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SYNTHESIS OF AN 8-DEAZA ANALOG OF THE INTERMEDIATE IN THE THYMIDYLATE SYNTHETASE REACTION

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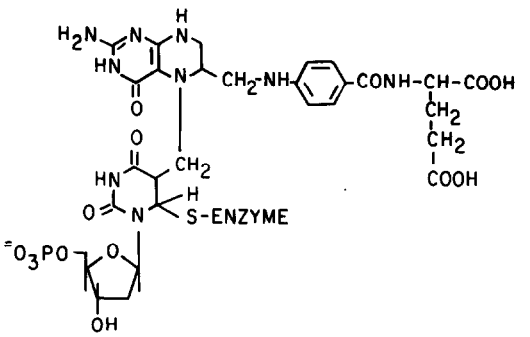
Abstract: Synthesis of an 8-deaza analog of the proposed intermediate in the thymidylate synthetase reaction was accomplished from diethyl 8-deazafolic acid and an unambiguous proof of the structure is provided.

Thymidylate synthetase catalyzes the conversion of 2'-deoxyuridine-5'-monophosphate (dUMP) to thymidine-5'-monophosphate (TMP) in the presence of the cofactor N^5,N^{10}-CH_2-tetrahydrofolic acid.[1] Based on model studies with 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) structure 1 was proposed as the initial enzyme-cofactor-substrate complex followed by redox rearrangement to give TMP.[2] The precise mechanism of the formation of TMP and 7,8-dihydrofolic acid is uncertain. Analogs of 1 could function as multisubstrate inhibitors of thymidylate synthetase and prevent TMP synthesis.

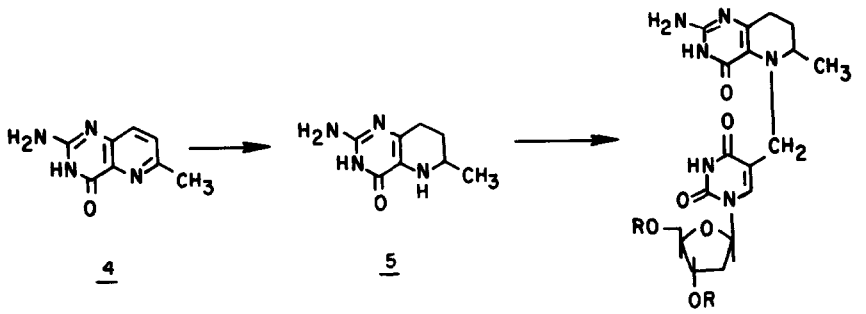


1

Compound 2, N-[4-[[(2-amino-3,4,5,6,7,8-hexahydro-4-oxo-5-(2'-deoxyuridin-5-yl)methyl-pyrido[3,2-d]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid can be considered as a multisubstrate analog of the proposed intermediate 1. Mertes and co-workers reported the synthesis and activity of 5-[(N-methylpiperazinyl)methyl]- and 5-[4-(1,2,3,4-tetrahydro-quinoxalyl)methyl]-2'-deoxyuridine-5'-phosphates as possible multisubstrate analog inhibitors of the enzyme.[3] These compounds lack the pyrimidine and p-aminobenzoyl glutamate moieties and showed only modest inhibition of thymidylate synthetase.

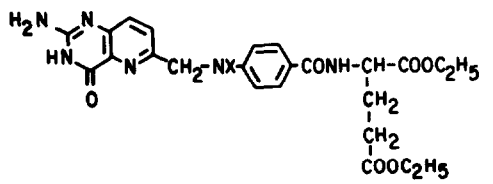
Prior to the synthesis of 2, the model compound 3 was prepared to test the feasibility of alkylation of a reduced pyridopyrimidine with 3',5'-di-O-acetyl-5-bromomethyl-2'-deoxyuridine.[4] Alkaline hydrolysis of 2,4-diamino-6-methyl-pyrido[3,2-d]pyrimidine[5] gave 2-amino-6-methyl-4-oxopyrido[3,2-d]pyrimidine 4.[6,7] Reduction of 4 in 0.1 N HCl over PtO_2 gave a quantitative yield

