Bull. Chem. Soc. Jpn., 73, 1621—1631 (2000)

Effect of the N^6-Methyl Group of the Adenine Ring on the Stacking Interaction

Toshio Itahara

Department of Bioengineering, Faculty of Engineering, Kagoshima University, Korimoto, Kagoshima 890-0065

(Received January 6, 2000)

In an effort to determine the effects of N^6-methylation of the adenine ring on stacking, the molecular aggregation of 9,9′-(α,ω-alkanediyl)bis[6-(dimethylamino)purine] (**1**) and of 9,9′-(α,ω-alkanediyl)bis[6-(methylamino)purine] (**2**) were compared with that of 9,9′-(α,ω-alkanediyl)bis[adenine] (**3**) by NMR spectroscopy. The obtained concentration effects indicate that N^6-methylation resulted in an increase in the population of intermolecular aggregates in the buffer solution at pD 7.0, and had an additive effect on the aggregation. It can be interpreted as a hydrophobic effect of the N^6-methyl groups. The aggregation of **1** and **2** depended on the length of the polymethylene chains in a similar manner as that of **3**, when the measurements were made on the solution of low concentrations. This suggests that the aggregates of **1**, **2**, and **3** were similar to each other through stacking between two adenine rings. Furthermore, the chemical shifts of the N^6-methyl groups were slightly influenced by the length of the polymethylene chains and the changes of temperature. The N^6-methyl group may be located outside the stacking. On the basis of these data, it may be concluded that the N^6-methylation enhanced the stacking, but had little influence on the conformation of the stacking.

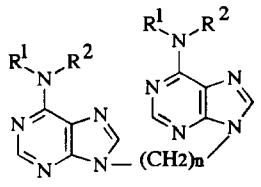
Since methyl-substituted bases are broadly found in nucleic acids,[1] the methylation of nucleic acid bases may have biological significance. N^6-Methyl- and N^6,N^6-dimethyladenines are known to be minor components in tRNA.[1] The effects of the N^6-methylation of the adenine ring on the major/minor conformations have been discussed from the standpoint of rotation of the methylamino groups.[2] On the other hand, the N^6-methylation of the adenine ring has been pointed out to play a role in stacking,[3,4] e.g., it has been reported to bring about a marked increase in the population of stacked conformers on the basis of a study of the thermodynamic behavior.[4] Tazawa et al.[4e] concluded that a hidden hydrophobic effect was of importance in stacking. Studies on the NMR of oligonucleotides containing N^6,N^6-dimethyladenine also lend some support to the effects of the N^6-methylation on stacking.[3b—f] Furthermore, puromycin, an antibiotic, forms an alternating stacking between the 6-dimethylaminopurine and the *p*-methoxyphenyl rings with an interplanar spacing of 3.4 Å.[5]

Stacking is one of molecular aggregations among aromatic molecules, such as nucleic acid bases[1] and chlorophylls,[6] and is commonly realized to be caused by a stacking interaction which is one of the π–π interactions.[1a,3,4] However, the nature of stacking interaction is still obscure.[7] NMR spectroscopy is one of the powerful tools used in the elucidation of stacking. The measurement of stacking is based on the fact that the ring current, owing to stacking between aromatic molecules, produces up-field shifts in the proton resonance compared with the isolated ones. The concentration effects of the aromatic molecules are indispensable to understanding stacking, and the effects of adenine derivatives have been investigated in detail.[3,8] On the other hand, we previously reported on the effects of the length of the polymethylene chain of 9,9′-(α,ω-alkanediyl)bis[adenine] (**3**),[9] in which it is placed between two adenine rings, in order to elucidate stacking between only two adenine rings. When the chemical shifts of **3** are measured in very low concentrations, intermolecular stacking is expected to be negligible. The length of the polymethylene chains corresponds to the distance between two adenine rings, which is correlated to an encounter frequency of two adenine rings. For instance, when the distance approaches infinity, the two molecules are isolated. If there is a stacking interaction between two molecules, the population of stacked conformers is expected to be relevant to the length of the polymethylene chains.

In the present investigation, the molecular aggregations of 9,9′-(α,ω-alkanediyl)bis[6-(dimethylamino)purine] (**1**) and 9,9′-(α,ω-alkanediyl)bis[6-(methylamino)purine] (**2**) were studied by NMR spectroscopy. In an effort to determine the effect of the N^6-methyl groups of the adenine ring, it was felt that an investigation concerning the aggregation of **1** and **2** was of importance for a comparison with that of **3** for which data have been reported.[9] Furthermore, the aggregations of 9-(5-bromopentyl)-6-(dimethylamino)purine (**4d**) and 9-(5-bromopentyl)-6-(methylamino)purine (**5d**) were compared with that of 9-(5-bromopentyl)adenine (**6d**) (Chart 1).

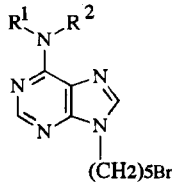
Results

Preparation and NMR Measurement. 9,9′-(α,ω-Alkanediyl)bis[6-(dimethylamino)purine] (**1**) and 9,9′-(α,ω-alkanediyl)bis[6-(methylamino)purine] (**2**) were prepared according to the procedure described for 9,9′-(α,ω-alkanediyl)bis[adenine] (**3**),[9] although **1a**,[10] **1b**,[11,12] **1c**,[11] **1e**,[11] **2a**,[11] and **2b**[12] were already known. The treatment of 6-(dimeth-



	R^1	R^2
1	Me	Me
2	Me	H
3	H	H

	n
a:	2
b:	3
c:	4
d:	5
e:	6
f:	7
g:	8
h:	9



	R^1	R^2
4d	Me	Me
5d	Me	H
6d	H	H

Chart 1.

ylamino)purine, 6-(methylamino)purine, and adenine with excess amounts of 1,5-dibromopentane gave the corresponding 9-(5-bromopentyl)purines: (**4d**), (**5d**), and (**6d**).[13]

^{1}H NMR measurements of the adenine derivatives were performed in buffer solutions at pD 7.0 and at pD 1.0[14] containing sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 and in organic solvents, such as CD_3OD and $CDCl_3$, containing tetramethylsilane as references. The hydrogen atoms of the adenine ring at the 2- and 8-positions of **1**—**6** were assigned on the basis of the ^{1}H NMR spectra of compounds containing the 8-deuterioadenine moiety, which were prepared according to a procedure described earlier.[9]

Concentration Effects. The concentration effects of **4d**, **5d**, and **6d** were studied. Figure 1 shows the relationship between the chemical shifts of the adenine ring protons at the 2- and 8-positions of **4d**, **5d**, and **6d** and their concentrations [log (concn in M, 1 M = 1 mol dm^{-3})] in the buffer solution at pD 7.0 at 27 °C. The chemical shifts of these compounds were to higher fields along with an increase in the concentrations. Since intermolecular aggregation generally depends on the concentration,[3,8] the up-field shifts of the chemical shifts are thought to be attributable to the formation of intermolecular aggregates. The extent of the shifts was dependent on the number of the N^6-methyl groups.

Although the chemical shifts of **1b**, **c** and **2b**, **c** showed a further concentration dependence, those of **3b**, **c** were little influenced by the concentration changes from 0.02 to 2.7 mmol dm^{-3}.[9] Figure 2 shows the relationship between the chemical shifts of the adenine ring protons of **1b**, **2b**, and **3b** (carbon number = 3) and their concentrations in a buffer solution at pD 7.0 at 27 °C. The up-field shifts of **1b** and **2b** began gradually above 0.1 and 0.15 mmol dm^{-3}, respectively. Furthermore, as can be seen from the concentration dependence of **1c**, **2c**, and **3c** (carbon number = 4) shown in Fig. 3, the aggregation of **1c** and **2c** occurred above 0.15 and 0.3 mmol dm^{-3}, respectively. These data suggest that **1** and **2** were influenced by concentrations less than 1 mmol dm^{-3}. On the other hand, when the measurments were made of solutions at their lower concentrations, the chemical shifts were almost constant. The order of the aggregation on the basis of the extent of the up-field shifts was **1b**, **c** > **2b**, **c** ≫ **3b**, **c**.

In order to compare the extent of the up-field shifts of the above compounds, the chemical-shift differences between log M = −4.5 and log M = −2.5 were determined by utilizing Figs. 1, 2, and 3. The chemical shifts of **1b**, **c**, **2b**, **c**, and **3b**, **c** at a concentration of log M = −2.5 were estimated on the basis of the curves shown in Figs. 2 and 3. The chemical-shift differences of H-2 and H-8 of the adenine ring were as follows: **1b** (ca. 0.13 and ca. 0.11 ppm); **1c** (ca. 0.12 and ca. 0.10 ppm); **2b** (ca. 0.065 and ca. 0.055 ppm); **2c** (ca. 0.06 and ca. 0.05 ppm); **3b** (less than 0.01 ppm and less than 0.01 ppm), **3c** (less than 0.01 ppm and less than 0.01 ppm), **4d** (ca. 0.06 and ca. 0.05 ppm); **5d** (ca. 0.045 and ca. 0.03 ppm); **6d** (less than 0.01 ppm and less than 0.01 ppm).

