Stereoelectronic Effect on One-Electron Reductive Release of 5-Fluorouracil from 5-Fluoro-1-(2′-oxocycloalkyl)uracils as a New Class of Radiation-Activated Antitumor Prodrugs

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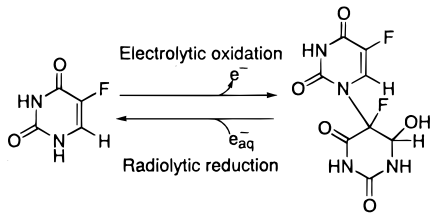
A series of 5-fluoro-1-(2′-oxocycloalkyl)uracils (**3**−**11**) that are potentially novel radiation-activated prodrugs for the radiotherapy of hypoxic tumor cells have been synthesized to evaluate a relationship between the molecular structure and the reactivity of one-electron reductive release of antitumor 5-fluorouracil (**1**) in anoxic aqueous solution. All the compounds **3**−**11** bearing the 2′-oxo group were one-electron reduced by hydrated electrons (e_{aq}^-) and thereby underwent C(1′)−N(1) bond dissociation to release 5-fluorouracil **1** in 47−96% yields upon radiolysis of anoxic aqueous solution, while control compounds (**12**, **13**) without the 2′-oxo substituent had no reactivity toward such a reductive C(1′)−N(1) bond dissociation. The decomposition of 2-oxo compounds in the radiolytic one-electron reduction was more enhanced, as the one-electron reduction potential measured by cyclic voltammetry in *N*,*N*-dimethylformamide became more positive. The efficiency of 5-fluorouracil release was strongly dependent on the structural flexibility of 2-oxo compounds. X-ray crystallographic studies of representative compounds revealed that the C(1′)−N(1) bond possesses normal geometry and bond length in the ground state. MO calculations by the AM1 method demonstrated that the LUMO is primarily localized at the π^* orbital of C(5)−C(6) double bond of the 5-fluorouracil moiety, and that the LUMO + 1 is delocalized between the π^* orbital of 2′-oxo substituent and the σ^* orbital of adjacent C(1′)−N(1) bond. The one-electron reductive release of 5-fluorouracil **1** in anoxic aqueous solution was presumed to occur from the LUMO + 1 of radical anion intermediates possessing a partial mixing of the antibonding C(2′)═O π^* and C(1′)−N(1) σ^* MO's, that may be facilitated by a dynamic conformational change to achieve higher degree of ($\pi^* + \sigma^*$) MO mixing.

Introduction

We recently reported electrochemical synthesis of a novel N(1)−C(5′)-linked dimer, 1-(5′-fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-fluorouracil (**2**), by anodic one-electron oxidation of 5-fluorouracil (**1**) in Ar-purged aqueous solution containing NaCl as a supporting electrolyte (Scheme 1).[1] The dimer **2** was also identified to show a formally reverse reactivity, undergoing one-electron reduction to release 5-fluorouracil **1** upon radiolysis in anoxic aqueous solution (Scheme 1).[1] Among the water radicals generated in the radiolysis,[2] the reducing species of hydrated electrons (e_{aq}^-) are responsible for the reductive release of **1**.[1] Thus, the yield of **1** is significantly diminished in the radiolysis of aerobic aqueous solution,[1] since the hydrated electrons e_{aq}^- are readily scavenged by dissolved oxygen to form less-active superoxide radical ions ($O_2^{-\cdot}$).[2] In view of a well-specified antitumor action of **1**,[3] the characteristic one-electron reduction reactivity of releasing **1** may display a potential that the N(1)−C(5′)-linked 5-fluorouracil dimer **2** can be a radiation-activated prodrug of exhibiting selective toxicity toward hypoxic tumor cells[4] under radiation treatment. Actually, as demonstrated by a tumor growth-delay assay, **2** had no antitumor effect in contrast to **1**, but could potentiate the radiotherapy to inhibit the tumor growth to considerable extent.[1]

Scheme 1



In attempting the molecular design by reference to the prototype compound **2** for a new class of radiation-activated prodrugs that release more efficiently antitumor 5-fluorouracil **1** under anoxic or hypoxic irradiation, our attention has been directed to the presence of an electron-accepting carbonyl group in the α position with respect to the leaving 5-fluorouracil-1-yl group in the compound **2**. This aspect is associated with the previous

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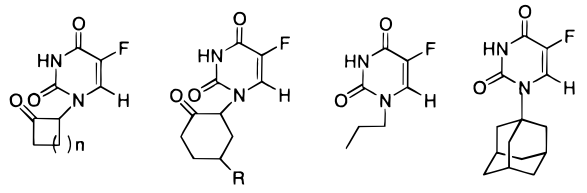
Chart 1



3-8 (n = 0-4, 9) **9**: R = Me **12** **13**

10, **11**: R = *t*-Bu

reports by Rossi and co-workers[5,6] that bridgehead chlorides of 2-oxo-substituted bicyclic compounds are replaced with diphenyl phosphide ions (Ph_2P^-) by a radical nucleophilic substitution ($S_{RN}1$) mechanism[7] under photoirradiation in liquid ammonia, while analogues without the 2-oxo substituent are unreactive under similar conditions. Most commonly the $S_{RN}1$ mechanism involves the formation of a radical anion intermediate of the substrates in the initiation step.[7] It has been postulated that the antibonding π^* MO of 2-oxo substituent as the LUMO of substrates accepts an electron to form the C═O π^* radical anion, then transferring the electron intramolecularly to the antibonding C−Cl σ^* MO which results in an electron-capturing dissociation of C−Cl bond to form the carbon-centered radical and chloride ion.[5,6] There is another possibility that a mixing of the C═O π^* MO and the C−Cl σ^* MO may be predominant and thereby the radical anion intermediate favors spontaneous dissociation of the C−Cl bond.[8]

