(11), 137.58 (8), 150.07 (13); MS (m/z) 282 ($M^{\cdot+}$, 100), 254, 253, 225, 130; HRMS 282.2032; calcd for $C_{19}H_{26}N_2$ 282.2094.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of **2-5**, **9**, **10b**, **11**, and **12** and ^1H NMR spectrum of **10a** (9 pages). Ordering information is given on any current masthead page.

Synthesis of Huperzine A and Its Analogues and Their Anticholinesterase Activity

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Huperzine A is a new alkaloid isolated from the club moss *Huperzia serrata* (Thunb.) Trev., a Chinese folk medicine. This alkaloid exhibits potent activity as an inhibitor of acetylcholinesterase. Consequently, the compound is presently being investigated in China for the treatment of individuals suffering from various forms of memory impairment including Alzheimer's dementia. Details of the total synthesis of (±)-huperzine A are described as well as the preparation of a variety of huperzine analogues including its presumed pharmacophore. The extent of these new compounds to inhibit acetylcholinesterase is presented along with a discussion of the effects of the structural changes on biological activity.

Introduction

Huperzine A (1) and B (2) are two new alkaloids isolated from *Huperzia serrata* (Thunb.) Trev. = *Lycopodium serratum* Thunb., a Chinese folk medicine (Qian Ceng Ta).[1] The structures of 1 and 2 have been determined by chemical and spectroscopic studies to be as shown in Figure 1. While huperzine A would appear to be closely related to another pyridone-containing alkaloid, selagine (3), a compound whose structure was elucidated in 1960 by Wiesner and co-workers,[2] recent studies have revealed the earlier structural assignment, i.e., 3, to be incorrect. The alkaloid isolated from *L. selago* L. and named selagine is, in fact, *identical* with huperzine A.[2]

Pharmacologically, huperzines A and B have been found to exhibit potent anticholinesterase activity: the pI_{50}s (negative logarithm of the molar concentration causing 50% inhibition) of huperzine A toward erythrocyte membrane and caudate nuclei acetylcholinesterase are 7.2 and 7.9, respectively.[3] Huperzine A is thus about 3 times more potent than physostigmine as an inhibitor of acetylcholinesterase but is less potent than physostigmine when tested against butyrylcholinesterase. The pI_{50}s of huperzine B toward erythrocyte and caudate acetylcholinesterase are 6.1 and 6.2, respectively. The rank order of antiacetylcholinesterase activity of huperzines A and B, physostigmine, and neostigmine are huperzine A > physostigmine > neostigmine > huperzine B.

Inhibitors of AChE range from some of the most toxic agents ever synthesized by man (VX, Sarin, and Soman) to the useful therapeutic agents physostigmine and neostigmine, compounds that find use in the treatment of glaucoma and myasthenia gravis, respectively (Figure 2).[4] Since a deficiency in the cholinergic system is believed to constitute one of the hallmarks of Alzheimer's dementia, reversible inhibitors of AChE that can make their way into the central nervous system may serve as palliative agents in the treatment of the disease.[5] In this regard a number of promising studies have begun to appear in the literature that support the clinical utility of huperzine A.[6c,d]

In experiments using the Y-maze, 167 μg/kg intraperitoneal administration of huperzine A was found to facilitate learning and retrieval in rats.[6a] In squirrel monkeys the intramuscular injection of huperzine A was found to improve the accuracy of retention by 5-13% at doses of 0.003-0.03 mg/kg.[6b] Furthermore, compound 1 has been tested clinically in the treatment of human memory impairment[6c] and myasthenia gravis.[6d] In a clinical study of 100 individuals (46-87 years of age) suffering from various forms of memory impairment including Alzheimer's dementia, huperzine A improved memory 1-4 h after injection and exhibited a duration of action of ~8 h. In 128 cases of myasthenia gravis, 99% of patients treated with

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(3) Wang, Y. E.; Yue, D. X.; Tang, X. C. *Acta Pharmacol. Sin.* **1986**, *7*(2), 110.
(4) For a review on acetylcholinesterase, see: Quinn, D. M. *Chem. Rev.* **1987**, *87*, 955. Also see: Brossi, A. *J. Med. Chem.* **1990**, *33*, 2311.
(5) Ashford, J. W.; Sherman, K. A.; Kumar, V. *Neurobiol. Aging* **1989**, *10*, 99. Bowen, D. M. *Monogr. Dev. Biol.* **1984**, *17*, 42. Summers, W. K.; Majovski, L. V.; Marsh, G. M.; Tachiki, K.; Kling, A. *New Engl. J. Med.* **1986**, *315*, 1241.
(6) (a) Tang, X. C.; Han, Y. F.; Chen, X. P.; Zhu, X. D. *Acta Pharm. Sin.* **1986**, *7*(6), 507. (b) Vincent, G. P.; Rumennik, L.; Cumin, R.; Martin, J.; Sepinwall, J. *Soc. Neurosci. Abstr.* **1987**, *13*(2), 844. (c) Zhang, S. L. *New Drugs Clin. Rem.* **1986**, *5*(5), 260. (d) Cheng, Z. S. et al. *New Drugs Clin. Rem.* **1986**, *5*(4), 197.

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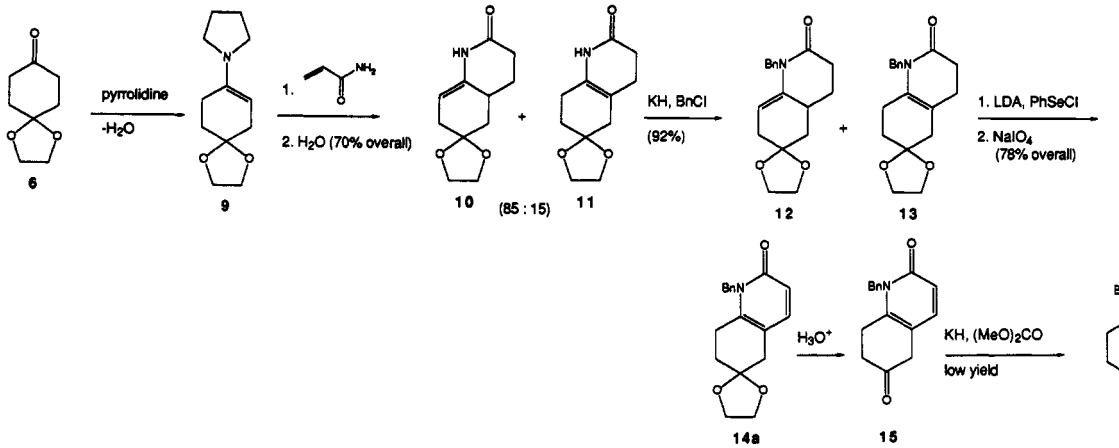
Scheme I. Synthesis of Fused-Ring Pyridone 5



6 9 10 11 12 13 14a 15 5 (R = Bn) 16

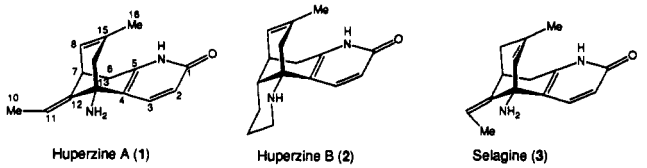


Figure 1.

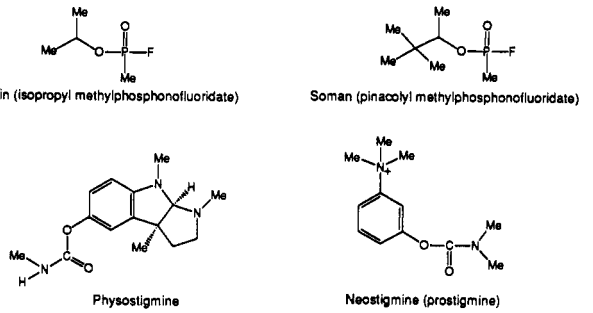


Figure 2. Other cholinesterase inhibitors.

1 in place of neostigmine had the clinical manifestations of their disease controlled or improved. Except for nausea, the side effects of huperzine A (e.g., fasciculation, dizziness, sweating, vision blurring, etc.) were milder than those induced by prostigmine.

In view of the tremendous need to identify agents capable of alleviating some of the symptoms of Alzheimer's disease, and because of the relative difficulty in extracting significant quantities of huperzine A from its natural source, we embarked upon a program to develop an efficient synthetic route to this molecule. We were also interested in preparing a variety of huperzine A analogues so that we might discover the extent to which its structure could be simplified yet still retain anti-AChE activity. Additionally, we wished to identify structural changes that might lead to analogues with greater anti-AChE activity and possibly duration of action.

Herein we report the total synthesis of huperzine A and selected analogues.[7] The action of these compounds on isolated rat brain cholinesterase is presented, along with a rationale for their relative inhibitory potencies.

Chemistry

Our retrosynthetic analysis of huperzine A is shown in Figure 3. The main feature of the synthesis plan is the

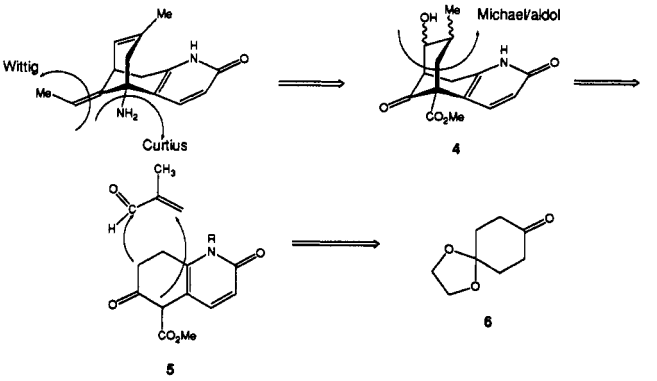
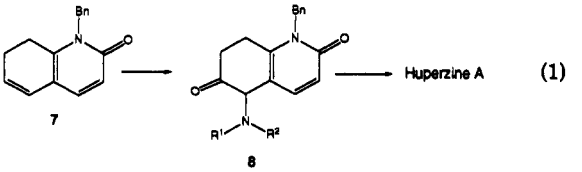


Figure 3. Simplification of the huperzine A structure.

projected use of the ring-fused pyridone structure 5, which provides an appropriate scaffolding upon which to construct the unsaturated carbon bridge. The carbomethoxy group of this substrate serves not only as an activating group for incorporation of the carbon bridge by a combined Michael/aldol process but, moreover, it also provides a handle for introduction of the amino group through execution of a Curtius rearrangement near the end of the synthesis. Subsequent to generation of the endocyclic double bond by dehydration of the intermediate aldol product 4, it was anticipated that the exocyclic olefin at C_{11}–C_{12} could be introduced through a Wittig reaction.[8] Lastly, the ring-fused pyridone 5 needed to initiate this project could come from the monoethylene ketal of cyclohexane-1,4-dione (6) by way of an enamine intermediate.

