Synthetic Spectroscopic Models Related to Coenzymes and Base Pairs. II. Evidence for Intramolecular Base–Base Interactions in Dinucleotide Analogs[1-3]

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Abstract: In order to study interactions of bases found in nucleic acids in the absence of complicating factors associated with hydrogen bonding or the usual carbohydrate and phosphodiester linkages, we have synthesized a series of 12 dinucleotide analogs in which the bases are connected by a trimethylene chain: B–$(CH_2)_3$–B′ or B–C_3–B′, where B and B′ are 9-substituted adenine or guanine or 1-substituted cytosine, thymine, or uracil residues. These compounds were studied optically at concentrations low enough to preclude formation of intermolecular complexes so that the perturbations associated with the 1:1 interaction of a pair of bases could be characterized, namely, by ultraviolet spectra in aqueous solution at room temperature and by emission spectra in 1:1 ethylene glycol–water glass in the vicinity of 77°K. In the series of B–C_3–B′, the order of interaction in neutral aqueous solution is purine–purine > purine–pyrimidine > pyrimidine–pyrimidine, as judged by hypochromism [decrease in integrated ultraviolet absorption intensity of B–C_n–B′ compared with equimolar B–$(CH_2)_2CH_3$ + B′–$(CH_2)_2CH_3$]. The divisions between the categories were not sharp, but the trend was unmistakable. The same order of interaction was confirmed by low-temperature fluorescence emission studies. Emission from singlet excimer states was observed for analogs with relatively strong base–base interaction, while pyrimidine–pyrimidine pairs had fluorescence emission similar to that characteristic of isolated chromophores. The fluorescence emission of 9,9′-trimethylenebisadenine (Ad–C_3–Ad), for example, was characteristic of an eximer state, the emission of Ad–C_3–Cy and Gu–C_3–Th occurred from both eximer states and from excited singlet states, and the fluorescence emission of Th–C_3–Th, Cy–C_3–Th, and Cy–C_3–Cy occurred only from excited singlet states similar to those found for the isolated chromophores of the molecules. Both hypochromism and excimer formation decreased when the pH was varied so as to result in ionization of the bases. Base–base interaction, as judged by hypochromism, was greatly diminished when 95% ethanol was substituted for water as the solvent. In a series of 9,9′-polymethylenebisadenines, the order of interaction deduced from hypochromism and emission studies was $n = 3 > 2$ and 6. Reduced interaction at $n = 2$ reflected the impossibility of this molecule assuming folded, parallel-plane conformations which would allow maximal interaction, as in Ad–C_3–Ad. An entropy effect was probably responsible for decreased interaction in the $n = 6$ compound relative to $n = 3$. The phosphorescence emission from all neutral dinucleotide analogs is characteristic of the base with the lower lying triplet state. This shows that the triplet energy transfer is an efficient mechanism in these molecules and confirms the order of the triplet levels of the bases, which is (in decreasing energy) C, G, A, T. 1,1′-Trimethylenebisthymine (Th–C_3–Th) underwent intramolecular photodimerization at 3.5 times the rate for TpT, presumably reflecting a greater time-average separation of the thymine rings in the latter case. The greater hypochromisms of the analogs compared with the corresponding ribodinucleoside phosphates in general suggest that average base–base separation is smaller in our analogs. The accumulated data indicate that the B–$(CH_2)_3$–B′ analogs serve as useful spectroscopic models for situations in which the nucleic acid bases are held in relatively close proximity, such as in aggregates of bases in aqueous solutions or in the native forms of nucleic acids.

Interactions between adjacent bases in a nucleic acid strand[5] contribute significantly to the structural stability of both single- and double-stranded nucleic acids.[6] In order to study these interactions in the absence of complicating factors associated with hydrogen bonding or the usual carbohydrate and phosphodiester linkages, we have synthesized a series of dinucleotide analogs in which the bases are connected by a polymethylene chain: B–$(CH_2)_n$–B′, or, more simply, B–C_n–B′, where B and B′ are 9-substituted adenine or guanine or 1-substituted cytosine, thymine, or uracil residues.

Purines or pyrimidines, as well as some nucleosides and nucleotides, are known to associate to varying degrees in aqueous solution.[7] With the exception of GMP[8] and isoguanosine,[9] these derivatives evidently

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(2) Taken from the Ph.D. Thesis of D. T. Browne, University of Illinois, 1968. Presented in part at the 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967, Abstracts C48.

(3) The following abbreviations are used: Ad, aden-9-yl; Gu, guan-9-yl; Cy, cytos-1-yl; Th, thym-1-yl; Ur, urac-1-yl; –C_n–, α,ω-disubstituted *n*-alkyl chain [–$(CH_2)_n$–]; C_3, *n*-propyl; GMP, guanosine 5′-phosphate; TpT, thymidylyl-(3′–5′)-thymidine; UpU, uridylyl-(3′–5′)-uridine; ApA, adenylyl-(3′–5′)-adenosine; TFA, trifluoroacetic acid; TMS, tetramethysilane; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; NAD^+, nicotinamide–adenine dinucleotide; TMP, thymidine 5′-phosphate; poly A, polyadenylic acid; poly d(A–T), poly[deoxyadenylyl-(3′–5′)-thymidylic acid].

(4) National Science Foundation Predoctoral Fellow, 1964–1968.

(5) H. De Voe and I. Tinoco, Jr., *J. Mol. Biol.*, **4**, 500 (1962).

(6) For recent reviews see (a) G. Felsenfeld and H. T. Miles, *Ann. Rev. Biochem.*, **36**, 407 (1967); (b) J. Josse and J. Eigner, *ibid.*, **35**, 789 (1966); (c) K. S. Kirby and T. L. V. Ulbricht, *Ann. Rept. Chem. Soc.*, **63**, 536 (1966); (d) T. L. V. Ulbricht, *ibid.*, **62**, 402 (1965); (e) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, N. Y., 1963, Chapter 8; (f) D. T. Browne, Ph.D. Thesis, University of Illinois, 1968.

(7) (a) M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *J. Am. Chem. Soc.*, **90**, 1042 (1968), and references therein; (b) G. K. Helmkamp and N. S. Kondo, *Biochim. Biophys. Acta*, **145**, 27 (1967); (c) O. Jardetzky, *Biopolymers Symp.*, **1**, 501 (1964); (d) K. E. Van Holde, Jr., and G. P. Rossetti, *Biochemistry*, **6**, 2189 (1967); (e) G. P. Rossetti and K. E. Van Holde, *Biochem. Biophys. Res. Commun.*, **26**, 717 (1967); (f) R. C. Lord and H. J. Thomas, Jr., *Biochim. Biophys. Acta*, **142**, 1 (1967); (g) J. N. Solie and J. A. Schellman, *J. Mol. Biol.*, **33**, 61 (1968); (h) E. L. Farquhar, M. Downing, and S. J. Gill, *Biochemistry*, **7**, 1224 (1968).

(8) (a) M. Gellert, M. N. Lipsett, and D. R. Davies, *Proc. Natl. Acad.*

form "stacks," in which the planes of the bases are approximately parallel and interbase hydrogen bonding does not occur to a significant extent. However, there is no guarantee that the relative orientation of the bases in such stacks is at all similar to that found in the native forms of nucleic acids, so that studies of these base–base interactions may not serve as a satisfactory basis for the understanding of analogous interactions in polymeric arrays.

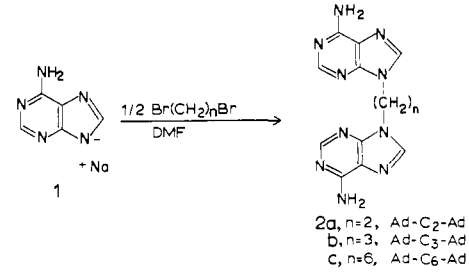
Investigations of certain single-stranded poly-, oligo-, and dinucleotides have been useful in evaluating base–base interaction in the absence of the interbase hydrogen bonding found in double-stranded structures.[6] However, many factors other than base–base interaction are clearly important in determining the structures of these single-stranded species. Electrostatic interactions between negatively charged phosphate groups undoubtedly contribute. The carbohydrate residue can also exert important, specific influences as shown, for example, by comparative studies of otherwise identical structures in the ribo and deoxyribo series.[10]

In the analogs B–C_n–B′ the effects of interbase linkage on base–base interaction are hopefully minimized. In the models in which the bases are connected by a trimethylene chain, this chain is of sufficient length to allow (but not dictate) vertical ring stacking similar to that found in nucleic acids but is too short to allow formation of intramolecular hydrogen bonds of the Watson–Crick[11] or Hoogsteen[12] types. Shorter chains[1b,13–15] would not allow the rings to lie in parallel planes, while longer chains would presumably result in decreased interaction due to an entropy effect. These compounds were studied optically at concentrations low enough to preclude formation of intermolecular complexes so that perturbations associated with the 1:1 interaction of a pair of bases could be characterized, namely, by ultraviolet spectroscopy in aqueous solution at room temperature and by emission spectroscopy in 1:1 ethylene glycol–water glass in the vicinity of 77°K.

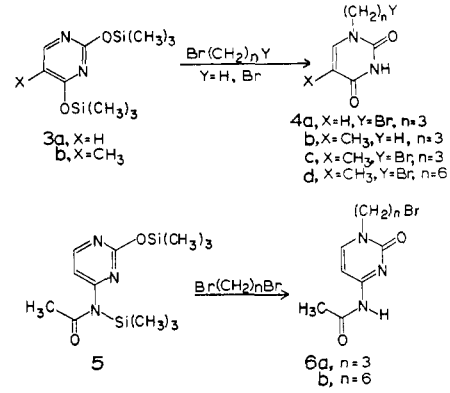
Synthesis of Dinucleotide Analogs and Related N-Propyl Bases

Synthesis of the desired dinucleotide analogs could in principle be accomplished either along routes involving alkylation of preformed nucleic acid bases or through sequences involving formation of these bases by ring closure reactions. Attention was initially focused on the direct alkylation approach, because of its potentially greater simplicity. The principal disadvantage of this approach arises from the fact that each of the nucleic acid bases has several sites susceptible to alkylation. It was therefore necessary to employ procedures that gave alkylation predominately at the desired site and to prove the structures of the products.

Alkylation of a suspension of sodium adenide (**1**) in dimethylformamide has been shown[1b,13,15,16] to give primarily 9-substituted products. Synthesis of the analogs **2a–c** was achieved by treatment of sodium adenide with 0.5 equiv of a polymethylene dibromide. The structures of **2a–c** were confirmed by ultraviolet spectroscopy.[17]



The bis(trimethylsilyl) derivatives of N^4-acetylcytosine (**5**), uracil (**3a**), and thymine (**3b**) have been employed in the synthesis of 1-substituted pyrimidine nucleosides.[18] The synthesis of 1-methylthymine by treatment of **3b** with methyl iodide in the presence of silver perchlorate has also been reported.[19] It was found[20] that treatment of the trimethylsilyl derivatives **3a,b** or **5** with an excess of an unactivated alkyl bromide at room temperature afforded the corresponding 1-substituted pyrimidine. The syntheses of 1-(ω-bromoalkyl)pyrimidines require mild conditions because of the possibility of competing intramolecular cyclization. The structures **4a–d** and **6a,b** were assigned on the basis of uv spectroscopy. Comparison of the spectra of **4a–d**



with spectra representative of the four possible monoalkyl derivatives of uracil[21] demonstrated unequivocally that alkylation had occurred at the 1 position. Similarly, the electronic absorption spectra of **6a,b** agreed

Sci. U. S., **48**, 2013 (1962); (b) J. Iball, C. H. Morgan, and H. R. Wilson, *Nature*, **199**, 688 (1963); (c) D. R. Davies, *Ann. Rev. Biochem.*, **36**, 321 (1967); (d) R. B. Homer and S. F. Mason, *Chem. Commun.*, 332 (1966).

(9) R. V. Ravindranathan and H. T. Miles, *Biochim. Biophys. Acta*, **94**, 603 (1965).

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(11) J. D. Watson, "Molecular Biology of the Gene," W. A. Benjamin, Inc., New York, N. Y., 1965.

(12) (a) K. Hoogsteen, *Acta Cryst.*, **12**, 822 (1959); (b) K. Hoogsteen, *ibid.*, **16**, 907 (1963).

(13) P. C. Huang, Ph.D. Thesis, University of Illinois, 1966.

(14) R. F. Lambert, Ph.D. Thesis, University of Illinois, 1967.

(15) R. F. Lambert, G. Weber, and N. J. Leonard, in preparation.

(16) (a) K. L. Carraway, Ph.D. Thesis, University of Illinois, 1966; (b) N. J. Leonard, K. L. Carraway, and J. P. Helgeson, *J. Heterocyclic Chem.*, **2**, 291 (1965); (c) K. L. Carraway, P. C. Huang, and T. G. Scott, in "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, W. W. Zorbach, Ed., Interscience Publishers, New York, N. Y., 1968.

(17) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *J. Am. Chem. Soc.*, **86**, 5320 (1964).

(18) T. Nishimura and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **12**, 357 (1964).

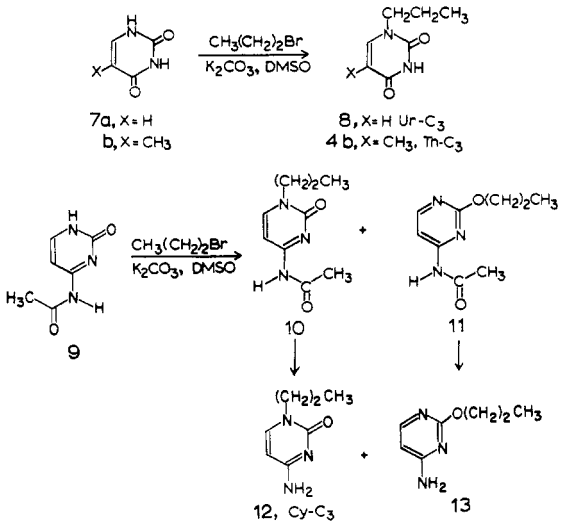
(19) E. Wittenburg, *Chem. Ber.*, **99**, 2380 (1966).

(20) D. T. Browne in ref 16c.

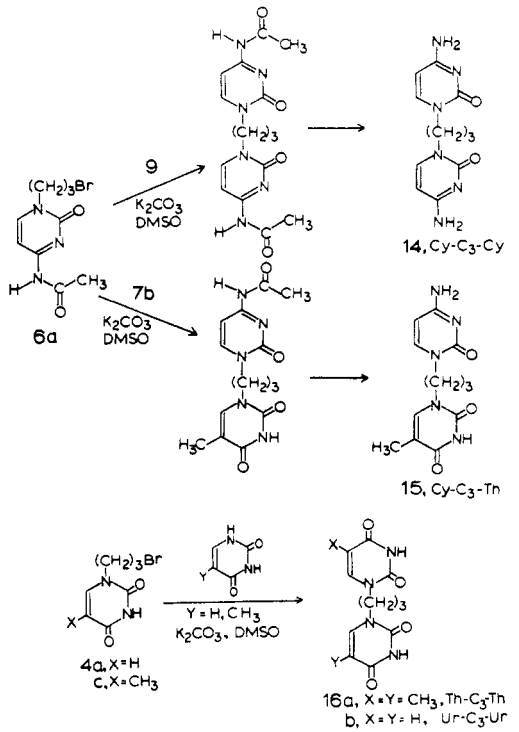
(21) (a) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952); (b) J. J. Fox and I. Wempen, *Advan. Carbohydrate Chem.*, **14**, 283 (1959).

well with that of N^4-acetyl-1-methylcytosine,[22] and deacetylation of **6a,b** yielded monosubstituted cytosines, the spectral properties of which were consistent only with those characteristic of 1 substitution.[21,23]

Compounds **4a,c,d** and **6a,b** were synthesized as potential agents for alkylation on other pyrimidine bases. Baker, Jackson, and Chheda[24] obtained a low yield of 1,1′-pentamethylenebisuracil (Ur–C_5–Ur) by treatment of a dimethyl sulfoxide solution of uracil with 0.33 equiv of 5-chloropentyl methanesulfonate in the presence of potassium carbonate. 1-(5-Chloropentyl)uracil is presumably an intermediate in the reaction. It was found that treatment of uracil (**7a**), thymine (**7b**), or N^4-acetylcytosine (**9**) with *n*-propyl bromide under conditions similar to those employed by Baker, *et al.*,[24,25] for synthesis of 1-substituted uracils yielded the corresponding 1-propylpyrimidines. Alkylation of N^4-acetylcytosine (**9**) also gave a minor amount (*ca.* 20% of monoalkylated material) of the O-alkylated species, 4-acetamido-2-propoxypyrimidine (**11**), in addition to the desired N^4-acetyl-1-propylcytosine (**10**). Compound **11** was separated from **10** by silica gel column chromatography and was treated with methanolic ammonia to yield 4-amino-5-propoxypyrimidine (**13**). The structure of **13**, which was isolated only as a mixture with dimethyl sulfoxide, could be assigned unequivocally on the basis of uv[21a] and nmr data. Pure **12** was easily obtained by treating the mixture of **10** and **11** with methanolic ammonia, followed by recrystallization.

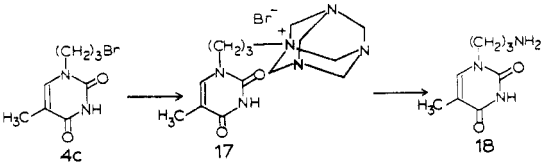


Use of **4a**, **4c**, and **6a** as alkylating agents was complicated by the fact that these compounds are capable of undergoing intramolecular cyclization reactions as well as intermolecular alkylation reactions (giving rise to dialkylated species). Solutions of **4c** or **6a** in dimethyl sulfoxide in the presence of potassium carbonate showed changes in uv and nmr spectra which suggested that inter- and intramolecular reactions were occurring. However, these reactions could be suppressed by addition of an excess of the appropriate pyrimidine base, resulting in the efficient synthesis of Cy–C_3–Cy (**14**), Cy–C_3–Th (**15**), and Th–C_3–Th (**16a**) or Ur–C_3–Ur (**16b**). Compounds **14** and **15** were separated from excess cytosine or thymine by silica gel[26] column chromatography. Separation of **16a** from excess thymine was accomplished by conversion of thymine to soluble (in acetic anhydride) 1-acetylthymine.[27] Ur–C_3–Ur (**16b**) was separated from excess uracil by conversion of uracil to volatile bis(trimethylsilyl)uracil.



The direct alkylation approach was unfortunately not readily applicable to the synthesis of purine–pyrimidine pairs. Reaction of **4c** with sodium adenide yielded only products of intramolecular cyclization, as did a variety of attempts to cause reaction of thymine or thymine derivatives with 9-(3-chloropropyl)- or 9-(3-bromopropyl)adenine.[13,14,16c] Attention was therefore turned to the synthesis of purine-containing analogs by routes involving ring closure.

Compounds **4c** and **6a** were first converted to the corresponding amines. The thymine amine **18** was synthesized by means of the Delépine (Sommelet) reaction[28] and was isolated as the formate salt in order to prevent intramolecular cyclization. The Delépine reaction was not readily applicable in the cytosine series; accordingly, **6a** was converted to the corresponding azide **19**, which was deacetylated and reduced to the desired amine **21**.



(22) G. W. Kenner, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 855 (1955).

(23) (a) I. Wempen and J. J. Fox, *J. Am. Chem. Soc.*, **86**, 2474 (1964); (b) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 1348 (1962); (c) T. Ueda and J. J. Fox, *J. Org. Chem.*, **29**, 1770 (1964).

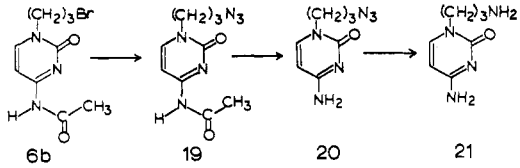
(24) B. R. Baker, G. D. F. Jackson, and G. B. Chheda, *J. Pharm. Sci.*, **54**, 1617 (1965).

(25) B. R. Baker and G. B. Chheda, *ibid.*, **54**, 25 (1965).

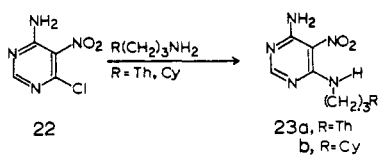
(26) Grade 923 silica gel, 100–200 mesh, obtained from Davidson Chemical, was used throughout.

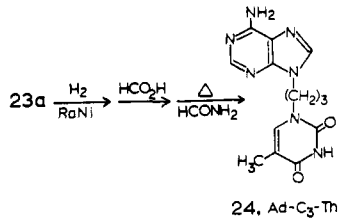
(27) M. Hoffer, *Chem. Ber.*, **93**, 2777 (1960).

(28) (a) S. J. Angyal, *Org. Reactions*, **8**, 197 (1954); (b) A. Galat and G. Elion, *J. Am. Chem. Soc.*, **61**, 3585 (1939); (c) C. Mannich and F. L. Hahn, *Chem. Ber.*, **44**, 1542 (1911).

