

Molecular Recognition via Base Pairing: Amine-Containing, Cytosine-Based Ditopic Receptors That Complex Guanosine Monophosphate

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Abstract: The synthesis and binding properties of two types of amine-containing, cytosine-based ditopic receptors, 4-amino-1-[4-*[N,N*-bis[2-(*N,N*-diethylamino)ethyl]amino]butyl]-2(1*H*)-pyrimidinone (**1**) and 4-amino-1-[4-(*N,N*-diethylamino)butyl]-2(1*H*)-pyrimidinone (**2a**), 4-amino-1-[5-(*N,N*-diethylamino)pentyl]-2(1*H*)-pyrimidinone (**2b**), and 4-amino-1-[6-(*N,N*-diethylamino)hexyl]-2(1*H*)-pyrimidinone (**2c**), are described. These systems were prepared by the functionalization of the trityl-protected iodoalkyl-substituted cytosine derivatives of general structure **9**, with the appropriate secondary amines [bis(diethylaminoethyl)amine and diethylamine, in the cases of **1** and **2a-c**, respectively] and subsequent deprotection with trifluoroacetic acid. The nitrogen-free cytosine derivative, 4-amino-1-(5-ethylheptyl)-2(1*H*)-pyrimidinone (**3**), was synthesized directly from the sodium salt of cytosine by reaction with 5-ethyl-1-iodoheptane in DMF. The four cytosine derivatives **1** and **2a-c** contain both base pairing and ammonium electrostatic binding subunits. As such, they were expected to act as efficient ditopic receptors for the complexation of guanosine 5'-monophosphate (GMP), a prototypical purine-derived substrate. Quantitative binding studies carried out in DMSO-*d*₆ indicate that compounds **1**, **2a**, **2b**, and **2c** in their neutral forms bind GMP free acid with first association constants, K_{11} , of $26\,000 \pm 3900$, 820 ± 120 , 1000 ± 150 , and $1300 \pm 200\text{ M}^{-1}$ and second association constants, K_{12} (for **1**) and K_{21} (for **2a-c**), of 1500 ± 230 , 660 ± 100 , 720 ± 110 , and $1200 \pm 180\text{ M}^{-1}$, respectively (where K_{11} , K_{21} , and K_{12} represent the formation of 1:1, 2:1, and 1:2 receptor-to-GMP complexes, respectively), whereas the ammonium-free system **3** was found to bind this same substrate as a 1:1 complex with a K_{11} of only $5 \pm 2\text{ M}^{-1}$. The greater binding affinity of receptor **1**, relative to that of **2a-c** (and **3**), is ascribed to the additional electrostatic interactions possible in the supramolecular GMP-receptor complex derived from this more precisely elaborated system.

The recognition and binding of mononucleotides and oligonucleotides are of utmost importance in biological systems. Complementary base pairings, for instance, are known to play a critical role in defining DNA double-helix formation and in regulating a variety of well-known information-transfer functions.¹ Moreover, ribozymes have been shown to bind oligonucleotides via base pairing,² while oligonucleotides are known to be recognized sequence specifically by restriction enzymes.³ In addition, the specific recognition of coenzymatic nucleotides such as ATP, FAD, and NADH is known to be necessary for the smooth function of a wide range of enzymatic systems.⁴ Complementary base pairings, directed multisite hydrogen bondings, specific stackings, and generalized electrostatic interactions, individually or in concert, may all contribute to the requisite nucleotide-nucleotide or protein-nucleotide recognition in these biological systems.⁵

In recent years, in part to understand these binding forces, a number of interesting synthetic receptors for nucleobases, nucleosides, and nucleotides have been prepared and studied.⁶⁻¹³

However, few of these have relied on specific base-pairing interactions (as opposed to stacking and more generalized hydrogen bondings) to achieve the desired molecular recognition.¹¹⁻¹³ In our own early efforts,^{12,13} much of the problem seemed to stem from the fact that in polar solvents (in which functionalized purine- and pyrimidine-derived systems are generally most soluble) the energies of binding interactions derived from simple base pairings are quite small.^{14,15} This led us to consider that a ditopic approach,

(1) Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. *The Molecular Biology of the Cell*; Garland Publishing: New York, 1983; pp 437-439.

(2) Cech, T. R. *Science* **1987**, *236*, 1532-1539.

(3) McClerin, J. A.; Frederick, C. A.; Wang, B.-C.; Greene, P.; Boyer, H. W.; Grable, J.; Rosenberg, J. M. *Science* **1986**, *234*, 1526-1536.

(4) Reference 1, pp 129-131.

(5) Recently, the X-ray crystal structure of a complex formed between GDP and an oncogene product protein (p21) was reported. Phosphate is involved in hydrogen bonding to the imidazole of His. See: Pai, E. F.; Kabsch, W.; Krenzel, U.; Holmes, K. C.; John, J.; Wittinghofer, A. *Nature* **1989**, *341*, 209-214.

(6) (a) Hamilton, A. D.; Van Engen, D. *J. Am. Chem. Soc.* **1987**, *109*, 5035-5036. (b) Hamilton, A. D.; Pant, N.; Muehldorf, A. *Pure Appl. Chem.* **1988**, *60*, 533-538. (c) Muehldorf, A. V.; Van Engen, D.; Warner, J. C.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 6561-6562. (d) Goswami, S.; Hamilton, A. D.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 3425-3426. (e) Hamilton, A. D.; Little, D. *J. Chem. Soc., Chem. Commun.* **1990**, 297-300.

(7) (a) Rebek, J., Jr.; Askew, B.; Ballester, P.; Buhr, C.; Jones, S.; Nemeth, D.; Williams, K. *J. Am. Chem. Soc.* **1987**, *109*, 5033-5035. (b) Rebek, J., Jr.; Askew, B.; Ballester, P.; Buhr, C.; Costero, A.; Jones, S.; Williams, K. *J. Am. Chem. Soc.* **1987**, *109*, 6866-6867. (c) Rebek, J., Jr.; Williams, K.; Parris, K.; Ballester, P.; Jeong, K.-S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1244-1245. (d) Askew, B.; Ballester, P.; Buhr, C.; Jeong, K.-S.; Jones, S.; Parris, K.; Williams, K.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1082-1090. (e) Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 245-225.

(8) For examples of adenosine 5'-triphosphate (ATP) and other phosphate anion recognition achieved through the use of linear and cyclic polyammonium cationic receptors see: (a) Tabushi, I.; Kobuke, Y.; Imuta, J. *J. Am. Chem. Soc.* **1980**, *102*, 1744-1745. (b) Kimura, E.; Kodama, M.; Yatsunami, T. *J. Am. Chem. Soc.* **1982**, *104*, 3182-3187. (c) Umezawa, Y.; Kataoka, M.; Takami, W.; Kimura, E.; Koike, T.; Nada, H. *Anal. Chem.* **1988**, *60*, 2392-2396. (d) Hosseini, M. W.; Lehn, J.-M.; Mertes, M. P. *Helv. Chim. Acta* **1983**, *66*, 2454-2466. (e) Yohannes, P. G.; Mertes, M. P.; Mertes, K. B. *J. Am. Chem. Soc.* **1985**, *107*, 8288-8289. (f) Hosseini, M. W.; Lehn, J.-M.; Maggiora, L.; Mertes, K. B.; Mertes, M. P. *J. Am. Chem. Soc.* **1987**, *109*, 537-544. (g) Hosseini, M. W.; Lehn, J.-M. *J. Am. Chem. Soc.* **1987**, *109*, 7047-7058. (h) Blackburn, G. M.; Thacher, G. R. J.; Hosseini, M. W.; Lehn, J.-M. *Tetrahedron Lett.* **1987**, *28*, 2779-2782. (i) Hosseini, M. W.; Lehn, J.-M.; Jones, K. C.; Plute, K. E.; Mertes, K. B.; Mertes, M. P. *J. Am. Chem. Soc.* **1989**, *111*, 6330-6335. (j) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896-3904. (k) Wilson, H. R.; Williams, R. J. P. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 1885-1892. (l) Bianchi, A.; Micheloni, M.; Paoletti, P. *Inorg. Chim. Acta* **1988**, *151*, 269-272. (m) Marecek, J. F.; Burrows, C. J. *Tetrahedron Lett.* **1986**, *27*, 5943-5946. (n) Behr, J.-P. *Tetrahedron Lett.* **1986**, *27*, 5861-5864. See also refs 6 and 7.

(9) For examples of molecular recognition involving phosphate-free nucleobase analogues see: (a) Chen, C.-W.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 4921-4922. (b) Lindsey, J. S.; Kearney, P. C.; Duff, R. J.; Tjivikua, P. T.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1988**, *110*, 6575-6577. (c) Brienne, M.-J.; Gabard, J.; Lehn, J.-M.; Stibor, I. *J. Chem. Soc., Chem. Commun.* **1989**, 1868-1870. (d) Lehn, J.-M.; Mascal, M.; DeCian, A.; Fischer, J. *J. Chem. Soc., Chem. Commun.* **1990**, 479-481. (e) Zimmerman, S. C.; Wu, W. *J. Am. Chem. Soc.* **1989**, *111*, 8054-8055. (f) Adrian, J. C., Jr.; Wilcox, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 8055-8057.

(10) For an example of enhanced adenine-thymine base pairing induced by an intercalating agent see: Constant, J. F.; Fahy, J.; Lhomme, J.; Anderson, J. E. *Tetrahedron Lett.* **1987**, *28*, 1777-1780.

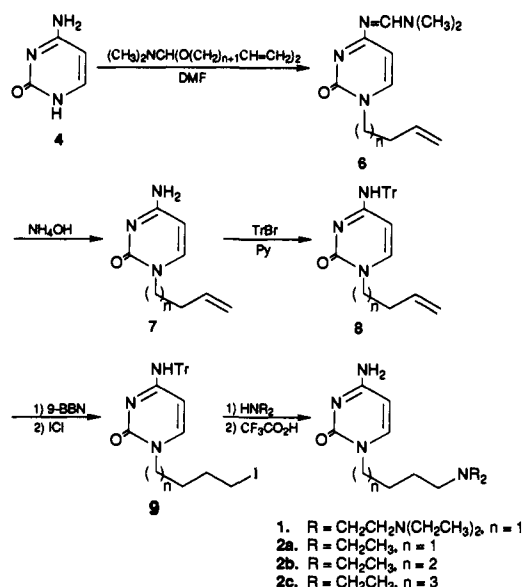
(11) Kim, M.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1987**, 1686-1688.

(12) Sessler, J. L.; Magda, D. M.; Hugdahl, J. *J. Inclusion Phenom.* **1989**, *7*, 19-26.

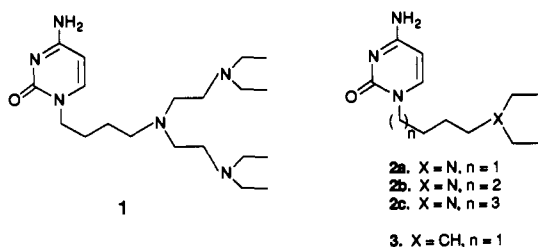
(13) Sessler, J. L.; Magda, D. In *Inclusion Phenomena and Molecular Recognition*; Atwood, J. L., Ed.; Plenum: New York, 1990; pp 17-26.

