0.01 N HCl) sh 220 (30900), λ_{max} 271 (5400). (See also Table III.) Anal. ($C_{14}H_{18}N_4O$) C, H, N.

Acknowledgment. The authors thank Dr. George H. Hitchings for his valuable encouragement and advice during the course of this investigation. The dihydrofolate reductase enzyme assays were carried out by Robert Ferone and his staff. The antibacterial assays were performed under the able aegis of Dr. S. R. M. Bushby. Renee Laube and Robert Johnson rendered valuable technical assistance in the preparation of a number of the compounds described here. We thank Dr. Robert Bryan, University of Virginia, for the X-ray analyses of trimethoprim salts. The senior author thanks her colleague Dr. Lee Kuyper for helpful stereochemical discussions.

Synthesis, Biological Activity, and Structure–Activity/Toxicity Relationships of a Series of Terphenyl Analogues of Hemicholinium-3 and Acetyl-*seco*-hemicholinium-3.[1] 3[2]

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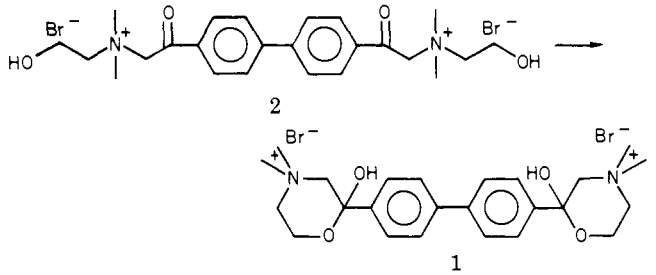
Further work on the development and investigation of activity and site of action of inhibitors which act presynaptically on neuromuscular function is reported. Terphenyl HC-3 (**5c**) and a series of six new terphenyl analogues of hemicholinium-3 (HC-3, 1) and acetyl-*seco*-hemicholinium-3 (AcHC-3, **3a**) all having a common terphenyl central nucleus were synthesized. The seco form of terphenyl HC-3 (**5c**) was altered at the choline moieties' oxo terminal to give the acetate **6a**, ether **6b**, ketone **6c**, alkane **6d**, thioacetate **6e**, and thiol **6f** analogues which, along with **5c**, are stable in slightly acidic H_2O. Ester hydrolysis of **6a** and enolization of **6c** slowly occurs at pH 7.4, with subsequent cyclization to form **5c** and a hemiacetalene **8**, respectively. Reaction or decomposition at pH 7.4 is insignificant for all seven terphenyl compounds for 4 to 5 h, but at pH 9.4 greater than 10% decomposes. In the presence of acetyl- or butyrylcholinesterase in H_2O at pH 7.4, contrary to its biphenyl analogue, **6e** does not hydrolyze; like their biphenyl analogues, all the other compounds are stable except **6a**, which reacts within seconds, apparently by an irreversible binding to the esterase, without hydrolysis and subsequent cyclization to **5c**. Compared to their respective biphenyl analogues, mouse toxicity studies (LD_{50}) show comparable lethalities of the terphenyl compounds, except for **6b** and **6e**, which are 9 and 23 times less toxic, respectively. Choline and neostigmine only slightly altered the toxicity of all compounds except **6a**, whose toxicity was effectively antagonized. Structure–activity/toxicity relationships of **5c** and **6a–f** are discussed relative to each other and their biphenyl analogues.

The synthesis of hemicholinium-3 (HC-3, 1), a prototypical prejunctional neuromuscular inhibitor, was reported in 1954 by Long and Schueler.[3] HC-3 (1) is synthesized as the seco form **2**, but rapidly undergoes intramolecular cyclization in H_2O to form the hemiacetal (1).[3,4] The pharmacological significance of cyclization was first evaluated with acetyl-*seco*-hemicholinium-3 (AcHC-3, **3a**), which, however, slowly undergoes hydrolysis in H_2O with subsequent cyclization to form HC-3 (1).[5–10] Alteration of the choline or acetylcholine moiety of **1** or **3a**, respectively, and further analysis of cyclization vs. noncyclization were studied in our laboratory with the analogs **3b–f**[2,6,7,11,12] and **4**.[2,11] The type and potency of pharmacological activity and toxicity varied with R group and cyclization. Replacement of the aromatic nucleus with an aliphatic (hexamethylene) chain, compound **5a**,[13] was reported in 1962, while substitution of a norphenyl nucleus, compound **5b**, was studied in 1966 in our laboratory.[14] Most of the



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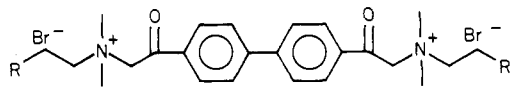
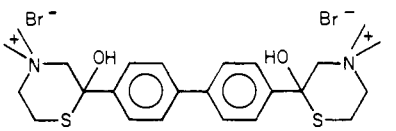


