Synthesis of Aminomethyl and Amino Analogs of 5-Benzylacyclouridine and 5-Benzyloxybenzylacyclouridine

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Amino analogs of BAU (5-benzylacyclouridine) and BBAU (5-benzyloxybenzylacyclouridine) and their 2'-hydroxymethyl derivatives were synthesized for evaluation as inhibitors of uridine phosphorylase and hence potential cancer chemotherapeutic agents. Both aminomethyl analogs were found to be potent inhibitors of this enzyme and good potentiators of the anti-tumor action of FUdR.

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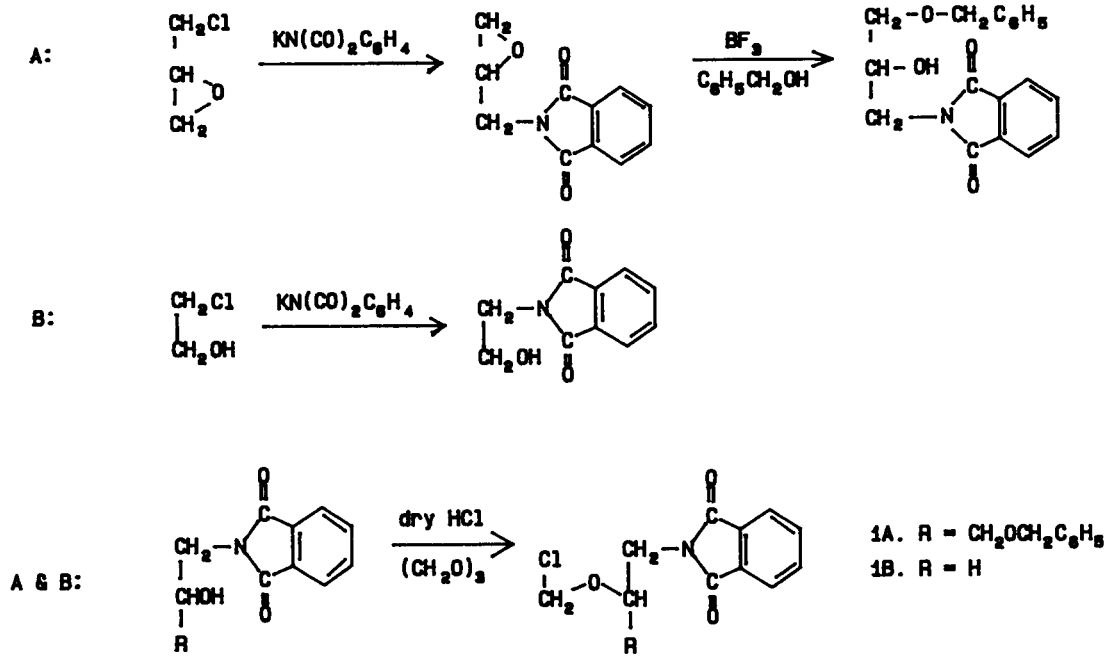
Our study of pyrimidine nucleoside analogs as potential cancer chemotherapeutic agents has been focussed on inhibitors of uridine phosphorylase, one of two pyrimidine phosphorylases known to be important in the salvage pathway of nucleic acid synthesis during which the bases are recycled. Inhibitors of this enzyme should not only be expected to affect cell replication, but might also serve to potentiate the action of drugs such as 5-fluoro-2'-deoxyuridine (FdUrd, formerly FUdR) against resistant human solid tumors, as the current clinical use of FdUrd is limited by phosphorolytic cleavage by this enzyme to the more toxic drug 5-fluorouracil (FUra, formerly 5-FU). In 1982 we reported the synthesis of two benzyluridine analogs, BAU (5-benzyl-1-[(2'-hydroxyethoxy)methyl]uracil, 5-benzylacyclouridine) and BBAU (5-(*m*-benzyloxybenzyl)-1-[(2'-hydroxyethoxy)methyl]uracil, or 5-benzyloxybenzylacyclouridine) which proved to be the most potent inhibitors of uridine phosphorylase known at the time [1-3]. Further studies in this series were directed toward modification of these two drugs in order to study the effect of adding different functional groups, to find the most effective variants, and to improve water solubility.

Variation of the acyclo moiety by adding a second functional group was suggested by the successful modification of the anti-viral agent Acyclovir (9-acycloguanine) [4-7] to improve water solubility without loss of activity by doubling the terminal CH_2OH of the side chain. We found the dihydroxy variants HM-BAU and HM-BBAU [8] (1'-hy-