(14) For instance, the equilibrium constant for cytidine-guanosine dimer formation in DMSO at 37 °C has been reported to be $3.7 \pm 0.6\text{ M}^{-1}$.¹⁵

Scheme I



wherein weak base-pairing interactions can be complemented by other binding forces, might constitute a more fruitful approach to nucleotide recognition. In this paper we report the synthesis of the ditopic receptors **1** and **2**, which contain both base-pairing



and amine-derived binding subunits,^{8,16} and show that they act as effective complexing agents for the binding of guanosine 5'-monophosphate free acid (GMP) in DMSO, under conditions where the amine-free control system **3** does not.

Results and Discussion

The synthesis of compounds **1** and **2a-c** is shown in Scheme I. Cytosine (**4**) was alkenylated¹⁷ to give the protected system **6**. After the protecting group was changed from DMF to trityl, hydroboration with 9-BBN and subsequent iodination with ICl¹⁸ then gave the iodide **9**. Coupling with the appropriate secondary amine, bis(diethylaminoethyl)amine¹⁹ and diethylamine, respectively, followed by deprotection with trifluoroacetic acid and subsequent neutralization then gave targets **1** and **2a-c**, respectively. The amine-free cytosine derivative **3** was synthesized directly from the sodium salt of cytosine by reaction with 5-ethyl-1-iodoheptane²⁰ in DMF.

Systems **1** and **2a-c** were specifically designed so as to incorporate both base pairing and electrostatic binding subunits within the same molecule. As such, they were expected to act as efficient ditopic receptors for the complexation of guanine-derived nucleotides in aprotic solvents. For the purposes of the present initial study, it was decided to consider guanosine 5'-phosphate (GMP) as the prototypical guanine-derived nucleotide substrate and DMSO as the aprotic solvent. The choice of GMP as the substrate

was made on the basis of its ready availability and the fact that, to the best of our knowledge, no synthetic receptors currently exist which are capable of complexing this substrate with high affinity.⁸ The choice of DMSO stems from the fact that GMP is soluble in it and the realization that guanine-cytosine hydrogen-bonding interactions, although weak, are appreciable in this solvent.¹⁴ Within the constraints imposed by these choices of solvent and substrate then, the question became whether compounds **1** and **2** would function as GMP receptors. More specifically, the problems were to determine the stoichiometry of GMP complexation for each cytosine-based system, quantify the degree of recognition (i.e., determine the relevant binding constants), and assess the relative extent to which base pairing and phosphate-to-amine electrostatic ion-pairing interactions contribute to the overall recognition process. As discussed in detail below, ¹H NMR methods were used to address these issues.

First, qualitative binding studies were carried out in an effort to probe the nature of the electrostatic interaction between the amino components of receptors **1** and **2** and the phosphate groups of GMP. For these studies, 1 equiv of GMP free acid was added to a DMSO-*d*₆ solution of the putative receptor, and the changes in the receptor signals were monitored by using ¹H NMR spectroscopy at 360 MHz. In the case of the monoamine receptors **2a-c**, the chemical shift of the methylene protons attached to the alkylamine nitrogens shifted to lower field (for instance, from 2.60 to 3.05 ppm in the case of **2a**), while the positions of the other receptor-based resonances (including the cytosine NH₂ peak which was shifted slightly to lower field) remained essentially unaltered. These results are consistent with ion pair formation between the GMP phosphate and the tertiary amine groups present in systems **2a-c**. This conclusion is supported by the fact that no shifts in the alkyl region were observed upon the addition of GMP to the alkyl-bearing control system **3** and by the observation that, when treated with the free-acid form of GMP in DMSO-*d*₆, shifts to lower field were observed in the α -methylene signals of triethylamine (which may be considered the "cytosine-free" analogue of receptor **2**). When similar additions were carried out in the case of **1**, changes were only observed in the six methylene groups adjacent to the "outer" diethylamino nitrogens (shifting from 2.46 to 3.00 ppm for the ethyl 8 H and from 2.46 to 2.76 ppm for the ethylene 4 H) with no significant changes being seen in any of the other receptor-based signals, including those associated with the flanking methylenes of the "central" linking amine. In this case, ion pairing between the phosphate and the amines of the receptor appears to be largely limited to the "outer" two diethylamine-like centers and apparently does not involve significant interaction between the phosphate and the central amine. Such a binding scenario appears reasonable on the basis of molecular modeling studies. The two outer nitrogens in **1** are favorably disposed to wrap around the phosphate and bind it in a chelating manner. Moreover, using these two nitrogens alone for ion pairing (i.e., as opposed to involving the central amino group) would allow for the greatest separation of charge in the complexed species.

Proton NMR methods were also used for quantitative binding studies. For these, however, the conditions of the experiment were modified. Now, increasing aliquots of the receptor (in DMSO-*d*₆) were added to a 5 mM solution of GMP free acid in DMSO-*d*₆ at ambient temperature and the changes in the guanine imine (NH) and exocyclic amino (NH₂) signals recorded as a function of ligand concentration. The observed changes in resonance position were then analyzed by using a standard curve fitting program and the relevant binding constants obtained.^{21,24} Under

(15) Newmark, R. A.; Cantor, C. R. *J. Am. Chem. Soc.* **1968**, *90*, 5010-5017.

(16) Polyammonium cations are known to complex the phosphate groups of DNA and RNA and thereby stabilize their tertiary structure. See, for instance: Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 343-346.

(17) Helfer, D. L.; Hosmane, R. S.; Leonard, N. J. *J. Org. Chem.* **1981**, *46*, 4803-4804.

(18) Kabalka, G. W.; Gooch, E. E. *J. Org. Chem.* **1981**, *46*, 2582-2584.

(19) Marxer, A.; Miescher, K. *Helv. Chim. Acta* **1951**, *34*, 924-931.

(20) Prout, F. S.; Cason, J. *J. Org. Chem.* **1949**, *14*, 132-136.

(21) Binding constants were determined by using either the Scatchard plot^{22,23} or the least-squares NMR curve fitting program of Whitlock and co-workers.²⁴ We are grateful to Professor Anshyn of this department for making this program available to us and for his assistance in carrying out these analyses.

(22) Connors, K. A. *Binding Constants*; Wiley: New York, 1987; Chapter 5.

(23) Sessler, J. L.; Murai, T.; Lynch, V. *Inorg. Chem.* **1989**, *28*, 1333-1341.

(24) Whitlock, B. J.; Whitlock, H. W. *J. Am. Chem. Soc.* **1990**, *112*, 3910-3915.

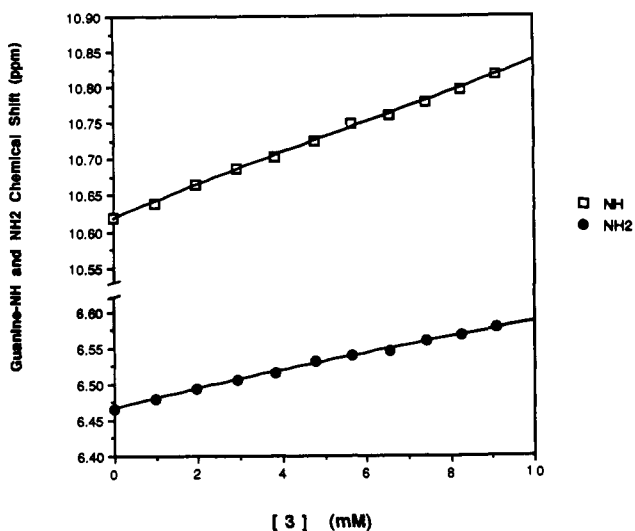


Figure 1. Observed chemical shifts in the guanine NH and NH₂ proton signals obtained upon the addition of increasing aliquots of **3** to a 5 mM solution of guanosine 5'-monophosphate free acid in DMSO-*d*₆ at 23 °C.

these conditions, the degree of GMP-GMP self-association is negligible.²⁵

When the above titration experiments were carried out with **3**, the simple alkyl-substituted control system, the results proved completely straightforward. Both the guanine NH and NH₂ signals were found to shift to lower field, with the change in chemical shift observed for the NH signals ($\Delta\delta_{\text{NH}}$) being roughly twice that obtained for the NH₂ protons (Figure 1). Such downfield shifts and 2:1 $\Delta\delta_{\text{NH}}$ to $\Delta\delta_{\text{NH}_2}$ ratios are characteristic of Watson-Crick base pairing in DMSO-*d*₆,¹⁵ suggesting that simple 1:1 base pairing is taking place in this control system. Moreover, the derived association constant of $5 \pm 2 \text{ M}^{-1}$ for this recognition process is in good agreement with the values reported for simple guanine-cytosine base pairing in this solvent.¹⁴

More complex binding behavior was observed in the case of the ditopic receptors **2a-c**. As was true for **3**, the addition of receptors **2a-c** to GMP gave rise in all cases to monotonic downfield shifts in the GMP guanine NH and NH₂ signals. In contrast to what was observed with **3**, however, the observed guanine NH₂ shifts were now in all cases larger than those expected for simple Watson-Crick base pairing (i.e., $2\Delta\delta_{\text{NH}_2} > \Delta\delta_{\text{NH}}$). Since the only chemical difference between, e.g., **2a** and the control system **3** is the presence of a donating amine, this result indicates that the observed ion-pairing interactions between the GMP 5'-phosphate and the receptor amine give rise to an additional H-bonding interaction involving the exocyclic guanine NH₂ protons, which is over and above that caused by cytosine-guanine base pairing. Consistent with this supposition is the observation that the addition of triethylamine to the free-acid form of GMP (under identical experimental conditions) also induces significant but slightly less dramatic downfield shifts in the guanine NH₂ resonances *without causing substantial shifts in the corresponding imine signals*. In particular, the guanine NH₂ proton signals were shifted from 6.47 to 6.54 and 6.58 ppm in the presence of 1 and 2 molar equiv of triethylamine, respectively (Figure 2). These results suggest that under the conditions of the experiment (i.e., in DMSO-*d*₆) electrostatic binding (ion pairing) between the added tertiary amine and the 5'-phosphate serves to enhance an intramolecular hydrogen-bonding interaction between the GMP phosphate oxygen and the exocyclic guanine NH₂ group. Presumably this reflects an increased relative stabilization of the *syn*-GMP conformer wherein the ribose and its 5'-phosphate lie directly "under" the purine nucleus. To the extent that this is true,

(25) The self-association constant for guanosine-guanosine dimerization has been reported to be 0.18 M^{-1} in DMSO at 37 °C.¹⁵ In control dilution experiments with GMP, we did not observe significant chemical shift changes for either the guanine imino or amino protons at concentrations below 6 mM in DMSO solution.

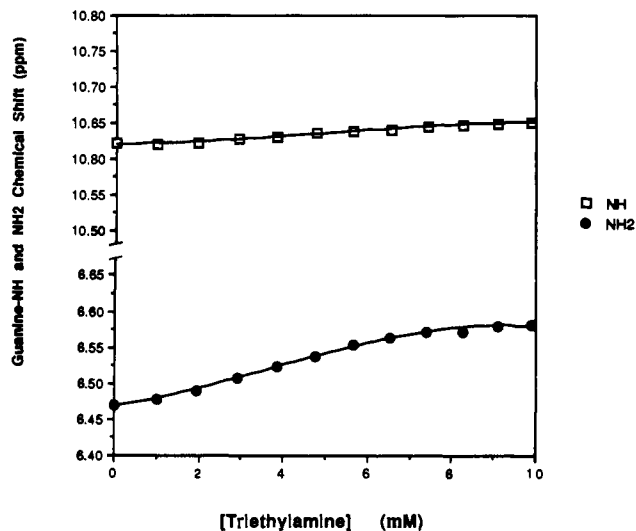


Figure 2. Observed chemical shifts in the guanine NH and NH₂ proton signals obtained upon the addition of increasing aliquots of triethylamine to a 5 mM solution of guanosine 5'-monophosphate free acid in DMSO-*d*₆ at 23 °C.

