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The Synthesis and Chemistry of A Simplified, Functional Analogue of Neocarzinostatin Chromophore: Identification of an Intramolecular 1,5-Hydrogen Atom Transfer Relevant to the Mechanism and Cleavage Selectivity of Diyl-Based DNA Cleaving Agents

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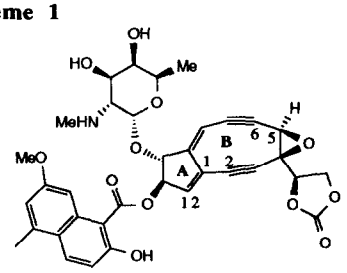
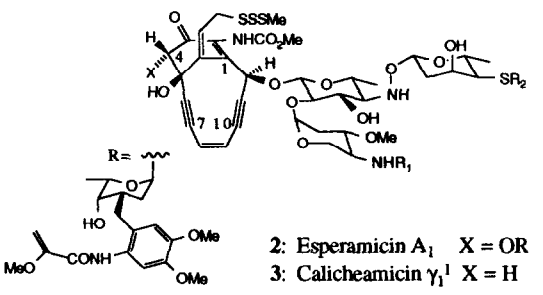
Abstract: The synthesis and chemistry of a monocyclic analogue of neocarzinostatin chromophore are described. This analogue is activated through a Michael addition process and provides cycloaromatized products similar to those obtained in the activation of neocarzinostatin chromophore. Mechanistic studies on this analogue have led to the first identification of a diyl self-quenching pathway based on a 1,5-hydrogen atom transfer that provides a novel hypothesis about the relationship between thiol structure and DNA single and double strand cleavage selectivity for neocarzinostatin chromophore and diyl-based cleaving agents.

The genesis of research on diyl-mediated DNA cleaving agents is found in the seminal studies of Ishida *et al.* who, in 1965, reported the isolation of neocarzinostatin (NCS) from *Streptomyces carzinostaticus* F-41.[1] Subsequent studies revealed that NCS exhibits a wide range of medicinally interesting activities including *in vitro* and *in vivo* antitumor activity, findings which propelled its further development as a human anticancer agent.[2-4] In the late seventies, an important step in establishing the molecular basis for the biological activity of NCS was made when it was found that NCS is actually a complex of a protein (NCS apoprotein) and a highly unsaturated chromophoric compound (NCS Chrom).[5] Of particular significance was the discovery that NCS Chrom exhibits almost all of the biological activity of NCS itself, including the ability to cleave DNA upon chemical activation.[6-8] Global efforts to establish the structure of the NCS Chrom reached fruition in 1985 when Edo *et al.* proposed the novel structure **1**, incorporating an unprecedented bicyclo[7.3.0]undecadienediyne subunit.[9]

Scheme 1



1: NCS Chrom



2: Esperamicin A_1 X = OR
3: Calicheamicin γ_1^1 X = H

The broader significance of NCS became more evident in 1987 with the publication of the structures of esperamicin A_1 (**2**) [10-12] and calicheamicin γ_1^I (**3**),[13,14] compounds with structural and functional similarities to NCS Chrom. Like NCS Chrom, these compounds possess a highly unsaturated medium ring core and associated carbohydrate residues. Moreover, these compounds also exhibit an ability to cleave DNA upon chemical activation. The mechanism for this cleavage process was insightfully suggested to involve conversion of the trisulfide functionality to a thiol followed by Michael addition of the latter to the ß-carbon of the enone.[10] The attendant rehybridization of C-1 (esperamicin A_1 numbering system) allows for facile cycloaromatization of the enediyne to an arene-1,4-diradical, a process first identified by the Bergman group in their seminal studies on simpler enediynes.[15,16] Subsequent abstraction of deoxyribosyl hydrogens of DNA by the resultant arene diradical serves to generate a radical site on DNA which upon reaction with oxygen leads to a hydroperoxide and ultimately strand scission.[17,18] Contemporaneously, it was proposed that NCS Chrom also produces a reactive diradical intermediate through the thiol induced generation and subsequent cycloaromatization of a highly unsaturated subunit, a cumulene-ene-yne.[19] In the past few years, this field has expanded further to include the DNA cleaving agents dynemicin,[20-22] kedarcidin,[23-25] and C-1027.[26-28] While further discoveries in this relatively new area can be anticipated, it is clear from the current group of six structural types that Nature has once again demonstrated an impressive ability to generate fascinating mechanistic and medicinal leads, in this case, providing a blueprint for the rational design and synthesis of a wide range of molecular cutting devices.

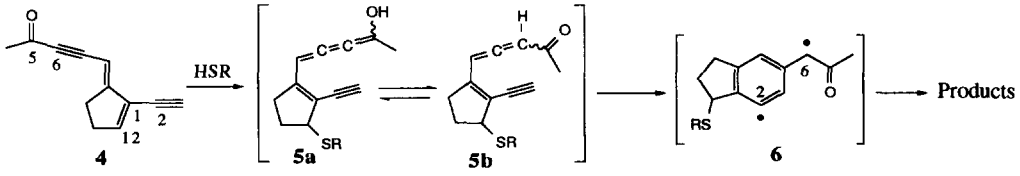
Our own research in this area started in 1985 with the report of the structure of NCS Chrom. At the time, this structure was without precedent and little was known about its molecular mode of action and how its analogues could be synthesized. As a consequence, our studies focused on developing a flexible strategy for the synthesis of analogues which could be used to corroborate the structure of the chromophore, to systematically study the role of its subunits in the mechanism of DNA cleavage, and most importantly to exploit this lead in the design and development of new and more effective DNA cleaving agents. In 1988, we reported the first synthesis of the carbobicyclic core of NCS Chrom and established the spectroscopic similarity of the two systems.[29] Involving the attachment of two appendages to a pre-formed A-ring (2-bromo-2-cyclopentenone or more highly functionalized systems), closure of the introduced appendages to form a relatively unstrained macrocycle, and ring contraction of the macrocycle to form the nine-membered B-ring, the strategy underlying this synthesis has proven to be effective for the preparation of several NCS Chrom analogues in our[30,31] and other laboratories.[32-34] Research in this area continues to be very active.[35-45]

In further studies, the ring contraction strategy, based originally on a photo-extrusion of sulfoxide, was extended to include a ring contractive Wittig rearrangement[31] and later augmented by a third more flexible and efficient approach to the nine-membered ring involving a relatively little studied intramolecular variant of the Hiyama-Nozaki reaction.[46] Proceeding presumably through the formation and contraction of a thirteen-membered oxymetallocycle, the latter method[31] efficiently produces analogues incorporating the nine-membered B ring of NCS Chrom with functionalities strategically located (C-4 and C-5) for modification as needed for activation and DNA recognition studies. More recently, aspects of our original strategy have been exploited in the synthesis of monocyclic analogues,[47,48] compounds which are activated for diyl formation through a Michael-type addition and which have provided much needed information on the role of the B ring in the chemistry of NCS Chrom. This work has also provided the first mechanistic proposal[48,49] and experimental

evidence for a radical translocation process pertinent to the thiol dependent strand cleavage selectivity (single versus double) exhibited by NCS Chrom.[50-54] Further studies bearing on the synthesis and chemistry of highly simplified, monocyclic analogues of NCS Chrom and additional mechanistic studies related to our previous proposal[48] on the cleavage selectivity of NCS and other diyl-based DNA cleaving agents is described below.

