

# Accurate prediction of the bound conformation of galanthamine in the active site of *Torpedo californica* acetylcholinesterase using molecular docking

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The alkaloid (−)-galanthamine is known to produce significant improvement of cognitive performances in patients with the Alzheimer's disease. Its mechanism of action involves competitive and reversible inhibition of acetylcholinesterase (AChE). Herein, we correctly predict the orientation and conformation of the galanthamine molecule in the active site of AChE from *Torpedo californica* (TcAChE) using a combination of rigid docking and flexible geometry optimization with a molecular mechanics force field. The quality of the predicted model is remarkable, as indicated by the value of the RMS deviation of ~0.5 Å when compared with the crystal structure of the TcAChE-galanthamine complex. A molecular model of the complex between TcAChE and a galanthamine derivative, SPH1107, with a long chain substituent on the nitrogen has been generated as well. The side chain of this ligand is predicted to extend along the enzyme active site gorge from the anionic subsite, at the bottom, to the peripheral anionic site, at the top. The docking procedure described in this paper can be applied to produce models of ligand-receptor complexes for AChE and other macromolecular targets of drug design. © 2001 by Elsevier Science Inc.

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## INTRODUCTION

Nicotinic cholinergic neurotransmission is known to be intimately associated with the recognition, learning, and memory.<sup>1</sup> Degeneration of cholinergic neurons in the basal forebrain causes a neuropathological disorder known as Alzheimer's disease (AD).<sup>2</sup> It has been shown that the density/activity of brain nicotinic acetylcholine receptors (nAChR) is substantially reduced in AD patients compared with a control group of the same age.<sup>3</sup> These abnormalities are closely associated with increased levels of neuritic plaques and neurofibrillary tangles that are usually considered the primary histopathological changes in AD patients.<sup>4</sup>

Based on these well-established findings, the majority of current drug therapeutic approaches to AD follow the cholinergic hypothesis. These approaches are aimed at elevating the transient levels of acetylcholine in the brain that may be achieved by inhibiting acetylcholinesterase with reversible inhibitors.<sup>5–7</sup> Inhibition of AChE is considered one of the most promising strategies for the treatment of AD and related diseases.<sup>8,9</sup> There are also possible therapeutic applications in the treatment of Parkinson's disease,<sup>10</sup> aging,<sup>10</sup> and myasthenia gravis.<sup>11</sup> Over the years, hundreds of compounds have been synthesized and tested for anticholinesterase activity, and several of them have found clinical applications.<sup>12</sup> The chemical structures of these inhibitors are very different, ranging from bis-quaternary compounds such as decamethonium (DME),<sup>13</sup> to simple mono cationic compounds such as edrophonium (EDR),<sup>14</sup> and formally neutral tricyclic compounds such as

tacrine (THA).<sup>13</sup> Due to its important therapeutic role, the enzyme has been a subject of many recent experimental<sup>15–20</sup> and theoretical<sup>21–26</sup> investigations.

Among the different classes of AChE inhibitors reported to date,<sup>13,14,27–29</sup> galanthamine (Figure 1) stands out as a very potent and promising drug for treatment of the cholinergic deficit in AD patients. It exhibits very low toxicity and side effects and presently is in the final stages of clinical evaluation.<sup>5</sup>

Galanthamine is an *amaryllidaceae* alkaloid that was first isolated in Russia<sup>30</sup> and structurally elucidated in Japan.<sup>31</sup> The difficulty of isolating galanthamine from its natural source severely hindered its use as a commercial drug or even as a starting material for the synthesis of derivatives. There are, however, a number of total synthesis procedures presently available, of which those via (−)-narwedine<sup>32</sup> are the most convenient and have been extensively employed for large-scale industrial preparation.<sup>33</sup> In addition, a number of galanthamine derivatives have been synthesized<sup>34</sup> and tested against TcAChE and human AchE (HuAChE) to understand the underlying mechanism of galanthamine action.

The successful design of novel potent inhibitors of AChE could be greatly facilitated by rational analysis of experimental structure–activity relationships among the enzyme inhibitors and accurate prediction of their conformations in the enzyme bound form. Recent X-ray crystallographic analysis of AChE from *Torpedo Californica* (EC 3.1.1.7)<sup>35</sup> followed by X-ray determination of the complexes of the enzyme with several structurally diverse inhibitors, such as THA,<sup>13</sup> EDR,<sup>14</sup> E2020,<sup>28</sup> and decamethonium,<sup>13</sup> provided crucial information with respect to the orientation of these inhibitors in the active site of the enzyme. More recently, the structure of TcAChE galanthamine complex was also elucidated by X-ray crystallography to 2.5 Å resolution (1QTI).<sup>36</sup> The crystallographic data indicated that most of these inhibitors have unique, largely non-overlapping binding orientations in the active site of the enzyme. The results of the structural analysis of these and several other AChE-ligand complexes<sup>18,27,29</sup> suggest that accurate prediction of the bound conformation and orientation of the AChE ligands represents a challenging task for molecular modelers.

Several molecular docking strategies have been developed over the years, following the seminal work of Kuntz et al.<sup>37</sup> (e.g., DOCK,<sup>38</sup> CAVEAT<sup>39</sup>, FlexX,<sup>40</sup> HAMMERHEAD<sup>41</sup>, BUILDER,<sup>42</sup> MSMC,<sup>43</sup> AUTODOCK,<sup>44</sup> and QXP<sup>45</sup>). Although some of these methods have been more popular than

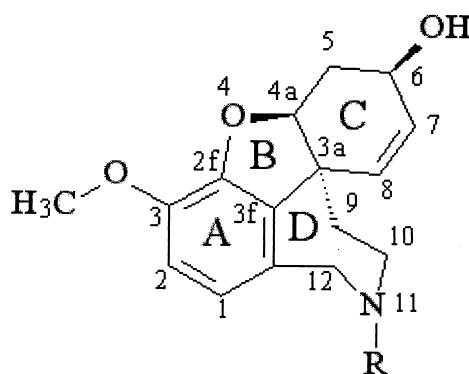
others, the abundance of alternative algorithms and methods stresses that there is no single best procedure applicable in all cases.<sup>46</sup>

In this article, we report an accurate prediction of the bound conformation of galanthamine (~0.5 Å RMS deviation from the X-ray crystal structure of the TcAChE-galanthamine complex for all heavy atoms) using an ad hoc docking strategy. The work reported herein was accomplished well before the crystal structure of the TcAChE-galanthamine complex was elucidated,<sup>36</sup> and is based on structural information derived from available crystal structures of a number of TcAChE-drug complexes. We have used a combination of rigid docking procedure of AUTODOCK<sup>44</sup> with flexible optimization of resulting complexes with the Tripos force field.<sup>47</sup> In addition, we have evaluated the ability of the recently developed SCORE method<sup>48</sup> to select most accurate binding orientation of galanthamine from multiple predictions generated from AUTODOCK runs and optimized with the Tripos forcefield. Using this ad hoc methodology, we have also predicted the bound conformation of a galanthamine derivative, SPH1107, which exhibits a long chain substituent at the nitrogen atom of ring D (see Figure 1).

