7-Methylguanine Nucleotides and Their Structural Analogues; Protolytic Equilibria, Complexing with Magnesium(II) Ion and Kinetics for Alkaline Opening of the Imidazole Ring

Edward Darzynkiewicz,[a] Imre Labadi,[b] Dorota Haber,[a] Kalman Burger[b] and Harri Lönnberg[c,*]

[a]Department of Biophysics, University of Warsaw, 02-089 Warsaw, Poland, [b]Department of Inorganic and Analytical Chemistry, Attila Jozsef University, H-6701 Szeged, Hungary and [c]Department of Chemistry, University of Turku, SF-20500 Turku, Finland

Darzynkiewicz, E., Labadi, I., Haber, D., Burger, K. and Lönnberg, H., 1988. 7-Methylguanine Nucleotides and Their Structural Analogues; Protolytic Equilibria, Complexing with Magnesium(II) Ion and Kinetics for Alkaline Opening of the Imidazole Ring. – Acta Chem. Scand., Ser. B 42: 86–92.

First-order rate constants for the alkaline opening of the imidazole ring of several 7-methylguanine nucleotides and their structural analogues were determined. The results obtained suggested that intramolecular interaction between the negatively charged 5′-phosphate group and the positively charged imidazole ring markedly retard the attack of hydroxide ion on the C8 atom of the 7-methylguanine ring. In contrast, hardly any influence on the acidities of the interacting base and phosphate moieties was detectable. No effect on the complexing of the phosphate group with magnesium(II) ion could be detected.

The 5′-terminus of mRNA's of eukaryotic cells consists of 7-methylgyuanosine linked by a 5′,5′-triphosphate bridge to the next nucleoside.[1,2] This cap structure binds specifically to proteins known as cap-binding proteins, which enhance the attachment of mRNA to the 40S ribosomal subunits.[3] 7-Methylguanosine 5′-monophosphate and several related compounds have been shown to compete with capped mRNAs for the cap-binding proteins, binding most probably to the same site.[4–8] These cap analogues all exhibit a common structural feature. An electrostatic interaction between the positively charged imidazole ring and negatively charged 5′-substituent has been assumed to stabilize a particular spatial configuration needed for optimal binding.[4,8] ^{1}H NMR spectroscopic studies have provided considerable evidence for the proposed conformational rigidity.[7–10] In contrast, data elucidating the influences on the chemical properties of the interacting base and phosphate moieties are limited to the observations of Hendler *et al.*, according to which phosphate groups on the ribofuranosyl ring lower the acidity and the rate of alkaline ring-opening of 7-methylguanosine.[11] The aim of the present study was to provide more extensive data on the effects that mutual interactions between the base and glycone moieties may have on their chemical behaviour. For this purpose the following aspects were studied: (i) the effect of glycone moiety structure on the rate of the alkaline ring-opening, (ii) the effect of ionic strength on the rate-retardations caused by negatively charged 5′-substituents, (iii) the effects of glycone moiety structure on the acidity of the base moiety in the absence and the presence of magnesium(II) ion, (iv) the effect of 7-methylation on the acidity of the glycone moiety phosphate groups, and (v) the effect of 7-methylation on the complexing of the phosphate groups with magnesium(II) ion. The strength of the intramolecular interaction is discussed on the basis of the data obtained.

*To whom correspondence should be addressed.

Results and discussion

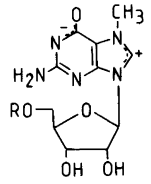
The alkaline decomposition of 7-methylguanosine has been shown to proceed by initial attack of hydroxide ion on the C8 atom of the base moiety, followed by rapid subsequent opening of the imidazole ring.[12–14] HPLC analyses of aliquots withdrawn at different invervals from alkaline solutions of 7-methylguanosine revealed that this first partial reaction of the multi-step pathway is practically irreversible. The starting material had completely disappeared before the concentration of the resulting intermediate, viz. a mixture of N^5 and N^6 formylated 2-amino-5-methylamino-6-ribosylaminopyrimidin-4(3*H*)-one,[14] began to decrease. As seen from Table 1, the reaction is first-order with respect to hydroxide ion. No catalysis by buffer constituents was observed. The rate constants obtained using 1:1 buffers of triethylamine and triethylammonium chloride (at an ionic strength of 0.50 mol dm^{-3}, adjusted with sodium chloride) were independent of the buffer concentration between 0.020 and 0.40 mol dm^{-3}.

