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Synthesis of High Specific Activity Tritium Labelled 1*S*,2*S*-(-)-*trans*-2-Isothiocyanato-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide, a Specific Irreversible Ligand for Kappa Opioid Receptors.

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SUMMARY

Optically pure tritium labeled 1*S*,2*S*-(-)-*trans*-2-isothiocyanato-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide, an affinity ligand specific for the kappa opioid receptor was synthesized from optically pure 1*S*,2*S*-(-)-*trans*-2-amino-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide via the sequence of dibromination (57%) followed by catalytic tritiation of the dibromide. The resulting tritium labelled aniline (14% yield, specific activity 31.2 Ci/mmol) was transformed to the title compound in 13.3% yield and 99+% radiochemical purity by treatment with thiophosgene.

KEY WORDS: Optically Pure Enantiomer, Affinity Ligand, Kappa Opioid Receptor, Tritium Labeled Affinity Ligand

INTRODUCTION

Site directed irreversible drugs have found much use in the elucidation of the structure and function of receptors in the CNS.[1-3] These agents are drug analogs that contain a reactive functional group positioned such that it can form a covalent bond with the receptor(s) to which the drug is bound. In radiolabelled form, irreversible drugs are extremely valuable research tools since they can radiolabel the receptor

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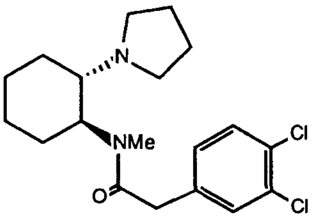
to which they become attached and thus allow its identification and purification.

Irreversible drugs which covalently label drug receptors can be classified as either photoaffinity or electrophilic ligands. We have been most interested in developing ligands in the latter class because of their usefulness *in vivo* and in situations where receptors are photolabile.

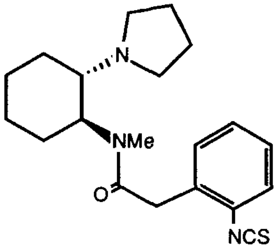
Thus, in the opioid field, we have developed site-directed electrophilic affinity ligands for specific and irreversible inhibition of mu,[4] delta[4] and kappa[5] opioid receptor subtypes. Using the etonitazene derived affinity ligand, BIT, we were able to specifically and irreversibly inhibit mu receptor binding *in vitro*.[4] The fentanyl-derived affinity ligand, FIT, although interacting with both mu and delta opioid receptors, was shown by us to deplete delta opioid receptor subtypes without effect on mu receptors.[4] SUPERFIT, a more potent and enantioselective analog of FIT was developed with the aim of using it for delta receptor identification and isolation.[6] In tritium labeled form, SUPERFIT proved to be instrumental in the identification and purification of delta receptors on both neuroblastoma x glioma hybrid cells,[6] as well as cells of the immune system.[7] Similarly, [^{3}H]-BIT[8] is presently being used to isolate and identify mu receptors. The electrophilic functionality used throughout these studies was the isothiocyanate (NCS) group; this has also served well in a number of other studies that include irreversible ligands for benzodiazepine,[9] phencyclidine[10] and sigma receptors.[11] Radiolabelled irreversible agents have also found use in the isolation of other receptor types. For instance, the photoaffinity labeling agent [^{3}H]-*m*-azidophencyclidine was useful in visualizing proteins corresponding to the phencyclidine binding site[12]; and an azido photoaffinity label derivative of [^{3}H]-di-N,N'-(o-tolyl)guanidine (DTG) found use in covalently labeling proteins corresponding to the sigma receptor.[13]

Kappa opioid receptors are of considerable interest because their activation is thought to produce analgesia with reduced addiction liability, tolerance and respiratory depression compared to mu receptors and also kappa receptors mediate other effects such as diuresis.[14] U50,488 [(±)-**1**] was developed by the Upjohn company as the first highly selective kappa opioid receptor agonist.[15,16] It was with (±)-**1** that we decided to base our kappa selective irreversible agent. Since the enantiomers in a racemic drug can have different, and in some cases opposite effects at receptors[17], it was necessary for us to synthesize our irreversible ligand in enantiomerically pure form. Thus, we determined that the kappa active enantiomer of (±)-**1** was 1*S*,2*S*-(-)-**1**.[18] In terms of radiolabelled reversible kappa receptor ligands, we recently reported the synthesis of high specific activity, optically pure [^{3}H]*S*,*S*-(-)-**1** via saturation with tritium gas of an unsaturated form of *S*,*S*-(-)-**1**.[19]

