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Propargylic Sulfone-Armed Lariat Crown Ethers: Alkali Metal Ion-Regulated DNA Cleavage Agents

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Alkali metal ion concentrations within cells are regulated during the cell cycle and may be significantly altered in cancer cells versus normal cells. Thus, the selective destruction of cancer cells might be achieved by agents that marry alkali metal ion recognition elements, such as crown ethers, with DNA-cleaving moieties. We have prepared a series of propargylic sulfone-armed lariat crown ethers, which bind sodium and potassium ions and exhibit little affinity for lithium ions, as determined by a picrate extraction assay. We have investigated the supercoiled DNA cleavage efficiency of these lariat crown ethers in the presence of various alkali metal ions. Monomeric propargylic sulfone-armed crown ethers **9b** and **10b** cleave DNA at physiologically relevant pH and exhibit modest increases in DNA cleavage efficiency in the presence of potassium and sodium as compared to lithium. In Tris-containing buffer, the monomeric lariat crown ether **10b** cleaves DNA in a sodium ion-dependent fashion, producing 66% more DNA cleavage in the presence of 5.7 mM sodium than in the absence of added sodium. The dimeric propargylic sulfone-armed lariat crown ether **11** cleaves DNA over 10-fold better in the presence of potassium than in the presence of lithium. While the level of metal ion discrimination exhibited by these compounds is rather modest, they do represent the first successful attempt to marry molecular recognition of specific alkali metal ion with covalent modification of DNA.

A number of structurally diverse DNA interactive agents form complexes with metal ions, and metal ion complexation plays an important role in the association of these molecules with DNA. DNA binding natural products, such as the aureolic acids mithramycin (*1*) and chromomycin A_3 (*2*) and the recently isolated cytotoxic bis(benzoxaole) UK-1 (*3*) bind to DNA as drug-Mg^{2+} complexes. The quinobenzoxazines, a class of antitumor fluoroquinolone analogs, form 2:2 drug–Mg^{2+} dimers in solution, and this metal ion-induced drug dimerization is required for the DNA binding (*4*), topoisomerase II inhibition (*5*), and photochemical DNA cleavage (*6*) exhibited by these compounds. The glycopeptide-derived antitumor antibiotic bleomycins cleave double-stranded DNA in a process that requires specific metal ions, especially Fe^{2+} (*7*). Given the established role of metal ion recognition in these anticancer compounds, a number of workers have attempted to design metallo-regulated DNA interactive agents (*8*).

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Dervan and Griffin have reported the synthesis of a bis-distamycin analog containing an oligoethyleneglycol linker (*9*). This DNA affinity cleavage agent displays a unique DNA sequence cleavage pattern in the presence of Ba^{2+} or Sr^{2+}, presumably due to the ability of the DNA-bound host to form a specific metal ion recognition site.

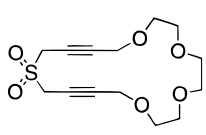
Less well studied are systems in which alkali metal ion recognition is coupled to DNA binding. Fukuda and coworkers have demonstrated that an anthracene-bearing 15-crown-5 analog displays increased DNA affinity in the presence of K^+ or Na^+ (*10*). Tsukube has proposed the use of lariat crown ethers in the supramolecular assembly of DNA interactive agents on DNA (*11*). The polyelectrolyte nature of DNA results in a high, local concentration of cations in the immediate vicinity of the DNA phosphate backbone (*12*). This locally high concentration of cations may serve to drive the complexation of weak alkali metal ion ligands attached to DNA interactive moieties. The effect of complexation can be to increase the affinity of the ligand for DNA or to induce a reactive conformation of a DNA-cleavage moiety (*13*).

Alkali metal ion concentrations within cells are regulated during the cell cycle (*14*), and may be significantly altered in cancer cells versus normal cells (*15–17*). This latter observation may be related to the overexpression (*18*) or activation (*19*) of certain sodium channel proteins within tumor cells. Thus, the selective destruction of cancer cells might be achieved by agents that marry alkali metal ion recognition elements, such as crown ethers, with DNA-cleaving moieties.

Nicolaou and coworkers have described propargylic sulfone-containing molecules as novel DNA-cleaving agents (*20*). Propargylic sulfones isomerize in mildly basic medium to allenic sulfones and consequently may serve as reactive electrophiles that alkylate DNA nucleophiles such as the N-7 of guanine. These alkylated bases may then undergo Maxam-Gilbert chemistry to effect DNA strand scission (*21*). Several groups have investigated the feasibility of modulating the DNA cleavage potential of propargylic sulfones by controlling the propargylic to allenic isomerization or by the incorporation of DNA-recognition elements that might increase the affinity of these molecules for DNA (*22*).

We previously reported the synthesis and DNA cleavage chemistry of the novel bis(propargylic) sulfone crown ether **1** (*23*). Dai and coworkers subsequently reported the synthesis and cytotoxicity of the propargylic sulfone-armed lariat crown ether **2** (*24*). Crown ether **2** does not display enhanced cytotoxicity relative to noncrown ether containing analog **3,** and the effect of alkali metal ions on the DNA cleavage due to this crown ether was not reported. More recently, this same group has reported cleavage due to the propargylic sulfone-armed lariat crown ether **4** (*25*). Surprisingly, these authors report no effect of added 1mM Na^+, K^+, or Sr^{2+} acetate on the cleavage of supercoiled ϕX174 DNA by **4,** and an inhibition of DNA cleavage in the presence of 1 mM $Ba(OAc)_2$.

Herein we report the preparation and metal ion binding properties of propargylic sulfone-armed lariat crown ethers **(9b, 10b)** and a model propargylic sulfone (**9a,** Scheme 1) that is devoid of alkali metal ion recognition capacity. We also report the preparation of the potential DNA double strand-cleaving, dimeric bis(propargyl) sulfone **11** (Eq. [3]). We additionally report a method for investigating the DNA cleavage efficiency of propargylic sulfones in the presence of various alkali metal ions. The crown ethers **9b, 10b,** and **11** are the first compounds reported to possess alkali metal



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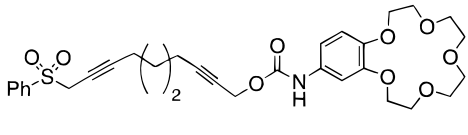

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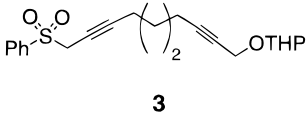

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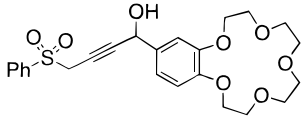

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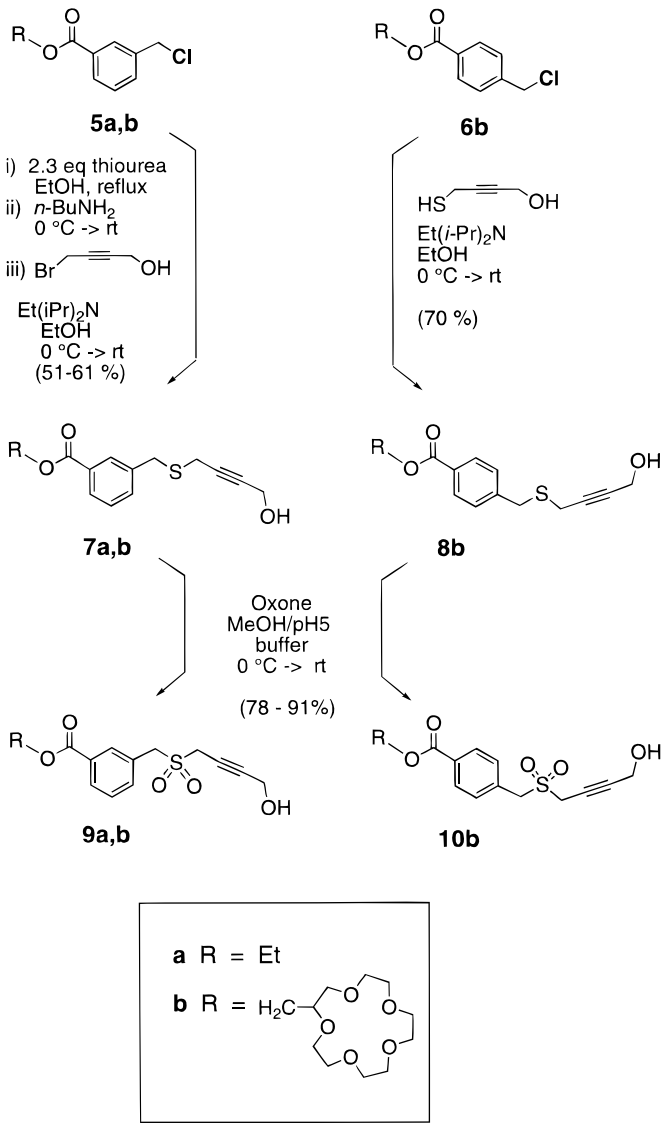
ion-enhanced DNA cleavage activity. The ability of these propargylic sulfone-armed lariat crown ethers to effect metallo-regulated DNA cleavage contrasts with results reported by Dai and coworkers, and we offer potential explanations for this disparity.

EXPERIMENTAL SECTION (37)

3-Chloromethylbenzoic acid, ethyl ester (*5a*) (*38*). To a solution of 3-chloromethylbenzoyl chloride (98% w/w, 0.502 g, 2.6 mmol) in 3 ml of THF was added dropwise via cannula with stirring a solution of EtOH (470 ml, 8.0 mmol), DMAP (0.066 g, 0.54 mmol), and pyridine (215 µl, 2.66 mmol) in 2 ml of THF. The reaction mixture was heated under reflux for 11 h, allowed to cool to room temperature, and transferred to a separatory funnel containing 50 ml of EtOAc and 25 ml of 15:5:5 brine/saturated aqueous NaH_2PO_4/water. The layers were mixed and then separated, and the aqueous layer was extracted with EtOAc (3 × 15 ml). The combined organic layers were washed with water (15 ml) and brine (15 ml). The organic layer was dried, concentrated, and the residue was purified by flash column chromatography on silica gel (20% EtOAc in hexanes) to afford ester **5a** (0.426 g, 83%) as a colorless oil: R_F 0.59 (20% EtOAc in hexanes); ^{1}H NMR δ 1.38 (t, *J* = 8.6 Hz, 3H), 4.35 (q, *J* = 8.6 Hz, 2H), 4.59 (s, 2H), 7.4 (t, *J* = 9.6 Hz, 1H), 7.55 (d, *J* = 9.6 Hz, 1H), 7.97 (d, *J* = 9.6 Hz, 1H), 8.03 (s, 1H); ^{13}C NMR d 14.21, 45.44, 61.04, 128.74, 129.4, 129.5, 130.94, 132.79, 137.71, 165.93; IR 1719, 723 cm^{-1}; MS 199 (MH^+), 163; HRMS *m/e* calcd for $C_{10}H_{12}ClO_2$: 199.0526; found 199.0528.