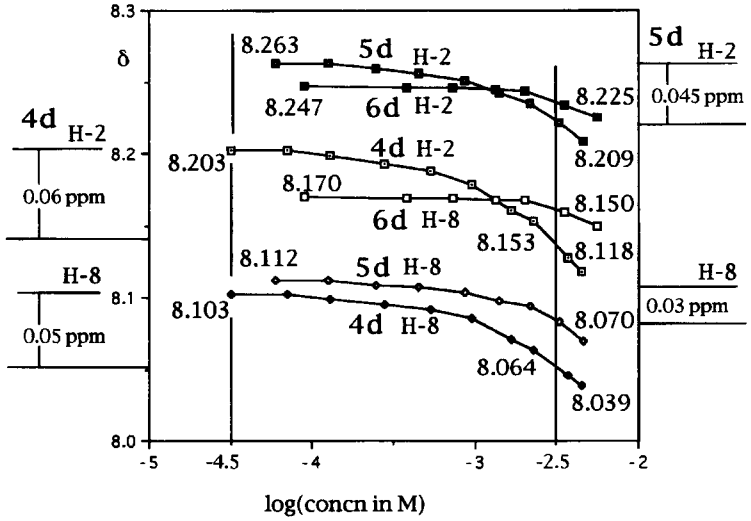


Fig. 1. Relationship between the chemical shifts (δ) of H-2 and H-8 of adenine ring of **4d**, **5d**, and **6d** and the concentrations [log (concn in M)] (1 M = 1 mol dm^{-3}) in the buffer solution at pD 7.0 at 27 °C.

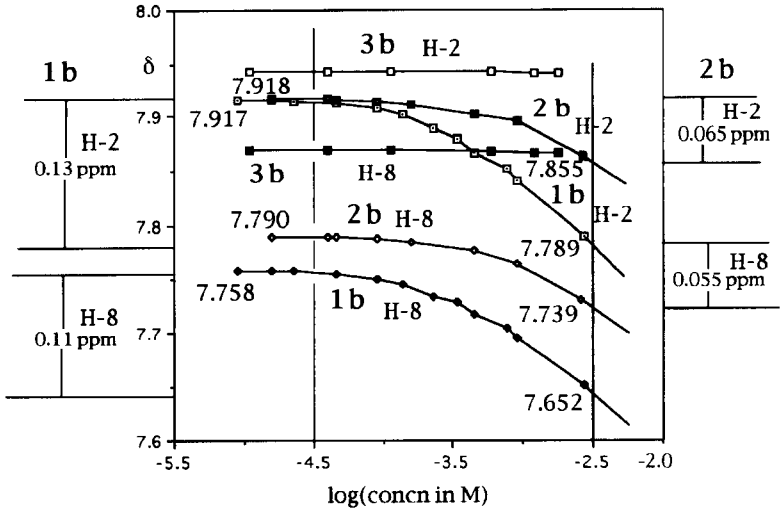


Fig. 2. Relationship between the chemical shifts (δ) of H-2 and H-8 of adenine ring of **1b**, **2b**, and **3b** and the concentrations [log (concn in M)] in the buffer solution at pD 7.0 at 27 °C. The values of the chemical shifts of **3b** were reported in Ref. 9.

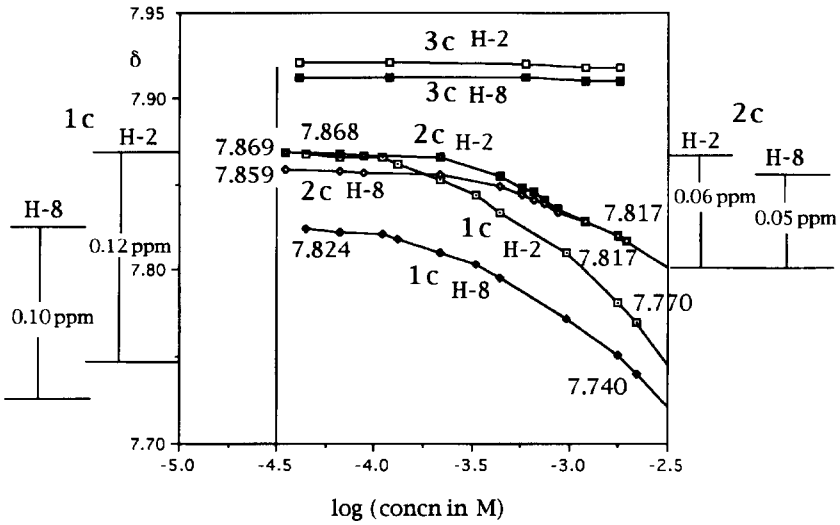


Fig. 3. Relationship between the chemical shifts (δ) of H-2 and H-8 of adenine ring of **1c**, **2c**, and **3c** and the concentrations [log (concn in M)] in the buffer solution at pD 7.0 at 27 °C. The values of the chemical shifts of **3c** were reported in Ref. 9.

The chemical shifts of the N^6-methyl groups of **1b**, **c** and **2b**, **c** were also to higher fields with an increase in the concentrations. Figure 4 shows the relationship between the chemical shifts of the dimethylamino groups of **1b**, **1c**, **1d**, and **4d** and the concentrations. The chemical-shift differences between $\log M = -4.5$ and $\log M = -2.5$ were as follows: **1b** (ca. 0.11 ppm); **1c** (ca. 0.10 ppm); **4d** (ca. 0.05 ppm), although the chemical shift of **1d** at a concentration of $\log M = -2.5$ was not determined because of the poor solubilities. These data shown in Figs. 1, 2, 3, and 4 suggest that the effects of the N^6-methyl groups on the aggregation had an additive property.

In contrast to the results in the buffer solution at pD 7.0, the chemical shifts of **1c**, **1d**, and **2c** in the buffer solution at pD 1.0 and in CD_3OD at 27 °C were little influenced by the concentrations (Fig. 5), although the chemical shifts of H-2 and H-8 of **1c** in the buffer solution at pD 1.0 were somewhat shifted to lower fields as the concentration was increased. It has already been presented in a previous paper that similar down-field shifts of the adenine ring protons in the buffer solution at pD 1.0 were caused by protonation at the 1-position of the adenine ring.[9] These results suggest that the adenine rings of **1** and **2** did not associate to form intermolecular aggregates in the buffer solution at pD 1.0 and in organic solvents.

These experiments leave no doubt that the N^6-methylation of the adenine ring results in a remarkable increase in the population of the intermolecular aggregates among the adenine rings in the buffer solution at pD 7.0, but only slightly enhances aggregation in organic solvents. On the basis of these data, it seemes reasonable to assume that the effects of the N^6-methyl groups on the aggregation are attributable to a hydrophobic interaction. Therefore, the obtained concentration effects of **1**, **2**, and **3** are explained in terms of an increase in the hydrophobicity of the adenine ring by N^6-methylation.

Effects of the Length of Polymethylene Chains. The chemical shifts of the adenine ring protons and the N^6-methyl

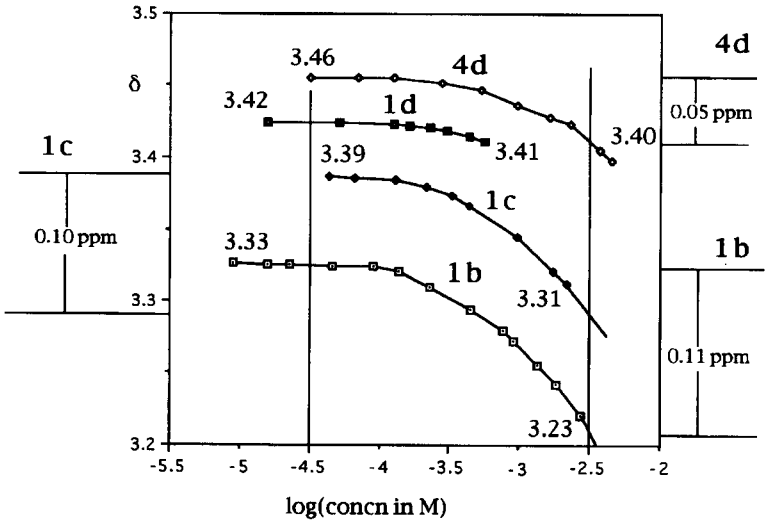


Fig. 4. Relationship between the chemical shifts (δ) of N^6-methyl group of adenine ring of **1b**, **1c**, **1d**, and **4d** and the concentrations [log (concn in M)] in the buffer solution at pD 7.0 at 27 °C. The values of the chemical shifts were calculated down to two decimal places.

