A FACILE SYNTHESIS OF [^{14}C]ENADOLINE [(5*R*)-(5α,7α,8β)]-N-METHYL-N-[7-(1-PYRROLIDINYL)-1-OXASPIRO[4.5] DEC-8-YL]-4-BENZOFURANACETAMIDE*

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Summary

4-Chloromethylbenzofuran (**10**) was synthesized from 2,3-dimethylanisole in 7 steps. The corresponding Grignard reagent prepared from magnesium-anthracene complex reacts with $^{14}CO_2$, $SOCl_2$, and PD130812 successively to give [^{14}C]enadoline (**2**), a non-peptide, selective kappa opioid receptor agonist. This method could be readily modified for the rapid, one-pot synthesis of [^{11}C]enadoline.

Key Words: C-14 Label, Enadoline, Analgesic, Opioid Receptors.

Introduction

The identification of three distinct opioid receptor subtypes, *mu*, *kappa* and *delta* in 1976 by Martin (1) has stimulated a great deal of interest in the opioid research for more than a decade. The expectation lies in the fact that selective *kappa* receptor agonists could be potential analgesics devoid of many of undesired side effects associated with activation of *mu* and *delta* receptors. U-50488 (**1**), a novel, non-peptide N-(2-aminocyclohexyl) arylacetamide class of selective κ receptor agonists, emerged from such a drug screening in 1982 (2). Subsequently enadoline, [(5*R*)-(5α,7α,8β)]-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5] dec-8-yl]-4-benzofuranacetamide (**2**), was synthesized at Parke-Davis and was shown to be a more selective agonist than U-50488 *in vitro* and thus a more potent

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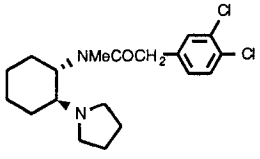
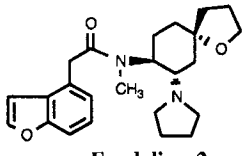
analgesic in rodent tests (3). More recently, it was demonstrated that enadoline significantly reduced the volume of ischemic brain damage in rodents (4,5). Although the mechanism of this neuroprotective effect of enadoline has been investigated and the earlier evidence presented suggested that an enadoline-mediated presynaptic inhibition of glutamate release could be a major contributor, a precise mechanism for the neuroprotective effect of kappa agonists is presently unknown.



