Letter - spectral assignment

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Proton NMR investigations on 6-alkylamino-2-alkylthioadenosine derivatives

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Introduction

Purine nucleosides have taken up an important role in living systems. Not only are purines the basic subunits of the nucleic acids, but they also interact with enzymes and other proteins as components of cofactors and signal molecules.[1–3] Their modified nucleosides and nucleotides are very important classes of compounds used in the therapy of a wide variety of diseases, because they can act as antiviral,[4] antitumor[5] and antimicrobial agents.[6] Purine nucleosides in which the alkylthio group at C-2 position and the alkylamine substituent at C-6 position typically excel in antiplatelet activities[7–13] (Fig. 1). So the synthesis and characterization of the modified purine nucleosides have attracted the attention of our and other many scientific teams.[14–18]

The distribution of electrons around the purine skeleton affects not only its chemical properties and reactivity but also the nuclear magnetic resonance (NMR) parameters. The nature of the substituent is reflected in the NMR chemical shifts and nuclear spin–spin coupling constants, which makes NMR spectroscopy an excellent tool for investigating and interpreting the structure, reactivity and intermolecular interactions in terms of the electron distribution.[19,20] The use of proton NMR spectroscopy could be a helpful tool to achieve it. Recently, we have published a complete analysis of proton NMR data for 6-alkylamino-2-alkylthioadenosine derivatives, and we observed that two protons of SCH_2 at C-2 of purine skeleton are split into one/two 'dt' or 'dq' coupling peaks, rather than canonical 't' or 'q' peaks,[9] but the reason is not clear.

To reveal the general behaviors of the chemical shifts signals of SCH_2 group depending on the configuration for SCH_2-containing adenosine derivatives, two series of 6-alkyl(aryl)amino-2-alkyl(aryl) thioadenosines and 6-alkyl(aryl)amino-2-alkylthiopurines were synthesized, and their proton NMR spectroscopic properties were described.

Experiment

The proton and carbon NMR spectra were recorded with Varian Mercury plus 200 (200 MHz), Varian Mercury plus 300 (300 MHz), Bruker 400 AMX (400 MHz) or Bruker 600 AMX (600 MHz) spectrometer in $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane as an internal standard at 298 K. Chemical shifts are reported in ppm on δ scale, and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br s (broad).

Results and discussion

The 6-alkyl(aryl)amino-2-alkyl(aryl)thioadenosines (**5a–l**) and 6-alkyl (aryl)amino-2-alkylthiopurines (**5m–o**) were synthesized according to our previous reported procedures,[9,10] which were outlined in Scheme 1. 6-Alkyl(aryl)amino-2-alkyl(aryl)thioadenosines (**5a–l**) was synthesized from the starting material guanosine (**1**) via acetylation, chlorination, diazotization–alkanethiolation and ammonolysis. 6-Alkyl(aryl)amino-2-alkylthiopurines (**5m–o**) were prepared by treating with 2-bromoethyl acetate, diazotization–alkanethiolation and ammonolysis from 2-amino-6-chloropurine (**6**). All of the 6-alkyl(aryl)amino-2-alkyl(aryl)thioadenosines and 6-alkyl(aryl)amino-2-alkylthiopurines derivatives, except **5a**, **5c**, **5e**, **5 k** and **5 m**, are new compounds (Table 1).

Analysis of the proton NMR data of 6-alkyl(aryl)amino-2-alkyl (aryl)thioadenosines (Table 2), we found that NH at C-6 of purine skeleton is a broad peak around 8.20 ppm, C-8 hydrogen is close to 8.50–7.90 ppm as a single peak; and then the chemical shifts of the nine protons in ribose are assigned in the region of 5.9–3.4 ppm. The SCH_2 at C-2 is assigned in the region of 3.10–2.90 ppm. However, if the substituent is SCH_3, the chemical shift is assigned at 2.5 ppm, which is often buried in the signals (two methyl peaks) of deuterated dimethylsulfoxide (DMSO-d_6) when it was used as the solvent (see ref. 9, compounds $5b_1$, $5b_7$, $5b_{10}$, $5b_{16}$ and $5b_{20}$).

