First Gallamine−Tacrine Hybrid: Design and Characterization at Cholinesterases and the M_2 Muscarinic Receptor

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Gallamine and tacrine are allosteric antagonists at muscarinic M_2 acetylcholine receptors and inhibitors of acetylcholinesterase. At both acetylcholine-binding proteins, gallamine and tacrine are known to occupy two different binding sites: in M_2 receptors within the allosteric binding area and in acetylcholinesterase at its catalytic and its peripheral site. To find new ligands of both targets, we designed a gallamine−tacrine dimer and several derived hybrid compounds to address the two binding sites. Their M_2 receptor allosteric and acetylcholinesterase inhibitory potential was determined. The hybrid compounds revealed an allosteric potency in the low nanomolar range exceeding the allosteric potency of gallamine and tacrine by factors of 100 and 4800, respectively. Cholinesterase inhibition was augmented by hybrid formation, and all compounds exhibited IC_{50} values in the lower nanomolar range. Thus, gallamine−tacrine hybrid formation is a valuable approach toward high affinity ligands concurrently targeting these acetylcholine-binding proteins.

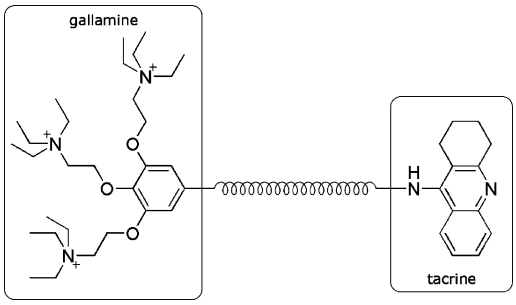
Introduction

Acetylcholine is the linchpin of neurotransmission in parasympathetically innervated target cells, autonomic ganglia, the neuromuscular junction and the CNS as it carries the nervous signal across the synaptic cleft and to the final effector cells. The level of acetylcholine inside the synaptic cleft is therefore subject to sensitive regulation mechanisms. Released at the presynaptic terminal, acetylcholine diffuses toward the postsynaptic side. There it may either bind to muscarinic receptors that are G-protein coupled or to nicotinic acetylcholine receptors, representing a group of ion channels. To terminate the resulting signal, the synaptic cleft needs to be freed of acetylcholine, a task that is mainly accomplished by acetylcholinesterase, a serine hydrolase of therapeutic importance.[1−3] Tacrine is a prototypal cholinesterase inhibitor, but it is also an allosteric modulator of muscarinic receptors. Gallamine is an archetypal muscarinic allosteric agent but at high concentrations it also inhibits cholinesterases.

At both targets, tacrine and gallamine can bind at topographically distinct places. A previous study on acetylcholinesterase inhibition by tacrine−trimethoxybenzene heterodimers[4] was based on the dual binding site model, that involves the peripheral and the active site.[5,6] While tacrine commonly binds to the active site of acetylcholinesterase, the trisquaternary phenolic ether gallamine is a long known ligand of the peripheral site.

Since its discovery[7] in 1947, gallamine has been used as a muscle relaxant (Flaxedil) and was additionally found to allosterically modulate muscarinic receptors.[8] Among the five muscarinic receptor subtypes classified until today, gallamine exhibits a selectivity for the M_2 subtype.[9] Gallamine belongs to the group of typical allosteric modulators that bind in the core region of the allosteric site.[10,11] Tacrine is an atypical muscarinic allosteric agent.[12,13] Recently, the atypical molecular interaction of tacrine at M_2 receptors was elucidated by applying a tacrine homodimer.[14] The homodimer had a higher allosteric affinity than the monomer. This finding suggested that the allosteric site has room to accommodate two tacrine molecules simultaneously, one in the core region of the allosteric site and one in its periphery.[14]

Chart 1. Two Building Blocks, Gallamine and Tacrine, To Be Linked by a Suitable Spacer



Inspired by this analogy of two distinct binding sites for gallamine and tacrine at both, acetylcholinesterase and the allosteric site of the M_2 muscarinic receptor, we envisaged hybrids of tacrine and gallamine or gallamine-related moieties (Chart 1) held together by a previously established linker.[4] The commonly utilized diaminoalkane linker of seven or eight methylene groups[5] was not suitable to carry the gallamine unit. However, our linker was chosen to stretch a comparable distance, bringing the gallamine moiety close to the peripheral binding site. These novel hybrid compounds were thought to exhibit increased affinity toward the M_2 muscarinic receptor and acetylcholinesterase by simultaneously addressing the respective tacrine and gallamine binding sites.

All compounds were evaluated as inhibitors of several acetylcholinesterases with IC_{50} values in the nanomolar concentration range. Comparable potency was observed for human butyrylcholinesterase, whose inhibition is expected to provide additional benefits in Alzheimer's disease.[3,15] The allosteric potential of the hybrids was assessed by their ability to bind to M_2 receptors whose acetylcholine binding site was blocked by the radiolabeled antagonist [^{3}H]*N*-methyl-scopolamine ([^{3}H]-NMS). In comparison to their building blocks, gallamine and

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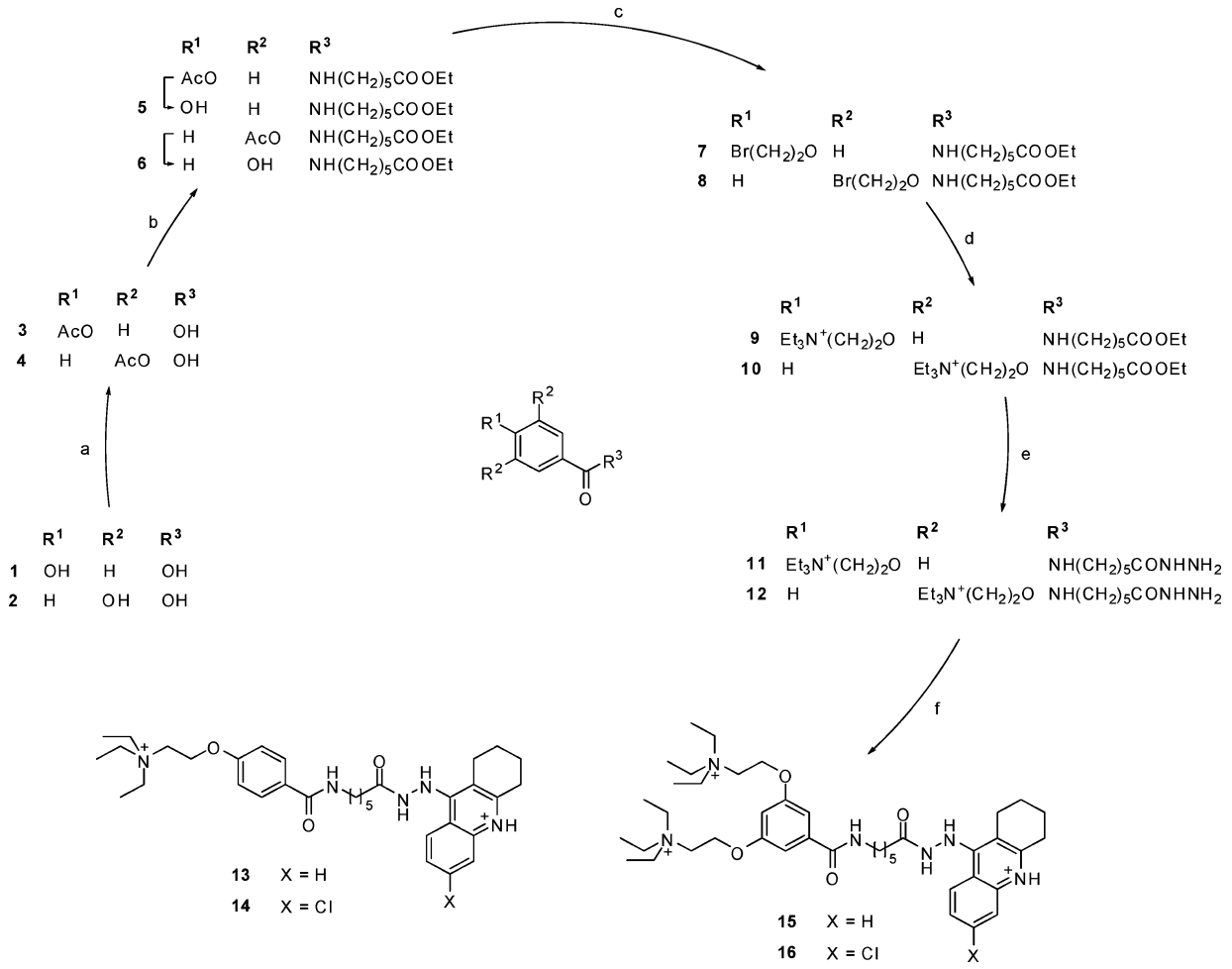
Scheme 1. Synthesis of the Mono- and Bisquaternary Gallamine−Tacrine Hybrids **13**−**16**[a]



[a] Reagents and conditions: (a) Ac_2O, Py, RT, 1 h; (b) (1) $(COCl)_2$, CH_2Cl_2, RT, 2 h; (2) $H_2N(CH_2)_5COOEt{\cdot}HCl$, DIEA, CH_2Cl_2, RT, 1 h; (3) K_2CO_3, MeOH, RT, 12 h; (c) K_2CO_3, 18-crown-6, $(CH_2Br)_2$, 80 °C, 36 h; (d) Et_3N, $MeNO_2$, 60 °C, 24 h; (e) N_2H_4, EtOH, 80 °C, 72 h; (f) 6,9-dichloro-/9-chloro-1,2,3,4-tetrahydroacridine, EtOH, 140 °C, 24 h.

