Dehydrooligopeptides. IX. Syntheses and Conversions of α-Dehydroglutamine Derivatives to *N*-Carboxy-α-dehydroglutamine Anhydride and Δ^1-Dehydroglutaminyldipeptides[1)]

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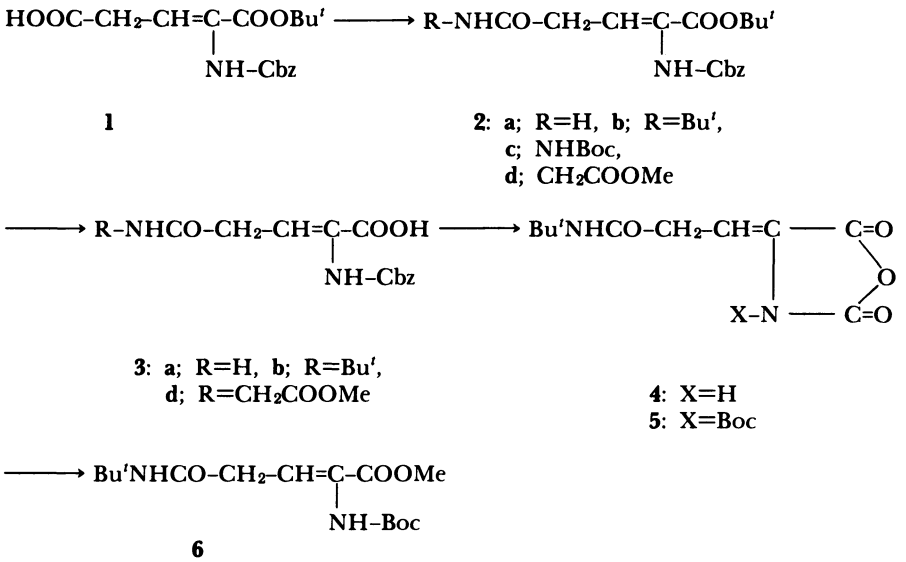
Synopsis. Various kinds of Cbz-α-dehydroglutamine derivatives were synthesized by the reaction of Cbz-α-dehydroglutamic acids with a few primary amines, including L-α-amino acid ester, and subsequently transformed to *N*-carboxy-α-dehydroglutamine anhydride and several Δ^1-dehydroglutaminyldipeptides.

In connection with the syntheses and reactions of α-dehydroamino acids (DHA) and dehydrooligopeptides (DHP), *N*-carboxy-α-dehydroamino acid anhydride (ΔNCA), derived by the cyclization of *N*-benzyloxycarbonyl (Cbz)-DHA with $SOCl_2$, were found to be a very important substrate for the transformation to various kinds of other DHA and DHP.[2-4)] So far, we reported on the syntheses of various kinds of ΔNCAs, derived from neutral (alkyl and aryl), acidic, and basic DHAs, which corresponded to the proteinic L-α-amino acid (AA).[5-8)] However, at present, the DHA and its ΔNCA corresponding to α,β-unsaturated derivatives of asparagine and glutamine, for example, α-dehydroglutamine (ΔGln) and its *N*-carboxy-α-dehydroglutamine anhydride (ΔGln·NCA) have never been synthesized.

In this paper, we wish to report on the syntheses of various kinds of Cbz-α-dehydroglutamine derivatives [Cbz-ΔGln(R)-OY: **2** (Y=Bu^t) and **3** (Y=H); **a**; R=H, **b**; R=Bu^t, **c**; R=NHBu^t, **d**; R=CH_2COOMe] and X-ΔGln(Bu^t)·NCA derivatives (**4**; X=H and **5**; X=Boc), by utilizing (*Z*)-isomer of Cbz-α-dehydroglutamic acid *t*-butyl ester (Cbz-ΔGlu-OBu^t) (**1**).[6)] Moreover, the coupling of DHA (**3b**) with an appropriate L-α-AA-OMe was also tried successfully to give the Δ^1-dehydrodipeptides.

