N(1)−C(5′)-Linked Dimer Hydrates of 5-Substituted Uracils Produced by Anodic Oxidation in Aqueous Solution

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Received July 25, 2000

Electrochemical dimerization reactivity has been studied for 5-substituted uracils (5XU) including thymine (**1a**: X = Me) and 5-halouracil derivatives (**1b**: X = F; **1c**: X = Cl; **1d**: X = Br; **1e**: X = I). Upon galvanostatic electrolysis of Ar-saturated aqueous solution **1a** underwent anodic oxidation to produce N(1)−C(5′)- and N(1)−C(6′)-linked dimer hydrates, 1-(6′-hydroxy-5′,6′-dihydrothymin-5′-yl)thymine (**5a**) and 1-(5′-hydroxy-5′,6′-dihydrothymin-6′-yl)thymine (**6a**), as the major products. These N−C-linked dimerizations were accompanied by the formation of novel stereoisomeric C(5)−C(5′)-linked dimers (meso isomer: **13a**[***meso***]; racemic isomer: **13a**[***rac***]) with a condensed tetrahydrofuran ring skeleton. Similar electrolyses of 5-fluorouracil (**1b**) and 5-chlorouracil (**1c**) also afforded the corresponding N(1)−C(5′)-linked dimer hydrates, 1-(5′-fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-fluorouracil (**5b**) and 1-(5′-chloro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-chlorouracil (**5c**), respectively, while resulting in neither N(1)−C(6′)-linked dimer analogues nor C(5)−C(5′)-linked dimers, unlike the reactivity of **1a**. In contrast to **1a**−**c**, no dimeric products were obtained from 5-bromouracil (**1d**) and 5-iodouracil (**1e**). The present electrochemical method was applicable to the cross-dimerization into N(1)−C(5′)-linked heterodimer hydrates composed of binary 5-substituted uracils that occurred in competition with the formation of homodimer hydrates. A mechanism of the N(1)−C(5′)-linked dimerization of **1a**−**c** has been proposed, by which allyl-type radical intermediates with limiting mesomeric forms of N(1)-centered and C(5)-centered pyrimidine radicals (**2a**−**c** [N(1)]/**2a**−**c** [C(5)]) are generated via anodic one-electron oxidation and subsequent deprotonation at N(1) and undergo a head-to-tail coupling.

Introduction

Pyrimidine bases undergo dimerization to form various modes of chemical linkages by photochemical,[1,2] radiation chemical,[3,4] and electrochemical[5−7] reactions. A formal [2 + 2] cycloaddition between the C(5)−C(6) double bonds of adjacent thymine moieties, producing the toxic cyclobutane photodimer, is the most typical example of the biologically relevant DNA photochemistry.[1] In the DNA-base model reactions in aqueous solution, there is considerable mechanistic evidence of the pyrimidine dimerizations involving successive transfers of an electron and a proton: (1) one-electron reduction of the pyrimidine followed by protonation to generate a carbon-centered free radical which results in a dihydrothymine dimer with a single C−C linkage; (2) one-electron oxidation of the pyrimidine followed by deprotonation to generate a carbon-centered or nitrogen-centered free radical which results in pyrimidine dimer with a single C−C or N−C linkage.

By the one-electron reduction mechanism, pyrimidines readily react with hydrated electrons to form the electron adducts (radical anions) upon radiolysis of aqueous solution under oxygen-free conditions.[8,9a] Such electron adducts of thymine derivatives are irreversibly protonated at C(6) to produce more stable 5,6-dihydrothymin-5-yl radicals[9,10] and thereby dimerize to give the C(5)−C(5′)-linked dihydrothymine dimers in considerable yields.[4] Pyrimidine dimers with a similar single C−C linkage are derived more generally from the electrochemical reduc-

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10.1021/jo0011282 CCC: $20.00
Published on Web 03/13/2001

tion.[5a] On the other hand, pyrimidine radical cations as produced by the one-electron oxidation have been a subject of extensive studies by pulse radiolysis and laser photolysis, because they are intermediate species of biological importance that would result from the direct action of ionizing radiation to cause oxidative damage of DNA.[8] Nevertheless, only a few studies on the pyrimidine dimerization by a one-electron oxidation mechanism involving radical cation intermediates have been documented,[5b] in contrast to the one-electron reductive dimerization. In an illustration of a C−C-linked dimerization, Johnson et al. reported that electrochemical oxidation of barbituric acid at a platinum electrode in basic aqueous solution produces two kinds of C(5)−C(5′)-linked dimers of hydurilic acid (4% yield) and alloxantin (25%) along with alloxan (50%) as a monomer oxidation product.[7a] Kato et al. characterized in further detail the C(5)−C(5′)-linked dimeric products derived from the electrochemical oxidation of barbituric acid derivatives,[7b,c] proposing a mechanism by which barbituric acids undergo one-electron oxidation followed by deprotonation to give the corresponding C(5)-centered radicals. As a different mechanism, direct evidence for transformation of 1-methylthymine radical cations to N(3)-deprotonated radicals and thereafter carbon-centered radicals by the addition of OH^- has been recently obtained by Fourier transform EPR on a nanosecond time scale range.[11c] Instead of such a C(5)−C(5′)-linked dimerization, Wagner et al.[2] first confirmed the formation of N(1)−C(5′)- and N(1)−C(6′)-linked dimer hydrates in the photooxidation of thymine (**1a**) sensitized by 2-methyl-1,4-naphthoquinone in aqueous solution, which could be derived from an intermediate of thymine radical cation as produced possibly by electron transfer to the electronically excited sensitizer.[12]

Previously, we found similar N(1)−C(5′)-linked dimerization of **1a** into 1-(6′-hydroxyl-5′,6′-dihydrothymin-5′-yl)thymine (**5a**) that occurs in much higher yield by anodic one-electron oxidation in Ar-purged aqueous solution containing NaCl as a supporting electrolyte.[6] This electrochemical method was also successfully applicable to synthesis of a novel N(1)−C(5′)-linked dimer hydrate, 1-(5′-fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-fluorouracil (**5b**) from 5-fluorouracil (**1b**) in aqueous solution.[6] The dimer **5b** was identified to show a formally reverse reactivity of undergoing one-electron reduction and release efficiently a well-specified antitumor agent of 5-fluorouracil **1b** by γ-radiolysis in anoxic aqueous solution.[6] Such a characteristic reactivity indicates that **5b** can be a radiation-activated prodrug of exerting selective cytotoxicity toward hypoxic tumor cells[13] under radiation treatment. This potentiality was verified by an in vivo assay using C3H/He mice that **5b** itself had no antitumor function in contrast to **1b**, but could potentiate the radiation therapy to inhibit growth of an SCCVII tumor.[6] These features of **5b** as a radiation-activated prodrug may provide a promising strategy of overcoming highly toxic side-effect of 5-fluorouracil **1b** in the clinical application.[14]

In this paper, we present a full account of the N(1)−C(5′)-linked pyrimidine dimerization by an anodic oxidation in the galvanostatic electrolysis of aqueous solution. For understanding of the one-electron oxidation reactivity of 5-substituted uracils (5XU) including thymine **1a** (X = Me) and 5-halouracil derivatives (**1b**: X = F; **1c**: X = Cl; **1d**: X = Br; **1e**: X = I), attempts were made to characterize the competitive formation of homo- and heterodimer hydrates.

