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One-Electron Reduction Characteristics of N(3)-Substituted 5-Fluorodeoxyuridines Synthesized as Radiation-Activated Prodrugs

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Abstract—We designed and synthesized N(3)-substituted 5-fluorodeoxyuridines as radiation-activated prodrugs of the antitumor agent, 5-fluorodeoxyuridine (5-FdUrd). A series of 5-FdUrd derivatives possessing a 2-oxoalkyl group at the N(3)-position released 5-FdUrd in good yield via one-electron reduction initiated by hypoxic irradiation. Cytotoxicity of the 5-FdUrd derivative possessing the 2-oxocyclopentyl group (**3d**) was low, but was enhanced by hypoxic irradiation resulting in 5-FdUrd release.

Introduction

The antitumor agent 5-fluorodeoxyuridine (5-FdUrd) is widely used for cancer chemotherapy.[1–3] However, clinical application of 5-FdUrd is limited because of its high toxicity toward normal cells as well as tumor cells. Therefore, the development of 5-FdUrd prodrugs that can be activated by ionizing radiation, photoirradiation,[4] or cancer associated proteases[5–7] are of great interest.

Recently, we demonstrated efficient one-electron reductive release of 5-fluorouracil (5-FU), an antitumor drug, from 5-fluoro-1-(2′-oxoalkyl)uracils upon hypoxic irradiation in aqueous medium where hydrated electrons are generated as active species for drug release.[8–11] The structure–reactivity relationships reported in our previous study revealed that one-electron reductive release of 5-FU was strongly affected by the carbonyl group and molecular flexibility of the substituent.[10]

Herein, we report a similar family of radiation-activated prodrugs of 5-FdUrd. 5-FdUrd is generally more toxic than 5-FU.[12–17] We synthesized 5-FdUrd derivatives possessing a 2-oxoalkyl group at the N(3)-position as prodrugs of 5-FdUrd and evaluated their reactivity following hypoxic irradiation. 5-FdUrd derivatives possessing a 2-oxocycloalkyl group released 5-FdUrd efficiently after irradiation, and laser flash photolysis studies of these compounds revealed that the generation of a stable radical anion was essential for the efficient release of 5-FdUrd. In addition, a biological assay using P388 T cells revealed that cytotoxicity of 5-FdUrd derivatives was enhanced by hypoxic irradiation resulting in the release of 5-FdUrd.

Results and Discussion

The general strategy for preparation of 5-FdUrd derivatives with a 2-oxoalkyl group at the N(3)-position is summarized in Scheme 1. Corresponding *a*-bromoketones were coupled with 2′-deoxy-3′,5′-di-*O*-acetyl-5-fluorouridine **1**[18] to obtain **2**. Hydrolysis of **2** under basic conditions furnished the desired N(3)-substituted 5-FdUrd **3a**–**d**. A control compound without a carbonyl group at the N(3)-position (**3e**) was prepared by reaction of **1** with 1-bromopropane.

We examined the one-electron reduction of **3a**–**d** during radiolysis in argon-purged aqueous solution containing 2-methyl-2-propanol to scavenge hydroxyl radicals. Under conditions of this radiolysis, hydrated electrons (e_{aq}^-) are generated as the major active species. As shown in Table 1, **3c** and **3d**, each possessing a 2-oxocycloalkyl

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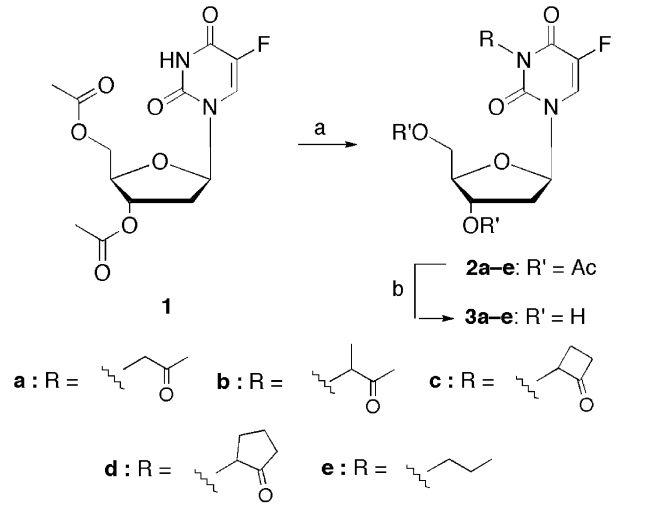