As can be seen from the proton NMR spectrum of 6-chloro-2-propylthio- 9-(2′,3′,5′-tri-*O*-acetyl-β-*D*-ribofuranosyl)-9*H*-purine (**4b**, Fig. 2) in DMSO-d_6 solution, the two proton signals of SCH_2 were not shown as canonical 't' peak, but as 'dt' coupling peak, and the

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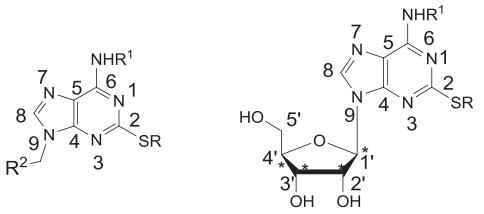
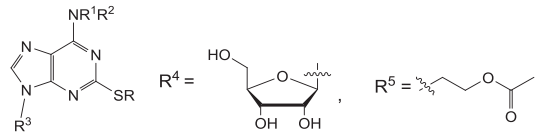


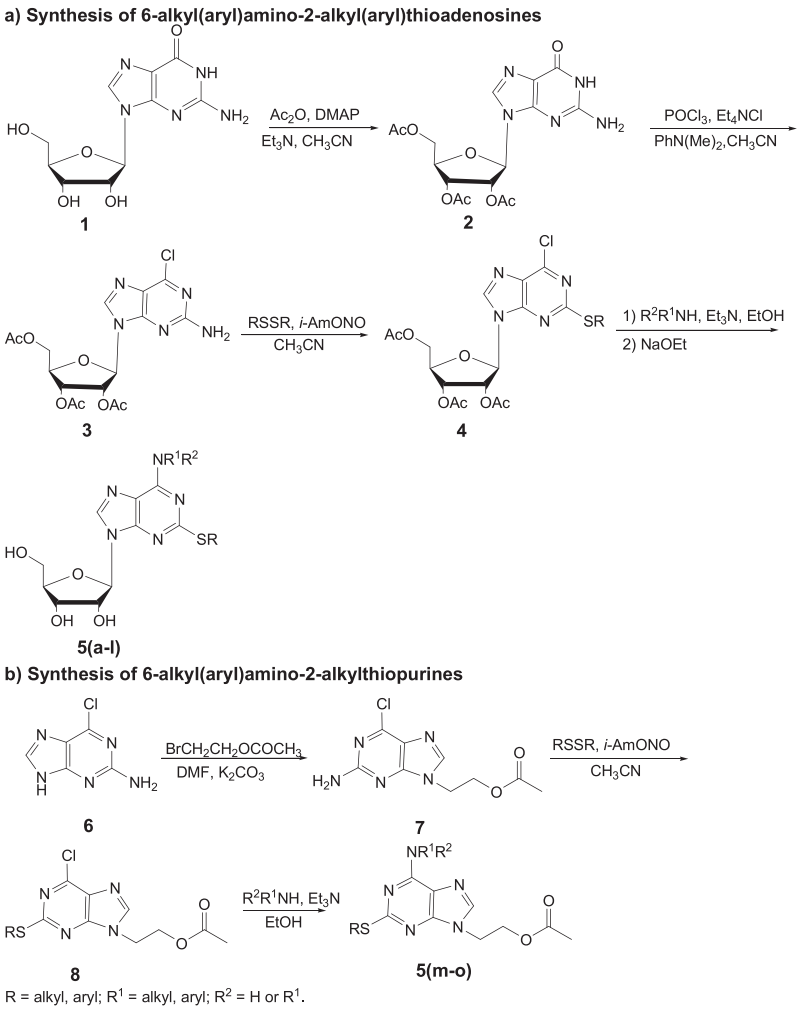
Figure 1. Chemical structures and numberings of 6-alkylamino-2-alkylthio-9-substitutedpurines.

assignment of the $J(SCH_2)$ values was 13.1 and 7.3 Hz. To our surprise, the multiplicity of CH_2 contiguous to sulfur (*S*) of compounds **5a**, **5c**, **5e**, **5 k**, **5j** and **5 l** in DMSO-d_6 is not the 't' or 'q' peaks, either, but the 'dt' or 'dq' coupling peaks, and the assignment of the $J(SCH_2)$ values was still 13.1 and 7.3 Hz (Fig. 2). The results demonstrate that two protons of SCH_2 are diastereomerism and their chemical shifts non-equivalence. Each of protons signal is split twice, which are first coupled each other as doublet peaks, and then is split by their adjacent substituent CH_2 or CH_3 into triplet or quartet peaks. However, when the ^1H NMR spectrum was recorded in $CDCl_3$, the two protons of SCH_2 were split into two separated 'dt' or 'dq' coupling peaks and the $J(SCH_2)$ values were still 13.1 and 7.3 Hz (Fig. 3). It indicates

Table 1. Chemical structures of 6-alkyl(aryl)amino-2-alkyl(aryl)thioadenosines (**5a–l**) and 6-alkyl(aryl)amino-2-alkylthiopurines (**5m–o**)



