with cooling (0–5°) and stirring a solution of 5 ml of thionyl chloride in 10 ml of pyridine. After 1 hr the mixture was heated under reflux for 2 hr and cooled to room temperature, 50 ml of 50% NaOH was added, and the heating was continued for an additional hour. The mixture was poured into H_2O, extracted (Et_2O), and washed, the solvent was removed, and the residue was recrystallized several times.

3-(5-Xanthyl)-2,3-dehydroquinuclidine (5a). A mixture of 9.3 g (0.03 mol) of carbinol 7 (Table I) and 50 ml of thionyl chloride was heated on the steam bath for 2 hr and the excess solvent removed in vacuo. The residue was dissolved in H_2O, 50 ml of 25% NaOH solution was added, and the mixture was heated at 80° for 1 hr. After cooling, the product was extracted (Et_2O) and washed and Et_2O was removed. The residue was recrystallized from *i*-Pr_2O: yield 1.5 g (17%); mp 117–119°. Anal. ($C_{20}H_{19}NO$) C, H, N.

Alternate Synthesis of 5b. To 5.3 g (0.02 mol) of 5-bromoxanthene[5] was added 3.6 g of triethyl phosphite. There was an immediate reaction which was moderated by cooling. The mixture was allowed to stand overnight at room temperature and then heated under reflux for 1 hr, and the low-boiling materials were removed in vacuo on a steam bath. To the light yellow viscous residue, 6 ml of DMF and 1.5 g of NaOMe were added, followed by the dropwise addition of a solution of 2.6 g of 3-quinuclidinone in 15 ml of DMF. The mixture was stirred at room temperature for 2 hr and poured into H_2O and the product was filtered and recrystallized from benzene–hexane: yield 2.4 g (49%); mp 196–197°.

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References and Notes

(1) (a) F. J. Villani, P. J. L. Daniels, C. A. Ellis, T. A. Mann, K. C. Wang, and E. A. Wefer, *J. Med. Chem.*, **15**, 750 (1972); (b) F. J. Villani, T. A. Mann, E. Wefer, J. Hannon, L. L. Larca, M. J. Landon, W. Spivak, D. Vashi, S. Tozzi, G. Danko, M. delPrado, and R. Lutz, *ibid.*, 18, 1 (1975).

(2) See, for example, and references therein: (a) E. L. Engelhardt, H. C. Zell, W. S. Saari, M. C. Christy, C. D. Colton, C. A. Stone, J. M. Stavorski, H. C. Wenger, and C. L. Ludden, *J. Med. Chem.*, 8, 829 (1965); (b) J. M. Bastian, A. Ebnöther, E. Jucker, E. Rissi, and A. P. Stoll, *Helv. Chem. Acta*, **49**, 214 (1966); (c) E. L. Engelhardt and H. C. Zell, U.S. Patent 3,275,640 (1966); (d) E. E. Galantay, U.S. Patent 3,458,522 (1969).

(3) L. N. Yakhontov, *Adv. Heterocycl. Chem.*, 11, 473 (1970).

(4) M. Gomberg and L. H. Cone, *Justus Liebigs Ann. Chem.*, **376**, 192 (1910).

(5) R. Tondeur and M. Urbain, *Chim. Ther.*, 207 (1966).

Synthesis and Biological Activity of Spin-Labeled Analogs of Biotin, Hexamethonium, Decamethonium, Dichlorisoproterenol, and Propranolol

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Spin-labeled analogs of biotin (vitamin H), hexamethonium, decamethonium, dichlorisoproterenol, propranolol, and primaquine containing the nitroxide free radical have been synthesized and tested for biological activity. The four spin-labeled analogs of biotin, 4-biotinamido-2,2,6,6-tetramethyl-1-piperidinyloxy (IV), 3-biotinamido-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (V), 3-biotinamidomethyl-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (VI), and 4-(biotinylglycyl)amino-2,2,6,6-tetramethyl-1-piperidinyloxy (VII), all interacted with avidin, a specific biotin binding protein found in raw egg white, at the same sites as did biotin itself. An unsymmetrical decamethonium spin label (XVIII) in which one of the quaternary methyl groups had been replaced by the 4-(2,2,6,6-tetramethyl-1-piperidinyloxy) moiety was 13 times more potent as an inhibitor of *Torpedo californica* acetylcholinesterase than the parent drug. The symmetrical decamethonium (XVI) and hexamethonium (XIV) spin labels were 18 and 1.8 times as active as decamethonium in the same assay system. The substitution of the 4-(2,2,6,6-tetramethyl-1-piperidinyloxy) group for the isopropyl groups of β-adrenergic blocking drugs dichlorisoproterenol and propranolol, to give spin labels XXI and XXII, caused a 45 and 54% reduction, respectively, in the ability of these compounds to inhibit the isoproterenol-stimulated activity of rat fat cell membranes. Finally, modification of primaquine by the introduction of the 4-(2,2,6,6-tetramethyl-1-piperidinyloxy) substituent into the amino group of the butyl side chain completely abolished the ability of the drug to bind to nucleic acids. These results suggest that the incorporation of the nitroxide group into drug molecules may be a useful approach to the synthesis of more specific spin labels for biological systems, such as egg white avidin, acetylcholinesterase, and the β-adrenergic receptor.

