Preparation of 2-Aminooxyethyliminodiacetic Acid, a Bifunctional Chelating Agent for Carbonyl Labeling

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2-Aminooxyethyliminodiacetic acid, designed as a possible bifunctional chelating agent with a hydroxylamino group that can bind a carbonyl group of biomolecules, was synthesized by three different methods. Its oxime derivatives with some carbonyl compounds were readily prepared.

Keywords 2-aminooxyethyliminodiacetic acid; bifunctional chelating agent; carbonyl-labeling

A number of bifunctional chelating agents (BCA's) with both metal-chelating and labeling sites in a single molecule have been used to label biomolecules with radioactive or stable metal ions for preparation of radiopharmaceuticals and immunomaterials. The labeling site can bind biomolecules such as plasma proteins, enzymes, antibodies and haptens. Most BCA's reported so far have a labeling group that reacts with amino or carboxylate groups of biomolecules.[1] We have designed BCA's with a labeling group that binds a carbonyl group.

The present paper deals with a possible BCA for carbonyl labeling, 2-aminooxyethyliminodiacetic acid, which has an iminodiacetic acid group as the metal chelating site and a hydroxylamino group as the labeling site.

Results and Discussion

2-Aminooxyethyliminodiacetic acid (**1**) was synthesized by three methods, A, B and C, as outlined in Chart 1. Table I lists physical and analytical data of **1** and new compounds synthesized in this work.

In method A, 2-bromoethyliminodiacetonitrile (**2**) was prepared by Mannich's reaction of 2-bromoethylamine, formaldehyde and sodium cyanide and converted to dimethyl 2-bromoethyliminodiacetate (**3**) by bubbling gaseous hydrogen chloride in methanol.[2] Dimethyl 2-*N*-phthalimidoxyethyliminodiacetate (**4**),[3] prepared by the reaction of **3** and *N*-hydroxyphthalimide in the presence of triethylamine, was hydrolyzed in alkaline and acid solutions to give **1**.[4]

In method B, **1** was prepared by acid hydrolysis[5] of dimethyl 2-(ethoxycarbonylaminooxy)ethyliminodiacetate (**5**), obtained by the reaction of **3** and ethyl *N*-hydroxycarbamate.[6]

In method C, diethyl 2-*N*-phthalimidoxyethyliminodiacetate (**7**) was prepared by reaction of *N*-(2-bromoethoxy)phthalimide (**6**) and diethyl iminodiacetate.[7] The hydrolysis of **7** gave **1**. The method gave the best yield among the three.

The oxime derivatives of **1** were readily prepared by the conventional method with carbonyl compounds such as benzaldehyde, vanillin, pyridoxal, progesterone and testosterone. The physical and analytical data of the oximes are summarized in Table II.

