Synthesis and Characterization of Optically Pure [^{3}H](+)-Azidophenazocine ([^{3}H](+)-AZPH), a Novel Photoaffinity Label for Sigma Receptors

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SUMMARY

[^{3}H](+)-cis-N-(2-(4-Azidophenyl)ethyl)-2'-hydroxy-2,6-dimethyl-6,7-benzomorphan ([^{3}H]**1**) ([^{3}H](+)-AZPH), a novel high affinity and high selectivity benzomorphan based photoaffinity label for sigma receptors was synthesized in 4 steps starting with optically pure (+)-normetazocine and 2-(*p*-azidophenyl)ethyl methanesulfonate ester. Condensation of these compounds in DMF in the presence of $NaHCO_3$ afforded **1** in 77% yield. The tritiation precursor (+)-cis-N-(2-(4-azidophenyl)ethyl)-2'-hydroxy-1',3'-dibromo-2,6-dimethyl-6,7-benzomorphan (**4**) for medium specific activity [^{3}H]**1** was obtained in 96% yield by bromination of **1** with 2 equivalents of bromine. The sequence of catalytic tritiation of **4**, treatment of the resulting aniline ([^{3}H]**5**) (13.5% radiochemical yield, specific activity 22.9 Ci/mmol) with nitrous acid followed by sodium azide afforded the target compound in 78% chemical yield.

Key Words: [^{3}H](+)-cis-N-(2-(4-Azidophenyl)ethyl)-2'-hydroxy-2,6-dimethyl-6,7-benzomorphan ([^{3}H](+)-AZPH), Novel Benzomorphan-Based Photoaffinity Label, Sigma Receptors, Optically Pure (+)-Normetazocine

INTRODUCTION

The sigma receptor has recently elicited much interest because of its association with the movement disorder dystonia [1], its possible involvement in psychotic behavior [2], as well as its association with the neuroprotective properties of certain drugs [3]. As part of our program to identify, isolate and further characterize this receptor, we wished to synthesize a high specific activity radiolabelled photoaffinity ligand for such purposes.

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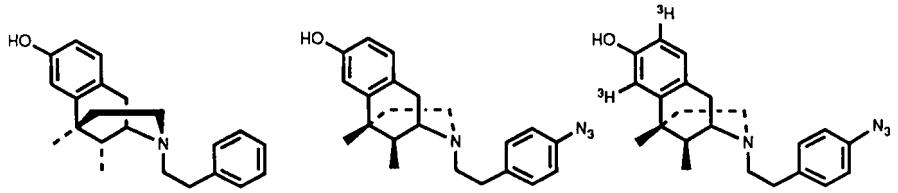
We and others have successfully utilized electrophilic affinity ligands in the identification and purification of receptors in the CNS [4] and immune system [5]. However, one disadvantage in the use of electrophilic ligands in certain cases is the high degree of non-specific labelling as well as the difficulty experienced in controlling the extent of labelling. These problems can frequently be circumvented by the use of radiolabelled photoaffinity ligands [6] since the intensity, duration and wavelength of the incident radiation can be finely tuned. Furthermore, non-specifically bound ligand can be washed out from crude receptor preparations prior to photoactivation, resulting in a higher specific labelling. Another advantage is that binding parameters can be determined since true equilibrium binding experiments can be performed in the absence of photoactivation; this is not necessarily the case with electrophilic ligands. Photoaffinity probes have been successfully employed in the isolation and purification of a variety of CNS receptor types that include cholinergic [7], opioid [8], and phencyclidine [9].

Kavanaugh et al. [10] have identified a sigma receptor polypeptide in guinea pig brain using the photoaffinity ligand [^{3}H]azido-DTG [N-(4-azido-2-tolyl)-N'-(2-tolyl)guanidine] [10]. However, recent evidence supports the notion that [^{3}H]azido-DTG and its parent compound [^{3}H]DTG label two distinct subclasses of sigma receptor [11]. Since these two classes have different affinities for (+)-benzomorphans, we surmised that a benzomorphan-based photoaffinity ligand would be a more selective probe for the site labelled preferentially by (+)-benzomorphans.



