NEW SYSTEM FOR PEPTIDE SYNTHESIS USING *N*-ACYLPYRAZOLES†

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Abstract——New system of peptide synthesis was described. The extension of one amino acid unit on the peptide chain was constituted from only 2 reaction steps, the conversion from esters to the corresponding *N*-acylpyrazoles and the subsequent aminolysis with amino esters. This new system was distinctive from the conventional peptide synthesis, which was consisted of 3 steps of the deprotection, the activation and the condensation. Moreover, the key intermediate *N*-acylpyrazoles exhibited the excellent properties of high sensitivity and separability for the chiral column on HPLC using the UV detector.

We have recently developed a method of preparation for 3-phenyl-*l*-menthopyrazole (**1**) as a new chiral auxiliary,[1] which has unique structure and properties that are different from the conventional chiral auxiliaries.[2] The most important characteristics of this auxiliary are that the acyl substrate terminates in the nitrogen atom of a heteroaromatic pyrazole ring in a chiral environment. The steric hindrance of **1** is especially relaxed by the twisting of the benzene ring, which overlaps one side of the terminal nitrogen atom.[1] This structural feature causes a diastereofacial effect in the reactions on the substrate moiety. Moreover, the lone pair of electrons on the adjacent nitrogen plays the role of a Lewis base, causing the chelation of N···Li-O in the lithium enolate derived from *N*-acylpyrazoles. These chelations freeze the bond rotation of the acyl group so that it is fixed in a *syn* configuration. As a result, the chirality of the (4*R*)-methyl group of **1** causes a highly asymmetric induction on the acyl group of 2-acyl-3-phenyl-*l*-menthopyrazoles in the reactions with alkyl halides,[3] diphenyl disulfide,[4] acyl chloride,[5] aldehydes,[6] and C=N compounds.[7] A similar chelation of N···Mg···O=C, which is observed in

† This paper is dedicated to Professor Teruaki Mukaiyama on the occasion of his 73rd birthday for his brilliant achievement in the field of heterocyclic chemistry.

the mixture of *N*-acylpyrazoles and $MgBr_2 \cdot OEt_2$,[8] induces the asymmetric addition of Grignard reagents,[9] 1,3-dipolar compounds,[10] and dienes[11] on *N*-(α,β-unsaturated) acylpyrazoles.

In the meantime, *N*-acylheteroaromatics such as *N*-acylimidazoles are utilized as the activated acyl moiety in the wide varieties of organic syntheses.[12] As an analogue of these *N*-acylheteroaromatics, *N*-acylpyrazoles are easily converted into acyl derivatives by the action of nucleophiles such as alcohols,[13] amines,[14] Grignard reagents,[15] or organozinc compounds[16] under basic or acidic conditions. Moreover *N*-acylpyrazoles are comparably stable and are easily prepared either from acid chlorides and pyrazoles or from β-diketones and acyl hydrazides.[13]

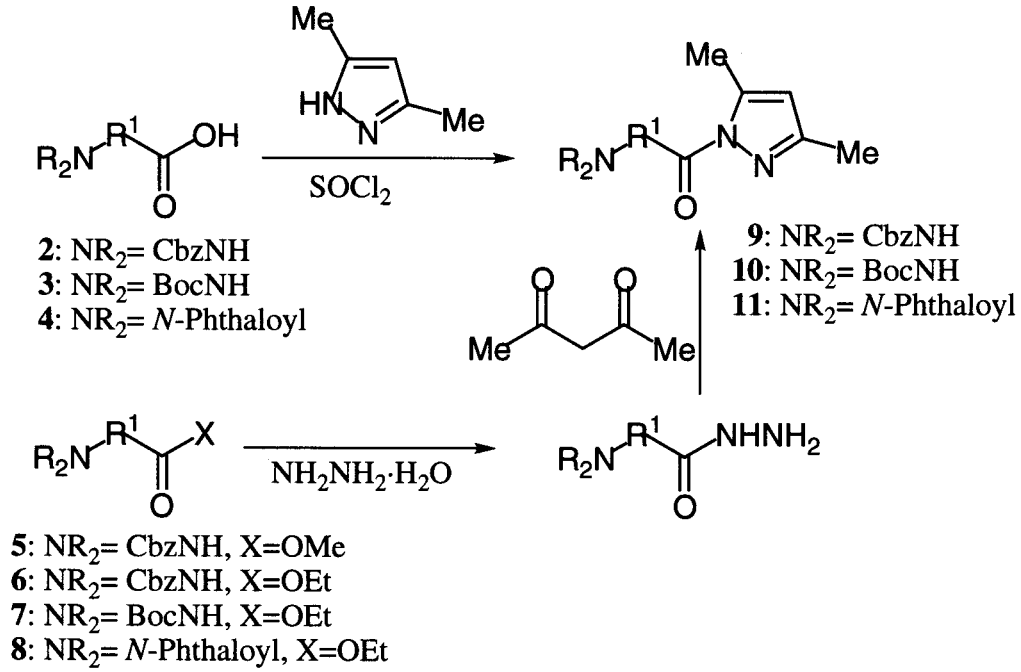
Since the peptide synthesis is one of the most important reactions, a large number of methodologies have been reported in the literature. Almost all of these methodologies are consisted of 3 steps; the deprotection and the activation of the terminal function, and the condensation with amino acid derivatives protected on another terminate. For example, ester function of peptide esters is deprotected to carboxylic acids, which are treated with the activating agent such as DCC (dicyclohexylcarbodiimide) and finally form the peptide bond with amino esters. Subsequently one amino acid unit is extended on the peptide chain.

