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EFFECT OF THE N^6-METHYL GROUP ON ADENINE RING ON THE INTRAMOLECULAR BASE-PAIRING OF ADENINE WITH THYMINE IN VARIOUS SOLVENTS

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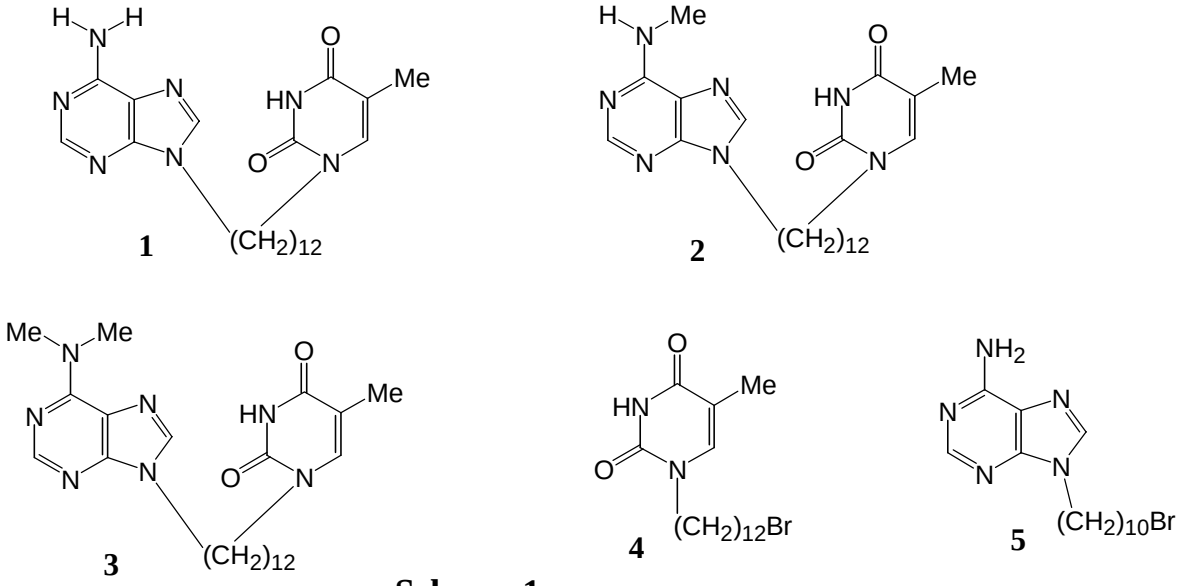
Abstract _ In order to study an intramolecular hydrogen bond between the adenine and thymine rings of 9-[12-(thymin-1-yl)dodecyl]adenine and N^6-methyl-9-[12-(thymin-1-yl)-dodecyl]adenine, their NMR chemical shifts of NH of thymine ring at 3-position were compared with those of N^6,N^6-dimethyl-9-[12-(thymin-1-yl)dodecyl]adenine in various solvents. The thermodynamic parameters of the intramolecular hydrogen bond in chloroform and benzene were estimated. The hydrogen bond was confirmed by the IR spectroscopy.

Nucleobase-pairing, which is a hydrogen-bonded assembly, plays an important role in the structure formation of DNA.[1] The intramolecular nucleobase-pairing may be of interest in connection with the formation of the hairpin loop of not only RNA but also DNA.[2] The intramolecular base-pairing of adenine and thymine connected with some linkers has been reported by several groups of workers.[3] Although the solvent effect on hydrogen bond is of importance in connection with the biological function,[4] little attention has been paid to the solvent effect on the base-pairing. On the other hand, N^6-substituted adenines such as kinetin (N^6-furfuryladenine), N^6-methyladenine,[5] and N^6,N^6-dimethyladenine[6] are widely

found in natural products. It is known that the hydrogen bond between 9-ethyladenine and 1-cyclohexyluracil is affected by the N^6-methyl group on the adenine ring in chloroform.[7] This work was undertaken to determine the effect of the N^6-methyl group on the adenine ring on the intramolecular base-pairing in various solvents.

