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STUDIES ON DNA - ACTIVE AGENTS: THE SYNTHESIS OF THE PARENT CARBOCYCLIC SUBUNIT OF NEOCARZINOSTATIN CHROMOPHORE A

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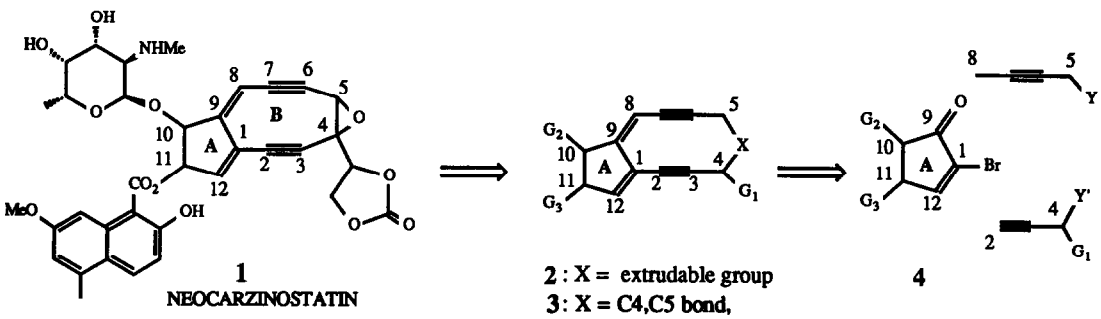
Summary: A synthesis of the parent carbocyclic subunit of NCS Chrom A (**1**) is described which provides corroborative support for the assigned but unprecedented structure of the active subunit of **1**, reference data for future synthetic and mechanism of action studies, and a general strategy for analogue and total synthesis.

Neocarzinostatin (NCS) is an antitumor antibiotic isolated from *Streptomyces carzinostaticus* var. F-41 by Ishida and coworkers in 1965[2] and subsequently shown to consist of a protein subunit, with a molecular weight of 10,700 daltons, non-covalently complexed to a highly labile non-protein chromophore (NCS Chrom A:**1**).[3,4]

SCHEME I



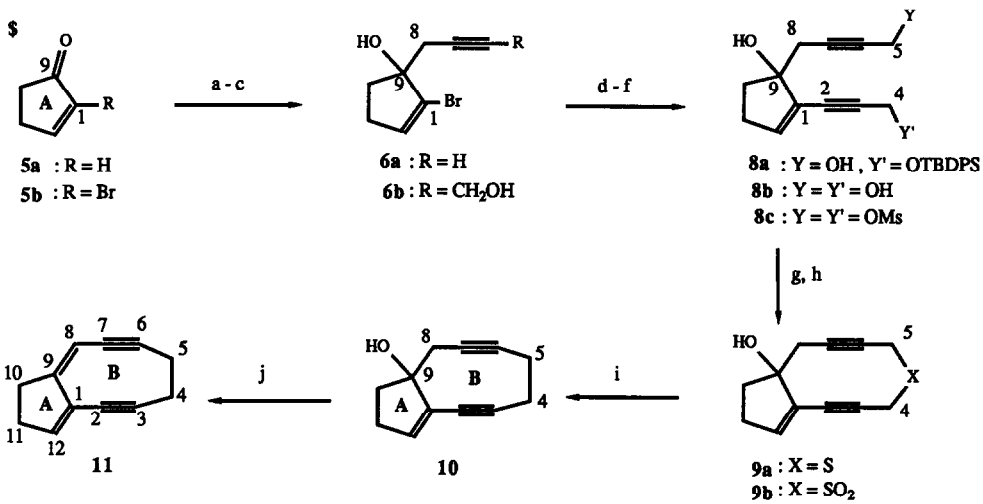
1
NEOCARZINOSTATIN
CHROMOPHORE A (NCS CHROM A)

