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Photoactive Barbiturate Receptors: An Ultimate Lock-and-Key System in Which the Key Unlocks the Lock

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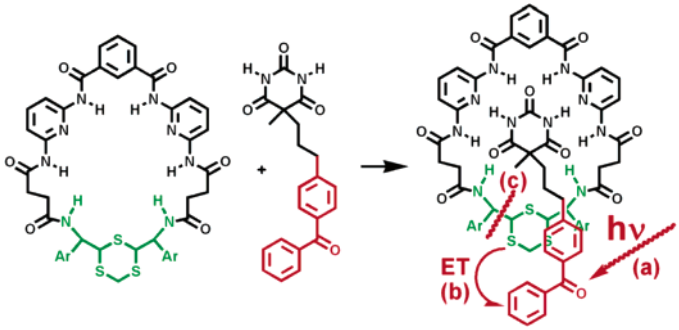
ABSTRACT



Conditional photofragmentation is achieved with binary systems incorporating the isophthaloyl bis-aminopyridine barbiturate recognition motif and dithiane- or trithiane-based photolabile modules, which cleave only in the presence of an external sensitizer. The components of the host–guest molecular recognition pair were each outfitted with either the sensitizer or the photocleavable module. In these pairs, photoinduced fragmentation is contingent on a molecular recognition event, which brings the sensitizer into the immediate proximity of the photolabile latch.

Photoinduced release of caged compounds has increasingly been utilized in molecular biology and electrophysiology to trigger concentration jumps of biological effectors under conditions of accurate spatial (mm/μm) and temporal (ms/μs) control.[1] As it is with the *top-down* approach in microfabrication and photolithography, the spatial addressability of the photorelease will eventually reach the diffraction limit, necessitating alternative *bottom-up* methodologies based on self-assembly and molecular recognition for ultimate spatial precision. One promising direction is the binary photolabile systems, which are not photolabile per se but become photocleavable once an external event brings their respective parts into the proximity of each other. Molecular recognition can be such an external event, with either the energy or electron transfer (ET) being the nature of the external sensitization. In our previous studies we have designed photolabile systems based on di- or trithiane adducts of carbonyl compounds and their derivatives, which require external ET sensitization.[2] In this Letter we describe *conditional* photofragmentations in artificial barbiturate receptors, inspired by Hamilton's isophthaloyl bis-aminopyridine motif.[3] In these systems the photorelease is contingent on a molecular recognition event, i.e., the docking of the barbiturate to the receptor. Our approach is critically different

(1) Goeldner, M.; Givens, R., Eds. *Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules*; Wiley-VCH: Weinham, 2005.

(2) (a) McHale, W. A.; Kutateladze, A. G. *J. Org. Chem.* **1998**, *63*, 9924. (b) Mitkin, O.; Wan, Y.; Kurchan, A.; Kutateladze, A. *Synthesis* **2001**, *8*, 1133. (c) Mitkin, O. D.; Kurchan, A. N.; Wan, Y.; Schiwal, B. F.; Kutateladze, A. G. *Org. Lett.* **2001**, *3*, 1841. (d) Vath, P.; Falvey, D. E.; Barnhurst, L. A.; Kutateladze, A. G. *J. Org. Chem.* **2001**, *66*, 2886. (e) Li, Z.; Kutateladze, A. G. *J. Org. Chem.* **2003**, *68*, 8236. (f) Kurchan, A. N.; Mitkin, O. D.; Kutateladze, A. G. *J. Photochem. Photobiol. A* **2005**, *171*, 121. (g) Gustafson, T. P.; Kurchan, A. N.; Kutateladze, A. G. *Tetrahedron* **2006**, *62*, 6574.

(3) (a) Chang, S.-K.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 1318. (b) Chang, S.-K.; Van Engen, D.; Fan, E.; Hamilton, A. D. *J. Am. Chem. Soc.* **1991**, *113*, 7640.

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from the approach of Bassani-Tucker et al.[4] where the molecular recognition is modulated by photochemistry (with the receptor being altered by *intra*molecular [4 + 4] photocyclization), but the photochemistry itself is not affected by molecular recognition. Other examples of molecular recognition affecting photoreactivity include Bach's enantioselective reactions driven by photoinduced electron transfer.[5]

Figure 1 shows the barbiturates and their receptors, carrying either the sensitizer or the dithiane/trithiane-based

