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Synthesis and Protein Degradation Capacity of Photoactivated Enediynes

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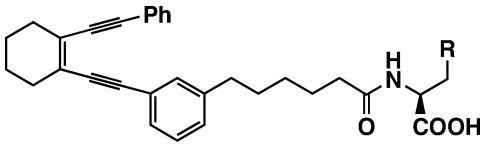

The viability of proteins as targets of thermally and photoactivated enediynes has been confirmed at the molecular level. Model studies using a labeled substrate confirmed the efficacy of atom transfer from diyl radicals produced from enediynes to form captodatively stabilized carbon centered aminoacyl radicals, which then undergo either fragmentation or dimerization. To exploit this finding, a family of enediynes was developed using an intramolecular coupling strategy. Derivatives were prepared and used to target specific proteins, showing good correlation between affinity and photoinduced protein degrading activity. The findings have potential applications in the design of artificial chemical proteases and add to our understanding of the mechanism of action of the clinically important enediyne antitumor antibiotics.

Introduction

The enediynes are a substantial class of antitumor agents, with spectacular biological profiles and proven clinical efficacy.[1] Nearly 20 discrete enediynes have been discovered, and clinical trials of a number of these are ongoing. An antibody conjugate of one enediyne (calicheamicin) was the first to gain approval by the FDA and shows promise in the treatment of acute myeloid leukemia (AML).[2] Although 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] In the case of calicheamicin, this involves a cascade of reactions which results in the formation of a postactivated 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.[2]

Depending on the local environment, however, it is also possible for other events to occur and, in the case of the enediyne C-1027, under anaerobic conditions, recombination of the ribosyl radical and enediyne-derived diyl has been observed, leading to DNA adduction.[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.[3] This has prompted a search for additional targets, the most likely being proteins.[4] Excepting water, proteins are the most abundant constituents of cells and extracelluar fluids by weight. Interest in this class of target was stimulated

* To whom correspondence should be addressed. Phone: 617-373-8619. Fax: 617-373-8795.

(1) Smith, A. L.; Nicolaou, K. C. *J. Med. Chem.* **1996**, *39*, 2103. Xi, Z.; Goldberg, I. DNA Damaging enediyne compounds. In *Comprehensive Natural Products Chemistry*; Barton, D. H. R., Nakanishi, K., Eds.; Pergamon: Oxford, NY, 1999, *7*, 553.

(2) For an overview of recent clinical findings, see: Schor, N. F. In *Cancer Therapeutics: Experimental and Clinical Agents*; Teicher, B., Ed.; Humana Press: Totowa, NJ, 1996; pp 229−240. Schmitt, D. A.; Kisanuki, K.; Kimura, S.; Oka, K.; Pollard, R. B.; Maeda, H.; Suzuki, F. *Anticancer Res.* **1992**, *12*, 2219. Takahashi, T.; Yamaguchi, T.; Kitamura, K.; Noguchi, A.; Mitsuyo, H.; Otsuji, E. *Jpn. J. Cancer Res.* **1993**, *53*, 976. Oda, M.; Sato, F.; Maeda, H. *JNCI* **1987**, *79*, 1205.

(3) Cell death induced by the suicide repair enzyme poly(ADP ribose polymerase) has been suggested. See: Durkacz, B. W.; Omidiji, O.; Gray, D. A.; Shall, S. *Nature* **1980**, *283*, 593. Zhao, B.; Konno, S.; Wu, J. M.; Oronsky, A. L. *Cancer Lett.* **1990**, *50*, 141. Nicolaou, K. C.; Stabila, P.; Esmaeli-Azad, B.; Wrasidlo, W.; Hiatt, A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3142. Nicolaou, K. C.; Smith, A. L.; Yue, E. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5881. For an example of base-selective DNA modification via synthetic enediynes, see: Tuntiwechapikul, W.; David, W. M.; Kumar, D.; Salazar, M.; Kerwin, S. M. *Biochemistry* **2002**, *41*, 5283.

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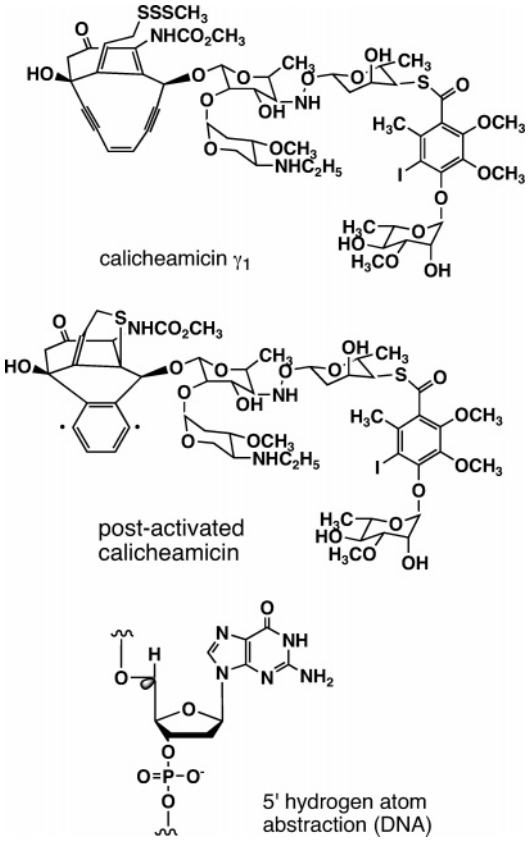
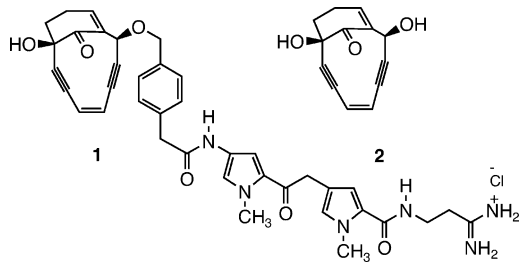
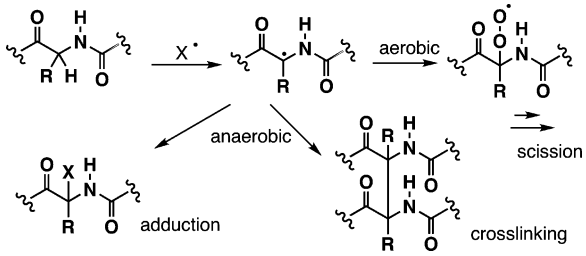


by reports from the Bristol-Myers-Squibb laboratories, which confirmed that a synthetic analogue of calicheamicin-induced[5] (**2**) protein degradation, whereas its biologically inactive derivative, which instead targeted DNA (**1**), did not.[5]