3-Chloromethylbenzoic acid, 1,4,7,10,13-pentaoxacyclopentadec-2-ylmethyl ester **(5b)**. To an argon flushed, chilled (-78°C) 10-ml round bottom flask containing a small stir bar, 30, 4 Å molecular sieves and a solution of 2-(hydroxymethyl)-15-crown-5 (95% w/w, 333 mg, 1.26 mmol) and TMEDA (115 µl, 0.762 mmol) in 4.8 ml of CH_2Cl_2 was added dropwise with stirring via canula a solution of 3-chloromethylbenzoyl chloride (98% w/w, 252 mg, 1.31 mmol) in 1.2 ml of THF. Stirring was continued for an additional 21.5 h as the reaction mixture warmed to room temperature. The reaction mixture was transferred to a separatory funnel containing 50 ml of EtOAc and 40 ml of 30:10 water/saturated aqueous KH_2PO_4. The layers were mixed and then separated, and the aqueous layer was extracted with EtOAc (4 × 50 ml). The combined organic layers were washed with 1:1 water/aqueous saturated $KHCO_3$ (2 × 35 ml). This aqueous wash was extracted with EtOAc (2 × 50 ml) and the pooled EtOAc layers were dried (K_2SO_4), concentrated and the residue was purified by flash column chromatography on silica gel (10% MeOH in $CHCl_3$) to afford ester **5b** (336 mg, 66%) as a pale



SCHEME 1

yellow oil: R_F 0.56 (10% MeOH in $CHCl_3$); ^{1}H NMR d 3.35–3.62 (m, 16H), 3.62–3.77 (m, 2H), 3.77–3.86 (m, 1H), 4.16 (dd, *J* = 11.8, 6.5 Hz, 1H), 4.31 (dd, *J* = 11.8, 4.7 Hz, 1H), 4.44 (s, 2H), 7.26 (t, *J* = 7.7 Hz, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.82 (d, *J* = 7.7 Hz, 1H), 7.88 (s, 1H); ^{13}C NMR d 44.99, 64.58, 69.89, 69.98, 70.06 (3C), 70.23, 70.39 (2C), 70.71, 77.00, 128.39, 129.02, 129.14, 130.19, 132.55, 137.41, 165.25; IR 1730, 714 cm^{-1}; MS 403 (MH^+), 367; HRMS *m/e* calcd for $C_{19}H_{28}ClO_7$: 403.1524; found 403.1519.

4-Chloromethylbenzoic acid, 1,4,7,10,13-pentaoxacyclopentadec-2ylmethyl ester **(6b)**. To a dry ice-acetone bath-cooled solution of 2-(hydroxymethyl)-15-crown-5 (95% w/w,

0.278 g, 1.057 mmol), TMEDA (96 μl, 1.057 mmol), and 4 Å molecular seives (25–30) in CH_2Cl_2 (4 ml) was added a solution of of 4-chloromethylbenzoyl chloride (97% w/w, 0.244 g, 1.254 mmol) in 1 ml of CH_2Cl_2. The resulting solution was allowed to slowly warm to room temperature over 35 min and stirred at room temperature for an additional 24 h. The reaction mixture was diluted with 35 ml of EtOAc and 30 ml of 5:25 saturated aqueous KH_2PO_4/water, the layers were mixed and then separated, and the aqueous layer was extracted with EtOAc (4 × 40 ml). The combined organic layers were washed with 1:1 saturated aqueous $KHCO_3$/water (2 × 30 ml), and these combined aqueous washes were reextracted with EtOAc (2 × 30 ml). The combined organic layers were dried (K_2SO_4), concentrated, and the residue was purified by flash column chromatography on silica gel (10% MeOH in $CHCl_3$) followed by preparative TLC (10% MeOH in $CHCl_3$) to afford ester **6b** (0.340 g, 80%) as a light pink oil: R_F 0.54 (10% MeOH in $CHCl_3$); ^{1}H NMR d 3.50–3.72 (m, 16H), 3.72–3.85 (m, 2H), 3.85–3.97 (m, 1H), 4.26 (dd, *J* = 11.8, 6.5 Hz, 1H), 4.41 (dd, *J* = 11.8, 4.7 Hz, 1H), 4.53 (s, 2H), 7.38 (d, *J* = 9.4 Hz, 2H), 7.96 (d, *J* = 9.4 Hz, 2H); ^{13}C NMR d 45.24, 64.87, 70.29, 70.42 (3C), 70.44, 70.58, 70.66, 70.81, 71.01, 77.44, 128.38, 129.95 (2C), 142.21, 165.78; IR 1724, 722 cm^{-1}; MS 403 (MH^+), 367; HRMS *m/e* calcd for $C_{19}H_{28}ClO_7$: 403.1524; found 403.1516.

3-[S-(4-hydroxybut-2-ynyl)thiomethyl]benzoic acid, ethyl ester **(7a)**. A solution of compound **5a** (0.229 g, 1.15 mmol) and thiourea (0.097 g, 1.27 mmol) in 3 ml of EtOH was heated with stirring under reflux for 21 h. The reaction mixture was then cooled with an ice-water bath, and *n*-butylamine (165 μl, 1.67 mmol) was added with stirring. The reaction mixture was allowed to stir for 2 h as the ice-water bath melted and warmed to room temperature. The reaction mixture was diluted with 30 ml of $CHCl_3$ and 25 ml of 15:5:5 saturated aqueous KCl/saturated aqueous KH_2PO_4/water, the layers were mixed, separated, and the aqueous layer was extracted with $CHCl_3$ (3 × 10 ml). The organic layer was dried (K_2SO_4), concentrated, and the residue was purified by flash column chromatography on silica gel (20% EtOAc in hexanes) to afford the thiol (0.166 g, 73%) as a colorless oil: R_F 0.63 (20% EtOAc in hexanes); ^{1}H NMR δ 1.35 (t, *J* = 8.0 Hz, 3H), 1.76 (t, *J* = 8.3 Hz, 1H), 3.72 (d, *J* = 8.3 Hz, 2H), 4.33 (q, *J* = 8.0 Hz, 2H), 7.34 (t, *J* = 8.5 Hz, 1H), 7.47 (d, *J* = 7.47 Hz, 1H), 7.87 (d, *J* =8.5 Hz, 1H), 7.95 (s, 1H); ^{13}C NMR δ 14.17, 28.46, 60.86, 128.06, 128.56, 128.90, 130.73, 132.33, 141.34, 166.10; IR 2574, 1721 cm^{-1}; MS 197 (MH^+), 163; HRMS *m/e* calcd for $C_{10}H_{13}O_2S$: 197.0636; found 197.0633. To a solution of this thiol (0.097 g, 0.495 mmol) in 3 ml of EtOH was added 4-bromo-2-butynl-*ol* (*38*) (99 μl of a 1.13 mg/μl solution in EtOH, 0.95 mmol) with stirring and cooling via ice-water bath. Diisopropylethylamine (105 μl, 0.092 mmol) was added and, after 5 min, the cooling bath was removed and the reaction mixture was stirred an additional 21 h at room temperature. The reaction mixture was diluted with 25 ml of EtOAc and 25 ml of 15:5:5 saturated aqueous KCl/saturated aqueous KH_2PO_4/water, the layers were mixed, separated, and the aqueous layer was extracted with EtOAc (3 × 15 ml). The combined organic layers were washed with water (10 ml) and saturated aqueous KCl (15 ml). The organic layer was dried (K_2SO_4), concentrated, and the residue was purified by flash column chromatography on silica gel (1:1 EtOAc/hexanes) to afford sulfide **7a** (0.110 g, 84%, or 61% overall from **5a**) as a pale yellow oil: R_F 0.55 (1:1 EtOAc/hexanes); ^{1}H NMR δ 1.38 (t, *J* = 8.0 Hz, 3H), 2.13 (s(br), 1H), 3.09 (t, *J* = 2.2 Hz, 2H), 3.88 (s, 2H), 4.30 (t, *J* = 2.2 Hz, 2H), 4.36 (q, *J* = 8.0 Hz, 2H), 7.37 (t, *J* =8.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 8.03 (s, 1H); ^{13}C NMR d 14.27, 18.91, 35.15, 51.15, 61.14,

81.28, 81.90, 128.30, 128.59, 130.29, 130.62, 133.39, 138.00, 166.56; IR 3443, 1716 cm^{-1}; MS 265 (MH^+), 247, 219; HRMS *m/e* calcd for $C_{14}H_{17}O_3S$: 265.0898; found 265.0896.

