Tetrahedron Letters, Vol.32, No.37, pp 4863-4866, 1991
Printed in Great Britain

0040-4039/91 $3.00 + .00
Pergamon Press plc

Studies on DNA Cleaving Agents: Synthesis and Chemically Induced Cycloaromatization of a Monocyclic Neocarzinostatin Chromophore Analogue

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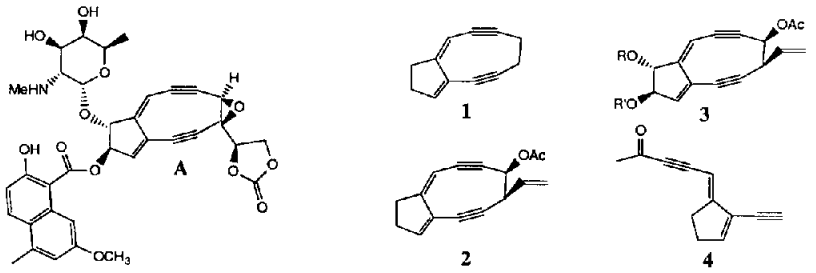
Abstract: The synthesis of an acyclic analogue of neocarzinostatin chromophore is described. This analogue is found to undergo cycloaromatization in the presence of thiols; the process involves a previously undetected internal hydrogen abstraction reaction.

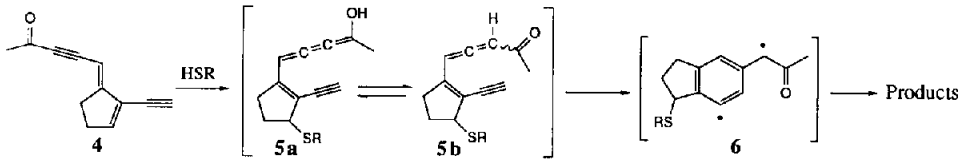
Neocarzinostatin (NCS)[1] is the original member of an emerging class of mechanistically and structurally novel DNA cleaving agents that now includes esperamicin,[2] calichemicin,[3] and dynemicin A.[4] Previous and ongoing research in these laboratories[5] has resulted in the first synthesis of the parent dienediyne system (**1**) and C5 functionalized analogues (**2** and **3**) of NCS Chrom I (**A**), the biologically active subunit of NCS. These analogues were designed to provide mechanistic information bearing on the role of strain, leaving group ability, and DNA recognition elements in the activation of NCS Chrom I surrogates. We describe below a synthetic route to monocyclic NCS Chrom I analogues and a study of their chemically induced cycloaromatization, as a reference point for studies on the bicyclic systems (**A**, **1-3**).

Scheme I



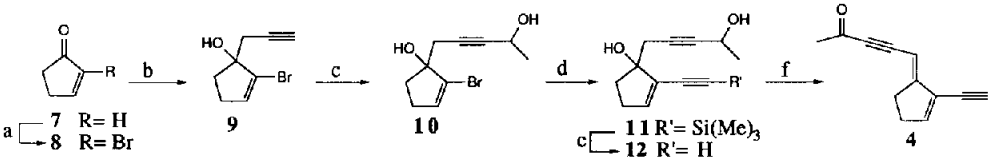
Our studies in this area were guided by the view that a dienediyne and a C5 leaving group or electron acceptor are minimally required for the conversion of NCS Chrom I to its corresponding diyl.[5] Monocyclic analogues of the type represented by **4** were thus expected to serve as diyl progenitors. By analogy to the proposed mode of NCS Chrom I activation,[1c] these dienediyne analogues would be expected to undergo thiol addition, affording a tetraenyne (**5a**) or a trienyne (**5b**) which upon cycloaromatization[6] would generate diyl **6** or its enol tautomer. Information on the viability and facility of these transformations and the behavior of **4** was sought in order to establish a much needed reference point for assessing the influence of structure on the chemistry of NCS Chrom I and a foundation for the design of new diyl generating devices.