The results of this study provide an important insight into the mode of esterase inhibition by galanthamine and its derivatives. Furthermore, the success of the strategy employed in this work provides a foundation for future docking studies of existing and de novo designed galanthamine derivatives. Finally, the docking strategy described in this paper can be applied to other classes of ligands and their pharmacological receptors.

## METHODS

The protein crystallographic database<sup>49</sup> currently contains seven structures of TcAChE-ligand complexes, including 1ACJ (tacrine),<sup>13</sup> 1ACL (decamethonium),<sup>13</sup> 2ACK (edrophonium),<sup>14</sup> 1VOT (huperzine A),<sup>27</sup> 1EVE [donepezil (E2020)],<sup>28</sup> 1AMN (the transition state model m-(*N,N,N*-trimethylamino)-trifluoroacetophenone),<sup>29</sup> and 1OCE (acylated TcAChE by the physostigmine analogue MF268).<sup>19</sup> The catalytic triad, which is formed by residues Ser200, His440, and Glu327, is located at the bottom of a 20 Å-deep gorge lined by hydrophobic residues. The active site of the enzyme is very wide, and the structural analysis of existing complexes indicates that all inhibitor molecules interact at the bottom of the gorge, with additional contacts, for some of them, with residues along the



galanthamine     $R = \text{CH}_3$   
SPH 1107         $R = \text{CH}_2\text{CH}_2\text{CH}_2-\text{N}(\text{Cyclohexyl})$

Figure 1. Structure of galanthamine and the *N*-alkyl derivative SPH1107.

gorge. On the basis of these observations and the fact that galanthamine inhibits AChE via a competitive like mechanism,<sup>50</sup> we first hypothesized that the galanthamine binding site is also located at the bottom of the gorge.

A second major postulate was made with respect to the side chain orientation of Phe330, which is responsible for substrate trafficking down the gorge (Color Plate 1). From a comparison of the published crystal structures of various TcAChE complexes it appears that this residue is the only site of conformational flexibility in contrast with the relative rigidity of the other side chains lining the gorge. Three major orientations of Phe330 have been observed experimentally. The TcAChE-tacrine complex (1ACJ) is characterized by the “closed gate” conformation whereas the TcAChE complexes with decamethonium (1ACL) and donepezil (1EVE) show an “open gate” conformation. In other known TcAChE complexes the Phe330 side chain is orientated in between these two extreme positions; for instance, the half open conformation is observed in the AChE/edrophonium complex (2ACK<sup>14</sup>).

Docking calculations were done both for galanthamine and its N-alkyl derivative SPH1107. We noticed that if the long N-alkyl group of derivative extends into the gorge, only an open gate conformation is possible. Thus, if one assumes that both galanthamine and its derivative bind via a similar mechanism, only this open conformation of the gorge should be assumed for both molecules. However, in principle, galanthamine, which lacks the long side chain, could bind to the active site in all three conformations of the gorge. Thus, for the completeness of this study and in light of three dominant orientations of Phe330 side chain (open gate, half open gate, and closed gate) observed for other inhibitors of AChE, we have considered all three possibilities in separate docking runs for galanthamine and only open gate conformation for SPH1107. As a starting point, we chose the TcAChE coordinates in complexes with decamethonium (1ACL), edrophonium (2ACK), and tacrine (1ACJ) for open, half open, and closed gate docking calculations, respectively. The crystal structure of the TcAChE-galanthamine complex later revealed the orientation of Phe330 to be in an open gate state.<sup>36</sup>

Two additional assumptions with respect to the galanthamine conformation were made in our studies. First, although galanthamine is a fairly rigid molecule, conformational flexibility of the cyclohexenol ring (ring C) and of the seven-member ring (ring D) cannot be neglected. Thus, it seemed reasonable to perform a conformational search on the galanthamine molecule. To this end we have employed a simulated annealing protocol that, in our experience, can efficiently overcome conformational rotational barriers in cyclic saturated systems.<sup>51</sup> This procedure resulted in a conformation with the lowest energy, which closely reproduced the crystal structure of (−)-galanthaminium bromide<sup>52</sup> and therefore was chosen for the ensuing docking studies.

Second, galanthamine contains a tertiary methylamine moiety as part of the seven-member ring (ring D). This group can adopt either an axial or equatorial orientation (Color Plate 2), characterized by practically the same value of molecular mechanics energy. *A priori*, both conformers could account for the biological activity of galanthamine. Therefore, both of them were considered independently in our docking study and are referred to as (eq)- and (ax)- conformers, respectively, in the following discussion.

Docking calculations were performed with the AUTO-

DOCK program<sup>44</sup> as described in the EXPERIMENTAL section using a subset of 42 amino acids that form the complete gorge and the active site of TcAChE. The dimensions of the active site box were set to be 22.5 Å × 22.5 Å × 22.5 Å to consider all gorge residues, including the part that connects the active site pocket at the bottom of the gorge with the peripheral anionic site at the top (see Color Plate 4). Similar docking run analyses were carried out for the open, half open, and closed AchE structures, for both the (eq)- and (ax)-conformers, yielding a total of six different AUTODOCK calculations. Further refinement of the resulting structures of TcAChE-galanthamine complexes was done using molecular mechanics optimization with the Tripos forcefiled.<sup>47</sup> In the course of these calculations, the orientation of Phe330 and Tyr121 (which opposes Phe330 in the gorge) was frozen in runs with half-open and closed state to preserve the respective gate conformations, while all other side chains of the 42 amino acids subset were allowed to relax.

## DOCKING OF GALANTHAMINE

In all calculations, galanthamine was initially placed at or near the catalytic triad (Ser200, His440, Glu327). Five hundred independent AUTODOCK runs for each of the different conformational states of the gorge were performed.

### Open Gate Conformation of the Gorge

As a result of the simulations, only 57 (eq)- and 22 (ax)-galanthamine conformers resided inside the gorge. In all other runs galanthamine was located outside of the gorge at or near the peripheral anionic site. Because of the established competitive binding behavior of galanthamine, only the former structures were considered and classified. Based on the energy values and galanthamine orientation in the TcAChE-galanthamine complexes, AUTODOCK generated six clusters of binding orientation for the galanthamine (eq)-conformer (Color Plate 4a) and four clusters for the (ax)-conformer (Color Plate 4b). The average AUTODOCK energies for all clusters are given in Table 1.