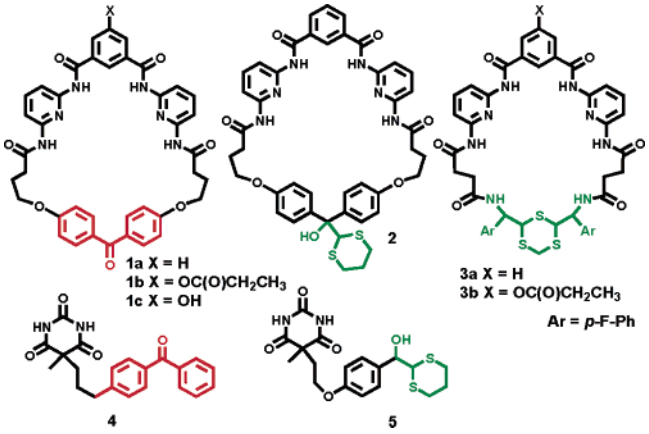


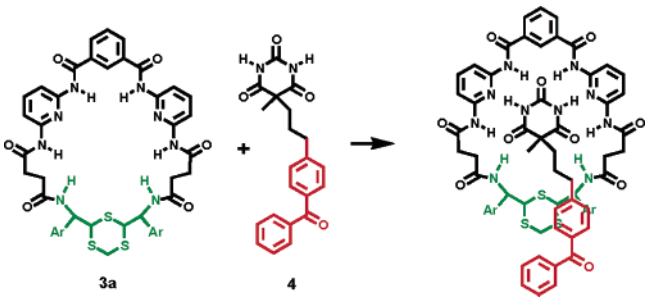
Figure 1. Model barbiturates and their receptors.

photocleavable module, synthesized as described in Supporting Information. The yields for receptors **1**−**3** after purification were 12−18%.[6]

In receptors **1a**−**c** it is the benzophenone (BP) moiety that completes the macroheterocycle, whereas the host **3** has the photolabile trithiane latch as a part of the macrocycle, adding two amide hydrogen bond contacts to the existing network of six H-bonds of the isophthaloyl bis-aminopyridine receptor. Host **1a** is converted into its dithiane adduct **2** with excess lithiated dithiane. The barbiturates carrying either a sensitizer (**4**) or a photolabile dithiane-based pendant (**5**) were used as the guest molecules for receptors **1**−**3**. Photolyses were carried out at 350−360 nm as the wavelength of irradiation is dictated by the sensitizers' absorption.

Conditional photoinduced fragmentation in macrocycle **3a** (Scheme 1) exemplifies the ultimate Lock and Key system,

Scheme 1



where irradiation of the "key" **4** causes fragmentation of the macrocycle, effectively *unlocking the lock*. The upper bound estimate of the K_D for the complex {**3a**·**4**} is 2−5 μM. At concentrations below 0.5−1 mM free benzophenone does not sensitize fragmentation in **3a**, whereas sensitizer **4**, outfitted with the guest barbiturate, does it very efficiently in the full range of concentrations above the K_D.

The unlocking is accompanied by the release of the barbiturate, which can be monitored by NMR. We and others have shown that the barbiturate binding constants for the acyclic bis-aminopyridine receptors are orders of magnitude lower than those of cyclic hosts.[2c,3]

Another mode of operation is the *conditional release of dithiane* from **2** or **5**. At higher concentrations the fragmentation caused by collisional quenching of the free sensitizer in solution successfully competes with the photoinduced fragmentation in the bound complex. However, with dilution, bimolecular quenching becomes much less efficient, whereas the quantum efficiency of the fragmentation occurring in the bound complex stays constant, i.e., the overall quantum yield of dithiane release becomes a function of K_D. The dissociation constants in pairs {**1**·**5**}, {**2**·**4**}, and {**3**·**4**} vary from 1 μM to 0.5 mM. Even for weaker complexes, such as {**1c**·**5**} (K_D = 0.48 mM), at lower concentrations dithiane release from the complex is much more efficient than the release sensitized by free (unbound) benzophenone in solution. Figure 2a shows the GCMS intensity (***I*** represents total ion

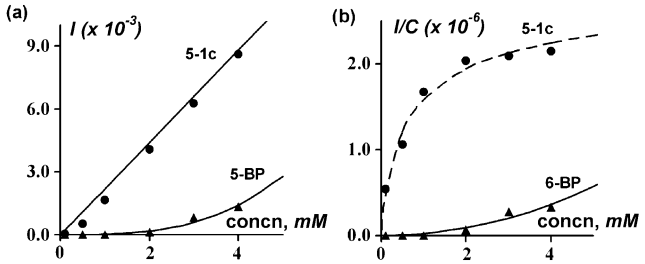


Figure 2. Dithiane release from **5** sensitized by **1c** (●) or by free benzophenone (BP) (▲): (a) dithiane GCMS peak area (kcounts) as a function of the initial concentration of [**5**] = [**1c**]; (b) the peak area divided by the initial concentration of the host−guest pair.

count in kilocounts) of the released dithiane detected in the solution plotted against the initial concentration of **5**.

The concentration of host **1c** was kept equal to that of **5** and of free benzophenone for fair comparison. The relative amount of the released dithiane, i.e., its detected intensity normalized by the initial concentration of the sensitizer (***I***/***C***), is the quantity much better reflecting the quantum efficiency of the fragmentation. As the concentration decreases, ***I***/***C*** decreases steadily for the free benzophenone−**5**

(4) Molard, Y.; Bassani, D. M.; Desvergne, J.-P.; Horton, P. N.; Hursthouse, M. B.; Tucker, J. H. R. *Angew. Chem., Int. Ed.* **2005**, *44*, 1072.
(5) Bauer, A.; Westkämper, F.; Grimme, S.; Bach, T. *Nature* **2005**, *436*, 1139.
(6) Comparable to the yields of the original Hamilton's barbiturate receptors, 12−14%.[3b]

pair; no dithiane is detected after photolysis at concentrations below 1 mM. In contrast, the ***I***/***C*** ratio stays relatively constant for the {**1c**·**5**} pair in the wide range of concentrations, decreasing considerably only in the vicinity of K_D. The fitted dashed line in Figure 2b represents a simulation obtained with $K_D = 0.48$ mM. At low concentrations, only the bonded pairs produce dithiane upon photolysis.