3-[S-(4-hydroxybut-2-ynyl)thiomethyl]benzoic acid, 1,4,7,10,13-pentaoxacylopentadec-2-ylmethyl ester **(7b)**. Following the same general procedure as in the preparation of **7a** above, chloride **5b** (0.45 g, 1.12 mmol) gave a residue after workup that was purified by flash column chromatography on silica gel (10% MeOH in $CHCl_3$) to afford the thiol (0.277 g, 62%) as a light yellow oil: R_F 0.60 (10% MeOH in $CHCI_3$); ^1H NMR d 1.60–1.94 (s(br), 1H), 3.40–3.62 (m, 16H), 3.64 (s, 2H), 3.65–3.77 (m, 2H), 3.77–3.89 (m, 1H), 4.18 (dd, *J* = 11.8, 6.5 Hz, 1H), 4.34 (dd, *J* = 11.8, 4.7 Hz, 1H), 7.25 (t, *J* = 9.7 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 7.84 (s, 1H); ^{13}C NMR d 28.14, 64.54, 69.98, 70.05, 70.15 (2C), 70.32 (2C), 70.44, 70.54, 70.76, 77.13, 127.85, 128.36, 128.76, 130.10, 132.29, 141.16, 165.65; IR 2550, 1724 cm^{-1}; MS 401 (MH^+), 367; HRMS *m/e* calcd for $C_{19}H_{29}0_7S$: 401.1634; found 401.1632. This thiol (0.051 g, 0.128 mmol) was alkylated as in the preparation of **7a** above to give a residue after workup that was purified by preparative TLC (1-mm silica gel plate, 10% MeOH in $CHCl_3$) to afford sulfide **7b** (0.049 g, 83%, or 51% overall from **5b**) as a light pink oil: R_F 0.50 (10% MeOH in $CHCl_3$); ^1H NMR d 2.9 (s(br), 1H), 3.06 (t, *J* = 2.2 Hz, 2H), 3.51–3.75 (m, 16H), 3.75–3.91 (m, 2H), 3.86 (s, 2H), 3.91–4.02 (m, 1H), 4.28 (dd, *J* = 11.8, 6.5 Hz, 1H), 4.28 (s(br), 2H), 4.41 (dd, *J* = 11.8, 4.7 Hz, 1H), 7.37 (t, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 8.0 (s, 1H); ^{13}C NMR d 18.61, 34.70, 50.63, 64.52, 70.04, 70.11, 70.18, 70.27, 70.34, 70.43, 70.55, 70.71, 70.86, 77.33, 80.38, 82.45, 128.30, 128.60, 129.96, 130.13, 133.49, 138.02, 166.13; IR 3361, 1727 cm^{-1}; MS 469 (MH+), 403; HRMS *m/e* calcd for $C_{23}H_{33}O_8S$: 469.1896; found 469.1899.

4-[S-(4-hydroxybut-2-ynyl)thiomethyl]benzoic acid, 1,4,7,10,13-pentaoxacylopentadec-2-ylmethyl ester **(8b)**. To a 5-ml round bottom flask containing a suspension of thiourea (28.1 mg, 0.37 mmol) in 400 ml of EtOH was added 4-bromo-2-butyn-1-*ol*(*39*) (45 µl of a 1.13 mg/ml solution in EtOH, 0.343 mmol) with stirring. An argon-flushed reflux condenser equipped with a Teflon sleeve over the male joint was attached to the reaction vessel, and the reaction mixture was heated for 14 h at 48°C and then allowed to cool to room temperature. The reflux condenser was replaced with a septum, and an additional 100 µl of EtOH was added. The reaction mixture was cooled to 0°C via ice-water bath. *n*-$BuNH_2$ (34 μl, 0.343 mmol) was added with stirring as the reaction mixture was allowed to warm to room temperature over 45 min. A solution of chloride **6b** (0.138 g, 0.343 mmol) in 400 μl of EtOH was added slowly via cannula with stirring followed by Hünig's base (60 μl, 0.343 mmol). The reaction mixture was allowed to stir at room temperature for an additional 22 h and was then transferred to a separatory funnel containing 70 ml of EtOAc and 62 ml of 42:10:10 saturated aqueous KCl/saturated aqueous KH_2PO_4/water. The layers were mixed, allowed to separate, and the aqueous layer was extracted with 2 × 40 ml of EtOAc. The combined organic layers were washed with 15 ml of water and 25 ml of saturated aqueous KCl and dried. The residue upon concentration of the organic layer was purified by preparative TLC (1-mm silica gel plate, 10% MeOH in $CHCl_3$) to afford two sulfide **8b**-containing fractions, A and B: fraction A (lower R_F, 75.5 mg, pale yellow oil) contained pure sulfide **8b;** fraction B (36.9 mg) contained 15% w/w sulfide **8b** contaminated with starting material **6b.** The total yield of compound **8b** based on recovered **6b** was 65%. Analytical data for sulfide **8b:** R_F 0.52 (10% MeOH in $CHCl_3$); ^1H NMR d 2.25 (s(br), 1H), 3.08 (t, *J* = 2.2 Hz, 2H), 3.52–3.70 (m, 16H), 3.70–3.87 (m, 2H), 3.84 (s, 2H), 3.87–4.0 (m, 1H), 4.27 (dd, *J* = 11.8, 6.5 Hz, 1H), 4.27 (s(br), 2H), 4.44 (dd, *J* = 11.8, 4.7

Hz, 1H), 7.36 (d, *J* = 8.7 Hz, 2H), 7.95 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR d 18.85, 35.16, 51.05, 64.77, 70.36, 70.43 (2C), 70.51 (2C), 70.70, 70.79, 70.96, 71.10, 77.58, 81.02, 81.79, 128.79, 128.94, 129.81, 143.00, 166.10; IR 3576, 1725 cm^{-1}; MS 469 (MH^+), 451, 401; HRMS *m/e* calcd for $C_{23}H_{33}O_8S$: 469.1896; found 469.1904.

General procedure for sulfone formation: 4-[S-4-hydroxybut-2-ynyl)sulfonylmethyl]benzoic acid, 1,4,7,10,13-pentaoxacyclopentadec-2-ylmethyl easter **(10b)**. To an ice-water bath-cooled solution of sulfide **8b** (0.014 g, 0.03 mmol) in 350 µl of MeOH was added dropwise with vigorous stirring a solution of Oxone (49.5% w/w $KHSO_5$, 0.13 g, 0.105 mmol) in 350 μl of water and 150 μl of 2.5M, pH 5.5, potassium citrate buffer, and the resulting heterogeneous reaction mixture was allowed to stir overnight as the ice-water bath melted. The reaction mixture was diluted with 25 ml of water and this was extracted with $CHCl_3$ (3 × 15 ml). The combined organic extracts were washed with water (10 ml) and saturated aqueous KCl (15 ml). The residue upon drying (K_2SO_4) and concentration of the organic layer afforded sulfone **10b** (0.014 g, 91%) as a pale pink oil. Sulfone **10b** exhibited some isomerization to the allene after chromatographic purification (silica gel) was attempted via flash column or preparative TLC. When necessary, compound **10b** could be purified via C-18 derivatized silica gel, employing 60:40 MeOH/water as the eluant. Analytical data for sulfone **10b:** R_F 0.40 (10% MeOH in $CHCl_3$); ^{1}H NMR d 3.33 (s (br), 1H), 3.55–3.74 (m, 16H), 3.70 (s(br), 2H), 3.74–3.87 (m, 2H), 3.87–4.0 (m, 1H), 4.32 (dd, *J* = 11.8, 6.5 Hz, 1H), 4.35 (s(br), 2H), 4.48 (dd, *J* = 11.8, 4.7 Hz, 1H), 4.49 (s, 2H), 7.55 (d, *J* = 9.2 Hz, 2H), 8.05 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR d 43.84, 50.86, 57.19, 64.89, 70.16 (3C), 70.34 (2C), 70.43, 70.50, 70.75, 70.93, 72.78, 77.36, 87.34, 130.28, 130.97 (2C), 132.43, 165.72; IR 3332,1722, 1329 cm^{-1}; MS 501 (MH^+), 433, 369; HRMS *m/e* calcd for $C_{23}H_{33}O_{10}S$: 501.1794, found 501.1786.

3-[S-(4-hydroxybut-2-ynyl)sulfonylmethyl]benzoic acid, ethyl ester **(9a)**. Following the general procedure (see compound **10b**), sulfide **7a** (0.02 g, 0.076 mmol) gave a residue after workup that was purified by flash column chromatography on silica gel (1:1 EtOAc/hexanes) to afford sulfone **9a** (0.018 g, 82%) as a colorless, crystalline solid: m.p. 79.5–80.5∞C; R_F 0.29 (1:1 EtOAc/hexanes); ^{1}H NMR d 1.39 (t, *J* = 8.4 Hz, 3H), 2.44 (s(br), 1H), 3.67 (t, *J* = 2.2 Hz, 2H), 4.36 (t, *J* = 2.2 Hz, 2H), 4.37 (q, *J* = 8.4 Hz, 2H), 4.49 (s, 2H), 7.50 (t, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 8.05 (d, *J* = 8.8 Hz, 1H), 8.18 (s, 1H); ^{13}C NMR d 14.25, 43.77, 50.97, 57.35, 61.52, 73.08, 87.42, 127.93, 129.30, 130.26, 131.23, 132.25, 135.01, 166.11; IR 3494, 1718, 1316 cm^{-1}; MS 297 (MH^+), 279, 251, 163; HRMS *m/e* calcd for $C_{14}H_{17}O_5S$: 297.0797; found 297.0798.

3-[S-(4-hydroxybut-2-ynyl)sulfonylmethyl]benzoic acid, 1,4,7,10,13-pentaoxapentadec-2-ylmethyl ester **(9b)**. Following the general method (see compound **10b**), compound **7b** (0.048 g, 0.103 mmol) afforded after workup sulfone **9b** (0.04 g, 78%) as a colorless oil. Sulfone **9b** exhibited partial isomerization to the allene when chromatographic purification involving silica gel (preparative silica gel TLC) was attempted. When necessary, compound **9b** could be purified via C-18 derivatized silica gel, employing 60:40 MeOH/water as the eluant. Analytical data for sulfone **9b:** R_F 0.35 (10% MeOH in $CHCl_3$); ^{1}H NMR d 3.30 (s(br), 1H), 3.53–3.73 (m, 18H), 3.73–3.90 (m, 2H), 3.90–4.04 (m, 1H), 4.33 (dd, *J* = 11.8, 6.5 Hz, 1H), 4.36 (t, *J* = 2.2 Hz, 2H), 4.43 (dd, *J* = 11.8, 4.7 Hz, 1H), 4.48 (s, 2H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 8.17 (s, 1H); ^{13}C NMR d 43.48, 50.58, 56.85, 64.77, 70.10, 70.15, 70.32, 70.44, 70.62, 70.95, 72.55, 77.31, 88.00, 128.17, 129.33, 130.39, 130.79, 131.89, 135.37, 165.62; IR 3354, 1727, 1331 cm^{-1}; MS 501 (MH^+), 369; HRMS *m/e* calcd for $C_{23}H_{33}O_{10}S$: 501.1794; found 501.1802.

