Synthesis of High Specific Activity (3,4-[^{3}H]Cyclohexyl)-N-[1 (2-benzo[b]thienyl)cyclohexyl]piperidine {[^{3}H]BTCP}: a Selective Probe for the Dopamine-Reuptake Complex

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SUMMARY

High specific activity [^{3}H]BTCP, a radioligand for the dopamine-reuptake complex was synthesized in 7-steps starting with the readily available starting materials, cyclohexane-1,4-dione monoethylene ketal and benzo[b]thiophene; the tritium label was introduced in the final step on the 3- and 4- positions of the cyclohexyl ring by catalytic tritiation of N-[4-(2-benzo[b]thienyl)cyclohexenyl]piperidine to give [^{3}H]BTCP in 7.3% yield with a specific activity of 29.8 Ci/mmol (51.4% isotopic incorporation).

Key Words: [^{3}H]BTCP, Radioligand, Dopamine Reuptake Complex, Cyclohexane-1,4-dione Monoethylene Ketal, Benzo[b]thiophene, N-[4-(2-Benzo[b]thienyl)cyclohexenyl]piperidine, Catalytic Tritiation

INTRODUCTION

Cocaine is a major drug of abuse in the United States resulting in a number of hospital emergencies and fatalities among the abusing population. Its exact mode of action is continuing to be an important area of research in the drug of abuse field. Among the sites in the brain with which cocaine is known to interact, the main one is at the dopamine (DA)-reuptake complex. Thus, a selective radiolabelled probe to study the interaction of cocaine and other drugs at the DA-reuptake complex would add to currently available ligands [1] and hopefully facilitate the study of this important CNS binding site.

In this regard, the drug of abuse, phencyclidine (PCP) is a moderate inhibitor of the DA-reuptake complex [2] and produces a variety of behavioral effects, some of which are induced through interaction with the dopaminergic pathways in the central nervous system (CNS) [3]. It was

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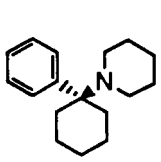
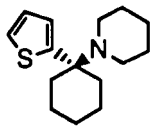
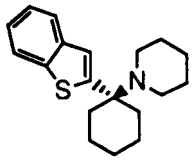
shown that modification or substitution of the aromatic moiety of PCP yielded compounds with various inhibitory potencies for both PCP and DA-reuptake sites [4].



PCP



TCP



1 (BTCP)

For example, with incorporation of the thienyl ring to give N-[1-(2-thienyl)cyclohexyl]piperidine (TCP), a 10-fold increase in the affinity for the PCP receptor and a 2-fold decrease in the affinity for the DA-reuptake complex was observed [5]. Conversely, incorporation of a benzo[b]thienyl ring gave N-[1-(2-benzo[b]thienyl)cyclohexyl]piperidine (BTCP) which exhibited a 20- to 30-fold decrease in affinity for the PCP receptor with a corresponding 70-fold increase in affinity for the DA-reuptake site [5].

Koek et al. compared the behavioral effects of BTCP with those of cocaine, PCP, ketamine and MK-801 and determined that the behavioral effects of BTCP are mediated through the DA-reuptake site while the effects of the other compounds are mediated through their action at the PCP receptor [6]. Vignon et al. [5] were the first to describe the use of [^{3}H]BTCP (tritium labelled on the piperidine moiety) in radioreceptor binding assays. They found a good correlation between the ability of drugs to displace [^{3}H]BTCP and their capacity to inhibit [^{3}H]DA transport [5].

