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Synthesis of a DNA-Cleaving Bis(propargylic) Sulfone Crown Ether.[1]

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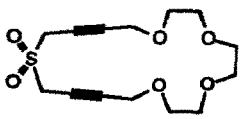
Summary: The prototypical bis(propargylic) sulfone crown ether 5 was synthesized in six steps and 26 % overall yield from readily available starting materials. In alkaline aqueous buffer, compound 5 cleaves supercoiled DNA.

DNA-reactive compounds play an important role in chemotherapy as well as biophysical studies. Recently, a new class of synthetic DNA reactive agents that incorporates a propargylic sulfone moiety has been reported.[2,3] The DNA cleavage due to these propargylic sulfones arises from their facile conversion to allenylic sulfones under basic conditions. The allenylic sulfones then either alkylate DNA-centered nucleophiles and cause DNA cleavage in analogy with Maxam-Gilbert chemistry,[4] or undergo cyclization involving proximal double or triple bonds to produce diradical intermediates[3] that may abstract hydrogen atoms from the DNA backbone leading to DNA strand scission.

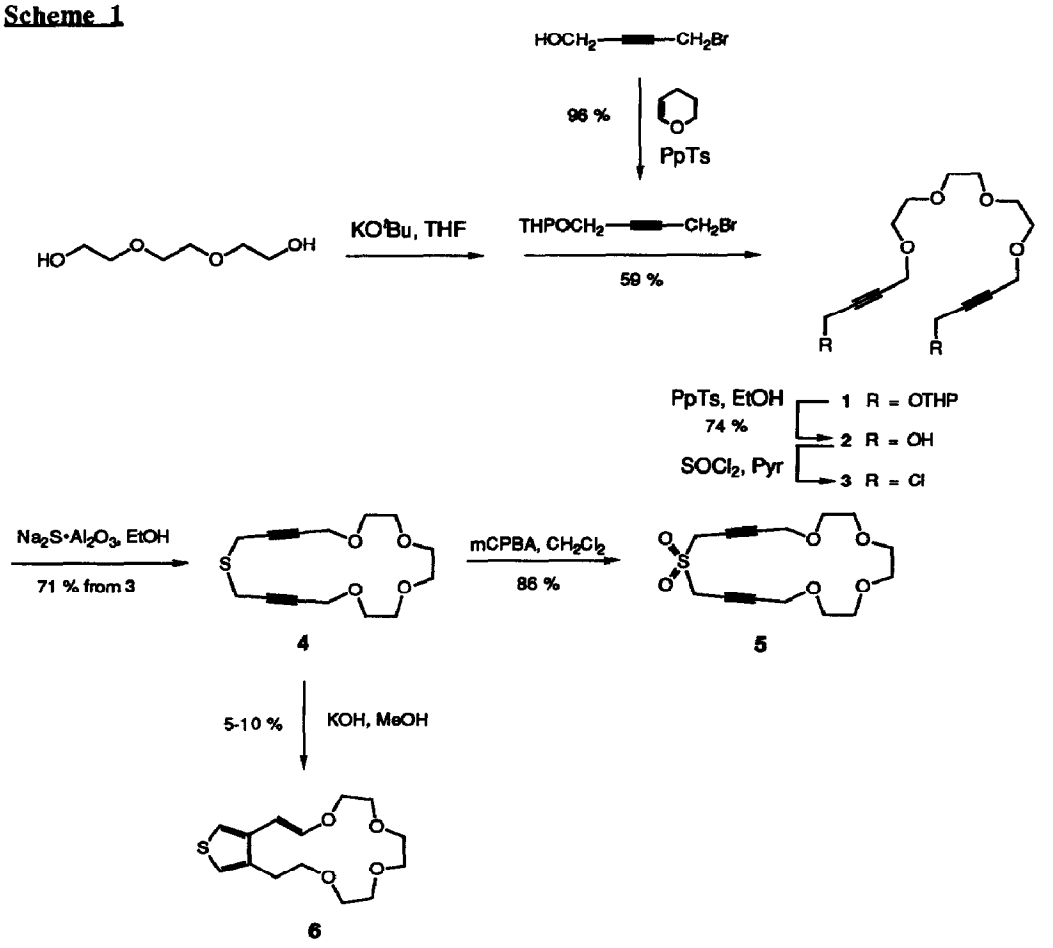
There have been a number of reports of the metalloregulation of DNA binding evidenced by various molecules possessing a crown ether or other metal-binding moiety covalently attached to a DNA-binding group.[5] The enhanced DNA binding of these compounds in the presence of the appropriate metal ion presumably results from a favorable interaction of the cationic metal-complexed crown ether moiety with the polyanionic DNA. By analogy, the incorporation of a bis(propargylic) sulfone moiety into a crown ether might give rise to a metalloregulated DNA cleavage reagent. Additionally, the state of complexation of the crown ether ring could affect the free radical / ionic pathway partitioning of the DNA cleavage reactions of these novel crown ethers.