Before describing the successful synthetic route to huperzine A, we wish to note for the sake of completeness that attempts were taken to explore the use of the α-amino ketone 8 as the substrate for the Michael/aldol process.[9] Such efforts, however, proved fruitless, for we were unable to transform olefin 7 to the ketone 8 (eq 1).

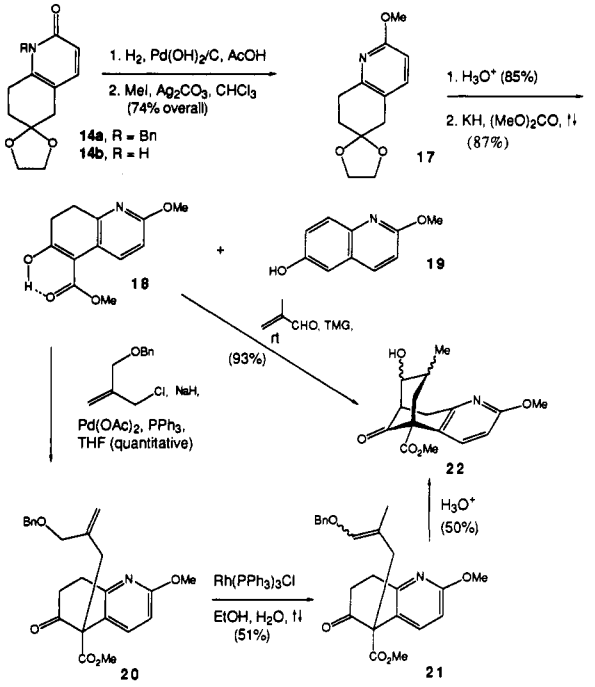


(7) For a preliminary account of the huperzine A synthesis, see: Xia, Y.; Kozikowski, A. P. *J. Am. Chem. Soc.* **1989**, *111*, 4116. For another synthesis of hupezine A, see: Qian, L.; Ji, R. *Tetrahedron Lett.* **1989**, *30*, 2089.

(8) Sreekumar, C.; Darst, K. P.; Still, W. C. *J. Org. Chem.* **1980**, *45*, 4260.

(9) Olefin 7 was available from the ketone prepared according to the method of Speckamp et al.: Dubas-Sluyter, M. A. T.; Speckamp, W. N.; Huisman, H. O. *Recl. Trav. Chim. Pays-Bas* **1972**, *91*, 157.

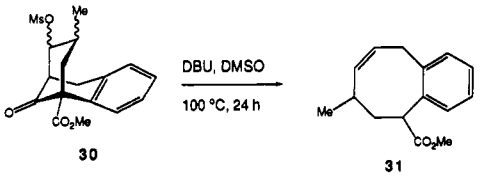
Scheme II. Synthesis of β-Keto Ester 18 and Two Possible Routes to Bridged Intermediate 22



To prepare the ring-fused pyridone **5**, we applied Stork's "aza-annelation" method to the mono-protected diketone **6** (Scheme I).[10] The pyrrolidine enamine of **6** was heated with acrylamide followed by hydrolysis to provide an 85:15 mixture of the lactams **10** and **11**. After protection of the lactam nitrogen by benzylation, a dehydrogenation sequence was brought about to afford pyridone **14a**. While the dehydrogenation reaction can be accomplished directly by the use of DDQ in benzene, the yield is only 40%. A higher yielding, multistep process consisting of selenylation, oxidative elimination, and isomerization (for **12**) was thus adopted.[11] Next, intermediate **14a** was hydrolyzed and the ketone **15** reacted with potassium hydride and dimethyl carbonate to produce the β-keto ester **5**.[12] Unfortunately, the yield of this reaction was quite poor with substantial amounts of the benzenoid compound **16** being isolated. Since in model reactions employing β-tetralone the carbomethoxylation was found to proceed cleanly and in yields of >95%, we suspected that the pyridone ring was responsible for the result.[13] Proton abstraction is possible at C-8, an event that can initiate a subsequent dehydrogenation step.[14] The pyridone ring was protected on oxygen rather than nitrogen to prevent this deprotonation reaction from occurring, thus leading to an alkoxypyridine derivative. Protection of pyridone as pyridine also leads to the further advantage that pyridines are often less polar than their pyridone counterparts, thus making them easier to isolate and purify. The α-methoxypyridine **17** was synthesized from **14b** by hydrogenolysis of its *N*-benzyl group, followed by O-methylation with methyl iodide and silver carbonate. This time the α-carbomethoxylation reaction gave an 87% yield of the desired β-keto ester **18** plus a very small amount of the hydroxyquinoline **19**.

At this stage, the Michael/aldol reaction sequence could be studied. A variety of Michael reaction catalysts, which included NaOMe, $(n\text{-Bu})_4NF$, Et_3N, $ZnCl_2$, and HOAc, were thus examined in order to bring about the reaction of **18** with methacrolein. Unfortunately, no reaction was observed.[15] While several alternative strategies for introducing the carbon bridge were examined such as the multistep strategy (**18** → **20** → **21** → **22**) displayed in Scheme II,[16] we eventually returned to the original Michael/aldol process because of its brevity. Tetramethylguanidine (TMG) has been reported to catalyze the Michael reactions of nitroalkanes with electron-deficient olefins.[17] Since β-keto esters have a pK_a (≅10) comparable to that of nitroalkanes, we examined the reaction of **18** with methacrolein using TMG as the base catalyst. The cyclic ketol **22** was formed as a mixture of stereoisomers in a single step in greater than 90% yield. To our knowledge, this reaction represents the first example of a TMG-catalyzed Michael addition of a β-keto ester to an electron-deficient double bond. The TMG-catalyzed Michael/aldol sequence must owe its success not only to the higher basicity of TMG relative to triethylamine but also to the ability of the resonance-delocalized TMG-H^+ cation to pair with and thus to stabilize the resonance-delocalized enolate anion intermediate generated during the course of the "bridging" process.

The ketol mixture **22** was dehydrated to the alkene **24** by reaction of its derived mesylates with sodium acetate and acetic acid at 120 °C.[19] In model studies, attempts to effect mesylate elimination from **30** with DBU in DMSO led to the formation of the fragmentation product **31** along with other side products (eq 2).



(2)

With the endocyclic double bond in position, introduction of huperzine's exocyclic double bond was carried out. Although the ketone carbonyl group of **24** is somewhat sterically encumbered, its Wittig reaction with ethylidenetriphenylphosphorane took place smoothly in THF at 0 °C → rt to provide a 90:10 mixture of the (*Z*)- and (*E*)-alkenes **25** in 73% yield. This olefin mixture consists largely of the incorrect isomer needed to assemble huperzine A, and an isomerization reaction was therefore

(10) Stork, G. *Pure Appl. Chem.*, **1968**, *17*, 383. Also see: Ninomiya, I.; Naito, T.; Higuchi, S.; Mori, T. *J. Chem. Soc., Chem. Commun.* **1971**, 457.

(11) Reich, H. J.; Renga, J. M.; Reich, I. L. *J. Am. Chem. Soc.* **1975**, *97*, 5434.

(12) Colvin, E. W.; Martin, J.; Shroot, B. *Chem. Ind. (London)* **1966**, 2130.

(13) Xia, Y.; Reddy, E. R.; Kozikowski, A. P. *Tetrahedron Lett.* **1989**, *30*, 3291.

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(15) For reviews on the Michael reaction, see: House, H. O. *Modern Synthetic Reactions*, 2nd ed.; W. A. Benjamin, Inc., **1972**; pp 595–623. Gawley, R. E. *Synthesis* **1976**, 777. Jung, M. E. *Tetrahedron* **1976**, *32*, 3. For the fluoride ion catalyzed Michael reaction, see: Clark, J. H.; Miller, J. M. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1743.

(16) Bäckvall, J.-E. *Pure Appl. Chem.* **1983**, *55*, 1669. Nyström, J.-E.; Bäckvall, J.-E. *J. Org. Chem.* **1983**, *48*, 3947. Kende, A. S.; Battista, R. A.; Sandoval, S. B. *Tetrahedron Lett.* **1984**, *25*, 1341. Kende, A. S.; Roth, B.; Sanfilippo, P. J. *J. Am. Chem. Soc.* **1982**, *104*, 1784.

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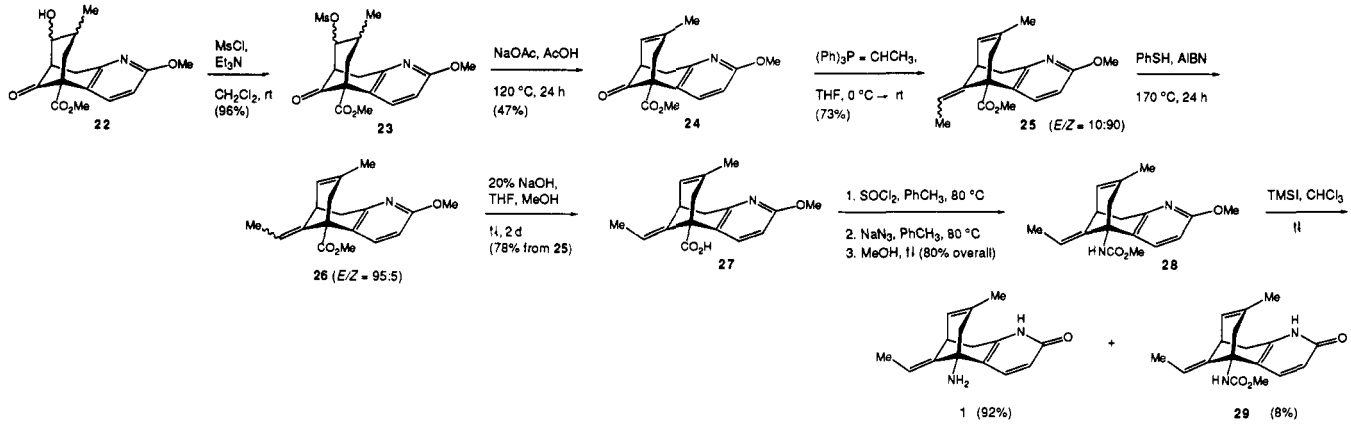
(18) The bicyclic amidine base DBU was also found to catalyze the conversion of **18** to **22**. For the use of DBU as a catalyst for the aldol reaction, see: Corey, E. J.; Andersen, N. H.; Carlson, R. M.; Paust, J.; Vedejs, E.; Vlattas, I.; Winter, R. E. K. *J. Am. Chem. Soc.* **1968**, *90*, 3245.