During recent years spin-labeled drug molecules have played an increasingly important role in studies of the interaction of drugs with their cellular targets or "receptor" macromolecules.[1–5] Spin-labeled drugs have also been used to determine the levels of morphine, barbiturates, sulfonamides, and other drugs in biological fluids.[6,7] The most commonly employed spin labels utilize the nitroxide group, since this free radical is very stable in aqueous solutions at physiological pH values. In an earlier paper,[8] we described procedures for synthesizing spin-labeled analogs of sulfonamides, acetylcholine, and barbituric acid. We now report the synthesis of spin-labeled analogs of (a) the vitamin biotin (IV–VII) for studies of egg white avidin, (b) decamethonium and hexamethonium (XIII–XIX) as potential probes for acetylcholinesterase and the acetylcholine receptor, (c) the β-adrenergic blocking drugs propranolol (XXII) and dichlorisoproterenol (XXI) for studies of the β-receptor and its interrelationship with membrane-bound adenylyl cyclase, and (d) the antimalarial drug primaquine (XXIII) for binding studies with nucleic acids.

The synthesis of the biotin analogs IV–VII was achieved by condensing the corresponding spin-labeled amines, prepared by previously reported methods, with biotin in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). For example, V was prepared by the condensation of biotin with amine II. We were unable to prepare biotinylglycine using either the biotinyl acid chloride technique described by Woli and coworkers[9] or the mixed an-

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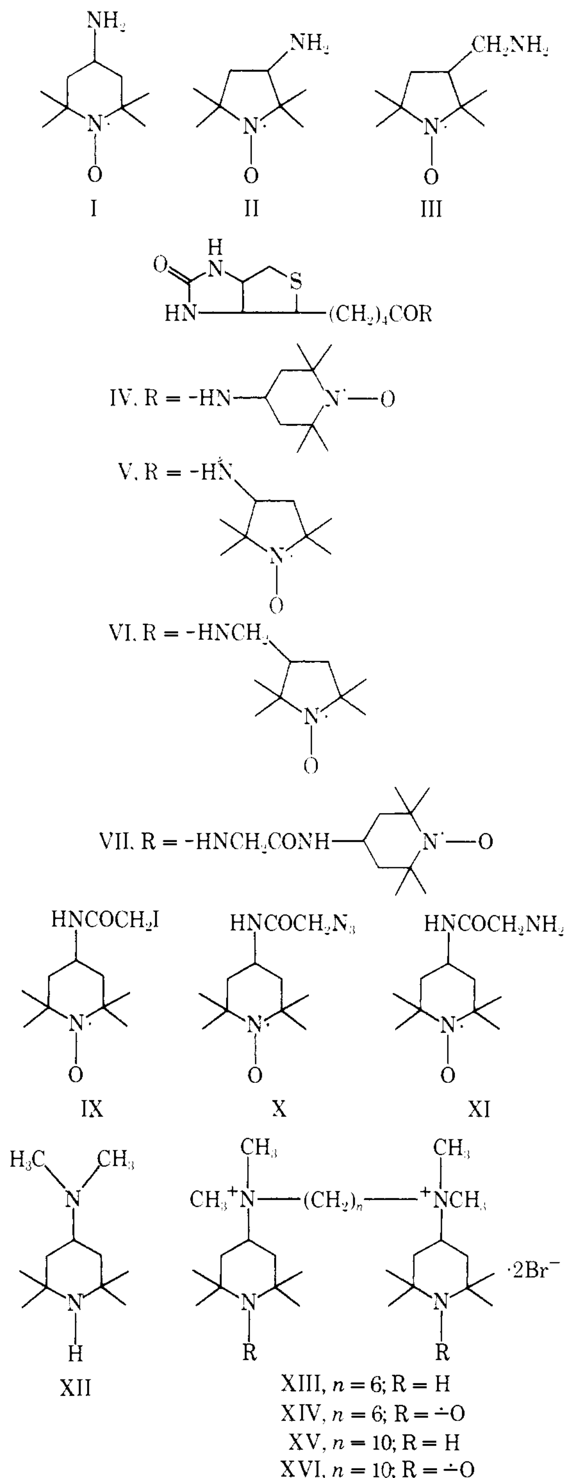
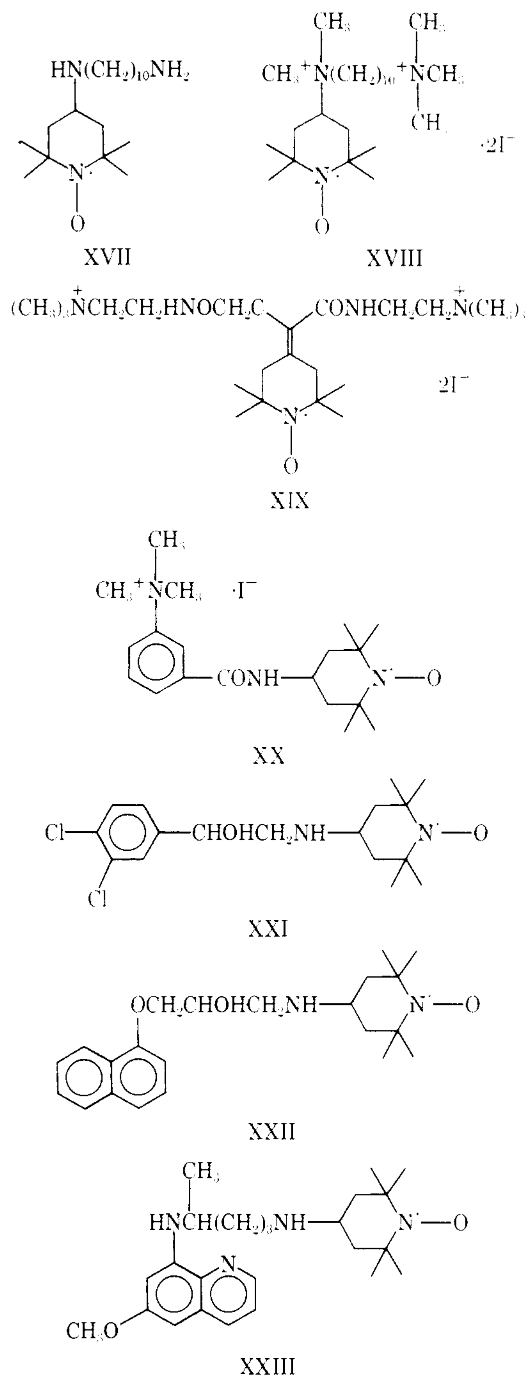
hydride procedure of Green et al.[10] As an alternate approach to the synthesis of biotin spin label VII, we displaced iodide from IX with azide to give X, which was then easily reduced with $NaBH_4$ to XI. Intermediate XI was