Table 1 summarizes the pseudo first-order rate

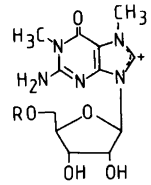
Table 1. Rate constants for the opening of the imidazole ring of 7-methylguanosine cap analogues in alkaline solutions at 298.2 K.[a]

Compound	[OH]/10^{-3} mol dm^{-3}	k^b/10^{-3} s^{-1}	$k(OH^-)$/dm^3 mol^{-1} s^{-1}	k(rel.)
1a	20	22.0(2)	1.10(1)	1
	10	11.0(1)		
	5.0	5.72(9)		
	0.93	1.08(3)		
	0.19	0.308(2)		
	0.032	0.052(1)		
1b	20	11.6(1)	0.58(1)	0.53
1c	20	5.88(6)	0.29(1)	0.26
1d	20	5.58(12)	0.28(1)	0.25
	10	3.07(3)		
	5.0	1.64(2)		
1e	20	2.19(3)	0.110(2)	0.10
	10	1.05(2)		
	5.0	0.525(11)		
1f	20	3.13(4)	0.158(4)	0.14
	5.0	0.710(7)		
1g	20	2.02(2)	0.102(3)	0.093
	5.0	0.448(5)		
2a	0.19	12.8(3)	68(1)	62
	0.032	1.95(3)		
2b	0.93	6.28(5)	6.6(4)	6.0
	0.19	1.67(2)		
3	20	1.10(3)	0.056(2)	0.051
	5.0	0.240(2)		
4a	20	1.27(2)	0.064(3)	0.058
	5.0	0.262(4)		
4b	20	2.84(3)	0.142(2)	0.13
5a	20	8.54(10)	0.43(1)	0.39
	5.0	1.89(3)		
5b	20	10.4(2)	0.52(2)	0.47
	5.0	2.29(3)		
6	20	30.2(3)	1.51(2)	1.4

[a]The concentration of hydroxide ion was adjusted with sodium hydroxide (0.005–0.02 mol dm^{-3}), a triethylamine/triethylammonium chloride buffer (1.9–9.3×10^{-4} mol dm^{-3}), or a glycine/glycine hydrochloride buffer (3.2×10^{-5} mol dm^{-3}). The ionic strength was adjusted to 0.10 mol dm^{-3} with sodium chloride. [b]Observed first-order rate constant. [c]Second-order rate constant.



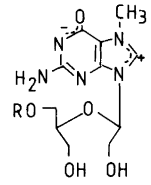
1a: R = H
1b: R = $PO(NH_2)_2$
1c: R = $PO(CH_3)O^-$
1d: R = $PO(OCH_3)O^-$
1e: R = $PO(O^-)_2$
1f: R = $PO(O^-)OPO(O^-)OCH_3$
1g: R = $PO(O^-)OPO(O^-)OPO(O^-)_2$



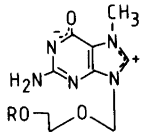
2a: R = H
2b: R = $PO(O^-)_2$

constants obtained spectrophotometrically for the alkaline ring-opening of the 7-methylguanosine cap analogues investigated. The data clearly indicate that negatively charged 5′-substituents markedly retard the attack of hydroxide ion on the C8 atom of the base moiety. For example, the relative rate constants for the cleavage of 7-methylguanosine (**1a**) and its 5′-$OPO(NH_2)_2$ (**1b**), 5′-$OPO(CH_3)O^-$ (**1c**), 5′$OPO(OCH_3)O^-$ (**1d**) and 5′-$OPO(O^-)_2$ (**1e**) derivatives are 1, 0.53, 0.26, 0.25 and 0.10, respectively. Analogously, 1,7-dimethylguanosine 5′-monophosphate (**2b**) undergoes alkaline ring-opening 10 times more slowly than 1,7-dimethylguanosine (**2a**). The rate-retardation thus correlates with the charge type rather than with the size of the 5′-substituent, although the lower reactivity of **1b** than of **1a** suggests that steric factors may contribute. The 60-fold rate-acceleration on going from **1a** to **2a** is expected, since the latter compound does not contain an ionizable proton at N1; hence, its base moiety is in fact positively charged and not a zwitterion as with **1a**.

