resentation;[35,36] parameters for the drug molecules were developed in previous studies in this laboratory. A distance-dependent dielectric constant of the form $\epsilon = 4r_{ij}$ was used.[37] The energy minimization calculations were performed using the EMPMDS program[38] on a VAX 11/750 computer. Refinements for the DNA–ligand complexes, and the separate DNA and ligands, were judged to have reached convergence in total energy when the rms value of the first derivative was ≤ 0.15 kcal mol^{-1} Å^{-1}. This was achieved within approximately 2000 cycles of energy refinement.

Biological Activity in Vitro. Three cell lines, leukemia L1210, Walker 256 (WS) carcinoma, and V79 Chinese hamster ovarian, were used. In each case, cells at a density of ca. 5×10^3 cells mL^{-1} were grown for 24 h, counted to confirm logarithmic growth, and treated with the compound (as the acetic acid addition salt or free base forms for 5–17) in aqueous solution. Incubation was continued for 2 days for the L1210 and WS assays and 6 days for the V79 cells. The surviving cells were then counted and the percentage inhibition in growth was calculated as compared to untreated controls.

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Supplementary Material Available: Tables of hydrogen atom coordinates and non-hydrogen atom anisotropic thermal parameters (2 pages). Ordering information is given on any current masthead page.

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Syntheses, Resolution, and Structure–Activity Relationships of Potent Acetylcholinesterase Inhibitors: 8-Carbaphysostigmine Analogues

Yuhpyng L. Chen,* Jann Nielsen, Kirk Hedberg, Audrey Dunaiskis, Shawn Jones, Lorena Russo, Jonathan Johnson, Jeffrey Ives, and Dane Liston

Medicinal Chemistry and Neuroscience Departments, Central Research Division, Pfizer Inc., Groton, Connecticut 06340.
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The synthesis of a series of 1,2,3,3a,8,8a-hexahydroindeno[2,1-*b*]pyrrole 5-alkylcarbamates and their resolution are reported. These compounds are structurally related to physostigmine with substitution of a methylene group in place of the NMe group at position 8 of physostigmine. Many of these 8-carbaphysostigmine analogues are more potent acetylcholinesterase inhibitors in vitro and less toxic in vivo than physostigmine. The (−)-enantiomer (e.g., **1d** and **1g**) possessing the same absolute configuration at C_{3a} and C_{8a} as that of physostigmine, is about 6 to 12-fold more potent at inhibiting acetylcholinesterase than the corresponding (+)-enantiomer (e.g., **1e** and **1h**).

Clinical studies suggest that acetylcholinesterase (AChE) inhibitors, such as THA (tetrahydro-9-aminoacridine) and physostigmine, may be useful in enhancing memory in patients with Alzheimer's disease.[1–4] However, THA induces a high incidence of liver toxicity.[5] The liver toxicity produced by THA may be structure-dependent rather than mechanism-related since physostigmine reportedly has not produced liver toxicity in clinical trials.[6,7] Physostigmine suffers from a short half-life, variable bioavailability, and a narrow therapeutic index,[8] which may account for the inconsistent clinical efficacy. A controlled-release formulation of physostigmine (Cogmine) is currently in phase III clinical trials for the treatment of Alzheimer's disease.[6] The related analogue, heptylphysostigmine (MF-201), was reported to have significantly less toxicity than physostigmine while retaining its in vitro potency in inhibiting AChE.[7] We envisioned replacing the *N*-methyl group at N_8 of the physostigmine nucleus with a methylene group to increase its chemical and metabolic stability by modification of the less stable aminal group to a more stable amino group. Since the cationic protonated amine at N_1 of physostigmine interacts with the anionic site of AChE, the resulting structure, 8-carbaphysostigmine **1**, would be expected to show better interaction with the enzyme because of its increased basicity. Furthermore, modifications

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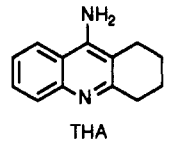
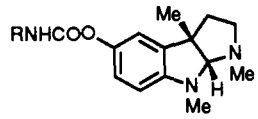
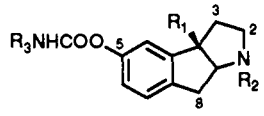


THA



R = Me, physostigmine
R = *n*-heptyl, heptylphysostigmine



1

8-carbaphysostigmine

of the substituents at the N_1, C_{3a}, and C_5 positions were explored in series 1. Resolutions of these 1,2,3,3a,8,8a-hexahydro-3a-methyl- and 1,2,3,3a,8,8a-hexahydroindeno[2,1-*b*]pyrrole carbamates were achieved. The syntheses and resolutions, as well as structure–activity relationships for in vitro AChE inhibition, elevation of brain acetylcholine (ACh) levels in vivo, and acute toxicity of this series of compounds are described herein.

Chemistry

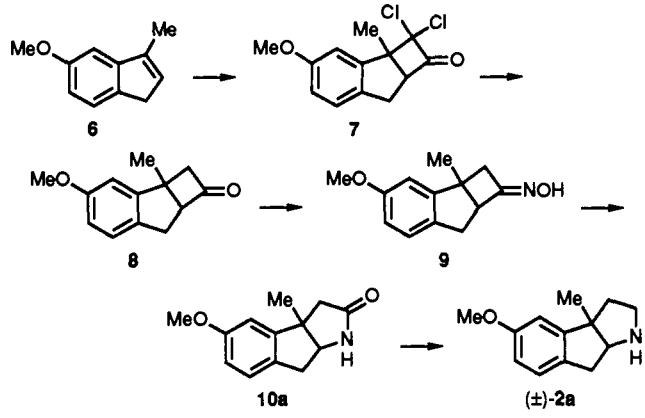
8-Carbaphysostigmine analogues 1 were synthesized via a key synthetic intermediate 2. The racemic tricyclic amine 2 was prepared via either a [2+2] cycloaddition followed by Beckmann rearrangement, as shown in Scheme I, or reductive cyclization followed by hydrogenation, as shown in Scheme II. Resolution of amine 2 as well as the synthesis of the final racemic and resolved 8-carbaphysostigmine analogues 1 are outlined in Scheme III.