(19) Colvin, E. W.; Martin, J.; Parker, W.; Raphael, R. A.; Shroot, B.; Doyle, M. *J. Chem. Soc., Perkin Trans. 1* **1972**, 860.

Scheme III. Completion of the Huperzine A Synthesis

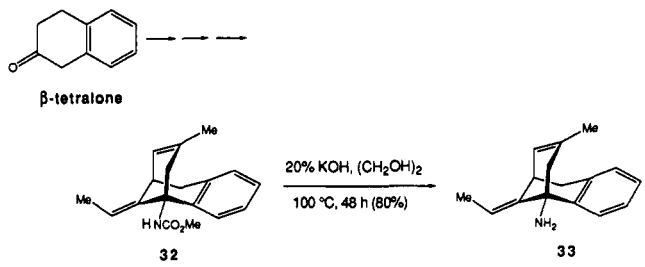


brought about using AIBN and thiophenol.[20] The extent of isomerization was dependent on the reaction temperature and at 130 °C led to an 80:20 mixture of the *E* and *Z* isomers, respectively, and to a 95:5 mixture of (*E*)- and (*Z*)-**26** at 170 °C.

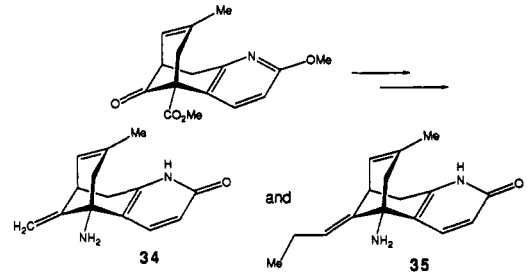
The last operations remaining to complete the synthesis of (±)-huperzine A were the conversion of the ester group of **26** to an amino group and cleavage of the *O*-methyl ether to reveal the pyridone ring. As shown in Scheme III the hindered ester group of **26** was first saponified to acid by being heated with 20% aqueous sodium hydroxide in THF/MeOH. Under these conditions we observed conversion of only the isomer of (*E*)-olefin stereochemistry to acid, whereas the more hindered *Z*-olefinic ester did not react. The acid **27** was converted in a single-pot operation to the carbamate **28** by acid chloride synthesis, acyl azide formation with concomitant Curtius rearrangement, and methanolysis of the resulting isocyanate.[21] Lastly, cleavage of both the methyl ether and the methyl carbamate of **28** was accomplished by the use of iodotrimethylsilane. A small amount (~8%) of the partially deprotected carbamate **29** was also isolated. The synthetic huperzine A was identical with a sample of the natural material by ^{1}H NMR, IR, mass spectra, and TLC comparisons.[22]

While the overall scheme presented above offers a reasonably efficient route to (±)-huperzine A, we felt that further improvements were necessary in order to facilitate scale-up procedures as well as analogue synthesis. In particular, the steps needed to obtain the pyridone **14b** (Scheme II) were unduly numerous and required the use of several expensive reagents [PhSeCl and $Pd(OH)_2$]. Consequently, we examined several alternate approaches to **14b** and eventually discovered that by heating ketone **6**, methyl propiolate, and methanolic ammonia in a Parr reactor at 100 °C for 10 h (200 psi internal pressure), the crystalline pyridone **14b** could be obtained in 70% yield in a single step! This reaction bears some resemblance to a pyridone synthesis reported by Speckamp,[9] but unlike the earlier procedure does not require prior formation and isolation of the enamine intermediate. The use of this new pyridone-forming route thus eliminates three of the synthetic steps required to produce huperzine A in the laboratory and allows one to obtain multigram amounts of the bridged ketol **22** within a week's time.[23]

Scheme IV. Synthesis of a Benzenoid Analogue of Huperzine A



Scheme V. Synthesis of Methylene and Propylidene Analogues of Huperzine A



Below we detail modifications in the synthesis that allow the preparation of several analogues of huperzine A, and we also describe the synthesis of the "presumed" pharmacophore of huperzine A.

Synthesis of Huperzine A Analogues

During our efforts to develop a total synthesis route to huperzine A, β-tetralone was employed as a readily available model compound for use in studying several critical reaction processes. Consequently, an analogue (**33**) of huperzine A possessing a benzene ring in place of the pyridone ring became available for biological evaluation.[13] The synthesis of this benzenoid analogue proceeded through a sequence of steps completely identical with those used to prepare huperzine A, with the exception that the final amine deprotection step was accomplished by base hydrolysis (Scheme IV).

In order to evaluate the contribution of huperzine A's ethylidene group to its biological activity, we synthesized both the methylene analogue **34** and the propylidene analogue **35** (Scheme V). These compounds were prepared through the huperzine A synthetic scheme by using methylene- and propylidenetriphenylphosphorane in place of the ethylidenetriphenylphosphorane. The thiophenol/AIBN-induced thermal isomerization of the *E*/*Z* mixture of olefin formed during the synthesis of the pro-

(20) Bhalerao, U. T.; Rapoport, H. *J. Am. Chem. Soc.* **1971**, *93*, 4835.
(21) Sunagawa, M.; Katsube, J.; Yamamoto, H. *Tetrahedron Lett.* **1978**, 1281.
(22) Natural huperzine A was obtained as a gift from Dr. Liu of the Shanghai Institute of Materia Medica.
(23) Kozikowski, A. P.; Reddy, E. R.; Miller, C. P. *J. Chem. Soc., Perkin Trans. I* **1990**, 195.

Scheme VI. Synthesis of a Phenyl-Bearing Analogue of Huperzine A

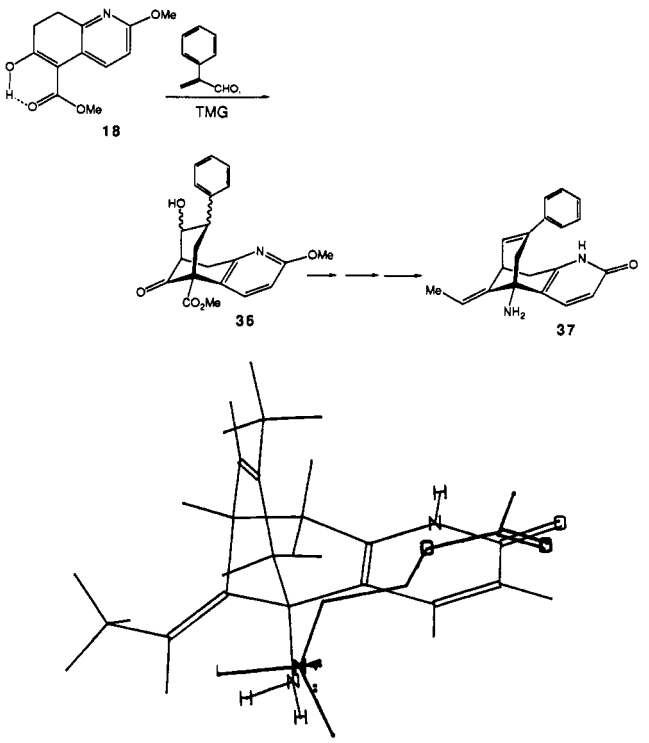
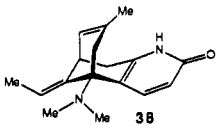


Figure 4. MacroModel-generated overlay of huperzine A and the nearly extended form of acetylcholine.

pylidene analogue gave rise to a 95:5 mixture of the (*E*)- and (*Z*)-olefins, just as in the case of the huperzine A synthesis.

To probe the ability of the unsaturated carbon bridge (C-8, C-14, and C-15) to accommodate additional functionality, we synthesized analogue **37** containing a phenyl substituent at C-15 in place of the methyl group. This compound was conveniently prepared by slight modification of the established scheme. The β-keto ester **18** was reacted with 2-phenylacrolein[24] and TMG at 0 °C for 1 h and then at room temperature overnight to provide the bridged adduct **36** in 97% yield (Scheme VI). The remaining steps in the synthesis of **37** were identical with those employed in the huperzine A synthesis.

The huperzine A molecule was also modified in the vicinity of its NH_2 group. The N,N-dimethylated derivative **38** was prepared from huperzine A by reaction with formic acid and formaldehyde in accord with a reported procedure.[25] The urethane derivative **29** was available as a byproduct of the huperzine synthesis, whereas the one-carbon homologue **42** was prepared from the ester **26** by a sequence of reactions involving LAH reduction, mesylate formation, azide displacement and reduction, urethane formation, and TMSI-promoted deprotection (Scheme VII). Demethylation of the methoxypyridine by TMSI failed when attempted on the unprotected amine **41**.



(24) Crossland, I. *Org. Synth.* **1981**, *60*, 6.
(25) Liu, J. S.; Yu, C. M.; Zhou, Y. Z.; Han, Y. Y.; Wu, F. W.; Qi, B. F.; Zhu, Y. L. *Acta Chim. Sin.* **1986**, *44*, 1035. Pine, S. H.; Sanchez, B. L. *J. Org. Chem.* **1971**, *36*, 829.

Table I. Extent of AChE Inhibition by the Compounds Tested[a]

compd no.	IC_{50} (M)
natural huperzine A	10^{-7}
(±)-huperzine A	3×10^{-7}
29	$>10^{-4}$
33	$>10^{-4}$
34	5×10^{-6}
35	2×10^{-5}
37	8×10^{-4}
38	4.5×10^{-4}
40	no activity
41	no activity
42	9.5×10^{-4}
46	9.5×10^{-4}
48	3×10^{-3}

[a] All compounds were tested by using rat hippocampal crude homogenates over a concentration range of 10^{-11} M to 10^{-3} M. These substances were all dissolved in 10% DMSO made up in the incubation buffer medium. In addition, those substances that did not dissolve readily were treated with mild hydrochloric acid and sonicated briefly until they dissolved in the solution. In each case the control medium always consisted of the same ingredients as those that were used to dissolve the compound under investigation. AChE was measured as described in ref 31.

