Synthesis and Pharmacology of Galantamine

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Contents

1. Introduction 116
2. Synthesis of Galantamine 116
2.1. Synthesis Using Phenolic Oxidative Coupling: The Biomimetic Approach 116
2.1.1. Synthesis of Racemic Galantamine 117
2.1.2. Synthesis of (−)-Galantamine 121
2.2. Synthesis Using the Intramolecular Heck Reaction 123
2.2.1. Synthesis of 6-Deoxygalantamine Derivatives 123
2.2.2. Synthesis of Racemic Galantamine 124
2.2.3. Synthesis of (−)-Galantamine 124
3. Synthesis of Galantamine Analogues 125
4. Pharmacological Profile 126
4.1. Acetylcholinesterase Inhibition 128
4.2. Galantamine as an Allosteric Potentiating Ligand of Nicotinic Acetylcholine Receptors 128
4.3. Enhancement of Neurotransmitter Release 128
4.4. Effects on Behavior and Memory Performance in Animal Models 128
4.5. Clinical Efficacy in Alzheimer's Disease Patients 129
4.6. The Neuroprotectant Effects 129
4.7. Neuroprotection in the Clinic 130
4.8. Metabolism and Excretion of Galantamine 131
5. Conclusions and Perspectives 131
6. Acknowledgments 131
7. References 131

1. Introduction

(−)-Galantamine (**1**) {(4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]-benzazepin-6-ol} (Chart 1), an alkaloid isolated from the Caucasian snow-drop (*Galanthus woronowii*; Figure 1) and from the bulbs of different species of the *Amaryllidaceae* family,[1] is a centrally acting, selective, reversible, and competitive acetylcholinesterase (AChE) inhibitor,[2] as well as an allosteric modulator of the neuronal nicotinic receptor for acetylcholine.[3] Galantamine, commercially available as Razadyne, galantamine hydrobromide, is the most recently approved AChE inhibitor in Europe by the European registration bureau and in the USA by the FDA for the symptomatic treatment of Alzheimer's disease (AD).[4]

Owing to the scarce supplies from threatened[5] botanical sources[6] and the high cost (about $50 000 per kilogram) of its isolation from daffodils (0.1−2% dry weight),[7] several total syntheses have been reported to produce this drug.

Since the last two reviews, one by Oshino in 1998[8a] and one by Martin in 1987,[8b] dedicated to the *Amaryllidaceae* family of natural products, no review has been published on this subject.[8c] In the last years, the extent of reports regarding galantamine has significantly increased, and therefore, an updated review may be of interest. In this review, we will summarize the current state of the art concerning the chemistry and biology of galantamine.[9] We will show and comment on the different reported semisynthetic approaches from related natural products, the total syntheses of galantamine in racemic or in enantiomerically pure form, and some recent articles on the syntheses of galantamine analogues, targeted to develop into more biologically active molecules. Finally, we will describe the pharmacology of galantamine, focusing on the most important details of its possibilities as an AChE inhibitor.

2. Synthesis of Galantamine

First, we must explain that all the current synthetic approaches to galantamine rely on two key reaction protocols: (a) the biomimetic approach via the phenolic oxidative coupling in the presence of metal oxidants[10] and (b) the intramolecular Heck reaction.[11] Moreover, a very few reports have disclosed the asymmetric synthesis of this molecule. In this section, we will describe a few synthetic approaches of galantamine, and in each case, we will highlight the processes that lead to compound **1** in enantiomerically pure form.

2.1. Synthesis Using Phenolic Oxidative Coupling: The Biomimetic Approach

In the 1960s, Barton and co-workers[12a] recognized that *Amaryllidaceae* alkaloids, including galantamine, could be regarded as derived from a common precursor, norbelladine (**2**, Chart 1) via intramolecular oxidative phenol coupling. Experiments using α-^{14}C-labeled norbelladine derivatives as precursors experimentally established norbelladine as the

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Maria do Carmo Carreiras received her degree in Pharmacy from University of Lisbon in 1977, her Master Sci. in chemistry from University Nova of Lisbon in 1987, and her Ph.D. in Pharmaceutical chemistry from University of Lisbon in 1989. In 1985, she was a fellow at the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, working under the direction of Prof. Henry-Philippe Husson. From 1986 to 1988, she was a Ph.D. student at Instituto de Química Orgánica General (IQOG) of Madrid. Since 1989, she has been Lecturer at Faculty of Pharmacy, University of Lisbon. As a member of the staff of the Pharmaceutical and Phytochemistry department, she has been involved in teaching organic chemistry and medicinal chemistry at the graduate and post-graduate level. From 1997 to 1999, in different periods, she was a postdoctoral fellow with Prof. Marco-Contelles at IQOG of Madrid, and in 2003 (June-August), she was visiting Professor at the same Institute. Her present research interests include the rational design of cholinesterase inhibitors, as well as dual cholinesterase/monoamine oxidase inhibitors, targeted for Alzheimer's disease.

biogenetic precursor for galantamine biosynthesis.[12b,c] After the oxidative phenol coupling, a dienone was assumed to be the key intermediate giving narwedine, formed by un unknown mechanism and postulated as the precursor of galantamine **1** (Scheme 1). Subsequent studies from the Kirby[12d] and Fuganti[12e] laboratories confirmed and supported this hypothesis. In 1998, Zenk[12f] reported new insights on this mechanism leading to galantamine by application of radioactive and heavy isotope-labeled potential precursors to parts of *Leucojum aestivum* plants. As shown (Scheme 2), these results have been confirmed only in part. A new scheme for the galantamine biosynthesis involves the oxidative phenol coupling of 4′-*O*-methylnorbelladine to a dienone,

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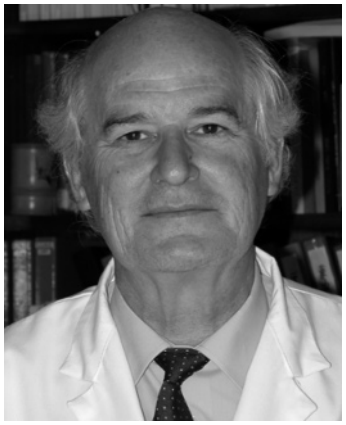
which undergoes intramolecular ring closure of the ether bridge to give *N*-demethylnarwedine, which after stereoselective reduction and N-methylation yields galantamine.

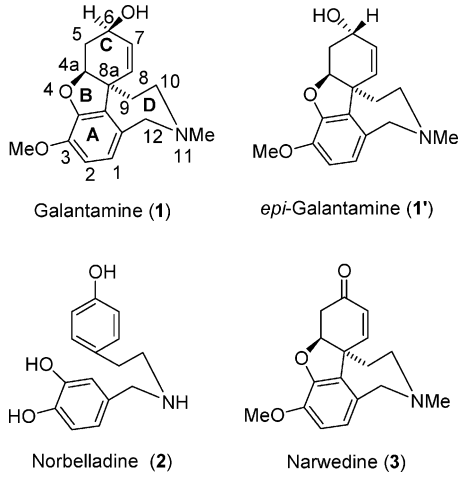
2.1.1. Synthesis of Racemic Galantamine

A considerable effort was devoted to prove the synthetic feasibility of the proposed biogenetic pathway. Barton and Kirby[12b] prepared racemic narwedine in 1.4% yield by phenol oxidation of diphenolic amine **4** using potassium ferricyanide (Scheme 3); subsequent reduction of narwedine (**3**) with lithium aluminum hydride represented the first published synthesis of racemic galantamine and *epi*-galantamine.[12b] The synthesis of compound **4** was straitghforward as depicted in Scheme 3. Starting from readily available *p*-hydroxyphenylacetic acid (**5**) and *O*-benzylisovanillin (**6**), the corresponding acyl chloride **7** and the *N*-methylamine **8** were easily combined into the aimed precursor **4** for the oxidative phenol coupling reaction.



Antonio G. García holds an M.D. and Ph.D. from Universidad Complutense de Madrid (Spain) 1970. He received postdoctoral training at the State University of New York, Health Science Center, 1971–1974. He is Professor and Chairman of the Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid (Spain) and author of 237 papers in international journals.

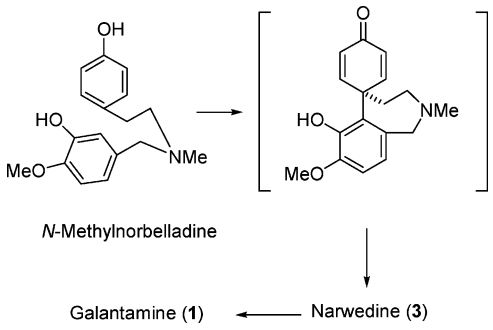
Chart 1



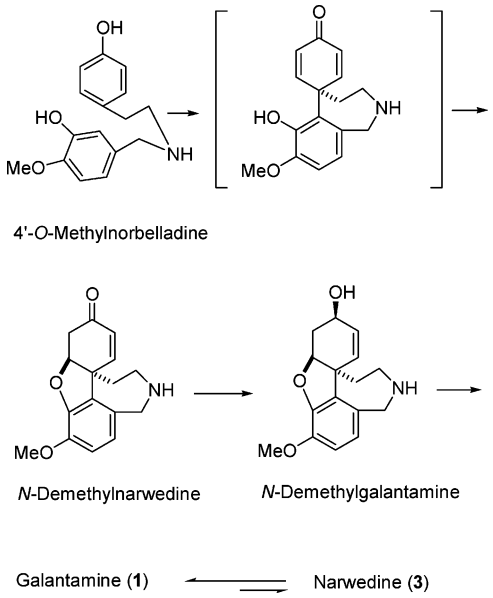
Based on the landmark that the biomimetic synthesis of galantamine represented, in the following years several groups made significant contributions on this topic improving Barton synthesis. Major modifications were directed to (a)

Figure 1. *Galanthus woronowii.* Reprinted with permission from Paul Tyerman.

