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Investigación

Assignment of the ^{1}H and ^{13}C NMR spectra of N^2,N^6-dibenzoyl-N^2,N^9-bis(2',3'-di-*O*-benzoyl-(α)-L-threofuranosyl)-2,6-diaminopurine

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Abstract. The complete ^{1}H- and ^{13}C NMR-chemical shifts assignments of the title 2,6-diaminopurine nucleoside are described. Spectroscopic analysis confirmed the *R*- configuration at the anomeric carbons and that both the threo-furanoses exist in a conformational equilibrium at room temperature.

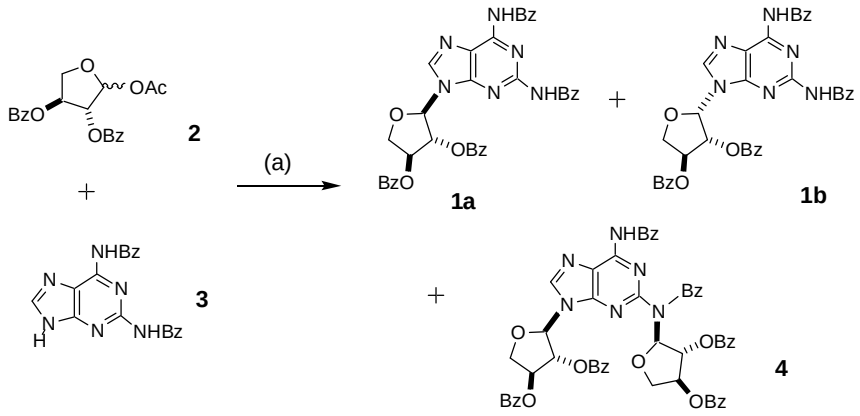
Keywords: ^{1}H and ^{13}C NMR spectroscopy; 2,6-diaminopurine; nucleosidation; (α)-L-threofuranose; nucleosides, N^2,N^6-dibenzoyl-N^2,N^9-bis(2',3'-di-*O*-benzoyl-(α)-L-threofuranosyl)-2,6-diaminopurine; TNA.

Resumen. Se describen las asignaciones de RMN ^{1}H y ^{13}C del nucleósido derivado de 2,6-diamino-purina nombrado en el título del trabajo. El análisis espectroscópico confirmó la configuración *R*- de los carbonos anoméricos, y ambas treo-furanosas existen en un equilibrio conformacional a temperatura ambiente.

Palabras clave: Espectroscopía de RMN ^{1}H y ^{13}C; 2,6-diaminopurina; nucleosidación; (α)-L-treofuranosa; nucleósidos, N^2,N^6-dibenzoil-N^2,N^9-bis(2',3'-di-*O*-benzoil-(α)-L-treofuranosil)-2,6-diaminopurina; ATN.

Introduction

The systematic study of the base-pairing properties of various sugar-modified-backbone analogs of nucleic acids, carried out by Eschenmoser and co-workers [1], has led to the discovery of α-L-threofuranosyl-(3' → 2')-oligonucleotides ("TNA") as an informational pairing system in an antiparallel strand orientation that is also capable of cross-pairing with complementary sequences of RNA and DNA [2]. TNA is derived from threose-sugar containing only four carbon atoms and is one of the simplest potentially natural nucleic acid alternatives investigated, in the context of a chemical etiology of nucleic acid structure. In this context it was further shown, by Eschenmoser and coworkers [3], that replacing adenine with 2,6-diaminopurine nucleobases in TNA-sequences results in enhanced thermal- and thermodynamic-stability of TNA/TNA-, TNA/RNA- and TNA/DNA-duplexes and accelerates template directed ligation of TNA ligands. The 2,6-diaminopurine-threose-nucleoside **1a**, which is the initial building block required for the automated solid-support synthesis of TNA-sequences, was synthesized by nucleosidation of the peracylated threose **2** with N^2,N^6-dienzoyl-2,6,-diaminopurine **3** ($D^{(Bz)2}$) under the standard Hilbert-Johnson-Vorbrüggen conditions (Scheme 1) [4]. Thus, an initial exploratory reaction of **2** with silylated **3** in the presence of 1.2 eq. of $SnCl_4$ as Lewis acid [5,6] afforded, apart from the epimeric mixture of N^9-nucleosides **1a** (24 %) and **1b** (8 %), a compound **4** which is formed in minor amounts (6 %).



