1150-cm^{-1} region than in the ground-state spectrum. We attribute these additional bands to symmetry lowering resulting from Jahn-Teller distortion, and these bands can be correlated to non totally symmetric modes (b_{1g} or b_{2g}) in the ground state.

Assignments for most of the vibrational bands in the T_1 state are found in Table I. We have already assigned the 1594-cm^{-1} mode to the $C_{ph}C_{ph}$ stretch (ϕ_4) and the 1236-cm^{-1} band to the C_mC_{ph} stretch (ν_1). If we assume that all phenyl modes in the T_1 state are unshifted, we can assign the 1181-cm^{-1} band to a $C_{ph}C_{ph}H$ bend (ϕ_6), in accord with the calculated ground-state assignment.[8] Likewise, the T_1 1264-cm^{-1} band is correlated to the 1269-cm^{-1} band (ν_{27}) of b_{2g} symmetry in the S_0 state (of NiTPP and CuTPP) which has C_mC_{ph} character. Consideration of the porphyrin molecular orbital diagrams[14] permits prediction of the direction of frequency shifts for the porphyrin skeletal modes[15] upon excitation to the T_1 state; the porphyrin modes involving predominantly C_aC_m or C_bC_b stretching motions should decrease, and modes primarily associated with C_aC_b or C_aN stretches should increase in frequency. In Table I, the T_1 1517-, 1495-, 1427-, and 1389-cm^{-1} bands are assigned to porphyrin modes. The T_1 1357- and 1289 cm^{-1} bands are probably due to b_{1g} or b_{2g} modes (D_{4h} symmetry designations) which become active through the Jahn-Teller effect.

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(14) Gouterman, M. *J. Mol. Spectrosc.* **1961**, *6*, 138-163. Predictions were made by using the a_{2u} molecular orbital coefficients of Maggiori: Maggiori, G. M. *J. Am. Chem. Soc.* **1973**, *95*, 6555-6559.

(15) A similar approach was successfully applied in the case of metalloporphyrin cation radicals. See, for example: Oertling, W. A.; Salehi, A.; Chang, C. K.; Babcock, G. T. *J. Phys. Chem.* **1989**, *93*, 1311-1319. See also ref 10.

A Novel Ribose C-4′ Hydroxylation Pathway in Neocarzinostatin-Mediated Degradation of Oligonucleotides

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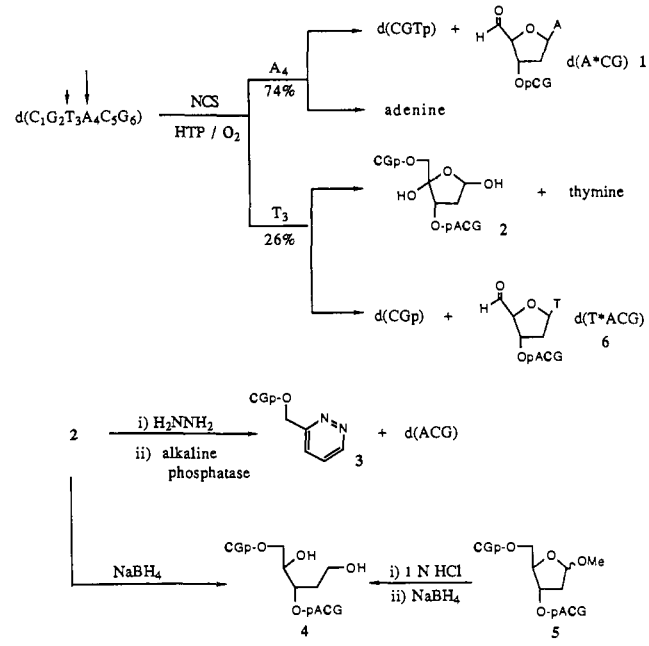
Currently, there is considerable interest in the mechanism of sequence-selective DNA damage by the action of antitumor antibiotic neocarzinostatin (NCS).[1] Upon incubation with thiol, NCS chromophore generates a highly reactive species, plausibly a biradical species derived from an NCS chromophore thiol adduct,[2] which abstracts a hydrogen normally from C-5′ of DNA deoxyribose.[1,3] Recent demonstration that synthetic hexanucleotides can act as a sequence-selective substrate for NCS[4] or NCS chromophore[5] has provided a particularly useful tool for establishing the chemical structure of the DNA lesion induced by NCS and clarifying the mechanism leading to its formation. Here we report that previously unobserved C-4′ hydroxylation of deoxyribose occurs significantly at T_3 in competition with normal C-5′ hydrogen abstraction from deoxyribose at A_4 in NCS-mediated degradation of self-complementary hexanucleotide d(CGTACG).[5,6]

