Studies on Synthesis of Antibacterial Agent (NM441). I. Kinetics and Mechanism of the Reaction of 4-(Bromomethyl)-5-methyl-1,3-dioxol-2-one with 1-Substituted Piperazine (NM394)

Hiroshi NISHIDA,* Tatsuya FUJII, Yoshiaki ABIRU, Katsuya YATSUKI, Masashi YAMAMOTO, Naoki SHIMIZU, Kazuo KAKEMI, Miyako MIKAWA, and Masahiro KISE

Research Laboratories, Nippon Shinyaku Company, Ltd., Nishioji Hachijo, Minami-ku, Kyoto 601

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When a tertiary amine (**4**,NM441) is synthesized from 4-bromomethyl-5-methyl-1,3-dioxol-2-one (DMDO-Br) and a secondary amine (**3**,NM394) in *N*,*N*-dimethylformamide (DMF), the quaternary ammonium salt **5**, the ring-opened compound **6**, and the 1,2-adduct **7** are formed as by-products. The tertiary amine **4** is formed by nucleophilic attack of **3** on the α-carbon to the bromine atom of DMDO-Br. The ring-opened compound **6** is formed by nucleophilic attack of **3** on carbonyl carbon of DMDO-Br. The quaternary ammonium salt **5** is formed by the reaction of DMDO-Br with **4** (the Menshutkin reaction). Main pathway for the formation of **7** is the Michael addition of **3** to **6**. Kinetics of the reactions have been studied and the methods to obtain **4** suppressing the formations of **5**, **6**, and **7** have been proposed based on the kinetic results.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (norfloxacin, **1**) has been known as an effective antibacterial agent.[1] It has also been found that lipophilicity of the drug is enhanced and its efficiency in oral absorption is improved when the secondary amine moiety in piperazinyl part of **1** is masked by a (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl (DMDO) group. Thus, 1-ethyl-6-fluoro-1,4-dihydro-7-[4-[(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl]-1-piperazinyl]-4-oxo-3-quinolinecarboxylic acid (**2**) was prepared.[2] 6-Fluoro-1-methyl-7-(1-piperazinyl)-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid (**3**) was also converted into its corresponding DMDO derivative, **4**.[3] The processes are shown in Scheme 1.

Unfortunately, however, the reactions of **1** and **3** with DMDO-Br are associated with certain side reactions and the yields of **2** and **4** are, respectively, unsatisfactory. A drug has to be highly pure. When by-products are formed from the reaction, it becomes difficult to obtain a drug in satisfactory purity. It is also required to elucidate the structures of by-products and mechanisms of their formation to eliminate these impurities before **4** is claimed as a drug.

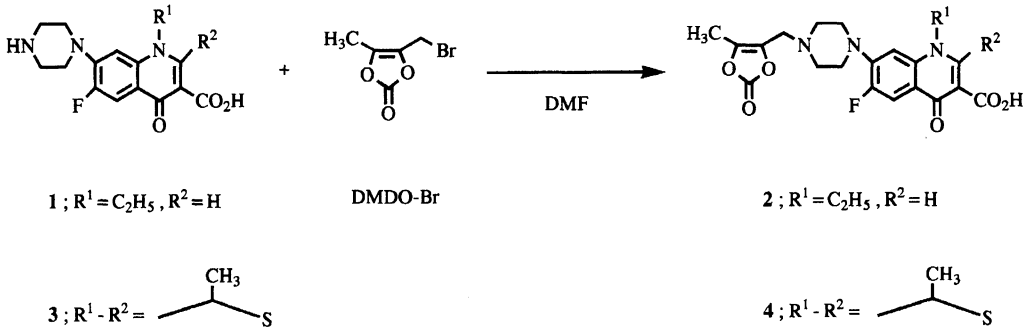
For establishing optimal conditions to obtain **4** in pure state, we studied kinetics of the main reaction as well as those of side reactions. The present report will describe detailed analyses of by-products and kinetics for their formations. The results has been applied to elucidate the optimal conditions for the formation of the major product, **4**, as pure as possible.

Results and Discussion

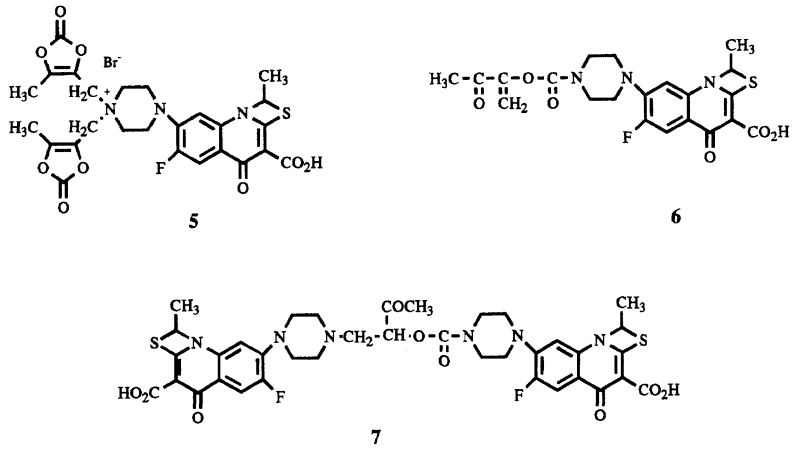
Determination of the Structures of By-Products. Three minor products are formed by the reaction of **3** with DMDO-Br in addition to **4**, the major product. They were isolated by means of preparative HPLC and the structures were identified to be a quaternary ammonium salt, **1**, 1-bis[(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl]-4-(3-carboxy-6-fluoro-1-methyl-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-7-yl)-1-piperazinium bromide (**5**), a ring-opened compound, 6-fluoro-1-methyl-4-oxo-7-[4-[(2-oxo-1-methylenepropyl)-oxycarbonyl]-1-piperazinyl]-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid (**6**), and a 1,2-adduct, 7-[4-[1-[4-(3-carboxy-6-fluoro-1-methyl-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinolin-7-yl)]-1-piperazinylmethyl]-2-oxopropoxycarbonyl]-1-piperazinyl]-6-fluoro-1-methyl-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid (**7**), by comparing their respective IR, ^{1}H NMR, and MS spectra, and HPLC chromatograms with those of independently synthesized authentic samples (Scheme 2). Yields of products from the reaction of **3** with DMDO-Br are listed in Table 1.

