Synthesis, Optical Resolution, Absolute Configuration, and Preliminary Pharmacology of (+)- and (-)-*cis*-2,3,3a,4,5,9b-Hexahydro-1-methyl-1*H*-pyrrolo-[3,2-*h*]isoquinoline, a Structural Analog of Nicotine

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Title compound, 8, has been synthesized from isoquinolinone, 1 (an improved preparation for which is presented) and separated into its antipodes with D- and L-di-*p*-toluoyltartaric acids. These antipodes and the racemic precursor have been evaluated (and found active) in two in vivo systems for their effects. The most potent of the three, (+)-8, has an ED_{50} of 7.13 μmol/kg for inhibition of spontaneous activity and 7.45 μmol/kg for antinociception compared to 4.44 and 4.81 μmol/kg, respectively, for (*S*)-(-)-nicotine. Compounds (-)-8 and 7 are about one-fourth as potent. Isomer (+)-8 has the 3a*R*,9b*S* configuration, the latter corresponding to (*S*)-(-)-nicotine as determined by X-ray crystallography. However, (+)-8 failed to compete for [^{3}H]-nicotine binding, and its pharmacological effects were not blocked by mecamylamine. These bridged nicotine analogs either are binding to an as-yet-unidentified nicotinic receptor or they represent a novel class of non-nicotinic analgesics.

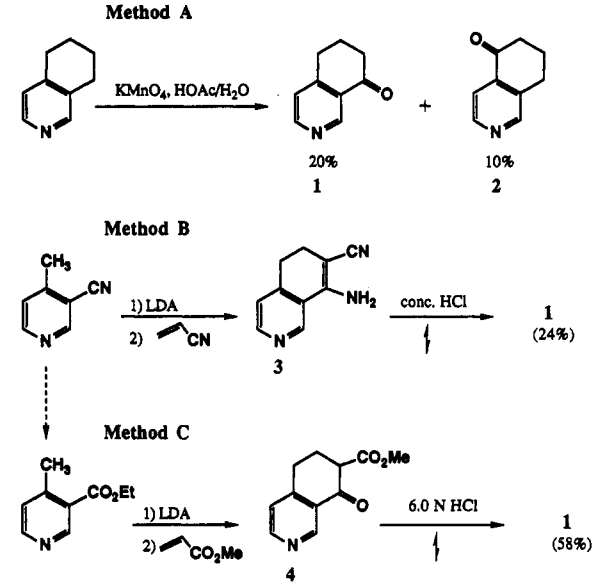
Introduction

In the study of nicotinic agents one question of interest has been what conformation nicotine assumes when it binds at its site(s) of action.[1] A technique pursued by this research group[2] and others[3] has been to synthesize conformationally restricted analogs of nicotine which, if potent, should shed light on the "active" conformation of nicotine and, by inference, on the internal dimensions of the receptor. An additional point of interest in this research is the possibility of multiple subtypes of the nicotinic receptor in the CNS. Protein and gene identification and sequencing have already demonstrated the existence of multiple nicotine subtypes;[4] the task then becomes one of finding pharmacological ligands that can distinguish among them. A starting point in this task is that the subtypes of a receptor share a similar pharmacophore. With this in mind, if the core ligand structure is flexible and possesses multiple conformations of similar energy, as is the case for nicotine, it may be possible to differentiate between the subtypes by restricting the core ligand to specific conformations. Of course this presumes that the ligand will assume specific (and different) conformations when interacting with the binding sites of the different receptor subtypes. However, the bridging units or additional substituents used to restrict the conformational flexibility of the core ligand may themselves interact with portions of the binding site, either favorably or unfavorably, and in turn impart some selectivity for one subtype.

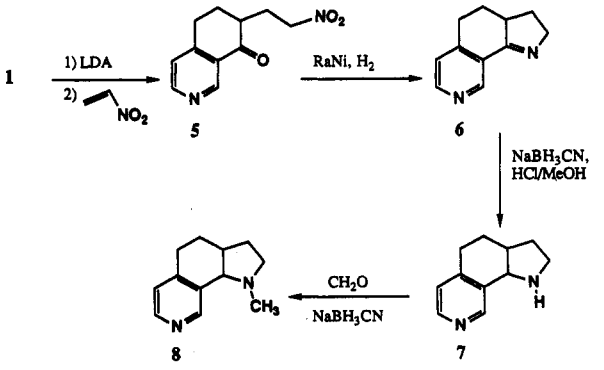
Chemistry

The synthesis of 8 began with 6,7-dihydro-8(5*H*)-isoquinolinone, 1, which had heretofore been reported only once[5] in a procedure requiring harsh conditions, a difficult isolation, and a poor yield (10%). We initially sought a modified version of the literature oxidation procedure (Scheme I, method A). This modification, while improving the yield (2-fold) still produces the isomeric 7,8-dihydro-5(6*H*)-isoquinolinone (2) as a side product. Two alternate approaches were developed, as shown in Scheme I (meth-

