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REMARKABLE TETHERING EFFECT ON DNA CLEAVAGE OF PROPARGYLIC SULFONE CONJUGATES WITH INTERCALATING MOIETIES

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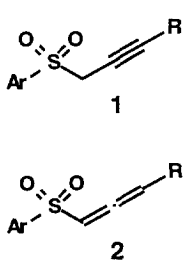
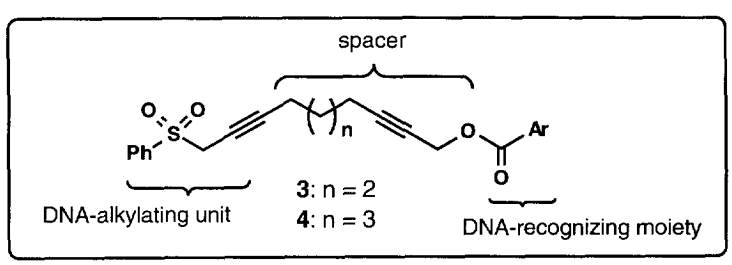
Abstract: A number of novel propargylic sulfone conjugates **3** and **4** with intercalating moieties were synthesized and evaluated for DNA cleavage activity through nucleic base alkylation. A remarkable enhancement in DNA cleaving potency was observed with those conjugates **3** possessing a suitable spacer, a right attachment point at the aromatic ring, and a good intercalator.

Interaction of small organic molecules with DNA usually results in damage and/or malfunctioning of the genetic biopolymers and it has attracted considerable efforts in the development of cytotoxic agents for chemotherapy of neoplastic diseases.[1] Nucleic base alkylation by electrophiles is one of the mechanisms for DNA strand cleavage; a variety of naturally occurring antibiotics [such as daunorubicin, mitomycin C, anthramycin, and (+)-CC-1065] and synthetic organic substances [such as nitrogen mustards, aziridines, and chloroethylnitrosoureas] are known to alkylate nucleic bases and cause DNA strand breakage.[2] Propargylic and allenic sulfones of the types **1** and **2** are a new class of nucleic base alkylating agents which were first reported by the Nicolaou's group to exhibit pH-dependent DNA cleavage activity.[3] A mechanism of action[3b] was proposed, featuring a base-induced isomerization of propargylic sulfones **1** into allenic sulfones **2** followed by nucleophilic addition of nucleic base toward **2**. Recently, others[4] and we[5] have reported the synthesis and DNA cleavage of various propargylic and allenic sulfones. Conjugations of propargylic sulfones with a quinoxaloyl[4b]



1

2



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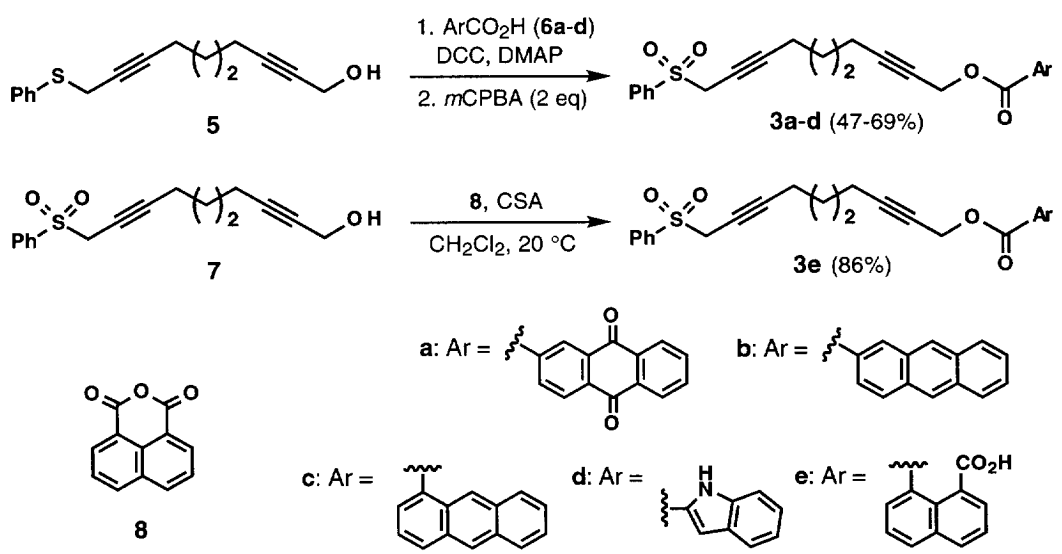
or lexitropsin[6] moiety have been attempted; but, no significant improvement in DNA cleavage potency was observed. In this paper, we disclose our recent results on the synthesis of propargylic sulfone conjugates of the types **3** and **4** possessing an intercalating group (Ar) and the remarkably enhanced DNA cleaving potency of those conjugates with a suitable tethering device.

