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Oxa-Enediynes: Probing the Electronic and Stereoelectronic Contributions to the Bergman Cycloaromatization

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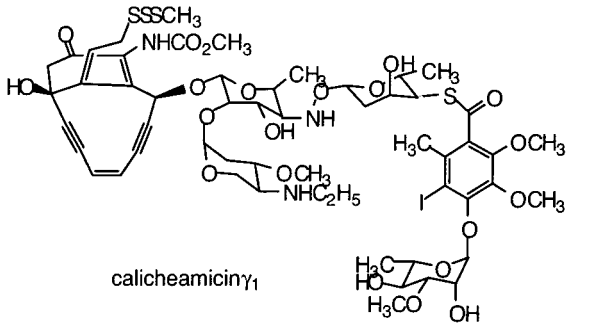
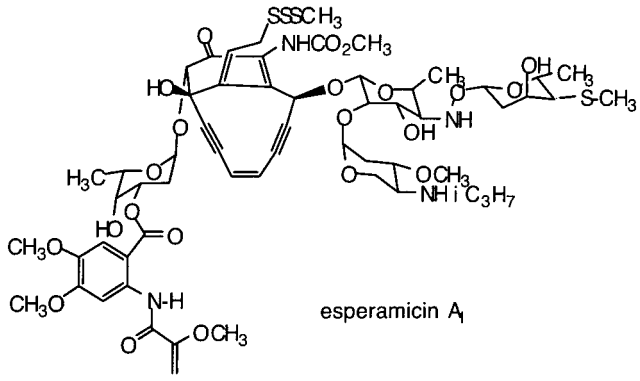
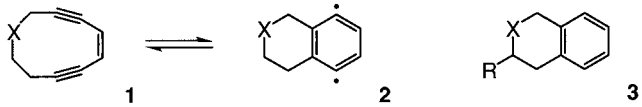
Efficient routes to three classes of 10-membered oxa-enediynes are presented. The electronic and stereoelectronic contributions to half-lives are supported by density functional theory calculations. One member of this class cyclizes to give an isochroman which binds to and degrades the aryl hydrocarbon receptor (AhR).

The enediynes are a promising class of antitumor agents, with spectacular biological profiles.[1] Over 20 natural enediynes have been discovered, and currently, clinical development of neocarzinostatin analogues continues in Japan and Europe. U.S. trials of a monoclonal antibody (MoAb) conjugate of calicheamicin (Mylotarg) are entering phase III, and esperamicin is entering phase II trials.[2] Interest in calicheamicin was heightened because the MoAb conjugate induced remission in up to 40% of patients with acute myeloid leukemia (AML) who had previously relapsed following other chemotherapies. While the in vitro and in vivo effectiveness of enediynes against certain cancers is unquestioned, the exact mechanism(s) of biological activity are complex and remain to be fully understood.[1] Enediynes per se are biologically inactive but undergo cycloaromatization reactions which give rise to cytotoxic diradicals, which are capable of inducing DNA strand scission at low concentration.[1] The basic pharmacophore of many of the natural enediynes is a cyclodec-3-ene-1,5-diyne unit (**1**, X = CH_2), biological or thermal activation of which results in Bergman cyclization of the core to yield the reactive diyl radical





species **2**, which then participates in atom-transfer chemistry.[3] In a cellular environment, these atom-



transfer events can result in formation of ribosyl radicals on DNA,[1,4] and it has also been suggested that they may participate in the generation of peptide radicals,[5] in part

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(1) Xi, Z.; Goldberg, I. DNA Damaging Enediyne Compounds. In *Comprehensive Natural Products Chemistry*; Barton, D. H. R., Nakanishi, K., Eds.; Pergamon: Oxford, 1999; Vol. 7, p 553. Smith, A. L.; Nicolaou, K. C. *J. Med. Chem.* **1996**, *39*, 2103. Grissom, J. W.; Gunawardena, G. U.; Klingberg, D.; Huang, D. *Tetrahedron* **1996**, *52*, 6453. Maier, M. E. *Synlett* **1995**, 13.

(2) 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**, *84*, 976. Oda, M.; Sato, F.; Maeda, H. *J. Natl. Cancer Inst.*, **1987**, *79*, 1205.

(3) Bergman, R. G. *Acc. Chem. Res.* **1973**, *6*, 25.

(4) Dedon, P. C.; Goldberg, I. H. *Chem. Res. Toxicol.* **1992**, *5*, 311.

