SYNTHESIS OF CARBON-14 LABELED PD 117,302 AND PD 126,212, POTENTIAL NEW ANALGESIC AGENTS.

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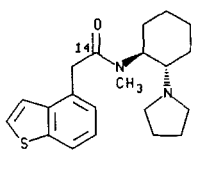
SUMMARY

Two kappa opiate agonists 1 and 2 have been labeled from bromo-[1-^{14}C]-acetic acid and sodium [1-^{14}C]acetate respectively.

Keywords: Benzo[b]thiophene-4-[carboxyl-^{14}C]acetic acid, 1,1-Dimethylethyl-[1-^{14}C]acetate, Benzofuran-4-[carboxyl-^{14}C]acetic acid, (±)-*trans*-N-Methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzo[b]thiophene-4-acetamide (PD 117,302), (±)-(5α,7α,8β)-N-Methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-4-benzofuranacetamide (PD 126,212), analgesic

INTRODUCTION

(±)-*trans*-N-Methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzo[b]thiophene-4-acetamide (PD-117,302) (1) and (±)-(5α,7α,8β)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl-4-benzofuranacetamide (PD-126,212) (2) have high affinity for the kappa opiate receptor and are potential new analgesics.[1,2] The pharmacokinetic and metabolic studies of these new drugs required the carbon-14 labeled compounds. The synthesis of these labeled compounds is described herein.



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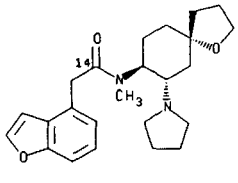

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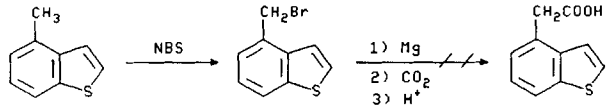
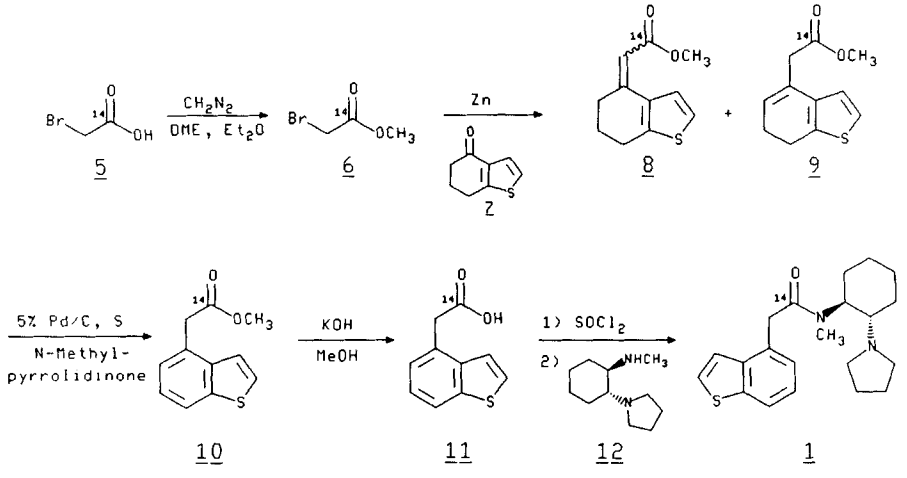
RESULTS AND DISCUSSION

The syntheses of 1 and 2 were designed to place the carbon-14 label in the metabolically stable carbonyl position. For the synthesis of 1, the original synthetic scheme as shown in Scheme 1 utilized 4-methylbenzo[b]thiophene 3 as a starting material.[3] There was literature precedent for the bromination of 3 with N-bromosuccinimide[4] and the spectral data from the crude reaction mixture suggested that the reaction was forming the required brominated product. However, attempts to isolate the labile bromide 4 and subsequently form the Grignard were unsuccessful.