Molecular design. Intercalation of coplanar extended aromatic compounds with DNA is one of the important mode of actions in DNA-drug interaction.[7] There are many structurally diversified organic substances, such as the anthracycline antitumor antibiotics,[8] known to interact with DNA through intercalation, an event leading to the inhibition of topoisomerase II enzyme and growth of tumor cells.[9] It is very common that antitumor antibiotics recognize DNA target through intercalation of the extended aromatic ring and meanwhile cause DNA strand cleavage near the intercalating site through a chemical process. This can be illustrated by the recently discovered enediyne antitumor antibiotic, dynemicn A[10] which possesses an anthraquinone moiety as the DNA-recognizing functionality and a 10-membered ring enediyne core as the DNA cleaver. The mode of intercalation and DNA cleavage of dynemicin A has been examined both experimentally and by computational modeling.[11] In this study, we designed the conjugation structures of **3** and **4** in which the DNA-alkylating unit (phenyl propargylic sulfone) and the DNA-recognizing moiety are linked by a spacer group with flexible chain length. Anthraquinone-2-carboxylic acid and other related aromatic carboxylic acid were chosen as the DNA-recognizing moieties [-OC(O)Ar] based on the following consideration: (a) intercalation of the conjugates with DNA should enhance the DNA cleavage potency comparing to the parent phenyl propargylic sulfones; and (b) the overall cytotoxicity of the conjugates should be further increased by contribution from the inhibition of topoisomerase II enzyme due to intercalation with DNA. Furthermore, we expect some difference in potency of the two series of conjugates **3** and **4** because the anchored conjugate on DNA target should have a proper chain length to reach the nucleic base for alkylation to take place.

Chemical synthesis. The propargylic sulfone conjugates **3a-e** were synthesized from the known hydroxyl propargylic sulfide **5** and sulfone **7**,[5a] respectively (Scheme 1).[12] Ester bond formation between the