Scheme 1. [a]Reagents and conditions: (a) bromoacetone (for **2a**), 3-bromo-2-butanone (for **2b**), 2-bromocyclobutanone (for **2c**), 2-bromocyclopentanone (for **2d**), 1-bromopropane (for **2e**), NaH, DMF, 49% (for **2a**), 21% (for **2b**), 17% (for **2c**), 27% (for **2d**), 60% (for **2e**); (b) NaOH, H_2O, MeOH, 84% (for **3a**), 94% (for **3b**), 94% (for **3c**), 81% (for **3d**), 70% (for **3e**).

Table 1. G-Values for X-radiolysis of **3a–e** (1 mM) in aqueous solution containing 2-methyl-2-propanol (100 mM) under hypoxic conditions

Starting material	G×10^7 mol/J		Selectivity (%)[a]
	Consumption of starting material	Formation of 5-FdUrd	
3a	3.0	1.8	60
3b	3.4	1.6	49
3c	2.8	1.9	70
3d	1.9	1.5	78
3e	2.1	0	0

[a]Percentage ratio G(formation of 5-FdUrd)/G (consumption of starting material).

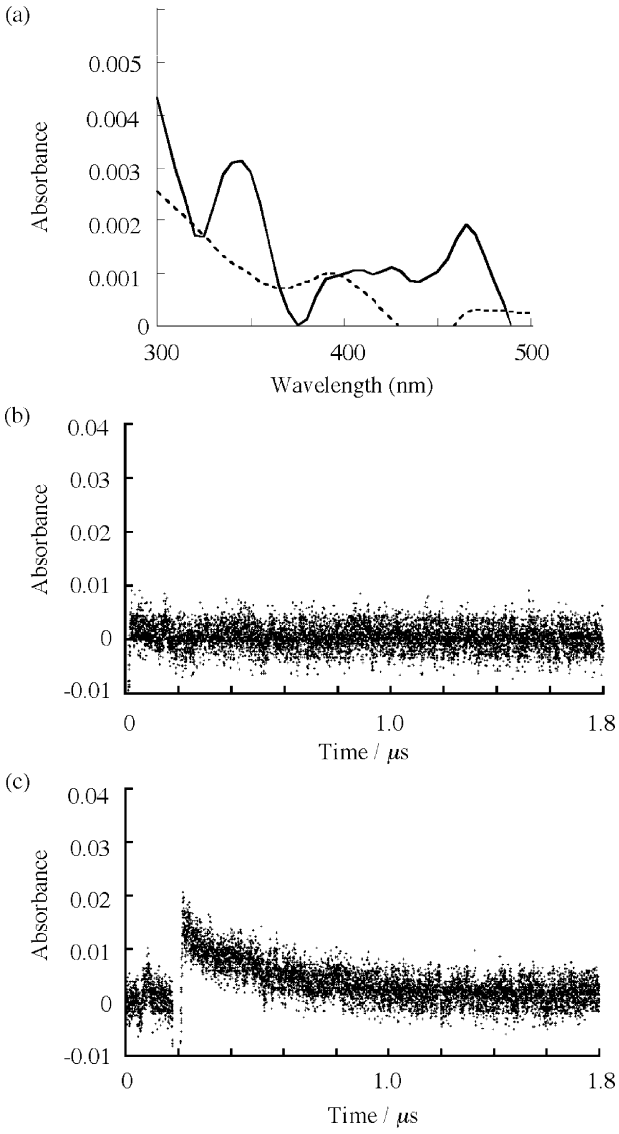


Figure 1. (a) Transient absorption spectra as observed (solid curve) 0.26 μs and (dotted curve) 1.8 μs after 266 nm-laser flash photolysis of 0.1 mM **3d** in the presence of 2.5 μM DMA. Time-courses of transient absorptions (b) for decay of radical anion of **3a** at 330 nm, (c) for decay of radical anion of **3d** at 330 nm.