3a, R = $OOCCH_3$ **3c**, R = CH_2COCH_3 **3e**, R = $SOCCH_3$
b, R = OC_2H_5 d, R = n-C_3H_7 f, R = SH



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(1) Presented in preliminary form at the meetings of the American Society for Pharmacology and Experimental Therapeutics, New Orleans, La., Aug 17, 1976.
(2) For Paper 2, see F. R. Domer, D. M. Chihal, H. C. Charles, and A. B. Rege, *J. Med. Chem.*, **20**, 59 (1977).
(3) J. P. Long and F. W. Schueler, *J. Pharm. Sci.*, **43**, 79 (1954).
(4) F. W. Schueler, *J. Pharmacol. Exp. Ther.*, **115**, 127 (1955).
(5) M. E. Maggio, "The Pharmacological Activity of Acetyl-*seco*-hemicholinium No. 3", *Diss. Abstr. B*, **29**, 1780 (1968–1969).
(6) F. R. Domer, V. B. Haarstad, A. B. Rege, H. C. Charles, and D. M. Chihal, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **34**, 752 (1975).
(7) V. B. Haarstad, F. R. Domer, D. M. Chihal, A. B. Rege, and H. C. Charles, *J. Med. Chem.*, **19**, 760 (1976).
(8) F. DeBalbian Verster, V. B. Haarstad, and E. C. White, *Pharmacologist*, **10**, 223 (1968).
(9) F. DeBalbian Verster, V. B. Haarstad, and E. C. White, *Pharmacologist*, 11, 291 (1969).
(10) E. F. Domino, M. E. Mohrman, A. E. Wilson, and V. B. Haarstad, *Neuropharmacology*, **12**, 549 (1973).
(11) F. R. Domer, H. C. Charles, D. M. Chihal, and R. C. Koch, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **35**, 801 (1976).
(12) F. R. Domer, H. C. Charles, D. M. Chihal, and R. C. Koch, *Arch. Int. Pharmacodyn. Ther.*, **229**, 276 (1977).
(13) M. F. Powers, S. Kruger, and F. W. Schueler, *J. Pharm. Sci.*, **51**, 27 (1962).
(14) S. N. Thampi, F. R. Domer, V. B. Haarstad, and F. W. Schueler, *J. Pharm. Sci.*, **55**, 381 (1966).

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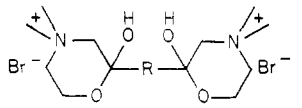
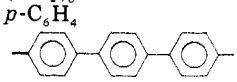
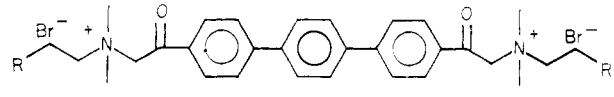


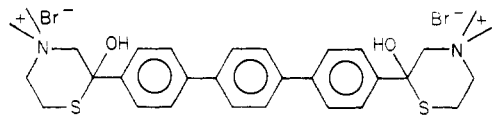
5a, R = $(CH_2)_6$
b, R = p-C_6H_4
c, R = 

pharmacological properties of the parent HC-3 (1) were maintained. The fluorescent properties of terphenyl HC-3 (5c) were used in 1974 to study the uptake of 5c by cochlear hair cells.[15] Therefore, logically fluorescent analogues of 3a–f and 4 that retain the characteristic activity of the parent compounds should be obtainable by substituting terphenyl for the biphenyl nucleus. Thus, as a continuation of our interest in both configurational and conformational structure–activity relationships of cholinergic compounds, an investigation of several terphenyl derivatives of analogues of HC-3 (1) and AcHC-3 (3a) was initiated. Herein, we describe the synthesis of 6a–f and



6a, R = $OOCCH_3$ 6c, R = CH_2COCH_3 6e, R = $SOCCH_3$
b, R = OC_2H_5 d, R = n-C_3H_7 f, R = SH

compare and contrast the chemistry and biological activity of these compounds and 5c with each other and the parent compounds 1 and 3a–f. The failure of 6e to hydrolyze and cyclize to terphenyl thio-HC-3 (7) when treated with



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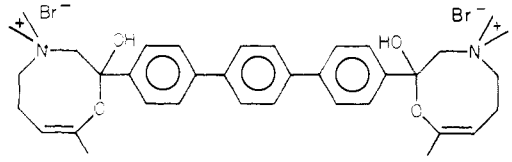
acetylcholinesterase or butyrylcholinesterase is contrasted with previously reported[2] hydrolysis and cyclization of the biphenyl analogue 3e to 4. The enolization of 6c in neutral or basic aqueous solution with subsequent cyclization to 8 is discussed.



