Dehydrooligopeptides. XII.[1] Convenient Synthesis of Various Kinds of *N*-Benzyloxycarbonyl-α-dehydroamino Acid Methyl Esters

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Various *N*-benzyloxycarbonyl-α-dehydroamino acid methyl esters were newly synthesized by the condensation of α-oxocarboxylic acids with benzyl carbamate followed by methyl esterification. Others were obtained by the Wittig–Horner reaction of aldehydes with diethoxyphosphinylglycine and by the base-catalyzed β-elimination of β-acetoxy or β-halo-α-amino acids.

Keywords synthesis; α-dehydroamino acid; condensation reaction; Wittig–Horner reaction; β-elimination

In the course of our work on the synthesis of α-dehydroamino acids (DHA; ⊿AA) and dehydrooligopeptides, various kinds of DHA derivatives, which correspond to the proteinic α-amino acids (AA), have so far been synthesized.[1-5] However, a general synthetic method for DHA derivatives has not yet been developed. In order to examine thoroughly the novel enzymatic hydrolysis and peptide bond formation of DHA esters, which we used to achieve the highly selective enzymatic hydrolysis of methyl γ-methyl-α-dehydroglutamate for the first time,[6,7] we required various new DHA esters as substrates.

In this paper, we wish to report the convenient synthesis of *N*-benzyloxycarbonyl (Cbz)-DHA methyl esters (**1**), whose side chain functional groups of $-N^{in}$, $-NH_2$, $-CONH_2$, and –COOH were protected with suitable protecting groups. The syntheses of **1** were substantially accomplished by the following three methods; *i. e.*, the condensation of 2-oxoalkanoic acids with benzyl carbamate using $POCl_3$, followed by methyl esterification (method A), the Wittig–Horner reaction of aldehydes with *N*-Cbz-(diethoxyphosphinyl)glycine methyl ester (**3**)[8] using *tert*-BuOK (method B), and the base-catalyzed β-elimination of 3-halo- or 3-acyloxy-2-(*N*-Cbz)-aminoalkanoic acid methyl esters (method C), according to Chart 1. In the cases of α-dehydroasparagine (⊿Asn) and α-dehydrotryptophan (⊿Trp) derivatives, the synthetic methods were slight modifications of methods C and B, respectively.

The β-amidation of (*Z*)-Cbz-α-dehydroaspartic acid methyl ester [(Z)-Cbz–⊿Asp–OMe; **1a**], which was derived from 2-(*N*-Cbz)-3-hydroxyaspartic acid in three steps according to method C,[2] was performed by the active esterification of the 3-carboxyl group with *N*-hydroxysuccinimide (HOSu) in the presence of dicyclohexylcarbodiimide (DCC), followed by treatment of the obtained (*Z*)-Cbz–⊿Asp(OSu)–OMe (**4**) with NH_3 or *tert*-$BuNH_2$. The expected dehydroasparagine derivatives, Cbz–⊿Asn(R)–OMe [R = H (**1d**) and R = *tert*-Bu (**1e**)], were obtained in 75% yield (Chart 2). Similarly, α-dehydroglutamine derivatives [Cbz–⊿Gln(R)–OMe; R = H (**1f**) and R = *tert*-Bu (**1g**)] were synthesized from Cbz–⊿Glu–OMe (**1h**) *via* (*Z*)-Cbz–⊿Glu(OSu)–OMe (**5**), although the synthesis of other α-dehydroglutamine derivatives with different protecting groups has already been achieved by the direct γ-amidation of α-dehydroglutamic acids with amines.[5]

On the other hand, as illustrated in Chart 2, N^{α}-Cbz-α-dehydrotryptophan methyl esters [Cbz–⊿Trp (R)–OMe; R = H (**1p**), R = Cbz (**1q**), and R = *tert*-butoxycarbonyl (Boc) (**1r**)] were also synthesized by the condensation of **3** with *N*-substituted indole-3-carboxaldehydes, which were derived by the acylation of indole-3-carboxaldehyde with benzyloxycarbonyl chloride (Cbz–Cl) and di-*tert*-butyl dicarbonate [$((CH_3)_3COCO)_2O$; $(Boc)_2O$], respectively, according to method B. A report on the synthesis of Boc–⊿Trp–OMe by the reduction of methyl 2-nitro-3-indoleacrylate has also recently appeared.[9]

