

## Chapter 8

### EXPERIMENTAL PROCEDURES

#### 8.1 Introduction

The excited-state proton-transfer reaction of 1AC was studied here using steady-state absorption and emission spectroscopy and time-correlated single-photon counting techniques. Experimental details are gathered in this chapter.

#### 8.2 Reagents

1AC was synthesized following the recipe of Stephenson and Warburton.<sup>1</sup> The complexing agents used in the isolated complexes experiment were purchased from the Aldrich Chemical Company and were used as received: acetamide [99+%], acetic acid (AA) [99.7+%, ACS reagent grade], deuterated acetic acid (AA-D) [98 atom % D], benzamide [99.5+%, sublimed], 2-cyanoacetamide [99%], 3,4,5,6,7,8-hexahydro-2(1H)-quinoline (HHQ) [97%], methylcyclohexane [99%, spectrophotometric grade], N-methylformamide [99%], 2,3,4,5,6-pentafluorobenzamide [99%], succinimide [98%], 2,2,2-trifluoroacetamide [97%], and  $\delta$ -valerolactam ( $\delta$ -VL) [99%]. N-methylformamide was either used as received or distilled under nitrogen. Dry methylcyclohexane for the

complexation studies was prepared by refluxing for 30 minutes over calcium hydride followed by distillation under nitrogen.

Most reagents for the bulk protic solvent work were also used as obtained from Aldrich Chemical Company: (anhydrous) benzyl alcohol [99+%], deuterium oxide [99.9 atom % D], ethylene glycol [99+%, spectrophotometric grade], ethylene glycol-D2 [98 atom % D], formamide [99+%, spectrophotometric grade], methanol [99.9%, HPLC grade], methanol-OD [99.5+ atom % D], 1-pentanol [99+%; stored over molecular sieves], 1-propanol [99.5%, HPLC grade], 2-propanol [99.5%, HPLC grade], 1,2-propanediol [99.5+%, ACS reagent grade] and 2,2,2-trifluoroethanol [99+%]. The t-butanol was from Mallinckrodt. Ultrapure water [18 M $\Omega$ .cm] or distilled water [~0.5 M $\Omega$ .cm] was found to yield similar results. N-methylformamide and benzyl alcohol were either used as received or distilled under nitrogen. 2,2,2-Trifluoroethanol was distilled under nitrogen immediately prior to use. The aprotic solvents N,N-dimethylformamide (DMF) [99.9%, HPLC grade] and methylcyclohexane [99%, spectrophotometric grade] were from Aldrich Chemical Company. The DMF was distilled once under nitrogen. The aprotic solvents used in experiments were typically high quality solvents from Aldrich Chemical Company, and unless noted, were used as received without special drying procedures.

### **8.3 Instruments: Steady-State and Time-Resolved Spectrometers**

Absorption spectra were recorded on a Hitachi U-3000 spectrophotometer with 1 nm resolution, and steady-state fluorescence measurements were made with 2 nm

resolution on a Spex 212E Fluorolog corrected for instrument response.<sup>2</sup> Time-correlated single-photon counting was used to measure the time-resolved fluorescence emission.<sup>3,4</sup> This spectrometer has been described earlier.<sup>5-7</sup> Very briefly, picosecond light pulses (8-12 ps, 3.8 MHz, ~10 nJ / pulse) of wavelength 580-700 nm are generated by a cavity-dumped dye laser (modified Coherent 599) that is synchronously-pumped by the doubled output of a mode-locked Nd:YAG laser (Coherent Anteres 76). Most of the visible red light is frequency doubled to provide suitable UV excitation with vertical polarization, and it is focused into the sample cuvette maintained at constant temperature. Fluorescence emission is collected at a right angle, is passed through a polarizer set at the magic angle, and is focused onto the entrance slit of a 0.25-m single monochromator (Instruments SA, Inc., Model H-10). A Hamamatsu 3908U 6- $\mu$ m MCP-PMT detects the fluorescence photons, and its amplified (Philips 6954-S100) and conditioned (Tennelec 454 Constant Fraction Discriminator, modified) signal provides the start pulse for the time-to-amplitude converter (Tennelec 864 TAC / Biased Amp). A portion of the visible red light from the dye laser is split to trigger a fast photodiode (Optoelectronics PD-30) whose conditioned signal (Tennelec 454, modified) is delayed and is used as a stop signal for the TAC. A multichannel analyzer (Oxford Nucleus PCA-II) organizes the TAC signal (<1% start/stop) into a fluorescence decay, which is fit iteratively to deconvolute the instrument response function generated by a scattering solution of non-dairy creamer in water (absorbance < 0.1). The fitting algorithm employed here is “upcvfit”.<sup>8</sup> Good fits satisfied the statistical test  $\chi^2 < 1.2$  and displayed no significant ripples in the residuals.

