Amino–Imino Tautomerization of N^2-(4-*n*-Butylphenyl)-2′-deoxy-3,5′-cycloguanosine

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N^2-(4-*n*-Butylphenyl)-2′-deoxy-3,5′-cycloguanosine (cBuPdG) has been studied by one-dimensional 1H and 13C, two-dimensional TOCSY, HMQC, and HMBC, and long range selective INEPT NMR experiments. In DMF and DMSO solutions cBuPdG exists as a mixture of two isomers involved in a slow rate of interconversion which have been identified as N^2 amino–imino tautomers, while in chloroform only the imino tautomer is present. It has been proved that the tautomeric form of the parent nucleoside, N^2-(4-*n*-butylphenyl)-2′-deoxyguanosine (BuPdG), is N^2 amino. The selective INEPT experiment can be used efficiently in assignment of tautomeric structures of nucleosides.

N^2-Phenylguanines and their nucleoside derivatives show interesting and important biological properties.[1,2] For example N^2-(4–*n*-butylphenyl)-2′-deoxyguanosine triphosphate (BuPdGTP) is a potent and selective inhibitor of α-type DNA polymerases.[3]

In one step of our recent synthesis of the α,β-methylene analog of BuPdGTP we identified a byproduct as N^2-(4–*n*-butylphenyl)-2′-deoxy-3,5′-cycloguanosine (cBuPdG).[4] 3,5′-Cyclopurine ribonucleosides have been synthesized from the corresponding nucleosides bearing a good leaving group at the 5′ position.[5] These types of compounds have fixed *syn* glycosidic conformation and have been widely used as model compounds for structural, conformational, and mechanistic studies.[6] However, except for 2′-deoxy-3,5′-cycloadenosine[7] no other 2′-deoxy-3,5′-cyclopurine nucleosides have been synthesized and characterized.

The proton NMR spectrum of cBuPdG in DMSO-d_6 at 20 °C (Figure 1) shows two sets of lines, many of them considerably broadened, suggesting a medium rate of interconversion between two isomers. Here we present NMR evidence that the observed isomerization is an amino–imino tautomerization. It is well established that guanine and its derivatives exist in the N^2 amino tautomeric form.[8] It has also been assumed that N^2-phenylguanines exist in the amino form, and models of their DNA polymerase inhibitory activity[9] are based on this assumption. Since the interaction of the phenyl ring with the N^2 amino group and especially its conjugation with nonbonding electrons on N can change the stability of the amino versus the imino forms, we sought evidence for the tautomeric structure of BuPdG.

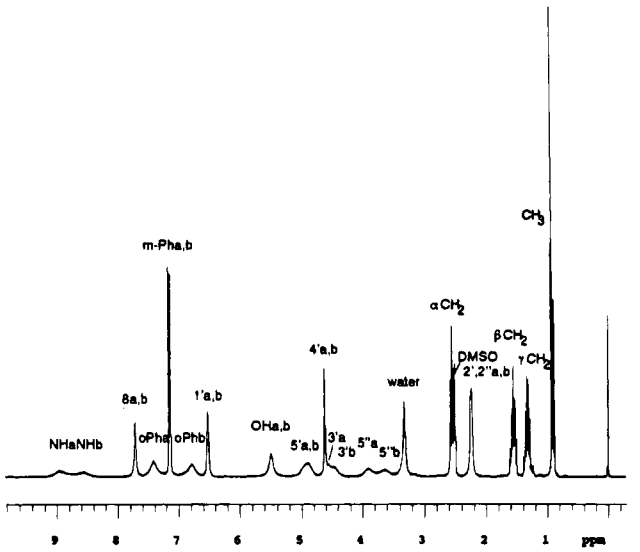

Figure 1. Proton NMR spectra of cBuPdG in DMSO-d_6 at 20 °C. Resonances assigned to forms **A** and **B** (Scheme 1) are labeled a and b, respectively.

In this paper we also present the selective INEPT experiment as an efficient and convenient alternative to the classical approach in studying the tautomeric structure of nucleosides, which requires syntheses of N-methylated or ^{15}N enriched derivatives.