Our approach to the design of simplified, monocyclic NCS Chrom analogues was guided by the view that the dienediyne and C-5 leaving group (or electron acceptor) are the minimally required functionalities for the conversion of NCS Chrom to a diradical. This suggests that the bicyclic structure of NCS Chrom could be simplified, in principle, without loss of function to monocycles of the type represented by **4**. By analogy to the proposed mode of activation of NCS Chrom[19,55] in which thiol addition to C-12 proceeds with cleavage of the epoxide at C-5 to produce a tetraenyne, the C-5 carbonyl group in analogue **4** was expected to serve as a functional equivalent of the epoxide, allowing for an activation process based on conjugate (Michael) addition of a thiol. Cycloaromatization of the resultant tetraenyne **5a** or trienyne **5b** would then lead to diyl **6** or its enol tautomer. Computer modeling suggests that this design could be extended to more functionalized diyl progenitors equipped with DNA recognition elements at positions C-10 and C-11 as needed to direct delivery and to guide cleavage selectivity.[56]

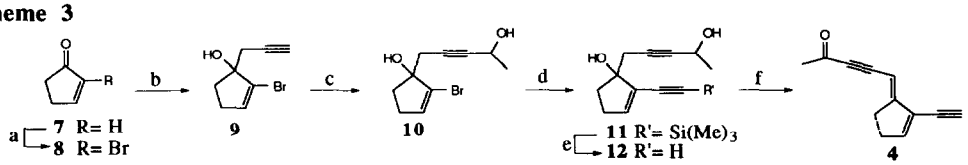
Scheme 2



4 **5a** **5b** **6**

In order to test the above concept, analogue **4** was synthesized through an adaptation of a strategy originally developed by this group (Scheme 3).[29-31,48] Accordingly, propargylmagnesium bromide addition to 2-bromo-2-cyclopentenone[57] provided bromoenyne **9** in 90% yield. Reaction of the dianion of **9** with acetaldehyde gave the diol **10** (89% yield), which upon coupling[58] with trimethylsilyl acetylene (91% yield) and subsequent deprotection produced diol **12** in 76% yield. Swern-Moffatt oxidation of **12** proceeded with concomitant dehydration to give the *E*-alkene **4** (38%), whose stereochemistry was determined by NOE studies.[59] This conversion was also achieved in a two step process involving oxidation of the secondary alcohol to the ketone with TPAP and elimination of the tertiary mesylate (MsCl/Et_3N). This process gave the same overall yield as the one step procedure, and as such, the one step procedure was preferentially utilized. The desired analogue **4** obtained from this procedure was found to be quite labile. However, it could be stored for a period of weeks without significant decomposition when kept in degassed hexane at -20 °C.

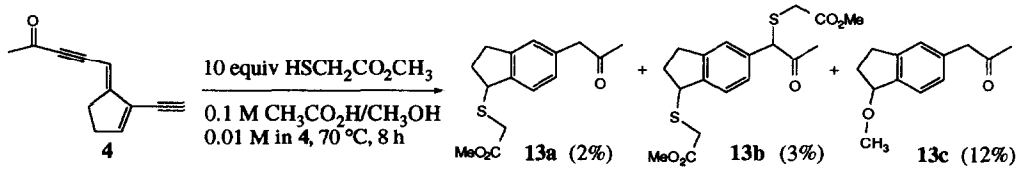
Scheme 3



(a) Br_2, Et_3N, CH_2Cl_2, 0 °C to room temp.; (b) $HCCCH_2MgBr$, Et_2O, room temp.; (c) EtMgBr (2.5 equiv), THF, acetaldehyde, room temp.; (d) $PdCl_2(PPh_3)_2$, CuI, $(i\text{-}Pr)_2NH$, HCCTMS (2.0 equiv), THF, room temp.; (e) K_2CO_3, MeOH, 4 h, room temp.; (f) $(COCl)_2$ (5.0 equiv), DMSO (10.0 equiv), Et_3N (15.0 equiv), CH_2Cl_2, -78 °C to 0 °C.

Activation-cycloaromatization studies on **4** were first examined by using the standard conditions previously employed for the activation of NCS Chrom.[55,60] When treated with methyl thioglycolate (10.0 equiv) in methanolic acetic acid (0.1M) at 70 °C, ketone **4** proved to be a functional NCS Chrom analogue, producing three cycloaromatized compounds, **13a**, **13b**, and **13c**, in 2%, 3%, and 12% yield, respectively, (Scheme 4). Products **13a** and **13b** presumably result from the expected conjugate addition of thiol to the unsaturated ketone **4**. Simple addition products corresponding to intermediates **5** (Scheme 2) did not accumulate under these conditions, indicating that the rate of their cycloaromatization was comparable to or faster than that of the conjugate addition.

Scheme 4

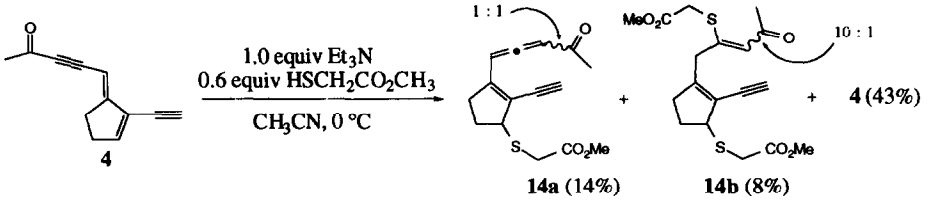


Several solvents and additives were examined in order to optimize the cycloaromatization reaction of **4**. Of the solvents tried (THF, benzene, *t*-butanol, ethanol, and methanol), cycloaromatized products were obtained only in methanol and ethanol. Alternative Michael donors including thiophenol, 4-hydroxythiophenol, *t*-butyl thiol, 1,2-ethanedithiol, and thioglycolic acid were also examined, however, only the latter two served to activate the analogue (**4**) for cycloaromatization. In some of these reactions, the dimethyl ketal of **4** was isolated as a byproduct, a result that prompted an examination of whether the addition of water would suppress the ketal formation. The effect of water proved to be significant. Addition of 15-25% water (by volume) to the reaction mixture led to an increase in yield of the methanol addition product, i.e., **13a** (<1%), **13b** (2%), **13c** (20%). The rate of reaction was also found to increase under these conditions by a factor of 2-3. These results bode well for the use of simplified analogues related to **4** under physiological conditions since the cycloaromatization of **4** proceeds in 25% water (by volume) with a half life of ~2.5 h at 37 °C. Finally, the addition of 10-20 equiv of 1,4-cyclohexadiene did not improve the efficiency of the reaction. In fact, the activation and cycloaromatization were more complex in the presence of this hydrogen atom source.

Basic conditions were also examined in order to explore the mechanism and efficiency of the activation-cycloaromatization process. The initial conditions chosen for these studies were the same as those shown in Scheme 4 except that Et_3N was used in place of acetic acid. Under the original temperature conditions (70 °C), only uncharacterizable materials were produced with this modification. However, when the temperature was reduced to 0 °C and the equivalents of thiol were decreased to 2.0, product **14b** (Scheme 5) was formed as well as another material that was derived from **14b** (as the temperature raised above 0 °C during the workup, TLC showed the disappearance of **14b** and the appearance of a new spot[61] slightly below **14b**). Attempts to use less thiol in order to promote the more efficient formation of Michael addition products produced little change in the reaction. Other solvents (THF, toluene, DMSO) and conditions (varying the amount of Et_3N or using NaH) also had little effect on the course of the reaction producing only **14b**. However, when CH_3CN was used as the solvent, along with the bisthiol adduct **14b**, a new product identified as the cycloaromatization precursor **14a** was formed. Monitoring of the reaction revealed that **14a** (1,8-addition) is the first formed product which then suffers competitive 1,4-addition of thiol with subsequent olefin isomerization leading to **14b**.

Optimization of the reaction conditions showed allene **14a** was most efficiently isolated at low conversion of **4**, thereby allowing for the recycling of starting material.