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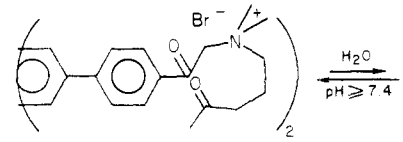
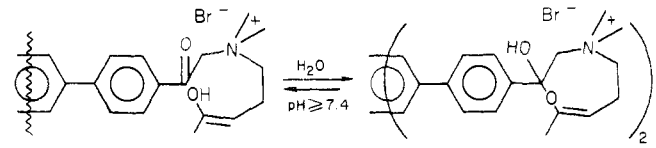
Chemistry and Initial Structure–Activity Relationships. The terphenyl compounds 5c and 6a–f were synthetized as bromide salts by the reaction of 4,4″-(1,1′:4′,1″-terphenylene)bis(1-bromo-2-oxoethylene) (9), prepared by Friedel–Crafts acylation, with the appropriate amines. The requisite *N*,*N*-dimethylethanolamine (10), 2-(dimethylamino)ethyl acetate (11), *N*,*N*-dimethylpentylamine (12), 5-(dimethylamino)-2-pentanone (13), and 2-(dimethylamino)ethanethiol (14) for synthesis of 5c and 6a,d,c,f respectively, were obtained commercially. Tammelin's[16] method for acetylation of 14 with acetic anhydride was used to synthesize 2-(thioacetyl)ethyldimethylamine (15), the precursor of 6e. Dimethylamino-2-ethoxyethane (16) for the synthesis of 6b was produced by reacting dimethylamine (17) with 1-bromo-2-ethoxyethane (18). The structures of the terphenyl compounds were confirmed by the method of syntheses, satisfactory combustion elemental analytical data, and ^1H NMR, IR, and UV spectral data.

The stability of the seven terphenyl compounds was investigated in H_2O at pH 4.0, 7.4, and 9.4 with UV spectrophotometry. At pH 4.0, none exhibited a change in the UV spectrum and thus appear stable for an extended time. At normal blood pH of 7.4 and at pH 9.4, compounds 5c and 6b,d–f showed a slow decrease in absorption λ_{max} (H_2O) with time without formation of a new absorption maximum, indicating gradual decomposition of negligible magnitude for at least 4 to 5 h. However, 6c at pH 7.4 and 9.4 showed a decrease in absorption at its original λ_{max} (H_2O) of 333 nm with the buildup of a new absorption λ_{max} (H_2O) of 293 nm. All of the terphenyl-*seco* compounds having a ketone group conjugated to aromatic nuclei have a λ_{max} (H_2O) between 326 and 333 nm, while the hemiacetal 5c, which lacks a conjugated ketone group, has a λ_{max} (H_2O) of 289 nm. It is therefore likely that 6c is undergoing slow enolization at pH 7.4 to produce a compound containing the moiety 19, which then cyclizes



6c



19 8

to form ultimately the hemiacetalene 8 at a rate producing negligible quantities during 4 to 5 h. However, after 24 h, or rapidly at pH 9.4, the production of 8 is significant. Slow hydrolysis of 6a occurs at pH 7.4 followed by cyclization to 5c, producing negligible quantities of 5c in 4 to 5 h. After 24 h, or at pH 9.4, significant amounts of 5c are produced.

As expected by structure and analogy with biphenyl analogues,[2,6,7,11] terphenyl compounds 5c and 6b–d,f do not react in vitro in aqueous pH 7.4 solutions of acetyl- or butyrylcholinesterase with activities equivalent to those in blood, i.e., approximately 4 units/mL. The terphenyl thioacetate 6e, in contrast to its biphenyl analogue 3e which hydrolizes and cyclizes to thio-HC-3 (7),[2,11] has been found to be stable under these conditions. Evidently, the added bulk of a third phenyl ring in the central portion of the molecule 6e, along with the transoid[2] conformation of the thioester moiety, interferes with the action of either esterase on the molecule, possibly by decreasing the molecule's affinity for the enzyme's receptor site. However, in analogy to its biphenyl analogue, 3a,[6,7] the terphenyl acetate 6a undergoes rapid reaction with these enzymes under identical conditions. Evidently, a third phenyl ring in the central portion of 6a, along with the cisoid[2] conformation of the oxoester moiety, is insufficient to interfere with the action of either esterase on the molecule. That this reaction is apparently an irreversible binding to esterase without subsequent hydrolysis is supported by a hypsochromic shift from λ_{max} (H_2O, pH 7.4) of 330 to 307 nm in the UV spectrum. The hemiacetal 5c, which would be produced from hydrolysis of 6a with subsequent release into solution from the esterase, has a λ_{max} (H_2O, pH 7.4)

(15) P. S. Guth, V. B. Haarstad, and S. D. Comis, *Acta Oto-Laryngol.*, 77, 251 (1974).
(16) L. E. Tammelin, *Acta Chem. Scand.*, 11, 487 (1957).

