Total Syntheses and Anticholinesterase Activities of (3a*S*)-*N*(8)-Norphysostigmine, (3a*S*)-*N*(8)-Norphenserine, Their Antipodal Isomers, and Other *N*(8)-Substituted Analogues†

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N(8)-Benzylesermethole (**6**) was prepared from 5-methoxytryptamine (**1**) in five steps. Resolution of compound **6** by dibenzoyl- and ditoluyltartaric acid provided enantiomers (−)- and (+)-**7**. After demethylation, reaction with isocyanates and catalytic debenzylation over hydrogen, the total syntheses of (−)- and (+)-*N*(8)-norphysostigmine [(−)- and (+)-**11**] and (−)- and (+)-*N*(8)-norphenserine [(−)- and (+)-**12**] were accomplished. (−)-*N*(8)-Norphysostigmine [(−)-**11**] and (−)-*N*(8)-norphenserine [(−)-**12**] were also obtained by transformations of natural physostigmine [(−)-**13**] and phenserine [(−)-**14**] prepared from (−)-**13**. The absolute configurations and optical purity of compounds (−)-**11**, (−)-**12**, (+)-**11**, and (+)-**12** were confirmed by a comparison of their optical rotations with those of the compounds synthesized from physostigmine [(−)-**13**]. The anticholinesterase activities of *N*(8)-nor- and *N*(8)-substituted analogues, (−)- and (+)-**9**, **-10**, **-11**, **-12**, **15**, and **16**, were compared with those of physostigmine [(−)- and (+)-**13**] and phenserine [(−)- and (+)-**14**] and are reported.

The alkaloid of the Calabar bean physostigmine [(−)-**13**] has been used medically to treat cholinergic disorders such as Alzheimer's disease[1] and has been the subject of numerous chemical syntheses.[2,3] Recent reviews include and describe the phenylcarbamate analogue, phenserine [(−)-**14**], which readily enters the brain and demonstrates a high selectivity of action against acetyl- versus butyrylcholinesterase (AChE and BChE), the two subtypes of the cholinesterase enzyme.[2] Additionally, phenserine is significantly less toxic and longer acting than physostigmine and, as a consequence of its dramatic effects on both cognition and β-amyloid precursor protein levels in animal studies, is presently proceeding toward clinical trials.[2,4] *N*(8)-Norphysostigmine, a minor alkaloid of the Calabar bean and obtained from physostigmine by chemical oxidation,[5] was expected to have similar anticholinesterase activity *in vitro* as physostigmine,[6] but a comparison of its action to that of physostigmine is lacking. It is known that N-demethylation is a well-recognized metabolic pathway,[7] and thus a practical synthesis of the enantiomers of *N*(8)-norphysostigmine and *N*(8)-norphenserine and an evaluation of their chemical and biological character seemed warranted and are reported herein. Furthermore, the synthesis and characterization of these novel compounds extended our investigation of the parameters optimal in influencing the interaction between physostigmine and the cholinesterase enzymes in the development of new drugs for neurological diseases.[2−4]

Following a strategy developed for the syntheses of the amphibian alkaloids pseudophrynaminol and pseudophrynamine A,[8,9] our route was used to prepare the intermediate indolopyrrolidine **6**, from commercially available 5-methoxytryptamine in five steps with a total yield of 40.5%, as shown in Scheme 1. The *N*(8)-benzylesermethole (**6**) was readily resolved into its enantionmers by dibenzoyl- and ditoluyltartaric acids. According to the procedure used in the synthesis of (+)-physostigmine,[10] both (−)- and (+)-*N*(8)-benzylphysostigmine [(−)- and (+)-**9**] and *N*(8)-benzylphenserine [(−)- and (+)-**10**] were obtained. The debenzylations of the above *N*(8)-benzyl-substituted compounds were not as easy as that of *N*(1)-benzylphysostigmine.[11] After studying a series of reactions we found conditions for a selected catalytic hydrogenative debenzylation for the N-debenzylation of compounds (−)- and (+)-**9** and **-10**. The carbamate moiety remained intact, and the C ring was not opened. Furthermore, we additionally synthesized the (−)-*N*(8)-norphysostigmine [(−)-**11**] and (−)-*N*(8)-norphenserine [(−)-**12**] from natural physostigmine (**13**) and phenserine (**14**), respectively, prepared from the former by Takano's method[5] in order to obtain samples for a comparison. A comparison of both the physical data and optical rotations of (−)-**11** and (−)-**12** prepared by total synthesis with those obtained from physostigmine [(−)-**13**] confirmed the complete identity of the products. In addition (+)-**11** and (+)-**12** were found to be the optically pure unnatural enantiomers.

The anticholinesterase activities of compounds (−)- and (+)-**9**, **-10**, **-11**, **-12**, **15**, and **16** were assessed against human acetyl- and butyrylcholinesterase *in vitro* and were compared to those of physostigmine [(−)-**13**] and phenserine [(−)-**14**].

† This paper is dedicated to Dr. Dieter Seebach, Laboratory of Organic Chemistry, ETH, Zurich, Switzerland, on the occasion of his 60th birthday.

