CHEMISTRY ON THE DAMAGE AND REPAIR OF THYMINE AND THYMIDINE DERIVATIVES†

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Abstract-----This review describes on the oxidative damage and repair studies of nucleic acids, particularly of thymine and thymidine derivatives.

CONTENTS

I. Introduction

Nucleic acids are important compounds to transmit genetic informations, as shown in the central dogma (DNA→RNA→ protein) of molecular biology. Therefore, the damages of nucleic acids are considered to have relation to mutagenesis, carcinogenesis, and aging. From these viewpoints, many studies including physical and chemical studies on the damages of nucleic acids have been done. Physical studies contain the methods of γ-ray, X-ray,[1-28] UV irradiations,[27, 29-39] and sonolysis.[40, 41] Chemical studies contain the methods of $KMnO_4$,[42-52] OsO_4,[53-56] hydroperoxides of lipids,[57] halogens,[58-60] and active oxygen species (hydrogen peroxide,[61-63] superoxide,[64-66] hydroxy radical,[67-69] and singlet oxygen[70-74]) oxidations.

These results are summarized as follows taking notice of each component of nucleic acids from the standpoint of organic chemistry.[1] The nucleotides damaged easily by γ-ray and X-ray are pyrimidine nucleotides.[1] The main active species in water is hydroxy radical. About 80 % of hydroxy radical attacks the bases of nucleosides and the residual 20 % reacts with suger moieties.[2] It is thought that the damages of pyrimidine bases start by the addition of hydroxy radical to the

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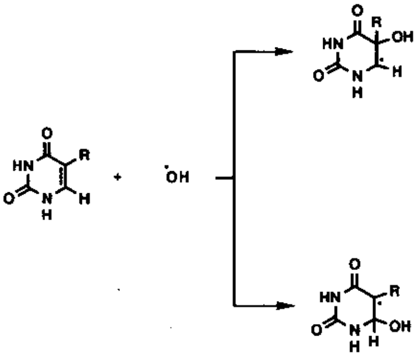
†Dedicated to Professor Edward C. Taylor on the occasion of his 70th birthday.

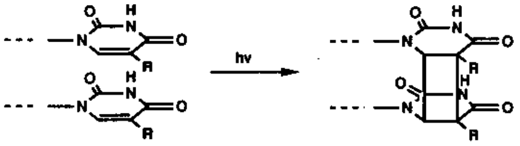
pyrimidine 5, 6-double bond as shown in Scheme 1.[28] Pyrimidines tend to be dimerized by UV irradiation (Scheme 2).[27] It was also reported that cross-linkings of L-amino acids to thymidine by UV irradiation induced damage in biological systems (Scheme 3).[37] 2) Pyrimidines are damaged oxidatively by $KMnO_4$ (Scheme 4).[51] The reactivities of 5'-nucleotides decrease according to pdT> pU> pC> purine nucleotides in their $KMnO_4$ oxidation.[46] OsO_4 oxidation is more specific than $KMnO_4$, because OsO_4 oxidizes only pyrimidine bases, especially thymine derivatives,[53] and does not purin bases. In contrast with the facts described above, the nucleic acid components damaged easily by $^1O_2$ are purines, especially guanine derivatives are damaged fast.[70]

On the basis of the above background, we have focused on thymine and thymidine derivatives which are thought to be damaged most easily among nucleic acids constituent elements. In this review we would like to describe the work which had been done in our laboratory at Kyoto in Paragraphs II and III.

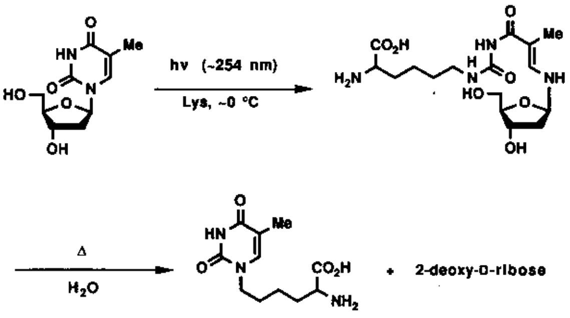
In the meantime, oxidative damages in biological systems may be caused mainly by active oxygen species. However there are some protective systems against the damages in live bodies. They include the



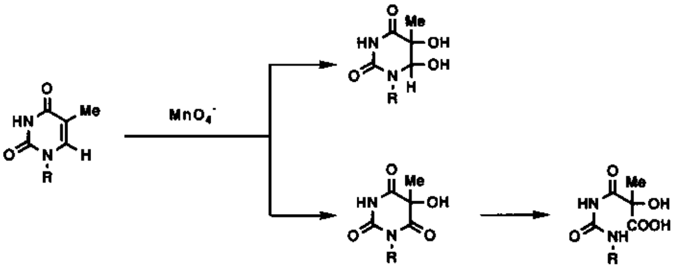
Scheme 1



Scheme 2



Scheme 3



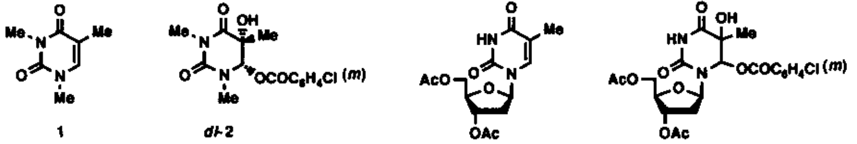
Scheme 4

enzymes such as superoxide dismutase, catalase, selenium-containing glutathione peroxidase, and the antioxidants such as α-tocopherol and ascorbic acid in biological systems. It has been repoted by Ames[75] that uric acid is a powerful antioxidant as well as a scavenger of singlet oxygen and radicals. Moreover after nucleic acids have been damaged, there are repair abilities of the participation of enzymes, for example, photoreactivation, excision repair, and post-replication repair.[76-83] Although there are many studies about them, the investigations of organic chemistry on molecular level are little. Therefore we have examined the possibility of nonenzymatic repair reactions using the model compounds, which will be described in Paragraphs IV and V.