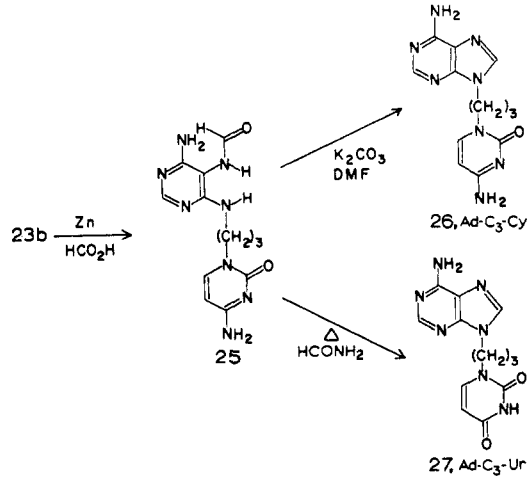


Condensation of **18** or **21** with 4-amino-6-chloro-5-nitropyrimidine (**22**) in the presence of triethylamine gave the 4,6-diamino-5-nitropyrimidines **23a,b**. Compounds of type **23** were readily converted into 9-substituted adenines by successive reduction of the nitro group, formylation, and cyclic dehydration.[29] Ring closure of the formylated intermediate could, in principle, yield either 6- or 9-substituted adenines. However, ring closure by dehydration in hot formamide almost invariably gives predominantly the 9-substituted species.[29] The Ad–C_3–Th analog **24** was synthesized from **23a**. Compound **23b** could be converted to either Ad–C_3–Cy (**26**) or Ad–C_3–Ur (**27**), depending upon the method of ring closure. Reduction and formylation were accomplished simultaneously with zinc dust–formic acid.



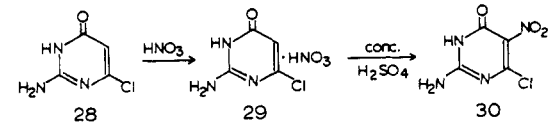


The structures of compounds **24**, **26**, and **27** were established by uv spectroscopy. The position of substitution on the adenine ring was further confirmed by nmr (TFA). The signal of the methylene adjacent to the adenine ring (Ad–CH_2) occurs at τ 5.22 ± 0.03 for all three compounds as compared with τ 5.37 in 9-propyladenine, 5.20 in Ad–C_3–Ad, and 6.15 (AdC_6–NH–CH_2) in 6-propylaminopurine.

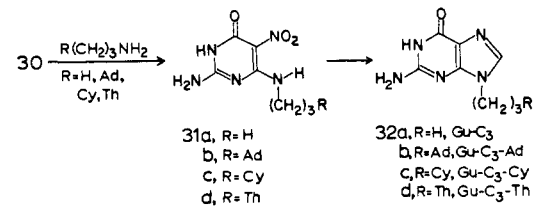


(29) (a) J. H. Lister, *Rev. Pure Appl. Chem.*, **11**, 178 (1961); (b) R. K. Robins in "Heterocyclic Compounds," Vol. 8, R. C. Elderfield, Ed., John Wiley & Sons, Inc., New York, N. Y., 1967, pp 208–244.

Syntheses of guanine-containing analogs involved a similar ring-closure sequence. It was found[20] that published procedures[30] for the synthesis of 2-amino-6-chloro-4(3H)-pyrimidinone (**30**) gave irreproducible yields. A simpler procedure, involving nitration of **28** by dissolving the nitrate salt **29** in concentrated sulfuric acid, was therefore adopted.[20]

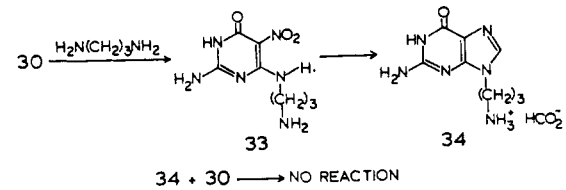


Condensation of **30** with *n*-propylamine, 9-(3-aminopropyl)adenine,[14,15] 1-(3-aminopropyl)cytosine, and 1-(3-aminopropyl)thymine formate salt gave precursors (**31a–d**) of Gu-C_3 (**32a**), Gu–C_3–Ad (**32b**), Gu–C_3–Cy (**32c**), and Gu–C_3–Th (**32d**), respectively.

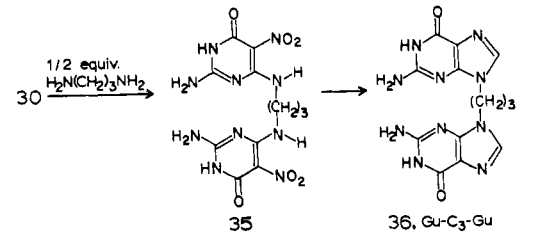


Details of the conversion **31** to **32**[30a] varied depending on the nature of the substituent R. Reduction and formylation were usually accomplished with zinc dust–formic acid. Reduction in methanol with Raney nickel–hydrogen followed by formylation with 98–100% formic acid was employed in several cases (**31a,d**) where the intermediate **31** was sufficiently soluble. Ring-closure dehydration was accomplished by potassium carbonate–DMF for **31b** and **c** and by heating in formamide for **31d**. 9-Propylguanine was synthesized by both of these ring-closure methods.

Synthesis of Gu–C_3–Gu (**36**) was initially approached *via* 9-(3-aminopropyl)guanine (**34**). However, **34**



failed to react with **30** under a variety of conditions. A successful but low-yield synthesis of Gu–C_3–Gu was eventually achieved by a sequence involving two ring closures in the final step (**35** → **36**).



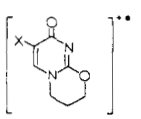
(30) (a) J. Davoll and D. D. Evans, *J. Chem. Soc.*, 5041 (1960); (b) W. Pfleiderer and H. Walter, *Ann. Chem.*, 677, 113 (1964); (c) A. Stuart and H. C. S. Wood, *J. Chem. Soc.*, 4186 (1963); (d) A. Stuart, D. W. West, and H. C. S. Wood, *ibid.*, 4769 (1964); (e) R. Lohrmann and H. S. Forrest, *ibid.*, 460 (1964).

Purification and Characterization of the Dinucleotide Analogs

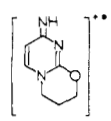
In order to obtain meaningful values of hypochromism, it was necessary to obtain the analogs in high purity. Most of them were therefore subjected to a final purification by silica gel column chromatography.[26] Many of the analogs were insoluble in the solvents commonly used for silica gel chromatography. It was found that they could be applied to the columns as concentrated solutions in 98–100% formic acid and eluted with mixtures of chloroform, glacial acetic acid, and methanol. Acetic acid and methanol were usually used in the ratio 2:1 (v:v).

Microanalysis, uv and nmr spectroscopy, and mass spectrometry were used in characterizing the analogs. As stated earlier, uv data alone often constituted an adequate structure proof. Nuclear magnetic resonance spectroscopy in trifluoroacetic acid (TFA) provided a valuable confirmation of structural assignments. All signals exhibited by the analogs could be related unambiguously to analogous signals in model compounds of known structure. For example, sharp singlets attributable to the C-2 and C-8 protons of adenine appeared at 526 and 559 Hz downfield from TMS in Ad–C_3 as compared with 524–527 and 556–564 Hz in the five adenine-containing analogs in the trimethylene series.

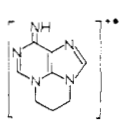
Mass spectrometry provided further evidence for the correctness of the structural assignments. Molecular ions were observed for most analogs and all N-propyl bases. Fragmentation patterns involving purine or pyrimidine nuclei agreed well with those reported[31] for derivatives of the nucleic acid bases. Fragmentation of the polymethylene chain was usually favored over cleavage involving a purine or pyrimidine nucleus. Intense peaks corresponding to a purine or pyrimidine nucleus plus C_3H_5 were observed in the spectra of many analogs. These ions may be analogous to the products of chemical intramolecular cyclization discussed previously (*e.g.*, **37–40**). It should be emphasized that many of these compounds had to be heated to very high temperatures in order to obtain their spectra. It is therefore likely that thermal processes as well as fragmentation induced by electron bombardment were observed in some cases.



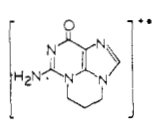
37a X=H, m/e 152
b X=CH_3, m/e 166



38 m/e 151



39 m/e 175



40 m/e 191

Ultraviolet Absorption Studies

A well-known feature of the ultraviolet spectra of nucleic acids is the so-called "hypochromic effect,"[6e] which refers to decreased ultraviolet absorption of the polymeric array relative to that of the constituent mononucleotides measured at a single wavelength, usually 260 mμ. Even dinucleotides exhibit substantially reduced absorption compared with that of the constituent monomers.[6e,32] This phenomenon was first satisfactorily explained by Tinoco,[33] who showed that coulombic interaction between the dipoles induced in the chromophores by light could result in either an increase (hyperchromism) or decrease (hypochromism) in light absorption, depending on the relative orientation of the transition moments of the chromophores in the ordered array. An equivalent treatment was provided by Rhodes.[34] Charney and Gellert[35] showed that hypochromism in monomeric nucleosides can be induced by changes in solvent, in the sense that some nucleosides have less intense uv absorption in acetonitrile than in water. Since the bases in a helical polynucleotide will have a largely nonaqueous environment, it is probable that nucleic acid hypochromism does not result entirely from specific oscillator interactions. A related observation was made by Leonard, Scott, and Huang,[1b] who showed that the presence of a trimethylammonium group in close proximity to an adenine chromophore caused decreased uv absorption by the adenine.

As exemplified in Figure 1, which shows the uv spectra of neutral, aqueous solutions of Ad–C_3–Ad and of Ad–C_3, equimolar in adenine residues, optical perturbations were observed for the dinucleotide analogs. Interaction of the bases in Ad–C_3–Ad produced not only a decrease in intensity near the absorption maximum of Ad–C_3, but also an increase in absorption at long wavelengths and a shift of the absorption maximum of trimethylenebisadenine about 5 mμ to the blue of that of 9-propyladenine. These changes are shown more clearly in Figure 2, which is a difference spectrum of Ad–C_3–Ad *vs.* Ad–C_3. Negative values of $\Delta\epsilon$ correspond to a hypochromic effect and positive values to a hyperchromic effect, or increase in absorption of the analog relative to its isolated, constituent chromophores. It should be noted, in particular, that the greatest decrease in absorption occurs at about 265 mμ, which is to the red of the absorption maxima of both Ad–C_3–Ad (256 mμ) and Ad–C_3 (261 mμ). It is therefore obvious that a comparison of absorption intensities at only one wavelength, such as 260 mμ, would not give an accurate estimate of the extent of electronic perturbation arising from base–base interaction. A more meaningful measure of the perturbation is provided by the quantity hypochromism, H,[33] which corresponds to an integrated hypochromic effect. Hypochromism is defined[33a] in terms of oscillator strengths, f, which are measures of the probability or "allowedness" of a transition as a whole.

Oscillator strengths for the dinucleotide analogs and corresponding N-propyl bases were determined by com-

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(33) (a) I. Tinoco, Jr., *J. Am. Chem. Soc.*, **82**, 4785 (1960); (b) I. Tinoco, Jr., *ibid.*, **83**, 5047 (1961); (c) I. Tinoco, Jr., *J. Chem. Phys.*, **33**, 1332 (1960); (d) I. Tinoco, Jr., *ibid.*, **34**, 1067 (1961).

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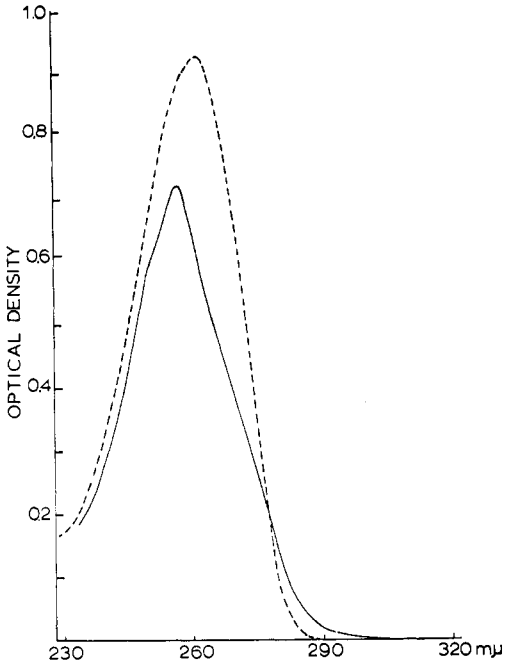

Figure 1. Comparative ultraviolet spectra of Ad–C_3–Ad (—) and Ad–C_3 (×2) (- - - -) in water, pH 7.

puter integration of the electronic absorption spectra from a cutoff wavelength in the vicinity of the absorption minimum to a point of zero absorption at long wavelength,[1b,32b] as described in the Experimental Section. Values of hypochromism were calculated from oscillator strengths, $f = 4.32 \times 10^{-9} \int (\epsilon(\lambda)/\lambda^2) d\lambda$, of the dinucleotide analogs and the appropriate N-propyl bases using the same integration cutoff. For example, in calculating the hypochromism, $\%\ H = \{1 - [f_{AB}/(f_A + f_B)]\}100$, of A–$C_3$–Th, f_{AB} is the oscillator strength of the analog and $f_A + f_B$ is the sum of the oscillator strengths of 9-propyladenine and 1-propylthymine. Hypochromism values for the dinucleotide analogs appear in Table I. A striking correlation

Table I. Hypochromism Values (% H) of Dinucleotide Analogs

Compound	Aqueous solution: Neutral	0.1 *N* HCl	0.1 *N* NaOH	95% ethanol
Ad–C_3–Ad	16.5	2.9	15.7	0.2
Gu–C_3–Gu[a]	15.8	2.3	8.5	
Ad–C_3–Gu	14.9	6.2	10.9	
Gu–C_3–Cy[b]	13.9	6.5	12.4	
Ad–C_3–Th	11.7	4.3	8.2	
Gu–C_3–Th	11.1	7.2	4.4	
Ad–C_3–Cy	9.9	0.6	10.6	
Ad–C_3–Ur	8.0	2.8	4.3	
Th–C_3–Th	7.0	6.6	5.6	−1.3
Th–C_3–Cy	5.5	1.0	7.7	
Cy–C_3–Cy	4.3	−2.7	4.7	
Ur–C_3–Ur	1.2	1.3	−0.7	
Ad–C_2–Ad	11.3	2.1	10.4	
Ad–C_6–Ad	6.1	−0.8	5.6	

[a] Compound hygroscopic and quite insoluble. The possible error is therefore larger than for other analogs. [b] Compound very hygroscopic. It is therefore possible that values of H reported can be up to 1.5% too high.

between the identity of the bases and the hypochromism values in neutral, aqueous solution is seen in the $n = 3$ series. As judged by hypochromism, the order of base–

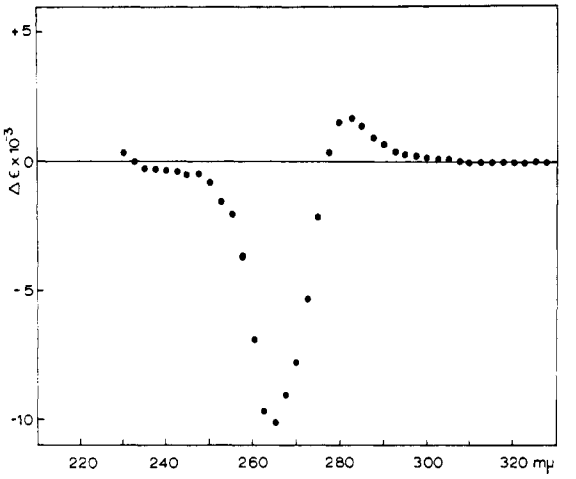

Figure 2. Difference uv spectrum for Ad–C_3–Ad *vs.* Ad–C_3 in water, pH 7.

base interaction is purine–purine > purine–pyrimidine > pyrimidine–pyrimidine. Divisions between the categories are not sharp, but the trend is unmistakable.

The order of interaction purine–purine > purine–pyrimidine > pyrimidine–pyrimidine had also been inferred from studies of the "stacking" interaction of monomeric bases, nucleosides, and nucleotides.[7,36] The published studies did not rely on hypochromism as a means of judging the strength of interaction. "Stacking" interactions of monomeric derivatives of nucleic acid bases should resemble interactions in the analogs to some extent, since the usual carbohydrate and phosphodiester linkages are not present in either case. Of course, the bases in the analogs are prevented from assuming some relative orientations by the trimethylene chain, while the bases in the monomeric derivatives are, in principle, capable of assuming almost any spatial relationship.

The correspondence of analog hypochromism values with the order of base stacking interaction in monomeric purine and pyrimidine derivatives seems unlikely to be fortuitous. It is therefore tentatively concluded, first, that base–base interactions in the analogs resemble interactions in stacks of bases in aqueous solution and in the presumably helical[37] complexes of mononucleosides or nucleotides with polynucleotides, and second, that hypochromism is at least a semiquantitative measure of the strength of base–base interaction. Support for the latter conclusion is provided by the studies of Warshaw and Tinoco,[32a,b] who have shown that the extent of structure in dinucleoside phosphates from RNA as judged from hypochromism usually agrees reasonably well with that inferred from optical rotation. However, use of hypochromism as a *precise* measure of this interaction is probably not justified since the magnitude of the hypochromism is a function of the relative orientation of transition moments[33a] and of

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the identity of the bases as well as the thermodynamic strength of interaction.

Warshaw and Tinoco[32a,b] have classified the 16 principal (*e.g.*, containing combinations of guanine, adenine, cytosine, and uracil) dinucleotides from RNA as "stacked" or "unstacked" on the basis of hypochromism and ORD data. In neutral, aqueous solution, all four ribodinucleotides containing two purines were judged to be stacked as compared with five out of eight purine–pyrimidine pairs and two of four pyrimidine–pyrimidine dinucleoside phosphates. The hypochromism of UpU was the lowest observed. There is thus some indication of the same interaction order, purine–purine > purine–pyrimidine > pyrimidine–pyrimidine, in the ribodinucleotides, although there are many individual exceptions to the trend. The exceptions, which are not surprising in view of the different connection between the bases for the ribodinucleotides and the B–C_3–B′ models, suggest specific roles of the sugar and phosphate linkages in determining the conformational preferences of ribodinucleotides.

In most of the dinucleotide analogs, guanine and adenine appear to have comparable capacities for interaction with each other and with other bases. Values of hypochromism of the three purine–purine analogs are similar, as are values for the pair Ad–C_3–Th and Gu–C_3–Th. The sole exception to this generalization is the pair Gu–C_3–Cy and Ad–C_3–Cy. Thymine seems to be capable of stronger interaction than cytosine, except when connected to guanine. These generalizations do not hold well, if at all, for the dinucleoside phosphates.[32a,b]

A comparison of the hypochromisms at pH 7 of Ad–C_3–Th and Th–C_3–Th with those of Ad–C_3–Ur and Ur–C_3–Ur, respectively, shows that the presence of a methyl group at the 5 position of uracil increases the strength of base–base interaction. Similar effects have been observed in polynucleotides.[38] Some possible interpretations of the stabilizing effect of the pyrimidine 5-methyl group have been given by Shugar, *et al.*,[38a-d,39a] and Helmkamp and Kondo[39b] have implicated a hydrophobic component in the energy of association of alkylpurines.

In the present study, the effect of charge on base–base interaction was assessed by determining hypochromism values of the analogs of aqueous solutions 0.1 *N* in hydrochloric acid or sodium hydroxide. Guanine, adenine, and cytosine residues are positively charged in the acidic solutions, and guanine, thymine, and uracil groups are ionized in 0.1 *N* sodium hydroxide.[40] By analogy with the behavior of adenylyl-(2′–5′)-adenosine and ApA,[32d,41] it is likely that the effect of a charge on one base on the ionization of the other base will not be sufficiently strong to prevent formation of dianionic or dicationic forms of the analogs. Analogs carrying two positive or negative charges showed substantially reduced hypochromism as compared to that at pH 7, presumably because of charge repulsion and increased solvation of the charged rings. With one exception (Th–C_3–Cy in 0.1 *N* NaOH), analogs carrying a single charge also showed a decrease in hypochromism, although this was usually less dramatic than for those cases in which both bases were charged. Negative charges appeared to be less effective than positive in reducing hypochromism (*e.g.*, Gu–C_3–Gu at pH 1 and 13). A similar difference in the effects of positive *vs.* negative charge was observed in the RNA dinucleoside phosphates,[32b] but the origin of the effect is not known with certainty.

In several studies of stacking interactions,[32a,b,42] attempts have been made to divide nucleic acid bases into two categories, those that stack and those that do not. Stacked molecules are said to have the bases in close contact most of the time, whereas the bases in unstacked molecules are usually separated by solvent.[32b] It was concluded[32a,b] that adenine, guanine, and cytosine stack while uracil does not and that two adjacent bases carrying a like charge become unstacked regardless of their identity. Dinucleoside phosphates with hypochromism of 3% or less were classified as unstacked; a hypochromism greater than 4% was taken as evidence of a stacked conformation.[32b] Similarly, trinucleotides have been inferred to be stacked, partially stacked, or unstacked on the basis of hypochromism[42b] or hypochromicity at 260 mμ.[42c]

The hypochromism data for the dinucleotide analogs were therefore examined to see whether a given analog could be classified as stacked or unstacked on the basis of optical perturbation. The distribution of hypochromism values for 36 states (three pH values for the 12 analogs in the $n = 3$ series) is shown in Table II. There

Table II. Distribution of Hypochromism Values

% H	<0	0–3	3–6	6–9	9–12	12–15	15–18
No. of states	2	7	7	9	5	3	3

is no break in the distribution of values that would justify classifying a given state as unstacked or stacked. Rather, a reasonably *continuous* spectrum of interaction seems to be possible. It may be that the analogs have a greater range of energetically favorable conformations available to them than do the naturally occurring dinucleoside phosphates, so that a more continuous range of interaction is possible. However, we are inclined to agree with Brahms, Maurizot, and Michelson[43] that classification of states as stacked or unstacked is an oversimplification in either case. The appearance of a "natural break" in the distribution of hypochromism values of Warshaw and Tinoco[32b] is probably fortuitous, since the break disappears when the values are grouped in a slightly different fashion.