U-50488, 1



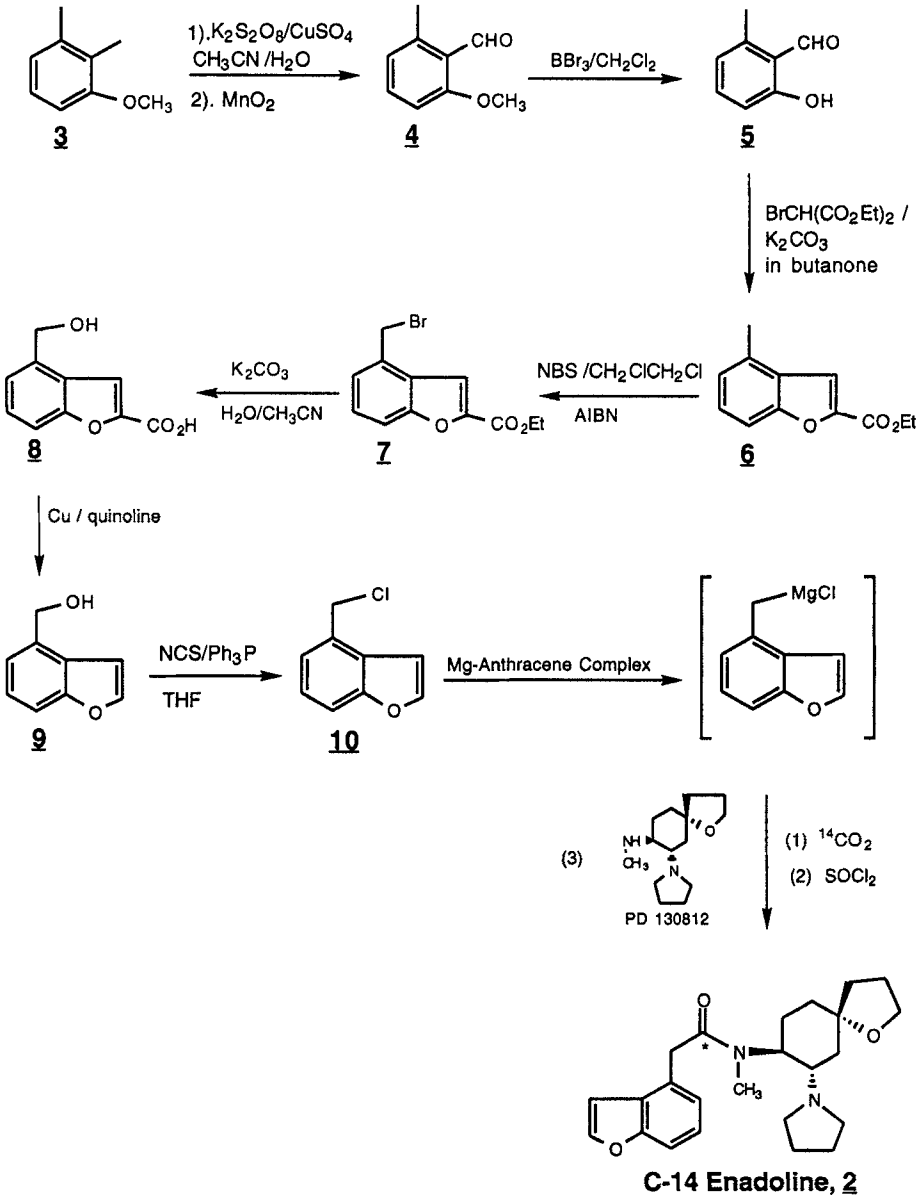
Enadoline, 2

	Opioid Receptor Binding Affinity Ki (nM)(9)		
	kappa (κ),	mu (μ),	κ /μ
U-50488, 1	10	880	88
Enadoline, 2	0.83	1260	1520

Positron emission tomography (PET) originally developed for the evaluation of cerebral blood flow and metabolism has been widely used for the study of CNS receptors. The κ specific PET ligands with high specific activity (C-11) would provide invaluable tools for investigating the functional characteristics of the binding site of κ receptors in their native or unaltered states, and possibly offer useful information on antinociceptive and neuroprotective actions of enadoline (2). Therefore, we became interested in developing the methodology suitable for the preparation of C-11 labeled enadoline (6). A ^{14}C-synthesis of enadoline as a model for future C-11 synthesis has been developed. The ^{14}C-labeled compound would be useful for further metabolism studies as well.

Racemic [^{14}C][(5α,7α,8β)]-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl] 4-benzofuranacet amide (PD126212) was synthesized several years ago in Parke-Davis from sodium [1-^{14}C]acetate and a C-14 label was introduced in the metabolically stable carbonyl position (7). Since the half life of carbon-11 isotope is only 22 min., the synthesis of the enadoline precursor that is readily amenable to labeling with C-11 becomes important. De-N-methyl enadoline could conveniently be labeled with $^{11}CH_3I$ under alkaline conditions. However, the fact that N-methyl group might be metabolically unstable has prompted us to search for an alternative precursor for labeling. Herein, we

wish to report the synthesis of 4-chloromethylbenzofuran (**10**) and subsequent labeling with carbon-14 isotope at the carbonyl position (Scheme 1).



Scheme 1: Synthesis of C-14 Labeled Enadoline

Chemistry

4-Chloromethylbenzofuran (**10**) was synthesized in 7 steps from 2,3-dimethylanisole (**3**). 2-Methoxy-6-methylbenzaldehyde (**4**) was obtained by refluxing 2,3-dimethylanisole in a suspension of potassium persulfate and copper sulfate and subsequent conversion of the resulting 2-methoxy-6-methylbenzyl alcohol to the corresponding aldehyde with manganese dioxide. De-O-methylation was carried out with boron tribromide in dichloromethane to afford 2-hydroxy-6-methylbenzaldehyde (**5**). Ethyl 4-methylcoumarilate (**6**) was readily prepared by refluxing a mixture of **5** and diethyl 2-bromomalonate in K_2CO_3 and butanone. Treatment of **6** with N-bromosuccinimide and AIBN in dichloroethane yielded ethyl 4-bromomethylcoumarilate (**7**), which was saponified quantitatively to **8**. Heating of 4-hydroxymethyl coumarilic acid at reflux in quinoline led to a decarboxylation product **9**. 4-Chloromethylbenzofuran (**10**) was prepared by stirring **9**, N-chlorosuccinimide, and triphenylphosphine in THF.

