and some of their derivatives suggest identical *R* configurations of (-)-**36** and (+)-**40**.[46]

Registry No. **3**-2-*d*, 84143-95-3; (+)-**3'**, 84235-33-6; **6**-2-*d*, 84143-96-4; (-)-**6'**, 84235-34-7; **9**, 38397-34-1; **10**-2-*d*, 84143-99-7; **12**-2-*d*, 84144-00-3; (-)-**36**, 84143-97-5; (±)-**36**, 84235-39-2; (±)-**40**, 84235-41-6; (-)-**41**, 4835-96-5; **42**, 84235-35-8; **43**, 67999-53-5; **44**, 84235-37-0; **45**, 84235-36-9; **46**, 58001-99-3; **47**, 72203-34-0; (-)-**48**, 84143-98-6; (±)-**52**, 84144-01-4; (±)-**53**, 84144-02-5; **54** (isomer 1), 84144-03-6; **54** (isomer 2), 84235-42-7; (±)-bicyclo[3.2.1]oct-2-ene, 823-02-9; [1*S*-(1α,2β,4α)]methyl 2-norbornylcarbamate, 84144-04-7; [1*S*-(1α,2α,4α)]methyl 2-norbornylcarbamate, 84235-40-5; cyclopentadiene, 542-92-7; (±)-bicyclo[3.2.1]oct-2-ene, 84235-38-1.

(46) Snatzke, G., unpublished results. We are grateful to Professor Snatzke for communication of his preliminary data.

Photoreaction of Thymidine with Alkylamines. Application to Selective Removal of Thymine from DNA

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Abstract: A new type of photoreaction between thymidine (Thd) and alkylamines has been described. Irradiation of Thd and methylamine in $NaHCO_3$ aqueous solution at 0 °C provided ring-opened adduct **15** quantitatively, which on subsequent heating gave 1-methylthymine (**8**). High selectivity toward Thd has been observed when the photoreaction of a mixture of dAdo, dCyd, dGuo, and Thd with methylamine was carried out at 0 °C in the presence of diazabicyclo[2.2.2]octane (0.2 mM), and then the photolysate was kept at 20 °C after irradiation. Irradiation of calf thymus DNA and methylamine under the specified conditions followed by heating at 70 °C led to the efficient release of **8** from DNA. The extent of DNA modification is readily determined by the absorbance change at 300 nm, which corresponds to the amount of the ring-opened adduct between methylamine and thymine in DNA. Model experiments using thymidylyl(3'-5')-2'-deoxyadenosine demonstrate that the 3'-5' phosphodiester linkage is cleaved efficiently in this photoreaction. The present results indicate the irradiation of DNA with methylamine induces an exceedingly facile removal of thymine from DNA with the strand cleavage at the reacting thymine, providing a convenient and potentially useful method for thymine selective modification of DNA.

Chemical modification of nucleic acid components is one of the promising approaches for studying the structure and function of nucleic acids.[1] The developments of base-specific chemical reactions of nucleic acid constituents is extremely important for modification of nucleic acids. The most prominent example so far known for the use of base-specific chemical reaction is the Maxam-Gilbert method for sequencing DNA.[2] In our study exploring the chemical basis of UV-induced nucleic acid-protein cross-links,[3] we have found that photoexcited thymidine reacts with primary amines to produce a ring-opened adduct, which on subsequent heating is readily converted to N(1)-substituted thymine in high yields.[4] We felt that this novel type of photochemical conversion would be used for a specific modification of thymine moieties in DNA. In the present paper, we describe the details of the photochemistry of thymidine in the presence of amines and its application to selective removal of thymine from DNA.[5]

Photochemistry of nucleic acid bases has been studied extensively for many years in connection with photobiology of nucleic acids.[6] The formation of pyrimidine photodimers and photohydrates has been recognized as one of the major reactions induced by UV irradiation of nucleic acids.[6] In view of the importance of photo-cross-linking of biopolymers, especially nucleic acid-protein cross-links, photochemical reactions of nucleic acid components with a variety of compounds have also been thoroughly investigated.[7] Despite many approaches to pyrimidine photochemistry, very little is known about the photoreaction in the presence of amines.[7c] The only study in which products have been fully characterized is that of photoaddition of 1,3-dimethyluracil with *n*-propylamine,[8] although photoaddition of 4-thiouracils[3c] and free radial initiated reaction of purines[9] with alkylamines have previously been reported. This is surprising since basic amino groups on the side chains of lysine and arginine are suggested to be involved in the binding of proteins to the backbones of nucleic acids in nucleic acid-protein complexes such as histone-DNA complexes,[10] and hence photochemical reaction with amino groups is expected to play an important role in the cross-linking processes.[4a,11] We therefore investigated the photochemistry of nucleic acid bases in the presence of amines in detail in order to get a clearer picture of the molecular aspects of UV-induced nucleic acid-protein cross-linking.

Results and Discussion

Photoreaction of Thymidine with Alkylamines. We first examined the direct irradiation of nucleosides including 2'-deoxyadenosine (dAdo), 2'-deoxyguanosine (dGuo), 2'-deoxycytidine (dCyd), and thymidine (Thd) with 254-nm light in the presence

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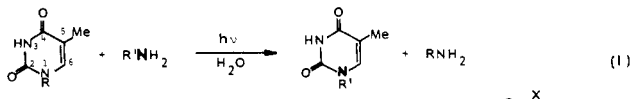
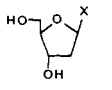
of alkylamines in aqueous solutions. Among these nucleosides, Thd was the only one to react smoothly with alkylamines. For example, irradiation of an aqueous solution (pH 9.5) of Thd (**1**) and *n*-butylamine with a low-pressure mercury lamp through



(1)

1, R = β-D-deoxyribofuranosyl
2, R = *n*-heptyl
3, R = ethyl

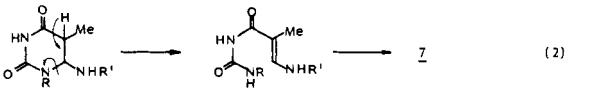
4, R' = *n*-butyl
5, R' = cyclohexyl
6, R' = hydroxyethyl
7, R' = *tert*-butyl
8, R' = methyl



9, X = OH
10, X = NH_2

Vycor filter (mainly 254 nm) at ambient temperature (~35 °C) gave rise to clean formation of 1-*n*-butylthymine (**4**) in 56% yield (based on consumed **1**) as the sole isolable product except the unreacted Thd. The existence of 2-deoxy-D-ribose (**9**) in the reaction mixture was confirmed by means of TLC analysis. Under similar conditions, irradiation of **1** with cyclohexylamine, 2-aminoethanol, and *tert*-butylamine produced the corresponding N(1)-substituted thymines **5**, **6**, and **7**, respectively. The structures of these products were confirmed by independent syntheses.[12] Photoreaction of simple analogues, 1-alkylthymines, with primary amines provided N(1)-substituted thymines in a similar fashion. On irradiation of 1-*n*-heptylthymine (**2**) and *n*-butylamine, both **4** and *n*-heptylamine were obtained in approximately the same yield. Photoreaction of 1-ethylthymine (**3**) with *n*-butylamine gave **4** together with liberation of ethylamine. These observations indicate that the N(1) nitrogen of **2** or **3** is extruded as *N*-alkylamine with the incorporation of the nitrogen of *n*-butylamine into the N(1) of the photoproduct **4**. In the case of **1**, 2-deoxyribose (**9**) is presumed to form from hydrolytically sensitive 2-deoxyribosylamine (**10**) under the aqueous conditions. Thus, the overall process is regarded as a "photochemical exchange reaction" between Thd N(1) nitrogen and alkylamine nitrogen.