Compound 11. A stirring solution of sulfide **7b** (0.139 g, 0.297 mmol), DMAP (0.018 g, 0.147 mmol) and diisopropylethylamine (48 μl, 0.276 mmol) in 1.1 ml of THF was cooled with an ice-water bath and malonyl dichloride (97% w/w, 16 μl, 0.16 mmol) was added dropwise. After 10 min the cooling bath was removed and the reaction mixture was heated under reflux for 13 h. Upon cooling to room temperature, the reaction mixture was diluted with 75 ml of EtOAc and 50 ml of 30:10:10 saturated aqueous KCl/saturated aqueous KH_2PO_4/water. The layers were mixed, separated, and the aqueous layer was extracted with EtOAc (3×30 ml). The combined organic layers were washed with water (20 ml), saturated aqueous $KHCO_3$ (2×20 ml), and saturated aqueous KCl (35 ml). The organic layer was dried (K_2SO_4), concentrated, and the residue was purified by preparative TLC (2-mm silica gel plate, 10% MeOH in $CHCl_3$) to give two product-containing fractions that were each resubjected to purification via preparative TLC (1-mm silica gel plate, 10% MeOH in EtOAc). The subsequent product-containing fractions were pooled to afford the corresponding bis(sulfide) (0.029 g, 26% based on recovered **7b** (0.035 g)) as a colorless oil: R_F 0.52; 0.07 (10% MeOH in $CHCl_3$; 10% MeOH in EtOAc); ^{1}H NMR δ 3.08 (t, J = 2.2 Hz, 4H), 3.50 (s, 2H), 3.55–3.70 (m, 3H), 3.70–3.87 (m, 4H), 3.85 (s, 4H), 3.87–4.0 (m, 2H), 4.29 (dd, J = 11.8, 6.5 Hz, 2H), 4.44 (dd, J = 11.8, 4.7 Hz, 2H), 4.77 (t, J = 2.2 Hz, 4H), 7.37 (t, J = 8.0 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 8.0 Hz, 2H), 7.97 (s, 2H); ^{13}C NMR δ 18.71, 35.06, 40.89, 53.48, 64.83, 70.34, 70.42, 70.50, 70.53, 70.68 (2C), 70.79, 70.95, 71.09, 76.63, 77.53, 83.10, 128.45, 128.60, 130.11, 130.45, 133.54, 137.91,165.44, 166.10; IR 1770, 1726 cm^{-1}; MS (FAB) 1005 (MH^+); HRMS (FAB) *m/e* calcd for $C_{49}H_{65}O_{18}S_2$: 1005.3612; found 1005.3603. Following the general procedure for sulfone formation (see Compound **10b;** requires 7 equivalents of oxidant to oxidize both sulfur atoms), Oxone oxidation of the bis(sulfide) (0.017 g, 0.17 mmol) afforded after workup bis(sulfone) **11** (0.016 g, 93%) as a colorless oil: ^{1}H NMR δ 3.54–3.75 (m, 32H), 3.58 (s, 2H), 3.71 (s(br), 4H), 3.75–3.88 (m, 4H), 3.88–4.02 (m, 2H), 4.32 (dd, J = 11.8, 6.5 Hz, 2H), 4.46 (dd, J = 11.8, 4.7 Hz, 2H), 4.48 (s(br), 4H), 4.83 (s, 4H), 7.48 (t, J = 7.5 Hz, 2H), 7.67 (d, J = 7.5 Hz, 2H), 8.05 (d, J = 7.5 Hz, 2H), 8.11 (s, 2H); ^{13}C NMR d 40.76, 43.52, 53.08, 57.02, 65.10, 70.31 (3C), 70.48 (2C), 70.61, 70.71, 70.78, 71.07, 75.00, 77.43, 82.08, 127.93, 127.93, 129.28, 130.35, 131.09, 131.98, 135.24, 165.45, 135.59; IR 1770, 1726, 1334 cm^{-1}; MS (FAB) 1069 (MH^+); HRMS (FAB) *m/e* calcd for $C_{49}H_{65}O_{22}S_2$: 1069.3409; found 1069.3396.

Di(4-hydroxybut-2-ynyl)sulfone **(*12*)** (*18*). To a solution of the THP-protected 4-bromo-2-butyn-1-*ol (11)* (0.594 g, 2.6 mmol) in 1.7 ml of CH_2Cl_2 and 0.3 ml of EtOH was added $Na_2S{\cdot}Al_2O_3$ (21% w/w Na_2S, 0.714 g, 1.9 mmol) in one portion and the reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was filtered through Celite, the solids were washed with CH_2Cl_2, and the solvent was evaporated. The residue was purified by flash column chromatography on silica gel (20% EtOAc in hexanes) to afford the sulfide (0.182 g, 42%) as a pale yellow oil: R_F 0.34 (20% EtOAc in hexanes); ^{1}H NMR δ 1.42–1.87 (m, 12H), 3.40 (t, J = 2.1 Hz, 4H), 3.46–3.56 (m, 2H), 3.74–3.86 (m, 2H), 4.24 (dt, J = 16.3, 2.1 Hz, 2H), 4.33 (dt, J = 16.3, 2.1 Hz, 2H), 4.76 (t(br), J = 3.7 Hz, 2H); ^{13}C NMR d 18.95, 19.37, 25.24, 30.14, 54.39, 61.88, 79.07, 81.05, 96.69; IR 1120, 1033 cm^{-1}; MS 339 (MH^+), 253, 237, 152; HRMS *m/e* calcd for $C_{18}H_{27}O_4S$: 339.1630; found 339.1634. To an ice-water bath-cooled solution of sulfide **3.19** (0.055 g, 0.16 mmol) in 4 ml of CH_2Cl_2 was added *m*-CPBA (50% w/w, 0.158 g, 0.46 mmol) in one portion and the reaction mixture was allowed to stir at 0°C for 0.5 h and then

at room temperature for 5.5 h. The reaction mixture was diluted with EtOAc (50 ml), washed with saturated aqueous Na_2SO_3 (2 × 15 ml), saturated aqueous $NaHCO_3$ (2 × 15 ml), and brine (20 ml) and dried. The solvent was evaporated and the residue was purified by flash column chromatography on silica gel (1:1 EtOAc in hexanes) to afford sulfone (0.027 g, 45%) as a colorless solid: m.p. 47–49°C; R_F 0.57 (1:1 EtOAc/hexanes); ^{1}H NMR δ 1.42–1.87 (m, 12H), 3.46–3.53 (m, 2H), 3.74–3.83 (m, 2H), 4.07 (t, J = 2.1 Hz, 4H), 4.24 (dt, J = 15.9, 2.1 Hz, 2H), 4.33 (dt, J = 15.9, 2.1 Hz, 2H), 4.76 (t(br), 2H); ^{13}C NMR d 18.96, 25.25, 30.15, 43.63, 54.21, 62.09, 72.78, 84.68, 97.18; IR 1342, 1133, 1035 cm^{-1}; MS 371 (MH^+), 285, 269; HRMS *m/e* calcd for $C_{18}H_{27}O_6S$: 371.1528; found 371.1509. To a solution of this sulfone (0.015 g, 0.04 mmol) in 1.5 ml of EtOH was added PPTS (0.024 g, 0.096 mmol) and the resulting reaction mixture was heated to 50°C with stirring for 7 h. Upon cooling to room temperature, the reaction mixture was diluted with EtOAc (20 ml) and 5:1 brine/water (12 ml). The layers were mixed and separated, and the aqueous layer was extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with brine (15 ml) and dried (Na_2SO_4), and the solvent was evaporated. The residue was triturated with a small amount of $CHCl_3$ and this was removed to afford sufone **12** (5 mg, 63%) as a colorless solid: R_F 0.56 (EtOAc); ^{1}H NMR d 1.65 (s(br), 2H), 4.07 (t, J = 2.0 Hz, 4H), 4.33 (t, J = 2.0 Hz, 4H); ^{13}C NMR (MeOH-d_4) δ 44.39, 50.75, 73.04, 87.65; MS 203 (MH^+), 185; HRMS *m/e* calcd for $C_8H_{11}O_4S$: 203.0378; found 203.0365.

Alkali metal picrates. Sodium and potassium picrate were prepared according to the reported method **(***40***)**. Lithium picrate was prepared by a modification of this method using lithium hydroxide. The salts were vacuum-dried (300 mm Hg) at 175°C for 2 days and ^{1}H-NMR analysis (DMSO-d_6) indicated that lithium and potassium picrate were anhydrous while sodium picrate was a monohydrate.

Alkali metal picrate extraction. The general procedure employed was that developed by Cram **(***27***)**. Distilled, demineralized water and spectrophotometric grade $CHCl_3$ and MeCN were used. $CHCl_3$ and water were saturated with each other prior to solution preparations as a means of preventing volume changes of the phases during the extractions. All glassware was washed with nonionic detergent, rinsed well with tap water, demineralized water, and methanol followed by drying in an oven (115°C) or a vacuum dessicator over P_2O_5. All operations were conducted at 23–24°C. In a typical extraction, 350 µl of 3.0 mM aqueous metal picrate and 350 µl of 3.0 mM host in $CHCl_3$ were placed in a 0.5 dram vial and this was immediately stoppered with a screw-cap. The vials were centrifuged for 1 min to drive the $CHCl_3$ layer to the bottom of the vial. The vials were then vortexed for 1 min with a Baxter S/P vortex mixer and centrifuged at high speed for 15 min with an International Clinical Centrifuge to effect complete phase separation. A 50-µl aliquot (gas tight syringe, volume measured by difference) of the $CHCl_3$ layer was removed from the middle and bottom of the vial and placed in a 1.0 ml volumetric tube and the volume was brought up to the mark with MeCN (dilution factor = 20). To ensure no contamination by the aqueous layer, the syringe needle was washed with a stream of demineralized water and dried with a Kimwipe before the aliquot was dispensed. The diluted $CHCl_3$ phase aliquot was homogenized by several inversions and transferred to a quartz cuvette dedicated for either lithium, sodium, or potassium extractions. Absorbance was measured at 380 nm against a blank prepared in a manner analogous to the extraction procedure above using demineralized water in place of aqueous metal picrate and host-free $CHCl_3$. The extractability of metal picrates by $CHCl_3$ in the absence of host was determined as above with $CHCl_3$ that

was free of host. The absorbance of the $CHCl_3$ layer (after dilution and against the aforementioned blank) for each metal picrate extracted was found to be 0.002 and this value was subtracted from all absorbance readings obtained from host-containing extraction experiments. An aliquot of the host solution in $CHCl_3$ was similarly diluted with MeCN and its absorbance was measured. This value was also subtracted from the absorbance value obtained from the extraction experiments.