The effect of varying the separation between the rings on the magnitude of hypochromism was investigated in the series Ad–C_n–Ad. As expected, the pH 7 hypochromism for $n = 6$ (6.1%) was considerably less than for $n = 3$ (16.5%), reflecting the greater time-average

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(43) J. Brahms, J. C. Maurizot, and A. M. Michelson, *J. Mol. Biol.*, **25**, 481 (1967).

separation of the two rings connected by the longer chain. In both the $n = 3$ and $n = 6$ cases, the observed hypochromism might be considered a consequence of conformations in which the bases lie in parallel planes. For the $n = 2$ case, however, the dihedral angle formed by the planes of the heterocycles can be about 39° at the lower limit. Since a relatively large hypochromism (11.3%) was observed for $n = 2$, it is obvious that base–base interaction and consequent optical perturbation are not limited to systems in which the bases can assume parallel plane conformations. Substantial hypochromisms were also observed at the $n = 2$ level in model systems related to flavin–adenine dinucleotide[14,15] and nicotinamide–adenine dinucleotide.[1b,13] However, in the Ad–C_3–Ad series and in the NAD^+ analogs,[1b,13] the hypochromism of $n = 2$ was sufficiently smaller than for $n = 3$[44] to suggest that *maximal* interaction may be associated with parallel plane conformations.

The influence of ethanol, an effective denaturing agent for DNA[45] and single-stranded poly A,[46] was determined for Ad–C_3–Ad and Th–C_3–Th. It was found (Table I) that virtually no interaction was detectable in 95% ethanol in either case, despite the fact that the bases are held rather close together by the trimethylene chain. It is therefore probable that the effect of denaturing solvents on nucleic acids is not only to increase electrostatic repulsion of phosphates,[47] but also to greatly diminish interactions of the bases. This observation is in accord with the fact that monomeric nucleic acid derivatives form hydrogen-bonded dimers, rather than stacks, in nonaqueous solutions[48] and with the Hanlon[49] interpretation of the reasons for DNA denaturation by organic solvents. Of course, formation of intramolecular hydrogen-bonded complexes is precluded in our analogs. The low values of hypochromism observed in 95% ethanol serve to emphasize the unique role, discussed by Weber,[50] *inter alia*, that water apparently plays in maintaining the conformation of biological macromolecules. The denaturing power of ethanol could reasonably be associated with a more negative enthalpy of ethanol–base contact (as compared to water–base contact), a less positive entropy change associated with base–base association in ethanol, or both. It is to be emphasized that "hydrophobic bonding" in the Kauzmann[51] or Némethy–Scheraga[52] sense is clearly *not* a major source of stability of the nucleic acids[6a,49] as is often assumed. Association of nucleic acid bases in aqueous solution is characterized by *negative* enthalpy and entropy changes,[46a,47,53] whereas association of hydrocarbons in water results in a large *positive* entropy change and a small positive or zero enthalpy change.[51,52a]

The shape (as well as the intensity and position of λ_{max}) of the ultraviolet absorption spectrum of Ad–C_3–Ad in neutral, aqueous solution (Figure 1) is dramatically different from that of Ad–C_3 in the same medium. The *ca.* 260-mμ band is sharpened near the maximum and slight shoulders appear on either side of the peak. These shoulders appear in better definition in an ultraviolet spectrum taken at −60° in water containing 25% LiCl (pH 7.3).[54] Exciton splitting[7g,55] is probably largely responsible for the anomalous shape of the Ad–C_3–Ad spectrum.[56] Well-defined exciton splitting has indeed been observed in the low-temperature uv spectrum of dimethylthymine molecules separated by about 2.8 Å.[57] Another (less likely) explanation of the shape of the Ad–C_3–Ad spectrum is that shielding of the bases from the solvent improves resolution of vibronic structure.[56]

In all but one (Cy–C_3–Cy) of the analogs containing identical bases (Ad–C_n–Ad, Gu–C_3–Gu, Cy–C_3–Cy, Th–C_3–Th, and Ur–C_3–Ur), the position of λ_{max} was clearly blue-shifted relative to the corresponding N-propyl base. Analogous blue shifts are well known in the uv spectra of polynucleotides[53a,55] and dinucleotides[32b,42a,53a] and may also be accounted for by exciton theory.[55] However, it is generally accepted[58,59] that interactions between degenerate or nearly degenerate transitions in the 260-mμ region do not result in *net* hypochromism of the 260-mμ band. The hypochromisms observed for the analogs are thus presumably largely a consequence of interaction of the 260-mμ transitions with transitions in the far-uv region,[58,59] which are difficult to study experimentally at the present time.

In order to obtain a detailed picture of the electronic perturbations accompanying base–base interactions, difference spectra were calculated for the analogs at pH 7 (*cf.* Figure 2). The calculations were carried out by computer, employing the digital data used to obtain oscillator strengths. For example, in calculating the difference spectrum of Ad–C_3–Th, the sum of the ε values for Ad–C_3 and Th–C_3 at a particular wavelength was subtracted from the ε value of Ad–C_3–Th at that

(44) (a) The values of ε for Ad–C_2–Ad at pH 13 and 1 determined in this study (24.6_5 and $26.2_4 \times 10^3$, respectively) are substantially higher than those reported by Lister (21.0 and 24.6×10^3).[44b] The most probable reason for this discrepancy is the fact that solutions of Ad–C_2–Ad with OD_{cm} of greater than *ca.* 0.25 are supersaturated in neutral or basic aqueous solution. Lister's uv data may have been obtained on solutions with OD_{cm} greater than 0.25. (b) J. H. Lister, *J. Chem. Soc.*, 3394 (1960).

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wavelength. Values of $\Delta\epsilon$ were calculated at intervals of 2.5 mμ starting from the integration cutoff of the analog. Negative values of $\Delta\epsilon$ correspond to hypochromicity and positive values to hyperchromicity. Since the ϵ values used were averages of at least three independent determinations, the calculated difference spectra reported are equivalent to the average of at least three experimentally determined difference spectra. The computer method has the added advantage of eliminating the problem of increased (relative to 10-mm cells) scattering encountered with commonly used 20-mm tandem cells.

Wavelength values of the difference spectra maxima, λ ($\Delta\epsilon_{max}$), are given in Table III along with values of λ_{max}. Comparison of these spectra with the corresponding electronic absorption spectra shows that the profiles of the difference spectra are not in general similar to those of the uv spectra. For example, λ ($\Delta\epsilon_{max}$) is almost always at longer wavelength than λ_{max}.

Table III. Comparison of Ultraviolet and Difference Spectra in Neutral, Aqueous Solution

Compound	λ_{max}[a]	λ ($\Delta\epsilon_{max}$)[b]	$\Delta\epsilon_{max}$[c]
Ad–C_3–Ad	256.0	265.0	−10,160
Gu–C_3–Gu	250.0	252.5	−4,420
	270.0 (sh)	277.5	−4,050
Ad–C_3–Gu	253.0	265.0	−5,420
Gu–C_3–Cy	270.5	277.5	−3,360
Ad–C_3–Th	261.5	270.0	−4,360
Gu–C_3–Th	267.0	277.5	−3,740
Ad–C_3–Cy	262.5	272.5	−3,270
Ad–C_3–Ur	260.5	270.0	−3,380
Th–C_3–Th	269.0	277.5	−2,860
Th–C_3–Cy	271.5	277.5	−1,860
Cy–C_3–Cy	274.0	272.5	−1,150
Ur–C_3–Ur	266.5	272.5	−1,150
Ad–C_2–Ad	257.0	265.0	−6,150
Ad–C_6–Ad	258.0	265.0	−3,290

[a] mμ, ±0.5. [b] Wavelength at which maximum difference between ϵ_{AB} and $\epsilon_A + \epsilon_B$ occurs, mμ ±2.5. [c] $\epsilon_{AB} - (\epsilon_A + \epsilon_B)$.

Most of the analogs were slightly hyperchromic in the region *ca.* 280–300 mμ. Hyperchromism at long wavelengths is a well-known phenomenon in polynucleotides[38c,60] and was observed in many of the dinucleoside phosphates.[32b,42a] The origin of the effect is, however, not well understood. According to Tinoco's theory,[33] a colinear array of transition moments will exhibit hyperchromism. The n–π* transition moments in a nucleic acid helix should be arrayed in this fashion, and Rich and Kasha[60a] suggested on the basis of dichroic studies on oriented 1:1 poly A–poly U that n–π* transitions were responsible for the long-wavelength hyperchromism observed in polynucleotide helices. However, subsequent studies have shown that in adenine derivatives[61] and other 6-substituted purines[61b] the n–π* transition(s) probably lie under the strong π–π* bands. A comparison between the observed and calculated fluorescence lifetime of AMP and poly A indicates that the n–π* level lies below the π–π* level and is the emitting level.[62a] Possible sources of long-wavelength hyperchromism in addition to interactions of n–π* transitions[62b] include changes in relative intensities of individual (unresolved) vibrational bands[62] and exciton splitting.[55]

Ur–C_3–Ur is the only analog for which positive and negative regions of the difference spectrum make comparable contributions to the observed hypochromism. Hyperchromicity is observed below *ca.* 262.5 mμ, which is close to the position of λ_{max} (266.5 mμ). As pointed out by Donovan, Laskowski, and Scheraga,[63] a small shift in the position of an entire transition not accompanied by a change in intensity will produce a difference spectrum closely resembling the first derivative curve of the absorption spectrum. A positive lobe will be observed at short wavelength if the spectrum of the perturbed species is blue shifted relative to the spectrum of the reference compound. A small blue shift (*ca.* 0.5–1.0 mμ) was indeed observed in Ur–C_3–Ur when compared with Ur–C_3, and the difference spectrum of the analog is a reasonable approximation in shape to the first derivative curve of either uv spectrum, taking into account the fact that small intensity changes have also taken place.

The difference spectra of Ad–C_3–Ad, Ad–C_2–Ad, and Ad–C_6–Ad differ in intensity but have very similar shapes and identical values of λ ($\Delta\epsilon_{max}$) at 265 ± 2.5 mμ. This similarity of shape shows that details of ground-state geometry (*e.g.*, the existence of parallel plane conformations) cannot be inferred from features of difference spectra.

The difference spectrum of Gu–C_3–Gu shows two well-resolved peaks at 252.5 and 277.5 mμ. These evidently reflect perturbations of the 252 and *ca.* 270 mμ π–π* transitions in Gu–C_3.[64] The longer wavelength band is reduced in intensity more (on a percentage basis) than the 252-mμ band, which is consistent with the fact that difference spectra maxima are in general to the red of the maxima of the corresponding absorption spectra.

The general features of the difference spectra of analogs containing two different bases could be predicted from the difference spectra of the analogs containing two identical bases. For example, the principal peaks of Ad–C_3–Ur, Ad–C_3–Th, and Ad–C_3–Cy difference spectra apparently correspond to the 265-mμ maximum in the Ad–C_n–Ad series, and the long-wavelength shoulders, to the Ur–C_3–Ur, Th–C_3–Th, and Cy–C_3–Cy difference spectra, respectively. Difference spectra of the mixed pairs could not in general be accurately generated by simple addition of the difference spectra of the two corresponding analogs containing identical bases. However, there is no reason to assume that the *magnitude* of perturbation, for example, of uracil transitions by a neighboring uracil should be the same as for perturbation by a neighboring adenine. If the magnitudes (but *not* the wavelength dependencies) of the B–C_3–B and B′–C_3–B′ difference spectra were adjusted, the spectra of B–C_3–B′ could be generated with reasonable accuracy by simple addition. Thus, the form (*i.e.*, wavelength dependence) of spectral perturbations

(60) (a) A. Rich and M. Kasha, *J. Am. Chem. Soc.*, **82**, 6197 (1960); (b) J. Drobnik and V. Kleinwächter, *Biochem. Biophys. Res. Commun.*, **21**, 366 (1965); (c) M. Gellert, *J. Am. Chem. Soc.*, **83**, 4664 (1961).

(61) (a) B. J. Cohen and L. Goodman, *ibid.*, **87**, 5487 (1965); (b) V. Kleinwächter, J. Drobnik, and L. Augenstein, *Photochem. Photobiol.*, **6**, 113 (1967).

(62) (a) J. Eisinger, to be published; (b) W. Rhodes, *Radiation Res.*, **20**, 132 (1963).

(63) J. W. Donovan, M. Laskowski, Jr., and H. A. Scheraga, *J. Am. Chem. Soc.*, **83**, 2686 (1961).

(64) P. R. Callis, E. J. Rosa, and W. T. Simpson, *ibid.*, **86**, 2292 (1964).

associated with base–base interaction does not appear to depend significantly on the identity of the neighboring base causing the perturbation.

Emission Properties

Emission from the excited states of nucleic acids and related systems has recently been the object of thorough investigation.[65] Quantum yields for emission are usually very low in aqueous solution at ambient temperature. Most studies of emission have therefore been carried out at low temperatures in an aqueous glass.

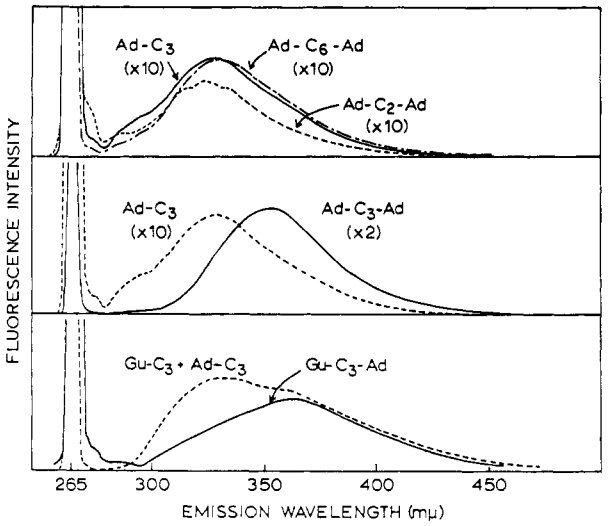

Figure 3. Fluorescence spectra.

Perhaps the most interesting results of the emission studies have been observations indicating interaction between the bases in the excited state. In contrast to the uv absorption spectrum of DNA, which qualitatively resembles that of a mixture of constituent nucleotides, the fluorescence emission from DNA and many polynucleotides and dinucleotides is qualitatively different from the fluorescence of the corresponding mononucleotides.[36a,65a,b,f] The fluorescence emission spectra of these polymeric species are broad and red shifted as compared to those of the monomers and show no vibrational fine structure. These observations strongly suggest[65a] that the emission observed is characteristic of an excited dimer, or excimer[66] (a complex between a molecule in the singlet excited state and a neighboring ground-state molecule). The equilibrium distance between the neighboring molecules is shorter in the excimer state than in the ground state.[66a,b,d–g]

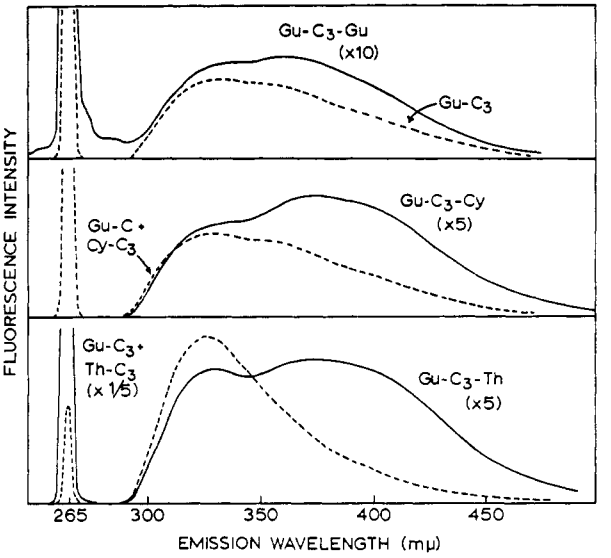

Figure 4. Fluorescence spectra.

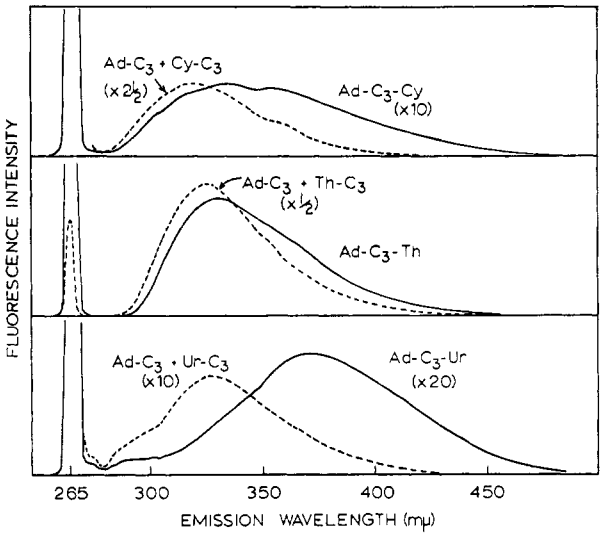

Figure 5. Fluorescence spectra.

Comparison of fluorescence emission and low-temperature ORD spectra of a series of dinucleotides has led to the conclusion[65a] that excimer formation is associated only with dinucleotides having their bases relatively close together in the ground state. Indeed, studies on a variety of different planar, aromatic molecules have strongly suggested that ability to assume a stacked or sandwich configuration is a necessary condition for excimer formation.[67]

Emission spectra of the dinucleotide analogs and N-propyl bases (Figures 3–9) were determined in the vicinity of 77°K with excitation at 265 mμ for samples dissolved in an ethylene glycol–water (1:1, v:v) glass. The wavelength maxima and quantum yields of fluorescence and phosphorescence are listed in Table IV.

By analogy with the behavior of dinucleotides[65a] and DNA,[45b,68] it is likely that at a given temperature interaction between the bases in a dinucleotide will be smaller

(65) For example, (a) J. Eisinger and R. G. Shulman, *Proc. Natl. Acad. Sci. U. S.*, **55**, 1387 (1966); (b) M. Guéron, R. G. Shulman, and J. Eisinger, *ibid.*, **56**, 814 (1966); (c) R. O. Rahn, T. Yamane, J. Eisinger, J. W. Longworth, and R. G. Shulman, *J. Chem. Phys.*, **45**, 2947 (1966); (d) A. A. Lamola, M. Guéron, T. Yamane, J. Eisinger, and R. G. Shulman, *ibid.*, **47**, 2210 (1967); (e) M. Guéron, J. Eisinger, and R. G. Shulman, *ibid.*, **47**, 4077 (1967); (f) J. Eisinger and R. G. Shulman, *J. Mol. Biol.*, **28**, 445 (1967); (g) K. Imakubo, *J. Phys. Chem. Soc.* (Japan), **24**, 1124 (1968); (h) J. Eisinger and R. G. Shulman, *Science*, **161**, 1311 (1968).

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(68) L. Levine, J. A. Gordon, and W. P. Jencks, *Biochemistry*, **2**, 168 (1963).

Table IV. Emission Spectra of Nucleotide and Dinucleotide Analogs

Compound	Fluorescence λ_{max}, mμ	Fluorescence Φ_F[a]	Phosphorescence λ_{max}, mμ	Phosphorescence τ_d, sec	Phosphorescence Φ_P[a]
Ad-C_3	328	0.01	403	2.9	0.02
AdH^+-C_3	~310	0.002	411[b]	3.5[b]	<0.001
Gu-C_3	330	0.13	402	1.5	0.12
Th-C_3	325	0.28	436		~0.001
Th^--C_3	320	0.28	428	0.5	0.015
Cy-C_3	316	0.02	407	0.5	0.004
TMP	322	0.21	440		~0.001
			Phosphorescence Characteristics		
Ad-C_2-Ad	324 M[c]	0.01	Ad		0.01
Ad-C_3-Ad	354 E[c]	0.045[d]	Ad		0.04
Ad-C_6-Ad	331 ~M	0.01	Ad		0.01
AdH^+-C_3-AdH^+	~310 M	0.015	AdH^+		0.007
Ad-C_3-Gu	362 E	0.12	Ad (97%)		0.01
Ad-C_3-Cy	327 M/(E)[c]	0.006	Ad		0.03
Ad-C_3-Th	330 M/(E)[c]	0.01	Th (99%)		0.03
Ad-C_3-Th^-	322 M	0.01	Ad + Th^- (1:1)		0.03
Gu-C_3-Gu	330/360 M/E	0.01[d]	Gu		0.06
Gu-C_3-Cy	330/370 M/E	0.03	Gu		0.03
Gu-C_3-Th	330/370 M/E	0.03	Th (99%)		0.02
Th-C_3-Th	327 M	0.25	Th		0.005
Th-C_3-Cy	326 M	0.18	Th		0.002
Th^--C_3-Th^-	321 M	0.28	Th^-		0.01
Th^--C_3-Cy	325 M	0.08	Th^-		0.02
Cy-C_3-Cy	316 M	0.015	Cy		0.002
TpT	331 M/E	0.14	T		0.01

[a] The fluorescence and phosphorescence quantum yields, Φ_F and Φ_P, were obtained at 80°K in ethylene glycol–water (1:1) glass and have experimental errors of approximately 50%. The exciting wavelength was 265 mμ. [b] Measured for AdH^+-C_3-AdH^+, since phosphorescence of AdH^+ is too weak. [c] C, M, and E refer to monomer- and excimer-like fluorescence spectra, M/E indicates approximately equal contributions from each, (E) indicates a minor excimer contribution. [d] Some uncertainty due to solubility difficulties.

in ethylene glycol–water mixtures than in pure water. Increased interaction will occur on lowering the temperature.[43,65a] It has been shown[65a] that the ORD spectra of a series of dinucleotide analogs in ethylene glycol–water at −30°, a temperature slightly above that at which the solvent mixture solidifies, are comparable to ORD spectra obtained in water at ambient temperature.[32b] The conformations of poly A in water at 25° and in ethylene glycol–water glass at 77°K are apparently similar.[65c] The dinucleotide analogs, therefore, probably have similar conformations in water at ambient temperature and in the ethylene glycol–water glass used for emission studies.

