A Novel Synthesis of New Antineoplastic 2′-Deoxy-2′-substituted-4′-thiocytidines

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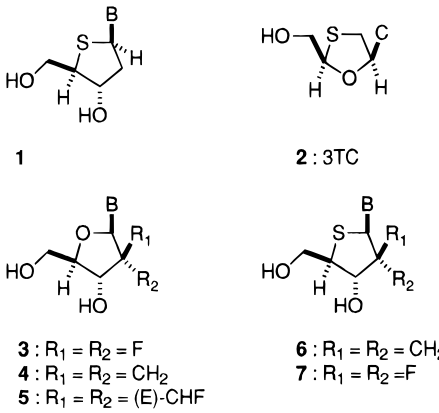
Nucleosides containing sulfur atoms instead of lactol oxygen have been the focus of much recent research because of their potent biological activity. Walker[1] and Secrist[2] independently reported that 2′-deoxy-4′-thiopyrimidine nucleosides (**1**) have antiviral and cytotoxic effects. 2′,3′-Dideoxy-3′-thiacytidine (3TC, **2**) has been shown to have potent anti-human immunodeficiency virus (HIV) activity[4] and anti-human hepatitis B virus activity.[5,6] Furthermore, new antineoplastic cytidine analogues having various 2′-substituents, 2′-deoxy-2′,2′-difluorocytidine[7] (Gemcitabine, **3**), 2′-deoxy-2′-methylenecytidine[8] (DMDC, **4**), and 2′-deoxy-2′(*E*)-(fluoromethylene)cytidine (**5**)[9] have been described.

The specific properties of 4′-thionucleosides and potent cytotoxicity of 2′-substituted cytidine analogues prompted us to synthesize 4′-thioDMDC (**6**) and 4′-thiogemcitabine (**7**) (Chart I). Since the first synthesis of 4′-thionucleosides was described in 1964,[10] several alternate synthetic methods have been reported.[1−3,11−14] These procedures are not optimal, however, due to a lengthy manipulation[3,10−12] and limitation to the use of 2′-deoxy derivatives.[1,2,13] Thus, a new synthetic strategy utilizing more generally available compounds would increase the success of this synthesis. Recent progress was made in this regard by Chu *et al.*,[6] in the synthesis of 3TC. These results led us to synthesize the title compounds employing an anhydrothiosugar as a key intermediate. In the present study, we describe a novel synthesis of 4′-thiocytidines originating from D-glucose.

Chart 1



In four steps, diisopropylideneglucose **8** was converted to 3-benzylxylose **9**, which was then subjected to acidic methanolysis to produce an anomeric mixture of 1-*O*-methyl-3-*O*-benzylxylose (**10**) with a high yield. Anomers were easily separated by a silica gel column. The separated α- and *β*-anomers of **10** were mesylated, producing α- and *β*-**11**, followed by treatment with sodium sulfide in DMF to yield bicyclic α- and *β*-**12** at 78% and 73% yield, respectively. Acid hydrolysis and hydride reduction of α,*β*-**12** produced 1,4-anhydro-4-thioarabinitol **13** with a 90% yield.[15] The primary alcohol of **13** was selectively protected with a *tert*-butyldiphenylsilyl (TBDPS) group to produce **14**, which was oxidized with DMSO−Ac_2O, giving **15**. The Wittig reaction of **15** yielded **16** (efficiency: 74% of **14**). A reaction with boron trichloride (BCl_3) effectively deprotected the benzyl group of **16** to yield **17** at over 90% efficiency.

Pioneering works of Kita *et al.* led to application of Pummerer reaction for the synthesis of C−C bond at the α-position of sulfoxides.[16] O'Neil and Hamilton also reported the syntheses of a tetrahydrothienylthymine and other derivatives using TMSOTf as a catalyst under similar reaction conditions.[17] On the basis of this, we designed the synthesis of the 4′-thiocytidine utilizing sulfoxide **18** obtained from *m*-CPBA oxidation of **17**. The compound **18** was treated with 3 equiv of the silylated *N*-acetylcytosine and 2 equiv of TMSOTf producing the 4′-thiocytidine derivative α,*β*-**19** (Scheme 1) with a 74% yield (α:*β* = 2.5:1).[18] The reaction conditions have not