Furthermore, besides ⊿Asn, ⊿Gln, and ⊿Trp derivatives, other previously unknown Cbz–⊿AA–OMe, *i.e.*, 2-amino-2-butenoic acid [⊿Abu (**1b**)], ⊿Ala (**1c**), ⊿Ile (**1j**), ⊿Nva (**11**), ⊿Lys (Boc) (**1k**), ⊿Orn (Boc) (**1m**), ⊿Phe (**1n**), and ⊿Val (**1s**) derivatives were newly synthesized, according to the appropriate method described above (Chart 1). A few of these DHAs have already been prepared as the α-free acid (Cbz–⊿AA– OH; **2**) by the condensation of 2-oxoalkanoic acids with benzyl carbamate or by the hydrolysis of the corresponding ⊿AA esters except for the methyl ester.[10,11]

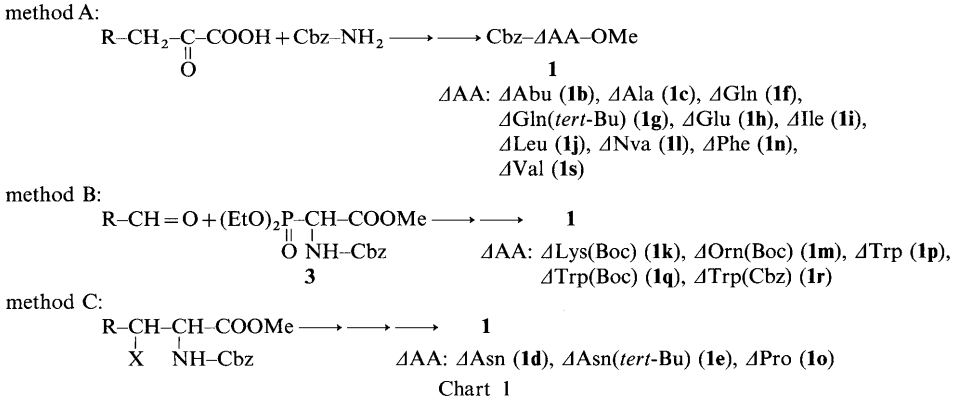

Chart 1

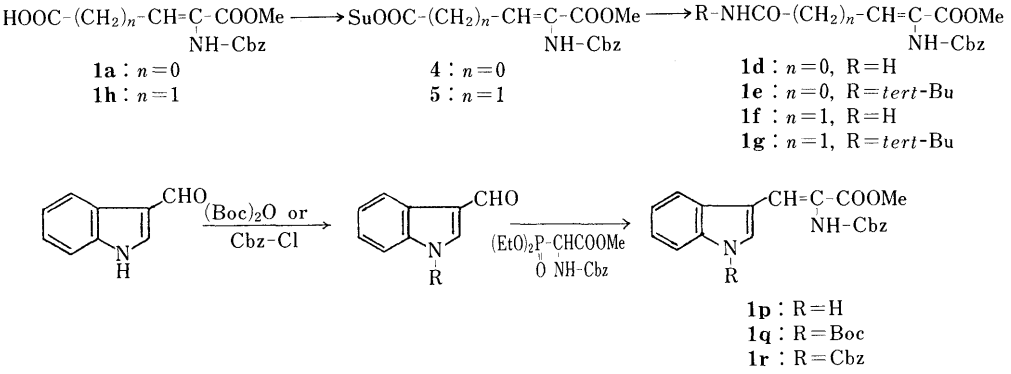

Chart 2

TABLE I. Yields, Melting Points, and Spectral Data (IR and ^{1}H-NMR) of **1**

Compd. No.	Synthetic method	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	IR ν (KBr) cm^{-1} C=C	^{1}H-NMR δ (CDCl$_3$, J, Hz) –CH=	^{1}H-NMR δ (CDCl$_3$, J, Hz) –NH– (br s)
1b	A	80	66—67	$C_{13}H_{15}NO_4$	62.64	6.07	5.62	62.86	6.07	5.53	1660	6.63, q (7.0, 14.0)	6.26
1c	A	91	73—74	$C_{12}H_{13}NO_4$	61,27	5.57	5.96	61.41	5.57	6.01	1665	7.26, m (+Ph)	6.98
1d	C	77	131—132	$C_{13}H_{14}N_2O_5$	56.11	5.07	10.07	56.37	4.85	10.31	1630	5.64, s	7.79, 10.58
1e	C	73	Syrup	$C_{17}H_{22}N_2O_5$	61.06	6.63	8.38	61.01	6.53	8.29	1655	5.31, s	5.73, 10.41
1f	A	57	159—160	$C_{14}H_{16}N_2O_5$	57.53	5.52	9.59	57.34	5.72	9.48	1630	6.60, t (7.0)	6.99,[a] 8.87
1g	A	94	Syrup	$C_{18}H_{24}N_2O_5$	62.05	6.94	8.04	62.21	6.77	7.98	1645	6.69, t (7.0)	6.28, 6.96
1h	A	54	96—98	$C_{14}H_{15}NO_6$	57.33	5.16	4.78	57.15	5.15	4.96	1650	6.71, t (7.0)	6.30
1i	A	91	Syrup	$C_{15}H_{19}NO_4$	64.96	6.91	5.05	65.09	6.79	5.11	1640	—	6.14
1j	A	93	60—63	$C_{15}H_{19}NO_4$	64.96	6.91	5.05	64.67	6.78	5.00	1660	6.39, d (10.0)	6.40
1k	B	70	75—76	$C_{20}H_{28}N_2O_6$	61.21	7.19	7.14	61.19	6.98	7.42	1660	6.48, t (8.0)	4.92, 6.68