Enadoline can be synthesized from the carboxylation of **10** *via* the corresponding Grignard reagent followed by coupling with the diamino side chain (PD 130812). The preparation of the Grignard reagent of **10** using conventional methods proved to be difficult. The reaction could be initiated with difficulties, and once started it gave only a coupling product (dimer). To circumvent this difficulty, an alternative procedure to prepare the Grignard reagent at lower temperature was investigated. Magnesium-anthracene complex, a yellow-orange colored solid, has been largely used for the preparation of benzyl type Grignard reagents from organic chlorides or bromides at very low temperature (8). When 4-chloromethylbenzofuran (**10**) was added slowly to a stirred Mg-anthracene solution at -45°C, the corresponding Grignard reagent formed in 70%. After CO_2 was bubbled through, thionyl chloride and PD130812 were added successively. The resulting product was isolated and characterized as enadoline in comparison of MS and NMR with those from an authentic sample. Both the unlabeled and ^{14}C-labeled syntheses using excess CO_2 and limiting $[^{14}C]CO_2$, respectively were carried out. The low radiochemical yield (34%) in the labeled synthesis as compared to the chemical yield of 80% in the unlabeled synthesis was probably attributed to the limiting labeled CO_2 used.

In conclusion, an improved, facile synthesis of $[^{14}C]$ enadoline has been developed. Conceivably, it may be possible to modify this method further in the microscale synthesis of $[^{11}C]$enadoline using large excess of the Grignard reagent and PD130812, and limiting amount of $[^{11}C]CO_2$.

Experimental Section

Materials and Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Electron ionization mass spectra (EIMS) were obtained using a Hewlett-Packard 5970A GC-MS spectrometer. Electron spray mass spectra were taken on a Fisons Trio 2000 spectrometer. ^{1}H-NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 solutions using a Varian XL-300 spectrometer. All chemicals were used as obtained. Solvents were distilled prior to use.

2-Methoxy-6-methylbenzaldehyde (4). To a mechanically stirred suspension of potassium persulfate (86.4 g, 0.32 mole) and copper (II) sulfate pentahydrate (8.0 g, 0.032 mole) in 560 mL of N_2-saturated water was added 2,3-dimethylanisole (21.8 g, 0.16 mole) in 560 mL of N_2-saturated acetonitrile and 26 mL of pyridine. The blue solution was slowly heated to reflux and maintained for 1 h, and then cooled to rt. The organic layer was separated, and the aqueous layer was back-extracted with ether (2x300 mL). The combined organic layers were washed with 5% Na_2CO_3 and brine, dried (Na_2SO_4), and concentrated. The dark oil in CH_2Cl_2 (20 mL) was flash chromatographed over silica gel on a short-path column eluting with hexane:ethyl acetate (9:1) to remove the dark polar impurities. The solvent was stripped off, and the yellow-orange oil in 100 mL of benzene was heated at reflux with activated manganese dioxide (15.0 g) for 3.5 h. The mixture was filtered and washed with benzene, and the filtrate concentrated to dryness to yield a yellow-orange oil. The oil was distilled *in vacuo* to afford a pale-yellow oil (12.0 g, 50%) which solidified on standing: mp 40-41^{o}C; ^{1}H-NMR ($CDCl_3$) δ 10.64 (1H,s, CHO), 7.35 (1H, t, J=7.8 Hz), 6.83 (1H,d, J=7.8 Hz), 6.79 (1H,d, J=7.8Hz); GC-MS (EI): m/e 150 (95%), 133 (30%).

2-Hydroxy-6-methylbenzaldehyde (5). To an 83 mL solution of 1 M boron tribromide in CH_2Cl_2 at -30^{o}C was added dropwise 2-methoxy-6-methyl benzaldehyde (12.5 g, 0.083 mole) in 80 mL of dry CH_2Cl_2 over 30 min. The orange-colored mixture was allowed to stir at 0^{o}C for 1.5 h. The reaction was quenched with 100 mL of water carefully. The organic phase was washed with 5% aq. $NaHCO_3$ solution, dried over magnesium sulfate, and concentrated *in vacuo*. The crude product was then purified by column chromatography (hexane:ethyl acetate 9:1) to afford 9.4 g (83%) of **5** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$) δ 11.92 (1H,s, OH), 10.33 (1H,s, CHO), 7.38 (1H, t, J=8.0 Hz), 6.82 (1H,d, J=8.1Hz), 6.71 (1H,d, J=8.1 Hz), 2.61 (3H,s, CH_3); GC-MS (EI) m/e 136 (95%), 135 (100%), 118 (16%), 107 (20%).