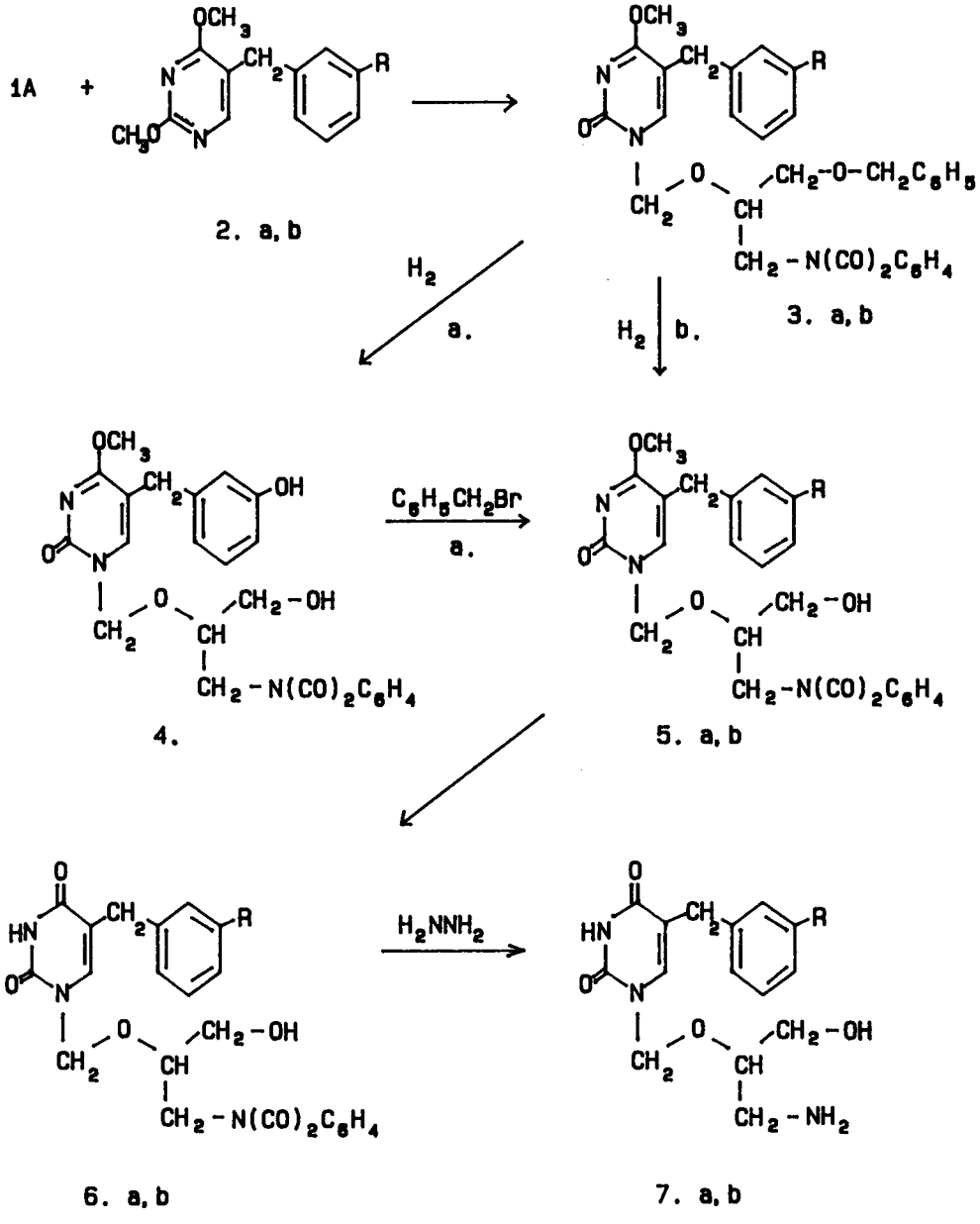
Scheme I



droxymethyl-2′-hydroxyethoxymethylbenzyl uracils) to be potent inhibitors of uridine phosphorylase, comparable to the parent compounds BAU and BBAU or better by a factor of 3 (as indicated by apparent K_i [8]); and better potentiators of the action of FUdR against tumor cells in culture or as xenograft (M. Y. Chu, unpublished). The addition of a 1′-aminomethyl group, a second function capable of hydrogen bonding, to the acyclo moiety was investigated by Siegel and Lin [11] and Lin and Liu [12], who synthesized aminomethyl-BAU (AHPBU), and found that this compound was a better inhibitor of uridine phosphorylase, prepared from Sarcoma 180 cells, than the corresponding dihydroxy analog by a factor of 5.

As BBAU variants in general are several-fold more ac-

Scheme II



tive inhibitors than the corresponding BAU variants, we now report the synthesis of 1'-aminomethyl BBAU, the most potent inhibitor found to date, and a synthesis of 1'-aminomethyl-BAU by the same procedure (AM series, Scheme II). Physical characteristics of the latter product were identical with those of a sample of AHPBU synthesized by Lin's procedure [12]. Our procedure differed from Lin's mainly in the order of steps. We have modified the preparation of the requisite acyclo reagent (Scheme I), and used the carbonate method [13] for the Hilbert-Johnson synthesis in preference to the trimethylsilyl method [14], as we find the carbonate method more adaptable to large scale preparations and higher yielding in the case of most of the benzyl analog intermediates. Other modifications are shown in Scheme II.

Catalytic hydrogenolysis to remove the benzyl blocking group of the acyclo moiety presents a problem with the BBAU analog, as the terminal benzyloxy group at the 5-position is removed at the same time to yield a *m*-hydroxy-BAU variant, less active than BBAU by more than an order of magnitude. Rather than change the hydroxy-protecting group to benzoate and risk rearrangement to a secondary ester [15], we found it simplest to realkylate the phenolic hydroxyl and defer deblocking of the 4-methoxyl group to the next to last step. Realkylation at the phenolic hydroxyl [16], rather than at N-3, was assured by the presence of the methoxyl group in the 4-position and verified by NMR spectra and elemental analysis. The AM analogs proved to be as potent or even more potent than the corresponding hydroxymethyl analogs as inhibitors of uridine phosphorylase, depending on the source of the enzyme [8,10,12].

The nmr spectra of the free amino products **7a**, **7b**, **10a** and **10b** universally lack a chemical shift of 11.3 ppm for N_3-H, although this peak is present in each case in the phthalimido precursor. Instead, there was very broad, characteristically shaped single peak in the region of 3.2-3.8 ppm, encompassing the 1' and 2' aliphatic protons, CH_2 at C_5, hydroxyl, presumably the amino and imino protons, and any moisture present in compound or solvent. When **7a** was converted to its hydrochloride, shifts for N_3-H at 11.4 and NH_3^+ at 7.96 were present as would be expected. The NMR spectra were also interesting in that the asymmetry of the tertiary carbon atom in the acyclo portion was invariably reflected in the splitting of the CH_2 at N_1 shift despite an intervening oxygen atom. The CH_2NH_2 and CH_2OH (OBzl) peaks were also split and to a small extent the CH_2 peaks of the benzyl blocking group which we have reported as singlets or very close doublets. This splitting was not seen in the DA series which lacks an asymetric carbon atom.

Compounds **6b**, **7b** and **8a** were isolated containing a molecule or partial molecule of the recrystallizing solvent (ethanol). The proportionate quantity of ethanol could be derived from integration of the CH_3CH_2 triplet at 1.06 ppm in the nmr spectrum, and was reflected in the chemical analysis. Other spectra did not display shifts for ethanol greater than 1/6 equivalent of a proton.

Potentiation of the action of FdUrd against two human tumors in culture, Pancreatic Carcinoma DAN or Lung Carcinoma LX-1, is shown in Table 1. Details of the method are described in reference [9]. Pretreatment of cultures with AM-BAU or AM-BBAU at 10 μM (final concentration) 5 minutes before adding FdUrd at a series of concentrations substantially increased growth inhibition by FdUrd over that of controls. AM-BBAU was found to be generally a better potentiator than AM-BAU and the best potentiator found to date. AM-BAU and especially AM-BBAU were to a small extent cytotoxic to cultures of DAN and LX-1 in the absence of any FdUrd.

