

## Electronic Transition Moments of 2-Aminopurine

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**Abstract:** The electronic spectrum of the unnatural nucleic acid base 2-aminopurine is important in order to understand the spectroscopy and photophysics of the normal DNA bases. Knowledge of transition moment directions and effective absorption components is also a prerequisite for the interpretation of emission spectra of 2-aminopurine incorporated into DNA as a probe of nucleic acid base motion or excitation energy transfer processes. Using linear dichroism, fluorescence anisotropy, and ordinary and magnetic circular dichroism techniques, the near-UV spectrum of 2-aminopurine is resolved into contributions from five electronic transitions. Four moderately strong  $\pi \rightarrow \pi^*$  transitions (I, III, IV, and V) are observed, polarized at  $+53^\circ$  (I),  $-72^\circ$  (III),  $-77^\circ$  (IV), and  $+57^\circ$  or  $-67^\circ$  (V) relative to the short molecular axis. In addition, one weak  $n \rightarrow \pi^*$  transition (II) polarized perpendicular to the molecular plane has been identified. Semiempirical INDO/S calculations support these assignments.

### Introduction

2-Aminopurine (2AP) is a fluorescent adenine isomer well suited to be inserted in polynucleotides without obstructing the base pairing. It can readily replace adenine (6-aminopurine) in base-pairs with thymine, but also form moderately stable pairing with cytosine.<sup>1–5</sup> Several studies of DNA dynamics using 2AP as an intrinsic probe of the helix motion have been performed with fluorescence anisotropy.<sup>6,7</sup> 2AP has a lower excitation energy than the natural nucleic acid bases, and therefore it can be selectively excited. 2AP can also be used as an excitation energy trap since its lowest excited singlet state has lower energy than any of the excited singlet states of the common bases and this feature has been exploited in investigations of excitation energy transfer and the localization of photoinduced damage in DNA.<sup>8</sup> The duplex melting point of oligomers containing 2AP has also been studied<sup>9</sup> as well as the effect of replacing adenine with 2AP in a specific recognition site.<sup>10</sup>

It was noted early that substitution by an electron donating group on C2 (Figure 1) of purine causes a substantial red-shift in the UV absorption.<sup>11</sup> The lowest excited singlet state of purine is of  $n\pi^*$  character,<sup>12</sup> and in 2-substituted purines it is most likely a  $\pi\pi^*$  state. This has a dramatic effect on the luminescence properties of these molecules; purine has an intense phosphorescence and very weak fluorescence whereas 2AP is strongly fluorescent and shows no phosphorescence. Both these molecules are contrasted by adenine (6-aminopurine) for which

both fluorescence and phosphorescence of similar magnitude are observed, but the total emission intensity is much smaller, indicating a very efficient non-radiative return to the ground state. Some observations on the spectroscopic properties of 2-aminopurine were made by Drobnik and Augenstein.<sup>13</sup> They studied the behavior of the absorption and emission spectra in different solvents and assigned the lowest excited state of 2AP to be of  $\pi\pi^*$  character based on the type of luminescence detected. Later Stryer and co-workers investigated the use of 2AP as fluorescent probe in polynucleotides and also characterized its fluorescence.<sup>14</sup>

To our knowledge no experimental determination of the transition moments of 2AP has appeared in the literature and so far the only information on individual transitions (excitation energies, oscillator strengths, and transition moment directions) has been derived from semiempirical molecular orbital calculations.<sup>15</sup>

In order to provide basic information for future biophysical studies using 2AP we have decided to experimentally and computationally investigate the low-lying excited electronic states of 2AP by determining the electronic transition moments and the intensity distribution among the different electronic transitions in the near-UV spectrum. This is valuable information needed for, for example, interpreting fluorescence anisotropy, energy transfer, and linear and circular dichroism measurements on oligo- or polynucleotide molecules modified with 2AP. The careful characterization of the excited states of 2AP is also part of another goal of ours, *viz.* to investigate the excited states of purine chromophores and to understand the interplay between their electronic structure and photophysical properties.<sup>16</sup>

This paper presents polarized UV spectra for 2AP, 2-amino-9-methylpurine (2A9MP), and the 2-aminopurine riboside (2APr) oriented in stretched films of poly(vinyl alcohol). Parameters characterizing the orientation of these molecules in the polymer film are determined from polarized IR measurements. In addition magnetic circular dichroism, fluorescence anisotropy, and, in the case of 2APr, also circular dichroism measurements support the UV band assignments and the

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transition moment polarizations. The reason to include the 9-substituted species in the investigation is 2-fold. Firstly, replacing the hydrogen at position 9 excludes the possibility for the 7-H/9-H tautomeric equilibrium, known to be important in purine<sup>17</sup> and adenine.<sup>18,19</sup> Secondly, such a substitution will perturb the orientation of the 2AP chromophore in the polymeric film and, thus, give additional information on the directions of the transition moments (UV and IR) within the molecular framework. The use of substitutional perturbation has been quite successfully employed in a number of earlier stretched film experiments to solve sign ambiguities regarding the polarization angles.<sup>20</sup> The experimental assignments of the UV spectrum are finally correlated with semiempirical INDO/S calculations.

## Materials and Methods

**Chemicals.** 2-aminopurine (2AP) and 2-aminopurine riboside (2APr) were purchased from Sigma Chemical Co. and used without further purification. 2-Amino-9-methylpurine (2A9MP) was synthesized from 2-aminopurine according to the method described by Hedayatullah<sup>21</sup> and purified by chromatography on silica gel with chloroform/methanol/ammonia (90/10/1) as eluant and by subsequent recrystallization from ethanol/water. The purity of 2A9MP was checked by <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopy and mass spectrometry. Poly-(vinyl alcohol) (PVA) was obtained as powder from E. I. du Pont de Nemours Co. (Elvanol). All aqueous solutions were prepared from deionized water (Millipore). All organic solvents were of spectrophotometric grade.

**Linear dichroism (LD)** is defined as

$$LD(\bar{\nu}) = A_{\parallel}(\bar{\nu}) - A_{\perp}(\bar{\nu}) \quad (1)$$

where  $A_{\parallel}(\bar{\nu})$  and  $A_{\perp}(\bar{\nu})$  are the absorbances measured with plane polarized light respectively parallel and perpendicular to the macroscopic sample axis (the stretching direction). The reduced linear dichroism,  $LD^r$ , is defined as

$$LD^r(\bar{\nu}) = (A_{\parallel}(\bar{\nu}) - A_{\perp}(\bar{\nu}))/A_{\text{iso}}(\bar{\nu}) \quad (2)$$

where  $A_{\text{iso}}(\bar{\nu})$  is the absorbance of a corresponding isotropic sample. For a uniaxial orientation of the sample molecules, such as in a polymer matrix,  $A_{\text{iso}}(\bar{\nu})$  can be calculated from the polarized components as<sup>22</sup>

$$A_{\text{iso}}(\bar{\nu}) = \frac{A_{\parallel}(\bar{\nu}) + 2A_{\perp}(\bar{\nu})}{3} \quad (3)$$