5

The bis(propargylic) sulfone crown ether **5** was synthesized in six steps and 26 % overall yield from 4-bromo-2-butyn-1-ol,[6] as shown in Scheme 1.[7] Tetrahydropyranyl acetal-protected 4-bromo-2-butyn-1-ol was used to alkylate the bis(potassium alkoxide) of triethyleneglycol. Deprotection of the tetrahydropyranyl protecting groups afforded the diol **2**, which was converted to the dichloride **3**. The crude dichloride, which appeared to be somewhat sensitive, was not

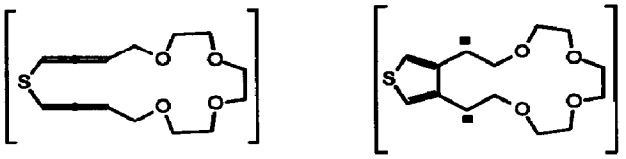
Scheme 1



purified, but rather subjected immediately to cyclization conditions using alumina-supported sodium sulfide reagent[8] to afford the cyclic sulfide **4**. Oxidation of sulfide **4** with excess *m*-chloroperoxybenzoic acid affords the crystalline sulfone **5** in high yield.[9]

Tan and co-workers have recently reported on the facility of macrocyclization reactions employing the alumina-supported sodium sulfide reagent, which obviates the need for high dilution conditions.[10] When freshly prepared sodium sulfide reagent was employed in our synthesis, the yield of sulfide **4** was quite good; however, a lower yield of sulfide **4** was obtained, and variable amounts of thiophene **6** were isolated from the reaction mixture, if the alumina-supported reagent was not newly prepared.[10] In our hands, the characteristic pink color of the freshly prepared reagent is lost when the reagent is stored in a desiccator for a few weeks. In this case the sodium sulfide reagent may have undergone reaction with oxygen and carbon dioxide in the air to produce sodium thiosulfate and sodium carbonate. The sodium carbonate could then serve as a base to promote the rearrangement of the bis(propargylic) sulfide **4** to the bis(allenylic) sulfide **7**.

Braverman and Duar have shown that bis(allenylic) sulfides can undergo a facile reaction to produce 3-vinylic substituted thiophenes, presumably via a diradical intermediate (8).[11] Indeed, treatment of bis(propargylic) sulfide 4 with KOH/methanol also produces the thiophene 6.



7 8

When incubated in Tris buffer, pH 8.5, bis(propargylic) sulfone crown ether 5 cleaves supercoiled double-stranded DNA (Form I) to produce circular relaxed (Form II) and linear (Form III) double-stranded products (Figure 1). Neither the addition of the radical scavenger cimetidine,[12] nor the exclusion of oxygen from the reaction inhibit the ability of compound 5 to cleave DNA (data not shown). As expected, the thiophene 6 is devoid of any DNA-cleaving properties. From these results, it appears that the DNA cleavage reaction of the bis(propargylic) sulfone crown ether 5 proceeds exclusively from nucleophilic attack by DNA on the allenylic sulfone moiety derived from base-catalyzed isomerization of 5. The resulting alkylated DNA adducts can undergo DNA strand scission under the prolonged alkaline reaction conditions. This is in accord with the conclusion of Nicolaou and co-workers, who have employed gel electrophoresis to determine that bis(propargylic) sulfone **10** cleaves specifically at guanine residues, presumably by alkylation of the N-7 position.[4]