RESULTS AND DISCUSSION

Previously we reported that the treatment of adenine with 1-(12-bromododecyl)-thymine **(4)** gave 9-[12-(thymin-1-yl)dodecyl]adenine **(1).**[3d] Under similar conditions the reaction of N^6-methyladenine and of N^6,N^6-dimethyladenine with **4** gave N^6-methyl-9-[12-(thymin-1-yl)dodecyl]adenine **(2)** and N^6,N^6-dimethyl-9-[12-(thymin-1-yl)dodecyl]adenine **(3)**, respectively (Scheme 1).



Scheme 1

Since the chemical shifts of Thy-NH (NH of thymine ring at 3-position) are remarkably to lower fields when the hydrogen bond is formed,[1,3] the NMR chemical shifts of Thy-NH of **1** and **2** were compared with those of **3** in various solvents. The concentration dependence on the chemical shifts of Thy-NH of **1**, **2**, and **3** in $CDCl_3$ is shown in Figure 1. Furthermore, the concentration dependence on the chemical shift of Thy-NH of **4** was similar to that of **3**. The chemical shifts of

Thy-NH of **1**, **2**, **3**, and **4** were to lower fields along with an increase in the concentrations because of an intermolecular interaction, but were little shifted in lower concentrations than 1 mM. This suggests that the intermolecular interaction were almost negligible when the measurements were carried out at lower concentrations than 1 mM. As can be seen from Figure 1, the chemical shifts of **1** and **2** were largely different from those of **3**. The differences are explained in terms of the formation of the intramolecular hydrogen bond between adenine and thymine rings of **1** and **2**. It is conceivable that the intramolecular hydrogen bond of **1** and **2** is built basing on the Watson-Crick or Hoogsteen base-pairing.[1] On the other hand, **3** can not exist in not only the Watson-Crick but also Hoogsteen base-pairing because of the presence of the N^6,N^6-dimethylamino group, although the thymine ring of **3** resulted in the thymine-thymine aggregation along with an increase of the concentration (Figure 1).

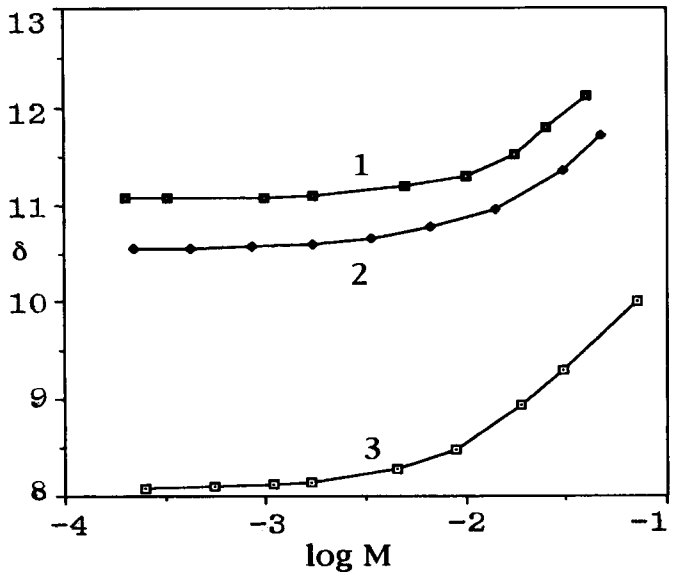
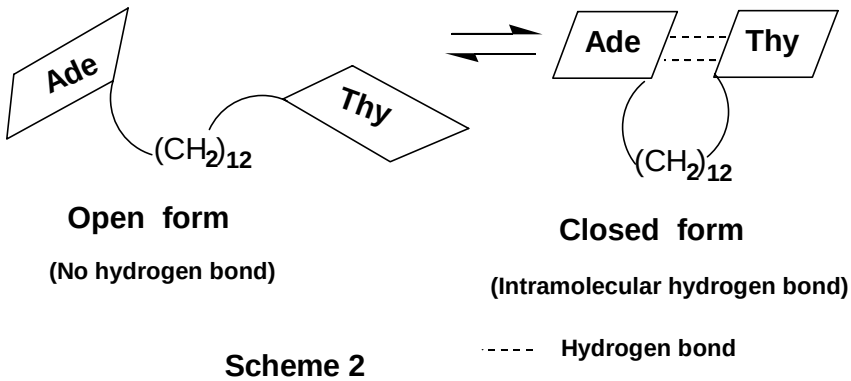


