Synthesis of Unlabelled and Tritium Labelled 4-Isothiocyanato-1-(1-phenylcyclohexyl)piperidine (Fourphit), Tools for the Study of the Dopamine Reuptake Complex.

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Summary

The synthesis of high specific activity tritium labelled 4-isothiocyanato-1-(1-phenylcyclohexyl)piperidine ([^{3}H]fourphit), a probe for the autoradiographic study of the dopamine reuptake complex, is described. An improved, facile synthesis of unlabelled fourphit in high overall yield is also presented. Detailed study of the interaction of unlabelled and tritium labelled fourphit with the dopamine reuptake complex may lead to a better understanding of the actions of cocaine.

Key words: Dopamine Reuptake Complex, Cocaine, Affinity Label, Autoradiography, Tritium Labelled 4-isothiocyanato-1-(1-phenylcyclohexyl)piperidine, Fourphit.

Introduction

Cocaine, one of the major drugs of abuse in the USA [1,2], exhibits a variety of pharmacological effects which are the result of its interaction with an array of neurotransmitter systems [2,3]. The reinforcing properties of cocaine are thought to be mediated through its inhibition of the dopamine (DA) reuptake complex [4]. Some of the behavioral effects of PCP (phencyclidine, 1-(1-phenylcyclohexyl)piperidine), another drug of abuse, can also be attributed to interactions with dopaminergic pathways [2,5]. PCP binds with high affinity both to a site associated with the N-methyl-D-aspartate (NMDA) receptor [6], and to the DA-reuptake complex [7-9]. Modification or substitution of the aromatic ring of PCP affords compounds which possess increased selectivity either for the NMDA/PCP binding site or the DA-reuptake complex [7-8]. For example,

0362–4803/92/090671–13$11.50

Received 20 April, 1992
Revised 11 June, 1992

TCP (1-(2-thienylcyclohexyl)piperidine) is a highly selective ligand for the NMDA/PCP-site [10], while its benzo analog (1-(2-benzothienylcyclohexyl)piperidine, BTCP) shows high selectivity for the DA-reuptake complex [8,11].

Ligands containing an electrophilic moiety such as an isothiocyanato group have been used to label a variety of receptors [12]. For example, metaphit (1-(3-isothiocyanatophenyl)cyclohexylpiperidine), a PCP-derivative, was successfully utilized as an irreversible ligand for the characterization of the NMDA/PCP binding site [13]. However, it was later discovered that metaphit also irreversibly inhibits the in vitro binding of both [^{3}H]cocaine [14] and [^{3}H]methylphenidate [15], thus identifying it as a probe for study of the DA-reuptake complex. Recently, fourphit (**1**, 4-isothiocyanato-1-(1-phenylcyclohexyl)piperidine) [16], a structural isomer of metaphit with the isothiocyanato moiety on the piperidine ring, was shown to bind rapidly and irreversibly to the [^{3}H]methylphenidate site [13,17]. In contrast to metaphit, **1** exhibits only reversible binding for the NMDA/PCP site, making it more useful than metaphit for the study of the DA-reuptake complex. We therefore desired tritium labelled **1** for the autoradiographic visualization of the components of the DA-reuptake complex with which fourphit interacts.

Fourphit (**1**) was originally [16] synthesized in low overall yield starting from carbinol **3**. Oppenauer oxidation gave the corresponding ketone in 25% yield [18], which, upon reaction with hydroxylamine and reduction of the resulting oxime with metallic sodium in ethanol, was converted into primary amine **5**. Reaction of **5** with thiophosgene afforded **1**.

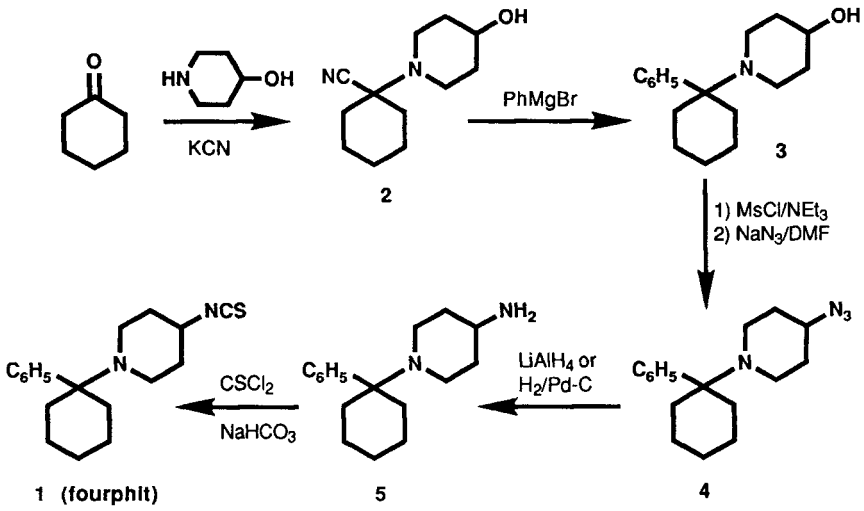
In this study, we report the synthesis of [^{3}H]fourphit ([^{3}H]**1**) starting from 1,4-cyclohexadione monoethylene ketal (**6**), and an improved, high-yield synthesis for fourphit (**1**). The synthetic approach for **1** and [^{3}H]**1** is based on the synthesis of PCP and many of its derivatives using the Bruylants reaction of an suitable aminonitrile with an arylmagnesium bromide as the key step [19]. The nitrogen function on the piperidine ring is introduced by means of a nucleophilic displacement of a methanesulfonate ester with azide.

