Synthesis of Novel Phenserine-Based-Selective Inhibitors of Butyrylcholinesterase for Alzheimer's Disease†

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Four novel analogues (**8**−**11**) of cymserine (**2**) were synthesized by methods similar to those recently developed for the total syntheses of N^8-norphenserine (Yu, Q. S.; et al. *J. Med. Chem.* **1997**, *40*, 2895−2901) and N^1,N^8-bisnorphenserine (Yu, Q. S.; et al. *J. Med. Chem.* **1998**, *41*, 2371−2379). As our structure−activity studies predicted, these compounds are highly potent and selective inhibitors of human butyrylcholinesterase (BChE) and will test the novel hypothesis that BChE inhibitors are useful in the treatment of Alzheimer's disease. In a similar manner, the same modifications that provided BChE selectivity were applied to the acetylcholinesterase (AChE)-selective inhibitor, tolserine (**5**), to provide the novel tolserine analogues **12**−**15**. As predicted, these modifications altered the AChE-selective action of tolserine (**5**) to favor a lack of cholinesterase enzyme subtype selectivity.

Introduction

Alzheimer's disease (AD) is the most common progressive dementia associated with aging, with β-amyloid plaques, neurofibrillary tangles, and synaptic loss being the major neuropathological hallmarks of the disease.[1] The cholinergic system is the earliest and most profoundly affected neurotransmitter system in AD, with substantial losses in the forebrain, cortex, and hippocampus.[2] This neurotransmitter together with these brain regions are critical in the acquisition, processing, and storage of memories and have supported the use of cholinomimetics in the treatment of AD.[3,4] Thus far, the agents that have demonstrated the greatest activity in AD therapy are cholinesterase inhibitors.[3] Two forms of cholinesterase coexist ubiquitously throughout the body, acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8), and although highly homologous, >65%, they are products of different genes on chromosomes 7 and 3 in humans, respectively.[5] Both subtype unselective cholinesterase and AChE-selective inhibitors have been used in AD to amplify the action of acetylcholine (ACh) at remaining cholinergic synapses within the AD brain,[3,4] and this has promoted the synthesis and development of novel inhibitors of AChE with favorable characteristics for in vivo use by the pharmaceutical industry.[3] The role of BChE in normal, aging, and diseased brain remains largely unknown, and there has been minimal interest in the design, synthesis, and development of selective inhibitors of BChE, except in the agricultural industry where toxic irreversible BChE inhibitors have long been used as insecticides.[5]

Interestingly, recent research has shown that overexpression of BChE occurs in neuritic Aβ plaques in the AD brain[6] and, furthermore, that the presence of BChE with Aβ dramatically amplifies the toxicity of Aβ in vitro.[7] Additionally, BChE is now known to be elevated in the AD brain, and a specific mutation in the gene encoding BChE, producing a functioning K variant form of BChE, together with APOE 4 results in an increase in the susceptibility of sporadic AD by 30-fold.[8] These facts suggest that inappropriate BChE activity increases the risk and/or progression of AD. It is hence possible that well-tolerated inhibitors of BChE may have utility in the treatment of AD.

To test this hypothesis, we initiated studies to design and synthesize novel, potent, and highly selective reversible inhibitors of BChE to provide a candidate for therapeutic development. Our prior quantitative structure−activity studies identified that specific 4′-substitutions in the phenylcarbamates of eseroline,[9] N^1-noreseroline,[9] and C-ring heterocongeners[10−12] provided the resulting compounds with a selectivity that favored BChE versus AChE. In this regard, the unsubstituted phenylcarbamate of eseroline, phenserine (**1**) (Chart 1), is a potent and 65-fold selective inhibitor of AChE versus BChE, whereas cymserine (**2**) has a 15-fold selectivity for BChE.[9,11,12] In contrast, 2′-substitutions in the phenylcarbamates of eseroline and derivatives provide compounds such as tolserine (**5**) that favor AChE-selective inhibitory activity.[9,11,12] In light of our former studies which demonstrated that alterations in the N^1 position of physostigmine and carbamates alter the relative selectivity of the resulting compounds for AChE and BChE,[13] we modified the N^1 and N^8 positions of cymserine (**2**), both separately and together, to provide analogues with improved BChE selectivity. We,

† This paper is dedicated to Dr. Arnold Brossi, Scientist Emeritus National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, and Department of Chemistry, School of Pharmacy, University of North Carolina, on the occasion of his 75th birthday and with whom we look forward to collaborating for many years more.