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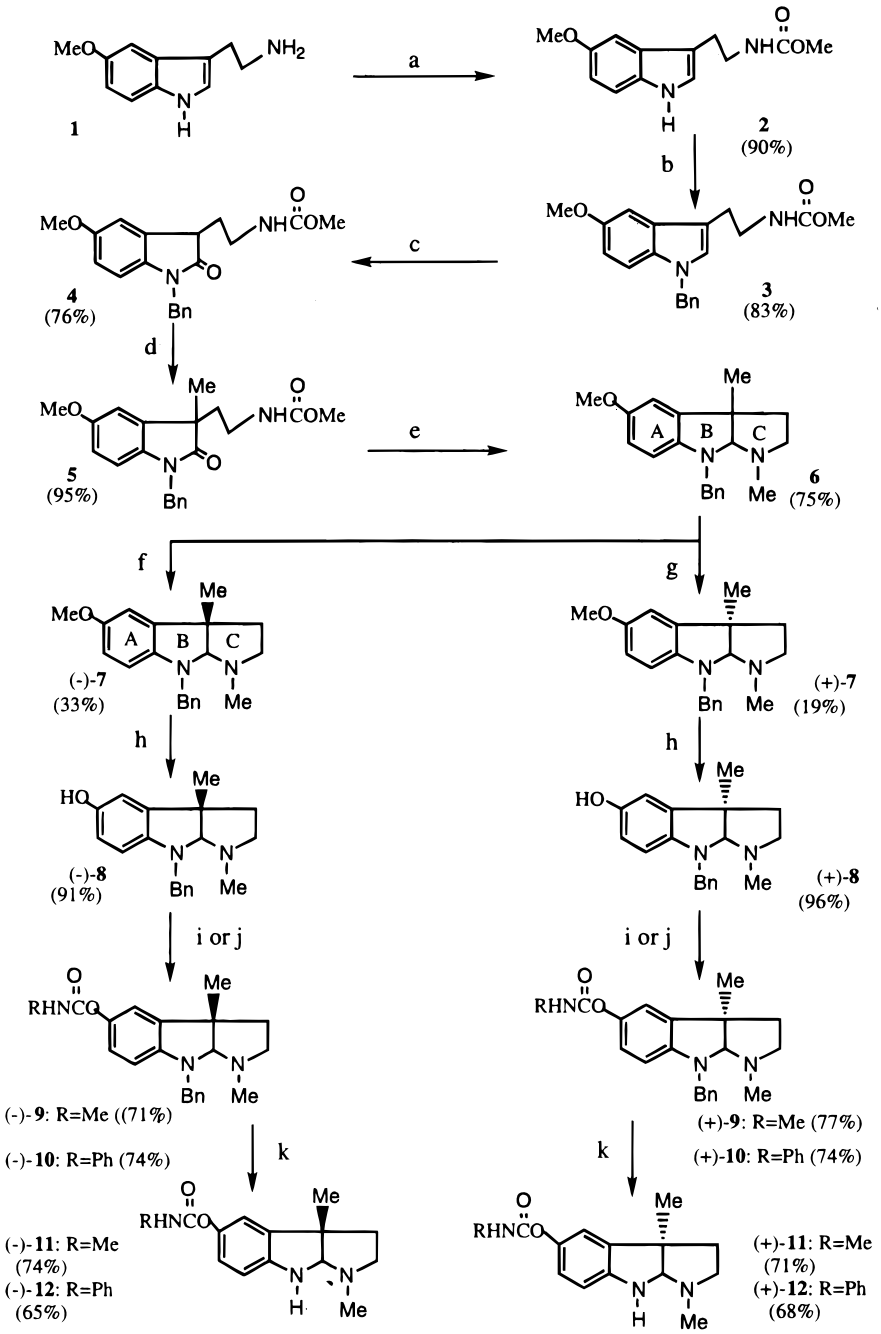
Scheme 1[a]

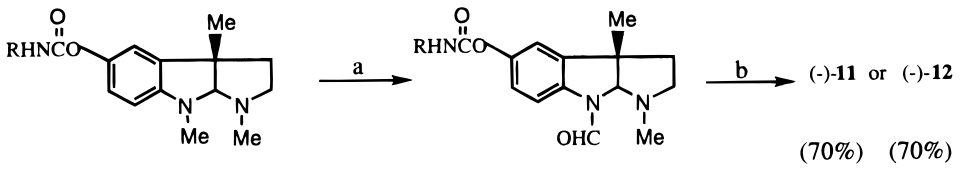


[a] Reagents: (a) $ClCO_2Me$, Et_3N, CH_2Cl_2; (b) BnBr, NaH, DMSO; (c) DMSO, concentrated HCl; (d) MeI, 20% NaOH, BTMAB, CH_2Cl_2; (e) Red-Al, benzene; (f) dibenzoyl-D-tartaric acid, THF; (g) ditoluyl-L-tartaric acid, THF; (h) BBr_3, CH_2Cl_2; (i) MeNCO, Na, Et_2O; (j) PhNCO, Na, Et_2O; (k) palladium hydroxide on carbon, H_2O, CH_3OH, TFA.

Results

Chemistry. Carbamate **2**, obtained from **1** on reaction with methyl chloroformate, was protected by a benzyl group to give *N*-benzylindole **3**. Oxidation of compound **3** with DMSO/concentrated HCl gave oxindole **4** in a yield of 76%.[12] Phase transfer methylation of **4** with MeI catalyzed by benzyltrimethylammonium bromide (BTMAB) gave compound **5** quantitatively. Instead of $LiAlH_4$,[13] using Red-Al (vitride)[14,15] as a reductive reagent at room temperature and benzene as a solvent, oxindole **5** was reduced and cyclized to give *N*(8)-benzylesermethole (**6**) (75%). There was no overreduced byproduct found. To provide the two series of antipodes with 3a*R* and 3a*S* configurations, respectively, for biological assessment, esermethole **6** was resolved with optically active acids, and the optically pure (−) and (+) antipodes [(−)- and (+)-**7**] were recovered. Compound **6** was mixed with 1.2 mol equiv of dibenzoyl-D-tartaric acid and crystallized from THF (2×) giving (−)-(3a*S*)-esermethole (−)-**7** with 100% ee in 37.5% yield. The optical purity was checked by HPLC on a chiral OD column.[16] From the combined mother liquor, a base enriched in (+)-(3a*R*)-esermethole was extracted and mixed with 1.2 equiv of ditoluyl-L-tartaric acid to form a salt which was recrystallized from THF (2×) to give (+)-(3a*R*)-esermethole (+)-**7** with 99% ee in 19.2% yield. From the mother liquor a further 16.3% of a mixture of (−)- and (+)-**7** was extracted. Compounds (−)- and (+)-**7** were transformed into *N*(8)-benzyl carbamates (−)-**11**, (−)-**12**, (+)-**11**, and (+)-**12** by

Scheme 2[a]



(-)-**13**: R=Me, Physostigmine
(-)-**14**: R=Ph, Phenserine

(-)-**15**: R=Me (28%)
(-)-**16**: R=Ph (35%)

[a] Reagents: (a) PDC, CH_2Cl_2; (b) aqueous 10% HCl, room temperature.

