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DNA Cleavage by Acyclic Eneyne-Allene Systems Related to Neocarzinostatin and Esperamicin-Calicheamicin

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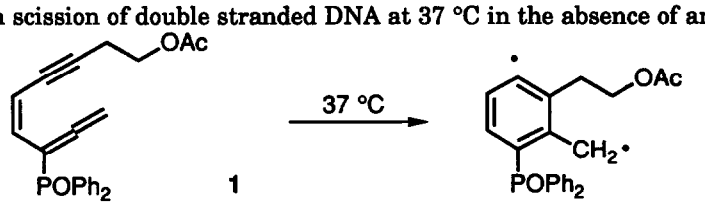
Summary: Substituted eneyne-allenes 1, 6, and 7 underwent Bergman type cyclization at 37 °C, whereas 5 was almost stable at the temperature. Compounds 1 and 7 showed DNA-cleaving activities.

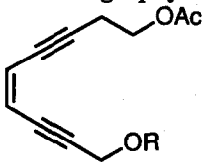
Recently, we have reported that acyclic eneyne-allene system **1** undergoes Bergman type cyclization smoothly at 37 °C to produce biradical **2**[1] and that this is in marked contrast to the case of acyclic enediyne system which required thermal activation of more than 130 °C for such cyclization.[2,3] At almost same time, A. G. Myers and co-workers also reported a similar cycloaromatization of simple unsubstituted eneyne-allenes such as (Z)-1,2,4-heptatrien-6-yne at higher temperatures, followed by very recent publication on a chemically triggered biradical formation via (Z)-1,2,4-heptatrien-6-yne system.[4] Since Bergman type cyclization has been proposed to play a crucial role in DNA-cleaving process mediated by a new class of cyclic enediyne antibiotics, esperamicins,[5] calicheamicins,[6] and neocarzinostatin,[7] the use of new biradical forming reaction of acyclic eneyne-allene system as a key reaction for DNA cleavage would be of considerable interest. While DNA-cleaving properties of synthetic strained enediyne systems as a mimic of esperamicin-calicheamicin have been described,[8] DNA cleavage by such non-strained acyclic eneyne-allene systems has not been accomplished. Here, we demonstrate that introduction of a substituent at C-3 position of eneyne-allene system decreases the activation energy for cyclization and indeed causes clean scission of double stranded DNA at 37 °C in the absence of any additives.



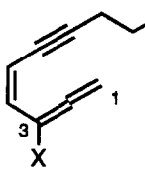
1 2

Eneyne-allene systems having -H, -CH_3, and -SOPh groups at C-3 position (**5**, **6**, and **7**, respectively) were synthesized from **3**.[1] Alcohol **3** was mesylated with mesyl chloride-triethylamine in CH_2Cl_2 at 0 °C to give **4** in 91% yield. Mesylate **4** was reduced with excess Zn-Cu couple in ethanol at 0 °C under sonication for *ca.* 1 h to afford **5** in 31% yield.[9] Eneyne-allene **6** was prepared in 48% yield by reaction of MeMgBr (1.5 equiv) with mesylate **4** in THF in the presence of CuCN (1.5 equiv) at -40 °C for 2 h.[10] Sulfoxide **7** was prepared directly from alcohol **3** in 56% yield by [2,3]-sigmatropic rearrangement of phenyl sulfenate generated *in situ* by reaction with benzenesulfenyl chloride (1.0 equiv) in the presence of triethylamine (1.0

equiv) in CH_2Cl_2 at -78 °C and then at 0 °C. Compounds **5** and **6** were stable enough to be handled at ambient temperature and purified by silica gel column chromatography. Compound **7** was relatively unstable at room temperature and quickly purified by short silica gel column chromatography at low temperature.

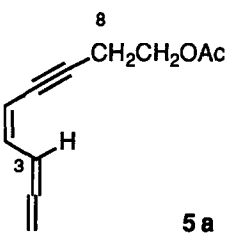


3 R = H
4 R = Ms



5 X = H
6 X = Me
7 X = SOPh

Each of eneyne-allenes **1**, **5**, **6**, and **7** was heated in THF-d_8 at various temperatures in a sealed tube and the progress of reaction was monitored by ^1H nmr spectroscopy. In all cases, the signals at an olefinic region gradually disappeared with a clean first-order kinetic behavior and new signals appeared concomitantly at an aromatic region, indicating the formation of aromatized compounds.[11] These observations suggest that the eneyne-allene systems undergo Bergman type cyclization as a major event. Activation parameters for cyclization of each compound were obtained from Arrhenius plot of the first-order rate constants. As shown in Table I, introduction of a substituent at C-3 position considerably lowered the activation energy. Indeed, substituted eneyne-allenes **1**, **6**, and **7** cyclized at 37 °C to generate biradical with useful rates (half lives of 80, 100 and 16 min, respectively),[12] whereas compound **5** was almost stable at 37 °C (half life of 66 h).[12] Activation energy for cyclization of **5** is *ca.* 4 kcal/mol higher than that of methyl-substituted **6**. The results can be reconciled with the difference in the ground state conformations of **5** and **6**. NOE relationship between C-3 proton and C-8 methylene of **5** was observable by means of NOE difference spectroscopy, whereas there was no NOE between C-3 methyl and C-8 methylene for **6**. This implies that in the ground state, **5** would exist as s-*trans* (**5a**) or twisted s-*trans* conformer, whereas s-*cis* or twisted s-*cis* conformation would be more favorable for **6**. Therefore, on the cyclization of **5** extra activation energy for the conformation change must be required. In fact, *ab initio* calculation estimates that s-*trans* conformer **5a** is 3.6 kcal/mol more stable than s-*cis* conformer.[13] Thus, the steric effect rather than the electronic effect appears to control the cyclization rate strongly.