[2 + 2] cycloaddition of indene 6 and dichloroketene afforded dichlorocyclobutanone derivative 7, which was subsequently dechlorinated with zinc and ammonium chloride in methanol at 40–45 °C to give cyclobutanone 8. Treatment of 8 with hydroxylamine and sodium acetate in methanol afforded the oxime 9 as a mixture of *Z* and *E* isomers. The best conditions for the Beckmann rearrangement for conversion of the oxime mixture to the desired regioisomer 10 were sodium hydride and tosyl chloride in methylene chloride at 0 °C, followed by stirring at room temperature for 15 h. The desired lactam 10 was obtained as a white solid in 60% yield after triturating with diethyl ether. Reduction of the lactam 10 with $LiAlH_4$ gave amine 2a. Tricyclic amines 2a and 2b can also be prepared using the method analogous to the literature[9] starting from indanone acetate 11a[9,10] and 11b as shown in Scheme II. Treatment of 11 with *tert*-butyl nitrite afforded the oxime 12. Hydrogenation of the oxime 11 at room temperature, followed by heating the filtrate in acetic acid to reflux gave the cyclized keto lactam 13. Hydrogenation of 13 with Pd/C at 55 psi and 70 °C gave the tricyclic lactam 10 which is reduced with $LiAlH_4$ to give tricyclic amine 2.

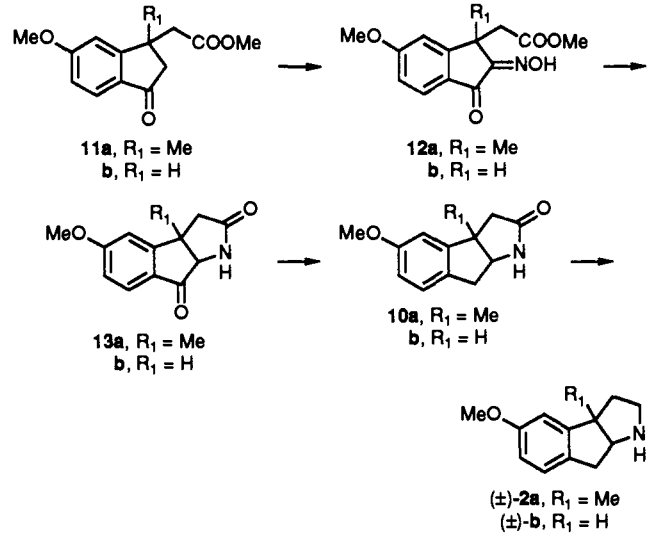
Resolution of 2a was achieved by recrystallizations of the corresponding di-*p*-toluoyl-L-tartaric acid salt with 2-propanol (Scheme III). The first crop of crystals was recrystallized several times until the optical rotation of the corresponding free base 4a reached a constant value of $[\alpha]^{25}_{589}$ +97° (methylene chloride, c = 1). The filtrate from the first recrystallization was recrystallized several times until the optical rotation of the corresponding free base 5a reached a constant value of $[\alpha]^{25}_{589}$ −93° (methylene chloride, c = 1). Both enantiomers were converted to the corresponding N_1-carbamate diasteromers 14a and 15a, by reaction with (*S*)-1-phenylethyl isocyanate, and the optical purity was determined by ^1H NMR. The methyl groups at the benzylic position of 14a and 15a show doublets at 1.49 ppm and 1.41 ppm, respectively, each with greater than 95% purity. Resolution of 2b was achieved by several recrystallizations of the corresponding (*R*)- and (*S*)-mandelic acid salts until the optical rotations of the corresponding free bases 4b and 5b reached a constant value of $[\alpha]^{25}_{589}$ −80° and +83° (methylene chloride, c = 1), respectively. The optical purity of 4b and 5b was determined by the method described for 4a and 5a. The absolute configurations of 4a and 5b were determined by X-ray crystal structural analysis of the corresponding chiral acid salt.

Both racemic amine 2 and enantiomers 4 and 5 were independently converted to the final 8-carbaphysostigmine analogue 1. *N*-Alkyl derivative 3 was obtained by acylation, followed by reduction with borane–methyl sulfide complex. Demethylation of 3, followed by reaction with alkyl isocyanate afforded 8-carbaphysostigmine 1. The corresponding di-*p*-toluoyl-L-tartrate salts were prepared as a form suitable for biological studies. The physicochemical properties of the synthetic intermediates and the final 8-carbaphysostigmine derivatives are listed in Tables I and II, respectively.

Scheme I



Scheme II



Biological Results and Discussion

The in vitro enzyme inhibition data and acute toxicity in mice of physostigmine, heptylphysostigmine, and 8-carbaphysostigmine analogues are presented in Table III.