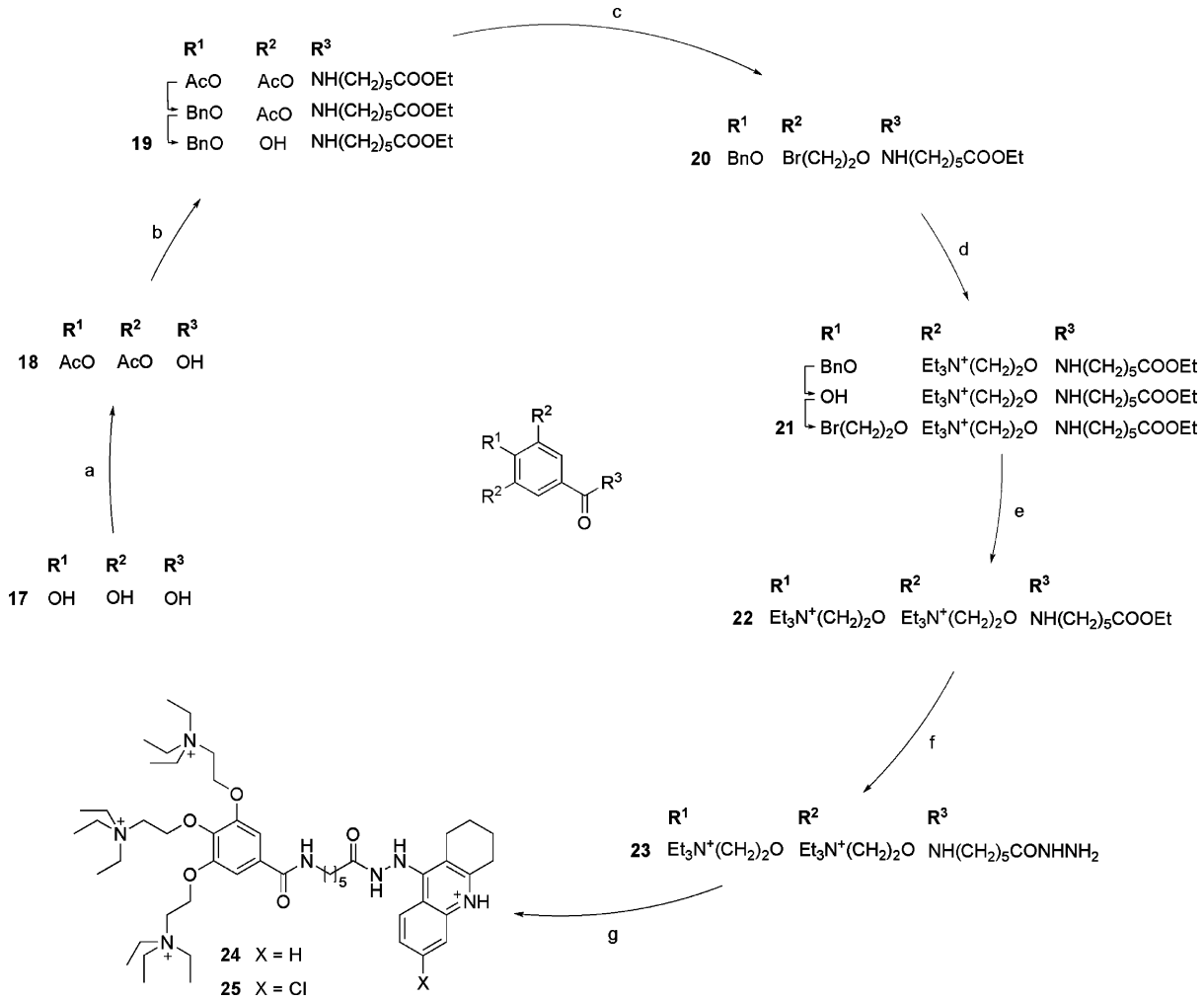
tacrine, the hybrids behaved as M_2 receptor modulators of remarkably high potency.

Results and Discussion

Synthesis. To gain a straightforward access toward compounds **24** and **25** shown in Scheme 2 and to study the importance of the substituents in the gallamine scaffold, the synthetic route was initially established for the simplified compounds **13**−**16** (Scheme 1). Starting from hydroxy-substituted benzoic acids **1** and **2**, the anticipated linker was introduced at first. A prerequisite was the protection of the phenolic groups to allow activation of the carboxylic acid for coupling with ethyl 6-aminohexanoate. Thus, 4-hydroxybenzoic acid (**1**) and 3,5-dihydroxybenzoic acid (**2**) were acetyl-protected by treatment with acetic anhydride in pyridine. Both 4-acetoxybenzoic acid (**3**) and 3,5-bis(acetoxy)benzoic acid (**4**) were subsequently reacted with ethyl 6-aminohexanoate by classical acyl chloride activation. Selective deprotection was achieved using potassium carbonate in methanolic solution to obtain intermediates **5** and **6**. During the fourth step, the phenolic groups were alkylated with 1,2-dibromoethane following a procedure of Sarkar et al.[16] to yield the 2-bromoethoxy-substituted compounds **7** and **8**. These were quaternized with triethylamine, and bromide salts **9** and **10** precipitated when a concentrated solution in anhydrous ethanol was added to ethyl acetate. The precipitation from ethyl acetate proved to be a valuable method to obtain these quaternary intermediates as well as the final compounds as solid substances. The mono- and bisquaternary carboxylic esters **9** and **10** were then converted into their corresponding carboxylic hydrazides **11** and **12** by reaction with aqueous hydrazine. The reaction conditions established in the course of previous investigations[4] had to be adjusted to achieve an almost complete reaction of **9** and **10**. Only complete conversion to **11** and **12** allows their collection by precipitation from ethyl acetate without further purification. The final seventh step was the introduction of a tacrine moiety by treatment with either 9-chloro- or 6,9-dichloro-1,2,3,4-tetrahydroacridine in sealed reaction vessels. The desired compounds **13**−**16** were obtained as amorphous yellow powders of notable hygroscopicity in overall yields between 20 and 36%.

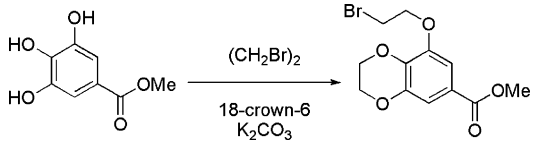
Following this procedure, efforts were undertaken to acquire the envisaged gallamine−tacrine heterodimers **24** and **25** (Scheme 2). Preliminary investigations concerning the alkylation of methyl 3,4,5-trihydroxybenzoate with 1,2-dibromoethane unfortunately revealed the almost quantitative formation of a benzo[*b*][1,4]dioxine (Chart 2). To avoid this ring closure, the *para*-hydroxy group had to be selectively protected, and a two-step alkylation process was employed. A benzyl protection was considered the most practical solution as its removal conditions are suitable for quaternary compounds. Thus, starting from 3,4,5-trihydroxybenzoic acid (**17**), the first two steps were again the acetyl protection of all three phenolic groups to give **18** and

Scheme 2. Synthesis of the Trisquaternary Gallamine−Tacrine Hybrids **24** and **25**[a]



[a] Reagents and conditions: (a) Ac_2O, Py, RT, 1 h; (b) (1) $(COCl)_2$, CH_2Cl_2, RT, 2 h; (2) $H_2N(CH_2)_5COOEt{\cdot}HCl$, DIEA, CH_2Cl_2, RT, 1 h; (3) BnCl, K_2CO_3, KI, Me_2CO, reflux, 18 h; (4) K_2CO_3, EtOAc, MeOH, H_2O, reflux, 1 h; (c) K_2CO_3, 18-crown-6, $(CH_2Br)_2$, 80 °C, 36 h; (d) (1) Et_3N, $MeNO_2$, 60 °C, 24 h; (2) H_2, Pd/C, MeOH, 6 h; (3) K_2CO_3, 18-crown-6, $(CH_2Br)_2$, MeCN, 80 °C, 36 h; (e) Et_3N, MeCN, 100 °C, 48 h; (f) N_2H_4, EtOH, 80 °C, 72 h; (g) 6,9-dichloro-/9-chloro-1,2,3,4-tetrahydroacridine, EtOH, 140 °C, 24 h.

Chart 2. Protection of the *para*-Hydroxy Group is Essential Because Direct Alkylation of Gallic Acid Derivatives with 1,2-Dibromoethane Leads Benzo[*b*][1,4]dioxins



coupling with ethyl 6-aminohexanoate. Following a method adapted from Pearson et al.,[17] the *para*-acetoxy moiety was exchanged by a benzyl group and the two remaining *meta*-acetoxy groups were cleaved to obtain **19**, which was subsequently treated with 1,2-dibromoethane to yield intermediate **20**. Reaction with triethylamine produced a bisquaternary carboxylic ester that was deprotected by hydrogenolysis and again alkylated with 1,2-dibromoethane. This second alkylation was found extremely sensitive to oxidation and had to be carefully protected by argon to achieve **21** free from any degradation products. The subsequent quaternization toward the trisquaternary carboxylic ester **22** was another crucial step as the use of nitromethane led to constant impurities that could not be removed. However, acetonitrile dissolved both educt and product at higher temperatures, and pure **22** precipitated upon cooling. The following hydrazide formation and coupling of **23** with 6-chloro- or 6,9-dichloro-1,2,3,4-tetrahydroacridine proceeded smoothly, and the final compounds **24** and **25** were obtained after 11 steps in 15% overall yield each.

Molecular Docking and Cholinesterase Inhibition. Possible binding modes of the quaternary target compounds **13**, **15** and **24** were investigated using the AutoDock software suite.[18] The 1ZGB[19] crystal structure of acetylcholinesterase from *Torpedo californica* in complex with a tacrine heterodimer was used as the docking template, because it already accommodates a hybrid inhibitor that stretches the entire gorge, binding simultaneously to both binding sites. The tacrine moiety was found to bind to the active site as in the crystal structures 1ACJ[20] (TcAChE−tacrine) or 1ZGB (TcAChE−tacrine-hupyridone), while interactions of the hydrazide linker with Asp72 and Tyr121 were observed as similarly reported before[21] (residue numbering throughout this report will refer to the organism of the complex discussed). The main emphasis was put on possible interaction partners at the utmost gorge entrance. The propidium−MmAChE (PDB 1N5R[22]) complex showed, that the quaternary side chain is pointing out of the gorge in two distinct orientations. It is either bound to an outer surface loop, comprised by Leu289−Ser293, or it interacts with His287. These orientations

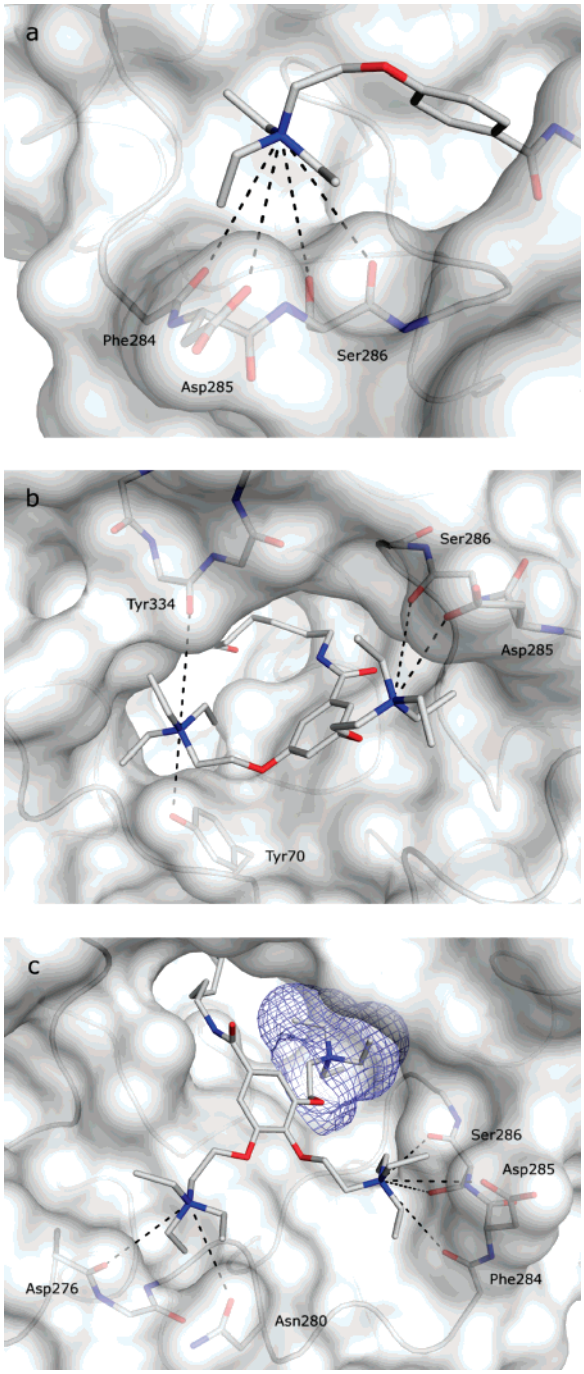


Figure 1. Compounds **13**, **15**, and **24** docked into acetylcholinesterase (PDB 1ZGB[19]) using AutoDock.[18] Graphics were created using PyMOL[40] and amino acid residue numbering refers to TcAChE.