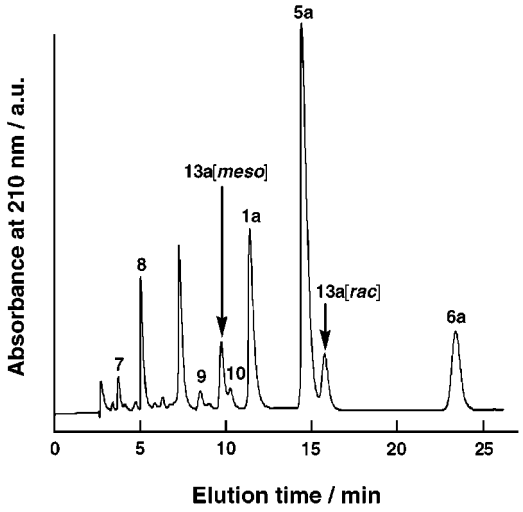

Figure 1. Representative HPLC analysis of aqueous solution of thymine (**1a**; 1 mM) after galvanostatic electrolysis for 150 min at 5 mA under Ar, as observed by delivering phosphate buffer (10 mM, pH 3.0) containing 3 vol % methanol at a flow rate of 0.6 mL min^{-1}.

Results and Discussion

Electrochemical N−C- and C−C−Linked Dimerizations. In a similar manner as reported previously,[6] galvanostatic electrolysis (5 mA) of thymine **1a** (1 mM) in aqueous solution (100 mL, pH 7.0) containing NaCl (5 mM) as a supporting electrolyte was carried out in a one-compartment glass cell (4 cm in diameter, 11 cm high) with two Pt electrodes (14 cm^2 in area, 1.6 cm separation) under Ar-bubbling. The reaction mixture was analyzed by HPLC at appropriate time intervals during the electrolysis. Figure 1 illustrates a representative HPLC chromatogram of the reaction mixture after electrolysis for 150 min, as monitored by UV absorbance at 210 nm. For a product characterization, the eluents from the respective major peaks were collected by preparative HPLC and fractionation. By reference to the authentic samples thus fractionated and identified, the electrolysis products were quantified using the analytical HPLC.

Table 1 summarizes the yields of dimeric products (Scheme 1) in the electrolysis of **1a**, which were evaluated from the HPLC chromatogram shown in Figure 1. As identified previously,[6] the electrolysis afforded N(1)−C(5′)- and N(1)−C(6′)-linked dimer hydrates, 1-(6′-hydroxy-5′,6′-dihydrothymin-5′-yl)thymine (**5a**) and 1-(5′-hydroxy-5′,6′-dihydrothymin-6′-yl)thymine (**6a**), as the major products in a yield ratio of ca. 3.5 to 1.0 independent of electrolysis time. In the present study, we further confirmed simultaneous formation of novel stereoisomeric

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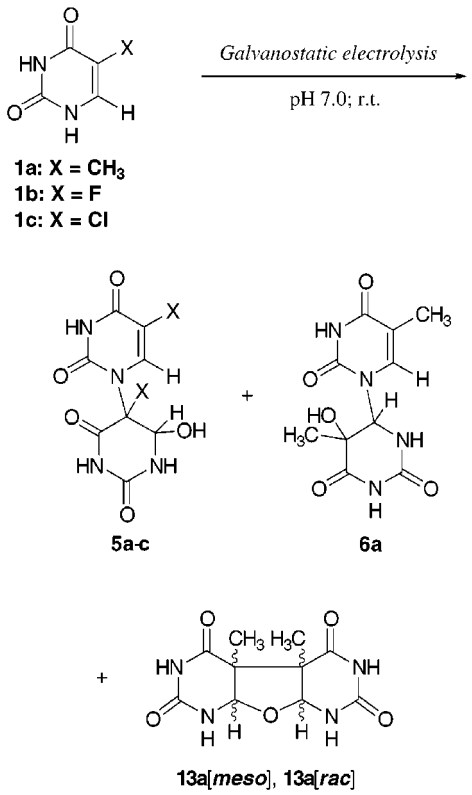
Table 1. One-Electron Oxidation of 5-Substituted Uracils (5XU; 1 mM) to Produce Various Dimeric Products in Aqueous Solution

				yield[d]/%				
5XU	oxidation process	time/min	conversion/%	**5**	**6**	**13**[***meso***]	**13**[***rac***]	total dimer
1a	anodic oxidation[a]	150	81	48	14	9	8	79
1b		180	77	52	0	0	0	52
1c		90	75	71	0	0	0	71
1a	photosensitized oxidation[b]	60	60	12	4	0	0	16
1a	γ-radiolytic oxidation[c]	600	34	11	1	0	0	12

[a] Ar-saturated aqueous solution containing NaCl (5 mM) was galvanostatically electrolyzed at 5 mA. [b] Aqueous solution containing 2-methyl-1,4-naphthoquinone (0.08 mM) in Pyrex glass tube (λ_{ex} > 280 nm) was photoirradiated with forced aeration. [c] Ar-saturated aqueous solution containing 2-methyl-2-propanol (100 mM) and $K_2S_2O_8$ (10 mM) were γ-irradiated at a dose rate of 1.43 Gy min^{-1}. [d] HPLC yield based on converted **1a**−**c**.

Scheme 1



C(5)−C(5′)-linked dimers (meso isomer: **13a**[***meso***]; racemic isomer: **13a**[***rac***]) among the major products in the electrolysis, both of which possess a condensed tetrahydrofuran ring skeleton (Scheme 1, Table 1). In view of the NOE difference spectra between C(5)-methyl and C(6)-H protons of the two pyrimidine rings that increased 12.8% for **13a**[***meso***] and 13.2% for **13a**[***rac***], respectively, both the C(5)−C(5′)-linked dimers **13a**[***meso***] and **13a**[***rac***] would be composed of a 5,6-*cis*-stereostructure of dihydropyrimidine ring. These N−C- and C−C-linked dimeric products accounted for 59−79% of the electrolyzed thymine **1a** and were accompanied by several minor products[6] involving thymine glycol (**7**), 5-hydroxymethyluracil (**8**), N^1-formyl-N^2-pyruvylurea (**9**), and 5,6-dihydrothymine (**10**). The products **7**−**9** are essential in the photosensitized and radiation-induced oxidations of **1a**, while 5,6-dihydrothymine **10** is attributable to reduction.[2,8,15]

Galvanostatic electrolyses of 5-fluorouracil **1b** and 5-chlorouracil (**1c**) under similar conditions also produced the corresponding N(1)−C(5′)-linked dimer hydrates, 1-(5′-fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-fluorouracil (**5b**) and 1-(5′-chloro-6′-hydroxy-5′.6′-dihydrouracil-5′-yl)-5-chlorouracil (**5c**), respectively, while resulting in neither N(1)−C(6′)-linked nor C(5)−C(5′)-linked dimer analogues unlike the reactivity of **1a** (Table 1). In contrast to **1a**−**c**, analogous N(1)−C(5′)-linked dimer hydrates (**5d**,**e**) were failed to be isolated from the electrolyzed aqueous solutions of 5-bromouracil (**1d**) and 5-iodoracil (**1e**), although the analytical HPLC and FAB-MS indicated the formation of N−C-linked dimeric products in much smaller amounts which were highly reactive to undergo decomposition during the electrolysis.[16]

