N-Alkylated Derivatives of 5-Fluorouracil

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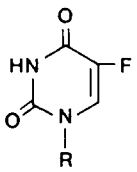
Abstract □ Some *N*-alkyl derivatives of 5-fluorouracil were designed to act as latent depot forms of 5-fluorouracil. A general and efficient method for the syntheses of the alkylated derivatives is described. As expected, the alkylated derivatives of 5-fluorouracil did not show any cytotoxicity in cell culture systems even up to 10^{-4} *M* concentration. The synthesis of 1,3-dimethyl-5-fluoro-5,6-dihydrouracil is also described.

Keyphrases □ 5-Fluorouracil—*N*-alkylated derivatives, synthesis, cytotoxicity □ Derivatives—*N*-alkylated, 5-fluorouracil, synthesis, cytotoxicity □ Cytotoxicity—5-fluorouracil, *N*-alkylated derivatives, synthesis

5-Fluorouracil (I) and its deoxyribonucleoside derivative, flouridine (II), have been found to be highly effective compounds for the treatment of various solid tumors (1–3). The tetrahydrofuryl derivative (III) of 5-fluorouracil has also been found to be clinically active (4). Efforts have been made to improve upon the efficacy of these drugs (5–11).

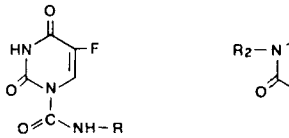


I, R = H

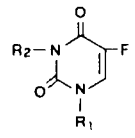
II, R = 2-DEOXYRIBOSE

III, R = 

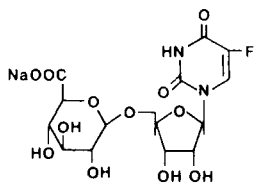
Recently, 1-alkyl carbamoyl derivatives (IV) of 5-fluorouracil have been tested as masked forms of 5-fluorouracil and several of them have been found to be promising (12, 13). The synthesis and antitumor properties of various *N*-acyl and *N*-(alkoxycarbonyl)-5-fluorouracil derivatives (V) which probably act as depot forms of 5-fluorouracil have been reported (14). 5′-*O*-Glucuronide of 5-fluorouridine (VI) has been described recently (15). This is expected to be activated by the β-glucuronidase activity in tumor cells.



IV, R = CH_3, C_2H_5, C_3H_7, C_4H_9 etc.

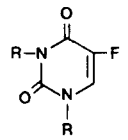


V, R_1, R_2, H, acyl, or, alkoxycarbonyl



VI

In view of the success of the various acyl, alkoxycarbonyl, and carbamoyl derivatives of 5-fluorouracil, the authors became interested in suitable *N*-alkyl derivatives of 5-fluorouracil in which an activated methylene group is attached to the nitrogen atom of the fluorouracil ring. These compounds could act as latent depot forms of 5-fluorouracil (Scheme I). In this report, a general method for the syntheses of various N_1, N_3-dialkyl (VII–X) and N_1-monoalkyl (XI and XII) derivatives of 5-fluorouracil and the study of their toxicities in cell culture systems are reported. In addition, the synthesis of N_1, N_3-dimethyl-5-fluoro-5,6-dihydrouracil (XIII), a model for 5-fluoro-5,6-dihydrouracil, which is an intermediate in the catabolism of 5-fluorouracil, is also described.

