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SYNTHESIS AND ACTIVITY OF A TETRAHYDROFOLATE INHIBITOR OF THE ENZYME N^{10}-FORMYL-H_4-FOLATE-MET-tRNA^{fMet} TRANSFORMYLASE

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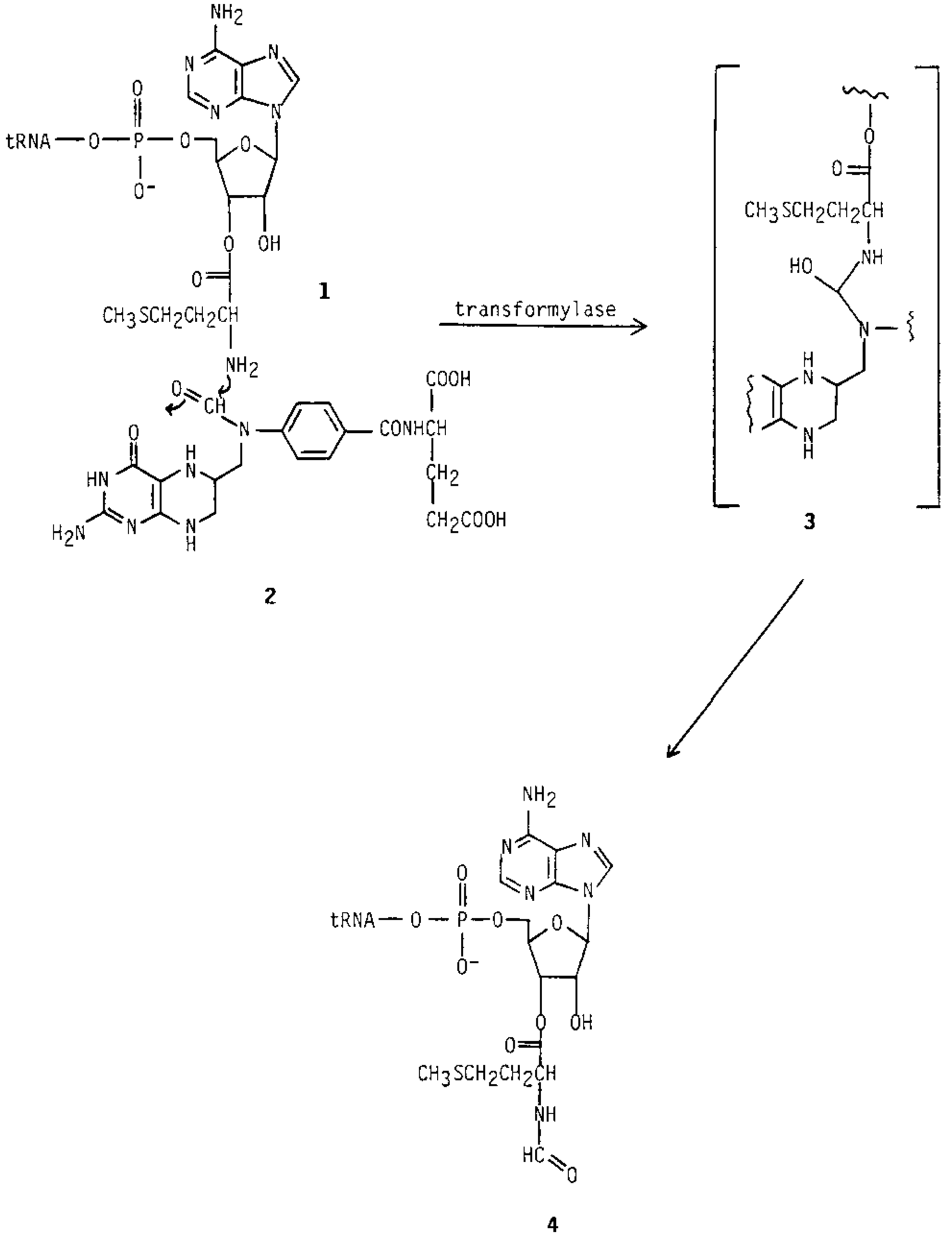
Abstract - The N-formylmethionine-tRNA^{fMet} is involved in the initiation of protein biosynthesis in procaryotic organisms and is formed via the transfer of a formyl group from N^{10}-formyltetrahydrofolic acid to the amino group of methionine in Met-tRNA^{fMet}. Based on a proposed intermediate for this formylation step, a transition state analog was designed and synthesized and was shown *in vitro* to be an excellent inhibitor of the transformylase reaction.