Table 1. 50% Inhibitory Concentration ± SEM[a] of Compounds versus Human Erythrocyte AChE and Plasma BChE

compound	IC_{50} (nM) AChE	IC_{50} (nM) BChE
(−)-**13**, (−)-physostigmine	27.9 ± 2.4	16.0 ± 2.9
(+)-**13**, (+)-physostigmine	9887.0 ± 6.0	2489.0 ± 290
(−)-**14**, (−)-phenserine	24.0 ± 1300.0	1300.0 ± 85.0
(+)-**14**, (+)-phenserine	3500.5 ± 54.8	23490.0 ± 300.0
(−)-**9**, (−)-*N*(8)-benzylnorphysostigmine	160.8 ± 40.9	21.8 ± 10.9
(+)-**9**, (+)-*N*(8)-benzylnorphysostigmine	2847.5 ± 6.0	1589.5 ± 356.0
(−)-**10**, (−)-*N*(8)-benzylnorphenserine	245.3 ± 10.0	85.3 ± 3.4
(+)-**10**, (+)-*N*(8)-benzylnorphenserine	11223.0 ± 283.2	7086.0 ± 1459
(−)-**11,** (−)-*N*(8)-norphysostigmine	56.7 ± 4.4	6.8 ± 2.2
(+)-**11**, (+)-*N*(8)-norphysostigmine	2193.5 ± 111.4	1120.5 ± 23.0
(−)-**12,** (−)-*N*(8)-norphenserine	40.8 ± 3.4	516.3 ± 60.9
(+)-**12**, (+)-*N*(8)-norphenserine	5654.5 ± 607.0	1745.5 ± 322.8
15, (−)-*N*(8)-formylnorphysostigmine	387.5 ± 46.0	46.0 ± 2.5
16, (−)-*N*(8)-formylnorphenserine	780.4 ± 1473.0	1473.0 ± 186.5

[a] Standard error of the mean.

demethylation and reaction with methyl isocyanate and phenyl isocyanate, respectively, according to known procedures.[10] The last step, the N-debenzylation, proved critical in this total synthesis. At first we chose $Pd(OH)_2$ on carbon as a catalyst and MeOH as a solvent for the reductive N-debenzylation, as explored in the synthesis of *N*(1)-norphysostigmine.[11] After 24 h hydrogenation, only a minimal amount of the desired product, besides some unknown polar compounds and mostly starting material, was found. With Pd/C, HCl in MeOH, the N-debenzylation was successful but the carbamate moiety was also reduced, and the pyrrolidine ring C opened in this acidic medium,[17] automatically giving a β-substituted indoline. After numerous trials we found the use of $Pd(OH)_2$/TFA as a catalyst, H_2O/MeOH as a solvent mixture, room temperature, and a reaction time of 1.5 h most suitable to accomplish the N-debenzylation without yielding byproducts.

Physostigmine [(−)-**13**] and phenserine [(−)-**14**], made from natural physostigmine,[16] were transformed to *N*(8)-norphysostigmine [(−)-**11**] and *N*(8)-norphenserine [(−)-**12**] by the method of Takano,[5] as shown in Scheme 2. First, (−)-**13** and (−)-**14** were selectively oxidized with pyridinium dichromate to form the *N*(8)-formyl derivatives **15** and **16** in yields of 28% and 35%, respectively. The ^1H NMR showed that the formamides **15** and **16** existed in two stable conformations, rotamers, in a ratio of 1:1. We chose not to separate these compounds since their hydrolysis yielded the same final products (−)-**11** and (−)-**12**. It may, indeed, have proved difficult to have separated **15** and **16** as they coeluted by TLC. The formamides **15** and **16** on hydrolysis with dilute hydrochloric acid (10%) at room temperature were transformed into (−)-*N*(8)-norphysostigmine [(−)-**11**] and (−)-*N*(8)norphenserine [(−)-**12**] in approximately 70% yields. The physical data of total synthesized (−)-**11** and (−)-**12** were identical with those of (−)-**11** and (−)-**12** obtained from (−)-**13**, and the optical rotations also were practically identical. Thus the structures and absolute configurations of the products of total synthesis, compounds (−)-**11** and (−)-**12**, and their unnatural enantiomers, (+)-**11** and (+)-**12**, were confirmed.

Biological Evaluation. Table 1 illustrates the anticholinesterase activity of compounds (−)- and (+)-**9**, **-10**, **-11**, **-12**, **15**, and **16** against human AChE and BChE, compared to those of physostigmine [(−)-**13**] and phenserine [(−)-**14**]. A comparison is additionally made to previously prepared and characterized (+)-physostigmine and (+)-phenserine.[18] As reported previously, these latter compounds lack anticholinesterase action compared to their 3a*S* enantiomers.

(−)-*N*(8)-Norphysostigmine [(−)-**11**] demonstrated potent AChE and BChE inhibitory activity which was comparable to that of physostigmine [(−)-**13**], with a similar slight selectivity for BChE versus AChE (7-fold). (−)-*N*(8)-Norphenserine [(−)-**12**], likewise, demonstrated potent AChE inhibitory action and, similar to phenserine [(−)-**14**], lacked activity against BChE (13-fold). In contrast, and similar to unnatural physostigmine and phenserine, their 3a*R* enantiomers lacked activity against either cholinesterase subtypes.

(−)-*N*(8)-Benzylnorphysostigmine [(−)-**9**] also demonstrated substantial AChE and BChE inhibitory action, which was slightly lower than that achieved by its debenzylated analogue (−)-**11** and by physostigmine [(−)-**13**]. The pattern of activity of (−)-*N*(8)-benzylnorphenserine [(−)-**10**] was dissimilar to that of (−)-*N*(8)-norphenserine [(−)-**12**] and phenserine [(−)-**14**]. It possessed significant but lower AChE action and potent BChE activity, losing the selectivity of AChE action prominent in most of the phenylcarbamates as exemplified by (−)-**14** and (−)-**12**. The 3a*R* enantiomers (+)-**9** and **-10** lacked anticholinesterase action. The *N*(8)-formyl derivative of physostigmine (**15**) possessed low AChE and moderate BChE inhibitory activity, whereas

the derivative of phenserine (**16**) lacked anticholinesterase action.

Discussion

We report, herein, the first total practical synthesis of both natural and unnatural *N*(8)-norphysostigmine (**11**) and *N*(8)-norphenserine (**12**) and confirm their structures and absolute configurations. The 3a*S* enantiomers of these are potential metabolites of physostigmine and phenserine. The former is utilized in the treatment of patients with Alzheimer's disease,[1] and a slow-release oral preparation (Synapton) has now reached phase III clinical trials.[19] The latter possesses far superior characteristics and is about to enter clinical trials.[2,4] (−)-*N*(8)-Norphysostigmine [(−)-**11**] and (−)-*N*(8)-norphenserine [(−)-**12**] both possessed potent anticholinesterase activities, which are similar in potency and selectivity to their *N*(8)-methyl analogues. Thus their production *in vivo*, potentially as a consequence of metabolism within the brain, likely would extend and potentiate the anticholinesterase actions of both physostigmine [(−)-**13**] and phenserine [(−)-**14**], probably affecting the former more than the latter as physostigmine's duration of action is far shorter (*in vivo* half-lives approximately 30−60 min and 8 h, respectively).[1,2,4]

In contrast the *N*(8)-formamides **15** and **16**, potential products of phase I oxidative metabolism[7] of physostigmine [(−)-**13**] and phenserine [(−)-**14**], respectively, lacked AChE inhibitory action. The former possessed potent BChE action which, if produced *in vivo*, likely would extend and/or potentiate physostigmine's actions.

