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A synthetic approach to GFP chromophore analogs from 3-azidocinnamates. Role of methyl rotors in chromophore photophysics†

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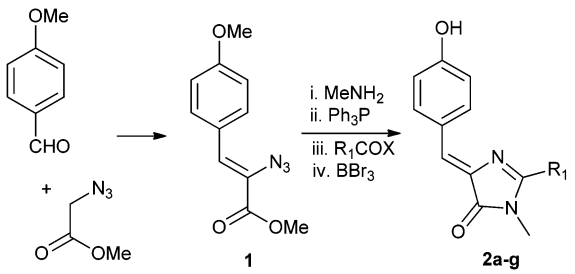
We have suggested a novel combinatorial approach for synthesis of otherwise inaccessible GFP chromophore analogs, and studied the influence of aliphatic substituents on their pH-dependent spectral properties. We found that the demethylation at C or N positions of the imidazolone ring leads to a decrease in the excited state lifetime.

Recently, the chromophore of Green Fluorescent Protein (GFP) and its synthetic analogs have attracted much interest due to the unique combination of their spectral and photochemical properties. Synthetic GFP chromophore (*p*-hydroxybenzylidene imidazolone, *p*-HOBDI, **2** in Scheme 1) derivatives were used to gain a more detailed understanding of spectral behavior,[1] chromophore maturation,[2] enhanced state proton transfer in fluorescent proteins,[3] and as a platform for novel fluorescent[4–6] and fluorogenic[7] dyes. Several synthetic approaches to different functionalized analogs of the GFP chromophore were developed,[8] including classical Erlenmeyer azlactone synthesis,[9] cyclization with aromatic aldimines,[10] condensation of saturated imidazolones with aldehydes,[11] and copper-catalyzed condensation of amidines with 2-bromocinnamic acid.[12]



Scheme 1 Synthesis of GFP chromophore analogs **2a–g**.

In the course of our systematic synthetic studies on the range of GFP chromophore analogs, we were interested in obtaining GFP chromophores bearing H and CF_3 substituents at position 2 of the imidazolone core (**2f** and **2g**). Utilization of the abovementioned methods was unsuccessful, so we looked for some alternative combinatorial synthetic approaches to develop a library of C-modified GFP chromophores.

One example of the construction of 4-arylideneimidazolone, which has a structure identical to that of the GFP core, based on the reaction of SEM-protected 2-azido-3-(indol-3-yl)acrylamide with methyldiphenylphosphine and 2-(indol-3-yl)-2-oxoacetyl chloride, is known in the literature. This method was applied in the synthesis of marine alkaloid rhopaladin D.[13] We have utilized this approach to the synthesis of the GFP chromophore analogs.

We have studied the reaction of (*Z*)-2-azido-3-(4-methoxyphenyl)-*N*-methylacrylamide (obtained by aminolysis of ester **1**, see ESI†) with triphenylphosphine and carboxylic anhydrides or acyl chlorides (Scheme 1).‡

We have found that among acyl chlorides, only pivaloyl chloride gave moderate yield (34%) in this reaction. Furthermore, we found that isobutyryl chloride gave poor yield (17%), while propionyl and acetyl chloride gave no desired product. This difference can be attributed to side reactions associated with deprotonation at the alpha-position of the acyl chloride.[14] At the same time, carboxylic anhydrides demonstrated reverse applicability: the best yields were obtained with sterically non-hindered radicals, Me, Et, CF_3 and H, while the secondary radical (^{i}Pr) gave poor yield and the tertiary radical (^{t}Bu) did not react. At the final stage of the synthesis, standard phenol demethylation conditions (BBr_3, CH_2Cl_2) were used to obtain the desired GFP chromophore analogs (Table 1).

The reported combinatorial synthetic approach appears to be useful for the synthesis of otherwise inaccessible GFP chromophore analogs. This approach allows introduction of a CF_3 substituent into an imidazolone core as well as providing the ability to obtain a parent heterocycle in this range, which bears a hydrogen at position 2.

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Table 1 Reactivity of carboxylic anhydrides and acyl chlorides in synthesis of C-derivatized GFP chromophores **2a–g**

Product	R_1	Yield at stage iii/%		Overall yield (4 stages)/%
		Anhydride	Acyl chloride	
2a	Me	61	0	42
2b	Et	35	Traces	26
2c	Pr	26	—	16
2d	^{i}Pr	15	17	12
2e	^{t}Bu	Traces	34	28
2f	CF_3	45	—	27
2g	H	30[a]	—	22

[a] Mixed anhydride with acetic acid $CH_3COOCHO$ was used.

