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Dehydrooligopeptides. X. Useful Synthetic Method for (*E*)- and (*Z*)-Isomers of Dehydroaspartic Acids, and Their $\Delta^{1,2}$-Dehydrodipeptides by the Base-Catalyzed β-Elimination[1]

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Various kinds of *N*-benzyloxycarbonyl (*E*)- and (*Z*)-dehydroaspartic acid (Cbz-ΔAsp) derivatives were successfully synthesized by both a base-catalyzed β-elimination of β-hydroxyaspartic acid esters (HyAsp) via their β-mesyloxy Asp derivatives and several modes of the cleavages of α-(*N*-Cbz)-aminomaleic acid anhydride. Moreover, for high-yield synthesis of $\Delta^{1,2}$-dehydrodipeptides (DHP) containing a ΔAsp residue, a similar β-elimination was applied to Δ^{1}-DHP with a C-terminal HyAsp residue. The configuration of all the new ΔAsp derivatives and their $\Delta^{1,2}$-DHPs were confirmed mainly on the basis of ^{1}H NMR spectral data.

In the course of our work regarding the synthesis of α-dehydroamino acid (DHA; ΔAA) and dehydrooligopeptide (DHP), in particular, various kinds of DHA derivatives, which corresponded to the proteinic α-amino acid (AA), have so far been synthesized.[2-7] Recently, the reason why much attention has been directed on the synthesis and the chemical property of DHA is because the *N*-carboxy α-dehydroamino acid anhydride (ΔNCA), derived from *N*-benzyloxycarbonyl (Cbz)-DHA and thionyl chloride, has very effective and extensive coupling abilities with H-AA-OH and oligopeptide for the DHP synthesis, as already reported.[3,5,8-10]

A synthesis of the (*Z*)-isomer of *N*-protected dehydroaspartic acid dimethyl ester [ΔAsp(OMe)-OMe] has already been reported by Kolasa and Gross.[11] However, simple synthetic routes to the various kinds of (*E*)- and (*Z*)-ΔAsp derivatives have not yet appeared in the literature. Particularly, in order to synthesize the important partial skeleton of phomopsin A containing (*E*)-ΔAsp moiety (Fig. 1), isolated from the culture of *Phomopsis leptostromiformis*,[12] a high-yield syntheses of (*E*)-ΔAsp derivatives and their $\Delta^{1,2}$-dehydrodipeptides (DHP)[13] containing dehydroisoleucine (ΔIle) residue must be accomplished by all means.

In the present paper, we wish to report on a useful syntheses of (*E*)- and (*Z*)-ΔAsp derivatives from both β-hydroxyaspartic acids (HyAsp-OH) and α-(*N*-Cbz)-aminomaleic acid anhydride, and those of $\Delta^{1,2}$-DHP (ΔAA-ΔAsp) by the coupling of appropriate ΔNCA with HyAsp-OMe, followed by a base-catalyzed β-elimination. Moreover, based on ^{1}H NMR spectral data, the configurational structure of ΔAsp derivatives and the corresponding $\Delta^{1,2}$-DHPs could be clearly determined.

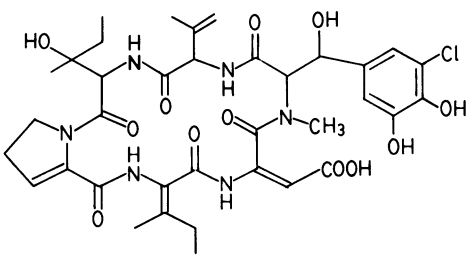


Fig. 1. Phomopsin A.

Results and Discussion

Syntheses of (*E*)- and (*Z*)-ΔAsp Derivatives. It has already been reported that the β-elimination of Cbz-β-(tosyloxy)aspartic acid dimethyl ester, derived from Cbz-β-(hydroxy)aspartic acid dimethyl ester [Cbz-HyAsp(OMe)-OMe] (**1a**) and *p*-toluenesulfonyl (tosyl) chloride, with organic base such as 1,4-diazabicyclo-[2.2.2]octane (Dabco) or 1,8-diazabicyclo[5.4.0]undec-5-ene (DBU) gave only the (*Z*)-isomer of Cbz-ΔAsp(OMe)-OMe [(*Z*)-**3a**].[11] In addition, detailed formation on the mechanism of (*Z*)-**3a** and its DHP was also discussed. However, syntheses of not only the (*E*)-isomer of **3a** but also its peptides have not been described.

