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Chemical and pharmacological characterization of galanthamine, an acetylcholinesterase inhibitor, and its derivatives. A potential application in Alzheimer's disease?

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Summary — We conducted structural and pharmacological studies of galanthamine, a cortical acetylcholinesterase (AChE) inhibitor, and 19 structural analogs. Systematic derivatization of galanthamine at the cyclohexene ring, tertiary amino, hydroxyl and methoxyl functions indicated that these structural features are essential for biological activity. Molecular modeling studies suggested that the low energy conformations of the analogs are similar to that of the parent. One derivative, galanthamine n-butyl carbamate, had an LD_{50} of over 100 mg/kg (ip) in mice. In a passive avoidance paradigm, this analog improved performance in a dose-dependent fashion with a peak effect at 0.1 mg/kg in control and 0.5 mg/kg in basal forebrain lesioned mice. In the same paradigm, the peak effect of the parent compound is a 6-fold higher dose. With this surprinsingly high therapeutic ratio, this compound may be of interest in treating cholinergic deficits of the central nervous system such as Alzheimer's disease.

galanthamine derivatives / molecular modeling / acetylcholinesterase inhibitor / Alzheimer's disease / passive avoidance / basal forebrain lesion

Introduction

Alzheimer's disease is the fourth leading cause of death among the elderly, and this devastating illness appears to be on the increase [1]. Palliative therapy, cure, or prevention of this disease are being actively investigated. One of the more promising palliative approaches relates to potentiating the activity of the central cholinergic system [2]. A decrease in central nervous system cholinergic markers is the most consistent and well-documented neurochemical change in Alzheimer's disease [2–5]. Accordingly, several pharmacological strategies to enhance central cholinergic function are being explored: muscarinic agonists [6], acetylcholine releasing agents [7], and cholinesterase inhibitors [8].

A reasonable therapeutic approach is to treat Alzheimer's disease with cholinesterase inhibitors [9]. However, few of these inhibitors can pass into the brain and remain effective without severe side effects. Physostigmine is the most widely studied cholinesterase inhibitor [10–12]. While this drug can produce memory improvement in a research setting, the clinical application of physostigmine is not without problems [13]. Another drug, tacrine (tetrahydro-9-aminoacridine, also known as THA), although less potent than physostigmine, has been reported to be more useful in the treatment of Alzheimer's disease [14]. It produces improvements in orientation, global assessment, and name-learning tests, however it can also cause undesirable side effects [15–19]. Structure–activity studies of acetylcholinesterase inhibitors have been conducted in order to design active pharmacological agents without severe side effects. Heptylphysostigmine, a modified physostigmine, shows some effect on passive avoidance learning, but produces greater inhibition in plasma than in brain, suggesting that its peripheral effects will be as great as those of physostigmine [9]. A new tacrine derivative, racemic 9-amino-1,2,3,4-tetrahydroacridin-1-ol maleate, is currently in phase II clinical trials [20].

Galanthamine (**1**, scheme 1), a long-acting, centrally-active competitive cholinesterase inhibitor, has shown considerable promise [21–23]. This natural

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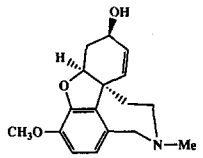
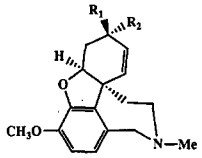


(1)



a, 96% → (2), $R_1 = OCOCH_3$, $R_2 = H$

b, 50% → (3), $R_1 = H$, R_2, $H = OCOCH_3$

c, 100% → (4), $R_1 = OOS(i\text{-}Pr)_3$, $R_2 = H$

d, 100% → (5), $R_1 = OCONHC_4H_9$, $R_2 = H$

e, 80% → (6), $R_1 = OCONHC_6H_5$, $R_2 = H$

f, 60% → (7), $R_1 = OCONHC_{10}H_7$, $R_2 = H$

g, 86% → (8), R_1, $R_2 = O$

g, h, 55% → (9), R_1, $R_2 = NNHCONH_2$

i, 53% → (10), $R_1 = H$, $R_2 = Cl$

[a]Ac_2O, DMAP, CH_2Cl_2, 0°C to rt; [b]Ph_3P, DEAD, glacial HOAc, THF, 0°C to rt; [c]$(iPr)_3SiOTf$, 2,6-lutidine, CH_2Cl_2, 0°C to rt; [d]*n*-BuN = C = O, THF, reflux; [e]PhN = C = O, THF, reflux; [f]1-$C_{10}H_7N$ = C = O, THF, reflux; [g]PCC, CH_2Cl_2, rt; [h]$H_2NCONHNH_2$·HCl, NaOAc, EtOH, H_2O, rt to 100°C; [i]*p*-TsCl, pyridine, CH_2Cl_2, 0°C to rt.

Scheme 1.

product, an alkaloid of the *Amaryllidaceae* family, is hydrolysis-resistant, only moderately toxic, and more readily absorbed than physostigmine [24]. The animal data suggest that this compound might be effective in treating the central cholinergic deficits in Alzheimer's disease. A recent clinical study found that **1** was a well-tolerated drug during long-term treatment [25]. Some patients showed improvement in almost all tests (short-term and long-term memory, speed-attention capacity, etc) and in the clinical rating scales. The potential therapeutic importance of **1** led us to undertake a systematic study of its solid-state and solution conformations [26]. Computer-assisted molecular modeling techniques were then used to determine and compare the lowest energy conformations of **1** and to compare the results with those obtained from ^1H NMR NOE studies, and a single crystal X-ray analysis. These studies afforded valuable information on the structural and conformational features essential to the biological activity. To further clarify how galanthamine interacts with AChE, we conducted structure-activity studies. The structural studies of galanthamine led to the selection of four sites to be modified. A series of structural analogs of **1** was synthesized and tested for AChE inhibition. The *in vitro* studies indicated that the potency of 1 of these analogs was similar to that of the parent compound. This analog was more lipophilic, and therefore potentially more accessible to the central nervous system. Consequently, preliminary behavioral screening was carried out on this derivative.

Chemistry

There are four sites which may contribute to the receptor-binding ability of galanthamine. These sites may affect the spatial arrangement of the molecule, its hydrogen-bonding properties, or its polarity. To better understand the influence of these sites on the competitive binding of **1** to the AChE molecule, we decided to derivatize the molecule in a systematic way at the hydroxyl function of the cyclohexene ring, at the cyclohexenol ring, at the tertiary amine site, and at the methoxyl group of the benzofuran ring.

Modifications of the hydroxyl function

The role of the postulated intramolecular hydrogen bonding in galanthamine and its salts has been a subject of interest as it has been accepted that such a bond has a major role in determining the biological activity. As this electrostatic attraction requires the presence of a hydroxyl group in an appropriate configuration, we decided to carry out several modifications on this functionality to examine the results of such changes on cortical AChE inhibition.

Modifications of the cyclohexenol ring

An obvious derivatization of galanthamine was the conversion of its rigid structure to a more flexible one, a modification that would favor intermolecular hydrogen bonding over intramolecular bonding. This change was accomplished by reducing the double bond of the cyclohexene ring.

Modifications at the tertiary amine site

Both demethylation and quaternization of the tertiary amine function were important modifications. Demethylation afforded an alternative site for hydrogen

bonding, and quaternization changed the properties of galanthamine by producing increased polarity, and decreased solubility in organic solvents. While these modifications reduce these derivatives' potential as centrally active AChE inhibitors, they provided critical information regarding the importance of different polar groups of galanthamine on the binding to the AChE molecule.

Modification of the methoxyl function

O-Demethylation of galanthamine was one of the most important modifications carried out on this compound as it introduced a new site for hydrogen bonding. The hydrogen of the phenolic hydroxyl function is closer to the furan oxygen than the hydrogen attached to the hydroxyl function on the cyclohexene ring.

Molecular modeling studies

To confirm the results of our previous structural studies [26] and to further ascertain the importance of hydrogen bonded conformers, we carried out molecular mechanics calculations on **1** and selected derivatives. The rationale used for the choice of compounds to be modeled was the same as that used for the synthesis of derivatives. The compounds modeled were galanthamine (**1**) and the following derivatives:

1) Acylated derivatives of the allylic alcohol: galanthamine acetate (**2**), 3-α-acetoxygalanthamine (**3**), *n*-butyl (**5**), phenyl (**6**), and α-naphthyl (**7**) carbamates. These compounds are unlikely to achieve intramolecular hydrogen bonding.

2) 1,2-Dihydro derivatives: lycoramine (**13**) and 3-amino-3-dehydroxy-1,2-dihydrogalanthamine (**17**). The flexibility of these derivatives favors intermolecular hydrogen bonding.

3) N-Demethylated derivatives: N-demethylgalanthamine (**22**). Demethylation increases the polarity of the molecule and increases intermolecular hydrogen bonding.

4) O-Demethylated derivatives: O-demethylgalanthamine (**21**). This modification introduces an alternate site for intramolecular hydrogen bonding.

Pharmacology [27]

The biological activities of galanthamine and 19 of its structural analogs were examined. Since we were interested in the potential use of these compounds as therapeutic agents in central cholinergic disorders such as Alzheimer's disease, we chose to examine the ability of these compounds to inhibit cortical AChE activity.

Based on the results of the *in vitro* biological activity studies, one of these compounds was chosen for preliminary behavioral analysis. The compound selected, galanthamine *n*-butyl carbamate (**5**), had a similar potency to that of the parent compound (85% inhibition of AChE, as compared to galanthamine) and was less polar than the parent. Theoretically, this reduced polarity would result in a greater ability to cross the blood-brain barrier following an intraperitoneal (ip) administration and hence increase the activity of this compound in the central nervous system with respect to the parent. The general toxicity of **5** was assessed following increasing ip doses. The behavioral activity of **5** was examined on passive avoidance (acquisition/retention) paradigm in control mice and mice that had received a lesion in the basal forebrain (BF). BF lesions in experimental animals replicate some of the neurochemical and cognitive deficits seen in Alzheimer's disease. Additionally, this lesion model is sensitive to attenuation by AChE inhibitors such as physostigmine [11, 12] and galanthamine [21, 23]. Dose-response curves for both control and BF-lesioned mice were determined for the galanthamine analog.

Results and discussion

Chemistry

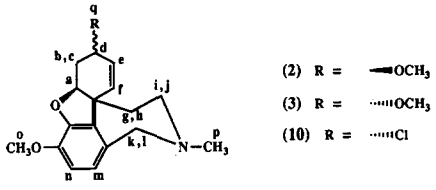
Previous structural studies of galanthamine (**1**) were carried out both in solution and in the solid state, using spectroscopic methods (IR, ^{1}H NMR, ^{13}C NMR, and X-ray analysis) [26]. In the solid state, galanthamine showed no evidence of intramolecular hydrogen bonding either by X-ray crystallography or IR (KBr disk). The X-ray analysis of galanthamine clearly showed intermolecular hydrogen bonding. In solution, in polar solvents such as DMSO-d_6, galanthamine exhibited external hydrogen bonding to the solvent, as shown by ^{1}H NMR temperature studies [26]. In nonpolar solvents (CCl_4), galanthamine showed intramolecular hydrogen bonding, as observed in IR dilution studies, and in accordance with the results of Barton and Kirby [28]. A ^{1}H NMR study of a natural sample of galanthamine, in $CDCl_3$, supported the infrared results [29]. In solution, the polarity of the environment determines the type of hydrogen bonding that occurs.