1l[8]	A	81	Syrup	$C_{14}H_{17}NO_4$	63.86	6.51	5.32	63.72	6.66	5.29	1660	6.69, t (7.0)	6.56
1m	B	82	90—91	$C_{19}H_{26}N_2O_6$	60.30	6.93	7.40	60.36	7.13	7.25	1665	6.56, t (8.0)	4.90, 6.48
1n	A	91	Syrup	$C_{18}H_{17}NO_4$	69.44	5.50	4.50	69.52	5.39	4.59	1645	7.33, m (+Ph)	6.49
1o	C	79	Syrup	$C_{14}H_{15}NO_4$	64.36	5.79	5.36	64.58	5.47	5.44	1620	5.75, t (3.0)	—
1p	B	66	191—192	$C_{20}H_{18}N_2O_4$	68.56	5.18	8.00	68.60	5.06	7.71	1630	7.92, m (+Ph)	8.75,[a] 11.61
1q[8]	B	80	98—99	$C_{25}H_{26}N_2O_6$	66.65	5.82	6.22	66.42	5.62	6.12	1640	7.17, m (+Ph)	6.42
1r	B	55	121—122	$C_{28}H_{24}N_2O_6$	69.41	4.99	5.78	69.24	4.92	5.82	1640	7.21, m (+Ph)	6.56
1s	A	91	49—50	$C_{14}H_{17}NO_4$	63.86	6.51	5.32	63.57	6.53	5.31	1645	—	6.18

a) Measured in DMSO-d_6.

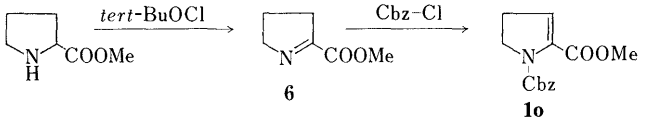

Chart 3

Subsequently, the desired **1** was readily obtained by the new esterification of **2** with MeOH in the presence of DCC and dimethylaminopyridine (DMAP). Cbz–ΔPro–OMe (**1o**) was also obtained by the addition of Cbz–Cl to 1,2-didehydroproline methyl ester (**6**), which was derived from H–Pro–OMe and *tert*-BuOCl,[12] followed by treatment with 1,8-diazabicyclo[5.4.0]undec-5-ene (DBU), according to Chart 3.

The yields, physical constants, and spectral data (infrared and proton nuclear magnetic resonance (IR and ^{1}H-NMR)) of the newly obtained Cbz-ΔAA-OMe (**1**) are summarized in Table I. The yields of **1** were found to reach *ca.* 78% and the structures of all the products could be confirmed by the spectral data and satisfactory elemental analyses.

In the IR spectrum of **1**, the characteristic absorptions of NH and C=C functions appear at 3200—3370 and 1630—1660 cm^{-1}, respectively. Furthermore, in the ^{1}H-NMR spectra, all the olefin protons of **1** resonate in the δ 7.92—5.31 region with various splitting patterns. The ^{1}H-NMR spectral data allowed the configurations of **1** to be tentatively assigned as (*Z*).