Scheme 1

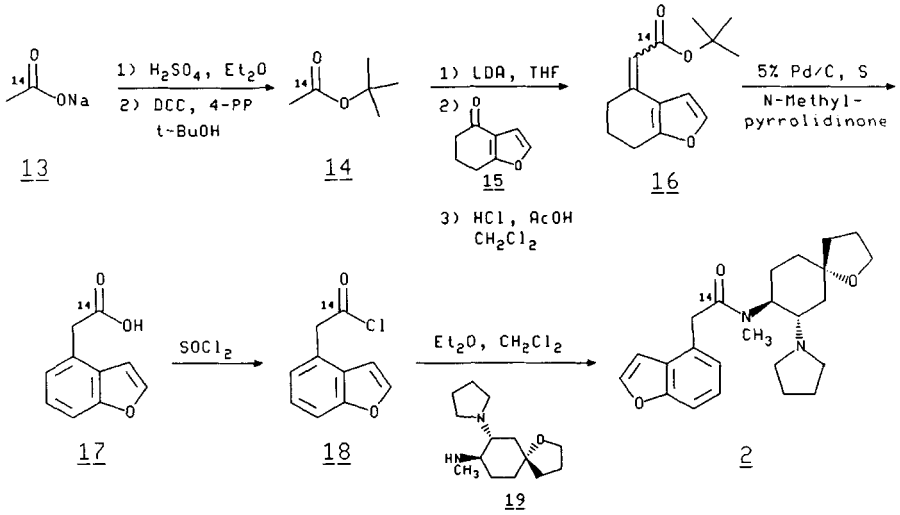
The target compound PD 117,302 was then synthesized by the route shown in Scheme 2. Bromo-[1-^{14}C]acetic acid 5 was esterified with diazomethane to yield the methyl ester 6. 6,7-Dihydrobenzo[b]thiophen-4(5H)-one 7 underwent a Reformatsky reaction with the methyl bromo-[1-^{14}C]acetate 6 to produce the β-hydroxyester which was subsequently dehydrated to form a mixture of *endo* and *exo* olefins, 8 and 9 respectively.



Scheme 2

Treatment of the olefin mixture with sulfur and 5% palladium on carbon afforded the fully aromatized product 10. The ester 10 was hydrolyzed with potassium

hydroxide to yield the acid 11 which was converted to the final product 1, by reaction of the corresponding acid chloride with 12. PD 117,302 was synthesized in an overall 19.6% radiochemical yield in five steps.



Scheme 3

In contrast to the work done to produce 1, an alternate route was developed for the synthesis of the benzofuran derivative 2 (Scheme 3). This was necessary due to the variable yields experienced in the Reformatsky reaction in the benzofuran system. It was found that the treatment of 1,1-dimethylethylacetate with lithium diisopropylamide in tetrahydrofuran at -78° C gave an excellent yield in this system. Labeled 1,1-dimethylethylacetate was formed by treatment of [1-^{14}C]acetic acid with 1,1-dimethylethanol in the presence of dicyclohexylcarbodiimide and 4-(1-pyrrolidinyl)pyridine. The resulting ester was treated with lithium diisopropylamide followed by 15 to give a mixture of the olefin 16 and the corresponding alcohol which was readily dehydrated under acidic conditions. ^{1}H NMR studies of the product from unlabeled experiments showed only the *exo* double bond formed. Oxidation of 16 to the fully aromatized product 17 was achieved under the same conditions as for the formation of 10. Under these conditions the 1,1-dimethylethyl ester was cleaved giving 17 directly. When large scale unlabeled experiments were done on this reaction, small amounts of benzo[b]thiophene-4-acetic acid were identified as an impurity. This was due to sulfur exchanging with oxygen in the benzofuran ring during the

reaction. There was HPLC evidence that a small amount of 11 was formed in the labeled experiment. The acid chloride 18 was produced by heating 17 with thionyl chloride/dimethylformamide. The crude yield was quite good but substantial decomposition occurred due to the high temperature during distillation of the product. Thus the resulting yield of the isolated material was 20%. The final product 2 was made by reacting the acid chloride 18 and amine 19.