Each of the N(1)−C(5′)-linked dimer hydrates **5a**−**c** reported herein should be a mixture of *cis*- and *trans*-isomers. With regard to this we investigated in further detail the electrochemical formation of 5-fluorouracil dimer hydrate **5b**. In the galvanostatic electrolysis at 5 mA of **1b** (1 mM) in Ar-saturated aqueous solution containing 100 mM NaCl, the racemic compounds of (5′*R*,6′*S*)- and (5′*S*,6′*R*)-1-(5′-fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-fluorouracils (*cis*-**5b**) were isolated almost exclusively as the gross sample from the electrolyzed aqueous solution (see also Table 1), although the crystal was composed of a chiral structure.[17] At higher galvanostatic current of 50 mA under separate conditions (10 mM **1b**; 300 mM NaCl; 330 min), however, a small amount (3.4% yield) of *trans*-isomer, (5′*R*,6′*R*)- and (5′*S*,6′*S*)-1-(5′-fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-fluorouracils (*trans*-**5b**), was also obtained as a byproduct along with a major product of *cis*-**5b** (46% yield). The apparent molar ratio of *trans*-**5b**/*cis*-**5b** increased with increasing the galvanostatic currents, although the yields of both isomers decreased significantly: e.g. 8.5% *cis*-**5b**

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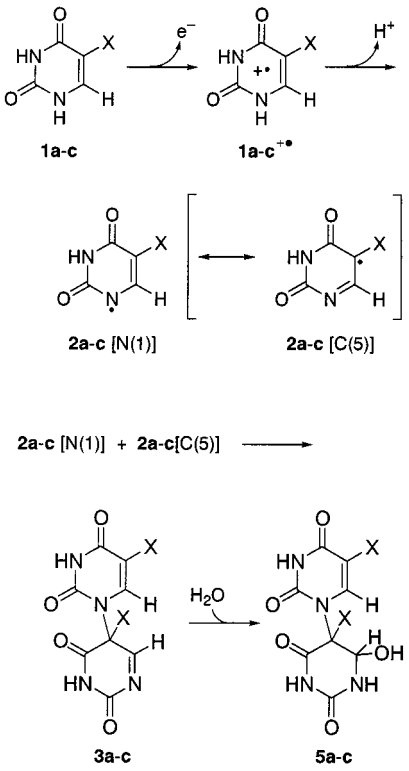
(16) Galvanostatic electrolysis (5 mA) of 5-bromouracil **1d** (1 mM) in aqueous solution containing NaCl (20 mM) afforded possible N(1)−C(5′)-linked dimers of 1-(5′-bromo-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-bromouracil (**5d**; FAB-MS (glycerol matrix) *m*/*z* 397, 399, and 401 [(M + H)$^+$] in ca. 1:2:1 relative abundance) and 1-(5′-chloro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-bromouracil (**5dc**; FAB-MS (glycerol matrix) *m*/*z* 353and 355 [(M + H)$^+$] in ca. 1:1 relative abundance). Under similar electrolysis (5 mA, 20 mM NaCl) of 5-iodouracil **1e** (1 mM), 1-(5′-chloro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-iodouracil (**5ec**; FAB-MS (glycerol matrix) *m*/*z* 401 [(M + H)$^+$]) but not 1-(5′-iodo-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-iodouracil (**5d**) could be detected. The formations of chlorohydrins **5dc** and **5ec** suggest that a bromohydrin **5d** and an iodohydrin **5e** as the expected N(1)−C(5′)-linked dimer hydrates may readily undergo HBr and HI eliminations, respectively, followed by HCl addition in aqueous NaCl solution.

(17) The X-ray crystallographic characterization was performed to get structural insight into their reactivity toward radiolytic one-electron reductive release of **1b** and will be reported elsewhere (Mori, M.; Teshima, S.; Hatta, H.; Fujita, S.; Taniguchi, R.; Nishimoto, S.).

Scheme 2



and 1.3% *trans*-**5b** were obtained at 200 mA for 108 min. It is therefore plausible that the electrolysis of 5-substituted uracils **1a**−**c** in aqueous solution under mild conditions with lower galvanostatic currents favors the formation of N(1)−C(5′)-linked *cis*-dimer hydrate.

Mechanism of Electrochemical Dimerizations. To get insight into the dimerization mechanism, **1a** (1 mM) was electrolyzed in aqueous NaCl (5 mM) solution using a two-compartment glass cell, in which anodic and cathodic compartments were separated by a glass filter. All of the dimers were produced exclusively in the anodic compartment, while about 90% of **1a** was recovered from the cathodic compartment after galvanostatic (10 mA) electrolysis for 60 min. Neither the conversion of **1a** nor the yields of dimers in the anodic oxidation were influenced by the addition of *t*-BuOH (200 mM) as a hydroxyl (OH) radical scavenger.[6,8a] In this context, the dimers characterized herein were not produced by γ-radiolysis of N_2O-saturated aqueous solution of **1a**, in which OH radicals are almost exclusively involved as an active species along with minor amount of hydrogen atoms.[8a] According to these observation, anodic one-electron oxidation of **1a** to generate a radical cation intermediate (**1a**$^{+\bullet}$, see Scheme 2), but not OH radical addition to **1a**, is provably a key reaction step leading to the electrochemical dimerization. In association with this mechanistic aspect, the anodic electrode potential (E_a) was confirmed to be almost constant (1.61 ± 0.01 V vs Ag/AgCl) during the galvanostatic electrolysis of **1a** in aqueous NaCl solution, irrespective of the shape of cell whether one-compartment cell or two-compartment cell. This E_a(**1a**) value is slightly more positive than the aqueous phase redox potential reported for **1a** (1.53 V Ag/AgCl at pH 7.0).[18] Thus, the diffusion of **1a** through an electrode/solution interface to undergo one-electron oxidation at the anode should be the most likely rate-determining step in the electrolytic N(1)−C(5′)-linked dimerization. Distinct from the constant E_a value, on the other hand, the cathodic electrode potential (E_c) that showed the initial value of −0.67 V vs Ag/AgCl at pH 7.0 became more positive with decreased pH in the electrolysis of **1a** using the one-compartment cell, while it became more negative with increased pH in the cathodic compartment using the two-compartment cell. This behavior of pH-dependent E_c values are accounted for by the one-electron reduction of protons (H^+) to evolve hydrogen (H_2) at the cathode.