Kinetics for the Formation of 4. The reaction of **3** with DMDO-Br was followed by observing the decrease in the concentration of DMDO-Br. Pseudo-first-order rate constants, k_{obsd}, under various concentrations of **3** are listed in Table 2. In Table 2, are also listed second-order rate constants, k_{calcd}, calculated by dividing k_{obsd} by the concentration of **3**. The result clearly indicates that the reaction kinetics is second-order; first-order in each of **3** and DMDO-Br.

The yield of **4**, however, does not exceed 50% when equimolar amounts of **3** and DMDO-Br are subjected to the reaction (Fig. 1). On the other hand, when an appropriate base such as potassium hydrogencarbonate is added to the reaction system or in the presence of excess **3**, the yield of **4** increases up to more than 90%. Thus, stoichiometry of the reaction is unimolecular in DMDO-Br and bimolecular in **3**. The ^{1}H NMR spectrum of the reaction mixture of **3** and DMDO-Br in DMF-d_7 revealed a dioxol methylene signal at δ=4.40 in addition to two dioxol methylene signals (δ=3.48 and 4.79) which arise from **4** and DMDO-Br respectviely. A hydrochloride salt of **4** (**4**·HCl) which is synthesized from **4** and HCl showed a dioxol methylene signal at δ=4.40 in the ^{1}H NMR spectrum. These observations indicate that **4**·H$^+$ is formed when **3** reacts with DMDO-Br.



Scheme 1.



Scheme 2.

Table 1. A Typical Composition of Products[b)]

Molar ratio	Time	$[\mathbf{3}]_0$	Products, yield/%[a)]					
3 : DMDO-Br : $KHCO_3$	h	mol dm^{-3}	**3**	**4**	**5**	**6**	**7**	Others
1.00 : 1.22 : 2.32	2	0.171	2.0	93.6	1.1	2.0	0.5	0.8
1.00 : 1.05 : 2.32	3	0.0171	1.4	95.9	0.6	1.0	0.1	0.8

a) Analyzed on HPLC. Conditions are described in the experimental section. b) Reaction temperature is 30 °C.

Table 2. Pseudo-First-Order Rate Constants, k_{obsd}, and Second-Order Rate Constants, k_{calcd}, for the Reaction of **3** with DMDO-Br

$[\text{DMDO-Br}]\times10^5$	$[\mathbf{3}]\times10^3$	$k_{obsd}\times10^3$	$k_{calcd}\times10$
mol dm^{-3}	mol dm^{-3}	s^{-1}	dm^3 mol^{-1} s^{-1}
6.51	3.07	0.915±0.012	2.98±0.04
6.27	4.24	1.22±0.047	2.88±0.11
6.21	6.85	2.08±0.10	3.04±0.15
5.77	8.81	2.60±0.097	2.95±0.11

It is apparent, therefore, that **4**·H$^+$ is formed at the initial and rate-determining step, which is followed by deprotonation by a base in the second and fast step as depicted in Scheme 3. The equilibrium in the second step might be shifted largely toward the formation of protonated **3** (**3**·H$^+$). It is expected that **3** has larger basicity than **4**.

Rate equations based on the reaction scheme are given by Eqs. 1, 2, 3, 4, and 5. These equations are normalized conditions suggested by Eq. 6.

$$\frac{dz_3}{dm} = -\frac{1}{N}z_3 z_{\text{DMDO-Br}} - z_3 z_{4\cdot H^+} + \frac{1}{K}z_{3\cdot H^+} z_4 \quad (1)$$

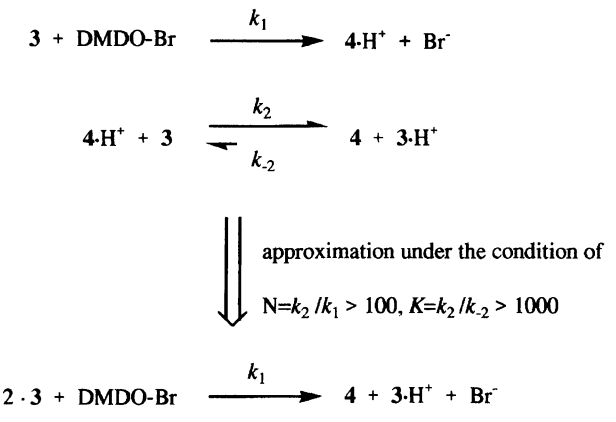
$$\frac{dz_{\text{DMDO-Br}}}{dm} = -\frac{1}{N}z_3 z_{\text{DMDO-Br}} \quad (2)$$

$$\frac{dz_{4\cdot H^+}}{dm} = \frac{1}{N}z_3 z_{\text{DMDO-Br}} - z_3 z_{4\cdot H^+} + \frac{1}{K}z_{3\cdot H^+} z_4 \quad (3)$$

$$\frac{dz_4}{dm} = \frac{dz_{3\cdot H^+}}{dm} = z_3 z_{4\cdot H^+} - \frac{1}{K}z_{3\cdot H^+} z_4 \quad (4)$$

$$z_3 = 1,\ z_{\text{DMDO-Br}} = L, \text{ and}$$
$$z_{4\cdot H^+} = z_4 = z_{3\cdot H^+} = 0 \quad (\text{at } m = 0) \quad (5)$$

$$m = k_2[\mathbf{3}]_0 t,\quad z_i = [i]_0/[\mathbf{3}]_0,\quad N = k_2/k_1,$$
$$K = k_2/k_{-2},\quad L = [\text{DMDO-Br}]_0/[\mathbf{3}]_0 \quad (6)$$



Scheme 3.

