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Identification of Purine Deoxyribonucleoside Anomers by Two Dimensional NOESY NMR

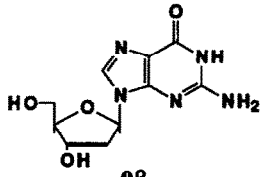
Joseph Gambino, Te-Fang Yang and George E. Wright*

Department of Pharmacology, University of Massachusetts
Medical School, Worcester, MA 01655

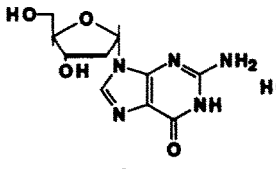
Abstract: Patterns of NOE crosspeaks in two dimensional ^{1}H NOESY spectra of purine deoxyribonucleosides are characteristic for the β and α anomers. Certain ^{1}H chemical shifts are characteristic for 7 and 9 regioisomers. NOE intensities, although qualitatively consistent with interproton distance ranges in N and S conformers, are insufficient for complete conformational analysis of nucleosides.

Synthesis of nucleosides by glycosylation of heterocycles typically results in multiple isomeric products. For example, purine 2'-deoxyribonucleosides may be isolated as α and β anomers of 7 and 9 regioisomers.[1-3] Structural assignment of anomers has been based empirically on the appearance of the 1'-H resonance in the ^{1}H NMR spectrum, and that of regioisomers on the relative chemical shifts of glycosidic and base protons.[4,5] For example, 1'-H of β anomers generally appears as a pseudotriplet ($J_{1'\text{-}2'} \approx J_{1'\text{-}2''}$), but that of α anomers is a doublet of doublets ($J_{1'\text{-}2'} > J_{1'\text{-}2''}$). However, the solvent dependence of chemical shifts and coupling constants, based, in part, on changes in conformational equilibria, makes such generalizations risky.[6] Recently, Seela's group applied one dimensional NOE difference NMR spectroscopy to this problem, resulting in general rules that can be used to distinguish between α and β anomers of ribo-, arabino-, 2'-deoxyribo- and 2',3'-dideoxyribo-nucleosides.[7] The convenience of two dimensional NOESY NMR to simultaneously display all distance-related cross relaxations between protons prompted us to apply this method to isomeric purine deoxyribonucleosides.

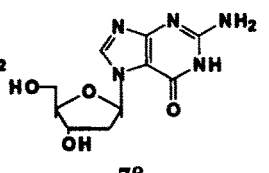
The method was tested with four isomers related to 2'-deoxyguanosine (structures). One dimensional NMR data for the deoxyguanosines obtained in Me_2SO-d_6 are summarized in Table 1. Conditions for acquisition of NOESY spectra were established with 9-β 2'-deoxyguanosine (see Experimental). Variation of the mixing time from 50 to 400 msec resulted in linearity of crosspeak intensities to 250-300 msec. All NOESY spectra were subsequently acquired with a mixing time of 300 msec to provide a balance between linearity and maximum intensity of weak crosspeaks. NOESY spectra of the four deoxyguanosine isomers



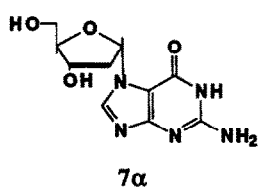
9β



9α



7β



7α

are displayed in Figure 1 and show characteristic patterns of crosspeaks for the β and α anomers, consistent with distance ranges calculated for assumed conformers (see below). In general, the β anomers display crosspeaks between H8-H3' and H8-H2', while α anomers show crosspeaks between H8-H4' and H8-H2". Within the deoxyribose ring only relative crosspeak intensities differed. In β anomers H1'-H2" > H1'-H2',

Table 1. ^{1}H NMR Spectra of 2'-Deoxyguanosine Isomers.

^{1}H	9-β	9-α	7-β	7-α
Chemical shifts, δ (ppm):				
1'	6.09	6.09	6.43	6.44
2'	2.50	2.67	2.47	2.66
2"	2.21	2.20	2.33	2.22
3'	4.33	4.26	4.33	4.26
4'	3.82	4.06	3.88	4.16
5'	3.54	3.43	3.62	3.47
5"	3.48	3.40	3.54	3.45
3'-OH	5.26	5.45	5.24	5.31
5'-OH	4.95	4.81	4.92	4.82
1-NH	10.61	10.61	10.82	10.92
NH_2	6.45	6.43	6.13	6.17
8	7.95	8.00	8.30	8.18
Coupling constants, J (Hz):				
1'2'	7.70	7.68	6.58	6.97
1'2"	6.16	2.80	6.08	1.99
2'2"	-13.38	-14.20	-13.27	-14.41
2'3'	5.79	7.06	6.73	6.87
2"3'	3.03	3.01	3.62	2.32
3'4'	2.86	2.90	2.99	2.79
4'5'	4.36	4.44	3.84	4.03
4'5"	4.57	4.77	4.58	5.06
5'5"	-11.25	-11.66	-11.67	-12.18
3'3'-OH	3.9	3.8	4.0	n.o.
5'5"/5"5"-OH	5.5	5.6	5.4	n.o.

