Conformers of Guanosines and their Vibrations in the Electronic Ground and Excited States, as Revealed by Double-Resonance Spectroscopy and Ab Initio Calculations

Eyal Nir,[c] Isabel Hünig,[b] Karl Kleinermanns,[b] and Mattanjah S. de Vries*[a]

Because of their biological importance the DNA bases have been the subject of many theoretical and experimental investigations.[1–4] In the gas phase the intrinsic properties of the bases can be studied at vibrational or even rotational resolution without intermolecular interactions.[5–15] Though small, these molecules are difficult to vaporize without extensive decomposition. Recently, the vibronic spectrum of laser-desorbed, jet-cooled guanine was obtained by resonance-enhanced two-photon ionization (R2PI) with detection at the parent mass.[16] This pioneering study was followed by a number of investigations on guanine,[5, 16–20] guanine base pairs,[5, 19, 20] guanosine,[5, 21] adenine,[22–25] and cytosine[25] by resonant two-photon ionization. The results showed that nucleobases and complexes composed of paired bases can be studied in great detail in the absence of the external effects of solvent interactions and the collective modes of DNA. Here we show by means of double-resonance laser spectroscopy and ab initio calculations that, in the investigated wavelength range around 290 nm, guanosine (Gs), 2′-deoxyguanosine (2′-deoxyGs), and 3′-deoxyguanosine (3′-deoxyGs) each exhibit only the spectrum of one stable isomer, which is stabilized by a strong intramolecular sugar(5′-OH)···enolguanine(3-N) hydrogen bond.

Figure 1 shows a possible structure of guanosine (Gs) with guanine in its enol form; atoms which are important for the following discussion are numbered. The R2PI spectra of laser-desorbed Gs, 2′-deoxyGs, 3′-deoxyGs have already been published.[5, 21] In the earlier paper we speculated that two conformers might contribute to the vibronic spectra of Gs and 3′-deoxyGs. To test this hypothesis we performed UV–UV spectral hole burning (SHB) measurements. Figure 2 shows that each of the three spectra originates from only one conformer, because every single peak in the R2PI spectrum is reflected in the corresponding SHB spectrum. For comparison the R2PI spectrum of 2′,3′-diisopropylguanosine is shown as well.

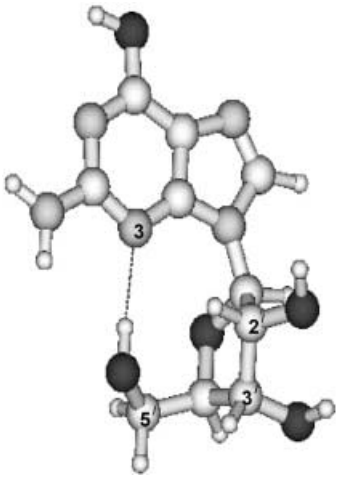

Figure 1. *A possible structure of guanosine with guanine in its enol form; the atoms which are most important for the discussion here are numbered.*

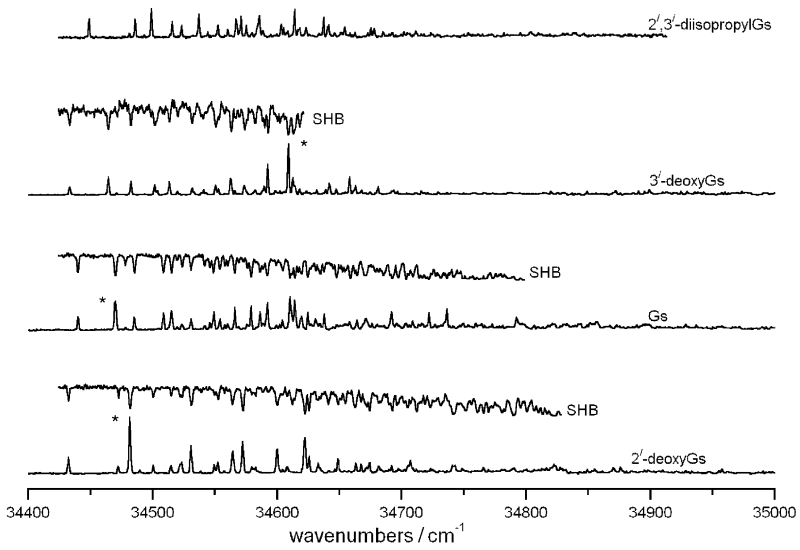

Figure 2. *R2PI and UV–UV SHB spectra of Gs, 2′-deoxyGs, and 3′-deoxyGs. The R2PI spectra are displayed in the respective lower traces. The upper traces show the SHB spectra with the probe laser set to the most intense vibronic peak, indicated by an asterisk. The trace shows the decrease in the ion intensity when the pump laser is scanned. Every peak in the R2PI spectrum is also observed in the corresponding SHB spectrum, and hence each of the three spectra comes from only one conformer. For comparison the R2PI spectrum of 2′,3′-diisopropylGs is also shown.*