The concentration of metal picrate in the $CHCl_3$ layer at equilibrium was determined from the absorbance of the diluted $CHCl_3$ layer with each metal picrate exhibiting an extinction coefficient (ε) of 16,900 $M^{-1}cm^{-1}$ at 380 nm in MeCN as determined previously by Cram and coworkers (*41*). Complex association constants (K_a) were determined by the method of Cram (*27*) from extraction constants (K_e; derived from absorbance measurements made on the $CHCl_3$ layer) and distribution constants (K_d; previously determined by Cram and coworkers (*27*)*;* i.e., lithium picrate, 1.42×10^{-3} M^{-1}; sodium picrate, 1.74×10^{-3} M^{-1}; potassium picrate, 2.55×10^{-3} M^{-1}). The values presented in Fig. 1 represent the mean plus or minus one standard deviation from two separate extractions.

Distribution of crown ether 9b. Equal volumes (2.53 ml) of a 3mM solution of crown ether **9b** in water-saturated $CHCl_3$ and 3 mM aqueous sodium picrate were homogenized in a screw-top vial via vortexing for 2 min and the mixture was allowed to stand overnight to allow complete phase separation. A 2.0-ml aliquot of the upper aqueous layer was carefully removed and dried *in vacuo;* the yellow solid residue was resuspended in $CDCl_3$ and subsequent ^1H NMR analysis revealed no trace of crown ether **9b.** Similar ^1H NMR analysis of a dried aliquot of the bottom $CHCl_3$ layer indicated the presence of crown ether **9b.**

Preparation of alkali metal ion DNA. A strain of DH5α *Escherichia coli* that contained the pGAD424 plasmid was incubated in Luria-Bertani media containing 400 μg/ml sodium ampicillin. Cells were collected and lysed, and the plasmid DNA was isolated with a QIAGEN miniprep spin kit according to the manufacturer's instructions. This afforded the DNA

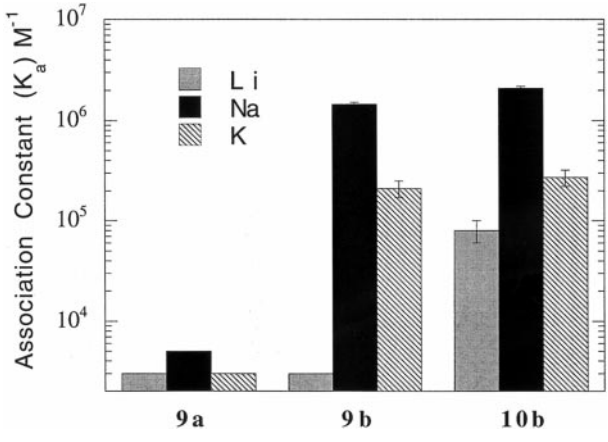


FIG 1. Alkali metal ion binding by propargylic sulfone-armed lariat crown ethers **9b** and **10b** and model compound **9a.** Metal ion binding association constants were determined in chloroform using the picrate extraction procedure. The values for the Li^+ ion association for compounds **9a** and **9b** are upper limits, as no detectable picrate salt was extracted into chloroform by these compounds.

as an aqueous solution in distilled, sterile water. Aliquots (10 μl) of this solution were gently mixed with 212.2 µl of sterile, pH 7.4, aqueous alkali metal phosphate solution. The plasmid DNA so obtained was determined spectrophotometrically to be 17 µM base pair and typically contained 75–85% supercoiled (Form I) DNA. For EC_{25} experiments, the concentration of alkal metal ion before addition of the aqueous DNA solution was 20 mM. For all other experiments, the concentration of lithium or sodium ions prior to addition of the aqueous DNA solution was either 0, 1.5, 3.0, or 6.0 mM in 18–20 mM Tris phosphate. It is worth noting that DNA solutions that were prepared using aqueous alkali metal acetates exhibited significantly less cleavage upon incubation with propargyic sulfones than solutions prepared as above which employed aqueous alkali metal phosphates.

Quantification of DNA cleavage by propargylic sulfones. In a typical cleavage experiment, 2 µl of a freshly prepared propargylic sulfone solution in d_6-DMSO (or, for control reactions, d_6-DMSO that was free of propargylic sulfone) was added to a small, sterile eppindorf tube followed by 14 μl of an aqueous solution of alkali metal ion-containing DNA prepared as above. The contents were mixed by brief (5 s) centrifugation at 6000 rpm and allowed to stand at room temperature for 19 h. Tubes were then heated for 90 s at 70°C. Upon cooling, 2 μl of 8× loading dye (13 mg/ml each of bromophenol blue and xylene cyanol in 30% aqueous glycerol) was added to each tube, the contents were gently homogenized, and 5 μl of the resulting solution was loaded onto a 0.7% w/v agarose gel. The DNA cleavage products were seperated by electrophoresis in 1× TBE running buffer at 45 V for 2.25 h. The agarose gel was stained for 15 min in TBE buffer that contained 0.25 μg/ml ethidium bromide and then destained in distilled water for 15–30 min. The gel was scanned with a Molecular Dynamics Fluorimager and the quantities of Forms I, II, and III DNA were assessed with the ImageQuaNT software program. The degree of cleavage of Form I DNA was determined using Eq. [1].

$$\text{Percent cleavage} = \frac{(2\times[\text{Form III}]+[\text{Form II}]}{(2\times[\text{Form III}]+[\text{Form II}]+[\text{Form I}]}\times 100 \qquad [1]$$

The reported, normalized percentage cleavage accounts for cleavage in control samples under the reaction conditions employed and this was calculated according to Eq. [2].

$$\text{Normalized percent cleavage} = \frac{\%\ \text{cleavage (drug)} - \%\ \text{cleavage (control)}}{100 - \%\ \text{cleavage control}} \qquad [2]$$

The values of normalized percent cleavage of DNA presented in Figs. 3 and 4 represent the mean plus or minus one standard deviation from three or four separate determinations. The reported EC_{25} values were obtained by interpolation from plots of normalized percentage cleavage versus concentration. The EC_{25} values reported represent the average ± standard deviation for three or more separate determinations.

RESULTS AND DISCUSSION

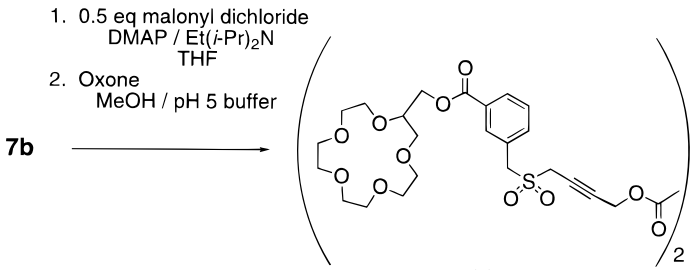
Synthesis of propargylic sulfone-armed lariat crown ethers **9b, 10b,** *and* ***11*** *and model propargylic sulfones* **9a** *and* **12.** The synthesis of the propargylic sulfone-armed lariat crown ethers began with esterification of the commercially available racemic 2-hydrox-

ymethyl-15-crown-5. The ester **5b** (Scheme 1) was prepared in 66% yield by the esterification procedure of Oriyama and coworkers (*26*), in which the alcohol is treated with TMEDA in methylene chloride in the presence of 4 Å molecular sieves at -78C, followed by addition of 3-chloromethylbenzoyl chloride. The preparation of the *para*-isomer **6b** proceeded under similar conditions to afford the ester in 80% yield. The model ester **5a** was prepared in 83% yield using standard DMAP-catalyzed esterification procedure.

Transformation of esters **5a, 5b,** and **6b** to the corresponding benzylic thiols was accomplished in fair to good yield by nucleophilic displacement by thiourea and subsequent isothiouronium decomposition with *n*-butylamine (Scheme 1) (*27*). The thiol derived from ethyl ester **5a** was alkylated with 4-bromobutyn-2-1-*ol* in the presence of diisopropylethylamine base to afford the thioether **7a** in 61% overall yield from **5a** (Scheme 1). Similarly, chloride **5b** was converted to the thioether **7b** in 51% overall yield. Unfortunately, the basic reaction conditions required for isothiouronium salt decomposition (*n*-butylamine in EtOH) caused extensive oxidation of the thiol derived from **6b** to the disulfide dimer. This observation led us to attempt a one-pot procedure in which the benzylic chloride **6b** was treated in the presence of diisopropylethylamine with the *in situ*-formed thiol derived from the treatment of 4-bromo-2-butyn-1-*ol* with thiourea followed by *n*-butylamine. This one-pot, three-step procedure afforded the thioether **8b** in 65% yield.

Oxidation of the propargylic thioethers **7a, 7b,** and **8b** was accomplished with the chemoselective reagent Oxone in citrate-buffered aqueous methanol in excellent yield (Scheme 1). The resulting sulfones **9b** and **10b** could not be purified by column chromatography on silica gel, as isomerization to the inseparable allenic species occurred, even when acetic acid-containing eluant was employed. Fortunately, the desired propargylic sulfone-armed crown ethers **9b** and **10b,** as well as the model compound **9a** were obtained essentually pure after aqueous workup, and when necessary, purification could be accomplished by column chromatography using C-18 derivatized silica gel.

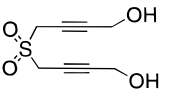
The dimeric crown ether **11** was prepared from the thioether **7b** as shown in Eq. [3]. The coupling of **7b** to malonyl dichloride was followed by Oxone oxidation to afford **11** in modest overall yield, primarily as a result of incomplete esterification during the coupling step. As in the case of sulfones **9a, 9b,** and **10b,** dimer **11** was unstable to normal phase chromatography, but could be purified by reverse phase column chromatography.



11 [3]

The model bis(propargylic) sulfone **12** was originally reported by Nicalou and coworkers (*20*). We prepared this compound from the previously reported tetrahydropyranyl acetal-protected 4-bromo-2-butyn-1-*ol* (*21*), which was treated with alumina-supported

NaS_2 reagent (*21*), followed by oxidation with *m*CBPA and deprotection of the alcohol protecting groups.



