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Protein Degradation with Photoactivated Enediyne-Amino Acid Conjugates

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Abstract—A series of photoactivated enediynes was prepared, and successfully employed for the selective degradation of target proteins.
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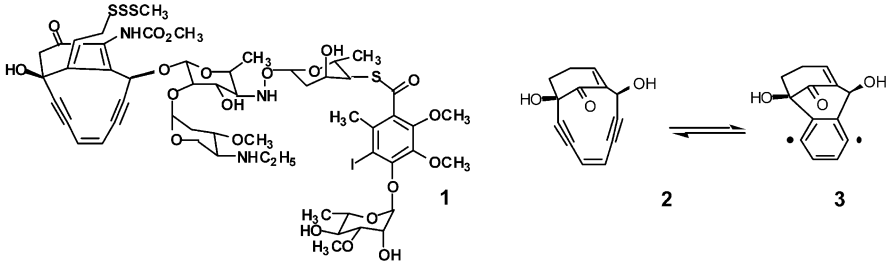
The enediynes are an important class of antitumor agents, with spectacular biological profiles.[1] Nearly 20 discrete enediynes have been discovered, and clinical trials of a number of these including calicheamicin **1** are ongoing.[2] Enediynes per se are biologically inactive, but undergo cycloaromatization reactions which give rise to cytotoxic diyl radicals, which are capable of inducing DNA strand scission at low concentration.[1] In the case of calicheamicin this involves a cascade of reactions which results in formation of a post-activated diyl core, which abstracts hydrogen atoms from the DNA backbone. On interception by molecular oxygen, an intermediate peroxide is formed which ultimately leads to strand scission by generation of a 5′ aldehyde.[1] Though the generally accepted target of the naturally occurring enediynes is believed to be DNA, there is often poor correlation between enediyne induced DNA damage and observed antitumoral activity. This has prompted a search for additional targets, including proteins. Interest in protein targets was stimulated by reports from the Bristol-Myers-Squibb laboratories, which confirmed that a synthetic analogue of calicheamicin with antitumoral activity, **2**, induced protein degradation, presumably via its cycloaromatized diyl **3**.[3] To provide proof of principle for the concept of proteinogenic substrates for enediynes, we previously demonstrated that the enediyne carboxylate derived from **4** undergoes Bergman cycloaromatization in the presence of labeled aminoacid **5**, producing arene **6**, and in the process converting **5** to dimer.[4] This implies generation of an intermediate captodatively stabilized radical, and has precedent based on the reported susceptibility of glycine residues to radical induced scission.[5] Though encouraging, a limiting factor for application of enediynes as reagents against protein targets would be the poor affinity of such lipophilic agents. We therefore elected to investigate enediyne-protein conjugates as a class, and study both their affinity and protein degrading ability. Our initial candidate was glycine derivative **7** (Scheme 1), produced by unmasking the alkynes of **4**[6] followed by carbodiimide coupling with a glycine derivative, and finally low temperature saponification (0 °C) to avoid



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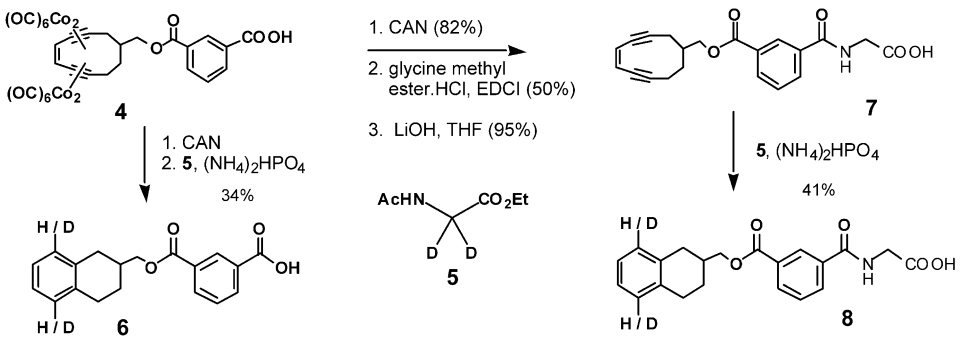
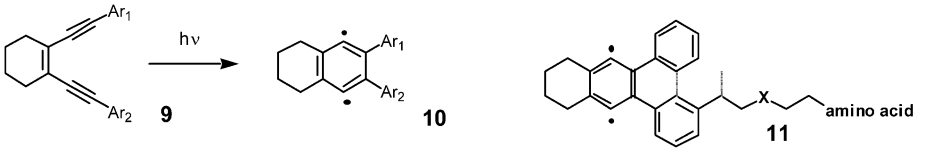


