Acyclo Analogs of Formycin A [1]

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Starting from 7-chloro-3-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**1**) by reaction with methanol/hydrogen chloride, subsequent methylation and reaction with *N*-bromosuccinimide 3-bromomethyl-7-methoxy-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**6**) is prepared. Treatment with ethylene glycol in the presence of potassium carbonate/potassium iodide and subsequent reaction with ethanolic ammonia afforded 7-amino-3-(2-hydroxyethoxymethyl)-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**11**) an acyclo analog of formycin A.

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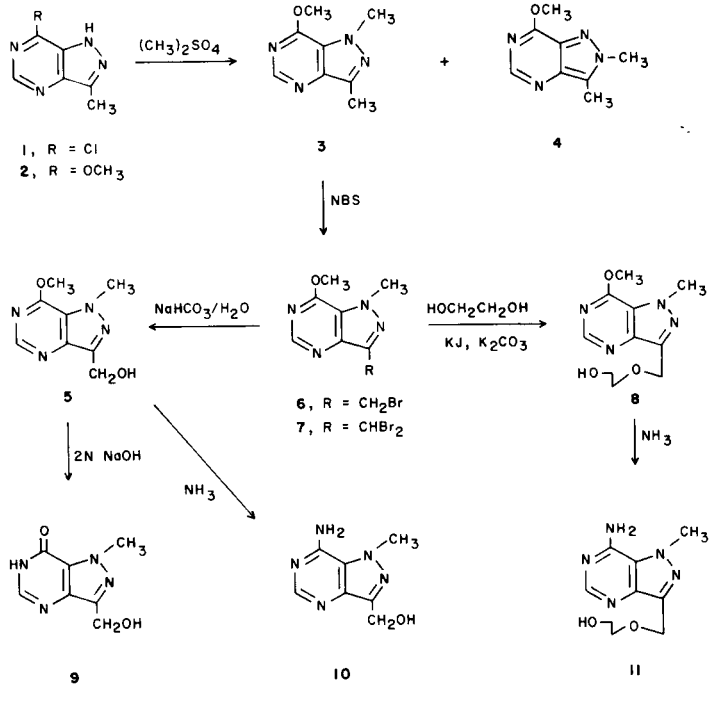
Formycin A exerts antiviral and antitumor properties but also shows relatively high toxicity [2]. In view of the recent discovery of acyclic nucleoside analogs with remarkable biological properties [3] a synthetic approach to acycloanalogs of Formycin A has been sought. Recently the synthesis of another acyclo nucleoside analog with the pyrazolo[4,3-*d*]pyrimidine skeleton has become known [4]. Since 1-methylformycin A is less toxic and is slower deaminated to biologicaly less active 1-methylformycin B than Formycin A itself [2d,2e] this methylated derivative has been chosen as the parent compound for the intended synthesis of the acyclic analog.

Synthesis.

For the preparative access to the target molecule **11** contrary to the literature methods for related substrates [4,5] a new synthetic strategy has been developed where the pyrazolo[4,3-*d*]pyrimidine system is formed first and afterwards the acyclo substituent is attached at the preformed activated binding site.

Starting from 7-chloro-3-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**1**) [6] the methoxy derivative **2** is prepared by refluxing with methanol. The subsequent methylation has been achieved with dimethyl sulphate whereby **3** and **4** are obtained in a 4:1 ratio. The isomers can be separated by selective extraction, the structural assignment follows from the ^{13}C-nmr spectra (see below). Reaction with *N*-bromosuccinimide under radiation with a photo lamp (100 W) gives the bromomethyl compound **6** beside a small amount of the dibrominated derivative **7**. By hydrolysis with acetone/aqueous sodium hydrogen carbonate the

Scheme I

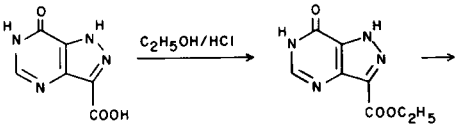


hydroxymethyl product **5** is obtained. Removal of the methoxy group is accomplished with 2*N* sodium hydroxide at room temperature, leading to 1-methyl-7-oxo-6,7-dihydropyrazolo[4,3-*d*]pyrimidin-3-methanol **9**. By reaction with ethanolic ammonia at 160° the corresponding amino compound **10** is obtained, but only low yields have been achieved caused in part by traces of water with subsequent formation of **9** which has to be removed by chromatography.

