Pourines. XLV. : Syntheses and Cytokinin Activities of the Cis Isomers of (1'R)-1'-Methylzeatin and Its 9-β-D-Ribofuranoside

メタデータ	言語: eng 出版者: 公開日: 2017-10-03 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	http://hdl.handle.net/2297/7597

Purines. XLV.[1] Syntheses and Cytokinin Activities of the *Cis* Isomers of (1′*R*)-1′-Methylzeatin and Its 9-β-D-Ribofuranoside

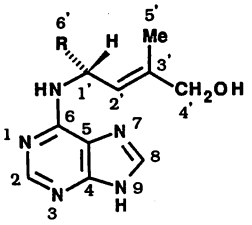
Tozo FUJII,*[,a] Masashi OHBA,[a] Miwa SAKARI,[a] and Satoshi MATSUBARA[b]

Faculty of Pharmaceutical Sciences, Kanazawa University,[a] Takara-machi, Kanazawa 920, Japan and Laboratory of Applied Biology, Kyoto Prefectural University,[b] Shimogamo Hangi-cho, Sakyo-ku, Kyoto 606, Japan. *Received April 18, 1990*

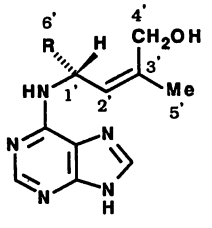
(1′*R*)-1′-Methyl-*cis*-zeatin (2b) and its 9-β-D-ribofuranoside (4b) have been synthesized for the first time from D-alanine (5) in 7 steps. The new *cis*-zeatin derivatives 2b and 4b, together with known *cis*-zeatin (2a), were tested for cytokinin activity in the tobacco callus and the lettuce seed germination bioassays. In both bioassays, the cytokinin activity was found to follow the order 2a > 2b > 4b, indicating that 2b and 4b were less active than the corresponding *trans* isomers 1b and 3b (natural cytokinins from *Pseudomonas syringae* pv *savastanoi*), respectively.

Keywords (1′*R*)-1′-methyl-*cis*-zeatin; (1′*R*)-1′-methyl-*cis*-zeatin 9-riboside; chiral synthesis; alanine; α-amino aldehyde; Horner–Wadsworth–Emmons reaction; *Z* stereoselectivity; diisobutylaluminum hydride reduction; cytokinin activity; ^{13}C-NMR

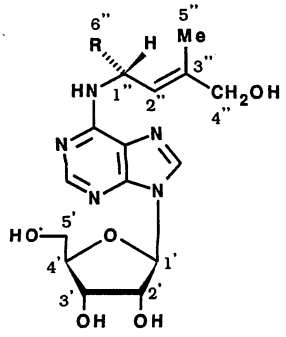
Cytokinins are a group of phytohormones characterized primarily by the ability to promote cell division in plant tissue cultures or secondarily by the ability to promote seed germination, leaf and cotyledon growth, or lateral bud development, to inhibit chlorophyll degradation, or to induce buds on moss protonema.[2] Apart from a large number of synthetic cytokinins whose activity varies from highly active to almost inactive, more than 30 natural cytokinins have so far been isolated from plants and microorganisms, and their chemical structures identified.[2c,3-6] Interestingly, all these natural cytokinins are N^6-substituted adenines with or without substituent(s) on the purine nucleus,[7] and *trans*-zeatin (**1a**) (which is often referred to simply as zeatin), *cis*-zeatin (**2a**), and the corresponding 9-β-D-ribofuranosides (**3a** and **4a**) are among them. The isolation of (1″*R*)-1″-methyl-*trans*-zeatin 9-β-D-ribofuranoside (**3b**)[3] in 1985 and its aglycone, (1′*R*)-1′-methyl-*trans*-zeatin (**1b**),[4] in 1986 from the culture filtrate of the gall-forming phytopathogenic bacterium *Pseudomonas syringae* pv *savastanoi* and the establishment of their structures by means of chemical synthesis[5a,b] exemplify the recent addition of new members to the natural cytokinin group. The occurrence of both the *cis* and *trans* isomers in the 1′-unsubstituted zeatin series suggests that the *cis* isomers of the new cytokinins **1b** and **3b** may also occur in nature, and the availability of synthetic reference samples would greatly facilitate the search for these *cis* isomers as natural products. This was the reason why we investigated the syntheses and cytokinin activities of (1′*R*)-1′-methyl-*cis*-zeatin (**2b**) and its 9-β-D-ribofuranoside (**4b**) in the present work. Brief accounts of the chemical results presented here have been published in preliminary form.[8]



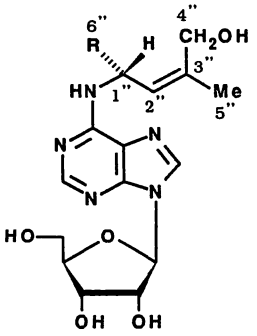
1a,b



2a,b



3a,b



4a,b

a: R = H **b**: R = Me

The synthetic routes to **2b** and **4b** started with the *N*-protected D-alanine methyl ester **7**,[5a,b,9] which was prepared from D-alanine (**5**) through the amino ester hydrochloride **6**[5a,b,10] according to the literature. The conversion of **7** to the (+)-aldehyde **8** had previously been effected in two steps consisting of $LiBH_4$ reduction of **7** and Me_2SO oxidation of the resulting *N*-protected amino alcohol using SO_3–pyridine complex in the presence of Et_3N.[5a,b] In the present study, however, reduction of **7** with diisobutylaluminum hydride (DIBAH) in CH_2Cl_2–hexane at −78 °C for 1 h was found to give **8** in one step in 66% yield. This was an application of the known method to reduce *N*-alkoxycarbonyl α-amino acid esters to the corresponding aldehydes without accompanying racemization.[11]

