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5′-(2-Nitrophenylalkanoyl)-2′-deoxy-5-fluorouridines as Potential Prodrugs of FUDR for Reductive Activation☆

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Abstract—Four 5′-(2-nitrophenylalkanoyl)-2′-deoxy-5-fluorouridines (**1a**–**d**) were designed and synthesized as potential prodrugs of FUDR for reductive activation. Two methyl groups were introduced α to the ester carbonyl to increase both the rate of cyclization activation and the stability of the conjugates towards serum esterases. Chemical reduction of the nitro group into an amino leads to cyclization and release of the active FUDR. Kinetic analysis of the cyclization activation process indicates that the two methyl groups α to the ester carbonyl restrict the rotational freedom of ground state molecule and promote the cyclization reaction. However, the two methyl groups also were found to render the conjugates as poor substrates of *E. coli* B nitroreductase. Conjugate **1c**, without the two methyl groups, was reduced by *E. coli* B nitroreductase ($t_{1/2}=8$ h) to give two products, a *N*-hydroxyl lactam and the drug FUDR, suggesting that the enzymatic reduction and subsequent cyclization activation proceeded through the hydroxylamine intermediate. These results indicate that cyclization activation will occur once the nitro group is reduced either to an amino or to a hydroxylamino group. The fact that the amino intermediates cyclized easily to release the incorporated drug FUDR suggests the feasibility of using peptide-linked acyl 2-aminophenylalkanoic acid esters as potential prodrugs for proteolytic activation.

Introduction

One of the characteristics of many solid tumors is the insufficient supply of oxygen to the core tissue due to poorly developed vasculature. These oxygen-deficient tumor cells are refractory to radiation therapy and most chemotherapeutic agents. As a result, they are capable of proliferating and causing tumor re-growth after treatments.[1] On the other hand, hypoxic tumor cells are known to have a greater capacity for reductive reactions as compared to normal well-oxygenated cells. This unique feature differentiates neoplastic tissues from the normal tissues and provides an attractive target for selective anticancer chemotherapy. Several bioreductively activated nitro compounds, quinines, and aromatic *N*-oxides are currently in clinical trials as hypoxia-selective cytotoxins and could potentially be developed into selective anticancer prodrugs.[2]

It is important to understand that bioreductions are found in both oxygenated and hypoxic cells. Ideally, the presence of oxygen would promote a redox cycle that suppresses the net reduction of a hypoxia-selective prodrug to its cytotoxic species, whereas the reaction cascade leads to the formation of the cytotoxic species in the absence of sufficient oxygen tension.[3]

Activation of aromatic nitro compounds via bioreduction to form cytotoxic species has been the subject of many investigations over the past 20 years.[2,4] There has been much interest in exploiting this transformation to activate prodrugs in the hypoxic region of solid tumors or by nitroreductases introduced site-specifically into tumor cells.[5] The nitro group has been shown to undergo up to six-electron (6e) reduction by flavin containing enzymes in cells. As shown in Figure 1, the formation of a nitro anion radical is the early process in this reduction. The hypoxia selectivity arises from the fact that in the normoxic tissues, the radical intermediate can react with molecular oxygen, leading to the regeneration of the parent nitro compound. Consequently, the reduction to generate highly cytotoxic species will only occur in the hypoxic tissue.[3]

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Also, nitroreduction by nitroreductases is of current interest because new methods such as the antibody-directed enzyme prodrug therapy (ADEPT) and gene-directed enzyme prodrug therapy (GDEPT) have been developed to introduce a specific enzyme into tumors. In ADEPT, a conjugate of a tumor specific antibody and an enzyme is administered, and the antibody–enzyme conjugates accumulate selectively on the surface of the antigen-expressing tumor cells. After a sufficient time interval for its clearance from the normal (non-target) tissue and the localization of the antibody–enzyme conjugates into the tumor tissue, the prodrug of an anticancer agent is administered and activated specifically at the tumor site. The active drug can be of low molecular weight, which would allow rapid diffusion and reach tumor regions not accessible to the antibody–enzyme conjugate, producing a bystander effect.[6] GDEPT is a related approach to ADEPT, where the DNA encoding for a prodrug-activating enzyme is selectively expressed and translated within a tumor cell.

An aerobic nitroreductase from *Escherichia coli* B is currently under evaluation for use in both ADEPT and GDEPT. *E. coli* B nitroreductase can reduce certain aromatic nitro groups into their corresponding hydroxylamines in the presence of NADH or NADPH as a cofactor. Prodrugs which are activated by this enzyme fall into two classes.[7] The first class is exemplified by 2,4-dinitrobenzamides (e.g., CB1954) and 2,4-dinitrophenyl related nitrogen mustards (e.g., SN23862), for which the nitro reduction has the potential to increase the alkylating reactivity. The second class of substrates includes 4-nitrobenzyloxycarbamyl derivatives of a range of amine-bearing cytotoxins. The enzyme reduces the nitro group into the corresponding hydroxylamine in the molecule, which then undergoes fragmentation to give the effector cytotoxin.[8,9] In this paper, we report the design, synthesis, and evaluation of nitro-containing prodrugs of FUDR, where nitro reduction would lead to a cyclization activation process and the release of the active drug FUDR.

Results and Discussion

Design

Our hypothesis is to use the nitro group as an electronic trigger, which upon reduction in hypoxic tissues or by a nitro reductase can initiate an intramolecular cyclization reaction and thereby result in the release of the active drug. Four potential prodrugs of FUDR (**1a–d**) have been designed based on the bioreductive activation mechanism shown in Scheme 1. The free 5′-hydroxyl group of the deoxyribose sugar in FUDR (**6**) is required for its cytotoxic activity. The hydroxyl group must be phosphorylated by thymidine kinase to FdUMP and other metabolites to affect DNA synthesis. In compounds **1a–d**, the introduction of an ester linkage masks this hydroxyl group leading to deactivation of FUDR.

Scheme 1 illustrates the proposed mechanism of activation upon reduction for the FUDR prodrugs **1a–d** designed. The reduction of the aromatic nitro group in **1a–d** could occur in hypoxic tumor tissues or by a nitroreductase such as the one from *E. coli*. The products of these bioreductions are believed to be either the corresponding hydroxylamines **2a–d** or the corresponding amines **3a–d**. This conversion of an electron-withdrawing nitro group into a nucleophilic hydroxylamine or amine is expected to trigger a spontaneous intramolecular cyclization. The hydroxylamino group in **2a–d** or the amino group in **3a–d** would attack the electrophilic ester carbonyl carbon, leading to the formation of the corresponding *N*-hydroxyl lactam **4a–d** or lactam **5a–d** and the release of the effector anticancer drug FUDR (**6**).