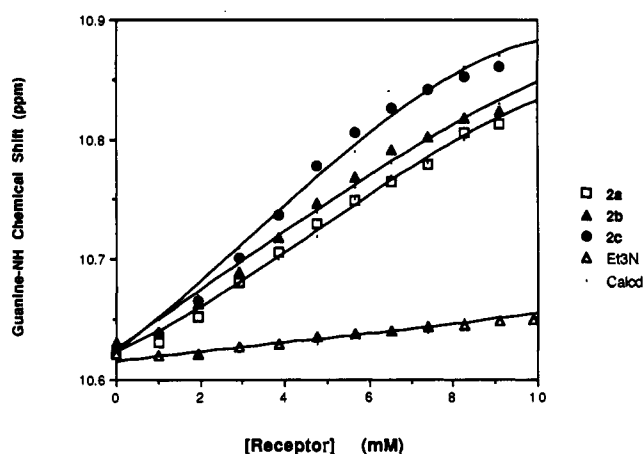


Figure 3. Calculated and observed chemical shifts in the guanine NH proton signals obtained upon the addition of increasing aliquots of receptors **2a-c** to a 5 mM solution of guanosine 5'-monophosphate free acid in DMSO-*d*₆ at 23 °C.

Table I. Complexation Data for the Complexation of GMP Free Acid in DMSO-*d*₆ at 23 °C^a

receptor	K_{11}, M^{-1}	K_{21}, M^{-1}	K_{12}, M^{-1}
1	26000	1500	
2a	820		660
2b	1000		720
2c	1300		1200
3	^b 5		
triethylamine	560		540
<i>N,N</i> -tetramethylbutyldiamine	9700 ^c	53 ^c	

^a Estimated errors $\pm 15\%$. ^b Calculated from Scatchard plot. ^c Calculated with NH₂.

the magnitude of the electrostatic binding interaction will reflect the free-energy changes associated with stabilizing this conformation.^{26,27}

An important consequence of the above control experiments is that changes in the chemical shift of the guanine imine (NH)

(26) This could account for why the observed K_{11} and K_{21} values for, e.g., triethylamine, are so close. Unfortunately, the barrier for *syn/anti* GMP interconversion is not known in DMSO; however, it is known that in H₂O the rotational barrier for GMP *syn/anti* interconversion is lower than that of other purine nucleotides due to an H-bonding interaction between the phosphate and exocyclic amino group.²⁷

(27) Son, J.-D.; Guschlbauer, W.; Gueron, M. *J. Am. Chem. Soc.* **1972**, *94*, 7903-7911.

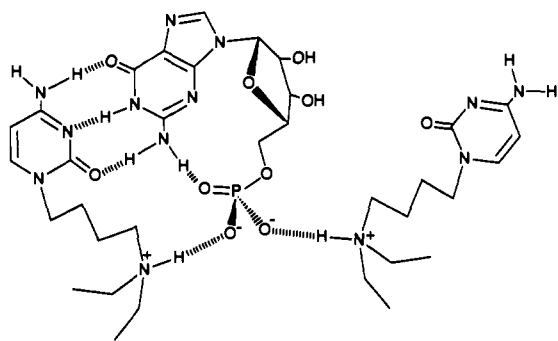


Figure 4. Proposed structure for the 1:2 supramolecular complex formed between guanosine 5'-monophosphate (GMP) free acid and receptor **2a**.

signals would be expected to provide a more reliable basis for quantitative analyses involving the amine-containing receptors **2a-c** (and *inter alia* **1**). Conversely, monitoring the changes in the guanine NH₂ signals as a function of concentration of triethylamine could provide a means of determining the extent of ion pair induced binding. Once determined, the value of the latter could, of course, serve as a "baseline" for assessing the relative extent to which electrostatics and base pairing contribute to the overall GMP binding capability of receptors **2a-c**.

Figure 3 shows the calculated and observed chemical shifts of the GMP guanine NH signals as a function of added binding agent for the cases of triethylamine and receptors **2a-c**, respectively, and Table I summarizes the derived association constants obtained from guanine imino proton shifts. A reasonable fit to experiment could only be obtained by assuming the formation of a 2:1 receptor to GMP complex, with values for the first (K_{11}) and second (K_{21}) association constants of $K_{11} = 560 \pm 85$, $K_{21} = 540 \pm 80$ and $K_{11} = 1300 \pm 200$, $K_{21} = 1200 \pm 180$ being determined for triethylamine and, for instance, **2c**, respectively. This favored 2:1 receptor to GMP binding stoichiometry, which was independently confirmed by Job plots (involving plots of chemical shift vs mole fraction of receptor), is presumably a direct consequence of the dibasic nature of GMP: Two different amines can bind to the same free-acid phosphate group, and in the case of triethylamine and receptors **2a-c** these would necessarily have to be provided by two separate amine-containing molecules. This is illustrated in Figure 4, which gives a schematic representation of the putative 2:1 complex formed between receptor **2a** and GMP free acid at higher relative receptor concentrations.

The association constants given in Table I indicate that the presence of the cytosine subunit in receptors **2a-c** serves to enhance the degree of binding due solely to the electrostatic amine to phosphate interaction. It is also clear from these data, however, that the contribution due to base pairing is relatively small. Nonetheless, it is apparent that H-bonding does play an appreciable role in stabilizing the receptor to GMP complexes and that this degree of stabilization is structure dependent. For instance, in the case of **2a**, which bears the shortest cytosine base to amine alkyl chain, the first and second binding constants, K_{11} and K_{21} , are not appreciably larger than those obtained with triethylamine (cf. Table I). However, in the case of **2c**, where this linking alkyl chain is longer and a better orientation for cytosine-guanine Watson-Crick hydrogen bonding can be sustained in the ion-paired complex (as judged by CPK models), base pairing contributes about a factor of 2.5 to the overall association constants.²⁸

On the basis of the above conclusion, it is reasonable to predict that receptor **1**, which contains a built-in two-point chelating polyamine, would be a significantly improved GMP complexing agent. This indeed appears to be the case. Here, as shown in Figure 5, the observed and calculated ¹H NMR binding profiles

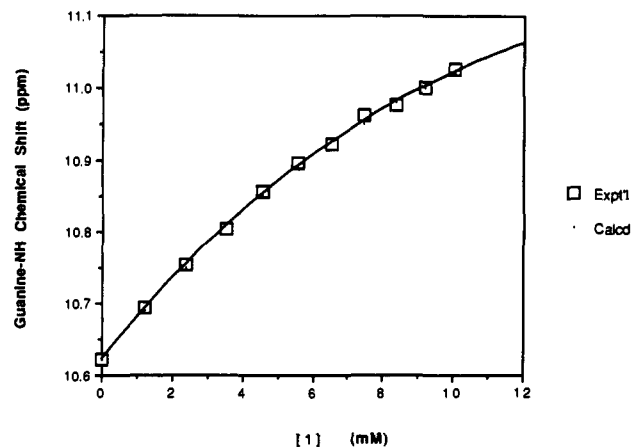


Figure 5. Calculated and observed chemical shifts in the guanine NH proton signals obtained upon the addition of increasing aliquots of receptor **1** to a 5 mM solution of guanosine 5'-monophosphate free acid in DMSO-*d*₆ at 23 °C.

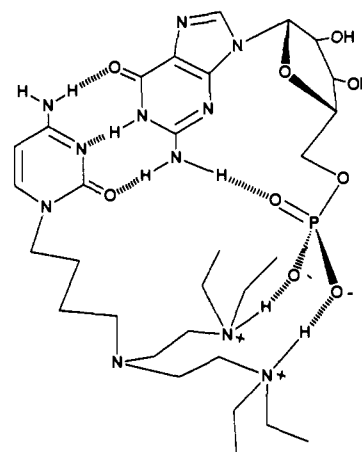


Figure 6. Proposed structure for the supramolecular complex formed between guanosine 5'-monophosphate (GMP) free acid and the ditopic receptor **1**.

indicate the preferential formation of a 1:1 receptor to GMP complex under near stoichiometric conditions. Now, presumably, the chelating polyamine portion of the receptor can wrap around and bind to both ion-pairing (hydroxyl) sites of the GMP free-acid phosphate so as to form the well-stabilized 1:1 complex depicted schematically in Figure 6. In specific quantitative terms (Table I), the K_{11} value for receptor **1** (ca. $26\,000 \pm 3900\text{ M}^{-1}$) is found to be approximately 17 times larger than the corresponding second association constant ($K_{12} = 1500 \pm 230\text{ M}^{-1}$) and roughly 20 times larger than the highest first association constant observed in the series of receptors **2a-c** (namely $1300 \pm 200\text{ M}^{-1}$ for **2c**). However, as was true for **2c**, the contribution due to base pairing is still relatively small. In the case of **1**, a comparison with the cytosine-free control system *N,N*-tetramethylbutyldiamine, for which $K_{11} = \text{ca. } 9500\text{ M}^{-1}$, indicates that this cytosine-guanine base pairing contributes a factor of roughly 2.7 to the overall GMP binding affinity. Thus, at least in this solvent system, base-pairing interactions serve more to "fine tune" the overall nature and magnitude of the GMP recognition rather than to define the basic complexation process. Nonetheless, the present results do indicate that in suitably designed synthetic systems base-pairing interactions can play an important role in enhancing a particular molecular recognition process.

Conclusion

To the best of our knowledge, compounds **1** and **2** represent the first rationally designed ditopic receptors capable of complexing GMP in an organic solvent. In these systems, the alkyl chain length and the number of nitrogens appear to be the important factors that determine the strength of binding. Through an it-

(28) Not surprisingly, this base-pairing interaction contributes most significantly to the first association constant rather than to the second. This is because the enthalpic contribution to the overall free energy is manifest during this first association; the binding by ion pairing of a second potentially base-pairing substrate can only contribute to the entropic term in the formation of a 2:1 complex.

erative interplay between synthesis and binding analysis, therefore, it should be possible to extend this work and design analogues of **1** and **2** that display yet greater affinity for GMP or, by appropriate modification of the nucleobase and/or polyammonium binding subunits, other nucleotides as well. The involvement of the nucleobase in complexation suggests that this approach may also be extended to the development of agents which exhibit transport properties as well. This approach could prove particularly beneficial in providing adjuncts for the delivery of non-natural, synthetic nucleotide analogues.^{29,30} Here, a receptor such as **1** could serve to neutralize the charged substrate and facilitate transport across the cell membrane or the lipidlike blood-brain barrier and aid in the area of drug delivery.³¹ Work directed toward achieving these goals is currently in progress.

Experimental Section

General Information. Proton and ¹³C NMR spectra were obtained in CDCl₃, DMSO-*d*₆, or CD₃OD with residual CHCl₃ (δ = 7.26 ppm for ¹H and 77.0 ppm for ¹³C), DMSO (δ = 2.49 ppm for ¹H), and CH₃OH (δ = 4.78 ppm for ¹H and 49.0 ppm for ¹³C) signals being used as the internal standards, respectively. Proton and carbon NMR spectra were recorded on either a Nicolet NT-360 (360 MHz) or a General Electric QE-300 (300 MHz) spectrometer. Routine electron impact (EI) mass spectra were measured with a Finnigan-MAT 4023 instrument. Fast atom bombardment mass spectrometry (FAB-MS) was performed by using a Finnigan-Mat TSQ-70 instrument and glycerol as the matrix. Elementary analyses were performed by Atlantic Microlab, Inc. Electronic spectra were measured in CHCl₃ on a Beckmann Instruments DU-7 spectrophotometer. Melting points were measured on a Mel-temp apparatus and are uncorrected.

