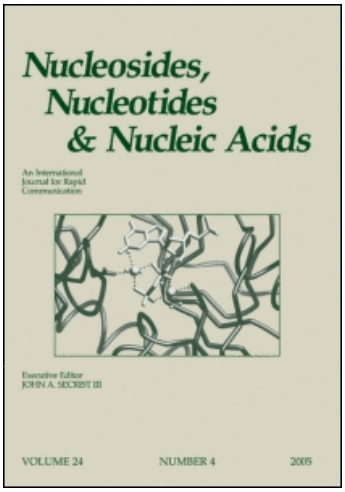


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthetic Studies on 2'-Substituted-4'-thiocytidine Derivatives as Antineoplastic Agents

Yuichi Yoshimura[a]; Mikari Endo[a]; Kenji Kitano[a]; Kohei Yamada[a]; Shinji Sakata[a]; Shinji Miura[a]; Haruhiko Machida[a]
[a] Biochemicals Division, Yamasa Corporation, Chiba, Japan

To cite this Article Yoshimura, Yuichi , Endo, Mikari , Kitano, Kenji , Yamada, Kohei , Sakata, Shinji , Miura, Shinji and Machida, Haruhiko(1999) 'Synthetic Studies on 2'-Substituted-4'-thiocytidine Derivatives as Antineoplastic Agents', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 815 — 820
To link to this Article: DOI: 10.1080/15257779908041569
URL: http://dx.doi.org/10.1080/15257779908041569

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 815–820 (1999)

SYNTHETIC STUDIES ON 2'-SUBSTITUTED-4'-THIOCYTIDINE DERIVATIVES AS ANTINEOPLASTIC AGENTS

Yuichi Yoshimura,* Mikari Endo, Kenji Kitano, Kohei Yamada, Shinji Sakata, Shinji Miura, and Haruhiko Machida

Biochemicals Division, Yamasa Corporation, 2-10-1 Araoicho, Choshi, Chiba 288-0056, Japan

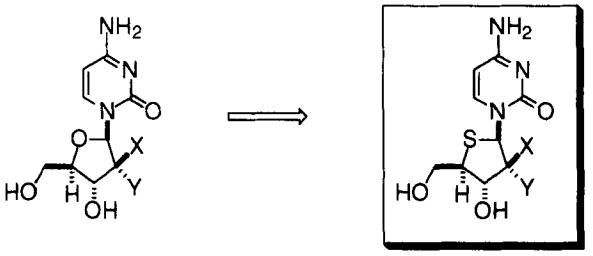
ABSTRACT: As potential antineoplastic agents, we have synthesized 4'-thioFAC and 4'-thiocytarazid by developing an alternative synthetic method. 4'-ThioFAC showed potent antineoplastic activities *in vivo* as well as *in vitro*.

The 4'-thionucleosides are attractive compounds as both antiviral and antitumor agents. Since the several 2'-substituted cytidine analogues, such as DMDC **1** and gemcitabine **2**, have already been known as potent antitumor agents,[1,2] the 4'-thio analogues of these compounds seem to be promising antitumor agents. When we started their synthesis, reports concerning 2'-substituted 4'-thionucleoside were quite limited.[3] Thus, we developed a novel method for synthesizing them, and found 4'-thioDMDC **5** had potent antitumor activities, as expected.[4,5] In contrast, the activity of 4'-thiogemcitabine **6** was rather disappointing, and was hundred times less active than 4'-thioDMDC.[4,5] These results suggested the effects of 2'-substituents of 4'-thionucleosides on antitumor activities might be different from those of usual 4'-oxy counterparts. To investigate the further structure-activity relationship of 2'-substituted-4'-thiocytidines, we have selected 1-(2-deoxy-2-fluoro-4-thio-β-D-arabinofuranosyl)cytosine (4'-thioFAC, **7**) as a third target: its 4'-oxy congener, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine (FAC, **3**) had been synthesized in 1970 by Wright and Fox, and reported to have antileukemic activity *in vitro*.[6] 4'-ThioFAC also seems to be promising as antitumor agent.