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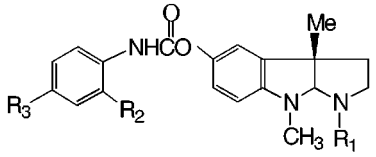
Chart 1. Chemical Structures of Compounds **1**−**7**



1, R_1= CH_3	R_2= H	R_3=H	Phenserine
2, R_1= CH_3	R_2= H	R_3= $(CH_3)_2CH$-	Cymserine
3, R_1= H	R_2= H	R_3= $(CH_3)_2CH$-	N^1-Norcymserine
4, R_1= CH_2CH_2Ph	R_2= H	R_3= $(CH_3)_2CH$-	N^1-Phenethylnorcymserine
5, R_1= CH_3	R_2= CH_3	R_3= H	Tolserine
6, R_1= H	R_2= CH_3	R_3= H	N^1-Nortolserine
7, R1= CH_2CH_2Ph	R_2= CH_3	R_3= H	N^1-Phenethylnortolserine

additionally, undertook parallel syntheses to apply successful modifications to provide BChE selectivity to the AChE-selective inhibitor tolserine (**5**) as a negative control to assess whether such modifications would reduce ensuing AChE inhibitory action, as would be predicted.

We synthesized cymserine (**2**), tolserine (**5**),[9,13] and their analogues N^1-norcymserine (**3**),[13] N^1-phenethylnorcymserine (**4**),[13] N^1-nortolserine (**6**),[13] N^1-phenethylnoryolserine (**7**),[13] and we reconfirmed their AChE and BChE inhibitory action. Additionally, we undertook total syntheses of the novel cymserine analogues N^8-norcymserine (**9**) and N^1,N^8-bisnorcymserine (**11**) and the novel tolserine analogues N^8-nortolserine (**13**) and N^1,N^8-bisnortolserine (**15**). Their potency and selectivity, together with those of their intermediates, N^8-benzylnorcymserine (**8**), N^1,N^8-bisbenzylnorcymserine (**10**), N^8-benzylnortolserine (**12**), and N^1,N^8-bisbenzylnortolserine (**14**), are reported herein.

Results

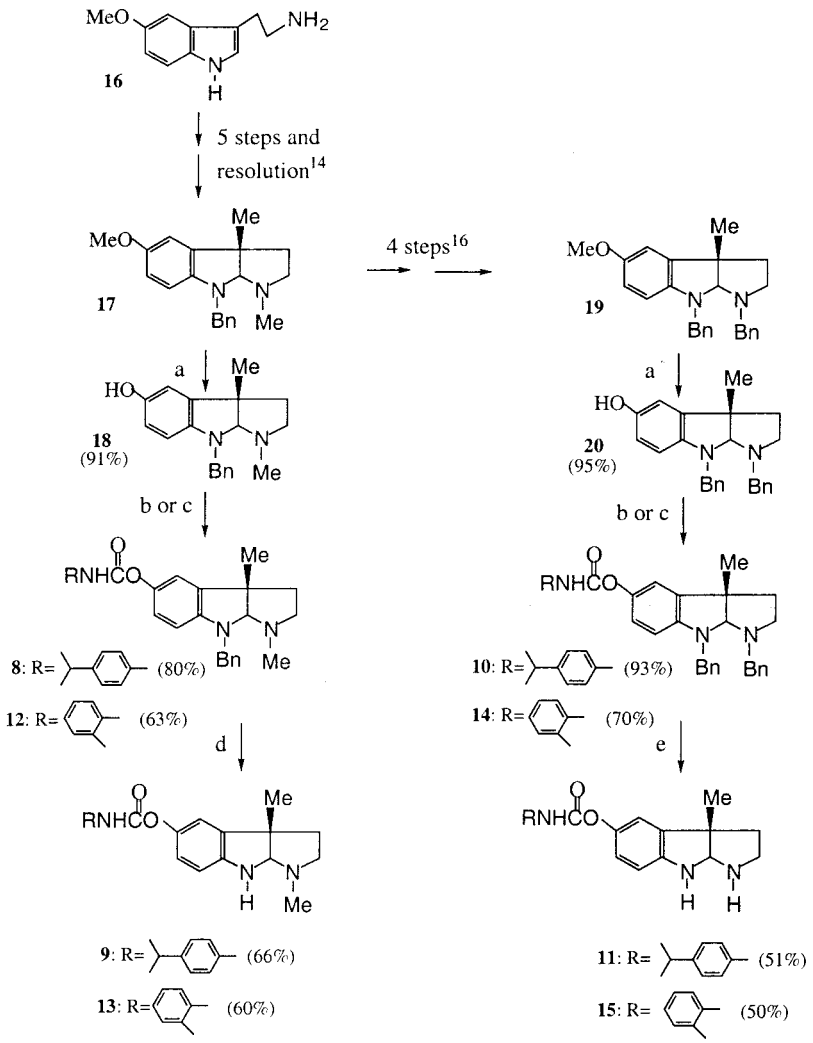
Chemistry. The important starting material, optically pure (3a*S*)-N^8-benzylnoresermethole (**17**), was made from commercially available 5-methoxytryptamine (**16**) by five steps and with a final chemical resolution which was described in our previous publication (Scheme 1).[14] Demethylation of compound **17** gave N^8-benzylnoreseroline (**18**) by treatment with BBr_3 in CH_2Cl_2. Reaction of either 4-isopropylphenyl isocyanate or *o*-tolyl isocyanate with compound **18** afforded N^8-benzylnorcymserine (**8**) or N^8-benzylnortolserine (**12**), respectively, according to the known procedure.[15] *N*-Debenzylation of compounds **8** and **12** was accomplished by catalytic hydrogenation in an acidic medium using Pd-$(OH)_2$/C as a catalyst to give N^8-norcymserine (**9**) and N^8-nortolserine (**13**),[14] respectively. Thereafter, the N^1-benzylnoresermethole (**17**) was transformed to N^1,N^8-bisbenzylnoresermethole (**19**) by the carbinolamine route, as described in our recent publication.[16] N^1,N^8-Bisbenzylnoreseroline (**20**), the demethyl product of compound **19**, then was reacted with either 4-isopropyl isocyanate or *o*-tolyl isocyanate to give N^1,N^8-bisbenzylnorcymserine (**10**) and N^1,N^8-bisbenzylnortolserine (**14**), respectively. Finally, catalytic debenzylation of compounds **10** and **14** in a neutral medium (2-propanol) gave the desired products N^1,N^8-bisnorcymserine (**11**) and N^1,N^8-bisnortolserine (**15**), respectively.