Table 1

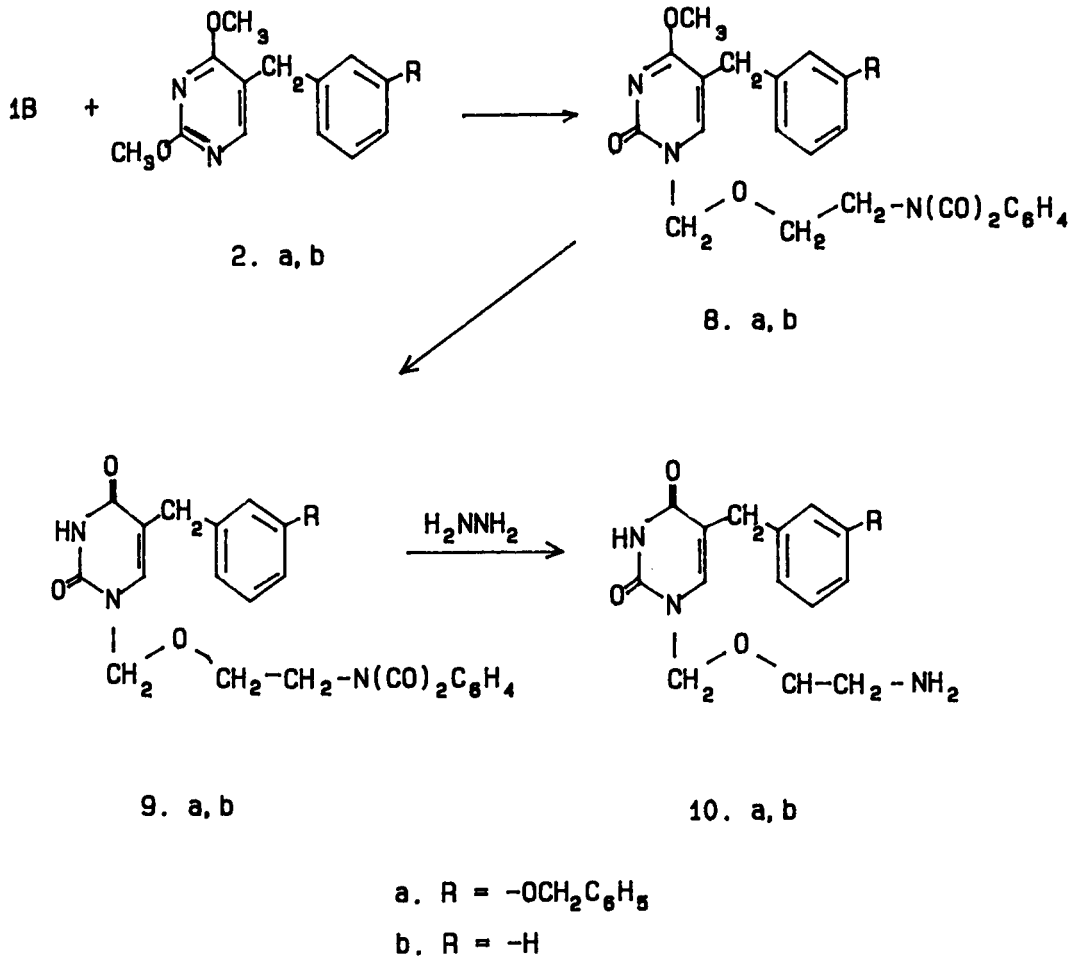
Potentiation by AM-BAU and AM-BBAU of the Inhibitory Effect of FUdR upon the growth of cells of two tumors in culture, Human Pancreatic Carcinoma DAN and Human Lung Carcinoma LX-1.

		% Inhibition of Cell Growth [a]		
			Potentiator	
Cell line	FUdR (μM)	None	AM-BAU 10 μM	AM-BBAU 10 μM
DAN	0	0	5.5 ± 0.2 [b]	1.83 ± 1.0 [b]
	0.3	21.0 ± 4.5 [c]	66.0 ± 4.1	71.0 ± 0
	1.0	41.0 ± 5.9	86.0 ± 0	95.0 ± 1.9
LX-1	0	0	0	9.6 ± 1.06
	0.3	11.1 ± 1.6	29.0 ± 4.7	75.3 ± 7.07
	1.0	20.0 ± 2.5	49.0 ± 5.5	85.8 ± 2.04

[a] Growth inhibition was calculated as the reduction in the number of cell doublings after 72 hours incubation as determined by the trypan blue exclusion method. Cells were pretreated with potentiator for 5 minutes before the addition of FUdR. In each experiment the non-treated control cells reached at least 3 doublings. [b] Significantly different at $P < 0.001$ from control value and $P < 0.01$ from one another. [c] Mean ± S.E.

The similar and more simple DA series (2'-deoxy-2'-amino acyclobenzyluridines) was synthesized for comparison purposes using the same methods (Scheme III). The preparation of the requisite acyclo reagent IB [16] is shown in Scheme I. The DA series was less active than its respective parent compounds BAU and BBAU by approximately a factor of 3 as indicated by apparent inhibition constants.

Scheme III



EXPERIMENTAL

Melting points were determined on a Gallenkamp apparatus and are uncorrected. The uv absorption maxima and extinction coefficients were obtained using a Perkin-Elmer Model 402 recording spectrophotometer, and ^1H nmr spectra were run on a Bruker WM-250 or WM-400 instrument in DMSO-d_6 or deuteriochloroform using trimethylsilane as an internal standard. Analytical tlc was run on Merck silica gel 60 F-254 plates using methylene chloride-ethanol solvent systems; and preparative TLC on Analtech silica gel GF plates (2 mm). Analyses were performed by the Baron Consulting Co. of Orange, Conn, and the Galbraith Laboratories of Knoxville, Tenn.

Preparation of Acyclo Reagent **1A**.

The starting material for the acyclo reagent, 1-phthalimido-2,3-epoxypropane, was made by reacting epichlorohydrin with potassium phthalimide. The characteristics of the product of this reaction, mp 92-94°, analysis and nmr were consistent with the structure of 3-phthalimidoepoxypropane; nmr (deuteriochloroform): δ 2.72 (m, 2H, CH_2-N), 3.33 (br s, 1H, CH tert), 3.85 (q, 2H, CH_2-OBzl, J_a = 9 Hz, J_b = 10 Hz), 7.54-8.00 (m, 4H, ArH of Phth).