The method developed for the synthesis of 4'-thioDMDC and 4'-thiogemcitabine should be generally applicable to the synthesis of other 2'-substituted 4'-thionucleosides, and was applied to the synthesis of 4'-thioFAC.[5] The 5-silylated 4-thiosugar derivative **9**, which was prepared from diacetoneglucose as reported previously,[4,5] was treated with DAST to give a 2-fluorinated compound **11** as a sole product.[5]

* Corresponding author. Tel: +81 479 220095, Fax: +81 479 229821,
E-mail: chem2yms@choshinet.or.jp

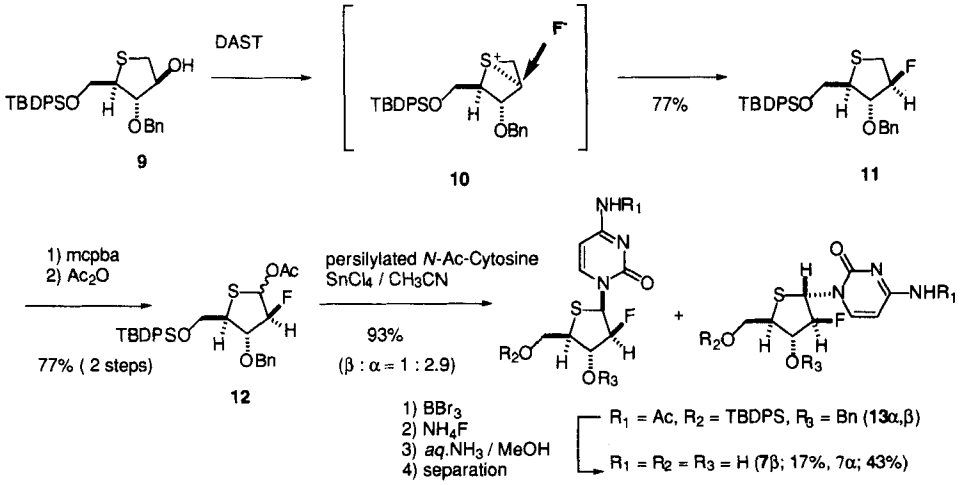
Chart 1



1: DMDC : X = Y = CH_2
2: Gemcitabine : X = Y = F
3: FAC : X = F, Y = H
4: Cytarazid : X = N_3, Y = H

5: 4'-ThioDMDC : X = Y = CH_2
6: 4'-Thiogemcitabine : X = Y = F
7: 4'-ThioFAC : X = F, Y = H
8: 4'-Thiocytarazid : X = N_3, Y = H