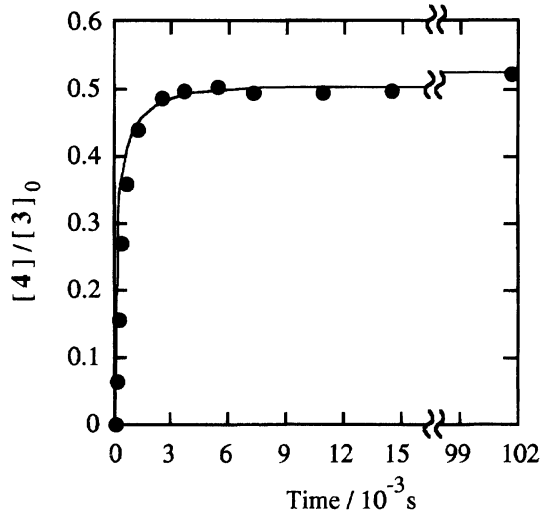


Fig. 1. Time course of the reaction of **3** with DMDO-Br at 304.0 K. $[\mathbf{3}]_0=4.01\times10^{-3}$ mol dm^{-3}, $[\text{DMDO-Br}]_0/[\mathbf{3}]_0=1.5$.

The simultaneous differential equations [Eqs. 1, 2, 3, 4, and 5] were solved at various values of N $(=k_2/k_1)$ and K $(=k_2/k_{-2})$, using a numerical method composed of the Euler routine. Figure 2 demonstrates the simulated result, which indicates that the reaction stops at 50 % conversion when k_1 is less than 1/100 of k_2, and the equilibrium constant K is over 1000. The simulation based on the data shown Fig. 1 predicts the following rate constants; $k_1=0.302\pm0.03$ dm^3 mol^{-1} s^{-1}, $k_2=30.2\pm3.0$ dm^3 mol^{-1} s^{-1}, and $k_{-2}=(3.02\pm0.3)\times10^{-2}$ dm^3 mol^{-1} s^{-1}. The value of k_1 thus elucidated agrees excellently with that experimentally obtained as the second-order rate constant ($k_1^{\text{exp}}=0.287\pm0.01$ dm^3 mol^{-1} s^{-1}) at 304.0 K. Arrhenius plot for the k_1-step affords the activation energy to be 29.1 ± 1.6 kJ mol^{-1}.

Kinetics for the Formation of 5. The Menshutkin reaction of **4** with DMDO-Br affords quaternary ammonium salt **5**. The reaction was followed by observing the decrease in the concentration of **4**. Pseudo-first-order rate constants, k_{obsd}, under various concentrations of DMDO-Br are listed in Table 3. In Table 3, are also listed second-order rate constants, k_{calcd}, calculated by dividing k_{obsd} by the concentration of DMDO-Br. The result clearly indicates that the reaction kinetics is first-order in both **4** and DMDO-Br.

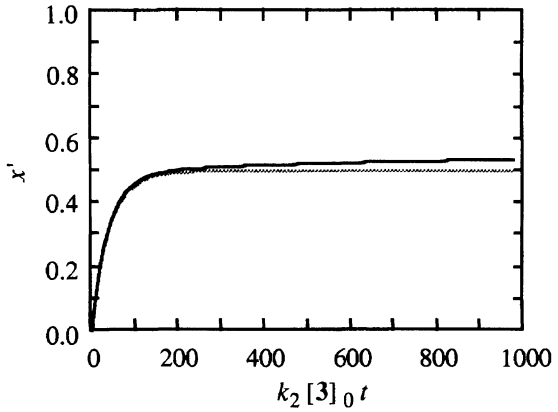


Fig. 2. Dependence of x' on $k_2[\mathbf{3}]_0 t$ calculated from Eqs. 1, 2, 3, 4, and 5 and the second-order rate equation. The solid curve is the calculated line from Eqs. 1, 2, 3, 4, and 5 for $L=1.5$, $K=1000$, and $N=100$. The dotted curve is the calculated line from the second-order rate equation which is obtained from an approximate equation in Scheme 3. $x'=([\mathbf{4}\cdot H^+]+[\mathbf{4}])/[\mathbf{3}]_0$.

The second-order rate constant of the reaction, k_3, has been elucidated to be $(4.35\pm0.20)\times10^{-5}$ dm^3 mol^{-1} s^{-1} at 304.0 K. Thus, it has been elucidated that the rate constant for the formation of **5**, k_3, is about 6600 times as small as that for the formation of **4**, k_1.

Kinetics for the Formation of 6. The secondary amine **3** attacks carbonyl carbon of DMDO-Br to afford the ring-opened compound, **6**. It is expected that the formation of **6** is first-order in **3** and first-order in DMDO-Br as observed for the formation of **4** (vide supra), because these two reactions are different in the reaction center only. In other words, the ratio [**6**]/[**4**] should remain constant throughout the reaction. Surprisingly, as is shown in Fig. 3, however, the ratio is not a constant. Instead, it decreases linearly as the reaction proceeds.

The observation can be explained by assuming that the reaction is second-order in either **3** or DMDO-Br.

Table 3. Pseudo-First-Order Rate Constants, k_{obsd}, and Second-Order Rate Constants, k_{calcd}, for the Reaction of DMDO-Br with **4**

$[\mathbf{4}]\times10^3$ / mol dm^{-3}	$[\text{DMDO-Br}]\times10$ / mol dm^{-3}	$k_{\text{obsd}}\times10^5$ / s^{-1}	$k_{\text{calcd}}\times10^5$ / dm^3 mol^{-1} s^{-1}
9.71	2.88	1.30±0.067	4.51±0.23
9.85	3.74	1.64±0.087	4.39±0.23
9.89	4.78	2.10±0.080	4.40±0.17
10.42	7.09	2.92±0.11	4.11±0.15

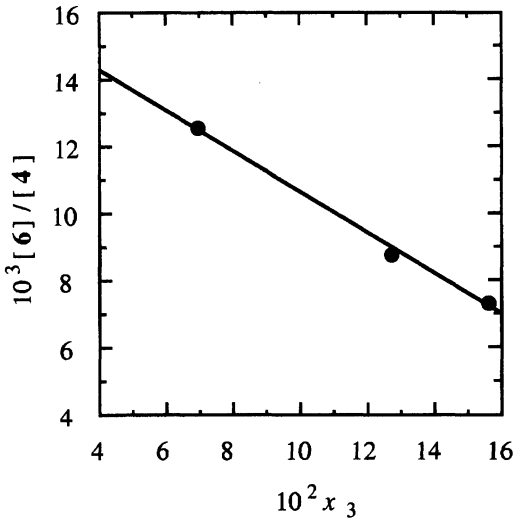


Fig. 3. Plots of [6]/[4] vs. x_3 for the reaction of **3** with DMDO-Br. $[\mathbf{3}]_0=2.49\times10^{-3}$ mol dm^{-3}, $[\text{DMDO-Br}]_0=2.09\times10^{-3}$ mol dm^{-3}. $x_3=([\mathbf{3}]_0-[\mathbf{3}])/[\mathbf{3}]_0$.

