The Journal of
Organic
Chemistry

VOLUME 58, NUMBER 22 OCTOBER 22, 1993

Communications

A Photochemically Triggered DNA Cleaving Agent: Synthesis, Mechanistic and DNA Cleavage Studies on a New Analog of the Antitumor Antibiotic Dynemicin

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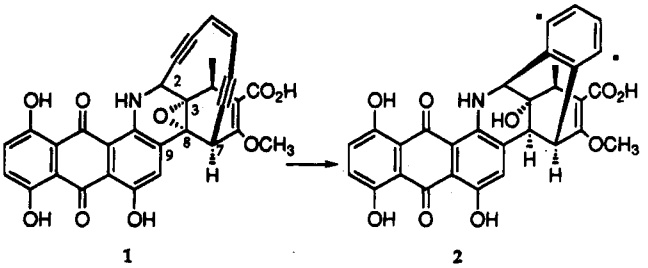
Received July 7, 1993•

Summary: An improved procedure for the fluoride-induced desilylative condensation of a silyl-protected alkyne with an aldehyde provides the first example of dynemicin analogs (11) that can be activated by photochemical deprotection on nitrogen, leading to DNA cleavage under neutral conditions in the absence of chemical activators.

Dynemicin (1) along with neocarzinostatin, esperamicin, calicheamicin, kedarcidin, and C-1027 constitute a fascinating class of naturally occurring antitumor antibiotics whose synthesis, novel mode of action, and medicinal potential have attracted widespread interest.[2,3] The



pronounced cytotoxic activity of these compounds is attributed to their ability to undergo chemically induced cycloaromatization to a diradical (diyl)[2d,f] which initiates DNA cleavage through hydrogen abstraction at deoxyribosyl sites.[2,3] For dynemicin, this cycloaromatization is induced by reduction of its anthraquinone subunit,[4] which by increasing electron density at C-9 leads to heterolysis of the proximate oxirane bond. As a consequence, C-2 and C-7, once confined to an antilike arrangement, can

• Abstract published in *Advance ACS Abstracts*, September 15, 1993.

(1) (a) Merck Sharp and Dohme Research Laboratories Academic Development Program Postdoctoral Fellow. (b) National Science Foundation Predoctoral Fellow. (c) Support from the German Academic Exchange Service for E.-M.H. is gratefully acknowledged. This work was presented in part at the 32nd National Organic Chemistry Symposium (June 1991), Minneapolis, MN and at the 205th National ACS Meeting (March 28–April 2, 1993), Denver, CO.

(2) For lead references see the following. Dynemicin: (a) Konishi, M.; Ohkuma, H.; Tsuno, T.; VanDuyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1990**, *112*, 3715. Neocarzinostatin: (b) Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331. Esperamicin: (c) Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3461. (d) Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *Ibid.* 3462 Calicheamicin: (e) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C.; Morton, G. O.; Borders, D. B. *Ibid.* 3463. (f) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *Ibid.* 3466. Kedarcidin: (g) Leet, J. E.; Schroeder, D. R.; Hofstead, S. J.; Golik, J.; Colson, K. L.; Huang, S.; Klohr, S. E.; Doyle, T. W.; Matson, J. A. *Ibid.* **1992**, *114*, 7496. C-1027. (h) Minami, Y.; Yoshida, K.; Azuma, R.; Saeki, M.; Otani, T. *Tetrahedron Lett.* **1993**, *34*, 2633. (i) Yoshida, K.; Minami, Y.; Azuma, R.; Saeki, M.; Otani, T. *Ibid.* **1993**, *34*, 2637.

(3) (a) For a review of natural and nonnatural enediyne compounds, see: Nicolaou, K. C.; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *42*, 1449. For lead references related to dynemicin modeling and analog synthesis see: (b) Langley, D. R.; Doyle, T. W.; Beveridge, D. L. *J. Am. Chem. Soc.* **1991**, *113*, 4395. (c) Wender, P. A.; Kelly, R. C.; Beckham, S.; Miller, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8835. (d) Nicolaou, K. C.; Dai, W.-M.; Tsay, S.-C.; Estevez, V. A.; Wrasidlo, W. *Science* **1992**, *256*, 1172. (e) Wood, J. L.; Porco, J. A., Jr.; Taunton, J.; Lee, A. Y.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 5898. (f) Tokiwa, Y.; Miyoshi-Saitoh, M.; Kobayashi, H.; Sunaga, Rie; Konishi, M.; Oki, T.; Iwasaki, S. *J. Am. Chem. Soc.* **1992**, *114*, 4107. (g) Magnus, P.; Fortt, S. M. *J. Chem. Soc., Chem. Commun.* **1991**, 544. (h) Maier, M. E.; Brandstetter, T. *Tetrahedron Lett.* **1992**, *33*, 7511. For earlier syntheses of enediyne compounds, see ref 3a.

