Synthesis and Reactivity of 5-Fluorouracil/Cytarabine Mutual Prodrugs

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Two mutual prodrugs, in which two different anti-cancer drugs are attached to the same molecule *via* labile linkages, are synthesized and examined kinetically. One of the mutual prodrugs loses a drug component under physiological conditions within an hour, but the other mutual prodrug (having a longer spacer between the two drugs) is stable to chemical degradation even at higher pH values. Thus, enzymatic hydrolysis alone will release the two anti-cancer drugs. The potential value of anti-cancer mutual prodrugs is discussed.

Introduction

During the course of his studies on the selective toxicity of chemotherapeutic agents in the late 1950s, Professor Adrien Albert, an Australian medicinal chemist, wrote the following: "Sometimes the substance, as administered, is only a 'prodrug' which has to be broken down to give the true drug. Examples of this kind are phenacetin, chloral hydrate, pentavalent arsenicals, and two of the antimalarials; pamaquin and proguanil. But this seems to be a rare phenomenon, and it appears that most substances act in the form in which they are given".[1] Albert's prodrug concept was later deliberately incorporated into drug design, and prodrugs are now commonplace.

A prodrug is defined as a pharmacologically inactive compound that is converted into an active drug by a metabolic biotransformation.[2] For example, a drug bearing a hydroxyl group might be deactivated by acetylation. After administration to a patient, the ester group is hydrolyzed *in vivo* either by spontaneous chemical hydrolysis or by the action of esterases which are prevalent in cells (eq 1). In this manner the drug is regenerated.

$$\underset{\substack{\text{prodrug}\\(\text{inactive})}}{\text{drug-OH} \rightarrow \text{drug-OAc}} \xrightarrow{\textit{in vivo}} \text{drug-OH} \qquad (1)$$

Enzymatic activation is preferred over chemical activation because the latter often involves inherently unstable compounds. Prodrugs can be used to overcome many problems including poor solubility, absorption, and patient acceptability as well as instability and toxicity. Prodrugs can also lead to prolonged release. Often prodrugs are classified into two types, namely those with a removable attachment ("carrier-linked") and those that are "bioprecursors", which are metabolically modified into active compounds.[3] The work herein concerns only the former type.

A mutual prodrug consists of two different synergistic drugs joined together. The two drugs may be connected directly or by means of a linker. Ordinarily, when two synergistic drugs are administered individually but simultaneously, they will be transported to the site of action with different efficiencies. When it is desirable to have the two drugs reach a site simultaneously, the mutual prodrug strategy may be used to advantage provided: "(a) the mutual prodrug is well absorbed; (b) both components are released concomitantly and quantitatively after absorption; (c) the maximal effect of the combination of the two drugs occurs at a 1:1 ratio; and (d) the distribution and elimination of the two components are similar".[4] A review of the subject appeared in 1994.[5] The concept of a mutual prodrug is, clearly, well established.

The work described herein involves a mutual prodrug composed of two different anti-cancer drugs. A cancer that is not totally eliminated by administration of a single drug will, sooner or later, become resistant to that drug. Administration of two drugs enhances the chance of successful therapy because the likelihood of resistance developing to two drugs is much less than to a single drug.[6] This has given rise to a large number of therapeutic regimes involving drug combinations. Two drugs can be administered either sequentially or simultaneously. In a sequential treatment, there still exists a possibility that cancer cells become resistant to the first drug prior to being exposed to the second. This population of resistant cells, albeit reduced in size from the original burden, can in turn become resistant to the second drug as well. Considerable guesswork is involved in the timing with which a patient receives the two drugs so as to minimize the chance of double-resistance.

Alternatively, the two drugs can be given simultaneously. Although this would seem to reduce the probability of double-resistance, there are also problems with the approach. Toxicity is, for example, a concern. If dosages are reduced to compensate for toxicity, then this could defeat the benefit of simultaneous administration. Even if dosages remain unaltered, one cannot be certain that simultaneous drug administration will kill 100% of the cells. A cell in the center of a poorly vascularized solid tumor might, for example, have a low probability of receiving one "hit", much less two. A resistance would develop, therefore, to a single drug prior to a cell's exposure to a lethal dose of the second. In effect, the tumor cells impose a time lag despite the simultaneous

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(1) Albert, A. *Nature* **1958**, *182*, 421−423.
(2) Silverman, Richard B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: San Diego, 1992; p 352.
(3) Silverman, Richard B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: San Diego, 1992; pp 352−355.
(4) Silverman, Richard B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: San Diego, 1992; p 377.
(5) Singh, G.; Dev Sharma, P. *Indian J. Pharm. Sci.* **1993**, *56(3)*, 69−79.
(6) Marx, J. L. *Science* **1986**, *234*, 818−820.

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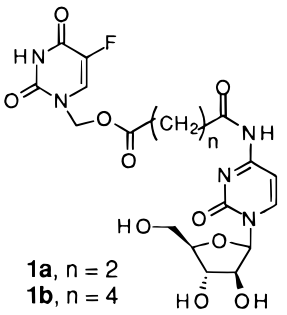
bulk administration of two different drugs. An enhanced chance of double-resistance by the cancer could also arise if there were a cross-resistance as would occur when a hit by one drug impairs the entry of the second into the cell.

Now consider a mutual prodrug. Entry of a mutual prodrug into a cancer cell necessarily exposes the cell to the ravishes of two drugs simultaneously. Double-resistance would be unlikely unless a lethal dose requires the entry of multiple drug molecules that can occur, under the given dosages, only over the course of a long time period. Note that the cytotoxicity of a mutual prodrug might exceed that provided by the corresponding two drugs administered sequentially or simultaneously. Thus, it is conceivable that a cancer cell is killed by a dual hit from a mutual prodrug while surviving a hit from one drug and then a hit by a second drug, say, 10 h later. By way of analogy, a person might succomb to a simultaneous attack of malaria and flu while surviving the two diseases when spaced 10 months apart.