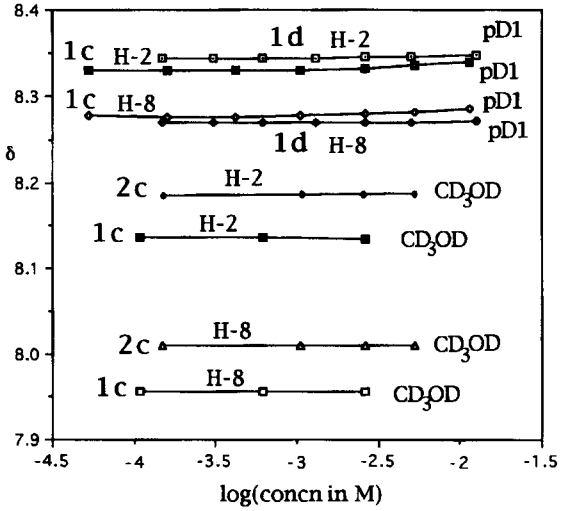


Fig. 5. Relationship between the chemical shifts (δ) of H-2 and H-8 of adenine ring of **1c**, **1d**, and **2c** and the concentrations [log (concn in M)] in the buffer solution at pD 1.0 and in CD_3OD at 27 °C.

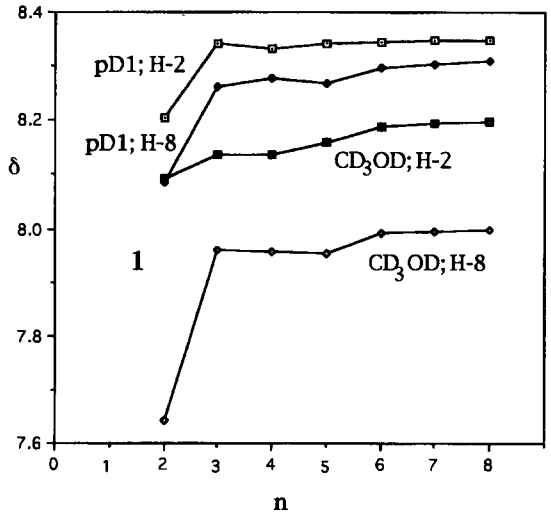


Fig. 6. Relationship between the chemical shifts (δ) of H-2 and H-8 of adenine ring of **1** and the carbon numbers (n) of the polymethylene chains in the buffer solution at pD 1.0 and CD_3OD at 27 °C. The values of the chemical shifts are shown in Table 1.

groups of **1** and **2** in the buffer solutions at pD 7.0 and at pD 1.0 and the organic solvents are given in Tables 1 and 2. From a consideration of the concentration effects, the intermolecular aggregation of **1** and **2** in the buffer solution at pD 7.0 is expected to be practically negligible at concentrations less than 0.1 mmol dm^{-3}. Therefore, measurements at concentrations less than 0.1 mmol dm^{-3} were required to determine an alteration in the chemical shifts, which was caused only by an intramolecular aggregation. On the other hand, the concentrations in the buffer solution at pD 1.0 and in organic solvents (Tables 1 and 2) were 1.0—1.5 mmol dm^{-3}, because the chemical shifts were little affected by the concentrations. The signals of the N^6-methyl groups of **1** and **2** were broad, compared with the signals of H-2 and H-8, and thus their values were calculated down to two decimal places. Furthermore, the values of the chemical shifts of the N^6-methyl groups of **1** and **2** in the buffer solution at pD 1.0 were not determined because the signals were extremely broad.[15]

Figures 6 and 7 show the effects of the length of the polymethylene chains of **1** and of **2**, respectively, in the buffer solution at pD 1.0 and in CD_3OD. The chemical shifts of H-2 and H-8 of **1** and **2** in the buffer solution at pD 1.0 and in organic solvents, such as CD_3OD and $CDCl_3$ (see Tables 1 and 2), were slightly influenced by the length of the polymethylene chains, except for **1a** and **2a**. On the other hand, the chemical shifts of H-2 and H-8 of **1** and **2** in the buffer solution at pD 7.0 were plainly affected by the length of the polymethylene chains. Figures 8 and 9 show the relationships between the chemical shifts of the adenine-ring protons of **1**, **2**, and **3** at concentrations less than 0.1 mmol dm^{-3} and the carbon numbers of the polymethylene

Table 1. Chemical Shifts of Adenine Ring Protons and N^6-Methyl Groups of **1a**—**g**[a] and **4d**

Solvent Temp		**1a** (n = 2)	**1b** (n = 3)	**1c** (n = 4)	**1d** (n = 5)	**1e** (n = 6)	**1f** (n = 7)	**1g** (n = 8)	**4d**
pD 7.0[b]	2-H	7.964	7.918	7.871	7.924	8.000	8.058	8.103	8.202
27 °C	8-H	7.770	7.758	7.826	7.857	7.924	7.974	8.007	8.102
	N^6-Me_2	3.35	3.33	3.38	3.41	3.38	3.36	3.34	3.46
pD 7.0	2-H	7.908	7.939	7.898	7.961	8.065	8.097	8.140	8.218
50 °C	8-H	7.823	7.750	7.815	7.842	7.935	7.975	8.009	8.093
	N^6-Me_2	3.36	3.33	3.39	3.42	3.41	3.39	3.38	3.46
pD 1.0[c]	2-H	8.205	8.342	8.333	8.343	8.344	8.347	8.349	8.362
27 °C	8-H	8.086	8.262	8.279	8.269	8.298	8.304	8.309	8.338
pD 1.0	2-H	8.211	8.341	8.335	8.348	8.350	8.354	8.356	8.368
50 °C	8-H	8.094	8.254	8.270	8.259	8.289	8.296	8.302	8.331
CD_3OD[d]	2-H	8.093	8.138	8.136	8.159	8.188	8.194	8.198	8.218
27 °C	8-H	7.643	7.960	7.956	7.953	7.993	7.996	7.999	8.019
	N^6-Me_2	3.45	3.47	3.49	3.50	3.50	3.50	3.50	3.51
$CDCl_3$[d]	2-H	8.423	8.356	8.334	8.329	8.335	8.342	8.347	8.352
27 °C	8-H	7.208	7.896	7.667	7.664	7.680	7.689	7.692	7.719
	N^6-Me_2	3.51	3.54	3.53	3.53	3.53	3.53	3.54	3.54

a) The ^1H NMR spectra of **1** were measured at least twice and the chemical shifts of the adenine ring protons at 2- and 8-positions were reproduced within ±0.004 ppm. b) The concentrations of **1** in the buffer solution at pD 7.0 were 0.04—0.1 mmol dm^{-3}. c) The concentrations of **1** in the buffer solution at pD 1.0 were 1.0—1.5 mmol dm^{-3}. The values of the chemical shifts of the N^6-methyl groups of **1** in the buffer solution at pD 1.0 were not determined because the signals were extremely broad. d) The concentrations of **1** in CD_3OD and in $CDCl_3$ were 1.0—1.5 mmol dm^{-3}.

Table 2. Chemical Shifts of Adenine Ring Protons and N^6-Methyl Groups of **2a**—**h**[a] and **5d**

