Fluorescent protein red Kaede chromophore; one-step, high-yield synthesis and potential application for solar cells[†]

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The one-step condensation between the Green Fluorescence Protein (GFP) chromophore and 1*H*-imidazole-5-carbaldehyde catalyzed by ZnCl₂ proved to be a facile method to synthesize the red Kaede chromophore and its derivatives (1a–d, 2, and 3 > 70%), among which 1a then demonstrated its potential as a dye in solar cell, with a moderate conversion efficiency (η) of 3.04%.

Kaede is a new fluorescent protein isolated from the stony coral Trachyphyllia geoffroyi. It contains a tripeptide, His 62-Tyr 63-Gly 64, the scaffold of a green chromophore, which is photoconvertible to a red chromphore following UV irradiation. Maximal illumination results in a 2000-fold increase in the ratio of red-to-green fluorescence. Kaede has the superiority in that the excitation light used to elicit green fluorescence does not induce photoconversion, such that photoconversion can be carried out selectively with appropriate targeting of UV excitation. As with the family of green fluorescence proteins (GFP), Kaede can be genetically encoded in cells and is easily detectable by standard fluorescence microscopy techniques.¹ Accordingly, Kaede has wide application in molecular and cell biology and provides a simple technique for marking individual cells, organelles and proteins for live cell imaging applications.²

Although the GFP chromophore (4-(4-hydroxybenzylidene)-1,2-dimethyl-1*H*-imidazol-5(4*H*)-one, *p*-**HBDI**, see Scheme 1) and numerous derivatives have been synthesized,³ it was not until 2008 that synthetic work on the Kaede chromophore (see Scheme 1 for structure) was reported.⁴ Unfortunately, the four step synthetic route incorporating *p*-**HBDI** as the starting material rendered a very poor yield of <3% for the Kaede core chromophore (**1a**).

With the aim to investigate the fundamental properties and applications of red Kaede core chromophores, we initially carried out a synthetic protocol similar to that reported in ref. 4. Unfortunately, the rather low yield made the subsequent workup procedure very difficult and hence hampered any further applications. To circumvent this obstacle, we expended great effort on improving the methodology. Herein, we report a facile synthetic route to prepare red Kaede chromophore (1a) from GFP chromophore *via* one-step condensation. In sharp contrast to the <3% yield of 1a,⁴ this simple and one-pot synthetic protocol renders a much higher yield of 70% for product 1a (see Scheme 1).

In this synthetic protocol, the GFP chromophore, *i.e. p*-HBDI, was prepared *via* an Erlenmeyer azlactone reaction.⁵ 1a was then synthesized through a condensation between p-HBDI and 1H-imidazole-5-carbaldehyde catalyzed by a Lewis acid. Detailed synthetic procedures and characterization, such as ¹H NMR, ¹³C NMR, MS and HRMS data, are provided in the ESI.[†] In this study, various types and amounts of Lewis acids have been used to optimize the reaction. The yield of 1a was then measured and the results are listed in Table 1. Accordingly, ZnCl₂^{3b} turns out to be the best suited catalyst. The optimum loading for ZnCl₂ seems to be in the range of 10 mol%, affording a vield of 70% for 1a. However, despite a decrease in yield, other ZnCl₂ amounts still lead to decent isolated product yields of >50% (see Table 1). Other catalysts rendered low yields of <30%. The results indicate that the degree of complexation on the intermediate may play a role in the yield of condensation. If we take into account the 60% vield for producing *p*-HBDI in this study, an overall yield of 42% was obtained for compound 1a synthesized from the commercially available starting materials.

Extension of the above condensation protocol using different functional groups R (see Scheme 1) is possible, as supported by the successful synthesis of Kaede-like chromophores **1b**, **1c** and **1d** with a yield of 72, 85 and 81%, respectively (see ESI). \dagger



Scheme 1 Structures and synthetic routes of 1a-d, 2 and 3.

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S. No.	Lewis acid used	Amount used in mol (%)	Isolated product yield (%)	
Ι	ZnCl ₂	(a) 1 (b) 10 (c) 100	(a) 55 (b) 70 (c) 65	
II	MgCl ₂	10	20	
III	$Cu(OTf)_2$	10	10	
IV	BF ₃ ·OEt ₂	10	25	
V	AlČl ₃	(a) 10 (b) 100	(a) 30 (b) 10	

Table 1 Synthetic yields of 1a under different Lewis acidic catalysts

In theory, other derivatizations of the vinyl double bond at the C(1) position of *p*-**HBDI** are also feasible through this facile condensation process (*vide infra*).

