Photoamplification

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Photoamplification and Multiple Tag Release in a Linear Peptide-Based Array of Dithiane Adducts**

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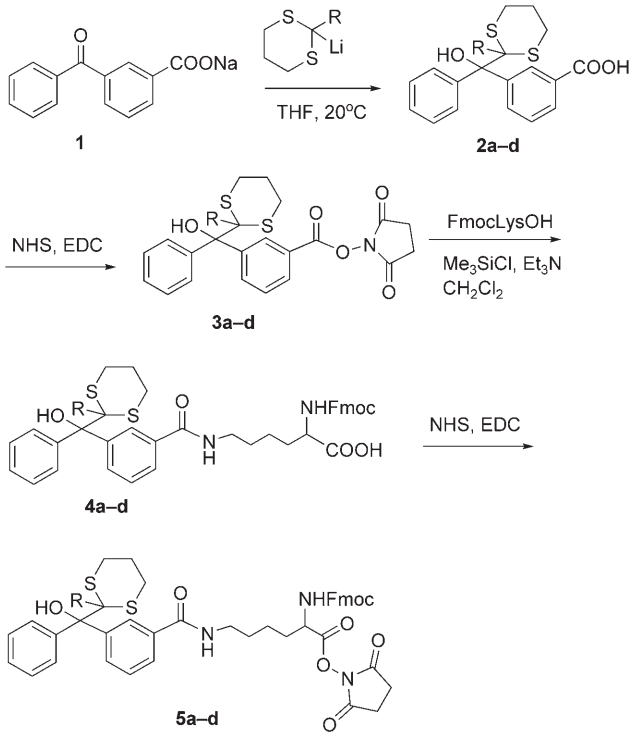
Externally sensitized photoinduced fragmentation in dithiane adducts of carbonyl compounds[1] requires the presence of an electron-transfer sensitizer, and therefore can be made contingent on a molecular recognition event. This event "arms" the binary photoactive system by bringing the adduct and the sensitizer into the immediate proximity of each other.[2] Such conditional photorelease of dithianes, which are readily detectable at subpicomolar levels, presents new opportunities for useful bioanalytical applications.

We have recently developed this concept into a fundamentally novel methodology for direct screening of solution-phase combinatorial libraries, in which various 2-alkyl-substituted dithianes are used as encoding digits.[3] By immobilizing dithiane-masked benzophenone units on polymeric beads or dendrimers, we have further shown that one molecular recognition event can trigger the release of multiple copies of the encoding dithiane tags, which amounts to amplification on the surface.[4] Such photoamplification is possible because each externally sensitized fragmentation of the benzophenone–dithiane adducts unmasks more sensitizer which, in turn, unmasks its neighbors carrying the amplification chain and therefore boosting the sensitivity.

The next logical question is whether this photoamplification methodology can be implemented in a *linear* array of masked sensitizers, and—if such amplification is possible—whether the propagation of the effect and the release of dithiane tags occurs sequentially (that is, not unlike the one-dimensional propagation in the Bickford fuse), randomly, or in some other unusual order. Herein, we report on photoamplification in linear polypeptide scaffolds.

The synthesis of masked benzophenone sensitizers tethered to Fmoc-protected lysine is outlined in Scheme 1. We chose four 2-substituted dithianes, ethyl (a), propyl (b), pentyl (c), and octyl (d), to positionally encode the lysine residues in the polypeptide chain. The lithiated dithianes were reacted with 3-benzoylbenzoic acid sodium salt (**1**), thus furnishing adducts **2a–d**, which were converted into *N*-hydroxysuccinimide (NHS) esters **3a–d** and tethered to Fmoc-protected lysine. The modified lysines **4a–d**, in the form of their NHS



Scheme 1. Synthesis of photoactive lysine conjugates. EDC = 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide; Fmoc = 9-fluorenylmethoxycarbonyl.

esters **5a–d**, were utilized in solid-state peptide synthesis on TentaGel beads (0.48 mmol g^{-1}). The peptides were capped with lysine-tethered benzophenone as the photoinitiator. As linear amplification in a single strand was the focus of our study, we chose the low-loading beads as a scaffold mimicking infinite dilution in solvent (that is, preventing interstrand sensitization; see Supporting Information).

