Dehydrooligopeptides. XIV. Convenient Coupling of *N*-Carboxy-α-dehydroamino Acid Anhydride with Both Amine and Carboxyl Components[1)]

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Synopsis. In order to further develop and expand the usefulness of *N*-carboxy-α-dehydroamino acid anhydride (ΔNCA), the optimum conditions of the acylation of ΔNCA with *N*-protected α-amino acids (AA) were thoroughly examined. Furthermore, various kinds of AA or dipeptides as a C-component were utilized in the acylation of ΔNCA, followed by condensation with an AA methyl ester as an *N*-component.

In connection with the application of *N*-carboxy-α-dehydroamino acid anhydride (ΔNCA; **1**),[2)] we have already reported the syntheses of various kinds of dehydrooligopeptides (DHP).[3—7)] At present, however, the isolation of *N*-protected AA–ΔNCA (**2**) from **1** and *N*-protected AA–OH (AA=α-amino acids) has been unsuccessful. Here, we wish to report the isolation of **2** in a pure form and new one-pot syntheses of a few DHP by the fragment condensation of **1** with both amine (N) and carboxyl (C) components.

Results and Discussion

In order to obtain **2** in a pure form by the acylation of **1** with *t*-butoxycarbonyl (Boc)–AA–OH, various procedures were examined extensively. Besides the condensing agent, the combination of the organic solvent and base were altered variously, as shown in Table 1. As a result, the acylation of equimolar *N*-carboxy-dehydrophenylalanine anhydride (ΔPhe·NCA; **1b**) with Boc–Phe–OH in CH_2Cl_2 in the presence of dicyclohexylcarbodiimide (DCC) and an organic base, such as pyridine, proceeded smoothly to give the expected Boc–Phe–ΔPhe·NCA (**2k**). In particular, as Tables 1 and 2 show, when **1b** and pyridine were used in a 2:1 ratio, the yield and the specific rotation of **2k** were found to be the highest (81% and −67.5°, respectively).

Table 1. Effect of the Catalyst and Solvent on the Yield of Boc–Phe–ΔPhe·NCA (**2k**)

Catalyst	Solvent	Time/h[a)]	Yield/%
Et_3N	CH_2Cl_2	24	18.4
NMM[b)]	CH_2Cl_2	24	31.3
DMAP[c)]	CH_2Cl_2	10	67.6
Pyridine	CH_2Cl_2	10	81.5
Pyridine	THF[d)]	12	62.3

a) At room temperature. b) *N*-Methylmorpholine. c) 4-Dimethylaminopyridine. d) Tetrahydrofuran.

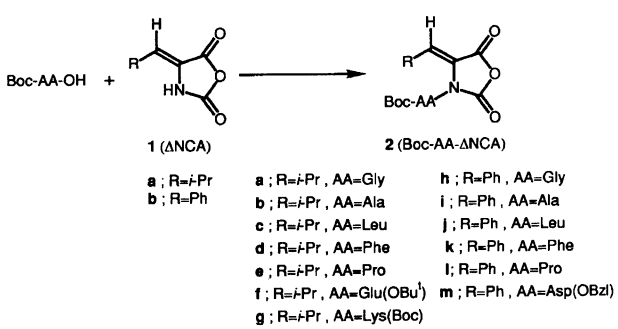
Table 2. Effect of the Molar Ratio of Pyridine to **1b** on the Specific Rotation of **2k**

Ratio of pyridine to **1b** (mol/mol)	$[\alpha]_D^{25}$/° (*c* 1.0, MeOH)
5.0	−57.2
3.0	−61.8
1.0	−64.6
0.5	−67.5
0.25	−64.0

Furthermore, in the case of *N*-carboxy-dehydroleucine anhydride (ΔLeu·NCA; **1a**) and pyridine in a 1:1 ratio, it was found that the similar condensation of **1a** with Boc–Phe–OH also proceeded to give Boc–Phe–ΔLeu·NCA (**2d**) in the highest yield of 85.5%, according to Scheme 1. Consequently, similar *N*-acylations of **1a** and **1b** with various kinds of Boc–AA–OH were worked up to give the expected Boc–AA–ΔPhe·NCA (**2a**—**g**) as colorless needles and Boc–AA–ΔLeu·NCA (**2h**—**m**) as colorless syrups in 85 and 78% yields, respectively.

