The neural network, on the contrary, does not require such a comprehensive term selection. This is a merit as well as a shortcoming of this method. Namely, one can analyze the given data without knowing special techniques. However, even if the appropriate results are obtained, the definitive reason may not be given.

The neural network studied here performs two processes at the same time: the process to convert the input data to the effective form and the process to classify the converted data referring to the characteristics. The former process is carried out by the first and second layers and the latter, by the second and third layers. Since these two processes are optimized to the training patterns, it is very probable for such a neural network to exceed the level of the multiregressional analysis as shown in this paper. Especially, the neural network becomes superior in such cases that the analysis includes a large number of the structural parameters or expansion terms compared to the number of the obtained biological data. However, one may wonder why it should be possible to determine, for example, 96 weights in model A or 420 in model C in Table I when a considerably small number of experimental data is used.

The operation of the neural network is very different from that of the usual multiregression analysis. The information in the given data is accumulated in the weight matrices as the number of the input data is increased. The decision by the network is very much like that of the brain of human: the number of given data seems to be how much a man experienced the situation. Namely, the larger the number of data and the better the quality of the data, the better the network gives the decision. Unlike the multiregression analysis, however, the reliability of the decision cannot be treated statistically at present.

Finally, it should be mentioned that one of the unfamiliar situations in the network is the uncertainty of weight matrices. Namely, the operation can be exactly defined by the mathematical expressions (eqs 1–5). However, the matrices do not always take the same definitive elements even if they given the definitive decision. For example, consider the case that the weight between the first and second layers which coagulates into neuron j_0 (of the second layer) can be dispersed into j_1 and j_2 (by simply increasing the number of neurons in the second layer). Then,

$$W_{ij0} = \lambda_1 W_{ij1} + \lambda_2 W_{ij2} \quad (13)$$

where λ_1 and λ_2 are coefficients. Noticeably, W_{ij1} and W_{ij2} are indefinite although they are controlled by the λ values. Therefore, the weight matrices do not always take the fixed elements even if they give the same results.

Cyclization-Activated Prodrugs. Basic Esters of 5-Bromo-2′-deoxyuridine

Walfred S. Saari,* John E. Schwering, Paulette A. Lyle, Steven J. Smith, and Edward L. Engelhardt

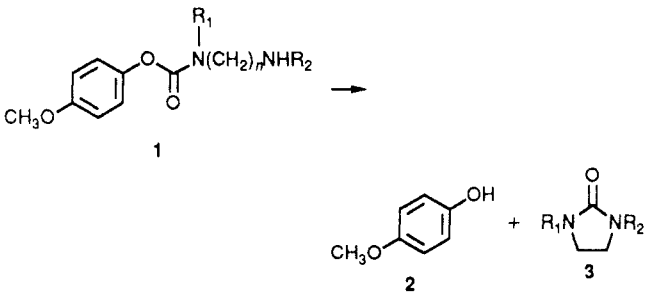
Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486. *Received February 20, 1990*

Some 3′- and 5′-[[(alkylamino)ethyl]glycyl] esters of 5-bromo-2′-deoxyuridine were prepared and evaluated in vitro as progenitors of the parent alcohol. The esters proved to be relatively stable at low pH but released 5-bromo-2′-deoxyuridine cleanly at rates which were pH and structure dependent. These basic esters are examples of cyclization-activated prodrugs in which generation of active drug is not linked to enzymatic cleavage but rather results from an intramolecular cyclization–elimination reaction.

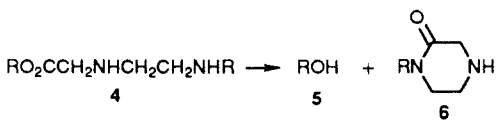
Ester prodrugs of alcohols are frequently utilized to circumvent adverse physicochemical limitations or to extend the duration of action of the parent drug.[1-3] Generally, ester prodrugs have depended upon chemical or enzymatic hydrolysis of the ester bond for conversion of prodrug to drug. However this strategy can only be successful in those cases where the alcohol is generated from the ester at a practical rate under physiological conditions. When this requirement is not attainable, this approach will fail or be of limited value. In addition, generation of drug by enzymatic mechanisms may be subject to much variability between species or even among individual members of a particular species.

A previous report[4] described some basic carbamate prodrugs (1) of the melanocytoxic agent 4-hydroxyanisole which generated the parent phenol 2 by a cyclization–activated mechanism under physiological conditions (Scheme I). In this approach, prodrug is converted to active drug by an intramolecular cyclization–elimination reaction and not through mechanisms involving intermolecular hydrolysis of the ester bond. By this method, ideally, drug formation is not dependent upon the host environment but instead solely upon the rate of the intramolecular cyclization reaction.

