Purines. XXXIII.[1] Syntheses and Cytokinin Activities of Both Enantiomers of 1′-Methylzeatin and Their 9-β-D-Ribofuranosides

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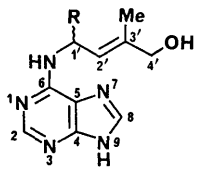
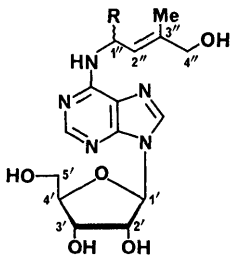
The structures and absolute configurations of the cytokinins 1′-methylzeatin and its 9-riboside, both secreted by *Pseudomonas syringae* pv *savastanoi*, have been established as [*R*-(*E*)]-2-methyl-4-(9*H*-purin-6-ylamino)-2-penten-1-ol [(1′*R*)-2] and [*R*-(*E*)]-*N*-(4-hydroxy-1,3-dimethyl-2-butenyl)adenosine [(1″*R*)-4], respectively, as a result of the chiral syntheses of (1′*R*)- and (1′*S*)-1′-methylzeatins [(1′*R*)-2 and (1′*S*)-2] and of their 9-β-D-ribofuranosides [(1″*R*)-4 and (1″*S*)-4] from D- and L-alanines. These zeatin derivatives were tested for cytokinin activity in the tobacco callus and the lettuce seed germination bioassays. The "natural" enantiomer (1′*R*)-2 was found to be as active as zeatin (1) itself and more active than its 9-riboside [(1″*R*)-4]. The "unnatural" enantiomer (1′*S*)-2 and its 9-riboside [(1″*S*)-4] were less active than the corresponding natural cytokinins, (1′*R*)-2 and (1″*R*)-4, respectively.

Keywords 1′-methylzeatin; 1′-methylzeatin 9-riboside; chiral synthesis; alanine; Wittig reaction; α,β-unsaturated ester; cytokinin activity; ^{1}H-NMR; ^{13}C-NMR; CD

Pseudomonas syringae pv *savastanoi*, the causative organism of olive, oleander, privet, and ash knot, secretes several cytokinins,[2-5] a type of phytohormone characterized primarily by the ability to promote cell division in plant tissue cultures.[6] In 1985, Surico *et al.*[3] reported the isolation of two new cytokinins and two other known cytokinins, zeatin (**1**) and zeatin 9-β-D-ribofuranoside (**3**), from AcOEt extracts of the culture filtrate of this gall-forming phytopathogenic bacterium. They proposed the gross structures **2**[7] and **4**[3] for the two new cytokinins on the basis of spectroscopic data and comparison with related adenine derivatives including **1** and **3**. The new cytokinins, 1′-methylzeatin (**2**) and its 9-β-D-ribofuranosyl derivative (**4**), are unique in that their N^6-substituents consist of a branched allyl alcoholic C_6-unit with an asymmetric center adjacent to the N^6 atom. However, the absolute stereochemistry at the asymmetric center remained unknown for both compounds at the time when the present study was undertaken. Our continuing interest[8] in synthesizing N^6-substituted adenine derivatives possessing cytokinin activity thus led us to synthesize both enantiomers [(1′*R*)-**2** and (1′*S*)-**2**] of 1′-methylzeatin (**2**) and their 9-β-D-ribofuranosides [(1″*R*)-**4** and (1″*S*)-**4**] in order to determine the absolute configurations of the natural samples by comparison. We also followed our plan to test the synthetic samples for cytokinin activity in the tobacco callus and the lettuce seed germination bioassays. A brief account of the chemical results recorded here has been published in a preliminary form.[9]



1: R = H
2: R = Me



3: R = H
4: R = Me

The key intermediates selected for the syntheses of the candidate structures were the chiral amine salts (*R*)-**14** and (*S*)-**14**, and we planned to incorporate the skeletons and chirality of D- and L-alanines [(*R*)-**5** and (*S*)-**5**] into them. In the (*R*)-series synthesis (Chart 1), D-alanine [(*R*)-**5**] was first converted into the *N*-protected amino ester (*R*)-**7**[10] through the amino ester hydrochloride (*R*)-**6**[11] according to the literature, and $LiBH_4$ reduction of (*R*)-**7** and Me_2SO oxidation of the resulting *N*-protected amino alcohol using SO_3–pyridine complex in the presence of Et_3N were effected in a manner similar to that employed by Shioiri's group[12] for the (*S*)-series, giving the aldehyde (*R*)-**9** in 71% overall yield [from (*R*)-**5**]. Wittig reaction of (*R*)-**9** with methyl 2-(triphenylphosphoranylidene)propionate[13] in CH_2Cl_2 at 22 °C for 1 h furnished a 95 : 5 mixture of (*R*)-**10** and its (*Z*)-isomer[8i] in 98% yield, from which (*R*)-**10** was isolated in 88% yield by recrystallization (from hexane). The assignment of geometry in (*R*)-**10** was based on the facts that it was the major isomer formed[14] and that its olefinic proton [δ 6.53 (dq, J=8.5 and 1.5 Hz)] resonated in $CDCl_3$ at lower field than that [δ 5.80 (dq, J=8.5 and 1.5 Hz)][8i] of the (*Z*)-isomer. The ester (*R*)-**10** was then hydrolyzed in MeOH with 2 N aqueous NaOH at 30 °C for 30 min to afford the acid (*R*)-**11** in 99% yield. The operation required in the next step was selective reduction of the carboxy group to the hydroxymethyl group, and it was achieved by application of the method of Yamada and co-workers.[15] Thus, acylation of (*R*)-**11** with ethyl chloroformate in the presence of Et_3N and $NaBH_4$ reduction of the resulting mixed anhydride produced (*R*)-**12** in 89% yield. The carbamate (*R*)-**12** was hydrolyzed with 10% aqueous HCl at room temperature for 1 h, and the amino alcohol hydrochloride that formed was first converted into the free base [by the use of Amberlite IRA-402 (HCO_3^-)], which was then isolated in the form of the oxalate [(*R*)-**14**] in 79% yield [from (*R*)-**12**].