Entry	Compound **5**	R	R^1	R^2	R^3
1	**5a**[9]	*n*-Pr	$CH_2(CH_2)_4CH_3$	H	R^4
2	**5b**	*i*-Pr	$CH_2(CH_2)_4CH_3$	H	R^4
3	**5c**[9]	*n*-Bu	$CH_2(CH_2)_4CH_3$	H	R^4
4	**5d**	*n*-Bu	$CH_3OCH_2CH_2$	H	R^4
5	**5e**[9]	Et	*c*-C_6H_{11}	H	R^4
6	**5f**	*n*-Pr	*p*-$MePhCH_2$	H	R^4
7	**5g**	*n*-Bu	*p*-$MePhCH_2$	H	R^4
8	**5h**	*n*-Bu	*p*-$MeOPhCH_2$	H	R^4
9	**5i**	CH_2Ph	*p*-$MeOPhCH_2CH_2$	H	R^4
10	**5j**	*n*-Pr	$CH(CH_3)Ph$	H	R^4
11	**5k**[9]	Et	*n*-Bu	*n*-Bu	R^4
12	**5l**	*n*-Pr	*n*-Bu	*n*-Bu	R^4
13	**5m**[10]	Et	*n*-Pr	H	R^5
14	**5n**	*n*-Bu	$PhCH_2CH_2$	H	R^5
15	**5o**	*n*-Pr	*n*-Bu	*n*-Bu	R^5



Scheme 1. Synthesis of 6-alkyl(aryl)amino-2-alkyl(aryl)thioadenosines and 6-alkyl(aryl)amino-2-alkylthiopurines.

Table 2. ^{1}H NMR chemical shifts (ppm) for 6-alkyl(aryl)amino-2-alkyl(aryl)thioadenosines (**5**)

Compound **5**	H-8	NH-6	H-1′	OH-2′	OH-3′	OH-5′	H-2′	H-3′	H-4′	H-5′$_a$	H-5′$_b$	SCH$_2$-2	
5a[9]	8.19	7.92	5.80	5.37	5.13	5.06	4.58	4.13	3.92	3.64	3.53	3.07	3.05
5c[9]	8.19	7.90	5.80	5.37	5.12	5.04	4.58	4.12	3.92	3.64	3.53	3.09	3.08
5d	8.23	7.88	5.81	5.43	5.18	5.08	4.58	4.12	3.92	3.63	3.56	3.07	3.08
5e[9]	8.21	7.73	5.81	5.41	5.18	5.07	4.58	4.13	3.92	3.64	3.53	3.07	3.06
5f	8.23	8.47	5.81	5.40	5.15	5.05	4.59	4.12	3.92	3.64	3.53	2.99	
5g	8.24	8.49	5.81	5.43	5.18	5.08	4.60	4.13	3.92	3.65	3.53	3.02	
5h	8.23	8.47	5.81	5.42	5.17	5.07	4.58	4.13	3.92	3.60-3.43		3.03	
5j	8.24	8.42	5.80	5.39	5.14	5.04	4.56	4.12	3.92	3.63	3.52	2.98	2.95