Though the prospect of developing protein-targeted antitumor agents remains of interest, if the process could be controlled on demand, an enediyne-based *reagent* capable of inducing protein degradation could be of use as a tool for molecular biology applications. Before engaging in this, however, a number of background studies were in order. Though enediyne-induced protein degradation had been suggested,[4,5] there were no studies confirming the molecular mechanism of such a process, and this would be a requirement if we were to be successful in the development of enediyne-based proteases.[6]

SCHEME 1. Potential Pathways for Peptide Degradation



Results and Discussion

In principle, there are several mechanisms by which an enediyne-derived diyl radical could interact with a protein. It is known that 1,4-diyls can undergo a variety of atom transfer reactions to produce neutral arene products, with hydrogen donors (including DNA), halogens, or reactive carbon species.[7] The analogous reaction with peptides would result in generation of an intermediate peptide radical, whose fate would be determined by environment (Scheme 1). Possibilities include scission, via formation of an intermediate peroxy radical (as in the case of DNA ribosyl radicals), peptide cross-linking, or adduction with the radical source (X•). Inspection of the chemical literature reveals that all of these pathways are in fact precedented.[8]

At the most basic level, it is known that carbon centered glycyl radicals are generated on exposure of glycine to peroxide sources and that they are relatively stable, a consequence of captodative stabilization.[8] Indeed, it has been shown that glycyl radicals can be selectively generated in preference to others, when peptides are exposed to peroxy radicals.[8] In one study, a synthetic polypeptide composed of glycine/alanine showed >30:1 selectivity for abstraction to form glycyl radicals,[9] and a similar preference for glycine has also been observed in proteins.[10,11] Of additional relevance, glycyl radicals themselves can persist under biological conditions.[12] In the anaerobic environment of *Escherichia coli*, both pyruvate formate lyase and ribonuclease reductase rely on generation of intermediate glycyl radicals for proper function.[13]

Proof of Principle Studies. On the basis of the reported susceptibility of glycine to radical-induced scission, we wished to demonstrate atom transfer to a diyl from a labeled mimic of this amino acid. Though a variety

(4) Zein, N.; Schroeder, D. R. In *Advances in DNA Sequence Specific Agents*; Jones, G. B., Ed.; JAI Press Inc.: Greenwich, CT, 1998, *3*, 201. Zein, N.; Reiss, P.; Bernatowic, M.; Bolgar, M. *Chem. Biol.* **1995**, *2*, 451.

(5) Zein, N.; Solomon, W.; Casazza, A. M.; Kadow, J. F.; Krishnan, B. S.; Tun, M. M.; Vyas, D. M.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1351. Lee, S.; Bain, A.; Sulikowski, G. A.; Solomon, W.; Zein, N. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1261. Wittman, M. D.; Kadow, J. F.; Langley, D. R.; Vyas, D. M.; Rose, W. C.; Solomon, W.; Zein, N. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1049.

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(7) Semmelhack, M. F.; Neu, T.; Foubelo, F. *J. Org. Chem.* **1994**, *59*, 5038. Grissom, J. W.; Gunawardena, G. U. *Tetrahedron Lett.* **1995**, *36*, 4951.

(8) Sperling, J. *J. Am. Chem. Soc.* **1969**, *91*, 5389. Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1971**, *93*, 967. Elad, D.; Sperling, J. *J. Chem. Soc. C* **1969**, 1579.

(9) Elad, D.; Schwarzberg, M.; Sperling, J. *J. Chem. Soc., Chem. Commun.* **1970**, 617.

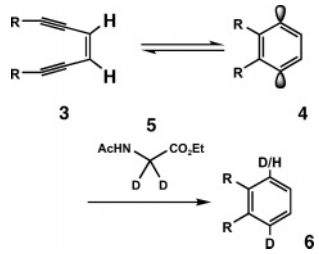
(10) Easton, C. J. *Chem. Rev.* **1997**, *97*, 53.

(11) Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1971**, *93*, 3839.

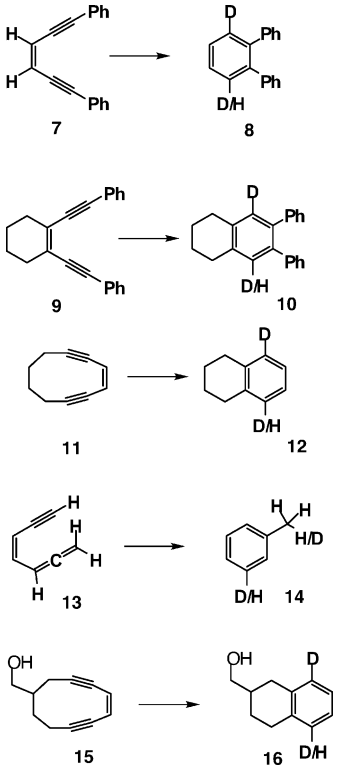
(12) Marsh, E. N. G. *BioEssays* **1995**, *17*, 431. Pedersen, J. Z.; Finazzi-Agro, A. *FEBS* **1993**, *325*, 53.

(13) Stubbe, J.; van der Donk, W. A. *Chem. Biol.* **1995**, *2*, 793. *Free Radicals in biology and medicine*; Halliwell, B., Gutteridge, J. C., Eds.; Oxford Science: New York, 1999.

SCHEME 2. Atom Transfer Using d_2-Gly Mimic



SCHEME 3. *d*-Atom Transfer from 5 to Diyls



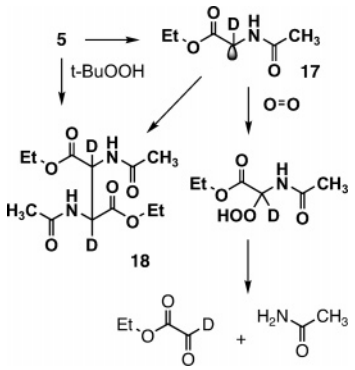
of options are available, we elected to use the dideuterioglycine **5** because an efficient synthesis is available[14] and its solubility in organic media would allow flexibility in the choice of enediynes. Accordingly, we prepared a number of synthetic enediyne core structures **3** designed to undergo cycloaromatization to yield diyls **4** in the presence of **5** (Scheme 2). The enediynes were selected on the basis of their differing modes of activation and propensity to undergo cycloaromatization and are all available using an efficient metallohalocarbenoid route developed in our laboratories.[15] Thermal cyclization of *Z*-enediyne **7** proceeded at 280 °C to give the corresponding arenes **8** isolated as a mixture of mono- and dideuterated species (Scheme 3, Table 1). Alternatively, photochemical Bergman cycloaromatization can be initiated with suitable substrates, and enediyne **7** likewise gave arenes **8** in moderate yield following 3 h of irradiation using a low-pressure mercury lamp, presumably involving *Z*−*E* photoisomerization and subsequent capture.[6] Similar behavior was observed for enediyne **9**. By moving to more reactive precursors, enediyne **11** and eneyne