Biological Evaluation. Table 1 illustrates the anticholinesterase activities of compounds **8**−**15** and of the previously synthesized compounds **3**, **4**, **6**, and **7** against human AChE and BChE, compared to those of phenserine (**1**), cymserine (**2**), and tolserine (**5**).

In accord with our previous reports,[9,11,12,14,16] phenserine (**1**) and tolserine (**5**) possessed potent anticholinesterase action with a high selectivity for AChE. In contrast, cymserine (**2**) possessed potent and selective BChE inhibitory action. This BChE selectivity was heightened in N^1-norcymserine (**3**) and dramatically further heightened by lipophilic N^1 substitution, as in N^1-phenethylnorcymserine (**4**)[13] (as compared to cymserine (**2**), $p < 0.05$). Interestingly, compounds **3** and **4** were 6- and 9-fold more potent as BChE inhibitors than was cymserine (**2**). Substitutions in the N^8 position alone resulted in compounds **8** and **9**. These, likewise, possessed a greater selectivity for BChE than did cymserine (**2**), but this occurred as a consequence of their reduced AChE inhibitory activity ($p < 0.05$ versus **2**) as their potency for BChE inhibition was in the same order of magnitude as that of cymserine (**2**). Of greatest interest, combined N^1,N^8 substitution conferred a high BChE selectivity to compounds **10** and **11**, with the activity of the latter being 50-fold more potent as a BChE inhibitor than cymserine (**2**).

In contrast to analogues of cymserine (**2**), replacement of a methyl by a hydrogen in the N^1 position of tolserine (**5**) resulted in compound **6** that maintained potent AChE inhibitory action which was slightly reduced compared to that of **5** ($p < 0.05$). Lipophilic N^1 substitution, however, rendered compound **7** largely inactive against both AChE and BChE. Similarly, replacement of a methyl by a hydrogen in the N^8 position of tolserine (**5**) resulted in compound **13** that maintained potent AChE inhibitory action but whose activity and hence AChE selectivity were reduced compared to that of **5** ($p < 0.05$). Lipophilic N^8 substitution, rendering compound **12**, further reduced potency against AChE to provide a moderately potent but unselective cholinesterase inhibitor. Combined N^1,N^8 substitution, as in **14** and **15**, further reduced AChE inhibitory potency compared to that of their monosubstituted analogues (**6**, **13**, **7**, **12**), to provide **15** with moderate anticholinesterase activity devoid of enzyme subtype selectivity.

Scheme 1[a]



[a] Reagents: (a) BBr_3, CH_2Cl_2; (b) 4-isopropylphenyl isocyanate, Na, Et_2O; (c) *o*-tolyl isocyanate, Na, Et_2O; (d) $Pd(OH)_2/C$, H_2O, CH_3OH, TFA; (e) $Pd(OH)_2/C$, *i*-PrOH.

