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Acyclic Analogues of Purine Nucleosides with Dimethylaminoethyl and Dimethylaminoethoxyalkyl Side Chains: Preparation, One- and Two-dimensional ^{1}H- and ^{13}C-NMR Studies

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The novel acyclic analogues of purine nucleosides containing 6-*N*-[2-(dimethylamino)ethyl] and 9-(2-hydroxyalkyl) substituents (**6**, **7**), or 9-[2-(2-(dimethylamino)ethoxy)alkyl] substituent and 6-amino group, free (**5**) or transformed into pyrrolo moiety (**11**, **12**), were prepared by reaction of protected 9-(2-hydroxyalkyl)adenines with 2-(dimethylamino)ethyl chloride hydrochloride. The substitution site in the purine ring was determined by ^{1}H- and ^{13}C-NMR, using chemical and substituent induced shifts, H-H and C-H coupling constants and connectivities in two-dimensional homo- and heteronuclear correlation spectra.

INTRODUCTION

A large number of open-chain nucleoside analogues has been made the object of intensive chemical and pharmacological investigation for the reason of their potential activity as antiviral and anti-HIV agents.[1,2] In this connection and in continuation of our programme on the synthesis of modified nucleoside analogues,[3–5] we have prepared a new series of purine acy-

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clonucleosides containing dimethylamino or dimethylamino and ether groups in aliphatic side chains (compounds **5–7**, **11** and **12** in Scheme 1). We report here the synthesis, one- and two-dimensional ^{1}H- and ^{13}C-NMR studies.

RESULTS AND DISCUSSION

Preparative Work

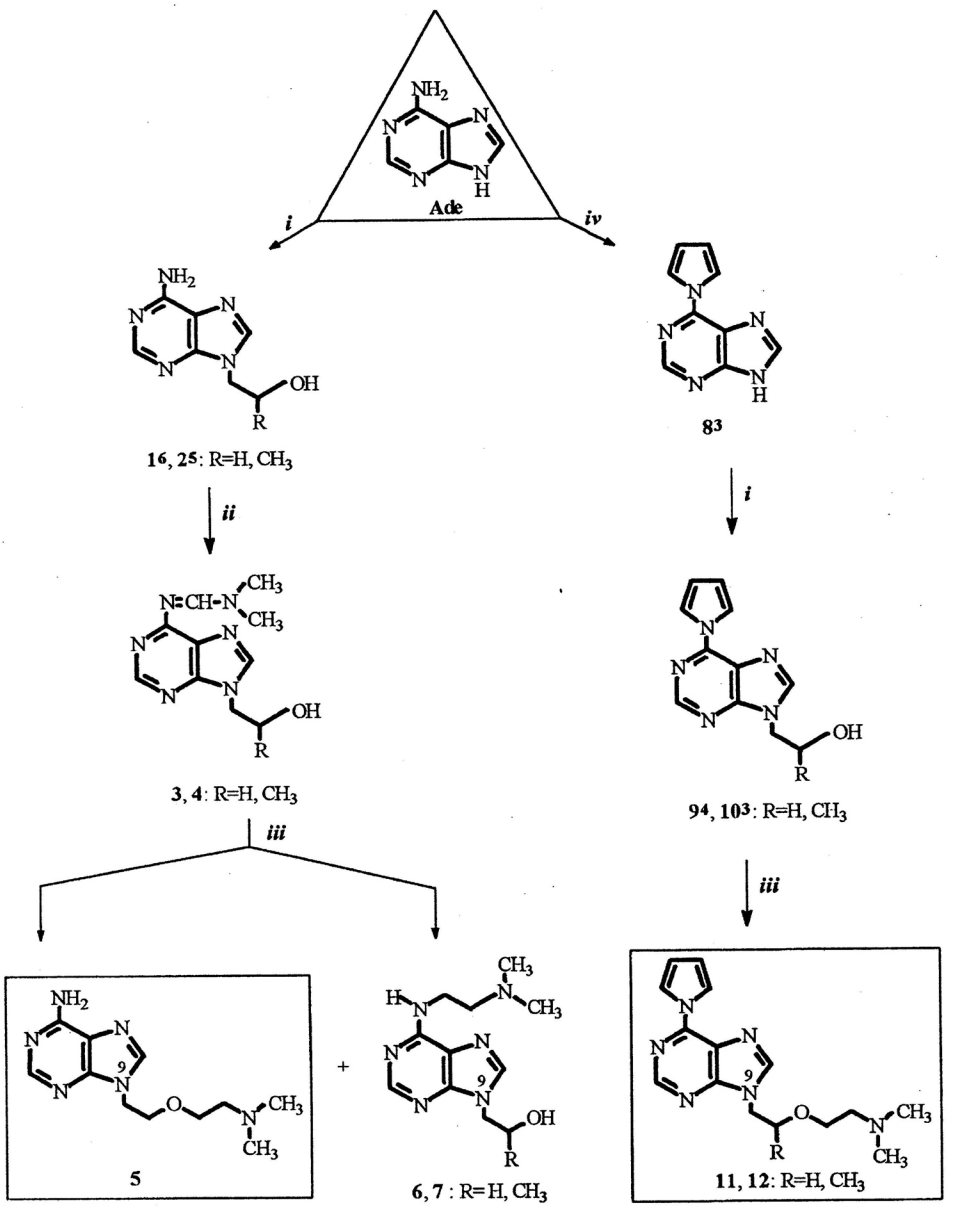
In order to prepare two types of 2-(dimethylamino)ethoxyalkyl derivatives of adenine with 6-amino group, free or transformed into the pyrrolo moiety, we designed two synthetic routes starting with adenine, as outlined in Scheme 1.

