

Accepted Article

- Title: Coumarin Photocages Modified with an Electron-Rich Styryl Moiety at the 3-Position: Long Wavelength Excitation, Rapid Photolysis and Photobleaching
- Authors: Linyong Zhu, Qiuning Lin, Lipeng Yang, Zhiqiang Wang, Yujie Hua, Dasheng Zhang, Bingkun Bao, Chunyan Bao, and Xueqing Gong

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: Angew. Chem. Int. Ed. 10.1002/anie.201800713 Angew. Chem. 10.1002/ange.201800713

Link to VoR: http://dx.doi.org/10.1002/anie.201800713 http://dx.doi.org/10.1002/ange.201800713

WILEY-VCH

COMMUNICATION

Coumarin Photocages Modified with an Electron-Rich Styryl Moiety at the 3-Position: Long Wavelength Excitation, Rapid Photolysis and Photobleaching

Qiuning Lin, ‡ Lipeng Yang, ‡ Zhiqiang Wang, Yujie Hua, Dasheng Zhang, Bingkun Bao, Chunyan Bao, Xueqing Gong and Linyong Zhu*

Abstract: A new class of coumarin photocages modified with an electron-rich styryl moiety at the 3-position was constructed. The large π -conjugated structure and stabilization of the carbocation intermediates by electron-donors endowed the new photocages excellent long wavelength absorption, large two-photon absorption cross-sections and high uncaging quantum yields. Moreover, the new photocages displayed unique photobleaching property after photocleavage as a result of the intramolecular cyclization rearrangement of carbocation intermediate to form five-membered ring byproducts and block the styryl conjugation at the 3-position. These superior properties of the new photocages are extremely beneficial for high concentration samples and thick specimens, thus extending the application of photocages in many fields.

Photocages, also termed as photoremovable protecting groups (PPGs), are molecules capable of releasing active groups with light activation at a specific wavelength.^{1,2} Due to the non-invasive, non-contamination and high spatiotemporal precision of light stimulus control, PPGs are widely used in various fields of chemistry, biology and materials science.³⁻⁵ The traditional PPGs such as o-nitrobenzyl,⁶ benzoinyl(desyl),⁷ 4-hydroxyphenacyl⁸ and coumarinyl⁹ moieties are all absorptive in the UV-light region that possesses low tissue penetration and causes unwanted photochemical side reactions.¹⁰

The new development of PPGs is expected to have longwavelength absorption that leads to deeper tissue penetration and matches the common laser wavelengths.^{3, 11} In these years, many efforts have been made to develop visible and near-IR lightinduced uncaging groups based on o-nitrobenzyl,¹² coumarin,¹³ BODIPY,¹⁴ cyanine¹⁵ and quinone¹⁶ derivates either by one- or two-photon excitation. Among them, the coumarin-based photocages attract much attentions due to the rapid photolysis and large two-photon (TP) absorption cross-section. Recently, Ellis-Davies and Jullien successfully extended the maximal absorption wavelength of coumarin-based photocages to the blue light region (450 nm) by 3-position π -conjugating with a acrylamide moiety¹⁷ or 2-position substituting by sulfur atom.¹⁸ However, further redshift absorption is accompanied by a drop in the uncaging efficiency.¹⁹ In addition, it is worth mentioning that

 [a] Dr. Q. Lin, L. Yang, Z. Wang, Y. Hua, D. Zhang, B. Bao, Prof. C. Bao, Prof. X. Xue, Prof. L. Zhu Key Laboratory for Advanced Materials East China University of Science and Technology 130# Meilong Road, Shanghai, 200237 (China) E-mail: linyongzhu@ecust.edu.cn
‡ These authors contributed equally.

Supporting information for this article is given via a link at the end of the document.

besides the absorption wavelength and uncaging efficiency, the competitive absorption by the photolysis byproducts would also affect the application of PPGs, especially for high concentration or thick samples due to the inner filtering of light rapidly attenuates the valid light intensity,²⁰ which significantly prolongs the uncaging time and decreases the overall conversion. Hence, the development of PPGs with long-wavelength absorption and a high uncaging efficiency as well as photobleaching at the irradiation wavelength is of great significance.

