HYDROPHILIC 1-(CARBOXYMETHYL)-5-FLUOROURACIL AMIDES: PREPARATION AND CYTOSTATIC ACTIVITY

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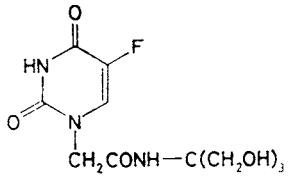
The following N-substituted amides *IV* were prepared by reaction of 1-(carboxymethyl)-5-fluorouracil *p*-nitrophenyl ester (*II*) with primary or secondary hydroxyalkylamines *III*: 2-hydroxypropyl (*IVa*), 3-hydroxypropyl (*IVb*), 2,3-dihydroxypropyl (*IVc*), 3-hydroxy-2-methyl-2-propyl (*IVd*), 1,3-dihydroxy-2-propyl (*IVe*), 1-deoxyglucitol-1-yl (*IVg*), 2-deoxyglucitol-2-yl (*IVh*), methyl--2,3-dihydroxypropyl (*IVi*), methyl-1-deoxyglucitol-1-yl (*IVk*), and n-butyl-2,3-dihydroxypropyl (*IVl*). None of these compounds had any cytostatic activity towards murine leukemia L-1210 cell growth in a tissue culture at 10^{-5} mol l^{-1}.

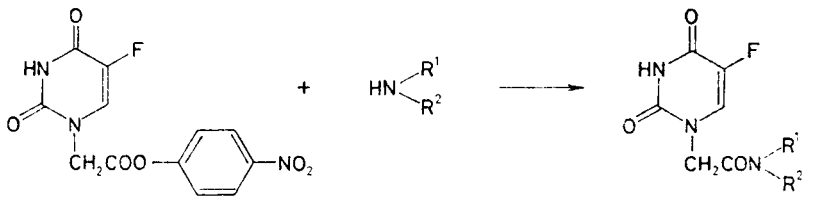
Some time ago, within the framework of cytostatic activity studies on 1-(carboxymethyl)-5-fluorouracil derivatives, we described the inhibitory effect of tris(hydroxymethyl)methylamide of this acid (*I*) on the growth of murine leukemia L-1210 cells (*in vitro*)[1]. Further investigation of N-tris(hydroxymethyl)methylamidocarbonylmethyl derivatives of pyrimidine bases (none of which was significantly active in the above test) has proven that the cytostatic effect is not solely due to the presence of the specific side chain[2]. Since structure–activity dependence in the series of 1-(carboxymethyl)-5-fluorouracil amides indicated that, generally, the amido or N-substituted--amidocarbonylmethyl groupings are not bearers of the cytostatic activity, it could be assumed that the biological effect is specifically related to the presence of hydro-



I

philic functionalities (hydroxyl groups) in the amide part of the molecule of *I*. Therefore, we decided to prepare a series of hydroxyalkylamides of 1-(carboxymethyl)-5-fluorouracil (*IV*) and to compare their cytostatic effect with that of compound *I* under the same conditions.

The general method of preparing these compounds starts from 1-(carboxymethyl)-5-fluorouracil *p*-nitrophenyl ester (*II*) accessible from the acid[3]. The reaction with the corresponding primary or secondary hydroxyalkylamine *III* was carried out in methanol, dimethylformamide or pyridine at room temperature*. We employed primary and secondary amines, substituted with an alkyl chain consisting of three to six carbon atoms carrying one to five hydroxyl functions (primary or secondary). In addition to the commercially available amines, this series also includes 2,3-dihydroxypropylamine derivatives *IIIc*, *IIIi* and *IIIl*; their 3-O-benzyl ethers were prepared by ammonolysis of 2-benzyloxymethyloxirane.