(-)-PHENAZOCINE

(+)-AZIDOPHENAZOCINE
((+)-AZPH, (+)-1)

[^{3}H](+)-AZIDOPHENAZOCINE
([^{3}H](+)-AZPH, [^{3}H](+)-1)

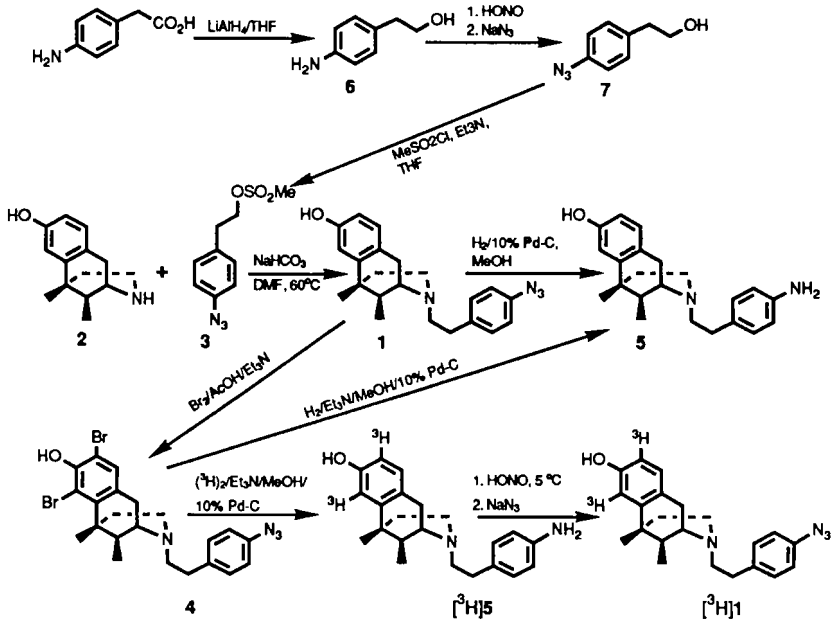
Thus, we synthesized (+)-azidophenazocine ((+)-AZPH, (+)-**1**). Compound (+)-**1** is structurally related to the benzomorphan analgesic phenazocine (NIH 7519) originally developed by May and Eddy [12]. Preliminary binding studies [13] with (+)-**1** indicated that it binds with high affinity and selectivity to sigma receptors, comparable to our previously reported benzomorphan sigma ligand, [^{3}H](+)-pentazocine [14]. Tritium labelled **1** seemed likely to be an excellent probe for photoaffinity labelling of this important receptor, and further investigation of the question of receptor heterogeneity. We wish to

report here the synthesis and characterization of high specific activity tritium labelled **1** ([^3H](+)-AZPH).

SYNTHESIS

The starting material for the synthesis of [^3H]**1** utilized the readily available optically pure (+)-normetazocine (**2**) [14] [15]. Condensation of this intermediate with 2-(*p*-azidophenyl)ethylmethanesulfonate (**3**) resulted in a high yield (77%) of (+)-AZPH (**1**). Intermediate **3** was obtained from *p*-aminophenylacetic acid by the sequence of LAH reduction (quantitative), diazotization followed by reaction with sodium azide (87%), and finally methanesulfonylation (99%). The tritiation intermediate **4** was synthesized in 96% yield by bromination of **3** with bromine in glacial acetic acid [14]; no tribromo biproducts were formed even with the use of excess bromine in acetic acid as we have observed previously with bromination of (+)-normetazocine [14]. In a trial hydrogenation experiment, treatment of **4** with hydrogen in the presence of 10% Pd-C and triethylamine resulted in a quantitative yield of aniline **5**. Similarly, catalytic hydrogenation of **1** also resulted in a quantitative yield of **5**. Thus, treatment of **4** with carrier free tritum gas [16] under the same conditions that were used for its hydrogenation, resulted in a 13.5% radiochemical yield of [^3H]**5**. No attempt was made to determine the specific activity of this intermediate at this stage.

