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Activation of Neocarzinostatin-Chromophore by 4-Hydroxythiophenol: Intramolecular Radical Trapping of Biradical Intermediate

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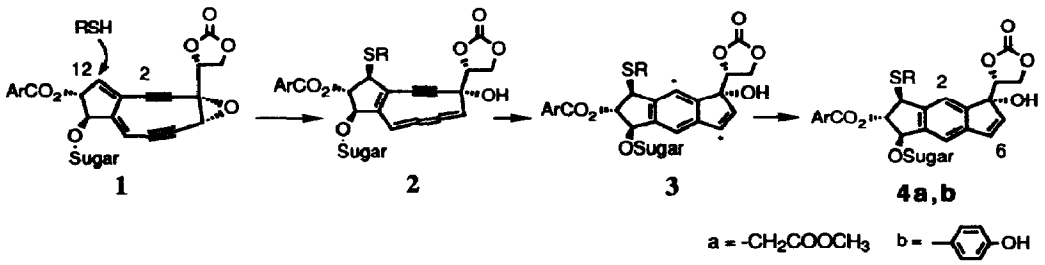
Abstract: The reaction of neocarzinostatin chromphore (1) with 4-hydroxythiophenol under DNA-cleaving conditions was investigated. It was found that a novel cyclization product (5) is formed as a major product together with a minor amount of normal cyclization product 4b. Tritium was incorporated into 4b and 5 from [^{3}H]-labeled poly(dA-dT), implying that both processes giving 4b and 5 are actually involved in the DNA cleavage reaction.

Neocarzinostatin is one of the first enediyne antitumor antibiotics shown to cleave DNA by hydrogen abstraction from deoxyribose backbone.[1] The activation mechanism of neocarzinostatin chromphore (NCS-C) (**1**) by methyl thioglycolate has been extensively studied, and it has been demonstrated that addition of thiol at C12 of the enediyne core unit produces cumulene **2** which spontaneously generates diradical **3** by a Bergman-type cycloaromatization.[2] Biradical **3** has also been shown to abstract hydrogen from DNA or other H donors to produce **4a**.[2] We have been investigating a NCS-induced DNA strand cleavage using 4-hydroxythiophenol (4-HTP) as an activator and demonstrated that diradical **3** competitively abstracts 4' and 5' hydrogens of DNA deoxyribose in a sequence dependent manner.[3] In our efforts to identify all products resulting from the reaction of **1** with 4-HTP under actual DNA-cleaving conditions, we now found that a novel cyclization product (**5**) is obtained as a major product together with a normal radical cyclization product (**4b**).

Scheme 1



A solution containing self-complementary oligonucleotide d(GCATGC)$_2$, **1** and 4-HTP in Tris-HCl buffer (pH 8.0) was incubated at 0 °C. HPLC analysis of the reaction mixture indicated the formation of a major product at 33 min and a minor product at 28 min (Figure 1). The minor product produced efficiently in Tris-HCl buffer (pH 8.0) containing 80 % isopropanol was identified as **4b** by ^{1}H NMR and FABMS (M+1, 788) as well as by a comparison with the reported spectroscopic data for other thiol-**1** adducts.[1d,2c,2e] The major product was assigned as **5** on the basis of spectral data including ^{1}H NMR and FABMS. The FABMS (M+1, 786) indicated that this product is a 1:1 adduct between **1** and 4-HTP. The ^{1}H NMR indicated the loss of two protons in the aromatic region and a strong NOE between H3''' and H13. These data in combination with the ^{1}H COSY and the NOESY experiment allow a complete assignment of all the ^{1}H signals. Table 1 shows the full assignment of ^{1}H signals for **5** and **4b**.