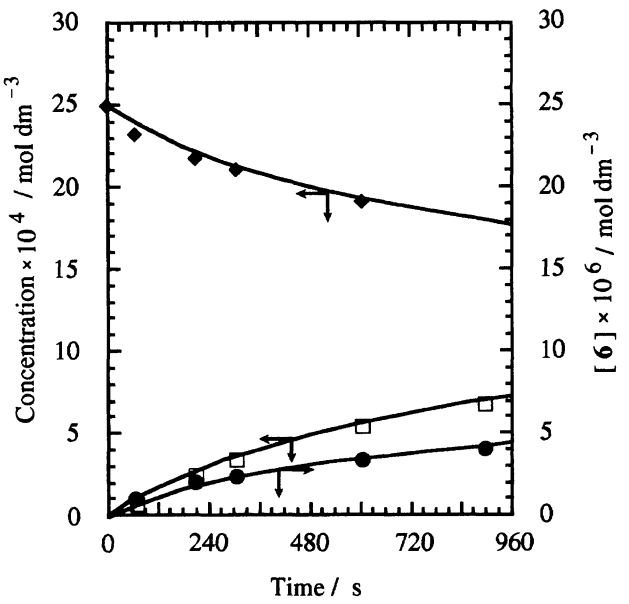


Fig. 4. Calculated and experimental relationship between the concentration and time for the reaction of **3** with DMDO-Br. The solid curves are theoretical lines calculated from Eqs. 7, 8, 9, 10, and 11; the points are experimental. ◆, [3]+[3·H$^+$]; □, [4]; ●, [6].

It has been reported that the kinetics for aminolysis of *p*-nitrophenyl acetate with butylamine is second-order in the amine and first-order in the acetate.[4] Since the present reaction is similar to this reported one, we assumed that the kinetics for the present reaction is second-order in **3**. Then, the differential Eqs. 7, 8, 9, 10, and 11, based on this proposed kinetics, were elucidated and solved using a numerical method composed of the Euler routine (cf. Scheme 4; vide infra).

$$\frac{d[\mathbf{3}]}{dt} = -2k_1[\mathbf{3}][\text{DMDO-Br}] - 2k_4[\mathbf{3}]^2[\text{DMDO-Br}] - k_{5a}[\mathbf{3}]^2[\mathbf{4}] - k_{5b}[\mathbf{3}]^2[\mathbf{6}] \quad (7)$$

$$\frac{d[\text{DMDO-Br}]}{dt} = -k_1[\mathbf{3}][\text{DMDO-Br}] - k_3[\text{DMDO-Br}][\mathbf{4}] - k_4[\mathbf{3}]^2[\text{DMDO-Br}] \quad (8)$$

$$\frac{d[\mathbf{4}]}{dt} = k_1[\mathbf{3}][\text{DMDO-Br}] - k_3[\text{DMDO-Br}][\mathbf{4}] - k_{5a}[\mathbf{3}]^2[\mathbf{4}] \quad (9)$$

$$\frac{d[\mathbf{3}\cdot\mathrm{H}^+]}{dt} = k_1[\mathbf{3}][\text{DMDO-Br}] + k_4[\mathbf{3}]^2[\text{DMDO-Br}] \quad (10)$$

$$\frac{d[\mathbf{6}]}{dt} = k_4[\mathbf{3}]^2[\text{DMDO-Br}] - k_{5b}[\mathbf{3}]^2[\mathbf{6}] \quad (11)$$

Figure 4 shows that experimentally observed concentrations of the components at appropriate time intervals agree excellently with those simulated through the calculation, from which the third-order rate constant, k_4, for the formation of **6** has been calculated to be 0.928 ± 0.047 dm^6 mol^{-2} s^{-1} at 304.0 K supporting that the reaction is indeed second-order in **3** and first-order in DMDO-Br.

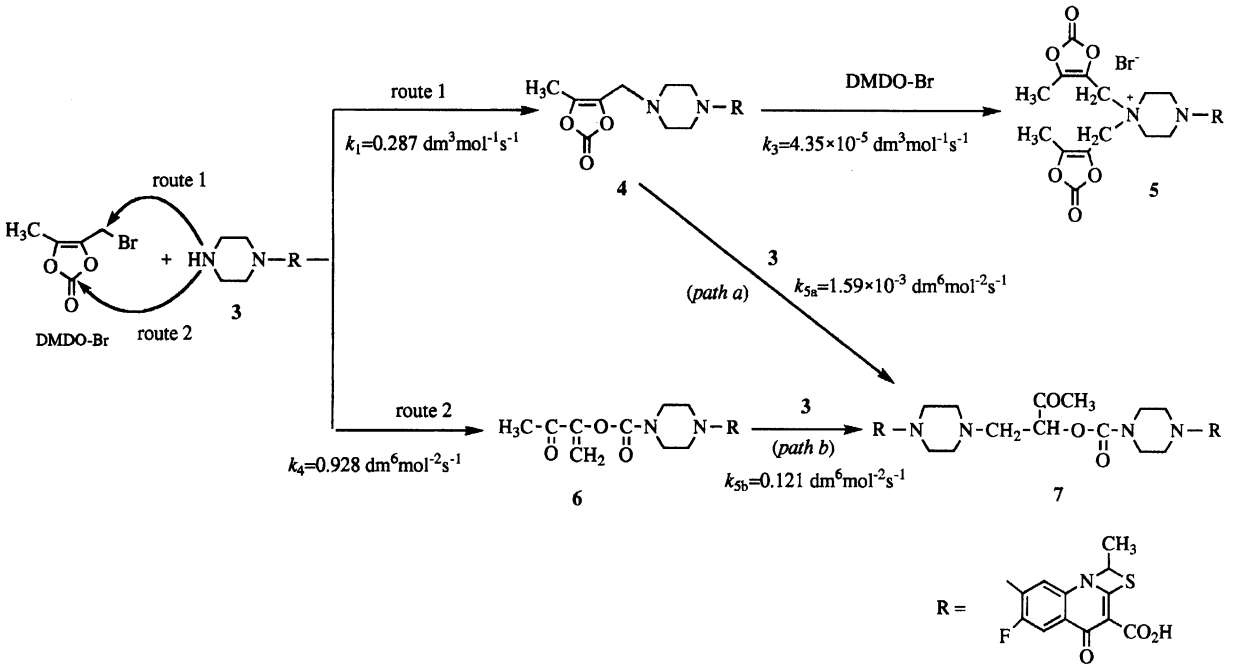
Kinetics for the Formation of 7. There are two possible pathways of the formation of the 1,2-adduct, **7**. That is, nucleophilic attack of **3** on the carbonyl carbon of **4** will result in the formation of **7** (path *a*), and, at the same time, the Michael addition of **3** onto the double bond in **6** will give the same product (path *b*) (cf. Scheme 4; vide infra). The former reaction was followed by observing the decrease in the concentration of **4**. Pseudo-first-order rate constants, k_{obsd}, and third-order rate constants, k_{calcd}, calculated by dividing k_{obsd} by the square of the concentration of **3** are listed in Table 4.