To get structural insight into the mechanism by which radiation-activated prodrugs analogous to the N(1)−C(5′)-linked dimer **2** undergo one-electron reduction to release 5-fluorouracil **1**, we synthesized 2-oxopropane (**3**) and a series of 2-oxocycloalkanes (**4**−**11**) with a common leaving 5-fluorouracil-1-yl group and evaluated their one-electron reduction reactivity in comparison with those of control compounds (**12** and **13**) bearing no 2′-oxo substituent (see Chart 1). For better understanding of a possible overlapping or mixing between the C(2′)═O π^* and the C(1′)−N(1) σ^* MO's in the radical anion intermediate, X-ray crystallographic and computational studies of representative 2-oxo compounds were also performed. In this paper, we disclose a definitive function of the 2′-oxo substituent of 5-fluoro-1-(2′-oxocycloalkyl)uracils in the radiation-activated reductive release of 5-fluorouracil **1** in anoxic aqueous solution, that may occur from the LUMO + 1 of radical anion intermediates with a partial mixing of the antibonding C(2′)═O π^* and C(1′)−N(1) σ^* MO's. The efficiency of 5-fluorouracil release is also shown to be enhanced by a dynamic conformational change to achieve higher degree of ($\pi^* + \sigma^*$) MO mixing.

Results and Discussion

Synthesis and Reduction Reactivity. 5-Fluoro-1-(2′-oxopropyl)uracil **3** and 5-fluoro-1-(2′-oxocycloalkyl)-uracils **4**−**10** were easily prepared by the reaction of 5-fluoro-2,4-bis(trimethylsiloxy)pyrimidine with 1-chloro-2-oxo compounds (for **5**, **7**, and **8**) or 1-bromo-2-oxo compounds (for **3**, **4**, **6**, **9**, and **10**).[9] *trans*-5-Fluoro-1-(5′-*tert*-butyl-2′-oxocyclohexyl)uracil **11** was synthesized in a similar manner, but was obtained only from *cis*-2-bromo-4-*tert*-butylcyclohexanone. Control compounds **12** and **13** without the 2′-oxo group were prepared by the reaction of 5-fluoro-2,4-bis(trimethylsiloxy)pyrimidine with 1-chloropropane and 1-adamanthyl trifrate,[10] respectively.

Table 1. X-Radiolysis of 2-oxo Compounds 3−11 (1 mM) in Phosphate Buffer Solution Containing 2-Methyl-2-propanol (1 M)

	$G \times 10^7$, mol J^{-1}			
substrate	−substrate	5-fluorouracil	% selectivity[a]	$E_{red}^{1/2}$, V[b]
3	2.6	1.6	59	−0.48
4	2.3	1.1	47	−0.61
5	2.2	2.1	96	−0.63
6	2.0	1.9	93	−0.74
7	2.5	1.5	58	−0.55
8	2.5	1.5	59	−0.56
9	2.1	1.3	61	−0.72
10	2.4	1.3	54	−0.54
11	2.7	1.4	53	−0.71

[a] Defined as the ratio G(5FU)/G(-substrate). [b] One-electron reduction potential vs Ag/Ag^+ in DMF as measured by cyclic voltammetry.

Reduction potentials ($E_{red}^{1/2}$ vs Ag/Ag^+) of the 2-oxo compounds **3**−**11** were measured by means of cyclic voltammetry in Ar-purged *N*,*N*-dimethylformamide (DMF) solution containing 0.1 M tetra-*n*-butylammonium bromide (TBAB) as a supporting electrolyte. The cyclic voltammograms were recorded upon scanning the working electrode potentials between −1.5 and 0 V vs Ag/Ag^+ at a rate of 500 mV s^{-1}. In this potential range all the 2-oxo compounds, except for **5** and **6**, displayed a single reversible redox process assigned to a redox couple of each 2-oxo compound and its one-electron reduced anion radical form, from which the corresponding one-electron reduction potentials were evaluated as listed in Table 1. In contrast to these 2-oxo compounds, 5-fluoro-1-(2′-oxocyclopentyl)uracil **5** and 5-fluoro-1-(2′-oxocyclohexyl)-uracil **6** showed irreversible cathodic reduction peak potentials (Ep_c) at −0.74 V ($E_{red}^{1/2} = -0.63$ V) and −0.86 V ($E_{red}^{1/2} = -0.74$ V) vs Ag/Ag^+, respectively (Table 1). A common behavior of all compounds including **5** and **6** was that an oxidation peak appeared at −0.38 V vs Ag/Ag^+ in the reverse anodic scan. This characteristic peak is most likely attributable to the oxidation of N(1)-deprotonated 5-fluorouracil anion (**1**(-H)$^-$) that could be a common product derived from the one-electron reductive C(1′)−N(1) bond dissociation of 2-oxo compounds **3**−**11** in an aprotic solvent of DMF. According to the above electrochemical characterization, it is plausible that one-electron reduction of the 2-oxo compounds **3**−**11** may release N(1)-deprotonated 5-fluorouracil anion **1**(-H)$^-$, while producing rather stable radical anion intermediates at least in aprotic solvents. In the latter regard, a separate pulse radiolysis study[11] demonstrated that the N(1)−C(5′)-linked dimer **2** is one-electron reduced by hydrated electrons in aqueous solution to form a consid-

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(7) (a) Bunnet, J. F. *Acc Chem Res.* **1978**, *11*, 413−420. (b) Bowman, W. R. *Chem. Soc. Rev.* **1988**, 17, 283−316.
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(9) Akashi, M.; Beppu, K.; Kikuchi, I.; Miyauchi, N. *J. Macromol. Sci., Chem.* **1986**, *A23*, 1233−1249.
(10) Takeuchi, K.; Moriyama, T.; Kinoshita, T.; Tachino, H.; Okamoto, K. *Chem. Lett.* **1980**, 1395−1398.

erably stable electron adduct at the 5-fluorouracil moiety with a lifetime of 8 ms.

