The Malleable Excited States of Benzothiadiazole Dyes and Investigation of their Potential for Photochemical Control

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Abstract

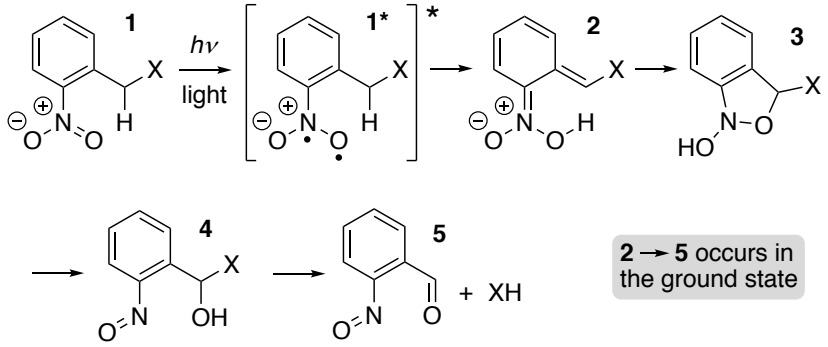
A strategy to control the efficiency of a photocleavage reaction based on changing the nature of the excited state is presented. A novel class of photoactive compounds has been synthesized by combining the classical *o*-nitrobenzyl scaffold with an environmentally sensitive dye, 4-amino-nitrobenzothiazole. Irradiation in a polar solvent lead to an excited state that is inoperative for photochemistry whereas excitation in a nonpolar solvent lead to an excited state that is photochemically active. A photochemical degradation appears to be the preferred process in contrast to the intended photocleavage process.

Introduction

Photocleavable protecting groups (PPGs, sometimes called photocages) offer the ability to unveil different reactive groups in a non-invasive manner using light.[1] The ability to independently address different functional groups, however, requires the capability to trigger distinct PPGs in a selective manner. PPGs that can be activated with different wavelengths of light provide one avenue to control and manipulate multiple functional groups.[1b] Unfortunately, attempts to identify pairs of PPGs for these purposes have only realized up to 70% differentiation between absorption maxima and selective photo-deprotection of different PPGs.[2] Alternatively, distinct PPGs that display differences in their quantum yield of released cargo upon excitation—a competition of release rates—similarly provide an avenue to selectively release specific functional groups.[3]

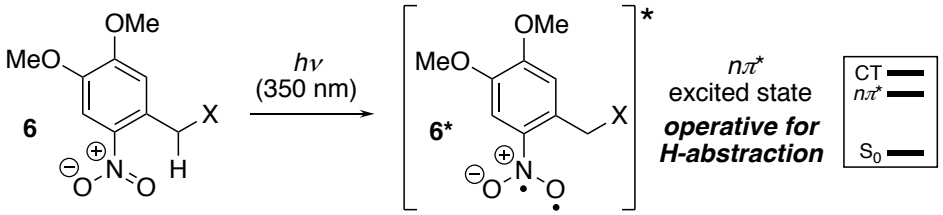
We were inspired by a different approach in which the user could selectively turn the photocleavage reaction ON or OFF. In this manner, the PPG could be turned OFF to allow activation of seemingly any other photo-sensitive feature in the system. Following this step, the PPG could then be turned back ON and subsequently photocleaved in a traditional manner. This work details our efforts towards this goal.

One of the most widely used and efficient PPGs is based on the *o*-nitrobenzylic scaffold (**1**).[1a-c] Following excitation of **1** to produce **1*** in the excited state (Scheme 1), rapid abstraction of the benzylic hydrogen (< 2 ns) by the nitro group can occur from both the singlet and triplet excited states to give **2**.[4] A series of ground-state steps then follow (**2** → **5**) to finally release the generic leaving group X and produce **5**; however, the H-abstraction process (**1*** → **2**) is the critical photochemical step.[4a, 5]



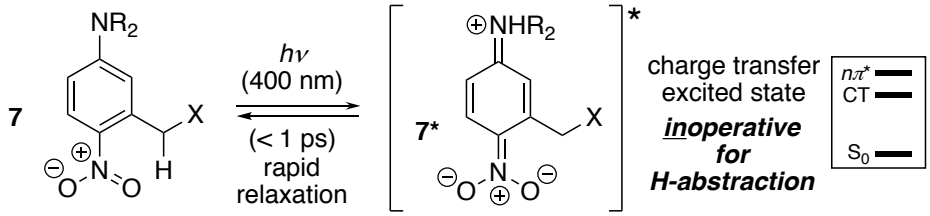
Scheme 1. Mechanism of photochemical reaction and release of X, where X is a leaving group.