Scheme 1. Nucleosidation Reaction Catalyzed by $SnCl_4$ (a): 0.25 M (1.2 eq.) $D^{(Bz)2}$, 0.9 M (3.9 eq.) BSA, 0.29 M (1.2 eq.) $SnCl_4$, MeCN / 80 °C / 2h.

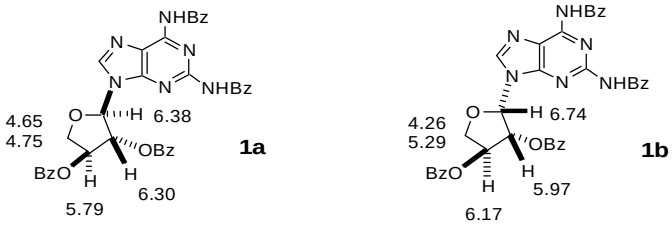


Fig. 1. Comparision of ^{1}H-Chemical Shifts (δ, $CDCl_3$) between Nucleosides **1a** and **1b**.

Table 1. ^{1}H and ^{13}C NMR data of the purine-nucleobase in **4**.

	N^2,N^6-dibenzoyl-N-2,6-diaminopurine	
H and C	δ_H	δ_C
2		155.133
4		152.568
5		121.108
6		149.494
8	8.155 brs	141.188
C(6)N*H*	8.648 s	

Compound **4** turned out to be, unexpectedly, a N^2,N^9-bisnucleoside derivative of 2,6-diaminopurine. Here, we report on the structure determination of **4** and its ^{1}H- and ^{13}C-NMR assignments with the aid of 2D-NMR measurements (^{1}H-^{1}H COSY, DEPT, HMQC, HMBC and NOESY).

Results and Discussion

Structure of compound **1a** was established on the basis of multidimensional NMR spectroscopic data and by an X-ray structure analysis of the 2',3'-diol derivative obtained by the selective hydrolysis the 2',3'-benzoate groups of **1a** [5]. Compounds **1a** and **1b** exhibited the same MS-data corresponding to the formula $C_{37}H_{28}N_6O_7$; they also displayed the same connectivity in the HMBC experiments, and the C-1'-N-9 linkage was determined by the presence of H-1'-C-4 crosspeaks, thus ruling out the possibility that **1b** could be the regioisomeric N^7-nucleoside. Comparison of the ^{1}H-chemical shifts between **1a** and **1b** (Fig. 1) coupled with MS-data further confirmed the epimeric relationship between the two nucleosides. A couple of noteworthy differences in the spectroscopical behaviour between the two nucleosides are: (a) NOESY correlation was observed between H-8 and H-1' for nucleoside **1b** which was absent in nucleoside **1a** and (b) the coupling constant between H-1' and H-2' for nucleoside **1a** ($J_{1',2'}$) = 2 Hz, while for nucleoside **1b** ($J_{1',2'}$) = 5 Hz.

Tables 1-4 list the ^{1}H- and ^{13}C-NMR data for compound **4**. ESI-MS for compound **4** indicated a molecular formula of $C_{55}H_{42}N_6O_{12}$. This molecular formula agreed with the structure of a N^2,N^6-dibenzoyl-2,6-diaminopurine ($C_5H_2N_6$(C_7H_5O)$_2$=$C_{19}H_{12}N_6O_2$) linked to two dibenzoylated threoses (2($C_{18}H_{15}O_5$)=$C_{36}H_{30}O_{10}$). The ^{13}C-NMR spectrum of **4** showed 43 signals, 8 of which correspond to the two threoses (rings A and B, see Fig. 2), 5 to the purine nucleus, 6 to the carbonyl groups, and the remaining 24 signals to the corresponding non-equivalent carbons of the six benzene rings. The individual spin systems were discerned from the HMQC and HMBC experiments and the ^{1}H- and ^{13}C-NMR assignments of the N^2,N^6-dibenzoyl-2,6-diaminopurine fragment are shown in Table 1. The amide proton (δ_H 8.648, exchanges with D_2O) showed HMBC correlation with C-5 (δ_C 121.108), which in turn correlated with H-8 (δ_H 8.155). This last proton showed a cross-peak with the secondary carbon located at δ_C 141.188 in the HMQC experiment.