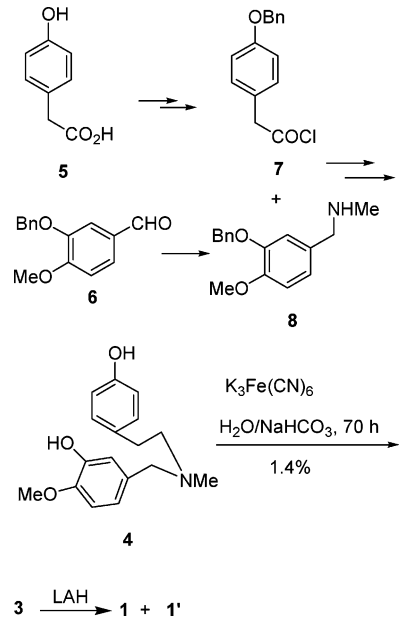
Scheme 1



Scheme 2



Scheme 3

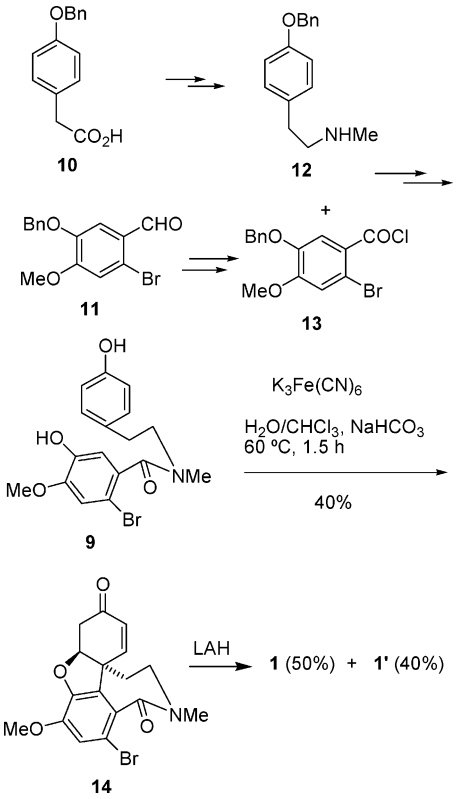


the protection of the para position to promote the intended oxidative phenol coupling more efficiently, (b) the introduction of a third phenol functional motif to avoid problems of regioselectivity in the aromatic ring functionalization, and (c) the use of other oxidants (PIFA [phenyliodine(III)bis-(trifluoroacetate)], $Mn(OAc)_3$) in looking for reactions that

might afford products in higher chemical yields and in milder experimental conditions.

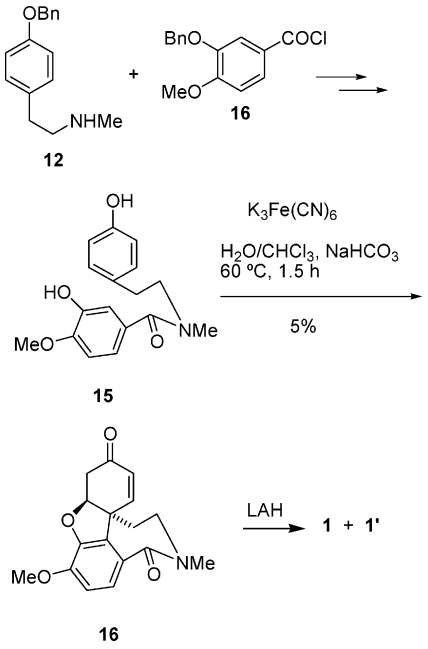
In 1969, Kametani started to publish a series of papers on the synthesis of galantamine and related derivatives.[13] On the basis of some previous synthetic works reported by Burchard[14] or by Abramovitch[15] and taking into account the low yields of the oxidative phenol coupling reported by Barton,[12] Kametani proposed diphenol **9** (Scheme 4) as the

Scheme 4



key precursor with the assumption that the bromine atom would prevent the para coupling to the hydroxy group and favor the ortho coupling. This compound was easily prepared by routine transformations starting from *p*-*O*-benzylhydroxyphenylacetic acid (**10**) and 2-bromo-*O*-benzylisovanillin (**11**), via *N*-methylamine **12** and acyl chloride **13**, respectively (Scheme 4). This hypothesis was fulfilled since the phenol oxidation of compound **9** afforded a narwedine-type compound (**14**) in 40% yield. Finally, treatment of compound **14** with lithium aluminum hydride (LAH) reduced the carbon−bromine bond, transformed the amide to an amine, and promoted the unselective reduction of the keto group to afford a mixture of galantamime (**1**) and *epi*-galantamine (**1′**) in 50% and 40% yield, respectively (Scheme 4).[13a,b] In 1971, Kametani proposed an alternative total synthesis of racemic galantamine based on the oxidative phenol coupling of compound **15** (Scheme 5),[13c] an amide of the precursor (**4**), previously used by Barton (Scheme 3).[12c] Compound **15** was synthesized from 4-benzyloxy-*N*-methylamine (**12**) and 3-benzyloxy-4-methoxybenzoyl chloride (**16**). However, and not unexpectedly, the key oxidative phenol coupling proceeded in a poor 5% yield, but the authors assumed that this approach was simpler and more efficient than their first alternative since the synthesis of the required amides was easier (Scheme 4). Eventually, Kametani and co-workers reported the synthesis of racemic *N*-norgalantamine following similar protocols on bromime-containing intermediates.[13d]

Scheme 5



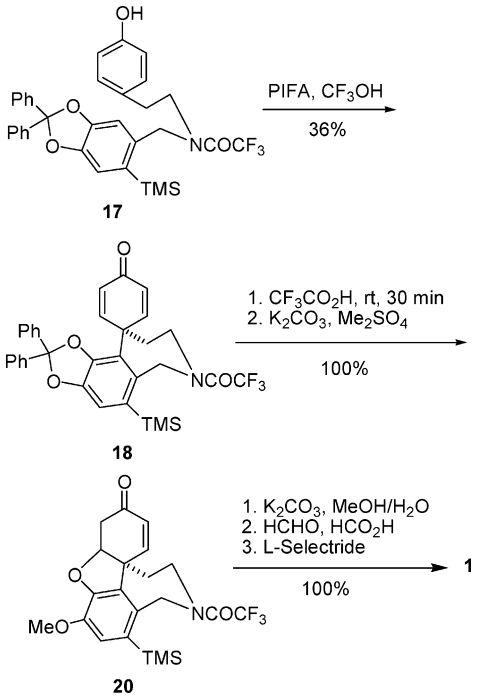
Bulgarian researchers have also been very active on this topic.[16] In an initial study, the synthesis of the tetracyclic ring system of galantamine was investigated by using the intramolecular para−ortho coupling of conveniently functionalized diaryl ethers by anodic oxidation.[16a] In a subsequent communication, Vlahov and colleagues[16b] have reported the synthesis of the same compound **9** (Scheme 4) previously prepared by Kametani and have submitted it to cyclization,[12b] but interestingly, they have found that under the same experimental conditions the yield was quite lower, not superior to 15%.

In 1998, Kita and co-workers made an important contribution to the synthesis of galantamine-type *Amaryllidaceae* alkaloids when they reported the use of PIFA as a suitable oxidant agent to promote the diphenol coupling on trifluroacetamide **17** (Scheme 6)[17] in a convenient chemical yield (36%). The use of trifluoroethanol as a solvent was critical for the accomplishment of this reaction. Finally, the acid hydrolysis of the acetal, followed by O-methylation, N-deprotection, and N-methylation, afforded galantamine exclusively in a very stereoselective ketone reduction using L-Selectride, in an overall quantitative chemical yield.

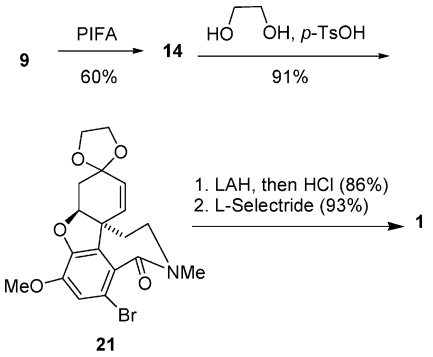
Krikorian[16c] has also shown the efficiency of PIFA in the key oxidative coupling reaction for the transformation of amide **9** into the tetracyclic derivative **14** (Scheme 4). Then a higher 60% yield was observed (Scheme 7). After the keto group was protected, the acetal **21** was reduced with LAH to eliminate the bromine atom, and the resulting product was submitted to acid hydrolysis to regenerate the ketone. Finally, the reduction with L-Selectride afforded only racemic galantamine in good chemical yield.

Carroll and collaborators[18] have also described the synthesis of racemic galantamine using as key intermediates formamides **23**[18a] and **24**[18b] (Scheme 8), prepared by selective and controlled bromination reactions [(Br_2, −65 °C) for **23**; (Br_2, rt) for **24**] from a common amide precursor (**22**). Their oxidative coupling reaction using potassium ferricyanide afforded moderate yields of compounds **25** (21%) or **26** (38−43%), respectively. The best yield was obtained from dibromide **24**. Finally, as expected, the reduction of com-

Scheme 6



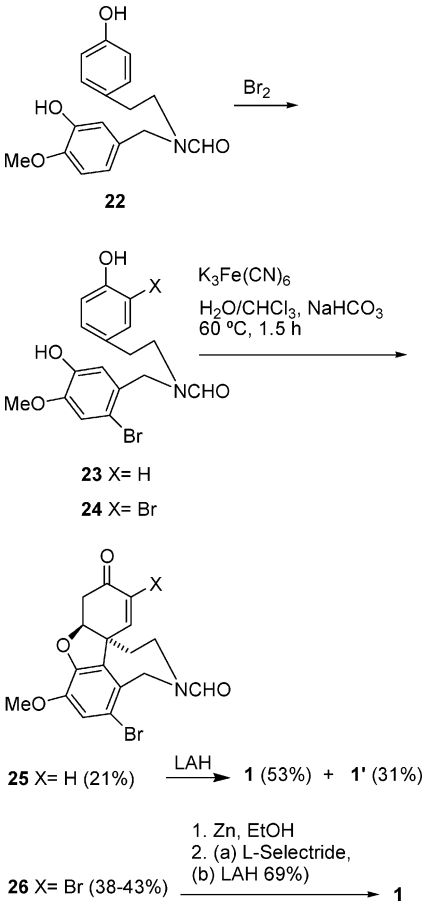
Scheme 7



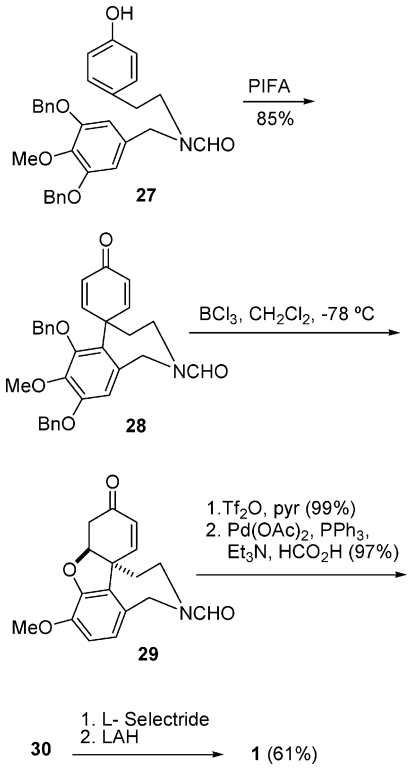
pound **25** with LAH provided mixtures of galantamine and *epi*-galantamine.[18a] In summary, galantamine was produced in 11% overall yield, starting from commercially available isovanillin and tyramine. Regarding compound **26**, the final steps consisted of the reduction of the C−Br bond with zinc in ethanol, the stereoselective reduction with L-Selectride, and the LAH-promoted reduction of the second C−Br bond. It is important to note that the use of L-Selectride for the reduction of the keto group, now a standard process in this chemistry, was really pioneered by the authors in this work.[18b] In this case, galantamine was produced in a higher 20% overall yield, starting from isovanillin and tyramine.

