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MODELS OF FOLATE COENZYMES 16[1]. CHEMICAL MODELLING OF THE THYMIDYLATE SYNTHASE REACTION. EVIDENCE FOR AN "EXOCYCLIC METHYLENE INTERMEDIATE" ANALOGUE, WHICH IS REDUCIBLE TO A THYMINE DERIVATIVE, IN THE REACTION OF 6-AMINOURACILS WITH A 5,10-METHYLENETETRAHYDROFOLATE MODEL

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Abstract - Reactions of 6-amino-, 6-alkylamino- and 6-anilino-1,3-dimethyluracils (1a-e) with 3,4-diphenyl-1-tosylimidazolidine (2), in the presence of acid, lead to the formation of products which are derived from an "exocyclic methylene intermediate" analogous to the one formed in the thymidylate synthase reaction. The intermediate has been identified by (a) spectral studies, (b) formation of adducts with dihydropyridine and dihydroquinoline derivatives and (c) its reduction to the corresponding thymine derivative. These results provide chemical precedence for the carbon transfer step of the thymidylate synthase reaction in a reaction between models of both the apoenzyme-substrate complex and the coenzyme 5,10-methylenetetrahydrofolate.

INTRODUCTION

The thymidylate synthase (dTMP synthase) catalyzed conversion of dUMP to dTMP involves the overall transfer of a "CH_3" group from the coenzyme 5,10-methylenetetrahydrofolate (5,10-CH_2-H_4folate) to the uridine derivative.[3] The currently accepted mechanism of action of the enzyme invokes the following sequence of reactions: (a) an initial attack of a nucleophile of the apoenzyme (a cysteine--SH) on the 6-position of the uracil moiety of dUMP, causing the generation of a powerful nucleophilic centre at C-5; (b) reaction of the latter with the activated form ($CH_2=\overset{+}{N}(5)H_4$folate) of the coenzyme and subsequent fragmentation of the resulting (apoenzyme-substrate-coenzyme) ternary complex into an exocyclic methylene intermediate and H_4folate (Chart I) and finally (c) reduction of the last mentioned intermediate by H_4folate, to give dTMP and H_2folate.

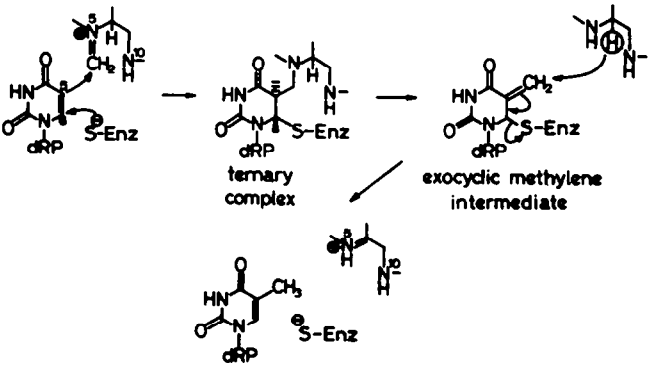


Chart I

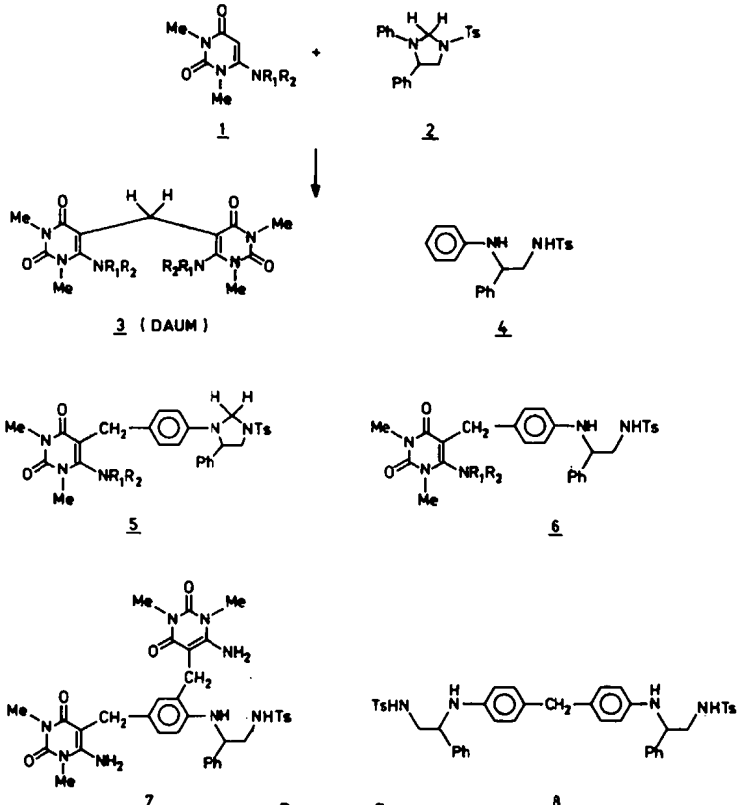


Chart II

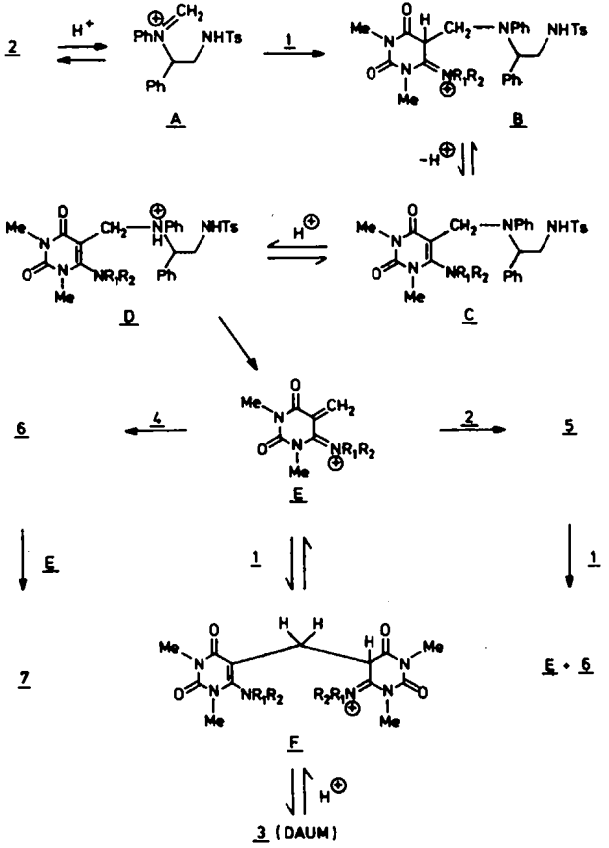


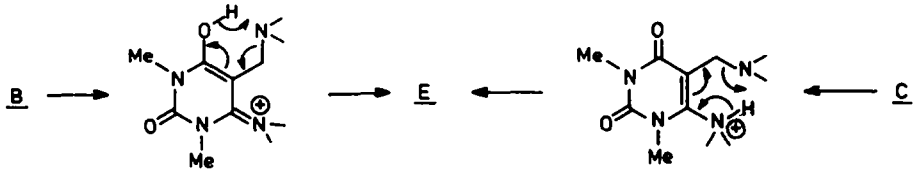
Chart III

Various steps of the mechanism described in Chart I have received support from model studies[4] and studies of mechanism-based inhibitors.[5] In order to derive further information on the details of the methylene transfer and the reduction steps, we have undertaken the development of chemical models (mimics) of dTMP synthase which closely parallel the enzymic reaction. In particular we have envisaged the study of the reaction of C(5)-activated uracil derivatives -as analogues of the apoenzyme-substrate adduct- with models of 5,10-CH_2-H_4folate.[6a] In this communication we present results of the reaction of 6-aminouracil derivatives (1a-e) with imidazolidine 2, in the presence of acid, which throw light upon mechanistic aspects of the methylene (carbon) transfer process, especially, as these relate to the facile formation of the putative exocyclic methylene intermediate. The aforementioned dTMP synthase mimics can in principle be extended to systems in which the generated exocyclic methylene intermediate may be reduced by the H_4folate analogue.