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accounting for the typically high biological activity of most cyclic enediynes. Since the Bergman cycloaromatization reaction is the key triggering event in the biological cascade, a great deal of effort has been spent studying the process and devising efficient means to control the process.[1] The relief of strain experienced when the C-10 ring undergoes cyclization has been examined and determined to play a dominant role and has been exploited in the form of triggering devices.[6] Additionally, a rule of thumb has been adopted by correlating the intra-acetylenic distance in the enediyne (the so-called "c−d" distance) with experimentally determined half-lives.[7] Other contributing factors involve the electronics of the process, and several reports point to subtle effects observed with substituted enediynes.[8] For our own studies, we wished to examine the effects of heteroatoms in the process, since (i) they may impart subtle strain and/or electronic contributions, (ii) the effects could then be modulated further by appropriate substitution α to the heteroatom, and (iii) synthesis of the agents could be expected to be straightforward, invoking disconnections adjacent to the heteroatom.[9,10]

SCHEME 1. BLYP Analysis of Cycloaromatization Barrier for Oxa-Enediyne 4

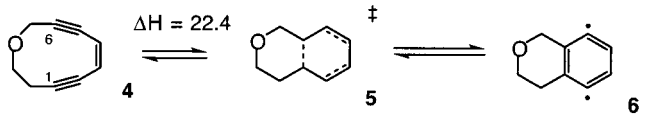


TABLE 1. Comparison of Intra-Acetylenic Distance and Enthalpic Barriers to Cycloaromatization[a]

entry	substrate	$r_{1,6}$-(diyne)	$r_{1,6}$(ts)	ΔH_{rel}-(diyne)	ΔH_{rel}(ts)	$t_{1/2}$(obsd)[b] (h)
1	**1**, X = CH_2	3.413	1.973	0.0	23.7	18
2	**4**	3.365	1.985	0.0	22.4	17
3	**20**	3.346	1.990	0.0	21.3	15
4	**28**	3.170	2.002	0.0	16.0	1

[a] All enthalpies in kcal/mol based on BLYP/6-311+G**//BLYP/6-31-G* electronic energies and BLYP/6-31G* rovibrational corrections. [b] 37 °C, 0.1 M 1,4-cyclohexadiene.

For an initial test case, we elected to study oxa-enediyne **4** and its expected conversion to diyl **6** through transition state **5**. Prior to embarking on synthetic studies, we probed the cyclization barrier using computational analyses and examined the intra-acetylenic "1−6" distance in the ground state and transition state (Scheme 1).[11]The results indicated a shortening of the 1−6 distance for **4** and a consequent earlier transition state (longer 1−6 distance) and decreased enthalpy of activation relative to that of the carbocyclic parent enediyne **1**, X = CH_2 (Table 1). The structural differences between **4** and **1**, X = CH_2, are due to the shorter CO versus CC bond distances. One could expect the $\Delta H^{\#}$ difference to translate to an experimental half-life of less than the 18 h/37 °C reported for **1**, X = CH_2,[12] and accordingly, **4** became a synthesis target.

Initially, the synthesis of **4** was investigated using popular Pd-mediated coupling chemistry, and two independent routes were examined, as shown in Scheme 2. Following formation of the unsymmetrical dialkynyl ether **7**, we had hoped to effect direct conversion to **4** via in situ tandem vinylation. Unfortunately, this coupling gives rise to a mixture of products **8/9**, and even under ideal conditions (stoichiometry, solvent, addition rates), not even a trace of **4** is formed. In an effort to effect closure in a stepwise manner, **7** was converted to masked alkyne **10**, and monovinylation was effected, followed by deprotection to give substrate **11**. Again, under a variety of different conditions, direct conversion to **4** could not be effected; however, over extended periods, polymeric materials were recovered which may have resulted from cyclization and in situ cycloaromatization. This suggested that a Pd-mediated route to **4** may not be desirable, and we sought a more rapid means to effect closure. Remedy was found in the form of a Williamson ether synthesis (Scheme 3). Chlorovinylation of **12** to give **13** was followed by propargylation/deprotection to give **14**. Bromination then gave **15** in good yield and was followed by mild unmasking to give bromo alcohol **16**. Exposure to base and tetrabutylammonium sulfate gave **4** directly; however, because of the predicted thermal instability of **4**, we elected to protect the enediyne as its cobalt carbonyl

(5) Zein, N.; Solomon, W.; Cassazza, A. M.; Kadow, J. F.; Krishnan, B. S.; Tun, M. M.; Vyas, D. M.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1351. 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. Jones, G. B.; Wright, J.; Plourde, G.; Purohit, A.; Wyatt, J.; Hynd, G.; Fouad, F. *J. Am. Chem. Soc.* **2000**, *122*, 9872. Jones, G. B.; Plourde, G. W.; Wright, J. M. *Org. Lett.* **2000**, *2*, 811. Jones, G. B.; Wright, J. M.; Hynd, G.; Wyatt, J. K.; Yancisin, M.; Brown, M. A. *Org. Lett.* **2000**, *2*, 1863. Jones, G. B.; Hynd, G.; Wright, J. M.; Purohit, A.; Plourde, G. W., II; Huber, R. S.; Mathews, J. E.; Li, A.; Kilgore, M. W.; Bubley, G. J.; Yancisin, M.; Brown, M. *J. Org. Chem.* **2001**, *66*, 3688.