For the experiments reported here, the spectrometer was operated with the following improvements. To minimize the dead-time of the time-to-amplitude converter [TAC] and thereby increase data acquisition rates, the experiment was run in the “reverse mode”<sup>3</sup>: the start signal for the TAC was provided by first fluorescence photon detected by a Hamamatsu 3908U MCP-PMT with Philips 6954-S100 preamplifier for the CFD, and the stop signal for the TAC was generated by an Optoelectronics PD-30 fast photodiode monitoring a portion of the cavity-dumped laser output. The 0.25-m single-monochromator (Instruments SA, Inc., Model H-10) with 2 mm slits allowed bandpass of 20 nm. Fluorescence decays were recorded over time ranges of 7 ns [3.51 ps/channel], 14 ns [6.72 ps/channel], or 27 ns [13.33 ps/channel] selected to best capture the emission decay. The instrument response of the spectrometer is 50-60 ps (FWHM) on the shortest time base, providing lifetime resolution of 25-30 ps.<sup>9</sup> The measured lifetime of a dilute solution of 2,5-diphenyloxazole [PPO] in methanol at 298 K is 1.52 ns and single-exponential, in acceptable agreement with  $1.4 \pm 0.2$  ns<sup>10</sup> and  $1.61 \pm 0.02$  ns<sup>11</sup> noted for this reference compound in ethanol. The absolute uncertainty in the measured fluorescence lifetimes is 10%, although the precision of the experiment is better than 5%.

#### **8.4 Sample Preparation**

The dilute 1AC:complex solutions were prepared in a glovebag that was evacuated twice before the final nitrogen fill to ensure satisfactory removal of oxygen and moisture. All glassware, cuvettes, and pipette-capillary tubes were baked at 120 °C for several hours prior to use. This precaution was an especially important procedure in

the formation of 1AC:AA-D. The nonpolar alkane solvent methylcyclohexane was chosen for this work to maximize the driving force for complex formation by minimizing extraneous interactions between the complexing agents and solvent.<sup>12</sup> Dilute solutions containing  $<18 \mu\text{M}$  1AC showed little tautomer emission from dimers prior to the addition of the complexing agents. Time-resolved tautomer emission from dimers was measured at higher concentrations [ $> 60 \mu\text{M}$ ] of 1AC in methylcyclohexane.

The preparation of 1AC complexes with deuterated acetic acid required considerable care since absorbed moisture on the surfaces of unbaked glassware, cuvettes, and pipette-capillary tubes was noted to reduce the observed kinetic isotope effect.<sup>13</sup> Neat deuterated acid was added directly to the 1AC solution in methylcyclohexane to minimize the exchange between the deuterons and protons from contamination.

Complexes of 1AC with the lactams or with acetic acid were created by spectrophotometric titration using 0.02 M lactam stock solutions or neat acetic acid. Because the solid amides are insoluble in methylcyclohexane, these complexes were formed by sonicating a  $15 \mu\text{M}$  solution of 1AC in methylcyclohexane with excess amide. The remaining insoluble amide was separated in a closed polycarbonate tube spun in a centrifuge (4000 or 20000 r.p.m. for 20 minutes). The liquid amides are also insoluble in methylcyclohexane, and therefore sonication was applied to form a dilute emulsion from a mixture of 5% v/v N-methylformamide in methylcyclohexane. A dilute 1AC solution was then spectrophotometrically titrated with this emulsion. Since the 1AC fluorescence

is quenched by oxygen, the samples were prepared in inert environments or were bubbled with nitrogen prior to measurements.