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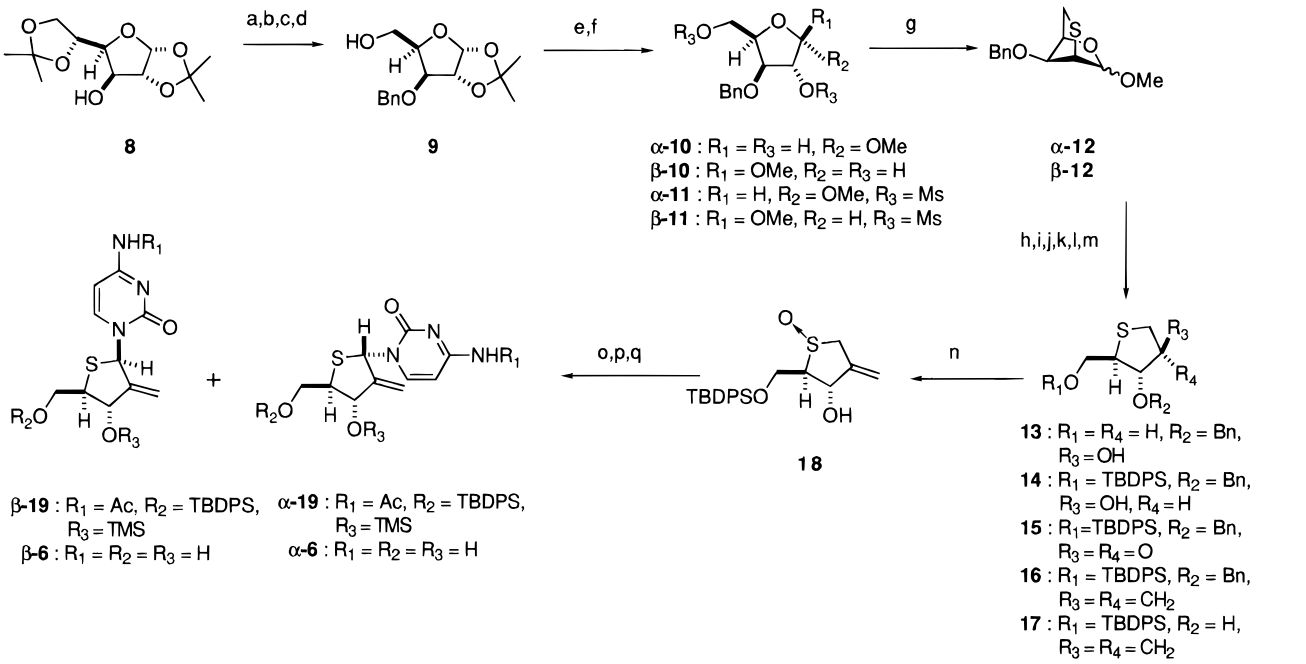
(1) Dyson, M. R.; Coe, P. L.; Walker, R. T. *J. Med. Chem.* **1991**, *34*, 2782−2786.
(2) Secrist, J. A.; Tiwari, K. N.; Riordan, J. M.; Montgomery, J. A. *J. Med. Chem.* **1991**, *34*, 2361−2366.
(3) Tiwari, K. N.; Secrist, J. A.; Montgomery, J. A. *Nucleosides Nucleotides* **1994**, *13*, 1819−1828.
(4) (a) Schinazi, R. F.; Chu, C. K.; Peck, A.; McMillan, A.; Mathis, R.; Cannon, D.; Jeong, L.-S.; Beach, J. W.; Choi, W.-B.; Yeola, S.; Liotta, D. C. *Antimicrob. Agents Chemother.* **1992**, *36*, 672−676. (b) Coates, J. A. V.; Cammack, N.; Jenkinson, H. J.; Jowett, A. J.; Jowett, M. I.; Pearson, B. A.; Penn, C. R.; Rouse, P. L.; Viner, K. C.; Cameron, J. M. *Antimicrob. Agents Chemother.* **1992**, *36*, 733−739.
(5) Doong, S.-L.; Tsai, C.-H.; Schinazi, R. F.; Liotta, D. C.; Chen, Y.-C. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 8495−8499.
(6) Beach, J. W.; Jeong, L. S.; Alves, A. J.; Pohl, D.; Kim, H. O.; Chang, C.-N.; Doong, S.-L.; Schinazi, R. F.; Cheng, Y.-C.; Chu, C. K. *J. Org. Chem.* **1992**, *57*, 2217−2219.
(7) (a) Hertel, L. W.; Kroin, J. S.; Misner, J. W.; Tustin, J. M. *J. Org. Chem.* **1988**, *53*, 2406−2409. (b) Hertel, L. W.; Boder, G. B.; Kroin, J. S.; Rinzel, S. M.; Poore, G. A.; Todd, G. C.; Grindey, G. B. *Cancer Res.* **1990**, *50*, 4417−4420.
(8) Yamagami, K.; Fujii, A.; Arita, M.; Okumoto, T.; Sakata, S.; Matsuda, A.; Ueda, T. *Cancer Res.* **1991**, *51*, 2319−2323.
(9) Bitonti, A. J.; Dumont, J. A.; Bush, T. L.; Cashman, E. A.; Cross-Doersen, D. E.; Wright, P. S.; Matthews, D. P.; McCarthy, J. R.; Kaplan, D. A. *Cancer Res.* **1994**, *54*, 1485−1490.