The enzyme Met-tRNA^{fMet} transformylase from *E. coli* has been described[1] and shown to catalyze the transfer of a formyl group[2] from N^{10}-formyltetrahydrofolic acid (**2**) to the free amino group of methionine in Met-tRNA^{fMet} (**1**), (Scheme I). The resulting N-formylmethionine-tRNA^{fMet} (**4**) is involved in the initiation of protein biosynthesis in procaryotic organisms.[3] The significance of this transformylation process for bacterial gene expression makes the inhibition of this process an interesting target for the potential development of antibacterial agents.

The transfer of the formyl group from **2** to **1** is unique for bacterial protein synthesis but it should be recognized that both **1** and **2** have functions in mammalian biochemical transformations[4] although the transformylase enzyme is lacking from eukaryotic cell extracts.[5] Therefore to reduce the possibility of toxicity, any analog designed to inhibit this process should closely resemble the transition state involved in the formyl transfer reaction catalyzed by the transformylase. Although a stable fMet-tRNA^{fMet} enzyme has been isolated, there is no evidence of a formyl transfer to the enzyme yielding an acid stable formylated enzyme.[6] Our results suggest that a formyl transfer may in fact take place directly from compound **2** to **1** implying the intermediacy of a structure

Scheme I



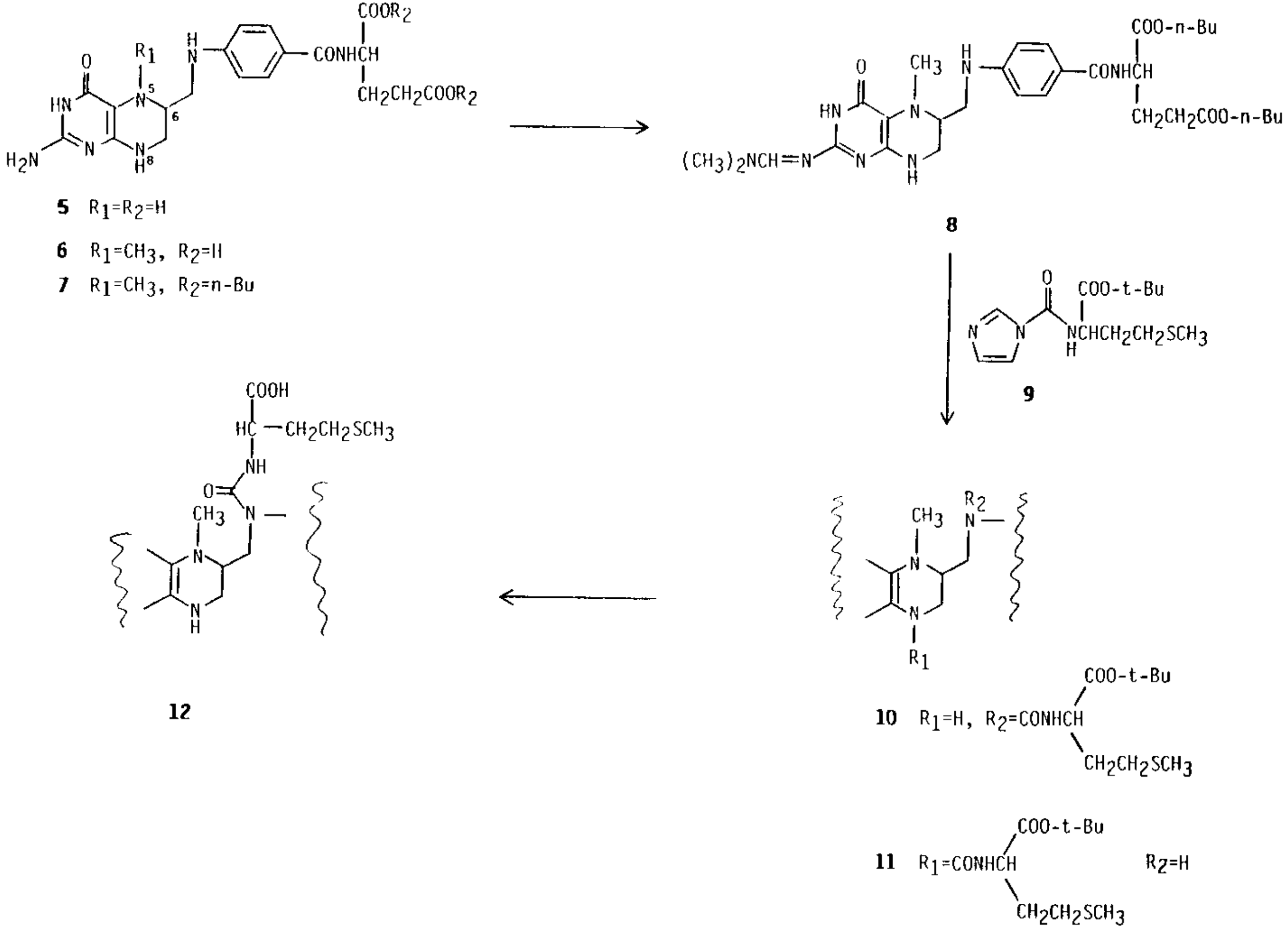
such as **3**[7] in the bacterial system. With this in mind, we envisaged a compound such as **15** as a possible stable transition state analog, which, although lacking a tetrahedral equivalent to the α-amino alcohol of intermediate **3**, might still function as an inhibitor of this one carbon transfer reaction.