For characterizing the one-electron reduction reactivity, X-radiolyses of 2-oxo compounds **3**−**11** (1 mM) in Ar-purged phosphate buffer solutions containing excess amount (1 M) of 2-methyl-2-propanol were carried out at room temperature. In the radiolysis of a diluted aqueous solution, water radicals such as oxidizing hydroxyl radicals (•OH), reducing hydrated electrons (e_{aq}^-), and reducing hydrogen atoms (H•) are primarily generated with the *G* values[12] of $G(^{\bullet}OH) = 2.8 \times 10^{-7}$ mol J^{-1}, $G(e_{aq}^-) = 2.8 \times 10^{-7}$ mol J^{-1}, and $G(H^{\bullet}) = 0.6 \times 10^{-7}$ mol J^{-1} (reaction 1). Under the present conditions, the OH radicals are scavenged by excess 2-methyl-2-propanol into substantially unreactive 2-methyl-2-propanol radicals ($^{\bullet}CH_2(CH_3)_2COH$) as in reaction 2. Thus, the hydrated electrons e_{aq}^- ($E(nH_2O/e_{aq}^-) = -2.9$ V vs NHE[13] at pH 7.0) are involved as the reductants for **3**−**11**. Smaller amount of the hydrogen atoms H• ($E(H^+/H^{\bullet}) = -2.4$ V vs NHE[13] at pH 7.0) would make relatively minor contribution to the reaction of **3**−**11**, although they add to the double bonds of pyrimidines.[14]

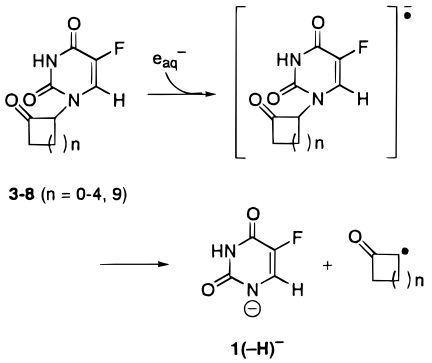
$$H_2O \rightarrow {}^{\bullet}OH + H^{\bullet} + e_{aq}^- \quad (1)$$

$$^{\bullet}OH + (CH_3)_3COH \rightarrow H_2O + {}^{\bullet}CH_2(CH_3)_2COH \quad (2)$$

In the case of water-insoluble compound **13**, Ar-purged methanol solution was similarly X-irradiated. This reaction system involves solvated electrons e_{MeOH}^- and reducing radicals $^{\bullet}CH_2OH$ for the reduction of **13**, since the concomitant primary radicals such as $CH_3O^{\bullet}$, H•, •OH, and $CH_3^{\bullet}$ react rapidly with methanol to produce additionally the reducing radicals $^{\bullet}CH_2OH$.[15]

Analytical HPLC of the X-irradiated solutions by reference to authentic samples indicated that all the 2-oxo compounds **3**−**11** release 5-fluorouracil **1** as a major product upon reduction by hydrated electrons e_{aq}^-. A few fragments with higher polarity were also produced in each reaction, as eluted faster in the HPLC. These products may be derived from degradation such as pyrimidine ring opening reaction, although they could not be identified. The residual 2-oxoalkane moiety resulted from the 5-fluorouracil release is also a possible product, but could not be detected by NMR analysis of each reaction mixture obtained after evaporation. Table 1 lists the *G* values for the decomposition of **3**−**11** and the formation of 5-fluorouracil **1** in the X-radiolytic reduction. In a separate experiment we also confirmed that 5-fluoro-1-propyluracil **12**, as a non-2-oxo analogue of **3**, did not release **1** upon X-irradiation up to 80% decomposition under similar conditions. Furthermore, X-radiolysis of **13** in Ar-purged methanol up to 90% decomposition resulted in no free 5-fluorouracil **1**. For comparison, representative X-radiolysis of **6** in Ar-purged methanol was performed to obtain the *G* values for the decomposition of **6** and the formation of **1** as 1.2 and 0.78 (65% selectivity), respectively. These results may be rationalized by a mechanism involving one-electron reductive dissociation of the C(1′)−N(1) bond of **3**−**11** into N(1)-deprotonated 5-fluorouracil anion **1**(-H)$^-$ and 2-oxocycloalkyl radical (2-oxopropyl radical in the case of **3**), as illustrated in Scheme 2. Evidently, 2′-oxo group is an essential part of the radiation-activated antitumor prodrugs that undergo one-electron reductive C(1′)−N(1) bond dissociation to release 5-fluorouracil **1** in sufficient yield.

