Brief Articles

Design and Synthesis of a Novel Photoaffinity Ligand for the Dopamine and Serotonin Transporters Based on 2β-Carbomethoxy-3β-biphenyltropane

Amy Hauck Newman,*[,†] Joo Hwan Cha,[†] Jianjing Cao,[†] Theresa Kopajtic,[‡] Jonathan L. Katz,[‡] M. Laura Parnas,[§] Roxanne Vaughan,[§] and John R. Lever[||]

Medicinal Chemistry and Psychobiology Sections, National Institute on Drug Abuse—Intramural Research Program, National Institutes of Health, Department of Health and Human Services, Baltimore, Maryland, University of North Dakota School of Medicine, Grand Forks, North Dakota, University of Missouri—Columbia, Departments of Radiology and Medical Pharmacology and Physiology, and Harry S. Truman Veterans Administration Medical Center, Columbia, Missouri

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Tropane-based photoaffinity ligands covalently bind to discrete points of attachment on the dopamine transporter (DAT). To further explore structure−activity relations, a ligand in which the photoactivated group was extended from the 3-position of the tropane ring was synthesized from cocaine via a Stille or Suzuki coupling strategy. 3-(4′-Azido-3′-iodo-biphenyl-4-yl)-8-methyl-8-aza-bicyclo[3.2.1]octane-2-carboxylic acid methyl ester (**11**; $K_i = 15.1 \pm 2.2$ nM) demonstrated high binding affinity for the DAT. Moreover, this compound showed moderate binding affinity for the serotonin transporter (SERT, $K_i = 109 \pm 14$ nM), suggesting the potential utility of [^{125}I]**11** in both DAT and SERT protein structure studies.

Introduction

Site-directed mutagenesis and structure−activity relationship (SAR) studies suggest that structurally divergent inhibitors of the dopamine transporter (DAT) bind to different sites or binding domains on the DAT.[1−3] The development of molecular probes that include irreversible (N_3, NCS) and radiolabeled (^{3}H, ^{125}I) ligands have provided important tools with which binding sites for both DAT substrates and inhibitors can be identified at a molecular level. The first tropane-based photoaffinity label for the DAT, [^{125}I]**1**, covalently labels transmembrane (TM) region 4−7, in contrast to [^{125}I]**2**, a photolabel based on another tropane-based DAT inhibitor, 3α-diphenylmethoxytropane; benztropine, which binds to TM 1-2 (Figure 1).[4−6] Based on divergent SAR between these classes of DAT inhibitors, we have reasoned that their pharmacophores might be binding to distinct domains on the DAT that could affect their behavioral profiles in animal models of cocaine abuse.[2]

SAR studies demonstrated that the N-position of 2β-carbomethoxy-3β-phenyl tropane could potentially be modified to incorporate a 3′-I, 4′-N_3-phenyl substitution without adversely affecting DAT binding affinity. To this end, [^{125}I]**3** was prepared[7,8] and discovered to covalently attach to TM 1-2, in contrast to [^{125}I]**1**, but similar to the benztropine-based [^{125}I]**2** (Figure 2).[9] These studies suggested that depending on the position of azide substitution on the tropane ring system, covalent attachment can occur in different TM regions and that not all tropane-based DAT inhibitors bind to the same recognition site on the DAT. Based on these early studies, we hypothesized that appending azido groups at various positions on the two tropane-based DAT inhibitors, cocaine and benztropine, would provide the opportunity to identify points of attachment on the DAT, elucidate three-dimensional (3D) arrangement of the TM domains, and direct future drug design.

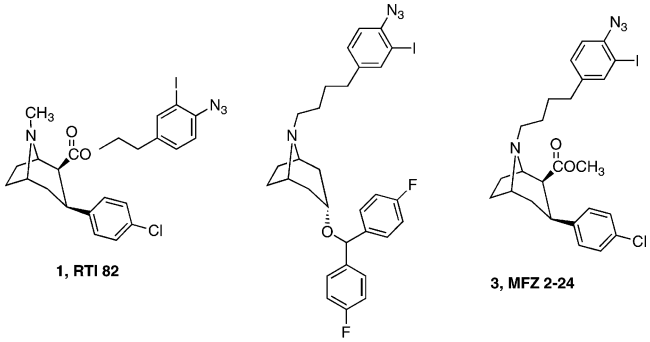

Figure 1. Chemical structures of tropane-based photoaffinity labels for the DAT.