Since a variety of nucleophiles are known to attack the C_6 positions of pyrimidine bases under thermal[13] and photochemical conditions,[7,14] we initially thought that nucleophilic attack of the alkylamino group on C_6 of the photoexcited **1** would first occur in this photoreaction to give 6-alkylamino-5,6-dihydrothymidine (**11**).[4a] Ring-opening of **11** may produce **12**, which on cyclization

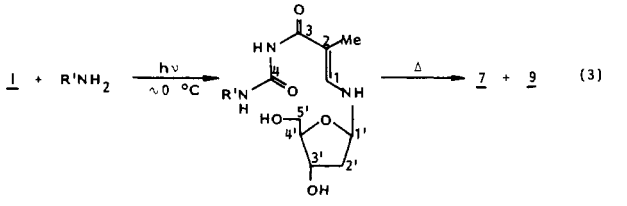


(2)

11

12, R = β-D-deoxyribofuranosyl, R' = *tert*-butyl
13, R' = *tert*-butyl, R = H

gives **7** under the basic reaction conditions. In order to get support of this hypothesis, we have attempted to characterize the intermediate(s) such as **12** at low-temperature irradiation. When a solution of **1** and *tert*-butylamine in distilled water was irradiated at ca. 0 °C, surprisingly, ring-opened adduct **14** was obtained in



(3)

14, R' = *tert*-butyl
15, R' = methyl

70% isolated yield in place of the expected **12**. Heating of the

(12) Cusak, N. J.; Hildick, H.; Robinson, D. H.; Rung, P. H.; Shaw, G. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1720.
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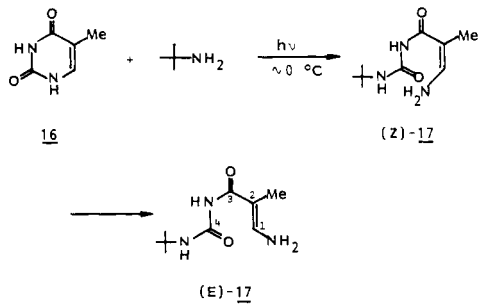
Table I. ^{13}C NMR Chemical Shifts (δ, CD_3OD) for Ring-Opened Adducts **14**, **15**, and **17**

atom	**14**	**15**	(*E*)-**17**[a]
C-1	146.3	146.4	145.1
C-2	98.9	98.9	94.9
C-3	172.2	171.9	170.7
C-4	155.6	157.8	154.0
C-Me	9.9	9.9	9.0
R'	29.6, 51.6 (R' = *t*-Bu)	26.5 (R' = Me)	29.1, 49.9 (R' = *t*-Bu)
C-1'	69.6	69.5	
C-2'	35.4	35.3	
C-3'	68.5	68.4	
C-4'	85.5	85.5	
C-5'	66.9	66.7	

[a] In Me_2SO-d_6.

aqueous solution of **14** rapidly produced **7** quantitatively, indicating that **14** is indeed the precursor of **7**. Similarly, irradiation of thymine (**16**) and *tert*-butylamine at 0 °C followed by preparative



(4)

16 (Z)-**17** (E)-**17**

high-performance liquid chromatography (HPLC) at the same temperature produced a 5:1 *E*–*Z* mixture of **17**. Irradiation of **1** and methylamine under the same conditions proceeded more efficiently to give less stable **15**, which again transformed into 1-methylthymine (**8**) on subsequent heating.

The structures of these ring-opened products are evident from their spectral and chemical properties. In the ^1H NMR of (*E*)-**17** there are an olefinic proton triplet (δ 7.48) and a two-amino proton doublet (δ 4.30), each having a large coupling constant (J = 10 Hz), indicating a partial structure >C=CHNH$_2$ and eliminating an alternative structure **13**. The *E* geometry of the carbon–carbon double bond was assigned by comparison of the chemical shifts of *C*-methyl and olefinic protons with those of the *Z* isomer: The olefinic proton of the *E* isomers appeared at 0.78 ppm lower field, with the *C*-methyl signal being 0.05 ppm at higher field. In support of this assignment, (*Z*)-**17** gradually isomerized to the more stable *E* isomer via enamine–imine tautomerization in alkaline aqueous solution. The ^{13}C NMR spectrum is also consistent with the assigned structure of (*E*)-**17**. The structures of both **14** and **15** were determined by their spectroscopic data including ^1H and ^{13}C NMR spectra in a similar way (Table I). In particular, the chemical shifts of *C*-methyl (δ 1.68) and olefinic (δ 7.36) protons in the ^1H NMR in comparison with those of (*E*)-**17** indicate the *E* geometry of the carbon–carbon double bond for **14**. All of the ring-opened adducts (**14**, **15**, **17**) are readily converted to the corresponding *N*(1)-alkylthymines upon being heated in slightly alkaline aqueous solutions. The UV absorption spectra of these products have considerably red-shifted maxima (~290 nm) compared to that of Thd.

Mechanistic Aspects. In these biomolecular photoreactions, the solution pH plays a crucial role. As shown in Figure 1, irradiation of **1** and methylamine at 0 °C did not give **15** at acidic or neutral pH, while the quantum yield of **15** increased with increasing pH in alkaline pH region (pH 8.0–11). The result suggests that a photoexcited state of Thd anion **19** (pK_a = 9.8) is involved in the photoaddition with methylamine. In support of this, N(3)-protected thymines such as 1,3-dimethylthymine did not undergo photoaddition with alkylamines under these conditions. Additional support for the involvement of the anion **19** is furnished

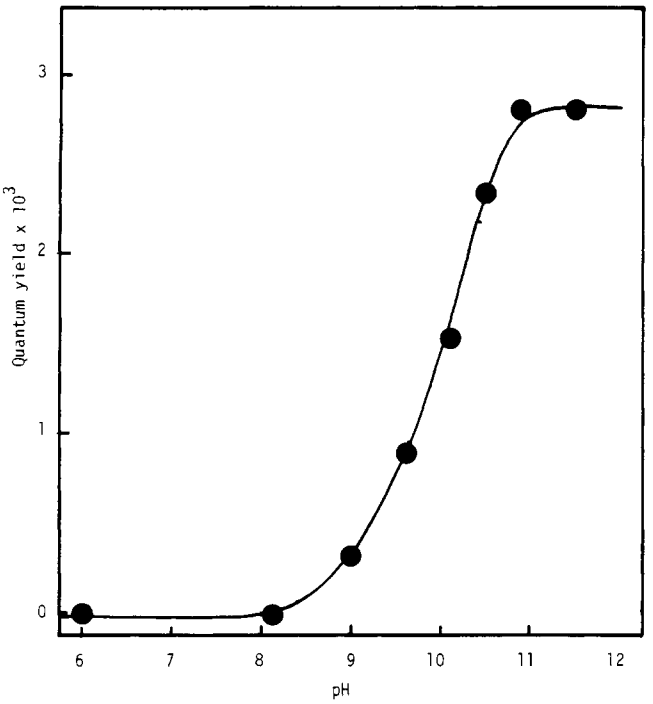
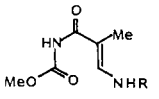


Figure 1. pH dependence of the quantum yield of the ring-opened adduct **15** in the photoreaction of Thd and methylamine at 0 °C.

by the fact that irradiation of methanolic solution of **1** in the presence of excess sodium methoxide gave a similar type of ring-opened product **18**. Irradiation of **1** and *n*-butylamine in methanol or acetonitrile never produced **4**, and **1** was recovered unchanged.

