NMR study of 9,9′-(alkane-α,ω-diyl)diadenine

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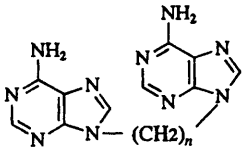
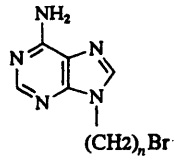
The relationship between NMR chemical shifts of adenine ring protons of low concentrations of 9,9′-(alkane-α,ω-diyl)diadenine and the length of the polymethylene chains has been investigated in buffer solutions at pD 1.0, 7.0 and 13.0 and in organic solvents such as CD_3OD, $(CF_3)_2CDOD$, [$^2H_6$]dimethyl sulfoxide and CF_3COOD. The chemical shifts were compared with those of 9-(ω-bromoalkyl)adenine.

The base-stacking interaction is one of the major stabilizing forces of the tertiary structure of nucleic acids and can be observed in aqueous solutions as well as in crystals. Many approaches to elucidate the stacking interaction in aqueous solutions have been reported. In the classical study by nuclear magnetic resonance (NMR) spectroscopy, several groups of workers have demonstrated the usefulness of NMR methods in elucidating the stacking conformations of nucleic acid bases on the basis of the changes in their chemical shifts by the concentration effect: as the solute concentrations are increased, the proton resonances in the bases are shifted to higher fields.[1] The concentration effect is interpreted in terms of vertical stacking of the bases, because the ring current due to the stacking interaction of aromatic bases produces an upfield shift in the proton resonance compared with the isolated bases. However, the interaction of the bases is explained as an isodesmic model of non-cooperative stacking among bases,[2] so that the changes in the chemical shifts of the base protons by these interactions do not correspond to only the relationship of two aromatic bases. Furthermore, self-association of molecules may affect NMR chemical shifts which are usually measured at concentrations of more than 1 mmol dm^{-3}, although it is generally neglected at the low concentrations used in UV and CD measurements. On the other hand, when ^{1}H NMR spectra of low concentrations of two nucleic acid bases linked by polymethylene chains are measured, each of the protons in the two bases is expected to be primarily affected by the ring current of the other base. Consequently almost only the interaction between the two bases may be observed.

9,9′-(Alkane-α,ω-diyl)diadenines **1a–j** are of interest as the model compounds to study the interaction between two adenine rings and **1b**,[3a,4a,5] **1c**,[4–7] **1d**,[5,7] **1e**[5] and **1f**.[3a,4a,5] have already been prepared. The UV and emission spectra of **1b–f** were investigated by Leonald and co-workers[4a] and Inaki *et al*.[5b] in a study of the stacking interaction. However, little attention has been paid to the NMR study of **1** in D_2O solutions in this connection. On the other hand, we have studied the relevancy between the chemical shifts of 7,7′-(alkane- α,ω-diyl)-ditheophyllines in D_2O and the length of the polymethylene chains and the results lend some support to the stacking interaction of the theophylline ring.[8] Therefore, the study of **1a–j** in aqueous solutions is expected to be of interest in connection with the stacking interaction of the adenine ring. The chemical shifts of **1** have been further compared with those of 9-(ω-bromoalkyl)adenines **2**.



1



2

	n		*n*
a:	1	**g**:	7
b:	2	**h**:	8
c:	3	**i**:	9
d:	4	**j**:	10
e:	5	**k**:	12
f:	6		

Results and discussion

9,9′-(Alkane-α,ω-diyl)diadenines **1a–j** and 9-(ω-bromoalkyl)-adenines **2b,f,g,j** were prepared from adenine and α,ω-dibromoalkanes similar to a method described before.[3–7] Since the structure of the adenine ring is known to be pH dependent,[2] the chemical shifts of the adenine ring protons of **1a–j** were measured in buffer solutions such as the Na_2HPO_4–NaH_2PO_4 (sodium phosphate) buffer solution at pD 7.0,[9] the KCl–HCl (Clark and Lubs) buffer solution at pD 1.0[9] and the KCl–NaOH buffer solution at pD 13.0.[9]

Fig. 1 shows the concentration dependence of the chemical shifts of the adenine ring of **1c** and **1d** in buffer solution at pD 7.0 and 27 °C. The measurements were made on solutions ranging in concentration from 0.04 to 1.8 mmol dm^{-3}. In spite of the well known pronounced concentration effect of nucleic acid bases and nucleotides,[1] the differences in the chemical shifts, as can be seen from Fig. 1, were within 0.003 ppm over the range of the concentrations studied. Therefore, self-association of **1** may be neglected at concentrations of less than 1 mmol dm^{-3}.