Protection of the amino group in 9-(2-hydroxyethyl)adenine (**1**)[6] and 9-(2-hydroxypropyl)adenine (**2**)[5] was achieved by reaction with *N,N*-dimethylformamide dimethyl acetal to give 6-*N*-(dimethylamino)methylene derivatives **3** and **4**, respectively. The same reagent has been successfully applied as a temporary protecting group for amino functions on nucleosides.[7,8] Reaction of **3** with 2-(dimethylamino)ethyl chloride hydrochloride (DMAEC · HCl) by a modified procedure to that for the preparation of 4-[2-(dimethylamino)ethoxy]bromobenzene[9] gave 9-[2-(2-(dimethylamino)ethoxy)ethyl]adenine (**5**) and 9-(2-hydroxyethyl)-6-*N*-[2-(dimethylamino)ethyl]adenine (**6**), while analogous reaction of **4** gave only 9-(2-hydroxypropyl)-6-*N*-[2-(dimethylamino)ethyl]adenine (**7**). However, in both reactions adenine derivatives **1** and **2**, respectively, were isolated in relatively high yields (40-60%). Formation of 6-N, N-9 disubstituted derivatives **6** and **7** may be explained by subsequent alkylation of the amino group in 9-(2-hydroxyalkyl)adenines **1** and **2**, respectively, formed after cleavage of the amidine group. Direct formation of **5**, without isolating the intermediate with the protected 6-amino group, might also be due to the relative lability of the amidine group. It means that the amidine group is found not to be an efficient protecting group for ether formation reactions of hydroxyalkyl adenine derivatives.

On the contrary, analogous etherification reactions of hydroxyalkyl derivatives **9**[4] and **10**[3], containing 6-*N*-pyrrolyl group, furnished, though in modest yields, the corresponding dimethylaminoethoxyalkyl derivatives **11** and **12**. Compounds **4, 7, 10** and **12** possess chiral centres and exist as racemic mixtures of enantiomers.

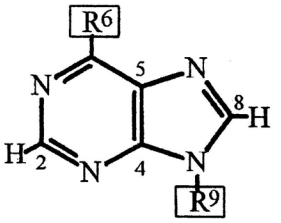
^{1}H- and ^{13}C-NMR Analysis

The one- and two-dimensional ^{1}H- and ^{13}C-NMR spectra have shown that in all molecules investigated here substitution of the purine ring took place at the N-9 position. In compounds **3, 4, 6** and **7,** additional substitu-



Scheme 1. Reagents: *i,* NaOH, ethylene or propylene carbonate in dry DMF for **1**, **9** or **2**, **10**, respectively; *ii, N,N*-dimethylformamide dimethyl acetal in dry DMF; *iii,* NaH and 2-(dimethylamino)ethyl chloride hydrochloride in dry DMF; *iv,* 2,5-dimethoxytetrahydrofuran in acetic acid.

tion at 6-N occurred as well. This was concluded from the pattern of chemical and substituent induced shifts and on the basis of the magnitude and multiplicity of H-H and C-H spin-spin coupling constants as well as connectivities in two-dimensional homo- and heteronuclear correlation spectra. The ^{1}H-NMR data are collected in Tables I and II (*c.f.* Scheme 2).



	R^6	R^9
1	NH_2	$CH_2(1')CH_2(2')OH$
2	NH_2	$CH_2(1')CH_2(2')OHCH_3$
3	$N{=}CH(\alpha)N(CH_3)_2$	$CH_2(1')CH_2(2')OH$
4	$N{=}CH(\alpha)N(CH_3)_2$	$CH_2(1')CH_2(2')OHCH_3$
5	NH_2	$CH_2(1')CH_2(2')OCH_2(3')CH_2(4')N(CH_3)_2$
6	$NHCH_2(3')CH_2(4')N(CH_3)_2$	$CH_2(1')CH_2(2')OH$
7	$NHCH_2(3')CH_2(4')N(CH_3)_2$	$CH_2(1')CH(2')OHCH_3$
11		$CH_2(1')CH_2(2')OCH_2(3')CH_2(4')N(CH_3)_2$
12		$CH_2(1')CH(2')CH_3OCH_2(3')CH_2(4')N(CH_3)_2$

Scheme 2.

The general characteristic of ^{1}H-NMR spectra is that H-2 is more deshielded than H-8, which was proved by HETCOR spectra. This is a common feature in N-9 substitution, since in N-7 substitution H-8 is more deshielded than H-2. The ^{1}H chemical shifts in purine skeleton of **1** and **2** are similar to those in adenine, except for amino protons, which are slightly deshielded in the former molecules. The same was found in other purine analogues and explained by the substituent effect of the side-chain and the possibility of self-association and hydrogen bonding.[3–5,10] The N-9 side-chain H-H coupling patterns in **1** are triplets (H-1′ and OH) and quartet (H-2′), while in **2** they are more complex due to the methyl group substitution at C-2′. The ^{1}H spectra confirmed that **3** and **4** are the 6-*N*-(dimethylamino)methylene derivatives of **1** and **2**. In the former molecules, H-2 (*ca.* 8.40 ppm) and H-8 (*ca.* 8.18 ppm) are more deshielded than in the latter ones (H-2 is 8.15 ppm and H-8 is *ca.* 8.08 ppm) as a consequence of electronic effects of the protective imino group at 6-N. COSY spectra of **3** and **4** show cross-peaks, arising from three-bond couplings in the N-9 side-chain and from four-bond coupling of imino proton, H-α, with *N*-methyl protons of 6-N moiety. In **4**, COSY spectra also revealed four-bond couplings between CH_3 and OH, as well as CH_3 and H-1′ of the N-9 side-chain. The azomethine proton, *i.e.*, imino proton H-α, in **3** and **4**, showed NOE cross-peaks with H-2 and H-8. These NOE's and the absence of NOE's between $N(CH_3)_2$ and H-2, as well as H-8, respectively, show that the H-α is closer to purine moiety

than the $N(CH_3)_2$, confirming the *E*-configuration at the imino double bond. In both **3** and **4**, the NOE cross-peaks between purine H-8 and *N*-methylene protons (H-1′) also exist. In acyclic analogues of purine nucleosides the NOE's between the H-8 and *N*-methylene protons are a well accepted probe for N-9 and N-7 substitution.[3–5] However, the N-7 substitution has been disregarded for all molecules here on the basis of ^1H chemical shifts, but also ^{13}C chemical and substituent induced shifts and the magnitude and multiplicity of C–H coupling constants. The *N*-methyl protons in **3** and **4** display two signals due to chemical nonequivalency, which arises from the hindered rotation of $N(CH_3)_2$ group at imino moiety. In addition, $N(CH_3)_2$ protons in **3** and **4** are much more deshielded than the corresponding protons in other molecules here, mainly due to the effect of π-electrons and anisotropy of imino moiety. In contrast to the $N(CH_3)_2$ group, protons of *N*-methylene (H-1′) and *O*-methylene groups (H-2′) in N-9 acyclic side-chain of **3** are chemi-

