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# SYNTHESIS OF 10-THIOAMINOPTERIN: A POTENT ANTI-BACTERIAL AGENT[1,2]

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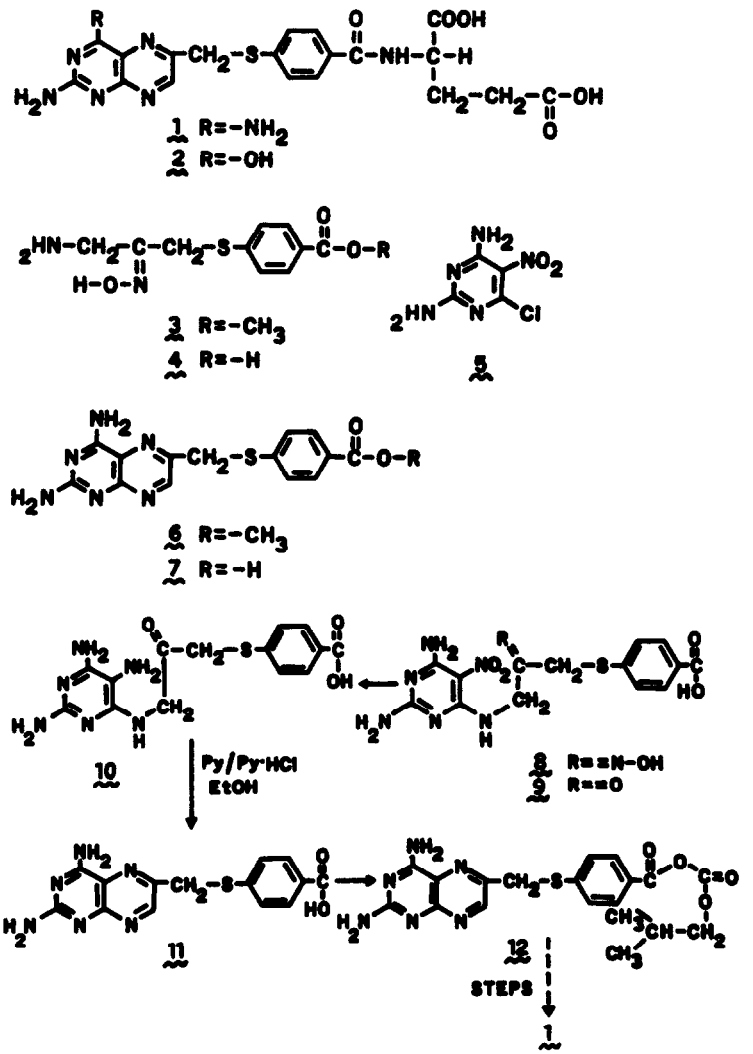
A number of folate analogs, which are altered in the $C^9$-$N^{10}$ bridge region of the vitamin folic acid, and their antifolate activities have been the subject of recent investigations from our laboratory.[3-6] We now wish to report the synthesis and antifolate properties of 10-thioaminopterin 1, which is the 4-amino-4-deoxy analog of 10-thiofolic acid[4], or the 10-thio analog of aminopterin.[7] Commercially available N-(2,3-epoxy)-propyl phthalimide, and p-carbomethoxy thiophenol prepared according to the procedure of Wiley[8] has been used as the starting material for the preparation[4] of the amino acetonyl oxime 3. Hydrolysis of this material with 1 N methanolic sodium hydroxide at room temperature resulted in the formation of the amino acid 4. The preparation of 6-chloro-2,4-diamino-5-nitropyrimidine 5 has been carried out according to published procedures.[4,9]

Reaction of 4 with an alcoholic solution of 5 under reflux conditions, in the presence of one equivalent of N-methyl morpholine as a proton acceptor, gave the necessary intermediate 8 in excellent yield after workup. The reductive cyclization using catalytic hydrogenations, a procedure that has been successfully employed by Montgomery and co-workers[10,11] for the preparation of several folate analogs, could not be applied to 8 due to the presence of sulfur. An alternate cyclization procedure was subsequently developed. This involved the deprotection of the carbonyl function of 8 using a 1:1 mixture of trifluoroacetic acid and 1 N HCl at 60° for 20 minutes. The intermediate 9 thus obtained in ~90% yield was dissolved in aqueous DMF and reduced with sodium dithionite under carefully controlled conditions. The progress of the reduction was monitored by the disappearance of the ultraviolet absorption due to the 5-nitro group of the pyrimidine ring and was complete in 15 minutes at 40°. The anticipated, spontaneous cyclization of 10 to 2,4-diamino-4-deoxy 7,8-dihydro-10-thiopteroic acid did not take place under these conditions. The reduction product 10 degraded rapidly on exposure to air, and on attempted oxidations with potassium permanganate or ferri cyanide. Therefore, this compound was subjected to a new cyclization oxidation technique using a pyridine, pyridine hydrochloride buffer system (pH ~5) in ethanol. The procedures involved initial cyclization of 10 to the desired 7,8-dihydropteroic acid analog by refluxing the compound in a nitrogen atmosphere in the buffer for 2 hours. The ultraviolet absorption spectrum of the cyclized material showed absorption peaks in 0.1 N NaOH at 334 and 280 nm in a 1:2 ratio, which is typical of a 7,8-dihydropteridine. On stirring this reaction mixture under aerobic conditions for 48 hours, spontaneous oxidation of the dihydro