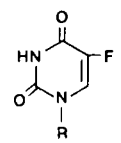


VII, R = CH_3

VIII, R = $CH_2C_6H_5$

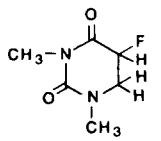
IX, R = $CH_2-CH=CH_2$

X, R = $CH_2-C\equiv CH$



XI, R = $CH_2C_6H_5$

XII, R = $CH_2-CH=CH_2$



XIII

RESULTS

Chemistry—The direct *N*-alkylation of 5-fluorouracil has been studied previously (16). It was observed that by using dimethyl sulfoxide as solvent and potassium carbonate as base, alkylation of 5-fluorouracil with unreactive halides led to N_3-alkylation, whereas with reactive halides, alkylation was found to be N_1-alkylation. However, formation of N_1, N_3-dialkyl derivatives was not described. Since among the acyl derivatives of 5-fluorouracil, the diacylated derivative, N_1-acetyl-N_3-*O*-toluyl-5-fluorouracil was found to be the most promising (14), there has been a greater interest in studying the dialkylated species of 5-fluorouracil. Preformation of the potassium salts[1] of 5-fluorouracil and subsequent reaction of the potassium salts with different alkylating agents led to excellent yields of the alkylated species. The formation of the potassium salts of 5-fluorouracil was accomplished in dimethylformamide as a solvent in the presence of potassium carbonate as a base. The process was found to be relatively slow at room temperature and was completed by overnight stirring when a thick gel of the potassium salts of 5-fluorouracil was formed. Reaction of the gel with different alkylating agents yielded either a mixture of monoalkyl and dialkyl products or, exclusively, the dialkyl products dependent upon the amount of the alkylating agents used. However, in all cases the total yields of the alkylated species (as shown in Table I) were very high compared with the other reported methods (16–18) of alkylation. In Table II are shown the different proportions of mono- and dialkylated species which could be obtained by using different amounts of the alkylating agents.

[1] Probably a mixture of N_1-mono, N_3-mono, and N_1, N_3-dipotassium salts is formed.

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Table I—*N*-Alkylated Derivatives of 5-Fluorouracil

Compound	Melting Point°	Yield, %		Molecular Formula		Analysis, % Calc.	Found
VII	130–131	90		$C_6H_7N_2O_2F$	C	45.57	45.48
					H	4.43	4.33
					N	17.72	17.52
VIII	oil	84	90	$C_{18}H_{15}N_2O_2F$	C	69.68	69.40
					H	4.84	4.64
					N	9.03	8.95
					F	6.13	6.25
XI	166–168	6		$C_{11}H_9N_2O_2F$	C	60.00	60.17
					H	4.09	4.25
					N	12.73	12.75
					F	8.64	8.83
IX	38	41.5	73.9	$C_{10}H_{11}N_2O_2F$	C	57.14	57.36
					H	5.24	5.31
					N	13.33	13.32
					F	9.05	9.36
XII	125	32.4		$C_7H_7N_2O_2F$	C	49.41	49.61
					H	4.12	4.32
					N	16.47	16.48
					F	11.18	11.47
X	98	92		$C_{10}H_7N_2O_2F$	C	58.25	58.26
					H	3.40	3.53
					N	13.59	13.50
					F	9.22	9.44
XIII	51	98.5		$C_6H_9N_2O_2F$	C	45.00	45.28
					H	5.63	5.80
					N	17.50	17.54
					F	11.18	11.84

The structures of the alkylated species were established by elemental analyses and by spectroscopic methods. The compounds gave satisfactory carbon, hydrogen, nitrogen, and fluorine analyses (Table I). In mass spectral measurements (see Experimental) all the compounds showed the molecular ions, and their fragmentation patterns confirmed their structures. The UV absorption spectra (Table III) of the dialkylated species showed them to be the N_1, N_3-dialkylated species. The monoalkylated species were found to be the N_1-alkylated derivatives by comparison of their melting points with those reported in the literature (16, 17) and also from the independence of their UV absorptions in neutral, acidic, and basic media (Table III). No N_3-alkylated compounds were isolated under the reaction conditions. A comparison of the PMR spectra of the mono- and dialkylated derivatives (Table IV) showed that N_1-CH_3 and N_1-CH_2- groups have higher field chemical shifts than the corresponding N_3-CH_3 and N_3-CH_2-groups. This provides an independent method for the identification of N_1- and N_3-substituted alkyl derivatives of 5-fluorouracil.

5-Fluoro-5,6-dihydrouracil is an intermediate in the catabolic degradation of 5-fluorouracil, and has been synthesized (2) in an extremely small yield by the catalytic hydrogenation of 5-fluorouracil. Lithium tri-*sec*-butyl borohydride has been used for the reduction of α,β-unsaturated ketones and esters to the corresponding saturated ketones and esters (19, 20). Recently, this reagent has been used successfully for the reduction of the 5,6-double bond of uracil and 5-fluorouracil derivatives (21). When 1,3-dimethyl-5-fluorouracil was reduced with lithium tri-*sec*-butyl borohydride, a quantitative yield of crystalline 1,3-dimethyl-5-fluoro-5,6-dihydrouracil (XIII) was obtained. Its structure was established by elemental analysis, lack of UV absorption, and its PMR spectrum. In the mass spectrum, a very strong peak due to the parent molecular ion was seen, confirming the structure of the compound.

