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Synthesis and Biological Activity of Fluorinated Intermediates of the Methionine Salvage Pathway

Michael E. Houston Jr.,[a] David L. Vander Jagt,[b] and John F. Honek[a*]

[a]Department of Chemistry, (GWC)[2], University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

[b]Department of Biochemistry, University of New Mexico, School of Medicine, Albuquerque, New Mexico USA 87131

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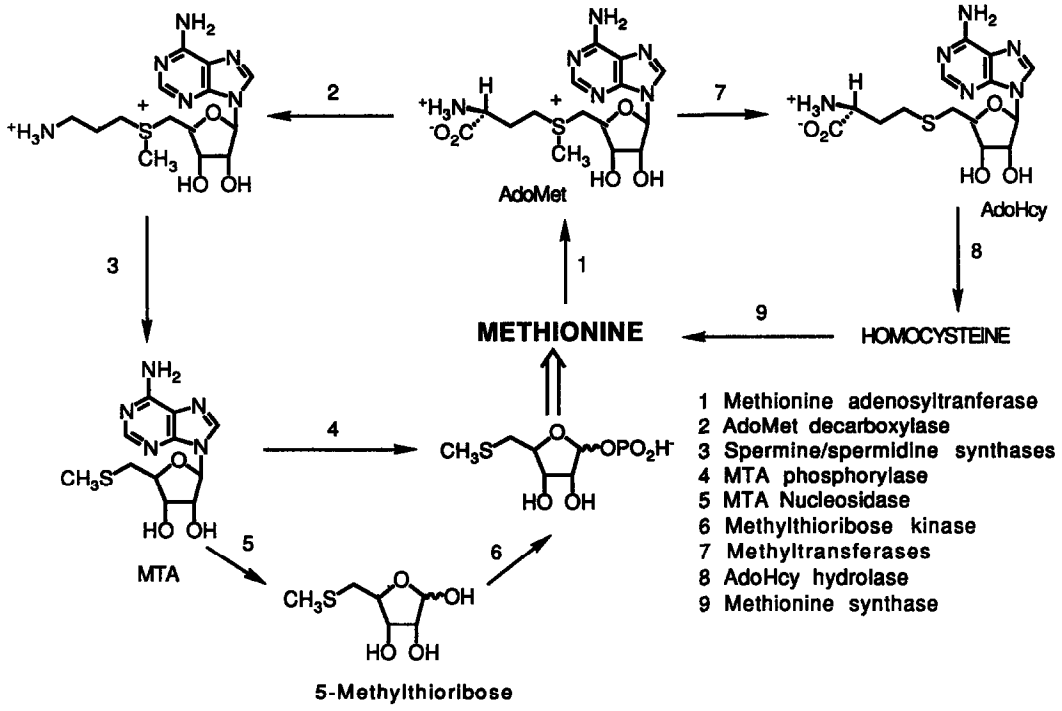
Abstract: Fluorinated analogs of several of the intermediates of the methionine salvage pathway were synthesized and tested as inhibitors of the malarial parasite *Plasmodium falciparum* (FCB1, clone NC-1). Several compounds exhibit good activity against this chloroquine resistant strain. In addition, several of the analogs show antitumor activity.

The world wide incidence of malaria has been estimated to be 110 million clinical cases annually, with approximately 270 million humans infected[1] and over one million malaria related deaths occurring each year.[2] The development of *Plasmodium falciparum* strains resistant to chloroquine or to other antimalarial agents such as the dihydrofolate reductase inhibitor pyrimethamine has underscored the importance for the development of new agents against this protozoa.[3]

The amino acid methionine's role in essential methylation reactions[4] and polyamine biosynthesis[5] is crucial to cellular viability. Methionine is the direct precursor to S-adenosylmethionine (AdoMet) which is utilized in various cellular methylation and polyamine biosynthetic reactions (Figure 1). Salvage pathways exist for recycling S-adenosylhomocysteine (AdoHcy)[6], a feedback inhibitor of various methylases, and 5'-deoxy-5'-methylthioadenosine[7] (MTA) back into methionine. Depending on the organism methionine can be salvaged from MTA by two different pathways (Figure 1). Certain pathogenic protozoa and bacteria (*P. falciparum*, *Klebsiella pneumoniae*) utilize a nucleosidase which converts MTA to 5-methylthioribose which is further transformed into 5-methylthioribose-1-phosphate by a specific kinase. However in mammals 5-methylthioribose-1-phosphate is directly produced from MTA by MTA phosphorylase. The completion of the pathway involves the multi-step conversion of 5-methylthioribose-1-phosphate into methionine and these individual steps have been investigated in some detail.[8] The absence of the MTA nucleosidase and kinase in mammals has very recently been regarded as a possible difference between mammals and some protozoa worthy of drug development.[9]