Experimental

Melting points were determined with a Yamato Mp-21 micro melting-point apparatus, and are uncorrected. The IR spectra were recorded with a Hitachi EPI-G2 spectrometer. The ^{1}H-NMR spectra were measured with a JEOL LMN PS-100 spectrometer in CDCl$_3$ or dimethyl sulfoxide-d_6 (DMSO-d_6) solution with tetramethylsilane as the internal standard.

Cbz–ΔAA–OMe (1b, 1c, 1i, 1j, 1l, 1n, and 1s) A solution of an appropriate 2-oxoalkanoic acid (RCH$_2$COCOOH: **b**; R=CH$_3$, **c**; R=H, **i**; R=CH$_2$CH$_3$, CH$_3$, **j**; R=CH (CH$_3$)$_2$, **l**; R=CH$_2$CH$_3$, **n**; R=C$_6$H$_5$, **s**; R=CH$_3$, CH$_3$) (50 mmol) and benzyl carbamate (50 mmol) in the presence of *p*-toluenesulfonic acid (2 g) in dry benzene (70 ml) was refluxed for 4 h, then allowed to stand at room temperature. The collected crystals were recrystallized from CHCl$_3$ or a mixture of cyclohexane and benzene to give **2** as colorless needles. Subsequently, MeOH (3 ml), DCC (70 mmol), and DMAP (6.5 mmol), were added successively to a solution of **2** in tetrahydrofuran (THF) (15 ml) with stirring at −10 °C. The reaction solution was stirred for 30 min at the same temperature, then for 24 h at room temperature. After removal of the solvent, the residue was dissolved in ethyl acetate (10 ml). The DCC urea was allowed to separate out

below −10 °C and filtered off. The filtrate was made up to 150 ml by adding ethyl acetate and washed successively with 10% citric acid, saturated $NaHCO_3$ aqueous solution, saturated NaCl aqueous solution, and then water, and finally dried over anhydrous Na_2SO_4. Concentration under reduced pressure gave colorless crystals or a syrup, which was recrystallized from a mixture of ethyl acetate and hexane or purified on a silica gel column using a mixture of $CHCl_3$ and acetone (20 : 1, v/v) as the eluent to give **1b**, **1c**, **1j**, or **1s** as colorless needles and **1i**, **1l**, or **1n** as a colorless syrup, respectively.

Cbz–⊿Asp(OSu)–OMe (4) DCC (11.8 mmol) was added to a solution of **1a**[2] (9.9 mmol) and HOSu (11.8 mmol) in 1,2-dimethoxyethane (DME) (30 ml) with stirring at −10 °C. Stirring was continued for 1 h, then the reaction solution was returned to room temperature for 24 h. Concentration of the solution under reduced pressure gave a residue, which was dissolved in ethyl acetate (15 ml) and then chilled to 0 °C. The DCC urea that separated out was filtered off and the filtrate was made up 200 ml by adding ethyl acetate. The resulting solution was washed successively with 1 M HCl, saturated $NaHCO_3$ aqueous solution, saturated NaCl aqueous solution, and then water and finally dried over anhydrous Na_2SO_4. Evaporation of the ethyl acetate gave crude colorless crystals (**4**), which were then subjected to the next reaction. Yield *ca.* 65%, mp 113—116 °C. ^{1}H-NMR δ: 9.15 (br s, 1H, NH), 5.52 (s, 1H, –CH=). IR ν_{max}^{KBr} cm^{-1}: 1625 (C=C), 1744 (C=O), 3315 (NH).

Cbz–⊿Glu(OSu)–OMe (5) Similarly, the reaction mixture of **1h** (12 mmol) with HOSu (14 mmol) in THF (30 ml) in the presence of DCC (14 mmol) was worked up to give **5** as a yellowish syrup almost quantitatively. ^{1}H-NMR δ: 7.34 (br s, 1H, NH), 6.75 (t, 1H, J=7.0 Hz, –CH=), 3.67 (d, 2H, 4-H).