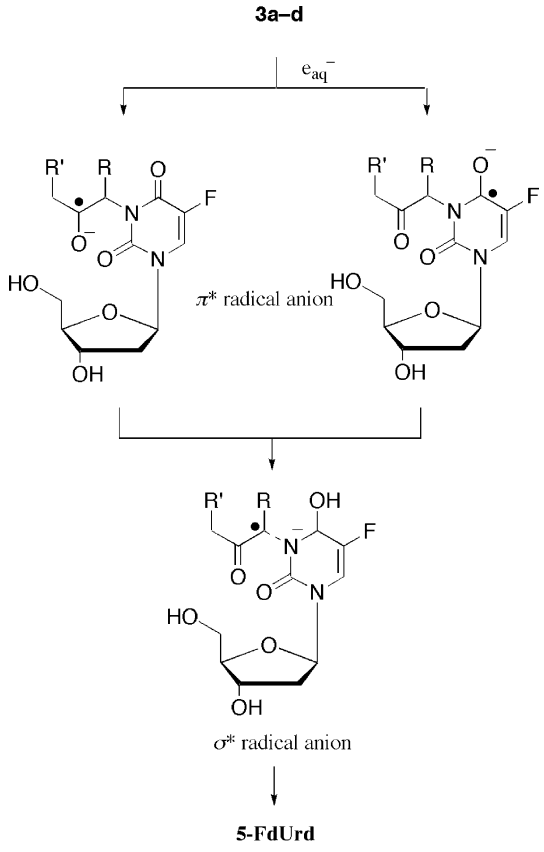
ring, exhibited efficient release of 5-FdUrd with 70–80% selectivity,[19] while **3a** and **3b**, which do not contain cycloalkyl rings showed lower efficiency of 50–60% selectivity.[19] To elucidate the reason for this difference, we investigated electrochemical and transient properties of these compounds. We measured reduction potentials ($E^{1/2}_{red}$ vs Ag/Ag^+) of **3a** and **3d** by cyclic voltammetry in DMF solution containing lithium perchlorate trihydrate as a supporting electrolyte. The cyclic voltammograms were recorded upon scanning the working electrode potentials between −2.5 and 0 V verses Ag/Ag^+. The half-wave reduction potentials of **3a** and **3d** were −1.83 and −1.79 V, respectively, indicating that these compounds have similar reactivity of capturing hydrate electrons. Laser flash photolysis of **3a** and **3d** also was conducted in the presence of dimethylaniline (DMA) as an electron donor to evaluate the stability of the intermediates of the one-electron reduction. Nanosecond laser flash photolysis was performed with argon-purged solutions containing the 5-FdUrd derivatives and DMA. The transient properties of DMA and the 5-FU derivatives have already been studied, and the radical cation of DMA (λ_{max} = 460 nm) and radical anion of 5-FU (λ_{max} = 330 nm) have been identified by laser flash photolysis[20] and pulse radiolysis.[21] As shown in Figure 1, laser flash photolysis of **3a** and **3d** with DMA showed characteristic absorption maxima at 330 nm assigned to the radical anion of **3a** and **3d**, and at 450 nm assigned to the radical cation of DMA. The radical anion of **3d** decayed into secondary transients with a rate constant of $k = 1.22\times10^6$ s^{-1} (Fig. 1c). In contrast, the radical anion of **3a** decayed rapidly with a rate constant of $k = 85.3\times10^6$ s^{-1} (Fig. 1b). These results suggest that the radical anion of **3d** is more stable than that of **3a**. Therefore, it appears that the generation of a stable radical anion is essential for the efficient release of 5-FdUrd from an N(3)-substituted 5-FdUrd.