(4) Sugiura, Y.; Shiraki, T.; Konishi, M.; Oki, T. *Proc. Nat. Acad. Sci. U.S.A.* **1990**, *87*, 3831.

(5) (a) Semmelhack, M. F.; Gallagher, J.; Cohen, D. *Tetrahedron Lett.* **1990**, *31*, 1521. (b) Snyder, J. P.; Tipsword, G. E. *J. Am. Chem. Soc.* **1990**, *112*, 4040.

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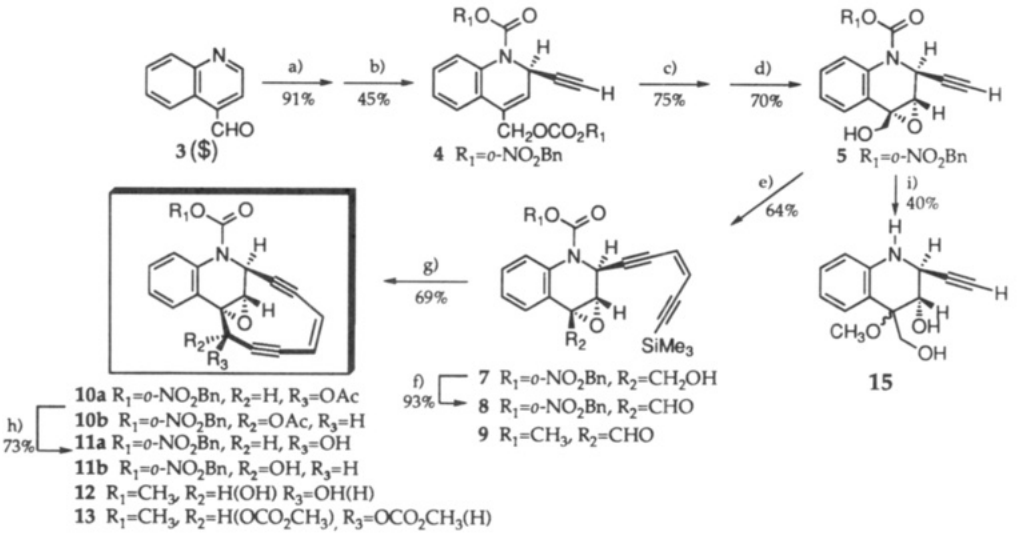
Scheme I[a]



[a] Key: (a) $NaBH_4$, MeOH, room temperature, 2 h; (b) 2.5 equiv of HCCMgBr and then 3.0 equiv of $O_2NC_6H_4CH_2OCOCl$, THF, 0° C, 30 min; (c) K_2CO_3, MeOH, room temperature, 1 h; (d) *m*-CPBA, CH_2Cl_2, 0 °C, 20 min; (e) 0.15 equiv of $Pd(PPh_3)_4$, 0.4 equiv of CuI, 3.0 equiv of *n*-$BuNH_2$, *cis*-ClCH=CHCCTMS (**6**), THF, room temperature, 2 h; (f) Dess–Martin periodinane, CH_2Cl_2, room temperature, 2 h; (g) CsF, Ac_2O, CH_3CN, room temperature, 3 h; (h) $Ba(OH)_2$, MeOH, room temperature, 20 min; (i) Hanovia/Pyrex, 1 equiv of NH_4Cl, THF/MeOH 4:1, 4 h.

assume a gauche-like conformation, allowing for facile cyclization of the enediyne moiety to a diyl (**2**).[3b,c,5]

We recently reported a concise route (seven steps) to dynemicin analogs based on an intramolecular desilylative alkyne addition to a carbonyl group,[6] a new method for the synthesis of cyclic enediynes that avoids the use of strongly basic conditions involved in the corresponding alkynylide condensations.[7] While these analogs effect cleavage of plasmid DNA,[8] the bimolecular requirement for their activation, which also applies to other analogs and the natural product leads,[9] complicates if not precludes kinetic analysis of the activation process and the as yet unaddressed goal of direct spectroscopic study of the proposed diyl intermediates. In order to address these important mechanistic issues as well as to achieve greater control over the activation process and consequently over the performance of these compounds as cleavage reagents or drugs, we have examined strategies that would allow for the photochemical activation of our readily accessible analogs. Toward this end, we report the synthesis of novel dynemicin analogs (**11**) which are activated toward cycloaromatization in the absence of additives and under neutral conditions by irradiation at wavelengths >300 nm. This study also describes a new modification of the intramolecular desilylative condensation, which greatly improves the utility of this method for the construction of enediyne based DNA cleaving agents.