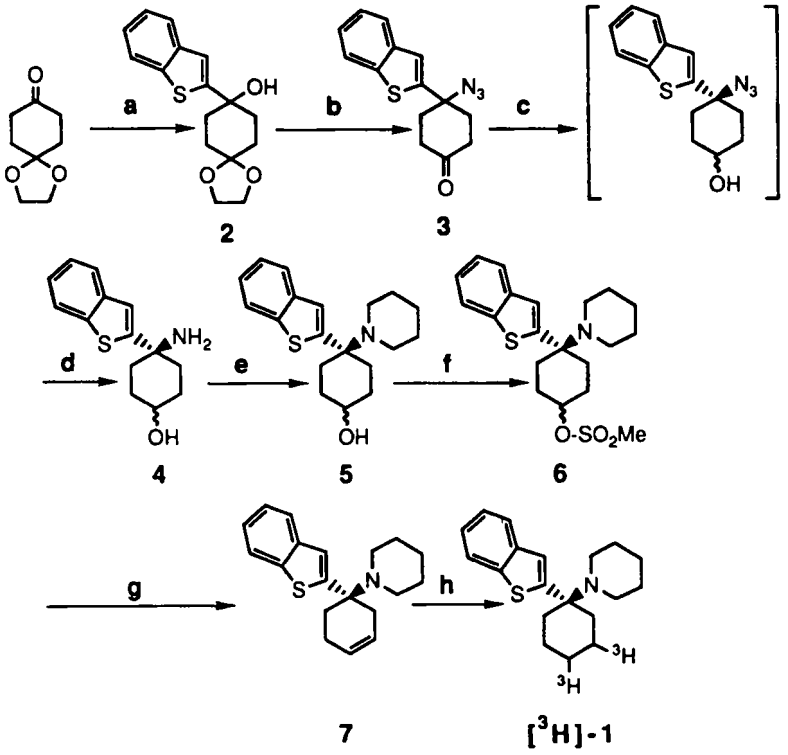
Herein is reported the first synthesis and characterization of high specific activity [^{3}H]BTCP (labelled on the 3- and 4- positions of the cyclohexyl ring) starting from readily available precursors.

SYNTHESIS

The synthetic route to [^{3}H]BTCP commenced with the readily available cyclohexane-1,4-dione monoethylene ketal (Scheme 1). Condensation of this ketone with 2-lithiobenzo[b]thiophene [7] afforded the corresponding tertiary alcohol **2** in quantitative yield. Solvolysis of **2** by treatment with CF_3COOH and sodium azide in $CHCl_3$ resulted in a simultaneous deketalization and solvolysis to give azide **3** in high yield [8]. No attempt was made to further purify or characterize **3**. Reduction of **3** by treatment with $LiAlH_4$ at -15 °C resulted in a stereoselective reduction of the carbonyl group; the second step involving $LiAlH_4$ reduction at 25 °C afforded aminoalcohol **4** as an insoluble crystalline solid in 90% overall yield from **2**. No attempt was made to assign the configuration of the hydroxyl group. Treatment of **4** with 1,5-dibromopentane [8] at 60 °C in

DMF in the presence of potassium carbonate afforded N-[1-(2-benzo[b]thienyl)-4-hydroxycyclohexyl]piperidine (**5**) in 83% yield. Treatment of **5** in THF with methanesulfonyl chloride in the presence of triethylamine afforded the crystalline methanesulfonate ester **6** in 96% yield. A facile elimination of the methanesulfonate group was achieved by treatment of **6** with excess potassium *t*-butoxide in DMSO at room temperature to give **7**. Interestingly, the elimination product **7** exhibited a considerably reduced (doubling of the Rf value) polarity on TLC (silica gel) compared with the corresponding saturated compound **1** (BTCP). Catalytic hydrogenation of **7** in MeOH in the presence of 10% Pd/C afforded **1** (BTCP), identical in all respects with an authentic sample made by a different method [9]. Thus, catalytic tritiation of **7** by reaction with carrier-free tritium gas [10] in the presence of 10% Pd/C afforded [^{3}H]BTCP in 7.3% radiochemical yield with a specific activity of 29.8 Ci/mmol (51% isotopic incorporation).