Previously 1-pyrazolecarboxylates, which were some kinds of *N*-acylpyrazoles, were reported to display the analogous behaviors toward nucleophiles despite high stability in the aqueous solution.[17] 1-Pyrazolecarboxylates are also regarded as a new facile alkoxycarbonylating agent particularly for the protection of amino acids and amino esters. On the bases of these chemical attributes, the utilities of *N*-acylpyrazoles are expected for the peptide bond formation. In this paper, we will report the preparation of *N*-acylpyrazoles derived from amino acids and their esters. These *N*-acylpyrazoles will constitute the new system of peptide bond formation, which is consisted of only two steps of the activation and the condensation.

Preparation of *N*-Acylpyrazoles Derived from α-Amino Acids and Esters

According to the general preparation of *N*-acylpyrazoles, 1-(CBZ-glycyl)-3,5-dimethylpyrazole (**9a**) was obtained from the mixture of Cbz-glycine (**2a**) and 3,5-dimethylpyrazole by the treatment with thionyl chloride in the presence of triethylamine (Method A). Similarly various Cbz-amino acids (**2**) gave 1-(α-Cbz-aminoacyl)-3,5-dimethylpyrazoles (**9**) in good yields. Accessible phthaloyl and *t*-butoxycarbonyl (Boc) groups are applicable for the protection of *N*-terminal of amino acids in this preparative method. During these preparations, the optical purities of amino acids were retained completely.

Since the direct acylation of pyrazoles was failed by the use of amino esters, the preparation of N-acylpyrazoles was undertaken from α-amino ester by the conversion into the corresponding hydrazide and subsequent reaction with β-dicarbonyl compounds such as 2,4-pentanedione. The reaction of



2: NR_2= CbzNH
3: NR_2= BocNH
4: NR_2= *N*-Phthaloyl

9: NR_2= CbzNH
10: NR_2= BocNH
11: NR_2= *N*-Phthaloyl

5: NR_2= CbzNH, X=OMe
6: NR_2= CbzNH, X=OEt
7: NR_2= BocNH, X=OEt
8: NR_2= *N*-Phthaloyl, X=OEt

a: R^1= -CH_2-
b: R^1= -CHMe-
c: R^1= -CHBn-
d: R^1= -CH(*i*-Pr)-
e: R^1= -CH(*i*-Bu)-
f: R^1= -CH_2-CONH-CH_2-
g: R^1= -CH_2-CONH-CHMe-
h: R^1= -CH_2-CONH-CHBn-
i: R^1= -CH_2-CONH-CH(*i*-Pr)-
j: R^1= -CH_2-CONH-CH(*i*-Bu)-
k: R^1= -CHMe-CONH-CH_2-
m: R^1= -CHBn-CONH-CH_2-
n: R^1= -CH(*i*-Pr)-CONH-CH_2-
p: R^1= -CH(*i*-Bu)-CONH-CH_2-
q: R^1= -CHMe-(CONH-CH_2)$_2$-

Scheme 1

Cbz-glycine ethyl ester (**6a**) with hydrazine hydrate in methanol afforded Cbz-glycyl hydrazide, which was treated with 2,4-pentanedione under weakly acidic conditions to give 1-(α-Cbz-glycyl)-3,5-dimethylpyrazole (**9a**) in good yield (Method B). Similarly various Cbz-amino esters (**5** and **6**) were successfully converted into 1-(α-Cbz-aminoacyl)-3,5-dimethylpyrazoles (**9**) step by step in one pot, as listed in Table 1. By this preparation, dipeptide esters and tripeptide esters (**5h-i**, **6f-g** and **6j-q**) were also converted into the corresponding *N*-acylpyrazoles (**9f-q**). As expected from the deprotecting method of phthaloyl group, the conversion from amino esters into the corresponding *N*-acylpyrazoles proceeded without any racemization.

The consequent *N*-acylpyrazoles (**9-11**) were extractable from aqueous reaction mixture with ordinary organic solvent. Further these compounds were very stable crystalline compounds and easily purified by means of chromatography or recrystallization. On the HPLC using the chiral column, the optical resolution was observed with remarkable separability factors. Moreover the strong UV absorption of these compounds led to the high sensitivity on the UV detector of HPLC as summarized in Table 2,

compared with those of *N*-protected amino acid derivatives. From these characteristics, pyrazole moiety of *N*-protected α-aminoacylpyrazoles was practically regarded as the useful marker for the quantitative analysis of amino acid and peptide derivatives especially for the evaluation of enantiomer ratio.