Based on the AUTODOCK energy, the Teq1 orientation should be chosen as the best prediction since it had the lowest energy among all clusters. However, one of the major drawbacks of the AUTODOCK version used in our studies is its inability to deal with the conformational flexibility of both receptor and ligand. To overcome this limitation, we employed the Tripos force field (Pullman-charges) and submitted one representative from each of the resulting clusters from AUTODOCK runs to a geometry optimization. During this process, the subset of 42 amino acids employed in the docking studies was also kept flexible. The results of these calculations are also given in Table 1.

The data in Table 1 show that the models Teq26 and Tax24 correspond to the orientations of galanthamine in the TcAChE-binding site with the lowest Tripos energy, which is different from the ranking based on the AUTODOCK energy. Due to this discrepancy, we have also exploited a recently developed SCORE method<sup>48</sup> to assess the binding free energy of ligands. This method uses an empirical scoring function derived from the analysis of available crystal structures of enzyme-inhibitor complexes and the respective experimentally determined binding constants. The highest SCORE energy corresponds to the highest free energy of binding. The resulting SCORE ranking

**Table 1.** Average energies (kcal/mol), SCORE value, and accuracy of matching (RMSD) the galanthamine x-ray structure in its complex with TcAChE for different orientation clusters of galanthamine in the active site of TcAChE in the open gate configuration. The data are shown only for the lowest energy clusters resulting from 500 AUTODOCK runs for each of the (eq)- and (ax) conformers of galanthamine.

Cluster name	No of structures in cluster	AUTODOCK Energy of complex	Tripos					SCORE value	RMSD (Å)
			Energy of complex	Energy of ligand	Energy of enzyme	Binding enthalpy			
Teq01	7	-49.34	-4001.07	22.10	-3965.35	-57.82	5.65	2.073	
Teq02	19	-46.66	-3981.1	20.35	-3964.21	-37.34	4.77	3.493	
Teq03	11	-45.54	-3990.97	21.40	-3966.37	-46.01	5.45	3.287	
Teq04	5	-43.64	-3983.67	23.62	-3966.52	-40.77	4.97	3.693	
Teq26	14	-35.07	-4002.57	22.18	-3963.29	-61.46	7.21	0.581	
Teq27	1	-28.32	-3984.36	21.93	-3962.13	-44.16	5.84	3.140	
Tax01	4	-44.1	-3989.91	18.53	-3964.30	-44.15	4.81	2.197	
Tax02	2	-42.46	-3983.69	20.99	-3962.99	-41.69	6.25	2.222	
Tax16	6	-36.61	-3980.72	19.83	-3965.08	-35.47	5.91	3.893	
Tax24	10	-32.88	-4003.49	19.62	-3963.02	-60.09	7.07	0.459	

of the galanthamine structures optimized with the Tripos force field is given in Table 1. Again the models Teq26 and Tax24 emerged as the most likely orientations of galanthamine in their complexes with TcAChE.

To evaluate how well the predicted orientations of galanthamine compare with that found in the crystal structure of the TcAChE-galanthamine complex,<sup>36</sup> the polypeptide backbones of each of the energy minimized models were superimposed onto the X-ray structure of the complex by a rigid RMS fit routine. The RMS deviations of the various predicted orientations of galanthamine from that found in the X-ray crystal structure of the TcAChE-galanthamine complex were then calculated independently and are reported in the last column of Table 1. Apparently, the galanthamine in Teq26 and Tax24 models with the highest ranking based both on Tripos and SCORE calculations also had the lowest RMS deviations from the X-ray structure of 0.58 Å and 0.46 Å, respectively.

### Half Open and Closed Gate Conformations of the Gorge

Both (ax)- and (eq) conformers of galanthamine were placed into the half open and closed gate conformations of TcAChE and the same procedure as described above for the open gate conformation of TcAChE was applied. AUTODOCK runs followed by geometry optimization with the Tripos force field resulted in several lowest energy clusters. These clusters are characterized in Table 2, including the accuracy of prediction evaluated by RMSD from the crystal structure of galanthamine-TcAChE complex. Again, the relative ranking of docking configurations based on the AUTODOCK energy did not correlate well with the Tripos binding enthalpy. However, in this case there was no correlation between the Tripos energy and SCORE values as well. In fact, in several occasions we have observed structures with reasonably high SCORE values that were clearly outside the active site.

As indicated by the comparison with the X-ray structure, the use of the half-open and closed gate conformations of TcAChE for docking yielded relatively correct prediction of the galanthamine orientation in the TcAChE active site as the lowest Tripos energy structure (cf. Table 2). However, the accuracy of the best predictions are almost twice as poor as those obtained with the open gate conformation of the enzyme, with only two clusters (c-Teq03 in the closed gate conformation and ho-Teq02 in the half-open conformation) having RMSD values of just under 1 Å. For each of the gate conformations, only the (eq)-conformer had a reasonable agreement with the crystal structure while the (ax)-conformer could not adopt an orientation in the active site similar to the actual structure.

The comparison between all calculations (Tables 1 and 2) suggests that only ranking based on the Tripos energies of geometry optimized complexes obtained from AUTODOCK calculations are in consistent agreement with experimental results. SCORE ranking agreed with Tripos calculations only in the case of open conformation of the enzyme. It is important to note that the Tripos energies of the complexes were higher than those obtained with the open gate conformation (cf. respective columns in Tables 1 and 2). Thus, on the basis of the Tripos energy values after optimization of AUTODOCK generated complexes, we would rule out both the half-open and closed conformations of the gorge and choose the open gate conformation as the correct one. This conclusion is in complete agreement with the experimental structure of the TcAChE-galanthamine complex.<sup>36</sup>

### DOCKING OF THE GALANTHAMINE DERIVATIVE SPH1107.

We have made the assumption that galanthamine derivatives with long chain substituents at the ring-nitrogen (ring D) could only bind to the enzyme in the open gate conformation, with

**Table 2.** Average energies (kcal/mol), SCORE value, and accuracy of matching (RMSD) the galanthamine x-ray structure in its complex with TcAChE for different orientation clusters of galanthamine in the active site of TcAChE in the “closed” and “half open” gate conformations with fixed positions of Phe330 and Tyr121. The data are shown only for the three lowest energy clusters resulting from 500 AUTODOCK runs for each of the (eq)- and (ax) conformers of galanthamine.

