Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 24, pp. 2925-2928, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00443-9

THE EFFECT OF PROPARGYLIC SUBSTITUTION ON THE ACTIVATION AND AROMATIZATION OF ENEDIYNES

Mark D. Wittman*, John F. Kadow, Kahnie Pham, Kinwa Leung,
Harold A. Mastalerz, Dolatrai M.Vyas[1],
William C. Rose, Wyle Solomon and Nada Zein[2]

Bristol-Myers Squibb Pharmaceutical Research Institute
[1] Division of Chemistry, 5 Research Pkwy, Wallingford, CT 06492-7660
[2] Oncology Drug Discovery, P. O. Box 4000, Princeton, NJ 08543-4000

Abstract: The thiol activation and aromatization of bicyclo[7.1.0]enediynes was found to be dependent of the nature of the propargyl substituent. These effects are correlated to antitumor activity.

The structural novelty and potent cytotoxicity of the natural products esperamicin, calicheamicin and dynemicin have served to focus interest in this growing class of enediynes.[1] These natural products undergo an intramolecular triggering event which leads to diradical formation at physiological pH and temperature. We have been involved in the search for synthetic mimics of these natural products which would incorporate different mechanisms for triggering. As a result of this program BMY-46108 (1'),[2] which is activated by an intermolecular mechanism, was identified as a lead structure.[3] During the course of this work we have been interested in uncovering the structural features of these natural products that enable facile activation and cycloaromatization. The following study of bicyclic enediynes bearing different propargylic substituents was undertaken to correlate thiol activation and ease of cycloaromatization with antitumor activity *in vitro* and *in vivo*.

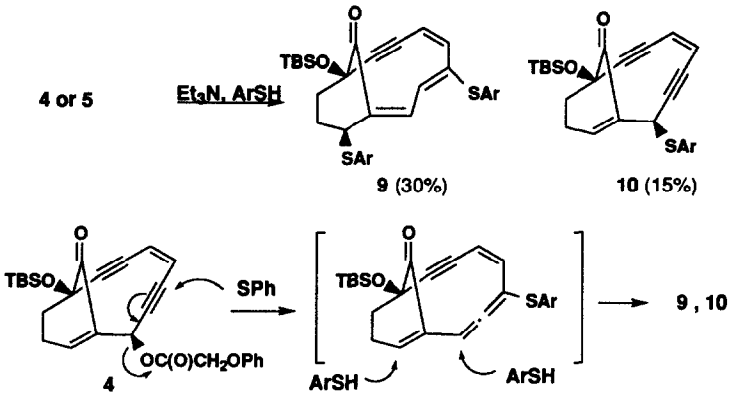
Several tertiary silyl enediyne analogs with different propargylic substituents were prepared for study. We chose to work with the silyl ethers because they were more stable than the unprotected enediynes but did not behave any differently than the unprotected enediynes we examined.

1 X=OH, Y=H
2 X=H, Y=H
3 X=H, Y=OH
4 X=OC(O)CH_2OPh, Y=H
5 X=F, Y=H

6 X=OH, Y=H (55%)
7 X=H, Y=H (47%)
8 X=H, Y=OH (29%)

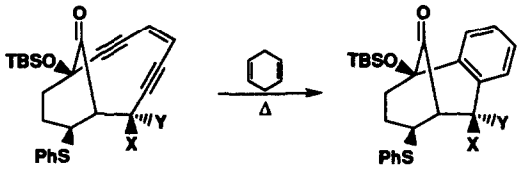
The enediyne **1**,[3] deoxygenated enediyne **2**[4] and epimeric alcohol **3**[5,6] were all prepared by total synthesis. The phenoxyacetyl ester **4** was prepared from **1** by acylation with phenoxyacetyl chloride and pyridine in THF to give the ester in 92% yield. Fluoride **5** was prepared in 74% yield by reacting **1** with DAST at -78 °C.

Enediyne **1** and the deoxygenated analog **2** were stirred with benzenethiol and triethylamine (THF, 25°C, 30 minutes) to give the Michael adducts **6** and **7** in 55% and 47% yield respectively (Stereochemistry determined by NOE measurements). The epimeric alcohol **3** reacted very sluggishly under identical conditions requiring greater than 9 hours to give **8** in 29% yield. The slow reaction of enediyne **3** is most likely due to the steric repulsion of the thiophenol and the hydroxyl which are both pseudoequatorial in the low energy twist boat conformation of thiol adduct **8**. When the analogs with electron withdrawing substituents, **4** and **5**, were treated with thiol in THF at 25°C, the expected Michael adducts were not obtained but rather the same inseparable mixture of two new compounds was obtained. By treating ester **4** with 4-methoxybenzenethiol and triethylamine the mixture could be resolved and the major product identified as triene **9** (30%) and the minor component identified as sulfide **10** (15%), in which the β-stereochemistry of the propargylic ester was retained.[7] The formation of **9** and **10** probably arise from a common allenyl intermediate resulting from S_N2' attack on the acetylene of the enediyne displacing the propargylic substituent. This allene could then undergo a second attack of thiol at two possible sites. The β-stereochemistry observed in **10** results from attack on the allene from the less hindered face.