II. Oxidative damage of thymine and thymidine derivatives

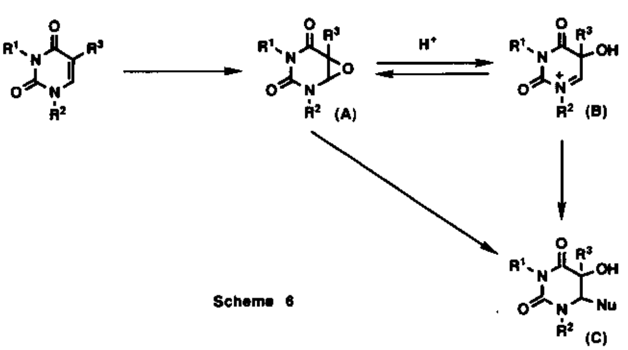
II-1. Oxidative damage of thymine and thymidine derivatives with *m*-chloroperbenzoic acid[84, 85]

Considering the similarity to the reaction *in vivo*, a model reaction for the oxidation of thymine and thymidine with peroxide was carried out by the use of 1, 3-dimethylthymine with *m*-chloroperbenzoic acid (MCPBA). Thus, oxidation of 1, 3-dimethylthymine (**1**) with MCPBA in CH_2Cl_2 under reflux gave the hydroxy ester (**2**) in 76 % yield (Scheme 5). The *cis* relationship of the C_5-hydroxy group and the C_6-acyloxy group in **2** was established by an X-ray analysis of the compound (**2**). Oxidation of diacetylthymidine (**3**) with MCPBA in CH_2Cl_2 under reflux gave the hydroxy esters (**4a** and **4b**) in 59 % and 17 % yields, respectively. The above results seem to suggest that the newly introduced hydroxy and acyloxy groups in **4a** and **4b** have a *cis* relationship.



1 *dl*-2 3 4a 4b

Scheme 5



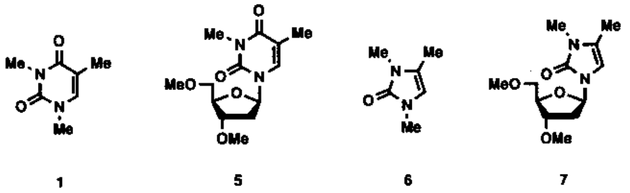
Scheme 6

The formation of these products could be readily explained by assuming that an initially formed epoxide (**A**) or a cationic intermediate (**B**) was attacked by nucleophiles to give thier adducts (**C**) as shown in Scheme 6. Furthermore, the predominant formation of *cis* product (**2**) is explicable in terms of a *gauche* effect, as it has been demonstrated that the *gauche* comformer is more stable than the *trans* comformer in certain highly electronegatively substituted systems (*gauche* effect).[72, 86, 87] Hence, it would be reasonable to assume that the nucleophile attacks the intermediate (**B**) from an energetically favorable direction to yield the *cis* product.

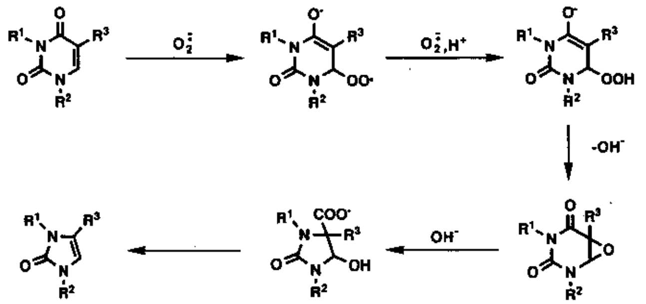
Thus, the formation of such oxidative products as **2** strongly suggested the possibility of cross-coupling of pyrimidine bases with amines and amino acids by way of the reactivity of the epoxide (**A**) or its equivalent (**B**).

II-2. Oxidative damage of thymine and thymidine derivatives with superoxide ion[88]

In connection with our model studies on the oxidative damage of nucleic acids, we have investigated the oxidation of the thymine and thymidine derivatives (**1**) and (**5**) with potassium superoxide (KO_2) (Scheme 7). Compounds (**1**) and (**5**), in which their active hydrogens were protected by alkylation, reacted with KO_2 in the presence of 18-crown-6 under argon atmosphere to produce the corresponding ring contracted imidazolone derivatives (**6**)[89] and (**7**) in 15-53 % yield. This ring contraction is a novel type of reaction in nucleic acid chemistry.



Scheme 7



Scheme 8

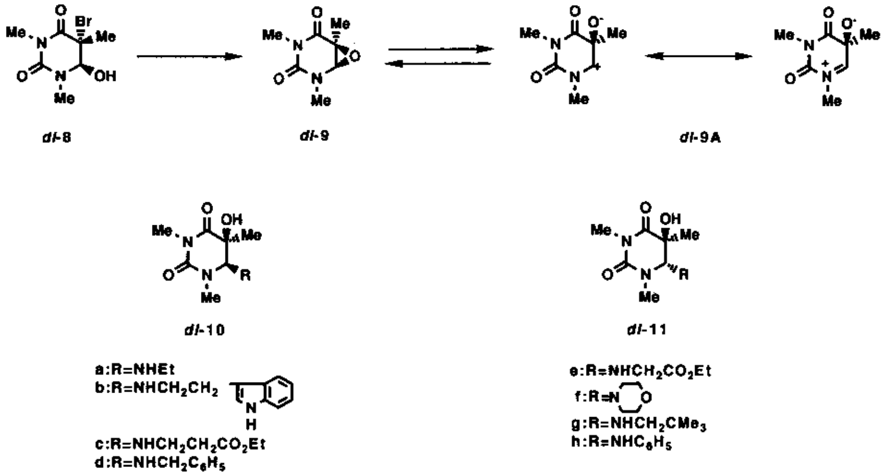
A plausible mechanism for the formation of the imidazolones (**6**) and (**7**) from the thymine derivatives (**1**) and (**5**) is shown in Scheme 8.[90]

It is considered that in the double-strand DNA there are no active hydrogens on the thymidine units, because they are included inside the double helix in a hydrogen-bonded state. Therefore we propose that this type of transformation of thymidine by superoxide ion might take place in biological systems under certain circumstances.

III. Reaction of thymine and thymidine epoxide derivatives with amines and L-amino acid derivatives

III-1. Reaction of 1, 3-dimethylthymine epoxide with amines[91, 92]

It should be considered that the intermediates epoxide (**A**) or its equivalent (**B**) mentioned in Paragraph II may also react with nucleophiles such as amino acids or nucleic acid components. Therefore the reactions of 1, 3-dimethythymine epoxide (**9**) with achiral amines as a model reaction for nucleic acid-protein cross-links have been pursued (Scheme 9).



