Identification of a More Potent Analogue of the Naturally Occurring Alkaloid Huperzine A. Predictive Molecular Modeling of Its Interaction with AChE

Alan P. Kozikowski,*,† Giuseppe Campiani,‡ Li-Qiang Sun,§ Shaomeng Wang,† Ashima Saxena,⊥ and Bhupendra P. Doctor⊥

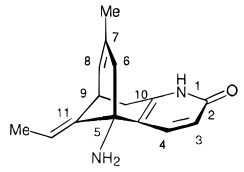
Contribution from the Georgetown University Medical Center, Institute for Cognitive and Computational Sciences, 3970 Reservoir Road NW, Washington, DC 20007-2197, Dipartimento Farmaco Chimico Tecnologico, Siena University, Banchi di Sotto 55, 53100 Siena, Italy, Department of Chemistry, Ohio State University, Columbus, Ohio 43210, and Division of Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307

Received July 5, 1996[⊗]

Abstract: Huperzine A (HA), a potent reversible inhibitor of acetylcholinesterase (AChE), is an important psychotherapeutic agent for improving cognitive function in Alzheimer's patients through the enhancement of central cholinergic tone. This molecule takes on added value in that it has recently been shown to exhibit neuroprotective properties (glutamate toxicity blocking activity) *in vitro*. Based upon our cumulative SAR information and to some extent the predicted binding site of HA within *Torpedo* AChE, we chose to investigate the synthesis and biology of certain C-10 substituted analogues. The important finding was made that introduction of an axial methyl group into the C-10 position of huperzine A increased the potency for AChE inhibition 8-fold; the corresponding equatorial isomer was about 1.5-fold less active than huperzine A. The introduction of substituents larger than methyl resulted in a drop in activity. For example, the ethyl analogue was found to be about 100-fold less active than huperzine A, indicating that while it is still capable of binding to *Torpedo* AChE, some steric interaction with the "walls" of the active site gorge must result. Through the use of molecular modeling methods involving the docking of these analogues to the reported X-ray crystal structure of *Torpedo* AChE, it is clearly evident that the C-10 axial methyl group points into a hydrophobic region of the enzyme, while the equatorial methyl group is directed to a less favorable hydrophilic region. Substituents larger than methyl were found to result in a conformational energy penalty. The ready explanation of this structure−activity relationship data provides further evidence in support of our modeling studies aimed at establishing huperzine A's binding site in AChE. This knowledge should facilitate the identification of other structural analogues of huperzine A likely to exhibit an improved therapeutic profile.

Introduction

Huperzine A (HA), a potent reversible inhibitor of acetylcholinesterase (AChE), is an important psychotherapeutic agent for improving cognitive function in Alzheimer's patients by enhancement of central cholinergic tone.[1] Because of the tremendous promise this alkaloid holds for the palliative treatment of a disease that afflicts millions of individuals worldwide, we have been engaged in an intensive effort to explore the structure−activity relationships of this alkaloid.[2] From biological studies with mammalian AChE, Torpedo AChE, mammalian BChEs (butyrylcholinesterases), and mouse AChE mutants together with molecular modeling studies, mammalian Tyr337(330) and Trp86(84) have been implicated in the binding of HA to AChE.[3] This particular interaction is of the cation (NH_3^+)-π type,[4] while other amino acid residues appear to participate in hydrogen bonding to the pyridone NH and the carbonyl group.[5] The superior inhibition properties of huperzine A have been attributed to the very slow dissociation ($t_{0.5}$ = 35 min) of the AChE−huperzine A complex in solution.[6]



Huperzine A (HA)

Chemical Synthesis

During our efforts to discover HA analogues of improved potency, we set about to explore in a systematic manner the

† Georgetown University Medical Center.
‡ Siena University.
§ Ohio State University.
⊥ Walter Reed Army Institute of Research.
⊗ Abstract published in *Advance ACS Abstracts,* November 1, 1996.

(1) Liu, J.-S.; Zhu, Y.-L.; Yu, C.-M.; Zhou, Y.-Z.; Han, Y.-Y.; Wu, F.-W.; Qi, B.-F. *Can. J. Chem.* **1986,** *64,* 837. Xia, Y.; Kozikowski, A. P. *J. Am. Chem. Soc.* **1989,** *111,* 4116 and references cited therein; Xu, S.-S.; Gao, Z.-X.; Weng, Z.; Du, Z.-M.; Xu, W.-A.; Yang, J.-S.; Zhang, M.-L.; Tong, Z.-H.; Fang, Y.-S.; Chai, X.-S.; Li, S.-L. *Acta Pharmacologica Sinica* **1995**, *16*, 391.

(2) Campiani, G.; Sun, L.-Q.; Kozikowski, A. P.; Aagaard, P.; McKinney, M. *J. Org. Chem.* **1993**, *58*, 7660; Kozikowski, A. P.; Campiani, G.; Nacci, V.; Sega, A.; Saxena, A.; Doctor, B. P. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1287.

(3) Saxena, A.; Qian, N.; Kovach, I. M.; Kozikowski, A. P.; Pang, Y. P.; Vellom, D. C.; Radic, Z.; Quinn, D.; Taylor, P.; Doctor, B. P. *Protein Science* **1994**, *3*, 1770. The dual numbering system provides the residue number in the species designated followed by the corresponding residue in *Torpedo* AChE.

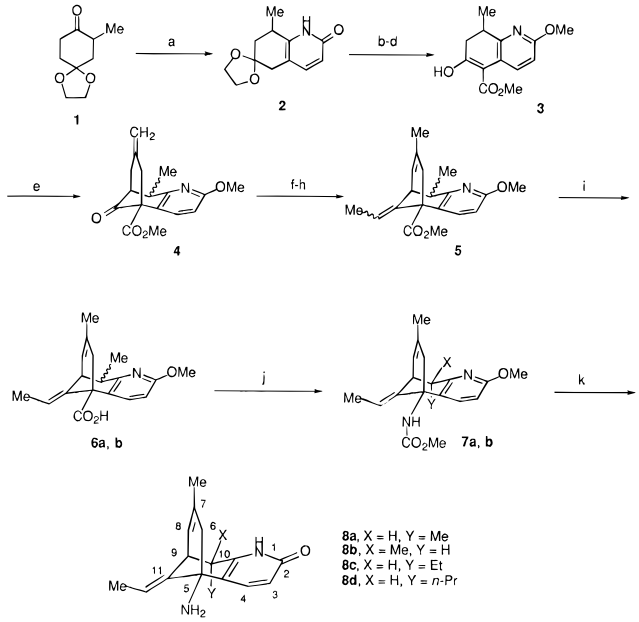
(4) Dougherty, D. A.; Stauffer, D. A. *Science* **1990**, *253*, 872.