then coupled with biotin using EEDQ. All of the spin-labeled biotin analogs were hygroscopic and crystallized with water and solvent of crystallization. Similar difficulties have been reported by Green and coworkers in their syntheses of a series of bis(biotinyl)diamines.[10]

Spin labels XVII and XXI–XXIII were prepared by reductive alkylation of the respective amines with 4-keto-2,2,6,6-tetramethyl-1-piperidinyloxy and sodium cyanoborohydride according to the procedure of Borch and coworkers.[11] Rosen has shown[12] that this procedure does not result in the reduction of the spin label.



Discussion

Egg white avidin is a tetrameric protein (mass 68,000 daltons) which binds four molecules of biotin[13,14] (vitamin H). Green has shown[15] that when 2-(4′-hydroxyazobenzene)benzoic acid (HABA) binds to avidin the absorption maximum of the dye shifts from 348 to 480 nm. Each avidin molecule has four binding sites for HABA from which the dye can be displaced by biotin.[15] The addition of biotin spin label IV to a HABA–avidin complex resulted in the displacement of an equivalent amount of dye. Similar results were obtained with labels V–VII. The interaction of biotin spin labels IV–VII was also monitored by means of optical absorption and fluorescence spectroscopy.[16] These experiments clearly demonstrated that the biotin spin labels occupied the same binding sites as did biotin.

The spin-labeled decamethonium analogs XVI and XVIII are more potent inhibitors of acetylcholinesterase

Table I. Inhibition of *Torpedo californica* Acetylcholinesterase by Quaternary Ammonium Compounds

Compound	K_I, $\times 10^6$ M
XIV	3.30[a]
XVI	0.18[a]
XVIII	0.25[a]
XIX	*a, b*
Decamethonium iodide	6.00[c]
XX	100[a]
Phenyltrimethylammonium iodide	38[d]

[a]These values were calculated from averages of duplicate determinations that did not differ by more than 5%. [b]No inhibition observed at 5×10^{-4} M. [c]K_D value determined from fluorescence titration: P. Taylor and N. M. Jacobs, *Mol. Pharmacol.*, 10, 93 (1974). [d]Determined by Wilson and Quan[18] for *Electrophorus electricus* acetylcholinesterase.