The derivatization of galanthamine was carried out as follows. The hydrobromide salt of natural galanthamine (Nivalin) (available from Pharmachim State Economie Association, 16 Iliensko, Chaunsee, Sofia, Bulgaria) was neutralized with ammonium hydroxide to produce pure galanthamine (**1**) in 85% yield. As shown in scheme 1, galanthamine (**1**) was treated with acetic anhydride (Ac_2O) and 4-N,N-dimethylaminopyridine (DMAP) in dichloromethane (CH_2Cl_2) to produce compound **2** in 96% yield. 3-α-Acetoxygalanthamine (**3**) was synthesized from galanthamine under Mitsunobu reaction conditions [30] with triphenylphosphine (Ph_3P), diethyl azodicarboxylate (DEAD), and glacial acetic acid (AcOH) in 50% yield. Conversion of the hydroxyl group to a bulky silyl protected derivative (**4**) was achieved in quantitative yield by treatment with triisopropylsilyl triflate

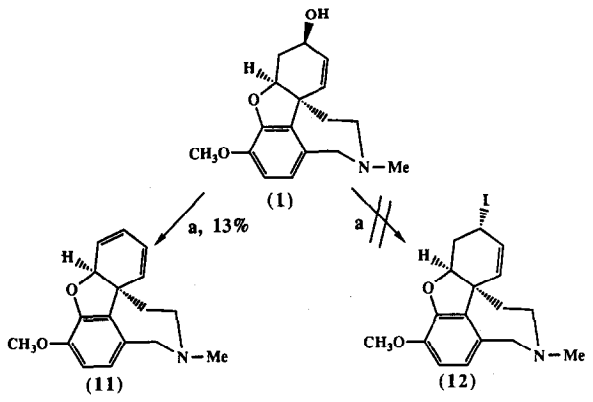
and 2,6-lutidine in dichloromethane. Galanthamine was also derivatized to its corresponding carbamates (**5-7**) by treatment with *n*-butyl, phenyl, and α-naphthyl isocyanate in tetrahydrofuran (THF), respectively, in good yields. Oxidation of the hydroxyl group of **1** with pyridinium chlorochromate (PCC) in dichloromethane resulted in narwedine (**8**) in 86% yield. Further treatment of compound **8** with semicarbazide hydrochloride afforded the corresponding semicarbazone (**9**) in 64% yield. Treatment of **1** with *p*-toluenesulfonyl chloride (*p*-TsCl) and pyridine (Py) in dichloromethane did not afford the expected tosylate but produced chloride **10** by the S_N2 displacement of the initially formed tosylate by chloride ion. Extensive ^1H NMR studies were conducted to confirm the structure of compound **10** using homonuclear decoupling, NOE difference and two dimensional COSY experiments. The ^1H NMR data of compound **10** were also compared with those of compounds **2** and **3** (table I) for further support of its structure. At first the ^1H NMR data of compound **2** were compared with those of its epimer **3** which was produced using the Mitsunobu reaction. Since the Mitsunobu conditions were known to give inversion of configuration [30], the ^1H data comparison of compounds **3** and **10** were then used to support the stereo- and regio-chemistry of **10** as seen in table I. Attempts to convert the hydroxyl group into an iodo group [31] using triphenylphosphine and triiodoimidazole [Im(I)$_3$] in toluene resulted in dehydration and produced diene derivative **11** in low yield as shown in scheme 2. The expected iodo derivative (**12**) could not be obtained.

In another transformation (scheme 3), galanthamine (**1**) was reduced to lycoramine (**13**) by treatment with 10% palladium on carbon as a catalyst under a hydrogen atmosphere, in 93% yield. After reduction, the molecule was further functionalized. The acetate (**14**) was obtained under standard reaction conditions from compound **13** in 89% yield. The iodide (**15**) was produced from lycoramine (**13**) by treatment with triphenylphosphine and triiodoimidazole in 35% yield. Compound **15** was converted to the azide (**16**) by S_N2 displacement with sodium azide (NaN_3) in dimethylformamide (DMF) in 98% yield, and further

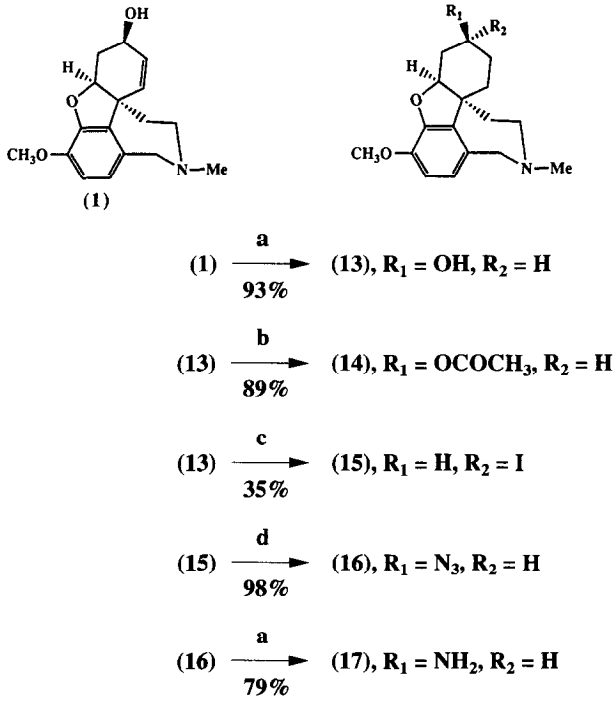
Table I. ^1H NMR data comparison for compounds **2**, **3** and **10**.



	(2)	(3)	(10)
H_a	4.58 (m, 1H)	4.63 (m, 1H)	4.57 (m, 1H)
H_b	2.09 (ddd, 1H, J = 16.3, 5.6, 3.5 Hz)	1.82 (m, 1H)	2.09 (ddd, 1H, J = 13.8, 10.7, 2.4 Hz)
H_c	2.69 (br d, 1H, J = 15.0 Hz)	2.83 (ddd, 1H, J = 18.0, 9.6, 4.3 Hz)	2.97 (m, 1H)
H_d	5.34 (br t, 1H, J = 5.2 Hz)	5.65 (m, 1H)	4.86 (ddd, 1H, J = 10.8, 3.9,1.9 Hz)
H_e	5.92 (dd, 1H, J = 10.3, 4.8 Hz)	5.79 (d, 1H, J = 10.4 Hz)	5.88 (br d, 1H, J = 10.4 Hz)
H_f	6.25 (d, 1H, J = 10.3 Hz)	6.07 (d, 1H, J = 10.3 Hz)	6.06 (d, 1H, J = 10.4 Hz)
H_g	1.66 (m, 1H)	1.80 (m, 1H)	1.74 (br dd, 1H, J = 14.0, 2.1 Hz)
H_h	2.11 (m, 1H)	2.23 (br t, 1H, J = 13.4 Hz)	2.19 (dt, 1H, J = 13.6, 2.7 Hz)
H_i	3.13 (m, 1H)	3.19 (m, 1H)	3.11 (br d, 1H, J = 14.4 Hz)
H_j	3.40 (m, 1H)	3.43 (m, 1H)	3.31 (br t, 1H, J = 13.2 Hz)
H_k	3.77 (br d, 1H, J = 14.9 Hz)	3.80 (d, 1H, J = 14.9 Hz)	3.70 (d, 1H, J = 15.1 Hz)
H_l	4.22 (br d, 1H, J = 15.0 Hz)	4.24 (d, 1H, J = 15.1 Hz)	4.11 (d, 1H, J = 15.1 Hz)
H_m	6.60 (d, 1H, J = 8.1 Hz)	6.68 (d, 1H, J = 8.2 Hz)	6.60 (d, 1H, J = 8.2 Hz)
H_n	6.68 (d, 1H, J = 8.1 Hz)	6.62 (d, 1H, J = 8.2 Hz)	6.66 (d, 1H, J = 8.2 Hz)
H_o	3.85 (s, 3H)	3.86 (s, 3H)	3.85 (s, 3H)
H_p	2.45 (s, 3H)	2.47 (s, 3H)	2.42 (s, 3H)
H_q	2.04 (s, 3H)	2.08 (s, 3H)	–



Scheme 2. [a]Ph_3P, $Im(I)_3$, toluene, reflux.



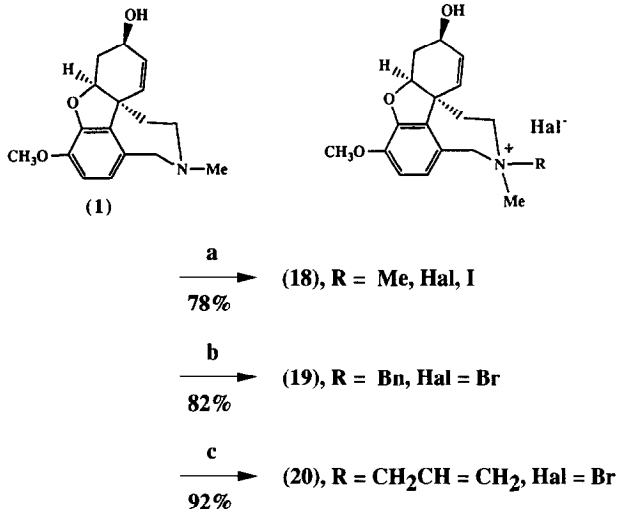
Scheme 3. [a]10% Pd/C, H_2, MeOH, rt; [b]Ac_2O, DMAP, CH_2Cl_2, 0°C to rt; [c]Ph_3P, $Im(I)_3$, toluene reflux; [d]NaN_3, DMF, 50°C.

reduced to the amine (**17**) by treatment with 10% palladium on carbon under a hydrogen atmosphere in 79% yield.

Quaternization of the tertiary amine function was carried out as shown in scheme 4. Galanthamine (**1**) was converted to its corresponding salts (**18-20**), in high yields, with methyl iodide, benzyl bromide, and allyl bromide, respectively. Selective N-demethylation of **1** and some of its derivatives could not be accomplished under a variety of conditions. The reason for this failure may be explained by the presence of benzylic nitrogen in the tetrahydroazepine ring. It is well-known, from dealkylation experiments, that the relative ease of removal of different groups is in the order: N-benzyl > N-allyl > N-cyclohexyl > N-alkyl, in agreement with their ability to stabilize electron-deficiency [32]. Therefore the N-demethylation of galanthamine is not a viable route to N-demethylgalanthamine (**22**). We obtained **22** from a different source. Compound **22** was purified by preparative thin layer chromatography on 0.05 nm precoated silica plates, using a 1:1 MeOH:Me_2CO solvent mixture.

We attempted O-demethylation using *in situ* generated trimethylsilyl iodide in acetonitrile, or 48% aqueous hydrobromic acid, but these protocols were not successful (scheme 5). O-Demethylation of lycoramine (**13**) with the same reagents also failed. The successful O-demethylation of galanthamine was carried out with boron tribromide in 31% yield, under the reaction conditions developed by Rice [33] convert codeine to morphine.