TABLE I

^1H-NMR chemical shifts (δ/ppm)[a] and H-H coupling constants (J/Hz)[b] for **Ade** and compounds **1–4** (*cf.* Schemes 1 and 2)

Comp.		**Ade**[c]	**1**	**2**[d]	**3**	**4**
H-2	δ	8.12(s, 1H)	8.15(s, 1H)	8.15(s, 1H)	8.42(s, 1H)	8.41(s, 1H)
H-8	δ	8.09(s, 1H)	8.09(s, 1H)	8.06(s, 1H)	8.20(s, 1H)	8.17(s, 1H)
NH_2	δ	7.13(s, 2H)	7.23(s, 2H)	7.26(s, 2H)		
H-1′	δ		4.20(2H)	4.13–3.99	4.24(2H)	4.17–4.04
	J		5.28(t)	(2H) (m)	5.47(t)	(2H) (m)
H-2′	δ		3.75(2H)	4.03(1H)	3.77(2H)	4.05(1H)
	J		5.24(q)	(m)	5.38(q)	(m)
OH	δ		5.03(1H)	5.06(1H)	5.02(1H)	5.05(1H)
	J		5.22 (t)	3.5(d)	5.25(t)	4.12(d)
H-α	δ				8.93(s, 1H)	8.93(s, 1H)
CH_3	δ			1.07(3H)		1.07(3H)
	J			5.6(d)		5.1(d)
$N(CH_3)_2$	δ				3.19(s, 3H)	3.20(s, 3H)
	δ				3.13(s, 3H)	3.13(s, 3H)

[a] DMSO-d_6 solutions, chemical shifts referred to TMS. Multiplicity of coupling and the number of protons are given in brackets: s = singlet, d = doublet, t = triplet, q = quartet, m = complex multiplet.

[b] Digital resolution 0.28 Hz.

[c] Signal of NH at 12.87 ppm.

[d] Reported in Ref. 5.

cally equivalent due to free rotation, which is perturbed in **4** by substitution of methyl group in the N-9 substituted side-chain. In ^{1}H-NMR spectra of **5**, besides signals of purine skeleton with chemical shifts similar to those in **1** and **2**, four triplets corresponding to CH_2 groups were observed (Table II). From shifts, H-H couplings and connectivities in COSY, NOESY and HETCOR spectra it was determined that these methylenes belong to the N-9

TABLE II

^{1}H-NMR chemical shifts (δ/ppm)[a] and H-H coupling constants (J/Hz)[b] for compounds **5–7** and **11** and **12** (*cf.* Schemes 1 and 2)

Comp.		**5**	**6**	**7**	**11**[e]	**12**[e]
H-2	δ	8.14(s, 1H)	8.22(s, 1H)[d]	8.21(s, 1H)[d]	8.75(s, 1H)	8.74(s, 1H)
H-8	δ	8.11(s, 1H)	8.09(s, 1H)	8.05(s, 1H)	8.61(s, 1H)	8.59(s, 1H)
NH_2/NH[c]	δ	7.22(s, 2H)	7.43(s, 1H)[d]	7.42(s, 1H)[d]		
H-1′	δ	4.29(2H)	4.21(2H)	4.15–3.98	4.47(2H)	4.39(1H)
	J	5.22(t)	5.50(t)	(2H) (m)	5.08(t)	14.3;3.6(dd)
	δ					4.26(1H)
	J					14.3;7.2(dd)
H-2′	δ	3.76(2H)	3.75(2H)	4.03(1H)	3.84(2H)	3.90(1H)
	J	5.22(t)	5.50(t)	(m)	5.08(t)	(m)
H-3′	δ	3.47(2H)	3.60(2H)[d]	3.59(2H)[d]	3.48(2H)[d]	3.52(1H)
	J	5.77(t)			5.77(t)	10.5;5.8(dt)
	δ					3.33(1H)
	J					10.5;5.8(dt)
H-4′	δ	2.32(2H)	2.49(2H)	2.48(2H)	2.31(2H)	2.24(2H)
	J	5.77(t)	6.60(t)	6.73(t)	5.77(t)	5.77(t)
OH	δ		5.20(1H)[d]	5.05(1H)[d]		
	J		(m)	(m)		
CH_3	δ			1.07(3H)		1.12(3H)
	J			5.22(d)		5.1(d)
$N(CH_3)_2$	δ	2.07(s, 6H)	2.21(s, 6H)	2.19(s, 6H)	2.05(s, 6H)	2.00(s, 6H)

[a] DMSO-d_6 solutions, chemical shifts referred to TMS. Multiplicity of coupling and the number of protons are given in brackets: s = singlet, d = doublet, t = triplet, q = quartet, m = complex multiplet.

[b] Digital resolution 0.28 Hz.

[c] In compounds **6** and **7**, NH is present at C-6 instead of NH_2.

[d] Signal is broadened.

[e] Signals of pyrrolo moiety: H-2″,5″ in **11** at 8.31 ppm (t, 2H) and in **12** at 8.31 ppm (t, 2H), while H-3″,4″ in **11** at 6.45 ppm (t, 2H) and in **12** at 6.44 ppm (t, 2H).