For a planar molecule, the  $LD^r$  for a transition  $i$  can be written<sup>23</sup>

$$LD_i^r = 3(S_{yy} \sin^2 \theta_i + S_{zz} \cos^2 \theta_i) \quad (\text{in-plane transition}) \quad (4)$$

$$LD_i^r = 3S_{xx} \quad (\text{out-of-plane transition}) \quad (5)$$

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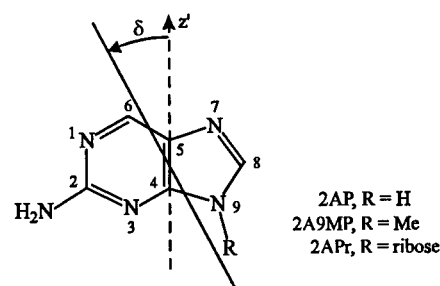
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**Figure 1.** The 2-aminopurine chromophore with atomic numbering and definition of an in-plane angle ( $\delta$ ).  $z'$  is a molecule-fixed reference axis parallel to the  $C_4-C_5$  bond.

where  $S_{zz}$ ,  $S_{yy}$ , and  $S_{xx}$  are the Saupe orientation parameters<sup>24</sup> for the diagonal in-plane axes  $z$  and  $y$ , and the out-of-plane axis  $x$ , characterizing the orientation of the solute molecules and  $\theta_i$  is the angle between the transition dipole moment and the preferred molecular orientation axis  $z$ . For an in-plane polarized transition the  $LD^r$  can have values between  $3S_{zz}$  and  $3S_{yy}$  and the orientation parameters are interrelated according to

$$3S_{yy} \leq LD^r(\text{in-plane}) \leq 3S_{zz} \quad (6)$$

$$S_{xx} + S_{yy} + S_{zz} = 0 \quad (7)$$

For overlapping transitions the observed  $LD^r$  is a weighted average of the  $LD_i^r$  values of the contributing transitions<sup>23</sup>

$$LD^r(\bar{\nu}) = \frac{\sum_i \epsilon_i(\bar{\nu}) LD_i^r}{\sum_i \epsilon_i(\bar{\nu})} \quad (8)$$

where  $\epsilon_i(\bar{\nu})$  is the molar absorptivity associated with transition  $i$  at wavenumber  $\bar{\nu}$ . The pure reduced linear dichroism  $LD_i^r$  is determined by the TEM method originally proposed by Thulstrup, Eggers, and Michl.<sup>25</sup> This method is based on forming linear combinations of the type  $A_{\parallel}(\bar{\nu}) - dA_{\perp}(\bar{\nu})$  with varying values of the reduction coefficient,  $d$ . The reduction coefficient,  $d_i$ , for which a specific spectral feature,  $i$ , disappears is related to the  $LD_i^r$  of the transition containing that feature by<sup>26</sup>

$$LD_i^r = 3 \frac{d_i - 1}{d_i + 2} \quad (9)$$

When the reduction coefficient is known it can be used to obtain the reduced absorption spectrum for a specific transition as outlined by Michl and Thulstrup.<sup>22</sup> This approach has been used by us to obtain approximate band shapes for the observed transitions. It should be noted that the resolution of band shapes is made under the assumption that there is only pair-wise overlap between neighboring (in energy) transitions and that the resolved band shapes correspond to the purely polarized transitions.

Given the orientation parameters and the direction of the orientation axis we can obtain the directions of transition moment  $i$  within the molecular framework according to

$$\delta_i = \alpha \pm \theta_i \quad (10)$$

where  $\delta_i$  and  $\alpha$  are the angles between the  $z'$  axis (Figure 1) and the transition dipole moment direction and the orientation axis, respectively.

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The experimental details of polarized UV measurements and preparation of PVA films have been reported previously.<sup>27</sup>

**Fluorescence emission measurements** were performed on a SPEX fluorolog  $\tau 2$  spectrofluorimeter. Quantum yields of fluorescence ( $\phi_f$ ) were determined relative to the quantum yield of an argon-purged solution of 2,5-diphenyloxazole (PPO) in cyclohexane ( $\phi_f = 0.85$ ).<sup>28</sup> The fluorescence excitation anisotropy (FA) spectra were measured with the samples in EPA (a 5/5/2 mixture of diethyl ether, 2-methylbutane, and ethanol) glass at 80 K, with Glan polarizers both in the excitation beam and in the emission beam. The fluorescence intensities were measured with polarizers set either vertically (v) or horizontally (h) in the excitation and emission beams. The excitation band pass was set to 2 nm and the emission was measured at 450 nm with a band-pass of 4 nm. The degree of anisotropy,  $r$ , was calculated as<sup>29</sup>

$$r(\bar{\nu}) = \frac{I_{vv}(\bar{\nu}) - I_{vh}(\bar{\nu})G(\bar{\nu})}{I_{vv}(\bar{\nu}) - 2I_{vh}(\bar{\nu})G(\bar{\nu})} \quad (11)$$

where  $G(\bar{\nu})$  is the ratio  $I_{hv}(\bar{\nu})/I_{hh}(\bar{\nu})$  used for instrumental correction. The first index refers to the excitation polarizer and the second to the emission polarizer. The theoretically limiting value of the anisotropy for any pure electronic transition,  $i$ , is reached when no molecular reorientation occurs during the excited state lifetime. This limiting value,  $r_{0i}$ , is given by<sup>29</sup>

$$r_{0i} = r_{01}(3 \cos^2 \beta_i - 1)/2 \quad (12)$$

where  $\beta_i$  is the angle between the  $i$ th transition moment of absorption and the emitting moment. The  $r_{01}$  is the observed limiting anisotropy for the lowest lying transition. For overlapping bands a method analogous to the TEM method is used, based on linear combinations of polarized intensities. The pure anisotropy is related to the reduction parameter  $d_i$  by<sup>26</sup>

$$r_{0i} = \frac{d_i - 1}{d_i + 2} \quad (13)$$

Time-resolved fluorescence measurements were performed with the SPEX fluorimeter using the phase and modulation technique.<sup>30</sup> A diluted silica sol scattering solution was used as a reference. Each measurement involved 15 modulation frequencies on a logarithmic scale in the frequency range 2–200 MHz and each point was an average of 5 measurements of the phase and the demodulation. The samples were excited at 300 nm and the emission collected through a 375-nm cut-off filter. The data were evaluated using the method of nonlinear least squares with the phase error set to 0.5° and the demodulation error set to 0.005.<sup>30</sup> For all three compounds studied in this work the data could be fitted nicely to single exponential decays with a maximum goodness-of-fit parameter  $\chi^2$  equal to about 0.7.

**Circular Dichroism (CD).** The CD is defined as the differential absorption of two circularly polarized light beams:

$$\text{CD}(\bar{\nu}) = A_l(\bar{\nu}) - A_r(\bar{\nu}) \quad (14)$$

where  $A_l(\bar{\nu})$  and  $A_r(\bar{\nu})$  are the absorption intensities with left and right circularly polarized light. A useful dimensionless quantity is the dissymmetry factor  $g$  defined as

$$g(\bar{\nu}) = \frac{A_l(\bar{\nu}) - A_r(\bar{\nu})}{A_{\text{iso}}(\bar{\nu})} \quad (15)$$

The CD was measured for 2APr dissolved in water. The measurements were made on a JASCO J-720 spectropolarimeter.