Molecular mechanics calculations allowed us to compare the low energy conformers of the galanthamine derivatives. These results confirmed that the conformations of the tetracyclic portions of these molecules were similar. In most cases, the tetrahydroazepine ring is puckered below the plane of the



Scheme 4. [a]CH_3I, Et_2O, rt; [b]BnBr, Et_2O, rt; [c]CH_2 = $CHCH_2Br$, Et_2O, rt.

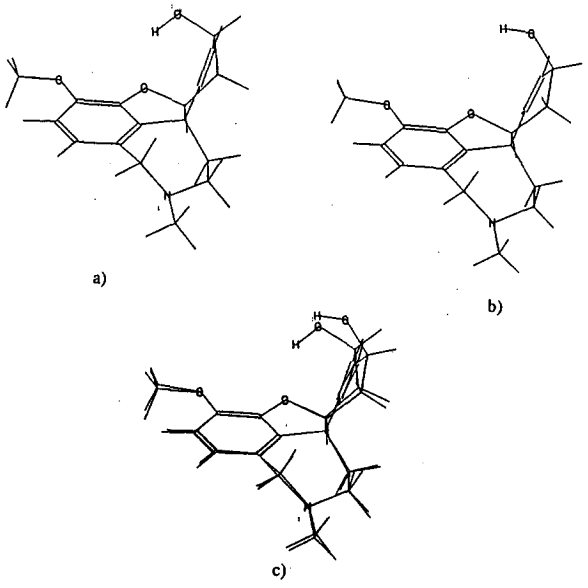
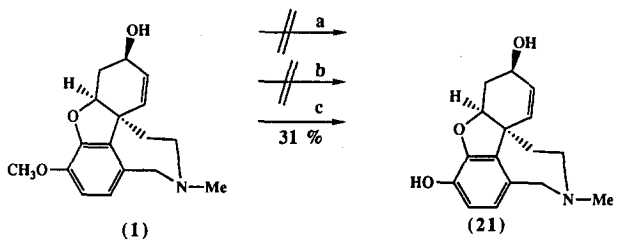


Fig 1. a) Computer-minimized structure of galanthamine (1a); b) X-ray structure of galanthamine (1b); c) superimposition of a) and b).



Scheme 5. [a]TMS-Cl, NaI, CH_3CN, rt to reflux; [b]48% HBr, 140°C; [c]BBr_3/CH_2Cl_2, $CHCl_3$, 0°C to rt; NH_4OH, 0°C.

benzofuran ring, while the allylic alcohol lies above it (**1a**, fig 1). The flexibility of the side chains of the acylated derivatives (acetate and various carbamates) permits a large number of accessible low-energy conformations.

The lowest energy conformer of **1** exhibited a geometry that agreed with the results of the NOE studies and X-ray data we had previously obtained [26]. The conformational differences exhibited between the energy-minimized structure of **1** (**1a**), and the X-ray

Table II. Structure–activity relationship of galanthamine analogs.

Compound	*Number*	*Percent cholinesterase Inhibition (at 10^{-5} M)*
O-Demethylgalanthamine	**21**	100+
10-Benzylgalanthaminium bromide	**19**	100+
Galanthamine methiodide	**18**	97
10-Allylgalanthaminium bromide	**20**	96
Galanthamine	**1**	93
N-Demethylgalanthamine	**22**	92
Galanthamine *n*-butylcarbamate	**5**	75
3-Deoxy-3-chlorogalanthamine	**10**	73
Galanthamine α-naphthyl carbamate	**7**	60
Galanthamine phenyl carbamate	**6**	37
Galanthamine acetate	**2**	32
3,4-Didehydro-3-deoxygalanthamine	**11**	31
1,2-Dihydrogalanthamine (lycoramine)	**13**	30
3-Azido-3-deoxy-1,2-dihydrogalanthamine	**16**	25
3-α-Acetoxygalanthamine	**3**	21
O-Triisopropylsilylgalanthamine	**4**	17
3-Amino-3-dehydroxy-1,2-dihydrogalanthamine	**17**	17
3-Deoxy-1,2-dihydro-3-iodogalanthamine	**15**	15
Galanthaminone (narwedine)	**8**	10
3-[(Aminocarbonyl)hydrazono]-3-deoxygalanthamine	**9**	0

Table III. IC_{50}'s for most potent galanthamine analogs.

Compound	*Number*	*IC_{50} ± SEM*	*$(10^{-7}$ M)[a] $K_i(10^{-7}$ M)[b]*
O-Demethyl-galanthamine	**21**	0.78 ± 0.1	0.17
10-Benzyl-galanthaminium bromide	**19**	1.76 ± 0.7	0.38
Galanthamine methiodide	**18**	2.8 ± 0.3	0.61
Galanthamine	**1**	3.97 ± 0.9	0.86
N-Demethyl-galanthamine	**22**	7.9± 0.1	1.72
Galanthamine *n*-butylcarbamate	**5**	10.9 ± 0.1	2.37
3-Deoxy-3-chlorogalanthamine	**10**	17.6 ± 1.1	3.83

[a][46], [b][46] (analogues assumed to be competitive inhibitors, similar to the parent compound)

determination (**1b**) appear in the cyclohexenol portion of the molecule (fig 1). These differences are shown in the superimposition of **1a** and **1b** as seen in **1c** (fig 1). These discrepancies are due to the manner in which the modeling software executes the minimization (the molecule is assumed to be *in vacuo*). The molecular modeling studies showed that, in general, the low energy conformations of the galanthamine derivatives had a very similar orientation in space as the galanthamine low energy conformer. This similarity suggests that a common ring conformation may be essential but not sufficient to retain the biological activity of the molecule.

In vitro structure–activity studies [27]

Twenty compounds, including galanthamine and its derivatives, were examined using *in vitro* AChE inhibition assays. To determine the relative potencies of the various compounds to inhibit cortical AChE, all the compounds were initially screened at a concentration of 10^{-5} M. The structure-activity relationship of the galanthamine analogs is summarized in table II. For those compounds that exhibited the greatest inhibition in the initial screening assay, full dose-response curves were obtained which consisted of 4–5 points in the range of 10^{-9}–10^{-5} M. IC_{50} values (the concentration of the drugs that inhibited 50% of AChE activity) were estimated (table III) and compared to the IC_{50} value for the parent compound. The IC_{50} values for these most potent compounds ranged from 0.78 10^{-7} M for O-demethylgalanthamine to 17.6 10^{-7} for 3-deoxy-3-chlorogalanthamine. The IC_{50} value reported here for galanthamine (3.97 10^{-7} M) is consistent with those previously reported [22]. Representative dose-response curves for these most potent analogs are shown in figure 2.

Modifications at the methoxyl group and at the tertiary amine function produced more polar compounds that showed greater enzyme (AChE) inhibitory activity than galanthamine. These products (**18-21**) were either quaternary amine salts or hydrophilic derivatives, which would prevent CNS penetration. While these compounds would not be relevant clinically, these studies highlighted the importance of these two functional groups on the binding of the derivatives to the AChE molecule and consequent biological activity. Alterations of the hydroxyl group to its corresponding ester, carbamates, and chloride (**2**, **5-7**, **10**) resulted in a slight loss of activity. Derivatization of the hydroxyl group to its epimeric acetoxyl group (**3**), bulky silyl group (**4**), ketone (**8**), or semicarbazone (**9**) resulted in a severe decrease of the drug's ability to inhibit cortical AChE. A similar loss of activity was observed when the cyclohexenol moiety was converted either to the 3,4-didehydro-3-deoxy (**11**) or cyclohexanol (**13**) derivatives. Conversion of the hydroxyl group of lycoramine to its

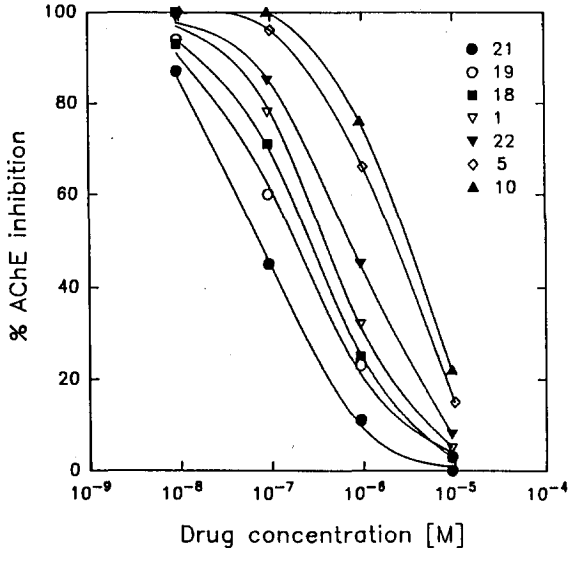


Fig 2. Dose-response curves for galanthamine and its most potent analogs: drug concentrations (in M) *vs* % inhibition of AChE with respect to controls.

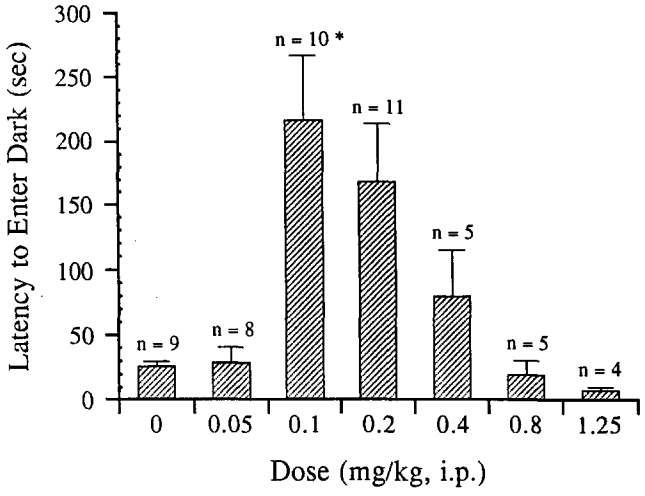


Fig 3. Compound **5** injected 3.5 h before acquisition improves the 24-h retention of control mice on a passive avoidance task. Mean scores (± SEM) and the number of subjects per dose are indicated. The latencies varied significantly with the drug dose (F = 5.3, P = 0.0004*) and the 0.1 mg/kg dose was significantly better than other doses (Scheffe's F-test = 2.45, $P < 0.05$).

epimeric iodide (**15**), azide (**16**), or amine (**18**) decreased the inhibitory activity as compared to lycoramine, suggesting that the C-O bond is partially responsible for the AChE inhibitory activity, but that the H-bonding ability (such as in **18**) is less important.

The *in vitro* studies show that the overall configuration of the tetracycle is important for AChE inhibition as evidenced by the drastic decrease in activity which occurs when the cyclohexene ring is reduced. In general, *in vitro* AChE inhibitory activity increases with an increase in the overall polarity of the molecule. The cyclohexenol sp^3 center in the proper configuration promotes activity. The structure–activity studies suggest that properly placed hydrophilic and lipophilic groups contribute to the effective binding of galanthamine to the AChE molecule. Since galanthamine is a competitive inhibitor of AChE [34, 35], we assume that these derivatives function as competitive inhibitors based on their conformational similarities calculated by molecular mechanics.

In vivo testing of galanthamine n-butyl carbamate

The *in vitro* studies indicated that the potency of AChE inhibition of one of these analogs, compound **5**, was 85% of that of the parent compound. The *n*-butylcarbamate derivative (**5**) was particularly interesting, because it was less polar than galanthamine and theoretically should cross the blood-brain barrier more readily following intraperitoneal (ip) administration. Therefore, *in vivo* studies were conducted on compound **5**.

