

**PROPOSED STRUCTURE OF THE NEOCARZINOSTATIN CHROMOPHORE-METHYL THIOLYCOLATE
ADDUCT; A MECHANISM FOR THE NUCLEOPHILIC ACTIVATION OF NEOCARZINOSTATIN**

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Summary: Structure 1 is proposed to represent the product of reaction of methyl thioglycolate with the neocarzinostatin chromophore. A mechanism for the formation of 1 is presented which provides a rationale for the nucleophilic activation of neocarzinostatin.

Neocarzinostatin (NCS) is an antitumor antibiotic consisting of separable protein and nonprotein (chromophore) components.^{1,2} The cytotoxicity and DNA-cleaving ability associated with the drug complex are fully reproduced in the isolated chromophore.³ In the absence of protein, the chromophore is exceedingly unstable, undergoing rapid decomposition at elevated pH ($t_{1/2} = 0.6$ min at 0°, pH 8)⁴ and upon incubation with thiols.⁵ The structure of NCS chromophore was recently determined (2, Scheme I) and features an epoxybicyclo[7.3.0]dodecadienyne nucleus.⁶

Reversible binding of 2 to DNA most likely occurs by intercalation of the naphthoate ester, positioning the nonaromatic portion of the chromophore in the minor groove of DNA.⁷ In a reaction markedly accelerated ($\cong 1000$ -fold) by added thiols,^{8,9} and requiring uptake of one molar equivalent of dioxygen,¹⁰ 2 selectively cleaves deoxythymidine and deoxyadenosine residues of DNA.¹¹⁻¹³ The cleavage reaction generates a 5'-aldehyde DNA fragment which derives its carbonyl oxygen from molecular oxygen.¹⁴ Experiments with 5'-[³H]-thymidine-labeled poly(dA-dT)-poly(dA-dT) (but not 1', 2' or 5-CH₃-[³H]-thymidine) have shown that tritium is covalently incorporated into the chromophore-derived product. Both tritium incorporation and DNA cleavage were shown to increase linearly with thiol concentration. Anaerobic incubation produces a similar profile of tritium uptake into chromophore, but the formation of a covalent DNA-chromophore adduct supplants DNA strand scission.¹⁵ Kinetic studies show that reaction of 2 with thiols precedes oxygen uptake.¹⁰ Together, these facts suggest that NCS activation is an oxygen-independent event.

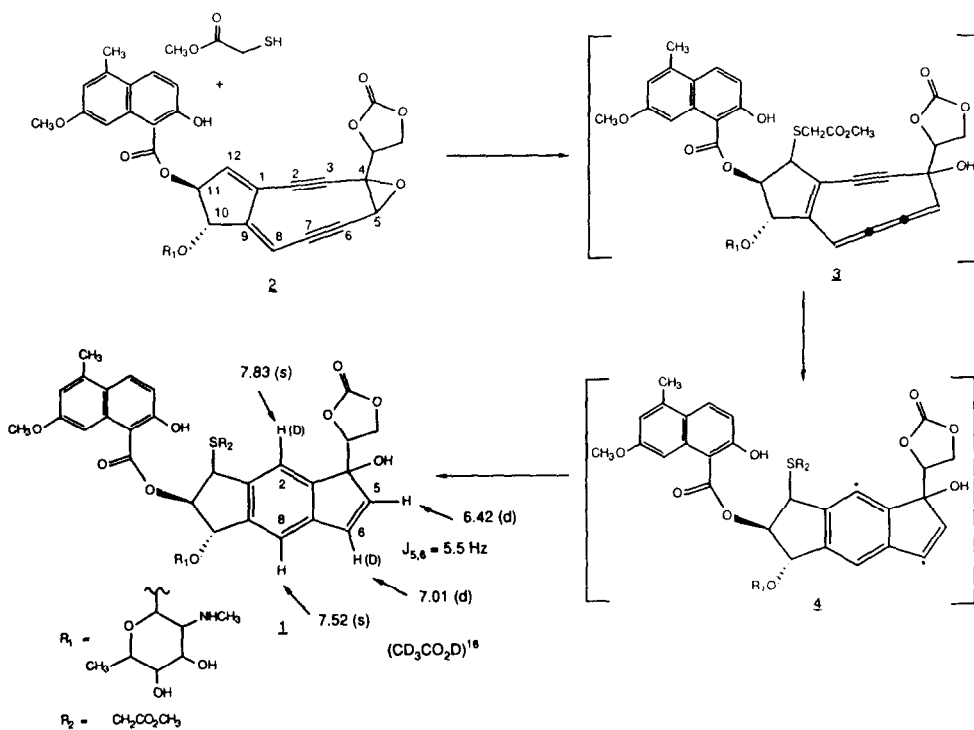
Kappen and Goldberg have presented a scheme for the cleavage of DNA by NCS in which it is proposed that thiol activation of 2 produces a free radical intermediate which abstracts a 5'-hydrogen atom from DNA.¹⁵ Addition of dioxygen to the incipient 5'-radical then initiates a cascade of reactions leading to 5'-aldehyde formation and DNA cleavage. Neither the structure of the chromophore-derived reaction product nor the mechanism for nucleophile-induced activation of 2 are known.

