EJ52-1998-475

*Journal of the Chinese Chemical Society*, **1998**, *45*, 475-479



Invited Paper

# Allene-eneyne Related Cycloaromatization: Design and Synthesis of New DNA-cleaving Compounds

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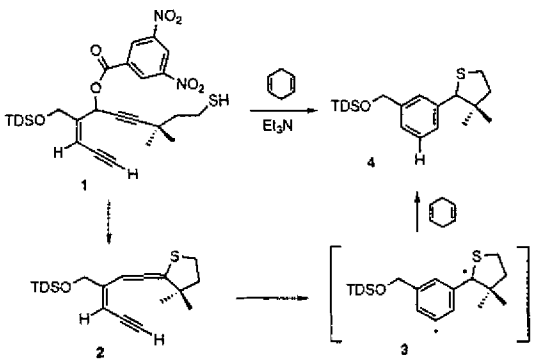
(Z)-7-Sulfonyl-3-hexen-1,5-diyne containing molecules are stable at room temperature and isomerized to eneyne-allene-sulfones under alkaline conditions. These eneyne-allene-sulfones were not isolable, spontaneously cyclized to form biradical intermediates under mild conditions, and exhibited good DNA-cleaving and human tumor cell line growth inhibition properties. Further studies indicated that compounds bearing on aromatic ring at C(3) and C(4), such as **25**, proved to be more active against those tumor cell lines. Removal of acetylene unit at C(1) and C(2), made the resulting compound less active. A related compound, (Z,Z)-12-(2-tetrahydropyranyl)oxy-1-phenylsulfonyldodeca-4,8-diene-2,6,10-triyne (**37**), was synthesized. Upon treatment of triethylamine at refluxing benzene, this compound undergoes double cycloaromatization to form a naphalene adduct, which possesses excellent DNA-cleaving activity.

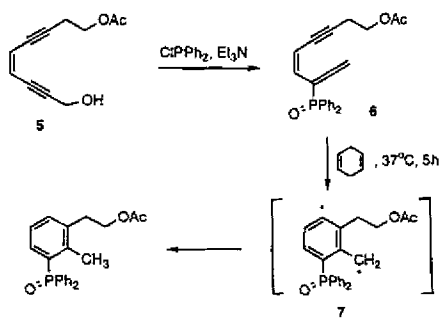
## INTRODUCTION

The enediyne antitumor antibiotics, represented by neocarzinostatin,[1] the calicheamicins,[2] esperimicins,[3] and dynemicins,[4] have attracted much attention due to their unusual molecular architecture and mode of activation leading to the formation of benzenoid diradicals and the resulting cleavage of DNA. The thermal cyclization of (Z)-3-hexen-1,5-diynes to 1,4-didehydrobenzene diradicals reported by Bergman[5] is considered to be the major mode of formation of biradicals in enediyne antitumor antibiotics. In studies on the mechanism of the DNA-cleaving activity of neocarzinostatin chromophore, Myers reported the cyclization of (Z)-1,2,4-heptatriene-6-ynes to α,3-didehydrotoluene diradicals.[6] Several potent DNA-cleaving agents have been developed based on Myers cyclization.[7] For some earlier examples, Myers prepared compound **1**.[6c] Treatment of compound **1** with triethylamine, in the presence of 1,4-cyclohexadiene, in dimethyl sulfoxide gave tetrahydrothiophene **4**. The formation of compound **4** suggested the following mechanism: in the presence of triethylamine, compound **1** could undergo intramolecular $S_N2'$ reaction to give the allen-enyne **2**, which undergoes cycloaromatization reaction to give diradial **3**. Hydrogen abstraction would lead **3** to compound **4** (Scheme I). Saito and coworkers[7c,7d] reported that the thermal decomposition of allenylphosphine oxide **6**, which is prepared by the reaction of propargyl alcohol **5** with chlorodiphenylphosphine, actually involves a formation of biradical intermediate **7** (Scheme II). Similar allenylphosphine oxide analogs were reported by Nicolaou.[7b] Thermolysis of **8** at 37 °C in the presence of 1,4-cyclohexadiene led to a formation of **10**. Biradical intermediate **9** was suggested as a intermediate (Scheme III).

