Notes

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Acetic Acid-Catalyzed Diketopiperazine Synthesis[1)]

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Optically pure diketopiperazines were obtained in good yields when dipeptide esters were refluxed in 2-butanol containing 0.1 M acetic acid for 3 hours. When prolyl-amino acid ester was used as the starting material, 1 to 2 M acetic acid was the most effective concentration.

Keywords——dipeptide esters; *cyclo*(–Val–Val–); <Glu–Arg(NO_2)–OBzl(NO_2); *cyclo*(–Gly–Gln–); *cyclo*[–Asn–Arg(NO_2)–]; *cyclo*[–Asp(OBzl)–Ala–]; *cyclo*[–Tyr(Bzl)–Arg^+–]Cl^-; racemization test

Diketopiperazines or 2,5-dioxopiperazines are readily formed from dipeptide esters by base-catalyzed[3)] or autoaminolysis reaction.[4)] However, these methods[5)] of diketopiperazine preparation have various disadvantages, *i. e.*, racemization,[3)] poor yield[3,4)] and long reaction time.[3,4)] Diketopiperazine formation by carboxyl-catalyzed intramolecular aminolysis of dipeptide ester was reported in a study of side reaction in solid phase peptide synthesis.[6)]

In this work, we have investigated the acetic acid-catalyzed intramolecular aminolysis of dipeptide esters for the preparation of diketopiperazines and found that it represents a preparation procedure superior to others reported to date.

The N-protected dipeptide esters shown in Table I were treated with 4 N hydrogen chloride in dioxane, 2 M *p*-toluenesulfonic acid in dioxane or 25% hydrogen bromide in acetic acid to remove the amino-protecting groups. To about 0.067 M solution of the resulting dipeptide ester in 2-butanol was added one equivalent of NMM. Acetic acid was added to this solution to make 0.1 M acid concentration according to Gisin and Merrifield.[6)] The reaction mixture was refluxed for several hours. The progress of the reaction was monitored by paper electrophoresis. In most cases, starting materials were no longer detectable in 3 hours and the diketopiperazines formed were crystallized out from the solution, but diketopiperazine formation from prolyl-amino acid esters, H–Pro–Leu–OMe and H–Pro–Val–OBzl, was incomplete during this period. However, the cyclization occured effectively in 1 to 2 M acetic acid. On the other hand, H–Leu–Pro–OMe was transformed to *cyclo*(–Leu–Pro–) in 0.1 M acid in good yield during 3 hours.

Analyses of racemates in prepared diketopiperazines, *cyclo*(–Leu–Leu–), *cyclo*(–Val–Leu–), *cyclo*(–Val–Ala–) and *cyclo*(–Val–DAla–) were carried out by TLC according to the procedure reported by Nitecki *et al.*[4a)] In this analysis, no racemate was detectable in any sample tested. Racemates in isoleucine-containing diketopiperazines, *cyclo*(–Val–Ile–), *cyclo*(–Ile–Ile–), *cyclo*(–Gly–Ile–) and *cyclo*(–Ile–Gly–) were also examined by using an amino acid analyzer[7)] after acid hydrolysis. The amount of D-alloisoleucine in each sample was less than 1%.

Since easy isomerization of *cyclo*(–Pro–Phe–) at the proline α carbon in sodium hydroxide solution[8)] is well known, for reference purposes, an attempt was made to prepare *cyclo*(–Pro–Phe–) by Fischer's method.[3)] Cyclization of H–Pro–Phe–OMe by ammonia in methanol resulted in almost complete isomerization of the proline residue. However, the desired product was obtained with high optical purity by the method described here, as shown in Table II.

For the preparation of arginine-containing diketopiperazines, arginine dipeptide esters