Scheme 2



X-ray Crystal Structure and Calculated Electronic Structure. X-ray studies of single crystals were carried out to elucidate the detailed structures of some representative compounds **3**−**5** and **7**−**9**. As illustrated in Figure 1, the X-ray structures revealed that the C(1′)−N(1) bonds of all compounds determined possess normal geometry and bond lengths ranging from 1.41 to 1.49 Å. The distortion angles of these 2-oxo compounds between the C(1′)−N(1) bond and the C(2′)═O bond were −3.5° (**3**), −4.8° (**4**), 35.6° (**5**), −28.0° (**7**), 135.9° (**8**), and 3.8° (**9**), respectively. Thus, comparing these compounds with each other, there would be no great difference in the extent of overlapping between the C(2′)═O π MO and the C(1′)−N(1) σ MO in the crystal. In fluid solution, however, conformational changes will be allowed for the 2-oxo compounds under the influence of their molecular structures, causing various extents of overlapping of hypothetical C(2′)═O π^* radical anion with C(1′)−N(1) σ^* MO. Such an accessible conformational change in fluid solution may account for the observation that both the one-electron reductive decomposition and the release of 5-fluorouracil **1** varied depending on the molecular structures of 2-oxo compounds (Table 1).

For better understanding of the electronic structural characteristics of 2′-oxo substituent that is crucial for promoting the one-electron reductive release of 5-fluorouracil **1**, MO calculations by the AM1 method[16] were performed for the 2-oxo compounds **3**−**11**. Figure 2 shows representatively the LUMO and LUMO + 1 of compound **6**. Each compound has similar LUMO that is primarily localized at the π^* orbital of C(5)−C(6) double bond of the 5-fluorouracil moiety, as in Figure 2, therein accepting hydrated electron to form the most stable radical

(11) (a) Mori, M.; Teshima, S.; Hatta, H.; Fujita, S.; Taniguchi, R.; Nishimoto, S. Unpublished data. In the pulse radiolysis of **2** in Ar-purged aqueous solution containing 2-methyl-2-propanol using pulsed electron beam with a half-width duration 1 μs, we observed a transient absorption spectrum with a maximum wavelength at 340 nm which was almost identical with that of 5-fluorouracil radical anion (see also ref 11b). (b) Rivera, E.; Schuler, R. H. *J. Phys. Chem.* **1983**, *87*, 3966−3971.

(12) The number of molecules produced or changed per 1 J of radiation energy absorbed by the reaction system (see also ref 2).

(13) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637−1755.

(14) (a) Neta, P.; Schuler, R. H. *Radiat. Res.* **1971**, *47*, 612−627. (b) Das, S.; Deeble, D. J. *Z. Naturforsch.* **1985**, *40c*, 292−294.

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(16) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909. The AM1 calculations were performed using the CAChe software.

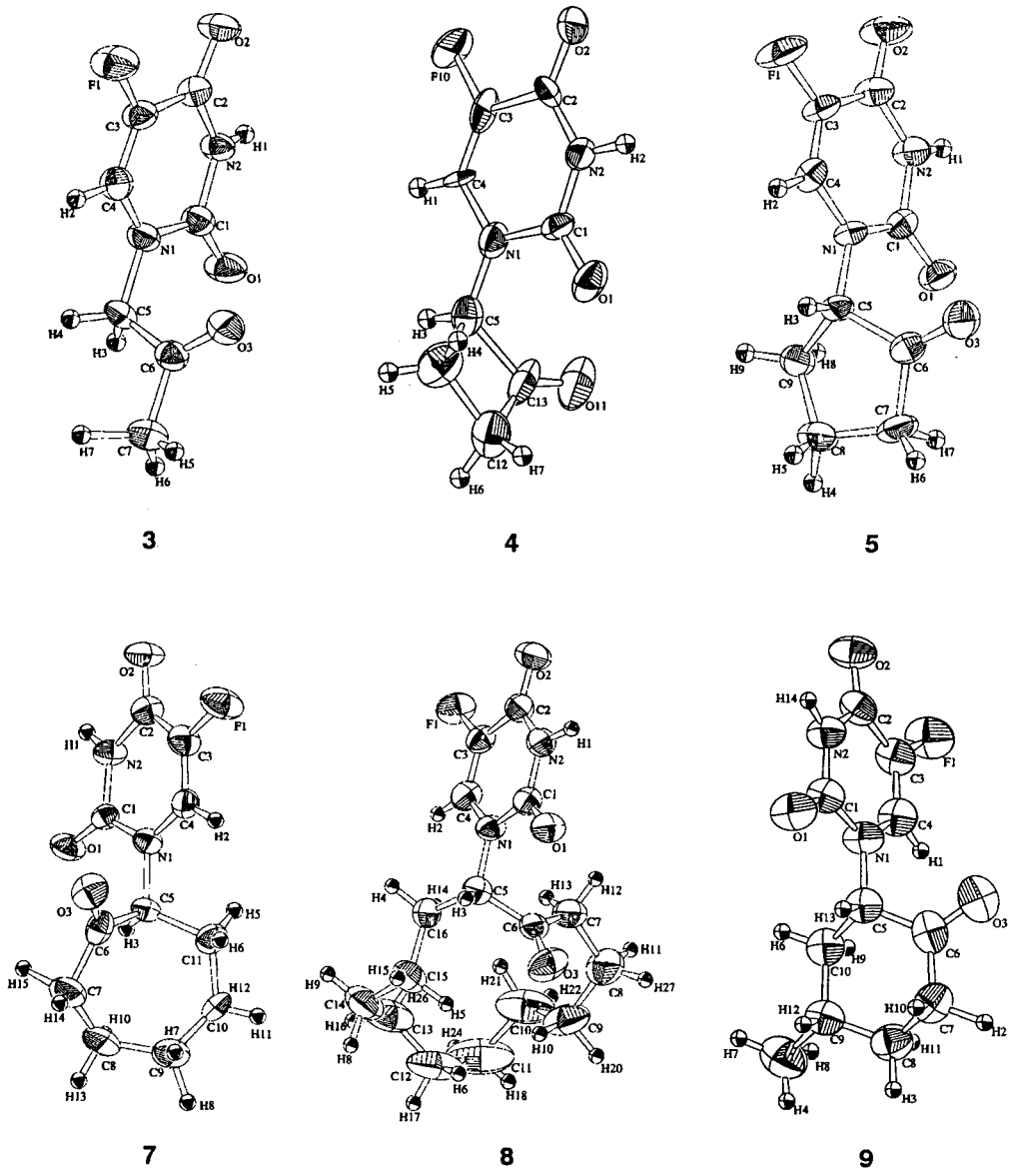

Figure 1. ORTEP drawings of **3**, **4**, **5**, **7**, **8**, and **9**.