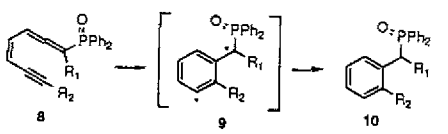
**Scheme I**




**Scheme II**

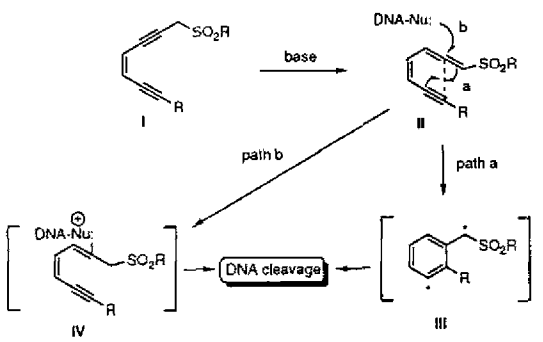


**Scheme III**



In designing of a new class of DNA cleavage agents, we proposed that molecules with (Z)-7-sulfonyl-3-hexene-1,5-diyne functionalities could undergo base-catalyzed isomerization to sulfonyl-allene-enyne **II**. Structure **II** was then expected to undergo either Myers cyclization to form biradical **III** or nucleophilic attack from DNA to form **IV** and cause the cleavage of DNA as shown in Scheme IV.

**Scheme IV**

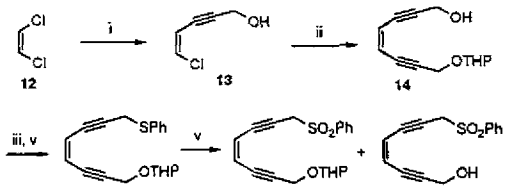


## RESULTS AND DISCUSSIONS

The synthesis of the representative compound **11** is outlined in Scheme V.[8] The commercially available (Z)-1,2-dichloroethylene **12** was coupled with propargyl alcohol, using bis(triphenylphosphine)palladium(II) chloride as catalyst to give (Z)-vinyl chloride **13** in 65% yield. Subsequent coupling of **13** with protected propargyl alcohol using tetrakis(triphenylphosphine)palladium(0) as the catalyst under the same conditions gave (Z)-enediyne **14** in 50% yield. Compound **14** was then converted into the corresponding mesylate by the treatment of mesyl chloride in the presence of triethylamine. Subsequent reaction of the mesylate with thiophenol in the presence of sodium hydroxide in aqueous tetrahydrofuran afforded sulfide **15** in 44% yield. Finally, oxidation of sulfide **15** with *m*-chloroperbenzoic acid provided sulfone **16** in 45% yield along with 32% of compound **11**. The protecting group of **16** was removed using camphor sulfonic acid to give **11** in 92% yield.

The degased solution of **16** in benzene (0.01 M) in the presence of 1,4-cyclohexadiene (1.5 M) was treated with $Et_3N$ at 30 °C for 10 h. After extractive isolation and flash

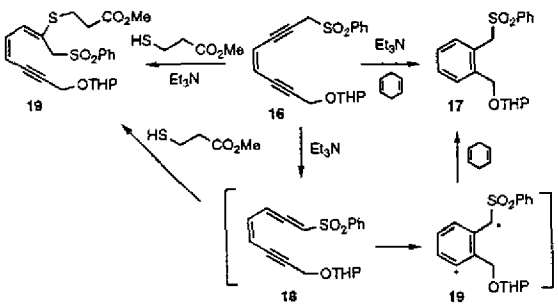
**Scheme V**



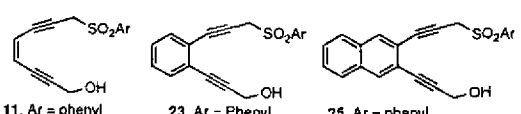
**Reagents and conditions:** i) $HCCCH_2OH$, $Pd(PPh_3)_2Cl_2$, $Et_2O$, CuI, $BuNH_2$, 65%; ii) $HCCCH_2OTHP$, $Pd(PPh_3)_4$, $Et_2O$, CuI, $BuNH_2$, 50%; iii) $CH_3SO_2Cl$, $Et_3N$, $CH_2Cl_2$; iv) HSPh, NaOH, THF-$H_2O$, 44%; v) mCPBA, $CH_2Cl_2$, 45% of16 and 32% of11.

chromatography, the aromatized compound **17** was obtained in 45% yield (Scheme VI). These results strongly suggested that the enyne-allene-sulfone **18** and biradical intermediate **19** are actually involved in the transformation of enediyne **16** to compound **17**. On the other hand, the reaction of **16** with methyl 3-mercaptopropionate in the presence of triethylamine in benzene afforded the nucleophilic addition adduct **19** in 53% yield. These results also suggested that enyne-allene-sulfone **18** serves as an excellent Michael acceptor and possibly possess DNA-cleavage and anti-tumor activities.