RESULTS AND DISCUSSION

The reactions of aminouracils 1a-e with imidazolidine 2, in presence of acid, result in the formation of products 3-8 described in Chart II. The rates of formation of these products and their distribution during the course of the reaction is dependent upon both, the nature of the groups R_1 and R_2 (in 1) and the concentration of acid in the reaction mixture. Furthermore, as will be discussed in the sequel, several of the products are transient in character, their appearance, build-up and breakdown being followed conveniently by analytical HPLC. The relevant compounds 3-8 have been isolated and their structures established by spectroanalytical data (vide experimental).

The formation of products 3-8 can be discussed on the basis of the reactions described in Chart III and IV. Under acidic conditions, imidazolidine 2 is expected to undergo a ring opening reaction leading to the formation of iminium salt intermediate A.[7] That this indeed is the case has been demonstrated by reduction of 2 (via A) to 9 by sodium cyanoborohydride, in the presence of acetic acid (Chart IV). According to one proposed pathway, A reacts with aminouracil derivative 1 to give, via intermediates B and C, the ammonium salt D, the latter subsequently fragments into the exocyclic methylene iminium intermediate E and diamine 4. It is recognized by us that E can also be formed via cyclic processes G and H, from B and C, respectively. The transfer of a methylene



unit from 2 to the uracil substrate (1) is analogous to similar carbon transfer reactions observed for a variety of imidazolidines which have been studied as 5,10-CH_2-H_4folate models.[6a-c] The intermediate E is analogous to the "exocyclic methylene intermediate" proposed in the enzyme catalyzed conversion of dUMP to dTMP.[3] Most of the observed products described in Chart II are derived from further reactions of intermediate E. Thus, an electrophilic substitution at the para position of the anilino moiety of 2, by E, leads to compound 5. However, since 5 itself is an imidazolidine derivative it can undergo a sequence of reactions analogous to that of 2 and, via reaction with 1, generate a molecule of uracil derivative 6 plus an equivalent amount of E. The product 6 is, in addition, derived from E by its reaction with diamine 4. Furthermore, 6a is susceptible to a second electrophilic substitution at the ortho position, by E, to yield 7. Yet another pathway involving E, is its reaction with 1 to give a di(aminouracilyl)methane derivative 3 (DAUM), via intermediate F. In fact, in reactions of 6-aminouracils with imidazolidines lacking an anilino function, DAUM type products are formed exclusively.[7a]

It would be anticipated that the rate of formation of E and its fate with respect to further transformations shall depend upon the nature of groups R_1 and R_2 and the conditions of the reaction. This can best be illustrated by discussing the results of the reactions of selected aminouracil derivatives in detail.

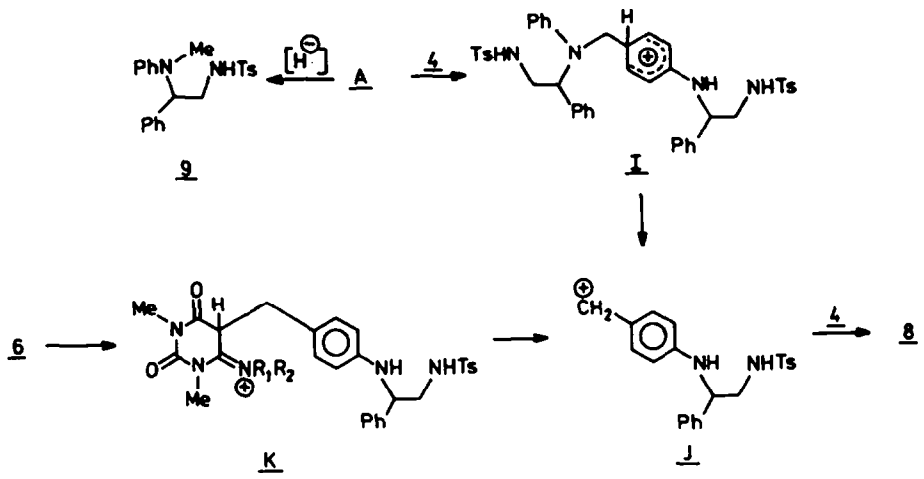


Chart IV

When 1b is allowed to react with 2 (1:1) in CF_3COOH/CH_3CN (1:3), at room temperature, after 1 hour the imidazolidine has almost disappeared (HPLC) and the mixture is found to consist of 1b, 4 and 5b in the ratio 1:1:1 and small amounts of 6b. Monitoring of this mixture (HPLC) with time shows that all three components decrease in concentration with the concurrent formation, at their expense, of product 6b (Fig. 1). After 48 hours, the latter compound constitutes about 90 p.c. of the total products.

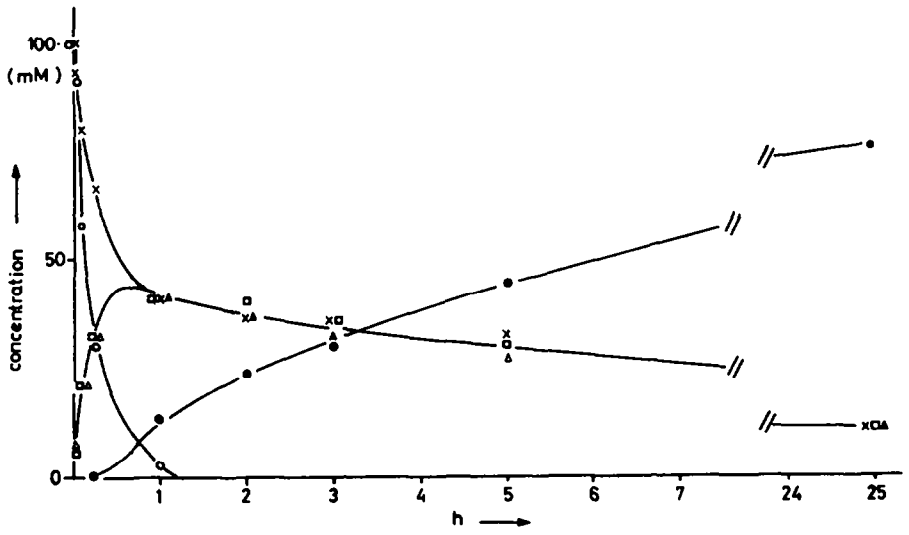


Fig.1. Time-dependent concentrations of 1b (-x-), 2 (-o-), 4 (-Δ-), 5b (-□-) and 6b (-●-) during reaction of 1b with 2.

