dimeric Vinca alkaloids, VLB, VCR, vinrosidine, and vinleurosine, and the results are presented as IC_{50} (50% inhibitory concentrations) in Table II.

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Studies on Prodrugs. 10. Possible Mechanism of N-Dealkylation of N-Masked Norfloxacins Having Several Active Methylene Groups

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As a prodrug approach to norfloxacin (NFLX, **2**), we have prepared several N-masked NFLXs (**1a–f**) and studied the cleavage mechanism of the C–N bond of N-masked NFLXs utilizing the following experiments: (1) the oxidation of N-masked NFLXs (**1a–f**) with *m*-chloroperbenzoic acid (MCPBA) and their subsequent cleavage to **2** in chloroform at room temperature or at 50 °C; (2) the liberation of NFLX from N-masked NFLXs after oral administration in mice. It was found that the chemical oxidative dealkylation of N-masked NFLXs proceeded when anion-stabilizing groups (e.g., CN, COR, COOR) are present on the α carbon of the nitrogen atom. In in vivo experiments, N-masked NFLXs having acidic hydrogens on the α carbon to the nitrogen atom also liberated NFLX (**2**) after oral administration.

Norfloxacin (NFLX, **2**) has been widely used as a clinically effective antibacterial agent,[1] but it has been shown that the blood level and the urinary recoveries after oral administration of NFLX were not sufficient for use as an effective oral antibiotic.[2] We have applied the prodrug technique to NFLX.[3-5] Recently, we have reported that in in vivo experiments, *N*-(2-oxopropyl)NFLX (**1a**) was absorbed efficiently and transformed into **2** whereas *N*-(2-hydroxypropyl)NFLX (**1f**) failed to metabolize into **2**[4] (Scheme I). Definition of such a metabolic difference of compounds **1a** and **1f** may open up the possibility for developing a new prodrug approach to amines.

The metabolic N-dealkylation of alkylamines is known to be catalyzed by flavin and cytochrome P-450 monooxygenases,[6] and several conceptual pathways for enzymatic dealkylation of amines should be considered; direct hydroxylation of the methyl carbon, with or without formation of an intermediate *N*-oxide, or electron-transfer oxidation of the nitrogen is the most probable of the proposed mechanisms.[6] Recently, Burka et al. found N-dealkylation of amines via the *N*-oxide to only be a minor pathway.[7] Other workers also have studied the mechanism of N-dealkylation of amines.[8] However, no definitive work has appeared on the mechanism of oxidative cleavage of amine derivatives **1**.

We focused our interest on the structure–metabolism relationship of compounds **1a** and **1f** and have hypothesized that acidic hydrogens on the α carbon to the nitrogen atom play an important part in the N-dealkylation of **1a**. To clarify our hypothesis, we synthesized several N-masked NFLXs (**1a–f**), with or without acidic hydrogens on the α carbon atom, and measured their metabolic conversion in serum after oral administration in mice. This paper describes the possible N-dealkylation mechanism of N-masked NFLXs having acidic hydrogens on the α carbon.

Results and Discussion

Chemical Oxidation of 1a–f with MCPBA. NFLX (**2**) was synthesized in accordance with the report of Koga et al.[1a] The N-masked NFLXs **1a–f** were prepared according to a recently described method.[4]

As shown in Scheme II, the oxidation of *N*-(2-oxopropyl)NFLX (**1a**) (0.53 mM) with MCPBA (1.06 mM) in dry chloroform (20 mL) at room temperature under an argon atmosphere afforded NFLX (**2**) and *N*-formyl NFLX **3**. We also tried the oxidation of other N-masked NFLXs (**1b–f**) under similar conditions. As summarized in Table I, the oxidation of **1b–d** also afforded **2**, and in the case of **1a** and **1b**, *N*-formylNFLX (**3**) was simultaneously produced. However, no formation of **2** and **3** was observed by oxidation of **1e** and **1f**.

We focused our interest on the C–N bond cleavage by chemical oxidations of **1a–d**.