5a

DNA cleavage by the eneyne-allene systems indeed took place at 37 °C, although their efficiencies were not very high. ΦX 174 replicated form I DNA (80 μM based on base pair) was incubated with each 500 μM of **1**, **6**, or **7** in 50 mM Tris acetate buffer (pH 8.0) at 37 °C for 12 h. While **6** did not show appreciable DNA-cleaving activity, probably because of its poor solubility in the buffer, single strand break (form II) and, to some extent, double strand break (form III) were observed as determined by agarose gel electrophoretic analysis in the case of **1** or **7** (Table II).[14, 15] When DNA cleavage by **7** was carried out in sodium acetate buffer (pH 4.6), the strand breaks (form II and III) were more prominent compared to those observed at

pH 8.0 (Table III).[14] Furthermore, even at 27 °C a similar DNA-cleaving activity was observed for **7**.

Table I. Activation Parameters, Rate Constants and Half Lives for Cyclization of Eneyne-Allenes **1**, **5**, **6**, and **7** in THF-d_8[a]

compound	Ea (kcal/mol)	log A (s^{-1})	rate constant (s^{-1})[b]	half life (min)[b]
1	17.3 ± 1.3	8.6	4.70×10^{-4}	24.6
5	21.3 ± 0.7	9.4	3.02×10^{-5} (60 °C)	383 (60 °C)
6	17.8 ± 0.5	8.3	3.40×10^{-4}	34.0
7	15.9 ± 1.5	8.1	1.78×10^{-3}	6.5

[a]The rate constants were measured over a temperature range (30 ~ 75 °C) by ^{1}H nmr spectroscopy. [b]Experimental values measured at 50 °C were shown except for **5** where the values at 60 °C were shown.

Table II. Cleavage of ΦX 174 Replicated Form I DNA by Various Eneyne-Allenes[a]

compound	form I (%)	form II (%)	form III (%)
1	62	33	5
6	83	17	—
7	62	25	13
DNA alone[b]	87	13	—

[a]ΦX174 replicated Form I DNA (80 μM per base pair) was incubated with each eneyne-allene **1**, **6**, and **7** (500 μM) in 50 mM Tris acetate (pH 8.0) at 37 °C for 12 h and analyzed by agarose gel electrophoresis. The values were determined by densitometry after ethidium bromide staining and not rigorously quantified. [b]The DNA used was contaminated by form II DNA.

Table III. Cleavage of ΦX 174 RFI DNA by **7** at Different pH and Temperatures[a]

concn. of **7** (μM)	conditions	form I (%)	form II (%)	form III (%)
0[b]	pH 8.0, 27 °C[c]	72	28	—
100		63	32	5
500		53	42	5
0[b]	pH 4.6, 37 °C[d]	77	23	—
100		67	33	—
500		5	91	4

[a]ΦX 174 replicated form I DNA (60 μM per base pair) was used. The incubation mixture was analyzed as shown in Table II. [b]The DNA used was contaminated by form II DNA. [c]Incubations were carried out for 6 h in 50 mM Tris acetate. [d]Incubations were carried out for 2 h in 50 mM sodium acetate.

It is surprising that even such a highly simplified acyclic model is capable of cleaving DNA. The intriguing possibilities obtaining a more practical DNA-cleaving molecule based on eneyne-allene system might be realized by further modification of the basic structure as well as the introduction of a DNA-binding group.[16]

References and notes

(1) R. Nagata, H. Yamanaka, E. Okazaki, and I. Saito, *Tetrahedron Lett.*, **30**, 4995 (1989).

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(3) The theoretical studies on the different reactivities of eneyne-allene and enediyne systems have been carried out: N. Koga and K. Morokuma, to be submitted for publication.

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(9) A simple reduction product at C-1 was also formed in a similar amount. Without sonication, the progress of the reaction was sluggish.

(10) Formation of S_N2 product was negligible under these conditions. However, when PhMgBr was used instead of MeMgBr, a mixture of allene (S_N2' product) and S_N2 product was formed in a ratio of 1 : 3.

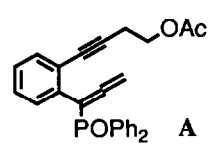
(11) See ref. 1. Analogous aromatized compounds to those obtained from **1** were isolated from thermolysis of **5**, **6**, and **7** in benzene-1,4-cyclohexadiene or in THF.

(12) These values were calibrated using the physical parameters obtained here.

(13) *Ab initio* calculation was carried out using SCF/MIDI1, a private communication from Dr. N. Koga.

(14) DNA cleavage experiments were repeated more than two times and in each case, a similar trend for DNA-cleaving pattern was observed.

(15) The active species responsible for DNA cleavage cannot be concluded to be a 1,4-biradical like **2** at present stage. Involvement of polar reaction pathway as well as biradical pathway has been proposed in the thermal cyclization of (Z)-1,2,4-heptatrien-6-yne.[4] The effect of oxygen and other factors on cutting DNA cannot be ruled out. Recently, DNA cleavage by allenyl sulfones has been reported; K. C. Nicolaou, G. Skokotas, P. Maligres, G. Zuccarello, E. J. Schweiger, K. Toshima, and S. Wendeborn, *Angew. Chem. Int. Ed. Engl.*, **28**, 1272 (1989). However, compound **A** which cannnot undergo cycloaromatization at 37 °C, did not show any detectable DNA cleaving activities (unpublished results).



A

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