Bifunctional Involvement of the Hydroxynaphthoate Ester Moiety in the Activation of Neocarzinostatin Chromophore in DNA-Mediated Site-Specific Cleavage

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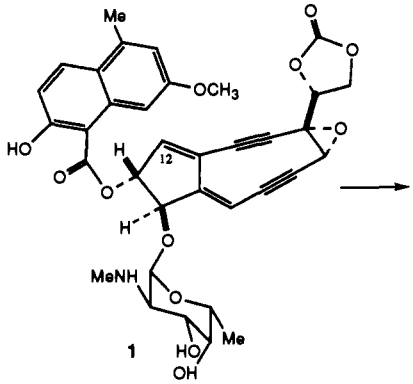
Thiol reagents activate the enediyne antibiotic neocarzinostatin chromophore (NCS-Chrom, **1**) for duplex DNA cleavage by greater than 1000-fold.[1] Nucleophilic addition of thiol takes place at C12 of NCS-Chrom, resulting in a Bergman-type cycloaromatization reaction to form the thiol adduct **2** with methyl thioglycolate, characterized by the tetrahydroindacene skeleton.[2]



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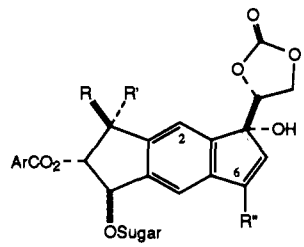
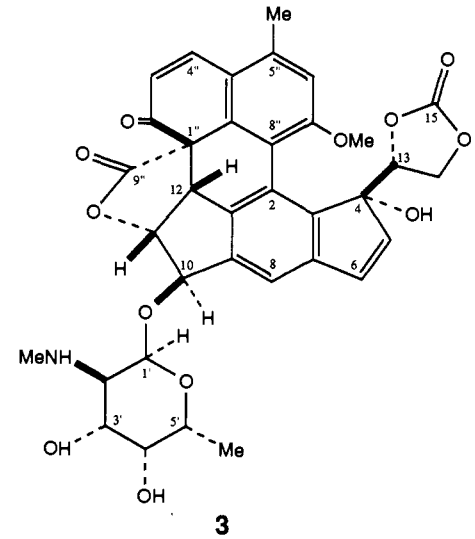


2 R=$SCH_2CO_2CH_3$ R'=R"=H

4 R=H R'=OCH_3 R"=$SCH_2CO_2CH_3$

The proposed intermediate 2,6-diradical species corresponding to thiol adducts such as **2** (cf. Scheme I) is believed to be responsible for sequence-specific hydrogen abstraction from the C5′, C1′, and/or C4′ positions of the deoxyribose on the complementary strands in the minor groove of duplex DNA.[1] Single-stranded DNA is not a substrate whether or not thiol is present. Recently, however, it was found that NCS-Chrom induces the highly efficient site-specific cleavage at the 3′-side of a bulge in single-stranded DNA in the absence of thiol, involving general base catalysis.[3] Only a reaction containing bulged DNA generates a new UV-absorbing and fluorescent postactivated product of **1**, labeled with ^3H abstracted from C5′ of the target nucleotide. We describe herein details of the structure elucidation including the absolute stereochemistry of the novel postactivated drug **3**, whose mode of formation involves the naphthoate ester moiety as both nucleophile and radical quencher.



3

Spectroscopic data (UV,[4] ^1H NMR,[5] MS) of the isolated yellow material (~150 μg)[3] suggested a rearranged structure isomeric with NCS-Chrom having the molecular formula $C_{35}H_{33}NO_{12}$ (FAB-MS found *m/z* 660.2220, calculated *m/z* 660.2090 [M + H]$^+$). EI-MS data of the trimethylsilylated product indicated the formation of a tri-TMS and a very weak tetra-TMS derivative (the C2′-$NHCH_3$ group silylates with difficulty) and several sugar fragments reported previously for NCS-Chrom and transformation products.[2c,6] These account for the one and three active protons in the aglycone and N-methylfucosamine moieties, respectively. ^1H NMR comparison with the thiol adduct **2**[2] indicated considerable similarity but with the conspicuous absence of two aromatic singlet resonances. From COSY, delayed COSY, and phase-sensitive NOESY experiments, these were assigned to protons missing at the C2 and C8″ positions (Table I), which also allowed assignment of all protons around the periphery of the indacenyl skeleton (H5 to H12). HMQC data[5] allowed assignment of all protonated carbons including that at 48.3 ppm ($^1J_{CH}$ ~ 137 Hz) for C12. Nucleophilic attack at C12 by the phenoxide anion of the naphthoate moiety could therefore be ruled out.