Anal. Calcd. for $C_{11}H_9NO_3$: C, 65.02; H, 4.43; N, 6.88. Found: C, 65.31; H, 4.33; N, 7.12.

The epoxide ring was then opened with benzyl alcohol in the presence of boron trifluoride [19] to product a 1,3- doubly blocked glycerol; nmr (DMSO-d_6): δ 3.19 (m, 1H, tert H), 3.58 (d, 2H, CH_2NPhth), 3.89 (d, 2H, CH_2-OBzl), 4.11 (br s, 1H, aliph OH), 4.57 (s, 2H, CH_2 of Bzl), 7.36 (br s, 5H, ArH of Bzl), 7.78 (m, 4H, ArH of Phth).

The product was chloromethylated in the 2-position with anhydrous hydrogen chloride and paraformaldehyde to yield a colorless oil. The sequence of reactions is shown in Scheme I.

5-Benzyloxybenzyl-1-[(1'-phthalimidomethyl-2'-benzyloxyethoxy)methyl]-2-oxo-4-methoxypyrimidine (**3a**).

2,4-Dimethoxy-5-(3'-benzyloxybenzyl)uracil **2a** (16 g, 48 mmoles) was added to a solution of 19 g (53 mmoles) of Acyclo Reagent **1A** in 150 ml of dry methylene chloride, containing 12 g of finely ground anhydrous sodium carbonate in suspension. The mixture was stirred overnight at room temperature and for 5 hours more with an additional 5 g of sodium carbonate. After filtering, the solution was spin-evaporated to dryness. The semisolid residue was taken up in 150 ml of ethanol, refiltered, heated to boiling and allowed to cool. The crystalline precipitate of **3a** showed a single spot on tlc and was analytically pure. It weighed 21 g (68%), mp 140-142°; uv (ethanol): λ max 282 nm (8100); nmr (deuteriochloroform): δ 3.40 (d, 2H, CH_2-NPhth, J = 3 Hz), 3.47-3.67 (m, 4H, CH_2OBzl and CH_2 at C_5), 3.84 (s, 3H, CH_3O), 4.30 (m, 1H, CH tert), 4.51 (m, 2H, CH_2 of Bzl block), 5.06 (s, 2H, CH_2 of term Bzl), 5.21 (d, 1H, CH_2 at N_1, J = 7 Hz), 5.36 (d, 1H, CH_2 at N_1, J = 7 Hz), 6.69-6.72 (m, 3H, *o* and *p*-H of inner Bzl), 7.10 (s, 1H, *m*-H of inner Bzl), 7.17-7.46 (m, 11H,

ArH of term Bzl (5) and Bzl block (5), C_6-H), 7.67-7.85 (sym m, 4H, ArH of Phth).

Anal. Calcd. fo $C_{38}H_{35}N_3O_7$: C, 70.69; H, 5.46; N, 6.51. Found: C, 70.85; H, 5.26; N, 6.37.

5-(3'-Hydroxybenzyl)-1-[(1'-phthalimidomethyl-2'-hydroxyethoxy]methyl]-2-oxo-4-methoxypyrimidine (**4**).

Hydrogenolysis was carried out at 3 atmospheres pressure, using 5% palladium on charcoal as the catalyst. Compound **3a** (6 g, 9.3 mmoles) in a mixture of 70 ml of methylene chloride and 100 ml of methanol containing 2 ml of 6 *N* hydrochloric acid was hydrogenated until the product showed only a single spot on tlc. The reaction mixture was evaporated to dryness under reduced pressure, and the residue taken up in methylene chloride, washed with water, dried over anhydrous magnesium sulfate, and re-evaporated to yield 3.61 g of white crystalline solid **4**, mp 106-108° (83%); uv (ethanol): λ max 281 nm (8900); nmr (DMSO-d_6): δ 3.18 (s, 2H, CH_2-NPhth), 3.41-3.78 (m, 8H, CH_2-OH, CH_3O, CH_2 at C_5 and CH tert), 4.06 (br s, 1H, CH tert), 4.89-5.10 (m, 1H, aliph OH), 5.03 (d, 1H, CH_2 at N_1(1), J = 8 Hz), 5.30 (d, 1H, CH_2 and N_1(1), J = 8 Hz), 6.48-6.65 (m, 3H, *o* and *p*-H of HO-Bzl), 7.00-7.10 (t, 1H, *m*-H of HO-Bzl, J = 6 Hz), 7.56 (s, 1H, C_6-H), 7.69-8.00 (m, 4H, ArH of Phth), 9.27 (s, 1H, ArOH).

Anal. Calcd. for $C_{24}H_{23}N_3O_7 \cdot H_2O$: C, 59.62; H, 5.21; N, 8.69. Found: C, 59.37; H, 5.17; N, 8.41.

5-Benzyloxybenzyl-1-[(1'-phthalimidomethyl-2'-hydroxyethoxy)methyl]-2-oxo-4-methoxypyrimidine (**5a**).

Compound **4** (2.5 g, 5 mmoles) was dissolved in 30 ml of dry methanol. To this solution there was added 10 ml of a solution of sodium methoxide prepared by dissolving 1 g of sodium in 75 ml of methanol (5.8 mmoles). After 15 minutes, 0.7 ml (5.9 mmoles) of benzyl bromide was added and the mixture stirred overnight at room temperature. The solution was evaporated under reduced pressure and the residue taken up in 150 ml of methylene chloride, washed with water until neutral, dried over anhydrous magnesium sulfate, concentrated, loaded onto four preparative tlc plates and developed with methylene chloride-methanol, 100:3. Compound **5a**, 1.5 g, (50%) was obtained as a pale yellow solid, mp 146-148°; uv (ethanol): λ max 282 nm (8750); nmr (DMSO-d_6): δ 3.17-3.29 (m, 2H, CH_2-NPhth), 3.43-3.68 (m, 5H, CH_2-OH and CH_3O), 3.56 (s, 2H, CH_2 at C_5 overlap), 4.05 (br s, 1H, CH tert), 4.95-5.05 (m, 1H, aliph OH), 5.01 (d, 1H, CH_2 at N_1, J = 11 Hz), 5.08 (s, 2H, CH_2 of term Bzl), 5.29 (d, 1H, CH_2 at N_1, J = 11 Hz), 6.69-6.85 (m, 3H, *o* and *p*-H of inner Bzl), 7.14-7.20 (t, 1H, *m*-H of inner Bzl, J = 5 Hz), 7.30-7.46 (m, 5H, ArH of term Bzl), 7.58 (s, 1H, C_6-H), 7.73-7.87 (m, 4H, ArH of Phth).