Scheme 1: Synthesis of Tritium Labelled BTCP
(N-[1-{2-Benzo[b]thienyl}cyclohexyl]piperidine)



Key: (**a**) (1) n-butyllithium, (2) benzo[b]thiophene; (**b**) CF_3COOH, NaN_3, $CHCl_3$; (**c**) $LiAlH_4$, ether, -15 °C; (**d**) $LiAlH_4$, ether, 25 °C; (**e**) pentane-1,5-dibromide, K_2CO_3, DMF, 60 °C; (**f**) $MeSO_2Cl$, Et_3N, THF; (**g**) *t*-BuOK, DMSO, 25 °C; (**h**) T_2, 10% Pd/C, MeOH

DISCUSSION

The high specific activity obtained in [^{3}H]**1** (BTCP) indicates that **7** served as an efficient tritiation precursor for [^{3}H]BTCP. The less than theoretical specific activity of [^{3}H]**1** can be attributed to Pd-catalyzed dehdrogenation of the tritiation solvent (MeOH) resulting in lowered specific activity of the final product as we have observed previously [11]. Because of the established position of unsaturation in intermediate **7**, the position of labelling of [^{3}H]**1** can be localized to the 3- and 4- and 5- positions of the cyclohexyl ring in a 1 : 2 : 1 ratio. The specific labelling of [^{3}H]**1** should prove useful in metabolism studies that involve the cyclohexyl ring moiety.

GENERAL EXPERIMENTAL DETAILS

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. Electron ionization mass spectra (EIMS) were obtained using a V. G. Micro mass 7070F 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. Infrared (IR) spectra were obtained from $CHCl_3$ solutions of compounds using a Beckman 4230 IR spectrometer. Ultra violet (UV) spectra were recorded from MeOH solutions (unless otherwise stated) using a Hewlett-Packard 8450 UV/VIS spectrophotometer. Analytical thin layer chromatography (TLC) was performed on 250 μM Analtech GHLF (Analtech, Inc., Newark, DE) silica gel plates. Solvent system A refers to $CHCl_3$-MeOH-NH_4OH (90:9:1). Solvent system B refers to $CHCl_3$-MeOH-NH_4OH (95:4.5:0.5). TLC plates were analysed for radioactivity using a Bethold model LB 2760 TLC scanner. Radioactivity determinations were carried out using a Packard model 2200 CA "Tri-Carb" liquid scintillation analyser; tritium labelled compounds were counted in Hydrofluor scintillation cocktail (National Diagnostics, Manville, NJ) with a counting efficiency of 45%. All synthetic and analytical operations were initially performed with unlabelled compounds and the structures were confirmed spectroscopically.

1-[2-(Benzo[b]thienyl)]-4,4-ethylenedioxycyclohexanol (2)

To a stirred solution of benzo[b]thiophene (33.6 g, 0.25 mol) in dry ether (200 mL) was added dropwise during 15 min at ambient temperature, a solution of n-butyllithium in hexane (172 mL, 1.1 eq of a 2.5 M solution). The exothermic reaction mixture began to reflux vigorously during the addition and therefore required periodic cooling in an ice-bath. After complete addition, a solution of cyclohexane-1,4-dione monoethylene ketal (39.12 g, 0.25 mol) in dry ether (200 mL) was added dropwise at ambient

temperature. Subsequently, the reaction was poured into cold water (200 mL) and the resulting solid was dissolved by addition of $CHCl_3$ (500 mL). The organic layer was retained and the aqueous layer was discarded. The organic layer was washed with a further 200 mL of water and the solvent was evaporated *in vacuo* to give **2** in quantitative yield. Analytically pure material was readily obtained by one recrystallization from 300 mL of hot 2-propanol: mp 157-158 °C; ^{1}H-NMR δ 7.80 (dd, J=1.7 Hz, J=7.3 Hz, 1H), 7.70 (dd, J=1.7 Hz, J=7.3 Hz, 1H), 7.25-7.36 (complex m, 2H), 7.21 (s, 1H), 3.98 (t, J=3.2 Hz, 4H), 2.20-2.31 (m, 2H), 2.03-2.20 (m, 4H), 1.70-1.96 (m, 2H); EIMS: M^+ calc for $C_{16}H_{18}O_3S$=290; Found: M^+=290; Anal calc for $C_{16}H_{18}O_3S$: C 66.18, H 6.25%; Found: C 66.02; H 6.28%.