Different lengths of the side chain can result in the formation of five- or six-membered lactam ring, which could influence the rate of cyclization. The two methyl groups attached to the α-position of the carbonyl were expected to restrict the rotational freedom of the ground-state conformation of the molecule, and therefore, place the amine or hydroxylamine in a more favorable position with respect to the ester carbonyl, a concept termed as 'stereopopulation control'. In addition, the methyl substitution has been found to accelerate the rate of lactonization and lactamization, which is known as the 'Thorpe–Ingold' effect.[10] This has also been used in other strategies based on intramolecular cyclization reactions and has been reviewed.[11]

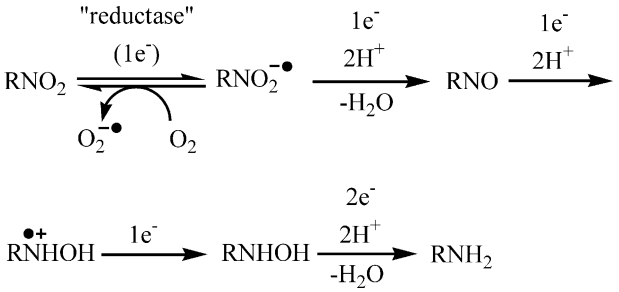
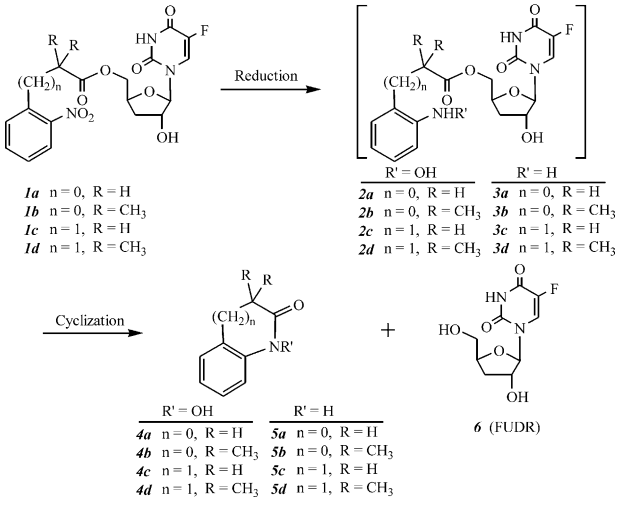
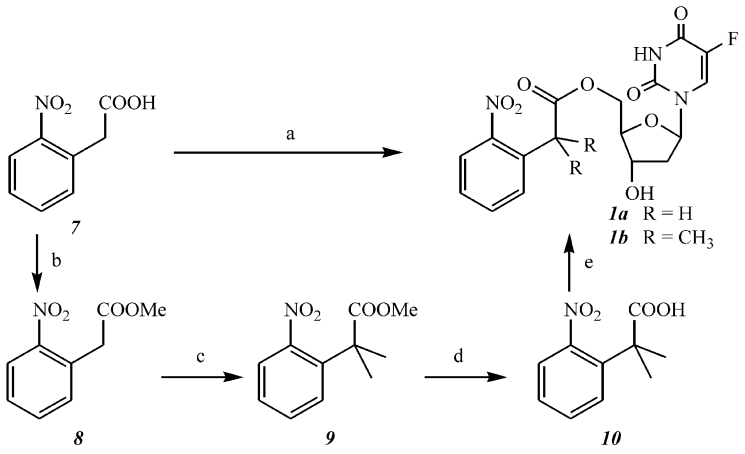


Figure 1. Selectivity of nitroaromatics towards hypoxia.



Scheme 1. Proposed mechanism of activation for the FUDR prodrugs upon reduction.



Scheme 2. Synthesis of conjugates **1a** and **1b**: (a) FUDR, DEAD, PPh_3, THF, rt 20 h, 83%; (b) $SOCl_2$, MeOH, 0 °C–rt, 100%; (c) MeI, NaH, 15-crown-5, DMF, −4 to 0 °C, 100%; (d) 1 N NaOH, MeOH, reflux, 6 h, 93%; (e) FUDR, DEAD, PPh_3, dioxane, rt 18 h, 53%.

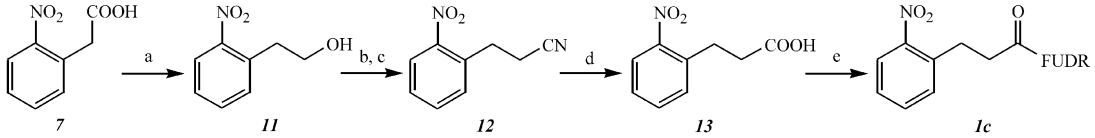
One concern of using prodrugs containing ester bonds is the stability of such linkages in the presence of esterases found in human serum. The two methyl substitutions would provide some steric hindrance around the ester bond to slow down such esterase-catalyzed hydrolysis and the resulting prodrugs might be sufficiently stable under physiological condition to be useful.

Synthesis

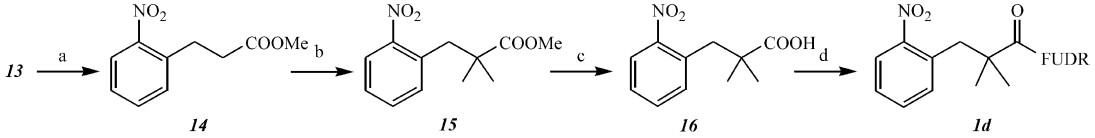
5′-(2-Nitrophenyl)acetyl-2′-deoxy-5-fluorouridine **1a** was prepared directly from the commercially available 2-nitrophenylacetic acid (**7**) and FUDR (Scheme 2). The conjugation between the acid and the 5′-hydroxyl group of the deoxyribose in FUDR was accomplished using diethyl azodicarboxylate (DEAD) and triphenylphosphine (PPh_3) in 83% yield. The Mistunobu reaction condition is very mild and highly regioselective for the primary hydroxyl over the secondary hydroxyl group in the deoxyribose sugar of FUDR.[12,13] The structure of conjugate **1a** was confirmed by ^1H NMR, COSY, and HRMS.

The route towards the synthesis of compound **1b** started with the esterification of 2-nitrophenylacetic acid (**7**) with thionyl chloride in methanol.[14] The methyl ester **8** was further dialkylated using methyl iodide and sodium hydride in the presence of catalytic amount of 15-crown-5. Sodium hydroxide mediated hydrolysis converted the dialkylated ester **9** to the corresponding carboxylic acid **10** in 93% yield for the three steps. Final coupling of the acid **10** with FUDR under the Mistunobu reaction conditions afforded the target compound **1b** in 53% yield. The overall yield for the synthesis of conjugate **1b** was 49%.

The synthesis of target compounds **1c** and **1d** was accomplished as shown in Schemes 3 and 4, respectively. Reduction of 2-nitrophenylacetic acid (**7**) using borane in THF[15] gave the corresponding alcohol **11**, which was converted to its tosylate and treated with potassium cyanide in NMP at 90–100 °C. The resulting nitrile **12** was hydrolyzed using 50% sulfuric acid to give the carboxylic acid **13**, which was coupled to FUDR under the same Mitsunobo conditions to afford the desired conjugate **1c** in 39% overall yield. As shown in Scheme 4, treatment of acid **13** with thionyl chloride in methanol afforded the methyl ester **14** in 92% yield, which was dialkylated with methyl iodide and LDA at −78 °C. This step turned out to produce a very complex product mixture, and the desired compound **15** was obtained in only 9% yield after purification by flash column chromatography. The low yield might be attributed to the strong electron withdrawing nitro group, which led to difficulty in selectively dialkylating the α-position on the side chain. The methyl ester **15**



Scheme 3. Synthesis of conjugate **1c**: (a) BH_3/THF, rt, 90%; (b) TsCl, pyridine, rt, 3 h, 82%; (c) KCN, NMP, 90–100 °C, 1.0 h, 86%; (d) 50% H_2SO_4, 130–135 °C, 4.5 h, 100%; (e) FUDR, DEAD, PPh_3, THF, rt, 16 h, 61%.



Scheme 4. Synthesis of conjugate **1d**: (a) $SOCl_2$, MeOH, 0 °C–rt, 1 h, 92%; (b) LDA, CH_3I, THF, −78 °C to rt, 9%; (c) LiOH, MeOH/H_2O (3:1), 5 °C, 15 h, 100%; (d) FUDR, DEAD, PPh_3, THF, rt, 32 h, 53%.

was further hydrolyzed using lithium hydroxide in methanol and water at 0 °C to produce the acid **16** in quantitative yield. Mistunobu reaction was again employed for the conjugation of acid **16** with FUDR, which afforded the desired compound **1d** in 53% yield.

