Synthesis and DNA Cleavage Study of a 10-Membered Ring Enediyne Formed via Allylic Rearrangement

Wei-Min Dai,*,† Kin Chiu Fong,† Chi Wai Lau,† Ling Zhou,‡ Wataru Hamaguchi,‡ and Sei-ichi Nishimoto‡

Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China, and Department of Energy and Hydrocarbon Chemistry, Graduate School of Engineering, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan

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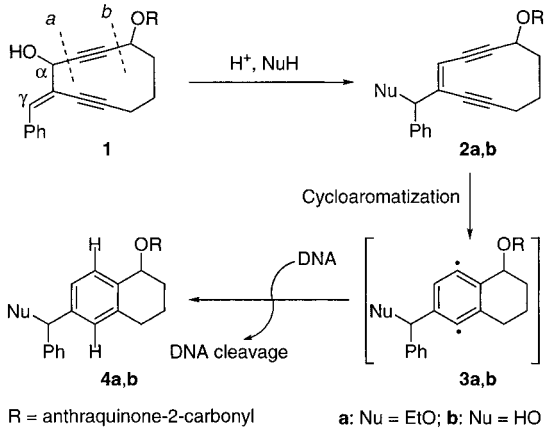
The naturally occurring enediynes[1] represent a novel class of antitumor antibiotics that feature a (*Z*)-hex-3-ene-1,5-diyne moiety constrained in a 9- or 10-membered ring. Coupled with other structural domains responsible for drug activation and delivery, the enediyne antitumor antibiotics present challenging targets for chemical synthesis.[1a−c] Stimulated by the intriguing mechanism of action and promising biological activity, extensive chemical and biological investigations on enediynes have been carried out during the past decade.[1] It is known that cycloaromatization[2] of enediynes such as **2** will give the diradical species **3**, which can damage DNA through hydrogen atom abstraction from the deoxyribose residue (Scheme 1).[1a,d,e,3] This event is regarded as the origin of the biological activity of enediynes. However, the extreme lability of simple 9- or 10-membered ring enediynes presents an obstacle for the development of synthetic enediyne drugs. In our recent work, we have established an efficient methodology for conversion of the thermally stable 1,2-diynyl-substituted allyl alcohols into acyclic enediynes by rearrangement of the allylic double bond.[4] A relatively unstrained 11-membered ring enediyne was synthesized similarly.[4b] Our methodology is conceptually related to the intramolecular allylic rearrangement proposed for the action of artifacts of the maduropeptin chromophore[5,6] and represents one of the emerging strategies[7] for enediyne prodrug design and synthesis. In this paper, we disclose the synthesis and DNA cleavage activity of the 10-membered ring enediyne **2**, formed in situ from precursor **1** via the allylic rearrangement (Scheme 1).

Scheme 1



In our previous studies,[4] we realized that the phenyl group attached at the exocyclic double bond of the precursor is essential for successful conversion into the enediyne. Thus, we decided to synthesize compound **1** according to the bond disconnection b shown in Scheme 1. The previously used pathway (a) for 11-membered ring formation[4b] failed to give 10-membered ring product. Starting from the known compound **5** readily available from α-bromocinnamaldehyde in three steps,[4a] alcohol **6** was prepared by protection of the allylic hydroxyl group (DHP, PPTS, CH_2Cl_2, 20 °C, 4 h) and subsequent removal of the silyl groups (*n*-Bu_4NF, THF, 20 °C, 4 h) in 66% yield (Scheme 2). Oxidation of **6** using PDC (4 Å MS, CH_2Cl_2, 20 °C, 1 h) gave aldehyde **7**, which cyclized in the presence of LDA−$CeCl_3$ (2 equiv each, THF, HMPA, −78 °C, **7** → **8**) under high dilution conditions (0.01 M). Compound **8** was obtained in 10% yield from **7** along with a byproduct (20%) resulting from an intermolecular addition of the lithium acetylide of **7**. At this stage, we envisaged the introduction of a DNA-recognition moiety into **8** using the propargylic hydroxyl group as the tethering point. Ester **9** was synthesized from **8** and anthraquinone-2-carboxylic acid under the DCC−DMAP conditions (CH_2Cl_2, 20 °C, 12 h) in 60% yield. The THP ether in **9** was then unmasked using PPTS in MeOH (20 °C, 24 h) to furnish alcohol **1** in 58%

* To whom correspondence should be addressed. Fax: int. + 2358-1594. E-mail: chdai@ust.hk.
† The Hong Kong University of Science and Technology.
‡ Kyoto University.

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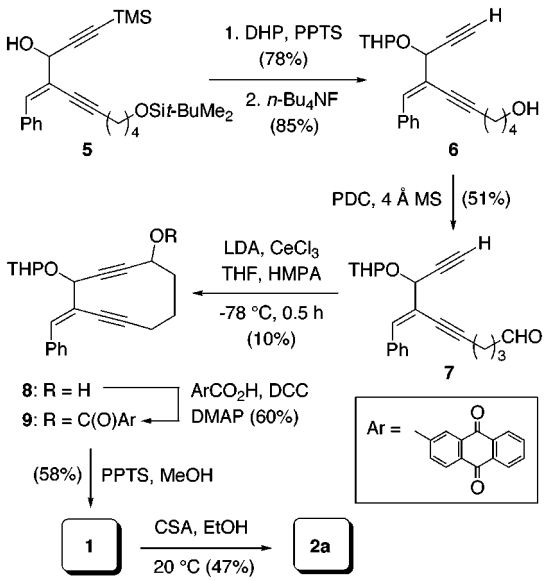
Scheme 2



yield. Finally, we prepared the 10-membered ring enediyne **2a** by a carefully designed allylic rearrangement under acid catalysis. Exposure of **1** to 3 mol equiv each of CSA (0.29 M) and EtOH in CH_2Cl_2 at 20 °C for 26 h gave enediyne **2a** in 47% yield at 83% conversion of **1**. With a dilute CSA solution (0.06 M) of the same reaction, only 80% of **1** was converted after 96 h at 20 °C, and **2a** was obtained in 31% yield. The diminished yield of **2a** is attributed to its decomposition after prolonged reaction time.