Scheme 5



The availability of intermediate **14a** through the base mediated addition of methyl thioglycolate to **4** allowed for the determination of a rate constant for its cycloaromatization. For this study, **14a** was dissolved in deoxygenated 0.1 M acetic acid-d_4 in methanol-d_4 and monitored at 40 °C in the probe of a high-field ^1H NMR spectrometer (400 MHz). Under these conditions, **14a** underwent smooth first-order decay [k_{obsd} = (3.7 ± 0.1) x 10^{-4} s^{-1}, $\Delta G^{\ddagger}$ = 23.3 kcal/mol][62-73] with concomitant appearance of signals corresponding to cycloaromatized products. Of further note, the efficiency of the formation of these products from **14a** was markedly improved over that observed in the activation of **4**. When **14a** was cycloaromatized in 0.1 M acetic acid in methanol at 40 °C compounds **13a** and **13c** were isolated in 1% yield and 85% yield, respectively. Overall, these results suggest that if greater efficiency could be achieved in the activation step (Michael addition), analogues based on prototype **4** could serve as easily accessible and efficient sources of diradicals and potential DNA cleaving agents.

A major additional finding of these studies pertains to the origin of **13c**. Related products proposed to arise from a 1,3-hydrogen shift have been observed previously in studies on NCS Chrom[74] and its analogues.[33] In 1991, we reported studies[47,48] which suggested that **13c** arises through a radical translocation pathway that corresponds to a potentially new mechanistic feature of the DNA cleavage process involving NCS Chrom and related enediyne based DNA cleaving agents. In these studies, it was found that when **4** was subjected to the reaction conditions in the absence of thiol, **13c** was not formed. Instead, **4** was recovered unchanged even after two days at 70 °C. Thus, **13c** is not derived from a simple Michael addition of methanol to **4**. Secondly, product **13a** when resubjected to the reaction conditions was recovered unchanged, ruling out the possibility that **13c** resulted from methanolysis of **13a**. These results suggest that **13c** is derived from the capture of an intermediate along the reaction path between **4** and **13a/13b**. Given the mechanistic constraints established in the above control experiments, the search for a precursor to **13c** turned to diyl **15** (Scheme 6). Possessing abstractable hydrogen atoms in the methyl thioglycolate subunit that are five atoms removed from the radical formed at C-2 (aryl radical), this diyl could partition between paths leading to **13a/13b** and to **13c**. To test this hypothesis, several labeling studies were conducted (Table 1). When the cycloaromatization of **4** was carried out in fully deuterated solvents in the presence of unlabeled thiol (Table 1, line 1), compounds **13a'** (~0.3%),[75] **13b'** (2%), and **13c'** (12%) were obtained. It is noteworthy that **13a'** and **13b'** (the thiol containing products) incorporated a deuterium atom at C-2 while **13c'** had a hydrogen at this position. A complementary experiment involving the use of labeled thiol ($HSCD_2CO_2CH_3$) produced the complementary labeling pattern, i.e., **13a"** (3%) and **13b"** (3%) had hydrogen incorporated at C-2 while **13c"** (12%) was labeled with deuterium at this position (Table 1, line 2).

Scheme 6

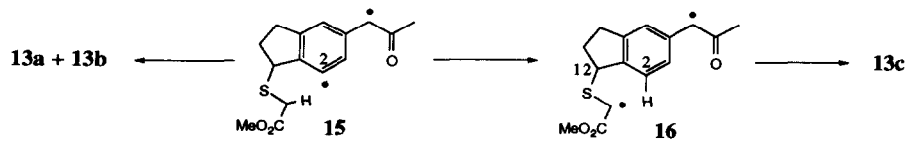
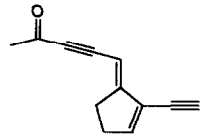


Table 1. Solvents and Labeling Results for Studies of 1,5-Hydrogen Transfer.[76]



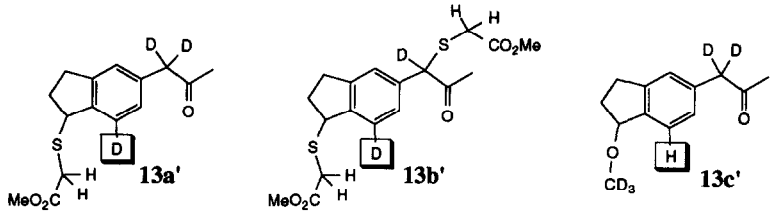
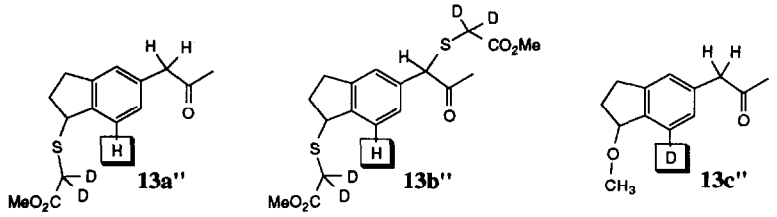
4

10 equiv $HSC(R)_2CO_2CH_3$

70 °C, 8-12 h

Solvent

Cycloaromatized Products

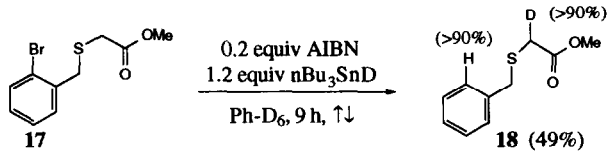
Solvent	Cycloaromatized Products
0.1 M CD_3CO_2D in CD_3OD R = H	 13a' 13b' 13c'
0.1 M CH_3CO_2H in CH_3OH R = D	 13a'' 13b'' 13c''

The percentage of hydrogen or deuterium incorporated at C-2 in the above compounds is: **13a'** (~85%); **13a''**, **13b'** (>95%); **13b''**, **13c'**, **13c''** (>97%). Values determined by ^1H NMR.

The contrasting incorporation of label and the isotopic effect on product ratios (**13a**:**13c** 1:6; **13a'**:**13c'** ~1:40, line 1; **13a''**:**13c''** ~1:4, line 2) in these experiments are both consistent with the proposed path in Scheme 6. Specifically, the activation and cycloaromatization of **4** would follow a common path to diyl **15**. At this juncture, the radical center at C-2 could be quenched through either an *inter*molecular or *intra*molecular abstraction process. An *inter*molecular atom abstraction reaction between diyl **15** and solvent would produce products **13a** and **13b** incorporating the solvent label at C-2 (deuterium in deuterated solvents or hydrogen in non-deuterated solvents); whereas, the *intra*molecular abstraction of the hydrogen or deuterium atoms adjacent to the ester group would lead to **16**. Homolytic or heterolytic cleavage of the C-12 sulfur bond in **16** would lead to a *p*-xylylene or a benzylic cation, respectively, which upon capture by methanol would produce **13c**. The viability of this internal hydrogen abstraction path is also supported by the growing body of related

intramolecular hydrogen abstraction processes observed in radical reactions in general[77-87] and the more specific studies outlined in Scheme 7 involving internal abstraction between an independently generated aryl radical and a thioglycolate hydrogen atom source.