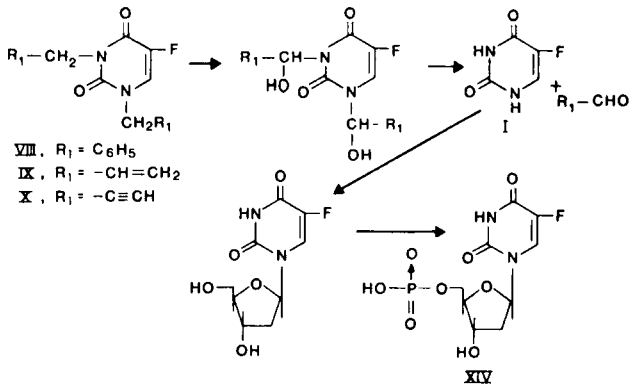
Table II—Ratios of N_1, N_3-Diallyl and N_1-Allyl 5-Fluorouracils Formed

5-Fluorouracil	Potassium Carbonate	Allyl Bromide	Diallyl/Monoallyl
1 mole	1.04 mole	1.30 mole	1.28
1 mole	1.6 mole	1.63 mole	3.3

Table III—UV Spectroscopic Data for *N*-Alkylated Derivatives of 5-Fluorouracil

Compounds	λ_{max} (ϵ) 95% Ethyl Alcohol	0.1 *M* HCl	0.1 *M* NaOH
VII	274 (7413)	271 (7260)	271 (7820)
VIII	276 (7560)	[a]	[a]
XI	274 (8930)	275 (9220)	273 (6760)
IX	273 (6570)	275 (7410)	271 (7070)
XII	274 (8240)	273 (8640)	272 (6270)
X	267 (7330)	271 (7980)	264 (7600)
XIII	—	—	—

[a] The absorptions could not be determined due to a solubility problem.



Scheme I

Growth Inhibition Studies—The growth inhibitory effects of the compounds (VII–XII) were studied against L-1210 (22) and CCRF-CEM leukemic cells in culture (23). As expected, none of the compounds showed any significant cell inhibitory effects even at concentrations up to 10^{-4} *M*.

DISCUSSION

N_1, N_3-Dialkylated (VII–X) and N_1-monoalkylated (XI and XII) derivatives of 5-fluorouracil were synthesized by a simple and highly efficient method. These compounds were chosen since they either have a small alkyl group (*e.g.*, methyl) or an active methylene group (attached to an unsaturated center) on nitrogen. These compounds, as expected, were found to have very little toxicity against leukemic cells in culture. However, like many anticancer agents (24, 25), these compounds could be activated by an oxidative mechanism (26, 27) in the whole animal as shown in Scheme I[2]. A slow formation of 5-fluorouracil (I) could provide the active compound (XIV) in a small steady state. Also, since the alkylated derivatives are much more soluble in nonpolar solvents in contrast to 5-fluorouracil, they are expected to have different pharmacological characteristics compared with 5-fluorouracil. Further biological studies of these compounds are in progress.

[2] Alternative metabolic transformation leading probably to inactive products are not ruled out by Scheme I.