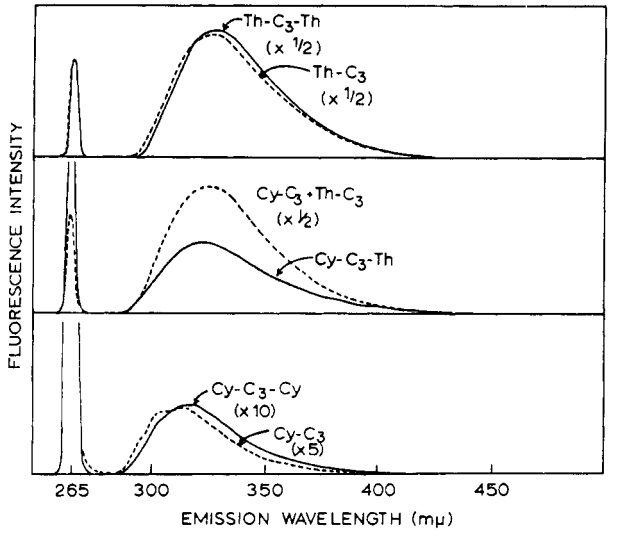

Figure 6. Fluorescence spectra.

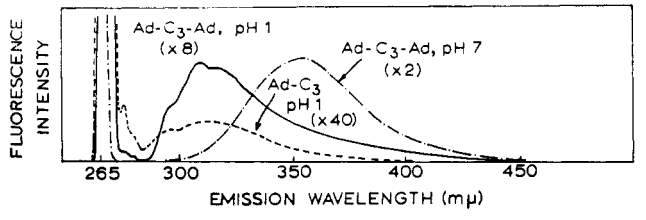

Figure 7. Fluorescence spectra.

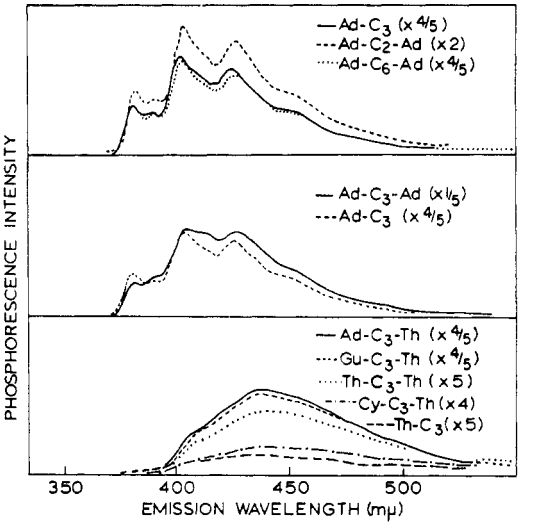

Figure 8. Phosphorescence spectra.

In discussing the emission spectra of the nucleic acid analogs, we shall first describe their fluorescence spectra and then their phosphorescence properties. Since the trimethylene linkage is shorter than the sugar–phos-

phate–sugar linkage of the dinucleotides it may be expected that the number of possible conformations for the B–C_3–B′ analogs, which obtains in the solid glass, is smaller. Presumably the distribution of conformations in the glass is representative of the conformations in solution.

Ad–C_3, Ad–C_2–Ad, and Ad–C_6–Ad all had fluorescence emission spectra of similar shape, wavelength maximum, and intensity (Figure 3). These spectra stand in striking contrast to that of Ad–C_3–Ad, for which the position of the maximum is shifted about 30 mμ to the red of the Ad–C_3 maximum, the intensity is increased by a factor of about five, and there is no indication of vibrational structure. Fluorescence emission from Ad–C_3–Ad is therefore[65a,b,f,66a] probably characteristic of an excimer state. Ad–C_6–Ad does not form an excimer because the rings are relatively widely separated in most of the conformations of the molecule. In Ad–C_2–Ad, excimer formation is not possible because the ethylene linkage prevents the rings from assuming the required[67] plane-parallel conformation. Hirayama[66i] refers to an "$n = 3$ rule" in connection with intramolecular eximer formation in diphenyl end-substituted alkanes, $Ph(CH_2)_nPh$. In the Ad–C_n series, the results of fluorescence emission and uv absorption studies were gratifyingly consistent in showing maximal interaction for $n = 3$, and reduced interactions, for different reasons, for $n = 2$ and 6.

Fluorescence emission from Gu–C_3–Ad and Ad–C_3–Ur was also predominantly from an excimer state (Figures 3 and 5). In contrast, fluorescence emission from Gu–C_3–Gu, Gu–C_3–Cy, Gu–C_3–Th, Ad–C_3–Cy, and Ad–C_3–Th appeared to originate from *both* excimer states and excited singlet states similar to those of the isolated monomers (Figures 4 and 5). In all these cases except Ad–C_3–Th, two bands were present, the one at longer wavelength arising from excimer emission. Some indication of a long-wavelength band was also present in the Ad–C_3–Th fluorescence spectrum, although this case is not as certain as the other four.

Three analogs with two pyrimidine rings (Th–C_3–Th, Th–C_3–Cy, and Cy–C_3–Cy) had fluorescence emission spectra quite similar in shape and position of maxima to the fluorescence of the isolated, constituent bases (Figure 6). Emission from excimer states was not observed. The fluorescence spectra of Ur–C_3–Ur and Ur–C_3 were too weak to record.

The ability of the dinucleotide analogs to form excimers thus paralleled the order of interaction deduced from absorption spectroscopy and provided further support for the validity of the hypothesis that hypochromism is a measure of base–base interaction. One purine–pyrimidine and two purine–purine analogs had emission mainly from excimer states. One purine–purine and five purine–pyrimidine analogs had mixed emission, and none of the three pyrimidine–pyrimidine pairs exhibited excimer fluorescence. Also in accord with hypochromism results, it was possible to suppress excimer formation by varying pH. As shown in Figure 7, the emission maximum of Ad–C_3–Ad was shifted about 50 mμ to the blue upon addition of acid. The pH 1 spectrum of Ad–C_3–Ad resembles that of Ad–C_3 at the same pH, although the latter is of too low intensity to permit a precise comparison of shape. It will be recalled that acidification of Ad–C_3–Ad aqueous solutions reduced hypochromism from 16.5 to 2.9%.

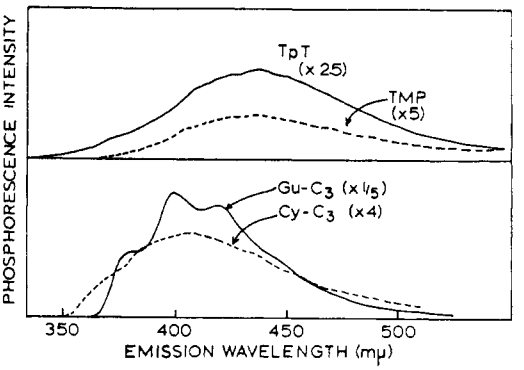

Figure 9. Phosphorescence spectra.

The fluorescence quantum yields for the B–C_3 compounds are within experimental error (about 50%) the same as for the corresponding mononucleotides. The dinucleotide analogs which have monomer-like fluorescence spectra have quantum yields of about the same magnitude as would be expected if the two bases absorbed and emitted independently of each other. The excimers, on the other hand, have fluorescence quantum yields that bear no simple relationship to the monomer yields; they may have smaller or greater yields reflecting greater or smaller nonradiative deexcitation rates of excimers compared with those of monomers.

In contrast to the fluorescence spectra, phosphorescence emission of the dinucleotide analogs was characteristic, in spectral shape and decay rate, of emission from isolated chromophores. Similarity of dimer and monomer phosphorescence emission has also been observed in studies on the dinucleoside phosphates.[65b] As shown in Figure 8, the phosphorescence spectra of Ad–C_3, Ad–C_2–Ad, and Ad–C_6–Ad are essentially identical in shape and position. In Ad–C_3–Ad, the intensity of weak intermediate peaks, which may correspond to a breathing mode,[65e] is somewhat enhanced, but the spectrum is otherwise quite similar to that of Ad–C_3.

The energies of the lowest lying triplet states of the nucleotides have been determined previously from the thresholds of their phosphorescence spectra and their order, decreasing in energy, is[65d] C, AH^+, G, T^-, A, T. Triplet energy transfer from higher to lower lying triplet states is expected to be rapid compared to the donor triplet-state lifetime, if the donor and acceptor molecules are close enough for an exchange interaction to be appreciable. This condition seems to be well satisfied in practically all the dinucleotide analogs; in all cases only the base which has the lower lying triplet state emits, as may be seen from Table IV. The identification of the phosphorescent species was made on the basis of the observed spectra and decay times.

The only exception to the rule given above appears in Ad–C_3–Th^-, where phosphorescence from Ad as well as from Th^- is observed. This observation probably reflects a near degeneracy of the triplet energies of Ad and Th^-. In this connection, it is interesting to note that the triplet energy is sometimes raised by the proximity of another base. Thus, the phosphorescence

threshold of $Th^{-}-C_3-Th^{-}$ is red shifted by about 30 Å compared to that of $Th^{-}-C_3$.

Singlet transfer by way of the excimers[65b,f] may of course occur before the triplet is formed. The final equilibrium population appears, however, to be established as a result of triplet transfer. The phosphorescence quantum yields are also given in Table IV. The ratio Φ_P/Φ_F depends on the nonradiative deexcitation rates as well as on the intersystem crossing rate and shows no simple relationship to the character of the fluorescence spectrum.

A case of considerable interest because of its analogy to DNA[65d] is that of the dinucleotide analogs which contain thymine. The phosphorescence emission is invariably from the thymine triplet which has the lowest energy of all the bases, even though the isolated thymine molecule, or Th–C_3, has a negligible intersystem crossing rate and phosphorescence yield. Intersystem crossing may be enhanced by the presence of a second base in close proximity, as suggested by a comparison of phosphorescence intensities of the pairs Th–C_3–Th and Th–C_3, TpT and TMP, and Ad–C_3–Ad and Ad–C_3. Similar effects have been observed in concentrated aqueous solutions of 1,3-dimethylthymine[65d] and in aggregates of thymine and its derivatives in ethylene glycol–water glass[65d] or in frozen water.[69] An effective explanation of the enhancement of intersystem crossing is lacking at the present time.[65d,70]

Photochemistry

The photochemistry of nucleic acids and related compounds[71] has been the subject of many recent investigations, largely because of its relation to the molecular interpretation of radiation damage. Thymine, cytosine, and cytosine–thymine dimers have been isolated after hydrolysis of the primary radiation products of DNA[71d] and other polynucleotides.[71c] These dimers are presumably formed from adjacent residues in the same nucleic acid chain and are of the cyclobutane type.[71c] The major thymine photoproduct from native DNA[71e] has been shown to be identical (after hydrolysis) with the *cis-syn* dimer[72] obtained as the major product of irradiation of thymine in ice.[73] From irradiated, *denatured* DNA, Ben-Hur and Ben-Ishai[74] have identified both the *cis-syn*- and the *trans-syn*-thymine dimers, the latter being produced as the minor component, and have concluded that the double helical structure imposes certain restrictions on photodimerization. The geometry of the microcrystalline arrays of thymine in ice[78c] and of 1-methylthymine and 3-methylthymine in ice limits, in these cases, the number of dimers which can be formed.[75] The relative proportions of *cis-syn*- and *trans-syn*-thymine dimers in irradiated, denatured DNA and in irradiated TpT[76] are similar.[74]

Since both *cis-syn* and *trans-syn* dimers are produced in TpT irradiation and the dimerization occurs rapidly in comparison to fluorescence emission,[57,77] it is probable that there are at least two stacked conformations of TpT in the ground state, at least one of which, the precursor of *trans-syn* product, does not correspond to a portion of the DNA double helix. Haschemeyer and Rich[78] have concluded that purine, and possibly pyrimidine, nucleosides can exist in two stable conformations with respect to rotation about the glycosyl linkage and have suggested that such isomerism should be considered in analysis of polynucleotide structures.[8c,79]

Photochemical studies were carried out in the spectrometer used for emission studies. Aqueous solutions of Th–C_3–Th and TpT were irradiated under identical conditions at ambient temperature. The reactions were followed by measuring optical density at the λ_{max} (*ca.* 270 mμ) of Th–C_3–Th or TpT. Figure 10 shows that irradiation of both TpT and Th–C_3–Th at 280 mμ led to a decrease in OD at λ_{max}. The rate of decrease for Th–C_3–Th was about 3.5 times that for TpT. Since the quantum yield for production of photoproducts from TpT is about 0.012 at 280 mμ,[76b] the corresponding quantum yield for Th–C_3–Th is about 0.04. Since thymine in aqueous solution is known to be almost completely insensitive to small doses of uv,[80] it is notable that Th–C_3–Th undergoes rapid photoreaction, reflecting ring proximity and favorable conformations in the latter. The faster rate of photoreaction of Th–C_3–Th compared with TpT probably reflects a smaller time-average separation of the thymine rings in the analog, since Hosszu and Rahn[81] showed that a decrease in vertical interaction between adjacent bases in DNA results in a decreased yield of thymine photodimers formed upon irradiation.

Products of the Th–C_3–Th irradiation have not been isolated in this preliminary study,[82] but there are positive indications that cyclobutane-type photodimers are the major reaction product. When an aqueous solution of Th–C_3–Th previously irradiated at 280 mμ was reirradiated at 246 mμ, optical density at λ_{max} increased substantially. Cleavage of thymine photodimers by light of short wavelength is a well-established phe-

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(72) Also referred to as the *cis* head-to-head (chh) dimer.[57]

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(82) Further studies of the photochemistry of Th–C_3–Th and related systems are currently in progress.

nomenon[71b,c] and is a consequence of the fact that the dimers have appreciable uv absorption only at short wavelength.[71c] An aqueous solution of Th–C_3–Th previously irradiated at 280 mμ showed no change in optical density when heated at 100° for 5 min. These conditions would presumably[73] result in at least partial dehydration if any Th–C_3–Th photohydrates had formed. Thymine photohydrates were thus evidently not major photoproducts, which is quite reasonable in view of the fact that hydrates have not been detected in the thymine case.[76b] Examination of space-filling molecular models suggests that Th–C_3–Th can form products analogous to the *cis-syn-* and *trans-syn-*thymine dimers. The trimethylene bridge is too short to allow formation of *anti*-type intramolecular dimers.

It has been suggested[57,77] that singlet excimer states are likely intermediates in the formation of thymine photodimers "since the excimer is well along the way to the dimer."[77] Emission from the excimer is not observed, but dimerization is fast compared to fluorescence emission in cases where two thymine molecules are correctly oriented for dimer formation. It is therefore possible that the complete absence of excimer fluorescence in the emission spectra of Th–C_3–Th, Th–C_3–Cy, and Cy–C_3–Cy results from the occurrence of rapid photoreactions which compete with excimer fluorescence. However, making the reasonable[65a,c] assumption that the rate of photoreaction of Th–C_3–Th in ethylene glycol–water glass is comparable to that in water at ambient temperature, it is clear that excimer formation is not as important in Th–C_3–Th as in the purine-containing analogs (with the possible exception of Ad–C_3–Th). Similar arguments suggest that excimer formation is a relatively minor process in Th–C_3–Cy and Cy–C_3–Cy as well.

Conclusion

Since the spectroscopic properties of the dinucleotide analogs resemble those of naturally occurring nucleotide arrays in many respects, it is appropriate to comment on the relevance of our results to an understanding of the spectroscopic and physical properties of nucleic acids.

Tinoco and coworkers[42b,83] have shown that the optical properties (hypochromism and optical rotation) of oligoribonucleotides and ribonucleic acids, including those having double helical regions, may be satisfactorily accounted for by considering only nearest-neighbor interactions. Study of pairwise interactions of nucleic acid bases could therefore account rather well for interactions in polymeric species.

The bases in a given strand of a hydrogen-bonded double helix face each other *syn* and *cis* in the sense that rotation of the appropriate base in a dinucleotide segment by 36° would bring the two bases into superposition. We have not yet obtained evidence in favor of a particular direction of facing in the analogs and expect that both directions of facing (*cis-syn* and *trans-syn*) will contribute to ground-state conformations. The distribution of cyclobutane-type products in the photolysis of TpT[76] suggests that both *cis-syn* and *trans-syn* facing is permitted in the natural

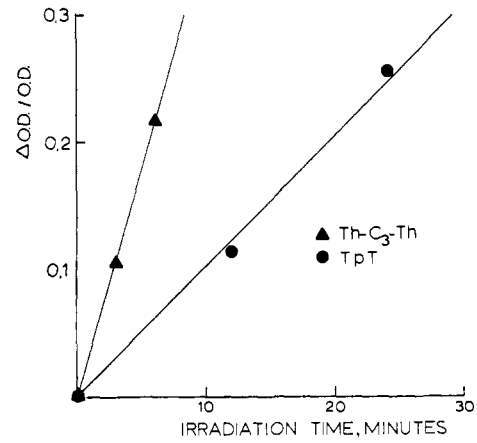

Figure 10. Irradiation of TpT and Th–C_3–Th at 280 mμ.

dinucleotide, the former being slightly favored. It is therefore possible that the properties of both natural dinucleotides and the synthetic analogs will reflect to some extent conformations not possible for dinucleotide segments incorporated into double helical structures.

While there is evidence that the conformations of some dinucleotides, *e.g.*, ApA,[84] represent the beginning of a right-handed helix, it does not necessarily follow that the properties of dinucleotides constitute a completely satisfactory basis for inferring the nature of base–base interactions in higher polymeric arrays. For example, there can be little doubt that the bases in a dinucleoside phosphate interact less strongly than they would if incorporated into a double helical structure. In view of the fact that the interbase linkage in general and a 2′-hydroxyl in particular can have a large effect on conformation, it is also possible that the time-average conformations of dinucleotides may differ significantly from the conformation of a dinucleotide segment in a regular helical array. For example, formation of an intramolecular hydrogen bond involving the 2′-hydroxyl in a ribodinucleoside phosphate might result in a different relative orientation of the bases than would be found in a system where conformational restrictions associated with helical structure must be satisfied. This is perhaps particularly true for double helical structures. Indeed, an intramolecular hydrogen bond involving the 2′-hydroxyl[10d,e,g] was *not* observed in double helical RNA.[85]

In nearly every case where comparison is possible, the hypochromisms characteristic of the dinucleotide analogs ($n = 3$) are *greater* than those exhibited by the corresponding ribodinucleoside phosphates.[32b] The rate of photoreaction of Th–C_3–Th is 3.5 times that of TpT. It is therefore likely that the time-average separation of the bases in the analogs is less than in the naturally occurring dinucleotides. This closer proximity of bases and the fact that their conformations are not influenced by factors such as intramolecular hydrogen bonding, which may not be as important in helical structures as in dinucleoside phosphates, indicate that these analogs do serve as useful models for inferring the contribution of base–base interaction to the structural and spectral properties of nucleic acids.