Figure 3 shows the IR spectra obtained from IR–UV measurements with the UV laser fixed to the most intense vibronic transition of the respective guanosine, indicated by asterisks in Figure 2. For comparison the IR spectra of 2′,3′-diisopropylGs and one enol and one keto guanine (G) tautomer are also shown. In 2′,3′-diisopropylGs, the OH groups of the sugar moiety in the 2′- and 3′-positions are blocked. Therefore, its IR spectrum does not exhibit the corresponding OH vibrations but agrees well with

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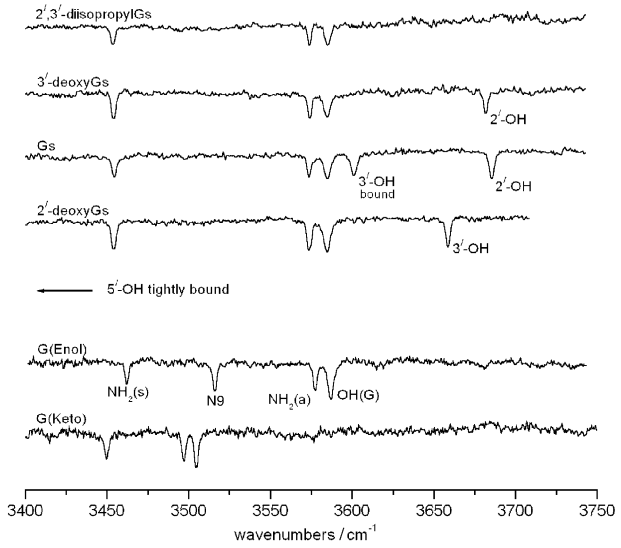

Figure 3. *IR – UV double-resonance spectra of Gs, 2′-deoxyGs, and 3′-deoxyGs. For comparison the IR spectra of 2′,3′-diisopropylGs and one enol and one keto tautomer of guanine (G) are also shown. For notation of the vibrations see text.*

that of G(Enol), aside from the N9H stretching vibration, which is absent because of substitution by the sugar residue in that position. Clearly the enol and not the keto tautomer of guanine is the chromophore in 2′,3′-diisopropylGs and, as a closer inspection shows, in all four Gs species. The OH group in the 5′-position of 2′,3′-diisopropylGs is not blocked by substitution and should therefore show an IR band. Therefore, we conclude that 5′OH is involved in a tight hydrogen bond, so that its stretching frequency is strongly red-shifted, probably to outside the investigated spectral range, which was limited by the tuning range of our crystals. The calculations presented below indeed show that the most stable Gs conformer has a strong intramolecular hydrogen bond between 5′-OH of the sugar and 3′-N of guanine (see Figure 1).

The IR spectra of the other guanosines support the Gs structure proposed in Figure 1, which is also the most stable structure according to the ab initio calculations presented below. In 3′-deoxyGs we observe an additional OH vibration compared to 2′,3′-diisopropylGs at higher frequency, which results from the free (not hydrogen-bonded) 2′-OH group of the sugar moiety. In 2′-deoxyGs there is an additional OH band near the 2′-OH band, which can be attributed to the 3′-OH stretching vibration. The IR spectrum of Gs in the range of the OH and NH stretching vibrations consists of the G(Enol) spectrum without the N9H band, the additional 2′-OH band of the sugar moiety, and a band somewhat red-shifted relative to that of free 3′-OH, which probably results from the involvement of 3′-OH in a weak intramolecular hydrogen bond with 2′-OH ("bound 3′-OH"). A short description of these findings was given in a recent review.[5]

The most stable conformers calculated at the HF 6-31G(d,p) level are displayed in Figure 4. Figures 5, 6, and 7 compare the IR spectra of Gs, 2′-deoxyGs, and 3′-deoxyGs with the vibrational spectra of their most stable conformers calculated at the HF 6-31G(d,p) level. The relative stabilization energies ΔE of the different conformers are also given. The comparison shows that the qualitative assignment given above is supported by the calculations.

In the case of 2′-deoxyGs, assignment to a conformer in which the 5′-OH group is involved in hydrogen bonding to N3 of guanine (structure 1 or 2 in Figure 4b) is straightforward, because the 2′-position is not available for hydrogen bonding. However, from the frequencies alone it is not possible to determine whether the experimental spectrum arises from structure 1 or 2.