Scheme I



Scheme II

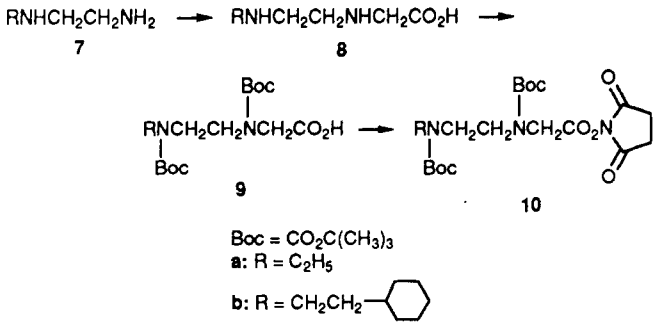


Although basic carbamates of phenols are sufficiently activated to generate phenol at useful rates under physiological conditions, the corresponding carbamates of al-

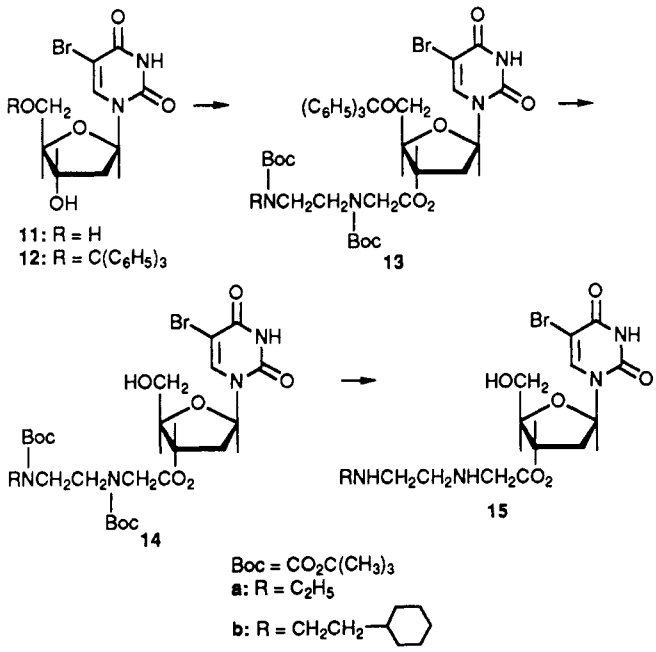
(1) Stella, V. J.; Charman, W. N. A.; Naringrekar, V. H. *Drugs* **1985**, *29*, 455.
(2) Stevenson, L-A.; Tunek, A. *Drug Metab. Rev.* **1988**, *19*, 165.
(3) Bundgaard, H.; Falch, E.; Jensen, E. *J. Med. Chem.* **1989**, *32*, 2507.
(4) Saari, W. S.; Schwering, J. E.; Lyle, P. A.; Smith, S. J.; Engelhardt, E. L. *J. Med. Chem.* **1990**, *33*, 97.

0022-2623/90/1833-2590$02.50/0 © 1990 American Chemical Society

Scheme III



Scheme IV



cohols cyclize too slowly to be of practical value. However basic esters are more reactive and can be designed to be effective precursors of alcohols. For example, basic esters such as **4** would be predicted to liberate the desired alcohol **5** with concomitant formation of piperazinone **6** (Scheme II). To test this concept, some basic esters of the clinically relevant radiation sensitizer 5-bromo-2′-deoxyuridine[5,6] have been synthesized and evaluated as progenitors of this alcohol by an intramolecular cyclization reaction.

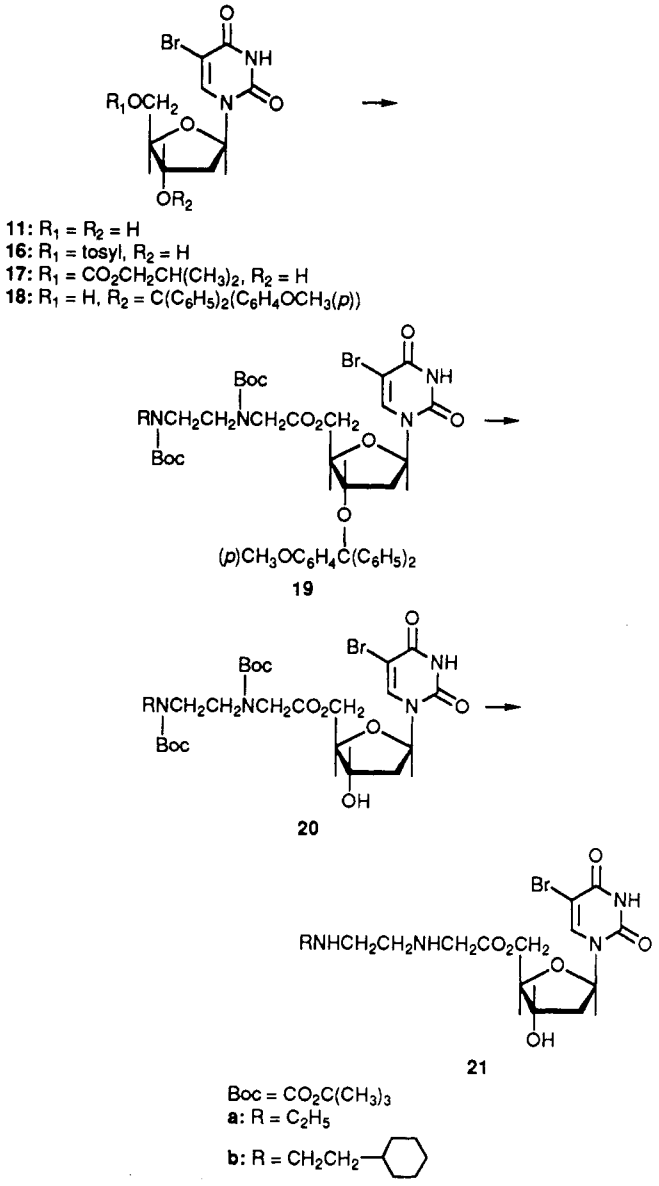
Chemistry

Syntheses of the desired 3′- and 5′-esters of 5-bromo-2′-deoxyuridine required protected diamino acid intermediates which could ultimately be deblocked at the last step without destruction of the ester function. The Boc (*tert*-butoxycarbonyl) group proved to be compatible with this approach.