Scheme 1



Treatment of **5** at 5 °C with nitrous acid during 3h followed by excess sodium azide resulted in a high yield of **1**. Identical treatment of

[^{3}H]**5** afforded [^{3}H]**1** in 78% yield. The specific activity of [^{3}H]**1** was found to be 22.9 Ci/mmol as determined by UV analysis of a solution in EtOH; this corresponds to a 39.5% incorporation of the tritium label. All manipulations of the azido compounds were carried out in the dark to avoid photodecomposition.

DISCUSSION

Bromination of **1** with excess bromine only resulted in the dibromo intermediate **4**; no bromination of the side-chain phenyl ring was observed as expected because of its deactivation by the electron-withdrawing azido group as we observed previously with arylazido compounds [17]. Catalytic hydrogenation of **1** or **4** in the presence of 10% Pd-C resulted in amine **5**. Thus, catalytic hydrogenation of **4** with carrier free tritium [16] resulted in [^{3}H]**5** (13.5% radiochemical yield; specific activity 22.9 Ci/mmol). This low radiochemical yield can be attributed to radiochemical decomposition of the product, incomplete tritiolysis of the bromine atoms of **4** and a less (39.5%) than theoretical percentage isotopic incorporation. The lowered isotopic incorporation is a result of Pd-catalysed dehydrogenation of the tritiation solvent (MeOH) as we have previously observed with catalytic tritiolyses of aromatic bromo-precursors [14] [18].

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 ionisation 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 KBr pellets (unless otherwise stated) using a Beckman 4230 IR spectrometer. Ultraviolet (UV) spectra were recorded from absolute ethanol (spectrophotometric grade) solutions using a Hewlett-Packard 8450A UV/VIS spectrophotometer. Analytical thin layer chromatography (TLC) was performed on 250 μ Analtech GHLF silica gel plates. TLC solvent system A refers to concentrated aqueous ammonia-methanol-chloroform (1:9:90), TLC system B refers to concentrated aqueous ammonia-methanol-chloroform (0.5:5:95) and TLC solvent system C refers to ethyl acetate-hexane (1:1). TLC plates were analysed for radioactivity with a Bertold model LB 2760 TLC scanner. Radioactivity determinations were carried out using a Packard model 2200 CA "Tri-Carb" liquid scintillation counter; tritium labeled compounds were

counted in Hydrofluor scintillation cocktail (National Diagnostics) with a counting efficiency of 45%. All synthetic and analytical operations were initially performed with unlabeled compounds and the structures were confirmed spectroscopically. All operations with azido compounds were performed in the dark or under subdued light.

2-(*p*-Aminophenyl)ethyl alcohol (6). To a stirred suspension of *p*-aminophenylacetic acid (25.6 g, 169 mmol) in dry THF (500 mL) was added portionwise at 0 °C, $LiAlH_4$ (12.85 g of 97% pure reagent) and the solution was boiled under reflux overnight under an argon atmosphere until TLC (solvent system A) indicated the reaction to be complete. The reaction mixture was cooled to 20 °C and treated dropwise (with ice-bath cooling) with water (12.8 mL), 5% aqueous NaOH (12.8 mL) water (38.4 mL) and stirred for 45 min at room temperature. The reaction mixture was filtered and the filter-cake was washed with a further amount of THF (50 mL) and then pressed dry. Evaporation of the solvent *in vacuo* afforded the crude **6** as an off-white solid in quantitative yield; pure **6** was obtained by crystallization from 2-propanol/isooctane (1:1): mp 103-105 °C (lit. [20] mp 107 °C); ^{1}H-NMR ($CDCl_3$) δ 7.02 (d, 2H, J=8.2 Hz), 6.65 (d, 2H, J=8.2 Hz), 3.79 (t, 2H , 6.6 Hz), 3.57 (br s, 2H, $\underline{NH_2}$), 2.76 (t, 2H, J=6.6 Hz), 1.60 (br. s, 1H, $\underline{OH}$); CIMS ($C_8H_{11}NO$) M + H requires: 138, M + M + NH_4^+ requires: 155, M + H found: 138, M + NH_4^+ found: 155; Anal. calcd. for $C_8H_{11}NO$: C 70.04, H 8.08, N 10.21; Anal. found: C 70.09, H 8.11, N 10.12.