The preparation of several *N*^{5}- and *N*^{5},*N*^{10}-disubstituted tetrahydrofolic acid analogs have been described,[8] whereas only one unstable *N*^{10}-substituted analog has been reported.[9] Because tetrahydrofolic acid **5**[10] is known to undergo rapid air oxidation,[11] we decided to choose the more stable *N*^{5}-methyltetrahydrofolic acid (**6**)[12] (racemic at C6) as our starting material for the preparation of the target intermediate molecule **12** (Scheme II). Because the *N*^{5}-methyl-*dl*-*L*-H_4-folate has previously been shown to be an effective inhibitor of the transformylation reaction (although noncompetitive with the formyl donor),[13] it was anticipated that substitution at *N*^{5} with a methyl group would not interfere with the inhibition properties of the target compound **15**. Due to the insolubility of **6**[14] the acid was first converted to its di-*n*-butyl ester **7** (1.5N HCl-^{n}BuOH, 80°C, 16 h) in 29% overall yield from folic acid. Treatment of **7** with *N*,*N*-dimethylormamide dimethylacetal[14] (DMF, 25°C, 4 h, argon) proceeded smoothly to yield the more soluble derivative **8**[15] in 83% yield (mp 104-108°C) after florisil column chromatography.

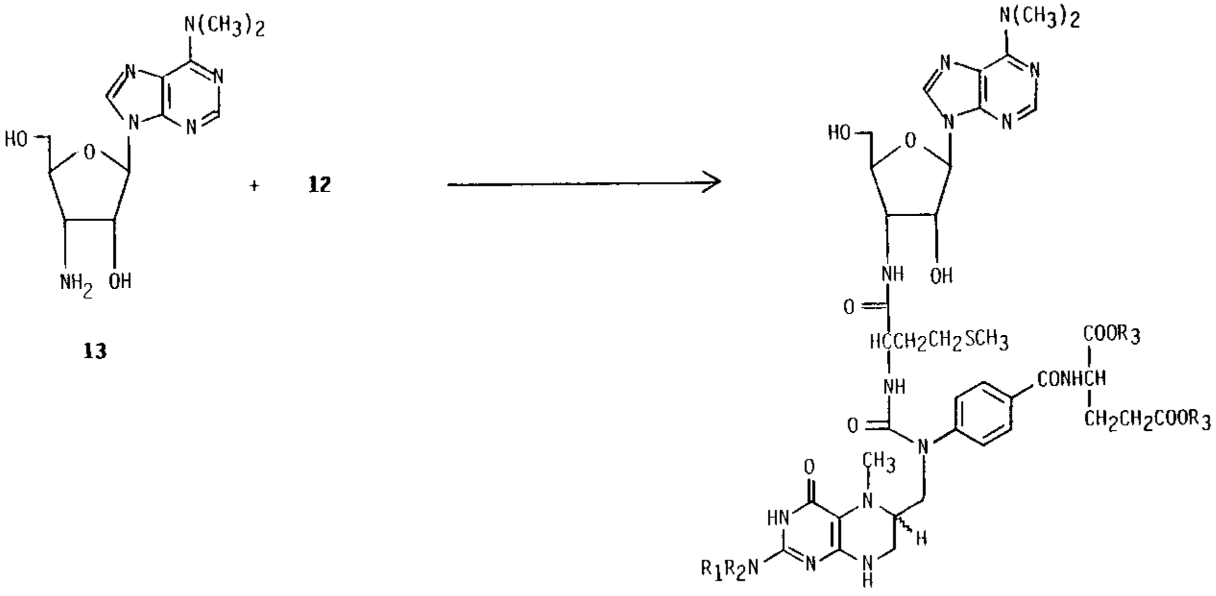
Treatment of the *N*^{5}-methyltetrahydrofolate **8** with the methionyl synthon **9**[16] (3 eq., DME, 85°C, 18 h, argon) produced the *N*^{10}-methionyl derivative **10** (64% yield) and a minor amount of the *N*^{8}-methionyl analog **11**[15] (11%) (separated by silica gel column chromatography). The *t*-butyl ester of compound **10** was then selectively cleaved (CF_3COOH, 25°C, 1 h) to produce the desired carboxylic acid derivative **12** (90% yield, as mono TFA salt). The final phase of the synthesis was accomplished (Scheme III) by coupling the acid **12** with the readily available puromycin aminonucleoside[17] **13** in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline[18] (EEDQ) (DMF, 40°C, 16 h, argon). Thus the tetrahydrofolate transition state analog[15] **14** was obtained in 32% yield after purification by silica gel column chromatography. It has been demonstrated that the antibiotic puromycin is capable of inhibiting protein synthesis due to the fact that it can successfully compete with the aminoacyl-tRNA for the ribosome.[19] The puromycin aminonucleoside **13** was, for this reason, considered an appropriate substitute for adenosine and would allow for the selective acylation of the C3 amino group necessary for the construction of the potential transformylase inhibitor **15**.

