Synthesis of 7-Aza-5,8,10-trideazafolic Acid and its 4-Amino-derivative as Potential Antifolates

Günther Wollein and Reinhard Troschütz*

Department of Medical Chemistry, Emil Fischer Center, Friedrich–Alexander University, Schuhstr.19, D-91052 Erlangen, Germany

Received April 29, 2002

Dedicated to Professor Kurt Eger (Leipzig) on the occasion of his 60th birthday

o-Aminoamide **8**, an intermediate in our multistep synthesisof the title compounds was prepared from 1,3-diketone **3**. The following condensation of **8** with chloroformamidine-HCl (**9**) gave pyrido[3,4-*d*]pyrimidine **10**. Dehydratisation of amide **8** led to *o*-aminonitrile **15**, which was cyclocondensated with guanidine (**16**) to yield pyrido[3,4-*d*]pyrimidine-2,4-diamine **17**. Coupling of the acids **11** and **18** with diethyl L-glutamate (**12**) and following saponification provided 7-aza-5,8,10-trideazafolic acid **14** and its 4-amino-derivative **20**.

J. Heterocyclic Chem., **39**, 1195(2002).

In the last decades many efforts have been made to obtain structural variations of folic acid as possible antitumor agents with antifolate activity [1].

As a result, some very promising attempts lead to methotrexate (MTX) (inhibition of mammalian dihydrofolatereductase) [2], which had already been synthesized in 1947, but is still the method of choice in the treatment of mammalian carcinoma in a combination therapy. More recent compounds with antitumor potency are pemetrexed [3] (mainly inhibition of thymidilate synthetase) [4] and lometrexol (inhibition of glycineamideribonucleotidformyltransferase) [5], which are currently under investigation in clinical trials.

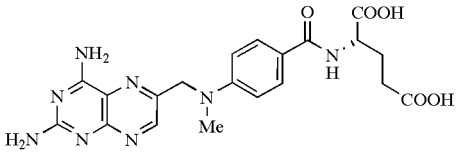
Unfortunately each of these therapeutic agents have disadvantages. Beside the common toxicity for healthy cells, some types of cancer have a naturally or acquired resistance against methotrexate [6], or – in the case of lometrexol - a cumulative toxicity [7]. For that reason it is necessary to synthesize new structural variations of antifolates having a better activity – toxicity ratio than do the existing ones.

With this intention we have planned to modify the pyrido[2,3-*d*]pyrimidine nucleus, which is present in the non classical antifolate piritrexim [8] and lometrexol. We formally shifted N^8 to position 7 in order to modulate the polarity and basicity of the anellated pyridine ring and come to a pyrido[3,4-*d*]pyrimidine as the basic heterobicycle for new antifolates, on one hand 4-amino-4-deoxy-7-aza-5,8,10-trideazafolic acid (**20**) with structural similarity to methotrexate, on the other hand 7-aza-5,8,10-trideazafolic acid (**14**), which in our opinion could posses antitumor activity.

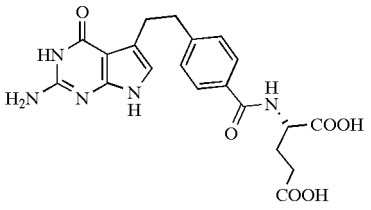
In literature only a pteroic acid analogue exists with a pyrido[3,4-*d*]pyrimidine structure [9].

Generally only few entries to substituted pyrido[3,4-*d*]-pyrimidines have been described and most of the existing routes did not seem suitable for our synthesis of 6-alkyl-substituted derivatives.[10-13]. A short retrosynthetic view, which explains our approach is outlined in scheme 2.