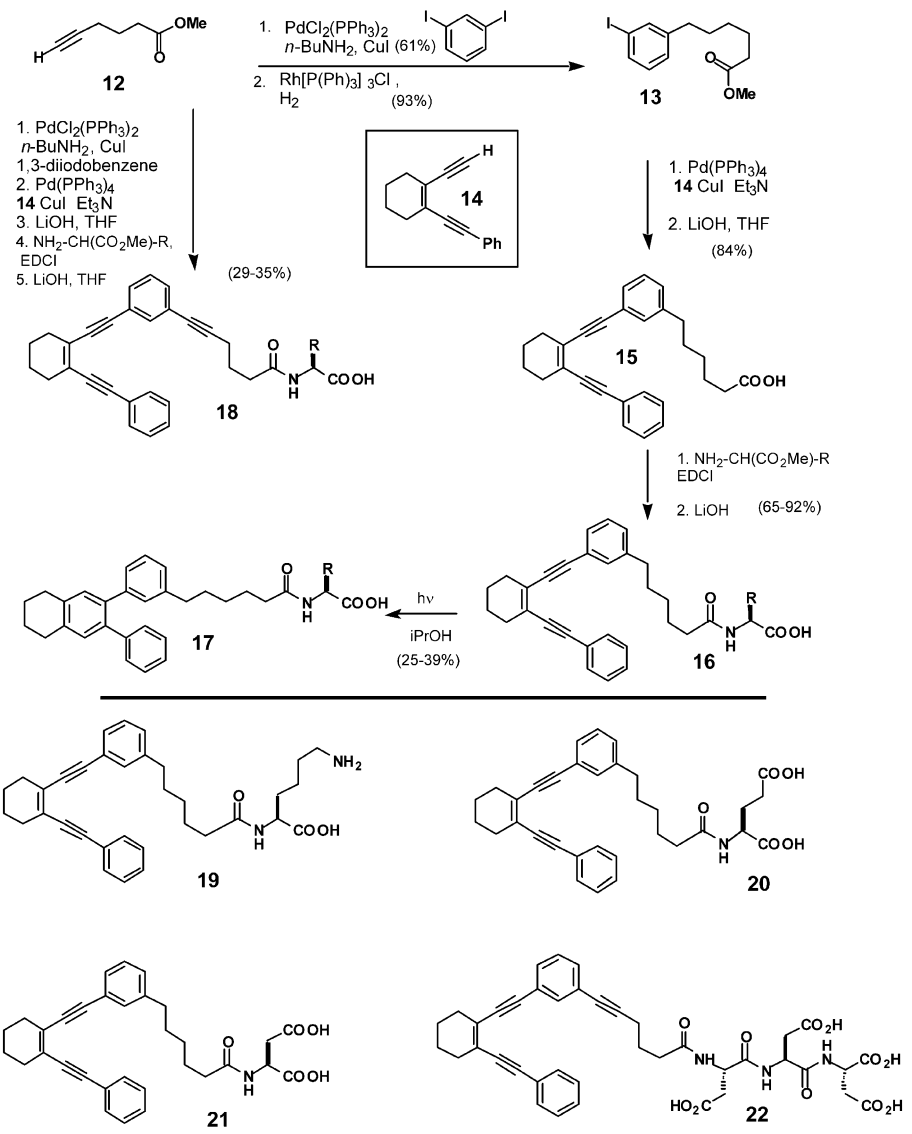
Scheme 1. Atom transfer chemistry of thermally activated enediyne–amino acid conjugate.



Scheme 2. The photo-Bergman cycloaromatization.

premature cycloaromatization. At physiological temperature **7** underwent Bergman cycloaromatization (1,4-cyclohexadiene) to give the neutral arene (70%) with an estimated $t_{1/2}$ of 17 h. As previously the diyl could be trapped with **5** (10 M, buffer) to give adduct **8** (3:2:1 D_0:D_1:D_2).[4] In contrast to the enediyne derived from **4** however, incubation of **7** with a panel of proteins indicated that it is capable of inducing degradation at elevated concentration, yielding a combination of agglomerated adducts and protein fragments.[7] Though encouraging, a drawback for use as a molecular biology reagent lies in the prolonged half-life of the enediyne core, and we elected to investigate photochemically activated variants. Since pioneering work in the 1990s several examples of the photo-Bergman cyclization have been reported,[8] including the cyclohexylenediynes **9**, which produce arenes derived from **10** on irradiation (Scheme 2).[9] We thus sought to incorporate this motif into a substrate with affinity for a specific protein target. In the case of bovine serum albumin (BSA), binding of amphiphilic substrates containing a single amino acid tethered to a polyaromatic core has been reported, and on this basis we elected to produce photoactivated enediyne *precursors* of generic diyl **11**.[10] Accordingly, our initial targets became enediyne **16**, and its structural variants (Scheme 3, **19**–**22**). Methyl-1-hex-5-ynoate **12** was coupled with 1,3-diiodobenzene, and subjected to selective hydrogenation to give alkyl arene **13** (Scheme 3). Enediyne **14** was then coupled,[9] and the carboxylate function revealed to give key building block **15**. Aminoacids were introduced using standard coupling methodology, followed by saponification to give substrate(s) **16**. Though stable indefinitely at ambient temperature, photo-irradiation (450 W medium pressure Hg lamp, 12 h) resulted in smooth cycloaromatization, giving arenes **17** in the presence of an appropriate hydrogen atom donor (iPrOH, MeOH). Likewise, modification of the synthesis allowed preparation of alkynyl linked analogues **18**. Of the enediynes assembled, the lysine derivative **19**, glutamic acid derivative **20** and aspartic acid derivative **21** were all assessed for binding affinity to BSA. Acid derivatives **20** and **21** had elevated affinity relative to **19**,[11] and photo-induced protein cleavage was accordingly assessed. The results were encouraging, with **21** capable of inducing cleavage of BSA into two discrete fragments (Fig. 1). Scatchard analysis[10,11] suggested that a single binding site exists, and presumably accounts for the single cleavage locus. Control studies confirmed that the photoactivated enediyne is responsible for the cleavage, and, when coupled with the binding data, suggests a tentative correlation between affinity and cleavage. In an attempt to extend the utility of the template, a number of more elaborate derivatives were assembled. Of these, triaspartic agent **22** was most promising, having been designed with affinity for histones in mind. From the work of Zein et al.[12] it is known that histone H1, the most basic of histones, is a preferential substrate for members of the acidic family of naturally occuring enediyne apoproteins, thus **22** was screened for affinity. Near micromolar affinity for H1 was confirmed, and photocleavage studies were then initiated. Remarkably, following 12 h irradiation, H1 is degraded into one principal component, which complements well the results obtained with the enediyne chromoprotein kedarcidin (Fig. 2).[13] These findings are significant in that they confirm that (i) controlled photodegradation of proteins by designed enediynes can be accomplished and (ii) that selectivity can be introduced by subtle structural variation.[14] Though preliminary, many applications of this general method may be envisioned, and the expeditious route to the templates **16** and **18** will help accelerate the search.