TABLE 1. Yields and Isotopic Ratios for Atom Transfer[a]

entry	adduct	method	yield (%)	D0:D1:D2[b]
1	**8**	thermal	10	31:2:1
2	**8**	hν	18	17:2:1
3	**10**	thermal	8	19:2:1
4	**10**	hν	20	14:2:1
5	**12**	thermal	11	3:2:1
6	**12**	hν	20	2:2:1
7	**14**	thermal	10	2:2:1
8	**14**	hν	15	7:2:1
9	**16**	thermal	40	4:2:1
10	**16**	hν	50	3:2:1

[a] Yields based on GC analysis, calibrated using internal (*n*-decane) and external standards in reactions at 0.01 M (mesitylene) using 10−100 equiv **5**. [b] Ratio determined by H/D NMR and GC/MS analysis using an authentic dideutero adduct prepared by incubation of enediyne with cyclohexadiene-d_8 or THF-d_8.

SCHEME 4. Fate of Glycyl Radical



allene **13**,[16] whose half-lives for cycloaromatization are approximately 18 and 24 h at 37 °C, respectively, cyclized readily at physiological temperature, giving low yields of labeled cycloaromatization adducts **12** and **14**, which could be increased slightly under photochemical conditions. Finally, the hydroxymethyl enediyne **15** was employed. The marked increase in chemical conversion to **16** suggested that electrostatic effects between the donor group and the pendant functionality might contribute to the efficiency of diyl capture. The higher ratios of D1 over D2 adducts observed presumably reflect the increased stability and discriminatory capacity of the diyl, in comparison to the more reactive monoradical which may abstract from a more diverse range of (H) atom donors.

Atom transfer from **5** implies generation of a captodatively stabilized radical **17**, which could be expected to either dimerize (**18**) or react with molecular oxygen to form peroxides which could ultimately result in fragmentation via a transient iminium ion (Scheme 4). To probe these scenarios, an authentic sample of **18** was prepared using an organic peroxide. Reanalysis of the crude reaction mixtures (Scheme 3) confirmed formation of **18** as the principal byproduct following atom transfer. Unsurprisingly, because all reactions were conducted under deoxygenated conditions, products derived from hydroperoxides were not isolated. Though the experiments showed clear evidence of atom-transfer chemistry from **5**, the relevance of these events in an aqueous

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(15) Jones, G. B.; Wright, J. M.; Plourde II, G. W.; Hynd, G.; Huber, R. S.; Mathews, J. E. *J. Am. Chem. Soc.* **2000**, *122*, 1937.
(16) Myers, A. G.; Parish, C. A. *Bioconjugate Chem.* **1996**, *7*, 322; Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493.

SCHEME 5. Atom Transfer in Aqueous and Organic Media

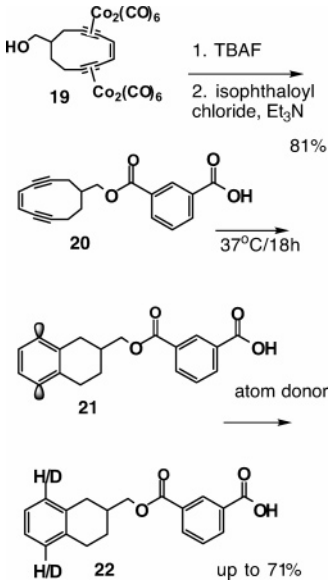


TABLE 2. Isotopic Ratios for Atom Transfer with 20[a]

entry	solvent	donor	% **22**	D0:D1:D2
1	CH_3OH		15	
2	CH_3OD		17	5:4:1
3	H_2O	glycine	<5	
4	$H_2O/(NH_4)_2HPO_4$	glycine	28	
5	$H_2O/(NH_4)_2HPO_4$	glycine-d_5	30	10:2:1
6	$H_2O/(NH_4)_2HPO_4$	**5**	42	3:2:1

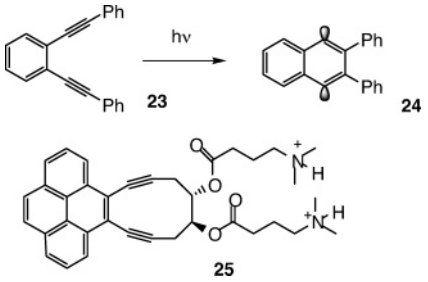
[a] Ratio determined by H/D NMR and GC/MS analysis using an authentic dideutero adduct.

environment remained in question. To address this issue, a water-soluble enediyne **20** was prepared, by reacting the shelf-stable cobalt complex **19** with isophthaloyl chloride (Scheme 5)[15] and then immediately unmasking the enediyne moiety. Cycloaromatization of **20** in neat 1,4-cyclohexadiene gave **22** in >70% yield; however, our primary interest was with aqueous applications. Though carboxylate **20** was freely soluble in polar organic solvents, phosphate buffer was required to achieve full homogeneity in aqueous conditions. To our delight, it was discovered that the cycloaromatization is indeed viable in aqueous media, and the corresponding atom-transfer adducts formed at 37 °C under aerobic conditions. The process was more efficient using **5** rather than d_5 (or H_5) glycine as the atom-transfer agent, suggesting that the abstracting ability in the diyls hydrophobic microenvironment is enhanced with this neutral donor and that this has a positive impact on the rate of cycloaromatization (Table 2).

Though a complex mixture of other byproducts was formed in addition to dimer **18**, both glyoxylate and acetamide were confirmed, suggesting the intermediacy of a peroxide under aerobic conditions (Scheme 4). Through repetition of the incubations under deoxygenated conditions (triple freeze−thaw cycles), the efficiency of formation of the peroxy-derived byproducts was reduced, suggesting some control may be possible in governing the fate of radical **17**.

Developing Photochemically Triggered Reagents. Though the Bergman cycloaromatization is a thermally controlled process, the naturally occurring enediynes all possess elaborate triggering functions,[17] which induce cycloaromatization on interaction with a cofactor. An interesting variant is the photochemically activated Bergman cyclization, as first demonstrated by Turro and Nicolaou in the form of enediyne **23**, which converts to diyl **24** on irradiation (Scheme 6).[18] Capitalizing on this process, Funk and Williams designed the pyrene enediyne **25**, which by virtue of the aminoalkyl side chain had affinity for DNA. Irradiation of this agent in the presence of pBR322 DNA resulted in degradation, thus constituting the first example of an enediyne-based photocleaver.[19] Spurred by these findings, a number of other systems have been demonstrated,[20] in what is becoming a rapidly maturing field.[21] Our challenge thus became to design a similar system for photochemical protein degradation.