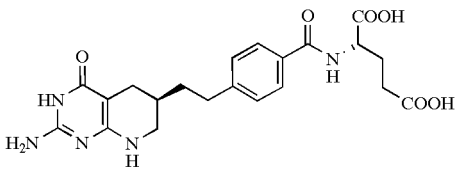
Scheme 1



Methotrexate

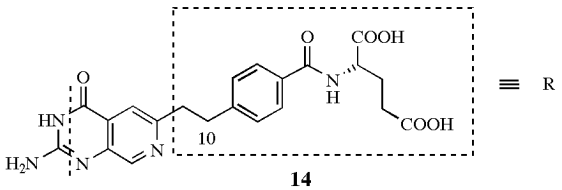


Pemetrexed

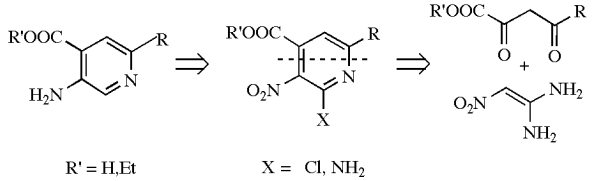


Lometrexol

Scheme 2



14



R' = H,Et X = Cl, NH_2

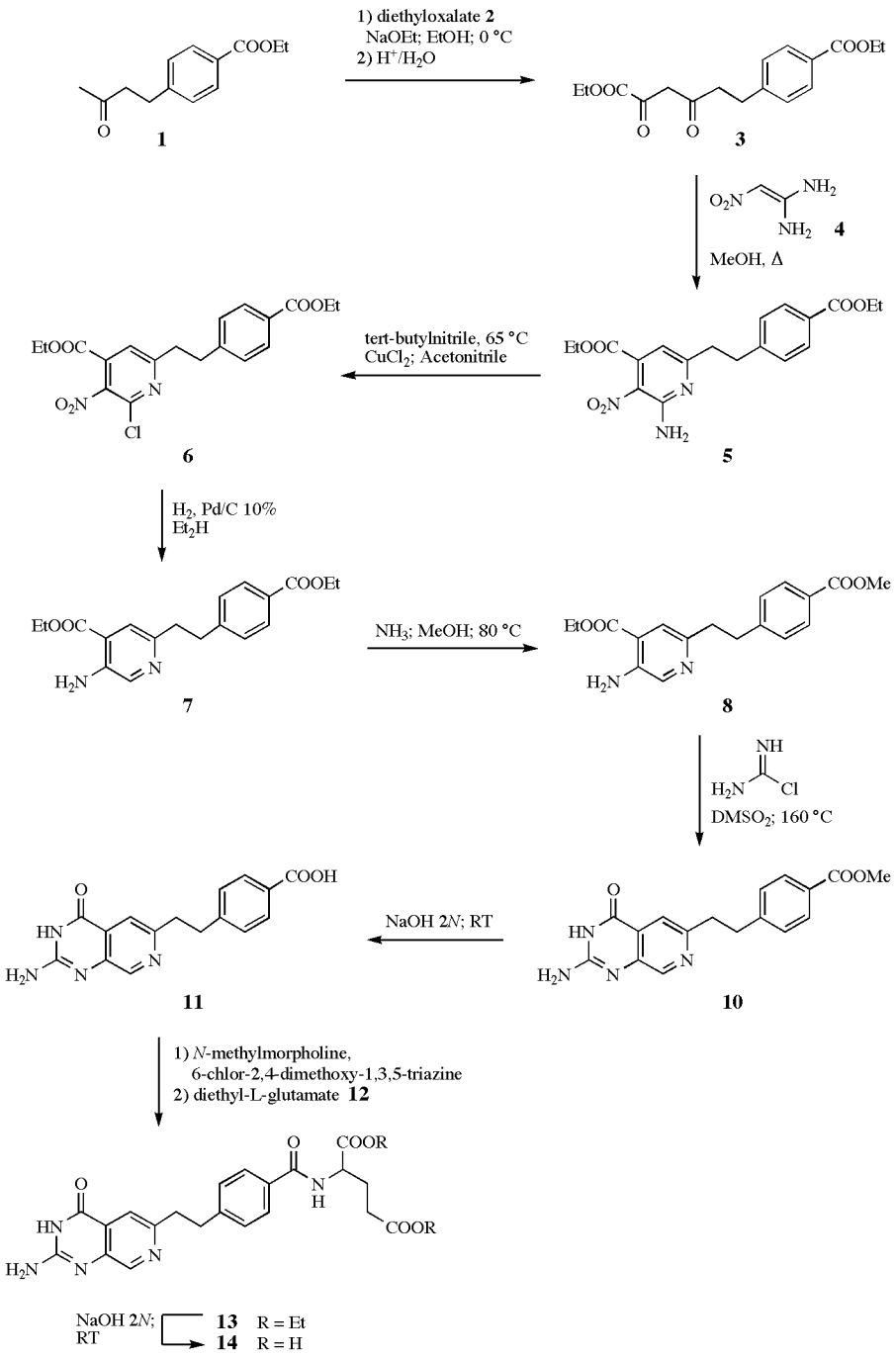
Cleavage of strategic bonds in the target compound 7-aza-5,8,10-trideazafolic acid (**14**) leads to a 3-amino-isonicotinic acid derivative as the key intermediate and guanidine. Further retrosynthesis gives rise to a 3-nitropyridine that could originate from a nitroendiamine and a 1,3-diketone [14].

One recent access to 3-amino-6-chloroisonicotinic acid exists in the directed lithiation of boc-protected 6-chloropyridine-3-ylamine with butyllithium followed by quenching with carbon dioxide [15]. We used this reaction with a boc-protected 6-styrylpyridine-3-ylamine as a model compound, but unfortunately did not get the desired 3-amino-6-styrylisonicotinic acid.

For this reason we decided to tread an alternative path.

Improving the method of Maguire and McKee [9], who synthesized ethyl 6-methyl-3-nitro-2(1*H*)pyridone-4-car-

Scheme 3



boxylate as a precursor of a 3-aminoisonicotinic acid derivative from nitroacetamide and ethyl acetopyruvate in a low yield of 13 %, we replaced nitroacetamide by 2-nitroethene-1,1-diamine (**4**) (Scheme 2) – in our hands a versatile building block for an easy entry to 3-nitropyridine-2-amines [16,17].

Our sequence for the synthesis of 7-aza-5,8,10-trideazafolic acid (**14**) starts with the preparation of the necessary 1,3-biselectrophile ethyl 4-(5-ethoxycarbonyl-3,5-dioxopentyl)benzoate (**3**) from claisen condensation of ketone **1** with diethyloxalate (**2**) [18] which proceeds in good yields after purification by Kugelrohr destillation.