II *III* *IV*

In formulae *III*, *IV*:

a, $R^1 = H$; $R^2 = CH_2CH(OH)CH_3$ (*RS*)
b, $R^1 = H$; $R^2 = CH_2CH_2CH_2OH$
c, $R^1 = H$; $R^2 = CH_2CH(OH)CH_2OH$ (*RS*)
d, $R^1 = H$; $R^2 = C(CH_3)_2CH_2OH$
e, $R^1 = H$; $R^2 = CH(CH_2OH)_2$
f, $R^1 = H$; $R^2 = CH_2CH(OH)CH_2OCH_2C_6H_5$ (*RS*)
g, $R^1 = H$; $R^2 = CH_2(CHOH)_4CH_2OH$ (D-*gluco*)
h, $R^1 = H$; $R^2 = CH(CHOH)_3CH_2OH$ (D-*gluco*), CH_2OH
i, $R^1 = CH_3$; $R^2 = CH_2CH(OH)CH_2OH$ (*RS*)
j, $R^1 = CH_3$; $R^2 = CH_2CH(OH)CH_2OCH_2C_6H_5$ (*RS*)
k, $R^1 = CH_3$; $R^2 = CH_2(CHOH)_4CH_2OH$ (D-*gluco*)
l, $R^1 = C_4H_9$; $R^2 = CH_2CH(OH)CH_2OH$ (*RS*)
m, $R^1 = C_4H_9$; $R^2 = CH_2CH(OH)CH_2OCH_2C_6H_5$ (*RS*)

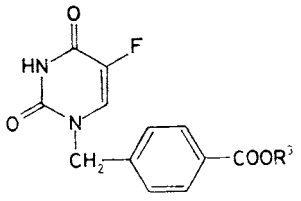
After the reaction, the excess amine *III* was removed by adding an ion exchanger (H^+ form), and *p*-nitrophenol formed in the reaction was separated by chromatography on silica gel or an anion exchange resin. The last-mentioned method was used in most cases also for the final purification of partially purified compounds *IV*. All the obtained derivatives *IV* were chromatographically homogeneous and in most cases crystalline. The 3-benzyloxy derivatives *IVf*, *IVj*, and *IVm* were hydrogenolyzed

* When the synthesis was performed in methanol, methyl esters instead of amides *IV* were often formed as side-products, particularly in cases of sterically hindered amines such as *IIId*, *IIIe*, *IIIh*, and *IIIk*.

in an acidic medium to afford the 2,3-dihydroxypropyl amides *IVc*, *IVi*, and *IVl* which were isolated by chromatography on an anion exchanger. All the compounds *IV* were subjected to an additional purification by preparative chromatography on octadecyl-silica gel in order to remove traces of possible impurities and the products were isolated only from completely homogeneous fractions (HPLC). Only this material was then used in the biological tests.

In addition to elemental analyses and mass spectra (some of the compounds *IV* gave molecular peaks, other gave fragments arising by loss of water or CH_2OH group; see ref.[2]) (Table I), compounds *IV* were characterized by paper electrophoresis; the mobilities of about 0·50 (related to uridine 3'-phosphate) result from the presence of the 5-fluorouracil ring. Ultraviolet spectra of the compounds *IV* exhibit absorption maximum at about 270 nm, characteristic of 1-substituted derivatives of 5-fluorouracil (data not shown).

Besides compound *I*, in the series of 1-(carboxymethyl)-5-fluorouracil amides the N-benzylamide is reported also to have a certain cytostatic activity[1]. Therefore, we have tried to prepare a derivative containing both the mentioned structural features, *i.e.* the tris(hydroxymethyl)methyl and the benzyl groupings: tris(hydroxymethyl)-methylamide of 1-(*p*-carboxybenzyl)-5-fluorouracil. We started from 1-(*p*-carboxybenzyl)-5-fluorouracil (*Va*) which was obtained by hydrolysis of the already described[4] *p*-cyanobenzyl derivative. Compound *Va* was esterified to the methyl ester *Vb* which, however, did not react with tris(hydroxymethyl)aminomethane to the desired TRIS--amide (although, under the same conditions, the analogous reaction of methyl ester of 1-(carboxymethyl)-5-fluorouracil takes place).