As predicted from previous studies involving modification of the N(1)-position of natural physostigmine,[2,3,20] both (−)-*N*(8)-benzylnorphysostigmine [(−)-**9**] and (−)-*N*(8)-benzylnorphenserine [(−)-**10**] demonstrated potent anticholinesterase activity. Although this was reduced compared to physostigmine [(−)-**13**], phenserine [(−)-**14**], and their debenzylated analogues [(−)-**11** and -**12**], their activity was similar to that achieved by the clinically approved anticholinesterase tacrine (AChE IC_{50} = 190 nM).[2]

We have previously demonstrated that inhibition of AChE and BChE by *N*(1)-methyl-substituted carbamates, as illustrated in Table 1, is highly enantioselective and rests entirely on the 3a*S* enantiomers. This does not hold for either the N(1)-nor series[18] or ring C heterocongeners.[2,3,21] These studies suggested that a basic N(1) nitrogen does not need to be present in the tricyclic molecule of physostigmine for cholinesterase action and that reduced steric factors associated with *N*(1)-methyl substitution in the nor-compounds, physosvenols and thiaphysovenols, allow potent activity in the antipodal isomers. 3a*S* Enantioselectivity is clearly maintained in the N(8)-nor series, suggesting the importance of the N(1) site of physostigmine and analogues in the binding and inhibition of AChE and BChE.

Interestingly, N(8)-benzylation of phenserine negated the selectivity of action associated with phenserine in a manner similar to that achieved by *N*(1)-benzyl and N(1)-phenylethyl analogues, which modified the selectivity to favor BChE.[22] There are a number of extensive reviews describing the hydrolysis of acetylcholine and choline esters by AChE and BChE.[23−25] The active catalytic sites of both enzyme subtypes share a high degree of homology, although they are products of different genes on different chromosomes (3 and 7, respectively) and, simplistically, are formed of two subsites. An esteratic subsite involves an active serine residue that hydrolyzes choline esters through electron transfer within a catalytic triad of amino acids, to form a highly transient acylated enzyme complex which rapidly hydrolyzes to rejuvenate active enzyme. The carbamylation of this conformation by physostigmine and analogues creates an enzyme complex which is hydrolyzed at a considerably slower rate, resulting in its reversible inhibition. Close to the esteratic site, other amino acids have been postulated to form an "anionic" subsite involved in the correct orientation of a substrate to the esteratic subsite for hydrolysis. Bulky groups at the N(1)- and N(8)-positions of physostigmine and analogues [(−)-**9**] likely alter the positioning of the compound so that it can no longer conveniently optimally occupy the esteratic site of AChE, reducing its AChE affinity and selectivity. These bulky substituents, additionally, increase the lipophilicity of the analogues and allow them to readily interact with BChE. We speculate that the interaction of these two phenomena contributes, together with substitutions that we have previously described in the phenylcarbamate,[2−4] to provide compounds with specific characteristics that allow them to exploit critical differences in the three-dimensional structures surrounding the active catalytic site between AChE and BChE to provide compounds with their subtype selectivity of action. Our further studies are elucidating this and are providing us compounds to both treat neurological diseases and elucidate the role of BChE within the nervous system.

Experimental Section

Chemistry. 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 Finnigan-1015D mass spectrometer; Optical rotations were measured by JASCO, Model DIP-370 (Japan, Spectroscopic Co., Ltd.); HPLC: Rainin 81-2XM Macintosh controlled HPLC system, Chiralcel OD column (25 cm × 0.46 cm i.d.) (Daicel Chemical Industries, Ltd.). Elemental analyses were performed by Atlantic Microlab, Inc. Unless otherwise indicated, all separations were carried out under flash column chromatography (silica gel 60, 230−400 mesh) using the described solvents. All reactions involving nonaqueous solutions were performed under an inert atmosphere.

5-Methoxy-3-[2′-[(methoxycarbonyl)amino]ethyl]indole (2). 5-Methoxytryptamine (10 g, 52.6 mmol) was dissolved in CH_2Cl_2 (360 mL), and Et_3N (10 mL) was added. The mixture was cooled to 0 °C, and with stirring, methyl chloroformate (5.2 mL, 67.3 mmol) in CH_2Cl_2 (40 mL) was added dropwise over 10 min. After warming to room temperature, stirring was continued for 20 min. The mixture was washed with 2% HCl (300 mL) and brine (150 mL), dried over Na_2SO_4, and evaporated in vacuo. The residue was flash chromatographed (CH_2Cl_2/MeOH = 20/1) to give **2** (11.75 g, 90.0%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.30 (bs, 1H, N1-H), 7.20−6.72 (m, 4H, Ar-H), 4.75 (bs, 1H, N-H), 3.85 (s, 3H, O-CH_3), 3.70 (s, 3H, O-CH_3), 3.38 (m, 2H, C2'-H_2), 2.80 (t, J = 8.0 Hz, 2H, C1'-H_2); CI-MS (NH_3) *m*/*z* 249 (MH^+). Anal. ($C_{13}H_{16}N_2O_3$) C, H, N.

1-Benzyl-5-methoxy-3-[2′-[(methoxycarbonyl)amino]ethyl]indole (3). Compound **2** (11.7 g, 47.4 mmol) was dissolved in DMSO (120 mL), and NaH (2.3 g, 51.3 mmol, 60% oil dispersion) was added. The mixture was stirred at room temperature for 20 min, and BnBr (8.78 g, 51.3 mmol) in DMSO (20 mL) was added dropwise over 30 min. After the addition, stirring continued for 2 h; then H_2O (400 mL) was

added. The mixture was extracted with AcOEt (2 × 300 mL). The combined AcOEt layers were washed with brine (200 mL) and H_2O (200 mL) and dried over Na_2SO_4. After the removal of solvent, the residue was flashed chromatographed (CH_2Cl_2/MeOH = 20/1) to give **3** (13.3 g, 82.9%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.40−6.70 (m, 9H, Ar-H), 5.20 (s, 2H, Ph-CH_2), 3.80 (s, 3H, O-CH_3), 3.70 (s, 3H, O-CH_3); CI-MS (NH_3) *m*/*z* 339 (MH^+). Anal. ($C_{20}H_{22}N_2O_3$) C, H, N.