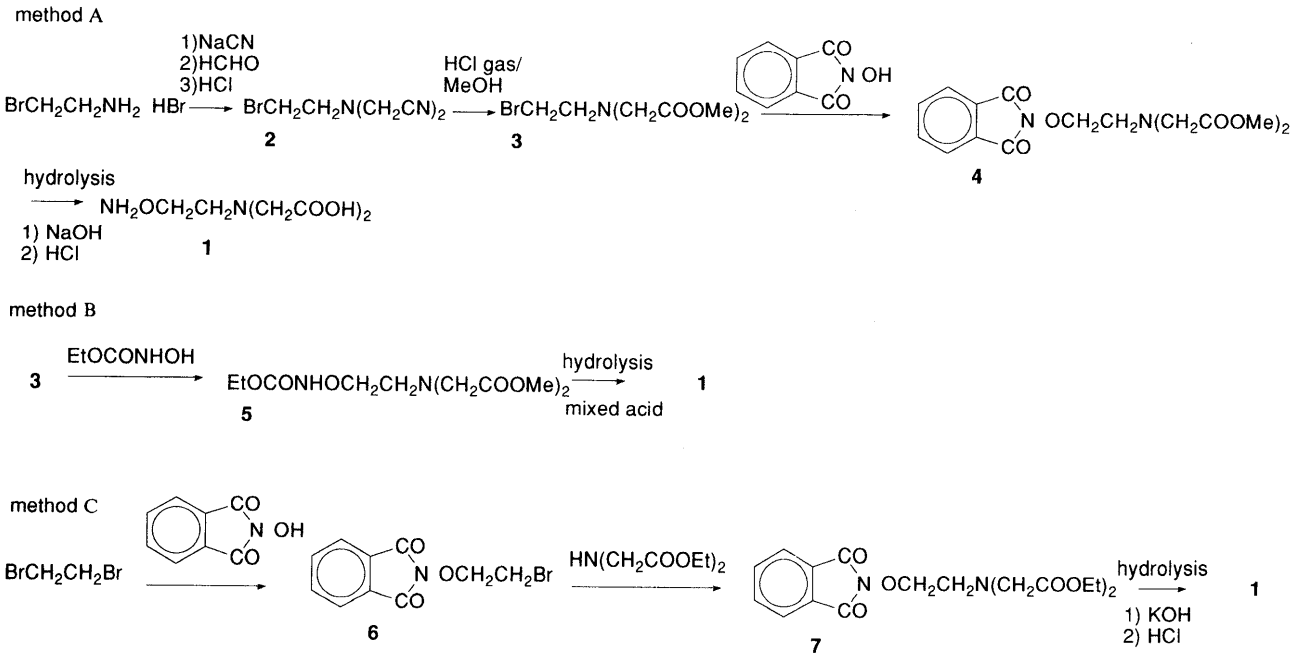

Chart 1. Syntheses of 2-Aminooxyethyliminodiacetic Acid (**1**)

The oximes formed metal chelates with various metal ions and the chelates may be used as metal-labeled haptens. Further studies are under way.

TABLE I. Physical Properties of **1** and Related Compounds

Compd. No.	Formula MW mp (recryst. solvent)	UV $\lambda_{max}^{2\text{-PrOH}}$ nm ($\varepsilon \times 10^3$)	Analysis (%) Calcd (Found) C	H	N	Mass FAB-MS *m/z*
1	$C_6H_{12}N_2O_5$ MW 192.17 Hygroscopicity					259 ($M+3Na-2H)^+$, 237 ($M+2Na-H)^+$, 215 ($M+Na)^+$
2	$C_6H_8BrN_3$ MW 202.06 mp 54 °C (EtOH)		35.67 (35.49	3.99 3.93	20.80 20.77)	203, 201 (M^+), 177, 175 (M^+-CN), 122 (M^+-Br)
3	$C_8H_{14}BrNO_4$ MW 268.11 bp 118 °C (0.35 mmHg)					270, 268 (M^+), 224, 223, 166, 164
4	$C_{16}H_{18}N_2O_7$ MW 350.33 mp 69.6 °C (Ether–EtOH)	219 (30.4) 240 sh 294 (1.9)	54.86 (54.77	5.18 5.01	8.00 8.04)	350 (M^+), 318 (M^+-CH_3OH), 291 (base, M^+-CH_3COO), 190 ($C_6H_4(CO)_2N-OCH_2CH_2^+$)
6	$C_{10}H_8BrNO_3$ MW 270.08 mp 95.9 °C (EtOH)	226 (17.4) 240 sh 295 (1.7)	44.47 (44.76	2.99 3.04	5.19 5.13)	271, 269 (M^+), 190 (M^+-Br), 163 ($C_6H_4(CO)_2N-OH^+$)
7	$C_{18}H_{22}N_2O_7$ MW 378.38 Oil	220 (16.0) 240 sh 296 (0.9)				378 (M^+), 332 (M^+-EtOH), 305 (base, $M^+-EtCOO$)

MW: molecular weight.

Experimental

General Procedures UV spectra were recorded on a Shimadzu UV-200 UV-visible recording spectrophotometer and IR spectra (KBr disks) on a Hitachi EPI-G3 IR spectrometer. FAB-MS were measured with a JEOL JMS-DX303 mass spectrometer. ^1H- and ^{13}C-NMR spectra (data summarized in Table III) were taken on a JEOL JNM-GX270 spectrometer. Chemical shifts are expressed in ppm on the δ scale from tetramethylsilane (TMS) or sodium 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionate (TSP-d_4) as an internal standard. TMS was used in dimethylsulfoxide-d_6 (DMSO-d_6) and $CDCl_3$ solutions, and TSP-d_4 in D_2O. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet and m=multiplet. The coupling constants are expressed in hertz (Hz). Melting points were determined with a Yanaco micro melting point apparatus and are uncorrected. For TLC, precoated Silica gel 5724 (Merck) was used. Elemental analyses were performed in the Faculty of Pharmaceutical Sciences, University of Tokyo. Chemicals were obtained from commercial sources and were of reagent grade.