TABLE I. Physical Constants of Dipeptide Derivatives used as Starting Materials

Compound	mp (°C)	$[\alpha]_D$[a] (deg.)	Formula	Elemental Anal.[b] C	H	N	Yield (%)
Boc–Pro–Leu–OMe	80—81[c]	− 76.5[c]					87
H–Leu–Pro–OMe·HCl	174—175	− 93.8	$C_{12}H_{22}N_2O_3 \cdot HCl$	51.69 (51.84	8.32 8.60	10.05 9.44)	85[d]
H–Pro–Val–OBzl·HCl	45—48	− 93.3	$C_{17}H_{24}N_2O_3 \cdot HCl$	59.89 (59.50	7.93 7.44	8.22 8.19)	80[d]
Boc–Leu–Leu–OMe	136—137[e]	− 55.0[e]					78
Boc–Val–Leu–OMe	126—128[f]	− 54.2[f]					80
Boc–Val–Ala–OBzl	82—83	−114.0	$C_{20}H_{30}N_2O_5$	63.47 (63.35	7.99 8.24	7.40 7.82)	67
Boc–Val–DAla–OBzl	81—82	− 15.4	$C_{20}H_{30}N_2O_5$	63.47 (63.10	7.99 8.03	7.40 7.61)	70
Boc–Val–Val–OBzl	48—50	− 80.0	$C_{22}H_{34}N_2O_5$	65.00 (64.60	8.43 8.78	6.89 6.99)	80
Boc–Val–Ile–OMe	144—145[g]	− 5.2[g]					82
Boc–Ile–Ile–OMe	154—155[h]	− 10.8[h]					87
Boc–Gly–Ile–OBzl(NO_2)	50—51	− 19.0	$C_{20}H_{29}N_3O_7$	56.72 (56.46	6.90 7.09	9.92 9.88)	86
Boc–Ile–Gly–OBzl(NO_2)	91—92	− 40.2	$C_{20}H_{29}N_3O_7$	56.72 (57.15	6.90 7.11	9.92 10.04)	85
Boc–Pro–Phe–OMe	71—73[i]	− 60.0[i]					87
H–Gly–Phe–OMe·HCl	35—40	+ 20.1	$C_{12}H_{16}N_2O_3 \cdot HCl$	52.84 (52.68	6.28 6.44	10.27 9.87)	75[d]
Boc–Glu(OBzl)–Tyr–OMe	38—41	− 38.6	$C_{27}H_{34}N_2O_8$	63.02 (62.62	6.66 6.89	5.44 5.82)	98
Boc–Gly–Gln–OBzl(NO_2)	60—62	− 35.3	$C_{19}H_{26}N_4O_8$	52.05 (52.33	5.98 6.11	12.78 13.01)	82
Z–Gln–Arg(NO_2)–OBzl–(NO_2)	193[j]	− 13.1[j] (DMF)					88[j]
Z–Asn–Arg(NO_2)–OBzl	180—182[k]	− 20.3[k] (DMF)					63[k]
H–Tyr(Bzl)–Arg–OMe·2 HCl	189—192	+ 21.1 (DMF)	$C_{23}H_{31}N_5O_4 \cdot 2HCl$	53.69 (53.65	6.47 6.56	13.61 13.53)	90[d]
Boc–Asp(OBzl)–Ala–OBzl	70—73	− 25.0	$C_{26}H_{32}N_2O_7$	64.65 (64.61	6.66 6.81	5.78 6.00)	74
Boc–Tyr–Gly–OEt	112—114	− 18.3	$C_{18}H_{26}N_2O_6$	59.00 (59.15	7.15 7.30	7.65 7.76)	86
Boc–Met–Tyr–OMe	28—35	− 37.0 (AcOH)	$C_{20}H_{30}N_2O_6S$	56.32 (56.40	7.09 7.43	6.57 6.79)	66
Boc–Tyr(Bzl)–His–OMe	115—118	− 26.7	$C_{28}H_{34}N_4O_6$	64.35 (64.35	6.56 6.80	10.72 10.98)	92
Boc–Trp–Leu–OMe	94—97	− 35.0	$C_{23}H_{33}N_3O_5$	64.01 (64.00	7.71 7.51	9.54 9.54)	98

a) Optical rotations were measured in methanol (c=1) at 20—23° unless otherwise noted.
b) Found values in parentheses.
c) Lit.[4a] mp 80—81°, $[\alpha]_D^{25}$−78.7° (c=1, MeOH).
d) Overall yield of the coupling reaction for the protected dipeptide ester and subsequent removal of Boc group.
e) Lit.[4a] mp 132—133°, [α] −50.4° (c=1, MeOH).
f) Lit.[4a] mp 126—128°, $[\alpha]_D^{25}$−53.2° (c=1, MeOH).
g) Lit. [K. Lloyd and G.T. Young, *J. Chem. Soc.* (*C*), **1971**, 2890.] mp 147.5—148.5°, $[\alpha]_D^{20}$−3°(c=1, EtOAc).
h) Lit. [K. Lloyd and G.T. Young, *J. Chem. Soc.* (*C*), **1971**, 2890.] mp 155°, $[\alpha]_D^{20}$−7°(c=1, EtOAc).
i) Lit. [R. Paul and G.W. Anderson, *J. Org. Chem.*, **27**, 2094 (1962).] mp 74—76°, $[\alpha]_D^{25}$−53° (c=1, $CHCl_3$).
j) K. Suzuki, T. Abiko, N. Endo, Sasaki and J. Arisue, *Chem. Pharm. Bull.*, **21**, 2627 (1973).
k) Y. Sasaki, *Chem. Pharm. Bull.*, **22**, 2188 (1974).