The starting compound **1** was derived from α-oxoglutaric acid in six steps, according to a method reported previously.[6)] For the subsequent amidation of γ-carboxyl group of the side chain in ΔGlu, first a treatment of **1** with *N*-hydroxysuccinimide (HOSu) in tetrahydrofuran (THF) was carried out in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) to form the expected γ-active ester as an intermediate. It was found that the γ-ester, thus yielded, reacted smoothly with aqueous ammonium to give Cbz-α-dehydroglutamine *t*-butyl ester (Cbz-ΔGln-OBu^t) (**2a**) in 86% yield. In a similar manner, the reaction of the active ester with *t*-butylamine was worked up to give Cbz-ΔGln(Bu^t)-OBu^t (**2b**) in 74% yield. Furthermore, the coupling of the above ester with H-AA-OMe, e.g., glycine methyl ester in the presence of both DCC and HOSu also proceeded to give Cbz-ΔGln(CH_2COOMe)-OBu^t (**2d**) in 84% yield. In the case of *t*-butyloxycarbonylhydrazine, however, compound **1** was directly treated in the presence of DCC to give Cbz-ΔGln(NHBoc)-OBu^t (**2c**) in 85% yield.

On the other hand, in order to obtain α-acid free Cbz-ΔGln derivatives, which are anticipated to convert to the corresponding ΔNCA, the α-ester cleavage of ΔGln-OBu^t (**2**) was carried out. When compounds **2a, b, d** were treated, respectively, with gaseous hydrogen chloride in THF or ethyl acetate, the corresponding Cbz-ΔGln-OH derivatives **3a, b, d** were readily obtained in ca. 87% yield.



Scheme 1.

Table 1. *N*-Blocked α-Dehydroglutamine Derivatives (**2** and **3**)

Compound[a] No.	Yield %	Mp θ_m/°C	IR, ν/cm^{-1} in KBr -NHCO-	IR, ν/cm^{-1} in KBr -C=C-	^{1}H NMR, δ in $CDCl_3$ -CH= (*J*/Hz)	^{1}H NMR, δ in $CDCl_3$ -NH- (bs) (*J*/Hz)
2a	86	145—146[b]	1665	1625	6.64t	6.60
			1540		(7.0)	5.96
2b	74	syrup[c]	1660	1650	6.68t	6.98
			1520		(7.0)	6.43
2c	85	syrup[d]	1685	1650	6.52t	8.70
			1550		(7.0)	7.06, 6.92
2d	84	syrup[c]	1665	1660[f]	6.68t	7.16t
			1520		(7.0)	(7.0)
3a	85	144—145[e]	1660	1620	6.66t	7.47
			1520		(7.0)	7.00
3b	89	160—161[e]	1665	1620	6.56t	8.72
			1540		(7.0)	7.61
3d	85	135—136[c]	1640	1640[g]	6.60t	8.52t
			1515		(7.0)	(7.0)

a) All the compounds were analyzed for C, H, and N, and were within ±0.3% of theoretical values. b) Colorless needles from diisopropyl ether–ethyl acetate. c) Pale yellow. d) Yellow. e) Colorless prisms from diisopropyl ether–ethyl acetate. f) Shoulder. g) Overlapped.

Table 2. (*Z*)-Cbz-ΔGln(Bu^t)-AA-OMe (**7**)

Compound[a] No.	AA	Yield %	Mp θ_m/°C	IR, ν/cm^{-1} in KBr -CONH-	^{1}H NMR, δ in $CDCl_3$ -CH= (*J*/Hz)	^{1}H NMR, δ in $CDCl_3$ -NH-CH-CO- (*J*/Hz)	$[\alpha]_D^{20}$ in MeOH (*c* 1)
7a	Gly	70	syrup[b]	1670	4.49t	4.06d	—
				1540	(7.0)	(7.5)	
7b	Ala	75	syrup[b]	1670	6.34t	4.52dq	−2.5°
				1550	(7.0)	(7.5, 4.5)	
7c	Leu	78	96—97[c]	1670	6.42t	4.64m	−12.6°
				1530	(7.0)		
7d	Phe	73	154—155[c]	1690	6.24t	4.84dt	−6.1°
				1530	(7.0)	(8.0, 5.0)	

a) All the compounds were analyzed for C, H, and N, and were within ±0.3% of theoretical values. b) Colorless. c) Colorless needles from hexane–ethyl acetate.