This reactivity pattern is also observed for aminouracils 1c and 1d, with the principal difference that the latter compounds react progressively slower with the imidazolidine 2. A comparison of the time-dependent disappearance of 2, shows the following results for the three uracils: 1b (1h), 1c (4 h) and 1d (24 h). Since the basicities of the amine components of these substrates are not very different, steric factors must dominate the reactivity of 2 with the aminouracils. This is understandable in terms of the increase in the bulk of R_1, R_2 which will cause increasing steric interactions with the incipient methylene group during the conversion from D to E (Chart III) and, thereby, progressively enhance the energy of the respective transition states. Consistent with

this reasoning is the observation that under the same reaction conditions 1a is consumed, in the reaction with 2, in less than 30 seconds. Even considering the slightly lower basicity of the amine component, the reaction of 1a is impressively rapid. The mixture at this stage comprises of 3a, 4 and unreacted 2 in equal amounts (1:1:1). Upon standing for about 10 minutes, 2 disappears and 3a, 4 and 5a (ca 1:2:1) are recognized in the mixture. Finally, after 24 hours, 6a is present as the major product (> 80%), while minor quantities of 7 can also be identified. All these transformations, although explicable on the basis of intermediate E, require an additional assumption; namely, that formation of product 3a (DAUM) is reversible under acidic conditions. This is, for example, necessary to account for the formation of 5a and 6a at the cost of 3a during the reaction. Confirmation of the aforementioned assumption is derived from the reaction of 3a with 2 (1:1, CF_3COOH/CH_3CN 1:3, room temperature) whereupon 7 is formed as the final product. This establishes the sequence: (i) breakdown of 3a into 1a and E, (ii) reaction of E with 2 to yield 5a, (iii) methylene group transfer from 5a to 1a to form 6a and E and finally, (iv) reaction of E with 6a to give 7 via an electrophilic substitution at the ortho position of the anilino moiety.

The observation that no DAUM derivative (3) is observed in the reaction of 1b–d with 2, under the relatively high acidic conditions [CF_3COOH/CH_3CN (1:3)], can be understood by recognizing that these uracil derivatives, being derived from the more basic amines, are largely in the protonated forms [most probably at C(5)][8] and, consequently, not available for reaction with intermediate E. Support for this rationale is obtained from the following three experiments. (a) When 1b,c are allowed to react with 2 in a mixture of CF_3COOH/CH_3CN (1:100), initially formed reaction mixtures are shown to contain DAUM derivatives 3b,c; (b) Reaction of the poorly nucleophilic 1e with 2 in CF_3COOH/CH_3CN (1:3) occurs slowly and the initial, almost exclusive, product involving carbon transfer is DAUM derivative 3e and (c) When 1e and 2 are allowed to stand in pure CF_3COOH, uracil derivative 6e is formed. The latter result implies that conversion of 3e to the corresponding intermediate E (R_1 = phenyl, R_2 = H) requires, as expected, strongly acidic conditions.

A characteristic feature of the reaction between 1e and 2 in CF_3COOH/CH_3CN (1:3) is the formation of a considerable amount of tetramine 8. The latter presumably arises from an electrophilic substitution reaction of 4 with intermediate J, which may be formed either from A via intermediate I, or from 6 via intermediate K (Chart IV). The formation of 8 is favoured, since the reaction of 4 with intermediate E is slowed down due to the lower concentration of E (R_1 = phenyl, R_2 = H) under the reaction conditions.

The salient role of intermediate E in the aforementioned reactions required that the formation of this crucial intermediate be attested independently. This has been achieved as follows. A direct method of recognizing E would be its generation (e.g. via DAUM 3) and spectroscopic identification. Comparison of the UV spectra of 3a and 10, in presence of CF_3COOH, suggests that a new chromophore, such as is incorporated in E, is generated (Fig. 2).

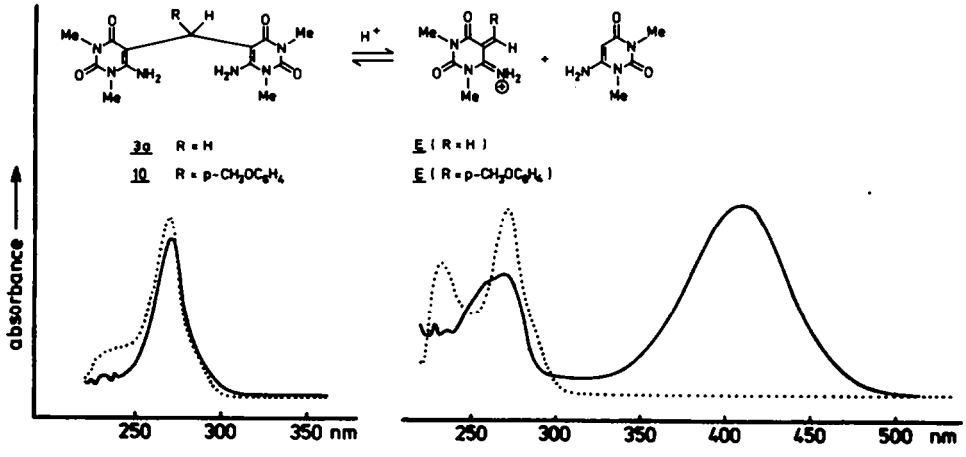


Fig. 2. UV spectra of 3a (left) and 10 (right) in CH_3CN before (·····) and after (——) addition of CF_3COOH.

Since the precise UV maxima of the intermediates are not correlatable with simply available models, the NMR spectrum of 10 in CF_3COOD was also examined. The data (vide experimental) fully support the formation of E [R = $C_6H_4OCH_3$(p)] in the acid mediated fragmentation of 10.

Further evidence for E (R_1 = H, R_2 = Me) is derived by its trapping at room temperature as 1:1 adducts 13 and 14, with dihydroquinoline and dihydropyridine derivatives 11 and 12a, respectively (Chart V).

The structure of adduct 13 is established by its spectral and X-Ray data and while the gross structure of adduct 14 is attested by its ^{1}H NMR spectrum, the stereochemistry of the B/C ring junction is not yet defined. In contrast to the pattern of reaction of E (R_1 = H, R_2 = Me) with 12a, at room temperature, when the components were heated at 80°C, the reduction product 15a was formed quantitatively. Furthermore, the same thymine derivative (15a) was obtained upon heating 14 at 80°C in CH_3COOH/CH_3CN. These results are accounted for by the reversibility of the kinetically formed 14 into E (R_1 = H, R_2 = Me) and Hantzsch ester (12a) at higher temperature, followed by reduction of the exocyclic methylene intermediate. Use of 4,4-dideuterated Hantzsch ester (12b) led to the formation of 15b in which the C(5)-methyl group incorporated one atom of deuterium (vide experimental).