Solvent Temp		**2a** (n = 2)	**2b** (n = 3)	**2c** (n = 4)	**2d** (n = 5)	**2e** (n = 6)	**2f** (n = 7)	**2h** (n = 9)	**5d**
pD 7.0[b]	2-H	7.980	7.918	7.870	7.966	8.080	8.138	8.176	8.264
27 °C	8-H	7.811	7.790	7.859	7.887	7.962	8.012	8.053	8.113
	N^6-Me	3.04	3.05	3.11	3.13	3.12	3.08	3.04	3.12
pD 7.0	2-H	7.924	7.954	7.920	8.015	8.138	8.171	8.210	8.275
50 °C	8-H	7.874	7.798	7.856	7.882	7.967	8.006	8.057	8.106
	N^6-Me	3.05	3.06	3.12	3.14	3.13	3.09	3.06	3.13
pD 1.0[c]	2-H	8.225	8.368	8.371	8.389	8.395	8.399	8.401	8.412
27 °C	8-H	8.113	8.248	8.260	8.270	8.302	8.313	8.323	8.345
pD 1.0	2-H	8.232	8.366	8.367	8.384	8.393	8.397	8.400	8.411
50 °C	8-H	8.125	8.248	8.265	8.268	8.299	8.310	8.320	8.344
CD_3OD[d]	2-H	8.104	8.202	8.189	8.213	8.232	8.237	8.244	8.261
27 °C	8-H	7.716	8.041	8.011	8.007	8.035	8.036	8.045	8.065
	N^6-Me	3.08	3.11	3.12	3.12	3.13	3.13	3.12	3.13
$CDCl_3$[d]	2-H	8.423	8.423	8.399	8.399	8.406	8.409	8.416	8.419
27 °C	8-H	7.203	7.896	7.685	7.674	7.690	7.696	7.708	7.731
	N^6-Me	3.21	3.22	3.22	3.22	3.22	3.22	3.21	3.22

a) The ^1H NMR spectra of **2** were measured at least twice and the chemical shifts of the adenine ring protons at 2- and 8-positions were reproduced within ±0.003 ppm. b) The concentrations of **2** in the buffer solution at pD 7.0 were 0.04—0.1 mmol dm^{-3}. c) The concentrations of **2** in the buffer solution at pD 1.0 were 1.0—1.5 mmol dm^{-3}. The values of the chemical shifts of the N^6-methyl groups of **2** in the buffer solution at pD 1.0 were not determined because the signals were extremely broad. d) The concentrations of **2** in CD_3OD and in $CDCl_3$ were 1.0—1.5 mmol dm^{-3}.

chains in the buffer solution at pD 7.0 at 27 °C. The chemical shifts of H-8 in Fig. 9 were to higher fields as the length of the polymethylene chains decreased, except for **1a** and **2a** (n = 2). On the other hand, among the chemical shifts of H-2 in Fig. 8, those of **1c**, **2c**, and **3c** (n = 4) were to the highest fields. The up-field shifts are thought to be attributable to the formation of intramolecular aggregates.

The relationships of H-2 and H-8 of **1**, **2**, and **3** were similar to each other. That is to say, the aggregation of **1** and **2** depended on the length of the polymethylene chains in a similar

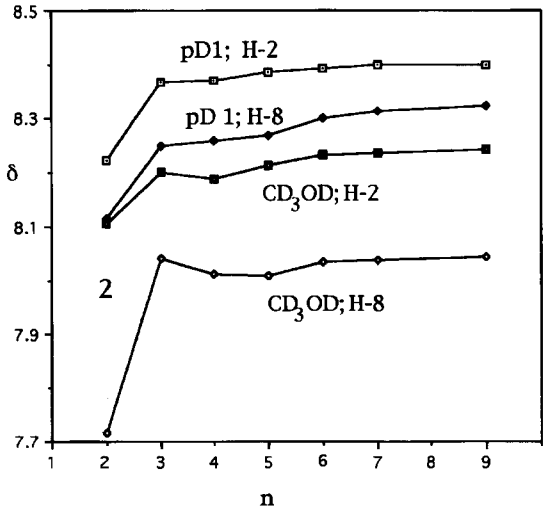


Fig. 7. Relationship between the chemical shifts (δ) of H-2 and H-8 of adenine ring of **2** and the carbon numbers (n) of the polymethylene chains in the buffer solution at pD 1.0 and CD_3OD at 27 °C. The values of the chemical shifts are shown in Table 2.

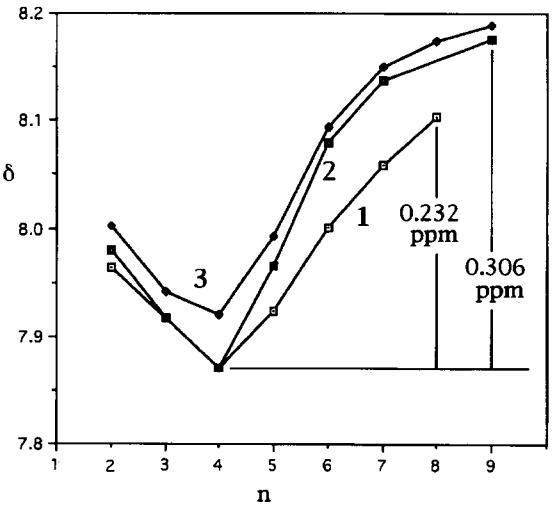


Fig. 8. Relationship between the chemical shifts (δ) of H-2 of adenine ring of **1**, **2**, and **3** and the carbon numbers (n) of the polymethylene chains in the buffer solution at pD 7.0 at 27 °C. The values of the chemical shifts of **1** and **2** are shown in Tables 1 and 2 and those of **3** were reported in Ref. 9.

manner as that of **3**. These observations are interpreted by assuming that the conformations of the intramolecular aggregates of **1**, **2**, and **3** are similar to each other. The intramolecular aggregation seems to bring about conformational motion in one restricted direction, compared with random conformational motions. Therefore, it is thought that the aggregation is stacking between two adenine rings. Geissner–Prett and Pullman[16] reported that the caluculated up-field shifts of H-2 and of H-8 due to the stacking of two adenine rings by 3.4 Å were 0.30—0.45 and 0.15—0.25 ppm, respectively. The chemical-shift differences of H-2 and of H-8 of **1**, which were determined by a comparison of the chemical shifts of **1c** (n = 4) with those of **1g** (n = 8), were 0.232 and 0.181

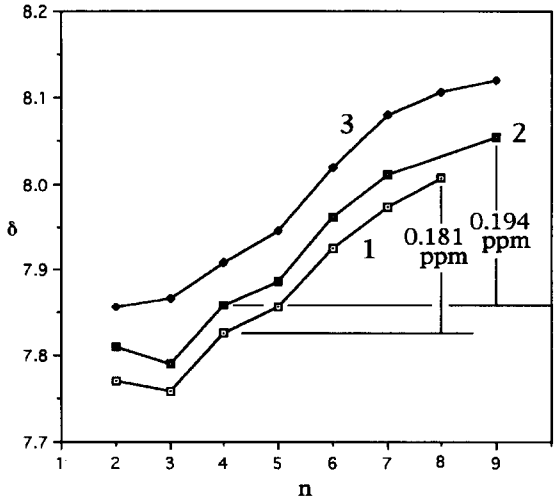


Fig. 9. Relationship between the chemical shifts (δ) of H-8 of adenine ring of **1**, **2**, and **3** and the carbon numbers (n) of the polymethylene chains in the buffer solution at pD 7.0 at 27 °C. The values of the chemical shifts of **1** and **2** are shown in Tables 1 and 2 and those of **3** were reported in Ref. 9.

ppm, respectively. Also, the differences in the shifts of H-2 and of H-8 between **2c** (n = 4) and **2h** (n = 9) were 0.306 and 0.194 ppm, respectively. These observed shifts seem to be roughly consistent with the calculated ones.[16] The tendency shown in Fig. 8 was different from that in Fig. 9 in the case of shorter polymethylene chains (n = 2 and 3). This may be attributable to connecting adenine rings at the 9-position with the polymethylene chains. The connection with the shorter polymethylene chains (n = 2 and 3) may bring especially two protons at the 8-position into a closer relationship because of steric factors.

We see subtle differences among **1**, **2**, and **3** in Fig. 8. When the carbon numbers of the polymethylene chains are more than 6, the values of the chemical shifts of H-2 of **2** approached those of **3**, but the values of **1** did not approach those of **3**. These results may result from a difference in the hydrophobicity between the dimethylamino group and the methylamino group. That is to say, the remarkable hydrophobic effect of the dimethylamino group of **1** may have an effect on the increase in the population of the intramolecular stacked conformers, even in the case of more than 6 carbon atoms of the polymethylene chains.

Figure 10 shows the relationship between the chemical shifts of the N^6-methyl groups and the length of the polymethylene chains. The relationship of the N^6-methyl groups was different from those of the adenine-ring protons shown in Figs. 8 and 9. Furthermore, the chemical-shift differences of the H-2, H-8, and N^6-methyl groups between **1c** and **1g** were 0.232, 0.181, and −0.04 ppm, respectively. Similarly the differences of the H-2, H-8, and N^6-methyl groups between **2c** and **2h** were 0.306, 0.194, and −0.07 ppm, respectively. The chemical shifts of the N^6-methyl groups, compared with those of H-2 and H-8, were slightly affected by the length of the polymethylene chains. These data are in contrast to the

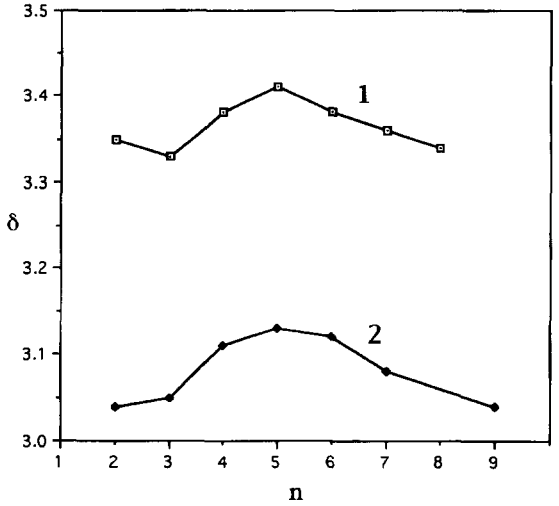


Fig. 10. Relationship between the chemical shifts (δ) of N^6-methyl groups of adenine ring of **1** and **2** and the carbon numbers (n) of the polymethylene chains in the buffer solutions at pD 7.0 at 27 °C. The values of the chemical shifts are shown in Tables 1 and 2.

results that the chemical shifts of the N^6-methyl groups were apparently affected by the concentrations (Fig. 4).

