Synthesis of *S*-Formycinyl-L-homocysteine and Its 3′-Deoxy Derivative

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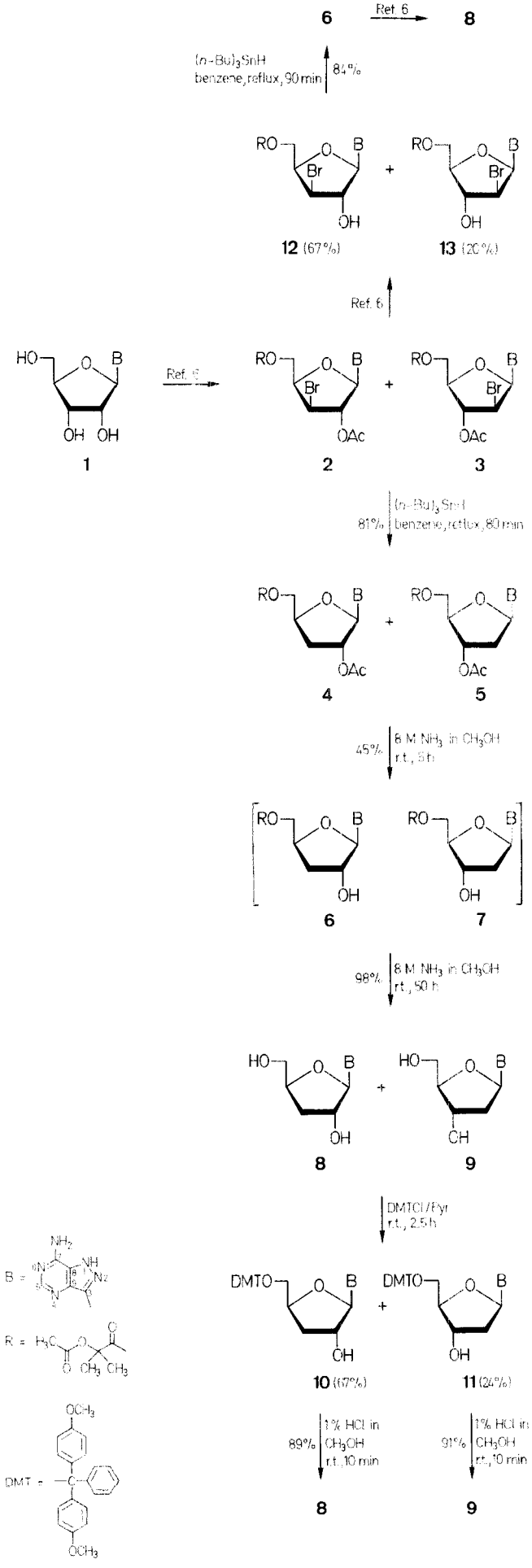
3′-Deoxyformycin was prepared by transformation of the formycin A with 2-acetoxyisobutyryl bromide. Both formycin A and its 3′-deoxy analogue were converted into their 5′-chloro-5′-deoxy derivatives with thionyl chloride. Finally 5′-chloro-5′-deoxy formycin A and 5′-chloro-3′,5′-dideoxyformycin A were condensed with L-homocysteine sodium salt to give *S*-formycinyl-L-homocysteine and *S*-3′-deoxyformycinyl-L-homocysteine in good yields.

S-Adenosyl-L-homocysteine and some of its congeners have shown a wide spectrum of biological activity which stems from their ability to inhibit *S*-adenosylmethionine dependent transmethylations.[1] Two approaches to the synthesis of these compounds have been reported. The first approach involves condensation of 2′,3′-*O*-isopropylidene-5′-*O*-tosyladenosine[2] or 5′-chloro-5′-deoxyadenosine[3] with L-homocysteine sodium salt. The other is based on the condensation of suitably protected L-homocystine with a free nucleoside in the presence of tri-*n*-butylphosphine.[4,5] *S*-Formycinyl-L-homocysteine and *S*-3′-deoxyformycinyl-L-homocysteine were prepared in order to carry out both structure-activity and metabolic studies. These compounds are envisaged to be resistant to purine nucleoside phosphorylase due to the presence of the C-glycosyl bond between the aglycone and the sugar moiety. Preparation of these analogues was carried out *via* condensation of appropriate 5′-chloro-5′-deoxynucleosides with L-homocysteine sodium salt. The synthesis which is based on condensation of formycin A or 3′-deoxyformycin A with suitably protected L-homocystine in the presence of tri-*n*-butylphosphine is under investigation and the results will be published elsewhere.