SCHEME 6. Photo-Bergman Cyclization



de Novo Class of Photoenediynes. With the atom-transfer chemistry of enediyne-derived diyls with peptide mimic **5** proven, under both thermal and photochemical activation conditions, we turned our attention to protein targets. The preliminary results served to illustrate the impact that hydrophilic functionality has on abstraction efficiency, and it became clear that, for application to protein targets, considerable flexibility would need to be built into the system. We elected to design enediyne systems where alkyne termini could be derivitized with appropriate protein recognition functions and the photoactivity of the Bergman cycloaromatization could be tuned. Specifically, because most photoactivated enediynes studied to date have the vinyl moiety embedded in an arene, we wished to design a family of differentially capped alicyclic enediynes **26** and investigate their photochemical activation to diyls **27**, as a function of ring-strain and electronic effects (Scheme 7).[22]

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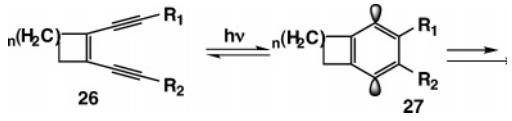
(22) Jones, G. B.; Warner, P. M. *J. Am. Chem. Soc.* **2001**, *123*, 2134.

SCHEME 7. Photochemical Bergman Cycloaromatization



SCHEME 8. Synthesis of Alicyclic Photo-Bergman Candidates

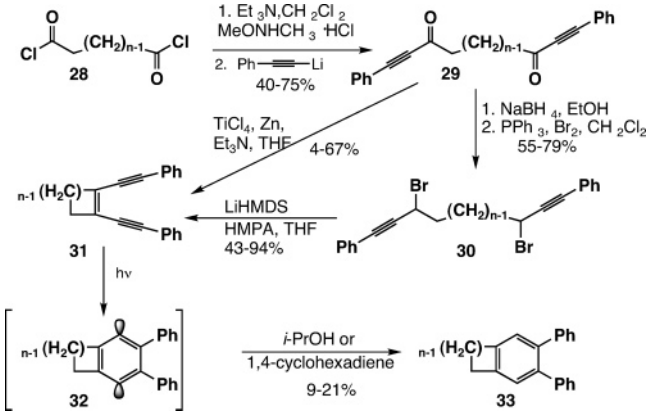


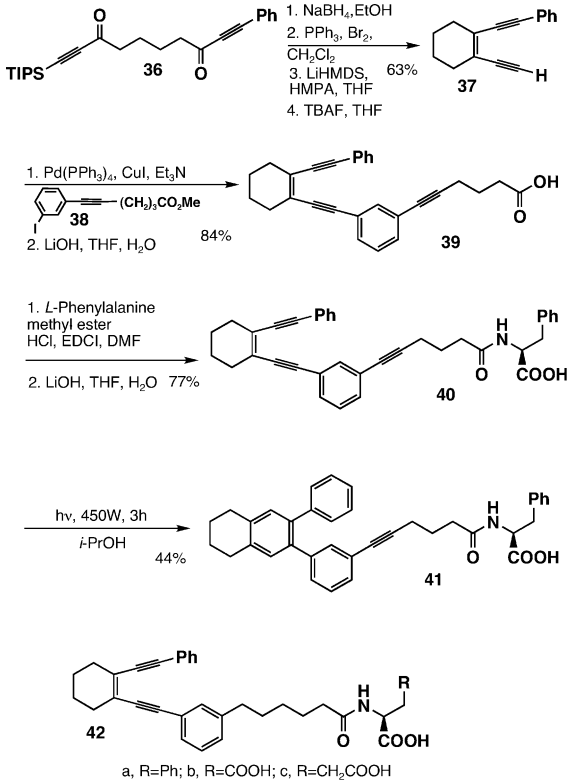
TABLE 3. Synthesis and Cycloaromatization of 31

entry	*n*	c−d (Å)[a]	time (h)[b]	conv[c]	% **33**[d]
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 *n*-2-propanol in a quartz vessel and irradiated using a 450 W lamp. [c] % conversion as determined by GC/MS. [d] Isolated yields (those based on recovered starting material). [e] Decomposition and formation of polymeric adducts ensues in <1 h.

Commencing from commercially available acyl chlorides **28**, we produced the corresponding diketones **29** via the intermediate Weinreb amides (Scheme 8).[23] Low valent Ti-mediated coupling gave **31** directly, but the yields were variable, in part, because of problems encountered recovering product from complex mixtures. Alternatively, conversion to bromides **30** followed by carbenoid coupling gave **31** ($n = 1-5$) in good yield on a preparative scale.[24] Though stable at room temperature, photochemical Bergman cycloaromatization gave adducts **33** in moderate yield, presumably via diyls **32** (Table 3). As had been found previously, the nature of the hydrogen donor plays an important role in the conversion to an arene adduct.[25] 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 3).[1] Though photo-Bergman cycloaromatization yields are modest, the synthesis of this class of enediynes (four- through eight-membered) is noteworthy and may lead to many new applications in materials and polymer chemistry.[26]

SCHEME 9. Preparation and Photo-Bergman Cyclization of Enediyne Hybrids



Applications. (i) Bovine Serum Albumin. With a route to photoactivated enediynes secure, we wished to investigate interaction of the intermediate diyl radicals **27** 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,[27] who reported that an alkyl pyrenyl derivative of phenylalanine (**34**) recognizes the protein's bovine serum albumin (BSA) and lysozyme, inducing photocleavage following irradiation in the presence of an electron acceptor.[27] Molecular modeling studies indicated that diyls **35** would be close mimics of this agent and could presumably be generated via irradiation of an enediyne precursor. Accordingly, enediyne **40** became a logical candidate for proof-of-principle studies. First, diketone **36** was prepared from adipoyl chloride using a mixed coupling procedure. Subsequent functional group interconversion and carbenoid coupling gave the differentially functionalized C-6 enediyne, which was unmasked to give **37** (Scheme 9).