A parallel sequence of conversions starting with L-alanine [(*S*)-**5**] yielded (*S*)-**6**,[16] (*S*)-**7**,[17] (*S*)-**8**,[12a] (*S*)-**9**[12a] [71% overall yield from (*S*)-**5**], (*S*)-**10** (87%),[18] (*S*)-**11** (99%), (*S*)-**12** (89%), and (*S*)-**14** (84%). Since the enantiomeric purity[19] of the above sample of (*R*)-**10** was determined to be more than 96% by means of proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy using the

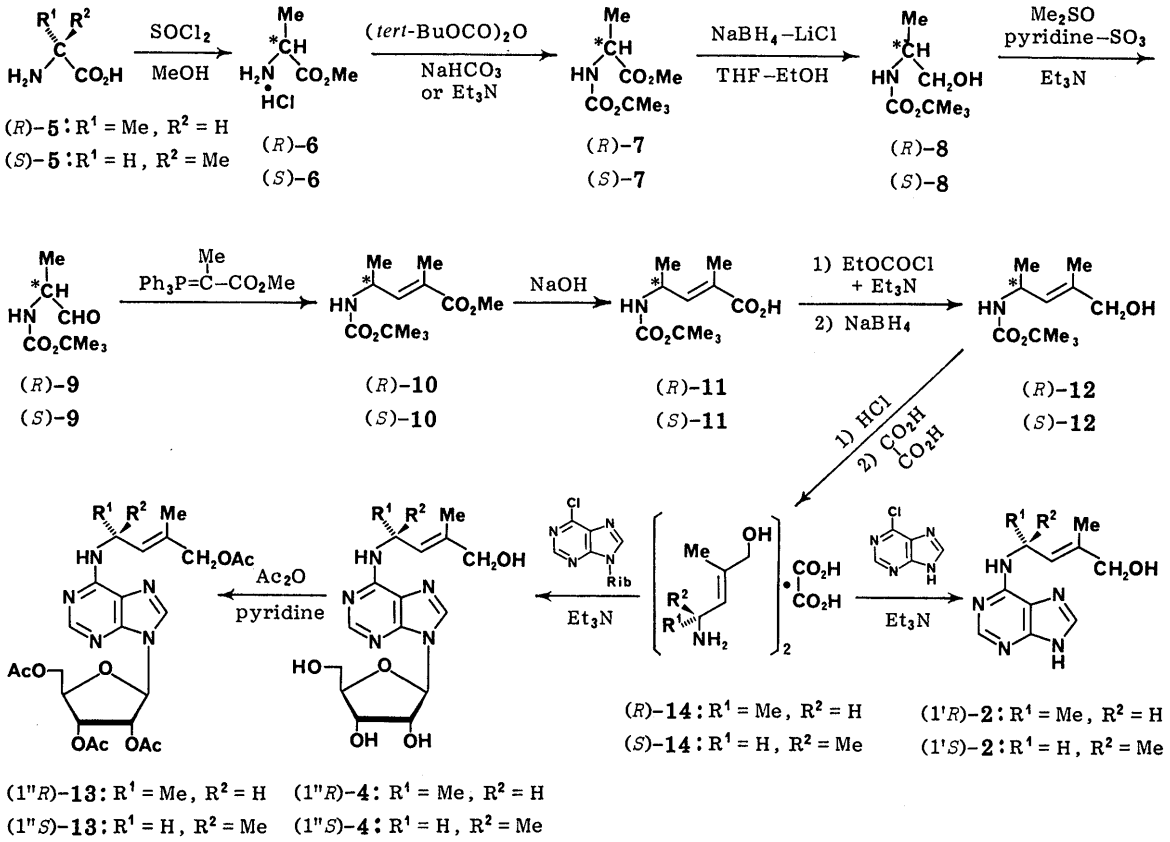


Chart 1

chiral shift reagent[20] tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) [Eu(hfc)$_3$],[21] those of the amine oxalates (*R*)-**14** and (*S*)-**14** should be equally high, provided that each of the conversions beyond **10** involved no racemization.

Purinylation of (*R*)-**14** with 6-chloropurine in boiling 1-butanol containing Et_3N for 10 h gave the (−)-base (1′*R*)-**2** in 70% yield. A similar purinylation of (*S*)-**14** afforded the (+)-enantiomer (1′*S*)-**2** in 70% yield. Of the two optical isomers, the (−)-enantiomer (1′*R*)-**2** was identical with natural 1′-methylzeatin by comparison of their chiroptical properties, mass (MS), ultraviolet (UV), and ^{1}H- and ^{13}C-NMR spectra, and thin-layer chromatographic (TLC) mobilities.

The syntheses of the corresponding 9-β-D-ribofuranosides were accomplished by condensations of 6-chloro-9-β-D-ribofuranosylpurine[22] with (*R*)-**14** and with (*S*)-**14** in boiling 1-butanol containing Et_3N for 8 h, giving (1′′*R*)-**4** and (1′′*S*)-**4** in 88% and 89% yields, respectively. The two nucleosides were separately acetylated with acetic anhydride and pyridine at 30 °C for 2 h to provide the tetra-*O*-acetyl derivatives (1′′*R*)-**13** and (1′′*S*)-**13** in 95% and 80% yields, respectively. Unfortunately, differentiation of the synthetic (1′′*R*)-**4** and (1′′*S*)-**4** and natural 1′-methylzeatin 9-riboside from each other by means of MS, UV, and ^{1}H- and ^{13}C-NMR spectroscopy and TLC or even differentiation at the tetra-*O*-acetyl level by means of MS, UV, infrared (IR) (in $CHCl_3$), and ^{1}H-NMR spectroscopy and TLC was not possible because of their structural resemblances. Nevertheless, comparison of their chiroptical properties clearly indicated that the synthetic (1′′*R*)-**4** and (1′′*R*)-**13** are identical with natural 1′-methylzeatin 9-riboside and its tetra-*O*-acetyl derivative, respectively.