Results

Temperature and Solvent Dependence of the Equilibrium. The temperature and the nature of the

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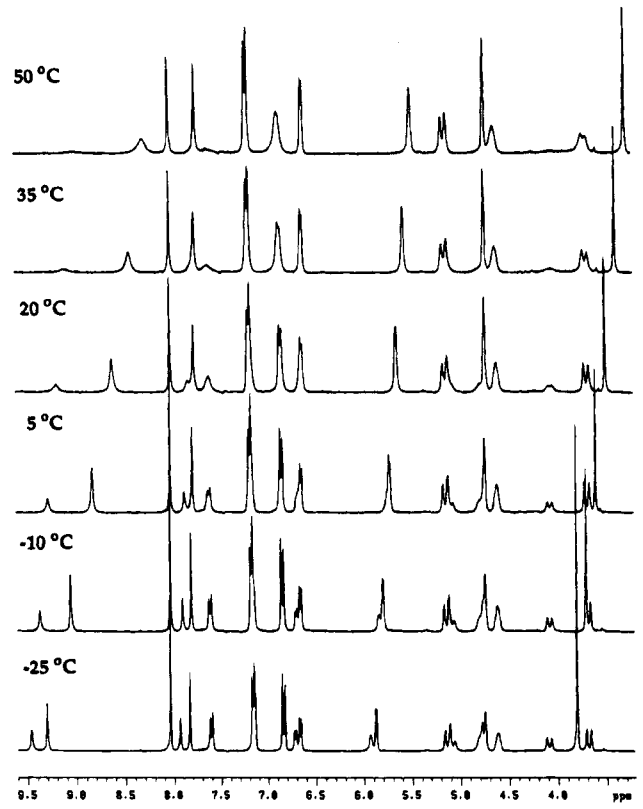
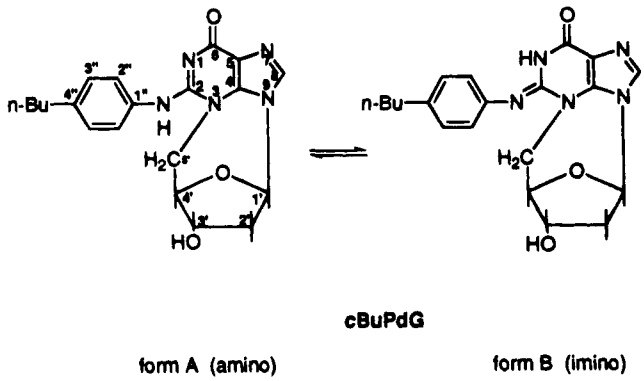


Figure 2. Proton NMR spectra of cBuPdG in DMF-d_7 at different temperatures.

Scheme 1



solvent influenced the ratio and the rate of interconversion between the two forms of cBuPdG, **A** and **B** (See Scheme 1). For instance, in DMSO-d_6 at 20 °C they are almost equally populated (Figure 1), whereas at 115 °C the **B** form is present almost exclusively. Similar behavior was observed in DMF-d_7 where the rate of exchange is slower and the lines are sharper. The addition of $CDCl_3$ to a solution of cBuPdG in DMSO-d_6 slows down the exchange and shifts the equilibrium toward the **B** form. In pure $CDCl_3$ only form **B** is present.

Figure 2 illustrates the temperature dependence of the ^1H NMR spectrum of the compound in DMF-d_7. In Figure 3 is shown the Van't Hoff plot of the temperature dependence of the equilibrium constant, $K = [\mathbf{A}]/[\mathbf{B}]$, in DMF-$d_7$, where **B** is the predominant isomer at room temperature. The equilibrium constants were calculated by integration of the signals at 7.61 and 6.85 ppm (Figure 2), which are well separated and represent the phenyl protons *ortho* to the amino group (see the following text). A least squares fit of the data resulted in $\Delta H° = -10.9$ ± 1.8 kJ mol^{-1} and $\Delta S° = -47 \pm 7$ J mol^{-1} deg^{-1} (for the **B** → **A** transition).

