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Intramolecular Cation−π Interactions As the Driving Force To Restrict the Conformation of Certain Nucleosides

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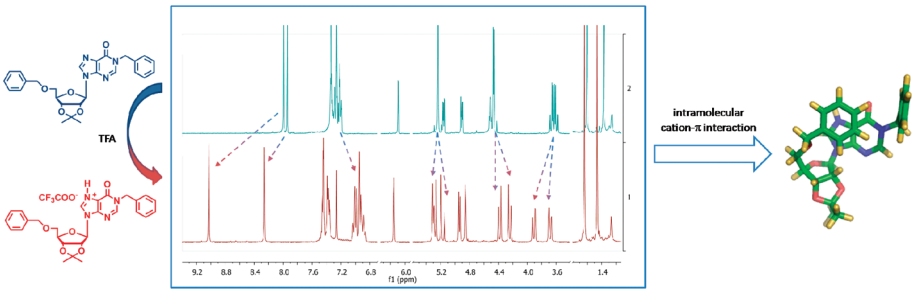

Despite the well-established importance of intermolecular cation−π interactions in molecular recognition, intramolecular cation−π interactions have been less studied. Here we describe how the simultaneous presence of an aromatic ring at the 5′-position of an inosine derivative and a positively charged imidazolium ring in the purine base drive the conformation of the nucleoside toward a very major conformer in solution that is stabilized by an intramolecular cation−π interaction. Therefore, the cation−π interaction between imidazolium ions and aromatic rings can also be proposed in the design of small molecules where this type of interaction is desirable. The imidazolium ion can be obtained by a simple acidification of the pH of the media. So a simple change in pH can shift the conformational equilibrium from a random to a restricted conformation stabilized by an intramolecular cation−π interaction. Thus the here described nucleosides can be considered as a new class of pH-dependent conformationally switchable molecules.

Introduction

Cation−π interactions in proteins take place between positively charged groups and aromatic residues[1−3] and they have been recognized as an important component of noncovalent binding forces in biological systems.[4,5] The most relevant interactions occur between the side chain of aromatic amino acids (Phe, Trp, Tyr) and metal or organic ions, including, in the latter group, the imidazolium ring of a protonated histidine. A particular example of organic ion in the nucleoside field is the 5′-cap structure in mRNA (m7GpppN) that consists of a 7-methylguanosine (m7G) that is linked via a 5′−5′ triphosphate bridge (ppp) to the 5′-end of the transcribed RNA where N represents the initiating nucleotide of the encoded sequence (Figure 1).[6] The presence of the 5′-cap structure in mRNAs is specifically recognized by a number of enzymes involved in mRNA translation (including the eukaryotic initiation factor eIF4E,[7,8] the CBC nuclear cap-binding complex,[9] the vaccinia virus

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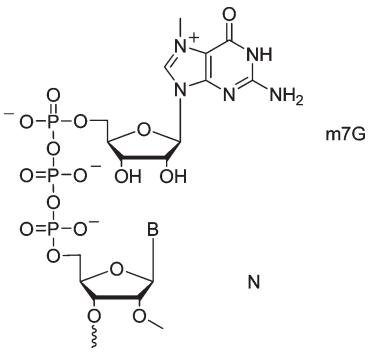

FIGURE 1. 5′-Cap structure of mRNA.

methyltransferase VP39[10,11]) or in RNA transcription (as in polymerase basic protein (PB2) of the influenza virus[12]). The structural insights provided by both X-ray crystallography and NMR spectroscopy within some of the 5′-capped complexes and their target enzymes reveal that the positively charged 7-methylguanine establishes a sandwich-type cation$-\pi$ interaction with the side chain of aromatic residues such as Trp, Tyr, or Phe.[13−15] Complexation of 7mGTP with the eIF4E factor, with a dissociation constant in the nM range, represents an excellent example of the importance of cation$-\pi$ interactions in molecular recognition.[8]

Cation$-\pi$ interactions are also finding an increasingly important role in guest−host recognition and supramolecular chemistry.[16−19] Quite recently, intramolecular cation$-\pi$ interactions, and in particular pyridinium$-\pi$ interactions, are being successfully applied in organic synthesis[20] or to study isomer populations.[21] Most reported cases of intramolecular pyridinium$-\pi$ interactions include some restrictions in the conformation of the substrate to favor the occurrence of stacking interactions, although there are some very recent examples that overcome this limitation.[22]

Here we report on a series of inosine derivatives whose conformational freedom is constrained in solution, as shown by ^{1}H NMR, and for which we propose that the driving force for this behavior is an intramolecular interaction between the imidazolium of the purine base and the phenyl ring of the benzyl substituent at the 5′-position of the sugar. This study started within our research program on purine nucleosides.[23] While performing the synthesis of 1-benzyl-5′-*O*-benzyl-2′,3′-*O*-isopropylideneinosine[23] (**1**, Figure 2a), the desired compound was obtained together with a novel derivative identified as 1,7-dibenzyl-5′-*O*-benzyl-2′,3′-*O*-isopropylideneinosine bromide[24] (**2**, Figure 2b).

The ^{1}H NMR spectra of both compounds in $CDCl_3$ showed significant differences particularly affecting in compound **2** the splitting of signals corresponding to geminal protons (the CH_2 of the benzyl groups at the 5′-position of the sugar and at the N1 of the purine; the protons at the 5′ position of the sugar H5′ and H5″) and the "upfield" shift of the aromatic protons of the benzyl group at the 5′-position (Figure 2b). Therefore we decided to investigate how the introduction of other substituents at position N7 in the inosine or other benzyl groups at the 5′-position of the nucleoside affected the appearance of these "fingerprint" signals in the ^{1}H NMR spectra. This was followed by a detailed conformational study in solution by means of ^{1}H NMR techniques and complemented with computational studies. With the information collected, we hypothesized that the conformational restrictions in this series of nucleosides required the simultaneous presence of a positive charge at the imidazole of the purine ring and an aromatic substituent at the 5′-position of the sugar. Therefore, the conformational freedom of the dibenzyl derivative **1** should be driven to a more restricted conformation by a simple change in the pH of the medium through protonation at N7 of the purine, and this was shown to be the case, as reported in the final section of this paper.

