Synthesis and Photochemical Activity of Designed Enediynes

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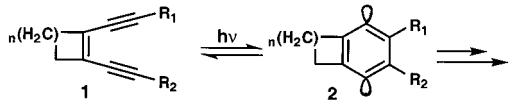
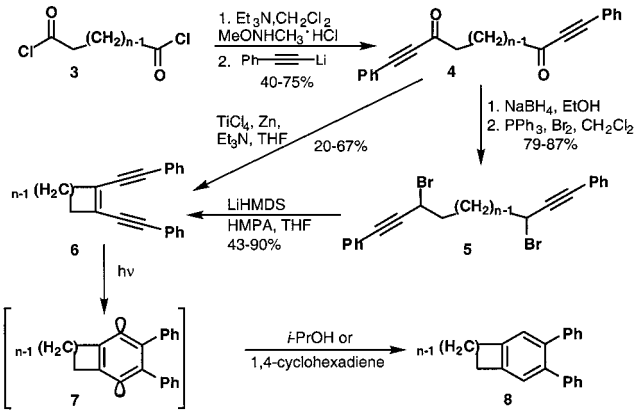
The enediynes are a growing class of antitumor agents, with spectacular biological profiles.[1] Nearly 20 discrete enediynes have been discovered, and clinical trials of a number of these are ongoing.[2] While the in vitro and in vivo effectiveness of enediynes against certain cancers is unquestioned, the exact mechanism(s) of biological activity remains to be fully resolved. 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] Depending on local environment, however, it is also possible for other events to occur. One potential class of targets is proteins.[3] Excepting water, proteins are the most abundant constituents of cells and extracellular fluids by weight. We recently demonstrated that simple amino acids are viable targets for diyl radicals, resulting in both degradation and dimerization via intermediate carbon centered amino acyl radicals.[4] Conventionally, the controlled degradation of proteins is often accomplished through affinity cleavage systems, typically containing a metal-redox center tethered to an entity recognized by the protein of interest.[5] Application of enediynes for this purpose could be an attractive proposition but would require two conditions be met: (i) the system must have affinity for a protein of interest and (ii) the enediyne needs to be thermally stable until activated "on demand". An attractive method for activation lies in the photochemical triggering of an otherwise unreactive enediyne. Indeed, examples of the direct "photochemical Bergman" cyclization have been reported.[6−9] In most examples studied to date the vinyl moiety of the enediyne is embedded in an arene, and mindful of this restriction, we wished to design a family of *alicyclic enediynes* **1**, and investigate their photochemical activation to diyls **2**, as a function of ring strain and electronic effects.[10]

Scheme 1. Photochemical Bergman Cycloaromatization



Scheme 2. Synthesis of Alicyclic Photo-Bergman Candidates



Commencing from commercially available acyl chlorides **3**, the corresponding diketones **4** were produced via the intermediate Weinreb amides (Scheme 2).[11] Low-valent Ti-mediated coupling gave **6** directly, but the yields were variable, in part due to problems recovering product from complex mixtures. Alternatively, conversion to bromides **5** followed by carbenoid coupling gave **6** ($n = 1-5$) in good yield on a preparative scale.[12] Though stable at room temperature, photochemical Bergman cycloaromatization gave adducts **8** in moderate yield, presumably via diyls **7** (Table 1). As had been found previously, the nature of the hydrogen donor plays an important role in the conversion to arene adduct.[7] Thus, although consumption of enediyne was often rapid using 1,4-cyclohexadiene, optimal yields of cycloaromatization products were obtained using 2-propanol, the balance of material typically composed of uncharacterized polymeric byproducts. Evidently ring strain effects play a role in the cycloaromatization, with lower conversion efficiency observed with the C-7 and C-8 analogues despite the appreciable reduction in intramolecular "c−d" distances relative to the six-membered analogue (Table 1).[1] Though photo-Bergman cycloaromatization yields are modest, the synthesis of this class of enediynes [4- through 8-membered] is noteworthy and may lead to many new applications in materials and polymer chemistry.[13]