side-chain. The chemical shifts of CH_2 protons in **5** are in the following order: δ(H-1′) > δ(H-2′) > δ(H-3′) > δ(H-4′), corresponding to enhanced shielding with increasing distance of CH_2 group from the purine π-system. Contrary to the situation in the imino moiety of **3** and **4**, the *N*-methyl protons of the N-9 side-chain in **5** show only one signal, obviously due to the free rotation of $N(CH_3)_2$ group. For compounds **6** and **7**, proton spectra showed that substitution took place both at N-9 and at 6-N. This was deduced as follows: integration of signals revealed only one proton at 6-N, that is, the presence of the NH group, while in the case of only N-9 substitution, the NH_2 group would exist. COSY spectra show cross-peaks for three-bond coupling between the following protons: NH and H-3′, H-3′ and H-4′, H-1′ and H-2′, H-2′ and OH, and in **7** also between H-2′ and CH_3. The COSY spectrum of **6** is given in Figure 1. In addition, long-range enhanced COSY of **6** (Figure 2) displays four-bond couplings between H-8 and H-1′, as well as H-4′ and $N(CH_3)_2$. All these findings confirm that two separated side-chains exist and that methylenes H-1′ and H-2′ belong to the N-9 side-chain, whereas H-3′ and H-4′ to the 6-N chain. NOE contacts between H-8 and H-1′, H-8 and H-2′, H-1′ and H-2′, as well as between NH and H3' and NH and H-4′, are in agreement with the N-9, 6-N disubstituted structure as well. From Tables I and II one can also recognize that chemical shifts of H-1′and H-2′ in **1**, **3** and **6** (also in **2**, **4** and **7**) are similar, since these protons belong to the same type of the N-9 side-chain. On the other hand, one can see that chemical shifts of H-3′ and H-4′ in **6** and **7** are somewhat greater than those in **5** and **11**, as well as **12**, respectively. This reflects different positions of methylene groups in the former and latter molecules, namely their presence in 6-N and N-9 side-chains, respectively. Like in **5**, in **6**, **7**, **11** and **12**, it also applies that the δ(H-1′) > δ(H-2′) > δ(H-3′) > δ(H-4′). The $N(CH_3)_2$ protons are more deshielded in the 6-N than in N-9 side-chain. In both **6** and **7**, the methylene group at 6-N, adjacent to the purine skeleton (H-3′), shows a broadened signal due to unresolved couplings and less free rotation than the methylene group (H-4′) more remote from the purine ring, which displays a triplet. The corresponding three-bond H-H coupling at H-4′ is somewhat greater (*ca.* 6.7 Hz) than the coupling at H-4′ of the N-9 side-chain in **5**, **11** and **12** (*ca.* 5.8 Hz). ^{1}H-NMR variable temperature measurements in the range 20-100 °C were not successful in resolving the H-3′ signal. In **11** and **12**, the 6-*N*-pyrrolo moiety significantly changes the proton chemical shifts of purine H-2 and H-8 (in average shifts increase by *ca.* 0.5 ppm) in comparison to the corresponding protons in other molecules here, while shifts in the N-9 side-chain do not alter so much. This is in agreement with reported results on the resonance contribution of pyrrolo moiety to shifts and couplings in purine analogues.[3,4] Signals of methylene groups are well resolved in **11**, as they are also in **3** and **5**, and split in triplets due to the chemical equivalency of geminal protons. Chemical shifts of H-1′ and H-2′ are greater than in any other molecule here due to the effect of pyrrolo moiety, while shifts of H-3′ and

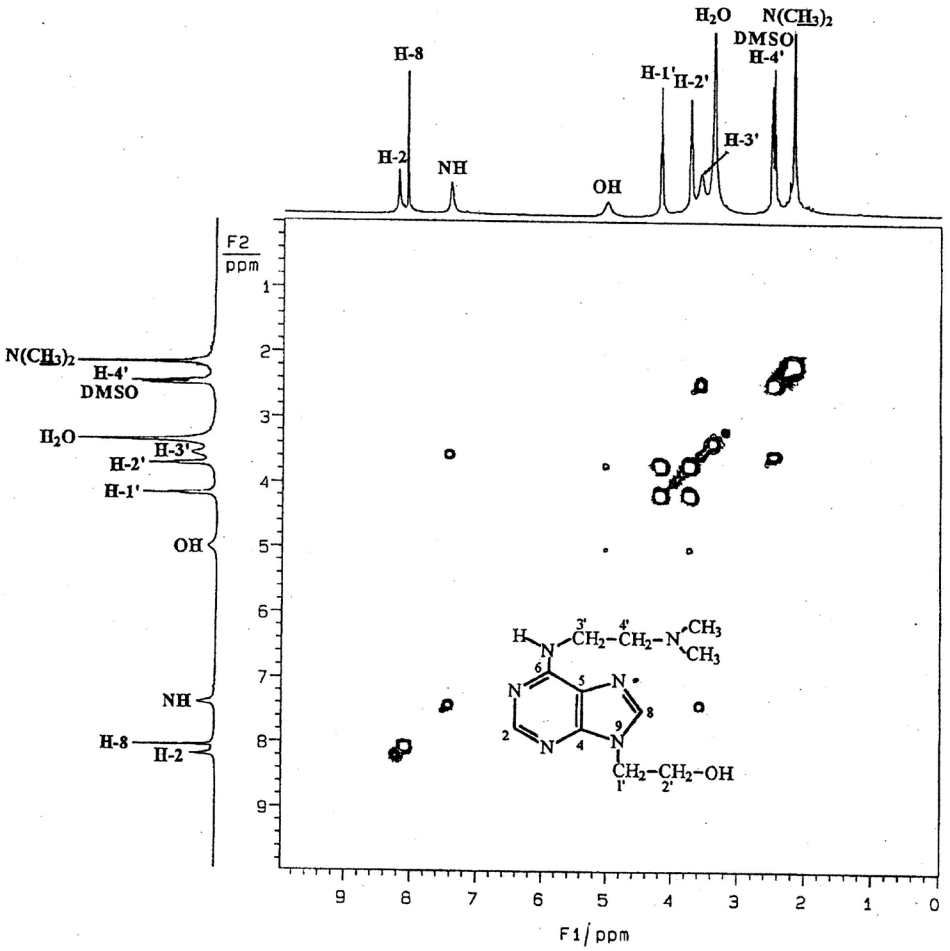


Figure 1. The COSY-45 spectrum of **6**, displaying three-bond H-H couplings.

H-4′, which are more remote from the pyrrolo substituted purine ring, are similar to those in **5**. Like in **5**, the $N(CH_3)_2$ group shows only one signal due to free rotation. In **12**, the methylene geminal protons at C-1′ and C-3′ are chemically nonequivalent due to methyl substitution at C-2′. Two groups of signals, centred at 4.39 ppm and 4.26 ppm, representing doublets of doublets, correspond to H-1′ methylene protons, while other two groups, centred at 3.52 ppm and 3.35 ppm, representing doublets of triplets, correspond to H-3′ methylene protons. The most shielded methylene protons (2.34 ppm) are at C-4′, adjacent to the $N(CH_3)_2$ group; due to chemical equivalency they display only one signal.