The data in Table 1 also shows that the charge shielding is not enhanced with increasing negative charge of the 5′-substituent if the charge is distributed over several phosphate groups. Accordingly, 7-methylguanosine 5′-diphosphate β-methyl ester (**1f**) and 5′-triphosphate (**1g**) react with hydroxide ion approximately as rapidly as the 5′-monophosphate (**1e**). In contrast, it is interesting to note that the rate-retarding effect is enhanced when the ribofuranosyl moiety is replaced by an open-chain structure. For example,



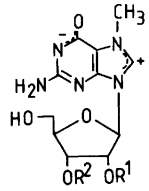
3: R = $PO(O^-)_2$



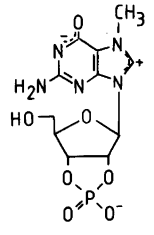
4a: R = $PO(O^-)_2$
4b: R = $PO(CH_3)O^-$

the *seco* (**3**) and *acyclo* (**4a**) analogues of 7-methylguanosine 5′-monophosphate exhibit relative rate constants of 0.046 and 0.052, respectively. A comparable decrease in reactivity takes place on going from **1c** to the corresponding *acyclo* derivative (**4b**). Accordingly, the electronic interaction between the cationic imidazole ring and anionic 5′-substituent seems to be strong enough to keep the latter in the proximity of the C8 atom even when the glycone moiety is acyclic. With **1e** and some of its analogues a strong preference for this kind of g^+ conformation of the exocyclic hydroxymethyl group has been demonstrated by NMR spectroscopy.[7–10]

The rate-retarding influence of the 5′-monophosphate group is not transmitted through bonds. As seen from Table 1, and reported earlier by Hendler *et al.*,[11] 7-methylguanosine 2′- and 3′-monophosphates (**5a**,**b**) also undergo al-



5a: $R^1 = PO(O^-)_2$, $R^2 = H$
5b: $R^1 = H$, $R^2 = PO(O^-)_2$



6

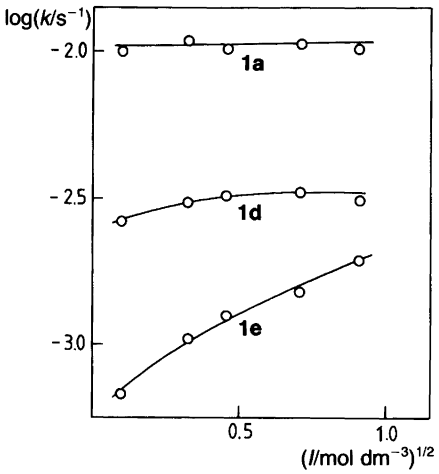


Fig. 1. The effect of ionic strength on the first-order rate constants for the disappearance of 7-methylguanosine (**1a**), its 5′-monophosphate (**1e**) and 5′-monophosphate methyl ester (**1d**) in aqueous sodium hydroxide (0.010 mol dm^{-3}) at 298.2 K. The ionic strength was adjusted with sodium chloride.

Table 2. Acidity constants, K_1, for the base moiety of 7-methylguanosine cap analogues in the absence and presence of magnesium(II) ion at 298.2 K[a]

Compound	$-\log(K_1/\text{mol dm}^{-3})$	
	Absent[b]	Present[c]
1a	7.19(2)	7.15(2)
1d	7.19(3)	7.13(2)
1e	7.37(3)	7.25(3)
5a	7.28(6)	7.33(6)
5b	7.33(6)	7.31(6)
6	7.10(3)	7.08(4)

