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MODELS OF FOLATE COFACTORS 17.[1] METHYLATION OF 1,3-DIMETHYL-6-METHYLAMINO-URACIL BY A 5,10-CH_2-H_4FOLATE MODEL. THE FIRST MIMIC OF THE OVERALL dTMP SYNTHASE REACTION

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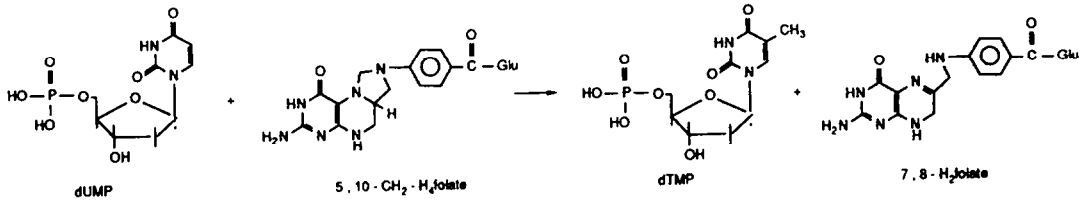
Abstract - 2-Tosyl-1,2,3,12b-tetrahydroimidazo[1,5-f]phenanthridine (6) was prepared in five steps from 6-chloromethylphenanthridine (1) in an overall yield of 50%. The reaction of 6 with 1,3-dimethyl-6-methylaminouracil (8) in CH_3CN/TFA (100:1) at 50-80°C, gave a mixture of products from which 1,3-dimethyl-6-methylaminothymine (9a) could be isolated. This constitutes a mimic of the overall dTMP synthase reaction in transferring both a methylene unit and a hydride equivalent from the cofactor model 6 to the 5-position of a uracil derivative. The other products which are formed in the reaction of 6 with 8 are derived from the reaction of the exocyclic intermediate C, formed in the carbon transfer step, with nucleophiles present in the reaction mixture.

INTRODUCTION

The enzyme dTMP synthase (thymidylate synthase, EC 2.1.1.45) catalyses the methylation of 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP) in a two step process, in which transfer of the methylene group from the cofactor 5,10-methylene-5,6,7,8-tetrahydrofolate to the 5-position of dUMP is followed by reduction of the methylene unit by the C(6)-hydrogen of the cofactor (Scheme I).



Scheme I

The currently accepted mechanism of the enzymic reaction[2] involves: (1) the formation of a covalent apoenzyme-substrate-cofactor ternary complex, by nucleophilic attack of a cysteine residue of the apoenzyme on the 6-position of dUMP, followed by reaction of the resulting nucleophilic centre at C(5) with the activated form of the coenzyme ($CH_2=\overset{+}{N}(5)H_4$folate); (2) fragmentation of the ternary complex into an exocyclic methylene intermediate and H_4folate and (3) reduction of the last

mentioned intermediate by H_4folate with concomitant expulsion of the apoenzyme resulting in the formation of dTMP and 7,8-H_2folate.

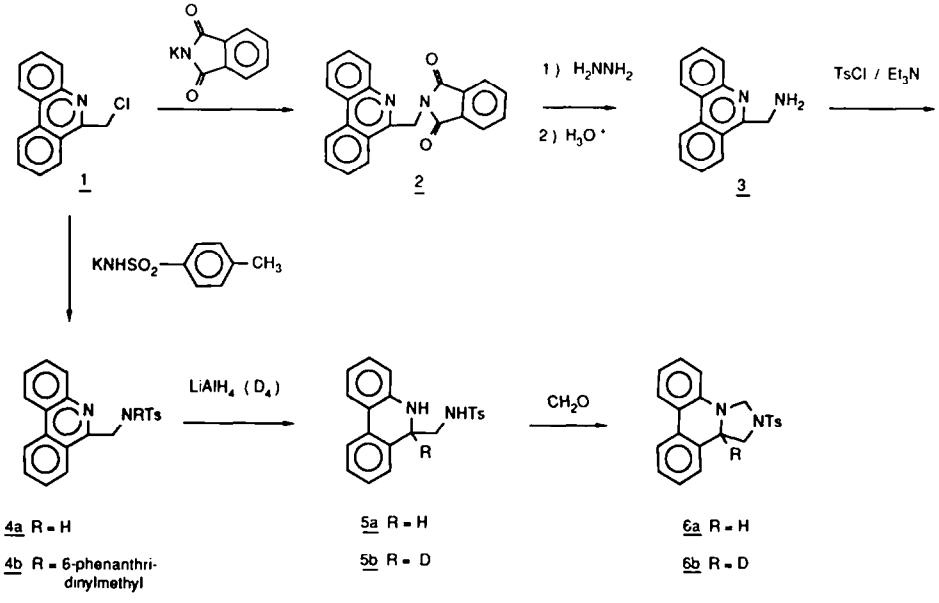
The carbon transfer step of this mechanism has received support from model studies[3a] and studies of mechanism-based inhibitors.[3b] In addition, a recent report claims the isolation of the native covalent ternary complex.[4] The postulated reduction step has, however, received little attention thus far and the nature of the transferred hydrogen moiety (i.e. a direct hydride transfer (H^-) or a two step radical mechanism (e^-+$H^\cdot$)) remains to be elucidated.[5]