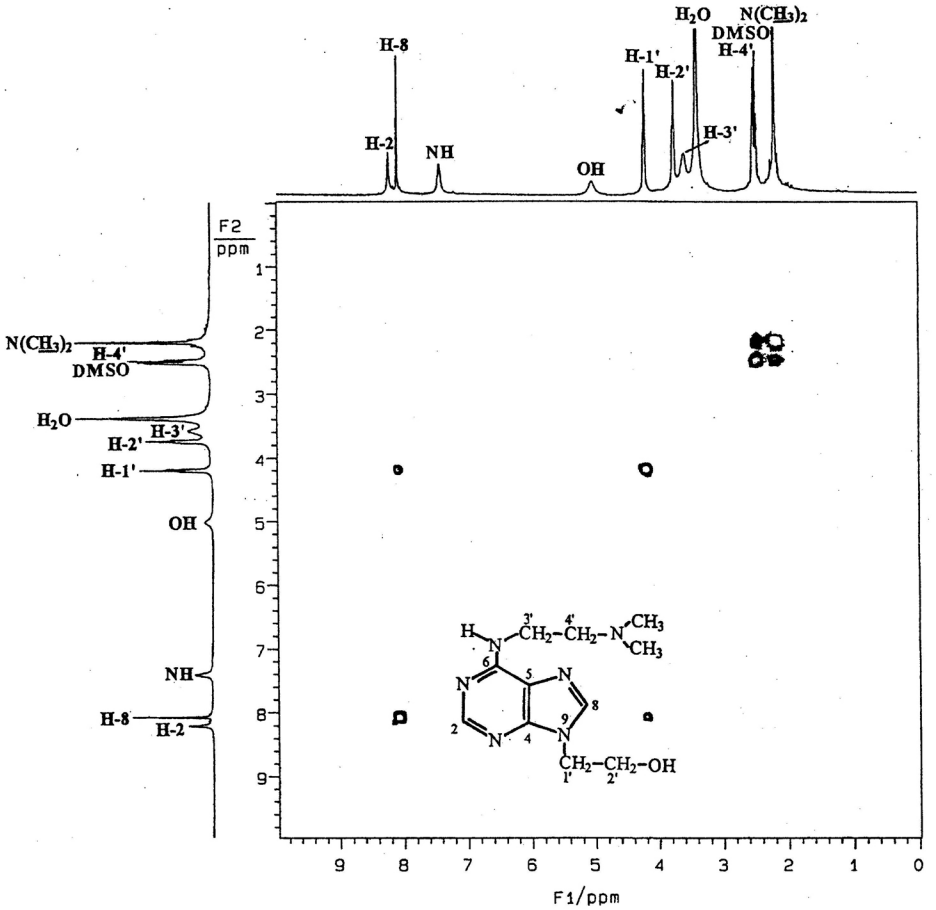


Figure 2. The delayed COSY-45 spectrum of **6**, displaying only four-bond H-H couplings.

The ^{13}C-NMR data are collected in Tables III and IV. For **1**, **2** and **5** chemical shifts and substituent induced shifts (SCS) are completely in agreement with the N-9 substitution of adenine.[5] SCS in **3** and **4** as well as **6** and **7** are quite different from those in the N-9 and N-7 substituted adenines.[3–5] The differential SCS obtained by subtracting the SCS of N-9 substituted derivatives **1** and **2** from SCS of the related **3**, **4**, **6** and **7**, respectively, revealed the effect of additional 6-N substitution in the latter molecules. The analysis of average SCS values gave the following results: SCS at C-2 in **3** and **4** is (–0.65 ppm) twice as much as in N-7 derivatives (–0.30 ppm)[5] and even four times greater (–0.15 ppm) than in the corresponding N-9 substituted adenine derivatives and **1** and **2**.[5] SCS at C-4 in N-7 derivatives[5] is small and

TABLE III

^{13}C-NMR chemical shifts (δ/ppm)[a], substituent shifts (SCS/ppm)[b] and one-bond C-H coupling constants (J/Hz)[c] for **Ade** and compounds **1**–**4**

Comp.		**Ade**	**1**	**2**	**3**	**4**
C-2	δ	152.69	152.53	152.56	151.89	151.90
	SCS		−0.16	−0.13	−0.80	−0.79
	J	198.1	198.5	197.9	200.0	199.6
C-4	δ	150.50	149.84	150.01	151.94	152.10
	SCS		−0.64	−0.49	1.44	1.60
C-5	δ	118.77	118.94	118.75	125.44	125.25
	SCS		0.17	−0.02	6.67	6.48
C-6	δ	156.16	156.21	156.20	159.28	159.25
	SCS		0.05	0.04	3.12	3.09
C-8	δ	139.13	141.60	141.86	143.51	143.69
	SCS		2.47	2.73	4.38	4.56
	J	212.4	211.7	211.7	211.7	211.2
C-1′	δ		45.81	50.15	45.85	50.22
C-2′	δ		59.39	64.58	59.28	64.66
C-α	δ				158.29	158.30
CH_3	δ			20.91		20.90
$(CH_3)_2N$	δ				40.65	40.66
					34.51	34.50

[a] DMSO-d_6 solutions, chemical shifts referred to TMS.

[b] SCS in **1**–**4** referred to **Ade**.