[a]The ionic strength was adjusted to 1.0 mol dm^{-3} with tetramethylammonium chloride. The values reported are the means of the results obtained by spectrophotometric and potentiometric measurements. [b]In the absence of magnesium(II) chloride. [c]In the presence of magnesium(II) chloride (0.10 mol dm^{-3}).

kaline ring-opening less readily than **1a**, but the influence of the 2′- and 3′-phosphates is considerably smaller than that of the 5′-phosphate. A cyclic 2′,3′-phosphate group (**6**) is even slightly rate-accelerating. Conformational analysis by ^{1}H NMR spectroscopy has shown that the proportion of the g^+ rotamers with this compound is smaller[15] (53 %) than with **5a**, **5b** or **1e** (61, 68 and 87 %, respectively), and the steric hindrance to the attack of hydroxide ion may thus be reduced.

The influence of ionic strength on the rate of the alkaline ring-opening of **1a**, **1d** and **1e** lends further support to the proposal that intramolecular interactions are responsible for the rate-retardations observed. Fig. 1 shows the rate constants obtained at different concentrations of sodium chloride. The rate of reaction of **1a** with hydroxide ion is rather insensitive to an increase in ionic strength, as expected for a reaction between a neutral and a charged species. In contrast, the alkaline ring-opening of **1e** exhibits a large positive salt effect. It is probable that intermolecular interactions between 5′-phosphate group and sodium ions weaken the intramolecular interactions, and thereby diminish the rate-retarding effect of the 5′-phosphate. However, reactions between negatively charged species, such as **1e** and hydroxide ion, usually show positive salt effects. Accordingly, the data in Fig. 1 are consistent with, but not a conclusive proof of, the suggested rate-retarding effect of intramolecular interactions.

The intramolecular interactions that retard the attack of hydroxide ion could also be expected to affect the electron density of the interacting base and phosphate moieties, and thus their acidity. Table 2 lists the acidity constants, K_1, for the base moieties of several derivatives of 7-methylguanosine. As seen, 2′-, 3′- and 5′-phosphate groups all reduce the acidity of the N1 proton by 0.1 to 0.2 logarithmic units, the influence of the 5′-group being largest. The effects of a cyclic 2′,3′-phosphate group and 5′-phosphate methyl ester are negligible. Accordingly, the effects on the base moiety deprotonation correlate with those on the alkaline ring-opening, but the differences between the individual acidity constants are too small to allow firm conclusions to be drawn.

Magnesium(II) chloride (0.10 mol dm^{-3}) had practically no effect on the acidity of the base moiety (Table 2), indicating that magnesium(II) ion does not coordinate to the 7-methylguanine ring. Only with 7-methylguanosine 5′-monophosphate (**1e**) did the acidity constants obtained in the absence and presence of magnesium(II) chloride differ appreciably (by 0.1 logarithmic units).

Table 3. Acidity constants, K_2, for the phosphate groups of guanine and 7-methylguanine nucleotides, and the stability constants, K_3, of their magnesium(II) ion complexes at 298.2 K.[a]

Compound	$-\log(K_2/\text{mol dm}^{-3})$	$-\log(K_3/\text{mol dm}^{-3})$
1e	6.15(2)	1.29(5)
2b	6.15(5)	1.28(5)
5a	5.85(5)	1.30(8)
5b	5.96(5)	1.22(7)
7a	5.99(5)	1.18(5)
7b	5.90(2)	1.24(7)
7c	6.38(5)	1.18(5)

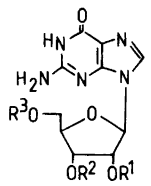
[a]The ionic strength was adjusted to 1.0 mol dm^{-3} with tetramethylammonium chloride. The values reported were obtained by potentiometric measurements.

It is possible that complexing of the 5′-phosphate dianion with magnesium(II) ion weakens its interaction with the base moiety, thereby decreasing the electron density of the purine ring and enhancing deprotonation.