In Figs. 2 and 3 the relationship between the chemical shifts of the protons of the adenine ring at the 2- and 8-positions of **1** and **2** in the buffer solution and at pD 7.0 and at 27 °C (Fig. 2) and 50 °C (Fig. 3) and the length of the polymethylene chains (n = 1–10) is shown. The ^{1}H NMR measurement of 9,9′-(dodecane-1,12-diyl)diadenine (**1k**) in the buffer solution at pD 7.0 (accumulation of 2000 times) did not give clear peaks of the adenine ring because of the very low solubility. The effect of temperature (27–80 °C) on the chemical shifts of **1a–e** in the buffer solution at pD 7.0 is summarized in Table 1.

In an effort to assign the aromatic absorptions of **1** to the hydrogen atoms of the adenine ring at the 2- and 8-positions, the compounds **1c–e** containing about 35% of the 8-deuterioadenine moiety were prepared by the reaction of a mixture of

Table 1 Effect of temperature on the chemical shifts of the protons of the adenine ring at the 2- and 8-positions of **1a–e**[a]

	δ									
	1a		**1b**		**1c**		**1d**		**1e**	
T/°C	H-2	H-8	H-2	H-8	H-2	H-8	H-2	H-8	H-2	H-8
27	8.438	8.315	8.002	7.857	7.942	7.867	7.918	7.910	7.993	7.945
40	—	—	7.977	7.887	7.957	7.868	7.930	7.917	8.008	7.947
50	8.438	8.315	7.967	7.908	7.967	7.878	7.958	7.927	8.040	7.949
60	—	—	7.950	7.925	7.996	7.887	7.985	7.936	8.055	7.954
70	—	—	7.943	7.935	8.015	7.896	8.010	7.944	8.075	7.958
80	8.423	8.323	7.950	7.935	8.032	7.904	8.035	7.952	8.093	7.961
$\Delta\delta$[b]	−0.015	+0.008	−0.052	+0.078	+0.090	+0.037	+0.117	+0.042	+0.100	+0.016

[a] Chemical shifts of H-2 and H-8 of **1a–e** in the sodium phosphate buffer solution at pD 7.0. Concentrations: <1.0 mmol dm^{-3}. [b] $\Delta\delta = \delta(80\ °C) - \delta(27\ °C)$.

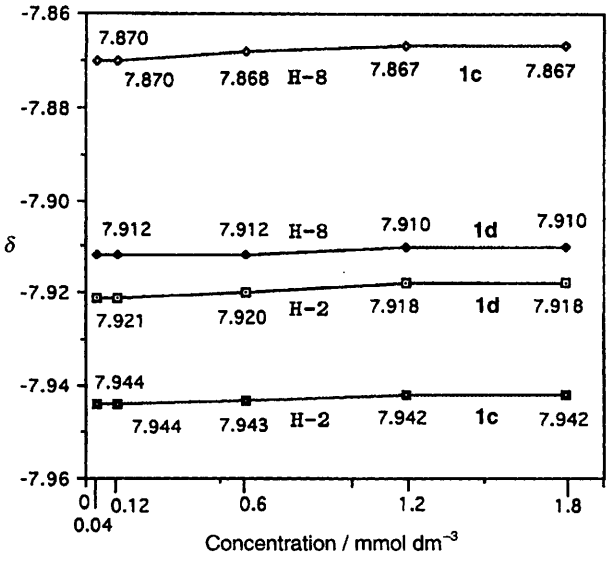


Fig. 1 Relationship between concentration (mmol dm^{-3}) of **1c** and **1d** in the buffer solution at pD 7.0 and the chemical shifts (δ) at 27 °C. The precision was within ±0.001 ppm.