Scheme I



Scheme II



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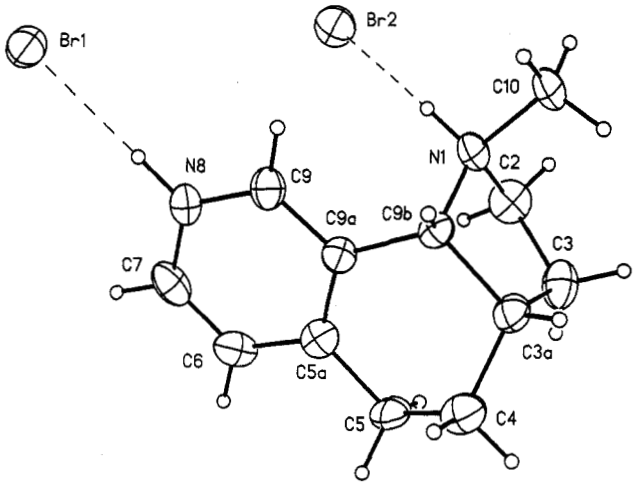

Figure 1. Thermal ellipsoid plot of one of the two conformational isomers present in (−)-8. Dashed lines are hydrogen bonds.

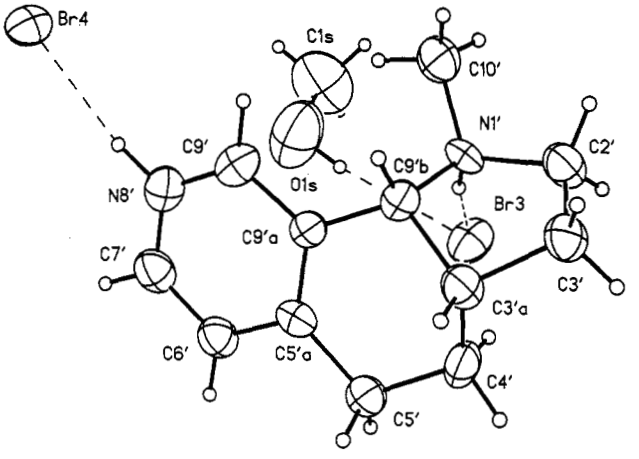

Figure 2. Thermal ellipsoid plot of the second of the two isomers of (−)-8.

ods B and C), based on the work of Tarnchompoo et al.,[6] both of which improved the yield (3–4 fold) in the absence of the side product. With the starting ketone in hand, the synthesis of 8 (Scheme II) followed the procedure described by Chavdarian and co-workers[3a] for the isomeric 2,3,3a,4,5,-9b-hexahydro-1-methyl-1*H*-pyrrolo[2,3-f]quinoline. As with the literature synthesis, we have found no evidence of the *trans* isomer in the product. X-ray studies have confirmed the geometry of 8. Optical resolution of 8 was effected with the D and L forms of di-*p*-toluoyltartaric acid; the antipodes were converted to the dihydrobromide salts for testing and for X-ray diffraction studies.

Results and Discussion.

X-Ray Diffraction of (+)- and (−)-8. X-ray study of the two optical antipodes allowed their absolute configurations to be determined: (−)-8 was found to possess the 3a*S*,9b*R* absolute configuration, while (+)-8 possesses the 3a*R*,9b*S* absolute configuration. The structures of the two enantiomers differ only slightly aside from the chirality. Each crystallized with two conformational isomers (Figures 1 and 2), four Br ions, and methanol solvate in the asymmetric unit. The conformers differ primarily around the C3a–C9b bridge (torsion angle C4–C3a–C9b–N1 = −143.7° and −76° in the isomer in which atom labels are primed). The central ring is in an envelope conformation with C4 out of the base plane and directed away from the five-membered ring which is also in an envelope conformation with N1 out of the base plane and *cis* to C4 (Figure 1). In the second isomer the central ring forms a half chair with C4′ directed toward the five-membered ring which is twisted such that N1′ is *cis* to C4′ (Figure 2).

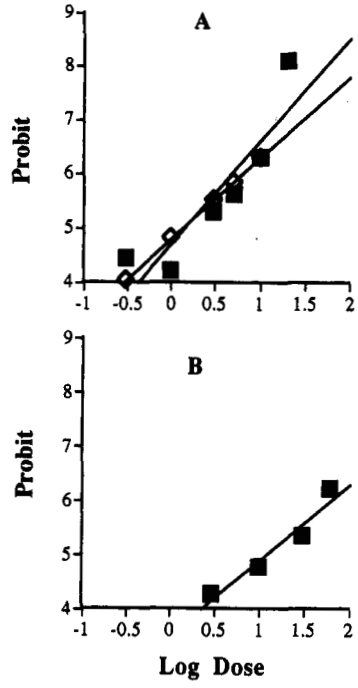

Figure 3. Effects of (+)-8 on spontaneous activity (open squares) and tail-flick (closed squares) are shown in panel A, whereas the effects of (−)-8 on tail-flick response are depicted in panel B.

