Synthesis of [^{3}H]-1-[1-(3-Isothiocyanatophenyl) cyclohexyl]piperidine (METAPHIT), an Acylating Agent for Phencyclidine Receptors.

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SUMMARY

The preparation of [^{3}H]-labelled 1-[1-(3-isothiocyanatophenyl) cyclohexyl]piperidine (METAPHIT), an electrophilic acylating agent for the phencyclidine (PCP) site is described. Synthesis of [^{3}H]METAPHIT was accomplished in three steps starting from 1-[1-(3-nitrophenyl)cyclohexyl]piperidine. Introduction of the tritium-label in 20.6% radiochemical yield was achieved in the penultimate step.

Key words: Phencyclidine, PCP Receptor, METAPHIT, Electrophilic Acylating, Tritium labelling.

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INTRODUCTION

The illicit use of phencyclidine (PCP) in the USA has become a major problem that is now rivaling the abuse of drugs such as heroin and cocaine. Phencyclidine was originally developed by the Parke-Davis company as an anesthetic agent. Subsequent use of PCP in humans resulted in its withdrawal from the market after the observation that bizarre dissociative effects occurred when the patients emerged from the anesthesia. These dissociative effects and the increasing abuse of PCP stimulated widespread interest in the biochemical pharmacology of PCP, and eventually led to the discovery of saturable, stereospecific binding sites in rat brain.[1-3] A high degree of correlation between binding affinity and in-vivo effects for several PCP like compounds gives credence to the hypothesis that the PCP site may be a pharmacological receptor.[1,2] However, the use of [^{3}H]-1-[1-(3-azidophenyl)cyclohexyl]piperidine to photoaffinity label rat brain PCP sites suggested that these sites were heterogeneous.[4] The discovery of an endogenous ligand with higher binding affinity for the PCP receptor than PCP itself suggests that there may be a physiological role for these sites in the central nervous system.[5]

Recent synthetic efforts in our laboratory led to the development of 1-[1-(3-isothiocyanatophenyl)cyclohexyl]piperidine (METAPHIT), the first electrophilic acylating agent for the PCP site.[6] Treatment of rat brain homogenates with a 10μM concentration of METAPHIT followed by washing revealed a significant loss in the binding of [^{3}H]-TCP, but no change in the affinity (K_d).[6] METAPHIT has also been shown to irreversibly antagonize the effects of PCP in-vivo,[7-11] and it has been reported that METAPHIT can exert a long lasting PCP agonist effect on PCP receptors coupled to N-methyl-D-aspartate receptors in brain slice experiments.[12] METAPHIT has been shown to

irreversibly inhibit binding sites other than the PCP binding site: pretreatment of rat striatal tissue with METAPHIT resulted in irreversible inhibition of [^{3}H]threo-(±)-methylphenidate binding.[13] In a more recent study, it was shown that METAPHIT irreversibly inhibited the binding of both sigma and PCP receptors.[14]

The availability of [^{3}H]METAPHIT would therefore provide a valuable tool for tritium labelling of PCP and sigma receptors, and in the subsequent study of the structure and function of these receptor types.

