and to explore questions of mechanism.[27,30,34,35] Those that depend on a prior estimate of the energy requirements for cyclization to biradical are advised to consider together both extremes of the potential energy surface rather than either alone.

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General Methodology for the Synthesis of Neocarzinostatin Chromophore Analogues: Intramolecular Chromium-Mediated Closures for Strained-Ring Synthesis

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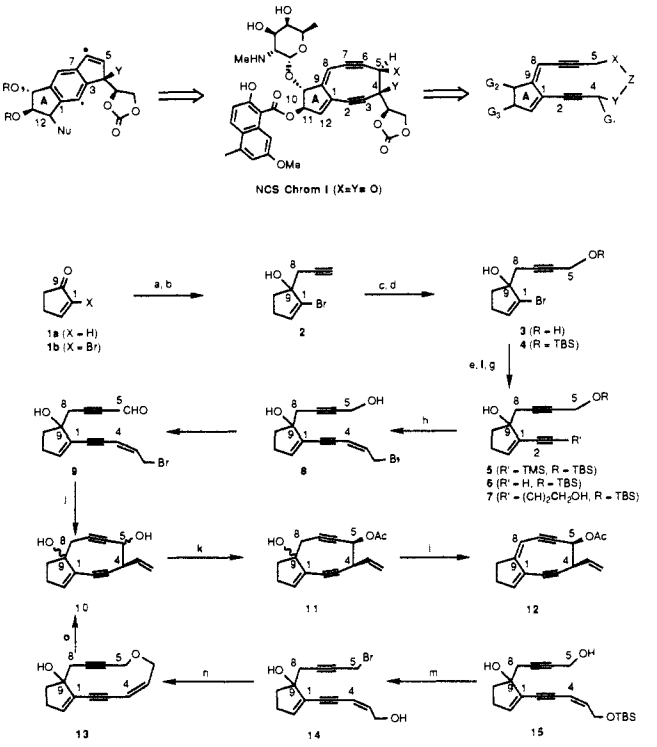
Neocarzinostatin (NCS) is an antitumor antibiotic that has been used for the treatment of pancreatic cancer, gastric cancer, and leukemia in humans.[3] The drug consists of a structurally unprecedented, non-protein chromophore (NCS Chr I)[4] stabilized through noncovalent association with a single-chain polypeptide (MW = 10700). NCS is proposed to function through the selective cleavage of DNA, involving deoxyribosyl hydrogen abstractions[5a,b] by a diyl formed upon thiol addition to NCS Chr I.[5c] Mechanistic studies[5] and the finding that NCS Chr I and its halohydrin analogue NCS Chr II exhibit similar biological activity[6] suggest that the bicyclic diene–diyne subunit and a leaving group at C5 are essential for diyl formation. Thus far, only one synthetic route to the bicyclic core of NCS Chr I has been reported,[7] while a less strained, homologous ring system has recently been elegantly assembled by Hirama and co-workers.[8] As part of our continuing effort to explore the fundamental utility and chemotherapeutic potential of this novel system for DNA cleavage, we have developed as described herein a convergent route to NCS Chr I analogues that possess the complete bicyclic core and functionality array required for diyl generation.

Our synthetic plan for bicyclic NCS Chr analogues involves three stages: attachment of appendages to the C1 and C9 positions of a preformed a ring, closure of the termini of these appendages by a ring-contraction strategy, and introduction of the C8–C9 double bond. This design allows for the attachment of various DNA recognition elements to the A ring, the segregation of the entropic and enthalpic problems associated with formation of the strained nine-membered B ring, and the installation of the unstable diyl progenitor functionality at a late synthetic stage. Central to the success of this plan is the construction of the strained cyclononadiyne subunit, which we have efficiently achieved through an internal chromium-mediated condensation.[9]

Scheme I[a]



