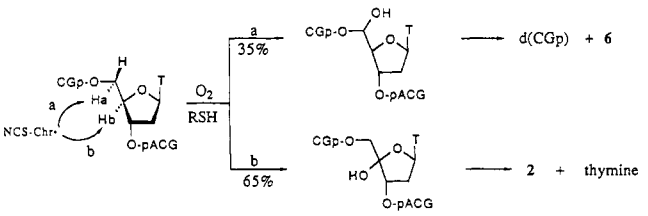
Scheme II



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.

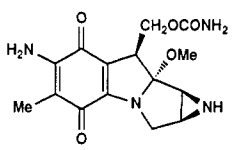
Practical Total Synthesis of (±)-Mitomycin C

Tohru Fukuyama* and Lihu Yang

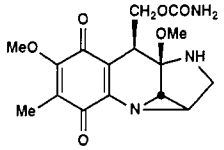
Department of Chemistry, Rice University
Houston, Texas 77251

Received May 12, 1989

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.

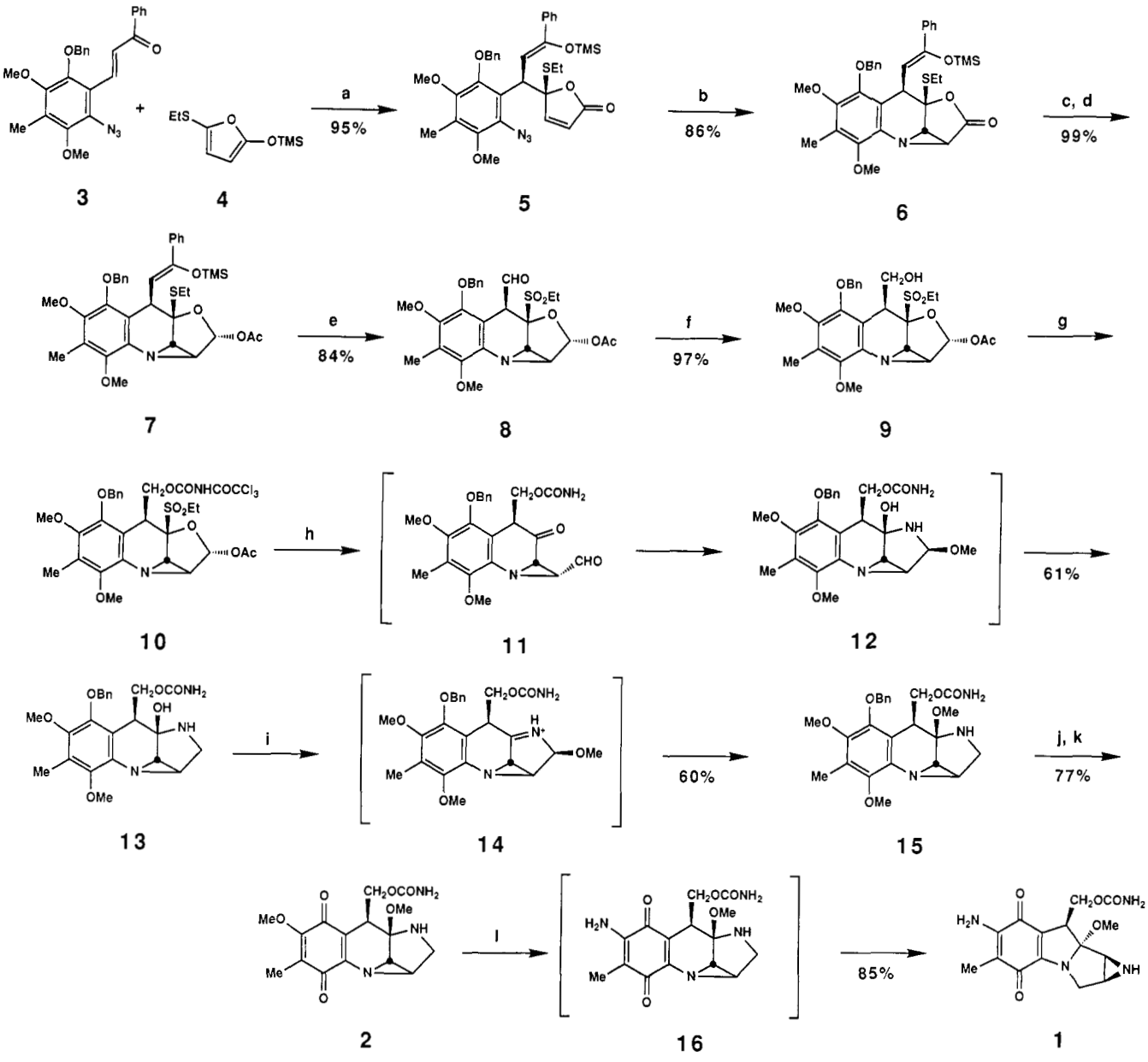
(2) (a) Nakatsubo, F.; Fukuyama, T.; Cocuzza, A. J.; Kishi, Y. *J. Am. Chem. Soc.* **1977**, *99*, 8115. (b) Fukuyama, T.; Nakatsubo, F.; Cocuzza, A. J.; Kishi, Y. *Tetrahedron Lett.* **1977**, 4295. (c) Kishi, Y. *J. Nat. Prod.* **1979**, *42*, 549.

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

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

0002-7863/89/1511-8303$01.50/0

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%.

Acknowledgment. This work was supported by the National Institutes of Health (Grant CA28119) and the Robert A. Welch Foundation. We thank Drs. T. Hirata and M. Kasai of Kyowa Hakko Kogyo for valuable information and for providing a sample of natural mitomycin C.

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.

(6) Kono, M.; Saitoh, Y.; Shirahata, K.; Arai, Y.; Ishii, S. *J. Am. Chem. Soc.* **1987**, *109*, 7224.

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