Temperature Effects. Tables 3 and 4 show the temperature dependence of the chemical shifts of **1a—d** and of **2b—d**, respectively. The chemical shifts of H-2 of **1** and **2** were greatly dependent on the temperature. Stacking may be responsible for the pronounced temperature dependence of H-2 of the adenine ring. On the other hand, the chemical shifts of the N^6-methyl group were little affected by the temperature. The data in these Tables, when considered with the relationship shown in Fig. 10, indicate that the N^6-methyl groups may be located outside the intramolecular stacking between two adenine rings. On the other hand, the adenine-ring protons at the 2- and 8-positions seem to participate in the stacked conformation. Thus, a tentative stacked conformation of **1** and **2** is shown as (I) or (II) in Chart 2. In view of the concentration effects of the chemical shifts of adenines, Ts'o et al.[8a] reported stacking orientations similar to (I) and (II) and Evans and Sarma[8c] proposed one similar to (I); still,

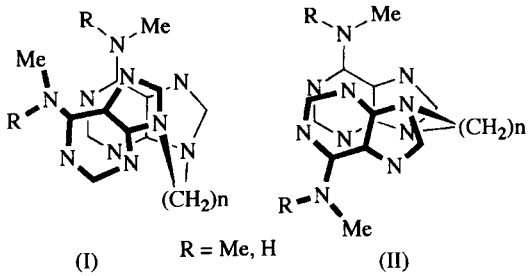


Chart 2.

Table 3. Effect of Temperature of the Chemical Shifts of Protons of Adenine Ring at 2- and 8-Positions and Dimethylamino Group at 6-Position of **1a—d**[a)]

Concn	**1a** (1.0 mmol dm^{-3})			**1b** (1.0 mmol dm^{-3})			**1c** (0.7 mmol dm^{-3})			**1d** (1.0 mmol dm^{-3})		
°C	H-2	H-8	Me_2N-6	H-2	H-8	Me_2N-6	H-2	H-8	Me_2N-6	H-2	H-8	Me_2N-6
24	7.955	7.751	3.35	7.830	7.688	3.27	7.815	7.781	3.37	7.898	7.842	3.41
30	7.944	7.765	3.35	7.851	7.699	3.28	7.834	7.791	3.37	7.913	7.841	3.41
40	7.925	7.792	3.35	7.879	7.711	3.29	7.857	7.795	3.38	7.936	7.841	3.41
50	7.904	7.817	3.36	7.904	7.722	3.30	7.877	7.798	3.38	7.957	7.840	3.42
60	7.885	7.840	3.36	7.924	7.729	3.31	7.898	7.801	3.39	7.976	7.840	3.42
70	7.862	7.862	3.36	7.940	7.733	3.32	7.919	7.805	3.39	7.996	7.840	3.42
80	7.845	7.885	3.37	7.954	7.736	3.33	7.939	7.809	3.40	8.014	7.839	3.42
$\Delta\delta$[b)]	−0.110	+0.134	+0.02	+0.124	+0.058	+0.06	+0.124	+0.028	+0.03	+0.116	−0.003	+0.01

a) Sodium phosphate buffer solution at pD 7.0. b) $\Delta\delta = \delta(80\ °C) - \delta(24\ °C)$.

Table 4. Effect of Temperature of the Chemical Shifts of Protons of Adenine Ring at 2- and 8-Positions and Methylamino Group at 6-Position of **2b—d**[a)]

Concn	**2b** (3.2 mmol dm^{-3})			**2c** (0.9 mmol dm^{-3})			**2d** (3.1 mmol dm^{-3})		
°C	H-2	H-8	MeN-6	H-2	H-8	MeN-6	H-2	H-8	MeN-6
24	7.854	7.739	3.08	7.831	7.831	3.10	7.910	7.850	3.11
30	7.875	7.751	3.09	7.849	7.835	3.10	7.934	7.854	3.11
40	7.907	7.766	3.10	7.879	7.841	3.11	7.967	7.862	3.12
50	7.932	7.777	3.11	7.908	7.844	3.11	7.998	7.868	3.13
60	7.954	7.787	3.12	7.935	7.849	3.12	8.025	7.873	3.13
70	7.975	7.794	3.13	7.962	7.853	3.12	8.049	7.877	3.14
80	7.995	7.801	3.14	7.989	7.860	3.13	8.071	7.881	3.14
$\Delta\delta$[b)]	+0.141	+0.062	+0.06	+0.158	+0.029	+0.03	+0.161	+0.031	+0.03

a) Sodium phosphate buffer solution at pD 7.0. b) $\Delta\delta = \delta(80\ °C) - \delta(24\ °C)$.

it may be difficult to determine whether the conformation is (I) or (II) on the basis of the present NMR data.

Discussion

Much evidence for the stacking of adenine rings has come from NMR studies[3,8,9,12,17] and other methods.[4] The results of the present study lend some support to this hypothesis. From the standpoint of stacking, the adenine rings of **1**, **2**, and **3** are speculated to exist in three situations (Chart 3), i.e., no stacking (**A**), intramolecular stacking (**B**), and intermolecular stacking such as (**C**), (**D**), etc. When the NMR spectra of **1**, **2**, and **3** are measured in low concentrations, the intermolecular stacking is negligible. The up-field shifts of the adenine-ring protons at low concentrations (Figs. 8 and 9) can be regarded as an increase in the population of (**B**), compared with (**A**).

Takemoto et al.[12,18] previously reported the NMR, UV, and emission spectra of **1b**, **2b**, and **3b** (n = 3), but did not discuss the effects of the N^6-methylation on stacking. From a consideration of the concentration effects (Figs. 1, 2, 3, 4, and 5), the effect of the N^6-methyl groups may be interpreted as a hydrophobic effect. On the other hand, the connection with the polymethylene chains draws two adenine rings within the length of the chains, so that the effects of the length of the polymethylene chains at low concentrations (Figs. 8, 9, and 10) are looked upon as a concentrated effect between only two adenine rings at a shorter distance. The chemical shifts of the N^6-methyl groups were little affected by the length of the polymethylene chains (Fig. 10) and the change in temperature (Tables 3 and 4). Therefore, the N^6-methyl groups may be located outside the stacking between two adenine rings, as shown in Chart 2. The tentative conformations, (I) and (II), are similar to those of adenine reported[8a,c] in spite of the presence of the N^6-methyl groups.

Although the chemical shifts of the N^6-methyl groups were little affected by the change in temperature and the length of the polymethylene chains, they were apparently influenced by the concentrations (Fig. 4). This may be attributable to the distinction between the intramolecular stacking between only two adenine rings and the intermolecular stacking. The concentration effects are generally caused by an average of molecular aggregates among several molecules. Furthermore, the problem may be related to the interplanar angles and deviations from fully stacked conformations, although in Chart 3 the stacked conformations are depicted as fully stacked structures. For example, a speculated scheme of stacking of three aromatic molecules is shown in Chart 4. The stacked conformation between two aromatic molecules is (**E**). When one aromatic molecule is stacked on (**E**), molecular aggregates such as (**F**), (**G**), and (**H**) may consist of three molecules. The concentration effects result from an average of the molecular aggregates such as (**E**), (**F**), (**G**), and (**H**), and therefore, it is thought that the chemical shifts of the N^6-methyl groups as well as the adenine-ring protons were influenced by the concentrations.

As can be seen from Figs. 8 and 9, a greater degree of population of the intramolecular stacking was observed in **1** and **2** than in **3**, but the intramolecular stacked conformations of **1**, **2**, and **3** were similar to each other. Therefore, the hydrophobic effect of the N^6-methyl groups has an effect on the increase in population of the stacked conformers, but may only little affect the conformation of the stacking. This suggests that another effect may play an important role in the stacking of adenine rings.