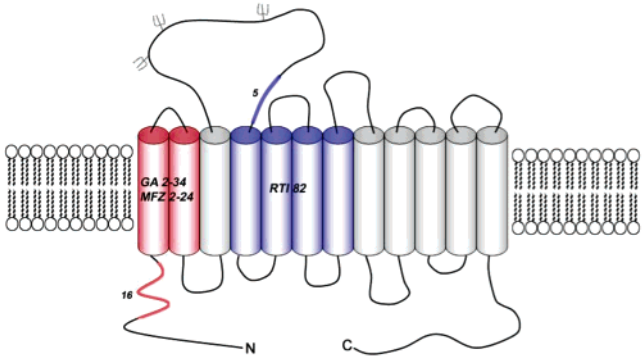

Figure 2. Schematic diagram of DAT showing TM domains (cylinders) and antibody epitopes (bold, numbered lines). Sites of attachment of indicated photoaffinity labels are shown for TMs 1-2 (red) or TMs 4−7 (blue).

* Corresponding author. Phone: (410)-550-6568, ext. 114. Fax: (410)-550-6855. E-mail: anewman@intra.nida.nih.gov.
† Medicinal Chemistry, National Institutes of Health.
‡ Psychobiology Sections, National Institutes of Health.
§ University of North Dakota School of Medicine.
|| University of Missouri—Columbia.

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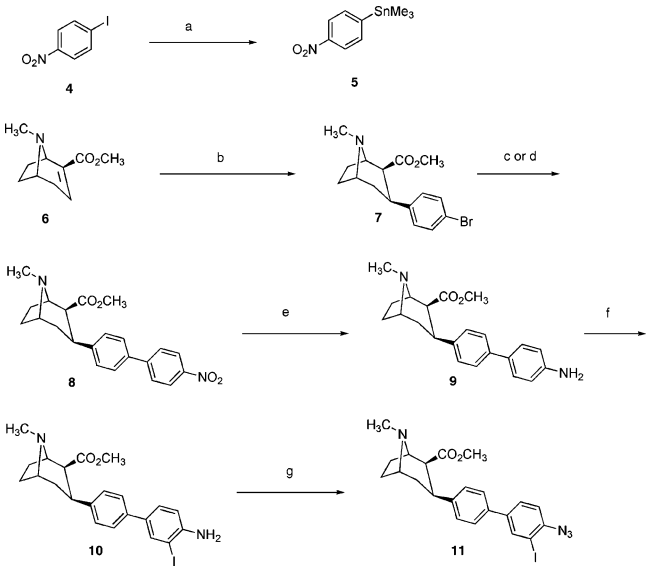
Scheme 1[a]



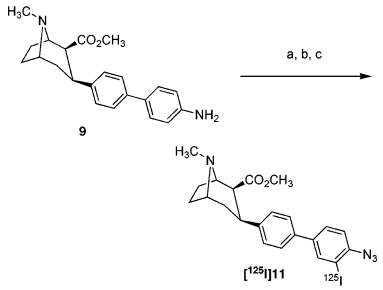
[a] Reagents and condition: (a) $(Sn(CH_3)_3)_2$, $Pd(PPh_3)_4$, toluene, reflux; (b) (i) 1-MgBr, 4-Br-benzene, anhyd. ether, −40 °C;[15] (ii) TFA, −78 °C; (c) **5**, $Pd(PPh_3)_4$, toluene, reflux; (d) 4-nitrophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 80 °C; (e) H_2, 10% Pd/C, MeOH; (f) ICl, HOAc, rt; (g) (i) $NaNO_2$, HOAc, 0 °C; (ii) NaN_3, 0 °C.