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**Magnetic circular dichroism (MCD)** measurements were made on the JASCO instrument equipped with a permanent horse shoe magnet. The magnetic field was calibrated to be 1.1 T by using the MCD signal at 510 nm of a 1 M solution of  $\text{CoSO}_4$  ( $\Delta\epsilon_{510} = -1.88 \times 10^{-2} \text{ M}^{-1} \text{ cm}^{-1} \text{ T}^{-1}$ ).<sup>31</sup> The MCD was measured for 2A9MP and 2AP in water solutions.

## Quantum Chemical Calculations

The molecular orbital (MO) calculations of electronic absorption spectra were performed with the INDO/S model Hamiltonian.<sup>32–34</sup> The two-center electron repulsion integrals were calculated using the Mataga–Nishimoto scheme.<sup>35</sup> In the configuration interaction (CI) calculation all singly excited (singlet) configurations using the 15 highest occupied and 5 lowest unoccupied MO's were included. The CI space thus includes all  $\pi$ ,  $n$ , and  $\pi^*$  orbitals and also the  $\sigma$  orbitals contributing to electronic transitions with an energy less than 50 000  $\text{cm}^{-1}$ . Expansion of the CI space was tested but the effects on calculated spectra were negligible. We also performed calculations in order to simulate the electronic spectrum of 2A9MP in water solution. This was performed in a self consistent reaction field (SCRF) calculation on a 2A9MP/water complex where five water molecules were placed at plausible hydrogen bond positions<sup>36</sup> ( $N_1$ ,  $N_3$ ,  $N_7$ , and the two amino hydrogens). The distances between hydrogen bonded atoms were all set to 2.0 Å. In the SCRF calculation the 2A9MP/water complex is embedded in a spherical cavity which is surrounded by a solvent continuum characterized by the dielectric constant and refractive index of water ( $\epsilon = 78$ ,  $n = 1.333$ ). We used the SCRF model B and the mass density approach to determine the cavity radius ( $a_0 = 4.56$  Å) both developed by Karelson and Zerner to be used in connection with the INDO/S model Hamiltonian.<sup>37</sup> The molecular geometries used in the INDO/S calculations were obtained from SCRF DFT(B3LYP)/6-31G(d,p) calculations. The SCRF DFT(B3LYP)/6-31G(d,p) geometry of 2A9MP will be reported elsewhere.<sup>38</sup> The used DFT method has proven to yield reliable structures for several purine derivatives including 2AP.<sup>39</sup>

## Results

We have experimentally and computationally investigated the near-UV spectrum of the 2-aminopurine chromophore. Prominent features in the polarized absorption spectrum are assigned to the four lowest  $\pi \rightarrow \pi^*$  transitions. In addition, we have found experimental evidence for a hidden low-lying  $n \rightarrow \pi^*$  transition.

The material is organized as follows. (i) The isotropic absorption ( $A_{\text{iso}}$ ), reduced linear dichroism ( $\text{LD}^r$ ), fluorescence anisotropy (FA), and magnetic circular dichroism (MCD) spectra are presented for 2-aminopurine (2AP) and 2-amino-9-methylpurine (2A9MP). The two sets of spectra are very similar and reveal the existence of several differently polarized electronic transitions. (ii) The UV absorption spectrum of 2-aminopurine riboside (2APr) is resolved into five different electronic transitions based on the linear dichroism measurements. Circular dichroism measurements on the chiral 2APr further support the assignments. (iii) The orientation parameters and the preferred orientation axis of 2AP and 2A9MP in the stretched PVA films are estimated from polarized IR measurements and used to determine the directions of the electronic transition

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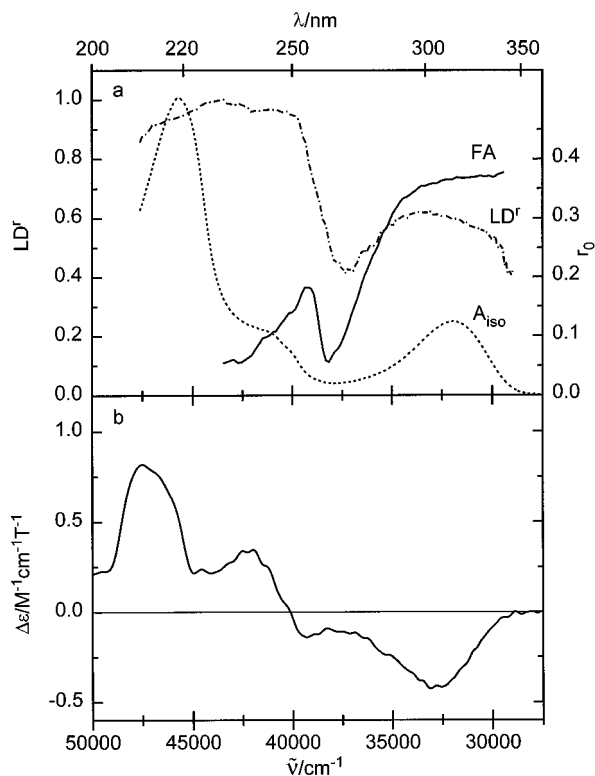
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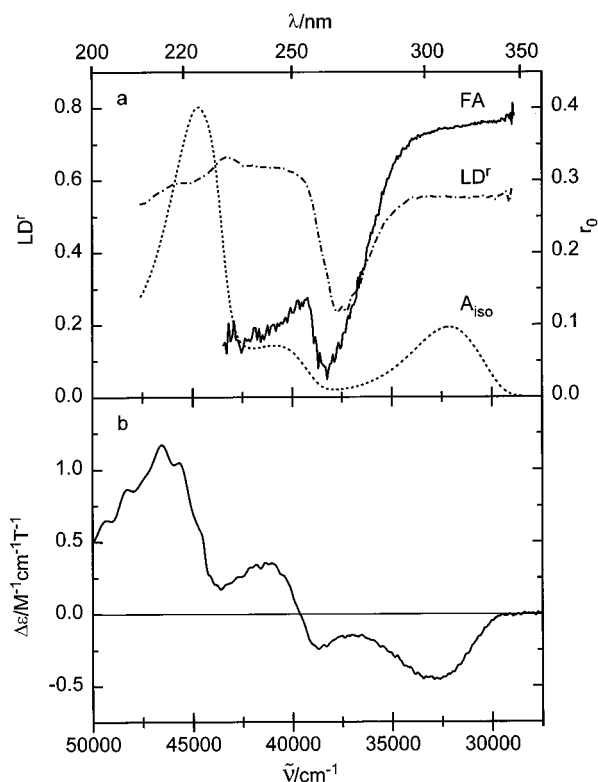


**Figure 2.** (a) Isotropic absorption ( $A_{\text{iso}}$ ,  $\cdots$ ), reduced linear dichroism ( $\text{LD}^r$ ,  $-$ ), and fluorescence anisotropy (FA,  $-\cdot-$ ) spectra of 2AP.  $A_{\text{iso}}$  and  $\text{LD}^r$  were determined from measurements in stretched PVA film and FA in EPA glass at 80 K. (b) Magnetic circular dichroism (MCD) of 2AP in water.