A control experiment confirmed that hypoxic irradiation of an N(3)-substituted 5-FdUrd without a carbonyl group (**3e**) in aqueous solution failed to release 5-FdUrd, indicating that the carbonyl group of **3a–d** is indis-

pensable for release of 5-FdUrd. A previous mechanistic study of the radiolytic release of 5-FU from N(1)-substituted 5-FU demonstrated that an N(1)-substituted 5-FU without a carbonyl group did not release 5-FU and formation of the C=O π^* radical anion is essential for radiation-induced release of 5-FU in sufficient yield.[10] Based on these results, it is reasonable to presume that radiolytic reduction of N(3)-substituted 5-FdUrd derivatives would proceed by a similar mechanism to release 5-FU, as shown in Scheme 2.

To investigate the effect of molecular oxygen on the radiolytic reactivity of the N(3)-substituted 5-FdUrd derivatives, we examined radiolysis of **3a** in aerobic aqueous solution. Despite the efficient release of 5-FdUrd from N(3)-substituted 5-FdUrd derivatives under hypoxic conditions, the release of 5-FdUrd from **3a** under oxic conditions was unsuccessful, resulting in decomposition of the starting material **3a**. This result indicates that the active species, hydrated electrons (e_{aq}^-) that are scavenged by dissolved molecular oxygen, are necessary for release of 5-FdUrd.

Understanding the biological activity of N(3)-substituted 5-FdUrd derivatives is important for determining their clinical relevance. We examined the cytotoxicity of 5-FdUrd derivative **3d** against P388 T cells. As shown in Figure 2, cytotoxicity of **3d** was low before irradiation, and was enhanced by hypoxic irradiation, suggesting that 5-FdUrd derivatives possessing a 2-oxoalkyl group at the N(3)-position are not toxic,



Scheme 2.

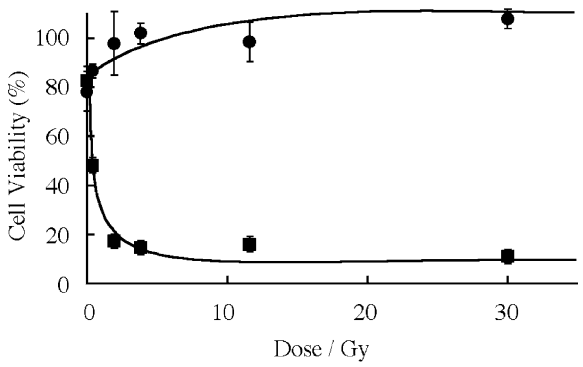


Figure 2. Radiation-induced cytotoxity of **3d** against P388 T cells. RPMI 1640 medium containing 0.04 mM **3d** (■) or without **3d** (●) was irradiated, and then added to P388 T cells.

but become toxic only upon hypoxic irradiation as 5-FdUrd is released. Thus, these 5-FdUrd derivatives possessing a 2-oxoalkyl group at the N(3)-position were useful as radiation-activated prodrugs of 5-FdUrd.

Conclusion

In summary, we investigated the radiolytic release of 5-FdUrd from N(3)-substituted 5-FdUrd derivatives. A series of 5-FdUrd derivatives, **3a**–**d**, released 5-FdUrd upon hypoxic irradiation in fair yields. The N(3)-substituted 5-FdUrd derivatives possessing 2-oxocycloalkyl groups exhibited especially efficient release of 5-FdUrd. Studies on transient properties revealed that 5-FdUrd derivative possessing a 2-oxocycloalkyl group, such as **3d**, formed a stable radical anion intermediate upon hypoxic irradiation, resulting in efficient release of 5-FdUrd. Furthermore, hypoxic irradiation of **3d** was cytotoxic toward P388 T cells as a result of 5-FdUrd release. Thus, these N(3)-substituted 5-FdUrd derivatives are promising candidates as radiation-activated prodrugs of 5-FdUrd for the treatment of hypoxic tumor cells.

