N-Modified Analogues of Cocaine: Synthesis and Inhibition of Binding to the Cocaine Receptor

Philip Abraham, J. Bruce Pitner, Anita H. Lewin, John W. Boja,† Michael J. Kuhar,† and F. Ivy Carroll*

Chemistry and Life Sciences, Research Triangle Institute, Post Office Box 12194, Research Triangle Park, North Carolina 27709, and Neurosciences Branch, Addiction Research Center, National Institute on Drug Abuse, Baltimore, Maryland 21224. *Received June 13, 1991*

Cocaine methiodide (**2**), *N*-norcocaine (**1b**), *N*-benzyl-*N*-norcocaine (**1c**), and *N*-nor-*N*-acetylcocaine (**1d**) were synthesized and evaluated for their ability to inhibit binding of [^{3}H]-3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester (WIN 35,428) to the cocaine receptor. The study showed that removal of the *N*-methyl group to give **1b**, or replacement with the larger *N*-benzyl group to give **1c**, has a relatively small effect on binding potency. In contrast, replacement of the *N*-methyl group by the acetyl moiety to give **1d**, or the addition of a methyl group to give **2**, reduces affinity for the receptor by a large factor. In order to gain preliminary information concerning the importance of the nitrogen location on the tropane ring system, the receptor binding affinity of 8-methyl-8-azabicyclo[3.2.1]octan-3β-ol benzoate (**5**, β-tropacocaine) was compared to that of the isomeric 6-methyl-6-azabicyclo[3.2.1]octan-3β-ol benzoate (**4d**). The fact that both compounds have similar binding affinities for the cocaine receptor suggests that 3β-(benzoyloxy)-6-methyl-6-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester, which is isomeric with cocaine, may possess binding potency similar to cocaine.

Even though cocaine (**1a**) has a long history of use and abuse, little is known about its biochemical mechanism(s) of action. Present evidence suggests that the reinforcing properties of cocaine (**1a**) are related to its ability to inhibit dopamine reuptake.[1,2] Recently, binding site(s) which correspond to the cocaine (**1a**) receptor on the dopamine transporter have been identified with several radioligands.[1-11] An understanding of the structural features needed for binding to this site will provide valuable information for the design of cocaine (**1a**) analogues with potential therapeutic use.

In this report, we address the significance (or role) of the *N*-methyl group at the 8-position of the tropane ring. In particular, the synthesis and binding properties of analogues in which (a) the *N*-methyl group was modified and (b) the nitrogen was moved to another position on the tropane ring of model compounds were investigated.

Results

Synthesis. The structures of the compounds synthesized for study are shown in Figure 1. Cocaine methiodide (**2**) was obtained by heating an acetone solution of **1a** with methyl iodide.[12] N-Demethylation of **1a** provided *N*-norcocaine (**1b**),[13,14] which could be benzylated with benzyl bromide to give the *N*-benzyl-*N*-nor analogue **1c** and acetylated with acetyl chloride to provide the *N*-acetyl-*N*-nor derivative **1d**.

We previously reported that reduction of 6-methyl-6-azabicyclo[3.2.1]octan-3-one (**3**) with lithium aluminum hydride gave a mixture of the 3α- and 3β-alcohols **4a** and **4b**.[15] Acylation of this mixture with the appropriate acid chloride followed by flash chromatographic separation gave the racemic esters **4c-f**. More recently, we reported the synthesis of the pure (1*S*,3*R*,5*R*)-**4b** and (1*R*,3*S*,5*S*)-**4b**.[16] Benzoylation of these two alcohols with benzoyl chloride gave (+)- and (-)-**4d**, respectively. β-Tropacocaine (**5**) and α-tropacocaine (**6**) were prepared by reported procedures.[17]