Table I. Mouse Toxicity Studies

compd	terphenyl LD_{50}, mg/kg ip	terphenyl 95% CL	biphenyl LD_{50}, mg/kg ip	ratio of terphenyl LD_{50} doses to that of biphenyl HC-3	ratio of terphenyl LD_{50} doses to that of biphenyl analogue
HC-3	0.107	0.075-0.139	0.13[b]	0.82	0.82
AcHC-3	0.333	0.319-0.346	0.125[b]	2.56	2.66
thio-AcHC-3	3.05	2.87-3.23	0.133[b]	23.5	22.9
ketone	4.27	3.67-4.87	3.1[c]	32.8	1.38
alkane	5.32	4.64-6.01	5.6[c]	40.9	0.95
ether	16.6	16.0-17.3	1.84[c]	127	9.02
thio-*seco*-HC-3	*a*	*a*	29.4[b]	*a*	*a*

[a] This compound is the least toxic terphenyl analogue. Insolubility problems as well as the low toxicity prevented an accurate assessment of the LD_{50}. [b] See ref 2 and 11. [c] See ref 6 and 7.

of 289 nm. The magnitudes of the relative differences in the λ_{max} (H_2O, pH 7.4) for these situations are comparable to those observed for the biphenyl compound which has been shown not to produce free acetate in the presence of these enzymes by a Warburg procedure.[6,7] Solubility problems prevent similar accurate Warburg determinations on the terphenyl compound **6a**.

The compound 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) reacts with a free thiol group to produce a substance which absorbs light maximally at 412 nm. When DTNB is added to a buffered (pH 7.4) solution of thiol **6f**, a rapid reaction occurs between **6f** and DTNB, indicating **6f** is, in fact, the nonpolymerized, noncyclized terphenylthio-*seco*-HC-3 (**6f**). Conversely, when DTNB is added to an identically prepared solution of thioester **6e** no reaction is observed, indicating lack of a free thiol group in **6e**. Likewise, when a solution of DTNB containing either acetyl- or butrylcholinesterase is added to a solution of **6e**, no reaction is observed, confirming the inability of either esterase to hydrolyze **6e**. This, as discussed above, is in contrast to the results obtained for the biphenyl analogues, where the thioester **3e** is hydrolyzed with subsequent cyclization by the esterases.

The fluorescence of terphenyl compounds **5c** and **6a–f** was examined qualitatively in solution utilizing a Bausch & Lomb PB-251 Dynazoom microscope with a 31-33-28 fluorescent microsource, a 5-58 transmission filter, and a Y-8 barrier filter. Terphenyl HC-3 (**5c**) was used as a standard. The compounds **6a–c,e** exhibited fluorescence equal to or slightly less than **5c**. Little or no fluorescence was observed with **6d** and **6f**. The fluorescence of these compounds, which all have neuromuscular blocking activity of various types,[17] may lend these compounds to fluorescent microscopic studies of the sites of action of these blockades.

Pharmacology and Initial Structure–Toxicity Relationships. Table I summarizes LD_{50} studies in Charles River CD-1 strain adult male albino mice (20–30 g). Due to the previously observed variation in the LD_{50} of HC-3 (1) with respect to the time of day,[14] the staircase sampling method of Finney[19] was utilized. This method takes into account variations in animals due to circadian rhythm. Thus, an accurate average LD_{50} dose can be readily obtained. Doses of the respective compounds as a sodium carboxymethylcellulose (0.8%, w/v) suspension in normal saline (0.9% NaCl) were injected ip. The animals which died began to show effects, including exophthalmos, mild to moderate ataxia, respiratory difficulties, loss of righting response, mild SLUD syndrome,[14] and clonic convulsions, within 5 to 10 min postinjection. Immediate autopsy revealed that cardiac contractions continued and peristalsis of the small intestines had usually increased. The lungs and peritoneal cavity were normal. Thus, the apparent cause of death was respiratory failure due to neuromuscular blockage. In the case of **6f**, the lack of toxicity and insolubility prevented an accurate assessment of the LD_{50}. The animals which did not survive doses of **6f** required 24–72 h before death occurred. At autopsy, traces of undissolved **6f** were in the peritoneal cavity. It appears that the quantity of **6f** in solution was insufficient to be lethal; however, as more **6f** was absorbed from the peritoneal cavity, tissue levels surpassed the lethal level. With the other compounds, death characteristically occurred in less than 1 h.

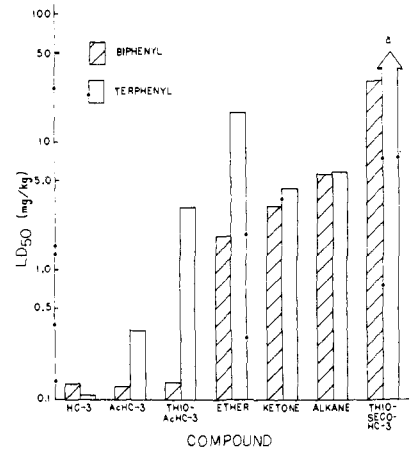


Figure 1. Toxicity vs. type of nonaromatic moiety. (a) The low toxicity and high insolubility of compound **6f** prevented an accurate determination of its LD_{50}.