Scheme 7

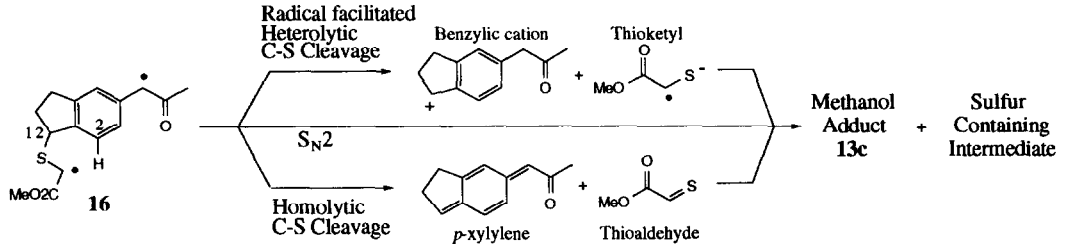


17 **18** (49%)

The above experiments firmly establish the facility of the intramolecular atom transfer pathway between an aryl radical and the hydrogen of an internal thioglycolate subunit. This finding is of considerable importance in the design and mode of action of NCS Chrom analogues and NCS Chrom itself[50,53] since any related intramolecular hydrogen transfer would reduce the efficiency of double strand cleavage by quenching one radical site of the intermediate diyl. Moreover, this finding provides the basis for the design of analogue probes to determine when and where activation occurs and the precise arrangement of the activating nucleophile to the diyl in the presence of DNA. Computer modeling studies done by this group[56] indicate that for certain sequences of DNA, the thioglycolate subunit of DNA-bound and activated NCS Chrom would be oriented so as to minimize internal hydrogen transfer; whereas, if activation occurs outside of the minor groove, such transfer would be expected to be facile. It is noteworthy that this internal quenching path could also apply to enediyne DNA cleaving agents in which a hydrogen at an anomeric center is positioned to quench a radical center of the intermediate diyl.[88-90]

A final point of interest is the fate of intermediate **16**. Two intriguing pathways leading to the methanol incorporated product (**13c**) can be envisioned (Scheme 8). The first involves radical facilitated heterolytic cleavage of the benzylic C-12 sulfur bond, resulting in the formation of a thioketyl directly and a benzylic carbocation which could then be captured by solvent. The second path involves homolytic cleavage and would necessitate the participation of both radicals. In this case, cleavage of the benzylic C-S bond would produce a thioaldehyde and a *p*-xylylene intermediate activated by a carbonyl group for conjugate addition of solvent. S_N2 displacement corresponding to these two step sequences could also be operative.[91] These pathways have interesting synthetic and mechanistic consequences within and beyond the scope of the NCS area.

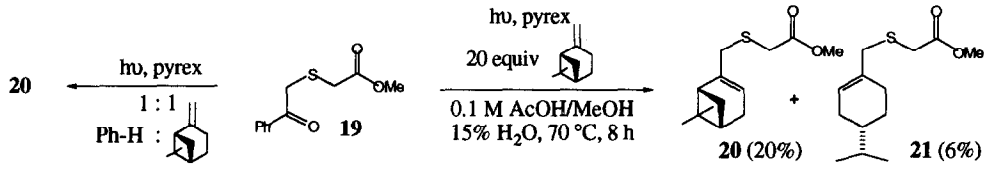
Scheme 8



Efforts to identify the fate of diyl **16** focused on the use of trapping agents to capture the newly formed sulfur containing reactive intermediate. Pertinent to this goal are the elegant studies of Vedejs *et al.*[92-94] which showed that the thioaldehyde in question (derived from **19**, left arrow Scheme 9) could be trapped with β-pinene through an ene reaction giving **20**. The Vedejs procedure was, therefore, repeated to generate an

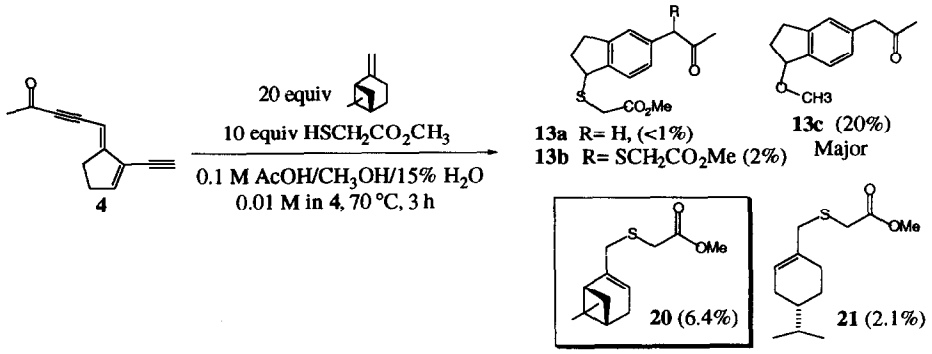
authentic sample of the thioaldehyde trapped product **20** and then modified (right arrow, Scheme 9) in order to emulate the procedure used in the activation studies of analogue **4**. Under the latter conditions, the thioaldehyde product was indeed trapped with an efficiency of 20%. In addition, product **21** (6% yield) derived from thiyl radical addition to the olefin and subsequent cyclobutane ring fragmentation was also detected.

Scheme 9

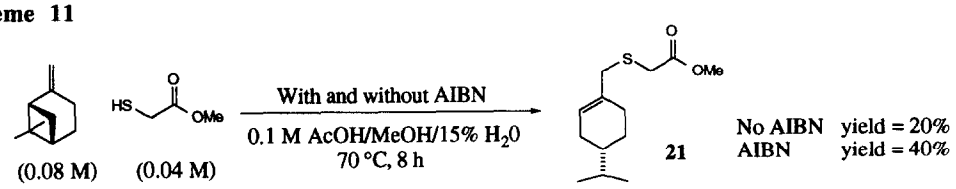


When the cycloaromatization reaction of the monocyclic analogue **4** was run using the conditions found to optimize the yield of the methanol adduct (**13c**, 15% water added) in the presence of 20 equiv of β-pinene (Scheme 10), the pinene-thioaldehyde ene reaction product (**20**) was isolated in 6.4% yield along with thiyl radical addition product **21** (2.1% yield). The methanol adduct **13c** was isolated in 20% yield indicating a 32% trapping efficiency of the thioaldehyde (assuming sole generation of a thioaldehyde intermediate) which is higher than that found in the photochemical model system. Furthermore, when allene-ene-yne **14a** was cycloaromatized in the presence of 20 equiv of β-pinene in 0.1 M CD_3CO_2D/CD_3OD, **20** was obtained in 40% yield which indicates a thioaldehyde trapping efficiency of 47% (based on an 85% yield of **13c** under these conditions). As a control, the trapping reaction was repeated without monocyclic analogue **4** (Scheme 11). In this case, only thiyl radical addition product **21** (20% yield) was obtained. Another control experiment was conducted with 0.5 equiv of AIBN as a radical source. This merely led to a higher yield of thiyl radical addition product **21** (40% yield). While these experiments do not eliminate the thioketyl pathway (or others related to it), they indicate that thioaldehyde is formed in the activation-cycloaromatization reaction of **4** under conditions in which methanol addition product **13c** is observed.

Scheme 10



Scheme 11



In summary, *this study demonstrates that simple monocyclic analogues of NCS Chrom can be used to generate diyls under mild conditions, indicating that ring strain is not a required feature in the design of related DNA cleaving agents.* The availability of compound **4**, its facile cyclization to diyl intermediates, and the potential of accommodating DNA recognition elements in such analogues, augurs well for the design of related simple, monocyclic DNA cleaving agents. Along with previous work from these laboratories, *this study has provided a first proposal and experimental evidence of a self quenching reaction involving an intramolecular 1,5-hydrogen atom shift that provides an explanation for the thiol dependent strand cleavage of DNA by NCS. This same pathway could also operate in enediyne agents for which a hydrogen atom at an anomeric center of an attached carbohydrate would serve to quench the proximate radical center.*[88-90] These studies provide the basis for the development of novel mechanistic probes that could figure in addressing the timing, location, and conformational characteristics of DNA cleavage effected both by NCS Chrom analogues and NCS Chrom itself since the internal quenching pathway uncovered in our work would be expected to proceed differently for a diyl generated in solution versus one generated in the structured environment of the DNA minor groove. Studies on these analogues and mechanistic issues are continuing.