Following ip administration of compound **5** at doses ranging from 0.1–30 mg/kg, no visible side effects were seen in the mice. At 50–100 mg/kg, the mice were wobbly and off balance with rapid heart rate beginning at 30 min and continuing until 4 h after drug administration. At doses up to 100 mg/kg, no lethality was observed and there were no visible changes in the animals' behavior or activity 24 h after drug administration. Galanthamine *n*-butylcarbamate is therefore considerably less toxic than other well-known centrally-active AChE inhibitors, such as physostigmine (LD_{50} = 4.5 mg/kg, ip) and galanthamine (LD_{50} = 10 mg/kg ip) [27].

Intraperitoneal administration of galanthamine *n*-butylcarbamate given 3.5 h before acquisition of the passive avoidance task improved performance of both control and BF lesioned mice in a dose-dependent fashion (figs 3 and 4 respectively). In control mice the optimal dose was 0.1 mg/kg and in the lesioned mice the optimal dose was 0.5 mg/kg. The inverted U-shaped dose-response curves seen in the control mice and suggested in the lesioned group are reminiscent of curves seen in BF-lesioned animals after administration of physostigmine [36, 37] and galanthamine [20]. Additionally, the shift to the right in the dose-response curve seen in the BF-lesioned mice (as compared to control) is consistent with a reduced choliner-

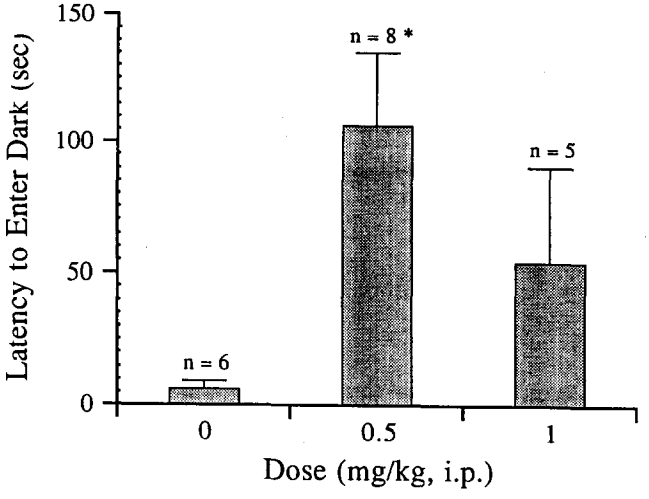


Fig 4. Compound **5** injected 3.5 h before acquisition improves the 24-h retention of BF-lesioned mice on a passive avoidance task. Mean scores (± SEM) and the number of subjects per dose are indicated. The latencies varied significantly with drug dose (F = 3.82, P = 0.041*) and the 0.5 mg/kg dose was significantly better than other dose (Scheffe's F-test = 3.88, $P < 0.05$).

gic innervation to the cortex in the BF-lesioned mice. It is interesting to note that the optimal dose of galanthamine *n*-butylcarbamate, (0.5 mg/kg in BF-lesioned mice: findings of this study), is 6-fold less than that of galanthamine (3 mg/kg in BF-lesioned mice: findings of Sweeney *et al*, 1990). Galanthamine *n*-butyl carbamate therefore has a remarkable therapeutic ratio (optimal effective dose/LD_{50}) that is over 200 in the BF-lesioned mice as compared to the lower therapeutic ratios for physostigmine (2.0) and galanthamine (6.6). Furthermore, galanthamine *n*-butyl carbamate administered 3.5 h before the acquisition trial improved performance of both control and BF-lesioned mice. This finding suggests that this compound may have a relatively long half-life as does galanthamine. Further studies on the pharmacokinetics and potential clinical relevancy of this compound are of considerable interest.

Conclusion

We have examined the structure–activity relationships of galanthamine and 19 of its derivatives. We systematically modified four different sites of the parent molecule. Molecular mechanics calculations showed that the low-energy conformations of galanthamine and its derivatives exhibited rigid ring connections and flexible side chains. The biological activities of a number of the derivatives may be due, in part, to structural similarities to the parent compound. Severe losses of biological activity resulted from altering the polarity of the hydroxyl group and changing the cyclohexenol ring structure. From all of the compounds tested, 1 was chosen for *in vivo* behavioral studies in mice because of its *in vitro* biological activity and relatively lower polarity (with an expected ability to enter the central nervous system). Galanthamine *n*-butylcarbamate was behaviorally active and improved performance of a passive avoidance task in a dose-dependent manner in both control and basal forebrain lesioned mice. Furthermore, this analog exhibited extremely low toxicity. This derivative, and potentially other analogs that have not yet been behaviorally tested, may be useful in augmenting central cholinergic function. A centrally-active, safe, long-acting AChE inhibitor could be of great clinical usefulness in conditions such as Alzheimer's disease.

Experimental protocols

Chemical synthesis: general

All solvents were reagent grade. Anhydrous ether (Et_2O), tetrahydrofuran (THF), benzene, and toluene were distilled from sodium/benzophenone. Dichloromethane (CH_2Cl_2) and dichloroethane were distilled from calcium hydride (CaH_2). Acetonitrile (MeCN) and N,N-dimethylformamide (DMF) were distilled from phosphorus pentoxide (P_2O_5). Methanol (MeOH) was distilled from either CaH_2 or I_2/Mg. Organic bases were reagent grade. 2,6-Lutidine and pyridine were distilled from CaH_2. Organic acids were reagent grade. Glacial acetic acid was distilled before use. Acetic anhydride (Ac_2O) was dried over P_2O_5 and then distilled. Methyl iodide (MeI) was distilled before use. Analytical thin layer chromatography (TLC) was performed on Merck silica gel (60 F 254) plates (0.25 mm), precoated with a fluorescent indicator. Visualization was effected with ultraviolet light, ninhydrin (3% w/v) in 95% ethanol containing 2% acetic acid, or phosphomolybdic acid (7% w/v) in 95% ethanol. Flash-column chromatography was carried out on Merck silica gel (60 particle size 0.040–0.063 mm). Melting points (mp) were determined with either a Thomas Hoover capillary melting point apparatus or a Fisher-Johns apparatus. They are expressed in degrees centigrade (°C), and are uncorrected. Proton and carbon magnetic resonance spectra (^1H, ^{13}C NMR) were recorded on either an IBM NR/250 AF (250 MHz) or a Bruker AM-500 (500 MHz) Fourier transform spectrometer, using $CDCl_3$, MeOD-d_4, or D_2O as solvents. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS) or chloroform as an internal standard. 3-(Trimethylsilyl)-1-propane sulfonic acid sodium salt hydrate was the internal standard for deuterium oxide. Coupling constants (*J* values) are in Hertz (Hz). Multiplicities are designated as singlet (s), doublet (d), triplet (t), multiplet (m), or broad (br). Infrared spectra (IR) were obtained on a Perkin-Elmer Model 281-B spectrometer. Samples were analyzed as potassium bromide (KBr) disks, or as chloroform solutions in sodium chloride cells. Absorptions are reported in wave numbers (cm^{-1}). The spectra are calibrated against the 1601 cm^{-1} band of a polystyrene film, and only the most prominent or characteristic absorptions were noted. Optical rotations (in degrees, °) were recorded on a Perkin–Elmer Model 241 polarimeter at the sodium D line. Ultraviolet spectra (UV) were obtained on a Perkin-Elmer Model 553 Fast Scan UV/VIS spectrophotometer with a Perkin-Elmer Model R 100A recorder. High-resolution mass spectra (HRMS) were obtained on either a VG 7070HS or a VG ZAB-E high-resolution double-focusing mass spectrometer, using either ammonia Chemical Ionization (CI), or Fast Atom Bombardment (FAB) with a Cesium (Cs) ion gun. The mass spectrometers were interfaced to VG/DEC 11-73 data systems. Elemental analyses were performed by Desert Analytics Organic Microanalysis Laboratory, Tucson, Arizona.

Preparation of galanthamine (1)

Galanthamine hydrobromide (6.80 g, 18.5 mmol) was dissolved in water (400 ml), the solution was cooled to 0°C and ammonium hydroxide (70 ml) was slowly added. The reaction mixture was stirred for 30 min at 0°C, and then for 2 h at ambient temperature. Ether (200 ml) was added, followed by addition of solid NaCl. Two layers were formed. The aqueous layer was extracted with ether (3 x 200 ml). The combined organic layers were washed with saturated sodium chloride solution, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude galanthamine free base was purified by silica gel flash-column chromatography using first acetone, then acetone: methanol (95:5) as eluants to afford pure galanthamine (**1**) as white crystals (4.52 g, 85% yield): TLC (MeOH:Me_2CO, 1:3) R_f 0.31; mp 127–128°C, lit [38] mp 125–126°; $[\alpha]_D^{22}$-131.4° (c 0.63, EtOH), lit [41] $[\alpha]_D^{24}$-114.4° (c 0.67, EtOH); UV (95% EtOH) λ_{max} 288 nm (log ε 3.5), lit [39] UV (EtOH) λ_{max} 288 nm (log ε 3.4); IR (KBr):

3450–3130, 3010, 2950, 2910, 2830, 2800, 1620, 1505, 1455, 1440, 1425, 1405, 1390, 1360, 1275, 1255, 1235, 1220, 1195, 1170, 1115, 1075, 1055, 1040, 1025, 990, 960, 915, 805, 765 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.57 (ddd, 1H, *J* = 13.7, 3.8, 1.6 Hz), 2.00 (ddd, 1H, *J* = 15.7, 5.0, 2.4 Hz), 2.08 (m, 1H), 2.40 (s, 3H), 2.41 (br s, 1H), 2.67 (ddd, 1H, *J* = 15.7, 4.8, 1.4 Hz), 3.04 (br d, 1H, *J* = 14.4 Hz), 3.26 (br dt, 1H, *J* = 13.6, 1.4 Hz), 3.67 (br d, 1H, *J* = 14.9 Hz), 3.82 (s, 3H), 4.08 (br d, 1H, *J* = 15.1 Hz), 4.13 (m, 1H), 4.60 (m, 1H), 5.99 (dd, 1H, *J* = 10.2, 5.0 Hz), 6.06 (d, 1H, *J* = 10.2 Hz), 6.61 (d, 1H, *J* = 8.2 Hz), 6.65 (d, 1H, *J* = 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 30.0, 33.9, 42.1, 48.2, 53.9, 55.9, 60.7, 62.1, 88.7, 111.2, 122.0, 126.9, 127.6, 129.4, 133.1, 144.1, 145.83; HRMS calcd for $C_{17}H_{22}NO_3$ (M^+ + H): 288.1599, found: 288. 1567.