There are significant differences in ranking of toxicity of the terphenyl and biphenyl series as shown in Table I and Figure 1. Toxicities of **5c** and **6a,c,d** are approximately equal to those of their biphenyl analogues, while toxicities of **6b** and **6e** are, respectively, 9 and 23 times less than those of the corresponding biphenyl analogue. For **6b**, the cause of the ninefold decrease in toxicity relative to **3b** is not apparent when considered in light of the almost unchanged toxicities of **5c** and **6a,c,d** relative to their

(17) F. R. Domer, R. C. Koch, H. C. Charles, and D. M. Chihal, *Arch. Int. Pharmacodyn. Ther.*, **229**, 251 (1977).
(18) D. J. Finney, "Probit Analysis", Cambridge University Press, New York, 1964, pp 226–232.
(19) Melting points of **5c** and **6a–f** were determined by heating the hot stage to an arbitrary temperature and adding a few crystals. At temperatures below the melting point, decomposition occurred. At the melting point and temperatures above, melting occurred before decomposition. The melting point was taken as that temperature at which the compound was first observed to melt before decomposing.

Table II. Antagonism by Choline and Neostigmine of Drug Toxicity in Mice

terphenyl compd	>LD_{95} of compd, mg/kg ip	antagonist[a] choline, mg/kg ip	antagonist[a] neostigmine, mg/kg ip	% mortality
HC-3 (5c)	0.338	20		70
			0.2	80
AcHC-3 (6a)	0.430	20		30
			0.2	20
thio-AcHC-3 (6e)	4.38	20		60
			0.2	80
ketone (6c)	8.57	20		100
			0.2	90
alkane (6d)	10.29	20		80
			0.2	100
ether (6b)	21.45	20		90
			0.2	90
thio-*seco*-HC-3 (6f)	*b*	*b*		*b*
			b	*b*
control		20		0
			0.2	0

[a] Antagonist was administered 1 min prior to compound. [b] This compound is the least toxic terphenyl analogue. Insolubility problems prevented determining a LD_{95} dose and thus prevented antagonism studies on this compound.

biphenyl analogues. The 23-fold decrease in toxicity of **6e** with respect to its biphenyl analogue is explainable in light of the differing interactions with acetyl- and butyrylcholinesterase. The biphenyl thioester **3e** has been shown to interact rapidly in vitro with either esterase, causing hydrolysis with subsequent cyclization to produce thio-HC-3 (**4**), a *thiohemiacetal*.[2,11] The toxicity of this compound then parallels that of the *hemiacetal* HC-3 (**1**). A similar interaction of **6e** with either esterase does not occur. Thus, the toxicity of **6e**, a seco compound, parallels that of the other seco bi- and terphenyl compounds, with the exception of the acetates **3a** and **6a** which bind irreversibly to either esterase and thus have a mechanism of action different from the other seco compounds investigated.

Studies of choline and neostigmine antagonism of drug toxicity in Charles River CD-1 adult male albino mice (20–30 g) are summarized in Table II. Choline (20 mg/kg) or neostigmine (0.2 mg/kg) (nonlethal doses in control animals) was administered ip 1 min prior to administration of an ip (>LD_{95}) dose of the test drug. The only compound which showed highly significant antagonism of toxicity with both choline and neostigmine was the terphenyl AcHC-3 (**6a**), which binds irreversibly to both acetyl- and butyrylcholinesterase, the most atypical mechanism of action in this series. Some antagonism by choline of the toxic effects of **5c** and **6e** was observed. This antagonism was, however, drastically less than that observed for **6a**. With the exception of **6f**, whose solubility and low lethality prevented antagonism studies, little or no antagonism was observed with the other compound–antagonist pairs studied.

Experimental Section

All melting points were determined on a Fisher-Jones hot stage melting point apparatus and are uncorrected.[19] Boiling points were observed during distillation and are also uncorrected. Infrared spectra were recorded on Perkin-Elmer 337 and 257 spectrophotometers and calibrated against polystyrene. Ultraviolet spectra and extinction coefficients were obtained on a Beckman DB spectrophotometer. Proton magnetic resonance spectra were determined on a Varian A-60 spectrometer using Me_2SO-d_6 as solvent and tetramethylsilane solution as external standard. Refractive indices were observed on a Bausch & Lomb refractometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., are indicated as empirical formulas, and are within ±0.4% of the theoretical values.

4,4″-(1,1′:4′,1″-Terphenylylene)bis(1-bromo-2-oxoethylene) (9). Anhydrous $AlCl_3$ (40.0 g, 0.300 mol) was added to *p*-terphenyl (25.0 g, 0.109 mol) in 200 mL of CS_2 at 0 °C, followed by the dropwise addition of bromoacetyl bromide (50.0 g, 0.248 mol). While stirring continuously, the mixture was warmed slowly (rate determined by HBr evolution) to reflux and maintained at reflux until HBr evolution had subsided. The mixture was cooled to ambient temperature, the CS_2 was decanted, and the remaining solid was added to a beaker containing 1 L of crushed ice, 250 mL of MeOH, and 250 mL of concentrated HCl. The solid was pulverized under solution, mixed well, filtered from solution, and washed with 4 L of H_2O. Material thus isolated was dried in a vacuum desiccator overnight and recrystallized from THF after decolorizing with activated carbon to give **9** (21.9 g, 0.047 mol, 43%): pale yellow needles; mp 214.0–215.8 °C.