Based on our knowledge of the kappa receptor active absolute configuration of (-)-**1**, we recently reported the synthesis and properties of 1*S*,2*S*-(-)-*trans*-2-isothiocyanato-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(-)-**2**], the first site-directed, enantiospecific acylating agent for irreversible inhibition of kappa opioid receptors *in vitro*.[5] Incubation of crude guinea pig brain membranes with a 1 μM concentration of **2** resulted in 90% depletion of kappa receptors labeled by the kappa selective reversible ligand, [^{3}H]-U69,593, without any affect on mu or delta receptor binding.[5] Compound (-)-**2** exhibited an IC_{50} against [^{3}H]-U69,593 of 52 nM in a reversible type binding assay.[5] Until now, an irreversible agent which can deplete kappa opioid receptors in the presence of mu and delta opioid receptors was unavailable in radiolabelled form.



(-)-**1** (U50,488 active enantiomer)



(-)-**2**

We now wish to report the synthesis of optically pure, high specific activity [^{3}H]-1*S*,2*S*-(-)-*trans*-2-isothiocyanato-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide {[^{3}H]-(-)-**2**}. This compound should prove useful in the radiolabelling and in the subsequent study of the structure and function of the kappa opioid receptor.

SYNTHESIS

The synthetic pathway for preparing tritium labeled (-)-**2** is shown in Scheme 1. Optically pure 1*S*,2*S*-(-)-*trans*-2-amino-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(-)-**3**][5] was used as the starting material. Dibromination of (-)-**3** in glacial acetic acid in the presence of triethylamine proceeded smoothly to give the tritiation precursor 1*S*,2*S*-(-)-*trans*-2-amino-3,5-dibromo-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(-)-**4**] in 57% yield after purification by column chromatography. Catalytic tritiation of (-)-**4** in methanol by overnight treatment with 50 Ci of carrier free tritium gas in the presence of 10% Pd-C afforded 14% radiochemical yield (based on amount of precursor) of radiochemically pure [^{3}H]-(-)-**3**, specific activity 31.2 Ci/mmol.

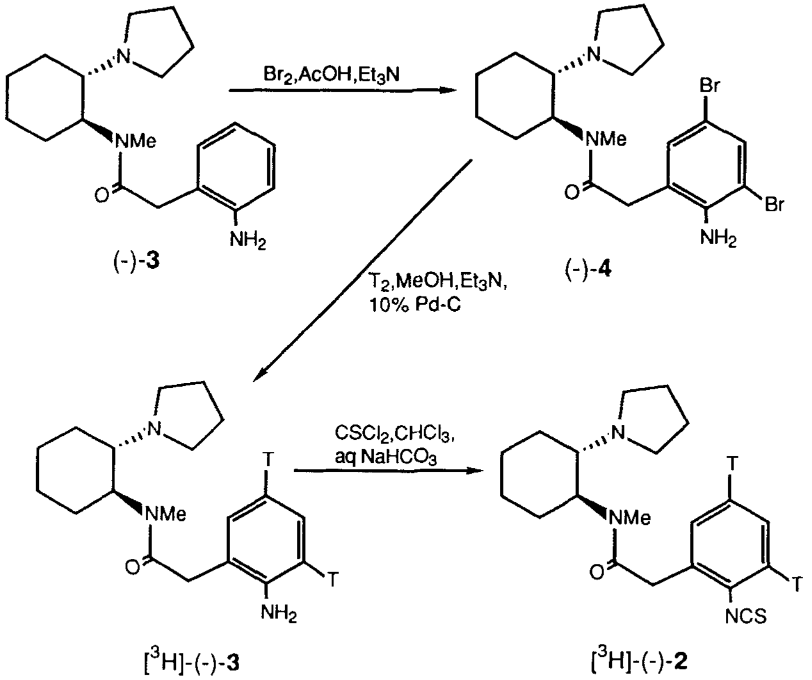
Treatment of [^{3}H]-(-)-**3** with freshly redistilled thiophosgene[12] in a chloroform/aqueous $NaHCO_3$ two phase system furnished 13.3% yield of the desired [^{3}H]-(-)-**2** (specific activity 31.2 Ci/mmol). The material was greater than 99% radiochemically pure as determined by TLC.

