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Polynucleotides. LIII.[1] Conformation of Dinucleoside Monophosphates containing 8-Methyladenosine Residue as studied by Proton Magnetic Resonance

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Conformation of dinucleoside monophosphates containing 8-methyladenosine (m^8A) residue, which prefer a *syn* conformation, was analyzed by proton magnetic resonance studies. The dimers are 2′-5′ and 3′-5′ isomers of Apm^8A, and m^8Apm^8A. The following results were obtained. The data for (2′-5′)Apm^8A are consistent with an *anti*(2′)-*anti*(5′), right-handed stacked conformation. The data for (3′-5′) Apm^8A are consistent with a *syn*(3′)-*syn*(5′) and a high *anti*(3′)-high *anti*(5′) stacked conformation. The data for (3′-5′)m^8Apm^8A can be explained by a *syn*(3′)-*syn*(5′) conformation with little stacking. The data for (2′-5′) m^8Apm^8A can be explained by an *anti*(2′)-*syn*(5′) stacked conformation. These results are in accordance with the results of ultraviolet and circular dichroism studies.

Keywords——8-methyladenylyl-adenosine; 8-methyladenylyl-8-methyladenosine; adenylyl-8-methyladenosine; proton magnetic resonance; *syn* conformation; stacking conformation

It is shown by various spectroscopic techniques and X-ray crystallographic analysis[3,4] that natural polynucleotides take a right-handed helical structure, in which the nucleoside residues take an *anti* (3′)-*anti* (5′), right-handed stacked conformation, in solution as well as in crystals. It is known that nucleoside derivatives with a bulky substituent at C(8) prefer a *syn* conformation both in solution[5,6] and crystal.[7] The polymer,[8] oligomer[9] and dinucleoside monophosphates[8,10] containing 8-bromoguanosine residue, the dinucleoside monophosphates of 8-bromoadenosine (br^8Apbr^8A),[11] the polymer of 8-bromoadenylic acid[12] and the polymer of 8-oxyadenylic acid[13] have been synthesized and examined mainly by ultraviolet (UV) absorption and circular dichroism (CD) techniques. However, further investigations are needed for elucidation of the stacking conformations in these oligo-and polynucleotides.

Recently we reported the synthesis and optical properties of 8-methyladenosine[14] and several dinucleoside monophosphates containing 8-methyladenosine (m^8A) and adenosines.[15] It is expected that perturbation by a methyl group to the adenine chromophore is smaller

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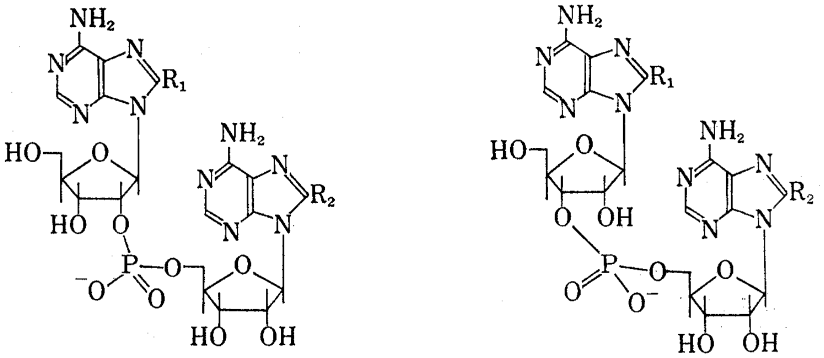
[1] Part LII: M. Ikehara, T. Fukui, and N. Kakiuchi, *Nucleic Acids Res.*, **5**, 1877 (1978).
[2] Location: *133-1 Yamadakami, Suita, Osaka 565, Japan.*
[3] S. Arnott, R. Chandrasekaran, and E. Selsing, "Structure and Conformation of Nucleic Acids and Protein-Nucleic Acid Interactions," ed. by M. Sundaralingam and S.T. Rao, University Park Press, Baltimore, 1975, pp. 577—596.
[4] A. Rich, *Accounts Chem. Res.*, **10**, 386 (1977).
[5] M. Ikehara, S. Uesugi, and K. Yoshida, *Biochemistry*, **11**, 830 (1972).
[6] S. Uesugi and M. Ikehara, *J. Am. Chem. Soc.*, **99**, 3250 (1977).
[7] S.S. Tavale and H.M. Sobell, *J. Mol. Biol.*, **48**, 109 (1970).
[8] A.M. Michelson, C. Monny, and A.M. Kapuler, *Biochim. Biophys. Acta*, **217**, 7 (1970).
[9] R. Yuki and H. Yoshida, *Biochim. Biophys. Acta*, **246**, 206 (1971).
[10] E.S. Gromova and W. Guschlbauer, *FEBS Letters*, **26**, 176 (1972).
[11] M. Ikehara, S. Uesugi, and M. Kobayashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2394 (1972).
[12] F.B. Howard, J. Frazier, and H.T. Miles, *J. Biol. Chem.*, **250**, 3951 (1975).
[13] J.O. Folayan and D.W. Hutchinson, *Biochim. Biophys. Acta*, **474**, 329 (1977).
[14] M. Ikehara, W. Limn, and T. Fukui, *Chem. Pharm. Bull.* (Tokyo), **25**, 2702 (1977).
[15] M. Ikehara, W. Limn, and S. Uesugi, *J. Carbohyd. Nucleosides. Nucleotides*, **5**, 163 (1978).

