PREPARATION AND BIOLOGICAL EFFECTS OF N-TRIS(HYDROXYMETHYL)METHYLAMINOCARBONYLMETHYL DERIVATIVES OF HETEROCYCLIC BASES

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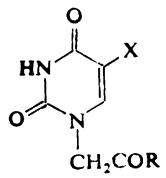
The title compounds were synthetized by the reaction of TRIS with *p*-nitrophenyl or alkyl esters of N-carboxymethyl derivatives of uracil, 5-chloro-, 5-bromo-, 5-iodouracil, thymine, cytosine, 6-azauracil, 2-pyridone, 2-pyrimidone, 3-pyridazone and orotic acid. The following novel N-carboxymethyl derivatives are also described: 6-azauracil derivative *VIIa* by condensation of 4-thio-6-azauracil with methyl bromoacetate followed by hydrolysis, 5-chlorouracil derivative *IIIa* by chlorination of uracil compound *IIa*, 2-pyrimidone (*IXa*) and 3-pyridazone derivative *Xa* by the reaction of the sodium salts of the bases with sodium chloroacetate. Of all the amides tested, only the 3-pyridazone derivative *Xd* and orotic acid derivative *XIId* inhibited the growth of L-1210 mouse leukemic cells *in vitro* with ID_{50} approx. 10^{-4} mol l^{-1}.

Recently we prepared[1] a series of N^1-aminocarbonylmethyl derivatives of 5-fluorouracil and studied their inhibition of growth of L-1210 mouse leukemic cells. Of all the compounds studied, only the tris(hydroxymethyl)methylaminocarbonylmethyl derivative *Id* showed a marked inhibitory effect: under the given experimental conditions its ID_{50} was similar to that of 5-fluorouracil. This, in itself interesting finding, was potentiated by the fact that compounds of this type should not be able to generate 5-fluorouracil under *in vivo* conditions and we therefore decided to study in detail the structure-activity relationship for derivatives analogous to *Id*. In this paper we investigate whether the biological activity has its origin in the tris(hydroxymethyl)-methylaminocarbonylmethyl structural unit bonded to the heterocyclic system.

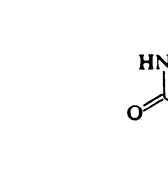
We prepared a series of compounds, derived from pyrimidine bases of three types: natural bases (uracil, cytosine, thymine), modified bases (2-pyridone, 3-pyridazone) and modified bases which alone or as their N^1-substituted derivatives show biological (antibacterial, cancerostatic) activity (5-halogenouracils, 6-azauracil, 2-pyrimidone). In addition to these compounds, orotic acid tris(hydroxymethyl)methylamide (*XIId*) was also prepared.

The compounds were synthesized starting from the corresponding N-carboxymethyl derivatives most of which had been prepared already previously. The hitherto unde-

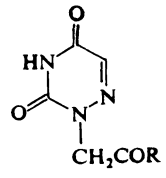
scribed 5-chlorouracil derivative *IIIa* was obtained by chlorination of 1-carboxymethyluracil (*IIa*) with N-chlorosuccinimide, 1-carboxymethyl-6-azauracil (*VIIa*) was prepared by reaction of 4-thio-6-azauracil with excess of methyl bromoacetate followed by hydrolysis, and the 1-carboxymethyl derivatives of 2-pyrimidone and 3-pyridazone (*IXa* and *Xa*, respectively) were obtained from sodium salts of the corresponding heterocyclic derivatives and sodium chloroacetate (Table I).