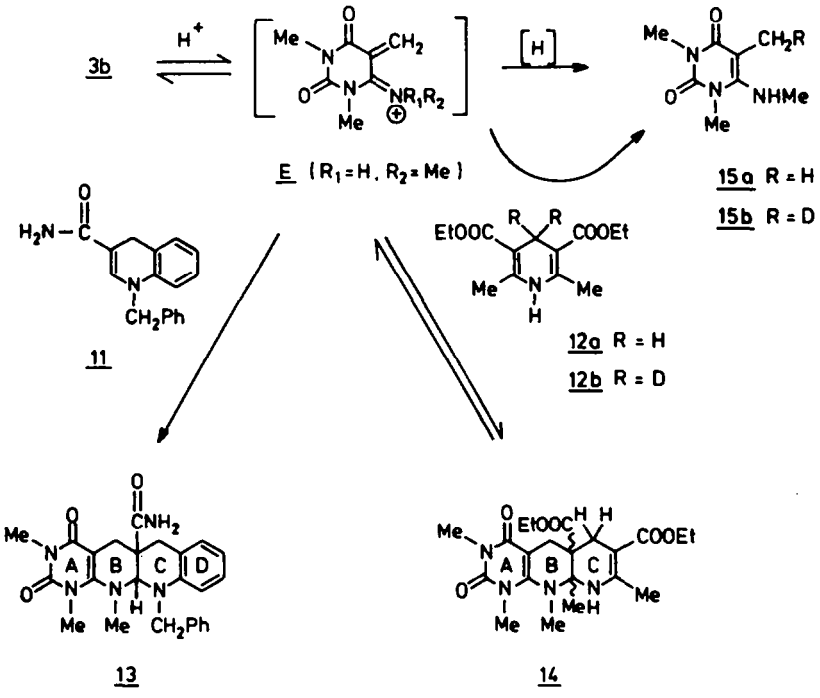


Chart V

Reduction of the in situ generated E (R_1 = H, R_2 = Me) by H_2/Pd or Et_3SiH led to the formation of aminothymine 15a. An equivalent amount of 1b was also produced in these reactions.

The overall process comprising the formation of intermediate E by methylene transfer from a 5,10-CH_2-H_4folate model and its subsequent reductive quenching to a thymine derivative, represents a mimic of the thymidylate synthase catalyzed conversion of dUMP into dTMP. In the enzymatic reaction, however, the folate coenzyme is the source of both the methylene unit and the hydride equivalent. To achieve this dual function in a non-enzymatic system, investigations directed at the development of suitable, sophisticated 5,10-CH_2-H_4folate coenzyme models are currently in progress.

EXPERIMENTAL

All melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 257 spectrometer. The absorptions are given in cm^{-1}. NMR spectra were determined with Varian A60 (60 MHz) and Bruker WM250 (250 MHz) instruments, using TMS as internal standard. The chemical shifts (δ) are given in ppm and spin-spin coupling constants (J) in Hertz. UV spectra were measured on a Hewlett-Packard 8451A spectrophotometer. Mass spectra were obtained with a Varian-Matt 711 spectrometer. HPLC analysis were performed using a reverse-phase column (PE-HS5-C18, 125x4.6 mm). Mobile phases used were: A: CH_3CN/H_2O 50:50; B: CH_3CN/H_2O 55:45 (reactions of 1a-e with 2); C: CH_3CN/H_2O 15:85 (reduction experiments)[7]. The eluents were pumped at 1-2 ml/min, using a Perkin-Elmer series 10 liquid chromatograph. The Holochrome variable wavelength detector used was set at 230 nm. Elemental analysis were carried out at the microanalytical laboratory, Department of Physical Organic and Analytical Chemistry, Organic Chemistry Institute, TNO, Zeist, The Netherlands. Flash chromatography was carried out according to the method described by Still,[9] using silica gel 60 (Merck) and CH_2Cl_2/CH_3OH 95:5 (v/v) as eluent. All solvents were destilled prior to use.[10] Uracil derivatives 1a-e were prepared from 6-chloro-1,3-dimethyluracil (Aldrich)[11] or 6-amino-1,3-dimethyluracil.[12] 1-Anilino-2-nitro-1-phenylethane was obtained as described by Leonard.[12] Compounds 10, 11 and 12 were synthesized as previously reported.[13,14,15]

2-Amino-1-anilino-1-phenylethane

To a refluxing suspension of 5.4 g (142 mmol) $LiAlH_4$ in 150 ml of dry THF was added dropwise a solution of 12 g (50 mmol) 1-anilino-2-nitro-1-phenylethane in 50 ml dry THF. The mixture was stirred for 15 min at reflux temperature. After cooling to room temperature 10.5 ml H_2O in 100 ml THF was added. The precipitate was removed by filtration, the filtrate was evaporated and the residue was dissolved in 100 ml CH_2Cl_2. The solution was extracted 3x with diluted hydrochloric acid (pH ∿ 3). The combined water layers were washed 5x with CH_2Cl_2, basified with ammonia (pH ∿ 9) and extracted 3x with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated, yielding 6.4 g (61%) of a light yellow oil, which was used without further purification; ^{1}H-NMR ($CDCl_3$): 1.50 (s, 2H, NH_2), 2.97 (dd, 1H, CH_2, J = 7.5 Hz, J = 12 Hz), 3.11 (dd, 1H, CH_2, J = 4.6 Hz, J = 12 Hz), 4.35 (dd, 1H, CH, J = 4.6 Hz, J = 7.5 Hz), 4.74 (s, 1H, NH), 6.55 (d, 2H, NAr, J = 7.9 Hz), 6.65 (t, 1H, NAr, J = 7 Hz), 7.08 (dd, 2H, NAr, J = 7 Hz, J = 7.9 Hz), 7.30 (m, 5H, CAr); MS: found 212.1309, calc. for $C_{14}H_{16}N_2$: 212.1310.

1-Anilino-1-phenyl-2-tosylaminoethane (4)

To a stirred solution of 0.9 g (4.2 mmol) of 2-amino-1-anilino-1-phenylethane and 0.5 g Et_3N in 15 ml CH_2Cl_2 (0°C), was added dropwise a solution of 0.9 g (4.7 mmol) of p-toluenesulfonylchloride in 10 ml CH_2Cl_2. The mixture was stirred under N_2 at room temperature for 30 min, washed with diluted hydrochloric acid (pH ∿ 4), brine (2x), dried over $MgSO_4$ and evaporated. The residue was crystallized from ethanol: 1.34 g (87%) of 4; m.p. 149.5-150.2°C; IR (KBr): 3375, 3285, 1600, 1500, 1330, 1150, 750, 695, 665; ^{1}H-NMR ($CDCl_3$): 2.40 (s, 3H, $ArCH_3$), 3.18 (m, 1H, CH_2), 3.32 (m, 1H, CH_2), 4.39 (dd, 1H, CH, J = 4.5 Hz, J = 7.5 Hz), 4.50 (s, 1H, ArNH), 5.05 (t, 1H, TsNH, J = 6.5 Hz), 6.46 (d, 2H, NAr, J = 7.9 Hz), 6.66 (t, 1H, NAr, J = 6.9 Hz), 7.07 (dd, 2H, NAr, J = 6.9 Hz, J = 7.9 Hz), 7.27 (m, 7H, CAr + SO_2Ar), 7.70 (d, 2H, SO_2Ar, J = 8.3 Hz); MS: found 366.1398, calc. for $C_{21}H_{22}N_2O_2S$: 366.1397; Anal. found C, 68.52; H, 6.12; N, 7.66; O, 8.72, calc. for $C_{21}H_{22}N_2O_2S$: C, 68.82; H, 6.05; N, 7.65; O, 8.73.

