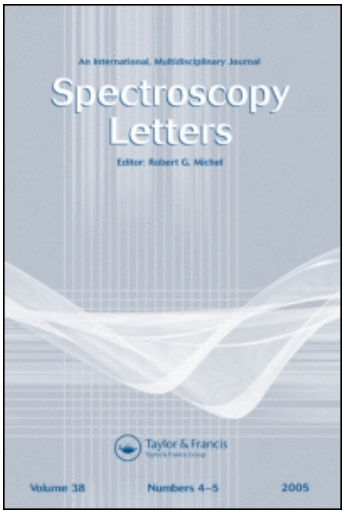

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NMR CHARACTERIZATION OF NOVEL PURINE NUCLEOSIDE ANALOGUES WITH 2,3-EPOXYPROPYL OR 3-AMINO-2-HYDROXYPROPYL MOIETY

Key words: *Purine Nucleoside Analogues, One- and Two-dimensional ^{1}H and ^{13}C NMR Correlation Spectroscopy*

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ABSTRACT

The structures of the title compounds were determined from their ^{1}H and ^{13}C NMR on the basis of chemical shifts, substituent induced shifts, H-H and C-H coupling constants, as well as connectivities in COSY, NOESY and HETCOR spectra. It has

been established that the purine skeleton is substituted at either *N*-9 or both *N*-9 and *N*-6 positions.

INTRODUCTION

Purine and pyrimidine nucleoside analogues have been of manifold pharmacological interest. Thus, a number of purine nucleoside analogues have shown potent antiviral activity, particularly against human immunodeficiency virus (HIV).[1] Some epoxide (*N*-glycidyl) derivatives of purine have been found to possess pronounced effect against P388 lymphocytic leukemia cells.[2] Furthermore, glycidyl ethers are used in the synthesis of drugs for the treatment of cardiovascular diseases.[3]

Searching for the compounds chemically related to such classes of compounds and related to our previous studies on acyclonucleosides[4-6] we have prepared the novel purine nucleoside analogues which contain 2,3-epoxypropyl (**1-4**) or 3-amino-2-hydroxypropyl (**5-11**) side-chains (SCHEME).[7]

It was found that 2,3-epoxypropyl ether derivative (**4**) and both compounds **4** and 2-hydroxy-3-isopropylaminopropyl derivative (**11**) showed inhibitory effect on growth of human malignant pancreatic carcinoma (*MiaPaCa2*) and B lymphocyte leukemia (*Raji*) cells, respectively.[7] We report here structure elucidation of those compounds performed using ^{1}H and ^{13}C NMR spectroscopy.

EXPERIMENTAL

The ^{1}H and ^{13}C one- and two-dimensional NMR spectra of compounds **1-11** were recorded on a Varian Gemini 300 spectrometer, operating at 75.46 MHz for the ^{13}C

resonance. The samples were dissolved in DMSO-d_6 and measured at 21 °C in 5 mm NMR tubes. The ^1H and ^{13}C chemical shift values (in ppm) are referred to TMS. Digital resolution in ^1H spectra was 0.28 Hz per point, while in ^{13}C spectra it was 0.70 Hz per point. The techniques used were the following: standard ^1H and ^{13}C (broadband proton decoupling), normal and inverse ^{13}C gated proton decoupling, APT, COSY, long-range COSY (delayed COSY with delay time of 0.2 s), NOESY and HETCOR.