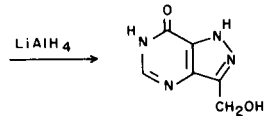
The conversion of **6** into the acyclo derivative **8** can be achieved by reaction with ethylene glycol in acetone in the presence of potassium iodide and potassium carbonate. By treatment with ethanolic ammonia at 130° for 16 hours the desired endproduct **11** is obtained.

For comparison purposes in the investigations of the biological properties also the already known 7-oxo-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidine-3-methanol (**14**) [7]

Scheme 2



12 **13**



14

has been synthesized by a new route starting from carboxylic acid **12** [8]. After conversion into the ester **13** and silylation with hexamethyldisilazane in order to obtain sufficient solubility in tetrahydrofuran, **14** is obtained by reduction with lithium aluminium hydride.

^{13}C-NMR Spectra.

In N-1 alkylated pyrazolo[4,3-*d*]pyrimidines the ^{13}C chemical shift of C-3 appears at lower field and the signal of C-4a at higher field than in the corresponding N-2 alkylated derivative **9**. On that basis (see Table 1) the side of methylation in **3** and **4** has been assigned. For comparison purposes also the ^{13}C-nmr data of 7-oxo-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidine-3-methanol (**14**) are included.

Table 1

^{13}C-NMR Shift (in DMSO-d_6) ppm (relative to TMS)

	3	**4**	**14**
C-3	140.5	132.7	137.3
C-3a	147.1	140.1	146.4
C-5	149.0	150.6	142.7
C-7	155.5	161.3	153.6
C-7a	121.4	131.0	127.7
C-CCH_3 (CH_2OH)	9.6	9.0	54.1
N-CH_3	37.4	38.2	
O-CH_3	52.9	53.8	

Biological Properties.

In order to evaluate potential antitumor properties **5, 8, 9, 10** and **11** have been tested for their ability to reduce the cloning efficiency of L 1210 leukemia cells. In this assay, compounds are considered active if the reduction in the cloning efficiency is more than 50% compared to the untreated control cells. Up to a concentration of 25 μg/ml all compounds tested were inactive whereas for comparison 1-(β-D-arabino)cytosine showed activity already at 2.5 μg/ml.

For the same compounds as above potential antiviral activity has been tested against Influenza A/Texas (MDCK cells), Influenza B/Hongkong (MDCK cells), Herpes simplex I (Vero cells), and Herpes simplex II (Vero cells). In no case any activity could be detected up to celltoxic concentrations.

EXPERIMENTAL

Melting points were determined on a Tottoli melting point apparatus and are uncorrected. Infrared spectra were determined on a Beckman IR-33 spectrometer, ^{1}H- and ^{13}C-nmr spectra were measured on a Bruker WH-90 spectrometer with tetramethylsilane as standard. Elemental analyses were performed by Institut für Organische Chemie, Universität Graz.

7-Chloro-3-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**1**) [6].

To 816 ml of freshly distilled phosphorus oxychloride were added 35 g (0.23 mole) of 3-methyl-7-oxo-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidine [6] and 70 ml of *N,N*-dimethylaniline. After heating under reflux for 2 hours, the excess phosporus oxychloride was removed *in vacuo* and the resulting dark oil was carefully poured on 420 g of ice. The solution was extracted 4 times with 500 ml of ethyl acetate and 250 ml of ether. The combined organic layers were washed with water, dried over sodium sulphate and taken to dryness. The residue was extracted several times with ether. After removal of the ether, 33.4 g of crude **1** was obtained; nmr (deuteriochloroform): δ 2.72 (s, CH_3, 3H), 8.89 (s, H-5, 1H), 12.8-13.2 (broad, NH, 1H). This product was used without further purification in the next step.

7-Methoxy-3-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**2**).