^{1}H-^{1}H COSY NMR experiments clearly indicated the presence of two sets (A and B) of the sugar protons (see Table 2). The signals at δ_C 88.730 (set A) and δ_C 91.557 (set B, determined as the anomeric carbons, due to the observed HMBC crosspeaks, *vide infra*) correlated with the hydrogens at δ_H 5.989 and 6.567, respectively, in the HMQC spectra, locating the anomeric hydrogens (Table 2). The connectivities of anomeric carbons of the threoses to N-9 and to the nitrogen at C-2 were established by the observed HMBC correlation between H-1'A and C-4 (δ_C 152.568) and between H-1'B and C-2 (δ_C 155.133). Selected HMBC correlations that allowed the full spectral assignments are shown in Fig. 2.

The stereochemistry at the anomeric carbons was determined by the presence of cross-peaks in the NOESY spectrum

Table 2. ^{1}H and ^{13}C NMR data of the threoses in **4**.

	Threofuranose A		Threofuranose B	
H and C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C
1'	5.989 d (2)	88.730	6.567 d (5.0)	91.557
2'	5.715 dd (2,2)	80.016	6.916 dd (5.0,5.0)	78.384
3'	5.657 ddd (5,2.5, 2)	75.980	5.750 ddd (7.5,5.0,5.0)	77.248
4'α	4.433 dd (11, 5)	73.343	4.493 dd (9.0,5.0)	70.763
4'ß	4.337 dd (11, 2.5)		4.415 dd (9.0,7.5)	

Table 3. ^{1}H and ^{13}C NMR data for the benzamides at N(6) and N(2) in **4**.

	N^2 and N^6-Benzamides			
	N(6)-CO-Ph		N(2)-CO-Ph	
H and C	δ_H(J, Hz)	δ_C	δ_H (J, Hz)	δ_C
1		164.167		172.298
2		133.086		136.787
3,7	7.874 dd (8,1)	127.986[a]	7.466 dd (7.5,1.5)	128.700
4,6	7.465 dd (8,8)	127.986[a]	7.018 dd (7.5,7.5)	128.219
5	7.562 dddd (8,8,1,1)	133.960	7.110 dddd (7.5,7.5,1.5,1.5)	130.478

[a] Overlapped signals.

Table 4. ^{1}H and ^{13}C NMR for the 2',- and 3'-*O*-benzoates in **4**.

	Benzoates							
	C(2'A)-OCO-Ph		C(3'A)-OCO-Ph		C(2'B)-OCO-Ph		C(3'B)-OCO-Ph	
H and C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C
1		164.634		165.304		166.135[f]		165.625[f]
2		128.511[e]		128.550[e]		129.414[g]		129.283[g]
3,7	8.006 dd (8.5,1)	130.012	7.816 dd (8.0,1.0)	129.735	7.992 dd (8.5,1.0)[b]	129.924[h]	7.998 dd (8.5,1.0)[b]	129.837[h]
4,6	7.504 dd (8.5,8.5)	128.788	7.439 dd (8.0,8.0)	128.788	7.327 dd (8.5,8.5)[c]	128.365[i]	7.371 dd (8.5,8.5)[c]	128.321[i]
5	7.643 dddd (8.5,8.5,1.5,1.5)	133.873	7.584 dddd (8.0,8.0, 1.0, 1.0)	132.824	7.508 dddd (8.5,8.5,1.0,1.0)[d]	133.246[j]	7.508 dddd (8.5,8.5,1.0,1.0)[d]	133.188[j]