Results and Discussion

Synthesis of fourphit

The starting carbinol **3** was obtained in 70% yield by Bruylants reaction of nitrile **2** [20] with phenylmagnesium bromide in tetrahydrofuran (THF)/diethyl ether. In contrast to an earlier report [18], we found protection of the alcohol as its trimethylsilyl ether unnecessary, provided an excess of phenylmagnesium bromide was used [20]. Treatment of **3** with methanesulfonyl chloride in the presence of triethylamine, followed by reaction of the resulting methanesulfonate ester with sodium azide in dimethylformamide (DMF) at 80 °C, gave azide **4** in 81% overall yield (Scheme 1). Reduction of **4** with lithium aluminum hydride or catalytic hydrogenation of **4** in the presence of acid gave amine **5**, which was transformed to fourphit (**1**) by treatment with

thiophosgene in the presence of sodium bicarbonate. Alcohol **3** could also be directly converted into azide **4** by a Mitsunobu-type reaction with zinc azide bispyridine complex [21]. However, in this case **4** was only obtained in low yield after chromatography.

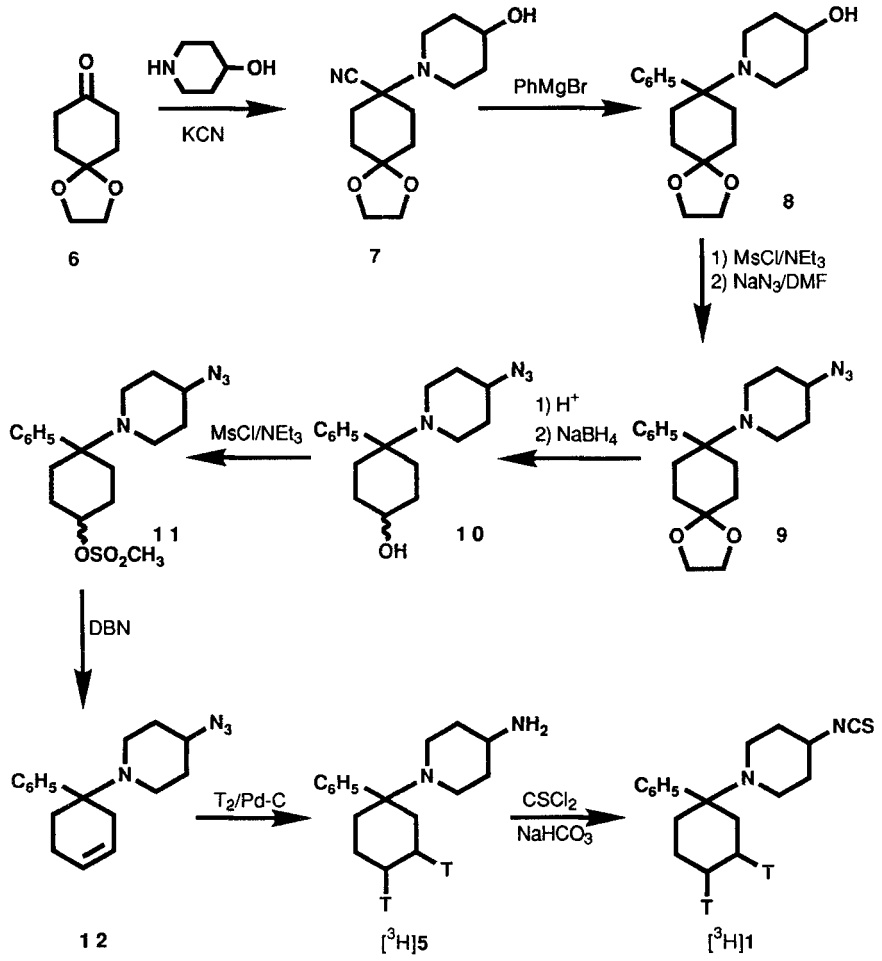


Scheme 1. Synthesis of fourphit (**1**)

