlabeled standard.

The distribution of radioactivity in tissues of Fisher female rats and mice was determined at 5, 30, and 120 min after intravenous injection of no-carrier added β-[^{103,106}Ru]ruthenocenylalanine (**5b**) (Tables I and II). The accumulation of radioactivity in the pancreas was low, and **5b** was rapidly excreted through the kidneys in both rats and mice. This excretion is even more rapid than that reported with $^{103}RuCl_3$.[7] There was also significant accumulation of radioactivity in the liver and lungs. In rats, uptake in the thymus and adrenals was low, but in mice the initial uptake (5 min) in these tissues was high with a rapid washout. The poor selectivity for the pancreas is probably a result of the presence of the bulky Ru–cyclopentadienyl group on one face of the aromatic ring. This may prevent recognition and utilization of this amino acid analogue for pancreatic protein synthesis. It is generally believed that acceptance for protein synthesis is necessary for localization of structurally modified amino acids in the pancreas.[24]

Our studies have shown that carrier-free $^{103,106}RuCl_3$ can be readily converted into the synthetically versatile ([^{103,106}Ru]ruthenocenylmethyl)trimethylammonium iodide in reasonable yield. Further elaboration to the amino acid analogue, β-[^{103,106}Ru]ruthenocenylalanine, was accomplished. These preliminary studies have shown that carrier-free ruthenium-radiolabeled ruthenocene can be readily prepared and that tissue-specific ruthenocene-substituted agents should be further pursued.

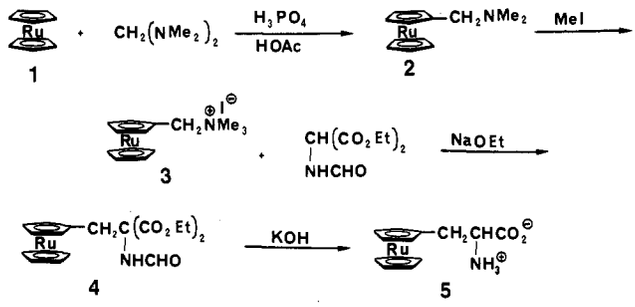
Experimental Section

Thin-layer chromatographic anlayses (TLC) were performed with 250-μm thick layers of silica gel GF or alumina GF coated on glass plates (Analtech., Inc.) Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (IR) were obtained with a Beckman Acculab 8 spectrophotometer. The proton nuclear magnetic resonance spectra were obtained on a JOEL FX900 11 with a multinuclear probe equipped with a JEC-980B computer system and FAFT 26/27/28 software system (Cranford, NJ). The ^{1}H chemical shifts were referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate or tetramethylsilane with a sweep width of 10000 Hz and an 8K transform, a pulse angle of 90°, and a spectrometer frequency of 80 MHz.

Gas chromatography/mass spectrometry (GC/MS) was performed on a Hewlett-Packard Model 5993 GC/MS system and were implemented with a coiled glass column (2 mm i.d. × 4 ft) packed with 3% OV-17 on Gas Chrom Q, 100–120 mesh. Helium (30 mL/min) was used as the carrier gas. The samples were

(21) Schlogl, K. *Monatsh. Chem.* **1957**, *88*, 601–621.
(22) Hauser, C. R.; Lindsay, J. K. *J. Org. Chem.* **1957**, *22*, 1246–1247.
(23) Osgerby, J. M.; Pauson, P. L. *J. Chem. Soc.* **1958**, 656–660.
(24) Taylor, D. M.; Cottrall, M. F. In "Radiopharmaceuticals and Labeled Compounds"; International Atomic Energy Agency: Vienna, 1973; Vol. I, pp 443–441.

Scheme I



chromatographed by temperature programming, 150–260 °C at 20 °C/min (injection port = 270 °C). Quantitation of yield was based on ^{103}Ru (keV = 497, $\epsilon = 3.58 \times 10^{-4}$, $I_0 = 0.89$).

Materials. Ruthenium(III) chloride hydrate and titanium(III) chloride, hydrogen reduced, were obtained from Alfa Inorganics, Danver, MA. All solvents were analytical grade and purified by standard procedures prior to use. The [^{103,106}Ru]ruthenium(III) chloride was obtained as a carrier-free solution in 2–5 N hydrochloric acid from the Isotope Sales Office, Oak Ridge National Laboratory.