Va, $R^3 = H$
Vb, $R^3 = CH_3$

The cytostatic activity of compounds *IV* was tested on tissue cultures of L-1210 cells under conditions described earlier[1,2]. The compounds were added to the growing culture and the cytostatic effect was evaluated after 72 h of growth in the presence of the tested compound. As seen from data in Table II, at the concentration used (10^{-5} mol l^{-1}, *i.e.* 2·5–4 μg/ml medium) the studied derivatives have no or a relatively small inhibitory effect.

From comparison of structure of the hydroxyalkyl groups in compounds *IV* with the compound *I* it is obvious that the density of hydrophilic groups around

TABLE I
Characterisation of compounds *IV*

Compound (m.p., °C)	Method (yield, %)	R_F			k^a	Mass spectrum	Formula (mol. weight)	Calculated/found			
		S1	S2	S3				% C	% H	% F	% N
IVa	*A*	0·58	0·13	0·36	0·20[b]	227	$C_9H_{12}FN_3O_4$	44·08	4·94	7·76	17·14
(205–211)	(80)					(M − H_2O)	(245·2)	44·03	4·66	7·64	16·81
IVb	*A*	0·53	0·10	0·29	0·22[b]	245	$C_9H_{12}FN_3O_4$	44·08	4·94	7·76	17·14
(210–213)	(80)					(M^+)	(245·2)	44·21	4·83	7·95	16·88
IVc	*D*	0·49	0·04	0·13	0·28[b]	261	$C_9H_{12}FN_3O_5$	41·38	4·63	7·27	16·09
(201–202)	(68)					(M^+)	(261·2)	41·67	4·88	7·17	16·16
IVd	*B*	0·47	0·19	0·50	0·40[b]	260	$C_{10}H_{14}FN_3O_4$	46·33	5·45	7·33	16·21
(186–191)	(72)					(M + 1)	(259·2)	46·50	5·23	7·11	15·98
IVe	*B*	0·51	0·05	0·15	0·30[b]	230	$C_9H_{12}FN_3O_5$	41·38	4·63	7·27	16·09
(213–214)	(65)					(M − CH_2OH)	(261·2)	40·97	4·32	7·34	16·12
IVf	*C*	0·76	0·30	—	0·19[c]	351	$C_{16}H_{18}FN_3O_5$	54·70	5·16	5·41	11·96
(143–144)	(75)					(M^+)	(351·3)	54·86	5·17	5·65	11·64

IVg[d]	*B*	0·41	—	0·20	0·26[b]	284 (M − 2 H_2O − − CH_2OH)	$C_{12}H_{18}FN_3O_8$ (351·8)	41·03 40·95	5·17 5·05	5·41 5·59	11·96 11·70
IVh (220)	*C* (20)	0·40	—	0·05	0·32[b]	307 (M − H_2O − − CH_2OH)	$C_{12}H_{18}FN_3O_8$ (351·8)	41·03 40·80	5·17 5·04	5·41 5·47	11·96 11·85
IVi[d]	*D* (64)	0·53	0·12	—	0·48[b]	275 (M^+)	$C_{10}H_{14}FN_3O_5$ (275·2)	43·68 43·80	5·13 5·05	6·90 7·12	15·27 15·40
IVj[d]	*C* (60)	0·82	0·65	—	0·27[c]	365 (M^+)	$C_{17}H_{20}FN_3O_5$ (365·3)	55·89 56·03	5·52 5·18	5·21 5·35	11·50 11·62
IVk (132–135)	*C* (30)	0·39	0·02	0·05	0·45[b]	348 (M + 1 − H_2O)	$C_{13}H_{20}FN_3O_8$ (365·3)	42·74 42·53	5·52 5·34	5·21 5·22	11·50 11·88
IVl[d]	*D* (62)	0·43	0·42	—	3·60[b]	317 (M^+)	$C_{13}H_{20}FN_3O_5$ (317·3)	49·20 49·13	6·35 6·47	5·98 6·13	13·25 13·05
IVm[d]	*C* (37)	0·77	0·80	—	1·45[c]	358 (M − H_2O − − CH_2OH)	$C_{20}H_{26}FN_3O_5$ (407·4)	58·95 59·12	6·44 6·70	4·67 4·45	10·31 10·20

[a] $k = (t_R - t_0)/t_0$, t_R retention time, t_0 HPLC hold-up time; [b] in S4; [c] in S5; [d] amorphous precipitate.

the amidic grouping in compound *I* is not achieved in any of the newly prepared derivatives. Probably, the cytostatic activity of *I* originates from the overall character of the amide group environment rather than from the presence of a hydrophilic functionality. Naturally, one also has to keep in mind that the parameters of the cell transport of *I* and compounds *IV* can be completely different.