3′-Deoxyformycin was prepared according to the route outlined in Scheme A. The mixture of 7-amino-3-[5-*O*-(2-acetoxyisobutyryl)-1-*O*-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl]pyrazolo[4,3-*d*]pyrimidine **(2)** and 7-amino-3-[5-*O*-(2-acetoxyisobutyryl)-2-bromo-2-deoxy-3-*O*-acetyl-β-D-arabinofuranosyl]pyrazolo[4,3-*d*]pyrimidine **(3)** in the ratio of 3:1 was obtained following the procedure described earlier.[6] It was allowed to react with tri-*n*-butyltin hydride in benzene in the presence of 2,2′-azobis-(2-methylpropionitrile). Like the compounds **2** and **3** the products 7-amino-3-[5-*O*-(2-acetoxyisobutyryl)-2-*O*-acetyl-3-deoxy-β-D-ribofuranosyl]pyrazolo[4,3-*d*]-pyrimidine **(4)** and 7-amino-3-[5-*O*-(2-acetoxyisobutyryl)-3-*O*-acetyl-2-deoxy-β-D-erythropentofuranosyl]pyrazolo[4,3-*d*]-pyrimidine **(5)** could not be separated by column chromatography on silica gel. Attempted selective removal of the 2′ or 3′-*O*-acetyl group from the mixture of **4** and **5** with 8 molar methanolic ammonia during 5 h at room temperature afforded 5′-*O*-(2-acetoxyisobutyryl)-3′-deoxyformycin **(6)** and 5′-*O*-(2-acetoxyisobutyryl)-2′-deoxyformycin **(7)** which had sufficiently different R_f values to be separated by column chromatography on silica gel. However, under the conditions described the deacetylation was accompanied by a deprotection of the 5′-position in about 15% yield whilst considerable amounts of the starting materials were still present. The reaction was therefore allowed to proceed for 50 h to afford the mixture of 3′-deoxyformycin **(8)** and 2′-deoxyformycin **(9)**. Separation of these compounds could be achieved by ion exchange chromatography on a Dowex 1-X2 (Cl^-) column.[7] It was found, however, more convenient to convert the compounds **8** and **9** into their 5′-*O*-dimethoxytrityl derivatives **10** and **11** with dimethoxytrityl chloride in pyridine in 91% yield. 5′-*O*-Dimethoxytrityl-3′-deoxyformycin **(10)** and its 2′-deoxy counterpart **11** had appreciably different R_f values (0.28 and 0.34, respectively, see experimental) and could be readily separated by column