For studies in bulk protic solvents, the 1AC concentrations were less than 30  $\mu\text{M}$  to keep the absorbance below  $A=0.15$  at the exciting wavelength for emission spectra. Fluorescence emission of 1AC in bulk protic solvents was excited in the region 305-335 nm, and these emission lifetimes were independent of the excitation wavelength. Solutions of 1AC in bulk protic solvents were typically prepared to have a peak absorbance of 0.3-1.0 for the absorbance measurements. Most data for the other bulk protic solvents was measured at least twice.

## 8.5 Quantum Yield Measurements

Quantum yields were determined with respect to quinine sulfate in 1.0 N  $\text{H}_2\text{SO}_{4(\text{aq})}$  [ $\phi_{\text{ref}}=0.546$ ]<sup>14,15</sup> or in 0.1 N  $\text{HClO}_{4(\text{aq})}$  [ $\phi_{\text{ref}}=0.59$ ]<sup>15</sup> and are estimated to have an uncertainty of  $\pm 10\%$ . The quantum yields were calculated using the following expression, which corrects for differences in absorption between the solvents used for the quantum standard ( $A_{r,\text{sol}}$ ) and for the sample ( $A_{s,\text{sol}}$ ):

$$\mathbf{j}_{\text{sample}} = \mathbf{j}_{\text{ref}} \cdot \left( \frac{1 - 10^{-A_r}}{1 - 10^{-A_s}} \right) \cdot \left( \frac{1 - 10^{-A_{r,\text{sol}}}}{1 - 10^{-A_{s,\text{sol}}}} \right) \cdot \left( \frac{\int I_s(\mathbf{l}) d\mathbf{l}}{\int I_r(\mathbf{l}) d\mathbf{l}} \right) \cdot \left( \frac{n_s^2}{n_r^2} \right) \quad (8.1)$$

In this expression,  $\phi$ ,  $A$ ,  $n$  denote the quantum yield, absorbance, and index of refraction for the sample (s) and quantum yield reference (r), respectively. The absorbance of the

sample and quantum yield standard at the exciting wavelength was measured with respect to their corresponding solvent blanks, while the absorbance of each solvent blank was determined with respect to air. The corrected fluorescence intensity was integrated over the entire emission range to calculate the total quantum yield for the 1AC samples, and the normal and tautomer quantum yields were estimated according to their relative areas in the dual fluorescence. Excitation of the 1AC and quinine sulfate was at 330 nm [bulk protic solvents] or 320 nm [1AC in methylcyclohexane]. Within experimental uncertainty, the quantum yields of 1AC in methylcyclohexane or in water were constant for excitation from the first  $S_1$  or second  $S_2$  absorption bands.<sup>16</sup>

Quantum yields of the relative tautomer emission from the complexes were estimated relative to the quantum yield 0.54 for 1AC in methylcyclohexane with 328 nm excitation and the estimated quantum yield 0.0038 for the tautomer emission of 1AC:acetamide with 328 nm or 348 nm excitation and assuming a complete reaction.

## **8.6 Fluorescence Measurements**

### **8.6.1 1AC Complex Study**

The 1AC complexes were excited on the red-edge of the first absorption band [348 nm] to excite fluorescence selectively from complexed species. Normal emission was monitored at 430-440 nm to avoid contributions from Raman scattering. Tautomer emission was recorded at 550-560 nm, which prevented contamination from the tail of

the normal emission.<sup>17</sup> Solvent blanks were examined for fluorescent impurities. Values reported for the decay characteristics of most of the complexes discussed here represent an average of two or three independent measurements. (The 1AC:NMF emulsion experiment was completed once. A second experiment was attempted but failed due to difficulty in controlling the composition of the emulsion. The results of the first experiment are presented as interesting observations, but this an example of the difficulty in controlling the composition of hydrogen-bonded liquids in nonpolar alkane solvents.) The absorption and fluorescence emission were measured at room temperature,  $295 \pm 2$  K, and the fluorescence lifetimes were typically determined at 298 K.