[c] Doublet at C-2, while doublet of triplets at C-8. Digital resolution ±0.7 Hz.

negative, *ca.* −0.30 ppm, while in N-9 derivatives it is *ca.* −0.60 ppm, and in N-9, 6-N disubstituted molecules **3** and **4** it is more greater and of opposite sign, amounting to 2.08 ppm. In the related N-9 substituted molecules, SCS at C-6 is *ca.* 0.10 ppm, while in the N-7 ones it is *ca.* −0.80 ppm, and in **3** and **4** it is as high as *ca.* 3.10 ppm. The SCS at C-8 is lower for 6-N substitution (*ca.* 1.87 ppm) than for N-9 one (*ca.* 2.60 ppm) and much lower than for N-7 substitution (*ca.* 5.33 ppm). The most striking SCS difference was observed at the C-5 atom. Namely, the corresponding SCS in N-9 derivatives is *ca.* 0.10 ppm, in N-7 ones it is *ca.* 1.80 ppm, while in **3** and **4** the SCS is as high as 6.67 ppm and 6.48 ppm, respectively. Differential SCS in **6** and **7** are of significantly lower magnitude and some of them are of different sign than those in **3** and **4**, although the through-bond distance from the substitution site is the same in both pairs of molecules. Thus, an aver-

TABLE IV

^{13}C-NMR chemical shifts (δ/ppm)[a], substituent shifts (SCS/ppm)[b] and one-bond C-H coupling constants (J/Hz)[c] for compounds **5–7**, **11** and **12**

Comp.		**5**	**6**	**7**	**11**[d]	**12**[d]
C-2	δ	152.63	152.60	152.50	151.88	151.85
	SCS	–0.06	–0.09	–0.19	–0.15	–0.18
	J	198.4	199.1	199.1	206.0	206.0
C-4	δ	149.81	149.19	149.19	146.61	146.59
	SCS	–0.69	–1.31	–1.31	0.02	0.00
C-5	δ	118.83	119.29	119.02	121.28	121.13
	SCS	0.06	0.52	0.25	0.10	–0.05
C-6	δ	156.23	154.86	154.70	153.53	153.74
	SCS	0.07	–1.30	–1.40	–0.97	–0.76
C-8	δ	141.43	141.54	141.52	146.36	146.64
	SCS	2.30	2.41	2.39	2.02	2.30
	J	211.2	212.1	212.1	214.7	215.3
C-1′	δ	42.86	45.92	50.19	43.36	47.90
C-2′	δ	68.23	59.46	64.71	67.89	73.09
C-3′	δ	68.53	37.82[e]	37.72[e]	68.49	66.28
C-4′	δ	58.30	58.23[e]	58.19[e]	58.25	58.62
CH_3	δ	–	–	20.88	–	17.25
$N(CH_3)_2$	δ	45.58	45.25	45.30	45.50	45.39

[a] DMSO-d_6 solutions, chemical shifts referred to TMS.

[b] SCS in **5–7** referred to **Ade**, while in **11** and **12** to 6-(*N*-pyrrolyl)purine.[3,4]

[c] Doublet at C-2, while doublet of triplets at C-8. Digital resolution ±0.7 Hz.

[d] Signals of pyrrolo moiety: C-2″,5″ 120.30 ppm in **11** and 120.28 ppm in **12**, and C-3″,4″ 112.61 ppm in **11** and 112.56 ppm in **12**.

[e] Signal is broadened.

age differential SCS at C-2 in **6** and **7** is almost zero, at C-4 *ca.* –0.70 ppm, at C-5 *ca.* 0.30 ppm, while at C-8 it is *ca.* –0.10 ppm. The only significant differential SCS was found at C-6, which is one bond away from the substitution site, amounting to *ca.* 1.40 ppm. These differences obviously reflect the type of 6-N substituent, which is the unsaturated imino group in **3** and **4**, while in **6** and **7** it is the saturated alkyl group. The ^{13}C spectra of **6** and **7** also showed that methylene signals C-3′ and C-4′ in the 6-N side-chain are rather broadened and significantly lower in intensity, while the C-3′ is the most shielded methylene carbon among the methylenes in the molecules investigated here (Table II). This is a consequence of the hindered rotation of

6-N methylene groups. The ^{13}C variable temperature measurements from 20 °C to 100 °C support this finding. The methyl carbons of 6-N side-chain in **6** and **7** show a sharp signal due to free rotation of the corresponding $N(CH_3)_2$ group. Contrary to this, ^{13}C-NMR spectra of **3** and **4** show two separated methyl signals for the $N(CH_3)_2$ group, confirming its hindered rotation. In addition, *N*-methyls in **3** and **4** are more shielded than the other $N(CH_3)_2$ in molecules investigated here, which is quite opposite to the situation observed in ^1H spectra.

The magnitudes of one-bond C-H coupling constants at C-2 (198–200 Hz) and C-8 (*ca.* 212 Hz) in **1–7** are in agreement with N-9 substitution, although the C-H splitting patterns at C-2 (doublet) and at C-8 (doublet of triplets) can arise from both the N-9 and N-7 substitution. For comparison, in N-7 derivatives of adenine, the magnitude of the one-bond C-H coupling at C-8 (*ca.* 208 Hz) is *ca.* 4 Hz lower than in N-9 regioisomers (*ca.* 212 Hz), while one-bond coupling at C-2 remains constant in a series of N-9/N-7 pairs (*ca.* 198 Hz). A slight increase (1–2 Hz) in magnitude of the one-bond C-H coupling at C-2 in **3**, **4**, **6** and **7** might be related to the effect of additional 6-N substitution in these compounds. SCS in **11** and **12** are changed due to the presence of the 6-*N*-pyrrolo moiety, but they display the characteristic features of N-9 substitution. The largest SCS change in **11** and **12** was found at C-8. Similarly to methylene C-1′ in **5**, the C-1′ in **11** is more shielded than that in **1**, **3** and **6**, which is connected with the longer N-9 side-chain in the former than in latter molecules. For the same reason, C-1′ in **12** is also more shielded than that in **2**, **4** and **7**. The β-effect of CH_3 group in the N-9 side-chain gives rise to a *ca.* 4 ppm greater chemical shift for C-1′ in **2**, **4**, **7** and **12** than in **1**, **3**, **6** and **11**. The pattern of C-H splitting at C-2 and C-8 in **11** and **12** might be related to both, the N-7 and N-9 substitution, but the magnitudes of these couplings (*cf.* Tables III and IV) are in agreement with those in N-9 regioisomers (206 Hz at C-2, while *ca.* 215 Hz at C-8), but not in the N-7 ones (*ca.* 202 Hz at C-2, while *ca.* 215 Hz at C-8). Unlike the adenine derivatives, where the main changes due to the N-9/N-7 substitution occur at C-8, in the case of the 6-(*N*-pyrrolyl)purine derivatives the greatest changes of one-bond C-H coupling occur at C-2.