H-2' and H-2" are *trans* and *cis* to 3'-OH, respectively; H-5' and H-5" are pro-R and pro-S, respectively. n.o., not observed.

but in α anomers H1'-H2' > H1'-H2". In β anomers H1'-H4' > H1'-H3', but in α anomers H1'-H3' > H1'-H4'. An additional distinction is the presence in α anomers of a crosspeak between H1'-H5',5".

The general applicability of NOESY spectra for anomer identification was tested with the four 3',5'-di-*p*-toluyl-2'-deoxyribofuranosyl derivatives of 2,6-dibromopurine.[3] The patterns observed in Figure 2 are fully consistent with those expected for β and α anomers based on the results of Figure 1. The advantage of 1D

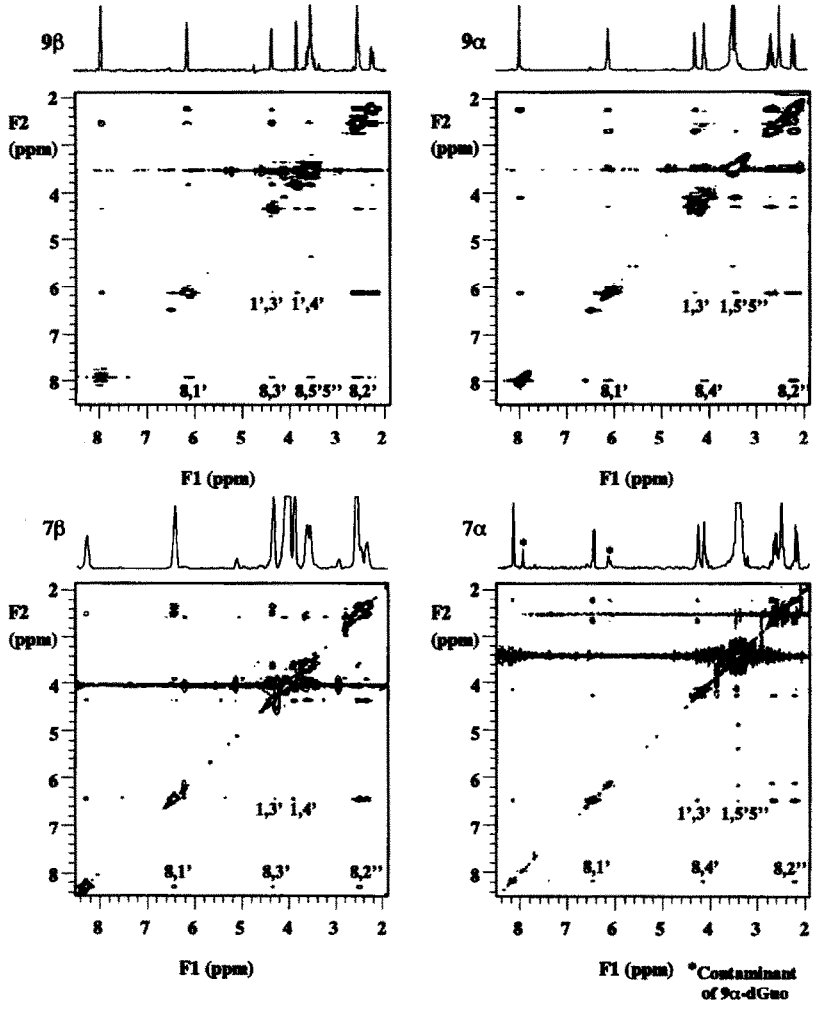


Figure 1. NOESY spectra of 2'-deoxyguanosines in Me_2SO-d_6

or 2D NOE measurements over coupling constant values in anomeric assignment is illustrated by the solvent-dependence of the H1' multiplet in the 9-α 2,6-dibromopurine nucleoside in Figure 2. The H1' of this nucleoside appeared as a doublet of doublets in $CDCl_3$ but a pseudotriplet in Me_2SO-d_6, leading to the original misassignment of its structure as a β anomer.[8]