Table 1. The Preparation of 1-(α-Aminoacyl)-3,5-dimethylpyrazoles

Run	Amino Acid Derivatives				Method	Product	Yield	Opt. Yield
		R_2N	R^1	X			(%)	(%)
1	**2a**	CbzNH	-CH_2-	OH	A	**9a**	42	--
2	**2b**	CbzNH	-CHMe-	OH	A	**9b**	67	100
3	**2c**	CbzNH	-CHBn-	OH	A	**9c**	66	100
4	**2d**	CbzNH	-CH(*i*-Pr)-	OH	A	**9d**	62	100
5	**2e**	CbzNH	-CH(*i*-Bu)-	OH	A	**9e**	93	100
6	**4b**	Pht[a]	-CHMe-	OH	A	**11b**	51	100
7	**6a**	CbzNH	-CH2-	OEt	B	**9a**	62	--
8	**5b**	CbzNH	-CHMe-	OMe	B	**9b**	50	100
9	**6b**	CbzNH	-CHMe-	OEt	B	**9b**	43	100
10	**7b**	BocNH	-CHMe-	OEt	B	**10b**	58	100
11	**5c**	CbzNH	-CHBn-	OMe	B	**9c**	42	100
12	**5d**	CbzNH	-CH(*i*-Pr)-	OMe	B	**9d**	62	100
13	**6e**	CbzNH	-CH(*i*-Bu)-	OEt	B	**9e**	35	100
14	**6f**	CbzNH	-CH_2-CONH-CH_2-	OEt	B	**9f**	66	--
15	**6g**	CbzNH	-CH_2-CONH-CHMe-	OEt	B	**9g**	63	100
16	**5h**	CbzNH	-CH_2-CONH-CHBn-	OMe	B	**9h**	47	100
17	**5i**	CbzNH	-CH_2-CONH-CH(*i*-Pr)-	OMe	B	**9i**	43	100
18	**6j**	CbzNH	-CH_2-CONH-CH(*i*-Bu)-	OEt	B	**9j**	52	100
19	**6k**	CbzNH	-CHMe-CONH-CH_2-	OEt	B	**9k**	72	100
20	**6m**	CbzNH	-CHBn-CONH-CH_2-	OEt	B	**9m**	39	100
21	**6n**	CbzNH	-CH(*i*-Pr)-CONH-CH_2-	OEt	B	**9n**	69	100
22	**6p**	CbzNH	-CH(*i*-Bu)-CONH-CH_2-	OEt	B	**9p**	55	100
23	**6q**	CbzNH	-CHMe-(CONH-CH_2)$_2$-	OEt	B	**9q**	57	100

a: Phthalimimo group was abbreviated as Pht.

Table 2. The UV Absorptions and Separability Factors of 1-(α-Aminoacyl)-3,5-dimethylpyrazoles

Run		R_2N	-R^1-	X	λ_{max}	ε_{max}	ε_{254}	HPLC Solvent	α^b
1	**2b**	CbzNH	-CHMe-	OH	207	1460	230	MeCN-H_2O (1:2)	1.10
2	**6b**	CbzNH	-CHMe-	OEt	203	1580	260	MeCN-H_2O (1:1)	1.00
3	**9b**	CbzNH	-CHMe-	DMP[a]	244	17800	10500	MeOH-H_2O (3:1)	1.17
4	**3b**	BocNH	-CHMe-	OH	193	84	0		c
5	**7b**	BocNH	-CHMe-	OEt	202	1010	94		c
6	**10b**	BocNH	-CHMe-	DMP[a]	239	17400	8920	MeOH-H_2O (2:1)	1.00
7	**4b**	Pht[a]	-CHMe-	OH	216	40300	570	MeCN-H_2O (1:4)	1.00
8	**8b**	Pht[a]	-CHMe-	OEt	218	36200	490	MeCN-H_2O (1:2)	1.00
9	**11b**	Pht[a]	-CHMe-	DMP[a]	220	41100	8560	MeOH-H_2O (2:1)	1.10
10	**2c**	CbzNH	-CHBn-	OH	212	1780	310	MeCN-H_2O (1:2)	1.00
11	**6c**	CbzNH	-CHBn-	OEt	215	1690	320	MeOH-H_2O (1:1)	1.07
12	**9c**	CbzNH	-CHBn-	DMP[a]	244	13600	9720	MeOH-H_2O (5:1)	1.12
13	**2d**	CbzNH	-CH(*i*-Pr)-	OH	209	2410	160	MeCN-H_2O (1:1)	1.03
14	**9d**	CbzNH	-CH(*i*-Pr)-	DMP[a]	243	21400	11500	MeOH-H_2O (5:1)	1.10
15	**3d**	BocNH	-CH(*i*-Pr)-	OH	201	480	91		c
16	**7d**	BocNH	-CH(*i*-Pr)-	OEt	202	550	160		c
17	**10d**	BocNH	-CH(*i*-Pr)-	DMP[a]	242	15300	8070	MeOH-H_2O (2:1)	1.13
18	**2e**	CbzNH	-CH(*i*-Bu)-	OH	209	790	165	MeCN-H_2O (1:2)	1.17
19	**9e**	CbzNH	-CH(*i*-Bu)-	DMP[a]	243	15600	8140	MeOH-H_2O (5:1)	1.41
20	**6k**	CbzNH	-CHMe-CONH-CH_2-	OEt	209	2070	160	MeOH-H_2O (1:1)	1.05
21	**9k**	CbzNH	-CHMe-CONH-CH_2-	DMP[a]	242	16200	8400	MeOH-H_2O (5:1)	1.12
22	**6q**	CbzNH	-CHMe-(CONH-CH_2)$_2$-	OEt	209	2780	150	MeOH-H_2O (1:1)	1.01
23	**9q**	CbzNH	-CHMe-(CONH-CH_2)$_2$-	DMP[a]	243	17900	9520	MeCN-H_2O (1:3)	1.04

