the gt rotamer becomes more probable. The introduction of the Gly residue to the amino or carboxyl side of L-Phe does not seem to affect the complexation-induced conformational change of the side chain, which provides additional confirmation that electrostatic interactions rather than steric ones play the dominant role.

In the case of complexation with α-CD, the penetration of the phenyl ring into the cavity is shallow[5] and the carboxyl group is sufficiently far away from the CD rim to avoid severe contact even in the tg and gg rotamers. We cannot find any enantiomeric differences for the complexation of L- and D-Phe with α- and β-CDs. This seems reasonable from examination of molecular models since no differences in structural features other than chirality are found. From this standpoint, it can be said that cycloamylose is a better, but not the best, model for enzymes.[10]

(10) We thank Professor R. Chûjô of this institute for helpful discussions. We are also grateful to Messers. K. Kosaka and K. Takahashi of JEOL for facilities for the 200-MHz NMR measurements. This work was partially supported by a Grant-in-Aid from the Ministry of Education, Science, and Culture, Japan (1980).

Selectivity in Binding a Phenanthridinium–Dinucleotide Derivative to Homopolynucleotides

Robert L. Letsinger* and Margaret E. Schott

Department of Chemistry, Northwestern Univeristy Evanston, Illinois 60201

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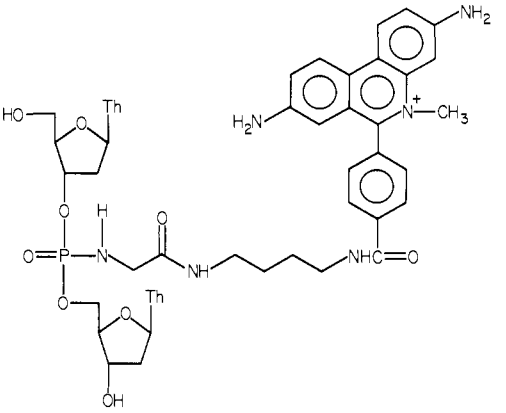
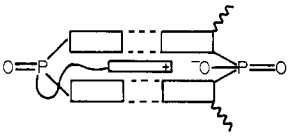
An oligonucleotide derivative possessing a structural feature that enhances binding of the oligomer to complementary sequences in a polynucleotide has interest as a potential site-specific inhibitor (or promoter) of enzymatic processes involving the polynucleotide. We describe in this Communication a model compound, **1**, designed to test the concept that a fragment capable of intercalating in the base pair pockets of DNA can serve as such a structural feature.

Selection of **1** for study was stimulated by the observations that the "melting temperature", T_m, of a double-stranded polynucleotide is increased when ethidium or related substances intercalate,[1] that ethidium intercalates even in dinucleotide pockets at sufficiently high concentrations,[2] and that an intercalator with two binding sites (i.e., a bis-intercalator) forms a much tighter complex with DNA than one with a single binding site (a mono-intercalator).[3] Molecular models indicate that the linker arm in **1** should permit the phenanthridinium moiety to fold back and insert into the pocket formed by the adjoined nucleoside bases and the complementary bases in a polynucleotide (see **2** for a schematic representation). In effect, the local concentration of the ethidium-like group is alway high in the vicinity of the dinucleotide. Compound **1** may therefore be viewed as a simple representative of an oligonucleotide derivative with two types of binding sites that could act cooperatively, the phenanthridinium ring and the pyrimidine bases. Alternatively, **1** is of interest as a model for a biologically active substance (the diaminophenanthridinium ring) with a covalently attached recognition system that could direct the active agent to a given nucleotide sequence.[4]