The following tetra-, hepta-, and decapeptides, which contain photoactive lysine residues and spacers, were synthesized:

Lys^{BP}-Lys^{DT1}-Lys^{DT2}-Lys^{DT3}-BEAD (**6a**)
Lys^{BP}-Gly-Lys^{DT1}-Gly-Lys^{DT2}-Gly-Lys^{DT3}-BEAD (**6b**)
Lys^{BP}-Met-Lys^{DT1}-Met-Lys^{DT2}-Met-Lys^{DT3}-BEAD (**6c**)
Lys^{BP}-Gly-Gly-Lys^{DT1}-Gly-Gly-Lys^{DT2}-Gly-Gly-Lys^{DT3}-BEAD (**6d**)

where Lys^{DTX} represents a lysine residue outfitted with benzophenone masked with 2-alkyldithiane, and Lys^{BP} is lysine that carries "free" tethered sensitizer. The standard synthetic Fmoc–piperidine protocol was used, except that to assure completion at each step of the peptide synthesis, the time for coupling of NHS esters **5a–d** was increased to 3 days

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per residue. Mono- and bis(glycyl) spacers were introduced to probe the distance dependence, whereas methionine was tested as a potential co-sensitizer or quencher. The beads were suspended in acetonitrile, degassed, and irradiated using a 330-nm long-pass filter to selectively excite the sensitizer. Subsequent release of dithianes into solution was monitored by GC–MS. After 10 min of irradiation all three dithianes were found in the solution, thus indicating that linear amplification and binary encoding in a linear array of dithiane tags are possible.

This finding is critical to the development of our methodology for encoding and screening of solution-phase combinatorial libraries. A single library member can now be readily encoded with a complete stringed set of tags, which are necessary for its subsequent identification. Beyond this, the unusual ordering of the initial relative efficiencies presented an interesting opportunity to probe the mechanism of the release. The bar graph in Figure 1 shows the relative dithiane

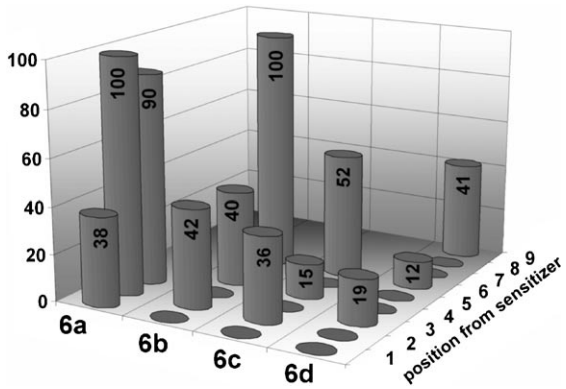


Figure 1. Release of dithianes after irradiation for 10 min as a function of their position in polypeptides **6a–d** (sensitizer is at position 0).

peak intensities after irradiation for 10 min as a function of their position in the polypeptide chain. The results illustrate that the overall efficiency of release from the position most distal to the sensitizer is generally higher.

A more detailed analysis of the release is shown in Figure 2. As a result of the secondary photooxidation of the released dithiane, its concentration over the course of photolysis reaches a maximum and then decreases. We approximate this release by a trinomial function $A = at^3 + bt^2 + ct$, where term c gives us $\partial A/\partial t$ at time zero, which allows an accurate comparison of the initial rates of release (values of c are bold in Figure 2). Again, as a rule, the most distal dithianes were released with higher initial efficiency.

We suggest that the outcome of photoamplification in peptides is governed by the migration of the formed radical cation from one dithianyl moiety to the next in the chain. Such charge transfer between photoactive residues should be unbiased and random, because the oxidation potentials of alkyl dithianes are nearly degenerate and the system does not have thermodynamic sinks, other than the wasteful back-electron-transfer (BET) trap. The probability of BET from the benzophenone radical anion is the lowest for the most distal dithiane, and accounts for the experimental observation