To throw light on various aspects of the mechanism of the enzymic transformation we have undertaken the development and investigation of chemical models of the dTMP synthase reaction. In a previous study[1] we have shown that the reaction of 1,3-dimethyl-6-methylaminouracil (8) (which can be regarded as a model of the apoenzyme-substrate complex) with imidazolidines (cofactor models), in the presence of acid, leads to the formation of the corresponding C(5)-exocyclic methylene intermediate, which, when generated in situ from di-(1,3-dimethyl-6-methylaminouracil-5-yl)methane (13), can be reduced by several reducing agents to 1,3-dimethyl-6-methylaminothymine (9). In this communication we demonstrate that the 5,10-CH_2-H_4folate model 6 is capable of transferring both a methylene unit and a hydride equivalent to the C(5)-position of the uracil derivative 8. This transformation can be regarded as the first mimic of the overall dTMP synthase reaction.[6] In the context of this comparison, however, it should be noted that 8 is at a higher oxidation state than the natural binary complex and as a consequence more nucleophilic at C(5).

RESULTS AND DISCUSSION

Synthesis of 6.

The 5,10-CH_2-H_4folate model 6 can be prepared from 6-chloromethylphenanthridine (1) in five steps, via the sequence 1 → 2 → 3 → 4a → 5 → 6, in an overall yield of 50% (Scheme II).



Scheme II

The Gabriel synthesis of 3 followed by its reaction with p-toluenesulfonylchloride, in the presence of triethylamine, yielded 4a in 82%. Since the isolation of 3 was laborious, it was usually converted to 4a without purification. Efforts to shorten the synthesis of 4a to one step (viz. 1 → 4a Scheme II) were not successful, since the reaction of 1 with potassium p-toluenesulfonamide in DMF

gave 4a in only 13% yield; the main product being the N,N-diphenanthridinyl-p-toluenesulfonamide 4b. It is noteworthy, however, that 4a is formed under these conditions since it was reported in the literature[7] that 4a was not formed in the reaction of 1 with p-toluenesulfonamide in a water/acetone mixture. Reduction of 4a with $LiAlH_4$ or $LiAlD_4$ gave the corresponding dihydrophenanthridine derivatives 5a,b which, upon subsequent reactions with formaldehyde yielded the folate models 6a,b, respectively. 4a was usually converted to 6(a,b) without isolation of 5(a,b). During the last step care should be taken to avoid a large excess of formaldehyde since that leads to the formation of the N(1),N(3)-dihydroxymethyl derivative of 5.

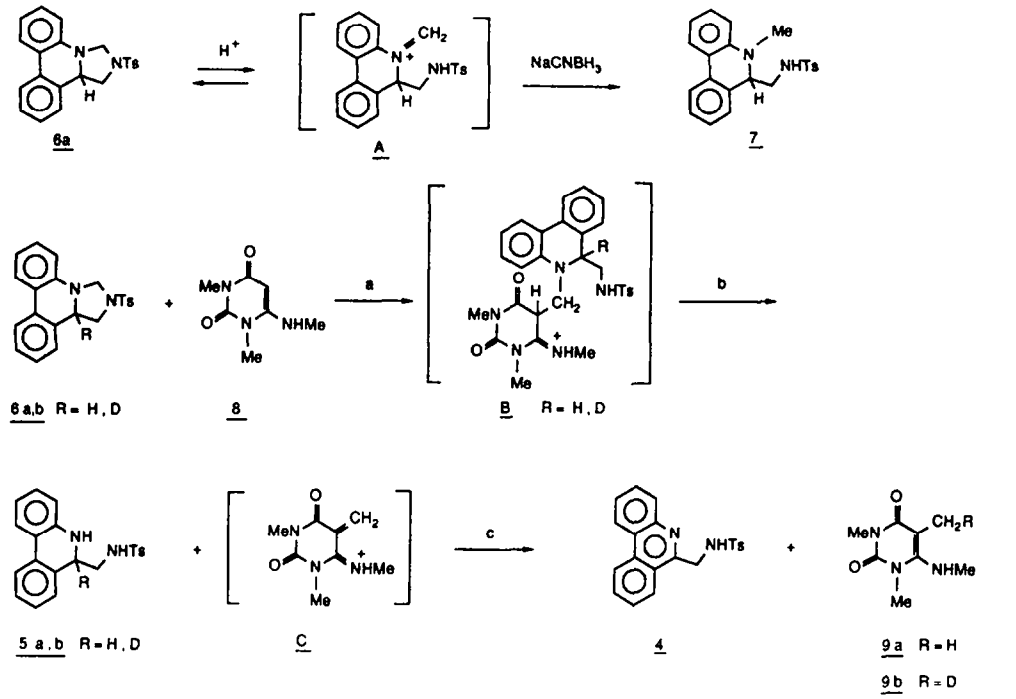
Acid catalyzed ring-opening of 6.