Binding studies were carried out at 23 °C in DMSO-*d*₆ by use of a 360-MHz ¹H NMR spectrometer (NT-360). Each spectrum of the continuous variation series was obtained with 16K data points over a sweep width of 4500 Hz. Further details of data reduction and binding constant determination are given below (vide infra).

Materials. Tetrahydrofuran (THF) was distilled from sodium benzophenone. Pyridine and *N,N*-dimethylformamide were distilled from MgO. Methylene chloride was distilled from CaH₂. Cytosine (Sigma, 98%) was dried by heating at 80 °C in vacuo overnight. Guanosine 5'-monophosphate free acid was obtained from Sigma. Dimethyl-*d*₆ sulfoxide was purchased from Isotec Inc. (99.9%). All other solvents and reagents were of reagent grade quality and used without further purification. Column chromatography was performed by using Merck silica gel 60. Membrane filters (Millex-HV 0.45 μ m) were purchased from Millipore Corp.

General Procedure for the Preparation of *N,N*-Dimethylformamide Dialkenyl Acetals (5a-c).³² A mixture of *N,N*-dimethylformamide dimethyl acetal (26.9 g, 0.23 mol) and alkenyl alcohol (~50 g, 0.5–0.7 mol) was heated at 100 °C for 3 h while the methanol formed was simultaneously removed by distillation through an attached straight-bore column. The moderately unstable products were in all cases isolated by fractional distillation under reduced pressure and used immediately in the subsequent synthetic steps.

(a) *N,N*-Dimethylformamide Dibutenyl Acetal (**5a**): yield 77%; bp 108 °C/25 mmHg; ¹H NMR (300 MHz, CDCl₃) δ 2.29 [6 H, s, N-(CH₃)₂], 2.34 (4 H, q, *J* = 6.7 Hz, CH₂CH=CH₂), 3.55 (4 H, m, OCH₂), 4.52 (1 H, s, NCH), 5.03 (2 H, d, *J* = 10.1 Hz, CH=CH₂), 5.09 (2 H, d, *J* = 15.7 Hz, CH=CH₂), 5.84 (2 H, m, CH=CH₂); ¹³C NMR (75 MHz, CDCl₃) δ 34.02 (CH₂CH=CH₂), 37.60 [N(CH₃)₂], 64.91 (OCH₂), 111.13 (NCH), 116.24 (CH=CH₂), 135.21 (CH=CH₂).

(b) *N,N*-Dimethylformamide Dipentenyl Acetal (**5b**): yield 70%; bp 137 °C/25 mmHg; ¹H NMR (300 MHz, CDCl₃) δ 1.68 (4 H, t of t, *J* = 7.2, 7.0 Hz, OCH₂CH₂), 2.14 (4 H, q, *J* = 7.2 Hz, CH₂CH=CH₂), 2.29 [6 H, s, N(CH₃)₂], 3.49 (4 H, m, OCH₂), 4.48 (1 H, s, NCH), 4.96 (2 H, d, *J* = 13.4 Hz, CH=CH₂), 5.02 (2 H, d, *J* = 17.2 Hz, CH=CH₂), 5.80 (2 H, m, CH=CH₂); ¹³C NMR (75 MHz, CDCl₃) δ 28.67

(OCH₂CH₂), 30.18 (CH₂CH=CH₂), 37.37 [N(CH₃)₂], 64.68 (OCH₂), 111.06 (NCH), 114.49 (CH=CH₂), 137.97 (CH=CH₂).

(c) *N,N*-Dimethylformamide Dihexenyl Acetal (**5c**): yield 50%; bp 150 °C/25 mmHg; ¹H NMR (300 MHz, CDCl₃) δ 1.48 (4 H, t of t, *J* = 7.0, 8.7 Hz, OCH₂CH₂CH₂), 1.59 (4 H, t of t, *J* = 7.1, 7.3 Hz, OCH₂CH₂), 2.08 (4 H, q, *J* = 7.0 Hz, CH₂CH=CH₂), 2.30 [6 H, s, N(CH₃)₂], 3.49 (4 H, m, OCH₂), 4.48 (1 H, s, NCH), 4.94 (2 H, d, *J* = 12.7 Hz, CH=CH₂), 5.00 (2 H, d, *J* = 15.9 Hz, CH=CH₂), 5.80 (2 H, m, CH=CH₂); ¹³C NMR (75 MHz, CDCl₃) δ 25.42 (CH₂CH=CH₂), 28.97 (OCH₂CH₂), 33.41 (CH₂CH₂CH=CH₂), 37.57 [N(CH₃)₂], 65.33 (OCH₂), 111.19 (NCH), 114.33 (CH=CH₂), 138.57 (CH=CH₂).

General Procedure for the Preparation of 1-Substituted-*N*-(dimethylamino)methylene]cytosines (6a-c).¹⁷ A mixture of dry cytosine (1.0 g, 9.0 mmol), *N,N*-dimethylformamide dialkenylacetal **5a-c** (50 mmol), and dry *N,N*-dimethylformamide (20 mL) was heated at 120 °C under nitrogen for 24 h. The reaction mixture was cooled and evaporated to dryness in vacuo, and the solid obtained was recrystallized from toluene.

(a) 1-Butenyl-*N*-(dimethylamino)methylene]cytosine (**6a**): yield 65%; mp 200–202 °C; ¹H NMR (300 MHz, CDCl₃) δ 2.51 (2 H, q, *J* = 6.8 Hz, CH₂CH=CH₂), 3.12 [3 H, s, N(CH₃)₂], 3.15 [3 H, s, N(CH₃)₂], 3.90 (2 H, d, *J* = 6.9 Hz, NCH₂CH₂), 5.06 (1 H, d, *J* = 11.5 Hz, CH₂CH=CH₂), 5.09 (1 H, d, *J* = 4.0 Hz, CH₂CH=CH₂), 5.76 (1 H, m, CH₂CH=CH₂), 6.00 (1 H, d, *J* = 6.9 Hz, C⁵-H), 7.35 (1 H, d, *J* = 6.9 Hz, C⁶-H), 8.81 (1 H, s, NCHN); ¹³C NMR (75 MHz, CDCl₃) δ 33.02 (NCH₂CH₂), 34.91 [N(CH₃)₂], 41.19 [N(CH₃)₂], 49.78 (NCH₂), 102.00 (C-5), 117.98 (CH=CH₂), 133.89 (CH=CH₂), 146.12 (C-6), 156.73 (C-2), 158.13 (N=CHN), 171.65 (C-4); MS, *m/e* (rel intensity) 220 (84, M⁺), 176 [100, [M - N(CH₃)₂]⁺]. HRMS Calcd for C₁₁H₁₆N₄O: 220.1324. Found: 220.1315.

(b) 1-Pentenyl-*N*-(dimethylamino)methylene]cytosine (**6b**): yield 29%; mp 194–196 °C; ¹H NMR (300 MHz, CDCl₃) δ 1.87 (2 H, q, *J* = 7.3 Hz, NCH₂CH₂), 2.10 (2 H, q, *J* = 7.0 Hz, CH₂CH=CH₂), 3.12 [3 H, s, N(CH₃)₂], 3.16 [3 H, s, N(CH₃)₂], 3.83 (2 H, t, *J* = 6.8 Hz, NCH₂CH₂), 5.00 (1 H, d, *J* = 9.0 Hz, CH₂CH=CH₂), 5.05 (1 H, d, *J* = 16.6 Hz, CH₂CH=CH₂), 5.80 (1 H, m, CH₂CH=CH₂), 6.02 (1 H, d, *J* = 6.9 Hz, C⁵-H), 7.35 (1 H, d, *J* = 6.9 Hz, C⁶-H), 8.84 (1 H, s, NCHN); ¹³C NMR (75 MHz, CDCl₃) δ 27.82 (CH₂CH=CH₂), 30.38 (NCH₂CH₂), 34.96 [N(CH₃)₂], 41.24 [N(CH₃)₂], 49.82 (NCH₂), 102.22 (C-5), 115.46 (CH=CH₂), 137.13 (CH=CH₂), 145.89 (C-6), 156.94 (C-2), 158.29 (N=CHN), 171.68 (C-4); MS, *m/e* (rel intensity) 234 (100, M⁺), 190 [94, [M - N(CH₃)₂]⁺]. HRMS Calcd for C₁₂H₁₈N₄O: 234.1480. Found: 234.1487.

(c) 1-Hexenyl-*N*-(dimethylamino)methylene]cytosine (**6c**): yield 22%; mp 180–182 °C; ¹H NMR (300 MHz, CDCl₃) δ 1.45 (2 H, m, NCH₂CH₂CH₂), 1.76 (2 H, m, NCH₂CH₂), 2.11 (2 H, q, *J* = 6.9 Hz, CH₂CH=CH₂), 3.13 [3 H, s, N(CH₃)₂], 3.15 [3 H, s, N(CH₃)₂], 3.82 (2 H, t, *J* = 7.27 Hz, NCH₂CH₂), 4.96 (1 H, d, *J* = 10.6 Hz, CH₂CH=CH₂), 5.01 (1 H, d, *J* = 17.0 Hz, CH₂CH=CH₂), 5.77 (1 H, m, CH₂CH=CH₂), 6.10 (1 H, d, *J* = 6.9 Hz, C⁵-H), 7.40 (1 H, d, *J* = 6.9 Hz, C⁶-H), 8.80 (1 H, s, NCHN); ¹³C NMR (75 MHz, CDCl₃) δ 25.58 (CH₂CH=CH₂), 28.31 (NCH₂CH₂CH₂), 33.06 (NCH₂CH₂), 35.06 [N(CH₃)₂], 41.44 [N(CH₃)₂], 50.30 (NCH₂), 102.25 (C-5), 114.79 (CH=CH₂), 137.91 (CH=CH₂), 146.01 (C-6), 158.58 (C-2), 158.58 (N=CHN), 171.65 (C-4); MS, *m/e* (rel intensity) 248 (100, M⁺), 204 [85, [M - N(CH₃)₂]⁺]. HRMS Calcd for C₁₃H₂₀N₄O: 248.1637. Found: 248.1635.

General Procedure for the Preparation of 1-Alkenylcytosines (7a-c).¹⁷ A mixture of **6a-c** (10.0 mmol) and concentrated ammonium hydroxide solution (50 mL) was stirred at room temperature for 20 h and then heated at 80 °C for 40 min to drive off excess ammonia. The solution was evaporated to dryness on a rotary evaporator, and the solid residue obtained was recrystallized from methanol with final cooling being effected at -15 °C in a freezer.