Scheme 1

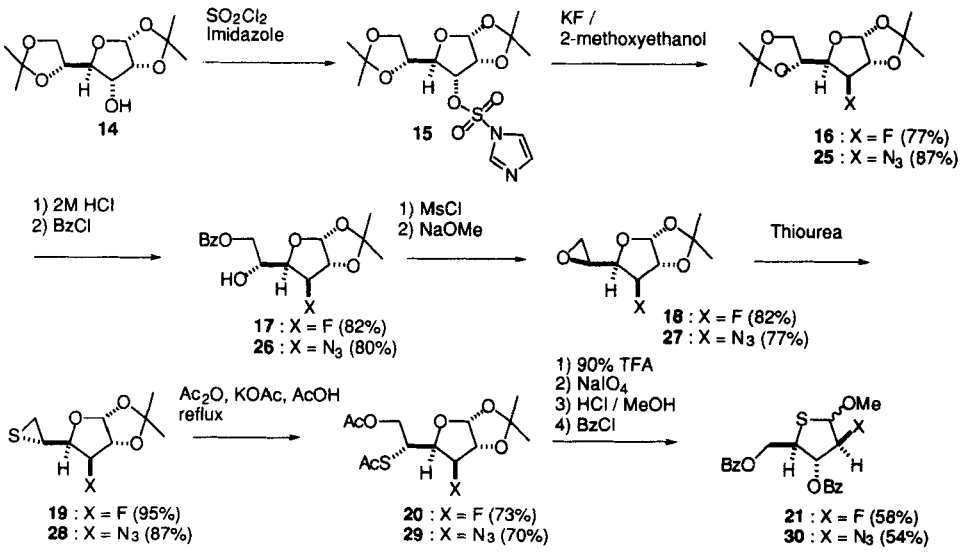


The structural elucidation of **11** at the latter stage of the synthesis clearly showed that it had 2'-up configuration as we expected. This is well-consistent with the results reported by Marquez and his co-workers; it is clear that the reaction proceeded *via* an episulfonium intermediate **10** as shown in Scheme 1.[7] The 2-fluoro derivative **11** was converted to 1-acetate **12** by the Pummerer rearrangement. The glycosylation reaction with persilylated N^4-acetylcytosine in the presence of stannic chloride gave a mixture of α- and β-anomers of 4'-thioFAC derivatives **13**. As in the case of 4'-thioDMDC and 4'-thiogemcitabine, the α-isomer was predominantly formed. Finally, compound **13** was deprotected in 3 steps, and the resulting isomers were separated by ODS column chromatography to give 4'-thioFAC **7** and its α-isomer. Evaluation of the biological activities of 4'-thioFAC showed potent antitumor activities against various solid tumors. The detail is discussed later.

Although we could achieve the synthesis of 4'-thioFAC, it remained several problems. Expensive and difficult-to-handle reagents have been used, such as DAST,TBDPSCl, BBr_3, and mcpba, which were unsuitable for a large-scale production. The most serious problem was unsatisfactory β-stereoselectivity of

the glycosylation step. This made the separation of the α- and β-isomers complicated. To remedy these drawbacks, we had to exploit an alternative synthetic method. To this end, we intended to apply the improved synthesis of FAC reported by Watanabe[8] to the synthesis of 4'-thioFAC. Thus, the synthesis from commercially available diacetoneallofuranose **14** was investigated.

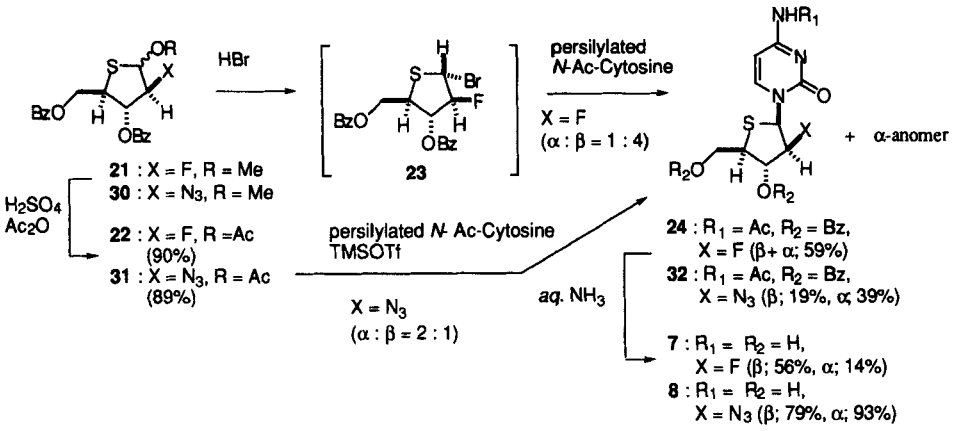
Scheme 2



For the *C*-3 fluorination of diacetoneallofuranose **14**, we used the method developed by Bristol-Myers group with slight modification,[9] instead of the original method. Diacetoneallofuranose **14** was treated with sulfuryl chloride and imidazole to give sulfuryl imidazole derivative **15**, which was fluorinated by the treatment with potassium fluoride in refluxing 2-methoxyethanol to give a 3-fluoro derivative **16** in 77% yield. The 3-fluoro derivative **16** was converted to a diacetate derivative **20** via an epoxide derivative **18** as shown in Scheme 2. 3,5-Dibenzoyl-2-fluoro-4-thioarabinose **21** was derived from the diacetate derivative **20** by acid hydrolysis, oxidation, treatment with acidic methanol, and benzoylation in 58% yield.