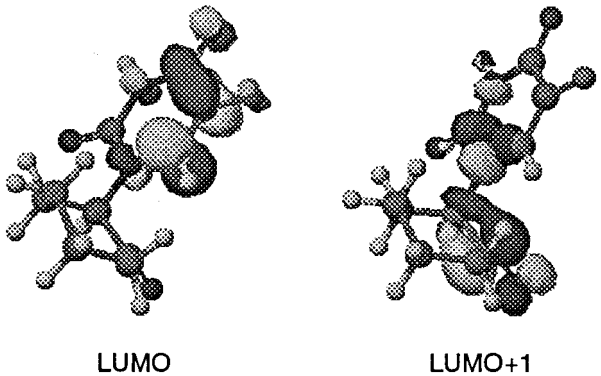

Figure 2. LUMO and LUMO + 1 of the 2-oxo compound **6** as calculated by AM1 method. The LUMO + 1 is delocalized by mixing of the π^* orbital of 2′-oxo substituent with the σ^* orbital of adjacent C(1′)−N(1) bond.

anion.[17] In contrast, the LUMO + 1 is delocalized between the π^* orbital of 2′-oxo substituent and the σ^* orbital of adjacent C(1′)−N(1) bond (see Figure 2), indicating a ($\pi^* + \sigma^*$) mixing of the antibonding orbitals. Such a mixing between π^* and σ^* orbitals is well-known for α-halo or α-amino ketones, causing the UV spectral perturbations and dominating the photochemical reactivity in the excited state.[18] Since the non-2-oxo compounds **12** and **13** cannot release 5-fluorouracil **1** upon one-electron reduction, the mixing of C(2′)═O π^* orbital as a possible electron-accepting site of secondary importance with the C(1′)−N(1) σ^* orbital at relatively higher energy level may be responsible for the promoted one-electron reductive C(1′)−N(1) bond dissociation of 2-oxo compounds **3**−**11**.

(17) A computational study on the electronic nature of radical anions generated from various N1-substituted 5-fluorouracil derivatives and their preferred fragmentation pathway has been recently reported (Borosky, G. L.; Nishimoto, S.; Pierini, A. B. *J. Mol. Struct. THEOCHEM* **2000**, *499*, 151−160). This indicated that while the most stable radical anion is of π-type for general N1-substituents, increase in the electron affinity of the N1-substituents can enhance the spin density at the C(1′)−N(1) bond favoring the formation of σ C(1′)−N(1) isomer of radical anion intermediates. According to this result, it seems plausible that the electron-adduct of 5-fluorouracil derivatives **3**−**11** bearing 2′-oxoalkyl substituents at N(1) could have a ($\pi^* + \sigma^*$) SOMO energy level closer to or even lower than the π^* level.

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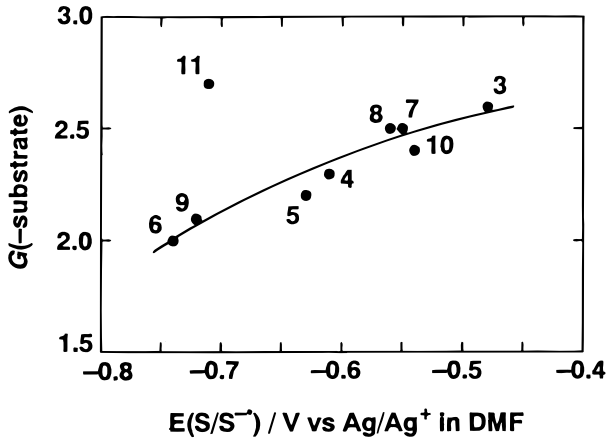

Figure 3. Correlation between the *G* value for decomposition of substrate (*G*(-substrate)) and the one-electron reduction potential ($E_{red}^{1/2}$ vs Ag/Ag^+) in DMF.

One-Electron Reduction Mechanism for Releasing 5-Fluorouracil. Figure 3 shows that the reactivity of all the 2-oxo compounds except **11** for radiolytic one-electron reduction in phosphate buffer, as measured by the *G* value for decomposition, increases as the reduction potential $E_{red}^{1/2}$ in DMF becomes more positive. Neither the *G* value nor the selectivity of 5-fluorouracil release has such a clear relationship with the $E_{red}^{1/2}$ value. As suggested by cyclic voltammetry and MO calculation, the $E_{red}^{1/2}$ value listed in Table 1 is attributable to one-electron reduction process involving the formation of π^* LUMO radical anion at the C(5)−C(6) double bond of 5-fluorouracil moiety, which produces a few fragments with higher polarity by pyrimidine ring opening reaction. In view of the calculated result that the LUMO + 1 energy levels are higher than the corresponding LUMO levels only by 0.32 to 0.58 eV, the 5-fluorouracil radical anion thus formed could intramolecularly transfer electron from the antibonding π^* orbital (LUMO) to the (π^* + σ^*) LUMO + 1 in competition with undergoing the pyrimidine ring fragmentation, thereby making considerable contribution to the 5-fluorouracil release which accounted for more than 47% of decomposed **3**−**11** (Table 1).