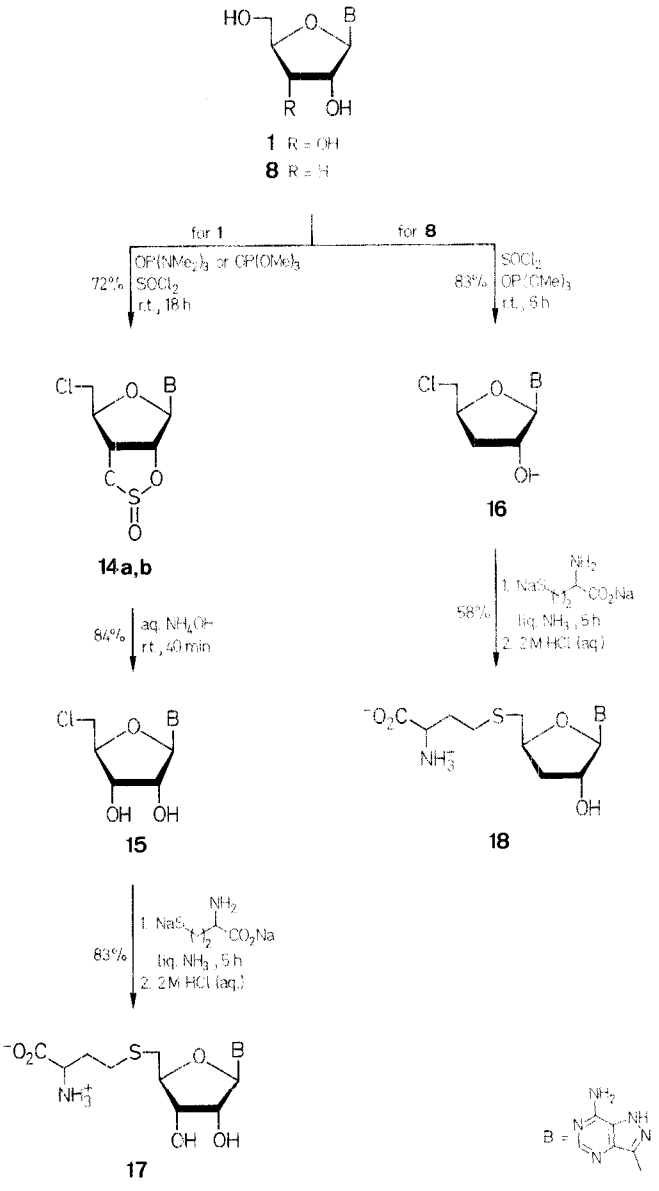


Scheme A

chromatography on silica gel. The subsequent detritylation of pure **10** and **11** with 1 % methanolic hydrogen chloride gave 3′-deoxyformycin **(8)** and 2′-deoxyformycin **(9)** in virtually quantitative yields as crystalline hydrochlorides.

3′-Deoxyformycin was also synthesised by an alternative route. 7-amino-3-[5-*O*-(2-acetoxyisobutyryl)-3-bromo-3-deoxy-β-D-xylofuranosyl]pyrazolo[4,3-*d*]pyrimidine **(12)** prepared according to the reported procedure[6] and purified by short column chromatography on silica gel, was reduced with tri-*n*-butyltin hydride in benzene in the presence of 2,2′-azobis-(2-methylpropionitrile) to give 5′-*O*-(2-acetoxyisobutyryl)-3′-deoxyformycin **(6)** in high yield (84 %). The removal of the 5′-*O*-protecting group from **6** with methanolic ammonia[6] afforded 3′-deoxyformycin **(8)**. The structure of the 3′-deoxyformycin was confirmed by its analytical and spectroscopic data which were in good agreement with those quoted earlier[6] as well as by crystallographic analysis.[8]

Formycin A **(1)** and 3′-deoxyformycin A **(8)** were subsequently chlorinated with thionyl chloride in trimethyl phosphate[9] or hexamethylphosphotriamide[10] (Scheme **B**). Reaction of the formycin A proceeded through diastereoisomeric mixture of 5′-chloro-2′,3′-*O*-sulphinyl derivatives **14a** and **14b** having the



Scheme B

epimeric chiral center located on sulphur atom. Both epimers were isolated and characterised, though absolute configurations of individual epimers were not determined. They had identical UV spectra but different ^{1}H-NMR spectra and optical rotations. Formation of such products was reported during chlorination of some diols[11] and nucleosides[12,13] under similar conditions. The mixture of compounds **14a** and **14b** could be deprotected *in situ* with aqueous ammonia to give crystalline 5′-chloro-5′-deoxyformycin **(15)** in 84% yield. Compounds of the type **14** were not detected during chlorination of the 3′-deoxyformycin **(8)**. Examination of the reaction mixture by HPTLC revealed instead the presence of small amount of side products with R_f values higher than that of **16** which could derive from the substitution at both 2′ and 5′-positions. 5′-Chloro-3′,5′-dideoxyformycin **(16)** was isolated in 83% yield following column chromatography on silica gel.

Finally compounds **15** and **16** were condensed with L-homocysteine sodium salt in liquid ammonia to afford *S*-formycinyl-L-homocysteine **(17)** and *S*-3′-deoxyformycinyl-L-homocysteine **(18)** respectively. The former was isolated in 83% yield by crystallisation whereas **18** was isolated by ion exchange chromatography on Sephadex A-25 column in 58% yield. Both **17** and **18** were homogeneous on HPLC and gave positive reactions with ninhydrin. The structures of **17** and **18** were established on the basis of spectroscopic and analytical data. The ^{1}H-NMR spectrum revealed the presence of signals which correspond to the amino acid and the nucleoside moiety. Anomeric protons appeared as doublets at $\delta = 5.00$ ($J = 5.4$ Hz) for **17** and at $\delta = 5.06$ ($J = 4.8$ Hz) for **18**. The anomeric protons of *S*-adenosyl-L-homocysteine and its 3′-deoxy counterpart appear as doublets at $\delta = 5.86$ ($J = 5.5$ Hz) and 5.89 ($J = 5.3$ Hz) respectively.[4,5] The characteristic differences between the chemical shifts corresponding to anomeric protons of **17** and **18** and those of their respective *N*-glycosyl analogues confirm the presence of the C-glycosyl bond between the aglycone and the sugar moiety in **17** and **18**.[14] The compounds are undergoing biochemical evaluation and the results will be published elsewhere.