The yields, physical constants, and spectral data (IR and ^{1}H NMR) of **2a**—**g** and **2h**—**m** are summarized in Tables 3 and 4 respectively.

The structures of all of the new compounds **2** thus obtained were determined spectroscopically and gave satisfactory results in elemental analyses. In particular, in the IR spectra of **2**, the appearance of the characteristic strong absorption band in the 1803—1827 cm^{-1} region due to the cyclic acid anhydride (–CO–O–CO–) clearly showed the maintenance of the ΔNCA ring system during the *N*-acylation. Furthermore, in the ^{1}H NMR spectra, the signals of the olefinic protons of **2a**—**g** appeared in the δ=6.68—6.15 region as a doublet



Scheme 1.

Table 3. The Yields and Melting Points of **2a**—**m**

Comd No.	Yield %	Mp[a] θ_m/°C	Formula	Found (Calcd)/% C	H	N
2a	79	Syrup	$C_{14}H_{20}N_2O_6$	53.77 (53.84)	6.37 6.45	9.02 8.97)
2b	92	Syrup	$C_{15}H_{22}N_2O_6$	55.53 (55.20)	6.72 6.80	8.69 8.58)
2c	93	Syrup	$C_{18}H_{28}N_2O_6$	58.79 (58.68)	7.45 7.66	7.56 7.60)
2d	86	Syrup	$C_{21}H_{26}N_2O_6$	62.89 (62.67)	6.32 6.51	7.11 6.96)
2e	94	Syrup	$C_{17}H_{24}N_2O_6$	58.12 (57.94)	6.67 6.87	8.07 7.95)
2f	77	Syrup	$C_{21}H_{32}N_2O_8$	57.41 (57.26)	7.03 7.32	6.51 6.36)
2g	71	Syrup	$C_{23}H_{37}N_3O_8$	57.39 (57.13)	7.54 7.71	8.79 8.69)
2h	79	121—122	$C_{17}H_{18}N_2O_6$	59.11 (58.95)	5.17 5.24	8.18 8.09)
2i	81	104—105	$C_{18}H_{20}N_2O_6$	60.07 (59.99)	5.60 5.77	7.81 7.77)
2j	80	127—128	$C_{21}H_{26}N_2O_6$	62.88 (62.67)	6.35 6.51	7.08 6.96)
2k	84	136—137	$C_{24}H_{24}N_2O_6$	66.21 (66.04)	5.32 5.54	6.66 6.42)
2l	75	90—91	$C_{20}H_{22}N_2O_6$	62.37 (62.16)	5.54 5.74	7.34 7.25)
2m	73	114—116	$C_{26}H_{26}N_2O_8$	63.32 (63.15)	5.41 5.30	5.77 5.67)

a) Colorless viscous syrups and colorless needles from hexane–ethyl acetate.

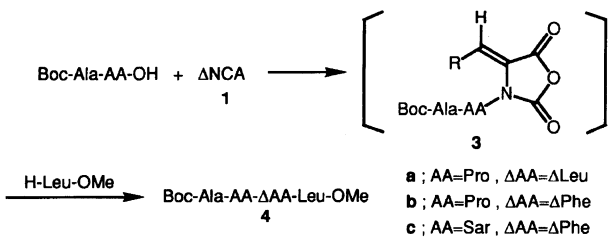
Table 4. The IR, ^{1}H NMR, and Specific Rotations of **2a**—**m**