Stability test

The stability of these potential prodrugs under physiological conditions is an important issue, since the ester linkage in these molecules might be susceptible to enzymatic hydrolysis by esterases present in human serum. Therefore, the stability of **1a–d** was tested by incubating each sample in sodium phosphate buffer (100 mM, pH 7.4) and in human serum at 37 °C. Acetonitrile was used to help dissolve the compounds, and the concentration of the organic solvent in the incubation mixture was kept below 5%. Aliquots (25 μL) were taken at different time intervals and analyzed by HPLC on a C-18 reversed-phase column using acetonitrile–water (0.1% trifluoroacetic acid) as the mobile phase. In the case of incubation in human serum, the active esterases were quenched with 7% $HClO_4$ solution (100 μL) immediately after the aliquots were withdrawn.[13]

All four compounds were found to be stable in the phosphate buffer at 37 °C over three days of incubation. However, their stability in human serum at 37 °C differs from each other as shown in Figure 2. Compounds **1b** and **1d** with the two methyl groups at the α-position to the ester carbonyl were found to be stable in human serum; there was no observable hydrolysis after 3 days of incubation. On the other hand, compounds **1a** and **1c** without the two methyl substitutions were subjected to hydrolysis catalyzed by esterases with half-lives of 68.6 and 12.4 h, respectively. These results indicate that the two methyl groups did protect the esters from enzymatic hydrolysis. The hydrolysis of methyl 2-nitrophenylacetate was also tested under the same conditions and was found to have a half-life of 19 h in human serum at 37 °C, which was much shorter than the 68.6 h half life for compound **1a**. Clearly, the two methyl groups at the α-position to the ester carbonyl as well as the deoxyribose sugar in FUDR contribute to the stability of FUDR conjugates **1b** and **1d**.

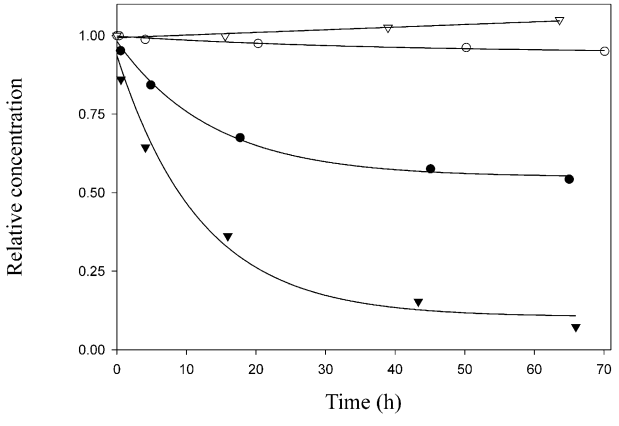


Figure 2. Stability of compounds **1a** (●), **1b** (○), **1c** (▼), and **1d** (▽) in human serum. Relative concentration against time: expressed by the ratio of the peak area at certain time interval versus peak area at time zero for each compound.

Chemical reduction

To test the feasibility of releasing the active drug FUDR from conjugates **1a–d**, two mild chemical reductions were carried out first on compound **1b**. One was hydrogenation using a hydrogen balloon and the other was sodium borohydride-mediated reduction. Both reactions were done in the presence of 10% Pd/C catalyst at room temperature.[16] These conditions should not affect the other functional groups present in the molecule while reducing the nitro group to the corresponding amine. Our results indicated that the cyclization did occur after reduction. The lactam **5b** and FUDR were the only products isolated under both reduction conditions. The amino intermediate **3b**, however, was not isolated, suggesting that the cyclization step was very fast.

Bioreduction in hypoxic tissues or reduction by nitroreductases would most likely stop at the hydroxylamine intermediate. It is known that cyclization via the hydroxylamine is faster than cyclization via the amine intermediate.[17,18] Thus, our chemical reduction conditions are simple and practical tests of the cyclization activation process.

Between the two methods of chemical reduction, hydrogenation was found to be milder than the sodium borohydride reduction; thus, hydrogenation was chosen to test cyclization activation process of compounds **1a**, **1c**, and **1d**. Reduction of compound **1d** gave a similar result as observed for compound **1b**; no amino intermediate **3d** but cyclized lactam **5d** and FUDR were isolated. On the other hand, the amino intermediates **3a** and **3c** were isolated after hydrogenation of compounds **1a** and **1c**, respectively. Incubation of the amino intermediates **3a** and **3c** in phosphate buffer (100 mM, pH 7.4) at 37 °C led to the cyclization and release of FUDR, which was analyzed by HPLC and confirmed by spectroscopic techniques.

In order to compare the rate of cyclization and release of FUDR from the conjugates **1a–d** upon reduction, we studied the kinetics of the cyclization reaction by monitoring the changes in UV–vis absorbance. In the case of compound **1a**, for which the amino intermediate **3a** was isolated, the maximum absorbance wavelength of amine **3a**, cyclized lactam **5a**, and FUDR were determined to be 271, 248, and 268 nm, respectively. The maximum change in UV absorption before and after cyclization of compound **3a** was at 249 nm. For the kinetic assay, we withdrew the filtrates directly after partial hydrogenation (4 min) of the nitro compounds, and immediately diluted them with phosphate buffer at 37 °C. The changes in UV absorbance at 249 nm were followed at different time intervals. The cyclization for compound **1b** upon reduction was found to be too fast to calculate the kinetic constant. The calculated half-lives for other compounds **1a**, **1c**, and **1d** upon reduction were 14, 4, and 2 min, respectively (Fig. 3). These results are con-

sistent with the role of two methyl groups in restricting the rotational freedom of the ground-state conformation of the molecule and facilitating the intramolecular cyclization process as outlined in Scheme 1.

Nitroreductase assay

Based on the standard conditions published in the literature,[19] compound **1c** was first tested using an incubation mixture containing compound **1c** (0.1 mM), nitroreductase (7 μg/mL), and cofactor NADH (1 mM) in phosphate buffer (100 mM, pH 7.0) at 37 °C. We found that the concentration of **1c** stopped changing after 1 h of incubation and only 20% of **1c** was consumed. It seemed as though the enzyme was losing activity or the cofactor NADH was decomposing. It has been reported in the literature that NADH was unstable under certain assay conditions.[20] When we added more NADH to the assay mixture after the 1-h incubation, the substrate was further reduced. This suggested to us that the problem was indeed due to the instability of NADH under this assay condition.

Rover and coworkers did a detailed study on factors that influence the stability of NADH that included pH, temperature and buffer ions.[21] They found that 13% of NADH decomposed after 40 min of incubation in 100 mM phosphate buffer. Since most of the nitroreductase assays were carried out in phosphate buffer, we decided to do our own test on the effect of phosphate buffer concentration on the stability of NADH. We incubated a 0.1 mM NADH solution in two different concentrations (100 and 10 mM) of phosphate buffer at pH 7.0 and 37 °C and monitored the absorbance change by UV–vis at 340 nm, which is the maximum absorbance wavelength for NADH. The half-life for the decomposition of NADH was found to be 4.5 h in 100 mM phosphate buffer, and it increased dramatically to 66 h in 10 mM phosphate buffer. Decreasing the incubation temperature from 37 °C to 25 °C resulted in a further increase in stability of NADH to a half-life of 138.6 h in 10 mM phosphate buffer (Fig. 4).

As a result, the reduction of compound **1c** by *E. coli* B nitroreductase was tested again in 10 mM phosphate buffer, at pH 7.0 and 25 °C (Fig. 5). Under this condition, the enzyme reduced compound **1c** steadily as shown by HPLC analysis. The formation of FUDR was confirmed by comparison with authentic sample, and the cyclized product *N*-hydroxyl lactam **4c** was confirmed by analytical LC–MS. The calculated half-life for the reductive activation is 8 h. A control experiment was carried out in parallel under the same conditions without the presence of nitroreductase, and no changes were observed within the same time interval.