Animal Tissue Distribution Experiments. The tissue distribution studies were performed with 10–12-week-old female Fischer 344 rats (170–200 g) and 7^1/$_2$-week-old female CD-1 mice (25–29 g) obtained from Charles River Laboratories. The animals were allowed food and water ad libitum prior to and during the course of the experiment. The radiolabeled amino acid (**5b**) was dissolved in physiological saline solution, the pH was adjusted to 7.4, and the solution was filtered through a 22-μm Millipore filter and injected via a laterial tail vein into ether-anethestized animals. After the times indicated in Tables I and II, the animals were killed by cervical fracture, and blood samples were obtained by cardiac puncture. The organs were then removed, rinsed with saline solution, and blotted dry to remove residual blood. The organs were weighed and counted in a NaI autogamma counter (Packard Instruments). Samples of the injected radioactive solutions were also assayed as a standard to calculate the percent injected dose per gram of tissue values.

[^{103,106}Ru]Ruthenocene (1b). In a typical synthesis, a 150-μL solution of $^{103}RuCl_3$ (5.56 mCi/mL) and $^{106}RuCl_3$ (0.60 mCi/mL) in 5 N HCl was evaporated to dryness under a stream of argon in a 2-mL conical vial. After the addition of 1.0 mL of anhydrous tetrahydrofuran (THF) to the residue, the solution was evaporated to dryness. The residue was dissolved in anhydrous THF and after the addition of 15 mg of $TiCl_3 \cdot H_2$ in a THF slurry, the mixture was stirred for 10 min. A solution of 1.0 mL of cyclopentadienylsodium (0.7 mmol/mL) in THF was added to the suspension, and the reaction was stirred at room temperature for 1 h. The mixture was evaporated to dryness under a stream of argon, quenched with 1 mL of MeOH, and again evaporated to dryness. The brown sludge remaining was triturated with diethyl ether and filtered through a short (1 cm) alumina column. The yellow eluate was concentrated almost to dryness and then redissolved in petroleum ether, and the solution then passed through a neutral alumina column (3 cm, activated, 100–120 mesh). Elution with petroleum ether gave 289.2 μCi of [^{103,106}Ru]ruthenocene in 35% yield. The radiochemical purity of **1b** was confirmed by TLC using petroleum ether on silica gel G (R_f 0.36) and alumina G (R_f 0.70). Authentic samples of ruthenocene were obtained from Pressure Chemical Co., Pittsburgh, PA, and also prepared by pretreatment of $RuCl_3$ with $TiCl_3 \cdot H_2$ as described for **1b**. The IR, ^{1}H NMR, and MS were consistent with the structure of ruthenocene. Preparation of the [^{103,106}Ru]ruthenocene in the absence of the $TiCl_3 \cdot H_2$ catalyst proceeded in only 8–11% yield.

[(Dimethylamino)methyl][^{103,106}Ru]ruthenocene (2b). The petroleum ether eluate containing **1b** was evaporated to dryness under argon in a 2-mL conical vial. After the addition of 2 mL of the Mannich reagent, which was prepared from 14 mL of HOAc, 1.4 mL of H_3PO_4, and 1.2 mL of *N,N'*-bis(dimethylamino)-methane, the vial was tightly capped and heated at 120 °C for 4 h. The vial was cooled, and the dark black reaction mixture was quenched by the addition of 3 mL of H_2O. The aqueous solution was extracted with diethyl ether (2 mL × 3) to remove the unreacted ruthenocene (13–50%). The aqueous fraction was made basic with 50% NaOH and extracted with diethyl ether, and the ether extract was dried over anhydrous Na_2SO_4. The ether fraction contained 101 μCi of ^{103,106}Ru (50% theoretical yield, range 40–70%). The radiochemical purity of **2b** was confirmed by TLC [5% MeOH in acetone, alumnia, (R_f 0.60)]. Authentic [(dimethylamino)methyl]ruthenocene was synthesized by the method of Hofer and Schlogl:[20] ^{1}H NMR ($CDCl_3$) δ 2.15 (s, 6 H, CH_3), 3.05 (s, 2 H, CH_2), 4.45 (s, 7 H, Cp-H and α-H), 4.59 (t, 2 H, β-H); the mass spectrum contained groups of ions characteristic of a ruthenocene nucleus with a parent peak at m/z 288, base peak at m/z 244 [$(RuCH_2)^+$], and a prominent group of ions at m/z 167 [$(CpRu)^+$].