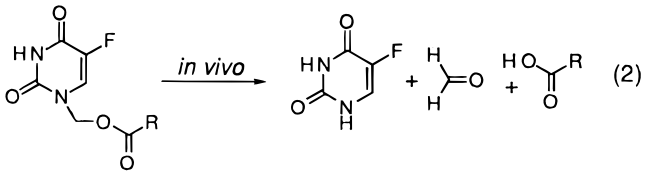
The simplistic nature of the above analysis should be appreciated. In actual fact, the lethality of drug therapy depends on numerous factors which are, for the most part, unknown: (a) the number of molecules per dose; (b) the number of cells per tumor; (c) the percent of utilizable drug molecules that survive metabolism, excretion, long-term storage, and entry into healthy cells; (d) the number of drug molecules required within a cancer cell to kill it; (e) the rate of resistance development. This is a complicated business best handled by making reasonable assumptions and by applying Poisson statistics to assess the percentage of cells receiving a lethal hit (an exercise in which we are now engaged). For the moment, however, it suffices to presume, without a firm basis, that anti-cancer mutual prodrugs are worthwhile candidates for study.

Two potential anti-cancer mutual prodrugs (**1a**, n = 2; **1b**, n = 4) synthesized in our laboratory differ only in the length of the spacer. They are seen to possess two



1a, n = 2
1b, n = 4

interconnected cytotoxic drugs, 5-fluorouracil (5-FU) and cytarabine (ara-C). 5-FU has been widely used with solid tumors including breast cancer, whereas ara-C has been used mainly in the treatment of acute leukemia and lymphomas. The cytarabine moiety is linked to the double prodrug through a hydrolyzable amide group. It is known, incidentally, that attaching an acyl group to the ara-C primary amine group decreases the drug's susceptibility to deactivation by cytidine deaminase;[7] the amide group therefore serves two functions. The 5-FU moiety is attached to the double prodrug *via* an (acyloxy)-methylene group known, from the work of others,[8] to be readily removable (eq 2).



(2)

Synthesis

The synthesis of **1a**,**b** is shown in Scheme 1. Several factors made the synthesis far more difficult than is apparent in the figures: (a) Attachment of the 5-FU moiety in **5a,b** created labile N−O ketal derivatives. These functionalities were sensitive to base (which would saponify the ester) and even to warm alcohol which can destroy the functionalities *via* transesterification. (b) All reactions subsequent to **5a,b** necessitated mild conditions and chemoselective reagents owing to the delicacy of the multifunctional compounds. Thus, use of heat, strong oxidizing or reducing agents, and harsh reagents (such as thionyl chloride) caused either yield-reducing side reactions or outright decomposition. This limitation greatly reduced the pool of usable reagents. (c) The polarity imparted by both the multiple heteroatoms required the use of highly polar solvents (DMF, DMSO, and glacial acetic acid) for the later reactions. To counter this problem, the synthetic intermediates were, with each step, deliberately imparted with alternating polarity (compare, for example, **4** and **5**, **5** and **6**, **6** and **7**, and **7** and **8**). This greatly facilitated purification by standard flash chromatography. Despite these problems, relatively simple syntheses were developed. Thus, with the exception of the first two steps in either synthesis, all transformations were carried out simply by stirring the reactants at room temperature. Yields ranged from moderate to excellent.

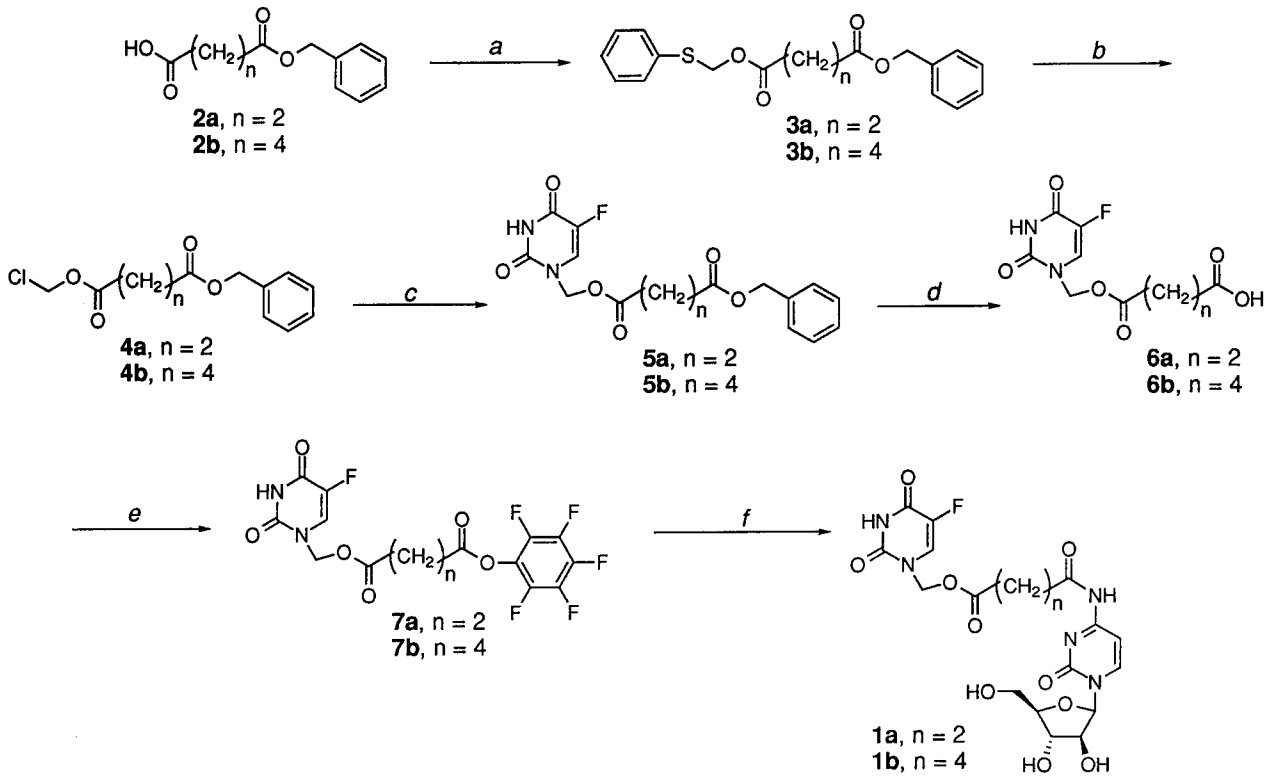
Formation of the desired phenylsulfanyl methyl ester **3a,b** proceeded well, and treatment of this compound with sulfuryl chloride, SO_2Cl_2, generated the desired chloromethyl ester (**4a,b**) plus the benzenesulfenyl chloride byproduct (**8**). Since **8** is highly reactive, it could not be carried through and had to be removed. This is normally accomplished by trapping with cyclohexene, to which **8** rapidly adds. The products are then normally separated by vacuum distillation. In our case, however, product **4a,b** and the cyclohexene addition product boiled at similar temperatures, so that separation by vacuum distillation was not possible. In order to circumvent this difficulty, we used higher boiling trapping agents for the benzenesulfenyl chloride (including *trans*-stilbene and squalene). This approach would have succeeded were it not for the fact that product yields suffered from thermal degradation during the high temperatures required for vacuum distillation of **4a,b**. A milder method of purification was needed.