Herein, we developed a new class of coumarin photocages modified with electron-rich (donor) styryl moieties (D-S) at the 3position (Figure 1a). Due to the 1) large π -conjugated structure, 2) stabilization of the carbocation intermediates by electrondonors, and 3) destruction of the 3-position π -conjugation during the photocleavage process, we first assembled the superior properties of long wavelength excitation, large TP absorption cross-section, high uncaging quantum yield and excellent photobleaching into a class of ideal photocages. We anticipate that these advantages will significantly promote the performance of PPGs, thus benefiting the application of PPGs in many areas.

First, as shown in Figure 1b and Figure S1, coumarin photocages conjugate at 3 positions with D-S moieties of pethoxystyryl (1a), 4'-N,N-dimethylaminostyryl (1b), or conformationally cycloalkyl-locked julolidinestyryl (1c), and its upgraded analogues with bis-julolidine (1d) or sulfur atom substitution (2b and 2d) were synthesized. In contrast, the coumarin photocages linked by bald styryl (1e) or electron withdrawing p-methylbenzotestyryl (1f) groups at the same position and the traditional 7-diethylaminocoumarin (DEAC) and o-nitrobenzyl (o-NB) PPGs were also prepared. The p-methoxybenzonic acid (p-mBA) was chosen as model cargo.

The photophysical and photochemical properties of all of the





WILEY-VCH

COMMUNICATION

Table 1. Photophysical and photochemical properties of PPGs 1a-1f, 2b, 2d, DEAC and o-NB. $^{\rm a}$

PPGs	λ_{max}	$\Phi_{\rm f}$	8 _{max}	Φu	ε _{max} Φ u	σ_{max}	$\sigma_{max} \Phi_u$
1a	430	0.47	3.0	0.45	13.5	58.6	26.4
1b	446	0.02	4.1	0.2	8.2	198	39.6
1c	456	Ν	4.4	0.19	8.36	Ν	Ν
1d	467	0.03	3.5	0.41	14.35	43.2	17.7
2b	490	Ν	3.0	0.4	12.0	Ν	Ν
		0.29 (570 nm)			1.16		
2d	515	Ν	2.5	0.7	17.5	Ν	Ν
		0.17 (600 nm)			1.19		
1e	428	0.53	3.2	0.05	1.6	67.8	3.39
1f	443	0.61	4.4	0.04	1.76	80.1	3.20
DEAC	380	0.14	2.0	0.07	1.4	30.8	2.16
o-NB	338	Ν	0.6	0.16	0.96	Ν	N

^aSymbols and abbreviations: λ_{max} , absorption maximum (nm); Φ_f , fluorescence quantum yield; ϵ_{max} , molar extinction coefficient (10⁴ M⁻¹cm⁻¹); Φ_u , uncaging quantum yield; $\epsilon_{max} \Phi_u$, uncaging efficiency upon one-photon excitation (10³ M⁻¹cm⁻¹); σ_{max} , maximum two-photon cross-section within 730-880 nm (GM, 1GM = 10⁻⁵⁰ cm⁴s/photon); $\sigma_{max} \Phi_u$, uncaging efficiency upon two-photon excitation (GM); and N, not detected.

PPGs were characterized and are summarized in Table 1. Compared to the traditional **DEAC**, the absorption maximum of PPGs **1a-1f**, **2b** and **2d** are significantly red-shifted to the blue to green light region of 430-515 nm (Figure 2a). For PPGs **1c** and **1d**, each julolidine ring contributes a ~10 nm red shift because of the greater conjugation of the amino group with the aromatic core.²¹ In addition, the enhanced intramolecular charge-transfer (ICT) through the 3d empty atomic orbitals of the sulfur atom¹⁸ made the thione PPGs of **2b** and **2d** red-shift 40-50 nm, thus further broadening the absorption band even to the red light region. Besides the absorption wavelength redshift, the molar extinction coefficients of the new PPGs (ranging from 25000 to 44000 M⁻¹cm⁻¹) were all larger than that of the **DEAC** coumarin parent.