Because of the unique steric and electronic properties of fluorine[10], our interest in the design of inhibitors of the above pathways currently involves the incorporation of fluorine into methionine and salvage pathway intermediates.[11] Therefore the corresponding fluorinated analogs at the methionine, nucleoside and ribose levels were synthesized and tested against a chloroquine resistant strain of *P. falciparum* (strain FCB1). We now wish to report our most recent results in this area including the good antimalarial and antitumor activity of several fluorinated analogs. Our approach was given added impetus by the recent reports by Riscoe and co-workers of the inhibitory activity of 5-trifluoromethylthioribose against a pyrimethamine resistant strain of *P. falciparum*[12] as well as *K. pneumoniae*.[13]

Figure 1. Methionine Salvage Pathways

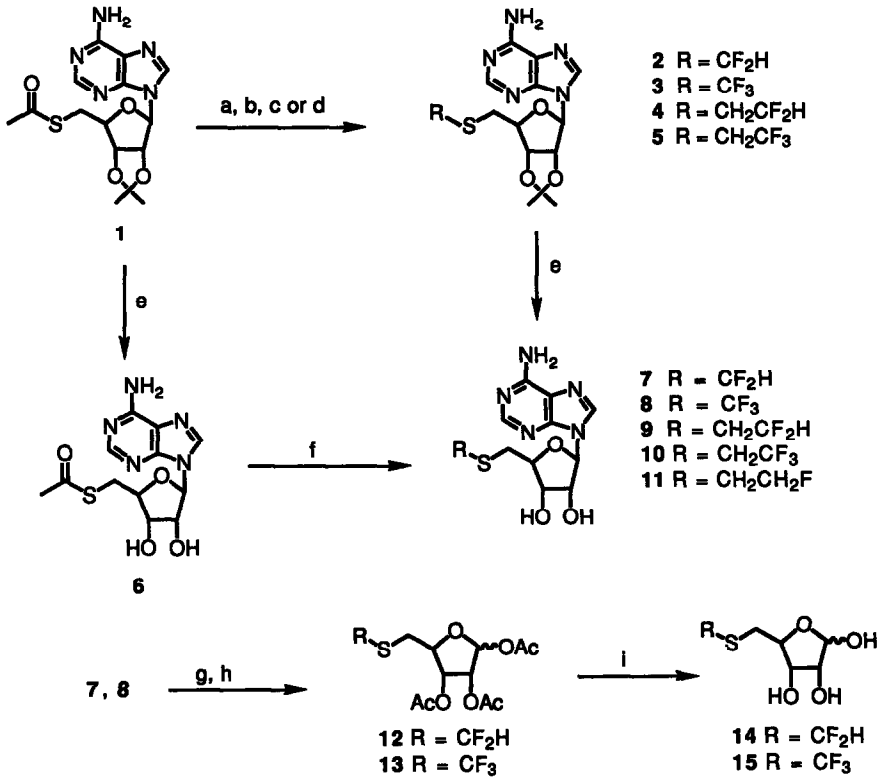


Our general synthetic approach to these compounds is applicable to both ribose and nucleoside analogs, several of which have been previously reported although the biological activity of these compounds have not been fully explored. The nucleoside analogs were prepared in the following manner (Scheme 1). Tosylation of 2', 3'-O-isopropylidene adenosine[14] followed by reaction with potassium thioacetate[15] gave rise to the thioacetate **1** Treatment of **1** with 4.5 equivalents of KOH in methanol/water 4:1 followed by alkylation with chlorodifluoromethane at 60 °C gave the corresponding 5'-difluoromethylthionucleoside **2** in 71 % yield.[16,17] The 5'-trifluoromethylthionucleoside **3** was prepared in 82 % yield by the reaction of **1** with iodotrifluoromethane in liquid ammonia under ultraviolet irradiation A complete lack of reactivity of the adenine base under these fluoroalkylation reactions was observed. The fluorinated ethylthio-nucleosides were prepared by alkylation of thioacetate **1** with the corresponding fluorinated ethyl triflates in greater than 60 % yield [18] Removal of the isopropylidene protecting groups with 0.1 N HCl in dioxane resulted in the quantitative production of the desired 5'-difluoromethylthioadenosine (**7**), 5'-trifluoromethylthioadenosine (**8**), 5'-difluoroethyl-thioadenosine (**9**) and 5'-trifluoroethylthioadenosine (**10**). Due to the decreased stability of the monofluoroethylthio-nucleoside to deprotection steps, the desired compound **11** was prepared from the deprotected thioacetate **6**.[19]