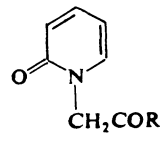
I, X = F
II, X = H
III, X = Cl



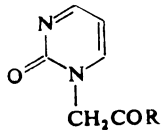
IV, X = Br
V, X = I
VI, X = CH_3



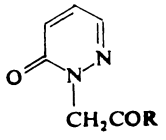
VII



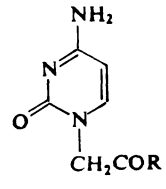
VIII



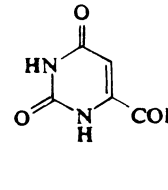
IX



X



XI



XII

In formulae *I*—*XII*: *a* R = OH; *b* R = OC_2H_5; *c* R = O—$C_6H_4(NO_2\text{-}p)$; *d* R = $NHC(CH_2OH)_3$.

The mentioned carboxylic acids were converted into their tris(hydroxymethyl)-methylamides by the procedure used in our previous communication[1], *i.e.* by transformation of the carboxylic acids into their activated *p*-nitrophenyl esters in the presence of N,N'-dicyclohexylcarbodiimide and subsequent *in situ* aminolysis of these intermediates with TRIS. However, in some cases this method was not satisfactory and was replaced by aminolysis of alkyl esters prepared by esterification of the starting carboxylic acids. Best results were obtained when the aminolysis with TRIS was carried out in methanol at temperatures higher than 100°C; however, the reaction can be performed also in other solvents such as *e.g.* dimethylformamide. The obtained tris(hydroxymethyl)methylamides were purified by chromatography on a weakly acidic cation-exchange resin. A complete purification was achieved by chromatography on octadecyl-silica gel in water. The obtained products were pure according to HPLC and were further used in the biological tests.

Structure of the prepared compounds was confirmed by elemental analysis and UV-spectra which only slightly differ from those of other N-substituted derivatives of the corresponding bases (Table II). Their mass spectra exhibit no molecular peaks but contain characteristic fragments at M−18 and M−31 due to loss of water or CH_2OH group.

TABLE I

Chromatography and electrophoresis

Compound	R_F		k^a		E_{Up}
	S1	S2	S4	S5	
Ia	0·38	—	1·38	—	1·17
IIa	0·49	0·48	0·86[b]	—	0·84
IIIa	0·35	—	2·45	—	0·97
Va	0·38	—	3·40	—	1·02
VIa	0·53	0·66	1·82	—	0·72
VIIa	0·35	—	—	—	1·62
VIIIa	0·60	0·95	—	—	0·87
IXa	0·50	—	2·04	—	0·86
Xa	0·47	—	—	—	0·74
XIa	0·42	—	0·72	—	0·25
XIIa	0·45	—	—	—	0·84
Vb	—	0·80	4·35[c]	—	0·02
VIIb	—	0·54	—	—	0·83
IXb	—	0·64	—	—	—
Xb	—	0·40	—	—	—
XIb	—	0·15	—	—	—
Id	0·48	0·15	—	—	0·37
IId	0·45	0·29	2·64	0·90	0
IIId	0·53	0·35	2·18	0·55	0·33
IVd	0·55	0·39	2·95	0·65	0·33
Vd	0·55	0·35	5·90	1·05	0·25
VId	0·67	0·40	2·95	0·55	0·05
VIId	0·49	0·05	—	—	0·29
VIIId	0·84	0·50	—	—	0
IXd	0·60	0·42	1·73	0·60	−0·10
Xd	0·47	0·24	—	—	0·10
XId	0·58	0·14[d]	0·50	—	−0·13
XIId	0·60	—	1·00	—	0·43

[a] Retention factor $k = (t_R - t_M)/t_M$ (t_R retention time, t_M hold-up time) (HPLC); [b] $k = 0·20$ in S5; [c] in S5; [d] in S3.

None of the studied amides *Id*–*XIId*, carboxylic acids *Ia*–*XIa* and their esters *VIIb*, *IXb*–*XIb* showed any significant antibacterial effect in tests with *Escherichia coli B*, cultivated on a synthetic medium with glucose. Of all twelve studied amides only two – the 3-pyridazone derivative *Xd* and the orotic acid derivative *XIId* – inhibited the growth of leukemic cells with ID_{50} approximately 10^{-4} mol l^{-1} (25 μg/ml medium). The other compounds, including the derivatives of biologically active heterocyclic bases, had no discernible inhibitory effect at concentration 10^{-4} mol l^{-1}.

