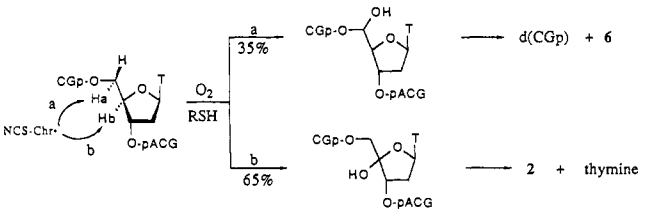
Scheme II



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).

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(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.

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Practical Total Synthesis of (±)-Mitomycin C

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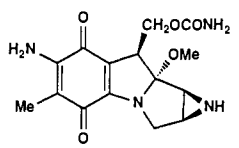
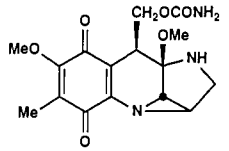
Mitomycin C (**1**) is a potent antitumor agent that is currently used extensively for cancer chemotherapy.[1] Almost 10 years after Kishi's first landmark total synthesis,[2] we reported a highly efficient synthesis of (±)-**1** via (±)-isomitomycin A (**2**) in 1987.[3] While our synthesis has significantly 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

(1) (a) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley: New York, 1979. (b) Carter, S. K.; Crooke, S. T. *Mitomycin C: Current Status and New Developments*; Academic Press: New York, 1979.

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(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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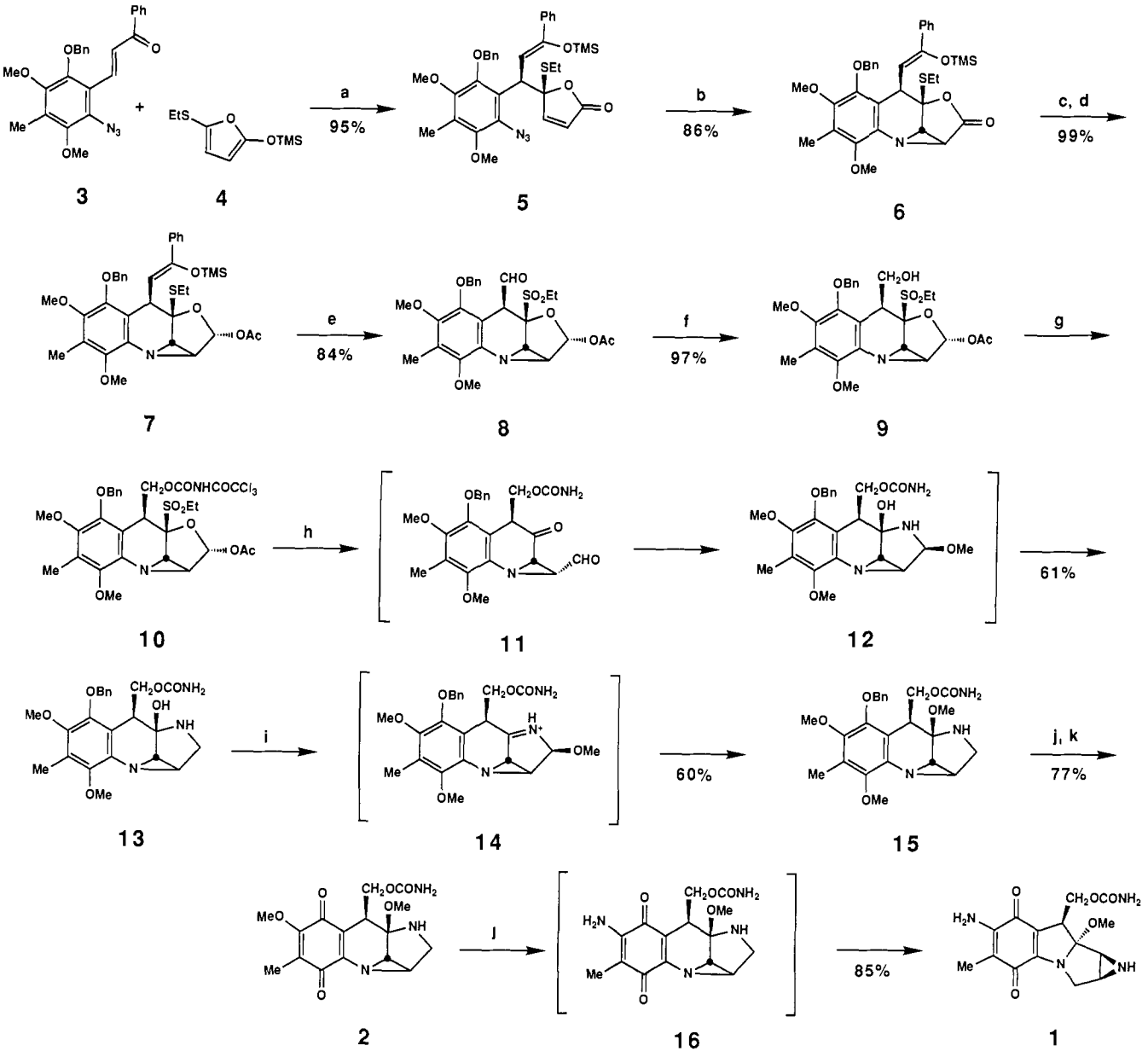
Scheme I.