With a route to photoactivated enediynes secure, we wished to investigate interaction of the intermediate diyl radicals **2** with protein targets, and accordingly sought to prepare a hydrophilic variant, which was capable of recognizing protein architecture. Our design was influenced by the work of Kumar,[14] who reported that an alkyl pyrenyl derivative of phenylalanine (Figure 1) recognizes the proteins bovine serum albumin (BSA) and lysozyme, inducing photocleavage following irradiation in the presence of an electron acceptor.[14] Molecular modeling studies indicated that arene **14**, the expected photocycloaromatization product of enediyne **13** (Scheme 3), bears a close structural resemblance to the reference probe, providing us with a logical candidate for proof-of-principle studies.

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Table 1. Synthesis and Cycloaromatization of Enediyne **6**

entry	*n*	c−d (Å)[a]	irradiation (h)[b]	conversion[c]	% **8**[d] (based on **6**)
1	2	4.941	3	100[e]	0 (0)
2	3	4.284	3	97	13 (13)
3	4	4.018	3	49	15 (31)
4	4	4.018	12	95	21 (22)
5	5	3.947	12	70	11 (16)
6	6	3.893	12	66	9 (14)

[a] Equilibrium geometry calculated using PM3 (PC Spartan Pro). [b] Reactions conducted at 0.4 g/L enediyne in 2-propanol in a quartz vessel, irradiated using a 450 W (Hanovia) lamp. [c] Based on recovered starting material. [d] Isolated yields. [e] Decompostion and formation of polymeric adducts ensues in <1 h.

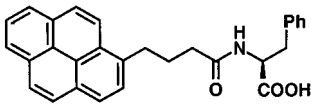
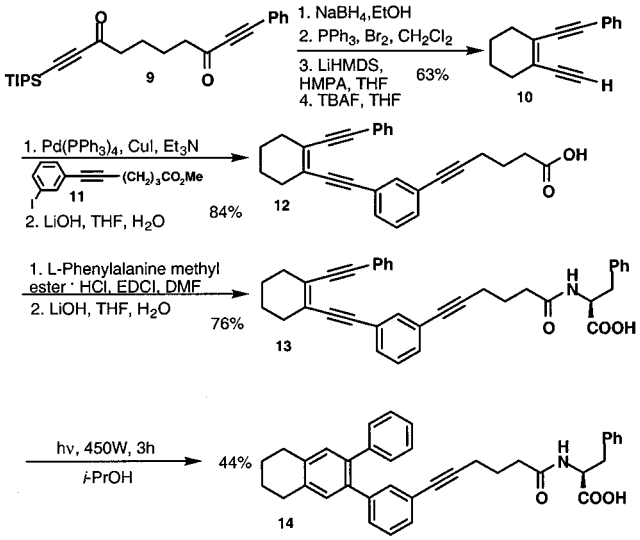


Figure 1.

Scheme 3. Preparation and Photo-Bergman Cyclization of the Phe-enediyne Hybrid



Accordingly, diketone **9** was prepared from adipoyl chloride using a mixed coupling procedure. Subsequent functional group interconversion and carbenoid coupling gave the differentially functionalized C6-enediyne, which was unmasked to give **10**. Photo-Bergman cycloaromatization of this enediyne (or the TIPS protected precursor) gave <10% of the corresponding arene product, suggesting the aryl groups play a key role in the (reversible) cyclization process.[7−9] The alkyne was then coupled with methyl-3-(3-iododphenyl)hexynoate **11**, followed by hydrolysis to give carboxylate **12**, which was then coupled with L-phenylalanine methyl ester and subjected to saponification to give conjugate **13** in good yield. In contrast to the model series, photochemical activation (450 W, 3 h) of this enediyne gave arene product **14** in appreciable yield, together with unreacted starting material, underscoring the influence of the conjugated arene unit.