(5) Kozikowski, A. P.; Pang, Y. P. In *Trends in QSAR and Molecular Modeling '92.* Proceedings of the 9th European Symposium on Structure−Activity Relationships: QSAR and Molecular Modeling, Wermuth, C. G., Ed.; ESCOM Science Publishers: Leiden, The Netherlands, 1993. Pang, Y.-P.; Kozikowski, A. P. *J. Comp. Aided-Mol. Design* **1994**, *8*, 669.

(6) Ashani, Y.; Peggins, J. O. III; Doctor, B. P. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 719.

S0002-7863(96)02282-2 CCC: $12.00

Scheme 1. Synthesis of the 10-Methyl Derivatives **8a** and **8b**[a]



[a] Reagents: (a) NH_3, MeOH, methyl propiolate, 135 °C, 12 h (57%); (b) Ag_2CO_3, MeI, $CHCl_3$, 87%; (c) 5% HCl, acetone (96%); (d) KH, $(MeO)_2CO$, (83%); (e) $Pd(OAc)_2$, Ph_3P, DBU, 2-methylene-1,3-propanediol diacetate (89%); (f) $EtPPh_3Br$, *n*-BuLi (79%); (g) CF_3SO_3H, dioxane, 93 °C (91%); (h) PhSH, AIBN, toluene, 85 °C (88%, *E*/*Z* = 95:5); (i) 20% NaOH, MeOH, THF (83%); (j) $(PhO)_2P(O)N_3$, Et_3N, toluene, 85 °C (82%); (*k*) TMSI, $CHCl_3$ (88%).

effect of various structural modifications to the parent molecule. Based upon our cumulative SAR information and to some extent the calculated binding site information, we now chose to explore the effect that substitution at HA's C-10 position might have on its activity. To prepare these C-10 methyl bearing analogues, cyclohexane-1,4-dione monoethylene ketal was methylated, and the resulting product **1** was converted to the ring fused pyridone **2** by reaction with ammonia and methyl propiolate in methanol (Scheme 1).[7] After conversion of the pyridone to methoxypyridine by *O*-methylation employing Ag_2CO_3/MeI, the methylated bicycle **3** was transformed to the corresponding tricycle **4** through use of the palladium catalyzed bicycloannulation strategy.[2] After further steps that follow our previously reported chemistry, the major, axial methyl isomer was obtained in pure form by fractional crystallization of **6**, while the pure equatorial isomer was obtained from the axial/equatorial mixture of **6** by prior conversion to the urethanes **7** followed by chromatographic separation and crystallization. These pure isomers were converted to the final products **8a** and **8b** using protocols previously reported.[2] The structure of these products was established rigorously through the use of NOESY and COSY experiments. For example, the irradiation of the C-10 methyl group in the urethane **7a** gives no enhancement in the signal of H-8, while irradiation of H-10 leads to an enhancement of H-8 of 4%. Likewise, irradiation of H-8 causes a 3.6% increase in the signal of H-10. In **7b**, irradiation of the C-10 methyl leads to an NOE of 4% for H-8, while irradiation of H-10 causes no change in this signal.

By carrying out an allylation reaction on the cyclohexane-1,4-dione monoethylene ketal and conducting similar chemistry, we were also able to acquire the axial 10-ethyl and 10-propyl derivatives through appropriate modifications to the allyl group. In these cases, the equatorial isomers were not isolated in pure form.

Table 1. K_I Values for the Inhibition of FBS AChE and Equine BChE by HA and Its C-10 Analogues

compound	K_I (μM) for FBS AChE[a]	K_I (μM) for equine BChE[a]
(±)-huperzine A	0.024	24
axial methyl **8a**	0.003	5.8
eq methyl **8b**	0.035	5.5
ethyl **8c**	2.04	>100
n-propyl **8d**	>200	>200
(±)-10,10-dimethylhuperzine A	0.017	9.5

[a] K_I is mean ± standard deviation; standard errors were all within 10% of the mean.

Biological Activity

K_I values for the inhibition of FBS AChE by the various inhibitors were determined by steady-state methods as reported previously.[6,9] K_I values for the inhibition of equine butyrylcholinesterase (BChE) by the various inhibitors were determined by analysis of kinetic data.[3] The results of the enzyme studies are provided in Table 1.

As can be discerned from these data, the C-10 axial methyl analogue of huperzine A is about 8-fold more potent than (±)-huperzine A. The equatorial methyl analogue, on the other hand, is about 1.5-fold less active. For purposes of comparison, data are also provided for the 10,10-dimethyl analogue.[10] As to be expected based upon the results obtained with the monomethyl analogues, this compound is comparable in activity to HA. As the size of the C-10 substituent is increased from methyl to ethyl and propyl, activity drops off dramatically, possibly indicating a serious steric interaction of this substituent with the "walls" of the active site gorge. Clearly, while some additional steric volume is available in the region of the AChE binding site occupied by the C-10 axial substituent of HA, the available space is small, with the methyl analogue serving as the optimal structure. Additionally, as is evident from the BChE activity that is presented, the methyl analogues of HA all retain their high specificity for AChE versus BChE, and, in fact, the axial methyl compound shows an improved selectivity ratio of 2000-fold relative to the 1000-fold selectivity exhibited by HA itself. The AChE versus BChE selectivity may be relevant to minimizing the peripheral effects of such agents in patients.

Molecular Modeling

To better understand the differences in activity found for the axial and equatorial methyl analogues, we carried out molecular modeling studies using as a starting point our reported binding site for HA in Torpedo AChE.[5] However, the previous binding model was refined by including crystallographic waters, a refinement that was considered essential in light of the X-ray results reported for edrophonium (EDR), tacrine (THA), and decamethonium (DECA), all of which include crystallographic waters in binding to the enzyme.[11] Through docking, energy minimization, and molecular dynamics studies, the most likely binding mode of HA in AChE is shown in Figure 1 (for details of the modeling studies and programs used, see the Experimental Section). The ammonium group of HA forms hydrogen bonds with Asp 72, Trp 84, and Asn 85 through two bridging water

(7) Kozikowski, A. P.; Reddy, E. R.; Miller, C. P. *J. Chem. Soc., Perkin Trans. 1* **1990**, 195.