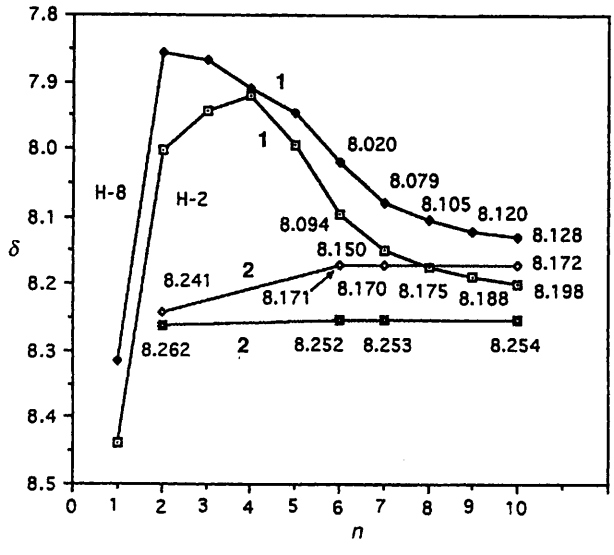


Fig. 2 Relationship between the chemical shifts (δ) of the adenine ring of **1** and **2** and the number of carbon atoms (n) of the polymethylene chains in the buffer solution at pD 7.0 and 27 °C. Concentrations: **1** (<1.0 mmol dm^{-3}) and **2** (<1.2 mmol dm^{-3}). The chemical shifts of **1a–e** are shown in Table 1.

8-deuterioadenine and adenine with α,ω-dibromoalkanes; 8-deuterioadenine was obtained by the heating of adenine in D_2O under reflux because purine derivatives such as adenine are

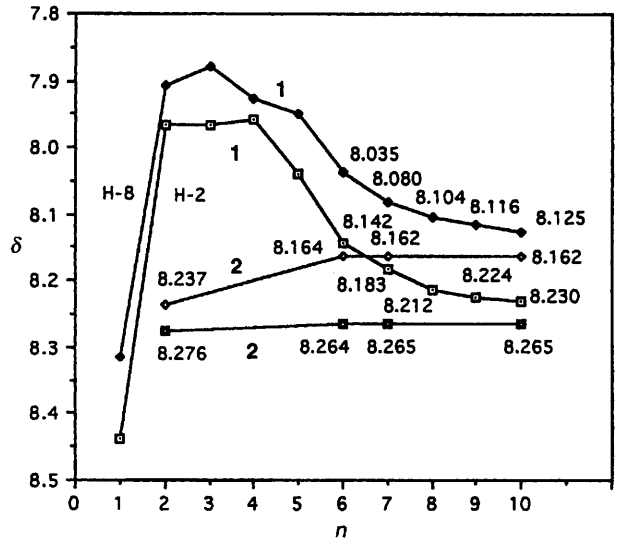


Fig. 3 Relationship between the chemical shifts (δ) of adenine ring of **1** and **2** and the number of carbon atoms (n) of the polymethylene chains in the buffer solution at pD 7.0 and 50 °C. Concentrations: **1** (<1.0 mmol dm^{-3}) and **2** (<1.2 mmol dm^{-3}). The chemical shifts of **1a–e** are shown in Table 1.

known to undergo the deuterium exchange at their C-8 position but not at their 2-position under the same conditions.[10] The assignments of the aromatic absorptions of **1a,b,f–j** in Figs. 2 and 3 and Table 1 were presumed on the basis of those of **1c–e** containing the 8-deuterioadenine moiety.

Fig. 2 shows that the proton resonances of the adenine ring at the 8-position of **1b–j** (n = 2–10) in the buffer solution at pD 7.0 and 27 °C were shifted to a higher field as the length of the polymethylene chains decreased, except for that of **1a** (n = 1). On the other hand, among the chemical shifts of the protons of the adenine ring at the 2-position of **1a–j**, the chemical shift of **1d** (n = 4) was shifted to the highest field. As the numbers of carbon atoms of **1** increased, the chemical shifts approached maximum values which were expected to be the same as those of **2**. It can be seen from Fig. 3 that similar behaviour was also observed at 50 °C except for **1b–d** (n = 2–4). That is, the chemical shifts of **1e–j** ($n \geq 5$) were shifted to a lower field with the increase in the carbon atoms, while the shifts of the chemical shifts of **1b–d** (n = 2–4) were different to those at 27 °C (Fig. 2).

Table 1 shows that the chemical shifts of the protons of the adenine ring at the 2-position of **1c–e** were largely influenced by the temperature, compared with those at the 8-position, while the temperature effect on **1a,b** was different to that of **1c–e**: the temperature effect of **1a** was very small and the chemical shift of H-2 of **1b** was shifted to a lower field as the temperature was lowered. The difference between **1a,b** and **1c–e** may be attributed to the length of the polymethylene chains: the shorter polymethylene chains such as the methylene and ethylene

groups of **1a** and **1b** may restrict the stacking interaction between the two adenine rings.