2-Bromoethyliminodiacetonitrile (2) 2-Bromoethylamine hydrobromide (143.4 g, 0.7 mol) was placed in a 1 l flask equipped with a condenser, and 30% formaldehyde solution (140.2 g) was added dropwise with stirring and cooling at 3—5 °C. Concentrated HCl (65 ml, 0.77 mol) was added dropwise to the mixture over 30 min and then 34% NaCN (208 g) was similarly added over 3 h. The reaction mixture was stirred for 18 h, then the white precipitate was collected, washed with a small amount of water and twice with 40 ml of cold ethanol, and dried over P_2O_5. The white product (85.1 g) was recrystallized from ethanol, mp 54—57 °C. IR, 2260 cm^{-1} (CN). Yield, 60.2%. *Rf* values (TLC): 0.70 (AcOEt : benzene = 1 : 1), 0.66 (acetone : $CHCl_3$ = 1 : 9).

Dimethyl 2-Bromoethyliminodiacetate (3) Dry HCl gas was passed into a solution of **2** (100 g, 0.495 mol) in 600 ml of dry methanol for 9 h under reflux with vigorous stirring. The reaction mixture was cooled, allowed to stand in a refrigerator overnight and filtered. The filtrate was evaporated to dryness at 55 °C. To the residue, 80 ml of ice water and 170 ml of 33% NaOH were added and the mixture was extracted with 800 ml of ether. The ether extract was dried over anhydrous Na_2SO_4 and the solvent was evaporated off. The residue was distilled under

TABLE II. Physical Properties of Oxime Derivatives with **1** HCl Salt

Carbonyl compd.	Formula MW mp (recryst. solvent)	UV λ_{max}^{MeOH} nm ($\varepsilon \times 10^3$)	Analysis (%) Calcd (Found) C	H	N	FAB-MS *m/z*
Benzaldehyde	$C_{13}H_{16}N_2O_5$ MW 280.28 mp 122 °C (2-PrOH–H_2O)	210 (14.2) 259 (14.0) 294 sh (1.2)				281 ($M+H)^+$, 267, 245, 222
Vanillin: 4-Hydroxy-3-methoxy-benzaldehyde	$C_{14}H_{18}N_2O_7$ MW 326.31 mp 177—181 °C (2-PrOH–H_2O)	216 (15.3) 226 sh (13.4) 276 (12.9) 305 (9.8)				349 ($M+Na)^+$, 371 ($M+2Na-H)^+$, 393 ($M+3Na-2H)^+$
Pyridoxal	$C_{14}H_{19}N_3O_7+H_2O$ MW 341.32	215 (12.6) 267 (8.5) 322 (3.9)				342 ($M+H)^+$
	$C_{14}H_{21}N_3O_8$ MW 359.335 mp 138.2 °C (MeOH)		46.80 (46.74	5.89 5.54	11.69 11.46)	
Progesterone	$C_{33}H_{50}N_4O_{10}+H_2O$ MW 662.78	207 (9.1) 249 (19.9)				685 ($M+Na)^+$, 707 ($M+2Na-H)^+$,
	$C_{33}H_{52}N_4O_{11}$ MW 680.80 mp 199.6 °C precipitate (MeOH–ether)		58.22 (57.86	7.70 7.42	8.23 8.19)	729 ($M+3Na-2H)^+$, 751 ($M+4Na-3H)^+$, 773 ($M+5Na-4H)^+$
Testosterone	$C_{25}H_{38}N_2O_6+H_2O$ MW 462.59	251 (12.6)				463 ($M+H)^+$, 485 ($M+Na)^+$
	$C_{25}H_{40}N_2O_7$ MW 480.60 mp 193—196 (MeOH–DMSO)		62.48 (62.77	8.39 8.14	5.83 5.83)	