In summary, routes to enediyne–amino acid conjugates have been developed, and the conjugates show efficacy in the photo-degradation of protein substrates. It is expected that the ready availability of the templates will now permit assembly and evaluation of both rationally designed and combinatorial libraries of conjugates, which may serve as valuable tools for proteomics.[15]



Scheme 3. Preparation of variable linker photoactivated enediyne–amino acid conjugates.

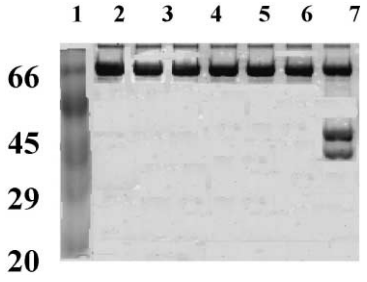


Figure 1. 10% SDS polyacrylamide gel of the reaction bovine serum albumin (BSA) (1 μM) with **21**. All assays conducted in 50 mM Tris–HCl, pH 7.0. Lane 1, molecular weight markers (KDa); lane 2, BSA no irradiation; lane 3, BSA — 3 h irradiation; lane 4, BSA — 12 h irradiation; lane 5, BSA + **21** (10 μM) no irradiation; lane 6, BSA + **21** (10 μM) 1 h irradiation; lane 7, BSA + **21** (10 μM) 12 h irradiation.

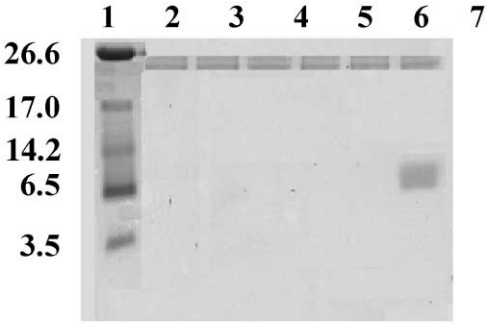


Figure 2. 16.5% SDS polyacrylamide Tris–tricine gel of reaction of histone H1 (1 μM) with **22**. All reations were conducted in 50 mM Tris–HCl, pH 7.0. Lane 1, molecular weight markers (KDa); lane 2, histone H1 no irradiation; lane 3, histone H1 — 1 h irradiation; lane 4, histone H1 — 12 h irradiation; lane 5, histone H1 + **22** (10 μM) no irradiation 12 h; lane 6, histone H1 + **22** (10 μM) 1 h irradiation; lane 7, histone H1 **22** (10 μM) 12 h irradiation.

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7. Proteins examined included BSA, lysozyme, and histones. Incubation of **7** for 24 h resulted in trace agglomeration at 10^{-6}M, and appreciable decomposition at 10^{-3} M. Details will be reported in a full account of this work.
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11. Binding constants obtained from best fit of data to Scatchard equation (Scatchard, G. *Ann. N.Y. Acad. Sci.* **1949**, *51*, 660). K_a (25 °C) BSA **19**: 1.58×10^5 M^{-1}, **20**: 5.75×10^5 M^{-1}, **21**: 6.71×10^5 M^{-1}.
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13. K_a **22** (25 °C) histone H1: 7.20×10^5 M^{-1}; H2: 1.69×10^5 M^{-1}. Analogous control reactions with **21** (K_a 0.4×10^5 M^{-1}) showed no detectable histone degradation after 12 h irradiation.
14. Compounds **19**–**21** produced no specific histone degradation products after 12 h irradiation (10 μM).
15. This work was supported by the National Institutes of Health (R01GM57123).