Since nucleic acid bases are stacked with interplanar distances of *ca.* 3.4 Å in the helical structure of β-DNA, Geissner-Prettre and Pullman[11] investigated the calculations for the chemical shift variations due to the base-stacking. The calculated chemical shift of H-2 due to the stacking of two adenine rings separated by 3.4 Å was 0.30–0.45 ppm and that of H-8 was 0.15–0.25 ppm.[11] The chemical shift differences of H-2 and H-8 of the adenine ring in the buffer solution at pD 7.0 and 27 °C, which were determined by the comparison of the chemical shifts of **1d** with those of **1j** (or **2j**), were 0.278 (0.334) and 0.219 (0.263) ppm, respectively. Also, the differences of the shifts of H-2 and H-8 of the adenine ring at 50 °C were 0.272 (0.307) and 0.198 (0.235) ppm, respectively. These values are roughly consistent with the calculated chemical shifts so that it seemed reasonable to assume that the upfield shifts of **1** resulted from the intramolecular base-stacking interaction.

The initial stage of the reaction of the adenine ring in aqueous solution at pH 1 instead of pH 7 is known to be the protonation at the 1-position.[2] The chemical shifts of the adenine ring protons of **1** in the KCl–HCl buffer solution at pD 1.0 and in the KCl–NaOH buffer solution at pD 13.0 and at 27 and 50 °C are shown in Table 2 in order to compare with those in the buffer solution at pD 7.0 (Figs. 2 and 3). The behaviour in the buffer solution at pD 7.0 resembled that in the buffer solution at pD 13.0, but was clearly different from that in the buffer solution at pD 1.0. The chemical shifts of **1c–j** in the buffer solution at pD 1.0 were influenced little by the temperature and were shifted to a higher field as the carbon chain length decreased, but the shifts to a higher field were small, compared with those in the buffer solutions at pD 7.0 and 13.0. The results in the buffer solution at pD 1.0 may be explained in terms of the protonation at the 1-position of the adenine ring and the repulsion between the resulting two cations. A similar effect on purine rings of the stacking interaction was reported in the literature concerning the X-ray analyses of the crystal structure of protonated purines[12] and the ^{1}H NMR study of 7-methylinosine[1h] and of N_1-oxide of adenosine monophosphate.[13] It is of interest that there was a stacking interaction between the two adenine rings even in the buffer solution at pD 1.0, if there might be repulsion between the two adenine rings.

In order to confirm the effect of solvent on the stacking interaction, the behaviour was examined in organic solvents such as [$^2H_6$]dimethyl sulfoxide ([$^2H_6$]DMSO), [$^2H_4$]methyl alcohol (CD_3OD), [$^2H_2$]hexafluoroisopropyl alcohol [$(CF_3)_2$-CDOD], and [^{2}H]trifluoroacetic acid (CF_3COOD) at 27 °C (Table 2). The chemical shifts of **1**, except **1a–c** (n = 1–3), remained almost constant in both [$^2H_6$]DMSO and CF_3COOD. In CD_3OD, the chemical shifts of **1d–j** (n = 4–10) shifted to a higher field as the numbers of carbon atoms decreased, although the shift was very small. The chemical shifts of **1b–j** (n = 2–10) in $(CF_3)_2CDOD$ were not shifted to a higher field, but slightly shifted to a lower field as the chain length decreased.

Table 3 shows the relationship between the chemical shifts of **1d** and the amount of organic solvent added to D_2O. In the mixture of D_2O and CD_3OD (protic solvent) and also the mixture of D_2O and [$^2H_6$]DMSO (aprotic solvent) the upfield shifts were dependent on adding amounts of D_2O. On the basis of these data, the effect of water as solvent on the stacking interaction of **1** was ascertained.

The stacking interactions of some compounds of **1** are known from their UV and emission spectra.[4a,5b] For example, Leonald and co-workers,[4a] reported that the order of the stacking interaction deduced from the hypochromism and emission studies was **1c** > **1b** and **1f**. The results presented in this paper represent the relationship between the upfield shifts of the adenine ring protons of **1** and the length of the polymethylene chains: the upfield shifts in aqueous solutions is definitely related to the length of the chains. From the results, it seemed reasonable to assume that the upfield shifts may roughly correspond to an increase in population of stacked conformers compared with a random conformational motion of the polymethylene chain. The shifts of the compounds **1e–j** with the longer polymethylene chains ($n \geq 5$) were consistent with this consideration. However, the upfield shifts of the protons at the 2-position were not necessarily similar to those at the 8-position when the number of carbon atoms in the chains were 2–4. The effect of the temperature on the chemical shifts of **1b–d** was also different to that of **1e–j**, although the steric effect of **1b** has already been described (Table 1). These data suggest that the stacked conformations were influenced by the polymethylene chains and the temperature.