Scheme 9

Table 1 The results of reactions of **9** with amines

Amine	Products		Ratio of **10**:**11**	Yield (%)
Ethylamine	—	**11a**	—	80.7
Tryptamine	**10b** ,	**11b**	1:5.6	95.0
β-Ala-OEt[a)]	**10c** ,	**11c**	1:3.9	89.7
Benzylamine	**10d** ,	**11d**	1:3.7	74.5
Gly-OEt[a)]	**10e** ,	**11e**	1:2.8	71.7
Morpholine	**10f** ,	**11f**	1:2.6	62.3
Neopentylamine	**10g** ,	**11g**	1:2.4	quant.
Aniline	**10h** ,	**11h**	1:1.3	95.4

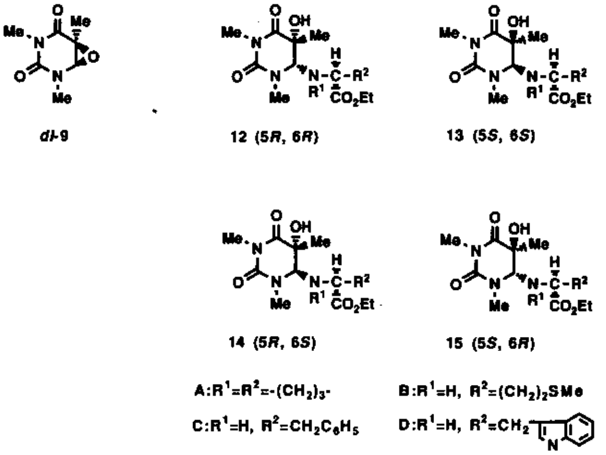
a) Abbreviations: β-Ala-OEt, β–alanine ethyl ester; Gly-OEt, glycine ethyl ester.

Table 2 The isomerization of *trans* adducts (**11**) to *cis* adducts (**10**)

Starting material	Product	Yield (%)
11a	**10a**	quant.
11b	**10b**	79.4
11c	**10c**	83.3
11d	**10d**	88.8
11e	**10e**	64.0
11f	**10f**	87.8
11g	**10g**	quant.
11h	**10h**	8.6

Ryang and Wang reported that *trans*-bromohydrin (**8**) was treated with triethylamine (Et$_3$N) to generate the epoxide (**9**),[87] reaction of **9** prepared by this procedure with nucleophiles was then examined. Thus, reaction of **9** prepared *in situ* from *dl-trans*-bromohydrin (**8**) and Et$_3$N with achiral amines in THF under reflux gave two products, a *cis* adduct (minor product) (**10**) and a *trans* adduct (major product) (**11**), except in the case of ethylamine. The results are summarized in Table 1. The stereostructures of the products were determined by X-ray analyses and by isomerization procedure of the *trans* adducts to the *cis* adducts by using boron trifluoride etherate (Table 2). Therefore, it seems reasonable to assume that the addition reaction proceeds *via* both the epoxide (**9**) which gives the *trans* adduct and the iminium intermediates (**9A**) which give the *cis* adduct selectively, by nucleophilic attack of amines as reported by Ryang and Wang.[72, 87] The observed regiospecificity of the epoxide ring opening might be attributed to the contribution of the lone-pair electrons on N$_1$.[87]

III-2. Reaction of 1, 3-dimethylthymine epoxide with L-amino acid derivatives[92, 93]

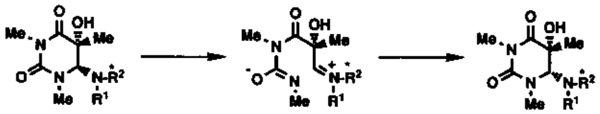


Scheme 10

Next we have investigated the reaction of 1, 3-dimethylthymine epoxide (**9**) with chiral L-amino acid derivatives as a model reaction for nucleic acid-protein interactions.

Reaction of epoxide (**9**) with L-amino acid derivatives (Pro-OEt, Met-OEt, Phe-OEt, and Trp-OEt) was investigated. Each reaction afforded four optically active diastereomers (**12**)-(**15**) as had been expected (Scheme 10). The stereostructures containing absolute configulation of the products were elucidated by an X-ray analysis, optical rotation, and isomerization of the *trans* adducts to *cis* adducts by using boron trifluoride etherate.

Incidentally we propose that the isomerization reaction by boron trifluoride etherate stated above proceeds as depicted in Scheme 11.

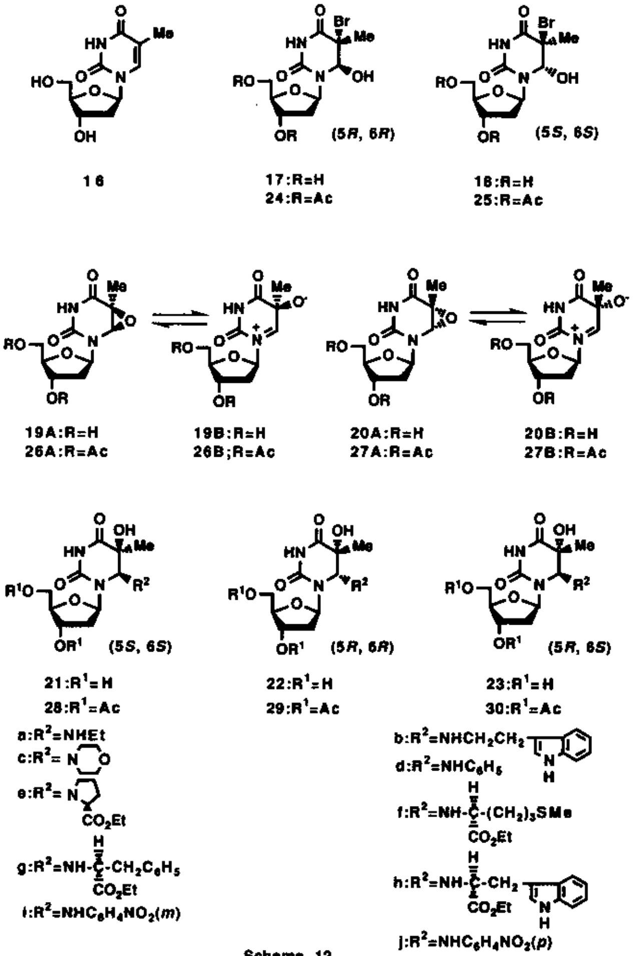


Scheme 11

III-3. Reaction of thymidine epoxide with amines and L-amino acid derivatives[94, 95]

Subsequently, the reaction of thymidine epoxides (**19**) and (**20**), which might be derived from one biological component thymidine (**16**), with amines and L-amino acid derivatives was explored (Scheme 12).

Reaction of thymidine with *N*-bromosuccinimide (NBS) afforded thymidine bromohydrins (**17**) and (**18**) in 66 and 31 % yields respectively.[58] Reaction of **19** prepared *in situ* from **17** and Et_3N with achiral amines or L-amino acid derivatives gave the cross-coupling product (**21**) in high yield (Table 3). On the other hand, reaction of **20** prepared from **18** gave cross-coupling products (**22**) and (**23**) (Table 4). The stereostructures containing absolute configuration of the products were elucidated by X-ray analysis, optical rotation, and isomerization of the *trans* adducts to *cis* adducts by using boron trifluoride etherate. In order to investigate whether the 5'-hydroxy group participates in forming a single product in a cross-coupling reaction of **17** or not, diacetyl bromohydrins (**24**) and (**25**) were prepared according to the literature.[58] Reaction of **24** with morpholine *via* **26** produced a single product (**28c**), which was identical with an acetylation product of **21c**.