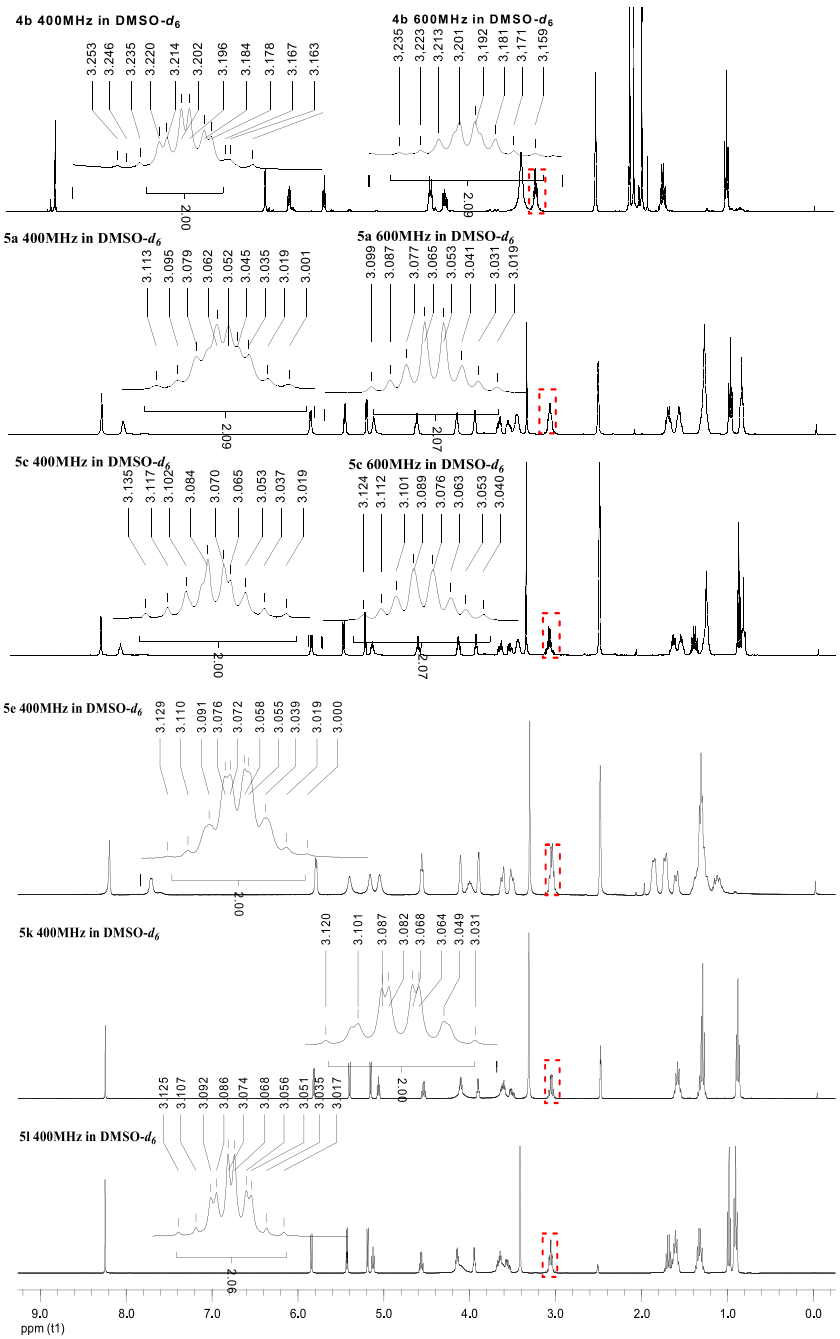


Figure 2. The SCH$_2$ splitting in ^{1}H-NMR spectra of compounds **4b**, **5a**, **5c**, **5j**, **5k** and **5l** in DMSO-d_6.

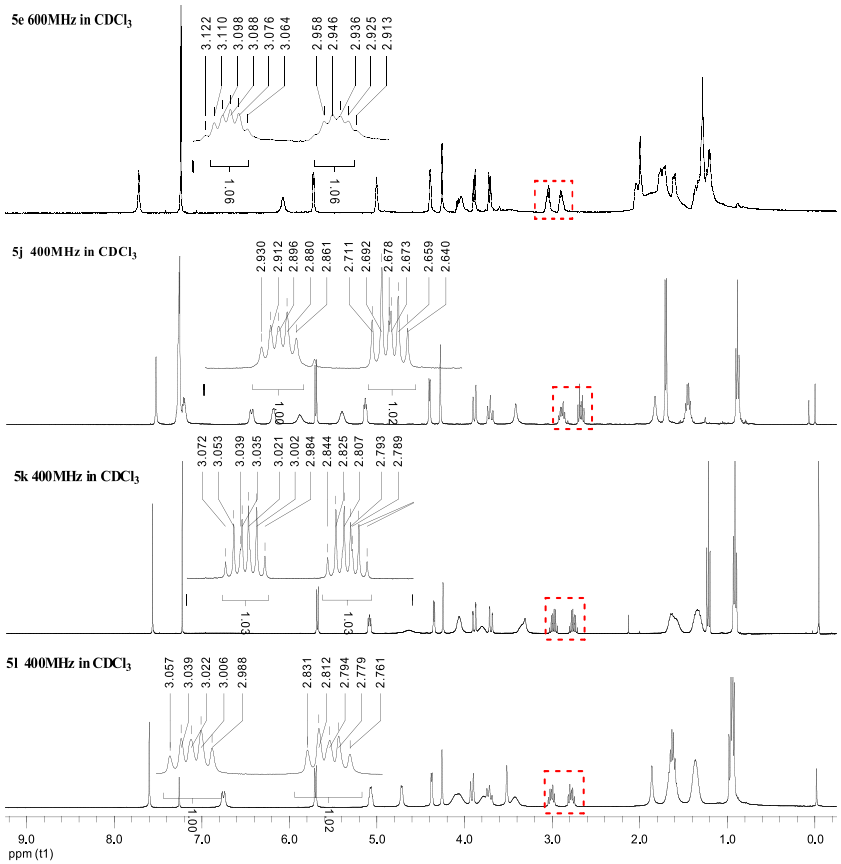


Figure 3. The SCH_2 splitting in ^{1}H-NMR spectra of compounds **5e**, **5k**, **5j** and **5l** in $CDCl_3$.