In the case of Gs and 3′-deoxyGs either 5′-OH is bound to the N3 atom (as in 2′-deoxyguanosine) or 2′-OH is bound to the N3 atom. The bands observed at about 3700 cm^{-1} can therefore be due to vibrations of free 2′-OH or of free 5′-OH. Comparison with the calculations cannot distinguish between these two possibilities. Given the energy differences between the two conformers (738 cm^{-1} for Gs 3 relative to Gs 1, and 369 cm^{-1} for 3′-deoxyGs 2 relative to 3′-deoxyGs 1), it is more likely that the structures found in the jet are those in which 5′-OH is involved in hydrogen bonding, but of course the order of stabilities may depend on the level of calculation. For 2′,3′-diisopropylGs the absence of the 5′-OH band from the spectrum is clearly due to its involvement in a strong hydrogen bond. On the other hand, if the band at around 3700 cm^{-1} is due to free 5′-OH, why does this band have different frequencies in GS and 2′-deoxyGs? There is no reason to believe that the other guanosines do not form this hydrogen bond involving 5′OH. It is more reasonable that the bands around 3700 cm^{-1} in guanosine and 3′-deoxyguanosine are due to the free 2′-OH stretching vibration and that the slight difference between the frequencies of these bands is due to interaction with 3′-OH in the case of guanosine. The magnitude of the frequency shift of the strongly bound 5′-OH cannot be accurately reproduced at the current level of theory. However, the main finding remains that we observe only one conformer of these rather complex biomolecules, which are folded by strong intramolecular hydrogen bonds into a preferred conformation.

Figure 8 shows the normal-mode vibrations of guanosine in the low-frequency range of the lowest $\pi - \pi^*$ state, as obtained from CIS/6-31G(d,p) calculations. Figure 9 compares the experimental vibronic frequencies with the frequencies calculated at the CIS level. The comparison allows a tentative assignment of the most intense vibronic bands of the REMPI spectrum. Most of the weaker bands can be assigned to overtone and combination bands. The low-frequency region of the spectrum is dominated by vibrations 1, 2, and 3 from Figure 8, which are mutual motions of the sugar and guanine rings and can be best characterized as torsion, butterfly, and gearing motions. The high vibronic activity of these modes in all investigated guanosines points to a substantial change in the mutual orientation of the two rings (guanosine folding) on electronic excitation. This finding also points to a guanosine structure with a strong noncovalent interaction between the sugar and guanine moieties that changes on electronic excitation. The high vibronic activity of guanosine contrasts to the very sparse vibronic spectra of 9-methyl-/9*H*-enolguanine, in which only bands at 60/66 and 438 cm^{-1} could be observed.[28]