Diamino acids **8a** and **8b** were easily prepared by reductive amination of glyoxylic acid with the appropriate diamine **7** in EtOH (Scheme III). Boc protective groups were introduced with di-*tert*-butyl dicarbonate in NaOH–THF solution and the protected acids were activated by conversion to the succinate esters **10a** and **10b**.

Condensation of unprotected 5-bromo-2′-deoxyuridine (**11**) with succinate ester **10a** in CH_3CN containing an equivalent of 4-(dimethylamino)pyridine led to a mixture of the two possible mono esters (**14a** and **20a**) in addition to diester. Pure Boc-protected 3′-ester **14a** could be isolated from this mixture by flash chromatography over silica gel.

Scheme V

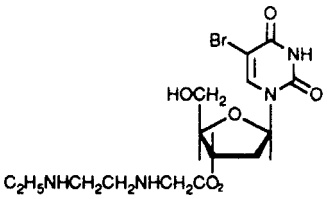
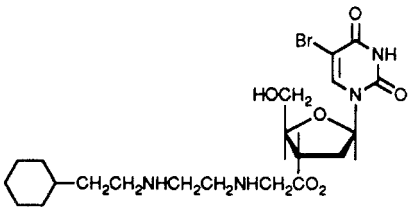
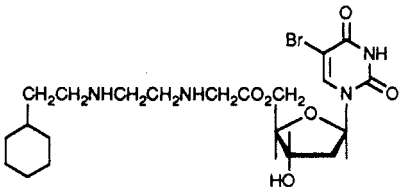


This same ester, as well as the cyclohexylethyl derivative **14b**, was selectively obtained from reaction of **10a** with the 5′-trityl derivative **12** followed by removal of the trityl protective group. Intermediate **13a** was also prepared by dicyclohexylcarbodiimide condensation of **12** and Boc acid **9a** (Scheme IV). Removal of the Boc groups of **14a,b** under acidic conditions allowed direct isolation of the basic esters **15a,b** as the stable HCl salts.

5′-Ester **21b** was synthesized for comparison with the corresponding 3′-ester **15b**. Initially, 5′-tosylate **16**[7] was reacted with the Na salt of the Boc-protected diamino acid **9b** to give a mixture of products from which **20b** could be isolated in low yield. However a better procedure proved to be dicyclohexylcarbodiimide-mediated esterification of the 3′-protected nucleoside **18** with Boc-protected diamino acid **9b** to give **19b** followed by removal of the meth-

(5) Kensella, T. J.; Mitchell, J. B.; Russo, A.; et al. *Int. J. Radiat. Oncol. Biol. Phys.* **1984**, *10*, 1399.
(6) Mitchell, J. B.; Russo, A.; Kinsella, T. J.; Glatstein, E. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1513.
(7) Lin, T-S.; Neenan, J. P.; Cheng, Y-C.; Prusoff, W. H.; Ward, D. C. *J. Med. Chem.* **1976**, *19*, 497.
(8) Blicke, F. F.; Zienty, F. B. *J. Am. Chem. Soc.* **1939**, *61*, 771.

Table I

compound	part. coeff	$t_{1/2}$, min (37 °C) pH 7.4	pH 6.8	pH 2.5	plasma, pH 7.4 rat	human
 **15a**	~0	23.0 ± 1.8	39.2 ± 1.8	no hydrolysis in 24 h	47.3 ± 6.8	70.0 ± 0.8
 **15b**	0.54	29.7 ± 1.3	45.8 ± 1.2		5.3 ± 0.4	47.3 ± 0.1
 **21b**	0.33	26.7 ± 1.0	41.9 ± 0.9		0.36 ± 0.01	26.7 ± 0.5

oxytrityl group. The 5′-ester **21b** was then obtained from **20b** by treatment with anhydrous HCl in EtOAc (Scheme V).

An authentic sample of piperazinone **22** was synthesized by alkylation of 4-(carbobenzyloxy)-2-piperazinone (**25**) with cyclohexylether tosylate (**24**) followed by removal of the CBZ group under catalytic hydrogenation conditions (Scheme VI).

Results and Discussion

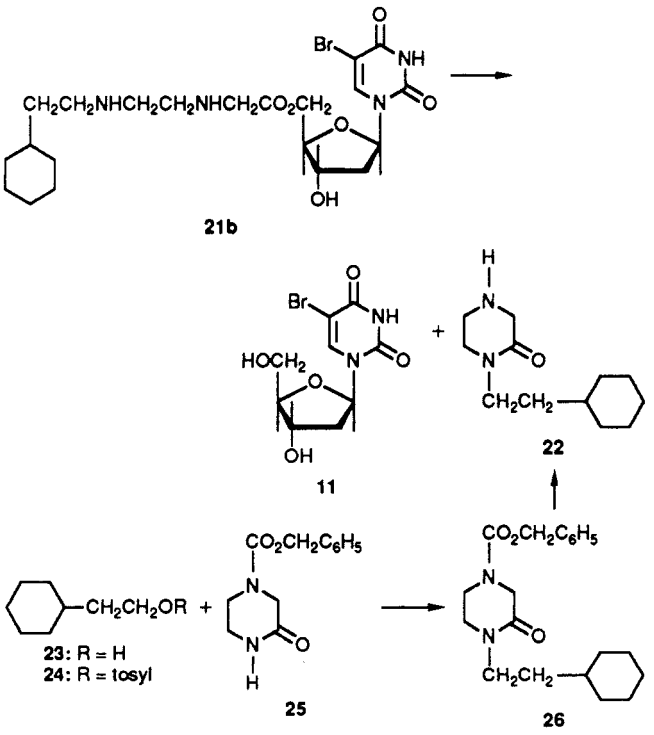
Stability of the 3′- and 5′-esters **15a,b** and **21b**, at 37 °C and various pH's, were determined conveniently by HPLC analysis. Since these esters are relatively stable at the pH of the HPLC mobile phase (~2), injection into the HPLC effectively stops the cyclization reaction. The cyclization is also halted upon quenching aliquots in dilute HCl or $HClO_4$. This latter method was particularly useful in the plasma experiments, as the dilute $HClO_4$ precipitated protein in addition to terminating further reaction.