[a] (a) Br_2, Et_3N, CH_2Cl_2, 0 °C to room temperature; (b) $HCCCH_2MgBr$, Et_2O, room temperature; (c) EtMgBr, HMPA, Et_2O, 50 °C, $(CH_2O)_n$, Et_2O, room temperature; (d) TBSCl, DMF, imidazole, 0 °C to room temperature; (e) $PdCl_2(PPh_3)_2$, CuI, $(i\text{-}Pr)_2NH$, HCCTMS, THF, room temperature: (f) K_2CO_3, CH_3OH, room temperature; (g) $PdCl_2(PPh_3)_2$, CuI, $(i\text{-}Pr)_2NH$, ICHCHCH$_2$OH, THF, room temperature; (h) Et_3N, MsCl, −78 °C; LiBr, $(CH_3)_2CO$, room temperature; 3:1:1 HOAc/THF/H_2O, room temperature; (i) MnO_2, room temperature; (j) $CrCl_2$, THF; (k) Ac_2O, Et_3N, DMAP, room temperature; (l) DMAP, MsCl, Et_3N, 0 °C; (m) MsCl, Et_3N, −50 °C to room temperature; LiBr, $(CH_3)_2CO$, room temperature; *n*-Bu_4NF, room temperature (47%); (n) NaH, HMPA, THF, reflux (52%); (o) *n*-BuLi, HMPA, −78 °C (<29%).

2-Bromocyclopentenone (**1b**) (Scheme I), prepared in one operation from cyclopentenone (**1a**),[10] served in the first stage of our plan as an excellent preformed A ring, possessing differentiated functionality for sequential bond formation to vicinally related centers C9 and C1. Addition of propargylmagnesium bromide to **1b** allowed for the formation of the C8–C9 bond, providing alcohol **2** (93% yield), from which diol **3** (80%) was obtained through a metalation and paraformaldehyde condensation se-

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quence. Conversion of the primary alcohol to its *tert*-butyldimethylsilyl ether **4** (90%)[11] completed the introduction of the protected C9 appendage.

Attachment of the remaining ring carbons of the NCS nucleus to C1 was then achieved in two ways, both based on palladium-mediated coupling. Addition of pent-2-en-4-ynol[12] (1 equiv) to an excess of bromide **4** (4 equiv) directly produced diyne **7** (97%). Unreacted **4** was readily recovered. Reactions conducted without an excess of **4** resulted in alkyne dimerization. As an alternative, bromide **4** was coupled first to (trimethylsilyl)acetylene to produce **5** (88%) which was then deprotected (**6**, 86%) and coupled to *cis*-3-iodo-2-propen-1-ol[13] to afford **7** (65%).

Modification of **7** as a prelude to B-ring formation entailed initially the conversion of its allylic alcohol functionality to an allylic bromide through mesylation and lithium bromide displacement. Without isolation, the resultant bromide was then desilylated (HOAc, THF, H_2O) to afford compound **8** in a combined yield of 75% for the three-step process. Oxidation of **8** with manganese dioxide provided aldehyde **9** (76%), possessing terminal functionalities required for an internal metal-mediated ring closure.

When aldehyde **9** was added to a suspension of chromium(II) chloride in THF, intramolecular closure producing the desired nine-membered ring occurred smoothly and efficiently (77–88%).[14] Three diastereomers were generated: a 1:1 ratio of two inseparable compounds epimeric at C9 and possessing a cis-substitution pattern about the newly formed bond (**10a** and **10b**, 67–76%) and a compound with a trans-substitution pattern about the C4–C5 bond (**10c**, 10–12%). **10** was also produced through the Wittig rearrangement of **13**,[15] although this process resulted in lower yields and products that were difficult to purify.

The efficacy of the above chromium-mediated closure for strained-medium-ring synthesis[9] is remarkable and unique among the reactions that we screened. Presumably, chromium first inserts into the carbon–bromine bond; coordinative activation of the aldehyde would then generate a 13-membered macrometallocyclic chelate.[16] Effective ring contraction would proceed through a six-centered transition state, producing the nine-membered carbocycle.