The photolysis of all the PPGs were then conducted under their corresponding excitation wavelength with a light intensity of 10 mW/cm². The photolysis evolution recorded by HPLC analysis indicated the smooth photolysis process of all of the PPGs with a high yield of **p-mBA** (Figure 2b and Figure S2). The PPGs **1a-1d**, **2b** and **2d** with D-S-conjugation displayed the behavior associated with a "fast" (t_{1/2} < 110 s) photorelease of **p-mBA** with a uncaging quantum yield Φ_u of 0.19 to 0.7, which are approximately 3 to 10 times higher than that of the common **DEAC** (0.07) and can rival or even exceed the **DEAC450** ($\Phi_u =$ 0.39) developed by Ellis-Davies recently.¹⁷ However, the electronwithdrawing-styryl conjugation analogue **1f** displayed the behavior associated with a "slow" photorelease with a timescale of 16 min ($\Phi_u = 0.04$). The photocage **1e** conjugated with a bald



Figure 2. a) Normalized absorbance spectra showed the long-wavelength absorption of D-S-conjugated PPGs **1a-1d**, **2b** and **2d**. b) Temporal evolution of the uncaging process indicated the faster photolysis of D-S-conjugated PPGs **2b** and **2d** than those of **DEAC** and **o-NB**. c) Large π -conjugation enhanced the two-photon absorption cross-sections of **1a**, **1b**, **1d**, **1e** and **1f** compared to common **DEAC**. d) The conversion rates of **1b** under TP excitation at 730 nm and 800 nm were much faster than those of **DEAC**. All of the tested samples (10⁵ M) were carried out in a 9:1 (v:v) mixture of methanol and DI water.

styryl group had a Φ_{μ} value of 0.05, which was closest to that of the DEAC coumarin parent. The above results demonstrated that the electron-rich effect in the 3-position substituent group of styryl is the key factor for the rapid photocleavage. In addition, the sulfur atom substitution also substantially enhanced the photolysis rate, as the Φ_{μ} values of the photocages **2b** and **2d** enlarged to 0.4 and 0.7, respectively. Combined with the enhanced molar extinction coefficients (ϵ), the uncaging efficiency $\epsilon \Phi_u$ values for most of the D-S-conjugated coumarin-based PPGs have approached or even exceeded 10000 M⁻¹cm⁻¹. Importantly, if the uncaging efficiency $\epsilon \Phi_u$ of 1400 M⁻¹cm⁻¹ for **DEAC** or 960 M⁻¹cm⁻¹ for **o-NB** was regarded as an usable standard of PPGs, the effective uncaging wavelengths of 2b and 2d can extend to 570 nm and 600 nm respectively (shown in Table 1 and Figure 2b), which easily matches the wavelength of commonly used lasers. Hence, the superiority of rapid photolysis and long wavelength excitation make the coumarin-based PPGs conjugated with electron-rich stryl moieties at the 3-position rank among the top families of photocages.

Next, the two-photon absorption properties of the new PPGs were determined by a TP-induced fluorescence method²² within 730-880 nm. As shown in Table 1 and Figure 2c, the TP absorption cross-sections of the examined molecules within the measured wavelength range were almost higher than that of **DEAC**. Then, the TP photolysis of photocages **1b** and **DEAC** were further carried out at 730 nm and 800 nm. Figure 2d showed that the TP-uncaging rate of **1b** was much faster than that of **DEAC** at both 730 nm and 800 nm. This result was consistent with the TP uncaging efficiency $\sigma \Phi_u$ of **1b** (39.6 GM at 730 nm and 7.3 GM at 800 nm) that was estimated to be 10-25 times higher than that of **DEAC** (1.7 GM at 730 nm and 0.73 GM at 800

WILEY-VCH

COMMUNICATION



Figure 3. a) Time course UV-Vis spectra during photolysis display the photobleaching behavior of **1a** (left) and **2b** (right). b) The absorbance ratio of after to before photolysis (A_{after}/A_{before}) revealed the photobleaching behavior of the new PPGs **1a-1f**, **2b** and **2d** compared to **DEAC** and **o-NB**. c) Partial ¹H NMR spectra of **1b** show that the peaks of the 3-position double bond disappeared after photolysis.

nm). Thus, the unique structure of styryl-conjugation also endowed our new PPGs with excellent two-photon photolysis properties.