The following cyclocondensation [16] of 1,3-diketone **3** with 2-nitroethene-1,1-diamine (**4**) was carried out in methanol under reflux. Of the two possible regioisomers – *i.e.* the isonicotinic acid derivative **5** and/or the α-picolinic acid derivative – we only isolated the desired 3-nitroisonicotinate **5** in a yield of 65% without further necessary purification. The isonicotinic ester structure of **5** was proved by heteronuclear bond correlation (hnbc). Only one coupling signal from the C1-hydrogen of the ethylene chain to the pyridine-C5 was detected. In case of the isomeric α-picolinic acid derivative, two correlation signals from C1-hydrogen of the ethylene bridge to C3- and C5 of the pyridine nucleus should have been observed.

The thus obtained 3-nitroisonicotinate **5** could be directly and efficiently converted to the 2-chloropyridine derivative **6** by substitutive deamination [19] with *tert*-butyl nitrite and anhydrous copper(II) chloride in dry acetonitrile.

Subsequent hydrogenation of 2-chloropyridine derivative **6** at atmospheric pressure resulted in the reduction of the nitro group and hydrogenolysis of the 2-chloro substituent, affording 3-aminoisonicotinate **7** in a yield of 58%.

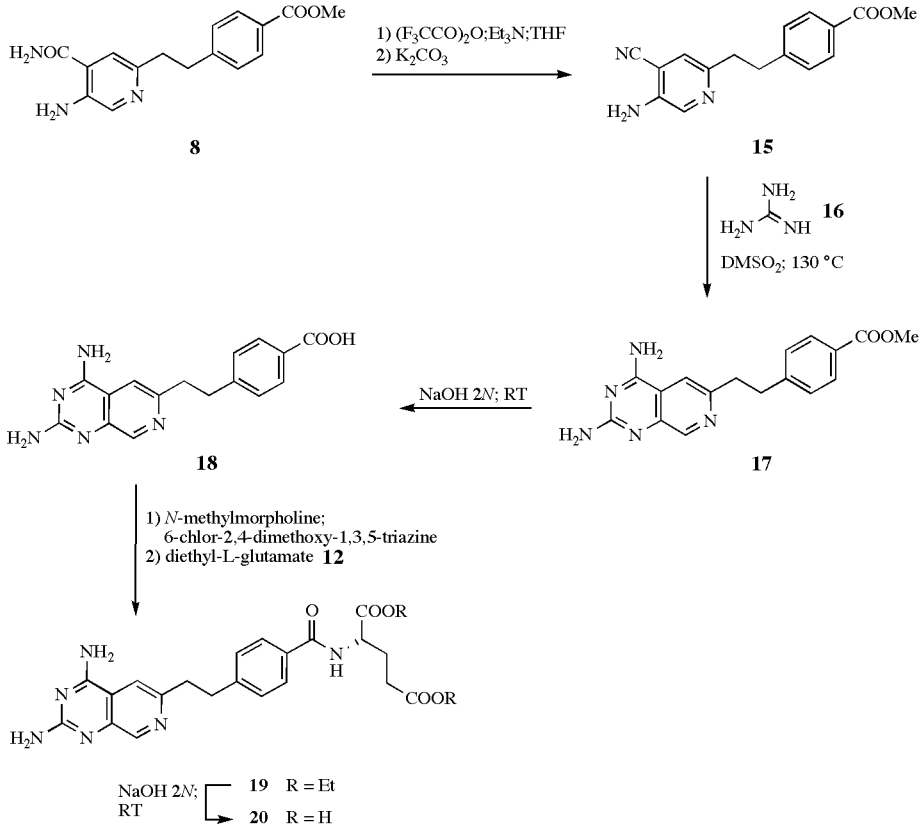
However, having no success in hydrogenation of **6** with a palladium on barium carbonate catalyst, we found palladium on charcoal 10% [20]- with a small amount of triethylamine – to be the exceptional catalyst.

The direct pyrimidine annellation to 2-amino-4-oxopyrido[3,4-*d*]pyrimidine **10** from *o*-aminoester **7** and guanidine or chloroformamidine-HCl [21] in various solvents and a variety of temperatures failed.

We solved the problem by treating of **7** with ammonia in methanol. The ammonolysis of **7** gave rise to *o*-aminoamide **8** (60%) and a diamide (15%), which were separated by column chromatography. The following ring closure of **8** with chloroformamidine hydrochloride in dimethylsulfone at 160 °C provided **10** [21], indicating that an *o*-aminoamide group has been formed by ammonolysis of **7**.

Saponification of **10** [22], followed by coupling of the resulting benzoic acid derivative **11** with diethyl L-glutamate

Scheme 4



(**12**) using 6-chloro-2,4-dimethoxy-1,3,5-triazine/*N*-methylmorpholine [23], gave rise to L-glutamate derivative **13**.

Final alkaline hydrolysis of **13** followed by acidification with acetic acid yielded the target compound *N*-[4-[2-(2-amino-4-oxopyrido[3,4-*d*] pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic acid (**14**).

The starting sequence for the synthesis of *N*-[4-[2-(2,4-diaminopyrido[3,4-*d*] pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic acid (**20**) is equal to that of L-glutamic acid derivative **14**.

In order to generate a amino function instead of the oxo function in position 4 of a pyrido[3,4-*d*]pyrimidine derivative **20** it was necessary to convert the 3-aminoisonicotinamide **8** into 3-aminoisonicotinonitrile **15**.

