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A practical conversion of natural physostigmine into the potent butyrylcholinesterase inhibitor N^1,N^8-bisnorcymserine

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Abstract

A rapid novel synthetic route to the potent reversible butyrylcholinesterase inhibitor (–)-N^1,N^8-bisnorcymserine (**1**) is reported from physostigmine (**2**) in a 20% total yield. Details on the formation of the imino-quinone **6** obtained in the oxidation of N^1-benzylnoresermethole (**4**) and its conversion into N^1-bisnoreseroline (**7**) are given. As expected, the product of this synthesis, (**1**), had identical biological activity to the same agent produced by total synthesis.

Keywords: butyrylcholinesterase; Alzheimer's disease therapeutic; physostigmine; anticholinesterase; N^1,N^8-bisnorcymserine.

Neuroscience and molecular biology studies show that inappropriate butyrylcholinesterase (BChE) activity increases the risk and/or progression of Alzheimer's disease.[1–3] Based on this, our group initiated studies to design and synthesize novel, potent, and highly selective reversible inhibitors of BChE to provide a candidate for therapeutic development and to test the novel hypothesis that BChE inhibitors are of benefit in the treatment of Alzheimer's disease.[4] (–)-N^1,N^8-Bisnorcymserine (**1**) proved to be one of the most potent and interesting inhibitors of human BChE in an initial pharmacological evaluation.[4] However, until now, synthesis of this optically pure compound could be accomplished only by tedious multiple step chemistry, which included the resolution of the required 3*S* enantiomer at an intermediate stage.[4–7] It was therefore desirable to develop an efficient synthetic method to rapidly gain a large quantity of this optically

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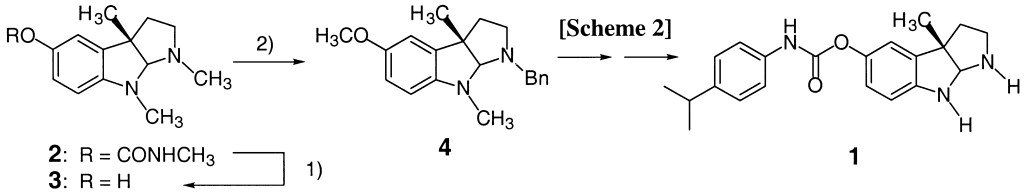
active compound for use in further pharmacological studies. We report, herein, the synthesis of (–)-N^1,N^8-bisnorcymserine (**1**) from the commercially available alkaloid, physostigmine (**2**).

(–)-Physostigmine (**2**) was treated with sodium *n*-butoxide in *n*-butanol to give eseroline (**3**).[8] (–)-Eseroline (**3**) then was purified and isolated as its fumarate salt, and, thereafter, was converted into N^1-benzylnoresermethole (**4**), according to a known procedure[9–11] (Scheme 1).



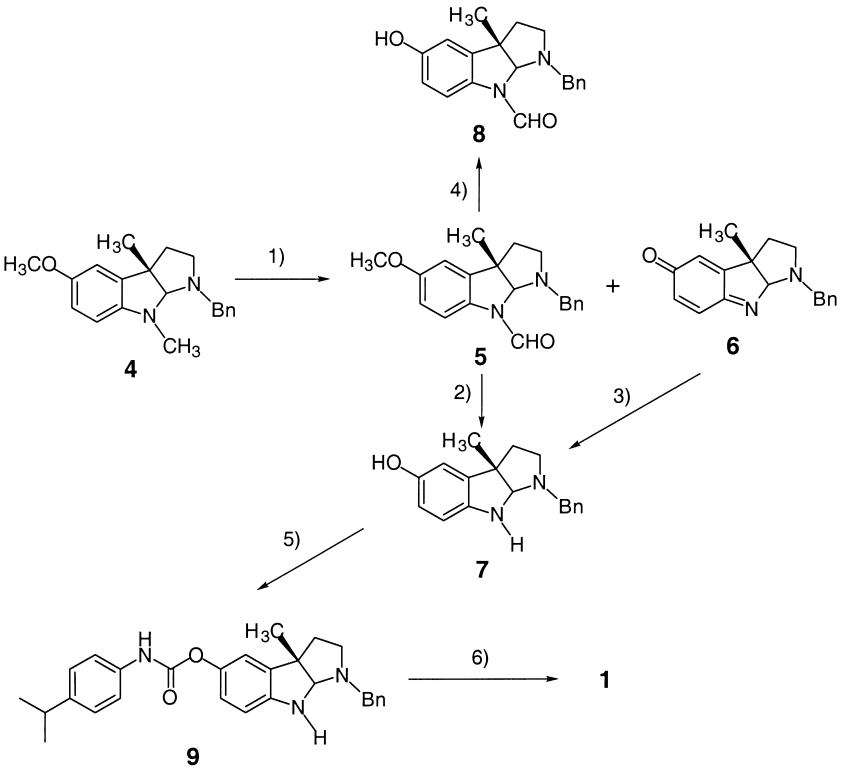
Scheme 1. (1) Ref. 7, 89%; (2) (a) CH_3I, KOH, DMSO (b) $C_6H_5CH_2NH_2$, total 66%