Gate configuration	Cluster name	No of structures in cluster	AUTODOCK energy of complex	Tripos energy of complex	Tripos binding enthalpy	SCORE value	RMSD [Å]
“closed”	c_Teq03	40	-43.6	-3828.2	-59.5	6.60	0.96
	c_Teq07	8	-40.9	-3827.0	-59.0	5.68	2.04
	c_Teq06	2	-41.0	-3823.3	-55.9	6.07	2.71
	c_Tax21	24	-31.5	-3826.8	-56.4	6.81	1.75
	c_Tax10	7	-36.1	-3821.3	-50.7	7.28	3.50
	c_Tax03	12	-33.6	-3819.1	-46.1	5.83	3.51
“half open”	ho_Teq02	73	-47.0	-3968.5	-54.5	6.81	0.94
	ho_Teq01	26	-50.2	-3966.3	-49.3	6.75	2.42
	ho_Teq13	8	-31.1	-3962.2	-48.4	4.85	3.64
	ho_Tax06	14	-38.5	-3969.7	-48.9	5.62	3.35
	ho_Tax03	12	-39.2	-3968.4	-42.9	5.14	2.83
	ho_Tax07	15	-38.4	-3962.5	-43.8	5.48	2.98

the side chain extending into the gorge toward the peripheral anionic site. Docking calculations were done with the galanthamine derivative SPH1107<sup>34</sup> (see Figure 1). The orientation and conformation of this derivative was predicted using the same procedure described for galanthamine itself. Again, both (eq)- and (ax)-conformers were considered since no dominant axial or equatorial orientation of the N-substituents in the bound state could be deduced from the molecular mechanics calculations. Results were evaluated by both the Tripos binding energy and the SCORE value, as described above (Table 3). The highest scoring models for the (eq)- and (ax)-conformers were superimposed onto the crystal structures of decamethonium, donepezil and MF268, which are known to bind with an extended conformation along the active site gorge (see Color Plate 5).

## COMPARISON OF THE MODELING RESULTS WITH CRYSTAL STRUCTURES.

The described modeling procedure discloses that there are only two distinct areas in which binding of galanthamine is allowed: the upper and the lower end of the gorge (Color Plate 6). This finding reflects the fact that the gorge narrows quite substantially from the mouth of the opening down into the protein before it opens up again at the catalytic site on the bottom of the gorge. The docking procedure clearly distinguishes the active site region from the peripheral anionic site at the mouth of the gorge. Galanthamine binding was detected only in these two locations. The deviation from the orientation observed in

**Table 3.** Average energy (kcal/mol), SCORE value, and deviation (RMSD) of the galanthamine moiety of derivative SPH1107 from the respective galanthamine ring structure as observed in the x-ray crystal structure of the TcAChE-galanthamine complex. Data are shown for the lowest energy clusters resulting from 500 AUTODOCK runs for each of the (eq)- and (ax) conformers of SPH1107.

Cluster name	No of structures in cluster	SYBYL energy of complex [kcal/mol]	SYBYL binding enthalpy [kcal/mol]	SCORE value	RMSD (core) [Å]
1107ax25	20	-4010.18	-76.27	8.30	0.50
1107ax22	42	-4006.79	-74.45	8.08	0.56
1107eq18	24	-4010.57	-75.22	7.77	0.41
1107eq37	5	-4006.55	-73.19	7.70	0.40

the X-ray crystal structure of the TcAChE-galanthamine complex<sup>36</sup> as revealed by the RMSD values also clearly separates the structures in the active site (RMSD < 4) from those at the periphery (RMSD > 6). No intermediate RMSD values in the range of 4–6 were found.

A comparison of the orientation and conformation of galanthamine in the crystal structure of the TcAChE-galanthamine complex with the best hits from our modeling study revealed an extremely good agreement for the models Teq26 and Tax24 (see Tables 1 and 2, respectively) with a resulting RMSD value of ~0.5 Å for both predicted structures. The orientation of the N-methyl group (ring D) is the only ambiguity present in our modeling studies that could not be resolved. Color Plate 7 displays an overlay of the corresponding predicted structures clearly showing that the experimental structure indeed has the equatorial conformation and thus matches our predicted model Teq26.

In addition, the orientation of 42 amino acids that were used to represent the TcAChE gorge in the modeling experiments also was in good agreement with the crystal structure (RMS deviation for all backbone atoms is 0.12 Å), even though modeling involved complete relaxation of the side chains in the final optimization steps.

The comparison of the hydrogen bonding pattern between galanthamine and TcAChE as revealed by the modeling study with that found in the crystal structure of the TcAChE-galanthamine complex is given in Table 4. These interactions include a hydrogen bond between the galanthamine O-CH<sub>3</sub> oxygen (ring A) acting as an acceptor, and the OH-group of Ser200 (O<sub>γ</sub>) acting as a donor, and a second hydrogen bond between the OH-group of the cyclohexenol (ring C) acting as a donor to the carboxyl-group of Glu199 (O<sub>ε1</sub>), acting as an acceptor.

## EXPERIMENTAL DETAILS

Docking studies were performed using the AUTODOCK 2.4 program to deduce conformations and orientations of galanthamine and the derivative SPH1107 in the TcAChE-binding site. AUTODOCK operates within pregenerated grid maps so that the conformational flexibility of the receptor is not considered at all during the docking process. The enzyme-conformation of the TcAChE-decamethonium complex (1ACL) was chosen for our docking study. Since the positions of most water molecules in the crystal structures of TcAChE-decamethonium and TcAChE-galanthamine complexes, respectively, are unlikely to be conserved, water molecules were excluded before the docking protocols. To study the influence

**Table 4. Distances of heteroatoms in hydrogen bonding of galanthamine with TcAChE active site amino acids.**

H-bond	Distance of heteroatoms [Å]		
	Teq26	Tax24	crystal structure
OH-CLU199	2.51	2.50	2.72
COC-HIS440	3.32	3.28	3.20
OCH <sub>3</sub> -SER200	2.50	2.50	2.97

of the Phe330 side chain orientation on the docking process, comparative AUTODOCK runs were performed also with fixed orientations of Phe330 and Tyr121 using an enzyme structure in the TcAChE-tacrin complex (1ACJ) and the TcAChE-edrophonium complex (2ACK) as well as the TcAChE-decamethonium complex (1ACL).

Residues with missing side chain atoms were rebuilt using the BIOSYM BIOPOLYMER module.<sup>53</sup> Hydrogen atoms were added to all amino acid residues. The side chains of glutamic and aspartic acids as well as the side chains of arginine, lysine, and histidine were assigned formal negative and positive charges, respectively. The total formal charge amounted to +4. The N- and C- terminus of the polypeptide chains were capped with an acetyl moiety (Ser4, Ser 490) and an N-methyl group (Glu484, Thr535), respectively. The resulting structure was subjected to geometry optimization employing the Tripos<sup>47</sup> force field as implemented in SYBYL 6.5 [Pullman charges, Powell Minimizer, 500 steps, convergence criteria: gradient 0.05 kcal/(mol2\*Å)]. In the first optimization step, only hydrogen atoms were relaxed while all heavy atoms were kept fixed. In the second step, side chains of all amino acids were allowed to move during the optimization process, keeping only the backbone atoms at their positions. In the final optimization step, all atoms were allowed to relax.