The series of compounds obtained by the above method made it possible to study the influence of aliphatic substituents in the GFP chromophore on its pH-dependent spectral properties. We have also included the N-unsubstituted chromophore **2h** (ESI†) in this comparative study.

Similarly to **2a**, studied by one of us earlier,[15] all homologs **2b–h** have three pH-dependent spectral forms: anionic, neutral and cationic (Fig. 1 and Table 2). Chromophores **2e**, **2g** and **2h** have lowered pK_a associated with protonation of the imidazole nitrogen, compared to a reference chromophore **2a**. This can be attributed to steric effects in the case of the ^{t}Bu substituent (**2e**) and to electronic effects in the case of dealkylated chromophores **2g** and **2h**. A significantly lowered phenolic pK_a (7.15 *vs.* 7.7 for **2a**) was observed in the case of **2f**, which carries the strong electronic withdrawing trifluoromethyl group. The latter chromophore is also characterized by an ~50 nm bathochromic shift in anionic form compared to **2a**.

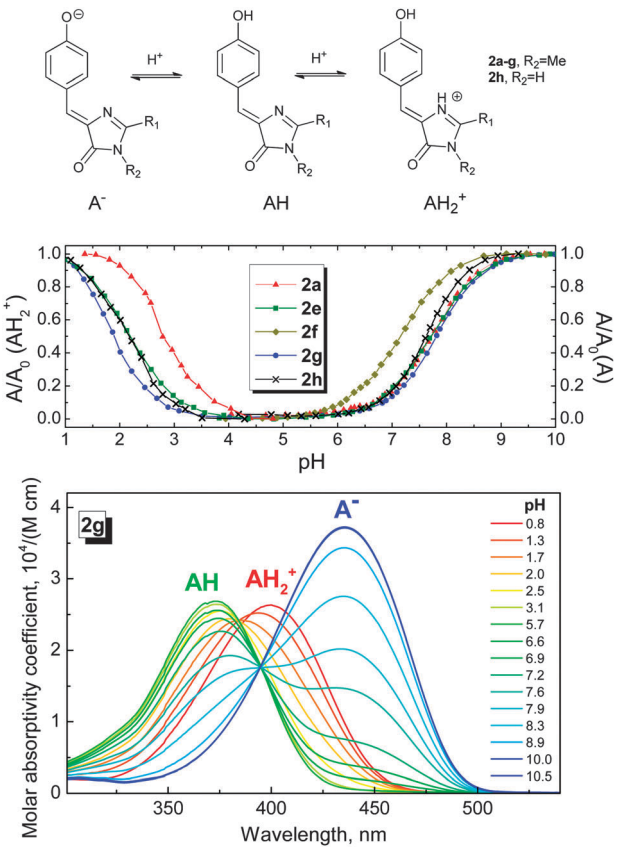

Fig. 1 pH-titration of chromophores **2a–h**.

Table 2 pK_a's and absorption maxima of novel GFP chromophore analogs compared with the known chromophore **2a** (measured in 0.2 M phosphate buffer)

Chromophore	R_1	R_2	pK_a		Abs/nm		
			Nitrogen	Phenol	Neutral form	Anionic form	Cationic form
2a	Me	Me	2.95	7.7	368	424	390
2e	^{t}Bu	Me	2.2	7.7	373	430	402
2f	CF_3	Me	dcmp	7.15	388	470	dcmp
2g	H	Me	1.9	7.80	374	434	399
2h	Me	H	2.2	7.7	365	422	386

Previously, one of us studied the subtle dependence of the *p*-HOBDI photophysics on the nature and position of alkyl substituents.[16] The availability of a wide variety of aliphatic derivatives of *p*-HOBDI prepared in this work made possible the testing of intriguing hypothesis of involvement of methyl rotors in the *p*-HOBDI deactivation mechanism proposed by Agmon *et al.*[17] They suggested that free barrierless rotation of C- and N-methyl groups on the imidazolone ring supplies "a dense thermal bath of rotational states that couple with the internal conversion. These modes can enhance the nonradiative transition by accepting the excess energy". In other words, demethylated derivatives of *p*-HOBDI were proposed to have slower rates of internal conversion and longer excited-state lifetimes.

We have compared the photoinduced behavior of the C- and N-demethylated derivatives of **2a** (**2g** and **2h**, correspondingly) and compared it with the behavior of **2a** and **2b**. To exclude possible selective hydrogen-bonding solute–solvent interactions, room-temperature acetonitrile was chosen as a solvent for these studies.