Ethyl 4-methylcoumarilate (6). To a suspension of 2-hydroxy-6-methylbenzaldehyde (5.0 g, 36.7 mmole) and potassium carbonate (10.2 g, 74 mmole) in 100 mL of butanone and 1.0 mL of water was added diethyl bromomalonate (9.4 g, 40.0 mmole). The reaction mixture was refluxed overnight, and cooled to rt. The solution was filtered and concentrated to dryness. The brown oil was treated with 2.0 g of P_2O_5 in dichloromethane for 30 min. The solution was filtered, washed with 5% $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (hexane:ethyl acetate 9:1) to afford 4.3 g (57%) of **6** as a white solid: mp 64-65°C; ^{1}H-NMR ($CDCl_3$) δ 7.57 (1H,s), 7.41(1H,d, J=8.3Hz), 7.34 (1H,t, J=8.3 Hz), 7.09 (1H,d, J=8.3 Hz), 4.45 (2H,q, J=7.1 Hz, OCH_2CH_3), 2.55 (3H,s, Ar-CH_3), 1.44 (3H,t, J=7.1 Hz, OCH_2CH_3).

Ethyl 4-bromomethylcoumarilate (7). A mixture of ethyl 4-methylcoumarilate (2.04 g, 10.0 mmole), N-bromosuccinimide (2.0 g, 11.0 mmole) and 0.5 g of AIBN in 50 mL of dichloroethane was heated at reflux for 2 h. Additional 0.20 g of NBS and 25.0 mg of AIBN were added to assure the completion of reaction, and the mixture was stirred for 15 min. The solution was poured into CH_2Cl_2 (50 mL), washed with 2% $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography to afford 2.7 g (95%) of **7** as a white solid: mp 83-84°C; ^{1}H-NMR ($CDCl_3$) δ 7.71 (1H,s), 7.56 (1H,d, J=8.3Hz), 7.40 (1H,t, J=8.3 Hz), 7.30 (1H,d, J=8.0 Hz), 4.74 (2H,s, CH_2Br), 4.47 (2H,q, J=7.1 Hz, OCH_2CH_3), 1.45 (3H,t, J=7.1 Hz, OCH_2CH_3); GC-MS (EI) m/e 284 (5%), 282 (5%), 237 (4%), 239 (4%), 203 (100%).

4-Hydroxymethylcoumarilic Acid (8). To a solution of ethyl 4-bromomethylcoumarilate (4.52 g, 16.0 mmole) in 64 mL of water and 16 mL of acetonitrile was added K_2CO_3 (6.64 g, 48 mmole). The reaction mixture was heated at reflux for 2 h., allowed to cool to rt, and filtered. Acetonitrile was removed at 40°C under a reduced pressure. The aqueous solution was acidified with conc. HCl (pH 1-2, paper). The precipitate was filtered off, washed with water, and dried under vacuum to give 2.9 g (95%) of **8**: mp 202-204°C; ^{1}H-NMR (DMSO) δ 13.54 (1H,s, CO_2H), 7.78 (1H,s), 7.57 (1H,d, J=8.3 Hz), 7.46 (1H,t, J=8.0 Hz), 7.30 (1H,d, J=8.0 Hz), 4.79 (2H,s, CH_2OH).

4-Hydroxymethylbenzofuran (9). 4-Hydroxymethylcoumarilic acid (192 mg, 1.0 mmole) and copper powder (100 mg) in quinoline (5.0 mL) were slowly heated at reflux for 1 h. The reaction mixture was poured into ether (30 mL), and filtered. The filtrate was

washed with 2.5 N HCl (3x50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by column chromatography (hexane : ethyl acetate 4:1) to afford 70 mg of **9** as a low melting solid: ^{1}H-NMR ($CDCl_3$) δ 7.63 (1H,d, J=2.2 Hz), 7.46 (1H,d, J=8.3 Hz), 7.27 (1H,t, J=8.0 Hz), 7.19 (1H,d, J=8.0 Hz), 6.88 (1H,d, J=2.2 Hz),4.86 (2H,s, CH_2OH); GC-MS (EI) m/e 148 (100%), 131 (100%), 119 (95%).