We reasoned that if the trapping agent for **8** contained a double bond plus a water-solublizing group, then the addition product could be removed by an aqueous wash. A number of such trapping agents were tested (including *trans*-cinnamic acid and 3-cyclohexenecarboxylic acid),

(7) Tsuro, T.; Iida, H.; Hori, K.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1981**, *41*, 4484−4488.

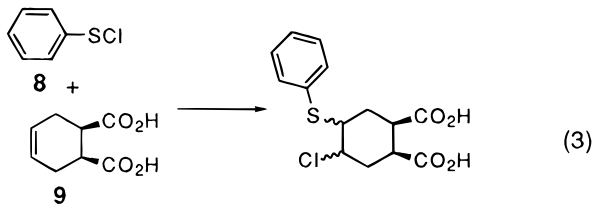
(8) (a) Ozaki, S.; Watanabe, Y.; Hoshiko, T.; Mizuno, H.; Ishikawa, K.; Mori, H. *Chem. Pharm. Bull.* **1984**, *32*, 733−738. (b) Mollgaard, B.; Hoelgaard, A.; Bundgaard, H. *Int. J. Pharm.* **1982**, *12*, 153−162. (c) Johansen, M.; Bundgaard, H.; Falch, E. *Int. J. Pharm.* **1983** *13*, 89−98.

Scheme 1[a]



[a]Reagents: (a) Cs_2CO_3, $PhSCH_2Cl$, DMF; (b) SO_2Cl_2, *cis*-4-cyclohexene-1,2-dicarboxylic acid, CH_2Cl_2; (c) 5-FU, Et_3N, DMF; (d) 10% Pd/C, glacial AcOH, 1,4-cyclohexadiene; (e) EDCI·HCl, pentafluorophenol, DMF; (f) ara-C, DMF.

but *cis*-4-cyclohexene-1,2-dicarboxylic acid (**9**) was found to be by far the most useful (eq 3). This procedure is recommended as an attractive variation on the published theme.[9]



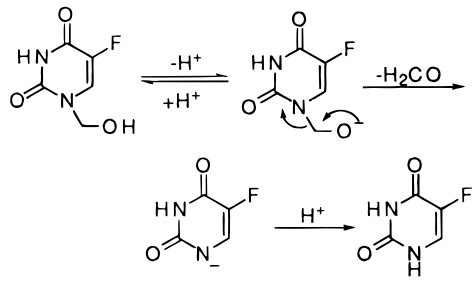
(3)

Attachment of 5-FU to **4a,b** in Scheme 1 according to a literature procedure[8a] proceeded as described. Only one product, **5a,b**, was obtained; there was no sign of the N^3 alkylation product. Catalytic transfer hydrogenation (CTH) debenzylation of **5a,b** in glacial acetic acid, using 1,4-cyclohexadiene as the hydrogen source,[10] gave **6a,b** in high yield. Esterification of **6a,b** with pentafluorophenol using a water-soluble carbodiimide, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl), gave activated ester **7a,b**. Immediate reaction of **7a,b** with free-base ara-C in DMF afforded the desired **1a,b**.[11]

Kinetics

Chemical stability of prodrugs at physiological pH values is of the essence. In its absence, the prodrug will revert to the active drug prematurely. It became necessary, therefore, to examine the hydrolysis kinetics of **1a** and **1b**. Note that these mutual prodrugs possess two labile groups connecting the drugs to the spacer. The questions thus arise as to which of the two drugs cleaves off first and how soon thereafter the second drug is freed. In order to answer such questions of reactivity, we first studied models of the mutual prodrugs in which each side could be examined without interference from the other.

With regard to the reactivity of the 5FU portion of the mutual prodrugs, mention should be made of the work from the Bundgaard group[8b,c] who measured the reactivity of similar 5-FU prodrugs. It was found that the observed rates of hydrolysis of N^1- or N^3-(hydroxylmethyl) 5-FU in the pH range 2−10 are directly proportional to the hydroxide ion activity. Thus plots of log k_{obs} *vs* pH were linear with a slope of 1.0 which is consistent with the mechanism in eq 4. Bundgaard used two methods



(4)

to determine the rate of hydrolysis of the formaldehyde-releasing 5-FU prodrugs: (a) direct measurement of the absorbance decrease at 245 nm and (b) colorimetric measurement of the formaldehyde concentration using

(9) Benneche, T.; Strande, P.; Wiggen, U. *Acta Chem. Scand.* **1988**, *43*, 74−77.
(10) Felix, A.M.; Heimer, E.P.; Lambros, T.J.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1978**, *43*, 4194−4196.
(11) Balajthy, Z.; Aradi, J.; Ildiko, T. K.; Elödi, P. *J. Med. Chem.* **1992**, *35*, 3344−3349.