The formation of radical cations of thymine derivatives via one-electron oxidation by strongly oxidizing sulfate radical anions ($SO_4^{-\bullet}$) has been studied by a pulse radiolysis method.[19] More recently, the radical cations generated by electron transfer from pyrimidines including thymine **1a** to anthraquinone-2,6-disulfonate in the excited triplet state have been detected by time-resolved Fourier transform EPR.[11a,b] In light of these one-electron oxidation processes, comparative experiments were performed to determine the dimerizations of **1a** in the photooxidation sensitized by 2-methyl-1,4-naphthoquinone and γ-radiolysis under conditions of generating sulfate radical anions $SO_4^{-\bullet}$. Similar to but much less efficiently than the electrolysis, both the photosensitized and radiolytic oxidations produced the N(1)−C(5′)- and N(1)−C(6′)-linked dimer hydrates, **5a** and **6a**, as shown in Table 1. These results provide a strong support for the above hypothetical intermediate of radical cation **1a**$^{+\bullet}$ that is involved in the electrolytic N−C-linked dimerization. In the separate experiments, similar anodic oxidations of 1-methylthymine (**14**) and thymidine (**15**) in aqueous NaCl solution were further confirmed to give neither N(1)−C(5′/6′)-linked dimer hydrates nor C(5)−C(5′)-linked dimers, probably because of the absence of deprotonation at N(1) in the corresponding radical cation intermediates. In accord with such a striking distinction in reactivity between thymine **1a** and its N(1)-substituted derivatives **14** and **15**, transformation of radical cation into a radical by irreversible deprotonation at N(1)[8a] should be essential to the oxidative pyrimidine dimerization. This was also supported by the observation that the pH value of the aqueous solution decreased gradually during the electrolysis in the one-compartment cell.

The electrolytic oxidative dimerization of 5-substituted uracils **1a**−**c** may be rationalized by a reaction pathways outlined in Scheme 2. The initial step involves one-electron oxidation of **1a**−**c** at the anode to generate the corresponding radical cations (**1a**−**c**$^{+\bullet}$). Comparing the anodic electrode potentials E_a, the reactivity of one-electron oxidation at the anode would increase in the order of **1b** (E_a = 1.68 ± 0.01 V vs Ag/AgCl) < **1c** (E_a = 1.61 ± 0.03 V) ≈ **1a** (E_a = 1.61 ± 0.01 V) ≤ **1d** (E_a = 1.60 ± 0.02 V) < **1e** (E_a = 1.54 ± 0.04 V). Despite higher reactivity, the anodic oxidations of 5-bromouracil **1d** and 5-iodouracil **1e** may be partly inhibited by the generation of bromide ions Br^- ($E°(Br°/Br^-)$ = 1.8 V vs Ag/AgCl)[20] and iodide ions I^- ($E°(I°/I^-)$ = 1.2 V vs Ag/AgCl),[20] respectively. The chloride ions Cl^- ($E°(Cl°/Cl^-)$ = 2.4 V vs Ag/AgCl)[20] involved as a component of supporting

(18) Kittler, L.; Löber, G.; Gollnick, F. A.; Berg, H. *Bioelectrochem. Bioenerg.* **1980**, *7*, 503−511. The reported redox potential vs NHE of **1a** was converted to the corresponding value vs Ag/AgCl.

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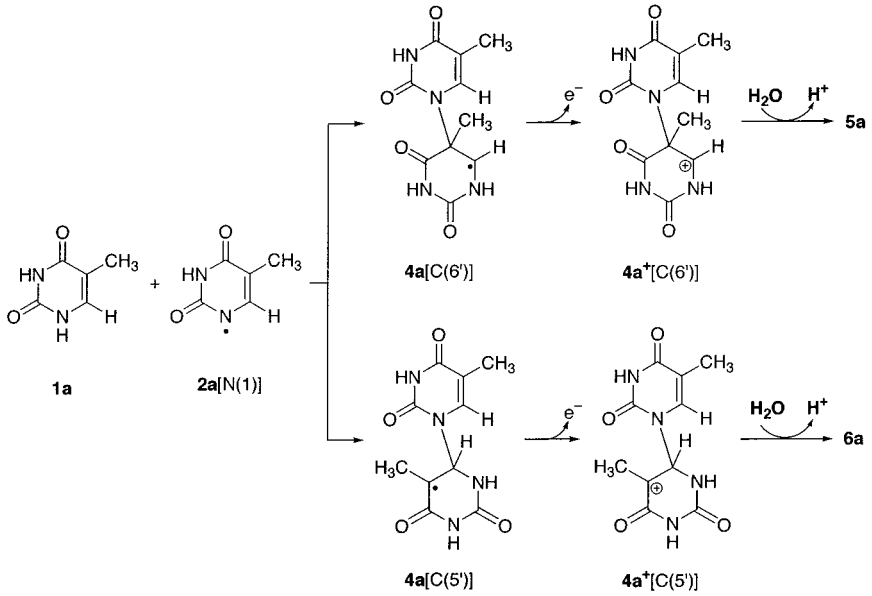
(20) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1711−1716. The reported redox potentials vs NHE of halide ions were converted to the corresponding value vs Ag/AgCl.

Scheme 3



electrolyte should not show such an inhibitory effect. These electrochemical reactivity accounts for the observation that the apparent rates of the decompositions of **1d**,**e** decreased to a considerable extent with increased electrolysis time, whereas those of **1a**−**c** were constant throughout the electrolyses.

The resulting radical cations **1a**−**c**$^{+\bullet}$ are successively deprotonated[11a,b,21] at N(1) to form allyl-type radical intermediates with limiting mesomeric forms of N(1)-centered radical (**2a**−**c**[N(1)]) and C(5)-centered radical (**2a**−**c**[C(5)]). It is most likely that such allyl-type radicals, **2a**−**c**[N(1)]/**2a**−**c**[C(5)], favor a head-to-tail coupling[8a] leading to a dimeric isopyrimidines (**3a**−**c**) which undergoes rapid hydration[22] to give the N(1)−C(5′)-linked dimer hydrates **5a**−**c**. In view of the electrochemical reaction characteristics that active species are generated in high density in the vicinity of an electrode, encounter of the radicals **2a**−**c**[N(1)]/**2a**−**c**[C(5)] could occur more efficiently and thereby resulted in higher yield of N(1)−C(5′)-linked dimer hydrates **5a**−**c**, compared with the photosensitized and radiolytic oxidative dimerizations involving mutual diffusion of the radicals as a rate-determining step (see Table 1). Thus, the anodic one-electron oxidation of **1a**−**c** may be the most probable rate-determining step for the electrolytic dimerization.