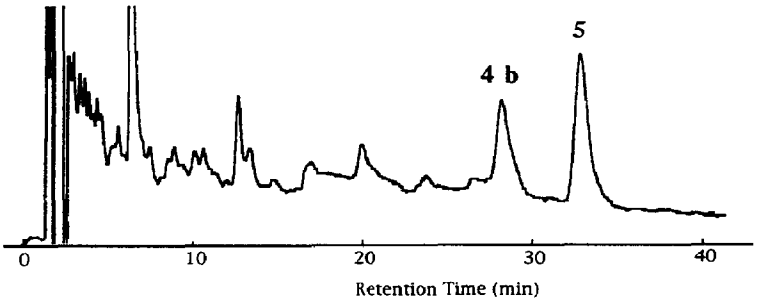


Figure 1. HPLC profile of the reaction mixture of **1**, 4-HTP and d(GCATGC)$_2$. The reaction mixture (total volume 150 μL) containing **1** (50 μM), 4-HTP (1 mM), d(GCATGC) (333 μM) and EDTA (1 mM) in 25 mM Tris-HCl (pH 8.0) was incubated at 0 °C for 2 h. Aliquot (100 μL) of the reaction mixture was analyzed by HPLC on CHEMCOBOND 5-ODS-H column (4.6 x 150 mm); elution was with 0.05 M ammonium acetate (pH 4.0), 33% acetonitrile isocratic, at a flow rate of 1.0 mL/min; detection at 340 nm.

Under the reaction conditions oligomer d(GCATGC)$_2$ was degraded to d(GCA)p and a 5'-aldehyde fragment d(T*GC) as reported.[3a] The amounts of **4b** and **5** and the total events occurred by NCS-C activated by 4-HTP under various conditions were summarized in Table 2. The total events were estimated by the amount of d(GCA)p after alkali treatment as described previously.[3c] Interestingly, product **5** was efficiently formed only in the presence of d(GCATGC)$_2$ (run 1 *vs* 2; run 3 *vs* 4). A selective formation of **5** was also observed in the presence of apoprotein (run 3). Since the addition of methanol to the reaction system enhanced the formation of **4b** (run 5), slightly enhanced production of **4b** in the reaction with isolated pure **1** is probably due to the hydrogen abstraction from methanol contained in a stock solution of **1** (run 1 or 2).

In order to know the role of **4b** and **5** in the DNA cleavage process, the incorporation of tritium into **4b** and **5** from [^{3}H]-labeled poly(dA-dT) was examined. It was found that approximately same amounts of tritium are incorporated into both **4b** and **5** (data not shown). These results clearly indicate that both processes giving **4b** and **5** are actually involved in the DNA cleavage reaction. The formation of **5** implies that C2 radical of **3b** undergoes a rapid intramolecular radical addition to the ortho position of the neighboring aromatic ring, whereas C6 radical is directly involved in the hydrogen abstraction from deoxyribose of d(GCATGC)$_2$ as shown in Scheme 2.[4] Preferred formation of **5** in the presence of DNA suggests that the binding orientation of biradical **3** in DNA duplex would facilitate a more enhanced intramolecular radical addition giving **5**.

Scheme 2

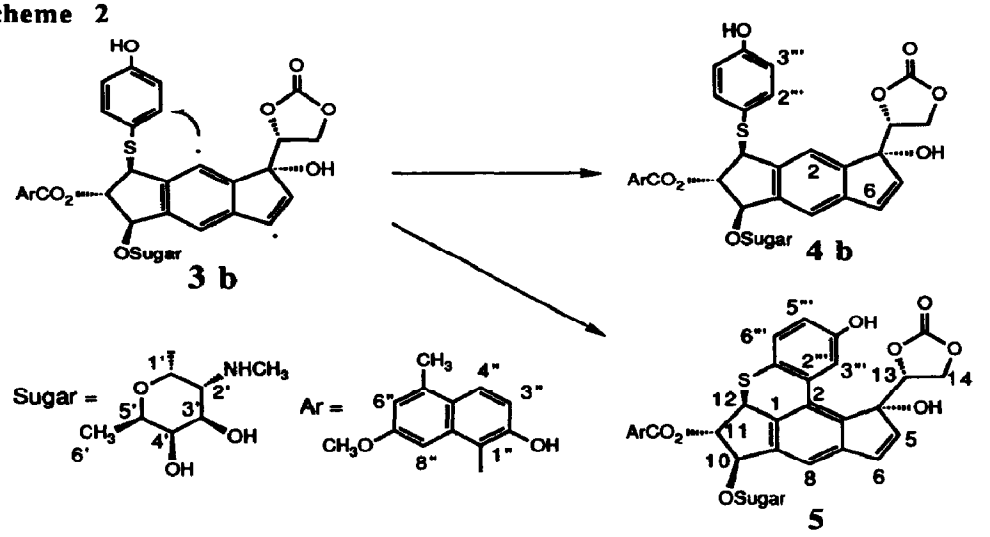


Table 1. Assignment of ^{1}H NMR (500 MHz) Data for **5** and **4b** in CD_3COOD-$CDCl_3$ (1:1)