The literature contains several references to the mechanism of stacking, which has been explained in terms of a hydrophobic interaction,[19] a charge-transfer interaction,[19b,20] an electrostatic interaction,[17,21] and a dispersion interaction.[19a,22] Electrical interactions containing charge-transfer interaction as well as electrostatic, induction, and dispersion interactions can be regarded as depending on the characteristics of the adenine ring. The hydrophobic effect is essential for the stacking of adenine rings, but the stacked conformation seems to primarily result from the characteristics of the adenine ring.

We give Chart 5 as a tentative model for the stacking between adenine rings. Although the effect of the N^6-methyl goups may contain a part of the electrical interactions, it seems reasonable to conclude that it is mainly caused by a hydrophobic interaction. Israelachvili and Pashley[23a] reported the exponential dependece of hydrophobic interaction on distance. The hydrophobic interactions of **1**, **2**, and **3**, which are expected to be dependent on the numbers of the N^6-methyl groups, are shown to be in conformity with a report,[23] although the hydrophobic interaction of the polymethylene chains leaves out the consideration in Chart 5. The hydrophobic effect is a major driving force for molecular aggregation when the molecules are far apart. However, the combination of the electrical and hydrophobic interactions, shown in Chart 5, may lead to the formation of stacking at a shorter distance. Although there are little data concerning the electrical interaction in this investigation, we shall presume the electrical interaction between two adenine rings as shown in Chart 5. The electrical interaction of the ade-

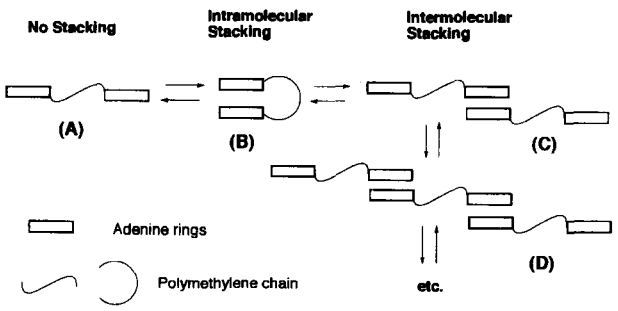


Chart 3.

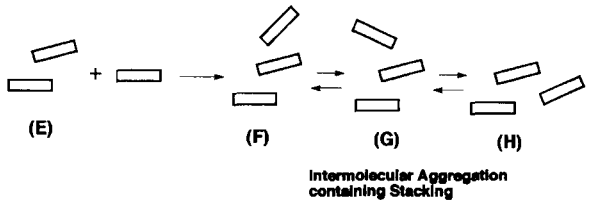


Chart 4.

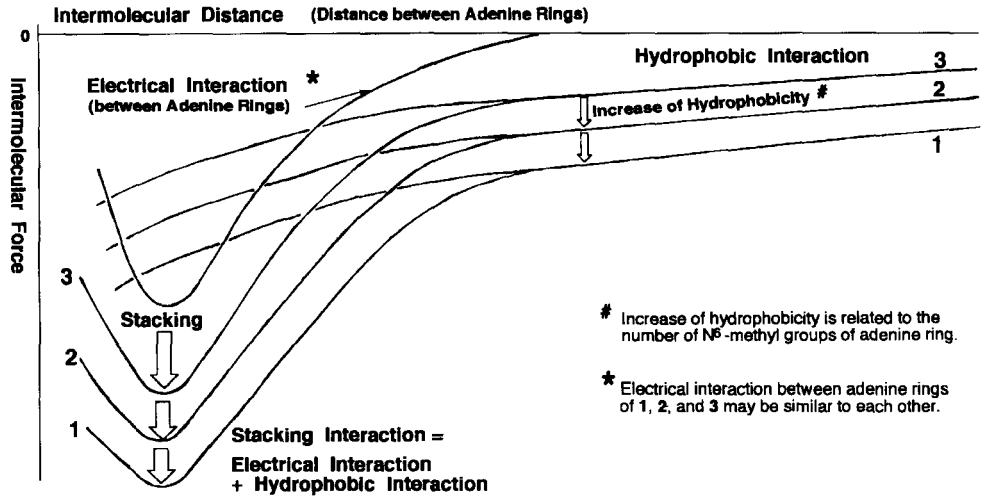


Chart 5.

nine ring of **1**, **2**, and **3** is expected to be similar to each other and an attractive force operating at a shorter distance between adenine rings. The total of the electrical and hydrophobic interactions, shown in Chart 5, may be called the stacking interaction between adenine rings. The hydrophobic effect of the N^6-methyl groups seems to work together with the electrical intercation and hydrophobic interaction of adenine, itself, and accommodates itself to the circumstance of stacking. Therefore, the N^6-methyl groups enhance the stacking, but may have little influence on the conformation of the stacking, because the conformation may depend on the combination of the electrical and hydrophobic interactions.

Experimental

The melting points were determined on a Yanagimoto micro melting-point apparatus and are uncorrected. The elemental analyses were performed by the Analytical Center of Kyoto University. 6-(Dimethylamino)purine and 6-(methylamino)purine were obtained commercially.

NMR Spectroscopy. The ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were obtained with a JEOL GSX400 spectrometer. The chemical shifts (δ-values) were measured in parts per million (ppm) downfield from sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 in the buffer solutions at pD 7.0 and 1.0 and from tetramethylsilane in CD_3OD, $CDCl_3$, and DMSO-d_6 as internal references. The concentrations of 3-(trimethylsilyl)propionate-2,2,3,3-d_4 were 0.6 mmol dm^{-3} in the sodium phosphate buffer solution at pD 7.0[14] and 0.8 mmol dm^{-3} in the HCl/KCl buffer solution at pD 1.0.[14] The ^{1}H NMR spectra were obtained from accumulation of 40—2000 free induction decays after each 45° pulse (5.7 μs) repeated every 5.73 s and were observed over a spectral width of 6002.4 Hz, corresponding to 32768 data points for acquisition time of 2.73 s. The ^{1}H NMR spectra of **1** and **2** in Tables 1 and 2 were measured at least twice and the chemical shifts of H-2 and H-8 of the adenine ring were reproduced within ±0.004 and ±0.003 ppm, respectively.

9,9′-(α,ω-Alkanediyl)bis[6-(dimethylamino)purine] (1) and 9,9′-(α,ω-Alkanediyl)bis[6-(methylamino)purine] (2). Into a solution of 6-(dimethylamino)purine or 6-(methylamino)purine (2 mmol) in DMF (50 ml), potassium carbonate (2 mmol) and α,ω-dibromoalkane (1 mmol) were added. The mixture was stirred at room temperature for 40 h. The resulting mixture was evaporated to give a residue, which was extracted with a mixture of chloroform and methanol. The extract was evaporated and chromatographed over silica gel. By elution of ethyl acetate containing 5% methanol, 6-(dimethylamino)purine or 6-(methylamino)purine was recovered. Further elution with a mixture of ethyl acetate containing 15—25% methanol gave (**1**) or (**2**). The yields and the spectral data of **1** and **2** are given below.

9,9′-Ethylenebis[6-(dimethylamino)purine] (1a): 47% yield; mp 232—234 °C (lit,[10] 226—227 °C); ^{1}H NMR ($CDCl_3$, 27 °C) δ = 8.36 (s, 2H), 7.16 (s, 2H), 4.70 (s, 4H), 3.51 (broad s, 12H); ^{13}C NMR ($CDCl_3$) δ = 155.02, 152.62, 150.27, 138.09, 120.29, 42.93, 38.55.

9,9′-Trimethylenebis[6-(dimethylamino)purine] (1b): 50% yield; mp 234—236 °C (lit,[11] 221—222 °C; lit,[12] 238—241 °C); ^{1}H NMR ($CDCl_3$, 27 °C) δ = 8.36 (s, 2H), 7.90 (s, 2H), 4.23 (t, 4H, J = 6.8 Hz), 3.55 (broad s, 12H), 2.51 (quintet, 2H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ = 155.02, 152.47, 150.62, 138.38, 120.25, 40.82, 38.55, 30.58.

9,9′-Tetramethylenebis[6-(dimethylamino)purine] (1c): 52% yield; mp 233—235 °C (lit,[11] 224—226 °C); ^{1}H NMR ($CDCl_3$, 27 °C) δ = 8.33 (s, 2H), 7.67 (s, 2H), 4.22 (broad, 4H), 3.53 (broad s, 12H), 1.91 (broad, 4H); ^{13}C NMR ($CDCl_3$) δ = 155.02, 152.45, 150.54, 138.01, 120.12, 42.83, 38.50, 27.16.