Compd No.	IR, ν/cm^{-1} in KBr O=C–O	NHCO	^{1}H NMR, δ[a] –CH= (J/Hz)	$[\alpha]_D^{25}$/° (c 1.0, MeOH)
2a	1815	1716 1515	6.68d (10.0)	—
2b	1812	1707 1518	6.15d (10.0)	−37.7
2c	1812	1716 1515	6.50d (10.0)	−30.4
2d	1809	1713 1503	6.40d (10.0)	−15.1
2e	1812	1707	6.44d (10.0)	−34.0
2f	1812	1725 1518	6.44d (10.0)	−27.7
2g	1812	1707 1524	6.44d (10.0)	−15.2
2h	1803	1683 1521	7.18s	—
2i	1803	1686 1521	7.13s	−130.1
2j	1803	1680 1518	7.16s	−91.4
2k	1806	1695 1525	7.1–7.9m	−67.2
2l	1810	1692	7.15s	−67.2
2m	1827	1722 1521	7.14s	−27.3

a) Measured in $CDCl_3$.

(J=10.0 Hz) and those of **2h**—**m** in the δ=7.50—7.13 region as a singlet. Based on the ^{1}H NMR data, the Z-configurational structures were also found to remain during the N-acylation.[2)]

In addition, the C-component Boc–alanylproline (Ala–Pro) was chosen for the similar N-acylation of **1**, according to Scheme 2. Although the isolation of Boc–Ala–Pro–ΔLeu·NCA (**3a**) and –ΔPhe·NCA (**3b**) in a pure form was unsuccessful, the desired Δ^3-dehydrotetrapeptides,[8,9)] Boc–Ala–Pro–ΔLeu–Leu–OMe (**4a**) and Boc–Ala–Pro–ΔPhe–Leu–OMe (**4b**), were obtained in 25.3 and 39.0% yields, respectively, by immediate treatment of **3a** and **3b** with H–Leu–OMe as the N-component. Thus, the one-pot syntheses of **4** were successful, although the yields were considerably low. In order to increase the yield of **4**, suitable combinations of



Scheme 2.

the basic catalyst and the solvent, and optimum ratios of the amount of the catalyst to the substrate **1** were studied under various conditions. It was found that 4-dimethylaminopyridine (DMAP) was superior to pyridine and the optimum molar ratio of DMAP to **1** was 1 : 1. Consequently, the compounds **4a** and **4b** were obtained in the highest yields of 68 and 78%, respectively (Table 5).

Furthermore, in place of Boc–Ala–Pro–OH, a similar work up of **1b** with consecutive Boc–alanylsarcocine (Ala–Sar) and H–Leu–OMe gave Boc–Ala–Sar–ΔPhe–Leu–OMe (**4c**) in a 79% yield.

Experimental

Melting points were determined with a Yamato (Model Mp-21) micro melting-point apparatus, and were not corrected. IR spectra were recorded with a Hitachi EPI-G2 grating spectrometer. ^{1}H NMR spectra were measured with a JEOL JMN PS-100 spectrometer in a $CDCl_3$ solution with tetramethylsilane as the internal standard. The specific rotations were measured in a 0.5-dm tube using a JASCO DIP-4 polarimeter (Japan Spectroscopic Co., Ltd.).

Boc–AA–ΔNCA (2). To a solution of an appropriate Boc–AA–OH (6.5 mmol) in CH_2Cl_2 (30 ml) was added DCC (7.7 mmol), with stirring, at −10 °C for 20 min followed by addition of **1** (6.5 mmol) and pyridine (7.2 mmol). After stirring for 1 h at −10 °C and at room temperature for 12

Table 5. The Yields and Physical Constants of **4a—c**

Compd No.	Yield %	Mp[a] θ_m/°C	Formula	Found (Calcd)/% C	H	N	$[\alpha]_D^{25}$/° (c 1.0)[b]
4a	68	167—168	$C_{26}H_{44}N_4O_7$	59.14 (59.52	8.24 8.45	10.33 10.68)	−25.3
4b	78	203—204	$C_{29}H_{42}N_4O_7$	62.78 (62.34	7.72 7.58	10.28 10.03)	−89.1
4c	79	108—109	$C_{27}H_{40}N_4O_7$	60.45 (60.88	7.47 7.57	10.37 10.52)	−21.6

a) Colorless needles from hexane–ethyl acetate. b) Measured in MeOH.

h, the solution was concentrated in vacuo. The residue was dissolved in a small amount of ethyl acetate and the precipitates were filtered off. The filtrate was diluted with ethyl acetate (300 ml), washed successively with 10% citric acid and brine, and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave crystals of syrups, which were purified by recrystallization from hexane–ethyl acetate or on a silica-gel column using benzene–acetone (4:1 v/v) as the eluent to give **2** as colorless needles or syrups (Tables 3 and 4).