Although the SAR of the 3α-diphenylmethoxy tropane-based DAT inhibitors suggested that neither of the phenyl rings in the 3-position could withstand a 3′-I, 4′-N_3 substitution without drastically reducing DAT affinity,[10,11] and this successful modification had been reported for the 3-phenyl tropanes.[12] The reported 3-(4′-azido-3′-iodo-phenyl)-8-methyl-8-aza-bicyclo-[3.2.1]octane-2-carboxylic acid methyl ester showed high affinity and wash-resistant binding to the DAT[12] but failed to label the DAT in subsequent immunoprecipitation and proteolysis attempts (Vaughan et al., unpublished observation). Thus, in an effort to discover a tropane-based photoaffinity ligand for the DAT wherein the photoactivated azide was placed in the 3*β*-position of the tropane ring, we designed and synthesized a 3*β*-biphenyl analogue. This synthesis was based on reports that the 3*β*-biphenyl analogues demonstrated high-affinity binding for the DAT[13,14] and our hypothesis that the azido group must be some distance away from the pharmacophore in order to covalently attach to the DAT protein near, but not within, the ligand binding domain.

Chemistry

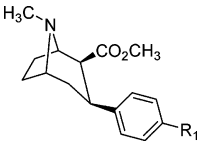
The synthetic strategy toward the desired product **11** initially employed a Stille coupling reaction using the prepared trimethyltin reagent **5** and following a modification of a procedure recently described (Scheme 1).[15] Although the nitrophenyl intermediate **8** had been previously reported,[14] we found the following modified procedure to be more efficient. Hence, intermediate **7**[15] was prepared from ecognine methyl ester and reacted with the trimethyltin reagent **5** to give the desired 4′-nitro-1,4-biphenyl intermediate (**8**) in 78% yield. Subsequently, an alternative and less-toxic Suzuki strategy was discovered using 4-nitrophenylboronic acid to give **8** in comparable yield. Catalytic hydrogenation gave the aniline **9** in moderate yield (63%) and was followed by iodination and azidization, following procedures previously reported.[7] The azide **11** was isolated as an oil and purified by column chromatography. A chemical purity of ∼95% was established by reversed-phase HPLC (254 nm) using several different mobile phase compositions and two dissimilar stationary phases for confirmation, namely, octadecylsilane- and cyanopropylsilane-covered silica. Attempts to prepare salts of the final product caused decomposition, and hence, the free base was used immediately and in the dark for binding characterization.

Scheme 2[a]



[a] Reagents and conditions: (a) [125I]-NaI, chloramine-T, NaOAc (0.25 M, pH 4.0)/MeOH, rt, 30 min; (b) HOAc (3.0 M), $NaNO_2$, (0.5 M), −10 °C; (c) NaN_3 (0.5 M), rt, 20 min, $Na_2S_2O_5$ (50 mM).

Table 1. DAT and SERT Binding Results



compd	R_1	DAT $K_i \pm$ SEM[a] (nM)	SERT $K_i \pm$ SEM[a] (nM)
7[b]	Br	0.42 ± 0.05	4.1 ± 0.28
8[c]	4′-NO_2−Ph	5.03 ± 0.72	51 ± 2.4
9	4′-NH_2−Ph	12.9 ± 0.49	1070 ± 110
10	3′-I,4′-NH_2−Ph	8.82 ± 0.73	134 ± 15
11	3′-I,4′-N_3−Ph	15.1 ± 2.2	109 ± 14
2		31.0 ± 4.5[d]	ND[e]

[a] Each K_i value represents data from at least three independent experiments, each performed in triplicate. K_i values were analyzed by GraphPad Prism. A detailed description of the binding assay methods has been previously published.[16,17] [b] Reference 15. [c] Reference 14. [d] K_i obtained from reported IC_{50}.[7] [e] ND = not determined in this assay.