Acknowledgments

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Experimental Section

All commercially available reagents were used without further purification unless otherwise noted. All reagents and starting materials were obtained from the Aldrich Chemical Co. unless otherwise noted. Hexane and EtOAc were purchased from J. T. Baker. THF, DME, and Et_2O were distilled from sodium-benzophenone ketyl. CH_2Cl_2, $(i\text{-}Pr)_2NH$, CH_3CN, and Et_3N were distilled from calcium hydride. Analytical thin layer chromatography (TLC) was performed on Merck pre-coated TLC plates (silica gel 60 F_{254}, 0.25 mm). Flash chromatography refers to column chromatography employing Merck Silica Gel 60 (40-63 μm) in the method of Still.[95] Concentration in vacuo refers to rotary evaporation. Melting points were taken on a Thomas Hoover apparatus and are uncorrected. NMR spectra were measured on a Varian Gemini-300 (^1H at 300 MHz, ^{13}C at 75 MHz) or a Varian XL-400 (^1H at 400 MHz, ^{13}C at 100 MHz) magnetic resonance spectrometer and are reported in parts-per-million (ppm) downfield from a tetramethylsilane internal standard (δ=0 ppm) as the reference in $CDCl_3$ unless otherwise noted. Infrared (IR) spectra were measured on a Perkin-Elmer model 1600 Fourier transform spectrometer as a thin film and are reported in wavenumbers (cm^{-1}). High resolution mass spectra (HRMS) were provided by the Mass Spectrometry Facility, University of California-San Francisco.

2-Bromo-1-(2-propynyl)-cyclopent-2-en-1-ol (9):
Magnesium turnings (13.8 g, 566 mmol, Fluka) and $HgCl_2$ (1.92 g, 7.07 mmol) were added to a dry 1000-mL three-neck round-bottom flask equipped with a stir bar, a glass stopper, a reflux condenser, and a 250-mL addition funnel. Et_2O (200 mL) was then added and the flask was placed in a 0 °C sonication bath. Propargyl bromide (42.7 mL, 566 mmol, Fluka) was added to the addition funnel along with Et_2O (50 mL). The sonicator was then turned on, and the propargyl bromide solution was allowed to slowly drip into the reaction. *Caution: The sonication sometimes takes a while to initiate the reaction. However, once this reaction starts, it is very exothermic. It is advisable to have a large ice bath ready to cool the reaction.* After the reaction had initiated, Et_2O (300 mL) was added. Sonication was continued as necessary until nearly all of the magnesium was consumed. At this point the reaction was a greenish color. This was allowed to stir an additional hour. Ketone **8** (57.0 g, 354 mmol) was added in Et_2O (100 mL) to the addition funnel. It was then slowly added to the reaction over 30 min. After an additional 30 min of stirring, the solution was poured into a separatory funnel

and diluted with Et_2O (200 mL). Saturated aqueous NH_4Cl (150 mL) was then slowly poured into the solution to quench the reaction. *Caution: This can be exothermic and large volumes of gas may be evolved.* The organic layer was washed with a second portion of saturated aqueous NH_4Cl (150 mL). The aqueous layers were then combined and reextracted with Et_2O (1 x 300 mL). The organic layers were combined, washed with brine (1 x 200 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to a dark red oil. Purification by flash chromatography (silica gel, 140 mm x 9", 3:1 hexane/EtOAc) gave **9** (64.0 g, 319 mmol, 90%) as an orange oil. ^1H NMR (300 MHz, $CDCl_3$) δ 6.12 (t, J = 2.6, 1 H), 2.68 (dd, J = 16.6, 2.6, 1 H, A of ABX), 2.52 (dd, J = 16.5, 2.5, 1 H, B of ABX), 2.44-2.52 (m, 2 H), 2.35-2.43 (m, 1 H), 2.19 (s, 1 H), 2.11 (t, J = 4.6, 1 H), 2.04 (t, J = 2.7, 1 H, X of ABX); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.6, 126.8, 84.2, 79.5, 70.3, 35.0, 29.6, 29.3; IR (thin film) 3395, 3296, 2941, 2852, 1615, 1425, 1064, 941, 642 cm^{-1}; HRMS calcd for C_8H_9BrO: 199.9837 (^{79}Br) 201.9816 (^{81}Br). Found 199.9852 (^{79}Br) 201.9815 (^{81}Br).

2-Bromo-1-(2-pentyn-4-ol)-cyclopent-2-en-1-ol (10):
Compound **9** (19.6 g, 97.5 mmol) was added in THF (50 mL) to a 1000-mL three-neck round-bottom flask equipped with a stir bar, a reflux condenser, an addition funnel, and a septa. THF (350 mL) was added, and the reaction cooled to 0 °C. EtMgBr (81.3 mL, 3.0 M in Et_2O, 244 mmol) was then added to the addition funnel and slowly added to the reaction. It was then allowed to warm to room temperature and stir for 2 h. The solution turned dark orange with a yellow precipitate. Acetaldehyde (16.3 mL, 293 mmol) was added to the addition funnel along with THF (20 mL). The reaction was cooled to 0 °C, and the acetaldehyde solution slowly dropped into the reaction. After stirring an additional 15 min, the reaction was poured into a separatory funnel and Et_2O (300 mL) was added. It was extracted with 1N HCl (2 x 150 mL) and brine (1 x 150 mL). The aqueous layers were combined and extracted again with Et_2O (2 x 300 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash chromatography (silica gel, 70 mm x 7", 3:1 hexane/EtOAc) gave **10** (21.3 g, 86.8 mmol, 89%) as an orange oil. ^1H NMR (300 MHz, $CDCl_3$) δ 6.07 (t, J = 2.4, 1 H), 4.51 (m, 1 H), 2.67 (d, J = 16.6, 1 H, A of AB), 2.48 (d, J = 17.0, 1 H, B of AB), 2.46 (s, 1 H), 2.37-2.50 (m, 2 H), 2.24-2.34 (m, 2 H), 2.03-2.12 (m, 1 H), 1.43 (d, J = 6.6, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.3, 127.4, 84.7, 84.3, 79.5, 58.3, 35.4, 29.8, 29.6, 24.3; IR (thin film) 3341, 2975, 2937, 1371, 1326, 1162, 1065, 1018, 892 cm^{-1}; HRMS calcd for $C_{10}H_{13}BrO_2$: 225.9993 (^{79}Br, M-H_2O) 227.9973 (^{81}Br, M-H_2O). Found 225.9952 (^{79}Br, M - H_2O) 227.9959 (^{81}Br, M - H_2O).

1-(2-pentyn-4-ol)-2-(2-(trimethylsilyl)ethynyl)cyclopent-2-en-1-ol (11):
Compound **10** (22.0 g, 89.4 mmol) and THF (250 mL) were added to a 500-mL round-bottom flask. $(PPh_3)_2PdCl_2$ (1.88 g, 2.68 mmol) was then added. This dissolved to give a bright yellow solution. *i*-Pr_2NH (125.0 mL, 894 mmol) was added, and after 10 min of stirring, CuI (1.50 g, 7.88 mmol) was added. After an additional 10 min, the addition of the trimethylsilylacetylene (19.0 mL, 134 mmol) was begun. The trimethylsilylacetylene was added over 3 h by a syringe pump. The reaction was allowed to stir for 2 h after the addition was complete. It was then poured into a separatory funnel, diluted with Et_2O (500 mL), and washed with saturated aqueous NH_4Cl (2 x 150 mL), 1 N HCl (1 x 150 mL), saturated aqueous $NaHCO_3$ (1 x 150 mL), and brine (1 x 150 mL). The aqueous layers were combined and extracted with Et_2O (2 x 500 mL). The organic layers were then combined, dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash chromatography (silica gel, 70 mm x 7", 1:1 hexane/EtOAc) gave **11** (21.3 g, 81.4 mmol, 91%) as a dark, red oil. ^1H NMR (300 MHz, $CDCl_3$) δ 6.25 (t, J = 2.6, 1 H), 4.53 (m, 1 H), 2.75 (d, J = 16.6, 1 H, A of AB), 2.52 (d, J = 16.3, 1 H, B of AB), 2.49-2.78 (m, 1 H), 2.25-2.44 (m, 2 H), 2.19 (s, 1 H), 1.96-2.05 (m, 1 H), 1.84 (d, J = 3.9, 1 H), 1.44 (d, J = 6.6, 3 H), 0.21 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.9, 130.2, 98.9, 98.6, 84.8, 84.3, 80.1, 58.1, 36.5, 30.0, 29.8, 24.2, -0.4; IR (thin film) 3357, 2962, 2151, 1322, 1250, 1167, 1069 cm^{-1}; HRMS calcd for $C_{15}H_{22}O_2Si$: 244.1283 (M-H_2O). Found 244.1285 (M-H_2O).