**Scheme VI**



According to the above described synthetic procedures, we have also prepared compounds **20**, **21**, **22**, **23**, **24** and **25** starting from (Z)-1,2-dichloroethylene, 1,2-diiodobenzene and 2,3-naphthalene bistriflate, respectively.[9] The incubation of compounds **11**, **20**, **21**, **22**, **23**, **24** and **25** with supercoiled ΦX 174 DNA (form I) aerobically at pH 8.0 and 37 °C for 14 h produced DNA rupture, leading to form II as shown in Fig. 1. The potencies were increased by the introduction of an aromatic ring at C(3) and C(4). Compounds **11**, **20**, **21**, **22**, **23**, **24** and **25** were evaluated in vitro against five human tumor cell lines (Colo 205, Hep G2, SK-



11. Ar = phenyl
20. Ar = *p*-$CH_3OC_6H_5$
21. Ar = *o*-$CH_3O_2CC_6H_4$
22. Ar = 2-naphthyl
23. Ar = Phenyl
24. Ar = 2-naphthyl
25. Ar = phenyl

Table 1. Inhibition of *in vitro* Human Tumor Cell[a] Growth by **11** and **20-25** ($IC_{50}$, μg/mL)[b]

| Compound | HepG2 | Colo 205 | SK-BR-3 | KB | Molt-4 |
|---|---|---|---|---|---|
| **11** | --- | + | + | + | |
| **20** | --- | + | + | + | |
| **21** | --- | + | + | + | |
| **22** | --- | + | + | + | |
| **23** | + | ++ | ++ | ++ | ++ |
| **24** | ++ | | | | |
| **25** | + | + | + | | ++ |

[a] Cell type: HepG2, larynx epidermoid cell line; Colo 205, colon cell line; SK-BR-3, melanoma cell line; KB, oral epidermoid cell line; Molt-4, leukemia cell line. [b] Relative potency of growth inhibition of cancer cell line was graded by concentration required for 50% inhibition: ++ ($IC_{50}$: < 4 μg/mL), + ($IC_{50}$: 4-10 μg/mL), --- ($IC_{50}$: > 10 μg/mL).

BR-3, KB and Molt-4). For each compound, dose-response curves for each cell line were measured with five different drug concentrations and the concentration causing 50% cell growth inhibition ($IC_{50}$) compared with the control was calculated. The results were summarized in Table 1. Again, compounds **23**, **24** and **25** bearing on aromatic ring at C(3) and C(4) proved to be active against these cell lines.

In order to have a better understanding of the mode of biological actions of this series of compounds caused either by alkylation prosess or via biradical intermediate, we have synthesized compound **26** by removing the 1,2-acetylene unit and compound **27** containing bis-propargyl sulfone moiety.[10] The synthesis of **26** is outlined in Scheme VII. Hydrogenation of **28** using palladium on charcol as catalyst afforded **29** in 31% yield. Protection of the hydroxyl group with 3,4-dihydro-2H-pyrane under acidic condition gave **30** in 41% yield. Palladium catalyzed coupling reaction of triflate **30** with propargyl alcohol gave **31** in 35% yield. Alcohol **31** was converted to sulfide **32** in 24% yield by the reaction of alcohol **31** with methanesulfonyl chloride to give the corresponding mesylate, followed by the reaction of mesylate with thiophenol under alkaline condition. Oxidation of sulfide **32** with *m*CPBA gave sulfone **33** in 46% yield. Finally, acid-catalyzed deprotection of **33** gave **26** in 80% yield.