2-(*p*-Azidophenyl)ethyl alcohol (7). To a stirred suspension of **6** (9.00 g, 65.7 mmol) in water (200 mL) was added concentrated aqueous HCl (10.9 mL, 131 mmol) after which a clear solution was formed. To this solution was added dropwise at 0 °C, a solution of $NaNO_2$ (4.99 g, 72.3 mmol) in water (20 mL) and stirring was continued at 0 °C for 2.5 h. To the solution was added a solution of NaN_3 (8.54 g, 131 mmol) in water (100 mL) and stirring was continued for a further 15 min at 0 °C. The reaction mixture was extracted with $CHCl_3$ (2 x 200 mL) and the combined $CHCl_3$ extract was extracted with $NaHCO_3$ (100 mL). Evaporation of the $CHCl_3$ afforded the crude product as a brown oil. High vacuum distillation (124 °C/1.5 mm Hg) of this crude product gave **7** (9.31 g, 87%). as a yellow oil (the synthesis of this compound has been previously reported [19] but no characterization is given): ^{1}H-NMR ($CDCl_3$) δ 7.22 (d, 2H, J=8.4 Hz), 6.98 (d, 2H, J=8.4 Hz), 3.84 (m, 2H), 2.85 (t, 2H, J=6.5 Hz); IR (film) 3350 (OH str.), 3030, 2940, 2870, 2100 (strong N_3 str.), 1605, 1580, 1505, 1280, 1040, 810 cm^{-1}; CIMS ($C_8H_9N_3O$) M + NH_4^+ requires: 181, M + H - N_2 requires: 136, M + NH_4^+ found: 181, M + H - N_2 found: 136; Anal. calcd. for $C_8H_9N_3O$: C 58.89, H 5.45, N 25.75; Anal. found: C 58.29, H 5.58, N 25.52.

2-(*p*-Azidophenyl)ethyl methanesulfonate ester (3). To a mixture of **7** (5.00 g, 30.7 mmol) and triethylamine (8.55 mL, 61.4 mmol) in ether (50 mL) was added dropwise with stirring at 0 °C, methanesulfonyl chloride (3.56 mL, 46.1 mmol). After the addition was complete, TLC (solvent system C) indicated that the reaction was complete. The reaction was diluted to 100 mL with ether and washed with saturated $NaHCO_3$ (50 mL), water (2 x 50 mL) and evaporated to an oil which was dried under high vacuum. The oil crystallized on standing under high vacuum to give **3** (7.3 g, 99%) which was of sufficient purity for use in the next step of the reaction sequence. Crystallization from 2-propanol afforded a pure sample: mp 46-47 °C; ^{1}H-NMR ($CDCl_3$) δ 7.23 (d, 2H, J=6.8 Hz), 6.99 (d, 2H, J=6.8 Hz), 4.39 (t, 2H, J=6.8 Hz), 3.04 (t, 2H, J=6.8 Hz), 2.89 (s, 3H, $OSO_2\underline{CH_3}$); CIMS ($C_9H_{11}N_3O_3S$) M + NH_4^+ requires: 259, M + NH_4^+ found: 259; Anal. calcd. for $C_9H_{11}N_3O_3S$: C 44.81, H 4.60, N 17.42; Anal. found: C 44.72, H 4.58, N 17.33.