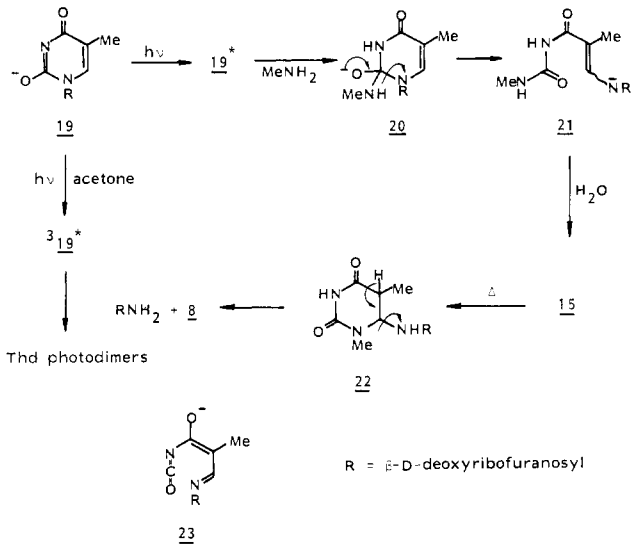


18, R = β-D-deoxyribofuranosyl

Quantum yield for the formation of the ring-opened adducts depends upon pH and amine concentrations. For example, photoreaction of **1** (0.2 mM) and methylamine (10 mM) in aqueous $NaHCO_3$–Na_2CO_3 solution at pH 10.5 gives **15** with a quantum yield of 2.4×10^{-3} (Figure 1). Thus the photoreaction with methylamine in alkaline pH region (pH 9–11) is an efficient process compared to the other photochemical processes reported for **1** and **16**, e.g., photodimerization of **1**[15] ($\phi = 5.6 \times 10^{-4}$) or **16**[15] ($\phi = 4.7 \times 10^{-4}$) and photohydration of **16**[16] ($\phi = 3 \times 10^{-6}$) in direct irradiation in neutral aqueous solutions. In fact, there was no indication for the formation of photohydrate or photodimers of **1** in our experimental conditions.

It has been known that acetone-sensitized irradiation of thymine in aqueous solution gives a mixture of thymine photodimers via the triplet state.[17] In contrast to direct irradiation leading to ring-opening reaction, acetone-sensitized irradiation of **1** in the presence of *n*-butylamine in aqueous solution never produced **4** but gave an inseparable mixture of Thd photodimers.[17b] The result indicates that the lowest triplet state (π,π^*) of Thd anion **19** is not responsible for the photoaddition. Therefore, the most likely candidate for the excited species responsible for the formation of **4** would be either the lowest excited singlet state or a vibrationally excited ground state of Thd anion. In view of the extremely short lifetime (a few picoseconds[18]) of the singlet state of **1** at room

Scheme I



temperature together with the bimolecular nature of the photoreaction, a vibrationally excited ground state of Thd anion rather than the singlet state is presumed to be involved in the photoreaction, although the excited species of **1** in polar protic solvents are more complicated because of the presence of tautomers[19] and should await further mechanistic study.

The formation of the ring-opened adducts **14** and **15** at low-temperature irradiation clearly indicates a nucleophilic attack of alkylamines on the C_2 position of **1**. We therefore propose a logical mechanism for the formation of **15** (Scheme I) by using an example of the reaction of **1** with methylamine. The first step is presumed to involve addition of methylamine to the carbon–nitrogen double bond of photoexcited anionic species **19** to give **20**. Subsequent ring-opening of **20** may yield anion **21** of greatest stabilization of negative charge by conjugation. Protonation of the anion **21** would give a *E*–*Z* mixture of the ring-opened adduct **17** as was the case of **16**. In the case of **1**, only the more stable *E* isomer **15** is isolable. Upon being heated in alkaline aqueous solution, **15** would undergo intramolecular cyclization and subsequent base-catalyzed β elimination to give **8** and **10** via **22**.[20]

Nucleophilic attack on the C_2 positions of pyrimidine bases has only few precedents.[21] For example, Komura et al.[21b] have recently suggested the attack of ribose 5-phosphate anion on C_2 carbonyl of the monoanion of barbituric acid for unusual spontaneous reaction between barbituric acid and D-ribose 5-phosphate in aqueous solution. However, we are unaware of any previous example of C_2 attack of nucleophiles in the photochemistry of pyrimidine bases.[7] An alternative mechanism involving isocyanate **23** formed by photochemical ring-opening[22] of **1** appears to be highly unlikely since aqueous solvent would intercept **23**: this was not the case. Furthermore, irradiation of **1** and methylamine in methanol did not give an appreciable amount of solvent-incorporated products such as **18**.

Selectivity of the Photoreaction. The conversion of **1** to **15** proceeds in high yield on irradiation with methylamine at low temperature and represents a key part of our method for the thymine-selective modification of DNA, while the isolated yield

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(18) (a) Daniels, M. Reference 6, Vol. I, p 23. (b) Hauswirth, W.; Daniels, M. *Chem. Phys. Lett.* **1971**, *10*, 140.
(19) Becker, R. S.; Kogan, G. *Photochem. Photobiol.* **1980**, *31*, 5 and references therein.
(20) For analogous reactions, see ref 12 and: (a) Shealy, Y. F.; O'Dell, C. A. *J. Heterocycl. Chem.* **1976**, *13*, 1041. (b) Anderson, G. L.; Broom, A. D. *J. Org. Chem.* **1977**, *42*, 4159.
(21) (a) Doerr, I. L.; Fox, J. J. *J. Am. Chem. Soc.* **1976**, *89*, 1760. (b) Komura, H.; Nakanishi, K.; Potvin, B. W.; Stern, H. J.; Krooth, R. S. *Ibid.* **1980**, *102*, 1208.
(22) For an analogous photoreaction leading to isocyanate, see: Swanson, B. J.; Crockett, G. C.; Koch, T. H. *J. Org. Chem.* **1981**, *46*, 1082. The formation of a Dewar pyrimidinone intermediate cannot explain the experimental observations; see: Hirokami, S.; Takahashi, T.; Nagata, M.; Hirai, Y.; Yamazaki, T. *Ibid.* **1981**, *46*, 1769.