EXPERIMENTAL

Bromo-[1-^{14}C]acetic acid at a specific activity of 25.2 mCi/mmol was purchased from Pathfinder Laboratories, St. Louis, Mo. Sodium [1-^{14}C]acetate at a specific activity of 56 mCi/mmol was purchased from American Radiolabeled Chemical, St. Louis, MO. 6,7-Dihydrobenzo[b]thiophen-4(5H)-one, Diazald®, 1,3-cyclohexanedione, N,N'-dicyclohexylcarbodiimide, and 4-(1-pyrrolidinyl)-pyridine were purchased from Aldrich Chemical Company, Milwaukee, WI. *trans*-N-Methyl-2-(1-pyrrolidinyl)cyclohexanamine (12) was synthesized as described previously.[1] 6,7-Dihydrobenzofuran-4(5H)-one was made as described by Matsumoto and Watanabe[5]. (±)-(5α,7α,8β)-N-Methyl-7-(1-pyrrolidinyl)-1-oxaspiro[4.5]decan-8-amine hydrochloride was synthesized by the Preparations Laboratory, Parke-Davis, Ann Arbor, MI using modifications of Horwell[2,6]. The free base was recovered by treatment with NaOH and extraction with CH_2Cl_2.

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined on a Varian XL-200 (200 MHz) spectrophotometer. Chemical shifts were reported in δ units downfield from tetramethylsilane. Mass spectra were recorded on a Finnigan Series 4000 GC-MS. Liquid scintillation counting was performed with a Packard Tricarb 4530 liquid scintillation counter using Mallinckrodt Handifluor liquid scintillation cocktail. Thin layer chromatography plates were analyzed for radiochemical purity (RCP) using a Berthold LB 2832 automatic TLC-analyzer. Silica gel plates (0.25 mm) were purchased from E. Merck. Reversed phase LKC-18F plates (0.20 mm) were purchased from Whatman. High pressure liquid chromatography (HPLC) was performed using a Spectra Physics SP8700 solvent

delivery system, Kratos Spectroflow 773 variable wavelength UV detector, Hewlett-Packard 3390A integrator and Packard RAM 7500 or Trace radioactivity monitor. Gas chromatographic analysis was performed using a Hewlett-Packard 5790 instrument with a 0.025 mm x 30 m J & W DB-5 column, injector @ 225° C, and FID @ 325° C.

Methyl Bromo-[1-^{14}C]acetate (6). To a 25 mL pear-shaped flask containing bromo-[1-^{14}C]acetic acid (276 mg, 1.98 mmol, 49.9 mCi) in 2 mL of dimethoxyethane was added an ethereal solution of diazomethane. The ethanol-free diazomethane was generated from Diazald® (1.51 g, 7.05 mmol) as described on the Diazald® packaging. Addition of diazomethane was stopped when the reaction solution maintained a slight yellow color. The excess Et_2O was distilled off using a short-path distillation head in a H_2O bath at 60° C. The 3.3 mL of liquid in the distillation flask was diluted to 4.2 mL with DME. GC analysis (isothermal @ 50° C) of the product showed the following composition: Et_2O (43.4%), CH_2Cl_2 (9.0%), DME (44.3 %), $BrCH_2{}^{14}COOMe$ (3.3%, t_R = 3.9 min). The methyl bromoacetate (47.5 mCi, 95% yield) was stored over 3A molecular sieves in a serum vial for 18 h and used in the next reaction without additional purification.

Methyl (6,7-Dihydrobenzo[b]thien-4(5H)-ylidene)-[1-^{14}C]acetate (8) and Methyl 6,7-Dihydrobenzo[b]thiophene-4-[carbonyl-^{14}C]acetate (9). 6,7-Dihydrobenzo[b]thiophen-4(5H)-one (636 mg, 4.18 mmol) in toluene (5 mL) and dimethoxyethane (2 mL) was added to freshly activated zinc (839 mg, 12.8 mmol) and an iodine crystal in a 25 mL 3-neck round bottom flask fitted with a condenser, a 5-mL dropping funnel, a septum and stirring bar under an N_2 atmosphere. The reaction was heated to reflux and when the iodine color had dissipated, the methyl bromo-[1-^{14}C]acetate was added slowly over a 35 min period. The reaction was refluxed for an additional 5 min after the addition was complete. The reaction was cooled to 25° C and 1 ml of formic acid was added. The reaction solution was washed with H_2O (2 x 15 mL). The toluene layer was treated with 15% HCl (10 mL) and stirred for 5 min. The organic layer was separated, dried (Na_2SO_4), filtered and concentrated under reduced pressure.