This assay condition was thus used to test compounds **1a**, **1b**, and **1d**. We found that compound **1b** was not a substrate of the *E. coli* B nitroreductase, and no significant changes could be observed after 3 days of incubation. Compound **1a** and **1d** were also poor substrates of the enzyme. Although the release of FUDR and the formation of cyclized lactams **4a** and **4d** could be detected by HPLC, the half-lives of both activations were longer than 3 days. Apparently, the two methyl groups in **1d** and the shorter spacing between phenyl and FUDR in **1a** adversely affected the substrate binding and/or reduction by the nitroreductase enzyme.

The slow activation of compounds **1a**–**d** by *E. coli* B nitroreductase indicates that these compounds won't be appropriate for *E. coli* B nitroreductase-mediated enzyme prodrug therapy. However, the fact that chemical reduction of all four conjugates, especially **1b**, to their corresponding amines leads to fast cyclization and release of the incorporated drug FUDR suggests the possibility of employing proteolysis to activate a tripartite conjugate such as **17** with a peptide acyl attached to the amino group. Cleavage of the peptide acyl group in **17** by a protease would produce the same amine intermediates as in the reduction of conjugates **1a**–**d**. These intermediates (**3a**–**d**) have been shown in this paper to undergo a facile intramolecular cyclization reaction leading to the release of the incorporated drug FUDR.

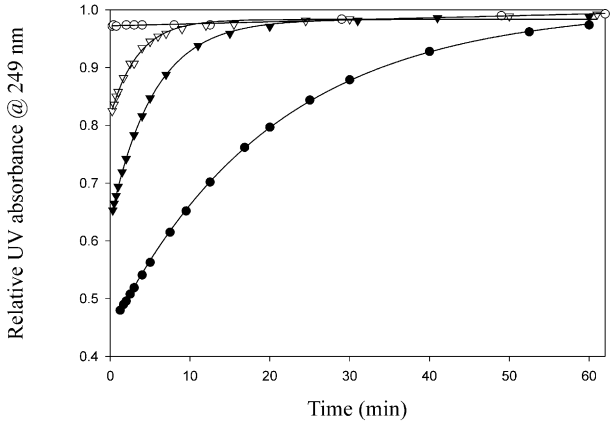


Figure 3. Kinetic study on the cyclization processes: Measuring UV absorbance at 249 nm of the filtrates from 4 min's hydrogenation of compounds **1a** (●), **1b** (○), **1c** (▼), and **1d** (▽) against time: expressed in the ratio of absorbance at certain time interval versus absorbance at $T = 120$ min when the absorbance for each compound has reached a constant.

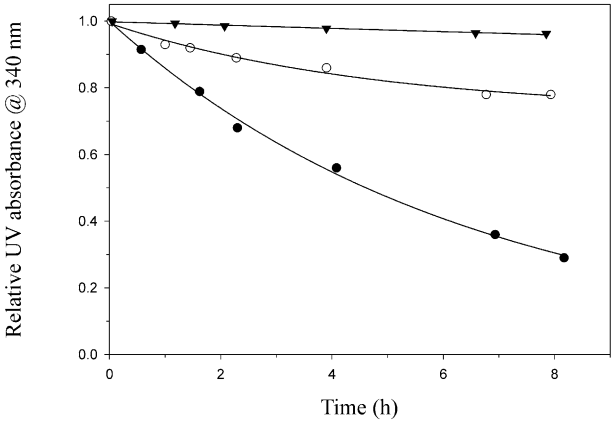


Figure 4. Stability of NADH under different incubating conditions. Relative UV absorbance of NADH against time: expressed by the ratio of the absorbance at certain time interval versus absorbance at time zero for each incubating condition. The conditions used were 100 mM phosphate buffer at 37 °C (●),10 mM phosphate buffer at 37 °C (○), and 10 mM phosphate buffer at 25 °C (▼).

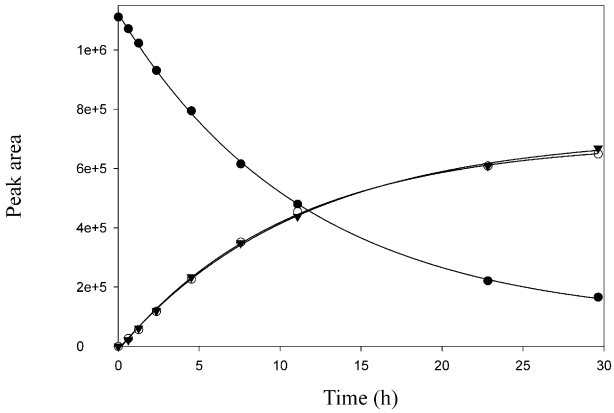
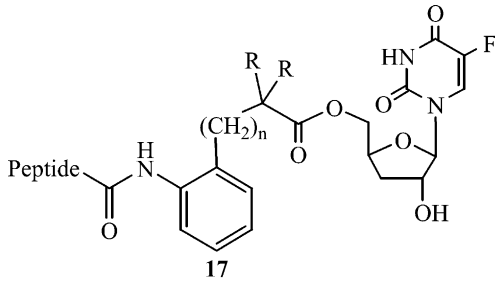


Figure 5. Reduction of compound **1c** by *E. coli* B nitroreductase: Composition of a solution of **1c** reductively activated by nitroreductase against time. Shown are the disappearance of **1c** (●) and appearance of **4c** (○) and FUDR (▼).



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In summary, four 5′-(2-nitrophenylalkanoyl)-2′-deoxy-5-fluorouridines (**1a–d**) were designed and synthesized as potential prodrugs of FUDR for reductive activation. Two methyl groups were introduced to increase the stability of the conjugates towards serum esterases. Chemical reduction of the nitro group to an amino functionality leads to cyclization and concomitant release of the active incorporated drug FUDR. Kinetic analysis of the cyclization activation process indicates that the two methyl groups α to the ester carbonyl act to restrict the rotational freedom of the ground state molecule and promote the cyclization reaction. However, the two methyl groups seem to adversely affect the affinity and/or catalysis of the conjugates as substrates of the *E. coli* B nitroreductase. Only conjugate **1c** was found to be a substrate of *E. coli* nitroreductase with a half life of around 8 h. **1a** and **1d** were poor substrates of the nitroreductase enzyme with half lives greater than 3 days while **1b** was not significantly affected by the enzyme over a three-day incubation. Two products were found from the enzymatic reduction of **1c**, a *N*-hydroxyl lactam and the drug FUDR, suggesting that the enzymatic reduction and subsequent cyclization activation proceed through the hydroxylamine intermediate. These results do indicate that once the nitro group is reduced either to the amine or the hydroxylamine, the cyclization activation is bound to occur. Although the poor activity of these conjugates as substrates of *E. coli* B nitroreductase limited their use as reductively activated prodrugs in ADEPT or GDEPT using the reductase, the amine conjugates **3a–d** might be linked to a peptide sequence known to be a good substrate of a tumor or tissue-specific protease and the resulting tripartite peptide conjugates might be developed into clinically useful prodrugs for proteolytic activation. This possibility is currently being explored in our laboratory.

Experimental

General methods

Solvents were either ACS reagent grade or HPLC grade. Unless otherwise stated, all reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using 0.25 mm Whatman precoated silica gel plates. TLC plates were visualized using either 7% (w/w) ethanolic phosphomolybdic acid or 1% (w/w) aqueous potassium permagnate containing 1% (w/w) $NaHCO_3$. Flash column chromatography was performed using silica gel (Merck 230–400 mesh). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise noted. All reagents were purchased at the highest commercial quality and used without further purification.

Infrared spectra were recorded with a Perkin-Elmer model 1600 series FTIR spectrometer using polystyrene as an external standard. Infrared absorbance is reported in reciprocal centimeters (cm^{-1}). All ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 or 200 MHz spectrometer at ambient temperature and calibrated using residual undeuterated solvents as the internal reference. Chemical shifts (300 MHz for ^{1}H and 75 MHz for ^{13}C or 200 MHz for ^{1}H and 50 MHz for ^{13}C) are reported in parts per million (δ) relative to CD_3OD (δ 3.3 for ^{1}H and 49.0 for ^{13}C). Coupling constants (*J* values) are given in hertz (Hz). The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; p = quintet; m = multiplet; br = broad. Mass spectral data were obtained from the University of Kansas Mass Spectrometry Laboratory (Lawrence, KS, USA).