The resulting enzyme structure was used as an input for the AUTOGRID program.<sup>44</sup> In the first step, AUTOGRID defines a fine-meshed grid (0.375 Å) that surrounds the area of the enzyme in which the ligand is expected to bind (size: 22.5 × 22.5 × 22.5 Å<sup>3</sup>). Since galanthamine is known to be a competitive inhibitor, the center of the box and the initial position of the ligand were placed at the bottom of the gorge.

For each ligand atom type AUTOGRID calculates the Van der Waals energies at each single grid point. The program generates separate grid maps for all atom types occurring in the ligand structure. A grid map for electrostatic interactions is computed by moving a point charge within the grid and calculating the resulting energies. In the case of galanthamine, grid maps for C, H, N, O, and point charge were used and the actual docking process was performed within this grid. AUTODOCK translates and rotates the ligand and simultaneously modifies user-defined torsion angles. The use of pre-generated grid maps affords a rapid energy evaluation of the enzyme/ligand complex. AUTODOCK uses a simulated annealing protocol to search for energetically most favorable orientation of ligand in the active site. One AUTODOCK simulation typically consists of 500 runs, 50 cycles, and 3000 accepted or rejected enzyme/ligand alignments, respectively. RMS tolerance during the clustering was set to 1 to reduce the number of clusters. All other values were used as default settings. Subsequently, AUTODOCK clusters the resulting orientations of the ligand, taking into account the total interaction energy between the ligand and enzyme and an RMSD criterion. Usually up to 30 clusters are obtained, representing a series of energetically meaningful enzyme/ligand arrangements.

These structures were submitted to a geometry optimization within the active site of the enzyme employing the Tripos force field. During the optimization process, the side chains of 42 amino acids surrounding the active site as well as the single bonds of the ligand (galanthamine and SPH1107, respectively) were kept flexible. When the influence of the orientation of Phe330 on the galanthamine docking was investigated, Phe330 and Tyr121 were fixed while the remaining 40 amino acids

again were kept flexible. The rest of the enzyme was held rigid, while long range interactions were still included. Dissociation constants ( $pK_D$ ) for the obtained ligand/enzyme complexes were estimated with the program SCORE,<sup>48</sup> and were the basis for the scoring of the ligand-enzyme-alignments suggested by the docking procedure.

## DISCUSSION

The aim of our docking studies was to develop a procedure that would allow the prediction of likely orientations of galanthamine and its derivative, SPH1107, in the active site of TcAChE. This structural information may be valuable for subsequent drug design studies using structure-based or 3D-QSAR methods. Examples of structure-based alignment for 3D QSAR analysis are provided by recent studies from our<sup>24</sup> and other laboratories.<sup>25,26</sup> Recently, various galanthamine derivatives were synthesized, which provide an excellent starting point for rational design and development of anti-cholinesterase drugs.<sup>34</sup> Derivatives that contain a long substituent on the nitrogen are of particular importance. Such structures potentially span the whole binding cavity of TcAChE and interact with several amino acids at the peripheral anionic site, which should lead to increased potency of AChE inhibition. We have included in our study the galanthamine derivative SPH1107, which has an N-propyl-piperidine substituent at the nitrogen atom rather than the methyl group.

Both axial and equatorial orientation of the nitrogen substituent in galanthamine and its SPH1107 derivative were considered separately in the AUTODOCK procedure. The incorporation of the SPH1107 derivative in the present study required the definition of a fairly large active site box in which the ligand is confined. Furthermore, it biased us to consider the open gate conformation of the active site gorge, found, for instance, in the TcAChE-decamethonium complex as the standard TcAChE structure. However, the closed gate structure (as in the TcAChE-tacrin complex) and the half open gate conformation (as in the TcAChE-edrophonium complex) were also included in our study.

Docking studies, especially with AUTODOCK usually identify a small number of potential binding orientations for a given ligand. It is quite difficult to unambiguously select the most probable binding site and the orientation of the ligand. Additional scoring procedures should be employed to rank the resulting structures. In our study we concentrated on two ranking criteria, the energy of the AChE-ligand complex obtained with the Tripos force field, and the energies calculated with the empirical SCORE method.<sup>48</sup>

As a consequence of using the large active site box in the docking study, a great portion of the resulting galanthamine structures are found near the peripheral anionic site at the top of the gorge. However, since galanthamine is known to be a competitive inhibitor, we assumed that binding actually occurs at the bottom of the gorge, similar to other known inhibitors of TcAChE. Most of the known inhibitors interact with both or either of the two residues, Ser200 and His440, that belong to the catalytic triad of the enzyme, with additional interaction with Glu199. This assumption substantially decreased the number of matches in the modeling study to 57 and 22 for the (eq)- and (ax)-conformers of galanthamine, respectively, out of 500 AUTODOCK results for each configuration. Furthermore, using the binding energies derived from the Tripos force field for

ranking the modeling results, the two highest scoring AUTODOCK clusters were predicted for the (eq)- and (ax)-conformers of galanthamine, respectively.

As a fortunate coincidence, a crystallographic investigation of the TcAChE-galanthamine complex was recently performed by Bartolucci et al<sup>36</sup> independently from the modeling studies reported in this article. This structure enabled us to test the accuracy of our predictions with respect to the orientation and conformation of galanthamine in the enzyme active site. Typical representatives of these (eq)- and (ax)-clusters nicely overlay with the galanthamine conformation in the crystal structure of the TcAChE-galanthamine complex, showing RMSD values of  $\sim 0.5 \text{ \AA}$ .

Comparably good overlap with the crystal structure was also achieved for the (eq)-structure when the closed gate and the open gate protein conformations were employed rather than the open gate state. However, in these cases the presence of the phenyl ring of Phe330 prohibits the (ax)-conformer of galanthamine to be oriented in the active site in a position that matches the crystal structure.

It should be noted that our computational docking experiments do not incorporate water molecules that might be located in the active site. This procedure is always a source of potential misinterpretation of docking studies as illustrated by recent docking calculations with the AChE inhibitor huperzine A.<sup>27</sup> From the crystallographic investigation,<sup>36</sup> it is evident that one water molecule does play an important role in the interaction between TcAChE and galanthamine. However, because of the very good overlap shown in this study between the predicted galanthamine conformation and that found by X-ray crystallography, water molecules do not seem to be essential for the accurate prediction of the bound ligand conformation.

The predicted structures show direct hydrogen bonding to the catalytic residue Ser200, which probably leads to the effective blockade of TcAChE by galanthamine. However, the docking study can not differentiate between the axial and equatorial conformer, since both conformers are characterized by similar score values and comparably good RMSD values. Considering that the two conformers should interchange rapidly in the naturally bound ligand, our findings appear reasonable.