The methylene transfer from the cofactor 5,10-CH_2-H_4folate (to the apoenzyme-substrate complex) is initiated by an acid catalyzed ring-opening of the imidazolidine moiety of the cofactor, to give the corresponding N(5) iminium cation. This finding is in agreement with model studies[8] involving unsymmetrically substituted 1,3-imidazolidines, which have been shown to undergo acid mediated ring-opening to the most thermodynamically favoured cations. In the case of folate cofactor model 6 it was anticipated that acid catalyzed ring-opening should lead to the formation of the N(3) iminium cation, i.e. the analogue of $CH_2=\overset{+}{N}(5)H_4$folate. That this indeed is the course of the reaction was attested by reductive trapping of the cation. When 6 was allowed to react with $NaCNBH_3$, in the presence of acetic acid, the N(3)-methyl derivative 7 was indentified as the exclusive product (Scheme III).

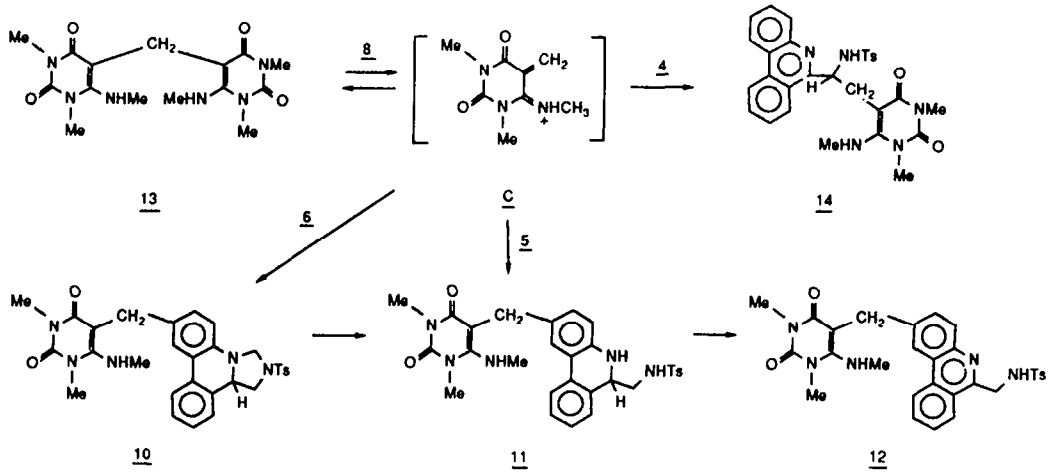
Reaction of 6a,b with 8.

Upon allowing a mixture of 1,3-dimethyl-6-methylaminouracil (8) and 6a, in acetonitrile and trifluoroacetic acid (TFA) (100:1 v/v), under argon, to reflux for 30 minutes, a mixture of products was obtained from which 1,3-dimethyl-6-methylaminothymine (9a) could be isolated in 10% yield. The formation of 9a can be rationalized on the basis of the reaction steps described in Scheme III. Intermediate A, derived by acid catalyzed ring-opening of 6a, reacts with 8 (step a) to result in the formation of adduct B. This adduct subsequently fragments into the exocyclic methylene intermediate C and dihydrophenanthridine derivative 5a (step b). Reduction of C by 5a, presumably via a hydride transfer process, results in the formation of 9a and 4a (step c). In agreement with this mechanism it was observed that the labelled thymine derivative 9b, containing a deuterium atom in the C(5)-methyl group (vide experimental, NMR), was formed, when 6b was employed instead of 6a. The transfer of one deuterium atom, presumably as D^-, is similar to the label transfer in models of the Friedkin mechanism, for which exocyclic methylene intermediates have been proposed.[9a,b]