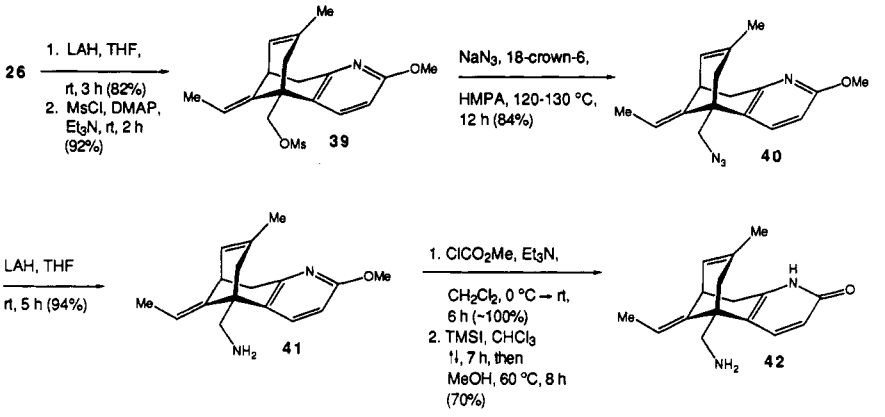
In Figure 4 we show a computer-generated overlay of huperzine A with the nearly extended conformation of acetylcholine. The huperzine A structure was built by using the INPUT mode of the MacroModel V2.5 program and was optimized by the BDNR energy minimization routine with the MM2 force field. The acetylcholine structure was built in the INPUT mode and the torsional angles of the structure so generated were set according to the values described in ref 26, which acetylcholine may assume during its enzymatic hydrolysis to choline and acetate ion. The superimposition of huperzine A and acetylcholine was produced by using the GEOMTR submode of the ANALYZ mode by overlaying the nitrogen atom, the carbon atom of the carbonyl group, and the ether oxygen atom of the ester group in the acetylcholine molecule with the corresponding nitrogen atom, carbon atom of the carbonyl group, and the nitrogen atom of the pyridone ring in huperzine A.

As is apparent from the overlay, a reasonable coincidence of structure can be found between the heteroatoms of acetylcholine in its {180°, 150°} conformation and those of huperzine A. The NH_2 group of huperzine is expected to be protonated at physiological pH and thus it mimics the Me_3N^+ group of acetylcholine. From such structural comparisons, it would appear reasonable to postulate that an aminomethyl-substituted pyridone might possess anticholinesterase activity. Pyridone **46** and the (*N,N*-dimethylamino)methyl-substituted pyridone **48**, which represents a "hybrid" of the acetylcholine and huperzine A structures, were therefore synthesized for biological assay.

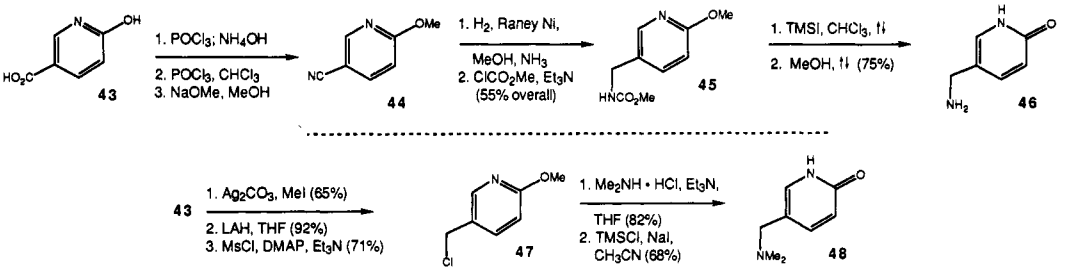
5-Cyano-2-methoxypyridine (**44**), prepared by standard methods from commercially available 2-hydroxy-5-pyridinecarboxylic acid (**43**), was converted to the urethane **45** by reduction and acylation.[27] Both protecting groups were cleaved simultaneously by the action of iodotrimethylsilane in chloroform to afford pyridone **46**. The *N,N*-dimethyl derivative of **46** was also assembled from **43** through a sequence of reactions involving O-

(26) Beveridge, D. L.; Radna, R. J. *J. Am. Chem. Soc.* **1971**, *93*, 3759. Chothia, C.; Pauling, P. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 3103. Chothia, F.; Pauling, P. *Nature (London)* **1969**, *223*, 919. For a caveat on the use of molecular modeling strategies in structure–activity studies, see: Behling, R. W.; Yamane, T.; Navon, G.; Jelinski, L. W. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6721.
(27) Forrest, H. S.; Walker, J. *J. Chem. Soc.* **1948**, 1939.

Scheme VII. Synthesis of an Aminomethyl Analogue of Huperzine A



Scheme VIII. Synthesis of Pyridones Related to the Huperzine A Pharmacophore



methylation, ester reduction, chloride formation, dimethylamine displacement, and deprotection (Scheme VIII).

Biological Results

All of the compounds synthesized were tested for their ability to inhibit the hydrolysis of labeled acetylcholine. The acetylcholine was obtained from rat brain hippocampal homogenates, and the compounds were tested over a concentration range of 10^{-11} M to 10^{-3} M. The IC_{50}'s for AChE inhibition by the 12 compounds are displayed in Table I together with the IC_{50} of natural huperzine A. As is apparent, (−)-huperzine A is the most active compound tested. Synthetic (±)-huperzine A is about one-half as potent, a result that is expected for a product "contaminated" by an equal proportion of the presumably inactive (+) isomer.

The benzenoid analogue **33** is at least 1000-fold less active than **1**, thus clearly demonstrating the contribution of the pyridone ring, the acetate-mimicking portion of huperzine A, to its AChE inhibitory activity.

Somewhat surprising is the 100-fold lower potency shown by the propylidene analogue **35**. Presumably the molecular volume of analogue **35** is too large for optimal binding to the enzyme. Removal of the methyl group from the exocyclic olefinic appendage also leads to a less active structure. When its racemic nature is taken into account, the methylene analogue **34** is ~30-fold less potent than (−)-huperzine A. This reduction in activity is in line with what is to be expected for the removal of a single methyl group, which leads to a compound of diminished surface area with a subsequent loss in hydrophobic binding and van der Waals interactions.[28]

The IC_{50} for the racemic phenyl-substituted analogue **37** is ~8000 times larger than that of (−)-huperzine A. It is thus apparent that substantial structural alterations in the area of the three-carbon bridge cannot be accommodated by the AChE binding site. An examination of the activity of the C-14 nor-methyl analogue of huperzine A would, therefore, be of some interest to explore, and efforts in this direction are underway.

The huperzine A analogues modified in the vicinity of the amino group, i.e., the *N,N*-dimethyl derivative **38**, urethane **29**, and the one-carbon homologue **42** are all poorly active. The methoxypyridine structures **40** and **41** failed to inhibit AChE at the highest concentrations tested (10^{-3} M).

Lastly, in spite of the reasonableness of the structural overlay presented in Figure 4, neither of the structurally simple (aminomethyl)pyridones **46** or **48** was found to exhibit useful AChE inhibitory activity. One may therefore conclude that the structural rigidity conferred upon huperzine A by its tricyclic skeleton and the contribution of the additional carbon atoms vis-à-vis **46** or **48** to hydrophobic binding forces and van der Waals interactions must play a significant role in huperzine A's interactions with the active site of the enzyme.[28]

Conclusions

Our efforts to synthesize analogues of huperzine A that are more active as inhibitors of AChE than huperzine A itself have not presently met with success. Since the analogues that have been synthesized to date represent rather trivial and obvious modifications to the parent structure, we remain hopeful that more "creative" alterations of huperzine A will provide more active analogues. The concentration of a drug needed to inhibit AChE in vitro is, of course, but one criterion of activity, for to be useful clinically the drug candidate must also pass certain hurdles relating to toxicity, metabolism, pharmacokinetics, and bioavailability.[28] In this regard, the development of huperzine A as a clinical candidate for the treatment of Alzheimer's dementia is more realistic, for this compound has already undergone significant testing in both human subjects and animals.

(28) *Modern Drug Research–Paths to Better and Safer Drugs*; Martin, Y. C., Kutter, E., Austel, V., Eds.; Marcel Dekker, Inc.: New York, 1989.

Because huperzine A is difficult to procure in large quantities from its natural sources, the present synthesis makes it possible to prepare the compound on the gram scale in the laboratory. Since the current synthetic approach leads to the racemic product, appropriate modifications are being pursued to develop an enantioselective route to 1.

Experimental Section

General experimental protocols can be found in ref 33.

(±)-1′,3′,4′,4′a,5′,7′-Hexahydrospiro[1,3-dioxolane-2,6′-(2′*H*)-quinolin]-2′-one (10) and 1′,3′,4′,5′,7′,8′-Hexahydrospiro[1,3-dioxolane-2,6′(2′*H*)-quinolin]-2′-one (11). In a 1000-mL round-bottomed flask equipped with a water separator and a condenser were placed 25.0 g (0.160 mol) of 1,4-cyclohexanedione monoethylene ketal, 27 mL (0.32 mol) of pyrrolidine, 1 g of *p*-toluenesulfonic acid, and 500 mL of benzene. The mixture was refluxed until no more water separated in the water separator. Benzene was evaporated, and the residue was dissolved in 500 mL of dioxane. To this solution was added 34 g (0.48 mol) of acrylamide, and the mixture was refluxed overnight. Water (100 mL) was added, and the solution was refluxed for 12 h. After being cooled down to rt, the dioxane was removed by rotary evaporation, and the aqueous residue was extracted with $CHCl_3$. The extracts were washed with brine, dried, and filtered. After evaporation of the solvent, the residue was chromatographed on silica gel (40% ethyl acetate in hexanes and then ethyl acetate) to give **10**/**11** as solids. The yield of **10** and **11** (ratio = 85:15) is 23.4 g (70%): R_f = 0.30 (ethyl acetate); IR 2900–3700 (br), 3211, 3063, 2951, 1676, 1473, 920, 733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.45 (br s, 0.85 H), 7.73 (br s, 0.15 H), 4.83–4.87 (m, 0.85 H), 3.90–4.03 (m, 4 H), 1.51–2.56 (four groups of multiplets, 9.15 H); mass spectrum, m/z 209 (M^+), 123, 86; exact mass calcd for $C_{11}H_{15}NO_3$ 209.1052, obsd 209.1051.

N-Benzylation of 10/11. A solution of the lactams **10**/**11** (11.70 g, 0.056 mol) in 200 mL of dry THF was added dropwise to a mixture of potassium hydride (3.36 g, 0.084 mol), benzyl chloride (12.9 mL, 0.112 mol), and tetrabutylammonium iodide (0.2 g) in 200 mL of dry THF. The mixture was stirred at rt overnight with the protection of a drying tube. Water was added dropwise to quench the excess KH, and the THF was removed by rotary evaporation. The aqueous residue was extracted with ethyl acetate. The extracts were washed with brine, dried, and filtered. Evaporation of the solvent and purification of the residue by flash chromatography (40%, 60%, and 80% ethyl acetate in hexanes, successively) gave 15.4 g (92%) of the N-benzylated product **12**/**13** (ratio = 70:30) as an oil: R_f = 0.46 (ethyl acetate); IR 2949, 2889, 1668, 1645, 947, 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.13–7.32 (m, 5 H), 5.41 (d, 0.7 H, J = 16.1 Hz), 4.84–4.87 (m, 1.3 H), 4.50 (d, 0.7 H, J = 16.1 Hz), 3.91–4.03 (m, 4 H), 1.58–2.81 (four groups of multiplets, 9.3 H); mass spectrum, m/z 299 (M^+), 213, 185, 91; exact mass calcd for $C_{18}H_{21}NO_3$ 299.1521, obsd 299.1521.