5′-(2-Nitrophenyl)acetyl-2′-deoxy-5-fluorouridine (1a). The commercially available 2-nitrophenylacetic acid (177 mg, 0.97 mmol), FUDR (200 mg, 0.81 mmol) and PPh_3 (250 mg, 0.95 mmol) were dried under vacuum overnight before 5 mL of anhydrous THF was introduced under argon atmosphere. DEAD (0.15 mL, 0.97 mmol) was then added and the reaction mixture was stirred at room temperature for 20 h. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel eluted with acetone/hexanes (1:5→1:2→1:1) to afford 277 mg of the desired product *1a* as a white foam solid (83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, 1H, J = 7.8 Hz), 7.72–7.42 (m, 4H), 6.18 (t, 1H, J = 6.0 Hz), 4.40 (dd, 1H, J = 3.3, 12.3 Hz), 4.31 (m, 1H), 4.11 (m, 2H), 3.34 (s, 2H), 2.25 (m, 1H), 1.95 (m, 1H); IR (KBr) 3446.1 (br), 3200.0, 3087.2, 1712.8, 1702.6, 1523.1, 1476.9, 1405.1, 1348.7, 1261.5, 1215.4, 1070.9, 1046.1, 784.6, 717.9 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 410.1 (MH^+, 7.1), 280.1 (12.9), 164.0 (6.6), 154.0 (100), 136.0

(91.2); HRMS calcd for $C_{17}H_{17}FN_3O_8$ (MH^+) 410.1000, found 410.1003.

2-Nitrophenylacetic acid methyl ester (8). To a solution of 2-nitrophenylacetic acid (40 g, 0.22 mol) in 240 mL of methanol was added thionyl chloride (36 mL) over 30 min while maintaining the temperature at 0–4 °C. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 18 h. After removal of the solvent in vacuo, the residue was partitioned between ethyl acetate and water. The organic phase was dried over anhydrous Na_2SO_4 and filtered. Removal of ethyl acetate in vacuo gave 43 g of methyl ester **8** as a yellow oil in quantitative yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.09–7.33 (m, 4H), 4.01 (s, 2H), 3.68 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.6, 148.9, 133.8, 133.5, 129.9, 128.8, 125.4, 52.4, 39. 7; IR (neat) 2953.8, 1738.5, 1613.7, 1579.8, 1523.1, 1430.8, 1408.3, 1348.7, 1215.4, 1169.2, 1000.0, 861.5, 784.6, 707.7 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 196.2 (MH^+, 100), 164.1 (55.5).

2-Methyl-2-(2-nitrophenyl)propionic acid methyl ester (9). A solution of 2-nitrophenylacetic acid methyl ester **8** (19.5 g, 0.1 mol), methyl iodide (14.3 mL, 0.23 mol) and 15-crown-5 (5.5 g, 0.025 mol) in 130 mL of DMF was stirred at −4 to 0 °C, to which was slowly added a small amount of sodium hydride (60% in oil) until the mixture suddenly turned blue. The remaining portion of sodium hydride (total 9.2 g, 0.23 mol) was added with stirring over 40 min while the temperature was maintained at 0–4 °C. Standing overnight after addition, the reaction mixture was diluted with ethyl acetate. The organic phase was washed sequentially with 1 N HCl solution, 1 N $KHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and filtered. Removal of ethyl acetate in vacuo afforded 22 g of the desired product **9** as a yellow oil in quantitative yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.93–7.38 (m, 4H), 3.66 (s, 3H), 1.68 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.8, 148.9, 139.4, 133.5, 128.2, 127.9, 125.7, 52.2, 46.5, 27.7; IR (neat) 2986.2, 2951.8, 1738.7, 1607.6, 1575.9, 1526.2, 1481.3, 1434.9, 1356.7, 1288.3, 1247.0, 1230.8, 1147.0, 1113.1, 1049.8, 988.4, 860.9, 785.6, 748.0, 709.5 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 224.2 (MH^+, 100), 192.2 (81.9), 164.2 (67.5).

2-Methyl-2-(2-nitrophenyl)propionic acid (10). A solution of 2-methyl-2-(2-nitrophenyl)propionic acid methyl ester **9** (8 g, 36 mmol) in 108 mL of methanol was treated with 1 N NaOH solution (108 mL) and was refluxed for 6 h. After removal of methanol in vacuo, the reaction mixture was adjusted with 1 N HCl solution to pH 2 and extracted with ethyl acetate. The organic phase was washed with brine and dried over anhydrous Na_2SO_4. Removal of ethyl acetate in vacuo gave 5.5 g of **10** as a yellow solid (93%). Mp (ethyl acetate) 146–148 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 11.91 (br s, 1H), 8.00–7.39 (m, 4H), 1.72 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 182.2, 148.5, 138.9, 133.6, 128.3, 128.1, 125.9, 46.6, 27.3; IR (KBr) 2992.4 (br), 2665.6, 1708.0, 1609.6, 1577.2, 1526.6, 1476.4, 1343.7, 1308.0, 1271.7, 1239.1, 1172.0, 1155.5, 944.5, 867.7, 789.5, 741.9, 703.3, 637.5 cm^{-1}; MS (EI) *m*/*z* (rel intensity): 164.3 (M^+–COOH, 3.6), 163.0 (100).

5′-(2-Methyl-2-(2-nitrophenyl)propionyl)-2′-deoxy-5-fluorouridine (1b). 2-Methyl-2-(2-nitrophenyl)propionic acid **10** (20 mg, 0.089 mmol), FUDR (20 mg, 0.081 mmol) and PPh_3 (25 mg, 0.095 mmol) were dried under vacuum for 16 h and dissolved in 0.5 mL of anhydrous dioxane, to which was introduced DEAD (20 μL, 0.13 mmol) at room temperature. The reaction mixture was stirred for 18 h. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel eluted with acetonehexanes (1:5→1:2→1:1) to afford 22 mg of the desired product **1b** as a white foam solid (53%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.09 (br s, 1H), 7.96 (d, 1H, J = 7.8 Hz), 7.65–7.37 (m, 4H), 6.18 (t, 1H, J = 6.0 Hz), 4.47 (dd, 1H, J = 3.3, 12.3 Hz), 4.24 (m, 1H), 4.15 (m, 2H), 2.30 (m, 1H), 1.72 (s, 3H), 1.67 (s, 3H), 1.59 (m, 1H); IR (KBr) 3456.4 (br), 3200.0, 3087.2, 2984.6, 2943.6, 2830.7, 1712.8, 1523.1, 1471.8, 1394.9, 1353.8, 1261.5, 1153.8, 1092.3, 1046.1, 892.3, 851.3, 789.7, 743.6 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 438.2 (MH^+, 5.4), 308.1 (13.5), 192.1 (5.7), 164.1 (10.3), 154.1 (100); HRMS calcd for $C_{19}H_{21}FN_3O_8$ (MH^+) 438.1313, found 438.1321.

2-(2-Nitrophenyl)ethanol (11). 2-Nitrophenylacetic acid (4.7 g, 26 mmol) was dissolved in 15 mL of anhydrous THF, to which was added slowly a solution of 1 N BH_3 in THF (52 mL, 52 mmol) under argon. After the addition, the reaction was allowed to proceed at room temperature for 20 h. Then the mixture was cooled to 0 °C, quenched by water, partitioned between saturated $NaHCO_3$ solution and ethyl acetate. The organic phase was washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel eluted with ethyl acetate/hexanes (1:10→1:5→1:3) to afford 3.9 g of **11** as a yellow oil (90%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.81–7.20 (m, 4H), 3.77 (t, 2H, J = 6.6 Hz), 3.02 (t, 2H, J = 6.6 Hz), 2.78 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 149.8, 133.9, 133.2, 133.0, 127.7, 124.9, 62.6, 36.2; IR (neat) 3361.8 (br), 2943.6, 2880.6, 1609.8, 1523.4, 1346.7, 1041.9, 860.4, 785.8, 740.5 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 168.0 (MH^+, 28.8), 153.9 (100).

