concerted reaction pathways on a surface that simply do not exist in the gas phase. These and other possibilities are currently under investigation in our laboratory.

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Registry No. 1, 1708-29-8; **2**, 110-00-9; **3**, 108-31-6; **4**, 20825-71-2; **5**, 100-40-3; **6**, 100-42-5; $H_2C{=}CHCH{=}CH_2$, 7782-44-7; Ag, 7440-22-4.

Communications to the Editor

Synthesis and DNA-Cleaving Abilities of Functional Neocarzinostatin Chromophore Analogues. Base Discrimination by a Simple Alcohol

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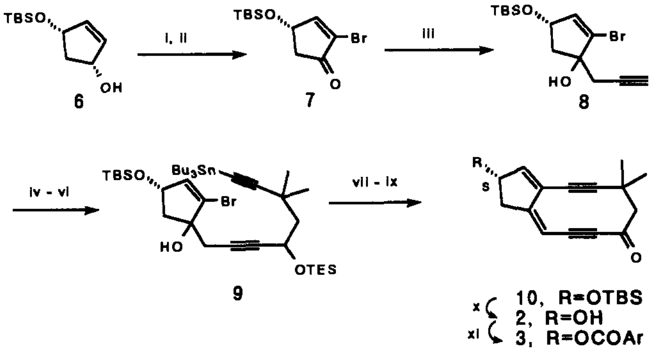
The precise roles of aglycon, carbohydrate, and intercalative aromatic moieties of DNA-binding antibiotics in site-specific DNA cleavage is a topic of intense current interest.[1,2] Neocarzinostatin (NCS) is a macromolecular antitumor agent that consists of biologically active chromophore **1**[3] and an apoprotein acting as a stabilizer and a carrier for **1**.[4] The labile dienediyne molecule **1** equipped with both a substituted naphthoic acid and an amino sugar moiety exhibits DNA-cleaving activities through carbon radical generation.[5] The base ($T > A \gg C > G$)[6] and sequence (GN_1T)[7] specificities in the cleavage of oligonucleotides by **1** have been attributed to the specific intercalation of the naphthoic acid moiety. Whereas we recently demonstrated that 10-membered-ring analogues **4** and **5**[8] undergo the thiol-triggered[5d] or radical-triggered[9] aromatization in a manner related to **1**,[10-12] both molecules were not capable of affording appreciable cytotoxic activities, probably due to the lack of hydrophilic and/or DNA-binding groups. We designed the second generation of NCS models, alcohol **2** and naphthoate **3**, to improve these points and have found **2** to possess striking guanine-selective DNA-cleaving ability.

Scheme I[a]



[a] (a) $(COCl)_2$, DMSO, Et_3N, −60 °C; (ii) Br_2, CH_2Cl_2, 0 °C; (iii) propargylmagnesium bromide, ether, −88 °C; (iv) BuLi, THF–HMPA, −78 °C, and then 3,3-dimethylpent-4-yn-1-al; (v) TESCl, pyr; (vi) BuLi, THF, −78 °C, and then Bu_3SnCl; (vii) $(Ph_3P)_4Pd$, THF, 60 °C, 89 h; (viii) K_2CO_3, MeOH; (ix) $(COCl)_2$, DMSO, Et_3N, −60 °C; (x) THF–H_2O–AcOH (1:1:1); (xi) $Me_2HN^+(CH_2)_3N{=}C{=}NEt,Cl^-$, $ArCO_2H$, CH_2Cl_2.

Key intermediate **7** was synthesized from readily available optically pure 3(*S*)-[(*tert*-butyldimethylsilyl)oxy]-5(*R*)-hydroxycyclopent-1-ene (**6**)[13] by the standard procedure (85%, Scheme I). Conversion of **7** to **2** and **3** essentially followed the synthetic scheme employed in the synthesis of **4**.[8a] Addition of propargylmagnesium bromide to **7** (91% yield) and condensation with 3,3-dimethylpent-4-yn-1-al by using BuLi at low temperature

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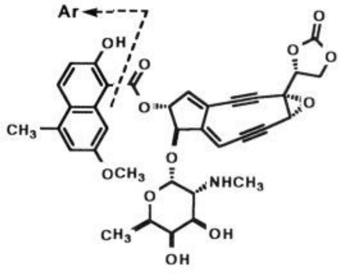
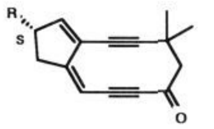
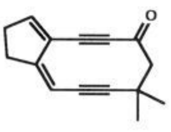