(6) Magnus, P.; Fortt, S.; Pitterna, T.; Snyder, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 4986. Snyder, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 5367. Nicolaou, K. C.; Sorensen, E. J.; Discordia, R.; Hwang, C. K.; Minto, R. E.; Bharucha, K. N.; Bergman, R. G. *Angew. Chem., Intl. Ed. Engl.* **1992**, *31*, 1044. Haseltine, J. N.; Cabal, M. P.; Mantlo, N. B.; Iwasawa, N.; Yamashita, D. S.; Coleman, R. S.; Danishefsky, S. J.; Schulte, G. K. *J. Am. Chem. Soc.* **1991**, *113*, 3850. Semmelhack, M. F.; Gallagher, J. J.; Minami, T.; Date, T. *J. Am. Chem. Soc.* **1993**, *115*, 11618. Myers, A. G.; Dragovich, P. S. *J. Am. Chem. Soc.* **1992**, *114*, 5859. Takahashi, T.; Tanaka, H.; Matsuda, A.; Doi, T.; Yamada, H.; Matsumoto, T.; Sasaki, D.; Sugiura, Y. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3303. Takahashi, T.; Tanaka, H.; Matsuda, A.; Doi, T.; Yamada, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3299. Nicolaou, K. C.; Dai, W. M.; Tsay, S. C.; Estevez, V. A.; Wrasidlo, W. *Science* **1992**, *256*, 1172. Wender, P. A.; Zercher, C. K.; Beckham, S.; Haubold, E.-M. *Org. Chem.* **1993**, *58*, 5867. Hay, M. P.; Wilson, W. R.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3417. Hay, M. P.; Wilson, W. R.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2829. Denny, W. A.; Wilson, W. R. *J. Pharm. Pharmacol.* **1998**, *50*, 387.

(7) Nicolaou, K. C.; Zuccarello, G.; Riemer, C.; Estevez, V. A.; Dai, W.-M. *J. Am. Chem. Soc.* **1992**, *114*, 7360.

(8) Nicolaou, K. C.; Smith, A. L.; Wendeborn, S. V.; Hwang, C. K. *J. Am. Chem. Soc.* **1991**, *113*, 3106. Maier, M. E.; Greiner, B. *Liebigs Ann. Chem.* **1992**, 855. Choy, N.; Blanco, B.; Wen, J.; Krishan, A.; Russell, K. C. *Org. Lett.* **2000**, *2*, 3761. David, W. M.; Kumar, D.; Kerwin, S. M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2509. Basak, A.; Bdour, H. M.; Shain, J. C.; Mandal, S.; Rudra, K. R.; Nag, S. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1321. Jones, G. B.; Plourde, G. W. *Org. Lett.* **2000**, *2*, 1757. Clark, A. E.; Davidson, E. R.; Zaleski, J. M. *J. Am. Chem. Soc.* **2001**, *123*, 2650. Funk, R. L.; Young, E. R. R.; Williams, R. M.; Flanagan, M. F.; Cecil, T. L. *J. Am. Chem. Soc.* **1996**, *118*, 3291. Turro, N. J.; Evenzahav, A.; Nicolaou, K. C. *Tetrahedron Lett.* **1994**, *35*, 8089.

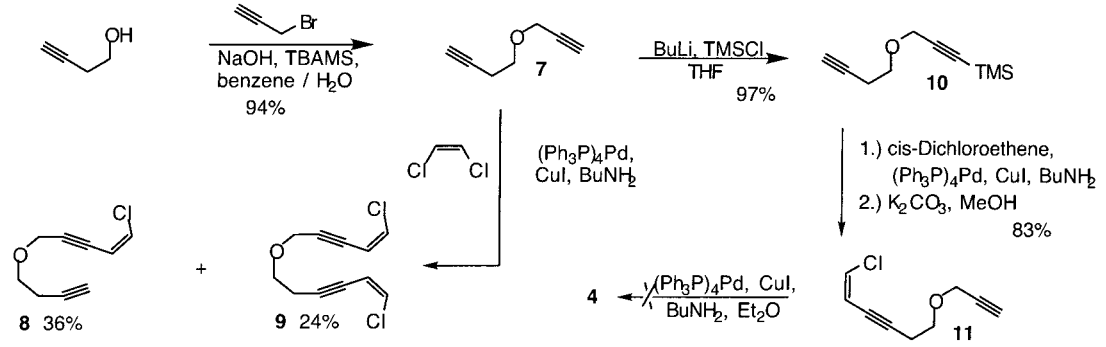
(9) For communication describing synthesis of **4** and **20** and bioassay of **20**, see: Jones, G. B.; Kilgore, M. W.; Pollenz, R. S.; Li, A.; Mathews, J. E.; Wright, J. M.; Huber, R. S.; Tate, P. L.; Price, T. L.; Sticca, R. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1971.