1′,5′,7′,8′-Tetrahydro-1′-(phenylmethyl)spiro[1,3-dioxolane-2,6′(2′*H*)-quinolin]-2′-one (14a). To a solution of diisopropylamine (35.2 mL, 0.252 mol) in 500 mL of dry THF at 0 °C under N_2 was added *n*-BuLi (135 mL of 1.6 M *n*-BuLi in hexanes, 0.216 mol). The solution was stirred at 0 °C for 20 min and then cooled to −78 °C. A solution of the benzyl-protected lactams **12**/**13** (21.5 g, 0.0719 mol) in 300 mL of dry THF was added at −78 °C. The color of the reaction mixture immediately turned deep blue. After stirring at −78 °C for 2 h, a solution of benzeneselenenyl chloride (27.5 g, 0.144 mol) in 200 mL of dry THF was added dropwise, and the resulting solution was stirred at −78 °C for 15 min.

The solution was quenched with methanol (50 mL) and poured into a mixture of $NaIO_4$ (61.5 g, 0.288 mol) in 1000 mL of H_2O–MeOH (1:1). The mixture was stirred at room temperature overnight.

THF and methanol were removed by rotary evaporation, and the aqueous residue was extracted with ethyl acetate. Concentration of the ethyl acetate solution gave a red syrup. Column chromatography (elution with 60% ethyl acetate in hexanes to remove the selenenylated side products and then with ethyl acetate) gave 16.7 g (78%) of the product **14a** as a light yellow syrup.

14a: R_f = 0.17 (ethyl acetate); IR 2957, 2887, 1664, 1593, 1545, 827, 733, 702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.06–7.34 (m, 6 H), 6.57 (d, 1 H, J = 9.3 Hz), 5.34 (s, 2 H), 3.97–4.02 (m, 4 H), 2.80 (t, 2 H, J = 6.6 Hz), 2.73 (s, 2 H), 1.83 (t, 2 H, J = 6.7 Hz); mass spectrum, m/z 297 (M^+), 206, 134, 91; exact mass calcd for $C_{18}H_{19}NO_3$ 297.1365, obsd 297.1364.

A Simplified Route to Pyridone 14b. To a solution of 1,4-cyclohexanedione monoethylene ketal (3 g, 0.019 mol) in 60 mL of ammonia-saturated methanol contained in a Parr reaction vessel was added 3.2 g (0.038 mol) of methyl propiolate. The reaction mixture was heated with stirring at 100 °C for 10 h. During this time the internal pressure reached a maximum of 200 psi. After cooling, the solvent was removed under reduced pressure, and the crude product was purified by flash chromatography with 15% MeOH–ethyl acetate as the eluent to afford 2.78 g (70%) of the pyridone **14b** as a light-yellow solid: mp dec above 250 °C; sublimes at 180 °C/0.4 Torr; IR 2930, 1639, 1620, 1554, 1506, 837, 696 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.56 (br s, 1 H), 7.14 (d, 1 H, J = 9.3 Hz), 6.40 (d, 1 H, J = 9.3 Hz), 4.02 (s, 4 H), 2.89 (t, 2 H, J = 6.6 Hz), 2.71 (s, 2 H), 1.93 (t, 2 H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 165.0, 143.4, 141.8, 117.3, 111.9, 107.3, 64.6, 36.2, 30.1, 25.7; mass spectrum, m/z 207 (M^+), 164, 134, 86, 69, 57; exact mass calcd for $C_{11}H_{13}NO_3$ 207.0895, obsd 207.0896. Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found: C, 63.69; H, 6.09; N, 6.71.

7′,8′-Dihydro-2′-methoxyspiro[1,3-dioxolane-2,6′(5′*H*)-quinoline] (17). (1) The benzyl-protected pyridone **14a** (4.67 g, 15.7 mmol) was stirred with 20% $Pd(OH)_2$ on carbon (2.34 g) in 300 mL of acetic acid under a H_2-filled balloon at room temperature for 36 h. The solution was filtered, and the acetic acid solvent was removed by rotary evaporation. Toluene and methylene chloride were added to the residue, and the resulting solution was evaporated to remove the final traces of acetic acid. The crude product **14b** was used directly in the following O-methylation reaction.

(2) The crude pyridone **14b** was stirred with a mixture of Ag_2CO_3 (8.67 g, 31.4 mmol) and iodomethane (9.8 mL, 0.157 mol) in chloroform (200 mL) in the dark at room temperature overnight. Filtration, concentration, and silica gel chromatography (40% ethyl acetate in hexanes) gave 2.57 g (74% for two steps) of product **17**: mp 77.5–78.5 °C; R_f = 0.48 (40% ethyl acetate in hexanes); IR 2942, 2885, 1601, 1581, 947, 817 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.22 (d, 1 H, J = 8.3 Hz), 6.52 (d, 1 H, J = 8.3 Hz), 4.03 (s, 4 H), 3.88 (s, 3 H), 3.01 (t, 2 H, J = 6.8 Hz), 2.89 (s, 2 H), 2.01 (t, 2 H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 162.1, 152.6, 139.7, 121.3, 108.0, 107.7, 64.5, 53.2, 37.5, 31.4, 30.7; mass spectrum, m/z 221 (M^+), 148, 134, 64; exact mass calcd for $C_{12}H_{15}NO_3$ 221.1052, obsd 221.1053. Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 64.95; H, 6.66; N, 6.24.

5,6,7,8-Tetrahydro-2-methoxy-6-oxo-5-quinolinecarboxylic Acid Methyl Ester (18). (1) The ketal **17** (1.71 g) was refluxed in 5% HCl–acetone (1:1) overnight. Acetone was removed on a rotary evaporator, and the aqueous layer was basified with solid $NaHCO_3$. The resulting mixture was extracted with ethyl acetate. The organic extracts were washed with brine, dried over anhydrous $MgSO_4$, and filtered. Concentration and flash chromatography (30% ethyl acetate in hexanes) gave 1.16 g (85%) of the ketone as a colorless solid, which decomposes upon storage: mp 49–50 °C; R_f = 0.44 (40% ethyl acetate in hexanes); IR 2945, 2916, 2891, 1712, 1604, 1582, 859, 825 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (d, 1 H, J = 8.3 Hz), 6.61 (d, 1 H, J = 8.3 Hz), 3.93 (s, 3 H), 3.51 (s, 2 H), 3.16 (t, 2 H, J = 6.9 Hz), 2.66 (t, 2 H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 209.4, 162.7, 153.5, 138.8, 120.2, 108.8, 53.4, 42.5, 38.0, 30.9; mass spectrum, m/z 177 (M^+), 162, 148, 134, 106; exact mass calcd for $C_{10}H_{11}NO_2$ 177.0790, obsd 177.0790. Anal. Calcd for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.82; H, 6.17; N, 7.87.

(2) The above ketone (1.16 g, 6.55 mmol) in 10 mL of dimethyl carbonate was added dropwise to a mixture of KH (1.05 g, 26.2 mmol) in 40 mL of dimethyl carbonate under nitrogen at room temperature. The mixture was refluxed for 3 h. The reaction was quenched with methanol, and the solution was neutralized with a saturated NH_4Cl solution. The methanol was removed by rotary evaporation, and the aqueous residue was extracted with ethyl acetate. The ethyl acetate extracts were washed with brine, dried, and filtered. Concentration and flash chromatography with

20% ethyl acetate in hexanes as eluent gave 1.34 g (87%) of **18** as a yellowish solid, which is stable only if stored in a freezer: mp 71–72 °C; R_f = 0.33 (20% ethyl acetate in hexanes); IR 2954, 2895, 2837, 1641, 1603, 640, 625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.16 (s, 1 H), 7.90 (d, 1 H, J = 8.7 Hz), 6.56 (d, 1 H, J = 8.7 Hz), 3.91 (s, 3 H), 3.90 (s, 3 H), 2.94 (t, 2 H, J = 7.8 Hz), 2.63 (t, 2 H, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$) δ 176.7, 171.9, 161.1, 151.1, 136.1, 119.8, 107.2, 98.2, 53.3, 51.7, 29.9, 29.0; mass spectrum, m/z 235 (M^+), 203, 148; exact mass calcd for $C_{12}H_{13}NO_4$ 235.0845, obsd 235.0845. Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.33; H, 5.54; N, 5.98.

(±)-7,8,9,10-Tetrahydro-8-hydroxy-2-methoxy-7-methyl-11-oxo-5,9-methanocycloocta[*b*]pyridine-5(6*H*)-carboxylic Acid Methyl Ester (22). The β-keto ester **18** (502 mg, 2.14 mmol) was stirred with methacrolein (1.76 mL, 21.4 mmol) and 1,1,3,3-tetramethylguanidine (54 μL, 0.42 mmol) in dry CH_2Cl_2 at room temperature overnight. Concentration and flash chromatography (40% ethyl acetate in hexanes) gave 604 mg (93%) of the bridged adduct **22**: the most polar product of this mixture of isomers crystallizes from CH_2Cl_2/hexanes to afford a colorless solid of mp 150–152 °C; R_f = 0.30–0.35 (40% ethyl acetate in hexanes); IR 3100–3600 (br), 2953, 1743, 1603, 758 cm^{-1}; ^1H NMR ($CDCl_3$) (one of the isomers) δ 7.02 (d, 1 H, J = 8.6 Hz), 6.60 (d, 1 H, J = 8.6 Hz), 3.91 (s, 3 H), 3.81 (s, 3 H), 3.62–3.69 (m, 2 H), 3.03–3.25 (m, 2 H), 2.23 (br s, OH), 1.98–2.04 (m, 2 H), 1.48–1.59 (m, 1 H), 1.03 (d, 3 H, J = 6.4 Hz); mass spectrum, m/z 305 (M^+), 273, 248, 188, 55; exact mass calcd for $C_{16}H_{19}NO_5$ 305.1263, obsd 305.1264. Anal. Calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 62.86; H, 6.07; N, 4.58.