In Scheme 2, [125I]**11** was prepared using a modification of procedures previously described.[8] This material displayed 99% radiochemical purity by HPLC and coeluted with the nonradioactive standard (**11**). HPLC data is included in the Supporting Information. The specific radioactivity of [125I]**11** was estimated as 2000 mCi/*μ*mol using HPLC to determine the mass associated with the UV absorbance peak area in a sample of known radioactivity. Samples of [125I]**11** were stable, and displayed 93% radiochemical purity after 12 weeks at −20 °C in the dark.

Compound **11** and its precursors (**7**−**10**) were evaluated for displacement of [3H]WIN 35 428 in rat striatal membranes or [3H]citalopram in rat brain stem or midbrain for binding affinities to DAT and SERT, respectively, using previously described methods.[16,17]

Results and Discussion

The precursor **7** demonstrated high affinity for both the DAT and SERT (Table 1). Replacing the 4′-Br group with the 4′-nitro-phenyl reduced binding affinities at both the DAT and SERT by ∼10-fold. Reduction to the aniline had little effect on DAT binding, but significantly decreased binding affinity for the SERT to ∼1 *μ*M. Addition of the 3′-I group again had little effect on DAT binding, but somewhat improved SERT binding. Likewise, replacing the aniline NH_2 with the N_3 group did not significantly change binding affinities at either the DAT or the SERT. Compared to previously reported **2**, compound **11** showed comparable affinity at the DAT. Hence, compound

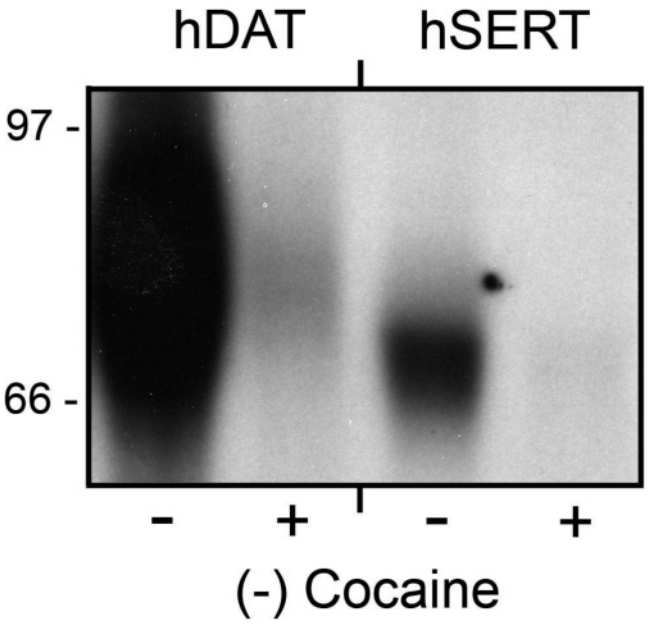


Figure 3. Photoaffinity labeling of hDAT and hSERT with [^{125}I]**11**. HEK 293 cells expressing hDAT or hSERT were photoaffinity labeled with [^{125}I]**11** in the presence or absence of 10 μM (−)cocaine. Cells were solubilized, and lysates were subjected to immunoprecipitation with antisera specific for DAT or SERT, followed by SDS-PAGE and autoradiography.[6,19,20]

11 (K_i = 15.1 nM) represents a new and high-affinity DAT photolabel in this series of tropane-based ligands and also demonstrates reasonably high affinity at the SERT (K_i = 109 nM).