(10) Reist, E. J.; Gueffroy, D. E.; Goodman, L. *J. Am. Chem. Soc.* **1964**, *86*, 5658−5663.
(11) Bobek, M.; Bloch, A.; Parthasarathy, R.; Whistler, R. L. *J. Med. Chem.* **1975**, *18*, 784−787.
(12) Leydier, C.; Bellon, L.; Barascut, J.-L.; Deydier, J.; Maury, G.; Pelicano, H.; El Alaoui, M. A.; Imbach, J.-L. *Nucleosides Nucleotides*, **1994**, *13*, 2035−2050.
(13) Uenishi and his co-workers reported an elegant synthesis of 2′-deoxy-4′-thionucleosides using Sharpless asymmetric epoxidation: (a) Uenishi, J.; Motoyama, M.; Nishiyama, Y.; Wakabayashi, S. *J. Chem. Soc. Chem. Commun.* **1991**, 1421−1422. (b) Uenishi, J.; Takahashi, K.; Motoyama, M.; Akashi, H.; Sasaki, T. *Nucleosides Nucleotides* **1994**, *13*, 1347−1361.
(14) Fluorine substituted derivatives and dideoxy analogues, including AZT, of 4′-thionucleosides were reported. Fluorine substituted derivatives: (a) Jeng, L. S.; Nicklaus, M. C.; George, C.; Marquez, V. E. *Tetrahedron Lett.* **1994,** *35*, 7569−7572. (b) Jeng, L. S.; Nicklaus, M. C.; George, C.; Marquez, V. E. *Tetrahedron Lett.* **1994,** *35*, 7573−7576. Dideoxy derivatives: (c) Secrist, J. A.; Riggs, R. M.; Tiwari, K. N.; Montgomery, J. A. *J. Med. Chem.* **1992**, *35*, 533−538. (d) Tber, B.; Fahmi, N.-E.; Ronco, G.; Villa, P.; Ewing, D. F.; Mackenzie, G. *Carbohydr. Res.* **1995**, *267*, 203−215.
(15) During the course of our investigation, the synthesis of **13** was independently reported by another group: Yuasa, H.; Kajimoto, T.; Wong, C.-H. *Tetrahedron Lett.* **1994**, *35*, 8243−8246.
(16) Kita, Y.; Tamura, O.; Yasuda, H.; Itoh, F.; Tamura, Y. *Chem. Pharm. Bull.* **1985**, *33*, 4235−4241.
(17) O'Neil, I. A.; Hamilton, K. M. *Synlett* **1992**, 791−792.