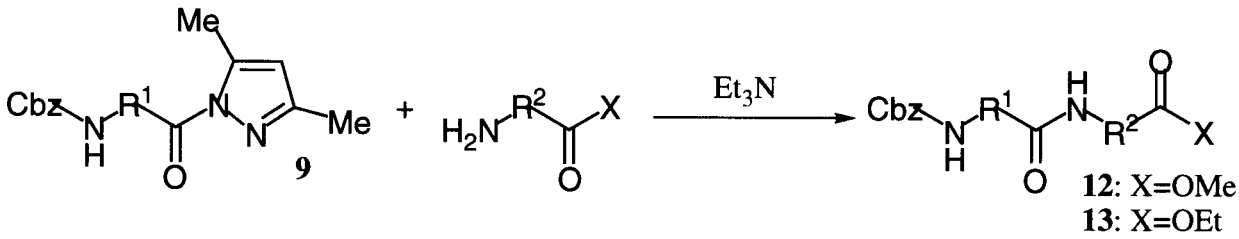
a: Phthalimino and 3,5-dimethylpyrazol-1-yl groups were abbreviated as Pht and DMP, respectively.

b: Separability factor of enantiomer on the chiral column (CHIRALCEL OD-R).

c: Undetectable by UV detector.

Peptide bond formation.

The previous paper reported that *N*-acylpyrazoles such as 3,5-dimethylpyrazole-1-carboxylates reacted with various amino compounds to form the amide bond.[17] Since analogous aminolysis was expected in *N*-protected α-aminoacylpyrazoles, the reaction of 1-(α-Cbz-glycyl)-3,5-dimethylpyrazole (**9a**) with free amino acids was undertaken. The acid function of free amino acids should be neutralized before the treatment with *N*-acylpyrazoles. In the presence of NaH, the reaction of **9a** with either glycine or alanine gave Cbz-glycine (**2a**). This fact was elucidated by the simple hydrolysis of **9a** without any action of amino acids. When triethylamine was used as a base, the peptide bond formation was unsuccessful with the recovery of starting materials.

