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A Latent Reaction in a Model GFP Chromophore Revealed upon Confinement: Photohydroxylation of *ortho*-Halo Benzylidene-3-methylimidazolidiones via an Electrocylization Process

Shampa R. Samanta,[†,∥] José P. Da Silva,[§] Anthony Baldridge,[‡,∥] Laren M. Tolbert,*[,‡] and V. Ramamurthy*[,†]

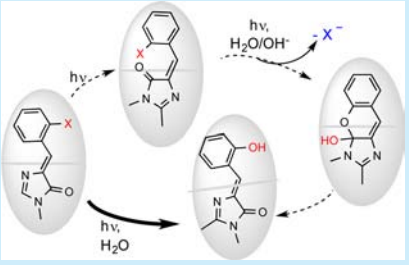
[†]Department of Chemistry, University of Miami, Coral Gables, Florida 33146, United States

[‡]School of Chemistry and Biochemistry, Georgia Institute of Technology, 901 Atlantic Drive, Atlanta, Georgia 30332-0400, United States

[§]Centro de Investigação em Química do Algarve, FCT, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

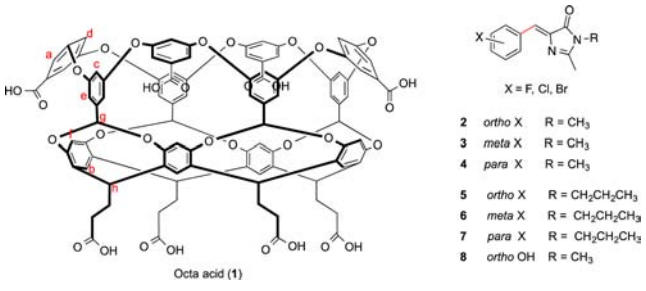
Supporting Information

ABSTRACT: Excited state behavior of halogen substituted model GFP chromophores was investigated in an acetonitrile solution and in a confined environment provided by an octa acid capsule in water. Of the *ortho*, *meta*, and *para* halogen substituted GFP chromophores only the *ortho* compounds gave a new product resulting from an unprecedented photosubstitution of halogens by the hydroxyl group. This unusual reaction highlights the importance of confined spaces in bringing about some unattainable photoreactions.



During the past decade there has been considerable interest in manipulating the excited state behavior of organic molecules.[1,2] Octa acid (OA, **1**, Scheme 1) is one of the hosts employed by us to control the excited state behavior of organic molecules in water through confinement and weak interactions.[3] Generally, OA forms 1:2 or 2:2 (guest to host) capsular assemblies with neutral organic molecules in sodium tetraborate buffered water (pH 8.7).[4,5] Recently, we demonstrated a strong emission of substituted *cis*-benzylidene-3-methylimidazolidiones (BMIs; Scheme 1), molecules having the basic skeleton of the chromophore (*para* hydroxybenzylidene-imidazolidone) responsible for the fluorescence of green fluorescent protein (GFP), within the OA capsule compared to that in solution.[6,7] We surmised this to be due to the arrest of single bond rotation (connecting aryl to the double bond; see the red colored bond in Scheme 1) within the confined OA capsule to prompt the enhanced fluorescence. BMIs when irradiated within the OA capsule preferred the *trans* geometry at the photostationary state (up to 98%) while in solution nearly equal amounts of both the *cis* and *trans* isomers were present.[6] Similar to *dicis* hexatriene, the *trans* isomer of BMI could undergo intramolecular 6e cyclization, a reaction hitherto not reported for BMIs.[8] Such a possibility was revealed upon examination of the excited state behavior of halogen (F, Cl, and Br) substituted BMIs **2**−**7** (Scheme 1) included within OA. The results of this study are presented below. Of the *ortho*, *meta*, and *para* halogen substituted BMIs **2**−**7** only the *ortho* ones (**2** and **5**) gave a new product resulting in substitution of halogen by the OH group (**8** in Scheme 1). To our knowledge, such a photosubstitution has not been reported in the case of any BMI.