0022-3263/96/1961-0822$12.00/0

Scheme 1[a]



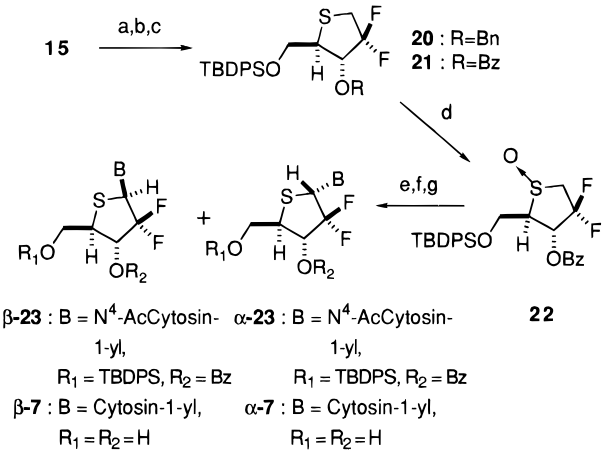
[a] (a) BnBr, NaH, DMF, THF; (b) 2 M HCl, THF; (c) $NaIO_4$, H_2O, MeOH; (d) $NaBH_4$, MeOH, 82% from **8**; (e) 5% HCl/MeOH, 91%; (f) MsCl, pyridine; (g) Na_2S, DMF, 100 °C, 78% (α-anomer) and 73% (β-anomer) from **10**; (h) 4 M HCl, THF; (i) $NaBH_4$, MeOH. 90% from **12**; (j) TBDPSCl, imidazole, DMF, 87%; (k) DMSO, Ac_2O; (l) $Ph_3P^+CH_3Br^-$, NaH, *tert*-amyl alcohol, THF, 74% from **14**; (m) BCl_3, CH_2Cl_2, −78 °C, then MeOH, pyridine. 92%; (n) *m*-CPBA, CH_2Cl_2, −78 °C; (o) silylated *N*-acetylcytosine, TMSOTf, $ClCH_2CH_2Cl$, 0 °C, 74% from **17**; (p) TBAF, THF; (q) aqueous NH_3, MeOH, then HPLC separation, 34% (α-anomer) and 13% (β-anomer) from **19**.

been optimized yet; β-selectivity remains a problem. Finally, the α,β-mixture of **19** was deprotected by tetrabutylammonium fluoride (TBAF) followed by aqueous ammonia in methanol. Pure α- and β- anomers of **6** were obtained from the mixture by HPLC (α; 34%, β; 13%).

The method described above was applied to the synthesis of 4′-thiogemcitabine. (Diethylamido)sulfur trifluoride (DAST) treatment[19] of ketone **15** produced the 2-deoxy-2,2-difluoro derivative **20** with a 48% yield. Compound **20** was simultaneously deprotected and benzoylated to give **21**, which was oxidized to produce **22**. The Pummerer type glycosylation of **22** resulted in a 57% yield of the protected 4′-thiogemcitabine **23** (Scheme 2) as an anomeric mixture (α:β = 2.6:1).[20] Deprotection of **23**, followed by HPLC separation, produced the α- and β-derivatives of 4′-thiogemcitabine (**7**).

We evaluated the antineoplastic properties of **6** and **7** against human T-cell leukemia (CCRF-HSB-2) and KB cells. None of the α-4′-thionucleosides had any measurable activity, whereas β-4′-thioDMDC (**6**) exhibited a potent antitumor activity against CCRF-HSB-2 cells (IC_{50} = 0.0091 μg/mL). In contrast, β-4′-thiogemcitabine (**7**) was only weakly active in the same cell line (IC_{50} = 1.5 μg/mL). β-4′-Thio-DMDC (**6**) was also effective against a solid tumor, KB cells (IC_{50} = 0.12 μg/mL). The activity was higher than that of DMDC **4** (KB cell; IC_{50} = 0.44 μg/mL). It is noteworthy that 4′-thioDMDC (**6**) had potent antineoplastic activity, but 4′-thiogemcitabine (**7**) did not, while both **3** and **4** were highly active.[7,8]

Scheme 2[a]



[a] (a) DAST, benzene, 0 °C, and then room temperature 48%; (b) BCl_3, CH_2Cl_2, −78 °C, and then MeOH, pyridine; (c) Bz_2O, Et_3N, DMAP, CH_3CN, 79% from **20**; (d) *m*-CPBA, CH_2Cl_2, −78 °C; (e) silylated *N*-acetylcytosine, TMSOTf, $ClCH_2CH_2Cl$, 0 °C, 57% from **21**; (f) TBAF, THF; (g) aqueous NH_3, MeOH, then HPLC separation, 36% (α-anomer) and 15% (β-anomer) from **23**.

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Supporting Information Available: Experimental procedures and characterization data (9 pages).

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(18) The stereochemistry of the anomeric carbon was determined by an NOE analysis of the free nucleoside **6** (minor isomer). It showed 7.1% NOE at the H-3′ proton when irradiated at H-6.

(19) An, S.-H.; Bobek, M. *Tetrahedron Lett.* **1986**, *27*, 3219−3222.

(20) The stereochemistry at the 1′-position was determined, after deprotection, by comparison of coupling constants of H-1′ with that of gemcitabin described in ref 7a.