The acidity constants, $K_{2'}$, for the phosphate groups of various guanosine monophosphates (**7a–c**) and their 7-methylated counterparts (**5a**, **5b**, **1e**) are listed in Table 3. It is clearly seen that methylation of N7 does not affect the acidity of the 2′- and 3′-phosphate groups. The deprotonation of the 5′-phosphate is facilitated slightly, but the effect on the acidity constant is only about 0.2 logarithmic units. The 5′-phosphate dianion probably interacts with the cationic imidazole ring more strongly than the 5′-hydrogen phosphate monoanion, and thus the deprotonation is facilitated.

Table 3 also lists the stability constants for the magnesium(II) complexes of the phosphate groups. The values obtained are all equal within the limits of experimental error. In other words, the complexing ability is not affected by intramolecular interactions with the base moiety.

In summary, the electrostatic interactions between the base and phosphate moieties of 7-methylguanosine 5′-monophosphate and its structural analogues appear to be strong enough to affect the conformation of the molecule in solution and thus the susceptibility of the C8 atom to nucleophilic attack. However, these interactions are too weak to alter significantly the acidity or the complexing ability of the interacting moieties. Accordingly, they can hardly play a dominant role in determining the binding specificity of 7-methylguanosine cap analogues.



7a: $R^1 = PO(O^-)_2$, $R^2 = R^3 = H$
7b: $R^1 = R^3 = H$, $R^2 = PO(O^-)_2$
7c: $R^1 = R^2 = H$, $R^3 = PO(O^-)_2$

Experimental

Materials. Of the compounds employed, **1a**, **1g**, **2a**, **7a**, **7b** and **7c** were commercial products from Sigma Chemical Company. The preparation of **1c**,[8] **1d**,[10] **1f**,[7] **3**,[8] **4a**[7] and **4b**[7] has been reported earlier. The other compounds were prepared as described below, and characterized by UV spectroscopy, ^{1}H NMR spectroscopy (Bruker AM-360) and TLC on cellulose plates (Merck) using a mixture of saturated aqueous ammonium sulfate, aqueous potassium phosphate buffer (0.1 mol dm^{-3}, pH 7.4) and 2-propanol (79:19:2 *v/v/v*) as eluent.

7-Methylguanosine 2′-, 3′- and 5′-monophosphates (**5a**, **5b**, **1e**) were obtained by treating the corresponding commercial guanosine monophosphates (**7a–c**), with methyl iodide in dimethyl sulfoxide. The crude products were purified by chromatography on a DEAE-Sephadex A25 (HCO_3^-)column (3.5×70 cm) using a linear gradient of triethylammonium bicarbonate (pH 7.5, from 0 to 0.5 mol dm^{-3}). The de-salted evaporation residues were converted to sodium salts by passing them through a Dowex 50W-X8 column (100/200 mesh, Na^+ form). The products obtained were dried over phosphorus pentoxide. The details of the experimental procedure have been described earlier.[7,8] The chromatographic and UV-spectroscopic properties of the compounds obtained were identical with those reported by Hendler *et al.*[11] **5a** exhibited ^{1}H NMR signals (D_2O, pD 6.8) at 6.08 (H1′, d), 5.10 (H2′, m), 4.50 (H3′, t), 4.29 (H4′, m), 4.09 (CH_3–N7, s), 3.90 (H5′, m) and 3.86 (H5″, m), and **5b** (D_2O, pD 7.7) at 6.04 (H1′, d), 4.72 (H2′, t), 4.66 (H3′, m), 4.37 (H4′, m), 4.10 (CH_3–N7, s), 3.97 (H5′, m) and 3.90 (H5″, m). The coupling constants J(P, H2′) and J(P, H3′) were 8.2 and 7.7

Hz, respectively. The ^{1}H NMR spectrum of **1e** was identical with that reported previously.[9]

7-Methylguanosine 5′-phosphordiamidate (**1b**) was prepared by methylating guanosine 5′-phosphordiamidate, obtained by the method of Bottka and Tomasz,[16] with methyl iodide in dimethyl sulfoxide. The product was purified on DEAE-Sephadex A25 (HCO_3^-), eluting with water. The compound obtained was homogeneous by TLC (R_F 0.65), showed UV absorption maximum at 258 nm (pH 2, $\varepsilon = 9.7\times10^3$) and ^{1}H NMR signals (D_2O, pD 8.4) at 6.05 (H1′, d), 4.65 (H2′, t), 4.44 (H3′, t), 4.39 (H4′, m), 4.34 (H5′, m), 4.22 (H5″, m) and 4.07 (CH_3–N7, s).