(+)-cis-N-(2-(4-Azidophenyl)ethyl)-2'-hydroxy-2,6-dimethyl-6,7-benzomorphan (1). To a stirred solution of (+)-normetazocine (**2**) base (0.50 g, 2.30 mmol) in dry DMF (10 mL) was added $NaHCO_3$ (1.00 g, 11.9 mmol) and 2-(*p*-azidophenyl)ethyl methanesulfonate ester (**3**) (0.67 g, 2.76 mmol) and the solution was heated to 60 °C and stirred at this temperature for 16 h under an N_2 atmosphere when TLC (solvent system A) indicated the reaction to be complete. The reaction mixture was poured into 10% aqueous citric acid (100 mL) and the aqueous solution was extracted with ether (3 x 50 mL) and the combined ether washings were discarded. The aqueous layer was basified by addition of excess concentrated aqueous ammonia solution and then extracted with ether (3 x 50 mL) and the combined ether extract was back-washed with water (50 mL). Evaporation of the solvent and high vacuum drying afforded **1** as a colorless foam (0.64 g, 77%). Crystallization of the hydrogen oxalate salt from 25 mL of 2-propanol afforded **1**.oxalate: softens 100 °C, decomposes at 140 °C; ^{1}H-NMR ($CDCl_3$) δ 7.19 (d, 2H, J=8.3 Hz), 6.95 (d, 2H, J=8.3 Hz), 6.93 (d, 1H, J=8.2 Hz), 6.72 (d, 1H, J=2.6 Hz), 6.60 (dd, 1H, J=8.2 Hz, J=2.6 Hz), 2.59-3.00 (complex m, 16H), 2.11 (td, 1H, J=3.2 Hz, J=12.4 Hz),1.78-1.95 (complex m, 2H), 1.34 (s, 3H, 5-$\underline{CH_3}$), 0.85 (d, 3H, J=7.1 Hz, 8-CH_3); UV (EtOH): λ_{max} 232 (11,290), 251 (16,530), 281 (5,750) nM; Anal. calcd. for $C_{24}H_{28}N_4O_5.0.25H_2O$: C 63.07, H 6.29, N 12.26; Anal. found: C 63.11, H 6.31, N 12.32; CIMS ($C_{22}H_{26}N_4O$) M + H requires: 363, M + H - N_2 requires: 335, M + H found: 363, M + H - N_2 found: 335; $[\alpha]_D$= +94 ° (c 0.826, MeOH).

(+)-cis-N-(2-(4-Aminophenyl)ethyl)-2'-hydroxy-2,6-dimethyl-6,7-benzomorphan (5). To a solution of **1**.oxalate (0.40 g) in MeOH (20

mL) was added 10% Pd-C (0.04 g) and the solution was vigorously stirred for 3 h at 20 °C under an atmosphere of hydrogen gas. TLC (solvent system A) indicated that the reduction was complete after this time. The reaction mixture was filtered through a pad of celite and the celite was washed with a further amount of MeOH (20 mL). The combined filtrate and washings were evaporated and the residue (0.41 g, quantitative) was converted to its free-base by partitioning between excess diluted aqueous ammonia (10 mL) and $CHCl_3$ (10 mL) to give aniline **5** as an off-white foam. A pure sample was obtained by crystallization from 2-propanol: mp 183.5-184.5 °C; ^{1}H-NMR ($CDCl_3$) δ 6.99 (d, 2H, J=8.3 Hz), 6.92 (d, 1H, J=8.3 Hz), 6.71 (d, 1H, J=2.6 Hz), 6.63 (d, 2H, J=8.3 Hz), 6.59 (dd, 1H, J=8.3 Hz, J=2.6 Hz), 3.56 (br. s, 2H, $\underline{NH_2}$), 2.59-3.04 (complex m, 16H), 2.05 (td, 1H, J=3.2 Hz, J=12.4 Hz), 1.79-1.97 (complex m, 2H), 1.34 (s, 3H, 5-$\underline{CH_3}$), 0.85 (d, 3H, J=7.1 Hz, 8-$\underline{CH_3}$); CIMS ($C_{22}H_{28}N_2O$) M + H requires: 337, M + H found: 337; Anal. calcd. for $C_{22}H_{28}N_2O$: C 78.53, H 8.39, N 8.33; Anal. found: C 78.48, H 8.43, N 8.27; $[\alpha]_D$= +132 ° (c 0.545, MeOH).