Experimental

General methods

^{1}H NMR spectra were measured with JEOL JNM-AL 300 (300 MHz), JEOL JMN-EX-400 (400 MHz) or JEOL JNM-A500 (500 MHz) spectrometers. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform ($\delta = 7.24$ in ^{1}H NMR) and residual dimethylsulfoxide ($\delta = 2.49$ in ^{1}H NMR) as internal standards. ^{13}C NMR spectra were measured with JEOL JNM-AL 300 (300 MHz), JEOL JMN-EX-400 (400 MHz) or JEOL JNM-A500 (500 MHz) spectrometers. Mass spectra were recorded on a JEOL JMS-SX102A spectrometer. Rigaku Radioflex-350 was used for X-radiolysis. A Wakogel C-200 was used for silicagel chromatography. Precoated TLC plates Merck silica gel 60 F_{254} was used for monitoring the reactions and also for preparative TLC. *N,N*-Dimethylformamide (DMF) was distilled under reduced pressure and acetonitrile was dried over P_2O_5

followed by distillation. All other reagents and solvents were used as received. Cyclic voltammetry were performed with Electrochemical Analyzer Model 660-A (BAS, Japan).

2′-Deoxy-3′,5′-di-*O*-acetyl-5-fluoro-3-(2′-propyl)uridine (2e). General procedure for alkylation of 2′-deoxy-3′,5′-di-*O*-acetyl-5-fluorouridine at N(3)-position. 2′-Deoxy-3′,5′-di-*O*-acetyl-5-fluorouridine[18] **1** (227 mg, 0.69 mmol) was added to a suspension of sodium hydride (53 mg, 1.33 mmol) in anhydrous DMF (30 mL) at 0 °C and the mixture was stirred at 0 °C for 40 min. To the resulting mixture was added 1-bromopropane (0.065 mL, 0.71 mmol) and the mixture was stirred at ambient temperature for 15 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (SiO_2, 5% MeOH/$CHCl_3$) to give **2e** (223 mg, 60%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, 3H, J = 7.5 Hz), 1.68 (quint, 2H, J = 7.5 Hz), 2.11 (s, 3H), 2.14 (s, 3H), 2.10–2.22 (m, 2H), 2.50–2.73 (m, 1H), 3.89–3.94 (m, 2H), 4.26–4.42 (3H), 5.19–5.24 (m, 1H), 6.33 (t, 1H, J = 6.3 Hz), 7.61 (d, 1H, J = 5.7 Hz); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 11.1, 20.6, 20.8, 37.8, 43.3, 63.6, 73.7, 82.2, 85.7, 121.0 (d, J = 33.1 Hz), 140.2 (d, J = 235.3 Hz), 149.0, 156.7 (d, J = 23.9 Hz), 170.0, 170.2; FABMS (glycerol) m/z 373 [(M + H)$^+$]; HRMS calcd for $C_{16}H_{22}N_2O_7F$ [(M + H)$^+$] 373.1411, found 373.1425.

2′-Deoxy-3′,5′-di-*O*-acetyl-5-fluoro-3-(2′-oxopropyl)uridine (2a). According to the method detailed for **2e** the reaction of bromoacetone (424 mg, 3.10 mmol) gave **2a** (586 mg, 49%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.03 (s, 3H), 2.07 (s, 3H), 2.10–2.17 (m, 1H), 2.19 (s, 3H), 2.46 (ddd, 1H, J = 14.0, 6.0, 2.4 Hz), 4.20–4.34 (3H), 4.69 (s, 2H), 5.13–5.15 (m, 1H), 6.20 (t, 1H, J = 6.4 Hz), 7.65 (d, 1H, J = 5.9 Hz); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 20.6, 20.7, 27.0, 37.8, 50.0, 63.5, 73.7, 82.4, 86.0, 121.8 (d, J = 33.1 Hz), 140.0 (d, J = 235.3 Hz), 148.7, 156.1 (d, J = 27.6 Hz), 170.0, 170.1, 199.2; FABMS (glycerol) m/z 387 [(M + H)$^+$]; HRMS calcd for $C_{16}H_{20}N_2O_8F$ [(M + H)$^+$] 387.1204, found 387.1207.

