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Design and Synthesis of a Chiral Hapten for a Radioimmunoassay of the Antidepressant (2S, 3S, 5R)-2-(3,5-Difluorophenyl)-3,5-dimethyl-2-morpholinol Hydrochloride

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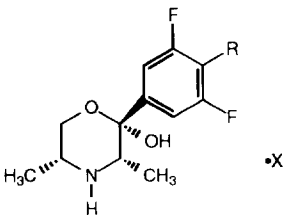
Abstract: The synthesis of a hapten for a radioimmunoassay of the antidepressant (2S, 3S, 5R)-2-(3,5-difluorophenyl)-3,5-dimethyl-2-morpholinol hydrochloride is described.

INTRODUCTION

(2S, 3S, 5R)-2-(3,5-Difluorophenyl)-3,5-dimethyl-2-morpholinol hydrochloride [**1**, BW 1555U88] is a potent, selective norepinephrine uptake inhibitor currently under evaluation as a potential antidepressant agent.[1] Due to the high potency of **1**, an analytical method capable of quantitating very low drug levels in unprocessed serum and plasma samples was required. Since radioimmunoassay (RIA) techniques provide a high level of sensitivity,[2] the development of an RIA for **1** was investigated.



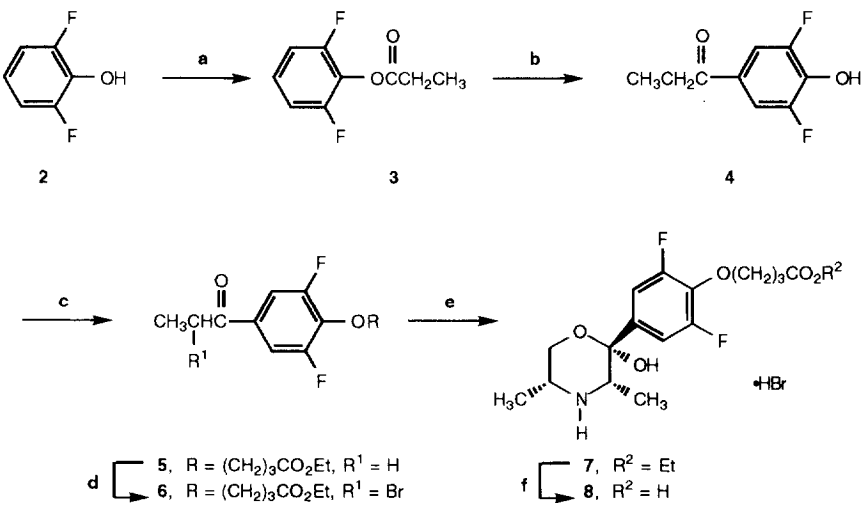
1; R = H, X = HCl
8; R = $O(CH_2)_3CO_2H$, X = HBr

Compound **1** does not contain a functional group that can be readily conjugated with a carrier protein to form an immunogen capable of eliciting an antibody response. It was necessary to design a specific hapten for the RIA of **1**. In designing a specific hapten with low cross reactivity with the parent drug's metabolites, it is important that "as many of the functional groups of the compound as possible remain unhindered in order to act as immunological discriminants".[2] The 4-oxobutyric acid derivative **8** was chosen. This paper describes the synthesis of the hapten **8**.

CHEMISTRY

The hapten **8** was synthesized by the route shown in Scheme 1. Reaction of propionyl chloride with **2** in acetonitrile gave a 61% yield of 2,6-difluorophenyl propionate (**3**). Fries rearrangement[3] of **3** with aluminum chloride gave 3',5'-difluoro-4'-hydroxypropiophenone (**4**) in 50% yield. Alkylation of **4** with ethyl 4-bromobutyrate and sodium ethoxide in ethanol in the presence of sodium iodide gave a 64% yield of ethyl 4-(2,6-difluoro-4-propionylphenoxy)butyrate (**5**). Bromination of **5** with dioxane dibromide[4] afforded a 92%

yield of ethyl 4-(4-(2-bromopropionyl)-2,6-difluorophenoxy)butyrate (**6**). Amination of **6** with (R)-(–)-2-amino-1-propanol in the presence of 2,6-lutidine gave (2S, 3S, 5R)-ethyl 4-(2,6-difluoro-4-(2-hydroxy-3,5-dimethyl-2-morpholinyl)phenoxy)butyrate hydrobromide (**7**) in 20% yield. Compound **7** crystallizes from the reaction mixture. Apparently, the diastereomer with the phenyl ring and the C-3 and C-5 methyl groups equatorial is the thermodynamically most stable isomer. No attempts were made to identify any other diastereomers in the reaction mixture. Hydrolysis of **7** with 48% aqueous hydrogen bromide afforded the 4-oxobutyric acid analogue **8** in 98% yield as the hydrobromide salt.