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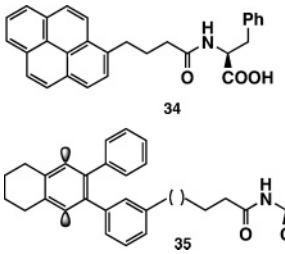


Photo-Bergman cycloaromatization of this enediyne (or the triisopropylsilyl (TIPS) protected precursor) gave <10% of the corresponding arene product, suggesting that the aryl groups play a key role in the (reversible) cyclization process.[22] The alkyne was then coupled with methyl-3-(3-iododphenyl)hexynoate **38**, followed by hydrolysis to give carboxylate **39**, which was then coupled with L-phenylalanine methyl ester and subjected to saponification to give conjugate **40** in good yield. In contrast to the model series, photochemical activation (450 W, 3 h) of this enediyne gave arene product **41** 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 **40** in aqueous buffer and using **41** and enediynes **39** and **31** as controls. In the case of 66 kD protein BSA, subsequent analysis (SDS−PAGE) showed some evidence of enediyne-induced degradation with one principal fragment (~40 kDa) visible (<5% relative to parent) when a 5:1 ratio of enediyne **40**/BSA was employed.[28] Encouraged by this initial finding, we sought analogues with improved affinity for BSA in the hope this would translate to improved photocleavage capacity. Modification of the synthesis could be affected, allowing preparation of alkyl-linked conjugates. This required hydrogenation of intermediate **38**, followed by a procedure identical to that employed for the preparation of **40**, giving access to enediynes **42a**−**c**. Of these agents, **42b** and **42c** showed enhanced affinity for BSA, and photolysis was conducted.[28] Significant cleavage into two principal fragments (31 and 35 kDa) was observed, tempting the correlation between affinity and cleavage efficiency (Figure 1).[28] Consistent with the outcome of the photodegradation experiment, Scatchard analysis suggested that only one binding site is involved.[28,27] In the case of the 14 kDa protein lysozyme, dimerization to form a species with *m/z* 28.6 kDa was observed following irradiation with **40** (Figure 2), suggesting the possibility of intermolecular diyl-mediated cross-coupling.[28] These initial findings encouraged us to search for photoproteases with specific targets of biological relevance, and we were encouraged to use carboxylate **39** as a building block.

(28) Plourde, G.; El-Shafey, A.; Fouad, F. S.; Purohit, A. S.; Jones, G. B. *Bioorg. Med. Chem. Lett.* **2002**, *12,* 2985. Binding constants of **40** and **42** were determined following Scatchard analysis: BSA **40** = 1.6×10^5 dm^3 mol^{-2}; **42a** = 1.8×10^5 dm^3 mol^{-2}; **42b** = 6.7×10^5 dm^3 mol^{-2}; **42c** = 5.8×10^5 dm^3 mol^{-2}; and lysozyme **40** = 0.3×10^5 dm^3 mol^{-2}; **42b** = 0.1×10^5 dm^3 mol^{-2}. Densitometric analysis of Coomassie stained gels (processed with Adobe Photoshop/NIH-image software) quantitated the BSA 29/37 kDa fragments (Figure 1) as 43% relative to the parent and the lysozyme dimer (28 kDa, Figure 2) at 8% relative to the parent. For procedures, see: Scatchard, G. *Ann. N. Y. Acad. Sci.* **1949**, *51,* 660. MS analyses were performed using desalted mixtures directly following incubation using a sinapinic acid (BSA) or an α-cyano-4-hydroxycinnamic acid matrix (lysozyme). For a discussion on binding sites in BSA, see ref 27.

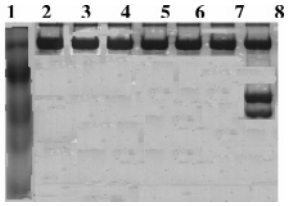


FIGURE 1. 10% SDS polyacrylamide gel of the reaction bovine serum albumin (BSA) (1 μM) with **42b**. All assays were conducted in 50 mM Tris−HCl, pH 7.0, at 37 °C. Lane 1: molecular weight markers (kDa). Lane 2: BSA, no irradiation. Lane 3: BSA, 3 h irradiation. Lane 4: BSA, 12 h irradiation. Lane 5: BSA + **41** (10 μM), 12 h irradiation. Lane 6: BSA + **42b** (10 μM), no irradiation. Lane 7: BSA + **42b** (10 μM), 1 h irradiation. Lane 8: BSA + **42b** (10 μM), 12 h irradiation.

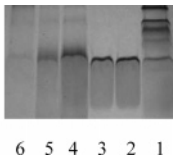
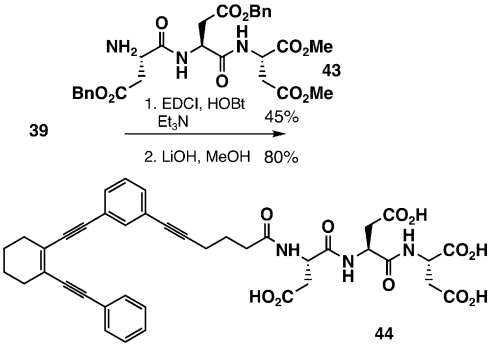


FIGURE 2. 15% SDS polyacrylamide gel of the reaction of lysozyme (Lyso) with **40** and **41**. All reactions were conducted in 50 mM Tris−HCl, pH 7.0, at 37 °C. Lane 1: molecular weight markers. Lane 2: Lyso (0.15 μM). Lane 3: Lyso (0.15 μM), 3 h irradiation. Lane 4: Lyso (0.15 μM) and **40** (0.75 μM), 6 h irradiation. Lane 5: Lyso (0.15 μM) and **40** (0.75 μM), 3 h irradiation. Lane 6: Lyso (0.15 μM) and **41** (15 μM), 12 h irradiation. The running gel was overlaid with a 4% stacking gel, and samples were run from top to bottom. Analogous reactions with **31** ($n = 4$) showed no change from the control (data not shown).

SCHEME 10. Synthesis of Tri(Asp) Conjugate



(ii) Histone H1. In the case of the naturally occurring chromoproteins kedarcidin, C-1027, and neocarzinostatin, reports that the apoprotein components have proteolytic ability have been made.[4] In the case of kedarcidin, studies suggested that the highly acidic apoprotein was capable of inducing selective degradation of histone H1.[4] As this is the most basic histone (34% arginine/lysine content), a natural conclusion was that affinity plays a role. Following proteolysis of the histone, it was suggested that the reactive enediyne chromophore could then more efficiently deliver to exposed DNA, contributing to the high efficiency of strand scission by kedarcidin.[4] On the basis of the present findings, we became interested in preparing an acidic enediyne−peptide conjugate to determine if enediyne-mediated histone degradation could be achieved and, if so, with what specificity. To investigate this, enediyne **39** was coupled with the triaspartic acid derivative **43** (Scheme 10). Deprotection of the