The third-order rate constant for the formation of **7** via path *a*, k_{5a}, has been calculated to be $(1.59\pm0.10)\times10^{-3}$ dm^6 mol^{-2} s^{-1} at 304.0 K ($k_{5a}/k_1=5.54\times10^{-3}$ dm^3 mol^{-1}) based on the reaction scheme of second-order in **3** and first-order in **4**.

Figure 5 demonstrates that the reaction of **3** with equimolar amount of **6** indeed results in the formation of **7** in quantitative yield. Kinetics was followed by observing the decrease in the concentration of **6**. In Table 5 are listed pseudo-first-order rate constants, k_{obsd}, and third-order rate constants, k_{calcd}, calculated by di-

Table 4. Pseudo-First-Order Rate Constants, k_{obsd}, and Third-Order Rate Constants, k_{calcd}, for the Reaction of **3** with **4**.

$[\mathbf{4}]\times10^4$ / mol dm^{-3}	$[\mathbf{3}]\times10^2$ / mol dm^{-3}	$k_{obsd}\times10^7$ / s^{-1}	$k_{calcd}\times10^3$ / dm^6 mol^{-2} s^{-1}
2.00	1.03	1.75±0.11	1.64±0.10
2.00	1.99	6.27±0.33	1.58±0.09
1.99	2.93	13.09±0.85	1.53±0.11



Scheme 4.

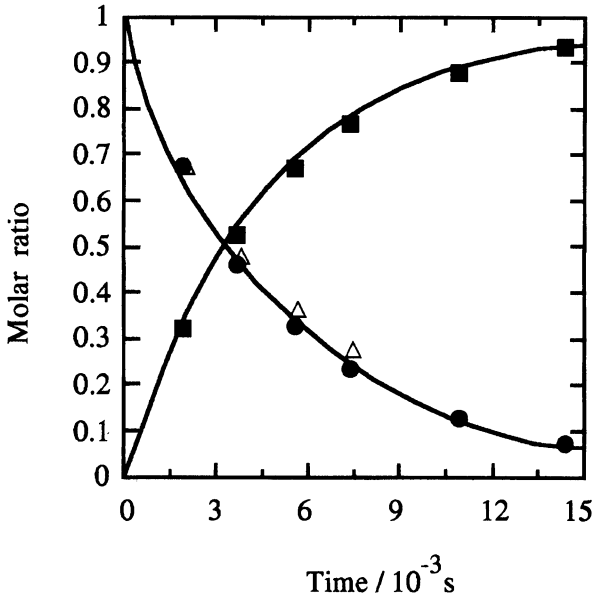


Fig. 5. Time course of the reaction of **3** with **6**. $[\mathbf{3}]_0 = 4.62\times10^{-2}$ mol dm^{-3}, $[\mathbf{6}]_0 = 8.26\times10^{-3}$ mol dm^{-3}. △, $1-([\mathbf{3}]_0-[\mathbf{3}])/[\mathbf{6}]_0$; ●, $[\mathbf{6}]/[\mathbf{6}]_0$; ■, $[\mathbf{7}]/[\mathbf{6}]_0$.

viding k_{obsd} by the square of the concentration of **3**. Here again, as observed for the formation of **6**, the reaction is second-order in **3** and first-order in **6**.

The reaction is similar to that reported for the Michael addition of pyrrolidine to *trans*-chalcone, where pyrrolidine participates in the reaction bimolecularly.[6] The third-order rate constant, k_{5b}, for the reaction via path *b* has thus been elucidated to be 0.121±0.003 dm^6 mol^{-2} s^{-1} at 304.0 K. It should be noted that k_{5b} is about 77 times larger than k_{5a}.

In order to elucidate relative importance between paths *a* and *b* at appropriate time intervals in the reaction of **3** with DMDO-Br, the rate Eqs. 12, 13, 14, 15, 16, and 17 were solved by means of a numerical method composed of the Euler routine. The results are depicted in Fig. 6.