The COSY and long-range COSY spectra were measured with a second pulse of 45° in magnitude mode with 1024 points in the F2 dimension and 256 increments in the F1 dimension, subsequently zero-filled to 1024 points. Each increment was recorded with 16 scans, relaxation delay of 1 s and 4500 Hz spectral width. The digital resolution was 8.9 Hz/point and 17.6 Hz/point in the F2 and F1 dimensions, respectively. The NOESY spectra were recorded under the same conditions as COSY spectra, but in phase-sensitive mode with the second pulse of 90° and mixing times in the range from 0.45 s to 0.80 s. The HETCOR spectra were recorded with 2048 points in the F2 dimension and 256 increments in the F1 dimension, which were zero-filled to 512 points. Increments were measured with 80 scans, relaxation delays of 1.2 s and spectral width of 19000 Hz in the F2 and 4500 Hz in the F1 dimensions, respectively. The digital resolution was 18.6 Hz/point for the F2 and 17.6 Hz/point for the F1 dimension. In all experiments the proton decoupling was performed using Waltz-16 modulation.

RESULTS AND DISCUSSION

^1H and ^{13}C NMR analysis

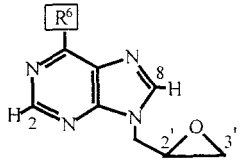
The ^1H and ^{13}C NMR spectra have shown that the substitution of the purine skeleton took place at N-9 or at both N-9 and N-6 positions. This was concluded from

the pattern of chemical and substituent shifts and on the basis of magnitude and multiplicity of H-H and C-H spin-spin coupling constants. Two-dimensional homo- and heteronuclear correlated spectra (COSY, NOESY, and HETCOR) corroborated these findings. The formation of theoretically possible N-1, N-3 and N-7 regioisomers was not observed. The ^{1}H NMR data of **1-11** are collected in TABLES 1 and 2. The general characteristic of ^{1}H NMR spectra is, that the H-2 (8.81-8.14 ppm) is more deshielded than the H-8 (8.67-7.97 ppm). This is a common feature in the N-9 substituted purine derivatives, whereas in the N-7 ones the H-8 is more deshielded than H-2. It is also in agreement with the previously reported results for other purine nucleoside analogues alkylated at the N-9 position.[4-6] The greater chemical shift of the H-2 than H-8 could be seen in FIG. 1, which displays the HETCOR spectrum of **2.**

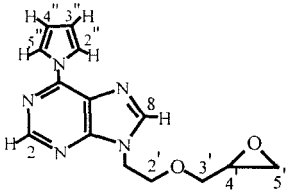
The ^{1}H and ^{13}C NMR spectra of compounds **1-4** confirmed that these molecules contain oxirane ring in a side-chain attached at N-9 of the purine ring. The ^{1}H data (TABLE 1) show nonequivalency of methylene protons, which is slightly greater for O-CH_AH_B than for N-CH_AH_B protons. This is due to the effect of the oxirane ring upon the rotation of the side-chain. In addition, the N-methylene protons are more deshielded (4.67-4.25 ppm) than the O-methylene ones (3.81-2.51 ppm). The chemical shift of methine proton and H-8 are very sensitive to the type of the side-chain bearing oxirane ring. In **2**, with shorter side-chain, these protons are more deshielded than in **4**, which contains longer side-chain. For example, the H-8 is at 8.60 ppm in **2** and at 8.18 ppm in **4**, while methine proton is at 3.48 ppm in **2** and at 3.10 ppm in **4**. The chemical shifts of methine proton, H-2 and H-8 depend also on the nature of the substituent at C-6.
In TABLE 1 a slight increase of these shifts can be seen, in a sequence from amino to chlorine substituent.

TABLE 1

^{1}H NMR chemical shifts (δ/ppm)[a] and H-H coupling constants (*J*/Hz) for compounds **1-4** (*c.f.* SCHEME).