TLC on silica gel eluted with hexane/EtOAc (9:1) indicated that the crude orange oil consisted of a major labeled product, the alcohol (87%) which had a lower R_f than the starting ketone. A small amount of the *endo* and *exo* olefins (5%) which had a higher R_f than starting material was also obtained. The crude product mixture was taken up in toluene (10 mL) and p-toluenesulfonic acid (4.7 mg) was added. The solution was stirred at 25° C for 1 h and then 50° C for 45 min. The toluene solution was concentrated in vacuo to 1.5 mL and the orange residue was chromatographed on a silica gel column eluted initially with hexane followed by hexane/EtOAc (9:1). A mixture of the two olefins was isolated as a yellow oil (305 mg, 35.2 mCi, 70.4% yield). TLC: RCP >95%, R_f = 0.41 and 0.45, Silica gel, hexane/EtOAc (9:1), same as authentic unlabeled standard.

Methyl Benzo[b]thiophene-4-[carbonyl-^{14}C]acetate (10). To a mixture of the *endo* and *exo* olefins 8 and 9 (305 mg, 35.2 mCi) in N-methylpyrrolidinone (2 mL) was added sulfur (100 mg, 3.13 mmol) and 5% Pd/C (4.4 mg). The reaction was refluxed for 2.5 h and then stored at 10° C for 18 h. The reaction was filtered through a Celite plug and the Celite was washed with toluene and H_2O. The two layers were separated and the toluene was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The black residue was chromatographed on a silica gel column eluted initially with hexane followed by hexane/EtOAc (9:1). The product was isolated as an orange oil (176 mg, 20.2 mCi, 57.4% yield). TLC: RCP >99%, R_f = 0.29, silica gel, Hexane/EtOAc (9:1), same as authentic unlabeled standard.

Benzo[b]thiophene-4-[carboxyl-^{14}C]acetic Acid (11). The methyl ester (176 mg, 20.2 mCi) was dissolved in MeOH (2 mL) and 30% KOH/MeOH (0.2 mL) was added. The solution was stirred at 25° C for 6 h. The reaction was monitored by TLC on silica gel, CH_2Cl_2/MeOH (4:1). Additional 30% KOH/MeOH (0.1 mL) was added until TLC showed an absence of starting ester. 5% $NaHCO_3$ (5 mL) was added to the reaction and the methanol was removed under reduced pressure. The residue was partitioned between H_2O and CH_2Cl_2. The aqueous layer was acidified with 15% HCl to pH 2 and extracted with CH_2Cl_2 (2 x 20 mL). The second CH_2Cl_2 layer was dried

(Na_2SO_4), filtered and concentrated to produce a pale yellow solid (148 mg). Unlabeled acid (51.4 mg) was added to the labeled material and the material was sublimed at 130° C at 2 torr to yield a yellow solid (194 mg, 15.0 mCi, 74.4% yield). TLC: R_f = 0.20, RCP >99%, silica gel, CH_2Cl_2/MeOH (4:1), same as authentic unlabeled standard.