Table 5. Pseudo-First-Order Rate Constants, k_{obsd}, and Third-Order Rate Constants, k_{calcd}, for the Reaction of **3** with **6**

$[\mathbf{6}]\times10^5$ / mol dm^{-3}	$[\mathbf{3}]\times10^2$ / mol dm^{-3}	$k_{obsd}\times10^5$ / s^{-1}	$k_{calcd}\times10$ / dm^6 mol^{-2} s^{-1}
9.79	3.41	13.9±0.47	1.19±0.04
9.92	2.47	7.28±0.23	1.19±0.04
9.65	1.54	2.91±0.07	1.22±0.03
9.99	1.10	1.51±0.03	1.25±0.03

$$\frac{d[\mathbf{3}]}{dt} = 2k_1[\mathbf{3}][\text{DMDO-Br}] - 2k_4[\mathbf{3}]^2[\text{DMDO-Br}] - k_{5a}[\mathbf{3}]^2[\mathbf{4}] - k_{5b}[\mathbf{3}]^2[\mathbf{6}] \quad (12)$$

$$\frac{d[\text{DMDO-Br}]}{dt} = -k_1[\mathbf{3}][\text{DMDO-Br}] - k_3[\text{DMDO-Br}][\mathbf{4}] - k_4[\mathbf{3}]^2[\text{DMDO-Br}] \quad (13)$$

$$\frac{d[\mathbf{4}]}{dt} = k_1[\mathbf{3}][\text{DMDO-Br}] - k_3[\text{DMDO-Br}][\mathbf{4}] - k_{5a}[\mathbf{3}]^2[\mathbf{4}] \quad (14)$$

$$\frac{d[\mathbf{6}]}{dt} = k_4[\mathbf{3}]^2[\text{DMDO-Br}] - k_{5b}[\mathbf{3}]^2[\mathbf{6}] \quad (15)$$

$$\frac{d[\mathbf{7}]}{dt} = k_{5a}[\mathbf{3}]^2[\mathbf{4}] \quad (path\ a) \quad (16)$$

$$\frac{d[\mathbf{7}]}{dt} = k_{5b}[\mathbf{3}]^2[\mathbf{6}] \quad (path\ b) \quad (17)$$

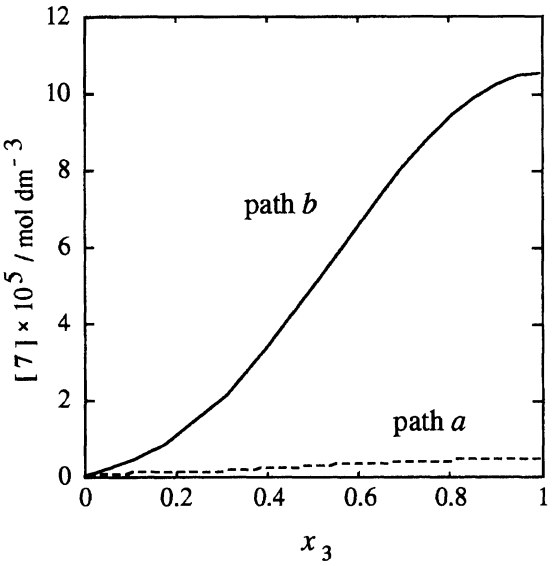


Fig. 6. Relationship between [**7**] and x_3 calculated from Eqs. 12, 13, 14, 15, 16, and 17 for $[\mathbf{3}]_0 = [\text{DMDO-Br}]_0 = 0.146$ mol dm^{-3}. **7** obtained from **3** and **4** (path *a*, ---). **7** obtained from **3** and **6** (path *b*, —). $x_3 = ([\mathbf{3}]_0 - [\mathbf{3}])/[\mathbf{3}]_0$.

Conclusion

The kinetics and analyses of products have revealed that the total scheme of the reaction can be summarized as those shown in Scheme 4. Based on these results, a couple of methods can be predicted to suppress the formation of undesired by-products, **5**, **6**, and **7**.

(i) DMDO-Br should not be exceed **3** in amount, because it reacts with the products, **4**, at the final stage of the reaction.

(ii) The presence of **3** should be monitored strictly and the reaction should be quenched immediately after it disappears.

(iii) The concentration of **3** should be kept low, because the formation of **4** is first-order in **3**, whereas the formation of **6** is second-order in **3**: Lower concentration of **3** prefers the formation of **4** over **6** kinetically.

(iv) When the concentration of **6** is kept low, the formation of **7** is suppressed simultaneously, because **7** is mainly resulted in through the reaction with **6** ($k_{5b}/k_{5a}=77$).

Thus, it is concluded that the concentration of **3** should be kept low and the concentration of DMDO-Br should exactly be equivalent or lower to **3** in order to obtain **4** in the most preferable yield suppressing the formations of undesired by-products, **5**, **6**, and **7**. Yields of products from the reaction of **3** with DMDO-Br under improved conditions are listed in Table 1 at the second line.

Experimental

Instruments. All melting points were determined in capillary tubes on a Yamato melting point apparatus MP-21 and were uncorrected. Elemental analyses were performed on a Yanaco CHN Corder MT-3 elemental analyzer. ^{1}H NMR spectra were recorded on a 200-MHz Varian XL-200 spectrometer using tetramethylsilane as an internal standard, and chemical shifts are given in ppm (δ). ^{1}H NMR spectra of all compounds are consistent with assigned structures. IR spectra were recorded on a Shimadzu IR-453-U-03 spectrometer. Mass spectra were recorded on a JEOL JMS-SX102 spectrometer at 70 eV ionization potential. Column chromatographic separations were carried out on Wako Gel C-200.

Materials. 6-Fluoro-1-methyl-7-(1-piperazinyl)-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid[3] (**3**), DMDO-Br,[5] 6-Fluoro-1-methyl-7-[4-[(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl]-1-piperazinyl]-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid[3] (**4**), and DMDO-Cl[5] were prepared according to the previous papers.

1,1-Bis[(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl]-4-(3-carboxy-6-fluoro-1-methyl-4-oxo-4*H*-[1,3]thiazeto-[3,2-*a*]quinoline-7-yl)-1-piperazinium Bromide (5): To a solution of **4** (1.18 g, 2.56 mmol) in DMF (40 cm^3), DMDO-Br (2.13 g, 14.34 mmol) was added at room temperature. After stirring at room temperature for 34 h, the reaction mixture was concentrated under reduced pressure at 75 °C and the concentate was poured into ice-water. The resulting precipitate was collected by filtration, washed three times with acetonitrile (10 cm^3), and dried to afford 1.14 g of a crude solid. The solid was dissolved in acetonitrile (2, 140 cm^3) at 85 °C. The solution was filtered and the filtrate was concentrated under reduced pressure until 2 dm^3 of acetonitrile was collected. The suspension was cooled on ice-water. The resulting precipitate was collected by filtration and dried to afford **5** (0.45g, 27%): Mp 185—192 °C (decomp). Found: m/z 574.1365. Calcd for $C_{26}H_{25}FN_3O_9S$: (M−Br), 574.1295. IR (KBr) 1825, 1705, 1628, and 1600 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ=2.17 (3H, d, J=6 Hz, 1-CH$_3$), 2.31 (6H, s, dioxol CH$_3$×2), 3.60—4.05 (8H, m, piperazine), 4.95 (4H, s, dioxol CH$_2$×2), 6.39 (1H, q, J=6 Hz, 1-H), 7.09 (1H, d, J=8 Hz, 8-H), 7.87 (1H, d, J=14 Hz, 5-H), and 14.00—15.00 (1H, br, CO$_2$H).