(8) De La Hoz, D.; Doctor, B. P.; Ralston, J. S.; Rush, R. S.; Wolfe, A. D. *Life Sci.* **1986**, *39*, 195.

(9) Ellman, G. L.; Courtney, D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *1*, 88. Doctor, B. P.; Toker, L.; Roth, E.; Silman, I. *Anal. Biochem.* **1987**, *166*, 399.

(10) Kozikowski, A. P.; Ding, Q.; Saxena, A.; Doctor, B. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 259.

(11) Harel, H.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P. H.; Silman, I.; Sussman, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9031.

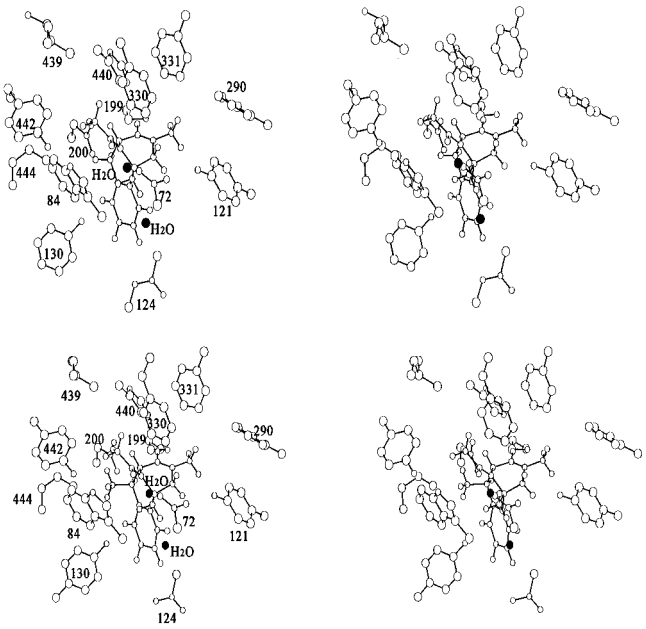


Figure 1. Stereoviews of the molecular modeling derived binding sites for huperzine A (top) and its C-10 axial methyl analogue (bottom) in complex with AChE.

molecules. These two water molecules were found to be present in all three X-ray crystal structures of AChE in complex with EDR, THA, and DECA.[11] The ammonium group of HA may also interact with the aromatic ring of Trp 84 through a cation-π interaction. The lactam NH forms an H-bond with the hydroxyl oxygen of Tyr 130. In addition, it also forms an H-bond to the carboxyl group of Glu 199. The carbonyl group of the lactam ring forms two H-bonds with the hydroxyl group of Tyr 130 and the backbone amide group of Leu 124. It is of interest to note that the site of the lactam carbonyl group of HA in the binding model was originally occupied by a water molecule in the X-ray crystal structures of AChE in complex with both EDR and THA, suggesting that this position is indeed ideal for the formation of H bonds. The hydrophobic interactions of HA with the enzyme occur primarily through five aromatic hydrophobic residues, Trp 84, Tyr 121, Phe 290, Phe 330, and Phe 331, and two aliphatic hydrophobic residues, Ile 439 and Ile 444.[12]

Upon modeling the C-10 substituted analogues of HA in AChE, we found that the presence of either one or two methyl groups at this position did not significantly alter their binding mode as compared to that of HA nor cause any significant conformational change in the protein. However, introduction of an axial ethyl group was found to cause some conformational change to Trp 84 and a significant conformational change to Glu 199. Larger alterations to the conformations of these residues were found when an *n*-propyl group was appended to the C-10 axial position of HA.

The C-10 equatorial methyl group was found to be in close contact with the amide group of Gly 117 and the carbonyl group on the backbone of His 440 as well as the side chains of Glu 199 and His 440. These groups are all hydrophilic in nature. Thus, although the equatorial methyl group does not cause any significant conformational changes in the protein, the hydrophobic−hydrophilic contact is not beneficial to the overall binding energy. In contrast, the C-10 axial methyl group was found to interact with the hydrophobic side chains of Trp 84 and Ile 444.

Taken together, these modeling results nicely explain the improved activity of the C-10 axial methyl derivative of HA compared to HA and its C-10 equatorial methyl counterpart. Within the vicinity of the C-10 equatorial face of HA, the enzyme is polar and hydrophilic in nature, whereas within the vicinity of the C-10 axial face of HA the enzyme is hydrophobic in nature. While the enzyme contains sufficient void volume to accommodate methyl substitution on either face of HA, only the axial methyl compound leads to favorable hydrophobic−hydrophobic interactions. While larger alkyl groups at the C-10 axial position would be capable of further enhancing such hydrophobic contacts, the void volume is limited, resulting in a conformational energy penalty that overwhelms these favorable hydrophobic interactions.

This work reveals that it is indeed possible to improve on the biological activity of a product derived from nature through a rather minor structural change. The work reveals the type of modification that confers increased AChE inhibitory potency to the product; specifically, a lipophilic substituent capable of providing additional hydrophobic contacts with the enzyme. Additionally, in view of the fact that the calculated log *P* of the methyl analogue is 0.82 versus 0.44 for huperzine,[13] the new analogue may exhibit an improved therapeutic profile through its increased ability to penetrate the blood brain barrier.[14]

The present work confirms and expands our molecular modeling based approach to the design of improved HA-based cholinesterase inhibitors. Based upon the binding model, we have designed ligands with additional enhanced binding features, and these results will be reported in due course.

Experimental Section

1. Molecular Modeling Methods. All the molecular modeling studies were carried out using the QUANTA molecular modeling package[15] running on a Silicon Graphics Indigo with IRIS5.3. All the energy calculations and molecular dynamics simulations were performed using a stand-alone version of the CHARMM program[16] (version 24), with the version 22 MSI parameter set, running on a Deck Alpha machine with two CPU processors. An adopted-basis Newton-Raphson algorithm, implemented in the CHARMM program, was used in the energy minimization. Energy was first minimized for 5000 steps for the whole system without any constraints. The residues 20 Å away from the surface of the inhibitor were then fixed, and the system was reminimized for another 5000 steps, or until convergence, defined as an energy gradient tolerance $\leq$0.001 kcal mol^{-1} Å^{-1}. A constant dielectric was used and set to 1. The nonbonded cutoff distance was set to 14.0 Å. A shifted smoothing function was used for the van der Waals interaction and a switch function for the electrostatic energy. The cutoff distance parameters used in the smoothing functions are as follows: CTOFNB = 12.0 Å; CTONNB = 8.0 Å.