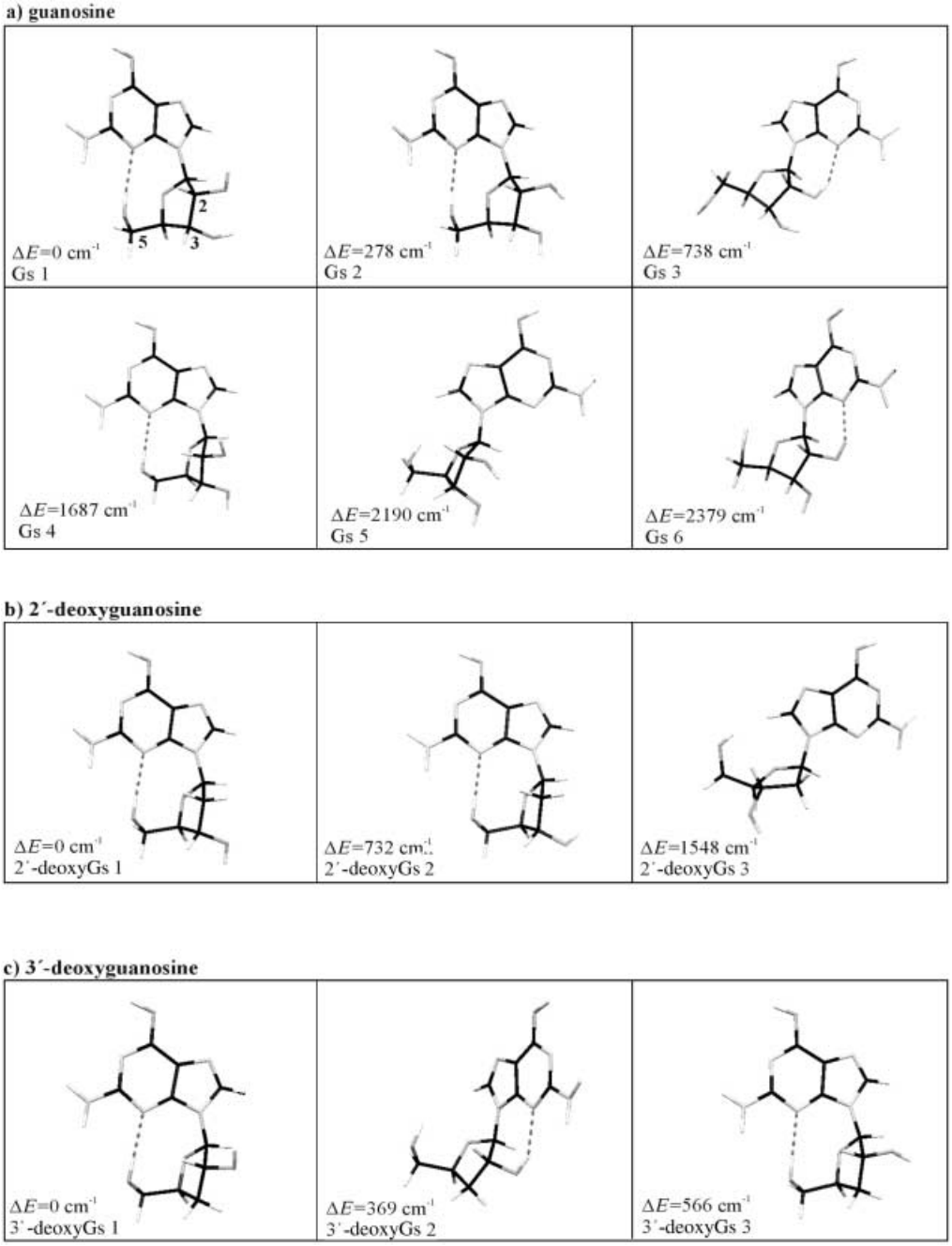

Figure 4. *The most stable conformer structures for Gs (a), 2′-deoxyGs (b) and 3′-deoxyGs (c), calculated at the HF 6-31G(d,p) level. The relative stabilization energies ΔE (including ZPE) of the different conformers are also given. The structures are numbered in order of relative stability.*

Although a rough assignment of the vibronic spectrum in the low-frequency range is possible, a number of bands cannot be assigned to the calculated fundamental vibrations and their overtone and combination bands (at least not at the level of a CIS calculation). For example, we observe vibronic bands at +30, +39, and +46 cm^{-1} built on the electronic origin of guanosine, while the calculated torsion and butterfly vibrations (vibrations 1 and 2 in Figure 8) are at 37 and 50 cm^{-1}, and the gearing vibration (vibration 3 in Figure 8) is at 80 cm^{-1}. While we observe only one conformer in the S_0 state, as unambigously shown by our SHB experiments, we cannot exclude the occurrence of a further conformer in the electronically excited state with a sufficiently small geometry change relative to the ground-state structure for a Franck–Condon transition or vibronic contributions from another electronic state (the lowest $n-\pi^*$ or the next $\pi-\pi^*$ state).

The conclusion that all the investigated guanosines are in the enol form is consistent with the fact that the origin of their electronic spectra is close to the origin of 9*H*-guanine in the enol tautomeric form.[28] This then suggests that substitution at the