Melting points were determined on a Reichert micro hot stage apparatus and are uncorrected. UV spectra were measured in 95% EtOH with a Pye-Unicam SP8-150 UV-Vis spectrometer. Observed rotations at the Na-D line were obtained at 25°C using a Perkin-Elmer 141 polarimeter. ^{1}H-NMR spectra were recorded at 250 MHz with a Bruker WH 250 spectrometer with TMS as an internal standard and DMSO-d_6 as a solvent unless otherwise indicated. The presence of water was confirmed by ^{1}H-NMR in cases where analytical data are given for hydrates; 2′-OH, 3′-OH, NH_2, N_1-H, and H_2O protons were exchangeable with D_2O. HPTLC was run on Merck silica gel 60 F_{254} analytical plates in the following solvent systems: (A) $CHCl_3$/EtOH (19:1), (B) $CHCl_3$/EtOH (9:1), (C) $CHCl_3$/EtOH (4:1) and on Merck DC-Alufolien Cellulose F sheets in system (D) *n*-BuOH/AcOH/H_2O (12:3:5) (upper layer). Column chromatography was carried out on silica gel 60 (230–400 mesh) (Merck) and short column chromatography on silica gel 60H (Merck). Solvent removal was performed *in vacuo* at 30–40°C unless otherwise indicated. L-Homocystine and formycin A monohydrate were purchased from SIGMA.

7-Amino-3-[2-*O*-acetyl-3-deoxy-5-*O*-(2-acetoxyisobutyryl)-β-D-ribofuranosyl]pyrazolo[4,3-*d*]pyrimidine (4) and 7-Amino-3-[2-deoxy-3-*O*-acetyl-5-*O*-(2-acetoxyisobutyryl)-β-D-erythropentofuranosyl]pyrazolo-[4,3-*d*]pyrimidine (5):

A mixture of **2** and **3** (1.50 g, 3 mmol) in the ratio of 3:1, which is prepared by reaction of the formycin A monohydrate **(1)** with 2-acetoxyisobutyryl bromide,[6] is dissolved in benzene (30 mL) under argon and Bu_3SnH (3.55 g, 12.15 mmol) and 2,2′-azobis-(2-methylpropionitrile) (0.08 g, 0.45 mmol) are added. The stirred reactants are heated under reflux for 80 min. The clear solution is cooled to ambient temperature and added dropwise to a stirred light petroleum (b.p. 30–40°C) (500 mL). The resulting colourless precipitate is collected by filtration and applied to a column of silica gel. The product is eluted with $CHCl_3$/EtOH (93:7) to give **4** and **5** in a ratio of 3:1 (^{1}H-NMR) as a colourless froth; yield: 1.03 g (81%). An analytical sample is obtained when the product is dissolved in a small amount of $CHCl_3$ and added dropwise to a stirred light petroleum (b.p. 30–40°C). The resulting colourless precipitate is collected by centrifugation and dried in a desiccator; $R_f = 0.20$ (B), 0.41 (C).

$C_{18}H_{23}N_5O_7 \cdot 0.25\ H_2O$	calc.	C 50.76	H 5.56	N 16.44
(425.9)	found	50.27	5.49	16.48

UV: $\lambda_{max} = 294$ nm (log $\varepsilon = 4.00$).

^{1}H-NMR (DMSO-d_6): $\delta = 1.38$, 1.41 [2s, $(CH_3)_2C$], for **4**; 1.46, 1.49 [2s, $(CH_3)_2C$] for **5**; 3.35 (s, H_2O); the integration indicates the ratio of **4**:**5** as 3:1.

3′-Deoxyformycin A (8) and 2′-deoxyformycin A (9):

The mixture of compounds **4** and **5** in the ratio of 3:1 (0.84 g, 2 mmol) is dissolved in 8 molar methanolic ammonia (25 mL) and the solution is stirred at room temperature. After 5 h examination of the mixture by HPTLC (system C) indicates the estimated amount of the starting material as 40% (single spot, $R_f = 0.41$), products of the deacetylation **6** and **7** as 45% ($R_f = 0.21$ and 0.13, respectively) and products of the full deprotection **8** and **9** as 15% (single spot, $R_f = 0.08$). After 50 h the solvent is removed *in vacuo* and the residue dissolved in water (150 mL). The aqueous solution is extracted with $CHCl_3$ (4 × 20 mL) and ether (25 mL). The organic extracts are discarded and the aqueous layer is concentrated under reduced pressure to give the mixture of **8** and **9** in the ratio of 3:1 (^{1}H-NMR) as a colourless glass; yield: 0.49 g, (98%). The mixture is separable on HPTLC by multiple development in system (B) and is used in the next stage (see below) without further purification.