At first, in order to investigate the synthesis of various (*E*)- and (*Z*)-isomers of ΔAsp derivatives **3**, instead of the above tosylation, the mesylation of **1a** was carried out, followed by a similar base-catalyzed β-elimination, by a previously reported method.[14]

The reaction of *threo*- and *erythro*-**1a**, respectively, which were freshly prepared (Table 1), with methanesulfonyl (mesyl; Ms) chloride in tetrahydrofuran (THF), was performed to yield the corresponding β-mesyloxy Asp derivatives (**2a**). Subsequently, the leaving mesyloxy group of **2a**, formed as an intermediate, was eliminated in situ with a base, such as triethylamine (TEA) or DBU, to give first an (*E*)-isomer of **3a** as a mixture with (*Z*)-**3a**. At the same time, the intermediate **2a** was also obtained purely. However, as can be seen in the summary given in Table 2, as long as the present method is used, the yield of (*E*)-**3a** was found to be less than 43%, although that of (*Z*)-**3a** was more than 72%. In any event, as a result, the expected (*E*)-**3a** was ultimately obtained, even though the yield was slightly low. Fortunately,

Table 1. β-Hydroxy- and β-Mesyloxyaspartic Acid Esters (**1** and **2**)

	Compound[a]	Yield %	Mp θ_m/°C	IR, ν/cm^{-1} in KBr -COO-	^{1}H NMR, δ in $CDCl_3$ (J/Hz) -NH-	-OH	α-H	β-H
1a	Threo[b]	77	129—130[c]	1760 1695	5.54d	3.44bs	4.44dd (9.0, 2.0)	4.55d (2.0)
	Erythro[b]	68	Syrup	1755 1740 1722	6.22d (9.0)	4.20d (4.5)	4.89dd (9.0, 2.5)	4.48dd (4.5, 2.5)
2a	Threo	70	Syrup	1760 1727	5.80—5.44m	—	5.08dd (9.5, 2.5)	5.50d (2.5)
	Erythro	73	98—99[d]	1775 1748 1690	5.76d (8.5)	—	4.96—5.24m	5.43d (3.0)
1b	Erythro	14	Syrup	1748 1740	6.08d (8.5)	3.91d (4.0)	4.80dd (8.0, 2.0)	4.40d (4.0, 2.0)
2b	Erythro	19	Syrup	1765 1730 1728	5.64d (8.0)	—	4.70dd (8.0, 2.0)	4.40d (2.0)
1c	Erythro	55	140—142[e]	1770 1745	7.52—7.12m (+Ph)		4.56dd (9.0, 4.0)	4.36d[f] (4.0)

a) All the compounds were analyzed for C, H, and N, and the results were within ±0.3% of theoretical values. b) Ref. 15. c) Colorless needles from benzene. d) Colorless needles from diisopropyl ether. e) Colorless needles from hexane-ethyl acetate. f) Measured in DMSO-d_6.

Table 2. Synthesis of **3** by the Base-Catalyzed β-Elimination of **1a** via **2a**

Substrate **1a**	Base	Time/h	Yield/% (*E*)-**3a**	(*Z*)-**3a**	**2a**	Recovery/% **1a**
Threo	TEA	2	2	27	70	—
	TEA	6	8	65	24	—
	TEA	16	12	72	15	—
	DBU	2	30	32	0	35
Erythro	TEA	1	1	2	73	—
	TEA	6	8	20	55	—
	TEA	24	11	49	34	3
	DBU	1	43	15	0	39
	DBU	2	29	29	0	38

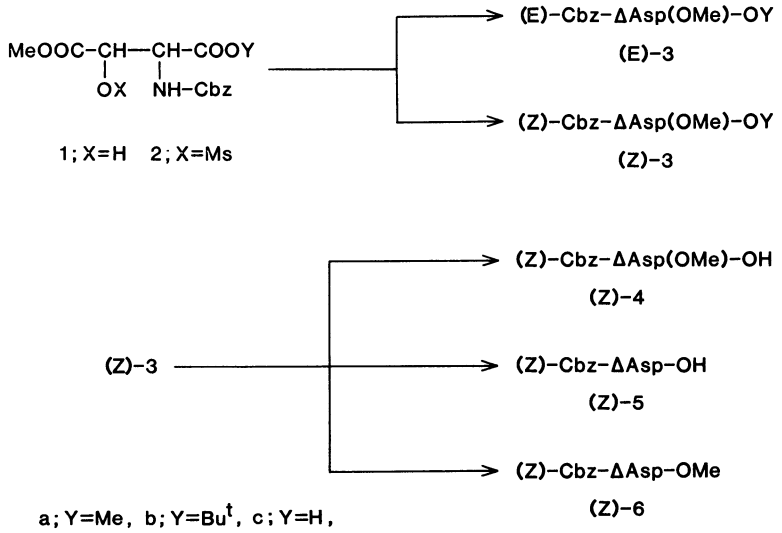
according to the another method described later, various (*E*)-isomer of ⊿Asp derivatives could be successfully synthesized in good yields.