4-Azido-4-[2-(benzo[b]thienyl)]cyclohexanone (3)

To a stirred solution of **2** (23 g, 79.3 mmol) in 250 mL of pentene stabilized chloroform (Fischer-Scientific, Fair Lawn, NJ) was added sodium azide (10.31 g, 158 mmol) followed by trifluoroacetic acid (18.3 mL, 237 mmol) and the solution was stirred for 21 h under an N_2 atmosphere. The reaction was quenched by pouring it into a cold (5 °C) aqueous solution of NaOH (15%). The organic layer was separated and the aqueous layer was extracted with a further amount (250 mL) of $CHCl_3$. The combined organic layer was back-washed with water (100 mL), dried over Na_2SO_4 and the solvent was evaporated *in vacuo* to yield a semi-crystalline solid. IR analysis of this crude product indicated the presence of some unreacted ethylene ketal. The crude product was dissolved in 200 mL of 88% formic acid and allowed to stand for 20 min at ambient temperature when IR analysis indicated that the hydrolysis of the ketal was complete. The reaction mixture was diluted to 800 mL with water and extracted with $CHCl_3$ (3 x 200 mL). The combined organic extract was washed with 10% potassium carbonate solution (2 x 200 mL), water (100 mL) and evaporated to a crystalline solid: IR (KBr) 2100 (azide str), 1730 (CO str) cm^{-1}. No attempt was made to further purify or characterize this material.

1-[2-(Benzo[b]thienyl)]-4-hydroxycyclohexylamine (4)

To a stirred solution of $LiAlH_4$ (237 mL of a 1.0 M solution, 237 mmol) at -15 °C, was added dropwise during 15 min, a solution of azide **3** in dry THF (100 mL). After the addition was complete, the reaction was allowed to warm to 20 °C. When the temperature reached 20 °C, an exothermic reaction ensued with vigorous evolution of N_2 gas. The reaction mixture was left to stir overnight at 20 °C and subsequently treated dropwise with water (9 mL), 15% aqueous NaOH (9 mL) and finally water (27 mL). After stirring for 45 min., the aluminum salts were filtered. Evaporation of the filtrated afforded 3.19 g, (16% yield from **2**) of

aminoalcohol **4**. The filter-cake was dissolved in 500 mL of 1.5M aqueous HCl and the solution was extracted with ether (2 x 200 mL) and the ether layer was discarded. The aqueous layer was basified by addition of excess concentrated aqueous ammonia solution and the resulting precipitate was filtered (slow). The filter-cake was washed with water until the washings were neutral to indicator paper. The filter-cake was air-dried to yield **4** (12.54 g, 74% yield from **2**). Further purification of **4** could be obtained through one crystallization of the fumarate salt from MeOH: mp 220-221 °C (dec); ^{1}H-NMR (fumarate salt in d_6-DMSO) δ 7.87 (d, J=7.4 Hz, 1H), 7.73 (d, J=7.4 Hz, 1H), 7.27-7.34 (complex m, 2H), 6.49 (s, 1H), 1.83-1.95 (complex m, 4H), 1.55-1.75 (complex m, 4H); EIMS: M^+ calc for $C_{14}H_{17}NOS$=247; Found: M^+=247, M^+-NH_3=230; Anal calc for $(C_{14}H_{17}NOS)_2.C_4H_4O_4$: C 62.92, H 6.27, N 4.59%; Found: C 62.73, H 6.28, N 4.51%.