The nature of the excited state of **1*** is also critical to realizing the key H-abstraction step (**1*** → **2**) and can best be understood by considering some examples from the literature (Schemes 2 and 3). The absorbance maximum of **1** is in the UV (300 nm). Substituents on the aromatic ring provide an avenue to red-shift the absorbance of *o*-nitrobenzyl derivatives. Thus, the installation of electron-donating methoxy substituents at the 3,4-positions of the benzene ring (**6**) can shift the absorbance to ~350 nm[6] (Scheme 2, an example of the common "push–pull" design strategy to red-shift chromophores).



Scheme 2. Substituent effects of *o*-nitrobenzyl derivatives.

Unfortunately, the installation of electron-donating groups also leads to a decrease in the quantum yield of released leaving group (X).[6] This strategy eventually pushes to a breaking point because, while stronger electron-donating groups such as $-NR_2$ in **7** further shift the wavelength of absorbance close to 400 nm, the photochemical reaction is shut down altogether (Scheme 3).[3a]

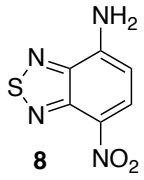


Scheme 3. Charge-transfer in *o*-nitrobenzyl derivatives.

The reason for the decrease in quantum yield of photocleavage of X in response to electron-donating substitution originates from the nature of the excited state (Scheme 2). It is known that an *nπ** excited state is responsible for H-abstraction.[5a, 7] As the ring substituent becomes more electron donating, however, the excited state takes on a charge transfer (CT) character that is inoperative for H-abstraction due to ultrafast relaxation (<1 ps) to the ground state.[8] Thus, this behavior indicates a potential avenue to selectively turn the photo-deprotection reaction ON and OFF by selectively controlling the nature of the excited state and the pathways available for

reaction. Indeed, protonation of the $-NR_2$ group in **7** with triflic acid removes its electron-donating ability which returns the photoactive $n\pi^*$ state.[3c] We hypothesized that modulation of the excited-state character could be achieved under milder conditions by employing an environmentally-sensitive dye (i.e. benzothiadiazole chromophores[9]).

Table 1. Emissive properties of **8**.



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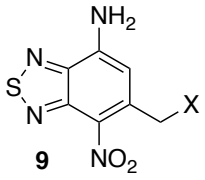


9

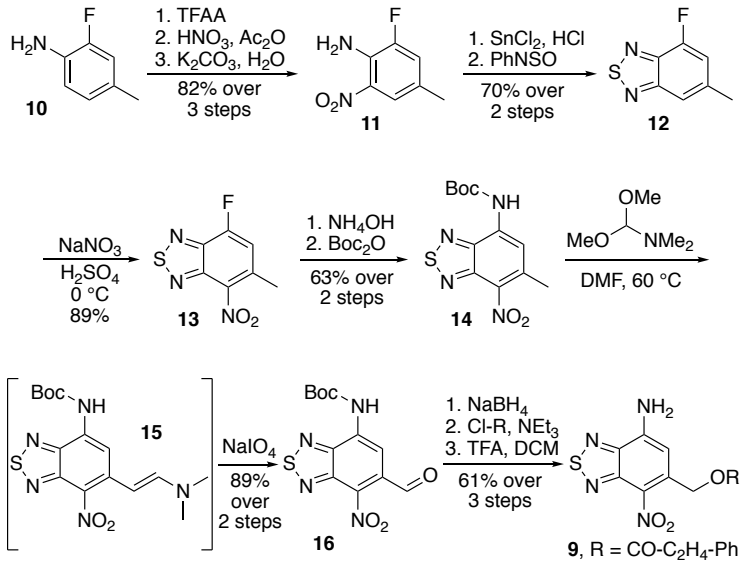
solvent	abs, λ_{max} (**8**, nm)	em, λ_{max} (**8**, nm)	Φ_F (**8**)[a]
benzene	421	504	0.15
acetonitrile	442	530	0.54

[a] Quantum yields determined relative to coumarin 153 in degassed ethanol (Φ_{std} = 0.38).

