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Coumarin-decorated Schiff base hydrolysis as an efficient driving force for the fluorescent detection of water in organic solvents

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Won Young Kim,^{a,‡} Hu Shi,^{b,‡} Hyo Sung Jung,^{a,‡} Daeheum Cho,^b Peter Verwilst,^a Jin Yong Lee,^{b,*} and Jong Seung Kim^{a,*}

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A coumarin based Schiff base was found to be an excellent indicator of moisture, *via* rapid *in situ* hydrolysis. A structure-relationship examination of a small library of Schiff bases revealed the critical importance of hydrogen bond acceptors in close proximity to the imine bond, and this observation was further supported by theoretical calculations as well as the solid state structure analysis. The most sensitive compound demonstrated a limit of detection and quantification of 0.18% and 0.54% v/v water in DMSO, respectively.

Water is a typical contaminant in a most organic solvents, which commonly represents an impediment to chemical reactions and industrial production processes, where dry operations should be guaranteed. Crucially, under the operation conditions of chemical production plants, water can induce the corrosion of metals, quench metal based reagents and cause the deactivation and deterioration of catalysts, especially so in the case of the refinery industry.^{1,2} As such, the detection and quantification of water in organic media is of vital importance.

Currently, traditional methods for the quantification of water content in gas streams or liquid phases are widespread, though are associated with drawbacks such as the cost of operation and instrumentation and their time-consuming nature.³ Recently, optical sensors for detecting water contents have received substantial attention due to the possibility of remote and *in situ* monitoring as well as their cost-effective fabrication.⁴

Fluorescent indicators, due to their innate high sensitivity, have received the lion's share of attention, with a lot of effort being devoted to the investigation of fluorescent sensors using sensing mechanisms such as photo-induced electron transfer, intramolecular charge transfer, proton transfer, water-induced decomplexation of dyes, water-induced interpolymer π -stacking aggregation, solvatochromism, etc.⁴⁻⁷

A particularly intriguing class of water liable chemicals are the Schiff bases. With their known instability in aqueous

environements,⁸ as well as their generally low cost and easy of manufacturing, sensors bearing this type of bond are a prime candidate in the design of water activatable fluorophores. Despite this, to date a sole example of a water-triggered chemodosimeter consisting of a 3,5-dichlorosalicylaldehyde Schiff base has been reported,⁹ and no in depth study exploring the influence of structural modification on the activity of this class of water sensors has been yet reported. In the current report, we therefore investigated the structure-activity relation of a small library of coumarin based fluorophores bearing a Schiff base, inducing water sensitivity through hydrolysis, and have rationalised our findings with the aid of theoretical calculations and crystal structure analysis.

In the current molecular design, coumarin was used as the fluorophore skeleton and derivatives of aniline and pyridine were introduced as Schiff bases, as they not only extend the conjugation inducing a fluorescence shift but also enhance the fluorescence intensity. It is thus expected that the presence of water in organic solvents containing probes **3-6** (Scheme 1) results in the dissociation of the Schiff-base, inducing observable changes in colour and fluorescence (Scheme 2).

Coumarin probes 3-6 were synthesized following the synthetic route depicted in Scheme 1. First, coumarin derivative 1 was synthesized from 4-(diethylamino)salicylaldehyde in a 93% yield, followed by the introduction of an aldehyde functionality using Vilsmeier–Haack conditions, providing a convenient handle to allow for further decoration, producing compound 2



^{a.} Department of Chemistry, Korea University, Seoul 136-701, Korea. E-mail: jongskim@korea.ac.kr; Fax: +82-2-3290-3121

^{b.} Department of Chemistry, Sungkyunkwan University, Suwon 16419, Korea. E-mail: jinylee@skku.edu

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Scheme 2 Conceptual illustration of the water-sensing probe.

in a 76% yield. After introducing the aldehyde group, probes possessing moisture sensitive imine bonds were obtained from the reaction of compound 2 with anilines and an amine bearing derivative of pyridine in anhydrous ethanol. All final compounds were fully characterized by ¹H, ¹³C NMR, and ESImass spectrometry and the probes were further characterised by the determination of their molar absorption coefficient (see Fig. S1-S10 and Table S1 in the ESI[†]).