Scheme 1. Chemical Structure of Octa Acid (1) and Guest Molecules Studied



The photochemistry of a number of BMIs (*ortho*, *meta*, and *para* halogen substituted at the aryl ring and *N*-methyl and *N*-propyl derivatives)[9,10] was examined in acetonitrile and as OA complexes in borate buffer solution. In the text we illustrate their photochemistry with the Cl/N-Me derivative as an exemplar. The data on fluoro and bromo derivatives mimicking the behavior of chloro derivatives are summarized in the Supporting Information (SI). Host−guest complexes of these BMIs were prepared by stirring an aliquot of DMSO-d_6 stock (see SI for details) solution of the guest with host OA in a borate buffer D_2O solution. ^{1}H NMR spectra of 1:2 OA complexes of **2** and free OA are presented in Figure 1. The

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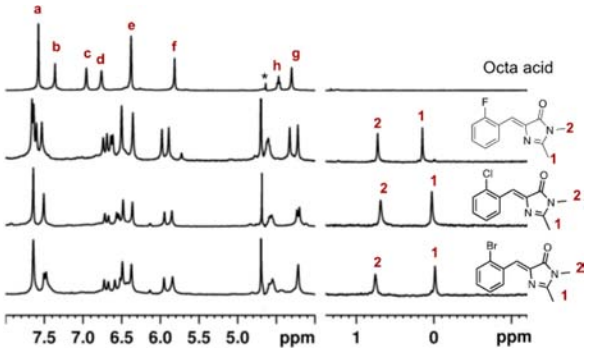
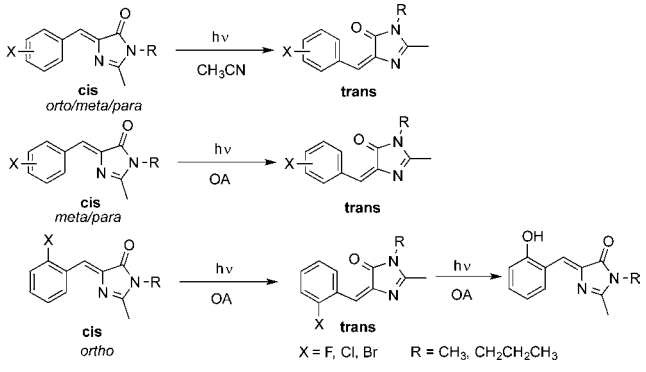


Figure 1. ^{1}H spectra of (i) octa acid, and capsular assembly of (ii) *ortho*-F/Me, (iii) *ortho*-Cl/Me, and (iv) *ortho*-Br/Me along with the assignment of the aliphatic protons of guests ([OA] = 1 mM in 10 mM borate buffer in D_2O and [guest] = 0.5 mM). Signals of octa acid "a–g" are assigned according to Scheme 1.

changes in the chemical shifts of the signals due to OA and the upfield shift of the signals due to the guest's alkyl groups indicated the inclusion of the guest within OA.[4,11,12] The inclusion is more apparent in the case of *N*-propyl derivatives where the methylene and methyl signals are shifted to below δ 0 ppm (Figures S1 and S2 in SI). When the guest amount exceeded the guest–host ratio of 1:2 the cloudy solution resulted in additional ^{1}H NMR signals due to free uncomplexed BMIs. These spectral observations suggested that the BMIs formed 1:2 capsular assemblies with OA. The single set of upfield shifted signals for guests during titration experiments suggested that there was no equilibrium between free and complexed guest molecules in solution and the BMIs remained complexed to OA in aqueous borate buffer solution under our experimental conditions.