These data lead to the following conclusions: *a*) the cytostatic effect of the 5-fluorouracil derivative *Id* (see ref.[1]) is not the result of its cleavage with formation of the biologically active 5-fluorouracil but *b*) it is also not caused solely by the tris(hydroxymethyl)methylaminocarbonylmethyl grouping in the molecule. With regard to the conclusions of our previous study[1], further biologically active compounds can be expected among 1-carboxymethyl-5-fluorouracil perhydroxyalkylamides.

TABLE II
Ultraviolet absorption spectra

Compound	pH 2, pH 7			pH 12		
	λ_{max}, nm	ε_{max}	λ_{min}, nm	λ_{max}, nm	ε_{max}	λ_{min}, nm
IIIa	276	8 600	239	278	6 500	250
VIIa	267	6 600	234	—	—	—
VIIIa	300	5 500	245	300	5 500	245
Vb	290	7 750	247	284	5 800	254
VIIb	266	7 000	234	—	—	—
IId	263	14 500	231	267	11 000	243
IIId	279	9 800	240	276	7 100	249
IVd	281	9 600	244	278	6 800	252
Vd	291	7 800	247	280	5 900	253
VId	269	10 000	236	267	7 600	246
VIId	268	6 200	234	—	—	—
VIIId	300	5 600	245	298	5 300	245
IXd	300	5 600	244	—	—	—
Xd	286	3 000	261	—	—	—
XId	283	15 600	244	—	—	—
XIId	277	6 850	238	316	6 700	270

EXPERIMENTAL

Unless otherwise stated, the solutions were taken down at 40°C/2 kPa and the compounds dried at 13 Pa over phosphorus pentoxide. The melting points were determined on a Kofler block and are uncorrected.

Dimethylformamide was kept over molecular sieve Potassit 3 and prior to use distilled from phosphorus pentoxide. Indicator-containing silica gel for thin-layer chromatography was a Kavalier (Czechoslovakia) product, octadecyl-silica gel was purchased from Laboratorní přístroje (Prague, Czechoslovakia). Paper chromatography was carried out on a paper Whatman No 1 in the system S1 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), thin-layer chromatography on Silufol UV 235 plates (Kavalier, Czechoslovakia) in the systems S2 chloroform–methanol (3 : 1), S3 chloroform–methanol (3 : 2). Paper electrophoresis was performed on a paper Whatman No 3 MM at 40 V/cm in 0·05 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5 (E_{Up} denotes mobility related to uridine-3′-phosphate). Preparative thin-layer chromatography was carried out on loose layers of silica gel (40 × 15 × 0·3 cm), chromatography on cellulose on a 80 × 4 cm column of microcrystalline cellulose (Macherey & Nagel) in the system S1 (20 ml/h, UV-detection with Uvicord). UV-Absorption spectra were taken on a Specord UV-VIS spectrometer (K. Zeiss, Jena, G.D.R.) in aqueous solutions, mass spectra on an AEI MS 902 instrument (temperature 120°C, 70 eV, direct inlet). HPLC analyses were performed on a high pressure column (3·3 × 150 mm) of Separon SIX C 18 (5 μ) with an LCD-254 UV detector and EZ 11 recorder (Laboratory Equipment, Prague, Czechoslovakia). Samples were applied with a sample valve injector (7 μl sample loop). Constametric I high pressure pump (LDC, USA) was used for elution (flow rate 0·5 ml/min). Analyses were run at room temperature with the systems S4, 0·1 mol l^{-1} triethylammonium hydrogen carbonate pH 7·5 and S5, 10% methanol (v/v) in the same buffer, detection at 254 nm (0·32 AUFS).