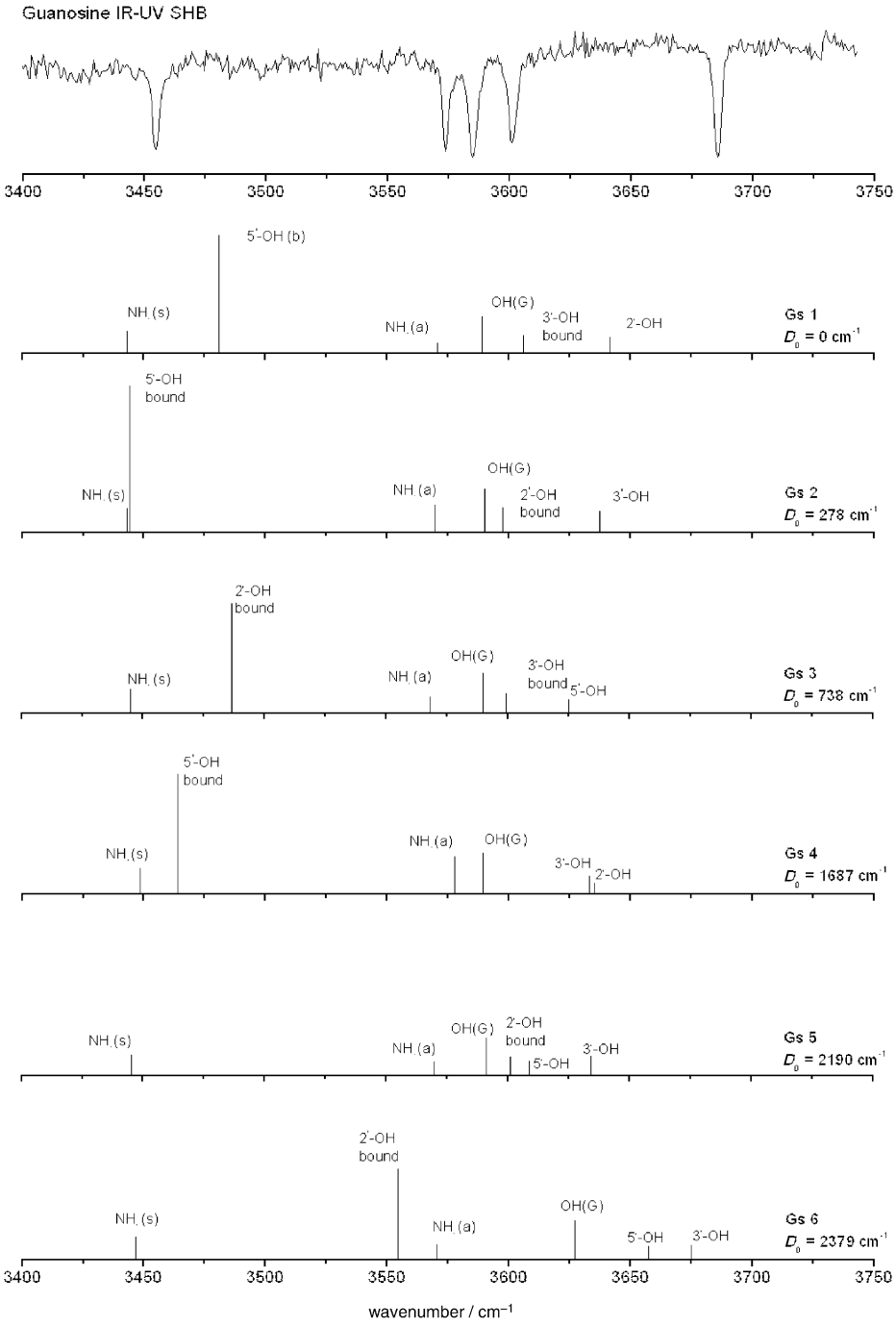

Figure 5. *IR – UV double resonance spectrum of Gs and vibrational spectra with relative stabilization energies ΔE of the most stable conformers calculated at the HF 6-31G(d,p) level. The vibrational frequencies are scaled by a factor of 0.893 for all NH_2 stretching frequencies, and by 0.867 for all OH stretch frequencies. Both scaling factors were obtained from the best fit of the calculated frequencies of enolguanine monomer to the corresponding experimental frequencies. The relative stabilities ΔE include the ZPE.*

N9 position of guanine and addition of the sugar moiety do not shift the electronic spectrum of the guanine chromophore very much (guanosine red shift of $-169/-312\,cm^{-1}$ relative to the spectra of 9-methyl/9*H*-enolguanine [28]).

The question thus arises why we do not observe guanosine in the keto form at wavelengths close to those of 9*H*-ketoguanine. One possible explanation might be a reduced lifetime of the excited state of ketoguanine substituted in the N9 position. The electronic spectrum of 9-methylketoguanine could also not be detected.[28]

Experimental and Theoretical Methods

The measurements on laser-desorbed nucleosides were performed with an apparatus described in detail elsewhere.[26] In short, neat

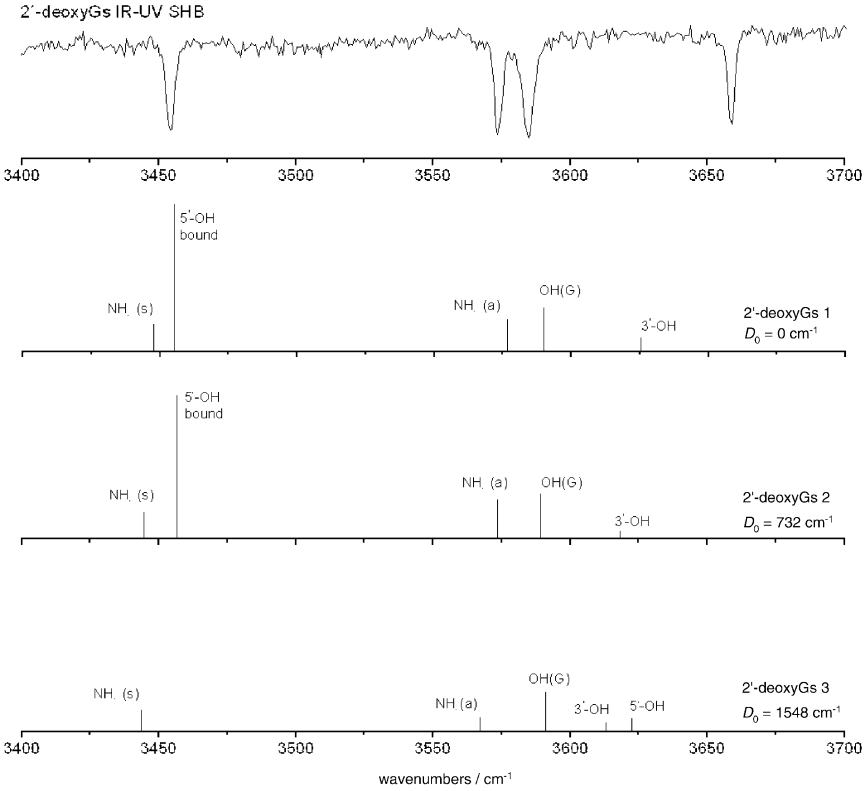

Figure 6. *IR – UV double-resonance spectrum of 2′-deoxyGs and vibrational spectra with relative stabilization energies ΔE of the most stable conformers calculated at the HF 6-31G(d,p) level. The scaling factors for the vibrational frequencies are the same as used for Gs.*