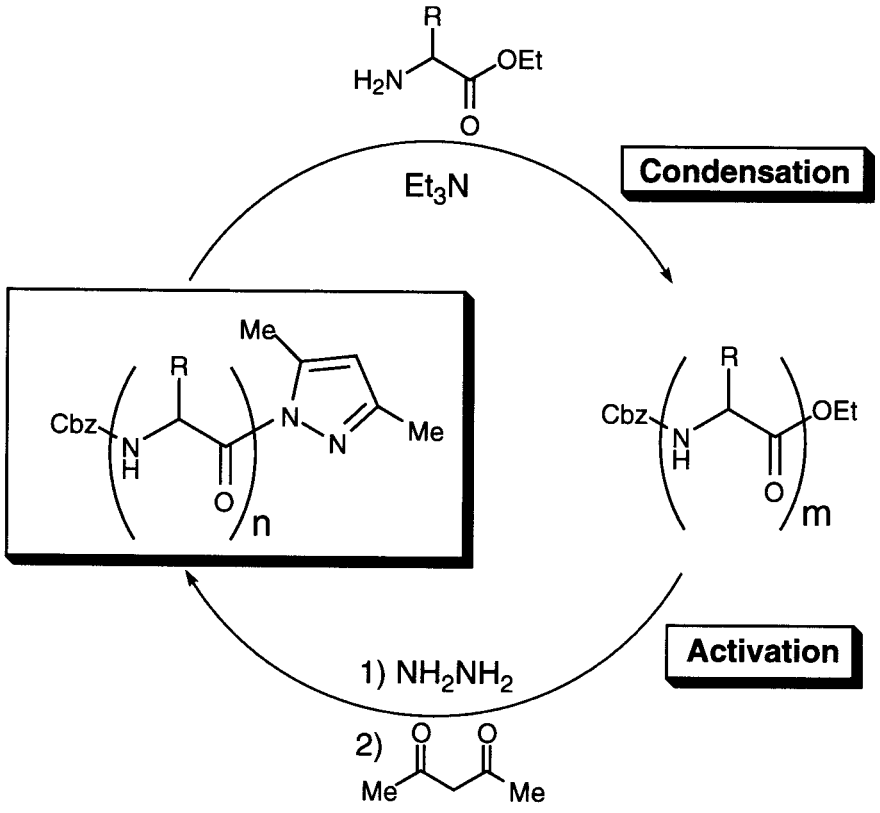


Scheme 2

Next **9a** was treated with glycine ethyl ester hydrochloride in the presence of triethylamine, and obtained Cbz-glycylglycine ethyl ester (**13f**) in 60 % yield. Similarly **9a** reacted with various amino ester to give the corresponding dipeptide esters (**12h**, **13g** and **13i-j**) with the retention of optical asymmetry in moderate to good yield, as listed in Table 3. Table 3 also showed the results of reactions of the glycine ethyl ester hydrochloride with 1-(α-Cbz-aminoacyl)-3,5-dimethylpyrazoles (**9b-e**) derived from various Cbz-amino acid derivatives. The reaction of 1-(α-Cbz-alanyl)-3,5-dimethylpyrazole (**9b**) and glycylglycine ethyl ester hydrochloride afforded tripeptide ester, Cbz-alanylglycylglycine ethyl ester (**13q**), which was also obtained from 1-(Cbz-alanylglycyl)-3,5-dimethylpyrazole (**9k**) and glycine ethyl ester hydrochloride. From these results, the peptide bond formation was readily attained by the reaction of amino esters with *N*-acylpyrazoles (**9**) derived from α-amino acid derivatives, where pyrazole moiety played a role of activation. The consequent peptide esters were applicable to the further conversion into the corresponding *N*-acylpyrazoles. When 1-phthaloylglycyl-3,5-dimethylpyrazole (**11a**) was treated with glycine ethyl ester hydrochloride, the desired dipeptide ester was also obtained. However, the deprotection of phthaloyl group should be caused by the action of hydrazine during the further conversion into *N*-acylpyrazole. Therefore the protecting group of *N*-terminal on peptide chain preferred to Cbz rather than phthaloyl.

Table 3. Peptide Bond Formation by 1-(α-Cbz-aminoacyl)-3,5-dimethylpyrazoles

Run	*N*-Aminoacylpyrazoles			Amino Acid Derivatives			Peptides	
		R_2N	R^1	R^2	X		Yield	Opt. Yield
1	**9a**	CbzNH	$-CH_2-$	$-CH_2-$	OH		0	--
2	**9a**	CbzNH	$-CH_2-$	$-CH_2-$	OEt	**13f**	60	--
3	**9a**	CbzNH	$-CH_2-$	-CHMe-	OEt	**13g**	92	100
4	**9a**	CbzNH	$-CH_2-$	-CHBn-	OMe	**12h**	67	100
5	**9a**	CbzNH	$-CH_2-$	-CH(*i*-Pr)-	OEt	**13i**	77	100
6	**9a**	CbzNH	$-CH_2-$	-CH(*i*-Bu)-	OEt	**13j**	81	100
7	**9b**	CbzNH	-CHMe-	$-CH_2-$	OEt	**13k**	59	100
8	**9c**	CbzNH	-CHBn-	$-CH_2-$	OEt	**13m**	69	100
9	**9d**	CbzNH	-CH(*i*-Pr)-	$-CH_2-$	OEt	**13n**	63	100
10	**9e**	CbzNH	-CH(*i*-Bu)-	$-CH_2-$	OEt	**13p**	77	100
11	**9k**	CbzNH	-CHMe-CONH-CH_2-	$-CH_2-$	OEt	**13q**	69	100
12	**9b**	CbzNH	-CHMe-	$-CH_2-$ CONH-CH_2-	OEt	**13q**	76	100



Scheme 3 New Peptide Synthetic System

Conclusion.

After all, new system of peptide synthesis was constituted of this peptide bond formation and the direct preparation of *N*-acylpyrazoles from the corresponding esters, as illustrated in Scheme 3. The extension of one amino acid unit on the peptide chain was required only 2 steps of independent reactions, the conversion from esters to *N*-acylpyrazoles and the subsequent aminolysis with amino esters. This new system was distinctive from the conventional peptide synthesis, which was consisted of 3 steps of the deprotection, the activation and the condensation. Moreover, the key intermediate *N*-acylpyrazoles exhibited the excellent properties of high sensitivity and separability for the chiral column on HPLC using the UV detector.

EXPERIMENTAL

NMR data were collected on a Varian NMR Gemini-200 (200 MHz) spectrometer in deuterochloroform with tetramethylsilane as an internal standard. UV spectra were measured in methanol by Shimadzu UV-3100PC spectrophotometer. Optical rotations were observed using a JASCO DIP-370 digital polarimeter. HPLC analysis was carried out by SIL-C18 (JASCO) column on JASCO BIP-I chromatograph and by CHIRALCEL OD-R (Daicel Chemical Industries) column on JASCO GULLIVER chromatograph series using aqueous methanol and acetonitrile. Melting points are uncorrected.