Boc–Ala–Pro–OH. To a solution of HCl·H–Pro–OMe (58 mmol) in CH_2Cl_2 (100 ml) was added Et_3N (58 mmol), with stirring, below 0 °C for 10 min. Boc–Ala–OH (53 mmol) and WSC·HCl (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride) (58 mmol) were added, with stirring, to the solution prepared above over 1 h. The solution was then stirred at room temperature for 12 h. After concentrating in vacuo, the residue was dissolved in ethyl acetate (300 ml) and washed successively with water, twice with 1 M HCl (140 ml) (1 M=1 mol dm^{-3}), water, saturated $NaHCO_3$ aqueous solution (70 ml), and brine (70 ml), and finally dried over anhydrous Na_2SO_4. After removal of the solvent in vacuo, crude Boc–Ala–Pro–OMe was obtained in an ca. 80% yield. Subsequently, to a solution of the obtained dipeptide ester (33 mmol) in MeOH (100 ml) was added 1 M NaOH (36 ml) under cooling and the resulting solution was stirred at room temperature for 2 h. After concentrating, the residue was dissolved in water (300 ml) and the aqueous solution was washed with ethyl acetate (100 ml) and then made acidic to pH 2 with 6 M HCl and extracted three times with ethyl acetate (100 ml). The combined extracts were washed with brine and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave crystals, which were recrystallized from hexane–ethyl acetate to give Boc–Ala–Pro–OH as colorless needles. Yield 67%, mp 147—148 °C. $[\alpha]_D^{25}$ −96.3° (c 1.0, MeOH). Found: C, 54.84; H, 7.84; N, 9.48%. Calcd for $C_{13}H_{22}N_2O_5$: C, 54.53; H, 7.75; N, 9.78%.

Boc–Ala–Sar–OH. In a similar manner, the coupling of Boc–Ala–OH with H–Sar–OMe and then ester hydrolysis of Boc–Ala–Sar–OMe were carried out to give a syrup, which was purified on a silica-gel column using $CHCl_3$–MeOH (10:1 v/v) as the eluent to give Boc–Ala–Sar–OH as a colorless viscous syrup. Yield 80%. $[\alpha]_D^{25}$ −33.8° (c 0.9, MeOH). Found: C, 50.87; H, 7.67; N, 10.66%. Calcd for $C_{11}H_{20}N_2O_5$: C, 50.76; H, 7.75; N, 10.76%.

Boc–Ala–AA–ΔLeu–Leu–OMe (4a—c). After adding DCC (4.2 mmol) to a solution of Boc–Ala–AA–OH (3.5 mmol) in CH_2Cl_2 (10 ml), with stirring, at 0 °C for 30 min, the resulting solution was further treated with an equimolar amount of **1b** (3.4 mmol) and DMAP for 1 h and at room temperature for 14 h. The solution was then treated with a solution of H–Leu–OMe (3.9 mmol) in CH_2Cl_2 (10 ml) and the pH was adjusted to 8 with *N*-methylmorpholine (NMM), with stirring, for 12 h. After concentrationg in vacuo, the residue was dissolved in a small amount of ethyl acetate. After stirring at −10 °C for 30 min and removing the insoluble material, the filtrate was diluted with ethyl acetate (300 ml) and washed successively three times with 10% citric acid (30 ml), brine (10 ml), and saturated $NaHCO_3$ aqueous solution (10 ml), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave crystals, which were recrystallized from hexane–ethyl acetate to give **4a—c** as colorless needles (Table 5).

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9) In this paper, the symbol Δ^3 indicates the position number of the double bond of the DHA residue from the *N*-terminus in sequence.