Results and Discussion

Synthesis and NMR Experiments. The tribenzyl derivative **2** (Scheme 1) can be obtained in 56% yield in a single step by reaction of 2′,3′-*O*-isopropylideneinosine (**3**) with an excess of benzyl bromide in the presence of NaH at room temperature for 1 h followed by heating at 80 °C for an additional hour. The synthesis of the N7-methyl derivative **4** was achieved by treatment of 1-benzyl-5′-*O*-benzyl-2′,3′-*O*-isopropylideneinosine (**1**)[23] with methyl iodide in DMF at 60 °C for 24 h and in the absence of base, as recently reported for other purine bases.[25]

On the other hand, treatment of 2′,3′-*O*-isopropylideneinosine (**3**) with benzyl bromide and DBU[26] in acetonitrile at rt afforded 1-benzyl-2′,3′-*O*-isopropylideneinosine (**5**) in 91% yield (Scheme 2). Reaction of **5** with *p*-methylbenzyl bromide in the presence of NaH in DMF at −20 °C for 30 min afforded the 5′-*O*-(4-methylbenzyl) derivative **6** in 63% yield. Further treatment of **6** with methyl iodide in DMF at 60 °C for 24 h yielded the N7-methyl nucleoside **7**.

The structure of the N7-substituted inosines **2**, **4**, and **7** was determined by ^{1}H NMR, ^{13}C NMR, gCOSY, gHMBC, and gHSQC experiments. The ^{1}H NMR spectra of compounds **4** and **7** showed the same pattern as for **2**, with similar splitting for the signals corresponding to the methylene groups and the upfield chemical shift for the aromatic protons of the

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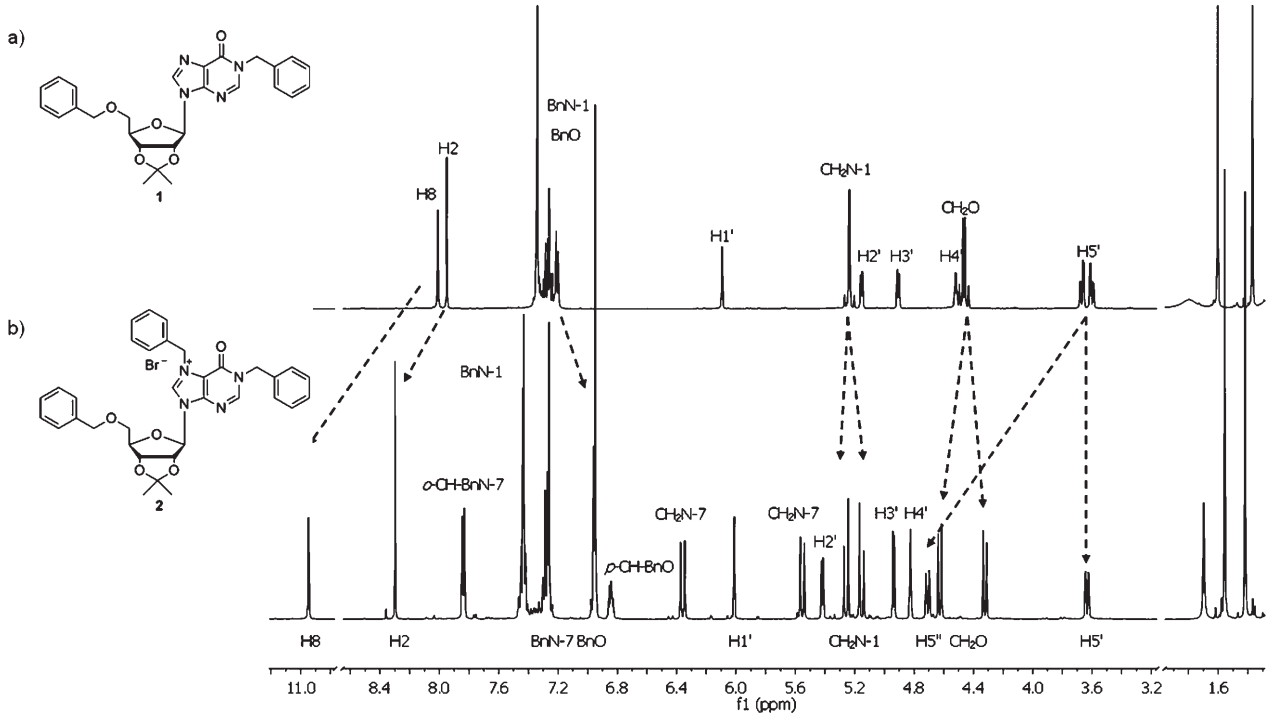
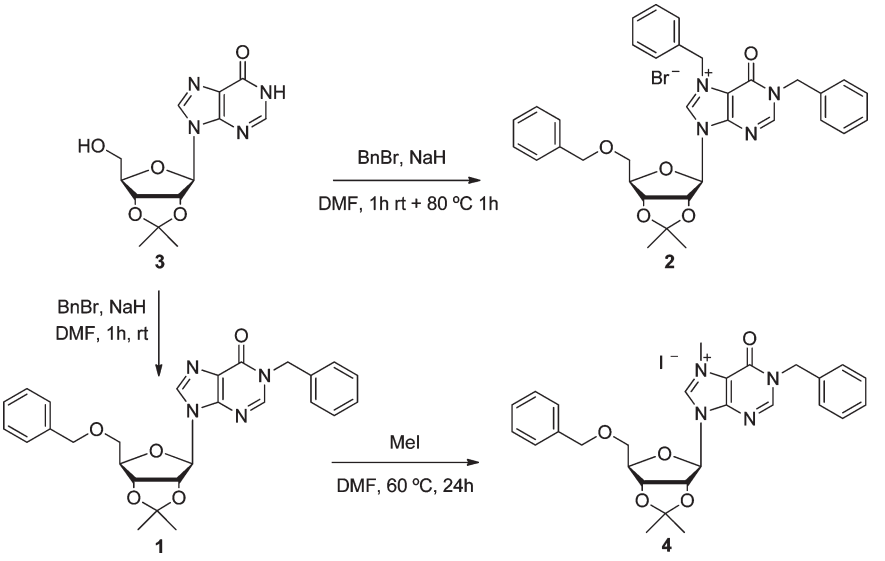


FIGURE 2. ^{1}H NMR spectra of compounds **1** and **2** in $CDCl_3$.

