The First Example of a Mimic of the Overall Thymidylate Synthase Reaction

Paul F. C. van der Meij, Tjoe B. R. A. Chen, Ellen Hilhorst, Eduard R. de Waard, and Upendra K. Pandit*

Organic Chemistry Laboratory, University of Amsterdam, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands

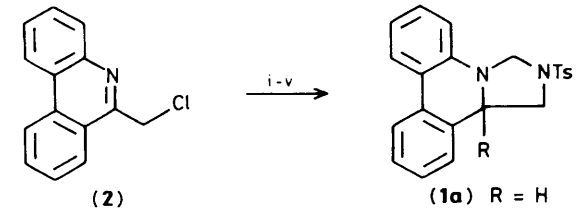
2-Tosyl-1,2,3,12b-tetrahydroimidazo[1,5-*f*]phenanthridine functions as a mimic of the cofactor 5,10-methylenetetrahydrofolate in transferring both a methylene moiety and a hydride equivalent (*i.e.* an overall methyl group) to the C-5 position of a uracil derivative.

The enzyme thymidylate synthase (E.C. 2.1.1.45) catalyses the conversion of 2'-deoxyuridinemonophosphate (dUMP) to 2'-deoxythymidinemonophosphate (dTMP) with the concomitant transformation of the cofactor 5,10-methylenetetrahydrofolate (5,10-CH_2-H_4folate) to 7,8-dihydrofolate. Attempts to mimic the enzymic reaction have been largely limited to the construction of models of the Friedkin intermediate† and the demonstration that these could be thermally fragmented to yield thymine derivatives.[1] Since the currently accepted mechanism of action of thymidylate synthase invokes a covalent apoenzyme–substrate–cofactor ternary complex,[2] rather than the Friedkin intermediate, we have recently studied the reactions of 6-aminouracil derivatives with 5,10-CH_2-H_4folate models and shown that these models are able to transfer a methylene unit to the activated C-5 position of the uracil substrate .[3] We now present the first example of an overall methyl transfer from a 5,10-CH_2-H_4folate cofactor model to a uracil derivative.

A 5,10-CH_2-H_4 folate model which was expected to possess the capacity to transfer both the methylene moiety and a hydride equivalent was recognized in the imidazophenanthridine derivative (**1a**). This was prepared from 6-chloromethylphenanthridine (**2**) in five steps in an overall yield of 48%. When $LiAlD_4$ was employed in place of $LiAlH_4$ in step iv, the sequence of reactions led to the corresponding deuterio analogue (**1b**). The structures of the compounds were confirmed by their spectral data.‡

When (**1a**) was allowed to react with 6-methylamino-1,3-dimethyluracil (**3**) [F_3CCO_2H in MeCN (1%), reflux under argon, 30 min] the resulting reaction mixture contained several compounds from which thymine derivative (**4a**) could be isolated and identified.§ The mechanism of formation of (**4a**) is visualized *via* the generation[3] and subsequent reduction of the methylene intermediate (**5**) by the dihydrophenanthridine derivative (**6**) (R = H). Wholly in agreement with the proposed mechanism, employment of labelled 5,10-CH_2-H_4folate model (**1b**) in a similar reaction led to the formation of the thymine derivative (**4b**)§ in which one deuterium atom was incorporated in the C-5 methyl group. In both experiments the formation of phenanthridine derivative (**7**) was demonstrated.

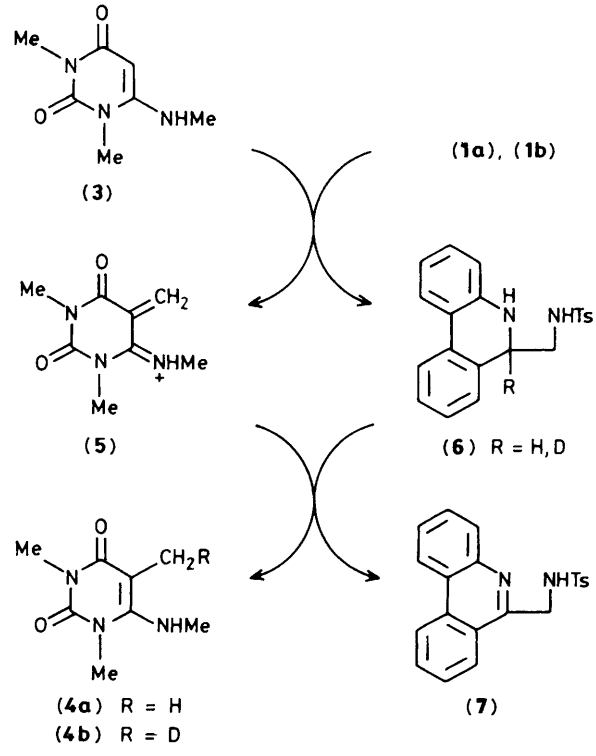
Further analysis of the reaction mixture [from (**1a**) and (**3**)] resulted in the isolation and characterization of products formed by the anticipated reactions[3] of methylene intermediate (**5**) with (**1a**), (**3**), (**6**), and (**7**). Since the formation of