Previously, Rossi and co-workers reported[5,6] that the LUMO belonging to the π^* orbital of 2-oxo substituent in 1-chlorobicyclic compounds with rigid molecular structures decreases the antibonding σ^* MO with higher energy of the C−Cl bond, thereby enhancing the reactivity of electron-capturing C−Cl bond dissociation. They also suggested that the reactivity of 2-oxo substituted 1-chlorobicyclic compounds correlates with the π^* MO order of C═O group but not with the σ^* MO order of C−Cl group, probably due to the most facilitated formation of C═O π^* radical anions. This implies that stabilization energy in the formation of π^* radical anion at 2-oxo substituent may determine the reactivity of C−Cl bond dissociation, which occurs spontaneously via intramolecular dissociative electron transfer from the C═O π^* without formation of the C−Cl σ^* radical anion. According to our calculations for the 2-oxo compounds **3**−**11**, there is a partial mixing of the C(2′)═O π^* MO and the relatively high energetic C(1′)−N(1) σ^* MO to generate lower energy (π^* + σ^*) LUMO + 1, while the LUMO is the most likely π^* MO of the C(5)−C(6) double bond of leaving 5-fluorouracil moiety (see Figure 2). Such an electronic structural feature of the 2-oxo compounds **3**−**11** is expected to be evolved as a result of conformational changes that are allowed to occur more freely than the 1-chloro-2-oxo-bicyclic compounds with more rigid molecular structures. Although the 2′-oxo substituent similarly plays a crucial function in the one-electron reductive release of 5-fluorouracil **1**, the reactivity of **3**−**11** showed no correlation with the (π^* + σ^*) LUMO + 1. This is not surprising in view of the molecular structures that the 2-oxo compounds **3**−**11** studied may be flexible enough to undergo conformational change within the lifetimes of the (π^* + σ^*) LUMO + 1 radical anion intermediates, distinct from the 1-chloro-2-oxo-bicyclic compounds. In this context, the axial conformer of α-chlorocyclohexanone dominates over the equatorial conformer in the photolytic C−Cl bond cleavage,[19] the reactivity being correlated to the degree of mixing between the C−Cl σ^* and C═O π^* orbitals in the LUMO which becomes a maximum at the dihedral angle of Cl−C−C═O = 90°.[20] It is thus reasonable to presume that the observed reactivity of one-electron reductive 5-fluorouracil release involves some contribution from a dynamic conformational change to achieve higher degree of MO mixing in the (π^* + σ^*) LUMO + 1 between the C(2′)═O π^* and C(1′)−N(1) σ^* orbitals.

Inspection of the data listed in Table 1 suggests strong influences of not only the overlapping between the C(2′)═O π^* and C(1′)−N(1) σ^* orbitals but also the structural flexibility of 2-oxo compounds on the efficiency of 5-fluorouracil release in the radiolytic one-electron reduction. The four-membered ring compound **4** that has probably the most rigid structure with rather small π^*-σ^* MO overlapping showed the lowest selectivity (47%) of 5-fluorouracil release among the tested 2-oxo compounds. On the other hand, the most flexible acyclic compound **3** or the relatively large ring compounds **7** and **8** which are unstrained and more flexible than the five- and six-membered ring compounds **5** and **6** also resulted in smaller selectivity (58−59%) of 5-fluorouracil release. Therefore, extreme flexibility is also unfavorable for the one-electron capturing C(1′)−N(1) bond dissociation, probably because the π^*- σ^* MO overlapped state of larger extent occurring with high frequency would have too short lifetime to allow intramolecular electron transfer from the C(2′)═O π^* radical anion to the antibonding C(1′)−N(1) σ^* orbital. In contrast to these two extremes, 5-fluoro-1-(2′-oxocyclopentyl)uracil **5** and 5-fluoro-1-(2′-oxocyclohexyl)uracil **6** released 5-fluorouracil **1** in nearly quantitative yields (96 and 97%) upon radiolytic one-electron reduction. These 2-oxo compounds may possess moderately flexible structures with accessible overlapping between the C(2′)═O π^* and C(1′)−N(1) σ^* orbitals.

The dependence of one-electron reductive 5-fluorouracil release on the molecular flexibility was examined in further details for a family of alkyl-substituted cyclohexanone derivatives**.** As indicated by comparing the reactivity among the 2-oxo compounds **6**, **9**−**11** (Table 1), the efficiency of C(1′)−N(1) bond dissociation to give 5-fluorouracil **1** is reduced to more extent with increasing the steric effect of 5′-alkyl substituent at cyclohexanone ring on the restriction of conformational change. Diasteroisomeric 2-oxo compounds with a *tert*-butyl-substituent, *cis*-

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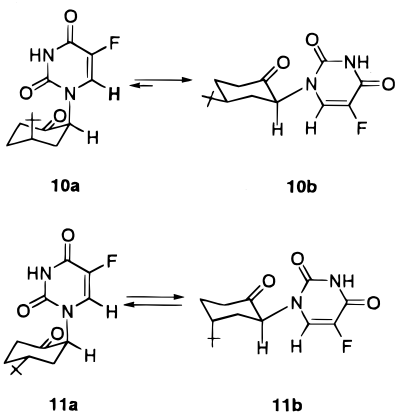
(20) Morrison, H.; Singh, T. V.; de Cardenas, L.; Severance, D. *J. Am. Chem. Soc.* **1986**, *108*, 3862−3863.