Synthesis of [^{3}H]fourphit

The synthesis of the tritium labelled fourphit ([^{3}H]**1**) followed a strategy analogous to that for **1** (Scheme 2). Reaction of 1,4-cyclohexanonedione monoethylene ketal (**6**) with 4-hydroxypiperidine and potassium cyanide gave **7**, which afforded aminoalcohol **8** in 70% yield upon treatment with excess phenylmagnesium bromide in THF/diethyl ether. As in the case of **3**, no protection of the alcohol function was necessary to obtain a good yield of the Bruylants product. Reaction of **8** with methanesulfonyl chloride, followed by treatment with sodium azide, gave **9** in 61% overall yield. Hydrolysis of the ketal function with 1N HCl at room temperature gave the oily ketone, which was reduced directly with sodium borohydride to give a 2:1 mixture of the epimeric alcohols **10**. Reaction of **10** with methanesulfonyl chloride, followed by treatment with 1,5-diazobicyclo[4.3.0]non-5-ene (DBN) in DMF afforded alkene **12** in 10% yield (based on alcohol **10**) after purification by column chromatography and crystallization.

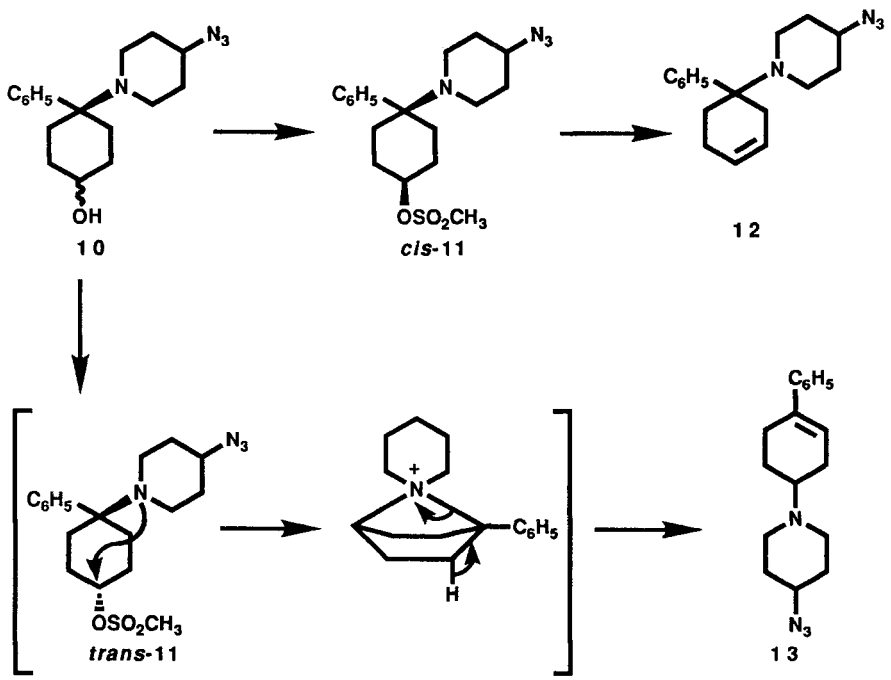
Catalytic hydrogenation of olefin **12** in methanol/chloroform (10:1) occurred with concomitant reduction of the azide function to give primary amine **5**. Catalytic tritiation of **12** with carrier-free tritium gas [22] under identical conditions gave [^{3}H]**5**, which afforded [^{3}H]fourphit ([^{3}H]**1**) upon treatment with thiophosgene. The specific activity of [^{3}H]**1** as determined by UV-spectroscopy at λ 249 nm (ε_{249} 1482 L.mol^{-1}.cm^{-1} as determined for a solution of unlabelled **1** in ethanol) was found to be 23 Ci/mmol (14% radiochemical and 36% chemical yield, 40% isotopic incorporation).



Scheme 2. Synthesis of [^{3}H]fourfit ([^{3}H]**1**)

As an unexpected side product from the elimination reaction of **11**, 1-phenyl-4-(4-azidopiperidinyl)cyclohex-1-ene (**13**) was isolated in various amounts depending on the reaction conditions. Methanesulfonation of the epimeric alcohols **10** afforded only one of the two possible esters, probably *cis*-**11**, upon aqueous work-up. Olefin **12** and styrene **13** were formed in a 3:1 ratio (determined from NMR through the integration of the vinylic signals), regardless whether the crude or purified methanesulfonate was utilized. When the methanesulfonation/elimination sequence was performed as a "one-pot"-procedure by reacting **10** first with methanesulfonyl chloride in DMF in the presence of triethylamine, followed by treatment with DBN, styrene **13** was the major product and only a small amount (10-15% according to NMR) of alkene **12** was formed. A possible reaction mechanism is depicted in Scheme 3. The unstable *trans*-**11**

undergoes intramolecular displacement of the methanesulfonate ester by the piperidine nitrogen. Upon treatment with base, styrene **13** is formed.



Scheme 3. Possible mechanism for the formation of **13**.