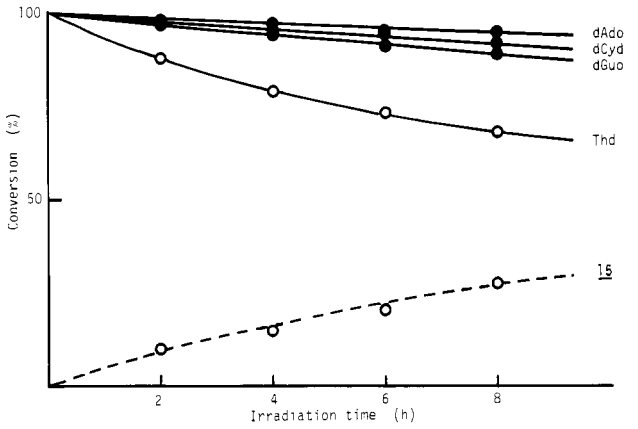

Figure 2. Photoreaction of a mixture of nucleosides (dAdo, dGuo, dCyd, and Thd) in the presence of methylamine at pH 10.5. After irradiation at 0 °C, the solution was kept at 20 °C for 20 min, and the disappearance of nucleosides (—) and the formation of **15** (---) were determined by HPLC and UV spectroscopy, respectively.

of **15** is not so high because of its thermal instability. When **1** and methylamine were irradiated at 0 °C in 0.1 M $NaHCO_3$ aqueous solution, a new absorption band of **15** appeared around 300 nm quantitatively. Under the same conditions, irradiation of dAdo and methylamine did not give any detectable product, and dAdo was recovered quantitatively. Irradiation of dCyd and methylamine in $NaHCO_3$ aqueous solution at 0 °C resulted in the formation of unstable dCyd photohydrate as reported previously.[23,24] However, on warming the photolysate to 20 °C, the photohydrate of dCyd reverted rapidly to dCyd in more than 95% yield as determined by HPLC.[23] Under the irradiation conditions, deamination of dCyd to dUrd has not been observed. Photoreaction between dGuo and methylamine under nitrogen atmosphere resulted in a considerable decrease of dGuo. Since dGuo is known to be susceptible to photooxidation,[25] disappearance of dGuo is probably due to oxidation with the trace of oxygen present in the reaction system. In fact, addition of diazabicyclo[2.2.0]-octane (Dabco), a singlet-oxygen quencher, to the reaction system considerably inhibited the disappearance of dGuo under these conditions. While the addition of Dabco cannot completely suppress the consumption of dGuo, the relative disappearance rate for Thd vs. dGuo is ca. 5:1 under the irradiation conditions (Figure 2). Thus, relatively high selectivity toward Thd (**1**) has been observed when photoreaction of the DNA components with methylamine was conducted at 0 °C in aqueous $NaHCO_3$ solution containing 0.2 mM of Dabco under nitrogen atmosphere, and the photolysate was then allowed to warm to 20 °C. Under the regulated conditions, irradiation of a mixture of four DNA components (dAdo, dCyd, dGuo, and Thd) in the presence of methylamine resulted in a preferential reaction with Thd as illustrated in Figure 2. It seems probable that much higher selectivity toward thymine would be expected in the photoreaction of polynucleotides such as DNA in the presence of methylamine as in the case of many other chemical modifications of polynucleotides.

Photoreaction of DNA with Methylamine. We next examined the photoreaction of DNA with methylamine. When heat-denatured calf thymus DNA in 0.1 M $NaHCO_3$ aqueous solution (pH 10.5) containing methylamine and Dabco was irradiated at 0 °C under the specified conditions and the progress of the reaction was monitored with UV spectroscopy at room temperature, a new absorption band around 300 nm was increased with increasing irradiation time with isosbestic points at 245 and 289 nm, in exactly the same manner as in the case of photoreaction between four DNA components and methylamine (Figure 3). The photolysate was then heated to 70 °C for 2 h. Upon this treatment, the absorption at 300 nm mainly due to the ring-opened product of thymine (**27**) was decreased with concomitant formation of 1-methylthymine (**8**), as evidenced by HPLC analysis. The amount of reacted thymine in DNA is readily calculated from the absorbance change at 300 nm (ΔAbs_{300}) before and after heating the photolysate at 70 °C by assuming that the ring-opened adduct of thymine in DNA (**27**) has the same molar extinction coefficient as that of **15** (ϵ 14 800 at 300 nm). 1-Methylthymine (**8**) thus released from DNA was determined directly by HPLC without any systematic degradations of DNA. As shown in Table II, external irradiation with a 10-W low-pressure mercury lamp through a Vycor filter for 6 h resulted in the release of **8** in 10% yield on the basis of thymine contained in DNA.[26]

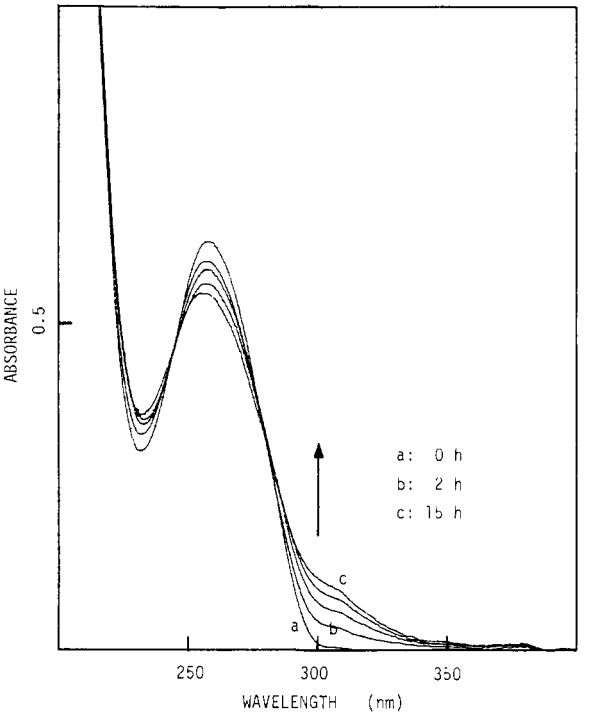

Figure 3. Progressive UV spectral change induced by irradiation of heat-denatured calf thymus DNA in 0.1 M aqueous $NaHCO_3$ at pH 10.5 in the presence of methylamine at 0 °C. In each run, UV absorption spectrum was measured after photolysate was kept at 20 °C for 20 min.

Table II. Variation of Product Yield with Irradiation Time in the Photoreaction of DNA in the Presence of Methylamine[a]

irradiation time, h	ΔAbs_{300} [b]	calculated yield of **27** in DNA,[c] %	yield of released **8**,[d] %
2	0.025	7.5	3.9
4	0.045	13.5	7.4
6	0.057	17.1	10.1
8	0.068	20.4	13.3
15	0.072	21.6	15.3

[a] Reaction conditions are the same as indicated in Figure 3. Yields are based on thymine contained in DNA (27.6%, 8.34×10^{-5} M). [b] Absorbance change at 300 nm before and after heating the photolysate at 70 °C. [c] The yield was calculated from the absorbance change (ΔAbs_{300}). [d] Yield determined by HPLC.