1-Carboxymethyl-5-chlorouracil (*IIIa*)

N-Chlorosuccinimide (2·02 g; 15 mmol) was gradually added at 60°C to a stirred suspension of 1-carboxymethyluracil (*IIa*, *cf.* ref.[2]; 1·70 g; 10 mmol) in a mixture of acetic acid and acetic anhydride (19 : 1; 300 ml). The mixture was stirred at 60°C to homogeneity and another portion (0·67 g; 5 mmol) of N-chlorosuccinimide was added. After stirring for 5 h at 60°C, water (10 ml) was added and the mixture was taken down *in vacuo*. The residue was codistilled with water, filtered, washed twice with a small amount of water and crystallized from the same solvent; yield 75%, m.p. 304–307°C. For $C_6H_5ClN_2O_4$ (204·6) calculated: 35·22% C, 2·47% H, 17·34% Cl, 13·70% N; found: 35·23% C, 2·70% H, 17·30% Cl, 13·55% N.

1-Carboxymethyl-6-azauracil (*VIIa*)*

Methyl bromoacetate (3·67 g; 24 mmol) was added to a solution of 2-thio-6-azauracil (*cf.*[3,4]; 1·29 g; 10 mmol) in 0·5 mol l^{-1} sodium ethoxide in ethanol (20 ml). After refluxing for 30 min, the solvent was evaporated *in vacuo*, the residue refluxed with 5% hydrochloric acid (20 ml), cooled and extracted with ether (4 × 50 ml). The ethereal extract was taken down and the residue mixed with benzene (20 ml). The separated crystals were collected on filter, washed with benzene and dried *in vacuo*. The aqueous portion was taken down *in vacuo*, the residue extracted with hot acetone (3 × 100 ml) and the material left after removal of acetone was combined with the first crystalline portion, mixed with water (20 ml) and dissolved by gradual addition of ammonia

* Compound *VIIa* has been mentioned in the literature[3] but without description of its preparation.

(to pH 8). The solution was applied on a column of Sephadex A-25 (500 ml) in 0·01 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5, and the column was washed with the same buffer to drop of UV-absorption. Further elution with a linear gradient of the same buffer (0·05 to 0·40 mol l^{-1}, à 2 l) afforded the main UV-absorbing fraction (at 0·25—0·35 mol l^{-1} buffer) which was taken down *in vacuo*. The volatile buffer was removed by repeated codistillation with methanol and the residue applied on a column of Dowex 50X8 (H^+-form; 100 ml). Elution with water afforded a UV-absorbing eluate which was taken down *in vacuo*. The residue was dried by codistillation with ethanol and crystallized from ethanol–ether (1 : 1) (with light petroleum added), affording 0·62 g (36%) of *VIIa*, m.p. 232°C. For $C_5H_5N_3O_4$ (171·1) calculated: 35·10% C, 2·95% H, 24·56% N; found: 35·40% C, 2·69% H, 24·58% N. $E_{Up} = 1·62$.

1-Carboxymethyl-2-pyrimidone (*IXa*)

A mixture of 2-pyrimidone[5] (4·80 g; 50 mmol), sodium hydroxide (4·0 g; 100 mmol), chloroacetic acid (4·73 g; 50 mmol) and water (40 ml) was refluxed for 1 h, cooled and acidified to pH 3 to 4 with conc. hydrochloric acid. After keeping in refrigerator, the product was collected on filter, washed with water and crystallized from 75% ethanol; m.p. 218—221°C (decomposition); yield 60%. For $C_6H_6N_2O_3$ (154·1) calculated: 46·75% C, 3·93% H, 18·18% N; found: 46·49% C, 3·73% H, 18·15% N. Mass spectrum: 154 (M^+), 110 ($M-CO_2$), 109 (M—COOH), 95 ($M-CH_2.CO_2H$).

2-Carboxymethyl-3-pyridazone (*Xa*)

was prepared from 25 mmol of 3-pyridazone[6] in 67% yield analogously as described for compound *IXa*. M.p. 165—170°C (dec.). For $C_6H_6N_2O_3$ (154·1) calculated: 46·75% C, 3·93% H, 18·18% N; found: 47·00% C, 3·86% H, 18·42% N.