Experimental

General experimental details

All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Gas chromatographic analysis was performed on a Hewlett-Packard 5890 instrument using a flame ionization detector. The ^{1}H NMR spectra were recorded on a Varian XL-300 instrument using deuterochloroform as solvent and tetramethylsilane as the internal standard. Chemical-ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. High-resolution mass spectra (HRMS) and electron ionization mass spectra (EIMS) were obtained using a VG-Micro Mass 7070F mass spectrometer. Infra-red (IR) spectra were measured using a Beckman 4230 IR spectrophotometer. Ultraviolet (UV) spectra were obtained from EtOH solutions using a Hewlett-Packard 8450 UV/VIS spectrophotometer. Thin-layer chromatography (TLC) was performed on 250 μ Analtech GHLF silica gel plates. TLC plates were analyzed for radioactivity with a Bertold model LB 2760 scanner. Radioactivity determinations were carried out using a Packard model 2200 CA

"Tri-Carb" liquid scintillation counter. Tritium labelled compounds were counted in a Hydrofluor scintillation cocktail (National Diagnostics) with a counting efficiency of 45%. All synthetic and analytical operations were initially performed with unlabelled compounds and the structures were confirmed spectroscopically.

4-Hydroxy-1-(1-phenylcyclohexyl)piperidine (3)

A solution of cyanopiperidine **2** [20] (10.0 g, 48 mmol) in 100 mL of anhydrous THF was added dropwise over a 1-h period to a cooled (5 °C), stirred solution of phenylmagnesium bromide (48 mL of a 3.0 M solution in diethyl ether, 144 mmol) in 100 mL of anhydrous THF. The solution was then allowed to warm to room temperature and was stirred under inert atmosphere for 14 h. The reaction mixture was poured onto a mixture of aqueous citric acid solution (200 mL, 1M) and ice (5 g) and the resulting solution was extracted with diethyl ether (2 x 100 mL). The aqueous layer was treated with aqueous NH_4OH to pH 9.5 and extracted with diethyl ether (2 x 100 mL). The combined ether extracts from the basic extraction were washed with brine, dried (Na_2SO_4), and concentrated to obtain 10.3 g of crude **3** as a white powder. Crystallization from ethyl acetate afforded 8.68 g (34 mmol, 70%) of **3** as a white, crystalline solid, m.p. 118-119 °C (m.p. 117-118 °C [20]); CIMS (NH_3) m/z 260 ($M^+ + 1$). Anal. Calcd for $C_{17}H_{25}NO$: C 78.72, H 9.71, N 5.40%. Found: C 78.78, H, 9.71, N 5.35%.

4-Azido-1-(1-phenylcyclohexyl)piperidine (4)

To a stirred solution of alcohol **3** (48.0 g, 185 mmol) and triethylamine (129 mL, 925 mmol) was added dropwise at ambient temperature during 10 min, methanesulfonyl chloride (15.8 mL, 20.4 mmol). TLC indicated the reaction to be complete after 10 min. The solution was filtered to remove triethylamine.HCl salt and the filter cake was washed with THF (100 mL). The combined filtrate and washings were evaporated to a colorless, crystalline residue. The residue was dissolved in CH_2Cl_2 (450 mL), the CH_2Cl_2 was removed by distillation and replaced with 2-propanol (450 mL). The crystallization flask was set aside to cool to ambient temperature and then seeded with a small crystal. The flask was cooled to 4 °C and the crystals were filtered and washed with cold 2-propanol and air-dried for 24 h, yield 39.5 g (62%): m.p. 117-118 °C; CIMS (NH_3) m/z 338 ($M^+ + 1$). Anal. Calcd for $C_{18}H_{17}NO_3S$: C 64.06, H, 8.06, N 4.15%. Found: C 64.05, H 8.10, N 4.11%.

A stirred mixture of the above crystalline methanesulfonate (30.0 g, 89 mmol) and sodium azide (28.9 g, 445 mmol) in dry DMF (150 mL) was heated at 60 °C for 24 h. The reaction mixture was cooled, diluted by addition of water (500 mL) and treated with concentrated aqueous NH_3 (ca. 100 mL). The aqueous mixture was extracted with diethyl ether (1000 mL) and the ether extract was back-washed with water (3 x 500 mL). Drying of the organic extract (Na_2SO_4) and evaporation of the solvent in vacuo afforded the product as an oil. This oil was dissolved in ethyl acetate (400 mL) and treated with a slight excess of a solution of HCl (g) in ethyl acetate to give **4**.HCl (16.4 g, 51 mmol, 58%): m.p. 193-194 °C; CIMS (NH_3) m/z 285 ($M^+ + 1$). Anal. Calcd for

$C_{17}H_{25}ClN_4.0.25\ H_2O$: C 62.76, H 7.90, N 17.22%. Found: C 62.84, H 7.88, N 16.83%.