SCHEME 1. Synthesis of 1-Benzyl-5′-*O*-benzyl-7-methyl-2′,3′-*O*-isopropylideneinosine Iodide (4)



benzyl group at the 5′-position (Figure 3 and Figure S1 in the Supporting Information). Thus, as a representative example, the ^{1}H NMR spectrum of **4** (Figure 3) showed two different signals for H-5′ and H-5″ protons (two doublets at δ 3.70 and δ 4.69 ppm, respectively). Also, the signals corresponding to the methylene unit of the benzyl group at the 5′ position appeared as two doublets (δ 4.47 and 4.72 ppm), and the same pattern was observed for the benzylic protons at N1 (doublets at 5.12 and 5.24 ppm). Moreover, the aromatic protons of the benzyl at 5′ were upfield shifted and appeared as three distinct sets of signals at δ 6.85, 6.96, and 7.02 ppm for the para, ortho, and meta protons, respectively. Finally, the H-8 from the inosine moiety was markedly downfield shifted.

The ^{1}H NMR spectrum of compound **4** was recorded at two different concentrations (10 mg/0.55 mL or 0.5 mg/0.55 mL) but no differences were observed. In addition, changing the solvent from $CDCl_3$ to the more polar DMSO-d_6 did not significantly modify the chemical shifts. Thus, these results pointed toward an intramolecular stabilization. The ^{1}H NMR spectrum of **4** in $CDCl_3$ was also registered at variable temperature (from 45 to −50 °C) (Figure S2 in the Supporting Information). By increasing the temperature, there was no evidence of coalescence, while by lowering the temperature, there was no splitting of signals, exclusively the upfield shift of some signals was observed (i.e, H8 and the aromatic protons at the 5′-position, in particular the

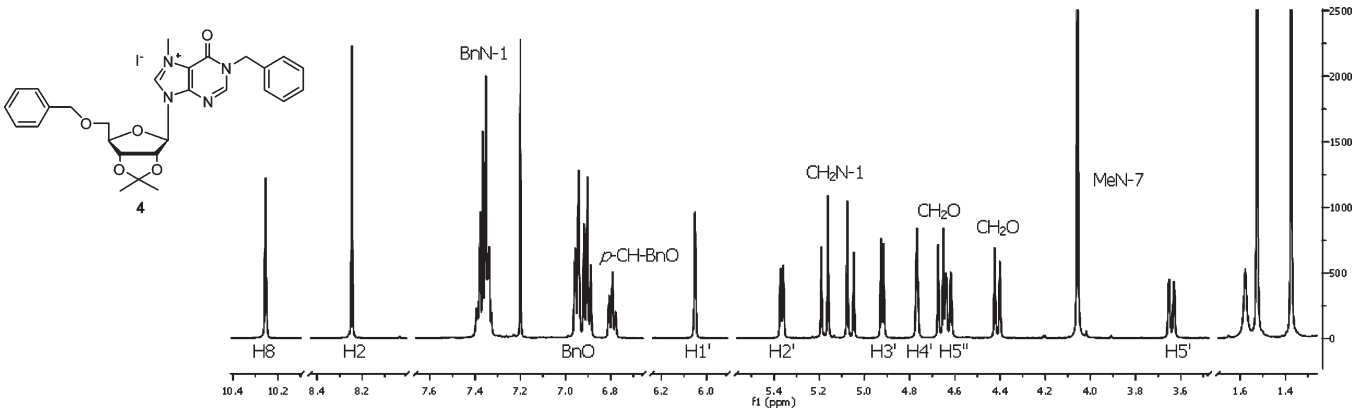
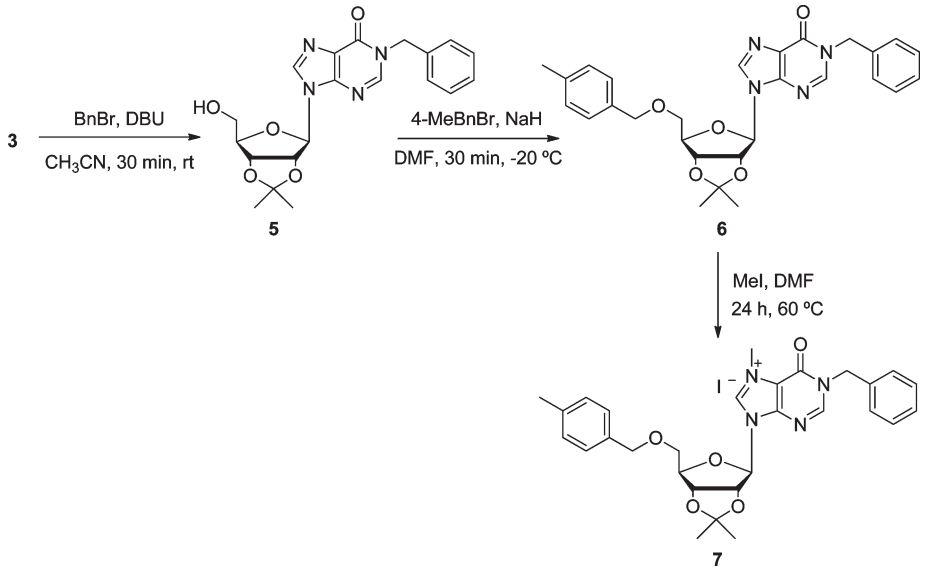


FIGURE 3. ^{1}H NMR spectrum of **4** in $CDCl_3$.

SCHEME 2. Synthesis of 1-Benzyl-7-methyl-5′-*O*-(4-methylbenzyl)-2′,3′-*O*-isopropylideneinosine Iodide (7)



proton at the para-position). This suggests the existence of a vastly populated conformer in a $CDCl_3$ solution.