3-(2-Nitrophenyl)propionitrile (12). A solution of 2-(2-nitrophenyl)ethanol **11** (1.7 g, 10 mmol) in 8 mL of pyridine was treated with *p*-toluenesulfonyl chloride (1.4 g, 11 mmol) at room temperature. After the reaction mixture was stirred at room temperature for 3 h, it was partitioned between 1 N HCl solution and ethyl ether. The organic phase was washed with 1 N $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$ and filtered. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel eluted with ethyl acetate/hexanes (1:20 →1:15→1:10) to afford 2.62 g of the desired tosylate as a white solid (82%). Mp (ethyl acetate/hexanes) 73.5–75 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95–7.25 (m, 4H), 4.34 (t, 2H, J = 7.2 Hz), 3.25 (t, 2H, J = 7.2 Hz), 2.42 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 149.2, 145.1, 133.6, 133.5, 132.8, 131.8, 130.0, 128.4, 127.9, 125.2, 69.6, 33.2, 21.8; IR (KBr) 3066.7, 2964.1, 1594.9, 1523.1, 1353.8, 1174.4, 1092.3, 969.2, 902.6, 810.3, 774.4, 656.4 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 322.1 (MH^+, 5.0), 150.1 (100).

To a solution of the tosylate (3.2 g, 10.0 mmol) in 30 mL of 1-methyl-2-pyrrolidinone was added potassium cyanide (0.98 g, 15 mmol) under argon atmosphere. The mixture was heated while stirring at 90–100 °C in an oil bath for 1.0 h. Then water was added and the reaction mixture was extracted with ethyl acetate (3×50 mL). The organic phase was washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel eluted with ethyl acetate/hexanes (1:15→1:10→1:5) to give 1.5 g of the desired nitrile **12** as a dark solid (86%). Mp (ethyl acetate/hexanes) 41–43 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.03–7.42 (m, 4H), 3.21 (t, 2H, *J* = 7.0 Hz), 2.82 (t, 2H, *J* = 7.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 145.0, 134.1, 133.3, 132.8, 128.9, 125.5, 119.0, 29.6, 18.6; IR (KBr) 3087.2, 2943.6, 2251.3, 1611.7, 1577.8, 1522.1, 1454.2, 1424.4, 1344.1, 1263.2, 1078.1, 864.0, 791.2, 749.4, 700.3 cm^{-1}; MS (EI) *m*/*z* (rel intensity): 176.0 (M^+, 4.6), 136.2 (18.3), 103.1 (100).

3-(2-Nitrophenyl)propionic acid (13). A solution of 3-(2-nitrophenyl)propionitrile **12** (1.0 g, 5.7 mmol) in aqueous sulfuric acid solution (5 mL, 50%) was heated at 130–135 °C for 4.5 h. The reaction mixture was diluted with 10 mL of saturated $(NH_4)_2SO_4$ solution and extracted with ethyl ether (3×40 mL). The organic phase was dried over anhydrous $MgSO_4$ and condensed in vacuo to afford 1.1 g of the desired acid **13** as a white solid in quantitative yield. Mp (acetone/hexanes) 111–113 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.00–7.37 (m, 4H), 3.25 (t, 2H, *J* = 7.4 Hz), 2.82 (t, 2H, *J* = 7.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.6, 135.4, 133.5, 132.3, 127.9, 125.1, 34.7, 28.2; IR (KBr) 2923.1 (br), 1697.4, 1517.9, 1435.9, 1338.5, 1307.7, 1266.7, 1220.5, 928.2, 856.4, 789.7, 723.1 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 196.1 (MH^+, 18.2), 178.1 (21.1), 150.1 (9.2), 154.1 (100).

5′-(3-(2-nitrophenyl)propionyl)-2′-deoxy-5-fluorouridine (1c). 3-(2-Nitrophenyl)propionic acid **13** (143 mg, 0.73 mmol), FUDR (150 mg, 0.61 mmol) and PPh_3 (192 mg, 0.73 mmol) were dried under vacuum for 16 h and dissolved in 5.5 mL of anhydrous THF, to which was introduced DEAD (115 μL, 0.74 mmol) at room temperature. The reaction mixture was stirred for 16 h. After concentration in vacuo, the residue was purified by flash column chromatography on silica gel eluted with acetone/hexanes (1:5→1:2→1:1) to afford 158 mg of the desired conjugate **1c** as a white foam solid (61%). ^{1}H NMR (200 MHz, acetone-d_6) δ 7.98 (dd, 1H, *J* = 8.0, 1.4 Hz), 7.86 (d, 1H, *J* = 7.0 Hz), 7.69–7.47 (m, 3H), 6.29 (dt, 1H, *J* = 1.5, 6.6 Hz), 4.48–4.28 (m, 3H), 4.12 (dd, 1H, *J* = 3.5, 8.3 Hz), 3.24 (t, 2H, *J* = 7.2 Hz), 2.86 (br s, 1H), 2.85 (t, 2H, *J* = 7.5 Hz), 2.36–2.30 (m, 2H); IR (KBr) 3446.1 (br), 3200.0, 3076.9, 2953.8, 2820.5, 1717.9, 1702.6, 1523.1, 1471.8, 1400.0, 1348.7, 1261.5, 1189.7, 1169.2, 1082.0, 1046.9, 784.6, 851.3, 784.6, 743.6 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 446.1 ($M + Na^+$, 100), 424.1 (MH^+, 16); HRMS calcd for $C_{18}H_{19}FN_3O_8$ (MH^+) 424.1156, found 424.1176.

3-(2-Nitrophenyl)propionic acid methyl ester (14). A solution of 3-(2-nitrophenyl)propionic acid **13** (140 mg, 0.72 mmol) in 2 mL of methanol was charged with thionyl chloride (0.12 mL) at 0 °C and the reaction mixture was stirred at this temperature for 1 h. After concentration in vacuo, the residue was dissolved in ethyl acetate, washed with water and dried over anhydrous Na_2SO_4. After removal of the solvent in vacuo, the product was purified by flash column chromatography on silica gel eluted with ethyl acetate/hexanes (1:20→1:15→1:10) to give 138 mg of the methyl ester **14** as a yellow oil (92%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.90–7.27 (m, 4H), 3.61 (s, 3H), 3.16 (t, 2H, *J* = 7.6 Hz), 2.67 (t, 2H, *J* = 7.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.0, 149.1, 135.8, 133.4, 132.4, 127.8, 125.1, 52.0, 34.8, 28.6; IR (neat) 2953.8, 2922.3, 2850.3, 1736.2, 1524.1, 1437.0, 1346.6, 1288.7, 1259.9, 1196.4, 1167.4, 858.3, 788.1, 746.7, 708.7 cm^{-1}; MS (ESI) *m*/*z* (rel intensity): 232.02 ($M + Na^+$, 38), 210.0 (MH^+, 34), 177.95 (80), 149.96 (100).