Table 1. Rate Constants for Hydrolysis of Ester in 10 at Various pH Values[a]

pH	k_{obs} (min^{-1})	$t_{1/2}$ (min)
4[b]	0.002	300
4.63[b]	0.009	75
5.0[b]	0.02	30
6.0[c]	0.2	3

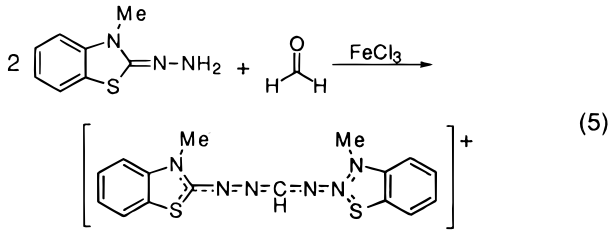
[a] 5×10^{-5} M substrate, 25 ± 1 °C, as determined by decrease in absorbance at 246 nm. [b] 0.1 M sodium acetate buffer. [c] 0.05 M potassium dihydrogen phosphate buffer.

Table 2. Rate Constants for Hydrolysis of Ester in 1a at Various pH Values[a]

pH	k_{obs} (min-1)	$t_{1/2}$ (min)
6.0[b]	0.005	~130
6.5[b]	0.016	~400
7.0[b]	0.03	~20
7.4[c]	0.073	~9

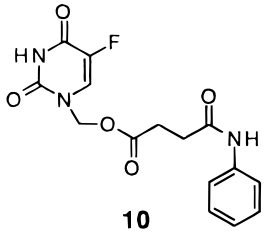
[a] 2×10^{-5} M substrate, 25 ± 1 °C, as determined by the colorimetric method. [b] 0.05 M potassium dihydrogen phosphate buffer. [c] 0.05 m sodium dihydrogen phosphate buffer.

a modification of the Sawicki, *et al.* method.[12] The method is based on the condensation of formaldehyde with 3-methyl-2-benzothiazolone hydrazone to form a blue, cationic dye (eq 5), which can be determined spectrophotometrically.



(5)

Following the results of Bundgaard, we synthesized a mutual prodrug analog with aniline replacing the ara-C unit (**10**). Rate constants for hydrolysis were obtained by monitoring the absorbance decrease at 246 nm in acidic buffers ranging from pH 4.0 to 6.0. The data, given in Table 1, give a linear log k_{obs} *vs* pH profile with a slope of unity. Above pH 6.0 the rates were too fast to measure by conventional means.



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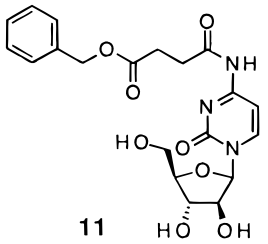
Cleavage of 5-FU from mutual prodrug **1a** was followed using the colorimetric assay for formaldehyde. The data are summarized in Table 2. It is seen that the hydrolysis rates are not as fast as with **10** (*e.g.* the half-lives at pH 6.0 for **1a** and **10** being 130 and 3 min, respectively). Nonetheless, the half-life for **1a** at a physiological pH of 7.4 is about 9 min, which is dangerously close to rendering the compound clinically useless. Fortunately, **1b** proved to be inert from pH 4.0 to 7.4 in sharp contrast to **1a**. Even at a high pH of 10.0, the mutual prodrug **1b** cleaves with a rate constant of only 3.3×10^{-3} min^{-1}, corresponding to a half-life of 210 min. From a clinical standpoint, this chemical stability is excellent.

The large reactivity difference between **1a** and **1b** led us to suspect that cleavage of 5-FU in **1a** accompanied by formaldehyde production was subject to intramolecular catalysis by the neighboring amide functionality. Apparently, the amide of **1b** is too distant to effect any anchimeric assistance. If this is true, then an ester-containing analog of **10**, intermediate **5a**, should be relatively inert, and indeed such was found to be the case. No hydrolysis was observed with **5a** at pH 8.0 after 1 h as judged by UV spectroscopy. We conclude, therefore, that intramolecular catalysis in **1a** and **10** by their amide groups greatly accelerates the 5-FU ejection. Intramolecular amide-catalyzed hydrolyses are well-known.[13]

Having disposed of the 5-FU end of things, we began looking at the ara-C side. Again, release of the drug was first examined with a model compound, **11**. No activity



11

was observed in pH 7.0 or pH 8.0 buffers at 25 °C as judged spectrophotometrically at 297 nm. Amide hydrolysis was forced upon the molecule only by subjecting it to pH = 10.0 buffer at 25 °C for several days; the corresponding rate constant is 1.77×10^{-3} min^{-1} or a half-life of about 400 min. Identical rate constants were obtained for **1a** and **1b**. As expected and hoped for, ara-C release *via* amide hydrolysis is inherently so slow that a cellular enzyme is required to effect the reaction.

In summary, mutual prodrugs **1a** and **1b** were found to have quite different reactivities. Prodrug **1a** is labile on its 5-FU side, but prodrug **1b** is chemically stable at the attachment sites of both 5-FU and ara-C. Biological testing with the compounds is now underway.