The absorption and emission spectra of Kaede 1a in aqueous solution are depicted in Fig. S1 of the ESI.[†] The absorption (emission) maxima of neutral and deprotonated $(-O^-)$ species are located at 435 nm (530 nm) and 495 nm (570 nm), respectively. In comparison, the tetrameric fluorescent protein Kaede^{2a} appears as an anionic form, which exhibits red shifted absorption and fluorescence maxima at 572 nm and 582 nm, respectively (*cf.* 495 and 570 nm for 1a in the anionic form). The significantly red-shifted absorption in protein red Kaede is plausibly due to the hydrogen-bond network formation between chromophore and peptide, inducing π -electron delocalization, while the smaller Stokes shift (572 \rightarrow 582 nm for Kaede *cf.* 435 \rightarrow 530 nm for 1a in H₂O) manifests the less polar and more rigid environment in protein red Kaede.

In aprotic solvents, compared to the generally observed extremely weak emission for *p*-HBDI ($\Phi \sim 10^{-4}$ and $\tau_{\rm f} \sim 1.7$ ps in toluene) at room temperature,^{6a} the emission yield of free red Kaede chromophore 1a in neutral form is as high as 0.13 with $\tau_{\rm f} \sim 575$ ps, the result of which indicates that the bulky imidazole group may, in part, hinder the exocyclic C–C bond rotation.^{3c} In sharp contrast, in protic solvents such as water, the emission yield of 2.8 × 10⁻³ (2.6 × 10⁻³) with $\tau_{\rm f} \sim 13$ ps (~12 ps) for the neutral (anion) form in water manifests a dramatic decrease in the quantum yields on going from aprotic to protic solvents. This result supports another role of solvent–solute hydrogen bonding in the nonradiative decay pathways, recently proposed by Yang *et al.*^{6b} and Tolbert and co-workers.^{6c}

Being able to produce large quantities of compound **1a** using this new synthetic protocol, its latent application should be of great interest. The anionic form of **1a** reveals a broad visible absorption with a high extinction coefficient $(8.0 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1} \text{ at } 495 \text{ nm}, \text{ see Fig. S1}).^{\dagger}$ This, in combination with its relatively low pK_a (7.7) of the hydroxyl group³ that is suitable for anchoring TiO₂ via hydroxide anion, leads us to propose the use of free red Kaede chromophore in organic-based dye sensitized solar cells (DSSCs).^{7a}

The E_{ox} of **1a** adsorbed on a 6 µm thick TiO₂ nanocrystalline film on TCO glass was measured with cyclic voltammetry (see ESI).† The results reveal that the redox potential of I^{-}/I_{3}^{-} (*ca.* 0.4 V *vs.* NHE) is more negative than the HOMO of **1a** and is able to regenerate the dye **1a** from electron donation. The LUMO level of **1a** is also sufficiently more negative than the conduction band edge of the TiO₂ electrode (-0.5 V *vs.* NHE at pH 7),^{7b} providing a favorable energy to inject electrons into the TiO₂ electrode. Using red Kaede chromophore **1a** as photosensitizer, a dye-sensitized nanocrystalline TiO₂ solar cell produces a 3.04% solarlight-to-electricity conversion

efficiency with a short-circuit current density of 6.6 mA $\rm cm^{-2}$, an open circuit photovoltage of 640 mV, and a fill factor of 0.72 under standard AM 1.5 irradiation (100 mW cm⁻²). **1a** was anchored via the anion form $(-O^{-})$ coordinated to Ti⁴⁺ on the TiO₂ surface. This viewpoint was further verified by replacing the -OH group in 1a with -OCH₃. Accordingly, a very low uptake of dye was observed in TiO₂, resulting in negligible photo-response. The incident photon-to-current conversion efficiencies (IPCEs) of 1a are shown in the inset of Fig. 1. The onset of the IPCE spectra based on 1a is ~ 670 nm, again confirming its origin from anion species. Visible light (400–700 nm) can be converted to a current with a maximum of 57% efficiency at 500 nm by the solar cell composed of 1a, producing a prominent photocurrent. These results strongly indicate the prospect of promising applications of red Kaede chromophores in DSSCs.