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Scheme III

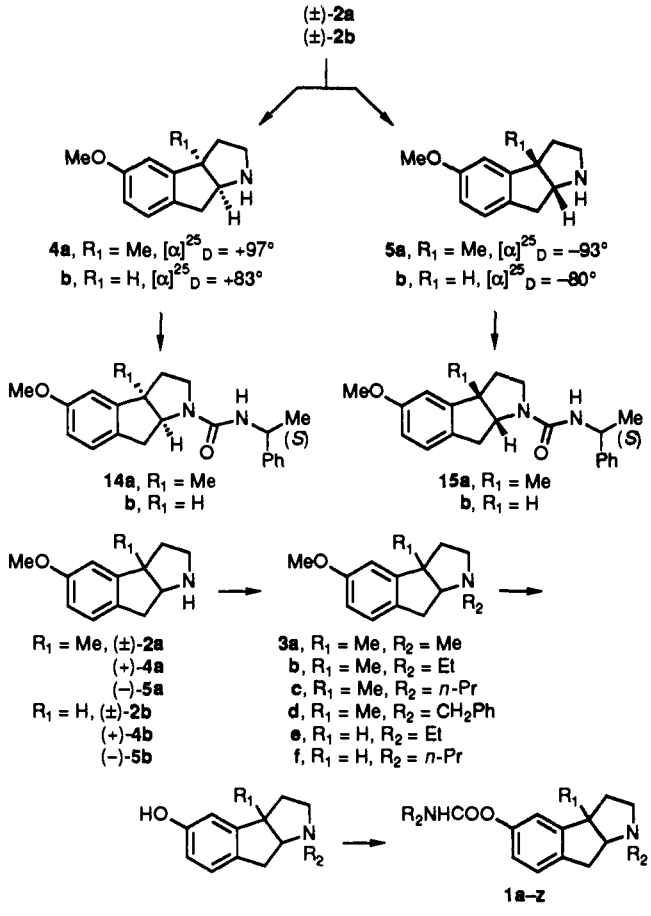

Table I. Physicochemical Properties of the Synthetic Intermediates

compd	mp (°C)	yield[a] (%)	formula	anal.
11a	oil	87	$C_{14}H_{16}O_4 \cdot 0.35H_2O$	C,H
12a	189–190	97	$C_{14}H_{15}NO_5$	C,H,N
13a	180–182	80	$C_{13}H_{13}NO_3$	C,H,N
2a	oil	90	$C_{13}H_{17}NO$	C,H,N
3c	oil	86	$C_{16}H_{23}NO \cdot C_{20}H_{18}O_8 \cdot 0.4H_2O$[b]	C,H,N
3d	oil	78	$C_{20}H_{23}NO \cdot C_{20}H_{18}O_8 \cdot 0.6H_2O$[b]	C,H,N
3e	oil	96	$C_{14}H_{19}NO \cdot C_{20}H_{18}O_8$[b]	C,H,N
3f	oil	75	$C_{15}H_{21}NO \cdot C_{20}H_{18}O_8 \cdot 0.3H_2O$[b]	C,H,N

[a] Yields were not optimized. [b] The parent compound is an oil; thus, the corresponding di-*p*-toluoyl-L-tartaric acid salt was prepared, and the resulting solid salt was washed with diethyl ether.

The elevations of brain acetylcholine levels in vivo induced by compound 1, THA, and heptylphysostigmine are shown in Table IV.

The active center of AChE consists of two subsites[11] which interact with ACh or the carbamate inhibitors: an anionic site which presumably attracts either the cationic form of ACh or the protonated-amine form of the inhibitors, and a serine residue which is acetylated by ACh or carbamoylated by the inhibitor. The structure–activity relationships in series 1 support this model as follows: (i) a bulky group at N_1 reduces activity, presumably by interfering with binding at the anionic site (**1r** vs **1a**). In general, the enzyme tolerates methyl, ethyl and *n*-propyl groups at N_1 (**1a**, **1b**, **1f**, **1p**, and **1q**); (ii) a sterically bulky carbamate side chain reduces activity, presumably by hindering the approach of the serine residue of the enzyme to the carbonyl group (**1j** and **1n**); (iii) in general, compounds with a methyl group at C_{3a} are 5 to 15 times more potent at inhibiting AChE than those without a substituent at C_{3a} (see Table III, example **1c–q** vs **1s–z**); (iv) the (−)-enantiomers (**1d** and **1g**), which possess the same absolute configurations at C_{3a} and C_{8a} positions as natural (−)-physostigmine, were 6 to 12 times more potent than the corresponding (+)-enantiomers (**1e** and **1h**); (v) the increased basicity[12] of the N_1-nitrogen in 1 (eq., **1l**) over that in physostigmine leads to improved potency, presumably due to a better interaction with the anionic site. While the methyl carbamate analogue showed potent AChE inhibitory activity but high toxicity, extending the chain length linearly to *n*-heptyl resulted in similar potency but diminished toxicity in mice (**1c** vs **1l**). A similar result was seen in the physostigmine series (physostigmine vs heptylphysostigmine).

Several compounds with potent in vitro activity were evaluated in vivo by measuring the increase in mouse brain ACh levels induced by systemically-administered inhibitor. THA and heptylphysostigmine served as controls and showed reproducible elevation of the ACh levels in mouse brain at doses equivalent to 20% of their LD50. Doses equivalent to this fraction of the LD50 were used to study the test compounds; data are shown in Table IV. All of the six novel compounds shown in Table IV produced statistically significant elevations in mouse brain ACh levels. Thus, potent in vitro AChE inhibitory activity translated into potent in vivo efficacy for many of the compounds in this series.

Conclusions

Replacement of the *N*-methyl group at position 8 of physostigmine with a methylene group has produced AChE inhibitors with greater potency and reduced toxicity. The SAR of this series of 8-carbaphysostigmine analogues can be explained in terms of currently accepted models for the active site of AChE. In addition, several compounds elevated mouse brain ACh levels at lower doses than THA and heptylphysostigmine.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Differential scanning calorimetry (DSC) was measured by a Perkin-Elmer DSC-4. ^1H NMR and ^{13}C NMR spectra were measured on a Varian XL-300 or Bruker 300 spectrometer, and chemical shifts are reported in δ with tetramethylsilane as internal reference. IR spectra were obtained on a Perkin-Elmer 1420 spectrophotometer. Elemental analyses were performed by the Analytical Laboratory of Pfizer Central Research, and the analytical results obtained for those elements were within ±0.4% of theoretical values. Optical rotations were taken on a Perkin-Elmer 241 MC polarimeter. pK_a values were determined according to the procedure of Clarke.[13]

1-Methyl-6-methoxy-3*H*-indene (6). A solution of 6-methoxy-1-indanone (8.100 g, 50 mmol) in dry THF was added dropwise to a solution of MeMgBr (3 N in ether, 20 mL, 60 mmol) in dry THF at 0 °C. After completion of addition, the mixture was stirred at room temperature for 0.5 h, quenched with aqueous

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(12) pK_a values of compounds in series 1 were not obtained because of their poor water solubility. By analogy of a pK_a value of 9.56 of the intermediate **3b**, compounds in series 1 are expected to be more basic than physostigmine which has a pK_a value of 8.29.