HMBC experiments[5] on a larger sample (~0.5 mg) proved critical. Most conspicuous was the presence of the indirectly detected carbonyl peak at 197.4 ppm coupled to the H3″ and H4″ protons (see Table I, **3**), which therefore readily accounts for the observed polarization of the C3″–C4″ double bond. Further connectivities between this carbonyl and H12 and between the quaternary carbon at 64.4 ppm (C1″) and H3″/H12 and correlations between the ester carbonyl at 169.9 ppm and H11 and H12 in particular provided strong evidence for a covalent

(1) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191 and references therein.
(2) (a) Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493. (b) Myers, A. G.; Proteau, P. J. *J. Am. Chem. Soc.* **1989**, *111*, 1146. (c) Hensens, O. D.; Dewey, R. S.; Liesch, J. M.; Napier, M. A.; Reamer, R. A.; Smith, J. L.; Albers-Schonberg, G.; Goldberg, I. H. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 538. (d) Hensens, O. D.; Goldberg, I. H. *J. Antibiot.* **1989**, *42*, 761.
(3) Kappen, L. S.; Goldberg, I. H. *Science*, **1993**, *261*, 1319.
(4) UV/visible (MeOH) λ_{max} 235, 250, 270, 305, ~385 nm; fluorescence excitation (MeOH) λ_{max} 530 nm; fluorescence emission (MeOH) λ_{max} 283, 320, 397 nm.
(5) ^1H NMR data were obtained at 400 and 500 MHz. ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectra were acquired in CD_3OD using 3-mm indirect detection and dual $^1H/^{13}C$ microprobes, respectively (Nalorac Cryogenics Corp.). ^1H-detected experiments (HMQC, HMBC, NOESY) were carried out with presaturation of either the solvent or the residual HOD peak. HMBC experiments were optimized for 4 and 7 Hz. A mixing time of 0.5 s was used in the NOESY experiments.
(6) (a) Albers-Schonberg, G.; Dewey, R. S.; Hensens, O. D.; Liesch, J. M.; Napier, M. A.; Goldberg, I. H. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1351. (b) Napier, M. A.; Goldberg, I. H.; Hensens, O. D.; Dewey, R. S.; Liesch, J. M.; Albers-Schonberg, G. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1703.

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Scheme I. Proposed DNA-Mediated Mechanism of Action of NCS-Chrom (**1**) in the absence of Thiol