Pharmacology. Nicotine produced depression of spontaneous activity and antinociception with ED_{50}'s of 4.44 and 4.81 μmol/kg, respectively. Evaluation of the (+)-enantiomer of 8 in these two assays revealed a pharmacological profile similar to that of nicotine. This enantiomer was also equally effective in these two pharmacolgical measures as shown in Figure 3A. The ED_{50}'s (CL) were determined to be 7.13 (2.34–21.6) and 7.45 (2.44–22.8) μmol/kg for spontaneous activity and antinociception, respectively. It appears that (+)-8 is somewhat less potent than nicotine. The (−)-enantiomer of 8 also produced antinociception which was dose responsive, as shown in Figure 3B. The ED_{50} (CL) was found to be 61.7 (24.8–152) μmol/kg, which indicates this enantiomer is approximately 10-fold less potent than its corresponding (+)-enantiomer. However, the (−)-enantiomer of 8 was distinctively different from its (+)-enantiomer and nicotine in that it failed to produce motor impairment. It was evaluated, using doses up to 160 μmol/kg on two separate occasions and the depression in spontaneous activity was less than 25% of control levels on both occasions. This separation of activity is unusual for nicotine analogs in that they typically alter spontaneous activity before exerting an antinociceptive effect. (±)-Nornicotine produced only 12% MPE in the antinociceptive assay at a dose of 68 μmol/kg and was toxic at the higher dose, while (±)-7 produced ED_{50}'s of 24.8 and 25.7 μmol/kg for depression of spontaneous activity and antinociception, respectively.

Since (+)-8 was a potent agonist exhibiting a pharmacological profile similar to that of nicotine, antagonism studies with mecamylamine were carried out to determine whether its actions are mediated through the nicotinic receptor. Pretreatment with mecamylamine (1.0 mg/kg)

failed to alter the effects of (+)-8 at a dose (3.0 mg/kg) which produced greater than 80% effect in both spontaneous activity and tail-flick assays.

The K_D for nicotine was found to be 1.41 ± 0.20 nM (means ± SEM, $N = 3$) and the B_{max} was 544 ± 34 fmol/mg of protein. Competition studies revealed that the (−)-enantiomer of 8 exhibited low affinity for nicotine binding with a K_I of 605 ± 217 nM whereas the (+)-8 failed to displace binding even at 10 μM concentrations. (±)-7 competed for binding with a K_I of 167 ± 19 nM.

These pharmacological data are intriguing for several reasons. First, preparation of conformationally restricted analogs resulted in compounds with relatively low affinity for the nicotine receptor. Even the compound [(±)-7] with the highest affinity was 100 times less potent than nicotine, and (+)-8 was completely devoid of affinity for the nicotine receptor. Yet, (+)-8 exhibited pharmacological properties and potencies similar to those of nicotine. It is a well-established fact that mecamylamine is capable of blocking almost all of the centrally mediated effects of nicotine and all other agents that bind to the nicotinic receptor. The failure of mecamylamine to antagonize the effects of (+)-8 provides further support that this analog is not binding to the nicotine receptor. One plausible explanation for these findings is that (+)-8 is producing nicotinic effects by acting at a nicotinic receptor that is not mecamylamine sensitive. On the other hand, it is not unreasonble to conclude that these bridged analogs are not nicotine-like because they are unable to bind to the nicotine site and are not blocked by mecamylamine. Considerable pharmacological experimentation will be required to answer these questions.

Experimental Section

All solvents were ACS reagent grade quality and were used without further purification, except for tetrahydrofuran (THF), which was dried by distillation over lithium aluminum hydride (LAH). Silica gel for column chromatography, as well as all other chemicals, was purchased from Aldrich Chemical Co., Milwaukee, WI, while TLC plates were purchased from Analtech, Inc., Newark, DE. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, and are within 0.4% of the theoretical values. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and have been corrected. IR spectra were determined with a Nicolet 5ZDX FT-IR spectrophotometer, and NMR spectra were recorded on either a JEOL FX90Q FT-NMR or a GE QE-300 FT-NMR. Electron impact (EI) and chemical ionization (CI) mass spectra were determined using a Hewlett-Packard 5988A GC/MS system equipped with a 30-m capillary glass GC column. Unless otherwise indicated, the mass spectra reported below are EI. All optical rotations were performed in MeOH using a Perkin-Elmer 141 digital polarimeter, and the concentration used in the measurement is given after the rotation in parentheses.