1





2, R = OH
3, R = OCOAr

5

4, R = H

in THF–HMPA (63%) followed by silylation (93% yield) and tributylstannation (90%) gave **9**. Palladium-mediated cyclization (60%),[14] selective hydrolysis of triethylsilyl (TES) ether (94%), and Swern oxidation (91%) produced *tert*-butyldimethylsilyl (TBS) ether **10**, which was hydrolyzed to unstable amorphous solid **2**[15] (59% yield) and then esterified with 2-hydroxy-7-methoxy-5-methylnaphthoic acid[16] to give **3** as a colorless solid, dec ca. 65 °C, in 54% yield.

Thiol-triggered cycloaromatization of **2** and **3** proceeded smoothly when **2** and **3** were treated with methyl thioglycolate (3 equiv),[8] to give the aromatized thiol adducts **13** (84:16 diastereomeric mixture) and **14** in 56% and 54% yields, respectively, possibly through the conjugated allene **11** and the succeeding radical intermediate **12** (Scheme II).[5d,12b,17] Alcohol **2** did afford the antibiotic and cytotoxic activities, as expected, without addition of thiol,[18] and cleaved the supercoiled pBR322 plasmid DNA (form I) to nicked circular form II in the presence of methyl thioglycolate.[19] On the other hand, the biological and DNA-cleaving activities of **3** have not been recognized, possibly because of its extremely low solubility in water. However, the suspension mixture of **3** in the aqueous solution of the NCS apoprotein did afford such activities.[20] Then their DNA-cleavage site specificity was examined with the 5′-end ^{32}P labeled 190-bp DNA fragment.[21]

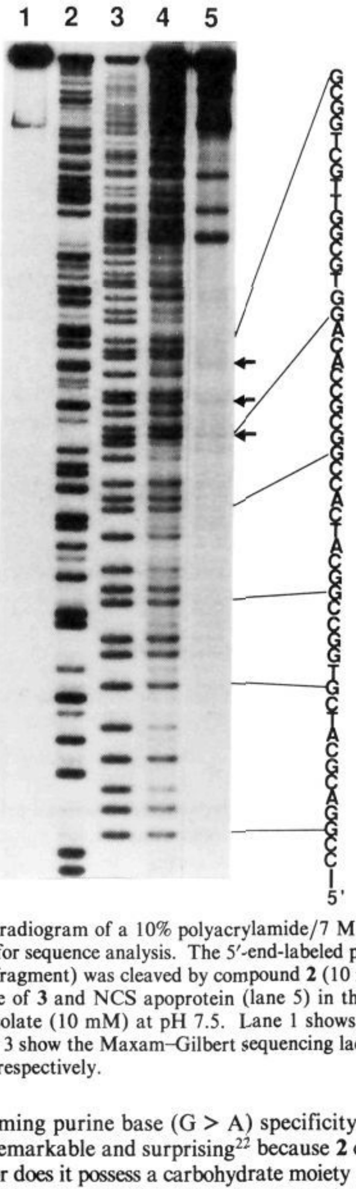


Figure 1. Autoradiogram of a 10% polyacrylamide/7 M urea slab gel electrophoresis for sequence analysis. The 5′-end-labeled pBR322 DNA (*Bam*HI-*Sph*I fragment) was cleaved by compound **2** (10 mM) (lane 4) and the mixture of **3** and NCS apoprotein (lane 5) in the presence of methyl thioglycolate (10 mM) at pH 7.5. Lane 1 shows intact DNA, and lanes 2 and 3 show the Maxam–Gilbert sequencing ladders for C + T and A + G, respectively.

The overwhelming purine base (G > A) specificity of **2** for the target site is remarkable and surprising[22] because **2** cannot be an intercalator nor does it possess a carbohydrate moiety (Figure 1).[1,2] Since the absence of methyl thioglycolate caused no lesion, the chiral alcohol is likely to discriminate the bases, and the C2 σ-radical instead of the resonance-stabilized C7 π-radical of the putative intermediate **12**[17] (Scheme II) would be responsible for this DNA scission, although the alkylation mechanism cannot be ruled out at present.[22b] The combination of **3** and the apoprotein in the presence of methyl thioglycolate appears to show some sequence selectivity: preferential attacks at T of 5′-GCT, which is reminiscent of **1**,[7a] and at G of 5′-GGT (see arrows in Figure 1).[23] The biological and DNA-cleaving activities of the mixture might derive from the complexation[20] and the transport of **3** by the apoprotein[4a] since the naphthoic acid moiety is essential for specific binding to the NCS apoprotein.[24]

Elements for the base recognition as well as the mode of action

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(15) Stored in solution below 0 °C in refrigerator.