As these compounds are highly lipophilic, wash-resistant binding experiments on the cold azido-compound frequently give false positives in the assessment of covalent attachment, in that the nonphotoactivated azide will often demonstrate wash-resistant binding.[4] Hence, we elected to synthesize [^{125}I]**11** directly and use the radiolabel to determine if covalent attachment did occur upon photoactivation. Under the labeled protein solubilizing conditions and subsequent gel electrophoresis, if covalent attachment of [^{125}I]**11** did not occur, the appropriate radiolabeled bands would not be present in the autoradiograph of the western blot. Solubilization of the protein from the membrane would free any reversibly bound ligand and it would be washed out as previously described.[18] Hence, [^{125}I]**11** was prepared (Scheme 2). HEK 293 cells expressing hDAT or hSERT were photoaffinity labeled with [^{125}I]**11** in the presence or absence of 10 μM (−)cocaine. Cells were solubilized, and lysates were subjected to immunoprecipitation with antisera specific for the DAT or SERT, followed by SDS-PAGE and autoradiography according to previously described procedures.[19,20] Radioactively labeled proteins of 80 or 66 kDa were obtained, demonstrating the incorporation of [^{125}I]**11** into DAT and SERT (Figure 3). Furthermore, in the presence of cocaine, the photolabeling is blocked, in further support of [^{125}I]**11** labeling a binding domain that overlaps with cocaine at both DAT and SERT.

Taken together, these studies demonstrate that [^{125}I]**11** binds covalently upon photoactivation and thus represents the first high-affinity photolabel for both the DAT and SERT, in which the azido group is placed in the 3β-position of the tropane ring. Moreover, the previously reported 3β-phenyl analogue demonstrated wash-resistant binding but failed to label the DAT using immunoprecipitation and proteolysis techniques. These results support our hypothesis that the photoactivated azido group may need to be extended away from the pharmacophore, as with photolabels **1**, **2**, **3**, and now **11**, to covalently attach to the protein and label the cocaine binding site on the DAT or SERT. As the crystal structure of the bacterial homologue LeuT can provide a template for the 3D structure of the DAT,[21] identification of amino acid residues that are involved in DAT inhibitor binding as well as the spatial arrangement of the DAT TMs should be ascertainable with these agents in the future. Thus, [^{125}I]**11** provides an important addition to our growing arsenal of irreversible ligands with which to characterize the 3D structure and function of these proteins.

Experimental Methods

All chemicals and reagents were purchased from Aldrich Chemical Co. and used without further purification. All column chromatography was performed using the silica gel (Merck, 230−400 mesh, 60Å) and eluting solvent mixtures as specified. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Plus 400 instrument. Proton chemical shifts are reported as parts per million (δ ppm) relative to tetramethylsilane (0.00 ppm) as an internal standard. Coupling constants are measured in Hz. Chemical shifts for ^{13}C NMR spectra are reported as δ relative to deuterated $CHCl_3$ ($CDCl_3$, 77.5 ppm, CD_3OD 49.3). Infrared spectra were recorded as a neat film on NaCl plates with a Perkin-Elmer Spectrum RX I FT-IR system. The HPLC system has been described previously.[8] A radioisotope dose calibrator (Capintec CRC-15W) was used for radioactivity measurements, and similar counting geometries were employed for each determination.

3β-(4-Bromo-phenyl)-8-methyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic Acid Methyl Ester (7). Following the procedure of Plisson et al.,[15] a suspended solution of magnesium chips (101 mg, 4.2 mmol) and a catalytic amount (~1 mg) of crystal iodine in anhydrous diethyl ether (0.6 mL) was added dropwise to a solution of 1,4-dibromobenzene (1.0 g, 4.2 mmol) in diethyl ether (3.5 mL), maintaining a gentle reflux. After completion of the addition, the reaction mixture was stirred at reflux for 1 h and then cooled to −40 °C. A solution of ecognine methylester (0.25 mg, 1.37 mmol) in diethyl ether/CH_2Cl_2 (5 mL/5 mL) was added dropwise. The reaction mixture was stirred at −40 °C for 3 h, then cooled to −70 °C and quenched with a solution of trifluoroacetic acid (TFA, 0.45 mL, 5.8 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was allowed to warm to room temperature and poured into a mixture of H_2O/ethyl acetate (v/v, 10 mL/10 mL). The aqueous layer was basified with NH_4OH (30%, w/w) and extracted with ethyl acetate (10 mL × 3). The collected organic solution was dried over $MgSO_4$, filtered, evaporated, and purified by column chromatography, eluting with diethyl ether−TEA (95:5) to afford 145 mg (31%) of product as a solid. ^{1}H NMR ($CDCl_3$): δ 7.28 (d, 2H, J = 8.4 Hz), 7.04 (m, 2H), 3.49−3.46 (m, 1H), 3.42 (s, 3H), 3.28−3.26 (m, 1H), 2.88−2.78 (m, 2H), 2.47 (t, 1H, J = 12.4 Hz), 2.15−2.00 (m, 5H), 1.66−1.49 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 172.1, 142.3, 131.1, 129.3, 127.5, 65.4, 62.3, 52.8, 51.4, 42.1, 34.1, 33.6, 26.1, 25.3.