The $-NH_2$ derivative of nitrobenzothiadiazole (**8**) displays an emissive excited-state that is dominated by charge-transfer character (Table 1).[10] Alternatively, Saha and Samanta have proposed that **8** can access an $n\pi^*$ excited state in nonpolar solvents such as benzene.[11],[12] This proposal was based on the observation that the quantum yield of fluorescence for **8** suddenly decreases in benzene compared with acetonitrile (Table 1). Saha and Samanta proposed that this sudden drop in emission intensity was due to "…the existence of two close-lying states (CT and $n\pi^*$) in the system. In the least polar solvent… emission originates from the $n\pi^*$ state, which is the lowest excited state. In relatively higher polarity solvents, the CT state is stabilized below the $n\pi^*$ states." In light of Schemes 2 and 3, the ability of **8** to alter the nature of its excited state depending on the solvent environment would provide an avenue to turn the photo-deprotection reaction ON and OFF. We hypothesized that if a benzylic leaving group ($-CH_2OCOR'$) were grafted onto **8**, it would create a new photocleavable protecting group with a malleable excited state (**9**). In polar solvents (acetonitrile), **9** would be excited to a CT excited state that is unable to undergo H-abstraction, rendering the photoreaction OFF. Conversely, in nonpolar solvents such as benzene, **9** could access an $n\pi^*$ excited state that is operative for H-abstraction, rendering the photoreaction ON. This approach represents a fundamentally new avenue to selectively activate and distinguish between PPGs (and functional groups), where differences in solvent polarity avoids the use of strong acids such as triflic acid.

Results and Discussion

To address design our hypothesis outlined above, we turned to the synthesis of **9** (Scheme 4). Starting from 2-fluoro-4-methylaniline (**10**), prior protection of the free amine was required to install the nitro group followed by deprotection of the intermediate product to give **11**.[13] Nitroamine **11** was reduced to give a diamine compound[14] that was subsequently converted to **12** via an oxidative ring closure with *N*-thionylaniline.[15] Benzothiadiazole **12** was then nitrated to install the critical photoactive $-NO_2$ group (**13**)[16]. Compound **13** was disposed towards installation of the amine electron donor through nucleophilic aromatic substitution.[17] The free amine was protected with a Boc group to give **14** in preparation for functionalization of the benzylic position.



Scheme 4. Synthesis of **9**.

We next turned our attention to installing the benzylic leaving group necessary to test our hypothesis described above. Numerous conditions to functionalize the benzylic position, such as radical halogenation and oxidation, failed to produce isolatable material, likely due to the strongly electron deficient nature of **14**. Capitalizing on this electron deficiency, however, we found that the benzylic CH_3 was readily deprotonated *in situ* during the reaction with *N*,*N*-dimethylformamide dimethyl acetal to produce enamine **15**.[18] Oxidation of the enamine double bond with sodium periodate provided aldehyde **16**.[19] Finally, the leaving group was installed by reduction of the aldehyde with sodium borohydride followed by esterification with 3-phenylpropionyl chloride. A final Boc deprotection led to the desired compounds **9** for evaluation.