Experimental Section

Preparation of Compounds 3. General Procedure. Aliphatic diacid monoester **2** (33 mmol) and cesium carbonate (33 mmol) were combined in 50 mL of DMF. The resultant suspension was stirred for 30 min at room temperature and then for 15 min in a 70 °C oil bath. After being cooled to room temperature, the suspension was stirred in an ice bath for 30 min, and chloromethyl phenyl sulfide (30 mmol) was then added *via* syringe. The mixture stirred for 3 h at room temperature and at 70 °C for 15 min. The mixture was cooled to room temperature, and 100 mL H_2O was added. After 15 min of stirring, the suspended cesium carbonate went into solution. The DMF/H_2O mixture was then extracted with diethyl ether (4 × 50 mL). The ether extracts were washed with 1 M NaOH (1 × 50 mL) and brine (4 × 50 mL) and dried over $MgSO_4$. The ether was reduced *in vacuo* to give a clear oil which was further purified (flash chromatography, 20% ethyl acetate/petroleum ether) to afford the desired product as a clear oil.

Succinic acid benzyl ester (phenylsulfanyl)methyl ester (3a): 80% yield; IR (neat) 3460, 3058, 3032, 2951, 1738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.69 (s, 4H), 5.12 (s, 2H), 5.41 (s, 2H), 7.29 (m, 10 H); ^{13}C NMR ($CDCL_3$) δ 29.00, 29.27, 66.61, 68.41, 127.41, 128.24, 128.29, 128.57, 129.14, 130.40, 134.63,

(12) Sawicki, E.; Hauser, T. R.; Stanley, T. W.; Elbert, W. *Anal. Chem.* **1961**, *33*, 93−96.

(13) Cohen, T.; Lipowitz, J. *J. Am. Chem. Soc.* **1961**, *83*, 4866−4877.

135.72, 171.56, 171.83. Anal. Calcd for $C_{18}H_{18}O_4S$ (330.33): C, 65.44; H, 5.49; S, 9.71. Found: C, 65.35; H, 5.51; S, 9.79.

Hexanedioic acid benzyl ester (phenylsulfanyl)methyl ester (3b): 94% yield; IR (neat) 3465, 3065, 3038, 2931, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.66 (m, 4H), 2.35 (t, J = 6.6 Hz, 4H), 5.09 (s, 2H), 5.39 (s, 2H), 7.33 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 24.32, 24.36, 33.96, 34.04, 66.34, 68.09, 127.49, 128.34, 128.69, 129.24, 130.49, 134.80, 136.10, 172.66, 173.14. Anal. Calcd for $C_{20}H_{22}O_4S$ (358.46): C, 67.02; H, 6.19; S, 8.93. Found: C, 66.89; H, 6.26; S, 8.80.

Preparation of Compounds 4. General Procedure. Diester **3** (20 mmol) was dissolved in 40 mL of CH_2Cl_2 in a three-neck round bottom flask equipped with an addition funnel and purged with argon. Sulfuryl chloride (24 mmol, 24 mL of a 1.0 M CH_2Cl_2 solution) was added to the addition funnel and slowly dropped into the reaction vessel. The mixture changed color, from clear to dark orange, upon addition of the sulfuryl chloride. After 1 h of stirring at room temperature, crystalline *cis*-4-cyclohexene-1,2-dicarboxylic acid (20 mmol) was added to the reaction mixture *via* powder funnel. The color changed again, after about 30 min of stirring, from dark orange back to the original clear. The mixture was stirred another 30 min, and a white precipitate formed. The precipitate was filtered and the solvent reduced *in vacuo* to produce an oily residue. The residue was dissolved in 50 mL of diethyl ether, and the ether solution was washed with 10% $NaHCO_3$ (1 × 50 mL) and H_2O (2 × 50 mL), dried over $MgSO_4$, filtered, and reduced *in vacuo* to afford **4** as a clear oil.

Succinic acid benzyl ester chloromethyl ester (4a): 90% yield; IR (neat) 1765, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.70 (s, 4H), 5.13 (s, 2H), 5.66 (s, 2H), 7.33 (s, 5H); ^{13}C NMR ($CDCl_3$) δ 28.77, 28.97, 66.73, 68.77, 128.27, 128.34, 128.57, 135.62, 170.44, 171.64. Anal. Calcd for $C_{12}H_{13}O_4Cl$ (256.69): C, 55.24; H, 5.15; Cl, 13.66. Found: C, 55.01; H, 5.12; Cl, 13.51.

Hexanedioic acid benzyl ester chloromethyl ester (4b): 98% yield; IR (neat) 1762, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.69 (m, 4H), 2.39 (m, 4H), 5.12 (s, 2H), 5.69 (s, 2H), 7.35 (s, 5H); ^{13}C NMR ($CDCl_3$) δ 24.07, 24.29, 33.72, 33.93, 66.40, 68.75, 128.30, 128.38, 128.72, 136.10, 171.39, 173.10. Anal. Calcd for $C_{14}H_{17}ClO_4$ (284.74): C, 59.14; H, 6.03; Cl, 12.31. Found: C, 59.03; H, 5.99; Cl, 12.48.

Preparation of Compounds 5. General Procedure. Into a 100 mL, three-neck flask equipped with stirring bar and addition funnel was placed 5-fluorouracil (8.11 mmol) and 10 mL of DMF. The mixture stirred for 10 min, and then a 3-fold excess of triethylamine (24.33 mmol) was added *via* syringe. The mixture stirred at room temperature for 30 min. Freshly prepared (less than 24 h old) **4** (8.11 mmol), dissolved in 10 mL of DMF, was added dropwise *via* addition funnel. A precipitate of triethylamine hydrochloride formed after about 1 h of stirring. The stirring continued overnight (16 h), and then the precipitate was filtered and the DMF removed by bulb-to-bulb distillation. The resultant crude solid gave **5** as a white solid after purification by flash chromatography (40% EtOAc/CH_2Cl_2, solid deposition on silica gel with THF).