3,4-Diphenyl-1-tosylimidazolidine (2)

A solution of 1.0 g (2.7 mmol) of 4 and 0.5 ml 37% CH_2O/H_2O in 15 ml ethanol was refluxed for 3 hours. The product crystallised upon cooling to room temperature: 0.57 g (94%) of 2; m.p. 125.2-126.5°C; IR (KBr): 1600, 1500, 1345, 1160, 750, 700, 670; ^{1}H-NMR ($CDCl_3$): 2.38 (s, 3H, $ArCH_3$), 3.43 (dd, 1H, CH_2, J = 5.2 Hz, J = 10.4 Hz), 3.90 (dd, 1H, CH_2, J = 7.4 Hz, J = 10.4 Hz), 4.52 (dd, 1H, CH, J = 5.2 Hz, J = 7.4 Hz), 4.74 (d, 1H, N-CH_2-N, J = 5.9 Hz), 5.02 (d, 1H, N-CH_2-N, J = 5.9 Hz), 6.37 (d, 2H, NAr, J = 7.9 Hz), 6.72 (t, 1H, NAr, J = 7.4 Hz), 7.11 (m, 4H, NAr + CAr), 7.23 (m, 5H, CAr + SO_2Ar), 7.67 (d, 2H, SO_2Ar, J = 8.3 Hz); MS: found 378.1389, calc. for $C_{22}N_{22}N_2O_2S$: 378.1397.

Reaction of uracil derivatives 1a-e with imidazolidine 2

General procedure

To a solution of 0.4 mmol of the uracil derivative in 4 ml CH_3CN/TFA (3:1, v/v) 0.4 mmol of 2 was added. The mixture was stirred at room temperature and the reaction was monitored by HPLC. Samples taken from the mixture (25 µl) were diluted with toluene and evaporated. The residue was dissolved in the mobile phase and analysed. DAUM derivatives 3 crystallized from the mixture and were isolated by filtration. All other products were isolated by flash chromatography. DAUM derivatives 3b,c could be isolated when the reaction was carried out in CH_3CN/TFA 100:1. Diamine derivative 6e was obtained as main product when the reaction was carried out in pure TFA.

"DAUM" derivatives 3a-c, e

3a: m.p. 356-357°C; IR (KBr): 3400, 3120, 1660, 1620, 1500, 785, 750, 680; ^{1}H-NMR (DMSO-d_6): 3.17 (s, 6H, 2x NCH_3), 3.29 (s, 6H, 2x NCH_3), 3.30 (s, 2H, CH_2), 7.51 (s, 4H, 2x NH_2); ^{1}H-NMR (pyridine-d_5): 3.45 (s, 6H, 2x NCH_3), 3.58 (s, 6H, 2x NCH_3), 3.77 (s, 2H, CH_2); MS (FD): 322 (M^+).
3b: m.p. 254.5-255°C; IR (KBr): 3260, 3210, 1682, 1640, 1595, 1495, 1450, 790, 755, 710; ^{1}H-NMR ($CDCl_3$): 2.89 (d, 6H, 2x $NHCH_3$, J = 5.4 Hz), 3.33 (s, 6H, 2x NCH_3), 3.41 (s, 6H, 2x NCH_3), 3.43 (s, 2H, CH_2), 7.55 (m, 2H, 2x $NHCH_3$); MS (FD): 350 (M^+).
3c: m.p. 204-205°C; IR (KBr): 3240, 3190, 1685, 1630, 1480, 1445, 790, 760, 700; ^{1}H-NMR ($CDCl_3$): 2.99 (s, 2H, CH_2), 3.30 (s, 6H, 2x NCH_3), 3.44 (s, 6H, 2x NCH_3), 4.22 (d, 4H, 2x CH_2NH, J = 6.6 Hz), 7.27 (m, 10H, 2x Ar), 7.84 (t, 2H, NH-CH_2, J = 6.6 Hz); MS (FD): 502 (M^+).
3e: m.p. 251.5-252.5°C; IR (KBr): 3260, 3170, 3090, 1690, 1620, 1595, 1475, 1430, 1250, 750, 730;

^{1}H-NMR ($CDCl_3$): 3.23 (s, 6H, 2x NCH_3), 3.42 (s, 2H, CH_2), 3.44 (s, 6H, 2x NCH_3), 6.86 (d, 4H, 2x NAr, J = 7.8 Hz), 6.99 (t, 2H, 2x NAr, J = 7.4 Hz), 7.29 (dd, 4H, 2x NAr, J = 7.4 Hz, J = 7.8 Hz), 10.08 (s, 2H, 2x NHAr); MS (FD): 474 (M^+).

Imidazolidine derivatives 5a-d

5a: ^{1}H-NMR ($CDCl_3$): uracil moiety: 3.32 (s, 3H, NCH_3), 3.34 (s, 3H, NCH_3), 4.30 (s, 2H, NH_2); anilino moiety: 3.58 (s, 2H, CH_2Ar), 6.27 (d, 2H, NAr, J = 8.5 Hz), 6.94 (d, 2H, NAr, J = 8.5 Hz); imidazolidine moiety: 3.37 (dd, 1H, CH_2, J = 5.2 Hz, J = 10.3 Hz), 3.85 (dd, 1H, CH_2, J = 7.3 Hz, J = 10.3 Hz), 4.48 (dd, 1H, CH, J = 5.2 Hz, J = 7.3 Hz), 4.64 (d, 1H, NCH_2N, J = 5.7 Hz), 4.96 (d, 1H, NCH_2N, J = 5.7 Hz), 7.06 (m, 2H, Ar), 7.22 (m, 3H, Ar); tosyl moiety: 2.39 (s, 3H, $ArCH_3$), 7.22 (2H, Ar), 7.65 (d, 2H, Ar, J = 8.2 Hz); MS (FD): 545 (M^+).
5b: ^{1}H-NMR ($CDCl_3$): uracil moiety: 2.59 (s, 3H, $NHCH_3$), 3.32 (s, 3H, NCH_3), 3.34 (s, 3H, NCH_3); anilino moiety: 3.64 (s, 2H, CH_2Ar), 6.28 (d, 2H, Ar, J = 8.5 Hz), 6.92 (d, 2H, Ar, J = 8.5 Hz); imidazolidine moiety: 3.36 (dd, 1H, CH_2, J = 5.3 Hz, J = 10.3 Hz), 3.87 (dd, 1H, CH_2, J = 7.3 Hz, J = 10.3 Hz), 4.49 (dd, 1H, CH, J = 5.3 Hz, J = 7.3 Hz), 4.67 (d, 1H, NCH_2N, J = 5.7 Hz), 4.97 (d, 1H, NCH_2N, J = 5.7 Hz), 7.05 (m, 2H, Ar), 7.21 (m, 3H, Ar); tosyl moiety: 2.38 (s, 3H, $ArCH_3$), 7.21 (2H, Ar), 7.65 (d, 2H, Ar, J = 8.2 Hz); MS (FD): 560 (M^++H).
5c: ^{1}H-NMR ($CDCl_3$): uracil moiety: 3.34 (s, 3H, NCH_3), 3.43 (s, 3H, NCH_3), 3.75 (t, 1H, $NHCH_2$, J = 6.4 Hz), 3.93 (d, 2H, $NHCH_2$, J = 6.4 Hz), 7.07 (m, 2H, Ar), 7.23 (m, 3H, Ar); anilino moiety: 3.43 (s, 2H, CH_2Ar), 6.23 (d, 2H, Ar, J = 8.6 Hz), 6.78 (d, 2H, Ar, J = 8.6 Hz); imidazolidine moiety: 3.38 (m, 1H, CH_2), 3.86 (dd, 1H, CH_2, J = 7.4 Hz, J = 10.3 Hz), 4.46 (dd, 1H, CH, J = 5.6 Hz, J = 7.4 Hz), 4.64 (d, 1H, NCH_2N, J = 5.8 Hz), 4.96 (d, 1H, NCH_2N, J = 5.8 Hz), 7.07 (m, 2H, Ar), 7.23 (m, 3H, Ar); tosyl moiety: 2.37 (s, 3H, $ArCH_3$), 7.23 (2H, Ar), 7.64 (d, 2H, Ar, J = 8.2 Hz); MS (FD): 635 (M^+).
5d: ^{1}H-NMR ($CDCl_3$): uracil moiety: 2.61 (s, 6H, $N(CH_3)_2$), 3.29 (s, 3H, NCH_3), 3.30 (s, 3H, NCH_3); anilino moiety: 3.63 (s, 2H, CH_2Ar), 6.26 (d, 2H, Ar, J = 8.6 Hz), 6.88 (d, 2H, Ar, J = 8.6 Hz); imidazolidine moiety: 3.35 (dd, 1H, CH_2, J = 5.0 Hz, J = 10.3 Hz), 3.85 (dd, 1H, CH_2, J = 7.4 Hz, J = 10.3 Hz), 4.48 (dd, 1H, CH, J = 5.0 Hz, J = 7.4 Hz), 4.64 (d, 1H, NCH_2N, J = 5.6 Hz), 4.97 (d, 1H, NCH_2N, J = 5.6 Hz), 7.04 (m, 2H, Ar), 7.21 (m, 3H, Ar); tosyl moiety: 2.38 (s, 3H, $ArCH_3$), 7.21 (2H, Ar), 7.65 (d, 2H, Ar, J = 8.3 Hz); MS (FD): 573 (M^+).