Now that both the "natural" and "unnatural" enantiomers [(1′*R*)-**2** and (1′*S*)-**2**] of 1′-methylzeatin, their 9-ribosides [(1′′*R*)-**4** ("natural") and (1′′*S*)-**4** ("unnatural")], and the tetra-*O*-acetyl derivatives [(1′′*R*)-**13** and (1′′*S*)-**13**] of the latter had become available, it was possible to test them for cytokinin activity in the tobacco callus and the lettuce seed germination bioassays. It may be seen from Table I that in the tobacco callus bioassay the "natural" aglycone (1′*R*)-**2** was the most active among the six test compounds: it was as active as the known 1′-unsubstituted cytokinin zeatin (**1**). Interestingly, the "unnatural" aglycone (1′*S*)-**2** was apparently less active than (1′*R*)-**2**. The maximal yield of the callus was obtained at 0.04 μM (1′*R*)-**2** and at 1 μM (1′*S*)-**2**. This stereochemistry/activity relationship also holds at the nucleoside and the tetra-*O*-acetyl derivative levels. As expected,[6d] the nucleoside (1′′*R*)-**4** and its tetra-*O*-acetyl derivative (1′′*R*)-**13** were all less active than the aglycone (1′*R*)-**2**. However, the corresponding triplets in the (*S*)-series had similar activities. Thus, the cytokinin activity follows the order: zeatin (**1**)≈(1′*R*)-**2**>(1′′*R*)-**4**≈(1′′*R*)-**13**>(1′*S*)-**2**≈(1′′*S*)-**4**≈(1′′*S*)-**13**. The same activity order was also found in the lettuce seed germination bioassay, as shown in Table II. All these results indicate that the introduction of a methyl group into zeatin (**1**) at the 1′-position with the (*R*) configuration does not alter its cytokinin activity, whereas that with the (*S*) configuration reduces the activity to a considerable extent. This suggests the importance of the three-dimensional structure around an asymmetric center in the N^6-substituent in determining the cytokinin activity.[6c,d,8f,g,23]

In summary, the above results have unequivocally estab-

TABLE I. Cytokinin Activity of Zeatin Analogues Tested by the Tobacco Callus Bioassay

Compound	Average fresh weight of tobacco callus (mg)											
	Concentration of test compound (μM)											
	0	0.0001	0.0004	0.001	0.004	0.01	0.04	0.1	0.4	1	4	10
(1′*R*)-**2**	17.3	29.2	52.8	120.3	828.1	1064.5	1489.7	1140.2	621.8	556.9	—	—
(1′*S*)-**2**	23.3	—	—	—	28.7	61.5	429.5	922.4	1024.5	1512.4	1005.5	—
(1″*R*)-**4**	30.1	—	—	—	—	164.7	435.8	1419.4	1664.6	1575.5	721.5	481.1
(1″*S*)-**4**	25.8	—	—	—	—	105.2	269.8	628.0	1300.5	1425.5	828.6	790.5
(1″*R*)-**13**	17.8	—	—	—	—	23.3	499.0	786.6	1540.6	1348.6	816.9	650.2
(1″*S*)-**13**	27.5	—	—	—	—	—	150.1	223.5	1477.9	1760.7	1408.7	820.0
Zeatin (**1**)	17.4	24.5	63.2	152.5	635.0	1053.0	1769.7	1448.5	1443.9	—	—	—

TABLE II. Cytokinin Activity of Zeatin Analogues Tested by Lettuce Seed Germination

Compound	Lettuce seed germination (%)											
	Concentration of test compound (μM)											
	0	0.001	0.004	0.01	0.04	0.1	0.4	1	4	10	40	100
(1′*R*)-**2**	3.8	—	—	9.6	15.0	19.4	21.4	32.4	57.4	65.7	—	88.2
(1′*S*)-**2**	3.8	—	—	—	4.4	5.8	4.3	8.1	11.7	16.0	27.3	30.3
(1″*R*)-**4**	5.9	—	—	—	—	7.6	6.9	10.5	20.2	28.2	35.7	59.7
(1″*S*)-**4**	5.9	—	—	—	—	5.6	5.0	5.7	5.1	7.3	5.7	12.8
(1″*S*)-**13**	3.7	—	—	—	—	5.2	2.8	3.9	4.9	6.4	12.2	10.6
Zeatin (**1**)	3.7	—	—	8.8	13.5	32.1	43.7	40.2	70.9	74.0	—	88.4
Kinetin[a]	4.1	—	—	—	8.6	9.6	16.3	44.1	73.8	79.5	79.3	—

a) An alternative name for N^6-furfuryladenine (6-furfurylaminopurine).

lished that the formulas (1′*R*)-**2** and (1″*R*)-**4** are complete expressions for the two new cytokinins from *Pseudomonas syringae* pv *savastanoi*. They have also served to characterize fully the new cytokinins through the use of the synthetic samples in place of the natural samples that were isolated only in minute amounts. Interestingly, (1′*R*)-**2** was found to be as active as zeatin (**1**) and more active than (1″*R*)-**4** in the two bioassay systems for cytokinin activity. The "unnatural" enantiomer (1′*S*)-**2** and its 9-riboside [(1″*S*-**4**] were less active than the corresponding "natural" cytokinins, (1′*R*)-**2** and (1″*R*)-**4**, respectively.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. Unless otherwise noted, each pair of enantiomers obtained in this work was identified by comparison of their IR spectra and TLC mobilities. Spectra reported herein were recorded on a Hitachi model 320 UV spectrophotometer [on solutions in 95% (v/v) aqueous EtOH, 0.1 N aqueous HCl (pH 1), 0.005 M phosphate buffer (pH 7 and 10), and 0.1 N aqueous NaOH (pH 13)], a JASCO A-202 IR spectrophotometer, a JASCO J-500C spectropolarimeter, a Hitachi M-80 mass spectrometer, or a JEOL JNM-FX-100 NMR spectrometer, equipped with a ^{13}C Fourier-transform NMR system, at 25 °C with Me_4Si as an internal standard. Optical rotations were measured with a JASCO DIP-181 polarimeter using a 1-dm sample tube. Elemental analyses were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, ddd = doublet-of-doublets-of-doublets, dq = doublet-of-quartets, m = multiplet, s = singlet.