Table 1. IC_{50} Values (nM) of Compounds versus Human Erythrocyte AChE and Plasma BChE[a]

		IC_{50} (nm) ± SEM		selectivity[b]	
no.	compound	AChE	BChE	AChE	BChE
1	phenserine	24 ± 6.0	1560 ± 45	65	
2	cymserine	758 ± 21	50 ± 1.0		15
3	N^1-norcymserine	324 ± 4.0	8.3 ± 1.0		39
4	N^1-phenethylnorcymserine	>30000	6.0 ± 1.0		>5000
8	N^8-benzylnorcymserine	13000 ± 1600	85 ± 11		152
9	N^8-norcymserine	2020 ± 284	78 ± 17		26
10	N^1,N^8-bisbenzylnorcymserine	>30000	132 ± 8.0		>227
11	N^1,N^8-bisnorcymserine	110 ± 15	1.0 ± 0.1		110
5	tolserine	10.3 ± 1.6	1950 ± 240	189	
6	N^1-nortolserine	17.0 ± 1.0	2165 ± 85	127	
7	N^1-phenethylnortolserine	550 ± 55	1790 ± 180	3	
12	N^8-benzylnortolserine	100 ± 3	600 ± 205	6	
13	N^8-nortolserine	40 ± 15	1430 ± 310	36	
14	N^1,N^8-bisbenzylnortolserine	1760 ± 126	641 ± 175		3
15	N^1,N^8-bisnortolserine	285 ± 21	396 ± 100	1	

[a] Minimum of four measurements per compound. [b] Because a smaller IC_{50} represents a higher activity, the selectivity is defined as: selectivity for AChE = IC_{50}(BChE)/IC_{50}(AChE), and selectivity for BChE = IC_{50}(AChE)/IC_{50}(BChE).

Discussion

We report, herein, the first synthesis and initial pharmacological evaluation of N^8-benzylnorcymserine and -tolserine (**8**, **12**), of N^8-norcymserine and -tolserine (**9**, **13**), of N^1,N^8-bisbenzylnorcymserine and -tolserine (**10**, **14**), and of N^1,N^8-bisnorcymserine and -tolserine (**11**, **15**). As predicted, the described novel analogues of cymserine (**2**), and in particular **10** and **11**, proved to be highly potent and selective inhibitors of BChE. Indeed, they were dramatically more potent than the known highly toxic irreversible BChE inhibitor, Iso-OMPA (IC_{50}: BChE, 980 nM; AChE, 340 000 nM; BChE

selectivity 346), a widely used standard for selective BChE inhibition.[5,17] The same analogues (**14**, **15**) of our potent and AChE-selective inhibitor, tolserine (**5**), rendered the compound unselective and dramatically less potent, reiterating the selectivity of these chemical manipulations for differential BChE activity.

Extensive biochemical analyses[5,18−22] have demonstrated that distinct regions within AChE and BChE, close to the bottom of a narrow 20 Å deep gorge that is invaginated into the surface of the enzymes, constitute binding domains for ACh and inhibitory ligands and provide the structural basis for specificity differences between the two enzyme subtypes. Three primary domains exist. These include (i) an acyl pocket that defines an active center involved in the catalysis of ACh and substrates through an active serine residue. This residue, similar to serine proteases, causes substrate hydrolysis through electron transfer in a catalytic triad. (ii) An active center choline subsite exists that is involved in the attraction and binding of the choline moiety of choline esters. (iii) A peripheral anionic site also exists which is uninvolved with ACh hydrolysis and lies closer to the entrance of the gorge.[23] Biochemical and structural data confirm that choline ester catalysis involves a nucleophilic attack on the substrate carbonyl by the activated active site serine (Ser_{203} and Ser_{198} in mammalian AChE and BChE and Ser_{200} in torpedo AChE, respectively).[5,18−22] In the proposed catalytic triad, the serine hydroxyl group is rendered nucleophilic via a charge relay involving the glutamate hydroxyl of close-by Glu_{334} (mammalian AChE) and the histidine imidazole of His_{447}. The deprotonation of serine allows the rapid subsequent attack on the substrate, causing substrate hydrolysis and subsequent release of the choline moiety in the case of ACh and choline esters. The resulting acetyl enzyme is highly labile to hydrolysis and rapidly reactivates.

Drugs that have a carbamoyl ester linkage, such as physostigmine and phenserine (**1**), act as alternative substrates and bind to the active centers in a similar orientation as ACh and choline esters. Nucleophilic attack by the active site serine, however, gives rise to a carbamoylated enzyme, with the carbamoyl moiety residing within the boundary of the acyl pocket. The carbamylated enzyme is far more stable than is the acetylated form, precluding further enzyme-catalyzed hydrolysis of ACh for a time that is dependent on the carbamate's structure.[23]

The limit of the acyl pocket and base of the gorge within AChE is delineated by two phenylalanines (Phe_{295} and Phe_{297}), which are replaced by valine and leucine within BChE. Our structure−activity studies predicted that the 4′ substitution in the phenylcarbamate moiety of cymserine (**2**) and analogues (**3**, **4**, **8**−**11**) would fit into the larger acyl pocket present in BChE, created by virtue of the smaller side chains associated with valine and leucine compared to phenylalanine, close to its active serine (Ser_{198}) binding site. This larger pocket allows the binding of the synthetic substrate butyrylcholine in BChE but not in AChE[5] and thus provides a means to design agents to differentially inhibit BChE. We predicted that not only would the 4′-isopropyl substitution in the phenylcarbamate of **2**−**4** and **8**−**11** fit into this extended pocket but that, additionally, lipophilic groups present at positions N^1 and N^8 of the compounds would interact with hydrophobic regions reported close to the choline subsite of BChE, which are not present within AChE.[5,18−22] The potent biological activity of compounds **2**, **4**, **8**, and **10** suggests that these predictions may have been correct, and we are planning to undertake X-ray crystallography studies to confirm them.