Scheme II



The synthesis of the desired precyclization system (Scheme III: **4**) was achieved through an adaptation of a strategy originally developed by this group.[5] Starting from commercially available cyclopentenone, α-bromination[7] (52% yield) followed by addition of a propargyl unit yielded bromoenyne **9** (90%). Trapping of the dianion of **9** with acetaldehyde gave the diol **10** (80% yield), which upon coupling[8] with trimethylsilyl acetylene and subsequent deprotection produced diol **12** (54% from **10**). Conversion of **12** to **4** was then accomplished using excess Swern reagent (30%).[9]

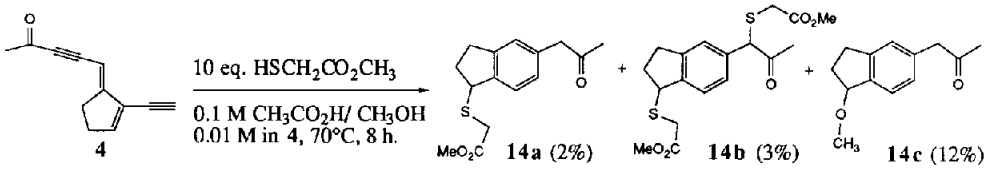
Scheme III



(a) Br_2, Et_3N, CH_2Cl_2, 0 °C to room temp.; (b) $HCCCH_2$-MgBr, Et_2O, room temp.; (c) EtMgBr (2.5 eq), THF, acetaldehyde, room temp.; (d) $PdCl_2(PPh_3)_2$, CuI, $(i\text{-}Pr)_2NH$, HCCTMS (2.0 eq), THF, room temp.; (e) K_2CO_3, MeOH, 4 h, room temp.; (f) $(COCl)_2$ (5.0 eq), DMSO (10.0 eq), Et_3N (14.0 eq), CH_2Cl_2, -78 °C to room temp.

The cyclization of **4** was examined under standard conditions previously employed in the activation of NCS Chrom I.[10] When treated with methyl thioglycolate (10.0 eq) in methanolic acetic acid (0.1M) at 70°C, analogue **4** did indeed undergo cycloaromatization, producing three cyclized compounds: **14a**, **14b**, and **14c** in 2, 3, and 12% yield, respectively (Scheme IV). Products **14a** and **14b** presumably result from conjugate addition of thiol to the extended enone **4**. Simple addition products corresponding to **5** (Scheme II) did not accumulate under these conditions, indicating that cycloaromatization proceeds at a rate at least comparable to that of the conjugate addition. This result and the facility of these reactions suggest that ring strain might not figure significantly in the design of simple, physiologically activatable analogues.