([^{103,106}Ru]Ruthenocenylmethyl)trimethylammonium Iodide (3b). An ether solution containing 192 μCi of **2b** was evaporated to dryness, and the residue was dissolved in 0.5 mL of freshly distilled CH_3I. The reaction was allowed to stand at room temperature for 0.5 h with stirring, and then excess CH_3I was removed under a stream of argon. The radiochemical purity of **3b** was confirmed by TLC using 5% MeOH in acetone on alumina G (R_f 0.15), which indicated that there was quantitative conversion to the quaternary ammonium compound. Authentic (ruthenocenylmethyl)trimethylammonium iodide was prepared by the method of Schlogl and Hofer:[20] ^{1}H NMR ($CDCl_3$) δ 4.95 (t, 2 H, α-CH_2), 4.75 (t, 2 H, β-CH_2), 4.70 (s, 5 H, Cp), 4.60 (s, 2 H, CH_2), 3.40 (s, 9 H, CH_3).

Diethyl (Ruthenocenylmethyl)formamidomalonate (4a). In a modification of the procedure by Osgerby and Pauson[23], the quaternary ammonium salt (150 mg, 0.36 mmol) was added to a solution of diethyl sodioformamidomalonate, prepared by dissolving diethyl formamidomalonate (100 mg, 0.49 mmol) in anhydrous EtOH containing NaOEt (0.46 mmol). The solution was refluxed overnight (12 h) under a nitrogen atmosphere, and the solvent was removed under reduced pressure to give a yellow solid. The residue was extracted with ether, dried over anhydrous Na_2SO_4, filtered, evaporated to dryness, and recrystallized from $EtOH/H_2O$ to give 125 mg of an off-white crystalline material (80% yield): ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 6 H, CH_3), 3.28 (s, 2 H, CH_2), 4.32 (q, 4 H, OCH_2), 4.45 (s, 5 H, Cp), 6.87 (s, 1 H, NH), 8.22 (s, 1 H, CHO). The mass spectrum contained groups of ions characteristic of an intact ruthenocene nucleus with a parent peak at m/z 447 and a base peak at m/z 245. A minor product (8–15%) was identified as ethyl 3-ruthenocenyl-2-formamidopropionate and evidently resulted from decarbethoxylation of the malonic ester. This product was identified based on its short retention time on GC/MS (1.12 min) and ions characteristic of a ruthenocene nucleus, with a parent ion at m/z 375 and a base peak at m/z 245.

Diethyl ([^{103,106}Ru]Ruthenocenylmethyl)formamidomalonate (4b). The quaternary ammonium salt, **2b**, was dissolved in 1.0 mL of anhydrous EtOH containing 0.25 mmol of diethyl sodioformamidomalonate, tightly capped, and heated at 90 °C for 15 h. TLC analysis of the reaction mixture (5% MeOH in acetone, alumina) indicated a single radioactive band corresponding to diethyl (ruthenocenylmethyl)formamidomalonate, R_f 0.66. The reaction mixture was evaporated to dryness under a stream of argon, the residue was extracted with ether (twice), and filtered through glass-wool. The eluate (9.2 mL) contained 29.38 μCi of ^{103,106}Ru (74% theoretical yield, range 74–90%) with TLC behavior identical with **4a** (5% MeOH in acetone on alumina, R_f 0.66).