TABLE III. NMR Data

Compd. No.	^{1}H-NMR	^{13}C-NMR
1 HCl salt	In D_2O, 3.85 (2H, t, J=4.2, CH_2N), 4.25 (4H, s, CH_2COO), 4.54 (2H, t, J=4.2, OCH_2)	In D_2O, 56.85 t (CH_2N), 58.63 t ($\underline{C}H_2COO$), 71.96 t (OCH_2), 171.27 s (COO)
	In DMSO-d_6, 3.72 (2H, t, CH_2N), 4.29 (4H, s, CH_2COO), 4.55 (2H, t, OCH_2)	In DMSO-d_6, 52.69 t (CH_2N), 53.93 t ($\underline{C}H_2COO$), 68.67 t (OCH_2), 167.36 s (COO)
2	In DMSO-d_6, 3.00 (2H, t, J=6.6, CH_2N), 3.60 (2H, t, J=6.6, $BrCH_2$), 3.91 (4H, s, CH_2CN)	In DMSO-d_6 (at 80 °C) 29.44 t ($BrCH_2$), 41.93 t ($\underline{C}H_2CN$), 54.47 t (CH_2N), 115.50 s ($CH_2\underline{C}N$)
3	In $CDCl_3$, 3.11 (2H, t, J=7, CH_2N), 3.60 (2H, t, J=7, CH_2Br), 3.63 (4H, s, CH_2COO), 3.71 (6H, s, OCH_3)	In $CDCl_3$, 42.17 t ($BrCH_2$), 51.67 q (OCH_3), 55.39 t ($\underline{C}H_2COO$), 56.44 t (CH_2N), 171.57 s (COO)
4	In $CDCl_3$, 3.25 (2H, t, J=5.3, CH_2N), 3.70 (6H, s, OCH_3), 3.74 (4H, s, CH_2COO), 4.36 (2H, t, J=5.3, OCH_2), 7.85—7.74 (4H, m, C_6H_4)	In $CDCl_3$, 51.59 q (OCH_3), 52.48 t (CH_2N), 55.23 t ($\underline{C}H_2COO$), 77.17 t (OCH_2), 123.54 d, 128.94 s, 134.50 d (C_6H_4), 163.40 s (CO–N–CO), 171.62 s (COO)
	In DMSO-d_6, 3.07 (2H, t, CH_2N), 3.58 (6H, s, OCH_3), 3.64 (4H, s, CH_2COO), 4.22 (2H, t, OCH_2), 7.86 (4H, s, C_6H_4)	In DMSO-d_6, (at 80 °C), 50.99 q (OCH_3), 52.34 t (CH_2N), 54.93 t ($\underline{C}H_2COO$), 76.65 t (OCH_2), 123.17 d, 128.78 s, 134.69 d (C_6H_4), 163.10 s (CO–N–CO), 171.11 s (COO)
5	In $CDCl_3$, 1.29 (3H, t, J=7.0, $C\underline{H}_3CH_2O$), 3.03 (2H, t, J=5.1, CH_2N), 3.63 (4H, s, CH_2COO), 3.72 (6H, s, OCH_3), 3.98 (2H, J=5.1, OCH_2), 4.21 (2H, q, J=7.0, $CH_3C\underline{H}_2O$), 8.49 (NH)	In $CDCl_3$, 14.52 ($\underline{C}H_3CH_2$), 51.67 (OCH_3), 52.13 (CH_2N), 54.96 ($\underline{C}H_2COO$), 61.60 ($CH_3\underline{C}H_2O$), 73.63 (OCH_2), 157.49 (CONH), 171.87 (COO)
6	In $CDCl_3$, 3.64 (2H, t, J=6.9, CH_2Br), 4.48 (2H, t, J=6.9, OCH_2), 7.88—7.76 (4H, m, C_6H_4)	In $CDCl_3$, 26.68 t (CH_2Br), 76.60 t (OCH_2), 123.71 d, 128.75 s, 134.71 d, (C_6H_4), 163.42 s (CO)
7	In $CDCl_3$, 1.27 (6H, t, J=7.0, $C\underline{H}_3CH_2$), 3.26 (2H, t, J=5.28, $C\underline{H}_2N$), 3.72 (4H, s, CH_2COO), 4.17 (4H, q, J=7.0, $CH_3C\underline{H}_2$), 4.37 (2H, t, J=5.28, OCH_2), 7.85—7.31 (4H, m, C_6H_4)	In $CDCl_3$, 14.25 q ($CH_2\underline{C}H_3$), 52.45 t (CH_2N), 55.45 t, ($\underline{C}H_2COO$), 60.52 t ($\underline{C}H_2CH_3$), 77.22 t (OCH_2), 123.52 d, 128.97 s, 134.50 d (C_6H_4), 163.42 s (CO–N–CO), 171.22 s (COO)
	In DMSO-d_6, 1.21 (6H, t, $C\underline{H}_3CH_2$), 3.12 (2H, t, CH_2N), 3.66 (4H, s, CH_2COO), 4.08 (4H, q, $CH_3C\underline{H}_2$), 4.28 (2H, t, OCH_2), 7.87 (4H, s, C_6H_4)	In DMSO-d_6, 14.08 q ($CH_2\underline{C}H_3$), 51.91 (CH_2N), 54.77 t ($\underline{C}H_2COO$), 59.87 t ($\underline{C}H_2CH_3$), 76.65 t (OCH_2), 123.19 d, 128.64 s, 134.71 d (C_6H_4), 163.18 s (CO–N–CO), 170.79 s (COO)