An alternative reaction pathway leading to the formation of N(1)−C(6′)-linked dimer hydrate **6a** should also be involved in the radical chemistry of dimerization, as shown in Scheme 3. In a similar manner to that of a typical OH radical reaction with uracil derivatives,[8a] N(1)-centered radicals **2a**[N(1)] may add to the C(5)/C(6) double bond of **1a**, producing N(1)−C(6′)-linked dimer C(5′) radical (**4a**[C(5′)]) and N(1)−C(5′)-linked dimer C(6′) radical (**4a**[C(6′)]). By reference to the reported rate constants for OH radical addition to **1a** in aqueous solution (4.6−7.6 × 10^9 M^{-1} s^{-1}),[23] the reaction route to N−C-linked dimerization shown in Scheme 3 seems to be sufficiently competitive with the diffusion-controlled radical coupling (Scheme 2). The dimer radicals **4a**[C(5′)] and **4a**[C(6′)] would be produced in the vicinity of the anode, therefore undergoing more readily successive anodic oxidation into the corresponding cations (**4a**$^+$-[C(5′)] and **4a**$^+$[C(6′)]), followed by hydrolytic OH addition to yield dimer hydrates **6a** and **5a**, respectively. Since pyrimidine C(5) and C(6) radicals have oxidizing and reducing properties,[24] respectively, the dimer C(6′) radicals **4a**[C(6′)] are expected to be more reactive toward anodic oxidation than the dimer C(5′) radicals **4a**[C(5′)]. On the other hand, the dimeric product distributions shown in Table 1 suggests that allyl-type radical intermediates **2b**,**c** bearing inductive 5-halo substituents may have less radical reactivity characteristic of limiting mesomeric structures **2b**,**c**[N(1)] and **2b**,**c**[C(5)] than thymine radical **2a**, thereby leading to neither N(1)−C(6′)-linked dimer hydrates nor C(5)−C(5′)-linked dimers.

On the other hand, a possible route to the formation of stereoisomeric C(5)−C(5′)-linked dimers **13a**[***meso***] and **13a**[***rac***] in almost equivalent yields may involve a bimolecular coupling of allyl-type C(5) radicals **2a**[C(5)] followed by hydration of the bis-isopyrimidine structure (**11a**) (Scheme 4). The resulting C(5)−C(5′)-linked dimer dihydrate (**12a**) is likely to undergo intramolecular condensation into the tetrahydrofuran ring skeleton during evaporation of the reaction mixture under reduced pressure.[25] The C(5) radical coupling reaction would also occur near the anode in competition with the head-to-tail coupling between **2a**[N(1)] and **2a**[C(5)]. Such a forced cage effect on the radical couplings is characteristic

(21) (a) Sevilla, M. D.; Van Paemel, C.; Nichils, C. *J. Phys. Chem.* **1972**, *76*, 3571. (b) Sevilla, M. D.; Van Paemel, C.; Zorman, G. *J. Phys. Chem.* **1972**, *76*, 3577. (c) Sevilla, M. D.; Suryanarayana, D.; Morehouse, K. M. *J. Phys. Chem.* **1981**, *85*, 1027. (d) Sevilla, M. D.; Swart, S.; Riederer, H.; Hüttermann, J. *J. Phys. Chem.* **1984**, *88*, 1601. (e) Novais, H. M.; Steenken, S. *J. Phys. Chem.* **1987**, *91*, 426.

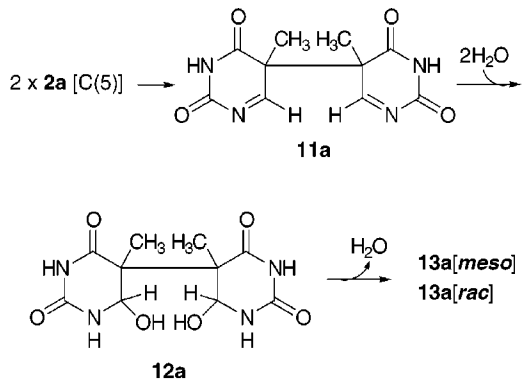
(22) Schuchmann, M. N.; Al-Sheikhly, M.; von Sonntag, C.; Garner, A.; Scholes, G. *J. Chem. Soc., Perkin Trans. 2* **1984**, 1777.

(23) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 676−759.

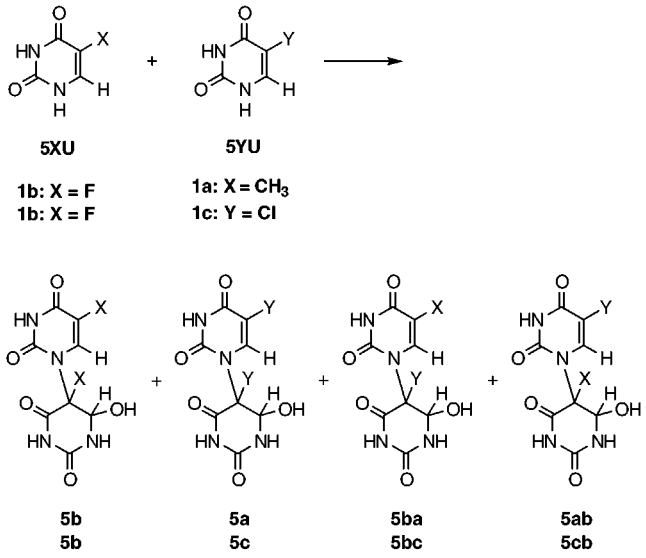
(24) (a) Steenken, S.; Neta, P. *J. Phys. Chem.* **1979**, *83*, 1134. (b) Fujita, S.; Steenken, S. *J. Am. Chem. Soc.* **1981**, *103*, 2540. (c) Schuchmann, M. N.; Steenken, S.; Wroblewski, J.; von Sonntag, C. *Int. J. Radiat. Biol.* **1984**, *46*, 225.

(25) As a similar condensation, we have recently found that *cis*-5,6-dihydro-5,6-dihydroxy-1,3-dimethylthymine (*cis*-1,3-dimethylthymine glycol) is dimerized almost quantitatively in the solid state to give dimers with a 1,4-dioxane structure upon evaporation under reduced pressure (unpublished data). See also: Ryang, H.-S.; Wang, S. Y. *J. Am. Chem. Soc.* **1978**, *100*, 1302.

Scheme 4



Scheme 5



of the electrochemical processes,[5a] and therefore both the N−C- and C−C-linked dimers were produced in higher yields, unlike the photosensitized and radiolytic oxidations resulting in only N−C-linked dimers in much lower yields. This illustrates an advantage of electrochemical synthesis of specific pyrimidine dimers by a radical coupling mechanism.

N(1)−C(5′)-Linked Cross-Dimerizations in the Binary Substrate Systems. Further attempts were made to synthesize N(1)−C(5′)-linked heterodimers composed of binary 5-substituted uracils, **1b** and **1a**,**c** (see Scheme 5) using the present electrochemical method. Typically, upon galvanostatic electrolysis at 20 mA under an Ar atmosphere, equimolar (0.5 mM) aqueous solution of **1b** and **1c** resulted in cross-dimerization to afford N(1)−C(5′)-linked heterodimer hydrates, 1-(5′-chloro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-fluorouracil (**5bc**) and 1-(5′-fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-chlorouracil (**5cb**), in competition with the formation of N(1)−C(5′)-linked homodimer hydrates **5b** and **5c**, as shown in the representative HPLC analysis (Figure 2). Under these conditions of electrolysis (20 mA), the decompositions of **1b**,**c** were apparently of the first-order kinetics with the formal rate constants of 0.023 min^{-1} for **1b** and 0.024 min^{-1} for **1c**. Figure 3 illustrates the time-course of the electrochemical dimerizations of **1b** and **1c**. Total amounts of the heterodimers **5bc** + **5cb** and the homodimers **5b** + **5c** increased to attain the respective maxima at 100 and 140 min (broken curves in Figure 3), thereafter decreasing gradually due to electrolyses of the