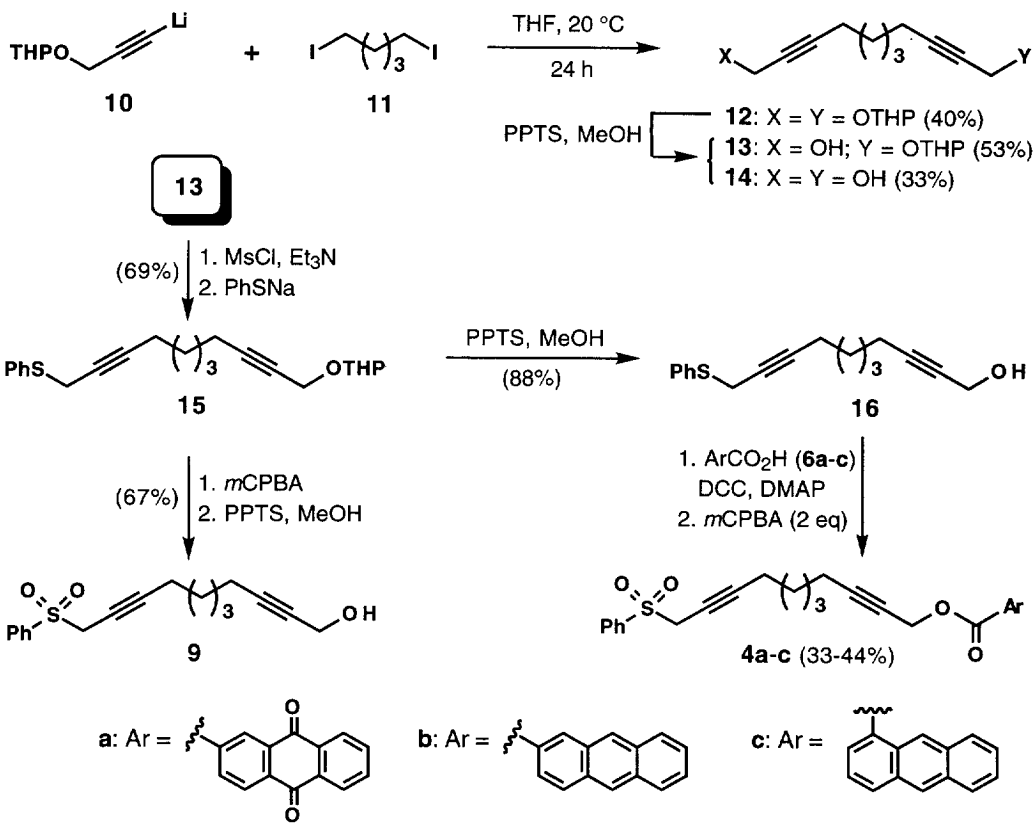
Scheme 1



alcohol **5** and anthraquinone-2-carboxylic acid (**6a**), 2-anthracenecarboxylic acid (**6b**), 1-anthracenecar-boxylic acid (**6c**), or indole-2-carboxylic acid (**6d**) was achieved under the standard DCC-DMAP conditions. Oxidation of the sulfide moiety in the resultant esters by excess *m*CPBA afforded sulfones **3a-d** in 47-69% overall yield. For the convenience of purification, the conjugate **3e** was prepared directly from the hydroxyl sulfone **7** and 1,8-naphthalic anhydride (**8**) catalyzed by (±)-10-camphorsulfonic acid (CSA) in 86% yield.

Scheme 2 illustrates the synthesis of the homologous hydroxyl propargylic sulfone **9** and its conjugates **4a-c**. Reaction of excess lithium acetylide **10** with 1,5-diiodopentane (**11**) at 20 °C for 24 h afforded the bis-alkylation product **12** in 40% yield (not optimized with recovery of starting materials). Selective removal of the THP ethers in **12** was attempted by treatment of **12** with a catalytic amount of PPTS in MeOH for a short time (2.5 h) to provide the mono alcohol **13** (53%) together with the diol **14** (33%). Even though the selectivity is not satisfactory, the diol **14** can be recycled by conversion into the bis-THP ether **12**. Mesylation of the mono

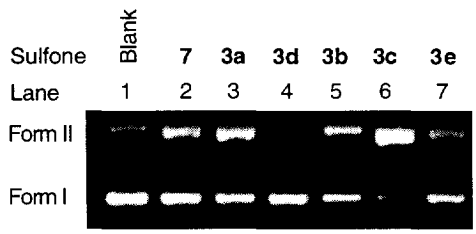
Scheme 2



alcohol **13** and subsequent nucleophilic substitution by PhSNa provided the sulfide **15** in 69% yield (two steps). Oxidation of **15** with *m*CPBA followed by removal of the THP ether furnished the hydroxyl propargylic sulfone **9** in 67% yield (two steps). Removal of the THP ether in **15** afforded the hydroxyl propargylic sulfide **16** in 88% yield. In the same manner, the conjugates **4a-c** were synthesized from **16** and **6a-c**, respectively, via the two-step procedure in 33-44% overall yield (not optimized).

DNA cleavage studies. In order to examine the tethering effect on DNA cleavage, the propargylic sulfone conjugates **3a-e** and **4a-c** together with controls **7** and **9** were studied for DNA cleavages using supercoiled ΦX174 Form I DNA and analyzed by agarose gel electrophoresis. The Form II band seen in the gel picture after ethidium bromide stain represents the single strand cleaved DNA fragments.[13] Figure 1 shows the DNA cleavage profiles of sulfones **7** and **3a-e** at 1.0 mM (final concentration)[14] after incubation at pH 8.5 and 37 °C for 72 h. In comparison with **7**, conjugates **3a** and **3c** (lanes 3 and 6) gave better DNA cleavage results

Figure 1

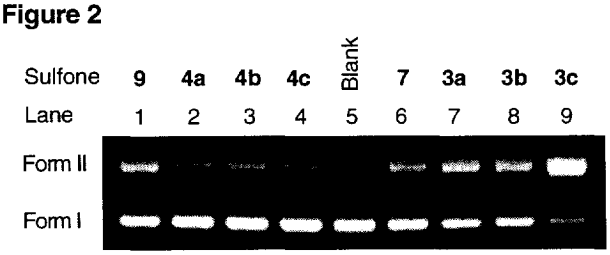


ΦX174 RFI DNA (54.3 μM/bp) was incubated with various propargylic sulfones at 1.0 mM in 20% DMSO containing TEA buffer solution (pH 8.5, 37 °C) for 72 h and then analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain).

as shown by the relative ratio of the Form II/Form I bands. In contrast to this, conjugates **3b**, **3d**, and **3e** exhibited either decreased (lane 4) or similar (lanes 5 and 7) potency comparing with **7**. These results suggest that the 2-anthracenecarboxylic ester, the indole-2-carboxylic ester, and the half ester of 1,8-naphthalic anhydride are not the suitable candidates for conjugation. It is surprising to note that both anthraquinone-2-carboxylic ester **3a** and 1-anthracenecarboxylic ester **3c** are better DNA cleavers than 2-anthracenecarboxylic ester **3b**. The remarkable difference in DNA cleaving potency of **3b** and **3c** may arise from much more favorable orientation of the allene moiety[15] in the "long tail" toward the nucleic base for the 1-anthracene ester **3c** than that of the 2-anthracene analog **3b**. The anthraquinone-2-carboxylic ester **3a** can be regarded as a much more potent intercalator than the 2-anthracene ester **3b**, thereby causing more Form II DNA formation. In the light of the high potency observed for the 1-anthracene ester **3c**, an anthraquinone-1-carboxylic ester of **7** should be very promising and it will be verified in our future study.