Of particular interest, and unpredicted from our prior studies of inducing BChE selectivity by providing lipophilicity at the N^1 and N^8 positions,[13] was the dramatic potency of N^1,N^8-bisnorcymserine (**11**) to inhibit BChE. Compared to cymserine (**2**), **11** was 50-fold more potent against BChE and only 7-fold more potent against AChE, providing a BChE selectivity of 110-fold versus 15-fold for **2**. Changing the N^1 and N^8 methyl groups within **2** to a H within **11** would increase the basic nature of the N^1 and N^8 nitrogen groups,[24] both separately as in **3** and **9** and combined as in **11**. This would also increase the hydrogen-bonding potential of these compounds, which may provide improved attraction to the proposed active center choline binding site. This modification would also reduce the lipophilicity of **3**, **9**, and **11** compared to **2** but nevertheless render them sufficiently lipid-soluble for blood−brain barrier penetration.[25] The mechanism by which this minor modification so dramatically alters the differential selectivity to favor BChE inhibitory action remains to be elucidated. It is possible, however, that conformational changes induced by drug−enzyme interactions,[26] which have not been defined in X-ray crystallographic analyses of the static enzyme, may account for the unpredicted selective potency of **11** for BChE and may additionally account for the inactivity of other compounds[27] that were designed to optimally fit the enzyme.

Preliminary studies, which will be published separately, demonstrate that compounds **2**, **4**, and **11**, similar to our experimental AD therapeutic phenserine (**1**), are well-tolerated in vivo, enter the brain, and improve cognitive performance in rodents. Of significant interest, however, is that whereas phenserine (**1**) produces cognitive improvement by selectively inhibiting AChE, the enzyme that degrades ACh, **2**, and particularly **4** and **11** appear to do so through BChE inhibitory action alone. Compared to tacrine (IC_{50} values: AChE, 190 nM; BChE, 47 nM; 4-fold BChE selectivity),[11,17] one of the only two drugs approved for the treatment of AD in the United States, compounds **4** and **11** are highly potent and selective. Recent studies have reported that subtype-selective cholinesterase inhibitors are not associated with the unfavorable side effect profile of unselective ones.[28] The present studies have thus provided us with biologically active and interesting compounds whose further pharmacological assessment will allow us to select a candidate for development to define the role of BChE in (i) the brain in development, health, and aging and (ii) the value of its inhibition in AD as a novel therapeutic strategy. These compounds represent the first available potent, reversible, and selective inhibitors of BChE to undertake this task.

Experimental Section

Chemistry. General. Melting points (uncorrected) were measured with a Fisher-Johns apparatus; ^{1}H NMR were recorded on a Bruker (Billerica, MASS) AC-300 spectrometer;

MS (*m*/*z*) were recorded on a Hewlett-Packard 5890 GC−MS (EI) and on a Finnigan-1015D mass spectrometer; optical rotations were measured by JASO, model DIP-370 (Japan, Spectroscopic Co., Ltd.).

(−)-(3a*S*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-4′-Isopropylphenylcarbamate (8). (−)-(3a*S*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-ol (**18**) (33 mg, 0.112 mmol), made from compound **17**,[14] was dissolved in ether (2 mL), and a piece of Na metal (approximately 1 mg) was added. The mixture was stirred at room temperature for 1 min; then 4-isopropylphenyl isocyanate (18.1 mg, 0.112 mmol) was added. The mixture was stirred at room temperature for 5 min. After the removal of solvent, the residue was chromatographed (CH_2Cl_2/MeOH = 20/1) to give **8** (40 mg, 80.0%) as a foam: $[\alpha]_D$ −60.0° (*c* = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.40−7.08 (m, 9H, Ar−H), 6.80 (d, *J* = 2.2 Hz, 1H, C4−H), 6.70 (dd, *J* = 2.2, 8.5 Hz, 1H, C6−H), 6.15 (d, *J* = 8.5 Hz, 1H, C7−H), 4.45 and 4.35 (AB, *J* = 16.6 Hz, 2H, Ph-CH_2), 4.25 (s, 1H, C8a−H), 2.80 (m, 1H, Ph-CH<), 2.68 (m, 2H, C2−H_2), 2.32 (s, 3H, N1−CH_3), 1.90 (m, 2H, C3−H_2), 1.35 (s, 3H, C3a-CH_3), 1.15 (d, *J* = 7.0 Hz, 6H, >CMe_2); EI-MS *m*/*z* (relative intensity) 294 (MH^+ − ArNHCO, 65), 280 (2.2), 265 (3.6), 237 (75), 207 (58), 160 (34), 91 (100); HR-MS *m*/*z* calcd for $C_{29}H_{33}N_3O_2$ 455.2573, found 455.2569.