Several attempts to prepare **15** by treatment of isonicotinamide **8** with cyanuric chloride [24], methyl (carboxysulfamoyl) triethylammonium hydroxide inner salt (Burgess reagent) [25] or $POCl_3$ [26] failed. In the end we achieved the nitrile **15** by using trifluoroacetic anhydride and excess triethylamine in tetrahydrofuran [27].

Ring closure of *o*-aminonitrile **15** to pyrido[3,4-*d*]-pyrimidin-2,4-diamine **17** was done successfully by reaction with guanidine in dimethylsulfone at 130 °C [28].

Saponification of benzoate **17** [22], followed by coupling of the resulting benzoic acid **18** with diethyl L-glutamate (**12**) using 6-chloro-2,4-dimethoxy-1,3,5-triazine/*N*-methylmorpholine [23], gave the intermediate L-glutamate derivative **19**.

Final alkaline hydrolysis of **19** followed by acidification with acetic acid yielded the MTX analogue *N*-[4-[2-(2,4-diaminopyrido[3,4-*d*] pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic acid (**20**).

EXPERIMENTAL

Melting points were determined on a Lindström capillary melting point apparatus from Büchi (type 530) and are uncorrected. The ir spectra were recorded on a Perkin-Elmer infrared-fourier-transform-spectrophotometer (type 1740). The ^{1}H nmr spectra and the ^{13}C nmr spectra were recorded on ft-nmr spectrometers: Bruker AC 250 (at 250.13 MHz), Bruker AM 360 (at 360.13 MHz). The ^{13}C nmr spectra were recorded at 60.6 and 90.6 MHz; the solvent was dimethylsulfoxide-d_6 (unless otherwise stated) with tetramethylsilane as the internal standard. The uv spectra were recorded on a Perkin-Elmer spectrophotometer (type Lambda 5). Solvent was methanol (UVasolâ Merck). Mass spectra were recorded on a Finnigan TSQ 70 (electron; ionisation energy 70 eV) and JEOL MS 700 (field desorption). Microanalyses were carried out on a Carlo Erba Elemental Analyzer Model 1106.

Ethyl 4-(5-Ethoxycarbonyl-3,5-dioxopentyl)benzoate (**3**).

To a stirred and cooled (0 °C) solution of 1.15 g (50 mmoles) sodium in 50 ml ethanol a mixture of 11 g (50 mmoles) ethyl 4-(3-oxobutyl)benzoate (**1**) and 14.6 g (100 mmoles) diethyloxalate (**2**) was added dropwise. After standing at room temperature overnight, it was neutralized with an equimolar amount of glacial acetic acid and poured on 300 ml ice. After extracting with ether (3 x 300 mL), the combined organic layers were washed with water, dried over sodium sulfate and evaporated to give 12 g (75%) of a colourless oil after distillation by Kugelrohr–apparatus; bp 140 °C (5*10^{-2} bar); ir (sodium chloride): 2985, 2938 and 2908 (CH), 1716 (ester CO), 1639 and 1608 cm^{-1} (ketone CO); ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.26-1.34 (m, 6H, 2x OCH_2-CH_3), 2.97 (s, 4H, CH_2-CH_2), 3.62 (s, 2H, O=C-CH_2-C=O), 4.23-4.34 (m, 4H, 2x OCH_2-CH_3), 7.37-7.43 (m, 2H, 3-H and 5-H), 7.84-7.92 ppm (m, 2H, 2-H and 6-H); ms: m/z 321 (M+1).

Anal. Calcd. for $C_{17}H_{20}O_6$: C, 63.75; H, 6.29. Found: C, 63.92; H, 6.39.

Ethyl 2-Amino-6-[2-(4-ethoxycarbonylphenyl)ethyl]-3-nitroisonicotinate (**5**).

A mixture of 980 mg (3 mmoles) ethyl 4-(5-ethoxycarbonyl-3,5-dioxo-pentyl)benzoate (**3**) and 320 mg (3 mmoles) 2-nitroethene-1,1-diamine (**4**) in 20 ml methanol was heated at reflux for 45 minutes and cooled to room temperature. Dropwise adding of water provided 755 mg (65%) of **5** as a bright yellow solid. mp 122-123 °C; ir (sodium chloride): 3521, 3455, 3382 and 3270 (NH_2), 2989, 2931 and 2865 (CH), 1720 (ester CO), 1631, 1565 and 1504 cm^{-1} (NO_2); uv (methanol): λ_{max} 390 nm (ε 6733); ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.26 (t, J = 7.1 Hz, 3H, OCH_2-CH_3), 1.31 (t, J = 7.1 Hz, 3H, OCH_2-CH_3), 2.96-3.10 (m, 4H, CH_2-CH_2), 4.29 (q, J = 7.1 Hz, 4H, 2x OCH_2-CH_3), 6.72 (s, 1H, 5-H), 7.37-7.41 (m, 2H, 3´-H and 5´-H), 7.85-7.90 (m, 2H, 2´-H and 6´-H), 7.95 ppm (broad s, 2H, NH_2 deuterium oxide exchangeable); ms: m/z 387 (M^+).

Anal. Calcd. for $C_{19}H_{21}N_3O_6$: C, 58.92; H, 5.46; N, 10.85. Found: C, 59.03; H, 5.46; N, 10.81.