A subsequent ester hydrolysis of (*Z*)-**3a**, thus obtained, with an equimolar 1 M LiOH (1 M=1mol dm^{-3}) was carried out in dioxane under cooling to give three kinds of products. The three compounds could be readily isolated by a silica-gel column chromatogram method and were identified as being (*Z*)-Cbz-⊿Asp(OMe)-OH [(*Z*)-**4**] and (*Z*)-Cbz-⊿Asp-OH [(*Z*)-**5**] in 45 and 3% yields, respectively, along with a trace of a by-product, also identified as (*Z*)-Cbz-⊿Asp-OMe [(*Z*)-**6**], as was illustrated in Scheme 1.

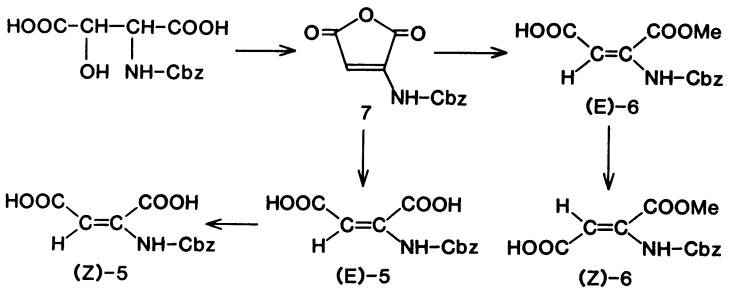
Furthermore, when three moles of 1 M LiOH was used, compound (*Z*)-**5** alone was found to be obtained in 71% yield. In the above case, although it was difficult to confirm whether the structure of mono ester of ⊿Asp (**4** and **6**) is α-methyl or β-methyl ester, an independent preparation of (*Z*)-**4** was successfully tried in the following manner.

First, according to the method mentioned above, (*E*)- and (*Z*)-Cbz-⊿Asp(OMe)-OBu^t (**3b**) were synthesized in 35 and 40% yields, respectively, by the successive mesylation and β-elimination of Cbz-HyAsp(OMe)-OBu^t (**1b**), which was derived from Cbz-HyAsp(OMe)-OH (**1c**)[15] and isobutene. On the other hand, it was found that a small amount of the expected Cbz-β-(Bu^{t}O)-Asp(OMe)-OBu^t was also obtained. Subsequently, the *t*-butyl ester decomposition of both (*E*)- and (*Z*)-**3b**, respectively, with HCl was carried out in ethyl acetate to give only one chemical species almost quantitatively, which was in complete accord with (*Z*)-**4**.

From the above results, the structural determination of **4** was exactly accomplished; it can be seen that the



Scheme 1.



Scheme 2.

isomerization of (*E*)-**3b** took place during the ester decomposition.

Moreover, in order to obtain the (*E*)-isomers of **4**—**6** in high yields, the authentic 2-(*N*-Cbz)-aminomaleic acid anhydride (**7**)[16] was cleaved with 1 M LiOH in the absence of methanol to give (*Z*)-**5** in an almost quantitative yield, while the treatment of **7** with base such as dicyclohexylamine (DCA) or TEA was carried out in methanol to give a hitherto unknown kind. Concerning the configurational structure of the unknown compound, thus obtained, from both the spectral data (IR and ^{1}H NMR) and the satisfactory elemental analysis ($C_{13}H_{13}NO_6$), it was supposed that a kind of the possible total four regio and geometric isomers of the corresponding ⊿Asp monomethyl esters [(*Z*)-**4**, (*E*)-**4**, (*Z*)-**6**, and (*E*)-**6**] was formed. Furthermore, by a comparison with various ⊿Asp derivatives already obtained (see above), it could be concluded that the expected (*E*)-Cbz-⊿Asp-OMe [(*E*)-**6**] was first synthesized in 84% yield. Subsequent isomerization of (*E*)-**6** with HCl was completely performed in ethyl acetate to yield (*Z*)-**6**, which was in accord with the product derived by the hydrolysis of (*Z*)-**3a**. In addition, (*Z*)-**6** was also found to be obtainable quantitatively by a treatment of **7** with methanol in the presence of TEA. Accordingly, the configurational structure of (*E*)-**6** could be unambiguously determined.

On the other hand, more interestingly, it was also found that a treatment of **7** with a saturated $NaHCO_3$ aqueous solution gave (*E*)-Cbz-⊿Asp-OH [(*E*)-**5**] in 95% yield, which was readily isomerized with 1 M LiOH in dioxane to give (*Z*)-**5** quantitatively, according to Scheme 2. Consequently, as summarized in Table 3, all of the (*E*)- and (*Z*)-isomers of **3**—**6**, except for (*E*)-**4**, were obtained purely.