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(18) (a) Inhibits the growth of *Bacillus licheniformis* by 5.3 × 10^{-2} μmol on pulp disk during a day of exposure (37 °C); cf. NCS, 1.1 × 10^{-5} μmol. (b) The growth of K562 chronic myelogenous leukemia cells was inhibited with an IC_{50} of 9.3 × 10^{-1} μmol L^{-1} during 3 days of exposure (37 °C); cf. IC_{50} of NCS, 1.1 × 10^{-2} μmol L^{-1}.

(19) Photograph of the agarose electrophoretic gel is included in the supplementary material.

(20) The amount of dissolved complex was apparently quite small due to the very low solubility of 3. Its exact concentration has not been determined.

(21) The cleavage reactions were initiated by addition of methyl thioglycolate (final concentration, 10 mM) to the reaction sample (total volume, 20 μL) of 2 (10 mM), the end-^{32}P-labeled pBR322 DNA (190-bp) fragment (1 nM), and sonicated calf thymus carrier DNA (5 μg mL^{-1}) in 20 mM Tris·HCl buffer (pH 7.5). After the incubation at 37 °C for 18 h, the DNA was extracted with chloroform and then precipitated with ethanol. The lyophilized sample was dissolved in 5 μL of loading buffer containing 5 M urea, 0.1% xylene cyanol FF, and 0.1 M NaOH and then heated at 90 °C before the loading into 10% polyacrylamide gel containing 7 M urea in 1× TBE buffer (89 mM Tris-borate, 2 mM Na_2EDTA, pH 8). DNA sequencing was carried out by the Maxam–Gilbert method (Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 449).

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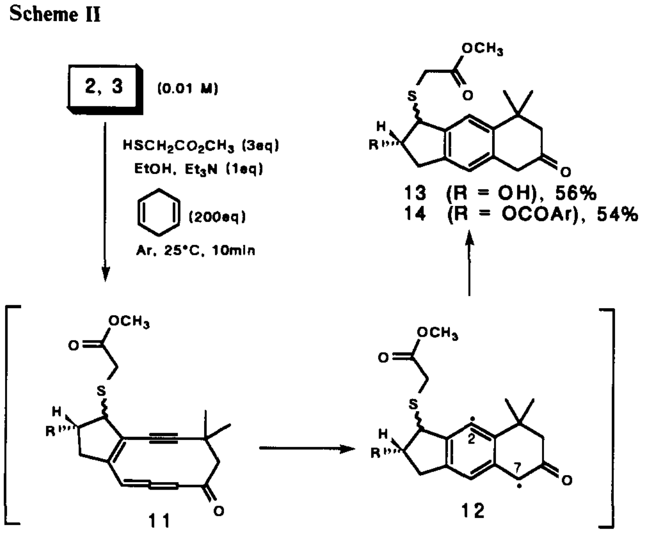
(23) The upper three strong bands in lane 5 are not reproducible.

(24) Unpublished results by Takahashi, K.; Suzuki, T.; and Tanaka, T. Also see: Edo, K.; Saito, K.; Akiyama-Murai, Y.; Mizugaki, M.; Koide, Y.; Ishida, N. *J. Antibiot.* **1988**, *41*, 554.

Scheme II



are currently under investigation in our laboratory.

Acknowledgment. We thank Dr. Hideo Komatsu, Toyohiko Miki, and Toshihiro Hinokitani, POLA Pharmaceutical R & D Laboratory, for testing the biological activities. Financial support by the Ministry of Education, Science and Culture, Japan, and the Mitsubishi, the CIBA-GEIGY, the Shorai, and the TERUMO Science Foundations are acknowledged.

Supplementary Material Available: Spectral and HRMS data for all new compounds, photograph of the electrophoretic gel for form I DNA cutting, and histogram of DNA cleavage pattern by **2** (8 pages). Ordering information is given on any current masthead page.