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Table V. Quantitative Electronic Absorption Data of Dinucleotide Analogs and N-Propyl Bases in Water[a]

Compound	Neutral				0.1 N HCl				0.1 N NaOH			
	λ_{max}	ϵ	λ_{min}	ϵ	λ_{max}	ϵ	λ_{min}	ϵ	λ_{max}	ϵ	λ_{min}	ϵ
Ad–C_3	261.0	14,360	227.0	2,470	259.5	14,080	231.0	2,820	261.0	14,340	232.5	2,380
Gu–C_3	252.0	12,510	225.0	3,160	252.0	11,860	225.0	2,570	255.0 (sh)	9,900	231.0	4,420
	270.0 (sh)	9,310			277.0	7,760	268.0	7,340	267.5	10,580		
Cy–C_3	230.0 (sh)	7,540	249.0	4,020	214.5	9,780	241.5	980	230.0 (sh)	7,720	249.0	4,060
	274.0	8,600			283.5	12,760			274.0	8,530		
Th–C_3	272.0	9,610	237.0	1,630	272.5	9,590	237.0	1,680	270.0	7,200	244.0	4,290
Ur–C_3	267.0	10,130	232.0	1,370	267.0	10,130	231.5	1,330	265.0	7,350	241.0	3,480
Ad–C_3–Ad	256.0	24,410	228.5	5,340	257.0	26,050	231.0	6,190	256.0	24,250	229.0	5,220
Ad–C_2–Ad	257.0	24,650	227.5	4,460	257.0	26,240	228.0	5,400	257.0	24,650	227.5	4,440
Ad–C_6–Ad	258.0	26,300	227.5	4,680	259.5	28,190	231.0	5,750	258.0	26,310	228.0	4,580
Ad–C_3–Gu	253.0	21,530	228.0	6,020	256.0	23,250	228.0	5,740	259.0	21,350	230.0	6,850
Gu–C_3–Gu	250.0	20,680	226.5	6,110	252.5	22,430	226.5	8,470	257.0 (sh)	17,800	231.5	8,540
	270.0 (sh)	15,200			275.0 (sh)	13,200			267.0	18,480		
Gu–C_3–Cy	253.5	14,720	227.5	10,200	256.5	14,150	232.0	5,840	269.0	15,690	237.5	9,800
	270.5	14,290	262.0	13,800	281.0	18,540	262.5	13,900				
Gu–C_3–Th	256.0	16,500	230.5	6,770	257.5	16,690	230.5	5,950	267.5	16,650	238.0	8,990
	267.0	16,090	263.5	16,000	270.0 (sh)	15,600						
Ad–C_3–Th	261.5	19,850	232.0	5,030	261.5	20,190	233.0	5,540	262.0	18,290	237.5	8,110
Ad–C_3–Cy	262.5	18,260	232.5	9,260	267.0	19,470	234.5	5,270	262.5	18,290	232.5	9,340
					285.0 (sh)	14,700						
Ad–C_3–Ur	260.5	21,630	229.5	4,180	260.5	22,320	231.5	4,880	261.0	20,020	234.0	7,650
Th–C_3–Th	269.0	17,360	236.0	3,530	269.5	17,380	236.0	3,570	269.5	13,280	244.0	6,450
Th–C_3–Cy	215.0 (sh)	20,400	242.0	7,120	277.0	19,990	237.5	3,070	230.0 (sh)	16,200	246.5	7,300
	271.5	16,490							271.0	13,690		
Cy–C_3–Cy	230.0 (sh)	14,600	249.0	8,270	213.0	18,710	241.0	2,160	230.0 (sh)	14,400	249.5	8,350
	274.0	15,910			283.0	25,560			273.5	15,840		
Ur–C_3–Ur	266.5	19,730	232.0	2,970	266.5	19,730	231.5	2,940	265.5	14,500	241.5	7,240

[a] Abbreviations: λ, wavelength in mμ; ϵ, molar extinction coefficient; sh, shoulder.

Experimental Section[86]

Electronic Absorption Studies. For quantitative measurements a specified amount of material, weighed to the nearest 0.01 or 0.002 mg, was placed inside a volumetric flask (usually 100 ml) and dissolved in the appropriate amount of 95% ethanol or water. The water employed was double distilled, the second distillation being carried out in an all-glass system under a carbon dioxide free atmosphere. If spectra were to be determined in water at several values of pH, three equal aliquots were withdrawn and placed in volumetric flasks. These were diluted to give three solutions of known, equal volume, one solution in water, one in 0.1 *N* aqueous HCl, and one in 0.1 *N* aqueous NaOH. When solubility properties permitted, all final solutions had peak OD_{cm} of approximately 0.7–0.8, corresponding to a concentration of *ca.* 3–8 $\times 10^{-5}$ *M*, depending on the compound being studied. Spectra of Gu–C_3–Gu at pH 7 and Ad–C_2–Ad were determined in 50-mm rather than 10-mm cells because of the low solubility of these compounds in water. In several representative cases, solutions of dinucleotide analogs or the correspondiug N-propyl bases having a peak OD of *ca.* 0.8 in 5-mm cells were shown to obey Beer's law in 10-mm and 50-mm cells on appropriate dilution. All spectra were determined against the appropriate blank (95% ethanol, water, 0.1 *N* HCl, or 0.1 *N* NaOH) using a matched set of sample and reference cells.

Spectra of dinucleotide analogs and the corresponding N-propyl bases were determined a minimum of three times, and average values of ϵ_{max} are reported. Individual values were reproducible to within $\pm 1.5\%$ for nonhygroscopic compounds and $\pm 3\%$ for the hygroscopic ones. Absorption spectra of intermediates were determined only once.

When oscillator strengths or difference spectra were to be obtained, the electronic absorption spectra were digitized at intervals of 2.5 mμ using a Benson-Lehner Corp. decimal converter Model F. Oscillator strengths were calculated from these data by an IBM 7094 computer using a program based on Simpson's rule. Difference spectra were also calculated by computer. Full details of these calculations and copies of all uv and difference spectra are available elsewhere.[6f]

Emission Studies. Fluorescence and phosphorescence data were taken in the vicinity of 77°K on a luminescence spectrometer which is described elsewhere.[62a] Compounds were dissolved in a 1:1 mixture (v:v) of water and ethylene glycol, which forms a glass at low temperature, to give solutions with peak $OD_{cm} \cong 10$. The solutions were placed in thin-walled quartz tubes, 2 mm o.d., which were sealed prior to determination of the emission spectra. The excitation wavelength was 265 mμ. The emission curves shown in Figure 3–9 were not corrected for the wavelength dependence of the sensitivity of the spectrometer and photomultiplier but the quantum yields given in Table IV contain the appropriate corrections. Quantum yields were obtained by the use of optically thick samples using *p*-terphenyl as a standard.[62a]

Photochemical Studies. Photolyses were performed at ambient temperature and in aqueous solution in the luminescence spectrometer used for emission studies. Absorption spectra of the solutions sealed in matched 2-mm o.d. quartz tubes were taken using a Bausch and Lomb Spectronic 505 spectrometer provided with a special sample holder. The tubes each contained 30 μl of solution, giving a 20-mm column, about 3 mm of which was irradiated. During irradiations the contents of the tube were shaken periodically to ensure that undimerized material was not being significantly depleted in the portion of the tube being irradiated, and, thus, that the extent of dimerization would be a linear function of the uv dosage.

1-Propylcytosine (1a, Cy–C_3).[20] To a solution of 5.00 g (32.6 mmol) of N^4-acetylcytosine (**9**)[87,88] in 800 ml of dry dimethyl sulfoxide were added 4.01 g (32.6 mmol) of 1-bromopropane and 4.95 g (35.8 mmol) of anhydrous potassium carbonate. The resulting suspension was stirred at ambient temperature for 14 hr and filtered, and solvent was removed from the filtrate *in vacuo*, leaving a pale yellow oil. The oil was suspended in 500 ml of water, and the suspension was extracted with four 300-ml portions of chloroform. The chloroform extracts were dried (Na_2SO_4) and evaporated to dryness *in vacuo*, leaving a pale yellow solid. This material was shown (tlc, silica gel column chromatography, nmr, and uv analysis) to consist of about 80% of the desired product, N^4-acetyl-1-propylcytosine (**10**), and about 20% of the O-alkylated product, 4-acet-

(86) Melting points are corrected. Electronic absorption spectra were recorded on a Cary Model 15 spectrophotometer. Nuclear magnetic resonance spectra were determined on a Varian Associates A-60, A-60A, or A-56/60 spectrometer using tetramethylsilane as an internal reference. Infrared spectra were determined on a Perkin-Elmer Model 337 or 521 grating spectrophotometer. Microanalyses were performed by Mr. Josef Németh and his staff, who also weighed samples for quantitative electronic absorption studies. Mass spectra were determined by Mr. Joseph Wrona using an Atlas Model CH_4 low-resolution spectrometer.

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amido-2-propoxypyrimidine (**11**). O-Alkylated material was removed in the recrystallization step. The pale yellow solid was dissolved in a mixture of 100 ml of methanol and 75 ml of methanol presaturated with ammonia at ambient temperature. The solution was allowed to stand at ambient temperature for 7.5 hr, at which time colorless platelets had separated. The suspension was evaporated to dryness *in vacuo*, leaving a colorless solid which was recrystallized from 95% ethanol to yield 2.76 g (55%) of colorless platelets, mp 256.5–259.5°; nmr (TFA) τ 1.95 (br s, 2, CyC_4–NH_2), 2.16 and 3.64 (2d, 2, J = 8 Hz, $CyC_{5,6}$–H), 6.00 (t, 2, J = 7 Hz, Cy–CH_2), 8.17 (m, 2, J = 7 Hz, C–CH_2–C), and 8.96 (t, 3, J = 7 Hz, CH_2–CH_3); mass spectrum (70 eV) *m/e* (relative intensity) 153 (53, M^+), 138 (23, Cy–$C_2H_4^+$), 125 (16, Cy–CH_3^+), 124 (25, Cy–CH_2^+), 111 (77, Cy–H^+), and 81 [100, Cy–CH_2^+ − (NH_2 and HCN)]. Quantitative electronic absorption data for this compound, other N-propyl bases, and the dinucleotide analogs are given in Table V.

Anal. Calcd for $C_7H_{11}N_3O$: C, 54.89; H, 7.24; N, 27.44. Found: C, 54.95; H, 7.39; N, 27.52.

1-Propylthymine (4b, Th–C_3).[20,89] **A. By Alkylation of Bis(trimethylsilyl)thymine.** A 9.20-g (0.034 mol) portion of bis(trimethylsilyl)thymine (**3b**)[90] was dissolved in 170 g (1.38 mol) of dry 1-bromopropane. The solution was kept at ambient temperature for 10 days and then poured into 300 ml of water. The water layer was extracted with three 300-ml portions of chloroform. The chloroform extracts were dried ($MgSO_4$), and solvent was removed *in vacuo* to leave a pale yellow solid. The solid was recrystallized from absolute ethanol to yield 2.78 g (48%) of colorless prisms, mp 134–136° (lit.[89] mp 138°); nmr ($CDCl_3$) τ 2.93 (s, 1, ThC_6–H), 6.30 (t, 2, J = 7 Hz, Th–CH_2), 8.08 (s, 3, ThC_5–CH_3), 8.33 (m, 2, J = 7 Hz, C–CH_2–C), and 9.07 (t, 3, J = 7 Hz, CH_2–CH_3); mass spectrum (70 eV) *m/e* (relative intensity) 168 (82, M^+), 139 (27, Th–CH_2^+), 126 (77, Th–H^+), 96 (100, Th–CH_2^+ − HNCO), 83 (16, Th–H^+ − HNCO), and 55 [28, Th–H^+ − HNCO and CO)].

Anal. Calcd for $C_8H_{12}N_2O_2$: C, 57.13; H, 7.19; N, 16.66. Found: C, 56.93; H, 7.07; N, 16.42.

B. By Alkylation of Thymine.[20] To a solution of 5.0 g (0.040 mol) of thymine (**7b**) in 135 ml of dry dimethyl sulfoxide were added 1.6 g (0.013 mol) of 1-bromopropane and 5.5 g (0.044 mol) of anhydrous potassium carbonate. The resulting suspension was stirred at ambient temperature for 11 hr and filtered, and solvent was removed *in vacuo* from the filtrate, leaving a colorless semisolid. The semisolid was suspended in 500 ml of water, and the water layer was extracted with three 300-ml portions of chloroform. The chloroform extracts were dried (Na_2SO_4) and evaporated to dryness *in vacuo*, leaving a pale yellow solid. Two recrystallizations from absolute ethanol yielded 0.98 g (44%, based on 1-bromopropane) of colorless prisms, mp 135–137°, identical by ir (Nujol) and mixture melting point with the product from section A.

1-Propyluracil (8, Ur–C_3). To a 10.0-g (0.089 mol) portion of uracil (**7a**) dissolved in 650 ml of dry dimethyl sulfoxide were added 3.14 g (0.0255 mol) of 1-bromopropane and 13.8 g (0.100 mol) of anhydrous potassium carbonate. The resulting suspension was stirred at ambient temperature for 20 hr. The viscous suspension was filtered, and dimethyl sulfoxide was removed from the filtrate *in vacuo*, leaving a pale yellow solid. The solid was partially dissolved in 1 l. of water, and the resulting suspension was extracted with six 500-ml portions of chloroform. The chloroform extracts were dried (Na_2SO_4) and concentrated *in vacuo*, leaving a pale yellow solid. Most of the solid was dissolved in 100 ml of 95% ethanol at ambient temperature. Insoluble material was removed by filtration, and the filtrate was evaporated to dryness *in vacuo*, leaving, after trituration with two 15-ml portions of diethyl ether, 1.7 g (44% based on 1-bromopropene) of a colorless solid, mp 113–121.5°. The solid was recrystallized from absolute ethanol to yield 1.16 g (30%) of colorless prisms, mp 121.5–123°. An additional recrystallization from isopropyl alcohol yielded analytically pure material, also colorless prisms, mp 121.5–123°; nmr (TFA) τ 2.28 and 3.82 (2d, 2, J = 8 Hz, $C_{5,6}$–H), 6.02 (t, 2, J = 7 Hz, Ur–CH_2), 8.20 (m, 2, J = 7 Hz, CH_2CH_3), and 8.98 (t, 3, J = 7 Hz, CH_2CH_3); mass spectrum (70 eV) *m/e* (relative intensity) 153 (46, M^+), 139 (3, Ur–$C_2H_4^+$), 125 (15, Ur–CH_2^+), 112 (57, UrH^+), 96 (8, Ur–$C_2H_4^+$ − HNCO), 82 (100, Ur–CH_2^+ − HNCO), 69 (24, Ur–H^+ − HNCO), and 54 [49, Ur–CH_2^+ − HNCO and CO)].

Anal. Calcd for $C_7H_{10}N_2O_2$: C, 54.23; H, 6.54; N, 18.17. Found: C, 54.34; H, 6.42; N, 17.93.

9-Propyladenine (Ad–C_3).[14,91] A sample of authentic 9-propyladenine, prepared by Lambert,[14] was recrystallized from isopropyl alcohol, yielding colorless needles, mp 175.5–177° (lit. mp 178–179°,[14] 175°[91]); nmr (TFA) τ *ca.* 0.7 (v br s, 2, AdC_6–NH_2), 0.72 and 1.24 (2s, 2, $AdC_{2,8}$–H), 5.37 (t, 2, J = 7 Hz, Ad–CH_2), 7.86 (m, 2, J = 7 Hz, C–CH_2–C), and 8.87 (t, 2, J = 7 Hz, CH_2–CH_3); mass spectrum (70 eV) reported by Lambert.[14]

Anal. Calcd for $C_8H_{11}N_5$: C, 54.22; H, 6.26; N, 39.52. Found: C, 54.28; H, 6.22; N, 39.71.

6-Propylaminopurine.[92] A 0.343-g (1.44 mmol) portion of 6-chloro-9-(2-tetrahydropyranyl)purine[93] was dissolved in 40 g (0.68 mol) of *n*-propylamine. The solution was kept at ambient temperature for 4 days. Excess *n*-propylamine was removed *in vacuo*, leaving a colorless foam which was dissolved in 20 ml of methanol. A 2-ml portion of 3 *N* aqueous hydrochloric acid was added, and the resulting solution was heated at reflux for 1 hr. The solution was neutralized with aqueous sodium bicarbonate, and solvents were removed *in vacuo*. The residue was washed with two 5-ml portions of water to remove sodium chloride, leaving 0.112 g (46%) of a colorless solid, mp 226–236°. The crude product was recrystallized from absolute ethanol, yielding colorless prisms, mp 244–245.5° (lit.[92] mp 240–241°); nmr (TFA) τ 0.44 (br s, 1, AdC_6–NH), 0.94 and 1.22 (2s, 2, $AdC_{2,8}$–H), 6.15 (m, 2, NH–CH_2), 7.97 (m, 2, J = 7 Hz, C–CH_2–C), and 9.88 (t, 2, J = 7 Hz, CH_2CH_3).

2-Amino-6-chloro-5-nitro-4(3H)-pyrimidinone (30).[20,30] Most of a 31.4-g (0.216 mol) portion of 2-amino-6-chloro-4(3H)-pyrimidinone (**28**)[94] was dissolved in 6 l. of methanol at ambient temperature. The remainder dissolved upon addition of 72 ml (1.1 mol) of concentrated nitric acid. The solution was concentrated to 1 l. *in vacuo*, cooled to −20°, and filtered, yielding 40.3 g (90%) of the nitrate salt of 2-amino-6-chloro-4(3H)-pyrimidinone (**29**) as a colorless, amorphous solid, mp 141–142° violent dec. The salt was added to 150 ml of vigorously stirred concentrated sulfuric acid kept at 0° with an ice bath, and the resulting suspension was stirred at 0° for 10 min. The ice bath was removed, and the mixture was stirred at ambient temperature for 2 hr. The homogeneous, pale yellow solution was poured over ice, and the resulting suspension was filtered while cold. The pale yellow precipitate was successively washed with cold water and anhydrous acetone and treated with 3.5 l. of anhydrous methanol at reflux. The suspension was filtered while hot, and the filtrate was cooled to −20°, yielding 25.4 g (69%) of yellow plates, mp 278° violent dec (lit.[30b] mp 275–276°).

2-Amino-5-nitro-6-propylamino-4(3H)-pyrimidinone (31a). A 2.26-g (0.014 mol) portion of 2-amino-6-chloro-5-nitro-4(3H)-pyrimidinone (**30**) was added, with stirring and external cooling, to 50 ml of *n*-propylamine. The homogeneous reaction mixture was kept at ambient temperature for 5 hr, and excess *n*-propylamine was removed *in vacuo*, leaving a pale yellow solid. The solid was stirred overnight with 10 ml of water and filtered, in order to remove *n*-propylamine hydrochloride. The residual colorless solid, mp 325° dec, weighed 2.31 g (84%). Quantitative electronic absorption data for this compound and other intermediates are given in Table VI.

Anal. Calcd for $C_7H_{11}N_5O_3$: C, 39.44; H, 5.20; N, 32.85. Found: C, 39.59; H, 5.25; N, 32.61.

9-Propylguanine (32a, Gu–C_3). A. Ring Closure in Formamide. To a 0.514-g (2.42 mmol) portion of 2-amino-5-nitro-6-propylamino-4(3H)-pyrimidinone (**31a**) suspended in 450 ml of 95% ethanol was added 3 g of Raney nickel. The resulting mixture was hydrogenated at ambient temperature with shaking at 45 psi for 21 hr, and filtered under nitrogen and the filtrate evaporated to dryness, leaving a white solid. The solid was immediately dissolved in 20 ml of deoxygenated 98–100% formic acid. The formic acid solution was allowed to stand at ambient temperature for 2 hr, and excess formic acid was removed *in vacuo*, leaving a white solid which was immediately dissolved in 10 ml of deoxygenated formamide. The formamide solution was heated at 180° for 1.5 hr under nitrogen. The resulting brown solution was diluted with 115 ml of absolute ethanol, treated with activated charcoal, and cooled to −20°, yielding 0.22 g (48%) of colorless prisms, mp >345°; nmr (TFA) τ 1.10 (s, 1, GuC_8–H), 5.60 (t, 2, J = 7 Hz, Gu–CH_2), 7.99

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Table VI. Quantitative Electronic Absorption Data of Intermediates in Water[a]

Compound	Neutral				0.1 N HCl				0.1 N NaOH			
	λ_{max}	ϵ	λ_{min}	ϵ	λ_{max}	ϵ	λ_{min}	ϵ	λ_{max}	ϵ	λ_{min}	ϵ
Th-C_3-Br	272.5	9,730	236.5	1,630	272.5	9,710	236.5	1,580	270.0	7,370	244.0	3,100
Th-C_6-Br	273.0	9,960	237.0	1,670	272.5	9,930	236.5	1,620	270.5	7,550	244.0	3,160
Th-C_3-$NH_3^+HCO_2^-$	270.0	9,460	235.0	1,770	270.0	9,490	235.0	1,820	269.5	7,270	240.0	3,260
Ur-C_3-Br	266.0	10,200	231.5	1,490	266.0	10,200	231.5	1,460	264.0	7,290	240.5	3,800
N^4Ac-Cy-C_3-Br	215.0	19,600	227.0	6,640	214.0	12,400	262.0	1,300				
	243.5	14,400	268.0	2,590	228.0 (sh)	8,770						
	298.0	8,450			311.5	15,500						
N^4Ac-Cy-C_6-Br	215.0	19,600	226.5	6,680	214.0	12,400	262.5	1,260				
	243.5	14,400	267.0	2,560	230.0 (sh)	8,790						
	297.5	8,480			311.5	15,800						
N^4Ac-Cy-C_3-N_3	214.5	19,500	227.5	6,620	214.0	13,100	262.5	1,650				
	244.5	14,400	267.5	2,840	230.0 (sh)	8,600						
	297.5	8,270			310.5	14,900						
Gu-C_3-$NH_3^+HCO_2^-$	252.0	13,000	225.0	3,210	252.5	11,600	226.0	2,610	257.0 (sh)	10,400	231.5	4,430
	270.0 (sh)	9,610			277.5	7,710	269.5	7,410	267.5	11,000		
ANOP-C_3	215.5	22,500	257.5	2,020	215.5	22,800	257.5	2,050	230.0 (sh)	23,900	278.0	3,260
	230.0 (sh)	16,600			235.0 (sh)	15,000			260.0 (sh)	3,750		
	285.0 (sh)	4,980			285.0 (sh)	5,010			346.0	16,000		
	335.0	13,800			335.0	13,700						
ANOP-C_3-NH_2	230.0 (sh)	14,100	257.5	2,820	215.0	23,400	257.5	2,230	230.0 (sh)	10,300	227.5	3,380
	280.0 (sh)	4,390			230.0 (sh)	16,300			260.0 (sh)	3,800		
	337.0	14,800			285.0 (sh)	4,920			346.0	16,600		
					334.0	14,700						
ANOP-C_3-Ad	238.0 (sh)	16,800	253.0	11,300	238.0 (sh)	17,200	252.0	12,500	235.0 (sh)	12,100	244.0	11,000
	264.0	12,900	291.0	4,850	263.0	13,600	295.0	5,060	262.0	14,800	289.0	3,460
	337.0	12,600			336.0	12,900			347.0	15,000		
ANOP-C_3-Th	235.0 (sh)	16,800	253.0	6,780	235.0 (sh)	16,600	253.0	6,800	269.0	10,100	249.0	7,600
	276.0	11,900	302.5	6,180	276.0	11,900	302.5	6,280	347.0	15,700	296.0	4,680
	335.0	13,100			335.0	13,000						
ANP-C_3-Cy	230.0 (sh)	20,900	253.0	4,880	217.5	20,700	227.5	20,400	230.0 (sh)	21,000	253.0	4,850
	275.0	8,320	298.5	2,930	239.5	23,800	259.0	7,440	275.0	8,350	299.0	2,950
	345.0	8,360			284.0	16,000	311.0	4,020	345.0	8,360		
					341.0	6,680						
ANP-C_3-Th	230.0 (sh)	17,700	250.0	4,700	215.0	20,800	224.0	20,100	230.0 (sh)	21,600	252.0	4,810
	274.0	9,110	301.0	3,450	241.0	25,600	260.0	10,600	271.0	7,340	295.0	2,720
	344.0	8,930			273.0	11,500	307.5	3,780	344.0	8,760		
					341.0	6,800						

[a] Abbreviations: λ, wavelength in mμ; ϵ, molar extinction coefficient; sh, shoulder; C_n-Br = ω-bromoalkyl; C_3-NH_2, ω-aminopropyl; C_3-N_3, ω-azidopropyl; N^4Ac-Cy, N^4-acetylcytos-1-yl; ANOP, 2-amino-5-nitro-4(3H)-oxopyrimid-6-ylamino; ANP, 4-amino-5-nitropyrimid-6-ylamino.

m, 2, J = 7 Hz, C-CH_2-C), and 8.87 (t, 3, J = 7 Hz, CH_2CH_3); mass spectrum (70 eV) *m/e* (relative intensity) 193 (100, M^+), 165 (20, Gu-CH_3^+), 164 (37, Gu-CH_2^+), 151 (50, Gu-H^+), 134 (18, Gu-H^+ − NH_3), and 109 (46, Gu-H^+ − H_2NCN).