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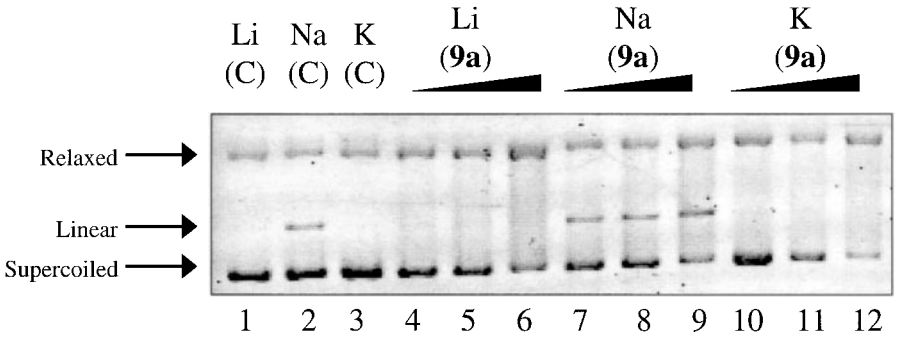
*Metal ion binding studies of propargylic sulfone-armed crown ethers **9b** and **10b** and model propargylic sulfone* **9a.** Cram's metal picrate extraction method (*28*) was used to assess the alkali metal ion association constants of propargylic sulfone-armed crown ethers **9b** and **10b** and model propargylic sulfone **9a.** The calculated alkali metal ion association constants for compounds **9a, 9b,** and **10b** are presented in Fig. 1. As expected, the model compound **9a** was found to be practically devoid of any metal ion binding affinity. In contrast, both lariat crown ethers **9b** and **10b** bind alkali metal ions, with an apparent selectivity of $Na^+ > K^+ >> Li^+$. This sodium ion selectivity is commensurate with that reported for picrate-extraction determined binding constants for other 15-crown-5-derived, (substituted)phenyl-pendent lariat crown ethers (*29*). Although the picrate extraction technique for estimating metal ion binding constants is operationally simple and requires minimal



FIGS. 2–5. Agarose gel of DNA cleavage reactions for compounds **9a, 9b, 10b,** and **11.** Li^+ -, Na^+-, or K^+ DNA was incubated alone or in the presence of various concentrations of compound for 19 h at room temperature, briefly heated at 70°C for 90 s, and then subjected to agarose gel electrophoresis. After electrophoresis, the gels were visualized with ethidium bromide staining and UV transillumination and the image was recorded on a fluorimaging system for quantification. Figure 2: Lanes 1–3, Li^+ -, Na^+ -, and K^+DNA in the absence of compound; lanes 4–6, Li^+DNA in the presence of 0.94, 1.88, and 4.38 mM **9a,** respectively; lanes 7– and 9, Na^+DNA in the presence of 0.94, 1.88, and 4.38 mM **9a,** respectively; lanes 10–12, K^+DNA in the presence of 0.94, 1.88, and 4.38 mM **9a,** respectively. Figure 3: Lanes 1–3, Li^+-, Na^+-, and K^+DNA in the absence of compound; lanes 4–6, Li^+DNA in the presence of 1.25, 3.12, and 6.25 mM **9b,** respectively; lanes 7–9, Na^+DNA in the presence of 1.25, 3.12, and 6.25 mM **9b,** respectively; lanes 10–12, K^+DNA in the presence of 1.25, 3.12, and 6.25 mM **9b,** respectively. Figure 4: Lanes 1–3, Li^+-, Na^+-, and K^+DNA in the absence of compound; lanes 4–6, Li^+DNA in the presence of 0.31, 0.62, and 0.94 mM **10b,** respectively; lanes 7–9, Na^+DNA in the presence of 0.31, 0.62, and 0.94 mM **10b,** respectively; lanes 10–12; K^+DNA in the presence of 0.31, 0.62, and 0.94 mM **10b,** respectively. Figure 5: Lanes 1–3, Li^+-, Na^+-, and K^+DNA in the absence of compound; lanes 4–6, Li^+DNA in the presence of 0.065, 0.125, and 0.25 mM **11,** respectively; lanes 7–9, Na^+DNA in the presence of 0.065, 0.125, and 0.25 mM **11,** respectively; lanes 10–12, K^+DNA in the presence of 0.065, 0.125, and 0.25 mM **11,** respectively.

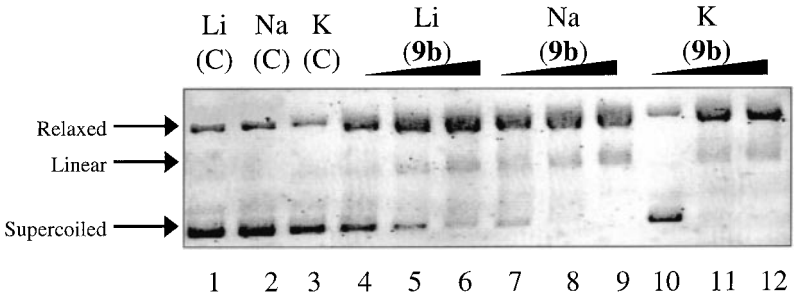


FIG. 3.

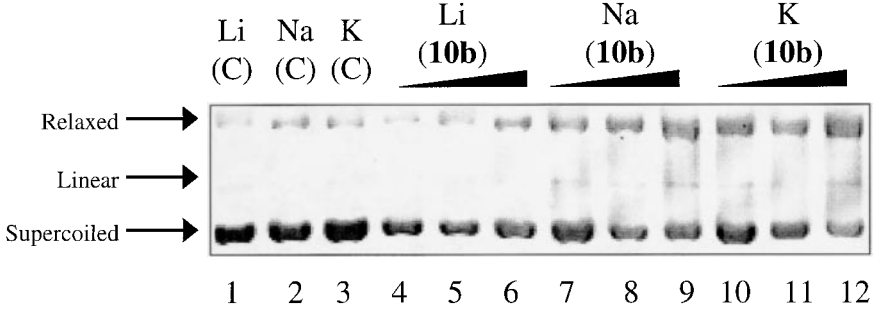


FIG. 4.

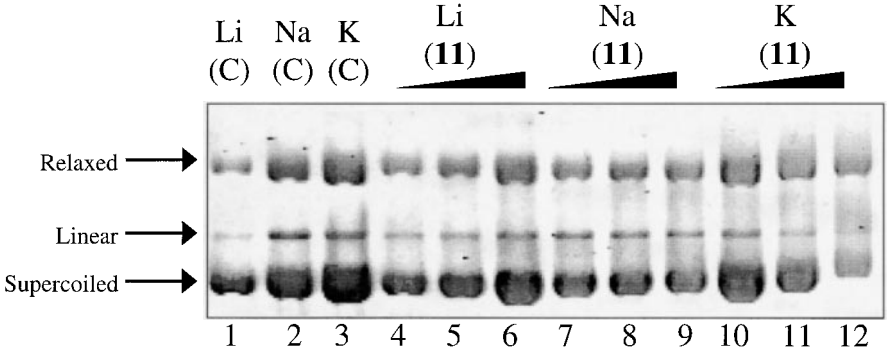


FIG. 5.

amounts of material, it suffers from the fact that the value of the binding constants obtained are a function of the lipophilicity of the crown ether as well as the stability of the metal ion complex in the organic medium (*27*). The slight variation in the K_a for sodium and potassium metal ions, when comparing the positional isomers **9b** and **10b** is probably more a reflection of variations in the extraction efficiency due to slight changes in the lipophilicity of the lariat crown ethers than altered participation of the lariat side chain in metal ion binding. The aqueous metal ion complex stability constants for these crowns are no doubt much less than those determined in chloroform using the picrate extraction data. For 15-crown-5 itself, the aqueous alkali metal ion complex stability constants for Li^+, Na^+, and K^+ are ~0.0, 5.23, and 8.51, respectively (*30*).

DNA cleavage studies. To assess the metallo-regulated DNA-cleaving capacity of these propargylic sulfones, aqueous solutions of supercoiled plasmid DNA in which the major alkali metal cation species is controlled were prepared. Commercially available supercoiled phage ΦX174 DNA contains a substantial amount of NaCl. Our repeated attempts to dialyze these commercially obtained DNA solutions against alkali metal ion phosphate buffers led to extensive nicking of the supercoiled DNA, presumably due to mechanical sheering during the dialysis and sample recovery. Therefore, supercoiled pGAD424 plasmid DNA was isolated from DF5α *E. coli* using a silica-based column (QIAGEN Mini-Prep). Elution of the DNA from the column with a minimal volume of distilled water and dilution of the resulting solution into the appropriate alkali metal ion phophate buffer (20 mM, pH 7.4) provided supercoiled plasmid DNA solutions in which the major alkali metal ion in solution is lithium, sodium, or potassium. We refer to these alkali metal cation-enriched DNA solutions as M^+DNAs (i.e., Li^+DNA, Na^+DNA, or K^+DNA).

The propagylic sulfone-armed lariat crown ethers **9b, 10b,** and **11,** as well as the model propargylic sulfone **9a** and bis(propargylic) sulfone **12** were incubated with each of these M^+DNAs at room temperature overnight (19 h), briefly (90 s) heated to 70°C to enhance the observed cleavage due to DNA alkylation, and then subjected to agarose gel electrophoresis. The separated DNA forms (supercoiled, relaxed, and linear) were visualized and quantified by ethidium bromide staining and fluorimaging. Figures 2–5 show representative DNA cleavage results for compounds **9a, 9b, 10b,** and **11** with Li^+DNA, Na^+DNA, and K^+DNA.

The concentration of compound necessary to effect cleavage of 25% of the supercoiled DNA (EC_{25}) was determined from plots of percent cleavage vs concentration for each compound. The EC_{25}s are reported as the mean ± standard deviation for three or more separate determinations.