(10) Related heterocyclic enediynes: Sakai, Y.; Nishiwaki, E.; Shishido, K.; Shibuya, M.; Kido, M. *Tetrahedron Lett.* **1991**, *32*, 4363. Basak, A.; Khamrai, U. K. *Tetrahedron Lett.* **1996**, *37*, 2475. Basak, A.; Khamrai, U. K.; Mallik, U. *J. Chem. Soc., Chem. Commun.* **1996**, 749. Singh, R.; Just, G. *Tetrahedron Lett.* **1990**, *31*, 185. Shain, J. C.; Khamrai, U. K.; Basak, A.; *Tetrahedron Lett.* **1997**, *38*, 6067.

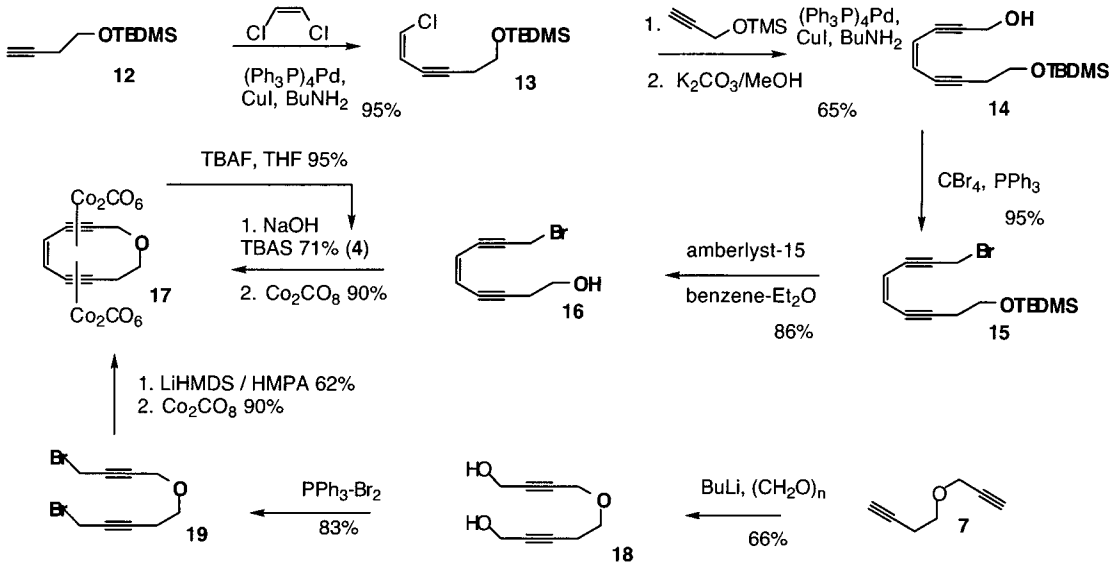
(11) Jones, G. B.; Warner, P. M. *J. Am. Chem. Soc.* **2001**, *123*, 2134.

(12) Jones, G. B.; Wright, J. M.; Plourde, G. W., II; Hynd, G.; Huber, R. S.; Mathews, J. E. *J. Am. Chem. Soc.* **2000**, *122*, 1937.