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(24) A similar result has been obtained in the irradiation of uridine in aqueous methylamine.[23e] No ring-opened adduct of type **15** was obtained with uridine.[8]

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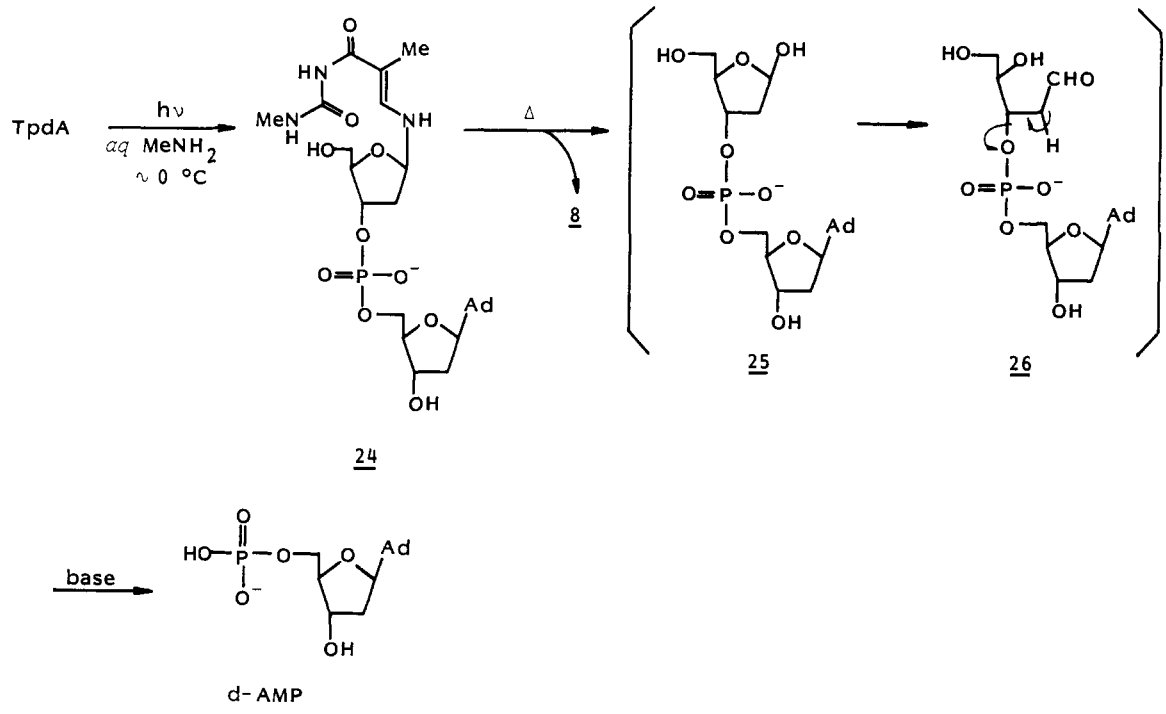
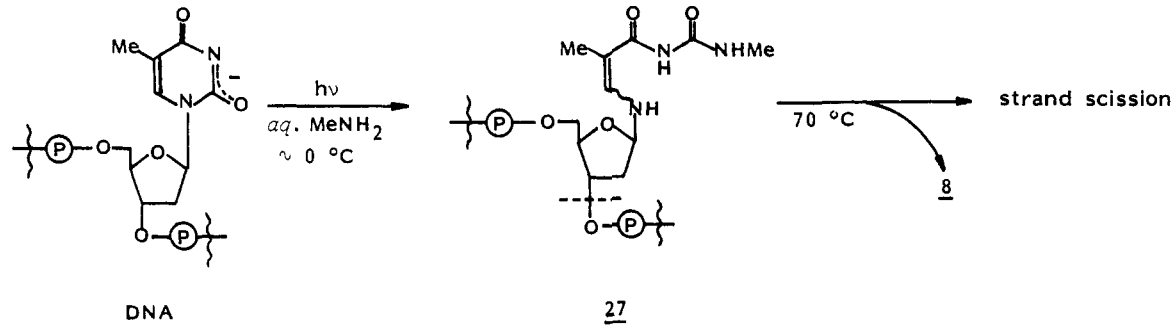
(26) Irradiation of native DNA with methylamine under the conditions gave a similar result but with a slightly decreased rate.

Scheme II



Scheme III



In order to ascertain whether the phosphodiester linkage of the DNA chain is cleaved in this photoreaction, we have carried out the photoreaction of a dinucleotide as a simple model. When thymidylyl(3′-5′)-2′-deoxyadenosine (TpdA) and methylamine were irradiated for 15 min at 0 °C, a new UV absorption due to the ring-opened adduct **24** appeared at ca. 300 nm in a quantitative yield, as estimated by the absorbance change (ΔAbs_{300}). After being heated at 70 °C for 2 h, the photolysate was analyzed by HPLC to reveal the presence of 8% of **8**, 12% of 2′-deoxyadenosine 5′-monophosphate (d-AMP), and 88% of unreacted TpdA. Irradiation for 45 min and successive heating produced **8**, d-AMP, and TpdA in 15, 24, and 68% yields, respectively (Table III). These results clearly indicate that the 3′-5′ phosphodiester linkage of TpdA is efficiently cleaved, giving rise to **8** and d-AMP by irradiation and subsequent heating in alkaline aqueous solution. The formation of **8** and d-AMP may be reasonably explained by the mechanism shown in Scheme II. The ring-opened adduct **24** formed by the reaction with methylamine would undergo intramolecular cyclization and β elimination to give **8** and **25** as mentioned earlier. The cleavage of the phosphodiester linkage would proceed via unmasked sugar aldehyde **26** by a well-known base-catalyzed β-elimination mechanism.[27] Thus, the present experiment suggests that irradiation of DNA with methylamine and subsequent heating of the photolysate may induce strand scission at the reacting thymine.

(27) Brown, D. M. "Basic Principles in Nucleic Acid Chemistry"; Ts'o, P. O. P., Ed.; Academic Press: New Work, 1974; Vol. II, Chapter 1.

Table III. Product Distribution in the Photoreaction of TpdA in the Presence of Methylamine[a]

irradiation time, min	TpdA,[b] %	product,[b] %	
		8	d-AMP
15	88	8	12
30	78	13	18
45	68	15	24

[a] Reaction conditions are indicated in Experimental Section. [b] Yield determined by HPLC.

Chemical reactions resulting in cleavage of certain base sequences of polynucleotides are of particular importance in investigating the biological function of nucleic acids and the mechanism of action of mutagens, carcinogens,[28] and anticancer agents.[29] For example, the anticancer agent neocarzinostatin has been demonstrated to induce DNA strand breaks with liberation of thymine from DNA.[29b,30] In the present work, we have demonstrated that irradiation of DNA with methylamine followed by heating at 70 °C results in an exceedingly facile removal of thymine from DNA. Model experiments using dinucleotide TpdA

(28) Searle, C. E. *ACS Monogr.* **1978**, *No. 174.*

(29) For recent examples, see: (a) Takeshita, M.; Grollman, A. P.; Ohtsubo, E.; Ohtsubo, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5983. (b) Hatayama, T.; Goldberg, I. H.; Takashita, M.; Grollman, A. P. *Ibid.* **1978**, *75*, 3603.