In the molecular dynamics simulations, the system, which includes the protein, the inhibitor, and crystallographic waters, was heated to 300 K in a period of 5 ps and equilibrated for 20 ps at 300 K. Finally, 100 ps constant temperature simulations were performed at 300 K with a step size of 0.001 ps. All the residues 10 Å away from the surface of the inhibitor were fixed in the dynamics simulations. Trajectories

(12) The molecular modeling studies appear consistent with our preliminary X-ray findings: Raves, M; Harel, H.; Pang, Y.-P.; Silman, I.; Kozikowski, A. P.; Sussman, J., unpublished results. For a recent paper on the X-ray structure of AChE in complex with a transition state analogue inhibitor, see: Harel, M.; Quinn, D. M.; Nair, H. K.; Silman, I.; Sussman, J. L. *J. Am. Chem. Soc.* **1996**, *118*, 2340.

(13) The log *P* values were calculated using the PALLAS FRAME MODULE, a prediction tool of physicochemical parameters supplied by CompuDrug Chemistry, Ltd., P.O. Box 23196, Rochester, NY 14692.

(14) Brewster, M. E.; Pop, E.; Bodor, N. In *Drug Design for Neuroscience*; Kozikowski, A. P., Ed.; Raven Press: New York, 1993.

(15) QUANTA, a molecular modeling system, is supplied by Molecular Simulations Inc., 200 Fifth Avenue, Waltham, MA 01803-5279.

(16) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.

were recorded every 0.1 ps during the final 100 ps simulations and analyzed. A shake algorithm was used to constrain bonds to hydrogen.[17]

The X-ray structure[11] of AChE (E.C.3.1.1.7) in complex with the inhibitor, decamethonium, was used as the initial structure in our docking, energy minimization, and molecular dynamics simulation studies. The previously reported structure of HA in complex with AChE, derived from a docking study,[5] served as a reference structure in the present study. All 66 crystallographic waters were retained in the modeling studies, except for two waters, whose positions were found to be occupied by HA. The TIP3P water model[18] was used to treat these water molecules in all the calculations. The protonation state for each residue of the protein was assigned according to Gilson *et al*.[19] The three-dimensional structures of HA and its analogues were built using the ChemNote module in the QUANTA program, and each structure was fully minimized using the Gaussian 92 program[20] with the 3-21G* basis set. The primary amine group in HA and its analogues was set to be protonated, as expected under conditions of physiological pH. The Mulliken atomic charges for HA and its analogues, which were used to build the Residue Topology File (RTF) for each inhibitor, were calculated using the Gaussian 92 program[20] with the 6-31G* basis set.

2. General Synthesis Methods. Melting points were determined using an Electrothermal 8103 apparatus and are uncorrected. IR spectra were taken with Perkin-Elmer 398 and FT 1600 spectrophotometers. ^{1}H NMR spectra were recorded on a Bruker 200 MHz spectrometer with TMS as internal standard; the values of chemical shifts (δ) are given in ppm. All reactions were carried out in an argon atmosphere. Progress of the reactions was monitored by TLC on silica gel plates (Riedel-de-Haen, Art. 37341). Merck silica gel (Kieselgel 60) was used for flash chromatography (230−400 mesh) columns. Extracts were dried over $MgSO_4$, and solvents were removed under reduced pressure. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer, and the results are within ±0.4% of the theoretical values. Yields refer to purified products and are not optimized.

(±)-5-(Ethylenedioxy)-1-methyl-2-oxocyclohexanecarboxylic Acid Methyl Ester. To a suspension of NaH (696 mg, 29 mmol) in anhydrous benzene (50 mL) and DMF (50 mL) cooled to 0 °C was slowly added a solution of 5-(ethylenedioxy)-2-oxocyclohexanecarboxylic acid methyl ester (6.0 g, 28 mmol) in the same solvent mixture under argon. The mixture was allowed to stir at room temperature for 1 h, then MeI (5.0 mL, 87 mmol) was added dropwise, and stirring was continued at room temperature for 2 h. The reaction was quenched with water (10 mL), and the solvent was evaporated. The residue was extracted with EtOAc, and the organic layers were washed with brine, dried, and concentrated. Flash chromatography (25% EtOAc in hexanes) gave 6.15 g (96%) of the title compound as a waxy solid: R_f 0.28 (25% EtOAc in hexanes); IR (neat) 1742, 1720, 1425 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.03 (m, 4 H), 3.72 (s, 3 H), 3.08 (m, 1 H), 2.68 (dt, 1 H, J = 1.4, 13.6 Hz), 2.50 (dt, 1 H, J = 4.0, 14.8 Hz), 2.01 (m, 2 H), 1.74 (d, 1 H, J = 13.8 Hz), 1.29 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 206.9, 173.5, 106.3, 64.6, 64.0, 54.3, 52.1, 43.4, 37.1, 34.9, 21.4. Anal. Calcd for $C_{11}H_{16}O_5$: C, 57.89; H, 7.07. Found: C, 57.63; H, 6.99.

(±)-2-Methylcyclohexane-1,4-dione 4-Monoethylene Ketal (1). A mixture of the above β-ketoester (2.00 g, 8.76 mmol), LiI (7.03 g, 52.5 mmol), and anhydrous freshly distilled DMF (30 mL) was heated at 130 °C for 6 h. After cooling to room temperature, the reaction mixture was poured onto crushed ice and stirred for 12 h. The mixture was extracted with EtOAc, and the organic layers were washed with brine, dried, and evaporated. The residue was purified by flash chromatography (40% EtOAc in hexanes) to give 1.28 g (86%) of **1** as a waxy solid: R_f 0.42 (40% EtOAc in hexanes); IR ($CHCl_3$) 1740, 1718 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.06 (m, 4 H), 2.67 (m, 2 H), 2.35 (ddd, 1 H, J = 2.9, 4.8, 14.6 Hz), 2.06 (m, 3 H), 1.75 (d, 1 H, J = 13.5 Hz), 1.03 (s, 3 H), 1.00 (s, 3 H). Anal. Calcd for $C_9H_{14}O_3$: C, 63.51; H, 8.29. Found: C, 63.58; H, 8.30.