result from two possible binding modes of the phenylphenanthridinium system, that forms a $\pi-\pi$ stacking with Trp286 and may be flipped by 180 degrees. For our compounds, a $\pi-\pi$ stacking of the benzamide unit with the corresponding Trp279 was not observed because the amide bond forms hydrogen bonds to mid-gorge amino acid residues, as was already found with a series of tacrine-trimethoxybenzene heterodimers.[23]

In all three compounds **13**, **15**, and **24**, one of the quaternary side chains is located at the same, fixed position. The backbone carbonyl oxygens of Phe284 and Ser286, and the side chain oxygens of Asp285 and Ser286 were found at the base of a pyramid, with the ammonium nitrogen at its top (Figure 1a). As this interaction is observed in all three docking sets, it is

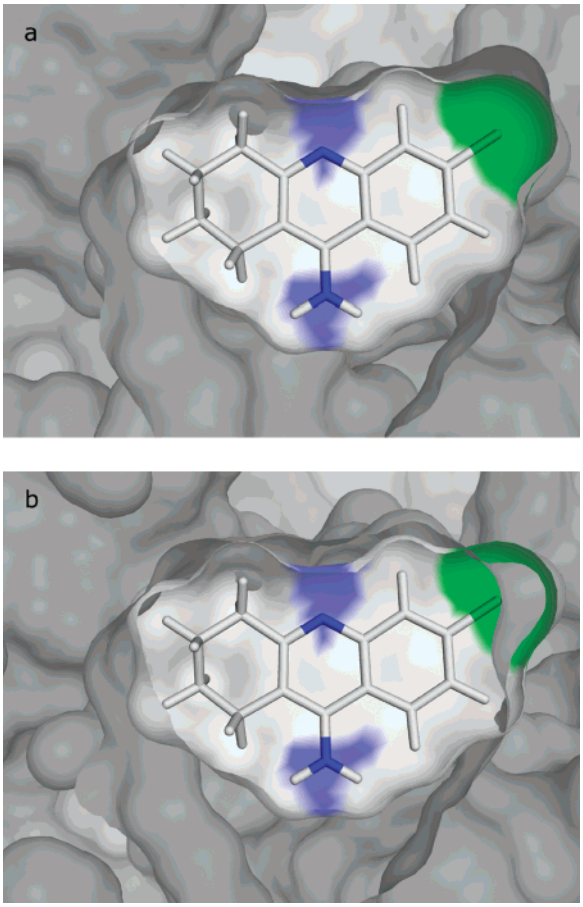


Figure 2. Cholinesterase selectivity: a chlorine at C6 fits perfectly into hAChE (a), while hBChE offers less space and impairs binding (b). Graphics were created using PyMOL.[40]

believed that the binding to the outer gorge lip plays a dominant role in ligand association. The second ammonium side chain of compound **15** (Figure 1b) is directed toward the gorge middle, where it is found on an axis connecting Tyr70 and Tyr334. This location allows the excellent accommodation of the ethyl substitutents, each of them pointing into a separate surface pocket. Compound **24** (Figure 1c) carrying three quaternary side chains was the most complex structure to dock with a high level of torsional constraints. Again, the triplet of Phe284, Asp285, and Ser286 is addressed by one ammonium group, this time in *para* position, while one of the *meta* side chains points into the gorge and the other toward the entrance. It is the inward pointing side chain that finds a perfect fit at the gorge center and forces the opposite side chain outward.

The inhibition of acetylcholinesterase was investigated for enzymes from *Electrophorus electricus*, *Torpedo californica*, and *Homo sapiens*, while butyrylcholinesterase inhibition was determined using the human enzyme. IC_{50} values derived from duplicate experiments are listed in Table 1, also including the selectivity between the two human cholinesterases. Data obtained for tacrine and gallamine in the presence of 6% acetonitrile differed notably from our results (Table 1) and literature reports[24−26] in the absence of acetonitrile. For two acetylcholinesterases, higher IC_{50} values were observed in the presence of acetonitrile, whereas the opposite applies to human butyrylcholinesterase inhibited by tacrine. These findings will be covered in more detail in a future report.

It was found that increasing substitution of the gallamine-derived moiety tends to reduce the inhibitory potency. In the

Table 1. Inhibition of Acetylcholinesterase from *Electrophorus electricus* (EeAChE) and *Torpedo californica* (TcAChE), Human Acetylcholinesterase (hAChE), and Human Butyrylcholinesterase (hBChE) by Compounds **13**−**16**, **24**, and **25**

	IC_{50} ± SEM (*nM, **μM)[a]				selectivity (IC_{50} hAChE/ IC_{50} hBChE)
cmpd	EeAChE	TcAChE	hAChE	hBChE	
13*	0.467 ± 0.032	17.6 ± 1.6	7.61 ± 0.41	1.50 ± 0.08	5.07
14*	26.0 ± 1.9	127 ± 3	5.44 ± 0.48	8.55 ± 0.42	0.64
15*	1.28 ± 0.05	8.59 ± 0.27	15.8 ± 0.8	1.42 ± 0.07	11.1
16*	48.0 ± 3.4	301 ± 10	6.75 ± 0.36	16.7 ± 1.9	0.4
24*	28.9 ± 2.4	27.7 ± 1.6	23.2 ± 1.2	2.40 ± 0.14	9.7
25*	90.0 ± 3.4	334 ± 41	6.54 ± 0.44	26.5 ± 0.9	0.3
tacrine*	300 ± 13	n.d.	926 ± 30	10.2 ± 0.3	91
gallamine**	3150 ± 110	n.d.	2110 ± 70	2390 ± 100	0.9
tacrine*	42.3 ± 2.0	n.d.	136 ± 4	28.9 ± 0.8	4.7
gallamine**	1070 ± 60	n.d.	1480 ± 50	235 ± 10	6.3

[a] IC_{50} values were determined in duplicate with at least four different inhibitor concentrations. Assays were performed in 0.1 M sodium phosphate buffer, pH 7.3, 0.1 M NaCl, 6% MeCN. The last two lines refer to measurements in the absence of MeCN. n.d. = not determined. For details, see Experimental Section.

Table 2. Parameters Describing the Allosteric Inhibition of [^{3}H]NMS Dissociation from Porcine Muscarinic M_2 Receptors by the Indicated Test Compounds

cmpd	$pEC_{50,diss}$[a]	$EC_{50,diss}$ (nM)	n_H
13	8.88 ± 0.06[b]	1.3	−1.08 ± 0.16
14	8.31 ± 0.03	5.0	−1.21 ± 0.10[c]
15	9.07 ± 0.09[b]	0.9	−0.97 ± 0.18
16	8.82 ± 0.06[b]	1.5	−0.88 ± 0.13
24	8.86 ± 0.04[b]	1.4	−0.88 ± 0.08
25	8.99 ± 0.02[b]	1.0	−0.91 ± 0.04
tacrine	5.20 ± 0.03	6300	−1.60 ± 0.23[c]
gallamine	6.88 ± 0.10[b]	130	−0.82 ± 0.21

[a] pEC_{50}: −log concentrations inducing a half-maximal reduction of the apparent rate constant k_{-1} of [^{3}H]NMS dissociation as a measure of binding affinity. For details, see Experimental Section. [b] Slope factor is not different from unity and was fixed to $n_H = 1$ for pEC_{50} evaluation. [c] Slope factor is different from unity (F-test, $p < 0.05$).

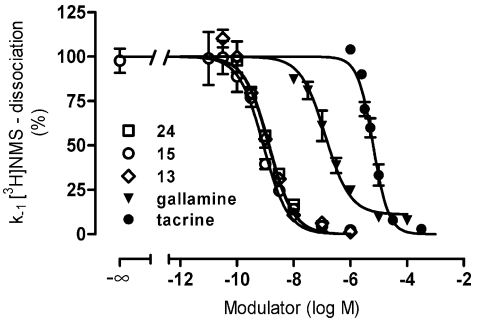


Figure 3. Interaction of the indicated test compounds with muscarinic M_2 receptors whose acetylcholine binding site is blocked by [^{3}H]NMS. Ordinate: apparent rate constant k_{-1} of [^{3}H]NMS-dissociation as a percentage of the control value determined in the absence of test compound. Curve inflection points of the hybrids showed no significant differences (one-way ANOVA, $p > 0.05$). Shown are means ± SEM derived from 3−12 dissociation experiments with duplicated values.

case of acetylcholinesterase from *Electrophorus electricus*, the affinity decreased by 2 orders of magnitude when comparing compounds **13**, **15**, and **24**. This loss in inhibitory potency due to increasing substitution was only 2- or 3-fold for the other three cholinesterases, as it was also observed in the case of the chloro-substituted derivatives **14**, **16**, and **25** with all studied cholinesterases. This might indicate the overall increase in energy consumption due to an increase of torsional constraints. In case of a chloro substitution at position 6 of the tacrine moiety (**14**, **16**, **25**), a significant loss in inhibitory potency toward acetylcholinesterase from *Torpedo californica* and a switch in selectivity from human butyrylcholinesterase to acetylcholinesterase was observed. Both phenomena are tightly linked to Pro446 in hAChE and its counterpart Met437 in hBChE or Ile439 in TcAChE. While the proline offers enough space to accommodate a chlorine at position 6, neither the methionine nor the isoleucine do so and thus impair the binding of a chloro-substituted tacrine to the active site (Figure 2). The 5-fold selectivity toward acetylcholinesterase achieved by the introduction of chlorine is in accordance with previous reports.[27,28]

Allosteric M_2 Receptor Modulation. The interaction of the building blocks and the hybrids **13**−**16**, **24**, and **25** with M_2 receptors was measured in receptors whose acetylcholine-binding site was blocked by the radioligand [^{3}H]NMS. As a consequence of ligand binding to the allosteric site, which is located in the extracellular entrance of the receptor's binding cleft, the dissociation of [^{3}H]NMS was inhibited. All compounds retarded [^{3}H]NMS dissociation concentration-dependently, as indicated by a reduction of the apparent rate constant k_{-1} (Figure 3). $pEC_{50,diss}$ values, that is, minus log concentrations for a half-maximum effect, correspond to test compound binding affinity for the allosteric site (Table 2). The $pEC_{50,diss}$ values found for gallamine and tacrine were in agreement with previous reports.[13,29] Hybrid formation increased the allosteric potency by factors of 100 relative to gallamine and 4800 relative to tacrine. This gain in potency is of particular interest since homodimerization of tacrine only resulted in a 20 fold increase in allosteric potency.[14] Remarkably, five hybrid substances exhibited $EC_{50,diss}$ values around 1 nM, only for **14** a slightly increased value of 5 nM was observed. Neither the introduction of chlorine (**14**, **16**, **25**) into the tacrine ring system, nor the successive simplification of the gallamine moiety had a significant effect on allosteric potency. For example, the removal of one *para*-(**15** vs **24**) or two *meta*-triethylammonioethoxy substituents (**13** vs **24**) retained the inhibition of [^{3}H]NMS dissociation. Thus, the number and position of the triethylammonioethoxy substituents is not decisive for the pronounced gain in potency.