1



2

As outlined in Scheme I, compound **1** was obtained by constructing the nucleotide portion (**5**) and the phenanthridinium unit (**8**) and then linking these two fragments via an amide bond. For synthesis of **5**, 2-chlorophenyl phosphorodichloridite (0.9 equiv) in 2:1 THF–C_5H_5N was treated successively with triazole (3 equiv, 5 min), 5′-*O*-(phenoxyacetyl)thymidine (1 equiv, −78 °C, 20 min), and 3′-*O*-(di-*p*-methoxytrityl)thymidine (0.5 equiv, −78 to 0 °C, 30 min).[5] The resulting phosphite was converted without isolation[6] to phosphoramidate **4** by reaction with excess ethyl azidoacetate and water in THF (room temperature, 40 h)[7], and **4** was converted to **5** by treatment with excess 1,4-diaminobutane in dioxane (40 °C, 40 h). The phenanthridinium unit was prepared from 2-aminobiphenyl by adaptation of reported procedures.[3f,g] Nitration (H_2SO_4 and KNO_3, 5 °C, 4 h), aroylation with 4-cyanobenzoyl chloride (90 min in refluxing C_6H_5Cl), and cyclization ($POCl_3$, 2 h in refluxing $C_6H_5NO_2$) gave phenanthridine **7**. Methylation (Me_2SO_4, 180 °C in $C_6H_5NO_2$ for 1 h), hydrolysis of the nitrile (75% aqueous H_2SO_4, 130 °C, 90 min), reduction of the nitro groups (Fe–0.03M HBr, 3 h at reflux), and esterification with nitrophenol (DCC in 3:1 DMF–C_5H_5N, 6 h) yielded the active ester **8**. Compound **1**[8] was then obtained by reaction of equimolar

(1) (a) LePecq, J. B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87. (b) Waring, M. J. *Biochem. J.* **1974**, *143*, 483. (c) Aktipis, S.; Martz, W. W. *Biochemistry* **1974**, *13*, 112. (d) Heller, M. J.; Tu, A. T.; Maciel, G. E. *Ibid.* **1974**, *13*, 1623. (e) Barton, J. K.; Lippard, S. J. *Ibid.* **1979**, *18*, 2661. (f) Phillips, D. R.; Roberts, G. C. K. *Ibid.* **1980**, *19*, 4795. (g) Kalisch, B. W.; van de Sande, J. H. *Nucleic Acids Res.* **1979**, *6*, 1881.

(2) (a) Reinhardt, C. G.; Krugh, T. R. *Biochemistry* **1978**, *17*, 4845. (b) Krugh, T. R.; Reinhardt, C. G. *J. Mol. Biol.* **1975**, *97*, 133. (c) Tsai, C.; Jain, S. C.; Sobel, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 628.

(3) (a) LePecq, J. B.; Lebret, M.; Barbet, J.; Roques, B. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2915. (b) Barbet, J.; Roques, B. P.; Commbrisson, S.; LePecq, J. B. *Biochemistry* **1976**, *15*, 2642. (c) Canellakis, E. S.; Shaw, Y. H.; Hanners, W. E.; Schwartz, R. A. *Biochim. Biophys. Acta* **1976**, *418*, 277. (d) Fico, R.; Chen, T. K.; Canellakis, E. S. *Science* **1977**, *198*, 53. (e) Delbarre, A.; Roques, B. P.; LePecq, J. B. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1977**, *284*, 81. (f) Dervan, P. B.; Becker, M. M. *J. Am. Chem. Soc.* **1978**, *100*, 1968. (g) Kuhlmann, K. F.; Charbeneau, N. J.; Mosher, C. W. *Nucleic Acids Res.* **1978**, *5*, 2629.

(4) Drugs that bind to DNA by intercalation include the trypanocidal agents ethidium bromide and aminoacridine, anticancer compounds adriamycin, daunomycin, and actinomycin, and the antimalarials chloroquine and quinacrine. Clearly, a recognition device that could direct an active pharmacological agent to a given DNA sequence or, closer to the present model, to a given RNA sequence offers interesting possibilities.

(5) Phosphite chemistry for oligonucleotide synthesis was followed up to the oxidation step. See: Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655. Melnick, B. P.; Finnan, J. L.; Letsinger, R. L. *J. Org. Chem.* **1980**, *45*, 2715.

(6) Some decomposition of the intermediate *o*-chlorophenyl phosphite was observed when an attempt was made to isolate this substance by preparative chromatography on silica gel; so the products of the reaction with dT(mmtr) were partitioned between CH_2Cl_2 and H_2O, the CH_2Cl_2 layer was concentrated, and the residual phosphite was treated directly with ethyl azidoacetate in THF.