1-Benzyl-1,3-dihydro-5-methoxy-3-[2′-[(methoxycarbonyl)amino]ethyl]-2*H*-indol-2-one (4). Compound **3** (13.3 g, 39.3 mmol) was dissolved in DMSO (15 mL). After addition of concentrated HCl (36%, 90 mL) dropwise, the solution became dark green and then stirred at room temperature for 1 h. H_2O (300 mL) was added, and the mixture was neutralized with $NaHCO_3$ and extracted with AcOEt (2 × 200 mL). The combined AcOEt layers were washed with an aqueous solution of $NaHCO_3$ (5%), dried over Na_2SO_4, and evaporated in vacuo to give a syrup which was recrystallized from AcOEt/hexane to give **4** (10.5 g, 77.5%) as white crystals: mp 112−113 °C; ^{1}H NMR ($CDCl_3$) δ 7.34−7.16 (m, 5H, benzyl-Ar-H), 6.68−6.56 (m, 3H, Ar-H), 5.33 (bs, 1H, NH), 4.90 (s, 2H, Ph-CH_2), 5.33 (broad, NH), 3.75 (s, 3H, O-CH_3), 3.68 (s, 3H, O-CH_3), 3.55 (m, 1H, C3-H), 3.45 (m, 2H, C2′-H_2), 2.15 (m, 2H, C1′-H_2); CI-MS (NH_3) *m*/*z* 355 (MH^+). Anal. ($C_{20}H_{22}N_2O_4$) C, H, N.

1-Benzyl-1,3-dihydro-5-methoxy-3-methyl-3-[2′-[(methoxycarbonyl)amino]ethyl]-2*H*-indol-2-one (5). Compound **4** (10.2 g, 28.7 mmol) was dissolved in CH_2Cl_2 (230 mL), and 20% NaOH (115 mL), benzyltrimethylammonium bromide (BTMAB) (1.15 g, 5.75 mmol), and MeI (8.1 g, 56 mmol) were added. The mixture was stirred vigorously at room temperature under N_2 for 2 h; then H_2O (300 mL) was added. The mixture was extracted with CH_2Cl_2 (3 × 300 mL). The combined CH_2Cl_2 layers were dried over Na_2SO_4. After the removal of solvent in vacuo, the residue was chromatographed (CH_2Cl_2/MeOH = 20/1) to give **5** (10.0 g, 94.9%) as a foam: ^{1}H NMR ($CDCl_3$) δ 7.34−7.16 (m, 5H, benzyl-Ar-H), 6.81 (d, *J* = 2.5 Hz, 1H, C4-H), 6.67 (dd, *J* = 2.5, 8.8 Hz, 1H, C5-H), 6.62 (d, *J* = 8.3 Hz, 1H, C7-H), 4.93 and 4.84 (AB, *J* = 15.6 Hz, 2H, Ph-CH_2), 4.75 (bs, 1H, NH), 3.76 (s, 3H, O-CH_3), 3.59 (s, 3H, O-CH_3), 3.10−2.80 (m, 2H, C2′-H2), 2.30−1.90 (m, 2H, C1′-H2), 1.42 (s, 3H, C3-CH_3); CI-MS (NH_3) *m*/*z* 369 (MH^+). Anal. ($C_{21}H_{24}N_2O_4$) C, H, N.

8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydro-5-methoxypyrrolo[2,3-*b*]indole (6). Compound **5** (9.0 g, 24.42 mmol) was dissolved in dry benzene (120 mL), and 17.12 g (60%) (50 mmol) of Red-Al was added at 0 °C. The mixture was stirried at room temperature for 5 h; then the reaction mixture was cooled again in an ice bath, and H_2SO_4 (10%) (120 mL) was added. After stirring for a while, the organic layer was separated. The aqueous solution was basified with Na_2CO_3, extracted by CH_2Cl_2 (3 × 200 mL), dried over Na_2SO_4, and evaporated under reduced presssure. The residue was flash chromatographed (CH_2Cl_2/MeOH = 20/1) to give **6** (5.66 g, 75.3%) as white crystals: mp 79−80 °C; ^{1}H NMR ($CDCl_3$) δ 7.33−7.23 (m, 5H, benzyl-Ar-H), 6.67 (d, *J* = 2.6 Hz, 1H, C4-H), 6.57 (dd, *J* = 2.6, 8.4 Hz, 1H, C6-H), 6.23 (d, *J* = 8.4 Hz, 1H, C7-H), 4.51 and 4.36 (AB, *J* = 16.6 Hz, 2H, Ph-CH_2), 4.27 (s, 1H, C8a-H), 3.75 (s, 3H, O-CH_3), 2.73−2.46 (m, 2H, C2-H_2), 2.43 (s, 3H, N-CH_3), 2.03−1.73 (m, 2H, C3-H_2), 1.43 (s, 3H, C3a-CH_3); CI-MS (NH_3) *m*/*z* 309 (MH^+). Anal. ($C_{20}H_{24}N_2O$) C, H, N.

Resolution of 8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydro-5-methoxypyrrolo[2,3-*b*]indole (6). Compound **6** (3.84 g, 0.0124 mol) was dissolved in THF (12 mL), and dibenzoyl-D-tartaric acid (5.37 g, 0.015 mol) was dissolved in THF (12 mL) also. The two solutions were mixed together, and crystallization gradually occurred. After filtration, 5.34 g of the crystalline salt of dibenzoyl-D-tartrate was obtained. The salt was dissolved in THF (50 mL) and left overnight to yield 2.68 g (32.5%) of dibenzoyl-D-tartrate of (−)-(3a*S*)-8-benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydro-5-methoxypyrrolo[2,3-*b*]indole **[(−)-7]** as white crystals: mp 126−127 °C; $[\alpha]^{20}_D$ −5.26° (*c* = 0.5, EtOH). Anal. ($C_{38}H_{38}N_2O_9$) C, H, N.