At the pH's examined, 7.4 and 6.8, each ester generated 5-bromo-2′-deoxyuridine and the corresponding piperazinone cleanly without any other detectable products formed. However the rate of alcohol formation was dependent upon ester structure and the pH. Kinetic analysis showed that the alcohol was formed following first-order kinetics.

All three esters of Table I generated alcohol at nearly the same rate, $t_{1/2}$ = 23, 26.7, and 29.7 min, at pH 7.4. The slightly slower rate observed with the cyclohexylethyl derivative **15b** compared to the *N*-ethyl analogue **15a** may be due to unfavorable steric interactions of the cyclohexyl group in the cyclization transition state. Similar steric considerations could also account for the increased rate of reaction of the primary 5′-ester **21b** compared to that of the secondary 3′-ester **15b**.

All three esters formed alcohol at a slower rate at pH 6.8 than at pH 7.4 and were stable at pH 2.5. This suggests that a nonprotonated amine function is critical for reaction

Scheme VI



to occur and is consistent with the idea that alcohol release is the result of a cyclization reaction. Confirmation that deoxyuridine alcohol is formed by the proposed cyclization mechanism of Scheme II was obtained by isolation of piperazinone **22** in 95.2% yield after heating **21b** at 37 °C and pH 7.4.

In contrast to these results, plasma experiments revealed quite different ester reactivities. In rat plasma, both *N*-cyclohexylethyl analogues **15b** and **21b** were converted to alcohol at much faster rates than in the nonplasma

experiments, indicating that these esters are good substrates for esterases present in rat plasma. However these same esters did not generate alcohol in human plasma faster than the nonplasma rates. The generally low esterase activity of human plasma, in comparison with rodent plasma, is well-documented.

It is surprising that formation of 5-bromo-2′-deoxyuridine from **15a,b** was actually slower in some of the plasma experiments than under nonenzymatic conditions. A similar result was also seen in the basic phenol carbamate series studied previously.[4] This apparent stabilizing effect of plasma could be explained by binding of the nucleoside ester to plasma proteins, which results in partial inhibition of the cyclization reaction.

This series of basic esters of 5-bromo-2′-deoxyuridine appears to satisfy the requirements for cyclization-activated prodrugs. That is, formation of drug from prodrug need not be linked to enzymatic activation but rather may result from a predictable, intramolecular cyclization–elimination reaction. However, since the rate of active drug formation is dependent upon alcohol structure, successful application of this prodrug strategy to other alcohols might require synthesis of different N-substituted esters than those used in this study.

Furthermore, this approach appears to allow variation of the prodrug partition coefficient in addition to selection of drug release rate through structure design. In this study, the rate of formation of drug from prodrug was not greatly affected by a change in partition coefficient of from ~0 to 0.54.

Another interesting aspect of the cyclization–activation mechanism is that generation of active drug should continue even after cellular uptake of prodrug. The release of a relatively lipophilic and neutral drug from a basic, hydrophilic prodrug offers intriguing possibilities which might be exploited for drug-distribution applications.

Experimental Section

All melting points were obtained on a Thomas-Hoover Unimelt capillary melting point apparatus using open capillaries and are uncorrected. Analytical results are indicated by atom symbols and are within 0.4% of theoretical values. ^{1}H NMR spectra were recorded for all intermediates and final products on either a Varian XL-300 or a GE NT-360 instrument using tetramethylsilane as an internal standard and are consistent with assigned structures. E. Merck silica gel (230–400 mesh) was used for the flash chromatographies.

Preparation of Boc-Protected Succinate Esters. *N*-(1,1-Dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-ethylamino]ethyl]glycine (9a). A solution of glyoxylic acid hydrate (9.2 g, 100 mmol) and *N*-ethylethylenediamine (8.82 g, 100 mmol) in EtOH (150 mL) was hydrogenated in a Parr apparatus at room temperature and an initial pressure of 51 psi with 5% Pd on C (2.0 g) as catalyst. After 18 h, hydrogen uptake was complete. Catalyst was removed by filtration through a pad of diatomaceous earth and the filtrate was concentrated under reduced pressure to give the diamino acid as an oil.

This diamino acid was dissolved in water (150 mL) and THF (100 mL) containing NaOH (4.0 g, 100 mmol) and stirred at room temperature while a solution of di-*tert*-butyl dicarbonate (48 g, 220 mmol) in THF (100 mL) was added over 1 h. After stirring at room temperature for 18 h, THF was removed under reduced pressure and the aqueous residue was extracted with Et_2O. The aqueous portion was then acidified with citric acid and extracted with two portions of CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel and elution with 20% MeOH–80% $CHCl_3$ gave 25.9 g (75%) of product: ^{1}H NMR ($CDCl_3$) δ = 1.08 (3 H, m), 1.43 (18 H, s), 3.3 (8 H, m), 3.85 (2 H, m).