(7) Use of an organic azide in forming phosphoramidate derivatives of dinucleoside phosphates was described by: Letsinger, R. L.; Heavner, G. A. *Tetrahedron Lett.* **1975**, 147. The P–N bond in **4** is stable in 80% aqueous HOAc (10 h, 25 °C) but is cleaved by CF_3COOH (100 °C, 20 min).

(8) λ_{max} (H_2O) 285 (ε 56 600), 495 nm (ε 5500).

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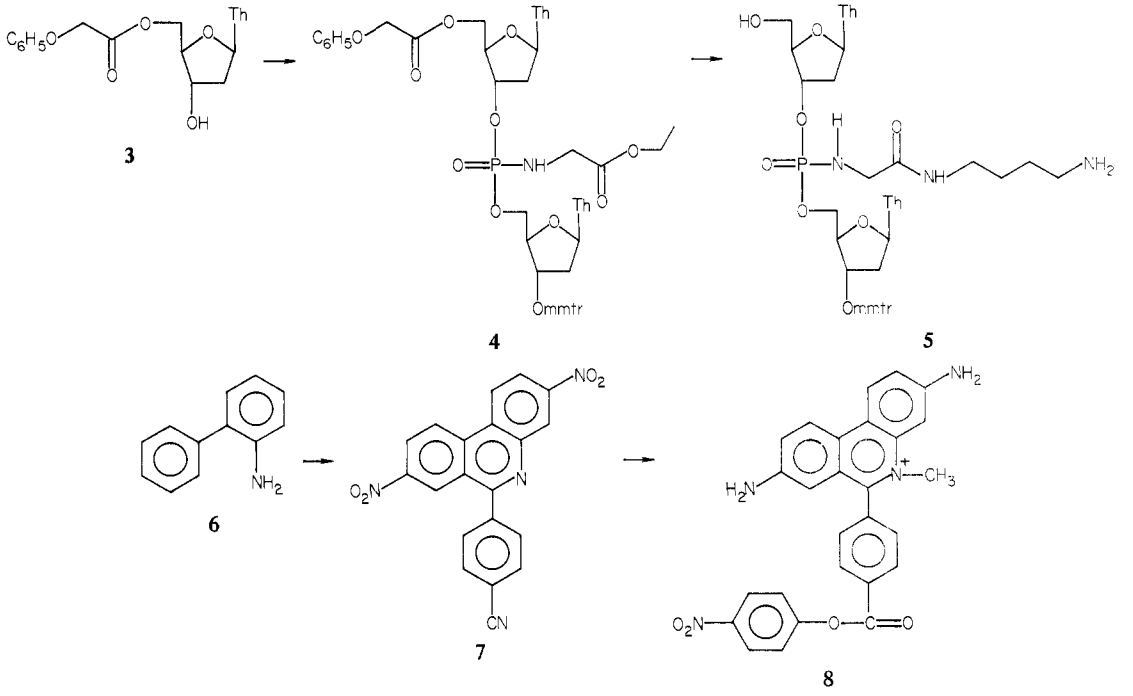
Scheme I



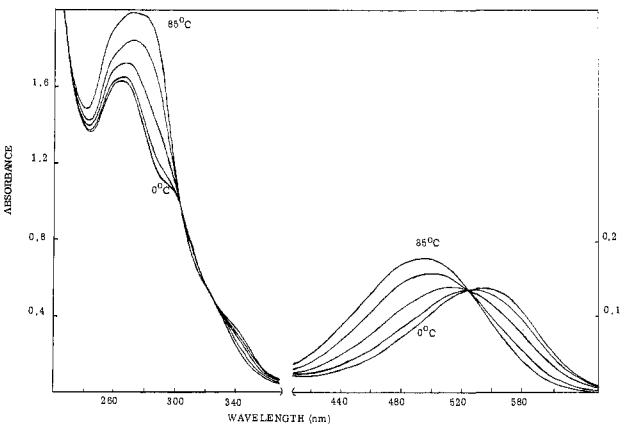


Figure 1. Absorption spectra of **1** (60 μM in dT) and poly-A (68 μM in A) in water, 0–85 °C. Read left ordinate for ultraviolet and right ordinate for visible region.

amounts of **5** and **8** in DMF followed by detritylation with 80% aqueous acetic acid (4 h).