The identity of regioisomers is not reflected in coupling constants or NOE results but in chemical shifts (Table 1).[9] For purine 2'-deoxyribonucleosides H8 and H1' are consistently found to be deshielded in 7-isomers by 0.2-0.4 ppm relative to 9-isomers.[4,5] The difference in chemical shifts between H4' and H5',5" is also greater in α anomers than in β anomers. This difference is more dramatic in protected, i.e. 3',5'-diacyl, deoxyribonucleosides in which the chemical shifts of H4' and H5',5" are nearly coincident in β anomers (for example, see Figure 2).

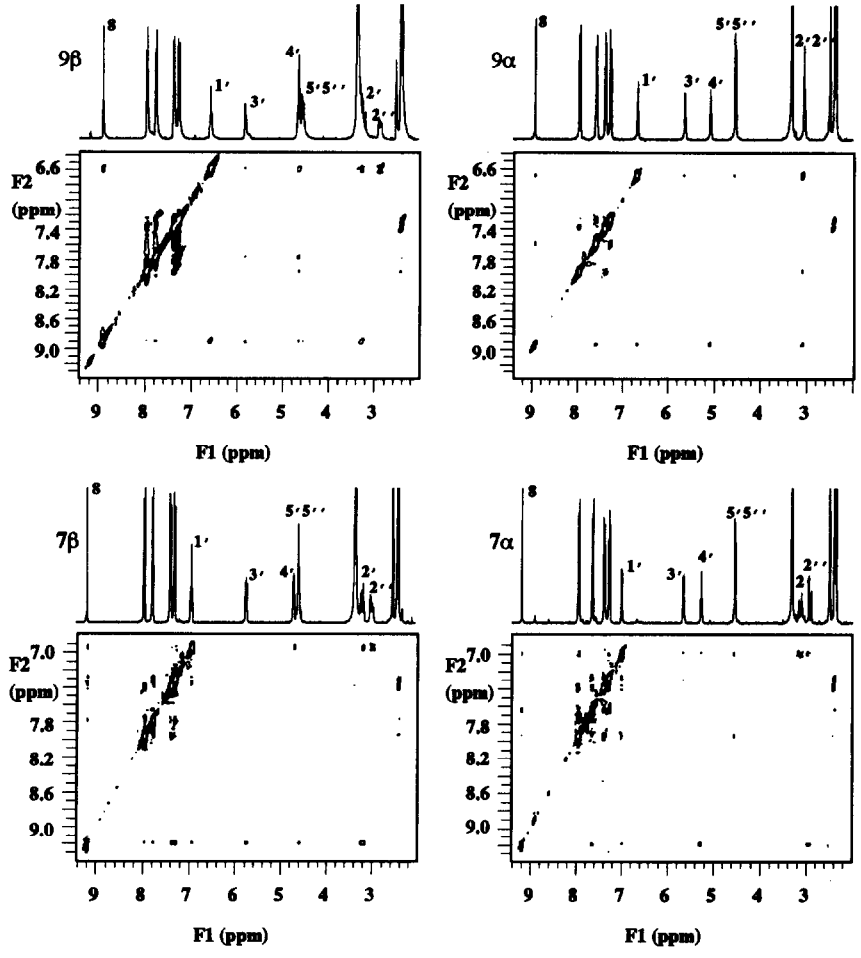


Figure 2. Partial NOESY spectra of 2′-deoxy-3′, 5′-di-*p*-toluyl-D-ribofuranosyl-2,6-dibromopurines in Me_2SO-d_6

The NOE patterns observed in α and β deoxyribonucleosides (Figures 1 and 2) are consistent with expected interproton distances in the range of conformations commonly occupied by furanosides.[10] Relative intensities of NOE crosspeaks for 9-β and 9-α deoxyguanosines, acquired at the 300 msec mixing time, agreed well with interproton distance ranges calculated for N (C3'-*endo*) and S (C2'-*endo*) conformers (Table 2). Attempts to use NOE crosspeak intensities of furanose protons to calculate N/S ratios for each anomer failed, however, suggesting the inadequacy of the two state ring puckering assumption (data not shown). Indeed, NOE intensities have been used in rigid nucleosides for estimation of *syn/anti* glycosidic bond ratios.[11] When vicinal coupling constants, $J_{H3'-H4'}$, were used to estimate the conformational distribution (see Experimental), the four 2'-deoxyguanosines gave nearly the same value, i.e. N/S ≈ 0.30 ±0.02. However, multiple conformers likely exist in solution involving variation in ring puckering, glycosidic bond

and C4'-5'CH_2OH torsion angles leading to many possible distance relationships between protons. The use of NOE intensities together with other structure constraints, such as ^1H-^1H and ^1H-^{13}C coupling constants, as a general approach to the conformational analysis of nucleosides is under study.