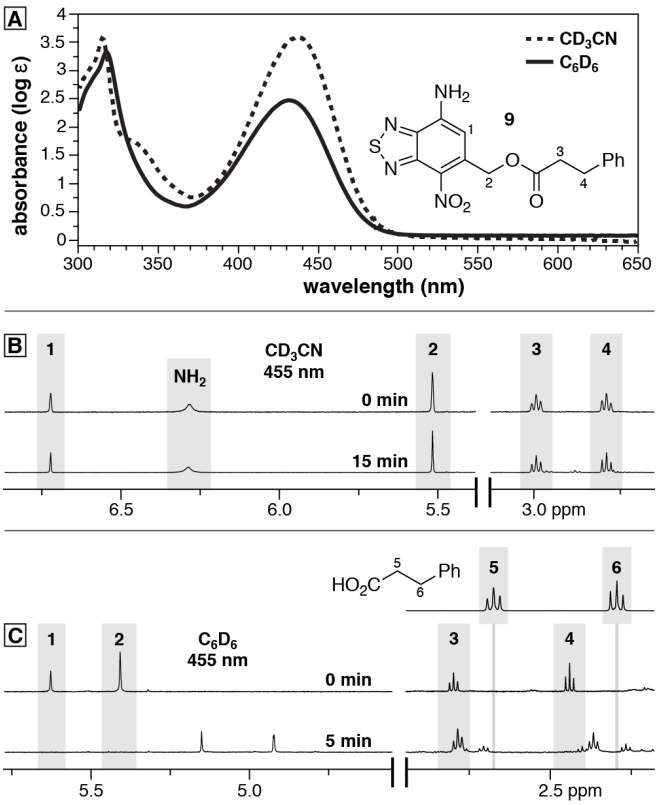


Figure 1. (A) UV-vis absorbance spectra of **9**. ^{1}H NMR (600 MHz) spectra of **9** in (B) CD_3CN and (C) C_6D_6 before and after irradiation at 455 nm (LED source) for times indicated. Irradiance of light source = 370 mW/cm^2.

To test our design hypothesis, we excited **9** at 455 nm in deuterated acetonitrile and benzene (Figure 1A). As anticipated, we did not observe any photochemical reaction in CD_3CN (Figure 1B). We ascribe this behavior to the benzothiadiazole chromophore adopting a CT excited state in acetonitrile, which is unable to undergo abstraction of the hydrogen at the benzylic position (akin to Scheme 3). Alternatively, a photochemical reaction was observed in C_6D_6 (Figure 1C).[20] We propose that this observation is consistent with an nπ* excited state and H-abstraction chemistry operating in benzene. Finally, as an aside, proton H1 in 9 appears below 6 ppm in benzene-d6, outside of the traditional "aromatic window" of where aromatic protons appear. This chemical shift is common for the proton ortho to the electron-donating NH2 group in this class of dyes (see Figure S17). We surmise this lower ppm shift is due, in part, to the lower aromatic character of the 6-membered ring in benzothiadiazoles (*vide infra*).[21]

While a clean photochemical reaction was observed in C_6D_6, we did not observe release of the 3-phenylpropionic acid leaving group (Figure 1C). Removal of the C_6D_6 and re-dissolving the material in a variety of polar-protic solvents to facilitate the ground-state deprotection failed to liberate the free 3-phenylpropoinic acid. Unfortunately, all attempts to isolate, purify and further characterize the photoproduct from Figure 1C have failed.

The efficiency of the photoreaction in Figure 1C suggests that the H-abstraction may be operating—to produce **17**—but perhaps the ensuing ground-state release of the leaving group is not occurring (Figure 2 and Scheme 1) and **17** decomposes by other means to give the product in Figure 1C. To investigate this possibility further, we initially considered that the lower aromaticity of the benzothiadiazole core[21] may be responsible for the seeming inability of putative **17** to progress along the ground-state pathway (Figure 2). The 6-membered ring of

benzothiadiazole is less aromatic than benzene according to several computed indices of aromaticity (Table 2, see SI for discussion).

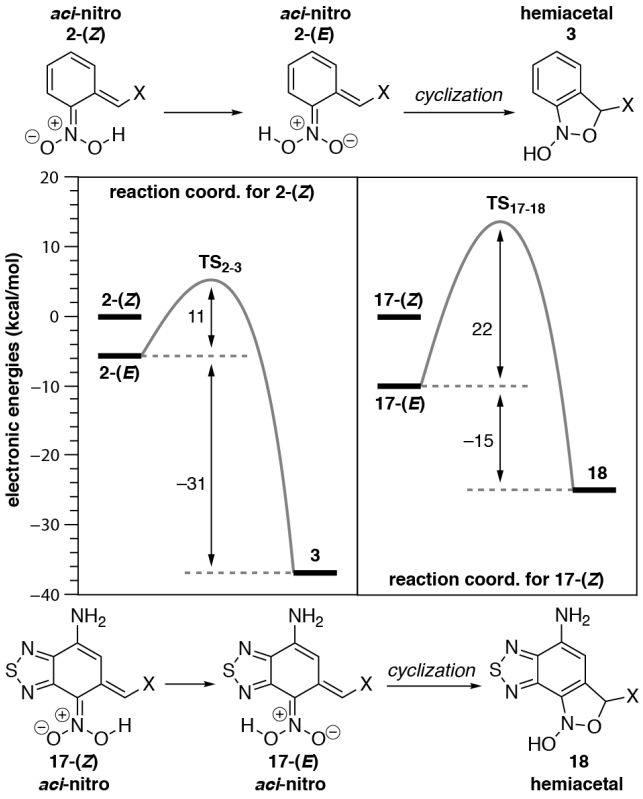
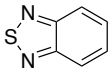


Figure 2. Potential energy surface for the thermal cyclization step. Energies are reported as electronic energies referenced to **2**. Structures and energies were determined at the M06-2X/6-311+g(d,p) level of theory.[22] X = formate as the leaving group.