The interaction of **1** with polyadenylate (poly-A)[9] in aqueous solution was examined spectrophotometrically. In dilute solution, ethidium bromide, a closely related phenanthridinium compound, exhibits a strong bathochromic shift (λ_{max} 478 → 518 nm) and a reduction in intensity of the visible absorption band on binding to duplex polynucleotides.[10] As shown in Figure 1, compound **1** exhibits a similar shift in the presence of *single-stranded* poly-A (λ_{max} 495 → 534 nm at 0 °C). With an increase in temperature from 0 to 85 °C the complex "melts out" over a rather broad range and the maximum returns to 497 nm. The midpoint of the transition, T_m, is near 47 °C as determined in either the visible (490 nm) or ultraviolet (280 nm) region (see Figure 2) and is relatively high (T_m = 25 °C) even for solutions 0.1 M in NaCl. For comparison, thymidine oligonucleotides smaller than the pentamer fail to bind to poly-A at 0 °C,[11] and neither of the

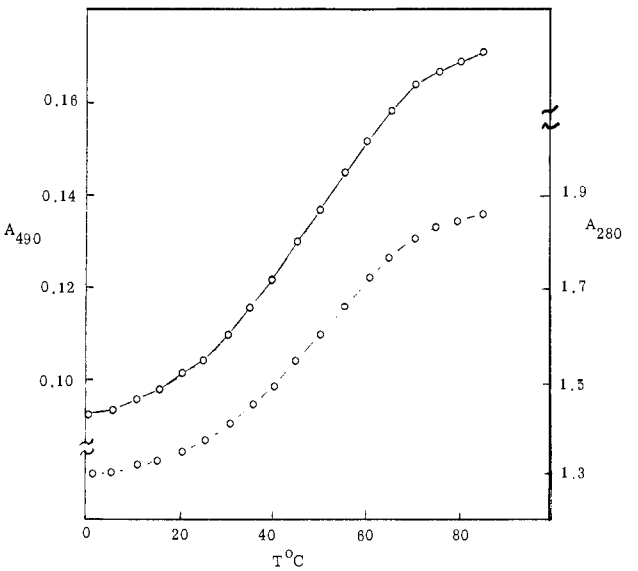


Figure 2. Effect of heating on absorbance of **1** (60 μM in dT) and poly-A (68 μM in A) in water, measured at 490 nm (—) (read left ordinate) and at 280 nm (– – –) (read right ordinate).

neutral triester derivatives $dT_{Et}T$ or $dT_{Me}T_{Me}T_{Me}T$[12] react with poly-A at comparable concentrations.

Titration of a solution of **1** in 0.1 M NaCl with poly-A indicated formation of a complex with a ratio of phenanthridinium (in **1**) to phosphorus (in poly-A) close to one (middle curve, Figure 3). Although very little ethidium binds directly to poly-A[13] (top curve, Figure 3), an interaction of ethidium with the complex of poly-A and **1** was observed (bottom curve, Figure 3). These data are consistent with a model for the complexes formed with limited poly-A in which molecules of **1** are aligned along the poly-A chain (as in **2**) with additional phenanthridinium moieties (from a second equivalent of **1** or from added ethidium) interspersed between them.

In control experiments it was found that addition of the ethyl

(9) The lyophilized potassium salt of poly(adenylic acid) was obtained from Boehringer Mannheim.
(10) Waring, M. J. *J. Mol. Biol.* **1965**, *13*, 269.
(11) Naylor, R.; Gilham, P. T. *Biochemistry* **1966**, *5*, 2722.
(12) These are the ethyl (Et) and methyl (Me) phospho triester derivatives of dTpT and dTpTpTpT, respectively.
(13) Waring, M. J. *Biochim. Biophys. Acta* **1966**, *114*, 234.

