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Spectral Assignments and Reference Data

Complete ^{1}H and ^{13}C NMR spectral assignment of α- and β-adenosine, 2′-deoxyadenosine and their acetate derivatives

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^{1}H and ^{13}C NMR chemical shifts of α- and β-anomers of adenosine, 2′-deoxyadenosine and their acetate derivatives were completely and definitely assigned using the concerted application of one- and two-dimensional experiments (gCOSY, gNOESY, gHSQC and gHMBC). The influence of the stereochemistry of the purine base on the NMR data of the hydrogen and carbon atoms of the furanose moiety was estimated. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: ^{1}H NMR; ^{13}C NMR; gCOSY; gNOESY; gHSQC; gHMBC; β- and α-adenine nucleosides; β- and α-adenine nucleoside acetates

INTRODUCTION

Although α-nucleosides are not found in nucleic acids, they are constituents of smaller molecules, e.g. vitamin B_{12}, present in living cells. Some of them exert biological activities equal to or even exceeding those of corresponding β anomers.[1] Recently it was demonstrated that α-2′-deoxyadenosine in DNA is a substrate of *E. coli*, human and *S. Cerevisiae* endonucleases.[2] Current projects in our laboratory involve the synthesis of modified purine nucleosides and derivatives with both anomeric configurations. ^{1}H and ^{13}C NMR spectroscopy can be a very useful tool to achieve the identification of the two anomers which have very similar chromatographic behaviors. The assignment of anomeric configurations is an important topic in nucleoside chemistry. Some NMR methods have already been used to detect spectral differences between natural and α anomers of purine nucleosides, such as (i) methyl ^{1}H resonances of isopropylidene derivatives;[3] (ii) C-1′ *versus* C-4′ chemical shifts for adenosine and base-modified adenosine analogues;[4] (iii) comparison of coupling constants of H-1′ for 2′-deoxyadenosine and derivatives, and (iv) 1D-nuclear Overhauser effect (NOE) difference spectroscopy.[5] To our knowledge, complete ^{1}H NMR data of α-adenosine and α-2′-deoxyadenosine are not reported in the literature. Moreover, no data are available for other nucleoside derivatives of our interest, i.e. acetates of α anomers. Chemical structures of compounds1-8 are shown in Fig. 1.

Here, we present the complete ^{1}H and ^{13}C NMR spectral assignment of adenosine (**1**), adenosine-2′, 3′, 5′-triacetate (**2**), 2′-deoxyadenosine (**3**), 2′-deoxyadenosine-3′, 5′-diacetate (**4**) and their α-anomers i.e. α-adenosine (**5**), α-adenosine-2′, 3′, 5′-triacetate (**6**), α-2′-deoxyadenosine (**7**) and α-2′-deoxyadenosine-3′, 5′-diacetate (**8**), comparing signals of relevance to distinguish between α and β anomers. Chemical structures of compounds 1–8 are shown in Fig. 1.