Chemistry. 6,7-Dihydro-8(5*H*)-isoquinolinone (1). Method A. 5,6,7,8-Tetrahydroisoquinoline (15.0 g, 112 mmol), AcOH (8.7 mL), and H_2O (500 mL) were stirred in a 2000-mL round bottom flask while potassium permanganate (40.0 g, 253 mmol) was introduced, in portions, over a 5-min period. After 35 min the resulting black slurry was filtered through a fritted glass funnel, and the filtrate was washed with CH_2Cl_2 (5 × 100 mL). The combined washes were dried (Na_2SO_4) and filtered, and the filtrate was concentrated in vacuo to an oil. The product was eluted from a column of silica (70–270 mesh) with ether, concentrated in vacuo to an oil, and resuspended in anhydrous ether. Precipitation with gaseous HCl and recrystallization from 2-PrOH/MeOH gave 3.15 g of 1; mp 194–197 °C. An analytical sample crystallized from MeOH melted at 230–232 °C (lit.[5] mp 230–232 °C). A second crop of crystals was isolated from the mother liquor to bring the yield to 4.1 g (20%): IR (free base, neat) 1688 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.1–2.3 (m, 2H, CH_2), 2.7 (t, 2H, $J = 6$ Hz, CH_2), 3.0 (t, 2H, $J = 6$ Hz, CH_2), 7.2 (d, 1H, $J = 5$ Hz, 4^{iso}-H), 8.6 (d, 1H, $J = 5$ Hz, 3^{iso}-H), 9.2 (s, 1H, 1^{iso}-H); MS m/z (% intensity) 147 (80), 119 (100), 91 (60). Anal. (C_9H_9NO·HCl) C, H, N.

7,8-Dihydro-5(6*H*)-isoquinolinone (2). The 2-PrOH/MeOH mother liquor from crystallization of 1 was concentrated in vacuo, redissolved in MeOH, and concentrated to a thick oil on a hot plate. The crystals that formed overnight were collected and recrystallized from 2-PrOH/MeOH: mp 237–239 °C (lit.[5] mp 235–236 °C); MS m/z (% intensity) 147 (100), 119 (70), 91 (80). Anal. (C_9H_9NO·HCl) C, H, N. The yield of 2, approximately 50% that of 1 based on GC/MS analysis of the crude product, was consistent with the literature[1] product ratio; the two products can be distinguished by TLC (ether saturated with 30% aqueous NH_4OH).

Method B. 8-Amino-7-cyano-5,6-dihydroisoquinoline (3). Lithium diisopropylamide (LDA, 19.2 mL, 38.5 mmol in pentane) was added to 100 mL of dry THF cooled to −78 °C, and 3-cyano-4-methylpyridine[7] (4.5 g, 38.1 mmol in 30 mL of THF) was introduced over an 18-min period. The solution was stirred for 15 min, and then acrylonitrile (4.44 g, 83.6 mmol) was added over 5 min.[4] The reaction was stirred for 1 h at −78 °C and at 0 °C for 2 h. H_2O (400 mL) and ether (100 mL) were added, and the mixture was shaken and allowed to separate overnight. The ether layer was removed, and the H_2O layer was washed with ether (3 × 100 mL). The combined extracts were dried (Na_2SO_4) and filtered, and the filtrate concentrated in vacuo to a brown oil. The oil was triturated with EtOH to yield 1.4 g (21%) of a yellow solid, 3, mp 171–173.5 °C; HCl salt, mp 252–255 °C. An additional 0.35 g was isolated from the EtOH filtrate by flash chromatography (EtOAc as eluent) to give a combined yield of 27%. IR (KBr) 3388, 3346 (NH), 2186 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5 (t, 2H, $J = 7$ Hz, CH_2), 1.8 (t, 2H, $J = 7$ Hz, CH_2), 4.8 (br s, 2H, D_2O exchangeable, NH_2), 7.2 (d, 1H, $J = 5$ Hz, 4^{iso}-H), 8.6 (d, 1H, $J = 5$ Hz, 3^{iso}-H), 8.7 (s, 1H, 1^{iso}-H); MS m/z (% intensity) 172 (10), 171 (100), 131 (90). Compound 3 failed to analyze correctly as either the free base or the HCl salt.

6,7-Dihydro-8(5*H*)-isoquinolinone (1). Compound 3 (0.38 g, 2.22 mmol) was heated at reflux in 6.0 M HCl (8 mL) for 19 h. The acid was neutralized with 6.0 M KOH and the aqueous solution was washed with ether (4 × 15 mL). The ether was dried (Na_2SO_4) and filtered, and the filtrate was concentrated in vacuo to an oil which crystallized on cooling. The yield was 0.30 g (92%) of 1, mp (free base) 37–39 °C, identical by TLC and GC/MS to an authentic sample of 1.