Finally, Node has reported a very interesting and efficient approach to galantamine (Scheme 9).[19a] The key points in this synthesis consisted of the use of 3,5-dibenzyloxy-4-methoxybenzaldehyde as a precursor, the PIFA-promoted oxidative coupling reaction of *N*-formamide **27** in trifluoroethanol at room-temperature rendering a dienone (**28**) in 85% yield, the selective O-debenzylation using BCl_3 at −78 °C, which provide the narwedine-type product **29** in a high yield, and, finally, the required O-deoxygenation of the extra phenol group on the corresponding triflate by palladium(0)-catalyzed reduction with formic acid. Note that in this approach the para position with respect to the phenol group in precursor **27** is not blocked but, alternatively, two

Scheme 8



Scheme 9



O-benzyloxy groups have been attached to the ortho positions of the methoxy group to make the para−ortho coupling the only possible reaction in the treatment with PIFA.

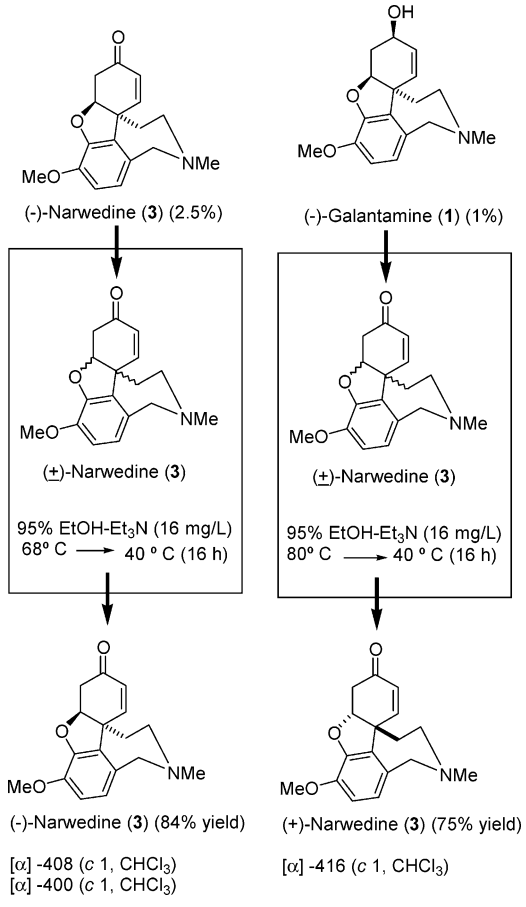
2.1.2. Synthesis of (−)-Galantamine

First, we will comment on the resolution techniques and thereafter on the characteristic asymmetric synthetic approaches described for the preparation of enantiomerically pure galantamine.

Galantamine and narwedine belong to the rare cases in the history of natural product synthesis, where the first synthesis of the racemic mixture was accompanied by the successful preparation of enantiomerically pure samples. Barton and Kirby[12b] were unable to resolve either (±)-galantamine or (±)-narwedine by using standard methods of resolution. However, they obtained (−)-galantamine (**1**) by reduction of (−)-narwedine (**3**) (Chart 1) achieved by chemical resolution. In the Barton method, (−)-narwedine is isolated by crystallization of a narwedine solution, which was mixed with 0.5 equiv of (+)-galantamine, the unnatural alkaloid.[12c] The main drawback was the availability of (+)-galantamine for a large scale preparation, and consequently, this moved other authors to find a solution.

Shieh and Carlson[7] solved this limitation and confirmed that because (±)-narwedine was a racemic conglomerate, a simple crystallization would allow isolation of enantiomerically pure samples without requiring the previous formation of diastereomeric derivatives (Chart 2). Thus, when a

Chart 2. Spontaneous Resolution of Racemic Narwedine



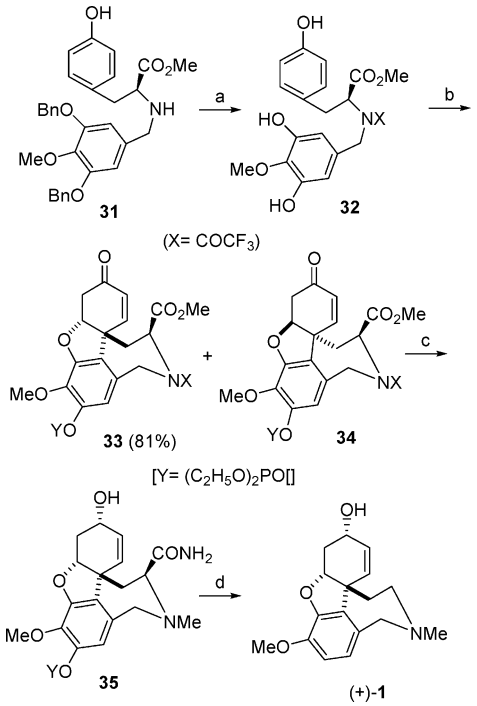
supersaturated solution of (±)-narwedine (16 mL/g) in 95% ethanol/triethylamine (9:1) at 68 °C was seeded with (−)-narwedine crystals (2.5%) and the suspension was cooled and maintained at 40 °C overnight, highly enantioenriched (−)-narwedine was isolated in 84% yield from (±)-narwedine (**3**). A similar process was used to prepare (+)-narwedine. They were also able to extend this methodology to a *total spontaneous* resolution of racemic narwedine by a small amount of a foreign substance such as galantamine. By the same protocol, (±)-narwedine (**3**) was dissolved in a solvent mixture (16 mL/g) of 95% ethanol/triethylamine (9:1) at 80 °C in the presence of a catalytic amount of (−)-galantamine (1%). After cooling at 40 °C for 16 h, enantiomerically pure (+)-narwedine was isolated in 75% yield. Similarly, when (±)-narwedine (**3**) was treated with a catalytic amount of (+)-galantamine (1%), enantiomerically pure (−)-narwedine was obtained in 76% yield.

Several hypotheses have been proposed to explain these phenomena,[7] but the most plausible explanation indicates that this process is a seeded total spontaneous resolution of enantiomers. Finally, Shieh and Carlson, in an independent work published one year before Carroll's paper,[18b] revealed that L-Selectride was the ideal high-yielding reducing agent for the total stereoselective reduction of narwedine to galantamine in racemic or in enantiomerically pure form.

However, since these reported resolutions did not appear to be practical for the preparation of the relatively large amounts of enantiomerically pure compounds, other methods have been investigated. Kametani described the first successful resolution of racemic galantamine by using optically active di-*p*-toluyl-D-tartaric acid as a resolving agent.[13e] Johnson has also reported the resolution of narwedine using the same agent and its transformation to (−)-galantamine.[20] Carroll noticed that the treatment of racemic galantamine with (−)-camphanic acid chloride gave a mixture of diastereomeric galanthamyl camphanate esters that could be separated by HPLC or by fractional recrystallization to render pure diastereomers. Final reduction with LAH afforded pure enantiomers.[18b]

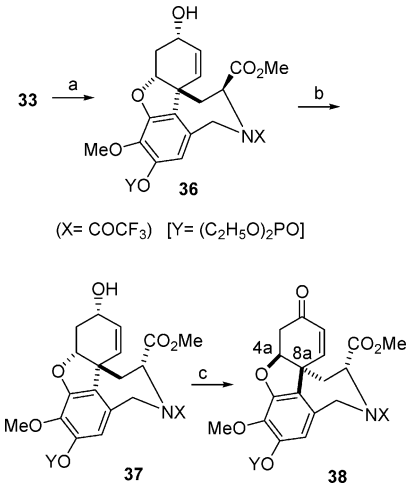
The first asymmetric synthesis of enantiomerically pure (+)- and (−)-galantamine has been reported from Koga's laboratory.[21] Compound **31**, obtained by reduction of the Schiff base produced from 3,5-dibenzyloxy-4-methoxybenzaldehyde and L-tyrosine methyl ester, followed by reduction with sodium borohydride, was protected as a trifluoroacetamide and submitted to hydrogenation to afford the key precursor (**32**) (Scheme 10) for the phenol oxidative para−ortho coupling reaction. This reaction was carried out with 5 equiv of manganic tris(acetylacetonate) in acetonitrile and proved quite efficient since the expected tetracyclic compound, isolated in 49% yield when submitted to phenol protection as the diethyl phosphonate, gave a mixture of compounds **33** (81%) and **34** (traces). The absolute configuration at the new stereogenic center, the quaternary spirocarbon, in major isomer **33** was established as shown in Scheme 10, after completion of the total asymmetric synthesis of the final product that resulted to be (+)-galantamine (**1**), the unnatural product. This was achieved in a series of simple reactions, in good overall yield, involving the reduction of the ketone, N-methylation, amidation, acetylation, dehydration of the amide **35** to render an unstable but not isolated amino nitrile, reduction with LAH, and final deoxygenation with sodium in liquid ammonia (Scheme 10). For the synthesis of the expected natural product (−)-**1**, a simple device was put in practice. As shown in Scheme 11, compound **33** (Scheme 10) was reduced with sodium borohydride and (*O*- and *N*-)-bis-trifluroacetylated, and then the *O*-trifluoroacetate was selectively hydrolyzed to produce amide **36**. Next, LDA-promoted epimerization gave compound **37** in poor yield (11%), which after oxidation by PCC afforded a ketone that slowly epimerized to the more

Scheme 10[a]



[a] Reagents and conditions: (a) i. $(CF_3CO)_2O$, pyr, ii. H_2, Pd/C (90%); (b) i. $Mn(acac)_3$ (49%), ii. $(C_2H_5O)_2POCl$, Et_3N; (c) (from **33**) i. $NaBH_4$, ii. 35% aq HCHO, 85% aq HCO_2H, ii. NH_3 (37%); (d) i. Ac_2O, pyr (89%), ii. $POCl_3$, pyr, then LAH (42%), iii. Na, NH_3 (72%).

Scheme 11[a]



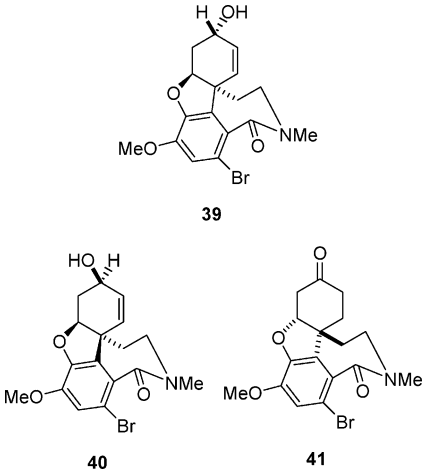
[a] Reagents and conditions: (a) i. $NaBH_4$, (99%), ii. $(CF_3CO)_2O$, pyr (92%), iii. 5% aq $KHCO_3$, MeOH (86%); (b) LDA, HMPT (11%); (c) PCC (72%).

stable ketone **38** with the correct and same configuration at carbons 4a and 12a as in the natural product (−)-**1**.