RESULTS AND DISCUSSION

Complete ^{1}H and ^{13}C NMR data of all compounds are shown in Tables 1–3. ^{1}H and ^{13}C resonances were unequivocally assigned based on combined information from 1D and 2D NMR (gCOSY, gNOESY, gHSQC and gHMBC) experiments. ^{1}H–^{1}H coupling constants were directly measured from resolution-enhanced 1D spectra and confirmed, when necessary, by homodecoupling. NOE analysis allowed the assignment of the nucleoside configuration, determination of the H-8 resonance and the assignment of H-2′α and H-2′β of 2′-deoxy derivatives. In particular, NOE correlations between H-1′ and H-4′ are present only in compounds **1**, **2**, **3** and **4** identified as β-anomers, in accordance with 1D-NOE difference spectroscopy studies referred to adenosine (**1**), α-adenosine (**5**)[5] and 2′-deoxyadenosine (**3**).[6] H-8 resonances were assigned according to NOESY correlations with H-1′ as well as HMBC correlations between H-1′ and C-8. Adenosine (**1**), 2′-deoxyadenosine (**3**), α-adenosine (**5**) and α-2′-deoxyadenosine (**7**) have H-8 resonance less shielded than H-2, in agreement with values reported for compounds **1** and **3**.[7] On the contrary, H-8 of the acetate derivatives **2**, **4**, **6** and **8** is more shielded than H-2 in contrast with literature results for adenosine-2′,3′,5′-triacetate (**2**)[8] and 2′-deoxyadenosine-3′,5′-diacetate (**4**).[9] In addition, NOESY correlations allowed the assignment of H-2′α and H-2′β of 2′-deoxyadenosine (**3**), 2′-deoxyadenosine-3′,5′-diacetate (**4**), α-2′-deoxyadenosine (**7**) and α-2′-deoxyadenosine-3′,5′-diacetate (**8**). In the β-compounds **3** and **4**, NOE-contacts between H-8 and the less shielded H-2′ were observed, whereas in the α-anomers **7** and **8** H-8 is in NOE-contact with the more shielded H-2′; these findings assigned H-2′β resonance to high frequency H-2′. The H-1′ resonances of β- and α-nucleosides could be useful to define anomeric configuration. Significant differences of chemical shifts were observed between β anomers adenosine (**1**), adenosine-2′,3′,5′-triacetate (**2**), and the corresponding α anomers **5** and **6**. In particular, H-1′ resonances of compounds **1** and **2** are more shielded than H-1′ of **5** and **6**. On the contrary, H-1′ resonances of β- and α-2′-deoxyribonucleosides show very similar chemical shifts (Tables 1 and 2) but different coupling constants. Natural 2′-deoxyadenosine (**3**) and 2′-deoxyadenosine-3′,5′-diacetate (**4**) show the coupling constants between H-1′ and H-2′α (both 6.0 Hz) to be larger than those of the α-anomers **7** and **8** (3.1 and 2.1 Hz respectively).

^{13}C resonance assignments were in accordance with data reported for adenosine (**1**),[4,10] α-adenosine (**5**)[4,11] and 2′-deoxyadenosine (**3**).[12] C-1′ and C-2′ resonances of the ribonucleosides **1**, **2**, **5** and **6** are significatively affected by the C-1′ configuration, whereas corresponding resonances of 2′-deoxyribose moiety in the compounds **3**, **4**, **7** and **8** are not (Table 3). C-1′ and C-2′ of **1** and **2** are less shielded than the corresponding atoms of the α-anomers **5**[4] and **6**.

Some of the previously reported NMR methods used to establish anomeric configuration of purine nucleosides are not straight, requiring chemical transformation to 2′, 3′-isopropylidene derivatives or ^{1}H NOE experiments. The finding that C-4′ is less shielded in α-anomers than C-1′ whereas it is more shielded in β-ones, is valid neither for the acetates **2** and **6** nor for the 2′-deoxy compounds **3**, **4**, **7** and **8**. The anomeric configuration of nucleosides **1**–**8** can be easily assigned by the analysis of the ^{1}H NMR spectrum. For the 2′-deoxynucleosides **3**, **4**, **7** and **8**, anomers can be identified assessing the coupling constants between H-1′ and H-2′α. In the case of ribonucleosides **1**, **2**, **5** and **6**, the magnitude of the H-1′ chemical shifts (Tables 1 and 2) or the evaluation of differences between δ-values of the H-2 and H-1′ protons can be used for identifying the anomer. The magnitude of these differences is larger than 2.15 ppm in β-derivatives (**1, 5**) and smaller than 1.85 ppm in the α-ones (**2, 6**)

EXPERIMENTAL

Materials

Adenosine (**1**), 2′-deoxyadenosine (**3**), α-adenosine (**5**) and α-2′-deoxyadenosine (**7**) are commercial products. All the acetylated compounds were prepared using acetic anhydride in pyridine. Usual work-up and purification by chromatographic column on silica gel afforded pure **2**, **4**, **6** and **8**.