(±)-1′,5′,7′,8′-Tetrahydro-8′-methylspiro[1,3-dioxolane-2,6′(2′*H*)-quinolin]-2′-one (2). A mixture of ketone **1** (100 mg, 0.58 mmol) and methyl propiolate (98.7 mg, 1.17 mmol) in concentrated methanolic ammonia (2 mL, approximately 8 M) was heated at 135 °C in a resealable tube under argon for 12 h. After cooling, the solvent was removed under reduced pressure, and the crude product was purified by flash chromatography (20% MeOH in EtOAc) to afford 73 mg (57%) of **2** as pale yellow crystals: mp 176−177 °C (EtOAc); IR (KBr) 3440, 1650, 1609 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 12.23 (br s, 1 H), 7.13 (d, 1 H, J = 9.5 Hz), 6.40 (d, 1 H, J = 9.2 Hz), 4.02 (m, 4 H), 3.11 (m, 1 H), 2.70 (q, 1 H, J = 16.0 Hz), 2.05 (dd, 1 H, J = 7.2, 5.6 Hz), 1.74 (dd, 1 H, J = 13.5, 5.5 Hz), 1.46 (d, 3 H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 164.8, 145.5. 143.3, 117.8, 111.1, 107.2, 64.6, 64.4, 38.7, 36.8, 31.3, 19.9. Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.33; H, 6.92; N, 6.12.

(±)-7′,8′-Dihydro-2′-methoxy-8′-methylspiro[1,3-dioxolane-2,6′-(5′*H*)-quinoline]. A mixture of Ag_2CO_3 (1.40 g, 5.0 mmol), pyridone **2** (0.93 g, 4.2 mmol), and MeI (1.54 mL, 25.2 mmol) in dry $CHCl_3$ (53 mL) was refluxed in the dark for 2 h. After cooling, the suspension was filtered with suction over Celite. The filtrate was concentrated, and the residue was chromatographed (25% EtOAc in hexanes) to give 850 mg (86%) of the title compound as a waxy solid: R_f 0.41 (25% EtOAc in hexanes); IR ($CHCl_3$) 2955, 2880, 1580 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.20 (d, 1 H, J = 8.3 Hz), 6.50 (d, 1 H, J = 8.2 Hz), 4.02 (m, 4 H), 3.90 (s, 3 H), 3.23 (m, 1 H), 3.00 (d, 1 H, J = 16.4 Hz), 2.81 (dd, 1 H, J = 16.4, 2.3 Hz), 2.12 (ddd, 1 H, J = 13.3, 6.6, 2.8 Hz), 1.74 (dd, 1 H, J = 13.3, 10.8 Hz), 1.43 (d, 3 H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 161.8, 156.1, 139.4, 120.7, 107.8, 107.5, 64.5, 64.4, 52.8, 40.1, 38.1, 34.8, 19.5. Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.58; H, 7.33; N, 6.04.

(±)-7,8-Dihydro-2-methoxy-8-methylquinolin-6(5*H*)-one. To a solution of the above quinoline (1.01 g, 4.35 mmol) in acetone (11.6 mL) was added 5% HCl (11.6 mL), and the mixture was refluxed for 3 h. The acetone was removed under vacuum, and the aqueous layer was neutralized with 10% $NaHCO_3$ solution and extracted with EtOAc. The organic layers were washed with brine, dried, and concentrated. The residue was purified by flash chromatography (20% EtOAc in hexanes) to give 800 mg (96%) of the title compound as colorless prisms: mp 82−83 °C (5% EtOAc in hexanes); R_f 0.28 (10% EtOAc in hexanes); IR (KBr) 1640, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.29 (d, 1 H, J = 8.1 Hz), 6.59 (d, 1 H, J = 8.2 Hz), 3.94 (s, 3 H), 3.51 (s, 2 H), 3.30 (m, 1 H), 2.79 (dd, 1 H, J = 15.8, 5.3 Hz), 2.42 (dd, 1 H, J = 15.8, 7.6 Hz), 1.36 (d, 3 H, J = 7.0 Hz). Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.11; H, 6.80; N, 7.33. Found: C, 68.82; H, 6.73; N, 7.19.

(±)-5,6,7,8-Tetrahydro-2-methoxy-8-methyl-6-oxoquinoline-5-carboxylic Acid Methyl Ester (3). To a refluxing suspension of KH (1.35 g, 11.8 mmol, 35% in oil) in anhydrous $(MeO)_2CO$ (19 mL) was added a solution of the above methylquinolin-6(5*H*)-one (0.75 g, 3.94 mmol) in anhydrous $(MeO)_2CO$ (4.7 mL) over 1 h. The reaction mixture was refluxed for an additional 2 h, cooled to room temperature, and quenched with MeOH (5 mL). The solvent was removed, and the residue was taken up in EtOAc. The solution was washed with brine, dried, and concentrated. Flash chromatography of the residue (8% EtOAc in hexanes) afforded 816 mg (83%) of **3** as a colorless oil: R_f 0.25 (8% EtOAc in hexanes); IR (neat) 2930, 1640, 827 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 13.14 (s, 1 H), 7.92 (d, 1 H, J = 8.8 Hz), 6.55 (d, 1 H, J = 8.7 Hz), 3.92 (s, 3 H), 3.90 (s, 3 H), 3.08 (m, 1 H), 2.76 (dd, 1 H, J = 16.7, 6.8 Hz), 2.39 (dd, 1 H, J = 16.7, 7.9 Hz), 1.32 (d, 3 H, J = 6.9 Hz). Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.65; H, 6.02; N, 5.62. Found: C, 62.73; H, 6.08; N, 5.58.

(±)-7,8,9,10-Tetrahydro-2-methoxy-10-methyl-7-methylene-11-oxo-5,9-methanocycloocta[*b*]pyridine-5(6*H*)-carboxylic Acid Methyl Ester (4). $Pd(OAc)_2$ (29 mg, 0.13 mmol) and PPh_3 (0.14 g, 0.52 mmol) were stirred at room temperature in dry dioxane (12 mL) for 30 min. A solution of the β-keto ester **3** (0.61 g, 2.45 mmol), DBU (0.51 mL, 2.74 mmol), and 2-methylene-1,3-propanediol diacetate (0.39 mL, 2.45 mmol) in dry dioxane (4.5 mL) was added dropwise to the complex thus obtained over a period of 10 min. After stirring for 20 min at room temperature, a solution of DBU (0.25 mL, 1.7 mmol) in dry

(17) (a) van Gunsteren, W. F. and Berendsen, H. J. C. *Mol. Phys*. **1977**, *34*, 1311.(b) Rychaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys*. **1977**, *23*, 327.