Probes 3-6 were subjected to a time-dependent colorimetric and fluorescence assay in an aqueous DMSO solution (Fig. 1 and S11, ESI[†]). The maximum of absorbance of probes 4-6 exhibited a blue shift upon hydrolysis (green to red line), in contrast to probe 3 demonstrating a red-shift upon hydrolysis. After complete cleavage of the imine bond, the released compound 2 shows strong absorption intensity at 436 nm. As can be seen from this figure, the reactivity of probe 6 clearly outperformed other probes. As the fluorescence spectra of the probes showed the same tendency (Fig. S11, ESI[†]), probe 6 was selected for further examination and assessment as a water sensing probe and the hydrolysis of this probe was further supported by ESI-MS, showing the disappearance of a peak at m/z 322.10 (corresponding to $[6 + H]^+$) and the emergence of a new peak at m/z 246.05 (corresponding to $[2 + H]^+$) (Fig. S12, ESI[†]).

The UV and fluorescence spectra of 6 were subsequently examined in different organic solvents (Fig. 2). The absorption band of 6 showed only a slight solvent dependency, however, the fluorescence spectra showed a dramatic change in wavelength and intensity. The probe stability is excellent in various organic solvents as assessed by UV/Vis and



Fig. 1 UV/Vis absorption spectra of water-sensing probes (10 µM) in an aqueous solution (2% DMSO) at rt.



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Fig. 2 UV/Vis spectra and fluorescence emission spectra of 6 (10 μ M) in various organic solvents (1% DMSO) at rt. λ_{ex} = 447 nm.

fluorescence spectra, which indicates that 6 could potentially be used as a water sensor in a range of organic solvents.

The time-dependent fluorescence intensity of compounds 3-6 was subsequently monitored (Fig. 3a) and products 4 and 6 were examined in greater detail to characterise and quantify their differential time-dependent behaviour. The kinetics of the hydrolysis of Schiff bases are known to follow a pseudo-first order kinetics profile. As such, considering the first order kinetics of the hydrolysis reaction of a reactant (**R**), generating a product (**P**), the concentration of reactant diminishes exponentially, as described in equation (1), with [R] and $[R]_0$ being the actual and initial concentration of the reactant.

$$[R] = [R]_0 e^{-kt} \tag{1}$$

$$[R]_0 = [R] + [P] \tag{2}$$

$$I_{\lambda} = \epsilon_{\lambda,R}[R] + \epsilon_{\lambda,P}[P] \tag{3}$$

The system is further characterised by its mass balance (equation (2)). For this time dependent reaction, the intensity of fluorescence I_{λ} at a certain wavelength (λ) and time comprises a contribution from the reactant and the product, depending on their relative concentrations and emissivities, as described in equation (3), with ϵ being the wavelength dependent emissivity of reactant and product.

For a system being characterised at two different wavelengths $(\lambda_1 \text{ and } \lambda_2)$, the concentration of reactant remaining at a given point in time can be written as a function of the intensities and the emissivities of reactant and product at these two wavelengths (equation (4)). Combining equations (4) and (1) resulting in equation (5), a nonlinear regression using the time dependent fluorescence allows for the determination of the rate constants (k).

$$[R] = \frac{\epsilon_{\lambda_1, P} I_{\lambda_2} - \epsilon_{\lambda_2, P} I_{\lambda_1}}{\epsilon_{\lambda_1, P} \epsilon_{\lambda_2, R} - \epsilon_{\lambda_1, R} \epsilon_{\lambda_2, P}}$$
(4)

$$[R]_0 e^{-kt} = \frac{\epsilon_{\lambda 1,P} I_{\lambda 2} - \epsilon_{\lambda 2,P} I_{\lambda 1}}{\epsilon_{\lambda 1,P} \epsilon_{\lambda 2,R} - \epsilon_{\lambda 1,R} \epsilon_{\lambda 2,P}}$$
(5)



Fig. 3 (a) Fluorescence spectra of probes (10 μ M) in water solution (2% DMSO) at rt. λ_{ex} = 402 nm (3); 456 nm (4); 456 nm (5); 447 nm (6). (b) $\ln(\epsilon_{499,2}I_{544^-}\epsilon_{544,2}I_{499})$ vs. time for the hydrolysis of **4** and **6** in distilled water. (c) pH dependence of rate constants for the hydrolysis of 4 and 6 in various buffer solutions.