(a) 1-Butenylcytosine (**7a**): yield 90%; mp 130–133 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 2.30 (2 H, q, *J* = 6.8 Hz, CH₂CH=CH₂), 3.68 (2 H, t, *J* = 7.0 Hz, NCH₂), 4.99 (1 H, d, *J* = 11.1 Hz, CH₂CH=CH₂), 5.00 (1 H, d, *J* = 16.2 Hz, CH₂CH=CH₂), 5.62 (1 H, q, *J* = 7.2 Hz, C⁵-H), 5.78 (1 H, m, CH₂CH=CH₂), 7.07 (2 H, br s, NH₂), 7.52 (1 H, d, *J* = 7.1 Hz, C⁶-H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 33.26 (NCH₂CH₂), 48.21 (NCH₂), 93.35 (C-5), 117.56 (CH=CH₂), 135.39 (C-H=CH₂), 146.45 (C-6), 156.14 (C-2), 166.23 (C-4); MS, *m/e* (rel intensity) 165 (69, M⁺), 124 [65, (M - C₃H₅)⁺], 111 [100, [M - C₄H₇]⁺], 81 (92). HRMS Calcd for C₈H₁₁N₃O: 165.0902. Found: 165.0899.

(b) 1-Pentenylcytosine (**7b**): yield 92%; mp 140–144 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.63 (2 H, t of t, *J* = 7.5, 6.9 Hz, NCH₂CH₂), 1.98 (2 H, q, *J* = 6.9 Hz, CH₂CH=CH₂), 3.61 (2 H, t, *J* = 7.1 Hz, NCH₂), 4.95 (1 H, d, *J* = 10.2 Hz, CH₂CH=CH₂), 5.02 (1 H, d, *J* = 17.2 Hz, CH₂CH=CH₂), 5.64 (1 H, d, *J* = 6.6 Hz, C⁵-H), 5.76 (1 H, m, CH₂CH=CH₂), 7.11 (2 H, br s, NH₂), 7.56 (1 H, d, *J* = 7.2 Hz,

(29) Examples include 9-(β -D-xylofuranosyl)guanosine monophosphate (xylo-GMP) and 9-(β -D-arabinofuranosyl)adenine (ara-A),³⁰ which are far more active in their chemically or enzymatically produced phosphorylated forms. For a further discussion of this point see: Holy, A. In *Approaches to Antiviral Agents*; Harden, M. R., Ed.; VCH Publishers: Deerfield Beach, FL, 1985; pp 101–134.

(30) Streissle, G.; Paessens, A.; Oedigert, H. *Adv. Virus Res.* **1985**, *30*, 83–138.

(31) *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 8th ed.; Dege, R. F., Ed.; Lippincott: Philadelphia, 1982; p 13.

(32) Arnold, Z.; Kornilov, M. *Collect. Czech. Chem. Commun.* **1964**, *29*, 645–651.

C⁶-H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 28.08 (CH₂CH=CH₂), 30.48 (NCH₂CH₂), 48.61 (NCH₂), 93.48 (C-5), 115.49 (CH=CH₂), 138.13 (CH=CH₂), 146.50 (C-6), 155.92 (C-2), 166.01 (C-4); MS, *m/e* (rel intensity) 179 (38, M⁺), 138 [17, (M - C₃H₅)⁺], 125 [100, (M - C₄H₆)⁺], 111 [32, (M - C₅H₈)⁺], 81 (57). HRMS Calcd for C₉H₁₃N₃O: 179.1059. Found: 179.1063.

(c) **1-Hexenylcytosine (7c)**: yield 95%; mp 160–162 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.29 (2 H, t, *J* = 7.6, 7.4 Hz, NCH₂CH₂), 1.53 (2 H, q, *J* = 7.6, 7.3 Hz, NCH₂CH₂CH₂), 1.98 (2 H, q, *J* = 6.9 Hz, CH₂CH=CH₂), 3.60 (2 H, t, *J* = 7.2 Hz, NCH₂), 4.93 (1 H, d, *J* = 11.2 Hz, CH₂CH=CH₂), 4.99 (1 H, d, *J* = 18.0 Hz, CH₂CH=CH₂), 5.59 (1 H, d, *J* = 7.0 Hz, C⁵-H), 5.78 (1 H, m, CH₂CH=CH₂), 6.93 (2 H, br s, NH₂), 7.54 (1 H, d, *J* = 7.0 Hz, C⁶-H); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 25.02 (CH₂CH=CH₂), 27.89 (NCH₂CH₂CH₂), 32.55 (NCH₂CH₂), 48.30 (NCH₂), 93.10 (C-5), 114.71 (CH=CH₂), 138.20 (CH=CH₂), 146.45 (C-6), 154.51 (C-2), 164.66 (C-4); MS, *m/e* (rel intensity) 193 (72, M⁺), 152 [67, (M - C₃H₅)⁺], 139 [48, (M - C₄H₆)⁺], 125 [82, (M - C₅H₈)⁺], 111 [100, (M - C₆H₁₀)⁺], 81 (92). HRMS Calcd for C₁₀H₁₅N₃O: 193.1214. Found: 193.1215.

General Procedure for the Preparation of 1-Alkenyl-*N*⁴-tritylcytosines (8a–c). A mixture of 7a–c (10.0 mmol) and trityl bromide (50.0 mmol) was dissolved in dry pyridine (100 mL) and heated at 90 °C for 24 h. After the solvent was evaporated, the product was isolated by column chromatography on silica gel (eluent: 3% CH₃OH in CH₂Cl₂).

(a) **1-Butenyl-*N*⁴-tritylcytosine (8a)**: yield 68%; mp 200–201 °C; ¹H NMR (300 MHz, CDCl₃) δ 2.44 (2 H, q, *J* = 6.9 Hz, CH₂CH=CH₂), 3.74 (2 H, t, *J* = 7.1 Hz, NCH₂), 4.93 (1 H, d, *J* = 7.3 Hz, C⁵-H), 5.02 (1 H, d, *J* = 13.9 Hz, CH₂CH=CH₂), 5.03 (1 H, d, *J* = 9.5 Hz, CH₂CH=CH₂), 5.71 (1 H, m, CH₂CH=CH₂), 6.77 (1 H, br s, NH), 6.86 (1 H, d, *J* = 7.3 Hz, C⁶-H), 7.23–7.34 (15 H, m, tr-H); ¹³C NMR (75 MHz, CDCl₃) δ 32.81 (NCH₂CH₂), 49.24 (NCH₂), 70.70 (CPh), 93.75 (C-5), 117.56 (CH=CH₂), 127.21, 127.98, and 128.46 (Ph), 133.80 (CH=CH₂), 143.89 (Ph), 144.96 (C-6), 155.54 (C-2), 165.40 (C-4); MS, *m/e* (rel intensity) 407 (100, M⁺), 393 [21, (M - CH₂)⁺], 330 [20, (M - Ph)⁺], 258 (23, NHtr⁺), 243 (82, tr⁺), 165 [82, (tr - Ph - H)⁺]. HRMS Calcd for C₂₇H₂₅N₃O: 407.1997. Found: 407.1993.

(b) **1-Pentenyl-*N*⁴-tritylcytosine (8b)**: yield 65%; mp 185–186 °C; ¹H NMR (300 MHz, CDCl₃) δ 1.79 (2 H, m, NCH₂CH₂), 2.03 (2 H, q, *J* = 7.0 Hz, CH₂CH=CH₂), 3.68 (2 H, t, *J* = 7.1 Hz, NCH₂), 4.95 (1 H, d, *J* = 7.3 Hz, C⁵-H), 4.96 (1 H, d, *J* = 10.1 Hz, CH₂CH=CH₂), 4.98 (1 H, d, *J* = 17.8 Hz, CH₂CH=CH₂), 5.78 (1 H, m, CH₂CH=CH₂), 6.78 (1 H, br s, NH), 6.89 (1 H, d, *J* = 7.3 Hz, C⁶-H), 7.23–7.34 (15 H, m, tr-H); ¹³C NMR (75 MHz, CDCl₃) δ 27.97 (NCH₂CH₂CH₂), 30.56 (NCH₂CH₂), 49.67 (NCH₂), 70.93 (CPh), 94.05 (C-5), 115.47 (CH=CH₂), 127.51, 128.28, and 128.74 (Ph), 137.21 (CH=CH₂), 144.16 (Ph), 144.99 (C-6), 155.91 (C-2), 165.62 (C-4); MS, *m/e* (rel intensity) 421 (100, M⁺), 407 [16, (M - CH₂)⁺], 344 [15, (M - Ph)⁺], 258 (17, NHtr⁺), 243 (63, tr⁺), 165 [68, (tr - Ph - H)⁺]. HRMS Calcd for C₂₈H₂₇N₃O: 421.2154. Found: 421.2152.

(c) **1-Hexenyl-*N*⁴-tritylcytosine (8c)**: yield 60%; mp 195–196 °C; ¹H NMR (300 MHz, CDCl₃) δ 1.34 (2 H, m, NCH₂CH₂CH₂), 1.66 (2 H, m, NCH₂CH₂), 2.04 (2 H, q, *J* = 6.9 Hz, CH₂CH=CH₂), 3.68 (2 H, t, *J* = 7.3 Hz, NCH₂), 4.94 (1 H, d, *J* = 7.4 Hz, C⁵-H), 4.94 (1 H, d, *J* = 10.5 Hz, CH₂CH=CH₂), 4.98 (1 H, d, *J* = 13.3 Hz, CH₂CH=CH₂), 5.74 (1 H, m, CH₂CH=CH₂), 6.79 (1 H, br s, NH), 6.89 (1 H, d, *J* = 7.4 Hz, C⁶-H), 7.23–7.35 (15 H, m, tr-H); ¹³C NMR (75 MHz, CDCl₃) δ 25.79 (NH₂CH₂CH₂CH₂), 28.45 (CH₂CH=CH₂), 33.17 (NCH₂CH₂), 50.02 (NCH₂), 70.76 (CPh), 94.05 (C-5), 114.88 (CH=CH₂), 127.47, 128.28, and 128.66 (Ph), 138.10 (CH=CH₂), 144.00 (Ph), 144.91 (C-6), 155.00 (C-2), 165.47 (C-4); MS, *m/e* (rel intensity) 435 (100, M⁺), 421 [25, (M - CH₂)⁺], 358 [15, (M - Ph)⁺], 258 (23, NHtr⁺), 243 (80, tr⁺), 165 [96, (tr - Ph - H)⁺]. HRMS Calcd for C₂₉H₂₉N₃O: 435.2310. Found: 435.2312.

General Procedure for the Preparation of 1-Iodoalkyl-*N*⁴-tritylcytosines (9a–c).¹⁸ The 1-alkenyl-*N*⁴-tritylcytosine (8a–c, 5.0 mmol) and 50 mL of dry THF were placed in a 250-mL N₂-flushed round-bottom flask equipped with a magnetic stirrer, septum inlet, and reflux condenser. The solution was cooled to 0 °C, and 36 mL of a 0.5 M solution of 9-borabicyclo[3.3.1]nonane (9-BBN) in THF was added slowly via a syringe. After 1 h, the ice bath was removed and the reaction mixture was stirred at room temperature for 5 h. To this organoborane solution, anhydrous methanol (30 mL) was added (to destroy the excess hydride). A methanolic solution of sodium acetate (25 mL, 1.0 M) was then added, followed by the dropwise addition of 25 mL of a methanolic solution of chloramine-T (8.15 g, 20 mmol) and then 25 mL of a methanolic solution of NaI (5.4 g, 20 mmol). Following these additions the mixture was stirred for 6 h at room temperature and then concentrated on a rotary evaporator. The residue was taken up into CHCl₃ and water (50 mL of each), and then the mixture was extracted with CHCl₃ (50 mL × 3). The combined CHCl₃ extracts were dried over MgSO₄ and taken to

dryness under reduced pressure. The product was isolated via column chromatography (silica gel, 2% CH₃OH in CH₂Cl₂, eluent).