The synthesis of analogs **11** (Scheme I), modeled after our preparation of a related analog,[6] started with reduction of commercially available quinoline carboxaldehyde (**3**) with $NaBH_4$ and treatment of the resulting alcohol with ethynylmagnesium bromide and *o*-nitrobenzyl chloroformate[10] to provide alkyne **4**. Hydrolysis of the carbonate moiety with K_2CO_3 in MeOH gave the corresponding carbinol which upon face-selective epoxidation afforded oxirane **5**. Pd(0)-catalyzed cross coupling of *cis*-chloro eneyne **6** with alkyne **5**[7c] yielded the highly functionalized alcohol **7**, from which aldehyde **8** was obtained by Dess–Martin periodinane oxidation.[11] The key closure reaction was affected by treatment of aldehyde **8** with CsF in dry MeCN but the desired alcohols **11** were formed in only 19% yield. Further study of this process revealed that the corresponding urethane **9**, which incorporates a more robust protecting group, behaves similarly, giving closure products **12** again in low yield. In this case, however, a clue bearing on the efficiency of the closure reaction was

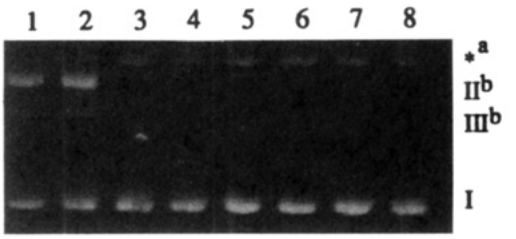


Figure 1. DNA (60 μm) nicking by **11** (210 μm) using irradiation from a 450-W Hanovia medium-pressure mercury arc lamp through a Pyrex filter for 15 min and analyzed by agarose gel electrophoresis: lane 1 (**11**, pH 6, *hν*); lane 2 (**11**, pH 7.5, *hν*); controls: lane 3 (no **11**, pH 6, *hν*); lane 4 (no **11**, pH 7.5, *hν*); lane 5 (**11**, pH 6, no *hν*); lane 6 (**11**, pH 7.5, no *hν*); lane 7 (no **11**, pH 6, no *hν*); lane 8 (no **11**, pH 7.5, no *hν*). Key: (a) This band is claimed to be a DNA catenane by the suppliers(Sigma). (b) These bands are identical to the bands derived from cleavage of pBR322 with NCS chromophore. In addition, the band corresponding to double-stranded cleavage (form III) is identical to that obtained by cleavage of pBR322 with BamH I.

(6) Wender, P. A.; Zercher, C. K. *J. Am. Chem. Soc.* **1991**, *113*, 2311.
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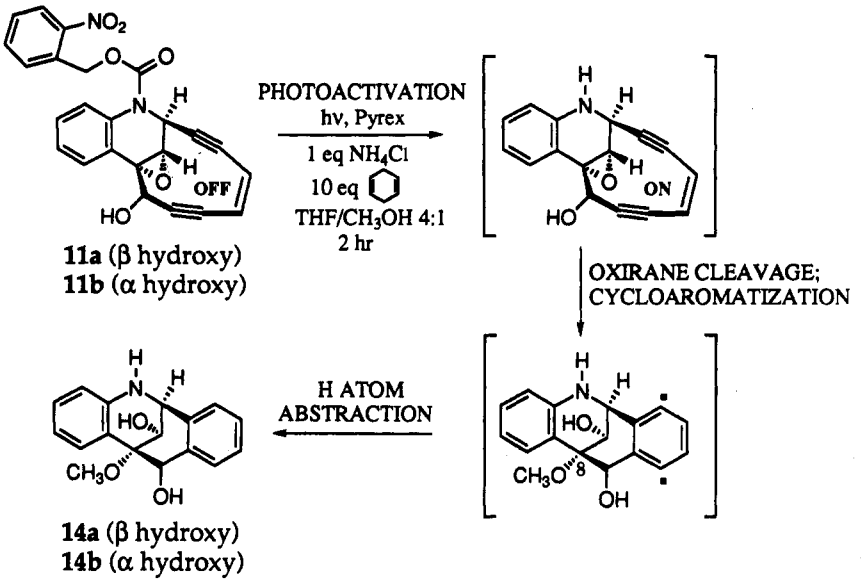
(8) Wender, P. A.; Zercher, C. K.; Beckham, S. Stanford University, unpublished results.
(9) For studies on the photo-induced activation of dynemicin and a dynemicin analog, see: (a) Shiraki, T.; Sugiura, Y. J. *Biochemistry* **1990**, *29*, 9795. (b) Nicolaou, K. C.; Dai, W. M.; Wendeborn, S. V.; Smith, A. L.; Torisawa, Y.; Maligres, P.; Hwang, C. K. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1032.
(10) *o*-Nitrobenzyl chloroformate was prepared in high yield by slow addition of triethylamine to a solution of the corresponding alcohol and triphosgene in ether.
(11) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.