(30) Kappen, L. S.; Goldberg, I. S.; Liesch, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 744.

suggest that the photoreaction of DNA with methylamine induces strand scission at the reacting thymine as illustrated in Scheme III. Observed high selectivity toward Thd in the photoreaction of DNA components strongly suggests that thymine is preferentially attacked by methylamine in the irradiation of DNA, although the selectivity in the photoreaction of DNA itself is the subject of further investigation. Owing to its simplicity and the easiness of monitoring the reaction, the present photoreaction may provide a convenient and potentially useful method for thymine-selective modification of DNA. We also observed that a similar type of photoreaction can take place between DNA and lysine residues of histones in DNA–histone complexes, which will be the subject of a forthcoming publication.

In summary, we have found a new type of efficient photoreaction between thymidine and alkylamines leading to ring-opened adducts. By utilizing this photochemical reaction as a key process, we have demonstrated a potentially useful method for selective removal of thymine from DNA. The method is unique in that it allows the extentent of DNA modification to be determined simply with UV spectroscopy without tedious degradations of modified DNA.

Experimental Section

Melting points are uncorrected. Elemental analyses were performed at the Analytical Center of Kyoto University. Ultraviolet spectra were recorded with a Shimadzu UV-200 spectrophotometer. ^1H and ^{13}C NMR spectra were measured on Varian HA-100 and FT-80A spectrometers, respectively, with Me_4Si as internal standard. High-performance liquid chromatography (HPLC) was performed on a Waters ALC/GPC 204 model equipped with a 254-nm or a 280-nm fixed-wavelength detector. Reverse-phase μBondapak C_{18} and Nucleosil $7C_{18}$ (1 × 25 cm) columns were used for analytical and preparative HPLC, respectively. TLC was performed on silica gel plates (Merck 60 PF_{254}).

Irradiations were carried out by using a 10-W low-pressure mercury lamp (method A) or a 400-W high-pressure mercury lamp (method B). Vycor filters were used to cut off wavelengths of $\lambda < 240$ nm in all cases. For preparative runs, sample solutions were irradiated internally with a 10-W low-pressure mercury lamp under nitrogen atmosphere at ambient temperature (~35 °C). The sample solutions were prepared by dissolving appropriate substrates in distilled water adjusted to the desired pH by adding alkylamines. For low-temperature irradiation, the reaction vessel was cooled in a refrigerated circulating bath at 0 °C. Yields for photochemical reactions were determined by HPLC analyses and are based on consumed starting materials.

Calf thymus DNA was purchased from P-L Biochemicals and used without purification. dAdo, dGuo, dCyd, Thd, d-AMP, and TpdA were obtained from Sigma. The following 1-alkylthymines were prepared according to the published procedures.[12,31,32] 1-*n*-Butylthymine (**4**): mp 136–138 °C (lit.[33] mp 140 °C); 1-cyclohexylthymine (**5**): mp 198–200 °C (lit.[34] mp 198 °C); 1-(hydroxyethyl)thymine (**6**): mp 179–181 °C (lit.[35] mp 179–181 °C); 1-methylthymine (**8**): mp 277–280 °C (lit.[36] mp 281 °C); 1-*tert*-butylthymine (**7**): mp 258 °C dec, ^1H NMR (CD_3OD) δ 1.59 (s, 9 H), 1.86 (d, 3 H, J = 1 Hz), 7.58 (q, 1 H, J = 1 Hz). Anal. ($C_9H_{13}N_2O_2$) C, H, N.

Irradiation of Thymidine (1) and Alkylamines at Ambient Temperature. A. With *n*-Butylamine. A solution of **1** (242 mg, 1 mmol) and *n*-butylamine (219 mg, 3 mmol) in distilled water (200 mL) at pH 9.5 was irradiated at ambient temperature (~35 °C) under the standard conditions (method A) for 36 h. Progress of reaction was monitored by HPLC and TLC. HPLC analysis as monitored at 254 nm revealed the presence of a single product except for the starting material **1**. After removal of the solvent, the residue was subjected to preparative HPLC (1 × 25 cm Nucleosil $7C_{18}$ column, methanol–water (6:4)). Collection of the fraction with retention time of 5 min gave 1-*n*-butylthymine (**4**) as colorless crystals (25 mg). ^1H NMR and chromatographic behaviors (TLC, HPLC) of the product **4** were identical with those of the authentic sample prepared independently. The yield of **4** determined by HPLC of the reaction mixture was 56% on the basis of consumed **1**.

The existence of 2-deoxy-D-ribose (**9**) in the reaction mixture was confirmed by TLC analysis (silica gel, 2-methyl-2-propanol–AcOH–water (4:1:1)). The spot at R_f 0.53 was detected by spraying a solution of diphenylamine–aniline–80% phosphoric acid in aqueous acetone.[37]

B. With *tert*-Butylamine. A solution of **1** (242 mg, 1 mmol) and *tert*-butylamine (730 mg, 10 mmol) in distilled water (100 mL) at pH 11.8 was irradiated under the standard conditions for 30 h. Removal of the solvent followed by preparative HPLC gave 1-*tert*-butylthymine (**7**) (20 mg). HPLC analysis of the mixture indicated the formation of **7** in 43% yield on the basis of consumed **1**.

C. With 2-Aminoethanol. A solution of **1** (242 mg, 1 mmol) and 2-aminoethanol (366 mg, 6 mmol) in distilled water (200 mL) at pH 10.8 was irradiated for 34 h as described above. After removal of the solvent, the residue was purified by preparative HPLC (methanol–water (1:9)) to give 1-(hydroxyethyl)thymine (**6**) (32 mg). The yield of **6** was 45% on the basis of consumed **1** as determined by HPLC.

D. With Cyclohexylamine. A solution of **1** (242 mg, 1 mmol) and cyclohexylamine (297 mg, 3 mmol) was irradiated under the standard conditions for 34 h. A similar workup gave 1-cyclohexylthymine (**5**) as colorless crystals (41 mg). The yield of **5** was 50% as determined by HPLC.

Irradiation of 1-*n*-Heptylthymine (2) and *n*-Butylamine. A solution of **2** (86 mg, 0.4 mmol) and *n*-butylamine (219 mg, 3 mmol) in distilled water (200 mL) was irradiated at ambient temperature under the standard conditions for 60 h. HPLC analysis of the reaction mixture as monitored at 254 nm revealed the formation of **4** in 17% yield. The existence of *n*-heptylamine in the mixture was confirmed by HPLC analysis after dansylation. To the reaction mixture (0.5 ml) were added successively Na_2CO_3 (10 mg) and 10% acetone solution of dansyl chloride (0.1 mL) and acetonitrile (1 mL), and the mixture was heated at 60 °C for 1 h. The yield of *n*-heptylamine as determined by HPLC was 16%.