1,7-Dimethylguanosine 5′-monophosphate (**2b**) was obtained by methylating 1-methylguanosine 5′-monophosphate with methyl iodide in dimethyl sulfoxide and purifying the product as described above for **1e**. 1-Methylguanosine 5′-monophosphate, used as a starting material, was synthesized by phosphorylating commercial 1-methylguanosine (Sigma) with phosphorus oxychloride in trimethyl phosphate according to Pal *et al.*[17] **2b** was homogeneous by TLC (R_F 0.68), UV-spectroscopically similar to 1,7-dimethylguanosine 5′-diphosphate[5] and exhibited ^{1}H NMR signals (D_2O, pD 6.7) at 6.08 (H1′, d), 4.66 (H2′, t), 4.48 (H3′, t), 4.37 (H4′, m), 4.14 (H5′, m), H5″, m), 4.13 (CH_3–N7, s) and 3.46 (CH_3–N1, s).

7-Methylguanosine 2′,3′-cyclic monophosphate (**6**) was prepared from guanosine 2′,3′-cyclic monophosphate as described above for **1e**. However, the upper limit for the concentration of triethylammonium bicarbonate during the chromatographic separation was 0.3 mol dm^{-3}.

Guanosine 2′,3′-cyclic monophosphate was obtained by cyclizing commercial guanosine 3′-monophosphate (Sigma) according to Smith *et al.*[18] **6** was homogenous by TLC (R_F 0.55) and showed ^{1}H NMR signals (D_2O, pD 7.3) at 6.25 (H1′, d), 5.42 (H2′, m), 5.15 (H3′, m), 4.55 (H4′, m), 4.09 (CH_3–N7, s), 3.93 (H5′, m) and 3.87 (H5″, m). The coupling constants J(P, H2′) and J(P, H3′) were 9.8 and 9.0 Hz, respectively.

The inorganic salts and buffer constituents employed were commercial products of reagent grade. The solutions were made in distilled, degassed water. The standard acid and base solutions used in potentiometric titrations were purchased from J. T. Baker.

Kinetic measurements. The first-order rate constants for the opening of the imidazole ring of 7-methylguanosine were obtained by following the disappearance of the starting material by the HPLC[19] and spectrophotometric[20] techniques described previously. The products were not analyzed, but the first step was assigned as opening of the imidazole ring on the basis of previous mechanistic studies.[12–14] With the other compounds, only the spectrophotometric method was used. The initial concentration of the starting material was 1×10^{-3} mol dm^{-3} in the HPLC and 1×10^{-4} mol dm^{-3} in the spectrophotometric measurements. The concentration of hydroxide ion in the reaction solutions was calculated from the acidity constants reported for water[21] and for the buffer constituents[22,23] at different ionic strengths.

Potentiometric titrations. The potentiometric titrations were carried out as described previously,[24] using a Radiometer combined glass electrode. Absorption of carbon dioxide during the titrations was prevented by passing a stream of nitrogen through the solutions. The ligand concentration employed varied from 5×10^{-4} to 5×10^{-3} mol dm^{-3}. The observed emf values, E, were transformed to oxonium ion concentrations via eqn. (1), where j_H and j_{OH} are fitting parameters which were adjusted with the aid of calibration titrations of hydrochloric acid with sodium hydroxide. E_o and C are the constants in the Nernst equation, and K_w is the acidity constant for water at the appropriate ionic strength.[25] A Gauss-Newton fitting method was applied to calculate the dependence of E on [H$^+$]. The equilibrium constants were calculated by use of the Bjerrum complex formation function using a Davidson-Fletcher-Powell method of minimization.[26]

$$E = E_o + C\log[H^w] + j_H[H^+] + j_{OH}K_w[H^+]^{-1} \quad (1)$$

Spectrophotometric measurements. The acidity constants for the base moiety of 7-methylguanosine and its derivatives were determined by recording their UV spectra (Cary 17D spectrophotometer) in triethanolamine buffers, the oxonium ion concentrations of which were determined potentiometrically by the method described above. The Bjerrum function was applied to obtain the acidity constants.