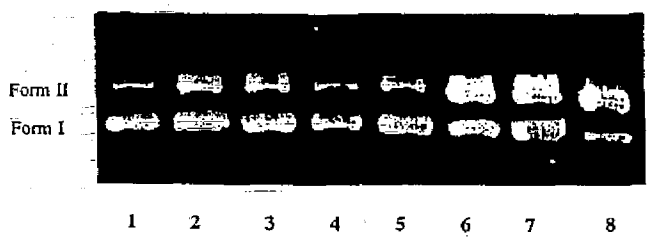
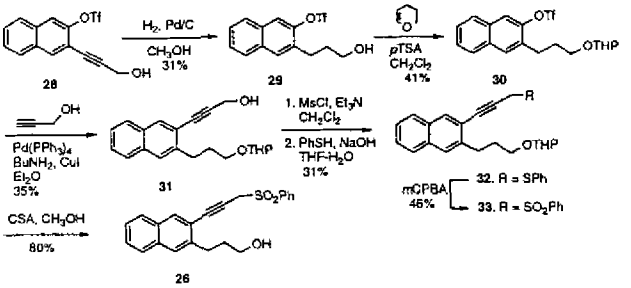


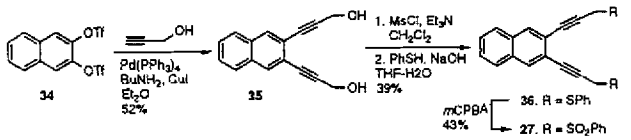

Fig. 1. DNA cleavage patterns on 1% agarose (ethidium bromide stain) of ΦX 174 (RFI) DNA (100 μM per base pair) incubated at 37 °C, 14 h at pH 8.0, 50 mM Tri-HCl, and the following additions. Lane 1. DNA plasmid as received; Lane 2. 50 μM **11**; Lane 3. 50 μM **20**; Lane 4. 50 μM **21**; Lane 5. 50 μM **22**; Lane 6. 50 μM **23**; Lane 7. 50 μM **24**; Lane 8. 50 μM **25**.

**Scheme VII**




Compound **27** was prepared starting from 2,3-napthalene-bistriflate **34**. Palladium-catalyzed coupling reaction of **34** with four equivalents of propargyl alcohol gave the bispropargyl alcohol **35** in 52% yield. Compound **35** was then converted to the corresponding sulfide **36** in 39% yield by the standard procedure. Finally, oxidation of **36** with *m*CPBA gave **27** in 43% yield (Scheme VIII).

**Scheme VIII**




Compounds **25**, **26** and **27** were evaluated *in vitro* against tumor cell lines (Colo 205, HepG2, HA22T, SK-BR-3 and Molt-4). The results were summarized in Table 2. Compound **26** lacking the 1,2-acetylene unit shows about ten times lower inhibition activity than compound **25**. Bispropargyl sulfone analog **27** bearing with enediyne moiety shows about equal activity to compound **25**.

Since the enyne-allene-sulfones undergo spontaneous cyclization to form biradical intermediates under mild conditions and exhibit good DNA-cleaving activity, we have extended our research in this area to thermal double cyclization of (Z,Z)-11-sulfonylundeca-3,7-diene-1,5,9-triyne system.[11] The synthesis of the prototype diene-triyne **37** is outlined in Scheme IX. Palladium-catalyzed coupling of *cis*-1,2-dichloroethylene **12** with protected propargyl alcohol **38** afforded **39** in 45% yield. Enyne **39** was subsequently coupled with (trimethylsilyl)acetylene using tetrakis-

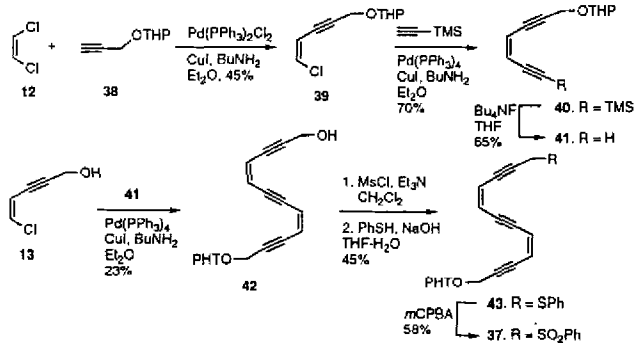
Table 2. Inhibition of *in vitro* Human Tumor Cell Growth by **25**, **26** and **27** ($IC_{50}$, μg/mL)[a]

| Compound | Colo 205 | HepG2 | HA22T | SK-BR-3 | Molt-4 |
|---|---|---|---|---|---|
| **15** | 5.63 | 5.40 | --- | 7.18 | 0.78 |
| **22** | 57.32 | --- | 65.91 | 70.00 | 9.45 |
| **25** | 8.44 | --- | 4.62 | 5.39 | 1.96 |

[a] Relative potency of growth inhibition of cancer cell line was graded by concentration required for 50% inhibition.