8-Methyl-3β-(4′-nitro-biphenyl-4-yl)-8-aza-bicyclo[3.2.1]octane-2β-carboxylic Acid Methyl Ester (8). Trimethyl-(4-nitrophenyl)-stannane (**5**, 329 mg, 1.15 mmol) was prepared from **4** via a Stille coupling strategy[15] and added to a stirred solution of compound **7** (300 mg, 0.88 mmol) in toluene (15 mL) and tetrakis(triphenylphosphine) palladium(0) ($Pd(Ph_3)_4$, 64 mg, 0.05 mmol). The reaction mixture was allowed to stir at reflux for 43 h under argon. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography, eluting with a gradient of hexanes−ethyl acetate−TEA (4/1 to 2/1) to give 260 mg (78%) of product as a foam. An alternative and less-toxic strategy was discovered using 4-nitrophenylboronic acid (593 mg, 3.55 mmol), which was added to a stirred solution of **7** (1.00 g, 2.96 mmol) in DEM (9 mL) and H_2O (3 mL), followed by addition of Na_2CO_3 (627 mg, 5.92 mmol) and $Pd(Ph_3)_4$ (102 mg, 0.08 mmol). The reaction mixture was allowed to stir at 80 °C for 3 h under argon. The solvent was removed under reduced pressure, and the

resulting residue was purified by column chromatography, eluting with a gradient of ether−TEA (97:3 to 95:5) to give 800 mg (71%) of product as a foam that was identical by TLC to the previously prepared product. ^{1}H NMR ($CDCl_3$): δ 8.25 (d, 2H, J = 9.2 Hz), 7.70 (d, 2H, J = 8.8 Hz), 7.53 (d, 2H, J = 8.8 Hz), 7.38 (d, 2H, J = 8.0 Hz), 3.61−3.59 (m, 1H), 3.52 (s, 3H), 3.40−3.38 (m, 1H), 3.09−3.03 (m, 1H), 2.98−2.97 (m, 1H), 2.63 (t, 1H, J = 12.8 Hz), 2.28−2.07 (m, 5H), 1.79−1.61 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 172.3, 147.7, 147.0, 144.6, 136.2, 128.3, 127.6, 127.1, 124.2, 65.5, 62.4, 52.8, 51.4, 42.2, 34.1, 33.8, 26.1, 25.4. Anal. ($C_{22}H_{24}N_2O_4$) for C, H, N.

3β-(4′-Amino-biphenyl-4-yl)-8-methyl-8-aza-bicyclo[3.2.1]-octane-2β-carboxylic Acid Methyl Ester (9). Compound **8** (115 mg, 0.30 mmol) was dissolved in 10 mL of MeOH to which a catalytic amount of Pd/C (10%, 5 mg) was added. The mixture was reduced on a Parr hydrogenator at 40 psi for 18 h and then filtered over celite. The filtrate was concentrated and dried *in vacuo* to afford 67 mg (63%) of product as a foam. ^{1}H NMR ($CDCl_3$): δ 7.43 (d, 2H, J = 8.0 Hz), 7.37 (d, 2H, J = 8.4 Hz), 7.27 (d, 2H, J = 8.4 Hz), 6.70 (d, 2H, J = 8.4 Hz), 3.68 (br s, 2H), 3.56−3.54 (m, 1H), 3.49 (s, 3H), 3.37−3.36 (m, 1H), 3.04−2.98 (m, 1H), 2.62 (t, 1H, J = 12.8 Hz), 2.22−2.05 (m, 5H), 1.76−1.57 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 172.5, 145.8, 141.2, 138.8, 131.6, 128.0, 127.8, 126.1, 115.5, 65.6, 62.5, 53.0, 51.4, 42.2, 34.3, 33.7, 26.2, 25.4. Anal. ($C_{22}H_{26}N_2O_2$ 0.5 H_2O) for C, H, N.