Ethyl 2-Chloro-6-[2-(4-ethoxycarbonylphenyl)ethyl]-3-nitroisonicotinate (**6**).

tert-Butyl nitrite (0.36 ml, 3 mmoles) was added to a stirred suspension of 322 mg (2.4 mmoles) anhydrous $CuCl_2$ in 30 ml anhydrous acetonitrile. The mixture was heated at 65 °C and then a solution of 330 mg (0.86 mmoles) **5** in 30 ml anhydrous acetonitrile was added over a period of 5 minutes to the reaction medium. During the addition the mixture turned completely black from the initial green colour. After complete gas evolution (15 minutes) the temperature was allowed to come to room temperature and the mixture was poured into 50 ml 20% aqueous HCl. Ethyl acetate (60 ml) was added and the organic phase separated, washed with 50 ml 20% aqueous HCl and dried (Na_2SO_4). The solvent was removed under reduced pressure and **6** was crystallized from methanol (0 °C) in a yield of 60% as colourless solid; mp 103-104 °C; ir (sodium chloride): 3066, 2992 and 2935 (CH), 1743 and 1700 (ester CO), 1592 and 1546 cm^{-1} (NO_2); ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.27 (t, J = 7.1 Hz, 3H, OCH_2-CH_3), 1.31 (t, J = 7.1 Hz, 3H, OCH_2-CH_3), 3.06-3.14 and 3.22-3.29 (m, 4H, CH_2-CH_2), 4.29 (q, J = 7.1 Hz, 2H, OCH_2-CH_3), 4.34 (q, J = 7.1 Hz, 2H, OCH_2-CH_3), 7.40-7.44 (m, 2H, 3´-H and 5´-H), 7.86-7.91 (m, 2H, 2´-H and 6´-H), 7.95 (s, 1H, 5-H); ms: m/z 406 (M^+).

Anal. Calcd. for $C_{19}H_{19}N_2O_6Cl$: C, 55.86; H, 4.69; N, 6.86. Found: C, 55.68; H, 4.98; N, 6.83.

Ethyl 5-Amino-2-[2-(4-ethoxycarbonylphenyl)ethyl]-isonicotinate (**7**).

To a solution of 100 mg (0.25 mmoles) **6** and 0.1 ml triethylamine in 8 ml ethanol was added 100 mg 10% palladium on

carbon and the mixture was hydrogenated for 2 hours under atmospheric pressure. The catalyst was filtered off and the filtrate was evaporated nearly to dryness. Storing at 0 °C yielded 60 mg (70%) colourless needles. mp 132 – 133 °C; ir (potassium bromide): 3451 and 3337 (NH_2), 2976 and 2937 (CH), 1712 cm^{-1} (ester CO); ^1H nmr (dimethylsulfoxide-d_6): δ 1.26-1.34 (m, 6H, 2x OCH_2-CH_3), 2.87 – 3.00 (m, 4H, CH_2-CH_2), 4.23-4.33 (m, 4H, 2x OCH_2-CH_3), 6.50 (broad s, 2H, NH_2 deuterium oxide exchangeable), 7.27 (s, 1H, 2-H), 7.31 – 7.37 (m, 2H, 3´-H and 5´-H), 7.82 – 7.88 (m, 2H, 2´-H and 6´-H), 8.21 ppm (s, 1H, 5H); ms: m/z 342 (M^+).

Anal. Calcd. for $C_{19}H_{22}N_2O_4$: C, 66.66; H, 6.48; N, 8.18. Found: C, 67.05; H, 6.74; N, 8.57.

Methyl 4-[2-(5-Amino-4-carbamoylpyridin-2-yl)ethyl]benzoate (**8**).

A solution of 500 mg (1.67 mmoles) **7** in 50 ml methanol was saturated with ammonia at 0 °C for 15 minutes. The mixture was kept in an autoclave at 80 °C overnight, cooled to room temperature and evaporated to dryness. The residue was separated by flash chromatography on silica gel (cyclohexane:ethyl acetate 5:5, v/v), yield 300 mg (60%). mp 173 – 175 °C; ir (potassium bromide): 3430, 3340 and 3186 (NH), 2952, 2925 and 2859 (CH), 1718 (ester CO), 1669 cm^{-1} (amide CO); ^1H nmr (dimethylsulfoxide-d_6): δ 2.84-2.93 and 2.98-3.07 (m, 4H, CH_2-CH_2), 3.83 (s, 1H, OCH_3), 6.38 (broad s, 2H, NH_2 deuterium oxide exchangeable), 7.31 (s, 1H, 2-H), 7.33-7.37 (m, 2H, 3´-H and 5´-H), 7.39 (broad s, 1H, CONH, deuterium oxide exchangeable), 7.84-7.89 (m, 2H, 2´-H and 6´-H), 7.94 (broad s, 1H, CONH, deuterium oxide exchangeable), 8.06 ppm (s, 1H, 5-H); ms: m/z 299 (M^+).

Anal. Calcd. for $C_{16}H_{17}N_3O_3$: C, 64.21; H, 5.72; N, 14.04. Found: C, 63.98; H, 5.68; N, 13.86.

Methyl 4-[2-(2-Amino-4-oxopyrido[3,4-*d*]pyrimidin-6-yl)ethyl]benzoate (**10**).