Succinic acid benzyl ester (5-fluoro-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)methyl ester (5a): 76% yield; mp 125-127 °C; IR (KBr) 3432, 3178, 3072, 2832, 1758, 1711, 1658 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.71 (s, 4H), 5.12 (s, 2H), 5.63 (s, 2H), 7.34 (s, 5H), 7.56 (d, J = 5 Hz, 1H), 9.45 (br, 1H); ^{13}C NMR ($CDCl_3$) δ 28.42, 28.44, 65.67, 70.55, 127.87, 128.00, 128.40, 129.47 (d, J_{CCF} = 34 Hz), 135.9, 139.35 (d, J_{CF} = 229 Hz), 149.19, 157.38 (d, J_{CCF} = 26 Hz), 171.66, 171.72; ^{19}F NMR (qualitative, no internal standard, $CDCl_3$) δ 9.93 (d, J_{FH} = 5 Hz). Anal. Calcd for $C_{16}H_{15}FN_2O_6$ (350.30): C, 54.84; H, 4.32; F, 5.42; N, 8.00. Found: C, 55.05; H, 4.39; N, 7.82.

Hexanedioic acid benzyl ester (5-fluoro-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)methyl ester (5b): 63% yield; mp 98 °C; IR (KBr) 3405, 3172, 3038, 2838, 1751, 1711, 1658 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.67 (m, 4H), 2.36 (q, J = 7 Hz, 4H), 5.11 (s, 2H), 5.63 (s, 2H), 7.35 (s, 5H), 7.62 (d, J = 5 Hz, 1H), 9.73 (br, 1H): ^{13}C NMR ($CDCl_3$) δ 24.06, 24.24, 33.54, 33.85, 66.46, 69.86, 128.38, 128.40, 128.70 (d, J = 34 Hz), 128.73, 136.07, 140.36 (d, J = 239 Hz), 149.58, 157.22 (d, J = 27 Hz), 173.19, 173.54. Anal. Calcd for $C_{18}H_{19}FN_2O_6$ (378.36): C, 57.12; H, 5.06; F, 5.02; N, 7.41. Found: C, 57.19; H, 5.07; N, 7.35.

Preparation of Compounds 6. General Procedure. Into a 100 mL round bottom flask was added **5** (3 mmol) and 17 mL of acetic acid. Complete dissolution occurred after about 30 min of stirring at rt. To the solution were added 10% Pd/C (1.0 g) and 1,4-cyclohexadiene (15.8 mmol, 1.5 mL). Periodic monitoring by TLC indicated that the reaction was complete after 1 h. THF (25 mL) was added to the reaction mixture (to make it less viscous and easier to filter). The mixture was filtered first through a cotton plug and then through a 0.45 μm nylon membrane filter to remove the Pd/C. The THF was removed *in vacuo*, and then 50 mL of heptanes was added. The acetic acid was removed by bulb-to-bulb distillation as an acetic acid/heptanes azeotrope. The white solid which remained after distillation was **6**. **6** often contained residual acetic acid by ^1H NMR.

Succinic acid Mono[(5-fluoro-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)methyl] ester (6a): 99% yield; mp 153-156 °C; IR (KBr) 3432, 3045, 2936, 2852, 1745, 1695, 1661 cm^{-1} ^1H NMR (CD_3OD) δ 2.62 (s, 4H), 5.66 (s, 2H), 7.89 (d, J = 6 Hz, 1H), (DMSO-d_6) δ 2.32−2.61 (m, combined with solvent signal, > 4H), 5.51 (s, 2H), 8.02 (d, J = 6 Hz, 1 H), 11.99 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 28.79, 28.66, 70.45, 129.50 (d, J_{CCF} = 34 Hz), 139.42 (d, J_{CF} = 229 Hz), 149.26, 157.45 (J_{CCF} = 25 Hz), 172.09, 173.33. Anal. Calcd for $C_9H_9FN_2O_6$ (260.18): C, 41.55; H, 3.49; F, 7.30; N, 10.77. Found: C, 41.35; H, 3.80; N, 10.07.

Hexanedioic acid mono[(5-fluoro-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)methyl] ester (6b): 60% yield; mp 171−173 °C; IR (KBr) 3430, 3138, 3038, 2951, 2838, 1751, 1735, 1700, 1660 cm^{-1}; ^1H NMR (DMSO-d_6) (1.49 (t, J = 3.6 Hz, 4H), 2.18 (t, J = 6.4 Hz, 2H), 2.34 (t, J = 6.4 Hz, 2H), 5.55 (s, 2H), 8.11 (d, J = 6.6 Hz, 1H), 11.00 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 23.64, 23.78, 32.84, 33.25, 70.49, 129.43 (d, J_{CCF} = 34 Hz), 139.42 (d, J = 230 Hz), 149.23, 157.40 (d, J_{CCF} = 26 Hz), 172.45, 174.24. Anal. Calcd for $C_{11}H_{13}FN_2O_6$ (288.23): C, 45.82; H, 4.55; F, 6.59; N, 9.51. Found: C, 46.05; H, 4.66; N, 9.51.

Preparation of Compounds 7. General Procedure. In a 100 mL round bottom flask was dissolved 6 (2mmol) in 5 mL of DMF. Pentafluorophenol (2 mmol) was added followed by EDCI·HCl (2 mmol). The mixture stirred at room temperature for 2 h. The DMF was removed by bulb-to-bulb distillation, and the resulting residue was purified (flash chromatography, 40% EtOAc/CH_2Cl_2, solid deposition on silica gel) to give **7**. **7** must be used immediately as it decomposes on standing.

Succinic acid 5-(fluoro-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)methyl ester 2,3,4,5,6-pentafluorophenyl ester (7a): 43% yield; mp 130−131 °C; ^1H NMR ($CDCl_3$) δ 2.85 (m, 2H), 3.04 (m, 2H), 5.69 (s, 2H), 7.59 (d, J = 5 Hz, 1H), 8.76 (br, 1H).

Hexanedioic acid 5-(fluoro-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)methyl ester 2,3,4,5,6-pentafluorophenyl ester (7b): 77% yield; mp 118 °C; ^1H NMR ($CDCl_3$) δ 1.79 (m, 4H), 2.47 (t, J = 6.9 Hz, 2H), 2.70 (t, J = 6.9 Hz, 2H), 5.66 (s, 2H), 7.62 (d, J = 5 Hz, 1H), 9.39 (br, 1H).