In the absence of DNA, 2 reacts irreversibly with nucleophiles to form a covalent adduct which will bind but not cleave DNA. The product of reaction of 2 with methyl thioglycolate (HSCH₂CO₂CH₃, MeOH; HPLC purification, 30% yield) has been isolated and evaluated spectroscopically by Goldberg and co-workers.¹⁶ ¹H NMR and FAB mass spectroscopic data have shown the product to be a 1:1 adduct with the added incorporation of two hydrogen atoms. Mass spectroscopy of the trimethylsilylated reaction product reveals the presence of an additional trimethylsilyl group (relative to trimethylsilylated 2), leading these authors to postulate that epoxide opening had occurred. ¹H NMR data established the site of reaction as the twelve-carbon nucleus; resonances for the naphthoate, *N*-methylfucosamine and ethylene carbonate subunits remained essentially unchanged. Replacing signals for the bicyclic ring were four sharp resonances: (CD₃CO₂D) δ 7.83 (s, 1H); 7.52 (s, 1H); 7.01 (d, *J*=5.5 Hz); 6.42 (d, *J*=5.5 Hz). Reaction of 2 with methyl *S*-[²H]-thioglycolate produced an adduct incorporating two carbon-bound deuterium atoms. PMR data for this adduct showed the absence of signals δ 7.83 and 7.01, and collapse of the δ 6.42-centered doublet to a singlet.

In this letter we assign the tetrahydroindacene structure 1 (Scheme I) to the NCS chromophore-methyl thioglycolate adduct, uniquely accommodating chemical and spectral data reported by Goldberg and co-workers.¹⁶ ¹H NMR data from a series of indene derivatives establishes a clear trend in chemical shift values which supports our assignment; the parent hydrocarbon, indene (5, Figure 1), is illustrative.^{17,18} Most compelling are spin-spin coupling constants (structure 6,¹⁹ Figure 1); the coupling of 5.5 Hz observed for protons 5-H and 6-H (NCS numbering) is diagnostic for the vinyl group of the indene ring.¹⁷

A proposed mechanism for the formation of 1 is presented in Scheme I. By this scheme, nucleophilic attack at C-12 and epoxide opening generate cumulene 3 which cyclizes to form the biradical 4. This mechanism is consistent with deuterium incorporation at C-2 and C-6 upon treatment of 2 with *S*-[²H]-methyl thioglycolate. The rearrangement of 3 to 4 is closely analogous to

the enediyne \rightarrow 1,4-benzenediyl rearrangement first demonstrated by Bergman²⁰ and recently invoked in the rearrangement of the related esperamicin and calicheamicin antibiotics.^{21,22} Importantly, this scheme provides a rationale for spontaneous nucleophile-induced biradical formation, a key step in the postulated mechanism for DNA cleavage by NCS.¹⁵



Scheme I

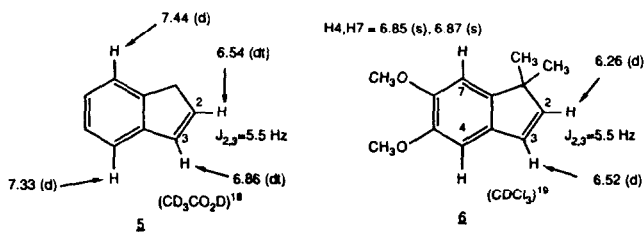


Figure 1

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