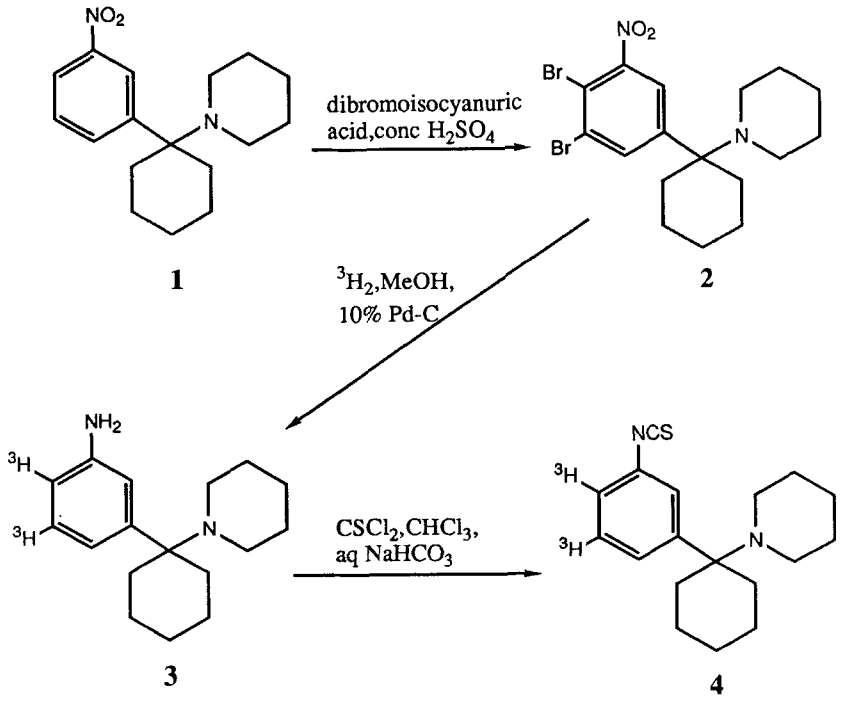
Synthesis

The synthetic pathway for preparing tritium labelled METAPHIT **(4)** is shown in Scheme 1. 1-[1-(3-Nitrophenyl)cyclohexyl]piperidine **(1)** available from nitration of 1-[1-phenylcyclohexyl]piperidine (PCP) was used as the starting material.[15] Dibromination of 1 with N,N´-dibromoisocyanuric acid (DBI) in concentrated H_2SO_4[16] afforded the desired tritiation precursor 1-[1-(3,4-dibromo-5-nitrophenyl)cyclohexyl]piperidine **(2)** in 50% yield. Addition of large molar excesses of DBI, in an attempt to tri- or tetrabrominate this compound, failed. Catalytic hydrogenation of 2 over 10% Pd-C during 1 hour afforded the known unlabelled 3.[15] Thus, catalytic tritiation of 2 over 10% Pd-C with 30 Ci of carrier free tritium gas followed by TLC purification afforded 1-[1-(5-amino-3,4-ditritiophenyl)cyclohexyl]piperidine **(3)** (20.6% radiochemical yield, 39.5 Ci/mmol) in greater than 99% radiochemical purity. Treatment of 3 with freshly redistilled thiophosgene ($CSCl_2$)[17] afforded the desired [^{3}H]-METAPHIT **(4)** in greater than 99.5% radiochemical purity (37% chemical yield) after purification by TLC. The purified material, when stored at -70 °C in absolute ethanol at a concentration of 1mCi/mL, showed no detectable

radiolysis after a 6 month period. The labelled compounds **(3)** and **(4)** co-migrated on TLC with the corresponding unlabelled reference compounds.

Scheme 1

Synthesis of Tritium Labelled METAPHIT
(1-[1(3-Isothiocyanatophenyl)cyclohexyl]piperidine)



DISCUSSION

Catalytic tritiation of intermediate **2** over 10% Pd-C afforded the desired aniline **3** with 61% incorporation of tritium in the 3 and 4 positions on the aromatic ring. Since this relatively high incorporation of tritium was repeatable, **2** represents a valuable tritiation precursor of high specific activity labelled **3**.

The substitution pattern of the bromine atoms on the aromatic ring of **2** was determined by selective INEPT

experiments.[18] Irradiating the two meta-situated protons remaining on the aromatic rings (7.52 and 7.71 ppm, J = 2.1 Hz) with a selective pulse elicited a response from C-1 of the cyclohexane ring in each case, confirming that each is three bonds away from this carbon atom. The result is consistent only with **2**, for in each of the alternative isomers, one of the protons is more than three bonds from C-1.

To demonstrate the positions of the tritium atoms in **3**, the carbon-13 spectrum of the unlabelled **3** was compared with that of the material prepared by catalytic deuteration (performed under identical conditions to the catalytic tritiation). Assignment of the carbon-13 signals of unlabelled **3** was readily made by reference to those of 2-phenyl-2-(1-piperidino)-adamantane,[19] the shifts characteristic of unlabelled **3** being obtained by addition of the increments anticipated from amino substitution:[20]

Carbon	Calculated	Observed
1	138.9	142.2
2	114.8	114.5
3	146.0	145.7
4	113.2	113.1
5	128.1	128.2
6	117.7	118.2

The only substantial difference between calculated and observed shifts is that of carbon-1, which, being held in an axial position in the adamantane derivative, suffers an upfield shift in this compound.

The carbon-13 spectrum of the deuterated material showed that carbon atoms 4 and 5 are approximately 50% deuterated- this result is consistent with the percentage of tritium incorporation. Carbon atons 4 and 5 appear as doublets much reduced in intensity, one branch of each doublet being shifted approximately 0.1 ppm upfield by the adjacent deuterium. Carbon-6 also appears as a doublet, one branch being shifted upfield by the deuterium on carbon-5. Carbon-3 appears as a doublet with a 0.05 ppm shift. Carbon atoms 1 and 2 are unchanged.

Reaction of the 3 with thiophosgene in the presence of aqueous sodium bicarbonate as described previously for the unlabelled compound,[6] afforded tritium labelled METAPHIT in 37% chemical yield. Addition of larger excesses of thiophosgene resulted in lower chemical yields.