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Fig. 4 Crystal structures of (a) 4 and (b) 6

Using this approach, the hydrolysis of the probe was monitored by fluorescence at 499 nm and 544 nm, and the emissivity of the probes at these wavelengths was determined to be $\epsilon_{544,4} = 30.53$, $\epsilon_{544,6} = 109.36$, $\epsilon_{544,2} = 30.06$, $\epsilon_{499,4} = 10.74$, $\epsilon_{499,6} = 50.30$, and $\epsilon_{499,2} = 97.64$. The rate constants were determined, revealing a nearly 2.6-fold increase in reaction rate in the case of 6 vs. 4 in distilled water.

The behaviour of the probes was further assessed across a wide range of pH values, but the reaction rate of **6** under acidic conditions was too fast in order to make accurate measurements (Fig. S13, ESI⁺), limiting the pH range for which rate constants of probe **6** could be determined as opposed to probe **4**, where the rate constants were reported in the pH = 3 to 13 range (Fig. 3c). The hydrolysis rate in basic solutions was strongly diminished, but greatly increased under acidic conditions for both **4** (with rates of $1.5 - 2 \text{ min}^{-1}$) and **6** (with an estimated rate of > 4 min⁻¹), following a trend previously reported for the pH dependent hydrolysis rates of 2-aminothiophenol Schiff bases.¹⁰

As shown in their crystal structures, compound **4** exhibits a perpendicular aromatic ring with the coumarin moiety, however, the pyridine ring of **6** is parallel with the imine bond of coumarin fluorophore (Fig. 4, Table S2, ESI†). This indicates that the nitrogen atom on pyridine ring of **6** can form hydrogen bonds (C(6)-H(11) with N(1) in Fig. 4b and Table S3, ESI†) and affect the imine bond hydrolysis by anchimeric assistance, an effect well known to accelerate the reaction rate of 1-hydroxy-7-azabenzotriazole based peptide coupling reagents.¹¹

In order to gain a better mechanistic insight into the pH dependent reaction rate as well as to quantify the effect of the potential anchimeric assistance, DFT and TDDFT calculations for the reactions of **6** and **4** with water in acidic, neutral, and basic solution conditions were performed. Initial structures were chosen from the crystal structures. All the calculations were performed with B3LYP exchange functional with 6-31G* basis sets using a suite of Gaussian 09 programs.¹² In the optimized structures, the dihedral angle between coumarin and the phenyl/pyridine ring moiety was calculated to be 12.2° (**6**) and 37.2° (**4**), which is consistent with the solid state structure, though more strongly pronounced in the solid state due to the packing effect in the crystal. The calculated maximum absorption wavelengths of **4**, **6**, and product **2** were 427, 431, and 394 nm, which are in excellent agreement with the experimental values of 450, 450, and 430 nm with oscillator



Fig. 5 Energy profiles for the hydrolysis of **4** (a) and **6** (b) in acidic, neutral, and basic solutions. Energies are given in kcal/mol.

strengths (f_{ab}) of 1.1848, 1.2420, and 0.8557, respectively. The calculated emission wavelengths of **4**, **6**, and product **2** were 504, 476, and 408 nm with oscillator strengths (f_{em}) of 1.1055, 1.4172, and 0.7805, respectively. The fluorescence intensity should be proportional to the product $(f_{ab} \times f_{em})$ of the oscillator strengths of absorption and emission. The values of $f_{ab} \times f_{em}$ for **4**, **6**, and **2** were 1.3098, 1.7602, and 0.6679 at their emission maximum wavelengths, respectively. Comparing these values with molar emissivity values, we can expect that the molecular photophysical property is retained in solution of **4** and **6**, but there is some additional solution chemistry in the case of **2**.