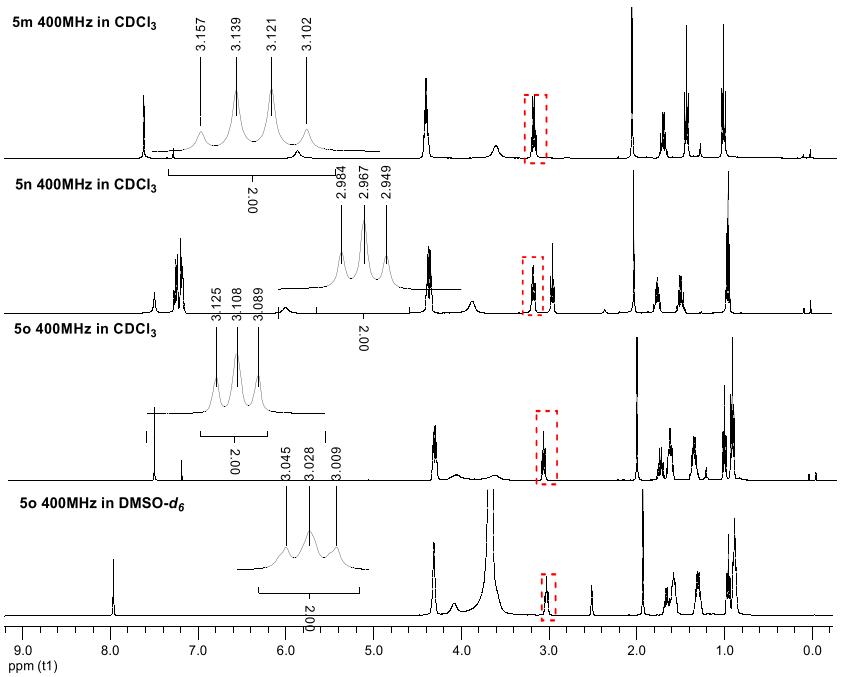


Figure 4. The SCH_2 splitting in ^{1}H-NMR spectra of compounds **5m**, **5n** and **5o**.

that the SCH_2 was also split into 'dt' or 'dq' coupling peaks, but as two separate parts.

The four chiral carbons in ribose, which connected with the 9-N of the purine skeleton, especially the 1′-carbon, the nearest chiral center, although there are seven chemical bonds between the SCH_2 and 1′-carbon, generate a long-range chiral effect resulting the two protons of SCH_2 chemical shift non-equivalence. However, when the non-chiral carbon-substituent at the 9-N of the purine skeleton, the two protons in SCH_2 were normally split into a 't' or 'q' peaks, rather than two 'dt' or 'dq' peaks (**5m–o**, Fig. 4). This proves that the four chiral carbons at the 9-N of purine skeleton result the two protons of SCH_2 diastereomerism, their chemical shift non-equivalence and split into two 'dt' or 'dq' coupling peaks.

Further analysis of the ^{1}H-NMR of the target compounds **5a–l**, suggesting that there is existence of solvent effect between $CDCl_3$ and DMSO-d_6. Such as the chemical shifts of NH and SCH_2, the NH chemical shift of compound **5j–l** in $CDCl_3$ was around 7.53 ppm but moved to 8.41 ppm in DMSO-d_6 (Figs. 2 and 3). It was assumed that a hydrogen bond was generated between solvent ($CDCl_3$) and the sample. The SCH_2 was split into two separated moieties in $CDCl_3$ as 'dt' coupling peaks but in DMSO-d_6 occurs in an integral for the coupling peaks (Fig. 2 and Fig. 3). In addition to the proton NMR data of SCH_2 and NH, the peaks of other groups also have a little change, but not very obviously.

Conclusions

In summary, the preparation and proton NMR spectroscopic characterization of several novel SCH_2-containing adenosine derivatives were described. The ^{1}H-NMR spectra of 6-alkylamino-2-alkylthioadenosine derivatives suggest that the two protons in the SCH_2 are diastereomerism, their chemical shifts non-equivalence and split into two 'dt' or 'dq' coupling peaks. However, the two protons of SCH_2 of 9-acetoxyethyl-6-alkylamino-2-alkylthiopurine derivatives are normally split into a canonical 't' or 'q' peak. This proves that the chiral carbons at the 9-N of purine skeleton have a long-range chiral effect on the two diastereotopic protons of SCH_2.

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