To a solution of 5.3 g (0.23 mole) of sodium in 640 ml of methanol was added 35 g (0.21 mole) of crude **1**. After boiling under reflux for 4 hours, the solution was cooled and neutralized with acetic acid. After removal of the solvents, the residue was stirred with 50 ml of water for 5 minutes, filtered and recrystallized from methanol to yield 20.5 g (60%) of **2**, mp 237°; nmr (DMSO-d_6): δ 2.52 (s, CH_3, 3H), 4.17 (s, OCH_3, 3H), 8.49 (s, H-5, 1H), 14.0-16.0 (broad, NH, 1H).

Anal. Calcd. for $C_7H_8N_4O$: C, 51.21; H, 4.91; N, 34.13. Found: C, 51.31; H, 4.93; N, 33.94.

1,3-Dimethyl-7-methoxy-1*H*-pyrazolo[4,3-*d*]pyrimidine (**3**) and 2,3-Dimethyl-7-methoxy-2*H*-pyrazolo[4,3-*d*]pyrimidine (**4**).

To an ice-cooled solution of 21 g of sodium hydroxide in 1.7 ℓ of water, 28.85 g (0.176 mole) of **2** was added in small portions. Dimethylsulphate (27.2 ml) was added dropwise and after the addition was complete, the solution was stirred at 0° for 2 hours and at room temperature for 1 hour. After neutralisation with hydrochloric acid, the solution was extracted 10 times with 120 ml of carbon tetrachloride. The combined organic

layers were washed 2 times with 90 ml of water, dried over sodium sulphate and taken to dryness. Recrystallisation from cyclohexane yields 25.6 g (82%) of **3**, mp 115-117°; nmr (DMSO-d_6): δ 2.47 (s, CH_3 at C-3, 3H), 4.12 (s, N-CH_3, 3H), 4.15 (s, O-CH_3, 3H), 8.52 (s, H-5, 1H).

Anal. Calcd. for $C_8H_{10}N_4O$: C, 53.91; H, 5.66; N, 31.44. Found: C, 54.01; H, 5.70; N, 31.30.

The combined water layers were extracted 4 times with 100 ml of chloroform. After drying over sodium sulphate, the chloroform was removed *in vacuo* and the residue recrystallized from cyclohexane to yield 4.7 g (15%) of **4**, mp 110-112°; nmr (DMSO-d_6): δ 2.56 (s, CH_3 at C-3, 3H), 4.1 (s, NCH_3 and OCH_3, 6H), 8.35 (s, H-5, 1H).

Anal. Calcd. for $C_8H_{10}N_4O$: C, 53.91; H, 5.66; N, 31.44. Found: C, 53.88; H, 5.71; N, 31.28.

3-Bromomethyl-7-methoxy-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**6**) and 3-Dibromomethyl-7-methoxy-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**7**).

A mixture of 5 g (28 mmoles) of *N*-bromosuccinimide and 0.3 g of dibenzoylperoxide was added to a solution of 5 g (28 mmoles) of **3** in 170 ml of carbon tetrachloride. The flask was equipped with a reflux condenser to put into an oilbath at 100°. The mixture was refluxed and irradiated with a 100 W photolamp for 90 minutes. After cooling, the succinimide formed was filtered off and the carbon tetrachloride was removed *in vacuo*. After column chromatography (silica gel) with benzene/ethyl acetate/methanol 30/10/1 as solvent, 3.37 g (47%) of **6** was obtained and recrystallized from cyclohexane, mp 129°; nmr (DMSO-d_6): δ 4.14 (s, NCH_3, 3H), 4.21 (s, OCH_3, 3H), 4.93 (s, CH_2Br, 2H), 8.57 (s, H-5, 1H).

Anal. Calcd. for $C_8H_9BrN_4O$: C, 37.38; H, 3.53; Br, 31.08; N, 21.79; O, 6.22. Found: C, 37.64; H, 3.51; Br, 31.17; N, 21.70; O, 6.30.

Additionally, 0.5 g (5.2 %) of **7** was obtained and recrystallized from cyclohexane, mp 130-131°; nmr (deuteriochloroform): δ 4.22 (s, NCH_3, 3H), 4.30 (s, OCH_3, 3H), 7.08 (s, $CHBr_2$, 1H), 8.64 (s, H-5, 1H).