3β-(4′-Amino-3′-iodo-biphenyl-4-yl)-8-methyl-8-aza-bicyclo-[3.2.1]octane-2β-carboxylic Acid Methyl Ester (10). Iodinemonochloride (82 mg, 0.50 mmol) in HOAc (1 mL) was cautiously added to a stirred solution of amine (**9**, 160 mg, 0.45 mmol) in HOAc (7 mL) at room temperature over 3 h. After evaporation of HOAc, the resulting residue was poured into a mixture of $CHCl_3$ (10 mL) and H_2O (10 mL). The aqueous layer was adjusted to pH 9 using a saturated solution of $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$ (10 mL × 3), and the combined organic fraction was dried over anhydrous $MgSO_4$, filtered, evaporated, and purified by column chromatography, eluting with $CHCl_3$/MeOH/NH_4OH (98:1:1) to afford 132 mg (61%) of the product as an oil. ^{1}H NMR ($CDCl_3$): δ 7.85 (d, 1H, J = 2.0 Hz), 7.39−7.33 (m, 3H), 7.28−7.24 (m, 3H), 6.75 (d, 2H, J = 8.8 Hz), 4.11 (br s, 2H), 3.57−3.55 (m, 1H), 3.50 (s, 3H), 3.37−3.36 (m, 1H), 3.04−2.98 (m, 1H), 2.61 (t, 1H, J = 12.4 Hz), 2.24−2.06 (m, 5H), 1.76−1.58 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 172.4, 146.0, 141.8, 137.3, 137.2, 133.3, 128.1, 127.9, 126.1, 115.0, 84.8, 65.5, 62.5, 53.0, 51.4, 42.2, 34.2, 33.7, 26.1, 25.4. Anal. ($C_{22}H_{25}IN_2O_2\cdot0.5H_2O\cdot0.25CHCl_3$) for C, H, N.

3β-(4′-Azido-3′-iodo-biphenyl-4-yl)-8-methyl-8-aza-bicyclo-[3.2.1]octane-2β-carboxylic Acid Methyl Ester (11; JHC 2−48). $NaNO_2$ (24 mg, 0.35 mmol) was added to a stirred solution of amine (**10**, 120 mg, 0.25 mmol) in HOAc (1.5 mL) and H_2O (1.5 mL) at 0 °C. After stirring for 30 min, NaN_3 (24 mg, 0.37 mmol) was added to the reaction mixture, and the solution was stirred at 0 °C for an additional 30 min. The solution was poured into a mixture of $CHCl_3$ (5 mL) and H_2O (5 mL). The aqueous layer was adjusted to pH 9 using a saturated solution of $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$ (5 mL × 3), and the combined organic fraction was dried over anhydrous $MgSO_4$, filtered, evaporated, and purified by column chromatography, eluting with $CHCl_3$/MeOH/NH_4OH (98:1:1) to afford 68 mg (54%) of product as an oil. ^{1}H NMR ($CDCl_3$): δ 7.99 (d, 1H, J = 2.4 Hz), 7.58 (dd, 1H, J = 2.0 Hz and 2.0 Hz), 7.43 (d, 1H, J = 8.0 Hz), 7.32 (d, 1H, J = 8.4 Hz), 7.16 (d, 2H, J = 8.0 Hz), 3.59−3.57 (m, 1H), 3.51 (s, 3H), 3.06−3.01 (m, 1H), 2.95−2.93 (m, 1H), 2.62 (t, 1H, J = 12.4 Hz), 2.24−2.04 (m, 5H), 1.78−1.59 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 172.0, 143.0, 140.2, 139.4, 138.2, 136.0, 128.0, 127.9, 126.3, 118.5, 88.1, 65.3, 62.2, 52.7, 51.2, 41.9, 34.0, 33.5, 25.9, 25.2. IR: 2107 (br, s), 1746 (s) cm^{-1}. Reversed-phase HPLC with detection at 254 nm was used to further ascertain purity. Mobile phases consisted of an organic component (MeOH/CH_3CN, 50:50) and an aqueous solution of Et_3N (2.1% v/v) and HOAc (2.8% v/v). Using a Waters C-18 Nova-Pak column (8 × 100 mm, 6 μm) at 4 mL/min with the organic component at 42%, **11** showed t_R = 18.7 min. Adjusting the mobile phase composition to 48% organic provided t_R = 9.9 min, with no evidence of more lipophilic contaminants. Using a Phenomonex IB-SIL Cyano column (4.6 × 150 mm, 5 μm) at 2 mL/min with the organic component at 20%, **11** had t_R = 10.7 min. The purity of **11** was 94−96% under all conditions. The "cut and weigh" method was used for quantification, with identical UV response factors assumed for each compound observed.