Biological. Table I summarizes the IC_{50} values of cocaine (**1a**) and of several analogues with various N-modifications to inhibit [^{3}H]-3β-(*p*-fluorophenyl)tropane-2β-carboxylic acid methyl ester {[^{3}H]-**7a**} binding to rat striatal membranes. Conversion of cocaine (**1a**) to *N*-norcocaine (**1b**) results in only a 3-fold lowering of potency whereas exchange of the methyl for the larger *N*-benzyl group to give **1c** lowers potency by approximately 7-fold. In contrast, replacement of the *N*-methyl group with the acetyl moiety to give **1d** or the addition of a second methyl group

Table I. Potencies of Cocaine and Analogues in Inhibiting Binding of [^{3}H]-**7a**[a]

compd	IC_{50} (μm)	compd	IC_{50} (μm)
1a	0.102[b]	(+)-**4d**	2.94 ± 0.627
1b	0.303 ± 0.059	(-)-**4d**	2.85 ± 0.272
1c	0.668 ± 0.067	**4e**	ND[c]
1d	3.37 ± 1.08	**4f**	9.03 ± 3.54
2	10.70 ± 1.53	**5**	5.18 ± 1.16
4c	19.8 ± 2.03	**6**	21.2 ± 2.51
4d	4.95 ± 1.39		

[a] All values are the mean of four to five experiments performed in triplicate. [b] Taken from ref 18. [c] ND = no displacement.

† National Institute on Drug Abuse.

(1) Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine Receptors on Dopamine Transporters Are Related to Self-Administration of Cocaine. *Science* **1987**, *237*, 1219.
(2) Bergman, J.; Madras, B. K.; Johnson, S. E.; Spealman, R. D. Effects of Cocaine and Related Drugs in Nonhuman Primates. III. Self-Administration by Squirrel Monkeys. *J. Pharm. Exp. Ther.* **1989**, *251*, 150.
(3) Reith, M. E. A.; Sershen, H.; Lajtha, A. Saturable [^{3}H]Cocaine Binding in Central Nervous System of Mouse. *Life Sci.* **1980**, *27*, 1055.
(4) Calligaro, D. O.; Eldefrawi, M. E. High Affinity Stereospecific Binding of [^{3}H]Cocaine in Striatum and Its Relationship to the Dopamine Transporter. *Membrane Biochem.* **1988**, *7*, 87.
(5) Madras, B. K.; Fahey, M. A.; Bergman, J.; Canfield, D. R.; Spealman, R. D. Effect of Cocaine and Related Drugs in Nonhuman Primates. I. [^{3}H]Cocaine Binding Sites in Caudate-Putamen. *J. Pharm. Exp. Ther.* **1989a**, *251*, 131.
(6) Madras, B. K.; Spealman, R. D.; Fahey, M. A.; Neumeyer, J. L.; Saha, J. K.; Milius, R. A. Cocaine Receptors Labeled by [^{3}H]2β-Carbomethoxy-3β-(4-fluorophenyl)tropane. *Mol. Pharmacol.* **1989b**, *36*, 518.
(7) Javitch, J. A.; Blaustein, R. O.; Snyder, S. H. [^{3}H]Mazindol Binding Associated with Neuronal Dopamine and Norepinephrine Uptake Sites. *Mol. Pharmacol.* **1984**, *26*, 35.
(8) Dubocovich, M. L.; Zahniser, N. R. Binding Characteristics of the Dopamine Uptake Inhibitor [^{3}H]Nomifensine to Striatal Membranes. *Biochem. Pharmacol.* **1985**, *34*, 1137.
(9) Anderson, P. H. Biochemical and Pharmacological Characterization of [^{3}H]-GBR 12935 Binding in Vitro in Rat Striatal Membranes: Labeling of the Dopamine Uptake Complex. *J. Neurochem.* **1987**, *48*, 1887.
(10) Kennedy, L. T.; Hanbauer, I. Sodium-sensitive Cocaine Binding to Rat Striatal Membrane: Possible Relationship to Dopamine Uptake Sites. *J. Neurochem.* **1983**, *41*, 172-178.
(11) Ritz, M. C.; Boja, J. W.; Grigoriadis, D. E.; Zacek, R.; Carroll, F. I.; Lewin, A. H.; Kuhar, J. M. [^{3}H]WIN 35,065-2: A Ligand for Cocaine Receptors in Striatum. *J. Neurochem.* **1990**, *55*, 1556-1562.