It is assumed that an allylic cation intermediate is generated from the acid-catalyzed dehydration of **1** followed by attack of EtOH at the cation exclusively at the γ carbon to give enediyne **2a**. Even though a strong acid was required for the formation of **2a** from **1** in CH_2Cl_2 at 20 °C, we expected that a similar allylic rearrangement may take place with heating under slightly basic pH in the presence of DNA[8] because a similar intramolecular event was reported for the maduropeptin chromophore derivative possessing a MeO as the leaving group.[6] DNA cleavage profiles of **1** and **2a** were examined together with two esters **10** and **11** of anthraquinone-2-carboxylic acid using ΦX174 RFI supercoiled DNA (form I). Figure 1A shows DNA cleavage results of **1**, **2a**, **10**, and **11** after 1% agarose gel electrophoresis, while Figure 1B represents the quantitative results obtained by scanning densitometry. In general, enediyne **2a** shows much more potent DNA cleavage activity than the precursor **1** at pH 8.5 and 7.5. This is consistent with the hypothesis that compound **1** exhibits its DNA damage via enediyne **2b** through in situ allylic rearrangement. Esters **10** and **11** are almost inactive at pH 8.5 and even inhibit DNA decomposition at pH 7.5 (lanes 9 and 10) compared with the control in lane 6 (Figure 1A).[9] By considering this effect, the actual % form II DNA formed at pH 7.5 in lanes 7 and 8 should be higher than the values given in Figure 1B. These results rule out the possibilities that **1** causes DNA cleavage through the anthraquinone ring (an intercalator) and the propargylic ester moiety (a possible alkylation site).

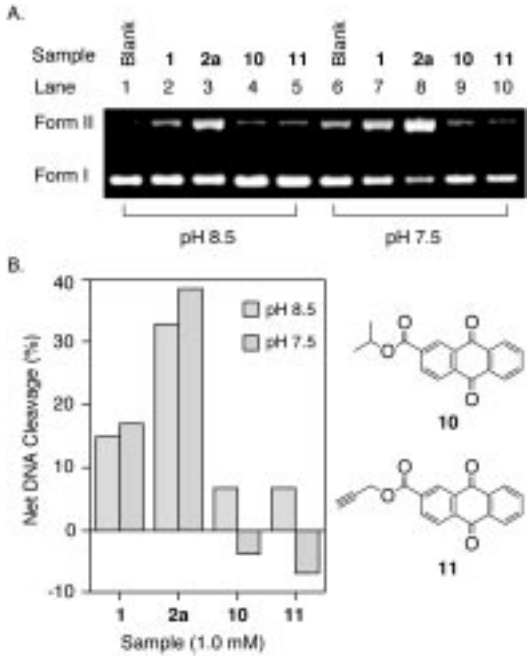


Figure 1. Results of DNA cleavage by allyl alcohol **1** and enediyne **2a** in comparison with esters **10** and **11**. (A) 1% agarose gel electrophoresis. ΦX174 RFI DNA (54.3 μM/bp) was incubated with the samples at 1.0 mM in 20% DMSO containing TEA buffer solution (pH 7.5 and 8.5) at 37 °C for 72 h and then analyzed by gel electrophoresis and ethidium bromide stain. (B) Scanning densitometry results of the gel picture shown in A. The percentage of net DNA cleavage was calculated by the following equation: $[(\text{form II})_s/[(\text{form I})_s + (\text{form II})_s] \times 100] - [(\text{form II})_c/[(\text{form I})_c + (\text{form II})_c] \times 100]$. The subscrips "s" and "c" refer to the samples and controls, respectively.

In summary, we have demonstrated that the acid-catalyzed allylic rearrangement can be applied to form a highly strained 10-membered ring enediyne in situ from thermally stable precursor **1**. This novel approach is conceptually related to the mechanism of action of the maduropeptin chromophore derivatives.[5] Modification of **1** by replacing the phenyl group at the exocyclic double bond with an electron-rich *p*-$MeOC_6H_4$ should provide a better molecular system for efficient generation of enediyne-based DNA cleaving antitumor agents under physiological conditions.[10] Research toward this goal is underway in our laboratories.

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Supporting Information Available: Synthetic procedures and spectral data of compounds **1**, **2a**, and **6**−**9**.

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(9) Logically, acidic pHs should favor the conversion of **1** into **2b**, and we observed a slightly increased potency of DNA cleavage by **1** when the pH was changed from 8.5 to 6.0. However, due to substantial decomposition of the DNA samples from two commercial sources in the acidic pHs, the observed pH effect on DNA cleavage of **1** is not very significant or convincing.

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