[4,4″-(1,1′:4′,1″-Terphenylylene)bis(2-oxoethylene)]bis(2-oxoethyldimethylammonium bromide) (5c). A solution of **9** (5.0 g, 0.011 mol) and **10** (20.0 g, 0.225 mol) at ambient temperature in a minimum of THF was stirred in a sealed flask overnight. The resulting precipitate was isolated by vacuum filtration, washed with THF and then Et_2O, and dried in a vacuum desiccator over 5 Å molecular sieves to yield 6.4 g (0.010 mol, 92%) of **5c**: mp 177–179 °C;[19] IR (KBr) ν 1615 (phenyl), 1080 (morpholinium ether), 818 cm^{-1} (*p*-phenyl); UV λ_{max} (H_2O) 289 nm (ϵ 26 500); ^1H NMR (Me_2SO-d_6) δ 7.63–7.45 (m, 12 H, aromatic), 3.53–2.89 (overlapping signals, 12 H, methylenes adjacent to nitrogen and oxygen), 2.10 (s, 2 H, hydroxyl), 1.96 (s, 12 H, nitrogen methyl). Anal. ($C_{30}H_{38}Br_2N_2O_4$) C, H, N.

[4,4″-(1,1′:4′,1″-Terphenylylene)bis(2-oxoethylene)]bis(2-acetoxyethyldimethylammonium bromide) (6a). Compound **6a** was synthesized from **9** (3.0 g, 0.0064 mol) and **11** (5.0 g, 0.038 mol) by a method analogous to that used for **5c**, yielding 3.6 g (0.0049 mol, 76%) of **6a**: mp 203.0–203.5 °C;[19] IR (KBr) ν 1695 (phenyl carbonyl), 1605 (phenyl), 1383 (carbonyl methylene), 808 cm^{-1} (*p*-phenyl); UV λ_{max} (H_2O) 330 nm (ϵ 41 500); ^1H NMR (Me_2SO-d_6) δ 7.92–7.57 (m, 12 H, aromatic), 4.41–3.13 (overlapping signals, 12 H, methylenes adjacent to nitrogen and carbonyl and one carbon removed from nitrogen), 3.27 (s, 12 H nitrogen methyl), 1.62 (s, 6 H, acetylmethyl). Anal. ($C_{34}H_{42}Br_2N_2O_6$) C, H, N.

Dimethylamino-2-ethoxyethane (16). In a sealed pressure vessel, **17** (25.0 g, 0.163 mol) and **18** (100 g, 2.2 mol) were stirred at ambient temperature for 2 h. The vessel was cooled to 0° C and opened, and excess **18** was distilled at ambient temperature. The resulting white crystalline hydrobromide salt of **16** was dissolved in 100 mL of H_2O, and the amine **16** was liberated by the addition of NaOH (6.5 g, 0.16 mol). This solution was extracted five times with ether, and the ethereal extract was dried over anhydrous Na_2SO_4. The free amine **16** (15 mL, 12.1 g, 0.103 mol, 63%) was isolated by distillation: bp 115–117 °C (lit. 116–118 °C).

[4,4″-(1,1′:4′,1″-Terphenylylene)bis(2-oxoethylene)]bis(2-ethoxyethyldimethylammonium bromide) (6b). The synthesis of **6b** from **9** (3.0 g, 0.0064 mol) and **16** (3.0 mL, 2.42 g, 0.021 mol) was accomplished with the method utilized for **5c**, yielding 3.9 g (0.0055 mol, 86%) of **6b**: mp 208.0–209.5 °C; IR (KBr) ν 1685 (phenyl carbonyl), 1610 (phenyl), 1393 (carbonyl methylene), 1117 (ether), and 806 cm^{-1} (*p*-phenyl); UV λ_{max} (H_2O) 329 nm (ϵ 48 000); ^1H NMR (Me_2SO-d_6) δ 7.90–7.52 (m, 12 H, aromatic), 3.73–3.33 (overlapping signals, 12 H, methylenes adjacent to nitrogen and carbonyl and one carbon removed from nitrogen), 3.20 (s, 12 H, nitrogen methyl), 3.17 (q, 4 H, J = 7.0 Hz, ethoxy methylene), 0.69 (t, 6 H, J = 7.0 Hz, ethoxy methyl). Anal. ($C_{34}H_{46}Br_2N_2O_4$) C, H, N.