2D-NOESY and 2D-ROESY experiments provide valuable information about the proximity of distal substituents. The most significant correlation signals corresponding to compound **4** are shown in Figure 4. Special attention should be paid to the strong cross-peak between H8 and H5″ (Figure 4a) indicating a close proximity between both. The correlations observed between the sugar protons H5′ and H5″ and the benzylic protons also at this position are very illustrative: H5″ shows a strong NOE only with one of the protons at the benzylic position, while H5′ shows NOE exclusively with the geminal proton also at the benzylic position. In addition, H5′ showed correlations with H3′ and with H4′ of the furanose ring, while H5″ showed a cross-peak only with H4′, suggesting there was no free rotation around the C4′−C5′ bond. Thus, these correlations point toward the lack of conformational freedom at this part of the molecule (Figure 4b). Some other peaks of medium or low intensity between the aromatic protons at the 5′-position and the methyl at N7 or the H2′ of the sugar (Figure 4c) may suggest that the aromatic ring at the 5′-position is frequently located over the sugar plane and quite close to the imidazole of the purine ring.

The 2D-NOESY and 2D-ROESY experiments carried out with compound **7**, with a 4-methylbenzyl group at the 5′-position, perfectly matched with the correlations described above for compound **4** (Figure S3 in the Supporting Information). In this case, 1D-NOESY experiments were performed by selective irradiation of the signal at 4.06 ppm, assigned to the methyl at N7 of the purine, and the signal at 2.20 ppm, which corresponds to the methyl at the position para of the benzyl group at 5′ (Figure S4 in the Supporting Information). From the irradiation of the signal at 4.06 ppm, NOE signals were observed for the ortho and meta protons of the benzyl group at 5′, pointing again to a close proximity between the imidazole of the base and the substituent at 5′. The irradiation of the signal at 2.20 ppm showed NOEs with the ortho and meta protons of the same substituent as expected, but also NOEs with the benzylic protons at N1, that should only be possible if the aromatic substituent at 5′, and in particular its para position, is located over the sugar and pointing toward the N1 of the base.

NMR Conformational Analysis. The conformation of nucleosides can be defined by three structural parameters: the puckering of the furanose ring, the conformation of the exocyclic bond C4′−C5′ (defined by the torsion angle γ), and

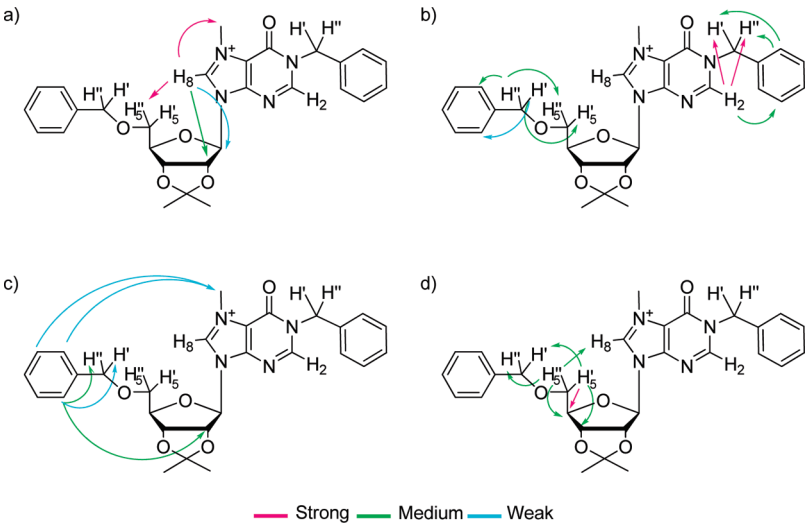


FIGURE 4. Correlations peaks from the 2D-NOESY and 2D-ROESY experiments for compound **4**.

TABLE 1. Comparison of the Pseudorotational Parameters for Compound 4 Based on Experimental and Calculated Values

	NMR data[a]	AMBER data[b]	^3J exptl (Hz)
$\theta_{H1'\text{-}C1'\text{-}C2'\text{-}H2'}$[c] (deg)	110.4	109.1	1.3
$\theta_{H2'\text{-}C2'\text{-}C3'\text{-}H3'}$[c] (deg)	−9.7	−13.0	5.4
$\theta_{H3'\text{-}C3'\text{-}C4'\text{-}H4'}$[c] (deg)	−96.0	−89.9	0
P furanose ring[d]	252 ($_O$T^4)	234 ($_4$E)	
τ_m[e] (deg)	33	30	

[a]Obtained from PSEUROT analysis. [b]Obtained after geometry optimization with HyperChem 7.5. [c]Dihedral angle. [d]Pseudorotational angle (P), the corresponding conformation in the pseudorotational circuit is represented in parentheses. [e]Maximum out-of-plane pucker.

the conformation of the glycosidic bond (defined by the torsion angle χ).[27,28] The puckering of the furanose ring in solution can be determined experimentally by ^{1}H NMR from vicinal proton−proton coupling constants using the concept of pseudorotation,[29−31] in which the conformation of the sugar ring is described by two parameters: the phase angle of pseudorotation (P) and the puckering amplitude (ν_m). These pseudorotational parameters have been calculated from the three experimental interprotonic coupling constants, $^3J_{H1',H2'}$, $^3J_{H2',H3'}$, $^3J_{H3',H4'}$, using the software PSEUROT-2.[32] Thus, the experimental data for compound **4** (Table 1) strongly support the presence of a major conformer in solution with calculated values for P and ν_m of 252° and 33°, respectively. These values comply with a twist-type conformation $_O$T^4.

The conformation around the exocyclic C4′−C5′ bond, in terms of three rotamers population, has been calculated from the experimental $^3J_{H4',H5'}$ and $^3J_{H4',H5''}$ coupling constants by using the appropriately parametrized Karplus equation.[33] Results obtained pointed to a population of 90% of the *gauche*+ rotamer, where the dihedral angles H4′−C4′−C5′−H5′ and H4′−C4′−C5′−H5″ adopt values of +60° and −60°, respectively. This is in agreement with the cross-peaks observed in Figure 4.