2-ethynyl-1-(2-pentyn-4-ol)cyclopent-2-en-1-ol (12):
Compound **11** (21.3 g, 81.4 mmol) and MeOH (500 mL) were combined in a 1000-mL round-bottom flask. K_2CO_3 (1.12 g, 8.14 mmol) was then added. The reaction was allowed to stir for 1.5 h. The MeOH was removed in vacuo. The resulting black oil was taken up in Et_2O (700 mL) and extracted with 1 N HCl (1 x 150 mL), saturated aqueous $NaHCO_3$ (1 x 150 mL), and brine (1 x 150 mL). The aqueous layers were combined and extracted with Et_2O (1 x 500 mL). The organic layers were then combined, dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash chromatography (silica gel, 70 mm x 6", 2:3 hexane/EtOAc) gave **12** (12.6 g, 66.1 mmol, 76%) as a brown oil. ^1H NMR (300 MHz, $CDCl_3$) δ 6.30 (t, J = 2.7, 1 H), 4.51 (q, J = 6.6, 1 H), 3.07 (s, 1 H), 2.75 (d, J = 16.5, 1 H, A of AB), 2.51 (d, J = 16.5, 1 H, B of AB), 2.51-2.61 (m, 1 H), 2.41-2.42 (m, 1 H), 2.19 (s, 1 H), 2.26-2.36 (m, 2 H), 1.97-2.05 (m, 1 H), 1.42 (d, J = 6.5, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.8, 129.2, 84.8, 84.5, 80.9, 80.0, 77.9, 58.1, 36.6, 30.1, 29.8, 24.2; IR (thin

film) 3265, 2982, 1362, 1326, 1167, 1062, 1012, 955 cm^{-1}; HRMS calcd for $C_{12}H_{14}O_2$: 172.0888 (M-H_2O). Found 172.0895 (M-H_2O).

***E*-1-(ethynyl)-5-(4-oxo-2-pentynylidene)cyclopentene (4):**
To a 250-mL round-bottom flask was added CH_2Cl_2 (100 mL). Oxalyl chloride (2.10 mL, 24.2 mmol, 5.0 equiv) was then added, and the reaction was cooled to -78 °C. DMSO (3.42 mL, 48.3 mmol, 10.0 equiv) was added. After 4 min, substrate **12** (0.918 g, 4.83 mmol) was added in CH_2Cl_2 (8 mL). The reaction was allowed to stir for 0.5 h at -78 °C. Et_3N (10.0 mL, 72.5 mmol, 15 equiv) was added, and the reaction was allowed to stir an additional 0.5 h. At this point, the cooling bath was removed, and the reaction was allowed to warm for 15 min. While the reaction was still cold (approximately -20 °C), it was diluted with CH_2Cl_2 (100 mL). It was then extracted with 1 N HCl (2 x 40 mL), saturated aqueous $NaHCO_3$ (1 x 40 mL), and brine (1 x 40 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification by flash chromatography at 4 °C in a cold room (silica gel, 40 mm x 7", 7:1 hexane/Et_2O) gave **4** (0.310 g, 1.82 mmol, 38%) as a yellow-white solid, (mp = 65-66 °C decomp). ^1H NMR (300 MHz, $CDCl_3$) δ 6.79 (t, *J* = 3.0, 1 H), 5.76 (s, 1 H), 3.18 (s, 1 H), 2.84-2.88 (m, 2 H), 2.65-2.69 (m, 2 H), 2.38 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.3, 166.3, 151.6, 127.1, 96.3, 94.4, 89.9, 82.0, 75.7, 32.3, 31.3, 29.1; IR (thin film) 3211, 2201, 1658, 1443, 1374, 877, 843, 703 cm^{-1}; HRMS calcd for $C_{12}H_{10}O$: 170.0732. Found 170.0744.

Cycloaromatization of 4, Procedure A:
To a 250-mL round-bottom flask was added the substrate **4** (0.210 g, 1.24 mmol) and 0.1 M acetic acid in methanol (125 mL) under aerobic conditions. Methyl thioglycolate (1.10 mL, 12.4 mmol) was added, and a reflux condenser was placed on top of the flask (open to the air). The reaction was placed in a 70 °C oil bath and followed by TLC. When the starting material was consumed (8-12 h), the reaction was concentrated in vacuo. The crude oil was purified by flash chromatography using a gradient elution (silica gel, 25 mm x 7", 100% hexane → 2:1 hexane/EtOAc). The gradient is slowly increased using 1, 2, 3, 5, 7, 10, 20, and 30% EtOAc. The order of elution is **13c**, **13a**, and **13b**. This yields **13a** (5.84 mg, 0.021 mmol, 1.7%) and **13b** (16.0 mg, 0.042 mmol, 3.4%) as light-yellow oils, and **13c** (30.0 mg, 0.148 mmol, 12%) as a clear oil. Analytically pure material was obtained by resubjecting each compound separately to a second flash chromatography (silica gel, 40:1 CH_2Cl_2/Et_2O). For compound **13a**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, *J* = 7.7, 1 H), 7.10 (s, 1 H), 7.04 (d, *J* = 7.8, 1 H), 4.47 (dd, *J* = 7.4, 4.2, 1 H), 3.75 (s, 3 H), 3.68 (s, 2 H), 3.26 (s, 2 H), 3.04-3.14 (m, 1 H), 2.81-2.91 (m, 1 H), 2.49-2.61 (m, 1 H), 2.11-2.25 (m, 1 H), 2.17 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.5, 171.4, 144.8, 141.7, 134.2, 128.0, 126.1, 125.3, 52.2, 50.8, 49.0, 33.7, 32.7, 30.7, 29.1; IR (thin film) 2951, 1735, 1717, 1434, 1356, 1278, 1157 cm^{-1}; HRMS calcd for $C_{15}H_{18}O_3S$: 278.0977. Found 278.0984. For compound **13b**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, *J* = 7.7, 1 H), 7.18 (s, 1 H), 7.13 (d, *J* = 7.8, 1 H), 4.92 (s, 1 H), 4.40 (dd, *J* = 7.4, 4.1, 1 H), 3.68 (s, 3 H), 3.65 (s, 3 H), 3.20 (s, 2 H), 3.12 (d, *J* = 14.8, 1 H, A of AB), 2.97-3.05 (m, 1 H), 2.93 (d, *J* = 14.8, 1 H, B of AB), 2.75-2.85 (m, 1 H), 2.45-2.53 (m, 1 H), 2.06-2.15 (m, 1 H), 2.09 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.4, 171.3, 170.9, 145.2, 143.4, 134.9, 127.6, 125.5, 125.3, 77.2, 60.7, 52.3, 48.7, 33.5, 32.7, 31.8, 30.6, 27.8; IR (thin film) 2950, 1734, 1437, 1280, 1153, 1011 cm^{-1}; HRMS calcd for $C_{18}H_{22}O_5S_2$: 382.0909. Found 382.0894. For compound **13c**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, *J* = 7.6, 1 H), 7.12 (s, 1 H), 7.07 (d, *J* = 7.7, 1 H), 4.81 (dd, *J* = 6.5, 3.9, 1 H), 3.70 (s, 2 H), 3.42 (s, 3 H), 3.02-3.10 (m, 1 H), 2.78-2.86 (m, 1 H), 2.31-2.39 (m, 1 H), 2.16 (s, 3 H), 2.06-2.14 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.1, 145.0, 141.8, 134.6, 127.7, 126.0, 125.5, 84.2, 56.1, 50.9, 31.9, 29.9, 29.1; IR (thin film) 2972, 2929, 2868, 1711, 1431, 1355, 1335, 1158, 1117, 1082 cm^{-1}; HRMS calcd for $C_{13}H_{16}O_2$: 204.1150. Found 204.1152.