EXPERIMENTAL

Unless stated otherwise, the solutions were evaporated at 40°C/2 kPa and compounds dried over phosphorus pentoxide at 13 Pa. The melting points were determined on a Kofler block and are uncorrected. Paper chromatography was performed on a paper Whatman No 1 in the system S1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), thin-layer chromatography was carried out on Silufol UV 254 plates (Kavalier, Czechoslovakia) in the systems S2, chloroform–methanol (9 : 1), and S3, chloroform–methanol (4 : 1). Paper electrophoresis was done on a Whatman No 3MM paper in the system S4, 0·05 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5, at 20 V/cm (mobility values related to uridine 3′-phosphate). The HPLC analyses were performed on Separon SIX C18 columns (200 × 4 mm) in the system S4 or S5, methanol–S4 (5 : 95), detection at 260 nm. Ultraviolet spectra were measured in aqueous solutions on a Specord UV-VIS instrument (Carl Zeiss, Jena, G.D.R.), mass spectra were taken on an AEI MS 902 spectrometer (120°C, 70 eV, direct inlet).

Chemicals. (*RS*)-1-Amino-2-hydroxypropane (*IIIa*) was a Merck (F.R.G.) product, 1-amino-3-hydroxypropane (*IIIb*) and 2-amino-2-methyl-1-propanol (*IIId*) were purchased from FERAK (West Berlin) and 1-deoxy-1-methylamino-D-glucitol (Meglumin) (*IIIk*) from VEB Fahlberg-List (Magdeburg, G.D.R.). 2-Amino-2-deoxyglucitol hydrochloride (*IIIb*) was obtained according to ref.[5] and 2-benzyloxymethyloxirane was prepared and kindly provided by Professor P. Nuhn, Sektion Pharmazie, Martin-Luther-Universität Halle (G.D.R.).

TABLE II

Inhibition of murine leukemia L-1210 cell growth by compounds *IV* at 10^{-5} mol l^{-1}, for experimental conditions see ref. 2

Compound	Inhibition[a], %	Compound	Inhibition[a], %
IVa	24	*IVg*	0
IVb	23	*IVh*	0
IVc	0	*IVi*	0
IVd	0	*IVk*	13
IVe	0	*IVl*	0
IVf	6	5-Flourouracil	95[b]

[a] Evaluated 72 h after addition of the compound; [b] at 10^{-6} mol l^{-1}.

(*RS*)-(3-Benzyloxy-2-hydroxypropyl)amine (*IIIf*)

2-Benzyloxymethyloxirane[6] (0·49 g; 3 mmol) was added to conc. aqueous ammonia (40 ml), precooled to 0°C. After standing at 5°C for 8 h and at room temperature for 16 h, the resulting solution was taken down *in vacuo* and the residue was codistilled with ethanol (5 × 10 ml). The obtained *IIIf* (95%) melted at 73–75°C. Hydrochloride m.p. 101–103°C (ethanol–ether). For $C_{10}H_{16}ClNO_2$ (217·7) calculated: 55·18% C, 7·42% H, 16·27% Cl, 6·44% N; found: 55·58% C, 7·29% H, 16·49% Cl, 6·47% N.