While the formation of 9 is mechanistically highly significant, its yield in these reactions is low on account of the competitive formation of products which are derived from the reactions of the electrophilic intermediate C with nucleophiles present in the reaction mixture (Scheme IV). Thus, the reaction of C with 8 leads to the reversible formation of 13.[1] The formation of 10, resulting from the reaction of C with 6, is also transient in character. Since 10 itself is an imidazolidine, it can react with 8, in a manner analogous to 6, to form additional amounts of intermediate C and product 11. The last mentioned compound is also formed in the reaction of C with 5. In fact 11 is the main component of the reaction mixture. Finally, oxidation of 11, under conditions of the reaction, leads to the formation of 12. In a separate experiment it could be shown that 12 is not formed as a result of the reaction of C with phenanthridine derivative 4a. Thus, when C, generated in situ from 13, was allowed to react with 4a, it did not yield 12, but gave 14 as the sole reaction product. This reaction is not unprecedented. In the literature[7] it is reported that 6-methylphenanthridine reacts with paraform and dimethylaminehydrochloride, under conditions of the Mannich reaction, to yield 6-(β-dimethylaminoethyl)phenanthridine. This reaction presumably proceeds via the enamine tautomer of 6-methylphenanthridine. A similar tautomerisation of 4a, followed by attack by C leads to 14. No 14 is formed in the reaction of 6a with 8 due to low concentrations of 4a and C in the reaction mixture.



Scheme III



Scheme IV

In order to study the influence of temperature on the course of the reaction, the compositions of the reaction mixtures, at refluxed temperature (80°C) and at 50°C were monitored (HPLC). The following differences were prominent. (i) In contrast to the reaction at 80°C, where no 6 could be

detected within 5 minutes; at 50°C, the presence of 6 could be demonstrated even after 25 minutes. (ii) At 50°C, the product profiles in the initial stages of the reaction exhibited higher concentrations of compounds 5, 10 and 13 relative to the reaction at 80°C (vide experimental). It should, however, be noted that since both 10 and 13 are transient with respect to stabler products, the compositions of the final mixtures of the reaction at the two temperatures are virtually the same. The study of the reaction at room temperature was thwarted by the poor solubility of 6 under the reaction conditions.

CONCLUSIONS

The present study has shown that reaction of 6 with 8 corresponds to a mimic of the dTMP synthase reaction in transferring both a methylene unit and a hydride equivalent from the cofactor model 6 to the 5-position of the uracil derivative 8. The carbon transfer step in the reaction leads to the formation of an exocyclic methylene intermediate which is reduced to the thymine derivative by the dihydrophenanthridine moiety of the model compound. Competitive reactions of the exocyclic methylene intermediate with nucleophiles present in the reaction mixture decrease the yield of the thymine derivative. The development of sophisticated mechanistically based models of the apoenzyme-substrate complex and studies aimed at the elucidation of the mechanism of the reduction step are currently in progress.

EXPERIMENTAL

NMR spectra were determined with Bruker WM 250 (250 MHz) and AC 200 (200 MHz) instruments. TMS was used as internal standard. The chemical shifts (δ) are given in ppm and spin-spin coupling constants in Hertz (Hz). Mass spectra were obtained with a Varian Matt 711 spectrometer (EI = electron impact, FI = field ionization, FD = field desorption). Infrared spectra were recorded on a Perkin Elmer 1310 spectrophotometer. The absorptions are given in cm^{-1}. Melting points were determined on a Leitz Wetzlar apparatus and are uncorrected.

Analytical HPLC was performed using a high speed reversed phase column (Pecosphere 3CR C18, 4.6 x 33 mm) and CH_3CN/H_2O 45:55 was used as mobile phase. The flow rate was set at 2 ml/min., using a Perkin Elmer 3D HPLC system (UV detection at 254 nm). Preparative HPLC was performed using a Polygosil 60 C18 column (16 x 250 mm). The mobile phase used to isolate the thymine derivatives consisted of $MeOH/H_2O$ 30:70 and was pumped at 7 ml/min using a Perkin-Elmer series 10 liquid chromatograph.The Holochrome variable wavelength detector used was set at 286 nm. Elemental analyses 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 using silica gel 60 (Merck) and mixtures of CH_3OH/CH_2Cl_2 as eluents. 1,3-Dimethyl-6-methylaminouracil was prepared from 6-chloro-1,3-dimethyluracil (Aldrich)[10] and 6-chloromethylphenanthridine was obtained in two steps from 2-aminobiphenyl (Aldrich)[11].

6-(Phthalimidomethyl)phenanthridine (2).
A mixture of 500 mg (2.2 mmol) of 6-chloromethylphenanthridine and 450 mg (2.4 mmol) of potassiumphthalimide in 5 ml DMF was stirred at 80°C for 15 minutes. After cooling to room temperature 100 ml of CH_2Cl_2 was added and the resulting suspension was filtered. The filtrate was evaporated and the white residue was crystallized from ethanol, yielding 720 mg of 2 (97%); m.p. 270.5-271.5°C; IR (KBr): 1765, 1710 (vs), 1420, 1395, 1110, 940, 745, 710; ^1H NMR ($CDCl_3$): δ 5.60 (s, 2H, CH_2), 7.56 (m, 2H, ArH), 7.70-7.96 (m, 7H, ArH), 8.25 (d, 1H, J = 8.0, ArH), 8.48 (m, 1H, ArH), 8.64 (d, 1H, J = 8.2, ArH); MS (EI): 338 (M^+, 100), 293 (100), 254 (15), 205 (28), 179 (22), 178 (22), 151 (18), 104 (20), 84 (20), 77 (31), 76 (25), 49 (30).