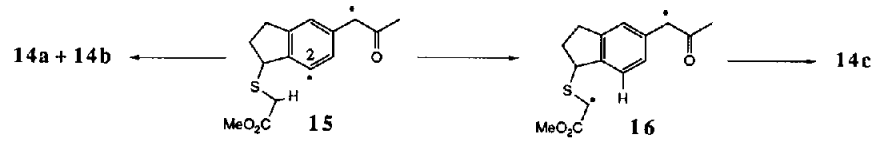
Scheme IV



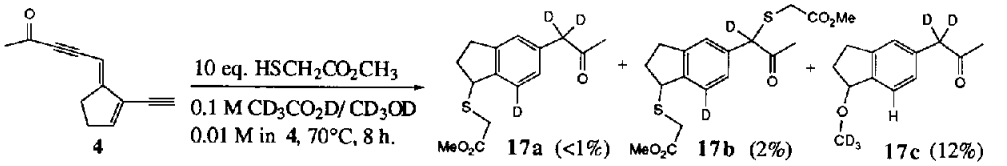
Of further interest in these studies is the origin of **14c**. While related apparent solvolysis products have been previously observed,[11] no explanation of their formation has heretofore been advanced. Several control experiments suggest that **14c** arises through an intriguing and previously undetected path. First, when subjected to the reaction conditions in the absence of thiol, **4** does not produce **14c** but is instead recovered unchanged even after two days at 70°C. Thus, **14c** is not derived from a simple Michael addition of solvent to **4**. Next, product **14a** was resubjected to the reaction conditions, but again **14c** was not observed, ruling out the possibility that **14c** resulted from solvolysis of **14a**. These results suggest that **14c** is derived from capture of an intermediate between **4** and **14a/14b**. In connection with this conclusion, it is interesting to note that diyl **15** (Scheme V) possesses abstractable hydrogen atoms in the methyl thioglycolate subunit that are six atoms removed from the radical formed at C2 (NCS Chrom I numbering) and could thus represent a branch point in the path leading to **14a/14b** versus **14c**. To test this hypothesis, the cyclization reaction of **4** was run in fully

deuterated solvents (Scheme VI). Compounds **17a**, **17b**, and **17c** were obtained where **17a** and **17b** had only deuterium at the C2 position while **17c** had only hydrogen atoms incorporated at C2 as determined by NMR. The incorporation of label and the isotopic effect on product ratio are both consistent with the proposed path in Scheme V.

Scheme V

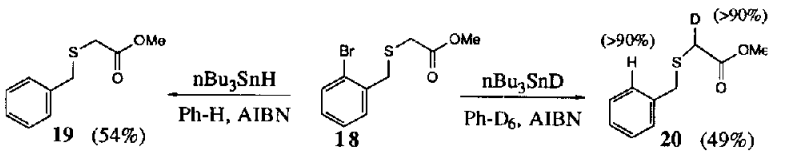


Scheme VI



In order to further test the feasibility of the proposed intramolecular hydrogen atom transfer, a simple analogue (**18**) was synthesized[12] and used to generate an intermediate similar to that proposed for the cyclization reaction. This substrate was treated separately with tri-n-butyltin hydride and deuteride (Scheme VII). In accord with the above results, *intra*molecular hydrogen transfer to the aryl radical is substantially favored (>90%) over intermolecular transfer, as evidenced by the observed labeling pattern in **20**.[13] This finding is of considerable importance in the design and mode of action of NCS Chrom I analogues and NCS Chrom I itself since any related intramolecular hydrogen transfer would reduce the efficiency of double strand cleavage by reducing the life time of the 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[14] indicate that for certain sequences of DNA, the thioglycolate subunit of DNA-bound and activated NCS Chrom I 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.

Scheme VII



Of further interest is the fate of intermediate **16**. Two intriguing pathways leading to the observed product **14c** can be envisioned. The first involves radical facilitated heterolytic cleavage of the benzylic C-S bond, resulting in the formation of a thio ketyl directly and a benzylic carbocation which could then be captured by solvent. The second path could involve a homolytic cleavage, again facilitated by the adjacent radical, but producing in this case a *p*-xylylene activated by a carbonyl group for conjugate addition of solvent. Both pathways, having interesting synthetic and mechanistic consequences within and beyond the scope of the NCS area, are under active investigation.

In summary, this study demonstrates that a strained ring system is not a required structural element for activatable NCS Chrom I analogues. The availability and stability of compound **4**, its facile cyclization to diyl intermediates, and its potential for accommodating DNA recognition elements, augurs well for the design of related simple, monocyclic DNA cleaving agents. This study also provides the basis for the development of novel mechanistic probes that could figure significantly in addressing the timing, location, and conformational characteristics of DNA cleavage effected both by NCS Chrom I analogues and NCS Chrom I itself. Work directed toward these goals is in progress.[15]

Acknowledgement: Support of this work by the National Institutes of Health through grant CA 31845 is gratefully acknowledged. Mass Spectra were provided by the Mass Spectrometry Facility, University of California-San Francisco supported by the NIH Division of Research Resources.

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(Received in USA 29 May 1991)