Aromaticity plays a key role in one of the steps in the ground-state release mechanism (Figure 3), where the remaining steps after ring closure to the hemiacetal do not directly involve the aromatic ring. To understand the effect that the reduced aromaticity of benzothiadiazole might have on the reaction of **9**, we calculated the energetics of the conversion of *aci*-nitro to hemiacetal for both **2** and **17** (Figure 2).[5c] The tautomeric *aci*-nitro forms (*Z* and *E*) can readily interconvert.[23]

Table 2 Differing aromaticity in the 6-membered ring.

computed aromaticity index [a]		
ASE (kcal/mol) [b]	–33.30	–16.27
NICS(1) [c]	–9.86	–7.91
HOMA [d]	0.99	0.60

[a] The lower the magnitude of the index, the lower the aromaticity of the cycle. [b] Aromatic stabilization energy.[24] [c] Nucleus independent

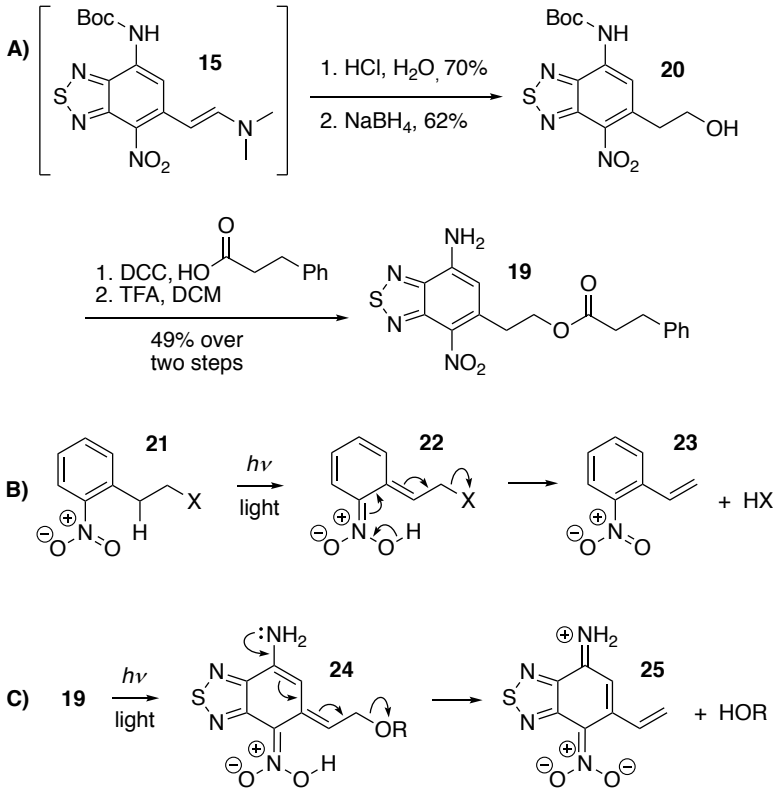
chemical shift.[25] [d] Harmonic oscillator model of aromaticity.[26]

The cyclization of *aci*-nitro **2** into hemiacetal **3** is driven, in part, by the re-aromatization of the benzene ring upon cyclization. Thus, there is a large thermodynamic driving force (–31 kcal/mol) for the formation of **3**. Similarly, aromatic stability builds in the transition state ($\mathbf{TS_{2\text{-}3}}$) to drive the cyclization required to form hemiacetal **3** (Figure 3).

Alternatively, the reduced aromatic character of benzothiadiazole in **17** means that there is a lower thermodynamic driving force to form **18**. Likewise, there is lower aromatic stabilization in the transition state ($\mathbf{TS_{17\text{-}18}}$), that does not compensate for the energy required for cyclization (Figure 2).[5c] We therefore hypothesized that the cyclization of **17** to **18** is unfavorable and **17** instead decomposes by other means. Subsequently, we synthesized **19** (Scheme 5A) to test our hypothesis that the lower aromaticity in **9** contributed to a low propensity for cyclization to the hemiacetal (**18**)—and in turn, the failure of **9** to release the leaving group.