Coupling constants (J) in Hz.

reduced pressure and 51.04 g of **3** was obtained (yield, 38.5%), bp (0.31 mmHg) 114 °C. The product was irritating to the skin and eyes, but was stable for over a year when kept in an ampule in a freezer. *Rf* values (TLC): 0.65 (AcOEt : benzene = 1 : 1), 0.74 (acetone : $CHCl_3$ = 1 : 9).

Dimethyl 2-*N*-Phthalimidoxyethyliminodiacetate (4) Triethylamine (20 g) was added dropwise to a mixture of **3** (30 g, 0.11 mol) and *N*-hydroxyphthalimide (17.38 g, 0.107 mol) in 165 ml of methanol under heating in a water bath. The mixture was heated at 60—80 °C for an additional 28 h and after cooling, the solvent was evaporated off under reduced pressure. To the residue, 100 ml of acetone was added and the resultant insoluble white powder was removed by filtration. The filtrate was evaporated to dryness and 120 ml of 0.2 N NaOH was added to the residue. The mixture was extracted three times with 100 ml of $CHCl_3$. The combined extract was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography with AcOEt : benzene = 1 : 1 and recrystallized from ether–EtOH. Yield, 16.3 g (43.5%), mp 69.6 °C. IR 1720 cm^{-1} (CO). *Rf* values (TLC): 0.55 (AcOEt : benzene = 1 : 1), 0.63 (acetone : $CHCl_3$ = 1 : 9).

2-Aminooxyethyliminodiacetic Acid (1) A solution of **4** (8 g, 0.023 mol) in 30 ml of 5 N NaOH was refluxed for 5 h. The mixture was cooled to 0 °C, then 100 ml of 0.2 N HCl and 22 ml of concentrated HCl were added dropwise and the resultant white precipitate was collected. The precipitate was suspended in a mixture of 22 ml of concentrated HCl and 150 ml of water and heated at 130 °C for 3 h. After further addition of 40 ml of concentrated HCl, heating was continued for 1 h. The mixture was cooled in a refrigerator overnight and filtered, and the filtrate was evaporated to dryness. The residue was dissolved in 40 ml of cold water and insoluble materials were removed by filtration. The pH of the solution was adjusted to 4.0 by addition of 33% NaOH and the solution was poured into 200 ml of absolute ethanol and cooled. The crystalline precipitate was collected by filtration and dried over P_2O_5 (yield, 4.12 g). The product (3 g) was purified by chromatography on a column of Dowex 50W × 8 cation exchange resin (H form, 100—200 mesh). Elution with 200 ml of water, 0.5, 1 and 3 N HCl afforded 1.65 g of the HCl salt of **1** from the 3 N HCl eluate as white hygroscopic crystals.

Dimethyl 2-(Ethoxycarbonylaminooxy)ethyliminodiacetate (5) An absolute methanol solution of **3** (10 g, 0.037 mol) and ethyl *N*-hydroxycarbamate (4 g, 0.038 mol) was heated at 50 °C. After dropwise addition of triethylamine, the mixture was heated at 60—90 °C for 4 h and allowed to stand at room temperature overnight. The solvent was evaporated off, acetone was added to the residue, and undissolved white solid was removed. After evaporation of the filtrate *in vacuo*, the residue was purified by silica gel chromatography with acetone : $CHCl_3$ = 1 : 9. Yellow oil. Yield 2.83 g (26.2%). *Rf* values (TLC): 0.4 (acetone : $CHCl_3$ = 1 : 9), 0.42 (AcOEt : benzene = 1 : 1).