(−)-(3a*S*)-1,3a-Dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-*b*]indol-5-yl *N*-4′-Isopropylphenylcarbamate (9). Compound **8** (22 mg, 0.048 mmol) was dissolved in a mixture of MeOH (1 mL), H_2O (1 mL), and TFA (0.5 mL). Palladium hydroxide on carbon (5 mg) was added. The reaction mixture was stirred under hydrogen at atmospheric pressure and room temperature for 1 h, and then the catalyst was filtered. The filtrate was evaporated in vacuo to provide a residue which was dissolved in H_2O, basified by Na_2CO_3, extracted with ether, and then dried over Na_2SO_4. After removal of solvent the residue was chromatographed on preparative TLC (silica gel) (CH_2CI_2 = 10/1) to give product **6** (12 mg, 65.7%) as a gum: $[\alpha]^{20}_D$ −73.8° (*c* = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.30 (d, *J* = 8.5 Hz, 2H, C2′-H and C6′-H), 7.10 (d, *J* = 8.5 Hz, 2H, C3′-H and C5′-H), 6.80−6.70 (m, 2H, C4−H and C6−H), 6.50 (d, *J* = 8.5 Hz, C7−H), 4.65 (s, 1H, C8a−H), 2. 85 (m, 2H, C2−H_2), 2.64 (m, 1H, −HC<), 2.48 (s, 3H, N1−CH_3), 2.00−1.90 (m, 2H, C3−H_2), 1.42 (s, 3H, C3a-CH_3), 1.20 (d, *J* = 7.0 Hz, 6H, >CMe_2); EI-MS *m*/*z* (relative intensity) 204 (MH^+ − ArNHCO, 99), 189 (25), 174 (8.3), 117 (10); HR-MS *m*/*z* calcd for $C_{22}H_{27}N_3O_2$ 365.2105, found 365.2100.

(−)-(3a*S*)-1,8-Dibenzyl-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-4′-Isopropylphenylcarbamate (10). (−)-(3a*S*)-1,8-Dibenzyl-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-ol (**20**) (68 mg, 0.18 mmol), made from compound **19**,[16] was dissolved in anhydrous ether (2 mL), and a piece of Na metal (approximately 1 mg) was added. The mixture was stirred at room temperature for 1 min; then 4-isopropylphenyl isocyanate (30 mg, 0.18 mmol) was added and stirred for 5 min. Evaporation of solvent gave a crude product which was directly chromatographed to give **10** (89 mg, 93.1%) as a gum: $[\alpha]^{20}_D$ −44.7° (*c* = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.50−7.10 (m, 14H, Ar−H), 6.78 (d, *J* = 2.2 Hz, 1H, C4−H), 6.70 (dd, *J* = 2.5, 8.5 Hz, 1H, C6−H), 6.15 (d, *J* = 8.5 Hz, 1H, C7−H), 4.48 (s, 1H, C8a−H), 4.30−4.15 (AB, *J* = 16.6 Hz, 2H, Ph-CH_2−N8), 3.73 (s, 2H, Ph-CH_2−N1), 2.80 (m, 1H, −HC<), 2.70 (m, 2H, C2−H_2), 1.90 (m, 2H, C3−H_2), 1.40 (s, 3H, C3a-CH_3), 1.15 (d, *J* = 7.0 Hz, 6H, >CMe_2); EI-MS *m*/*z* (relative intensity) 370 (MH^+ − ArNHCO-, 1.0), 294 (90), 279 (10), 237 (8.0), 174 (95), 160 (92), 132 (60), 104 (55), 91 (100); HR-MS *m*/*z* calcd for $C_{35}H_{37}N_3O_2$ 531.2888, found 531.2907.

(−)-(3a*S*)-3a-Methyl-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-*b*]indol-5-yl *N*-4′-Isopropylphenylcarbamate (11). Compound **10** (42 mg, 0.078 mmol) was dissolved in 2-propanol (1 mL), and $Pd(OH)_2$/C (5 mg) was added. The reaction mixture was stirred under hydrogen at atmospheric pressure and room temperature for 60 h; then the catalyst was filtered. Evaporation of solvent gave a residue which was chromatographed (CH_2Cl_2/MeOH = 10/1) to give as the most polar component compound **8** (14 mg, 51.0%) as a gum: $[\alpha]^{20}_D$ −71.1° (*c* = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.29 (d, *J* = 8.5 Hz, 2H, C2′-H and C6′-H), 7.10 (d, *J* = 8.5 Hz, 2H, C3′-H and C5′-H), 6.80 (m, 2H, C4−H and C6−H), 6.55 (d, *J* = 8.5 Hz, C7−H), 5.20 (s, 1H, C8a−H), 2.90 (m, 1H, Ph-CH<), 2.80 (m, 2H, C2−H_2), 2.13 (m, 2H, C3−H_2), 1.45 (s, 3H, C3a-CH_3), 1.18 (d, *J* = 7.0 Hz, >CMe_2); EI-MS *m*/*z* (relative intensity) 190 (MH^+ − ArNHCO, 98), 174 (10), 160 (70), 146 (100), 133 (11), 117 (15), 103 (5.0), 91 (14); HR-MS *m*/*z* calcd for $C_{21}H_{25}N_3O_2$ 351.1948, found 351.1941.