Galanthamine acetate (2)

To a stirred solution of galanthamine (**1**, 0.0700 g, 0.244 mmol) in dry dichloromethane (2.5 ml) was added acetic anhydride (0.030 ml, 0.317 mmol) followed by dimethylaminopyridine (0.0536 g, 0.439 mmol) at 0°C, under an argon atmosphere. The reaction mixture was stirred at 0°C for 10 min and at ambient temperature for 60 min. The solvent was removed on a rotary evaporator, and the residue diluted with ethyl acetate (5 ml). The solution was washed successively with water (5 ml), 10% sodium carbonate solution (5 ml), and saturated sodium chloride solution (5 ml). The organic layer was dried (Na_2SO_4), evaporated under reduced pressure, and the crude product purified by silica gel flash-column chromatography using acetone, and then methanol: acetone (1:10) as eluants. The pure compound **2** was obtained as white crystals (0.0773 g, 96% yield): TLC (MeOH: Me_2CO, 1:3) R_f 0.33; mp 126–128°C, lit [40] mp 129–130°C; $[\alpha]_D^{22}$-88° (c 0.45, MeOH); IR (KBr) 2980, 2920, 2890, 2840, 1730, 1510, 1450, 1440, 1420, 1375, 1290, 1270, 1250, 1220, 1170, 1155, 1133, 1115, 1050, 1030, 1015, 1000, 970, 925, 905, 810 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.66 (m, 1H), 2.04 (s, 3H), 2.09 (ddd, 1H, J = 16.3, 5.6, 3.5 Hz), 2.11 (m, 1H), 2.45 (s, 3H), 2.69 (br d, 1H, *J* = 15.0 Hz), 3.13 (m, 1H), 3.40 (m, 1H), 3.77 (br d, 1H, *J* = 14.9 Hz), 3.85 (s, 3H), 4.22 (br d, 1H, *J* = 15.0 Hz), 4.58 (m, 1H), 5.34 (br t, 1H, *J* = 5.2 Hz), 5.92 (dd, 1H, *J* = 10.3, 4.8 Hz), 6.25 (d, 1H, *J* = 10.3 Hz), 6.60 (d, 1H, *J* = 8.1 Hz), 6.68 (d, 1H, *J* = 8.1 Hz); HRMS calcd for $C_{19}H_{24}NO_4$ (M^+ + H) 330.1705, found 330.1688.

3-α-Acetoxygalanthamine (3)

To a solution of galanthamine (**1**, 0.120 g, 0.418 mmol) in THF (5 ml), in an ice-water bath and under an argon atmosphere, was added triphenylphosphine (0.143 g, 0.544 mmol) and diethyl azodicarboxylate (0.109 g, 0.100 ml, 0.627 mmol), followed by freshly distilled glacial acetic acid (0.0528 g, 0.060 ml, 0.878 mmol) at 0°C. The reaction mixture was brought to ambient temperature, and stirred for 24 h at this temperature. The resulting mixture was first treated with 5% sodium bicarbonate solution (5 ml), and then saturated sodium chloride solution (5 ml). The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and then purified by silica gel flash-column chromatography using acetone, then acetone: methanol (19:1) as eluants to afford compound **3** as pale yellow crystals (0.0690 g, 50% yield): TLC (MeOH:Me_2CO, 1: 3) R_f 0.36; mp 88–90°C; $[\alpha]_D^{22}$-233.2° (c 0.72, MeOH); IR (KBr) 3040, 2940, 2840, 1735, 1675, 1620, 1505, 1435, 1370, 1280, 1235, 1200, 1165, 1120, 1110, 1065, 1040, 1025, 1000, 970, 795 cm^{-1}; ^1H NMR ($CDCl_3$) 1.80 (m, 1H), 1.82 (m, 1H), 2.08 (s, 3H), 2.23 (br t, 1H, *J* = 13.4 Hz), 2.47 (s, 3H), 2.83 (ddd, 1H, *J* = 18.0, 9.6, 4.3 Hz), 3.19 (m, 1H), 3.43 (m, 1H), 3.80 (d, 1H, *J* = 14.9 Hz), 3.86 (s, 3H), 4.24 (d, 1H, *J* = 15.1 Hz), 4.63 (m, 1H), 5.65 (m, 1H), 5.79 (d, 1H, *J* = 10.4 Hz), 6.07 (d, 1H, *J* = 10.3 Hz), 6.62 (d, 1H, *J* = 8.2 Hz), 6.68 (d, 1H, *J* = 8.2 Hz); HRMS calcd for $C_{19}H_{24}NO_4$ (M^+ + H) 330.1705, found 330.1673.

O-Triisopropylsilylgalanthamine (4)

To a solution of galanthamine (**1**, 0.0800 g, 0.279 mmol) in dichloromethane (4 ml) was added triisopropylsilyl trifluoromethanesulfonate (0.121 g, 0.110 ml, 0.334 mmol), followed by 2,6-lutidine (0.0717 g, 0.080 ml, 0.669 mmol) at 0°C in an ice-water bath, under an argon atmosphere. The reaction mixture was brought to room temperature, stirred for 1 h, and then diluted with ether (20 ml). The ether layer was washed successively with 5% aqueous sodium bicarbonate solution (10 ml), then with saturated sodium chloride solution (10 ml). The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified by silica gel flash-column chromatography using first acetone, then acetone: methanol (19:1) as eluants to give compound **4** as pale green crystals (0.124 g, 100% yield): TLC (MeOH:Me_2CO, 1:3) R_f 0.38; mp 120–122°C; $[\alpha]_D^{22}$-73.2° (c 0.37, $CHCl_3$). IR ($CHCl_3$): 2970, 2950, 2880, 2860, 2500-2100, 1510, 1460, 1440, 1435, 1295, 1280, 1235, 1170, 1135, 1105, 1085, 1065, 1015, 995, 880 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03-1.10 (m, 21H), 1.60 (m, 1H), 2.12 (m, 1H), 2.14 (m, 1H), 2.43 (s, 3H), 2.47 (m, 1H), 3.11 (m, 1H), 3.42 (m, 1H), 3.76 (d, 1H, *J* = 15.1 Hz), 3.82 (s, 3H), 4.27 (d, 1H, *J* = 15.0 Hz), 4.38 (m, 1H), 4.61 (br t, 1H, *J* = 4.1 Hz), 5.95 (dd, 1H, *J* = 10.3, 4.1 Hz), 6.04 (d, 1H, *J* = 10.3 Hz), 6.57 (d, 1H, *J* = 8.1 Hz), 6.65 (d, 1H, *J* = 8.1 Hz); HRMS calcd for $C_{26}H_{42}NO_3Si$ (M^+ + H) 444.2925, found 444.2965.

Galanthamine n-butylcarbamate (5)

To a solution of galanthamine (**1**, 1.00 g, 3.48 mmol) in dry THF (17 ml) was added n-butyl isocyanate (0.864 g, 0.980 ml, 8.71 mmol), at ambient temperature, under an argon atmosphere. The reaction mixture was brought to reflux and stirred for 48 h. The solution was then cooled, concentrated under reduced pressure, and the residue purified by silica gel flash-column chromatography using first acetone, then acetone: methanol (19:1) as eluants to afford compound **5**, which solidified slowly to a white foam (1.34 g, 100% yield): TLC (MeOH: Me_2CO, 1:3) R_f 0.35; mp 47–49°C; $[\alpha]_D^{22}$-55.7° (c 1.25, $CHCl_3$); IR ($CHCl_3$) 3450, 3020, 2970, 2940, 2420, 2300, 1710, 1630, 1510, 1470, 1460, 1450, 1440, 1300, 1280, 1235, 1170, 1165, 1140, 1110, 1065, 1050, 1015 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91 (t, 3H, *J* = 7.2 Hz), 1.33 (br dd, 2H, *J* = 14.7, 7.2 Hz), 1.44 (m, 2H), 1.57 (br dd, 1H, *J* = 13.6, 1.7 Hz), 2.09 (m, 1H), 2.11 (m, 1H), 2.39 (s, 3H), 2.64 (br d, 1H, *J* = 9.8 Hz), 3.05 (br d, 1H, *J* = 14.5 Hz), 3.14 (br dd, 2H, *J* = 13.1, 6.6 Hz), 3.30 (br t, 1H, *J* = 13.5 Hz), 3.67 (d, 1H, *J* = 15.2 Hz), 3.84 (s, 3H), 4.12 (d, 1H, *J* = 15.1 Hz), 4.55 (br s, 1H), 4.76 (br s, 1H), 5.28 (br t, 1H, *J* = 5.0 Hz), 5.92 (dd, 1H, *J* = 10.2, 4.7 Hz), 6.24 (d, 1H, *J* = 10.3 Hz), 6.58 (d, 1H, *J* = 8.1 Hz), 6.65 (d, 1H, *J* = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 13.6, 19.8, 28.0, 31.9, 34.3, 40.6, 41.8, 47.8, 53.7, 55.6, 60.4, 63.0, 86.4, 111.0, 121.3, 123.5, 129.1, 129.9, 132.2, 143.9, 146.4, 156.2; HRMS calcd for $C_{22}H_{31}N_2O_4$ (M^+ + H) 387.229, found 387.228. Anal $C_{22}H_{30}N_2O_4$ 1/3 H_2O (C, H, N).

Galanthamine phenylcarbamate (6)

To a solution of galanthamine (**1**, 0.300 g, 1.05 mmol) in THF (5 ml) was added phenyl isocyanate (0.376 g, 0.340 ml, 3.14 mmol) in 1 portion at room temperature, under an argon atmosphere. The reaction mixture was heated at reflux for 24 h, and the solvent evaporated under reduced pressure. The crude

residue was purified by silica gel flash-column chromatography using first dichloromethane, then methanol: acetone (1:9) as eluants to afford compound **6** (0.339 g, 80% yield) as white crystals: TLC (MeOH:Me_2CO, 1:3) R_f 0.33; mp 85–87°C; $[\alpha]_D^{22}$-49.8° (c 0.46, MeOH); IR ($CHCl_3$) 3430, 3040, 2980, 2440, 2380, 2340, 1730, 1630, 1600, 1515, 1465, 1445, 1310, 1300, 1285, 1235, 1195, 1170, 1140, 1090, 1080, 1070, 1050, 1030, 1020 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 1.54 (br d, 1H, J = 13.7 Hz), 2.09 (m, 1H), 2.11 (m, 1H), 2.37 (s, 3H), 2.71 (br d, 1H, *J* = 16.1 Hz), 3.04 (br d, 1H, *J* = 14.5 Hz), 3.26 (br t, 1H, *J* = 13.6 Hz), 3.66 (d, 1H, *J* = 15.1 Hz), 3.80 (s, 3H), 4.10 (d, 1H, *J* = 15.1 Hz), 4.52 (m, 1H), 5.35 (br t, 1H, *J* = 4.8 Hz), 5.93 (dd, 1H, *J* = 10.3, 4.8 Hz), 6.24 (d, 1H, *J* = 10.3 Hz), 6.56 (d, 1H, *J* = 8.1 Hz), 6.63 (d, 1H, *J* = 8.1 Hz), 7.00 (m, 1H), 7.24 (m, 2H), 7.34 (d, 2H, *J* = 7.9 Hz), 7.37 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 27.9, 34.3, 37.0, 41.9, 47.9, 53.7, 55.7, 60.4, 63.7, 86.4, 111.1, 118.6, 121.5, 122.9, 123.1, 128.9, 129.3, 130.5, 132.1, 138.0, 143.8, 146.4; HRMS calcd for $C_{24}H_{27}N_2O_4$ (M^+ + H) 407.196, found 407.197.