Craig and co-workers have reported that *N*-oxides are formed by the oxidation of tertiary amines with MCPBA at low temperature (<0 °C).[9] When the oxidation of **1a** with MCPBA at low temperature (<0 °C) was carried out according to the Craig method, a colorless solid (**5a**) separated. The mass spectrum of **5a** showed ions at *m/e* 392 (MH^+), 376 ($MH^+ - 16$) [O]. Compound **5a** was redissolved in organic solvent to decompose to **2** and *N*-formylNFLX (**3**) was not obtained. However, the thermolysis of **5a** was carried out in the presence of MCPBA to afford **2** and **3**.

On the other hand, **5c** obtained by a similar manner was more stable compared to **5a**. Compound **5c** was charac-

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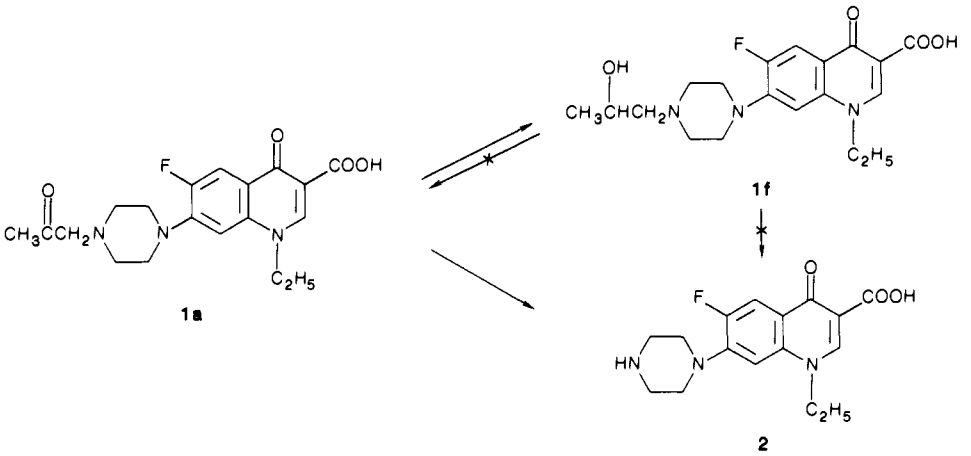
Scheme I



Scheme II. Chemical Oxidation of 1a and 1c

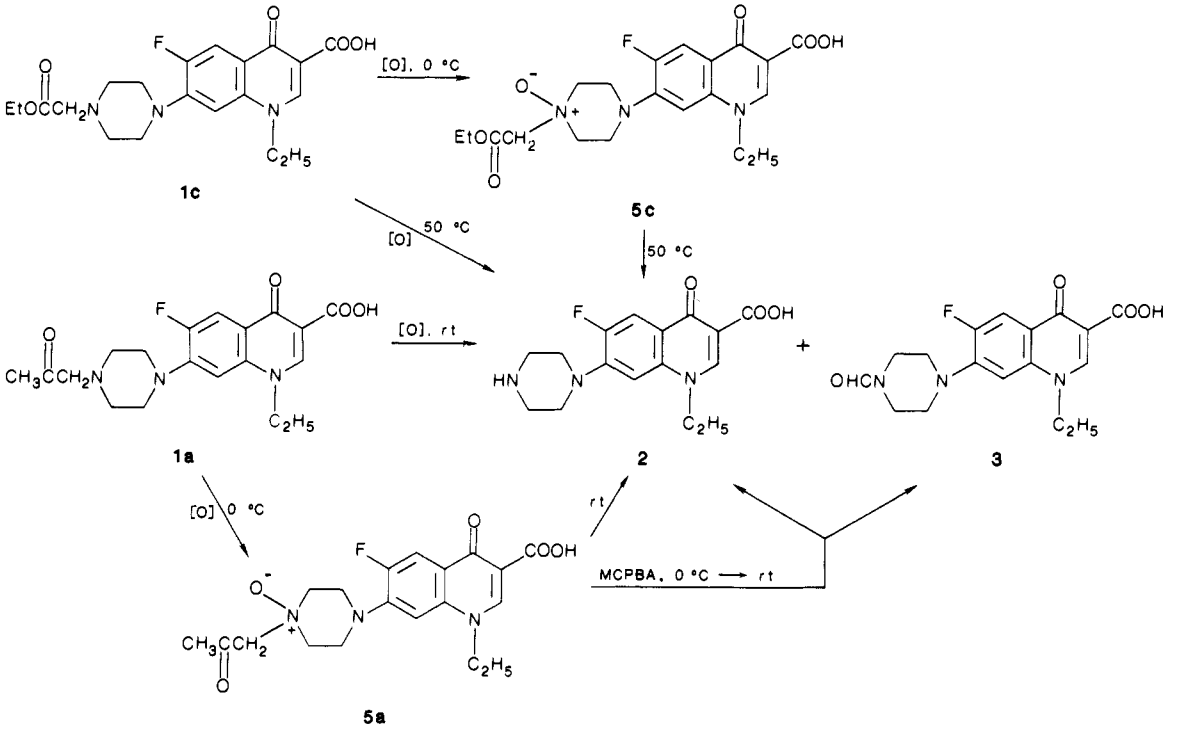
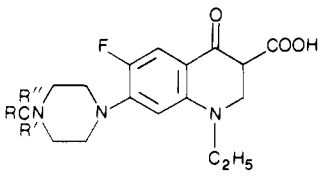