Scheme 12

Table 3 The results of reactions of **19** and **26** with nucleophiles

Nucleophile	Product	Yield (%)
Ethylamine	**21a**	98.4
Tryptamine	**21b**	95.0
Morpholine(A)	**21c**	quant.
Aniline	**21d**	61.6
Pro-OEt	**21e**	97.3
Met-OEt	**21f**	96.6
Phe-OEt	**21g**	81.0
Trp-OEt	**21h**	98.7
m-NO_2-aniline	**21i**	45.2
p-NO_2-aniline	**21j**	63.4
Morpholine(B)	**28c**	56.1

On the other hand, reaction of **25** with morpholine *via* **27** produced two cross-coupling products (**29c**) and (**30c**), which were identical with acetylation products of **22c** and **23c**, respectively. Therefore, it is clear that 5'-hydroxy group, at least, does not participate in forming a single product, although the reason why **19** gives a sole product still remains unclear. Cross-coupling reaction of thymidine epoxides (**19**) and (**20**) with amines and amino acid derivatives obviously produced more *cis* products than the reaction of 1, 3-dimethylthymine epoxide (**9**).

It is thought that suger moiety of thymidine epoxide contributed to this proportions. If thymine and thymidine epoxide are produced *in vivo*, they may react with amino acid and other nucleophiles to produce the corresponding cross-coupling products.

Table 4 The results of reactions of **20** and **27** with nucleophiles

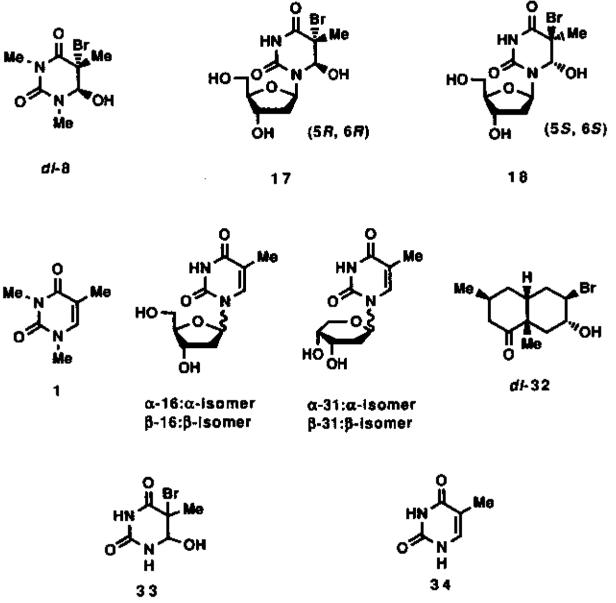
Nucleophile	Product	Yield (%)	(total yield)
Ethylamine	**22a**	53.3	
	23a	a)	
Tryptamine	**22b**	53.4	
	23b	a)	
Morpholine	**22c**	34.3	
	23c	46.7	(81.0)
Aniline	**22d**	22.0	
	23d	40.8	(62.8)
Pro-OEt	**22e**	31.7	
	23e	31.6	(63.3)
Met-OEt	**22f**	32.1	
	23f	12.1	(44.2)
Phe-OEt	**22g**	36.8	
	23g	24.1	(60.9)
Trp-OEt	**22h**	31.5	
	23h	a)	
Morpholine	**29c**	8.3	
	30c	25.4	(33.7)

a) Not isolable in a pure state because of its instability.

IV. Repair of thymine and thymidine bromohydrin derivatives, models of oxidatively damaged nucleosides

IV-1. Repair of thymine and thymidine bromohydrin derivatives, models of oxidatively damaged nucleosides, by sunlight or heat[96, 97]

In relation to the oxidative damage of nucleic acids, especially of thymidine, it has recently been reported that haloperoxidases such as chloroperoxidase and bromoperoxidase obtained from living cells activated a halide anion to the halonium cation in the presence of hydrogen peroxide and catalyzed halogenation of nitrogen-containing aromatic heterocycles such as pyrazol, uracil, thymine, and cytosine to yield the respective halogenated (oxidized) products. Chloroperoxidase, in particular, catalyzed the oxidation of thymine to thymine bromohydrin in the presence of potassium bromide and hydrogen peroxide.[59] This finding strongly suggests that thymidine in living cells can be oxidized to thymidine bromohydrin with the aid of haloperoxidases.



Scheme 13

During our studies on the reactions of 1, 3-dimethylthymine and thymidine epoxides with amines, L-amino acid derivatives, and other nucleophiles as model reactions for nucleic acid-protein cross-links mentioned above, we observed that treatment of the bromohydrins (**8**), (**17**), and (**18**) with nucleophiles such as thiophenol, *N*-acetyl-L-cysteine, benzoic acid, and L-ascorbic acid gave thymine derivatives (**1**), (**16**), and (**31**) corresponding to the repaired products (Scheme 13). However, the reproducibility of the reaction was very poor. Finally, we realized that the repair reaction could proceed without any reagent when expose to either sunlight or heat. As can be seen from the results in Table 5, the reaction proceeded with sunlight or heat (Entries 1 and 4), but not in the dark at room temperature. However, when a catalytic amount of azobisisobutylonitrile (AIBN) was added, the reaction in the dark proceeded smoothly (Entry 5) and that under sunlight was slightly accelerated (Entry 2). The results also showed that galvinoxyl, a radical scavenger, quenched the reaction with the starting material being recovered in good yield (Entries 3 and 6). These results show that the repair reaction proceeds by a radical mechanism.[26] Furthermore, this reaction seems to be characteristic of bromohydrins having an aminal (α-carbinolamine) moiety, because reaction of *trans*-bromohydrins (**32**)[98] under the same reaction conditions resulted in recovery of the starting material (**32**) in high yield.

