ORGANIC LETTERS

2000
Vol. 2, No. 6
811−813

Understanding Enediyne–Protein Interactions: Diyl Atom Transfer Results in Generation of Aminoacyl Radicals

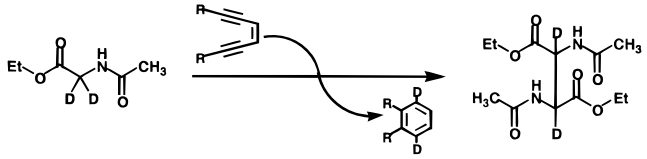
Graham B. Jones,* Gary W. Plourde II, and Justin M. Wright

Bioorganic and Medicinal Chemistry Laboratories, Department of Chemistry, Northeastern University, Boston, Massachusetts 02115

grjones@lynx.neu.edu

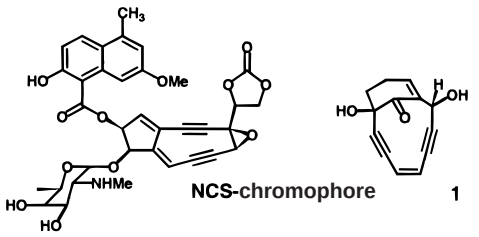
Received January 14, 2000

ABSTRACT



The origin of the protein modulating capacity of enediynes has been probed. A series of synthetic enediyne-derived diyls participated in atom transfer chemistry with a labeled amino acid. Subsequent experiments suggest that diyl radicals may modulate protein architecture via formation of captodatively stabilized radicals.

Interest in the chemistry of the enediyne family of antitumor antibiotics has grown steadily over the past decade, fueled both by reports of their remarkable biological activity and their intriguing and challenging molecular structures.[1] 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 clarified.[2] Though DNA is the recognized target of the naturally occurring enediyne chromophores, studies involving calicheamicin suggest that specific DNA strand scission is not directly related to cytotoxicity in this potent antitumor agent but that subsequent promotion of the DNA suicide repair enzyme poly(ADP-ribose)polymerase results in cellular death by means of depleting cellular NAD^+ and ATP levels.[3] Both the naturally occurring kedarcidin and the related enediyne neocarzinostatin (NCS) are *chromoproteins* that are capable of causing damage to histones, in addition to DNA.[4] In this class of



enediyne antibiotic, proteolytic ability is derived from the associated apoprotein component, tempting speculation that this may serve as a dynamic delivery vehicle for the DNA cleaving chromophore.[4] In related studies, the Bristol-Myers

(1) Reviews: Grissom, J. W.; Gunawardena, G. U.; Klingberg, D.; Huang, D. *Tetrahedron* **1996**, *52*, 6453−6518. Maier, M. E. *Synlett* **1995**, 13. Nicolaou, K. C.; Smith, A. L. *Acc. Chem. Res*. **1992**, *25*, 497.

(2) Xi, Z.; Goldberg, I. H. In *Comprehensive Natural Products Chemistry*; Barton, D. H. R., Nakanishi, K.; Eds.; Elsevier Science: Oxford, 1999; Vol. 7, pp 553−592. Lee, M. D.; Durr, F. E.; Hinman, L. H.; Haman, P. R.; Ellestad, G. A. The Calicheamicins. In *Advances in Medicinal Chemistry*; Maryanoff, B. E., Maryanoff, C. A., Eds.; Jai Press: Greenwich, 1993; Vol. 2.

(3) 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.

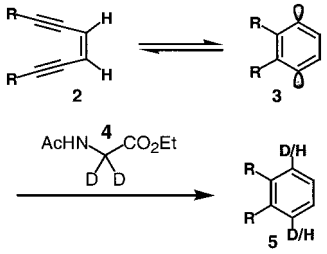
(4) Zein, N.; Schroeder, D. R. In *Advances in DNA Sequence Specific Agents*; Jones, G. B., Ed., JAI Press: Greenwich, 1998; Vol. 3, p 201. Zein, N.; Reiss, P.; Bernatowicz, M.; Bolgar, M. *Chem. Biol*. **1995**, *2*, 451. Zein, N.; Casazza, A. M.; Doyle, T. W.; Leet, J. E.; Schroeder, D. R.; Solomon, W.; Nadler, S. G.; *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8009.