Anal. Calcd for $C_8H_{11}N_5O_3$: C, 49.73; H, 5.74; N, 36.25. Found: C, 49.93; H, 6.02; N, 36.14.

B. Ring Closure with Potassium Carbonate–Dimethylformamide. To a solution of 0.311 g (1.41 mmol) of 2-amino-5-nitro-6-propylamino-4(3H)-pyrimidinone (**31a**) in 200 ml of 98–100% formic acid was added 7.0 g (0.11 g-atom) of zinc dust. The resulting suspension was stirred at ambient temperature for 15 min and filtered under nitrogen, and the filtrate was evaporated to dryness *in vacuo*, leaving a white solid which was immediately dissolved in 50 ml of dry, deoxygenated dimethylformamide. A 0.66-g (4.8 mmol) portion of potassium carbonate was added, and the resulting suspension was stirred at reflux under nitrogen for 17 hr. The yellow-brown suspension was filtered, and solvent was removed *in vacuo*, leaving a red solid. The solid was recrystallized from ethanol–water, yielding 0.15 g (55%) of pale yellow prisms, mp >345°, identical with the product described in section A as judged by ir (Nujol) and nmr (TFA) spectra.

2-Amino-6-(3-aminopropylamino)-5-nitro-4(3H)-pyrimidinone (33). A 1.28-g (6.73 mmol) portion of 2-amino-6-chloro-5-nitro-4(3H)-pyrimidinone was dissolved in 25 ml of 1,3-diaminopropane. The yellow solution was stirred overnight at ambient temperature, and excess 1,3-diaminopropane was removed *in vacuo*. The residue was recrystallized from ethanol–water (1:9), yielding 0.99 g (65%) of yellow needles, mp 268–270° dec; nmr (TFA) τ 0.2 (br s, 1, N-H), 2.9 (v br s, 2, CH_2NH_2), 6.0 (br s, 2, Py-CH_2), 6.5 (br s, 2, CH_2NH_2), and 7.6 (br s, 2, C-CH_2-C).

Anal. Calcd for $C_7H_{12}N_6O_3$: C, 36.84; H, 5.30; N, 36.83. Found: C, 36.55; H, 5.23; N, 35.66, 38.58 (av 37.12).

9-(3-Aminopropyl)guanine, Formate Salt (34). A. Reduction with PtO_2–H_2. To a solution of 0.399 g (1.75 mmol) of 2-amino-6-(3-aminopropylamino)-5-nitro-4(3H)-pyrimidinone (**33**) in 100 ml of 98–100% formic acid–methanol (1:3, v:v) was added 0.5 g of Adams catalyst. The resulting suspension was hydrogenated with shaking at 55 psi and ambient temperature for 4.0 hr. Catalyst was removed by filtration under nitrogen, and solvents were removed from the filtrate *in vacuo*, leaving a yellow oil which was dissolved immediately in 3.5 ml of deoxygenated formamide. The formamide solution was heated at 180° under nitrogen for 2.5 hr. A 10-ml portion of water was added, and the solution was cooled to 0°, yielding 0.169 g (38%) of an amorphous, off-white solid, mp *ca.* 231° dec. Further cooling of the supernatant yielded 49 mg (11%) of colorless platelets, mp 233–237° dec. The two crops were identical as judged by ir (Nujol) and mixture melting point; nmr (TFA) τ 0.95 (s, 1, GuC$_8$-H), 1.65 (s, 1, HCO_2H), 2.0 (br s, 2, CH_2NH_2), 5.52 (t, 2, J = 7 Hz, Gu-CH_2), 6.35 (br s, 2, CH_2-NH_2), and 7.60 (m, 2, J = 6 Hz, C-CH_2-C).

Anal. Calcd for $C_9H_{14}N_6O_3$: C, 42.52; H, 5.58; N, 33.06. Found: C, 42.82; H, 5.57; N, 33.04.

B. Reduction with Zinc Dust–Formic Acid. To a solution of 0.400 g (1.75 mmol) of 2-amino-6-(3-aminopropylamino)-5-nitro-4(3H)-pyrimidinone (**33**) in 100 ml of 98–100% formic acid was added 7.0 g (0.11 g-atom) of zinc dust. The resulting suspension was stirred at ambient temperature for 15 min and filtered under nitrogen, and excess formic acid was removed *in vacuo*, leaving a pale yellow oil which was dissolved in 3.5 ml of deoxygenated formamide. The formamide solution was heated under nitrogen

at 180–190° for 2.5 hr. An 11-ml portion of water was added, and the solution was cooled to 0°, yielding 0.25 g (56%) of a colorless, amorphous solid, mp 235–238° dec. The product was identical with that from section A on the basis of ir (Nujol) and mixture melting point.

1-(3-Bromopropyl)thymine (4c).[20] A 53-g (0.20 mol) portion of bis(trimethylsilyl)thymine (**3b**) was dissolved in 600 g (3.0 mol) of 1,3-dibromopropane under dry nitrogen. The solution was kept at ambient temperature for 10 days and poured into 1 l. of water and the water layer was extracted with four 800-ml portions of chloroform. The chloroform extracts were dried (Na_2SO_4) and concentrated *in vacuo*, leaving a pale yellow solid. The solid was recrystallized from absolute ethanol to yield 39.5 g (82%) of colorless platelets, mp 136–138°; nmr (TFA) τ 2.41 (s, 1, ThC_6–H), 5.83 (t, 2, J = 7 Hz, Th–CH_2), 6.50 (t, 2, J = 7 Hz, CH_2–Br), 7.54 (m, 2, J = 7 Hz, C–CH_2–C), and 7.94 (s, 3, ThC_5–CH_3).

Anal. Calcd for $C_8H_{11}BrN_2O_2$: C, 38.88; H, 4.49; N, 11.38. Found: C, 38.91; H, 4.66; N, 11.12.

1-(3-Aminopropyl)thymine, Formate Salt (18). To a solution of 2.14 g (15.2 mmol) of hexamethylenetetramine in 20 ml of chloroform heated to reflux was added a hot solution of 3.45 g (13.9 mmol) of 1-(3-bromopropyl)thymine (**4c**) in 20 ml of chloroform. The reaction mixture was stirred at reflux for 22 hr, cooled to 0°, and filtered to yield 4.81 g (90%) of the 1:1 adduct **17** as a colorless powder, mp 199–205° dec. A 12.20-g portion of the adduct was suspended with stirring in 85 ml of 95% ethanol–concentrated hydrochloric acid (8:1, v:v). The suspension was stirred for 2 days at ambient temperature and filtered, yielding 10.6 g of a colorless solid. The solid was dissolved in distilled water, and the solution was applied to a column containing 125 g (dry weight) of Dowex 1-X8, formate form. Elution with distilled water gave a deliquescent solid from which ammonium formate was removed by sublimation at 60° (0.1 mm), leaving 5.48 g (76% based on the 1:1 adduct) of a colorless solid, mp 157.5–160°. Recrystallization from absolute ethanol yielded analytically pure material as colorless prisms, mp 162–163°; nmr (TFA) τ 1.63 (s, 1, HCO_2H), 2.37 (s, 1, ThC_6–H), 2.9 (v, br s, 2, CH_2NH_2), 5.78 (t, 2, J = 7 Hz, Th–CH_2), 6.50 (m, 2, J = 7 Hz, CH_2NH_2), 7.58 (m, 2, J = 7 Hz, C–CH_2–C), and 7.88 (s, 3, ThC_5–CH_3).

Anal. Calcd for $C_9H_{15}N_3O_4$: C, 47.16; H, 6.60; N, 18.33. Found: C, 46.91; H, 6.71; N, 18.20.

1-(6-Bromohexyl)thymine (4d). A 45.8-g (0.170 mol) portion of bis(trimethylsilyl)thymine (**3b**) was dissolved in 480 g (1.97 mol) of dry 1,6-dibromohexane under nitrogen. The solution was kept at ambient temperature for 34 days and poured into 600 ml of water, and the water layer was extracted with five 500-ml portions of chloroform. The chloroform extracts were dried (Na_2SO_4) and concentrated *in vacuo*, leaving a pale yellow solution of the product in excess 1,6-dibromohexane. The solution was poured into 1.5 l. of *n*-pentane, resulting in precipitation of a colorless solid which was recrystallized from absolute ethanol to yield 43.3 g (88%) of colorless microcrystals, mp 111–113°; nmr ($CDCl_3$) τ 2.87 (s, 1, ThC_6–H), 6.22 (t, 2, J = 7 Hz, Th–CH_2), 6.53 (t, 2, J = 7 Hz, CH_2–Br), 8.05 (s, 3, ThC_5–CH_3), and 7.8–8.8 (v br m, 8, C–$(CH_2)_4$–C).

Anal. Calcd for $C_{11}H_{17}BrN_2O_2$: C, 45.68; H, 5.93; N, 9.69. Found: C, 45.80; H, 6.08; N, 9.59.

1-(3-Bromopropyl)uracil (4a). To a stirred suspension of 70 g (0.62 mol) of uracil (**7a**) and 140 g (1.29 mol) of trimethylsilyl chloride in 600 ml of dry dioxane was added, dropwise, a solution of 131 g (1.30 mol) of triethylamine in 200 ml of dry dioxane. The resulting suspension was stirred under nitrogen for 24 hr at ambient temperature and filtered under nitrogen. Removal of solvent from the clear, red filtrate left crude bis(trimethylsilyl)uracil[90] as a red oil which was dissolved, without further purification, in 1.6 kg (8.0 mol) of 1,3-dibromopropane. The solution was kept at ambient temperature for 7 days and was then poured into 2 l. of water. The resulting suspension was extracted with a total of 8 l. of chloroform. The chloroform extracts were dried (Na_2SO_4) and concentrated *in vacuo*, leaving a red solution of the product in excess 1,3-dibromopropane, which was applied directly to a column containing 1 kg of silica gel packed in chloroform. The desired product was eluted with methanol–chloroform (2.5:97.5, v:v) as a pale yellow solid, which was recrystallized from absolute ethanol to yield 33.51 g (23% based on uracil) of colorless prisms, mp 87–96°. Recrystallization from isopropyl alcohol yielded analytically pure material (30.2 g), colorless prisms, mp 87–96°; nmr (TFA) τ 2.28 and 3.82 (2d, 2, J = 8 Hz, $UrC_{5,6}$–H), 5.83 (t, 2, J = 7 Hz, Ur–CH_2), 6.49 (t, 2, J = 6 Hz, CH_2–Br), and 7.62 (m, 2, J = 6 Hz, C–CH_2–C).

Anal. Calcd for $C_7H_9BrN_2O_2$: C, 36.07; H, 3.89; N, 12.02. Found: C, 36.36; H, 4.00; N, 11.77.

N^4-Acetyl-1-(3-bromopropyl)cytosine (6a).[20] A 33.7-g (0.124 mol) portion of N^4-acetylbis(trimethylsilyl)cytosine (**5**)[90] was dissolved, under nitrogen, in 600 g (3.0 mol) of 1,3-dibromopropane. The solution was kept at ambient temperature for 10–20 days and poured into 500 ml of water, and the aqueous layer was extracted with six 500-ml portions of chloroform. The chloroform extracts were dried (Na_2SO_4) and concentrated *in vacuo*, leaving a dark red solution of the product in excess 1,3-dibromopropane, which was applied directly to a column containing 700 g of silica gel packed in chloroform. The desired product was eluted with methanol–chloroform (2.5:97.5, v:v) as 12.0 g (43%) of pale yellow needles, mp 139–142°; nmr (TFA) τ 1.51 and 3.34 (2d, 2, J = 7 Hz, $CyC_{5,6}$–H), 5.60 (t, 2, J = 7 Hz, Cy–CH_2), 6.50 (t, 2, J = 6 Hz, CH_2–Br), and 7.51 (s superimposed on m, 5, $COCH_3$ and C–CH_2–C).

Anal. Calcd for $C_9H_{12}BrN_3O_2$: C, 39.43; H, 4.41; N, 15.33. Found: C, 39.68; H, 4.48; N, 15.46.

N^4-Acetyl-1-(6-bromohexyl)cytosine (6b). A 39.7-g (0.133 mol) portion of N^4-acetylbis(trimethylsilyl)cytosine (**5**) was dissolved under nitrogen in 490 g (2.0 mol) of dry 1,6-dibromohexane. The solution was kept at ambient temperature for 16 days and poured into 1 l. of water. The resulting suspension was extracted with six 1-l. portions of chloroform. Filtration of the remaining aqueous suspension yielded 9.2 g (45%) of N^4-acetylcytosine (**9**). The chloroform extracts were dried (Na_2SO_4) and concentrated *in vacuo*, leaving a deep red solution in excess 1,6-dibromohexane, which was applied directly to a column containing 1 kg of silica gel packed in chloroform. The desired product was eluted with methanol–chloroform (2.5:97.5, v:v) as *ca.* 10 g of a yellow solid. Recrystallization of the solid from benzene–cyclohexane yielded 7.6 g (18%) of white needles, mp 131–133°; nmr (TFA) τ 1.59 and 3.36 (2d, 2, J = 7 Hz, $CyC_{5,6}$–H), 5.80 (t, 2, J = 7 Hz, Cy–CH_2), 6.54 (t, 2, J = 7 Hz, CH_2–Br), 7.48 (s, 3, $COOCH_3$), and 7.8–8.9 (br m, 8, C–$(CH_2)_4$–C).

Anal. Calcd for $C_{12}H_{18}BrN_3O_2$: C, 45.58; H, 5.74; N, 13.29. Found: C, 45.81; H, 5.69; N, 13.06.

N^4-Acetyl-1-(3-azidopropyl)cytosine (19). To a solution of 3.00 g (11.0 mmol) of N^4-acetyl-1-(3-bromopropyl)cytosine (**6a**) in 400 ml of acetonitrile was added 0.747 g (11.5 mmol) of sodium azide. The resulting suspension was heated at reflux with stirring for 22 hr, filtered to remove precipitated sodium bromide, and evaporated *in vacuo*, leaving a yellow solid. The solid was dissolved in methanol–ethyl acetate, and the solution was applied to a column containing 300 g of silica gel packed in ethyl acetate. The desired product was eluted with methanol–ethyl acetate (1:19, v:v) as a pale yellow solid which was recrystallized from absolute ethanol to yield 1.45 g (56%) of pale yellow prisms, mp 103–105° (after drying *in vacuo*, mp 133–134°); ir ($CHCl_3$) 2100 cm^{-1} (N_3); nmr (TFA) τ 1.47 and 3.13 (2d, 2, J = 8 Hz, $CyC_{5,6}$–H), 5.67 (t, 2, J = 7 Hz, Cy–CH_2), 6.40 (t, 2, J = 7 Hz, CH_2–N_3), 7.45 (s, 3, $COCH_3$), and 7.75 (m, 2, J = 7 Hz, C–CH_2–C).

Anal. Calcd for $C_9H_{12}N_6O_2$: C, 45.76; H, 5.13; N, 35.58. Found: C, 46.00; H, 5.12; N, 34.88.

1-(3-Aminopropyl)cytosine (21). To a 1.03-g (4.35 mmol) portion of N^4-acetyl-1-(3-azidopropyl)cytosine (**19**) dissolved in 50 ml of methanol was added 15 ml of methanol presaturated with ammonia at ambient temperature. The solution was allowed to stand at ambient temperature for 4 hr, at which time removal of the acetyl group was complete as judged by uv analysis. Solvent was removed *in vacuo*, leaving 0.84 g (99%) of a pale yellow solid (**20**), mp 165–171°; nmr (TFA) τ 1.89 (br s, 2, CyC_4–NH_2), 2.07 and 3.56 (2d, 2, J = 8 Hz, $CyC_{5,6}$–H), 5.83 (t, 2, J = 7 Hz, Cy–CH_2), 6.43 (t, 2, J = 7 Hz, CH_2N_3), and 7.85 (m, 2, J = 7 Hz, C–CH_2–C). The crude 1-(3-azidopropyl)cytosine (**20**) was dissolved in 200 ml of 95% ethanol. A 5-g portion of Raney nickel was added, and the mixture was hydrogenated with shaking at 45 psi for 70 min. Catalyst was removed by filtration and solvent by evaporation *in vacuo*, leaving 0.70 g (94%) of a pale yellow gum. The gum, which was difficult to recrystallize without causing decomposition, was of satisfactory purity for use in subsequent reactions. Analytically pure material was obtained by chromatography of a portion of the crude product on silica gel. Elution with methanol afforded colorless prisms, mp 138–140°; nmr (TFA) τ 1.95 (br s, 2, CyC_4–NH_2), 2.08 and 3.57 (2d, 2, J = 8 Hz, $CyC_{5,6}$–H), 2.9 (v br s, 2, CH_2–NH_2), 5.77 (t, 2, J = 7 Hz, Cy–CH_2), 6.6 (m, 2, CH_2NH_2), and 7.55 (m, 2, J = 7 Hz, C–CH_2–C); qualitative uv_{max} (H_2O) 274 (pH 7, 13), 283 mμ (pH 1).

Anal. Calcd for $C_7H_{12}N_4O$: C, 49.99; H, 7.19; N, 33.31. Found: C, 49.81; H, 7.20; N, 32.73.

9,9′-Trimethylenebisadenine (2b, Ad-C_3-Ad). A suspension of 10.1 g (74.5 mmol) of adenine and 3.48 g (78.0 mmol) of sodium hydride (54% dispersion in mineral oil) in 300 ml of dry dimethylformamide was stirred at ambient temperature under nitrogen for 1 hr. To the resulting suspension of sodium adenide (**1**) was added 7.88 g (39.0 mmol) of 1,3-dibromopropane. The suspension was stirred for 2 days and filtered, yielding 7.50 g (65%) of a colorless, amorphous solid, mp *ca.* 315° dec. A portion of the solid was successively washed with water, acetone, and chloroform and recrystallized from glacial acetic acid–absolute ethanol (1:1, v:v) to yield analytically pure material as a colorless amorphous solid, mp *ca.* 330° dec; nmr (TFA) τ 0.72 and 1.25 (2s, 4, $AdC_{2,8}$-H), *ca.* 0.7 (v br s, 4, AdC_6-NH_2), 5.20 (t, 4, J = 7 Hz, Ad-CH_2), and 7.06 (m, 2, J = 7 Hz, C-CH_2-C); mass spectrum (70 eV) *m/e* (relative intensity) 310 (3, M^+), 175 (41, Ad-$C_3H_5^+$), 162 (100, Ad-$C_2H_4^+$), 149 (44, Ad-CH_3^+), 148 (62, Ad-CH_2^+), 135 (23, Ad-H^+), and 108 (21, Ad-H^+ − HCN).