Irradiation of 1-Ethylthymine (3) and *n*-Butylamine. A solution of **3** (154 mg, 1 mmol) and *n*-butylamine (219 mg, 3 mmol) in distilled water (200 mL) was irradiated under the standard conditions for 12 h. HPLC analysis of the mixture revealed the presence of **4** (90% based on consumed **1**). A similar treatment with dansyl chloride followed by HPLC analysis showed the presence of *N*-ethylamine in 88% yield on the basis of consumed **1**.

Isolation of Ring-Opened Adducts at Low-Temperature Irradiation. A. Irradiation of 1 and *tert*-Butylamine. A solution of **1** (242 mg, 1 mmol) and *tert*-butylamine (730 mg, 10 mmol) in distilled water (100 mL) at pH 11.6 was irradiated at 0 °C by Method A for 24 h. The resulting solution was neutralized to pH 7 with 1 N HCl and lyophilized to dryness. The residue was subjected to preparative HPLC (methanol–water (1:1), 2 mL/min). During elution, the solvent was cooled in a ice–water bath. Two major fractions with retention times of 1.5 and 5 min were collected. The former fraction contained unreacted **1** (130 mg). The combined latter fractions were lyophilized to dryness to give **14** as hygroscopic syrup (100 mg, 70% based on consumed **1**). Attempts to obtain an analytically pure sample by recrystallization were unsuccessful because of its thermal instability. Purity of **14** was more than 95% as estimated by HPLC analysis.

***N*-[β-((2-Deoxy-D-ribofuranosyl)amino)-α-methylacryloyl]-*N'*-*tert*-butylurea (14)**: UV (H_2O) 291 nm (log ε 4.38); ^1H NMR ($CDCl_3$) δ 1.37 (s, 9 H), 1.68 (s, 3 H), 2.02 (m, 2 H), 3.40–4.40 (m, 6 H), 4.70 (m, 1 H, anomeric H), 6.04 (m, 1 H, NH), 7.35 (br d, 1 H), 7.94 (br s, 1 H), 8.88 (br s, 1 H).

A solution of **14** (2 mg) in aqueous $NaHCO_3$ (10 mL, pH 10.5) was heated at 70 °C for 2 h. HPLC analysis of the solution revealed complete conversion of **14** to **7** (more than 98%). The existence of **9** in the solution was confirmed by TLC analysis as described before.

B. Irradiation of Thymine (16) and *tert*-Butylamine. A solution of **16** (126 mg, 1 mmol) and *tert*-butylamine (730 mg, 10 mmol) in distilled water (100 mL) at pH 11.6 was irradiated at 0 °C as described above for 24 h. The resulting solution was neutralized to pH 7.0 with 1 N HCl and lyophilized to dryness. The residue was subjected to preparative HPLC (methanol–water (1:9), 2 mL/min). Three major fractions with retention times of 1.6, 3, and 4.7 min were collected. The first fraction was unreacted **1** (60 mg). The second fraction was lyophilized to dryness to give (*E*)-**17** as syrup (80 mg, 40% based on consumed **1**).

***N*-[(*E*)-β-Amino-α-methylacryloyl]-*N'*-*tert*-butylurea ((*E*)-17).** UV (H_2O) 293 nm (log ε 4.23); ^1H NMR ($CDCl_3$) δ 1.38 (s, 9 H), 1.70 (s, 3 H), 4.30 (br d, 2 H, J = 10 Hz, NH_2), 7.48 (t, 1 H, J = 10 Hz), 7.88 (br s, 1 H, NH), 8.87 (br s, 1 H, NH). Anal. ($C_9H_{17}N_3O_2$) C, H, N.

The third fraction on a similar workup gave unstable (*Z*)-**17** (16 mg, 10% based on consumed **1**).

***N*-[(*Z*)-β-Amino-α-methylacryloyl]-*N'*-*tert*-butylurea ((*Z*)-17).** UV (H_2O) 301 nm (log ε 4.15); ^1H NMR ($CDCl_3$) δ 1.38 (s, 9 H), 1.75 (s, 3 H), 6.22 (br d, 2 H, J = 10 Hz, NH_2), 6.70 (t, 1 H, J = 10 Hz), 7.00

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(br s, 1 H, NH), 8.75 (br s, 1 H, NH).

A solution of (*Z*)-**17** (0.1 mg) in aqueous $NaHCO_3$ (pH 11.4, 10 mL) was kept at room temperature overnight. HPLC analysis indicated complete conversion of (*Z*)-**17** to (*E*)-**17**. Heating of the solution of (*E*)-**17** (0.1 mg) in aqueous $NaHCO_3$ solution (pH 11.0, 10 mL) at 70 °C for 2 h gave **7** in 90% yield as determined by HPLC.

C. Irradiation of 1 and Methylamine. A solution of **1** (242 mg, 1 mmol) and 40% methylamine (2 mL, 20 mmol) in distilled water (200 mL) at pH 12.0 was irradiated at 0 °C for 7 h as described above. After removal of methylamine in vacuum, the resulting solution was neutralized and then lyophilized to dryness. Preparative HPLC (acetonitrile–water (15:85), 2 mL/min) of the residue at 0 °C yielded **15** (80 mg, 65% based on consumed **1**). Attempts to obtain an analytically pure sample were unsuccessful because of its thermal instability.

***N*-[(*E*)-β-((2-Deoxy-D-ribofuranosyl)amino)-α-methylacryloyl]-*N'*-methylurea ((*E*)-15)**: UV (H_2O) 289 nm (log ε 4.26); ^1H NMR ($C-D_3OD$) δ 1.72 (s, 3 H), 1.94 (m, 2 H), 2.82 (s, 3 H), 3.40–4.40 (m, 4 H), 4.60 (m, 1 H, anomeric H), 7.43 (s, 1 H).

A solution of **15** (0.1 mg) in aqueous $NaHCO_3$ (pH 10.5, 10 mL) was heated at 70 °C for 2 h. HPLC analysis revealed the formation of **8** in 74% yield.

Acetone-Sensitized Irradiation of 1 and *n*-Butylamine. A solution of **1** (48 mg, 0.2 mmol) and *n*-butylamine (44 mg, 0.6 mmol) in acetone–water (1:3, 40 mL) was irradiated under nitrogen atmosphere with a 100-W high-pressure mercury lamp through a Pyres filter at room temperature. Under this condition, acetone absorbs more than 90% of the incident light in the 313-nm region. After irradiation for 10 h, the solvent was removed, and the residue was analyzed by HPLC and ^1H NMR. The HPLC analysis indicated complete disappearance of **1** but no formation of **4**. ^1H NMR of the residue showed methyl signals at δ 1.2–1.4, which are ascribable to those of isomeric Thd photodimers.[15] Further attempts to separate Thd photodimers have not been made.

Irradiation of 1,3-Dimethylthymine and *n*-Butylamine. A solution of 1,3-dimethylthymine (150 mg, 1 mmol) and *n*-butylamine (439 mg, 6 mmol) in distilled water (200 mL) at pH 11.1 was irradiated by method A at ambient temperature for 48 h. HPLC analysis (methanol–water (1:1)) of the reaction mixture showed that it contains only the starting material.