Diamine derivatives 6a-e

6a: ^{1}H-NMR ($CDCl_3$): uracil moiety: 3.32 (s, 3H, NCH_3), 3.34 (s, 3H, NCH_3), 4.33 (s, 2H, NH_2); anilino moiety: 3.60 (s, 2H, $ArCH_2$), 4.44 (s, 1H, NH); 6.38 (d, 2H, Ar, J = 8.4 Hz), 6.90 (d, 2H, Ar, J = 8.4 Hz); aminoethane moiety: 3.15 (m, 1H, CH_2), 3.29 (m, 1H, CH_2), 4.33 (m, 1H, CH), 4.91 (t, 1H, NHTs, J = 6.5 Hz), 7.25 (m, 5H, Ar); tosyl moiety: 2.40 (s, 3H, $ArCH_3$), 7.25 (2H, Ar), 7.69 (d, 2H, Ar, J = 8.3 Hz); MS (FD): 533 (M^+).
6b: ^{1}H-NMR ($CDCl_3$): uracil moiety: 2.57 (d, 3H, $NHCH_3$, J = 5.7 Hz), 3.33 (s, 3H, NCH_3), 3.36 (s, 3H, NCH_3), 3.67 (q, 1H, $NHCH_3$, J = 5.7 Hz); anilino moiety: 3.64 (s, 2H, $ArCH_2$), 4.45 (s, 1H, NH), 6.38 (d, 2H, Ar, J = 8.4 Hz), 6.87 (d, 2H, Ar, J = 8.4 Hz); aminoethane moiety: 3.15 (m, 1H, CH_2), 3.31 (m, 1H, CH_2), 4.36 (m, 1H, CH), 4.97 (t, 1H, NHTs, J = 6.5 Hz), 7.24 (m, 5H, Ar); tosyl moiety: 2.40 (s, 3H, CH_3), 7.25 (2H, Ar), 7.68 (d, 2H, Ar, J = 8.2 Hz); MS (FD): 547 (M^+).
6c: ^{1}H-NMR ($CDCl_3$): uracil moiety: 3.35 (s, 3H, NCH_3), 3.44 (s, 3H, NCH_3), 3.83 (t, 1H, $NHCH_2$, J = 6.9 Hz), 3.92 (d, 2H, CH_2NH, J = 6.9 Hz), 7.25 (m, 5H, Ar); anilino moiety: 3.44 (s, 2H, $ArCH_2$), 4.43 (s, 1H, NH), 6.33 (d, 2H, Ar, J = 8.4 Hz), 6.74 (d, 2H, Ar, J = 8.4 Hz); aminoethane moiety: 3.14 (m, 1H, CH_2), 3.29 (m, 1H, CH_2), 4.33 (m, 1H, CH), 4.89 (t, 1H, NHTs, J = 6.5 Hz), 7.10 (m, 2H, Ar), 7.25 (m, 3H, Ar); tosyl moiety: 2.39 (s, 3H, $ArCH_3$), 7.28 (2H, Ar), 7.68 (d, 2H, Ar, J = 8.2 Hz); MS (FD): 624 (M^++H).
6d: ^{1}H-NMR ($CDCl_3$): uracil moiety: 2.60 (s, 6H, $N(CH_3)_2$), 3.29 (s, 3H, NCH_3), 3.31 (s, 3H, NCH_3); anilino moiety: 3.64 (s, 2H, $ArCH_2$), 4.35 (s, 1H, NH), 6.35 (d, 2H, Ar, J = 8.5 Hz), 6.82 (d, 2H, Ar, J = 8.5 Hz); aminoethane moiety: 3.14 (m, 1H, CH_2), 3.27 (m, 1H, CH_2), 4.33 (dd, 1H, CH, J = 4.5 Hz, J = 7.9 Hz), 5.03 (t, 1H, NHTs, J = 6.5 Hz), 7.22 (m, 5H, Ar); tosyl moiety: 2.39 (s, 3H, $ArCH_3$), 7.24 (2H, Ar), 7.67 (d, 2H, Ar, J = 8.3 Hz); MS (FD): 561 (M^+).
6e: ^{1}H-NMR ($CDCl_3$): uracil moiety: 3.15 (s, 3H, NCH_3), 3.40 (s, 3H, NCH_3), 5.60 (s, 1H, NHAr), 6.64 (d, 2H, Ar, J = 7.6 Hz), 6.99 (t, 1H, Ar, J = 7.4 Hz), 7.25 (m, 2H, Ar); anilino moiety: 3.60 (s, 2H, $ArCH_2$), 4.45 (s, 1H, NH), 6.39 (d, 2H, Ar, J = 8.3 Hz), 6.89 (d, 2H, Ar, J = 8.3 Hz); aminoethane moiety: 3.15 (m, 1H, CH_2), 3.32 (m, 1H, CH_2), 4.37 (dd, 1H, CH, J = 4.5 Hz, J = 7.2 Hz), 4.75 (t, 1H, NHTs, J = 6.5 Hz), 7.25 (m, 5H, Ar); tosyl moiety: 2.40 (s, 3H, $ArCH_3$), 7.25 (2H, Ar), 7.69 (d, 2H, Ar, J = 8.3 Hz); MS (FD): 609 (M^+).