Method C. Methyl 6,7-dihydro-8(5*H*)-isoquinolinone-7-carboxylate (4). Ethyl 4-methyl-3-pyridinecarboxylate[7] (6.6 g, 40. mmol in 40 mL of THF) was added over a 20-min period to a solution of LDA (generated from 44 mmol of diisopropylamine and 44 mmol of BuLi in 200 mL of THF) at −78 °C. The resulting red solution was stirred for 20 min, and methyl acrylate (8.61 g, 100. mmol in 20 mL THF) was added over a 15-min period. The reaction was stirred an additional 1.5 h, 10% AcOH (80 mL) was added, and the reaction was allowed to warm to room temperature. The organic layer was removed and the aqueous layer was washed with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to yield an orange oil which crystallized on cooling. A small sample of 4 was purified for analysis: picrate salt (out of EtOH) mp 148–150 °C; IR (picrate salt, KBr) 2706 (OH), 1638 (C=O) cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 2.4–2.8 (m, 4H, CH_2), 3.8 (s, 1H, CH), 3.9 (s, 3H, OCH_3), 7.0–7.2 (m, 1H, 4^{iso}-H), 8.5–8.7 (m, 1H, 3^{iso}-H), 8.9–9.2 (m, 1H, 1^{iso}-H); MS m/z (% intensity) 205 (1), 165 (50), 120 (100). Anal. ($C_{17}H_{14}N_4O_{10}$) C, H, N.

6,7-Dihydro-8(5*H*)-isoquinolinone (1). The crude 4 from three such runs (total crude weight of 29.0 g) was dissolved in 434 mL of 6.0 M aqueous HCl and heated at reflux for 24 h. The acidic solution was concentrated in vacuo, resuspended in water (100 mL), cooled in ice, and made basic with 6.0 M KOH. The now basic aqueous solution was washed with Et_2O (2 × 100 mL) and CH_2Cl_2 (3 × 100 mL), the combined organics were dried (Na_2SO_4) and concentrated to yield a brown oil. Kugelrohr distillation (110–120 °C, 0.4 mmHg) yielded 10.29 g (58%) of 1 as a colorless oil which crystallized on cooling; mp (free base) 37–38 °C; mp (hydrochloride from 2-PrOH/MeOH) 227–230 °C.

7-(2-Nitroethyl)-6,7-dihydro-8(5*H*)-isoquinolinone (5). A solution of diisopropylamine (2.38 g, 23.4 mmol) in THF (37 mL) was cooled to −30 °C under a nitrogen atmosphere, and butyl lithium (13.6 mL, 21.8 mmol in pentane) was added by syringe. The solution was stirred for 20 min, and then cooled to −78 °C. To this was added 1 (2.9 g, 20.4 mmol) in THF (12 mL). The resulting dark solution was stirred for 20 min, and then nitroethylene[8] (3.4 mL, 20.2 mmol in benzene) was added. The resulting yellow slurry was stirred at −78 °C for 1 h, and then allowed to warm to room temperature overnight. AcOH (11 mL of a 10% aqueous solution) was added, and the two layers were allowed to separate. The organic layer was removed, and the aqueous layer was washed with ether (3 × 50 mL) and CH_2Cl_2 (50 mL). The combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo to an oil which solidified on standing. The unreacted starting material was removed by kugelrohr distillation [oven temperature 165 °C (0.3 mmHg)] and the remaining residue in the distillation flask (crude yield 51%) was sufficiently pure for the next step. A small quantity was purified for characterization: 5 (HCl salt from 2-PrOH/MeOH) mp 164–167 °C; free base (out of EtOH) mp 79–81 °C; IR (KBr) 1687 (C=O), 1546, 1384 (NO_2) cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 1.8–2.8 (m, 7H, CH_2 + CH), 2.9–3.2 (m, 2H, CH_2), 4.5–4.8 (m, 2H, CH_2), 7.2 (d, 1H, J = 5 Hz, 4^{iso}-H), 8.7 (d, 1H, J = 5 Hz, 3^{iso}-H), 9.1 (s, 1H, 1^{iso}-H); MS m/z (% intensity) 220 (10), 190 (5), 146 (10), 119 (100). Anal. ($C_{11}H_{12}N_2O_3$·HCl) C, H, N.