[b,c,e-j] Interchangeable signals; [d]Overlapped signals

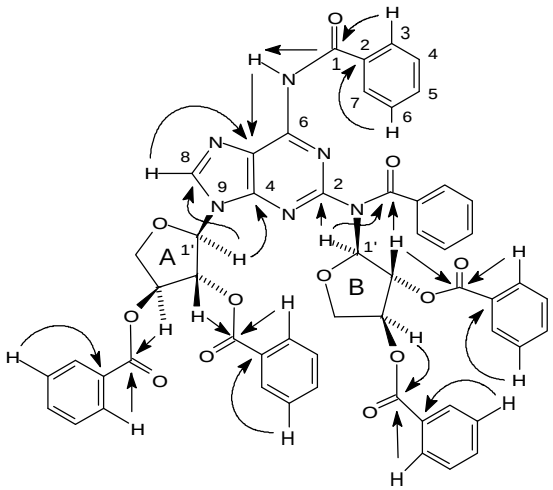


Fig. 2. Selected HMBC correlations for **4**.

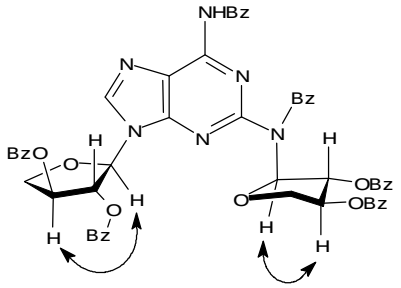
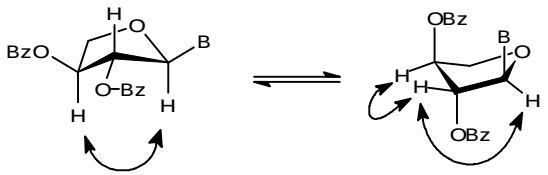


Fig. 3. NOESY cross-peaks between H-1' and H-3' for both threoses of **4**.



C(2')-*endo*-C(3')-*exo* C(2')-*exo*-C(3')-*endo*

Fig. 4. The two main puckering modes for threose indicating the observed NOESY correlations.

between H-1' and H-3' for both threoses (Fig. 3), determining their *cis*-relationship. This existence of NOESY between H-1' and H-3' could be possible only when the nucleoside is an α-anomer. Therefore the configuration at C-1'A and C-1'B is assigned to be *R*.

This NOESY correlation is weak for the ring A and strong for the ring B at room temperature, suggesting unequal conformational populations for each threose. The observed ^{1}H-^{1}H coupling constants between H-1' and H-2' are also different ($J_{1',2'}$ = 2.0 Hz for ring A, and J1',2' = 5.0 Hz for ring B, see Table 2), suggesting that the average conformations for both threoses are different. This is in agreement with the NOESY interactions between H-1' and H-2', between H-2' and H-3', and between H-1' and H-3', which were observed to be different for both threoses. These observations underline the limitations in assigning the relative configuration at C(1') considering only the magnitude of the $J_{1',2'}$ coupling constants. The schematic representations of the two principal puckering modes for the threose ring, namely, C(2')-*endo* (S) and the C(3')-*endo* (N) that explain the observed NOESY correlations for both threoses of **4**, are shown in Fig. 4.

This conformational interconversion [C(2')-*endo* (S)⇌ C(3')-*endo* (N)] is well documented for the ribofuranosyl series in the literature, and the barrier of the conformational equilibrium is relatively low [7,8]. The average activation energy has been estimated to be 4.7 ± 0.5 kcal/mole for purine ribonucleosides.