1 R=-NH2
2 R=-OH
3 R=-CH3
4 R=-H
5
6 R=-CH3
7 R=-H
8 R=N-OH
9 R=O
10
Py/Py·HCl
EtOH
11
12
STEPS
1

derivative to the pteroate analog 11 took place in very good yield. Purification of 11 was accomplished by ion exchange chromatography on columns of DEAE cellulose in the chloride form using a linear sodium chloride gradient from 0 to 0.5 M in 0.005 M phosphate buffer at pH 7.0 as eluate. All the tubes with spectral properties corresponding to 11 were pooled and concentrated in vacuum. On acidification of the concentrate with glacial acetic acid to pH 4.5, a bright yellow precipitate formed which was collected by filtration and dried in vacuum over $P_2O_5$. 4-Amino-4-deoxy-10-thiopteroic acid 11 showed Nmr signals (TFA) at δ ppm 9.09 (S, 1H, $C_7H$); 8.13 (d, 2H, $H_{2'6'}$, J = 9 Hz); 7.55 (d, 2H, $H_{3'5'}$, J = 9) and 4.65 (brs, $C_9$-methylene) in complete agreement with the desired structure.

Condensation of 3 with 5 in a similar manner gave the analogous intermediate 8 having a carbomethoxy group at the 4' position. Deprotection of the carbonyl function, dithionite reduction, cyclization and subsequent oxidation of this material as described for 8 gave 4-amino-4-deoxy-4'-carbomethoxy-10-thiopteroic acid 6 in good yield. However, under conditions which were required for the hydrolysis of the carbomethoxy function to the free acid, compound 6 underwent deamination at position 4 to yield 10-thiopteroic acid.[6]

The carboxyl group of 11 was activated as the mixed anhydride by reaction with one equivalent of isobutyl chloroformate at 0° in a 1:1 mixture of DMSO:THF, using 1.25 equivalents of N-methylmorpholine as a proton acceptor. t-Boc-L-glutamic acid α-benzylester was esterified to the Merrifield chloromethyl resin, through the γ-carboxyl group, and the amino group was subsequently deprotected with 20% TFA in $CH_2Cl_2$. The mixed anhydride 12 was then allowed to couple with the free amino group of the glutamate moeity on the resin and the resin-bound glutamate conjugate of 11 was hydrolyzed off with the simultaneous deprotection of the α-carboxyl group by procedures described previously.[12] The crude, cleaved product 1 thus obtained in ~85% yield based on 11 was purified by ion exchange chromatography as described for 11. The U.V. spectrum of 1 was very similar to that of aminopterin, and showed λmax at 377 (7,814); 285 (17,868) and 262 (30,116) in 0.1 N NaOH and as 341 (10,648), 283 (17,823) and 248 nm (24,723) in 0.1 N HCl respectively. The Nmr spectrum of 1 in 0.1 N NaOD/$D_2O$ using SDSS as an internal standard showed relevant signals at 8.65 S ($H_7$) 7.75 d (J = 9, $H_{2'6'}$) 7.38 d (J = 9, $H_{3'5'}$) 4.48 brs ($C_9$-methylene) and between 3.2 and 1.5 PPm C (glutamic acid), similar to those of folic acid, substantiating the correctness of the structure.

Compounds 11 and 1 were tested for their ability to inhibit the growth of two standard folic acid requiring bacteria: *Streptococcus faecium* (ATCC 8043) and *Lactobacillus casei* (ATCC 7469). For *S. faecium*, the concentration of 11 and 1 required for the 50% inhibition of growth were $3 \times 10^{-9}$ and $4 \times 10^{-10}$ g/ml and for *L. casei* the corresponding levels were $1 \times 10^{-8}$ and $1 \times 10^{-11}$ g/ml respectively. 10-Thioaminopterin was studied *in vitro* as an inhibitor of *L. casei*[13] dihydrofolate reductase. Using dihydrofolate as substrate at a concentration of

$10.2 \times 10^{-8}$ M, 1 produced 50% inhibition of the enzymatic reaction at a concentration of $4.5 \times 10^{-9}$ M. Under these standard assay conditions, 1 was equally as potent as methotrexate as inhibitor of this enzyme.

Acknowledgements

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References

1. This work was presented as part of "New Folate Analogs: Alterations in the $C^9$-$N^{10}$ Bridge Region" at the Vth International Symposium on Pteridines in Konstanz, W. Germany on April 16, 1975.
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   10-Thioaminopterin = N-[p-[[(2,4-diamino-4-deoxy-6-pteridinyl)-methyl]thio] benzoyl]-glutamic acid. Other abbreviations include: DHFR, dihydrofolate reductase; DEAE, diethylaminoethyl; t-Boc, tert-butyloxycarbonyl. All the compounds described here gave elemental analyses within acceptable limits.
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