CONCLUSIONS

The structure of novel purine nucleoside analogues **3–7**, **11** and **12** was determined by ^1H- and ^{13}C-NMR spectroscopy. The analysis performed in terms of chemical and substituent shifts, H-H and C-H coupling constants and connectivities in COSY, NOESY and HETCOR spectra, showed that all molecules are substituted at N-9, while in **3**, **4**, **6** and **7** additional substitution at 6-N occurred as well.

EXPERIMENTAL

Melting points (uncorrected) of compounds **3**, **4**, **6** and **7** were determined with a Büchi 535 instrument. Precoated Merck silica gel 60F-254 plates were used for thin layer chromatography (TLC) and the spots were detected under UV light (254 nm). Column chromatography was performed using silica gel (0,05–0,2 mm) Merck; the glass column was slurry-packed under gravity. Solvent systems used for TLC and column chromatography were as follows: S_1 = CH_2Cl_2–MeOH (3 : 1); S_2 = CH_2Cl_2–MeOH–NH_4OH (6 : 6 : 0.3); S_3 = CH_2Cl_2–MeOH–NH_4OH (6 : 6 : 0.1). DMF was dried by keeping it over type-4Å molecular sieves with occasional shaking.

The electron impact mass spectrum of **4** was recorded on the VARIAN MAT 311A instrument with ionizing energy 70 eV.

The ^1H- and ^{13}C-NMR 1D and 2D spectra of **1–7** and **11–12** were recorded on a Varian Gemini 300 spectrometer, operating at 75.46 MHz for the ^{13}C nucleus. All samples were measured in DMSO-d_6 solution, while some of them also in $CDCl_3$ solution, at 20 °C in 5 mm NMR tubes. Chemical shifts were referred to TMS. Variable temperature measurements were performed for compounds **6** and **7,** measured from 20 °C to 100 °C. Digital resolution in ^1H-NMR spectra was 0.28 Hz, while in ^{13}C-NMR spectra 0.70 Hz per point. The spectra were measured using the following techniques: broadband proton decoupling, gated decoupling, APT, COSY, delayed COSY, NOESY and HETCOR. All COSY and delayed COSY spectra were measured with second 45° pulse (COSY-45) and obtained in the magnitude mode. Delayed COSY, *i.e.* long-range enhanced COSY spectra were measured with delay time of 0.3 s. NOESY spectra were measured in the phase-sensitive mode. In both, COSY and NOESY, 1024 points in F2 dimension and 256 increments in F1 dimension were used. Data in F1 were subsequently zero-filled to 1024 points. Every increment was obtained with 16 scans, using 2750 Hz spectral width and a relaxation delay of 1 s. The resolution was 5.4 Hz/point and 10.7 Hz/point in F2 and F1 dimensions, respectively. The NOESY spectra were measured applying several mixing times (0.45–0.75 s). The HETCOR spectra were measured with 2048 points in F2 dimension and 256 increments in F1 dimension, which were zero-filled to 512 points. Increments were obtained with 64–128 scans, relaxation delay of 1.5 s and spectral width of 20000 Hz in F2 and 4500 Hz in F1 dimensions, respectively. The digital resolution was 19.53 Hz/point in F2 and 17.6 Hz/point in F1 dimension, respectively.

*9-(2-Hydroxyethyl)-6-*N*-[(dimethylamino)methylene]adenine (**3**)*

A suspension of **1**[6] (1.79 g, 10 mmol) and *N,N*-dimethylformamide dimethyl acetal (DMF-DMA) (4.77 g, 5.3 mL, 40 mmol) in dry *N,N*-dimethylformamide (DMF) (20 mL) was stirred with exclusion of atmospheric moisture at room temperature for approximately 5 hours until a solution was obtained. The solution was kept at room temperature overnight, then evaporated *in vacuo*. The residue was dissolved in dichloromethane and ethanol and reconcentrated. After drying (60 °C/0.01 Torr), a white solidified product of **3** was obtained: yield 93.0%, m.p. 172–174.5 °C. A portion of the crude product (1.08 g) was dissolved in warm chloroform (17 mL) and the solution was added dropwise under stirring to light petroleum (b. p. 40–70 °C; 200 mL). The precipitate was collected with suction and washed with light petroleum to

give colourless crystals of **3**: yield 92.6%, m.p. 173.5–174.5 °C, R_F 0.43 in S_1. Attempted purification of the crude product by column chromatography (S_1) was not successful because of the permanent presence of **1** in all fractions.

Anal. Calcd. for $C_{10}H_{14}N_6O$ (M_r = 234.26): C 51.27, H 6.02, N 35.88%; found C 51.23, H 6.04, N 35.83%.

*9-(2-Hydroxypropyl)-6-*N*-[(dimethylamino)methylene]adenine (4)*

Compound **2**[5] (2.90 g, 15 mmol) was treated with DMF-DMA (7.15 g, 8.0 mL, 60 mmol) in dry DMF (40 mL) in the same way as **1** in the preparation of **3**. The crude product (3.72 g) was recrystallized from chloroform (30 mL)/light petroleum (420 mL) to give colourless crystals of **4**: yield 92.5%, m.p. 111–113 °C, R_F 0.52 in S_1.

MS (70 eV) *m/z* (rel. intensity): 39.9 (15.05), 44.0 (32.54), 175.0 (59.34), 192.0 (41.99), 203.0 (29.59), 204.0 (33.20), 233.1 (68.68), 248.1 (100.00)

Anal. Calcd. for $C_{11}H_{16}N_6O$ (M_r = 248.29): C 53.21, H 6.50, N 33.85%; found C 53.17, H 6.52, N 33.82%.