Subsequently, in order to synthesize the ΔGln·NCA derivative by a method reported previously,[5] compound **3b** was chosen and then treated with $SOCl_2$ to give the desired ΔGln(Bu^t)·NCA (**4**) in 70% yield. Subsequent *N*-acylation of **4** with di-*t*-butyl dicarbonate [$(Boc)_2O$] was carried out to form Boc-ΔGln·NCA (**5**) as an intermediate, which was treated in situ with methanol to give Boc-ΔGln(Bu^t)-OMe (**6**) almost quantitatively, as illustrated in Scheme 1. Consequently, it was found that compound **4** also had a high reactivity with alcohol, such as the hitherto obtained ΔNCA derivatives.[5]

As summarized in Table 1, it can be seen that the yields of **2** and **3** were very high with an average value of 84%. In addition, the spectral data of **2** and **3** show the configurational structure of DHA to be a (*Z*)-isomer remain unchanged during the amidation and consecutive ester cleavage.

Futhermore, according to Scheme 2, the synthesis of Cbz-Δ^1-dehydroglutaminyldipeptides were successfully tried by the usual peptide synthetic method. The coupling of **3b** with an appropriate H-AA-OMe (AA=Gly, Ala, Leu, Phe) was carried out to give

3b + H-AA-OMe ⟶ Cbz-ΔGln(Bu^t)-AA-OMe

7: AA; **a**; Gly, **b**; Ala, **c**; Leu, **d**; Phe

Scheme 2.

Cbz-ΔGln(Bu^t)-AA-OMe (**7**) in a good yield, as summarized in Table 2.

In conclusion, it is firmly believed that the synthesis of the ΔGln·NCA derivative is surely available for the synthesis of various kinds of DHP containing the ΔGln residue.

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.).

General. To a solution of **1** (45.0 mmol) and HOSu (50.0 mmol) in diethylene glycol dimethyl ether (65 ml) was added portionwise DCC (51.0 mmol) under cooling. After stirring the resulting solution at room temperature for 12 h, the solution was again cooled at 5—10 °C. After removal of the *N,N'*-dicyclohexylurea separated out, the filtrate was concentrated under reduced pressure to give the corresponding active ester as an intermediate, which was utilized for the following amidation.

Preparation of 2a. To a solution of the active ester, thus obtained, in THF (50.0 ml) was added, with stirring, 28% aqueous ammonium (11.0 ml) at −10 °C. After continuing the stirring for 15 min and then at room temperature 15 min, the reaction solution was concentrated under reduced pressure. The residual product was dissolved with chilled ethyl acetate (30.0 ml) and then HOSu deposited was filtered off. The filtrate was further diluted with ethyl acetate (40 ml) and the resultant solution was washed twice with 15% NaCl aqueous solution and then dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the residual crystals were collected and then recrystallized from diisopropyl ether–ethyl acetate to give **2a** as colorless needles.

Preparation of 2b and 2d. To a solution of the active ester in CH_2Cl_2 (40 ml) was added drop by drop, with stirring, a solution of *t*-butylamine or glycine methyl ester (50.0 mmol) in CH_2Cl_2 (15 ml) under cooling. After stirring for 30 min and then at room temperature for 1 h, the reaction mixture was concentrated under reduced pressure. The residue, thus obtained, was dissolved with chilled ethyl acetate (30 ml) and then HOSu deposited was filtered off. The filtrate was again diluted with ethyl acetate (40 ml) and the resulting solution was washed successively with 10% citric acid, saturated $NaHCO_3$ aqueous solution, and then water and finally dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the residual syrup was purified on silica-gel column using a mixture of $CHCl_3$ and acetone (20:1 v/v) as the eluent. The fraction was concentrated to give **2b** or **2d** as a pale-yellow syrup.

Preparation of 2c. A solution of **1** (2.7 mmol) in CH_2Cl_2 (10 ml) was cooled at −5 °C and then DCC (3.0 mmol) was added, with stirring, for 30 min. To the resultant solution was added Boc–$NHNH_2$ (3.0 mmol) with stirring; then the reaction solution was stirred continuously at room temperature for 12 h. After removing the solvent and then adding chilled ethyl acetate (5 ml) to the residue, the *N,N'*-dicyclohexylurea deposited was filtered off. The filtrate was diluted with ethyl acetate (15 ml); then the resultant solution was washed successively with 10% citric acid, saturated $NaHCO_3$ aqueous solution, 15% NaCl aqueous solution, and then water and finally dried over anhydrous Na_2SO_4. After concentrating the solution under reduced pressure, the residual syrup was purified on a silica-gel column using a mixture of chloroform and acetone (20:1 v/v) as the eluent. The concentration of the fraction gave **2c** as a yellow syrup.