***N*-(1,1-Dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-ethylamino]ethyl]glycine *N*-Succinimide Ester (10a).** A solution of Boc-protected acid **9a** (25.9 g, 74.8 mmol) and *N*-hydroxysuccinimide (9.75 g, 82.1 mmol) in CH_2Cl_2 (350 mL) and DMF (17 mL) was cooled in an ice bath while a solution of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (34.8 g, 82.1 mmol) in CH_2Cl_2 (250 mL) was added over 75 min. After stirring in the ice bath for 1 h and then at room temperature for 18 h, the reaction mixture was washed successively with water, 10% citric acid, saturated $NaHCO_3$ solution, and water and then dried (Na_2SO_4). The filtered solution was concentrated under reduced pressure and the residue was recrystallized from EtOAc–hexane to give 17.7 g (53%) of the active ester **10a**, mp 116–119 °C. Anal. ($C_{20}H_{33}N_3O_8$) C, H, N.

***N*-(1,1-Dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-(2-cyclohexylethyl)amino]ethyl]glycine (9b).** A solution of *N*-(2-cyclohexylethyl)ethylenediamine **8** (8.52 g, 50 mmol) and glyoxylic acid hydrate (4.7 g, 50 mmol) in EtOH (200 mL) and water (30 mL) was hydrogenated in a Parr apparatus at room temperature and an initial pressure of 39 psi over a 5% Pd on C catalyst (2.0 g). After uptake of 1 equiv of H_2 was complete, catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give diamino acid **8b** (11.1 g).

A solution of this diamino acid (11.1 g, 48.6 mmol), NaOH (1.9 g, 48.6 mmol), and di-*tert*-butyl dicarbonate (21.8 g, 100 mol) in water (75 mL) and THF (75 mL) was stirred at room temperature over night. After removing THF under reduced pressure, the aqueous solution was acidified with solid citric acid and product was extracted into EtOAc. The organic extract was washed with brine, dried (Na_2SO_4), filtered, and concentrated. The residue was flash chromatographed over silica gel and pure protected diamino acid **9b** (20.8 g, 97%) was obtained as a gum: ^{1}H NMR ($CDCl_3$) δ = 0.9–1.7 (34 H, m), 3.15–3.45 (4 H, m), 3.95–4.1 (2 H, m).

***N*-(1,1-Dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-(2-cyclohexylethyl)amino]ethyl]glycine *N*-Succinimide Ester (10b).** A solution of the di-Boc diamino acid **9b** (4.0 g, 9.33 mmol), *N*-hydroxysuccinimide (1.1 g, 9.33 mmol), and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (3.95 g, 9.33 mmol) in CH_2Cl_2 (105 mL) was stirred at room temperature for 20 h. After washing with 10% citric acid and water, the organic layer was dried (Na_2SO_4), filtered, and concentrated to give 2.9 g (59%) of hydroxysuccinate ester **10b**. An analytical sample (mp 121–123.0 °C) was obtained upon recrystallization from EtOAc–hexane. Anal. ($C_{26}H_{43}N_3O_8$) C, H, N.

5-Bromo-5′-trityl-2′-deoxyuridine (12). A solution of (+)-5-bromo-2′-deoxyuridine (5.0 g, 16.3 mmol) and trityl chloride (5.6 g, 20 mmol) in pyridine (50 mL) was stirred at 120 °C for 1 h, cooled, and then concentrated under reduced pressure. After adding H_2O to the residue, product was extracted into CH_2Cl_2, which was then dried (Na_2SO_4), filtered, and concentrated. The residue was flash chromatographed over silica gel and eluted with 20% MeOH–80% $CHCl_3$. Recrystallization from EtOAc–hexane gave 4.25 g (44%) of analytically pure trityl derivative as the EtOAc solvate: mp 143–145 °C with softening at 110–115 °C. Anal. ($C_{28}H_{25}BrN_2O_5$·0.5EtOAc) C, H, N.

5-Bromo-5′-trityl-2′-deoxyuridine 3′-[*N*-(1,1-Dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-ethylamino]ethyl]glycyl ester] (13a). Method A. From Succinate Ester 10a. A solution of 5-bromo-5′-trityl-2′-deoxyuridine hemi(ethyl acetate) solvate (**12**; 1.0 g, 1.69 mmol), *N*-(1,1-dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-ethylamino]ethyl]glycine *N*-succinimide ester (**10a**; (0.89 g, 2.0 mmol), and 4-(dimethylamino)pyridine (0.21 g, 1.69 mmol) in CH_3CN (50 mL) was stirred at room temperature for 5 days. After concentrating under reduced pressure, the residue was flash chromatographed over silica gel. Elution with 2% MeOH–98% $CHCl_3$ gave 0.78 g (48%) of pure ester as an oil.

Method B. From DCC Coupling with Acid 9a. A solution of trityl alcohol **12** (1.6 g, 2.91 mmol), Boc-protected acid **9a** (1.04 g, 3.0 mmol), and 4-(dimethylamino)pyridine (0.71 g, 5.8 mmol) in CH_2Cl_2 (50 mL) was cooled in an ice bath under N_2 while a solution of dicyclohexylcarbodiimide (0.62 g, 3.0 mmol) in CH_2Cl_2 (15 mL) was added over 15 min. After addition was complete, the reaction mixture was stirred at ice-bath temperature for 30 min and then at room temperature overnight. $CHCl_3$ and 10%

citric acid were added. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel and elution with 5% MeOH–95% $CHCl_3$ gave a quantitative yield of protected 3′-ester **13a** identical (^1H NMR and TLC) with that from method A.