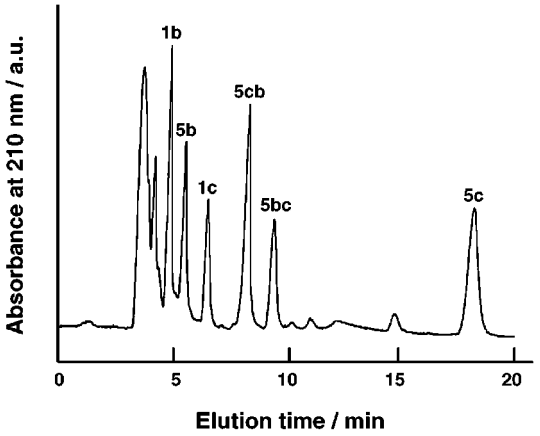


Figure 2. HPLC analysis of aqueous solution of 5-fluorouracil (**1b**; 0.5 mM) and 5-chlorouracil (**1c**; 0.5 mM) after galvanostatic electrolysis for 100 min at 10 mA under Ar, as observed by delivering phosphate buffer (10 mM, pH 3.0) containing 10 vol % methanol at a flow rate of 0.6 mL min^{-1}.

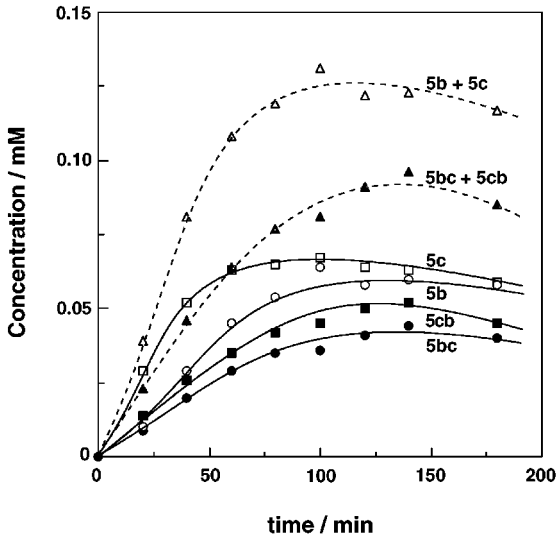


Figure 3. Time-course of N(1)−C(5′)-linked dimerization in the galvanostatic electrolysis at 20 mA of 5-fluorouracil (**1b**; 0.5 mM) and 5-chlorouracil (**1c**; 0.5 mM) in Ar-saturated aqueous solution: (○) **5b**; (□) **5c**; (●) **5bc**; (■) **5cb**; (△) total homodimers **5b** + **5c**; (▲) total heterodimers **5bc** + **5cb**.

primary dimeric products. At 140 min of the electrolysis 48% of the electrolyzed **1b** (93% conversion) was incorporated into homodimer **5b** and heterodimers **5bc**,**cb**, while 44% of the electrolyzed **1c** (98% conversion) into homodimer **5c** and heterodimers **5bc**,**cb**.

In a similar galvanostatic electrolysis at 10 mA, equimolar (0.5 mM) aqueous solution of **1a** and **1b** gave an N(1)−C(5′)-linked heterodimer hydrate of 1-(5′-fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)thymine (**5ab**) along with the N(1)−C(5′)- and N(1)−C(6′)-linked homodimer hydrates **5a**,**b** and **6a**, but there was practically no N(1)−C(5′)-linked heterodimer hydrate of 1-(6′-hydroxy-5′,6′-dihydrothymin-5′-yl)-5-fluorouracil (**5ba**) as a counterpart of **5ab** (Figure 4). In a separate experiment for aqueous solution of 0.18 mM **1a** and 0.64 mM **1b**, however, we observed the formation of **5ba** (0.01 mM) in addition to **5ab** (0.07 mM) upon electrolysis at 10 mA for 150 min. Since the reactivity toward anodic oxidation of **1a** (the formal rate constant of 0.019 min^{-1}) was higher than that of **1b** (0.013 min^{-1}) under these conditions (10

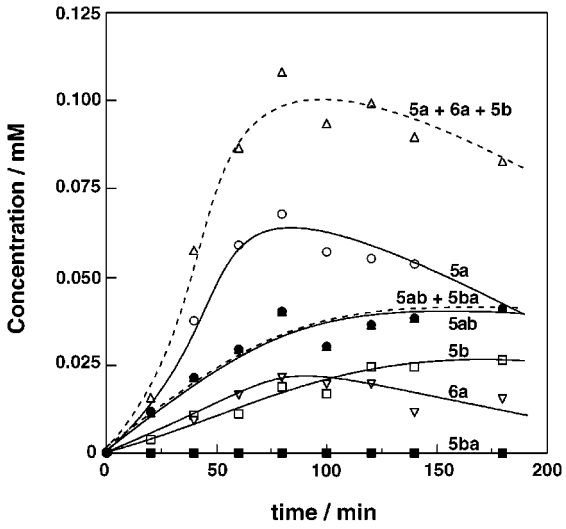


Figure 4. Time-course of N(1)−C(5′)- and N(1)−C(6′)-linked dimerizations in the galvanostatic electrolysis at 10 mA of thymine (**1a**; 0.5 mM) and 5-fluorouracil (**1b**; 0.5 mM) in Ar-saturated aqueous solution: (○) **5a**; (▽) **6a**; (□) **5b**; (●) **5ab**; (■) **5ba**; (△) total homodimers **5a** + **6a** + **5b**; (▲) total heterodimers **5ab** + **5ba**.

mA), the thymine dimerization to **5a** and **6a** were superior to the 5-fluorouracil-incorporated dimerization to **5b** and **5ab** in the earlier stage up to 80 min of electrolysis. Both **5a** and **6a** decomposed upon prolonged electrolysis, while **5b** and **5ab** still increased. At 80 min of the electrolysis when the yields of **5a** and **6a** attained their maxima, 58% of the electrolyzed **1a** (75% conversion) and 46% of the electrolyzed **1b** (34% conversion) were incorporated into the corresponding dimers, respectively.

The cross-dimerization behavior in the binary substrate systems is compatible with a radical coupling mechanism involving allyl-type radicals **2a**−**c**[N(1)]/**2a**−**c**[C(5)] (Scheme 2). Comparing the formal rates of competitive decompositions, the anodic one-electron oxidation reactivity of thymine **1a** is considerably higher than those of 5-halorouracils **1b** and **1c** which are of similar reactivity. It is also likely that the thymine radical **2a** favors limiting N(1)-centered structure **2a**[N(1)] much more than 5-fluorouracil radical **2b** and thereby produce exclusively an N(1)−C(5′)-linked heterodimer hydrate **5ab** upon their encounter. In the binary aqueous solution of **1b** and **1c**, 5-chlorouracil radical **2c** has a disposition to be limiting N(1)-centered structure **2c**[N(1)] relative to 5-fluorouracil radical **2b**, although almost random radical coupling may occur to give heterodimer hydrates **5bc** and **5cb** in comparable yields.