1-Methoxycarbonylmethyl-6-azauracil (*VIIb*)

A solution of 1-carboxymethyl-6-azauracil (*VIIa*; 0·51 g; 3 mmol) in methanolic 3M-HCl (12 ml) was set aside overnight at room temperature and concentrated to 3—4 ml *in vacuo* at 25°C. Upon addition of ether (5 ml) and cooling to 5°C, the separated product was filtered, washed with ether and dried *in vacuo*; yield 75% of the chromatographically pure product *VIIb*, m.p. 126—127°C (ethanol–light petroleum). For $C_6H_7N_3O_4$ (185·1) calculated: 38·92% C, 3·81% H, 22·70% N; found: 39·02% C, 3·85% H, 22·86% N. $E_{Up} = 0·86$.

1-Methoxycarbonylmethyl-2-pyrimidone (*IXb*)

was prepared from 1-carboxymethyl-2-pyrimidone (*IXa*; 5 mmol) in the same manner as described for the compound *VIIb*; yield 0·9 g (88%) of the easily water soluble hydrochloride of *IXb*, m.p. 152—153°C. For $C_7H_9ClN_2O_3$ (204·6) calculated: 41·08% C, 4·43% H, 17·33% Cl, 13·69% N; found: 41·03% C, 4·20% H, 17·17% Cl, 13·37% N. The free base *IXb* was prepared from the hydrochloride by careful neutralization of concentrated aqueous solution with ammonia. addition of an excess of ethanol and precipitation of the filtered solution with ether.

2-Methoxycarbonylmethyl-3-pyridazone (*Xb*)

was prepared from the compound *Xa* in the same way as *IXb* in 83% yield.

Hydrochloride: m.p. 154—156°C (dec.). For $C_7H_9ClN_2O_3$ (204·6) calculated: 41·08% C, 4·43% H, 17·33% Cl, 13·69% N; found: 40·91% C, 4·11% H, 17·46% Cl, 13·90% N.

1-Ethoxycarbonylmethylcytosine (*XIb*)

Dry hydrogen chloride was introduced into a cooled suspension of 1-carboxymethylcytosine (*XIa*, *cf.*[2]; 0·25 g) in ethanol (25 ml) until all dissolved. The mixture was kept at room temperature overnight, concentrated *in vacuo* to half of the original volume and mixed with an excess of ether. The hydrochloride of *XIb* was filtered, washed with ether and dried *in vacuo*; m.p. 183–191°C (ethanol); yield 85%. A 10% aqueous solution of this product was treated with ammonia to pH 9 and allowed to crystallize at 0°C. The product was collected on filter, washed with ice-cold water and crystallized from ethanol, affording *XIb* in 70% yield; m.p. 234–235°C (reported[7] m.p. 237–239°C). For $C_8H_{11}N_3O_3$ (197·2) calculated: 48·72% C, 5·62% H, 21·31% N; found: 49·02% C, 5·64% H, 21·43% N.

1-Tris(hydroxymethyl)methylaminocarbonylmethyluracil (*IId*)

Tris(hydroxymethyl)aminomethane (0·67 g; 5·5 mmol) was added under stirring to a suspension of 4-nitrophenyl ester of 1-carboxymethyluracil (*IIc*; see[8]; 1·46 g; 5 mmol) in methanol (30 ml). After dissolution the mixture was set aside at room temperature for 5 h and Dowex 50 X 8 (H^+-form; washed with methanol) was added to disappearance of the yellow colour. The suspension was filtered, the resin washed with methanol, the filtrate taken down *in vacuo* and the residue dissolved in water (50 ml) and extracted with ether (3 × 25 ml). Upon concentration of the aqueous phase *in vacuo*, the crystalline product *IId* was filtered, washed with ice cold water and dried *in vacuo*; m.p. 185–187°C; yield 75%. For $C_{10}H_{15}N_3O_6$ (273·2) calculated: 43·95% C, 5·54% H, 15·38% N; found: 44·45% C, 5·51% H, 15·43% N.