Scheme I



A typical reaction mixture (50 μL) containing NCS (250 μM), d(CGTACG) (42 μM strand concentration), and 4-hydroxythiophenol (HTP, 4 mM)[4] as an NCS activator in 50 mM Tris·HCl buffer (pH 7.2) was incubated at 0 °C for 12 h under aerobic conditions.[7] When the reaction mixture was treated with hot alkali (0.5 M NaOH, 90 °C, 5 min), formation of free thymine and adenine was detected by reverse-phase HPLC in a ratio of ca. 1:3, suggesting that NCS attacks T_3 and A_4 sites in a similar ratio.[5] Direct analysis of the reaction mixture by reverse-phase HPLC[8] indicated the formation of three major products, d(CGTp), 5′-aldehyde fragment d(A*CG) (**1**), and unknown product, together with several minor products including free adenine and thymine as indicated in Scheme I. The structure of **1** was established by quantitative reduction to d(ACG) by $NaBH_4$ (0.05 M, 0 °C, 15 min). Collection of the HPLC peak of the unknown product followed by enzymatic digestion with snake venom phosphodiesterase and alkaline phosphatase gave dG, dC, and dA in a ratio of 2:2:1. Treatment of the fraction[9] with aqueous hydrazine (0.1 M, 90 °C, 5 min) followed by alkaline phosphatase digestion cleanly produced pyridazine derivative **3**[10] and d(ACG). The structure of **3** was confirmed by comparison of its HPLC

(1) Goldberg, I. H. *Free Radical Biol. Med.* **1987**, *3*, 41 and references therein.

(2) (a) Myers, A. P.; Proteau, P. J.; Handel, T. M. *J. Am. Chem. Soc.* **1988**, *110*, 7212. (b) Myers, A. G.; Proteau, P. J. *Ibid.* **1989**, *111*, 1146.

(3) (a) Kappen, L. S.; Goldberg, I. H. *Biochemistry* **1983**, *22*, 4872. (b) Kappen, L. S.; Goldberg, I. H. *Nucleic Acids Res.* **1985**, *13*, 1637 and references therein.

(4) (a) Kawabata, H.; Sugiyama, H.; Tashiro, T.; Takeshita, H.; Matsuura, T.; Saito, I.; Ito, A.; Koide, Y. *Nucleic Acids Symp. Ser.* **1988**, *No. 20*, 69. (b) Kawabata, H.; Takeshita, T.; Fujiwara, H.; Sugiyama, H.; Matsuura, T.; Saito, I. *Tetrahedron Lett.* **1989**, *30*, 4263.

(5) Lee, S. H.; Goldberg, I. H. *Biochemistry* **1989**, *28*, 1019.

(6) Hydrogen abstraction at C-1′ of deoxyribose giving 2-deoxyribonolactone has recently been reported at the d(AGC) sequences in NCS chromophore mediated degradation of oligonucleotides: Kappen, L. S.; Goldberg, I. H. *Biochemistry* **1989**, *28*, 1027.

(7) In a control experiment in the absence of HTP or NCS, oxidation of the hexamer never proceeded even after 24-h incubation under the conditions. The disappearance rate of d(CGTACG) in the presence of NCS was ca. 50% of that observed for d(GCATGC)[4] upon incubation with HTP under identical conditions. HTP- and 2-mercaptoethanol-activated NCS showed exactly the same sequence selectivity in cleaving 5′-end labeled 261-bp DNA fragments.

(8) HPLC conditions: Wakosil $5C_{18}$ ODS column; 0.05 M triethylammonium acetate containing 3-14% acetonitrile, linear gradient, 20 min; flow rate 1.5 mL/min; retention time (d(CGTp)) 10 min, (**1**) 12 min, (**2**) 14.5 min.

(9) Evaporation of the fraction to dryness under reduced pressure below 10 °C resulted in a rapid decomposition of **2** to d(CGp), d(CG), d(pACG), and d(ACG) as revealed by HPLC.

(10) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M.; van del Marel, G. A.; van Boom, J. H. *Biochemistry* **1988**, *27*, 58.