The yields, physical constants, and spectral data of ⊿Asp derivatives **3**—**6** are summarized in Table 3. Particularly, based on the fact that the olefin proton signal of (*E*)-isomer (δ 6.17) of DHA shifted always at lower magnetic field than that of (*Z*)-isomer (δ 5.54), similar to the results reported previously,[17] the configuration of all the ⊿Asp derivatives, thus obtained, could be clearly confirmed.

Synthesis of $\Delta^{1,2}$-DHP Containing ⊿Asp Residue. It has already been reported that the successive mesylation and base-catalyzed β-elimination of Δ^1- or Δ^2-DHP containing N- and C-terminal threonine residues respectively were carried out to give $\Delta^{1,2}$-

Table 3. (*E*)- and (*Z*)-Cbz-⊿Asp-OH Derivatives **3**—**6**

Compound[a]	Yield %	Mp θ_m/°C	IR, ν/cm^{-1} in KBr -NHCO-	-C=C-	^{1}H NMR, δ in $CDCl_3$ (DMSO-d_6) -NH-	-CH=
(*E*)-**3a**	30	Syrup	1715 1540	1630	7.49bs	6.40s
(*Z*)-**3a**	72	Syrup	1695 1485	1640	9.63bs	5.44s
(*E*)-**3b**	36	Syrup	1715 1535	1628	7.09bs	6.64s
(*Z*)-**3b**	40	Syrup	1685 1480	1635	9.62bs	5.40s
(*Z*)-**4**	91	121—122[b]	1692 1495	1632	(9.85bs	5.67s)
(*E*)-**5**	95	161—162[c]	1710 1500	1628	(10.10bs	5.78s)
(*Z*)-**5**	quant.	148—149[d]	1695 1518	1630	(9.78bs	5.53s)
(*E*)-**6**	94	146—147[c]	1682 1545	1625	(10.59bs	5.81s)
(*Z*)-**6**	quant.	84—85[b]	1630	1635	(9.95bs	5.58s)

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

Table 4. The Yields and Melting Points of **10**

Compound	HyAsp	Yield %	Mp[c] θ_m/°C	Formula	Found (%) C	H	N	Calcd (%) (C	H	N)
10a	t[a]	30	92—93	$C_{15}H_{24}N_2O_8$	49.69	6.69	7.68	(49.99	6.71	7.77)
	e[b]	31	110—113		49.85	6.67	7.81	(49.99	6.71	7.77)
10b	t	42	132—136	$C_{16}H_{26}N_2O_8$	50.81	7.02	7.38	(51.33	7.00	7.48)
	e	30	136—137		50.95	7.04	7.42	(51.33	7.00	7.48)
10c	t	40	144—146	$C_{17}H_{28}N_2O_8$	52.40	7.25	7.18	(52.57	7.27	7.21)
	e	26	139—142		52.72	7.18	7.17	(52.57	7.27	7.21)
10d	t	53	128—129	$C_{20}H_{26}N_2O_8$	56.57	6.20	6.58	(56.86	6.20	6.63)
	e	38	146—147		56.77	6.24	6.59	(56.86	6.20	6.63)

a) Threo. b) Erythro. c) Threo=colorless prisms from hexane-ethyl acetate. Erythro=colorless needles from hexane-ethyl acetate.

DHP.[14] By taking advantage of the above-mentioned useful procedure, the desired various ⊿^{1,2}-dehydrodipeptides containing C-terminal ⊿Asp(OMe) residue were successfully synthesized as follows.

Firstly, for a high-yield synthesis of ⊿^{1}-DHP with a C-terminal HyAsp residue as the substrates, the so-called ⊿NCA method developed by us was carried out with very good results. Various (*Z*)-isomer of ⊿NCA derivatives (**8**: **a**; ⊿Abu,[18] **b**; ⊿Val, **c**; ⊿Ile)[2,3,5] were treated with di-*t*-butyl dicarbonate [$((CH_3)_3COCO)_2O$; $(Boc)_2O$] in the presence of pyridine and then with *threo*- or *erythro*-HyAsp(OMe)-OMe as an amine component in the presence of *N*-methylmorpholine (NMM) at pH 9 to give the corresponding (*Z*)-*t*-butoxycarbonyl (Boc)-⊿^{1}-dehydrodipeptide dimethyl ester [Boc-⊿AA-HyAsp(OMe)-OMe] (**10**) in ca. 36% overall yield.