(a) **1-(4-Iodobutyl)-*N*⁴-tritylcytosine (9a)**: yield 70%; ¹H NMR (300 MHz, CDCl₃) δ 1.79 (4 H, m, NCH₂CH₂ and CH₂CH₂I), 3.17 (2 H, t, *J* = 6.0 Hz, CH₂CH₂I), 3.70 (2 H, t, *J* = 6.1 Hz, NCH₂), 4.98 (1 H, d, *J* = 7.2 Hz, C⁵-H), 6.79 (1 H, br s, NH), 6.91 (1 H, d, *J* = 7.3 Hz, C⁶-H), 7.23–7.34 (15 H, m, tr-H); ¹³C NMR (75 MHz, CDCl₃) δ 5.20 (CH₂I), 29.39 (NCH₂CH₂), 47.99 (NCH₂), 70.40 (CPh), 93.27 (C-5), 126.86, 127.66, 128.12, and 143.54 (Ph), 144.37 (C-6), 155.23 (C-2), 164.92 (C-4); MS, *m/e* (rel intensity) 407 [4, (M - HI)⁺], 244 [100, (tr + H)⁺], 165 [78, (tr - Ph - H)⁺].

(b) **1-(5-Iodopentyl)-*N*⁴-tritylcytosine (9b)**: yield 69%; ¹H NMR (300 MHz, CDCl₃) δ 1.37 (2 H, m, CH₂CH₂CH₂I), 1.68 (2 H, m, CH₂CH₂I), 1.80 (2 H, m, NCH₂CH₂), 3.16 (2 H, t, *J* = 6.8 Hz, CH₂CH₂I), 3.67 (2 H, t, *J* = 7.3 Hz, NCH₂), 4.98 (1 H, d, *J* = 7.3 Hz, C⁵-H), 6.82 (1 H, br s, NH), 6.92 (1 H, d, *J* = 7.3 Hz, C⁶-H), 7.23–7.31 (15 H, m, tr-H); ¹³C NMR (75 MHz, CDCl₃) δ 6.23 (CH₂I), 27.28 (CH₂CH₂I), 27.80 (CH₂CH₂CH₂I), 32.65 (NCH₂CH₂), 49.79 (NCH₂), 70.89 (CPh), 94.18 (C-5), 127.44, 128.22, 128.65, and 144.02 (Ph), 144.80 (C-6), 155.80 (C-2), 165.50 (C-4), MS, *m/e* (rel intensity) 421 [1, (M - HI)⁺], 244 [100, (tr + H)⁺], 165 [96, (tr - Ph - H)⁺].

(c) **1-(6-Iodohexyl)-*N*⁴-tritylcytosine (9c)**: yield 65%; ¹H NMR (300 MHz, CDCl₃) δ 1.28 (2 H, m, NCH₂CH₂CH₂), 1.40 (2 H, m, CH₂CH₂CH₂I), 1.68 (2 H, m, CH₂CH₂I), 1.79 (2 H, m, NCH₂CH₂), 3.15 (2 H, t, *J* = 7.0 Hz, CH₂CH₂I), 3.67 (2 H, t, *J* = 7.5 Hz, NCH₂), 4.96 (1 H, d, *J* = 7.3 Hz, C⁵-H), 6.78 (1 H, br s, NH), 6.90 (1 H, d, *J* = 7.4 Hz, C⁶-H), 7.23–7.34 (15 H, m, tr-H); ¹³C NMR (75 MHz, CDCl₃) δ 6.30 (CH₂I), 25.40 (CH₂CH₂I), 28.80 (CH₂CH₂CH₂I), 29.90 (NCH₂CH₂CH₂), 33.17 (NCH₂CH₂), 49.95 (NCH₂), 70.90 (CPh), 94.14 (C-5), 127.48, 128.27, 128.69, and 144.07 (Ph), 144.81 (C-6), 155.87 (C-2), 165.54 (C-4); MS, *m/e* (rel intensity) 435 (2, M⁺), 244 [100, (tr + H)⁺], 165 [92, (tr - Ph - H)⁺].

Synthesis of *N*⁴-Tritylcytosine Triamine Derivative [4-[(Triphenylmethyl)amino]-1-[4-[(*N,N*-bis[2-(*N,N*-diethylamino)ethyl]amino)butyl]-2(1*H*)-pyrimidinone] (10). A mixture of 1-iodobutyl-*N*⁴-tritylcytosine (9a, 250 mg, 0.47 mmol), bis(diethylamino)ethylamine (276 mg, 0.28 mmol),¹⁹ and potassium carbonate (120 mg, 0.87 mmol) was dissolved in acetonitrile (30 mL) and stirred at room temperature for 24 h. The solvent was then removed under reduced pressure and the product isolated via column chromatography (silica gel, 0.5% concentrated NH₄OH in CH₃OH, eluent): 273 mg, 93%; ¹H NMR (300 MHz, CDCl₃) δ 0.88 (12 H, t, *J* = 7.2 Hz, CH₂CH₃), 1.31 (2 H, m, NCH₂CH₂CH₂), 1.55 (2 H, m, NCH₂CH₂), 2.34 (2 H, t, *J* = 7.2 Hz, CH₂NCH₂), 2.40 (8 H, q, NCH₂CH₃), 2.40 (8 H, m, NCH₂CH₂N), 3.55 (2 H, t, *J* = 7.2 Hz, NCH₂CH₂CH₂), 4.84 (1 H, d, *J* = 7.3 Hz, C⁵-H), 6.67 (1 H, br s, NH), 6.79 (1 H, d, *J* = 7.4 Hz, C⁶-H), 7.11–7.20 (15 H, m, tr-H); ¹³C NMR (75 MHz, CDCl₃) δ 11.34 (NCH₂CH₃), 24.08 (CH₂CH₂CH₂N), 26.64 (NCH₂CH₂CH₂), 47.00 (NCH₂CH₃), 49.77 (CH₂CH₂CH₂N), 50.68 (NCH₂CH₂N), 52.37 (NCH₂CH₂N), 54.24 (NCH₂CH₂CH₂), 70.37 (CPh), 93.67 (C-5), 127.71, 128.25, 127.10, and 143.58 (Ph), 144.70 (C-6), 155.55 (C-2), 165.12 (C-4); FABMS, *m/e* (rel intensity) 624 [100, (M + H)⁺]. HRMS Calcd for C₃₉H₅₅N₆O: 623.4437. Found: 623.4420.

Synthesis of Cytosine Triamine Derivative [4-Amino-1-[4-[(*N,N*-bis[2-(*N,N*-diethylamino)ethyl]amino)butyl]-2(1*H*)-pyrimidinone] (1). The *N*⁴-tritylcytosine triamine derivative (10, 285 mg, 0.46 mmol) was dissolved in trifluoroacetic acid (50 mL) and stirred at room temperature for 6 h. After removal of the solvent on a rotary evaporator, the resulting oil was dissolved in CHCl₃ (50 mL) and then neutralized with aqueous potassium carbonate. After pouring into a separatory funnel, the CHCl₃ layer was collected and washed with 2 N NaOH (4 × 20 mL). It was then dried over potassium carbonate and taken to dryness under reduced pressure. The product was isolated via column chromatography (silica gel, 2.5% concentrated NH₄OH in CH₃OH, eluent). The resulting oil was dissolved in CH₂Cl₂ (5 mL) and was filtered through a membrane filter (Millipore 0.45 μm) to remove any solids (inorganic salts, impurities from silica gel, etc.). The solvent was removed under reduced pressure, and the resulting oil was then dried in vacuo overnight to produce 130 mg of compound 1 as an oil (50%): ¹H NMR (360 MHz, DMSO-*d*₆) δ 1.08 (12 H, t, *J* = 7.2 Hz, CH₂CH₃), 1.18 (2 H, m, NCH₂CH₂CH₂), 1.36 (2 H, m, NCH₂CH₂CH₂), 2.68 (2 H, t, *J* = 7.2 Hz, NCH₂CH₂CH₂CH₂NCH₂), 2.80 (16 H, m, NCH₂CH₂N and NCH₂CH₃), 3.63 (2 H, t, *J* = 7.1 Hz, NCH₂CH₂CH₂), 5.62 (1 H, d, *J* = 7.4 Hz, C⁵-H), 6.90 (1 H, br s, NH₂), 7.10 (1 H, br s, NH₂), 7.56 (1 H, d, *J* = 7.4 Hz, C⁶-H); ¹³C NMR (75 MHz, CD₃OD) δ 11.22 (NCH₂CH₃), 24.72 (CH₂CH₂CH₂N), 27.79 (NCH₂CH₂CH₂), 47.96 (NCH₂CH₃), 50.43 (CH₂CH₂CH₂N), 51.15 (NCH₂CH₂N), 52.53 (NCH₂CH₂N), 55.44 (NCH₂CH₂CH₂), 95.36 (C-5), 147.12 (C-6), 158.69 (C-2), 167.53 (C-4); MS, *m/e* (rel intensity) 380 (100, M⁺). HRMS Calcd (M⁺): 380.3264. Found: 380.3249. Anal. Calcd for

C₂₀H₄₀N₆O: C, 63.12; H, 10.59; N, 22.08. Found: C, 63.22; H, 10.33; N, 22.10. UV-vis (CHCl₃) λ_{max} (ε) = 281.5 nm (6600).

General Procedure for the Preparation of 4-(N⁴-Tritylcytosine) Diethylamine Derivatives (11a-c). The 4-(N⁴-tritylcytosine) diethylamine derivatives (11a-c) were prepared from the 1-iodoalkyl-N⁴-tritylcytosines 9a-c (1.0 mmol), diethylamine (500 mg, 6.85 mmol), and potassium carbonate (280 mg, 2.03 mmol) according to the method described above for the triamine derivative 10. The products were isolated via column chromatography on silica gel using 15–30% CH₃OH in CH₂Cl₂ as the eluents.

(a) 4-[(Triphenylmethyl)amino]-1-[4-(N,N-diethylamino)butyl]-2-(1H)-pyrimidinone (11a): yield 95%; ¹H NMR (300 MHz, CDCl₃) δ 1.10 (6 H, t, J = 7.2 Hz, CH₂CH₃), 1.55 (2 H, m, NCH₂CH₂CH₂), 1.70 (2 H, t of t, J = 7.7, 7.1 Hz, NCH₂CH₂), 2.60 (2 H, t, J = 7.2 Hz, CH₂NCH₂), 2.64 (4 H, q, J = 7.5 Hz, NCH₂CH₃), 3.71 (2 H, t, J = 7.2 Hz, NCH₂CH₂), 4.97 (1 H, d, J = 7.3 Hz, C⁵-H), 6.77 (1 H, br s, NH), 6.95 (1 H, d, J = 7.33 Hz, C⁶-H), 7.22–7.31 (15 H, m, tr-H); ¹³C NMR (75 MHz, CDCl₃) δ 11.18 (NCH₂CH₃), 24.39 (NCH₂CH₂CH₂), 28.73 (NCH₂CH₂), 48.18 (CH₂CH₂N), 48.92 (NCH₂CH₂), 70.75 (CPh), 93.80 (C-5), 127.45, 128.24, 128.66, and 143.96 (Ph), 145.91 (C-6), 156.03 (C-2), 165.52 (C-4). HRMS Calcd for C₃₁H₃₆N₄O: 480.2889. Found: 480.2896.