Table IV—PMR Spectral Data for *N*-Alkylated Derivatives of 5-Fluorouracil

Compound	δ, ppm					
	H_6	N_1-CH_3	N_3-CH_3	N_1-CH_2-	N_3-CH_2	Other Hydrogens
VII	7.45 (d) ($J_{H_6,F}$ = 4.5 Hz)	3.4 (s)	3.5 (s)			
VIII	at 7.34	—	—	4.8 (s)	5.1 (s)	7.34 (aromatic)
XI	8.13 (d) ($J_{H_6,F}$ = 8 Hz)	—	—	4.84 (s)	—	7.38 (aromatic)
IX	7.32 (d) ($J_{H_6,F}$ = 6 Hz)	—	—	4.38 (d) (J = 6 Hz)	4.63 (d) (J = 6 Hz)	5.25 (m, CH_2 of vinyl) 5.87 (m, CH of vinyl)
XII	7.4 (d) ($J_{H_6,F}$ = 6 Hz)	—	—	4.33 (d) (J = 6 Hz)	—	5.32 (m, CH_2 of vinyl) 5.92 (m, CH of vinyl)
X	7.68 (d) ($J_{H_6,F}$ = 6 Hz)	—	—	4.65 (d) (J = 3 Hz)	4.75 (d) (J = 3 Hz)	2.2 (t, J = 3 Hz) and 2.57 (t, J = 3 Hz) (acetylenic Hs)
XIII	—	3.13 (s)	3.23 (s)	—	—	3.7 (m, 2H, C_6-H) 5.1 (2t, 1H, C_5-H, $J_{H_5,F}$ = 48 Hz)

5-Fluoro-5,6-dihydrouracil is an intermediate in the catabolism of 5-fluorouracil. There is no convenient method available for the synthesis of this compound. A highly efficient method for the synthesis of 1,3-dimethyl-5-fluoro-5,6-dihydrouracil has been developed. This compound in combination with 5-fluorouracil may show different toxicities to tumor systems. This aspect is also under extensive study.

EXPERIMENTAL[3]

***N_1, N_3*-Dimethyl 5-Fluorouracil (VII)**—5-Fluorouracil (2.0 g, 15.4 mmoles) was dissolved in dimethylformamide (50 ml) when a clear solution was formed. To this solution powdered anhydrous potassium carbonate (4.2 g, 30.4 mmoles) was added, and the mixture was stirred overnight at room temperature when a thick white gel of the potassium salts of 5-fluorouracil was formed. Methyl iodide (4.2 g, 29.6 mmoles) was added and the mixture was stirred for 3 days at room temperature. Dimethylformamide was removed under high vacuum and the residue was partitioned between chloroform (200 ml) and water (100 ml). The aqueous layer was again extracted with chloroform (2 × 100 ml). The combined chloroform extract was dried over anhydrous magnesium sulfate, filtered, and solvent was removed to yield a crystalline white solid (2.2 g, 13.9 mmoles, 90%), single spot on TLC, R_f = 0.12, 0.25, and 0.91 in Solvents A, B, and C, respectively. On crystallization from absolute ethanol this yields long white needles, mp 130–131° [lit. (18) mp 128–130°]; ν_{max} 3070, 1702, and 1650 cm^{-1}; MS m/z 158 (M^+, 100%).

***N_1,N_3*-Dibenzyl-5-fluorouracil (VIII) and *N_1*-Benzyl-5-fluorouracil (XI)**—Benzyl bromide (7.8 g, 45.6 mmoles) was added to the potassium salts of 5-fluorouracil made by stirring 5-fluorouracil (3 g, 23 mmoles) and anhydrous potassium carbonate (6.3 g, 45.6 mmoles) in dimethylformamide (80 ml). The mixture was stirred at room temperature for 6 days. Dimethylformamide was removed and the residue partitioned between chloroform and water. The chloroform extracts on removal of solvent yielded an oil which was chromatographed on silica gel. The column was washed with petroleum ether (bp 35–60°, 300 ml) and then the dibenzyl derivative (VIII) was eluted with chloroform–ethyl acetate (15:1 Fractions 2–4, total volume 250 ml) and was obtained on removal of solvent as a gum (6 g, 19.4 mmoles, 84%); TLC, R_f = 0.38, 0.90, and 0.95 in Solvents A, B, and C respectively; IR ν_{max} 3040, 3080, 1710, 1720, 1660, 1650 cm^{-1}; MS m/z 310 (M^+, 51.7%), 219 (M^+ − 91, 19.3%), 91 ($C_6H_7^+$, 100%).

***N_1*-Benzyl-5-fluorouracil (XI)**—N_1-Benzyl-5-fluorouracil was eluted with chloroform–methanol (10:1 fractions 6–7). On removal of solvent, a white solid (300 mg, 1.4 mmoles, 6%) was obtained. This was crystallized from absolute ethanol as white plates, mp 170° [lit. (16) mp 170–171°]; TLC R_f 0.06, 0.08, and 0.81 in solvents A, B, and C, respectively; IR ν_{max} 3070, 1720, 1665 cm^{-1}; MS m/z 220 (M^+), 91 ($C_7H_7^+$, 100%).