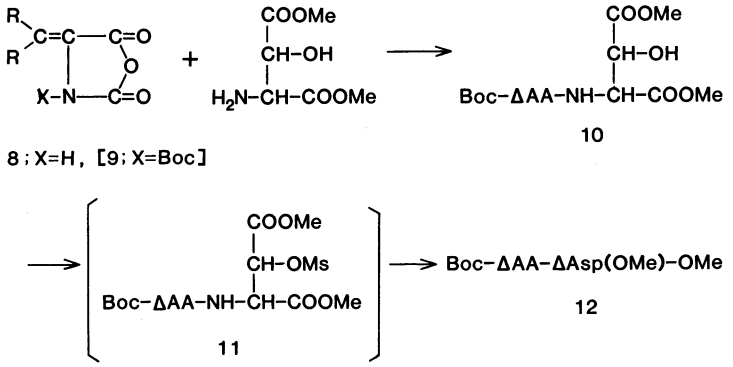
From the above result, it is obvious that the acylation of **8** with $(Boc)_2O$ occurred at first to form Boc-⊿NCA (**9**) as an intermediate, which was immediately coupled with HyAsp(OMe)-OMe in one-pot to give **10**. Even though the overall yield of **10** was lower (at present) these procedures seem to be very useful for the synthesis of ⊿^{1}-dehydrodipeptide esters (**10**), as summarized in Tables 4 and 5.

Subsequently, the mesylation of **10** with mesyl chloride and then the β-elimination of the mesyloxy derivatives (**11**), formed as unstable intermediates, were carried out in situ in THF in the presence of TEA or DBU to give first the expected Boc-⊿^{1,2}-dehydrodipeptide dimethyl ester [Boc-⊿AA-⊿Asp(OMe)-OMe] (**12**). In the case of **10a**—**c** containing a *threo*-HyAsp

Table 5. The IR and ^{1}H NMR Spectral Data of **10**

Compound	HyAsp	IR, ν/cm^{-1} in KBr -NHCO-		^{1}H NMR, δ in $CDCl_3$ (J/Hz) -CH=	-OH	NH-CH-CO
10a	t[a]	1685,	1545	6.48q (7.0)	3.96bs	5.12dd (2.5, 9.5)
	e[b]	1665,	1550	6.52q (7.0)	4.10d	5.16dd (2.0, 8.0)
10b	t	1680,	1540	—	3.88bs	4.96dd (2.0, 8.2)
	e	1665,	1540	—	4.22bs	5.16dd (3.0, 8.0)
10c	t	1680,	1535	—	4.08bs	4.92dd (2.0, 8.0)
	e	1670,	1540	—	4.22bs	5.18dd (2.0, 10.0)
10d	t	1650,	1542	6.90s	4.26bs	5.00dd (2.0, 10.0)
	e	1674,	1548	7.11s	4.02d	5.12dd (2.5, 7.5)

a) Threo. b) Erythro.



Scheme 3.

residue, the above-mentioned successive reactions were performed in the presence of TEA for 24 h to give only a (*Z*,*Z*)-isomer of **12a—c** in 51—92% yield, as summarized in Table 6. On the other hand, when compound **10b** containing *erythro*-HyAsp residue was similarly worked up in the presence of DBU for 4.5 h, Boc-⊿Val-(*E*)-⊿Asp(OMe)-OMe [(*E*)-**12b**] was obtained in a 20% yield, whereas the similar reaction of *erythro*-**10c** gave Boc-(*E*)- and (*Z*)-⊿Ile-(*Z*)-⊿Asp-(OMe)-OMe [(*E*,*Z*)- and (*Z*,*Z*)-**12c**] in a 23% yield. In this case, the N-terminal (*Z*)-⊿Ile of **10c** containing either *threo*- or *erythro*-HyAsp residue was found to be isomerized partially to give a mixture of (*E*,*Z*)- and (*Z*,*Z*)-**12** during β-elimination.

As Table 8 shows, the chemical shifts of the olefin proton of two isomers, (*Z*,*E*)- and (*Z*,*Z*)-**12**, are clearly distinguishable from each other; that is, the signal of (*E*)-⊿Asp (δ 6.72) shifts at a considerably lower magnetic field than that of (*Z*)-⊿Asp (δ 5.40) residue.[17] From this fact, the configurations of **12** could be readily confirmed. Furthermore, from the above results, it can be seen that the formation and yield of each geometric isomers of **12** results from differences between the structure of substrates, which contain *erythro*- or *threo*-HyAsp(OMe)-OMe residue, and the reaction conditions. Accordingly, if only product (*Z*,*Z*)-**12** is desired, the reaction time must necessarily to be prolonged in the presence of TEA, although only an example is shown. On the contrary, if an appropriate **12** containing (*E*)-⊿Asp residue is expected, it seems to be preferable to shorten the reaction time in the presence of DBU. Unfortunately, however, it is not understandable why the similar β-elimination of **10** containing *erythro*-HyAsp residue gave either (*Z*, *E*)- or (*Z*,*Z*)-**12**, as summarized in Tables 6, 7, and 8. Consequently, at present, it is difficult to