In view of the former transformation of compound **33** into (+)-**1** (Scheme 10), the synthesis of product **38** represented a formal total synthesis of natural (−)-**1**. In summary, Kogas' approach to galantamine is a very elegant example of the use of the chiral pool for the synthesis of natural products. Note also that, unfortunately in this case, the unnatural product was obtained from the natural starting material, L-tyrosine, but the synthesis of the natural galantamine is also possible taking into account the convenient availability of the D-tyrosine derivatives.

In 1989, Vlahov reported his investigations on the asymmetric reduction of compound **14** (Scheme 4) for the synthesis of advanced intermediates leading to enantiomerically pure galantamine.[16b] More than 400 species of microorganisms were screened, but only five gave reproducible results. *Septomyxa affinis* DSM 6737 produced pure compound **39** (Chart 3) in 50% yield. *Nematospora corylii* CBS

Chart 3

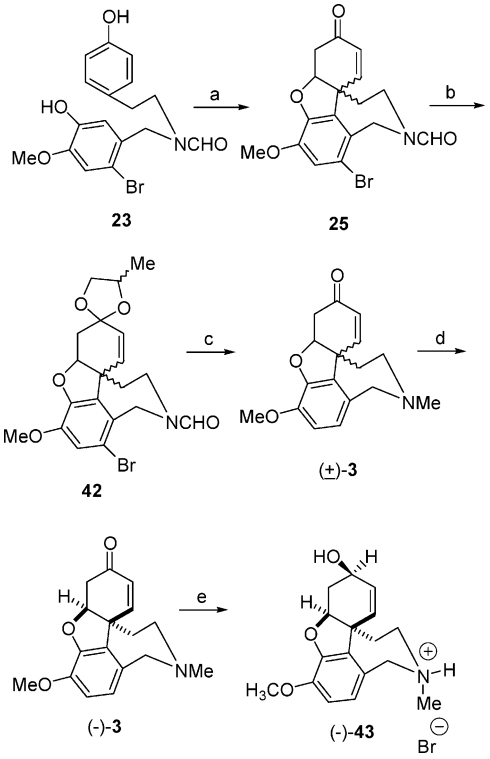


2608 rendered racemic **40** (Chart 3) in 50% chemical yield. *Ashybya gossypii* IFO 1355 afforded enantiomerically pure **40** and racemic **39** in a 1:2 ratio in total yield over 45%. Finally, *Nocardia alba* DSM 43130 and *Bacilus cereus* DSM 508 hydrogenated the double bond to impart enantiomerically pure (+)-lycoramine derivative **41** [Chart 3, the compounds shown in this chart, and in all other cases (see below), have been represented with the appropriate absolute configuration at the corresponding stereogenic centers, but the descriptors (*R*,*S*) were not used for simplicity)].

The Austrian group led by Jordis has achieved considerable improvements on the chemistry of galantamine and analogues.[22] Regarding the synthesis of (−)-galantamine, initially in a preliminary communication in 1998[22a] and later in a full paper,[22b] they have proposed a new kilogram synthesis at an industrial level starting from compound **23** previously reported by Carroll and associates[18a] (Scheme 8). As shown in Scheme 12, the synthetic sequence follows the known process related by Carroll[18b] and the resolution of racemic narwedine elucidated by Shieh and Carlson.[7] The protocol is simple proceeding in nine steps from 3,4-dimethoxybenzaldehyde and requires neither low-temperature reactions nor chromatographic purifications rendering an overall yield of 18−21%.[22a]

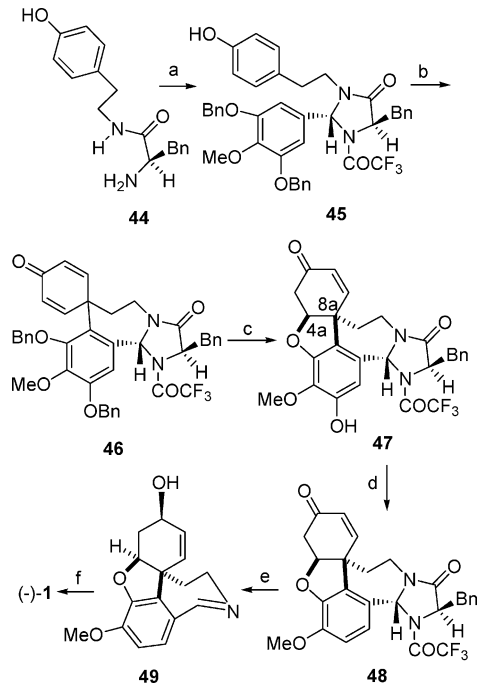
In 2004, a very creative synthesis of (−)-galantamine based on the *remote asymmetric induction* concept was published by Node and co-workers.[19b] The synthesis started with the reaction of tyramine with (*R*)-*N*-BOC-D-phenylalanine providing compound **44** (Scheme 13), whose reaction with 3,5-dibenzyloxy-4-methoxybenzaldehyde gave an imine, which after acid treatment cyclized to yield imidazolidinone **45**, isolated practically as the diastereomerically pure trans isomer. This outcome was ascertained by a nuclear Overhauser effect (NOE) experiment in the ^1H NMR spectrum. The oxidative phenol coupling reaction provided dienone **46** in a notorious 61% yield. The final steps of the synthesis regarding compounds **46**−**49**, via intermediate **48**, are based upon the previous report of this group on the synthesis of the racemate[19a] with the necessary adjustments for the elimination of the imidazolidinone residue. Note also that the chirality of the starting material, D-phenylalanine,

Scheme 12[a]



[a] Reagents and conditions: (a) $K_3Fe(CN)_6$, H_2O/toluene, Na_2CO_3 (45−50%); (b) 1,3-propanediol (89.5%); (c) i. LAH, ii. HCl (95%); (d) EtOH/Et_3N, cat (−)-**3** (80%); (e) i. L-Selectride, ii. HBr (99%).

Scheme 13[a]



[a] Reagents and conditions: (a) i. 3,5-dibenzyloxy-4-methoxybenzaldehyde, rt, ii. HCl, dioxane (80%), iii. $(CF_3CO)_2O$, pyr (94%); (b) PIFA, CF_3CH_2OH, −40 °C (61%); (c) i. BCl_3, −78 °C (95%); (d) i. Tf_2O, pyr (83%), ii. $Pd(OAc)_2$, PPh_3, HCO_2H, DMF (100%); (e) i. L-Selectride, THF, −78 °C (78%), ii. KOH, ETOH (96%); (f) i. $NaBH_4$, MeOH, then HCO_2ET (100%), ii. LAH, THF (94%).

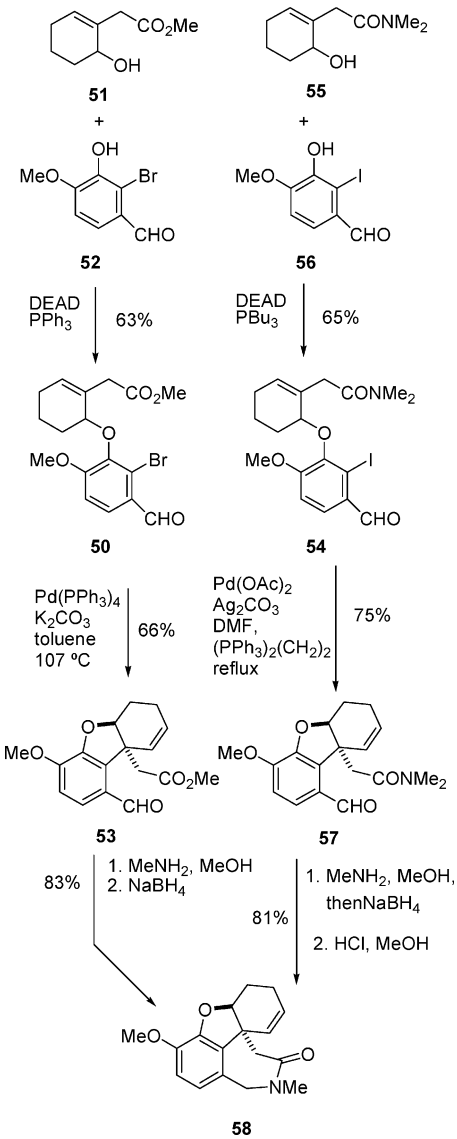
controlled the formation of the new stereocenters at C-8a and C-4a in a very efficient way during the selective deprotection of one of the *O*-benzyl ethers, since only one isomer was detected and isolated after the purification of compound **47** (Scheme 13). It is also worth mentioning that in contrast to Koga's approach[21] (see Schemes 10 and 11), the stereocenter present in the α-amino acid used as chiral auxiliary is outside the forming azepine ring system installed in an imidazolidinone, which despite the apparent remote distance, promotes a total asymmetric induction.

2.2. Synthesis Using the Intramolecular Heck Reaction

2.2.1. Synthesis of 6-Deoxygalantamine Derivatives

In 2000 Fels[23] and in 2001 Parsons[24] reported a stereoselective approach toward the galantamine ring system based on the intramolecular Heck reaction.[11] Fels prepared the cyclohexenyl,aryl ether **50** from β,γ-unsaturated ester **51** and benzaldehyde **52**. Afterward, compound **50** was submitted to reaction with tetrakis(triphenylphosphine) palladium(0) in the presence of potassium carbonate, and compound **53** was obtained in 66% yield (Scheme 14).

Scheme 14



Similarly, starting from β,γ-unsaturated amide **55** and benzaldehyde **56**, Parsons synthesized iodide **54**. Thereafter, benzofurane **57** was obtained in 75% yield by refluxing

iodide **54** with $Pd(OAc)_2$ and silver carbonate in DMF (Scheme 14). Finally, both compounds **53** and **57** were transformed into the same derivative **58**.

However, it is interesting to mention that these authors have not pursued a total synthesis of galantamine concerning this approach. This was reserved for Trost's group (see below) who reported a synthesis of (−)-galantamine in 2000[25a] and an improved version of this synthesis 2 years later,[25b] by using an intramolecular Heck reaction on chiral related substrates.