The energy profiles for the hydrolysis of 4 and 6 in acidic. neutral, and basic solutions were shown in Fig. 5. All the reactant complex (RC), transition state (TS), and product complex (PC) were confirmed from the frequency calculations and IRC calculations. In all cases, the hydrolysis proceeds in steps excluding equilibrium (protonation and two deprotonation) processes. The first step is the addition of water to the imine bond (C=N), and the second step is dissociation of the hemiaminal intermediate into an aldehyde and amine. In acidic solutions, the second dissociation step is barrierless, while in basic solutions, the first water addition step is barrierless. In neutral solutions, for both 4 and 6, the water addition is the rate determining step. It can clearly be seen that the activation barriers for the hydrolysis of 6 are lower than for 4, which is consistent with the kinetic data obtained from the time-dependent fluorescence changes. It can be therefore concluded that the hydrolysis can be much enhanced in acidic solutions as noted from the greatly reduced activation barrier as compared to neutral solutions, while non-catalytic reactions were observed in basic solutions. The experimentally observed non-catalytic feature in basic solution was also supported from the activation barriers of the dissociation (17.7 and 20.0 kcal/mol for 4 and 6, respectively) being virtually identical to those in neutral solution (17.3 and 19.9 kcal/mol for 4 and 6, respectively). The most striking feature is that the hydrolysis can be much enhanced in acidic solution, and 6 is much faster than 4. The calculated energy profile is in excellent agreement with experimentally observed pH dependent kinetics. Thus, the probe 6 would most effectively work as a water sensor in acidic solutions.



Fig. 6 (a) Fluorescence intensity at 536 nm and of **6** in DMSO with increasing water content in the range of 0-10% (ν/ν) (b) linear fit of the range (below 1.0% ν/ν). λ_{ex} = 459 nm, data recorded after 800s of equilibration.

In the first water addition step, the reactant complex (RC) of 4 shows two water molecules involved in an hydrogen bonding network with the imine bonds. The RC of 6 is similar to 4, but shows an additional water molecule interacting with the nitrogen atom of the pyridine ring through hydrogen bonding as shown in the SI (Fig. S14, ESI[†]). The additional water molecule in 6 plays a role as a blocking group, without which the nitrogen atom of pyridine destroys the hydrogen bonding network. Subsequently, the hemiaminal intermediate was formed through a transition state (TS1) containing a 6membered ring as shown in Fig. S14, ESI⁺. Finally, the intermediate dissociates into the final products, aldehyde and amine, through the transition state (TS2) also containing a 6membered ring. The natural bond orbital (NBO) population (charge) on the imine bond can be used as be a simple estimate for the reactivity of the water addition. The atomic charges of C and N of the imine bond were calculated to be 0.106e and -0.481e for 4, and 0.123e and -0.519e for 6, respectively. Thus, the water addition to the more positive carbon of 6 would be easier compared with 4. In acidic solutions, protonation at the nitrogen atom of the imine bond can induce some charge transfer from the carbon atom of the imine bond, making the carbon more electron deficient, hence prone to water addition. The atomic charges of C and N of the imine bond under these conditions were calculated to be 0.176e and -0.495e for 4, and 0.184e and -0.519e for 6, respectively. The protonation at N of the imine bond can also reduce the barrier by stabilizing the accumulated negative charge at the nitrogen atom after water addition. This charge redistribution is consistent with the calculated activation barriers.

Finally, the performance of probe **6** in the determination of the water content in DMSO solutions was assessed. Probe **6** was subjected to increased amounts of water in DMSO. Upon excitation at 459 nm, the fluorescence at 536 nm was monitored in the 0–10% v/v water in DMSO range (Fig. 6a). The probe showed a good linear response in the 0–1% (v/v) range (Fig. 6b), allowing for the determination of the limit of detection (LOD) and limit of quantitation (LOQ) as defined in equations 6 and 7, with σ being the standard deviation of the blank and *m* the slope of the calibration curve (Fig. 6b):

$$\text{LOD} = \frac{3.3\sigma}{m} \tag{6}$$

$$LOQ = \frac{10\sigma}{m} \tag{7}$$

Thus the LOD and the LOQ of **6** for water in DMSO were determined to be 0.18% and 0.54% v/v demonstrating the highly sensitive nature of this type of Schiff base bearing fluorophores for the determination of moisture in organic solvents.