Table 6. Base-Catalyzed β-Elimination of **10**

Compound **10**		Reaction conditions		Yield of **12**/%	
⊿AA	HyAsp	Base	Time/h	(*E*)-⊿AsP	(*Z*)-⊿AsP
⊿Val	Threo	TEA	24	0	92
⊿Ile	Threo	TEA	24	0	67
⊿Abu	Threo	TEA	24	0	51
⊿Val	Erythro	DBU	4.5	20	0
⊿Ile	Erythro	DBU	4.5	0	23

Table 7. The Melting Points of **12**

Compound No.	⊿AA	Geometry	Mp θ_m/°C	Formula	Found (%)			Calcd (%)		
					C	H	N	(C	H	N)
12a	⊿Abu	(*Z*, *Z*)	Syrup	$C_{14}H_{20}N_2O_7$	51.82	6.25	8.39	(51.21	6.14	8.53)
12b	⊿Val	(*Z*)	120—122[b]	$C_{15}H_{22}N_2O_7$	52.80	6.61	7.91	(52.62	6.48	8.18)
		(*E*)	144—146[c]		52.59	6.53	7.95	(52.62	6.48	8.18)
12c	⊿Ile[a]	(*E*, *Z*) (*Z*, *Z*)	128—131[b]	$C_{16}H_{24}N_2O_7$	53.67	7.04	7.74	(53.92	6.79	7.86)

a) (*E*, *E*)- and (*Z*, *Z*)-**12** obtained as a mixture of 2:3 ratio. b) Colorless needles from hexane–ethyl acetate. c) Colorless prisms from hexane–ethyl acetate.

Table 8. The IR and ^{1}H NMR Spectral Data of **12**

Compound	Geometry	IR, ν/cm^{-1} in KBr -NHCO-		^{1}H NMR, δ in $CDCl_3$ (J/Hz) -CH=	-CH=	-NH-
12a	(*Z*, *Z*)	1635,	1480	6.54q (7.5)	5.39s	10.76bs
12b	(*Z*)	1630,	1480	—	5.40s	10.72bs
	(*E*)	1640,	1540	—	6.72s	8.62bs
12c	(*E*, *Z*)	1635,	1480	—	5.41s	10.74bs
	(*Z*, *Z*)				5.39s	10.69bs

obtain selectively and quantitatively only one of the geometric isomers.

In conclusion, it is noteworthy that various kinds of (*E*)- and (*Z*)-⊿Asp derivatives and their dehydrodipeptides were readily synthesized from several routes and that their configurations could be completely determined. Moreover, it is firmly believed that these obtained results will contribute to the synthesis of the important partial skeleton of phomopsin A.

Experimental

General. Melting points were determined with a Yamato micro melting-point apparatus MP-21 model and were uncorrected. The IR spectra were recorded with a Hitachi EPI-G2 grating spectrometer. The ^{1}H NMR spectra were measured with a JEOL JMN PS 100 spectrometer in a $CDCl_3$ solution with tetramethylsilane as the internal standard.

Preparation of 1c. To a solution of β-HyAsp(OMe)-OH (4.0 g, 24.5 mmol) in a saturated $NaHCO_3$ aqueous solution (61 ml) was added a solution of Cbz-Cl (5 ml, 29.4 mmol) in dry diethyl ether (20 ml), with vigorous stirring, drop by drop under cooling. After continuous stirring at room temperature for 1.5 h, Cbz-Cl (2.7 ml, 16.0 mmol) and $NaHCO_3$ (2.6 g, 30.0 mmol) were further added to the resultant solution at room temperature for 2 h. The reaction solution was poured into water (100 ml) and washed with ethyl acetate. The aqueous layer was made to pH 2 with 1 M HCl and then extracted three times with ethyl acetate (30 ml). The combined extracts were washed with saturated NaCl aqueous solution and then dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the residue was solidified with hexane and then the crude crystals were collected. Recrystallization from a mixture of hexane and ethyl acetate gave **1c** as colorless needles.

Reaction of 1c with Isobutene. Into a suspension of **1c** (3.56 g, 1.2 mmol) in dry CH_2Cl_2 (50 ml) was introduced isobutene gas below −40 °C, until the suspension volume increased to about 75 ml. After adding concentrated sulfuric acid (0.12 ml), the resulting suspension was put into a sealed tube and then allowed to stand at room temperature for 10 days until becoming homogeneous. The reaction solution was poured into a saturated $NaHCO_3$ aqueous solution (150 ml) and the resultant solution was extracted three times

with $CHCl_3$. The combined extracts were washed with water and then dried over anhydrous Na_2SO_4. After removing the solvent, the residue was purified on a silica-gel column using a mixture of benzene and acetone (30:1 v/v) as the eluent. The first fraction gave **1c** (0.6 g) and the second **2b** (0.9 g) in 19% yield.