0022-2623/92/1835-0141$03.00/0

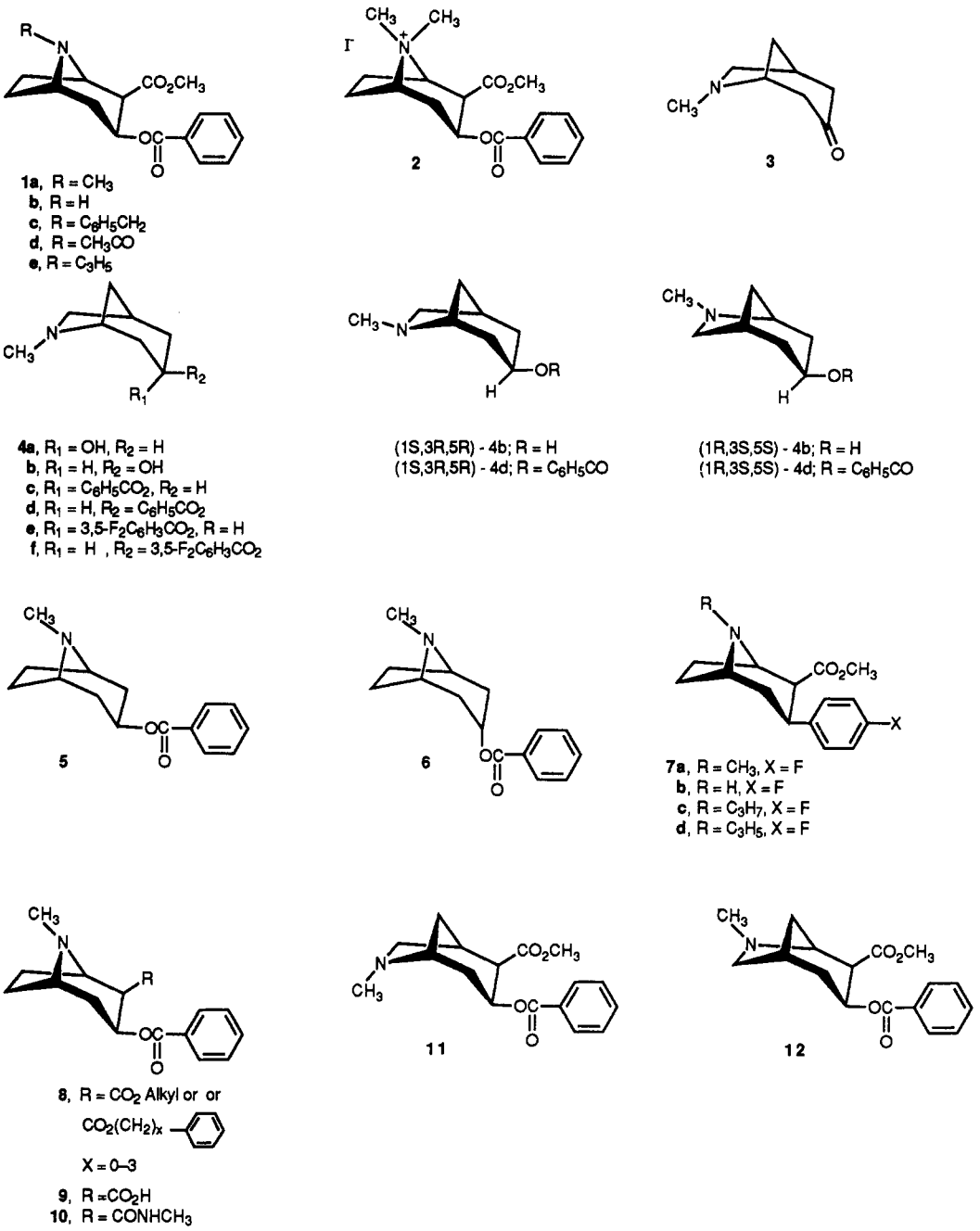

Figure 1. Structural formulas for cocaine and analogues. Structures with wedged bonds are optically active.