4-Amino-1-(1-phenylcyclohexyl)piperidine (5)

A. with lithium aluminum hydride (LAH)

A solution of azide **4** (4.60 g, 16 mmol) in THF (50 mL) was added dropwise over a 15-min period to a cooled (5 °C), stirred suspension of LAH (0.61 g, 16 mmol) in anhydrous THF (50 mL). The resulting suspension was stirred at ambient temperature under inert atmosphere for 10 additional minutes, the reaction mixture was cooled in an ice bath and 20 mL of a saturated ammonium chloride solution was cautiously added. After the bubbling ceased, 20 mL of diethyl ether was added and the top (organic) layer was removed. This procedure was repeated twice. The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated to yield 3.80 g (92 %) of a cream-coloured solid. The free base was crystallized from ethanol/H_2O to yield **5** (3.0 g, 15 mmol, 72 %) as a white, crystalline solid, m.p. 82-83 °C (m.p. 181-184 °C (di-HCl salt) [16]); CIMS (NH_3) m/z 259 ($M^+ + 1$). Anal. Calcd for $C_{17}H_{26}N_2.0.25\ H_2O$: C 77.66, H 10.16, N 10.65%. Found: C 77.65, H 10.16, N 10.61%.

B. by catalytic reduction

4.HCl (16.0 g, 50 mmol) in MeOH (200 mL) containing 10% Pd-C (1.6 g) was stirred under an atmosphere of hydrogen (1 atm pressure) for 4 h at ambient temperature when TLC ($CHCl_3$/MeOH/NH_4OH (90:9:1) indicated the reaction to be complete. The reaction mixture was filtered through celite to remove the catalyst, the celite was washed with MeOH (50 mL) and the solvent was evaporated to give an oily residue. The residue was dissolved in water (400 mL) and the pH was adjusted to 3 by addition of excess concentrated aqueous HCl solution. The aqueous solution was washed with diethyl ether (400 mL) and the ether extract was discarded. The aqueous layer was basified by the addition of excess concentrated aqueous NH_3 solution and extracted with CH_2Cl_2 (3 x 200 mL). The combined organic extracts were evaporated in vacuo to give a pale yellow oil. The oil was filtered through a 4 inch pad of silica gel (eluent: $CHCl_3$/MeOH/NH_4OH 95:4.5:0.5) and the solvents were evaporated to give **5** (7.6 g, 59%) as a colorless, crystalline solid identical to that from the LAH reduction procedure described above.

4-Isothiocyanato-1-(1-phenylcyclohexyl)piperidine (fourphit, 1)

To a vigorously stirred solution of **5** (7.6 g, 29 mmol) in a mixture of pentene-stabilized $CHCl_3$ (200 mL) and saturated aqueous $NaHCO_3$ (200 mL) was added in one portion, freshly redistilled thiophosgene (2.47 mL, 33 mmol). The reaction mixture was stirred for 10 min at ambient temperature when TLC ($CHCl_3$/MeOH/NH_4OH 90:9:1) indicated the reaction to be complete. The organic layer was separated and the aqueous layer was washed with $CHCl_3$ (200 mL). The combined organic extract was washed with water (100 mL), dried (Na_2SO_4) and the solvent was evaporated in vacuo to give the crude product as a pale yellow oil. This oil was dissolved in warm ethyl acetate (100 mL) and

treated with excess HCl (g) in ethyl acetate to give **1**.HCl (8.56 g, 86%), m.p. 197-197.5 °C (dec). (m.p. 176-180 °C (from 2-propanol/diisopropyl ether) [16]). ^{1}H-NMR ($CDCl_3$, free base) δ 1.23-1.51 (m, 4H), 1.72 (m, 4H), 1.92 (m, 4H), 2.03 (m, 4H), 2.74 (m, 2H), 3.43 (m, 1H, CHNCS), 7.29 (m, 5H, ArH). ^{1}H-NMR ($CDCl_3$, HCl salt) δ 1.21 (m, 2H), 1.47 (m, 2H), 1.84 (m, 2H), 1.96 (m, 2H), 2.57 (m, 4H), 3.60 (m, 2H), 4.04 (m, CHNCS), 7.54 (m, 5H, ArH). IR ($CHCl_3$, free base) 2101 (NCS) cm^{-1}. CIMS (NH_3) m/z 301 (M^+ + 1). Anal. Calcd for $C_{18}H_{25}ClN_2S$: C 64.17, H 7.48, N 8.31%. Found: C 64.22, H 7.40, N 8.25%.