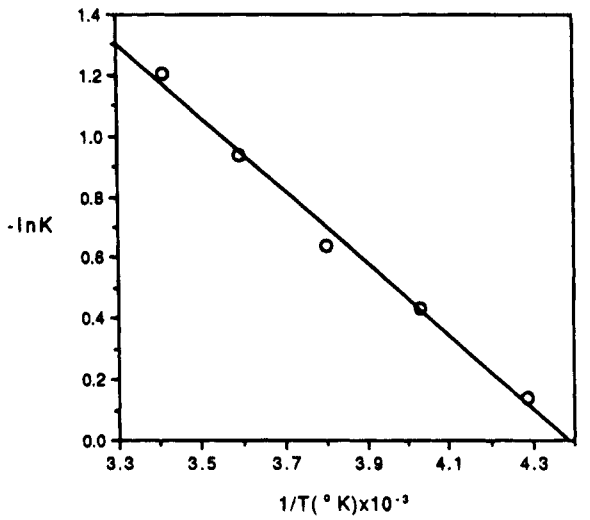


Figure 3. Van't-Hoff plot of temperature dependence of the equilibrium constant **A**/**B** in DMF-d_7 for forms **A** and **B** of cBuPdG.

Table 1. ^1H Chemical Shifts (δ) of cBuPdG in DMF-d_7 at −25 °C and in $CDCl_3$ at Room Temperature, and of AccBuPdG in $CDCl_3$ at Room Temperature

proton number	cBuPdG in DMF-d_7 at −25 °C form **A**	cBuPdG in DMF-d_7 at −25 °C form **B**	cBuPdG in $CDCl_3$	AccBuPdG in $CDCl_3$
1′	6.72	6.67	6.24	6.28
2′	2.46	2.46	2.50	2.52
2″	2.33	2.33	2.39	2.48
3′	4.81	4.62	4.77	5.34
4′	4.78	4.75	4.77	4.84
5′	5.08	5.14	5.25	5.31
5″	4.10	3.69	3.73	3.71
8	7.95	7.84	7.35	7.39
o-Ph	7.61	6.84	6.76	6.77
m-Ph	7.13	7.16	7.17	7.16
NH	9.47	9.32	7.62	7.62

Assignments. The assignment of ^1H resonances of the two forms of cBuPdG (Table 1) was made by means of the short mixing time (10 ms) TOCSY spectrum in DMF-d_7 at −25 °C. The spectrum showed two sets of sugar and aromatic spin systems, both of which have a similar connectivity pattern (topology) and unusually high unequivalency of the 5′,5″ protons. The latter effect is characteristic for a methylene group involved in a restrained, cyclic structure and is due to the different positions of the H atoms with respect to the anisotropic magnetic field of the purine ring, one in the plane experiencing a deshielding effect and the other above the plane experiencing a shielding effect. The main difference between the two forms is in the chemical shifts of NH protons, phenyl protons *ortho* to the nitrogen, and the 5′,5″ protons from the sugar ring.

These data could be explained by two possibilities: either hindered rotation around the C2−N^2 bond, in which case the two forms would represent *syn* and *anti* isomers, or amino−imino tautomerization, in which case the two forms would be N^2-amino- and N^2-imino tautomers as depicted in Scheme 1. The observed temperature dependence of the line shapes ruled out the possibility of having hindered rotation around the C2−N^2 bond as a basis for the isomerization. Even at 100 °C the two sets of lines did not coalesce completely, suggesting an

Table 2. ^{13}C Chemical Shifts (δ) of AccBuPdG in $CDCl_3$ at Room Temperature, cBuPdG in DMF-d_7 at −25 °C, and BuPdG in DMF-d_7 at Room Temperature

carbon number	AccBuPdG in $CDCl_3$	cBuPdG in DMF-d_7 at −25 °C		BuPdG in DMF-d_7
		form **A**	form **B**	
2	142.66	151.66	144.12	150.48
4	141.80	140.68	142.85	150.62
5	117.32	123.56	117.43	119.20
6	156.14	164.61	157.14	157.58
8	132.59	134.27	134.34	137.00
1′	87.52	88.73	89.04	84.18
2′	42.15	45.34	45.06	41.04
3′	73.78	71.25	71.25	71.97
4′	83.25	86.76	86.97	88.97
5′	51.84	55.86	53.01	62.89
1″	143.31	138.21	145.90	137.38
2″	121.50	122.26	122.48	120.33
3″	130.16	128.83	130.08	129.28
4″	148.66	137.96	137.30	137.81
α^a	35.01	*b*	*b*	35.18
β^a	33.66	*b*	*b*	34.28
γ^a	22.92	22.78	22.78	22.66
δ^a	13.94	14.20	14.20	14.07
CH_3CO	170.64			
CH_3CO	20.77			