Combined mother liquor was evaporated in vacuo; the residue was dissolved in H_2O (40 mL), basified with Na_2CO_3, and extracted into CH_2Cl_2 (3 × 100 mL). The extract was dried over Na_2SO_4 and evaporated in vacuo. The residue was chromatographed on silica gel (CH_2Cl_2/MeOH = 10:1) to give 1.82 g of (+)-(3a*R*) richer base [(−)- and (+)-**7**] which was dissolved in THF (6 mL) and mixed with a solution of ditoluyl-L-tartaric acid (2.22 g, 0.0057 mol) in THF (6 mL). Crystallization gradually occurred, and filtration gave 3.3 g of a crystalline salt which was dissolved in THF (50 mL) and left overnight to yield 1.65 g (19.2%) of ditoluyl-L-tartrate of (+)-(3a*R*)-8-benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexhydro-5-methoxypyrrolo[2,3-*b*]indole **[(+)-7]** as white crystals: mp 173−174 °C; $[\alpha]^{20}_D$ +4.76° (*c* = 0.5, EtOH). Anal. ($C_{40}H_{42}N_2O_9$) C, H, N.

(−)-(3a*S*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydro-5-methoxypyrrolo[2,3-*b*]indole [(−)-7]. Dibenzoyltartrate (1 g) of compound (−)-**7** was dissolved in H_2O (40 mL), basified by Na_2CO_3, and extracted by CH_2Cl_2 to give the base of compound (−)-**7** (460 mg, 100%) as white crystals: mp 39−40 °C; $[\alpha]_D$ −87.1° (*c* = 1, EtOH); 100% ee, estimated by HPLC analysis with a Chiracel OD column.[26] Anal. ($C_{20}H_{24}N_2O$) C, H, N.

(+)-(3a*R*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydro-5-methoxypyrrolo[2,3-*b*]indole [(+)-7]. Ditoluyl-L-tartrate (1.04 g) of compound (+)-**7** was dissolved in H_2O (40 mL), basified by Na_2CO_3, and extracted by CH_2Cl_2 to give the base of compound (+)-**7** (455 mg, 98%) as white crystals: mp 39−40 °C; $[\alpha]_D$ +87.0° (*c* = 1, EtOH); 99% ee, estimated by HPLC analysis on a Chiracel OD column.[26] Anal. ($C_{20}H_{24}N_2O$) C, H, N.

(−)-(3a*S*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-ol [(−)-8]. Compound (−)-**7** (460 mg, 1.49 mmol) was dissolved in $CHCl_3$ (10 mL), and BBr_3 (99.9%) (0.5 mL) was added dropwise for 5 min with stirring. The mixture was stirred at room temperature for 4 h, and MeOH (10 mL) was added slowly. After removal of solvent under vacuo, the residue was dissolved in H_2O (20 mL), basified with $NaHCO_3$, and extracted with ether (3 × 20 mL). After evaporation of solvent the residue was flash chromatographed (CH_2Cl_2/MeOH = 20:1) to give (−)-**8** (420 mg, 95.5%) as a light pink foam: $[\alpha]_D$ −102.7° (*c* = 0.95, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.33−7.23 (m, 5H, benzyl-Ar-H), 6.67 (d, *J* = 2.6 Hz, 1H, C4-H), 6.57 (dd, *J* = 2.6, 8.4 Hz, 1H, C6-H), 6.23 (d, *J* = 8.4 Hz, 1H), 5.12 (bs, 1H, NH), 4.51 and 4.36 (AB, *J* = 16.6 Hz, 2H, Ph-CH_2), 4.27 (s, C8a-H), 2.73−2.46 (m, 2H, C2-H_2), 2.43 (s, 3H, N1-CH_3), 2.03−1.73 (m, 2H, C2-H_2), 1.43 (s, 3H, C3a-CH_3); CI-MS (NH_3) *m*/*z* 295 (MH^+). Anal. ($C_{19}H_{22}N_2O$) C, H, N.

(+)-(3a*R*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-ol [(+)-8]. According to the procedure for making compound (−)-**8**, from compound (+)-**7** (392 mg, 1.27 mmol), 340 mg (91.1%) of compound (+)-**8** was obtained as a gum: $[\alpha]_D$ +102.1° (*c* = 0.2, EtOH); ^{1}H NMR and MS were identical with that of compound (−)-**8**. Anal. ($C_{19}H_{22}N_2O\cdot{}^1/_6H_2O$) C, H, N.

(−)-(3a*S*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Methylcarbamate [(−)-9]. Compound (−)-**8** (145 mg, 0.49 mmol) was dissolved in ether (10 mL), and Na (1 mg) was added. The mixture was stirred at room temperature for 1 min; then methyl isocyanate (28.1 mg, 0.49 mmol) was added. The mixture was stirred at room temperature for 10 min. After the removal of solvent, the residue was chromatographed (CH_2Cl_2/MeOH = 20/1) to give (−)-**9** (127 mg, 70.8%) as white crystals: mp 79−81 °C; $[\alpha]_D$ −86.3° (*c* = 0.7, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.37−7.26 (m, 5H, benzyl-Ar-H), 6.82 (d, *J* = 2.2 Hz, 1H, C4-H), 6.74 (dd, *J* = 2.2, 8.5 Hz, 1H, C6-H), 6.24 (d, *J* = 8.5 Hz, 1H, C7-H), 4.90 (d, *J* = 3.9 Hz, 1H), 4.55 and 4.41 (AB, *J* = 16.6 Hz, 2H, Ph-CH_2), 4.36 (s, 1H, C8a-H), 2.94 (d, *J* = 3.9 Hz, 3H, NH-CH_3), 2.80−2.75 (m, 2H, C1-H_2), 2.45 (s, 3H, N1-CH_3), 2.05−2.00 (m, 2H, C2-H_2), 1.44 (s, 3H, C3a-CH_3); CI-MS (NH_3) *m*/*z* 352 (MH^+). Anal. ($C_{21}H_{25}N_3O_2$) C, H, N.