3-(2-Nitrophenyl)-2,2-dimethylpropionic acid methyl ester (15). To a solution of 2.5 N *n*-butyl lithium in THF (5.8 mL, 14.5 mmol) was added freshly distilled diisopropylamine (2.0 mL, 14.5 mmol) dropwise at −50 °C. After reaction proceeded for 45 min, a solution of ester **14** (736.8 mg, 3.52 mmol) in 25 mL of THF was added and the reaction mixture quickly turned into a dark suspension. Then methyl iodide (4.39 mL, 70.4 mmol) was added slowly into the reaction mixture. The reaction proceeded at −78 °C for 1 h and was gradually warmed to room temperature. After being stirred for an additional 2.5 h, the reaction was quenched by saturated NH_4Cl solution at 0 °C and extracted with methyl *t*-butyl ether. The organic phase was washed with brine and dried over anhydrous $MgSO_4$. After concentration in vacuo, the residue was purified by flash column chromatography on silica gel eluted with ethyl acetate/hexanes (1:20→1:15→1:10) to give 73 mg of the methyl ester **15** as a yellow oil (9%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.25 (m, 4H), 3.64 (s, 3H), 3.32 (s, 3H), 1.17(s, 6H); IR (neat) 2978.6, 2953.8, 1731.4, 1528.8, 1474.1, 1453.8, 1354.5, 1279.7, 1194.5, 1146.8, 1122.4, 854.4, 786.1, 736.1 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 238.1 (MH^+, 9.5), 206.1 (6.7), 178.1 (8.8), 154.0 (100); HRMS calcd for $C_{12}H_{16}NO_4$ (MH^+) 238.1079, found 238.1079.

3-(2-Nitrophenyl)-2,2-dimethylpropionic acid (16). A solution of 3-(2-nitrophenyl)-2,2-dimethylpropionic acid methyl ester **15** (73 mg, 0.3 mmol) in 5 mL of methanol/water (3:1) was charged with lithium hydroxide (20 mg, 0.83 mmol) at 0 °C. The reaction mixture was stirred at this temperature for 15 h. After removal of methanol in vacuo, the residue was partitioned between methyl *t*-butyl ether and brine. The organic phase was dried over anhydrous $MgSO_4$ and filtered.

Removal of the solvent in vacuo gave 70 mg of the desired acid **16** as a yellow oil quantitatively. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.42 (br s, 1H), 7.86–7.37 (m, 4H), 3.38 (s, 2H), 1.21(s, 6H); IR (neat) 3418.2 (br), 2974.4, 2918.7, 1699.3, 1529.7, 1475.9, 1355.0, 1286.3, 1152.2, 854.1, 786.2, 730.6, 667.9 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 224.1 (MH^+, 5.3), 206.1 (8.8), 178.1 (19.7), 154.0 (100).

5′-(3-(2-nitrophenyl)-2,2-dimethylpropionyl)-2′-deoxy-5-fluorouridine (1d). 3-(2-Nitrophenyl)-2,2-dimethylpropionic acid *16* (69 mg, 0.31 mmol), FUDR (75.8 mg, 0.308 mmol) and PPh_3 (84.8 mg, 0.31 mmol) were dried under vacuum for 16 h and dissolved in 5 mL of anhydrous THF, to which was introduced DEAD (0.051 mL, 0.33 mmol) at room temperature. The reaction mixture was stirred for 32 h. After concentration in vacuo, the residue was purified by flash column chromatography on silica gel eluted with acetone/hexanes (1:5→1:2→1:1) to afford 52 mg of the desired conjugate **1d** as a white foam solid (53% based on the recovery of 20 mg of the starting material **16**). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.03 (br s, 1H), 7.84 (d, 1H, J = 7.2 Hz), 7.60–7.27 (m, 4H), 6.22 (t, 1H, J = 6.3 Hz), 4.32 (dd, 1H, J = 4.2, 10.3 Hz), 4.22 (d, 1H, J = 3.0 Hz), 4.16 (dd, 2H, J = 3.3, 5.1 Hz), 3.55 (br s, 1H), 3.32 (q, 2H, J = 13.5 Hz), 2.51 (m, 1H), 2.11 (m, 1H), 1.20 (s, 6H); IR (KBr) 3445.7 (br), 3195.4, 3087.7, 2980.3, 2828.5, 1694.0, 1681.8, 1526.3, 1472.6, 1403.8, 1354.9, 1264.6, 1195.8, 1093.7, 1051.3, 970.4, 859.4, 788.5, 738.3 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 452.1 (MH^+, 6.2), 322.1 (15.4), 178.0 (6.7), 154.0 (100); HRMS calcd for $C_{20}H_{23}FN_3O_8$ (MH^+) 452.1469, found 452.1474.

Stability determination in phosphate buffer. Each of the conjugates **1a–d** (1.5 mg) was dissolved in 200 μL of CH_3CN and stored at 0 °C prior to use. A 10 μL solution was withdrawn and quickly added to 190 μL of prewarmed 100 mM phosphate buffer (pH 7.4) at 37 °C to give a final concentration of 1 mM. The resulting solution was incubated at 37 °C while aliquots (25 μL) of sample were removed at different time intervals and injected directly into the HPLC injection port. HPLC analysis with C-18 reversed-phase column used either a gradient mobile phase from 28% CH_3CN to 52% CH_3CN over 10 min or isocratic elution with a mobile phase of 40% CH_3CN, at a flow rate of 1 mL/min and detection wavelengths at 220 and 280 nm.

Stability determination in human serum. A 10-μL stock solution was quickly added to 190 μL mixture of prewarmed human serum and phosphate buffer (4:1) at 37 °C to give a final concentration of 1 mM. The solution was incubated at 37 °C. At various time intervals, aliquots (25 μL) were withdrawn and quenched immediately by aqueous $HClO_4$ solution (0.1 mL, 7%) to stop enzyme hydrolysis. After centrifugation, the supernatant was analyzed by the same HPLC method mentioned above.

General procedure A: hydrogenation. A solution of the conjugates **1a–d** (0.05 mmol) in 3 mL of methanol underwent atmospheric hydrogenation in the presence of 10% Pd/C. Both HPLC and TLC were used to monitor the progress of reduction. At the end of reaction, the catalyst was removed by filtration, the residue was washed with methanol (3×5 mL). The combined organic phase was concentrated in vacuo, and the residue was purified by flash column chromatography on silica gel eluted with acetone–hexanes to afford the cyclized lactam, FUDR, and/or the amine intermediate. HPLC analysis was performed on a C-18 reversed-phase column (150×4.6 mm), using first an isocratic elution of 2% CH_3CN for 5 min followed by a gradient elution from 2% CH_3CN to 70% CH_3CN over 15 min and a final isocratic elution of 70% CH_3CN for 5 min, at a flow rate of 1 mL/min and detection wavelengths at 220 and 280 nm.

General procedure B: cyclization. A solution of the conjugates **1a–d** (0.05 mmol) in 3 mL of methanol underwent hydrogenation according to procedure A. If the product isolated was the amine intermediate, the isolated intermediate was then incubated in 100 mM phosphate buffer (pH 7.4) at 37 °C and monitored by HPLC. At the end of reaction, the solution was extracted with ethyl acetate, and the organic phase was washed with brine, dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent in vacuo afforded the lactam. FUDR in the aqueous phase was detected by HPLC and confirmed by comparison with an authentic sample.

General procedure C: $NaBH_4$ reduction. To a solution of $NaBH_4$ (20 mg) in 0.5 mL of water was added the conjugates **1a–d** (0.05 mmol) and a suspension of 10% Pd/C in 2 mL of methanol under nitrogen atmosphere. Both HPLC and TLC were used to monitor the progress of reaction. At the end of reaction, the reaction mixture was diluted with acetone, and the catalyst was removed by filtration. The residue after concentration was partitioned between CH_2Cl_2 and water, and both organic and aqueous phases were analyzed by HPLC (the same method as mentioned above). The organic phase was concentrated in vacuo and subjected to flash column chromatography to afford the cyclized lactam, FUDR, and/or the amine intermediate. FUDR was detected in the aqueous phase by HPLC.