Figure 1. Concentration Dependence on the Chemical Shift of Thy-NH of **1**, **2**, and **3** in $CDCl_3$ at 27 °C.

Table 1 shows the values of the chemical shifts of Thy-NH of **1**, **2**, and **3** in various solvents at lower concentrations than 0.5 mM except for those in methanol.[8] It is conceivable that the adenine and thymine rings of **1** and **2** exist in an equilibrium between an open form (no hydrogen bond) and a closed form (intramolecular hydrogen

bond) (Scheme 2), because the intermolecular interaction was almost negligible at lower concentrations than 0.5 mM. Therefore, the chemical shifts ($\delta_{observed}$) of **1** and **2** originated from the equilibrium between the open form and the closed form, while the chemical shifts of **3** came from those of only the open form. The chemical shifts of **1** and **2** in toluene-d_8, benzene-d_6, and $CDCl_3$ were remarkably to lower field, compared with those of **3.** The difference of the chemical shifts between **1** (or **2**) and **3** can be regarded as an increase of the closed form. On the other hand, it can be seen from Table 1 that the chemical shifts of **1** and **2** in DMSO-d_6, pyridine-d_5, and methanol were similar to those of **3.** These results



Scheme 2

Table 1. NMR Chemical Shift of Thy-NH of **1, 2,** and **3** at 27 °C[a)]

	1	**2**	**3**
solvent	chemical shift / δ		
toluene-d_8	13.54	13.50	8.31
benzene-d_6	13.50	13.45	8.29
pyridine-d_5	13.14	13.14	13.11
DMSO—d_6	11.16	11.16	11.16
$CDCl_3$	11.07	10.56	8.08
MeOH[b)]	11.12	11.13	11.07
acetone-d_6	10.49	10.34	9.88
1,4-dioxane-d_8	10.18	10.18	9.96

a) The concentrations were less than 0.5 mM except in MeOH.

b) Conditions: **1** (3.1 mM), **2** (5.6 mM), or **3** (6.0mM) in MeOH containing CD_3OD (12%).

suggest that the adenine and thymine rings of **1** and **2** hardly result in the formation of the intramolecular hydrogen bond in DMSO, pyridine, and methanol.

Table 2 shows the temperature dependence on the chemical shifts of **1**, **2**, and **3**. The chemical shift differences of **1** between 27 °C and 80 °C in toluene-d_8 and $CDBr_3$ were 2.31 and 1.71 ppm, respectively, and the differences of **2** were 2.29 and 1.57 ppm, respectively. However, the chemical shift differences of **1** and **2** in 1,4-dioxane-d_8 and DMSO-d_6 were smaller, compared with those in toluene-d_8 and $CDBr_3$. Furthermore, the chemical shift difference of **3** in toluene-d_8 (0.53 ppm) was smaller, compared with the differences of **1** and **2** in toluene-d_8. These results are consistent with the results of Table 1.