(±)-*trans*-N-Methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzo[b]thiophene-2-[carbonyl-^{14}C]acetamide Hydrochloride, PD 117302-^{14}C (1). The acid 11 (194 mg, 1.0 mmol, 15.0 mCi) was dissolved in thionyl chloride (2 mL, 3.26 g, 27 mmol) and refluxed for 30 min. The solution turned yellow and was then stirred for an additional hour at 25° C. The thionyl chloride was removed with dry CCl_4 (3 x 2 mL) and evaporated to dryness each time. The crude acid chloride was dissolved in CH_2Cl_2/Et_2O (1:1) (4.5 mL) and *trans*-N-methyl-2-(1-pyrrolidinyl)-cyclohexanamine (12) (184 mg, 1.0 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise. Within 5 minutes white crystals had appeared and after stirring an additional 20 min, the solution was filtered. The solid was washed with Et_2O and subsequently recrystallized from MeOH/Et_2O to yield a white solid 1 (240 mg, 0.66 mmol, 9.8 mCi, yield 65.3%, specific activity 14.9 mCi/mmol) m.p. 258-9° C, (authentic standard 252-4° C) TLC: RCP >99% R_f = 0.32 silica gel, CH_2Cl_2/MeOH/NH_4OH (19:1:0.1); R_f = 0.29 silica gel, EtOAc/EtOH/NH_4OH (5:5:0.1); R_f = 0.37 C_{18}, MeOH/H_2O/NH_4OH (9:1:0.1). HPLC: RCP >99%, t_R = 8.5 min; Alltech Licrosorb RP-2, 10 μ, 4.6 mm I.D. x 25 cm; MeOH/$(NH_4)_3PO_4$ buffer (pH 7.4, 0.032 *M*), 72:28; flow 1 mL/min, UV 214 nm. ^{1}H NMR (DMSO-d_6) δ 7.9 (d, 1H, arom.), 7.7 (m, 2H, arom.), 7.3 (t, 1H, arom.), 7.2 (d, 1H, arom.), 4.5 (m,1H, N$^{\oplus}$H), 4.3-4.0 (dd, 2H, arom.-CH_2C=O), 3.7-3.0 (m, 6H, NCH_2, NCH), 3.0 (s, 3H, NCH_3), 2.1-1.7 (m, 12H,C-CH_2-C). IR (KBr) 1640 cm^{-1} (C=O). Anal. calcd for $C_{21}H_{28}N_2OS \cdot HCl$: C, 64.18; H, 7.44; N, 7.13. Found: C, 64.33; H, 7.66; N, 7.00.

1,1-Dimethylethyl-[1-^{14}C]acetate (14). Sodium [1-^{14}C]acetate (300 mg, 3.57 mmole, 200 mCi) was mixed with concentrated H_2SO_4 (110 μl, 3.9 mmole) in Et_2O (5 mL) for 3 h. The mixture was filtered into a solution of N,N'-dicyclohexylcarbodiimide (2.711 g, 13.1 mmole), 4-(1-pyrrolidinyl)pyridine (193 mg,

1.3 mmole), 1,1-dimethylethanol (856 mg, 11.6 mmole) in Et_2O (20 mL). The solid was rinsed 3 times with Et_2O (5 ml). After stirring 1 h under N_2, unlabeled acetic acid (475 μl, 7.98 mmole) was added and the reaction stirred at room temperature for 16 h. The reaction mixture was filtered and rinsed with Et_2O. The volume of the Et_2O was reduced by atmosphere distillation with a pot temperature < 55° C. The remaining solution was vacuum distilled to give a solution which was 27% 14, 14% 1,1-dimethylethanol, and 57% Et_2O by GC (isothermal @ 40° C) This was used directly in the next step.

1,1-Dimethylethyl 2-(6,7-dihydro-4(5H)-benzofuranylidene)-[1-^{14}C]acetate (16). Lithium diisopropylamide was generated from n-butyllithium (11.6 mmole, 1.6 *M* in hexane) and diisopropylamine (1.68 mL, 11.6 mmole) in tetrahydrofuran (10 mL) at 0° C under N_2. The solution was cooled to -78° C. The solution of 14 was added keeping the internal temperature below -70° C. After 45 min, a solution of 15 (1.57 g, 11.6 mmole) in tetrahydrofuran (15 mL) was added again keeping the internal temperature below -70° C. The reaction mixture was stirred overnight, warming slowly to 10° C. The reaction was quenched with water, extracted with Et_2O, dried (Na_2SO_4) and evaporated to give an oil. The oil was dissolved in CH_2Cl_2 mixed with acetic acid (1 mL) and concentrated HCl (30 μL). After 30 min, K_2CO_3 was added to neutralize the excess HCl, then filtered. The filtrate was evaporated to give 1.065 g (40 % yield from acetic acid) of 16 as a white solid. mp = 36-40 °C, TLC: R_f = 0.50 RCP = 97.9 %, silica gel, hexane/EtOAc (4:1).