Anal. Calcd. for $C_{31}H_{29}N_3O_7 \cdot 0.5H_2O$: C, 65.96; H, 5.36; N, 7.45. Found: C, 65.87; H, 5.40; N, 7.42.

5-Benzyloxybenzyl-1-[(1'-phthalimidomethyl-2'-hydroxyethoxy)methyl]-uracil (**6a**).

A solution of 1.5 g (3.3 mmoles) of **5a** in a mixture of 100 ml of ethanol, 20 ml of water and 3 ml of concentrated hydrochloric acid was stirred for 4 hours at 50° and then overnight at room temperature. The progress of the reaction was monitored by the drop in the uv maximum from 282 to 268 nm. When no further drop occurred, the mixture was evaporated to dryness and the residue recrystallized from ethanol to yield 1.2 g of white crystalline solid (67%), mp 174-176°; uv (ethanol): λ max 269 nm (9100); nmr (DMSO-d_6): δ 2.94 (d, 1H, CH_2NPhth, J = 15 Hz), 3.10 (d, 1H, CH_2NPhth, J = 15 Hz), 3.44-3.51 (m, 1H, CH_2-OH), 3.55 (m, 2H, CH_2 at C_5), 3.66-3.73 (m, 1H, CH_2-OH), 3.94 (m, 1H, CH tert), 4.92 (d, 1H, CH_2 at N_1, J = 10 Hz), 4.93-5.00 (m, 1H, aliph OH), 5.04 (s, 2H, CH_2 of term Bzl), 5.20 (d, 1H, CH_2 at N_1, J = 11 Hz), 6.68-6.82 (m, 3H, *o* and *p*-H of inner Bzl), 7.14 (t, 1H, *m*-H of inner Bzl, J = 5 Hz), 7.27-7.46 (m, 6H, ArH of term Bzl and C_6-H), 7.76-7.86 (m, 4H, ArH of Phth), 11.07 (br s, 1H, N_3-H).

Anal. Calcd. for $C_{30}H_{27}N_3O_7 \cdot 0.25H_2O$: C, 65.99; H, 5.08; N, 7.70. Found: C, 65.88; H, 4.93; N, 7.54.

5-Benzyloxybenzyl-1-[(1'-aminomethyl-2'-hydroxyethoxy)methyl]uracil (**7a**).

To 0.6 g (1.1 mmoles) of **6a** dissolved in 50 ml of ethanol there was added 1.5 ml of 98% hydrazine. The mixture was heated under reflux for 2 hours, evaporated under reduced pressure and co-evaporated several times with ethanol to remove residual hydrazine. The residual oil was purified by applying it to 2 preparative tlc plates and developing with methylene chloride-methanol (20:3), to yield 0.15 g of compound **7a** (33%), as a white hygroscopic powder; uv (*p*H 1): λ max 264 nm (8000); (*p*H 12): λ max 264 nm (7800); nmr (DMSO-d_6): δ 2.50-2.69 (m, 2H, C$H_2$$NH_2$), 2.94-4.08 (v br m, >10H, NH_2, aliph OH, 5 aliph H and × H_2O), 3.50 (s, 2H, CH_2 at C_5 overlap), 5.04 (s, 2H, CH_2 of term Bzl), 5.13 (d, 1H, CH_2 at N_1, J = 10 Hz), 5.19 (d, 1H, CH_2 at N_1, J = 10 Hz), 6.80-6.93 (m, 3H, *o* and *p*-H of inner Bzl), 7.18 (t, 1H, *m*-H of inner Bzl, J = 8 Hz), 7.30-7.48 (m, 5H, ArH of term Bzl), 7.64 (s, 1H, C_6-H), no peak at 11.4 (N_3-H). As peaks could not be found for N_3-H and NH_2, the compound was converted to its hydrochloride and the NMR redetermined. Peaks for N_3-H and NH_3^+ were then present in the spectrum of the hydrochloride and integrated for the correct number of protons; nmr (DMSO-d_6): δ 2.83, 3.01 (2 br s, 1H each, C$H_2$$NH_2$), 3.44-3.60 (m, >5H, CH_2 at C_5, OH, CH_2OH and H_2O overlap), 3.81 (br s, 1H, CH), 5.06 (s, 2H, CH_2 of OBzl), 5.18 (q, 2H, CH_2 at N_1, J_a = 8 Hz, J_b = 8 Hz), 6.80-6.93 (m, 3H, *o* and *p*-H of inner Bzl), 7.19 (t, 1H, *m*-H of inner Bzl, J = 7 Hz), 7.30-7.47 (m, 5H, ArH of term Bzl), 7.72 (s, 1H, C_6-H), 7.96 (br s, 3 H, NH_3^+), 11.39 (s, 1H, N_3-H).

Anal. Calcd. for $C_{22}H_{25}N_3O_5 \cdot 0.75H_2O$: C, 62.19; H, 6.29; N, 9.89. Found: C, 62.40; H, 6.05; N, 9.79.

5-Benzyl-1-[(1'-phthalimidomethyl-2'-benzyloxyethoxy)methyl]-2-oxo-4-methoxypyrimidine (**3b**).

The alkylation of compound **2b** was carried out by the same procedure as for **2a**, which differs from that of Lin's [11] in regard to the method (carbonate) and the order of deblocking steps. 5-Benzyl-2,4-dimethoxypyrimidine (**2b**), 4 g (12 mmoles), was added to a solution of 6.9 g (29 mmoles) of Acyclo Reagent 1A in 100 ml of dry methylene chloride, containing 6 g of finely ground anhydrous potassium carbonate. After stirring at room temperature overnight and filtering, the solvent was spin-evaporated and the residue washed twice with hexane. The residue crystallized on standing. It was recrystallized from ethanol to yield 4.6 g of a white crystalline product, **3b** (72%), mp 131-132°; uv (ethanol): λ max 285 nm (8000); nmr (DMSO-d_6): δ 3.26 (s, 2H, CH_2NPhth), 3.51-3.73 (m, 7H, CH_3O, CH_2 at C_5 and CH_2OBzl), 4.25 (br s, 1H, CH), 4.53 (s, 2H, CH_2 of OBzl), 5.07 (d, 1H, CH_2 at N_1(1)), 5.30 (d, 1H', CH_2 at N_1 (1)), 7.10-7.38 (m, 10H, ArH of Bzls), 7.61 (s, 1H, C_6-H), 7.74-7.84 (sym m, 4H, phthalate).