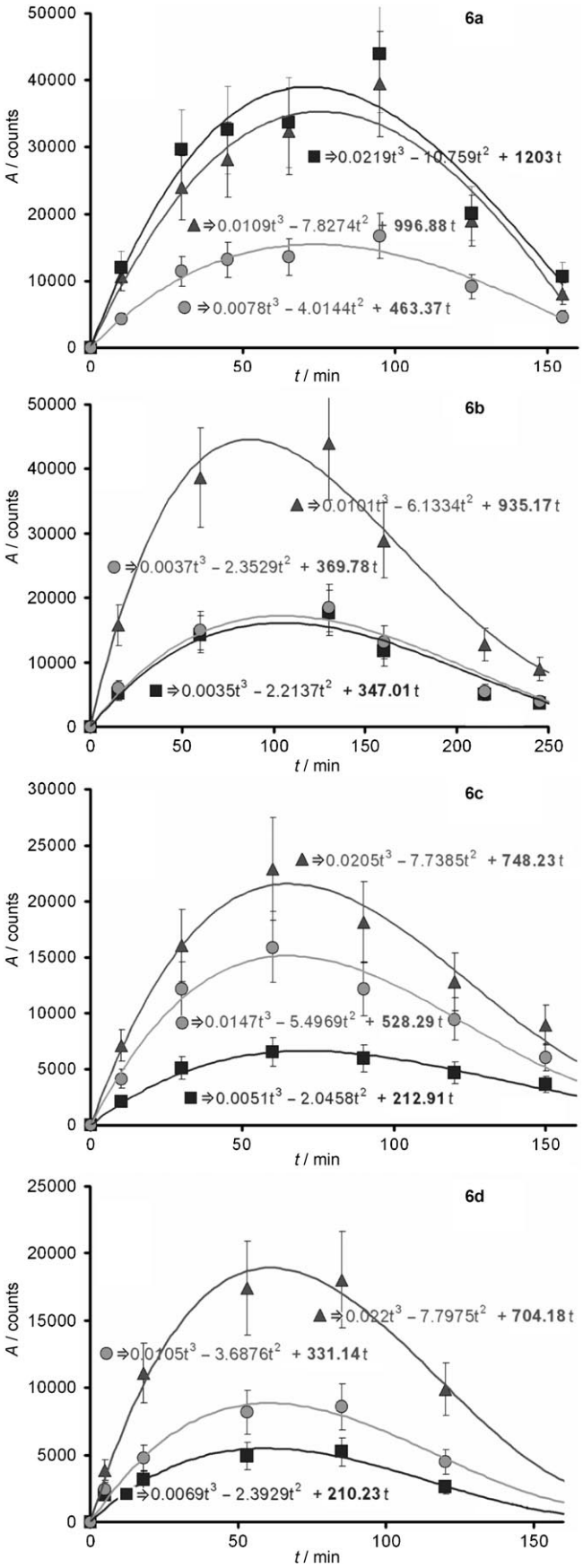
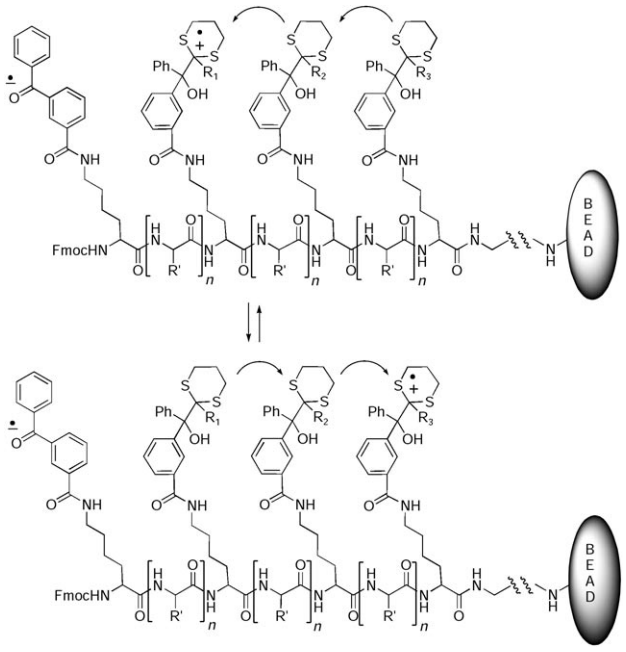


Figure 2. Release of dithianes from peptides **6a–d**; A = area. Position from the sensitizer: ● proximal, ■ middle, ▲ distal.

in which the most efficient release is from the distal position in **6b–d** (Scheme 2). As a result of BET, the proximal radical cation does not live long enough to fragment efficiently. This stochastic approach provides rationale for most of the