2 3 4

a) CH_3CH_2COCl, CH_3CN. b) $AlCl_3$, Δ. c) $Br(CH_2)_3CO_2Et$, EtONa, NaI, Δ.
d) dioxane dibromide. e) (R)-(–)-2-amino-1-propanol, 2,6-lutidine, CH_3CN. f) 48% aqueous HBr.

Scheme 1

CONCLUSIONS

We have developed a synthetic route for the preparation of a hapten of the potential antidepressant agent **1**. The carboxylic acid group of hapten **8** provides a handle for conjugation with a carrier protein. The hapten-protein conjugate should provide an immunogen suitable for development of an RIA of **1**.

EXPERIMENTAL SECTION

Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., and are within +/– 0.4% of the calculated values. The NMR spectra were recorded on a Varian Gemini 200 or XL 300 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The refractive indices were determined on an ABBE refractometer. The propionyl chloride, 2,6-difluorophenol, 4-bromobutyrate, and (R)-(–)-2-amino-1-propanol were purchased from Aldrich Chemical Co.

2,6-Difluorophenyl propionate (3). Propionyl chloride (22.2 g, 0.24 mole) was added dropwise to a solution of 2,6-difluorophenol (**2**) (30.0 g, 0.23 mole) in acetonitrile (300 mL) at room temperature, and the mixture was refluxed for 18 h. The mixture was concentrated in vacuo, and the residue was distilled under reduced pressure to give 26.0 g (61%) of **3** as a colorless oil, bp 61-65 °C at 5 mm Hg; ^{1}H nmr ($CDCl_3$): δ 1.30 (t, 3H, CH_3), 2.68 (q, 2H, CH_2), 6.92-7.20 (3H, ArH).

Anal. Calcd for $C_9H_8F_2O_2$ (m.w. 186.15): C, 58.06; H, 4.33. Found: C, 57.95; H, 4.31.

3',5'-Difluoro-4'-hydroxypropiophenone (4). Aluminum chloride (37.6 g, 0.282 mole) was added in portions over a 1.5 h period to **3** (25.4 g, 0.136 mole) heated to 80-90 °C under nitrogen. The mixture was heated for an additional 2 h at 90-95 °C. After the mixture was cooled to room temperature, ice was cautiously added followed by addition of a mixture of ice (50 g) and concentrated aqueous hydrochloric acid (60 mL). After 2h at ice-bath temperature, the resulting solid was filtered, washed with water and hexane, and dried to obtain 12.6 g (50%) of crude **4**. A 1.0 g sample of **4** was recrystallized from dichloromethane/pentane to give 0.77 g of **4** as a white solid, mp 127-129 °C; ^{1}H nmr (DMSO-d_6): δ 1.03 (t, 3H, CH_3), 2.95 (q, 2H, CH_2), 7.60-7.63 (m, 2H, ArH), 11.25 (br s, 1H, OH).

Anal. Calcd for $C_9H_8F_2O_2$ (m.w. 186.15): C, 58.06 H, 4.33. Found: C, 57.98; H, 4.36.

Ethyl 4-(2,6-difluoro-4-propionylphenoxy)butyrate (5). A solution of **4** (10.5 g, 0.056 mole) in ethanol (50 mL) was added dropwise rapidly to a solution of sodium (1.4 g, 0.061 mole) in ethanol (100 mL) at room temperature. After the solution was stirred for 5 min, a solution of ethyl 4-bromobutyrate (11.7 g, 0.06 mole) in ethanol (20 mL) was added dropwise and the mixture was heated. Before the mixture reached reflux temperature sodium iodide (9.0 g, 0.06 mole) was added in one portion. The mixture was heated at reflux for 18 h. The mixture was concentrated in vacuo, and ice and water were added to the residue. The pH was adjusted to 1.0 by the addition of 1N aqueous hydrochloric acid, and the resulting solution was extracted with diethyl ether. The diethyl ether extracts were washed successively with 1N aqueous sodium hydroxide and water, dried over sodium sulfate, filtered, and concentrated in vacuo to give 12.4 g of a yellow oil. Column chromatography on silica gel with dichloromethane as eluent gave 10.7 g (64%) of **5** as a colorless oil; $\eta_D^{21.5°}$ = 1.4905; ^{1}H nmr ($CDCl_3$): δ 1.23 (2 t, 6H, 2 CH_3), 2.09 (m, 2H, CH_2), 2.55 (t, 2H, CH_2), 2.91 (q, 2H, CH_2), 4.14 (q, 2H, CH_2), 4.29 (t, 2H, CH_2), 7.26-7.55 (2H, ArH).

Anal. Calcd for $C_{15}H_{18}F_2O_4$ (m.w. 300.29): C, 59.99; H, 6.04. Found: C, 60.07; H, 6.08.