Anal. Calcd. for $C_{31}H_{29}N_3O_6$: C, 69.00; H, 5.42; N, 7.79. Found: C, 69.17; H, 5.04; N, 7.80.

5-Benzyl-1-[(1'-phthalimidomethyl-2'-hydroxyethoxy)methyl]-2-oxo-4-methoxypyrimidine (**5b**).

Compound **3b**, 4 g (7.4 mmoles), in 200 ml of methanol containing 2 ml of 6 *N* hydrochloric acid was subjected to catalytic hydrogenolysis at 3 atmospheres pressure over 5% Pd on charcoal. One tenth of the filtered solution (20 ml) was immediately neutralized with sodium hydroxide and spin-evaporated to dryness. The residue was taken up in methylene chloride, washed with water and dried over anhydrous sodium sulfate. Removal of the solvent under vacuum and recrystallization of the solid product yielded 120 mg of **5b**, (36%, based on 1/10 of the starting material), mp 145-147°; uv (ethanol): λ max 282 nm (8500); nmr (DMSO-d_6): δ 3.26 (s, 2H, CH_2NPhth), 3.43-3.68 (m, 5H, CH_3O and CH_2OH), 3.55 (s, 2H, CH_2 at C_5 overlap), 4.04 (br s, 1H, CH), 4.94-5.06 (m, 2H, CH_2 at N_1(1) and OH), 5.28 (d, 1H, CH_2 at N_1(1), J = 10 Hz), 7.11-7.30 (m, 5H, ArH), 7.57 (s, 1H, C_6-H), 7.72-7.84 (sym m, 4H, phthalate).

Anal. Calcd. for $C_{24}H_{23}N_3O_6$: C, 64.14; H, 5.16; N, 9.35. Found: C, 64.00; H, 5.17; N, 9.58.

5-Benzyl-2-[(1'-phthalimidomethyl-2'-hydroxyethoxy)methyl]uracil (**6b**).

The remaining 9/10 of the preceding hydrogenated solution was heated to 60° and spin-evaporated at 60° without neutralization. The solid residue was recrystallized from ethanol to yield 2.5 g of **6b** as a white crystalline solid (86%, based on 9/10 of the original starting material, **3b**.

The uv maximum had shifted from 284 to 267 nm, mp 179-180°; uv (ethanol): λ max 267 nm (10,800); nmr (DMSO-d_6): δ 1.06 (t, ⅔ H, C*H*$_3$CH$_2$OH, J = 7 Hz), 2.97 (d, 1H, CH$_2$NPhth (1), J = 15 Hz), 3.12 (d, 1H, CH$_2$NPhth (1), J = 15 Hz), 3.42-3.73 (m, 4H, C*H*$_2$OH and CH$_2$ at C$_5$), 3.97 (br s, 1H, CH), 4.37 (t, ¼ H, OH of C$_2$H$_5$OH, J = 5 Hz), 4.91 (d, 1H, CH$_2$ at N$_1$(1), J = 11 Hz), 4.96 (t, 1H, OH, J = 5.5 Hz), 5.19 (d, 1H, CH$_2$ at N$_1$(1), J = 11), 7.08-7.25 (m, 5H, ArH), 7.28 (s, 1H, C$_6$-H), 7.77-7.85 (sym m, 4H, phthalate), 11.07 (s, 1H, N$_3$-H).

Anal. Calcd. for $C_{23}H_{21}N_3O_6 \cdot \frac{1}{3}CH_3CH_2OH$: C, 63.06; H, 5.14; N, 9.32. Found: C, 62.90; H, 5.41; N, 9.22.

5-Benzyl-1-[(1′-aminomethyl-2′-hydroxyethoxy)methyl]uracil (**7b**).

Compound **6b**, 1.8 g (4 mmoles), was heated to boiling in 200 ml of ethanol until it dissolved. To this there was added 5 ml of 98% hydrazine and heating continued for 6 hours under reflux. After allowing to cool overnight the solvent was evaporated under vacuum and co-evaporated several times to remove residual hydrazine. The residue was purified by preparative chromatography (ethyl acetate-ethanol, 4:1). Evaporation of the eluting solvent yielded 0.51 g (40%) of a white foam; uv (*p*H 1): λ max 266 nm (9100); (*p*H 11): λ max 269 nm (6700), (Lit [11], *p*H 1: 266 nm (9100); *p*H 13: 266 nm (6200); nmr (DMSO-d_6): δ 2.55-2.80 (m, 2H, C*H*$_2$NH$_2$, partly overlapped by DMSO), 3.10-3.75 (br m, >9H, C*H*$_2$OH, NH$_2$, N$_3$-H, CH tert), OH, CH$_2$ at C$_5$, overlapped by trace H$_2$O), 5.17 (q, 2H, CH$_2$ at N$_1$, J_a = 10 Hz, J_b = 10 Hz), 7.14-7.41 (m, 5H, ArH), 7.66 (s, 1H, C$_6$-H). No peaks between 8.0 and 13.0.

Anal. Calcd. for $C_{15}H_{19}N_3O_4 \cdot \frac{1}{3}CH_3CH_2OH$: C, 58.68; H, 6.60; N, 13.10. Found: C, 58.63; H, 6.39; N, 13.17.

Preparation of Acyclo Reagent 1B.

Acyclo Reagent 1B [18] was prepared by chloromethylating the product obtained from the reaction of potassium phthalimide with ethylene chlorohydrin. Ethylene chlorohydrin and potassium phthalimide in DMF were heated under reflux until the phthalimide had dissolved and potassium chloride was precipitated. After removing the solvent under vacuum, the solid phthalimidoethanol was washed with 2 *N* sodium hydroxide and recrystallized from ethanol. The product moved as a single spot on tlc. It was then chloromethylated in ice-cold methylene chloride solution with para-formaldehyde and dry gaseous hydrogen chloride [18] by the same procedure used for acyclo reagent 1A.