Recently the stacking interaction of nucleic acids has been of interest in connection with the nature of the π–π interaction between aromatic compounds.[14] From the results of this investigation, the stacking interaction of **1** in aqueous solutions seems to be explained in terms of an attraction (attractive interaction) between the two adenine rings mediated by water rather than repulsion between the organic molecule and water such as the hydrophobic effect.

Experimental

The melting points were determined on a Yanagimoto micro melting-point apparatus and are uncorrected. The ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were obtained with a JEOL GSX400 spectrometer. Chemical shifts are reported in ppm downfield from tetramethylsilane in organic solvents or from sodium [2,2,3,3-$^2H_4$]3-(trimethylsilyl)-propionate in D_2O and the buffer solutions; J values are given in Hz. The elemental analyses were performed by the Analytical Center of Kyoto University.

9,9′-(Alkane-α,ω-diyl)diadenine 1

To a solution of adenine (10 mmol) in *N*,*N*-dimethylformamide (DMF) (150 ml), potassium carbonate (10 mmol) and α,ω-dibromoalkane (5 mmol) were added. The mixture was stirred at room temperature for 40 h. The resulting mixture was poured into water (300 ml) to give **1** as a solid crude compound. Compound **1** was recrystallized with acetic acid and methanol. The spectral data are given below.

9,9′-Methylenediadenine 1a. Mp >300 °C; ^{1}H NMR ([$^2H_6$]DMSO, 27 °C) δ 8.35 (s, 2 H), 8.18 (s, 2 H), 7.28 (s, 4 H, NH_2), 6.55 (s, 2 H); ^{13}C NMR ([$^2H_6$]DMSO) δ 155.97, 152.96, 149.06, 140.72, 118.37, 49.32 (Found: C, 46.68; H, 3.46. Calc. for $C_{11}H_{10}N_{10}$: C, 46.70; H, 3.56%).

9,9′-Ethylenediadenine 1b. Mp >300 °C (lit.,[3a,4a] > 300 °C); ^{1}H NMR ([$^2H_6$]DMSO, 27 °C) δ 8.05 (s, 2 H), 7.78 (s, 2 H), 7.13 (s, 4 H, NH_2), 4.61 (s, 4 H); ^{13}C NMR ([$^2H_6$]DMSO) δ 155.83, 152.39, 149.54, 140.39, 118.48, 42.56.

9,9′-Trimethylenebis(adenine) 1c. Mp > 330 °C (lit.,[4a] 330 °C decomp.; lit.,[5a] 317–320 °C); ^{1}H NMR ([$^2H_6$]DMSO, 27 °C) δ 8.18 (s, 2 H), 8.13 (s, 2 H), 7.17 (s, 4 H, NH_2), 4.18 (t, 4 H, J 6.6), 2.39 (quint., 2 H, J 6.6); ^{13}C NMR ([$^2H_6$]DMSO) δ 155.86, 152.30, 149.45, 140.70, 118.65, 40.38, 29.77.

9,9′-Tetramethylenediadenine 1d. Mp > 300 °C (lit.,[5a] 312–317 °C); ^{1}H NMR ([$^2H_6$]DMSO, 27 °C) δ 8.12 (s, 2 H), 8.11 (s, 2 H), 7.15 (s, 4 H, NH_2), 4.17 (br, 4 H), 1.77 (br, 4 H); ^{13}C NMR ([$^2H_6$]DMSO) δ 155.83, 152.26, 149.41, 140.70, 118.62, 42.20, 26.57.

9,9′-(Pentane-1,5-diyl)diadenine 1e. Mp 280–382 °C (lit.,[5a] 278–280 °C); ^{1}H NMR ([$^2H_6$]DMSO, 27 °C) δ 8.11 (s, 2 H), 8.11 (s, 2 H), 7.13 (s, 4 H, NH_2), 4.11 (t, 4 H, J 7.0), 1.84 (quint, 4 H, J 7.0), 1.21 (quint, 2 H, J 7.0); ^{13}C NMR ([$^2H_6$]DMSO) δ 155.83, 152.24, 149.43, 140.68, 118.62, 42.55, 28.72, 22.97.