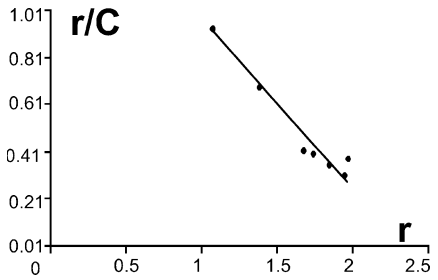


FIGURE 3. Scatchard plot of histone H1 with enediyne−triaspartic acid conjugate **44**. Solutions of enediyne **44** in a minimum quantity of methanol were diluted with 25 mM Tris−HCl (pH = 7.0) buffer. Binding was assessed spectrophotometrically over eight different concentrations of histone (0−50 μmol range) with fixed **44** (20 μmol). A curve was constructed via Scatchard analysis using: $r/C_f = K_b (n - r)$, where r is the number of moles enediyne bound per mole of protein, C_f is the free-enediyne concentration, n is the number of binding sites, and K_b is the binding constant. This figure illustrates r/C_f (y axis) vs r (x axis), revealing a K_b of 7.2×10^5 dm^3 mol^{-2}, and $n = 2.3$.

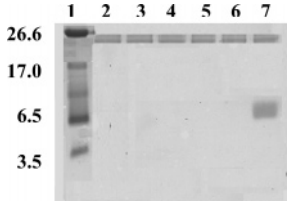
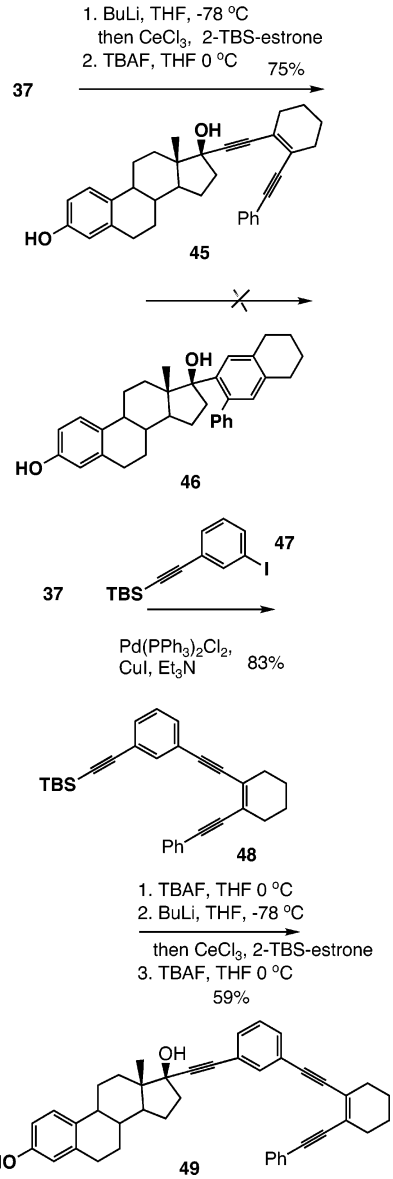


FIGURE 4. 16.5% SDS polyacrylamide Tris−tricine gel of the reaction of histone H1 (21.5 kDa, 1 μM) with **44**. All reactions were conducted in 50 mM Tris−HCl, pH 7.0. Lane 1: molecular weight markers (kDa). Lane 2: histone H1, no irradiation. Lane 3: histone H1, 1 h irradiation. Lane 4: histone H1, 12 h irradiation. Lane 5: histone H1 + **44** (10 μM), no irradiation, 12 h. Lane 6: histone H1 + **44** (10 μM), 1 h irradiation. Lane 7: histone H1 + **44** (10 μM), 12 h irradiation.

conjugate with LiOH gave the enediyne−triasp conjugate **44** in good yield. As expected, this conjugate had appreciable solubility in aqueous buffer. Binding of this agent to a variety of histones revealed that affinity, as expected, was greater for H1 versus others, and Scatchard analysis suggested that (in the case of H1) two binding sites are involved (Figure 3).[29] More significantly, irradiation of **44** in the presence of histone H1 (21.5 kDa) led to degradation of the protein into principal components in the 8−11 kDa range (Figure 4).[29] The application of a designed photoactivated enediyne with near micromolar affinity for a biological target is noteworthy and suggested even greater affinity might be attainable by coupling the photowarhead to the ligand with a complimentary protein receptor.

(iii) Human Estrogen Receptor α. As a final example of application, we elected to study agents with the capability of degrading the human estrogen receptor (hER), as lipophilic steroidal ligands with nanomolar affinity are well recognized. Though a multitude of substituted steroid templates are available via direct synthesis, we were particularly interested in commencing from a commercially available building block. Surveying numerous options, we decided to utilize estrone, introducing the enediyne functionality at the 17α position. Through quantitative structure−activity relationship (QSAR) analysis, it has been shown that 17α-alkynyl

SCHEME 11. High Affinity Carbocyclic Enediyne−Estrogens



derivatives of estradiol show comparable hER affinity to its endogenous ligand (estradiol),[30] and we decided to construct a hybrid with an alkynyl linker between the enediyne and the steroidal template. Accordingly, enediyne **37** was coupled to a protected (TBDMS) ether of estrone via its organocerium derivative, then unmasked to give **45** (Scheme 11). Unfortunately, this compound, which had a relative binding affinity (RBA) of >100 nM (Table 4), proved stable at physiological temperature and did not undergo the expected photo-Bergman cycloaromatization to give **46** even on prolonged irradiation. Following a strategy adopted previously, we elected to insert a spacer unit in the system between the enediyne and ligand and pursued *m*-disubstituted ligand **49**.

(29) Binding constants determined following Scatchard analysis: **44** histone H1 = 7.2×10^5 dm^3 mol^{-2} and histone H2 = 1.7×10^5 dm^3 mol^{-2}; **42b** histone H1 = 0.4×10^5 dm^3 mol^{-2}. Control reactions using **40** and **42** failed to produce any discernible photodegradation products (histone H1) after 12 h irradiation (10 μM).

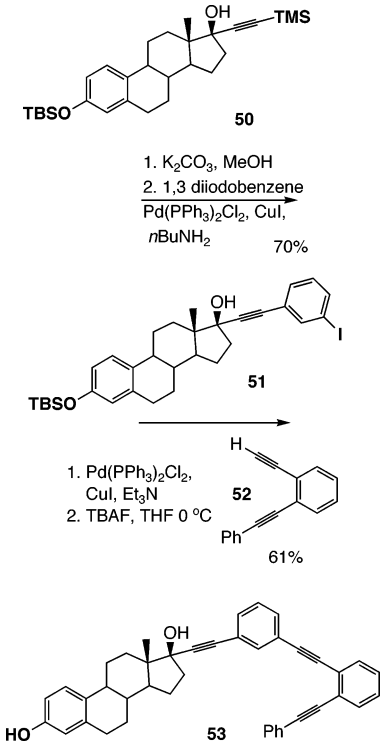
(30) Gao, H.; Katzenellenbogen, J. A.; Garg, R.; Hansch, C. *Chem. Rev.* **1999**, *99*, 723.