2′-Deoxy-3′,5′-di-*O*-acetyl-5-fluoro-3-(1′-methyl-2′-oxopropyl)uridine (2b). According to the method detailed for **2e** the reaction of 3-bromo-2-butanone (302 mg, 2.00 mmol) gave **2b** (168 mg, 21%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.49–1.52 (m, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.11–2.17 (m, 1H), 2.44–2.49 (m, 1H), 4.21–4.34 (3H), 5.13–5.18 (2H), 6.21 (t, 1H, J = 6.4 Hz), 7.64 (d, 1H, J = 5.6 Hz); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 13.5, 13.6, 20.6, 20.6, 26.1, 37.7, 57.0, 57.1, 63.5, 73.5, 82.3, 85.8, 121.8 (d, J = 34.9 Hz), 140.0 (d, J = 235.3 Hz), 148.4, 148.4, 156.1 (d, J = 25.7 Hz), 156.2 (d, J = 25.8 Hz), 170.1, 202.1, 202.2; FABMS (glycerol) m/z 401 [(M + H)$^+$]; HRMS calcd for $C_{17}H_{22}N_2O_8F$ [(M + H)$^+$] 401.1360, found 401.1370.

2′-Deoxy-3′,5′-di-*O*-acetyl-5-fluoro-3-(2′-oxocyclobutyl)uridine (2c). According to the method detailed for **2e** the reaction of 2-bromocyclobutanone (149 mg, 1.00 mmol) gave diastereomixture **2c** (72 mg, 17%) as a white oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.05–2.16 (m, 1H), 2.08, 2.08 (s×2, total 3H), 2.11, 2.12 (s×2, total 3H), 2.39 (ddd, 1H, J = 20.8, 10.8, 4.8 Hz), 2.43–2.55 (m, 2H), 2.93–3.03 (m, 1H), 3.20–3.30 (m, 1H), 4.24–4.40 (3H), 5.18 (m, 1H), 5.86 (m, 1H), 6.24 (m, 1H), 7.63, 7.65 (d×2, total 1H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 17.0, 17.1, 20.7, 20.8, 37.9, 38.0, 42.7, 62.8, 63.0, 63.6, 63.7, 73.7, 82.6, 82.6, 86.0, 86.2, 121.7 (d, J = 35.0 Hz), 121.8 (d, J = 35.0 Hz), 140.1 (d, J = 236.8 Hz), 140.2 (d, J = 237.6 Hz), 148.2, 148.2, 155.7 (d, J = 25.4 Hz), 155.8 (d, J = 26.2 Hz), 170.0, 170.3, 170.3, 202.1, 202.3; FABMS (glycerol) m/z 399 [(M + H)$^+$]; HRMS calcd for $C_{17}H_{20}N_2O_8F$ [(M + H)$^+$] 399.1204, found 399.1214.

2′-Deoxy-3′,5′-di-*O*-acetyl-5-fluoro-3-(2′-oxocyclopentyl)uridine (2d). According to the method detailed for **2e** the reaction of 2-bromocyclopentanone (212 mg, 1.30 mmol) gave **2d** (144 mg, 27%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.77–1.90 (m, 1H), 2.02 (s, 3H), 2.06 (s, 3H), 2.10–2.45 (m, 7H), 4.20–4.33 (3H), 4.87–5.14 (2H), 6.11–6.25 (m, 1H), 7.61 (brs, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 19.0, 20.6, 20.7, 25.6, 35.7, 37.7, 57.1, 58.6, 63.5, 73.7, 82.4, 85.6, 86.0, 121.6 (d, J = 35.0 Hz), 140.1 (d, J = 237.2 Hz), 147.7, 149.3, 155.9 (d, J = 143.4 Hz), 156.2 (d, J = 139.7 Hz), 169.9, 170.1, 211.3; FABMS (glycerol) m/z 413 [(M + H)$^+$]; HRMS calcd for $C_{18}H_{22}N_2O_8F$ [(M + H)$^+$] 413.1360, found 413.1365.