Galanthamine α-naphthylcarbamate (7)
1-Naphthyl isocyanate (0.531 g, 0.450 ml. 3.14 mmol) was added to a stirred solution of galanthamine (**1**, 0.300 g, 1.06 mmol) in THF (5 ml) at ambient temperature, under an argon atmosphere. The reaction mixture was heated to reflux for 24 h. After the solvent was evaporated under reduced pressure, the crude material was purified by silica gel flash-column chromatography using dichloromethane at first, and then methanol:acetone (1:9) as eluants to afford 0.287 g of the product (**7**) as white crystals (60% yield): TLC (MeOH: Me_2CO, 1:3) R_f 0.33; mp 203–204°C; $[\alpha]_D^{22}$-94.9° (c 0.35, $CHCl_3$); IR ($CHCl_3$) 3430, 2970, 2440, 2340, 1730, 1630, 1600, 1530, 1515, 1495, 1480, 1475, 1450, 1440, 1380, 1350, 1300, 1285, 1235, 1195, 1175, 1105, 1090, 1070, 1050, 1035, 1015 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.61 (br dd, 1H, *J* = 13.7, 2.0 Hz), 2.16 (m, 1H), 2.18 (m, 1H), 2.41 (s,3H),2.83(dt, 1H, *J* = 16.3, 1.4Hz), 3.08 (brd, 1H, *J* = 14.4Hz), 3.32 (br t, 1H, *J* = 13.3 Hz), 3.69 (d, 1H, *J* = 15.2 Hz), 3.86 (s, 3H), 4.14 (d, 1H, *J* = 15.1 Hz), 4.61 (m, 1H), 5.43 (br t, 1H, *J* = 5.1 Hz), 6.04 (dd, 1H, *J* = 10.2, 4.8 Hz), 6.34 (d, 1H, *J* = 10.3 Hz), 6.61 (d, 1H, *J* = 8.1 Hz), 6.68 (d, 1H, *J* = 8.2 Hz), 7.44–7.90 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 28.0, 31.0, 34.3, 41.9, 48.0, 53.8, 55.8, 60.5, 64.2, 86.4, 111.1, 118.9, 120.7, 121.5, 123.0, 124.7, 125.8, 126.0, 126.6, 128.6, 129.3, 130.7, 132.1, 132.7, 134.0, 144.0, 146.5, 154.1; HRMS calcd for $C_{28}H_{29}N_2O_4$ (M^+ + H) 457.2127, found 457.2185.

Galanthaminone (narwedine) (8)
Pyridinium chlorochromate (0.158 g, 0.732 mmol) was added to a stirred solution of galanthamine (**1**, 0.0700 g, 0.244 mmol) in dichloromethane at ambient temperature. The reaction mixture was stirred for 8 h, under an argon atmosphere, and then diluted with methanol, and filtered over Celite. The filtrate was evaporated under reduced pressure, and the crude product was purified by silica gel flash-column chromatography using acetone, then methanol: acetone (1:9) as eluants. The pure compound **8** was obtained as white crystals (0.0600 g, 86% yield): TLC (MeOH:Me_2CO, 1:3) R_f 0.26; mp 184–186°C, lit [41] mp 188–189°C; IR (KBr) 2950, 2915, 2840, 1680, 1500, 1435, 1280, 1260, 1250, 1220, 1205, 1190, 1180, 1160, 1045, 1020, 1000, 920, 810, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.02 (m, 1H), 2.32 (br t, 1H, *J* = 13.1 Hz), 2.54 (s, 3H), 2.76 (dd, 1H, *J* = 17.7, 3.8 Hz), 3.18 (dd, 1H, *J* = 17.9, 2.1 Hz), 3.28 (m, 1H), 3.43 (m, 1H), 3.86 (s, 3H), 3.90 (br d, 1H, *J* = 15.4 Hz), 4.28 (br d, 1H, *J* = 14.4 Hz), 4.76 (m, 1H), 6.08 (d, 1H, *J* = 10.4 Hz), 6.70 (d, 1H, *J* = 8.2 Hz), 6.74 (d, 1H, *J* = 8.2 Hz), 6.90 (d, 1H, *J* = 10.4 Hz); HRMS calcd for $C_{17}H_{20}NO_3$ (M^+ + H) 286.1443, found 286.1417.

3-[(Aminocarbonyl)hydrazono]-3-deoxygalanthamine (9)
To a solution of narwedine (**16**, 0.0790 g, 0.277 mmol) in ethanol (3 ml) was added water (1 ml) followed by semicarbazide hydrochloride (0.0587 g, 0.526 mmol) and sodium acetate (0.0863 g, 1.05 mmol) at ambient temperature. The reaction mixture was brought to 100°C and stirred for 5 h under a reflux condenser. The ethanol was evaporated under reduced pressure. The remaining aqueous solution was cooled and crystallization was induced by scratching. The semicarbazone crystals were removed by filtration, washed with cold water, and then recrystallized from methanol/ether to afford compound **9** as white crystals (0.0610 g, 64% yield): TLC (*i*-PrOH: AcOH:H_2O, 6:3: 1) R_f 0.32; mp 264°C (dec), lit [42] mp 254–255°C; IR (KBr): 3480, 3400, 3320, 3200, 3020, 2960, 2940, 2620, 2560, 2480, 1675, 1630, 1570, 1510, 1465, 1450, 1440, 1410, 1370, 1275, 1200, 1170, 1165, 1130, 1080, 1060, 1010 cm^{-1}; ^{1}H NMR (MeOD-d_4) δ 2.20 (br d, 1H, *J* = 13.8 Hz), 2.33 (br t, 1H, *J* = 14.7 Hz), 2.62 (dd, 1H, *J* = 17.8, 4.0 Hz), 2.99 (s, 3H), 3.31 (m, 1H), 3.42 (dd, 1H, *J* = 18.0, 2.0 Hz), 3.66 (m, 1H), 3.83 (s, 3H), 3.86 (d, 1H, *J* = 14.7 Hz), 4.36 (d, 1H, *J* = 15.0 Hz), 4.78 (m, 1H), 6.27 (d, 1H, *J* = 10.4 Hz), 6.34 (d, 1H, *J* = 10.4 Hz), 6.85 (d, 1H, *J* = 8.3 Hz), 6.89 (d, 1H, *J* = 8.4 Hz); HRMS calcd for $C_{18}H_{23}N_4O_3$ (M^+ + H) 343.1770, found 343.1815.

3-Deoxy-3-chlorogalanthamine (10)
To a solution of galanthamine (**1**, 0.143 g, 0.479 mmol) in dichloromethane (5 ml) was added *p*-toluenesulfonyl chloride (0.227 g, 1.19 mmol) and pyridine (0.787 g, 0.800 ml, 9.94 mmol) at 0°C in an ice-water bath, under an argon atmosphere. The reaction mixture was brought to ambient temperature and stirred for 24 h. It was then diluted with dichloromethane, washed with saturated sodium chloride solution, filtered, and separated. The resulting organic layer was dried (Na_2SO_4), concentrated under reduced pressure, and then purified by silica gel flash-column chromatography with acetone as an eluant to give compound **10** as a colorless oil (0.0810 g, 53% yield): TLC (MeOH:Me_2CO, 1:3) R_f 0.41; $[\alpha]_D^{22}$-282.8° (c 0.61, $CHCl_3$); IR ($CHCl_3$) 3040, 3010, 2940, 2840, 1630, 1510, 1440, 1325, 1290, 1260, 1240, 1195, 1165, 1120, 1065, 1050, 1035, 1010, 990, 890 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.74 (br dd, 1H, *J* = 14.0, 2.1 Hz), 2.09 (ddd, 1H, *J* = 13.8, 10.7, 2.4 Hz), 2.19 (dt, 1H, *J* = 13.6, 2.7 Hz), 2.42 (s, 3H), 2.97 (m, 1H), 3.11 (br d, 1H, *J* = 14.4 Hz), 3.31 (br t, 1H, *J* = 13.2 Hz), 3.70 (d, 1H, *J* = 15.1 Hz), 3.85 (s, 3H), 4.11 (d, 1H, *J* = 15.1 Hz), 4.57 (m, 1H), 4.86 (ddd, 1H, *J* = 10.8, 3.9, 1.9 Hz), 5.88 (br d, 1H, *J* = 10.4 Hz), 6.06 (d, 1H, *J* = 10.4 Hz), 6.60 (d, 1H, *J* = 8.2 Hz), 6.66 (d, 1H, *J* = 8.2 Hz); HRMS calcd for $C_{17}H_{21}ClNO_2$ (M^+ + H) 306.125, found 306.123.

3,4-Didehydro-3-deoxygalanthamine (11)
To a solution of galanthamine (**1**, 0.100 g, 0.348 mmol) in toluene (7 ml) was added triphenylphosphine (0.274 g, 1.05 mmol), followed by triiodoimidazole (0.235 g, 0.523 mmol). The reaction mixture was heated at reflux for 3 h, under an argon atmosphere. The reaction mixture was diluted with 7 ml of toluene, treated with saturated sodium bicarbonate solution (14 ml), and then stirred for 10 min. Iodine was added until the dark yellow color persisted. Excess iodine was reduced by addition of a saturated sodium bisulfite solution. The two layers were separated, and the aqueous layer was washed with ethyl acetate (2 x 10 ml). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent evaporated

under reduced pressure. Silica gel flash-column chromatography of the crude material using first acetone, then acetone: methanol (9:1) as the gradient solvent system gave the pure product (**11**) as a pale yellow oil (0.0123 g, 13% yield): TLC (Me_2CO:MeOH, 9:1) R_f 0.26; $[\alpha]_D^{22}$-323.9° (c 0.16, $CHCl_3$); IR ($CHCl_3$) 2990, 2930, 1625, 1505, 1460, 1435, 1280, 1275, 1255, 1160, 1110, 1095, 1075, 1050, 1005, 995, 900 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.67 (br d, 1H, J = 11.2 Hz), 2.00 (m, 1H), 2.39 (s, 3H), 3.02 (br d, 1H, J = 14.4 Hz), 3.35 (m, 1H), 3.67 (d, 1H, J = 15.1 Hz), 3.83 (s, 3H), 4.17 (d, 1H, J = 15.1 Hz), 4.81 (d, 1H, J = 5.4 Hz), 6.01 (dd, 1H, J = 9.7, 5.4 Hz), 6.02 (dd, 1H, J = 9.7, 5.4 Hz), 6.16 (d, 1H, J = 9.7 Hz), 6.25 (dd, 1H, J = 9.5, 5.4 Hz), 6.59 (s, 2H); HRMS calcd for $C_{17}H_{19}NO_2$ (M^+) 269.142, found 269.145.