8-Cyano-8-[1-(4-hydroxypiperidyl)]-1,4-dioxaspiro[4.5]decane (7)

A solution of 4-hydroxypiperidine (16.3 g, 0.16 mol) in 150 mL of water was adjusted to pH 3.5 with concentrated aqueous HCl. 1,4-Cyclohexanedione monoethylene ketal (**6**, 24.0 g, 0.15 mol) and potassium cyanide (11.0 g, 0.17 mol) were added and the resulting solution was stirred for 24 h at room temperature. The reaction mixture was extracted with ethyl acetate (500 mL, 2 x 100 mL), and the combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo. The residue was crystallized from ethyl acetate to give 27.7 g of **7** (0.10 mol, 68% yield based on **6**), m.p. 116-117 °C. ^{1}H NMR ($CDCl_3$) δ 1.50-2.15 (m, 8H), 2.35 (dd, 2H), 2.95 (m, 2H), 3.75 (m, 1H, CH(OH), 3.95 (s, 4H, -OCH_2CH_2O-). IR (KBr) 3300 cm^{-1} (OH). CIMS (NH_3) m/z 267 (M^+ + 1), 240 (-HCN). Anal. Calcd for $C_{14}H_{22}N_2O_3$: C 63.14, H 8.33, N 10.52%. Found: C 63.40, H 8.33 N 10.53%.

8-[1-(4-Hydroxypiperidyl)]-8-phenyl-1,4-dioxaspiro[4.5]decane (8)

A mixture of 50 mL of 3M phenylmagnesium bromide (0.15 mol) in diethyl ether and 150 mL of anhydrous THF was cooled to 0 °C under argon and a solution of aminonitrile **7** (12.6 g, 47 mmol) in 150 mL of anhydrous THF was added over a 1-h period. After stirring overnight at room temperature, the reaction was poured into 250 mL of ice-cold, 1N HCl. The layers were separated and the aqueous layer was washed with diethyl ether (3 x 50 mL). The aqueous layer was basified with 2 N KOH and extracted with diethyl ether (3 x 100 mL). The combined organic layers were washed with 50 mL of water, dried (Na_2SO_4), and evaporated to give an oil which solidified on the addition of ethyl acetate. Recrystallization from ethyl acetate gave 10.5 g (33 mmol, 70%) of **8**, m.p. 153-154 °C. ^{1}H NMR ($CDCl_3$) δ 1.25 (m, 1H), 1.39-1.63 (m, 5H), 1.64-2.09 (m, 7H), 2.36 (m, 2H), 2.80 (m, 2H), 3.44 (m, 1H, CHOH), 3.88-3.99 (m, -OCH_2CH_2O-), 7.24-7.32 (m, 5H, ArH). CIMS (NH_3) m/z 318 (M^+ + 1). Anal. Calcd for $C_{19}H_{27}NO_3$: C 71.89, H 8.57, N 4.41%. Found: C 72.03 H 8.75, N 4.68%.

8-[1-(4-Azidopiperidyl)]-8-phenyl-1,4-dioxaspiro[4.5]decane (9)

Amino alcohol **8** (8.00 g, 25 mmol) was dissolved in 200 mL of pentene-stabilized $CHCl_3$ and the solution was cooled to 0 °C. Triethylamine (7.0 mL, 50 mmol) and methanesulfonyl chloride (3.18 g, 28 mmol) were added and the mixture was stirred for 30 min. Saturated aqueous $NaHCO_3$ (200 mL) was added, the layers were separated, and the aqueous layer was extracted with chloroform (2 x 50 mL). The combined

organic extracts were dried (Na_2SO_4), and concentrated to give 9.90 g (25 mmol, 99%) of the pure methanesulfonate ester, which was used without further purification, m.p. 132-133 °C (dec). ^1H NMR ($CDCl_3$) δ 1.76-2.23 (m, 8H), 2.36 (m, 2H), 2.83 (m, 2H), 2.97 (s, 3H, SO_2CH_3), 4.01 (m, 4H, -OCH_2CH_2O-), 4.55 (m, 1H, C<u>H</u>OSO_2CH_3), 7.37 (m, 5H, ArH). CIMS (NH_3) m/z 396 (M^+ + 1), 217.

The methanesulfonate ester (9.80 g, 25 mmol) was dissolved in 100 mL of dry DMF, sodium azide (8.20 g, 0.13 mol) was added and the resulting mixture was heated to 60 °C for 16 h. The mixture was cooled to room temperature and water (200 mL) was added. Extraction with ethyl acetate (3 x 50 mL), drying of the combined organic extracts (Na_2SO_4), and evaporation of the solvents gave 8.50 g of a light brown solid. Crystallization from methanol gave 5.86 g (17 mmol, 62%) of azide **9**, m.p. 123-124 °C. ^1H NMR ($CDCl_3$) δ 1.53 (m, 4H), 1.79-2.01 (m, 8H), 2.32 (m, 2H), 2.78 (m, 2H), 3.09 (m, 1H, C<u>H</u>N_3), 3.90 (m, 4H, OCH_2CH_2O), 7.20-7.29 (m, 5H, ArH). CIMS(NH_3) m/z 343 (M^+ + 1), 217. Anal. Calcd for $C_{19}H_{26}N_4O_2$: C 66.64, H 7.65, N 16.36%. Found: C 66.26, H 7.76, N 16.07%.