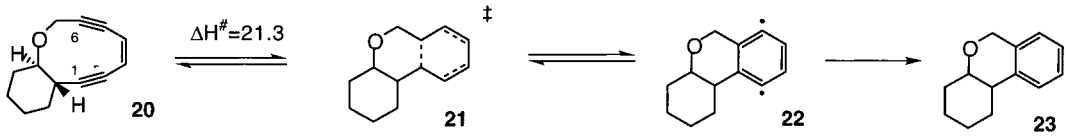
SCHEME 2. Attempted Pd-Mediated Routes to Oxa-Enediyne 4



SCHEME 3. Refined Routes to Oxa-Enediyne 4 and Its Cobalt Carbonyl Complex 17



SCHEME 4. BLYP Analysis of Bicyclic Oxa-Enediyne 20



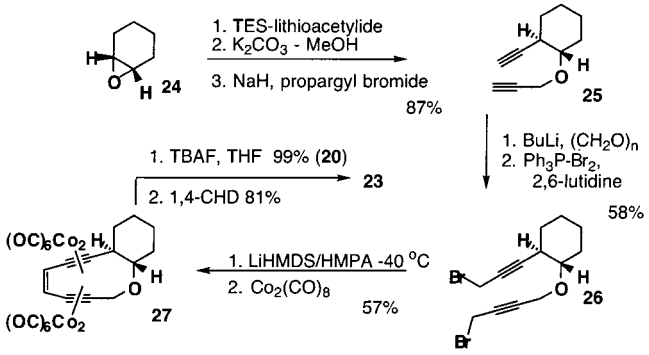
derivative **17**, which was stable for extended periods. As needed, smooth decomplexation was achieved by exposure to TBAF in THF.[13] Though this was effective, we wished to develop an even more direct route to **4** and succeeded; bis hydroxymethylation of **7** gave **18** in high yield, which was then followed by bis bromination to give **19**. Intramolecular carbenoid coupling/elimination followed by in situ complexation gave **17** in high yield.[14] Overall, the carbenoid route via dibromide **19** proved far superior in terms of both efficiency and cost-effectiveness, and could be conducted effortlessly on a multigram scale. With quantities of **4** in hand, Bergman cyclization was then investigated under controlled conditions; incubation of **4** at 37 °C in the presence of 1,4-cyclohexadiene (30 equiv) gave a nearly quantitative (>90%) yield of isochroman (**3**, X = O, R = H), presumably via the intermediacy of diyl **2**, X = O. Significantly, the half-life for the Bergman cycloaromatization was in agreement with that predicted by the computational studies (Table 1).

Having established a high yielding and economical route to oxa-enediynes of type **1**, we sought to incorporate the method in the syntheses of more complex systems which would allow us to gauge the impact of other effects on cycloaromatization. We envisioned that bicyclic oxa-enediyne **20** would provide an opportunity to gauge the effect of strain on the cycloaromatization and is also a target that might be accessible using the carbenoid method of synthesis. Accordingly, the BLYP analysis of **20** and its barrier to transition state **21** en route to diyl **22** was examined (Scheme 4). It was revealed that the barrier to cyclization was less than that for **4**, in accord with the shortened 1−6 distance; again, the transition state occurred earlier (Table 1).

(13) Jones, G. B.; Wright, J. M.; Rush, T. M.; Plourde, G. W., II; Kelton, T. F.; Mathews, J. E.; Huber, R. S.; Davidson, J. P. *J. Org. Chem.* **1997**, *62*, 9379.

(14) Jones, G. B.; Huber, R. S. U.S. Patent 5,436,361. Jones, G. B.; Huber, R. S.; Mathews, J. E. *J. Chem. Soc., Chem. Commun.* **1995**, 1791. Jones, G. B.; Huber, R. S. *Tetrahedron Lett.* **1994**, *35*, 2655. Hynd, G.; Jones, G. B.; Plourde, G. W.; Wright, J. W. *Tetrahedron Lett.* **1999**, *40*, 4481.

SCHEME 5. Carbenoid Coupling Route to Bicyclic Oxa-Enediyne

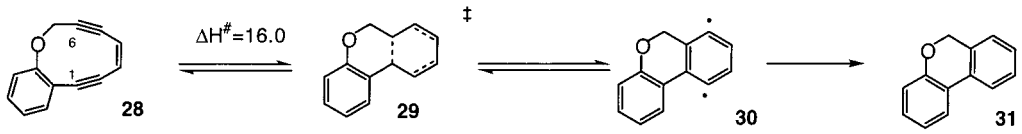


The synthesis of **20** commenced from cyclohexene oxide which was ring opened with lithiotriethylsilyl acetylide (Scheme 5). Acetylene desilylation proved more effective at this stage and was then followed by propargylation to give bis alkyne **25**. Hydroxymethylation, followed by bromination under mild conditions, then provided enediyne precursor **26**. The intramolecular carbenoid addition/elimination reaction was again employed, which gave a high yield of **20** directly. For practical purposes, however, the cyclization was followed by immediate enediyne protection using dicobalt octacarbonyl to give **27** that was stable at 25 °C for extended periods. Deprotection of **27** to liberate **20** was again easily achieved using TBAF. The half-life of **20** was found to be approximately 15 h at physiological temperature, in good empirical agreement with its calculated intra-acetylenic (c−d) distance of 3.346 Å. Incubation of **20** in the presence of 1,4-cyclohexadiene (30 equiv) led to conversion (>80%) to tricyclic product **23**,[15] presumably via the intermediacy of diradical **22**. As such, the process represents an efficient route to substituted isochromans.