(b) 4-[(Triphenylmethyl)amino]-1-[5-(N,N-diethylamino)pentyl]-2-(1H)-pyrimidinone (11b): yield 92%; ¹H NMR (300 MHz, CDCl₃) δ 0.96 (6 H, t, J = 7.3 Hz, NCH₂CH₃), 1.20 (2 H, m, NCH₂CH₂CH₂), 1.41 (2 H, m, CH₂CH₂NCH₂), 1.63 (2 H, m, NCH₂CH₂), 2.35 (2 H, t, J = 7.7 Hz, CH₂NCH₂), 2.47 (4 H, t, J = 7.2 Hz, NCH₂CH₂), 3.61 (2 H, t, J = 7.1 Hz, NCH₂CH₂), 4.92 (1 H, d, J = 7.2 Hz, C⁵-H), 6.86 (1 H, br s, NH), 6.85 (1 H, d, J = 7.2 Hz, C⁶-H), 7.18–7.25 (15 H, m, tr-H); ¹³C NMR (75 MHz, CDCl₃) δ 10.97 (NCH₂CH₃), 24.39 (NCH₂CH₂CH₂), 25.89 (CH₂CH₂N), 28.73 (NCH₂CH₂), 46.46 (NCH₂CH₂CH₂), 49.94 (CH₂CH₂N), 52.25 (NCH₂CH₂), 70.62 (CPh), 94.11 (C-5), 127.30, 128.10, 128.49, and 143.76 (Ph), 144.86 (C-6), 155.96 (C-2), 165.40 (C-4). HRMS Calcd for C₃₂H₃₈N₄O: 494.3045. Found: 494.3053.

(c) 4-[(Triphenylmethyl)amino]-1-[6-(N,N-diethylamino)hexyl]-2-(1H)-pyrimidinone (11c): yield 85%; ¹H NMR (300 MHz, CDCl₃) δ 0.99 (6 H, t, J = 7.3 Hz, NCH₂CH₃), 1.25 (4 H, m, NCH₂CH₂CH₂ and NCH₂CH₂CH₂CH₂), 1.41 (2 H, m, CH₂CH₂NCH₂), 1.63 (2 H, m, NCH₂CH₂), 2.37 (2 H, t, J = 7.7 Hz, CH₂NCH₂), 2.50 (4 H, t, J = 7.1 Hz, NCH₂CH₂), 3.63 (2 H, q, J = 7.1 Hz, NCH₂CH₃), 4.93 (1 H, d, J = 7.2 Hz, C⁵-H), 6.87 (1 H, br s, NH), 6.87 (1 H, d, J = 7.2 Hz, C⁶-H), 7.20–7.30 (15 H, m, tr-H); ¹³C NMR (75 MHz, CDCl₃) δ 11.12 (NCH₂CH₃), 26.32 (CH₂CH₂CH₂N), 26.41 (NCH₂CH₂CH₂), 27.10 (CH₂CH₂N), 28.91 (NCH₂CH₂), 46.58 (NCH₂CH₂), 50.23 (CH₂CH₂N), 52.53 (NCH₂CH₂), 70.71 (CPh), 94.12 (C-5), 127.40, 128.19, 128.57, and 143.88 (Ph), 144.88 (C-6), 156.00 (C-2), 165.45 (C-4). HRMS Calcd for C₃₃H₄₀N₄O: 508.3202. Found: 508.3195.

General Procedure for the Preparation of Cytosine Monoamine Derivatives (2a-c). The N⁴-tritylcytosine diethylamine derivatives 11a-c were deprotected with trifluoroacetic acid according to the method described above for the triamine derivative 10. The products were purified by column chromatography on silica gel using 2% concentrated NH₄OH in CH₃OH as the eluents and recrystallized from CHCl₃-hexane.

(a) 4-Amino-1-[4-(N,N-diethylamino)butyl]-2(1H)-pyrimidinone (2a): yield 46%; mp 220–221 °C; ¹H NMR (300 MHz, CDCl₃) δ 0.90 (6 H, t, J = 7.1 Hz, CH₂CH₃), 1.30 (2 H, m, NCH₂CH₂CH₂), 1.52 (2 H, m, NCH₂CH₂), 2.31 (2 H, t, J = 7.2 Hz, CH₂NCH₂), 2.39 (4 H, q, J = 7.1 Hz, NCH₂CH₃), 3.60 (2 H, t, J = 7.1 Hz, NCH₂CH₂), 5.60 (1 H, d, J = 7.2 Hz, C⁵-H), 6.90 (2 H, br s, NH₂), 7.54 (1 H, d, J = 7.2 Hz, C⁶-H); ¹³C NMR (75 MHz, CD₃OD) δ 11.25 (NCH₂CH₃), 24.18 (CH₂CH₂N), 28.30 (NCH₂CH₂), 47.59 (NCH₂CH₃), 50.72 (CH₂CH₂N), 53.27 (NCH₂CH₂), 95.71 (C-5), 147.38 (C-6), 158.99 (C-2), 167.85 (C-4); MS, *m/e* (rel intensity) 238 [24, (M + H)⁺], 209 [57, (M - C₂H₅)⁺], 166 [80, (M - N(C₂H₅)₂)⁺], 112 [54, (M - C₆H₁₃N(C₂H₅)₂)⁺], 86 (100). HRMS Calcd (M⁺) 238.1794. Found: 238.1797. Anal. Calcd for C₁₂H₂₂N₄O: C, 60.48; H, 9.30; N, 23.51. Found: C, 60.53; H, 9.30; N, 23.48. UV-vis (CHCl₃): λ_{max} (ε) = 281.5 nm (6110).

(b) 4-Amino-1-[5-(N,N-diethylamino)pentyl]-2(1H)-pyrimidinone (2b): yield 60%; mp 213–216 °C; ¹H NMR (300 MHz, CDCl₃) δ 0.91 (6 H, t, J = 7.1 Hz, CH₂CH₃), 1.20 (2 H, m, NCH₂CH₂CH₂), 1.35 (2 H, m, CH₂CH₂NCH₂), 1.53 (2 H, m, NCH₂CH₂), 2.30 (2 H, t, J = 7.2 Hz, CH₂NCH₂), 2.40 (4 H, q, J = 7.1 Hz, NCH₂CH₃), 3.58 (2 H, t, J = 7.2 Hz, NCH₂CH₂), 5.59 (1 H, d, J = 7.1 Hz, C⁵-H), 6.89 (2 H, br s, NH₂), 7.54 (1 H, d, J = 7.0 Hz, C⁶-H); ¹³C NMR (75 MHz, CD₃OD) δ 10.98 (NCH₂CH₃), 25.44 (CH₂CH₂CH₂N), 26.36 (CH₂CH₂N), 29.91 (NCH₂CH₂), 47.47 (NCH₂CH₃), 50.86 (CH₂CH₂N), 53.23 (NCH₂CH₂), 95.87 (C-5), 147.53 (C-6), 159.01 (C-2), 167.71 (C-4); MS, *m/e* (rel intensity) 253 [12, (M + H)⁺], 223 [41, (M - C₂H₅)⁺], 180 [16, (M - N(C₂H₅)₂)⁺], 112 [34, (M - C₅H₁₀N(C₂H₅)₂)⁺], 86 (100). HRMS

Calcd (M⁺): 252.1950. Found: 252.1969. Anal. Calcd for C₁₃H₂₄N₄O: C, 61.87; H, 9.59; N, 22.20. Found: C, 61.79; H, 9.56; N, 22.19. UV-vis (CHCl₃): λ_{max} (ε) = 281.5 nm (6960).

(c) 4-Amino-1-[6-(N,N-diethylamino)hexyl]-2(1H)-pyrimidinone (2c): yield 45%; mp 200–202 °C; ¹H NMR (CD₃OD) δ 0.91 (6 H, t, J = 7.1 Hz, CH₂CH₃), 1.21 (4 H, m, NCH₂CH₂CH₂ and CH₂CH₂CH₂NCH₂), 1.36 (2 H, m, CH₂CH₂NCH₂), 1.56 (2 H, m, NCH₂CH₂), 2.34 (2 H, t, J = 7.2 Hz, CH₂NCH₂), 2.45 (4 H, q, J = 7.1 Hz, NCH₂CH₃), 3.62 (2 H, t, J = 7.2 Hz, NCH₂CH₂), 5.72 (1 H, d, J = 7.1 Hz, C⁵-H), 7.42 (1 H, d, J = 7.0 Hz, C⁶-H); ¹³C NMR (75 MHz, CD₃OD) δ 11.10 (NCH₂CH₃), 26.82 (CH₂CH₂CH₂N), 27.43 (NCH₂CH₂CH₂), 28.32 (CH₂CH₂N), 30.12 (NCH₂CH₂), 47.62 (NCH₂CH₃), 50.88 (CH₂CH₂N), 53.52 (NCH₂CH₂), 95.65 (C-5), 147.45 (C-6), 159.06 (C-2), 167.83 (C-4); MS, *m/e* (rel intensity) 267 [4, (M + H)⁺], 251 [9, (M - CH₃)⁺], 237 [35, (M - C₂H₅)⁺], 194 [10, (M - N(C₂H₅)₂)⁺], 112 [11, (M - C₆H₁₂N(C₂H₅)₂)⁺], 86 (100). HRMS Calcd (M⁺): 266.2107. Found: 266.2099. Anal. Calcd for C₁₄H₂₆N₄O: C, 63.12; H, 9.84; N, 21.03. Found: C, 62.80; H, 9.78; N, 20.65. UV-vis (CHCl₃): λ_{max} (ε) = 281.5 nm (6660).

Synthesis of 4-Amino-1-(5-ethylheptyl)-2(1H)-pyrimidinone (3). The amine-free cytosine alkyl derivative, 4-amino-1-(5-ethylheptyl)-2(1H)-pyrimidinone (3), was obtained by coupling the sodium salt of cytosine with 5-ethyl-1-iodoheptane in DMF. 5-Ethyl-1-iodoheptane in turn was prepared from the corresponding alcohol via the mesylate.

5-Ethylheptanol was synthesized from the reaction of 2-ethyl-1-bromobutane (28.5 g, 0.172 mol), magnesium (4.60 g, 0.189 mol), and oxetane (10.0 g, 0.172 mol) in dry ether under conditions similar to those reported by Prout and Cason.²⁰ In this way, 3.0 g of 5-ethylheptanol was obtained (12%); bp 95–100 °C/18 mmHg; ¹H NMR (300 MHz, CDCl₃) δ 0.82 (6 H, t, J = 7.3 Hz, CH₂CH₃), 1.17–1.29 (9 H, m, CHCH₂CH₃, CH₂CH₂CH₂CH, and CH₂CH₃), 1.54 (2 H, t of t, J = 6.6, 6.8 Hz, CH₂CH₂OH), 2.16 (1 H, br s, OH), 3.63 (2 H, t, J = 6.5 Hz, CH₂OH); ¹³C NMR (75 MHz, CDCl₃) δ 10.82 (CHCH₂CH₃), 22.85 (CHCH₂CH₃), 25.28 (CH₂CH₂CH), 32.46 (CH₂CH₂CH), 33.14 (CH₂CH₂OH), 40.26 (CH), 62.90 (CH₂CH₂OH).