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Table II. Physicochemical Properties for 9-Carbaphysostigmine Derivatives

no.	isomer	R^1	R^2	R^3	mp (°C)	yield (%)[a]	formula[b]	anal.
1a	(±)	Me	Me	*n*-hep	95[g]	76	$C_{21}H_{32}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.5H_2O$	C,H,N
1b	(±)	Me	Me	*n*-hex	98[g]	50	$C_{20}H_{30}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.5H_2O$	C,H,N
1d	(-)	Me	Et	*n*-hep	89–92[c]	72	$C_{22}H_{34}N_2O_2 \cdot C_{20}H_{18}O_8$	C,H,N
1e	(+)	Me	Et	*n*-hep	81[g]	45	$C_{22}H_{34}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.4H_2O$	C,H,N
1f	(±)	Me	Et	*n*-hex	85[g]	92	$C_{21}H_{32}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.5H_2O$	C,H,N
1g	(-)	Me	Et	*n*-hex		60	$C_{21}H_{32}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.5H_2O$	C,H,N
1h	(+)	Me	Et	*n*-hex	122–123[c]	72	$C_{21}H_{32}N_2O_2 \cdot C_{20}H_{18}O_8$	C,H,N
1i	(±)	Me	Et	*n*-bu	95[g]	62	$C_{19}H_{28}N_2O_2 \cdot C_{20}H_{18}O_8$	C,H,N
1j	(±)	Me	Et	*t*-Bu	94–95[d]	62	$C_{19}H_{28}N_2O_2$	
1k	(±)	Me	Et	*n*-Pr	92[g]	89	$C_{18}H_{26}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.6H_2O$	C,H,N
1l	(±)	Me	Et	Me	107[g]	78	$C_{16}H_{22}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.8H_2O$	C,H,N
1m	(±)	Me	Et	Ph	144–116[e]	71	$C_{21}H_{24}N_2O_2$	
1n	(±)	Me	Et	(*S*)-CH(Me)Ph	107[g]	92	$C_{23}H_{28}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.6H_2O$	C,H,N
1o	(±)	Me	Et	CH_2Ph	102[g]	54	$C_{22}H_{26}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.5H_2O$	C,H,N
1p	(±)	Me	*n*-Pr	*n*-hep	81[g]	77	$C_{23}H_{36}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.3H_2O$	C,H,N
1q	(±)	Me	*n*-Pr	*n*-hex	84[g]	81	$C_{22}H_{34}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.6H_2O$	C,H,N
1r	(±)	Me	CH_2Ph	*n*-hex	82[g]	66	$C_{27}H_{36}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.8H_2O$	C,H,N
1s	(±)	H	Et	*n*-hep	103–106[f]	30	$C_{21}H_{32}N_2O_2$	C,H,N
1t	(±)	H	Et	*n*-hex	104–106[f]	61	$C_{20}H_{30}N_2O_2$	C,H,N
1u	(±)	H	Et	*n*-Bu	109–111[f]	67	$C_{18}H_{26}N_2O_2$	C,H,N
1v	(±)	H	Et	*n*-Pr	117–120[f]	57	$C_{17}H_{24}N_2O_2$	C,H,N
1w	(±)	H	Et	Me		85	$C_{15}H_{20}N_2O_2 \cdot C_{20}H_{18}O_8 \cdot 0.7H_2O$	C,H,N
1x	(±)	H	*n*-Pr	*n*-hep	106–108[f]	72	$C_{22}H_{34}N_2O_2$	C,H,N
1y	(±)	H	*n*-Pr	*n*-hex	103–105[f]	51	$C_{21}H_{32}N_2O_2$	C,H,N
1z	(-)	H	*n*-Pr	*n*-hex		38	$C_{21}H_{32}N_2O_2$	C,H,N

[a] Yields were not optimized. [b] $C_{20}H_{18}O_8$ = di-*p*-toluoyl-L-tartaric acid. [c] The parent compound is an oil, and the corresponding salt was prepared and recrystallized from ethyl acetate. [d] The parent compound was recrystallized from petroleum ether. [e] The parent compound was recrystallized from a mixture of ether and heptane. [f] The parent compound was recrystallized from heptane. [g] The parent compound is an oil. The corresponding salt was prepared and triturated with diethyl ether to give a white solid. The value given is the center of a broad endotherm as measured by DSC.

Table III. AChE IC50[a] and LD50[b] of 8-Carbaphysostigmine Analogues, Physostigmine, and Heptylphysostigmine

compd	isomer	R_1	R_2	R_3	IC50 (nM)	LD50 (mg/kg)
physostigmine	(-)				128 ± 14	0.88
heptylphysostigmine	(-)				110 ± 20	24
1a	(±)	Me	Me	*n*-hep	114 ± 1	21
1b	(±)	Me	Me	*n*-hex	77 ± 4	10
1c	(±)	Me	Et	*n*-hep	58 ± 4	9
1d	(-)	Me	Et	*n*-hep	36 ± 5	6
1e	(+)	Me	Et	*n*-hep	211 ± 13	18
1f	(±)	Me	Et	*n*-hex	45 ± 6	5
1g	(-)	Me	Et	*n*-hex	20 ± 7	4
1h	(+)	Me	Et	*n*-hex	233 ± 22	32
1i	(±)	Me	Et	*n*-Bu	90 ± 16	4
1j	(±)	Me	Et	*t*-Bu	12 487 ± 33	>100
1k	(±)	Me	Et	*n*-Pr	154 ± 17	6
1l	(±)	Me	Et	Me	38 ± 5	.88
1m	(±)	Me	Et	Ph	176 ± 29	8
1n	(±)	Me	Et	(*S*)-CH(Me)Ph	1330 ± 256	NT[c]
1o	(±)	Me	Et	CH_2Ph	69 ± 9	2
1p	(±)	Me	*n*-Pr	*n*-hep	93 ± 21	27
1q	(±)	Me	*n*-Pr	*n*-hex	62 ± 7	17
1r	(±)	Me	CH_2Ph	*n*-hep	506 ± 96	NT[c]
1s	(±)	H	Et	*n*-hep	381 ± 20	58
1t	(±)	H	Et	*n*-hex	208 ± 58	19
1u	(±)	H	Et	*n*-Bu	1076 ± 149	>32
1v	(±)	H	Et	*n*-Pr	2192 ± 689	NT[c]
1w	(±)	H	Et	Me	253 ± 83	NT[c]
1x	(±)	H	*n*-Pr	*n*-hep	629 ± 103	NT[c]
1y	(±)	H	*n*-Pr	*n*-hex	546 ± 11	NT[c]
1z	(-)	H	*n*-Pr	*n*-hex	518 ± 47	NT[c]