In order to prepare the desired N^1,N^8-bisnor derivatives, selective N^8-demethylation of compound **4** is a key step. It was reported[12] that oxidation of N^8-methyl to N^8-formyl, followed by hydrolysis with diluted hydrochloric acid afforded N^8-norphysostigmine. Following the previous methods, the oxidation of N^1-benzylnoresermethole (**4**) gave the N^8-formyl compound **5**[13] in 25 and 36% yields, respectively, when pyridinium dichromate (PDC) and Collins' reagent[14] were used as oxidants in dichloromethane. This low yield is an obstacle in the efficient conversion of physostigmine (**2**) to the target compound (**1**), particularly in light of the fact that the N^8-formyl compound **5** proved to be a mixture of geometrical isomers in a ratio of 1:3. These were clearly detected in the ^{1}H NMR spectrum, and showed as two close spots by TLC, but, nevertheless, were difficult to separate.[6,7]

With some modification, the yield rose to 45% when the oxidation of N^1-benzylnoresermethole (**4**) was undertaken with PDC in an ice bath and in the presence of a weak base, such as pyridine or sodium bicarbonate. We additionally found that a new compound, the imino-quinone **6**,[15] was simultaneously generated and we isolated it from the reaction mixture by column chromatography in a 19% yield.

The following is a detailed experimental procedure for the oxidation of N^1-benzylnoresermethole (**4**): To a solution of **4** (1.54 g, 4.99 mmol) in CH_2Cl_2 (75 mL) was added $NaHCO_3$ (1 g). The mixture was stirred vigorously and cooled in an ice bath. Pyridinium dichromate (3.76 g, 9.99 mmol) was then added, and the mixture was stirred for 2 h. The reaction mixture was filtered and the resulting solid was washed with CH_2Cl_2 (50 mL). The combined CH_2Cl_2 solution was washed with water (3×50 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (silica gel) using petroleum ether:ethyl acetate (10:1 and 10:2) as the eluent to obtain starting material **4** (0.15 g) and **5** (0.65 g, 45%), then using petroleum ether:ethyl acetate (4:1) as the eluent to obtain the more polar component **6** (0.235 g, 19%).

Compound **5** then was reacted with BBr_3 in dichloromethane for one hour at room temperature and methanol was added to destroy any remaining BBr_3. The resulting solution was evaporated to give a residue that was purified by flash chromatography to afford N^1-benzylbisnorseroline (**7**), with simultaneous hydrolysis of the *N*-formyl group. The latter was unexpected and provided a valuable one pot reaction to effect both *O*-demethylation and *N*-deformylation, resulting in compound **7** in a yield of 77% (Scheme 2).



Scheme 2. (1) PDC, $NaHCO_3$, CH_2Cl_2; (2) BBr_3, CH_2Cl_2, CH_3OH 77%; (3) $NaBH_4$, THF, 85%; (4) BBr_3, CH_2Cl_2, 91%; (5) Na, anhydrous ether, 4-isopropylphenyl isocyanate, 97%; (6) H_2, $Pd(OH)_2/C$, 70%

In the reaction of compound **5** with BBr_3, carefully controlling the addition of methanol to destroy remaining BBr_3 (specifically, by using approximately an equivalent of methanol and maintaining the temperature at 0°C in an ice bath), followed by dilution of the reaction mixture with dichloromethane and washing the CH_2Cl_2 solution with water to remove acid resulted in *O*-demethylation alone to give compound **8**[17] in 91% yield.

Compound **6** was reduced with sodium borohydride in THF to afford the useful intermediate N^1-benzylbisnorseroline (**7**)[16] in 85% yield.