Conclusion

The present study has shown that the electrolytic oxidation in aqueous solution is much more effective for preparing N(1)−C(5′)-linked dimer hydrates of 5-substituted uracils such as thymine **1a**, 5-fluorouracil **1b**, and 5-chlorouracil **1c**, in comparison with the photosensitized and radiolytic oxidations. This electrochemical method was applicable to synthesis of N(1)−C(5′)-linked heterodimer hydrates in aqueous solution containing any combination of binary substrates among **1a**−**c**. A possible mechanism of the electrolytic N(1)−C(5′)-linked pyrimidine dimerization may involve the generation of allyl-type radical intermediates with limiting mesomeric forms of N(1)-centered radical **2a**−**c**[N(1)] and C(5)-centered radical **2a**−**c**[C(5)] via anodic one-electron oxidation followed by deprotonation at N(1). A head-to-tail radical coupling between **2a**−**c**[N(1)] and **2a**−**c**[C(5)] to form an N(1)−C(5′)-linkage could be more facilitated in the electrochemical system, because the radical intermediates may be generated in higher density in the vicinity of an electrode.

Experimental Section

Materials and General Methods. Thymine (**1a**), 5-fluorouracil (**1b**), 5-chlorouracil (**1c**), 5-bromouracil (**1d**), 5-iodouracil (**1e**), 1-methylthymine (**14**), and thymidine (**15**) were used as purchased from Sigma Chemicals. All other reagents and solvents obtained from Wako Pure Chemical Industries or Nacalai Tesque were used without further purification. High-resolution positive FAB mass spectra (FAB−HRMS) were measured using glycerol matrix. In the HPLC analysis, sample solutions were injected onto a reversed phase column (Wakosil 5C18, ϕ 4.6 mm × 150 mm) containing C18 chemically bonded silica gel (5 μm particle size). The phosphate buffer solutions (10 mM, pH 3.0) containing varying contents (3−20 vol %) of methanol were delivered as the mobile phase at a flow rate of 0.6 mL min^{-1}. The column eluents were monitored by the UV absorbance at 210 nm. For isolation and purification of the products, the electrolyzed solutions were evaporated to a minimum volume and were subjected to a preparative HPLC system. The isolation was performed on a reversed-phase column (Wakosil 10C18, ϕ 10 mm × 300 mm) containing C18 chemically bonded silica gel (10 μm particle size) and ion-exchanged water (Corning Mega-Pure System MP-190 (>16 MΩ cm)) containing 5−10 vol % methanol was delivered at a flow rate of 3 mL min^{-1}.

Galvanostatic Electrolysis. Typically, aqueous solutions (100 mL, pH 7.0) of various 5-substituted uracils (**1a**−**e**; 1 mM) containing NaCl (5 mM) as a supporting electrolyte were electrolyzed with a constant current of 5 mA at room temperature under Ar-bubbling, using a one-compartment glass cell (4 cm in diameter, 11 cm high) with Pt electrodes (14 cm^2 in area, 1.6 cm distant). For cross-dimerization, aqueous solution (100 mL, pH 7.0, 50 mM NaCl) of a given pair of 5-substituted uracils was subjected to similar galvanostatic electrolysis at 10 mA or 20 mA. A mechanistic characterization of the electrolytic dimerization was performed with a two-compartment cell in which the anode and cathode compartments were separated by a glass filter. Electrode potentials vs Ag/AgCl of the anode and cathode were measured using a digital multimeter. Aliquots (10 μL) were withdrawn at appropriate intervals from the aqueous solution during the electrolysis and analyzed by HPLC.

Preparative Scale for Electrochemical Synthesis of Dimers. N(1)−C(5′)-Linked homodimer hydrates **5a**−**c** and C(5)−C(5′)-linked homodimers **13a** (racemic and meso isomers) were prepared by galvanostatic electrolysis (100 mA) for ∼300 min of **1a**−**c** (30 mM) in aqueous solution (100 mL, pH 7.0, 100 mM NaCl), using a one-compartment glass cell. Similarly, N(1)−C(5′)-linked heterodimer hydrates **5ba**, **5ab**, **5cb**, and **5bc** were prepared by galvanostatic electrolysis (300 mA) for 180−240 min of **1a**/**1b** and **1b**/**1c** pairs (30 mM/20 mM ratio) in aqueous solution (100 mL, pH 7.0, 100 mM NaCl). All the dimeric products were isolated and purified by the preparative HPLC as described above. The spectroscopic data for N(1)−C(5′)-linked dimer hydrates **5a** and **5b** (*cis*-**5b**), along with those for N(1)−C(6′)-linked dimer hydrate **6a**, have been reported previously.[6]

(5′*R*,6′*R*)-1-(5′-Fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-fluorouracils (*trans*-5b): mp > 218 °C dec; ^{1}H NMR (399.65 MHz, DMSO-d_6) δ 5.24 (1H, m), 7.24 (1H, d, J = 6.3 Hz), 8.02 (1H, d, J = 7.2 Hz), 8.49 (1H, s), 11.02 (1H, s), 12.09 (1H, s); ^{13}C NMR (100.40 MHz, DMSO-d_6) δ 75.98 (d, J = 37.2 Hz), 94.06 (d, J = 208.1 Hz), 125.70 (dd, J = 9.5, 36.9 Hz),

139.81 (d, J = 231.9 Hz), 148.23, 151.3, 156.90 (d, J = 26.3 Hz), 161.29 (d, J = 20.8 Hz); FAB−HRMS (glycerol matrix) m/z: calcd for $C_8H_7F_2N_4O_5$ [(M + H)$^+$] 277.0385, found 277.0381.

1-(5′-Chloro-6′-hydroxy-5′,6′-dihydeouracil-5′-yl)-5-chlorouracil (5c). mp >261 °C dec; ^{1}H NMR (399.65 MHz, DMSO-d_6) δ 5.59 (1H, d, J = 5.2 Hz), 7.21 (1H, d, J = 5.6 Hz), 8.19 (1H, s), 8.48 (1H, s), 10.88 (1H, s), 12.40 (1H, s); ^{13}C NMR (100.40 MHz, DMSO-d_6) δ 74.95, 82.12, 109.03, 138.47, 149.10, 150.74, 158.87, 162.55; FAB−HRMS (glycerol matrix) m/z: calcd for $C_8H_7Cl_2N_4O_5$ [(M + H)$^+$] 308.9794, found 308.9852. Anal. Calcd for $C_8H_6Cl_2N_4O_5 \cdot 0.5H_2O$: C, 30.21; H, 2.22; Cl, 22.29; N, 17.61. Found: C, 30.34; H, 1.88; Cl, 22.13; N, 17.82.

Meso Form of C(5)−C(5′)-Linked Dimer with a Condensed Tetrahydrofuran Ring Skeleton 13a[*meso*]. ^{1}H NMR (399.65 MHz, DMSO-d_6) δ 1.24 (6H, s), 4.98 (2H, d, J = 2.9 Hz), 8.26 (2H, s), 10.28 (2H, s); NOE difference spectrum between C(5)-methyl and C(6)-H protons of the two pyrimidine rings increased 12.8%; ^{13}C NMR (100.40 MHz, DMSO-d_6) δ 16.56, 50.36, 86.05, 151.29, 171.06; FAB−HRMS (glycerol matrix) m/z: calcd for $C_{10}H_{13}N_4O_5$ [(M + H)$^+$] 269.0886, found 269.0878.