Table 2. Relative NOE Intensities and Interproton Distances in 9-β and 9-α Deoxyguanosines.

	9-β		9-α	
Protons	NOE	Distances N-S (Å)	NOE	Distances N-S (Å)
1'-2'	227	2.8-3.1	3188	2.5-2.4
1'-2"	2575	2.4-2.5	607	3.1-2.8
1'-3'	26	3.9-3.8	157	2.7-4.1
1'-4'	361	3.3-3.2	n.o.	3.7-4.0
1'-5'5"	n.o.	4.4-4.2	318	3.5-3.6
8-1'	550	3.8-3.8	539	3.8-3.8
8-2'	1613	4.2-3.5	n.o.	4.8-4.9
8-2"	n.o.	4.9-4.6	1063	3.8-4.2
8-3'	211	3.2-5.3	n.o.	5.5-4.9
8-4'	n.o.	4.5-4.7	363	4.0-3.4
8-5'5"	96	3.6-4.0	n.o.	5.0-5.0

NOE intensities were measured at 300 msec mixing time, and are relative to 2'-2" NOE = 10,000; all values are negative. n.o., not observed. Distances were calculated with the program PCMODEL using structures with phase angles of 18°(N) and 162°(S) and a glycosidic torsion angle of -165° (*anti*). Distances to H5',5" are to the 5'-C.

EXPERIMENTAL

2'-Deoxyguanosine was obtained from Sigma, and its isomers were synthesized from the corresponding 6-chloropurine nucleosides derived from sodium salt glycosylation of 2-amino-6-chloropurine with 1-chloro-3,5-di-p-toluyl-β-D-ribofuranose.[12] Preparative HPLC (silica gel, 25% acetone in toluene) was used to separate the known 9-β, 9-α and 7-β protected nucleosides (48, 3.6 and 8.9% yields, respectively) from a fourth product, the previously unreported 7-α isomer (2.2% yield). Hydrolysis of the 9-α, 7-β and 7-α intermediates with 2-mercaptoethanol and sodium methoxide in ethanol after reflux for 3 days gave, respectively, authentic 9-α and 7-β 2'-deoxyguanosines and 64% of 7-α 2'-deoxyguanosine (MH^+: calcd. for $C_{10}H_{14}N_5O_4$ 268.1045; found [EF-FAB] 268.1042).

NMR spectra were obtained at 300 MHz in a 5 mm ^1H probe with a Varian Unity 300 instrument and processed on a Sun Sparcstation 1+. All spectra were obtained at 25°C with 12 mM nucleosides dissolved in Me_2SO-d_6 containing TMS as internal standard. 1D spectra were acquired in 30848 data points at a spectral width of 4116 Hz, and processed with 0.3 Hz line broadening. Chemical shifts and coupling constants in Table 1 are iterated values from spectral simulation with the VNMR software. NOESY spectra

(deoxyguanosines were previously exchanged with D_2O) were acquired using the States-Haberkorn hypercomplex method as 2048 data points in t2 (spectral width 2856 Hz) and at 2 x 256 increments in t1 with 32 scans per t1 value. The 90° pulse width was 7.2 μsec. Final spectra were processed with zero filling to 2048 x 2048 data points and with squared sinebell functions shifted by -0.358 sec in t2 and by -0.09 sec in t1.

Structure calculations were done with the program PCMODEL (Serena Software, Bloomington, IN) on a Sun Sparcstation 1+ using the default structure of 2'-deoxyguanosine as a starting point. (The standard conformational nomenclature is that of ref. 10.) The furanose rings of 9-β and 9-α isomers were driven to the N (phase angle 18°) and S (phase angle 162°) conformers with the dihedral driver in the MMX force field. The glycosidic bond torsion angles were -165° (*anti*) and the exocyclic 5'-CH_2OH groups were *gauche-gauche* in each final structure. N/S conformational ratios, X_N/X_S ($X_N + X_S = 1$), were calculated from $X_S = (J_N-J_{3'4'})/(J_N-J_S)$ where J_N = 8.8 Hz and J_S = 1.1 Hz.[13]

ACKNOWLEDGEMENTS

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