Ethyl 4-(4-(2-bromopropionyl)-2,6-difluorophenoxy)butyrate (6). A solution of dioxane dibromide (prepared by dropwise addition of bromine [5.4 g, 0.034 mole] to dioxane [40 mL] at room temperature) was added dropwise to a solution of **5** (10.3 g, 0.034 mole) in dioxane (30 mL). After stirring 3 h at room temperature, the mixture was poured into ice water (1 L) and the aqueous mixture was extracted with dichloromethane. The dichloromethane extracts were dried (sodium sulfate) and concentrated in vacuo to give 13.1 g of a yellow oil. Column chromatography on silica gel with dichloromethane as the eluent yielded three fractions: Fraction A gave 2.49 g and contained the major component (R_f = 0.33) plus a less polar impurity (R_f = 0.54) . Fraction B gave 8.3 g and contained the major component plus a faint origin impurity. Fraction C contained only the major component and gave 1.63 g of **6** as a pale yellow oil. Analytical data were obtained on fraction C. Fraction B could be used without further purification in the next step; $\eta_D^{21.2°}$ = 1.5181; ^{1}H nmr ($CDCl_3$): δ 1.26 (t, 3H, CH_3), 1.88 (d, 3H, CH_3), 2.12 (m, 2H, CH_2), 2.56 (t, 2H, CH_2), 4.15 (q, 2H, CH_2), 4.33 (t, 2H, CH_2), 5.12 (q, 1H, CH), 7.57-7.60 (2H, ArH).

Anal. Calcd for $C_{15}H_{17}BrF_2O_4$ (m.w. 379.20): C, 47.51; H, 4.52. Found: C, 47.39; H, 4.50.

(2S, 3S, 5R)-Ethyl 4-(2,6-difluoro-4-(2-hydroxy-3,5-dimethyl-2-morpholinyl)phenoxy)butyrate hydrobromide (7). A solution of (R)-(–)-2-amino-1-propanol (15.0 g, 0.20 mole) and 2,6-lutidine (23.6 g, 0.22 mole) in acetonitrile (175 mL) was added dropwise to a solution of crude **6** (76.3 g, ~ 0.18 mole) in acetonitrile (100 mL) at room temperature. After stirring 5 days at ambient temperature, the mixture was heated to 30 °C and diethyl ether (60 mL) was added to induce crystallization. After precipitation began, the mixture was placed in a refrigerator at 5 °C for 4.5 h, then the resulting solid was filtered and washed with cold acetonitrile to give 16.0 g (20%) of crude **7** hydrobromide. Recrystallization of a 2.5 g sample from ethanol/diethyl ether mixtures gave 1.49 g of **7** as a white solid, mp 191-192 °C; $[\alpha]_D^{20} = +20.29$ (c = 0.69, 95% ethanol); ^{1}H nmr (DMSO-d_6): δ 0.94 (d, 3H, CH_3), 1.17 (m, 6H, 2 CH_3), 1.93 (m, 2H, CH_2), 2.48 (t, 2H, CH_2), 3.51 (br m, 2H, 2 CH), 3.84 (br d, 2H, CH_2), 4.06 (q, 2H, CH_2), 4.14 (t, 2H, CH_2), 7.23-7.29 (2H, ArH), 7.58 (br d, 1H, OH), 8.75 (br s, 1H, NH), 9.25 (br s, 1H, HBr).

Anal. Calcd for $C_{18}H_{26}BrF_2NO_5$ (m.w. 454.31): C, 47.58; H, 5.77; N, 3.08. Found: C, 47.61; H, 5.81; N, 3.03.

(2S, 3S, 5R)-4-(2,6-Difluoro-4-(2-hydroxy-3,5-dimethyl-2-morpholinyl)phenoxy)butyric acid hydrobromide (8). A mixture of **7** (2.0 g, 0.004 mole) and 48% aqueous hydrogen bromide (20 mL) was stirred at ambient temperature for 3 h, concentrated in vacuo, and the residue was stirred in hexane for 18 h. The mixture was concentrated in vacuo, and the residue was stirred in diethyl ether for 1h. The diethyl ether was decanted and fresh diethyl ether was added to the residue. After 3 h the diethyl ether treatment was repeated, and the mixture was stirred at room temperature for 18 h. The resulting solid was filtered and dried to give 1.67 g (98%) of **8** as a white solid, mp 165-167 °C, $[\alpha]_D^{20} = +21.97$ (c = 0.80, 95% ethanol); ^{1}H nmr (DMSO-d_6): δ 0.94 (d, 3H, CH_3), 1.19 (d, 3H, CH_3), 1.89 (m, 2H, CH_2), 2.40 (t, 2H, CH_2), 3.49 (br m, 2H, 2 CH), 3.84 (br d, 2H, CH_2), 4.14 (t, 2H, CH_2), 7.22-7.29 (2H, ArH), 7.59 (br d, 1H, OH), 8.77 (br m, 1H, NH), 9.27 (br m, 1H, HBr), 12.15 (br, 1H, COOH).

Anal: Calcd for $C_{16}H_{22}BrF_2NO_5$ (m.w. 426.26): C, 45.08; H, 5.20; N, 3.29. Found: C, 45.08; H, 5.22; N, 3.32.

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