The thermodynamic parameters of the intramolecular base-pairing of **1** in $CDCl_3$ (ΔH = -21 ± 2 KJ mol^{-1}, ΔS = -69 ± 7 J K^{-1} mol^{-1}) was previously reported.[3d] In an effort to determine the effect of N^6-methyl group on the intramolecular hydrogen bond, it was felt that a comparison of the thermodynamic parameters of **1** and **2** was of importance. The chemical shifts (δ_{open}) of the open form of **1** and **2** were estimated from the chemical shifts of **3**. To determine the chemical shifts (δ_{closed}) of the

Table 2. Temperature Dependence on the Chemical Shift of Thy-NH of **1**, **2**, and **3**

Temp	**1** / chemical shift: δ				**2** / δ				**3** / δ		
°C	C_7D_8	$CDBr_3$	C_4D_8O	DMSO	C_7D_8	$CDBr_3$	C_4D_8O	DMSO	C_7D_8	C_4D_8O	DMSO
27	13.54	10.95	10.18	11.16	13.50	10.29	10.18	11.16	8.31	9.96	11.16
35	13.27	10.70	10.12	11.11	13.27	9.98	10.13	11.12	8.24	9.93	11.11
40	13.07	10.48	10.08	11.08	13.10	9.73	10.10	11.09	8.19	9.91	11.08
50	12.66	10.17	10.01	11.02	12.72	9.46	10.03	11.03	8.08	9.86	11.03
60	12.20	9.85	9.94	10.96	12.24	9.17	9.97	10.97	7.99	9.80	10.98
70	11.72	9.57	9.88	10.91	11.78	8.94	9.90	10.91	7.87	9.74	10.92
80	11.23	9.34	9.83	10.86	11.21	8.72	9.83	10.85	7.78	9.69	10.86
Δδ	2.31	1.71	0.35	0.30	2.29	1.57	0.36	0.31	0.53	0.27	0.30

C_7D_8 = toluene-d_8; C_4D_8O = 1,4-dioxane-d_8 ; DMSO = dimethyl sulfoxide-d_6

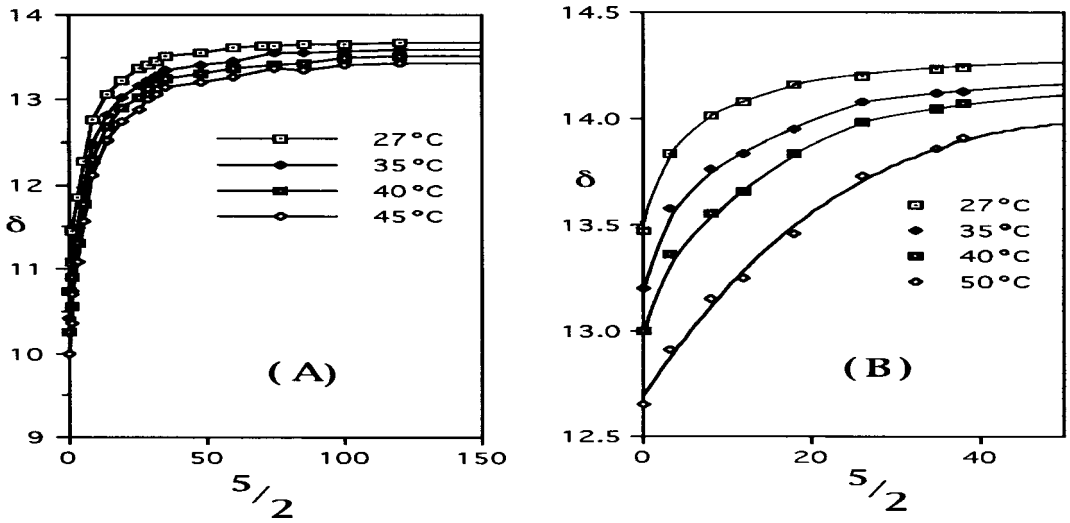


Figure 2. Titration Curves of **2** with **5:** the Chemical Shift of Thy-NH vs the Rate of **5** (mol) to **2** (mol). **(A) 2** (1.2 mM) in $CDCl_3$. **(B) 2** (0.6 mM) in benzene-d_6.