Table I. Oxidation of N-Masked NFLXs (1a–f) with MCPBA[a]

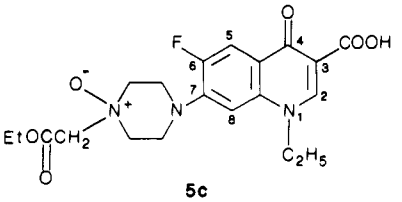


compd	R	R′	R″	MCPBA, equiv	time, h	temp, °C	products[b] (%) 2	3	recovered[c]
1a	$COCH_3$	H	H	2.0	6	rt[d]	59	32	–
1b	$COCH_3$	CH_3	H	2.0	12	rt	52	27	3
1c	COOEt	H	H	1.1	10	50	77	–	–
1d	CN	H	H	1.1	10	50	83	–	–
1e	$COCH_3$	CH_3	CH_3	1.1	24	50	–	–	64[e]
1f	$CH(OH)CH_3$	H	H	1.1	24	50	–	–	12[e]

[a] MCPBA = *m*-chloroperbenzoic acid. [b] Isolated yield. [c] Starting material. [d] rt = room temperature. [e] *N*-Oxide should be produced but it could not be isolated.

terized by ^1H NMR analysis and mass spectrometry (Table II). In particular, the methylene protons on the carbon adjacent to the nitrogen atom in **5c** were observed at lower field (δ 4.68) than the corresponding methylene protons in **1c** (δ 3.32). The ^1H NMR spectrum of **5c** exhibited signals for four methylene protons of the piperazine moiety as doublets at 3.59 ppm (2 H) and 3.86 ppm (2 H) and as triplets at 4.03 ppm (2 H) and 4.23 ppm (2 H). The mass