Irradiation (450 W medium pressure mercury lamp with Corning filter 4-69; >320 nm) of OA complexes of *ortho*, *meta*, or *para* halogen substituted BMIs (see SI for details) in aqueous borate buffer resulted in the corresponding *trans* isomer (Scheme 2; for LC-MS traces and ^{1}H NMR spectra of

Scheme 2. Photoreactions of *ortho*, *meta*, and *para* Halogenated BMIs in Acetonitrile Solution and as Host–guest Complexes with OA in Water



selected BMIs, see Figures S3–S12 in SI). The photoreaction that culminated with the formation of the corresponding *trans* isomers in the case of *meta* and *para* halogen substituted BMIs surprised us when a new product was formed, as evident from both the ^{1}H NMR spectra and LC-MS traces in the case of *ortho* halogen substituted *N*-Me and *N*-Pr BMIs (**2** and **5**). Progress of the photoreaction as followed by ^{1}H NMR in the case of *ortho*-Cl BMI **2** is presented in Figure 2 (for results on *ortho*-F and *ortho*-Br derivatives, see Figures S13 and S14 in SI).

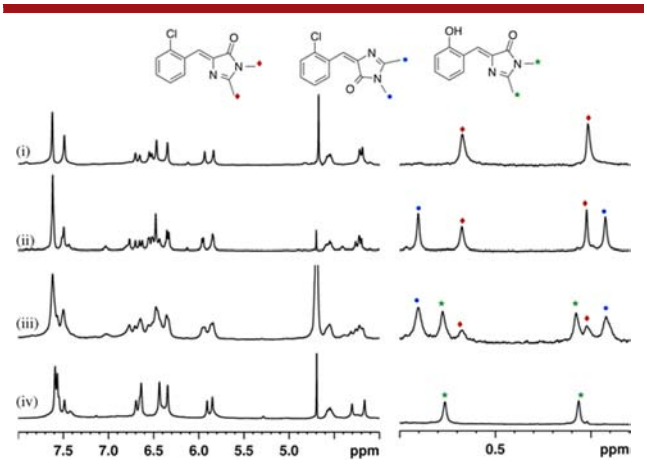


Figure 2. ^{1}H NMR spectra of photochemical reaction (>320 nm) of *ortho*-Cl/*N*-Me@OA_2 in aerated solution of borate buffer in D_2O ([OA] = 1 mM in 10 mM borate buffer in D_2O and [guest] = 0.5 mM) (i) *cis-ortho*-Cl/*N*-Me@OA_2, (ii) upon photolysis of (i) for 2 min, (iii) upon photolysis of (i) for 30 min, and (iv) *cis-ortho*-OH/*N*-Me@OA_2.

The signals due to the methyl groups changed from the initial two due to the *cis* isomer (**2**) to two new ones, identified by comparison with authentic samples, to be due to the *trans* **2**. Further irradiation led to the appearance of two additional signals with concomitant less intense signals due to the *trans* and the *cis* isomers.

Comparison of the ^{1}H NMR spectra of the new signals with an authentic sample confirmed the final photoproduct to be *ortho*-OH BMI **8** (Scheme 2).[13,14] It is clear from Figure 3 (plot

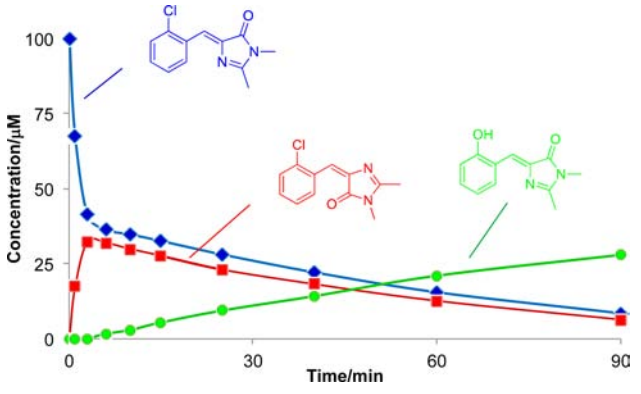


Figure 3. Phototransformation of *cis-ortho*-Cl/N-Me@OA_2 (100 μM:200 μM) in borate aqueous solution (10 mM) at pH 9, and formation of *trans-ortho*-Cl/N-Me and *cis-ortho*-OH/N-Me.

of concentration of products with respect to duration of irradiation as monitored by LC-MS) that the formation of BMI **8** requires accumulation of the *trans* isomer suggesting that the *trans-ortho* halogen substituted BMIs **2** are the precursors of BMI **8**. The identical product distribution inferred from LC-MS traces of irradiations conducted under degassed and aerated conditions in Figure S15 rule out any role for oxygen in the formation of **8** from **2**.[15–17]

The photochemical behavior of BMIs in acetonitrile (in the absence of OA) was probed to infer the confining role of OA.