Chemical depurination of compounds **7** and **8** was achieved utilizing a modified procedure of Robins and Robins [20] Acetylation with 10 equivalents of acetic anhydride in pyridine followed directly by acetolysis with glacial acetic acid at 110 °C gave rise to the protected ribose analogs **12** and **13** in 53 % and 55 % respectively. Deprotection with

catalytic sodium methoxide yielded 5-difluoromethylthioribose (14) and 5-trifluoromethylthioribose (15).[21] We have previously reported the synthesis of difluoromethylhomocysteine (16) and trifluoromethylhomocysteine (17).[11]

Scheme 1. Synthesis of Fluorinated Methylthioadenosine and Methylthioribose Analogs



Reagents: (a) 4.5 eq. KOH, CF_2HCl, 60 °C, MeOH/H_2O 4:1; (b) NH_3 (*l*), CF_3I, uv; (c) 2.1 eq. KOH, CF_2HCH_2OTf, MeOH; (d) 2.1 eq. KOH, CF_3CH_2OTf, MeOH; (e) 0.2 N HCl, dioxane/H_2O 1:1; (f) 2.1 eq. KOH, CFH_2CH_2OTf, MeOH; (g) 10 eq. Ac_2O, pyridine; (h) 3 eq. Ac_2O, HOAc, 110 °C; (i) cat. NaOMe, MeOH.

The biological data for the fluorinated analogs is presented in Table 1. Because of the requirement of *P. falciparum* for exogenous methionine,[22] it was felt that fluorinated analogs of methionine may be transported into the malarial cell and cause cell death. However this was not observed with fluorinated analogs under the assay conditions utilized in this study. In addition, although trifluoromethylthioribose has been shown to inhibit the pyrimethamine resistant strain of *P. falciparum* (IC_{50} = 50 μM)[13], it is interestingly less active against the chloroquine resistant strain (IC_{50} > 100μM). A similar lack of activity of the difluoromethylthioribose analog against this strain of *P. falciparum* was also observed (IC_{50} > 100μM). The known transport and metabolism of MTA into human erythrocytes[23] and nucleosides into *P. falciparum* [22,24] indicated that fluorinated analogs of MTA could be cytotoxic to this protozoa. Indeed, the corresponding MTA

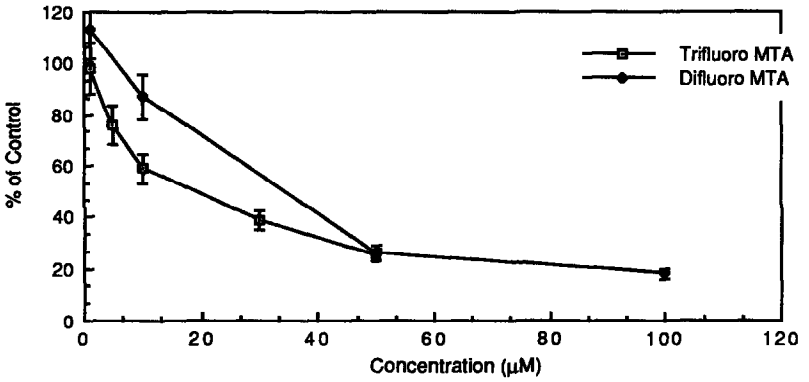
nucleoside analogs **7** and **8** are quite active against this strain (Figure 2). The fluorinated ethyl nucleosides were also found to be active (Table 1). Nucleoside transport may well be more efficient than transport of the ribose and methionine analogs and could account for the greater effectiveness of the nucleosides.