[4,4″-(1,1′:4′,1″-Terphenylylene)bis(2-oxoethylene)]bis(4-oxopentyldimethylammonium bromide) (6c). Using the method employed for **5c**, 10.0 g (0.078 mol) of **13** and 3.0 g (0.0064 mol) of **9** were reacted to give 4.0 g (0.0055 mol, 86%) of **6c**: mp 165–166 °C; IR (KBr) ν 1700 (phenyl carbonyl and carbonyl), 1610 (phenyl), 1401 (carbonyl methylene), 810 cm^{-1} (*p*-phenyl); UV λ_{max} (H_2O) 333 nm (ϵ 39 500); ^1H NMR (Me_2SO-d_6) δ 7.93–7.59 (m, 12 H, aromatic), 3.62–2.96 (overlapping signals, 12 H, me-

thylenes adjacent to nigrogen and carbonyl and one carbon removed from nitrogen), 3.18 (s, 12 H, nitrogen methyl), 1.95 (s, 6 H, carbonyl methyl). Anal. ($C_{36}H_{46}Br_2N_2O_4$) C, H, N.

[4,4′-(1,1′:4′,1″-Terphenylylene)bis(2-oxoethylene)]bis(1-pentyldimethylammonium bromide) (6d). By a method analogous to that used for the synthesis of **5c**, 3.0 g (0.0064 mol) of **9** and 10.0 g (0.086 mol) of **12** were allowed to react to yield 4.4 g (0.0063 mol, 98%) of **6d**: mp 263–264 °C;[19] IR (KBr) ν 1690 (phenyl carbonyl), 1605 (phenyl), 1400 (carbonyl methylene), 810 cm^{-1} (*p*-phenyl); UV λ_{max} (H_2O) 331 nm (ϵ 44 500); ^1H NMR (Me_2SO-d_6) δ 7.92–7.58 (m, 12 H, aromatic), 3.65–2.75 (overlapping signals, 12 H, methylenes adjacent to nitrogen and carbonyl and one carbon removed from nitrogen), 3.17 (s, 12 H, nitrogen methyl), 1.83–0.51 (three overlapping multiplets, 14 H, methylenes two and three carbons removed from nitrogen and terminal methyl). Anal. ($C_{36}H_{50}Br_2N_2O_2$) C, H, N.

2-(Thioacetyl)ethyldimethylamine (15). The method of Tammelin was utilized to prepare **15**:[16] bp 83–84 °C (22 mmHg), lit. bp 78 °C (14 mmHg); n^{25}_D 1.4747, lit. n^{25}_D 1.4763.

[4,4″-(1,1′:4′,1″-Terphenylylene)bis(2-oxoethylene)]bis-[2-(thioacetyl)ethyldimethylammonium bromide] (6e). Compounds **9** (3.0 g, 0.0064 mol) and **15** (7.5 g, 0.051 mol) were reacted by the method utilized for the synthesis of **5c** and yielded 3.6 g (0.0047 mol, 73%) of **6e**: mp 143–144 °C; IR (KBr) ν 1690 (phenyl carbonyl), 1615 (phenyl), 1402 (carbonyl methylene), 809 cm^{-1} (*p*-phenyl); UV λ_{max} (H_2O) 330 nm (ϵ 49 000); ^1H NMR (Me_2SO-d_6) δ 7.91–7.64 (m, 12 H, aromatic), 3.80–2.70 (overlapping signals, 12 H, methylenes adjacent to nitrogen and carbonyl and one carbon removed from nitrogen), 3.21 (s, 12 H, nitrogen methyl), 2.24 (s, 6 H, thioacetyl methyl). Anal. ($C_{34}H_{42}Br_2S_2N_2O_4$) C, H, N.

[4,4″-(1,1′:4′,1″-Terphenylylene)bis(2-oxoethylene)]bis(2-thioethyldimethylammonium bromide) (6f). Employing the method of synthesis of **5c** for the production of **6f**, 3.0 g (0.0064 mol) of **9** and 3.0 g (0.029 mol) of 14 were combined to produce 4.1 g (0.0060 mol, 93%) of **6f**: mp 238–239 °C; IR (KBr) ν 2730 (thiol), 1675 (phenyl carbonyl), 1615 (phenyl), 1397 (carbonyl methylene), 799 cm^{-1} (*p*-phenyl); UV λ_{max} (H_2O) 326 nm (ϵ 28000); ^1H NMR (Me_2SO-d_6) δ 7.91–7.59 (m, 12 H, aromatic), 3.38–2.72 (overlapping signals, 12 H, methylenes adjacent to nitrogen and carbonyl and one carbon removed from nitrogen), 2.66 (s, 12 H, nitrogen methyl), 2.27–2.17 (m, 2 H, thiol). Anal. ($C_{30}H_{38}Br_2N_2O_2S_2$) C, H, N.

Sodium Carboxymethylcellulose Stock Solution and Method of Preparing Uniform Drug Samples. Sodium carboxymethylcellulose (10.4 g) was dissolved in 200 mL of normal saline (0.9% NaCl) by heating to 90–100 °C. The solution was then maintained at 4 °C for 36–48 h to allow complete hydration of the carboxymethylcellulose, producing a 5.2% (w/v) stock solution usable for 1 month if stored at 4 °C. Prior to use, an aliquot of the stock solution was diluted with normal saline to 0.8% (w/v) and allowed to warm to room temperature. Samples of the slightly soluble terphenyl compounds were then suspended in the 0.8% solution by stirring for 1–2 h, a time which produced the most uniform particle size and thus the most even distribution of compound in the suspension.