Assignment	5: δ(ppm)	4b: δ(ppm)
2	-	7.26(s,1H)
5	6.24 (d, 1 H, J = 5.8 Hz)	6.11 (d, 1 H, J = 5.7 Hz)
6	6.80 (d, 1 H, J = 5.8 Hz)	6.72 (d, 1 H, J = 5.7 Hz)
8	7.17 (s, 1 H)	7.19 (s, 1 H)
10	5.65 (d, 1 H, J = 6.5 Hz)	5.05 (bs, 1 H)
11	5.87 (t, 1 H, J = 6.5 Hz)	5.75 (bs, 1 H,)
12	4.38(d, 1 H, J = 6.5 Hz)	4.42 (d, 1 H, J = 1.8 Hz)
13	5.28 (dd, 1 H, J = 8.6, 7.1 Hz)	4.55 (dd, 1 H, J = 8.4, 6.1 Hz)
14a	2.96 (dd, 1 H, J = 8.4, 7.1 Hz)	4.01 (dd, 1 H, J = 8.6, 6.1 Hz)
14b	3.68 (m, 1 H)	4.26 (dd, 1 H, J = 8.6, 8.4 Hz)
1'	5.51 (d, 1 H, J = 3.4 Hz)	5.48 (d, 1 H, J = 3.4 Hz)
2'	3.50 (dd, 1 H, J = 10.7, 3.4 Hz)	3.53 (dd, 1 H, J = 9.8, 3.4 Hz)
3'	4.43 (bd, 1 H, J = 10.7 Hz)	4.09 (bd,1 H, J = 9.8 Hz)
4'	3.77 (bs, 1 H)	3.62 (bs, 1 H)
5'	4.09 (m, 1 H)	3.38 (bd, 1 H, J =6.5 Hz)
6'	1.14 (d, 3 H, J = 6.5 Hz)	0.91 (d, 3 H, J = 6.5 Hz)
2'NCH_3	2.57 (s, 3 H)	2.74 (s, 3 H)
3"	6.85 (d, 1 H, J = 9.3 Hz)	6.82 (d, 1 H, J = 9.3 Hz)
4"	7.91 (d, 1 H, J = 9.3 Hz)	7.84 (d, 1 H, J = 9.3 Hz)
6"	6.70 (d, 1 H, J = 1.8 Hz)	6.58 (d, 1 H, J = 2.0 Hz)
8"	6.98 (d, 1 H, J = 1.8 Hz)	7.36 (d, 1 H, J = 2.0 Hz)
5"CH_3	2.42 (s, 3 H)	2.36 (s, 3 H)
7"OCH_3	3.68 (s, 3 H)	3.08 (s, 3 H)
2"'	-	7.13 (d, 2 H, J = 8.6 Hz)
3"'	8.48 (d, 1 H, J = 2.6 Hz)	6.56 (d, 2 H, J = 8.6 Hz)
5"'	6.61 (dd, 1 H, J = 8.6, 2.6Hz)	
6"'	7.16 (d, 1 H, J = 8.6 Hz)	

Table 2. The Reaction of d(GCATGC)$_2$ with **1** Activated by 4-HTP.

run	NCS	d(GCATGC)(μM)	Methanol content (%)	5(μM)	4b(μM)	Strand cleavage (μM)
1	**1**	333	10	4.4	2.4	10.9
2	**1**	0	10	0.6	2.6	-
3	**1**+apo	333	0.1[a]	3.7	0.5	11.2
4	**1**+apo	0	0.1[a]	0.6	1.0	-
5	**1**+apo	333	10	3.7	2.3	12.5

[a]To dissolve 4-HTP 0.1 % of methanol was added to the reaction mixture.

Double strand cleavage induced by NCS-C activated by normal thiols has been proposed to proceed via double hydrogen abstractions by C2- and C6 radicals of **3**. In contrast, double strand cleavage induced by NCS-C activated by 4-HTP was reported to be very inefficient compared to those employing other thiols such as glutathione or 2-mercaptoethanol.[5] The present observation of the efficient intramolecular trapping of the C2 radical would clearly explain this discrepancy.[6]

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