Matrix ESR Evidence for the Formation of the Bicyclo[3.2.0]hepta-2,6-diene Radical Cation Both from Ionized Quadricyclane and as an Intermediate in the Radical Cation Photoisomerization of Norbornadiene to Cycloheptatriene

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In previous matrix-isolation studies,[1-3] the only reported product of quadricyclane (**1**) ionization was the same species generated from norbornadiene (**2**) and characterized as $\mathbf{2}^{\bullet+}$. Similarly, the gas-phase ions formed from **1** and **2** were found to be mutually indistinguishable by mass spectrometry.[4] On the other hand, evidence for a distinct radical cation $\mathbf{1}^{\bullet+}$ that rapidly isomerizes to $\mathbf{2}^{\bullet+}$ has been obtained from CIDNP[5a-d] and pulse radiolysis[5e] studies in solution. Here we report that under matrix-isolation conditions of especially high dilution, ionization of **1** leads to the bicyclo[3.2.0]hepta-2,6-diene radical cation ($\mathbf{3}^{\bullet+}$), which is also shown to represent an important new intermediate on this much-studied $C_7H_8^{\bullet+}$ potential energy surface.[1-5]

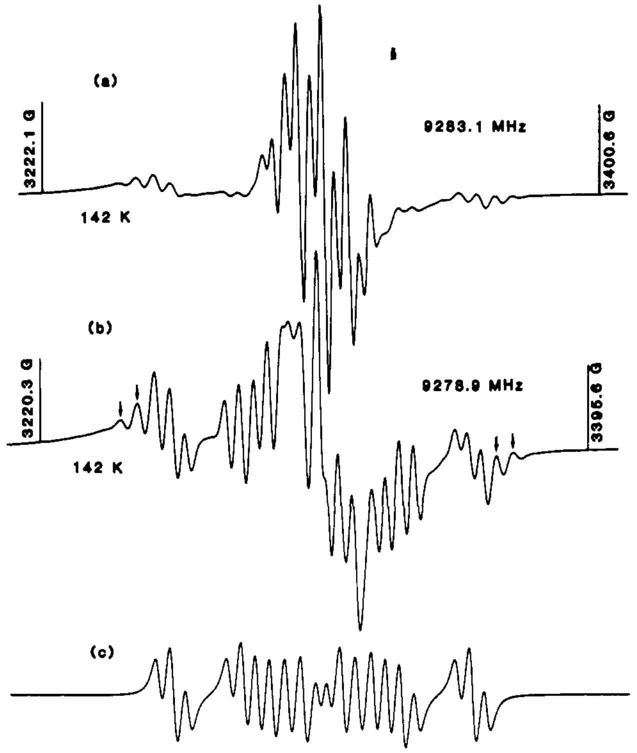
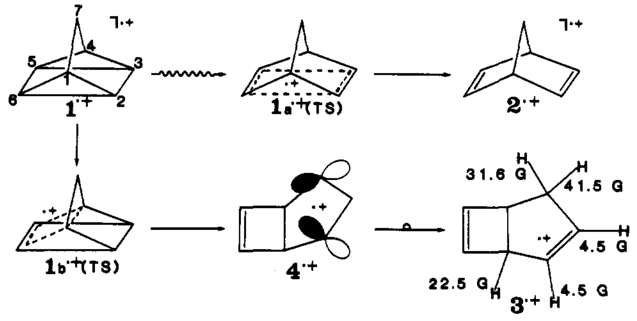


Figure 1. ESR spectra of γ-irradiated solid solutions containing (a) ca. 0.1 mol % and (b) ca. 0.03 mol % of quadricyclane in CF_3CCl_3 (dose, 0.3 Mrad). The main patterns in spectra a and b are assigned to $\mathbf{2}^{\bullet+}$ and $\mathbf{3}^{\bullet+}$, respectively; the extra components marked by arrows are from $\mathbf{5}^{\bullet+}$. Spectrum c was simulated using the coupling constants for $\mathbf{3}^{\bullet+}$ given in the text.

Scheme I



As shown in Figure 1a, the ESR spectrum generated by the radiolytic oxidation[6] of a ca. 0.1 mol % solid solution of **1** in CF_3CCl_3 is dominated by a quintet-of-triplets (a(4H) = 8.0 G; a(2H) = 3.1 G) pattern in the center which is clearly recognizable as that of $\mathbf{2}^{\bullet+}$.[3,7] The additional signals seen in the wings, however, are considerably stronger than any corresponding signal obtained from the oxidation of **2**, suggesting that a second species is formed from **1** but not from **2**. On lowering the concentration of **1** more than 3-fold, a dramatic change in the spectrum of the oxidized products was observed (Figure 1b). Here the outer signals are

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