Photochemically induced modification of BSA and lysozyme was examined by photolyzing **13** in aqueous buffer, using **14** and enediynes **6** ($n = 4$) as controls. In the case of 66 kD protein BSA, subsequent analysis (SDS-PAGE) showed evidence of enediyne induced degradation, with one principal fragment (~40 kDa) visible (Figure 2). Subsequent analysis by MALDI-TOF revealed the presence of fragments at 44.76, 11.28, and 10.22 kDa suggesting the possibility of two sites of cleavage.[15] In the case of the 14 kDa protein lysozyme, dimerization to form a species with *m*/*z* 28.63 kDa was observed following irradiation (Figure 3), suggesting the possibility of intermolecular diyl

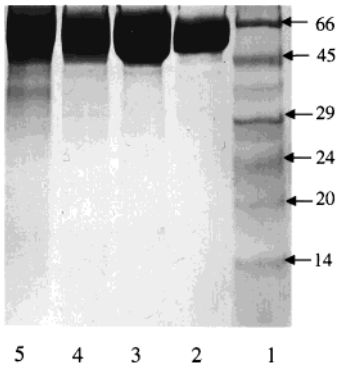


Figure 2. 10% SDS polyacrylamide gel of the reaction of bovine serum albumin (BSA) with **13** and **14**. All reactions were conducted in 50 mM Tris-HCl, pH 7.0 at 37 °C. From right to left; lane 1, molecular weight markers (KDa); lane 2, BSA control (1.5 μM); lane 3, BSA (1.5 μM), **13** (150 μM), no irradiation; lane 4, BSA (1.5 μM), **14** (150 μM), 3h irradiation; lane 5, BSA (1.5 μM), **13** (7.5 μM), 3h irradiation. The running gel was overlaid with a 4% stacking gel and samples were run top to bottom. Analogous reactions with **6** (n = 3) showed no change from control (data not shown).

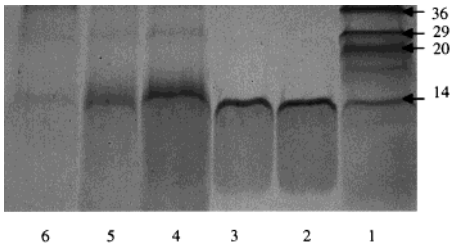


Figure 3. 15% SDS polyacrylamide gel of the reaction of Lysozyme (Lyso) with **13** and **14**. All reactions were conducted in 50 mM Tris-HCl, pH 7.0 at 37 °C. From right to left; lane 1, molecular weight markers (in KDa); lane 2, Lyso (0.15 μM); lane 3, Lyso (0.15 μM), 3h irradiation; lane 4, Lyso (0.15 μM), **13** (0.75 μM), 3h irradiation; lane 5, Lyso (0.15 μM), **13** (0.75 μM), 6h irradiation; lane 6, Lyso (0.15 μM), **14** (15 μM), 12h irradiation. The running gel was overlaid with a 4% stacking gel and samples were run top to bottom. Analogous reactions with **6** (n = 3) showed no change from control (data not shown).

mediated cross coupling.[15] Though the origins of the observed degradation and dimerization events require further study, generation of protein free radicals has precedent.[16] Furthermore, it has recently been reported that in the case of the naturally occurring enediyne chromoprotein C-1027, the enediyne chromophore induces proteolysis of its own apoprotein,[17] suggesting that protein targets of other enediynes may be found. The present findings might ultimately lead to the design of specific photoproteases, and we envision the ready accessibility and photochemical profile of carboxylate **12** will help expedite this process.[18]

Supporting Information Available: Synthetic procedures and spectroscopic data for the preparation of **13** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) MS analysis performed using desalted mixtures directly following incubation using a sinapinic acid (BSA) or α-cyano-4-hydroxy-cinnamic acid matrix (lysozyme). Binding constants of **13** for BSA (1.6×10^5 dm^3 mol^{-2}) and lysozyme (0.3×10^5 dm^3 mol^{-2}) were determined following Scatchard analysis. Densitometric analysis of Coomassie stained gels (Microtek Scanmaker 4, processed with Adobe Photoshop/NIH-image software) quantitated the BSA 44 kDa fragment as 4% relative to parent, and the lysozyme dimer (28 kDa) at 8% relative to parent.

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