Table 1. Growth Inhibition of *P. falciparum* (FCB1)[25]

COMPOUND	IC_{50} (μM)*
L-Difluoromethylhomocysteine (**16**)	> 100
L-Trifluoromethylhomocysteine (**17**)	> 100
5-Difluoromethylthioribose (**14**)	> 100
5-Trifluoromethylthioribose (**15**)	>100
5'-Difluoromethylthioadenosine (**7**)	35
5'-Trifluoromethylthioadenosine (**8**)	18
5'-Monofluoroethylthioadenosine (**11**)	43
5'-Difluoroethylthioadenosine (**9**)	25
5'-Trifluoroethylthioadenosine (**10**)	85

* Triplicate determinations. Values are +/- 10 % or less

Figure 2. Growth Inhibition of *P. falciparum* *



* Triplicate values +/- S. D.

In addition, the biological activities of several of these analogs against various cancer cell lines[26] and HIV[27] were investigated. Difluoromethylhomocysteine (**16**) showed activity against small cell lung cancer DMS114 (GI_{50} = 65 μM, concentration of compound inhibiting cell growth by 50 %) and COLO 205 colon cancer (GI_{50} = 92 μM) Fluorinated nucleosides **7**, **8** and **10** showed activity against non small cell lung cancer NCI-H522 with the difluoromethyl- and the trifluoroethylthionucleosides being more active (GI_{50} = 86 μM and 45 μM respectively). These analogs showed no toxicity to other cell lines at the dose levels tested. Analogs **7**, **8** and **10** were inactive against HIV infected T4 lymphocytes but showed toxicity (IC_{50}) to T4 lymphocytes of 222 μM , 117 μM and 245 μM respectively

The novel nucleoside **8** shows the best inhibitory activity against *P. falciparum* (FCB1) of all fluorinated salvage pathway intermediates prepared to date. Investigations to determine the exact mechanism of action of these nucleosides is currently in progress; however, the biological data reported herein appears to indicate that the nucleosides may not be active due to their conversion into either fluorinated ribose or methionine level intermediates as both these analog types are devoid of activity. A more detailed investigation of the site(s) of action of these agents appears warranted.[28]

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19. Spectral data for nucleoside analogs: (**7**): ^{1}H NMR (CD_3OD, 250 MHz): 8.19 (s, 1H, purine), 8.15 (s, 1H, purine), 6.99 (t, 1H, CF_2H, J_{HF} = 56.9 Hz), 5.93 (d, 1H, H-1'), 4.84 (m, 1H, H-2'), 4.29 (m, 1H, H-3'), 4.19 (dt, 1H, H-4'), 3.21 (m, 2H, CH_2S); ^{13}C NMR (DMSO): 155.9, 152.5, 149.5, 140.4, 122.0 (t, J_{CF} = 269.8 Hz), 119.1, 87.9, 83.7, 72.7, 72.5, 30.0. (**8**): ^{1}H NMR (CD_3OD): 8.19 (s, 1H, purine), 8.16 (s, 1H, purine), 5.94 (d, 1H, H-1'), 4.84 (m, 1H, H-2'), 4.33 (m, 1H, H-3'), 4.19 (m, 1H, H-4'), 3.33 (m, 2H, CH_2S); ^{13}C NMR (CD_3OD): 151.5, 149.7,