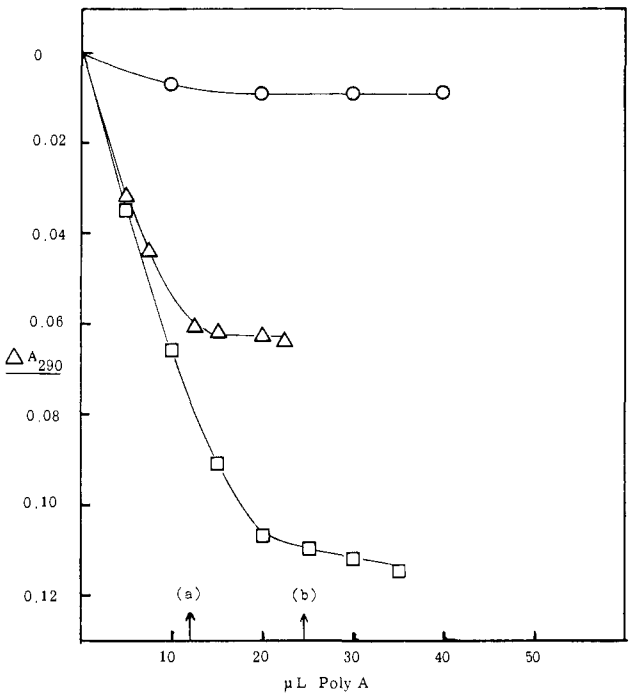


Figure 3. Decrease in absorbance at 290 nm of a solution of ethidium bromide (16 μM) (○), compound **1** (5 μM) (△), and compound **1** (5 μM) + ethidium bromide (14 μM) (□) in 925 μL of Tris buffer (0.01 M, pH 6.8) 0.1 M in NaCl on addition of 5-μL increments of an aqueous solution of poly-A (380 μM). The absorbances have been corrected for volume changes and the small absorbance of poly-A at 290 nm. At the arrows, the A (in poly-A)/d*T* (in **1**) ratio is (1) 0.5 and (2) 1.0.

ester of thymidylyl-(3′-5′)-thymidine ($dT_{Et}T$) (60 μM) has no effect on the spectrum of ethidium bromide (30 μM) in a solution of poly-A (60 μM) at 0 °C, either in the presence or absence of NaCl (0.1 M). Furthermore, spectral data show little or no interaction of **1** with poly-G, poly-C, and poly-U. We therefore believe that the spectral shifts exhibited on addition of poly-A to aqueous solutions of **1** demonstrate formation of a complex stabilized both by specific Watson–Crick base pairing and by an interaction involving a phenanthridinium group favorably positioned by covalent attachment to the thymidine nucleotide. Work is being continued with the objectives of further defining the nature of the interaction of **1** with poly-A and, by extending the oligonucleotide chain, of developing more effective and versatile recognition–delivery systems.

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Preparation and Spectral Properties of Lipophilic Fluorescein Derivatives: Application to Plasma Low-Density Lipoprotein

J. R. Falck,* Monty Krieger, Joseph L. Goldstein, and Michael S. Brown

Department of Molecular Genetics
University of Texas Health Science Center
Dallas, Texas 75235

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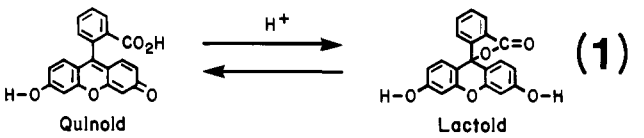
Carrier systems for delivery of analytical probes and pharmacologically active agents in a biological milieu have gained prominence in many applications.[1] However, limitations[2] in the carriers and their ability to shield the transported substance from the surroundings have spurred continued interest in improved systems. Recently, low-density lipoprotein (LDL), the major cholesterol-transport protein in human plasma, has been exploited[3] as a selective and rapid vehicle for delivery of certain classes[4] of lipophilic compounds to mammalian cells in tissue culture. This powerful technique satisfies many of the major objectives[2] for carriers by sequestering compounds inside the delipidated core of LDL during an ordered sequence[5] of binding at a specific cell surface receptor, endocytic internalization, and fusion with lysosomes wherein the LDL core contents are liberated. Molecules are incorporated into the LDL core most readily when they are attached to cis-unsaturated fatty acid esters of cholesterol. We report herein the preparation and some spectral properties of several lipophilic fluorescein derivatives specifically designed[4] for reconstitution into LDL. As a consequence of their unique structure and fluorescence characteristics, these probes are valuable tools[6] in the study of LDL and cholesterol metabolism.