3,3a,4,5-Tetrahydro-2*H*-pyrrolo[3,2-*h*]isoquinoline (6). Raney nickel (2.6 g of wet catalyst) was added to a solution of 5 (2.57 g, 11.7 mmol) in MeOH (100 mL), and the mixture was hydrogenated in a Parr apparatus at 55 psig for 12 h. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo to an oil. Kugelrohr distillation [oven temperature 125–130 °C (0.9 mmHg)] yielded 0.94 g (47%) of an oil which crystallized on cooling. A small quantity was converted to the dipicrate salt (from EtOH) for elemental analysis: IR (free base, neat) 1623, 1595, 1427 (C=N) cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 1.4–1.9 (m, 2H, CH_2), 2.1–2.5 (m, 2H, CH_2), 2.7–3.0 (m, 3H, CH + CH_2), 3.3–4.0 (m, 1H, CH), 4.0–4.4 (m, 1H, CH), 7.1 (d, 1H, J = 5 Hz, 4^{iso}-H), 8.5 (d, 1H, J = 5 Hz, 3^{iso}-H), 9.3 (s, 1H, 1^{iso}-H); MS m/z (% intensity) 172 (100), 144 (30), 130 (10). Anal. ($C_{11}H_{12}N_2$·$2C_6H_3N_3O_7$) C, H, N.

2,3,3a,4,5,9b-Hexahydro-1*H*-pyrrolo[3,2-*h*]isoquinoline (7). Sodium cyanoborohydride ($NaBH_3CN$, 1.22 g, 19.4 mmol) was added to a solution of 6 (2.44 g, 14.2 mmol) in MeOH (10 mL) containing a drop of bromocresol green indicator. The solution was stirred and 2.0 M HCl/MeOH (concentrated HCl diluted to 2 M concentration with MeOH) (12 mL) was added over 20 min to maintain the yellow end point. The solution was stirred an additional 3 h, and then excess 2.0 M HCl/MeOH was added. The solution was concentrated in vacuo to a white paste, dissolved in H_2O (40 mL), and cooled, and 6.0 M KOH was added until the solution became basic (>pH 9). The cloudy mixture was washed with ether (5 × 30 mL) and CH_2Cl_2 (2 × 30 mL), and the organic washes were combined and dried (Na_2SO_4). Filtration, concentration in vacuo of the filtrate, and Kugelrohr distillation [oven temperature 114–117 °C (0.1 mmHg)] gave 1.53 g (59%) of 7 as an oil: IR (neat) 3320 (NH), 1602 (C-N) cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 1.4–2.8 (m, 6H, CH_2), 2.9–3.2 (m, 1H, CH), 3.4 (s, 1H, NH), 4.1 (d, 1H, J = 6.3 Hz, CH), 7.1 (d, 1H, J = 5 Hz, 4^{iso}-H), 8.4 (d, 1H, J = 5 Hz, 3^{iso}-H), 8.7 (s, 1H, 1^{iso}-H); MS m/z (% intensity) 174 (28), 173 (84), 130 (100). A small sample was purified as the dihydrobromide salt from 2-PrOH; mp 197–199 °C. Anal. ($C_{11}H_{14}N_2$·2HBr) C, H, N.

2,3,3a,4,5,9b Hexahydro-1-methyl-1*H*-pyrrolo[3,2-*h*]isoquinoline (8). Sodium cyanoborohydride (1.12 g, 17.8 mmol) was added to a stirred, cooled (0 °C) solution of 7 (1.94 g, 11.1 mmol) and 37% aqueous formaldehyde (7.8 mL, 96 mmol) in acetonitrile (32 mL). AcOH (520 μL) was added after 20 min, and the reaction was stirred overnight. Excess 2.0 M HCl/MeOH was added and the resulting solution was concentrated to a slurry. The crude product was dissolved in H_2O (44 mL), made basic with 6.0 M KOH (pH >9), and washed with ether (5 × 25 mL) and CH_2Cl_2 (2 × 30 mL). The combined extracts were dried ($MgSO_4$) and filtered, and the filtrate concentrated in vacuo to an oil. Kugelrohr distillation [oven temperature 110 °C (0.4 mmHg)] gave 1.18 g of 8 (55%) as a clear oil. A small sample was purified as the dihydrobromide salt from 2-PrOH; mp 209–210 °C dec; IR (dihydrobromide salt, KBr) 2699 (NH), 1647, 1638 (CN) cm^{-1}; ^1H NMR (dihydrobromide salt in DMSO-d_6) δ 1.5–3.8 (m, 9H, CH_2 + CH), 3.0 (s 3H, NCH_3), 4.8 (d, 1H, J = 7.5 Hz, CH), 8.0 (d, 1H, J = 5.7 Hz, 4^{iso}-H), 8.9 (d, 1H, J = 5.7 Hz, 3^{iso}-H), 9.2 (s, 1H, 1^{iso}-H). MS m/z (% intensity) 188 (60), 187 (100), 173 (4), 130 (60); MS (CI) m/z (% intensity) 229 (4), 217 (20), 190 (15), 189 (100), 188 (18). Anal. ($C_{11}H_{16}N_2$·2HBr) C, H, N.