Diamine derivative 7

7: ^{1}H-NMR (CD_3CN/CD_3OD): uracil moiety (2x): 3.15 (s, 3H, NCH_3), 3.20 (s, 3H, NCH_3), 3.25 (s, 3H, NCH_3), 3.28 (s, 3H, NCH_3); anilino moiety: 3.40 (s, 2H, p-CH_2Ar), 3.46 (d, 1H, o-CH_2Ar, J = 15.8 Hz), 3.56 (d, 1H, o-CH_2Ar, J = 15.8 Hz), 5.95 (d, 1H, ArC_6-H, J = 8.2 Hz), 6.63 (dd, 1H, ArC_5-H, J = 1.9 Hz, J = 8.2 Hz), 6.93 (d, 1H, ArC_3-H, J = 1.9 Hz), aminoethane moiety: 3.18 (m, 2H, CH_2), 4.29 (dd, 1H, CH, J = 4.7 Hz, J = 7.7 Hz), 7.15 (m, 5H, Ar); tosyl moiety: 2.30 (s, 3H, $ArCH_3$), 7.22 (d, 2H, Ar, J = 8.3 Hz), 7.62 (d, 2H, Ar, J = 8.3 Hz); MS (FD): 700 (M^+).

Tetramine derivative 8

8: ^{1}H-NMR ($CDCl_3$): 2.39 (s, 6H, 2x $ArCH_3$), 3.14 (m, 2H, 2x CH_2), 3.28 (m, 2H, 2x CH_2), 3.63 (s, 2H, $ArCH_2Ar$), 4.33 (dd, 2H, 2x CH, J = 4.6 Hz, J = 7.5 Hz), 4.76 (t, 2H, 2x NHTs, J = 6.4 Hz), 6.34 (d, 4H, 2x NAr, J = 8.3 Hz), 6.82 (d, 4H, 2x NAr, J = 8.3 Hz), 7.23 (m, 14H, 2x CAr + 2x SO_2Ar), 7.69 (d, 4H, 2x SO_2Ar, J = 8.3 Hz); MS (FD): 745 (M^++H).

Reduction of intermediate A

A mixture of 100 mg (0.26 mmol) of 2 and 164 mg of $NaCNBH_3$ (3 eq) in 6 ml CH_3CN/CH_3COOH 1:1 was

stirred at 60°C for 1 hour. After cooling to room temperature chloroform and Na_2CO_3/H_2O were added. The water layer was extracted 2x with chloroform. The combined organic layers were dried over $MgSO_4$ and evaporated; 9: ^{1}H-NMR ($CDCl_3$): 2.43 (s, 3H, $ArCH_3$), 2.48 (s, 3H, $ArNCH_3$), 3.44 (m, 1H, CH_2), 3.63 (m, 1H, CH_2), 4.73 (m, 1H, CH), 4.95 (dd, 1H, NHTs, J = 5.3 Hz, J = 10.2 Hz), 6.74 (d, 2H, NAr, J = 7.9 Hz), 6.80 (t, 1H, NAr, J = 6.9 Hz), 7.02 (m, 2H, NAr), 7.24 (m, 7H, CAr + SO_2Ar), 7.74 (d, 2H, SO_2Ar, J = 8.3 Hz); MS (EI): 380 (M^+), 197, 196, 182, 181, 180, 107, 106, 91, 77.

Reductive trapping of intermediate E

With H_2-10% Pd/C

A mixture of 100 mg (0.29 mmol) of 3b and 10 mg 10% Pd/C in 10 ml CH_3COOH was shaken under H_2 at room temperature for 1 hour. HPLC analysis showed a complete conversion of 3b to 1b and 15a. The mixture was filtered over hyflo and the filtrate was evaporated. The reaction products were isolated by flash chromatography:
1b: m.p. 243-245°C (lit.[10] 244-245°C); ^{1}H-NMR ($CDCl_3$): 2.82 (d, 3H, $NHCH_3$, J = 4.8 Hz), 3.29 (s, 3H, NCH_3), 3.37 (s, 3H, NCH_3), 4.67 (m, 1H, $NHCH_3$), 4.81 (s, 1H, C(5)-H); IR (KBr): 3300, 1700, 1635, 1610, 1550, 1450, 770, 755; MS (EI): 169 (M^+), 141, 91, 82, 55, 42, 28.
15a: m.p. 161-162°C (lit.[16] 162-163°C); ^{1}H-NMR ($CDCl_3$): 1.95 (s, 3H, C(5)-CH_3), 2.86 (d, 3H, $NHCH_3$, J = 5.7 Hz), 3.32 (s, 3H, NCH_3), 3.42 (s, 3H, NCH_3), 3.60 (m, 1H, $NHCH_3$); IR (KBr): 3355, 1690, 1605, 1530, 1410, 1250, 1160, 1055, 1040, 750; MS: found 183.1028, calc. for $C_8H_{13}N_3O_2$: 183.1005.

With Et_3SiH

A solution of 200 mg (0.57 mmol) of 3b and 80 mg Et_3SiH (1.2 eq) in 10 ml CH_2Cl_2/TFA (9.5:0.5) was stirred at room temperature for 30 min. HPLC analysis showed a complete conversion of 3b to 1b and 15a. Water and 4N ammonia were added and the water layer was extracted 5x with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated. The reaction products 1b and 15a were isolated by flash chromatography.

With Hantzsch ester (12a)
A solution of 90 mg (0.26 mmol) of 3b and 70 mg (0.28 mmol) Hantzsch ester (12a) in 6 ml CH_3CN/CH_3COOH 3:2 was heated under N_2 at 80°C for 4 hours. HPLC analysis showed a complete conversion of 3b to 1b and 15a. Toluene was added to the reaction mixture, the solvents were evaporated and the reaction products 1b and 15a were isolated by flash chromatography.

With 4,4-dideutero-Hantzsch ester (12b)
A solution of 72 mg (0.20 mmol) of 3b and 60 mg of 12b(0.23 mmol) in 5 ml CH_3CN/CH_3COOH 3:2 was heated under N_2 at 80°C. After 4 hours HPLC analysis showed a complete conversion of 3b to 1b and 15b. Toluene was added to the reaction mixture, the solvents were evaporated and the reaction products 1b and 15b were isolated by flash chromatography.
15b: m.p. 161-162°C; ^{1}H-NMR ($CDCl_3$): 1.94 (t, 2H, CH_2D, J = 2.1 Hz), 2.86 (d, 3H, $NHCH_3$, J = 5.7 Hz), 3.33 (s, 3H, NCH_3), 3.43 (s, 3H, NCH_3), 3.61 (m, 1H, $NHCH_3$); MS: found 184.1076, calc. for $C_8H_{12}DN_3O_2$: 184.1068.

Reaction of intermediate E with 1-benzyl-3-carbamoyl-1,4-dihydroquinoline (11)
A solution of 80 mg (0.30 mmol) of 11 and 105 mg (0.30 mmol) of 3b in 6 ml CH_3CN/CH_3COOH 3:2 was stirred at room temperature during one night. HPLC analysis showed a complete conversion of 3b and 11 to 1b and 13. Toluene was added to the reaction mixture, the solvents were evaporated and the products were isolated by flash chromatography. 13 was crystallized from CH_3CN.
13: m.p. 247-249°C (dec.); ^{1}H-NMR ($CDCl_3$): 2.40 (s, 3H, NCH_3), 2.70 (d, 1H, C(5)H (or C(6)H), J = 18.7 Hz), 2.79 (d, 1H, C(5)H (or C(6)H), J = 18.7 Hz), 2.80 (d, 1H, C(6)H (or C(5)H), J = 16.2 Hz), 2.88 (s, 3H, NCH_3), 3.30 (s, 3H, NCH_3), 3.58 (d, 1H, C(6)H (or C(5)H), J = 16.2 Hz), 4.50 (d, 1H, CH_2Ar, J = 17.9 Hz), 4.60 (d, 1H, CH_2Ar, J = 17.9 Hz), 4.88 (s, 1H, C(11a)H), 5.52 (s, 1H, $CONH_2$), 6.08 (s, 1H, $CONH_2$), 6.41 (dd, 1H, C(10)H, J = 1.0 Hz, J = 8.3 Hz), 6.70 (dt, 1H, C(3)H, J = 1.0 Hz, J = 7.4 Hz), 7.02 (m, 4H, CH_2Ar + C(7)H + C(9)H), 7.15 (m, 3H, CH_2Ar); IR (KBr): 3440, 1685, 1665, 1645, 1610, 1485, 760; MS (FD): 445 (M^+).