Subsequently, the repair reaction of thymine bromohydrin (**33**) to thymine (**34**) was investigated, the results being summarized in Table 6. The repair reaction of **33**, as well as the bromohydrins (**8, 17**, and **18**), with sunlight proceeded

Table 5 Data on the repair reaction of bromohydrins (**8**), (**17**), and (**18**)

Entry	Reaction conditions a)	Bromohydrin (**8**)			Bromohydrin (**17**)				Bromohydrin (**18**)			
		Reaction time (h)	**1** (%)	Recovery (%)	Reaction time (h)	**16** (%)	**31** (%)	Recovery (%)	Reaction time (h)	**16** (%)	**31** (%)	Recovery (%)
1	A	2.5	81.7	—	1.5	42.7 (1:4.1)b)	16.1 (β)c)	—	1.0	32.7 (1:2.8)b)	8.1 (1:1.4)b)	—
2	A, AIBN	1.0	89.3	—	1.0	37.0 (1:4.0)b)	13.0 (β)c)	—	1.0	34.9 (1:3.0)b)	8.9 (1:1.7)b)	—
3	A, Galvinoxyl	17.0	—	66.6	17.0	—	—	92.5	22.0	—	—	84.2
4	B	10.0	92.4	—	3.0	59.8 (1:2.4)b)	13.1 (β)c)	—	3.0	66.8 (1:1.7)b)	22.4 (1:1.5)b)	—
5	C, AIBN	3.0	quant.	—	4.0	50.0 (1:2.6)b)	18.2 (β)c)	—	6.0	49.5 (1:2.9)b)	4.7 (β)c)	—
6	B, Galvinoxyl	10.0	—	81.0	6.0	—	—	90.0	8.0	—	—	91.7

a) Reaction Conditions: A-- under sunlight irradiation at room temperature; B--in the dark under reflux; C--in the dark at room temperature.

b) Values in parentheses indicate the proportions of α- and β- isomers. Isomers were isolated by preparative tlc in Entry 1. In other Entries, proportions of α and β were determined by nmr spectroscopy.

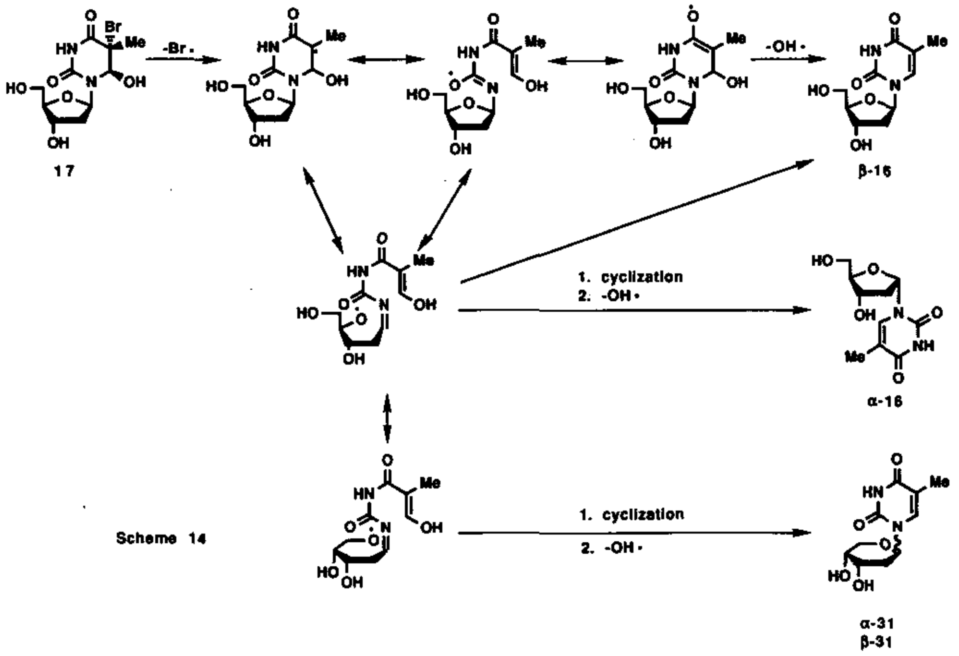
c) A trace amount of α-isomer (α-**31**) was present.

smoothly (Entries 1 and 2), whereas the reaction of **33** in THF with heat scarcely proceeded (Entries 3 and 4). The repair reaction with heat finally proceeded in dimethyl sulfoxide (DMSO) in the dark at 130 °C in the presence of a catalytic amount of AIBN (Entry 5). The difference of reactivity between the bromohydrins (**33**) and (**8**), (**17**), and (**18**) seems to be attributable to the presence of the N-1 substituent.

Table 6 Data on the repair reaction of thymine bromohydrin (**33**)

Entry	Reaction conditions[a)]	Reaction time (h)	**34** (%)	Recovery (%)
1	A	4.5	82.3	—
2	A, AIBN	2.5	93.2	—
3	B	23.0	—	quant.
4	B, AIBN	14.0	—	quant.
5	C, AIBN	0.8	72.9	—

[a)] *Reaction conditions:* A; *under sunlight irradiation at room temperature,* B; in the dark under reflux, C; DMSO was used instead of THF in the dark at 130 °C.



Scheme 14

Next, the repair reaction of the bromohydrin (**8**) with other reagents, especially biological compounds was examined. The results are summarized in Tables 7 and 8, indicating that addition of reagents affected the repair reaction in THF under heating in the dark (Table 7), but did not affect the reaction under sunlight (Table 8), although the reason for this remains to be unclarified.

In Scheme 14, we propose a plausible mechanism for the repair reaction of bromohydrin (**17**), which was initiated by radical scission of the C_5-Br bond.

We think that thymidine may protect the other components *in vivo* by oxidizing (halogenating) itself and then readily undergoing repair. We consider that thymidine plays an important role, functionally and structurally, in the damage and repair of nucleic acids.

Table 7 Data on the repair reaction of the bromohydrin (**8**) with heat and reagents

Reagnet	Equivalent	Reaction time (h)	**1** (%)
—	—	10-31	92-96
N-Ac-L-Cys-OMe	1.2	0.8	95
N-Ac-L-Cys	1.2	1.5	97
PhSH	2.4	2.3	96
Ascorbic acid	1.2	2.0	96
Ph3P	1.2+1.2[a]	5.0	88

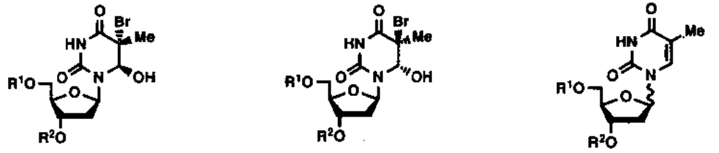
[a] The reagent (1.2 eq.) was added after 1 h.

Table 8 Data on the repair reaction of the bromohydrin (**8**) with sunlight and reagents

Reagent	Equivalent	Reaction time (h)	**1** (%)
—	—	2.5-6.5	81-96
N-Ac-L-Cys-OMe	1.2	6.0	81
N-Ac-L-Cys	1.2	6.5	87
PhSH	2.4	2.5	97

IV-2. Repair of thymidine phosphate bromohydrins, models of oxidatively damaged nucleotides, by sunlight or heat[99]

Subsequently, we investigated the repair reaction of bromohydrins (**35**, **36**, and **37**)[14] of thymidine diphenylphosphates as model compounds of nucleotides which possess phosphate linkage(s) as well as DNA, by sunlight and heat (Scheme 15).