Further tracking the UV-Vis absorption evolution of the photolysis process of the new PPGs, a typical photobleaching phenomenon was observed (Figure 3a and Figure S3). The maximum absorption band of the styryl-conjugated coumarin gradually disappeared. Simultaneously, absorption bands at 380 nm for ketone photocages 1a-1f and at 470 nm for thione photocages 2b and 2d were generated, which were corresponding to the absorption of the nuclear of ketones²³ or thiones¹⁸ coumarin with non-conjugation at the 3-position. The absorbance ratio of after to before photolysis (Aafter/Abefore) of the new PPGs 1a-1f, 2b and 2d decreased as high as 53-fold at the corresponding irradiation wavelength (Figure 3b). While for the traditional PPGs **DEAC** or **o-NB**, the absorbance ratio only slightly decreased (1.4-fold) or even oppositely increased (0.73-fold). The ¹H NMR spectrum of **1b** and **2b** (Figure 3c and Figure S4) showed that the peaks of the 3-position double bond of styryl moiety disappeared after photolysis, which indicated that the π conjugation at the 3-position was broken during the photocleavage process that led to the photobleaching behavior.

Based on the above results, we can speculate the photolysis mechanism of our new PPGs as shown in Figure 4a. According to the recognized photo- S_N 1-dependent photocleavage of coumarin-based PPGs,²⁴ first, the heterolysis of the 4-position C-O bond formed a free ion-pair of the **p-mBA** anion and carbocation intermediate (**IM1**) upon light excitation. Then, intramolecular cyclization rearrangement of the carbocation, due to the conformational flexibility of stilbene structure, was speculated to achieve a more stable intermediate (**IM1**→**IM2**), followed by trapping of the carbocation by the solvent (hydroxy) to give a probable five-membered ring byproduct **F** rather than the traditional 4-hydroxymethyl-coumarin byproduct.^{23, 24} Upon that, the large π -conjugation at the 3-position was blocked, thus finally



Figure 4. a) Speculation of the photolysis mechanism for the 3-position styrylconjugated coumarin-based PPGs. b) Calculated energy profiles of the photolysis process of 1b and 1f supported the photolysis promotion by the electron-rich effect and the assumption of intramolecular cyclization rearrangement to form five-membered ring byproduct. c) ¹H NMR spectra of the cyclized byproduct of 1b.

inducing the photobleaching behavior.

To better illuminate the above hypothesis, computational density functional theory (DFT) calculations of electron-donating (1b) and electron-withdrawing (1f) styryl-conjugated PPGs were first conducted. The calculated structures of the key states are illustrated in Figure S5. The calculated energy profiles of photolysis processes in Figure 4b show that photolysis of 1b gives a smaller stabilization energy of the carbocation intermediate IM1 (1.06 eV) compared with that of 1f (1.97 eV), which indicates that the electron-rich effect significantly stabilizes the carbocation intermediate and enables the D-S-conjugated coumarin undergoing photolysis more readily and much faster. Moreover, the energy level of the carbocation intermediate IM2 is much lower than that of IM1 for both PPGs 1b and 1f, which supports the assumption that IM1 would like to undergo intramolecular cyclization rearrangement to form a fivemembered ring intermediate. The Mulliken charges were further calculated to testify the carbocation rearrangement (Figure S6). Next, the photolysis byproduct of 1b was isolated and all of the HR-MS (ESI), H¹ NMR and C¹³ NMR spectra (Figure 4c, Figure S7 and Figure S8) testified the cyclized structure of the byproduct 1b-F. All of the above results effectively certify that the electronrich effect on 3-position promoted the photolysis rate and the large π-conjugation was finally blocked by cyclization rearrangement, thus changing the absorption performance of our new PPGs.