Transient absorption spectra of the GFP-synthetic chromophores are shown in Fig. 2. The spectra are very close to those of the parent *p*-HOBDI studied by Vengris *et al.*[18] and one of us[19] earlier. All spectra consisted of overlapped photoinduced absorption (PA) bands at 460, 570, and 770 nm, their ratio varied among the compounds. The analysis of the transient data of *p*-HOBDI and its derivatives was presented earlier.[18,19] It was demonstrated that upon photoexcitation the relaxation pathway is characterized by a sum of exponentials and involves a transition through a succession of states along a reaction coordinate that includes contributions from intramolecular twisting motion, solvation dynamics, and vibrational cooling. Therefore, the experimental time constants determined from the analysis of PA data can be considered as average values for a distribution of the population in both the excited and the ground state. In this work we will not analyse in detail the complex behavior of the subpicosecond photoinduced kinetics of the novel chromophores, but will perform a comparative analysis of the kinetics of a series of dealkylated derivatives. Based on the similarity of the ground- and excited-state absorption spectra of all homologs, we assumed that the excited-state deactivation mechanism is the same. Then we fitted the decay of the common 570 nm PA band using a polyexponential function.

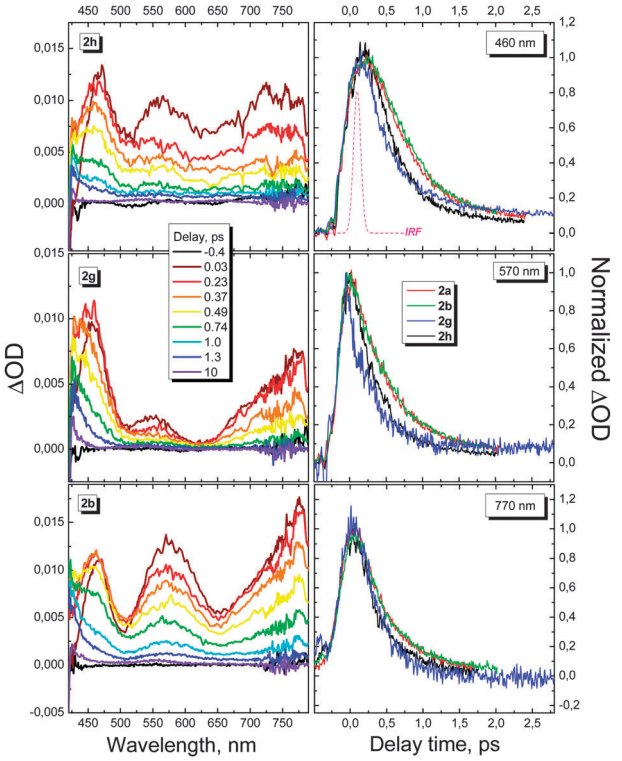

Fig. 2 Left column: transient absorption spectra of GFP chromophores in MeCN after excitation at 370 nm. The −0.4 ps trace corresponds to the spectral baseline in the absence of pump excitation. Right column: normalized kinetics of the TA signals. Color coding is the same for each column.

The PA kinetics of the GFP-synthetic chromophores are shown in Fig. 2. At all wavelengths the decays of the C- and N-dealkylated derivatives (**2g** and **2h**, correspondingly) were faster than those of **2a**. The characteristic decay times were 0.35 ps and 0.37 ps for **2g** and **2h** *vs.* 0.52 ps for **2a** and 0.57 ps for C-ethyl derivative **2b**.

In conclusion, in the present work we have reported a novel synthetic combinatorial approach to C-derivatized GFP chromophore analogs, which gives access to otherwise inaccessible structures, *e.g.* parent heterocycle **2g** and trifluoromethylated chromophore **2f**. Recently, GFP chromophore analogs have been demonstrated to be excellent fluorogenic probes to specifically label a target protein,[20] RNA[7] and cellular metabolites.[21] The new synthetic approach reported in this work will help to further develop these advanced labelling strategies. Spectral studies demonstrated that in contrast to Agmon's hypothesis, the demethylation at C or N positions of the imidazolone ring leads to a decrease in the S_1 lifetime at least at room temperature. This indicates that methyl substituents introduce an additional (but not large) barrier into the photoisomerization-induced deactivation pathway. We will expand our studies to the viscous and low-temperature solvents where the photo-induced isomerization would be inhibited, and the role of methyl rotors may be more pronounced.