The variation in the DNA cleavage EC_{25}s as a function of M^+DNA for propargylic sulfones **9a, 9b, 10b, 11,** and **12** are presented in Table 1 and Fig. 6. It is evident from Fig. 6 that crown ether-containing propargylic sulfones (**9b** and **10b**) exhibit greater cleavage of Na^+DNA and K^+DNA than Li^+DNA. The propargylic sulfone-armed lariat crown ethers **9b** and **10b** cleave Li^+DNA to a similar degree as model propargylic sulfone **9a,** which cleaves all M^+DNAs to the same extent, without regard to the dominant cation present. Thus, the cleavage efficiency of propargylic sulfone-armed lariat crown ethers **9b** and **10b** is significantly higher than the model propargylic sulfone only in the presence of the lariat crown-complexed Na^+ and K^+ ions. While regioisomeric pendent crown ether propargylic sulfones **9b** and **10b** cleave alkali metal ion-containing solutions with the same order of efficiency, i.e., Na^+DNA ~ K^+DNA >>

TABLE 1

DNA Cleavage by Propargylic Sulfone-Armed Lariat Crown Ethers

Compound	EC_{25} (mM)[a]		
	Li^+DNA	Na^+DNA	K^+DNA
9a	2.2 ± 0.2	2.5 ± 0.3	2.3 ± 0.1
9b	2.1 ± 0.2	0.68 ± 0.06	0.64 ± 0.09
10b	1.65 ± 0.01	0.45 ± 0.03	0.45 ± 0.02
11	1.1 ± 0.2	0.20 ± 0.01	0.09 ± 0.02
12	1.29 ± 0.07	0.8 ± 0.2	1.14 ± 0.05

[a] Concentration necessary to effect 25% cleavage of supercoiled DNA, normalized for purity of starting supercoiled DNA, expressed as an average ± standard deviation for three or more separate determinations.

Li^+DNA, the somewhat enhanced potency of **10b** versus **9b** for all M^+DNAs may be the result of increased binding or steric accessibility of **10b** for DNA (*22,23*).

The exact origin of the specific alkali metal-ion dependent DNA cleavage due to crown ethers **9b** and **10b** is not clear. The lack of an effect of the nature of the alkali metal ion on the DNA cleavage due to model compound **9a** argues against a general alkali metal ion-induced increase in the susceptibility of DNA to alkylation by these electrophiles. However, we reasoned that the observed alkali metal ion effects could be a reflection of increased formation of the ultimate allenic sulfone electrophilic species derived from these crowns in the presence of Na^+ and K^+ ions. In order to test this hypothesis, solutions of compounds **9a, 9b,** and **10b** in 1:1 MeOH/aqueous $KHPO_4$ buffer (100 mM, pH 7.4) were allowed to stand at room temperature for 4 h and the extent of isomerization to the corresponding allenes determined by ^1H NMR. Under

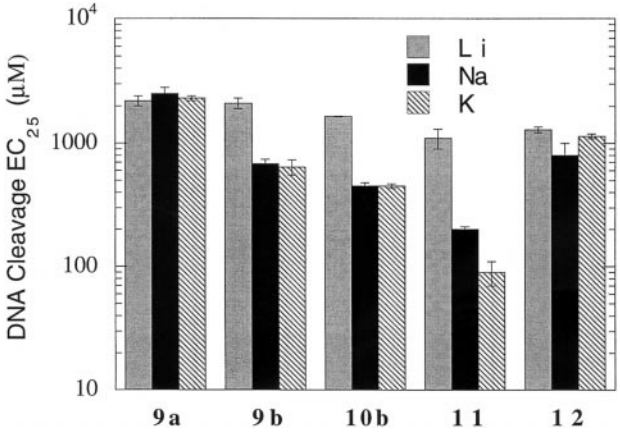


FIG. 6. Effect of alkali metal ions on the supercoiled DNA cleavage efficiency of propargylic sulfone-armed lariat crown ethers **9b, 10b,** and **11** and reference compounds **9a** and **12.** The EC_{25} is the micromolar concentration of each compound required to effect 25% cleavage supercoiled DNA when incubated in Li-, Na-, or K-phosphate buffer, pH 7.4, at 25°C for 19 h.

these conditions, there was no significant difference in the isomeraztion of the crowns **9b** and **10b** (50 and 52% isomerization, respectively) as compared to the model propargylic sulfone **9a** (54% isomerization). Thus, the alkali metal ion-facilitated DNA cleavage by these propargylic sulfone-armed lariat crown ethers must be due to specific interactions between these compounds and DNA that is enhanced in the presence of the appropriate metal ion.

The DNA cleavage efficiency of the dimeric crown ether **11** displays a distinct alkali metal ion-dependency. As has been reported for other bis(propargylic) sulfones (*18*), the dimeric sulfone **11** is a more effective DNA cleavage agent than the propargylic sulfones **9a, 9b,** or **10b.** Cleavage of Li^+DNA by **11** is twice as great as that observed for the model compound **9a** and similar to that observed for the model bis(propargylic) sulfone **12.** The increased DNA cleavage efficiency of dimeric crown ether **11** for Na^+DNA as compared to Li^+DNA is slightly greater than that observed for crown ethers **9b** and **10b;** however, the relative efficiency of DNA cleavage of **11** in the presence of K^+ ions is significantly greater. The K^+-dependent DNA cleavage enhancement observed for **11** is not observed for the simple bis(propargylic) sulfone **12,** which shows only a very modest increase in DNA cleavage efficiency for Na^+DNA as compared to either Li^+DNA or K^+DNA.

Certain 15-crown-5-containing molecules are known to form 2:1 "sandwich" complexes with potassium ions in aqueous solutions (*31*,*32*). The linker separating the two 15-crown-5 moieties in compound **11** is sufficiently flexible to allow the formation of a 1:1 **11**–K^+ complex in which a single K^+ ion is sandwiched between the two crown ether rings *(33).* The K^+ ion-enhanced DNA cleavage due to **11** may reflect the increased DNA cleavage efficiency of this type of sandwich complex. Alternatively, the bis(propargylicsulfone) **11** may, in the presence of potassium ions, preferentially associate with the DNA as a 2:1 **11**–K^+ complex in which a K^+ ion is sandwiched by 15-crown-5 moieties from two molecules of **11.** Once near the target DNA, the uncomplexed crown ethers units of the associated complex may recruit additional molecules of **11** to the DNA via formation of new 2:1 **11**–K^+ sandwich complexes. The net result may be cooperative binding of **11** to DNA in the presence of potassium ions and, thus, enhanced cleavage of K^+DNA versus Na^+DNA or Li^+DNA.

Having demonstrated that propargylic sulfone-armed lariat crown ethers exhibit preferential cleavage of M^+DNAs where M^+ is Na^+ or K^+, we next examined whether the extent of cleavage of supercoiled DNA by these lariat crown ethers could be regulated by varying the concentration of metal ions in solution. Supercoiled pGAD424 DNA solutions containing 0.0, 1.4, 2.9, or 5.7 mM alkali metal phosphate in Tris buffer (17–20 mM) were prepared. These DNA solutions were incubated with propargylic sulfones **10b** and **9a** and the DNA cleavage products were separated and quantified as previously described. The propargylic sulfone-armed lariat crown ether **10b** cleaved supercoiled DNA in a Na^+-dependent manner, but the DNA cleavage due to **10b** was independent of Li^+ ion concentration (Fig. 7). As shown in Table 2, the DNA cleavage efficiency of lariat crown ether **10b** increased 66% on increasing the Na^+ concentration in these DNA cleavage reactions from 0 to 5.7 mM. In contrast to these results, the DNA cleavage due to crown ether **10b** was completely unaffected by the addition of increasing concentrations of Li^+ ions (Table 2). Similarly, the DNA cleavage due to the model propargylic sulfone **9a,** which lacks any metal ion recognition moiety, is insensitive to increased Na^+ ion concentrations in the DNA cleavage reactions

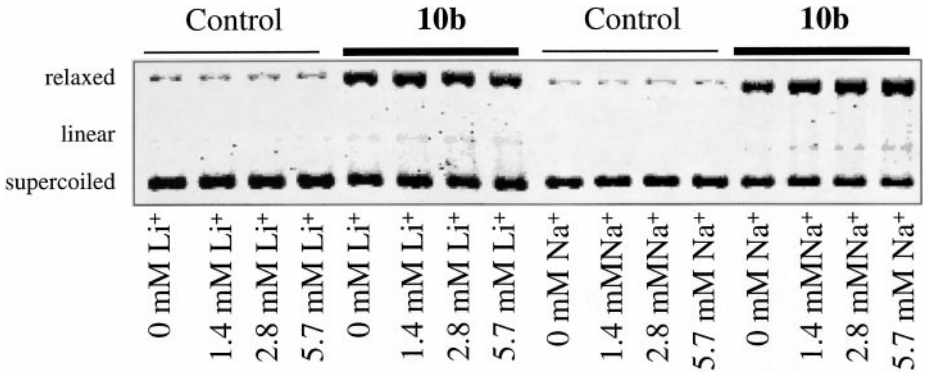


FIG. 7. Sodium-ion mediated DNA cleavage due to lariat crown ether **10b.** Compound **10b** and supercoiled DNA in TRIS buffer containing Li- or Na-phosphate (20 mM total buffer concentration, pH 7.4) were incubated for 20 h at 25ºC and the products separated by agarose gel electrophoresis, visualized with ethidium bromide, and analyzed by quantitative fluorescence imaging. Control lanes are from reactions containing no added compound, all other lanes are from reactions containing 1.6 mM of lariat crown ether **10b.** Significantly more relaxed and linear DNA are formed in the presence of **10b** and increasing concentrations of Na-phosphate than in the presence of Li-phosphate.

(Table 2). These results conclusively demonstrate that appropriate propargylic sulfone-armed lariat crown ethers can effect the alkali metal ion-regulated cleavage of DNA.