6-(p-Tosylamidomethyl)phenanthridine (4a) (1 → 2 → 3 → 4a).
A suspension of 2.5 g (7.4 mmol) of 2 and 0.5 ml of hydrazine monohydrate in 400 ml ethanol was refluxed until a clear colorless solution was obtained (∿ 24 hours). After stirring for an additional two hours at reflux temperature 2.5 ml of conc. HCl was added. The solution was cooled to room temperature and evaporated under reduced pressure. To the residue 200 ml CH_2Cl_2 and 3 ml triethylamine were added and the resulting suspension was filtered. To the colorless filtrate 1.4 g (7.3 mmol) of p-toluenesulfonylchloride and 2 ml of triethylamine were added and the solution was stirred overnight at room temperature. After filtration, the solution was washed with dil. HCl and brine, dried over $MgSO_4$ and evaporated. The residue was crystallized from ethanol, yielding 2.2 g of 4a (82%); m.p. 171.5-173°C; IR ($CHCl_3$): 3020, 1715, 1355, 1160; ^1H NMR ($CDCl_3$): δ 2.28 (s, 3H, CH_3), 4.79 (d, 2H, J = 4.3, CH_2), 6.99 (t, 1H, J = 4.3, NHTs), 7.17 (d, 2H, J = 8.0, ArH), 7.62-7.78 (m, 3H, ArH), 7.81-7.87 (m, 3H, ArH), 7.98 (d, 1H, J = 7.7, ArH), 8.08 (dd, 1H, J = 1.3, J = 8.1, ArH), 8.50 (dd, 1H, J = 1.5, J = 8.0, ArH), 8.60 (d, 1H, J = 8.2, ArH); MS (EI): 362 (M^+, 5), 207 (100), 179 (63), 151 (15), 91 (37).

6-(p-Tosylamidomethyl)phenanthridine (4a) (1 → 4a).
A solution of 1 g (4.3 mmol) of 1 in 5 ml dry DMF was added to a stirred suspension of 0.9 g (4.3 mmol) of p-$CH_3C_6H_4SO_2NHK$ in 45 ml dry DMF. The mixture was stirred at 50°C for 1 hour and evaporated in vacuo. To the residue 100 ml of CH_2Cl_2 was added, the resulting suspension was filtered,

the filtrate was evaporated and the residue was separated by flash chromatography (SiO_2, CH_2Cl_2/CH_3OH 95:5) yielding 200 mg (13%, based on 1) of 4a (after crystallization from ethanol) and 670 mg (56% based on 1) of 4b (after crystallization from ethanol): m.p. 168°C, dec. (lit.[7], 160-161°C); ^1H NMR ($CDCl_3$): δ 2.49 (s, 3H, $ArCH_3$), 5.07 (s, 4H, $2xCH_2$), 7.23-7.48 (m, 12H, ArH), 7.90-8.03 (m, 6H, ArH), 8.44 (d, 2H, ArH, J = 8.4); MS (FD): 553 (M^+), 554 (M^++H).