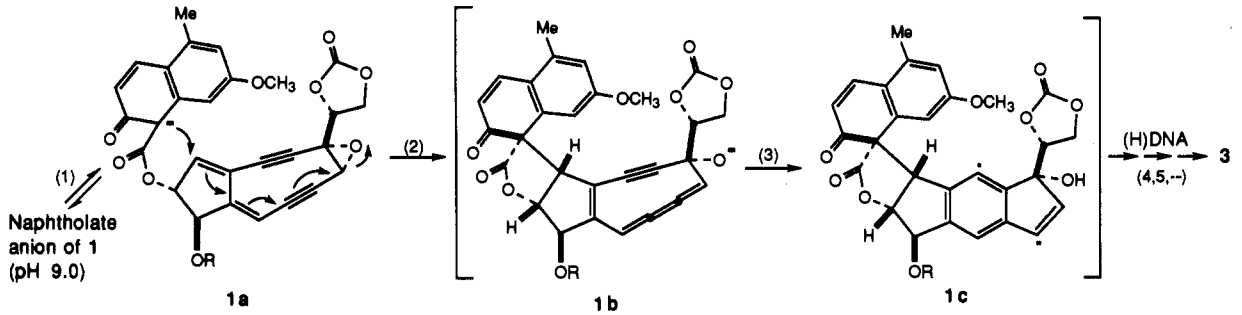

Table I. ^1H and ^{13}C NMR Data for **3** in CD_3OD

no.	^{13}C, ppm[a]	^1H, ppm[b] (mult, J(Hz))	HMBC (4 and 7 Hz)
1	142.8		
2	131.8		
3	144.2		
4	90.3		
5	140.2	6.37 (d, 5.9)	C3, C4, C6, C7
6	134.5	6.89 (d, 5.9)	C3, C4, C5, C7, C8
7	149.3		
8	123.5	7.40 (s)	C1, C2, C3, C9, C10
9	139.4		
10	83.9	5.08 (br s)	C8, C9, C11, C12, C1′
11	89.3	5.72 (d, 3.4)	C1, C9, C12, C9″
12	48.3	4.33 (d, 3.4)	C1, C2, C9, C1″, C2″, C9″
13	81.1	6.15 (dd, 6.8, 8.8)	C4, C5, C14, C15
14a	67.3	3.64 (dd, 6.8, 8.8)	C4, C13, C15
14b		4.40 (t, 8.7)	C4, C15
15	157.3		
1′	97.1	5.53 (br d, ~2)[c]	C10, C3′, C5′
2′	59.7	~3.28 (br m)[c]	
2′-NHMe	32.9	2.78 (br s)	C2′
3′	69.2	3.88 (br m)[c]	
4′	72.5	3.61 (m)	C2′, C3′
5′	68.5	3.77 (br q, 6.8)	C1′, C4′, 5′-Me
5′-Me	16.2	1.11 (d, 6.8)	C4′, C5′
1″	64.4		
2″	197.4		
3″	121.0	6.02 (d, 10.3)	C1″, C2″, C4a″, C8a″
4″	146.3	8.00 (d, 10.3)	C2″, C4″a, C5″, C8″
4a″	124.2		
5″	141.1		
5″-Me	19.3	2.54 (s)	C4a″, C5″, C6″
6″	114.9	7.08 (s)	C4a″, C5″, 5″-Me C7″, C8″, C8a″, C2
7″	159.6		
7″-OMe	56.0	3.97 (s)	C7″
8″	122.4		
8a″	135.9		
9″	169.9		

[a] At 125 MHz, CD_3OD signal at 49.0 ppm. [b] At 500 MHz, CD_3OD signal at 3.30 ppm. [c] Sharpens on spiking with CD_3COOD.

bond between C1″ and C12 and formation of the 5-membered spirolactone moiety. The lack of protons at the C8″ and C2 positions suggested a second cyclization step with covalent bond formation between these two positions. Corroboration came from the observed connectivities H6″ → C8″ and the 4-bond couplings H8 → C2 and H6″ → C2 (optimized for 4 Hz). All 35 carbons were indirectly detected via HMQC and HMBC experiments and confirmed directly via ^{13}C detection.[5]

The relative and absolute stereochemistry of **3** followed readily from NOESY and $^3J_{HH}$ data, modeling, and the known absolute stereochemistry of the thiol adduct **2**.[7] The trans,cis arrangement of the substituents at C10, C11, and C12 was evident from the proton–proton coupling constants ($^3J_{10,11} = 0.6$, $^3J_{11,12} = 3.3$ Hz).[8] The same configurational arrangement was previously observed for the rearranged chromophore **4**[9] but opposite that found at C12 in **2**.[2,7] The trans,cis arrangement was corroborated by weak and strong *n*Oes between H10/H11 and H11/H12, respectively. Only the cis arrangement of substituents at C11/C12 was feasible from modeling studies. Similarly, only the *S* configuration at C1″ allows for ring closure to occur between C2 and C8″ as depicted in **3**. The complete absolute stereochemistry of **3** is therefore defined as 1″*S*, 4*R*, 10*R*, 11*R*, 12*S*, 13*R*.

Formation of the nucleophile at C1″ (**1a**, Scheme I) in tautomeric equilibrium with the naphtholate anion ($pK_a \sim 8.5$) is consistent with general base catalysis of the DNA cleavage reaction having a pH optimum of about 9.0.[3] The mechanism of formation of diradical **1c** can thus be envisaged similarly to that proposed for thiols.[2a,b] proceeding by nucleophilic attack of C1″ at C12 with epoxide ring opening to generate the cumulene intermediate **1b**, which then undergoes a Bergman-type rearrangement to the 2,6-diradical **1c**. The C6 radical abstracts hydrogen specifically from C5′ of deoxyribose in DNA, whereas by contrast, the C2 radical is quenched by reaction with C8″.[10] Very recent neocarzinostatin model studies by Lamothe and Fuchs[11] suggest that formation of the spirolactone (step 2) may be a spontaneous process. Studies are in progress to clarify this point and to further elucidate the role of the bulged DNA in completing the ring-strained structure **3** by correctly orienting the naphthoate and indacenyl moieties for subsequent covalent bond formation between C2 and C8″.

Acknowledgment. This work was supported by NIH Grant CA44257.

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(8) Obtained in CD_3OD with addition of a trace amount of CD_3COOD.
(9) Tanaka, T.; Fujiwara, K.; Hirama, M. *Tetrahedron Lett.* **1990**, *31*, 5947.
(10) Alkenylaryl and arylaryl radical intramolecular addition reactions are well documented: Beckwith, A. L. J.; Ingold, K. U. In *Rearrangements in Ground and Excited States*; de Mayo, P., Ed.; Academic Press: New York, 1980; Vol. 1 pp 209–215 and references therein.
(11) Lamothe, M.; Fuchs, P. L. *J. Am. Chem. Soc.* **1993**, *115*, 4483.