to give the quaternary salt **2** results in 30- and 100-fold decreases in potency.

β-Tropacocaine (**5**) which is missing the 2-carbomethoxy group but retains the 3β-benzoyloxy group of cocaine (**1a**) has an IC_{50} of 5.18 μm. Its 3α-isomer, α-tropacocaine (**6**), is 4 times less potent. The benzoate esters of 6-methyl-6-azabicyclo[3.2.1]octan-3-ol show a similar pattern of activity. Thus, the β-benzoates (±)-, (+)-, and (−)-**4d** have potencies similar to that of β-tropacocaine (**5**) whereas the potency of the α-benzoate **4c** is almost identical to that of α-tropacocaine (**6**). Similarly, the β-3,5-difluorobenzoate **4f** has as IC_{50} of 9.03 μm with the α-3,5-difluorobenzoate **4e** showing no displacement of the radioligand.

Discussion

In recent studies we have reported on the synthesis and receptor binding properties of the seven stereoisomers of

(12) Shen, M.; Ruble, J. R.; Hite, G. Stereochemical Aspects of Local Anesthetic Action II. Crystal Structure of Cocaine Methiodide. *Acta Crystallogr. Sect. B* **1975**, *B31*, 706–2709.
(13) Baldwin, S. W.; Jeffs, P. W.; Natarajan, S. Preparation of Norcocaine. *Synth. Commun.* **1977**, *7*, 79.
(14) Borne, R. F.; Bedford, J. A.; Buelke, J. L.; Craig, C. B.; Hardin, T. C.; Kibbe, A. H.; Wilson, M. C. Biological Effects of Cocaine Derivatives I: Improved Synthesis and Pharmacological Evaluation of Nor-cocaine. *J. Pharm. Sci.* **1977**, *66*, 119.
(15) Carroll, F. I.; Abraham, P.; Parham, K.; Griffith, R. C.; Ahmad, A.; Richard, M. M.; Padilla, F. N.; Witkin, J. M.; Chiang, P. C. 6-Methyl-6-azabicyclo[3.2.1]octan-3α-ol 2,2-Diphenylpropionate (Azaprophen), a Highly Potent Antimuscarinic Agent. *J. Med. Chem.* **1987**, *30*, 805.
(16) Pitner, J. B.; Abraham, P.; Joo, Y. J.; Triggle, D. J.; Carroll, F. I. Synthesis and Stereoselective Reduction of (±)-, (+)-, and (−)-6-Substituted-6-azabicyclo[3.2.1]octan-3-one. *J. Chem. Soc., Perkins Trans. 1*, in press.
(17) Beyerman, H. C.; Siegmann, C. M.; Sixma, F. L. J.; Wisse, J. H. The Sterical Structure of Tropinol and Pseudo-tropinol. *Recl. Trav. Chem. Pay-Bas* **1956**, *75*, 1445.