Table 3. The Chemical Shift of Thy-NH for the Thermodynamic Parameters

	Solvent	Temp/ °C	δ_{open} [a]	$\delta_{Observed}$	δ_{closed} [b]
1	benzene-d_6	27	8.29	13.50	14.28
		35	8.21	13.19	14.17
		40	8.16	12.99	14.11
		50	8.05	12.62	14.00
	$CDCl_3$	27	8.07	11.07[c]	13.71[c]
		40	8.02	10.42[c]	13.50[c]
		45	8.00	10.20[c]	13.41[c]
2	benzene-d_6	27	8.29	13.45	14.26
		35	8.21	13.15	14.16
		40	8.16	12.95	14.10
		50	8.05	12.59	14.00
	$CDCl_3$	27	8.07	10.68	13.69
		35	8.04	10.35	13.59
		40	8.02	10.18	13.51
		45	8.00	9.96	13.43

a) The values of δ_{open} were estimated from the chemical shifts of **3.**
b) The values of δ_{closed} were determined from the titration of **1** and **2** with **5.**
c) The values of $\delta_{observed}$ and δ_{closed} of **1** in $CDCl_3$ were reported in the previous paper.[3b]

closed form of **1** and **2**, the titration of **1** and **2** with 9-(10-bromodecyl)adenine **(5)** was studied. Figure 2 shows titration curves of Thy-NH of **2** in $CDCl_3$ at 27 °C, 35 °C, 40 °C, and 45 °C and in benzene-d_6 at 27 °C, 35 °C, 40 °C, and 50 °C. The values of δ_{closed} of **1** in benzene-d_6 were similar to those of **2**. The values of the chemical shifts of Thy-NH for thermodynamic parameters are summarized in Table 3.

The equilibrium constant K was calculated as follows:

$K = [\text{closed form}]/[\text{open form}]$

$= (\delta_{observed} - \delta_{open})/(\delta_{closed} - \delta_{observed})$

The enthalpy and entropy change values of the intramolecular base-pairing of **1** and **2** were determined by plotting log K against (1/T) using

$\log K = (-\Delta H/2.3R)(1/T) + (\Delta S/2.3R)$

The thermodynamic parameters of **1** and **2** determined in the present experiments were as follows:

1 in C_6D_6: $\Delta H = -25 \pm 3$ KJ mol^{-1}, $\Delta S = -66 \pm 10$ J K^{-1} mol^{-1}

1 in $CDCl_3$: $\Delta H = -22 \pm 2$ KJ mol^{-1}, $\Delta S = -72 \pm 7$ J K^{-1} mol^{-1}

2 in C_6D_6: $\Delta H = -24 \pm 3$ KJ mol^{-1}, $\Delta S = -64 \pm 10$ J K^{-1} mol^{-1}

2 in $CDCl_3$: $\Delta H = -19 \pm 2$ KJ mol^{-1}, $\Delta S = -65 \pm 7$ J K^{-1} mol^{-1}

For reference the value of ΔH reported for the intermolecular hydrogen bond between 9-ethyladenine and 1-cyclohexyluracil in chloroform was -6.2 ± 0.6 kcal mol^{-1} (-25.9 ± 2.5 KJ mol^{-1}).[9] The value of ΔH of **2** determined in the present experiments was smaller, compared with that of **1** in $CDCl_3$ because of the effect of the N^6-methyl group on the adenine ring on the intramolecular base-pairing.[7] However, interestingly the values of ΔH and ΔS of **2** in benzene-d_6 were similar to those of **1**. This suggests that the intramolecular hydrogen bond was little affected by the N^6-methyl group on the adenine ring in benzene.