17: $R^1=R^2=H$
35A: $R^1=H$, $R^2=PO(OC_6H_5)_2$
36A: $R^1=PO(OC_6H_5)_2$, $R^2=H$
37A: $R^1=R^2=PO(OC_6H_5)_2$

18: $R^1=R^2=H$
35B: $R^1=H$, $R^2=PO(OC_6H_5)_2$
36B: $R^1=PO(OC_6H_5)_2$, $R^2=H$
37B: $R^1=R^2=PO(OC_6H_5)_2$

16: $R^1=R^2=H$
38: $R^1=H$, $R^2=PO(OC_6H_5)_2$
39: $R^1=PO(OC_6H_5)_2$, $R^2=H$
40: $R^1=R^2=PO(OC_6H_5)_2$

Scheme 15

The results shown in Table 9, indicate that the reaction proceeded with sunlight or heat in the presence of AIBN, and that the reaction is completely prohibited by addition of galvinoxyl, the starting material material being recovered in good yields. These fact suggest that the repair reaction of nucleotide bromohydrins (**35**, **36**, and **37**) as well as nucleoside bromohydrins (**17** and **18**) proceeds through a radical mechanism. Moreover, it should be noted that bromohydrins (**35** and **37**) possessing 3'-phosphate linkage were repaired smoothly to regenerate the corresponding phosphate derivatives (**38** and **40**) of β-thymidine. It is particularly interesting that bromohydrins (**37**) with the 3', 5'-diphosphate linkages of a nucleic acid type was repaired most efficiently. This finding strongly suggests the functional significance of phosphate linkage, in addition to the functional and structural significance of thymidine itself, in the repair reaction of oxidatively damaged thymidine derivatives.

Table 9 Data on the repair reaction of bromohydrins (**35**, **36**, and **37**)[a]

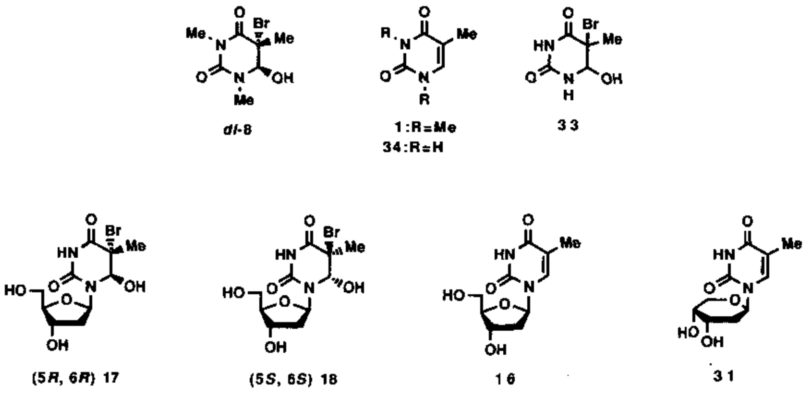
	Reaction conditions[b]	A	A AIBN	B	C	C AIBN	D
35[c]	Reaction time (h)	2.0	1.0	14.0	5.0	1.5	12.0
	38 (%)	71.9[d]	72.5[d]	—	25.2[d]	58.4[d]	—
	Recovery (%)	—	—	95.1	—	—	88.4
36A	Reaction time (h)	2.0	1.0	13.0	3.0	1.5	9.0
	39 (%)	49.8 (1:8.3)	62.3 (1:7.4)	—	32.7 (1:0.9)	34.4 (1:1.9)	—
	Recovery (%)	—	—	80.3	—	—	65.7
36B	Reaction time (h)	2.0	1.0	17.0	5.0	2.0	19.0
	39 (%)	48.7 (1:2.9)	51.8 (1:2.0)	—	37.9 (1:0.8)	44.9 (1:1.5)	—
	Recovery (%)	—	—	62.6	—	—	84.7
37A	Reaction time (h)	2.8	2.0	16.5	23.0	2.5	22.5
	40 (%)	87.4[d]	85.8[d]	—	—	63.1[d]	—
	Recovery (%)	—	—	80.3	54.0	—	86.2
37B	Reaction time (h)	2.3	2.3	19.5	22.5	0.8	22.5
	40 (%)	79.8[d]	90.4[d]	—	—[e]	75.1[d]	—
	Recovery (%)	—	—	89.0	—[e]	—	95.8

[a] Values in parentheses indicate the proportions of α- and β-isomers.
[b] Reaction conditions: A) under sunlight irradiation (2400 lux) at room temperature; B) under reaction conditions A in the presence of galvinoxyl ; C) in the dark under reflux; D) under reaction conditions C in the presence of galvinoxyl.[c] A mixture of **35A** and **35B** was used. [d] A trace amount of α-isomer was contained. [e] Decomposition.

IV-3. Repair of thymine and thymidine bromohydrin derivatives, models of oxidatively damaged nucleosides, by Copper (II) and ascorbic acid system[100]

The reaction wrote in Paragraph IV-1 and 2 hardly proceeded at all in water. Since this repair reaction included a radical mechanism,[8] we have tried the use of transition metals known to perform electron transfer in biological systems.

We explored the repair of 1,3-dimethylthymine bromohydrin (**8**) with metals (Cu, Co, Fe, Mo, or Mn), which are known to participate in redox reactions in living cells (Scheme 16). In a typical procedure, a suspension of **8**, $CuSO_4$, and ascorbic acid (AA) in water was stirred under argon atmosphere at room temperature. The results showed that the repair reaction did not occur with Co, Fe, Mo, Mn, or AA and the respective metal ions. However, as can be seen from Table 10, the reaction proceeded rapidly with 2.4 equiv. of $CuSO_4$ and AA (Entry 1), and proceeded even with a catalytic amount of $CuSO_4$ with 2.4 equiv. of AA (Entry 4). However, it did not proceed when AA or $CuSO_4$ alone was used (Entries 5 and 6). The repair reaction also proceeded smoothly within 5 min with AA and $Cu(OAc)_2$ which contained a counter anion other than SO_4^{2-} (Entry 7).



Scheme 16

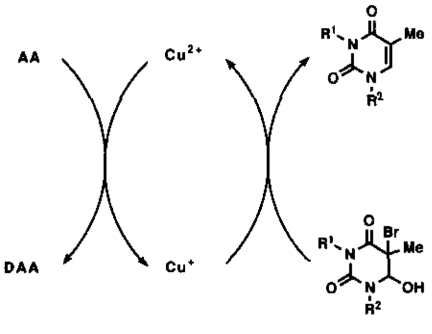
Table 10 The repair reaction of **8** by the Cu^{2+}-ascorbic acid (AA) system

Entry	CuSO4 (equiv.)	AA (equiv.)	Reaction time	TMU (1) (%)	Recovery (%)
1	2.4	2.4	5 min	99.0	—
2	1.2	2.4	14 h	73.6	9.2
3	0.5	2.4	21 h	72.3	13.2
4	0.1	2.4	36 h	64.5	21.0
5	—	2.4	36 h	—	quant.
6	2.4	—	36 h	—	quant.
7[a]	2.4	2.4	5 min	80.4	—

[a] $Cu(OAc)_2$ was used instead of $CuSO_4$.