Table II. ^{1}H NMR and Mass Spectral Data of 5c

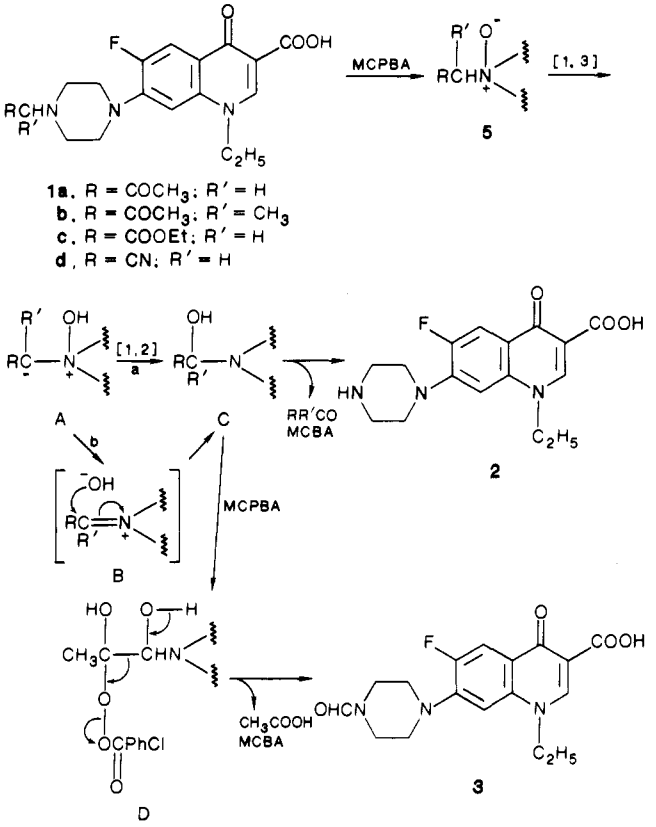


5c

compd	^{1}H NMR ($CDCl_3$, ppm, J = Hz)[a]	MS, m/e[b]
5c	1.34 (t, 3 H, $COOCH_2CH_3$, J = 7.0 Hz)	422 (MH^+)
	1.60 (t, 3 H, NCH_2CH_3, J = 7.2 Hz)	406 (MH^+ - 16) [O]
	3.59 (d, 2 H, piperazine-CH_2, J = 11 Hz)	362 (MH^+ - 16 - 44) [CO_2]
	3.86 (d, 2 H, piperazine-CH_2, J = 11 Hz)	
	4.03 (t, 2 H, piperazine-CH_2, J = 11 Hz)	333 (MH^+ - 16 - 73)
	4.23 (t, 2 H, piperazine-CH_2, J = 11 Hz)	[$COOCH_2CH_3$]
	4.31 (q, 2 H, $COOCH_2CH_3$, J = 7.0 Hz)	
	4.33 (q, 2 H, NCH_2CH_3, J = 7.2 Hz)	
	4.68 (s, 2 H, N(O)CH_2COOEt)	
	6.98 (d, 1 H, H_8, J = 6.8 Hz)	
	8.14 (d, 1 H, H_5, J = 12.6 Hz)	
	8.71 (s, 1 H, H_2)	
	14.9 (bs, 1 H, COOH)	

[a] ^{1}H NMR spectra were determined at 300 MHz on a Bruker AM-300 NMR spectrometer. [b] A Hitachi M-80B mass spectrometer and 0101 control system and M8061 SIMS apparatus.

Scheme III



spectrum of **5c** showed ions at m/e 422 (MH^+), 406 (MH^+ - 16) [O], 362 (MH^+ - 16 - 44) [CO_2], 333 (MH^+ - 16 - 73) [$COOC_2H_5$]. The N-oxide **5c** thus prepared was stirred at 50 °C in dry chloroform under an argon atmosphere to change into **2** in high yield (Scheme II). Although we attempted to react **1e** and **1f** with MCPBA at low temperature (<0 °C), their N-oxides could not be isolated. This strongly suggested that acidic hydrogens on the carbon atom adjacent to the nitrogen may be essential to the N-dealkylation of this class of amines **1**.

Thus, these results show that a possible mechanism for the N-dealkylation of N-masked NFLXs (**1a–d**) by oxidation with MCPBA is that indicated in Scheme III.

Table III. Serum Concentrations of the Liberated NFLX after Oral Administration of 1a–f and NFLX Itself in Mice

compd	time after administration, h 0.5	1.0	2.0	4.0	AUC[a]
1a (100 mg/kg, po)[b]	6.33	5.74	3.96	2.62	22.01
1b (100 mg/kg, po)	2.63	1.68	1.67	0.63	5.71
1c (100 mg/kg, po)	3.77	1.37	0.38	0.42	3.90
1d (100 mg/kg, po)	0.29	0.75	1.15	0.63	3.06
1e (100 mg/kg, po)	nd[c]	nd	nd	nd	-
1f (100 mg/kg, po)	nd	nd	nd	nd	-
2 (NFLX) (100 mg/kg, po)	3.47	2.02	1.12	0.56	6.49

[a] In (μg·h)/mL. [b] A dose equivalent to 100 mg/kg of NFLX. [c] Not detected.

Namely, the reaction could be initiated by the formation of N-oxide **5** having acidic hydrogens on the α carbon, followed by a proton transfer from the α carbon atom to the oxygen atom of the N-oxide to produce N-ylide A. The manner of transformation from the intermediate A to C may be interpreted via two paths (a, b) as shown in Scheme III. For example, (a) the [1,2]-sigmatropic migration of the resulting hydroxyl group spontaneously proceeds to generate the carbinolamine C and (b) the elimination of hydroxide from intermediate A to form an iminium ion B which could then be attacked by hydroxide to give the intermediate C. The intermediate carbinolamine C decomposes into **2**. Moreover, it may be assumed that the carbinolamine C reacts in part with MCPBA to afford N-formylNFLX (**3**) via intermediate D.