Preparation of 3a, b, d. A solution of **2** (10.0 mmol) in THF (50 ml) was saturated with HCl gas under cooling and then allowed to stand at room temperature for 1 h. After removing the solvent under reduced pressure, the residual substance was dissolved in $CHCl_3$ (30 ml) and the resultant solution was again concentrated. The crude crystals, thus obtained, were collected by using diisopropyl ether. Recrystallization from diisopropyl ether–ethyl acetate gave **3a, b, d** as colorless prisms.

Preparation of 4. To thionyl chloride (15 ml) was added a suspension of **3b** (5.0 mmol) in diethyl ether (15 ml), drop by drop, with stirring at −10 °C. After stirring vigorously for 30 min at −5—−8 °C. After removing the excess $SOCl_2$ under reduced pressure below 30 °C, the residue, thus obtained, was dissolved in carbon tetrachloride and the resultant solution was again condensed. This procedure was repeated three times and then the final residue, thus obtained, was purified on a silica-gel column using $CHCl_3$ to give crystalline product. Recrystallization from a mixture of benzene and ethyl acetate gave **4** as colorless prisms, yield 75%, mp 150—151 °C. ν_{max}^{KBr} 1840, 1780 (–CO–O–CO–), 1640 (C=C) cm^{-1}. ^1H NMR δ=5.86 (t, 1H, –CH=), 7.62 [s, 1H, –NH– (ring)]. Found: C, 53.28; H, 6.19; N, 12.45%. Calcd for $C_{10}H_{14}N_2O_4$: C, 53.09; H, 6.24; N, 12.38%.

Preparation of 6. To a solution of **4** (2.2 mmol) in THF (8 ml) was added $(Boc)_2O$ (2.6 mmol) and pyridine (30 µl) under cooling. After stirring for 4 h, the solution prepared was treated with methanol (8 ml) and then made to pH 8—9. The reaction mixture was further stirred at room temperature for 12 h and then concentrated under reduced pressure. The residual syrup, thus obtained, was dissolved in ethyl acetate (30 ml) and the resultant solution was washed successively with 10% citric acid, saturated $NaHCO_3$ aqueous solution, and then water, and finally dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the residue, thus obtained, was purified on a silica-gel column using a mixture of $CHCl_3$ and acetone (15:1 v/v) to give **6** as colorless syrup. Yield 95%. ν_{max}^{KBr} 1650, 1545 (–NHCO–), 1640 (C=C) cm^{-1}. ^1H NMR δ=6.56 (bs, 1H, 2 –NH–), 6.74 (t, 1H, –CH=). Found: C, 57.22; H, 8.53; N, 9.07%. Calcd for $C_{15}H_{26}N_2O_5$: C, 57.31; H, 8.34; N, 8.91%.

Preparation of 7. A suspension of an appropriate H–AA–OMe·HCl (1.80 mmol) in THF (6 ml) in the presence of TEA (1.80 mmol) was stirred under cooling for 30 min. To the prepared suspension was successively added **3b** (1.50 mmol), HOSu (1.70 mmol), and DCC (1.70 mmol); the resulting solution was returned to room temperature and then stirred for 2 h. After removing the solvent under reduced pressure, ethyl acetate (15 ml) was added to the residue obtained and the resultant solution was chilled and then filtered. The filtrate was washed successively with 10% citric acid, saturated $NaHCO_3$ aqueous solution, and water and then dried over anhydrous Na_2SO_4. After concentrating the solution under reduced pressure, the residual crystalline product was collected by using diisopropyl ether. Recrystallization from a mixture of hexane and ethyl acetate gave colorless needles. When the residue was syrup, purification on a silica-gel column using a mixture of $CHCl_3$ and ethyl acetate gave **7** as a colorless syrup. See Table 2.

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