(*R*)-(1-Methyl-2-oxoethyl)carbamic Acid *tert*-Butyl Ester [(*R*)-9] This compound was synthesized from (*R*)-(2-hydroxy-1-methylethyl)carbamic acid *tert*-butyl ester [(*R*)-**8**][12] [mp 59—61 °C (from hexane); $[\alpha]_D^{11}$ +8.1° (c = 1.00, CH_2Cl_2)], prepared from D-alanine [(*R*)-**5**] in 94% overall yield through (*R*)-**6**[11] and (*R*)-**7**[10] according to the literature procedures, in a manner slightly modified from that [12a] described for the preparation of the (*S*)-enantiomer: To a stirred solution of (*R*)-**8** (4.38 g, 25 mmol) and Et_3N (7.59 g, 75 mmol) in dry Me_2SO (75 ml) was added a solution of SO_3–pyridine complex (11.94 g, 75 mmol) in dry Me_2SO (75 ml) under an atmosphere of N_2 over 4 min at 20—22 °C with occasional ice-cooling. The mixture was stirred at the same temperature for 8 min and then poured onto crushed ice (*ca.* 600 ml), and the resulting aqueous mixture was extracted with hexane (100 ml). The aqueous layer was separated from the hexane layer and extracted successively with ether (200 ml) and CH_2Cl_2 (4 × 400 ml). The CH_2Cl_2 extracts were combined, washed successively with 10% aqueous citric acid (2 × 200 ml), H_2O (2 × 200 ml), and saturated aqueous $NaHCO_3$ (200 ml), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to leave (*R*)-**9** (3.16 g) as a colorless solid, mp 79—87 °C. A similar treatment of the above ethereal extracts and recrystallization of the resulting residue from a mixture of hexane (5 ml) and ether (0.5 ml) gave a second crop (0.12 g) of (*R*)-**9**, mp 85—87 °C. The total yield was 3.28 g (76%). Recrystallization of crude (*R*)-**9** from hexane afforded an analytical sample as colorless plates, mp 90—91 °C (lit.[24] mp 88 °C); $[\alpha]_D^{18}$ +35.2° (c = 1.00, MeOH)[25] [lit.[24] $[\alpha]_D^{20}$ −43.2° (c = 1.05, $CHCl_3$)]; IR ν_{max}^{Nujol} cm^{-1}: 3350 (NH), 1730 (CHO), 1690 (carbamate CO); ^1H-NMR ($CDCl_3$) δ: 1.34 (3H, d, J = 7 Hz, CHMe), 1.46 (9H, s, CMe_3), 4.20 (1H, m, CHMe), 5.04 (1H, br, NH), 9.56 (1H, s, CHO). *Anal.* Calcd for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.63; H, 8.99; N, 7.98.

(*S*)-(1-Methyl-2-oxoethyl)carbamic Acid *tert*-Butyl Ester [(*S*)-9] This was prepared from (*S*)-(2-hydroxy-1-methylethyl)carbamic acid *tert*-butyl ester [(*S*)-**8**][12a] [mp 59—60 °C (from hexane); $[\alpha]_D^{16}$ −8.8° (c = 1.00, CH_2Cl_2)], synthesized from L-alanine [(*S*)-**5**] in 94% overall yield through (*S*)-**6**[16] and (*S*)-**7**[17] according to the literature procedures, in 75% yield in a manner similar to that described above for (*R*)-**9** and recrystallized from hexane to give colorless plates, mp 89—90 °C (lit.[12a] mp 90—92 °C); $[\alpha]_D^{16}$ −34.3° (c = 1.00, MeOH) [lit.[12a] $[\alpha]_D^{20}$ −40.9° (c = 1, MeOH)].

[*R*-(*E*)]-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoic Acid Methyl Ester [(*R*)-10] Condensation of methyl 2-(triphenylphosphoranylidene)propionate[13] in CH_2Cl_2, work-up of the reaction mixture, and determination of the isomer ratio were performed as described below for (*S*)-**10**, giving a 95 : 5 mixture (mp 53—55 °C) of (*R*)-**10** and the (*Z*)-isomer[8i] in 98% yield. Recrystallization of the isomer mixture from hexane furnished pure (*R*)-**10** in 88% yield. Further recrystallization in the same manner gave an analytical sample as colorless

pillars, mp 79—80 °C; $[\alpha]_D^{15}$ +14.4° (c=1.00, MeOH).[21] *Anal.* Calcd for $C_{12}H_{21}NO_4$: C, 59.24; H, 8.70; N, 5.76. Found: C, 59.16; H, 8.92; N, 5.74. The ^{1}H-NMR spectrum of this sample in $CDCl_3$ was superimposable on that of (*S*)-**10**.

[*S*-(*E*)]-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoic Acid Methyl Ester [(*S*)-10] A solution of methyl 2-(triphenylphosphoranylidene)propionate[13] (6.90 g, 19.8 mmol) in CH_2Cl_2 (10 ml) was added to a stirred solution of (*S*)-**9** (3.12 g, 18 mmol) in CH_2Cl_2 (10 ml) over 8 min at 16—18 °C with occasional ice-cooling. The resulting mixture was stirred at 22 °C for 1 h and then concentrated to dryness *in vacuo*. The partly crystallized residue was extracted with hot hexane (5 × 30 ml). The hexane extracts were combined and concentrated *in vacuo* to leave an oil. Purification of the oil by means of flash chromatography[26] [column diameter, 30 mm; Silica gel 60 (E. Merck, No. 9385); hexane–AcOEt (3 : 1, v/v)] afforded a colorless solid (4.29 g, 98%), mp 52—56 °C. A single recrystallization of the solid from hexane yielded a pure sample of (*S*)-**10** (3.79 g, 87%), mp 79—80 °C. Concentration of the mother liquor of this recrystallization left a jelly, which was a 4 : 3 mixture of (*S*)-**10** and the (*Z*)-isomer as estimated by means of ^{1}H-NMR spectroscopy on the basis of the relative integral intensities of two doublets-of-quartets at δ 6.53 and 5.83 attributable to the olefinic protons of (*S*)-**10** and the (*Z*)-isomer,[8i] respectively. This made it possible to estimate the (*E*) : (*Z*) ratio in the product before recrystallization as 95 : 5. Further recrystallization of the above pure sample of (*S*)-**10** from hexane provided an analytical sample as colorless pillars, mp 79—80 °C; $[\alpha]_D^{16}$ −13.8° (c=1.00, MeOH); IR ν_{max}^{Nujol} cm^{-1}: 3370 (NH), 1712 (α,β-unsaturated ester CO), 1695 (carbamate CO); ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, d, J=6.5 Hz, CHMe), 1.43 (9H, s, CMe_3), 1.92 (3H, d, J=1.5 Hz, CH=CMe), 3.74 (3H, s, CO_2Me), 4.52 (2H, br, CHMe and NH), 6.53 (1H, dq, J=8.5, 1.5 Hz, CH=CMe). *Anal.* Calcd for $C_{12}H_{21}NO_4$: C, 59.24; H, 8.70; N, 5.76. Found: C, 59.21; H, 8.89; N, 5.66.