5-Benzyloxybenzyl-2′-deoxy-2′-phthalimidoethoxy-methyl-2-oxo-4-methoxypyrimidine (**8a**).

5-Benzyloxybenzyl-2,4-dimethoxypyrimidine **1a** (7.5 g, 22 mmoles) was added to a solution of 5.6 g (23 mmoles) of acyclo reagent 1B in 100 ml of methylene chloride containing 5 g of finely ground anhydrous potasssium carbonate. The reaction mixture was stirred overnight at room temperature, neutralized and the solvent removed under reduced pressure to yield 10.9 g of a colorless oil (crude yield 96%). A small sample was purified for analysis by preparative chromatography, followed by recrystallized from ethanol. The final product contained one molecule of ethanol according to the NMR spectrum and the elemental analysis, mp 125-126°; uv (ethanol): λ max 282 nm (6300); nmr (DMSO-d_6): δ 1.06 (t, 3H, C*H*$_3$CH$_2$OH, J = 10 Hz), 3.41-3.48 (m, 4H, CH$_2$NPhth and CH$_3$C*H*$_2$OH), 3.71-3.80 (m, 7H, C*H*$_2$CH$_2$NPhth, CH$_3$O, and CH$_3$C*H*$_2$OH), 4.37 (t, 1H, OH of C$_2$H$_5$OH), 5.06, 5.15 (2s, 2H each, CH$_2$ at N$_1$ and CH$_2$ of OBzl), 6.71-6.86 (m, 3H, *o* and *p*-H of inner Bzl), 7.17 (t, 1H, *m*-H of inner Bzl, J = 8 Hz), 7.29-7.45 (m, 5H, ArH), 7.77 (s, 1H, C$_6$-H), 7.84 (s, 4H, phthalate).

Anal. Calcd. for $C_{30}H_{27}N_3O_6 \cdot CH_3CH_2OH$: C, 67.24; H, 5.82; N, 7.36. Found: C, 67.09; H, 5.74; N, 7.79.

5-Benzyloxybenzyl-2′-deoxy-2′-phthalimidoethoxymethyluracil (**9a**).

Removal of the 4-methoxy blocking group was accomplished by stirring 4.5 g of compound **8a** (8.6 mmoles) overnight at room temperature in 100 ml of 6 *N* hydrochloric acid. After removal of the solvent, 3.4 g of a single product was obtained which showed a shift in the uv maximum from 282 to 266 nm (77%), mp 135-136°; uv (ethanol): λ max 268 nm (9200); nmr (DMSO-d_6): δ 3.29 (s, 2H, CH$_2$C*H*$_2$NPhth), 3.76 (s, 4H, C*H*$_2$CH$_2$NPhth and CH$_2$ at C$_5$), 5.04, 5.05 (2s, 4H, CH$_2$ at N$_1$ and CH$_2$ of term Bzl), 6.72-6.87 (m, 3H, *o* and *p*-H of inner Bzl), 7.14 (t, 1H, *m*-H of inner Bzl, J = 10 Hz), 7.29-7.47 (m, 5H, ArH), 7.51 (s, 1H, C$_6$-H), 7.85 (s, 4H, phthalate), 11.37 (s, 1H, N$_3$-H).

Anal. Calcd. for $C_{29}H_{25}N_3O_6$: C, 68.10; H, 4.93; N, 8.21. Found: C, 68.39; H, 4.62; N, 8.64.

5-Benzyloxybenzyl-2′-deoxy-2′-aminoethoxymethyluridine (**10a**).

Compound **9a** (3 g, 5.9 mmoles) was dissolved in 200 ml of ethanol by heating to reflux. Hydrazine (7.5 ml, 98%) was added, and heating continued for 4 hours. After cooling overnight the mixture was filtered, solvent evaporated and the residue evaporated several times with ethanol to remove residual hydrazine. The solid residue remaining was recrystallized from ethanol, to yield 1.5 g (67%), mp 148-149°; uv (*p*H 1): λ max 267 nm (8600); (*p*H 11): λ max 267 (7400); nmr (DMSO-d_6): δ 2.64 (t, 2H, CH$_2$C*H*$_2$NH$_2$, J = 6 Hz), 3.42 (t, 2H, C*H*$_2$CH$_2$NH$_2$, J = 6 Hz), 3.48 (br s, 3H, NH$_2$, N$_3$-H), 3.51 (s, 2H, CH$_2$ at C$_5$), 5.07, 5.09 (2s, 4H, CH$_2$ at N$_1$ and CH$_2$ of term Bzl), 6.79-6.97 (m, 3H, ArH, *o* and *p*-H of inner Bzl), 7.18 (t, 1H, *m*-H of inner Bzl, J = 8 Hz), 7.30-7.50 (m, 5H, ArH of term Bzl), 7.65 (s, 1H, C$_6$-H); no peak at 11.3 (N$_3$-H).

Anal. Calcd. for $C_{21}H_{23}N_3O_4$: C, 66.12; H, 6.08; N, 11.02. Found: C, 65.92; H, 6.01; N, 11.20.

5-Benzyl-2′-deoxy-2′-phthalimidoethoxymethyl-2-oxo-4-methoxypyrimidine (**8b**).

To a solution of 3.8 g (16 mmoles) of acyclo reagent 1B in 100 ml of methylene chloride there was added 4 g (17 mmoles) of 5-benzyl-2,4-dimethoxypyrimidine **2b** and 4 g of finely ground anhydrous potassium carbonate. The reaction mixture was stirred overnight at room temperature, filtered and the solvent evaporated under vacuum. Recrystallization of the solid residue from methylene chloride-ethanol yielded 3.8 g (57%), mp 181-182°; uv (ethanol): λ max 286 (8000); nmr (DMSO-d_6): δ 3.48 (s, 2H, CH$_2$ at C$_5$), 3.74 (s, 3H, CH$_3$O), 3.72-3.79 (m, 4H, CH$_2$CH$_2$), 5.15 (s, 2H, CH$_2$ at N$_1$), 7.13-7.29 (m, 5H, ArH), 7.77 (s, 1H, C$_6$-H), 7.85 (s, 4H, phthalate).