NCS is active against gram-positive bacteria, certain experimental tumors, and, of special interest, certain solid tumors and acute leukemia in humans.[3] The biological activity of NCS is retained by NCS Chrom A,[5] which is proposed to function in thiol-activated form by intercalation into the minor groove of DNA followed by hydrogen abstraction initiated cleavage of deoxyribosyl residues, preferentially at thymidine rich sites.[6] The structure of NCS Chrom A is unprecedented[7] and its reactions have received only very preliminary investigation. Consequently, the precise mechanism by which it effects DNA cleavage is not as yet established at the molecular level. However, the strained diyne subunit of NCS Chrom A suggests that the reactive intermediate could be a diradical[8] produced from **1** in a fashion analogous to that implicated in the mode of action of the recently reported antitumor agent esperamicin (calichemicin).[9,10] The synthesis of NCS Chrom A analogues should allow for the detailed investigation of this possibility, and, additionally, for an assignment of the as yet incompletely established stereochemistry of **1**, corroboration of its unusual carbon subunit, and further development of this significant

chemotherapeutic lead and potential tool for site specific DNA cleavage. We describe herein a general approach to the active carbocyclic subunit in NCS Chrom A (**1**) in the first synthesis of a member of this carbocyclic class, namely the parent hydrocarbon **11** (Scheme II; see also, **3**: Scheme I).

Two factors influenced our synthetic plans in this area: NCS Chrom A (**1**) is unstable at high pH, in the presence of certain nucleophiles, or upon exposure to air or light and its nine-membered (B) ring is highly strained.[3,4,11] The problems suggested by these observations were expected to be minimized by postponing 9-membered (B) ring closure until the end of the synthesis and by utilizing, at that point, a ring contraction strategy (Scheme I: **2**→**3**) so as to decouple enthalpic and entropic difficulties that would arise simultaneously if B-ring formation were attempted through direct closure of an acyclic precursor. As such, a potentially general strategy for the synthesis of **1** and its analogues was expected to arise through annelation of a ring larger than nine members onto a pre-formed A ring (Scheme I: **4**→**2**), followed by contraction of the former by an extrusion or rearrangement pathway (**2**→**3**). The successful execution of this general plan is detailed below (Scheme II).

SCHEME II



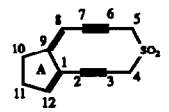
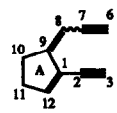
a) Br_2, Et_3N, CH_2Cl_2, 0° ➞r.t.; b) $HCCCH_2MgBr$,Et_2O, r.t.; c) EtMgBr, HMPA ,Et_2O, 50°C; CH_2O, Et_2O, r.t. ; d) $PdCl_2(PPh_3)_2$, CuI, $(i\text{-}Pr)_2NH$, $HCCCH_2OTBDPS$(**7**),THF, r. t. ; e)n-Bu_4NF, THF, -50°C➞r.t. ; f) MsCl, Et_3N, CH_2Cl_2,- 20°C ; g) Na_2S, aq. EtOH,r.t. ; h) m-CPBA, $NaHCO_3$, CH_2Cl_2, 0°C➞r.t. ; i) PhCOPh, MeCN/PhH(1:1), hv, r.t. ; j) MsCl, DMAP, CH_2Cl_2.

2-Bromocyclopentenone (**5b**), prepared in 1 operation from cyclopentenone (**5a**),[12] served in our plan as an excellent pre-formed A-ring, suitably and differentially functionalized for sequential bond formation to vicinally related centers C9 and C1. Thus, addition of propargyl magnesium bromide[13] to **5b** allowed for the formation of the C8, C9 bond, providing alcohol **6a** (93% yield) which was then converted to diol **6b** (80%) through a metallation and paraformaldehyde condensation sequence. Attachment of the remaining ring carbons of the parent system to C1 was then achieved by palladium mediated coupling of protected propargyl alcohol **7** with vinyl bromide **6b**.[15] It is noteworthy that this coupling occurs in high yield (94%) only when diisopropyl amine is

used as base; with diethyl amine, the coupled product is formed in only 52% yield. Deprotection of silyl ether **8a** was efficiently achieved in 97% yield with fluoride and the resultant triol **8b** was activated for double displacement by selective reaction with two equivalents of mesyl chloride (84% yield). Reaction of the dimesylate **8c** with Na_2S under medium dilution conditions gave the cyclic thiol ether **9a** in 69% yield. This product proved to be unstable in pure form but could be kept in CH_2Cl_2 solution maintained at -20°C.