According to the photolysis rate equation and Beer-Lambert law, either the photolysis rate or light intensity with depth shows an inverse exponential relationship with the competitive absorption of increasing byproducts.^{20, 25} That signifies the competitive absorption by byproducts severely bothers the photolysis process, especially for high concentration or thick samples. However, once the photocages possess the photobleaching property, the negative disturbance of competitive absorption can be eliminated. Thus, we demonstrated the superiority of our new PPGs for uncaging in high concentration

COMMUNICATION



Figure 5. a) Temporal evolution of the uncaging process showed the superiority of PPGs 1a and 2d with low competitive absorption compared to DEAC and o-NB at different initial concentrations $(2\times10^{-4}, 2\times10^{-3} \text{ and } 2\times10^{-2} \text{ M})$. b) i) Schematic illustration of photo-crosslinking gelation by 1a-HA or DEAC-HA with PEG-4MaI; ii) Photographs of the specimens before and after light irradiation (20 mW/cm²) revealed the deeper light penetration in 1a-HA hydrogel than that in DEAC-HA hydrogel; iii) The attenuation of light intensity (20 mW/cm²) after transmitting hydrogel specimens with a thickness of 0.5 cm.

samples due to their unique photobleaching behavior. Figure 5a shows the conversion curves of PPGs 1a and 2b as well as the traditional DEAC and o-NB at different initial concentrations $(2 \times 10^{-4}, 2 \times 10^{-3} \text{ and } 2 \times 10^{-2} \text{ M})$. Except for rapid photolysis, our new PPGs 1a and 2b showed extremely high conversion that remained up to 90% even when the initial concentration reached to 2x10⁻² M. However, the final conversions of DEAC and o-NB with severe competitive absorption by the photolysis byproducts are initial concentration-dependent, which sharply decreased to 15% and 11% at the high concentration of 2×10^{-2} M even after long-term irradiation. To further indicate that the photobleaching property also has benefits for uncaging in thick specimens, we followed the idea of our previous work²⁶ to prepare photocrosslinking hydrogel (Figure 5b i). Thiols-caged PPGs were synthesized from 1a and DEAC and grafted to hyaluronic acid (HA) to obtain photoresponsive polymer 1a-HA and DEAC-HA (Figure S9). Upon light irradiation, photo-uncaged thiols of 1a-HA and DEAC-HA induce rapid gelation with four-arm PEG tetramaleimide (PEG-4Mal) by thiol Michael addition (Figure S10 and S11). The final thickness of 1a-HA hydrogel (1.8 cm) is much higher than that of DEAC-HA hydrogel (0.5 cm) after enough light irradiation from the bottom of specimens at 450 nm and 405 nm respectively (Figure 5b ii). Meanwhile, the light intensity showed 99.8% attenuation after passing through the formed 0.5-cm **DEAC-HA** hydrogel, while in contrast, only 28.1% light intensity was attenuated under the same thickness of 1a-HA hydrogel (Figure 5b iii and Figure S12). Overall, the unique photobleaching behavior of our new PPGs successfully overcomes the inner filtering effect of the photolysis byproduct and thus extremely benefits the high concentration or thick samples.

In conclusion, we have successfully constructed a new class of coumarin photocages modified with electron-rich styryl moieties at the 3-position. Experimental measurements and DFT computational calculations demonstrated their excellent properties of long wavelength-excitation, rapid photolysis and photobleaching, thus indicating that the new coumarin-based PPGs are extremely beneficial for diverse applications in chemical synthesis, biological manipulation and material manufacturing.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (21425311 and 21774030).

Keywords: photoremovable protecting groups • coumarin photocages • photocleavage • photochemistry • photolysis