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BMIs **2** –**7** (100 mM) resulted in the conversion of the *cis* to the *trans* isomer (Scheme 2) (for LC-MS traces of selected samples, see Figures S3–S10 and S16–S17 in SI). In solution[6,7] at the photostationary state the amount of the *trans* isomer varied between 60% and 40% and, as expected, the ratio of the *trans* to the *cis* isomer varied with the structure of the BMI. The product distribution against time of irradiation in acetonitrile solution shown in Figure S18 in SI for the *para*-Cl derivative confirms that prolonged irradiation resulted in *cis* and *trans* isomers only. In the current context it is important to note that uncomplexed *ortho*-halogen substituted BMIs underwent only geometric isomerization upon irradiation in acetonitrile.

The above photoreaction could also be probed by the fluorescence measurements of the reactant and products. While the *cis* BMIs@OA (representing guest BMI included within the host OA) showed fluorescence in the region 390–540 nm, the *trans* isomer showed weak to no fluorescence. The noticeable fluorescence, in the region 540–700 nm, of the hydroxyl substituted BMI **8** within the OA capsule was weak to none when free in borate buffer solution (Figure 4 insert).[13,14] The

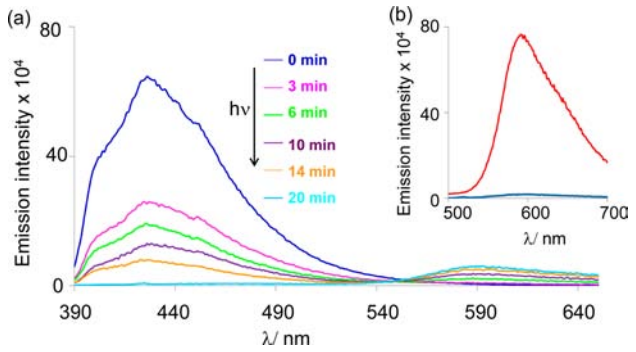
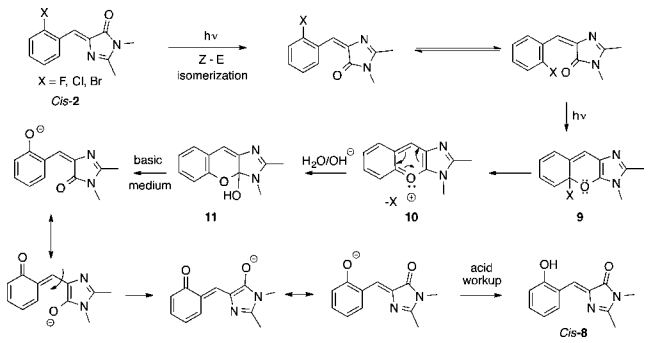


Figure 4. Emission spectra recorded at various times of photolysis (>320 nm) of *cis-ortho*-Cl/*N*-Me@OA_2 in aerated borate buffered water ([guest] = 10^{-5} M, [OA] = 2×10^{-5} M). λ_{ex} 370 nm, and (inset) emission spectra of *ortho*-OH/N-Me ([guest] = 10^{-4} M) in OA (red, [OA] = 2×10^{-4} M) and in borate buffer solution of H_2O (blue).

emission spectra of *cis-ortho* Cl *N*-methyl BMI (**2**) at different stages of irradiation presented in Figure 4 shows a decrease in the emission due to the *cis*-isomer with progress of irradiation and new emission appearing in the region 540–700 nm, the same region of the authentic BMI **8**'s fluorescence. A similar observation was made with *cis-ortho* F and *cis-ortho* Br isomers (Figures S19 and S20 in SI). These *cis-ortho* halogen BMIs were weakly fluorescent in benzene solution, and upon irradiation the intensity decreased and no new emissions appeared (Figures S21 and S22 in SI) consistent with the lack of formation of BMI **8** in benzene solution. Thus, from ^{1}H NMR, LC-MS, and fluorescence results we conclude that *ortho* halogen substituted BMIs exhibit unique behavior within the OA capsule.