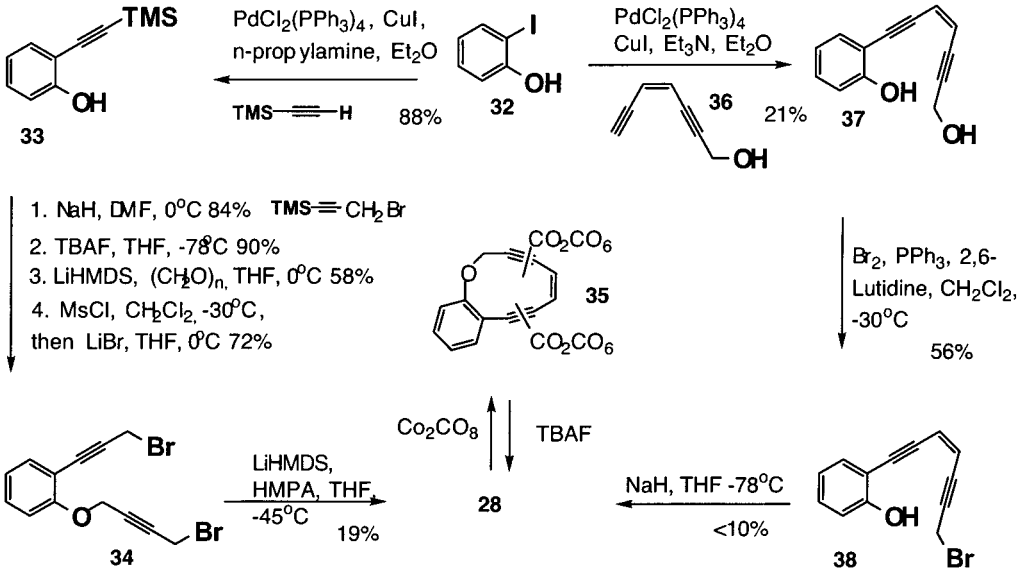
Finally, we elected to examine the contribution that arylation of the heteroatom would have on the cycloaromatization process. Enediyne **28** was identified as a target, in part, on the basis of computational analyses of its conversion to **29** (Table 1, Scheme 6). Surprisingly, the barrier proved minimal, although the relative effects of increased strain and electronics are not readily assessed. However, it became clear that any intended synthesis would require careful handling of the product. Two complimentary synthetic strategies were envisioned, either with a carbenoid coupling from **34** or, possibly, with a Williamson type etherification from **38** (Scheme 7). Both sequences began with Pd-mediated couplings of an alkynyl substrate to produce **33** and **37**. Substrate **33** was elaborated by etherification with trimethylsilyl propargyl bromide to give a bis TMS protected intermediate. Deprotection with TBAF followed by hydroxymethylation gave the corresponding diol, which was converted to dibromide **34** by employing mild bromination conditions with MsCl and LiBr. All attempts to prepare **28** and then conduct an in situ complexation to give **35** were unsuccessful, since the enediyne decomposed rapidly to give a complex mixture of products. However, trace quantities of **28** were obtained, allowing spectroscopic analysis and determination of its half-life, which, as predicted, was short (Table 1). The alternate method, achieved via bromination of **37** and then Williamson closure on **38**, likewise allowed isolation of trace quantities of **28**, but again, it proved impossible to prepare quantities of its derived complex **35**. Clearly, computational analyses had successfully predicted the thermal instability of this enediyne (Table 1).

One of the goals of our synthesis program is to explore new molecular targets for enediynes, and we have been

SCHEME 6. BLYP Analysis of Benzofused Oxa-Enediyne 28



SCHEME 7. Complimentary Routes to Oxa-Enediyne 28 and Its Cobalt Carbonyl Complex 35



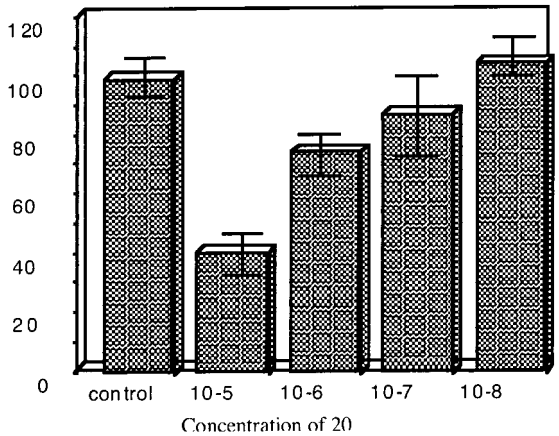


FIGURE 1. Protein (AhR) expression as a function of exposure to enediyne **20** in MCF-7 cells. Cells were incubated with either DMSO (control) or **20** at indicated concentrations (M) for 16 h. Cells were then harvested, lysed, and subjected to Western blot analysis.