Scheme II



obtained from the isolation of carbonates **13** as byproducts. Since **13** would arise from reaction of an alkoxide intermediate with the carbamate group of starting material or product, a process that would induce their decomposition and lower the reaction yield, the addition of an alkoxide trapping agent was expected to suppress this undesired reaction path. In accord with this plan, incorporation of acetic anhydride in the reaction more than tripled the efficiency of the closure, affording acetates **10** in 69% yield. The diastereoselectivity of this reaction(1:2 α/β) is the same as that observed in the absence of an alkoxide trap. This desilylative condensation offers several advantages for enediyne analog synthesis as it avoids the need for a separate alkyne deprotection step, effects closure in the absence of strong base, and allows protection or functionalization of the addition product in one operation. As needed, removal of the acetate moiety can be efficiently achieved by treatment of **10** with $Ba(OH)_2$ in MeOH.

The viability of the photoactivation process for diyl generation was initially supported by the finding that epoxide **5**, while hydrolytically stable in the absence of light, undergoes rapid hydrolysis to afford diol **15** (Scheme I) when irradiated at >300 nm. In a similar fashion, independent irradiation of a dilute solution of **11a** and **11b** in THF/MeOH (4:1) through a Pyrex filter with a 450-W Hanovia lamp gave the cycloaromatized product **14a** or **14b**, respectively, in 30–40% yield along with intractable, oligomeric material.[12] The facile formation of cycloaromatized products under these conditions is consistent with an initial photoinduced cleavage of the carbamate moiety to the free amine.[13] Analogous to reduction of the anthraquinone subunit of dynemicin, this photodeprotection serves to increase electron density proximate to the oxirane bond, thereby facilitating its hydrolytic opening as required for subsequent cycloaromatization of the enediyne. Cycloaromatization of **11a** and **11b** can also be induced by treatment of these compounds with 1 equiv of HCl, generated *in situ* (THF/MeOH) by the addition of 1 equiv of acetyl chloride. As a prelude to further mechanistic studies, the performance of these compounds as DNA cleaving agents was tested with circular, supercoiled DNA. Importantly, when **11a** (200–500 mM) was incubated with pBR322 DNA (60 mM/bp) and the mixture was irradiated at >300 nm, the formation of both form II and form III DNA cleavage products was observed (Figure 1).[14] The efficiency of this cleavage correlated with irradiation time. Moreover, no cleavage occurred in the absence of light or when the DNA was irradiated in the absence of **11**.

In summary, analogs (**11**) of the antitumor antibiotic dynemicin have been prepared through an intramolecular desilylative condensation that avoids the use of strong base and the need for a separate deprotection step for silyl-protected alkynes. This analog can be induced to undergo cycloaromatization under neutral conditions in the absence of additives through the use of a photochemically activatable switch. This is the first example of an dynemicin analog that can be activated by photochemical deprotection of nitrogen. Photoactivation of this analog in the presence of DNA leads to the formation of both form II and form III cleavage products. The ability to control the timing of activation and to generate diyls in the absence of additives under neutral conditions opens opportunities for the use of these analogs as mechanistic probes, reagents for nucleic acid modification, and potentially site-selective chemotherapeutic agents. Studies in these areas are in progress.

Acknowledgment. This research was supported by a grant (CA31845) from the National Institutes of Health. Mass spectra were provided by the Mass Spectrometry Facility, University of California—San Francisco, supported by the NIH Division of Research Resources.

Supplementary Material Available: IR, NMR, and mass spectrometry data for compounds, **5**, **7**, **8**, **11a,b**, and **14a** and additional DNA cleavage controls (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(12) The yields for this process have not been optimized and do not necessarily relate to the efficiency of DNA cleavage. Activation in a flask may be complicated by bimolecular processes that would not be operative when these molecules are associated with DNA.

(13) McCray, J. A.; Trentham, D. R.; Reid, G. P.; Walker, J. W. *J. Am. Chem. Soc.* **1988**, *110*, 7170.

(14) Characterization of cleavage products, efforts to determine whether double strand cleavage is attributable to one double cleavage event or two (or more) single cleavage events, and studies on the influence of DNA recognition elements on the cleavage selectivity are in progress.