5′-*O*-Dimethoxytrityl-3′-deoxyformycin (10) and 5′-*O*-dimethoxytrityl-2′-deoxyformycin (11):

The mixture of compounds **8** and **9** in the ratio of 3:1 (0.50 g, 2 mmol) is coevaporated with pyridine (3 × 25 mL). The residue is dissolved in dry pyridine (40 mL) and dimethoxytrityl chloride (0.82 g, 2.4 mmol) dissolved in pyridine (40 mL) is added dropwise during 1.5 h. The pale yellow solution is stirred at room temperature for further 1 h and then the reaction is quenched by addition of MeOH (30 mL). The solvents are largely removed *in vacuo* and the residue is dissolved in $CHCl_3$ (150 mL) and washed with water (30 mL), 5% aqueous $NaHCO_3$ (2 × 30 mL) and water (30 mL). The $CHCl_3$ is removed *in vacuo*, the residue coevaporated with toluene (2 × 30 mL) and applied to a column of silica gel which is initially eluted with $CHCl_3$. Subsequent elution with $CHCl_3$/EtOH (19:1) affords **10** as a colourless froth; yield: 0.74 g (67%); m.p. 139–143°C (EtOH); $R_f = 0.28$ (B), 0.49 (C).

$C_{31}H_{31}N_5O_5 \cdot 0.5H_2O$	calc.	C 66.17	H 5.73	N 12.45
(562.6)	found	65.90	5.57	12.36

UV: $\lambda_{max} = 283$ (log $\varepsilon = 3.99$), 294 nm (log $\varepsilon = 3.95$).

^{1}H-NMR (DMSO-d_6): $\delta = 1.95$ (m, 1H, H-3′); 2.34 (m, 1H, H-3″); 3.05 (m, 2H, H-5′, H-5″); 3.35 (s, H_2O), 3.71 (s, 3H, OCH_3); 3.72 (s, 3H, OCH_3); 4.42 (m, 1H, H-4′); 4.80 (m, 1H, H-2′); 5.06 (d, 1H, H-1′, $J = 2.60$ Hz); 5.27 (br s, 1H, OH-2′); 6.77, 7.20 (m, 13H_{arom}); 8.14 (s, 1H, H-5); 12.68 (br s, 1H, N_1-H).

Further elution of the column with $CHCl_3$/EtOH (9:1) affords **11** as a colourless foam; yield: 0.26 g (24%); m.p. 133–135°C (EtOH); $R_f = 0.14$ (B), 0.35 (C).

$C_{31}H_{31}N_5O_5 \cdot H_2O$	calc.	C 65.13	H 5.81	N 12.25
(571.6)	found	65.30	5.64	12.63

UV: $\lambda_{max} = 283$ (log $\varepsilon = 3.99$), 294 nm (log $\varepsilon = 3.95$).

^{1}H-NMR (DMSO-d_6): $\delta = 2.10$ (m, 1H, H-2′); 2.86 (m, 1H, H-2″), 3.05 (m, 2H, H-5′, H-5″); 3.34 (s, H_2O); 3.71 (s, 6H, OCH_3); 3.94 (m, 1H, H-4′); 4.34 (m, 1H, H-3′); 5.13 (d, 1H, OH-2′, $J = 3.88$ Hz); 5.42 (dd, 1H, H-1′, $J = 6.19$, 3.03 Hz); 6.81, 7.24 (m, 13H_{arom}); 8.10 (s, 1H, H-5); 12.68 (br s, 1H, N_1-H).

3′-Deoxyformycin (8):

5′-*O*-Dimethoxytrityl-3′-deoxyformycin (**10**; 0.56 g, 1 mmol) is dissolved in 1% methanolic HCl (30 mL) and the solution is stirred at room temperature for 10 min. The solvent is removed *in vacuo* and the residue partitioned between H_2O/$CHCl_3$ (5:1) (120 mL). The aqueous and organic layers are separated and the aqueous solution is further extracted with $CHCl_3$ (2 × 20 mL), and then concentrated *in vacuo*. The residue is coevaporated with 1% methanolic HCl (50 mL) and cry-

stallised from EtOH to give crystalline **8** as its hydrochloride; yield: 0.26 g (89%); m.p. 208–209°C (Lit.[6] m.p. 207–209°C); ^{1}H-NMR data agree with literature[6] values.

UV: λ_{max} = 233 (log ε = 3.81), 295 nm (log ε = 4.01).