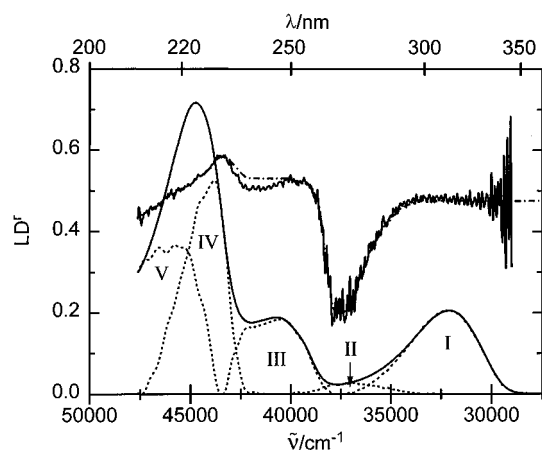
moments. (iv) The concluded electronic transitions for the 2AP chromophore are finally compared to the transition energies, oscillator strengths, and moment directions calculated by the INDO/S method.

**(i) Electronic Absorption Spectrum of 2AP.** Figure 2a shows the  $A_{\text{iso}}$ ,  $\text{LD}^r$ , and FA spectra of 2-aminopurine. The low-resolution absorption spectrum shows three major bands in the near-UV region with maxima near 33 000, 41 000, and 46 000  $\text{cm}^{-1}$ . The  $\text{LD}^r$  and FA spectra display the variation of the effective polarization with excitation energy. The  $\text{LD}^r$ , on the one hand, measures to what degree the transition moments of different electronic transitions align with the preferred molecular orientation axis, i.e. the projections of the transition moments on the orientation axis. The FA, on the other hand, measures the projections of the absorption transition moments onto the emitting moment. Since the FA for the lowest absorption band is close to its theoretical limit, +0.4, it can be concluded that the lowest absorption transition moment is almost parallel to the emitting moment. Knowing the degree of orientation and the direction of the orientation axis for the solute molecule in a stretched film experiment allows the calculation of the transition moment directions from the  $\text{LD}^r$  values (eq 4). However, the quantitative analysis will be deferred to a later section and the variation of the polarization ( $\text{LD}^r$  and FA) will at this point only be used qualitatively to find the number of different electronic transitions.

The  $A_{\text{iso}}$ ,  $\text{LD}^r$ , and FA spectra are very similar for 2AP, 2A9MP, and 2APr as seen in Figures 2a, 3a, and 4 (the FA spectrum for 2APr is omitted in Figure 4 for clarity). It is therefore logical to analyze the UV spectra of these molecules simultaneously, with the assumption that they share the same chromophoric core with small structural perturbations influencing neither the number of electronic transition nor their character.



**Figure 3.** (a) Isotropic absorption ( $A_{\text{iso}}$ ,  $\cdots$ ), reduced linear dichroism ( $\text{LD}^r$ ,  $-$ ), and fluorescence anisotropy (FA,  $-\cdot-$ ) spectra of 2A9MP.  $A_{\text{iso}}$  and  $\text{LD}^r$  were measured in stretched PVA film and FA in EPA glass at 80 K. (b) Magnetic circular dichroism (MCD) of 2A9MP in water.



**Figure 4.** Spectral resolution of the absorption spectrum of 2APr in PVA film into purely polarized absorption components (I–V,  $-\cdot-$ ). The band shapes correspond to the effective polarizations determined using the TEM method (see text). Measured ( $-$ ) and fitted ( $-\cdot-$ )  $A_{\text{iso}}$  and  $\text{LD}^r$  spectra.

The lowest absorption band has a constant polarization indicating that only one electronic transition contributes (transition I). The MCD spectra in Figures 2b and 3b also show a single band further supporting that a single electronic transition contributes to the lowest absorption band. At higher energy the polarization decreases dramatically and reaches a minimum at 37 000  $\text{cm}^{-1}$  between the two lowest absorption bands. This indicates a hidden weak transition (II) with a large angle both to the orientation axis and to the lowest electronic transition. The polarization increases again for the second absorption band at 41 000  $\text{cm}^{-1}$  and this peak is assigned to the third electronic transition (III). At around 46 000  $\text{cm}^{-1}$  we find the third prominent absorption band. The  $\text{LD}^r$  varies slightly over this

**Table 1.** Observed Electronic Transitions for 2AP, 2A9MP, and 2APr

|                    | trans (type)                    | $\bar{\nu}^a/\text{cm}^{-1}$ | $\epsilon^b/\text{M}^{-1} \text{cm}^{-1}$ | $\text{LD}^c$ | $\theta^d/\text{deg}$ | FA    | $\beta^d/\text{deg}$ |
|--------------------|---------------------------------|------------------------------|---|---------------|-----------------------|-------|----------------------|
| 2AP                | I ( $\pi \rightarrow \pi^*$ )   | 32700                        | 5400                                      | 0.45–0.60     | 48–39                 | 0.37  | 0                    |
| $S_{xx} = -0.33^e$ | II ( $n \rightarrow \pi^*$ )    | $\sim 36000$                 | $\sim 140$                                | 0.42          | oop                   | 0.057 | 49                   |
| $S_{yy} = 0.00^e$  | III ( $\pi \rightarrow \pi^*$ ) | 41400                        | 4200                                      | 0.96          | 11                    | 0.18  | 36                   |
| $S_{zz} = 0.33^e$  | IV ( $\pi \rightarrow \pi^*$ )  | 45400                        | 14900                                     | 1.0           | 0                     | 0.09  | 45                   |
|                    | V ( $\pi \rightarrow \pi^*$ )   | 48500                        | 10900                                     | 0.87          | 21                    |       |                      |
| 2A9MP              | I ( $\pi \rightarrow \pi^*$ )   | 32800                        | 5500                                      | 0.55          | 32                    | 0.38  | 0                    |
| $S_{xx} = -0.28^e$ | II ( $n \rightarrow \pi^*$ )    | $\sim 36000$                 | $\sim 140$                                | 0.23          | oop                   | 0.045 | 50                   |
| $S_{yy} = 0.04^e$  | III ( $\pi \rightarrow \pi^*$ ) | 40000                        | 3600                                      | 0.63          | 23                    | 0.14  | 40                   |
| $S_{zz} = 0.24^e$  | IV ( $\pi \rightarrow \pi^*$ )  | 44400                        | 16700                                     | 0.66          | 18                    | 0.065 | 48                   |
|                    | V ( $\pi \rightarrow \pi^*$ )   | 46700                        | 11300                                     | 0.59          | 28                    |       |                      |
| 2APr               | I ( $\pi \rightarrow \pi^*$ )   | 32700                        | 5500                                      | 0.47          | 28                    | 0.37  | 0                    |
| $S_{xx} = -0.21^e$ | II ( $n \rightarrow \pi^*$ )    | $\sim 36000$                 | $\sim 140$                                | 0.20          | oop                   | 0.058 | 49                   |
| $S_{yy} = 0.01^e$  | III ( $\pi \rightarrow \pi^*$ ) | 40000                        | 4700                                      | 0.53          | 21                    | 0.18  | 36                   |
| $S_{zz} = 0.20^e$  | IV ( $\pi \rightarrow \pi^*$ )  | 44400                        | 14100                                     | 0.58          | 11                    | 0.09  | 45                   |
|                    | V ( $\pi \rightarrow \pi^*$ )   | 46700                        | 9800                                      | 0.44          | 32                    |       |                      |