9,9′-(1,5-Pentanediyl)bis[6-(dimethylamino)purine] (1d): 50% yield; mp 137—138 °C; ^{1}H NMR ($CDCl_3$, 27 °C) δ = 8.33 (s, 2H), 7.66 (s, 2H), 4.15 (t, 4H, J = 7.0 Hz), 3.53 (broad s, 12H), 1.93 (quintet, 4H, J = 7.0 Hz), 1.36 (quintet, 2H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ = 155.03, 152.41, 150.54, 138.04, 120.21, 43.39, 38.57, 29.54, 23.75. Calcd for $C_{19}H_{26}N_{10}$: C, 57.85; H, 6.64%. Found: C, 58.07; N, 6.58%.

9,9′-(1,6-Hexanediyl)bis[6-(dimethylamino)purine] (1e): 48% yield; mp 179—181 °C (lit,[11] 226—227 °C); ^{1}H NMR ($CDCl_3$, 27 °C) δ = 8.34 (s, 2H), 7.68 (s, 2H), 4.15 (t, 4H, J = 7.0 Hz), 3.53 (broad s, 12H), 1.86 (broad quintet, 4H, J = 7.0 Hz), 1.37 (broad quintet, 4H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ = 155.01, 152.37, 150.51, 138.11, 120.19, 43.53, 38.55, 29.87, 26.125. Calcd for $C_{20}H_{28}N_{10}$: C, 58.80; H, 6.91%. Found: C, 58.59; 6.75%.

9,9′-(1,7-Heptanediyl)bis[6-(dimethylamino)purine] (1f): 43% yield; mp 113—114 °C; ^{1}H NMR ($CDCl_3$) δ = 8.34 (s, 2H), 7.69 (s, 2H), 4.14 (t, 4H, J = 7.0 Hz), 3.53 (broad s, 12H), 1.85 (quintet, 4H, J = 7.0 Hz), 1.40—1.27 (m, 6H); ^{13}C NMR ($CDCl_3$) δ = 154.97, 152.32, 150.52, 138.12, 120.18, 43.58, 38.50, 29.88, 28.52, 26.44. Calcd for $C_{21}H_{30}N_{10}$: C, 59.69; H, 7.16%. Found:

C, 59.73; H, 7.09%.

9,9′-(1,8-Octanediyl)bis[6-(dimethylamino)purine] (1g): 40% yield; mp 89—90 °C; ^{1}H NMR ($CDCl_3$, 27 °C) δ = 8.35 (s, 2H), 7.69 (s, 2H), 4.15 (t, 4H, J = 7.2 Hz), 3.53 (broad s, 12H), 1.85 (broad quintet, 4H, J = 7.2 Hz), 1.35—1.25 (m, 8H); ^{13}C NMR ($CDCl_3$) δ = 154.97, 152.31, 150.53, 138.14, 120.18, 43.65, 38.51, 29.95, 28.86, 26.50. Calcd for $C_{22}H_{32}N_{10}$: C, 60.53; H, 7.39%. Found: C, 60.69; H, 7.37%.

9,9′-Ethylenebis[6-(methylamino)purine] (2a): 32% yield; mp 290—294 °C (lit,[11] 285 °C decomp); ^{1}H NMR (DMSO-d_6, 27 °C) δ = 8.14 (s, 2H), 7.77 (s, 2H), 7.59 (s, 2H, NH), 4.93 (s, 4H), 2.94 (broad s, 6H); ^{13}C NMR (DMSO-d_6) δ = 154.71, 152.22, 148.43, 139.93, 118.84, 42.46, 26.72.

9,9′-Trimethylenebis[6-(methylamino)purine] (2b): 40% yield; mp 250—252 °C (lit,[12] 255—256 °C); ^{1}H NMR ($CDCl_3$) δ = 8.42 (s, 2H), 7.90 (s, 2H), 5.88 (s, 2H, NH), 4.25 (t, 4H, J = 6.6 Hz), 3.22 (broad s, 6H), 2.54 (quintet, 2H, J = 6.6 Hz); ^{1}H NMR (DMSO-d_6) δ = 8.22 (s, 2H), 8.17 (s, 2H), 7.65 (s, 2H, NH), 4.19 (t, 4H, J = 7.0 Hz), 2.96 (broad s, 6H), 2.40 (quintet, 2H, J = 7.0 Hz); ^{13}C NMR (DMSO-d_6) δ = 154.90, 152.31, 148.46, 140.39, 119.12, 40.41, 29.82, 26.87.

9,9′-Tetramethylenebis[6-(methylamino)purine] (2c): 41% yield; mp 267—270 °C; ^{1}H NMR ($CDCl_3$) δ = 8.40 (s, 2H), 7.69 (s, 2H), 5.84 (s, 2H, NH), 4.24 (broad, 4H), 3.22 (broad s, 6H), 1.93 (broad, 4H); ^{1}H NMR (DMSO-d_6) δ = 8.20 (s, 2H), 8.10 (s, 2H), 7.63 (s, 2H, NH), 4.18 (broad, 4H), 2.96 (s, 6H), 1.77 (broad, 4H); ^{13}C NMR (DMSO-d_6) δ = 154.91, 152.28, 148.50, 140.39, 119.07, 42.21, 26.92, 26.59. Calcd for $C_{16}H_{20}N_{10}$: C, 54.33; H, 5.65% Found: C, 54.53; H, 5.72%.

9,9′-(1,5-Pentanediyl)bis[6-(methylamino)purine] (2d): 43% yield; mp 236—238 °C; ^{1}H NMR ($CDCl_3$) δ = 8.40 (s, 2H), 7.67 (s, 2H), 5.87 (s, 2H, NH), 4.16 (t, 4H, J = 7.0 Hz), 3.22 (broad s, 6H), 1.95 (quintet, 4H, J = 7.0 Hz), 1.37 (quintet, 2H, J = 7.0 Hz); ^{1}H NMR (DMSO-d_6) δ = 8.19 (s, 2H), 8.08 (s, 2H), 7.62 (s, 2H, NH), 4.11 (t, 4H, J = 7.4 Hz), 2.95 (broad s, 6H), 1.83 (quintet, 4H, J = 7.4 Hz), 1.20 (quintet, 2H, J = 7.4 Hz); ^{13}C NMR (DMSO-d_6) δ = 154.89, 152.25, 148.41, 140.36, 119.06, 42.54, 28.74, 26.86, 22.93. Calcd for $C_{17}H_{22}N_{10}$: C, 55.72; H, 6.05%. Found: C, 55.42; H, 6.05%.

9,9′-(1,6-Hexanediyl)bis[6-(methylamino)purine] (2e): 44% yield; mp 273—276 °C; ^{1}H NMR (DMSO-d_6) δ = 8.19 (s, 2H), 8.10 (s, 2H), 7.59 (s, 2H, NH), 4.11 (t, 4H, J = 7.2 Hz), 2.96 (broad s, 6H), 1.78 (quintet, 4H, J = 7.2 Hz), 1.26 (quintet, 4H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ = 154.88, 152.24, 148.40, 140.36, 119.09, 42.63, 29.11, 26.86, 25.32. Calcd for $C_{18}H_{24}N_{10}$: C, 56.83; H, 6.37%. Found: C, 56.78; N, 6.41%.

9,9′-(1,7-Heptanediyl)bis[6-(methylamino)purine] (2f): 43% yield; mp 206—209 °C; ^{1}H NMR (DMSO-d_6) δ = 8.21 (s, 2H), 8.12 (s, 2H), 7.64 (s, 2H, NH), 4.11 (t, 4H, J = 7.2 Hz), 2.95 (broad s, 6H), 1.77 (quintet, 4H, J = 7.2 Hz), 1.31 (quintet, 2H, J = 7.2 Hz), 1.19 (quintet, 4H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ = 154.84, 152.18, 148.45, 140.41, 119.08, 42.68, 29.15, 27.67, 26.94, 25.70. Calcd for C H N : C, 57.85; H, 6.64%. Found: C, 58.13; H, 6.50%.

9,9′-(1,9-Nonanediyl)bis[6-(methylamino)purine] (2h): 43% yield; mp 189—191 °C; ^{1}H NMR ($CDCl_3$) δ = 8.42 (s, 2H), 7.71 (s, 2H), 5.84 (s, 2H, NH), 4.16 (t, 4H, J = 7.4 Hz), 3.21 (broad s, 6H), 1.86 (quintet, 4H, J = 7.4 Hz), 1.32—1.20 (m, 10H); ^{13}C NMR ($CDCl_3$) δ = 155.57, 153.16, 148.98, 139.47, 119.88, 43.85, 30.05, 29.14, 28.84, 27.64, 26.53. Calcd for $C_{21}H_{30}N_{10}$: C, 59.69; H, 7.16%. Found: C, 59.19; H, 7.10%.