N-[1-(2-Benzo[b]thienyl)cyclohexyl]-4-hydroxypiperidine (5)

To a stirred solution of **4** (base from 3.83 g, 12.6 mmol of fumarate salt by partitioning it between concentrated aqeous NH_4OH and $CHCl_3$) in dry DMF (30 mL) was added 1,5-dibromopentane (2.67 g, 11.6 mmol) and the solution was heated and stirred for 24 h at 60 °C. After this time, K_2CO_3 (1.60 g, 11.6 mmol) was added and stirring was continued for a further 24 h at 60 °C when TLC (solvent system A) indicated the reaction to be near complete. The reaction mixture was poured into 10% aqueous K_2CO_3 (200 mL) and extracted with $CHCl_3$ (2 x 100 mL). The organic layer was dried (Na_2SO_4) and evaporated to give the crude product as an oil in quantitative yield. To separate unreacted **4**, the crude product was dissolved in acetic anhydride (200 mL) and treated with a catalytic amount of 4-(N,N-dimethylamino)pyridine and allowed to stand at 20 °C for 1 h when TLC (solvent system B) indicated the reaction to be complete. The solvent was evaporated *in vacuo* and the residue was partitioned between ether (200 mL) and 10% aqueous citric acid (200 mL). The ether layer was separated and the aqueous layer was extracted with a further 2 x 200 mL of ether and the combined ether extract was discarded. The aqueous layer was basified by addition of excess concentrated aqueous ammonia solution, and the solution was extracted with $CHCl_3$(3 x 100 mL). The $CHCl_3$ extract was dried (Na_2SO_4) and the solvent was evaporated to give an oily residue. The crude ester was dissolved in 10% methanolic KOH (200 mL) and stirred for 10 min at 20 °C when TLC (solvent system B) indicated hydrolysis of the O-acetyl group to be complete. The solvent was evaporated and the residue was partitioned between water (200 mL) and ether (200 mL). The aqueous layer was extracted with a further 2 x 200 mL of ether and the combined ether extract was back-washed with water (50 mL) and dried over Na_2SO_4. Evaporation of the solvent *in vacuo* afforded **5** (2.75 g, 83%). An analytical sample was obtained by crystallization from MeOH.: mp 154-155 °C; ^{1}H-

NMR δ 7.78 (dd, J=1.5 Hz, J=7.5 Hz, 1H), 7.71 (dd, J=1.5 Hz, J=7.5 Hz, 1H), 7.25-7.35 (complex m, 2H), 7.02 (s, 1H), 3.75 (m, 1H, CHOH), 2.43-2.49 (complex m, 6H), 1.73-1.92 (complex m, 6H), 1.52-1.73 (complex m, 4H), 1.29-1.35 (m, 2H); EIMS: M^+ calc for $C_{19}H_{25}NOS$=316; Found: M^+=316; Anal calc for $C_{19}H_{25}NOS.0.25\ H_2O$: C 71.32, H 8.03, N 4.38%; Found: C 71.39, H 7.91, N 4.84%.

N-[1-(2-Benzo[b]thienyl)cyclohexyl]-4-(methanesulfonyl oxy)piperidine (6)

To a stirred solution of **5** (1.00 g, 3.17 mmol) in dry THF (40 mL) was added triethylamine (TEA) (2.21 mL, 15.8 mmol) followed by dropwise addition of methanesulfonyl chloride (0.29 mL, 3.74 mmol) in THF (10 mL). TLC (solvent system A) indicated that the reaction was complete after stirring for 10 min at 20 °C. The solvent was filtered to remove TEA.HCl and the filter-cake was washed with THF (5 mL). The combined filtrate and washings were evaporated *in vacuo* and the residue was crystallized from ethyl acetate/hexanes (1:2) (30 mL) to give **6** (0.89 g; the mother liquor afforded 0.30 g, total yield: 1.19 g, 96%): mp 145-146 °C (dec); ^{1}H-NMR δ 7.79 (dd, J=1.6 Hz, J=7.0 Hz, 1H), 7.73 (dd, J=1.6 Hz, J=7.0 Hz, 1H), 7.26-7.36 (complex m, 2H), 7.02 (s, 1H), 4.77 (m, 1H, $\underline{CH}OSO_2CH_3$), 3.04 (s, 1H, $OSO_2\underline{CH_3}$), 2.43-2.49 (m, 6H), 2.11-2.17 (m, 2H), 1.87-1.96 (m, 4H), 1.52-1.60 (m, 4H), 1.32-1.33 (m, 2H); EIMS: M^+ calc for $C_{20}H_{27}NO_3S_2$=393; Found: M^+=393, M^+-$MeSO_3H$=297, M^+-$MeSO_3H$-butadiene=243; Anal calc for $C_{20}H_{27}NO_3S_2$: C 61.04, H 6.92, N 3.56%; Found: C 60.93, H 6.98, N 3.55%.