9,9′-(Hexane-1,6-diyl)diadenine 1f. Mp 250–253 °C (lit.,[3a] 254–255 °C; lit.,[4a] 270–172 °C; lit.,[5a] 250–252 °C); ^{1}H NMR ([$^2H_6$]DMSO, 27 °C) δ 8.11 (s, 2 H), 8.11 (s, 2 H), 7.13 (s, 4 H,

Table 2 Chemical shifts of the protons of the adenine rings at the 2- and 8-positions of **1**[a]

		δ																			
		1a		**1b**		**1c**		**1d**		**1e**		**1f**		**1g**		**1h**		**1i**		**1j**	
Solvent	*T*/°C	H-2	H-8	H-2	H-8	H-2	H-8	H-2	H-8	H-2	H-8	H-2	H-8	H-2	H-8	H-2	H-8	H-2	H-8	H-2	H-8
pD 1.0[b]	27	8.784	8.485	8.245	8.182	8.387	8.293	8.386	8.295	8.404	8.320	8.412	8.334	8.417	8.347	8.422	8.352	8.425	8.355	8.428	8.361
	50	8.580	8.491	8.253	8.191	8.393	8.297	8.394	8.302	8.410	8.312	8.420	8.333	8.425	8.347	8.429	8.351	8.431	8.354	—	—
pD 13.0[c]	27	8.304	8.124	7.996	7.857	7.940	7.864	7.909	7.897	7.983	7.942	8.094	8.020	8.151	8.081	8.188	8.107	8.195	8.115	8.198	8.126
	50	8.310	8.128	7.957	7.907	7.982	7.880	7.958	7.928	8.037	7.951	8.140	8.031	8.182	8.082	8.211	8.105	8.215	8.113	—	—
CD_3OD	27	—	—	—	—	8.165	8.127	8.152	8.083	8.167	8.088	8.176	8.095	8.187	8.099	8.192	8.105	8.194	8.108	8.197	8.112
$(CF_3)_2CDOD$	27	—	—	8.252	8.058	8.266	8.039	8.253	7.953	8.246	7.936	8.244	7.925	8.246	7.923	8.246	7.922	—	—	8.246	7.924
$[^2H_6]DMSO$	27	8.349	8.175	8.051	7.778	8.176	8.126	8.116	8.109	8.112	8.105	8.113	8.110	8.117	8.112	8.119	8.114	8.120	8.115	8.123	8.118
CF_3COOD	27	9.113	8.703	9.045	8.632	9.370	8.782	9.337	8.779	9.375	8.802	9.373	8.797	9.388	8.810	9.390	8.810	—	—	9.386	8.812

[a] The concentrations of **1a**–**k** are less than 1.0 mmol dm^{-3} in the buffer solutions and less than 1.5 mmol dm^{-3} in organic solvents. [b] The buffer solution at pD 1.0. The chemical shifts of **1k** and **2f**,**g**,**j** (<1.2 mmol dm^{-3}) in the buffer solution at pD 1.0 are as follows: **1k**: (27 °C) δ 8.430, 8.366; (50 °C) 8.433, 8.364; **2f**: (27 °C) δ 8.431, 8.375; (50 °C) 8.438, 8.375; **2g**: (27 °C) δ 8.434, 8.375; (50 °C) 8.440, 8.376; **2j**: (27 °C) δ 8.434, 8.376; (50 °C) 8.441, 8.380. [c] The buffer solution at pD 13.0.

Table 3 The chemical shifts of the protons of the adenine ring at 2- and 8-positions of **1d** in the mixtures of D_2O and organic solvents at 27 °C[a]

	Mixtures of D_2O and CD_3OD		Mixtures of D_2O and $[^2H_6]DMSO$	
Organic solvent (%)[b]	H-2	H-8	H-2	H-8
0	7.921	7.913	7.921	7.913
2.5	7.916	7.911	7.923	7.914
5	—	—	7.929	7.921
7	7.930	7.924	—	—
10	—	—	7.953	7.948
12	7.941	7.941	—	—
17.5	—	—	7.973	7.973
20	7.953	7.953	—	—
23	—	—	7.991	7.991
30	7.988	7.979	—	—
40	8.030	8.010	—	—
50	8.072	8.038	8.068	8.068

[a] Concentrations of **1d**: <1.2 mmol dm^{-3}. [b] Percentage by volume of CD_3OD or $[H_6]DMSO$.

NH_2), 4.10 (t, 4 H, *J* 7.2), 1.78 (br, 4 H), 1.27 (br, 4 H); ^1H NMR ($[^2H_6]DMSO$, 50 °C) δ 8.12 (s, 2 H), 8.08 (s, 2 H), 7.0 (s, 4 H, NH_2), 4.10 (t, 4 H, *J* 7.0), 1.78 (br, 4 H), 1.27 (br, 4 H); ^{13}C NMR ($[^2H_6]DMSO$) δ 156.12, 152.53, 149.72, 140.97, 118.91, 42.92, 29.39, 25.62.