1-3



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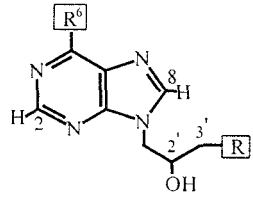
Comp.		1[b]	2[b]	3	4[b]
H-2	δ	8.15(s, 1H)	8.76(s, 1H)	8.81(s, 1H)	8.70(s, 1H)
H-8	δ	8.06(s, 1H)	8.60(s, 1H)	8.67(s, 1H)	8.18(s, 1H)
CH_2N	δ	4.45(1H)	4.64(1H)	4.65(1H)	4.49(2H)
	J	14.65;3.36(dd)	14.81;3,3(dd)	14.8;3.51(dd)	4.77(t)
	δ	4.25(1H)	4.43(1H)	4.42(1H)	
	J	14.80;5.70(dd)	14.77;5.49(dd)	14.8;5.65(dd)	
H-2'	δ	3.38(1H)	3.48(1H)	3.46(1H)	3.91(2H)
	J	(m)	(m)	(m)	(m)
H-3'	δ	2.79(1H)	2.84(1H)	2.83(1H)	3.81(1H)
	J	4.27(d)	4.26(t)	4.43(t)	11.52;2.53(dd)
	δ	2.51(1H)	2.58(1H)	2.57(1H)	3.35(1H)
	J		4.18;2.39(dd)	4.58;2.44(dd)	11.79;6.18(dd)
H-4'	δ	-	-	-	3.10(1H)
	J				(m)
H-5'	δ				2.78(1H)
	J	-	-	-	4.77;4.21(dd)
	δ				2.55(1H)
	J				4.91;2.67(dd)

[a] DMSO-d_6 solutions, chemical shifts referred to TMS. Multiplicity of coupling and number of protons are given in the brackets: s = singlet, d = doublet, t = triplet, m = multiplet, digital resolution ±0.28 Hz.

[b] Signals for NH_2 group at 8.27 ppm (s, 2H) in **1** and for pyrrolo ring: H-2",5" at 8.3 ppm (2H), *J*=2.3 Hz (t); H-3",4" at 6.4 ppm (2H), *J*=2.2 Hz (t) in **2** and **4**.

TABLE 2

^{1}H NMR chemical shifts (δ/ppm)[a] and H-H coupling constants (*J*/Hz) for compounds **5-11** (*c.f.* SCHEME).



5-11

Comp.		5[b]	6[b]	7[b]	8[b]	9[b]	10[b]	11[b]
H-2	δ	8.75(s, 1H)	8.15(s,1H)	8.75(s, 1H)	8.26(s, 1H)	8.74(s,1H)	8.73(s, 1H)	8.21(s, 1H)
H-8	δ	8.56(s, 1H)	8.10(s,1H)	8.56(s, 1H)	7.97(s, 1H)	8.60(s,1H)	8.54(s, 1H)	8.10(s, 1H)
CH_2N	δ	4.34(2H)	4.24(1H)	4.50(1H)	4.42(1H)	4.49(1H)	4.61(1H)	4.34(2H)
	J	10.55(d)	13.48(d)	11.6(d)	13.43(d)	13.65;2.55(dd)	(m)	(m)
	δ		4.07(1H)	4.18(1H)	4.22(1H)	4.16(1H)	4.41(1H)	
	J		(m)	8.20(d)	13.58;8.09(dd)	13.50;8.40(dd)	(m)	
H-2'	δ	4.03(1H)	3.92(1H)	4.12(1H)	4.40(1H)	4.07(1H)	4.40(1H)	4.18(1H)
	J	(m)	(m)	(m)	(m)	(m)	(m)	(m)
H-3'	δ	2.67(2H)	2.69(2H)	2.66(b, 2H)	2.70(2H)	2.61(2H)	3.28-3.19(2H)	3.30(2H)
	J	(m)	(m)		(m)	(m)	(m)	(m)
H-4'	δ	2.67(4H)	2.5(1H)	2.66(b, 4H)	2.63-2.51 (1H)	2.51(s, 4H)	3.28-3.19(4H)	3.07(1H)
	J	(m)	(m)				(m)	(m)
CH_3	δ	1.01(6H)	0.97(6H)	1.10 (6H)	0.99(s, 3H)		1.46(6H)	1.24(6H)
	J	7.03(t)	5.28(t)	6.87(t)		-	7.18(t)	9.96(d)
	δ				0.97(s, 3H)		1.36;1.30(6H)	1.22(6H)
	J						7.31(t);7.18(t)	6.23(d)

[a] DMSO-d_6 solutions, chemical shifts referred to TMS. Multiplicity of coupling and number of protons are given in the brackets: s = singlet, d = doublet, t = triplet, m = multiplet, b = broad signal, digital resolution ±0.28 Hz.