1-Tris(hydroxymethyl)methylaminocarbonylmethyl-5-chlorouracil (*IIId*)

N,N′-Dicyclohexylcarbodiimide (1·05 g; 5 mmol) was added at 5°C to a stirred solution of 1-carboxymethyl-5-chlorouracil (*IIIa*; 1·02 g; 5 mmol) and 4-nitrophenol (0·70 g; 5 mmol) in dimethylformamide (10 ml). After stirring for 2 h at room temperature, tris(hydroxymethyl)-aminomethane (0·72 g; 6 mmol) was added and the mixture was set aside overnight at room temperature. Ethanol (20 ml) was added and the mixture was filtered. The stirred filtrate was treated with dry Dowex 50 X 8 (H^+-form) until the mixture lost its yellow colour. The Dowex was filtered off, washed with ethanol and the filtrate was taken down *in vacuo*. The residue was dissolved in water (100 ml), filtered and the filtrate extracted with ether (3 × 25 ml) and concentrated *in vacuo*. The remaining syrupy residue crystallized on addition of ether, the ether was decanted and the crystals dissolved in ethanol (10 ml) and kept in a refrigerator overnight. The crystallized product was filtered, washed with ice-cold ethanol and dried *in vacuo*. A saturated aqueous solution of this material was applied on a column of octadecyl-silica gel (200 ml) and the column was washed with water. After a small fraction of impurities the principal UV-absorbing portion was obtained which was taken down *in vacuo*. Crystallization from water gave 0·50 g (32·5% from *IIIa*) of the product *IIId*; m.p. 213–215°C. For $C_{10}H_{14}ClN_3O_6$ (307·7) calculated: 39·03% C, 4·59% H, 11·52% Cl, 13·65% N; found: 39·14% C, 4·38% H, 11·32% Cl, 14·04% N.

1-Tris(hydroxymethyl)methylaminocarbonylmethyl-5-bromouracil (*IVd*)

was prepared from 4 mmol of 1-carboxymethyl-5-bromouracil (*IVa*; see[9]) as described for *IIId*. The product was purified by chromatography on octadecyl-silica gel and crystallization from methanol–ether; m.p. 204–206°C; yield 65%. For $C_{10}H_{14}BrN_3O_6$ (352·2) calculated: 34·10% C, 4·01% H, 22·69% Br, 11·93% N; found: 34·13% C, 3·78% H, 22·53% Br, 11·47% N.

1-Tris(hydroxymethyl)methylaminocarbonylmethyl-5-iodouracil (*Vd*)

was prepared from 1-carboxymethyl-5-iodouracil (*Va*; see[10]) by the procedure described for the compound *IIId*; however, because of low solubility of the product in water, 4-nitrophenol could not be removed by ether extraction. Therefore, the residue after evaporation of the reaction mixture was chromatographed on three plates of silica gel (*vide supra*) in chloroform–methanol (17 : 3). The product bands were eluted with methanol and the solvent was evaporated. Further purification on octadecyl-silica gel gave 30% of compound *Vd*, m.p. 212—213°C (80% aqueous ethanol–ether). For $C_{10}H_{14}IN_3O_6$ (399·1) calculated: 30·09% C, 3·54% H, 31·80% I, 10·53% N; found: 29·77% C, 3·43% H, 32·38% I, 10·29% N. The chromatography on octadecyl-silica gel afforded also 0·15 g of methyl ester of compound *Va* as the side-product; m.p. 199—200°C (water). For $C_7H_7IN_2O_4$ (310·1) calculated: 27·11% C, 2·28% H, 40·93% I, 9·04% N; found: 27·35% C, 2·42% H, 41·24% I, 8·98% N. Mass spectrum: 310 (M, $C_7H_7IN_2O_4$), 279 (M—OCH_3), 278, 251 (M—$COOCH_3$).