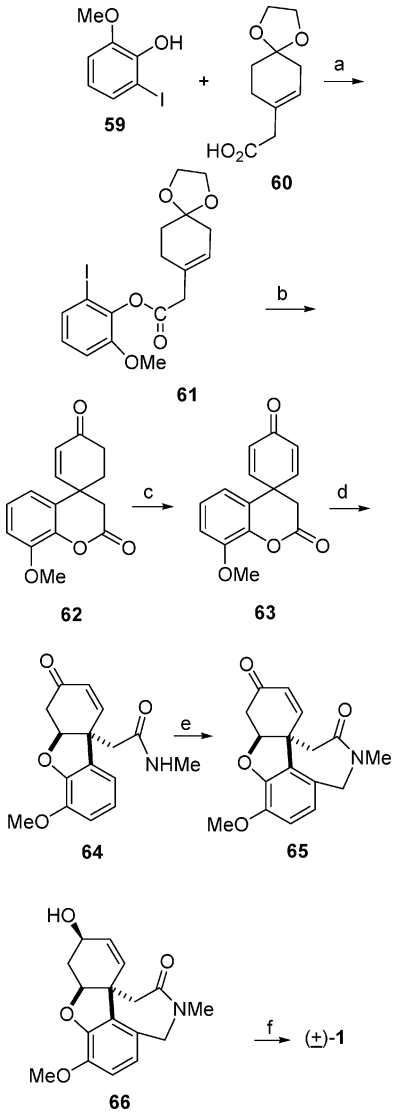
2.2.2. Synthesis of Racemic Galantamine

On the basis of their formal synthesis of lycoramine by using an intramolecular Heck reaction,[9c] in 2001 Guillou and Thal reported[26a] the total synthesis of racemic galantamine following a similar strategy.[26b] This group reported the first use of an intramolecular reaction as an alternative for creating the spiro quaternary carbon atom of galantamine type of alkaloids. In contrast with the other approaches[23−25] simultaneously developed, the aryl iodide partner **59** was devoid of any carboxaldehyde moiety and the β,γ-unsaturated ester **61** did not show an allylic alcohol but a protected ketone (Scheme 15). This selection of functional groups completely changed the structure of precursor **61** for the intramolecular Heck reaction. Moreover, it was also useful in the last steps of the synthesis since the problems observed by other authors in the allylic oxidation were excluded in this case (compare with the synthesis reported by Trost et al.,[25] see above). In addition, the known high stereoselectivity in the reduction of the ketone with L-Selectride secured the efficient formation of the expected allylic alcohol with the correct relative configuration. The Heck reaction, promoted by palladium-*trans*,*trans*-dibenzylideneacetone in the presence of dppe [1,2-bis(diphenylphosphanyl)ethane] and tallium acetate, afforded compound **62** in 67% yield. The formation of dienone **63** was more difficult than envisaged, but the use of $(PhSeO)_2O$ in the presence of molecular sieves solved the problem affording the product in 50% yield. The stereoselective transformation of dienone **63** into benzofurane **64** is one of the most interesting and original contributions of this synthetic approach. Thus, this product is the result of the reaction of lactone **63** with methylamine, the opening of the ring, and formation of the corresponding amide with a free phenol group that spontaneously attacked the α,β-unsaturated ketone. Finally, the tetracyclic ring system of galantamine was assembled by simple electrophilic aromatic substitution of an iminium ion formed in the reaction of amide **64** with paraformaldehyde in the presence of trifluoroacetic acid. The resulting ketone **65** was reduced with L-Selectride, and racemic galantamine was finally obtained by LAH reduction of amide **66**. As stated by the authors, the 2001 synthesis of galantamine was achieved in eight steps with an overall yield of 12%. It was the first efficient total non-biomimetic synthesis of galantamine.

2.2.3. Synthesis of (–)-Galantamine

Trost and Toste published the second asymmetric synthesis of (−)-galantamine in 2000[25a] and an improved version of this synthesis in 2002[25b] by using as key steps the formation of the O4−C4a bond by a palladium-catalyzed asymmetric allylic alkylation (AAA)[27] and an intramolecular Heck reaction to prepare the quaternary center C8a. Both syntheses started with the same intermediate (**67**), prepared in the reaction of 2-bromovanillin **52** with carbonate **68** in the presence of ligand **69**. In this process, compound **67** was obtained in 72% yield and in 87−88% ee on a 24 mmol scale. The absolute configuration at the newly formed stereocenter was confirmed after completion of the total synthesis of (−)-galantamine as shown in Scheme 16.

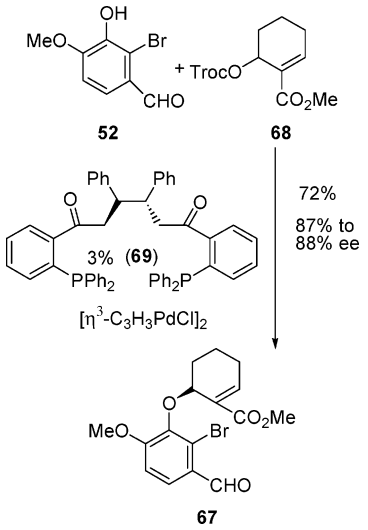
Scheme 15[a]



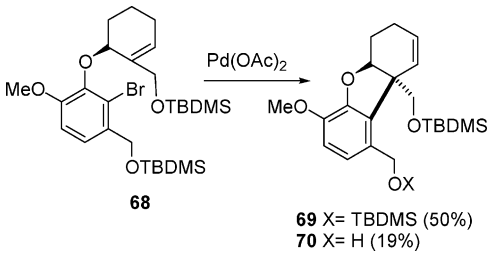
[a] Reagents and conditions: (a) EDCI, DMAP, CH_2CI_2 (80%); (b) i. $Pd_2(dba)_3$, dppe, TlOAc, CH_3CN (67%), ii. Ph_3CBF_4, CH_2Cl_2 (100%); (c) $(PhSeO)_2O$, CH_2Cl_2 (50%); (d) 40% aq $MeNH_2$, THF (100%); (e) i. $(CH_2O)_n$, TFA (63%), ii. L-Selectride, THF, −78 °C (93%), ii. KOH, EtOH (96%); (f) LAH, DME (80%).

All attempts to carry out the intramolecular Heck reaction on compound **67** failed, leading to phenol **52**. According to a statement from Larock,[28] it was argued[25a] that the presence of an electron-withdrawing group (CHO) favoring the palladium-promoted ionization was apparently the reason for this undesired reaction. Therefore, the authors prepared compound **68** by total reduction (DIBAL-H), followed by persilylation, whose Heck reaction was still problematic, but conditions were found to provide the expected product as a mixture of compounds **69** and **70** (Scheme 17). However, the simultaneous articles from Fels[23] and Parsons[24] clearly showed that the Heck reaction on precursors bearing a carboxaldehyde function worked satisfactorily. Thus, in 2002 Trost prepared nitrile (**71**) (Scheme 18) with the carboxaldehyde in the aromatic moiety, eliminating the electron-

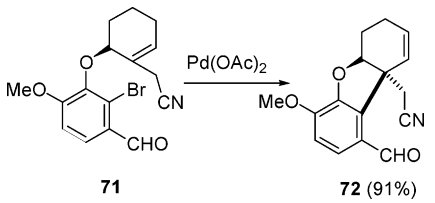
Scheme 16



Scheme 17



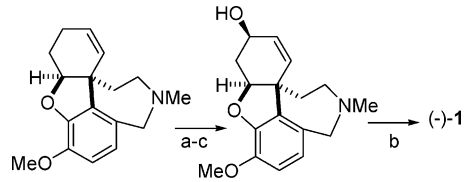
Scheme 18



withdrawing group (CO_2Me) directly bonded to the cyclohexenyl ring, and performing one-carbon homologation via the corresponding alcohol. As expected, compound **72** was obtained in 91% yield. In view of these results, it was surprising that the authors did not use the readily available compounds of type **51** or **55** (Scheme 14) at the AAA step.

Looking back to the first 2000 synthesis of (−)-galantamine,[25a] compounds **69** and **70** (Scheme 17) were submitted to a total desilylation reaction, followed by selective benzylic oxidation to generate an intermediate that was submitted to a series of reactions with the purpose of forming the tetracyclic core of (−)-**1**. First of all, they were treated with methylamine, followed by reduction with sodium cyanoborohydride and *N*-BOC protection. On the other hand, oxidation of the alcohol, Wittig olefination, and acid hydrolysis afforded an aldehyde that was submitted to a reductive Mannich amination protocol to produce compound **73** (Scheme 19) in 16% overall yield from compounds **69** and **70** (Scheme 17). At this stage of the synthetic sequence, it was only necessary to functionalize the C-ring. To do so, the allylic oxidation was investigated but without success. Thus, an alternative, tedious four-step protocol was developed to incorporate the C6 hydroxyl group in place, which ended with the synthesis of alcohol **74** (Scheme 19). Finally, when this compound was treated with Osborn's rhenium-

Scheme 19[a]

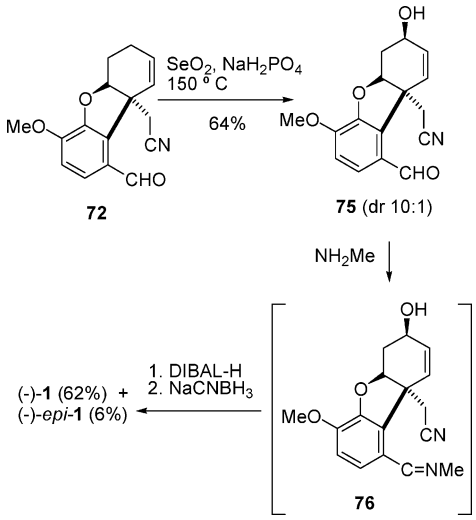


[a] Reagents and conditions: (a) i. TsOH, ii. DMDO/acetone, iii. DBU (45%); (b) PhseSePh, $NaBH_4$ (98%); (c) $NaIO_4$, then 80 °C (64%); (d) $Ph_3SiOReO_3$ (50%).

(VII) catalyst a product was obtained that was identical to natural galantamine (Scheme 19).

In the 2002 synthesis of (−)-galantamine, these problems were solved in a more efficient procedure. As shown in Scheme 20, the allylic oxidation of compound **72** using SeO_2

Scheme 20



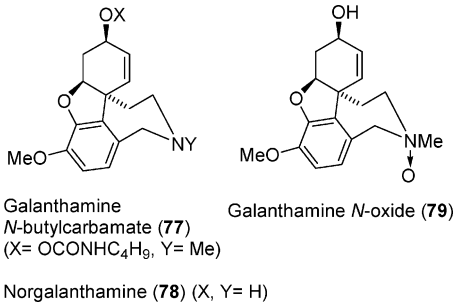
rendered compound **75** in moderate yield as a mixture of isomers in a 10:1 ratio, as a result of the preferred oxidation from the more hindered concave face through an ene mechanism. This mixture was converted to galantamine and to its epimer in one-pot process via reaction with methylamine, followed by reduction with DIBAL-H. Not only was the imine reduced in this reaction, but the nitrile was converted to an aldehyde and trapped in situ to yield a presumed hemiaminal, which was reduced with sodium cyanoborohydride to afford the final products (Scheme 20). As stated by the authors, the 2002 synthesis of galantamine[25b] is shorter (eight steps) and more efficient [96% ee, 14.8% overall yield from **52** and **68** (Scheme 16)] than the synthesis reported in 2000[25a] (14 steps, 88% ee, 1.5% overall yield).