than decamethonium itself (Table I). This suggests that the binding of these labels to acetylcholinesterase is stabilized by some interaction between the lipophilic piperidine moiety and a hydrophobic area at or near the enzyme active site. In contrast to labels XVI and XVIII, spin label XIX at a concentration of 5×10^{-4} M did not inhibit acetylcholinesterase. This result is somewhat surprising, since a Corey–Pauling–Koltun model of XIX, in its fully extended conformation, clearly shows that the quaternary nitrogens are separated by about the same distance as the quaternary nitrogens of decamethonium. One possible explanation for this anomalous result may be that the bulky piperidine group of XIX prevents the ligand from binding to acetylcholinesterase. The lower affinity of the hexamethonium spin label XIV (Table I) in comparison to XVI and XVIII is to be expected, since in this analog the quaternary nitrogen atoms are not far enough apart to permit the simultaneous two-point attachment of the inhibitor to the active center anionic site and the peripheral active site of acetylcholinesterase.[17] Spin label XX was also a very poor inhibitor of acetylcholinesterase (Table I). Wilson and Quan have synthesized a series of phenyltrimethylammonium analogs and have shown that a 3-OH group is necessary for maximal inhibition of acetylcholinesterase.[18]

The β-adrenergic blocking activity of spin labels XXI and XXII was assayed by measuring their ability to inhibit the isoproterenol-stimulated increase in adenylyl cyclase activity of rat fat cell membranes. It can be seen from Table II that the replacement of the N-isopropyl group of dichlorisoproterenol and propranolol by the more bulky piperidyl moiety caused a decrease in β-adrenergic blocking activity.

The electron spin resonance spectra of solutions containing the primaquine analog (XXIII) and either yeast RNA and calf thymus DNA clearly showed that the spin label did not bind to these nucleic acids. Possibly the introduction of the piperidine group into the drug abolishes its ability to interact with DNA and RNA. Experiments are presently in progress to determine whether spin-labeled acridines will be more suitable as probes for nucleic acids.

The introduction, into a drug molecule, of the nitroxide radical together with its bulky heterocyclic ring may (i) leave the biological activity of the drug unchanged, (ii) enhance the biological activity of the drug, or (iii) partially or completely abolish the biological activity of the drug. In this study, all three types of change were encountered. In the absence of data concerning the biological targets of drug molecules, it may not always be possible to predict precisely the biological potency of a spin-labeled drug molecule. Nevertheless, when such spin-labeled drugs are biologically active, and when it can be demonstrated that they interact with the same target sites as the parent drug molecules, then they should be useful tools for studying the topography of receptor macromolecules.

Table II. Inhibition of Isoproterenol-Stimulated Adenylyl Cyclase Activity in Rat Fat Cell Membranes by Spin Labels XXI and XXII, Dichlorisoproterenol, and Propranolol

Compound[a]	Isoproterenol-stimulated adenylyl cyclase[b] (nmol of cAMP/mg of protein/10-min incubation at 30°)	% inhibn
	1.97	0
XXI	1.59	19
Dichlorisoproterenol	1.39	29
XXII	1.17	41
Oxalic acid	1.97	0
Propranolol	0.22	89

[a]The final drug concentration was 6.6×10^{-5} M. [b]These values are averages of duplicate determinations which did not differ from each other by more than 5%.

Experimental Section

Melting points were obtained with a Thomas-Hoover melting point apparatus and are uncorrected. All elemental analyses were within ±0.4% of the theoretical values, except where indicated.

3-Amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (II) was prepared from the corresponding 3-carbamoyl compound by the method of Krinitskaya et al.[19] **3-Methylamino-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (III)** was prepared from the same intermediate by the method of Piette and Hsia[20] in 59% yield and was used without any further purification.

4-[[5-(Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl)-1-oxopentyl]amino]-2,2,6,6-tetramethyl-1-piperidinyloxy (IV) (Method 1). To a solution of 0.28 g (1.64 mmol) of 4-amino-2,2,6,6-tetramethyl-1-piperidinyloxy (I) in 25 ml of absolute EtOH, 0.4 g (1.64 mmol) of biotin (Sigma Chemical Co.) and 0.45 g (10% excess) of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, Aldrich) were added. The mixture was stirred at room temperature for 48 hr and filtered and the alcohol solution was concentrated under reduced pressure. The residual semisolid was dissolved in $CHCl_3$, and the $CHCl_3$ solution was washed with bicarbonate solution and H_2O and dried (Na_2SO_4). Removal of $CHCl_3$ afforded a pink-orange solid (0.42 g, 65%). Crystallization from $CHCl_3$–n-hexane gave pure IV, mp 197.5–198.5°. Anal. C, H, N.