<sup>a</sup> Wavenumber of absorption maximum in water solution. Estimated from resolution of band shapes in PVA film. <sup>b</sup> Molar absorptivities in water solution. <sup>c</sup> Angle between transition moment and the orientation axis. oop = out-of-plane polarization. <sup>d</sup> Angle between the *i*th transition moment and the transition moment of the lowest lying transition. <sup>e</sup> Orientation parameters from Holmén *et al.*<sup>38</sup>

band and two overlapping transitions (IV and V) of about equal magnitude are needed to explain the observed spectra.

Further evidence for the spectral assignments is found in the MCD spectra (Figures 2b and 3b). The MCD of the first absorption band is negative with a band shape similar to the absorption spectrum supporting that only one electronic transition contributes.<sup>40</sup> The MCD spectrum is difficult to analyze for the higher lying transitions, but it is reassuring to notice that prominent features of the absorption spectrum are found in the MCD as well.

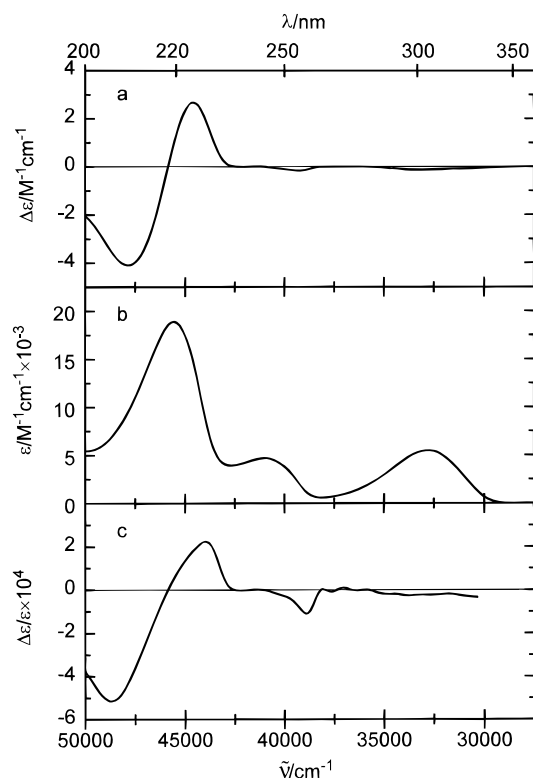
**(ii) Spectral Resolution and Circular Dichroism.** In order to quantify the contributions of the individual electronic transitions to the observed absorption spectrum, a resolution based on the assumption of purely polarized pair-wise overlapping transitions was performed. Linear combinations of the polarized components ( $A_{||}$  and  $A_{\perp}$ ) are formed in a trial and error fashion and the contribution from a given electronic transition is removed yielding the pure band shape of the remaining neighboring transition. For the lowest electronic transition this procedure directly yields the band shape, but for transitions having overlap on both sides from two different electronic transitions the band shapes have to be catenated from two different pieces. Figure 4 shows the absorption spectrum of 2APr resolved into five electronic transitions. Table 1 comprises the spectral parameters concluded from the fit such as band maxima, molar absorptivities, and intrinsic  $\text{LD}^c$  values for these electronic transitions. Figure 4 also shows the sum of the absorption components and the corresponding calculated  $\text{LD}^c$  spectrum (eq 8). The fits with respect to both these observations are almost perfect. However, it should be noted that the resolution of band shapes has been made under the assumption of only pair-wise overlap and that such a procedure is an oversimplification and could lead to artefacts. The high energy side of the third band (Figure 4) is probably not representative for the correct band shape of this transition which in turn indicates that it is not only transition IV that overlaps in this region but there might be contributions from higher lying transitions and/or that one or more weak transitions are present between 40 000 and 42 000  $\text{cm}^{-1}$ . There is also a slight deviation in  $\text{LD}^c$  at 42 000  $\text{cm}^{-1}$  which we will further comment on in the Discussion section.

(40) Numerous examples are found in the literature where two isolated overlapping electronic transitions give rise to a bisignate MCD spectrum. See for example: Voelter, W.; Records, R.; Bunnenberg, E.; Djerassi, C. *J. Am. Chem. Soc.* **1968**, *90*, 6163. Albinsson, B.; Kubista, M.; Nordén, B.; Thulstrup, E. W. *J. Phys. Chem.* **1989**, *93*, 6646. Albinsson, B.; Eriksson, S.; Lyng, R.; Kubista, M. *Chem. Phys.* **1991**, *151*, 149.

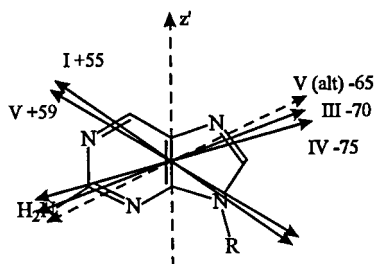
Figure 5 shows the CD,  $A_{\text{iso}}$ , and dissymmetry factor spectra of 2APr in neutral water solution. The CD is very weak for the two lowest absorption bands but shows a strong bisignate feature for the third band at 46 000  $\text{cm}^{-1}$ . This is another indication for the existence of, at least, two overlapping transitions in this region (IV and V). Noteworthy is also the sharp peak in the dissymmetry factor in the region between the first and second absorption bands. This might be associated with the weak transition (II) found from the resolution of the polarized absorption spectra.

**(iii) Molecular Orientation and Transition Moment Directions.** In order to use the concluded  $\text{LD}^c$  values of Table 1 to calculate the directions of the transition moments within the molecular framework we need to know the orientation parameters and the direction of the preferred molecular orientation axis. These properties are found from polarized IR measurements on the different solute molecules in stretched PVA films. IR linear dichroism measurements and *ab initio* calculations of the IR transition moments have been investigated in a study of the effects of substituents on the vibrational spectra of aminopurines.<sup>38</sup> The orientation parameters determined in that study are cited in Table 1. The direction of the orientation axes for 2AP and 2A9MP is assumed to be along their respective principal minimum moment of inertia axes which lie in the purine plane for both molecules as deduced from geometry optimizations at the DFT(B3LYP)/6-31G(d,p) level.<sup>38</sup> This leads to orientation axes directions for 2AP and 2A9MP equal to  $-76^\circ$  and  $-93^\circ$  (i.e.  $+87^\circ$ ), respectively (Figure 1). Further realignment of the molecular orientation axis is expected upon sugar substitution, and a  $10^\circ$  rotation of the effective orientation axis toward the sugar moiety is indeed observed when comparing 2A9MP and 2APr in the IR study. 2AP exhibits the highest degree of orientation, i.e. has higher absolute values of the orientation parameters  $S_{xx}$  and  $S_{zz}$ . That the orientation is better than those of the 9-substituted derivatives is expected since putting a substituent almost perpendicular to the initial molecular orientation axis should impair the orientation.