To prove that the gorge interior may be exploited for the docking studies of other inhibitors, we applied the same strategy to the galanthamine derivative SPH1107 that carries a long chain at the nitrogen atom. We found that the AUTODOCK clusters for either the (eq)- and (ax)-conformer are almost identical with respect to the orientation of the rigid galanthamine ring structure. The only minor difference is in the orientation of the N-substituent as a consequence of the axial and equatorial position, respectively. Both structures nicely superimpose on the corresponding galanthamine conformer and indeed showed the expected extension of the N-substituent towards the outside of the gorge. The galanthamine derivative SPH1107 displays interactions with the gorge residues that are very similar to those observed in crystal structures of the TcAChE complex with decamethonium (1ACL) and donepezil (1EVE). It is worth noting that important structural segments of both the galanthamine derivative and donepezil have almost exactly the same location in the active site. For instance, the nitrogen atom of galanthamine is positioned only  $1.26 \text{ \AA}$  (eq-conformer) and  $1.51 \text{ \AA}$  (ax-conformer) apart from the piperidine nitrogen of donepezil. Furthermore, the double bond of

the cyclohexenol ring (ring C) of the galanthamine derivative nicely overlays with the aromatic benzyl moiety of donepezil.

In summary, we have developed a combined docking procedure that uses AUTODOCK rigid docking followed by a flexible molecular mechanics optimization of resulting structures. AUTODOCK is used initially to generate several acceptable orientations of ligands. The resulting structures are subjected to the geometry optimization with the Tripos forcefield, and the ligand-receptor interaction energies are used for the final ranking of ligand receptor complexes. We showed that this procedure provides a reliable method for predicting the orientation and conformation of galanthamine type ligands in the active site of the TcAChE as judged by very low RMSD values between the predicted highest scoring orientations of galanthamine and the experimentally determined one. The success of the reported studies provides an excellent basis for the applications of the described docking procedures to a large number of de novo designed and synthesized galanthamine derivatives. The present docking studies may guide the design process as well as provide structure based 3D alignment of galanthamine type AChE inhibitors, which might prove relevant for a 3D QSAR analysis. Investigations along these lines are presently ongoing in our laboratories. **Note added in proof.** After completion of this study a second structure of the TcAChE/galanthamine complex was deposited in the PDB database (1DX6),<sup>54</sup> which shows an identical orientation and conformation of galanthamine in the TcAChE active site to the 1QTI-structure.<sup>36</sup>

## ACKNOWLEDGMENTS

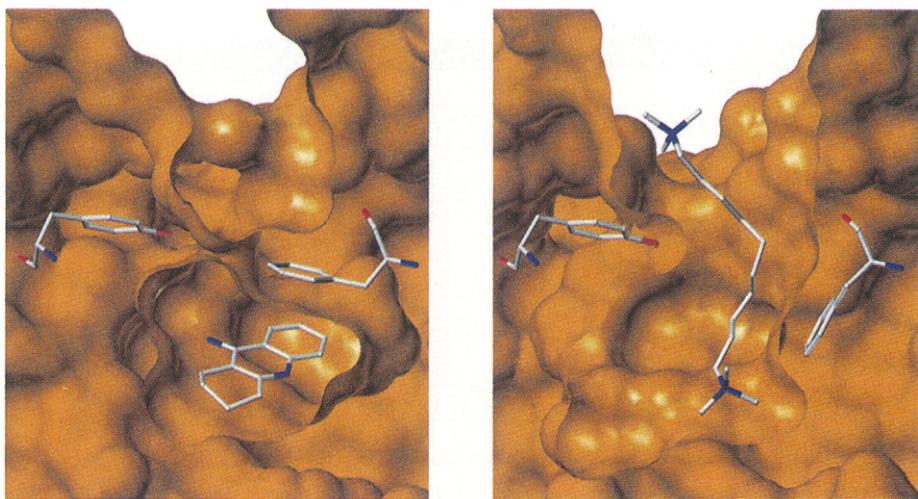
Support to C. Pilger by Fonds der Chemischen Industrie (FCI) is gratefully acknowledged. A. Tropsha acknowledges the support from NIH (SBIR Grant RR10687-02A1).

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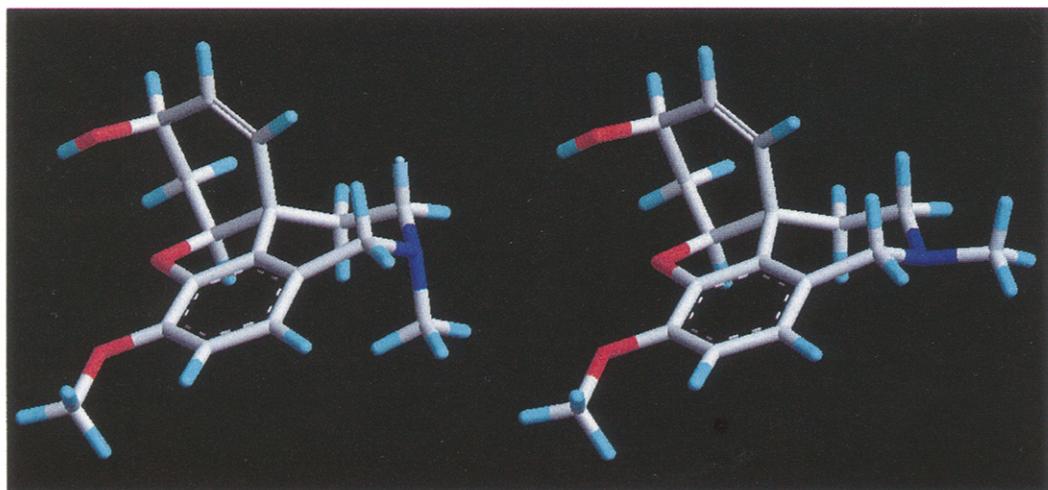
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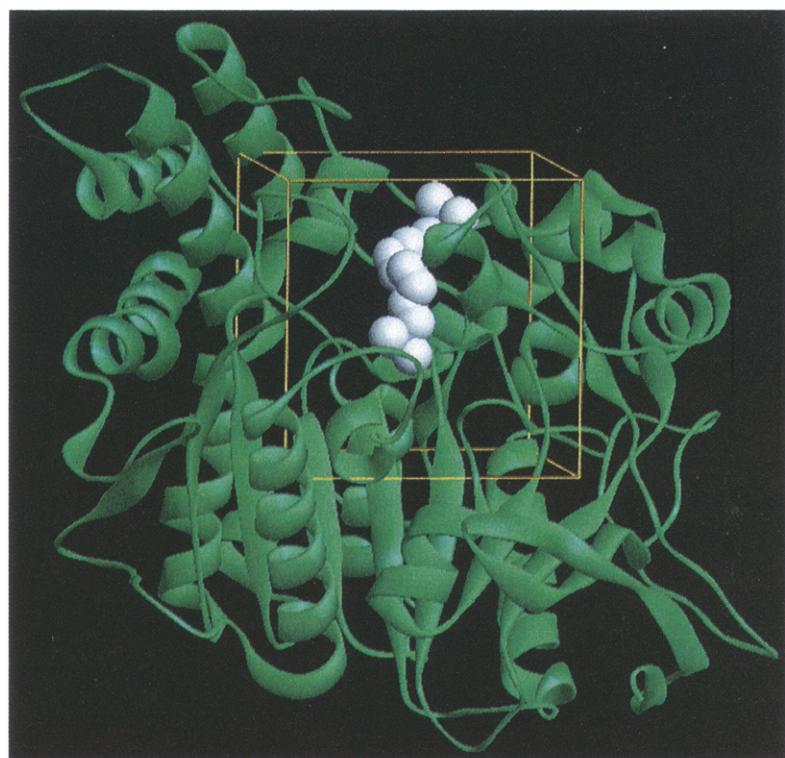
**Accurate prediction of the bound conformation of galanthamine in the active site of *Torpedo californica* acetylcholinesterase using molecular docking**