(−)-(3a*S*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Phenylcarbamate [(−)-10]. Compound (−)-**8** (215 mg, 0.73 mmol) was dissolved in ether (10 mL), and Na metal (1 mg) was added. The mixture was stirred at room temperature for 1 min; then phenyl isocyanate

(87.1 mg, 0.73 mmol) was added. After addition of phenyl isocyanate, the reaction mixture was immediately evaporated in vacuo; the residue was chromatographed (CH_2Cl_2/MeOH = 20/1) to give (−)-**10** (224 mg, 74.0%) as a light pink foam: $[\alpha]_D$ −83.2° (*c* = 0.3, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.46−7.08 (m, 10H, Ar-H), 6.88 (d, *J* = 2.5 Hz, 1H, C4-H), 6.80 (dd, *J* = 2.5, 8.5 Hz, 1H, C6-H), 6.50 (d, *J* = 8.5 Hz, 1H, C7-H), 4.55 and 4.42 (AB, *J* = 16.6 Hz, 2H, Ph-CH_2), 4.36 (s, 1H, C8a-H), 2.80−2.75 (m, 2H, C2-H_2), 2.45 (s, 3H, N1-H_3), 2.04−2.00 (m, 2H, C3-H_2), 1.45 (s, 3H, C3a-CH_3); CI-MS (NH_3) *m*/*z* 414 (MH^+). Anal. ($C_{26}H_{27}N_3O_2$) C, H, N.

(+)-(3a*R*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Methylcarbamate [(+)-9]. According to the procedure for making compound (−)-**9**, from 138 mg (0.47 mmol) of compound (+)-**8**, 122 mg (77.0%) of compound (+)-**9** was obtained as a white crystal: mp 79−81 °C; $[\alpha]_D$ +87.1° (*c* = 0.2, EtOH); ^{1}H NMR and MS were identical with that of compound (−)-**9**. Anal. ($C_{21}H_{25}N_3O_2 \cdot {}^1/_4H_2O$) C, H, N.

(+)-(3a*R*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Phenylcarbamate [(+)-10]. According to the procedure for making compound (−)-**10**, from 134 mg (0.46 mmol) of compound (+)-**8**, 140 mg (73.7%) of compound (+)-**10** was obtained as a foam: $[\alpha]_D$ +82.9° (*c* = 0.8, EtOH); ^{1}H NMR and MS were identical with that of compound **12**. Anal. ($C_{26}H_{27}N_3O_2 \cdot {}^1/_4CH_3OH$) C, H; N: Calcd, 9.96; found, 9.43.

(−)-(3a*S*)-1,3a-Dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Methylcarbamate [(−)-11]. Compound (−)-**9** (50 mg, 0.15 mmol) was dissolved in a mixture solution of MeOH (1 mL), H_2O (4.5 mL), and TFA (0.5 mL); then palladium hydroxide on carbon (6 mg) was added. The reaction mixture was stirred under a balloon of hydrogen at room temperature for 1.5 h; 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_2Cl_2/MeOH = 10/1) to give product (−)-**11** (28 mg, 73.5%) as white crystals (from petroleum ether): mp 154−155 °C; $[\alpha]^{20}_D$ −108.1° (*c* = 0.2, EtOH) [lit.[5] mp 152−153 °C, $[\alpha]^{28.5}_D$ −116° (*c* = 0.2, EtOH); lit.[27] mp 151 °C, $[\alpha]^{21}_D$ −108.6° (EtOH)]; ^{1}H NMR ($CDCl_3$) δ 6.80 (d, *J* = 2.2 Hz, 1H, C4-H), 6.74 (dd, *J* = 2.2, 8.5 Hz, 1H, C6-H), 6.24 (d, *J* = 8.5 Hz, 1H, C7-H), 4.90 (bs, 1H, NH), 4.32 (s, 1H, C8a-H), 4.00 (bs, 1H, NH), 2.94 (d, *J* = 3.9 Hz, 3H, N-CH_3), 2.64 (m, 2H, C2-H_2), 2,35 (m, 3H, N-CH_3), 1.90 (m, 2H, C3-H_2), 1.35 (s, 3H, C3a-CH_3); CI-MS (NH_3) *m*/*z* 262 (MH^+). Anal. ($C_{14}H_{19}N_3O_2$) C, H, N.

(−)-(3a*S*)-1,3a-Dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Phenylcarbamate [(−)-12]. Compound (−)-**10** (45 mg, 0.11 mmol) was dissolved in a mixture solution of MeOH (2 mL), H_2O (2 mL), and TFA (1 mL). Palladium hydroxide on carbon (10 mg) was added. The reaction mixture was stirred under a balloon of hydrogen at room temperature for 2 h; then the catalyst was filtered. The filtrate was evaporated in vacuo to give 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 a preparative TLC (silica gel) (CH_2Cl_2 = 10/1) to give product (−)-**12** (23 mg, 64.7%) as white crystals (from petroleum ether): mp 76−77 °C; $[\alpha]^{20}_D$ −54.5° (*c* = 0.2, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.60−7.12 (m, 5H, Ph-H), 6.92−6.58 (m, 3H, Ar-H), 4.52 (s, 1H, C8a-H), 2.80−2.60 (m, 2H, C2-H_2), 2.50 (s, 3H, N-CH_3), 2.15−2.00 (m, 2H, C3-H_2), 1.45 (s, 3H, C3a-CH_3); CI-MS (NH_3) *m*/*z* 324 (MH^+). Anal. ($C_{19}H_{21}N_3O_2$) C, H, N.

(+)-(3a*R*)-1,3a-Dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Methylcarbamate [(+)-11]. According to the procedure for making compound (−)-**11**, from compound (+)-**9** (44 mg, 0.13 mmol), 24 mg (70.7%) of compound (+)-**11** was obtained as white crystals: mp 154−155 °C; $[\alpha]^{20}_D$ +107.0 (*c* = 0.2, EtOH); MS and ^{1}H NMR were identical with that of compound (−)-**11**. Anal. ($C_{14}H_{19}N_3O_2$) C, H, N.

(+)-(3a*R*)-1,3a-Dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Phenylcarbamate [(+)-12]. According to the procedure for making compound (−)-**12**, from compound (+)-**10** (51 mg, 0.123 mmol), 27 mg (68.0%) was obtained as white crystals (from petroleum ether): mp 76−77 °C; $[\alpha]^{20}_D$ +54.0° (*c* = 0.2, EtOH); MS and ^{1}H NMR were identical with that of compound **(−)-12**. Anal. ($C_{19}H_{21}N_3O_2 \cdot {}^1/_2H_2O$) C, H, N.