Anal. Calcd for $C_{13}H_{14}N_{10}$: C, 50.31; H, 4.55; N, 45.14. Found: C, 50.09; H, 4.46; N, 45.32.

9,9′-Dimethylenebisadenine (2a, Ad-C_2-Ad).[44] A 7.29-g (38.8 mmol) portion of 1,2-dibromoethane was added to a mechanically stirred suspension of 74.0 mmol of sodium adenide (**1**) in 300 ml of dry dimethylformamide. The resulting suspension was stirred at ambient temperature for 18 hr and filtered, leaving a colorless solid which was stirred with 200 ml of water for 1 hr. The resulting suspension was filtered, leaving 4.55 g (41%) of an amorphous solid, mp >335°. A 1.06-g portion of the solid was dissolved in a minimum amount of 98–100% formic acid, and the formic acid solution was applied to a column containing 200 g of silica gel packed in chloroform–acetic acid–methanol (85:10:5, v:v). Elution with chloroform–acetic acid–methanol (85:10:5, v:v) yielded the desired product. A middle fraction, weighing 280 mg, was recrystallized from glacial acetic acid–methanol (1:1, v:v) to yield 240 mg of an amorphous, colorless solid, mp >335° (lit.[44] mp >350°); nmr (TFA) τ 1.02 and 1.38 (2s, 4, $AdC_{2,8}$-H), *ca.* 1.0 (v, br s, 4, AdC_6-NH_2), and 4.70 (s, 4, Ad-CH_2); mass spectrum (70 eV) *m/e* (relative intensity) 296 (11, M^+), 162 (100, Ad-$C_2H_4^+$), 161 (46, Ad-$C_2H_3^+$), 148 (16, Ad-CH_2^+), 136 (43, Ad-H_2^+), 135 (57, Ad-H^+), 134 (48, Ad$^+$), 108 (40, Ad-H^+ − HCN), and 94 (13, Ad-CH_2^+ − 2HCN).

Anal. Calcd for $C_{12}H_{12}N_{10}$: C, 48.64; H, 4.08; N, 47.28. Found: C, 48.74; H, 4.28; N, 46.73.

9,9′-Hexamethylenebisadenine (2c, Ad-C_6-Ad).[12,44] A 9.47-g (38.8 mmol) portion of 1,6-dibromohexane was added to a stirred suspension of 74.0 mmol of sodium adenide (**1**) in 300 ml of dry dimethylformamide. The mixture was stirred at ambient temperature for 18 hr and filtered. The insoluble solid was washed thoroughly with water, acetone, and chloroform, leaving 8.2 g (62%) of an amorphous, off-white solid, mp *ca.* 260° dec. A 3.1-g portion of the crude product was recrystallized from glacial acetic acid–methanol (1:1, v:v) to yield 2.1 g of a colorless, amorphous solid, mp 263–269°. A 1.17-g portion of the recrystallized product was dissolved in 98–100% formic acid, and the formic acid solution was applied to a column containing 200 g of silica gel packed in chloroform–acetic acid–methanol (92.5:5:2.5, v:v). The desired product was eluted with chloroform–acetic acid–methanol (85:10:5, v:v). A middle fraction, 0.44 g, was recrystallized from glacial acetic acid–methanol (1:1, v:v), yielding 0.40 g of a colorless, amorphous solid, mp 270–271° (lit.[44] mp 254–255°); nmr (TFA) τ *ca.* 0.5 (v br s, 4, AdC_6-NH_2), 0.68 and 1.23 (2s, 4, $AdC_{2,8}$-H), 5.30 (t, 4, J = 6 Hz, Ad-CH_2), 7.85 (br s, 4, Ad-CH_2-CH_2), and 8.32 (br s, 4, Ad-CH_2-CH_2-CH_2); mass spectrum (70 eV) *m/e* (relative intensity) 352 (46, M^+), 217 (20, Ad-$C_5H_{11}^+$), 204 (83, Ad-$C_5H_{10}^+$), 190 (40, Ad-$C_4H_8^+$), 176 (26, Ad-$C_3H_6^+$), 163 (26, Ad-$C_2H_5^+$), 162 (27, Ad-$C_2H_4^+$), 149 (77, Ad-CH_3^+), 148 (61, Ad-CH_2^+), 136 (47, Ad-H_2^+), 135 (100, Ad-H^+), and 108 (41, Ad-H^+ − HCN).

Anal. Calcd for $C_{16}H_{20}N_{10}$: C, 54.53; H, 5.71; N, 39.75. Found: C, 54.60; H, 5.70; N, 39.95.

N^6,$N^{6'}$-Trimethylenebis[2,6-diamino-5-nitro-4(3H)pyrimidinone] (35). To a solution of 2.00 g (1.05 mmol) of 2-amino-6-chloro-5-nitro-4(3H)-pyrimidinone (**30**) and 1.06 g (1.05 mmol) of triethylamine in 1.5 l. of methanol was added 0.35 g (0.475 mmol) of 1,3-diaminopropane. The initially homogeneous solution was stirred for 23 hr and filtered to yield 1.94 g (102% based on monohydrate, hygroscopic), of a yellow, amorphous solid, mp >325° (discolors over wide range beginning *ca.* 245°); qualitative uv_{max} (98–100% HCO_2H-C_2H_5OH; 1:5, v:v) 327 mμ and *ca.* 285 mμ (sh).

Anal. Calcd for $C_{11}H_{14}N_{10}O_{16}\cdot H_2O$: C, 33.00; H, 4.03; N, 34.99. Found: C, 33.10; H, 3.92; N, 34.69.

9,9-Trimethylenebisguanine (36, Gu-C_3-Gu). To a solution of 2.50 g (6.54 mmol) of N^6,$N^{6'}$-trimethylenebis[2,6-diamino-5-nitro-4(3H)-pyrimidinone] (**35**) dissolved in 600 ml of 98–100% formic acid was added 45 g (0.69 g-atom) of zinc dust. The resulting suspension was stirred at ambient temperature for 15 min and filtered under nitrogen, and the filtrate was reduced *in vacuo* to 50 ml. The resulting suspension was transferred under nitrogen to a flask containing 8 ml of deoxygenated formamide. Excess formic acid was removed *in vacuo*, leaving a yellow solution which was heated under nitrogen at 175–190° for 2.5 hr and poured into 35 ml of water. The cold solution deposited 0.498 g (22%) of crude 9,9′-trimethylenebisguanine (**36**) as a tan, amorphous solid, mp *ca.* 285° dec (darkens without melting starting at 215°).

The crude product could be purified substantially by digestion in concentrated aqueous ammonia, treatment with activated charcoal, and reprecipitation with glacial acetic acid. This sequence yielded about 35% recovery (8% over-all) of a pale tan solid, mp >345°. Microanalytical data on such material were close to the values expected for the desired product (**36**); nmr (TFA) τ 0.93 (s, 2, GuC_8-H), 5.33 (t, 4, Gu-CH_2), and 7.22 (br s, 2, C-CH_2-C); mass spectrum (70 eV) *m/e* (relative intensity) 342 (3, M^+), 191 (35, Gu-$C_3H_5^+$), 151 (100, Gu-H^+), 109 (27, Gu-H^+ − H_2NCN), and 81 [25, Gu-H^+ − (H_2NCN and CO)].

Material was prepared for microanalysis and spectral studies by dissolving the crude product (0.498 g) in a minimum of formic acid and applying the formic acid solution to a column containing 150 g of silica gel packed in chloroform–acetic acid–methanol (85:10:5, v:v). The desired product was eluted slowly with chloroform–acetic acid–methanol (77.5:15:7.5, v:v). Four middle fractions (1 l. of eluent), 35 mg of a pale yellow, amorphous solid, mp >345°, were dissolved in concentrated aqueous ammonia. The hot solution was treated with activated charcoal and filtered, and the filtrate was concentrated *in vacuo* until precipitation had begun. The cold suspension was filtered to yield 20 mg of a colorless, hygroscopic, amorphous solid, mp >345°.

Anal. Calcd for $C_{13}H_{14}N_{10}O_2$: C, 45.61; H, 4.12; N, 40.92. Found: C, 45.84; H, 4.23; N, 41.14.

6-[3-(Aden-9-yl)propylamino]-2-amino-5-nitro-4(3H)-pyrimidinone (31b). To a solution of 1.45 g (7.60 mmol) of 2-amino-6-chloro-5-nitro-4(3H)-pyrimidinone (**30**) and 0.77 g (7.60 mmol) of triethylamine in 1.5 l. of methanol was added 1.33 g (6.92 mmol) of 9-(3-aminopropyl)adenine. The initially homogeneous solution was kept at ambient temperature for 26 hr, at which time 1.58 g (67%) of an amorphous, colorless solid, mp >345°, had precipitated; nmr (TFA) τ 0.1 (br s, 1, N-H), 0.70 and 1.22 (2s, 2, $AdC_{2,8}$-H), *ca.* 1.0 (v br s, 2, AdC_6-NH_2), 5.2 (br s, 2, Ad-CH_2), 6.1 (br s, 2, Py-CH_2), and 7.4 (br s, 2, C-CH_2-C).

Anal. Calcd for $C_{12}H_{14}N_{10}O_3$: C, 41.61; H, 4.08; N, 40.45. Found: C, 41.71; H, 4.22; N, 40.69.

9-[3-(Aden-9-yl)propyl]guanine (32b, Ad-C_3-Gu). To a solution of 0.700 g (2.02 mmol) of 6-[3-(aden-9-yl)propylamino]-2-amino-5-nitro-4(3H)-pyrimidinone (**31b**) in 150 ml of 98–100% formic acid was added 14 g (0.21 g-atom) of zinc dust. The resulting suspension was stirred at ambient temperature for 15 min and filtered under nitrogen. The filtrate was evaporated *in vacuo*, leaving a pale yellow oil which was immediately suspended in 150 ml of dry, deoxygenated dimethylformamide. The suspension was reduced to 100 ml *in vacuo* to ensure complete removal of formic acid and water. A 0.840-g (6.06 mmol) portion of anhydrous potassium carbonate was added, and the resulting suspension was stirred at reflux under nitrogen for 23 hr. The red-brown suspension was evaporated to dryness *in vacuo*. The residue was taken up in 80 ml of 15% aqueous ammonia, the resulting solution was heated (steam bath) with activated charcoal and filtered, and the pH of the filtrate was adjusted to neutrality with acetic acid. The cold (4°) solution deposited 249 mg (38%) of a pale yellow, amorphous solid, mp *ca.* 335° dec. A 160-mg portion of the solid was dissolved in formic acid, and the formic acid solution was applied to a column containing 30 g of silica gel packed in chloroform–acetic acid–methanol (92.5:5:2.5, v:v). The desired product was eluted with chloroform–acetic acid–methanol as a white solid, which was dissolved in concentrated aqueous ammonia. The solution was reduced *in vacuo* to one-half the initial volume and cooled to 4°, yielding 80 mg (50% recovery) of an amorphous, hygroscopic, colorless solid, mp >345°; nmr (TFA) τ 0.73 and 1.22 (2s, 2, $AdC_{2,8}$-H), 0.88 (s, 1, GuC_8-H), *ca.* 1.0 (v br s, 2, AdC_6-NH_2), 5.17 and 5.30 (m, 4, Gu-CH_2 and Ad-CH_2), and 7.15 (br s, 2, C-CH_2-C); mass spectrum (70 eV) *m/e* (relative intensity) 326 (24, M^+), 191 (25, Gu-$C_3H_5^+$), 178 (96, Gu-$C_2H_4^+$), 176 (25, Ad-$C_3H_6^+$), 175 (48, Ad-$C_3H_5^+$), 165 (15, Gu-CH_3^+), 164 (17, Gu-CH_2^+), 162 (36,

Ad–$C_2H_4^+$), 149 (52, Ad–CH_3^+), 148 (100, Ad–CH_2^+), 135 (58, Ad–H^+), and 108 (36, Ad–H^+ − HCN).

Anal. Calcd for $C_{13}H_{14}N_{10}O$: C, 47.85; H, 4.33; N, 42.93. Found: C, 48.12; H, 4.63; N, 43.18.

2-Amino-6-[3-(cytos-1-yl)propylamino]-5-nitro-4(3H)-pyrimidinone (31c). To a solution of 1.58 g (8.28 mmol) of 2-amino-5-nitro-4(3H)-pyrimidinone (**30**) and 0.93 g (9.1 mmol) of triethylamine in 1 l. of methanol was added a solution in 250 ml of methanol of 1-(3-aminopropyl)cytosine (**21**), which had been prepared by deacetylation and reduction of 1.95 g (8.28 mmol) of N^4-acetyl-1-(3-azidopropyl)cytosine (**19**). The initially homogeneous solution was allowed to stand at ambient temperature for 2 days, at which time 1.86 g (70%) of a pale yellow, amorphous solid, mp >335°, had precipitated; nmr (1:1 TFA–DMSO-d_6) τ 0.1 (v br s, 1, N–H), 1.83 (br s, 2, CyC_4–NH_2), 2.17 and 3.73 (2d, 2, J = 7 Hz, $CyC_{5,6}$–H), 5.9 and 6.2 (2 br s, 4, Cy–CH_2 and Py–CH_2), and 7.8 (br s, 2, C–CH_2–C); uv_{max} (98–100% HCO_2H) 285 mμ (ε 19,000) and 322.5 mμ (ε 10,600); uv_{min} 310 mμ (ε 9900).

Anal. Calcd for $C_{11}H_{14}N_8O_4 \cdot H_2O$: C, 38.82; H, 4.74; N, 32.93. Found: C, 39.06; H, 4.52; N, 32.02.

9-[3-(Cytos-1-yl)propyl]guanine (32c, Gu–C_3–Cy). To a solution of 0.600 g (1.87 mmol) of 2-amino-6-[3-(cytos-1-yl)propylamino]-5-nitro-4-(3H)-pyrimidinone (**31c**) in 125 ml of 98–100% formic acid was added 12 g (0.18 g-atom) of zinc dust. The resulting suspension was stirred at ambient temperature for 15 min and filtered under nitrogen, and the filtrate was evaporated to dryness *in vacuo*. The residue was suspended in 90 ml of dry, deoxygenated dimethylformamide, and the suspension was concentrated to 60 ml *in vacuo* to ensure complete removal of formic acid and water. A 0.777-g (5.61 mmol) portion of anhydrous potassium carbonate was added, and the resulting suspension was stirred at reflux under nitrogen for 50 hr. The suspension was evaporated to dryness *in vacuo*, and the residue was dissolved in 40 ml of 15% aqueous ammonia, heated (steam bath) with activated charcoal, and filtered. The pH of the hot filtrate was adjusted to neutrality with glacial acetic acid. The filtrate, on standing at ambient temperature, deposited 328 mg (58%) of a pale yellow, amorphous solid, mp >335°. A 221-mg portion of the solid was dissolved in 1.5 ml of formic acid, and the formic acid solution was applied to a column containing 30 g of silica gel packed in chloroform–acetic acid–methanol (92.5:5:2.5, v:v). The desired product was eluted with chloroform–acetic acid–methanol (55:30:15, v:v) as 210 mg of a colorless, amorphous solid, mp >335°. The solid was dissolved in concentrated aqueous ammonia, and the resulting solution was reduced *in vacuo* until precipitation had begun. The suspension was stored overnight at 4° and filtered. Further cooling of the supernatant at 4° yielded 30 mg of analytically pure material as a colorless, hygroscopic amorphous solid, mp >335°; nmr (TFA) τ 0.91 (s, 1, GuC_8–H), 1.72 (br s, 2, CyC_4–NH_2), 2.05 and 3.55 (2d, 2, J = 8 Hz, $CyC_{5,6}$–H), 5.40 (t, 2, J = 7 Hz, Gu–CH_2), 5.77 (t, 2, J = 7 Hz, Cy–CH_2), and 7.38 (br m, 2, C–CH_2–C); mass spectrum (70 eV) *m/e* (relative intensity) 191 (34, Gu–$C_3H_5^+$), 151 (97, Cy–$C_3H_5^+$ and/or Gu–H^+), 150 (77, Gu^+ and/or Gu–$C_3H_6^+$ − H_2NCN), 135 (36, Gu–H^+ − NH_2), 122 (50, Gu^+ − CO), 111 (100, Cy–H^+), 95 (32, Cy–H^+ − NH_2), 81 [31, Cy–CH_2^+ − (NH_2 and HCN)], 69 (52, Cy–H^+ − NCO), and 55 [53, Cy–CH_2 − (NCO and HCN)].

Anal. Calcd for $C_{12}H_{14}N_8O_2$: C, 47.67; H, 4.67; N, 37.07. Found: C, 47.49; H, 4.75; N, 36.95.

2-Amino-5-nitro-6-[1-(3-thym-1-yl)propyl]-4(3H)-pyrimidinone (31d). To a solution of 0.75 g (3.92 mmol) of 2-amino-6-chloro-5-nitro-4(3H)-pyrimidinone (**30**) and 0.40 g (4.72 mmol) of triethylamine in 750 ml of methanol was added a solution of 0.90 g (3.92 mmol) of the formate salt of 1-(3-aminopropyl)thymine (**18**) in 50 ml of methanol. The yellow solution was allowed to stand at room temperature for 2 days, concentrated *in vacuo* to 300 ml, and cooled to −20°, yielding 0.85 g (65%) of a colorless, amorphous solid, mp 184–187°; nmr (TFA) τ 0.9 (br s, 1, N–H), 2.45 (s, 1, ThC_6–H), 5.6–6.4 (br s, 4, Th–CH_2 and Py–CH_2), 7.7 (br s, 2, C–CH_2–C), and 7.90 (s, 3, ThC_5–CH_3).

Anal. Calcd for $C_{12}H_{15}N_7O_5 \cdot 0.5H_2O$: C, 41.61; H, 4.66; N, 28.32. Found: C, 41.60; H, 4.77; N, 28.35.

9-[3-(Thym-1-yl)propyl]guanine (32d, Gu–C_3–Th). A 1.30-g (3.86 mmol) portion of 2-amino-5-nitro-6-[3-(thym-1-yl)propylamino]-4(3H)-pyrimidinone (**31d**) was partially dissolved in 350 ml of hot methanol. An 8-g portion of Raney nickel was added, and the warm suspension was hydrogenated with shaking at 50 psi for 1.5 hr without further heating. Catalyst was removed by filtration under nitrogen, and solvent was removed *in vacuo*, leaving a yellow oil which was dissolved in 30 ml of 98–100% formic acid. The solution was allowed to stand at ambient temperature for 25 min. A 5-ml portion of deoxygenated formamide was then added, and excess formic acid was removed *in vacuo*. The formamide solution was heated under nitrogen for 3 hr at 183–185° and poured into 20 ml of water and the resulting solution cooled to 4°, yielding 214 mg (18%) of an amorphous yellow solid, mp >335°. A 194-mg portion of the solid was dissolved in 98–100% formic acid, and the formic acid solution was applied to a column containing 30 g of silica gel packed in chloroform–acetic acid (93:7, v:v). The desired product was eluted with chloroform–acetic acid–methanol (77.5:15:7.5, v:v) as a colorless, amorphous solid. The solid was dissolved in concentrated aqueous ammonia, and the solution was reduced *in vacuo* until precipitation had begun. Filtration of the resulting suspension yielded 127 mg (59% recovery, 11% overall yield) of a colorless, amorphous solid. Analytically pure material was obtained by a second recrystallization from aqueous ammonia as a colorless, amorphous solid, mp >335°; nmr (TFA) τ 0.89 (s, 1, GuC_8–H), 2.46 (s, 1, ThC_6–H), 5.42 (t, 2, J = 7 Hz, Gu–CH_2), 5.82 (t, 2, J = 7 Hz, Th–CH_2), 7.36 (m, 2, J = 7 Hz, C–CH_2–C), and 7.93 (s, 3, ThC_5–CH_3); mass spectrum (70 eV) *m/e* (relative intensity) 317 (66, M^+), 231 [57, M^+ − 2HNCO or M^+ − (H_2NCN, HNCO, and H)], 165 (93, Gu–CH_3^+), 164 (76, Gu–CH_2^+), 126 (69, Th–H^+), 96 (49, Th–CH_2^+ − HNCO), and 55 [100, Th–H^+ − (HNCO and CO)].

Anal. Calcd for $C_{13}H_{15}N_7O_3$: C, 49.21; H, 4.76; N, 30.90. Found: C, 48.90; H, 4.74; N, 30.70.

4-Amino-6-[3-(cytos-1-yl)propylamino]-5-nitropyrimidine (23b). To a solution of 1-(3-aminopropyl)cytosine (**21**) in 200 ml of methanol, prepared by deacetylation and reduction of 0.675 g (2.86 mmol) of N^4-acetyl-1-(3-azidopropyl)cytosine (**19**), were added 0.35 g (3.43 mmol) of triethylamine and a solution of 0.50 g (2.86 mmol) of 4-amino-6-chloro-5-nitropyrimidine (**22**) in 50 ml of methanol. The initially homogeneous solution was stirred at ambient temperature for 5 hr, at which time 0.365 g (42% based on azide) of an amorphous, colorless solid, mp *ca.* 307° dec, had precipitated; nmr (TFA) τ −0.3, 0.6, and 1.8 (3 br s, *ca.* 3, N–H), 1.63 (s, 1, PyC_2–H), 1.8 (br s, 2, CyC_4–NH_2), 2.09 and 3.57 (2d, 2, J = 8 Hz, $CyC_{5,6}$–H), 5.85 (br s, 4, Cy–CH_2 and Py–CH_2), and 7.8 (br s, 2, C–CH_2–C).