145.8, 145.1, 132.3 (q, J_{CF} = 305.1 Hz), 120 5, 90.8, 84.4, 75.0, 73.9, 33.1. (**9**): ^{1}H NMR (CD_3OD): 8.18 (s, 1H, purine), 8.09 (s, 1H, purine), 5.92 (d, 1H, H-1'), 5.86 (tt, 1H, $\underline{CF_2H}CH_2$, J_{HF} = 56.5 Hz), 4.73 (t, 1H, H-2'), 4.27 (t, 1H, H-3'), 4.20 (m, 1H, H-4'), 2.93 (m, 2H, CH_2S) , 2.81 (dt, 2H, $CF_2H\underline{CH_2}$, J_{HF} = 16.7 Hz) ; ^{13}C NMR (CD_3OD): 157.3, 153.9, 150.6, 141.5, 121.1, 117.9 (t, J_{CF} = 240.9 Hz), 90.3, 85.9, 74.7, 74.0, 36.1 (t, J_{CF} = 24.0 Hz), 35.9 (**10**)· ^{1}H NMR (DMSO): 8 22 (s,1H, purine), 8.15 (s, 1H, purine), 5.93 (d, 1H, H-1'), 4.73 (m, 1H, H-2'), 4.36 (m, 1H, H-3'), 4.16 (m, 1H, H-4'), 2.95 (m, 4H, $CF_3\underline{CH_2}$, CH_2S); ^{13}C NMR (DMSO): 156.0, 152.5, 149.4, 140.2, 126.5 (q, J_{CF} = 276.0 Hz), 120.5, 90.7, 87.9, 83.8, 72.6, 35.1, 33.4 (q, J_{CF} = 31.4 Hz). (**11**): ^{1}H NMR (CD_3OD): 8.23 (s, 1H, purine), 8.13 (s, 1H, purine), 5.92 (d, 1H, H-1'), 4 71 (t, 1H, H- 2'), 4 42 (dt, 2H, J_{HF} = 47.4 Hz), 4.25, (t, 1H, H-3'), 4.14 (m, 1H, H-4'), 2.93 (m, 2H, CH_2S), 2.75 (dt, 2H, $CFCH_2\underline{CH_2}$, J_{HF} = 21 0 Hz). ^{13}C NMR (DMSO): 156.1, 152.6, 149.5,139.8, 119.2, 85.7 (d, J_{CF} = 217.8 Hz), 82.1, 81.3, 72 5, 67 5, 34.2, 31.7 (d, J_{CF} = 20.7 Hz). In addition all compounds had mass spectra and infrared characteristics in complete agreement with the assigned structures.

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21 Spectral data for ribose analogs. (**14**): ^{1}H NMR (CD_3OD): 7.14 (t, 1H, CF_2H α, J_{HF} = 57.3 Hz), 7.07 (t, 1H, CF_2H β, J_{HF} = 57.3 Hz), 5.21 (d, 1H, H-1α), 5.08 (d, 1H, H-1β), 4.14-3.79 (m, 3H, H-2, H-3, H4), 3.15-2.86 (m, 2H, CH_2S); ^{13}C NMR (DMSO): 122.9 (t, CF_2H α, J_{CF} = 269.0), 122.8 (t, CF_2H β, J_{CF} = 269.0 Hz),103.3 (C-1β), 98.1 (C-1α), 83.0 (C-4α), 82.6 (C-4β), 77.2 (C-2β), 74.9 (C-3β), 74.2 (C-3α), 72 3 (C- 2α), 32.7 (C-5β), 31 2 (C-5α). (**15**): ^{1}H NMR (CD_3OD): 5.20 (d, 1H, H-1α), 5.06 (d, 1H, H-1β), 4.11-3.79 (m, 3H, H-2, H-3, H-4), 3.36 (m, 2H, CH_2S); ^{13}C NMR (CD_3OD): 132 7 (q, CF_3 α, J_{CF} = 304.2 Hz),132.6 (q, CF_3 β, J_{CF} = 304.2 Hz), 103.4 (C-1β), 98.1 (C-1α), 81.4 (C-4α), 81.2 (C-4β), 77.1(C-2β), 75.1 (C-3β), 74.3 (C-3α), 72.2 (C-2α), 32.7 (C-5β), 31.2 (C-5α). ^{13} C assignments based on the assignments for α and β ribofuranoside obtained from; Bock, K. and Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27. In addition all compounds had mass spectra and infrared characteristics in complete agreement with the assigned structures

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28 Recent work has shown that both monofluoromethylthioadenosine[29] and difluoromethylthioadenosine[30] (prepared[31] from D-ribose) inhibit MTA phosphorylase from mouse and rat liver respectively. However the effects of these analogs on *P. falciparum* MTA nucleosidase and erythrocyte MTA phosphorylase have yet to be investigated. Sufrin and co-workers[32] have just recently reported the preliminary antitumor activity of **11** against MTA phosphorylase containing and deficient cell lines.

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