3. Synthesis of Galantamine Analogues

In 1992 Joullié and co-workers published a substantial work on the synthesis, molecular modeling, and acetylcholinesterase inhibitory activities of a series of galantamine analogues.[29a] Prior to this work, a paper on the spectroscopic studies of galantamine and galantamine methiodide was published.[29b] The structure−activity studies led these authors to select four sites for chemical modification: (a) the hydroxyl function, (b) the cyclohexanol ring, (c) the tertiary amine site, and (d) the methoxy function. A number of galantamine analogues were prepared accordingly, including modified C-ring, 3-carbamate, 3-ester, and ammonium

derivatives. From these compounds, galantamine *n*-butylcarbamate (**77**) (AChE inhibitory activity, $IC_{50} \pm$ SEM 10.9 $\pm$ 0.1 μM; compare with (−)-**1**, $IC_{50} \pm$ SEM 3.97 $\pm$ 0.9 μM) (Chart 4) was particularly interesting because it was

Chart 4



Galanthamine *N*-butylcarbamate (**77**) (X= $OCONHC_4H_9$, Y= Me)

Galanthamine *N*-oxide (**79**)

Norgalanthamine (**78**) (X, Y= H)

behaviorally active and improved performance of a passive avoidance task in a dose-dependent manner in both control and basal forebrain lesioned mice.[29a]

Of critical importance for the design of new galantamine analogues, Guillou and colleagues considered the synthesis of norgalantamine (**78**) (Chart 4).[26b] Previous attempts in other laboratories had been unsuccessful.[29a] At last, this was possible via a nonclassical Polonovski−Potier reaction,[30] after demethylation of the galantamine *N*-oxide (**79**) with hydrated ferrous sulfate in methanol at 10 °C, in 76% yield.[26b] From this compound, the way for the synthesis of the pursued bis-interacting ligands in the galantamine series was set up.[26c,d] The rationale for this project was based on the crystallographic structure of AChE from *Torpedo californica (Tc)*,[31a] as well as the structure of *Tc*AChE complexed with (−)-galantamine. [31b]

The AChE active site accommodates a catalytic triad (Ser200, His440, Glu327) located at the bottom of a deep and narrow gorge lined with aromatic residues and a subsite including Trp84 located near the bottom of the cavity. Trp84 has been identified as the binding site for ACh, decamethonium, and edrophonium. In addition, Trp279, located at the peripheral site at the opening of the gorge, is involved in the binding of the second quaternary group of decamethonium. The distance between Trp84 and Trp279 is 12 Å. The structure of the complex of (−)-galantamine and *Tc*AChE,[31b] solved by Sussman and co-workers by X-ray at 2.3 Å resolution (Figures 2 and 3), shows that (−)-galantamine binds at the base of the active site gorge of *Tc*AChE

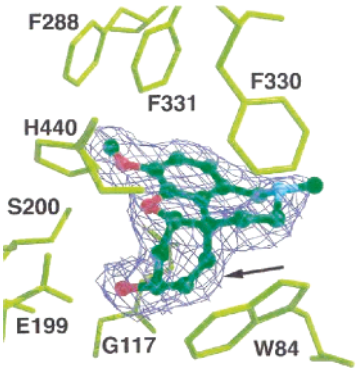


Figure 2. Electron density map from the galantamine *Tc*AChE complex. The double bond of the cyclohexene ring in galantamine (shown as a ball-and-stick model in green) is indicated by the arrow. Reprinted with permission from ref 31. Copyright 1999 Elsevier B.V.

interacting with both the acyl-binding pocket and the indole ring of Trp84. The tertiary amine group of (−)-galantamine did not interact with Trp84 as was expected; instead a $\pi-\pi$ interaction between the double bond of the cyclohexene ring was observed.

Therefore, with these results in mind it was assumed that bis-ligands could simultaneously interact with the active and peripheral sites; hence the activity of such ligands could be greatly improved.[32] Starting from norgalantamine (**78**) (Chart 4), using standard and simple chemistry, the French authors prepared and evaluated the AChE inhibitory activities of compounds **80**, **81**,[26c] and **82**[26d] (Chart 5). These compounds were found to be more active than galantamine and tacrine in inhibiting AChE, thus confirming the working hypothesis. Very recently, Sussman and co-workers have published the complexes of derivatives **80**, **81**, and **83**[26c] (Chart 5) with *Tc*AChE,[31c] solved by X-ray analysis, and have confirmed the postulated double interaction with both peripheral and catalytic sites of the enzyme, since the nitrogen atom is oriented up to the gorge. Cocrystallization of bis-interacting ligand **81**[31c] provided evidence for the so-called "back-door" hypothesis.[31d,e] This hypothesis was prompted by the fact that bulky reversible AChE inhibitors such as huperzine A and B or galantamine are able to reach the active site. The *Tc*AChE−compound **81** complex suggested the facile rearrangement of the Trp279−Ser291 loop, which may produce a significant increase in the diameter of the gorge, thus facilitating the entry of the inhibitors.[31c]

The iminium salt of galantamine (**83**) (Chart 5) was shown to enhance learning and memory in young and old rats confirming its ability to cross the blood−brain barrier (BBB).[26f,g] Thus, the presence of an iminium function on galantamine could be in equilibrium with its neutral carbinol amine form and favor the molecule to cross the BBB, whereas quaternary ammonium salts prepared by Joullié and co-workers did not cross the BBB.[29]

The French group reported the synthesis of the open D-ring galantamine analogues such as **84**−**86** (Chart 6) that manifested lower activity than the parent compound, galantamine or tacrine.[26e]

Similarly, the galantamine sulfur analogue (**87**) (Chart 6) described by Jordis and associates[22c] was devoid of any noticeable AChE or butyrylcholinesterase (BuChE) inhibitory activity. This group also transformed galantamine into compound **88** (Chart 6), which belongs to the well-known crinine-type of alkaloids, which showed no inhibitory activity toward AChE.[22d] More recently, Jordis communicated the synthesis of the 11-aza analogue of galantamine (**89**, Chart 6), using the oxidative phenol coupling reaction promoted by potassium ferricyanide[22a,b] on suitable adapted precursors. The biological activity of this compound remains to be published elsewhere.[22e] Jordis' group also published the synthesis of *N*-chiral quaternary *N*-alkyl galanthanium halides and their stereoselective dealkylation reaction promoted by L-Selectride or Super Hydride.[22f]

In conclusion, a Chinese group described the synthesis of the galantamine analogue **90** (Chart 6),[33] referring to its very poor activity as AChE inhibitor ($IC_{50} = 150$ μM).

4. Pharmacological Profile

In this section, we will review the effects of galantamine as an AChE inhibitor, an allosteric potentiator of neuronal nicotinic receptors for acetylcholine (nAChR), a modulator of neurotransmitter release, and an agent causing neuropro-

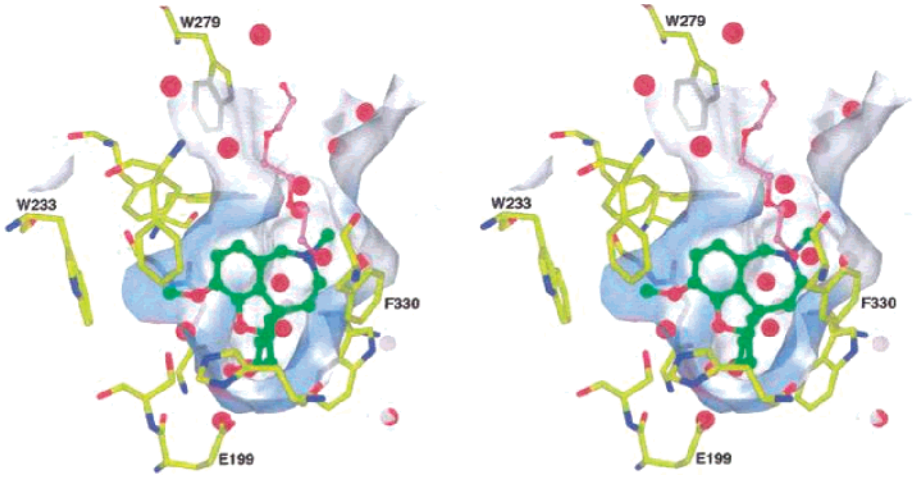
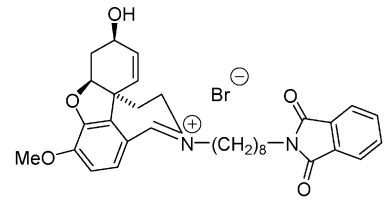
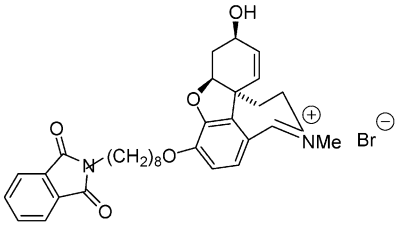


Figure 3. Stereoview of galantamine in the active site gorge of *Tc*AChE. Galantamine is shown as a ball-and-stick model in green. Reprinted with permission from ref 31. Copyright 1999 Elsevier B.V.

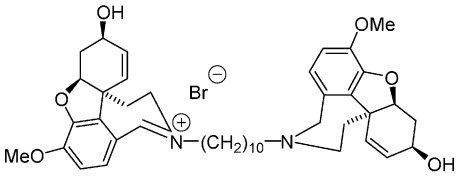
Chart 5



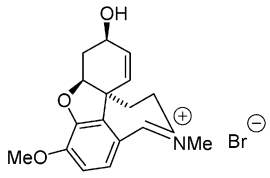
80 (IC_{50}= 0.1 x 10^{-7} ± 0.02)



81 (IC_{50}= 4.99 x 10^{-7} ± 99)

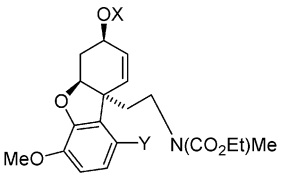


82 (IC_{50}= 12 x 10^{-9} ± 1)

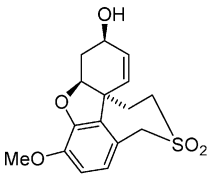


83 (IC_{50}= 7.02 x 10^{-7} ± 90)

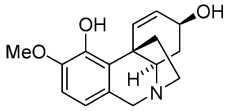
Chart 6



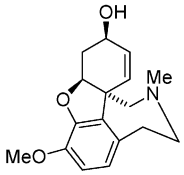
(**84**) X= CO_2Et, Y= CH_2Cl
(**85**) X= H, Y= CH_2OH
(**86**) X= H, Y= CHO



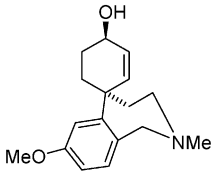
(**87**)



(**88**)



(**89**)



(**90**)

tection through an antiapoptotic action. In so doing, we will try to place galantamine in the context of the treatment of dementia, both of vascular origin and of AD type.