Adenosine-2′, 3′, 5′-triacetate (**2**): $[\alpha]^{25}_D$-30.7 (*c* 1, $CHCl_3$); *m/z* 394 [M + 1]; 416 [M + Na], 809 [M + M + Na]; 2′-deoxyadenosine-3′, 5′-diacetate (**4**): $[\alpha]^{25}_D$-24.4 (*c* 1, $CHCl_3$); *m/z* 394 [M + 1], 809 [M + M + Na]; α-adenosine-2′, 3′, 5′-triacetate (**6**): $[\alpha]^{25}_D$ + 25.1 (*c* 1,

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Table 1. ^{1}H-NMR data for compounds **1, 3, 5** and **7** (δ, ppm; J, Hz)[a,b] in DMSO-d_6 solutions

H	1	3	5	7
2	8.13 (s)	8.13 (s)	8.13 (s)	8.15 (s)
8	8.34 (s)	8.33 (s)	8.31 (s)	8.38 (s)
NH_2	7.33 (bs)	7.30 (bs)	7.20 (bs)	7.27 (bs)
1′α	5.87 (d)	6.34 (dd)	–	–
	6.2	*6.0; 8.0*	–	–
1′β	–	–	6.32 (d)	6.33 (dd)
	–	–	*5.4*	*3.1; 8.0*
2′α	–	2.25 (ddd)	–	2.33 (ddd)
	–	*2.7; 6.0; −13.1*	–	*2.7; 3.1; −14.2*
2′β	4.61 (ddd)	2.72 (ddd)	4.36 (ddd)	2.76 (ddd)
	5.1; 6.2; 6.3	*5.7; 8.0; −13.1*	*5.4; 5.4; 5.6*	*6.9; 8.0; −14.2*
3′	4.14 (ddd)	4.41 (dddd)	4.15 (ddd)	4.30 (dddd)
	3.0; 4.6; 5.1	*2.6; 2.7; 4.0; 5.7*	*4.9; 5.4; 5.6*	*2.7; 2.9; 4.6; 6.9*
4′	3.96 (ddd)	3.88 (ddd)	4.08 (ddd)	4.13 (ddd)
	3.0; 3.6; 3.7	*2.6; 4.2; 4.4*	*3.5; 5.2; −12.0*	*2.9; 4.5; 4.5*
5′a	3.67 (ddd)	3.62 (ddd)	3.58 (ddd)	3.40–3.48 (AB part of ABXY system)
	3.6; 4.4; −12.1	*4.4; 5.0; −12.0*	*3.5; 5.2; −12.0*	–
5′b	3.55 (ddd)	3.52 (ddd)	3.48 (ddd)	3.40–3.48 (AB part of ABXY system)
	3.7; 7.2; −12.1	*4.2; 6.7; −12.0*	*4.2; 6.2; −12.0*	–
2′-OH	5.43 (d)	–	5.46 (d)	–
	6.3	–	*5.6*	–
3′-OH	5.17 (d)	5.30 (d)	5.44 (d)	5.76 (d)
	4.6	*4.0*	*5.6*	*4.6*
5′-OH	5.41 (dd)	5.23 (dd)	4.86 (dd)	4.84 (dd)
	4.4; 7.2	*5.0; 6.7*	*5.2; 6.2*	*5.7; 5.7*

[a] Multiplicity in parentheses.
[b] Coupling constants (J) in italics.