Acknowledgements. Financial support from the Academy of Finland and the Polish Academy of Sciences (CPBR 3.13) is gratefully acknowledged. The authors also wish to thank Dr. Irene Ekiel for recording the NMR spectra, Dr. Harald Wiedner for a gift of GDP, and Mr. Jarmo Saimanen, B.Sc., for technical assistance.

References

1. Shatkin, A. J. *Cell 9* (1976) 645.
2. Rhoads, R. E. *Prog. Mol. Subcell. Biol. 9* (1985) 104.
3. Shatkin, A. J. *Cell 40* (1985) 223.
4. Hickey, E. D., Weber, L. A., Baglioni, C., Kim, C. H. and Sarma, R. H. *J. Mol. Biol. 109* (1977) 173.
5. Adams, B. L., Morgan, K., Muthukrishnan, S., Hecht, S. M. and Shatkin, A. J. *J. Biol. Chem. 253* (1978) 2589.
6. Grifo, J. A., Tahara, S. M., Morgan, M. A., Shatkin, A. J. and Merrick, W. C. *J. Biol. Chem. 258* (1983) 5804.
7. Darzynkiewicz, E., Ekiel, I., Tahara, S. M., Seliger, L. S. and Shatkin, A. J. *Biochemistry 24* (1985) 1701.
8. Darzynkiewicz, E., Ekiel, I., Lassota, P. and Tahara, S. M. *Biochemistry 26* (1987) 4372.
9. Kim, C. H. and Sarma, R. H. *J. Am. Chem. Soc. 100* (1978) 1571.
10. Darzynkiewicz, E., Antosiewicz, J., Ekiel, I., Morgan, M. A., Tahara, S. M. and Shatkin, A. J. *J. Mol. Biol. 153* (1981) 451.
11. Hendler, S., Furer, E. and Srinivasan, P. R. *Biochemistry 9* (1970) 4141.
12. Townsend, L. B. and Robins, R. K. *J. Am. Chem. Soc. 85* (1963) 242.
13. Chetsanga, C. J., Bearie, B. and Makaroff, C. *Chem.-Biol. Interact. 41* (1982) 217.
14. Chetsanga, C. J. and Makaroff, C. *Chem.-Biol. Interact. 41* (1982) 235.
15. Ekiel, I. and Darzynkiewicz, E. *Unpublished results.*
16. Bottka, S. and Tomasz, J. *Tetrahedron 35* (1979) 2909.
17. Pal, C. B., Schmidt, D. G. and Farrelly, J. G. In: Townsend, L. B. and Tipson, R. S., Eds., *Nucleic Acid Chemistry*, Wiley, New York 1978, Vol. 2, pp. 963–971.
18. Smith, M., Moffatt, J. G. and Khorana, H. G. *J. Am. Chem. Soc. 80* (1958) 6204.
19. Lönnberg, H. and Lehikoinen, P. *J. Org. Chem. 49* (1984) 4964.
20. Lönnberg, H. *Acta Chem. Scand., Ser. A 31* (1977) 265.
21. Harned, H. S. and Owen, B. B. *The Physical Chemistry of Electrolyte Solutions*, Reinold, New York 1943, p. 485.
22. Cox, M. C., Everett, D. H., Landsman, D. A. and Munn, R. J. *J. Chem. Soc. B* (1968) 1373.
23. King, E. J. *J. Am. Chem. Soc. 73* (1951) 155.
24. Veber, M., Katona-Balasz, J., Burger, K., Kaloy, K., Mermecz, I. and Meszaros, Z. *Inorg. Chim. Acta 135* (1987) 61.
25. Perrin, D. D. *Ionisation Constants of Inorganic Acids and Bases in Aqueous Solution*, 2nd ed., Pergamon, Oxford 1982.
26. Himmelblau, D. M. *Applied Nonlinear Programming*, McGraw-Hill, New York 1972.

Received September 2, 1987.