As mentioned above, the Lewis acid assisted glycosylation resulted in the poor β-selectivity. Thus, we investigated the nucleophilic substitution of 1-α-bromide of 4-thiosugar which could selectively be prepared from 1-acetate **22**, as in the synthesis of FAC and its related compounds.[8] The 1-acetate derivative **22**, obtained form dibenzoyl derivative **21** by acetolysis, was transiently converted to the corresponding 1-α-bromide **23**. However, our first attempt to obtain 4'-thioFAC by nucleophilic substitution of 1-α-bromide **23** was unsuccessful: the reaction in refluxing 1,2-dichloroethane gave only trace amounts of the glycosylated product. Quite interestingly, when this reaction was done without solvent under reduced pressure, the glycosylated product was formed in 5 h with predominant formation of the β-isomer. After deprotection and separation, the structure of the major isomer was confirmed by instrumental analysis, as was 4'-thioFAC **7**. (Scheme 3)

Scheme 3



Next, we chose 4'-thiocytarazid **8** as our target compound, because cytarazid **4**, 4'-oxy counterpart of the target, has been known to have antitumor activities.[10] However, our first attempt to synthesize 4'-thiocytarazid by the original method was unsuccessful. Against our expectation, the Mitsunobu reaction of 1-deoxy-4-thioarabinose **9** gave only a ribo-azide derivative.[5] Therefore, we tried to synthesize 4'-thiocytarazid by the alternative method mentioned above.

As in the case of 4'-thioFAC, diacetoneallofuranose **14** was converted to 2-azido-4-thioarabinose derivative **31** which was subjected to the glycosylation reaction. (Scheme 2) First, we have tried to prepare 1-α-bromide, as 4'-thioFAC was synthesized. However, the generated 1-bromide was unstable, and the reaction with persilylated N^4-acetylcytosine gave none of the desired product. Alternatively, the reaction of 1-acetate **31** with persilylated N^4-acetylcytosine in the presence of TMS triflate gave protected 4'-thiocytarazid **32** in 58% yield as a 1 : 2 mixture of β- and α-isomers. After silica gel column purification, the separated isomers were deblocked to give a β- and α-anomer of 4'-thiocytarazid **8**, respectively. (Scheme 3) 4'-Thiocytarazid showed only moderate activity against leukemic cell lines (CCRF-HSB-2; IC_{50} 7.8 μg/mL), and was inactive against solid tumor KB cells (IC_{50} 42 μg/mL). Thus, the incorporation of sulfur instead of 4'-oxygen resulted in the reduction of antitumor activity of cytarazid.

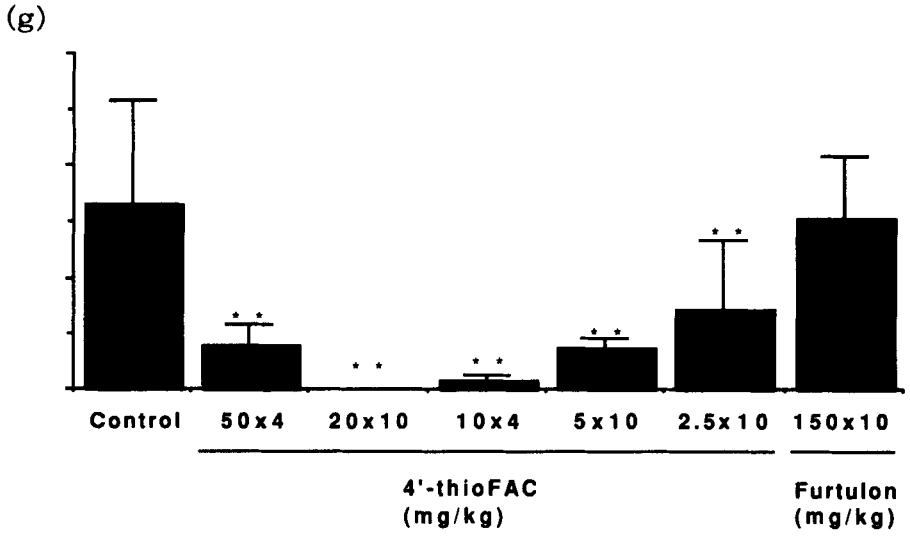
As mentioned above, 4'-thioFAC has shown potent antineoplastic activities against various solid tumor cell lines *in vitro*. The results are summarized in Table 1. 4'-ThioFAC has a similar antitumor spectrum to 1-(β-D-arabinofuranosyl)cytosine (araC), but, 4'-thioFAC was about several to 10 times more active than araC in most of the cell lines tested. Particularly, 4'-thioFAC has shown potent inhibitory activities against stomach and colon cancer cell lines. In contrast, FAC, 4'-oxy congener of 4'-thioFAC, did not show any antitumor activity except leukemic and one colon cancer cell line.