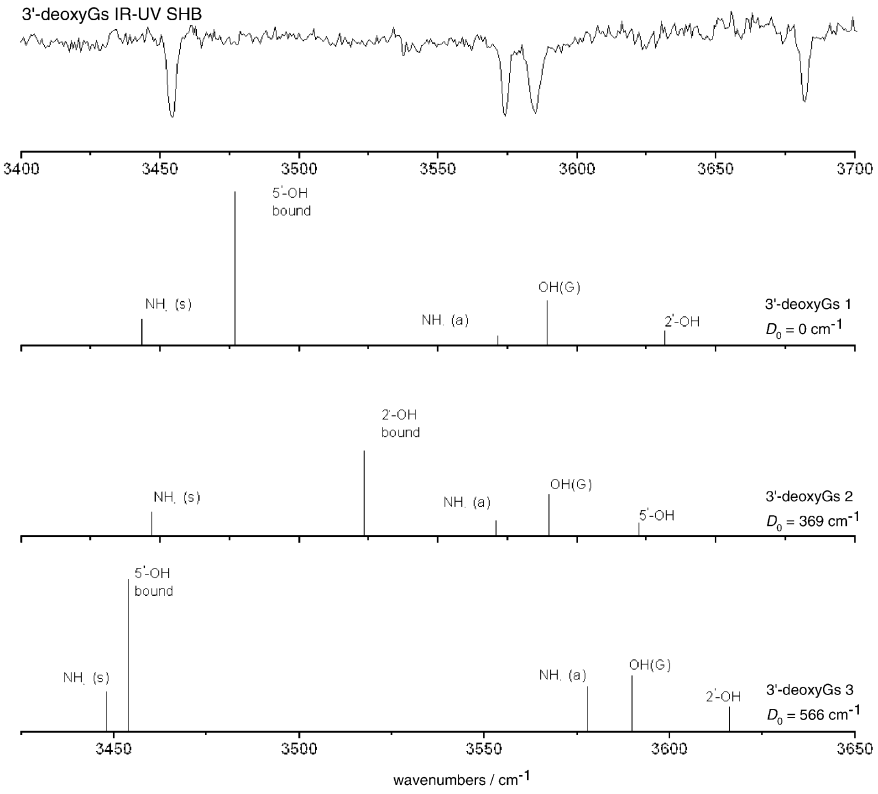

Figure 7. *IR – UV double resonance spectrum of 3′-deoxyGs and vibrational spectra with relative stabilization energies ΔE of the most stable conformers calculated at the HF 6-31G(d,p) level. The scaling factors for the vibrational frequencies are the same as used for Gs.*

material was laser-desorbed from a graphite substrate in front of a pulsed nozzle. The fluence of the Nd:YAG desorption laser, operated at 1064 nm (where graphite absorbs but guanosine does not), was typically about 1 mJ cm^{-2} or less, which is considerably lower than the fluences normally used for ablation. The laser was focused to a spot of about 0.5 mm diameter within 2 mm in front of the nozzle. We used a pulsed valve (General Valve, Iota One) with a nozzle diameter of 1 mm at a backing pressure of about 5 atm argon drive gas. The skimmed molecular beam is crossed by the ionization laser beams at right angles inside the source region of a reflectron time-of-flight (TOF) mass spectrometer. By monitoring the parent mass peaks of, for example, the guanosines while varying the two-photon, one-color R2PI ionization wavelength, we obtained mass-selected excitation spectra.

Spectral hole burning was performed by using two counterpropagating dye laser pulses with a delay of about 150 ns. This results in two peaks in the TOF spectrum: a first peak from the "burn" laser, and a second peak from the "probe" laser. When both lasers are tuned to a resonance of the same tautomer, the burn laser causes a decrease in the signal of the probe laser by depopulating the common ground state. Usually we scan the burn laser while the probe laser frequency is set to an intense band of a given isomer. If a significant band of the R2PI spectrum is absent in the burn spectrum it belongs to another isomer (or to a hot band, which, however, we do not observe in our guanosine spectra). In the next step we probe at this frequency while scanning the pump laser to reveal the spectrum of the next isomer.