***N_1, N_3*-Diallyl-5-fluorouracil (IX) and *N_1*-Allyl-5-fluorouracil (XII)**—The potassium salts of 5-fluorouracil (10 g, 76.6 mmoles) were made by stirring with anhydrous potassium carbonate (11 g, 79.7 mmoles) in dimethyformamide (50 ml). Allyl bromide (12 g, 100 mmoles) was added and the mixture was stirred for 1 week at room temperature. Dimethylformamide was removed under vacuum and the residue was treated with water (100 ml) and 6 *N* HCl to pH 4. The mixture was extracted with methylene chloride. The methylene chloride layer was washed with water, dried over anhydrous magnesium sulfate, and solvent was removed yielding a gummy semisolid residue which was chromatographed on silica gel (40–140 mesh). The column was washed with petroleum ether (bp 40–60°) and then diallyl-5-fluorouracil (IX, 6.7 g, 31.9 mmoles, 41.5%) was eluted with methylene chloride. The monoallyl-5-fluorouracil (XII, 4.24 g, 24.9 mmoles, 32.4%) was eluted with methylene chloride–ethyl acetate (15:1) and chloroform–methanol (10:1). N_1, N_3-Diallyl-5-fluorouracil was crystallized from absolute ethanol in the cold as long white needles, mp 38°; TLC, R_f = 0.27, 0.69, and 0.98 in solvents A, B, and C, respectively; IR ν_{max} 1650, 1680 cm^{-1}; MS m/z 210 (M^+, 26.5%). N_1-Monoallyl-5-fluorouracil was crystallized from absolute ethanol in small white needles, mp 125–126°; TLC, R_f = 0.07, 0.08, and 0.71 in Solvents A, B, and C, respectively; IR ν_{max} 1650, 1680 cm^{-1}; MS m/z 170 (M^+).

***N_1,N_3*-Dipropargyl-5-fluorouracil (X)**—To the potassium salts made from 5-fluorouracil (5 g, 38.5 mmoles) and anhydrous potassium carbonate (8.0 g, 57.9 mmoles) in dimethylformamide (160 ml), propargyl bromide (9.5 ml of 80% solution in toluene) was added and stirred at room temperature for 4 days. Dimethylformamide was removed under high vacuum and the residue was partitioned between water (10 ml) and methylene chloride (300 ml). The methylene chloride layer was washed with water, dried over anhydrous magnesium sulfate, and solvent removed to obtain a residue (7.3 g, 35.42 mmoles, 92%) which crystallized readily at room temperature. The material was crystallized from methylene chloride into long colorless needles, mp 98°; TLC, R_f 0.23, 0.67, and 0.98 in solvents A, B, and C, respectively; IR ν_{max} 3280 (≡CH), 1655, 1670, 1718 cm^{-1}; MS m/z 206 (M^+), 167 (M^+—CH_2—C≡CH).

***N_1,N_3*-Dimethyl-5-fluoro-5,6-dihydrouracil (XIII)**—N_1, N_3-Dimethyl-5-fluorouracil (316 mg, 2 mmoles) was dissolved in dry tetrahydrofuran (10 ml) and cooled in a dry ice–acetone bath under argon atmosphere. Lithium tri-*sec*-butyl borohydride (2.2 ml of 1 *M* solution in tetrahydrofuran) was injected and the mixture was stirred in a dry ice–acetone bath for 10 min. The mixture was decomposed with saturated ammonium chloride solution which was injected and stirred in the cold bath for another 10 min. Tetrahydrofuran was removed under aspirator and the residue was extracted with methylene chloride. The methylene chloride layer was washed with water, dried over anhydrous magnesium sulfate, and solvent was removed to obtain a thick liquid which was chromatographed over silica gel. Compound XIII was eluted with methylene chloride–ethyl acetate (15:1). On removal of solvent, a thick colorless liquid (315 mg, 1.97 mmoles, 98.5%) was obtained which crystallized from ethanol, mp 51°; TLC, R_f = 0.15, 0.33, and 0.94 in Solvents A, B, and C, respectively; IR ν_{max} 1728, 1690 cm^{-1}; MS m/z 160 (M^+, 100%).