cocaine,[18] a series of 3β-(substituted phenyl)tropane-2β-carboxylic acid methyl esters (7) where R = CH_3 and X = various substituents,[19,20] and cocaine analogues with modified C-2 substituents.[21] These investigations show that cocaine (1a) binding to the dopamine transporter is highly stereospecific[18] and that highly potent compounds could be obtained by adding para substituents to 3β-phenyltropane-2β-carboxylic acid methyl ester (i.e., 7, X = Cl, I, Br, and CH_3).[19,20] In addition, we found that the methyl group of the carbomethoxy group could be increased in size (see structure 8) with either no loss or little loss in binding potency.[21] However, conversion of cocaine to the acid (benzoylecgonine, 9) or to the monomethylamide analogue 10 resulted in large losses of potency.[21] Reith and co-workers[22] found that both norcocaine (1b) and N-allyl-N-norcocaine (1e) had lower binding affinities than cocaine in the striatum. In a recent study, Madras and co-workers[23] reported that the N-nor analogue 7b, as well as the N-propyl-N-nor and N-allyl-N-nor analogues 7c and 7d, respectively, showed potency slightly less than the parent N-methyl compound 7a. These two studies show that either removal of the N-methyl group or replacement with larger groups has relatively small effects on binding potency.

In this study, we found that replacement of the N-methyl group with a hydrogen (1b) or with an N-benzyl substituent (1c) resulted in 3–7-fold loss in potency. In contrast, we found that replacement of the N-methyl group by an acetyl moiety to give the amide 1d or addition of a methyl group to create the quaternary salt 2 have relatively large effects on binding affinity. Specifically, the potency of the amide 1d is reduced by a factor of 33 and the salt 2 has potency 1/111th that of cocaine (1a). These limited studies suggest that an amino functionality is required for potent binding to the dopamine transporter. This is probably part of the reason for the large decrease in potency observed for benzoylecgonine (9), which should have substantial zwitterionic character.[21]

The binding properties of 3β-(benzoyloxy)-6-methyl-6-azabicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (11) and/or 3β-(benzoyloxy)-7-methyl-7-azabicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (12), which are both isomeric with cocaine, would provide information concerning the importance of the nitrogen position for binding to the cocaine site. Since the synthesis of 11 and 12 would be a challenging undertaking, we devised a simpler model to gain information on the effect of nitrogen location.

β-Tropacocaine (5) is a cocaine analogue with the 2β-carbomethoxy group removed. Similarly, compound 4d is an analogue of 11 and/or 12 lacking the 2β-carbomethoxy group. If it is assumed that the loss of the 2β-carbomethoxy group will have a similar effect, at least qualitatively, on the binding properties of both 5 and 4d, a comparison of the binding affinities of 5 and 4d should provide information concerning the effect of nitrogen location on the tropane ring. The similar binding affinity of 5 (IC_{50} = 5.18 μm) to those of 4d (IC_{50} = 4.97 μm) and 4f (IC_{50} = 9.03 μm) suggests that the cocaine analogues 11 and/or 12 will have binding potency similar to that of cocaine. It is somewhat surprising that the (+)- and (−)-isomers of 4d showed similar potency. This might suggest that both compounds 11 and 12 will show binding potencies similar to cocaine. However, the presence of the 2β-carbomethoxy group itself might have a large effect on stereoselectivity. As would be predicted from the binding affinity of the stereoisomers of cocaine, the 3α-isomers 6, 4c, and 4e are less potent than their 3β-counterparts.

Conclusions

The nitrogen moiety in cocaine is important for potent binding to the cocaine site on the dopamine transporter. The binding data for N-methyl-modified cocaine analogues suggest or show that (a) a basic amino group is needed and (b) removal of the N-methyl group or its replacement with an N-benzyl group results in small losses in binding potency. In addition, binding data on model compounds suggest that the nitrogen of cocaine can be moved from the 8- to the 6- and/or 7-position of the azabicyclic ring system without loss in binding potency.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III polarimeter (1-dm cell). NMR spectra were recorded on a Bruker WM-250 spectrometer using tetramethylsilane as an internal standard. High-resolution mass spectra were obtained on a VG Analytical ZAB E spectrometer. Thin-layer chromatography was carried out on Whatman silica gel 60 TLC plates using $CHCl_3$–MeOH–concentrated NH_4OH (40:9:1) unless otherwise noted. Visualization was accomplished under UV or in an iodine chamber. Column chromatography was conducted using 230–400-mesh silica gel with $CHCl_3$–MeOH–concentrated NH_4OH (190:9:1) as eluant. Microanalyses were carried out by Atlantic Microlab, Inc. [^{3}H]-7a (3β-(p-fluorophenyl)tropane-2β-carboxylic acid methyl ester) with specific activity 83.1 Ci/mmol was purchased from Dupont-New England Nuclear (Boston, MA).