than that by a bromine group and the methyl hydrogens can be a probe for proton magnetic resonance (PMR) studies. It was found by UV, CD and carbon-13 nuclear magnetic resonance (^{13}C-NMR) studies of 8-methyladenosine that the 8-methyl group exerted less hindrance to the rotation of base around the glycosidic bond than 8-bromo atom.[6,14] From UV and CD studies of the dinucleoside monophosphates the following conclusions have been drawn: 1) the 2′-5′ isomers take more stable stacked conformation than the corresponding 3′-5′ isomers; 2) (2′-5′) m^{8}ApA takes predominantly *anti*(2′)-*anti*(5′), right-handed stacked conformation similar to that of ApA; 3) (2′-5′) Apm^{8}A takes less stable *anti*(2′)-*anti*(5′), right-handed stacked conformation; and 4) (2′-5′) m^{8}Apm^{8}A takes a high content of *anti*(3′)-*syn*(5′), right-handed stacked conformation. However, for further understanding of the conformation of these dimers, investigations by some other techniques such as PMR were needed.

Here we wish to report conformational analysis of these dimers by PMR and discuss the behavior of the *syn* preferring nucleoside residue in the dinucleoside monophosphates.



Ia: $R_1=CH_3$, $R_2=CH_3$
IIa: $R_1=H$, $R_2=CH_3$
IIIa: $R_1=CH_3$, $R_2=H$

Ib: $R_1=CH_3$, $R_2=CH_3$
IIb: $R_1=H$, $R_2=CH_3$

Fig. 1. Structures of the Dinucleoside Monophosphates containing 8-Methyladenosine Residue

Materials and Method

The synthesis of dinucleoside monophosphates containing m^{8}A residue and the monophosphates of m^{8}A are reported in the previous paper.[15] (2′-5′) and (3′-5′)ApA were synthesized according to the published procedure.[16]

The samples were prepared in D_2O after adjusting the pD with NaOD and lyophilizing from D_2O solution three times. PMR spectra were recorded with a Hitachi R-22 spectrometer (90 MHz, ambient probe temperature 34°). Chemical shifts were measured from an external tetramethylsilane capillary.

The H-8 proton of the adenosine residue was assigned by the deuterium exchange (90° for 30 min at pD 7.4).[17] Under this condition more than 50% of H-8 proton was exchanged.

Results and Discussion

Spectral Data and Assignments of Chemical Shifts of the Dinucleoside Monophosphates

The chemical shifts of the base, methyl and H-1′ protons of the dimers, 2′-5′ and 3′-5′ isomers of m^{8}Apm^{8}A and Apm^{8}A, as well as component monomers, together with the coupling constants of H-1′ and H-2′ protons, $J_{H\text{-}1',H\text{-}2'}$, are given in Table I. The data for (2′-5′) and (3′-5′) ApA's are also included for comparison. The PMR spectrum of (2′-5′) Apm^{8}A is shown in Fig. 2. The spectra of the dimers were measured in D_2O at pD 7.5. The spectra

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[16] K. Miura and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **19**, 2567 (1971).
[17] M.P. Schweizer, S.I. Chan, G.K. Helmkamp, and P.O.P. Ts'o, *J. Am. Chem. Soc.*, **86**, 696 (1964).