Cbz–⊿Asn–OMe (1d) Aqueous NH_3 (28%, 1.3 ml) was slowly added to a solution of **4** (6.3 mmol) in THF (16 ml) with stirring at 0 °C. The resulting solution was returned to room temperature and then stirred continuously for 2 h. Concentration of the reaction solution under reduced pressure gave a residue, which was dissolved in ethyl acetate (150 ml). The resulting solution was washed with saturated NaCl aqueous solution and then dried over anhydrous Na_2SO_4. Concentration of this solution under reduced pressure gave crude crystals, which were recrystallized from a mixture of ethyl acetate and benzene to give **1d** as colorless needles.

Cbz–⊿Gln–OMe (1f) Similarly, the treatment of **5** (15 mmol) with 28% aqueous NH_3 (2.5 ml) in THF (30 ml) for 1 h gave **1f** as colorless needles.

Cbz–⊿Asn(*tert*-Bu)–OMe (1e) *tert*-$BuNH_2$ (0.2 ml) was added dropwise to a solution of **4** (1.4 mmol) in DME (5 ml) with stirring at 0 °C. The reaction solution was returned to room temperature and stirred continuously for 5 h. After removal of the DME under reduced pressure, the residue was dissolved in ethyl acetate (50 ml) and the resulting solution was washed with saturated NaCl aqueous solution and then dried over anhydrous Na_2SO_4. Concentration of the solution under reduced pressure gave a crude syrup, which was purified on a silica gel column using a mixture of $CHCl_3$ and acetone (20 : 1, v/v) as the eluent to give **1e** as a yellowish syrup.

Cbz–⊿Gln (*tert*-Bu)–OMe (1g) Similarly, the treatment of **5** (34 mmol) with *tert*-$BuNH_2$ (41 mmol) for 1.5 h gave **1g** as a yellowish syrup.

Cbz–⊿Glu–OMe (1h) An aqueous solution of LiOH (3.3 mmol) in water (50 ml) was added dropwise to a solution of Cbz–⊿Glu(OMe)–OMe[3] (3.3 mmol) in dioxane (100 ml) with stirring, under cooling. Stirring was continued for 3 h, the solvent was removed *in vacuo*, and the residue was dissolved in saturated $NaHCO_3$ aqueous solution. The resulting solution was washed once with ethyl acetate. The aqueous layer was acidified to pH 2 with concentrated hydrochloric acid and then extracted three times with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4 and finally concentrated under reduced pressure. The crystalline residue thus obtained was recrystallized from benzene to give **1h** as colorless needles.

1-Boc-indole-3-carboxaldehyde A 1 M NaOH solution (21 ml) was added to a solution of indole-3-carboxaldehyde (21 mmol) in a mixture of dioxane (63 ml) and H_2O (21 ml) with stirring at 0 °C for 20 min, and then $(Boc)_2O$ (25 mmol) was added. The resulting solution was gradually brought to room temperature and then stirred continuously for 5 d. More H_2O (100 ml) was added and the whole was extracted twice with ethyl acetate. The combined extracts were washed with saturated NaCl aqueous solution and then dried over anhydrous Na_2SO_4. Concentration of the solution under reduced pressure gave crude crystals, which were recrystallized from diisopropyl ether to give colorless needles identified as 1-Boc-indole-3-carboxaldehyde. Yield 67%, mp 123—124 °C (lit.[14] mp 124.5—125.5 °C). *Anal.* Calcd for $C_{14}H_{15}NO_3$: C, 68.55; H, 6.08; N, 5.82. Found: C, 68.67; H, 6.16; N, 5.71.

1-Cbz-indole-3-carboxaldehyde Triethylamine (6 ml) was added dropwise to a solution of indole-3-carboxaldehyde (21 mmol) in $CHCl_3$ (50 ml) with stirring at 0 °C for 20 min and then Cbz-Cl (32 mmol) was added. Thre resulting solution was brought to room temperature and stirred continuously for 24 h. More $CHCl_3$ (50 ml) was added to the reaction solution, which was then washed with water and finally dried over anhydrous Na_2SO_4. Concentration of the solution gave a reddish syrup. Yield 95%. The product thus obtained was used in the following condensation without purification.