N-Benzyl-N-norcocaine (1c, 3β-(Benzoyloxy)-8-benzyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid Methyl Ester) Hydrochloride. A mixture of 1b (289 mg, 1.0 mmol), powdered K_2CO_3 (0.76 g), and benzyl bromide (188 mg, 1.1 mmol) in acetone (10 mL) was stirred overnight at room temperature. The solid was removed by filtration and washed with acetone. The filtrate and the washings were evaporated, and the residue was purified by chromatography on a silica gel column to give 294 mg (80%) of pure 1c. The hydrochloride was recrystallized from CH_2Cl_2–Et_2O: mp 187–190 °C; $[\alpha]^{23}_D$ +4.6° (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.15 (m, 4), 2.65 (m, 2), 3.35 (m, 2), 3.67 (s, 3, OCH_3), 3.89 (m, 2), 4.60 (m, 1), 5.49 (m, 3), 7.49–7.90 (m, 9, ArH). Anal. Calcd ($C_{23}H_{25}N_4O_4$·1.75HCl): C, H, N.

N-Acetyl-N-norcocaine (1d, 3β-(Benzoyloxy)-8-acetyl-8-azabicyclo[3.2.1]octane-2β-carboxylic Acid Methyl Ester). To a stirred solution of 1b (580 mg, 2.0 mmol) in pyridine (2 mL) at 0 °C was added acetyl chloride (188 mg, 2.4 mmol). After 4 h at 0 °C, the mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 2 N HCl and water. The solvents were removed after drying over Na_2SO_4 and chromatographed on a silica gel column to give 0.595 g (90%) of 1d as a clear oil which solidified when kept under vacuum for 24 h. The solid was triturated with ether

(18) Carroll, F. I.; Lewin, A. H.; Philip, A.; Parham, K.; Boja, K. W.; Kuhar, M. J. Synthesis and Ligand Binding of Cocaine Isomers at the Cocaine Receptor. *J. Med. Chem.* **1991**, *34*, 883.

(19) Carroll, F. I.; Gao, Y.; Rahman, M. A.; Philip, A.; Parham, K.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Synthesis, Ligand Binding, QSAR and CoMFA Study of 3β-(p-Substituted phenyl)tropane-2β-carboxylic Acid Methyl Esters. *J. Med. Chem.* **1991**, *34*, 2719.

(20) Boja, J. W.; Carroll, F. I.; Rahman, M. A.; Philip, A.; Lewin, A. H.; Kuhar, M. J. New, Potent Cocaine Analogs: Ligand Binding and Transport Studies in Rat Striatum. *Eur. J. Pharmacol.* **1990**, *184*, 329–332.

(21) Lewin, A. H.; Gao, Y.; Abraham, P.; Boja, J. W.; Kuhar, M. J.; Carroll, F. I. The Effect of 2β-Substitution on Binding Affinity at the Cocaine Receptor. *J. Med. Chem.*, in press.

(22) Reith, M. E. A.; Meisler, B. E.; Sershen, H.; Lajtha, A. Structural Requirements for Cocaine Congeners to Interact with Dopamine and Serotonin Uptake Sites in Mouse Brain and to Induce Stereotyped Behavior. *Biomed. Pharm.* **1986**, *35*, 1123.