4-Benzofuran-4-[carboxyl-^{14}C]acetic Acid (17). The ester 16 was mixed with 5% Pd/C (35 mg) and sulfur (410 mg) in N-methylpyrrolidinone (2.0 mL) and heated under vacuum (160 torr) at 165° C for 6 h. The temperature was then raised to 200° C for 30 min. The solvent was removed by vacuum distillation. The residue was dissolved in EtOAc and filtered through Celite. The filtrate was extracted with 1 *M* NaOH. The aqueous layer was washed with EtOAc, acidified with concentrated HCl and extracted with CH_2Cl_2. The solution was dried ($MgSO_4$), passed through a small column of silica gel, rinsing the column with 5% MeOH in CH_2Cl_2. The product 17 was isolated as a dark oil, 649 mg (81 % yield). TLC:

R_f = 0.47, RCP > 99%, silica gel, CH_2Cl_2:MeOH (9:1) co-chromatographed with unlabeled 17.

Benzofuran-4-[carbonyl-^{14}C]acetyl Chloride (18). The acid 17 from the previous step was mixed with $SOCl_2$ (3 mL) and DMF (20 μL) under N_2. After 1 h at room temperature the solution was heated to 55° C for 3 h. The excess $SOCl_2$ was removed in vacuum by co-distillation with toluene. The residue was vacuum distilled to give 13 mCi (20% yield) of 18 as a colorless oil.

(±)-(5α,7α,8β)-N-Methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-4-benzofuran-[carbonyl-^{14}C]acetamide Hydrochloride, PD 126212-^{14}C (2). Amine 19 (800 mg, 2.57 mmole) in Et_2O (3 mL) was placed in a flask under N_2. The acid chloride 18 in CH_2Cl_2 (10 mL) was added forming a white precipitate. Additional CH_2Cl_2 was added until dissolution was complete. The reaction was stirred for 16 h. The liquid was decanted from a white solid and evaporated. The residue was dissolved in Et_2O (25 mL) and CH_2Cl_2 (4 mL) and more 19 (800 mg) was added. After 1 h the resulting precipitate which formed was isolated, rinsed with diethyl ether several times and dried to give 208 mg of crude 2. The material was converted to the free base by treating an aqueous solution of 2 with K_2CO_3 and extraction with CH_2Cl_2. The free base was purified by flash column chromatography on silica gel eluted with 5% CH_3OH in CH_2Cl_2. The isolated pure free base was dissolved in Et_2O and treated with HCl gas. The salt was isolated and dried in vacuum at 65° C to give 61 mg (19 % yield, specific activity 13.4 mCi/mmol). TLC: RCP > 99 %, R_f = 0.26, silica gel, CH_2Cl_2/CH_3OH (9:1); R_f = 0.58, silica gel, EtOAc/EtOH/Et_3N (75:20:5); R_f = 0.62 C_{18}, CH_3OH/0.5 *M* NaCl (4:1). HPLC: Chemical purity and RCP >99 %, t_R = 6.2 min, Alltech Econosphere C_{18}, 10 μ, 4.6 mm ID x 25 cm, 0.05 *M* Et_3N adjusted to pH = 3.0 w/conc. H_3PO_4/THF/CH_3CN (78:11:11), flow rate 2.0 mL/min, UV @ 220 nm. ^{1}H NMR (DMSO-d_6) δ 9.80 (s, 1H), 7.93 (d, J = 2.2, 1H), 7.46 (d, J = 8.3, 1H), 7.24 (t, J = 7.8, 1H), 7.14 (d, J = 2.0, 1H), 7.06 (d, J = 6.8, 1H), 4.56(m, 1H), 4.04 (dd, J = 46.6, J = 16.2, 2H), 3.71 (m, 3H), 3.29-3.15 (m, 4H), 2.97 (s, 3H), 2.02-1.83 (m, 7H), 1.70 (t, J = 6.1, 2H), 1.56 (m, 3H).

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