[a] IC50 values shown are mean ± standard deviation of three assays against human acetylcholinesterase. [b] LD50 values are from lethality measured at 24 h in mice following ip administration. [c] NT = not tested.

Table IV. Percent Elevation of ACh Level in Mouse Forebrain Following Intraperitoneal Administration of Inhibitor

compound	dose (mg/kg)	ACh (% control[a])
vehicle		100
THA	6.6	136[b]
heptylphysostigmine	4.8	148[b]
1b	2.0	162[b]
1d	1.2	129[b]
1g	0.8	148[b]
1m	1.6	121[b]
1o	0.4	123[b]
1q	3.4	141[b]

[a] Refer to the Experimental Section for test procedures and methods of reporting results. [b] Statistically different from vehicle with $p < 0.05$ (Student's *t*-test).

HCl to pH 1, and extracted with EtOAc. The organic layer was washed with brine, dried, and concentrated to give 6.864 g (86%) of white crystals: mp 46–47 °C; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3 H), 3.25 (m, 2 H), 3.9 (s, 3 H), 6.25 (m, 1 H), 6.75 (dd, 1 H), 6.9 (d, 1 H), 7.35 (d, 1 H) ppm; ^{13}C NMR ($CDCl_3$) δ 13.0, 37.0, 55.6, 104.8, 110.3, 123.9, 130.2, 136.5, 139.8, 147.6, 158.95 ppm. Anal. ($C_{11}H_{12}O$) C, H.

2,2-Dichloro-2,2a,7,7a-tetrahydro-4-methoxy-2a-methyl-1*H*-cyclobuta[a]inden-1-one (7). To a stirred mixture of 1-methyl-6-methoxy-3*H*-indene (2.632 g, 16.45 mmol) and activated Zn (1.190 g, 18.23 mmol) in anhydrous ether was added a solution of Cl_3CCOCl (1.9 mL, 17 mmol) and $POCl_3$ (1.6 mL, 17 mmol) in ether. The mixture was refluxed for 2 h, then stirred at room temperature overnight. Workup afforded a brown oil which can be used directly for the next dehalogenation step. An amount of 0.500 g of material was purified by silica gel column purification to give white crystals: mp 65–67 °C; ^{1}H NMR ($CDCl_3$) δ 1.8 (s, 3 H), 3.0–3.3 (m, 2 H), 3.8 (s, 3 H), 3.96 (dd, 1 H), 6.84 (m, 2 H), 7.1 (m, 1 H), ppm. Anal. ($C_{13}H_{12}O_2Cl_2$) C, H.

2,2a,7,7a-Tetrahydro-4-methoxy-2a-methyl-1*H*-cyclobut[a]inden-1-one (8). A solution of the crude material of the above dichlorocyclobut[a]inden-1-one (3.955 g, 14.59 mmol) in MeOH was treated with Zn (8.000 g, 123 mmol) and NH_4Cl (6.400 g, 120 mmol) and heated to 45–50 °C overnight. The mixture was filtered through Celite, and the filtrate was concentrated to give a white solid which was purified by silica gel chromatography to give 2.084 g (67%) of white crystals: mp 71–72 °C; ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 3 H), 2.95–3.35 (m, 4 H), 3.55–3.65 (m, 1 H), 3.8 (s, 3 H), 6.7–6.8 (m, 2 H), 7.05 (d, 1 H) ppm; ^{13}C NMR ($CDCl_3$) δ 24.5, 32.5, 44.1, 55.5, 61.0, 68.7, 108.9, 113.7, 125.6, 134.2, 149.5, 160 ppm. Anal. ($C_{13}H_{14}O_2$) C, H.

2,2a,7,7a-Tetrahydro-4-methoxy-2a-methyl-1*H*-cyclobut[a]inden-1-one Oxime (9). A solution of the above cyclobutanone 8 (2.084 g, 10.3 mmol) in MeOH was treated with NH_2OH·HCl and sodium acetate and stirred at room temperature

overnight. Workup and purification through a silica gel column afforded 1.581 g (71%) of a mixture of *Z* and *E* isomers of oxime as a white solid, which was used directly for the next reaction: ^{1}H NMR ($CDCl_3$) δ 1.55 (s, 3 H), 2.9–3.3 (m, 4 H), 3.4–3.7 (m, 1 H), 3.78 (s, 3 H), 6.65–6.8 (m, 2 H), 7.05 (m, 1 H), 8.3 (brs, 1 H) ppm; ^{13}C NMR ($CDCl_3$) a mixture of two isomers, which shows one set of peaks at δ 24.4, 35.2, 44.8, 48.0, 54.1, 55.5, 108.6, 113.6, 125.8, 134.7, 150.0, 159.6, 160.2 ppm, and the other set of signals at δ 24.3, 35.0, 45.6, 46.9, 53.7, 55.5, 108.3, 113.5, 125.6, 134.9, 150.0, 158.7, 159.5 ppm.