In order to establish the nucleofugal potential of the C5 substituent as required for diyl formation and to protect the secondary alcohol from the ensuing dehydration conditions, **10a** and **10b** were acetylated to give **11a** and **11b** (52%). This mixture was then treated with 4-(dimethylamino)pyridine, methanesulfonyl chloride, and triethylamine to afford the functionalized NCS Chr I carbocycle (**12**: 25%).[17] As expected, this functionalized analogue was even more labile than the parent hydrocarbon.[7] The half-life of **12** at room temperature was ~8 h as compared to ~48 h for the desacetoxy compound.[7] The instability of these compounds in the absence of activating reagents is noteworthy, suggesting that unimolecular activation mechanisms may be available for dilyl generation in these systems and others such as NCS Chr II. This possibility and the chemistry and cleavage reactions of **12** and its analogues are currently under investigation.

In summary, this study has resulted in the first synthesis of a *functionalized* bicyclo[7.3.0]dodeca-1,8-diene-2,6-diyne system, suitably equipped for conversion to an NCS diyl analogue. This strategy, by virtue of its convergent nature and its potential to accommodate DNA recognition elements at C10 and C11 and various leaving groups at C5, offers much flexibility for NCS analogue design, as needed to explore and exploit systematically the chemistry of the biologically active bicyclic subunit of NCS. In addition to its effective service for NCS analogue synthesis, the mild and efficient internal chromium-mediated closure represents a potentially general solution to the problem of strained-medium-ring synthesis presented by other DNA cleaving agents. These studies are in progress.

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Supplementary Material Available: IR, ^1H NMR, ^{13}C NMR, and mass spectroscopic data for compounds **3**, **6–9**, and **11a,b**, ^1H NMR data for compounds **10a,b**, and ^1H and partial ^{13}C NMR data for compound **12** (5 pages). Ordering information is given on any current masthead page.

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(14) Beginning with **5**, compounds were stored in solution at −20 °C to avoid decomposition. Compounds **10a,b** were separated from **10c** by flash chromatography (silica gel; diethyl ether/hexane (3:1)).
(15) Compound **15** was obtained through the palladium-mediated coupling of compound **3** and the protected pent-2-en-4-ynol (67%). For a review of the [2,3] Wittig rearrangement, see: Nakai, T.; Mikami, K. *Chem. Rev.* **1986**, *86*, 885. For recent applications, see: Marshall, J. A.; Robinson, E. D.; Zapata, A. *J. Org. Chem.* **1989**, *54*, 5854.
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Pseudo-Four-Dimensional Nuclear Magnetic Resonance by Off-Resonance Decoupling. An Approach for Distinguishing Coupled Proton Pairs by the Frequencies of Their Attached Heteronuclei

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For large molecules (>10 kDa), many of the proton NMR signals overlap, hindering an assignment process based on the analysis of proton–proton *J*-correlated and NOE data.[1] By combination of a heteronuclear shift correlation (e.g., HMQC) and homonuclear 2D NMR experiment (e.g., COSY, NOESY) in a [$X-H_A-H_B$] 3D NMR experiment,[2] many of the proton–proton correlations can be resolved by editing with respect to the chemical shift of a heteronucleus (X) attached to one of the coupled protons (H_A). Although H_A may be uniquely defined by the chemical shift of X in this 3D experiment, the coupling partner, H_B, may be difficult to identify. In principle, H_B could be uniquely defined in a [$X-H_A-H_B-Y$] 4D NMR experiment by the chemical shift of the heteronucleus (Y) attached to H_B. However, a true 4D NMR experiment may be impractical due to the requirements for three independent, incrementable time periods and the large number of pulses and delays necessary to effect all of the coherence transfers.

In this communication, we describe an approach for identifying scalar or dipolar coupled proton pairs (H_A, H_B) by the chemical shifts of *both* of their attached heteronuclei (X, Y). The two frequencies of the coupled protons (ν_{H_A}, ν_{H_B}) and one of the heteronuclear frequencies (ν_X) are determined in a heteronuclear 3D NMR experiment (e.g., HMQC–NOESY, HMQC–COSY).[2] The other heteronuclear frequency (ν_Y) which is used to characterize the H_B spin is obtained by applying an off-resonance

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