[a] *n*-Butyl group. [b] Overlapped with solvent signal.

activation enthalpy for this process quite higher than the typical value of 11–18 kcal/mol for rotation around C6–N^6 and C4–N^4 of mono- and dimethyl derivatives of adenosine and cytosine, respectively.[10,11] Moreover, in the case of N^2 mono- and dimethyl guanosines there was no observed hindered rotation at all.[11] In addition, the rate of interconversion and the ratio of the two isomers of cBuPdG strongly depend on the polarity of the solvent (see above). Finally, the noncyclic parent nucleoside BuPdG showed no sign of isomerization even at −40 °C in DMF-d_7 (data not shown).

Although form **B** is the only form present in $CDCl_3$ further study of its structure by ^{13}C or two-dimensional experiments was not possible due to the marginally low solubility of cBuPdG in chloroform, sufficient only for 1D proton spectra. To overcome this problem, cBuPdG was acetylated with Ac_2O and DMAP to N^2-(4-*n*-butylphenyl)-3′-acetyl-2′-deoxy-3,5′-cycloguanosine (AccBuPdG). This compound has sufficient solubility in chloroform and the same behavior as cBuPdG, existing in two forms in DMSO-d_6 solution and only one form, analogous to form **B** of cBuPdG, in $CDCl_3$. The proton resonances of both forms in DMSO-d_6 were assigned by the COSY spectrum. Except for the H-3′ all the other resonances of both forms have chemical shifts similar to their counterparts in cBuPdG. In Table 1 are given the proton chemical shifts of AccBuPdG in $CDCl_3$ (the form analogous to form **B** of cBuPdG), and $^1H-^1H$ coupling constants are listed in the Experimental Section.

In Table 2 are the ^{13}C chemical shifts of AccBuPdG in $CDCl_3$. The ^{13}C assignments were made by the HMQC[12] experiment which provides one bond $^1H-^{13}C$ correlations, and the HMBC[13] experiment which provides multiple bond $^1H-^{13}C$ connectivities. Since in the HMBC spectrum of AccBuPdG in $CDCl_3$ (analogous to form **B** of cBuPdG) the NH proton at 7.62 ppm showed no cross peaks, the question of its position remained open. This result is not unexpected, taking into account that even after careful purification of the sample and drying of the solution the NH resonance is relatively broad (4 Hz line width), and its coupling constants to the adjacent carbons, especially the two-bond couplings, are expected to be quite small. Both factors contribute to the considerable loss of magnetization during the delay required to create antiphase magnetization along $^1H-^{13}C$ long range multiplets and lead to inefficient magnetization transfer.

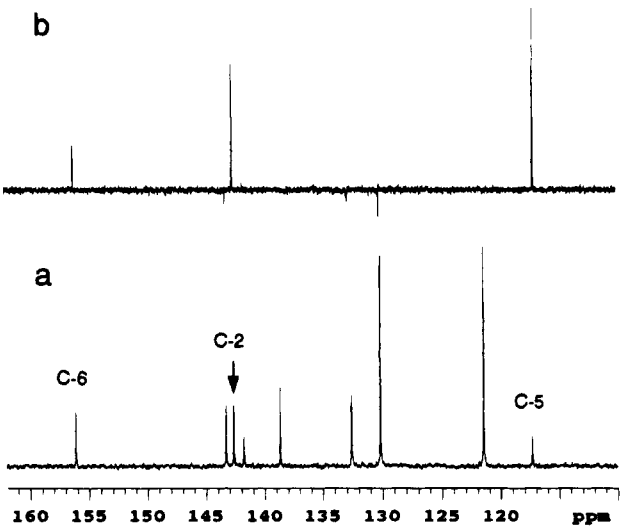


Figure 4. Selective INEPT experiment applied to AccBuPdG in $CDCl_3$ at 20 °C. (a) The relevant region of the normal ^{13}C spectrum. (b) Selective INEPT experiment after selective irradiation of the NH proton resonance at 7.62 ppm.