[*R*-(*E*)]-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoic Acid [(*R*)-11] A solution of (*R*)-**10** (1.046 g, 4.3 mmol) and 2 N aqueous NaOH (4.3 ml) in MeOH (10 ml) was stirred at 30 °C for 3 h. The reaction mixture was concentrated *in vacuo* to a volume of *ca.* 5 ml, brought to pH 2 by addition of 2 N aqueous HCl, and extracted with $CHCl_3$ (3 × 20 ml). The $CHCl_3$ extracts were dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to leave a colorless solid (974 mg, 99%), mp 122—124 °C. Recrystallization from benzene–hexane (1 : 1, v/v) and drying over P_2O_5 at 2 mmHg first at 50 °C for 12 h and then at 60 °C for 11 h yielded an analytical sample of (*R*)-**11** as colorless plates, mp 123—124 °C[27]; $[\alpha]_D^{26}$ +4.7° (c=1.02, MeOH); IR ν_{max}^{Nujol} cm^{-1}: 3270 (NH), 1691 (CO_2H and carbamate CO's); ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, d, J=6.5 Hz, CHMe), 1.43 (9H, s, CMe_3), 1.92 (3H, d, J=1.5 Hz, CH=CMe), 4.3—4.8 (2H, m, CHMe and NH), 6.65 (1H, dq, J=9, 1.5 Hz, CH=CMe). *Anal.* Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.64; H, 8.55; N, 6.13.

[*S*-(*E*)]-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoic Acid [(*S*)-11] Hydrolysis of (*S*)-**10** with 2 N aqueous NaOH in MeOH and work-up of the reaction mixture were carried out as described above for (*R*)-**11**, giving (*S*)-**11** (99% yield) as colorless plates, mp 123—124 °C; $[\alpha]_D^{17}$ −4.2° (c=1.00, MeOH). *Anal.* Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.60; H, 8.57; N, 5.99.

[*R*-(*E*)]-(4-Hydroxy-1,3-dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(*R*)-12] A solution of ethyl chloroformate (326 mg, 3 mmol) in dry tetrahydrofuran (THF) (1 ml) was added dropwise to a solution of (*R*)-**11** (688 mg, 3 mmol) and Et_3N (304 mg, 3 mmol) in dry THF (4 ml) with stirring at −15 °C. The mixture was stirred at temperatures of −15° to −5 °C for 30 min, and the precipitate that resulted was filtered off and washed with THF (3 × 1.5 ml). The filtrate and washings were combined and added dropwise to a stirred mixture of $NaBH_4$ (284 mg, 7.5 mmol) and H_2O (3 ml) at 5—10 °C over 20 min. The resulting mixture was stirred at room temperature for 3 h, concentrated *in vacuo* to a volume of *ca.* 3 ml, brought to pH 3—4 with 10% aqueous H_3PO_4, and extracted with ether (10 ml, then 2 × 5 ml). The ethereal extracts were washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to leave a colorless oil. The oil was purified by flash chromatography[26] [column diameter, 10 mm; Silica gel 60 (E. Merck, No. 9385); AcOEt–hexane (3 : 2, v/v)] to furnish (*R*)-**12** (576 mg, 89%) as a colorless oil, $[\alpha]_D^{16}$ +0.1° (c=1.00, MeOH); $[\alpha]_{365}^{16}$ −8.4° (c=1.00, MeOH); IR ν_{max}^{film} cm^{-1}: 3330 (OH and NH), 1690 (carbamate CO); ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, d, J=6.5 Hz, CHMe), 1.43 (9H, s, CMe_3), 1.73 (3H, d, J=1.3 Hz, CH=CMe), 1.85 (s, OH), 3.99 (2H, d, J=1.1 Hz, CH_2OH), 4.44 (2H, m, CHMe and NH), 5.30 (1H, m, CH=CMe).

[*S*-(*E*)]-(4-Hydroxy-1,3-dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(*S*)-12] This was synthesized in 89% yield from (*S*)-**11** in a manner similar to that described above for (*R*)-**12** and isolated as a colorless oil, $[\alpha]_D^{16}$ −0.2° (c=1.00, MeOH); $[\alpha]_{365}^{17}$ +7.9° (c=1.00, MeOH).

[*R*-(*E*)]-4-Amino-2-methyl-2-penten-1-ol Ethanedioate (2 : 1) (Salt) [(*R*)-14] A mixture of (*R*)-**12** (570 mg, 2.65 mmol) and 10% aqueous HCl (5 ml) was shaken at room temperature for 60 min, giving a clear solution. The solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (25 ml), and the column was eluted with H_2O. The eluate (50 ml) was concentrated to dryness *in vacuo* to leave an oil (305 mg), which was dissolved in EtOH (2 ml). The resulting ethanolic solution was exactly neutralized by addition of a solution of oxalic acid (119 mg, 1.32 mmol) in EtOH (1 ml) and, if necessary, with Et_3N. The precipitate that resulted was collected by filtration, washed with EtOH (4 × 1 ml), and dried to give (*R*)-**14** (336 mg, 79%) as a colorless solid, mp 217—218 °C (dec.). Recrystallization from 95% (v/v) aqueous EtOH produced an analytical sample as colorless needles, mp 222—223 °C (dec.); $[\alpha]_D^{18}$ −5.2° (c=1.00, MeOH); $[\alpha]_{365}^{20}$ −21.6° (c=1.00, MeOH); IR ν_{max}^{Nujol} cm^{-1}: 3380 (OH), 1580 (COO^- and NH_3^+); ^{1}H-NMR (Me_2SO-d_6) (at 50 °C) δ: 1.13 (3H, d, J=6 Hz, CHMe), 1.58 (3H, d, J=1.2 Hz, CH=CMe), 3.79 (2H, br s, CH_2OH), 3.86 (1H, dq, J=9, 6 Hz, CHMe), 5.15 (4H, br s, OH and NH_3^+), 5.33 (1H, m, CH=CMe).[28] *Anal.* Calcd for $C_{14}H_{28}N_2O_6$: C, 52.48; H, 8.81; N, 8.74. Found: C, 52.21; H, 9.10; N, 8.75.