(−)-(3a*S*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-2′-Methylphenylcarbamate (12). (−)-(3a*S*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-ol (**18**) (36 mg, 0.122 mmol), made from compound **17**,[14] was dissolved in ether (2 mL), and Na (1 mg) was added. The mixture was stirred at room temperature for 1 min; then 2-methylphenyl isocyanate (16.3 mg, 0.122 mmol) was added. The mixture was stirred at room temperature for 5 min. After the removal of solvent, the residue was chromatographed (CH_2Cl_2/MeOH = 20/1) to give **12** (33 mg, 63.4%) as a foam: $[\alpha]_D$ −59.7° (*c* = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.30−6.90 (m, 9H, Ar−H), 6.80 (d, *J* = 2.2 Hz, 1H, C4−H), 6.78 (dd, *J* = 2.2, 8.5 Hz, 1H, C6−H), 6.15(d, *J* = 8.5 Hz, 1H, C7−H), 4.45 and 4.35 (AB, *J* = 16.6 Hz, 2H, Ph-CH_2), 4.28 (s, 1H, C8a−H), 2.68 (m, 2H, C2−H_2), 2.35 (s, 3H, N1−CH_3), 2.25 (s, CH_3, Ph-CH_3), 1.90 (m, 2H, C3−H_2), 1.37 (s, 3H, C3a-CH_3); EI-MS *m*/*z* (relative intensity) 294 (MH^+ − ArNHCO, 90), 280 (1.0), 264 (2.0), 250 (20), 203 (9.2), 160 (33), 91 (100); HR-MS *m*/*z* calcd for $C_{27}H_{29}N_3O_2$ 427.2262, found 427.2258.

(−)-(3a*S*)-1,3a-Dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-*b*]indol-5-yl *N*-2′-Methylphenylcarbamate (13). Compound **12** (27 mg, 0.062 mmol) was dissolved in a solution containing MeOH (1 mL), H_2O (1 mL), and TFA (0.5 mL), and then palladium hydroxide on carbon (4 mg) was added. The reaction mixture was stirred under hydrogen at atmospheric pressure and room temperature for 1 h, and then the catalyst was filtered. The filtrate was evaporated to give a residue which was dissolved in H_2O, basified by Na_2CO_3, and extracted by ether. The ether solution then was dried over Na_2SO_4. After the removal of solvent, the residue was chromatographed on preparative TLC (silica gel) (CH_2CI_2/MeOH = 10/1) to give product **13** (13 mg, 60.1%) as a gum: $[\alpha]^{20}_D$ −75.5° (*c* = 0.2, $CHCl_3$) EtOH); ^{1}H NMR ($CDCl_3$) δ 7.20−6.90 (m, 4H, Ar−H), 6.70 (m, 2H, C4−H and C6−H), 6.50 (d, *J* = 8.5 Hz, 1H, C7−H), 4.65 (s, 1H, C8a−H), 2.90−2.55 (m, 2H, C2−H_2), 2.48 (m, 3H, N1−CH_3), 2.35 (s, 3H, Ph-CH_3), 2.05 (m, 2H, C3−H_2), 1.45 (s, 3H, C3a-CH_3); EI-MS *m*/*z* (relative intensity) 204 (MH^+ − ArNHCO, 95), 189 (25), 174 (8.0), 160 (100), 146 (87), 131 (8.0), 117 (9.0); HR-MS *m*/*z* calcd for $C_{20}H_{23}N_3O_2$ 337.1792, found 337.1789.