In order to confirm the hydrogen bond in $CDCl_3$ and in benzene, IR spectroscopy of **2** was studied at room temperature (Figure 3). In a benzene-d_6 solution of **2**, almost only one band was observed at 3317 cm^{-1} (hydrogen bonded Ade-NH and Thy-NH) in the range of 3100-3600 cm^{-1}, while **2** displayed three major bands at 3449 (non-bonded Ade-NH), 3395 (non-bonded Thy-NH), and 3320 cm^{-1} (hydrogen bonded

Ade-NH and Thy-NH) in $CDCl_3$. Furthermore, two carbonyl stretching bands were observed at 1703 (non-bonded carbonyl) and 1672 cm^{-1} (hydrogen bonded carbonyl) in the benzene-d_6 solution of **2.** These results suggest that the adenine and thymine rings of **2** mostly exist in the closed form in benzene at room temperature.

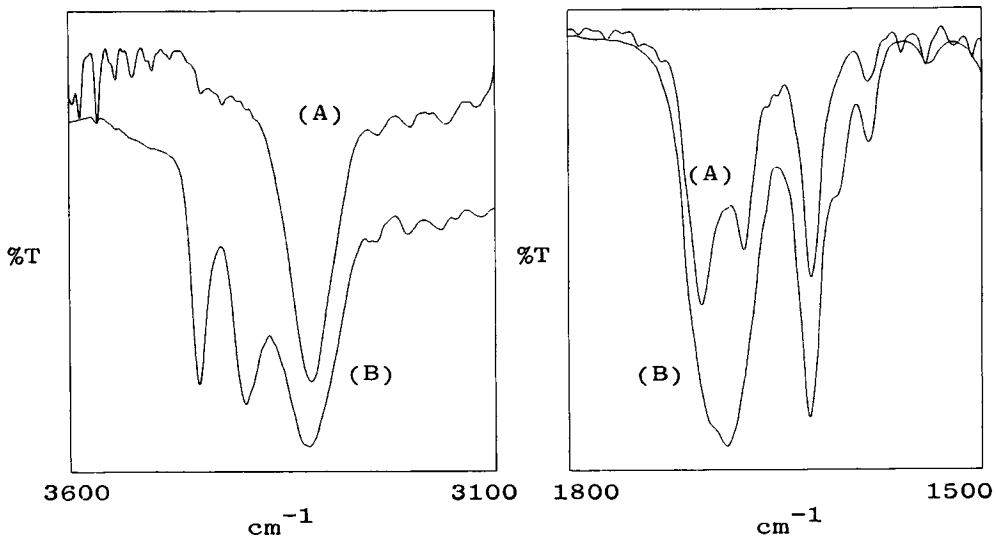


Figure 3. IR Spectra of **2** in Benzene-d_6 and $CDCl_3$.
(A) **2** (4 mM) in benzene-d_6: 3317, 1703, 1672, 1623 cm^{-1}.
(B) **2** (7 mM) in $CDCl_3$: 3449, 3395, 3320, 1700 (shoulder), 1685, 1625 cm^{-1}.

EXPERIMENTAL

The melting points were determined on a Yanagimoto micro melting-point apparatus and are uncorrected. The elemental analyses were performed in the Analytical Center of Kyoto University. The ^{1}H-NMR spectra (400 MHz) and ^{13}C-NMR spectra (100 MHz) were obtained with a JEOL GSX 400 spectrometer. The chemical shifts (δ-values) were measured in parts per million (ppm) down-field from tetramethylsilane as an internal reference. The deuterium solvents were obtained commercially. The IR spectra were recorded on a JASCO FT/IR-420 spectrometer. The measurements in $CDCl_3$ (Merck, >99.8%, H_2O <0.01%) and in benzene-d_6 (Merck, >99.6%, H_2O <0.02%) were made at room temperature with a 0.1 mm KBr cell. The preparation of 9-[12-(thymin-1-yl)dodecy]adenine **(1)** was previously reported.[3d]

N^6-Methyl-9-[12-(thymin-1-yl)dodecy]adenine (2).