[^{125}I]3β-(4′-Azido-3′-iodo-biphenyl-4-yl)-8-methyl-8-aza-bicyclo-[3.2.1]octane-2β-carboxylic Acid Methyl Ester (11; [^{125}I-JHC2−48). Treatment of a solution of the aniline **9** (50 μL, 3.0 mM) in aqueous NaOAc buffer (pH 4.0; 0.25 M) containing MeOH (33%, v/v) at ambient temperature with no-carrier-added [^{125}I]-NaI (20 μL, 2.05 mCi; ca. 1.0 nmol; Amersham Corp.) was followed by *N*-chloro-4-toluenesulfonamide (chloramine-T) trihydrate (15 μL, 3.5 mM). After 15 min, the mixture was chilled in an ice/MeOH bath and then treated sequentially with ice-cold HOAc (50 μL, 3.0 M) and $NaNO_2$ (25 μL, 0.5 M). After a 15-min incubation period, sodium azide (25 μL, 0.5 M) was added, the ice bath was removed, and the mixture was allowed to stand at ambient temperature for 30 min. The reaction was quenched with $Na_2S_2O_5$ (5 μL, 50 mM) and taken up in a syringe along with rinses (2 × 100 μL) of the vessel with the HPLC mobile phase: MeOH (21%), CH_3CN (21%), and an aqueous solution (58%) of Et_3N (2.1% v/v) and HOAc (2.8% v/v). The HPLC system was equipped with a UV absorbance detector (254 nm), a flow-through radioactivity detector, and a Waters C-18 Nova-Pak column (radial compression module, 8 × 100 mm, 6 μm). Using a flow rate of 4 mL/min, radioactive material (t_R = 21.0 min, k'= 25) corresponding to **11** was resolved from both nonradioactive and radioactive side products. The [^{125}I]**11** was collected (12.5 mL), diluted with distilled water (17.5 mL), and passed through an activated (MeOH/water) solid-phase extraction cartridge (Waters Sep-Pak Light *t*-C-18) that was flushed with water (2.5 mL), to remove salts, and then with air. Elution with MeOH (1.1 mL) gave [^{125}I]**11** (1.26 mCi) in 61% radiochemical yield. This material displayed 99% radiochemical purity by HPLC and coeluted with nonradioactive standards of **11**. The specific radioactivity of [^{125}I]**11** was estimated as 2000 mCi/μmol, using HPLC to determine the mass associated with the UV absorbance peak area in a sample of known radioactivity. Formulations were supplemented (1% v/v) with Tris HCl buffer (5 mM, pH 7.4) prior to storage at −20 °C in the dark.

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Supporting Information Available: HPLC analyses of compounds **11** and [^{125}I]**11** are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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