10.1021/ol0055566 CCC: $19.00
Published on Web 03/01/2000

Squibb group identified protein targets of a synthetic enediyne *chromophore* (**1**), which possesses the core framework of the enediyne esperamicin.[5] This agent, which has demonstrated in vitro and in vivo antitumor activity, induced damage to membrane extracts, tubulins, and histones, presumably via its derived diyl radicals, whereas significantly higher concentrations of **1** were required to achieve appreciable DNA cleaving activity.[6] In view of the potential significance of a selective enediyne based protein modulator,[7] we sought to examine the molecular basis for diyl−protein interactions using atom transfer chemistry.[8]

Accordingly, we prepared a number of synthetic enediyne core structures **2** designed to undergo cycloaromatization to yield diyls **3** in the presence of the labeled amino acid **4**, readily prepared from glycine (Scheme 1).[8] The enediynes

Scheme 1. Atom Transfer Chemistry Using Deuterated Glycine Mimics



were selected on the basis of their differing modes of activation, and propensity to undergo cycloaromatization, and were all prepared using an efficient metallohalocarbenoid route developed in our laboratories.[9] Thermal cyclization of *Z* enediyne **6** proceeded at 280 °C to give the corresponding arenes **7** isolated as a mixture of labeled and unlabeled species (Table 1). Alternatively, photochemical Bergman cycloaromatization can be initiated with suitable substrates,[10] and enediyne **6** likewise gave arenes **7** in moderate yield following 3 h irradiation using a low-pressure mercury lamp

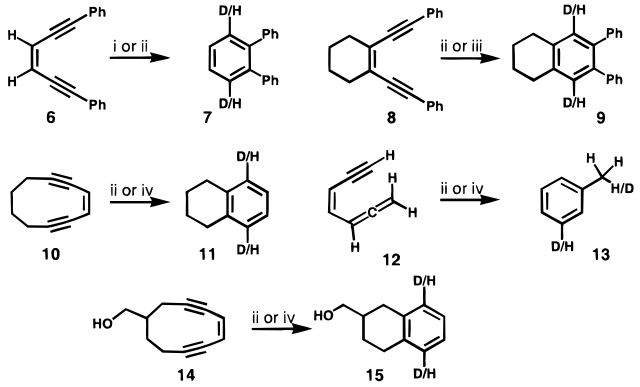
Table 1. Yields and Isotope Ratios of Atom Transfer Adducts

adduct	method	yield (%)[a,b]	D0:D1:D2[c]
7	thermal	10	31:2:1
	hν	18	17:2:1
9	thermal	8	19:2:1
	hν	20	14:2:1
11	thermal	11	3:2:1
	hν	20	2:2:1
13	thermal	10	2:2:1
	hν	15	7:2:1
15	thermal	40	4:2:1
	hν	50	3:2:1

[a] Yields based on GC analysis, calibrated using internal (*n*-decane) and external standards. [b] Mass balance composed of recovered starting material (10−30%) and unidentified oligomers. [c] Ratio determined by H/D NMR and GC/MS analysis using authentic dideutero adduct (>95% D2 incorporation at 20% conversion) prepared by incubation of enediyne with cyclohexadiene-d_8[12] or THF-d_8.

(Hanovia 450 W, quartz vessel). Similar behavior was observed for enediyne **8** (Scheme 2). Moving to more

Scheme 2. Deuterium Atom Transfer from Labeled Amino Acid to Enediyne-Derived Diyl Radicals



Conditions: i. 280°C, PhH, 4h, **4**; ii. hv, PhH, 3h, 37°C **4**; iii. 240°C, PhH, 3h, **4**; iv. 37°C, PhH, 48h, **4**

reactive precursors, enediyne **10** and eneyne allene **12**, whose half-lives for cycloaromatization are approximately 18 and 24 h at 37 °C, respectively,[1,11] cyclized readily at physiological temperature, giving low yields of labeled cycloaromatization adducts **11** and **13**, which could be increased slightly under photochemical conditions.

Finally, the hydroxymethyl enediyne **14** was employed. The marked increase in chemical conversion to **15** suggests that electrostatic effects between the donor (**4**) and pendant functionality contribute to the efficiency of diyl capture. Indeed, repeating the experiments using benzene-d_6 as solvent gave no increase in deuterium incorporation. Atom transfer from **4** implies generation of a capdodatively stabilized radical **16**, which could be expected to either dimerize (**17**) or react with molecular oxygen to form peroxides (e.g., **18)** which could ultimately result in fragmentation via a transient iminium ion (Scheme 4).[13] To probe these scenarios, an authentic sample of **17** was prepared (*tert*-butyl peroxide, h*ν*, PhH, 48 h, 55%). Reanalysis of the crude reaction mixtures (Scheme 2) confirmed formation of **17** as the principal byproduct following atom transfer. Not surprisingly, since all reactions were conducted under deoxygenated

(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.

(6) 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*, 1351.

(7) Jones, G. B.; Kilgore, M. W.; Pollenz, R. S.; Li, A.; Mathews, J. E.; Wright, J. M.; Huber, R. S.; Tate, P. L.; Price, T. L.; Sticca, R. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1971.

(8) Braslau, R.; Anderson, M. O. *Tetrahedron Lett.* **1998**, *39*, 4227.