3-[[5-(Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl)-1-oxopentyl]amino]-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (V). This was prepared by method 1 from II and biotin in MeOH at 40–50° for 2 hr and then for 48 hr at room temperature in 77% yield. Crystallization from $CHCl_3$–Et_2O gave V as yellow solid, mp 139–140°. Anal. H, N; C: calcd, 56.38; found, 55.71.

3-[[[5-(Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl)-1-oxopentyl]amino]methyl]-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (VI). This was prepared from III and biotin by method 1. Crystallization from $CHCl_3$–n-hexane gave VI as yellow solid, mp 181–183°. Anal. H, N; C: calcd, 54.92; found, 55.39.

4-(2-Iodoacetamido)-2,2,6,6-tetramethyl-1-piperidinyloxy (IX) was prepared according to the method of McConnell et al.[21] using dry $CHCl_3$ as solvent during acetylation.

4-(2-Azidoacetamido)-2,2,6,6-tetramethyl-1-piperidinyloxy (X). To a solution of 0.85 g (2.5 mmol) of IX in 15 ml of dry DMF, 1.6 g (24.6 mmol) of sodium azide was added. The reaction mixture was stirred at room temperature for 18 hr, diluted with water, and saturated with NaCl. The aqueous solution was extracted with ether (3 × 25 ml). The ether solution was washed with saturated NaCl solution, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. This afforded 0.63 g (99%) of a red liquid; ir ($CHCl_3$) showed the characteristic band for $-N_3$ at 2240 cm^{-1} and for >C=O at 1720 cm^{-1}.

4-(2-Aminoacetamido)-2,2,6,6-tetramethyl-1-piperidinyloxy (XI) was prepared by $NaBH_4$ reduction[22] of the azido group using 2-propanol as solvent. A solution of 0.6 g (2.3 mmol) of X in 10 ml of dry 2-propanol was added to 0.21 g (5.5 mmol) of $NaBH_4$ in 10 ml of dry *i*-PrOH with stirring. The reaction mixture was refluxed for 18 hr and cooled and the solvent was removed under reduced pressure. The residual oil was dissolved in $CHCl_3$, and the $CHCl_3$ solution extracted with dilute HCl. The acid solution was made basic with dilute NaOH and extracted with $CHCl_3$. The $CHCl_3$ solution was dried (Na_2SO_4) and the solvent removed to give 0.52 g (98%) of pure XI (GC–MS, ir) as a red oil.

4-[[[[5-(Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl)-1-oxopentyl]amino]acetyl]amino]-2,2,6,6-tetramethyl-1-piperidinyloxy (VII) was obtained by condensation of XI with biotin according to method 1 described earlier in 35–40% yield. Crystallization from $CHCl_3$–Et_2O afforded VII as pink solid, mp 132–135°. Anal. C, H, N.

4,4-Dimethylamino-2,2,6,6-tetramethylpiperidine (XII) was prepared according to the method of Hubbell et al.[23]

4,4′-[1,6-Hexanediylbis(dimethylimino)]bis[2,2,6,6-tetramethylpiperidine] Dibromide (XIII) from XII. To a solution of XII (2.76 g, 15 mmol) dissolved in 20 ml of absolute EtOH, 1.8 g (7.37 mmol) of 1,6-dibromohexane was added dropwise with stirring. After the addition was complete, the reaction mixture was refluxed for 6 hr, cooled, and poured into large excess of dry Et_2O. The white solids were filtered ahd three crystallizations from EtOH–Et_2O gave 2.6 g of analytically pure white crystals, mp 256–257°. Anal. C, H, N.

4,4′-[1,6-Hexanediylbis(dimethylimino)]bis[2,2,6,6-tetramethyl-1-piperidinyloxy] dibromide (XIV) was prepared by a method similar to one described by Rozantsev and Krinitskaya.[24] To a solution of XIII (1.25 g, 2 mmol) in 40 ml of H_2O, 0.5 g of sodium tungstate, 0.5 g of sodium EDTA, and 10 ml of 30% H_2O_2 were added. The pH of this solution was adjusted to 10, and the reaction was allowed to continue for 7 days in the dark at room temperature. The yellow solution was freeze-dried, and the solids were crystallized from hot EtOH to give 0.8 g (62.5%) of XIV as a pale yellow-orange solid, mp 258–258.5°. Anal. C, H, N.

4,4′-[1,10-Decanediylbis(dimethylimino)]bis[2,2,6,6-tetramethylpiperidine] dibromide (XV) was prepared from XII and 1,10-dibromodecane in 70% yield by a similar method to one described for XIII. Crystallization from EtOH gave XV monohydrate as an orange solid, mp 214–215°. Anal. C, H; N: calcd, 8.12; found, 7.46.