Serum Level and Metabolism. In order to confirm the liberation of NFLX (**2**) from N-masked NFLXs in vivo, we measured the blood level of NFLX and the biological transformation of N-masked NFLXs. First, the stability of **1a–f** at pH 7.4 and 37 °C was determined by high-performance liquid chromatography (HPLC). As a result, **1a–f** were found to be stable at physiological pH. The serum specimens were collected at regular time intervals (0.5, 1.0, 2.0, and 4.0 h) after oral administration, and the serum levels of some species were measured individually by HPLC (Table III). As shown in Table III, compounds **1a–d** having acidic hydrogens on the α carbon atom afforded **2** in in vivo experiments, whereas compounds **1e,f** without acidic hydrogens, were not transformed into **2**. The in vivo behavior of **1a–f** was approximately parallel

to the results of the chemical oxidation of **1a–f**. Thus, tertiary alkylamines having acidic hydrogens on the α carbon to the nitrogen atom were easily dealkylated by chemical oxidation, and similar cleavage of the C–N bond also proceeded in an in vivo system. The above results may have some bearing on the metabolic dealkylation of tertiary alkylamines having acidic hydrogens on the α carbon atom. This work may open up the possibility for developing a new prodrug approach to amines.

Experimental Section

Melting points were determined on a Yamato capillary melting point apparatus, Model MP-21, and all melting points are uncorrected. ^{1}H NMR spectra were determined at 300 MHz on a Bruker AM-300 NMR spectrometer using TMS as an internal standard. Mass spectra were measured with a Hitachi M-80B mass spectrometer and 0101 control system and M8061 SIMS apparatus. All compounds were analyzed for C, H, N, and the values were within ±0.4% of the calculated theoretical ones.

Oral Absorbability Test. The serum concentrations of NFLX in mice treated with compounds **1a–f** and NFLX itself were determined by HPLC. Test compounds were suspended in 0.5% sodium carboxymethylcellulose and administered orally at a dose of 100 mg/kg. After 0.5, 1.0, 2.0, and 4.0 h the mice were killed by bleeding. The collected blood was centrifuged, and the test serum was adjusted and then analyzed by HPLC (equipped with a Hitachi Model 655 pump, a Shimadzu Model SPD-2A spectrophotometric detector (at 280 nm), and a YMC A-312 column). The mobile phase consisted of 0.5% acetic acid–acetonitrile (90:10 v/v), and the flow rate was 2.0 mL/min.

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-[(ethoxycarbonyl)-methyl]-1-piperazinyl]-4-oxoquinoline-3-carboxylic Acid (1c). NFLX (0.4 g, 1.25 mmol) was added to a solution of ethyl bromoacetate (0.23 g, 1.1 mmol) and potassium bicarbonate (0.125 g, 1.0 mmol) in *N,N*-dimethylformamide (DMF, 4 mL). The solution was stirred at room temperature for 8 h. The DMF was then removed under reduced pressure. The residue was poured into water (8 mL) and extracted with chloroform (20 mL). The extact was washed with water, dried over anhydrous sodium sulfate, and evaporated. The residual solid was recrystallized from chloroform–ethanol to give **1c** (0.31 g, 61%) as a pale yellow crystalline solid: mp 229–231 °C; MS, *m/e* 405 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3 H, $COOCH_2CH_3$, J = 7.0 Hz), 1.59 (t, 3 H, NCH_2CH_3, J = 7.2 Hz), 2.83 and 3.40 (m, 8 H), 3.32 (s, 2 H, NCH_2COOEt), 4.25 (q, 2 H, $COOCH_2CH_3$, J = 7.0 Hz), 4.33 (q, 2 H, NCH_2CH_3, J = 7.2 Hz), 6.64 (d, 1 H, H_8, J = 6.9 Hz), 8.07 (d, 1 H, H_5, J = 13 Hz), 8.66 (s, 1 H, H_2), 15.1 (bs, 1 H, COOH). Anal. ($C_{20}H_{24}N_3O_5F$) C, H, N.