DL-β-Ruthenocenylalanine (5a). The crude alkylation product containing **4a** was dissolved in 20 mL of $EtOH/H_2O$ (1:1) containing 200 mg of KOH. The mixture was refluxed for 24 h, evaporated to dryness, redissolved in 20 mL of H_2O, acidified to pH 1.2 with H_3PO_4, and passed over a strong cation-exchange resin (Amberlite CG-120, 100–200 mesh) in the acid form. The β-ruthenocenylalanine was eluted with 1 N NH_4OH, which was evaporated to dryness. The amber powder remaining was dissolved in a minimum volume of H_2O. This solution (pH 12) was filtered and adjusted to pH 6–7 with HCl. The amino acid precipitate was filtered, washed with a minimum volume of H_2O, and dried under vacuum to yield a biege powder. The powder was recrystallized from $EtOH/H_2O$ and $MeOH \cdot H_2O$ to give a beige powder, mp 228–231 °C dec, in an overall yield of 39% from

ruthenocene: TLC (*n*-BuOH/H_2O/HOAc, 4:1:1) R_f 0.46 with positive color response when sprayed with ninhydrin; HPLC, LiChrosorb RF-18 (10 μm), mobile phase 0.01 M phosphate buffer, pH 3.0/MeOH (60:40), flow rate 1 mL/min, detection; UV max 245 nm; t_R = 9.92 min; IR (KBr) 3415 (br), 2050 (br), 1600 (s), 1510 (m), 1440 (w), 1415 (m), 1355 (m), 1190 (w), 1155 (w), 1110 (m), 1030 (m), 1045 (m), 1010 (m), 865 (m), 820 (s); ^1H NMR (D_2O, NaOD) δ 2.51 (t, 2 H, CH_2), 3.24 (dd, 1 H, CH), 4.51 (s, 2 H, α-H), 4.59 (s, 5 H, Cp), 4.6 (s, 2 H, β-H); the mass spectrum of the bis(trimethylsilyl) derivative contained groups of ions characteristic of an intact ruthenocene nucleus with a parent peak at *m/z* 463 and a base peak at *m/z* 245. Anal. ($C_{13}H_{15}N_2Ru \cdot H_2O$) C, H, N.

β-[^{103,106}Ru]Ruthenocenylalanine (5b). The EtOH solution of the crude alkylation product from the previous reaction was evaporated to 0.5 mL under a stream of argon, and 0.5 mL of 1 M NaOH was then added. The solution was refluxed for 2 h, cooled, acidified to pH 1–3 with H_3PO_4, and passed over a strong cation-exchange resin (Amberlite CG-120, 100–200 mesh). The first 5 mL of eluate contained the product, 106 μCi (40% theoretical yield), which chromatographed with authentic **5a** with *n*-BuOH/HOAc/H_2O (4:1:1) or *n*-BuOH/HOAc/pyridine/H_2O (4:1:1) on silica gel G.

Acknowledgment. Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc. The authors thank K. R. Ambrose, E. B. Cunningham, and J. A. Roberts of the Oak Ridge National Laboratory for technical assistance and Lynda Watson for typing the manuscript.

Registry No. 1 (^{103}Ru derivative), 12093-76-4; 1 (^{106}Ru derivative), 89462-67-9; **2**, 33293-45-7; **2** (^{103}Ru derivative), 89462-68-0; **2** (^{106}Ru derivative), 89462-69-1; **3**, 33292-36-3; **3** (^{103}Ru derivative), 89462-70-4; **3** (^{106}Ru derivative), 89462-71-5; **4**, 89462-72-6; **4** (^{103}Ru derivative), 89462-73-7; **4** (^{106}Ru derivative), 89462-74-8; **5**, 89462-75-9; **5** (^{103}Ru derivative), 89462-76-0; **5** (^{106}Ru derivative), 89462-77-1; $^{103}RuCl_3$, 65234-97-1; $^{106}RuCl_3$, 63767-78-2; cyclopentadienylsodium, 63936-05-0; *N,N'*-bis(dimethylamino)methane, 51-80-9; ethyl 3-ruthenocenyl-2-formamidopropionate, 89462-78-2; diethyl formamidomalonate, 6326-44-9.