Anal. Calcd. for $C_8H_8Br_2N_4O$: C, 28.60; H, 2.40; Br, 47.57; N, 16.64. Found: C, 28.40; H, 2.45; Br, 47.30; N, 16.44.

7-Methoxy-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine-3-methanol (**5**).

A solution of 2.5 g (9.7 mmoles) of **6** and 1 g (12 mmoles) of sodium hydrogen carbonate in 75 ml of acetone and 75 ml of water was refluxed for 4 hours. After removal of the solvents *in vacuo*, the residue was washed with 3 ml of cold water and recrystallized from ethanol to yield 1.3 g (68%) of **5**, mp 179-182°; nmr (DMSO-d_6): δ 4.17 (s, NCH_3, 3H), 4.20 (s, OCH_3, 3H), 4.72 (t, CH_2OH, 2H, J = 6 Hz), 5.27 (t, CH_2OH, 1 H, J = 6 Hz), 8.51 (s, H-5, 1H).

Anal. Calcd. for $C_8H_{10}N_4O_2$: C, 49.48; H, 5.19; N, 28.85. Found: C, 49.33; H, 5.14; N, 28.91.

3-(2-Hydroxyethoxymethyl)-7-methoxy-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**8**).

A solution of 2.5 g (10 mmoles) of **6**, 0.4 g (2.4 mmoles) of potassium iodide and 1.4 g (10 mmoles) of potassium carbonate in 15 ml of glycol and 15 ml of acetone was stirred at room temperature for 14 hours. After neutralisation with acetic acid and removal of the acetone *in vacuo*, the residue was taken up in 100 ml of water and extracted 4 times with 100 ml of chloroform. The combined organic layers were washed with water, dried over sodium sulphate and taken to dryness. After column chromatography (silicagel) with chloroform/methanol 12/1 as eluant, 0.43 g (18%) of **8** was obtained and recrystallized from a mixture of acetone an cyclohexane, mp 109-110°; nmr (deuteriochloroform): δ 3.79 (s, OCH_2CH_2O, 4H), 4.23 (s, NCH_3, 3H), 4.24 (s, OCH_3, 3H), 4.97 (s, $CH_2OCH_2CH_2O$, 2H), 8.53 (s, H-5).

Anal. Calcd. for $C_{10}H_{14}N_4O_3$: C, 50.41; H, 5.92; N, 23.52. Found: C, 50.31; H, 5.97; N, 23.33.

1-Methyl-7-oxo-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidine-3-methanol (**9**).

A solution of 0.5 g (2.57 mmoles) of **5** in 5 ml of 2*N* sodium hydroxide was stirred at room temperature for 19 hours. After dilution with 30 ml of water, the solution was neutralized with weak acidic ion exchange resin (Merck IV, H^+). The resin was filtered off, washed with water and, after removal of the water *in vacuo*, **9** was recrystallized from 2-propanol to yield 0.32 g (69%), mp 248-250°; nmr (DMSO-d_6): δ 4.18 (s,NCH_3, 3H), 4.64 (s, CH_2O, 2H), 5.19 (broad, OH, 1H), 7.85 (s, H-5, 1H), 12.28 (broad, NH, 1H); ir (potassium bromide): 1695 (C=O).

Anal. Calcd. for $C_7H_8N_4O_2$: C, 46.47; H, 4.48. Found: C, 46.40; H, 4.50.

7-Amino-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine-3-methanol (**10**).

A solution of 0.85 g (4.38 mmoles) of **5** in 30 ml of absolute ethanol, saturated with ammonia at 0°, was heated in an autoclave at 160° for 15 hours. After cooling and removal of the solvent *in vacuo*, the mixture was separated by preparative tlc (silicagel, Merck 60 F) with chloroform/methanol/aqueous ammonia 9/1/0.01 as solvent. Compound **10** was removed from the silicagel by repeated extraction with hot methanol. After removal of the methanol *in vacuo*, the residue was dissolved in hot water, filtered through neutral aluminium oxide and allowed to cool to obtain 0.1 g (12%) of **10**, mp 240°; nmr (DMSO-d_6): δ 4.19 (s, NCH_3, 3H), 4.68 (s, CH_2O, 2H), 5.01 (s, OH, 1H), 7.27 (s, NH_2, 2H), 8.115 (s, H-5, 1H).