Optical Resolution of 2,3,3a,4,5,9b-Hexahydro-1-methyl-1*H*-pyrrolo[3,2-*h*]isoquinoline, (−)-8. Di-*p*-toluoyl-D-tartaric acid (2.54 g, 6.3 mmol) in EtOH (35 mL) was stirred while (±)-8 (1.18 g, 6.3 mmol) in EtOH (38 mL) was added. The solution was concentrated to half its original volume and allowed to cool overnight to room temperature. The crystals that precipitated were collected and dried to give 1.1 g: mp 163–164 °C dec; $[\alpha]^{24}_D$ = +59.5° (8.36 mM). Recrystallization in EtOH (20 mL concentrated to half its original volume) gave 0.95 g of precipitate: mp 164–165 °C dec; $[\alpha]^{21}_D$ = +59.1° (7.86 mM). This solid was partially dissolved in H_2O (25 mL) and cooled in ice, while 6.0 M KOH was added to adjust the pH to >10. The aqueous layer was washed with ether (5 × 25 mL) and CH_2Cl_2 (2 × 30 mL), and the combined washes were dried ($MgSO_4$). The solid was removed by filtration, and the filtrate was concentrated in vacuo to an oil (0.44 g), which was dissolved in 2-PrOH (20 mL) and to which excess HBr (30–32% in AcOH) was added. The solution was concentrated in vacuo to reveal a brown solid which was recrystallized in 2-PrOH/MeOH to give 0.48 g of the dihydrobromide of (−)-8:[9] mp 245–247 °C dec; $[\alpha]^{21}_D$ = −48.5° (14.7 mM). Anal. ($C_{11}H_{16}N_2$·2HBr) C, H, N.

(+)-8. The original filtrate from the isolation of (−)-8 was concentrated in vacuo to a solid, partially dissolved in H_2O (30 mL) and made basic with 6.0 M KOH (pH >10). The aqueous solution was washed with ether (5 × 30 mL) and CH_2Cl_2 (2 × 30 mL), and the organic washes were dried ($MgSO_4$) and concentrated in vacuo to an oil. This residue was dissolved in EtOH (20 mL), and di-*p*-toluoyl-L-tartaric acid (1.95 g, 4.8 mmol, in 30 mL EtOH) was added. The solution was stirred at reflux, concentrated to two-thirds its original volume, capped, and allowed to cool to room temperature overnight. The resulting crystalline solid was isolated and dried to give 1.2 g: mp 164–165 °C dec; $[\alpha]^{24}_D$ = −59.3° (7.71 mM). Recrystallization in 2-PrOH (15 mL concentrated to two-thirds its original volume) gave 1.0 g: mp 165–166 °C dec; $[\alpha]^{25}_D$ = −58.7° (7.77 mM). Conversion to the dihydrobromide, in the manner described for (−)-8, yielded 0.53 g of (+)-8 dihydrobromide: mp 243.5–244.5 °C dec; $[\alpha]^{25}_D$ = +47.5° (17.3 mM). Anal. ($C_{11}H_{16}N_2$·2HBr) C, H, N.

Single-Crystal X-ray Analysis of (−)-8 and (+)-8 Dihydrobromide Salts. Crystals of (−)-8 and (+)-8 grown from methanol were selected for data collection in the $\theta/2\theta$ mode on a computer-controlled automated diffractometer (Siemens R3m/V). The space group was uniquely determined by the observed extinctions. The data were corrected for Lorentz, polarization and absorption effects. The absorption correction for (−)-8 used a face indexed numerical correction (min and max absorption, 0.16 and 0.65), and (+)-8 used a semiempirical correction based on the ϕ dependence of a number of reflections with χ ca. 90° (min and max transmissions, 0.36 and 0.96). Both structures were solved by direct methods with the aid of the program SHELXTL[10] and refined by full-matrix least-squares fit.[10] The parameters refined include the coordinates and anisotropic thermal parameters for all non-hydrogen atoms (C1S and O1s in (+)-8 were refined isotropically). Carbon hydrogens used a riding model in which the coordinate shifts of the carbons were applied to the attached hydrogens with C–H = 0.96 Å, H angles idealized, and U_{iso}(H) set at fixed values. The determination of the absolute configuration was based on a method suggested by Rogers.[11] The parameter η which multiplies all $\Delta f''$ values (imaginary component of the anomolous scattering factor) in the expression $f_o^{anom} = f_o + \Delta f' + i\eta\Delta f''$ refines to η = 1.24(10) and 1.11(14) for (−)-8 and (+)-8, respectively. The correct choice of enantiomers would give a value near 1.0 while an incorrect choice would give values near −1.0. Additional experimental and structural analysis details are given in Table I, and the tables of crystal coordinates, bond distances, and bond angles are available as supplmentary material.