Into a solution of N^6-methyladenine (6-methylaminopurine) (150 mg, 1 mmol) in

in N,N-dimethylformamide (DMF) (30 mL), 1-(12-bromododecyl)thymine **(4)**[3d] (373 mg, 1 mmol) and K_2CO_3 (138 mg, 1 mmol) were added. The mixture was stirred at rt for 15 h. The reaction mixture was evaporated to give a residue, which was submitted to chromatography over silica gel. Elution of a mixture of ethyl acetate and methanol (4:1) gave **2** (154 mg, 34%) : mp 80-81 °C (MeOH); ^{1}H-NMR ($CDCl_3$) δ 11.22 (br s, 1H, Thy-NH), 8.42 (s, 1H, Ade-2 or Ade-8), 7.90 (s, 1H, Ade-8 or Ade-2), 6.99 (s, 1H, Thy-6), 6.90 (br s, 1H, Ade-NH), 4.19 (t, 2H, J=7 Hz), 3.71 (t, 2H, J=7 Hz), 3.21 (br s, 3H, Ade-NMe), 1.94 (s, 3H, Thy-Me), 1.89 (br quintet, 2H, J=7 Hz), 1.68 (br quintet, 2H, J=7 Hz), 1.35-1.2 (br, 16H); ^{13}C-NMR ($CDCl_3$) δ 165.09, 155.50, 153.29, 151.41, 148.83, 140.35, 139.79, 119.43, 110.60, 47.97, 43.79, 29.80, 29.26, 29.22, 29.21, 29.16, 29.02, 28.80, 28.79, 27.53, 26.38, 26.12,12.35. IR (see Figure 3). Anal. Calcd for $C_{23}H_{35}N_7O_2$•1/2H_2O: C, 61.31; H, 8.05; N, 21.76. Found: C, 61.65; H, 8.05; N, 21.75.

N^6,N^6-Dimethyl-9-[12-(thymin-1-yl)dodecyl]adenine (3).

Into a solution of N^6,N^6-dimethyladenine (6-dimethylaminopurine) (163 mg, 1 mmol) in DMF (30 mL), 1-(12-bromododecyl)thymine **(4)** (374 mg, 1 mmol) and K_2CO_3 (140 mg, 1 mmol) were added. The mixture was stirred at rt for 15 h. The reaction mixture was evaporated to give a residue, which was submitted to chromatography over silica gel. Elution of a mixture of ethyl acetate and methanol (5:1) gave **3** (163 mg, 35%): mp 103-104 °C (MeOH); ^{1}H-NMR ($CDCl_3$) δ 8.36 (s, 1H, Ade-2 or Ade-8), 8.15 (br s, 1H, Thy-NH), 7.72 (s, 1H, Ade-8 or Ade-2), 6.96 (s, 1H, Thy-6), 4.17 (t, 2H, J=7 Hz), 3.67 (t, 2H, J=7 Hz), 3.54 (br s, 6H, Ade-NMe_2), 1.92(s, 3H, Thy-Me), 1.87 (br quintet, 2H, J=7 Hz), 1.66 (br quintet, 2H, J=7 Hz), 1.35-1.2 (br, 16H); ^{13}C-NMR ($CDCl_3$) δ 164.76, 154.97, 152.29, 151.16, 150.46, 140.50, 138.41, 120.11, 110.49, 48.54, 43.76, 38.58, 30.01, 29.38, 29.37, 29.34, 29.33, 29.13, 29.06, 29.02, 26.63, 26.40, 12.31. IR ($CDCl_3$) 3394 (non-bonded Thy-NH), 2929, 2856, *ca.* 1700 (shoulder), 1684 cm^{-1}. Anal. Calcd for $C_{24}H_{37}N_7O_2$•1/2H_2O: C, 62.04; H, 8.24; N, 21.10. Found: C, 62.24; H, 8.23; N, 21.10.

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8 Attempts to determine the chemical shifts of Thy-NH of **1**, **2**, and **3** in H_2O containing D_2O were unsuccessful because of the poor solubility in water.

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