Anal. Calcd. for $C_7H_9N_5O$: C, 42.64; H, 5.62; N, 35.51. Found: C, 42.65; H, 5.48; N, 35.23.

7-Amino-3-(2-hydroxyethoxymethyl)-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**11**).

A solution of 0.68 g (2.86 mmoles) of **8** in 50 ml of absolute ethanol, saturated with ammonia at 0°, was heated in an autoclave at 130° for 16 hours. After cooling, the solution was filtered and taken to dryness. The residue was separated by preparative tlc (silicagel) with chloroform/methanol/aqueous ammonia 9/1/0.001 as eluant. Compound **11** was removed from the silica gel by repeated extraction with hot ethanol. The alcoholic solutions were filtered hot through neutral aluminium oxide and concentrated to a small volume. On cooling, 0.25 g (39%) of **11** were obtained, mp 173-175°; nmr (DMSO-d_6): δ 3.39 (s, OH, 1H), 3.48 (s, OCH_2CH_2O, 4H), 4.21 (s,NCH_3, 3H), 4.67 (s, $CH_2OCH_2CH_2O$, 2H), 7.32 (s, NH_2, 2H), 8.16 (s, H-5, 1H).

Anal. Calcd. for $C_9H_{13}N_5O_2$: C, 48.42; H, 5.87; N, 31.37. Found: C, 48.24; H, 5.94; N, 31.09.

Ethyl 7-Oxo-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidine-3-carboxylate (**13**).

A suspension of 8 g (40.4 mmoles) of **12** [8] in 640 ml of absolute ethanol was treated with 55 ml of ethanol, saturated with hydrogen chloride at 0°, and 13.3 ml of triethylorthoformate. After heating under reflux for 2 hours, the solution was concentrated to about 200 ml and the hydrochloride of **13** was precipitated by the addition of ether. The hydrochloride was dissolved in 50 ml of water and neutralized with 10% sodium hydrogen carbonate solution. On cooling, 5 g (59%) of **13** was obtained and recrystallized from ethanol, mp 270-273°; nmr (DMSO-d_6): δ 1.37 (t, CH_2CH_3, 3H, J = 7 Hz), 4.29 (qu, CH_2CH_3, 2H, J = 7 Hz), 8.04 (s, H-5, 1H), 12.22 (broad, NH, 1H); ir: (potassium bromide): 3450, 3240, 3105 (NH), 1715, 1695 (C=O).

Anal. Calcd. for $C_8H_8N_4O_3$: C, 46.16; H, 3.87; N, 26.91. Found: C, 46.24; H, 3.99; N, 26.69.

7-Oxo-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidine-3-methanol (**14**).

A suspension of 1.4 g (6.73 mmoles) of **13** and 0.09 g of ammonium sulphate in 8.5 ml of hexamethyldisilazane was heated under reflux for 3 hours. The excess of hexamethyldisilazane was distilled off and the resulting colourless oil was dissolved in 25 ml of tetrahydrofuran. This solution was added dropwise under nitrogen to a solution of 0.5 g (13.17 mmoles) of lithium aluminium hydride in 70 ml of tetrahydrofuran. The reaction mixture was stirred at room temperature until no more **13** could be detected by gc. The excess lithium aluminium hydride was destroyed by the careful addition of 2.5 ml of water in 42 ml of dimethylformamide. The reaction mixture was brought to *p*H 7 with hydrochloric acid and filtered. The precipitate was extracted twice with 50 ml of dimethylformamide/water 1/1. All solutions were combined, taken to dryness and washed twice with 10 ml of ether. After recrystallization from 8 ml of water, 0.8 g (72%) of **14** was obtained, mp 267-268°; nmr (DMSO-d_6) δ 4.73 (s, CH_2O, 2H), 5.04 (s, OH, 1H), 7.81 (s, H-5, 1H), 11.0-12.0 (broad, pyrimidine-NH, 1H), 13.0-14.0 (broad, pyrazole-NH).

Anal. Calcd. for $C_6H_6N_4O_2$: C, 43.38; H, 3.64; N, 33.72. Found: C, 43.24; H, 3.68; N, 33.57.

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