6-(p-Tolylamidomethyl)-5,6-dihydrophenanthridines (5a and 5b)
A mixture of 150 mg (0.42 mmol) of 4a and 100 mg of $LiAlH_4$ (2.6 mmol) in 20 ml of dry THF was stirred at room temperature for three hours. After addition of water the precipitate was filtered and the filtrate was evaporated. The residue was purified by column chromatography (SiO_2, CH_2Cl_2/CH_3OH 95:5), whereupon 5a was obtained in 65% (98 mg) yield. The product was crystallized from ethanol. 5b was obtained according to the same procedure.
5a: m.p. 163-164.5°C; IR ($CHCl_3$): 3390, 3020, 1605, 1490, 1445, 1330, 1160, 1090; ^1H NMR ($CDCl_3$): δ 2.40 (s, 3H, CH_3), 2.91 (td, 1H, J = 4.7, J = 5.8, J = 13.6, CH_2), 3.09 (td, 1H, J = 7.2, J = 8.8, J = 13.6, CH_2), 4.49 (dd, 1H, J = 4.6, J = 8.8, CH), 4.4-4.7 (bs, 1H, NH), 4.80 (dd, 1H, J = 5.8, J = 7.2, NHTs), 6.68 (d, 1H, J = 7.8, ArH), 6.81 (dt, 1H, J = 1.0, J = 7.7, ArH), 7.06-7.38 (m, 6H, ArH), 7.62-7.73 (m, 4H, ArH); MS (EI): 364 (M^+, 1%), 335 (5), 207 (52), 181 (49), 180 (100), 179 (55), 152 (30), 91 (30); MS (FD): 364 (M^+).
5b: m.p. 164-165.5°C; IR ($CHCl_3$): 3390, 3010, 1605, 1490, 1445, 1330, 1160, 1090; ^1H NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3), 2.89 (dd, 1H, J = 5.6, J = 13.6, CH_2), 3.06 (dd, 1H, J = 7.0, J = 13.6, CH_2), 4.4-4.7 (bs, 1H, NH), 4.85 (dd, 1H, J = 5.9, J = 7.0, NHTs), 6.66 (d, 1H, J = 8.0, ArH), 6.79 (t, 1H, J = 7.3, ArH), 7.10-7.34 (m, 6H, ArH), 7.63-7.69 (m, 4H, ArH); MS (EI): 365 (M^+, 1%), 336 (5), 207 (27), 182 (90), 181 (100), 180 (45), 153 (27), 152 (24), 91 (25); MS (FD): 365 (M^+).

2-Tosyl-1,2,3,12b-tetrahydroimidazo[1,5-f]phenanthridines (6a and 6b).
A mixture of 250 mg (0.7 mmol) of 4a and 150 mg (4 mmol) of $LiAlH_4$ in 40 ml dry THF was stirred at room temperature for three hours. After addition of water the precipitate was filtered and to the filtrate 100 mg (1.2 mmol) of 37% CH_2O (in water) was added. The solution was refluxed for 30 minutes, cooled to room temperature and evaporated. The residue was purified by column chromatography (SiO_2, CH_2Cl_2): 163 mg of 6a (63%). The product was crystallized from ethanol. 6b was obtained according to the same procedure.
6a: m.p. 188-189°C; IR ($CHCl_3$): 3010, 1600, 1495, 1450, 1370, 1350, 1300, 1160, 1090; ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H, CH_3), 3.47 (dd, 1H, J = 8.6, J = 9.7, CH_2), 4.17 (dd, 1H, J = 5.7, J = 8.6, CH_2), 4.43 (dd, 1H, J = 5.7, J = 9.7, CH), 4.52 (d, 1H, J = 5.5, N-CH_2-N), 4.79 (d, 1H, J = 5.5, N-CH_2-N), 6.46 (d, 1H, J = 8.0, ArH), 6.91 (m, 2H, ArH), 7.27 (m, 5H, ArH), 7.70 (m, 4H, ArH); MS (EI): 376 (4), 222 (42), 218 (66), 180 (100), 91 (30), 42 (55); exact mass: found: 376.1231, calc. for $C_{22}H_{20}N_2O_2S$: 376.1245; anal. found: C, 69.70; H, 5.34; N, 7.52; O, 8.60; calc. for $C_{22}H_{20}N_2O_2S$: C, 70.19; H, 5.35; N, 7.44; O, 8.50.
6b: m.p. 190-191°C; IR ($CHCl_3$): 3015, 1600, 1490, 1445, 1370, 1350, 1160, 1090; ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H, CH_3), 3.46 (d, 1H, J = 8.6, CH_2), 4.17 (d, 1H, J = 8.6, CH_2), 4.52 (d, 1H, J = 5.5, N-CH_2-N), 4.79 (d, 1H, J = 5.5, N-CH_2-N), 6.46 (d, 1H, J = 8.0, ArH), 6.91 (m, 2H, ArH), 7.27 (m, 5H, ArH), 7.70 (m, 4H, ArH); MS (EI): 377 (6), 222 (52), 218 (53), 181 (100), 91 (32), 42 (45); exact mass: found: 377.1315, calc. for $C_{22}H_{19}DN_2O_2S$: 377.1308.