1-Tris(hydroxymethyl)methylaminocarbonylmethylthymine (*VId*)

was prepared in 80% yield from 1-carboxymethylthymine (*VIa*; see[2]; 5 mmol) as described for *IIId*. The chromatographically (HPLC) pure product was obtained directly by crystallization from water; m.p. 184—187°C (methanol–ether). For $C_{11}H_{17}N_3O_6$ (287·3) calculated: 45·99% C, 5·97% H, 14·63% N; found: 46·16% C, 5·79% H, 14·40% N.

1-Tris(hydroxymethyl)methylaminocarbonylmethyl-6-azauracil (*VIId*)

A mixture of compound *VIIb* (0·3 g; 1·62 mmol), tris(hydroxymethyl)aminomethane (1·8 g; 14·6 mmol) and methanol (25 ml) was heated in an autoclave to 100°C for 25 h. After evaporation *in vacuo*, the residue was dissolved in minimum amount of water, applied on a column of Amberlite IRC 50 (H^+-form, 100 ml), and eluted with water until the eluate no longer showed UV-absorption. This eluate was taken down *in vacuo*, the residue dissolved in water (25 ml; adjusted with ammonia to pH 8·5) and applied on a column of DEAE-Sephadex A-25 (100 ml). After washing with water to remove impurities, the product was eluted with linear gradient (0—0·2 mol . l^{-1}) of triethylammonium hydrogen carbonate, pH 7·5 (3 ml min^{-1}). The product fraction (0·05—0·06 mol l^{-1}) was taken down *in vacuo*, the residue codistilled with methanol (3 × 25 ml) and again passed through a column of Amberlite IRC 50 (H^+-form, 50 ml). The aqueous UV-absorbing eluate was taken down and the residue was codistilled with ethanol and crystallized from ethanol–ether to give 0·18 g (40%) of *VIId*, m.p. 186°C. For $C_9H_{14}N_4O_6$ (274·2) calculated: 39·42% C, 5·15% H, 20·44% N; found: 39·56% C, 5·30% H, 20·38% N. E_{Up} = 0·29. Mass spectrum: 257 (M—17). 243 (M—CH_2OH), 226 (243—OH), 208 (226—H_2O), 195 (225—CH_2O), 170 (BCH_2CONH_2).*

1-Tris(hydroxymethyl)methylaminocarbonylmethyl-2-pyridone (*VIIId*)

was prepared in 40% yield from compound *VIIIa* (see[11]; 5 mmol) *via* the 4-nitrophenyl ester *VIIIc* as described for *IIId*, except the purification on octadecyl-silica gel. The product was pure on HPLC; m.p. 167—169°C (ethanol–light petroleum). For $C_{11}H_{16}N_2O_5$ (256·3) calculated: 51·56% C, 6·30% H, 10·93% N; found: 51·57% C, 6·05% H, 10·48% N.

* B denotes the heterccyclic base, in this case 6-azauracil.

1-Tris(hydroxymethyl)methylaminocarbonylmethyl-2-pyrimidone (*IXd*)

A mixture of the methyl ester *IXb* (750 mg; 3·6 mmol of hydrochloride), tris(hydroxymethyl)-aminomethane (14·4 mmol) and methanol (25 ml) was heated to 100°C for 8 h in an autoclave. After evaporation of methanol *in vacuo* and chromatography on Amberlite IRC 50 (H^+-form; see preparation of *VIId*), the residue was chromatographed on a column of cellulose. The product-containing fractions (R_F 0·60, S1) were combined, taken down and dried *in vacuo* to give the amorphous yellowish product in 38% yield. For $C_{10}H_{15}N_3O_5$ (257·3) calculated: 46·68% C, 5·88% H, 16·34% N; found: 46·78% C, 5·98% H, 16·23% N. Mass spectrum: 208 ($M-H_2O-$ $-CH_2OH$), 190 ($208-H_2O$), 153 (B CH_2CONH_2).