5-Ethylheptanol (3 g, 0.021 mol) was then transformed into the corresponding mesylate in dry CH₂Cl₂ (50 mL) by treating with methanesulfonyl chloride (11.5 g, 0.10 mol) in the presence of triethylamine (10.1 g, 0.10 mol). The product, 5-ethylheptyl mesylate, was then isolated via column chromatography (silica gel, CH₂Cl₂, eluent): 2.7 g (70%); oil; ¹H NMR (300 MHz, CDCl₃) δ 0.82 (6 H, t, J = 7.3 Hz, CH₂CH₃), 1.10–1.40 (9 H, m, CHCH₂CH₃, CH₂CH₂CH₂CH, and CH₂CH₃), 1.72 (2 H, t of t, J = 6.6, 6.8 Hz, CH₂CH₂O), 2.97 (3 H, s, mesyl), 4.19 (2 H, t, J = 6.8 Hz, CH₂O); ¹³C NMR (75 MHz, CDCl₃) δ 10.72 (CHCH₂CH₃), 22.52 (CHCH₂CH₃), 25.14 (CH₂CH₂CH), 29.41 (CH₂CH₂CH), 32.00 (OCH₂CH₂), 37.17 (mesyl), 40.05 (CH), 70.17 (CH₂CH₂O).

5-Ethyl-1-iodoheptane was obtained by heating the acetone solution (50 mL) of 5-ethylheptyl mesylate (0.50 g, 2.6 mmol) with NaI (15.0 g, 0.10 mol) at reflux for 20 h. After cooling, the solvent was removed under reduced pressure and the residue was dissolved in CHCl₃ (30 mL), washed with water, and dried over sodium sulfate. The solvent was then removed on a rotary evaporator to give the product as an oil: 0.60 g (90%); ¹H NMR (300 MHz, CDCl₃) δ 0.84 (6 H, t, J = 7.3 Hz, CH₂CH₃), 1.10–1.40 (9 H, m, CHCH₂CH₃, CH₂CH₂CH₂CH, and CH₂CH₃), 1.80 (2 H, t of t, J = 6.5, 6.7 Hz, CH₂CH₂I), 3.18 (2 H, t, J = 7.2 Hz, CH₂I); ¹³C NMR (75 MHz, CDCl₃) δ 7.15 (CH₂I), 10.80 (CHCH₂CH₃), 25.23 (CHCH₂CH₃), 27.61 (CH₂CH₂CH), 31.49 (CH₂CH₂CH), 33.89 (ICH₂CH₂), 40.03 (CH).

4-Amino-1-(5-ethylheptyl)-2(1H)-pyrimidinone (3) was synthesized as follows: Dry cytosine (111 mg, 1.00 mmol) and sodium hydride (26.9 mg, 1.12 mmol) were dissolved in dry DMF (15 mL), and the resulting solution was stirred at room temperature for 1 h. After the addition of 5-ethyl-1-iodoheptane (0.31 g, 1.2 mmol), the solution was then heated at 80 °C for 20 h. After the solvent was removed by vacuum distillation, the resulting solid was purified by column chromatography (silica gel, 10% CH₃OH in CH₂Cl₂ as eluent) to provide 51 mg of compound 3 (22%); mp 247–248 °C; ¹H NMR [300 MHz, CDCl₃-CD₃O (1:1)] δ 0.79 (3 H, t, J = 7.3 Hz, CH₂CH₃), 1.19 (1 H, m, CHCH₂CH₃), 1.24 (8 H, m, NCH₂CH₂CH₂CH₂ and CH₂CH₃), 1.63 (2 H, m, NCH₂CH₂), 3.71 (2 H, t, J = 7.40 Hz, NCH₂), 5.35 (2 H, s, NH₂), 5.79 (1 H, d, J = 7.0 Hz, C⁵-H), 7.38 (1 H, d, J = 7.2 Hz, C⁶-H); ¹³C NMR (75 MHz, CDCl₃) δ 11.22 (CHCH₂CH₃), 24.80 (CHCH₂CH₃), 26.42 (CH₂CH₂CH), 30.65 (CH₂CH₂CH), 33.54 (NCH₂CH₂), 41.72 (CH), 51.07 (NCH₂CH₂), 95.60 (C-5), 147.48 (C-6), 159.11 (C-2), 167.87 (C-4); MS, *m/e* (rel intensity) 237 (78, M⁺), 222 [37, (M - CH₃)⁺], 208 [92, (M - C₂H₅)⁺], 166 [100, (M - CH(C₂H₅)₂)⁺], 152 [80, (M - C₆H₁₃)⁺], 138 [49, (M - C₇H₁₅)⁺], 125 [75, (M - C₈H₁₆)⁺], 112 [75, (M - C₉H₁₈)⁺]. HRMS Calcd (M⁺): 237.1841. Found: 237.1815. Anal. Calcd for C₁₃H₂₃N₃O: C, 65.79; H, 9.77; N, 17.70. Found: C, 65.73; H, 9.70; N, 17.64. UV-vis (CHCl₃): λ_{max} (ε) = 281.5 (6290).

Determination of Binding Constants. A typical experiment would consist of adding 10 separate 5- μ L aliquots of a 100 mM solution of **1** (or **2a-c** or **3**) in DMSO- d_6 to 500 μ L of a 5 mM solution of GMP free acid in DMSO- d_6 and recording the chemical shift changes in the guanine imino and amino protons. Data reduction for compounds **1** and **2a-c** was then effected by using the least-squares NMR curve fitting program of Whitlock.²⁴ As this program provides no built-in error analysis function, errors were estimated by carrying out a "dummy" calculation on the extreme range of experimental values obtained from two separate (and independent) titrations. Unless otherwise indicated, the reported values are considered to be accurate to within $\pm 15\%$. In the case of **3**, data reduction was effected by using a standard Scatchard plot.^{22,23} Now, however, because of the low value involved, the errors are considered to be significantly larger, being on the order of 40%.

Acknowledgment. This research was supported by funds pro-

vided by the Texas Advanced Research Program (Grants 3658-016 and 4549). J.L.S. acknowledges support from the Dreyfus Foundation in the form of a Teacher-Scholar Award, the Sloan Foundation in the form of a Fellowship, and the National Science Foundation (Presidential Young Investigator Award, 1986). We thank Prof. Eric Anslyn of this department for helpful discussions.

Registry No. **1**, 130798-30-0; **1-GMP**, 130798-36-6; **2a**, 130798-31-1; **2a-¹/₂GMP**, 130798-35-5; **2b**, 130798-32-2; **2**, 130798-33-3; **3**, 130798-34-4; **4**, 71-30-7; **5a**, 130798-15-1; **5b**, 130798-16-2; **5c**, 130798-17-3; **6a**, 130798-18-4; **6b**, 130798-19-5; **6c**, 130798-20-8; **7a**, 130798-21-9; **7b**, 130798-22-0; **7c**, 130798-23-1; **8a**, 130798-24-2; **8b**, 130798-25-3; **8c**, 130798-26-4; **9a**, 130798-27-5; **9b**, 130798-28-6; **9c**, 130798-29-7; **10**, 130798-37-7; **11a**, 130798-38-8; **11b**, 130798-39-9; **11c**, 130798-40-2; **GMP**, 85-32-5.

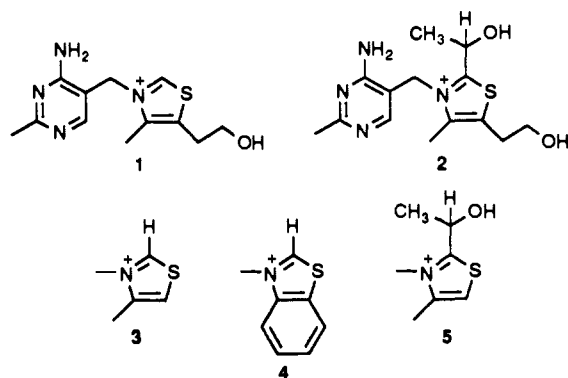
Acidities of C2 Hydrogen Atoms in Thiazolium Cations and Reactivities of Their Conjugate Bases

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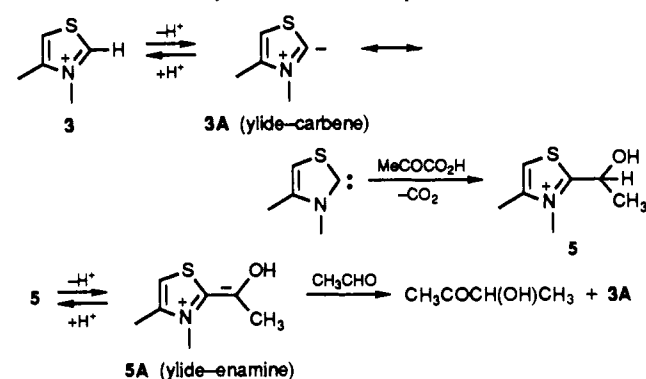
Contribution from the Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3113. Received January 8, 1990

Abstract: The equilibrium acidity for the C2-H bond in the 3,4-dimethylthiazolium cation (TZCH⁺), a model for thiamin, was estimated to be higher than 16 by direct titrations with indicators in DMSO solution. This result is in agreement with several earlier indirect estimates based on kinetic acidities and Brønsted plots, which place the acidity in the 16-20 pK_{HA}⁺ region in aqueous solution, but not with a direct titration in aqueous medium made under stopped-flow conditions, which placed the acidity in the region of pK_{HA}⁺ \approx 13. 3-Methylbenzothiazolium cation (BZCH⁺), which was found to be considerably more acidic, reacted with Et₃N in DMSO to give a dimer BZC=CZB. Evidence is presented to show that this and similar dimerizations occur by addition of the conjugate base of BZCH⁺ to the H-C=N⁺ bond of the BZCH⁺ thiazolium cation, followed by deprotonation. The conjugate base of BZCH⁺ adds to BZCH⁺ in preference to reacting with excess of electrophiles such as *t*-BuOH, PhCHO, or PhCH=CH₂. Amines, such as piperidine, add rapidly to less acidic thiazolium cations under conditions where little or no deprotonation occurs. These observations exclude a carbene mechanism for dimerization and amine adduct formation.

The conjugate bases obtained by removing the acidic C2 and C2 α hydrogen atoms, respectively, from the thiazolium cation moieties of thiamin (**1**) and 2-(α -hydroxyethyl)thiamin (**2**) are effective in both enzymic and nonenzymic catalysis.^{1,2} The acidities of **1** and **2** and those of related models, such as 3,4-dimethylthiazolium cation (**3**), 3-methylbenzothiazolium cation (**4**), and 2-(α -hydroxyethyl)-3,4-dimethylthiazolium cation (**5**), and the reactivities of their conjugate bases have therefore been a matter of considerable interest for the past three decades.²



Scheme I. Base-Catalyzed Reactions in Aqueous Buffers



The reactions by which the vitamin B₁ enzyme cofactors **1** and **2** exert their catalytic activity are illustrated with thiazolium ions **3** and **5** in Scheme I.

The first of these reactions is the catalytic decarboxylation of pyruvic acid, which is a primary function of thiamin pyrophosphate in nature. The product of this decarboxylation is 5, which can either react with base to eliminate acetaldehyde and regenerate the ylide-carbene **3A** or be transformed in several steps to acetoin with the regeneration of **3A**. The ability of thiazolium cation models, such as **3**, to play the catalytic roles of **1** and **2** in the presence of basic aqueous buffers has been demonstrated in several investigations.^{1,2} Furthermore, thiazolium cations, such as **3-**

(1) Breslow, R. *J. Am. Chem. Soc.* **1958**, *80*, 3719-3726.

(2) For reviews, (see: (a) Gallo, A. A.; Mical, J. J.; Sable, H. Z. In *Bioorganic Chemistry*; van Tamelen, E. E., Ed.; Academic Press: New York, 1978; Vol. 4, pp 147-177. (b) Kluger, R. *Chem. Rev.* **1987**, *87*, 863-876.