9,9′-(Heptane-1,7-diyl)diadenine 1g. Mp 225–227 °C; ^1H NMR ($[^2H_6]DMSO$, 27 °C) δ 8.12 (s, 2 H), 8.11 (s, 2 H), 7.13 (s, 4 H, NH_2), 4.10 (t, 4 H, *J* 6.8), 1.77 (quint, 4 H, *J* 6.8), 1.31 (quint, 2 H, *J* 6.8), 1.20 (quint, 4 H, *J* 6.8); ^{13}C NMR ($[^2H_6]DMSO$) δ 156.12, 152.51, 149.72, 140.99, 118.92, 42.97, 29.42, 27.98, 26.01 (Found: C, 53.40; H, 6.17. Calc. for $C_{17}H_{22}N_{10}\cdot H_2O$: C, 53.11; H, 6.29%).

9,9′-(Octane-1,8-diyl)diadenine 1h. Mp 255–258 °C; ^1H NMR ($[^2H_6DMSO$, 27 °C]) δ 8.12 (s, 2 H), 8.11 (s, 2 H), 7.13 (s, 4 H, NH_2), 4.10 (t, 4 H, *J* 7.2), 1.77 (quint, 4 H, *J* 7.2), 1.30–1.10 (m, 8 H), ^{13}C NMR ($[^2H_6]DMSO$) δ 155.90, 152.29, 149.50, 140.78, 118.71, 42.77, 29.24, 28.20, 25.82 (Found: C, 56.48; H, 6.26. Calc. for $C_{18}H_{24}N_{10}$: C, 56.82; H, 6.36%).

9,9′-(Nonane-1,9-diyl)diadenine 1i. Mp 200–202 °C; ^1H NMR ($[^2H_6]DMSO$, 27 °C) δ 8.12 (s, 2 H), 8.11 (s, 2 H), 7.13 (s, 4 H, NH_2), 4.11 (t, 4 H, *J* 7.2), 1.77 (quint, 4 H, *J* 7.2), 1.30–1.10 (m, 10 H); ^{13}C NMR ($[^2H_6]DMSO$) δ 155.90, 152.29, 149.51, 140.78, 118.71, 42.81, 29.24, 28.60, 28.23, 25.86 (Found: C, 55.45; H, 6.66. Calc. for $C_{18}H_{24}N_{10}\cdot H_2O$: C, 55.32; H, 6.70%).

9,9′-(Decane-1,10-diyl)diadenine 1j. Mp 239–241 °C; ^1H NMR ($[^2H_6]DMSO$, 27 °C) δ 8.12 (s, 2 H), 8.12 (s, 2 H), 7.15 (s, 4 H, NH_2), 4.10 (t, 4 H, *J* 7.2), 1.78 (quint, 4 H, *J* 7.2), 1.30–1.10 (m, 12 H); ^{13}C NMR ($[^2H_6]DMSO$) δ 156.11, 152.50, 149.72, 141.00, 118.92, 43.02, 29.46, 28.84, 28.47, 26.09 (Found: C, 58.57; H, 6.87. Calc. for $C_{20}H_{28}N_{10}$: C, 58.80; H, 6.91%).

9,9′-(Dodecane-1,12-diyl)diadenine 1k. Mp 218–220 °C; ^1H NMR ($CDCl_3$) δ 8.37 (s, 2 H), 7.79 (s, 2 H), 5.58 (s, 4 H, NH_2), 4.19 (t, 4 H, *J* 7.2), 1.89 (quint, 4 H, *J* 7.2), 1.40–1.20 (m, 16 H); ^1H NMR ($[^2H_6]DMSO$) δ 8.13 (s, 4 H), 7.17 (s, 4 H, NH_2), 4.12 (t, 4 H, *J* 7.2), 1.79 (quint, 4 H, *J* 7.2), 1.30–1.10 (m, 16 H); ^{13}C NMR ($[^2H_6]DMSO$) δ 155.83, 152.23, 149.45, 140.75, 118.64, 42.76, 29.21, 28.70, 28.69, 28.26, 25.83 (Found: C, 60.08; H, 7.33. Calc. for $C_{22}H_{32}N_{10}$: C, 60.53; H, 7.39%).

9-(ω-Bromoalkyl)adenine 2. Into a solution of adenine (10 mmol) in DMF (150 ml), potassium carbonate (10 mmol) and α,ω-dibromoalkane (12 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 chloroform. The extract was evaporated and chromatographed over silica gel. By elution with a mixture of ethyl acetate and methanol (85:15) **2** was given. The yields and the spectral data of **2** are given below.