Whereas the concentration−effect curve of tacrine was steep (Table 2), as expected for an atypical allosteric modulator, most hybrids showed curve slopes not different from unity, which is in agreement with a typical mode of allosteric action. Again, **14** was an exception in that its curve slope was slightly steeper than unity (Table 2).

Conclusion

The successful synthesis of the gallamine−tacrine hybrid **24** was accomplished through a multistep convergent synthesis based on a concise protection-deprotection strategy and the previously established linker chemistry. All compounds exhibited a profound inhibitory potential toward acetylcholinesterases as well as butyrylcholinesterase. The simplified derivative **13** was found to be a very potent inhibitor of acetylcholinesterase from *Electrophorus electricus* with an IC_{50} value of about 500 pM and showed a three times increased activity at human acetylcholinesterase compared to the trisquaternary compound **24**. The anticipated influence of a 6-chloro substitution of the tacrine moiety on cholinesterase selectivity was observed, though all compounds tend to be rather unselective inhibitors of both human cholinesterases. The allosteric potency of the gallamine−tacrine hybrids at M_2 receptors is strongly increased compared to their building blocks, gallamine and tacrine. As the orthosteric site was occupied by the radioligand in these experiments, the gain in potency is explained by simultaneous hybrid binding to the core region and the peripheral region of the allosteric site. The detailed elucidation of the allosteric modulation of muscarinic receptors, including the question of whether orthosterically free receptors might allow for an allosteric-orthosteric hybrid binding topography, will be the subject of further studies and reported in due course.

Experimental Section

General Methods and Materials. Melting points were determined on a Boëtius melting point apparatus and are uncorrected. Several compounds were prepared using the Büchi Glas Uster autoclave "TinyClave". Thin-layer chromatography was performed on Merck aluminum sheets, silica gel 60 F_{254}. Preparative column chromatography was performed on Merck silica gel 60, 70−230 mesh. ^{13}C NMR (125 MHz) and ^1H NMR spectra (500 MHz) were recorded on a Bruker Avance DRX 500 spectrometer; ^{13}C NMR signals were assigned on the basis of $^{13}C/^1H$ correlation experiments (HSQC, HMBC). Acetylcholinesterase was purchased from Fluka (Deisenhofen, Germany; *Electrophorus electricus*) and Sigma (Steinheim, Germany; *Homo sapiens*), and butyrylcholinesterase was purchased from Lee Biosolutions (St. Louis, U.S.A.; *Homo sapiens*). Acetylcholinesterase from *Torpedo californica* was a kind gift from the Institute for Physiological Chemistry, University of Bonn, Germany. Elemental analyses were performed with a Vario EL apparatus.

4-Acetyloxybenzoic Acid (3). 4-Hydroxybenzoic acid (**1**; 0.15 mol, 20.7 g) was dissolved in a mixture of pyridine (50 mL) and acetic anhydride (50 mL). After stirring at room temperature for 1 h, the reaction mixture was poured into water (500 mL) and the pH was adjusted to about 2 using concentrated hydrochloric acid. The solution was extracted three times with ethyl acetate (500 mL), the combined organic extracts were dried using anhydrous sodium sulfate, and the solvent was evaporated in vacuo. The remaining crude product was washed with petrol ether and recrystallized from ethyl acetate to yield **3** (19.5 g, 72%) as white needles, mp 195 °C, lit.[30] 187−192 °C. ^1H NMR (DMSO-d_6) δ 2.28 (s, 3 H), 7.25 (ddd, 2 H, J = 2.4, 2.5, 8.9 Hz), 7.98 (ddd, 2 H, J = 2.2, 2.5, 8.9 Hz), 12.96 (bs, 1 H); ^{13}C NMR (DMSO-d_6) δ 21.01, 122.18, 128.47, 130.99, 154.09, 166.74, 168.97. Anal. ($C_9H_8O_4$) C, H, N.

3,5-Bis(acetyloxy)benzoic Acid (4). 3,5-Dihydroxybenzoic acid (**2**; 0.15 mol, 23.1 g) was reacted as 4-hydroxybenzoic acid (**1**) to yield **4** (30.0 g, 84%) as a white powder, mp 164 °C, lit.[31] 161 °C. ^1H NMR (DMSO-d_6) δ 2.28 (s, 6 H), 7.26 (t, 1 H, J = 2.2 Hz), 7.57 (d, 2 H, J = 2.2 Hz), 13.34 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 20.92, 120.29, 120.50, 133.05, 151.06, 165.89, 169.05. Anal. ($C_{11}H_{10}O_6$) C, H, N.

Ethyl 6-((4-Hydroxybenzoyl)amino)hexanoate (5). 4-Acetyloxybenzoic acid (**3**; 30.0 mmol, 5.40 g) was dissolved in anhydrous dichloromethane (50 mL) with catalytic amounts of *N*,*N*-dimethylformamide. Oxalyl chloride (34.4 mmol, 3.0 mL) was added while stirring, and once the gas evolution had ceased, it was evaporated to dryness. The residue was again dissolved in dichloromethane (20 mL), and ethyl 6-aminohexanoate hydrochloride (30.0 mmol, 5.88 g) was suspended in the solution. After dropwise addition of *N*-ethyl-*N*,*N*-diisopropylamine (60.0 mmol, 10.5 mL) over 1 h, washing with water and a saturated solution of sodium hydrogen carbonate, drying with anhydrous sodium sulfate, and evaporation in vacuo yielded a white solid that was dissolved in anhydrous methanol (60 mL). After adding potassium carbonate (3.2 mmol, 0.44 g) and stirring for 12 h, the solution was poured into water (200 mL) containing 2 M hydrochloric acid (4 mL). The resulting suspension was extracted three times using ethyl acetate, and the combined organic phases were dried using anhydrous sodium sulfate. Compound **5** was obtained by evaporation in vacuo as a white powder (7.71 g, 92%), mp 75 °C. ^1H NMR (DMSO-d_6) δ 1.15 (t, 3 H, J = 7.1 Hz), 1.28 (tt, 2 H, J = 7.6 Hz), 1.48 (tt, 2 H, J = 7.4 Hz), 1.53 (tt, 2 H, J = 7.5 Hz), 2.26 (t, 2 H, J = 7.4 Hz), 3.19 (dt, 2 H, J = 6.5 Hz), 4.03 (q, 2 H, J = 7.1 Hz), 6.76 (ddd, 2 H, J = 2.4, 2.9, 9.5 Hz), 7.68 (ddd, 2 H, J = 2.4, 2.9, 9.5 Hz), 8.12 (t, 1 H, J = 5.7 Hz), 9.86 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 14.24, 24.37, 26.07, 29.04, 33.62, 38.98, 59.75, 114.81, 125.60, 129.11, 160.04, 165.92, 172.97. Anal. ($C_{15}H_{21}NO_4$) C, H, N.

Ethyl 6-((3,5-Dihydroxybenzoyl)amino)hexanoate (6). 3,5-Bis(acetyloxy)benzoic acid (**4**; 20.0 mmol, 4.76 g) was reacted as 4-acetyloxybenzoic acid (**3**), and **6** was obtained as a white precipitate by suction filtration (4.76 g, 81%), mp 78 °C. ^1H NMR (DMSO-d_6) δ 1.16 (t, 3 H, J = 7.1 Hz), 1.27 (tt, 2 H, J = 7.7 Hz), 1.47 (tt, 2 H, J = 7.5 Hz), 1.53 (tt, 2 H, J = 7.6 Hz), 2.26 (t, 2 H, J = 7.3 Hz), 3.16 (dt, 2 H, J = 6.6 Hz), 4.03 (q, 2 H, J = 7.2 Hz), 6.32 (t, 1 H, J = 2.2 Hz), 6.63 (d, 2 H, J = 2.2 Hz), 8.15 (t, 1 H, J = 5.7 Hz), 9.35 (s, 2 H); ^{13}C NMR (DMSO-d_6) δ 14.26, 24.35, 26.03, 28.87, 33.63, 39.00, 59.77, 105.00, 105.53, 137.19, 158.31, 166.49, 172.98. Anal. ($C_{15}H_{21}NO_5 \cdot H_2O$) C, H, N.

Ethyl 6-((4-(2-Bromoethoxy)benzoyl)amino)hexanoate (7). Ethyl 6-((4-hydroxybenzoyl)amino)hexanoate (**5**; 25.0 mmol, 6.98 g), potassium carbonate (150.0 mmol, 20.73 g), and 18-crown-6 (3.8 mmol, 1.00 g) were suspended in 1,2-dibromoethane (120 mL) and heated to 80 °C for 36 h while stirring. Subsequent removal of the inorganic salts by suction filtration, washing with dichloromethane, and evaporation in vacuo produced a crude residue that was subjected to column chromatography using petrol ether and ethyl acetate in a ratio of 1:2. Compound **7** was achieved from the first fractions as a white solid (8.74 g, 91%), mp 82 °C. ^1H NMR (DMSO-d_6) δ 1.15 (t, 3 H, J = 7.1 Hz), 1.29 (tt, 2 H, J = 7.6 Hz), 1.50 (tt, 2 H, J = 7.3 Hz), 1.54 (tt, 2 H, J = 7.6 Hz), 2.27 (t, 2 H, J = 7.4 Hz), 3.21 (dt, 2 H, J = 6.6 Hz), 3.80 (t, 2 H, J = 5.5 Hz), 4.03 (q, 2 H, J = 7.1 Hz), 4.37 (t, 2 H, J = 5.4 Hz), 7.00 (ddd, 2 H, J = 2.5, 2.8, 9.8. Hz), 7.80 (ddd, 2 H, J = 2.5, 2.8, 9.8 Hz), 8.26 (t, 1 H, J = 5.7 Hz); ^{13}C NMR (DMSO-d_6) δ 14.24, 24.36, 26.05, 28.97, 31.37, 33.60, 39.05, 59.75, 67.99, 114.19, 127.58, 129.09, 160.16, 165.58, 172.95. Anal. ($C_{17}H_{24}BrNO_4$) C, H, N.