Table II. Physical Constants of Diketopiperazines

Diketopiperazine	mp (°C)	$[\alpha]_D$[a] (deg.)	Formula	Elemental Anal.[b] C	H	N	Yield (%)	Literature mp (°C)	Literature $[\alpha]_D$(deg.)
–Pro–Leu–[c]	158—160	−135.0 (EtOH)					83	157—158[d]	−133[d]
–Leu–Pro–[e]	157—159	−132.8 (EtOH)					94		
–Pro–Val–[f]	169—171	−154.2					78	166—168[g]	
–Leu–Leu–	273—274 (dec.)	− 44.0					74	275—276[d]	−44.6[d]
–Va–Leu–	259—261 (dec.)	− 47.1					88	264—268[d]	−47.3[d]
–Val–Ala–	259—261 (dec.)	− 30.2					88	264—265[d]	−27.0[d]
–Val–DAla–	267—269 (dec.)	+ 26.7					94	272—273[d]	+24.4[d]
–Val–Val–	269—271 (dec.)	− 77.5	$C_{10}H_{18}N_2O_2$	60.58 (60.35	9.15 9.29	14.13 13.88)	79		
–Val–Ile–	276—278 (dec.)	− 53.3	$C_{11}H_{20}N_2O_2$	62.23 (62.46	9.50 9.88	13.20 13.19)	76		
–Ile–Ile–	259—262 (dec.)	− 60.0	$C_{12}H_{22}N_2O_2$	63.68 (63.88	9.80 9.83	12.38 12.06)	85		
–Gly–Ile–	249—251 (dec.)	+ 29.6	$C_8H_{14}N_2O_2$	56.45 (56.15	8.29 8.58	16.46 15.98)	86		
–Ile–Gly–	247—250 (dec.)	+ 29.8					80		
–Pro–Phe–	132—133	− 89.0 (c=0.3, H_2O)					89	133[h]	−83[h]
–Gly–Phe–	273—275 (dec.)	+100.8					83	268[d]	+97.8[d]
–Glu(OBzl)–Tyr–	196—198	− 75.0 (DMF)	$C_{21}H_{22}N_2O_5$	65.95 (65.80	5.80 5.83	7.33 7.25)	85		
–Gly–Gln–	244—246 (dec.)	+ 15.2	$C_7H_{11}N_3O_3$	45.40 (45.38	5.99 6.18	22.69 22.85)	78		
–Gln–Arg–(NO_2)–[i]									
–Asn–Arg–(NO_2)–	235—237 (dec.)	−108.0 (N–Me-2-pyrrolidone)	$C_{10}H_{17}N_7O_5$	38.09 (38.19	5.44 5.60	31.10 30.89)	87		
–Tyr(Bzl)–Arg·HCl–	177—182 (dec.)	− 27.4	$C_{22}H_{27}N_5O_3 \cdot HCl \cdot 1/2H_2O$	58.07 (57.74	6.42 6.44	15.39 14.98)	94		
–Asp(OBzl)–Ala–	189—192	− 20.0 (MeOH)	$C_{14}H_{16}N_2O_4$	60.86 (60.83	5.84 6.11	10.14 10.23)	97		
–Tyr–Gly–	270—275 (dec.)	+ 63.4 (DMF)					87	287—288.5[j]	
–Met–Tyr–	273—277 (dec.)	− 52.0 (N–Me-2-pyrrolidone)	$C_{14}H_{18}N_2O_3S$	57.12 (56.85	6.16 6.31	9.52 9.49)	86	294—295[j]	
–Tyr(Bzl)–His–	196—198	− 79.0	$C_{22}H_{22}N_4O_3$	67.67 (67.14	5.68 5.75	14.35 13.82)	80		
–Trp–Leu–	261—262 (dec.)	+ 45.6	$C_{17}H_{21}N_3O_2$	68.20 (68.00	7.07 7.16	14.04 13.92)	83	265—268[d]	+48.0[d]

a) Optical rotations were measured in acetic acid (c=1) at 18—22° unless otherwise noted.
b) Found values in parentheses.
c) Prepared by cyclization of H–Pro–Leu–OMe in 2 M acetic acid-2-butanol.
d) See ref. 4a.
e) Prepared by cyclization of H–Leu–Pro–OMe in 0.1 M acetic acid-2-butanol.
f) Cyclized in 2 M acetic acid-2-butanol.
g) P.E. Young, V. Madison and E.R. Blout, *J. Am. Chem. Soc.*, **98**, 5365 (1976).
h) See ref. 8a.
i) This product was not obtained by the method described here.
j) K.D. Kopple and H.G. Ghazarian, *J. Org. Chem.*, **33**, 862 (1968).

containing nitrated or protonated guanidine could be used as starting materials; the yields were good.