6-Fluoro-1-methyl-4-oxo-7-[4-[(2-oxo-1-methylene-propyl)oxycarbonyl]-1-piperazinyl]-4*H*-[1,3]thiazeto-[3,2-*a*]quinoline-3-carboxylic Acid (6): To a suspension of **3** (2.00 g, 5.72 mmol) and KHCO$_3$ (1.72 g, 17.18 mmol) in DMF (10cm^3) was added dropwise DMDO-Cl (2.13 g, 14.34 mmol) at room temperature. After stirring at room temperature for 22 h, the reaction mixture was poured into ice-water. The aqueous mixture was neutralized with 3% AcOH (pH 6—7). The resulting precipitate was collected by filtration, washed with water, and dried to afford a solid. The solid was chromatographed on silica gel with CHCl$_3$–CH$_3$OH (50 : 1) as an eluent to afford **6** (0.66 g, 25%): Mp 219—223 °C (decomp). Found: C, 54.46; H, 4.50; N, 9.06 %. Calcd for $C_{21}H_{20}FN_3O_6S$: C, 54.66; H, 4.37; N, 9.11%. IR (KBr) 1718, 1695, 1625, 1600, and 1500 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ=2.19 (3H, d, J=7 Hz, 1-CH$_3$), 2.39 (3H, s, CH$_3$CO), 3.20—3.95 (8H, m, piperazine), 5.69 (1H, d, J=2 Hz, one of C=CH$_2$), 5.90 (1H, d, J=2 Hz, one of C=CH$_2$), 6.10 (1H, q, J=7 Hz, 1-H), 6.45 (1H, d, J=7 Hz, 8-H), 7.95 (1H, d, J=12 Hz, 5-H), and 14.15 (1H, br, CO$_2$H).

7-[4-[1-[4-(3-Carboxy-6-fluoro-1-methyl-4-oxo-4*H*-

[1,3]thiazeto[3,2-*a*]quinolin-7-yl)]-1-piperazinylmethyl]-2-oxopropoxycarbonyl]-1-piperazinyl]-6-fluoro-1-methyl-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic Acid (7): To a suspension of **3** (12.20 g, 34.92 mmol) and $KHCO_3$ (6.10 g, 60.93 mmol) in DMF (30 cm^3) and acetone (30 cm^3), DMDO-Cl (5.00 g, 33.66 mmol) was added dropwise at room temperature. After stirring at room temperature for 22 h, the reaction mixture was poured into ice-water. The aqueous mixture was neutralized with 3% AcOH (pH 6—7). The resulting precipitate was collected by filtration, washed with water, and dried to afford a solid, which was recrystallized from $CHCl_3$–CH_3OH (10:1) to give **7** (5.99 g, 21%) as a pale yellow powder: Mp 197—205 °C (decomp); Found: C, 53.67; H, 4.62; N, 10.24%. Calcd for $C_{37}H_{36}F_2N_6O_9S_2 \cdot H_2O$: C, 53.62; H, 4.62; N, 10.14%. IR (KBr) 1705, 1625, 1600, and 1500 cm^{-1}; ^1H NMR (CF_3CO_2D) δ=2.11 (6H, d, J=7 Hz, 1-CH_3×2), 2.19 (3H, s, CH_3CO), 2.56—3.60 (18H, m, piperazine and $CHC\underline{H}_2N$), 5.19 (1H, t, J=4 Hz, $CH_3COC\underline{H}$), 6.37 (2H, q, J=7 Hz, 1-H×2), 6.90 (1H, d, J=6 Hz, one of 8-H), 6.96 (1H, d, J=6 Hz, one of 8-H), 7.74 (1H, d, J=12 Hz, one of 5-H), and 7.80 (1H, d, J=12 Hz, one of 5-H); MS m/z 811 $(M+H)^+$.

Isolation of By-Products 5, 6, and 7: To a suspension of **3** (10.00 g, 28.62 mmol) and $KHCO_3$ (5.70 g, 56.93 mmol) in DMF (30 cm^3) and acetone (30 cm^3), DMDO-Br (6.05 g, 31.35 mmol) was added dropwise at room temperature. After stirring at room temperature for 24 h, the reaction mixture was treated in a similar manner to that described on the synthesis of **7** and a solid was obtained. The solid was recrystallized from MeCN. The by-products **5**, **6**, and **7** were isolated from the mother liquid by means of preparative HPLC. The operating conditions were as follows. Apparatus: BIP-I system (Japan Spectroscopic Co.) equipped with UV detector (UVDEC-100-V), Rheodyne type 7125 injector, and integrated data analyzer (C-R3A, Shimadzu Co., Kyoto, Japan). Stationary phase: Nucleosil $5C_{18}$ packed in a 30 cm×10.0 mm i.d. stainless steel column (Macherey–Nagel Co.). Column temperature: 25 °C. Detection: UV 275 nm. Mobile phase: water : acetonitrile=2 : 3 (v/v). Flow rate : 4.0 $cm^3\,min^{-1}$.