Ethyl 6-((3,5-Bis(2-bromoethoxy)benzoyl)amino)hexanoate (8). Ethyl 6-((3,5-dihydroxybenzoyl)amino)hexanoate (**6**; 40.0 mmol, 11.81 g) was reacted as ethyl 6-((4-hydroxybenzoyl)amino)-hexanoate (**5**) to obtain **8** as a white powder (3.10 g, 64%), mp 98 °C. ^1H NMR (DMSO-d_6) δ 1.15 (t, 3 H, J = 7.3 Hz), 1.29 (tt, 2 H, J = 7.6 Hz), 1.50 (tt, 2 H, J = 7.6 Hz), 1.54 (tt, 2 H, J = 7.6 Hz), 2.27 (t, 2 H, J = 7.3 Hz), 3.21 (dt, 2 H, J = 6.5 Hz), 3.79 (t, 4 H, J = 5.4 Hz), 4.03 (q, 2 H, J = 7.2 Hz), 4.35 (t, 4 H, J = 5.4 Hz), 6.69 (t, 1 H, J = 2.2 Hz), 7.03 (d, 2 H, J = 2.2 Hz), 8.39 (t, 1 H, J = 5.5 Hz); ^{13}C NMR (DMSO-d_6) δ 14.24, 24.34, 26.03, 28.82, 31.44, 33.59, 39.17, 59.75, 68.19, 104.15, 106.52, 137.05, 159.08, 165.39, 172.95. Anal. ($C_{19}H_{27}Br_2NO_5$) C, H, N.

2-(4-(((6-Ethoxy-6-oxohexyl)amino)carbonyl)phenoxy)-*N*,*N*,*N*-triethylethanammonium Bromide (9). Ethyl 6-((4-(2-bromoethoxy)benzoyl)amino)hexanoate (**7**; 20.0 mmol, 7.73 g) was heated to 60 °C for 24 h in a solution of triethylamine (200 mmol, 27.9 mL) and anhydrous nitromethane (100 mL). After cooling to room temperature, the solvent and excess amine were evaporated in vacuo and the oily residue was taken up in absolute ethanol (10 mL).

Pouring the alcoholic solution into ethyl acetate (1000 mL) produced the final product **9** as a white and hygroscopic precipitate (7.54 g, 77%). ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3 H, J = 7.1 Hz), 1.23 (t, 9 H, J = 7.3 Hz), 1.29 (tt, 2 H, J = 7.6 Hz), 1.50 (tt, 2 H, J = 7.3 Hz), 1.54 (tt, 2 H, J = 7.5 Hz), 2.27 (t, 2 H, J = 7.4 Hz), 3.21 (dt, 2 H, J = 6.6 Hz), 3.38 (q, 6 H, J = 7.2 Hz), 3.69 (t, 2 H, J = 4.9 Hz), 4.03 (q, 2 H, J = 7.2 Hz), 4.45 (t, 2 H, J = 4.7 Hz), 7.03 (ddd, 2 H, J = 2.4, 2.8, 9.8 Hz), 7.85 (ddd, 2 H, J = 2.5, 2.9, 9.6 Hz), 8.30 (t, 1 H, J = 5.7 Hz); ^{13}C NMR (DMSO-d_6) δ 7.45, 14.25, 24.35, 26.05, 28.98, 33.60, 39.06, 53.09, 55.29, 59.75, 61.34, 114.22, 127.85, 129.06, 159.61, 165.46, 172.96. Anal. ($C_{23}H_{39}BrN_2O_4 \cdot 0.5H_2O$) C: calcd, 55.64; found, 55.17; H, N.

2-(3-(((6-Ethoxy-6-oxohexyl)amino)carbonyl)-5-((2-triethylammonio)ethoxy)phenoxy)-*N*,*N*,*N*-triethylethanammonium Dibromide (10). Ethyl 6-((3,5-bis(2-bromoethoxy)benzoyl)amino)-hexanoate (**8**; 20.0 mmol, 10.18 g) were reacted as ethyl 6-((4-(2-bromoethoxy)benzoyl)amino)hexanoate (**7**) to obtain **10** by precipitation of an alcoholic solution (50 mL) from ethyl acetate (900 mL) as a white and hygroscopic powder (11.5 g, 81%). ^{1}H NMR (DMSO-d_6) δ 1.16 (t, 3 H, J = 7.1 Hz), 1.24 (t, 18 H, J = 7.1 Hz), 1.29 (tt, 2 H, J = 7.8 Hz), 1.52 (tt, 2 H, J = 7.3 Hz), 1.54 (tt, 2 H, J = 7.4 Hz), 2.27 (t, 2 H, J = 7.4 Hz), 3.23 (dt, 2 H, J = 6.6 Hz), 3.39 (q, 12 H, J = 7.2 Hz), 3.69 (t, 4 H, J = 4.7 Hz), 4.03 (q, 2 H, J = 7.2 Hz), 4.46 (t, 4 H, J = 4.7 Hz), 6.75 (t, 1 H, J = 2.2 Hz), 7.15 (d, 2 H, J = 2.2 Hz), 8.52 (t, 1 H, J = 5.7 Hz); ^{13}C NMR (DMSO-d_6) δ 7.50, 14.26, 24.33, 26.04, 28.94, 33.59, 39.15, 53.11, 55.32, 59.77, 61.72, 104.48, 106.79, 137.08, 158.50, 165.30, 172.96. Anal. ($C_{31}H_{57}Br_2N_3O_5 \cdot H_2O$) C, H, N.

***N*,*N*,*N*-Triethyl-2-(4-(((6-hydrazino-6-oxohexyl)amino)carbonyl)phenoxy)ethanammonium Bromide (11).** 2-(4-(((6-Ethoxy-6-oxohexyl)amino)carbonyl)phenoxy)-*N*,*N*,*N*-triethylethanammonium bromide (**9**; 2.0 mmol, 0.97 g) was dissolved in a mixture of hydrazine (51.4 mmol, 2.5 mL hydrazine hydrate 100%) and absolute ethanol (20 mL), and the solution was heated to 80 °C for 72 h in a glass reactor. After cooling to room temperature, absolute ethanol (80 mL) was added and the solution evaporated in vacuo. The remaining residue was taken up in absolute ethanol (10 mL) and poured into ethyl acetate (300 mL) at −20 °C while stirring. Compound **11** was obtained by suction filtration as a white and very hygroscopic powder (0.81 g, 86%). ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 9 H, J = 7.3 Hz), 1.26 (tt, 2 H, J = 7.6 Hz), 1.50 (tt, 4 H, J = 7.2 Hz), 2.00 (t, 2 H, J = 7.4 Hz), 3.21 (dt, 2 H, J = 6.6 Hz), 3.38 (q, 6 H, J = 7.3 Hz), 3.69 (t, 2 H, J = 4.9 Hz), 4.12 (s, 2 H), 4.44 (t, 2 H, J = 4.7 Hz), 7.03 (ddd, 2 H, J = 2.4, 2.9, 9.8 Hz), 7.85 (ddd, 2 H, J = 2.5, 2.9, 9.8 Hz), 8.30 (t, 1 H, J = 5.7 Hz), 8.88 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 7.45, 25.13, 26.33, 29.12, 33.52, 39.19, 53.09, 55.29, 61.33, 114.22, 127.86, 129.07, 159.60, 165.44, 171.66. Anal. ($C_{21}H_{37}BrN_4O_3 \cdot 0.5H_2O$) C, H, N: calcd, 11.61; found, 11.12.

***N*,*N*,*N*-Triethyl-2-(3-(((6-hydrazino-6-oxohexyl)amino)carbonyl)-5-((2-triethylammonio)ethoxy)phenoxy)ethanammonium Dibromide (12).** 2-(3-(((6-Ethoxy-6-oxohexyl)amino)carbonyl)-5-((2-triethylammonio)ethoxy)phenoxy)-*N*,*N*,*N*-triethylethanammonium dibromide (**10**; 2.0 mmol, 1.42 g) was reacted as 2-(4-(((6-ethoxy-6-oxohexyl)amino)carbonyl)phenoxy)-*N*,*N*,*N*-triethylethanammonium bromide (**9**) to precipitate **12** from ethyl acetate (300 mL) using an alcoholic solution (10 mL) at room temperature. Compound **12** was obtained as a white and very hygroscopic powder (1.07 g, 77%). ^{1}H NMR (DMSO-d_6) δ 1.24 (t, 18 H, J = 7.1 Hz), 1.27 (m, 2 H), 1.51 (m, 4 H), 2.01 (t, 2 H, J = 7.4 Hz), 3.22 (dt, 2 H, J = 6.6 Hz), 3.40 (q, 12 H, J = 7.3 Hz), 3.69 (t, 4 H, J = 4.9 Hz), 4.42 (bs, 2 H), 4.47 (t, 4 H, J = 4.6 Hz), 6.76 (t, 1 H, J = 2.4 Hz), 7.16 (d, 2 H, J = 2.2 Hz), 8.53 (t, 1 H, J = 5.7 Hz), 8.95 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 7.50, 25.10, 26.30, 29.04, 33.49, 39.25, 53.11, 55.32, 61.74, 104.52, 106.80, 137.09, 158.50, 165.30, 171.65. Anal. ($C_{29}H_{55}Br_2N_5O_4 \cdot H_2O$) C, H, N.

***N*,*N*,*N*-Triethyl-2-(4-(((6-(2-(1,2,3,4-tetrahydroacridin-9-yl)-hydrazino)-6-oxohexyl)amino)carbonyl)phenoxy)ethanammonium Bromide Hydrochloride (13).** *N*,*N*,*N*-Triethyl-2-(4-(((6-hydrazino-6-oxohexyl)amino)carbonyl)phenoxy)ethanammonium bromide (**11**; 1.0 mmol, 0.47 g) and 9-chloro-1,2,3,4-tetrahydroacridine (1.0 mmol, 0.22 g) were dissolved in absolute ethanol (20 mL) and heated to 140 °C for 24 h in a glass reactor. After cooling to room temperature and evaporation of the solvent in vacuo, the remaining residue was taken up in absolute ethanol (10 mL) and poured into ethyl acetate (300 mL) while stirring to precipitate the final product. Compound **13** was obtained by suction filtration as a yellow, hygroscopic powder (0.62 g, 90%). ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 9 H, J = 7.1 Hz), 1.29 (tt, 2 H, J = 7.6 Hz), 1.51 (tt, 2 H, J = 7.3 Hz), 1.56 (tt, 2 H, J = 7.6 Hz), 1.80 (bs, 4 H), 2.29 (t, 2 H, J = 7.2 Hz), 2.69 (bs, 2 H), 3.09 (bs, 2 H), 3.20 (dt, 2 H, J = 6.2 Hz), 3.39 (q, 6 H, J = 7.3 Hz), 3.70 (t, 2 H, J = 4.9 Hz), 4.45 (t, 2 H, J = 4.7 Hz), 7.03 (d, 2 H, J = 8.9 Hz), 7.57 (ddd, 1 H, J = 1.0, 7.3, 8.6 Hz), 7.85 (ddd, 1 H, J = 1.0, 7.1, 8.6 Hz), 7.88 (d, 2 H, J = 8.9 Hz), 8.09 (dd, 1 H, J = 0.7, 8.7 Hz), 8.42 (t, 1 H, J = 5.2 Hz), 8.69 (d, 1 H, J = 8.8 Hz), 9.65 (s, 1 H), 11.15 (s, 1 H), 14.68 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 7.51, 20.26, 21.43, 24.18, 24.55, 26.32, 28.24, 29.08, 33.09, 39.13, 53.14, 55.35, 61.41, 111.13, 114.24, 115.10, 119.42, 124.27, 125.82, 127.83, 129.16, 132.96, 137.44, 152.61, 155.29, 159.64, 165.50, 171.90. Anal. ($C_{34}H_{49}BrClN_5O_3 \cdot 2H_2O$) C: calcd, 56.16; found, 55.73; H, N.