For more tightly bound complexes, such as {**2**·**4**} ($K_D = 86\ \mu$M), the ***I***/***C*** curve stays relatively level at concentrations high enough for the subsequent detection of the released dithiane, i.e., above the dithiane detection limit by GCMS (Figure 3). This effectively extends the dynamic range of

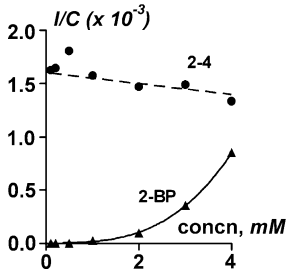
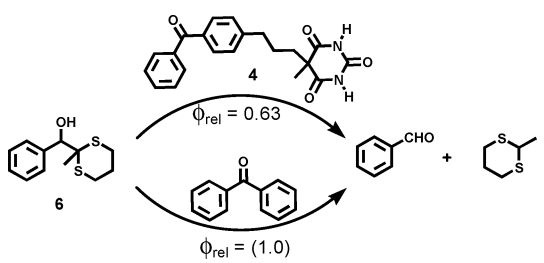


Figure 3. Normalized dithiane peak intensity ***I***/***C*** as a function of the initial concentrations: host **2** sensitized by barbiturate **4** (●) or by free benzophenone (BP) (▲).

concentrations at which the fragmentation in the bound state is overwhelmingly more efficient than the one caused by bimolecular collisional quenching. We hypothesize that the observed slight decline of the dithiane peak intensity at higher concentrations (dashed line) is due to secondary photooxidation of dithiane.[2g] The slow photodegradation of the dithiane markers affects the reactions of both the bound and the free sensitizers.

Expectedly, complexation does not guarantee quantum yields of fragmentation better than those of free collisional quenching at higher concentrations. In fact, at 5 mM, benzophenone releases more dithiane from receptor **2** than does the benzophenone-tethered barbiturate **4**. Depending on the structural features and conformational flexibility in the bound complex, its quantum efficiency of fragmentation can be both greater than the benzophenone-sensitized (as was observed for {**5**·**1c**} versus {**5**·BP}) or smaller, as is the case for {**2**·**4**} versus {**2**·BP} at 5 mM (not shown in Figure 3). The complexation, while increasing the initial ET rate, also makes back electron transfer more efficient. In some complexes this can lower the yield of the triplet charge-separated species, needed for the productive channel, i.e., the fragmentation. At a higher concentration bimolecular quenching of the triplet benzophenone can be very efficient, and yet the radical ion pairs have a chance of escaping the cage and diffusing apart to slow the wasteful back electron transfer. Another factor is the intramolecular quenching of the sensitizer in the tethered modules, which makes the sensitizer inherently less efficient, although we did not find this effect to be of significance in the studied systems. In a control experiment (Scheme 2) barbiturate **4** is only one-

Scheme 2



third less efficient than the unsubstituted benzophenone as a sensitizer in a fragmentation reaction of a model benzaldehyde−dithiane adduct **6** that, unlike **2**, is not capable of complexation with **4**.

Finally, an alternative proof of concept for the conditional (i.e., molecular recognition dependent) photoinduced release of dithiane at higher concentrations is to quench the bimolecular sensitization channel with an external quencher, for which we chose diethyl sulfide. The dithiane-bearing receptor **2** (5 mM) was sensitized, in the presence of 1 M diethyl sulfide, by free benzophenone or the guest **4**. All photolyses were run in a Rayonet carousel reactor with RPR 3500 lamps (∼350 nm). At this range of wavelengths there was no self (non-sensitized) cleavage observed in the dithiane adducts. GCMS analysis of irradiated samples showed no traces of released dithiane in the case when free benzophenone was used as the sensitizer. On the contrary, sensitization with the benzophenone−barbiturate conjugate **4** produced dithiane in amounts comparable to the amounts released in the absence of the quencher. Same observations were made for pairs {**5**·**1a**} (forms complex) and {**5**·BP} (does not form complex) in the presence of diethyl sulfide as the external quencher.

To conclude, using isophthaloyl bis-aminopyridines as barbiturate recognition elements, we designed binary photolabile systems capable of conditional fragmentation and release of dithiane tags. Photoinduced fragmentation in such binary systems is only possible when a molecular recognition event arms the system, making it light-sensitive. Although a number of useful applications can utilize this concept, we believe that it can be most beneficial for bioanalytical applications, where a molecular recognition event is detected and reported via photoinduced dithiane release in a bulk solution or in a spatially addressable manner on the surface of a chip. We are currently pursuing these directions.

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Supporting Information Available: Synthetic procedures and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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