General Preparation of 1-(α-Aminoacyl)-3,5-dimethylpyrazoles.

Method A. To the mixture of 3,5-dimethylpyrazole (2.0 g, 21 mmol), *N*-protected amino acid or peptide (19.6 mmol) and triethylamine (7.0 g, 69 mmol) in dry toluene (40 mL), thionyl chloride (2.6 g, 22 mmol) in dry toluene (10 mL) was added and the mixture was stirred for 2 h at 5°C. The mixture was washed with dilute hydrochloric acid (1N), water, saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and concentrated. The product was purified by recrystallization.

Method B. *N*-Protected amino ester or peptide ester (2.0 mmol) was refluxed with hydrazine hydrate (250 mg, 5.0 mmol) in methanol (2 mL) for 80 min. To the subsequent mixture, the methanol solution (2 mL) of 2,4-pentanedione (500 mg, 5.0 mmol) and *p*-toluenesulfonic acid (125 mg, 0.66 mmol) was added and stirred for 16 h at rt. The reaction was quenched by the addition of water, and the product was extracted with ethyl acetate. The organic layer was washed with water, saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel or recrystallization.

1-(Cbz-glycyl)-3,5-dimethylpyrazole (**9a**).

mp 49-53°C (from hexane-benzene); yield 67 %; ^{1}H-NMR ($CDCl_3$): δ 2.21 (3H, s), 2.51 (3H, s), 4.73

(2H, d, J=6 Hz), 5.14 (2H, s), 5.56 (1H, br s), 5.96 (1H, s), 7.27-7.35 (5H, m). *Anal.* Calcd for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.63. Found: C, 62.59; H, 6.01; N, 14.54.

1-(Cbz-alanyl)-3,5-dimethylpyrazole (**9b**).

mp 114-115°C; yield 48 %; ^{1}H-NMR ($CDCl_3$): δ 1.52 (3H, d, J=6.8 Hz), 2.23 (3H, s) 2.52 (3H, s), 5.12 (2H, s), 5.57 (2H, m), 5.96 (1H, s), 7.35 (5H, s). *Anal.* Calcd for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.36; N, 13.94. Found: C, 63.62; H, 6.52; N, 13.75.

1-(Boc-alanyl)-3,5-dimethylpyrazole (**10b**)

mp 101.0-102.0°C (from hexane-benzene); yield 42 %; ^{1}H-NMR ($CDCl_3$): δ 1.44 (9H, s), 1.45 (3H, d, J= 6.9), 2.22 (3H, s), 2.53 (3H, d, J=0.8 Hz), 5.25 (1H, m), 5.53 (1H, m), 5.96 (1H, s). *Anal.* Calcd for $C_{13}H_{21}N_3O_3$: C, 58.41; H, 7.92; N, 15.72. Found: C, 58.29; H, 7.77; N, 15.66.

1-(N-phthaloylalanyl)-3,5-dimethylpyrazole (**11b**)

mp 157.0-159.0°C (from hexane-AcOEt); yield 50 %; ^{1}H-NMR ($CDCl_3$): δ 1.89 (3H, d, J=7.4 Hz), 2.11 (3H, s), 2.53 (3H, s), 5.91(1H, q, J=7.4 Hz), 5.92 (1H, s), 7.70-7.88 (4H, m). *Anal.* Calcd for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13. Found: C, 64.59; H, 5.14; N, 14.16.

1-(Cbz-phenylalanyl)-3,5-dimethylpyrazole (**9c**).

mp 141-142°C (from hexane-benzene); yield 84 %; $[\alpha]_D$:+73.3° (c 0.488, MeOH); ^{1}H-NMR ($CDCl_3$): δ 2.26 (3H, s), 2.47 (3H, s), 3.09 (1H, dd, J=13.9, 7.3 Hz), 3.34 (1H, dd, J=13.5, 4.3 Hz), 5.07 (2H, AB, J=12.9 Hz), 5.48 (1H, d, J=8.6 Hz), 5.82-5.89 (1H, m), 5.98 (1H, s), 7.05-7.08 (2H, m), 7.18-7.36 (8H, m). *Anal.* Calcd for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 70.01; H, 6.18; N, 11.15.

1-(Cbz-valyl)-3,5-dimethylpyrazole (**9d**).

mp 57.5-59.0°C (from hexane-AcOEt); yield 62 %; ^{1}H-NMR ($CDCl_3$): δ 0.87 (3H, d, J=6.8 Hz), 1.03 (3H, d, J=6.8 Hz), 2.23 (3H, s), 2.37 (1H, m), 2.51 (3H, d, J=0.8 Hz), 5.12 (2H, s), 5.50 (2H, m), 5.96 (1H, d, J=1 Hz), 7.35 (5H, s). *Anal.* Calcd for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76. Found: C, 65.67; H, 6.94; N, 12.45.