[a] (a) $SnCl_4$ (0.1 equiv), CH_2Cl_2, −78 °C; Py (1 equiv). (b) Toluene, 110 °C 3 h. (c) DIBAL, THF, −78 °C. (d) Ac_2O, Py. (e) RuO_2 (0.05 equiv), $NaIO_4$ (5 equiv), EtOAc/H_2O (1:1), 23 °C. (f) $NaBH_4$, MeOH. (g) CCl_3CONCO, CH_2Cl_2, 23 °C. (h) NH_3, MeOH, 23 °C; $NaBH_4$. (i) CSA (0.3 equiv), MeOH, 23 °C. (j) H_2 (1 atm), 10% Pd/C, EtOH. (k) DDQ, acetone/H_2O (20:1), −78 °C. (l) NH_3, MeOH, 23 °C, 5 h.

following one-pot transformations without further purification. When treated with saturated NH_3 in MeOH at 23 °C for 1 h, **10** underwent facile ammonolysis to give the unstable intermediate **12**[5] via keto aldehyde **11**. Addition of $NaBH_4$ to the mixture gave the desired aminal **13** in 61% overall yield from **9**. While the bridgehead aminal **13** resisted $NaBH_4$ reduction, the required methoxy group could be introduced via highly strained iminium ion **14** under carefully controlled acidic conditions (camphorsulfonic acid, MeOH, 23 °C) to give **15** in 60% yield. Hydrogenolysis of the benzyl ether **15** (H_2, 10% Pd/C, EtOH, 23 °C) followed by oxidation of the resultant phenol with DDQ (acetone/H_2O (20:1), −78 °C) gave (±)-isomitomycin A (**2**) in 77% yield. Since equilibration of isomitomycin C (**16**) and mitomycin C (**1**) through mitomycin rearrangement[6] is much more facile than that of isomitomycin A with **1** being the predominant isomer,[7] isomitomycin A (**2**) was directly converted to (±)-mitomycin C (**1**) via isomitomycin C (**16**) in 85% yield by treatment with saturated NH_3 in MeOH at 23 °C. The synthetic mitomycin C was identical with an authentic sample in both TLC behavior and spectroscopic properties. The overall yield of (±)-**1** from commercially available 2,6-dimethoxytoluene is 10%.

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Supplementary Material Available: Copies of ^1H and ^{13}C NMR spectra of key intermediates **2**, **6–9**, **13**, and **15** and of synthetic and natural mitomycin C and high-resolution mass spectral data for **2**, **6–9**, **13**, and **15** (17 pages). Ordering information is given on any current masthead page.

(5) Due to the unstable nature of this intermediate, the structure has not been spectroscopically verified.

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(7) Private communication from Dr. M. Kasai, Kyowa Hakko Co., Ltd., Tokyo.