Reaction of intermediate E with Hantzsch ester (12a) and 4,4-dideutero-Hantzsch ester (12b)
A solution of 175mg (0.5 mmol) of 3b and 125 mg (0.5 mmol) of 12a (or 12b) in 6 ml CH_3CN/CH_3COOH 3:2 was stirred under N_2 at room temperature during one night. HPLC analysis showed a complete conversion of 3b and 12a (or 12b) to 1b and 14 (or 6,6-dideuterio-14). Toluene was added to the reaction mixture, the solvents were evaporated and the products were isolated by flash chromatography.
14: ^{1}H-NMR ($CDCl_3$): 1.18 (m, 6H, 2x CH_2CH_3), 1.53 (s, 3H, C(9a)CH_3), 2.16 (s, 3H, C(8)CH_3), 2.43 (m, 2H, C(5)H + C(6)H), 2.68 (d, 1H, C(5)H, J = 17.0 Hz), 2.72 (s, 3H, NCH_3), 2.86 (dd, 1H, C(6)H, J = 1.2 Hz, J = 17.5 Hz), 3.28 (s, 3H, NCH_3), 3.30 (s, 3H, NCH_3), 3.98-4.17 (m, 5H, 2x CH_2CH_3 + NH); IR (KBr): 3380, 2965, 1720, 1690, 1620, 1480, 1285, 1100, 785; MS (FD): 434 (M^+).

6,6-Dideuterio-14: ^{1}H-NMR ($CDCl_3$): 1.20 (m, 6H, 2x (CH_2CH_3), 1.56 (s, 3H, C(9a)CH_3), 2.19 (s, 3H, C(8)CH_3), 2.45 (d, 1H, C(5)H, J = 17.4 Hz), 2.71 (d, 1H, C(5)H, J = 17.4 Hz), 2.75 (s, 3H, NCH_3), 3.33 (s, 3H, NCH_3), 3.34 (s, 3H, NCH_3), 3.98 (s, 1H, NH), 3.99-4.23 (m, 4H, 2x CH_2CH_3); IR (KBr): 3380, 2965, 1720, 1690, 1620, 1480, 1285, 1100, 785; MS (FD): 436 (M^+).

Thermal transformation of 14a into 15a and oxidized Hantzsch ester
A solution of 34 mg of 14a in 2.5 ml CH_3CN/CH_3COOH 3:2 was heated under N_2 at 80°C. After 4 hours HPLC analysis showed a complete conversion of 14a to 15a and oxidized Hantzsch ester.

NMR spectra of 10 in (a) DMSO, (b) TFA and of 1a in TFA

10: ^{1}H-NMR (DMSO-d_6): 3.16 (s, 6H, 2x NCH_3), 3.37 (s, 6H, 2x NCH_3), 3.72 (s, 3H, OCH_3), 5.55 (s, 1H, CH), 6.77 (d, 2H, Ar, J = 8.6 Hz), 7.00 (d, 2H, Ar, J = 8.6 Hz), 7.47 (s, 4H, 2x NH_2).
10: ^{1}H-NMR (TFA-d, 60 MHz): 3.57 (s, 6H, NCH_3(E) + NCH_3(1a)), 3.70 (s, 3H, NCH_3 (1a)), 3.82 (s, 3H, NCH_3(E)), 4.08 (s, 3H, OCH_3), 7.21 (d, 2H, Ar, J = 8.5 Hz), 8.31 (d, 2H, Ar, J = 8.5 Hz), 8.78 (s, 1H, CH), 11.75 (TFA).
1a: ^{1}H-NMR (TFA-d, 60 MHz): 3.57 (s, 3H, NCH_3), 3.70 (s, 3H, NCH_3), 11.75 (TFA). C(6)-NH_2 and C(5)-H are rapidly exchanged by deuterium.

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REFERENCES

1. Part 15. A.R. Stoit and U.K. Pandit, Heterocycles, 1986, in press.
2. Taken in part from the forthcoming doctorate dissertation of P.F.C. van der Meij, University of Amsterdam.
3. a. D.V. Santi and P.V. Danenberg, in R.L. Blakley and S.J. Benkovic, Eds., Folates and Pterines, Vol. I, Wiley, New York, 1984, pp. 345-398;
   b. S.J. Benkovic, Acc. Chem. Res. 11, 314 (1978);
   c. S.J. Benkovic, Ann. Rev. Biochem. 49, 227 (1980);
   d. L.J. Slieker and S.J. Benkovic, J. Am. Chem. Soc. 106, 1833 (1984).
4. Ref. 3a, pp. 353-360, and references cited therein.
5. Ref. 3a, pp. 375-380, and references cited therein.
6. a. P.F.C. van der Meij, R.D. Lohmann, E.R. de Waard, T.B.R.A. Chen, and U.K. Pandit, J. Chem. Soc., Chem. Comm. 1229 (1985);
   b. H.C. Hiemstra, H. Bieräugel, M. Wijnberg and U.K. Pandit, Tetrahedron 39, 3981 (1983);
   c. H. Bieräugel, R. Plemp and U.K. Pandit, Tetrahedron 39, 3987 (1983);
   d. A. Stoit and U.K. Pandit, Tetrahedron 41, 3355 (1985).
7. a. H. Bieräugel, R. Plemp, H.C. Hiemstra and U.K. Pandit, Tetrahedron 39, 3971 (1983);
   b. T.H. Fife and A.M. Pellino, J. Am. Chem. Soc. 102, 3062 (1980);
   c. T.H. Fife and A.M. Pellino, J. Am. Chem. Soc. 103, 1201 (1981).
8. A.D. Broom, J.L. Shim and G.L. Anderson, J. Org. Chem. 41, 1095 (1976).
9. W.C. Still, M. Kahn and A. Mitra. J. Org. Chem. 43, 2923 (1978).
10. W. Pfleiderer and K.H. Schündehütte, Anal. 612, 158 (1958).
11. C.W. Whitehead and J.J. Traverso, J. Am. Chem. Soc. 82, 3971 (1960).
12. N.J. Leonard, G.W. Leubner and E.H. Burk, J. Org. Chem. 15, 979 (1950).
13. F. Yoneda, F. Takayama and A. Koshiro, Chem. Pharm. Bull. 27, 2507 (1979).
14. A. Singer and S.M. McElvain, Org. Synth. 14, 30 (1934).
15. S. Shinkai, H. Hamada, T. Ide and O. Manake, Chem. Lett. 685 (1978).
16. W. Pfleiderer and H. Ferch, Anal. 615, 52 (1958).

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