6-Fluoro-1-methyl-7-[4-[(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl]-1-piperazinyl]-4-oxo-4*H*-[1,3]thiazeto-[3,2-*a*]quinoline-3-carboxylic Acid Hydrochloride (4·HCl): To 1 mol dm^{-3} hydrochloric acid (100 cm^3), **4** (1.00 g, 2.17 mmol) was slowly added. After stirring at room temperature for 6 h, the resulting precipitate was collected by filtration, washed with water, and dried to afford **4**·HCl as a white solid (0.98 g, 82%): Mp 207—211 °C (decomp). Found: C, 48.18; H, 4.61; N, 8.01%. Calcd for $C_{21}H_{21}ClFN_3O_6S \cdot 3/2H_2O$: C, 48.05; H, 4.61; N, 8.05%. IR (KBr) 3420, 2910, 1810, 1705, 1625, 1600, and 1500 cm^{-1}; ^1H NMR (DMSO-d_6) δ=2.14 (3H, d, J=7 Hz, 1-CH_3), 2.24 (3H, s, dioxol CH_3), 3.20—3.95 (8H, m, piperazine), 4.40 (2H, s, dioxol CH_2), 6.39 (1H, q, J=7 Hz, 1-H), 7.07 (1H, d, J=6 Hz, 8-H), 7.89 (1H, d, J=12 Hz, 5-H), 11.2—11.8 (1H, br, N^+H), and 14.4—14.8 (1H, br, CO_2H).

^1H NMR Measurement of the Reaction Mixture of 3 and DMDO-Br: The substrates **3** (5.9 mg, 0.0169 mmol) and DMDO-Br (98 mg, 0.508 mmol) were dissolved in 0.5 ml of DMF-d_7. After the solution was placed on a constant temperature bath (304.0 K) for 0.5 h, the ^1H NMR spectrum was measured.

Kinetic Runs. Formation of 4 from 3 and DMDO-Br: The substrates 3 were dissolved in DMF at an appropriate concentration between 3.07×10^{-3}—8.81×10^{-3} mol dm^{-3}. DMDO-Br was dissolved in DMF separately. The solution of DMDO-Br was added to the solution of **3** in a flask which was equipped with a stirrer and a thermometer. The flask was placed in a thermostat at 304.0 K. In order to obtain the concentrations of DMDO-Br at various time intervals, aliquots were pipetted out and the solutions were diluted with DMF. The concentrations of DMDO-Br were determined by HPLC as described below. The rate constants were calculated by means of a linear least-squares fit. When the data were plotted as $\ln([\text{DMDO-Br}]_0/[\text{DMDO-Br}])$ vs. time, where $[\text{DMDO-Br}]_0$ is the initial concentration of DMDO-Br and [DMDO-Br] is the concentration of DMDO-Br at a particular time, a good linear correlation was observed, suggesting that the reaction rate follows a pseudo-first-order kinetics.

The Arrhenius plot for the k_1 afforded an activation energy to be 29.1±1.6 kJ mol^{-1} (Fig. 7).

Determination of Concentrations of DMDO-Br and 4 by HPLC Using an Internal Standard: The operating conditions were as follows. Apparatus: LC-6A system (Shimadzu Co., Kyoto, Japan) equipped with UV detector (SPD-6A, Shimadzu), Rheodyne type 7125 injector, and integrated data analyzer (C-R3A, Shimadzu). Stationary phase: COSMOSIL $5C_{18}$-AR packed in a 25 cm×4.6 mm i.d. stainless steel column (Nacalai Tesqe Co., Kyoto, Japan). Column temperature: 25 °C. Detection: UV 275 nm. Internal standard: ethyl 4-hydroxybenzoate. Mobile phase A: 1 mol dm^{-3} H_3PO_4 : 1 mol dm^{-3} sodium 1-pentanesulfonate : water : acetonitrile=22 : 20.3 : 507 : 1473 (v/v). Flow rate: 0.9 $cm^3\,min^{-1}$.

Formation of 5 from DMDO-Br and 4: The substrate **4** was dissolved in DMF at a concentration of 1×10^{-2} mol dm^{-3}. DMDO-Br was dissolved in DMF separately. The reaction solutions were mixed and the concentration of **4** was determined at various time intervals in a similar manner to that described in the section on the formation of **4** from **3** and DMDO-Br.

Formation of 6 from 3 and DMDO-Br: The reac-

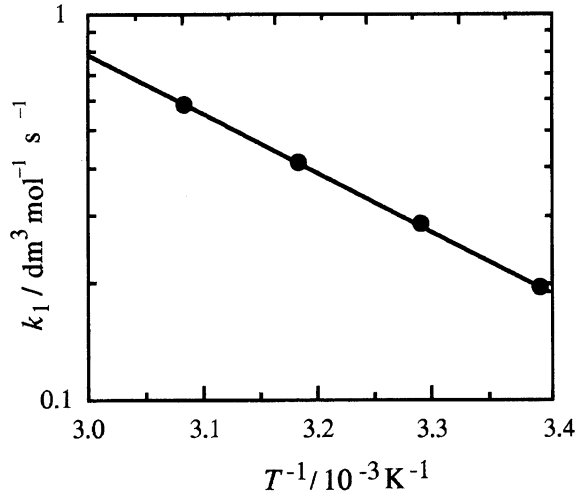


Fig. 7. Arrehenius plot for the formation of **4**.

tion solution was prepared and the concentrations of **3** and **4** were determined in a similar manner to that described on the formation of **4** from **3** and DMDO-Br. The concentrations of **6** were determined by HPLC as described below.

Determination of Concentrations of 6 and 7 by HPLC: The concentrations of **6** and **7** were determined in the same manner as that described in the section for determination of concentrations of DMDO-Br and **4** by HPLC, except for that butyl 4-hydroxybenzoate was used as an internal standard instead of ethyl 4-hydroxybenzoate and mobile phase B was used instead of mobile phase A. Mobile phase B: water : acetonitrile : 70% methanesulfonic acid=650 : 350 : 0.5 (v/v).

Formation of 7 from 3 and 4: The substrate **4** was dissolved in DMF at a concentration of 2×10^{-4} mol dm^{-3}. The substrate **3** was dissolved in DMF separately. The reaction solutions were mixed and the concentration of **4** was determined at various time intervals in a similar manner to that described in the section of the formation of **4** from **3** and DMDO-Br.

Formation of 7 from 3 and 6: The substrate **6** was dissolved in DMF at a concentration of 1×10^{-4} mol dm^{-3}. The substrate **3** was dissolved in DMF separately. The reaction solutions were mixed and the concentration of **6** was determined at various time intervals as described above.

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