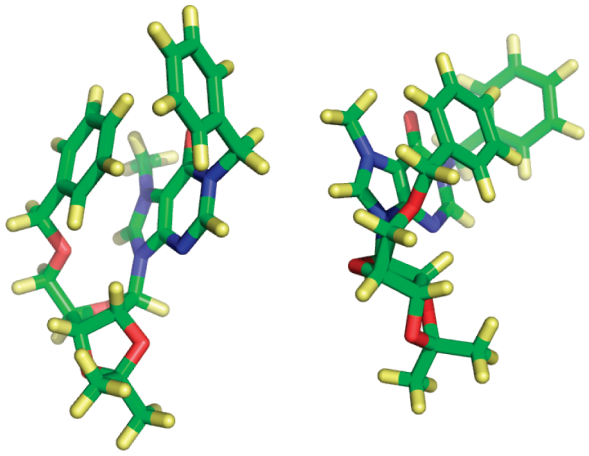


FIGURE 5. Two different views of the 3D structure obtained for compound **4** after geometry optimization.

Concerning the conformation of the glycosidic bond, this was determined from the cross-peaks observed for the signals corresponding to protons H2 and H8 of the purine in the NOESY/ROESY experiments. Thus, while H8 showed cross-peaks with some protons on the β-face of the furanose (H5″ and H2′), the H2 of the purine gave NOEs exclusively with the benzylic and aromatic protons of the substituent at N1, while no correlations were detected with any protons from the furanose. These data point to a major *anti* conformation for the glycosidic bond.

On the basis of these data, a preliminary 3D structure for **4** was constructed and subjected to geometry optimization by means of molecular mechanics by using the AMBER force field as implemented in HyperChem 7.5.[34] As a result, a conformer was obtained in which the benzyl group at the 5′-position faces the purine base (Figure 5). Comparison of

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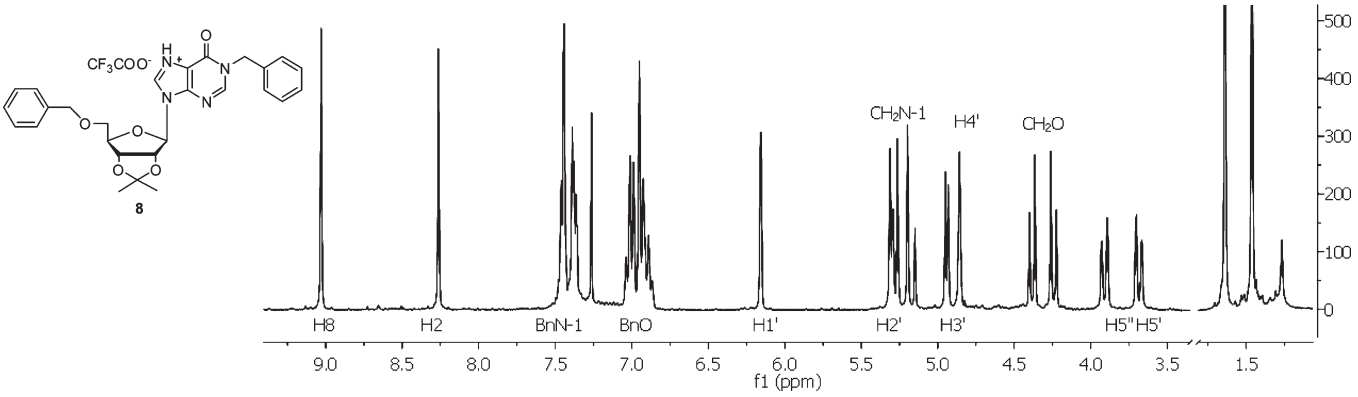

FIGURE 6. ^{1}H NMR spectrum of **8** in $CDCl_3$.

the angle values in this minimized conformer with those determined experimentally on the basis of the interprotonic coupling constants was in excellent agreement (Table 1). Moreover this conformer accounts for the correlations seen in the NOESY/ROESY experiments.

Can the Conformation of 5′-*O*-Benzylinosines Be Driven by a Change in pH?. From the experiments described above, it could be argued that the simultaneous presence of a benzyl group at the 5′-position of the nucleoside and a positive charge at the N7 of the imidazole ring lead to very significant conformational restrictions such that the benzyl group is located over the furanose ring and stacking with the positively charged base. Therefore an intramolecular cation$-\pi$ interaction could be the driving force for these conformational restrictions. If this is the case, a simple protonation of the N7 of the purine in the dibenzyl derivative **1** could also favor this type of interaction and result in a conformationally restricted nucleoside in solution. In this respect, it has been recently reported that purine nucleosides, when treated with TFA, are thermodynamically protonated at N7.[35] Thus, when a solution of the dibenzyl derivative **1** in $CDCl_3$ was acidified by addition of TFA (10 equiv), the ^{1}H NMR spectrum drastically changed. The new species (**8**) showed the "fingerprint" signals already observed for the other N7-substituted compounds, affecting in particular the signals corresponding to CH_2O5', NCH_2, H5′, and H5″ and aromatic signals (Figure 6). Although in our hands these differences in the ^{1}H NMR spectrum were already highly indicative of similar conformational restrictions to those observed for **2**, **4**, or **7**, compound **8** was subjected to the same battery of NMR experiments, conformational determinations, and geometry optimization already enumerated for the N7-methyl derivative (Figures S5 and S6, and Table S1 in the Supporting Information). The results obtained with compound **8** pointed again to an intramolecular stabilization between the aromatic ring at 5′ and the imidazolium ion of the purine base.

In addition, and based on a referee's suggestion, it was important to figure out if a new change in the pH toward neutral values would restore the conformation of the dibenzyl derivative to a random conformation. By adding a base as diisopropylethylamine to a solution of the protonated species **8** in $CDCl_3$, the ^{1}H NMR spectrum changed again and became almost identical with the spectrum of the unprotonated species **1** (Figure S7 in the Supporting Information). Thus the conformation of the dibenzyl derivative **1** switches from a random to a restricted conformation in the presence of TFA while the addition of base to the latest restores the random conformation.

Molecular Dynamics Simulations. To assess whether a molecular mechanics force field can account for these conformational preferences in solution, the behavior of the major conformers determined for compounds **4** and **8** on the basis of the NMR data was simulated by using molecular dynamics (MD). The dibenzyl derivative **1** was also included in these studies for comparative purposes. (See the Supporting Information for details.)