4-Chloromethylbenzofuran (10). A mixture of 4-hydroxymethylbenzofuran (148 mg, 1.0 mmole), N-chlorosuccinimide (134 mg, 1.0 mmole) and triphenylphosphine (265 mg, 1.0 mmole) was stirred in THF (2.0 mL) at rt. for 1 h. The solution was transferred into CH_2Cl_2 (10 mL), washed with water, dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethyl acetate 95:5) to afford 120 mg (72%) of **10** as an oil: ^{1}H-NMR ($CDCl_3$) δ 7.70 (1H,d, J=2.2 Hz), 7.50 (1H,d,J=8.0 Hz), 7.35-7.20 (2H,m), 6.95 (1H,d, J=2.2 Hz), 4.85 (2H,s, CH_2Cl); GC-MS (EI) m/e 168 (30%), 166 (100%), 131 (100%), 102 (85%).

Magnesium-Anthracene Complex: Magnesium (turning, crushed, 0.53 g, 22.0 mmole) in 2.0 mL of THF was activated with 0.10 mL of iodoethane for 5 min. Anthracene (2.0 g, 11.2 mmole) in 20 mL of hot THF was added. The reaction mixture was heated at reflux for 10 min, allowed to cool to rt. The mixture was stirred at rt. for 7 days during which a heavy orange precipitate formed. This orange suspension was diluted with additional 20.0 mL of THF.

Enadoline (2) (one-pot): To a 0.80 mL of the above Mg-anthracene complex (0.22 mmole) at -40°C was slowly added 4-chloromethylbenzofuran (33.0 mg, 0.20 mmole) in THF (1.0 mL). The blue color persisted until the addition was completed. The reaction mixture was allowed to warm to -10°C within 1.5 h, and bubbled with CO_2 for 5 min. Thionyl chloride (2 M, 60 uL) in CH_2Cl_2 was added and stirred for 5 min.; PD 130812 (30.0 mg, 0.126 mmole) in 0.20 mL of THF and triethylamine (20 uL) were added and stirred for 10 min. The mixture was poured into 3.0 mL of methanol, and concentrated *in vacuo*. The crude product was purified on preparative tlc plates (20 cm x 20cm, 0.5 mm thickness) (diisopropyl ether:methoxy ethanol:ammonium hydroxide 9:1:0.1) to yield 40.0 mg (80%) of CI-977 as a free base. TLC, HPLC, MS (ES) and NMR were consistent with those from an authentic sample.

C-14 Labeled enadoline (2): To a solution of the above Mg-anthracene complex (0.80 mL, 0.22 mmole) in a v-shaped vial at -40°C was slowly added 4-chloromethyl

benzofuran (33.0 mg, 0.20 mmole) in THF (1.0 mL). The reaction mixture was allowed to warm to -10°C within 1.5 h, and the v-shaped reaction vial was then quickly connected to a vacuum manifold. The $^{14}CO_2$ generated from 28.0 mg (0.14 mmole, 7.7 mCi) of $Ba^{14}CO_3$ and conc. H_2SO_4 was condensed in the vial which was immersed in a liquid nitrogen bath. The reaction mixture was allowed to warm to rt., and transferred to 8.0 mL of methanol. The solution was filtered, and concentrated *in vacuo*. The crude product was purified by preparative thin layer chromatography ($CHCl_3$:ethyl acetate:formic acid 50:50:1) to afford 14.0 mg of C-14 4-benzofuranacetic acid. To this radioactive 4-benzofuranacetic acid in CH_2Cl_2 : DMF (0.8 mL/0.050 mL) was added 2 M $SOCl_2$ (40 uL) in CH_2Cl_2. The solution was stirred at rt for 30 min. PD 130812 (25.0 mg) and TEA (15 uL) in 0.2 mL of CH_2Cl_2 were added, and the mixture was stirred for 30 min. The reaction mixture was poured into methanol (1.0 mL), and concentrated *in vacuo*. The crude product was purified by preparative thin layer chromatography (diisopropyl ether:methoxy ethanol :ammonium hydroxide 9:1:0.1) to give 14.0 mg (radiochemical yield: 34%) of enadoline as a free base and 4.0 mg of unreacted C-14 4-benzofuranacetic acid. The ^{14}C-labeled enadoline was analyzed by HPLC (chemical purity : 99.5%; radiochemical purity: 99.4%).

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