Compound **19** represents a related class of PPGs that, despite similarities with o-nitrobenzyl PPGs, undergoes a different ground-state mechanism for release of the leaving group (**21**–**23**, Scheme 5B).[1a] The initial photochemical H-abstraction step is the same for **21** and **1**, however, we hypothesized that expulsion of the leaving group from **19** would be facilitated by the electron-donating $–NH_2$ group (**24**–**25**, Scheme 5C). Wherein, lower aromaticity has been shown to contribute to greater rates of release of leaving groups.[27]

In analogy to **9**, compound **19** did not show any photochemical reactivity in CD_3CN (Figure B) but did show pronounced changes upon irradiation in C_6D_6 (Figure C). Unfortunately, we similarly did not observe the release of free leaving group (Figure 4C).



Scheme 5. (A) Synthesis of **19** and (Band C) proposed mechanism of release.

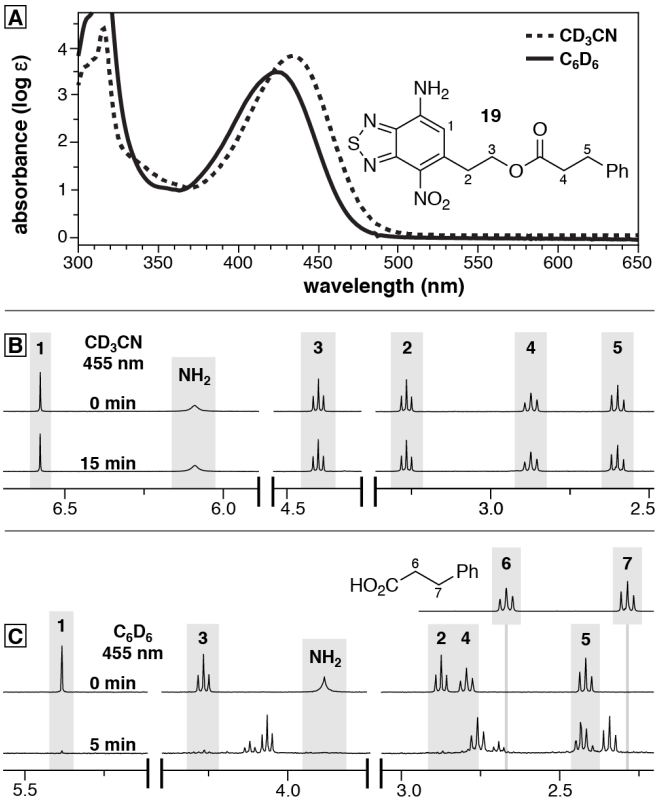
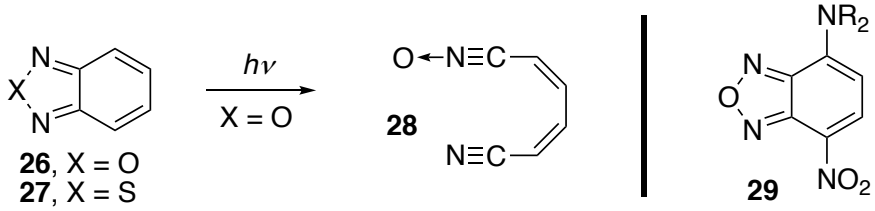


Figure 3. (A) UV-vis absorbance spectra of **9**. ^{1}H NMR (600 MHz) spectra of **9** in (B) CD_3CN and (C) C_6D_6 before and after irradiation at 455 nm (LED source) for times indicated. Irradiance of light source = 370 mW/cm^2.

Despite different mechanisms of ground-state release, the results of the photoirradiation of **9** and **19** in C_6D_6 are similar: production of a clean photoproduct without loss of leaving group. This result lead us to conclude that aromaticity was not likely to be a dominant factor in inhibiting the ground-state release of the leaving group for **9**.



26, X = O
27, X = S

Scheme 6. Fast photoreaction of benzofurazans.