Compound **7** is a key intermediate from which the N^1,N^8-bisnorcymserine was readily prepared. Reaction of 4-isopropylphenyl isocyanate with compound **7** afforded N^1-benzyl-N^8-norcymserine (**9**).[18] *N*-Debenzylation of compound **9** was accomplished by catalytic hydrogenation using $Pd(OH)_2/C$ as a catalyst and *iso*-propanol as a solvent to give N^1,N^8-bisnorcymserine (**1**).[4] As both of the products of the oxidation of N^1-benzylnoresermethole (**4**), compounds **5** and **6**, were converted into N^1-benzylbisnorseroline (**7**), the synthesis of N^1,N^8-bisnorcymserine (**1**) from physostigmine (**2**) was achieved in an overall yield that totalled 20%. Chemical characterization demonstrated that compound **1**, prepared as described herein, was chemically identical in every respect to the same material prepared by total synthesis.[4] An ex vivo assay[4–11] then was undertaken to quantify and compare the activity of N^1,N^8-bisnorcymserine (**1**), synthesized by both routes, to inhibit human BChE and AChE prepared freshly from plasma and erythrocytes, respectively. As expected, compound **1**, prepared as described herein and by total synthesis,[4] had

identical anticholinesterase activity (with IC_{50}, concentration required to inhibit 50% enzyme action, values for BChE and AChE being 1.0 and 110 nM, respectively).

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13. Compound **5** (pale brown crystal): mp 88–92°C; $[\alpha]_D^{22} = -42.2$ ($c = 0.102$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): 1.35 (s, 1/3 3H, *C*3a-CH_3), 1.41 (s, 2/3 3H, *C*3a-CH_3), 1.91–2.04 (m, 2H, H-3), 2.40–2.75 (m, 2H, H-2), 3.74 (s, 3H, *O*-CH_3), 3.85–4.15 (m, 2H, CH_2Ph), 4.86 (s, 2/3H, H-8a), 5.22 (s, 1/3 H, H-8a), 6.61–6.71 (m, 2H), 8.12 (s, 2/3H, *N*-CHO), 8.82 (s, 1/3H, *N*-CHO); HRMS: calcd: 322.1681 (M^+), found: 322.1679.
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15. Compound **6** (pale yellow syrup): $[\alpha]_D^{22} = -201.4$ ($c = 0.209$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): 1.32 (s, 3H, *C*3a-CH_3), 1.63–1.71 (m, 1H, H-3a), 1.85–1.96 (m, 1H, H-3b), 2.20–2.31 (m, 1H, H-2a), 2.15–2.24 (m, 1H, H-2b), 3.80 (d, J = 13.4 Hz, 1H, *N*-CH_2Ph), 4.21 (d, J = 13.4 Hz, 1H, *N*-CH_2Ph), 5.02 (s, 1H, H-8a), 6.12 (d, J = 1.7 Hz, 1H, H-4), 6.54 (dd, J = 1.7, 20 Hz, 1H, H-6); ^{13}C NMR (75 MHz, $CDCl_3$): 23.30, 39.79, 49.70, 50.90, 54.42, 101.47, 121.65, 126.91, 128.19 (2C), 128.51 (2C), 133.37, 135.59, 138.85, 160.84, 165.63, 167.87; UV (CH_2Cl_2): $\lambda_{max} = 268$ nm, $\lambda_{max} = 330$ nm; HRMS: calcd: 278.1419 (M^+), found: 278.1421.
16. Compound **7** (foam): $[\alpha]_D^{22} = -224.1$ ($c = 0.054$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): 1.39 (s, *C*3a-CH_3), 1.86–1.94 (m, 2H, H-3), 2.58–2.73 (m, 2H, H-2), 3.71–3.84 (m, 2H, CH_2Ph), 4.46 (s, 1H, H-8a), 6.40–6.53 (m, 3H, H-4, H-6 and H-7); HRMS: calcd: 280.1576 (αM^+), found: 280.1578.
17. Compound **8** (syrup): ^{1}H NMR (300 MHz, $CDCl_3$): 1.40 (s, 1/3 3H, *C*3a-CH_3), 1.45 (s, 2/3 3H, *C*3a-CH_3), 1.91–2.04 (m, 2H, H-3), 2.40–2.75 (m, 2H, H-2), 3.85–4.21 (m, 2H, CH_2Ph), 4.92 (s, 2/3H, H-8a), 5.34 (s, 1/3H, H-8a), 6.61–6.71 (m, 2H), 8.20 (s, 2/3H, *N*-CHO), 8.89 (s, 1/3H, *N*-CHO).
18. Compound **9** (foam): $[\alpha]_D^{22} = -157.3$ ($c = 0.066$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): 1.25 (d, J = 6.8 Hz, 6H, $(CH_3)_2$CH), 1.46 (s, 3H, *C*3a-CH_3), 1.98–2.11 (m, 2H, H-3), 2.70–2.98 (m, 3H, H-2 and $CHMe_2$), 3.80–3.90 (m, 2H, CH_2Ph), 4.54 (s, 1H, H-8a), 6.50–6.90 (m, 2H); HRMS: calcd: 441.2416 (M^+), found: 441.2418. Fumarate of compound **9**: white crystal, mp 137–139°C.