5-Bromo-2′-deoxyuridine 3′-[*N*-(1,1-Dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-ethylamino]ethyl]glycyl ester] (14a). Method A. From Deprotection of 13a. A solution of trityl derivative **13a** (0.78 g, 0.90 mmol) in 80% HOAc–20% H_2O (30 mL) was stirred at 60 °C for 90 min and then at room temperature for 4 h. After concentrating under reduced pressure, the residue was partitioned between water and $CHCl_3$. The organic extract was dried (Na_2SO_4), filtered, and concentrated. Flash chromatography of the residue over silica gel and elution with 5% MeOH–95% $CHCl_3$ gave 0.21 g (38%) of product as a waxy solid.

Method B. From Direct Esterification of 5-Bromo-2′-deoxyuridine. A solution of succinate ester **10a** (723 mg, 1.63 mmol), (+)-5-bromo-2′-deoxyuridine (500 mg, 1.63 mmol), and 4-(dimethylamino)pyridine (199 mg, 1.63 mmol) in CH_3CN (30 mL) and DMF (5 mL) was stirred at room temperature for 5 days. After concentrating under reduced pressure, the residue was flash chromatographed over silica gel. Elution with 2% MeOH–98% $CHCl_3$ gave 0.17 g (17%) of the 3′-ester identical with that of method A by TLC and ^1H NMR.

5-Bromo-2′-deoxyuridine 3′-[*N*-[2-(Ethylamino)ethyl]glycyl ester] Dihydrochloride (15a). A solution of the di-Boc ester **14a** (0.21 g, 0.34 mmol) obtained from method A in EtOAc (25 mL) was cooled in an ice bath and saturated with anhydrous HCl for 5 min. After stirring in the ice bath for 30 min and then at room temperature for 30 min, solvent was removed under reduced pressure. Recrystallization of the residue from MeOH-EtOAc-hexane gave 0.13 g (76%) of **15a**, mp 208–209 °C darken at 201 °C.

Deprotection of the di-Boc ester obtained from method B by this same procedure provided a product with the same melting point, mixed melting point, and ^1H NMR. Anal. ($C_{15}H_{23}BrN_4O_6{\cdot}2HCl$) C, H, N.

5-Bromo-5′-trityl-2′-deoxyuridine 3′-[*N*-(1,1-Dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-(2-cyclohexylethyl)amino]ethyl]glycyl ester] (13b). A solution of succinate ester **10b** (0.89 g, 1.69 mmol), 5-bromo-5′-trityl-2′-deoxyuridine **12** (1.0 g, 1.69 mmol), and 4-(dimethylamino)pyridine (0.21 g, 1.69 mmol) in CH_3CN (50 mL) was stirred at room temperature for 2 days. Additional hydroxysuccinate ester (0.45 g) was added to the reaction and stirring was continued for 20 h more. After concentrating under reduced pressure, the residue was flash chromatographed over silica gel and product (1.3 g, 80%) eluted with 2% MeOH–98% $CHCl_3$.

5-Bromo-2′-deoxyuridine 3′-[*N*-(1,1-Dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-(2-cyclohexylethyl)amino]ethyl]glycyl ester] (14b). A solution of the trityl derivative **13b** (1.3 g, 1.35 mmol) in 80% HOAc–20% H_2O (80 mL) was stirred at 60 °C for 2 h. After concentrating under reduced pressure, the residue was partitioned between CH_2Cl_2 and H_2O, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel and elution with 3% MeOH–97% $CHCl_3$ gave 0.50 g (61%) of **14b**.

5-Bromo-2′-deoxyuridine 3′-[*N*-[2-[(2-Cyclohexylethyl)amino]ethyl]glycyl ester] Dihydrochloride Hydrate (15b). A solution of the Boc-protected ester **14b** (0.5 g, 0.82 mmol) in EtOAc (20 mL) was cooled in an ice bath and saturated with HCl gas for 5 min. After stirring at ice-bath temperature for 15 min and then at room temperature for 30 min, solvent was removed under reduced pressure and the residue was recrystallized from MeOH–EtOAc–hexane to give 0.31 g (62%) of the 3′-ester, mp 142–152 °C dec. Anal. ($C_{21}H_{33}BrN_4O_6{\cdot}2HCl{\cdot}H_2O$) C, H, N.

5-Bromo-5′-[(isobutyloxy)carbonyl]-2′-deoxyuridine (17). Isobutyl chloroformate (2.32 g, 17 mmol) was added over 3 min to a stirred solution of (+)-5-bromo-2′-deoxyuridine (5.0 g, 16.3 mmol) in pyridine (100 mL) cooled in an ice bath. After stirring at room temperature for 18 h, solvent was removed under reduced pressure and the residue was flash chromatographed over silica gel. Elution with 5% MeOH–95% $CHCl_3$ gave 4.3 g (62%) of product. An analytical sample (mp 119–121 °C) was obtained by recrystallization from EtOAc–hexane: ^1H NMR ($CDCl_3$) δ = 0.97 (6 H, d), 2.0 (1 H, m), 2.2 (1 H, m), 2.48 (1 H, m), 3.98 (2 H, d), 4.19 (1 H, q), 4.42 (2 H, t), 4.5 (1 H, m), 6.31 (1 H, t), 7.94 (1 H, s). Anal. ($C_{14}H_{14}BrN_2O_7$) C, H, N.