We were able to elucidate the position of the NH proton of AccBuPdG by detecting its $^1H-^{13}C$ long range coupling in a one-dimensional selective INEPT experiment.[14] This is an INEPT experiment where all proton pulses are replaced by selective pulses on the proton of interest (32 Hz rf field in our experiments; see Experimental Section). Since the signals due to the natural population difference of ^{13}C levels are canceled by phase cycling, the detected signals are due only to a population transfer by coupling to the proton of interest. The defocusing and refocusing delays are optimized for detection of small, long range couplings. In Figure 4 is the result of the selective INEPT experiment applied to AccBuPdG in $CDCl_3$ when the NH proton resonance at 7.62 ppm is selectively irradiated. The signals detected at the chemical shifts of C-6, C-2, and C-5 of the purine ring clearly show that this proton is attached to N-1, indicating the imino structure for AccBuPdG in $CDCl_3$ (form **B**, see Scheme 1).

The selective INEPT experiment was also successfully used in proving the tautomeric structure of BuPdG. The ^1H spectrum of BuPdG in DMF-d_7 has two NH resonances at 10.76 and 9.07 ppm, tentatively assigned as H-1 and 2-NH, respectively.[3] In Figure 5 is the result of the selective INEPT experiment after irradiation of the resonance at 9.07 ppm. The observed connectivites between this proton and C-1″ and C-2″ in the phenyl ring and C-2 in the purine ring prove an amino tautomeric structure and the 2-NH assignment for BuPdG. The ^{13}C

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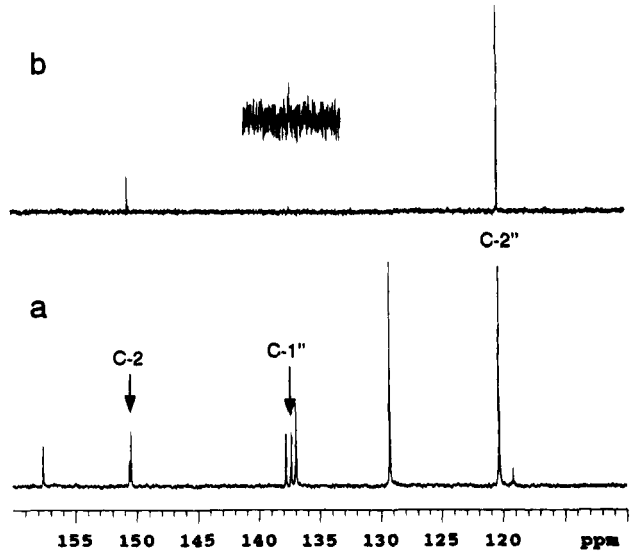


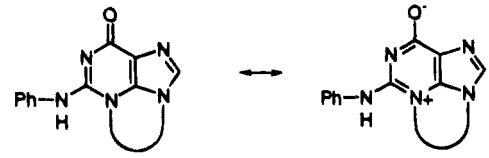
Figure 5. Selective INEPT experiment applied to BuPdG in DMF-d_7 at 20 °C. (a) The relevant region of the normal ^{13}C spectrum. (b) Selective INEPT experiment after irradiation of the NH proton resonance at 9.07 ppm. Insert, vertical scale ×5.

assignments (Table 2) necessary for the interpretation of the selective INEPT experiment of BuPdG have been made by comparison with the literature values for 2′-deoxyguanosine[15] and by analysis of the long range coupling pattern in a $^1H-^{13}C$ coupled spectrum (data not shown). Attempts to observe population transfer by selective irradiation of the other NH resonance at 10.76 ppm failed, possibly because of the very short T_2 of this nucleus (line width 17 Hz).