actively developing enediyne mimics of transcription factors, including the estrogen receptor.[16] Another interesting target is the orphan receptor referred to as the aryl hydrocarbon receptor (AhR). The AhR is a ligand-activated transcription factor which is responsible for regulation of the production of a number of cytochromes P-450.[17] The highest affinity ligands for the AhR are oxygenated heterocycles, including chlorodibenzofuran and TCDD. Because of the structural similarity with oxoenediyne products **23/31**, we sought means to investigate the potential interaction of the parent enediynes with this receptor. A number of natural and synthetic enediynes are reported to possess proteolytic activity, and it is very likely that diradicals derived from the enediynes contribute to the observed in vitro effects by interacting with specific protein targets.[5] Because of the instability of oxoenediyne **28**, we opted to restrict our initial study of biological targets to enediyne **20**.

First, we conducted experiments to monitor AhR *expression* in MCF-7 breast cancer cells over a range of concentrations of **20**. The results showed a nearly 60% reduction in protein at 10^{-5} M and a correlation between enediyne and protein at lower concentrations (Figure 1).[18]

Though AhR degradation mediated by **20** might be expected to yield various protein fragments, no smaller fragments were identified on Western analysis (N.B. the antibody used is against the first 416 amino acids of this 805aa protein and is thus incapable of detecting a C-terminal fragment[19]), suggesting that the enediyne behaves in a manner similar to that of xenobiotics which degrade the AhR, including TCDD.[18] The current model for this degradation pathway involves AhR ubiquitination and subsequent breakdown via the 26S proteasome, resulting in complete conversion to small oligopeptides. Significantly, preformed **23** was incapable of inducing AhR degradation even at the highest concentration used (within standard error margin). Thus, it is reasonable to assume that a 1,4-diyl (or potentially an oxa-allene intermediate) derived from **20** is responsible for the observed degradation.

Since the enediyne **20** has a half-life of 15 h at physiological temperature, additional assays were performed at 10^{-5} M enediyne, with increased incubation times before analysis for protein. The results showed (Figure 2) that depletion levels off by 16 h of incubation and AhR levels do not recover.

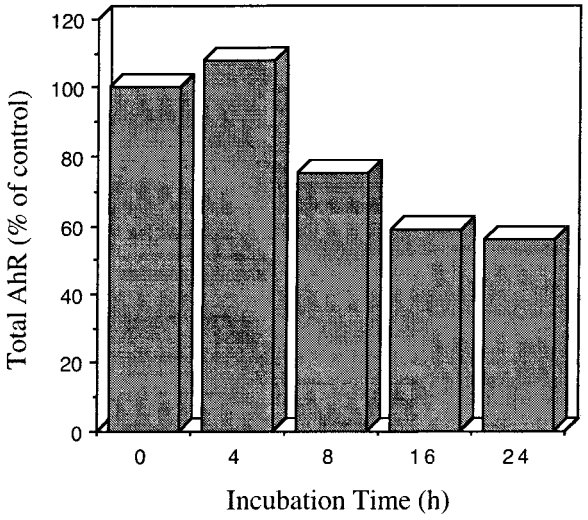


FIGURE 2. Effect of incubation time in the degradation of AhR by enediyne **20**. Triplicate plates of MCF-7 cells were treated with 2×10^{-5} M **20** for 4, 8, 16, or 24 h. Cells were then harvested, resolved by SDS−PAGE, blotted, and stained for AhR and actin.[19]

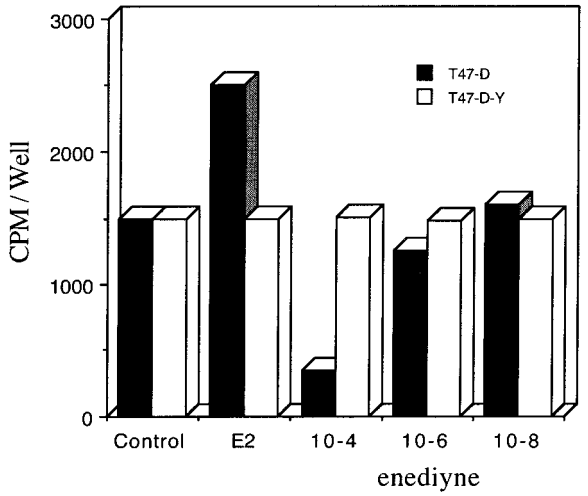


FIGURE 3. Antiproliferative effects of **20** versus T47-D and T47-D-Y breast cancer cells. Cells received estradiol (E2) at 1×10^{-9} M or enediyne **20** at indicated concentrations (M). All cells received ^{3}H thymidine (1×10^{-6} M); after 6 h, nucleii were recovered and incorporated ^{3}H thymidine was measured.