5-Bromo-3′-(4-methoxytrityl)-2′-deoxyuridine (18). A solution of 5-bromo-5′-(isobutylcarbonyl)-2′-deoxyuridine (4.2 g, 10.3 mmol) and (4-methoxyphenyl)diphenylmethyl chloride (3.4 g, 11 mmol) in pyridine (75 mL) was stirred at 100 °C for 3 h. Solvent was removed under reduced pressure and the residue was partitioned between EtOAc and water. The EtOAc extract was washed with brine, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel and elution with 20% EtOAc–80% *n*-butyl chloride gave 4.7 g (67%) of the 5′-protected methoxytrityl derivative.

This compound (4.7 g, 6.92 mmol) in dioxane (35 mL) containing water (8 mL) and 1 N NaOH (27.6 mL) was stirred at room temperature for 90 min. Citric acid was added to adjust the pH to 7.5 and the product was extracted with two portions of CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried (Na_2SO_4), filtered, and concentrated. The residue was flash chromatographed over silica gel. Elution with 2% MeOH–98% $CHCl_3$ gave 2.6 g of product: ^1H NMR ($CDCl_3$) δ = 3.29 (1H, d of d), 3.68 (1 H, d of d), 3.8 (3 H, s), 3.93 (1 H, m), 4.38 (1 H, m), 5.29 (1 H, s), 6.22 (1 H, d of d), 6.48 (2 H, d), 7.2–7.5 (12 H, m), 8.02 (1 H, s), 8.26 (1 H, br s).

5-Bromo-3′-(4-methoxytrityl)-2′-deoxyuridine 5′-[*N*-(1,1-Dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-(2-cyclohexylethyl)amino]ethyl]glycyl ester] (19b). A solution of methoxytrityl-protected alcohol **18** (1.9 g, 3.20 mmol), di-Boc acid **9b** (1.37 g, 3.20 mmol), and 4-(dimethylamino)pyridine (0.78 g, 6.40 mmol) in CH_2Cl_2 (50 mL) was cooled in an ice bath under N_2 while a solution of dicyclohexylcarbodiimide (0.66 g, 3.20 mmol) in CH_2Cl_2 (15 mL) was added over 20 min. After addition was complete, the reaction was stirred at ice bath temperature for 30 min and then at room temperature overnight. $CHCl_3$ and 10% aqueous citric acid were added. The organic layer was washed with saturated $NaHCO_3$ solution, brine, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel and elution with 2% MeOH–98% $CHCl_3$ gave a quantitative yield of the protected 5′-ester **19b**.

5-Bromo-2′-deoxyuridine 5′-[*N*-(1,1-Dimethylethoxycarbonyl)-*N*-[2-[*N*-(1,1-dimethylethoxycarbonyl)-*N*-(2-cyclohexylethyl)amino]ethyl]glycyl ester] (20b). Method A. From Deprotection of 19b. A solution of the methoxytrityl derivative **19b** (3.1 g, 3.23 mmol) in 80% HOAc–H_2O (100 mL) was stirred at 60 °C for 90 min. After concentrating under reduced pressure, the residue was partitioned between EtOAc and saturated $NaHCO_3$ solution. After washing the organic extract with brine, it was dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel and elution with 3% MeOH–97% $CHCl_3$ gave 1.3 g (66%) of **20b**.

Method B. From Displacement of Tosylate 16. To a solution of the di-Boc acid **9b** (0.86 g, 2.0 mmol) in DMF (5 mL) under N_2 was added 60% NaH (80 mg, 2.0 mmol). After stirring at 50 °C for 15 min, the NaH had all reacted. More DMF (10 mL) and tosylate **16** (0.92 g, 2.0 mmol) were added, and the reaction mixture was stirred at 80 °C for 20 h. DMF was then removed under reduced pressure and the residue was partitioned between EtOAc and brine. The organic extract was dried (Na_2SO_4), filtered, and concentrated. Flash chromatography of the residue over silica gel and elution with 5% MeOH–95% $CHCl_3$ gave 0.12 g (10%) of product identical with that obtained by method A by TLC and ^1H NMR.

5-Bromo-2′-deoxyuridine 5′-[*N*-[2-[(2-Cyclohexylethyl)amino]ethyl]glycyl ester] Dihydrochloride (21b). A solution of **20b** (1.3 g, 2.14 mmol) in EtOAc (25 mL) was cooled in an ice bath and saturated with HCl gas for 8 min. After stirring in the ice bath for 15 min and then at room temperature for 30 min, solvent was removed under reduced pressure and the residue recrystallized from MeOH–EtOAc to give 0.94 g (75%) of 5′-ester **21b**. Anal. ($C_{21}H_{33}BrN_4O_6{\cdot}2HCl$) C, H, N.

4-(Carbobenzyloxy)-1-(2-cyclohexylethyl)-2-piperazinone (26). Solid *p*-toluenesulfonyl chloride (3.81 g, 20 mmol) was added to a cooled solution of 2-cyclohexylethanol (2.56 g, 20 mmol) in pyridine (25 mL). After stirring at ice-bath temperature for 30 min and then at room temperature for 3 h, the reaction mixture was poured onto ice and extracted with EtOAc. The organic layer

was washed with 1 N HCl, brine, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and concentrated to yield 4.01 g (71%) of the tosylate.