Assignments of the ^{13}C spectrum of cBuPdG in DMF-d_7, where the two tautomers exist in equilibrium, are presented in Table 2. The two sets of signals were distinguished by recording two spectra: one at −25 °C, where the two forms exist in the ratio 1:1.35 and the other at room temperature where the ratio is 1:3. The assignments were made using the previous assignments of AccBuPdG in $CDCl_3$ (imino form, form **B**) and the BuPdG assignments (as representative for the amino tautomer, form **A**). The biggest differences in the ^{13}C spectra of the two forms of cBuPdG are in the chemical shifts of C-1″ of the phenyl ring and C-2, C-6, and C-5 of the pyrimidine part of the purine ring, the latter being shifted downfield in the amino form **A** by 7.54, 7.47, and 6.13 ppm, respectively. This result is expected, considering that in the imino form both N-1 and N-3 are taking part in the aromatic system with their nonbonding n-orbitals, contributing to the increase of electron density, whereas in the amino form N-1 has the opposite effect, being involved in the aromatic system with its low energy p-orbital, decreasing the electron density. This effect can not be fully compensated by NH-2, which is exocyclic. C-1″ moves as expected in the opposite direction, being shifted 7.69 ppm downfield in the imino form (**B**).

The assignments are also supported by the big difference of the temperature coefficients of the chemical shifts of NH protons of the two forms of cBuPdG in DMF-d_7: −7.3 ppb/deg for that in the amino (**A**) form versus −16.2 ppb/deg for the imino (**B**) form. In the latter case the NH proton is an "amide" type and is more likely to be involved in H-bonding with solvent.

Scheme 2



Discussion

In this work we have shown that cBuPdG undergoes a N^2 amino–imino tautomerization in polar solvents with a slow rate of interconversion and that in less polar solvents such as chloroform only the imino form is present. We have also shown that the parent noncyclic compound BuPdG exists solely as the N^2 amino tautomer in any solvent at room temperature. In fact, at temperatures as low as −40 °C no evidence of tautomerization of BuPdG was observed in DMF-d_7. We have found no literature report of amino–imino tautomerization among 3,5′-cyclopurine nucleosides. Apparently, both the alkylation of N-3 and phenyl substitution at N^2 are necessary to stabilize the imino form and to slow the exchange to an extent making this isomerization readily observable by NMR. The fact that the amino form of cBuPdG only appears in more polar solvents, such as DMSO or DMF, could be explained by its stabilization by partial charge distribution according to the resonance structures in Scheme 2.

The selective INEPT experiment was introduced by Bax et al.[14,16] and has been used in structural determination of natural and pharmaceutical products,[18] including nucleosides.[19] Gmeiner and Lown[19b] used it to monitor magnetization transfer from NH protons to tertiary carbons of nucleoside bases. Yet in recent years the selective INEPT experiment has been superceded by its two-dimensional rival, HMBC. In our opinion, when only one or a few connectivities are of interest and when one deals with very small long range $^1H-^{13}C$ couplings or broad proton signals with short T_2 values, the one-dimensional selective experiment has clear advantages. In a two-dimensional experiment the increase in the length of the experiment does not necessarily improve the signal-to-noise ratio, because it is mainly the t_1 noise due to the instability and nonlinearity of the hardware which determines the noise level. Obviously such problems do not exist in one-dimensional selective population transfer experiments allowing very weak connectivities to be detected.

Although the selective INEPT experiment lacks the sensitivity of the recently proposed selective inverse detection experiments[20] it has some important advantages. First, in an inverse detection experiment a majority of the signal is due to protons which do not interact

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with ^{13}C and has to be canceled by phase cycling. Different measures like BIRD pulse train or homospoil pulses could partially relieve this problem, yet it cannot be completely avoided. Incomplete canceling causes unwanted signals which in the 2D version can be easily distinguished, since they are not modulated with the F_1 frequency; in a 1D inverse experiment they can be a source of confusion. Second, in an inverse detection selective experiment a ^{13}C spectrum has to be acquired at the start of each experiment, in order to measure the exact offset of the ^{13}C resonance of interest. Finally, many instruments are not capable of inverse detection or require modification of the hardware.

The standard approach in studying the tautomeric structures of pyrimidines, purines, and their nucleosides in solution uses either comparison of the spectra (UV, IR, NMR) of the compounds with their N- and O-methylated derivatives, or ^{15}N NMR spectroscopy of isotopically enriched compounds. These approaches require additional synthetic work. The approach presented above provides a simple and straightforward alternative, which can be readily extended to other heterocyclic structures.