2′-Deoxy-5-fluoro-3-(2′-propyl)uridine (3e) (General procedure for hydrolysis). To a solution of **2e** (153 mg, 0.41 mmol) in methanol (1.5 mL) and water (4.1 mL) was added sodium hydroxide (16 mg, 0.41 mmol) and the mixture was stirred at 0 °C for 30 min. The reaction mixture was concentrated in vacuo and the crude product was purified by column chromatograpy (SiO_2, 5% MeOH/$CHCl_3$) to give **3e** (83 mg, 70%) as a white oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, 3H, J = 7.3 Hz), 1.65 (quint, 2H, J = 7.3 Hz), 2.10–2.34 (4H), 3.86–4.05 (5H), 4.57–4.61 (m, 1H), 6.31 (t, 1H, J = 6.4 Hz), 7.89 (d, 1H, J = 5.9 Hz); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 11.3, 20.8, 40.9, 43.4, 62.3, 71.6, 86.8, 86.9, 122.7 (d, J = 35.0 Hz), 140.2 (d, J = 233.5 Hz), 149.3, 157.1 (d, J = 25.7 Hz); FABMS (glycerol) m/z 289 [(M + H)$^+$]; HRMS calcd for $C_{12}H_{18}N_2O_5F$ [(M + H)$^+$] 289.1200, found 289.1213.

2′-Deoxy-5-fluoro-3-(2′-oxopropyl)uridine (3a). According to the method detailed for **3e** the reaction of **2a** (582 mg, 1.51 mmol) gave **3a** (383 mg, 84%) as a white oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.12–2.16 (m, 2H), 2.20 (s, 3H), 3.55–3.66 (m, 2H), 3.79–3.82 (m, 1H), 4.23–4.27 (m, 1H), 4.70 (s, 2H), 5.19 (t, 1H, J = 4.9 Hz), 5.27 (t, 1H, J = 4.0 Hz), 6.15 (t, 1H, J = 6.0 Hz), 8.38 (t, 1H, J = 7.3 Hz); ^{13}C NMR (DMSO–d_6, 400 MHz) δ 27.3, 40.1, 50.3, 61.1, 70.2, 85.8, 87.9, 124.2 (d, J = 34.9 Hz), 139.4 (d, J = 229.8 Hz), 148.8, 156.1 (d, J = 27.5 Hz), 201.2; FABMS (glycerol) m/z 303 [(M + H)$^+$]; HRMS calcd for $C_{12}H_{16}N_2O_6F$ [(M + H)$^+$] 303.0992, found 303.1006.

2′-Deoxy-5-fluoro-3-(1′-methyl-2′-oxopropyl)uridine (3b). According to the method detailed for **3e** the reaction of **2b** (162 mg, 0.40 mmol) gave **3b** (94 mg, 74%) as a white

oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.39 (d, 3H, J = 6.8 Hz), 2.04 (s, 3H), 2.15 (dd, 2H, J = 6.4, 4.8 Hz), 3.55–3.67 (2H), 3.80 (q, 1H, J = 3.5 Hz), 4.25 (quint, 1H, J = 3.5 Hz), 5.13–5.18 (2H), 5.24 (d, 1H, J = 3.5 Hz), 6.13 (m, 1H), 8.37 (m, 1H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 13.1, 25.9, 25.9, 39.9, 40.0, 56.4, 60.7, 60.7, 69.7, 69.8, 85.6, 85.6, 87.6, 124.1 (d, J = 35.1 Hz), 124.1 (d, J = 35.1 Hz), 139.3 (d, J = 228.9 Hz), 148.4, 156.0 (d, J = 26.7 Hz), 202.6, 202.6; FABMS (glycerol) m/z 317 [(M + H)$^+$]; RMS calcd for $C_{13}H_{18}N_2O_6F$ [(M + H)$^+$] 317.1149, found 317.1145.

2′ - Deoxy - 5 - fluoro - 3 - (2′ - oxocyclobutyl)uridine (3c). According to the method detailed for **3e** the reaction of **2c** (70 mg, 0.17 mmol) gave **3c** (50 mg, 94%) as a white oil: ^{1}H NMR (acetone-d_6, 400 MHz) δ –2.38 (3H), 2.40–2.49 (1H), 2.96–3.14 (2H), 3.78–3.88 (2H), 3.97 (ddd, 1H, J = 1.1, 2.9, 6.0 Hz), 4.43–4.45 (2H), 4.48–4.52 (1H), 5.86–5.92 (1H), 6.24–6.28 (1H), 8.38 (m, 1H); ^{13}C NMR (acetone-d_6, 400 MHz) δ 17.3, 17.3, 41.5, 41.5, 41.6, 41.6, 42.8, 62.2, 62.2, 62.3, 62.3, 63.8, 63.9, 71.4, 71.5, 71.6, 71.6, 87.1, 87.1, 88.9, 88.9, 124.6 (d, J = 35.1 Hz), 140.7 (d, J = 230.4 Hz), 149.4; FABMS (glycerol) m/z 315 [(M + H)$^+$]; HRMS calcd for $C_{13}H_{16}N_2O_6F$ [(M + H)$^+$] 315.0992, found 315.1000.