4,4′-[1,10-Decanediylbis(dimethylimino)]bis[2,2,6,6-tetramethyl-1-piperidinyloxy] dibromide (XVI) was prepared by a similar method, one described for XIV in 50% yield. Crystallization from EtOH gave XVI as a deep orange solid, mp 212–213° dec. Anal. C, H, N.

4-[(10-Aminodecyl)amino]-2,2,6,6-tetramethyl-1-piperidinyloxy (XVII). This compound was prepared by reductive alkylation of 1,10-diaminodecane with 4-keto-2,2,6,6-tetramethyl-1-piperidinyloxy using sodium cyanoborohydride[11,12] as reducing agent. To a solution of 4-keto-2,2,6,6-tetramethyl-1-piperidinyloxy (1.3 g, 7.6 mmol) in 25 ml of absolute MeOH, 6.5 g (38 mmol, Aldrich) of 1,10-diaminodecane was added. The mixture was stirred for 0.5 hr at room temperature, the pH of the solution was adjusted to 8.5, and 1.0 g (16 mmol) of sodium cyanoborohydride and some molecular sieves (Fisher Scientific Type 3A) were added. The mixture was stirred for 48 hr at room temperature and filtered. After the solvent had been removed under reduced pressure, the residual semisolid was taken up in H_2O; the pH was adjusted to 4–5 and extracted with $CHCl_3$. The aqueous solution was made basic with dilute NaOH and extracted with Et_2O (4 × 25 ml), dried (Na_2SO_4), and filtered and Et_2O was removed under reduced pressure. This afforded a red viscous oil which could not be crystallized. However, purification was achieved by chromatography over neutral alumina using $CHCl_3$–MeOH (95:5) as eluent. Removal of the solvent from fraction II and crystallization of the solids from MeOH–Et_2O afforded 0.9 g (36%) of pure XVII (GC–MS) as a pink-red solid, mp 107–109°.

4-[Dimethyl[10-(trimethylammonio)decyl]ammonio]-2,2,6,6-tetramethyl-1-piperidinyloxy diiodide (XVIII) was prepared by refluxing XVII (0.1 g) with large excess (10×) of methyl iodide and 0.1 g of solid sodium bicarbonate in absolute EtOH (20 ml) for 6 hr. The reaction mixture was cooled and filtered and dry Et_2O was added. Pink-red solids were filtered and crystallized (three times) from EtOH–Et_2O to give analytically pure XVIII as pink-red solid, mp 185–190°. Anal. C, H, N.

2,2,6,6-Tetramethyl-4-[3-oxo-3-[[2-(trimethylammonio)ethyl]amino]-1-[[[2-(trimethylammonio)ethyl]amino]carbonyl]propylidene]-1-piperidinyloxy Diiodide (XIX). To an ice-cold solution of 0.5 g (1.8 mmol) of 4-[(1,2-dicarboxy-2-ethylidene)]-2,2,6,6-tetramethyl-1-piperidinyloxy (prepared according to the method of Golubev and Rozantsev[25]) and 0.37 g (4.2 mmol, Aldrich) of *uns*-dimethylethylene diamine in 10 ml of $CHCl_3$ was added 0.81 g (4.2 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI). The reaction mixture was allowed to warm to room temperature and stirred overnight. The $CHCl_3$ solution was washed with H_2O, dried (Na_2SO_4), and filtered and the solvent removed under reduced pressure. A portion of the residue (0.4 g) was dissolved in 5 ml of absolute EtOH and refluxed for 0.5 hr with 0.25 ml of methyl iodide. The solvent was removed, and the residue was crystallized from EtOH–Et_2O. The solids (0.4 g) were filtered and recrystallized from EtOH and gave pure XIX as yellow rosette crystals, mp 225–226° dec. Anal. C, H, N.

2,2,6,6-Tetramethyl-4-[[3-(trimethylammonio)benzoyl]amino]-1-piperidinyloxy Iodide (XX). To an ice-cold solution of 0.5 g (3 mmol) of 3-dimethylaminobenzoic acid and 0.51 g (3 mmol) of I in 25 ml of $CHCl_3$ was added 0.69 g (3.6 mmol) of EDCI. The reaction mixture was warmed to room temperature and stirred overnight. The $CHCl_3$ solution was washed with H_2O, dried (Na_2SO_4), and filtered. Removal of $CHCl_3$ and chromatography over silica gel afforded 0.32 g of a solid which was refluxed with 0.2 ml of methyl iodide in absolute EtOH for 2.5 hr. The solvent was removed and crystallized from EtOH giving XX as pale yellow platelike crystals, mp 203–204°. Anal. C, H, N.