In order to effect the proposed ring contraction, we next converted thiol ether **9a** to the corresponding sulfone **9b** (91% yield) through carefully controlled oxidation with MCPBA/$NaHCO_3$. Photolysis of a solution of **9b** and benzophenone for 10 minutes at room temperature gratifyingly produced the highly unstable ring contracted product **10** in 9-15% yield.[16] Only limited attempts were made to optimize this process due to the instability and volatility of **10**, problems which are expected to be diminished in the application of the strategy to more complex, C4 and/or C5 substituted analogues of **1**. Nevertheless, the process proved adequate for our immediate objective, i.e., the initial synthesis of the parent ring system. Toward this end, attempts to effect dehydration of **10** with Burgess reagent [17] gave encouraging, but irreproducible results. However, after much screening of other methods for dehydration, again complicated by the instability of **10**, this problem was eventually solved by treatment of alcohol **10** with mesyl chloride in the presence of dimethylaminopyridine. Dienediyne **11**, the parent hydrocarbon ring system of NCS Chrom A (**1**), was thus obtained in high yield.[18]

Table I. ^{13}C NMR DATA FOR NCS Chrom A (**1**), NCS Chrom A Chlorohydrin, Parent Carbocycle (**11**), Analogues **12** and **13** (solvent)

Carbon #	NCS Chrom. A[7] (CD_3CO_2D, CD_3OD, 1:1)[a]	NCS Chrom A. HCl[7] (CD_3OD)[a]	PARENT (**11**)[18] (THF-D_6)[b]	 12 (C_6D_6)[a]	 13 (C_6D_6)[a]
9	160.2	156.2	163.7	155.3	160.4
12	139.4	136.2	139.3	147.7	148.5
1	129.8	130.9	129.3	c	126.6
8	106.5	107.8	99.2	98.5	97.7
7	90.7	93.0	92.8	83.8	81.5
6	99.7	100.5	99.0	84.9	82.5
3	97.6	99.9	98.2	84.4	82.3
2	87.5	89.9	87.7	83.0	76.4
11	81.6	82.6	31.6	30.6	31.4
10	82.2	82.5	30.8	30.2	28.8
4	63.8	80.1	20.7	48.7	
5	55.2	58.5	19.9	47.7	

a) Spectra recorded at room temperature; (b) Spectrum recorded at -30° to -35°C); (c) Signal obscured by solvent

As expected from studies on NCS Chrom A, the parent hydrocarbon (**11**) was found to be unstable at room temperature ($t_{1/2} \cong 48h$) or when exposed to air or to certain chromatographic supports. Therefore, for characterization purposes, the dehydration reaction mixture was rapidly passed through a base-washed silica gel chromatographic column and the effluent fractions containing only **11** were then combined and carefully concentrated at low temperature and immediately examined by low temperature NMR spectroscopy. For further comparison, ^{13}C data were also obtained for diene diynes **12** and **13**; the former was prepared by dehydration of **9b** and the latter from **6a** through an alkynylation/dehydration sequence. As given in the table, the data obtained for these analogues are in agreement with that reported for NCS Chrom A and its chlorohydrin derivative for atoms with similar substitution.

In conclusion, this study has resulted in the first synthesis of a bicyclo [7.3.0] dodeca -1, 8-dien-2, 6-diyne (**11**), the parent carbocyclic system of NCS Chrom A (**1**), and in a general strategy for the synthesis of NCS Chrom A analogues. This effort provides corroborative support for the assigned, but previously unprecedented, structure of **1** and critical reference data for future synthetic, spectroscopic, and mechanism of action studies directed at exploiting this exciting chemotherapeutic lead. Studies toward these goals are in progress.

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18. The isolated yield for this reaction could not be directly determined since the product (**11**) must be kept in solution to suppress polymerization. **11**: ^1H NMR (C_6D_6) δ 5.87 (1H, m); 5.18 (1H, m); 2.17 (2H, m); 2.13 (4H, bs), and 1.98 (2H, m).

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