The directions of the electronic transition moments relative to the molecular orientation axis are given in Table 1 (angle  $\theta$ ). These values are calculated from the intrinsic  $\text{LD}^c$  values (eq 4) using the orientation parameters from the IR measurements. With knowledge of the orientation axis direction it is now simple to calculate two possible directions for each electronic transition moment (eq 10). The sign ambiguity can be experimentally solved in favorable cases. From the FA values the angles between transition moments of the lowest



**Figure 5.** Circular dichroism (a), isotropic absorbance (b), and dissymmetry factor (c) of 2APr in neutral water solution.



**Figure 6.** Electronic transition moment directions of the 2-aminopurine chromophore.

electronic transition and the higher ones are obtained. These angles are also listed in Table 1 ( $\beta$ ). Comparison between the relative  $\theta$  values and the  $\beta$  values provides the possibility for disregarding one of the two directions of the transition moments. It is therefore concluded that in-plane polarized transitions III and IV are on the opposite side of the orientation axis compared to transition I.

The orientation axis changes direction upon 9-substitution as already stated. If the electronic transitions are unaffected by substitution, as can be expected for 9-substitution of the 2AP molecule with "inert" substituents, the change in angle  $\theta$  might be used to obtain the absolute directions of the transition moments. For example, the angle  $\theta$  for the first electronic transition in 2AP decreases about  $10^\circ$  upon 9 substitution. This is a strong indication that transition I is polarized at  $+55^\circ$  (Figure 6) rather than at the alternative solution  $-61^\circ$ . In the latter case the angle  $\theta$  would be expected to increase upon 9 substitution. The other electronic transitions in Table 1 are treated similarly yielding a complete list of absolute transition moment directions for the 2AP chromophore. These are given in Table 2 and are depicted in Figure 6.

**(iv) Comparison with Calculations—Assignments.** The 2AP chromophore is essentially planar.<sup>38</sup> Its valence molecular orbitals may therefore be considered to be of either  $\pi$  or  $\sigma$

symmetry and the possible electronic transitions are of  $\sigma \rightarrow \sigma^*$ ,  $\sigma \rightarrow \pi^*$ ,  $\pi \rightarrow \sigma^*$ , or  $\pi \rightarrow \pi^*$  type. All of these transitions except the  $\pi \rightarrow \pi^*$  are usually of too high energy to be readily observed in aromatic compounds. However, in molecules that possess lone-pair orbitals, as for example some N-heterocycles, high-lying nonbonded electrons could be excited into the  $\pi^*$  orbitals and these  $n \rightarrow \pi^*$  transitions are of course formally of the  $\sigma \rightarrow \pi^*$  type. Thus, we expect the near-UV spectrum of 2AP to be composed of a number of in-plane polarized transitions of  $\pi \rightarrow \pi^*$  character and also out-of-plane polarized, comparably weaker, transitions of  $n \rightarrow \pi^*$  character.

The INDO/S method was used to determine the molecular orbitals for 2A9MP. Solvent effects on the electronic spectrum were simulated by a self consistent reaction field calculation on a complex between five water molecules and one 2A9MP molecule. The properties of the excited states were modeled by a configuration interaction calculation where 76 singly excited configurations were included. The results from these calculations are summarized in Table 2. The results with and without water are very similar with respect to transition energies, oscillator strengths, and moment directions. There is a slight red-shift for a majority of the  $\pi \rightarrow \pi^*$  transitions and a blue-shift for many of the  $n \rightarrow \pi^*$  transitions when adding the solvent, in accordance with general experimental experience.

The lowest electronic transition ( $S_0 \rightarrow S_1$ ,  $\pi \rightarrow \pi^*$ ) is calculated to be moderately strong, centered at about  $33\,000\text{ cm}^{-1}$  and polarized at an angle  $+60^\circ$ . This is almost in perfect agreement with the experimental observations for transition I and leaves little doubt for its assignment.

The second calculated electronic transition is of  $n \rightarrow \pi^*$  character and is therefore weak and out-of-plane polarized. We assign it to be the second transition resolved in the spectrum (Figure 4). It is generally difficult to observe such a weak transition in the presence of strong  $\pi \rightarrow \pi^*$  transitions but in this case we believe that we have firm evidence of an  $n \rightarrow \pi^*$  transition in the valley between the first two absorption bands. Firstly, the pronounced drop in both LD<sup>r</sup> and FA shows the existence of a weak transition which is polarized with a large angle to the orientation axis and the lowest electronic transition moment, respectively. Secondly, comparison with purine where the position of the lowest  $n \rightarrow \pi^*$  transition is known<sup>12</sup> and using qualitative theory for the substituent effects predicts the existence of an  $n \rightarrow \pi^*$  transition in this region. Thirdly, the INDO/S calculations agree quantitatively with this assignment and there is no additional weak  $\pi \rightarrow \pi^*$  transition predicted in this region.

The next transition predicted ( $S_0 \rightarrow S_3$ ) is of  $\pi \rightarrow \pi^*$  character and is assigned to the experimentally observed transition III. The calculated oscillator strength, energy, and moment direction again agree almost exactly with the experimental observations.

The following four calculated transitions ( $S_0 \rightarrow S_4$ ,  $S_5$ ,  $S_6$ , and  $S_7$ ) are all too weak to be observed in the presence of other much stronger transitions. The two overlapping strong transitions (IV and V) contributing to the third absorption band at  $46\,000\text{ cm}^{-1}$  are assigned to the next two strong close-lying  $\pi \rightarrow \pi^*$  transitions ( $S_0 \rightarrow S_8$  and  $S_0 \rightarrow S_9$ ). The observed strengths, energies, and moment directions are in fair agreement with the calculated results. In the high-energy region it becomes increasingly difficult to make accurate experimental observations and e.g. the position, strength, and moment direction of transition V are less accurately determined than for the transitions at low energy.