9-(2-Bromoethyl)adenine 2b. 20% yield; mp 200–205 °C (lit.,[15] 195–200 °C); H NMR ($CDCl_3$) δ 8.36 (s, 1 H), 7.89 (s, 1 H), 5.74 (s, 2 H, NH_2), 4.62 (t, 2 H, *J* 6.0), 3.79 (t, 2 H, *J* 6.0); ^1H NMR ($[^2H_6]DMSO$) δ 8.19 (s, 1 H), 8.17 (s, 1 H), 7.30 (s, 2 H, NH_2), 4.58 (t, 2 H, *J* 6.0), 3.95 (t, 2 H, *J* 6.0); ^{13}C NMR ($[^2H_6]DMSO$) δ 155.75, 152.25, 149.37, 140.91, 118.59, 44.58, 31.45.

9-(6-Bromohexyl)adenine 2f. 28% yield; mp 138–139 °C; ^1H NMR ($CDCl_3$) δ 8.37 (s, 1 H), 7.80 (s, 1 H), 6.09 (s, 2 H, NH_2), 4.21 (t, 2 H, *J* 7.2), 3.39 (t, 2 H, *J* 7.2), 1.93 (quint, 2 H, *J* 7.2), 1.84 (quint, 2 H, *J* 7.2), 1.50 (quint, 2 H, *J* 7.2), 1.37 (quint, 2 H, *J* 7.2); ^{13}C NMR ($CDCl_3$) δ 155.65, 152.99, 150.11, 140.33, 119.69, 43.77, 33.52, 32.43, 29.94, 27.55, 25.81 (Found: C, 44.37; H, 5.41; N, 23.59. Calc. for $C_{11}H_{16}N_5Br$: C, 44.31; H, 5.41; N, 23.49%).

9-(7-Bromoheptyl)adenine 2g. 28% yield; mp 136–137 °C; ^1H NMR ($CDCl_3$) δ 8.37 (s, 1 H), 7.80 (s, 1 H), 6.05 (s, 2 H, NH_2), 4.20 (t, 2 H, *J* 7.2), 3.39 (t, 2 H, *J* 6.8), 1.91 (quint, 2 H, *J* 7.2), 1.83 (quint, 2 H, *J* 6.8), 1.45–1.34 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 155.64, 152.97, 150.11, 140.33, 119.67, 43.87, 33.74, 32.55, 29.99, 28.18, 27.91, 26.48 (Found: C, 46.16; H, 5.78; N, 22.42. Calc. for $C_{12}H_{18}N_5Br$: C, 46.16; H, 5.81; N, 22.43%).

9-(10-Bromodecyl)adenine 2j. 25% yield; mp 111–112 °C; ^1H NMR ($CDCl_3$) δ 8.38 (s, 1 H), 7.79 (s, 1 H), 5.70 (s, 2 H, NH_2), 4.19 (t, 2 H, *J* 7.2), 3.40 (t, 2 H, *J* 6.8), 1.90 (quint, 2 H, *J* 7.2), 1.84 (quint, 2 H, *J* 6.8), 1.41 (quint, 2 H, *J* 7.2), 1.35–1.25 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 155.45, 152.98, 150.15, 140.43, 119.67, 43.98, 34.00, 32.79, 30,07, 29.27, 29.27, 28.,99, 28.67, 28.11, 26.64 (Found: C, 50.68; H, 6.67; N, 19.80. Calc. for $C_{15}H_{24}N_5Br$: C, 50.85; H, 6.83; N, 19.77%).

^1H NMR study in aqueous solutions

Each sample of **1** (less than 10^{-3} mmol) was added to the buffer solutions (1.0 ml) containing the reference. After the mixture was heated and then cooled by standing in air, an insoluble material was removed by filtration. The concentrations of sodium [2,2,3,3-$^2H_4$]3-(trimethylsilyl)propionate as the reference were 0.6 mmol dm^{-3} in the sodium phosphate buffer solution at pD 7.0 and 0.8 mmol dm^{-3} in the buffer solutions at pD 1.0 and 13.0. The ^1H NMR spectra were obtained from accumulation of 40–8800 scans and observed over a range of 6002.4 Hz, corresponding to 32 768 data points. Chemical shifts (δ values) were measured in parts per million (ppm) downfield from the reference as an internal standard and estimated to an accuracy of ±0.001 ppm.

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