(23) Madras, B. K.; Kamien, J. B.; Fahey, M. A.; Canfield, D. R.; Milius, R. A.; Saha, J. K.; Neumeyer, J. L.; Spealman, R. D. N-Modified Fluorophenyltropane Analogs of Cocaine with High Affinity for Cocaine Receptors. *Pharm. Biochem. Behav.* **1990**, *35*, 949.

to give analytically pure **1d**: mp 104–107 °C; $[\alpha]^{23}_D$ +3.22° (*c* 1.99, $CHCl_3$); ^1H NMR ($CDCl_3$) [^1H NMR indicates the presence of two forms of the *N*-acetyl group] δ 1.95 (m, 4), 1.96 and 2.11 (2 s, 3, $COCH_3$ endo and exo), 2.46 and 2.60 (2 m, 1), 3.12 and 3.22 (2 m, 1), 3.68 and 3.69 (2 s, 3, OCH_3), 4.40 and 4.47 (2 m, 1), 4.95 and 5.08 (2 m, 1), 5.51 (m, 1, H-3), 7.45–8.0 (m, 5, ArH). Anal. Calcd ($C_{18}H_{21}NO_5$): C, H, N.

(1*R*,3*S*,5*S*)-6-Methyl-6-azabicyclo[3.2.1]octan-3β-ol Benzoate [(−)-4d] Hydrochloride. To a solution of (1*R*,3*S*,5*S*)-**4b** (150 mg, 1.06 mmol), 15 mg of (dimethylamino)pyridine, and 0.3 mL of triethylamine at 5 °C was added 0.246 mL (2.12 mmol) of benzoyl chloride via syringe. The mixture was stirred for 3 h at room temperature and partitioned between dilute aqueous NH_4OH and CH_2Cl_2. The aqueous layer was extracted twice with CH_2Cl_2 and the combined organic fractions were concentrated and purified by flash chromatography to give 260 mg of the β-benzoate (−)-**4d** free base as a waxy solid: TLC silica gel, $CHCl_3$–MeOH–NH_4OH (80:18:2) R_f 0.50. The hydrochloride salt was prepared and recrystallized from MeOH–Et_2O to give 190 mg (68%): mp 256 °C dec; ^1H NMR (250 MHz, $CDCl_3$) δ 1.82 (m, 3), 2.42 (m, 2), 2.72 (m, 2), 2.94 (s, 3, NCH_3), 3.08 (d, 1), 3.75 (m, 1), 3.93 (br s, 1), 5.40 (m, 1, H-3, $W_{1/2}$ = 21 Hz), 7.54 (m, 3, ArH), 8.00 (m, 2, ArH); $[\alpha]^{24}_D$ −9.0° (*c* 0.5, MeOH). Anal. Calcd ($C_{15}H_{20}ClNO_2$): C, H, N.

(1*S*,3*R*,5*R*)-6-Methyl-6-azabicyclo[3.2.1]octan-3β-ol Benzoate [(+)-4d]. This compound was obtained from alcohol (1*S*,3*R*,5*R*)-**4b** by a procedure analogous to that described for (−)-**4d** from (1*R*,3*S*,5*S*)-**4b**. The TLC and ^1H NMR spectrum were identical to that of (−)-**4d**. The hydrochloride salt was recrystallized from MeOH–Et_2O: mp 259 °C dec; $[\alpha]^{24}_D$ +8.8° (*c* 0.5, MeOH). Anal. Calcd for ($C_{15}H_{20}ClNO_2$): C, H, N.

(±)-6-Methyl-6-azabicyclo[3.2.1]octan-3β-ol Benzoate (4d) Hydrochloride. Compound **4d** was prepared in 59% yield from alcohol **4b** as described for (−)-**4d**: mp 260–261 °C; the TLC and ^1H NMR spectrum were identical to that of (−)-**4d**. Anal. Calcd ($C_{15}H_{20}ClNO_2$): C, H, Cl, N.