A mixture of 50 mg (0.16 mmoles) **8**, 60 mg (0.53 mmoles) of freshly prepared chloroformamidine hydrochloride (**9**) and 50 mg of dimethylsulfone was heated for 1 hour at 160 °C in an open flask with magnetic stirring. Water was added to the solid mass and, following warming this mixture, ammonium hydroxide 10% was added until neutralisation was achieved. The resulting white solid was isolated by filtration in a yield of 39 mg (40%); mp > 260 °C; ir (potassium bromide): 3334 and 3176 (NH), 1718 (ester CO), 1655 cm^{-1} (CO); ^1H nmr (dimethylsulfoxide-d_6): δ 3.04-3.12 (m, 4H, CH_2-CH_2), 3.82 (s, 3H, OCH_3), 6.62 (broad s, 2H, NH_2, deuterium oxide exchangeable), 7.34-7.40 (m, 2H, 3´-H and 5´-H), 7.52 (s, 1H, 8-H), 7.82-7.89 (m, 2H, 2´-H and 6´-H), 8.56 ppm (s, 1H, 5-H); ms: m/z 324 (M^+).

Anal. Calcd. for $C_{17}H_{16}N_4O_3$: C, 62.96; H, 4.97; N, 17.28. Found: C, 63.34; H, 5.25; N, 17.34.

Diethyl *N*-[4-[2-(2-Amino-4-oxopyrido[3,4-*d*]pyrimidin-6-yl)ethyl]-benzoyl]-L-glutamate (**13**).

The acid **11** was achieved from methyl 4-[2-(2-amino-4-oxopyrido[3,4-*d*]pyrimidin-6-yl)ethyl]benzoate (**10**) by stirring in 2 *N* sodium hydroxide at room temperature for 4 hours followed by neutralisation with glacial acetic acid. Compound **11** was obtained in a amorphous modification at 0 °C, collected by filtration, dried under vacuum and used without further purification.

To a suspension of 100 mg (0.33 mmoles) **11** in 7 ml dimethylformamide at 25 °C was added 0.042 ml (0.38 mmoles) *N*-methylmorpholine followed by 73 mg (0.39 mmoles) 6-chloro-2,4-dimethoxy-1,3,5-triazine and the resulting solution was stirred at 25 °C for 1 hour. *N*-methylmorpholine (0.06 ml, 0.54 mmoles) was added to the solution followed by 83 mg (0.35 mmoles) diethyl L-glutamate hydrochloride, and the resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated *in vacuo* and the residue was taken up in 7 ml dichloromethane. The dichloromethane layer was washed with 5% $NaHCO_3$, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel eluting with chloroform:methanol (95:5, v/v) to give 130 mg (80%) as a white solid. mp 215-217 °C; ir (sodium chloride): 3297 (NH), 2981 and 2935 (CH), 1735 (ester CO), 1662 cm^{-1} (CO); ^1H nmr (dimethylsulfoxide-d_6): δ 1.13-1.21 (m, 6H, 2x OCH_2-CH_3), 1.93-2.16 and 2.40-2.46 (m, 4H, ethyl glutamate CH_2-CH_2), 3.07 (s, 4H, aryl CH_2-CH_2), 3.99-4.14 (m, 4H, 2x OCH_2-CH_3), 4.37-4.46 (m, 1H, ethyl glutamate $HCNH_2$), 6.52 (broad s, 2H, NH_2, deuterium oxide exchangeable), 7.29-7.35 (m, 2H, 3´-H and 5´-H), 7.54 (s, 1H, 8-H), 7.75-7.79 (m, 2H, 2´-H and 6´-H), 8.56 (s, 1H, 5-H), 8.60-8.64 (d, 1H, CONH); 11.21 ppm (broad s, 1H, NH, deuterium oxide exchangeable); ms: m/z 495 (M^+).

Anal. Calcd. for $C_{25}H_{29}N_5O_6$: C, 60.60; H, 5.90; N, 14.13. Found: C, 60.60; H, 6.18; N, 14.42.

N-[4-[2-(2-Amino-4-oxopyrido[3,4-*d*]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic Acid (**14**).

A solution of 50 mg (0.1 mmoles) of **13** in 1 ml 2 *N* sodium hydroxide was allowed to stir at room temperature for 24 hours. The reaction mixture was acidified with glacial acetic acid and the solid product was collected by filtration, washed with water and dried to give 29 mg (65%) as a white solid. mp 254-255 °C (decomposition); ir (sodium chloride): 3316 (NH); 2981 and 2931 (CH); 1725 cm^{-1} (CO); ^1H nmr (F_3CCOOD): δ 2.37-2.59 and 2.65-2.90 46 (m, 4H, ethyl glutamate CH_2-CH_2), 3.15-3.24 and 3.75-3.88 (m, 4H, aryl CH_2-CH_2), 5.07-5.17 (m, 1H, ethyl glutamate $HCNH_2$), 7.45 (s, 1H, 8-H), 7.49-7.55 (m, 2H, 3´-H and 5´-H), 7.80-7.94 (m, 2H, 2´-H and 6´-H), 8.58 ppm (s, 1H, 5-H); ms: m/z 440 (M+1).

Anal. Calcd. for $C_{21}H_{21}N_5O_6$: C, 57.41; H, 4.82; N, 15.94. Found: C, 57.36; H, 4.73; N 16.15.