3,3a,8,8a-Tetrahydro-5-methoxy-3a-methylindeno[2,1-*b*]pyrrol-2(1*H*)-one (10a). A solution of the oxime **9** (434 mg) in CH_2Cl_2 was treated with NaH (88 mg) at 0 °C. After 5 min, the mixture was treated with tosyl chloride (419 mg) and stirred at 0 °C for 8 h and then room temperature overnight. The mixture was quenched with 2 N NaOH, extracted with CH_2Cl_2, dried, and concentrated to dryness. The residue was triturated with ether to give 260 mg (60%) of a white solid: ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 3 H), 2.58 (AB q, 2 H), 2.78 (d, 1 H), 3.16 (dd, 1 H), 3.77 (s, 3 H), 4.03 (d, 1 H), 6.51 (s, 1 H, NH), 6.69 (s, 1 H), 6.74 (d, 1 H), 7.06 (d, 1 H) ppm. Anal. ($C_{13}H_{15}NO_2$) C, H, N.

Methyl 5-Methoxy-1-oxoindan-3-acetate (11b). A solution of 5-methoxy-1-indanone-3-acetic acid (30.000 g, 0.136 mol) in methanol (200 mL) was treated with concentrated H_2SO_4 (4 mL), heated under reflux for 3 h, and then stirred at room temperature overnight. The resulting solution was concentrated to dryness. The residue was neutralized with saturated $NaHCO_3$ and extracted with ether. The organic layer was washed with water, dried, and concentrated to give 29.500 g (93%) of a yellow solid **11b**: ^{1}H NMR (DMSO-d_6) δ 2.48 (d, 2 H), 3.18 (m, 2 H), 3.46 (s, 3 H), 3.86 (s, 3 H), 4.24 (t, 1 H), 7.0 (dd, *J* = 1, 7 Hz, 1 H), 7.14 (d, *J* = 1 Hz, 1 H), 7.64 (d, *J* = 1, 7 Hz, 1 H) ppm.

Methyl 2,3-Dihydro-2-(hydroxyimino)-6-methoxy-3-oxo-1*H*-indene-1-acetate (12b). A solution of **11b** (29.150 g, 0.124 mol) in anhydrous diethyl ether (200 mL) was saturated with HCl gas and cooled to 5–10 °C in an ice bath, and butyl nitrite (21.01 g, 24 mL, 0.204 mol) was added dropwise and stirred for 3 h. The product was filtered off to yield 28.800 g (88%) of oxime **12b** as an orange solid. The material was used directly for the next step reaction. One gram of material was purified by silica gel column chromatography using 10% ethyl acetate in chloroform as eluent to give 0.875 g of the title compound as a yellow solid: mp 189–190 °C; ^{1}H NMR (DMSO-d_6) δ 2.46 (m, 2 H), 3.16 (m, 2 H), 3.42 (s, 3 H), 3.82 (s, 3 H), 4.2 (t, *J* = 4 Hz, 1 H), 6.96 (dd, *J* = 1, 7 Hz, 1 H), 7.12 (d, *J* = 1 Hz, 1 H), 7.64 (d, *J* = 7 Hz, 1 H) ppm. Anal. ($C_{13}H_{13}NO_5$) C, H, N.

1,3,3a,8a-Tetrahydro-5-methoxyindeno[2,1-*b*]pyrrole-2,8-dione (13b). A solution of oxime **12b** (1.000 g, 3.8 mmol) in AcOH (50 mL) saturated with HCl gas was treated with 10% Pd on carbon (0.500 g) and hydrogenated at 50 psi for 1 h. After removal of the catalyst, the filtrate was heated to reflux for 4 h, and the reaction was monitored by TLC (5% MeOH in $CHCl_3$). Removal of solvents gave a brown solid. The solid was triturated with ether to give 0.590 g (72%) of the desired dione **13b** as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 2.24 (dd, *J* = 2, 18 Hz, 1 H), 2.84 (dd, *J* = 8, 18 Hz, 1 H), 3.9 (s, 3 H), 4.04 (m, 1 H), 4.2 (d, *J* = 4 Hz, 1 H), 7.04 (dd, *J* = 1, 7 Hz, 1 H), 7.18 (d, *J* = 1 Hz, 1 H), 7.62 (d, *J* = 7 Hz, 1 H), 8.58 (s, 1 H, NH) ppm. Anal. ($C_{12}H_{11}NO_3$) C, H, N.

3,3a,8,8a-Tetrahydro-5-methoxyindeno[2,1-*b*]pyrrol-2(1*H*)-one (10b). A solution of dione **13b** (10.000 g, 0.046 mol) and 10% Pd on carbon (5.000 g) in AcOH (150 mL) was hydrogenated at 50 psi at 70 °C for 4 h. Catalyst was filtered off, and the filtrate was evaporated to dryness to give a solid which was triturated with ether to give 9.000 g (96%) of **10b** as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.22 (d, *J* = 14 Hz, 1 H), 2.64 (dd, *J* = 10, 14 Hz, 1 H), 2.72 (d, *J* = 18 Hz, 1 H), 2.98 (dd, *J* = 6, 18 Hz, 1 H), 3.68 (s, 3 H), 3.70 (m, 1 H), 4.3 (t, *J* = 6 Hz, 1 H), 6.72 (dd, *J* = 2, 8 Hz, 1 H), 6.8 (d, *J* = 2 Hz, 1 H), 7.08 (d, *J* = 8 Hz, 1 H) ppm. Anal. ($C_{12}H_{13}NO_2$) C, H, N.

1,2,3,3a,8,8a-Hexahydro-5-methoxyindeno[2,1-*b*]pyrrole (2b). To a suspension of $LiAlH_4$ (1.600 g, 42.6 mmol) in dry THF (10 mL) was added dropwise a solution of **10b** (2.900 g, 14.3 mmol) in 3 mL of THF. When the addition was complete, the mixture was heated to reflux for 5 h. The mixture was cooled and treated by successive dropwise addition of H_2O (1.6 mL), 15% NaOH solution (1.6 mL), and H_2O (4.8 mL). The mixture was stirred for 20 min, and granular precipitates were filtered off. The filtrate was dried and concentrated to yield 2.600 g (96%) of **2b** as a brown oil. The crude material was used directly for the next step without purification: ^{1}H NMR ($CDCl_3$) δ 1.9 (m, 1 H), 2.2 (m, 1 H), 2.82 (d, *J* = 15 Hz, 1 H), 2.84 (m, 2 H), 3.2 (dd, *J* = 6, 15 Hz, 1 H), 3.7 (m, 1 H), 3.98 (s, 3 H), 4.0 (t, *J* = 6 Hz, 1 H), 6.7 (m, 3 H), 7.04 (d, *J* = 7 Hz, 1 H) ppm.