2′ - Deoxy - 5 - fluoro - 3 - (2′ - oxocyclopentyl)uridine (3d). According to the method detailed for **3e** the reaction of **2d** (146 mg, 0.35 mmol) gave **3d** (93 mg, 81%) as a yellow oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.87–2.00 (1H), 2.12–2.45 (7H), 3.82 (m, 2H), 3.96–4.00 (1H), 4.42–4.51 (2H), 4.43 (t, 1H, J = 5.2 Hz), 4.90–5.20 (br, 1H), 6.18–6.42 (br, 1H), 8.34 (d, 1H, J = 6.8 Hz); ^{13}C NMR (acetone-d_6, 400 MHz) δ 19.6, 26.2, 36.3, 41.4, 41.5, 57.4, 59.0, 62.3, 71.6, 86.8, 87.2, 88.8, 124.4 (d, J = 34.9 Hz), 140.7 (d, J = 229.8 Hz), 148.8, 150.5; FABMS (glycerol) m/z 329 [(M + H)$^+$]; HRMS calcd for $C_{14}H_{18}N_2O_6F$ [(M + H)$^+$] 329.1149, found 329.1142.

Cyclic voltammetry experiment

Cyclic voltammetry were performed in a one component cell with a glassy carbon working electrode, a platinum counter electrode and a silver–silver chloride reference electrode. Measurements were made with a BAS 600 A electrochemical analyzer. The cell contained 2 mM prodrugs and 0.1 M lithium perchlorate as supporting electrolyte in dimethylformamide. The scan rate was 100 mV/s.

Radiolytic reduction

Aqueous solutions of the N(3)-substituted FdUrd **3a–e** (1 mM) containing 2-methyl-2-propanol (100 mM) were purged with argon for 20 min and then irradiated in a sealed glass ampoule at ambient temperature with an X-ray source (5 Gy min^{-1}). After the irradiation, the solution was immediately subjected to HPLC analysis.

Laser flash photolysis

The laser flash photolysis experiments were carried out with Unisoku TSP-601 flash spectrometer. A Continuum Surelite-I Nd-YAG (Q-switched) laser with the fourth harmonic at 266 nm was employed for the flash photoirradiation. The probe beam from Hamamatsu 150 W xenon short arc was guided with an optical fiber scope to be arranged in an orientation perpendicular to the exciting laser beam. The probe beam was monitored with a Unisoku MD200 photomultiplier tube through a Hamamatsu image intesifier controller DG535 (1024 photodiodes). Timing of the exciting pulse laser, the probe beam and the detection system was achieved through a tektronix model TDS 3012 digital phosphor oscilloscope that was interfaced to an NEC Windows98 computer. Aqueous solutions of **3a** (0.1 mM), **3d** (0.1 mM) and DMA (2.5 μM) were deaerated by argon bubbling prior to the laser flash photolysis.

Radiation-induced cytotoxity of 3d

P388 T cells (mouse leukemia T cell) was used for the cytotoxity test in vitro. RPMI 1640 medium containing 0.04 mM **3d** was irradiated and then added to P388 T cells which were grown in RPMI 1640 medium supplemented with 0.05 mM 2-mercaptoethanol and 10% FBS. After 3 days exposure to a drug at 37 °C in a 5% CO_2 incubator, 10 μL of MTT [3-(4′,5′-dimethylthiazol-2′ - yl) - 2,5 - diphenyltetrazolium bromide] solution was added to the suspension. The cytotoxicity was tested by a MTT assay.[22]

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