The water soluble trisodium salt **15** was prepared from the ester **14**, (6 eq. 0.35N aqueous NaOH in 1:1 DME/H_2O, 25°C, 20 h) purified via silica gel chromatography (eluent 3:1 propanol/ water) and lyophilized to give a white solid homogeneous by tlc (cellulose, 1:2 1% aqueous sodium bicarbonate/ propanol). The structure of **15** rests on the method of synthesis, the ^{1}Hnmr (in D_2O/CD_3OH) δ 2.07 (s, 3H, SCH_3), 2.53 (s, 3H, NCH_3), 3.44 [s, $N(CH_3)_2$], 3.2-3.7 [m, NCH; NCH_2, overlapping $N(CH_3)_2$]

Scheme II


5 R1=R2=H
6 R1=CH3, R2=H
7 R1=CH3, R2=n-Bu
8
9
10 R1=H, R2=CONHCH(COO-t-Bu)CH2CH2SCH3
11 R1=CONHCH(COO-t-Bu)CH2CH2SCH3 R2=H
12

Scheme III



14 $R_1+R_2=(CH_3)_2NCH=$, R_3=n-Bu

15 $R_1=R_2=H$, $R_3=Na$

3.78, 4.02 (br., AB, J gem=12 Hz, CH_2OH) 4.44 (m, 2H), 4.65-4.7 (m) 6.11 (br, s, 1H, NCHOC), 7.49-7.92 (AA'BB', 4H), 8.18 (s, 1H), 8.34 (s, 1H), the uv_{max} (H_2O) 215 nm (sh ε 42,300), 275 (30, 100) and amino acid analysis: Found Met (1.0), Glu(1.1).

The fact that **15** can serve as an inhibitor of the transformylase was demonstrated in vitro. In these experiments transformylase activity was assayed essentially as described by Dickerman et al.[1b] Compound **15** was found to be an excellent inhibitor (~70% inhibition of transformylase activity at a concentration of 10^{-6} M) while the reference compound Ca leucovorin had an inhibitory activity of ~50% at 1×10^{-4} M. More detailed enzymatic studies will be required to determine the mode of inhibition by compound **15** and whether it is truly functioning as a transition state inhibitor.

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15. All new compounds were homogeneous by tlc and gave satisfactory ^{1}H nmr, uv, and ir data.

16. N-Imidazocarbonylmethinonine t-butyl ester (**9**) was prepared from L-methionine t-butyl ester hydrochloride and N,N'-carbonyldiimidazole (Et_3N/CH_2Cl_2, 25°C, 2 h, argon), ^{1}H nmr ($CDCl_3$) 1.48 (s, 9H), 2.09 (s SCH_3), 2.15 (m, CH_2), 2.59 (t 2H), 4.64 (m, 1H), 7.51 (br, s, 1H), 7.46 (br s,1H), 7.67 (m, NH), 8.20 (br s, 1H).

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