[*S*-(*E*)]-4-Amino-2-methyl-2-penten-1-ol Ethanedioate (2 : 1) (Salt) [(*S*)-14] This was prepared in 84% yield from (*S*)-**12** in a manner similar to that described above for (*R*)-**14** and recrystallized from 95% (v/v) aqueous EtOH to form colorless needles, mp 222—223 °C (dec.)[29]; $[\alpha]_D^{17}$ +5.3° (c=1.00, MeOH); $[\alpha]_{365}^{17}$ +22.0° (c=1.00, MeOH). *Anal.* Calcd for $C_{14}H_{28}N_2O_6$: C, 52.48; H, 8.81; N, 8.74. Found: C, 52.37; H, 9.05; N, 8.75.

[*R*-(*E*)]-2-Methyl-4-(9*H*-purin-6-ylamino)-2-penten-1-ol [(1′*R*)-1′-Methylzeatin] [(1′*R*)-2] A stirred solution of 6-chloropurine (108 mg, 0.7 mmol) and (*R*)-**14** (135 mg, 0.42 mmol) in 1-butanol (7 ml) containing Et_3N (156 mg, 1.54 mmol) was heated under reflux for 10 h. The reaction mixture was concentrated *in vacuo* to leave a jelly, which was dissolved in a little H_2O. The resulting aqueous solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (7 ml), and the column was eluted with H_2O. The eluate (100 ml) was concentrated *in vacuo*, and the residual jelly was purified by flash chromatography[26] [column diameter, 20 mm; Silica gel 60 (E. Merck, No. 9385); $CHCl_3$–MeOH (8 : 1, v/v)] to give (1′*R*)-**2** (114 mg, 70%) as a colorless solid, mp 201—202 °C. Recrystallization from H_2O yielded an analytical sample as colorless prisms, mp 201—202 °C; $[\alpha]_D^{26}$ −109° (c=0.153, EtOH); CD (c=2.71 × 10^{-5} M, MeOH) $[\theta]^{25}$ (nm): −20300 (273) (neg. max.), +61300 (214) (pos. max.); MS *m/z* (relative intensity): 233 (M^+) (14), 216 (86), 202 (71), 174 (18), 162 (7), 160 (10), 148 (2), 136 (100), 135 (22); UV $\lambda_{max}^{95\%\,EtOH}$ 270 nm (ε 18500); $\lambda_{max}^{H_2O}$ (pH 1) 275 (17400); $\lambda_{max}^{H_2O}$ (pH 7) 269 (18800); $\lambda_{max}^{H_2O}$ (pH 10) 272 (17900); $\lambda_{max}^{H_2O}$ (pH 13) 275 (18200); ^{1}H-NMR (CD_3OD) δ: 1.36 (3H, d, J=6 Hz, CHMe), 1.79 (3H, d, J=1 Hz, CH=CMe), 3.96 (2H, br s, CH_2OH), 5.25 (1H, dq, J=9, 6 Hz, CHMe), 5.54 (1H, m, CH=CMe), 8.06 and 8.24 (1H each, s, purine protons); ^{13}C-NMR (CD_3OD) δ: 14.2 [C(3′)-Me],[30,31] 21.9 [C(1′)-Me],[31] 45.5 [C(1′)], 68.0 [C(4′)], 127.8 [C(2′)], 138.3 [C(3′)], 140.4 [C(8)], 153.8 [C(2)], 154.7 [C(6)]. *Anal.* Calcd for $C_{11}H_{15}N_5O$: C, 56.63; H, 6.48; N, 30.03. Found: C, 56.48; H, 6.48; N, 30.13. This sample was identical (by comparison of the MS, UV, and ^{1}H- and ^{13}C-NMR spectra, TLC mobility, and chiroptical properties) with a natural sample of 1′-methylzeatin[7] [CD (c=3.03 × 10^{-5} M, MeOH) $[\theta]^{25}$ (nm): −18800 (273) (neg. max.), +56500 (214) (pos. max.)].

[*S*-(*E*)]-2-Methyl-4-(9*H*-purin-6-ylamino)-2-penten-1-ol [(1′*S*)-1′-Methylzeatin] [(1′*S*)-2] Condensation of (*S*)-**14** with 6-chloropurine and work-up of the reaction mixture were carried out as described above for (1′*R*)-**2**, giving (1′*S*)-**2** in 70% yield as colorless prisms, mp 201—202 °C; $[\alpha]_D^{26}$ +103° (c=0.137, EtOH); CD (c=2.89 × 10^{-5} M, MeOH) $[\theta]^{25}$ (nm): +19700 (273) (pos. max.), −59900 (214) (neg. max.). *Anal.* Calcd for $C_{11}H_{15}N_5O$: C, 56.63; H, 6.48; N, 30.03. Found: C, 56.34; H, 6.52; N, 29.90.