Methyl 4-[2-(5-Amino-4-cyanopyridin-2-yl)ethyl]benzoate (**15**).

Triethylamine (126 mg, 1.25 mmoles) were added to a solution of 60 mg (0.20 mmoles) **8** at 0 °C in 2 ml tetrahydrofuran (THF) followed by 160 mg trifluoroacetic anhydride in 1 ml THF. After 30 minutes 2 ml water was added, and the mixture was extracted with 2 x 2 ml diethyl ether. The organic layer was dried and evaporated, and the residue was dissolved in 2 ml methanol-water (1:1) containing 100 mg K_2CO_3. The mixture was heated at 70 °C for 24 hours, cooled, and extracted with 2 x 2 ml ethyl acetate. The extracts were washed with water and brine, dried, and evaporated to give 50 mg (90%); mp 128-129 °C; ir (sodium chloride): 3436 and 3359 (NH), 2923 and 2854 (CH), 2217 (CN), 1712 cm^{-1} (ester CO); ^1H nmr (dimethylsulfoxide-d_6): δ 2.85-2.93 and 2.94-3.05 (m, 4H, CH_2-CH_2), 3.84 (s, 3H, OCH_3), 6.22 (broad s, 2H, NH_2, deuterium oxide exchangeable), 7.21 (s, 1H, 2-H), 7.30-7.44 (m, 2H, 3´-H and 5´-H), 7.82-7.91 (m, 2H, 2´-H and 6´-H), 8.20 ppm (s, 1H, 5-H); ms: m/z 281 (M^+).

Anal. Calcd. for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 68.85; H, 5.91; N, 14.17

Methyl 4-[2-(2,4-Diaminopyrido[3,4-*d*]pyrimidin-6-yl)ethyl]-benzoate (**17**).

Compound **15 (**35 mg, 0.12 mmoles) were dissolved at 130 °C in 250 mg dimethylsufone. After addition of 40 mg (0.68 mmoles) guanidine (**16**), the mixture was stirred at this temperature for 1.5 days. The hot solution was diluted with 0.5 ml water, the solid thus obtained was collected by filtration and chromatographed on silica gel eluting with chloroform:methanol (9:1, v/v) to give 20 mg (50%); mp 286-288 °C; ir (sodium chloride): 3548 and 3336 (NH), 2919 and 2854 (CH), 1751 cm^{-1} (CO); uv (methanol): λ_{max} 345 nm (ε 8591); ^{1}H nmr (dimethylsulfoxide-d_6): δ 2.84-2.92 and 3.27-3.40 (m, 4H, CH_2-CH_2), 3.72 (s, 3H, OCH_3), 6.26 (broad s, 2H, NH_2, deuterium oxide exchangeable), 6.80 (s, 1H, 8-H), 6.82-6.85 (m, 2H, 3´-H and 5´-H), 6.94 (broad s, 2H, NH_2, deuterium oxide exchangeable), 7.12-7.19 (m, 2H, 2´-H and 6´-H), 8.43 ppm (s, 1H, 5-H); ms: m/z 323 (M^+).

Anal. Calcd. for $C_{17}H_{17}N_5O_2$: C, 63.15; H, 5.30; N, 21.66. Found: C, 63.42; H, 5.18; N, 21.34.

Diethyl *N*-[4-[2-(2,4-Diaminopyrido[3,4-*d*]pyrimidin-6-yl)-ethyl]benzoyl]-L-glutamate (**19**).

Preparation and purification according to **11** from 50 mg (0.16 mmoles) **17** *via* the acid **18** yielded 51 mg (65%) **19** as a white solid; mp 220-221 °C; ir (sodium chloride): 3409 and 3286 (NH); 2981 and 2931 (CH); 1735 cm^{-1} (CO); ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.12-1.23 (m, 6H, 2x OCH_2-CH_3), 1.95-2.16 and 2.39-2.46 (m, 4H, ethyl glutamate CH_2-CH_2), 2.96-3.08 and 3.37-3.50 (m, 4H, aryl CH_2-CH_2), 4.01-4.15 (m, 4H, 2x OCH_2-CH_3), 4.38-4.48 (m, 1H, ethyl glutamate $HCNH_2$), 6.44 (broad s, 2H, NH_2, deuterium oxide exchangeable), 6.84 (s, 1H, 8-H), 7.15 (broad s, 2H, NH_2, deuterium oxide exchangeable), 7.28-7.36 (m, 2H, 3´-H and 5´-H), 7.75-7.83 (m, 2H, 2´-H and 6´-H), 8.44 (s, 1H, 5-H), 8.61-8.68 (d, 1H, CONH); ms: m/z 494 (M^+).

Anal. Calcd. for $C_{25}H_{30}N_6O_5$: C, 60.72; H, 6.11; N, 17.00. Found: C, 60.79; H, 5.94; N, 17.32.

N-[4-[2-(2,4-Diaminopyrido[3,4-*d*]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic Acid (**20**).