Table I. ^{1}H NMR Data for the Dimers containing m^{8}A and Related Compounds

Compound		Concn. (M)	pD	Chemical shift (ppm)$^{a)}$				$J_{\text{H-1}', \text{H-2}'}$ (Hz)
				H-8	H-2	CH$_3$	H-1′	
(2′-5′)m^{8}Apm^{8}A	m^{8}Ap–	0.075	7.5		8.68	3.06	6.53	6.2
	-pm^{8}A				8.37	3.00	6.28	6.4
(3′-5′)m^{8}Apm^{8}A	m^{8}Ap–	0.075	7.5		8.42	2.96	6.10	6.2
	-pm^{8}A				8.42	3.03	6.30	5.2
(2′-5′)Apm^{8}A	Ap–	0.1	7.5	8.54	8.13		6.49	5.3
	-pm^{8}A				8.40	2.83	6.13	6.4
(3′-5′)Apm^{8}A	Ap–	0.1	7.5	8.51	8.32		6.18	5.4
	-pm^{8}A				8.32	2.96	6.28	5.4
2′-m^{8}AMP		0.1	7.5		8.55	3.08	6.48	6.4
3′-m^{8}AMP		0.1	7.5		8.45	3.03	6.41	5.8
5′-m^{8}AMP		0.1	5.4		8.55	3.09	6.43	6.2
2′-AMP		0.1	5.4	8.71	8.42		6.57	7.4
3′-AMP		0.1	5.4	8.67	8.44		6.43	4.8
5′-AMP		0.1	5.4	8.78	8.47		6.44	4.8
(2′-5′)ApA	Ap–	0.1	7.5	8.64	8.21		6.59	4.2
	-pA			8.57	8.44		6.26	2.8
(3′-5′)ApA	Ap–	0.1	7.5	8.64	8.27		6.23	4.0
	-pA			8.53	8.39		6.35	4.6

a) Shifts are given relative to external TMS.

of the monomers were measured at pD 5.4 except for 2′-m^{8}AMP and 3′-m^{8}AMP, which were insoluble in D$_2$O at this pD, to minimize the secondary phosphate dissociation.

In the case of the heterodimers, assignments of the H-8 and methyl protons can be made unambiguously because the former is exchangeable with deuterium upon heating in D$_2$O and the latter gives an isolated signal at the highest field. In the case of (3′-5′) m^{8}Apm^{8}A and (3′-5′)Apm^{8}A, both H-2 protons have the same chemical shift and are assigned without argument. The assignments of H-1′ protons of 3′-5′ isomers and 2′-5′ isomers were made analogously to those of (3′-5′)ApA[18] and (2′-5′)ApA[18] respectively. In the case of (2′-5′) Apm^{8}A, the assignment of the two H-1′ resonances (6.49 and 6.13 ppm) was confirmed by a technique utilizing the specific signal broadening effect of Mn^{2+} upon binding.[19,20] In the presence of Mn^{2+} (3×10^{-5} M), the signal at 6.49 ppm was specifically broadened. This result suggests that this signal belongs to the H-1′ of the 2′-nucleotidyl unit (Ap-), in which the H-1′ is closer to the Mn^{2+} bound to the phosphate group than that in the 5′-nucleotidyl unit (-pm^{8}A). The assignments of the other protons were made considering the conformation proposed from CD study or the conformation suggested from tha data of the remaining assigned protons. The assignments for (3′-5′)ApA and (2′-5′) ApA were made in accordance with the published works.[18,21]

Conformation of (2′-5′)Apm^{8}A (IIa)

The dimerization shifts, $\Delta\delta=\delta(\text{monomer})-\delta(\text{dimer})$, according to the above assignments are presented in Table II. The results of the ApA isomers show a qualitative agreement with the published data at infinite dilution,[21] better agreement being obtained for (3′-5′) ApA. These results suggest that the present results at a moderate concentration of the dimers, which is chosen because of the limitation of our instrument, may be satisfactory at least to a qualitative understanding of the dimer conformations.