DISCUSSION

Catalytic tritiation of the intermediate [(-)-**4**] in methanol over 10% Pd-C afforded the desired aniline [^{3}H]-(-)-**3** in 14% radiochemical yield. Since the percentage incorporation of tritium into this intermediate was 53%, (-)-**4** represents a valuable precursor for the preparation of high specific activity [^{3}H]-**2**. It is likely that a theoretical yield of tritium incorporation was not possible in this case because of Pd-catalysed isotopic dilution with hydrogen (from the methanol solvent). The ^{1}H-NMR spectrum of (-)-**4** exhibited *meta* (J=1.6 Hz) coupled doublets at 7.10 (H-4) and 7.45 (H-6) ppm indicative of a 3,5-dibromo substitution. Because in a previous study involving catalytic tritiation of a related aromatic bromo precursor, we unequivocally proved that direct replacement of bromine with tritium was occurring,[20] it is highly likely that there is a 3,5-distribution of tritium in [^{3}H]-(-)-**3**, and thus in [^{3}H]-(-)-**2**.

Scheme 1

Synthesis of Tritium Labeled 1*S*,2*S*-(-)-*trans*-2-Isothiocyanato-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide



EXPERIMENTAL

Materials and Methods

Melting points were determined on a Thomas Hoover capillary apparatus and are uncorrected. Combustion Analyses were determined at Atlantic Microlabs, Atlanta, GA. Chemical ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were taken from $CDCl_3$ solutions of compounds using a Varian XL-300 spectrometer. Infra-Red (IR) spectra were obtained from $CHCl_3$ solutions of compounds using a Beckman 4230 IR spectrometer. Ultraviolet (UV) spectra were recorded from methanol solutions using a Hewlett-Packard 8450A UV/VIS spectrophotometer. Specific rotation ($[\alpha]_D$) values were determined at the sodium D-line (598 nm) using a Perkin-Elmer 241-MC polarimeter. Analytical thin layer chromatography (TLC) was performed on 250 μM Analtech GHLF silica gel plates. TLC system A refers to: concentrated aqueous ammonia-methanol-chloroform (1:9:90); TLC system B refers to methanol-ethyl acetate (3:7). For tritium labeled compounds, TLC plates were analyzed with a Berthold model LB 2760 TLC scanner. Radioactivity determinations were carried out using a Packard model 2200 CA Tri-Carb liquid scintillation analyzer; tritium labeled compounds were counted in hydrofluor scintillation cocktail with a counting efficiency of 45%. All synthetic and analytical operations were initially performed with unlabeled compounds and the structures were confirmed spectroscopically.