Cyclizations of H–Glu(OBzl)–Tyr–OMe and H–Gly–Gln–OBzl(NO_2) gave the corresponding diketopiperazines with satisfactory yields. When H–Gln–Arg(NO_2)–OBzl(NO_2) was cyclized, <Glu–Arg(NO_2)–OBzl(NO_2) was a major product. It seems that amino acyl glutamine or amino acyl γ-benzyl glutamic acid esters are preferable in general as starting materials to avoid possible formation of pyroglutaminyl amino acid ester, although a satisfactory yield of *cyclo*[–Glu(OBzl)–Tyr–] was obtained, as described above. There were no problems with asparagine or aspartic acid derivatives, as shown in Table II. In the preparation of tyrosine-containing diketopiperazines, both protected phenolic hydroxy and unprotected phenolic hydroxy derivatives can be used as starting materials. An acid-labile tryptophan-containing diketopiperazine, *cyclo*(–Trp–Leu–), could be prepared in good yield from Boc–Trp–Leu–OMe by the use of 2 M *p*-toluenesulfonic acid in dioxane[9] as a deblocking reagent for the Boc group.

Experimental

All melting points are uncorrected. Optical rotations were determined with an Atago Polax. Amino acid analyses were carried out on a Hitachi KLA-3B amino acid analyzer. TLC were performed on silica gel plates (Kieselgel GF_{254}, Merck) with iso-propyl ether-$CHCl_3$–AcOH (6:3:1).[4a] Paper electrophoresis was performed on Toyo Roshi No. 51 paper, in pH 6.20 pyridine–acetate buffer at 500 V for 45 min. The spot(s) was visualized with chlorine-*o*-tolidine reagents.[10]

Preparation of Protected Dipeptide Ester——The protected dipeptide esters listed in Table I were prepared by the N,N′-dicyclohexylcarbodiimide-1-hydroxybenztriazole method[11] in a usual manner except for Z–Asn–Arg(NO_2)–OBzl and Z–Gln–Arg(NO_2)–OBzl(NO_2), which were prepared by the active ester method.[12] In cases where the product was an oil, it was converted to the hydrochloride by treatment with 4 N HCl-dioxane for analysis (see Table I). Yields and physical constants of the dipeptide ester precursors are listed in Table I.

Preparation of Diketopiperazines——Boc-dipeptide ester (1 mmol) was treated with 4 N HCl–dioxane (3 ml) at room temperature for 30 min, then the excess HCl was removed by repeated evaporation with dioxane in a vacuum (×3). The resulting hydrochloride was dissolved in 0.1 M AcOH-2-butanol (15 ml), and NMM (0.11 ml, 1 mmol) was added. The resulting weakly acidic solution was refluxed in an oil bath (120°) for 3 hrs. In most cases, the diketopiperazines began to crystallize out from the hot or cold solution. After concentration of the reaction mixture to a small volume, the product was collected on a filter, washed with small amounts of cold H_2O, and then recrystallized from a suitable solvent, such as H_2O, 2-propanol or 2-butanol.

Z groups in Z–Asn–Arg(NO_2)–OBzl and Z–Gln–Arg(NO_2)–OBzl(NO_2) were removed by treatment with 25% HBr–AcOH for 30 min in a usual manner. The Boc group in Boc–Trp–Leu–OMe was removed by treatment with 2 M *p*-toluenesulfonic acid–dioxane containing 2% anisole according to the method reported by Suzuki *et al.*[9]

For the isolations of *cyclo*(–Pro–Leu–) and *cyclo*(–Pro–Val–), the reaction mixture was concentrated to dryness and the residue was extracted twice with EtOAc. The extract was washed with NaCl-saturated H_2O, dried over $MgSO_4$ and evaporated to dryness in a vacuum to yield a slightly brown product which was washed with cold abs. ether and dried in a vacuum.