A plausible mechanism for formation of **8** from **2** is presented in Scheme 3. We visualize the reaction sequence as follows: (a) Isomerization of *cis* **2** to *trans* **2** upon excitation within OA. (b) The *trans* isomer then adopts a conformation suitable for 6e cyclization and readily gives **9** upon excitation. (c) Aromatization of **9** leads to **10** via loss of the halogen substituent. (d) Exposure of **10** to water within the dynamic capsule[18,19] leads to addition of OH^- or H_2O to yield the intermediate **11**. (d) The unstable hemiacetal **11** in a basic

Scheme 3. Plausible Mechanism for the Formation of Hydroxylated Product from *ortho*-Halogen-BMIs Included within Octa Acid Capsule



environment results in ring opening and single bond rotation to yield the isolated product, *cis* **8**. Apparently, **8** prefers to exist as a *cis* rather than *trans* isomer within OA. The photostable *cis* **8**@OA upon irradiation did not isomerize while *trans* **8**@OA quantitatively isomerized to the *cis* isomer. We have already demonstrated that the oxygen of the OH did not come from atmospheric oxygen. Irradiation in D_2O resulted in OD instead of OH as per mass spectrometry. This suggested that hydroxylation occurs by the addition of the solvent water.

It is important to note that the time for quantitative conversion of *cis* **2** to *cis* **8** under identical irradiation conditions was dependent on the nature of the halogen at the *ortho* position and the *N*-alkyl group: 100% conversion was reached when 2:1 OA complexes of *ortho* bromo, chloro, and fluoro substituted **2** were irradiated, in 1 h 15 min, 2 h 30 min, and 6 h, respectively. As for the role of the alkyl group in influencing the rate of conversion, in the case of chloro derivative *N*-Me (**2**) 2 h 30 min were required while *N*-Pr (**5**) took only 25 min for quantitative conversion. It is quite likely the larger size of the *ortho* halogen (bromo vs fluoro) and *N*-alkyl (propyl vs methyl) groups favor the cyclization process. Our recent report of enhancement of the fluorescence with the bulkiness of the *N*-alkyl group and the *ortho* substituents in BMIs within the OA capsule and the similarity observed here prompts us to wonder if the "volume" effect is in play in the cyclization process as well.[6,7]

In summary, we have established the unusual behavior of the *ortho*-halogen substituted BMIs within the OA capsule while *meta* and *para* counterparts behave no differently than in organic solvents (*cis*–*trans* isomerization) (Scheme 2). The unprecedented photosubstitution of *ortho*-halogens by the hydroxyl group of BMIs within the OA capsule is an added feature of the role of confined spaces in bringing about some unattainable photoreactions. Photosubstitution of aromatics proceeds by a number of different mechanisms,[20−24] and the one we have proposed here is different from the previous ones and probably is restricted to confined spaces. The present studies thus herald the value of confinement in revealing a new photochemical facet of a GFP chromophore.

■ ASSOCIATED CONTENT

Supporting Information

Sample preparation and irradiation procedures, isolation and characterization of products, and additional NMR, LC-MS traces, and fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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AUTHOR INFORMATION

Corresponding Authors

*E-mail: murthy1@miami.edu.
*E-mail: tolbert@chemistry.gatech.edu.

Present Address

‖S.R.S. is currently at the Department of Chemistry, University of Pennsylvania, PA, and A.B. is at the Department of Chemistry, University of California, Berkeley, CA.

Notes

The authors declare no competing financial interest.

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