2-(4-(((6-(2-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)hydrazino)-6-oxohexyl)amino)carbonyl)phenoxy)-*N*,*N*,*N*-triethylethanammonium Bromide Hydrochloride (14). *N*,*N*,*N*-Triethyl-2-(4-(((6-hydrazino-6-oxohexyl)amino)carbonyl)phenoxy)ethanammonium bromide (**11**; 1.0 mmol, 0.47 g) and 6,9-dichloro-1,2,3,4-tetrahydroacridine (1.0 mmol, 0.25 g) were reacted as described above to obtain **14** as a yellow and hygroscopic powder (0.68 g, 94%). ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 9 H, J = 7.3 Hz), 1.29 (tt, 2 H, J = 7.6 Hz), 1.52 (tt, 2 H, J = 7.3 Hz), 1.56 (tt, 2 H, J = 7.3 Hz), 1.80 (bs, 4 H), 2.30 (t, 2 H, J = 7.3 Hz), 2.66 (bs, 2 H), 3.07 (bs, 2 H), 3.25 (dt, 2 H, J = 6.3 Hz), 3.39 (q, 6 H, J = 7.3 Hz), 3.70 (t, 2 H, J = 4.7 Hz), 4.45 (t, 2 H, J = 4.6 Hz), 7.02 (d, 2 H, J = 8.9 Hz), 7.64 (dd, 1 H, J = 2.2, 9.5 Hz), 7.87 (d, 2 H, J = 8.8 Hz), 8.19 (d, 1 H, J = 2.2 Hz), 8.41 (t, 1 H, J = 5.4 Hz), 8.73 (d, 1 H, J = 9.5 Hz), 9.77 (s, 1 H), 11.18 (s, 1 H), 14.88 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 7.49, 20.16, 21.28, 24.05, 24.49, 26.29, 28.25, 29.06, 33.10, 39.11, 53.14, 55.34, 61.40, 111.49, 113.64, 114.22, 118.27, 126.19, 126.68, 127.82, 129.13, 137.46, 138.23, 153.22, 155.11, 159.63, 165.50, 171.96. Anal. ($C_{34}H_{48}BrCl_2N_5O_3 \cdot 2H_2O$) C, H, N.

***N*,*N*,*N*-Triethyl-2-(3-(((6-(2-(1,2,3,4-tetrahydroacridin-9-yl)-hydrazino)-6-oxohexyl)amino)carbonyl)-5-((2-triethylammonio)-ethoxy)phenoxy)ethanammonium Dibromide Hydrochloride (15).** *N*,*N*,*N*-Triethyl-2-(3-(((6-hydrazino-6-oxohexyl)amino)carbonyl)-5-((2-triethylammonio)ethoxy)phenoxy)ethanammonium dibromide (**12**; 0.5 mmol, 0.35 g) and 9-chloro-1,2,3,4-tetrahydroacridine (0.5 mmol, 0.11 g) were reacted as described above to achieve **15** as a yellow and hygroscopic powder (0.33 g, 72%). ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 18 H, J = 7.1 Hz), 1.29 (tt, 2 H, J = 7.8 Hz), 1.54 (tt, 2 H, J = 7.6 Hz), 1.56 (tt, 2 H, J = 7.6 Hz), 1.81 (bs, 4 H), 2.29 (t, 2 H, J = 7.4 Hz), 2.69 (bs, 2 H), 3.09 (bs, 2 H), 3.22 (dt, 2 H, J = 6.6 Hz), 3.40 (q, 12 H, J = 7.2 Hz), 3.69 (t, 4 H, J = 4.9 Hz), 4.49 (t, 4 H, J = 4.6 Hz), 6.77 (t, 1 H, J = 2.2 Hz), 7.24 (d, 2 H, J = 2.3 Hz), 7.59 (ddd, 1 H, J = 1.0, 7.1, 8.5 Hz), 7.87 (ddd, 1 H, J = 1.0, 7.7, 8.4 Hz), 8.06 (dd, 1 H, J = 1.0, 8.5 Hz), 8.69 (d, 1 H, J = 8.5 Hz), 8.77 (t, 1 H, J = 5.7 Hz), 9.65 (s, 1 H), 11.11 (s, 1 H), 14.51 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 7.53, 20.27, 21.40, 24.18, 24.53, 26.30, 28.27, 28.99, 33.07, 39.20, 53.14, 55.33, 61.81, 104.60, 106.91, 111.22, 115.10, 119.45, 124.22, 125.88, 133.04, 137.02, 137.42, 152.67, 155.31, 158.51, 165.28, 171.89. Anal. ($C_{42}H_{67}Br_2ClN_6O_4 \cdot 3H_2O$) C, H, N.

2-(3-(((6-(2-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)hydrazino)-6-oxohexyl)amino)carbonyl)-5-((2-triethylammonio)ethoxy)phenoxy)-*N*,*N*,*N*-triethylethanammonium Dibromide Hydrochloride (16). *N*,*N*,*N*-Triethyl-2-(3-(((6-hydrazino-6-oxohexyl)amino)carbonyl)-5-((2-triethylammonio)ethoxy)phenoxy)ethanammonium dibromide (**12**; 1.0 mmol, 0.70 g) and 6,9-dichloro-1,2,3,4-tetrahydroacridine (1.0 mmol, 0.25 g) were reacted as described above to yield **16** as a yellow and hygroscopic powder (0.88 g, 93%). ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 18 H, J = 7.3 Hz), 1.30 (tt, 2 H, J = 7.9 Hz), 1.54 (tt, 2 H, J = 7.6 Hz), 1.56 (tt, 2 H, J = 7.6 Hz), 1.80 (bs, 4 H), 2.30 (t, 2 H, J = 7.3 Hz), 2.66 (bs, 2 H), 3.07 (bs, 2 H),

3.22 (dt, 2 H, $J = 6.5$ Hz), 3.40 (q, 12 H, $J = 7.2$ Hz), 3.69 (t, 4 H, $J = 4.6$ Hz), 4.49 (t, 4 H, $J = 4.3$ Hz), 6.76 (t, 1 H, $J = 2.1$ Hz), 7.23 (d, 2 H, $J = 2.2$ Hz), 7.65 (dd, 1 H, $J = 2.2$, 9.2 Hz), 8.18 (d, 1 H, $J = 2.2$ Hz), 8.74 (d, 1 H, $J = 9.2$ Hz), 8.76 (t, 1 H, $J = 5.5$ Hz), 9.77 (s, 1 H), 11.18 (s, 1 H), 14.79 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 7.55, 20.17, 21.28, 24.08, 24.49, 26.29, 28.25, 28.96, 33.10, 39.20, 53.16, 55.36, 61.82, 104.63, 106.93, 111.53, 113.65, 118.26, 126.23, 126.70, 137.03, 137.48, 138.22, 153.25, 155.16, 158.51, 165.29, 171.96. Anal. ($C_{42}H_{66}Br_2Cl_2N_6O_4 \cdot 4H_2O$) C, H, N.

3,4,5-Tris(acetyloxy)benzoic Acid (18). 3,4,5-Trihydroxybenzoic acid (**17**; 0.15 mol, 25.5 g) was reacted as 4-hydroxybenzoic acid (**1**) to yield **18** (38.8 g, 87%) as a white powder, mp 172 °C, lit.[32] 163 °C. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 6 H), 2.32 (s, 3 H), 7.74 (s, 2 H), 13.39 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 20.50, 122.06, 129.07, 138.42, 143.36, 165.51, 167.04, 168.12. Anal. ($C_{13}H_{12}O_8$) C, H, N.

Ethyl 6-((4-Benzyloxy-3,5-dihydroxybenzoyl)amino)hexanoate (19). 3,4,5-Tris(acetyloxy)benzoic acid (**18**; 30.0 mmol, 8.89 g) and ethyl 6-aminohexanoate hydrochloride (30.0 mmol, 5.88 g) were reacted following the standard procedure described before. Evaporation in vacuo produced a crude oil that was dissolved in anhydrous acetone (100 mL). Benzyl chloride (60.0 mmol, 6.9 mL), potassium carbonate (90.0 mmol, 12.4 g), and potassium iodide (9.0 mmol, 1.5 g) were added, and the reaction mixture was heated to reflux for 18 h. Subsequent removal of the inorganic salts by suction filtration and evaporation in vacuo led to an oily residue that was washed with *n*-hexane to remove excess benzyl chloride. A solution of potassium carbonate (30.0 mmol, 4.1 g) in water (40 mL) was added to a solution of the residue in a mixture of ethyl acetate (200 mL) and methanol (50 mL). After refluxing for 1 h and washing with water (150 mL) containing concentrated hydrochloric acid (5 mL), the reaction mixture was subjected to flash column chromatography using ethyl acetate. Recrystallization from chloroform and *n*-pentane produced **19** (7.47 g, 62%) as a white solid, mp 119 °C. ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3 H, $J = 7.1$ Hz), 1.27 (tt, 2 H, $J = 7.7$ Hz), 1.46 (tt, 2 H, $J = 7.3$ Hz), 1.53 (tt, 2 H, $J = 7.5$ Hz), 2.26 (t, 2 H, $J = 7.4$ Hz), 3.15 (dt, 2 H, $J = 6.5$ Hz), 4.03 (q, 2 H, $J = 7.0$ Hz), 5.00 (s, 2 H), 6.78 (s, 2 H), 7.28 (tt, 1 H, $J = 1.4$, 7.4 Hz), 7.33 (tt, 2 H, $J = 1.4$, 6.6 Hz), 7.50 (d, 2 H, $J = 6.9$ Hz), 8.09 (t, 1 H, $J = 5.5$ Hz), 9.24 (s, 2 H); ^{13}C NMR (DMSO-d_6) δ 14.25, 24.36, 26.03, 28.91, 33.63, 39.01, 59.77, 73.21, 106.95, 127.79, 128.11, 128.31, 130.38, 136.57, 138.04, 150.54, 166.26, 172.97. Anal. ($C_{22}H_{27}NO_6$) C: calcd, 65.82; found, 65.02; H, N.