Instead, we propose that some other photo-degradation process must be dominant for both **9** and **19** in benzene. This hypothesis is supported by previous work with certain derivatives of the benzofurazan (**26**, the oxygen congener of the benzothiadiazole **27**), which report a fast photoreaction to form **28** in both polar and non-polar (Scheme 6).[28] We note, however, that the photodegradation process in Scheme 6 has not been observed for benzofurazan chromophores (**29**) that display the same push-pull system present in **8**, **9** and **19** (NR_2 donor, NO_2 acceptor).[29] Similarly, such a photoreaction has not been documented with **27** and we specifically targeted the benzothiadiazole core (**27**) in **9** and **19** because of its reported photostability relative to benzofurazan (**26**).[9, 30] Thus, we must conclude that the reactive excited state in **9** and **19** leads to a preferred degradation process without release of the leaving group. Attempts to characterize

the photochemical products using NMR have failed due to instability and insolubility. However, mass spectral analysis of the reaction mixtures indicates a photochemical product common to both **9** and **19** is formed. (see SI for further discussion).

Conclusions

This work describes our design strategy to apply the malleable nature of the excited state of an environmentally sensitive fluorophore to the control of a photo-deprotection reaction. The stark contrast in photochemical reactivity between different solvents suggests that we were successful in selecting between a reactive excited state ($n\pi^*$) and an unreactive state (charge transfer). The photochemical reaction appears to operate cleanly, but unfortunately, does not appear to release the leaving group. We propose that a competing side reaction must divert the photochemical reaction (or ensuing ground-state release of the leaving group) down a non-productive path. Notably, while both **9** and **19** do not perform as intended, this is one of the few studies of *o*-nitrobenzyl reactivity that looks beyond the classical benzene scaffold,[1a] and towards novel dye scaffolds. We propose that further investigation of the photochemical designs described here could lead to a PPG with the longest wavelength of absorption (>400 nm) of any known *o*-nitrobenzylic derivative while maintaining a compact size.[31]

Acknowledgements

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Keywords: photochemistry • protecting groups • environmental sensitivity • benzothiadiazole • chromophore

References

[1] aP. Klan, T. Solomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov, J. Wirz, Chem Rev 2013, 113, 119-191; bM. J. Hansen, W. A. Velema, M. M. Lerch, W. Szymanski, B. L. Feringa, Chem. Soc. Rev. 2015, 44, 3358-3377; cH. T. Yu, J. B. Li, D. D. Wu, Z. J. Qiu, Y. Zhang, Chem. Soc. Rev. 2010, 39, 464-473; dP. F. Wang, Asian J Org Chem 2013, 2, 452-464.
[2] V. San Miguel, C. G. Bochet, A. del Campo, J. Am. Chem. Soc. 2011, 133, 5380-5388.
[3] aC. G. Bochet, Tetrahedron Lett. 2000, 41, 6341-6346; bA. Blanc, C. G. Bochet, J. Org. Chem. 2002, 67, 5567-5577; cE. Riguet, C. G. Bochet, Org Lett 2007, 9, 5453-5456.
[4] aT. Schmierer, S. Laimgruber, K. Haiser, K. Kiewisch, J. Neugebauer, P. Gilch, Phys Chem Chem Phys 2010, 12, 15653-15664; bT. Schmierer, F. Bley, K. Schaper, P. Gilch, J Photoch Photobio A 2011, 217, 363-368.
[5] aS. Fröbel, P. Gilch, J Photochem. Photobiol. A 2015, 318, 150-159; bT. Schmierer, G. Ryseck, T. Villnow, N. Regner, P. Gilch, Photochem Photobiol Sci 2012, 11, 1313-1321; cY. V. Il'ichev, J. Wirz, J Phys Chem A 2000, 104, 7856-7870; dY. V. Il'ichev, M. A. Schworer, J. Wirz, J Am Chem Soc 2004, 126, 4581-4595.
[6] aS. Charier, O. Ruel, J. B. Baudin, D. Alcor, J. F. Allemand, A. Meglio, L. Jullien, B. Valeur, Chem-Eur J 2006, 12, 1097-1113; bH. Görner, Photoch Photobio Sci 2005, 4, 822-828.
[7] aS. Laimgruber, T. Schmierer, P. Gilch, K. Kiewisch, J. Neugebauer, Phys Chem Chem Phys 2008, 10, 3872-3882; bA. Migani, V. Leyva, F. Feixas, T. Schmierer, P. Gilch, I. Corral, L. Gonzalez, L. Blancafort, Chem Commun 2011, 47, 6383-6385.