*9-[2-(2-(Dimethylamino)ethoxy)ethyl]adenine (5) and 9-(2-Hydroxyethyl)-6-*N*-[(dimethylamino)ethyl]adenine (6)*

A 55% mineral oil dispersion of sodium hydride (1.50 g, 34.5 mmol) was washed with three 6-mL portions of *n*-hexane. To this, a solution of **3** (2.70 g, 11.5 mmol) and 2-(dimethylamino)ethyl chloride hydrochloride (DMAEC · HCl) (2.48 g, 17.25 mmol) in dry DMF (85 mL) was added dropwise. The stirred mixture was heated at 120 °C for 3.5 h, cooled to room temperature and filtered. The filtrate was concentrated *in vacuo* and the residue dissolved in dichloromethane and methanol and reconcentrated. The residual oily product (3.42 g) was submitted to column chromatography (S_2) to give solidified products of **5** (yield 9.0%, R_F 0.31) and **6** (yield 27,1%, R_F 0.42) and **1** (yield 41.7%, R_F 0.66). Product **5**, which contained a small amount of **1** and **6**, was further purified by double column chromatography (S_2) providing a solidified product of **5** (yield 1.6%, m. p. 98–99.5°C, R_F 0.31). Final purification of **6** by double recrystallization from ethyl acetate gave colourless crystalls of **6**: yield 15.1%, m.p. 99.5–100.5 °C, R_F 0.42.

Anal. Calcd. for **5**, $C_{11}H_{18}N_6O$ (M_r = 250.30): C 52.78, H 7.25, N 33.58%; found C 52.74, H 7.22, N 33.62%.

Anal. Calcd. for **6**, $C_{11}H_{18}N_6O$ (M_r = 250.30): C 52.78, H 7.25, N 33.58%; found C 52.80, H 7.26, N 33.57%.

*9-(2-Hydroxypropyl)-6-*N*-[(dimethylamino)ethyl]adenine (7)*

Compound **4** (2.98 g, 12 mmol) was treated in the same way as **3** in the preparation of **5** and **6** using sodium hydride (1.57 g, 55% in oil, 36 mmol) and DMAEC · HCl (2.59 g, 18 mmol) in dry DMF (90 mL). The stirred reaction mixture was heated at 125 °C for 5 h. The cooled mixture was filtered, the solvent evaporated *in vacuo* and the residue dissolved in dichloromethane and reconcentrated. The residual dark

brown oil (3.80 g) was submitted to column chromatography (S_2) yielding a solidified product of **7** (yield 15.8%, R_F 0.38) and, as the main product, compound **2** (yield 58.7%, R_F 0.81). The crude product of **7** was purified by column chromatography (S_2) and recrystallization of the almost pure product from ethyl acetate to give colourless crystals of **7**: yield 3.0%, m. p. 111–112 °C, R_F 0.38.

Anal. Calcd. for $C_{12}H_{20}N_6O$ (M_r = 264.33): C 54.53, H 7.63, N 31.79%; found C 54.50, H 7.65, N 31.82%.

*9-[2-(2-(Dimethylamino)ethoxy)ethyl]-6-*N*-pyrrolyl)purine (**11**)*

A 55% mineral oil dispersion of sodium hydride (0.38 g, 8.8 mmol) was washed with three 3-mL portions of *n*-hexane. To this, a solution of **9**[4] (0.92 g, 4 mmol) and DMAEC · HCl (0.69 g, 4.8 mmol) in dry DMF (30 mL) was added dropwise. The stirred mixture was heated at 110–130°C for 9 h and then more DMAEC · HCl (0.23 g, 1.6 mmol) and sodium hydride (0.24 g, 5.6 mmol) were added. Heating at 125°C was continued for 8 h and, after addition of the same quantities of DMAEC · HCl (total 1.15 g, 8.0 mmol) and sodium hydride (total 0.86 g, 20 mmol), for another 8 h (total 25 h). The mixture was cooled to room temperature and filtered. The filtrate was concentrated *in vacuo* and the residue dissolved in methanol and reconcentrated (2x). The residual brown oil (1.09 g) was submitted to column chromatography (S_1), yielding yellowish oil of **11** (yield 20.8%; R_F 0.17) and the starting compound **9** (yield 12.0%, R_F 0.82). Analytical sample of **11** was prepared by another column chromatography (S_1): yield 15.8%, R_F 0.17 in S_1; 0.42 in S_3.

Anal. Calcd. for $C_{15}H_{20}N_6O$ (M_r = 300.36): C 59.98, H 6.71, N 27.98%; found C 60.00, H 6.70, N 27.95%.

*9-[2-(2-(Dimethylamino)ethoxy)propyl]-6-*N*-pyrrolyl)purine (**12**)*

To sodium hydride (0.65 g, 55% in oil, 15 mmol), washed with *n*-hexane (3×3 mL), a solution of **10**[3] (1.22 g, 5.0 mmol) and DMAEC · HCl (1.08 g, 7.5 mmol) in dry DMF (45 mL) was added dropwise. The stirred mixture was heated at 120 °C for 5 h. After the mixture was cooled and filtered, the solvent was evaporated. The residual brown oil (1.47 g) was submitted to column chromatography (S_1), yielding yellowish oil of **12** (yield 39.5%, R_F 0.23) and the starting compound **10** (yield 34.4%, R_F 0.78). Analytical sample of **12** was afforded by a repeated column chromatography (S_3): yield 24.8%, R_F 0.52.

Anal. Calcd. for $C_{16}H_{22}N_6O$ (M_r = 314.39): C 61.13, H 7.05, N 26.73%; found C 61.10, H 7.09, N 26.78%.

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SAŽETAK

Aciklički analozi purinskih nukleozida s dimetilaminoetil- i dimetilaminoetoksialkilnim bočnim lancima: priprava, jedno- i dvodimenzijska ^{1}H- i ^{13}C-NMR istraživanja

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Novi aciklički analozi purinskih nukleozida koji sadržavaju supstituente 6-*N*-[2-(dimetilamino)etil] i 9-(2-hidroksialkil) (**6**, **7**) ili pak supstituent 9-[2-(2-(dimetilamino)etoksi)alkil] i slobodnu 6-amino skupinu (**5**) ili transformiranu u pirolnu jezgru (**11**, **12**) pripravljeni su u reakciji zaštićenog 9-(2-hidroksialkil)adenina s 2-(dimetilamino)kloretan-hidrokloridom. Položaj supstitucije u purinskom prstenu određen je pomoću spektara ^{1}H- i ^{13}C-NMR na temelju kemijskih pomaka, pomaka induciranih supstituentima, konstanata sprege H-H i C-H, te povezanosti u dvodimenzijskim homo- i heteronuklearnim korelacijskim spektrima.