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Notes and references

‡ Typical synthetic procedure: (*Z*)-2-azido-3-(4-methoxyphenyl)-*N*-methylacrylamide, obtained by the action of $MeNH_2$ on azidoester **1** (ESI†) (5.2 g, 22.4 mmol), and triphenylphosphine (7.0 g, 26.7 mmol) were heated in dry toluene (100 mL) under argon at 65 °C for 30 min. A yellow precipitate separated and effervescence was observed. The reaction mixture was cooled to room temperature and trifluoroacetic anhydride (4.2 g, 20 mmol) and DIPEA (1.3 g, 10 mmol) were added. The mixture was heated to 40 °C and stirred for 2 h, then cooled, diluted with 200 mL $CHCl_3$, washed with aqueous $NaHCO_3$ (5%, 100 mL), water (2 × 100 mL), brine (2 × 100 mL) and dried over Na_2SO_4. The solvent was evaporated and the product ((*Z*)-4-(4-methoxybenzylidene)-1-methyl-2-(trifluoromethyl)-1*H*-imidazol-5(4*H*)-one) was purified using column chromatography ($CHCl_3$: EtOH = 50 : 1). Yellowish solid, 1.28 g (45%), mp 99–101 °C.

1 L. M. Tolbert, A. Baldridge, J. Kowalik and K. M. Solntsev, *Acc. Chem. Res.*, 2012, **45**, 171.
2 P. E. Ivashkin, K. A. Lukyanov, S. Lukyanov and I. V. Yampolsky, *J. Org. Chem.*, 2011, **76**, 2782.
3 M. S. Baranov, K. A. Lukyanov, A. O. Borissova, J. Shamir, D. Kosenkov, L. V. Slipchenko, L. M. Tolbert, I. V. Yampolsky and K. M. Solntsev, *J. Am. Chem. Soc.*, 2012, **134**, 6025.
4 I. V. Yampolsky, A. A. Kislukhin, T. T. Amatov, D. Shcherbo, V. K. Potapov, S. Lukyanov and K. A. Lukyanov, *Bioorg. Chem.*, 2008, **36**, 96.
5 W.-T. Chuang, B.-S. Chen, K.-Y. Chen, C.-C. Hsieh and P.-T. Chou, *Chem. Commun.*, 2009, 6982.
6 L. Wu and K. Burgess, *J. Am. Chem. Soc.*, 2008, **130**, 4089.
7 J. S. Paige, K. Y. Wu and S. R. Jaffrey, *Science*, 2011, **333**, 642.
8 P. E. Ivashkin, I. V. Yampolsky and K. A. Lukyanov, *Russ. J. Bioorg. Chem.*, 2009, **35**, 652.
9 I. V. Yampolsky, T. A. Balashova and K. A. Lukyanov, *Biochemistry*, 2009, **48**, 8077.
10 (*a*) A. Baldridge, J. Kowalik and L. M. Tolbert, *Synthesis*, 2010, 2424; (*b*) M. Ikejiri, M. Tsuchino, Y. Chihara, T. Yamaguchi, T. Imanishi, O. Obika and K. Miyashita, *Org. Lett.*, 2012, **17**, 4406.
11 A. Baldridge, K. M. Solntsev, C. Song, T. Tanioka, J. Kowalik, K. Hardcastle and L. M. Tolbert, *Chem. Commun.*, 2010, **46**, 5686.
12 X. Gong, H. Yang, H. Liu, Y. Jiang, Y. Zhao and H. Fu, *Org. Lett.*, 2010, **12**, 3128.
13 P. M. Fresneda, P. Molina and M. A. Sanz, *Synlett*, 2000, 1190.
14 I. Bosch, A. Gonzalez, F. Urpi and J. Vilarrasa, *J. Org. Chem.*, 1996, **61**, 5638.
15 J. Dong, K. M. Solntsev and L. M. Tolbert, *J. Am. Chem. Soc.*, 2006, **128**, 12038.
16 J. Conyard, M. Kondo, I. A. Heisler, G. Jones, A. Baldridge, L. M. Tolbert, K. M. Solntsev and S. R. Meech, *J. Phys. Chem. B*, 2011, **115**, 1571.
17 R. Gepshtein, D. Huppert and N. Agmon, *J. Phys. Chem. B*, 2006, **110**, 4434.
18 M. Vengris, I. H. M. van Stokkum, X. He, A. F. Bell, P. T. Tonge, R. van Grondelle and D. S. Larsen, *J. Phys. Chem. A*, 2004, **108**, 4587.
19 K. M. Solntsev, O. Poizat, J. Dong, J. Rehault, Y. Lou, C. Burda and L. M. Tolbert, *J. Phys. Chem. B*, 2008, **112**, 2700.
20 A. Baldridge, S. Feng, Y. T. Chang and L. M. Tolbert, *ACS Comb. Sci.*, 2011, **13**, 214.
21 J. S. Paige, T. Nguyen-Duc, W. Song and S. R. Jaffrey, *Science*, 2012, **335**, 1194.