EXPERIMENTAL

Materials and Methods

Melting points were determined on a Thomas Hoover capillary apparatus and are uncorrected. Elemental analyses were performed at Atlanta 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-NMR spectra were obtained from $CDCl_3$ solutions using a Varian XL-300 spectrometer. Infra-red (IR) spectra were obtained from KBr discs using a Beckman 4230 IR spectrophotometer. Ultra-Violet (UV) spectra were recorded using a Hewlett-Packard 8450A UV/VIS spectrophotometer. Thin layer chromatography (TLC) was performed on 250 μ Analtech GHLF silica gel plates. TLC system A refers to: concentrated aqueous ammonia-methanol-chloroform (0.5 : 4.5 : 95); TLC system B refers to ethyl acetate-hexane (1 : 5). For radiolabelled compounds, TLC plates were analysed with a Bertold model LB 2760 TLC scanner. Radioactivity determinations were carried out with a Packard model 2200 CA "Tri-Carb" liquid scintillation analyser using hydrofluor scintillation cocktail. All synthetic and analytical operations were initially carried out with unlabelled intermediates, and the structures assigned were confirmed spectroscopically.

1-[1-(3,4-Dibromo-5-nitrophenyl)cyclohexyl]piperidine (2). To a suspension of N,N′-dibromoisocyanuric acid (4.16 g, 14.5

mmol) in concentrated sulphuric acid (25 mL) was added 1 (2.88g, 10 mmol) in small portions during 10 minutes. The resulting homogeneous red solution was stirred for 1 hour at 20 °C, poured onto 100 g of crushed ice, neutralized by the addition of concentrated aqueous ammonia, and extracted with chloroform (3 x 100 mL). Drying (Na_2SO_4) of the combined organic extract followed by evaporation of the solvent afforded the crude product as an oil. Purification of the crude product by chromatography on silica gel, eluting with ammonia-methanol-chloroform (0.2 : 1.8 : 98) afforded 2 as an oil. Crystallization of the HCl salt of 2 from hot IPA containing 1% water afforded 2.HCl.0.5H_2O (3.51 g, 50%): mp 243-244 °C(dec.); IR 3050, 3000, 2930, 2600, 1530, 1440, 1350, 1020, 870, 720 cm^{-1}; ^{1}H-NMR 7.71 (d, 1H, J=2.1 Hz), 7.52 (d, 1H, J=2.1 Hz), 2.23 (m, 4H), 2.11-1.69 (m, 8H), 1.57-1.23 (m, 8H); MS (CIMS) m/z 447 (M+H for $C_{17}H_{22}{}^{79}Br^{81}BrN_2O_2$). Anal.(Found) C,41.38; H,4.96; N,5.65%. Anal. (Calculated for $C_{17}H_{23}ClBr_2N_2O_2 .0.5H_2O$) C,41.53; H,4.92; N,5.70%.

1-[1-(5-Amino-3,4-ditritiophenyl)cyclohexyl]piperidine (3). A solution of 2, 21.2 mg (0.044 mmol) in methanol (2 mL) containing 10% Pd-C (20 mg) was stirred overnight at room temperature under an atmosphere of carrier free tritium gas (30 Ci, 0.52 mmol). The solution was filtered, evaporated under a stream of argon (to remove labiles) and then rediluted to 25 mL with absolute ethanol for storage (activity of crude product = 2.18 Ci). Excess aqueous ammonia was added to the crude product (to liberate 3 free base) prior to evaporation of the solvent. Purification by TLC on one 20 cm x 20 cm x 0.5 mm plate eluting with solvent system A followed by extraction of the band corresponding to 3 with 30 mL of concentrated aqueous ammonia-methanol-chloroform (1 : 9 : 90) afforded 522.7 mCi (20.6%) of 3 in greater than 99% radiochemical purity as determined by TLC

analysis. The product was dissolved in 30 mL of absolute methanol for UV analysis (UV_{max} 289 nM, ε_{289} = 2210 cm.liter.mol^{-1} in MeOH). Specific activity = 39.5 Ci/mmol. Incorporation of tritium = 61%.

1-[1-(5-Isothiocyanato-3,4-ditritiophenyl) cyclohexyl]piperidine (METAPHIT) (4). To a stirred solution of **3** (522.7 mCi, 0.013 mmol) in a mixture of saturated sodium bicarbonate (2 mL) and chloroform (2 mL) was added via syringe, freshly redistilled thiophosgene (1.51 μL, 0.020 mmol) and the reaction stirred for 30 minutes at room temperature. The organic layer was removed and transferred to a 5 mL vial. The aqueous layer was extracted with a further 2 mL of chloroform and the combined organic layer evaporated under a stream of argon. TLC separation on one 20 cm x 20 cm x 0.5 mm TLC plate, eluting with solvent system B, and extraction (30 mL of ethyl acetate) of the band co-migrating with unlabelled METAPHIT, afforded 191.9 mCi (37%) of **4** in >99.5% radiochemical purity.

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