(18) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926.

(19) Glison, M. K.; Straatsma, T. P.; McCammon, J. A.; Ripoll, C. H.; Axelsen, P. H.; Silman, I.; Sussman, J. L. *Science* **1994**, *263*, 1276.

(20) Gussian 92/DFT, Revision G.4: Frisch, M. J.; Truck, G. W.; Schlegel, H. B.; Robb, M. A.; Head-Gordon, M.; Replogle, E. S.; Gomperts, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. Gaussian, Inc.: Pittsburgh, PA, 1993.

dioxane (2 mL) was added dropwise. After stirring at room temperature for 20 min, the mixture was refluxed for 3 h. Concentration and flash chromatography (10% EtOAc in hexanes) gave 0.66 g (89%) of **4** as a mixture of two diastereoisomers (70/30). Major diastereoisomer (ax-C(10)Me, less polar): mp 112−113 °C (from hexanes); R_f 0.18 (10% EtOAc in hexanes); IR (neat) 1741, 1728, 1589, 1479, 1116, 738 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.97 (d, 1 H, J = 8.7 Hz), 6.57 (d, 1 H, J = 8.7 Hz), 4.76 (m, 1 H), 4.43 (m, 1 H), 3.89 (s, 3 H), 3.79 (s, 3 H), 3.21 (q, 1 H, J = 7.3 Hz), 3.12 (d, 1 H, J = 12.2 Hz), 2.72 (m, 2 H), 2.58 (d, 2 H, J = 13.6 Hz), 1.30 (d, 3 H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 208.3, 171.4, 163.0, 156.1, 138.9, 137.5, 123.5, 115.8, 109.8, 62.3, 53.3, 53.1, 52.6, 47.8, 46.8, 43.0, 23.1. Anal. Calcd for $C_{17}H_{19}NO_4$: C, 67.77; H, 6.31; N, 4.65. Found: C, 67.90; H, 6.34; N, 4.59. Minor diastereoisomer (eq-C(10)Me, more polar): R_f 0.15 (10% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 6.94 (d, 1 H, J = 7.9 Hz), 6.57 (d, 1 H, J = 8.5 Hz), 4.75 (m, 1 H), 4.45 (m, 1 H), 3.89 (s, 3 H), 3.79 (s, 3 H), 3.44 (m, 1 H), 3.17 (d, 1 H, J = 14.2 Hz), 2.84 (m, 2 H), 2.59 (dd, 2 H, J = 13.8, 2.8 Hz), 1.51 (d, 3 H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 208.1, 171.3, 162.6, 154.9, 139.1, 137.1, 124.4, 115.7, 109.6, 63.1, 53.1, 52.5, 51.1, 48.2, 40.7, 36.5, 15.2.

(11*Z*)-(±)-11-Ethylidene-7,8,9,10-tetrahydro-10-methyl-7-methylene-2-methoxy-5,9-methanocycloocta[*b*]pyridine-5(6*H*)-carboxylic Acid Methyl Ester. To a suspension of $EtPPh_3Br$ (574 mg, 1.55 mmol) in dry THF (5.6 mL) was added *n*-BuLi (0.72 mL, 1.32 mmol, 2.5 M in hexane) within 10 min. The resulting orange-colored suspension was stirred at room temperature for 70 min. After cooling to 0 °C, a solution of β-ketoester **4** (100 mg, 0.33 mmol) in dry THF (1.5 mL) was slowly added over a period of 15 min. The resulting mixture was allowed to warm to room temperature and stirred at room temperature for 2 h. The reaction was quenched with water, the THF was removed by rotary evaporation, and the aqueous residue was extracted with EtOAc. The organic layers were washed with brine, dried, and concentrated. Flash chromatography of the residue (20% EtOAc in hexanes) gave 245 mg (79%) of the olefin as a colorless oil which was a mixture of diastereoisomers (E/Z = 10/90). Major diastereoisomer (less polar): R_f 0.49 (20% EtOAc in hexanes); IR (neat) 2945, 1730, 1661, 1601, 1578 cm^{-1}; ^{1}H NMR ((Z)-olefin, $CDCl_3$) δ 6.83 (d, 1 H, J = 8.8 Hz), 6.41 (d, 1 H, J = 7.9 Hz), 5.49 (q, 1 H, J = 7.6 Hz), 4.47 (m, 1 H), 4.19 (m, 1 H), 3.83 (s, 3 H), 3.67 (s, 3 H), 2.73 (d, 1 H, J = 7.3 Hz), 2.53 (m, 1 H), 2.38 (m, 2 H), 2.10 (s, 2 H), 1.51 (d, 3 H, J = 7.0 Hz), 1.31 (d, 3 H, J = 7.1 Hz). Anal. Calcd for $C_{19}H_{23}NO_3$: C, 72.84; H, 7.35; N, 4.47. Found: C, 73.03; H, 7.41; N, 4.59.

(11*Z*)-(±)-11-Ethylidene-9,10-dihydro-2-methoxy-7,10-dimethyl-5,9-methanocycloocta[*b*]pyridine-5(6*H*)-carboxylic Acid Methyl Ester. A mixture of the above olefin (11 mg, 0.035 mmol), CF_3SO_3H (46 μL, 0.055 mmol), and dry dioxane (0.3 mL) was heated at 93 °C in a resealable tube for 12 h. The solvent was removed, and the residue was partitioned between aqueous $NaHCO_3$ and EtOAc. The organic phase was washed with brine, dried, and filtered. Concentration and flash chromatography (20% EtOAc in hexanes) gave 10 mg (91%) of the title compound as a colorless oil which was a mixture of diastereoisomers. Major diastereoisomer: R_f 0.46 (20% EtOAc in hexanes); IR (neat) 1730, 1661, 1601, 1578 cm^{-1}; ^{1}H NMR ((Z)-olefin, CDC l$_3$) δ 7.02 (d, 1 H, J = 8.7 Hz), 6.44 (d, 1 H, J = 8.3 Hz), 5.28 (m, 2 H), 3.83 (s, 3 H), 3.62 (s, 3 H), 3.03 (m, 1 H), 2.85 (m, 1 H), 2.63 (d, 1 H, J = 4.7 Hz), 2.16 (d, 1 H, J = 11.3 Hz), 1.46 (d, 3 H, J = 6.8 Hz), 1.41 (s, 3 H), 1.22 (d, 3 H, J = 6.9 Hz). Anal. Calcd for $C_{19}H_{23}NO_3$: C, 72.84; H, 7.35; N, 4.47. Found: C, 72.87; H, 7.29; N, 4.41.