Table I. Crystal and Refinement Data

	(−)-8	(+)-8
formula	$2[C_{12}H_{18}N_2^{2+}\cdot 2Br^-]$	$2(C_{12}H_{18}N_2^{2+}\cdot 2Br^-)$
asymmetric unit	(CH_3OH)	(CH_3OH)
crystal system	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$
a, Å	8.098(2)	8.135(3)
b, Å	15.256(3)	15.295(8)
c, Å	23.926(5)	23.975(11)
V, Å^3	2.955.8(10)	2983(2)
Z	8	8
formula weight	366.1	366.1
$F(000)$	1464	1464
ρ(calc), g cm^{-3}	1.645	1.630
temp, °C	−50	22
crystal dim, mm	0.06 × 0.24 × 0.60	0.17 × 0.32 × 0.40
λ, wavelength, Å	1.54184	0.71073
μ, absorption coef, mm^{-1}	6.83	5.42
2θ max, deg	112	45
2θ scan speed, deg/min	variable, 7.5–30.0	variable, 4.0–30
2θ scan range, deg	$2.0 + \Delta_{\alpha 1\alpha 2}$	$2.4 + \Delta_{\alpha 1\alpha 2}$
data collected, hkl	0–8, 0–16, 0–26	0–8, 0–16, 0–25
unique data	2320	2219
R_{int}	0.010	0.011
unique data, $F_o > 3\sigma(F_o)$	2252	1179[e]
parameters refined	317	299
weighting function, g[a]	0.00023	0.00023
R[b], wR,[c] S[d]	0.047, 0.058, 2.11	0.075, 0.070, 1.88
Fourier excursions, e Å^{-3}	1.42, −0.72	0.88, −0.76

[a] $w^{-1} = \sigma^2(F_o) + gF_o^2$. [b] $\sum||\Delta|/\sum|F_o|$. [c] $\sum[(w\Delta^2)/\sum(wF_o^2)]^{1/2}$. [d] $[\sum w(\Delta^2)/(N_o - N_p)]^{1/2}$. [e] $F_o > 4\sigma(F_o)$.

Pharmacology. Nicotine and its analogs were tested for their ability to decrease spontaneous activity and inhibition of the tail-flick response (antinociception) in mice. Mice were placed into individual photocell activity cages immediately after sc administration of either 0.9% saline, (*S*)-(−)-nicotine di-L-tartrate, or one of the enantiomers of 8. They were allowed to acclimate for 10 min. Data were expressed as a percentage of the responses produced by the saline-treated group. The tail-flick reaction time to heat stimulus was determined following drug or saline administration using the method of D'Amour and Smith as modified by Dewey et al.[12] Preinjection control values (2–4 s) were determined for all animals. Saline or drug was administered sc 5 min prior to testing for tail-flick latency. A 10-s cut-off time was imposed. Data were expressed as percent maximum possible effect (% MPE) where % MPE = [(test latency - control latency)/(10 s-control latency)] × 100. The ED_{50} values and confidence limits were determined by unweighted least-squares linear regression analysis of the log dose versus probit plot.[13] Antagonism studies were conducted by administering mecamylamine hydrochloride (1.0 mg/kg, sc) 10 min before administration of nicotine or its analogs. Spontaneous activity and tail-flick response were measured as described above.

Nicotine Receptor Binding. The assay was conducted as described by Scimeca and Martin.[14] Whole mouse brain (minus cerebellum) were homogenized in 10 volumes of ice-cold 0.05 M Na-K phosphate buffer (pH 7.4) and centrifuged (17500*g*, 4 °C) for 30 min. The pellet was then resuspended in 20 volumes of ice-cold glass-distilled water and allowed to remain on ice for 60 min before being centrifuged as before. The resulting pellet was then resuspended to a final tissue concentration of 40 mg/mL of buffer. [^{3}H]Nicotine was incubated with 0.5 mL of tissue homogenate in a final incubation volume of 1 mL. The tissue was incubated for 2 h at 4 °C and rapidly filtered through Whatman GF/C filters. Specific binding was defined as the difference in the amount of binding in the presence and absence of 100 μM nicotine. Following three consecutive washes with ice-cold buffer, the filters were allowed to air dry and then placed in scintillation vials for determination of radioactivity. Following transformation of the data by the method of Scatchard (1949) the K_D and B_{max} values were determined using the LIGAND program.[15] Displacement of [^{3}H]nicotine binding at 1 nM was determined in the presence of increasing concentrations of various ligands and converted to percent displacement of specific binding. The IC_{50}'s were determined from a plot of the log concentration versus percent displacement and converted to K_i values by the method of Cheng and Prusoff.[16]

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Supplementary Material Available: Crystal coordinates, bond distances, and bond angles for (−)-8 and (+)-8 (10 pages). See any current masthead for ordering information.

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