Cbz–⊿Trp(Cbz)–OMe (1r) A solution of **3** (11 mmol) in CH_2Cl_2 (20 ml) was added dropwise to a solution of *tert*-BuOK (11 mmol) in CH_2Cl_2 (5 ml) with stirring at −70 °C. After the *tert*-BuOK had dissolved completely, a solution of 1-Cbz-indole-3-carboxaldehyde (11 mmol) in CH_2Cl_2 (5 ml) was added to the above prepared solution and the resulting solution was gradually brought to room temperature. Stirring was continued for 3 h, then the reaction solution was concentrated to give a crude residue, which was dissolved in ethyl acetate (100 ml). The resulting solution was washed with saturated NaCl aqueous solution and finally dried over anhydrous Na_2SO_4. Concentration of the solution under reduced pressure gave a reddish syrup, which was purified on a silica gel column using a mixture of $CHCl_3$ and acetone (20 : 1, v/v) as the eluent to give colorless crystals. Recrystallization from a mixture of ethyl acetate and hexane gave **1r** as colorless prisms.

Cbz–⊿Trp(Boc)–OMe (1q) In a similar manner, the condensation of an equimolar 1-Boc-indole-3-carboxaldehyde (11 mmol) with **3** gave **1q**[8] as colorless prisms.

Cbz–⊿Trp–OMe (1p) A 1 M LiOH solution (2.7 ml) was added dropwise to a solution of **1q** (2.1 mmol) in MeOH (15 ml) with stirring at 0 °C. The resulting solution was brought to room temperature and then stirred continously overnight. A saturated $NaHCO_3$ aqueous solution (15 ml) was added, and the resulting solution was extracted three times with diethyl ether (100 ml). The combined extracts were washed with saturated NaCl aqueous solution and then dried over anhydrous Na_2SO_4. Concentration of the solution under reduced pressure gave crude crystals, which were recrystallized from a mixture of ethyl acetate and hexane to give **1p** as colorless needles.

Cbz–⊿Orn(Boc)–OMe (1m) As in the case of **1c**, the condensation of equimolar 3-(*N*-Boc)amino-2-propanal[4] (1.6 mmol) with **3** at −60 °C for 30 min and then at room temeperature gave a syrup, which was purified on a silica gel column using a mixture of benzene and acetone (10 : 1, v/v) to give crystals, which were recrystallized from a mixture of ethyl acetate and hexane to give **1m** as colorless needles.

Cbz–⊿Lys(Boc)–OMe (1k) Similarly, the condensation of equimolar 4-(*N*-Boc)amino-2-butanal[4] (1.3 mmol) with **3** gave a syrup, which was purified on a silica gel column using a mixture of benzene and acetone (10 : 1, v/v) as the eluent to give colorless crystals. Recrystallization from a mixture of ethyl acetate and hexane gave **1k** as colorless needles.

1,2-Didehydroproline Methyl Ester (6) Triethylamine (72 mmol) was added to a solution of H–Pro–OH·HCl (60 mmol) in diethyl ether (100 ml) with stirring at room temperature. Stirring was continued for 2h, then the Et_3N·HCl salt deposited was filtered off and the filtrate was cooled at 0 °C. *tert*-BuOCl (30 mmol) was added dropwise to the resulting solution and then DBU (30 mmol) was added at 0 °C over 40 min. The reaction solution was brought to room temperature and stirred for a further 40 min. After removal of the insoluble meterial, the filtrate was concentrated under reduced pressure. The obtained oily residue was purified on a silica gel column using a mixture of ethyl acetate and hexane (1 : 1, v/v) as the eluent to give **6** as a yellow syrup. Yield 65%. ^{1}H-NMR δ: 3.87 (s, 3H, $COOCH_3$). IR ν_{max}^{KBr} cm^{-1}: 1630 (C=N), 1720 ($COOCH_3$). The product **6** was used in the following addition-elimination reaction without further purification.

Cbz–⊿Pro–OMe (1o) Cbz–Cl (24 mmol) was added to a solution of **6** (16 mmol) in $CHCl_3$ (20 ml) with stirring at 0 °C. The resulting solution was slowly brought to room temperature and then stirred for 2h. The prepared solution was again cooled to 0 °C and treated with DBU (24 mmol). The resulting solution was brought to room temperature, stirred continuously overnight, then washed successively with 1 M HCl and water, and dried over anhydrous Na_2SO_4. Concentration of the solution under reduced pressure gave a syrup, which was purified on a silica gel column using $CHCl_3$ to give **1o** as a yellowish syrup.

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