of 2-amino-6-methyl-4-oxo-5,6,7,8-tetrahydropyrido[3,2-d]pyrimidine 5.[6,7] Alkylation of 5 with 3',5'-di-O-acetyl-5-bromomethyl-2'-deoxyuridine in anhydrous dimethyl formamide gave the protected nucleoside 6 in 63% yield, which on treatment with ethanolic ammonia gave 3.

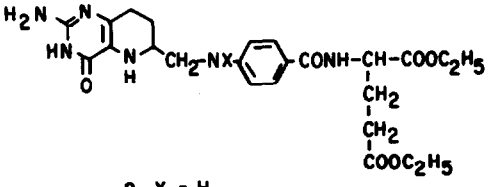


4 5 6; R = $COCH_3$ 3; R = H

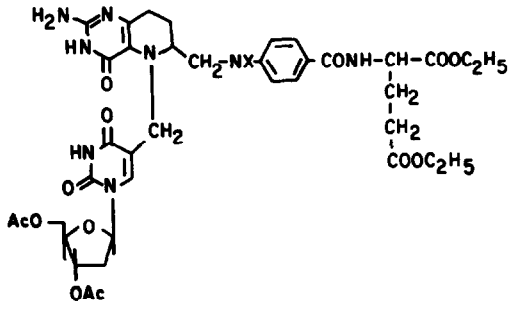
The requisite pyridopyrimidine derivative for the synthesis of 2, diethyl 8-deazafolate 7 was prepared from 2-amino-6-hydroxymethyl-4-oxopyrido[3,2-d]pyrimidine according to the procedure reported earlier.[8] Reduction of 7 in ethanol containing an equimolar amount of 0.1 N HCl over Adams catalyst gave diethyl 5,6,7,8-tetrahydro-8-deazafolate 8 in 35-40% yield.[9] The other products of reduction were 2-amino-6-methyl-4-oxo-5,6,7,8-tetrahydropyrido[3,2-d]pyrimidine 5 and diethyl p-aminobenzoyl-L-glutamate. Two other groups [7,10] reported the preparation of 8 (in the acid form) but in both instances no spectral properties were reported nor was the compound



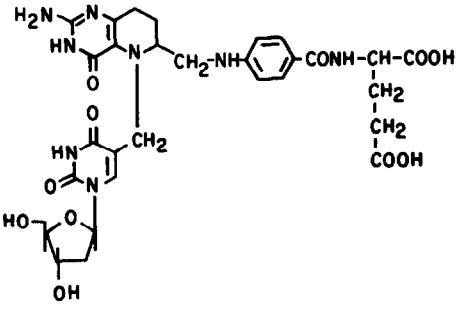
7 X = H
10 X = NO



8 X = H
11 X = NO



9 X = H
12 X = NO



2

isolated in a pure state. After chromatographic purification, compound 8 was alkylated with 3',5'-di-O-acetyl-5-bromomethyl-2'-deoxyuridine in anhydrous dimethyl formamide to give 9 in 61% yield, which on saponification gave 2.[11] The molecular weight of 684 was independently confirmed by fast atom bombardment (FAB) mass spectrometry, which showed MH^+ = 685.[12]

In order to prove that the alkylation had occurred at N^5 of 8 and not at N^{10}, the following set of compounds of compounds were prepared. Treatment of 7 in 6N HCl with $NaNO_2$ at 0-5°C, according to the procedure of Cosulich and Smith[13] gave diethyl N^{10}-nitroso-8-deazafolate 10 in 80% yield. Reduction of 10 under conditions similar to those described above gave diethyl N^{10}-nitroso-5,6,7,8-tetrahydro-8-deazafolate.[14] Unlike the case of 8, N^5 is the only site available for alkylation in compound 11. Alkylation of 11 with 3',5'-di-O-acetyl-5-bromomethyl-2'-deoxyuridine gave 12. Treatment of 9 with HNO_2 ($HCl+NaNO_2$) at 0-5°C gave 12, whose nmr spectrum was identical to the compound obtained from 11.[15] The fact that 9 can be converted 12 showed that the alkylation of 8 had occurred only at N^5 and not at N^{10}. All new compounds had satisfactory elemental analyses and spectral properties.