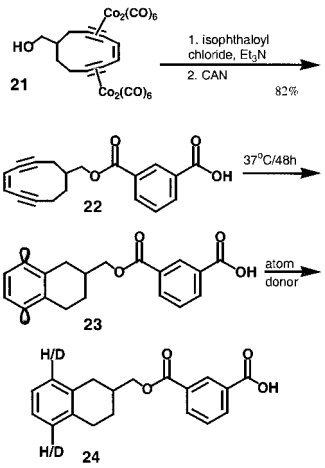
(9) Jones, G. B.; Huber, R. S.; Mathews, J. E. *J. Chem. Soc., Chem. Commun.* **1995**, 1791. Jones, G. B.; Wright, J. M. *Tetrahedron Lett.* **1999**, *40*, 7605.

(10) Turro, N. J.; Evenzhav, A.; Nicolaou, K. C. *Tetrahedron Lett.* **1994**, *35*, 8089. Funk, R. L.; Young, E. R. R.; Williams, R. F.; Flanagan, M. F.; Cecil, T. L. *J. Am. Chem. Soc.* **1996**, *118*, 3291.

(11) Myers, A G.; Dragovich, P. S. *J. Am. Chem Soc.* **1989**, *111*, 9130.

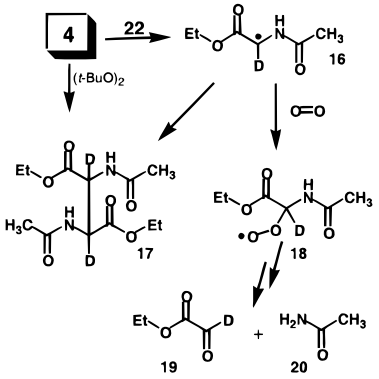
(12) Karel, K. J.; Brookhart, M.; Aumann, R. *J. Am. Chem. Soc.* **1995**, *103*, 2695.

Scheme 3. Atom Transfer Chemistry Using an Enediyne Carboxylate



conditions, products derived from hydroperoxide **18** were not isolated. Though the experiments show clear evidence of atom transfer chemistry from **4**, the relevance of these events in organic media remained in question. To address this issue water-soluble enediyne **22** was prepared, by reacting the shelf-stable cobalt complex **21** with isophthaloyl chloride (Scheme 4),[9] and then immediately unmasking the

Scheme 4. Potential Intermediates in the Activation of Amido Acyl Radicals



enediyne moiety. Though carboxylate **22** 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: the corresponding *atom-transfer adducts formed at 37 °C under aerobic conditions*. The process was more efficient using **4** rather than d_5 (or H_5) glycine as atom transfer agent. This may reflect a preference for the (hydrophobic) diyl to abstract from a neutral atom transfer agent, and this has a positive impact on the rate of cycloaromatization (Table 2, entry 6). Though a complex

Table 2. Atom Transfer Chemistry of Enediyne Carboxylate **22**

entry	solvent	donor[13]	yieldof 24 (%)	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	$H_2O/(NH_4)_2HPO_4$	**4**	42	3:2:1

mixture of other byproducts was formed in addition to dimer **17**, both glyoxylate **19** and acetamide **20** were detected (<10%), suggesting the intermediacy of peroxide **18** under aerobic conditions. Repeating the incubations under deoxygenated conditions (triple freeze−pump−thaw cycles, N_2), the efficiency of formation of the peroxy-derived byproducts was reduced (<1%), suggesting some control may be possible in governing the fate of radical **16**. On the basis of these preliminary findings, a likely extension of this work may be the design of enediynes capable of inducing inter- and intramolecular peptide cross-links, via generation of peptide radicals. Such radicals are not without precedent;[14] indeed, families of "radical enzymes" exist, including the well-studied examples from *Escherichia coli* ribonucleotide reductase and pyruvate-formate lyase, both of which involve intermediate glycyl radicals.[15]

In summary, a water-soluble enediyne has been prepared and used to investigate amino acid−diyl interactions. Evidence in support of diyl-mediated peptide damage at the molecular level has been gleaned, which has ramifications for the biology of enediyne antitumor agents. Application of this methodology in the design of target specific protein modulators will be reported in due course.[16]

OL0055566

(13) In all cases, 30 equiv of amino acid mimic was employed, since control reactions using the conventional hydrogen donor 1,4-cyclohexadiene gave optimal yields (>80%) under these conditions. Further increases in stoichiometry resulted in only negligible increases in chemical yield of the labeled (or control) adducts, but reduction (<10 equiv) resulted in a sharp decrease. For discussion on the influence of quench concentration on rate of abstraction, see: Semmelhack, M. F.; Neu, T.; Foubelo, F. *J. Org. Chem.* **1994**, *59*, 5038.

(14) Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*; Oxford University Press: 1999.
(15) Marsh, E. N. G. *BioEssays* **1995**, *17*, 431.

(16) We thank the NIH [1RO1GM57123] for generous financial support.