(±)-6-Methyl-6-azabicyclo[3.2.1]octan-3β-ol 3,5-Difluorobenzoate (4f) Hydrochloride. Compound **4f** hydrochloride was prepared by a procedure similar to that described for (−)-**4d**: mp 242–243 °C; ^1H NMR ($CDCl_3$–CD_3OD) δ 2.97 (s, 3, NCH_3), 5.38 (m, 1, H-3), 7.24–7.63 (m, 3, ArH). Anal. Calcd ($C_{15}H_{18}ClNO_2$): C, H, N.

(±)-6-Methyl-6-azabicyclo[3.2.1]octan-3α-ol Benzoate (4c) Hydrochloride. To a solution of a mixture of **4a** and **4b**[15] (1.13 g, 0.008 mol) in dry THF (20 mL) at 0 °C was added dropwise a solution of benzoyl chloride (0.565 g, 0.004 mol) in 5 mL of THF. The precipitate formed after stirring overnight was removed and the filtrate was evaporated to give 1.05 g of an oily residue which was purified by chromatography on a silica gel column to give 675 mg (69%) of pure **4c**. The hydrochloride salt was recrystallized from MeOH–Et_2O to give 580 mg of **4c**·HCl: mp 188–189 °C; ^1H NMR (CD_3OD) δ 2.91 (s, 3, NCH_3) 5.32 (t, 1, H-3), 7.54 (m, 3, ArH) 7.98 (m, 2, ArH). Anal. Calcd ($C_{15}H_{20}ClNO_2$): C, H, N, Cl.

(±)-6-Methyl-6-azabicyclo[3.2.1]octan-3α-ol 3,5-Difluorobenzoate (4e) Difluorobenzoate. To a solution of a mixture of **4a** and **4b**[15] (2.82 g, 0.02 mol) in dry THF (40 mL) was added dropwise a solution of 3,5-difluorobenzoyl chloride (1.79 g, 0.01 mol) in THF (5 mL) at 0 °C. The white precipitate formed after 4 h was separated by filtration and washed with cold THF. The filtrate and washings were evaporated under reduced pressure. The waxy solid was recrystallized from $CHCl_3$–Et_2O followed by recrystallization from CH_2Cl_2–Et_2O to give 0.63 g (24%) of **4e** as the difluorobenzoate salt: mp 142–144 °C; ^1H NMR (CD_3OD) δ 2.91 (s, 3, NCH_3) 5.37 (br s, 1, H-3), 6.75–7.58 (m, 6, ArH). Anal. Calcd ($C_{22}H_{21}F_4NO_4$): C, H, N.

Biological. [^{3}H]-7a Radioligand Binding. Rat striata from male Sprague–Dawley rats (250–350 g) were rapidly dissected, frozen, and stored at −70 °C until used. The frozen rat striata were homogenized in 20 volumes of 10 mM phosphate buffer (pH 7.4) containing 0.32 M sucrose using a polytron (setting 6) for 10 s. The homogenate was centrifuged for 10 min at 40000*g*, the resulting pellet was washed in buffer, recentrifuged, and resuspended to a tissue concentration of 1.0 mg/mL. Binding assays were carried out in a total volume of 0.5 mL containing 0.5 nM [^{3}H]-**7a**. The suspensions were incubated for 2 h on ice. Incubations were terminated by filtration with three 5-mL washes through Whatman GF/B filters previously soaked in 0.05% polyethylenimine. Radioactivity was counted in 5 mL of scintillation cocktail at an efficiency of 50–55%. Nonspecific binding of [^{3}H]-**7a** was defined by the presence of 30 μM (−)-cocaine. Under these conditions nonspecific binding was approximately 5–8% of total binding. IC_{50} values were determined from competition curves of 10–12 points utilizing the curve-fitting program EBDA.[24] Mean values and standard errors were calculated from 3–4 assays for each test drug.

Acknowledgment. This work was supported in part by the National Institute on Drug Abuse, Grant No. DA05477. We thank Theresa Kopojtic (ARC) and Karol Parham (RTI) for technical assistance.

(24) Biosoft Software, Ferguson, MO.