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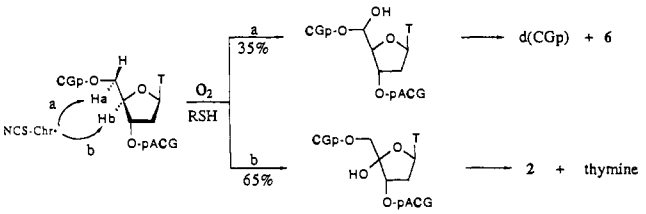
Scheme II



behaviors in several solvent systems with those of an authentic sample.[10,11] These results strongly suggest that the structure of the unknown product is **2**, which results from C-4′ hydroxylation of deoxyribose at T_3 with the release of free thymine. For further confirmation, the product was reduced with $NaBH_4$ to two diastereomers of pentanucleotide **4**, one of which comigrated in two solvent systems on reverse-phase HPLC with an authentic *R* isomer prepared by independent synthesis from **5**.[12] A similar C-4′ hydroxylation of deoxyribose leading to an alkaline labile site has been demonstrated in photoinduced DNA cleavage reaction by cobalt–bleomycin complexes.[14,15]

Given the structure of the alkaline labile abasic product, quantitative analysis was then effected under different HPLC conditions. The amount of abasic product **2** (3.0 μM concentration) was quantitated as **3** by direct treatment of the mixture with 0.1 M aqueous hydrazine (90 °C, 5 min) followed by alkaline phosphatase digestion and corresponded well to spontaneously released thymine (3.0 μM). The exact ratio of T_3 products vs A_4 products was determined to be 26:74 by quantification of the total amounts of thymine (4.6 μM) and adenine (13.0 μM) which were released by hot alkali treatment (0.5 M NaOH, 90 °C, 5 min). The formation of **2** via C-4′ hydroxylation amounted to 65% of the total oxidation products (4.6 μM) at T_3,[16] other T_3 products being d(CGp) and 5′-aldehyde fragment d(T*ACG) (**6**) (each 1.7 μM), both of which were derived from C-5′ oxidation at T_3 (Scheme II). Aldehyde **6** was quantitated as d(TACG) after $NaBH_4$ reduction. In contrast, the reaction at A_4 occurred selectively at C-5′, leading to d(CGTp) (12.0 μM) and d(A*CG) (**1**) (8.5 μM), together with spontaneous adenine release (1.9 μM). The ratio (83:17) of 5′-aldehyde formation vs free adenine release was exactly the same as that obtained in the reaction of d(GCATGC) with NCS.[4]

The present results demonstrate that C-4′ hydroxylation of deoxyribose leading to an alkaline labile abasic site with concomitant free base release is indeed a viable process at certain sequences in NCS-mediated DNA degradation. Biradical species derived from thiol-activated NCS chromophore[2b] could abstract H_a or adjacent H_b hydrogen competitively in the minor groove along the -CGT- sequence as illustrated in Scheme II. Of particular interest is that a similar C-4′ hydroxylation also occurs at T_4 of the longer self-complementary octanucleotide d(GCGTACGC) in competition with C-5′ oxidation at A_5, showing that such C-4′ hydroxylation is not limited to hexanucleotides. Further work to clarify the contribution of such a C-4′ hydroxylation pathway in NCS-mediated degradation of calf thymus DNA is currently underway and will be forthcoming.

Acknowledgment. This work was supported by a Grant-in-Aid for Priority Research from the Ministry of Education, Japan. We are grateful to Pola Kasei Corp. for providing NCS.

(11) HPLC conditions: Cosmosil $5C_{18}$ ODS column; 0.05 M ammonium formate containing 3% acetonitrile; flow rate 1.5 mL/min; retention time 18 min. Enzymatic digestion with calf spleen phosphodiesterase and alkaline phosphatase produced dG and dC in a 1:1 ratio.

(12) (*R*)-**4** was prepared as follows: 1-*O*-methoxy-5-*O*-dimethoxytrityl-2-deoxy-D-ribose was converted to 2-cyanoethyl phosphoramidite by the procedure of van Boom.[13] The solution was applied directly on an automatic solid-phase DNA synthesizer. Fully deblocked **5** was purified by reverse-phase HPLC. A solution of **5** was treated with 1 N HCl (20 °C, 4 h) and then followed by $NaBH_4$ reduction (0 °C, 15 min) after neutralization. HPLC purification provided (*R*)-**4** in 16% overall yield. HPLC conditions: YMS $5C_{18}$ ODS column; 0.05 M ammonium formate containing 4.4% acetonitrile; flow rate 1.5 mL/min; retention time ((*R*)-**4**) 187 min, ((*S*)-**4**) 205 min. Enzymatic digestion with snake venom phosphodiesterase and alkaline phosphatase produced dC, dG, and dA together with modified dG and d(CG).