Next we examined the conditions necessary to achieve cycloaromatization of enediynes **7** and **8** relative to **6**. Enediyne **6** was consumed within 1 hour at 37 °C in

cyclohexadiene to give the aromatized core **11** in 47% yield. When the deoxygenated enediyne **7** was heated at 37°C no aromatization was observed. In contrast to **6**, **7** required heating at 80 °C for 72 hours to give a 58% yield of **12**.[4] Interestingly, when the epimeric alcohol **8** was heated at 37°C only a retro Michael reaction was observed giving the enone **3**. Cyclization to **13** was achieved in 30% yield by heating at 80°C for 48 hours in the presence of 12 equivalents of benzenethiol and three equivalents of triethylamine. Analysis of the reaction by TLC revealed a small amount of enediyne **8** present during the reaction indicating an equilibrium between **8** and **3** favoring **3**. The reasons for the accelerated Bergman cyclization of the propargylic alcohol analog **6** relative to **7** are unclear at the present time since ring strain in both systems should be equal and calculations have failed to uncover electronic effects on the cycloaromatization. The observation that the stereochemistry of the propargylic hydroxyl is important is equally puzzling.



6 X=OH, Y=H
7 X=H, Y=H
8 X=H, Y=OH

11 X=OH, Y=H (47%)
12 X=H, Y=H (58%)
13 X=H, Y=OH (30%)

Enediynes **1-5** were desilylated using 48% HF in acetonitrile to give analogs **1'-5'** and evaluated both *in vitro* and *in vivo*. Cytotoxicity testing was done against the HCT-116 human colon tumor cell line[8] and *in vivo* testing performed in mice implanted with Madison 109 lung carcinoma (M109).[9] The compounds were administered in a single injection intraperitoneally (ip), at several dose levels, five days after ip tumor implantation.

Table I. Biological Activity of Desilylated Enediynes

	IC_{50} HCT 116[a]	%T/C M109 (mg/kg/inj)[b]
Esperamicin A_1	1.0×10^{-12}	143-162(0.04-0.05)
1'	1.0×10^{-7}	144 (16)
2'	$>2.0 \times 10^{-6}$	not tested
3'	1.0×10^{-6}	115 (128)
4'	1.9×10^{-7}	143 (64)
5'	2.4×10^{-8}	112 (32)

a) Molar concentration at which 50% of the cells have been killed
b) mean survival time of a treated group over a control group multiplied by 100. A value of 125 or greater is considered active.

The reason for the potent *in vitro* activity of the fluoro analog **5'** is unknown but this analog was inactive when tested *in vivo*. The unexpected *in vivo* activity of **4'** may be the result of esterase hydrolysis of some of **4'** to **1'** hence the higher dose required to achieve an antitumor effect similar to that achieved by administration of **1'**.[10] The lack of activity of enediyne **3'**, despite having achieved maximum tolerated dose levels, correlates with the higher temperature needed to effect its cycloaromatization. Although **2'** was not tested in the M109 model it has been shown to be less active than **1'** in other *in vivo* models as one would expect from the elevated temperature required for cycloaromatization.

In conclusion, this study demonstrates the role of the propargylic substituent in determining the reaction pathway of simple bicyclic enediynes with thiols and the temperature at which they cycloaromatize. First, we have found that enediynes equipped with an enone trigger and an electron withdrawing group at the propargylic position suffer from significant attack on the enediyne inactivating them to cycloaromatization. Secondly, while we currently do not understand the reason(s) why the cycloaromatization of enediyne **6** occurs at a much lower temperature than that required for **7** or **8**, the presence of a propargylic hydroxyl of the correct stereochemical configuration is necessary for the activation and cycloaromatization of enediynes under physiological conditions.

Acknowledgements The authors thank P. Magnus for helpful discussions and J. Golik and S. Huang for help in determining the structure of compounds **10** and **11.**

References and Notes

1. a) Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T.W. *J. Am. Chem. Soc.* **1987**, *109*, 3462. b) Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Seigel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464. c) Konishi, M.; Ohkuma, H.; Tsuno, T.; Oki, T.; VanDuyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1990**, *112*, 3715.
2. Zein, N.; Solomon, W.; Casazza, A.M.; Kadow, J.K.; Krishnan, B.S.; Tun, M. M.; Vyas, D.M.; Doyle, T.W. *Bioorg. Med. Chem. Letts.*, **1993**, *3*, 1351.
3. Kadow, J. F.; Tun, M. M.; Vyas, D. M.; Wittman, M. D.; Doyle, T. W. *Tetrahedron Letts.*, **1992**, *33*, 1423.
4. Pham, K.; Wittman, M. D. Unpublished results. See Magnus, P.; Lewis, R. *J. Am. Chem Soc.* **1988**, *110*, 6921.
5. Mastalerz, H.; Lueng, K. unpublished results.
6. All new compounds identified by ^{1}H and ^{13}C NMR, IR and MS.
7. Stereochemistry determined by NOE between the enone H and the propargylic H.
8. Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.;Seniff, D.; Boyd, M.R. *Cancer Research* **1988**, *48*, 4827-4833.
9. Rose, W. C. *Cancer Treat. Rep.* **1986**, *65*, 299. Rose, W. C.; Kamei, H.; Nishiyama, Y.; Bradner, W. T.; Stringfellow, D. A. *Investigational New Drugs* **1990**, *8*, 7.
10. For esterase acylation of the proparglic position see Yamashita, D.S.; Rocco, V.P.; Danishefsky, S.J. *Tetrahedron Letts.*, **1991**, *32*, 6667.

(*Received in USA* 26 *September* 1994; *accepted* 18 *November* 1994)