### **8.6.2 Temperature Studies of 1AC in Diols, Benzyl Alcohol, Water and Amides**

The temperature dependence of the 1AC reaction rate was examined in ethylene glycol and ethylene glycol-D<sub>2</sub> (EG; 1,2-ethanediol, m.p. -13 °C); propylene glycol (PG; 1,2-propanediol, m.p. -60 °C); benzyl alcohol (BzOH; m.p. -15 °C); water and deuterium oxide (m.p. 0 °C); and the amides formamide (FA; m.p. 2-3 °C) and N-methylformamide (NMF; m.p. -40 °C). Measurements over the temperature range 1 °C - 70 °C were repeated twice for ethylene glycol and once for the other solvents. The temperature was regulated to  $\pm 0.5$  °C by constant-temperature water flowing through a brass sample block. Experiments on 7AI in EG were repeated to provide a base of comparison with earlier results.<sup>17</sup> Fluorescence in the temperature studies was excited at 290 nm for 7AI and 1AC in the diols and water, at 306 nm for 1AC in BzOH, at 328 nm for 1AC in FA, and at 331 nm for 1AC in NMF.

For some of the time-resolved emission in the EG samples, a wider bandpass was used to assist data collection. The sample cuvettes containing the EG samples also employed pieces of black glass to help reduce scatter in the photon-counting experiments. Later experiments found this black glass to be unnecessary. For the steady-state emission measurements in the diols, a Corning O-54 cutoff filter removed the 2nd-order diffraction of the 290 nm excitation while uniformly passing visible light above 340 nm. The deuterated ethylene glycol solvent was acidic which induced some protonation of the 7AI probe, as reflected in the steady-state temperature series. The temperature-dependence of the kinetics in BzOH, water, FA and NMF was measured directly without corrections from steady-state emission spectra.

### 8.6.3 1AC pH Study

The pH range 1-13 was examined roughly prior to focusing on the acidic-neutral range. Controlling the pH by addition of HCl or NaOH was found to produce unstable readings on VWR (Cat. No. 34100-674) and Beckman  $\Phi$ 40 pH meters, especially near neutral pH values. Therefore, a ~2 mM buffer solution of MES (4-morpholine-ethanesulfonic acid,  $pK_a = 6.1$ ) was used in these measurements to stabilize the pH measurements. Time-resolved emission measurements with 306 nm excitation were recorded at wavelengths 370 nm, 400 nm, 480 nm, and 560 nm in the normal and tautomer regions over the range pH=3-8. The experiment was repeated with excitation at the isobestic point at 331 nm to monitor the emission at 400 nm and 480 nm.

#### **8.6.4 1AC in Methanol / Water Solvent Mixtures**

Steady-state and time-resolved emission spectra of 1AC were measured in the series of mixtures 100:0, 90:10, 70:30, 50:50, 30:70, 10:90, and 0:100 [methanol:water, by volume]. Emission at 380-410 nm (normal) and 560 nm (tautomer) was monitored following excitation at 290 nm. The reported values represent an average of at least two measurements.

#### **8.6.5 1AC in Methanol / Methanol-OD Solvent Mixtures**

The time-resolved emission of normal 1AC in 7 mixtures of methanol and methanol-OD was recorded at 375 nm or 390 nm following excitation at 306 nm or 331 nm using the time-correlated single-photon counting spectrometer described here. The trueness of single-exponential fits and the reproducibility of the experiment were confirmed for independent measurements made over a two month period using the standard PPO in methanol ( $\tau = 1.53$  ns at 25 °C). Time-resolved emission was recorded over a 13 ns window (6.72 ps/channel). The mixtures were prepared gravimetrically, and the solutions were saturated with nitrogen prior to fluorescence measurements.