[b] Signals for amino group at *ca.* 7.2 ppm (s, 2H) in **5** and **6** pyrrolo ring: H-2",5" at *ca.* 8.3 ppm (s, 2H) and H-3",4" at *ca.* 6.4 ppm (s, 2H) in **7**, **8** and **9** and for NH at 7.55 and 8.90 ppm in **11**. Signals of OH groups and other NH protons are overlapped.

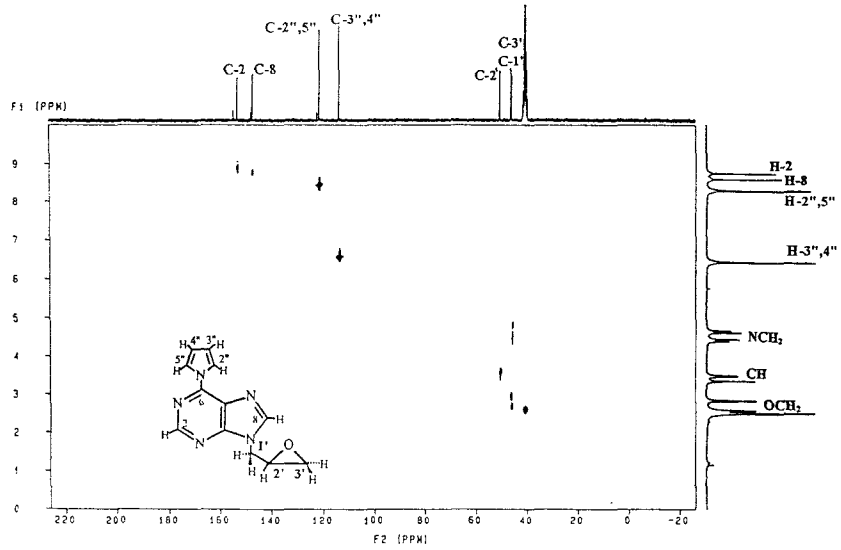


FIG 1. The ^{1}H-^{13}C heteronuclear correlated (HETCOR) spectrum of compound **2**.

The NOESY spectrum of **2** shows the cross-peak between H-8 and one of the N-methylene protons (H-1').

The ^{1}H NMR spectra of 3-amino-2-hydroxypropyl derivatives of purine nucleoside analogues (**5-11**) show, beside signals of purine moiety, five or six additional groups of signals. The ^{1}H NMR data for **5-11** are displayed in TABLE 2.

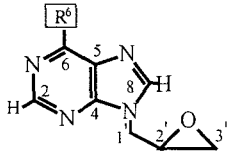
The substituent effect of the side-chain $N(C_2H_5)_2$ group on H-2 and H-8 is greater than the substituent effect of the $NHCH(CH_3)_2$ group. Therefore, these protons are more deshielded in **5** than in **6**. The same behavior was found in **7** and **8,** as well. Since observed substituent effects are of long range they probably arise due to combined action of inductive and resonance influence of the N-9 substituted group on purine skeleton. In addition, the bulkiness of side-chain has to be taken into account as well.

The ^{1}H NMR spectra of **10** and **11** showed that substitution took place at both the N-9 and the N-6 sites, *i.e.* two separated side-chains exist. This was substantiated by COSY and NOESY spectra, where two independent spin subsystems were resolved. One has to note that substituent effect of the $N(C_2H_5)_2$ group at the H-2 and H-8 sites is greater in **10** than the one of the $NHCH(CH_3)_2$ group in **11**. As mentioned before the same substituent effects were found for **5/6** and **7/8** pairs.