(+)-cis-N-(2-(4-Azidophenyl)ethyl)-2'-hydroxy-1',3'-dibromo-2,6-dimethyl-6,7-benzomorphan (4). To a stirred solution of **1**.oxalate (0.50 g, 1.11 mmol) and triethylamine ((0.79 mL, 5.67 mmol) in acetic acid (5.0 mL) was added dropwise at 20 °C a solution of bromine (freshly redistilled) (0.13 mL, 2.52 mmol) in acetic acid (5 mL). After the addition was complete, TLC (solvent system A) indicated the reaction to be complete. The reaction was poured into a mixture of ice (50 g) and excess concentrated aqueous ammonia solution. When all of the ice had melted, the still-cold solution was filtered and the filter cake was washed thoroughly with cold water and pressed dry. The filter-cake was then dried overnight *in vacuo* at 60 °C to give **4** (base) (0.55 g, 96%). An analytically pure sample was obtained by recrystallization of the free base from boiling MeOH: mp 179-180 °C (dec); ^{1}H-NMR ($CDCl_3$) δ 7.23 (s, 1H), 7.19 (d, 2H, J=8.4 Hz), 6.95 (d, 2H, J=8.4 Hz), 2.55-3.00 (complex m, 16H), 1.80-2.05 (complex m, 3H), 1.71 (s, 3H, 5-$\underline{CH_3}$), 0.87 (d, 3H, J=7.1 Hz); CIMS ($C_{22}H_{24}{}^{79}Br^{81}BrN_4O$) M + H requires: 521, M + H-N_2 requires: 423, M + H found: 521, M + H-N_2 found: 423; Anal. calcd. for $C_{22}H_{24}Br_2N_4O$: C 50.79, H 4.65, N 10.77; Anal. found: C 50.70, H 4.68, N 10.71; $[\alpha]_D$=+110 ° (c 0.971, $CHCl_3$) .

Hydrogenation of 4 to (+)-cis-N-(2-(4-Aminophenyl)ethyl)-2'-hydroxy-2,6-dimethyl-6,7-benzomorphan (5). To a mixture of **4** (base) (20 mg) and 10% Pd-C (20 mg) in MeOH (2.0 mL) was added triethylamine (50 μL) followed by an atmosphere of H_2. The reaction mixture was stirred for 40 min. at 20 °C after which time TLC (solvent

system A) indicated the reaction to be complete. The reaction mixture was filtered through a pad of celite and the filtrate was evaporated *in vacuo* to give a quantitative yield of product. The residue was partitioned between ether (5 mL) and dilute aqueous ammonia solution (5 mL) and the ether layer was evaporated *in vacuo* to give **5** (base) identical by TLC and NMR to an authentic sample of **5** as prepared above.