## Discussion

**Ground State Species—Tautomerism.** All purine derivatives have several conceivable tautomers. This has been known

**Table 2.** Calculated and Experimental Electronic Transitions for 2A9MP

| INDO/S <sup>a</sup>   |                           |                            |        |                        |                            |       |                        |   |              |                            |                   |                        |  |
|-----------------------|---------------------------|----------------------------|--------|------------------------|----------------------------|-------|------------------------|---|--------------|----------------------------|-------------------|------------------------|--|
| gas phase             |                           |                            |        |                        | water <sup>b</sup>         |       |                        |   | experimental |                            |                   |                        |  |
| trans                 | character                 | $\bar{\nu}/\text{cm}^{-1}$ | $f^c$  | $\delta^d/\text{deg.}$ | $\bar{\nu}/\text{cm}^{-1}$ | $f^c$ | $\delta^d/\text{deg.}$ | $\pi \rightarrow \pi^*$ main config <sup>e</sup>  | trans        | $\bar{\nu}/\text{cm}^{-1}$ | $f^c$             | $\delta^d/\text{deg.}$ |  |
| $S_0 \rightarrow S_1$ | $(\pi \rightarrow \pi^*)$ | 33550                      | 0.19   | +64                    | 33043                      | 0.20  | +60                    | H $\rightarrow$ L (0.86)<br>H $\rightarrow$ L + 1 (0.33)<br>H - 1 $\rightarrow$ L + 1 (0.24)    | I            | 32800                      | 0.10              | +55                    |  |
| $S_0 \rightarrow S_2$ | $(n \rightarrow \pi^*)$   | 34162                      | 0.009  | oop                    | 33983                      | 0.008 | oop                    |   | II           | ~36000                     | ~0.002            | oop                    |  |
| $S_0 \rightarrow S_3$ | $(\pi \rightarrow \pi^*)$ | 38094                      | 0.28   | -76                    | 38088                      | 0.29  | -76                    | H $\rightarrow$ L + 1 (0.85)<br>H - 1 $\rightarrow$ L (-0.33)<br>H $\rightarrow$ L (-0.39)      | III          | 40000                      | 0.06              | -70                    |  |
| $S_0 \rightarrow S_4$ | $(n \rightarrow \pi^*)$   | 38599                      | 0.005  | oop                    | 39278                      | 0.006 | oop                    |   |              |                            |                   |                        |  |
| $S_0 \rightarrow S_5$ | $(n \rightarrow \pi^*)$   | 41465                      | 0.02   | oop                    | 41655                      | 0.02  | oop                    |   |              |                            |                   |                        |  |
| $S_0 \rightarrow S_6$ | $(\pi \rightarrow \pi^*)$ | 43793                      | 0.006  | -35                    | 43697                      | 0.01  | -53                    | H $\rightarrow$ L + 2 (0.85)<br>H - 1 $\rightarrow$ L (-0.43)                                   |              |                            |                   |                        |  |
| $S_0 \rightarrow S_7$ | $(n \rightarrow \pi^*)$   | 45438                      | 0.0007 | oop                    | 45227                      | 0.005 | oop                    |   |              |                            |                   |                        |  |
| $S_0 \rightarrow S_8$ | $(\pi \rightarrow \pi^*)$ | 46442                      | 0.66   | -48                    | 45314                      | 0.65  | -50                    | H - 1 $\rightarrow$ L (-0.75)<br>H $\rightarrow$ L + 2 (-0.42)<br>H $\rightarrow$ L + 1 (-0.31) | IV           | 44400                      | 0.19              | -75                    |  |
| $S_0 \rightarrow S_9$ | $(\pi \rightarrow \pi^*)$ | 50810                      | 0.67   | +54                    | 50610                      | 0.60  | +54                    | H - 1 $\rightarrow$ L + 1 (-0.87)<br>H $\rightarrow$ L + 4 (-0.27)<br>H $\rightarrow$ L (0.23)  | V            | 46700                      | 0.13 <sup>f</sup> | +59 or -65             |  |

<sup>a</sup> 76 singly excited configurations were included in the CI calculation. <sup>b</sup> Water solution simulated in a SCRF calculation on a 2A9MP/5H<sub>2</sub>O complex (SCRF Model B<sup>37</sup>). <sup>c</sup> Oscillator strength. <sup>d</sup> In-plane angle defined according to Figure 1. oop = out-of-plane polarization. <sup>e</sup> The CI coefficients are given in parentheses. H = HOMO; L = LUMO. <sup>f</sup> Estimated from the relative molar absorptivities of transitions IV and V.

for quite some time, and experimental<sup>17,18a-c</sup> as well as theoretical<sup>19,41,42</sup> investigations on this subject have been performed. For the aminopurines, adenine and 2AP, only the prototropic 7-H and 9-H tautomers have low enough energy to be present at room temperature. Adenine is a mixture of about 20% 7-H form and 80% 9-H form in water<sup>18b</sup> and in dimethyl sulfoxide<sup>17b</sup> (DMSO), but in the gas phase<sup>43</sup> and in rare-gas matrices<sup>42</sup> the 9-H tautomer dominates. This dependence on solvent polarity has been explained by the 7-H tautomer having a substantially larger dipole moment than the 9-H tautomer.<sup>19,44</sup>

Naturally, the amount of published material is less exhaustive for 2-aminopurine. Very recent calculations show that the 9-H tautomer has about 18 kJ/mol lower enthalpy of formation than the 7-H tautomer of 2-aminopurine in the gas phase.<sup>39</sup> This predicts only a very small amount of the 7-H tautomer at room temperature (<1%). The effects of water on the heat of formations were also included but still only minor contributions of 7-H-2AP were predicted.<sup>39</sup> As far as we know, no experimental investigation on the tautomeric equilibrium of 2AP has been performed.

The electronic absorption spectra of 2AP and 2A9MP in stretched PVA and other solvents are similar. However, 2AP has a significant drop in the LD<sup>f</sup> (Figure 2) over the red edge of the first band which is not observed for either 2A9MP or 2APr (Figures 3 and 4). The INDO/S spectra of the 9-H tautomer of 2AP and 2A9MP are very similar while the calculated spectrum of the 7-H tautomer is slightly red shifted and has an  $n \rightarrow \pi^*$  transition calculated at 33 008 cm<sup>-1</sup> as the lowest lying transition. The second transition is a relatively strong ( $f = 0.20$ )  $\pi \rightarrow \pi^*$  transition calculated at 33282 cm<sup>-1</sup> with a polarization equal to +55° which is similar to the calculated polarization (+62°) of the lowest lying transition in the 9-H tautomer of 2AP. The drop in LD<sup>f</sup> below 33 000 cm<sup>-1</sup>

can be explained by a very small amount of the 7-H tautomer present in the PVA film, if it holds experimentally that it has an  $n \rightarrow \pi^*$  transition as the lowest transition in analogy with the 7-H and 9-H tautomers of purine.<sup>12</sup> The fluorescence from 2AP is that of a single ground state species with no dependence on band shape of the emission with excitation wavelength or vice versa both in room temperature aqueous solution and in low temperature EPA glass. Furthermore, the fluorescence decay is a clean single exponential with a lifetime close to the lifetimes found for 2A9MP and 2APr (Table 3). These data imply that the 7-H tautomer present has to have a very low quantum yield for fluorescence which in fact is expected if the lowest lying state is an  $n\pi^*$  state as in the case of purine,<sup>12</sup> or if the lowest  $n\pi^*$  and  $\pi\pi^*$  states are very close in energy.<sup>45</sup> In the following, 2AP will be treated as the single 9-H tautomer bearing in mind that there might be minor contributions from the 7-H tautomer.