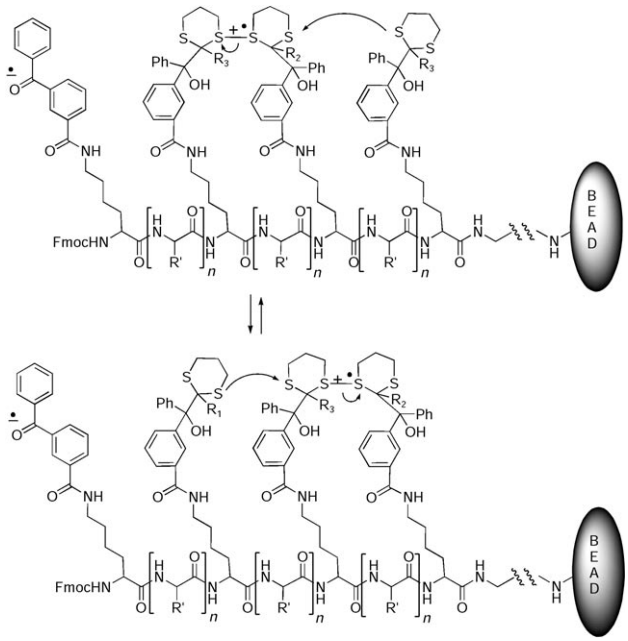


Scheme 2. Radical-cation hopping in the polypeptide chain.

experimental observations, if one recalls that as soon as the fragmentation occurs a fresh copy of the sensitizer is unmasked. The unmasked sensitizer is in turn excited, which initiates similar radical-cation hopping away from the radical anion, with the most distal position this time being the one closest to the original terminal benzophenone.

One peculiar experimental observation is the release from tetrapeptide **6a** (Figure 2), which has no spacers between the photoactive lysine residues. In this peptide, the release of the two distal dithianes seems to occur simultaneously. This result may point to a specific mode of radical-cation propagation in a system where dithiane adducts are spatially close to each other and, therefore, are capable of forming *inter*dithiane two-center, three-electron (2c3e) S–S bonds. Such 2c3e bonds are well-precedented in the literature.[5]

Previously, we have also shown that additional stabilization in bis(dithianyl) radical cations considerably increases their quantum yields of fragmentation.[6] We therefore suggest that in tight systems, such as **6a**, propagation of the radical cation may occur through the direct formation of 2c3e bonds, so that the charge density migrates in the form of a dimeric dithiane radical cation (Scheme 3). Release of either of the dithianes from these pairs has near-equal probability, which explains the comparable initial efficiencies of release from the second (1203) and third (997) adducts. The dimer formed by the first and second dithianes is again at a disadvantage of BET. The introduction of methionine (Met) as a spacer (cf. **6b** and **6c**) produced an overall effect of a competitive quencher,



Scheme 3. Charge-density migration through dimeric radical cations.

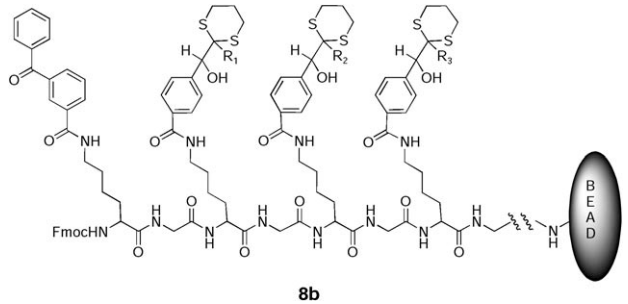
even though $Met^{+\bullet}$ is known to form 2c3e bonds with various heteroatoms.[7]

To assess the effect of the polymeric matrix on the amplified release, the photoactive dithiane adducts were immobilized in the reverse order of the glycine-separated heptapeptide **6b**:

Lys^{DT3}-Gly-Lys^{DT2}-Gly-Lys^{DT1}-Gly-Lys^{BP}-BEAD (**7b**)

that is, the lysine carrying the sensitizer was immobilized first. Irradiation of **7b** produced the same preferential release of the distal dithiane (from the terminal Lys^{DT3}), thus attesting to the generality of the described phenomenon.

The second control was to utilize the dithiane adducts of 4-formylbenzoic acid, which are not capable of amplification. Upon irradiation of **8b** (Figure 3) we see the most distal



8b

Figure 3. Peptide **8b** based on benzaldehyde adducts, which is not capable of amplification.

dithiane eventually released into solution, whereas the combined relative quantum efficiency of the release from the aldehyde-based linear array is an order of magnitude smaller. Most likely this is caused by the premature reduction of the only sensitizer in the strand during the extended photolysis needed to fragment all three dithiane adducts. In the amplified release from **6a–d** and **7b** every fragmentation

event replenishes the sensitizer, which carries the photoamplification chain.

In conclusion, we have demonstrated that binary dithiane encoding and amplification can be implemented in linear polypeptide-based arrays of photoactive, externally sensitized dithiane adducts. The variable efficiency of dithiane release also opens up an exciting opportunity for developing a positionally encoded molecular barcode system beyond simple binary encoding.

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