4-[[2-(3,4-Dichlorophenyl)-2-hydroxyethyl]amino]-2,2,6,6-tetramethyl-1-piperidinyloxy (XXI) was prepared by reductive alkylation of 2-(3,4-dichlorophenyl)hydroxyethylamine (0.97 g, 4.0 mmol) with 4-keto-2,2,6,6-tetramethyl-1-piperidinyloxy (0.22 g, 13 mmol) and 0.25 g (9 mmol) of sodium cyanoborohydride. Work-up as described for XVII afforded an orange residue which upon crystallization from $CHCl_3$–*n*-hexane gave XXI as orange prismatic crystals, mp 100–102°. Anal. C, H, N.

4-[[2-Hydroxy-3-(1-naphthalenyloxy)propyl]amino]-2,2,6,6-tetramethyl-1-piperidinyloxy (XXII). This was prepared by a similar method to one described for XVII from 2-hydroxy-3-(1-naphthaleneoxy)propylamine hydrochloride (2.0 g, 7.8 mmol), 4-keto-2,2,6,6-tetramethyl-1-piperidinyloxy (1.0 g, 5.88 mmol), and 0.4 g (5.88 mmol) of sodium cyanoborohydride. The reaction mixture was stirred at 50° for 72 hr. Work-up gave a dark liquid which was chromatographed over neutral alumina using $CHCl_3$ as eluent. This afforded 0.8 g (36%) of a red oil which was shown to be the desired compound XXII through GC–MS. It was converted into its oxalate salt which upon two crystallizations from MeOH–Et_2O gave a pink solid, mp 140–142° dec. Anal. C, H, N.

4-[[4-[(6-Methoxy-8-quinolinyl)amino]pentyl]amino]-2,2,6,6-tetramethyl-1-piperidinyloxy (XXIII) was prepared by a similar method to one described for XVII from primaquine diphosphate (5.0 g, 10.9 mmol), 4-keto-2,2,6,6-tetramethyl-1-piperidinyloxy (1.0 g, 5.88 mmol), and 0.4 g (5.88 mmol) of sodium cyanoborohydride. Work-up and chromatography over neutral alumina using $CHCl_3$–MeOH (95:5) as eluent gave 2.2 g (91%) of a red oil which was shown to be the desired XXIII through mass spectra.

A part of this red oil was converted to its methiodide salt by treating it with an excess of methyl iodide at room temperature for 18 hr. Crystallization from EtOH–Et_2O gave a yellow solid, mp 122–124°. XXIII was also converted into its hydrochloride salt by adding concentrated HCl to an MeOH solution of XXIII (pH 4.0) and removing MeOH. Crystallization of the yellow solid from EtOH–Et_2O gave a solid, mp 180–184° dec. The oxalate salt of XXIII was prepared and crystallization from *i*-PrOH–Et_2O gave yellow solid, mp 138–140°. Anal. C, H, N.

Pharmacological Evaluation. Avidin was purchased from the Worthington Biochemical Corp. and purified by chromatography over carboxymethylcellulose according to the procedure of Green and Toms.[26] The concentration of avidin was estimated either from the absorption of the solution at 280 nm ($E_{1\,cm}^{1\%}$ = 15.4)[26] or by titration with HABA according to the procedure of Green.[15] HABA was purchased from the Sigma Chemical Co. All other chemicals were of reagent grade. The displacement of HABA from avidin by spin labels IV–VII was measured by monitoring the change in absorption of the dye at 500 nm.[26] The molar extinction coefficients for free and avidin-bound HABA at 500 nm are 600 and 34,500, respectively.[26] All solutions contained 50 m*M* sodium phosphate buffer, pH 7.4.

Acetylcholinesterase was isolated from *Torpedo californica* by the procedure of Taylor et al.[27] Acetylcholinesterase activity was measured by the pH stat method of Taylor et al.[27] All solutions

contained acetylcholine (0.25 m*M*), NaCl (0.1 *M*), $MgCl_2$ (40 m*M*), and Tris–HCl buffer, pH 8.0 (10 m*M*). The initial rate of acetylcholine hydrolysis was measured at 22° in the absence of (k_0) and presence (k_i) of increasing inhibitor concentrations. The K_I value (i.e., the concentration of drug producing 50% inhibition of enzyme activity) for each spin label was determined graphically from a plot of log $[(k_0/k_i) - 1]$ against log [spin label]. The following concentration ranges were employed: XIV, 10^{-6}–10^{-4} *M;* XVI, 6×10^{-8}–6×10^{-6} *M;* XVIII, 10^{-7}–10^{-5} *M;* XX, 10^{-5}–10^{-3} *M*.