**Electronic Spectrum—Assignments.** The near-UV absorption spectrum of 2AP is here described by five electronic transitions. It should be realized that this is the minimum number of components needed to explain the present experimental observations ( $A_{\text{iso}}$ , LD<sup>f</sup>, FA, MCD, and CD) and additional electronic transitions, albeit much weaker, are suggested by the theoretical calculations. In spite of this discrepancy, the agreement between experiments and calculated excitation energies, oscillator strengths, and transition moment directions must be described as exceptional. Although the INDO/S method is known to produce relatively reliable results for hetero-atom-containing chromophores, the purine bases have been exceedingly difficult to model.<sup>46</sup>

The first absorption band is due to a single  $\pi \rightarrow \pi^*$  transition. This conclusion is supported by a constant polarization (Figures 2, 3, and 4), simple absorbance-like MCD spectra (Figures 2b and 3b), and the INDO/S calculation. It is also supported by the natural lifetimes,  $\tau_0 = \tau/\phi_f$ , as measured (Table 3) and compared to calculations based on the intensity of the lowest absorption band. Using the Strickler–Berg equation<sup>47</sup> and the resolution of Figure 4 yields a natural lifetime of  $\tau_0(\text{calc}) =$

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**Table 3.** Fluorescence Properties of 2AP, 2A9MP, and 2APr in Water

|                          | 2AP   | 2A9MP | 2APr  |
|--------------------------|-------|-------|-------|
| $\nu_r^a/\text{cm}^{-1}$ | 27510 | 27850 | 27820 |
| $\phi^b$                 | 0.66  | 0.72  | 0.65  |
| $\tau^c/\text{ns}$       | 11.8  | 11.1  | 10.4  |
| $\tau_0^d/\text{ns}$     | 17.9  | 15.4  | 16.0  |

<sup>a</sup> Wavenumber of fluorescence maximum. <sup>b</sup> Based on  $\phi_r = 0.85$  for 2,5-diphenyloxazole (PPO) in cyclohexane.<sup>28</sup> Excitation at 33333  $\text{cm}^{-1}$  (300 nm). <sup>c</sup> Observed fluorescence lifetime. <sup>d</sup> Natural lifetime.

13.0 ns for 2APr in close agreement with the measured value. Similar results are found for 2AP and 2APr.

The most remarkable result from an experimental viewpoint is the resolution of the  $n \rightarrow \pi^*$  band. The position of this transition in the adenine base has been discussed for a long time and it is probably of great consequence for the photo-physical behavior of adenine and other purine-based chromophores. In purine, two equally strong  $\pi \rightarrow \pi^*$  transitions contribute to the first absorption band centered at 260 nm and a weak  $n \rightarrow \pi^*$  transition is observed at the red edge of this band.<sup>12</sup> In this case the weak  $n \rightarrow \pi^*$  transition is easily identified at about 290 nm (34 500  $\text{cm}^{-1}$ ). Upon amino substitution at the 2 position to form 2AP, the  $n\pi^*$  transition blue shifts by about 2000  $\text{cm}^{-1}$  and one of the  $\pi \rightarrow \pi^*$  transitions red shifts by 4000  $\text{cm}^{-1}$ . This reversal of states changes the photophysical behavior of the purine molecule from non-fluorescent at room temperature and phosphorescent at 77 K to become strongly fluorescent (quantum yields and lifetimes for fluorescence are found in Table 3). In purine the lowest excited state is of  $n\pi^*$  character with the next  $\pi\pi^*$  state at 3000  $\text{cm}^{-1}$  higher energy, whereas in 2AP the  $\pi\pi^*$  state is about 4000  $\text{cm}^{-1}$  lower in energy than the lowest  $n\pi^*$  state (Table 2).

The strength of the  $n \rightarrow \pi^*$  transition in the 2AP chromophore is difficult to determine due to the rather strong overlap with transition I, but an estimate can be made from the observed  $\text{LD}^f$  value (0.23) at the wavenumber of this transition. This is the weighted average (eq 8) of the  $\text{LD}^f$  values of the pure electronic transitions I and II. If we use the  $\text{LD}^f$  value for transition I, 0.55 for 2A9MP, and the value corresponding to a pure out-of-plane transition (eq 5, Table 1),  $-0.84$ , a molar absorptivity of about 140  $\text{M}^{-1} \text{cm}^{-1}$  for the  $n \rightarrow \pi^*$  transition is needed in order to explain the observed value. The strength of this transition is in good agreement with previous observations for  $n \rightarrow \pi^*$  transitions in heterocyclic molecules.<sup>12,48</sup>

The observation of an  $n \rightarrow \pi^*$  transition among the  $\pi \rightarrow \pi^*$  transitions brings up the question whether the less negative reduced linear dichroism features reported early<sup>49</sup> for DNA in flow linear dichroism measurements could be caused by out-of-plane polarized intensity due to  $n \rightarrow \pi^*$  transitions in the natural nucleic acid bases. While Matsouka and Nordén<sup>50</sup> could fit their  $\text{LD}^f$  spectra of B and A form DNA in the 220–300-

nm region using standard geometries and transition moments with an out-of-plane component with  $\epsilon = 250 \text{ M}^{-1} \text{cm}^{-1}$  at about 233 nm, Johnson and co-workers<sup>51</sup> have interpreted the variations in  $\text{LD}^f$ , as far as to 180 nm, in terms of inclinations of the nucleic acid bases from the ideal almost perpendicular B form geometry. In DNA, as well as in stretched film measurements on the isolated nucleic acid bases (due to their more disk-like rod-like orientation behavior), even relatively weak out-of-plane intensity is expected to give a noticeable effect in the  $\text{LD}^f$  due to their orthogonality with respect to the  $\pi \rightarrow \pi^*$  transitions.<sup>23</sup> It is therefore interesting to note that at 42 000  $\text{cm}^{-1}$  (238 nm) for 2APr there is a weak but significant feature in the  $\text{LD}^f$  (Figure 4) which cannot be accounted for by the fitted  $\pi \rightarrow \pi^*$  transitions. It is thus quite possible that this feature is due to another  $n \rightarrow \pi^*$  transition (to fit the experimental spectrum it should have an intensity corresponding to roughly  $\epsilon = 150 \text{ M}^{-1} \text{cm}^{-1}$ ).

It appears to be a one-to-one relation of the electronic transitions in 2AP to the corresponding transitions in purine. The lowest energy  $\pi \rightarrow \pi^*$  transition in purine is substantially red shifted upon forming the 2-amino adduct but is otherwise not affected (same direction and oscillator strength). The second  $\pi \rightarrow \pi^*$  transition in purine is unaffected with respect to energy, transition moment direction, and strength. This holds approximately for the two higher energy  $\pi \rightarrow \pi^*$  transitions as well. As judged by the appearance of the molecular orbitals and their relative contributions to the calculated electronic transitions, the character of the excited states is not changed when going from purine to 2AP, it is merely the energy of some excitations that have changed. This conservation of excited state character does not seem to be experimentally verified for adenine, and a simple one-to-one relation with purine is probably not identifiable in that case.<sup>52</sup>

## Conclusions

The electronic absorption spectrum of the 2AP chromophore was resolved into five different electronic transitions. The absolute transition moment directions for these transitions were experimentally determined. INDO/S calculations support the spectral assignments, and the degree of conformity between experiment and theory is surprisingly high. The lowest excited state of 2AP is of  $\pi\pi^*$  character while the second excited state is of  $n\pi^*$  character. The order of these states is reversed in comparison with purine which explains the drastic differences in photophysics between purine and 2AP.

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