4-Azido-1-[4-hydroxy-1-phenyl(cyclohexyl)]piperidine (10)

Ketal **9** (3.76 g, 11 mmol) was dissolved in 150 mL of 1N HCl and stirred overnight at room temperature. The mixture was basified with concentrated aqueous ammonia and extracted with diethyl ether (3 x 50 mL). The combined ethereal layers were washed with water (2 x 50 mL), dried (Na_2SO_4), and evaporated to give 3.16 g (11 mmol, 97%) of the corresponding ketone as a light yellow oil, which was homogeneous on TLC. EIMS m/z 298 (M^+), 241. IR (neat) 2090 (-N_3), 1710 (C=O) cm^{-1}.

The crude ketone (3.16 g, 11 mmol) was dissolved in 100 mL of 95% ethanol and the solution was cooled to 0 °C. Sodium borohydride (1.20 g, 32 mmol) was added in small portions. After 30 min, 100 mL of water and 100 mL of diethyl ether were added and the layers were separated. The aqueous fraction was extracted with ether (2 x 25 mL) and the combined organic fractions were washed with 1 M aqueous ammonia (25 mL) and water (25 mL), dried (Na_2SO_4), and evaporated to give 2.50 g (8.3 mmol, 79%) of the crystalline, epimeric alcohols **10**. An analytical sample was obtained by crystallization from 2-propanol, m.p. 120-121 °C. ^1H NMR ($CDCl_3$) δ 1.26 (m, 2H), 1.49-1.97 (m, 11H), 2.35-2.57 (m, 2H), 2.86 (m, 2H), 3.13 (m, 1H, C<u>H</u>N_3), 3.68-3.87 (two m (ratio 2:1), 1H, C<u>H</u>OH), 7.23-7.37 (m, 5H, ArH). IR (KBr) 3300 (OH), 2090 (-N_3) cm^{-1}. EIMS m/z 300 (M^+), 241. Anal. Calcd for $C_{17}H_{24}N_4O.0.25H_2O$: C 66.97, H 8.10, N 18.37%. Found: C 67.06, H 8.06, N 18.29%.

4-Azido-1-[4-methanesulfonyloxy-1-phenyl(cyclohexyl)]piperidine (11)

Azidoalcohol **10** (1.01 g, 3.4 mmol) was dissolved in 60 mL of pentene-stabilized $CHCl_3$ and cooled to 0 °C. Triethylamine (0.90 mL, 6.5 mmol) and methanesulfonyl chloride (0.30 mL, 3.9 mmol) were added and the mixture was stirred for 30 min at room temperature. Saturated aqueous $NaHCO_3$ (100 mL) was added, the layers were separated, and the aqueous layer was extracted with $CHCl_3$ (2 x 20 mL). The combined organic layers were washed with water (25 mL), dried (Na_2SO_4), and evaporated to

give methanesulfonate ester **11** (0.85 g, 2.2 mmol, 66%) as a colorless oil which was used without further purification in the next step. ^{1}H NMR ($CDCl_3$) δ 1.55 (m, 6H), 1.80 (m, 4H), 2.10 (m, 2H), 2.47 (m, 2H), 2.81(m, 2H), 3.05 (s, 3H, SO_2CH_3), 3.15 (m, 1H, C<u>H</u>N_3), 4.75 (m, 1H, C<u>H</u>OSO_2CH_3), 7.20-7.45 (m, 5H, ArH). CIMS (NH_3) m/z 379 (M^+ + 1).

4-Azido-1-(1-phenylcyclohex-3-enyl)piperidine hydrochloride (12.HCl)
Crude **11** (0.60 g, 1.6 mmol) was dissolved in 10 mL of dry DMF, 1,5-diazabicyclo[4.3.0]non-5-ene (1.00 g, 8.0 mmol) was added and the mixture was heated under argon for 16 h (oil bath temperature 110 °C). After cooling to room temperature, the solvent was evaporated and the residue was partitioned between 10 mL of water and 10 mL of diethyl ether. The aqueous layer was extracted with diethyl ether (2 x 10 mL), and the combined organic extracts were dried (Na_2SO_4) and evaporated. Chromatography of the residue (SiO_2, hexanes/ethyl acetate 3:1) gave 110 mg of alkene **12** and 50 mg of styrene **13**. Compound **12** was converted into the HCl salt by treatment with ethanolic HCl and recrystallized from ethyl acetate, giving pure (TLC) **12**.HCl (110 mg, 0.35 mmol, 10% yield based on **10**), m.p. 180-182 °C (dec). ^{1}H NMR (free base, $CDCl_3$) δ 0.90 (m, 1H), 1.20-2.05 (m, 8H), 2.18 (m, 2H), 2.45 (bs, 2H), 2.55 (m, 1H), 3.05 (m, 1H, C<u>H</u>N_3), 3.27 (m, 1H), 5.52 (m, 1H, CH=), 5.80 (m, CH=), 7.25 (m, 3H, ArH), 7.45 (2H, ArH). IR (neat, free base) 2105 (N_3). EIMS m/z 282 (M^+), 228, 156. HRMS: Calcd for $C_{17}H_{22}N_4$: 282.1844. Found: 282.1839. Anal. Calcd for $C_{17}H_{22}N_4$.HCl: C 64.04, H 7.27, N 17.57%. Found: C 63.97, H 7.29, N 17.44%. Styrene **13** was crystallized as its HBr-salt from ethanol, m.p. 211-213 °C (dec). ^{1}H NMR (HBr-salt, $CDCl_3$) δ 1.933 (d, 2H), 2.51-2.67 (m, 4H), 2.68-2.86 (m, 4H), 2.99-3.13 (m, 2H), 3.31-3.40 (m, 2H), 4.02 (m, 1H, C<u>H</u>N_3), 5.88 (d, *J* 5.3 Hz, 1H, CH=), 7.20-7.40 (m, 5H, Ar-H). IR (KBr) 2105 (N_3). EIMS m/z 282 (M^+). HRMS: Calcd for $C_{17}H_{22}N_4$: 282.1844. Found: 282.1845. Anal. Calcd for $C_{17}H_{22}N_4$.HBr: C 56.20, H 6.38, N 15.42%. Found: C 56.16, H 6.39, N 15.38%.