2′-Deoxyformycin (9):

The compound **9** is prepared as its hydrochloride following removal of 5′-O-dimethoxytrityl group from **11** in the way which is identical to that described above for 3′-deoxyformycin **(8)**; yield: 91%; m.p. 196–197°C (EtOH) (Lit.[6] m.p. 194–196°C); ^{1}H-NMR data agree with literature[6] values.

UV: λ_{max} = 232 (log ε = 3.79), 294 nm (log ε = 4.00).

7-Amino-3-[3-bromo-3-deoxy-5-*O*-(2-acetoxyisobutyryl)-β-D-xylofuranosyl]pyrazolo[4,3-*d*]pyrimidine (12) and 7-amino-3-[2-bromo-2-deoxy-5-*O*-(2-acetoxyisobutyryl)-β-D-arabinofuranosyl]pyrazolo[4,3-*d*]pyrimidine (13):

The mixture of compounds **12** and **13** (3:1), which is prepared by reaction of compounds **2** and **3** (2.54 g, 5.07 mmol) with 8 molar methanolic ammonia,[6] is applied to a short column of silica gel. Elution of the column with $CHCl_3$/EtOH (29:1) affords **12** as a colourless foam; yield: 1.57 g (67%); ^{1}H-NMR data agree with literature values;[6] R_f = 0.25 (B), 0.37 (C).

Further elution of the column with $CHCl_3$/EtOH (29:1) affords **13** as a colourless powder; yield: 0.47 g (20%); ^{1}H-NMR data agree with literature values[6]; R_f = 0.21 (B), 0.31 (C).

7-Amino-3-[5-*O*-(2-acetoxyisobutyryl)-3-deoxy-β-D-ribofuranosyl]pyrazolo[4,3-*d*]pyrimidine [(5′-*O*-(2-acetoxyisobutyryl)-3′-deoxyformycin)] (6):

Compound **12** (1.37 g, 3 mmol) is dissolved in dry benzene (30 mL) under an atmosphere of argon and Bu_3SnH (3.55 g, 12.15 mmol) and 2,2′-azobis-(2-methylpropionitrile) (0.08 g, 0.45 mmol) are added. The mixture is heated under reflux with stirring for 90 min, and then cooled to ambient temperature. The solvent is removed *in vacuo* and the residue coevaporated with toluene (2 × 30 mL) and applied to a short column of silica gel. The product is eluted with $CHCl_3$/EtOH (47:3) to give **6** as a colourless foam; yield 0.96 g (84%); ^{1}H-NMR data agree with literature[6] values; R_f = 0.21 (C).

UV: λ_{max} = 294 nm (log ε = 3.94)

Epimeric 7-Amino-3-(5-chloro-5-deoxy-2,3-*O*-sulphinyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidines (14a and 14b):

Formycin A monohydrate (**1**; 0.53 g, 2 mmol) is added to a stirred solution of HMPA (5 mL) and thionyl chloride (0.38 mL), 5.2 mmol). The resulting pale-yellow solution is stirred at room temperature for 18 h, and then poured onto ice-water (10 mL). The mixture is neutralised carefully with conc. aqueous ammonia and extracted with $CHCl_3$ (5 × 10 mL). The combined $CHCl_3$ extract is dried (Na_2SO_4) and the solvent is removed *in vacuo*. The residue is applied to a silica gel column. Elution of the column with $CHCl_3$/EtOH (9:1) affords the less polar **14a** as a colourless foam; yield: 0.11 g (17%); m.p. 139–145°C partial melting, decomposes at > 200°C ($CHCl_3$); $[\alpha]_D^{25}$ = −51.7° (*c* = 0.53, MeOH); R_f = 0.22 (B), 0.43 (C).

$C_{10}H_{10}ClN_5O_4S$	calc.	C 36.21	H 3.03	N 21.11
(331.7)	found	35.87	2.90	20.81

UV: λ_{max} = 293 nm (log ε = 4.00)

^{1}H-NMR (DMSO-d_6): δ = 3.85 (m, 2H, H-5′, H-5″); 4.42 (m, 1H, H-4′); 5.34 (d, 1H, H-1′, *J* = 4.86 Hz); 5.74 (m, 1H, H-3′); 6.30 (m, 1H, H-2′); 7.40 (br s, 2H, NH_2); 8.24 (s, 1H, H-5); 13.11 (br s, 1H, N_1-H).