[^{3}H](+)-cis-N-(2-(4-Aminophenyl)ethyl)-2'-hydroxy-2,6-dimethyl-6,7-benzomorphan ([^{3}H]5). To a stirred solution of **4** (base) (20 mg, 0.038 mmol) in MeOH (2.0 mL) was added 10% Pd-C (20 mg) followed by triethylamine (50 μL) and the solution was stirred for 2 h at 20 °C under an atmosphere of carrier-free tritium gas (30 Ci, 0.52 mmol). The catalyst was filtered and the labiles were removed from the filtrate by evaporation of the MeOH solvent under a stream of N_2 gas. The residue was reconstituted to a volume of 25 mL with MeOH containing 1 mL of glacial acetic acid and the crude product was stored at -80 °C until further use: The solvent was evaporated under a stream of N_2 gas and the residue was redissolved in MeOH (5 mL) and transferred to another vial. Two drops of concentrated aqueous ammonia was added and the solvent was evaporated under a stream of N_2. The residue was dissolved in MeOH (1.0 mL) and applied to one 20 cm x 20 cm x 0.5 mm preparative TLC plate and plate was also spotted with an authentic sample of unlabelled **5**. The plate was eluted with solvent system A and the band comigrating with unlabelled **5** was scraped off and extracted for 30 min with solvent system A (20 mL). The extract was filtered through a plug of glass wool and the filtrate was evaporated under a stream of N_2 gas to give pure [^{3}H]**5**. The residue was dissolved in acetic acid-MeOH (1:24) for storage prior to use: yield=301.8 mCi (13.5%); specific activity=22.9 Ci/mmol; [^{3}H]**5** was identical by UV (MeOH) and TLC (solvent system A) to an authentic sample of unlabelled **5** as prepared above.

[^{3}H](+)-cis-N-(2-(4-Azidophenyl)ethyl)-2'-hydroxy-2,6-dimethyl-6,7-benzomorphan ([^{3}H]1) ([^{3}H](+)-AZPH). The solution of [^{3}H]**5** (301.8 mCi, 0.013 mmol) from above was evaporated under a stream of N_2 gas and the residue was dissolved in 2.0 mL of MeOH containing 3-drops of concentrated aqueous HCl. The solution was evaporated to dryness under a stream of N_2 to afford [^{3}H]**5**. HCl (301.8 mCi, 0.0132 mmol). This salt was dissolved in 0.9 mL of 1.0 M aqueous HCl and the solution was cooled to 5 °C with stirring. To the stirred and cooled solution was added $NaNO_2$ (9.0 mg, 0.13 mmol). and the reaction mixture was stirred for 3h at this temperature. After this time NaN_3 (40 mg, 0.61 mmol) was added in one portion and the solution was stirred for 5 min. in

the dark. The reaction was quenched by addition of 6 drops (excess) of concentrated aqueous ammonia solution and the pH was checked to make sure that it was basic (pH 9.5). The basified solution was extracted with ether (2 x 2 mL) and the combined ether extract was evaporated under a gentle stream of N_2. The residue was applied to one 20 cm x 20 cm x 0.5 mm preparative TLC plate and the plate was also spotted with an authentic sample of unlabelled **1** as prepared above. The plate was eluted with solvent system B and the band comigrating with unlabelled **1** was scraped off and extracted for 45 min. in the dark with solvent system A. The extract was filtered through glass wool and evaporated under a stream of N_2. The residue was dissolved in $CHCl_3$ (10 mL) and the solution was transferred to a new vial (this step removes silica gel from the product) and the solvent was evaporated under a stream of N_2 in the dark. The residue was made up to 100 mL with 100% EtOH for storage and UV analysis; addition of 5 μL of concentrated aqueous HCl to this stock solution served to further stabilize the [^{3}H]**1** by protonation of its amino group: yield=235 mCi (78%): UV (EtOH): λ_{max} 231 (A=1.143), 251 (A=1.692), 280 (A=0.5556) nM; specific activity=22.9 Ci/mmol (by UV analysis of the solution and calculation of its concentration by measurement of the absorbance at 251 nM and use of ε_{251}=16530 liter $mol^{-1}cm^{-1}$). The solution was adjusted to a final volume of 235 mL (1 mCi/mL) and stored at -80 °C in the dark for increased shelf-life of the [^{3}H]**1**.

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