We performed IR – UV SHB by the same method, employing a difference frequency IR laser as the burn laser. The radiation from an infrared dye (a mixture of Styryl 8 and Styryl 9) was aligned colinearly with the perpendicularly polarized Nd:YAG fundamental (1064 nm) and directed through a MgO-doped $LiNbO_3$ crystal to generate IR radiation tunable in the range 3300 – 4000 cm^{-1}. Suitable dielectric mirrors separate the Nd:YAG fundamental and the dye laser beam behind the crystal. We typically used 50 mJ of the YAG fundamental and 10 mJ of the dye laser to obtain around 1 mJ per pulse of IR radiation between 3300 and 4000 cm^{-1} with a bandwidth of less than 0.1 cm^{-1}. The IR laser was calibrated by recording the spectrum of water vapor. Color centers in the $LiNbO_3$ crystal decrease the IR intensity in the range of 3515 – 3550 cm^{-1}. In this spectral range we used another $LiNbO_3$ crystal with an intensity gap in a different region.

The calculations were carried out with the Gaussian 98 program package[27] and were performed at the HF 6-31G(d,p) level. All structures were fully optimized with 10^{-8} hartree as SCF convergence criterion, and $1.5 \cdot 10^{-5}$ hartree/bohr and $1.5 \cdot 10^{-5}$ hartree/degree as convergence cri-

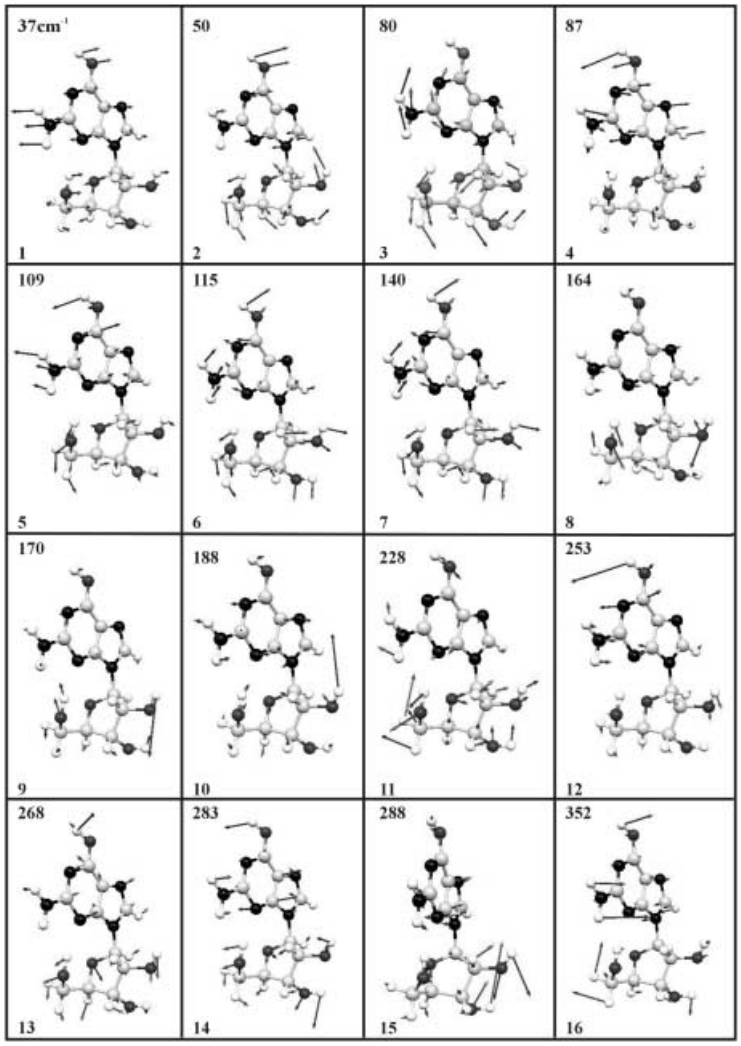

Figure 8. *Normal-mode vibrations of Gs in the low-frequency region, obtained from CIS/6-31G(d,p) calculations on the lowest $\pi-\pi^*$ state.*