Methyl-(RS)-(3-benzyloxy-2-hydroxypropyl)amine (IIIj) was prepared similarly to *IIIf* from 30% aqueous methylamine (30 ml) and 2-benzyloxymethyloxirane[6] (0·65 g; 4 mmol); yield 95% of an oil. Hydrochloride m.p. 82–85°C (ethanol–ether). For $C_{11}H_{18}ClNO_2$ (231·7) calculated: 57·01% C, 7·83% H, 15·31% Cl, 6·04% N; found: 56·87% C, 7·56% H, 15·08% Cl, 5·85% N.

n-*Butyl-(RS)-(3-benzyloxy-2-hydroxypropyl)amine* (IIIm) was prepared similarly to *IIIj* by heating 2-benzyloxymethyloxirane (3 mmol) and 30% aqueous n-butylamine (30 ml) on a steam bath for 2·5 h. The crude product was used in the reaction with *II* without further purification.

Serinol (*IIIe*)

A solution of serinol oxalate (1·5 mmol, 3 mmol of the base) in water (5 ml) was applied on a column of Dowex 50X8 (H^+ form) and the column was washed with water until the eluate was no longer acidic. The base was eluted with 1 mol l^{-1} ammonia (200 ml) and after evaporation *in vacuo*, the residue was dried by repeated codistillation with ethanol (3 × 20 ml). This product was used in the reaction with *II* without further purification.

Analogously, 2-amino-2-deoxy-D-glucitol (*IIIh*) was prepared from its hydrochloride.

D-Glycamine (*IIIg*)

A mixture of D-glucose oxime (14·3 g; 73 mmol), 10% aqueous methanol (600 ml) and Raney nickel (5 g) was hydrogenated in an autoclave at 100°C and 120 atmospheres for 12 h. After filtration, the solvent was evaporated *in vacuo* and the residue was dissolved in water (100 ml) and acidified by addition of Dowex 50X8 (H^+ form). The suspension was applied on the same ion exchanger (150 ml) and the column was washed with water (1 l). The resin was resuspended in water (300 ml) and adjusted to pH 11 with aqueous ammonia. The mixture was filtered and the Dowex washed with 1% aqueous ammonia (200 ml). The filtrate was taken down *in vacuo* and the residue (9 g) was dissolved in water (10 ml). Concentrated hydrochloric acid (5 ml) and ethanol (300 ml) were added, the supernatant was decanted and the residue was triturated with methanol to give 7 g (44%) of hydrochloride *IIIg*, m.p. 158–159°C. For $C_6H_{16}ClNO_5$ (217·7) calculated: 33·11% C, 7·41% H, 16·29% Cl, 6·44% N; found: 33·25% C, 7·32% H, 16·43% Cl, 6·56% N.

Preparation of Compounds *IV*

A) Free base *III* (1·5 mmol) was added to a suspension of *II* (0·31 g; 1 mmol) in methanol (2 ml). After standing overnight at room temperature, the mixture was diluted with methanol (5 ml) and Dowex 50X8 (H^+ form) was added with stirring until the yellow colour disappeared. The Dowex was filtered off, washed with boiling methanol (4 × 3 ml) and the filtrate was stripped of the solvent *in vacuo*. The residue was triturated with ether and crystallized from ethanol–light petroleum. This procedure was used for the preparation of *IVa*, *IVb*, and *IVf* (Table I).

B) The reaction was performed in the same manner as described under *A* in pyridine (5 ml) (compounds *IVd* and *IVg*) or dimethylformamide (4 ml) (compounds *IVe* and *IVh*). The obtained

crude product in a small amount of water was made weakly alkaline with aqueous ammonia and applied on a column of Dowex 1X2 (acetate form). The column was washed with water until UV-absorption and conductivity of the eluate dropped. The product was eluted with linear gradient of acetic acid (0—0·5 mol l^{-1}; 0·5 l each). The combined product fractions were taken down *in vacuo*, the residue was codistilled with water (3 × 20 ml) and ethanol and crystallized from ethanol–ether.