1-(Boc-valyl)-3,5-dimethylpyrazole (**10d**)

bp 125-130°C/ 6 mmHg; yield 29 %; ^{1}H-NMR ($CDCl_3$): δ 0.86 (3H, d, J=6.8 Hz), 1.02 (3H, d, J=6.8 Hz), 1.44 (9H, s), 2.22 (3H, s), 2.31 (1H, m), 2.52 (3H, s), 5.22 (1H, m), 5.22 (1H, m), 5.95 (1H, s). *Anal.* Calcd for $C_{15}H_{25}N_3O_3$: C, 60.99; H, 8.53; N, 14.23. Found: C, 60.86; H, 8.51; N, 14.19.

1-(Cbz-leucyl)-3,5-dimethylpyrazole (**9e**).

mp 86-87°C; yield 93 %; ^{1}H-NMR ($CDCl_3$): δ 0.93 (3H, d, J=6.0 Hz), 1.04 (3H, d, J=5.6 Hz), 1.55 (1H, m), 1.76 (2H, m), 2.22 (3H, s), 2.50 (3H, s), 5.11 (2H, s), 5.57 (2H, m), 5.95 (1H, s), 7.34 (5H, s). *Anal.* Calcd for $C_{19}H_{25}N_3O_3$: C, 66.45; H, 7.34; N, 12.24. Found: C, 66.47; H, 7.53; N, 12.12.

1-(Cbz-glycylglycyl)-3,5-dimethylpyrazole (**9f**).

mp 11[illegible]°C (from hexane-benzene); yield 66 %; ^{1}H-NMR ($CDCl_3$): δ 2.22 (3H, s), 2.51 (3H, s), 3.99

(2H, d, J=5.6 Hz), 4.80 (2H, d, J=5 Hz), 5.13 (2H, s), 5.70 (1H, s), 5.97 (1H, s), 6.90 (1H, s), 7.34 (5H, s). *Anal.* Calcd for $C_{17}H_{20}N_4O_4$: C, 59.29; H, 5.85; N, 16.27. Found: C, 59.04; H, 5.93; N, 15.90.

1-(Cbz-glycylalanyl)-3,5-dimethylpyrazole (**9g**).

mp 125.0-126.0°C (from hexane-benzene); yield 55 %; ^{1}H-NMR ($CDCl_3$): δ 1.52 (3H, d, J=7 Hz), 2.23 (3H, s), 2.51 (3H, s), 3.94 (2H, d, J=4.4 Hz), 5.15 (2H, s), 5.75 (1H, quint, J=7.4 Hz), 5.98 (1H, s), 7.36 (5H, s). *Anal.* Calcd for $C_{18}H_{22}N_4O_4$: C, 60.32; H, 6.19; N, 15.63. Found: C, 60.36; H, 6.34; N, 15.53.

1-(Cbz-glycylphenylalanyl)-3,5-dimethylpyrazole (**9h**).

mp 116.0-118.0°C (from hexane-AcOEt); yield 47 %; ^{1}H-NMR ($CDCl_3$): δ 2.25 (3H, s), 2.45 (3H, s), 3.10 (1H, ABX, J=7.0, 13.4 Hz), 3.32 (1H, ABX, J=4.7, 13.8 Hz), 3.86 (2H, s), 5.10 (2H, s), 5.56 (1H, m), 5.98 (1H, s), 6.04 (1H, q, J=6.3 Hz), 6.86-7.36 (10H, m). *Anal.* Calcd for $C_{24}H_{26}N_4O_4$: C, 66.34; H, 6.03; N, 12.89. Found: C, 66.14; H, 6.03; N, 12.81.

1-(Cbz-glycylvalyl)-3,5-dimethylpyrazole (**9i**).

bp 155-160°C/ 6 mmHg; yield 52 %; ^{1}H-NMR ($CDCl_3$): δ 0.83 (3H, d, J=6.8 Hz), 0.98 (3H, d, J=6.8 Hz), 2.22 (3H, s), 2.38 (1H, sept, J=6.3 Hz), 2.50 (3H, s), 3.95 (2H, d, J=5.4 Hz), 5.14 (2H, s), 5.61 (1H, broad s), 5.73 (1H, q, J=4.7 Hz), 5.96 (1H, s), 6.92 (1H, d, J=9.2 Hz), 7.34-7.36 (5H, m). *Anal.* Calcd for $C_{20}H_{26}N_4O_4$: C, 62.16; H, 6.78; N, 14.5. Found: C, 61.90; H, 6.82; N, 14.17.

1-(Cbz-glycylleucyl)-3,5-dimethylpyrazole (**9j**).

mp 49-50.5°C (from hexane-AcOEt); yield 43 %; ^{1}H-NMR ($CDCl_3$): δ 0.90 (3H, d, J=5.8 Hz), 1.01 (3H, d, J=5.6 Hz), 1.51 (3H, m), 2.21 (3H, s), 2.45 (3H, s), 3.95 (2H, d, J=4.4 Hz), 5.13 (2H, s), 5.60 (1H, m), 5.85 (1H, t, J=9.3 Hz), 5.95 (1H, s), 6.87 (1H, d, J=8.8 Hz), 7.35 (5H, d, J=4 Hz). *Anal.* Calcd for $C_{21}H_{28}N_4O_4$: C, 62.98; H, 7.05; N, 13.99. Found: C, 63.35; H, 7.2; N, 13.95.