Chart 2



5-fluoro-1-(5′-*tert*-butyl-2′-oxocyclohexyl)uracil **10** and *trans*-5-fluoro-1-(5′-*tert*-butyl-2′-oxocyclohexyl)uracil **11,** which possess the least flexible ring structures are of particular interest. Since the one-electron reductive C(1′)−N(1) bond dissociation should favor a conformation in which dihedral angle between the C(2′)═O bond and C(1′)−N(1) bond is orthogonal, the *cis*-isomer **10** in a more stable conformation with 5-fluorouracil-1-yl and *tert*-butyl groups in the equatorial positions needs flipping for the efficient release of 5-fluorouracil **1**. This structural restriction may account for the reactivity that the *cis*-isomer **10** had significantly smaller selectivity (54%) of the C(1′)−N(1) bond dissociation than the nonsubstituted cyclohexanone analogue **6** (93%) with less strain energy for the conformational change. Thus, strain energy of the 2′-oxocycloalkane moiety seems to be at least one of the dominant factors in determining the C(1′)−N(1) bond dissociation reactivity of 5-fluoro-1-(2′-oxocycloalkyl)uracils. In contrast to the *cis*-isomer **10** isolated as the sole structure, there existed two distinct conformers for the *trans*-isomer **11** arising from bulky 5-fluorouracil-1-yl and *tert*-butyl groups, as confirmed by NMR. The ^{1}H NMR study demonstrated that **11a** with axial 5-fluorouracl-1-yl group and **11b** with axial *tert*-butyl group are in dynamic equilibrium with a molar ratio of 1.3:1 at room temperature in $CDCl_3$, as evaluated from doublet−doublet peak areas of the C(1′) protons at 5.39 and 5.15 ppm, respectively (Chart 2). These conformers could not be separately isolated. In light of the overlapping between the C(2′)═O π* MO and the C(1′)−N(1) σ* MO, the conformer **11a** should undergo one-electron reductive dissociation of the C(1′)−N(1) bond to release 5-fluorouracil **1**, in preference to the counterpart **11b**. It is also noted that there is no substantial difference in the selectivity of 5-fluorouracil release between the *cis* isomer **10** (54%) and *trans* isomer **11** (53%), while the latter showed somewhat higher reactivity for radiolytic decomposition than the former. This observation implies that the energy barrier for the conformational change from the less reactive **11b** to the more reactive **11a** may be kinetically similar to that of **10** involving dynamic change from the conformer **10b** (isolated as the sole stable product) to a possible conformer **10a** (not detectable in this work probably due to much smaller fraction) (Chart 2).

Conclusions

The present study demonstrated that the 2′-oxo substituent is crucial for the efficient C(1′)−N(1) bond dissociation to release 5-fluorouracil **1** by the radiolytic one-electron reduction of 2-oxo compounds **3**−**11** in anoxic aqueous solution. The reactivity of one-electron reductive releasing 5-fluorouracil **1** was strongly affected by the molecular flexibility of 2-oxo compounds **3**−**11**. In view of the X-ray crystal data and the MO calculations, the 5-fluorouracil release is presumed to occur from the LUMO + 1, but not LUMO, of radical anion intermediates with a partial mixing of the antibonding C(2′)═O π* and C(1′)−N(1) σ* MO's, that may be facilitated by a dynamic conformational change to achieve higher degree of (π* + σ*) MO mixing. The structure−reactivity relationship reported herein may provide a guiding principle of developing a family of radiation-activated prodrugs that release antitumor 5-fluorouracil sufficiently in the radiotherapy of hypoxic tumor cells. In addition, our approach would be applicable to molecular design of similar prodrugs[21] consisting of various antitumor agents with more potent toxicity than 5-fluorouracil.

Experimental Section

Materials. 5-Fluorouracil (**1**) was used as purchased from Tokyo Kasei Chemicals. *N*,*N*-Dimethylformamide (DMF) was distilled under reduced pressure and acetonitrile was dried over P_2O_5 followed by distillation. Tetra-*n*-buthylammonium bromide (TBAB, Nacalai Tesque) of reagent grade was recrystallized from benzene−hexane and stored in a vacuum desiccator prior to use. All the other reagents were of the best available grades and used as received.

General Methods. The high-resolution fast atom bombardment mass spectrometry (FAB-HRMS) were performed using glycerol matrix. Reaction products in the irradiated solution (5 μL) were analyzed by high-performance liquid chromatography (HPLC). Sample solutions were injected onto a reversed phase column (YMC-Pack ODS-AM, ϕ 6.0 mm × 150 mm) containing C_{18} chemically bonded silica gel (5 μm particle size). The phosphate buffer solution (10 mM, pH 3.0) containing 5 vol % methanol or the 0.05 vol % trifluoroacetic acid buffer solution containing 30 vol % acetonitrile was delivered as the mobile phase at a flow rate of 0.6 mL min^{-1} at 40 °C. A normal phase column (5SL Nakarai Tesqu, ϕ 4.5 mm × 250 mm) was used for HPLC analysis of substrate **13** and chloroform was delivered as the mobile phase at a flow rate of 0.6 mL min^{-1} at room temperature. The column eluents were monitored by the UV absorbance at 210 nm.

Radiolytic Reduction. Aqueous solutions were prepared with water ion-exchanged using Corning Mega-Pure System MP-190 (>16 MΩ cm). Typically, aqueous solutions (2 mL) of the 2-oxo compounds **3**−**13** (1 mM) containing excess amount (1 M) of 2-methyl-2-propanol were buffered at pH 7.0 ± 0.1 with phosphate buffer (KH_2PO_4 and Na_2HPO_4, 10 mM), purged with Ar for 20 min and then irradiated in a sealed glass ampule at room temperature with an X-ray source (8.0 Gy min^{-1}).