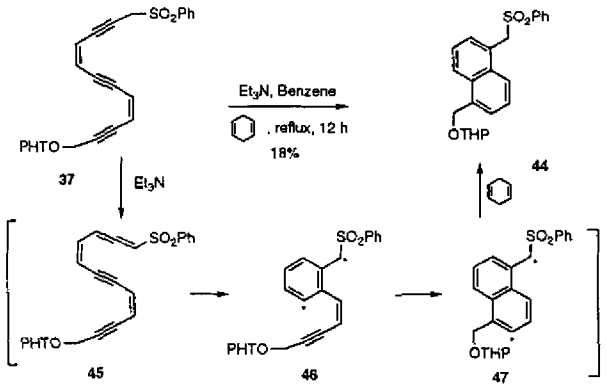
(triphenylphosphine)palladium(0) as the catalyst to give **40** in 70% yield. The silyl group of **40** was removed with tetrabutylammonium fluoride in THF to afford **41** in 65% yield. Coupling of (Z)-vinyl chloride **13** with **41** gave **42** in 23% yield. The hydroxyl group of **42** was converted to sulfide **43** in 45% yield by the standard procedures. Finally, sulfide **43** was oxidized with *m*CPBA to furnish sulfone **37** in 58% yield.

**Scheme IX**




Thermolysis of **37** with triethylamine (4 equiv.) in diluted, degassed benzene solution (0.01 M, 80 °C, 12 h) containing 1,4-cyclohexadiene (1.5 M) afforded **44** in 18% yield. The isolation of naphthalene **44** strongly suggested that the diradical, α,6-didehydro-α-methylnaphthalene **47**, is formed either by the concerted cyclization of allenyl sulfone **45** or by a stepwise pathway through diradical **46** (Scheme X).

**Scheme X**



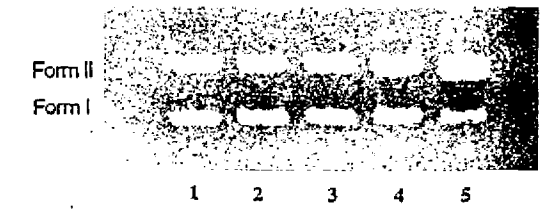




Fig. 2. Results of DNA cleavage by **37**. ΦX 174 Form I DNA (50 mM/bp) was incubated for 48 h at 47 °C with **37** in TBE buffer solution (pH 8.3) containing 20% DMSO and analyzed by electrophorisis (1% agarose gel, ethidium bromide stain). Lane 1. DNA control without incubation; Lane 2. DNA control with incubation. Lane 3-6. DNA with **1**, 10, 100 and 500 μM of **37**, respectively.

The DNA cleaving activity of **37** was examined by incubation with supercoiled ΦX 174 DNA (form I) at pH 8.3 and 47 °C for 48 h. The agarose gel picture shown in Fig. 2 indicated that the proportion of single stranded cleavage product (Form II) increased with increasing amount of **37**. At concentration of 100 μM (lane 5), more than 90% of form I DNA was converted into form II.

In conclusion, molecules with (Z)-7-sulfonyl-3-hexen-1,5-diyne functionalities proceeded base-catalyzed conversion to (Z)-enyne-allene-sulfones and subsequent Myers cyclization to form aromatized products under mild conditions. Most of these molecules show good DNA-cleaving properties and inhibition activity of the growth of tumor cell lines. These results might provide an opportunity for the development of a new class of DNA-cleaving antitumor agents.

## ACKNOWLEDGMENT

We thank the National Science Council of the Republic of China for generous support of this program.

Received February 7, 1998.

**Key Words**

(Z)-7-Sulfonyl-3-hexen-1,5-diyne; Eneyne-allene-sulfone; DNA-cleavage; Antitumor antibiotics.

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