Preparation of (*E*)- and (*Z*)-3a. To a solution of **1a** (2g, 6.43 mmol) and MsCl (1.1 g, 9.7 mmol) in dry THF (20 ml) was added drop by drop a solution of DBU or TEA (19.3 mmol) in dry THF (10 ml) at 0 °C. After stirring at 0 °C for 1 h, the resulting chilled solution was returned to room temperature and then allowed to stand. After removing the solvent, the residue was dissolved in ethyl acetate (100 ml) and the resultant solution was washed successively with 10% citric acid aqueous solution, saturated $NaHCO_3$ solution, water and finally dried over anhydrous $MgSO_4$. After concentrating the solution under reduced pressure, the residual syrup was chromatographed on a silica-gel column using a mixture of benzene and acetone (30:1 v/v) as the eluent. Three or four fractions were always obtained, as summarized in Table 2. The expected (*E*)- and (*Z*)-**3a** and intermediates Cbz-β-(MsO)Asp(OMe)-OMe (**2a**) are listed in Table 3.

Preparation of (*E*)- and (*Z*)-3b. In a similar manner, a treatment of **1b** with MsCl in the presence of DBU was worked up to give (*E*)- and (*Z*)-**3b**, no accompanying the corresponding β-mesyloxy derivative (**2b**). See Table 3.

Hydrolysis of 3a with LiOH. To a solution of (*Z*)-**3a** (4.26 g, 14.5 mmol) in dioxane (15 ml) was added 1 M LiOH (15 ml) drop by drop at 5—8 °C. The resulting solution was slowly returned to room temperature and then stirred continuously for 3.5 h. The reaction solution was poured into water (100 ml) and then washed with diethyl ether. The aqueous layer was acidified to pH 1 with 6 M HCl and extracted three times with ethyl acetate (50 ml). The combined extracts were washed with saturated NaCl aqueous solution and then dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure the residual crude crystals obtained were recrystallized from benzene to give (*Z*)-**4** as colorless needles. The filtrate was concentrated under reduced pressure to give a viscous syrup, which was chromatographed on a silica-gel column using a mixture of $CHCl_3$, methanol and ethyl acetate (200:1:1 v/v) to give three fractions. Compound (*Z*)-**4** was further obtained in a 45% yield from the first fraction and small amounts of (*Z*)-**5** and **6** were also obtained in 3 and 2% yields, respectively, from the second and the final fractions. On the other hand, from the ether layer (*Z*)-**3a** was recovered in 40% yield.

Similarly, the hydrolysis of (*Z*)-**3a** was worked up with 1 M LiOH (45 ml) at room temperature for 24 h to give (*Z*)-**5** in 71% yield. In addition, in a similar manner, treatment of (*E*)-**3a** was performed to give (*Z*)-**4**, **5**, and **6** in almost the same ratio obtained above.

Ester Decomposition of 3c. A solution of (*Z*)- or (*E*)-**3c** (1.0 g, 3.3 mmol) in ethyl acetate (30 ml) saturated with gaseous HCl was stirred at room temperature for 1.5 h. After removing the solvent and excess HCl under reduced pressure, the residue was dissolved in ethyl acetate (30 ml) and the resulting solution was again concentrated under reduced pressure. The residue,thus obtained, was crystallized by treating with hexane. The collected crystals were recrystallized from small quantity of ethyl acetate to give (*Z*)-**4** alone in 91% yield.

Preparation of (*E*)-5 from 7. a) Method A: A solution of **7** (2.47 g, 10.0 mmol) in saturated $NaHCO_3$ aqueous solution (50 ml) was stirred at room temperature for 1 h. The reaction solution was washed with diethyl ether and the aqueous layer was made to pH 2 with 1 M HCl and then extracted three times with ethyl acetate (50 ml). The combined extracts were washed with a saturated NaCl aqueous solution and then dried over anhydrous Na_2SO_4. After concentrating the solution under reduced pressure, the residue, thus obtained was crystallized by treating with diisopropyl ether. The collected crude crystals were recrystallized from a mixture of diisopropyl ether and ethyl acetate (5:2 v/v) to give (*E*)-**5** as colorless needles quantitatively.

b) Method B: To a solution of **7** (2.47 g, 10.0 mmol) in dioxane (30 ml) was added 1 M LiOH (13.5 ml) under cooling. After stirring for 1 h, ice-water (100 ml) was added to the reaction solution and the resulting solution was washed with chilled diethyl ether and then made to pH 2 with 1 M HCl. The acidified solution was similarly worked up to give (*E*)-**5**, according to Method A.