Allene-ene-yne intermediate (14a) and bisthiol adduct (14b):
To a 200-mL round-bottom flask was added ketone **4** (0.2884 g, 1.70 mmol) in CH_3CN (90 mL). The reaction was cooled to 0 °C. Methyl thioglycolate (0.0911 mL, 1.02 mmol, 0.6 equiv) was added over 20 min in CH_3CN (10 mL) with a syringe pump. The reaction was then concentrated in vacuo to give a dark oil. Purification by flash chromatography at 4 °C in a cold room (silica gel, 23 mm x 6", 2:1 hexane/Et_2O) gave **4** (0.124 g, 0.729 mmol, 43%), **14a** (0.066 g, 0.239 mmol, 14%), and **14b** (0.051 g, 0.134 mmol, 8%). For the higher R_f diastereomer of **14a**: ^1H NMR (300 MHz, $CDCl_3$) δ 6.82 (d, *J* = 6.3, 1 H), 6.06 (d, *J* = 6.0, 1 H), 4.14-4.18 (m, 1 H), 3.76 (s, 3 H), 3.53 (d, *J* = 15.0, 1 H, A of AB), 3.46 (s, 1 H), 3.34 (d, *J* = 15.1, 1 H, B of AB), 2.42-2.70 (m, 3 H), 2.27 (s, 3 H), 2.00-2.08 (m, 1 H). For the lower R_f diastereomer of **14a**: ^1H NMR (300 MHz, $CDCl_3$) δ 6.83 (d, *J* = 6.1, 1 H), 6.07 (d, *J* = 6.0, 1 H), 4.14-4.18 (m, 1 H), 3.76 (s, 3 H), 3.51 (d, *J* = 14.9, 1 H, A of AB), 3.46 (s, 1 H), 3.32 (d, *J* = 15.0, 1 H, B of AB), 2.35-2.70 (m, 3 H), 2.25 (s, 3 H), 2.00-2.09 (m, 1 H); ^{13}C NMR (100 MHz, 0.1 M CD_3CO_2D/CD_3OD, mixture of diastereomers) δ 220.22, 220.19, 199.3, 172.8, 144.2, 125.0, 100.6, 94.8, 88.0, 78.8, 54.7, 52.9, 33.2, 32.7, 32.1, 27.2; IR (thin film) 3200, 2948, 1922, 1735, 1680, 1434, 1278, 1224, 1151, 1014 cm^{-1}; HRMS calcd for

$C_{15}H_{16}O_3S$: 276.0820. Found 276.0816. For compound **14b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (s, 1 H), 4.05 (m, 1 H), 3.76 (s, 3 H), 3.73 (s, 3 H), 3.62 (s, 2 H), 3.57 (d, J = 10.2, 2 H), 3.47 (d, J = 15.0, 1 H, A of AB), 3.44 (s, 1 H), 3.28 (d, J = 15.0, 1 H, B of AB), 2.71-2.79 (m, 1 H), 2.52-2.62 (m, 1 H), 2.35-2.48 (m, 1 H), 2.24 (s, 3 H), 1.96-2.05 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.1, 170.9, 169.1, 147.7, 133.3, 123.4, 122.8, 86.1, 78.9, 52.7, 52.4, 48.1, 34.6, 33.0, 32.7, 31.7, 29.2; IR (thin film) 3265, 3000, 2952, 2086, 1736, 1677, 1600, 1435, 1293, 1195, 1156, 1008 cm^{-1}; HRMS calcd for $C_{18}H_{22}O_5S_2$: 382.0909. Found 382.0902.

Cycloaromatization of 4, Procedure B (deuterated solvents):
Same general procedure as A. Ketone **4** (0.237 g, 1.39 mmol) and methyl thioglycolate (1.25 mL, 13.9 mmol) were combined in a 250-mL round-bottom flask with 0.1 M acetic acid-d_4 in methanol-d_4 (139 mL) as the solvent. After 12-15 h at 70 °C, workup and purification according to procedure A gave **13a'** (1.20 mg, 0.00418 mmol, 0.3%), **13b'** (11.2 mg, 0.0293 mmol, 2.1%), and **13c'** (35.8 mg, 0.171 mmol, 12.3%) as oils. Selected analytical data for compound **13a'**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (s, 1 H), 7.04 (s, 1 H), 4.47 (dd, J = 7.4, 4.2, 1 H), 3.75 (s, 3 H), 3.26 (s, 2 H), 3.04-3.14 (m, 1 H), 2.81-2.91 (m, 1 H), 2.49-2.61 (m, 1 H), 2.11-2.25 (m, 1 H), 2.17 (s, 3 H); GC/MS for $C_{15}H_{15}D_3O_3S$, selected peaks,(relative intensity): 281.00 (4%), 176.10 (47%), 133.10 (33%). For compound **13b'**: ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.25 (s, 1 H), 7.19 (s, 1 H), 4.97 (s, 1 H, D exchanged for H on silica), 4.48 (dd, J = 7.4, 4.4, 1 H), 3.72 (s, 3 H), 3.69 (s, 3 H), 3.28 (s, 2 H), 3.17 (d, J = 15.0, 1 H, A of AB), 3.06-3.11 (m, 1 H), 3.00 (d, J = 15.1, 1 H, B of AB), 2.83-2.93 (m, 1 H), 2.52-2.59 (m, 1 H), 2.12-2.21 (m, 1 H), 2.14 (s, 3 H); HRMS calcd for $C_{18}H_{20}D_2O_5S_2$: 384.1034. Found 384.1025. For compound **13c'**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, J = 7.7, 1 H), 7.05 (s, 1 H), 6.99 (dd, J = 7.7, 0.8, 1 H), 4.73 (dd, J = 6.6, 4.1, 1 H), 2.95-3.05 (m, 1 H), 2.68-2.78 (m, 1 H), 2.21-2.33 (m, 1 H), 2.08 (s, 3 H), 1.97-2.06 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.2, 145.0, 141.8, 134.6, 127.6, 126.0, 125.5, 84.1, 55.2 (septet, $J_{C,D}$= 20), 50.9, 50.3 (p, $J_{C,D}$= 21), 31.8, 29.9, 29.0; HRMS calcd for $C_{13}H_{11}D_5O_2$: 209.1464. Found 209.1463.