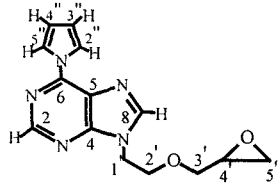
The ^{13}C NMR data for **1-11** are collected in TABLES 3 and 4. The ^{13}C chemical shifts and one-bond C-H coupling constants of the compounds **1-4** and **5-9**, as well as **10** and **11** are completely in agreement with substitution in the N-9,[4,5] and both the N-9 and the N-6 positions,[6] respectively. The general feature of ^{13}C NMR spectra is that the C-2 is always more deshielded than the C-8, while the magnitude of the one-bond C-H coupling constant at the C-2 is always lower (*ca.* 200-205 Hz) than at the C-8 (*ca.* 210-215 Hz). The $^{13}C\{^1H\}$ gated decoupled spectra of the purine moiety show doublet for the C-2 and doublet of triplets for the C-8 resonances, which is in accord with substitution at either N-9 or N-7, but not at the N-1 or N-3 sites of the purine ring. The N-7 substitution was disregarded on the basis of chemical shifts and magnitudes of one-bond C-H coupling constants.[4-6] The additional triplet splitting at the C-8 arises from three-bond C-H coupling with two N-methylene protons of the acyclic residue at the N-9, which was confirmed by comparison with C-H coupled spectrum of the parent molecule 6-(*N*-pyrrolyl)purine, where only a doublet for the C-8 was observed. In **1-4** (TABLE 3) the greatest substituent effect (SCS) of the oxirane side-chain was observed at the C-8, ranging from 1.35 to 2.05 ppm, while the lowest SCS was found at the C-2, ranging from –0.07 to 0.18 ppm.

TABLE 3

^{13}C NMR chemical shifts (δ/ppm)[a], substituent induced chemical shifts (SCS/ppm)[b] and one-bond C-H coupling constants (*J*/Hz)[c] for **6PyPu**[b] and compounds **1-4**.



1-3



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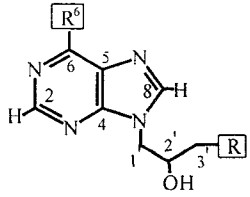
COMP.		**6PyPu**[b]	**1**	**2**[d]	**3**	**4**[d]
C-2	δ	152.03	152.77	152.16	151.98	151.96
	SCS		0.08	0.13	0.18	-0.07
	J	205.4	198.7	206.3		206.0
C-4	δ	146.59	149.83	146.77	149.40	146.70
	SCS		-0.67	0.18	1.31	0.11
C-5	δ	121.18	118.61	121.26	130.90	121.35
	SCS		-0.16	0.12	1.34	0.17
C-6	δ	154.50	156.10	153.61	152.38	153.57
	SCS		-0.06	-0.89	-2.03	-0.93
C-8	δ	144.34	140.97	146.12	147.86	146.39
	SCS		1.74	1.78	1.35	2.05
	J	212.8	211.7	215.2		214.8
C-1'	δ	-	44.44	44.92	45.20	43.41
C-2'	δ	-	49.66	49.55	49. 7	68.36
C-3'	δ	-	45.11	45.21	45.24	71.21
C-4'	δ	-	-	-	-	50.26
C-5'	δ	-	-	-	-	43.41

[a] DMSO-d_6 solution, chemical shifts referred to TMS.
[b] SCS for compound **1** referred to adenine, **2** and **4** to 6-(*N*-pyrrolyl)purine (**6PyPu**) and **3** to 6-chloropurine.
[c] Doublet for C-2, while for C-8 doublet of triplet, digital resolution ±0.7 Hz.
[d] Chemical shifts for pyrrolo moiety: C-2",5" at 120,3 ppm and C-3",4" at 112,7 ppm for **2** and **4**.