The ^{1}H- and ^{13}C-NMR for the two benzamides and the four benzoates are shown in Tables 3 and 4, respectively, and were assigned by identifying the HMBC correlations for each carbonyl group with the corresponding *o*,*m*-hydrogens (H-3, H-7 and H-4, H-6), and in the case of the benzoates, also identifying the interactions with the sugar hydrogens. The carbonyl of the benzamide at C-6 (δ_C 164.167) showed a cross-peak with the amide hydrogen, while the benzamide at C-2 (δ_C 172.298) showed a cross-peak with the anomeric hydrogen H-1'B, as shown in Fig. 2. Some assignments can be interchanged and several signals appeared overlapped, as indicated in Tables 3 and 4.

The observed downfield chemical shift of H-C(2') in threose B (H-2'B, δ 6.92) of **4** deserves a comment. One possible explanation is the proximity of this hydrogen to a hete-

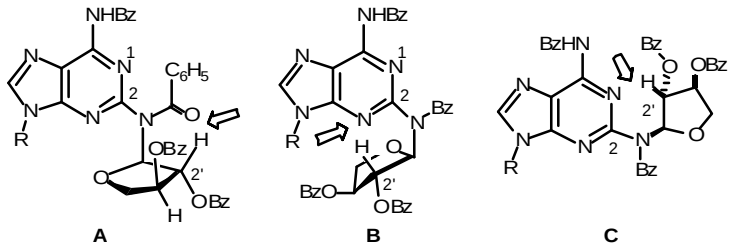


Fig 5. Three possible conformers of **4** explaining the proximity of H-2'B to a heteroatom.

roatom; and in principle, there are at least 3 conformational possibilities, which would result in such proximity. These are (a) the carbonyl group of the benzamide linked to C(2) close to H-2'B (conformer A); (b) H-2'B close to N(3) (conformer B), and free rotation along the sigma C(2)-N bond generates another arrangement in which (c) H-2'B close to N(1) (conformer C). These conformational possibilities are shown in Fig. 5.

Simple hand held molecular model considerations reveal that it is not possible with any degree of certainty to single out one conformer out of the three possibilities considered here; though there seems to be a preference for the sterically less hindered conformer C. However, the observed data correspond to an average structure rather than a single conformer, since the NOESY interactions show evidence of the conformational mobility of **4** at room temperature.

Experimental

***N²,N⁶*-dibenzoyl-*N⁹*-(2',3'-di-*O*-benzoyl-(α)-L-threofuranosyl)-2,6-diaminopurine (1a), *N²,N⁶*-dibenzoyl-*N⁹*-(2',3'-di-*O*-benzoyl-(β)-L-threofuranosyl)-2,6-diaminopurine (1b), *N²,N⁶*-dibenzoyl-*N²,N⁹*-bis(2',3'-di-*O*-benzoyl-(α)-L-threofuranosyl)-2,6-diaminopurine (4).** In a dried three necked round bottom flask equipped with a condenser and a septum was placed 1.1 g (2.96 mmol, 1.02 eq.) of *N²,N⁶*-dibenzoyl-2,6-diaminopurine **3** (previously dried in the high vacuum at 70 °C, using the Büchi gun) and solution of 1.1 g (2.90 mmol, 1.0 eq.) of peracylated sugar **2** in 9 mL of MeCN was added by a syringe. Additional 3 mL of MeCN was used to rinse the flask. The reaction mixture was heated to 80 °C with stirring under Ar. After 10 min, 2.78 mL (11.2 mmol, 3.86 eq.) of bis(trimethylsilyl)acetamide (BSA) was added, obtaining a clear solution. After 30 min, 415 μL (3.5 mmol, 1.22 eq.) of $SnCl_4$ was added and the heating was maintained for 2 h. The solution turned dark brown. The reaction mixture was cooled to room temp., poured to a stirred solution of EtOAc and satd. aq. $NaHCO_3$ (150 mL each). The phases were separated, and the aqueous phase was extracted two times with additional portions of EtOAc. The organic phases were combined, washed with $NaHCO_3$, brine, and dried ($MgSO_4$). The organic residue was concentrated to dryness to afford a residue (1.4 g), which was separated by column chromatography (60 g of silica gel packed with hexane-EtOAc (55:45), and increasing the polarity to 1:1, 3:2, and 7:3). From the fractions eluted with hexane-EtOAc (1:1) was obtained a residue (320 mg), and part of this (81 mg) was rechromatographed with silica (7 g) suspended in hexane/EtOAc (85:15) and increasing the proportion of EtOAc to 75:25, 70:30, 65:35, 60:40, 1:1. This procedure afforded 43 mg of **4** (6 %) as a yellow solid. From the fractions eluted with hexane/EtOAc (7:3) of the original column chromatography, a residue (717 mg) was obtained, which was purified by column chromatography (∅ 2.5 × 40 cm, 100 g of silica gel) and a mixture of hexane/benzene/EtOAc/CH_2Cl_2 (10:10:2.5:2.5) with gradually increasing polarity by the addition of methanol (0.1 → 0.4 %) to this mixture) to afford 470 mg of **1a** (24 %) and 150 mg of **1b** (8 %).