[8] S. A. Kovalenko, R. Schanz, H. Hennig, N. P. Ernsting, J Chem Phys 2001, 115, 3256-3273.
[9] A. Thooft, K. Cassaidy, B. VanVeller, J. Org. Chem. 2017, 82, 8842-8847.
[10] S. Fery-Forgues, J. P. Fayet, A. Lopez, J. Photochem. Photobiol. A: Chem. 1993, 70, 229-243.
[11] S. Saha, A. Samanta, J Phys Chem A 1998, 102, 7903-7912.
[12] The Saha and Samanta work above concerned the study of the oxygen variant, nitrobenzoxadiazole. However, the identity of the chalcogen in the 5-membered ring heterocycle does not have a large impact on the properties of the dye. Thus, for clarity we only discuss the nitrobenzothiadiazole, for which the arguments of Saha and Samanta still hold.
[13] N. Zhang, X. Zhang, J. Zhu, A. Turpoff, G. Chen, C. Morrill, S. Huang, W. Lennox, R. Kakarla, R. Liu, C. Li, H. Ren, N. Almstead, S. Venkatraman, F. G. Njoroge, Z. Gu, V. Clausen, J. Graci, S. P. Jung, Y. Zheng, J. M. Colacino, F. Lahser, J. Sheedy, A. Mollin, M. Weetall, A. Nomeir, G. M. Karp, J. Med. Chem. 2014, 57, 2121-2135.
[14] Y. Yasui, D. K. Frantz, J. S. Siegel, Org. Lett. 2006, 8, 4989-4992.
[15] K. Kawamoto, S. Uchiyama, Chem. Lett. 2012, 41, 1451-1452.
[16] K. S. Sharma, V. Singh, R. P. Singh, Indian J. Chem. 1978, 16B, 892-894.
[17] Y. Yan, Z. Liu, J. Zhang, R. Xu, X. Hu, G. Liu, Bioorg. Med. Chem. Lett. 2011, 21, 4189-4192.
[18] M. Edin, S. Grivas, ARKIVOC 2000, 1-5.
[19] E. C. Riesgo, X. Jin, R. P. Thummel, J. Org. Chem. 1996, 61, 3017-3022.
[20] The low solubility of 9 in benzene made determination of the quantum yield of reaction impractical because we could not capture all light hitting the sample.
[21] Y. Nguyen, B. Lampkin, A. Venkatesh, E. Arkady, A. Rossini, B. VanVeller, J. Org. Chem. 2018, 83, 9850–9857.
[22] Y. Zhao, D. G. Truhlar, Theor Chem Acc 2008, 120, 215-241.
[23] M. Schworer, J. Wirz, Helv Chim Acta 2001, 84, 1441-1458.
[24] P. V. Schleyer, F. Puhlhofer, Org Lett 2002, 4, 2873-2876.
[25] R. Gershoni-Poranne, A. Stanger, Chem Soc Rev 2015, 44, 6597-6615.
[26] T. M. Krygowski, H. Szatylowicz, O. A. Stasyuk, J. Dominikowska, M. Palusiak, Chemical Reviews 2014, 114, 6383-6422.
[27] K. M. Schmid, S. T. Phillips, Journal of Physical Organic Chemistry 2013, 26, 608-610.
[28] aW. Heinzelmann, P. Gilgen, Helvetica Chimica Acta 1976, 59, 2727-2737; bS. Uchiyama, K. Takehira, S. Kohtani, T. Santa, R. Nakagaki, S. Tobita, K. Imai, Physical Chemistry Chemical Physics 2002, 4, 4514-4522; cI. Yavari, S. Esfandiari, A. J. Mostashari, P. W. W. Hunter, The Journal of Organic Chemistry 1975, 40, 2880-2883.
[29] S. Uchiyama, K. Takehira, S. Kohtani, K. Imai, R. Nakagaki, S. Tobita, T. Santa, Organic & Biomolecular Chemistry 2003, 1, 1067-1072.
[30] S. Uchiyama, K. Kimura, C. Gota, K. Okabe, K. Kawamoto, N. Inada, T. Yoshihara, S. Tobita, Chem.–Eur. J. 2012, 18, 9552-9563.
[31] L. Donato, A. Mourot, C. M. Davenport, C. Herbivo, D. Warther, J. Leonard, F. Bolze, J. F. Nicoud, R. H. Kramer, M. Goeldner, A. Specht, Angew Chem Int Ed Engl 2012, 51, 1840-1843.