TABLE 4

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



5-11

COMP.		**5**	**6**	**7**[d]	**8**[d]	**9**[d]	**10**	**11**
C-2	δ	152.61	152.53	151.87	151.80	151.78	152.13	152.61
	SCS	-0.08	-0.16	-0.15	-0.23	-0.25	-0.48	0.08
	J			206.0	206.5	206.0		
C-4	δ	149.98	149.9	146.60	146.58	146.56	150.31	149.24
	SCS	-0.52	-0.60	-0.03	0.00	0.03	0.33	-0.66
C-5	δ	118.80	118.7	121.40	121.26	121.28	119.22	119.09
	SCS	0.03	0.00	0.22	0.08	0.10	0.42	0.39
C-6	δ	156.21	156.23	153.76	153.76	153.70	153.84	154.16
	SCS	0.05	0.07	-0.74	-0.74	-0.80	-2.37	-2.07
C-8	δ	141.95	141.91	146.84	146.80	146.76	139.80	141.45
	SCS	2.82	2.78	2.50	2.46	2.42	-2.15	-0.46
	J			212.1	214.1	214.6		
C-1'	δ	56.27	47.38	48.20	47.94	48.06	47.33	46.77
C-2'	δ	66.28	68.25	66.27	68.14	66.95	65.26	65.39
C-3'	δ	47.55	50.47	56.42	50.53	54.10	56.25	47.37
C-4'	δ	47.30	48.38	47.26	48.38	59.51	48.31	49.95
C-5'	δ	10.67	22.68	10.94	22.76	23.03	42.88	22.52

[a] DMSO-d_6 solution, chemical shifts referred to TMS.
[b] SCS for **5** and **6** referred to adenine, for **7**, **8** and **9** to **6-PyPu**, while for **10** to **5** and for **11** to **6**.
[c] Doublet for C-2, while for C-8 doublet of triplet. Digital resolution ±0.7 Hz.
[d] Signals for pyrrolo moiety: C-2",5" at *ca.* 120.30 ppm and C-3",4" at *ca.* 112.61 ppm for **7**, **8** and **9**.

In compound **2** signals of the C-1' (NCH_3) and C-3' (oxirane OCH_2 group) are very close, while in **4** corresponding signals (C-1' and C-5') are even overlapped. However, they were distinguished on the basis of the difference in magnitude of their one-bond C-H coupling. Thus, one-bond C-H coupling for NCH_2 is *ca.* 142 Hz, while it is even *ca.* 177 Hz for the oxirane OCH_2 group.

In **5-9** (TABLE 4) the greatest substituent effect was observed at the C-8, like it is in **1-4**. Great differences of SCS at the C-8 in **10** and **11** could be related to different influence of the corresponding substituents at the N-9 and, the N-6 positions, as it was already discussed for ^{1}H NMR spectra. As expected the greatest substituent effect in **10** and **11** was observed at the C-6, which was one bond apart from the attached substituent. The lowest SCS in **5-11** were observed for the C-2 and the C-5.

CONCLUSIONS

The structure of purine nucleoside analogues containing 2,3-epoxypropyl (glycidyl) (**1-4**), 3-diethylamino-2-hydroxypropyl (**5** and **7**), 2-hydroxy-3-isopropylaminopropyl (**6** and **8**) and 2-hydroxy-3-(*N*-pyrrolidinyl)propyl (**9**) groups in side-chains was determined by ^{1}H and ^{13}C NMR spectroscopy. The analysis performed in terms of chemical and substituent shifts, H-H and C-H coupling constants and connectiveties in COSY, NOESY and HETCOR spectra showed that purine derivatives are substituted either at the N-9 (in **1-9**) or both at N-9 and N-6 (in **10** and **11**).

ACKNOWLEDGEMENT

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