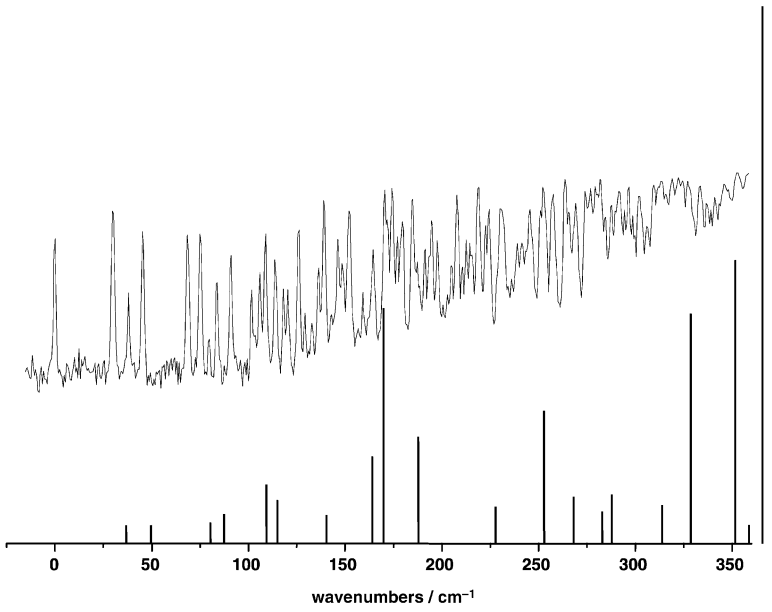

Figure 9. *The vibrational spectrum of Gs calculated at the CIS/6-31G(d,p) level for the lowest $\pi-\pi^*$ state, in comparison to the UV–UV SHB spectrum of Gs. The calculated frequencies are unscaled.*

teria for the gradient optimization of the structures. The vibrational frequencies were obtained by performing a normal-mode analysis on the optimized geometries by using analytical gradients of the energy. The stabilization energies were corrected for the zero-point energy (ZPE) by using the harmonic frequencies.

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Keywords: ab initio calculations · IR spectroscopy · laser spectroscopy · nucleosides · UV/Vis spectroscopy

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Tetrachloro-substituted Perylene Bisimide Dyes as Promising n-Type Organic Semiconductors: Studies on Structural, Electrochemical and Charge Transport Properties

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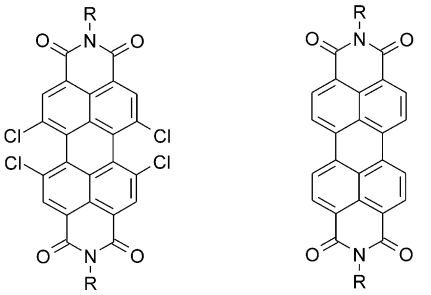
Perylene tetracarboxylic acid bisimides belong to the most interesting functional materials. They exhibit photoconductivity[1] and n-type semiconducting properties[2] which provide prospects for application in photovoltaic devices,[3] organic field-effect transistors (OFETs),[4] and light emitting diodes (LEDs).[5] In contrast to the extensively studied p-type semiconductors, the number of organic compounds with good n-type semiconductivity is still very limited. As pointed out previously,[4] n-type semiconducting organic materials require molecules with large electron affinities to facilitate the injection of electrons and formation of stable radical anions ideally even in the presence of air and humidity. Increasing electron affinity of molecules can be achieved by introduction of electron-withdrawing functional groups such as halogens or cyano groups. Naphthalene tetracarboxylic bisimides[6] and oligothiophenes[7] with perfluoroalkyl substituents, phthalocyanines[8] and oligothiophenes[9] with cyano substituents, and perhalogenated phthalocyanines[8] are examples of n-type semiconductors for which improved semiconductivities were achieved with this simple chemical concept.

The second feature that must be optimized in an organic charge transport material is the molecular order in the solid state. For crystalline materials, high charge carrier mobilities are feasible if the conjugated systems exhibit sufficient overlap with adjacent molecules in the crystal lattice.[4c] For perylene bisimides, it has been shown that increased ordering of perylene molecules leads to an increase in exciton diffusion length and an improvement of charge carriers mobilities.[2a, 10] However, all those compounds studied previously are based on the functionalization at N atoms of imide groups rather than at perylene core, and the influence of the latter factor on the molecular packing in solid state is still rarely reported. Additionally, perylene bisimides functionalized at bay positions (1, 6, 7, and 12 positions in the perylene core) show a twisted skeleton,[11] which could be utilized to avoid the formation of dimeric "sandwich pairs" that have shown to act as traps for charge carriers, as pointed out in a recent review.[2b] With these facts in mind, we synthesized tetrachloro-substituted perylene bisimides **1a–c** (Scheme 1) and unsubstituted perylene bisimide **2**[12] as reference material to elucidate the effect of the bay-positioned chlorine atoms on the electron affinity, the solid-state ordering and the charge-transport properties of these materials.



Scheme 1. *Chemical structures of perylene bisimides* ***1a–c*** *and* ***2****.*

An enhancement of the electron affinity of perylene bisimides by four chlorine substituents was observed by cyclic voltammetry of **1a**. Two reversible reduction waves were detected at half-wave potentials of -0.87 V and -1.07 V vs. Fc/Fc^+ (Fc: ferrocene) corresponding to the formation of radical anions and dianions, but no reversible oxidation could be observed up to 1.2 V. For comparison, perylene bisimides without bay substituents are reduced at around -0.98 V and -1.2 V and

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