The NMR-determined structures for compounds **4** and **8** were used as the starting structures for the simulation. The torsional restraints compatible with the experimental values for $^3J_{H-H}$ were kept for 10 ns and this period was followed by 90 ns of unrestrained simulations. For the dibenzyl derivative **1**, and since no hints of conformational restrictions had been observed in the ^{1}H NMR spectrum, the simulation was performed unrestrained for the total time of 100 ns. All simulations were carried out in an explicit solvent, that is, in a $CHCl_3$ box at 300 K and 1 atm. The AMBER force field was employed as implemented in the Amber 8.0 release.[36−38] Different conformational parameters were monitored as a function of time for the three compounds by using the PTRAJ module in Amber 8.0,[38] including some dihedral angles and distances between relevant atoms belonging to distal substituents.

Regarding the conformation around the C4′−C5′ bond in compounds **4** and **8**, MD simulations monitoring the dihedral angle H4′−C4′−C5′−H5″ confirmed the *gauche*+ conformation, with an average value of −66° for both compounds, as shown in Figure 7 for compound **8**. These values are in excellent agreement with the experimental values determined from the NMR studies that indicated between 90% and 93% of the conformers with such a dihedral angle value.

To determine the relative spatial disposition of the substituent at the 5′ position and the purine base, the distance

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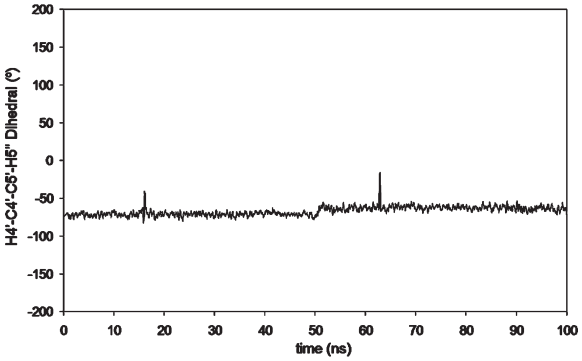

FIGURE 7. MD simulations. H4′−C4′−C5′−H5″ dihedral angle (deg) for compound **8**.

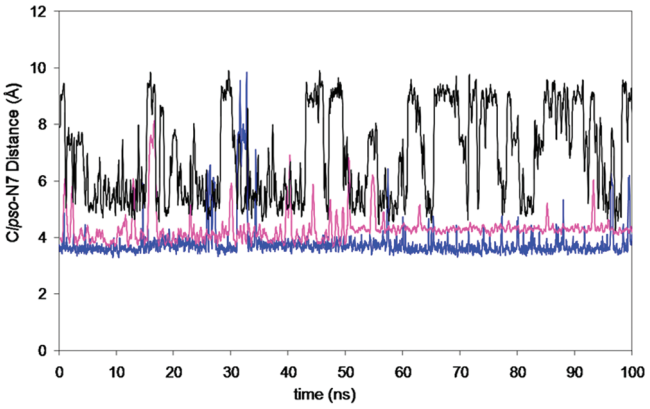

FIGURE 8. MD simulations. Distances (expressed in Å) between the *ipso* carbon of the aromatic ring at 5′ and the N7 of the purine base from the MD trajectories calculated for **1** (black), **4** (magenta,) and **8** (blue) in a $CHCl_3$ solution.

between the *ipso* carbon of the aromatic ring at 5′ and the N7 of the purine base was monitored. As shown in Figure 8, this distance for **1** (black line) showed great variations, with values oscillating between 4 and 10 Å, as expected for a conformationally unrestrained compound. However, this distance remained almost constant for **4** (displayed in magenta) and **8** (displayed in blue) along the whole simulation, with average values of 4.3 and 3.8 Å, respectively, in agreement with the lack of conformational freedom of both substituents and as an indication of the proximity between the inosine base and the aromatic ring at the 5′ position. Since the only structural difference between compounds **1** and **8** is the presence of a proton at N7, leading to the transformation of the purine imidazole into an imidazolium ion, it can be proposed that the conformational restrictions observed for the protonated compound **8** are due to an intramolecular cation−π interaction between the aromatic substituent at 5′ and the imidazolium ion in the purine base. It should be noticed that the distance of 3.8 Å monitored in the MD calculations closely resembles the reported intermolecular distances between the cationic 7-methylG of the 5′-cap and the side chains of Trp102B and Trp56B in the cap binding protein eIF4E[8] and other systems involving cation−π interactions.[4]

Conclusions

The conformational behavior of a series of N7-substituted inosines with an aromatic ring at the 5′-position of the ribose has been investigated by NMR techniques and molecular modeling, including molecular dynamics simulations. These compounds behave as conformationally restricted nucleosides and we propose that the driving force for this restriction is the stabilization through an intramolecular cation−π interaction between the aromatic ring at the 5′-position of the ribose and the positively charged imidazolium ring in the purine base. To the best of our knowledge there are no previous examples involving imidazolium ions in intramolecular cation−π interactions in small molecules. Thus, the cation−π interaction between imidazolium ions and aromatic rings can be proposed in the design of small molecules involved in guest−host interactions or organic synthesis.

The imidazolium ion required for the above-described interaction can be obtained by an acidification of the pH of the medium. Thus, by a simple change in pH the conformation of the nucleoside switches from a random to a restricted conformation, as shown for compound **8**. Therefore, these inosine derivatives can be considered as a new class of pH-dependent conformationally switchable molecules.