(11*E*)-(±)-11-Ethylidene-9,10-dihydro-2-methoxy-7,10-dimethyl-5,9-methanocycloocta[*b*]pyridine-5(6*H*)-carboxylic Acid Methyl Ester (5). To a solution of the above olefin mixture (E/Z = 10/90, 270 mg, 0.86 mmol) in dry toluene (1.9 mL) were added AIBN (105 mg, 0.62 mmol) and PhSH (134 μL, 1.29 mmol). The resulting solution was heated at 85 °C for 21.5 h. Evaporation of the solvent afforded a residue which was dissolved in CH_2Cl_2, washed with brine, and dried. After concentration, the crude product was chromatographed (15% EtOAc in hexanes) to afford 237 mg (88%) of **5** as a colorless oil, which by ^{1}H NMR analysis consisted of a 95/5 mixture of the (E)- and (Z)-alkenes. Major diastereoisomer: R_f 0.42 (15% EtOAc in hexanes); ^{1}H NMR ((E)-olefin, $CDCl_3$) δ 7.05 (d, 1 H, J = 8.1 Hz), 6.50 (d, 1 H, J = 8.8 Hz), 5.42 (m, 1 H), 5.18 (q, 1 H, J = 6.6 Hz), 3.89 (s, 3 H), 3.73 (s, 3 H), 3.31 (d, 1 H, J = 4.6 Hz), 3.10 (m, 2 H), 2.18 (d, 1 H, J = 16.9 Hz), 1.68 (d, 3 H, J = 6.8 Hz), 1.51 (s, 3 H), 1.27 (d, 3 H, J = 6.9 Hz).

(11*E*)-(±)-11-Ethylidene-9,10-dihydro-2-methoxy-7,10-dimethyl-5,9-methanocycloocta[*b*]pyridine-5(6*H*)-carboxylic Acid (6). Ester **5** (237 mg, 0.76 mmol, E/Z = 95/5) was dissolved in 1.8 mL of MeOH/THF 2:1, and 20% NaOH (0.6 mL) was added. The mixture was heated under reflux for 26 h. After cooling, the solution was adjusted to pH 5−6 with 5% HCl, and MeOH and THF were evaporated. The aqueous residue was extracted with EtOAc. The organic layers were washed with brine, dried, and concentrated. The crude product was purified by flash chromatography (EtOAc) to give 211 mg (83%) of the acid **6** as colorless prisms. Crystallization from acetone/hexanes afforded 83 mg of the pure major ax-C(10)Me diastereoisomer: mp 220 °C (dec) (from EtOAc); R_f 0.58 (EtOAc); IR (KBr) 2941, 1706, 1599, 1425, 746 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.22 (d, 1 H, J = 8.8 Hz), 6.55 (d, 1 H, J = 8.8 Hz), 5.47 (m, 2 H), 3.90 (s, 3 H), 3.34 (d, 1 H, J = 5.2 Hz), 3.00 (m, 2 H), 2.13 (d, 1 H, J = 16.9 Hz), 1.72 (d, 3 H, J = 6.3 Hz), 1.52 (s, 3 H), 1.29 (d, 3 H, J = 7.0 Hz); ^{13}C NMR δ 179.2, 162.8, 157.4, 137.8, 134.7, 132.2, 126.5, 124.9, 116.6, 108.5, 54.8, 53.3, 45.2, 44.0, 39.3, 22.7, 20.3, 13.2. Anal. Calcd for $C_{18}H_{21}NO_3$: C, 72.24; H, 7.02; N, 4.68. Found: C, 72.47; H, 7.19; N, 4.46.

(11*E*)-(±)-[11-Ethylidene-9,10-dihydro-2-methoxy-7,10-dimethyl-5,9-methanocycloocta[*b*]pyridin-5(6*H*)-yl]carbamic Acid Methyl Ester, Axial Isomer (7a). A mixture of ax-C(10)Me acid **6** (30 mg, 0.1 mmol), dry Et_3N (13.7 μL, 0.1 mmol), $(PhO)_2P(O)N_3$ (21.3 μL, 0.1 mmol), and dry toluene (0.36 mL) was heated at 85 °C for 3 h. After cooling, the solvent was removed, and the residue was dissolved in dry MeOH (0.36 mL). The resulting solution was heated under reflux for 18 h. Evaporation and flash chromatography (10% EtOAc in CH_2Cl_2) gave the urethane **7a** (27 mg, 82%) as a colorless solid: mp 173−174 °C (from hexanes); R_f 0.29 (10% EtOAc in CH_2Cl_2); IR (KBr) 3325, 1712, 1599, 1531, 1475, 1041 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.51 (d, 1 H, J = 8.6 Hz), 6.52 (d, 1 H, J = 8.8 Hz), 5.53 (q, 1 H, J = 6.8 Hz), 5.48 (m, 1 H), 4.99 (s, 1 H), 3.88 (s, 3 H), 3.61 (s, 3 H), 3.38 (d, 1 H, J = 4.8 Hz), 2.94 (dq, 1 H, J = 5.4, 1.4 Hz), 2.49 (d, 1 H, J = 13.4 Hz), 2.15 (d, 1 H, J = 15.5 Hz), 1.71 (d, 3 H, J = 6.8 Hz), 1.49 (s, 3 H), 1.27 (d, 3 H, J = 6.9 Hz); MS m/z 328 (48%, M$^+$), 313, 281, 253, 238 (100%), 224. Anal. Calcd for $C_{19}H_{24}N_2O_3$: C, 69.49; H, 7.37; N, 8.53. Found: C, 69.77; H, 7.52; N, 8.66.