Ethyl 6-((4-Benzyloxy-3,5-bis-(2-bromoethoxy)benzoyl)amino)hexanoate (20). Ethyl 6-((4-benzyloxy-3,5-dihydroxybenzoyl)-amino)hexanoate (**19**; 15.0 mmol, 6.02 g), potassium carbonate (150.0 mmol, 20.73 g), and 18-crown-6 (3.8 mmol, 1.00 g) were suspended in 1,2-dibromoethane (120 mL) and heated to 80 °C for 36 h while stirring. Subsequent removal of the inorganic salts by suction filtration, washing with dichloromethane, and evaporation in vacuo resulted in a crude product that was subjected to column chromatography using petrol ether and ethyl acetate in a ratio of 1:2. Compound **20** was achieved from the first fractions as a white solid (8.33 g, 90%), mp 89 °C. ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3 H, $J = 7.3$ Hz), 1.30 (tt, 2 H, $J = 7.7$ Hz), 1.51 (tt, 2 H, $J = 7.6$ Hz), 1.55 (tt, 2 H, $J = 7.6$ Hz), 2.27 (t, 2 H, $J = 7.4$ Hz), 3.23 (dt, 2 H, $J = 6.6$ Hz), 3.84 (t, 4 H, $J = 5.4$ Hz), 4.03 (q, 2 H, $J = 7.2$ Hz), 4.37 (t, 4 H, $J = 5.4$ Hz), 5.07 (s, 2 H), 7.18 (s, 2 H), 7.30 (tt, 1 H, $J = 1.6$, 7.3 Hz), 7.36 (tt, 2 H, $J = 1.3$, 7.0 Hz), 7.53 (m, 2 H), 8.35 (t, 1 H, $J = 5.7$ Hz); ^{13}C NMR (DMSO-d_6) δ 14.24, 24.36, 26.07, 28.99, 31.64, 33.58, 39.21, 59.76, 68.97, 74.35, 106.59, 128.00, 128.23, 128.51, 130.19, 137.67, 139.27, 151.68, 165.32, 172.94. Anal. ($C_{26}H_{33}Br_2NO_6$) C, H, N.

2-(5-(((6-Ethoxy-6-oxohexyl)amino)carbonyl)-2-(2-bromoethoxy)-3-((2-triethylammonio)ethoxy)phenoxy)-*N*,*N*,*N*-triethylethanammonium Dibromide (21). Ethyl 6-((4-benzyloxy-3,5-bis-(2-bromoethoxy)benzoyl)amino)hexanoate (**20**; 10.0 mmol, 6.15 g) was heated to 60 °C for 24 h in a solution of triethylamine (100.0 mmol, 14.0 mL) and anhydrous nitromethane (100 mL). After cooling to room temperature, the solvent and excess amine were thoroughly evaporated in vacuo to allow the deprotection of the intermediate. Hydrogenolysis was carried out using Pd/C (0.8 g) and hydrogen at balloon pressure in anhydrous methanol for 6 h. Subsequently, the catalyst was filtered off and the reaction mixture was evaporated in vacuo. Potassium carbonate (150.0 mmol, 20.73 g) and 18-crown-6 (3.8 mmol, 1.00 g) were added to the remaining oil, and the reaction apparatus was protected by argon, followed by the addition of 1,2-dibromoethane (60 mL) and anhydrous acetonitrile (60 mL). The suspension was heated to 80 °C for 36 h and the inorganic salts were removed afterward. Evaporation of the acetonitrile produced a suspension that was diluted by ethyl acetate (50 mL) to obtain the precipitate **21** by suction filtration (4.20 g, 50%) as a white and hygroscopic powder. ^{1}H NMR (DMSO-d_6) δ 1.16 (t, 3 H, $J = 7.1$ Hz), 1.24 (t, 18 H, $J = 7.1$ Hz), 1.30 (tt, 2 H, $J = 7.6$ Hz), 4.03 (q, 2 H, $J = 7.2$ Hz), 1.54 (tt, 2 H, $J = 7.3$ Hz), 1.54 (tt, 2 H, $J = 7.6$ Hz), 2.27 (t, 2 H, $J = 7.4$ Hz), 3.25 (dt, 2 H, $J = 6.6$ Hz), 3.42 (q, 12 H, $J = 7.2$ Hz), 3.71 (t, 2 H, $J = 6.2$ Hz), 3.74 (t, 4 H, $J = 4.9$ Hz), 4.23 (t, 2 H, $J = 6.0$ Hz), 4.50 (t, 4 H, $J = 4.7$ Hz), 7.42 (s, 2 H), 8.62 (t, 1 H, $J = 5.8$ Hz); ^{13}C NMR (DMSO-d_6) δ 7.55, 14.26, 24.35, 26.05, 29.03, 31.93, 33.60, 39.13, 53.18, 55.53, 59.77, 62.57, 72.70, 107.02, 130.49, 138.42, 150.98, 165.17, 172.97. Anal. ($C_{33}H_{60}Br_3N_3O_6 \cdot H_2O$) C, H, N.

2-(5-(((6-Ethoxy-6-oxohexyl)amino)carbonyl)-2,3-bis((2-triethylammonio)ethoxy)phenoxy)-*N*,*N*,*N*-triethylethanammonium Tribromide (22). 2-(5-(((6-Ethoxy-6-oxohexyl)amino)carbonyl)-2-(2-bromoethoxy)-3-((2-triethylammonio)ethoxy)phenoxy)-*N*,*N*,*N*-triethylethanammonium dibromide (**21**; 2.0 mmol, 1.67 g) was suspended in a mixture of triethylamine (36.0 mmol, 5.0 mL) and anhydrous acetonitrile (20 mL). The reaction mixture was heated to 100 °C for 48 h in a glass reactor. At first the educt dissolved as the temperature raised, but soon the final product precipitated. After cooling to room temperature **22** was collected by suction filtration (1.30 g, 69%) as a white and hygroscopic powder and washed once with cooled acetonitrile. ^{1}H NMR (DMSO-d_6) δ 1.16 (t, 3 H, $J = 7.1$ Hz), 1.23 (t, 9 H, $J = 7.3$ Hz), 1.25 (t, 18 H, $J = 7.3$ Hz), 1.30 (tt, 2 H, $J = 7.9$ Hz), 1.51−1.59 (m, 4 H), 2.27 (t, 2 H, $J = 7.4$ Hz), 3.25 (dt, 2 H, $J = 6.7$ Hz), 3.45 (q, 12 H, $J = 7.3$ Hz), 3.47 (q, 6 H, $J = 7.3$ Hz), 3.61 (t, 2 H, $J = 4.9$ Hz), 3.73 (t, 4 H, $J = 5.5$ Hz), 4.03 (q, 2 H, $J = 7.2$ Hz), 4.26 (t, 2 H, $J = 4.7$ Hz), 4.55 (t, 4 H, $J = 5.4$ Hz), 7.48 (s, 2 H), 8.71 (t, 1 H, $J = 5.7$ Hz); ^{13}C NMR (DMSO-d_6) δ 7.57, 7.59, 14.26, 24.34, 26.05, 28.99, 33.59, 39.13, 53.20, 55.06, 55.83, 59.76, 62.19, 65.94, 106.72, 131.10, 137.45, 150.97, 165.05, 172.96. Anal. ($C_{39}H_{75}Br_3N_4O_6 \cdot H_2O$) C, H, N.

***N*,*N*,*N*-Triethyl-2-(5-(((6-hydrazino-6-oxohexyl)amino)carbonyl)-2,3-bis((2-triethylammonio)ethoxy)phenoxy)-ethanammonium Tribromide (23).** 2-(5-(((6-Ethoxy-6-oxohexyl)-amino)carbonyl)-2,3-bis((2-triethylammonio)ethoxy)phenoxy)-*N*,*N*,*N*-triethylethanammonium tribromide (**22**; 1.0 mmol, 0.94 g) was dissolved in a mixture of hydrazine (51.4 mmol, 2.5 mL hydrazine hydrate 100%) and absolute ethanol (20 mL), and the solution was heated to 80 °C for 72 h in a glass reactor. After cooling to room temperature, absolute ethanol (80 mL) was added and the solution was evaporated in vacuo. The remaining residue was taken up in absolute ethanol (10 mL) and poured into ethyl acetate (300 mL) while stirring to precipitate **23** as a white and very hygroscopic powder (0.84 g, 91%) that was collected by suction filtration. ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 9 H, $J = 7.2$ Hz), 1.25 (t, 20 H, $J = 6.9$ Hz), 1.51 (tt, 2 H, $J = 7.6$ Hz), 1.54 (tt, 2 H, $J = 7.6$ Hz), 2.01 (t, 2 H, $J = 7.4$ Hz), 3.25 (dt, 2 H, $J = 6.7$ Hz), 3.45 (q, 12 H, $J = 7.0$ Hz), 3.47 (q, 6 H, $J = 7.3$ Hz), 3.61 (t, 2 H, $J = 4.9$ Hz), 3.72 (t, 4 H, $J = 5.5$ Hz), 4.11 (bs, 2 H), 4.26 (t, 2 H, $J = 4.9$ Hz), 4.55 (t, 4 H, $J = 5.4$ Hz), 7.47 (s, 2 H), 8.68 (t, 1 H, $J = 5.8$ Hz), 8.90 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 7.57, 7.59, 25.14, 26.32, 29.10, 33.52, 39.26, 53.20, 55.07, 55.83, 62.18, 65.94, 106.70, 131.14, 137.43, 150.98, 165.07, 171.66.

***N*,*N*,*N*-Triethyl-2-(5-(((6-(2-(1,2,3,4-tetrahydroacridin-9-yl)-hydrazino)-6-oxohexyl)amino)carbonyl)-2,3-bis((2-triethylammonio)ethoxy)phenoxy)ethanammonium Tribromide Hydrochloride (24).** *N*,*N*,*N*-Triethyl-2-(5-(((6-hydrazino-6-oxohexyl)-

amino)carbonyl)-2,3-bis((2-triethylammonio)ethoxy)phenoxy)-ethanammonium tribromide (**23**; 0.4 mmol, 0.37 g) and 9-chloro-1,2,3,4-tetrahydroacridine (0.4 mmol, 0.09 g) were dissolved in absolute ethanol (10 mL) and heated to 140 °C for 24 h in a glass reactor. After cooling to room temperature the reaction mixture was poured into ethyl acetate (300 mL) while stirring to precipitate **24** as a yellow, hygroscopic powder (0.45 g, 98%) that was collected by suction filtration. ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 9 H, J = 7.0 Hz), 3.75 (t, 4 H, J = 5.5 Hz), 1.24 (t, 18 H, J = 7.3 Hz), 1.30 (tt, 2 H, J = 7.4 Hz), 1.52−1.61 (m, 4 H), 1.81 (bs, 4 H), 2.29 (t, 2 H, J = 7.3 Hz), 2.70 (bs, 2 H), 3.09 (bs, 2 H), 3.24 (dt, 2 H, J = 6.6 Hz), 3.46 (q, 12 H, J = 7.2 Hz), 3.48 (q, 6 H, J = 7.1 Hz), 3.63 (t, 2 H, J = 4.7 Hz), 4.27 (t, 2 H, J = 4.7 Hz), 4.58 (t, 4 H, J = 5.4 Hz), 7.60 (s, 2 H), 7.60 (ddd, 1 H, J = 1.0, 5.4, 7.6 Hz), 7.87 (ddd, 1 H, J = 0.7, 7.1, 8.4 Hz), 8.05 (dd, 1 H, J = 0.7, 8.5 Hz), 8.70 (d, 1 H, J = 8.8 Hz), 9.06 (t, 1 H, J = 5.7 Hz), 9.65 (s, 1 H), 11.09 (s, 1 H), 14.46 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 7.60, 7.62, 20.26, 21.40, 24.18, 24.55, 26.31, 28.23, 28.98, 33.08, 39.20, 53.22, 55.10, 55.89, 62.31, 66.01, 106.92, 111.20, 115.09, 119.39, 124.26, 125.89, 131.02, 133.04, 137.39, 137.47, 150.99, 152.64, 155.35, 164.98, 171.89. Anal. ($C_{50}H_{85}Br_3ClN_7O_5 \cdot 3H_2O$) C, H, N.