For the isolation of *cyclo*(–Pro–Phe–), the concentrated reaction mixture was extracted with CH_2Cl_2. The extract was washed well with NaCl-saturated H_2O, dried over $MgSO_4$ and evaporated to dryness in a vacuum. The oily residue was crystallized from EtOAc-abs. ether (1:1). Yields and physical constants of all diketopiperazines prepared are listed in Table II.

<Glu-Arg(NO_2)-OBzl(NO_2)——This compound was obtained from H–Gln–Arg(NO_2)–OBzl(NO_2)·HBr in the manner described above: recrystallized from H_2O; yield 83%; mp 196—197°; $[\alpha]_D^{23}$ −40° (c=0.5, DMF); *Anal.* Calcd for $C_{18}H_{23}N_7O_8$: C, 46.45; H, 4.98; N, 21.07. Found: C, 46.61; H, 4.99; N, 20.73.

Analysis of Racemate——Four kinds of diketopiperazines prepared were examined by TLC.[4a] Each of them gave a single spot negative to ninhydrin and positive to chlorine-*o*-tolidine reagents. Their *Rf* values were as follows: *cyclo*(–Leu–Leu–), 0.53; *cyclo*(–Val–Leu–), 0.50; *cyclo*(–Val–Ala–), 0.28; *cyclo*(–Val–D-Ala–), 0.36.

Four isoleucine-containing diketopiperazines, *cyclo*(–Val–Ile–), *cyclo*(–Ile–Ile–), *cyclo*(–Gly–Ile–) and *cyclo*(–Ile–Gly–) were hydrolyzed with 6 N constant-boiling HCl at 110° for 20 hrs and the hydrolysates (about 1 μmol) were applied to an amino acid analyzer. The amount of D-alloisoleucine[9] in each sample was less than 1%. In the acid hydrolysate of Boc–Ile–Ile–OMe, a little D-alloisoleucine was detected (less than 1%).

Cyclization of H-Pro-Phe-OMe by Fischer's Method——Boc–Pro–Phe–OMe (250 mg) was treated with 4 N HCl–dioxane as described above. A solution of the resulting hydrochloride in ammonia-saturated MeOH (10 ml) was stirred at room temperature for 20 hrs. After removal of the solvent, the residue was

worked up as described above. wt. 60 mg (37%); mp 145—146°; $[\alpha]_D^{22}$ +89° (c=0.2, H_2O) {*cyclo*(–D-Pro–Phe–)[8a]: mp 148—150°; $[\alpha]_D^{20}$ +92° (c=0.2, H_2O)}.

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References and Notes

1) Amino acids, peptides and their derivatives are of the L-configuration unless otherwise stated. Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **11**, 1726 (1972). Other abbreviations: Boc=*tert*-butoxycarbonyl, Z=benzyloxycarbonyl, Me=methyl, Et=ethyl, Bzl=benzyl, Bzl(NO_2)=*p*-nitrobenzyl, NMM=N-methylmorpholine, TLC=thin-layer chromatography.
2) Location: *Komatsushima, Sendai 983, Japan.*
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Synthesis of Furan Derivatives. LXXXVII. Kinetic Studies of the Thermal Curtius Rearrangement of 2-Benzofuroyl Azide and Related Compounds[1)]

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The kinetics of the thermal Curtius rearrangement of benzoheteroaroyl azides, *i.e.*, 2-benzofuroyl azide (**2**), 2-benzothenoyl azide (**4**), 2-indolecarbonyl azide (**6**), 2-, 3-, 4-, 5-, 6-, and 7-quinolinecarbonyl azides (**8**, **10**, **12**, **13**, **14**, and **15**), and 1- and 2-naphthoyl azides (**17** and **18**), in toluene were studied by infrared spectrophotometry to determine how the annelation of the benzene ring to side *b* of 2-furoyl, 2-thenoyl, and 2-pyrrolecarbonyl azides (**1**, **3**, and **5**) and 2-, 3-, and 4-pyridinecarbonyl azides (**7**, **9**, and **11**) affects the rearrangement and its rate.

The annelation effect slightly promotes the rearrangement; the effect on the thiophene ring and pyrrole ring is greater than that on the furan ring, and the effect on the pyridine ring of 4-pyridinecarbonyl azide (**11**) is greater than that on the pyridine ring of 2- and 3-pyridinecarbonyl azides (**7** and **9**).

Keywords——acyl azide; thermal Curtius rearrangement; annelation effect; infrared spectrophotometric method; activated three-membered intermediate; compensation effect; structure-activity relationship; PMO method; intramolecular electrophilic reagent; electron-deficient nitrogen