N-[4-(2-Benzo[b]thienyl)cyclohexenyl]piperidine (7)

To a stirred solution of **6** (300 mg, 0.76 mmol) in dry DMSO (5 mL) was added potassium *tert*-butoxide (500 mg) and the solution was allowed to stand overnight at at ambient temperature under an atmosphere of argon. The product was diluted with water (50 mL) and extracted with ether (50 mL). The ether layer was back-washed with water (2 x 25 mL) and evaporated to give an oily residue. Purification of the crude product by column chromatography on silica gel, eluting with ethyl acetate/hexane (2:23) afforded 200 mg (88%) of **7** as a colorless oil. Crystallization of **7**.HCl from ethyl acetate/ether (1:1) (3 mL) afforded 150 mg of **7**.HCl: mp 177-178 °C(dec); ^{1}H-NMR (base) δ 7.76 (dd, J=1.5 Hz, J=6.2 Hz, 1H), 7.63 (dd, J=1.5 Hz, J=6.2 Hz, 1H), 7.23-7.30 (complex m, 2H), 7.05 (s, 1H), 5.82 (m, 1H, olefinic), 5.65 (m, 1H, olefinic), 2.44-2.64 (complex m, 6H), 2.01-2.12 (m, 2H), 1.39-1.86 (complex m, 8H); EIMS: M^+ calc for $C_{19}H_{23}NS$=297; Found: M^+=297, M^+-butadiene=243; Anal calc for $C_{19}H_{24}ClNS.0.25H_2O$ C 67.43, H 7.30, N 4.14%; Found C 67.13, H 7.44, N 4.40%.

[^{3}H]N-[1-(2-Benzo[b]thienyl)cyclohexyl]piperidine ([^{3}H]BTCP)

A solution of **7**.HCl (20 mg, 0.060 mmol) in MeOH (2.0 mL) containing 10% Pd/C (20 mg) was stirred overnight under an atmosphere of carrier-free tritium gas (20 Ci, 0.345 mmol). The solution was filtered to remove catalyst and the labile tritium was removed by evaporation of the solvent under a stream of N_2. The residue was made up to 25 mL with MeOH for storage prior to purification. To this crude reaction mixture was added 2 drops of concentrated aqueous NH_3 and the solvent was evaporated under a stream of N_2. The residue was applied to one 20 cm x 20 cm x 1 mm preparative TLC plate and the plate was eluted with concentrated aqueous NH_3/MeOH/$CHCl_3$ (0.3:2.7:97). The band co-migrating with an authentic sample of unlabelled **1** [9] was removed and extracted with 50 mL of absolute ethanol. The silica gel was filtered by passage of the extract through a plug of glass wool and the solvent was adjusted to a final volume of 200 mL: yield=255 mCi (7.3% radiochemical yield); radiochemical purity>99.5%; specific activity=29.8 Ci/mmol (from UV analysis of the solution at λ=270 nM; ε_{270}=10500 liter^{-1}.cm^{-1}.mol); percent isotopic incorporation=51.4%. The compound showed no significant deterioration after several months at -80 °C.

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