2-(5-(((6-(2-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)hydrazino)-6-oxohexyl)amino)carbonyl)-2,3-bis((2-triethylammonio)-ethoxy)phenoxy)-*N*,*N*,*N*-triethylethanammonium Tribromide Hydrochloride (25). *N*,*N*,*N*-Triethyl-2-(5-(((6-hydrazino-6-oxohexyl)amino)carbonyl)-2,3-bis((2-triethylammonio)ethoxy)phenoxy)ethanammonium tribromide (**23**; 0.4 mmol, 0.37 g) and 6,9-dichloro-1,2,3,4-tetrahydroacridine (0.4 mmol, 0.10 g) were reacted as described above to obtain **25** as a yellow, hygroscopic powder (0.46 g, 98%). ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 9 H, J = 7.3 Hz), 1.24 (t, 18 H, J = 7.1 Hz), 1.30 (tt, 2 H, J = 7.9 Hz), 1.52−1.62 (m, 4 H), 1.81 (bs, 4 H), 2.30 (t, 2 H, J = 7.4 Hz), 2.66 (bs, 2 H), 3.07 (bs, 2 H), 3.24 (dt, 2 H, J = 6.5 Hz), 3.46 (q, 12 H, J = 7.0 Hz), 3.48 (q, 6 H, J = 7.1 Hz), 3.63 (t, 2 H, J = 4.8 Hz), 3.74 (t, 4 H, J = 5.5 Hz), 4.27 (t, 2 H, J = 4.6 Hz), 4.57 (t, 4 H, J = 5.2 Hz), 7.59 (s, 2 H), 7.67 (dd, 1 H, J = 2.2, 9.1 Hz), 8.14 (d, 1 H, J = 2.2 Hz), 8.74 (d, 1 H, J = 9.5 Hz), 9.03 (t, 1 H, J = 5.7 Hz), 9.77 (s, 1 H), 11.13 (s, 1 H), 14.64 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 7.59, 7.61, 20.16, 21.26, 24.06, 24.47, 26.29, 28.26, 28.97, 33.08, 39.20, 53.21, 55.09, 55.88, 62.29, 65.99, 106.90, 111.57, 113.65, 118.27, 126.27, 126.67, 131.02, 137.46, 137.51, 138.19, 150.98, 153.27, 155.20, 164.98, 171.95. Anal. ($C_{50}H_{84}Br_3Cl_2N_7O_5 \cdot 3H_2O$) C, H: calcd, 7.39; found, 7.86; N.

Cholinesterase Inhibition Assays. Cholinesterase inhibition was assayed spectrophotometrically in duplicate on a minimum of at least four different inhibitor concentrations at 25 °C according to a literature method.[33] Assay buffer was 100 mM sodium phosphate, 100 mM NaCl, pH 7.3. Stock solutions of acetylcholinesterase (*Electrophorus electricus*, ∼100 U/mL; *Torpedo californica*, ∼3 U/mL; *Homo sapiens*, ∼3 U/mL) and butyrylcholinesterase (*Homo sapiens*, ∼10 U/mL) in assay buffer were kept at 0 °C. Appropriate dilutions were prepared immediately before starting the measurement. Acetyl- or butyrylthiocholine (10 mM) and 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB; 7 mM) were dissolved in assay buffer and kept at 0 °C. Stock solutions of the inhibitors were prepared in assay buffer. IC_{50} values were calculated from the linear steady-state turnover of the substrate using eq 1

$$IC_{50} = \frac{[I]}{\frac{v_0}{v} - 1} \quad (1)$$

where [I] is the inhibitor concentration and v_0 and v are the rates in the absence and presence of the inhibitor, respectively. Into a cuvette containing 825 μL of assay buffer, 50 μL of the DTNB solution, 60 μL of acetonitrile, 10 μL of an inhibitor solution, and 10 μL of a cholinesterase solution (∼3 U/mL) were added and thoroughly mixed. After incubation for 15 min at 25 °C, the reaction was initiated by adding 50 μL of the acetyl- or butyrylthiocholine solution.

Molecular Docking Studies. Crystal structures of several acetylcholinesterase complexes (1ACJ,[20] 1ZGB,[19] and 1N5M[22]) were retrieved from the Protein Data Bank. Unnecessary information from the PDB files was dropped by keeping only the ATOM entries, missing side chain atoms were generated using the repair feature of the ADT suite,[34] and polar hydrogens were added. Using the CORINA[35] web service, three-dimensional coordinates were obtained for the ligands. OpenBabel[36] was used to convert these PDB files to MOL2 format, which allows the addition of partial charges that were calculated using MOPAC.[37] It can be observed from the crystal structures above and one obtained for the precursor 6,9-dichloro-1,2,3,4-tetrahydroacridine[38] that only one tacrine conformer is energetically favored. Therefore, the right conformer and the protonation at the endocyclic nitrogen has to be considered when generating these coordinates. As a result of the energy minimization, the structures from the CORINA algorithm tend to be stretched, which gives a good bias toward the anticipated binding mode.

AutoDock[18] was used to discover possible binding modes, as it implements a more meticulous scoring procedure than the GOLD[39] software used in previous investigations.[4] In addition to the evaluation of hydrophobic and hydrogen-bonding contacts, there are additional terms regarding electrostatic interactions, the ligands' torsional entropy, and desolvation upon binding. This was thought to drive the docking process into the right direction with respect to the quaternary side chains. The docking grid (180 000 points, 50 × 60 × 60, 0.375 Å) was centered in between Tyr121 and Phe330 (PDB 1ZGB, *Torpedo californica*) to stretch the complete distance from the active to the peripheral binding site. A population of 100 chromosomes and a set of 100 000 generations with the rigid root placed at the center of mass was chosen for 100 runs. This resulted in negative binding energies ΔG_{bind} for all compounds, with a stepwise decrease of freed energy as the substitution pattern increased. This is probably due to the increasing amount of torsional energy, which exceeds the energy estimation for the binding process.

To validate the applied docking procedures, a classical redocking approach was undertaken. Tacrine and gallamine were removed from their corresponding crystal structures (1ACJ, 1N5M) and rebuilt by the same procedure as applied for ligand preparation. They were then docked into the gorge without any bias toward the active or peripheral binding site, and the top-scored poses were compared with their original position in the crystal structure. The predicted positioning of tacrine comes very close to the one observed in 1ACJ. The rmsd calculated by PyMOL[40] adds up to 0.561 Å, which is accounted by the literature[41] to be a very good fit. Looking at gallamine, the matching is not as good as with tacrine but still acceptable. The crystal structure of acetylcholinesterase in complex with gallamine (1N5M) does not state the conformation of the quaternary side chains. The rmsd comparison refers therefore only to the tris-substituted benzene moiety and gives a value of 2.498 Å. From a total of 100 runs, 87% located the gallamine ligand at the peripheral binding site, which demonstrates its clear bias to bind peripherally.

Radioligand Binding Assay. Membranes were prepared from porcine hearts at 4 °C as described before.[14,29] Ventricular tissue (40 g) was homogenized in a 0.32 M sucrose solution and centrifuged for 11 min at 300 *g* (2000 rpm in a Beckman rotor 35). Thereafter, the supernatant was centrifuged for 40 min at 80 000 *g* (32 000 rpm in a Beckman rotor 35), and the resulting pellet was resuspended in 4 mM Na_2HPO_4, 1 mM KH_2PO_4, pH 7.4 (4 mL/g original tissue wet weight). Aliquots (0.5 mL) were shock frozen in liquid nitrogen and stored at −80 °C; the protein content ranged between 0.3 and 0.9 mg/mL.

For measurement of [^{3}H]NMS binding cardiac membranes (60−100 μg protein/mL) were incubated with 0.2 nM [^{3}H]NMS in 5 mM Na,K,P_i-buffer, pH 7.4 at 23 °C. Atropine (1 μM) served to define nonspecific binding. Under control conditions specific binding of [^{3}H]*N*-methylscopolamine was characterized by a pK_D = 9.8 ± 0.1 (0.15 nM) and B_{max} = 98 ± 8 fmol/mg protein (means ± SEM, n = 12). The assays aimed to measure radioligand dissociation ($t_{0.5,diss,control}$ = 5.1 ± 0.2, mean ± SEM, n = 42) were prepared in a larger volume; after a preincubation period with

radioligand of 30 min, the time course of [^{3}H]NMS dissociation was visualized by adding 1 μM atropine. Test compounds were added together with atropine. At time intervals suited to describe the time course of dissociation, 1 mL aliquots were separated and filtered rapidly (glass fiber filters No. 6, Schleicher and Schüll, Dassel, FRG). After washing the filters (twice with 5 mL of ice cold incubation buffer), they were placed into scintillation vials, and the radioactivity was determined in the presence of 5 mL of Ready Protein (Beckman) by liquid scintillation counting in a Beckman LS 6500. Data of individual experiments were analyzed by nonlinear regression analysis using the Prism software (ver. 4.0, Graph Pad, San Diego, U.S.A.). Dissociation data was fitted to a monoexponential decay function; biexponential curve fitting did not yield better results (F-test, $p \geq 0.05$, data not shown). The rate constant of radioligand dissociation was calculated from the half-lives using eq 2

$$k_{-1} = \frac{\ln 2}{t_{0.5}} \tag{2}$$

Concentration effect curves for the effect of the modulators on the rate constant k_{-1} of [^{3}H]NMS dissociation were analyzed by a four-parameter logistic equation. It was checked whether the curve slope deviated from unity (F-test). Multiple comparisons were carried out by one-way ANOVA with Tukey's multiple comparison test. A p value < 0.05 was taken as the criterion for statistical significance.

Supporting Information Available: Elemental analysis results for **13**−**16**, **24**, and **25** as well as for intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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