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5. V. Oakes, H. N. Rydon and K. Undheim, J. Chem. Soc, 4678 (1962).
6. Two different procedures for the preparation of 4 were recently reported in the following publication. J. L. Kelley and E. W. McLean, J. Heterocyclic Chem. 18, 671 (1981). One of the procedures is similar to the one reported by us in an earlier publication, see reference 8.
7. C. Temple, C. L. Kussner, D. L. Smithers, L. L. Bennett and J. A. Montgomery, J. Med. Chem. 24, 1254 (1981) reported the preparation of 4 by the ammonolysis of 2-chloro-6-methyl-4-oxopyrido[3,2-d]pyrimidine.
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9. Spectral data for compound 8: UV λ_{max} (pH 7) 300 nm (ε 27,000). ^1H NMR (270 MHz, DMSO-d_6)δ 8.24 (d, glu NH, 1, J=8.8 Hz), 7.68 and 6.63 (d each, 4, C_6H_4, J=8.6 Hz), 6.61 (t(concentration dependent), 1, 10-NH), 5.86 (br s, 2, 2-NH_2), 4.37 (m, 1, CH-$COOC_2H_5$), 4.05 (m, 4, CH_2-CH_3), 3.15 (m, 3, 9-CH_2 and 6-CH), 2.37 (m, 4, CH_2-$COOC_2H_5$ and 8-CH_2), 2.00 (m, 3, CH-CH_2 and 7-CH), 1.6 (m, 1, 7-CH) and 1.17 (m, 6, CH_2-CH_3).
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11. Saponification of 9 was carried out at room temperature in a mixture of ethanol and N NaOH. For experimental procedure see reference 8. The yield of 2 from 9 was 55%. Spectral data

for 2: UV λ_{max} (pH 7) 278 nm (ε 24,000), 299 nm (ε 23,500). ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.09 (2, pyrimidine 6-CH and glu NH), 7.59 and 6.50 (d each, 4, C_6H_4, J=8.8 Hz), 6.05 (br s, 2, 2-NH_2), 5.91 (t(concentration dependent), 1, 10-NH), 6.23 (t, 1, anomeric 1'-CH, J=6.8 Hz), 4.36 (m, 1, CH-COOH), 4.22 (m, 1, 3'-CH), 4.6-4.81 (m, 5, 5-NCH_2, 5'-CH_2 and 4'-CH), 2.88 (m, 2, 9-CH_2), 2.35 (m, 6, glu CH_2, 2'-CH_2, 2'-CH_2 and 8-CH_2), 2.01 (m, 3, CH-CH_2 and 7-CH) and 1.8 (m, 1, 7-CH).

12. The Fast Atom Bombardment (FAB) mass spectrum of 2 was determined on a Kratos MS-50 instrument, using 6 kV Ar atoms at the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, Nebraska. For details of FAB mass spectral technique see M. Barber, R. S. Bordoli, R. D. Sedgwick and A. N. Tyler, Nature, 273, 270 (1981).
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14. Reduction and formylation N^{10}-nitrosofolic acid was reported by von E. Khalifa, A. N. Ganguly, J. H. Bieri and M. Viscontini, Helv. Chim. Acta. 63, 2254 (1980). Only traces of hydogenolysis products were observed along with 5-10% of 8. The isolated yield of 11 (UV (pH 7) λ_{max} 282 nm (ε 16,000)) was 72%.
15. There were traces of decomposition products.

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