Three clear features are patent in areas such as the parietal and temporal cortices, the hippocampus, the entorhinal cortex, and the amygdala in the brain of AD patients, that is, amyloid plaques, neurofibrillary tangles, and a loss of cholinergic neurons. Amyloid plaques are composed of β-amyloid (Aβ) fragments; Aβ1−40 and Aβ1−42 peptides are neurotoxic and are probably responsible for the loss of brain cholinergic neurons and ACh in the cholinergic nucleus basalis of Meynert and medial septum, the serotonergic raphe nuclei, and the noradrenergic locus coeruleus of the basal forebrain, leading to a loss of the cholinergic innervation of the cerebral cortex.[34−36] In addition, there is a severe loss of nAChRs, which correlates with the severity of the disease at the time of death.[37] These observations have been the basis for the development of cholinomimetic drugs during the last 10 years. Tacrine and physostigmine were members of the first generation of AChE inhibitors, which blocked with similar potency both AChE and BuChE.[38,39] However, they exhibited a high incidence of adverse effects such as hepatotoxicity, and their use in the treatment of AD has been

Table 1. Summary of the Effects of Clinically Relevant Acetylcholinesterase (AChE) Inhibitors Described so Far in the Literature

drugs	AChE inhibition (IC_{50}, nM)		potentiation of purinergic twitches (% E_{max})[g]	other effects
	brain	vas deferens[g]		
tacrine	77,[a] 80,[b] 93,[c] 450[d]	100 ± 20 (n = 3)	43 ± 6	
physostigmine	0.7,[a] 18,[d] 60,[b] 251[c]	700 ± 80 (n = 3)	68 ± 11	
rivastigmine		2100 ± 220 (n = 3)	7 ± 5	blockade of BuChE[e,h]
donepezil	7,[a] 13[c]	4300 ± 510 (n = 3)	4 ± 1	
galantamine	1995[c]	15800 ± 3270 (n = 3)	89 ± 12	blockade of K^+ channels[f]

[a] Rat brain.[52] [b] Mouse brain.[53] [c] Rat cortex.[54] [d] Human cortex.[55] [e] Human cerebrospinal fluid.[56] [f] Chromaffin cells.[57] [g] Reference 58. [h] BuChE = butyrylcholinesterase.

abandoned.[40] Currently, rivastigmine, donepezil, and galantamine are the AChE inhibitors approved for treatment of AD patients. Donepezil and galantamine selectively and reversibly inhibit AChE,[41,100] whereas rivastigmine inhibits both AChE and BuChE[42] in a pseudo-irreversible mode. Furthermore, galantamine is an allosteric modulator of nicotinic acetylcholine receptors (nAChRs).[43,44] Recently, donepezil was reported to inhibit nAChRs with a parallel desensitization effect of the receptor.[45]

Moreover, the noncompetitive and low-to-moderate-affinity *N*-methyl-D-aspartate (NMDA) antagonist memantine[46] is currently approved for the treatment of moderate to severe dementia of the Alzheimer's type. NMDA receptors have an important physiological role in learning and memory. However, NMDA receptor over activation, after increased glutamate release, leads to excessive calcium influx, triggering neuronal death.[47] Due to the rapid unblocking kinetics of memantine, it blocks the pathological but not the physiological activation of NMDA receptors.[46] Regarding the previous properties, Moriguchi[48,49] and collaborators demonstrated that galantamine and donepezil act on NMDA receptors of rat cortical neurons potentiating their activity, and this action together with cholinesterase inhibition would contribute to improvement of learning, memory, and cognition in Alzheimer's patients.

Next, we will focus on the pharmacological profile of galantamine.

4.1. Acetylcholinesterase Inhibition

Mashkovskii[50] first noted that galantamine reversed tubocurare-induced muscle paralysis. Later studies proved that this effect was due to AChE inhibition.[51] These authors estimated that galantamine was more potent than pyridostigmine and less effective than neostigmine in canine muscle homogenates. In the cat brain, they found an IC_{50} of 20 μM.

Table 1 shows the potencies of various AChE inhibitors. Note that physostigmine was the most effective in the brain, followed by donepezil and tacrine and, last, by galantamine. Galantamine was also the least potent AChE inhibitor in the rat vas deferens[58] compared with other compounds currently used for AD treatment. Thus, galantamine seems to be only a modest AChE inhibitor.

4.2. Galantamine as an Allosteric Potentiating Ligand of Nicotinic Acetylcholine Receptors

Galantamine, a good ligand for nAChRs, has been shown to act as an agonist generating single-channel currents in α-bungarotoxin-insensitive α4β2-type nAChRs of cultured rat hippocampal neurons.[59] However, galantamine did not produce a detectable whole-cell current in M10 cells expressing α4β2 receptors.[60] It increases ACh-induced whole-cell currents in PC12 cell α3-type nAChRs, hippocampus α7-type nAChRs,[61,62] α4β2 nAChRs expressed in human embryonic kidney cells,[63] and α7-type nAChR in α7/5-HT_3 chimera.[64] These data point to the concept of galantamine as an allosteric potentiating ligand (APL). By binding to nAChRs at a different site from the ACh binding site, galantamine causes an allosteric change that allows ACh to elicit a greater receptor response. However, the ability of galantamine to elicit single-channel receptor currents, as mentioned above, casts some doubts on the exact interaction of galantamine with nAChRs. Thus, under certain experimental conditions, it may also behave as a partial agonist.

4.3. Enhancement of Neurotransmitter Release

It was expected that with promotion of cholinergic neurotransmission through the APL effect of galantamine an improvement of neurotransmitter release could emerge at certain brain synapses. Thus, galantamine has been shown to enhance γ-aminobutyric acid (GABA) and glutamate release in hippocampus slices,[65] as well as purinergic neurotransmission.[58] In addition, galantamine has been recently proven to enhance the NMDA receptor whole-cell current in cortical neurons.[66] Galantamine also enhances the release of dopamine in mouse striatal slices.[67] These galantamine effects may be the basis for the improvement of behavior in AD patients since dopamine and serotonin, rather than ACh, are involved in these behavioral symptoms.

4.4. Effects on Behavior and Memory Performance in Animal Models

Sweeney and colleagues[68] used a surgical mouse model of memory impairment. Thus, the mice were put through tasks of reference memory and working memory following previous training 3, 5, and 24 h after being given galantamine intraperitoneally. In mice with impaired memory, galantamine enhanced the working memory, which was evidenced by a reduction of time in task performance.

Another animal study[69] was performed in rats that were treated similarly to the mouse model formerly described. The task presented to the rats was passive avoidance, in which they were to remain on a wooden platform surrounded by an electrified grid floor. The rats were given increasing doses of galantamine, tacrine, physostigmine, or solvent. The time spent in passive avoidance increased with all three drugs. Galantamine produced significant improvements at the earliest times and with the lowest doses when compared to physostigmine or tacrine. These data suggested that galantamine could possibly improve some of the deficits in AD patients. Therefore, trials in patients were performed.

Table 2. Summary of Various Placebo-Controlled Trials with Galantamine in Patients with Mild to Moderate Alzheimer's Disease (See Ref 72)[a]

type of design	patients (*n*)	regimens	efficacy parameters	results	ref
PC,DB,R	636	G24 (212) G32 (211) placebo (213)	Δ ADAS-cog Δ CIBIC-plus	Δ ADAS-cog G24 = G32 > placebo[b] Δ CIBIC-plus G24 = G32 > placebo[c]	61
PC,DB,R	978	G8 (140) G16 (279) G24 (273) placebo (286)	Δ ADAS-cog Δ CIBIC-plus	Δ ADAS-cog G24 = G16 > G8 > placebo[c] Δ CIBIC-plus G24 = G16 > G8 > placebo[c]	62
PC,DB,R	653	G24 (220) G32 (218) placebo (215)	Δ ADAS-cog Δ CIBIC-plus	Δ ADAS-cog G24 = G32 > placebo[b] Δ CIBIC-plus G24 = G32 > placebo[c]	37

[a] ADAS-cog = Alzheimer's disease Assessment Scale, cognitive subset; CIBIC-plus = Clinician's Interview-Based Impression of Change plus Caregiver Input; DB = double blind; G8 = galantamine, 8 mg/d; G16 = galantamine, 16 mg/d; G24 = galantamine, 24 mg/d; G32 = galantamine, 32 mg/d; PC = placebo-controlled; R = random; Δ = change in. [b] $p < 0.001$. [c] $p < 0.05$.

4.5. Clinical Efficacy in Alzheimer's Disease Patients

The first report on the clinical efficacy of galantamine emerged in 1990. Thomsen[70] and co-workers gave galantamine to a 60-year woman with Alzheimer's disease. At 50−70% of AChE inhibition, a marked reduction in episodes of confusion associated with an improvement of the outward appearance as well as in her performance of daily tasks was observed after 140 days of treatment.

Rainer[71] conducted an open-label study giving galantamine 15 mg/day to nine AD patients for 8 weeks. Strong trends toward improvement in the psychometric tests were observed. All the clinical general impressions reported at least moderate recovery in all nine patients. In another similar open-label study,[72] 18 patients were given galantamine 30 mg/day. Six patients continued the treatment for 1 year. According to their caregivers, these six patients showed considerable progress in their daily living and emotional stability. Three of the six patients experienced improvements in their neuropsychiatric test performance.

These pilot studies provided the impetus for the study of galantamine in larger populations of AD patients. Three well-designed double-blind randomized placebo-controlled trials were performed. They are summarized in Table 2. The study accomplished by Raskind and associates[73] included 636 patients with mild to moderate AD. Patients treated with galantamine had sustained improvements over the 6-month double-blind phase that were different from placebo. The placebo recipients who were switched to galantamine 24 mg/day gained enough cognitive ability to parallel those patients initially treated with galantamine.