(−)-(3a*S*)-8-Formyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Methylcarbamate (15). Physostigmine [(−)-**13**] (350 mg, 1.09 mmol) and pyridinium dichromate (PDC) (700 mg, 1.86 mmol) were dissolved in CH_2Cl_2 (60 mL). The reaction mixture was stirred for 12 h at room temperature; then another 700 mg of PDC was added and the mixture stirred for a further 12 h. After filtration the dark precipitate which was formed during the reaction was dissolved in H_2O, basified by Na_2CO_3, and extracted with CH_2Cl_2. The combined filtrates and extracts were evaporated in vacuo to give a residue which was partitioned between H_2O and Et_2OH. The separated latter solution was dried over Na_2SO_4 and then evaporated in vacuo to give a residue which was chromatographed on silica gel (EtOAc/petroleum ether = 1/2) to give product **15** as a foam (100 mg, 28%): $[\alpha]^{20}_D$ −21.7° (*c* = 0.2, EtOH); ^{1}H NMR ($CDCl_3$) δ 8.97 (s, 1/2H, O=CHN), 8.57 (s, 1/2H, O=CNH), 7.25−6.87 (m, 3H, Ar-H), 4.95 (s, 1/2H, C8a-H), 4.70 (s, 1/2H, C8a-H), 2.95 (d, *J* = 3.0 Hz, 1/2 3H, HN-CH_3), 2.85 (d, *J* = 3.0 Hz, 1/2 3H, HN-CH_3), 2.80−2.70 (m, 2H, C2-H_2), 2.65 (s, 1/2 3H, N1-CH_3), 2.55 (s, 1/2 3H, N1-CH_3), 2.20−2.00 (m, 2H, C2-H_2), 1.55 (s, 1/2 3H, C3a-CH_3), 1.45 (s, 1/2 3H, C3a-CH_3); CI-MS (NH_3) *m*/*z* 324 (MH^+).

(−)-(3a*S*)-8-Formyl-1,3a-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Phenylcarbamate (16). Phenserine [(−)-**14**] (138 mg, 0.41 mmol) and PDC (376 mg, 1.0 mmol) were dissolved in CH_2Cl_2 (30 mL). The reaction mixture was stirred for 12 h at room temperature; then another 376 mg (1.0 mmol) of PDC was added and the mixture stirred for a further 12 h. After filtration the dark precipitate formed during the reaction was dissolved in H_2O, basified with Na_2CO_3, and extracted by CH_2Cl_2. The combined filtrates and extracts were evaporated in vacuo to give a residue which was partitioned between H_2O and Et_2O. The latter solution was separated, dried over Na_2SO_4, and then evaporated in vacuo to give a residue which was chromatographed on silica gel (EtOAc/petroleum ether = 1/2) to give product **16** (50.4 mg, 35.0%) as a foam: $[\alpha]^{20}_D$ −25.7° (*c* = 0.5, EtOH); ^{1}H NMR ($CDCl_3$) δ 8.92 (s, 1/2H, O=CHN), 8.50 (s, 1/2H, O=CNH), 7.50−6.87 (m, 8H, Ar-H), 4.95 (s, 1/2H, C8a-H), 4.75 (s, 1/2H, C8a-H), 2.48 (s, 1/2 3H, N-CH_3), 2.15−2.00 (m, 2H, C3-H_2), 1.48 (s, 1/2 3H, C3a-CH_3), 1.46 (s, 1/2 3H, C3a-CH_3); CI-MS (NH_3) *m*/*z* 352 (MH^+).

(−)-*N*(8)-Norphysostigmine [(−)-11] from Compound 15. Compound **15** (80 mg, 0.25 mmol) was dissolved in 2 mL of 10% HCl. The reaction mixture was stirred for 4 h at room temperature and then basified by Na_2CO_3, extracted by CH_2Cl_2, and dried over Na_2SO_4. After the removal of solvent, the residue was chromatographed on silica gel (CH_2Cl_2/MeOH = 10/1) to give a pale yellow gum which was crystallized from petroleum ether to give product (−)-**11** as white crystals: mp 154−155 °C; $[\alpha]^{20}_D$ −108.4° (*c* = 0.3, EtOH); ^{1}H NMR and MS were identical with that of compound (−)-**11** made from compound (−)-**9**.

(−)-*N*(8)-Norphenserine [(−)-12] from Compound 14. Compound **14** (28 mg, 0.079 mmol) was dissolved in 2 mL of 10% HCl; the reaction mixture was stirred for 4 h at room temperature and then basified by Na_2CO_3, extracted by CH_2Cl_2, and dried over Na_2SO_4. After evaporation of solvent, the residue was chromatographed on silica gel (CH_2Cl_2/MeOH = 10/1) to give a pale yellow gum which was crystallized from petroleum ether to give product (−)-**12** as white crystals: mp 75−76 °C; $[\alpha]^{20}_D$ −54.1° (*c* = 0.3, EtOH); ^{1}H NMR and MS were identical with that of compound (−)-**12** made from compound (−)-**10**.

Quantitation of Anticholinesterase Activity. The action of compounds (−)- and (+)-**9**, **-10**, **-11**, **-12**, **15**, and **16** 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-methylthiocholine and *s*-butyrylthiocholine (0.5 mmol/L) (Sigma Chemi-

cal Co., St. Louis, MO) was determined. Compounds were dissolved in Tween 80/EtOH, 3:1 (v:v; <150 μL total volume), and diluted in 0.1 M Na_3PO_4 buffer (pH 8.0) in half-log steps to provide a final concentration range spanning 0.3 nM to 30 mM. The Tween 80/EtOH was diluted in excess of 1 in 5000 and had no effect on either AChE or BChE activity.

Freshly collected blood was centrifuged (10000*g*, 10 min, 4 °C) and the plasma 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 diluted with a further 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 ($\lambda = 412$ nm). To correct for nonspecific substrate hydrolysis, aliquots were coincubated under conditions of complete enzyme inhibition (by addition of 1×10^{-5} M physostigmine [(−)-**13**]), and the change in absorbance was subtracted from those observed with the varying concentrations of test compounds. Compounds were analyzed on four separate occasions and assayed along side physostigmine [(−)-**13**] and phenserine [(−)-**14**], as control and external standards whose activity we have previously reported.

The enzyme activity at each concentration of test compound is expressed as a percent of activity in the absence of compound, transformed to a logit format (logit = %activity/100 − %activity) and plotted as a function of its log concentration. Inhibitory activity was calculated as an IC_{50}, defined as the concentration of compound (nM) required to inhibit 50% enzymatic activity, and determined from the correlation between log concentration and logit activity.

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