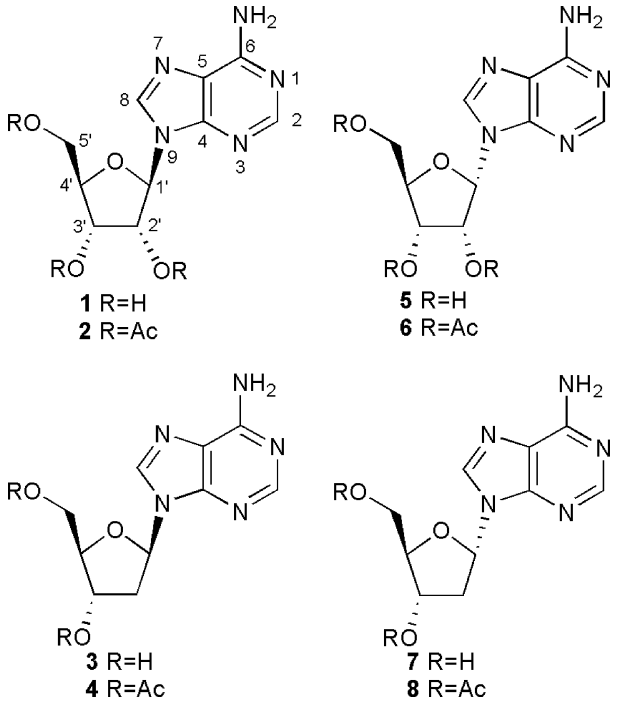

Figure 1. Chemical structure and numbering for compounds **1–8**.

CHCl$_3$); m/z 336 [M + 1], 358 [M + Na], 693 [M + M + Na]; α-2′-deoxyadenosine-3′, 5′-diacetate (**8**): $[\alpha]^{25}_D$ + 33.0 (c 1, CHCl$_3$); m/z 336 [M + 1], 358 [M + Na], 693 [M + M + Na].

NMR spectroscopy

NMR spectra were recorded on a Bruker AVANCE 500 spectrometer equipped with a 5-mm broadband reverse probe with field z-gradient, operating at 500.13 and 125.76 MHz for ^{1}H and ^{13}C, respectively. DMSO-d_6 (isotopic enrichment 99.95%) and CDCl$_3$ (isotopic enrichment 99.95%) were used as solvents for nucleosides (**1, 3, 5** and **7**) and for acetates (**2, 4, 6** and **8**), respectively. The samples contained 20 mmol of product dissolved in 0.5 ml of solvent (40 mM). The central peak of DMSO-d_6 signals (2.50 ppm for ^{1}H and 39.50 ppm for ^{13}C) and of CDCl$_3$ signals (7.26 ppm for ^{1}H and 77.7 ppm for ^{13}C) were used as internal reference standard. The sample temperature was maintained constant at 298 K. For 1D acquisition, the parameters were as follows: ^{1}H spectral width of 5000 Hz and 32 K data points providing a digital resolution of *ca* 0.305 Hz per point, relaxation delay 2 s; ^{13}C spectral width of 29 412 Hz and 64 K data points providing a digital resolution of *ca* 0.898 Hz per point, relaxation delay 2.5 s. The experimental error in the measured ^{1}H–^{1}H coupling constants is ±0.5 Hz. The experiments were performed through standard pulse sequences. gCOSY-45 and phase sensitive gNOESY experiments were acquired with 512 t_1 increments; 2048 t_2 points; spectral/spectrum width 10.0 ppm. The g-NOESY experiments were performed on degassed samples and with a mixing time of 800 ms. gHSQC and gHMBC experiments were acquired with 512 t_1 increments; 2048 t_2 points; spectral/spectrum width 10.0 ppm for ^{1}H and 180 ppm for ^{13}C.

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Table 2. ^{1}H data for compounds **2, 4, 6** and **8** (δ, ppm; J, Hz)[a,b] in $CDCl_3$ solutions