Anal. Calcd. for $C_{23}H_{21}N_3O_5$: C, 65.86; H, 5.05; N, 10.02. Found: C, 65.91; H, 4.94; N, 10.23.

5-Benzyl-2′-deoxy-2′-phthalimidoethoxymethyluracil (**9b**).

Compound **8b** was deblocked at the 4-position by dissolving 3 g (7 mmoles) in 200 ml of ethanol and 20 ml of 6 *N* hydrochloric acid, heating to reflux temperature for 10 minutes and allowing to cool overnight. The crystalline precipitate was filtered and recrystallized from ethanol, to yield 2.8 g (96%), mp 171-172°; uv (ethanol): λ max 267 nm (7900); nmr (DMSO-d_6): δ 3.40-3.50 (m, 2H, CH$_2$NPhth), 3.74 (m, 4H, CH$_2$ at C$_5$ and C*H*$_2$CH$_2$NPhth), 5.05 (s, 2H, CH$_2$ at N$_1$), 7.13-7.26 (m, 5H, ArH), 7.51 (s, 1H, C$_6$-H), 7.86 (s, 4H, phthalate), 11.33 (s, 1H, N$_3$-H).

Anal. Calcd. for $C_{22}H_{19}N_3O_5 \cdot 0.5H_2O$: C, 63.76; H, 4.86; N, 10.14. Found: C, 63.56; H, 5.22; N, 9.96.

5-Benzyl-2′-deoxy-2′-aminoethoxymethyluracil (**10b**).

Compound **9b** (1.2 g, 3 mmoles) in 200 ml of ethanol containing 6 ml of 98% hydrazine was heated to reflux overnight and then cooled. After filtering, the solution was spin-evaporated and co-evaporated several times with ethanol and water to remove excess hydrazine. The residue was placed on a silica gel column and eluted with ethanol, followed by ammoniacal ethanol. The product crystallized out on evaporation of the solvent and was recrystallized from alcohol, yield, 0.65 g (80%), mp 116-118°, mixed mp with a sample prepared by reducing the 2′-azido analog of BAU (S. H. Chu, unpublished) (mp 120-121°) was 117-119°; uv (*p*H 1): λ max 266 (9800); (*p*H 11): λ max 266 nm (7300); nmr (DMSO-d_6): δ 2.65 (t, 2H, CH$_2$C*H*$_2$NH$_2$, J = 6 Hz), 3.43 (t, 2H, C*H*$_2$CH$_2$NH$_2$, J = 6 Hz), 3.44 (br s, 3H, NH$_2$ and N$_3$-H), 3.57 (s, 2H, CH$_2$ at C$_5$), 5.09 (s, 2H, CH$_2$ at N$_1$), 7.16-7.30 (m, 5H, ArH), 7.65 (s, 1H, C$_6$-H), no peak at 11.3 (N$_3$-H).

Anal. Calcd. for $C_{14}H_{17}N_3O_3 \cdot 0.25H_2O$: C, 60.09; H, 6.30; N, 15.02. Found: C, 60.31; H, 6.70; N, 14.68.

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REFERENCES AND NOTES

[1] S. H. Chu, M. Y. Chu, F. N. M. Naguib, M. H. el Kouni, S. Cha and P. Calabresi, *Proc. Am. Assoc. Cancer Res.*, **24**, 305, #1208 (1983).

[2] J. G. Niedzwicki, M. H. el Kouni, S. H. Chu and S. Cha, *Biochem. Pharmacol.*, **30**, 2097 (1981).

[3] J. G. Niedzwicki, S. H. Chu, M. H. el Kouni, E. C. Rowe and S. Cha, *Biochem. Pharmacol.*, **31**, 857 (1982).

[4] J. C. Martin, C. A. Dvorak, D. F. Smee, T. R. Matthews and J. P. H. Verheyden, *J. Med. Chem.*, **26**, 759 (1983).

[5] W. T. Ashton, J. D. Karkas, A. K. Field and R. L. Tolman, *Biochem. Biophys. Res. Commun.*, **105**, 1716 (1982).

[6] K. O. Smith, K. S. Galloway, W. L. Kennell, K. K. Ogilvie and B. K. Radatus, *Antimicrob. Agents Chemother.*, **22**, 55 (1982).

[7] H. J. Schaeffer, in "Nucleosides, Nucleotides and their Biological Applications", J. L. Rideout, D. W. Henry and L. M. Beacham, III, eds, Academic Press, New York, 1983, pp 1-17.

[8] S. H. Chu, Z. H. Chen, E. C. Rowe, F. N. M. Naguib, M. H. el Kouni and M. Y. Chu, *Nucleosides Nucleotides*, **3**, 303 (1984).

[9] M. Y. Chu, F. N. M. Naguib, M. H. Iltsch, M. H. el Kouni, S. H. Chu and S. Cha, *Cancer Res.*, **44**, 1852 (1984).

[10] S. A. Siegel and T. S. Lin, *Biochem. Pharmacol.*, **34**, 1121 (1985).

[11] T. S. Lin and M. C. Liu, *J. Med. Chem.*, **28**, 971 (1985).

[12] F. N. M. Naguib, M. H. el Kouni, S. H. Chu and S. Cha, *Biochem. Pharmacol.*, in press.

[13] C. W. Noell and C. C. Cheng, in "Nucleic Acid Chemistry", Part 1, L. B. Townsend and R. S. Tipson, eds, Wiley Interscience, New York, NY, 1978, pp 57-66.

[14] M. J. Robins and P. W. Hatfield, *Can. J. Chem.*, **80**, 547 (1982).

[15] H. Hibbert and N. M. Carter, *J. Am. Chem. Soc.*, **51**, 1601 (1929).

[16] S. H. Chu, Z. H. Chen, Z. Y. Weng, E. C. Rowe, E. Chu and M. Y. Chu, *J. Heterocyclic Chem.*, in press.

[17] K. S. Park, M. H. el Kouni, T. A. Krenitsky, S. H. Chu, and S. Cha, *Biochem. Pharmacol.*, **35**, 3853 (1986).

[18] J. L. Kelley, M. P. Krochmal and H. J. Schaeffer, *J. Med. Chem.*, **24**, 472 (1981).

[19] J. Berecoechea and J. Anatol., *J. Oleagineux*, **24**, 347 (1969); *Chem. Abstr.*, **71**, 90725h (1969).