(13) Nielsen, J.; Taagaard, M.; Marugg, J. E.; van Boom, J. H.; Dahl, O. *Nucleic Acids Res.* **1986**, *14*, 7391.

(14) (a) Chan, C. H.; Meares, C. F. *Biochemistry* **1982**, *21*, 6332. (b) Wensel, T. G.; Chang, C. H.; Meares, C. F. *Ibid.* **1985**, *24*, 3060. (c) Saito, I.; Morii, T.; Sugiyama, H.; Matsuura, T.; Meares, C. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1989**, *111*, 2307.

(15) In fact, photoirradiation (366 nm) of d(CGTACG) in the presence of green Co(III)–peplomycin complex[14c] also provided **2** together with other products. The details will be published elsewhere.

(16) In contrast to the oxidation with the bleomycin–Fe(II)–O_2 system,[17] formation of only a small amount (<3%) of d(CGp)glycolate was detected, probably due to the presence of a large excess of HTP in the reaction system.

(17) (a) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608. (b) Murugesan, N.; Xu, C.; Ehrenfeld, G. M.; Sugiyama, H.; Kilkuskie, R. E.; Rodriquez, L.; Hecht, S. M. *Biochemistry* **1985**, *24*, 5735.

Practical Total Synthesis of (±)-Mitomycin C

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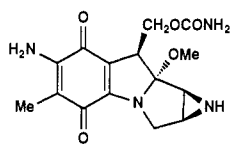
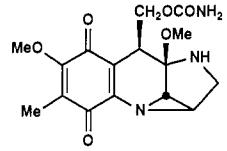
Mitomycin C (**1**) is a potent antitumor agent that is currently used extensively for cancer chemotherapy.[1] Almost 10 years after Kishi's first landmark total synthesis,[2] we reported a highly efficient synthesis of (±)-**1** via (±)-isomitomycin A (**2**) in 1987.[3] While our synthesis has significantly broadened the prospect of mitomycin synthesis, substantial improvement needs to be made before it can be used for a total synthesis of a large amount of mitomycins. In this communication we report a practical total synthesis of (±)-mitomycin C that involves a highly reactive bridgehead iminium species in a key step. This efficient route may be used for a synthesis of a wide variety of hitherto inaccessible mitomycin analogues.



1: Mitomycin C



2: Isomitomycin A

As in our previous synthesis,[3] the readily available chalcone **3** and 5-(ethylthio)-2-(trimethylsiloxy)furan (**4**) were coupled in the presence of 0.1 equiv of $SnCl_4$ at −78 °C to give, after addition of pyridine, the desired silyl enol ether **5** in 95% yield (Scheme I). When heated at 110 °C in toluene, the intramolecular azide–olefin cycloaddition of **5** occurred smoothly to give exclusively the tetracyclic aziridine **6** in 86% yield. Partial reduction of the lactone **6** with DIBAL in THF and subsequent acetylation of the resultant lactol furnished the acetate **7** in 99% yield. While ozonolysis of the silyl enol ether **7** resulted in a complex mixture, oxidation with RuO_4 (RuO_2, $NaIO_4$, EtOAc, H_2O, 23 °C) furnished the aldehyde **8** in 84% yield with concomitant oxidation of the sulfide to sulfone. The aldehyde **8** was then reduced with $NaBH_4$ to give the alcohol **9** in 97% yield.

Upon treatment with trichloroacetyl isocyanate,[4] **9** gave the *N*-(trichloroacetyl)carbamate **10**, which was subjected to the

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(2) (a) Nakatsubo, F.; Fukuyama, T.; Cocuzza, A. J.; Kishi, Y. *J. Am. Chem. Soc.* **1977**, *99*, 8115. (b) Fukuyama, T.; Nakatsubo, F.; Cocuzza, A. J.; Kishi, Y. *Tetrahedron Lett.* **1977**, 4295. (c) Kishi, Y. *J. Nat. Prod.* **1979**, *42*, 549.

(3) Fukuyama, T.; Yang, L.-H. *J. Am. Chem. Soc.* **1987**, *109*, 7881.

(4) Kocovsky, P. *Tetrahedron Lett.* **1986**, *27*, 5521.

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