X-ray Crystallography. The colorless needle-shaped crystals of **3**−**5** and **7**−**9** were grown from acetone solutions (~10 mg mL^{-1}). The crystals were mounted directly on a glass fiber. The data were collected at 23 °C with graphite-monochromated Mo Kα radiation (λ = 1.54178 Å). Details of crystal data, data collection, and structure refinement are summarized in the Supporting Information. The intensities of three representative reflections which were measured after every 150 reflections remained constant throughout data collection, indicating electronic stability of the sample crystals. The data were corrected for Lorentz and polarization effects, but not for absorption. The structures were solved by direct methods

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(SHELXS-97[22] for compounds **3**, **7**, and **8**; SHELXS86[23] for compounds **9**; SAPI91[24] for compound **4**; SIR88[25] for compound **5**) and refined anisotropically for non-hydrogen atoms. Hydrogen atoms were refined with isotropic temperature factors. Tables of positional and thermal parameters, bond lengths, bond angles, torsion angles, and nonbonding atom distances are listed in the Supporting Information.

Cyclic Voltammetry. Cyclic voltammograms were recorded using a Pt working electrode, Pt wire for the common electrode and aqueous Ag/AgCl as a reference in a five-necked jacketed cell (20 mL). The redox potentials of 2′-oxo compounds (5 mM) were determined at room temperature in Ar-purged DMF solution containing 0.1 M TBAB as a supporting electrolyte.

5-Fluoro-1-(2′-oxocycloalkyl)uracil (4−10). Compounds **4**−**10** were prepared following the method reported by Akashi et al.[9] Typically, to a stirred solution of 5-fluoro-2,4-bis(trimethylsiloxy)pyrimidine (1.8 mL, 7.2 mmol) and 2-bromocyclobutanone (1.07 g, 7.2 mmol) in dry MeCN (10 mL) cooled in an ice bath for 15 min under nitrogen was added 0.08 mL of $SnCl_4$. The solution was stirred for 3 h at room temperature and for 15 h at 40 °C. After cooling to room temperature and adding 1 mL of MeOH, the resulting reaction mixture was stirred in an ice bath for 30 min and evaporated. The residue was purified by medium-pressure column chromatography (Chloroform-MeOH 20:1) to afford **4** that was recrystallized into yellow crystal from acetone in 45% yield: ^{1}H NMR (400 MHz, acetone-d_6) δ 2.40−2.47 (m, 1H), 2.75 (d, 2H, J = 13.2 Hz), 2.88−3.05 (m, 1H), 5.33 (m, 1H), 7.74 (d, 1H, J = 6.8 Hz), 10.54 (br s, 1H); ^{13}C NMR (100 MHz, acetone-d_6) δ 19.1 (CH_2), 42.9 (CH_2), 71.6 (CH), 129.7 (d, CH, J = 34.0 Hz), 141.6 (d, CF, J = 231.6 Hz), 150.3 (C), 158.1 (d, C, J = 25.7 Hz), 202.6 (C); FAB-HRMS m/z: calcd for $C_8H_8N_2O_3F$ [(M + H)$^+$] 199.0519, found 199.0514.

***trans*-5-Fluoro-1-(5′-*tert*-butyl-2′-oxocyclohexyl)uracil (11).** In a similar manner, compound **11** was prepared from 5-fluoro-2,4-bis(trimethylsiloxy)pyrimidine (1.7 mL, 5.4 mmol) and *cis*-2-bromo-4-*tert*-butylcyclohexanone[26] (1.26 g, 5.4 mmol) as yellow oil in 6% yield. As confirmed by ^{1}H NMR the resulting oil consisted of a mixture of two conformers, **11a** with axial 5-fluorouracil-1-yl group and **11b** with axial *tert*-butyl group, that could not be separately isolated: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (s, 18H), 1.54−1.77 (m, 4H), 2.01−2.14 (m, 4H), 2.25−2.42 (m, 4H), 2.64−2.74 (m, 2H), 5.15 (for **11b**) (dd, 1H, J = 6.4, 12.2 Hz), 5.39 (for **11a**) (dd, 1H, J = 6.8, 11.7 Hz), 7.28 (d, 1H, J = 4.5 Hz), 7.31 (d, 1H, J = 4.4 Hz), 8.87 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.2 (CH_2), 25.5 (CH_2), 27.5 (CH_3), 29.9 (CH_2), 30.3 (CH_2), 32.6 (C), 40.0 (CH_2), 40.1 (CH_2), 46.0 (CH), 46.1 (CH), 58.0 (CH), 59.8 (CH), 122.9 (d, CH, J = 32.2 Hz), 123.6 (d, CH, J = 32.2 Hz), 140.2 (d, CF, J = 233.5 Hz), 140.7 (d, CF, J = 234.4 Hz), 149.9 (C), 151.3 (C), 156.4 (d, C, J = 25.7 Hz), 157.7 (d, C, J = 25.7 Hz), 202.5 (C), 203.3 (C); FAB-HRMS m/z: calcd for $C_{14}H_{20}N_2O_3F$ [(M + H)$^+$] 283.1458, found 283.1458.

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Supporting Information Available: Full details of X-ray crystallographic data for compounds **3**, **4**, **5**, **7**, **8**, and **9**, procedures for the synthesis and NMR data of compounds **5**−**10** is available free of charge via the Internet at http://pubs.acs.org.

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