Table 11 The repair reaction of **8** by Cu^+

Entry	CuCl (equiv.)	AA (equiv.)	Reaction time	TMU(1) (%)	Recovery (%)
1	2.4	2.4	20 h	89.3	4.4
2	2.4	—	36 h	74.3	10.6



AA, ascorbic acid
DAA, dehydroascorbic acid

Scheme 17

To examine the active species of this reaction, we used CuCl instead of Cu^{2+}-AA. Table 11 shows that the reaction proceeded *via* either Cu^+-AA together or Cu^+ alone, but was slower than that with Cu^{2+}-AA. Moreover, water-soluble Cu^+ is unstable in water and lead immediately to disproportionation to Cu and Cu^{2+}. Since the reaction with Cu^{2+}-AA was very fast, it was thought that the active species was fresh Cu^+ generated *in situ* in the media (Scheme 17). DNA and nucleic acid bases are known to be damaged by Cu^{2+}, AA, and O_2,[68, 69, 101-107] where a hydroxy radical is thought generally to be the active species. To determine whether the active species of this repair reaction is the hydroxy radical or not, the reaction of **8** with Fenton's reagent ($FeSO_4$-H_2O_2) was examined. Since there was no repair product (**1**) at all, the hydroxy radical was excluded as an active species in the repair reaction by

Cu^{2+}-AA.

Next, we investigated the repair reaction of thymine bromohydrin (**33**), (5*R*, 6*R*)-thymidine bromohyrin (**17**), and (5*S*, 6*S*)-thymidine bromohydrin (**18**). Thymine bromohydrin (**33**) gave thymine (**34**) in high yield, and thymidine bromohydrins (**17**) and (**18**) gave thymidine (**16**) accompanied by thymine (**34**) and pyranose-form thymidine (**31**). The reaction of thymidine (**16**) with Cu^{2+}-AA did not give **31** at all but a small amount of thymine (**34**) (Table 12); therefore **31** might be produced in the repair process of **17** and **18** as described above.

Table 12 The repair reaction of **33**, **17**, **18**, and **16** by the Cu^{2+}-AA system

Starting material	CuSO$_4$ (equiv.)	AA (equiv.)	Reaction time	**16** (%)	**31** (%)	**34** (%)
33	2.4	2.4	5 min	—	—	92.9
17	2.4	2.4	5 min	61.9	14.0	15.1
18	2.4	2.4	5 min	57.5	16.8	14.3
16	2.4	2.4	2 h	90.7	—	8.3

In conclusion, thymine and thymidine bromohydrins turned out to be readily repaired in water at room temperature by the Cu^{2+} and AA system. Since both Cu^{2+} and AA are widely distributed in biological systems, this type of repair of thymine and thymidine bromohydrins might occur in living cells.

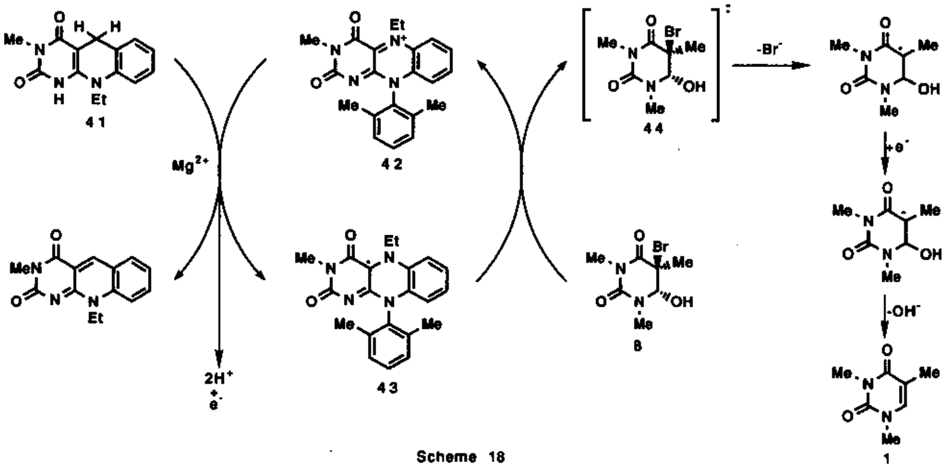
IV-4. Repair of thymine and thymidine bromohydrin derivatives, models of oxidatively damaged nucleosides, by 1,5-dihydro-5-deazaflavin and flavinium[108]

NADH and flavin work co-operatively as an electron bridge in many biological systems.[109] NADH apparently acts as a two-electron carrying shuttle, whereas flavin acts as both a one- and a two- electron carrying shuttle. Flavin can therefore function as an electron switch between NADH and iron, *e.g.* in a respiratory system, transferring one electron from NADH to iron. After consideration of this natural system, we attempted to construct an effective one-electron transfer system for use in synthetic organic chemistry. We have found that a combination of 1,5-dihydro-5-deazaflavin[110] and flavinium perchlorate[111] in the presence of magnesium perchlorate can accomplish one-electron transfer very efficiently.

Since this repair reaction mentioned above included one-electron chemistry, it occurred to us that the use of the 1,5-dihydro-5-deazaflavin-flavinium system as an electron bridge may accomplish the reductive repair of **8** (Scheme 18).

As can be seen from Table 13, the repair reaction proceeded very smoothly to give the original **1** in high yield (Entry 1). The results also showed that galvinoxyl could completely quench the reaction, the starting material being recovered in good yield (Entry 4). The reaction did not proceed on omission of magnesium perchlorate (Entry 2) or **42** (Entry 3) from the system. The role of magnesium perchlorate appears to be to facilitate electron transfer from **41** to **42**, because the reduced flavin radical (**43**) or its equivalent generated *in situ* by sodium hydrosulfite (Na$_2$S$_2$O$_4$) reduction of **42** could repair **8** under the same conditions, thouth in low yield.