Reductive trapping of intermediate A.
A mixture of 36 mg (0.1 mmol) of 6a and 25 mg of $NaCNBH_3$ (4 eq.) in 5 ml of CH_3CN/CH_3COOH (3:2) was stirred at 70°C for 1 hour. After cooling to room temperature the solution was neutralized with $NaHCO_3/H_2O$ and extracted with CH_2Cl_2 (3x). The organic layer was dried over $MgSO_4$ and evaporated. TLC analysis (SiO_2, CH_3OH/CH_2Cl_2 5:95) showed only one reaction product, viz. 7; IR ($CHCl_3$): 3395, 3020, 1600, 1490, 1450, 1405, 1330, 1160, 1090; ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H, CH_3), 2.85 (td, 1H, J = 5.7, J = 7.3, J = 13.4, CH_2), 3.06 (s, 3H, NCH_3), 3.10 (td, 1H, J = 6.1, J = 7.5, J = 13.4, CH_2), 4.27 (dd, 1H, J = 5.7, J = 7.5, NHTs), 4.37 (dd, 1H, J = 6.1, J = 7.3, CH), 6.65 (d, 1H, J = 8.1, ArH), 6.81 (dt, 1H, J = 1.0, J = 7.5, ArH), 7.11-7.36 (m, 6H, ArH), 7.58 (d, 2H, J = 8.3, ArH), 7.63-7.71 (m, 2H, ArH). MS (EI): 378 (M^+, 1%), 195 (37), 194 (100), 179 (15), 152 (12), 91 (21); MS (FD): 378 (M^+).

Reaction of 6a with 8 at 80°C.
A solution of 95 mg (0.25 mmol) of 6a and 45 mg (0.26 mmol) of 8 in 10 ml CH_3CN was stirred at 80°C under argon. After 15 minutes 90 µl of TFA was added and the reaction was monitored by HPLC (see text). Analysis of the mixture showed that several products which were intially present (viz. 5a, 6a, 10 and 13) disappeared almost completely after 5 minutes. At this stage the mixture mainly consisted of 4a, 8, 9a, 11 and 12. After 30 minutes, the mixture was cooled to room temperature, toluene was added and the solution was evaporated. From the residue 9a, 11 and 12 were isolated by flash chromatography (CH_2Cl_2/CH_3OH 95:5). The first separation yielded a fraction (13.5 mg) consisting of 9a and 12, which was separated a second time to yield 4 mg (∿ 10%) of 9a. 4a, 5a, 10 and 13 were not isolated from the reaction mixture, but were identified by comparison with authentic samples (retention time HPLC). The product 10 was prepared from 11 (reaction with 1.1 eq. CH_2O in THF) and 13 was prepared from 8 according to literature procedure.[1]
9a: ^1H NMR ($CDCl_3$): δ 1.95 (s, 3H, C(5)CH_3), 2.86 (d, 3H, J = 5.3, $NHCH_3$), 3.33 (s, 3H, NCH_3), 3.43 (s, 3H, NCH_3), 3.61 (m, 1H, $NHCH_3$); MS (EI): 183 (M^+, 100%), 154 (35), 153 (15), 125 (20), 97 (26), 96 (57), 71 (22), 69 (30), 68 (24), 56 (16); exact mass: calc. for $C_8H_{13}N_3O_2$: 183.1005, found: 183.1010.
10: ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H, $ArCH_3$), 2.67 (d, 3H, J = 5.7, $NHCH_3$), 3.38 (s, 3H, NCH_3), 3.40 (s, 3H, NCH_3), 3.44 (m, 1H, CH_2), 3.70 (m, 1H, $NHCH_3$), 3.79 (s, 2H, $ArCH_2$), 4.15 (dd, 1H, J = 5.9, J = 8.6, CH_2), 4.37 (dd, 1H, J = 5.9, J = 9.5, CH), 4.47 (d, 1H, J = 5.5, NCH_2N), 4.77 (d, 1H, J = 5.5, NCH_2N), 6.38 (d, 1H, J = 8.1, ArH), 6.93 (d, 1H, J = 7.4, ArH), 7.02 (dd, 1H, J = 1.6, J = 8.2, ArH), 7.20-7.35 (m, 4H, ArH), 7.52 (d, 1H, J = 1.4, ArH), 7.60-7.80 (m, 3H, ArH). MS (FI): 557 (M^+).
11: ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H, $ArCH_3$), 2.66 (d, 3H, J = 5.6, $NHCH_3$), 2.72-2.89 (m, 1H, CH_2),

2.93-3.10 (m, 1H, CH_2), 3.36 (s, 3H, NCH_3), 3.40 (s, 3H, NCH_3), 3.75 (s, 2H, $ArCH_2$), 3.90 (q, 1H, J = 5.6, $NHCH_3$), 4.40 (dd, 1H, J = 4.1, J = 9.0, CH), 4.55-4.70 (bs, 1H, NH), 5.34 (dd, 1H, NHTs), 6.55 (d, 1H, J = 8.0, ArH), 6.88 (dd, 1H, J = 1.5, J = 8.0, ArH), 7.04 (d, 1H, J = 7.2, ArH), 7.09-7.32 (m, 5H, ArH), 7.44 (d, 1H, J = 1.2, ArH), 7.62 (d, 2H, J = 8.2, ArH); MS (FI): 545 (M^+).
12: ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H, $ArCH_3$), 2.67 (d, 3H, J = 5.8, $NHCH_3$), 3.40 (s, 3H, NCH_3), 3.42 (s, 3H, NCH_3), 3.84 (q, 1H, J = 5.8, $NHCH_3$), 4.08 (s, 2H, $ArCH_2$), 4.7 (d, 2H, J = 4.0, CH_2NHTs), 6.93 (t, 1H, J = 4.0, NHTs), 7.17 (d, 2H, J = 8.1, ArH), 7.55 (dd, 1H, J = 1.7, J = 8.5, ArH), 7.64 (dt, 1H, J = 0.7, J = 7.7, ArH), 7.77-7.82 (m, 3H, ArH), 7.92 (d, 1H, J = 8.2, ArH), 7.97 (d, 1H, J = 8.4, ArH), 8.29 (d, 1H, J = 1.0, ArH), 8.53 (d, 1H, J = 8.2, ArH); MS (FI): 543 (M^+).