In order to investigate the influence of chain length *n* in the spacer on DNA cleavage, the homolog **9** and its conjugates **4a-c** were assayed together with **7** and **3a-c** at 1.0 mM concentration[14] (Figure 2). It was found

Figure 2



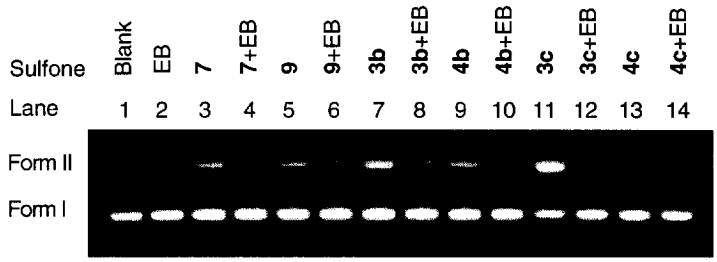
ΦX174 RFI DNA (54.3 μM/bp) was incubated with various propargylic sulfones at 1.0 mM in 20% DMSO containing TEA buffer solution (pH 8.5, 37 °C) for 72 h and then analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain).

that the parent propargylic sulfones **7** and **9** behaved similarly (lanes 1 and 6) regardeless the chain length, however, the homologous conjugates **4a-c** completely lost their reactivity with DNA. These results imply that while the active allenic sulfone moiety *in situ* formed from the intercalated **3a-c** are located at the right sites for nucleic base alkylation, the extended spacer in conjugates **4a-c** separates the allene moiety far from the nucleic base, thus, resulting in negligible DNA cleavage. The anthracene-1-carboxylic ester **3c** (lane 9) was confirmed

to be the best DNA cleaver among all compounds examined in Figures 1 and 2.

Next, we performed a competition experiment[16] using ethidium bromide (EB) which is a well-known DNA intercalator. As shown in Figure 3, EB itself did not cause any effect on DNA substrate (lane 2), but, the EB-pretreated DNA is insensitive to the propargylic sulfones **7**, **9**, **3b**,**c**, and **4b**,**c** (lanes 4, 6, 8, 10, 12, and 14).[3b,5b] Since the parent hydroxyl propargylic sulfones **7** and **9** were also effected by EB, inhibition on intercalation of **3b**,**c**, and **4b**,**c** by EB might not be the sole cause. Perhaps, the intercalated EB effectively blocks the approach of the sulfones and prevents alkylation to take place at the EB-modified nucleic bases. For the untreated DNA substrate, the potency of **3c** remains very promising even at 0.1 mM (lane 11).

Figure 3



ΦX174 RFI DNA (54.3 μM/bp) was incubated with various propargylic sulfones (at 0.1 mM) in the presence or absence of ethidium bromide (EB, at 0.1 mM) in 20% DMSO containing TEA buffer solution (pH 8.5, 37 °C) for 72 h and then analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain).

In summary, two series of propargylic sulfone conjugates possessing an intercalator were synthesized and their DNA cleavage activities were examined. Remarkably enhanced potency was observed with conjugate **3c** possessing a suitable spacer (n = 2), and a right attachment point (C-1) at the anthracene ring. From this study, it concludes that orientation of the alkylating unit within the intercalated DNA substrate seems essential for attaining high potency. Further studies on the conjugates of propargylic sulfone with DNA-recognizing moiety is in progress in our laboratories.

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12. All new compounds gave satisfactory ^{1}H and ^{13}C NMR spectra and MS data.
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14. Due to minor precipitation, the final concentration for **3b,c** or **4c** may be slightly lower than 1.0 mM.
15. The allenes formed from the propargylic sulfones are chiral and the two enantiomers should interact with DNA differently. For our discussion here, the same enantiomers are compared. Currently, we are working on the molecular modeling in order to obtain 3D structures of the allenes in the bound DNA.
16. The DNA was treated with 0.1 mM ethidium bromide for 2 h before incubating with the sulfones.