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18) P.O.P. Ts'o, N.S. Kondo, M.P. Schweizer, and D.P. Hollis, *Biochemistry*, **8**, 997 (1969).

19) S.I. Chan and J.H. Nelson, *J. Am. Chem. Soc.*, **91**, 168 (1969).

20) K.N. Fang, N.S. Kondo, P.S. Miller, and P.O.P. Ts'o, *J. Am. Chem. Soc.*, **93**, 6647 (1971).

21) N.S. Kondo, H.M. Holmes, L.M. Stempel, and P.O.P. Ts'o, *Biochemistry*, **9**, 3479 (1970).

Table II. Dimerization Shifts

Compound		δ(monomer)−δ(dimer) (ppm)			
		H-8	H-2	CH_3	H-1′
(2′-5′)m^{8}Apm^{8}A	m^{8}Ap–		−0.13	0.02	−0.05
	-pm^{8}A		0.18	0.09	0.15
(3′-5′)m^{8}Apm^{8}A	m^{8}Ap–		0.03	0.07	0.31
	-pm^{8}A		0.13	0.06	0.13
(2′-5′)Apm^{8}A	Ap–	0.17	0.29		0.08
	-pm^{8}A		0.15	0.26	0.30
(3′-5′)Apm^{8}A	Ap–	0.16	0.12		0.25
	-pm^{8}A		0.23	0.13	0.15
(2′-5′)ApA	Ap–	0.07	0.21		−0.02
	-pA	0.21	0.03		0.18
(3′-5′)ApA	Ap–	0.03	0.17		0.20
	-pA	0.25	0.08		0.09

(2′-5′) Apm^{8}A shows CD spectrum similar in pattern but much smaller in magnitude with respect to that of ApA.15) From this result, it has been proposed that (2′-5′) Apm^{8}A takes an unstable *anti*(3′)-*anti*(5′), right-handed stacking conformation. This conformation is illustrated in Fig. 2. The PMR spectrum of Apm^{8}A is also included in Fig. 2. The H-8 of Ap residue was assigned by deuterium exchange procedure17) and the H-1′ of both residues were assigned by the Mn^{2+} binding experiment.19,20) The H-1′ of pm^{8}A is much more shielded than that of Ap residue. This trend is the same as that of (2′-5′) ApA suggesting a right-handed stack. The large shielding (0.26 ppm) on the methyl protons of pm^{8}A residue suggests that this residue takes predominantly an *anti* conformation22) receiving the shielding from the adenine ring of Ap residue. Relatively small shielding (0.17 ppm) on H-8 of Ap residue suggests that this residue may also take an *anti* conformation locating the H-8 away from the 8-methyladenine ring of m^{8}A residue. These considerations lead to the assignments of

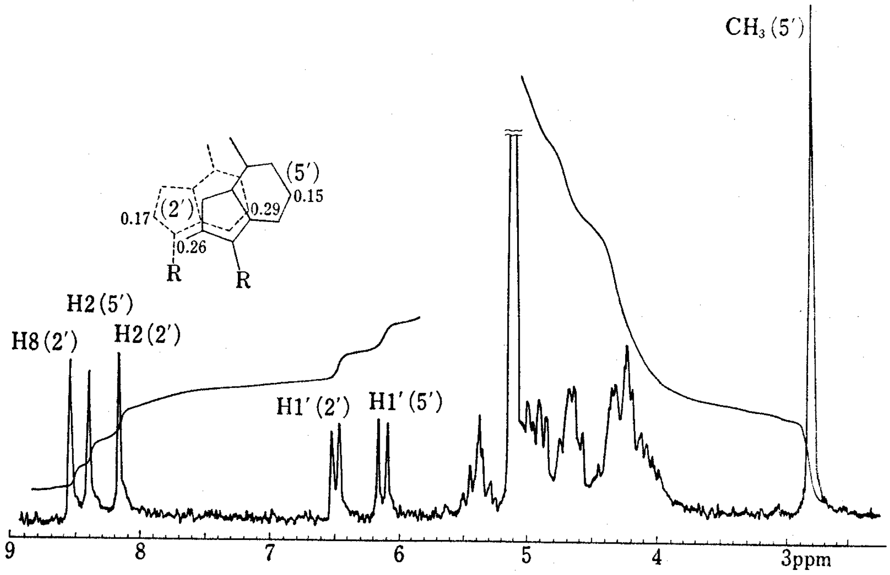

Fig. 2. The 90 MHz PMR Spectrum and the Proposed Stacking Conformation of (2′-5′) Apm^{8}A

The spectrum was taken in D_2O at 34°, 0.1 M, pD 7.5. The chemical shifts are expressed in ppm from external TMS.