Further elution of the column with $CHCl_3$/EtOH (9:1) gives the mixture of **14a** and **14b**; yield: 0.30 g (45%) and pure **14b** as a white froth; yield: 0.07 g (10%); m.p. 140–148°C partial melting, decomposes at > 200°C (EtOAc); $[\alpha]_D^{25}$ = −99.8° (*c* = 0.52, MeOH); R_f = 0.19 (B), 0.40 (C).

$C_{10}H_{10}ClN_5O_4S$	calc.	C 36.21	H 3.03	N 21.11
(331.7)	found	35.69	2.96	20.70

UV: λ_{max} = 293 nm (log ε = 3.99)

^{1}H-NMR (DMSO-d_6): δ = 3.87 (m, 2H, H-5′, H-5″); 4.62 (m, 1H, H-4′); 5.63 (m, 2H, H-1′, H-3′); 6.20 (m, 1H, H-2′); 7.48 (br s, 2H, NH_2); 8.24 (s, 1H, H-5); 13.12 (br s, 1H, N_1-H).

7-Amino-3-(5-chloro-5-deoxy-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine(5′-chloro-5′-deoxyformycin A) (15):

Formycin A monohydrate (**1**; 0.53 g, 2 mmol) is added to a stirred solution of trimethylphosphate (5 mL) and thionyl chloride (0.38 mL, 5.2 mmol). The resulting pale-yellow solution is stirred at room temperature for 18 h, and then poured onto ice water (7.5 mL). The mixture is brought to pH 9 with conc. aqueous ammonia and stirred at room temperature for about 40 min and the extracted with $CHCl_3$ (7.5 mL). $CHCl_3$ and water layers are separated and stored at 0°C for 24 h. A colourless crystalline precipitate from both the water and $CHCl_3$ layer is collected by filtration to give **15**; yield: 0.48 g (84%). An analytical sample is obtained by recrystallisation from water; m.p. 143–150°C partial melting, decomposes at > 200°C (Lit.[15] no m.p. quoted); R_f = 0.11 (C).

$C_{10}H_{12}ClN_5O_3 \cdot 0.75\ H_2O$	calc.	C 40.14	H 4.54	N 23.40
(299.2)	found	39.80	4.54	23.53

UV: λ_{max} = 294 nm (log ε = 3.97); Lit.[15] UV (H_2O); λ_{max} = 295 nm (log ε = 4.0).

^{1}H-NMR (DMSO-d_6): δ = 3.34 (s, H_2O); 3.75 (m, 1H, H-5′); 3.85 (m, 1H, H-5″); 4.01 (m, 1H, H-4′) 4.23 (m, 1H, H-3′); 4.64 (m, 1H, H-2′); 5.05 (d, 1H, H-1′, *J* = 5.57 Hz); 5.17, 5.19 (2 br s, 2H, OH-2′, OH-3′); 7.40 (br s, 2H, NH_2); 8.18 (s, 1H, H-5); 13.00 (br s, 1H, N_1-H).

7-Amino-3-(5-L-homocysteinyl-5-deoxy-β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine(*S*-formycinyl-L-homocysteine) (17):

L-Homocystine (0.27 g, 1 mmol) and sodium (0.092 g, 4 mmol) are added portionwise to a stirred liquid ammonia (30 mL). The blue colour of the resulting solution is discharged with a small amount of NH_4Cl and 5′-chloro-5′-deoxyformycin A (**15**; 0.29 g, 1 mmol) is added. The stirring is continued for about 5 h until ammonia evaporates. The residual ammonia is removed under vacuum and the resulting white solid residue is dissolved in water (80 mL) and stirred at room temperature for 15 min. Insoluble particles are filtered off and the filtrate acidified to pH 6.5 with 2 molar HCl, concentrated under reduced pressure to about 20 mL and stored at 0°C for 16 h. The resulting colourless crystals are collected by filtration. Concentration of the mother liquors affords further batches of the crystalline material, which is essentially pure on TLC (D) and gives positive test with ninhydrin; yield: 0.32 g (83%). Recrystallisation from water gives analytically pure product which is homogeneous on HPLC; m.p. > 210°C (dec.), R_f = 0.08 (D).

$C_{14}H_{20}N_6O_5S.0.75\ H_2O$	calc.	C 42.25	H 5.44	N 21.15
(397.9)	found	42.13	5.03	21.31

UV: λ_{max} = 294 nm (log ε = 3.89).