Experimental Section

N^2-(4-*n*-Butylphenyl)-2′-deoxyguanosine (BuPdG) and N^2-(4-*n*-butylphenyl)-2′-deoxy-3,5′-cycloguanosine (cBuPdG) were synthesized as described in refs 17 and 4, respectively.

N^2-(4-*n*-Butylphenyl)-3′-acetyl-3,5′-cyclo-2′-deoxyguanosine (AccBuPdG). cBuPdG (30 mg; 0.078 mmol) and DMAP (30 mg, 0.24 mmol) were dissolved in 0.6 mL of dry pyridine. Acetic anhydride (50 μL, 0.53 mmol) was added dropwise through a septum. After stirring for 1 h the reaction mixture was diluted with 50 mL of $CHCl_3$ and washed sequentially with water, 2% citric acid, 5% $NaHCO_3$, and water. Column chromatography on silica gel with chloroform:methanol (25:1) gave 27.1 mg (81%) of glassy residue: mp 128–131 °C. Anal. Calcd for $C_{22}H_{25}N_5O_4 \cdot 0.33H_2O$: C, 61.53; H, 6.02; N, 16.31. Found: C, 61.46; H, 5.85; N, 16.10. ^1H and ^{13}C NMR chemical shifts are listed in Tables 1 and 2. $^1H-^1H$ coupling constants (Hz): $J_{1'2'} = 6.28$, $J_{1'2''} = 0.83$, $J_{2'2''} = -14.56$, $J_{2'3'} = 5.07$, $J_{2''3'} = 7.84$, $J_{3'4'} = 1.77$, $J_{4'5'} = 2.78$, $J_{4'5''} = 5.28$, $J_{5'5''} = -14.59$.

NMR Spectroscopy. The solutions used for ^{13}C, two-dimensional, and heteronuclear experiments contained 15 mg of cBuPdG or 30 mg of BuPdG dissolved in 0.35 mL of DMF-d_7, or 25 mg of AccBuPdG dissolved in 0.35 mL of $CDCl_3$.

HMQC and HMBC spectra were acquired at 500 MHz with a reverse detection 5 mm probe. All other spectra were acquired at 300 MHz with a 5 mm broadband probe. One dimensional ^1H and COSY experiments were acquired using the decoupler coil. The TOCSY spectrum was acquired with a 5 mm ^1H probe. The chemical shifts are reported in ppm relative to TMS. For ^{13}C spectra the center of the solvent resonance was used as internal reference: 77.0 ppm for $CDCl_3$ and 30.10 ppm for the higher field methyl resonance of DMF-d_7.

^1H Spectral Simulation and Assignments. Estimated coupling constants and chemical shifts were measured from the relevant multiplets and were used as starting point for spectral simulation with the spectral simulation software from the instrument software package (an upgraded LAOCOON 3 program).

The **TOCSY spectrum** was acquired with 10 ms mixing time and the data were processing with zero filling and apodization with a squared $\pi/2$ shifted sine bell in both dimensions.

The **^1H, ^{13}C HMQC spectrum and ^1H, ^{13}C HMBC spectra** were obtained using standard pulse sequences[12,13] and the following: 2k × 2k size; 256 increments, 32 acquisitions per increment for HMQC, and 384 increments, 256 acquisitions per increment for HMBC. Both spectra were processed with zero filling and apodization with a squared $\pi/2$ shifted sine bell in both dimensions.

Selective INEPT Experiment. The standard refocused INEPT pulse sequence from the instrument software package was modified by placing the first 90° ^{13}C pulse at the end instead of at the middle of the 90° ^1H pulse. Defocusing delay, $D_1 = {}^1/_4J_{lr} - 3 \times 90°(^1H)$, and refocusing delay, $D_2 = {}^1/_4J_{lr} - 2 \times 90°(^1H)$, where J_{lr} is the expected long range coupling constant, were set in two separate experiments to 71.6 and 75.5 ms, respectively (optimal for 3 Hz $^1H-^{13}C$ coupling), or 29.9 ms and 33.9 ms, respectively (optimal for 6 Hz $^1H-^{13}C$ coupling). The width of the selective 90° ^1H pulse, 7800 μs, was calibrated by optimizing population transfer from the aromatic proton to the methyl carbon of mesitylene (50% solution in $CDCl_3$). The spectra were acquired without spinning the sample.

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