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22) J. Donohue and K.N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).

the H-2 protons as shown in Fig. 2. Thus the H-2 of Ap residue is more shielded than that of pm^{8}A residue (0.29 ppm *vs.* 0.15 ppm). The reversed assignments for the H-2 protons resulting an anomalously large shielding (0.42 ppm) of the H-2 of pm^{8}A residue are unlikely. After all, the present PMR data are consistent with an anti(2′)-anti(5′), right-handed stack which has been proposed from CD data.

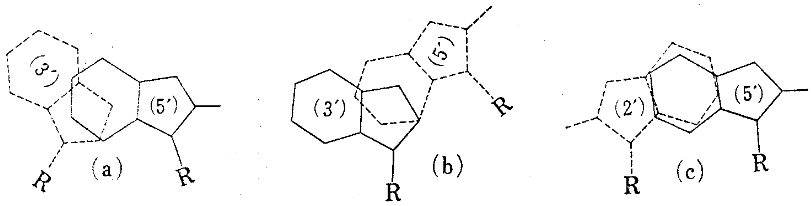

Fig. 3. Schematic Illustrations of the Proposed Modes of Stacking Conformation

The bases drawn by the dotted line are located at the bottom.
(a) and (b): for (3′-5′) Apm^{8}A. (c): for (2′-5′) m^{8}Apm^{8}A.

Conformation of (3′-5′)Apm^{8}A (IIb)

The H-8 of Ap residue was assigned by deuterium exchange procedure. The H-2 protons of both residues have the same chemical shift. Therefore the all base protons can be assigned unambiguously. Thus in Ap residue the H-8 is more shielded than H-2 and in pm^{8}A residue the H-2 is more shielded than the methyl protons. These results are consistent with a *syn*(3′)-*syn*(5′), right-handed stack as illustrated in Fig. 3a. However, examination with the Corey–Pauling–Koltun (CPK) models reveals that a left-handed stack with both residues in a high-*anti*(intermediate between *anti* and *syn*) region[23] can be also a probable conformation (see Fig. 3b). The CD spectrum of (3′-5′) Apm^{8}A is very similar to that of the monomers (m^{8}A and A).[14,15] Since (3′-5′) Apm^{8}A has a considerable hypochromicity (9%) it may take some stacked conformation(s), most probably an equilibrium mixture of the right-handed and the left-handed stacked conformations.

Conformation of (3′-5′)m^{8}Apm^{8}A (Ib)

The PMR data of 3′-m^{8}AMP at pD 5.4 were not available because of a solubility problem but the chemical shift change of the base protons between pD 5.4 and 7.5 is assumed to be samll as observed in the case of 3′-AMP.[24] The H-2 protons of both residues of (3′-5′) m^{8}Apm^{8}A have the same chemical shift. The $\Delta\delta$ of the H-2 of m^{8}Ap residue and pm^{8}A residue are $\approx$0.03 ppm and 0.13 ppm respectively. The very small $\Delta\delta$ value of the H-2 of m^{8}Ap residue suggests that this residue takes a *syn* conformation. We can assume that the pm^{8}A residue takes also a *syn* conformation as in the case of (3′-5′) Apm^{8}A. These considerations lead to the assignments of the methyl protons as indicated in Table I. Thus the methyl protons of m^{8}Ap and pm^{8}A show $\Delta\delta$'s of 0.07 ppm and 0.06 ppm respectively. The CD spectrum of (3′-5′) m^{8}Apm^{8}A is almost the same as that of the monomer, m^{8}A, suggesting no stacking conformation or no preference for a special conformation.[15] Since all $\Delta\delta$ values of the protons of this dimer are relatively small, the present PMR results are consistent with the conclusion from CD study.