Reaction of 6b with 8 at 80°C.
After stirring a mixture of 6b (150 mg) and 8 (67 mg) in CH_3CN (10 ml, 80°, 30 min, Ar), 100 µl of TFA was added and the mixture was further stirred for 2 hours. The mixture was cooled to room temperature, toluene was added and the solution was evaporated. The residue was chromatographed (SiO_2, CH_2Cl_2:CH_3OH 95:5) and the fractions containing 9b were collected. 9b was isolated by preparative HPLC, yielding 4 mg of 9b: ^{1}H NMR ($CDCl_3$): δ 1.95 (t, 2H, CH_2D, J = 2.1), 2.88 (d, 3H, $NHCH_3$, J = 5.7), 3.35 (s, 3H, NCH_3), 3.45 (s, 3H, NCH_3), 3.60 (m, 1H, $NHCH_3$); MS (EI): 184 (M^+, 100%), 155 (27), 154 (19), 126 (16), 98 (19), 97 (51), 94 (41), 71 (24), 70 (24), 69 (32), 57 (20); exact mass: calc. for $C_8H_{12}DN_3O_2$: 184.1068, found: 184.1067.

Reaction of 6a with 8 at 50°C.
A solution of 140 mg (0.37 mmol) of 6a and 70 mg (0.41 mmol) of 8 in 15 ml CH_3CN was stirred at 50°C under argon. After 15 minutes 50 µl TFA was added and the reaction was monitored by HPLC. After 2 minutes 4a, 5a, 6a, 8, 9a, 10, 11, 12 and 13 could be identified in the mixture, though 5a and 13 were the main products. After 10 minutes, a considerable amount of 6a was still present, the concentration of 13 had decreased and that of 10, 11 and 12 had increased. After 25 minutes, 6a had almost disappeared and the main products, in addition to small amounts of 4a, 9a, 10 and 13 were 5a, 8, 11 and 12. After 1.5 hours 10 and 11 had almost disappeared and the main products were 5a, unreacted 8, 11 and 12. Besides these, small amounts of 4a and 9a and traces of unidentified products could be recognized (HPLC). 9a could be isolated by flash chromatography and preparative HPLC.

Reaction of 13 with 4a.
A solution of 70 mg (0.2 mmol) of 4a and 70 mg (0.2 mmol) of 13 was stirred at 80°C in a mixture of 5 ml CH_3CN and 50 µl of TFA for 1 hour. After cooling to room temperature the TFA salt of 14 crystallized out from the reaction mixture and the yellow crystals were collected by filtration. HPLC analysis of the filtrate showed the presence of 8 besides minor amounts of unreacted 4 and 13 and 14. The solid was dissolved in CH_2Cl_2, washed with $NaHCO_3$ and the organic layer was dried over $MgSO_4$ and evaporated. The residue was crystallized from ethanol to yield 60 mg of 14 (55%).
14: m.p. 232.5-233.5°C; ^{1}H NMR ($CDCl_3$): δ 2.06 (s, 3H, $ArCH_3$), 2.35 (dd, 1H, J = 10.4, J = 14.2, CH_2), 2.78 (d, 3H, J = 5.9, $NHCH_3$), 3.14 (dd, 1H, J = 2.9, J = 14.4, CH_2), 3.33 (s, 3H, NCH_3), 3.40 (s, 3H, NCH_3), 4.57 (q, 1H, J = 5.9, $NHCH_3$), 5.37 (m, 1H, CH), 6.76 (d, 2H, J = 8.1, ArH), 7.48 (d+bm, 3H, J = 8.1, ArH + NHTs), 7.60-7.74 (m, 2H, ArH), 7.79-7.84 (m, 2H, ArH), 8.04 (dd, 1H, J = 1.2, J = 8.2, ArH), 8.49 (dd, 1H, J = 1.1, J = 8.1, ArH), 8.55 (m, 1H, ArH), 8.79 (m, 1H, ArH); MS (FI): 543 (M^+).

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