This tosylate (2.82 g, 10 mmol) was added to a solution of 4-(carbobenzyloxy)-2-piperazinone[9] **25** (2.33 g, 10 mmol) and 60% NaH (0.40 g, 10 mmol) in DMF (50 mL) under N_2, and the solution was stirred at room temperature overnight. After concentrating under reduced pressure, the residue was partitioned between water and EtOAc. The EtOAc extract was washed with brine, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel and elution with 1% MeOH–99% $CHCl_3$ gave 2.83 g (82%) of cyclohexylethyl derivative **26**: ^1H NMR ($CDCl_3$) δ = 0.95 (2 H, m), 1.22 (4 H, m), 1.45 (2 H, m), 1.7 (6 H, m), 3.33 (2 H, m), 3.41 (2 H, m), 3.7 (2 H, m), 4.13 (2 H, s), 5.16 (2 H, s), 7.35 (5 H, s).

1-(2-Cyclohexylethyl)-2-piperazinone Hydrochloride (22). A solution of CBZ derivative **26** (2.8 g, 8.13 mmol) in absolute EtOH (150 mL) was hydrogenated over a 5% Pd on C catalyst in a Paar apparatus at 40 psi and room temperature for 2 h. After filtering through a pad of diatomaceous earth, the filtrate was concentrated under reduced pressure and the residue was flash chromatographed over silica gel. Elution with 5% MeOH–95% $CHCl_3$ gave 0.65 g of pure deblocked piperazinone **23** as an oil. Conversion to the HCl salt with anhydrous HCl in EtOH and recrystallization from MeOH–EtOAc–hexane gave 0.65 g (33%) of the HCl salt, mp 180–183 °C dec. Anal. ($C_{12}H_{22}N_2O{\cdot}HCl$) C, H, N.

Reaction of 21b at pH 7.4. A solution of the 5′-[*N*-[2-[(2-cyclohexylethyl)amino]ethyl]glycyl ester] **21b** (59 mg) in pH 7.4 phosphate buffer (30 mL) was stirred at 37 °C for 7 h. Some 10% NaOH solution was added to maintain a pH of 7.4. HPLC analysis showed that all of the ester had been converted to 5-bromo-2′-deoxyuridine by comparison with an authentic sample. After lyophilization, the residue was taken up in EtOAc, washed with saturated $NaHCO_3$ solution and brine, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel and elution with 5% MeOH–95% $CHCl_3$ gave 20 mg (95.2%) of 1-(2-cyclohexylethyl)-2-piperazinone (**22**) by TLC and ^1H NMR comparison with an authentic sample. Conversion to the HCl salt (mp 180–183 °C) further confirmed the structure by mixed melting point with an authentic sample.

Determination of Ester Half-Lives in Buffer. Buffer solution (2.0 mL) preheated to 37 °C was added quickly to approximately 0.5 mg of the ester to give a final concentration of 1 mM. The resulting solution was heated at 37 °C while 20-mL samples were removed at intervals and injected directly into the HPLC injection port. In some cases, 0.10-mL aliquots were quenched in 1 N HCl (1.8 mL) prior to injection. Unreacted ester and 5-bromo-2′-deoxyuridine concentrations were determined by HPLC analysis with a C-18 reverse-phase column using a gradient of 98% pH 2.4 H_3PO_4–2% CH_3CN to 70% pH 2.4 H_3PO_4–30% CH_3CN over 10 min, flow = 3.0 mL/min. The esters are stable at the pH of the mobile phase, and therefore injection into the HPLC or quenching in acid effectively stops the reaction. The detector was set at 280 nm. The half-life is the time required for 50% conversion of ester to alcohol and was calculated by using first-order kinetics. The results in Table I are the average and standard deviation of at least two separate determinations.

Determinations of Ester Half-Lives in Plasma. A solution of the ester (5×10^{-4} M) in fresh plasma (1.6 mL) and pH 7.4 phosphate buffer (0.40 mL) was heated at 37 °C and the pH was maintained at 7.4 with a Radiometer Copenhagen pH STAT. At various intervals, aliquots (25 μL) were removed, quenched in 7% $HClO_4$ (0.20 mL) to stop the reaction, and shaken. After centrifugation (14000*g*, 8 min) clear supernatent was pipetted from the insoluble pellet and analyzed by the same HPLC method used in the buffer reactions. The results in Table I are the average and standard deviation of at least two separate determinations.

Determination of Partition Coefficients. Approximately 0.5 mg of ester was dissolved in pH 7.4 buffer (1 mL) and quickly added to a rapidly stirred mixture of *n*-octanol (5 mL) and pH 7.4 buffer (4 mL) at 23 °C. Aliquots of each layer were removed every 2 min and frozen in dry ice–acetone until analyzed by the HPLC method described above. Although the esters are not stable under these conditions, the amount of ester present in each phase can be used to calculate an approximate partition coefficient. Calculated values became constant after 5 min of mixing.

Acknowledgment. We are indebted to J. Moreau for elemental analyses, S. Pitzenberger, J. Murphy, and S. Varga for ^1H NMR spectra, D. Frankenfield for rat plasma, and R. Gould for human plasma. We also thank W. Randall for helpful advice on determining half-lives of the esters and V. Finley for preparation of the manuscript.

(9) Tomatis, R.; Solvadori, S.; Sarto, G. P. *Eur. J. Med. Chem.* 1981, *16*, 229.