The facile equilibrium (reaction 1) between fluorescein's



(1)

fluorescent quinoid and nonfluorescent lactoid tautomers is reflected in its well-known pH dependent fluorescence.[7] To ensure maximum fluorescence following release from the LDL core into a lysosomal environment[8] (pH ~4), we sought to lock the fluorophore into its quinoid form by esterifying the carboxyl. All attempts, however, to esterify fluorescein with cholesteryl ricinoleate (**1**) using a wide variety of carboxyl activating reagents resulted in complex product mixtures.[9] In contrast, 3-*O*-methylfluorescein (**2**) is esterified readily with **1** using diethyl azodicarboxylate/triphenylphosphine furnishing **3** (78%) as a bright yellow oil[10] [NMR ($CDCl_3$) δ 0.62–2.12 (70 H, complex m), 2.32 (2 H, t, J = 7 Hz), 3.88 (3 H, s), 4.36–5.02 (2 H, m), 5.12–5.44 (3 H, m), 6.38–7.96 (6 H, m), 7.16–7.34 (1 H, m),

(1) For recent reviews, consult: Gregoriadis, G., Ed. "Drug Carriers in Biology and Medicine"; Academic Press: New York, 1979. Gregoriadis, G. *Nature (London)* **1977**, *265*, 407–411.

(2) Gregoriadis, G. In "Liposomes in Biological Systems"; Gregoriadis, G., Allison, A. C., Eds.; Wiley: New York, 1980; Chapter 2. Yoshicami, S.; Nöll, G. N. *Science (Washington, D.C.)* **1978**, *200*, 1393–1394.

(3) Krieger, M.; Goldstein, J. L.; Brown, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5052–5056. Krieger, M.; Brown, M. S.; Faust, J. R.; Goldstein, J. L. *J. Biol. Chem.* **1978**, *253*, 4093–4101.

(4) Some structural guidelines for compounds to be reconstituted into LDL have been suggested. Krieger, M.; McPhaul, M. J.; Goldstein, J. L., Brown, M. S. *J. Biol. Chem.* **1979**, *254*, 3845–3853.

(5) For a review of LDL and its relation to atherosclerosis and cholesterol metabolism, see: Goldstein, J. L.; Brown, M. S. *Annu. Rev. Biochem.* **1977**, *46*, 897–930.

(6) Naphthalene and pyrene containing sterol probes are restricted in some applications because of a requirement for fluorescence excitation in the UV range. The hydrophobic probe dioleyl fluorescein (DOF) is excited in the visible region; however, reconstituted LDL preparation of DOF displays receptor-independent uptake by cells. For a study using DOF and a pyrene bisnorcholenic ester as well as a discussion concerning the need for refinements in fluorescent probes, see: Krieger, M.; Smith, L. C.; Anderson, R. G. W.; Goldstein, J. L.; Kao, Y. J.; Pownall, H. J.; Gotto, A. M., Jr.; Brown, M. S. *J. Supramol. Struct.* **1979**, *10*, 467–478. Naphthalene: Kao, Y. J.; Soutar, A. K.; Hong, K.-Y.; Pownall, H. J.; Smith, L. C. *Biochemistry* **1978**, *17*, 2689–2696. For a recent example using the nitrobenzoxadiazole fluorophore, consult: Craig, I. F.; Via, D. P.; Mantulin, W. W.; Pownall, H. J.; Gotto, A. M., Jr.; Smith, L. C. *J. Lipid Res.* **1981**, *22*, 687–696.

(7) Martin, M. M.; Lindquist, L. *J. Lumin.* **1975**, *10*, 381–390. Fothergill, J. E. In "Fluorescent Protein Tracing", 3rd ed.; Nairn, R. C., Ed.; Williams and Wilkins Co.: Baltimore, 1969; Chapter 2.

(8) Vaes, G. In "Lysosomes and Storage Diseases"; Hers, H. G., Van Hoof, F., Eds.; Academic Press: New York, 1973; Chapter 2.

(9) The chemistry of fluorescein has been reviewed. Wawzonek, S. In "Heterocyclic Compounds"; Elderfield, R. C., Ed.; Wiley: New York, 1951; Vol. 2, Chapter 13.

(10) Satisfactory IR, NMR, and elemental analysis data were obtained on chromatographically homogeneous samples unless otherwise noted. Mass spectral data were obtained for compounds with M_r 1000.

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