α-d_2-Methyl thioglycolate:
To a 250-mL round-bottom flask was added methyl thioglycolate (7.5 mL, 70.8 mmol) and CH_3OD (100 mL). This solution was degassed with nitrogen bubbling for 10 min. Et_3N (11.8 mL, 84.9 mmol) was added and the reaction was allowed to stir for 12 h. It was then concentrated in vacuo. ^{1}H NMR at this point showed the deuterium content in the α position to be 88%. The material was then resubjected to the same conditions (same amount of CH_3OD and Et_3N added). After it was concentrated the second time it was purified by distillation (bp = 55 °C at 18 mmHg, Lit. bp = 42 °C at 10 mmHg). ^{1}H NMR now showed a deuterium incorporation of 98.7% at the α position and 85% at the SH. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.76 (s, 3 H), 1.99 (s, 1 H).

Cycloaromatization of 4, Procedure C (deuterated thiol):
Same general procedure as A. Ketone **4** (0.057 g, 0.335 mmol) and α-d_2-methyl thioglycolate (0.283 mL, 3.35 mmol) were combined in a 200-mL round-bottom flask with 0.1 M acetic acid in methanol (60 mL) as the solvent. After 8 h at 70 °C, workup and purification according to procedure A gave **13a''** (2.8 mg, 0.010 mmol, 3%), **13b''** (3.8 mg, 0.010 mmol, 3%), and **13c''** (8.2 mg, 0.040 mmol, 12%). For compound **13a''**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, J = 7.7, 1 H), 7.10 (s, 1 H), 7.04 (d, J = 7.8, 1 H), 4.47 (dd, J = 7.4, 4.2, 1 H), 3.75 (s, 3 H), 3.68 (s, 2 H), 3.04-3.14 (m, 1 H), 2.81-2.91 (m, 1 H), 2.49-2.61 (m, 1 H), 2.11-2.25 (m, 1 H), 2.17 (s, 3 H). For compound **13b''**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, J = 7.7, 1 H), 7.18 (s, 1 H), 7.13 (d, J = 7.8, 1 H), 4.92 (s, 1 H), 4.40 (dd, J = 7.4, 4.1, 1 H), 3.68 (s, 3 H), 3.65 (s, 3 H), 2.97-3.05 (m, 1 H), 2.75-2.85 (m, 1 H), 2.45-2.53 (m, 1 H), 2.06-2.15 (m, 1 H), 2.09 (s, 3 H). For compound **13c''**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (s, 1 H), 7.06 (s, 1 H), 4.81 (dd, J = 6.5, 3.9, 1 H), 3.70 (s, 2 H), 3.42 (s, 3 H), 3.02-3.10 (m, 1 H), 2.78-2.86 (m, 1 H), 2.31-2.39 (m, 1 H), 2.16 (s, 3 H), 2.06-2.14 (m, 1 H).

Cycloaromatization of 4, Procedure D (thioaldehyde trapping agent present):
Same general procedure as A. Ketone **4** (0.060 g, 0.353 mmol), 0.1 M acetic acid in methanol (34 mL), and H_2O (6.0 mL, 15% by volume) were combined in a 100-mL round-bottom flask. β-pinene (1.12 mL, 7.06 mmol) and methyl thioglycolate (0.315 mL, 3.53 mmol) were then added. After 4 h at 70 °C, workup and purification according to procedure A gave, in order of elution, **20** (0.0054, 0.0225 mmol, 6.4%), **21** (0.0018 g, 0.0074 mmol, 2.1%), **13c** (0.0144 g, 0.0706 mmol, 20%), **13a** (0.0010 g, 0.00353 mmol, 1%), and **13b** (0.0027 g, 0.00706 mmol, 2.0%). The analytical data for compounds **13a**, **13b**, and **13c** were consistent with those shown in procedure A. For compound **20**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.43 (dd, J = 2.8, 1.40, 1 H), 3.74 (s, 3 H), 3.17 (dq, J = 13.4, 1.2, 2 H), 3.16 (s, 2 H), 2.10-2.45 (m, 5 H), 1.30 (s, 3 H), 1.12 (d, J = 8.7, 1 H), 0.83 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 142.3, 121.2, 52.2, 45.0, 40.4, 38.5, 38.1, 31.8, 31.7, 31.3, 26.1, 21.0; IR (thin film) 2918, 1737, 1435, 1275, 1154, 1128, 1013 cm^{-1}; HRMS calcd for

$C_{13}H_{20}O_2S$: 240.1184. Found 240.1173. For compound **21**: ^1H NMR (300 MHz, $CDCl_3$) δ 5.59 (m, 1 H), 3.73 (s, 3 H), 3.17 (m, 2 H), 3.14 (s, 2 H), 2.03-2.12 (m, 3 H), 1.76-1.87 (m, 2 H), 1.48 (q, J = 6.6, 1 H), 1.17-1.29 (m, 2 H), 0.90 (d, J = 6.7, 3 H), 0.89 (d, J = 6.8, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 132.3, 126.3, 52.2, 39.9, 39.5, 32.1, 31.8, 28.9, 27.3, 26.1, 19.9, 19.6; IR (thin film) 2935, 1735, 1430, 1280, 1134, 1008 cm^{-1}; HRMS calcd for $C_{13}H_{22}O_2S$: 242.1341. Found 242.1335.

Carbomethoxymethyl *o*-bromobenzyl sulfide (17):
To a 200-mL round-bottom flask was added methyl thioglycolate (1.0 mL, 11.2 mmol) and THF (100 mL). The reaction was cooled to 0 °C, and Et_3N (1.56 mL, 11.2 mmol) was added. *o*-Bromobenzyl bromide (2.0 g, 8.00 mmol) was then added in THF (10 mL). The reaction was allowed to warm to room temperature and stir for 4 h. It was then diluted with Et_2O (100 mL) and extracted with 1 N HCl (1 x 25 mL), saturated aqueous $NaHCO_3$ (1 x 25 mL), and brine (1 x 25 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash chromatography (silica gel, 40 mm x 6", 9:1 hexane/EtOAc) gave **17** (1.72 g, 6.24 mmol, 78%) as a clear oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 8.0, 1 H), 7.39 (dd, J = 7.5, 1.6, 1 H), 7.28 (t, J = 7.4, 1 H), 7.14 (td, J = 7.7, 1.7, 1 H), 3.96 (s, 2 H), 3.74 (s, 3 H), 3.16 (s, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 136.7, 133.4, 131.1, 129.0, 127.5, 124.7, 52.2, 36.4, 32.1; IR (thin film) 2998, 2950, 1732, 1468, 1437, 1280, 1132, 1025, 763, 735 cm^{-1}; HRMS calcd for $C_{10}H_{11}BrO_2S$: 273.9663 (^{79}Br) 275.9643 (^{81}Br). Found 273.9672 (^{79}Br) 275.9641 (^{81}Br).

Carbomethoxy-d_1-methyl benzyl sulfide (18):
To a 200-mL round-bottom flask was added **17** (0.256 g, 0.931 mmol) and benzene (15 mL). Tri-*n*-butyltin deuteride (0.301 mL, 1.12 mmol) and AIBN (0.030 g, 0.186 mmol) were then added. The reaction was placed in an oil bath and refluxed for 9 h. The reaction was cooled and diluted with Et_2O (40 mL) and extracted with saturated aqueous KF (3 x 10 mL) and brine (1 x 10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash chromatography (silica gel, 20 mm x 6", 9:1 hexane/EtOAc) gave **18** (0.0899 g, 0.456 mmol, 49%) as a clear oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.27-7.36 (m, 5H), 3.84 (s, 2H), 3.73 (s, 3H), 3.08-3.10 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 137.4, 129.3, 128.7, 128.6, 127.4, 52.2, 36.1, 31.8, 31.6 (t, $J_{C,D}$= 20); IR (thin film) 3002, 2950, 1736, 1494, 1435, 1273, 1010 cm^{-1}; HRMS calcd for $C_{10}H_{11}DO_2S$: 197.0621. Found 197.0621.

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