[*R*-(*E*)]-*N*-(4-Hydroxy-1,3-dimethyl-2-butenyl)adenosine [(1′*R*)-1′-Methylzeatin 9-β-D-Ribofuranoside] [(1″*R*)-4] A stirred solution of 6-chloro-9-β-D-ribofuranosylpurine[22] (289 mg, 1 mmol) and (*R*)-**14** (192 mg, 0.6 mmol) in 1-butanol (10 ml) containing Et_3N (233 mg, 2.3 mmol) was heated under reflux for 8 h. The reaction mixture was concentrated *in vacuo* to leave a yellow oil, which was dissolved in a little H_2O. The resulting aqueous solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (10 ml), and the column was eluted with H_2O. The eluate (80 ml) was concentrated *in vacuo*, and the residue was purified by flash chromatography[26] [column diameter, 20 mm; Silica gel 60 (E. Merck, No. 9385); AcOEt–EtOH (4 : 1, v/v)] to furnish (1″*R*)-**4** (321 mg, 88%) as a foamy glass, which solidified on standing at room temperature.

Recrystallization from MeOH and drying over P_2O_5 at 2 mmHg and 50 °C for 6 h provided an analytical sample of a hemihydrate of (1″*R*)-**4** as colorless pillars, mp 130—132 °C; $[\alpha]_D^{14}$ −117° (*c* = 0.102, MeOH); $[\alpha]_{365}^{15}$ −573° (*c* = 0.102, MeOH); CD (*c* = 5.20 × 10⁻⁵ M, MeOH) $[\theta]^{25}$ (nm): −24300 (277) (neg. max.), +50400 (217) (pos. max.); UV $\lambda_{max}^{95\%\,EtOH}$ 270 nm (ε 18700); $\lambda_{max}^{H_2O}$ (pH 1) 265 (19600); $\lambda_{max}^{H_2O}$ (pH 7) 269 (19700); $\lambda_{max}^{H_2O}$ (pH 13) 269 (19800); ^{1}H-NMR (CD_3OD) δ: 1.35 (3H, d, *J* = 6.5 Hz, CHMe), 1.77 (3H, d, *J* = 1 Hz, CH=CMe), 3.74 and 3.88 [1H each, dd, *J* = 12.5, 2.5 Hz, C(5′)-H's],[32] 3.94 [2H, br s, C(4″)-H's], 4.17 [1H, ddd, *J* = 2.5 Hz, each, C(4′)-H], 4.32 [1H, dd, *J* = 5, 2.5 Hz, C(3′)-H], 4.74 [1H, dd, *J* = 6.5, 5 Hz, C(2′)-H], 5.27 (1H, dq, *J* = 9, 6.5 Hz, CHMe), 5.53 (1H, m, CH=CMe), 5.94 [1H, d, *J* = 6.5 Hz, C(1′)-H], 8.21 and 8.23 (1H each, s, purine protons); ^{13}C-NMR (CD_3OD) δ: 14.1 [C(3″)-Me],[31,32] 21.8 [C(1″)-Me],[31] 45.6 [C(1″)], 63.5 [C(5′)], 68.0 [C(4″)], 72.7 [C(2′)], 75.4 [C(3′)], 88.2 [C(4′)], 91.3 [C(1′)], 121.3 [C(5)], 127.7 [C(2″)], 138.3 [C(3″)], 141.3 [C(8)], 149.3 [C(4)], 153.5 [C(2)], 155.3 [C(6)]. *Anal.* Calcd for $C_{16}H_{23}N_5O_5 \cdot 1/2H_2O$: C, 51.33; H, 6.46; N, 18.71. Found: C, 51.49; H, 6.47; N, 18.76. This sample was identical [by comparison of the fast-atom-bombardment (FAB) MS, UV, and ^{1}H- and ^{13}C-NMR spectra, TLC behavior, and chiroptical properties] with a natural sample of 1′-methylzeatin 9-riboside[3] [CD (*c* = 2.49 × 10⁻⁵ M, MeOH) $[\theta]^{25}$ (nm): −16900 (277) (neg. max.), +38800 (217) (pos. max.)].

[*S*-(*E*)]-*N*-(4-Hydroxy-1,3-dimethyl-2-butenyl)adenosine [(1′*S*)-1′-Methylzeatin 9-β-D-Ribofuranoside] [(1″*S*)-4] Condensation of (*S*)-**14** with 6-chloro-9-β-D-ribofuranosylpurine[22] in 1-butanol in the presence of Et_3N and work-up of the reaction mixture were accomplished as described above for (1″*R*)-**4**, affording (1″*S*)-**4** in 89% yield as a colorless foamy glass, $[\alpha]_D^{18}$ −2.2° (*c* = 0.50, MeOH); $[\alpha]_{365}^{19}$ +133° (*c* = 0.50, MeOH); CD (*c* = 2.88 × 10⁻⁵ M, MeOH) $[\theta]^{25}$ (nm): +15800 (275) (pos. max.), −47100 (216) (neg. max.); UV $\lambda_{max}^{95\%\,EtOH}$ 270 nm (ε 18400); $\lambda_{max}^{H_2O}$ (pH 1) 265 (19200); $\lambda_{max}^{H_2O}$ (pH 7) 269 (19300); $\lambda_{max}^{H_2O}$ (pH 13) 269 (19400); ^{1}H-NMR (CD_3OD) δ: 1.35 (3H, d, *J* = 6.5 Hz, (CHMe), 1.78 (3H, br s, CH=CMe), 3.75 and 3.88 (1H each, dd, *J* = 12.5, 2.5 Hz, C(5′)-H's],[32] 3.94 [2H, br s, C(4″)-H's], 4.17 [1H, ddd, *J* = 2.5 Hz each, C(4′)-H], 4.32 [1H, dd, *J* = 5, 2.5 Hz, C(3′)-H], 4.74 [1H, dd, *J* = 6, 5 Hz, C(2′)-H], 5.28 (1H, dq, *J* = 9, 6.5 Hz, CHMe), 5.53 (1H, m, CH=CMe), 5.94 [1H, d, *J* = 6 Hz, C(1′)-H], 8.22 and 8.23 (1H each, s, purine protons); ^{13}C-NMR (CD_3OD) δ: 14.2 [C(3″)-Me],[32,33] 21.8 [C(1″)-Me],[33] 45.5 [C(1″)], 63.4 [C(5′)], 67.9 [C(4″)], 72.5 [C(2′)], 75.3 [C(3′)], 88.1 [C(4′)], 91.1 [C(1′)], 121.0 [C(5)], 127.4 [C(2″)], 138.0 [C(3″)], 141.0 [C(8)], 149.0 [C(4)], 153.2 [C(2)], 155.0 [C(6)]. The UV and ^{1}H- and ^{13}C-NMR spectra and TLC mobility of this sample were virtually identical with those of (1″*R*)-**4**; the solution IR spectra of the two diastereomers were not available because of the poor solubility. This made differentiation between them unfeasible by these means alone.