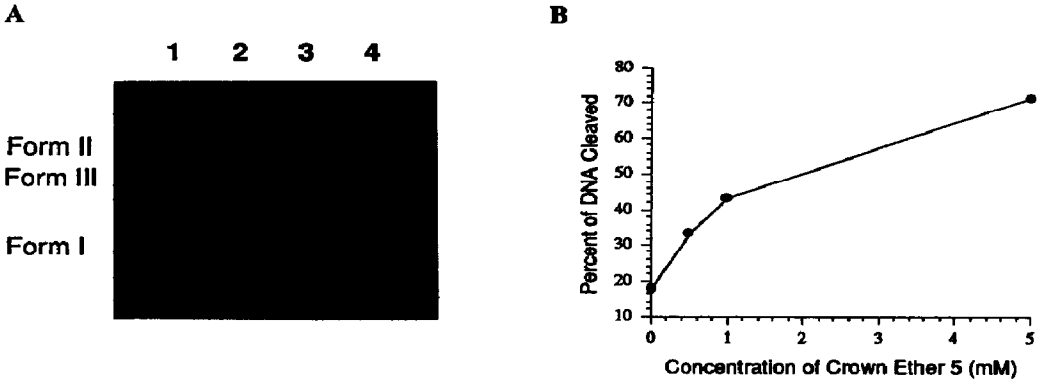
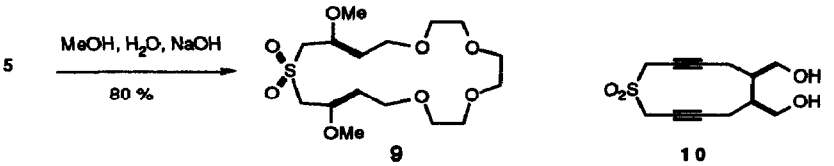


FIGURE 1. Cleavage of supercoiled ØX174 plasmid DNA by crown ether 5. A. ØX174 Form I DNA (160 μM per base pair) in 50 mM Tris, pH 8.5, was incubated for 24 h at 37 °C with crown ether 5 and analyzed by electrophoresis (0.7 % agarose gel, 60 V, 1.25 h, ethidium bromide stain). Lane 1, 0 mM 5; lane 2, 0.5 mM 5; lane 3, 1 mM 5; lane 4, 5 mM 5. B. A negative image of the gel was scanned by a laser densitometer to provide an estimate of the amounts of Forms I, II, and III. The percent cleavage was calculated by the equation $\frac{[\text{Form II}] + 2[\text{Form III}]}{[\text{Form I}] + [\text{Form II}] + 2[\text{Form III}]} \times 100$. The Form I DNA used was contaminated with approximately 15 % Form II.

The proposed mechanism for DNA cleavage by sulfone **5** requires that the allenylic form of **5** serve as an electrophile without competing Braverman-Duar cyclization. In order to test this proposal, a methanolic solution of the bis(propargylic) sulfone crown ether **5** was treated with aqueous sodium hydroxide. Under these conditions, the bis(enol ether) crown ether **9** is obtained in high yields.[13] Bis(enol ether) **9** appears to be a single, but as yet undefined, isomer by ^1H NMR. No thiophene dioxide-derived products were detected, indicating that nucleophilic addition to the allenylic sulfone can occur to the exclusion of Braverman-Duar cyclization.



Investigations of the metal ion binding of crown ether **5** and metalloregulated DNA cleavage are underway.[14]

References and Notes

1. Portions of this work were presented as a poster at the Thirty-Third National Organic Chemistry Symposium, Montana State University, June 13-17, 1993.
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14. This work was supported by a grant from the American Association of Colleges of Pharmacy. Mr. Zheng Chai is acknowledged for technical assistance.

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