Furthermore, antitumor activities of 4'-thioFAC were evaluated in nude mice bearing human colon cancer SW48 xenografts. 4'-ThioFAC was administered intravenously or orally, and its antitumor activities were evaluated by measuring tumor weights on days at 40 or 45. 4'-ThioFAC was highly active

Table 1: *In vitro* Antitumor activities of 4'-thioFAC and the related compounds

Cell Lines	Origin	IC_{50} (μg/mL)		
		Ara-C	FAC	4'-ThioFAC
MKN-45	Stomach	1.5	>100	0.057
NUGC-4		2.6	>100	0.17
Colo320DM	Colon	0.027	0.19	0.025
SW48		0.27	>100	0.018
PC-8	Lung (NSCLC)	>100	>100	>100
PC-9		>100	>100	>100
KB	Head & Neck	0.21	100	0.067
CCRF-HSB-2	Leukemia	0.056	0.15	0.086

Chart 2 Antitumor activities of 4'-thioFAC against human colon carcinoma SW48 xenograft (s.c.-p.o.)



**: p<0.01 (Dunnett's multiple camparison test)

both in intravenous and oral administration. It is noteworthy that the oral administration showed potent inhibitory activities against tumor growth, which was comparable to those by the intravenous administration, and even at the 2.5 mg treatment, 4'-thioFAC had significant antitumor activity (Chart 2).

In conclusion, as potential antitumor agents, we have synthesized 4'-thioFAC and 4'-thiocytarazid by developing an alternative synthetic method. 4'-ThioFAC showed potent antitumor activities against solid tumors *in vivo* as well as *in vitro*. Especially, 4'-thioFAC showed more potent antitumor activities in the oral administration than the intravenous administration.

ACKNOWLEDGMENT: The authors are grateful to Prof. A. Matsuda, Hokkaido University, for useful suggestions during this work. The authors also acknowledge Dr. K. Kodama, Yamasa Corporation, for his encouragement.

REFERENCES

1. Yamagami, K.; Fujii, A.; Arita, M.; Okumoto, T.; Sakata, S.; Matsuda, A.; Ueda, T. *Cancer Res.* **1991**, *51*, 2319-2323.
2. Hertel, L. W.; Border, G. B.; Kroin, J. S.; Rinzel, S. M.; Poore, G. A.; Todd, G. C.; Grindey, G. B. *Cancer Res.* **1990**, *50*, 4417-4420.
3. Ototani, N.; Whistler, R. L. *J. Med. Chem.* **1974**, *17*, 535-537.
4. Yoshimura, Y.; Kitano, K.; Satoh, H.; Watanabe, M.; Miura, S.; Sakata, S.; Sasaki, T.; Matsuda, A. *J. Org. Chem.* **1996**, *61*, 822-823.
5. Yoshimura, Y.; Kitano, K.; Yamada, K.; Satoh, H.; Watanabe, M.; Miura, S.; Sakata, S.; Sasaki, T.; Matsuda, A. *J. Org. Chem.* **1997**, *62*, 3140-3152.
6. Wright, J. A.; Wilson, D. P.; Fox, J. J. *J. Med. Chem.* **1970**, *13*, 269-272.
7. Jeong, L. S.; Nicklause, M. C.; George, C.; Marquez, V. E. *Tetrahedron Lett.* **1994**, *35*, 7569-7572.
8. Reichman, U.; Watanabe, K. A.; Fox, J. J. *Carbohydr. Res.* **1975**, *42*, 233-240.
9. Tann, C. H.; Brodfuehrer, P. R.; Brundidge, S. P.; Sapino, Jr., C.; Howell, H. G. *J. Org. Chem.* **1985**, *50*, 3644-3647.
10. Matsuda, A.; Yasuoka, J.; Sasaki, T.; Ueda, T. *J. Med. Chem.* **1991**, *34*, 999-1002.