***N²,N⁶*-dibenzoyl-*N⁹*-(2',3'-di-*O*-benzoyl-(α)-L-threofuranosyl)-2,6-diaminopurine (1a).** For complete characterization see reference 5.

***N²,N⁶*-dibenzoyl-*N⁹*-(2',3'-di-*O*-benzoyl-(β)-L-threofuranosyl)-2,6-diaminopurine (1b).** Mp. 116-118°C, TLC (benzene / EtOAc / CH_2Cl_2 / MeOH 8:1:1:0.2): R_f 0.45. ^{1}H NMR (500 MHz, $CDCl_3$): 4.26 (*dd*, *J* = 9.5, 2.5, H-C(4′a)); 5.19 (*dd*, *J* = 9.5, 5.0, H-C(4′b)); 5.97 (*dd*, *J* = 5.0, 2.5, H-C(2′)); 6.17 (br *s*, H-C(3′)); 7.2-8.2 (*m*, 20 arom. H); 8.06 (*s*, H-C(8)); 9.38 and 9.53 (br *s*, HNCO). ^{13}C NMR (125 MHz, $CDCl_3$): 72.53 (*t*, C(4′)); 74.67 (*d*, C(2′)); 77.17 (*d*, C(3′); 84.82 (*d*, C(1′)); 119.58 (s, C(5)); 141.64 (*d*, C(8)); 149.75 (*s*, C(6)); 152.34 (s, C(4)); 152.68 (*s*, C(2)); 165.19 (*s*, 2*CO* benzamido); 165.54, 165.81 (2*s*, *CO*). MALDI-FTMS, *m/z*: 691.1892 (100, $[M + Na]^+$).

***N²,N⁶*-dibenzoyl-*N²,N⁹*-bis(2',3'-di-*O*-benzoyl-(α)-L-threofuranosyl)-2,6-diaminopurine (4).** Mp. 122-124 °C, TLC (hexane / EtOAc 1:1): R_f 0.35. ^{1}H NMR and ^{13}C NMR: see Tables 1-4. ESI-MS (pos.), *m/z*: 979 (100, $[M + H]^+$); ESI-MS (neg.), *m/z*: 977 (38, $[M - H]^-$).

Spectra. All the experiments were performed at room temperature on a Varian Unity Plus 500 spectrometer operating at 499.89 MHz for ^{1}H and 125.71 MHz for ^{13}C. TMS was used as an internal reference for the spectra, and $CDCl_3$ as solvent. The COSY spectra were acquired with a spectral width of 4827 Hz in f_2 and f_1, with 16 transients and 128 t_1 increments. The pulse programs of COSY, HMQC, HMBC and NOESY experiments were taken from the Varian software library and

were obtained using an indirect detection probe. The HMQC and HMBC (9 Hz) were collected using a spectral width of 4827 Hz for f_2 (^{1}H) and 21751 Hz for f_1 (^{13}C) and 26109 Hz for HMBC. With 1024 data points, a relaxation delay of 1s using 32 transients (HMQC) and 128 transients (HMBC) both with 256 increments. Data were processed with linear prediction to 2048 points in the indirect detected frequency.

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