Resolution of Racemic 1,2,3,3a,8,8a-Hexahydro-5-methoxyindeno[2,1-*b*]pyrrole (2b). To a solution of racemic 1,2,3,3a,8,8a-hexahydro-5-methoxyindeno[2,1-*b*]pyrrole (**2b**) (1.200 g, 6.34 mmol) in 2-propanol (5 mL) was added a solution of (*S*)-mandelic acid (0.960 g, 6.34 mmol) at room temperature, and it was stirred for 1 h. White precipitate formed and was filtered. This white solid was collected and washed with 2-propanol. The solid was dissolved in water, basified to pH 13, and extracted with CH_2Cl_2. The organic layer was dried and concentrated to give 0.320 g of an oil, $[\alpha]^{25}_{589}$ −76° (methylene chloride, *c* = 1). The oil was converted to the (*S*)-mandelic acid salt, recrystallized, and basified to give 0.191 g of oil **5b**, $[\alpha]^{25}_{589}$ −80° (methylene chloride, *c* = 1). The absolute configuration of the (*S*)-mandelic acid salt of **5b** was determined by the X-ray crystal structure analysis, and the stereochemistry of both hydrogens at C_{3a} and C_{8a} are of the β configuration.

The filtrate was basified to give an oil, which was converted to the (*R*)-mandelic acid salt, recrystallized from 2-propanol, and basified to give 0.24 g of an oil, $[\alpha]^{25}_{589}$ +74.5° (methylene chloride, *c* = 1). The resulting oil was subjected three times to salt formulation, recrystallization, and basification to give 0.13 g of oil **4b**, $[\alpha]^{25}_{589}$ +83.0° (methylene chloride, *c* = 1). Anal. ($C_{12}H_{15}NO{\cdot}C_8H_8O_3$) C, H, N.

Both enantiomers were converted to the corresponding N_1-carbamate diasteroisomers **14b** and **15b** by reaction with 1 eq of (*S*)-1-phenylethyl isocyanate in CH_2Cl_2 and evaporated to dryness. The optical purity was determined by ^{1}H NMR ($CDCl_3$). The methyl groups at the benzylic position of **14b** and **15b** show doublets at an δ 1.49 ppm and 1.45 ppm, respectively, each with greater than 95% purity.

Resolution of Racemic 1,2,3,3a,8,8a-Hexahydro-5-methoxy-3a-methylindeno[2,1-*b*]pyrrole (2a). The racemic compound **2a** (8.100 g, 39.9 mmol) was treated with di-*p*-toluoyl-L-tartaric acid in 2-propanol and allowed to crystallize, and then it was filtered. The first crop of crystals was recrystallized several times until the optical rotation of the corresponding free base **4a** reached a constant value of $[\alpha]^{25}_{589}$ +97° (methylene chloride, *c* = 1). The filtrate from the first recrystallization was recrystallized several times until the optical rotation of the corresponding free base **5a** reached a constant value of $[\alpha]^{25}_{589}$ −93° (methylene chloride, *c* = 1). This resolution experiment afforded 3.560 g of **4a** and 2.500 g of **4b**. The absolute configuration of di-*p*-toluoyl-L-tartaric acid salt of **4a** was determined, and the stereochemistry of C_{3a}-methyl and C_{8a}-H are of the α configuration.

Both enantiomers were converted to the corresponding N_1-carbamate diasteroisomers **14a** and **15a** by reaction with 1 eq of (*S*)-1-phenylethyl isocyanate in CH_2Cl_2 and evaporated to dryness. The optical purity was determined by ^{1}H NMR ($CDCl_3$). The methyl groups at the benzylic position of **14a** and **15a** show doublets at at δ 1.49 ppm and 1.41 ppm, respectively, each with greater than 95% purity.

1,2,3,3a,8,8a-Hexahydro-5-methoxy-1,3a-dimethylindeno[2,1-*b*]pyrrole (3a). A solution of 1,2,3,3a,8,8a-hexahydro-5-methoxy-3a-methylindeno[2,1-*b*]pyrrole (**2a**) (0.500 g, 2.46 mmol) in EtOH (25 mL) was treated with 37% formaldehyde (1 mL) and 10% Pd on charcoal (0.500 g) and hydrogenated at 50 psi for 2.5 h. The catalyst was filtered off through Celite, and the filtrate was concentrated and dried in vacuo to give 0.476 g (89%) of **3a** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.3 (s, 3 H), 1.9–2.15 (m, 2 H), 2.36 (s, 3 H), 2.35–2.5 (m, 1 H), 2.5–2.7 (m, 1 H), 2.7–3.05 (m, 3 H), 3.75 (s, 3 H), 4.7–4.9 (m, 1 H), 6.6–6.7 (m, 2 H), 7.0 (m, 1 H) ppm. The corresponding di-*p*-toluoyl-L-tartaric acid salt was prepared as a solid, mp 140–144 °C. Anal. ($C_{14}H_{19}NO{\cdot}C_{20}H_{18}O_8{\cdot}0.3H_2O$) C, H, N.