Preparation and purification according to **14** from 50 mg (0.10 mmoles) **19** yielded 31 mg (71%) **20** as a white solid; mp 268-269 °C (decomposition); ir (sodium chloride): 3374 and 3313 (OH, NH), 2981 and 2935 (CH), 1715 cm^{-1} (CO); ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.89-2.15 and 2.25-2.40 (m, 4H, ethyl glutamate CH_2-CH_2), 2.90-3.09 and 3.35-3.50 (m, 4H, aryl CH_2-CH_2), 4.26-4.43 (m, 1H, ethyl glutamate $HCNH_2$), 6.54-6.80 (broad s, 1H, NH_2, deuterium oxide exchangeable), 6.89 (s, 1H, 8-H), 7.25-7.39 (m, 2H, 3´-H and 5´-H), 7.74-7.85 (m, 2H, 2´-H and 6´-H), 8.41-8.54 ppm (m, 2H, 5-H and CONH); ms: m/z 439 (M+1).

Anal. Calcd. for $C_{21}H_{22}N_6O_5$: C, 57.53; H, 5.06; N, 19.17. Found: C, 57.38; H, 5.26; N, 19.04.

REFERENCES AND NOTES

[1] A. L. Jackson, Antifolate Drugs in Cancer Therapy, Humana Press, Totowa, New Jersey,1999.

[2] A. Rosowski, Progress in Medical Chemistry, Edt. by G. Ellis and G. B. West, Elsevier, Amsterdam, **26**, 1 (1989).

[3] A. Graul, M. Tracy and J. Castañer, *Drugs Fut.*, **23**, 498 (1998).

[4] P. Karlson, Biochemie, 13. Auflage, Georg Thieme Verlag, Stuttgart - New York 1988, pp 94-97.

[5] E. C. Taylor, P. J. Harrington, S. R. Fletcher, G. P. Beardsley and R. G. Moran, *J. Med. Chem.*, **28**, 914 (1985).

[6] J. R. Piper, G. S. McCaleb, J. A. Montgomery, R. L. Kisliuk, Y. Gaumont and F. M. Sirotnak, *J. Med. Chem.*, **29**, 1080 (1986).

[7] M. S. Ray, F. M. Muggia, G. C. Leichman, R. L. Nelson, R. W. Dyke and R. G. Moran, *J. Natl. Cancer Inst.*, **85**, 1154 (1993).

[8] E. M. Grivsky, S. Lee, C. W. Sigel, D. S. Duch and C. A. Nichol, *J. Med. Chem.*, **23**, 327 (1980).

[9] J. H. Maguire and R. L. McKee, *J. Heterocyclic Chem.*, **16**, 133 (1979).

[10] Z. Ozdowska and B. Szczyinski, *Rocz. Chem.*, **50**, 1771 (1976).

[11] R. G. Glushkov, O. Ya Belyaeva, V. G. Granik, M. K. Polievktova, A. B. Girgorev, V. E. Serokhvostova and T. F. Vlasova, *Khim. Geterotsikl. Soedin.*, 1640 (1976).

[12] D. J. Berry, J. D. Cook and B. J. Wakefield, *J. Chem. Soc., Perkin Trans. 1*, 2190 (1972).

[13] R. Madhav, *Org. Prep. Proced. Int.*, **14**, 403 (1982).

[14] D. G. Batt and G. C. Houghton, *J. Heterocyclic Chem.*, **32**, 963 (1995).

[15] G. W. Rewcastle, W. A. Denny, R. T. Winters, N. L. Colbry, and H. D. Hollis Showalter, *J. Chem. Soc., Perkin Trans. 1*, 2221 (1996).

[16] R. Troschütz and A. Lückel, *Arch. Pharm. (Weinheim)*, **324**, 73 (1991).

[17] R. Troschütz and A. Karger, *J. Heterocyclic Chem.*, **33**, 1815 (1996).

[18] J. J. Parlow, D. A. Mischke and S. S. Woodard, *J. Org. Chem.*, **62**; 17; 5908 (1997).

[19] V. Ojea, C. Peinador and J. M. Quintela, *Synthesis*, 798 (1992).

[20] A. Tanaka, T. Terasawa, H. Hagihara, N. Ishibe, M. Sawada, Y. Sakuma, M. Hashimoto, H. Takasugi and H. Tanaka, *J. Med. Chem.*, **41**, 4408 (1998).

[21] S. W. Schneller and A. C. Ibay, *J. Org. Chem.*, **51**, 4067 (1986).

[22] E. C. Taylor and P. M. Harrington, *J. Org. Chem.*, **55**, 3222 (1990).

[23] E. C. Taylor, H. H. Patel, G. Sabitha and R. Chaudhari, *Heterocycles*, **43**, 349 (1996).

[24] G. A. Olah, S. C. Narang, A. P. Fung and B. G. B. Gupta, *Synthesis*, 657 (1980).

[25] D. A. Claremon and B. T. Phillips, *Tetrahedron Letters*, **29**, 2155 (1988).

[26] K. E. Andersen and E. B. Pedersen, *Liebigs. Ann. Chem.*, 1012 (1986).

[27] M. Rowley, J. J. Kulagowski, A. P. Watt, D. Rathbone, G. I. Stevenson, R. W. Carling, R. Baker, G. R. Marshall, J. A. Kemp, A. C. Foster, S. Grimwood, R. Hargreaves, C. Hurley, K.L. Saywell, M. D. Tricklebank and P. D. Leeson, *J. Med. Chem.*, **40**, 4053 (1997).

[28] F. Schönfeld and R. Troschütz, *Heterocycles*, **55**, 1679 (2001).