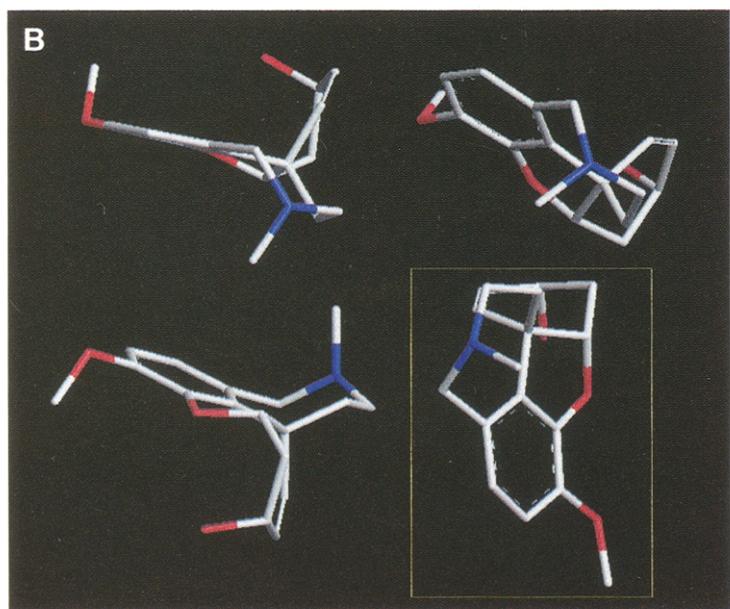
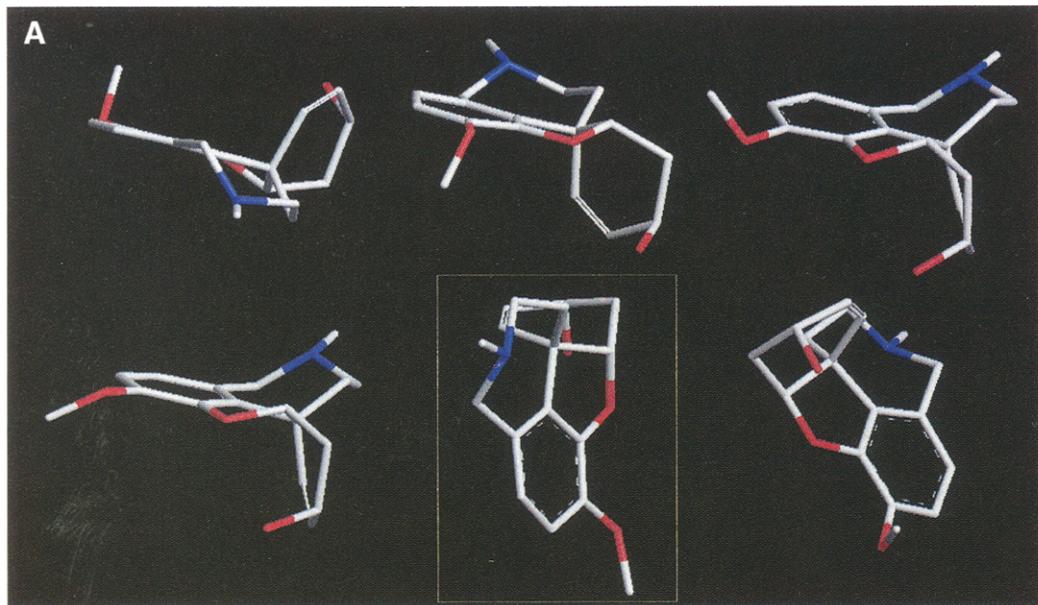
Color Plate 1. Comparison of the side chain orientation of PHE 330 with a closed gate conformation in the TcAChE-tacrine complex (left) and an open gate conformation in the TcAChE-decamethonium complex (right).



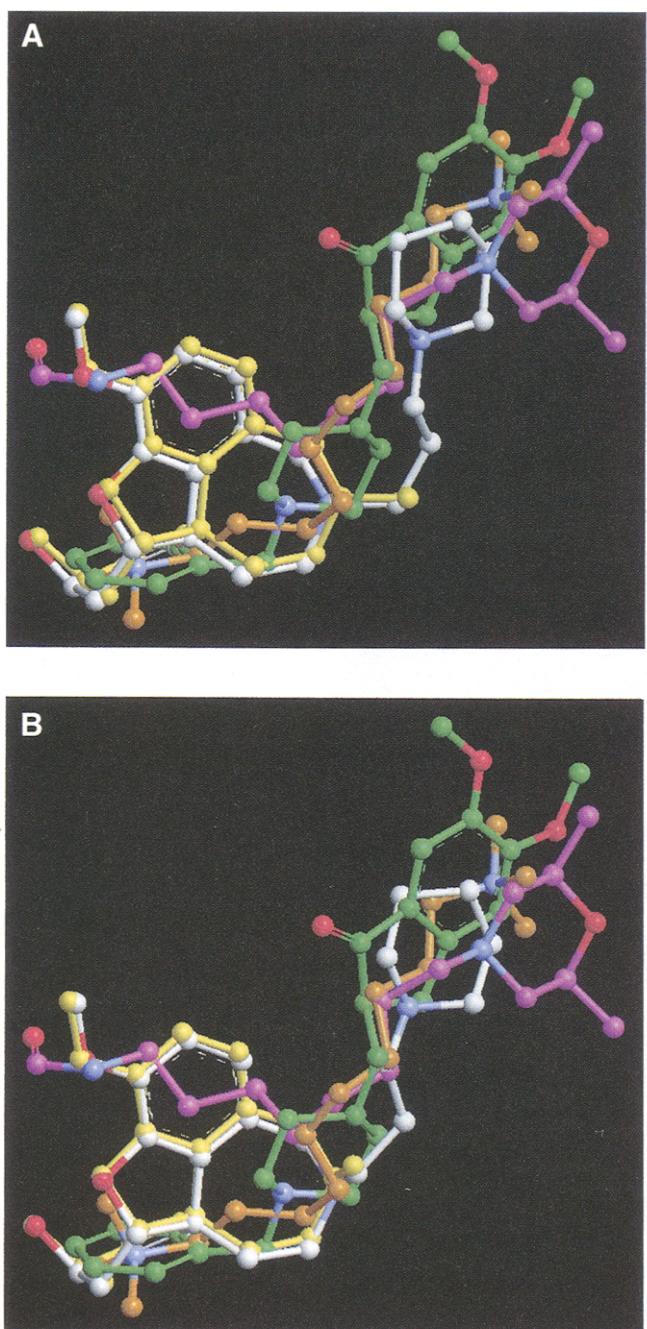
Color Plate 2. Global minimum structures of galanthamine with tertiary methylamine group in (ax)-conformer (left) and (eq)-conformer (right).