Experimental Section

1,7-Dibenzyl-5′-*O*-benzyl-2′,3′-*O*-isopropylideneinosine Bromide (2). To a solution of 2′,3′-*O*-isopropylideninosine (**3**, 200 mg, 0.63 mmol) in dry DMF (4 mL) was added NaH (60% in mineral oil, 81 mg, 2.10 mmol). The reaction was stirred at room temperature for 15 min. Then, benzyl bromide (0.24 mL, 2.03 mmol) was added and stirring was continued for 1 h at room temperature and 1 h at 80 °C. Volatiles were removed, and the residue was purified by flash column chromatography (CH_2Cl_2:MeOH, 100:1) to yield **2** (235 mg, 56%) as a white solid. Mp (CH_2Cl_2:MeOH) 82−84 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43, 1.63 (s, 6H, $C(CH_3)_2$), 3.64 (dd, J = 10.8, 2.2 Hz, 1H, H-5′), 4.33 (d, J = 11.2 Hz, 1H, CH_2O), 4.66 (d, J = 11.2 Hz, 1H, CH_2O), 4.75 (dd, J = 10.9, 1.8 Hz, 1H, H-5″), 4.85 (m, 1H, H-4′), 4.94 (d, J = 5.8 Hz, 1H, H-3′), 5.11 (d, J = 14.3 Hz, 1H, CH_2N-1), 5.25 (d, J = 14.3 Hz, 1H, CH_2N-1), 5.41 (dd, J = 5.7, 1.4 Hz, 1H, H-2′), 5.54 (d, J = 13.6 Hz, 1H, CH_2N-7), 6.01 (d, J = 1.4 Hz, 1H, H-1′), 6.39 (d, J = 13.6 Hz, 1H, CH_2N-7), 6.83 (m, 1H, *p*-CH-OBn), 6.96 (m, 4H, OBn), 7.25−7.33 (m, 3H, 7-NBn), 7.41−7.49 (m, 5H, 1-NBn), 7.84−7.87 (m, 2H, *o*-CH-7-NBn), 8.24 (s, 1H, H-2), 10.96 (s, 1H, H-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.2 (CH_3), 26.9 (CH_3), 50.3 (CH_2N-1), 51.9 (CH_2N-7), 70.2 (C-5′), 72.25 (CH_2O), 82.8 (C-3′), 85.9 (C-2′), 88.7 (C-4′), 96.6 (C-1′), 113.8 ($C(CH_3)_2$), 114.2 (C-5), 127.0, 127.5, 127.9, 134.0 (7-NBn), 129.0, 129.2, 129.3, 134.0 (1-NBn), 129.0, 129.2, 129.7, 137.5 (OBn), 139.6 (C-8), 144.3 (C-4), 150.6 (C-2), 151.7 (C-6); MS (ES, positive mode) m/z 579 $[M]^+$. Anal. Calcd for $C_{34}H_{35}BrN_4O_5 \cdot H_2O$: C, 60.27; H, 5.50; N, 8.27. Found: C, 59.89; H, 5.13; N, 8.58.

1-Benzyl-5′-*O*-benzyl-7-methyl-2′,3′-*O*-isopropylideneinosine Iodide (4). To a solution of 1-benzyl-5′-*O*-benzyl-2′,3′-*O*-isopropylideneinosine (**1**, 87 mg, 0.18 mmol) in dry DMF (1.5 mL) was added methyl iodide (22 μL, 0.35 mmol). The reaction was stirred at 60 °C overnight. Volatiles were removed, and the residue was purified by CCTLC in the Chromatotron (CH_2Cl_2:MeOH, 20:1) to yield **4** (76 mg, 69%) as a yellow solid. Mp (CH_2Cl_2:MeOH) 186−188 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43, 1.59 (s, 6H, $C(CH_3)_2$), 3.70 (dd, J = 10.8, 1.8 Hz, 1H, H-5′), 4.12 (s, 3H, CH_3-N7), 4.47 (d, J = 11.1 Hz, 1H, CH_2O), 4.69 (dd, J = 10.8, 2.0 Hz, 1H, H-5″), 4.72 (d, J = 11.1 Hz, 1H, CH_2O), 4.83 (m, 1H, H-4′), 4.98 (d, J = 5.8 Hz, 1H, H-3′), 5.12 (d, J = 14.4 Hz, 1H, CH_2N-1), 5.24 (d, J = 14.4 Hz, 1H, CH_2-N-1), 5.43 (dd, J = 5.8, 1.4 Hz, 1H, H-2′), 6.11 (d, J = 1.4 Hz,

1H, H-1′), 6.85 (m, 1 H, *p*-CH-OBn), 6.96 (m, 2H, *m*-CH-OBn), 7.02 (m, 2H, *o*-CH-OBn), 7.39−7.46 (m, 5H, NBn), 8.31 (s, 1H, H-2), 10.31 (s, 1H, H-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.2 (CH_3), 26.9 (CH_3), 36.8 (CH_3-N7), 50.3 (CH_2N-1), 70.5 (C-5′), 72.4 (CH_2O), 82.7 (C-3′), 86.1 (C-2′), 88.7 (C-4′), 96.4 (C-1′), 113.9 ($C(CH_3)_2$), 115.2 (C-5), 126.9, 127.6, 128.0, 137.5 (OBn), 128.9, 129.3, 129.3, 133.8 (NBn), 139.3 (C-8), 144.1 (C-4), 150.5 (C-2), 151.8 (C-6); MS (ES, positive mode) m/z 503 $[M]^+$. Anal. Calcd for $C_{28}H_{31}IN_4O_5$: C, 53.34; H, 4.96; N, 8.89. Found: C, 53.18; H, 5.02; N, 8.87.

1-Benzyl-2′,3′-*O*-isopropylideneinosine (5).[39,40] To a solution of 2′,3′-*O*-isopropylideneinosine (**3**, 500 mg, 1.62 mmol) in CH_3CN (10 mL) were added benzyl bromide (0.17 mL, 1.41 mmol) and DBU (208 μL, 2.14 mmol). The reaction was stirred at room temperature for 30 min. Volatiles were removed, and the residue was purified by flash column chromatography (AcOEt:MeOH, 10:1) to yield **5** (585 mg, 91%) as an amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36, 1.64 (s, 6H, $C(CH_3)_2$), 3.78 (m, 1H, H-5′), 3.93 (m, 1H, H-5″), 4.49 (m, 1H, H-4′), 5.03−5.13 (m, 3H, H-3′, H-2′, OH), 5.16 (m, 2H, CH_2N), 5.84 (d, J = 4.2 Hz, 1H, H-1′), 7.35 (m, 5H, Ph), 7.87 (s, 1H, H-8), 8.04 (s, 1H, H-2); MS (ES, positive mode) m/z 399 $[M + 1]^+$.