[*R*-(*E*)]-*N*-(4-Acetyloxy-1,3-dimethyl-2-butenyl)adenosine 2′,3′,5′-Triacetate [(1″*R*)-13] A solution of (1″*R*)-**4** · 1/2H_2O (75 mg, 0.2 mmol) and acetic anhydride (0.8 ml) in pyridine (1.4 ml) was stirred at 30 °C for 2 h. After addition of EtOH (1 ml), the reaction mixture was concentrated *in vacuo* to leave a colorless oil. The oil was dissolved in $CHCl_3$ (10 ml), and the $CHCl_3$ solution was washed successively with 10% aqueous citric acid (2 × 10 ml) and saturated aqueous $NaHCO_3$ (10 ml), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to leave (1″*R*)-**13** (102 mg, 95%) as a colorless glass, $[\alpha]_D^{14}$ −54.3° (*c* = 0.50, $CHCl_3$); $[\alpha]_{365}^{15}$ −319° (*c* = 0.50, $CHCl_3$); MS *m/z* (relative intensity): 533 (M^+) (2), 474 (54), 259 (3), 216 (100), 200 (4), 157 (2), 139 (15), 97 (8); UV $\lambda_{max}^{95\%\,EtOH}$ 268 nm (ε 18100); IR $\nu_{max}^{CHCl_3}$ (0.2 M solution) cm^{-1}: 3430 (NH), 1750 (ester CO); ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, d, *J* = 6 Hz, CHMe), 1.81 (3H, d, *J* = 1 Hz, CH=CMe), 2.08 (6H, s, two OAc's), 2.13 and 2.14 (3H each, s, two OAc's), 4.2—4.6 [5H, m, two CH_2's and C(4′)-H],[32] 5.25 (1H, m, CHMe), 5.48 (1H, m, CH=CMe), 5.67 [1H, dd, *J* = 5.5, 4 Hz, C(3′)-H], 5.70 (1H, d, *J* = 8 Hz, NH), 5.93 [1H, dd, *J* = 5.5 Hz each, C(2′)-H], 6.17 [1H, d, *J* = 5.5 Hz, C(1′)-H], 7.88 and 8.38 (1H each, s, purine protons). This sample was identical (by comparison of the MS, UV, and ^{1}H-NMR spectra and specific rotation) with a sample of the tetra-*O*-acetyl derivative[3] of natural 1′-methylzeatin 9-riboside.

[*S*-(*E*)]-*N*-(4-Acetyloxy-1,3-dimethyl-2-butenyl)adenosine 2′,3′,5′-Triacetate [(1″*S*)-13] This was prepared from (1″*S*)-**4** in 80% yield in a manner similar to that described above for (1″*R*)-**13** and isolated as a colorless glass, $[\alpha]_D^{15}$ −3.5° (*c* = 0.82, $CHCl_3$); $[\alpha]_{365}^{17}$ +64° (*c* = 0.82, $CHCl_3$); MS *m/z* (relative intensity): 533 (M^+) (6), 474 (100), 259 (11), 216 (100), 200 (8), 157 (6), 139 (51), 97 (27); UV $\lambda_{max}^{95\%\,EtOH}$ 268 nm (ε 18400); IR $\nu_{max}^{CHCl_3}$ (0.2 M solution) cm^{-1}: 3430 (NH), 1750 (ester CO); ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, d, *J* = 6 Hz, CHMe), 1.81 (3H, br s, CH=CMe), 2.09 (6H, s, two OAc's), 2.13 and 2.14 (3H each, s, two OAc's), 4.2—4.6 [5H, m, two CH_2's and C(4′)-H],[32] 5.26 (1H, m, CHMe), 5.49 (1H, m, CH=CMe), 5.67 [1H, dd, *J* = 5.5, 4 Hz, C(3′)-H], 5.76 (1H, d, *J* = 8 Hz, NH), 5.92 [1H, dd, *J* = 5.5 Hz each, C(2′)-H], 6.17 [1H, d, *J* = 5.5 Hz, C(1′)-H], 7.89 and 8.38 (1H each, s, purine protons). The MS, UV, IR ($CHCl_3$), and ^{1}H-NMR spectra and TLC mobility of this sample were virtually identical with those of (1″*R*)-**13** so that differentiation between the two diastereomers was impossible by these means only.

Bioassay Procedure For tobacco callus (*Nicotiana tabacum* L. cv Wisconsin No. 38) bioassay, the previously reported method[8g,j,34] was used. The basal culture medium was the Linsmaier and Skoog medium[34] containing mineral salts, 30 g/l sucrose, 10 g/l agar, 100 mg/l *myo*-inositol, 2 mg/l indole-3-acetic acid (IAA), and 0.4 mg/l thiamine hydrochloride. Aqueous solutions of the test compounds were filter-sterilized and added to the autoclaved basal media in 50-ml conical flasks, shortly before solidification. Each flask contained 20 ml of medium and 3 pieces of tobacco callus (5—8 mg each, fresh weight) implanted on the agar surface. Each experimental treatment included 4 replicates of flasks. The flasks were maintained at 28 °C in the dark for 30 d, and then the fresh weight of tissues was determined. The results are summarized in Table I.

The cytokinin assay by lettuce seed germination was carried out as described previously.[8g,j,35] Lettuce (*Lactuca sativa* L. cv New York 515) seeds were sown on a sheet of filter paper, wetted with 4 ml of an aqueous solution of test compound, in a Petri dish (7 cm in diameter). After a 48 h incubation in darkness at 27 °C, the germination percentages were determined. Table II summarizes the results.

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31) This assignment was based on comparison with that of the (*Z*)-isomer,[8i] and the details will be published elsewhere shortly.
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