*Lycoramine (1,2-Dihydrogalanthamine) (**13**)*

To a solution of galanthamine (**1**, 0.100 g, 0.348 mmol) in 2 ml of dry methanol was added 0.0200 g of 10% palladium on carbon, and the solution flushed three times with hydrogen gas. The reaction mixture was shaken for 10 h at ambient temperature in a Parr hydrogenator (at 40 psi). The catalyst was removed by filtration over Celite and washed thoroughly with methanol. The solution was concentrated under reduced pressure, and the residue purified by silica gel flash-column chromatography using acetone: methanol (90:10, 80:20) as eluants. The pure product (**13**) was obtained as white crystals (0.0938 g, 93% yield): TLC (MeOH:Me_2CO, 1:3) R_f 0.18, mp 110–112°C, lit [43] mp 121°C; $[\alpha]_D^{22}$-112.1° (c 0.56, MeOH), lit [46] $[\alpha]_D^{27}$-98° (alcohol); IR (KBr) 3220, 2960, 2920, 2880, 2860, 1500, 1450, 1430, 1340, 1320, 1310, 1260, 1240, 1210, 1200, 1160, 1140, 1110, 1080, 1050, 1030, 1020, 1000, 950, 945, 770, 640 cm^{-1}; ^1H NMR (MeOD-d_4) δ 1.45 (m, 1H), 1.69–1.88 (m, 4H), 1.94 (m, 1H), 2.01 (ddd, 1H, J = 16.0, 5.3, 4.0 Hz), 2.31 (ddd, 1H, J = 15.8, 2.4, 1.8 Hz), 2.36 (s, 3H), 3.01 (br d, 1H, J = 14.2 Hz), 3.17 (dt, 1H, J = 14.3, 1.7 Hz), 3.62 (d, 1H, J = 14.8 Hz), 3.82 (s, 3H), 3.99 (d, 1H, J = 14.7 Hz), 4.05 (dt, 1H, J = 5.3, 2.6 Hz), 4.31 (t, 1H, J = 3.4 Hz), 6.61 (d, 1H, J = 8.1 Hz), 6.72 (d, 1H, J = 8.2 Hz); HRMS calcd for $C_{17}H_{23}NO_3$ (M^+) 289.167, found 289.171.

*3-Acetoxy-1,2-dihydrogalanthamine (**14**)*

To a solution of 1,2-dihydrogalanthamine (**13**, 0.300 g, 1.04 mmol) in 10 ml of dichloromethane was added acetic anhydride (0.138 g, 0.130 ml, 1.35 mmol) and dimethylaminopyridine (0.228 g, 1.87 mmol) at 0°C, in an ice-water bath, under an argon atmosphere. The reaction mixture was stirred for 10 min at 0°C, and then for 1 h at ambient temperature. After the evaporation of the solvent under reduced pressure, the residue was diluted with ethyl acetate (15 ml), then washed with water (17 ml), 10% sodium carbonate solution (7 ml), and saturated sodium chloride solution (7 ml), successively. The organic layer was dried over anhydrous Na_2SO_4, evaporated, and the residue purified by silica gel flash-column chromatography using first acetone, then methanol: acetone (1:10) as the eluants. The product (**14**) was obtained as white crystals (0.306 g, 89% yield): TLC (MeOH:Me_2CO, 1:3) R_f 0.22; mp 84–85°C; $[\alpha]_D^{22}$-109.8° (c 0.81, MeOH); IR (KBr) 3040, 2940, 2930, 2900, 2870, 2840, 1730, 1505, 1450, 1430, 1370, 1285, 1260, 1230, 1220, 1200, 1190, 1160, 1075, 1065, 1055, 1020, 950, 790 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60–1.75 (m, 4H), 1.87 (br d, 1H, J = 13.3 Hz), 1.93 (m, 1H), 1.99 (br d, 1H, J = 12.5 Hz), 2.03 (s, 3H), 2.35 (s, 3H), 2.52 (br d, 1H), 3.03 (br d, 1H, J = 14.6 Hz), 3.20 (br t, 1H, J = 13.9 Hz), 3.60 (d, 1H, J = 15.0 Hz), 3.86 (s, 3H), 4.03 (d, 1H, J = 15.0 Hz), 4.35 (m, 1H), 5.09 (m, 1H), 6.56 (d, 1H, J = 8.1 Hz), 6.65 (d, 1H, J = 8.1 Hz); HRMS calcd for $C_{19}H_{26}NO_4$ (M^+ + H) 332.1862, found 332.1841.

*3-Deoxy-1,2-dihydro-3-iodogalanthamine (**15**)*

To a solution of 1,2-dihydrogalanthamine (**13**, 0.500 g, 1.73 mmol) in toluene (35 ml), was added triphenylphosphine (1.36 g, 5.19 mmol), followed by triiodoimidazole (1.17 g, 2.60 mmol). The reaction mixture was refluxed for 2 h under an argon atmosphere. Additional triphenylphosphine (0.182 g, 0.692 mmol) and triiodoimidazole (0.155 g, 0.346 mmol) were added and the reaction mixture stirred for 1 h at reflux. To the reaction mixture were added additional toluene (35 ml) and saturated sodium bicarbonate solution (70 ml). The reaction mixture was stirred for 10 min at ambient temperature, and then iodine was added until the dark yellow color persisted. Saturated sodium bisulfite solution was added to reduce the excess iodine, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (50 ml), and the combined organic layers were dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue purified by silica gel flash-column chromatography using at first acetone and then acetone:methanol (9:1) as eluants. The pure product (**15**) was obtained as pale yellow crystals (0.242 g, 35% yield): TLC (MeOH:Me_2CO, 1:3) R_f 0.34, mp 123–125°C; $[\alpha]_D^{22}$-169.3° (c 0.14, $CHCl_3$); IR (KBr) 2930, 2890, 2830, 1615, 1500, 1440, 1430, 1420, 1310, 1280, 1270, 1245, 1230, 1210, 1190, 1180, 1165, 1150, 1100, 1050, 1030, 985, 960, 955, 790 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (br dt, 1H, J = 14.0, 3.0 Hz), 1.85–1.99 (m, 3H), 2.08 (br d, 1H, J = 14.6 Hz), 2.20 (dd, 1H, J = 8.3, 3.3 Hz), 2.31 (ddd, 1H, J = 15.8, 12.5, 3.7 Hz), 2.39 (s, 3H), 3.02 (br d, 1H, J = 12.9 Hz), 3.05 (ddd, 1H, J = 15.1, 4.3, 2.1 Hz), 3.20 (br t, 1H, J = 12.4 Hz), 3.65 (d, 1H, J = 15.1 Hz), 3.86 (s, 3H), 3.96 (d, 1H, J = 15.0 Hz), 4.10 (m, 1H), 4.41 (m, 1H), 6.57 (d, 1H, J = 8.2 Hz), 6.65 (d, 1H, J = 8.2 Hz); HRMS calcd for $C_{17}H_{23}INO_2$ (M^+ + H) 400.078, found 400.080.

*3-Azido-3-deoxy-1,2-dihydrogalanthamine (**16**)*

To a solution of 3-deoxy-1,2-dihydro-3-iodogalanthamine (**15**, 0.0500 g, 0.125 mmol) in DMF (1 ml) was added sodium azide (0.0204 g, 0.313 mmol) and the reaction mixture stirred for 3 h at 50°C, under an argon atmosphere. The reaction mixture was treated with a saturated sodium chloride solution, and then extracted with ethyl acetate (5 x 10 ml). The combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure, and the residue purified by silica gel flash-column chromatography using methanol:acetone (1:9) as eluants to afford compound **16** as a yellow oil (0.0385 g, 98% yield): TLC (MeOH:Me_2CO, 1:9) R_f 0.14; $[\alpha]_D^{22}$-141.4° (c 1.64, $CHCl_3$); IR ($CHCl_3$) 3010, 2940, 2850, 2100, 1670, 1630, 1595, 1510, 1465, 1450, 1440, 1290, 1275, 1260, 1160, 1110, 1090, 1070, 1050, 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57-2.18 (m, 7H), 2.35 (s, 3H), 2.51 (br d, 1H, J = 16.4 Hz), 3.02 (br d, 1H, J = 14.7 Hz), 3.19 (m, 1H), 3.60 (d, 1H, J = 15.6 Hz), 3.85 (s, 3H), 3.90 (m, 1H), 4.02 (d, 1H, J = 15.0 Hz), 4.34 (br t, 1H, J = 3.3 Hz), 6.56 (d, 1H, J = 8.1 Hz), 6.65 (d, 1H, J = 8.1 Hz); HRMS calcd for $C_{17}H_{23}N_4O_2$ (M^+ + H) 315.182, found 315.180.

*3-Amino-3-dehydroxy-1,2-dihydrogalanthamine (**17**)*

To a solution of 3-azido-3-deoxy-1,2-dihydrogalanthamine (**16**, 0.0327 g, 0.104 mmol) in dry methanol (1 ml) was added 10% palladium on carbon (0.0033 g). The mixture was placed in a Parr apparatus. Hydrogen gas was flushed through the reaction vessel three times. The reaction mixture was then shaken for 10 h at room temperature and 40 psi. The reaction mixture was filtered through a pad of Celite. The pad was then washed thoroughly with methanol, the filtrate dried over anhydrous

Na_2SO_4, and the solvent evaporated under reduced pressure. The crude material was purified by silica gel flash-column chromatography using methanol: acetone as the gradient solvent system (1:4, 1:1) to afford compound **17** as a thick oil which slowly solidified (0.0238 g, 79% yield): TLC (MeOH: AcOH: H_2O, 8:1:1) R_f 0.32; mp 125-130°C; $[\alpha]_D^{22}$-129.3° (c 0.08, MeOH); IR (KBr) 3500–3160, 2930, 2880, 2850, 2800, 1660, 1620, 1590, 1505, 1435, 1385, 1360, 1265, 1220, 1165, 1110, 1030, 950, 790 cm^{-1}; ^{1}HNMR($CDCl_3$) δ 1.18-2.03 (m, 7H), 2.17 (br d, 1H, *J* = 15.6 Hz), 2.33 (s, 3H), 2.99 (br d, 1H, *J* = 13.2 Hz), 3.14 (m, 1H), 3.30 (m, 1H), 3.59 (d, 1H, *J* = 14.6 Hz), 3.82 (s, 3H), 3.97 (d, 1H, *J* = 14.6 Hz), 4.36 (m, 1H), 6.62 (d, 1H, *J* = 7.6 Hz), 6.73 (d, 1H, *J* = 7.4 Hz); HRMS calcd for $C_{17}H_{25}N_2O_2$ (M^+ + H) 289.192, found 289.189.

*Galanthamine methiodide (**18**)*

Galanthamine (**1**, 0.250 g, 0.871 mmol) was placed in a 100 ml flame-dried, round-bottomed flask, and dissolved in dry ether (50 ml). Iodomethane (0.185 g, 0.081 ml, 1.31 mmol) was added to the clear solution, and the reaction mixture was stirred on a magnetic stir plate for 12 h, at room temperature, under an argon atmosphere. The white precipitate that formed was removed by filtration, washed with ether, and then recrystallized from hot water/methanol to afford compound **18** as white crystals (0.290 g, 78% yield): TLC (*i*-PrOH:AcOH:H_2O, 6:3:1) Rf 0.16; mp 286–291°C, lit [39] mp 280-284°C; $[\alpha]_D^{22}$-96° (c 0.55, H_2O), lit [39] $[\alpha]_D^{22}$-94.4°; UV (95% EtOH) λ_{max} 291 nm (log ε 3.30); IR (KBr) 3561, 3490-3200, 2940, 1630, 1510, 1480, 1435, 1370, 1275, 1235, 1215, 1195, 1170, 1060, 1000, 960, 885, 865, 820, 800 cm^{-1}; ^{1}H NMR (D_2O) δ 2.11 (m, 1H), 2.20 (ddd, 1H, *J* = 16.3, 5.3, 3.1 Hz), 2.37 (m, 1H), 2.53 (dt, 1H, *J* = 16.3, 1.5 Hz), 2.95 (s, 3H), 3.38 (s, 3H), 3.65 (m, 1H), 3.88 (s, 3H), 4.10 (m, 1H), 4.31 (br t, 1H, *J* = 5.0 Hz), 4.38 (br d, 1H, *J* = 13.4 Hz), 4.78 (br d, 1H, *J* = 3.4 Hz), 4.98 (br s, 1H), 6.07 (dd, 1H, *J* = 9.8, 4.7 Hz), 6.20 (br s, 1H), 6.91 (br s, 1H), 6.98 (d, 1H, *J* = 8.2 Hz); HRMS calcd for $C_{17}H_{21}NO_3$ (M^+ - I - Me): 287.1521, found: 287.1508.