- P. Klán, T. Šolomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov, J. Wirz, *Chem. Rev.* 2013, *113*, 119-191.
- [2] G. C. R. Ellis-Davies, Nat. Methods. 2007, 4, 619-628.
- [3] M. J. Hansen, W. A. Velema, M. M. Lerch, W. Szymanski, B. L. Feringa, *Chem. Soc. Rev.* 2015, 44, 3358-3377.
- G. Mayer, A. Heckel, Angew. Chem. 2006, 118, 5020-5042; Angew. Chem. Int. Ed. 2006, 45, 4900-4921.
- [5] C. Yang, M. W. Tibbitt, L. Basta, K. S. Anseth, *Nat. Mater.* 2014, 13, 645-652.
- [6] A. Patchornik, B. Amit, R. B. Woodward, J. Am. Chem. Soc. 1970, 92, 6333-6335.
- [7] J. C. Sheehan, R. M. Wilson, J. Am. Chem. Soc. 1964, 86, 5277-5281.
- [8] C.-H. Park, R. S. Givens, J. Am. Chem. Soc. 1997, 119, 2453-2463.
- [9] R. S. Givens, B. Matuszewski, J. Am. Chem. Soc. 1984, 106, 6860-6861.
- [10] K. Kalka, H. Merk, H. Mukhtar, J. Am. Acad. Dermatol. 2000, 42, 389-413.
- [11] G. Bort, T. Gallavardin, D. Ogden, P. I. Dalko, Angew. Chem. 2013, 125, 4622-4634; Angew. Chem. Int. Ed. 2013, 52, 4526-4537.
- [12] H. K. Agarwal, R. Janicek, S.-H. Chi, J. W. Perry, E. Niggli, G. C. R. Ellis-Davies, J. Am. Chem. Soc. 2016, 138, 3687-3693.
- [13] Y. Chitose, M. Abe, K. Furukawa, J.-Y. Lin, T.-C. Lin, C. Katan, Org. Lett. 2017, 19, 2622-2625.
- [14] T. Slanina, et al. J. Am. Chem. Soc. 2017, 139, 15168-15175.
- [15] R. R. Nani, A. P. Gorka, T. Nagaya, H. Kobayashi, M. J. Schnermann, *Angew.Chem.* 2015, 127,13839-13842; *Angew. Chem. Int. Ed.* 2015, 54, 13635-13638.
- [16] D. P. Walton, D. Dougherty, A. J. Am. Chem. Soc. 2017, 139, 4655-4658.
- [17] J. P. Olson, H.-B. Kwon, K. T. Takasaki, C. Q. Chiu, M. J. Higley, B. L. Sabatini, G. C. R. Ellis-Davies, *J. Am. Chem. Soc.* **2013**, *135*, 5954-5957.
- [18] L. Fournier, I. Aujard, T. L. Saux, S. Maurin, S. Beaupierre, J.-B. Baudin, L. Jullien, *Chem. Eur. J.* **2013**, *19*, 17494-17507.
- [19] A. Gandioso, M. Palau, A, Nin-Hill, I. Melnyk, C. Rovira, S. Nonell, D. Velasco, J. García-Amorós, V. Marchán, *ChemistryOpen* 2017, 6, 375-384.
- [20] B. D. Fairbanks, M. P. Schwartz, C. N. Bowman, K. S. Anseth, *Biomaterials* **2009**, *30*, 6702-6707.
- [21] B. J. Coe, et al. *Dyes Pigm.* **2009**, *8*2, 171-186.
- [22] B. R. Cho, K. H. Son, S. H. Lee, Y.-S. Song, Y.-K. Lee, S.-J. Jeon, J. H. Choi, H. Lee, M. Cho, J. Am. Chem. Soc. 2001, 123, 10039-10045.
- [23] Q. Lin, C. Bao, S. Cheng, Y. Yang, W. Ji, L. Zhu, J. Am. Chem. Soc. 2012, 134, 5052-5055.
- [24] T. Eckardt, V. Hagen, B. Schade, R. Schmidt, C. Schweitzer, J. Bendig, J. Org. Chem. 2002, 67, 703-710.
- [25] S. Asmusen, G. Arenas, W. D. Cook, C. Vallo, Dent. Mater. 2009, 25, 1603-1611.
- [26] Z. Liu, Q. Lin, Y. Sun, T. Liu, C. Bao, F. Li, L. Zhu, Adv. Mater. 2014, 26, 3912-3917.

WILEY-VCH

COMMUNICATION

Entry for the Table of Contents

COMMUNICATION

Coumarin photocages: Based on the 1) large π -conjugation, 2) carbocation stabilization and 3) intramolecular cyclization rearrangement, we constructed a new class of coumarin-based photocages with electron-rich styryl moiety at the 3-position. The long wavelength excitation, rapid photolysis and photobleaching make the new photocages benefit for high concentration and thick specimens, thus extending the application of photocages to more areas.



Qiuning Lin, ‡ Lipeng Yang, ‡ Zhiqiang Wang, Yujie Hua, Dasheng Zhang, Bingkun Bao, Chunyan Bao, Xueqing Gong and Linyong Zhu*

Page No. – Page No.

Coumarin Photocages Modified with an Electron-Rich Styryl Moiety at the 3-Position: Long Wavelength Excitation, Rapid Photolysis and Photobleaching