(±)-9,10-Dihydro-2-methoxy-7-methyl-11-oxo-5,9-methanocycloocta[*b*]pyridine-5(6*H*)-carboxylic Acid Methyl Ester (24). (1) Mesyl chloride (1.89 mL, 24.5 mmol) was added dropwise to a solution of the alcohols **22** (1.87 g, 6.13 mmol), triethylamine (8.46 mL, 61.3 mmol), and a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine in 50 mL of dry CH_2Cl_2 at rt. The solution was stirred for 6 h at rt. The solution was diluted with CH_2Cl_2, washed with saturated NH_4Cl, dried, and concentrated. Flash chromatography (40% ethyl acetate in hexanes) gave 2.26 g (96%) of the mesylate **23**, which crystallizes from CH_2Cl_2/hexane as a mixture of stereoisomers: mp 167–173 °C; R_f = 0.34–0.36 (40% ethyl acetate in hexanes).

(2) The mesylate **23** (2.26 g, 5.90 mmol) was heated with anhydrous NaOAc (0.48 g, 5.9 mmol) in AcOH at 120 °C under N_2 for 24 h. The acetic acid was removed by rotary evaporation at 55 °C. The residue was dissolved in ethyl acetate, washed with saturated Na_2CO_3 and brine, and dried. Evaporation of the ethyl acetate and flash chromatography of the residue (20% and then 40% ethyl acetate in hexanes) gave 521 mg (31%, or 47% based on 66% conversion) of **24** and 0.76 g (34%) of the starting material.

24: mp 120–121 °C (CH_2Cl_2/hexane); R_f = 0.27 (20% ethyl acetate in hexanes); IR 2947, 1745, 1603, 1576, 831 cm^{-1}; ^1H NMR (500 MHz) δ 7.11 (d, 1 H, J = 8.6 Hz), 6.62 (d, 1 H, J = 8.6 Hz), 5.42–5.43 (m, 1 H), 3.92 (s, 3 H), 3.76 (s, 3 H), 3.36–3.42 (m, 2 H), 3.18 (d, 1 H, J = 18.2 Hz), 3.15 (m, 1 H), 2.53 (d, 1 H, J = 17.5 Hz), 1.60 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 207.5, 171.4, 163.2, 150.7, 137.7, 133.6, 126.4, 123.8, 109.6, 60.1, 53.4, 52.7, 46.9, 46.0, 40.4, 22.3; mass spectrum, m/z 287 (M^+), 255, 228, 200, 184; exact mass calcd for $C_{16}H_{17}NO_4$ 287.1158, obsd 287.1157. Anal. Calcd for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.87. Found: C, 67.03; H, 5.99; N, 4.88.

(*Z*)-(±)-11-Ethylidene-9,10-dihydro-2-methoxy-7-methyl-5,9-methanocycloocta[*b*]pyridine-5(6*H*)-carboxylic Acid Methyl Ester (25). *n*-BuLi (2.57 mL, 3.80 mmol) was added dropwise to a mixture of ethyltriphenylphosphonium bromide (1.59 g, 4.28 mmol) in 15 mL of dry THF at rt under nitrogen. The reaction mixture was stirred at rt for 30 min and then cooled to 0 °C. The ketone **24** (273 mg, 0.951 mmol) in 5 mL of dry THF was added dropwise to this mixture at 0 °C. The resulting mixture was allowed to warm to rt and stirred at rt for 4 h. The reaction was quenched with water. The THF was removed by rotary evaporation, and the aqueous residue was extracted with ethyl acetate. The ethyl acetate extracts were washed with brine, dried, and concentrated. Flash chromatography (hexanes and then 10% ethyl acetate in hexanes) gave 208 mg (73%) of olefin **25** as white solid (E/Z = 10/90): mp 128–130 °C; R_f = 0.39 (20% ethyl acetate in hexanes); IR ((*Z*)-olefin) 2909, 1732, 1601, 1578, 1558, 735, 638 cm^{-1}; ^1H NMR ((*Z*)-olefin, $CDCl_3$) δ 7.09 (d, 1 H, J = 8.5 Hz), 6.54 (d, 1 H, J = 8.6 Hz), 5.51 (q, 1 H, J = 7.3 Hz), 5.40–5.42 (m, 1 H), 3.89 (s, 3 H), 3.71 (s, 3 H), 2.99–3.19 (m, 3 H), 2.81 (d, 1 H, J = 16.5 Hz), 2.21 (d, 1 H, J = 17.0 Hz), 1.57 (s, 3 H), 1.51 (d, 3 H, J = 7.3 Hz); mass spectrum, m/z 299 (M^+), 240, 57; exact mass calcd for $C_{18}H_{21}NO_3$ 299.1521, obsd 299.1521.

(*E*)-(±)-11-Ethylidene-9,10-dihydro-2-methoxy-7-methyl-5,9-methanocycloocta[*b*]pyridine-5(6*H*)-carboxylic Acid Methyl Ester (26). The olefin mixture **25** (E/Z = 10/90, 295 mg, 0.987 mmol) was heated with azobis(isobutyronitrile) (0.32 g, 2.0 mmol) in 25 mL of thiophenol at 170 °C under nitrogen for 24 h. Thiophenol was distilled off at 105 °C and aspirator pressure. The residue was dissolved in CH_2Cl_2 and washed with 10% NaOH (2 times) and brine. After drying with $MgSO_4$ and concentration, the crude product was used directly in the next hydrolysis reaction. ^1H NMR analysis revealed olefins **26** to be comprised of an 95/5 mixture of the (*E*)- and (*Z*)-alkenes, respectively. ^1H NMR (in part) of crude **26** ((*E*)-olefin, $CDCl_3$): δ 5.15 (q, 1 H, J = 6.7 Hz), 3.74 (s, 3 H), 3.62 (br s, 1 H), 1.70 (d, 3 H, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, ~1:5 E/Z mixture) 176.6, 175.4, 162.4, 152.6, 137.6, 137.4, 136.7, 136.2, 132.7, 132.5, 128.0, 127.6, 126.0, 124.3, 115.8, 114.2, 108.7, 108.4, 53.2, 52.0, 50.7, 45.4, 45.2, 43.4, 39.7, 39.6, 32.8, 22.7, 12.7, 12.2. Anal. Calcd for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. Found: C, 72.10; H, 7.13; N, 4.66.

(*E*)-(±)-(11-Ethylidene-9,10-dihydro-2-methoxy-7-methyl-5,9-methanocycloocta[*b*]pyridin-5(6*H*)-yl)carbamic Acid Methyl Ester (28). The crude ester **26** (~0.987 mmol, E/Z = 95/5) was dissolved in 40 mL of 20% NaOH and THF (1:1). Enough MeOH was added to convert the heterogeneous mixture into a homogenous one, and this solution was refluxed under nitrogen for 2 days. THF and MeOH were removed by rotary evaporation, and the aqueous residue was extracted with CH_2Cl_2. These organic extracts were washed with brine, dried, and concentrated to give the unhydrolyzed *Z* ester, which can be recycled through the isomerization step. The aqueous residue was adjusted to a pH of ~7 with concentrated HCl. Extraction with CH_2Cl_2, drying, and concentration gave the crude acid which was further purified by column chromatography (20% ethyl acetate in hexanes to remove thiophenol and then ethyl acetate) to afford 220 mg (78% from **25**) of the pure acid **27**: R_f = 0.39 (ethyl acetate); IR 2500–3500 (br), 2932, 2594, 1705, cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.25 (d, 1 H, J = 8.5 Hz), 6.57 (d, 1 H, J = 8.5 Hz), 5.40–5.42 (m, 1 H), 5.31 (q, 1 H, J = 6.7 Hz), 3.89 (s, 3 H), 3.62 (m, 1 H), 2.84–3.12 (m, 3 H), 2.18 (d, 1 H, J = 17.0 Hz), 2.74 (d, 3 H, J = 6.8 Hz), 1.54 (s, 3 H); mass spectrum, m/z 285 (M^+), 240, 84, 69; exact mass calcd for $C_{17}H_{19}NO_3$ 285.1365, obsd 285.1365.

Thionyl chloride (72 μL, 0.90 mmol) was added dropwise to a solution of the acid **27** (86 mg, 0.30 mmol) in 10 mL of toluene under nitrogen at rt. The solution was heated at 80 °C until the solids dissolved (ca. 2 h) and then cooled to rt. Sodium azide (0.12 g, 1.8 mmol) was added, and the mixture was heated at 80 °C for 8 h. The toluene was removed by rotary evaporation, 10 mL of MeOH was added, and the resulting mixture was refluxed overnight. The methanol was removed by rotary evaporation, and the residue was dissolved in ethyl acetate. The solution was washed with brine, dried, and concentrated. Flash chromatography (20% ethyl acetate in hexanes) gave 76 mg (80% from the pure acid) of the urethane **28**: R_f = 0.15 (20% ethyl acetate in hexanes); IR 3331 (br), 2930, 1716, 1597, 1581, 1558, 777, 733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.56 (d, 1 H, J = 8.6 Hz), 6.55 (d, 1 H, J = 8.6 Hz), 5.54–5.56 (m, 1 H), 5.36 (q, 1 H, J = 6.8 Hz), 4.98 (s, carbamate NH), 3.88 (s, 3 H), 3.66 (br s, 1 H), 3.62 (s, 3 H), 3.07 (br d, 1 H, J = 17.4 Hz), 2.82 (dd, 1 H, J = 16.7, 1.6 Hz), 2.57 (br d, 1 H, J = 15 Hz) 2.23 (d, 1 H, J = 15.6 Hz), 1.72 (d, 3 H, J = 6.8 Hz), 1.51 (s, 3 H); mass spectrum, m/z 314 (M^+), 239, 224, 84, 69; exact mass calcd for $C_{18}H_{22}N_2O_3$ 314.1630, obsd 314.1630.

(±)-Huperzine A (1). Iodotrimethylsilane (0.39 mL, 2.7 mmol) was added dropwise to a solution of the carbamate **28** (86 mg, 0.27 mmol) in 10 mL of chloroform under nitrogen at rt. The solution was then refluxed for 8 h. Methanol (10 mL) was added, and the solution was refluxed overnight. Concentration and flash chromatography on silica gel half-saturated with ammonia with 2% methanol in chloroform as eluent gave 60 mg (92%) of huperzine A (**1**) and a small amount of the partially deprotected carbamate **29**.

Synthetic huperzine A: R_f = 0.10 (basic SiO_2, $CHCl_3$–acetone–MeOH 50/45/5); IR 3277, 2928, 1655, 1616, 1558, 1458, 1406, 1377, 1306, 1174, 1118, 912, 833, 769, 731, 659 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.42 (br s, pyridone NH), 7.90 (d, 1 H, J = 9.3 Hz), 6.42 (d, 1 H, J = 9.6 Hz), 5.49 (q, 1 H, J = 6.7 Hz), 5.42 (m, 1 H), 3.61 (m, 1 H), 2.89 (dd, 1 H, J = 16.8, 5.1 Hz), 2.70 (d, 1 H, J = 15.9 Hz), 2.14 (br s, 2 H), 1.68 (d, 3 H, J = 6.6 Hz), 1.61 (br s, NH_2), 1.55 (s, 3 H); mass spectrum, m/z 242 (M^+), 227, 187, 57; exact mass calcd for $C_{15}H_{18}N_2O$ 242.1419, obsd 242.1419. Anal. Calcd for $C_{15}H_{18}N_2O$: C, 74.35; H, 7.49; N, 11.56. Found: C, 73.93; H, 7.53; N, 11.30.