Preparation of (*Z*)-5. a) Method A: To a solution of **7** (2.47 g, 10.0 mmol) in dioxane (25 ml) was added 1 M LiOH (25 ml) at room temperature. After stirring for 1 h, the reaction solution was made to pH 2 with 1 M HCl and then extracted three times with ethyl acetate (50 ml). The combined extracts were washed with a saturated NaCl aqueous solution and dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the residue was crystallized by treating with hexane. Recrystallization from a mixture of ethyl acetate and hexane gave (*Z*)-**5** as colorless needles quantitatively.

b) Method B: The treatment of the solution of (*E*)-**5** (2.65 g, 10.0 mmol) in dioxane (30 ml) with 1 M LiOH (25 ml) was similarly worked up to give (*Z*)-**5** quantitatively by the isomerization.

Preparation of (*E*)-6. a) Method A: To a solution of **7** (1.0 g, 4.05 mmol) in dry methanol (10 ml) was added drop by drop a solution of DCA (0.8 ml, 4.05 mmol) in dry methanol (5 ml). After stirring for 30 min and then for 1.5 h at room temperature, the reaction solution was concentrated under reduced pressure. The crystalline residue, thus obtained, was dissolved successively in 10% citric acid (30 ml) and ethyl acetate (30 ml); then the resulting solution was vigorously shaken. The aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the crude crystals collected by using diisopropyl ether were recrystallized from small amount of ethyl acetate to give (*E*)-**6** as colorless needles. 83% yield.

b) Method B: To a solution of **7** (1.0 g, 4.05 mmol) in dry methanol (10 ml) was added drop by drop a solution of TEA (0.57 ml, 4.05 mmol) in dry methanol (5 ml) under cooling. The resultant solution was stirred for 30 min and then at room temperature for 30 min. The treatment of the reaction solution was similarly worked up to give (*E*)-**6** almost quantitatively.

Preparation of (*Z*)-6. a) Method A: To a solution of **7** (1.0 g, 4.05 mmol) in dry methanol (10 ml) was added drop by drop TEA (0.86 ml, 6.08 mmol) under cooling. The

resulting solution was allowed to stand at room temperature and then stirred continuously for 12 h. After removing the solvent, the residue was dissolved in 10% citric acid (30 ml) and the resultant solution was extracted twice with ethyl acetate (30 ml). The combined extracts were washed with saturated NaCl aqueous solution and then dried over anhydrous Na_2SO_4. The concentration of the solution under reduced pressure gave crude crystals, which were collected by using hexane and then recrystallized from a mixture of hexane and ethyl acetate to give (*Z*)-**6** as colorless needles quantitatively.

b) Method B: A solution of (*E*)-**6** (1.0 g, 4.06 mmol) in ethyl acetate (30 ml) saturated with gaseous HCl was stirred at room temperature for 6 h. The reaction solution was concentrated under reduced pressure to give crude residue, which was again dissolved in ethyl acetate (30 ml). This procedure was repeated until HCl gas was completely taken off. The crude crystals collected by using hexane were similarly worked up to give (*Z*)-**6** quantitatively.

Preparation of 10. To a solution of ΔNCA (7.04 mmol) and $(Boc)_2O$ (8.45 g, 8.45 mmol) in THF (7 ml) was added drop by drop pyridine (0.15 ml, 1.85 mmol) under cooling. The resulting solution was slowly returned to room temperature and then stirred continuously overnight. To the solution,thus prepared, was further added a solution of HyAsp(OMe)–OMe·HCl (2.25 g, 10.6 mmol) and TEA (1.48 ml, 10.6 mmol) in dry THF (7 ml) at room temperature. After making the solution pH 9 with NMM (1.0 ml, 9.1 mmol), the resulting solution was stirred for more 2.5—4.0 h and then concentrated under reduced pressure to give a crude residue, which was dissolved in ethyl acetate (100 ml). This solution was successively washed with 10% citric acid, water, saturated aqueous $NaHCO_3$ solution and water and finally dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the crude residue obtained was crystallized and collected by using diisopropyl ether. In the case of a syrup, which did not crystallize, purification on a silica-gel column using a mixture of $CHCl_3$ and acetone (20:1 v/v) as the eluent was carried out to give crystals, which were recrystallized from a mixture of petroleum ether and ethyl acetate to give **10**. The dipeptide containing a *threo*-HyAsp residue, thus obtained, was colorless prisms; the one with an *erythro*-HyAsp residue was colorless needles.

Preparation of 12. To a solution of **10** (1.60 mmol) and MsCl (0.19 ml, 2.40 mmol) in dry THF (7 ml) below 0 °C. After stirring for 1 h and then at room temperature for 24 h in the case of TEA, or for 4.5 h in the case of DBU, the reaction solution was concentrated under reduced pressure. The residue, thus obtained, was dissolved in ethyl acetate (50 ml) and the resulting solution was washed successively with 10% citric acid, water, saturated aqueous $NaHCO_3$ solution, and then water and finally dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the obtained residual crystals were recrystallized from a mixture of petroleum ether and ethyl acetate to give **12** as colorless prisms [(*Z*)-isomer] or colorless needles [(*E*)-isomer].

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