H	2	4	6	8
2	8.36 (s)	8.35 (s)	8.35 (s)	8.36 (s)
8	7.96 (s)	7.98 (s)	8.10 (s)	8.08 (s)
NH_2	5.82 (bs)	5.78 (bs)	5.64 (bs)	5.82 (bs)
1′α	6.18 (d)	6.43 (dd)	–	–
	5.3	*6.0; 8.1*	–	–
1′β	–	–	6.68 (d)	6.53 (dd)
	–	–	*5.6*	*2.1; 7.4*
2′α	–	2.62 (ddd)	–	2.77 (ddd)
	–	*2.5; 6.0; −16.1*	–	*2.1; 2.1; −15.3*
2′β	5.92 (dd)	2.95 (ddd)	5.76 (dd)	2.93 (ddd)
	5.3; 5.4	*6.3; 8.1; −14.1*	*5.6; 5.7*	*7.2; 7.4; −15.3*
3′	5.67 (dd)	5.43 (ddd)	5.52 (dd)	5.33 (ddd)
	4.3; 5.4	*2.4; 2.5; 6.3*	*4.3; 5.7*	*2.1; 2.1; 7.2*
4′	4.44	4.35	4.65 (ddd)	4.63 (ddd)
	overlaps5′a	overlaps5′b	*3.5; 4.0; 4.3*	*2.1; 4.1; 4.4*
5′a	4.45 (dd)	4.42 (dd)	4.38 (dd)	4.30 (dd)
	3.2; −13.1	*5.4; −13.0*	*3.5; −12.5*	*4.1; −12.1*
5′b	4.37 (dd)	4.36 (dd)	4.24 (dd)	4.24 (dd)
	5.5; −13.1	*4.4; −13.0*	*5.5; −12.5*	*4.4; −12.1*
2′-CH_3(CO)[c]	2.08 (s)	–	1.88 (s)	–
3′-CH_3(CO)[c]	2.14 (s)	2.13 (s)	2.11 (s)	2.12 (s)
5′-CH_3(CO)[c]	2.12 (s)	2.09 (s)	2.16 (s)	2.00 (s)

[a] Multiplicity in parentheses.
[b] Coupling costants (J) in italics.
[c] Assigned based on gHMBC spectra.

Table 3. ^{13}C-NMR data (δ, ppm) for compounds **1, 3, 5** and **7** in DMSO-d_6 solutions; for **2**, **4**, **6** and **8** in $CDCl_3$ solutions

C	1	2	3	4	5	6	7	8
2	152.4	153.9	152.4	153.7	152.2	154.0	152.4	153.6
4[a]	149.0	150.4	148.9	150.3	149.5	150.8	148.9	150.2
5	119.3	120.8	119.3	120.8	118.0	119.7	118.9	120.6
6	156.2	156.1	156.1	156.1	155.8	156.0	156.0	156.0
8	139.9	139.5	139.5	139.3	141.1	140.1	139.7	139.2
1′	87.9	86.9	83.9	85.3	83.3	83.3	83.5	86.0
2′	73.4	73.8	39.4	38.3	70.6	71.0	39.9	38.8
3′	70.6	71.3	71.0	75.2	70.6	71.9	70.8	75.2
4′	85.9	81.0	88.0	83.2	84.9	81.1	88.5	84.6
5′	61.6	63.8	61.9	64.5	61.4	64.0	61.6	64.4
2′-CO[a]	–	170.1	–	–	–	169.3	–	–
3′-CO[a]	–	170.3	–	171.0	–	170.0	–	170.8
5′-CO[a]	–	171.1	–	171.1	–	171.0	–	171.1
2′-CH_3(CO)	–	21.1	–	–	–	20.8	–	–
3′-CH_3(CO)	–	21.2	–	21.6	–	21.2	–	21.5
5′-CH_3(CO)	–	21.5	–	21.5	–	21.5	–	21.5

[a] Assigned based on gHMBC spectra.

Delay values were optimized for $^{1}J_{C,H}$ 140.0 Hz and $^{n}J_{C,H}$ 3.0 Hz. Zero filling in F_1 to 1 K, $\pi/2$ shifted sine-bell squared (for gHSQC) or sine-bell (for gHMBC) apodization functions were used for processing.

Mass spectroscopy and optical rotations

Mass spectra were recorded on Finnigan LCQ-Deca (Termoquest) in ESI positive-ion mode, KV 5.00, 225 °C, 15 V. Optical rotations were measured on a Perkin-Elmer 241 polarimeter (sodium D line at 25 °C).

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