Irradiation of 1 in the Presence of Sodium Methoxide in Methanol. A solution of **1** (242 mg, 1 mmol) and sodium methoxide (1.01 g, 5 mmol) in methanol (50 mL) was irradiated at 0 °C by method A for 24 h. After addition of 0.6 N HCl (20 mL), the resulting solution was evaporated to 2 mL. Preparative HPLC (methanol–water (4:6)) gave **18** (53 mg) as syrup. The yield of **18** as determined by analytical HPLC was 30% on the basis of consumed **1**.

Methyl [β-((2-deoxy-D-ribofuranocyl)amino)-α-methylacryloyl]carbamate (18): UV (H_2O) 290 nm (log ε 4.10); ^1H NMR ($CDCl_3$) δ 1.69 (s, 3 H), 2.00 (m, 2 H), 3.20–4.20 (m, 6 H), 3.67 (s, 3 H), 4.50–4.90 (m, 2 H), 5.84 (dd, 1 H, *J* = 12, 10 Hz, NH), 7.37 (d, 1 H, *J* = 12 Hz). Anal. ($C_{11}H_{18}N_2O_6$) C, H, N.

Quantum-Yield Measurements at Different pH. Quantum-yield measurements were carried out at 0 °C in a merry-go-round apparatus by using 5-bromouracil actinometry ($\phi = 1.8 \times 10^{-3}$).[38] Irradiation was made with a 400-W high-pressure mercury lamp through a Vycol filter (cutoff < 240 nm). Solutions of **1** (0.2 mM) and methylamine (10 mM) in 40 mM $NaHCO_3$–Na_2CO_3 buffer (each 10 mL) were adjusted to the pH values indicated in Figure 1 in quartz tubes. For pH 6.0, the solution was acidified with 1 N HCl. The sample solutions and the actinometer solution (2×10^{-4} M) were purged with nitrogen. The irradiation was stopped at less than 15% conversion of **1**. The formation of **15** was assayed by UV spectroscopy monitored at 300 nm. The results are shown in Figure 1.

Selectivity of the Photoreaction. A solution of dAdo (0.2 mM), 40% methylamine (1 mL, 10 mM), and Dabco (0.2 mM) in 50 mM aqueous $NaHCO_3$ at pH 10.5 was irradiated at 0 °C by method A for 100 min. HPLC analysis (acetonitrile–water–acetic acid (40:60:1)) of the mixture at room temperature indicated no loss of dAdo.

A solution of dCyd (0.2 mM) and methylamine (20 mM) in 50 mM $NaHCO_3$ was irradiated at 0 °C under the same conditions for 30 min. Progress of reaction was monitored by UV spectroscopy and HPLC. The UV spectrum of the photolysate immediately after irradiation showed the disappearance of dCyd absorption (270 nm). HPLC analysis indicated the presence of a new peak ascribable to dCyd photohydrate[23] with retention time of 6 min in addition to unreacted dCyd. When the photolysate was kept at 20 °C for 20 min, the peak of the photohydrate completely disappeared with the enhancement of the dCyd peak. The reversibility is more than 95% as determined by HPLC.

When a solution of dGuo (0.2 mM) and methylamine (20 mM) was irradiated at 0 °C under nitrogen atmosphere for 30 min, 10% of the dGuo was consumed, as determined by HPLC. However, the same irradiation in the presence of Dabco (0.2 mM) resulted in 96% recovery of dGuo.

A solution containing dAdo (0.70 mM), dGuo (0.58 mM), dCyd (0.54 mM), and Thd (0.69 mM) was irradiated at 0 °C by method A in the presence of methylamine (0.1 M) and Dabco (0.2 mM) in 0.1 M aqueous $NaHCO_3$ at pH 10.5 under nitrogen atmosphere. After irradiation, the solution was kept at 20 °C for 20 min, and the disappearance of nucleosides and the formation of **15** were determined by HPLC (acetonitrile–water–acetic acid (40:60:1)) and UV spectroscopy, respectively. The results are shown in Figure 2.

Irradiation of Calf Thymus DNA in the Presence of Methylamine. A solution of heat-denatured calf thymus DNA (50 mg), 40% methylamine (1 mL, 10 mmol), and Dabco (2.2 mg, 0.02 mmol) in 0.1 M aqueous $NaHCO_3$ (100 mL) at pH 10.5 was irradiated externally with a 10-W low-pressure mercury lamp through a Vycol filter at 0 °C under nitrogen atmosphere. Equivalent volumes (5 mL) of the photolysate were withdrawn periodically, and UV absorption spectra were measured after the solution was kept at 20 °C for 20 min. The solutions were then heated at 70 °C for 2 h, and their UV spectra were recorded again. Absorbance changes at 300 nm (ΔAbs_{300}) before and after heating at 70 °C were calculated in each case. After being passed through a membrane filter (Millipore FH, 0.5 μm) to remove DNA, the sample solutions were subjected to HPLC analyses (methanol–water (1:9), 2 mL/min). The amounts of **8** with retention time of 5 min were determined by using a Waters Data Module 730. Identification of **8** was made by comparison of chromatographic behaviors (TLC, HPLC) and UV spectrum with those of authentic sample after collection of the HPLC peak. The results are shown in Figure 3 and Table II.

Irradiation of Thymidylyl(3'–5')-2'-deoxyadenosine (TpdA) and Methylamine. A solution of TpdA (0.353 mg, 59 μmol) and 40% methylamine (10 μL) in distilled water (10 mL) at pH 11.8 was irradiated internally by method B at 0 °C. Progress of reaction was monitored by the appearance of 300-nm absorption. After irradiation, aliquots of the photolysate were withdrawn and heated at 70 °C for 2 h. The solutions were analyzed by HPLC under the following conditions: d-AMP (retention time 3 min; μBondapak C_{18} column; methanol–0.01 M KH_2PO_4 (1:9), 2 mL/min); 1-methylthymine (**8**) (retention time 5 min; μBondapak C_{18} column; methanol–water (1:9), 2 mL/min). The results are shown in Table III.

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Registry No. 1, 50-89-5; **2**, 76849-30-4; **3**, 21472-94-6; **4**, 15236-33-6; **5**, 21031-74-3; **6**, 22441-51-6; **7**, 76849-31-5; **8**, 4160-72-9; **9**, 36792-88-8; **14**, 84129-13-5; (*E*)-**15**, 84073-39-2; **16**, 65-71-4; (*Z*)-**17**, 84073-40-5; (*E*)-**17**, 80585-58-6; **18**, 84073-41-6; dAdo, 958-09-8; dGuo, 961-07-9; dCyd, 951-77-9; butylamine, 109-73-9; *tert*-butylamine, 75-64-9; 2-aminoethanol, 141-43-5; cyclohexylamine, 108-91-8; methylamine, 74-89-5; acetone, 67-64-1; 1,3-dimethylthymine, 4401-71-2; sodium methoxide, 124-41-4; TpdA, 19192-40-6; diazabicyclo[2.2.2]octane, 280-57-9.

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