(**2**) (**1a**) R = H (**1b**) R = D

Ts = *p*-$MeC_6H_4SO_2$-

Reagents: i, K-phthalimide; ii, NH_2NH_2, H^+; iii, *p*-$MeC_6H_4SO_2Cl$; iv, $LiAlH_4$ or $LiAlD_4$; v, CH_2O.



† For 'Friedkin intermediate,' (5-thimidinyl-H_4 folate) see ref. 2, Figure 1.

‡ *Selected spectroscopic data:* (**1a**) m.p. 187—188 °C; ^1H n.m.r. ($CDCl_3$, 250 MHz): δ 2.36 (s, 3H, *p*-Me), 3.47 (dd, 1H, CH_2, *J* 8.6, 9.7 Hz), 4.17 (dd, 1H, CH_2, *J* 5.7, 8.6 Hz), 4.43 (dd, 1H, CH, *J* 5.7, 9.7 Hz), 4.52 (d, 1H, NCH_2N, *J* 5.5 Hz), 4.79 (d, 1H, NCH_2N, *J* 5.5 Hz), 6.46 (d, 1H, ArH, *J* 8.0 Hz), 6.91 (m, 2H, ArH), 7.27 (m, 5H, ArH), 7.70 (m, 4H, ArH); mass (electron impact): found 376.1231, calc. for $C_{22}H_{20}N_2O_2S$: 376.1245.

(**1b**) M.p. 190—191 °C; ^1H n.m.r. ($CDCl_3$, 250 MHz): δ 2.36 (s, 3H, *p*-Me), 3.46 (d, 1H, CH_2, *J* 8.6 Hz), 4.17 (d, 1H, CH_2, *J* 8.6 Hz), 4.52 (d, 1H, NCH_2N, *J* 5.5 Hz), 4.79 (d, 1H, NCH_2N, *J* 5.5 Hz), 6.46 (d, 1H, ArH, *J* 8.0 Hz), 6.91 (m, 2H, ArH), 7.27 (m, 5H, ArH), 7.70 (m, 4H, ArH); mass (electron impact): found 377.1315, calc. for $C_{22}H_{19}DN_2O_2S$: 377.1308.

§ Compound (**4a**) was isolated in *ca.* 10% yield after chromatographic separation. The ^1H n.m.r. spectrum of the product was identical to that of an authentic sample: ($CDCl_3$): δ 1.95 (s, 3H, C-5-Me), 2.86 (d, 3H, NH*Me*, *J* 5.3 Hz), 3.33 (s, 3H, NMe), 3.43 (3, 3H, NMe), 3.61 (m, 1H, N*H*Me).

(**4b**) ^1H N.m.r. ($CDCl_3$): δ 1.95 (t, 2H, CH_2D, *J* 2.1 Hz), 2.88 (d, 3H, NH*Me*, *J* 5.7 Hz), 3.35 (s, 3H, NMe), 3.45 (s, 3H, NCH_2), 3.60 (m, 1H, N*H*Me).

these compounds is not directly related to the model reaction of thymidylate synthase, they will be described elsewhere.

This work was carried out in part under auspices of the Stichting Scheikundig Onderzoek Nederland (S.O.N.) with financial support of the Netherlands Organization for Fundamental Research (Z.W.O.).

Received, 8th December 1986; Com. 1740

References

1 R. S. Wilson and M. P. Mertes, *Biochemistry*, 1973, **12**, 2879; R. Plemp and U. K. Pandit, *Heterocycles*, 1979, **12**, 1137; P. A. Charlton and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1363.

2 M. A. Moore, F. Ahmed, and R. B. Dunlap, *Biochemistry*, 1986, **25**, 3311.

3 P. F. C. van der Meij, R. D. Lohmann, E. R. de Waard, T. B. R. A. Chen, and U. K. Pandit, *Tetrahedron*, 1986, **42**, 3921.