1*S*,2*S*-(-)-*trans*-2-Amino-3,5-dibromo-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(-)-4]. A solution of freshly redistilled bromine (0.33 g, 2.06 mmol) in acetic acid (10 mL) was added dropwise at room temperature during 20 min to a stirred solution of (-)-**3**·HBr[5] (0.41 g, 1.03 mmol) and triethylamine (0.43 mL, 3.09 mmol) in acetic acid (50 mL) to afford a pale yellow solution. TLC (system A) indicated one major product. The reaction mixture was poured into a mixture of ice (200 g) and concentrated aqueous ammonia solution (excess) and extracted with ether (3 x 50 mL). The organic phase was dried (Na_2SO_4) and the solvent evaporated to give the crude product as an oil. The crude product was purified by column chromatography on silica gel, eluting with concentrated aqueous ammonia-MeOH-$CHCl_3$ (0.5:4.5:95) to afford 0.28 g (57%) of pure (-)-**4** as a colorless oil: IR 3460, 3320, 3200, 2940, 1615, 1580, 1510, 1410, 1245, 1080, 910 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.45 (d, 1H, J=1.6 Hz), 7.10 (d, 1H, J=1.6 Hz), 5.16 (br s, 2H, NH_2), 4.42 (m, 1H), 3.65 (s, 1H), 3.60 (s, 1H), 2.94 (s, 3H, NMe), 2.51 (m, 2H), 2.39 (m, 2H), 1.86-1.11 (m, 13H) ppm; MS (CIMS) m/z 474 (MH^+ for $C_{19}H_{27}{}^{79}Br^{81}BrN_3O$). The (-)-**4** oxalate salt crystallized from 10 mL of ethanol: mp 203-204 °C (dec); $[\alpha]_D$ =17.1° (c, 0.33, MeOH); Anal. (Found): C 44.72, H 5.17, N 7.36%. Anal. (Calculated for $C_{21}H_{29}Br_2N_3O_5$) C 44.77, H 5.19, N 7.46%.

1*S*,2*S*-(-)-*trans*-2-Amino-3,5-ditritio-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide ([^{3}H]-(-)-3). To a solution of (-)-**4** oxalate (20 mg, 0.0355 mmol) in methanol (2.0 mL) was added triethylamine (0.20 mL) followed by 10% Pd/C (20 mg) and the reaction mixture was stirred overnight at room temperature under an atmosphere of carrier-free tritium gas (50 Ci, 0.847 mmol). The reaction mixture was filtered and a few drops of 28% aqueous ammonia were added. The solvent was removed by evaporation under a stream of nitrogen, and the residue was applied to one 20 cm x 20 cm x 1 mm preparative TLC plate and the plate run with solvent system A. The band comigrating with unlabeled [(-)-**3**] was removed and extracted with 50 mL of 2:18:80 concentrated aqueous ammonia-MeOH-$CHCl_3$ for 40 min and the extract filtered through a glass wool plug. Evaporation of the solvent under a stream of nitrogen and reconstitution of the residue with 100 mL of MeOH for scintillation counting and UV analysis afforded 283.1 mCi of [^{3}H]-(-)-**3** in greater than 99% radiochemical purity as determined by TLC analysis (14% radiochemical yield based on 20 mg of **4**.oxalate). UV analysis of the solution (UV_{max} 290 nM, ε_{290} = 2249 L $mol^{-1}cm^{-1}$) afforded a specific activity value of 31.2 Ci/mmol (53% incorporation). The solution was diluted with 500 mL of MeOH containing 1 mL of acetic acid (stabilizer) for final storage.

1*S*,2*S*-(-)-*trans*-2-Isothiocyanato-3,5-ditritio-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide ([^{3}H]-(-)-2). To a stirred solution of [^{3}H]-(-)-**3** (50 mCi, 1.603 x 10^{-3} mmol) in a mixture of saturated aqueous $NaHCO_3$ (0.5 mL) and $CHCl_3$ (0.5 mL) was added, via syringe, 18.3 µL of a solution of thiophosgene in $CHCl_3$ (2.40 x 10^{-3}mmol) (prepared by diluting 18.3 µL of freshly redistilled thiophosgene to 1.83 mL of $CHCl_3$), and the solution stirred for 1h at room temperature in a darkened room. The lower organic layer was diluted to 2 mL with $CHCl_3$ and the organic layer separated. The aqueous layer was washed with a further 2 x 2 mL of $CHCl_3$ and the combined organic extract was evaporated under a stream of nitrogen. The residue was taken up in 1 mL of $CHCl_3$ and applied to one 20 cm x 20 cm x 1 mm preparative silica gel TLC plate. The plate was eluted with TLC system B and the band comigrating with unlabeled (-)-**2** was removed and extracted with 40 mL of 95% ethanol. The extract was filtered through glass wool and the volume accurately adjusted for counting and storage to 40 mL by addition of 95% ethanol: yield 6.66 mCi (13.3%); radiochemical purity was determined to be 99+% after cospotting with a large excess of unlabeled (-)-**2** (carrier) and chromatographing on analytical TLC (solvent system A).

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