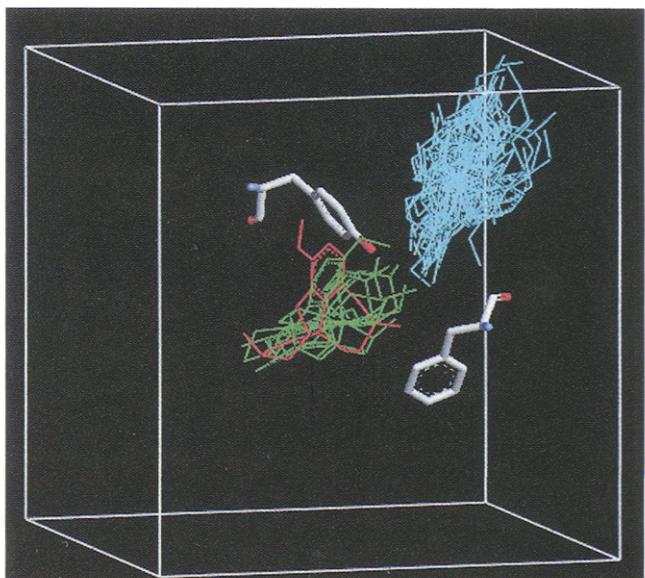
Color Plate 3. Location of the active site box (yellow) used in the AUTODOCK procedure to predict galanthamine binding in the active site. For comparison the structure of decamethonium (white) is placed in the enzyme according to the known crystal structure (1ACL).



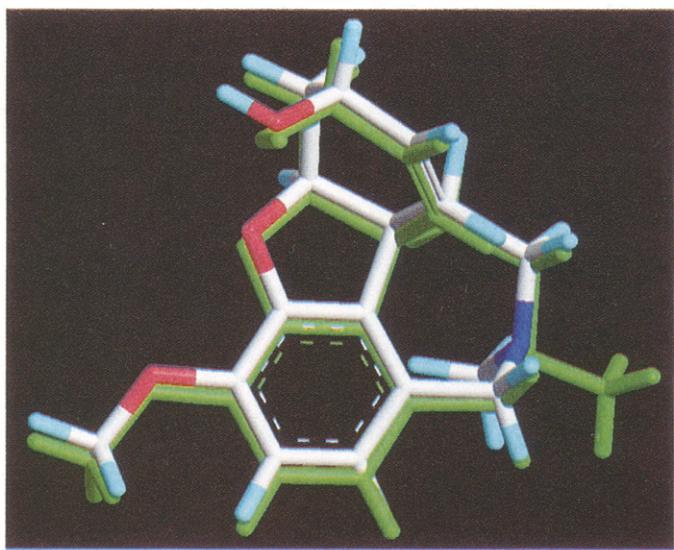
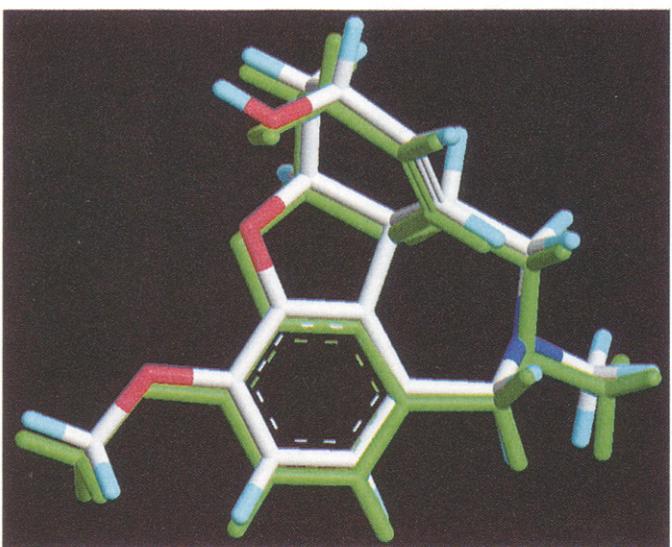
Color Plate 4. Typical orientations of galanthamine in the active site of AChE: (A) (eq)- conformers, (B) (ax)- conformers) found by AUTODOCK. Structures are oriented as found in the active site of the enzyme (not shown) . Yellow boxes denote the preferred orientation as found by the modeling study.



Color Plate 5. Structural overlay of the highest scoring (ax)-conformer (**A**) and (eq)-conformer (**B**) of galanthamine derivative SPH1107 (white) with the respective galanthamine (ax)- and (eq)-conformer as revealed by the modeling study (yellow). For comparison the crystal structures of decamethonium (1ACL, brown), donapecil (1EVE, green) and Mf268 (1OCE, yellow) are shown.



Color Plate 6. Binding location of all AUTODOCK clusters found with the (eq)-conformer of galanthamine. For comparison, the active site box as used in the AUTODOCK process and the amino acids Phe330 and Tyr121 are shown. The red structure depicts cluster T-eq-26 (see Table 2, green structures are located in the active site and typically have RMSD values <4 compared with the crystal structure, while blue structures have RMSD values >6 and clearly are positioned at the mouth of the gorge.



Color Plate 7. Structural overlay of galanthamine models Teq26 (**A**), and Tax24 (**B**) as revealed by the docking study with that of galanthamine present in the crystal structure of the TcAChE-galanthamine complex<sup>45</sup> (green).