3,3-Dimethyl-1,3-dihydroindol-2-one (5b). Reduction of 2-methyl-2-(2-nitrophenyl)propionic acid-FUDR conjugate **1b** according to procedure A or C afforded the lactam **5b** as a white solid. Mp 149.5–151 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.42 (br s, 1H), 7.28–6.96 (m, 4H), 1.44(s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 184.8, 140.2, 136.5, 127.8, 122.7, 122.6, 110.2, 45.0, 24.5; IR (KBr) 3157.9, 3097.4, 2969.7, 1714.7, 1676.4, 1622.0, 1485.2, 1474.1, 1461.1, 1411.3, 1385.3, 1339.1, 1226.3, 1213.3, 1174.1, 1014.2, 945.3, 812.9, 787.2, 754.8, 739.1, 715.0, 658.5, 619.2 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 162.2 (MH^+, 100), 161.2 (M^+, 65); HRMS calcd for $C_{10}H_{12}NO$ (MH^+) 162.0919, found 162.0898.

5′-(2-Aminophenyl)acetyl-2′-deoxy-5-fluorouridine (3a). Compound **3a** was obtained as a white solid from reduction of 2-nitrophenylacetic acid–FUDR conjugate

1a according to procedure A. Mp 78–80 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.69 (d, 1H, J = 6.3 Hz), 7.64–6.57 (m, 4H), 6.22 (t, 1H, J = 6.8 Hz), 4.4 (m, 2H), 4.24 (dd, 1H, J = 12.3, 2.7 Hz), 4.11 (dd, 1H, J = 7.3, 3.3 Hz), 3.65 (s, 2H), 2.18 (ddd, 1H, J = 14, 5.7, 2.4 Hz), 1.94 (dd, 1H, J = 14, 7.2 Hz); IR (KBr) 3415.4 (br), 3200.0, 3066.6, 2953.8, 2820.5, 1702.6, 1687.2, 1620.5, 1471.8, 1359.0, 1261.5, 1097.4, 1051.3, 882.0, 753.8 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 380.2 (MH^+, 4.2), 154.1 (100); HRMS calcd for $C_{17}H_{19}FN_3O_6$ (MH^+) 380.1258, found 380.1296.

1,3-Dihydroindol-2-one (5a). Lactam **5a** was obtained from 2-aminophenylacetic acid–FUDR conjugate **3a** according to procedure B as a white solid. Mp 126–127 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.21 (br s, 1H), 7.17–6.81 (m, 4H), 3.47 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.4, 142.8, 128.1, 125.5, 124.8, 122.5, 110.1, 36.5; IR (KBr) 3216.1, 3076.9, 3035.9, 1701.8, 1619.2, 1472.5, 1387.2, 1333.9, 1304.5, 1239.5, 1176.0, 749.8 cm^{-1}; MS (EI) *m*/*z* (rel intensity): 133.0 (M^+, 100), 105.1 (11.1).

5′-(3-(2-Aminophenyl)propionyl)-2′-deoxy-5-fluorouridine (3c). Compound **3c** was obtained from reduction of 3-(2-nitrophenyl)propionic acid–FUDR conjugate **1c** according to procedure A as a white foam solid. ^{1}H NMR (300 MHz, acetone-d_6) δ 7.86 (d, 1H, J = 7.0 Hz), 7.02–6.54 (m, 4H), 6.28 (dt, 1H, J = 6.6, 1.6 Hz), 4.45–4.24 (m, 3H), 4.11 (m, 1H), 2.92–2.69 (m, 4H), 2.33–2.25 (m, 2H); IR (neat) 3372.1 (br), 3210.3, 3087.6, 1713.7, 1498.4, 1455.9, 1359.1, 1265.2, 1199.6, 1091.2, 1051.3, 751.8 cm^{-1}.

3,4-Dihydro-1*H*-quinolin-2-one (5c). Lactam **5c** was obtained from 3-(2-aminophenyl)propionic acid–FUDR conjugate **3c** according to procedure B as a white solid. Mp 151–153 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.30 (br s, 1H), 7.15–6.70 (m, 4H), 2.91 (t, 2H, J = 7.5 Hz), 2.57 (t, 2H, J = 7.5 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.9, 137.4, 128.2, 127.7, 123.9, 123.3, 115.5, 30.9, 25.6; IR (KBr) 3195.9, 3093.6, 2975.6, 2912.5, 1683.5, 1594.4, 1492.2, 1437.9, 1386.5, 1341.4, 1282.0, 1247.1, 1012.3, 1199.2, 1033.1, 816.2, 748.5, 682.0 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 148.1 (MH^+, 100.0), 120.0 (10.5).

3,3-Dimethyl-3,4-dihydro-1*H*-quinolin-2-one (5d). Reduction of 3-(2-nitrophenyl)-2,2-dimethylpropionic acid–FUDR conjugate **1d** according to procedure A afforded compound **5d** as a white solid. Mp 123–125 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.70 (br s, 1H), 7.11–6.65 (m, 4H), 2.74 (s, 2H), 1.41 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 176.6, 137.0, 128.7, 127.5, 122.5, 123.1, 114.7, 40.5, 37.5, 24.6; IR (KBr) 3198.4, 3071.8, 2965.2, 2925.4, 1671.1, 1596.6, 1492.7, 1389.5, 1258.5, 1094.0, 863.8, 758.4, 670.3 cm^{-1}; MS (FAB, *m*-NBA) *m*/*z* (rel intensity): 176.0 (MH^+, 100); HRMS calcd for $C_{11}H_{14}NO$ (MH^+) 176.1075, found 176.1056.

Kinetic study of cyclization at 37 °C. First, the UV spectra of FUDR, 2-aminophenylacetic acid–FUDR conjugate **3a** and 1,3-dihydroindol-2-one **5a** were acquired using 80 μM of each in 100 mM phosphate buffer (pH 7.4). The λ_{max} for FUDR is 268 nm. The λ_{max} for **3a** is 271 nm. The λ_{max} for **5a** is 248 nm. The maximum change in UV spectra before and after cyclization were found to be around 249 nm. Thus, subsequent kinetic studies on the cyclization of conjugates **1a–d** upon reduction were performed by measuring the absorbance change at 249 nm. A solution of the conjugates **1a–d** (10 mg) and 10% Pd/C (1 mg) in 4 mL of methanol underwent atmospheric hydrogenation for 4 min at room temperature. 1 mL of the reaction mixture was withdrawn into a cuvette and centrifuged. A 5-μL aliquot of the supernatant solution was diluted 200 times with pre-warmed phosphate buffer, pH 7.4 at 37 °C. The changes of the UV absorbance at 249 nm were monitored. The data was fitted to first-order decay kinetics ($y = y_0 + ae^{-kt}$) to calculate the kinetic constant and half-life of the cyclization activation process for each compound.

Determination of NADH stability in the incubation solution. NADH (0.1 mM) was incubated in 100 mM phosphate buffer (pH 7.0) at 37 °C, NADH (0.1 mM) in 10 mM phosphate buffer (pH 7.0) at 37 °C, and NADH (0.1 mM) in 10 mM phosphate buffer (pH 7.0) at 25 °C and the stability was monitored by UV–vis at 340 nm. The data were fitted to first order decay kinetics ($y = y_0 + ae^{-kt}$) to calculate the kinetic constants and half-lives of decay.

Reduction with *E. coli* B nitroreductase. The enzyme (1.5 μL of 1.18 mg/mL stock solution, 7 μg/mL) was added to the solution of the conjugates **1a–d** (2.5 μL of 10 mM stock solution in DMSO, 0.1 mM) and NADH (2.5 μL of 100 mM stock solution in phosphate, 1 mM) in 10 mM phosphate buffer (243.5 μL, pH 7.0) at 25 °C. Aliquots were withdrawn and analyzed by HPLC. The half life of reduction by *E. coli* nitroreductase was calculated based on the disappearance of the substrate. Control experiment was carried out under identical conditions without the enzyme. The products were confirmed by comparison with authentic samples and analytical LC–MS.

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