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In conclusion, we synthesized coumarin-based fluorescent moisture sensors 3-6. Their UV/Vis and fluorescence spectra confirmed that these probes are stable in various organic solvents, yet sensitive to moisture content in organic solvents and aqueous solutions. We monitored the time-dependent fluorescence changes for the most efficient probes 4 and 6, and found that they follow pseudo-first order kinetics as evidenced from the single exponential decay. The kinetics study in buffer solutions at various pH levels revealed that the hydrolysis of 4 and 6 was remarkably enhanced in acidic solutions, in contrast to basic solutions, not exhibiting catalytic effects. Thus, the probe 6 is expected to be an efficient potential fluorescent moisture sensor at neutral or acidic pH. The experimentally observed kinetics were rationalised by the analysis of the solid state conformation in the crystal structure as well as pH dependent theoretical calculations. The probe was further determined to exhibit a limit of detection (LOD) and limit of quantitation (LOQ) of 0.18% and 0.54% v/v water in DMSO. Taken together these results indicate the strong potential of Schiff base bearing fluorophores as highly sensitive water sensors in organic solvents via a hydrolysis mechanism.

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Notes and references

- M. Araujo, L. M. Santos, M. Fortuny, R. L. F. V. Melo, R. C. C. Coutinho and A. F. Santos, *Energy Fuels*, 2008, 22, 3450.
- N. S. Foster, J. E Amonette, T. Autrey and J. T. Ho, Sens. Actuator B-Chem., 2001, 77, 620.
- W. Chen, Z. Zhang, X. Li, H. Ågren and J. Su, RSC Adv., 2015, 5, 12191.
- (a) H. S. Jung, P. Verwilst, W. Y. Kim and J. S. Kim, *Chem. Soc. Rev.*, 2016, **45**, 1242. (b) P. Verwilst, K. Sunwoo and J. S. Kim, *Chem. Commun.*, 2015, **51**, 5556. (c) J. Wu, B. Kwon, W. Liu, E. Ansyln, P. Wang and J. S. Kim, *Chem. Rev.*, 2015, **115**, 7893. (d) M. H. Lee, J. S. Kim and J. L. Sessler, *Chem. Soc. Rev.*, 2015, **44**, 4185. (e) Z. Yang, J. Cao, Y. He, J. Yang, T. Kim, X. Peng and J. S. Kim, *Chem. Soc. Rev.*, 2014, **43**, 4563. (f) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2008, **37**, 1465.
- 5. M. Bai and W. R. Seiz, *Talanta*, 1994, **41**, 993.
- Citterio, K. Minamihashi, Y. Kuniyoshi, H. Hisamoto, S. Sasaki and K. Suzuki, *Anal. Chem.*, 2001, 73, 5339.
- H. Mishra, V. Misra, M. S. Mehata, T. C. Pant and H. B. Tripathi, J. Phys. Chem. A, 2004, 108, 2346.
- (a) S. Wang, B. Wu, F. Liu, Y. Gao and W. Zhang, *Polym. Chem.*, 2015, 6, 1127; (b) Z. Yang, M. She, J. Zhang, X. Chen, Y. Huang, H. Zhu, P. Liu, J. Li and Z. Shi, *Sens. Actuator B-Chem.*, 2013, 176, 482.
- G. Men, G. Zhang, C. Liang, H. Liu, B. Yang, Y. Pan, Z. Wang and S. Jiang, *Analyst*, 2013, 138, 2847.
- H. B. Hassib, N. S. Abdel-Kader and Y. M. Issa, J. Solution Chem. 2012, 41, 2036.
- 11. L. A. Carpino, J. Am. Chem. Soc., 1993, 115, 4397.
- Gaussian 09 (Revision D.01). M. J. Frisch, et al. Gaussian, Inc., Wallingford CT, 2009.

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