TABLE 4. Relative Binding Affinity of Steroid−Enediynes for ER[a]

entry	substrate	RBA (nM)
1	**45**	103
2	**49**	14
3	**53**	12

[a] Candidate + 3H β-estradiol incubated with cytosol at 4 °C. Unbound agents were removed (DCC), and bound 3H β-estradiol was measured by a scintillation counter. RBA corresponds to the concentration required to reduce hER bound 3H β-estradiol by 50%.[32]

SCHEME 12. High Affinity Photoactivated Enediyne−Estrogens



Coupling of **37** with iodoalkyne **47** gave **48**, which was unmasked and subjected to 1,2 addition to estrone TBS ether and, finally, followed by phenolic deprotection to give **49**. To our surprise, the affinity of **49** was greatly superior to that of **45**, supporting the notion that the ligand binding domain (LBD) of hERα has considerable tolerance for lipophilic functionality at the steroidal 17α position.[31] Though the binding affinity of **49** was spectacular, to our disappointment, its photo-Bergman cycloaromatization profile was poor, giving only trace amounts of the expected cycloaromatization product even after prolonged irradiation. We elected to improve the efficiency of the critical process by extending the conjugation of the ligand and pursued **53** as a refined target. Though this required complete resynthesis, an efficient process was developed via preassembly of enediyne template **52** (Scheme 12). This was coupled in turn to iodoarene **51**, produced by arylation of the unmasked alkyne derived from **50**, which could be produced from either estrone TBS ether or the commercially available 17α-ethynyl estradiol. Though the binding affinity of **53** did not differ markedly from **49** (Table 4),[32] this ligand underwent smooth photocycloaromatization to give complete conversion to cycloaromatized product within 12 h.

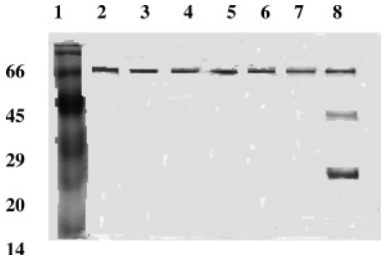


FIGURE 5. 10% SDS polyacrylamide gel of the reaction of the estrogen receptor (ER) (1.5 μg/mL) with **53** (550 μM). All reactions were conducted in 20 mM Tris−HCl buffer at pH 8.0. Lane 1: molecular weight markers (kDa). Lane 2: ER, no irradiation. Lane 3: ER, 6 h irradiation. Lane 4: ER, 12 h irradiation. Lane 5: ER + **53**, no irradiation. Lane 6: ER + **52**, 12 h irradiation. Lane 7: ER + **53**, 1 h irradiation. Lane 8: ER + **53**, 12 h irradiation.

Irradiation in the presence of recombinant hER (66 kDa) resulted in marked degradation to produce two discrete fragments within 12 h (31 and 35 kDa) and control reactions confirming the effect of the enediyne warhead (Figure 5). The ability to induce on demand degradation of a nuclear receptor using a lipophilic warhead is of significance and underscores the potential use of such entities as molecular reagents.

In summary, the viability of proteins as targets of enediynes has been confirmed at the molecular level. A new series of photochemically activated enediynes has been prepared using an intramolecular coupling strategy, and derivatives are used for specific protein targeting. On the basis of molecular architecture, three independent classes of enediynes with defined protein targets have been identified (albumin, histone, and estrogen receptor) and show correlation between affinity and protein degrading activity. On the basis of these findings, coupled with expeditious synthetic routes to the core warhead, enediyne reagents capable of inducing specific protein modulation can now be designed. Such affinity cleavage systems may offer advantages over conventional methods because they do not function via metal ion redox chemistry,[33] presenting instead a lipophilic warhead. Furthermore, the biological significance of proteins as targets for enediyne-derived diyls has been strengthened by recent reports. Thorson has demonstrated that an organism which produces one of the naturally occurring enediyne chromophores is self-protected by an associated protein-based suicide substrate, which intercepts diyls produced by enediyne degradation resulting in formation of a *glycyl radical* in the protein.[34] Hirama has also demonstrated that the C-1027 apoprotein is slowly degraded by the

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(33) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1991**, *113*, 1859. Hoer, D.; Cho, H.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 3249. Cuenoud, B.; Tarasow, T. M.; Schepartz, A. *Tetrahedron Lett.* **1992**, *33*, 895. Hegg, E. L.; Burstyn, J. N. *J. Am. Chem. Soc.* **1995**, *117*, 7015. Wu, J.; Perrin, D. M.; Sigman, D.; Kaback, H. R. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9186. Parac, T. N.; Kostic, N. M. *J. Am. Chem. Soc.* **1996**, *118*, 5946. Kumar, C. V.; Buranaprapuk, A. *J. Am. Chem. Soc.* **1999**, *121*, 4262. Miyake, R.; Owens, J. T.; Xu, D.; Jackson, W. M.; Meares, F. *J. Am. Chem. Soc.* **1999**, *121*, 7453.

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diradical derived from its enediyne chromophore.[35] Accordingly, the prospect of designing synthetic enediynes to incapacitate specific protein targets resulting from the human proteome project has become a realistic goal. The findings may also prove important to our understanding of the naturally occurring enediyne chromoproteins and, specifically, to the interactions of chromophore and apoprotein components of these clinically relevant antibiotics.[1,36]

Experimental Procedures[15]

1,10-Diphenyl-1,9-decadiyne-3,8-dione (29; *n* = 4). A suspension of *N*,*O*-dimethylhydroxylamine hydrochloride (3 g, 30.75 mmol) and Et_3N (10 mL, 70 mmol) in CH_2Cl_2 (50 mL) was cooled to 0 °C.[15] Adipoyl chloride (14 mmol) was added, and the mixture was left to stir at 0 °C for 1 h, warmed to 25 °C, stirred for an additional 1 h, and then quenched with brine (50 mL). The product was extracted with CH_2Cl_2 (2 × 100 mL), washed with brine (3 × 100 mL), and dried ($MgSO_4$). Solvents were removed in vacuo, and the residue was purified via a short column (silica gel, 1% Et_3N in ether) to give the corresponding crude amide:[37] ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67 (s, 6H), 3.16 (s, 6H), 2.44 (bs, 4H), 1.67 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 60.9, 31.9, 31.4, 24.1.