The second study directed by Tariot and collaborators[74] included 978 patients with mild to moderate AD. Galantamine given at 16 and 24 mg/day resulted in a significant benefit after 5-month treatment. Placebo patients revealed deterioration after 5 months. The third large trial enrolled 653 patients with mild to moderate AD.[37] The results were similar to those of the two previous trials; that is, patients who received galantamine 24 or 32 mg/day presented better cognitive ability than those who received placebo. A later study performed in patients with "advanced moderate" AD demonstrated that galantamine provided sustained benefits, as previously shown for mild to moderate patients. It is interesting that 51% of those patients improved their cognition scores, which were maintained above baseline for as much as 1 year.[75] Another study was also completed in patients with probable vascular dementia or AD combined with cerebrovascular disease. This was a double-blind, multicenter, placebo-controlled, and randomized study. The authors concluded that galantamine revealed therapeutic efficacy on all essential areas of cognitive and noncognitive abilities in this group of dementia patients.[76] A Cochrane review concluded that there was evidence for galantamine demonstrating efficacy on global ratings, cognitive tests, activity assessments of daily living, and behavior. The magnitude for the cognitive effect is similar to other cholinesterase inhibitors including donepezil, rivastigmine, and tacrine.[77,78]

Results of cholinesterase inhibitors on behavior and caregiver distress confirm a benefit elicited by galantamine.[79] Other cholinesterase inhibitors present similar effects. Thus, rivastigmine reduced mood disorders and hallucinations in a 2-year open-label extension of a double-blind clinical trial.[80] Furthermore, donepezil therapy was associated with a reduced total Neuropsychyatric Inventory score in patients with moderate-to-severe AD.[81]

4.6. The Neuroprotectant Effects

Agonists for nAChRs increase the synthesis of neurotrophic factors[82] and protect neuronal cells against the cell toxic effects of glutamate,[83] trophic factor deprivation,[84] hypoxia,[85] and β-amyloid.[86] In addition, they improve memory performance and learning in rodents and nonhuman primates, as well as alertness and rapid information processing in humans.[87] In this context, emerged the hypothesis that galantamine, by increasing the effects of ACh at nicotinic receptors, could afford neuronal protection against several neurotoxic stimuli.

Table 3 presents an overview of the "in vitro" studies showing the neuroprotectant effects of galantamine. Upon exposure of neuroblastoma cells or chromaffin cells to Aβ or thapsigargin to disrupt Ca^{2+} homeostasis, apoptotic cell death increased 4.3-fold. Galantamine at 0.3 μM significantly reduced the extent of apoptotic cell death. This protective effect disappeared in the presence of α-bungarotoxin, an α7 nAChR blocker. Furthermore, galantamine enhances the expression of α7 receptors and the antiapoptotic protein Bcl-2,[88] suggesting that the antiapoptotic effects of galantamine are mediated via activation of α7 nAChRs (Figure 4). Galantamine has also been shown to protect neurons against glutamate-induced neurotoxicity[89] and against glutamate plus β-amyloid-induced cell death.[90]

Table 3. Summary of the "in Vitro" Studies Showing Neuroprotectant Effects of Galantamine on Apoptotic Death Caused by Two Different Toxic Stimuli[88,90]

model	galantamine (μM)	toxic stimulus	% protection
human neuroblastoma SH-SY5Y line	0.3	thapsigargin, 3 μM	60
chromaffin cells	0.3	thapsigargin, 3 μM	80
human neuroblastoma SH-SY5Y line	0.3	β-amyloid, 10 μM	80
fetal rat cerebral cortex	0.1−10	β-amyloid and glutamate	52−100

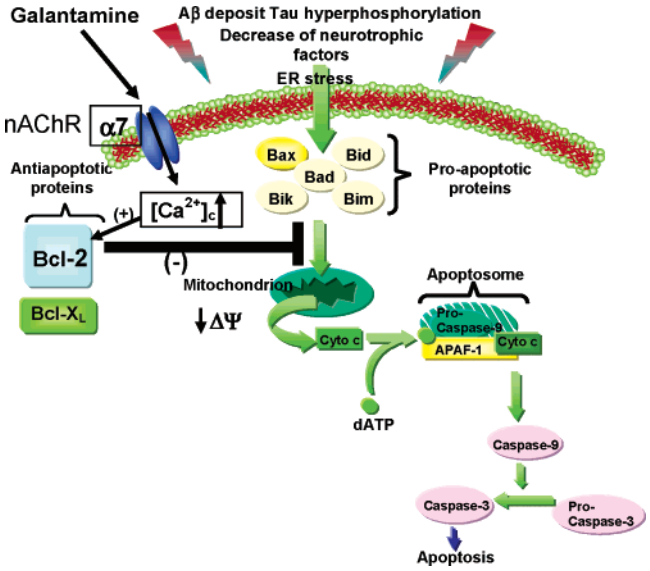


Figure 4. Mechanism of the antiapoptotic effect of galantamine.

In a model of glucose and oxygen deprivation in rat hippocampal slices, galantamine preserves metabolic activity and affords neuroprotection in the micromolar range.[91] These results suggest that the allosteric modulation of nAChRs is a viable option for neuroprotection in a model resembling vascular dementia. At first sight, this may seem a contradictory finding since galantamine has been reported to enhance glutamate release[65] and excess glutamate is neurotoxic during brain ischemic damage. However, this finding is consistent with clinical data showing that continuous NMDA receptor antagonism leads to increased mortality in stroke trials. In contrast, modest sustained activation of NMDA receptors might actually enhance neuronal survival in stroke models, especially in the penumbra area.[92] Moreover, a modest increase of glutamate release through the galantamine APL effect on α7 nAChRs[65] also activates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, leading to enhanced secretion of brain-derived neurotrophic factor (BDNF), which is fundamental for the survival of glutamatergic pyramidal neurons.[93]

The last study was performed "in vivo", in anti-neuronal growth factor (NGF) AD11 transgenic mice. The mice were treated with galantamine (3.5 mg/kg) from 2 weeks to 2 months. In this model of AD, overexpression of the anti-NGF removes free NGF, effectively reducing the amount of NGF available for normal activity of cholinergic neurons, and consequently, the number of those neurons is reduced drastically. Treatment with galantamine restored the number of cholinergic neurons in the anti-NGF mice, almost to the level of the age-matched controls. The mice also developed amyloid precursor protein (APP) deposits along the brain vasculature, and galantamine reduced these APP deposits by 80%. It is suggested that these effects are most likely associated with allosteric activation of α7 receptors, since the most potent AChE inhibitors, tacrine and physostigmine, are unable to afford neuroprotection. Furthermore, at the dose of 3.5 mg/kg galantamine, only a small amount of the brain AChE is inhibited.[94]

Recent clinical data strengthen the validity of the anti-NGF mouse as a model of AD.[95] The insertion of NGF-secreting cells into the brain of patients with mild AD substantially reduces cognitive deterioration over 18 months.[96] Another relevant clinical issue deals with the concentration of galantamine used in laboratory experiments and the levels reached in the brain of animals and patients. The brain levels for galantamine can be deduced from PET imaging studies using PMP or MP4A as tracers, specific to monitor brain AChE inhibition.[97,98] In patients treated for 12 months with flexible doses of 16−24 mg/day galantamine, the inhibition of brain AChE amounts to 37%.[99] This suggests a functional brain galantamine concentration in the range of 0.5−1.2 μM, depending on whether the IC_{50} for galantamine AChE inhibition is considered to be 0.8 μM[100] or 2.3 μM.[101]

In mice, galantamine doses between 1 and 5 mg/kg achieve brain levels in the range of 0.2−1 μM.[94] Therefore, all preclinical studies have used doses and concentrations of galantamine in the range of those reached in clinical conditions.

4.7. Neuroprotection in the Clinic

It is difficult to show neuroprotection in the clinic using functional scales.[102,103] A horizontal slope in any functional scale, which is different from a deteriorating downward slope for a placebo, can indicate a neuroprotective effect. Today, this type of clinical trial is unethical since the placebo arm has to be extended one year at least. On the other hand, symptomatic treatment with the current AChE inhibitors yields a transient improvement of cognitive scales during the first 3 months, followed by a decline where the slope becomes parallel to the placebo slope. The upregulation of AChE during chronic treatment[104] may, additionally, mask the neuroprotection measured with functional scales; this is particularly true for donepezil since it causes quite substantial upregulation of AChE.

A 3 year study of galantamine in AD patients has been recently disclosed.[105] For ethical reasons, no placebo arm was included; the placebo effect was calculated with the Stern equation.[106] One factor to consider is the period to reach the placebo ADAS-Cog value, over which period the treated patients maintain a better quality of life, compared to the gradual deterioration of untreated patients. This period increased from 12 months in a 1-year study to 18 months in a 3-year study. Thus, after 3 years, the treated patients show a decline equivalent to untreated patients after 18 months. This suggests that the slope of deterioration in the treated arm is more moderate than that in the extrapolated placebo arm. In addition, the authors explain that this effect was not due to dropout of nonresponders since the slope of the patients that prematurely abandoned the trials was similar to the slope of treated patients. This is often a confusing factor since only responders are retained in such long-term clinical trials.

This result has been confirmed in a 4 year extension study[107] where 185 patients treated with galantamine lost on

average only 13 points on the ADAS-Cog scale, compared to a 25 point decline for the extrapolated placebo group. In this case, the galantamine effect is equivalent to the course of an untreated patient after 24 months, suggesting a 24 month delay in the progress of the disease.

4.8. Metabolism and Excretion of Galantamine

In studies carried out with different species (rats, dogs, and humans), galantamine and its metabolites were predominantly excreted in the urine (from 60% in male rats to 93% in humans). In extensive metabolizers for CYP2D6, urinary metabolites resulting from O-demethylation represented 33.2% of the dose compared with 5.2% in poor metabolizers, which showed correspondingly higher urinary excretion of unchanged galantamine and its *N*-oxide. Genetic polymorphism in the expression of CYP2D6 is not expected to affect the pharmacodynamics of galantamine.[108]

5. Conclusions and Perspectives

Galantamine is an alkaloid that has attracted the interest of both organic chemists and pharmacologists, in a collaborative effort aimed at designing, preparing, and evaluating novel biologically active compounds for AD treatment. In this review, we have shown how an apparently complex molecule such as galantamine can be very efficiently prepared in a few steps, in racemic or in enantiomerically pure form, by using basically two key reaction protocols, the phenol oxidative coupling reaction and an intramolecular Heck reaction. The first method is not as sophisticated as the second one, but the yields of the resulting product range from low to moderate.

From the pharmacological point of view, galantamine is not a mere AChE inhibitor, since its surprising and clinically relevant neuroprotectant effects are not exclusively explained by its enzyme inhibitory activity. Neuroprotection afforded by galantamine is mediated by α7 nAChRs and by the overexpression of the antiapoptotic protein Bcl-2. This effect is probably linked to its APL response on such receptors. A correlation between these laboratory studies and the clinical course of AD patients treated with galantamine seems to exist. A number of functional long-term clinical studies with high retention rates of patients indicate a substantial delay in cognitive deterioration in the long term (up to 4 years). This is consistent with a neuroprotective effect beyond the mere symptomatic effects attributed to AChE inhibitors. These data suggest the existence of a window of therapeutic opportunity to test and validate novel neuroprotective agents in AD patients and other types of dementia. The experience with galantamine may pave the way for improved clinical development of new nAChRs ligands with more suitable properties than galantamine, that is, disease modifying or neuroprotective compounds.

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