## ENDNOTES

- <sup>1</sup> (a) L. Stephenson and W. K. Warburton, *J. Chem. Soc. C*, **1970**, 1355-1364.
- (b) The synthesis was completed by S. J. Boryschuk, M.S. Thesis, The Pennsylvania State University, 1993.
- <sup>2</sup> (a) The instrument correction files for the emission and excitation scans (mcorr896.spt, xcor0896.spt) were created by J. A. Gardecki and M. Maroncelli.
- (b) Earlier instrument correction files (mcorr991.fin, xcorr991.fin) are discussed in R. S. Fee, Ph. D. Thesis, The Pennsylvania State University, 1994.
- <sup>3</sup> See, for example, J. R. Lakowicz, Ed., *Topics in Fluorescence Spectroscopy. Volume 1: Techniques*. (New York, Plenum Press, 1991).
- <sup>4</sup> A second introduction to TCSPC is G. R. Holtom, *SPIE Vol. 1204: Time-Resolved Laser Spectroscopy in Biochemistry II*, pp. 2-12 (1990).
- <sup>5</sup> C. F. Chapman, R. S. Fee, and M. Maroncelli, *J. Phys. Chem.*, **94**, 4929, (1990).
- <sup>6</sup> C. F. Chapman, Ph. D. Thesis, The Pennsylvania State University, 1993.
- <sup>7</sup> R. S. Fee, Ph. D. Thesis, The Pennsylvania State University, 1994.
- <sup>8</sup> This is an unpublished computer program written by M. Maroncelli.
- <sup>9</sup> (a) This resolution corresponds to HWHM of the instrument response function. It was confirmed by the measurements of the reaction in the 1AC: $\delta$ -valerolactam hydrogen-bonded complex: the decay in the normal region of  $\sim 30$  ps was resolved as a rise time of  $\sim 30$  ps in the tautomer region. Simulations of emission decays using a measured instrument response function reveal that rise times greater than 20 ps and decay times greater than 10 ps may be reasonably resolved. (b) The instrument response of this spectrometer based on a cavity-dumped, single-jet dye laser synchronously pumped by a mode-locked Nd:YAG laser is limited in part by the electronic detection and in part by the width ( $\sim 8$ -12 ps) of the excitation pulses. A similar spectrometer exploiting a Ti:Sapphire laser whose pulse widths are shorter ( $\sim 1$ -2 ps) operates with an instrument response function of  $\sim 25$ -30 ps FWHM.
- <sup>10</sup> J. R. Lakowicz, *Principles of Fluorescence Spectroscopy* (New York, Plenum Press, 1983). pp. 88-89.

- <sup>11</sup> D. J. S. Birch and R. E. Imhof in *Topics in Fluorescence Spectroscopy, Volume 1: Techniques*. [J. R. Lakowicz, Ed.] (New York, Plenum Press, 1991). p. 54.
- <sup>12</sup> J. W. Walmsley, *J. Phys. Chem.*, **85**, 3181-3187 (1981). This study on 7AI in several nonpolar solvents provides a discussion of complications with solvents such as benzene and carbon tetrachloride.
- <sup>13</sup> Other researchers have commented on the care required to form deuterated complexes. See, for example, T. Fiebig, M. Chachisvilis, M. Manger, A. H. Zewail, A. Douhl, I. Garcia-Ochoa, A. de La Hoz Ayuso, *J. Phys. Chem. A.*, **103**, 7419-7431 (1999).
- <sup>14</sup> J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, **75**, 991 (1971).
- <sup>15</sup> R. A. Velapoldi and K. D. Mielenz, *NBS Special Publication 260-64*, pp. 50-52. (1980)
- <sup>16</sup> These preliminary measurements indicate the photophysical behavior of 1AC may be contrasted with the monophotonic ionization reported for indole and its derivatives. See, for example, F. Gai, R. L. Rich, J. W. Petrich, *J. Am. Chem. Soc.*, **116**, 735 (1994).
- <sup>17</sup> R. S. Moog and M. Maroncelli, *J. Phys. Chem.*, **95**, 10359-10369 (1991).