***N*-(1-β-D-Arabinofuranosylcytosyl)succinamic Acid (5-Fluoro-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)methyl Ester (1a).** Ara-C (0.15 g, 0.62 mmol) and activated ester **7a** (0.46 g, 1.08 mmol) were combined in 2 mL of DMF. The mixure stirred at room temperature for 72 h. The DMF was removed by bulb-to-bulb distillation, and a clear viscous residue remained. The residue was dissolved in a minimum (2−3 mL) of methanol and then rapidly precipitated out of solution by addition of 500 mL of $CHCl_3$ (it is critical that contact with methanol be kept to a minimum). The precipitate was then immediately filtered through a 0.45 μm nylon membrane and dried in a vacumn dessicator at 60 °C. In order to remove persistent $CHCl_3$ after drying, the precipitate was dissolved in 2−3 mL of deionized H_2O, rapidly frozen solid, and lyophilized. This process was repeated twice to afford 284 mg of analytically pure **1a** (93%): mp 126 °C dec; IR (KBr) 3412 (br), 3078, 2931, 1721, 1654, 1565, 1491, 1391, 1314,

1131, 1050 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.61 (t, J = 5 Hz, 2H), 2.69 (t, J = 5 Hz, 2H), 3.61 (d, J = 4 Hz, 2H), 3.82 (s, 1H), 3.91 (s, 1H), 4.05 (s, 1H), 5.10 (br, 1H), 5.47 (br, 2H), 5.56 (s, 2H), 6.04 (d, J = 4 Hz, 1H), 7.12 (d, J = 4 Hz, 1H), 8.03 (d, J = 7 Hz, 1H), 8.08 (d, J = 6 Hz, 1H), 10.92 (s, 1H), 11.90 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 27.98, 31.05, 61.07, 71.83, 74.64, 76.20, 85.80, 87.05, 94.29, 129.51 (d, J_{CCF} = 34 Hz), 139.42 (J_{CF} = 229 Hz), 146.73, 149.30, 154.51, 157.50 (d, J_{CCF} = 25 Hz), 162.05, 172.05, 172.41; ^{19}F NMR (qualitative, no internal standard, DMSO-d_6) δ 9.93 (d, J_{FH} = 5 Hz). Anal. Calcd for $H_{18}H_{20}FN_5O_{10}\cdot H_2O$ (503.40): C, 42.95; H, 4.41; F, 3.77; N, 13.91. Found: C, 42.84; H, 4.38; N, 13.92.

5-[(1-β-D-Arabinofuranosylcytosyl)carbamoyl]-pentanoic Acid (5-Fluoro-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)methyl Ester (1b). Activated ester **7b** (0.44, 200 mg) and ara-C (0.44 mmol, 107 mg) were combined in 5 mL of DMF and stirred at room temperature. The reaction was monitored periodically by TLC (1:4 MeOH/$CHCl_3$). After 4 days, the DMF was removed by bulb-to-bulb distillation, and the residue was purifed (flash chromatograhy, solid deposition on silica with 1:1 THF/CH_3CN plus a few drops MeOH, gradient elution, 1:1 THF/$CHCl_3$ followed by 100% THF) to give 150 mg of **1b** (66% yield): mp 105 °C dec; IR (KBr) 3430, 3078, 2945, 1720, 1665, 1645, 1565, 1490, 1388, 1314, 1124 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.54 (m, 4H), 2.37 (m, 4H), 3.62 (m, 2H), 3.83 (m, 1H), 3.92 (m, 1H), 4.06 (m, 1H), 5.07 (br, 1H), 5.49 (d, J = 4.8 Hz, 2H), 5.63 (s, 2H), 6.05 (d, J = 3.9 Hz, 1H), 7.22 (d, J = 3 Hz, 1H), 8.05 (d, J = 7.5 Hz, 1H), 8.13 (d, J = 6.6 Hz, 1H), 10.84 (s, 1H), 11.97 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 23.59, 23.79, 32.84, 35.94, 61.05, 70.53, 74.63, 76.15, 85.79, 87.02, 94.32, 129.49 (d, J = 34 Hz), 139.47 (d, J = 229 Hz), 146.73, 149.27, 154.52, 157.44 (d, J = 26 Hz), 162.17, 172.48, 173.58. Anal. Calcd for $C_{20}H_{24}FN_5O_{10}\cdot H_2O$ (531.46): C, 45.20; H, 4.93; F, 3.57; N, 13.18. Found: C, 45.32; H, 4.88; N, 12.99.

***N*-Phenylsuccinamic Acid (5-Fluoro-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)methyl Ester (10).** Succinate half-acid **6a** (0.26 g, 1 mmol) was dissolved in 5 mL of DMF. Pentafluorophenol (0.184 g, 1 mmol) was added and allowed to dissolve, followed by EDCI·HCl (0.19 g, 1 mmol). The mixture stirred at room temperature for 2 h. Aniline (0.93 g, 1 mmol) was then added and the mixture stirred for ~16 h. The DMF was removed by bulb-to bulb distillation and the residue purified (flash chromatography, two separate columns, first eluant 90% EtOAc/hexanes, second eluant 1:1 acetone/chloroform) to give 200 mg of **10** (59% yield): mp 155 °C; IR (KBr) 3138, 3080, 2838, 1728, 1658,1257 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.62 (s, 4H), 5.58 (s, 2H), 7.00 (t, J = 8 Hz, 1H), 7.26 (t, J = 8 Hz, 2H), 7.54 (d, J = 8 Hz, 2H), 8.08 (d, J = 7 Hz, 1H), 9.98 (s, 1H), 11.97 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.53, 30.64, 70.43, 118.90 122.97, 128.64, 129.48 (d, J_{CCF} = 34 Hz), 139.13, 139.37 (d, J_{CF} = 26 Hz), 149.22, 157.38 (d, J_{CCF} = 26 Hz) 169.56, 172.19.