1-Benzyl-5′-*O*-(4-methylbenzyl)-2′,3′-*O*-isopropylideneinosine (6). To a solution of **5** (150 mg, 0.38 mmol) in dry DMF (2.5 mL) were added NaH (60% in mineral oil, 50 mg, 1.24 mmol) and 4-methybenzyl bromide (63 mg, 0.34 mmol). The reaction was stirred at −20 °C for 30 min, and then it was neutralized by the addition of AcOH. Volatiles were removed, and the residue was purified by CCTLC in the Chromatotron (CH_2Cl_2:MeOH, 20:1) to yield **6** (120 mg, 63%) as an amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35, 1.59 (s, 6H, $C(CH_3)_2$), 2.32 (s, 3H, CH_3-Ph), 3.57 (dd, J = 10.5, 3.8 Hz, 1H, H-5′), 3.64 (dd, J = 10.5, 3.1 Hz, 1H, H-5″), 4.42 (d, J = 11.7 Hz, 1H, CH_2O), 4.46 (d, J = 11.7 Hz, 1H, CH_2O), 4.52 (m, 1H, H-4′), 4.89 (dd, J = 6.1, 2.2 Hz, 1H, H-3′), 5.12 (dd, J = 6.1, 2.7 Hz, 1H, H-2′), 5.22 (d, J = 14.7, 1H, CH_2N-1), 5.27 (d, J = 14.7, 1H, CH_2N-1), 6.10 (d, J = 2.7 Hz, 1H, H-1′), 7.10−7.13 (m, 4H, OBn), 7.29−7.35 (m, 5H, NBn), 7.98 (s, 1H, H-8), 8.02 (s, 1H, H-2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.1 (CH_3-Ph), 25.3 (CH_3), 27.2 (CH_3), 49.1 (CH_2N-1), 69.9 (C-5′), 73.5 (CH_2O), 81.8 (C-3′), 85.3 (C-2′), 85.8 (C-4′), 91.5 (C-1′), 114.1 ($C(CH_3)_2$), 124.8 (C-5), 128.0, 129.1, 134.0, 137.7 (OBn), 128.2, 128.3, 129.0, 135.9 (NBn), 138.5 (C-8), 146.9 (C-4), 147.1 (C-2), 156.4 (C-6); MS (ES, positive mode) m/z 503 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{30}N_4O_5$: C, 66.92; H, 6.02; N, 11.15. Found: C, 66.80; H, 6.14; N, 11.09.

1-Benzyl-7-methyl-5′-*O*-(4-methylbenzyl)-2′,3′-*O*-isopropylideneinosine Iodide (7). To a solution of **6** (100 mg, 0.20 mmol) in dry DMF (1.0 mL) was added methyl iodide (25 μL, 0.40 mmol). The reaction was stirred at 60 °C overnight. Volatiles were removed, and the residue was purified by CCTLC in the Chromatotron (CH_2Cl_2:MeOH, 10:1) to yield **7** (50 mg, 49%) as a yellow solid. Mp (CH_2Cl_2:MeOH) 99−101 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43, 1.58 (s, 6H, $C(CH_3)_2$), 2.20 (s, 3H, CH_3-Ph), 3.70 (dd, J = 10.9, 2.0 Hz, 1H, H-5′), 4.06 (s, 3H, CH_3-N7), 4.48 (d, J = 11.0 Hz, 1H, CH_2O), 4.61 (dd, J = 10.9, 2.2 Hz, 1H, H-5″), 4.69 (d, J = 11.1 Hz, 1H, CH_2O), 4.81 (m, 1H, H-4′), 4.99 (d, J = 5.9 Hz, 1H, H-3′), 5.13 (d, J = 14.5 Hz, 1H, CH_2N-1), 5.29 (d, J = 14.5 Hz, 1H, CH_2N-1), 5.45 (dd, J = 1.4, 5.8 Hz, 1H, H-2′), 6.13 (d, J = 1.0 Hz, 1H, H-1′), 6.90 (m, 2H, *m*-CH-OBn), 6.96 (m, 2H, *o*-CH-OBn), 7.33−7.45 (m, 5H, NBn), 8.29 (s, 1H, H-2), 10.31 (s, 1H, H-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.0 (CH_3-Ph), 25.2 (CH_3), 26.8 (CH_3), 36.7 (CH_3-N7), 50.0 (CH_2N-1), 70.4 (C-5′), 72.4 (CH_2O), 82.6 (C-3′), 86.1 (C-2′), 88.7 (C-4′), 96.1 (C-1′), 113.8 ($C(CH_3)_2$), 115.1 (C-5), 127.0, 128.7, 134.7, 137.9 (OBn), 128.5, 129.1, 129.3, 134.2 (NBn), 139.3 (C-8), 144.21 (C-4), 150.7 (C-2), 151.8 (C-6); MS (ES, positive mode) m/z 517 $[M]^+$. Anal. Calcd for $C_{29}H_{33}IN_4O_5 \cdot H_2O$: C, 52.57; H, 5.32; N, 8.46. Found: C, 52.36; H, 5.10; N, 8.65.

Treatment of 1 with TFA. 1-Benzyl-5′-*O*-benzyl-2′,3′-*O*-isopropylideneinosine (**1**, 7.0 mg, 0.014 mmol) was dissolved in $CDCl_3$ (0.55 mL) and introduced in a NMR tube. A ^{1}H 1D NMR spectrum was recorded at 298 K. Then, TFA (10.6 μL, 0.143 mmol) was added and a new ^{1}H−1D NMR spectrum was recorded that corresponds to compound **8**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.46, 1.64 (s, 6H, $C(CH_3)_2$), 3.69 (dd, J = 10.9, 1.5 Hz, 1H, H-5′), 3.91 (dd, J = 10.9, 1.7 Hz, 1H, H-5″), 4.24 (d, J = 10.8 Hz, 1H, CH_2O), 4.38 (d, J = 10.8 Hz, 1H, CH_2O), 4.86 (m, 1H, H-4′), 4.94 (d, J = 5.7 Hz, 1H, H-3′), 5.17 (d, J = 14.4 Hz, 1H, CH_2N-1), 5,28 (d, J = 14.4 Hz, 1H, CH_2N-1), 5.29 (d, J = 1.4 Hz, 1H, H-2′), 6.16 (d, J = 1.4 Hz, 1H, H-1′), 6.89−7.04 (m, 5, OBn), 7.36−7.46 (m, 5H, NBn), 8.26 (s, 1H, H-2), 9.03 (s, 1H, H-8).

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Supporting Information Available: General chemical procedures and conditions for NMR studies and molecular dynamics simulations are provided, Figures S1−S7 and Table S1, and spectra of compounds **2** and **4**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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