29: R_f = 0.19 (basic SiO_2, $CHCl_3$–acetone–MeOH 50:45:5); IR 3277 (br), 2926, 1734, 1716, 1684, 1653, 1616, 1578, 1558, 1541, 1522, 1506, 1489, 1456, 1437, 1375, 1302, 1248, 1192, 1107, 1070, 1039, 933, 835, 777 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.92 (br s, pyridone NH), 7.48 (d, 1 H, J = 9.6 Hz), 6.43 (d, 1 H, J = 9.3 Hz), 5.45 (m, 1 H), 5.36 (q, 1 H, J = 6.9 Hz), 4.92 (s, carbamate NH), 3.65 (br s, 1 H + 3 H), 2.95 (br d, 1 H), 2.65 (d, 1 H, J = 16.2 Hz), 2.45 (br d, 1 H), 2.23 (d, 1 H, J = 14.1 Hz), 1.69 (d, 3 H, J = 6.9 Hz), 1.57 (s, 3 H); mass spectrum, m/z 300 (M^+), 242, 227, 210, 57; exact mass calcd for $C_{17}H_{20}N_2O_3$ 300.1474, obsd 300.1474.

Benzene Isostere 33. This compound was prepared by methods analogous to those used to prepare huperzine A, and therefore only spectral data are provided: R_f = 0.25 (40% ethyl acetate in hexanes); IR 3381, 2962, 2919, 2860, 1485, 1448 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.77 (dd, 1 H, J = 7.8, 1.2 Hz), 7.11–7.28 (m, 2 H), 7.02 (d, 1 H, J = 7.6 Hz), 5.52 (q, 1 H, J = 6.7 Hz), 5.40–5.42 (m, 1 H), 3.66 (t, 1 H, J = 4.7 Hz), 3.04 (dd, 1 H, J = 16.3, 5.1 Hz), 2.83 (dd, 1 H, J = 16.2, 1.4 Hz), 2.26 (br s, 2 H), 1.84 (br s, NH_2), 1.73 (d, 3 H, J = 6.8 Hz), 1.52 (s, 3 H); mass spectrum, m/z 225 (M^+), 210, 170, 130; exact mass calcd for $C_{16}H_{19}N_2$ 225.1517, obsd 225.1517.

Methylene Analogue 34. This compound was prepared by methods analogous to those used to prepare huperzine A, and therefore only spectral data are provided: R_f = 0.11 (basic SiO_2, $CHCl_3$–acetone–MeOH 50:45:5); IR 3365, 3271, 3096, 1655, 1610, 908, 823 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.77 (br s, 1 H), 7.91 (d, 1 H, J = 9.3 Hz), 6.42 (d, 1 H, J = 9.3), 5.41 (br s, 1 H), 4.95 (s, 1 H), 4.91 (s, 1 H), 3.31 (br s, 1 H), 2.98 (dd, 1 H, J = 16.8, 5.1 Hz), 2.77 (d, 1 H, J = 16.2 Hz), 2.20 (s, 2 H), 1.55 (s, 3 H); ^{13}C NMR (MeOH-d_4) δ 156.2, 141.9, 134.8, 131.9, 125.3, 116.0, 113.9, 108.7, 94.7, 45.8, 40.3, 31.8, 28.0, 13.1; mass spectrum m/z 228 (M^+) 213, 198, 97, 69, 57; exact mass calcd for $C_{14}H_{16}N_2O$ 228.1263, obsd 228.1263.

Propylidene Analogue 35. This compound was prepared by methods analogous to those used to prepare huperzine A, and therefore only spectral data are provided: R_f = 0.11 ($CHCl_3$–acetone–MeOH 50:45:5); IR 3400, 3305, 2959, 2926, 1734, 1655, 1614, 1554, 733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.94 (br s, 1 H), 7.92 (d, 1 H, J = 9.6 Hz), 6.41 (d, 1 H, J = 9.3 Hz), 5.40 (m, 2 H), 3.59 (m, 1 H), 2.90 (dd, 1 H, J = 5.1 Hz and 17.1 Hz), 2.69 (dd, 1 H, J = 6.9 and 16.8 Hz), 2.16–2.03 (m, 3 H), 1.60 (m, 1 H), 1.55 (s, 3 H), 0.99 (t, 3 H, J = 7.5 Hz); mass spectrum, m/z 256 (M^+) 234, 201, 97, 91, 69, 57; exact mass calcd for $C_{16}H_{20}N_2O$ 256.1576, obsd 256.1576.

Phenyl Analogue 37. This compound was prepared by methods analogous to those used to prepare huperzine A, and therefore only spectral data are provided: R_f = 0.1 (basic SiO_2, $CHCl_3$–acetone–MeOH 50:45:5); IR 3280, 2924, 1699, 1657, 1616, 1558, 1522, 910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.6 (br s, 1 H), 7.95 (d, 1 H, J = 9.3 Hz), 7.26–7.17 (m, 5 H), 6.41 (d, 1 H, J = 9.4 Hz), 6.10 (m, 1 H), 5.58 (q, 1 H, J = 6.4 Hz), 3.83 (s, 1 H), 3.04 (dd, 1 H, J = 4.9, 16.4 Hz), 2.85 (d, 1 H, J = 16.3 Hz), 2.74 (d, 1 H, J = 16.5 Hz), 2.53 (d, 1 H, J = 16.4 Hz), 1.73 (d, 1 H, J = 6.7 Hz); mass spectrum, m/z 304 (M^+) 289, 275, 261, 187, 91; exact mass calcd for $C_{20}H_{20}N_2O$ 304.1576, obsd 304.1577.

Preparation of Mesylate 39. (1) To an ice-cold solution of the ester **26** (0.075 g, 0.25 mmol) in THF (3 mL) was added LAH (0.019 g, 0.5 mmol), and stirring was continued for 3 h at rt. The excess LAH was treated with ethyl acetate (4 mL), and the reaction was quenched with a 10% aqueous Na_2SO_4 solution. The solvent was removed, and the residue was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography to afford the alcohol in 82% yield (0.055 g): R_f = 0.42 (20% ethyl acetate in hexanes); IR 3362, 2928, 1595, 1578, 1506, 1473, 825, 789 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.55 (d, 1 H, J = 8.6 Hz), 6.58 (d, 1 H, J = 8.6 Hz), 5.52 (q, 1 H, J = 6.7 Hz), 5.44 (m, 1 H), 4.15 (d, 1 H, J = 11.9 Hz), 4.05 (d, 1 H, J = 11.9 Hz), 3.87 (s, 3 H), 3.59 (m, 1 H), 3.03 (dd, 1 H, J = 5.3, 17.2 Hz), 2.83 (dd, 1 H, J = 1.7, 17.2 Hz), 2.14 (d, 1 H, J = 16.5 Hz), 1.80 (d, 1 H, J = 16.5 Hz), 1.74 (d, 3 H, J = 6.7 Hz), 1.25 (s, 3 H); mass spectrum, m/z 271 (M^+), 240, 198; exact mass calcd for $C_{18}H_{21}NO_2$ 271.1572, obsd 271.1573.

(2) To the alcohol (25 mg, 0.092 mmol) in dichloromethane (3 mL) was added triethylamine (0.064 mL, 0.46 mmol) and a catalytic amount of 4-(dimethylamino)pyridine followed by methanesulfonyl chloride (15 μL, 0.18 mmol). The reaction mixture was stirred at rt for an additional 2 h, and then it was quenched by the addition of ice. The mixture was further diluted with dichloromethane, and the organic layer was washed with 10% $NaHCO_3$ and brine and dried. Purification of the crude compound by column chromatography (25% ethyl acetate in hexanes) afforded mesylate **39** (30 mg) in 92% yield: R_f = 0.58 (30% ethyl acetate in hexanes); ^1H NMR ($CDCl_3$) δ 7.51 (d, 1 H, J = 8.6 Hz), 6.58 (d, 1 H, J = 8.6 Hz), 5.49 (m, 1 H), 5.45 (q, 1 H, J = 6.7 Hz), 4.69 (d, 1 H, J = 10.3 Hz), 4.63 (d, 1 H, J = 10.3 Hz), 3.89 (s, 3 H), 3.61 (m, 1 H), 3.03 (dd, 1 H, J = 5.0, 17.1 Hz), 2.93 (s, 3 H), 2.84 (dd, 1 H, J = 1.1, 17.1 Hz), 2.26 (d, 1 H, J = 16.4 Hz), 1.89 (d, 1 H, J = 16.4 Hz), 1.47 (d, 3 H, J = 6.6 Hz), 1.53 (s, 3 H).

Preparation of Azide 40. To the mesylate **39** (28 mg, 0.088 mmol) in dry HMPA (3 mL) were added sodium azide (57 mg, 0.88 mmol) and a catalytic amount of 18-crown-6. The reaction mixture was stirred at 120–130 °C for 12 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with water, dried, and concentrated. Purification of the crude compound by flash chromatography (15% ethyl acetate in hexanes as eluent) afforded azide **40** (21 mg) in 84% yield: R_f = 0.65 (20% ethyl acetate in hexanes); IR 2928, 2096, 1595, 1475, 983 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.49 (d, 1 H, J = 8.5 Hz), 6.59 (d, 1 H, J = 8.6 Hz), 5.46 (m, 1 H), 5.39 (q, 1 H, J = 6.7 Hz), 3.89 (s, 2 H), 3.88 (s, 3 H), 3.61 (m, 1 H), 3.05 (dd, 1 H, J = 5.3, 17.1 Hz), 2.85 (dd, 1 H, J = 1.2, 17.2 Hz), 2.23 (d, 1 H, J = 16.3 Hz), 1.90 (d, 1 H, J = 16.3 Hz), 1.74 (d, 3 H, J = 6.6 Hz), 1.25 (s, 3 H); mass spectrum, m/z 296 (M^+), 268, 253, 240, 212, 198, 57; exact mass calcd for $C_{17}H_{20}N_4O$ 296.1637, obsd 296.1636.