(15) Negishi, E.; Nguyen, T.; O'Connor, B.; Evans, J. M.; Silveira, A. *Heterocycles* **1989**, *28*, 55.
(16) Jones, G. B.; Purohit, A.; Wright, J. W.; Hynd, G.; Wyatt, J.; Swamy, N.; Ray, R. *Tetrahedron Lett.* **2001**, *42*, 8579. Jones, G. B.; Huber, R. S.; Mathews, J. E.; Li, A. *Tetrahedron Lett.* **1996**, *37*, 3643.
(17) Chaloupka, K.; Krishnan, V.; Safe, S. *Carcinogenesis* **1992**, *13*, 2233. Wilhelmsson, A.; Whitelaw, M. L.; Gustafsson, J.-A.; Poellinger, L. *J. Biol. Chem.* **1994**, *269*, 19028. Merchant, M.; Morrison, V.; Santostefano, M.; Safe, S. *Arch. Biochem. Biophys.* **1992**, *298*, 389.
(18) Pollenz, R. S. *Mol. Pharmacol.* **1996**, *49*, 391. Pollenz, R. S.; Santostefano, M. J.; Klett, E.; Richardson, V.; Necela, B.; Birnbaum, L. S. *Toxicol. Sci.* **1998**, *42*, 117. Davarions, N. A.; Pollenz, R. S. *J. Biol. Chem.* **1999**, *274*, 28708. Pollenz, R. S.; Barbour, E. R. *Mol. Cell. Biol.* **2000**, *20*, 6094.
(19) Holmes, J.; Pollenz, R. S. *Mol. Pharmacol.* **1997**, *52*, 202.

Since the enediyne clearly has an impact on receptor levels, the ramifications of such degradation in the cell cycle were then studied. It has been reported that ligands with an affinity for AhR cause a decrease in nuclear estrogen receptor (hER) levels and, as such, can behave as antiestrogens.[17] To investigate this, we studied the cytotoxicity of **20** using two breast cancer cell lines: one that is known to contain both AhR and functional human estrogen receptor (T-47D) and one that is deficient of the hER (T-47D-Y). Using a conventional thymidine uptake assay, while the T-47D-Y cells grew at rates apparently unaffected by enediyne concentrations 10^{-8} through 10^{-4} M, *the T-47D cells were sensitive, with agent* ***20*** *proving cytotoxic at concentrations as low as* 10^{-6} *M and causing 83% growth inhibition at* 10^{-4} *M* (Figure 3). As expected, when stimulated with estradiol (E2, 10^{-9} M) only the T-47D cell line showed enhanced proliferation, suggesting that the observed antiproliferative effects of **20** may be due to its interaction with the transcriptional machinery.

Monitoring for AhR protein in MCF-7 cells showed a clear correlation between enediyne concentration and the depletion of cellular protein (Figure 1), and it is tempting to speculate that this impacts cell proliferation (Figure 3). Using affinity-driven synthesis, it may be possible to produce oxa-enediynes with an even higher activity than that of the present example, as exemplified by our recent findings on the degradation of the estrogen receptor by designed estramycins.[16] Such agents may be useful as probes of transcriptional events or even as antitumoral agents in their own right.[20]

In summary, a series of thermally activated oxa-enediynes were produced using an efficient carbenoid coupling/elimination procedure. The impact of the heteroatom on enediyne cycloaromatization showed good correlation with computational analyses. Since even one of the simple members of this class of enediynes is capable of inducing receptor degradation, it is likely that more advanced members can be designed for use as biological modulators. It is expected that the interplay of chemical synthesis, computational chemistry, and bioassay will permit identification of optimal candidates for in-depth evaluation.

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Supporting Information Available: Synthetic procedures and spectroscopic data for the preparation of all compounds and calculation coordinates for compounds **4**, **20**, and **28** (23 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) For enediyne−estrogen synthesis, see: Wang, J.; DeClercq, P. J. *Angew. Chem., Intl. Ed. Engl.* **1995**, *34*, 1749.