*10-Benzylgalanthaminium bromide (**19**)*

To a solution of galanthamine (**1**, 0.250 g, 0.871 mmol) in dry ether (50 ml) was added benzyl bromide (0.224 g, 0.160 ml, 1.31 mmol) at room temperature, under an argon atmosphere. The reaction mixture was stirred for 24 h at ambient temperature. The white precipitate that formed was collected by filtration, washed thoroughly with cold water, and recrystallized from methanol/ether to give compound **19** as white crystals (0.327 g, 82% yield): TLC (*i*-PrOH:AcOH:H_2O, 6:3:1) R_f 0.48; mp 192°C (dec); $[\alpha]_D^{22}$-126.8° (c 0.39, H_2O); IR (KBr) 3620–3180, 3020, 2900, 2940, 2920, 2850, 1625, 1600, 1510, 1480, 1460, 1440, 1400, 1370, 1360, 1290, 1280, 1220, 1170, 1150, 1070, 1065, 1010, 960, 850, 820, 780, 760, 740, 735, 710, 705 cm^{-1}; ^{1}H NMR (MeOD-d_4) δ 2.01 (m, 1H), 2.16 (ddd, 1H, *J* = 15.7, 5.2, 3.4 Hz), 2.28 (m, 1H), 2.48 (br d, 1H, *J* = 15.7 Hz), 2.86 (s, 3H), 3.52 (m, 1H), 3.84 (s, 3H), 4.09 (m, 1H), 4.20 (m, 1H), 4.50 (br s, 1H), 4.68 (m, 1H), 4.85 (s, 2H), 5.07 (br d, 1H), 6.05 (m, 1H), 6.17 (br s, 1H), 6.82 (br s, 1H), 6.88 (d, 1H, *J* = 7.8 Hz), 7.54-7.68 (m, 5H); HRMS calcd for $C_{24}H_{28}NO_3$ (M^+ - Br) 378.2069, found 378.2057.

*10-Allylgalanthaminium bromide (**20**)*

To a stirred solution of galanthamine (**1**, 0.300 g, 1.05 mmol) in dry ether (50 ml) was added allyl bromide (0.190 g, 0.140 ml, 1.57 mmol) at ambient temperature, under an argon atmosphere. The reaction mixture was stirred for 6 h under these conditions. The precipitate formed was collected by filtration, washed thoroughly with ether, and then with dichloromethane. The resulting compound was dried under reduced pressure to give a pure product (**20**) as white crystals (0.393 g, 92% yield): TLC (*i*-PrOH:AcOH:H_2O, 6:3:1) R_f 0.24; mp 264-266°C; $[\alpha]_D^{22}$-120.7° (c 0.15, H_2O); IR (KBr) 3600-3180, 3020, 2960, 1620, 1510, 1440, 1290, 1270, 1210, 1170, 1070, 1050, 1010, 950, 850, 820, 800 cm^{-1}; ^{1}H NMR (D_2O) δ 2.13 (m, 1H), 2.20 (ddd, 1H, *J* = 16.3, 5.2, 3.1 Hz), 2.37 (m, 1H), 2.54 (dt, 1H, *J* = 16.3, 1.4 Hz), 2.88 (s, 3H), 3.65 (m, 1H), 3.88 (s, 3H), 4.03 (m, 1H), 4.20 (d, 2H, *J* = 7.3 Hz), 4.32 (br t, 1H, *J* = 4.8 Hz), 4.37 (d, 1H, *J* = 14.4 Hz), 4.78 (m, 1H), 4.90 (d, 1H, *J* = 12.9 Hz), 5.80 (d, 1H, *J* = 16.9 Hz), 5.84 (d, 1H, *J* = 10.2 Hz), 6.07 (dd, 1H, *J* = 10.1, 4.8 Hz), 6.18 (d, 1H, *J* = 9.6 Hz), 6.21 (m, 1H), 6.92 (d, 1H, *J* = 7.4 Hz), 6.98 (d, 1H, *J* = 8.3 Hz); HRMS calcd for $C_{20}H_{27}NO_3$ (M^+ - Br + H) 329.199, found 329.202.

*O-Demethylgalanthamine (**21**)*

To a solution of galanthamine (**1**, 0.500 g, 1.74 mmol) in chloroform (17 ml) was added 1M BBr_3 in CH_2Cl_2 (10.5 ml, 10.5 mmol) in one portion, at 0°C. The reaction mixture was stirred for 2 h at 0°C, and then for 30 min at ambient temperature, under an argon atmosphere. It was then slowly poured into 30 ml of NH_4OH solution containing ice, and was stirred for 1 h. The organic layer was separated, and then the aqueous layer extracted with chloroform (4 x 15 ml). The combined organic layers were dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude solid was purified by silica gel flash-column chromatography using acetone:methanol (19:1, 9:1) as eluants to afford compound **21** as white crystals (0.148 g, 31% yield): TLC (MeOH:Me_2CO, 3:2) R_f 0.39; mp 220–222°C, lit [44] mp 210.5–213°C; $[\alpha]_D^{22}$-138.8° (c 0.48, EtOH), lit [44] $[\alpha]_D$ -133° (c 0.23, EtOH); IR (KBr) 3475, 3040, 2950, 2930, 2900, 2880, 1620, 1600, 1475, 1455, 1435, 1420, 1400, 1385, 1345, 1330, 1310, 1250, 1230, 1200, 1170, 1135, 1060, 1035, 980, 950, 940, 930, 805, 775 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.57 (br dd, 1H, *J* = 13.7, 2.3 Hz), 2.01 (ddd, 1H, *J* = 15.6, 4.8, 2.2 Hz), 2.10 (dt, 1H, *J* = 13.3, 2.8 Hz), 2.40 (s, 3H), 2.66 (m, 1H), 3.06 (br d, 1H, *J* = 14.4 Hz), 3.27 (br t, 1H, *J* = 13.2 Hz), 3.67 (br d, 1H, *J* = 15.2 Hz), 4.08 (br d, 1H, *J* = 15.1 Hz), 4.17 (br t, 1H, *J* = 4.5 Hz), 4.59 (m, 1H), 5.99 (dd, 1H, *J* = 10.2, 5.0 Hz), 6.07 (d, 1H, *J* = 10.2 Hz), 6.54 (d, 1H, *J* = 8.1 Hz), 6.61 (d, 1H, *J* = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 20.0, 33.5, 41.9, 48.4, 53.7, 60.5, 62.2, 88.7, 115.7, 122.6, 127.2, 127.2, 128.2, 132.7, 140.4, 144.7; HRMS calcd for $C_{16}H_{19}NO_3$ (M^+) 273.1365, found 273.1346.

Molecular modeling

Molecular mechanics calculations were carried out using MacroModel version 2.5 on a VAX 11/750 or 11/785 computer. Sixteen conformers of **1** were generated by hand, and minimized using Allinger's MM2 force field employing either the Block Diagonal Newton Raphson (BDNR) or the Polak-Ribiere Conjugate Gradient (PRCG) minimization option, followed by minimization to a low gradient (< 0.006 kJ/mol·Å) using the Full Matrix Newton Raphson (FMNR) option. The lowest energy conformer of **1** was also determined by a Monte Carlo search of the structure. Ten-thousand conformations were generated and minimized. Of the 33 unique conformers found, the lowest in energy was the same as that found by hand. Derivatives of **1** were generated by appropriately modifying the corresponding minimized conformations of galanthamine itself. These derivatives were minimized as indicated above, and their conformational energies further studied by energy mapping dihedral angle rotations about selected bonds on added side chains. The dihedral angles were set to values that showed the

lowest energy, then the individual conformers were minimized again (using MM2, BDNR/PRCG, followed by FMNR) to a low gradient (RMS<0.006 kJ/molÅ).

Biological evaluation: in vitro AChE inhibition assays
Mice were killed by cervical dislocation. The brains were rapidly removed onto an ice-cooled metal plate and immediately frozen on dry ice. Cortical AChE activity was measured according to the acetylthiocholine method of Ellman [45]. All drugs were initially dissolved in 100 µl of absolute ethanol, and then diluted to the proper concentrations in phosphate-buffered saline. Ethanol-saline dilute concentrations were ranged from 1:200 to 1:1000 (v:v). The drug and cortical homogenates were incubated at room temperature for 30 min before the addition of the substrate for the AChE assay. The percent AChE inhibition was calculated for each of the compounds in comparison to an absolute ethanol-saline diluted control. The ethanol-saline solution did not result in significant loss of activity in the AChE assay. Only assays that fulfilled the requirements of linearity were used.

All drugs were initially screened for inhibition of cortical AChE at 10^{-5} M. For the most potent compounds, full dose response curves were obtained (for drug ranges between 10^{-5} and 10^{-9} M). The Sigmaplot 4.0 curve-fitting program and the logistic function of DeLean [46] were used to fit dose-response curves and to calculate IC_{50} values. Each experiment was repeated 3–4 times. Since we assumed that the analogues were competitive inhibitors similar to the parent compound, K_i values were estimated using the method of Cheng and Prusoff (1973) [47] where

$$K_i = \frac{IC_{50}}{1 + \frac{C}{K_M}}$$

C = concentration of acetylthiocholine
$K_M = 1.4\ 10^{-4}$ M of AChE [45].

Behavioral testing of galanthamine n-butylcarbamate (5)
Eighty-one adult male BALB/cByJ mice were kept on a 16/8 hp light/dark cycle with food and water available *ad libitum*. Nineteen halothane-anesthetized mice received 0.6 µl of ibotenic acid (10 µg/µl) bilaterally into the basal forebrain region [23]. Following a 2-week, post-operative recovery period, the lesioned mice, in parallel with 52 non-lesioned controls, were tested using a passive avoidance paradigm. The accuracy of the lesion was confirmed histologically. Mice (controls and BF-lesioned mice) received either vehicle or compound **5** (0.05-1.25 mg/kg, ip) 3.5 h before an acquisition trial. During acquisition, the mouse was placed in a lighted compartment. After 20 s, a divider between the light and dark compartments was opened. Upon entering the dark compartment, the mouse received an inescapable footshock (2.0 mA for 3 s). After 20 min, a second acquisition trial was given, conducted exactly as the first trial. Retention (latency to enter the dark compartment up to a maximum of 360 s) was measured 24 h later. Passive avoidance data were analyzed using ANOVA and a post-noc Scheffe F-test to compare individual means. To determine behavioral side effects, ten non-lesioned mice were administered various doses of compound **5**, ranging from 1-100 mg/kg, ip. General activity of the animals was monitored for the 24 h that followed.

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