2-Tris(hydroxymethyl)methylaminocarbonylmethyl-3-pyridazone (*Xd*)

was prepared from compound *Xb* (3·2 mmol) as described for the preparation of compound *IXd*. Chromatography on cellulose in S1 afforded the amorphous product *Xd* in 37% yield; m.p. 136°C (dec.). For $C_{10}H_{15}N_3O_5$ (257·3) calculated: 46·68% C, 5·88% H, 16·34% N; found: 46·54% C, 5·97% H, 16·09% N.

1-Tris(hydroxymethyl)methylaminocarbonylmethylcytosine (*XId*)

A mixture of ethyl ester *XIb* (1 mmol), tris(hydroxymethyl)aminomethane (2 mmol) and dimethylformamide (2 ml) was heated to 150°C for 3 h, the solvent was evaporated at 50°C/13 Pa, the residue dissolved in water (20 ml) and applied on a column of octadecyl-silica gel (200 ml). Elution with water (after washing out the salts) afforded the product *XId*, m.p. 206–207°C. Yield 40%. For $C_{10}H_{16}N_4O_5$ (272·3) calculated: 44·11% C, 5·93% H, 20·58% N; found: 44·37% C, 5·96% H, 20·82% N. Mass spectrum: 223 ($M-H_2O-CH_2OH$; $C_9H_{11}N_4O_3$), 205 ($223-H_2O$), 152 (BCH_2CO), 112 (BH_2).

Orotic Acid Tris(hydroxymethyl)methylamide (*XIId*)

A solution of orotic acid (*XIIa*; 0·78g; 5 mmol), 4-nitrophenol (0·7 g; 5 mmol) and N,N'-dicyclohexylcarbodiimide (1·05 g; 5 mmol) in dimethylformamide (20 ml) was stirred for 15 min. Tris(hydroxymethyl)aminomethane (0·72 g; 6 mmol) was added and the stirring was continued for 6 h at room temperature. The mixture was processed as described for the preparation of compound *IId*. A weakly alkaline solution of the residue after evaporation of the aqueous phase was applied on a column of Dowex 1X2 (acetate; 100 ml). Neutral impurities were removed by washing with water and then the product was eluted with a linear gradient (0–0·5 mol l^{-1}) of acetic acid (1 l each). The first fractions (0·02–0·05 mol l^{-1}), containing the product, were taken down *in vacuo* and the residue was crystallized from 80% ethanol with addition of ether; m.p. 214–215°C; yield 0·32 g (25%). For $C_9H_{13}N_3O_6$ (259·2) calculated: 41·70% C, 5·06% H, 16·22% N; found: 41·86% C, 5·27% H, 16·13% N. E_{Up} = 0·43 (*XIIa* 0·84).

Cell Growth Experiments

were performed on L-1210 leukemic cells in RPMI 1640 medium containing 15% horse serum, penicillin (50 units/ml), streptomycin (50 μg/ml) and 2-mercaptoethanol (10^{-6} ml l^{-1}), using multiwell tissue culture plates (Nunc Product, Roskilde, Denmark). For measurement of the effect of drugs on cell growth, 0·15 ml cell suspension (10^5 cells/ml) was placed in each well and a freshly prepared solution of the drug in physiological saline (end concentration 10^{-4} mol l^{-1}, autoclaved for 20 min) was added. Each sample was dispensed into 2 or 3 separate wells, and incu-

bated in 10% CO_2 at 37°C in a humid atmosphere. After 72 h the cells were counted and incubated in a Bürker hemocytometer. During 72 h the L-1210 cells in control wells underwent 4 1/2 mitotic cycles. The reproducibility was ±10%. Under these conditions, compound *Xd* caused 49% inhibition, compound *XIId* 38% inhibition of cell growth. The other compounds tested were inactive.

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