^{1}H-NMR (DMSO-d_6): δ = 1.98, 2.05 (m, 2H, H-β); 2.68 (t, 2H, H-γ, *J* = 6.60 Hz); 2.78 (m, 2H, H-5′, H-5″); 3.40 (br s, H_2O); 3.42 (m, 1H, H-α); 3.93 (m, 1H, H-4′); 4.11 (m, 1H, H-3′); 4.62 (m, 1H, H-2′); 5.00 (d, 1H, H-1′, *J* = 5.41 Hz); 5.17 (br s, 1H, OH); 7.78 (br s, 2H, NH_2); 8.15 (s, 1H, H-5), 14.00 (br s, N_1-H).

7-Amino-3-(5-chloro-3,5-dideoxy-β-D-ribofuranosyl)pyrazolo[4,3-*d*]-pyrimidine(5′-chloro-3′,5′-dideoxyformycin) (16):

3′-Deoxyformycin (**8**; 0.25 g, 1 mmol) is added to a solution of thionyl chloride (0.19 ml, 2.6 mmol) in trimethylphosphate (2.5 mL). The reactants are stirred at room temperature for 6 h and then poured onto ice-water (25 mL). The aqueous solution is neutralised with Bio-Rad AG-1-X-2 (100–200 mesh) OH^- resin, the resin is filtered off and washed with aqueous methanol (1:1) (4 × 15 mL). The combined aqueous and methanolic solutions are concentrated under reduced pressure. The residue is coevaporated with pyridine (3 × 15 mL) and toluene (3 × 15 mL), and applied to a short column of silica gel. The product is eluted with $CHCl_3$/EtOH (89:11) to give **16** as a colourless froth; yield: 0.22 g (83%); m.p. 140–150°C partial melting, decomposes at > 200°C (H_2O); R_f = 0.21 (C).

$C_{10}H_{12}ClN_5O_2.0.75\ H_2O$	calc.	C 42.41	H 4.80	N 24.73
(283.2)	found	41.96	4.63	24.83

UV: λ_{max} = 294 nm (log ε = 3.97).

^{1}H-NMR (DMSO-d_6): δ = 2.06 (m, 1H, H-3′); 2.28 (m, 1H, H-3″); 3.49 (s, H_2O); 3.78 (m, 2H, H-5′, H-5″); 4.40 (m, 1H, H-4′); 4.68 (m, 1H; H-2′); 5.05 (d, 1H, H-1′, *J* = 3.15 Hz); 7.43 (br s, 2H; NH_2); 8.17 (s, 1H, H-5); 13.04 (br s, N_1-H).

7-Amino-3-(5-L-homocysteinyl-3,5-dideoxy-β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine (*S*-3′-deoxyformycinyl-L-homocysteine) (18):

L-Homocystine (0.27 g, 1 mmol) and sodium (0.092 g, 4 mmol) are added portionwise to a stirred liquid ammonia (30 mL). The blue colour of the resulting solution is discharged with a small amount of NH_4Cl and 5′-chloro-3′,5′-dideoxyformycin (**16**; 0.27 g, 1 mmol) is added. The stirring is continued for about 5 h until ammonia evaporates. The

residual ammonia is removed under vacuum and the resulting white solid residue is dissolved in water (100 mL) and stirred at room temperature for 15 min. Insoluble particles are filtered off and the filtrate acidified to pH 6.5 with 2 molar HCl, concentrated under reduced pressure and applied to a column of Sephadex A-25 (2.5 × 20 cm). The product is eluted with 0.01 molar aqueous $Et_3NH_2CO_3$ buffer. The fractions containing the product (UV absorbing, ninhydrin positive) are combined, concentrated and lyophilised to give **18** as a colourless crystalline precipitate which is homogeneous on TLC (D) and HPLC; yield: 0.21 g (58%); m.p. > 200°C (dec.); $R_f = 0.15$ (D).

$C_{14}H_{20}N_6O_4S \cdot H_2O$	calc.	C 43.52	H 5.74	N 21.74
(386.4)	found	43.15	5.49	21.68

UV: λ_{max} = 294 nm (log ε = 3.70).

^{1}H-NMR (DMSO-d_6): δ = 1.95–2.37 (m, 4H, H-3′, H-3″, H-β); 2.64 (t, 2H, H-γ, J = 7.32 Hz); 2.77 (m, 2H, H-5′, H-5″); 3.45 (br s, H_2O); 3.46 (m, 1H, H-α); 4.37 (m, 1H, H-4′); 4.71 (m, 1H, H-2′); 5.06 (*d*, 1H, H-1′, J = 2.95 Hz); 7.81 (br s, 2H, NH_2); 8.14 (s, 1H, H-5); 14.30 (br s, N_1-H).

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