Trial Hydrogenation of 12
A solution of **12**.HCl (10.0 mg, 0.033 mmol) in a mixture of MeOH (1.0 mL) and $CHCl_3$ (0.1 mL) was stirred at room temperature and 10.0 mg of 10% Pd/C was added. The reaction mixture was stirred for 16 h under an atmosphere of hydrogen. The solution was filtered and evaporated in vacuo to give pure **5**, identical to an authentic sample prepared as described above.

[^{3}H]-4-Amino-1-(1-phenylcyclohexyl)piperidine ([^{3}H]5)
A solution of **12**.HCl (10.0 mg, 0.033 mmol) in a mixture of MeOH (1.0 mL) and $CHCl_3$ (0.1 mL) was stirred at room temperature and 10.0 mg of 10% Pd/C was added. The reaction mixture was stirred for 16 h under an atmosphere of carrier-free tritium gas (10 Ci, 0.17 mmol). The solution was filtered, evaporated under a gentle stream of N_2 gas to remove labile tritium and then reconstituted to 25 mL with MeOH for storage [22]. The solution was basified with concentrated aqueous ammonia, and

evaporated under a stream of N_2 gas. The residue was taken up in 0.5 mL of MeOH and applied to a 1.0 mm preparative TLC plate (20 x 20 cm). The plate was eluted with $CHCl_3$/MeOH/NH_4OH (85:15:0.1) and the band comigrating with reference **5** was scraped off and extracted with 10 mL of $CHCl_3$/MeOH/NH_4OH (80:20:0.1). After stirring for 10 min, the extract was filtered through glass wool and the filtrate was evaporated under a stream of N_2 gas. The residue was dissolved in 5 mL of MeOH and stored at -70 °C.

[^{3}H]-4-Isocyanato-1-(1-phenylcyclohexyl)piperidine ([^{3}H]Fourphit, [^{3}H]1) The methanolic solution of [^{3}H]**5**, obtained as described above, was evaporated under a stream of N_2 gas and the residue was dissolved in 1 mL of pentene-stabilized $CHCl_3$. An aqueous solution of 1 M $NaHCO_3$ (1 mL) was added. With vigorous stirring, 40 µL of a 0.52 M solution of freshly distilled thiophosgene in pentene-stabilized $CHCl_3$ was added, and the resulting mixture was stirred for 30 min. The layers were separated, and the aqueous fraction was extracted with $CHCl_3$ (2 x 1 mL). The combined organic extracts were concentrated under a stream of N_2, redissolved in 1 mL of $CHCl_3$, and applied to a 1.0 mm preparative TLC plate (20 x 20 cm). The plate was eluted with $CHCl_3$/MeOH/NH_4OH (95 : 5 : 0.1) and the band comigrating with reference **1** was scraped off and extracted with 10 mL of $CHCl_3$/MeOH/NH_4OH (95:5:0.1). The solution was evaporated to dryness, and reconstituted with toluene to 200 mL. Yield of [^{3}H]**1**: 261 mCi (14% radiochemical yield); radiochemical purity > 99% (by TLC analysis); specific activity 23 Ci/mmol (from ε_{249} 1482 L.mol^{-1}.cm^{-1} as found for a solution of **1** in ethanol.); 40% incorporation of tritium; 36% chemical yield.

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22. This reaction step was performed by Amersham Corporation, 2636 South Clearbrook Drive, Arlington Heights, IL 60005-4692, USA.

Acknowledgements

J.T.M.L., B.R. de C. and N.A.G. gratefully acknowledge partial support from G. D. Searle and the Fogarty Foundation. We thank Mr. Noel Whittaker and Mr. Wesley White for determination of mass and NMR spectra.