1,2,3,3a,8,8a-Hexahydro-5-methoxy-3a-methyl-1-ethylindeno[2,1-*b*]pyrrole (3b). A solution of **2a** (3.000 g, 14.78 mmol) in 200 mL of CH_2Cl_2 was treated with acetyl chloride (1.279 g, 16.3 mmol) and pyridine (1.288 g, 16.3 mmol) and stirred at room

temperature for 1 h. The mixture was washed with H_2O and extracted with CH_2Cl_2. The organic layer was washed with dilute HCl, then neutralized with saturated $NaHCO_3$ solution, dried, and concentrated to give 3.644 g (100%) of the the corresponding amide as a brown semisolid. To a solution of the amide in dry THF was added dropwise a solution of 2 M BH_3–Me_2S in THF (18.6 mL, 37.18 mmol). After addition, the mixture was heated at reflux for 3 h and cooled to 0 °C, and MeOH was added. The resulting solution was treated with concentrated HCl and stirred at room temperature overnight. Solvent was evaporated, and the residue was washed with saturated K_2CO_3 and H_2O, and extracted with $CHCl_3$. The organic layer was dried and concentrated to yield 3.140 g (92%) of compound **3b** as an oil, which is reasonably pure and can be used directly for the next step without purification. The oil was purified by silica gel column chromatography to give 2.700 g (79%) of pure **3b**: ^1H NMR δ 1.15 (t, 3 H), 1.35 (s, 3 H), 1.86–2.1 (m, 2 H), 2.2–2.4 (m, 2 H), 2.62–2.92 (m, 3 H), 2.92–3.12 (m, 2 H), 3.76 (s, 3 H), 6.65 (d, J = 2 Hz, 1 H), 6.68 (d, J = 2 Hz, 2 H), 7.0 (d, J – 7 Hz, 1 H) ppm. Anal. ($C_{15}H_{21}NO$·0.4H_2O) C, H, N.

(±)-1,2,3,3a,8,8a-Hexahydro-1-ethyl-3a-methylindeno[2,1-*b*]pyrrol-5-ol, Heptylcarbamate (1c). A solution of 1,2,3,3a,8,8a-hexahydro-5-methoxy-1-ethyl-3a-methylindeno[2,1-*b*]pyrrole (**3b**) (0.910 g, 3.94 mmol) in 48% HBr (25 mL) was heated at reflux for 4 h and evaporated to dryness. The residue was treated with saturated K_2CO_3 to pH 9 and extracted with CH_2Cl_2, and the organic phase was dried and concentrated to give 0.758 g (89%) of the corresponding 5-hydroxy derivative as an oil, which can be used directly for the next step. The corresponding HBr salt was prepared as white crystals: mp 188–190 °C. Anal. ($C_{14}H_{19}NO$·HBr) C, H, N.

A solution of the 5-hydroxy derivative (0.750 g, 3.46 mmol) in dry benzene was treated with NaH (5 mg, 0.2 mmol) and *n*-heptyl isocyanate (536 mg, 3.80 mmol) at room temperature and stirred for 2 h. The mixture was quenched with H_2O and brine, extracted with $CHCl_3$, dried, and concentrated to give 1.050 g (85%) of the carbamate **1c** as an oil: ^1H NMR ($CDCl_3$) δ 0.84 (m, 3 H), 1.13 (t, 3 H), 1.2–1.4 (m, 11 H), 1.4–1.6 (m, 2 H), 1.9–2.1 (2 H), 2.3–2.5 (m, 2 H), 2.78–3.16 (m, 5 H), 3.2 (q, 2 H), 5.0 (t, 1 H, NH), 6.8–6.9 (m, 2 H), 7.06 (d, 1 H) ppm. The corresponding di-*p*-toluoyl-L-tartaric acid salt was prepared as a white solid. Anal. ($C_{22}H_{34}N_2O_2$·$C_{20}H_{18}O_8$·0.7H_2O) C, H, N.

Biological Methods. Acetylcholinesterase Inhibition. AChE activity was determined as described by Ellman.[14] The assay solution consists of a 0.1 M sodium phosphate buffer, pH 8.0, with the addition of 100 μM tetraisopropylpyrophosphoramide, 100 μM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 0.02 units/mL AChE (Sigma Chemical Co., derived from human erythrocytes), and 200 μM acetylthiocholine iodide. The final assay volume was 0.25 mL. Test compounds were added to the assay solution prior to enzyme addition, and a 20-min preincubation period with enzyme was followed by addition of substrate. Changes in absorbance at 412 nM were recorded for 5 min. The reaction rates were compared, and the percent inhibition due to the presence of test compounds was calculated.

Acute Toxicity. Male CD-1 mice (15–27 g) (Charles River) were allowed tap water and Agway Prolab RMH 3000 Chow (Agway, Syracuse, NY) ad libitum. The animal rooms were 21 ± 1 °C with lights on 0700 to 1900. Animals were allowed to acclimate to the facility for at least 4 days before testing. Animals were injected (10 mL/kg body weight, ip) and lethality was measured over a 24-h time period. The LD50 was determined using at least four dose levels with nine animals per dose. LD50 was calculated using the Spearman–Karber statistics.

Mouse Brain Acetylcholine Measurement. AChE inhibitors were given intraperitoneally. Animals were sacrificed at 1 h post dosing, and the forebrains were removed and homogenized in 20 mM sodium phosphate buffer, pH 5.3. Homogenates were centrifuged 20 min at 12000*g*; supernatants (10–20 μL) were used for determination of ACh with an ACh analysis system from Bioanalytical Systems (West Lafayette, IN). A polymeric anion-exchange column resolves ACh from choline, and a post-column reactor column containing immobilized AChE and choline oxidase converts ACh and Ch to betaine and hydrogen peroxide; the hydrogen peroxide is readily measured with an electrochemical detector at +500 mV vs Ag/AgCl reference electrode and a platinum working electrode. Sensitivity was approximately 3–5 pmol of ACh. Typical ACh control values were 18–25 nmol/g in mouse forebrain. Data for drug treated animals are reported as percent control values. In general, the treatment consisted of eight animals per group. Statistical significance was determined by Student's one-tailed *t*-test.

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Supplementary Material Available: Proton NMR spectral data and physical properties of compounds **1a,b,d–y,2a,3c–f,11a,12a**, and **13a**; for compounds **4a** and **5b**, tables of crystal and refinement parameters, atomic coordinates, bond lengths and angles, and thermal parameters (38 pages). Ordering information is given on any current masthead page.

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