1-(Cbz-alanylglycyl)-3,5-dimethylpyrazole (**9k**).

mp 142-143°C (from hexane-AcOEt); yield 72 %; ^{1}H-NMR ($CDCl_3$): δ 1.44 (3H, d, J=7.2 Hz), 2.22 (3H, s), 2.51 (3H, s), 4.36 (1H, m), 4.78 (2H, d, J=5.4 Hz), 5.12 (2H, s), 5.97 (1H, s), 7.34 (5H, m). *Anal.* Calcd for $C_{18}H_{22}N_4O_4$: C, 60.32; H, 6.19; N, 15.63. Found: C, 60.07; H, 6.19; N, 15.54.

1-(Cbz-phenylalanylglycyl)-3,5dimethylpyrazole (**9m**).

mp 124.0-126.0°C (from hexane-AcOEt); yield 39 %; ^{1}H-NMR ($CDCl_3$): δ 2.21 (3H, s), 2.49 (3H, s), 3.12 (2H, d, J=6.8 Hz), 4.57 (1H, m), 4.72 (2H, dd, J=5.2 Hz), 5.07 (2H, s), 5.96 (1H, s), 7.25 (10H, m). *Anal.* Calcd for $C_{24}H_{26}N_4O_4$: C, 66.34; H, 6.03; N, 12.89. Found: C, 66.36; H, 6.16; N, 12.89.

1-(Cbz-valylglycyl)-3,5-dimethylpyrazole (**9n**).

mp 180-181°C (from hexane-AcOEt); yield 44 %; ^{1}H-NMR ($CDCl_3$): δ 0.98 (6H, dd, J=2.2, 2.0 Hz), 1.27 (1H, m), 2.22 (3H, s), 2.52 (3H, s), 4.14-4.21 (1H, m), 4.80 (2H, s), 5.12 (2H, s), 5.59 (1H, d, J=9 Hz), 5.97 (1H, s), 6.85 (1H, s), 7.34 (5H, s). *Anal.* Calcd for $C_{20}H_{26}N_4O_4$: C, 62.16; H, 6.78; N, 14.5.

Found: C, 62.02; H, 6.77; N, 14.24.

1-(Cbz-leucylglycyl)-3,5-dimethylpyrazole (**9p**).

mp 129.0-131.0°C (from hexane-benzene); yield 55 %; ^{1}H-NMR ($CDCl_3$): δ 0.96 (6H, d, J=3.6 Hz), 1.57-1.90 (3H, m), 2.23 (3H, d, J=2.8 Hz), 2.53 (3H, s), 4.79 (2H, d, J=2 Hz), 5.13 (2H, s), 5.35 (1H, d, J=7.2 Hz), 5.98 (1H, s), 6.85 (1H, m), 7.35 (5H, s). *Anal.* Calcd for $C_{21}H_{28}N_4O_4$: C, 62.98; H, 7.05; N, 13.99. Found: C, 62.82; H, 7.14; N, 14.11.

1-(Cbz-alanylglycylglycyl)-3,5-dimethylpyrazole (**9q**).

mp 162.0-164.0°C (from hexane-AcOEt); ^{1}H-NMR ($CDCl_3$): δ 1.41 (3H, d, J=7 Hz), 2.22 (3H, s), 2.48 (3H, s), 4.06 (2H, m), 4.278 (1H, quint, J=6.9 Hz), 4.78 (2H, d, J=5.4 Hz), 5.08 (2H, d, J=5.4 Hz), 5.60 (1H, d, J=6.8 Hz), 5.96 (1H, s), 7.13 (2H, br s), 7.32 (5H, s). *Anal.* Calcd for $C_{20}H_{25}N_5O_5$: C, 57.82; H, 6.07; N, 16.86. Found: C, 57.56; H, 6.13; N, 16.62.

The Properties of 1-(*N*-Protected α-Aminoacyl)-3,5-dimethylpyrazoles (9-11) on HPLC.

The racemic mixture of **9-11** was resolved on the HPLC on chiral column (CHIRALCEL OD-R) with aqueous methanol or acetonitrile, and the separability factor was evaluated from the chromatogram. The UV absorption spectra were also measured in methanol. The results were summarized in Table 2.

General Peptide Bond Formation.

The THF solution (6 mL) of α-amino ester hydrochloride (3.0 mmol), 1-(α-Cbz-aminoacyl)-3,5-dimethylpyrazole (**9**, 1.5 mmol) and triethylamine (380 mg, 3.8 mmol) was refluxed for 80 min. After quenching the reaction with dilute hydrochloric acid (1 N), the product was extracted with ethyl acetate. The organic layer was washed with water, saturated $NaHCO_3$ and brine, and dried over anhydrous $MgSO_4$. The solvent was removed and the residue was recrystallized from hexane-ethyl acetate mixture. Optical purity of the resulting peptide ester was evaluated by means of HPLC on chiral column (CHIRALCEL OD-R).

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