(11*E*)-(±)-5-Amino-11-ethylidene-5,6,9,10-tetrahydro-7,10-dimethyl-5,9-methanocycloocta[b]pyridin-2(1*H*)-one, Axial Isomer (8a). Me_3SiI (94 μL, 0.67 mmol) was added to a solution of carbamate **7a** (22 mg, 0.067 mmol) in dry $CHCl_3$ (2.4 mL) at room temperature, and the solution was refluxed for 6 h. After cooling and evaporation, the residue was dissolved in MeOH (2.4 mL), and the solution refluxed for 18 h. Evaporation of the solvent afforded a residue which was partitioned between 10% $NaHCO_3$ solution and $CHCl_3$. The organic layers were washed with brine, dried, and concentrated. Flash chromatography (15% MeOH in $CHCl_3$) gave 15 mg (88%) of **8a** as colorless prisms: mp 289−290 °C (EtOAc); R_f 0.22 (15% MeOH in $CHCl_3$); IR (KBr) 3420, 1656, 1618 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.87 (d, 1 H, J = 9.0 Hz), 6.42 (d, 1 H, J = 9.7 Hz), 5.66 (q, 1 H, J = 6.8 Hz), 5.43 (d, 1 H, J = 5.1 Hz), 3.30 (d, 1 H, J = 5.1 Hz), 2.93 (br q, 1 H, J = 6.4 Hz), 2.08 (s, 2 H), 1.70 (d, 3 H, J = 6.7 Hz), 1.52 (s, 3 H), 1.26 (d, 3 H, J = 7.1 Hz); a better resolved signal for 10-H was obtained in $CDCl_3/CD_3OD$ 1:1: δ 2.70 (dq, 1 H, J = 6.8, 1.3 Hz); ^{13}C NMR ($CDCl_3$) δ 165.5, 147.6, 140.4, 140.3, 133.7, 124.7, 121.4, 117.4, 113.1, 54.6, 49.7, 40.5, 40.1, 22.5, 20.0, 13.0; ^{13}C NMR (CD_3OD) δ 165.9, 148.8, 141.9, 139.7, 135.0, 125.6, 123.7, 118.1, 115.0, 55.5, 41.8, 40.9, 22.6, 20.2, 13.2; MS m/z 256 (82%, M$^+$), 241 (100%), 227, 226, 211, 201, 174, 161. Anal. Calcd for $C_{16}H_{20}N_2O$: C, 74.97; H, 7.86; N, 10.93. Found: C, 75.22; H, 8.00; N, 10.92.

(11*E*)-(±)-[11-Ethylidene-9,10-dihydro-2-methoxy-7,10-dimethyl-5,9-methanocycloocta[*b*]pyridin-5(6*H*)-yl]carbamic Acid Methyl Ester, Equatorial Isomer (7b). Starting from the stereoisomeric mixture of carboxylic acids **6**, a stereoisomeric mixture of methyl carbamates was obtained following the same procedure as above. From this mixture, **7b** was isolated in 28% yield by crystallization from hexane: colorless prisms, mp 189−190 °C (hexane); R_f 0.31 (10% EtOAc in CH_2Cl_2); IR (KBr) 1715, 1601, 1531, 1028 cm^{-1}; ^{1}H NMR

($CDCl_3$) δ 7.54 (d, 1 H,J = 8.2 Hz), 6.53 (d, 1 H, J = 7.9 Hz), 5.49 (m, 1 H), 5.32 (q, 1 H, J = 6.7 Hz), 4.98 (s, 1 H), 3.88 (s, 3 H), 3.61 (s, 3 H), 3.51 (m, 1 H), 3.07 (m, 1 H), 2.62 (d, 1 H, J = 15.4 Hz), 2.24 (d, 1 H, J = 15.7 Hz), 1.71 (d, 3 H, J = 6.8 Hz), 1.52 (s, 3 H), 1.43 (d, 3 H, J = 7.4 Hz). Anal. Calcd. for $C_{19}H_{24}N_2O_3$: C, 69.49; H, 7.37; N, 8.53. Found: C, 69.73; H, 7.41; N, 8.49.

(11*E*)-(±)-5-Amino-11-ethylidene-5,6,9,10-tetrahydro-7,10-dimethyl-5,9-methanocycloocta[*b*]pyridin-2(1*H*)-one, Equatorial Isomer (8b). Starting from **7b** (14 mg, 0.043 mmol), the same procedure as for the axial isomer was followed to obtain 8 mg (74%) of the product: colorless prisms, mp 264−265 °C (EtOAc); R_f 0.25 (15% MeOH in $CHCl_3$); IR (KBr) 3420, 1653, 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.61 (br s, 1 H), 7.87 (d, 1 H, J = 9.8 Hz), 6.40 (d, 1 H, J = 9.7 Hz), 5.45 (m, 2 H), 3.41 (t, 1 H, J = 4.7 Hz), 2.96 (m, 1 H), 2.15 (ABq, 2 H, J = 16.8 Hz), 1.67 (d, 3 H, J = 6.8 Hz), 1.59 (s, 3 H), 1.42 (d, 3 H, J = 7.1 Hz); MS *m/z* 256 (M^+, 90%), 241 (100%), 227, 224, 201, 174, 161. Anal. Calcd. for $C_{16}H_{20}N_2O$: C, 75.13; H, 7.80; N, 10.97. Found: C, 75.22; H, 8.00; N, 10.92.

Stereochemical Assignments. The stereochemistry of the methyl group at C-10 of **6**, **7**, and **8** was established by ^1H NMR decoupling and COSY experiments in addition to the NOESY experiments discussed in the text and tabulated at the end of this section. In **8**, the protons at C-6 were assigned by their diagnostic chemical shifts (^1H NMR in CD_3OD shows an ABq at 2.22 ppm, while in $CDCl_3$ the same protons appear as a singlet at 2.08 ppm). In 1/1 $CDCl_3$:CD_3OD as a solvent, a correlation between 9-H and 8-H was observed. These protons exhibit broad doublets with J values of 5.0 (9-H, 3.30 ppm) and 4.9 (8-H, 5.43 ppm) Hz, respectively. Furthermore, a long range coupling exists between 8-H and one of the protons at C(6). One of the protons at C(10) appeared as a doublet of quartets at 2.70 ppm. The smaller (doublet) coupling is of the magnitude expected for equatorial protons at C(9) and at C(10) (1.3 Hz); if the proton at C(10) was in the axial position, no coupling with 9-H would have been observed, due to a dihedral angle of about 80°. In consequence, the C(10) methyl group is axial.

The NOE data are shown in Table 2.

Table 2.

irradiation	observed	NOE%
Me-Axial, Urethane **7a**		
Me(10)	H-8	
H-10	H-8	4.3_5
H-9	H-8	11.1_5
H-8	H-10	3.6
Me-Equatorial, Urethane **7b**		
Me(10)	H-8	4.0
H-10	H-8	
H-9	H-8	9.8
H-8	H-10	

Acknowledgment. We are indebted to the National Institute on Aging and Interneuron Pharmaceuticals for partial support of this work. We also thank Professor Alessandro Sega of the Instituto di Chimica Organica, Siena University for his assistance with the NMR studies.

JA9622822