Anal. Calcd for $C_{13}H_{14}N_{10}O_2$: C, 43.13; H, 4.61; N, 36.59. Found: C, 43.45; H, 4.76; N, 36.31.

9-[3-(Cytos-1-yl)propyl]adenine (26, Ad–C_3–Cy). To a 0.700-g (2.28 mmol) portion of 4-amino-6-[3-(cytos-1-yl)propylamino]-5-nitropyrimidine (**23b**) dissolved in 100 ml of 98–100% formic acid was added 14 g (0.21 g-atom) of zinc dust. The resulting suspension was stirred for 15 min at ambient temperature and filtered under an atmosphere of dry nitrogen. Excess formic acid was evaporated *in vacuo*, leaving a pale yellow oil which was immediately suspended in 80 ml of dry, deoxygenated dimethylformamide. The suspension was concentrated to 60 ml *in vacuo* to ensure complete removal of formic acid and water. A 0.945-g (6.84 mmol) portion of anhydrous potassium carbonate was added, and the resulting suspension was stirred at reflux under nitrogen for 5.5 hr. The suspension was evaporated to dryness, yielding a red-brown solid. The solid was triturated with 30 ml of water and filtered, and the insoluble residue was washed with an additional 10 ml of water. The water washings were concentrated *in vacuo* to 5 ml. On standing at 4°, this solution deposited 186 mg (29%) of a pale yellow, amorphous solid, mp 280–285° dec. A 112-mg portion of the solid was dissolved in 2 ml of 98–100% formic acid, and the solution was applied to a column containing 30 g of silica gel packed in chloroform–glacial acetic acid–methanol (92.5:5:2.5, v:v). The desired product was eluted with chloroform–glacial acetic acid–methanol (7:2:1, v:v) as a colorless, amorphous solid, mp *ca.* 295° dec. Recrystallization of the solid from distilled water gave 68 mg (61% recovery, 17% over-all) of a colorless, amorphous solid, mp *ca.* 301° dec. An additional recrystallization from water yielded analytically pure material, mp *ca.* 305° dec; nmr (TFA) τ 0.72 and 1.27 (2s, 2, $AdC_{2,8}$–H), *ca.* 1.0 (br s, 2, AdC_6–NH_2), 1.86 (br s, 2, CyC_4–NH_2), 2.06 and 3.56 (2d, 2, J = 7 Hz, $CyC_{5,6}$–H), 5.22 (t, 2, Ad–CH_2), 5.78 (t, 2, Cy–CH_2), and 7.33 (m, 2, C–CH_2–C); mass spectrum (70 eV) *m/e* (relative intensity) 286 (4, M^+), 243 [3, M^+ − HNCO or M^+ − (HCN and NH_2)], 175 (85, Ad–$C_3H_5^+$), 162 (32, Ad–$C_2H_4^+$), 151 (54, Cy–$C_3H_5^+$), 149 (50, Ad–CH_3^+), 148 (88, Ad–CH_2^+), 138 (100, Cy–$C_2H_4^+$), 135 (45, Ad–H^+), 125 (25, Cy–CH_3^+), 108 (54, Ad–H^+ − HCN), 95 (24, Cy–H^+ − NH_2), and 81 [24, Ad–H^+ − 2HCN or Cy–CH_2^+ − (HCN and NH_2)].

Anal. Calcd for $C_{12}H_{14}N_8O$: C, 50.34; H, 4.93; N, 39.14. Found: C, 50.25; H, 5.00; N, 39.11.

9-[3-(Urac-1-yl)propyl]adenine (27, Ad–C_3–Ur). To a solution of 1.30 g (4.43 mmol) of 4-amino-6-[3-(cytos-1-yl)propylamino]-5-ni-

tropyrimidine (**23b**) dissolved in 200 ml of 98–100% formic acid was added 25 g (0.38 g-atom) of zinc dust. The resulting suspension was stirred at ambient temperature for 15 min and filtered under nitrogen. The filtrate was concentrated *in vacuo* and transferred to a flask containing 5 ml of deoxygenated formamide. Excess formic acid was removed *in vacuo*, leaving a yellow solution in formamide which was heated at 184–185° under nitrogen for 2.5 hr. The orange solution, diluted with 20 ml of water and cooled to 0°, deposited 251 mg (21%) of a pale yellow, amorphous solid, mp *ca.* 315° dec. A 166-mg portion of the solid was dissolved in formic acid, and the formic acid solution was applied to a column containing 30 g of silica gel packed in chloroform–acetic acid–methanol (92.5:5:2.5, v:v). The desired product was eluted with chloroform–acetic acid–methanol (85:10:5, v:v) as a colorless solid which was dissolved in 15% aqueous ammonia. The solution, reduced to one-half the original volume *in vacuo* and cooled to 4°, deposited 102 mg (62% recovery) of an amorphous, colorless solid, mp *ca.* 330° dec; nmr (TFA) τ 0.71 and 1.29 (2 s, 2, $AdC_{2,8}$–H), 2.26 and 3.78 (2d, 2, J = 7 Hz, $UrC_{5,6}$–H), 5.22 (t, 2, J = 7 Hz, Ad–CH_2), 5.83 (t, 2, J = 7 Hz, Ur–CH_2), and 7.35 (m, 2, J = 7 Hz, C–CH_2–C); mass spectrum (70 eV) *m/e* (relative intensity) 287 (10, M^+), 176 (8, Ad–$C_3H_6^+$), 162 (14, Ad–$C_2H_4^+$), 149 (100, Ad–CH_3^+), 148 (79, Ad–CH_2^+), 135 (26, Ad–H^+), 108 (12, Ad–H^+ − HCN), 82 (14, Ur–CH_2^+ − HNCO), and 54 [13, Ur–CH_2^+ − HNCO and CO)].

Anal. Calcd for $C_{12}H_{13}N_7O_2$: C, 50.17; H, 4.56; N, 34.14. Found: C, 50.02; H, 4.37; N, 34.00.

4-Amino-5-nitro-6-[3-(thym-1-yl)propylamino]pyrimidine (23a). To a solution of 0.762 g (4.37 mmol) of 4-amino-6-chloro-5-nitropyrimidine (**22**) and 0.93 g (9.17 mmol) of triethylamine in 50 ml of methanol was added a solution of 1.00 g (4.37 mmol) of the formate salt of 1-(3-aminopropyl)thymine (**18**) in 30 ml of methanol. The initially homogeneous solution was stirred at ambient temperature for 3 hr, yielding 1.28 g (92%) of an amorphous, colorless solid, mp 249–252°; nmr (TFA) τ −0.5 and 0.5 (v br s, *ca.* 2, N–H), 1.57 (s superimposed on v br s, 2, N–H and PyC_2–H), 2.45 (s, 1, ThC_6–H), 5.9 (m, 4, Th–CH_2 and Py–CH_2), 7.7 (m, 2, C–CH_2–C), and 7.90 (s, 3, ThC_5–CH_3).

Anal. Calcd for $C_{12}H_{15}N_7O_4$: C, 44.86; H, 4.70; N, 30.52. Found: C, 44.95; H, 4.91; N, 30.18.

9-[3-(Thym-1-yl)propyl]adenine (24, Ad–C_2–Th). A 0.58-g (1.8 mmol) portion of 4-amino-5-nitro-6-[3-(thym-1-yl)propylamino]-pyrimidine (**23a**) was suspended in 300 ml of 95% ethanol, and 3 g of Raney nickel was added. The resulting mixture was hydrogenated with shaking at 45 psi and ambient temperature for 70 min and filtered, and the filtrate was evaporated to dryness *in vacuo* leaving a pale yellow solid which was immediately dissolved in 98–100% formic acid. The formic acid solution was allowed to stand overnight at ambient temperature and was then heated under reflux for 2 hr under nitrogen. Removal of excess formic acid *in vacuo* left a yellow oil, which was immediately dissolved in 20 ml of deoxygenated formamide. The formamide solution was heated for 2 hr at 180° under nitrogen. The formamide solution, cooled to ambient temperature, deposited 0.129 g of an amorphous, colorless solid, mp 303–305° dec. Addition of 20 ml of water to the supernatant yielded an additional 98 mg of material identical with the first crop; total yield 0.227 g (42%); nmr (TFA) τ *ca.* 0.7 (v br s, 2, AdC_6–NH_2), 0.61 and 1.22 (2s, 2, $AdC_{2,8}$–H), 2.45 (s, 1, ThC_6–H), 5.22 (t, 2, J = 7 Hz, Ad–CH_2), 5.83 (t, 2, J = 7 Hz, Th–CH_2), 7.25 (m, 2, C–CH_2–C), and 7.90 (s, 3, ThC_5–CH_3); mass spectrum (70 eV) *m/e* (relative intensity) 301 (10, M^+), 176 (6, Ad–$C_3H_6^+$), 162 (13, Ad–$C_2H_4^-$), 149 (100, Ad–CH_3^+), 148 (81, Ad–CH_2^+), 135 (12, Ad–H^+), 108 (8, Ad–H^+ − HCN), and 96 (6, Th–CH_2^+ − HCNO).

Anal. Calcd for $C_{13}H_{15}N_7O_2$: C, 51.83; H, 5.02; N, 32.55. Found: C, 51.57; H, 4.95; N, 32.38.

1,1′-Trimethylenebisthymine (16a, Th–C_3–Th). To a solution of 12.8 g (0.101 mol) of thymine (**7b**) and 5.00 g (0.0202 mol) of 1-(3-bromopropyl)thymine (**4c**) in 100 ml of dry dimethyl sulfoxide was added 3.36 g (0.0242 mol) of anhydrous potassium carbonate. The resulting suspension was stirred at ambient temperature for 16 hr. The viscous suspension was filtered, and solvent was removed *in vacuo* from the filtrate, leaving 16.6 g of a colorless solid. An 11.4-g portion of the solid was suspended with stirring in a mixture of 30 ml of acetic anhydride and 0.45 g of pyridine. The suspension was heated at 140° with stirring for 35 min and filtered while hot to remove the soluble 1-acetylthymine. The insoluble residue was washed with hot acetic anhydride, leaving 3.21 g (79%) of a colorless solid, mp 322–325°. A single recrystallization from water yielded analytically pure material as colorless microcrystals, mp 330–332°; nmr (TFA) τ 2.36 (s, 2, ThC_6–H), 5.90 (t, 4, J = 7 Hz, Th–CH_2), 7.56 (m, 2, J = 7 Hz, C–CH_2–C), and 7.92 (s, 6, ThC_5–CH_3); mass spectrum (70 eV) *m/e* (relative intensity) 292 (18, M^+), 167 (52, Th–$C_3H_6^+$), 166 (100, Th–$C_3H_5^+$), 153 (65, Th–$C_2H_4^+$), 140 (61, Th–CH_3^+), 126, (37, Th–H^+), 110 (84, Th–$C_2H_4^+$ − HNCO), 96 (77, Th–CH_2^+ − HNCO), and 55 [65, Th–H^+ − (HNCO and CO)].

Anal. Calcd for $C_{13}H_{16}N_4O_4$: C, 53.42; H, 5.52; N, 19.17. Found: C, 53.17; H, 5.57; N, 19.32.

1-[3-(Thym-1-yl)propyl]cytosine (15, Cy–C_3–Th). To a solution of 4.61 g (36.6 mmol) of thymine (**7b**) and 5.00 g (18.3 mmol) of N^4-acetyl-1-(3-bromopropyl)cytosine (**6a**) in 40 ml of dry dimethyl sulfoxide was added 2.02 g (14.6 mmol) of anhydrous potassium carbonate. The resulting suspension was stirred at ambient temperature for 22 hr. The viscous suspension was filtered (Celite), and solvent was removed from the filtrate *in vacuo*, leaving a yellow gum which was triturated with three 150-ml portions of diethyl ether to remove residual dimethyl sulfoxide. The gum was dissolved in 300 ml of methanol–water (2:1, v:v), and 60 ml of concentrated aqueous ammonia was added. The solution was allowed to stand at ambient temperature for 2 hr and was then evaporated to dryness *in vacuo*, leaving a pale yellow solid. The solid was nearly completely dissolved in 50 ml of hot dimethylformamide, and the hot solution was applied to a column containing 1 kg of silica gel packed in methanol–chloroform (1:19, v:v). The desired product was eluted with methanol–chloroform (1:9, v:v) as 0.70 g (14%) of a colorless solid, mp 267–270° dec. Later fractions contained an additional 1.9 g (37%) of material, which consisted largely of the desired product but which was shown (nmr) to be contaminated by an unidentified material which did not contain a thymine nucleus. Two recrystallizations of the earlier fractions from water yielded analytically pure material as a colorless solid, mp *ca.* 299° dec; nmr (TFA) τ 1.8 (br s, 2, CyC_4–NH_2), 1.96 and 3.54 (2d, 2, J = 8 Hz, $CyC_{5,6}$–H), 2.44 (s, 1, ThC_6–H), 5.82 and 5.93 (2 overlapping t, 4, Cy–CH_2 and Th–CH_2), 7.63 (m, 2, J = 7 Hz, C–CH_2–C), and 7.92 (s, 3, ThC_5–CH_3); mass spectrum (70 eV) *m/e* (relative intensity) 277 (15, M^+), 234 [3, M^+ − HNCO or M^+ − (NH_2 and HCN)], 166 (9, Th–$C_3H_5^+$), 151 (51, Cy–$C_3H_5^+$), 138 (35, Cy–$C_2H_4^+$), 125 (100, Cy–CH_3^+), 112 (16, Cy–H_2^-), 108 (17, Cy–CH_2^+ − NH_2), 96 (34, Th–CH_2^+ − HNCO), 95 [44, Cy–H^+ − NH_2 or Th–CH_2^+ − (HNCO and H)], 83 (23, Th–H^+ − HNCO), 81 [36, Cy–CH_2^+ − (HCN and NH_2)], and 68 [18, Cy–H^+ − (NH_2 and HCN)].

Anal. Calcd for $C_{12}H_{15}N_5O_3$: C, 51.98; H, 5.45; N, 25.26. Found: C, 52.15; H, 5.34; N, 25.50.

1,1′-Trimethylenebiscytosine (14, Cy–C_3–Cy). To a solution of 5.00 g (32.6 mmol) of N^4-acetyltosine (**9**) and 4.42 g (16.3 mmol) of N^4-acetyl-1-(3-bromopropyl)cytosine (**6a**) in 800 ml of dry dimethyl sulfoxide was added 2.48 g (18.0 mmol) of anhydrous potassium carbonate. The resulting suspension was stirred at ambient temperature for 7 hr, and filtered, and solvent was removed from the filtrate *in vacuo*, leaving a yellow semisolid. The semisolid was suspended at ambient temperature in a mixture of 1.2 l. of methanol and 400 ml of methanol presaturated with ammonia at ambient temperature. The suspension was stirred for 5 hr, at which time nearly all material had dissolved. Removal of solvent *in vacuo* left a pale yellow solid which was dissolved in methanol. The methanol solution was applied to a column containing 800 g of silica gel packed in methanol–chloroform (1:9, v:v). The desired product was eluted with methanol–chloroform (1:1) as 2.7 g (63%) of a colorless solid, mp *ca.* 315° dec. The solid was dissolved with heating in 15% aqueous ammonia. The solution was filtered, and the filtrate was reduced *in vacuo* until precipitation had begun. The cold solution deposited 1.90 g of colorless needles, mp *ca.* 325° dec; nmr (TFA) τ 1.9 (br s, 4, CyC_4–NH_2), 2.02 and 3.57 (2d, 4, J = 8 Hz, $CyC_{5,6}$–H), 5.83 (t, 4, J = 7 Hz, Cy–CH_2), and 7.61 (m, 2, J = 7 Hz, C–CH_2–C); mass spectrum (70 eV) *m/e* (relative intensity) 151 (100, Cy–$C_3H_5^+$), 138 (44, Cy–$C_2H_4^+$), 125 (78, Cy–CH_3^+), 112 (39, Cy–H_2^+), 111 (40, Cy–H^+), 108 (35, Cy–CH_2^+ − NH_2), 95 (43, Cy–H^+ − NH_2), 81 [71, Cy–CH_2^+ − (NH_2 and HCN)], and 68 [39, Cy–H^+ − (NH_2 and HCN)].

Anal. Calcd for $C_{11}H_{14}N_6O_2$: C, 50.37; H, 5.38; N, 32.05. Found: C, 50.42; H, 5.52; N, 31.83.

1,1′-Trimethylenebisuracil (16b, Ur–C_3–Ur). To a solution of 14.4 g (0.129 mol) of uracil (**7a**) in 200 ml of dry dimethyl sulfoxide were added 6.00 g (0.0257 mol) of 1-(3-bromopropyl)uracil (**4a**) and 4.27 g (0.308 mol) of anhydrous potassium carbonate. The resulting suspension was stirred at ambient temperature for 6.5 hr and filtered (Celite). The filtrate was evaporated *in vacuo*, leaving a gummy white residue, which was triturated with three 200-ml

portions of diethyl ether, leaving 22 g (some dimethyl sulfoxide still present) of a colorless solid. A 17-g portion of the solid was suspended in 300 ml of dry dioxane. To the stirred suspension were added 30.4 g (0.280 mol) of trimethylsilyl chloride and, dropwise, a solution of 28.4 g (0.280 mol) of triethylamine in 40 ml of dioxane. The resulting suspension was stirred at ambient temperature for 20 hr and filtered under nitrogen, and the filtrate was concentrated *in vacuo*, leaving a viscous yellow oil. An 18.6-g portion of bis(trimethylsilyl)uracil, bp 68–70° (0.2 mm) [lit. bp 116° (12 mm)], was removed from the oil by distillation, leaving a red-brown pot residue. The residue was dissolved in concentrated aqueous ammonia. Partial removal of solvent *in vacuo*, followed by overnight standing at ambient temperature, led to deposit of 1.53 g (29%) of a pale yellow, amorphous solid, mp 277–287°. The solid was dissolved in formic acid, and the formic acid solution was applied to a column containing 200 g of silica gel packed in chloroform. The desired product was eluted with methanol–chloroform (1:9, v:v) as 1.34 g (25%) of an amorphous, colorless solid, mp 280–290° dec. A 140-mg portion of a middle fraction was recrystallized twice from water to yield 100 mg of an amorphous solid, mp 287–293° dec. A final recrystallization from methanol–water gave analytically pure material as colorless needles, mp 289–293° dec; nmr (TFA) τ 2.24 and 3.80 (2d, 4, J = 8 Hz, $UrC_{5,6}$–H), 5.89 (t, 4, J = 7 Hz, Ur–CH_2), and 7.65 (m, 2, J = 7 Hz, C–CH_2–C); mass spectrum (70 eV) *m/e* (relative intensity) 264 (4, M^+), 221 (2, M^+ − HNCO), 152 (100, Ur–$C_3H_5^+$), 139 (35, Ur–$C_2H_4^+$), 126 (32, Ur–CH_3^+), 112 (28, Ur–H^+), 109 (23, Ur–$C_3H_5^+$ − HNCO), 69 (24, Ur–H^+ − HNCO), and 55 [31, Ur–CH_3^+ − HNCO and CO)].

Anal. Calcd for $C_{11}H_{12}N_4O_4$: C, 49.99; H, 4.56; N, 21.20. Found: C, 49.86; H, 4.54; N, 21.12.

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Interaction of Metal Ions with Polynucleotides and Related Compounds. XII. The Relative Effect of Various Metal Ions on DNA Helicity[1]

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Abstract: The ability of Cu(II) and Zn(II) to bring about the unwinding and rewinding of the DNA double helix under appropriate conditions has been previously demonstrated. The present study reveals that other divalent metal ions have similar effects upon DNA and can be placed in a sequence that indicates the magnitude of their influence on DNA structure, as follows: Mg(II), Co(II), Ni(II), Mn(II), Zn(II), Cd(II), Cu(II). This sequence is deduced from the following phenomena. (1) The melting temperature (T_m) of DNA increases with increasing metal concentration for the metals at the left of the series, whereas T_m passes through a maximum for the metals to the right, the maximum occurring at lowest concentration at the far right. (2) Mg(II) at the far left cannot induce any detectable rewinding of the double helix; Co, Ni, and Mn produce partial rewinding, whereas the metals on the extreme right bring about complete renaturation of DNA. (3) These metals produce an increasing shift in the absorption maximum of DNA, from left to right. The placement of the metals in the same sequence by these three criteria is readily explained by their ability to bind both phosphate and heterocyclic "base" sites with the ratio of affinity for "base" to phosphate increasing from left to right.

The ability of metal ions to react with a variety of electron-donor sites on polynucleotides has received considerable attention. There are two main sites of interaction, the phosphate moieties of the ribose phosphate backbone, and the electron-donor groups on the bases.[2,3] Two types of interaction carry with them some rather interesting consequences. Reaction with phosphate means stabilization of ordered structures,[4–8] but cleavage of phosphodiester bonds with polyribonucleotides at high temperature.[9–17] Reaction with bases means destabilization of ordered structures.[2]

The differences in the behavior of various metal ions with polynucleotides have made it apparent that some metal ions prefer the phosphate sites and other metal

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