Dai and coworkers have previously reported the synthesis of the propargylic sulfone-armed lariat benzo-15-crown-5 **4.** These researchers similarly assayed for metallo-regulated cleavage of supercoiled DNA (in this case, ΦX174 phage DNA) by **4** by the addition of alkali or alkaline earth metal salt solutions (sodium, potassium, barium, or strontium acetate) to solutions containing DNA and **4.** When sodium, potassium, or strontium salt solutions were applied, no change in the extent of DNA cleavage by **4** was observed; the addition of barium salts, however, served to decrease the cleavage of DNA by **4.** Some commercially available preparations of ΦX174 are supplied as solutions that contain 12 mM sodium ions. Even after dilution to the working concentration for these cleavage assays, the supercoiled DNA may have contained up to 1–2 mM NaCl. In the presence of this Na^+-containing DNA solution, Dai's lariat

TABLE 2

Sodium Ion-Dependent DNA Cleavage by A Propargylic Sulfone-Armed Lariat Crown Ether

Compound	Normalized Percent DNA Cleavage			
10b(1.6 mM)	0 mM Li^{+a}	1.4 mM Li^{+a}	2.8 mM Li^{+a}	5.7 mM Li^{+a}
	36 ± 2	36 ± 3	36 ± 1	31 ± 1
	0 mM Na^{+a}	1.4 mM Na^{a}	2.8 mM Na^{+a}	5.7 mM Na^{+a}
	33 ± 2	43 ± 3	50 ± 3	55 ± 3
9a (2.5 mM)	51 ± 2	51 ± 2	51 ± 2	48 ± 5

[a] Supercoiled DNA in constant ionic strength (20 mM) buffer consisting of TRIS and 0, 1.4, 2.8, or 5.7 mM Li- or Na-phosphate, pH 7.4.

crown may not have responded to further increases in alkali metal ion concentration, or the changes may have been too subtle to detect without the careful fluorimaging quantification that we performed. Alternatively, as demonstrated here with dimeric crown **11** and lariat crowns **9b** and **10b,** the DNA cleavage efficiency and metallo-regulation of DNA cleavage exhibited by this class of molecules is somewhat sensitive to structural changes, although the structure activity relationship for this class of compounds is not yet apparent. Dai's lariat crown **4** may simply not be an efficient metallo-regulated DNA cleavage agent, as opposed to compounds **9b, 10b,** and **11.** Finally, the inhibition of DNA cleavage due to **4** in the presence of $Ba(OAc)_2$ may be due to the trapping of the reactive allenic sulfone intermediate by acetate. We have observed such an effect on the DNA cleavage chemistry of bis(propargylic) sulfone crown ether **1,** in which acetate salts tend to inhibit the DNA cleavage reaction (*34*).

CONCLUSIONS

Propargylic sulfone-armed lariat crown ethers **9b** and **10b,** dimer **11** and model propargylic sulfone **9a** were prepared in four to five steps and modest overall yield. Propargylic sulfone-armed lariat crown ethers **9b** and **10b** bind sodium and potassium ions and exhibit little, if any, affinity for lithium ions as determined by a picrate extraction assay. Model propargylic sulfone **9a** did not bind lithium, sodium, or potassium ions to an appreciable extent under the conditions of the picrate extraction assay.

DNA solutions (M^+DNAs) have been prepared which contain primarily a single species of alkali metal ion. Using these DNA solution, we have shown that lariat crown ethers **9b, 10b,** and **11** are effective metallo-regulated DNA cleavage agents. 15-crown-5-containing propargylic sulfones **9b, 10b,** and **11** preferentially cleave Na^+DNA and K^+DNA versus Li^+DNA. The role of specific metal ion recognition in the metallo-regulated DNA cleavage displayed by **9b, 10b,** and **11** is demonstrated by the equivalent cleavage of Li^+DNA by these compounds when compared to the model propargylic sulfone **9a,** which does not recognize metal ions and which displays nearly equipotent cleavage of all M^+DNA solutions. Most notably, the DNA cleavage due to lariat crown ether **10b** is modulated by the addition of 1–5 mM sodium phosphate to cleavage reactions containing 20 mM Tris buffer. In comparison, the addition of lithium phosphate has no effect on DNA cleavage due to **10b.**

These propargylic sulfone lariat crown ethers display different metal ion selectivities in their metallo-regulated DNA cleavage effects. While crowns **9b** and **10b** both display over three-fold enhanced DNA cleavage in the presence of either Na^+ and K^+, when compared to Li^+, the bifunctional bis(propargyl sulfone) **11** cleaves K^+DNA over tenfold more efficiently than Li^+DNA, and twofold better than Na^+DNA. While this level of metal ion discrimination is not nearly as great as that reported for alkali metal ion-selective ligands (*35*), it does represent the first successful attempt to marry molecular recognition of specific alkali metal ions with covalent modification of DNA.

The propargyl sulfone crown ether **11** is a modestly potent DNA cleavage agent. It is important to note that our DNA cleavage assays are done at a physiologically relevant pH (7.4) in contrast to the high pH (8.0–8.5) typically employed by others studying DNA cleavage chemistry of propargylic sulfones (*18,19,23,36*). Under our more physiologically relevant conditions, compound **11** is an effective DNA cleavage agent

at micromolar concentrations. This compound has been selected by the NCI for further evaluation as a potential anticancer agent.

ACKNOWLEDGMENTS

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REFERENCES

1. Theim, J., and Meyer, B. (1981) *Tetrahedron* **37,** 551.
2. Ramakrishna, N., Miller, D. M., and Sakai, T. T. (1990) *J. Antibiot.* **43,** 1543.
3. Reynolds, M. B., DeLuca, M. R., and Kerwin, S. M. (1999) *Bioorg. Chem.* **27,** 326.
4. Fan, J-Y.; Sun, D.; Yu, H.; Kerwin, S. M.; Hurley, L. H. J. (1995) *Med. Chem.* **38,** 408.
5. Zeng, Q. P.; Kwok, Y.; Kerwin, S. M.; Mangold, G.; Hurley, L. H (1998) *J. Med. Chem.* **41,** 4273−4278.
6. Yu, H-T.; Hurley, L. H.; Kerwin, S. M. (1996) *J. Am. Chem. Soc.* **118,** 7040−7048.
7. Stubbe, J.; Kozarich, J. W. (1987) *Chem Rev.* **87,** 1107–1135.
8. (a) Sigman, D. S. (1986) *Acc. Chem. Res.* **19,** 180. (b) Barton, J. K. (1986) *Science* 233, 727. (c) Pratiel, G.; Bernadou, J.; Meunier, B. (1996) *Metal Ions Biol. Syst.* **33,** 399.
9. Griffin, J. H.; Dervan,. P. B. (1987) *J. Am. Chem. Soc.* **109,** 6840.
10. Fukuda, R.; Takenaka, S.; Takagi, M. (1990) *J. Chem. Soc., Chem. Commun.* 1028.
11. Tsukube, H. (1996) *Coord. Chem. Rev.* **148,** 1.
12. Manning, G. S. (1978) *Quart. Rev. Biophys. II* **2,** 179.
13. McPhee, M. M.; Kerwin, S. M. (1996) *J. Org. Chem.* **61,** 9385.
14. Stephen, J.; Osborne, M. P.; Spencer, A. J.; Warley, A. (1990) *Scan. Microsc.* **4,** 781.
15. Zs.-Nagy, I. (1989) *Scan. Microsc.* **3,** 473.
16. Roomans, G.; Von Euller, A. (1996) *Cell Biol. Int.* **20,** 103.
17. Cameron, I. L.; Smith, N. K. R. (1989) *Magnesium* **8,** 31.
18. Smith, P.; Rhodes, N. P.; Shortland, A. P.; Fraser, S. P.; Djamgoz, M. B. A.; Ke, Y.; Foster, C. S. (1998) *FEBS Lett.* **423,** 19.
19. Besson, P.; Gore, J.; Vincent, E.; Hoinard, C.; Bougnoux, P. (1996) *Biochem. Pharmacol.* **51,** 1153.
20. Nicolaou, K. C.; Skokotas, G.; Maligres, P.; Zuccarello, G.; Schweiger, E. J.; Toshima, K.; Wenderborn, S. (1989) *Angew. Chem. Int. Ed. Eng.* **28,** 1272.
21. Nicolaou, K. C.; Wendeborn, S.; Maligres, P.; Isshiki, K.; Zien, N.; Ellestad, G. (1991) *Angew. Chem. Int. Ed. Eng.* **30,** 418.
22. Gupta, R.; Xie, G.; Lown, J. W. (1994) *Gene* **149,** 81.
23. Kerwin, S. M. (1993) *Tetrahedron Lett.* **35,** 1023.
24. Dai, W.-M.; Fong, K. C. (1995) *Tetrahedron Lett.* **36,** 5613.
25. Dai, W.-M..; Fong, K. C.; Danjo, H.; Nishimoto, S.-I.; Solow, M.; Mak, W. L.; Yeung, M. L. (1996) *Bioorg. Med. Chem. Lett.* **6,** 1093.
26. Sano, T.; Ohashi, K.; Oriyama, T. (1999) *Synthesis* 1141.
27. Attempts to decompose the isothiouronium species with bicarbonate or mild acid failed to yield the desired thiols.
28. Helgeson, R. C.; Weisman, G. R.; Toner, J. L.; Tarnowski, T. L.; Chao, Y.; Mayer, J. M.; Cram, D. J. (1979) *J. Am. Chem. Soc.* **101,** 4928.
29. Arnold, K. A.; Echegoyen, L; Gokel, G. W. (1987) *J. Am. Chem. Soc.* **109,** 3713.
30. Izatt, R. M.; Bradshaw, J. S.; Nielson, S. A.; Lamb, J. D.; Christensen, J. J. (1985) *Chem. Rev.* **85,** 271.
31. Van Remoortere, F. P. (1974) *Inorg Chem* **13,** 2826. Beer, P. D. (1986) *J. Chem. Soc., Chem. Comm.* 1678.
32. Saiful Islam, M.; Pethrick, R. A.; Pugh, D.; Wilson, M. J. J. (1998) *Chem. Soc., Faraday Trans.* **94,** 39.
33. Modeling was done with PCModel (Serena software, Bloomington, IN) using the MMX force field and explicit coordination of K^+ ions by all 10 oxygen atoms of the two crown ether rings.
34. McPhee, M. M, and Kerwin, S. M. Manuscript in preparation.
35. Bradshaw, J. S.; Izatt, R. M. (1997) *Acc. Chem. Res.* **30,** 338.

36. Dia, W.-M.; Li, Q.; Fong, K. C.; Chow, C. W.; Zhou, L.; Hamaguchi, W.; Nishimoto, S. (1998) *Bioorg. Med. Chem. Lett.* **8,** 169.
37. For general experimental procedures, see Ref. 14.
38. Morgan, G. T.; Porter, C. R. (1926) *J. Chem. Soc.* 1258.
39. Bailley, W. J.; Fujiwara, E. (1955) *J. Am. Chem. Soc.* **77,** 165.
40. Silberrad, O.; Phillips, H. A. (1908) *J. Chem. Soc.* 474.
41. Newcomb, M.; Timko, J. M.; Walba, D. M.; Cram, D. J. (1977) *J. Am. Chem. Soc.* **99,** 6404.