Racemic Form of C(5)−C(5′)-Linked Dimer with a Condensed Tetrahydrofuran Ring Skeleton 13a[*rac*]. ^{1}H NMR (399.65 MHz, DMSO-d_6) δ 1.39 (6H, s), 4.77 (2H, d, J = 4.4 Hz), 8.52 (2H, dd, J = 4.2, 1.5 Hz), 10.53 (2H, s); NOE difference spectrum between C(5)-methyl and C(6)-H protons of the two pyrimidine rings increased 13.2%; ^{13}C NMR (100.40 MHz, DMSO-d_6) δ 16.44, 50.45, 85.47, 151.57, 171.34; FAB−HRMS (glycerol matrix) m/z: calcd for $C_{10}H_{13}N_4O_5$ [(M + H)$^+$] 269.0886, found 269.0878.

1-(6′-Hydroxy-5′,6′-dihydrothymin-5′-yl)-5-florouracil (5ba). ^{1}H NMR (399.65 MHz, DMSO-d_6) δ 1.51 (3H, s), 5.35 (1H, d, J = 4.6 Hz), 6.60 (1H, d, J = 4.9 Hz), 7.92 (1H, s), 8.16 (1H, d, J = 7.0 Hz), 10.39 (1H, s), 12.06 (1H, s, br); ^{13}C NMR (100.40 MHz, DMSO-d_6) δ 15.50, 66.56, 74.64, 127.92 (d, J = 35.1 Hz), 139.91 (d, J = 229.6 Hz), 149.4, 151.22, 157.02 (d, J = 25.9 Hz), 169.50; FAB−HRMS (glycerol matrix) m/z: calcd for $C_9H_{10}FN_4O_5$ [(M + H)$^+$] 273.0635, found 273.0641. Anal. Calcd for $C_9H_9FN_4O_5$: C, 39.71; H, 3.33; F, 6.98; N, 20.58. Found: C, 39.20; H, 3.32; F, 6.85; N, 20.36.

1-(5′-Fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)thymine (5ab). mp >263 °C dec; ^{1}H NMR (399.65 MHz, DMSO-d_6) δ 1.81 (3H, d, J = 0.9 Hz), 5.40 (1H, br), 7.15 (1H, s), 7.51 (1H, q, J = 0.9 Hz), 8.48 (1H, s), 10.85 (1H, s), 11.66 (1H, s); ^{13}C NMR (100.40 MHz, DMSO-d_6) δ 12.12, 74.28 (d, J = 29.8 Hz), 93.8 (d, J = 220.8 Hz), 111.58, 135.33 (d, J = 4.6 Hz), 149.53, 151.78 (d, J = 4.5 Hz), 162.15 (d, J = 24.4 Hz), 163.30; FAB−HRMS (glycerol matrix) m/z: calcd for $C_9H_{10}FN_4O_5$ [(M + H)$^+$] 273.0635, found 273.0634. Anal. Calcd for $C_9H_9FN_4O_5$: C, 39.71; H, 3.33; F, 6.98; N, 20.58. Found: C, 39.51; H, 3.12; F, 7.07; N, 20.83.

1-(5′-Fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-chlorouracil (5cb). mp >271 °C dec; ^{1}H NMR (399.65 MHz, DMSO-d_6) δ 5.41 (1H, d, J = 5.5 Hz), 7.16 (1H, d, J = 4.9 Hz), 8.17 (1H, s), 8.50 (1H, s), 10.91 (1H, s), 12.25 (1H, br); ^{13}C NMR (100.40 MHz, DMSO-d_6) δ 74.27 (d, J = 28.2 Hz), 93.85 (d, J = 220.5 Hz), 109.79, 137.0, 148.63, 151.65, 156.49, 161.62 (d, J = 24.4); FAB−HRMS (glycerol matrix) m/z: calcd for $C_8H_7ClFN_4O_5$ [(M + H)$^+$] 293.0089, found 293.1017. Anal. Calcd for $C_8H_6ClFN_4O_5$: C, 32.84; H, 2.07; Cl,12.12; F, 6.49; N, 19.15. Found: C, 32.51; H, 1.97; Cl, 12.09; F, 6.23; N, 19.28.

1-(5′-Chloro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-fluorouracil (5bc). mp >278 °C dec; ^{1}H NMR (399.65 MHz, DMSO-d_6) δ 5.56 (1H, d, J = 4.6 Hz), 7.19 (1H, J = 5.8 Hz), 8.29 (1H, d, J = 7.3 Hz), 8.48 (1H, s), 10.85 (1H, s), 12.33 (1H s); ^{13}C NMR (100.40 MHz, DMSO-d_6) δ 74.96, 82.09, 126.42 (d, J = 36.3 Hz), 140.16 (d, J = 233.5 Hz), 148.44, 150.75, 156.77 (d, J = 25.9 Hz), 162.28; FAB−HRMS (glycerol matrix) m/z: calcd for $C_8H_7ClFN_4O_5$ [(M + H)$^+$] 293.0089, found 293.0098. Anal. Calcd for $C_8H_6ClFN_4O_5$: C, 32.84; H, 2.07; Cl,-12.12; F, 6.49; N, 19.15. Found: C, 32.64, H, 1.84; Cl, 11.87; F, 6.30; N, 19.15.

Photooxidation of Thymine (1a) Sensitized by 2-Methyl-1,4-naphthoquinone. Aqueous solution of **1a** (1 mM, 10 mL, pH 7.0) containing 2-methyl-1,4-naphthoquinone (0.08 mM) in Pyrex glass tube was photoirradiated with forced aeration under magnetic stirring (1000 rpm) at 24 °C with a high-pressure Hg arc (450 W).

γ-Radiolytic Oxidation of Thymine (1a). Aqueous solution of **1a** (1 mM, 10 mL, pH 7.0) containing 2-methyl-2-propanol (100 mM) and $K_2S_2O_8$ (10 mM) was purged with Ar and γ-irradiated in a sealed ampule at room temperature with a ^{60}Co γ-ray source at a dose rate of 1.43 Gy min^{-1}. Under these conditions of aqueous radiolysis, strongly oxidizing sulfate radical anions ($SO_4^{-\bullet}$) are generated from the reaction of peroxodisulfate ion ($S_2O_8^{2-}$) with hydrated electron (e_{aq}^-),[8a] thus one-electron oxidizing **1a**.

Acknowledgment. This work was supported by the Grant-in-Aids for Scientific Research (B) (No. 07455339) from the Ministry of Education, Science, Sports, and Culture of Japan. We are grateful to Dr. T. Ueno for performing fast atom bombardment mass spectral analyses and Mr. H. Fujita for his assistance with nuclear magnetic resonance spectral measurements. We also thank Dr. M. Inaba for helpful discussions on the electrochemical analysis.

JO0011282