In Scheme 18, we propose a plausible mechanism for the repair of **8** *by this novel one-electron reduction system.* The reaction would be rationalised by the elimination of bromide ion from the bromohydrin radical anion (**44**) initially formed by one-electron transfer. There is a precedent for this type of carbon-bromine bond scission in the uracil series.[112]



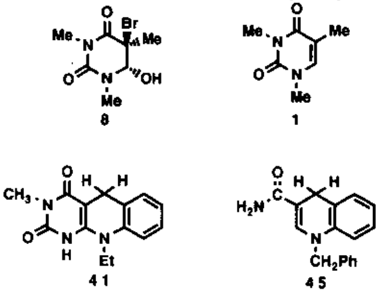
Scheme 18

Table 13 The repair reactions of 1,3-dimethylthymine bromohydrin (**8**) by the 1,5-dihydro-5-deazaflavin (**41**)-flavinium (**42**) system

Entry	Reagent	1 (%)	Recovery (%)
1	**41** **42** Magnesium perchlorate	88.7	0
2	**41** **42**	0	69.5
3	**41** Magnesium perchlorate	0	72.5
4	**41** **42** Magnesium perchlorate Galvinoxyl	0	58.0

The same repair reaction proceeds using an electron bridge consisting of natural NADPH and FMN (riboflavin 5'-phosphate), although in less than half the yield of our artificial system. These results suggest that this type of repair of thymine and thymdine bromohydrins may occur in living cells. Furthermore, this one-electron reduction may have considerable utility in organic synthesis as well as in biomimetic reactions, because of the mildness of the conditions and the good yields obtained.

IV-5. Repair of thymine and thymidine bromohydrin derivatives, models of oxidatively damaged nucleosides, by 1,5-dihydro-5-deazaflavin or 1,4-dihydroquinoline[113]



Scheme 19

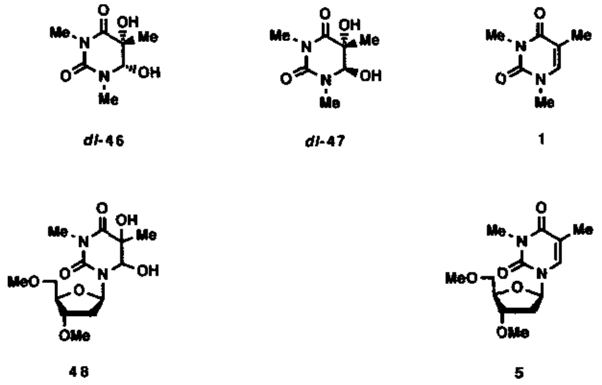
The reductive conversion of 1,3-dimethylthymine bromohydrin (**8**) into 1,3-dimethylthymine (**1**) occurred by 10-ethyl-1,5-dihydro-3-methyl-5-deazaflavin (**41**)[114] in the presence of trifluoroacetic acid in high yield (Scheme 19). Without **41** or trifluoroacetic acid, this reaction did not proceed at all, giving the starting material (**8**) (34-49 %) and decomposition products even after 3 days.

Additionally, another NADH model, 1-benzyl-3-carbamoyl-1,4-

dihydroquinoline (**45**),[115] can also reduce **8** into **1** under the same conditions. Furthermore, the combination of sodium hydrosulfite and trifluoroacetic acid converted **8** into **1** in high yield under the same conditions.

V. Repair of thymine and thymidine diol derivatives, models of oxidatively damaged nucleosides[116]

It has been reported that the *cis* isomer of thymine and thymidine diols are released in human and rat urine as the result of excision repair of oxidatively damaged DNA.[76] Although the several kinds of excision repairs for the abnormal nucleic acids by endonucleases are known in biological systems, we considered that there would possibly be other types of repair mechanism for oxidatively modified nucleic acids. Thus, we have examined the repair reactions of thymine and thymidine diols as models of damaged nucleic acids using a wide variety of reducing agents.



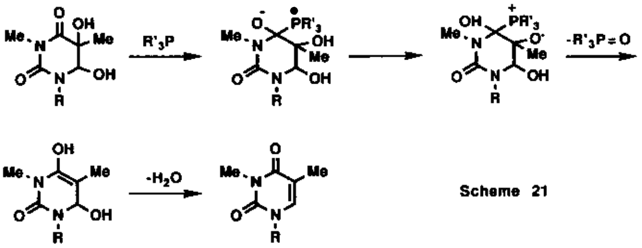
Scheme 20

A mixture of 1,3-dimethythymine diol (*cis* **46** or *tras* **47**)[17] (Scheme 20) and phosphorus compounds was treated under the conditions indicated in Table 14.

Table 14. The results of reaction of **46** and **47** with phosphorus compounds

Compound	Reagent	Equivalent	Solvent	Conditions		Yield of **1** (%)
46	PBr_3	10.0	THF	Room temp.	20 h	61.0
47						74.4
46	PCl_3	2.4	THF	Reflux	20 h	73.2
47						72.0
46	$(PhO)_3P$	Neat	—	100 °C	3 h	86.7
47						85.7
46	$(MeO)_3P$	Neat	—	100 °C	16 h	86.0
47						85.3
46	n-Bu_3P	Neat	—	100 °C	24 h	20.0
47						20.0
46	PCl_5	2.4	THF	Reflux	20 h	0
47						0
46	$POCl_3$	2.4	THF	Reflux	40 h	0
47						0

In both **46** and **47**, the reaction proceeded at the same rate to give almost the same yield of **1** in spite of their different stereochemistry, therefore the same reaction intermediate could be considered. The results using several tri and pentavalent phosphorus compounds, sammarized in Table 14, showed that only trivalent phosphorus compounds were effective for this repair reaction. The reaction of thymidine diol derivative (**48**) with triphenylphosphite gave β-thymidine derivative (**5**)[117] in 89% yield, and not α-thymidine isomer. Therefore it seemed reasonable to assume that the movement of the lone pair of N_1 followed by the scission of the C_1'-O bond in ribosyl moiety did not take place.



Scheme 21

The nucleophilic attack on carbonyl carbon by trivalent phosphorus compounds at the initial stage is generally accepted as the mechanism of the Percow reaction (reaction of α-halocarbonyl compopunds with trivalent phosphorus compounds), although the details are still not completely resolved.[118-120] Therefore, we would like to propose tentatively a Perkow type of reaction mechanism shown in Scheme 21 for the reductive repair reaction. To our knowledge, natural and stable trivalent phosphorus compounds have not yet been reported in the field of biochemistry. However, if a trivalent phosphorus derivative is brought about transiently in living cells, this type of the repair reaction for oxidatively modified nucleic acids might occur under certain conditions in live bodies.

We consider that the results described in Paragraphs II to V can offer useful knowledges to understand chemistry about the oxidative damages of thymidine *in vivo*, the cross-coupling reactions with nucleophiles which are present in biological systems, and the repair reactions of oxidatively damaged thymidines to the normal thymidines. These reactions might be brought about from inherent structure and function of thymidine. It is strongly suggested that thymidine may play an important role with regard to the damage and repair of nucleic acids in biological systems.

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