To test the stability of the 1a solar cell under thermal stress and visible-light soaking, the evolution of photovoltaic parameters has been measured under irradiance of AM 1.5G sunlight during visible-light soaking at 60 °C. Using low-volatile electrolytes (a mixture of BMII/I2/NBB/GNCS in molar ratio of 1/0.15/0.5/0.1, see detail in the ESI),† the resulting data show that photovoltaic parameters of compound **1a** are 4.62 mA cm⁻² (J_{sc}), 618 mV (V_{oc}), 0.636 (FF), and 1.84% (η). Over a period of ~72 h aging, as shown in Fig. S3,† the current density of the device slightly decreased by 0.2 mA cm^{-2} , which is plausibly due to the desorption of a non-negligible amount of dye from the TiO₂ surface. However, it seems that the loss was compensated by the dispersion of 1a and hence the decrease of π - π stacking of compound 1a adsorbed onto the TiO2 surface, resulting in an increase of fill factor (see Fig. S3).^{†7c} After 72 h of light soaking, the net



Fig. 1 Photocurrent density vs. voltage curves for DSSCs based on 1a (--), 2 (- Φ -) and 3 (- Δ -) under AM 1.5 G simulated solar light (100 mW cm⁻²). Performances of DSSCs were measured with 0.16 cm² working area and 0.6 M 1-propyl-3-methylimidazolium iodide (PMII), 0.1 M LiI, 0.05 M I₂, 0.5 M 4-*tert*-butylpyridine (TBP) in dry acetonitrile as electrolyte. Inset: Photocurrent action spectrum of 1a.

Table	2	Photophysical	properties	(in	CH ₃ CN)	and	photovoltaic
performance data of various derivatives of GFP core chromophores							

Dye	λ_{ab}/nm	$J_{\rm sc}/{ m mA~cm^{-2}}$	$V_{\rm oc}/{ m mV}$	FF	η (%)
1a	435 ^a , 495 ^b	6.6	640	0.72	3.04
1b	$436^{a}, 497^{b}$	0.31	472	0.51	0.07
1c	$438^{a}, 498^{b}$	0.29	466	0.44	0.06
1d	$495^{a}, 512^{b}$	1.26	496	0.68	0.43
2	470^{a}	6.63	453	0.65	1.95
3	517 ^a	6.47	526	0.69	2.34
^a Neut	ral from. ^b And	ionic form.			

photovoltaic parameters were measured to be 4.42 mA cm⁻² (J_{sc}), 613 mV (V_{oc}), 0.651 (FF), and 1.76% (η). The results provide evidence of decent stability of the Kaede core chromophore upon exploitation as a dye for the solar cell.

The DSSC performance of 1b-1d was also examined under the same cell configuration. Unfortunately, as shown in Table 2, the photovoltaic parameters are all significantly lower than that of 1a. We believe that this inferiority is mainly due to the low solubility of 1b-1d in CH₃CN, as evidenced by low absorbance (<1.0 at λ_{ab}) for **1b–1d** under saturated CH₃CN (or MeOH) and consequently the pale yellow color appearance on the prepared cell. The lower FF and V_{oc} values also point to a possible aggregation effect for 1b-1d. Attempts have been made to increase dye solubility in CH₃CN by adding tetrabutyl ammonium hydroxide; unfortunately the cell performance was not improved. Although formation of 1b-1d/ammonium salt increases the solubility of 1b-1d, the deprotonation of hydroxy group caused lower dye uptake onto TiO₂ surface.^{7d} To optimize the solar cell performance by using other analogues, we then performed the similar condensation method between the methoxylated GFP core (p-MBDI, see Scheme 1) and R = thiophene-2,5-dicarbaldehyde, followed by attachment of 2-cyanoacetic acid in the presence of piperidine to make compound 2. We also subsequently reacted 3a (see Scheme 1) via a Sonogarshira reaction, followed by condensation with 2-cyanoacetic acid, to afford 3 with a high yield of 83% (see ESI for details).[†] Both 2 and 3 possess carboxylic groups and can thus anchor on TiO_2 . The resulting I-V curve and DSSC data are listed in Fig. 1 and Table 2, respectively. Clearly, although 2 holds a J_{sc} similar to that of 1a, both V_{oc} and FF are inferior, giving lower conversion efficiency of 1.95%. Compared with 2, diphenylamine substituted 3 shows a 1.16-fold increase in voltage and a conversion efficiency of 2.34%. The results reveal the effectiveness upon incorporating a bulky diphenylamine group, which might decrease the π - π stacking onto the TiO₂ surface, rendering a larger V_{oc} (cf. 2, see Table 2).

We thus present herein a facile, one-step reaction protocol to synthesize red Kaede core chromophores, 1a-d, 2 and 3, with a product yield of >70%. We also demonstrated its versatility in strategic design of various kinds of functionalized

red Kaede chromophores suited for application in *e.g.* DSSCs. Using the –OH form for **1a** or –COOH group for **2** and **3** to coordinate TiO₂, moderate conversion efficiency has been achieved. These, together with the feasibility in its derivatization, should attract great attention as well as pave a new avenue for researchers working on bio-organic molecules and/or energy relevant subjects. At the current stage, the efficiencies are lower than those reported for other organic,⁸ ruthenium⁹ and certain porphyrin¹⁰ dyes applied in DSSCs. Further work in this area will aim to match the performance of these other dyes.

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