Phenyl acetylene (2.11 mL, 1.97 g, 19.27 mmol) was dissolved in THF (80 mL), and the solution was cooled to −10 °C. *n*-BuLi (19.27 mmol, 2.8 mL of 2.5 M in hexanes) was added dropwise over 10 min, and the mixture was warmed to room temperature (0.5 h), cooled to 0 °C, and cannulated onto a cold solution (0 °C) of the crude Weinreb amide (8.76 mmol) in THF (90 mL). The reaction was warmed to room temperature (40 min) and quenched with HCl (5%, 50 mL), and then brine (50 mL) was added. The mixture was extracted with ethyl acetate (3 × 150 mL), and the organic layers washed with brine (2 × 100 mL), dried (Na_2SO_4), and condensed in vacuo. The residue was then purified with SGC (10% EtOAc/hexanes) to give **29**, *n* = 4 (2.063 g, 75%), as a light brown oil:[38] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (m, 4H), 7.32 (m, 6H), 2.21 (m, 4H), 1.91 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.4, 133.2, 131.0, 128.9, 120.0, 90.59, 88.0, 43.2, 23.6. Anal. Calcd for $C_{22}H_{18}O_2$: C, 84.05; H, 5.77. Found: C, 84.10; H, 5.89.

1,10-Diphenyl-1,9-decadiyne-3,8-diol. To a 50 mL round-bottom flask was added ketone **29**, *n* = 4 (1.0 g, 2.4 mmol), and MeOH (10 mL). The resulting solution was cooled to −15 °C, and $NaBH_4$ (0.181 g, 4.8 mmol) was added in one portion. The mixture was allowed to warm to room temperature over 1.0 h, then carefully poured onto cold HCl (2%, 20 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were treated with 5% HCl (2 × 20 mL) and brine (3 × 50 mL), dried over $MgSO_4$, and finally concentrated in vacuo. Purification of the residual oil using SGC (30:70, EtOAc/hexanes) afforded the title compound (0.600 g, 79%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (m, 4H), 7.25 (m, 6H), 4.60 (t, *J* = 5.7 Hz), 3.24 (br s, 2H), 1.82 (m, 4H), 1.55 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 131.7, 128.3 (2C), 122.7, 90.4, 84.7, 62.69, 37.7, 24.9; IR (neat) 3350, 2936, 2250, 1450, 1010, 740 cm^{-1}. $C_{22}H_{22}O_2$ Requires: C, 82.99; H, 6.96. Found: C, 83.27; H, 7.14.

3,8-Dibromo-1,10-diphenyl-1,9-decadiyne (30, *n* = 4). Triphenylphosphine (1.23 g, 4.71 mmol) was dissolved in CH_2Cl_2 (25 mL), the resulting solution was cooled to 0 °C, and then bromine (0.753 g, 4.71 mmol, 0.243 mL) was added dropwise over 5 min. The resulting suspension was allowed to stir for 0.5 h, and then 2,6-lutidine (1.51 g, 14.13 mmol, 1.64 mL) was added. The resulting solution was stirred for an additional 0.5 h, and then a solution of 1,10-diphenyl-1,9-decadiyne-3,8-diol (0.500 g, 1.57 mmol), dissolved in CH_2Cl_2 (10 mL), was added dropwise via cannula. The reaction mixture was allowed to slowly warm to 10 °C over 2 h, diluted with *n*-pentane (30 mL), and stirred an additional 10 min at 0 °C. The suspension was filtered through a bed of silica gel and rinsed with *n*-pentane (3 × 50 mL). The combined organic eluents were concentrated in vacuo, and the resulting oil was purified via SGC (100% hexanes) to yield the title compound (0.48 g, 69%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (m, 4H), 7.38 (m, 6H), 4.81 (2H, t, *J* = 6.6 Hz), 2.15 (m, 4H), 1.71 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.0, 129.0, 128.5, 122.2, 88.0, 87.3, 39.6, 37.8, 26.7; IR (neat) 3010, 2960, 2240, 1430, 1330, 1209, 1145, 910, 621 cm^{-1}. $C_{22}H_{20}Br_2$ Requires: C, 59.49; H, 4.54. Found: C, 59.67; H, 4.82.

1,2-Di(2-phenyl-1-ethynyl)-1-cyclohexene (31, *n* = 4). In a 100 mL round-bottom flask was placed dibromide **30**, *n* = 4 (0.100 g, 0.226 mmol), THF (23 mL), and HMPA (1.01, 5.65 mmol, 0.983 mL). The resulting solution was cooled to −78 °C, and LiHMDS (0.565 mmol, 0.565 mL of 1.0 M in THF) in THF (5 mL) was added via cannula over the course of 2.0 h. On completion of the base addition, the reaction mixture was allowed to stir for an additional 0.5 h and then poured onto a saturated solution of NH_4Cl (25 mL). The organic material was extracted into Et_2O (3 × 25 mL), and the combined extracts were treated with 10% HCl (2 × 25 mL), water (2 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL), and brine (1 × 25 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified via radial chromatography (100% hexanes) to afford the title compound (0.593 g, 94%) as a white solid, mp 72 °C, that was spectroscopically identical to that reported:[39] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (m, 4H), 7.31 (m, 6H), 2.39 (m, 4H), 1.71 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 131.5, 128.3, 128.1, 126.5, 123.6, 93.6, 90.4, 30.0, 21.8.

6,7-Diphenyl-1,2,3,4-tetrahydronaphthalene (33, *n* = 4). A photochemical reaction vessel (250 mL) was charged with bisphenylenediyne **31**, *n* = 4 (0.100 mg, 0.354 mmol), and spectroscopic grade *i*PrOH (200 mL). The resulting solution was deoxygenated with Ar for 30 min, then irradiated using a 450 W low-pressure mercury lamp, maintaining a slow purge of Ar throughout the entire 12 h irradiation. The solution was concentrated in vacuo, and the residue was purified by preparative thin layer chromatography (TLC) (2 mm, 100% hexanes) to yield the title compound (19 mg, 20%) as a white solid, mp 109 °C, spectroscopically identical to that reported:[40] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (m, 6H), 6.99 (m, 4H), 2.86 (m, 4H), 1.85 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.8, 138.0, 136.7, 131.5, 130.0, 127.9, 126.3, 269.3, 23.4; MS (EI) *m/z* 284 (M+), 254, 241, 228; HRMS calcd for $C_{22}H_{20}$ 284.1565, found 284.1520.

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Supporting Information Available: Synthetic procedures and spectroscopic data for the preparation of all reagents (31 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

JO051403Q

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