9-(5-Bromopentyl)-6-(dimethylamino)purine (4d) and 9-(5-Bromopentyl)-6-(methylamino)purine (5d). Into a solution of 6-(dimethylamino)purine or 6-(methylamino)purine (1 mmol) in DMF (50 ml), potassium carbonate (1 mmol) and 1,5-dibromopentane (4 mmol) were added. The mixture was stirred at room temperature for 14 h. The resulting mixture was evaporated to give a residue which was extracted with a mixture of chloroform and methanol (9 : 1). The extract was evaporated and chromatographed over silica gel. Elution with ethyl acetate gave **4d** (30%) or **5d** (38%).

4d: Mp 63—64 °C; ^{1}H NMR ($CDCl_3$) δ = 8.35 (s, 1H), 7.72 (s, 1H), 4.19 (t, 2H, J = 7.2 Hz), 3.54 (broad s, 6H), 3.38 (t, 2H, J = 7.2 Hz), 1.92 (quintet, 2H, J = 7.2 Hz), 1.90 (quintet, 2H, J = 7.2 Hz), 1.49 (quintet, 2H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ = 154.99, 152.37, 150.54, 138.09, 120.23, 43.49, 38.55, 33.18, 32.05, 29.23, 25.24. Calcd for $C_{12}H_{18}BrN_5$: C, 46.16; H, 5.81; N, 22.43%. Found: C, 46.43; H, 5,74; N, 22.45%.

5d: Mp 138—139 °C; ^{1}H NMR ($CDCl_3$) δ = 8.42 (s, 1H), 7.73 (s, 1H), 5.96 (broad s, NH, 1H), 4.21 (t, 2H, J = 7.2 Hz), 3.38 (t, 2H, J = 7.2 Hz), 3.22 (broad s, 3H), 1.94 (quintet, 2H, J = 7.2 Hz), 1.91 (quintet, 2H, J = 7.2 Hz), 1.50 (quintet, 2H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ = 155.57, 153.19, 149.08, 139.47, 119.97, 43.63, 33.18, 32.00, 29.29, 27.60, 25.21. Calcd for $C_{11}H_{16}BrN_5$: C, 44.31; H, 5.41; N, 23.49%. Found: C, 44.54; H, 5,40; N, 23.48%.

The author thanks Dr. Takehiko Ueda (Associate Professor of Kagoshima University, Faculty of Engineering) for useful discussion.

References

1 a) W. Springer, "Principles of Nucleic Acid Structure," Springer Verlag, New York (1984). b) R. L. P. Adams, J. T. Knowler, and D. P. Leader, "The Biochemistry of the Nucleic Acids," Chapman and Hall, London and New York (1986).

2 a) D. M. G. Martin and C. B. Reese, *J. Chem. Soc., Chem. Commun.*, **1967**, 1275. b) J. D. Engel and P. H. von Hippel, *Biochemistry*, **13**, 4143 (1974).

3 a) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, **89**, 3612 (1967). b) S. M. Olsthoorn, C. A. G. Haasnoot, and C. Altona, *Eur. J. Biochem.*, **106**, 85 (1980). c) A. J. Hartel, G. Wille, J. H. van Boom, and C. Altona, *Nucleic Acids Res.*, **91**, 1405 (1981). d) C. S. M. Olsthoorn, J. Doornbos, H. P. M. de Leeuw, and C. Altona, *Eur. J. Biochem.*, **125**, 367 (1982). e) P. P. Lankhorst, C. M. Groeneveld, G. Wille, J. H. van Boom, and C. Altona, *Recl. Trav. Chim. Pays-Bas*, **101**, 253 (1982). f) Y. T. can den Hoogen, P. P. Lankhorst, P. Gijsman, A. J. Hartel, J. H. van Boom, and C. Altona, *Eur. J. Biochem.*, **171**, 143 (1988).

4 a) D. Pörschke and F. Eggers, *Eur. J. Biochem.*, **26**, 490 (1972). b) R. Bretz, A. Lustig, and G. Schwarz, *Biophys. Chem.*, **1**, 237 (1974). c) L. P. Vickers and G. K. Ackers, *Arch. Biochem. Biophys.*, **174**, 747 (1976). d) M. P. Heyn, C. U. Nicola, and G. Schwarz, *J. Phys. Chem.*, **81**, 1611 (1977). e) I. Tazawa, T. Koike, and Y. Inoue, *Eur. J. Biochem.*, **109**, 33 (1980).

5 M. Sundaralingam and S. K. Arora, *J. Mol. Biol.*, **71**, 49 (1972).

6 G. McDermott, S. M. Prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell, and N. W. Isaacs, *Nature*, **374**, 517 (1995).

7 C. A. Hunter, *Angew. Chem., Int. Ed. Engl.*, **32**, 1584 (1993).

8 a) M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *J. Am. Chem. Soc.*, **90**, 1042 (1967). b) T-D. Son and C. Chachaty, *Biochim. Biophys. Acta*, **335**, 1 (1973). c) F. E. Evans and R. H. Sarma, *Biopolymers*, **13**, 2117 (1974). d) Y-F. Lam and

G. Kotowycz, *FEBS Lett.*, **78**, 181 (1977).

9 T. Itahara, *J. Chem. Soc., Perkin Trans. 2*, **1996**, 2695.

10 J. H. Lister, *J. Chem. Soc.*, **1960**, 3394.

11 J. H. Lister, *J. Chem. Soc.*, **1963**, 2228.

12 F. Kawabata, K. Kondo, and K. Takemoto, *Makromol. Chem.*, **169**, 37 (1973).

13 T. Itahara, *J. Chem. Soc., Perkin Trans. 2*, **1998**, 1455.

14 H. A. McKenzie, "Data for Biochemical Research," ed by R. M. Dawson, D. C. Elliott, W. H. Elliott, and K. M. Jones, Clarendon Press, Oxford (1969), Chap. 20. The values of pD of the buffer solutions were determined by means of a pH meter and were uncorrected.

15 The spectra of **1a—g**, **2a—h**, **4d**, and **5d** in the buffer solution at pD 1.0 at 27 °C showed two broad signals of the N^6-methyl groups because of the restricted rotation of the methylamino groups, although the restricted rotation had already been reported.[2] The spectra in the buffer solution at pD 1.0 at 50 °C showed one very broad signals and the signals sharpened at 80 °C.

16 C. Giessner-Prettre and B. Pullman, *J. Theor. Biol.*, **27**, 87 (1970).

17 a) L. F. Newcomb and S. H. Gellman, *J. Am. Chem. Soc.*, **116**, 4993 (1994). b) L. F. Newcomb, T. S. Haque, and S. H. Gellman, *J. Am. Chem. Soc.*, **117**, 6509 (1995).

18 Y. Inaki, T. Renge, K. Kondo, and K. Takemoto, *Makromol. Chem.*, **176**, 2683 (1975).

19 a) S. Claude, J-M. Lehn, F. Schmidt, and J-P. Vigneron, *J. Chem. Soc., Chem. Commun.*, **1991**, 1183. b) S. B. Ferguson, E. M. Sanford, E. M. Seward, and F. Diederich, *J. Am. Chem. Soc.*, **113**, 5410 (1991). c) K. M. Guckian, B. A. Schweitzer, R. X-F. Ren, C. J. Sheils, P. L. Paris, C. Tahmassebi, and E. C. Kool, *J. Am. Chem. Soc.*, **118**, 8192 (1996).

20 a) T. Ishida, M. Doi, H. Ueda, M. Inoue, and G. M. Scheldrick, *J. Am. Chem. Soc.*, **110**, 2286 (1988). b) S. C. Zimmerman, C. M. VanZyl, and G. S. Hamilton, *J. Am. Chem. Soc.*, **111**, 1373 (1989). c) Y. Yamagata, M. Kato, and S. Fujii, *Chem. Pharm. Bull.*, **42**, 2385 (1994).

21 a) A. V. Muehldorf, D. Van Engen, J. C. Warner, and A. D. Hamilton, *J. Am. Chem. Soc.*, **110**, 6561 (1988). b) A. Sarai, J. Mazur, R. Nussinov, and R. J. Jernigen, *Biochemstry*, **27**, 8498 (1988). c) C. A. Hunter and J. K. M. Sanders, *J. Am. Chem. Soc.*, **112**, 5525 (1990). d) J. Zhang and J. S. Moore, *J. Am. Chem. Soc.*, **114**, 9701 (1992). e) F. Cozzi, M. Cinquini, R. Annuziata, and J. S. Siegel, *J. Am. Chem. Soc.*, **115**, 5330 (1993).

22 W. L. Jorgensen and D. L. Severance, *J. Am. Chem. Soc.*, **112**, 4768 (1990).

23 a) J. N. Israelachvili and R. Pashley, *Nature*, **300**, 341 (1982). b) J. N. Israelachvili, "Intermolecular and Surface Forces," Academic Press, London (1992).