Conformation of (2′-5′)m^{8}Apm^{8}A (Ia)

From CD study it has been suggested that (2′-5′) m^{8}Apm^{8}A favors an *anti*(2′)-*syn*(5′), right-handed stacked conformation as illustrated in Fig. 3c.[14] The relatively large hypochromicity (14%) also suggests fairly stable stacked conformation of this dimer. We can make tentative assignments which is consistent with the *anti*(2′)-*syn*(5′) stack as shown in

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23) P. Prusiner, T. Brennan, and M. Sundaralingam, *Biochemistry*, **12**, 1196 (1973).
24) M.P. Schweizer, A.D. Broom, P.O.P. Ts'o, and D.P. Hollis, *J. Am. Chem. Soc.*, **90**, 1042 (1968).

Table I. The dimerization shifts based on the assignments show that the H-2 of the pm^{8}A residue is more shielded than the methyl protons of the same residue (this is true even if the assignments of the methyl protons are reversed). The methyl protons of the m^{8}Ap residue are not shielded and the H-2 of the same residue shows a negative $\Delta\delta$. The negative $\Delta\delta$ of the H-2 may result from inadequate monomer data or specific deshielding effect upon formation of a stable stacked conformation. The former reason is less likely because it is reported that the H-2 of 2′-AMP shows upfield shift upon changing pD from 7.5 to 5.4.[24] If 2′-m^{8}AMP has the same property, the corrected $\Delta\delta$ for the H-2 of m^{8}Ap residue will be more negative. If the latter reason is correct, the deshielding may be caused by some heteroatom ($O_{1'}$ or N_7) of the pm^{8}A residue. Negative $\Delta\delta$ values have been observed in the H-2 protons of dAp residues in d-ApC and d-ApT.[25]

When the assignments of the H-2 protons are reversed, the *anti*(2′)-*syn*(5′) conformation may be also necessary to produce a negative $\Delta\delta$ on the H-2 of pm^{8}A residue. In this case, the source of deshielding can be the phosphate group. Examination with CPK models also suggests this possibility.

Conclusions

The PMR studies of the isomers of Apm^{8}A and m^{8}Apm^{8}A have led us to the following conclusions. 1) The dimerization shift data for (2′-5′) Apm^{8}A are very similar to those for (2′-5′) ApA which is assumed to take an *anti*(2′)-*anti*(5′), right-handed stacked conformation. 2) The dimerization shift data for (3′-5′) Apm^{8}A are consistent with *syn*(3′)-*syn*(5′) and high *anti*(3′)-high *anti*(5′) stacked conformation. 3) The data for (3′-5′) m^{8}Apm^{8}A can be explained by a *syn*(3′)-*syn*(5′) conformation. 4) The data for (2′-5′) m^{8}Apm^{8}A can be explained by *anti*(2′)-*syn*(5′) stacked conformation. In the case of (2′-5′) Apm^{8}A and (2′-5′) m^{8}Apm^{8}A, the PMR results give a support to the suggested conformations from CD and UV studies. In the case of (3′-5′) Apm^{8}A and (3′-5′) m^{8}Apm^{8}A, they show CD spectra very similar to those of the monomer spectra. The PMR studies suggest that (3′-5′) Apm^{8}A does take some stacked conformation(s), most probably an equilibrium mixture of the right-handed stack and the left-handed stack and that (3′-5′) m^{8}Apm^{8}A having relatively small dimerization shifts does not take a stable stacked conformation. These conclusions are also in accordance with those from UV and CD studies.

As to the factors which affect the conformation of these dimers, the following points may be noted when all the data are taken into account. Firstly, the conformational stability of these dimers depends on the type of phosphodiester bond. The 2′-5′ isomer has more stable stacking conformation than that of the 3′-5′ isomer. Secondly, the presence of m^{8}A residue(s) in a dimer reduce the stability of stacking and the destabilizing effect is specially remarkable in the case of 3′-5′ isomers. Thirdly, the conformation of a particular dimer may be governed by a combined effect of relative stability of glycosidic conformations of the monomer components and relative stability of mode of stacking. If stabilization by stacking interaction is greater, A and m^{8}A residues may change their glycosidic conformations.

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25) D.M. Cheng and R.H. Sarma, *J. Am. Chem. Soc.*, **99**, 7333 (1977).