Rat fat cells were isolated by the procedure of Pawlson et al.[28] Adenylyl cyclase was assayed by the method of Krishna and coworkers[29] as modified by Manganiello and Vaughan.[30] The final incubation mixture contained [^{3}H]-ATP (3.3 m*M*), cyclic AMP (0.8 m*M*), $MgCl_2$ (6.6 m*M*), phosphoenol pyruvate (0.17 m*M*), pyruvate kinase (1.7 μg/ml), bovine serum albumin (0.27%), and Tris–HCl buffer, pH 8.0 (33 m*M*). Adenylyl cyclase activity was stimulated by the addition of isoproterenol (100 μ*M*). The final concentration of added drugs and spin labels was 6.6×10^{-5} *M*.

Calf thymus DNA and yeast RNA were purchased from the Sigma Chemical Co. Electron spin resonance measurements were made at 25° with a Varian E-4 spectrometer operating at 9.5 GHz. Samples, containing the nucleic acid (10 mg/ml) dissolved in 0.05 *M* sodium phosphate buffer (pH 7.4) and spin label XXIII (10^{-6} *M*), were introduced into the cavity in a quartz aqueous sample cell.

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References and Notes

(1) H. M. McConnell and B. G. McFarland, *Q. Rev. Biophys.*, **3**, 91 (1970).
(2) O. H. Griffith and P. Jost, *Methods Pharmacol.*, **2**, 223–276 (1972).
(3) J. D. Morrisett, C. A. Broomfield, and B. E. Hackley, Jr., *J. Biol. Chem.*, **244**, 5758 (1969).
(4) R. H. Erlich, D. K. Starkweather, and C. F. Chignell, *Mol. Pharmacol.*, **9**, 61 (1973).
(5) D. D. Koblin, S. A. Kaufmann, and H. H. Wang, *Biochem. Biophys. Res. Commun.*, **53**, 1077 (1973).
(6) R. Leute, E. F. Ullman, and A. Goldstein, *J. Am. Med. Assoc.*, **221**, 1231 (1972).
(7) C. F. Chignell and D. K. Starkweather, *Pharmacology*, **8**, 368 (1972).
(8) C. F. Chignell, D. K. Starkweather, and R. H. Erlich, *J. Med. Chem.*, **15**, 876 (1972).
(9) D. Wolf, J. Valiant, and K. Folkers, *J. Am. Chem. Soc.*, **73**, 4142 (1951).
(10) N. M. Green, L. Konieczny, E. J. Toms, and R. C. Valentine, *Biochem. J.*, **125**, 781 (1971).
(11) (a) R. F. Borch and H. D. Durst, *J. Am. Chem. Soc.*, **91**, 3996 (1969); (b) R. F. Borch, M. D. Bernstein, and H. D. Durst, *ibid.*, **93**, 2897 (1971).
(12) G. M. Rosen, *J. Med. Chem.*, **17**, 358 (1974).
(13) N. M. Green, *Biochem. J.*, **89**, 585 (1963).
(14) N. M. Green, *Biochem. J.*, **89**, 609 (1963).
(15) N. M. Green, *Biochem. J.*, **94**, 23C (1965).
(16) C. F. Chignell, D. K. Starkweather, and B. K. Sinha, *J. Biol. Chem.*, in press.
(17) J. P. Changeux, *Mol. Pharmacol.*, **2**, 369 (1966).
(18) I. B. Wilson and C. Quan, *Arch. Biochem. Biophys.*, **73**, 131 (1958).
(19) L. A. Krinitskaya, E. G. Rozantsev, and M. B. Neiman, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 115 (1965).
(20) J. C. Hsia and L. H. Piette, *Arch. Biochem. Biophys.*, **129**, 296 (1969).
(21) H. M. McConnell, W. Deal, and R. T. Ogata, *Biochemistry*, **8**, 2580 (1969).
(22) P. A. S. Smith, J. H. Hall, and R. O. Kan, *J. Am. Chem. Soc.*, **84**, 485 (1962).
(23) W. L. Hubbell, J. C. Metcalfe, S. M. Metcalfe, and H. M. McConnell, *Biochim. Biophys. Acta*, **219**, 415 (1970).
(24) E. G. Rozantsev and L. A. Krinitskaya, *Tetrahedron*, **21**, 491 (1965).
(25) V. S. Golubev and E. G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 716 (1965).
(26) N. M. Green and E. J. Toms, *Biochem. J.*, **118**, 67 (1970).
(27) P. Taylor, J. W. Jones, and N. M. Jacobs, *Mol. Pharmacol.*, **10**, 78 (1974).
(28) L. G. Pawlson, C. J. Lovell-Smith, V. C. Manganiello, and M. Vaughan, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1639 (1974).
(29) G. Krishna, B. Weiss, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **163**, 379 (1968).
(30) V. C. Manganiello and M. Vaughan, manuscript in preparation.