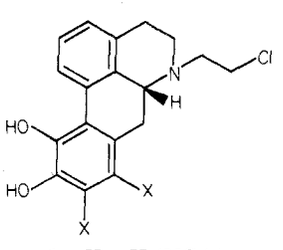
Aporphines. 58. *N*-(2-Chloroethyl)[8,9-^{2}H]norapomorphine, an Irreversible Ligand for Dopamine Receptors: Synthesis and Application[1]

Ji-Hua Guan,[†] John L. Neumeyer,*[,†] Crist N. Filer,[‡] David G. Ahern,[‡] Leslie Lilly,[§] Masayuki Watanabe,[§] Dimitri Grigoriadis,[§] and Philip Seeman[§]

Section of Medicinal Chemistry, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, Massachusetts 02115, New England Nuclear, Boston, Massachusetts 02118, and Department of Pharmacology, University of Toronto, Canada M5S 1AB. *Received August 30, 1983*

The synthesis of the title compounds (**1c** and its ^{2}H isomer **1b**) from *N*-(2-hydroxyethyl)norapomorphine was carried out by ring bromination, followed by chlorination to the 2-chloroethyl compound **6**. Further reduction with $^2H_2$ or $^3H_2$ and Pd/C gave **1b** or **1c**. Radiochemically pure (97%) **1c** was obtained with a specific activity of 16.3 Ci/mmol. The purity of **1c** was determined by LC, HPLC, UV, and NMR. [^{3}H]NCA was shown to label the D_2 receptor; however, the D_2 signal appears to be only a small portion of the total signal, which may include binding to other dopamine receptor subtypes (D_1 and D_3).

Tritiated catechol aporphines, such as apomorphine (APO) and *N*-*n*-propylnorapomorphine (NPA), are known to bind selectively at sites believed to represent dopamine (DA) receptors in the central nervous system (CNS).[2,3] The synthesis[4] and demonstration that (−)-*N*-(2-chloroethyl)-10,11-dihydroxynoraporphine [*N*-(2-chloroethyl)-norapomorphine, NCA, **1a**] causes persistent DA receptor



1a, X = H (NCA)
b, X = ^{2}H
c, X = ^{3}H

blockage suggested that this inhibition of DA receptor function may involve covalent bonding of a receptor binding site.[4-8] NCA blocked DA-sensitive adenylate cyclase activity in a noncompetitive and apparently irreversible manner.[5] This effect was prevented by coincubation with DA or APO but not with norepinephrine. Analogues of NCA with low affinity for DA receptor sites defined by binding of 1 nM [^{3}H]APO[3] also had much weaker effects against the DA-sensitive cyclase activity. These included the 10-O-methylated derivative (analogue of apocodeine) and a 10,11-diester derivative of NCA.

Additional pharmacological actions of NCA have been described.[9-14] Mustafa et al.[9] reported that NCA (57 μmol) locally injected into the nucleus accumbens of rat brain can block the behavioral excitation produced by similarly

[†] Northeastern University.
[‡] New England Nuclear.
[§] University of Toronto.

(1) Abstracted in part from the thesis of J.H.G., which was submitted in June, 1982, to Northeastern University in partial fulfillment of the requirements for the M.S. degree in Medicinal Chemistry.
(2) Seeman, P. *Pharmacol. Rev.* **1980**, *32*, 229.
(3) Arana, G. W.; Baldessarini, R. J.; Harding, M. *Biochem. Pharmacol.* **1981**, *30*, 3171.
(4) Neumeyer, J. L.; Law, S. J.; Baldessarini, R. J.; Kula, N. S. *J. Med. Chem.* **1980**, *23*, 594.
(5) Baldessarini, R. J.; Kula, N. S.; Arana, G. W.; Neumeyer, J. L.; Law, S. J. *Eur. J. Pharmacol.* **1980**, *67*, 105.
(6) Costall, B.; Fortune, D. J.; Law, S. J.; Naylor, R. J.; Neumeyer, J. L.; Nohria, V. *Nature (London)* **1980**, *285*, 571.
(7) Costall, B.; Fortune, D. H.; Granchelli, F. E.; Law, S. J.; Naylor, R. J.; Neumeyer, J. L.; Nohria, V. *J. Pharm. Pharmacol.* **1980**, *32*, 571.
(8) Cohen, S. A.; Neumeyer, J. L. *J. Med. Chem.* **1983**, *26*, 1348.
(9) Mustafa, A. A.; Sharma, R. P.; Woodruff, G. N. *Br. J. Pharmacol.* **1982**, *75*, 39P.

0022-2623/84/1827-0806$01.50/0