Hydrolysis of 5 A solution of **5** (1.66 g, 6 mmol) in a mixture of 3 ml each of concentrated HCl, 85% formic acid and water was heated at 120 °C for 1 h. After cooling, the solution was treated as described above and 0.8 g of the HCl salt of **1** was obtained.

***N*-(2-Bromoethoxy)phthalimide (6)** Dimethylformamide (DMF) solutions of 103.7 g of 1,2-dibromoethane and 30 g of *N*-hydroxyphthalimide were mixed and 30 g of triethylamine was added dropwise. The mixture was stirred at room temperature for 30 min, heated stepwise at 65 °C for 30 min, 70 °C for 1 h and 75 °C for 30 min to avoid rapid evaporation of triethylamine, then cooled and filtered. The filtrate was evaporated under reduced pressure and 200 ml of water was added to the residue. Undissolved white precipitate was collected by filtration (yield, 47 g). The product was purified by silica gel chromatography with AcOEt : benzene = 1 : 4 followed by recrystallization from ethanol. Yield, 28.1 g (56.6%), mp 95.9 °C. *Rf* value (TLC): 0.71 (AcOEt : benzene = 1 : 4).

Diethyl 2-*N*-Phthalimidoxyethyliminodiacetate (7) A 100 ml volume of an absolute xylene solution of **6** (10 g, 0.037 mol) and diethyl iminodiacetate (15.4 g, 0.08 mol) was refluxed for 16 h. The precipitate was separated by decantation, then the xylene layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography with AcOEt : benzene = 1 : 4. Pale yellow oil. Yield, 7.5 g (53.6%). *Rf* values (TLC): 0.47 (AcOEt : benzene = 1 : 4), 0.64 (AcOEt : benzene = 1 : 1). Hydrolysis of **7** to form **1** was carried out by an analogous method to that used in the case of **4**.

Oxime of 1 A methanol solution of a carbonyl compound and the HCl salt of **1** was neutralized with NaOH and refluxed for 0.5—1 h. After cooling, the precipitate was collected by filtration and purified by crystallization from an appropriate solvent.

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References and Notes

1) C. H. Chang, C. F. Meares, D. A. Goodwin, *Appl. Nucl. Radiochem.*, **1982**, 103; T. G. Wensel, C. F. Meares, *Radioimmunoimaging Radioimmunother.*, **1983**, 185; C. F. Meares, M. J. McCall, D. T. Reardan, D. A. Goodwin, C. I. Diamanti, M. McTigue, *Anal. Biochem.*, **142**, 68 (1984); C. F. Meares, D. A. Goodwin, *J. Protein Chem.*, **3**, 215 (1984); M. D. Richard, M. L. Thakur, J. Stedman, A. Damjanov, I. Damjanov, *Nucl. Med. Biol.*, **17**, 181 (1990); K. Kina, *Bunseki*, **1991**, 704; M. Studer, C. F. Meares, *Bioconjugate Chem.*, **3**, 420 (1992).
2) G. Schwarzenbach, G. Anderegg, W. Schneider, H. Senn, *Helv. Chim. Acta*, **38**, 1147 (1955).
3) A. F. McKay, G. L. Garmaise, G. Y. Paris, S. Gelblum, *Can. J. Chem.*, **38**, 343 (1960).
4) G. Barger, T. E. Weichselbaum, "Organic Synthesis," Coll. Vol. II, ed. by A. H. Blatt, John Wiley and Sons, Inc., New York, 1943, pp. 384—386.
5) P. E. Gagnon, K. Savard, R. Gaudry, E. M. Richardson, *Can. J. Res.*, **25B**, 28 (1947).
6) R. T. Major, F. Dürch, H-J. Hess, *J. Org. Chem.*, **24**, 431 (1959).
7) L. H. Amundsen, J. J. Sanderson, "Organic Synthesis," Vol. 24, ed. by N. L. Drake, John Wiley and Sons, Inc., New York, 1944, pp. 44—46.