***N*-(1-β-D-Arabinofuranosylcytosyl)succinamic Acid Benzyl Ester (11). 2a** (1.04 g, 5 mmol) was dissolved in 10 mL of DMF. EDCI·HCl (0.96 g, 5 mmol) and pentafluorophenol (0.58 g, 5 mmol) were added, and the mixture was stirred overnight. The intermediate succinic acid benzyl ester 2,3,4,5,6-pentafluorophenyl ester was separated from the reaction mixture (silica gel plug, 25%EtOAc/petroleum ether), and a portion (0.61 g, 2 mmol) dissolved in 5 mL of DMF. Ara-C (0.243 g, 1 mmol) was added, and the mixture stirred was overnight. The DMF was removed *in vacuo* and the residue purified (flash chromatography, 3:1 tetrahydrofuran/chloroform) to afford 0.33 g of **11** (76% yield): mp 88−90 °C; IR (KBr) 3330 (br), 1724, 1644 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.67 (dt, J = 3, 13 Hz, 4H), 3.59 (dd, J = 5, 7 Hz, 2H), 3.81 (dd, J = 3, 5 Hz, 1H), 3.92 (d, J = 3 Hz, 1H), 4.04 (d, J = 5 Hz, 1H), 5.06 (s, 3H), 5.50 (t, J = 4 Hz, 2H), 6.05 (d, J = 4 Hz, 1H), 7.15 (d, J = 8 Hz, 1H), 7.33 (s, 5H), 8.05 (d, J = 8 Hz, 1H), 10.92 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 29.39, 32.49, 62.55, 67.21 76.27, 77.87, 87.20, 89.16, 96.72, 128.97, 129.04, 129.41, 137.38, 147.70, 157.49, 163.79, 173.61, 173.94. Anal. Calcd for $C_{20}H_{23}N_3O_8$ (433.42): C, 54.52; H, 5.35; N, 9.69. Found: C, 54.31; H, 5.44; N, 9.47.

Kinetics. Ultraviolet and visible spectral measurements were performed with a Varian DMS 200 spectrophotometer or a Varian DMS 300 spectrophotometer. Measurement of pH was carried out with a Corning pH meter 130 at the temperature of study. Aqueous buffers were either purchased from Fisher Scientific or made in our laboratory. Buffers were made in our laboratory by adjustment of a 0.1 M sodium acetate solution (pH = 3.7−5.6), 0.05 M sodium dihydrogen phosphate solution (pH = 6−8), or 0.025 M sodium carbonate/sodium bicarbonate solution (pH = 10) to the desired pH with 1 M NaOH. UV method. The UV method is defined as recording the absorbance changes over time for reactions in which the absorbance of substrate and product differ maximally at a particular wavelength. This method was used to study ara-C activation of **1a**, **1b**, and model **11**, as well as 5-FU activation of model **10**. Reactions were performed in 3 mL aliquots of buffer solution in a temperature-controlled quartz cuvette after initiation by addition of 50 μL of a substrate stock solution in acetonitrile. Reaction rates were determined for hydrolysis of the ara-C amide for both the mutual prodrugs (**1a,b**) and the model (**11**) by recording the the decrease in absorbance at 297 nm. Reaction rates were determined for 5-FU model **10** by recording the decrease in absorbance at 246 nm. Initial concentration of all substrates studied by the UV method was 5×10^{-5} M. Psuedo-first-order rate constants were calculated from plots of ln ($Abs_t - Abs_\infty$) *vs* time, where Abs_∞ and Abs_t are the absorbance at infinity and at time, t, respectively. A pH measurement of the reaction buffer solution was taken after each reaction had reached completion, and no change in pH was observed.

Colorimetric Method. 5-FU activation of **1a**, **1b**, and model **5a** was studied using this method. Formaldehyde released by activation of the 5-FU side of the mutual prodrug or model was determined using a modification[8c] of a colorimetric method described by Sawicki *et al.*[12] A sample (~15 mg) of the solid prodrug or model was weighed out on an analytical balance and added to 25 mL of buffer in a temperature-controlled, water-jacketed, covered beaker equipped with a stirring bar. All reactions were run at 25 ± 1 °C except for **5a**, which was run at 37 ± 1 °C (physiological temperature). The sample dissolved in less than 5 s to give a solution of ~1.2×10^{-3} M substrate. At appropriate intervals, 1 mL samples were withdrawn and diluted to 10 mL with water. A 500 μL aliquot of the diluted test solution (~1.2×10^{-4} M substrate) was mixed with 400 μL of a 0.1 M acetate buffer (pH = 4.0) and 100 μL of a 0.5% aqueous solution of 3-methyl-2-benzothiazolone hydrazone hydrochloride hydrate. After the mixture was allowed to stand for 25−30 min at room temperature (20−25 °C), 500 μL of a 0.25% aqueous iron(III) chloride hexahydrate solution was added. After 10 min, 1500 μL of water was added, and the absorbance of the mixture (~2×10^{-5} M substrate) was read *vs* a reagent blank at 625 nm. The concentration of formaldehyde in the solution was calculated by referring to a standard curve. The formaldehyde standard curve was constructed using the aforementioned colorimetric method to analyze seven aqueous formaldehyde solutions in the range $(2-20) \times 10^{-6}$ M. The seven aqueous formaldehyde solutions were made from either solid paraformaldehyde or a 37% aqueous formaldehyde solution analyzed for analytical use by the gravimetric method of Yoe and Reid.[14] The molar absorptivity was found to be 4.44×10^4 L/mol·cm. Pseudo-first-order rate constants were calculated from the slopes of linear plots of ln ($Abs_\infty - Abs_t$) vs time, where Abs_∞ and Abs_t are the absorbance at infinity and at time, t, respectively. Extent of reaction at infinity was determined using the standard curve for formaldehyde. A pH measurement of the buffer solution was taken after each reaction had reached completion and no pH change was observed.

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