Stability of 5c and 6a–f in H_2O at pH 4.0, 7.4, and 9.4. Water solutions of **5c** and **6a–f** (1.0×10^{-5} M) at pH 4.0 or buffered to pH 7.4 with phosphate buffer or to pH 9.4 with carbonate–bicarbonate buffer were prepared. The UV absorption spectrum of each was immediately recorded utilizing an identically buffered H_2O sample as a reference. Each sample was maintained at ambient temperature for 48 h, and UV spectra were recorded at intervals. At pH 4.0 all the compounds were stable for the entire 48 h. For **5c** and **6a,b,d–f** at pH 7.4 and 9.4, a slow decrease in absorbance at λ_{max} (H_2O) with no new λ_{max} (H_2O) formation was observed. This decomposition was negligible for at least 4 to 5 h at pH 7.4. However, different results were obtained with **6c**, which at pH 7.4 showed a slow decrease in λ_{max} (H_2O) of 333 nm with the buildup of a new λ_{max} (H_2O) of 293 nm, indicating that the compound slowly underwent enolization with subsequent cyclization to hemiacetalene **8**. At pH 7.4, the reaction produced a negligible quantity of **8** during the first 4 to 5 h. However, after 24 h or at pH 9.4 the amount of **8** produced was significant.

Interaction of 5c and 6a–f in H_2O at pH 7.4 with Acetyl- and Butyrylcholinesterase. Procedure A. Phosphate buffered (pH 7.4) aqueous solutions of **5c** and **6a–f** (1.0×10^{-5} M) were prepared. Employing an identically buffered H_2O sample as a reference, the UV absorption spectrum of a 1-mL aliquot was immediately recorded. A 0.1-mL aliquot of a 40 unit/mL phosphate buffered (pH 7.4) H_2O solution of acetylcholinesterase was added to sample and reference to produce an enzyme concentration similar to that found in blood. The UV absorption spectrum was immediately recorded. An examination of the spectra of **5c** and **6b–f** recorded before and after enzyme addition showed no shift in λ_{max} (H_2O, pH 7.4). However, an examination of equivalent spectra for **6a** revealed a hypsochromic shift of λ_{max} (H_2O, pH 7.4) from 330 to 307 nm. Thus, no reaction of **5c** and **6b–f** with the enzyme was observed. This is analogous to what was observed for biphenyl analogues, except terphenyl thio-AcHC-3 (**6e**). Its biphenyl analogue was observed to hydrolyze and cyclize to thio-HC-3 (**4**). Although reaction of **6a** with enzyme did occur, the *hemiacetal* form of terphenyl HC-3 (**5c**), which has a λ_{max} (H_2O, pH 7.4) of 288 nm, was not produced, a result analogous to that observed with the biphenyl analogue. Each solution was subsequently incubated at 37 °C for 6 h, and the UV absorption spectrum was again recorded. No significant changes occurred in any spectra after 6 h, indicating that **5c** and **6b–f** do not react with acetylcholinesterase, while **6a** reacts apparently irreversibly at pH 7.4 and 37 °C with the enzyme.

Procedure B. The experiment described in procedure A was repeated, substituting butyrylcholinesterase for acetylcholinesterase. Equivalent results were obtained.

Interaction of 6e and 6f in H_2O at pH 7.4 with 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB). Procedure A. Aqueous buffered (pH 7.4) solutions of thio compounds **6e** and **6f** (1.0×10^{-5} M) were prepared. The absorbance at 412 nm was recorded, employing an identically buffered aqueous sample as a reference. To each sample and reference was added 100 μL of a 0.01 M 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) solution. In the sample containing **6f**, an immediate reaction was observed between **6f** and DTNB, indicating the presence of a free thiol group in **6f**. Conversely, no reaction was observed between the thioester group of **6e** and DTNB.

Procedure B. Solutions and references identical with those utilized in procedure A were prepared, and the absorbance at 412 nm was recorded. A total of 100 μL of a solution 0.01 M in DTNB and containing 0.025 units/100 μL of acetylcholinesterase was added to sample and reference and the absorbance again recorded at 412 nm. No change in rate of reaction of **6e** or **6f** with DTNB from that found in procedure A was observed, indicating that **6e**, in contrast to its biphenyl analogue, does not hydrolyze in the presence of acetylcholinesterase.

Procedure C. The experiment described in procedure B was repeated, substituting butyrylcholinesterase for acetylcholinesterase. Equivalent results were obtained.

Fluorescence of 5c and 6a–f. The fluorescence of **5c** and **6a–f** was examined qualitatively in solution utilizing a Bausch & Lomb PB-251 Dynazoom microscope with a 31-33-28 fluorescent microsource, a 5-58 transmission filter, and a Y-8 barrier filter. Terphenyl HC-3 (**5c**) was used as a standard for fluorescence. The fluorescence of **6a–c,e** was equal to or slightly less than that of **5c**. Little or no fluorescence of **6d** and **6f** was observed.

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