Preparation of Amine 41. To the cooled azide **40** (18 mg, 0.061 mmol) in THF (2.5 mL) was added LAH (5 mg, 0.122 mmol), and the resulting mixture was stirred at rt for 5 h. The excess LAH was treated with ethyl acetate, and the mixture was quenched with a 10% Na_2SO_4 solution. The product was extracted with ethyl acetate, and the organic layer was washed with water, dried over K_2CO_3, and concentrated. Purification of the crude product by column chromatography (75% ethyl acetate in hexanes) afforded the amine **41** (15 mg) in 94% yield: R_f = 0.08 (ethyl acetate); IR 3250–3400 (weak br peak), 2926, 2855, 1576, 1473, 1321, 1307, 1261 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46 (d, 1 H, J = 8.5 Hz), 6.60 (d, 1 H, J = 8.5 Hz), 5.42 (m, 1 H), 5.38 (q, 1 H, J = 6.6 Hz), 3.89 (s, 3 H), 3.60 (m, 1 H), 3.28 (d, 1 H, J = 14.1 Hz), 3.13 (d, 1 H, J = 14.3 Hz), 3.02 (dd, 1 H, J = 5.3, 17.1 Hz), 2.83 (dd, 1 H, J = 17.0 Hz), 2.16 (d, 1 H, J = 16.0 Hz), 1.82 (d, 1 H, J = 16.5 Hz), 1.74 (d, 3 H, J = 6.5 Hz), 1.25 (s, 3 H); mass spectrum, m/z 270 (M^+).

Preparation of Pyridone 42. (1) To an ice-cold solution of amine **41** (12 mg, 0.04 mmol) in dichloromethane (2 mL) were added triethylamine (20 μL, 0.16 mmol) and methyl chloroformate (7 μL, 0.08 mmol). The reaction mixture was stirred for an additional 6 h at rt. The solvents were removed by rotary evaporation, and the residue was dissolved in water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated. Flash chromatography (25% ethyl acetate in hexanes) of the crude product afforded the carbamate in nearly quantitative yield: R_f = 0.16 (20% ethyl acetate in hexanes); IR (neat) 3331 (br), 2926, 1724, 1595, 1558, 825 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45 (d, 1 H, J = 8.6 Hz), 6.56 (d, 1 H, J = 8.5 Hz), 5.43 (m, 1 H), 5.33 (d, 1 H, J = 6.6 Hz), 4.37 (br, 1 H, NH), 3.88 (s, 3 H), 3.84 (d, 1 H, J = 7.1 Hz), 3.73 (m, 1 H), 3.67 (dd, 1 H, J = 3.6, 13.8 Hz), 3.58 (s, 3 H), 2.97 (dd, 1 H, J = 4.9, 17.0 Hz), 2.83 (d, 1 H, J = 16.9 Hz), 2.24 (d, 1 H, J = 16.5 Hz), 1.89 (d, 1 H, J = 16.5 Hz), 1.71 (d, 3 H, J = 6.6 Hz), 1.50 (s, 3 H); mass spectrum, m/z 328 (M^+), 253, 240, 226, 212,

198, 123, 88; exact mass calcd for $C_{19}H_{24}N_2O_3$ 328.1787, obsd 328.1788.

(2) To the carbamate (13 mg, 0.039 mmol) in chloroform (7 mL) was added iodotrimethylsilane (56 μL, 0.4 mmol), and the resulting solution was refluxed for 7 h. Methanol (5 mL) was added, and reflux was continued for 8 h. The solvents were removed by rotary evaporation, and the crude product was purified by flash chromatography (silica gel half-saturated with ammonia) using 3% methanol in chloroform as eluent and then 50% methanol in chloroform to afford pyridone **42** (7 mg) in 70% yield: R_f = 0.025 (basic SiO_2, $CHCl_3$–acetone–MeOH 50:45:5, two runs); IR (Nujol) 3149, 3121, 1651, 1600, 1152, 1119 cm^{-1}; ^1H NMR (MeOH-d_4) δ 7.66 (d, 1 H, J = 9.4 Hz), 6.44 (d, 1 H, J = 9.4 Hz), 5.46 (m, 1 H), 5.36 (q, 1 H, J = 6.6 Hz), 3.64 (br, 1 H), 3.57 (dd, 2 H, J = 8.5, 14.2 Hz), 2.86 (dd, 1 H, J = 4.8, 17.2 Hz), 2.64 (d, 1 H, J = 16.9 Hz), 2.20 (d, 1 H, J = 16.5 Hz), 2.02 (d, 1 H, J = 16.6 Hz), 1.75 (d, 3 H, J = 6.6 Hz), 1.55 (s, 3 H); mass spectrum, m/z 256 (M^+), 239, 224, 212, 200, 184, 128; exact mass calcd for $C_{16}H_{20}N_2O$ 256.1570, obsd 256.1576.

5-(Aminomethyl)-2(1*H*)-pyridinone (46). 5-Cyano-2-methoxypyridine (**44**, 2 g, 14.9 mmol), prepared by the procedure of Forrest and Walker,[27] was dissolved in 40 mL of ammonia-saturated methanol and hydrogenated over 0.8 g of Raney nickel at a pressure of 45 psi. After 3 h the catalyst was filtered, the solvent evaporated, and the crude product dissolved in 20 mL of dichloromethane. The solution was cooled (ice bath), and 4 mL of triethylamine and 2.3 mL of methyl chloroformate were added. After 2 h at rt, the solvent was removed by rotary evaporation, and the residue was dissolved in water and extracted with 3 × 10 mL of ethyl acetate. The combined organic layers were washed with water, dried, and concentrated to afford 1.6 g (55% overall yield) of the crude carbamate **45**. The carbamate (116 mg, 0.59 mmol) was dissolved in 10 mL of chloroform and refluxed for 7 h with 1.68 mL (11.8 mmol) of iodotrimethylsilane. Methanol (5 mL) was then added and gentle reflux maintained for 8 h. The reaction mixture was concentrated, and the crude product was purified by flash chromatography over ammonia-saturated silica gel, using first 3% methanol in ethyl acetate and then 25% methanol in ethyl acetate to furnish 47 mg (75%) of the pyridone **46**: R_f = 0.1 (basic SiO_2, 7% methanol in ethyl acetate); IR (Nujol) 3387, 2922, 2856, 1653, 1606, 904 cm^{-1}; ^1H NMR (D_2O) δ 7.75 (dd, 1 H, J = 2.4, 9.3 Hz), 7.66 (s, 1 H), 6.67 (d, 1 H, J = 9.3 Hz), 4.03 (s, 2 H); ^{13}C NMR (MeOH-d_4) δ 155.0, 136.3, 128.7, 110.5, 106.4, 31.2; mass spectrum, m/z 124 (M^+), 108, 96, 78, 53; exact mass calcd for $C_6H_8N_2O$ 124.0637, obsd 124.0637.

5-[(Dimethylamino)methyl]-2(1*H*)-pyridinone (48). Since the transformations employed to prepare compound **48** are routine and in part related to the methods described above, only spectral data follow: R_f = 0.15 (acetone–$CHCl_3$–MeOH 9:10:1): IR (Nujol) 2922, 1672, 1593, 1543, 1522, 1377, 1153, 1005, 949, 721 cm^{-1}; ^1H NMR (D_2O) δ 7.75 (d, 0.5 H, remaining portion of dd obscured by proton at 7.71), 7.71 (br s, overlapping signals, 1.5 H), 6.67 (dd, 1 H, J = 8.5, 1.5 Hz), 4.15 (s, 2 H), 2.62 (s, 6 H); ^{13}C NMR (MeOH-d_4) δ 155.6, 135.4, 130.4, 112.0, 100.9, 48.9, 33.1; mass spectrum, m/z 152 (M^+), 128, 108, 80, 69, 58; exact mass calcd for $C_8H_{12}N_2O$ 152.0950, obsd 152.0949.

Determination of AChE Activity. Rats were killed by decapitation. Brains were extirpated rapidly. The hippocampus was dissected out on ice according to the method of Glowinski and Iverson.[29] Samples were homogenized in ice-cold 0.32 M sucrose. Homogenates were centrifuged at 1000*g* for 10 min to remove cell nuclei and heavy debris. The supernatant was then aspirated off and spun again (12000*g*) for 20 min to form a pellet (Whittaker's P_2 fraction) that contained synaptosomes and mitochondria.[30] The pellet was resuspended in 0.32 M sucrose. A portion of this synaptosome-rich fraction was added in triplicate to ice-cold pH 7.4 Krebs–Ringer medium.

Assay of AChE was carried out according to the method described in Mantione et al.[31] Tissue homogenate was incubated for 30 min at 30 °C in a final volume of 20 μL containing 75 mM sodium phosphate buffer, pH 7.0, containing 1.5 mM [^{14}C]-acetylcholine (1.9 mCi/mmol). To each sample was added 25 μL of cold water, followed by 150 μL of tetraphenylboron solution.[32] The tubes were vortexed for 10 s and then centrifuged for 1 min. The bottom aqueous layer was quickly frozen in a dry ice/acetone bath, and the top organic layer was aspirated off. Finally, the buffer was allowed to thaw, and a 25-μL portion was counted for the amount of [^{14}C]acetate formed. The amount of residual [^{14}C]acetylcholine left in the buffer by the extraction step alone was determined by subtracting from the tissue sample values of [^{14}C]acetylcholine measured in a blank sample that contained buffer and substrate, but no tissue.

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Supplementary Material Available: ^1H NMR for **48**, **46**, **42**, **41**, **40**, **39**, **37**, **35**, **34**, **29**, **28**, **25**, and **10**/**11** and ^{13}C NMR for **25**, **24**, **18**, **17**, and **14b** (18 pages). Ordering information is given on any current masthead page.

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Reaction of Aminopropanedinitrile 4-Methylbenzenesulfonate (Aminomalononitrile *p*-Toluenesulfonate (Tosylate)) with Isothiocyanates

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Aminopropanedinitrile 4-methylbenzenesulfonate (ammoniopropanedinitrile or aminomalononitrile *p*-toluenesulfonate (tosylate)) reacts with alkyl and aryl isothiocyanates in 1-methyl-2-pyrrolidinone (NMP) to give 5-amino-2-(alkylamino)-4-cyanothiazoles and 5-amino-2-(arylamino)-4-cyanothiazoles (2,5-diaminothiazole-4-carbonitriles), respectively, which react with amidines or ortho esters to afford 7-amino-2-(alkylamino)thiazolo[5,4-*d*]pyrimidines and 7-amino-2-amino-2-(arylamino)thiazolo[5,4-*d*]pyrimidines.

Propanedinitrile (malononitrile, dicyanomethane, $CH_2(CN)_2$) and its derivatives are important compounds for the preparation of diverse substrates and for the synthesis of a wide variety of heterocycles.[1-7] Of particular

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