Growth Inhibition Assay—L-1210 cells and CCRF-CEM cells were grown in medium[4] supplemented with 10% dialyzed fetal calf serum with

[3] Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The UV spectra were recorded on a Beckman model 25. Spectra were taken in 95% ethanol unless otherwise mentioned. The IR spectra were done on a Beckman 4210 in a fluorinated hydrocarbon. PMR spectra (δ) were recorded on a Varian EM-390 90-MHz NMR spectrometer in deuterated chloroform, using tetramethylsilane as internal reference. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., or Spang Microanalytical Laboratory, Ann Arbor, Mich. Mass spectra were taken on a Hewlett-Packard, model 5985 spectrophotometer. TLC was performed on an Eastman Chromagram sheet (6060 silica gel with fluorescent indicator) in the indicated solvents: Solvent A, chloroform; Solvent B, methylene chloride–ethyl acetate (15:1); Solvent C, chloroform–methanol (10:1); R_f's for 5-fluorouracil are 00.0, 00.0, and 0.26 in Solvents A, B, and C, respectively.

[4] Roswell Park Memorial Institute (RPMI) 1640.

a doubling time of 10–12 and 18–24 hr, respectively. The solutions were diluted to a stock solution of 10^{-3} *M* with phosphate-buffered saline, sterilely filtered, and aseptically diluted by half-log increments. Each concentration (0.7 ml) was added to duplicate 13- × 75-mm test tubes. Cells from logarithmically growing stock culture were suspended in prewarmed medium[4] supplemented with 10% dialyzed fetal calf serum, 10 m*M* (morpholine-propanesulfonic acid, and 20 m*M* [*N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid]. Cell suspension (1.8 ml) was added to each tube. The tubes were incubated upright at 37° in a warm room or dry incubator. Under these conditions, L-1210 cells grew exponentially 15- to 25-fold from an initial density of 2–2.5 × 10^4/cm^3; CCRF-CEM cells grew exponentially 8- to 10-fold. After 48 hr (for L-1210 cells), or 72 hr (for CCRF-CEM cells), the incubation was terminated and the cell densities were determined[5]. The degree of proliferation of each 2-ml culture was expressed as the ratio of the final cell density to the initial cell density; this index was plotted against the drug concentration employed. The concentration of drug which depresses the ratio to 50% of control (the IC_{50}) was graphically determined. For the clinically effective drug, 5-fluorouracil, IC_{50} = 1.9 × 10^{-6} *M* for CCRF-CEM cells in culture.

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[5] Determined by using a Coulter Counter.

High-Pressure Liquid Chromatographic Assay of Cloxacillin in Serum and Urine

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Abstract □ Two rapid, specific, and sensitive high-pressure liquid chromatographic (HPLC) assays were developed for cloxacillin in serum and urine. A reversed-phase column (RP-8) was selected for use with two different sets of HPLC conditions and sample pretreatment procedures. Cloxacillin extraction efficiencies are reported from serum and urine. Equations are presented for linear relationships between peak height or peak area ratios of cloxacillin to nafcillin (internal standard) and the cloxacillin concentration over a range of 0–80 μg/ml. The sensitivity limit of these assays was ~0.3 μg/ml of a standard solution for one method and 0.05 μg/ml for the other HPLC assay.

Keyphrases □ Cloxacillin—high-pressure liquid chromatographic assay, serum, urine, nafcillin □ Nafcillin—cloxacillin, high-pressure liquid chromatographic assay, serum, urine □ High-pressure liquid chromatography—cloxacillin in serum and urine □ Penicillins—high-pressure liquid chromatography of cloxacillin

Cloxacillin sodium (I), [3-(*o*-chlorophenyl)-5-methyl-4-isoxazoyl] penicillin sodium, is a semisynthetic penicillin synthesized in 1962 (1). It can be administered both parenterally and orally. Like other penicillins, I is sensitive to nucleophilic and electrophilic attack catalysed by general bases and acids, respectively. Maximum stability of

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