(−)-(3a*S*)-1,8-Dibenzyl-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-2′-Methylphenylcarbamate (14). (−)-(3a*S*)-1,8-Dibenzyl-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-ol (**20**) (64 mg, 0.17 mmol), made from compound **19**,[16] was dissolved in anhydrous ether (2 mL), and a piece of Na metal (approximately 1 mg) was added. The mixture was stirred at room temperature for 1 min, and then *o*-tolyl isocyanate (23 mg, 0.17 mmol) was added and stirred for 5 min. Evaporation of solvent gave a crude product which was directly chromatographed to give **14** (60 mg, 70.0%) as a gum: $[\alpha]^{20}_D$ −47.9° (*c* = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.30−6.90 (m, 14H, Ar−H), 6.83−6.65 (m, 2H, C4−H and C6−H), 6.23 (d, *J* = 8.5 Hz, 1H, C7−H), 4.58 (s, 1H, C8a−H), 4.35−4.25 (AB, *J* = 16.6 Hz, 2H, Ph-CH_2−N8), 3.85 (s, 2H, Ph-CH_2−N1), 2.80 (m, 2H, C2−H_2), 2.30 (s, 3H, Ph-CH_3), 2.05 (m, 2H, C3−H_2), 1.40 (s, 3H, C3a-CH_3); EI-MS *m*/*z* (relative intensity) 370 (MH^+ − ArNHCO-, 31), 294 (80), 280 (30), 237 (8.0), 207 (90), 174 (82), 160 (81), 132 (50), 104 (30), 91 (100); HR-MS *m*/*z* calcd for $C_{33}H_{37}N_3O_2$ 503.2566, found 503.2568.

(−)-(3a*S*)-3a-Methyl-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-*b*]indol-5-yl *N*-2′-Methylphenylcarbamate (15). Compound **14** (28 mg, 0.05 mmol) was dissolved in 2-propanol (1 mL), and $Pd(OH)_2$/C (5 mg) was added. The reaction mixture

was stirred under hydrogen at atmospheric pressure and room temperature for 60 h; then the catalyst was filtered. Evaporation of solvent gave a residue which was chromatographed (CH_2Cl_2/MeOH = 10/1) to give, as the most polar component, compound **15** (8 mg, 50.0%) as a gum: $[\alpha]^{20}_D$ −78.5° (c = 0.2, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 7.70−6.85 (m, 7H, Ar−H), 4.15 (s, 1H, C8a−H), 2.70−2.50 (m, 2H, C2−H_2), 2.30 (s, 3H, Ph-CH_3), 1.65 (m, 2H, C3−H_2), 1.30 (s, 3H, C3a-CH_3); EI-MS m/z (relative intensity) 232 (M^+ − tolyl, 100), 217 (70), 188 (94), 174 (92), 160 (38), 133 (10), 91 (13); HR-MS (NH_3) m/z calcd for $C_{19}H_{21}N_3O_2$ 323.1635, found 323.1630.

Pharmacology. Quantitation of Anticholinesterase Activity. The action of compounds **1**−**15** to inhibit the ability of freshly prepared human AChE and BChE, derived from plasma and erythrocytes, respectively, to enzymatically degrade the specific substrates acetyl-(β-methyl)thiocholine and *s*-butyrylthiocholine (each 0.5 mmol/L) (Sigma Chemical Co., St. Louis, MO) was quantified. Compounds were dissolved in Tween 80/EtOH (3:1) (v:v; <150 μL total volume) and were diluted in 0.1 M Na_3PO_4 buffer (pH 8.0) in half-log concentrations to provide a final concentration range that spanned from 0.3 nM to 30 mM. Tween 80/EtOH was diluted to in excess of 1 in 5000 and possessed no inhibitory action on either AChE or BChE.

Freshly collected blood was centrifuged (10000*g*, 10 min, 4 °C), and the plasma was removed and diluted 1:125 with 0.1 M Na_3PO_4 buffer (pH 7.4). Erythrocytes were washed five times in isotonic saline, lysed in 9 volumes of 0.1 M Na_3PO_4 buffer (pH 7.4) containing 0.5% Triton-X (Sigma), and then diluted with an additional 19 volumes of buffer to a final dilution of 1:200. Analysis of anticholinesterase activity, utilizing a 25-μL sample of each enzyme preparation, was undertaken at their optimal working pH, 8.0, in 0.1 M Na_3PO_4 buffer (0.75 mL total volume). Compounds were preincubated with enzymes (30 min, room temperature) and then were incubated with their respective substrates and 5,5′-dithiobis-(2-nitrobenzoic acid) (25 min, 37 °C). Production of a yellow thionitrobenzoate anion was measured by spectrophotometer at 412 nm λ. To correct for nonspecific substrate hydrolysis, aliquots were coincubated under conditions of absolute enzyme inhibition (by the addition of 1×10^{-5} M physostigmine) and the associated alteration in absorbance was subtracted from that observed through the concentration range of each test compound. Each compound was analyzed on four occasions and assayed alongside (−)-physostigmine, as a control and external standard whose activity we have previously reported.[9−17,23]

The enzyme activity at each concentration of test compound was expressed as a percent of activity in the absence of compound, transformed into a logit format (logit = % activity/100 − % activity), and then plotted as a function of its log concentration. Enzyme inhibitory activity was calculated as an IC_{50}, defined as the concentration of compound (nM) required to inhibit 50% of enzymatic activity, and determined from a correlation between log concentration and logit activity. A two-tailed Student's *t*-test was carried out to compare between the values of two means. When more than two means were compared, one-way analysis of variance and the Bonferroni multiple test were used.[29]

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