C) The reaction was performed as described under *A* with *II* (1·5 mmol) and *III* (3 mmol) in pyridine (5 ml) at room temperature for 3 h. Evaporation of the solvent *in vacuo* and further work-up according to *A* afforded the crude product which was further purified by preparative thin-layer chromatography on silica gel plates (20 × 20 cm) in chloroform–methanol (compounds *IVf*, *IVj*, and *IVm*). Bands of the products were eluted with methanol, the solvent was evaporated and the product was crystallized from ethanol–light petroleum. The crude product *IVk* was worked up analogously and purified in the system S1; in this case the product was precipitated with ether from the concentrated methanolic eluate.

D) Concentrated hydrochloric acid (0·5 ml), 30% palladium chloride (0·3 ml), and 10% Pd/C (0·5 g) were successively added to a solution of *IVf*, *IVj* or *IVm* (1 mmol) in ethanol (60 ml). The mixture was hydrogenated at atmospheric pressure and room temperature overnight, filtered and the solvent was evaporated *in vacuo*. The residue was further purified by chromatography on Dowex 1 (see Method *B*). This procedure was used for the preparation of *IVc*, *IVi*, and *IVl* (Table I).

Purification of Compounds *IV* by Hydrophobic Chromatography

Prior to the biological tests, samples of compounds *IV* (100 mg) in water (5 ml) were applied on a column (20 × 3 cm) of octadecyl-silica gel (20 μ) preequilibrated with water. The column was first washed with water (300 ml) and then with a linear gradient of methanol (0—5%, à 1 l). The main UV-absorbing fractions were individually tested by HPLC (*vide supra*) and those containing pure *IV* were combined. After evaporation *in vacuo*, the residue was crystallized from ethanol–ether. Yields of the purified products ranged from 70% to 80%.

1-(*p*-Carboxybenzyl)-5-fluorouracil (*Va*)

A solution of 1-(*p*-cyanobenzyl)-5-fluorouracil[4] (1·05 g; 4·3 mmol) in a mixture of acetic acid (10 ml) and 6 mol l^{-1} hydrochloric acid (10 ml) was refluxed for 15 h. After cooling to 5°C, the product was filtered and washed with water; yield 0·92 g (82%) of *Va*, m.p. 290—295°C. For $C_{12}H_9FN_2O_4$ (264·2) calculated: 54·55% C, 3·44% H, 7·19% F, 10·60% N; found: 54·07% C, 3·26% H, 7·19% F, 11·04% N. $R_F = 0·50$ (S1), $E_{Up} = 0·96$ (S4).

1-(*p*-Methoxycarbonylbenzyl)-5-fluorouracil (*Vb*)

A mixture of *Va* (0·40 g; 1·5 mmol) and 3 mol l^{-1} methanolic hydrogen chloride (20 ml) was refluxed for 30 min, cooled and methanol was evaporated *in vacuo*. The residue was codistilled with methanol (4 × 10 ml) and crystallized from ethanol to give *Vb* (80%), m.p. 214—216°C. For $C_{13}H_{11}FN_2O_4$ (278·2) calculated: 56·11% C, 3·99% H, 6·83% F, 10·07% N; found: 56·38% C, 4·15% H, 7·19% F, 10·24% N. $R_F = 0·80$ (S1).

Reaction with TRIS: A mixture of *Vb* (278 mg; 1 mmol), TRIS (363 mg; 3 mmol), and methanol (20 ml) was heated in an autoclave to 120°C for 12 h. According to chromatography in the systems S1 and S2, *Vb* did not react under these conditions.

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REFERENCES

1. Pischel H., Holý A., Veselý J., Wagner G., Cech D.: Collect. Czech. Chem. Commun. *47*, 2806 (1982).
2. Pischel H., Holý A., Veselý J., Wagner G.: Collect. Czech. Chem. Commun. *49*, 2541 (1984).
3. Pischel H., Holý A., Wagner G.: Collect. Czech. Chem. Commun. *44*, 1634 (1979).
4. Wagner G., Hanfeld V., Pischel H.: Pharmazie *32*, 668 (1977).
5. Karrer P., Meyer J.: Helv. Chim. Acta *20*, 626 (1937).
6. Ulbricht V., Makeš J., Jureček M.: Collect. Czech. Chem. Commun. *29*, 1466 (1964).

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