1-Ethyl-6-fluoro-1,4-dihydro-7-[4-(cyanomethyl)-1-piperazinyl]-4-oxoquinoline-3-carboxylic Acid (1d). NFLX (0.4 g, 1.25 mmol) was added to a solution of cyanomethyl chloride (0.1 g, 1.32 mmol) and potassium bicarbonate (0.125 g, 1.25 mmol) in DMF (20 mL). The solution was stirred at room temperature for 12 h. The DMF was then removed under reduced pressure. The residue was poured into water (30 mL) and extracted with chloroform (45 mL). The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated. The residual solid was recrystallized from chloroform–ethanol to give **1d** (0.22 g, 49%) as a pale yellow crystalline solid: mp 220–225 °C dec; MS, *m/e* 358 (M^+); ^{1}H NMR (DMSO-d_6) δ 1.41 (t, 3 H, NCH_2CH_3, J = 7.0 Hz), 2.50 and 3.33 (m, 8 H), 3.37 (bs, 2 H, NCH_2CN), 4.60 (q, 2 H, NCH_2CH_3, J = 7.0 Hz), 7.20 (d, 1 H, H_8, J = 7.3 Hz), 7.94 (d, 1 H, H_5, J = 13.3 Hz), 8.96 (s, 1 H, H_2), 15.3 (bs, 1 H, COOH). Anal. ($C_{18}H_{19}N_4O_3F \cdot 0.25\ H_2O$) C, H, N.

Other N-masked NFLXs were prepared in accordance with our previous report.[4]

Oxidation of 1a. General Procedure. To a solution of *N*-(2-oxopropyl)NFLX (0.2 g, 0.53 mmol) in chloroform (20 mL) was added MCPBA (0.18 g, 1.06 mmol) at room temperature under argon atmosphere, and the solution was stirred for 6 h. The solvent was then removed under reduced pressure. The residue was chromatographed on a column of silica gel with chloroform/methanol (2/1 v/v) as an eluent under medium pressure to give **2**[1a] (0.1 g, 59%) and **3** (0.06 g, 32%) as pale yellow needles: mp 165–172 °C dec; IR (KBr) cm^{-1} 1712, 1678, 1630, 1614, 1458; ^{1}H NMR (DMSO-d_6) δ 1.44 (t, 3 H), 3.10–3.50 (m, 4 H), 3.55–3.70 (m, 4 H), 4.56 (q, 2 H), 7.16 (d, 1 H), 7.82 (d, 1 H), 8.06 (s, 1 H), 8.84 (s, 1 H), 15.04 (bs, 1 H); MS, *m/e* 347 (M^+). Anal. ($C_{17}H_{18}N_3O_4F$) C, H, N.

The oxidation of other N-masked NFLXs (**1b–f**) has been carried out by the same procedure as described above.

Preparation of *N*-Oxide 5c. A solution of MCPBA (255 mg, 1.47 mmol) in dry chloroform (50 mL) was added gradually at 0 °C to an ice-cooled, stirred solution of **1c** (500 mg, 1.23 mmol) in chloroform. Stirring was continued for 3 h. The obtained solid was filtered off and washed with chloroform and ether to give the *N*-oxide **5c** (470 mg): mp 157–161 °C dec; ^{1}H NMR spectral data shown in Table II.

Conversion from *N*-Oxide 5c to NFLX (2). The solution of *N*-oxide **5c** (400 mg) in chloroform (50 mL) under argon atmosphere was stirred for 5 min at room temperature and for 6 h at 50 °C. The solvent was then removed under reduced pressure. The residual solid was recrystallized from DMF to give NFLX **2** (239 mg): mp 219–224 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.44 (t, 3 H), 2.90 and 3.24 (m, 8 H), 4.59 (q, 2 H), 7.16 (d, 1 H), 7.88 (d, 1 H), 8.90 (s, 1 H), 15.6 (bs, 1 H). This agreed with the analytical data in the literature.[1a]

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Registry No. **1a**, 103175-73-1; **1b**, 103258-03-3; **1c**, 118476-20-3; **1d**, 99726-81-5; **1e**, 103240-26-2; **1f**, 103240-28-4; **2**, 70458-96-7; **3**, 70459-04-0; ethyl bromoacetate, 105-36-2; cyanomethyl chloride, 107-14-2.