The conversion of the (+)-aldehyde **8** into the (*Z*)-ester **9** with excellent *Z* stereoselectivity was a key process in the present synthetic scheme. We recently reported that the Wittig reaction[12] of **8** with methyl 2-(triphenylphosphoranylidene)propionate in CH_2Cl_2 at 22 °C for 1 h produced a 5:95 mixture of the (*Z*)-ester and its (*E*)-isomer in 98% yield.[5a,b] In order to reverse such stereoselectivity, **8** was subjected to the Still–Gennari modification[13] [$(CF_3CH_2O)_2P(O)CH(Me)CO_2Me$, $KN(SiMe_3)_2$, 18-crown-6/MeCN, tetrahydrofuran, −78 °C, 30 min] of the Horner–Wadsworth–Emmons reaction.[12] As expected, this modification afforded a 99:1 mixture of the desired (*Z*)-ester **9** and its (*E*)-isomer[5a,b] in 68% yield, from which **9** [mp 54—54.5 °C; $[\alpha]_D^{22}$ −71.7° (MeOH)] was isolated in 61% yield by recrystallization (from hexane). The assignment of geometry in **9** was based on the facts that it was the major

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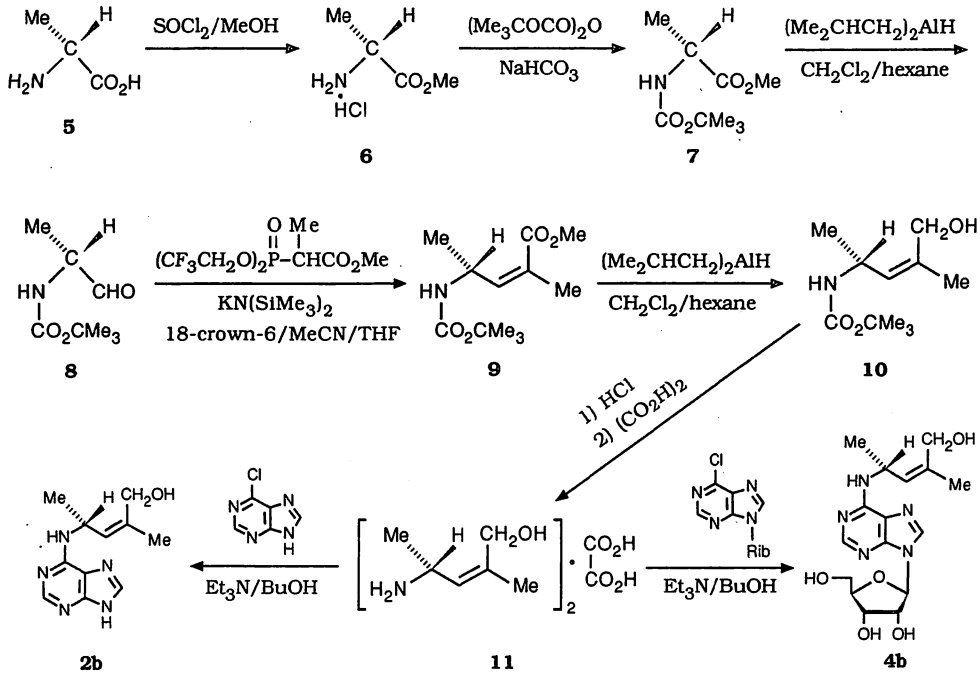


Chart 1

TABLE I. ^{13}C Shieldings of Both Geometrical Isomers of 1'-Methylzeatin and Their 9-β-D-Ribofuranosides

Carbon	Chemical shift[a]			Carbon	Chemical shift[a]		
	1b[b]	**2b**			**3b**[b]	**4b**	
	in CD_3OD	in CD_3OD	in $(CD_3)_2SO$		in CD_3OD	in CD_3OD	in $(CD_3)_2SO$
C(2)	153.8	153.5	152.1	C(2)	153.5	153.4	152.1
C(4)	151.1	151.0	—[c]	C(4)	149.3	149.1	148.5
C(5)	118.8	118.8	—[c]	C(5)	121.3	121.0	119.4
C(6)	154.7	154.5	153.0	C(6)	155.3	155.2	153.5
C(8)	140.4	140.6	138.6	C(8)	141.3	141.5	139.5
C(1')	45.5	45.4	43.2	C(1'')	45.6	45.5	43.2
C(2')	127.8	130.5	128.8	C(2'')	127.7	130.4	128.5
C(3')	138.3	137.9	135.5	C(3'')	138.3	138.0	135.6
C(4')	68.0	62.2	59.9	C(4'')	68.0	62.2	59.9
C(5')	14.2[d]	21.7[d]	21.0[d]	C(5'')	14.1[d]	21.7[d]	21.0
C(6')	21.9[d]	22.1	22.0	C(6'')	21.8[d]	22.1	21.9
				C(1')	91.3	91.4	87.9
				C(2')	75.4[d,e]	75.5	73.4
				C(3')	72.7[d,e]	72.7	70.6
				C(4')	88.2	88.2	85.8
				C(5')	63.5	63.5	61.6

a) In ppm downfield from internal Me_4Si. *b*) Data taken from ref. 5*d*. *c*) An unmeasurably small peak resulting from the poor solubility of **2b**. *d*) This assignment is based on a selective proton decoupling experiment. *e*) The previous C(2') and C(3') shift assignments for **3b**[3,5b] and its (1''*S*)-isomer[5b] should be reversed accordingly.

isomer formed[13] and that its olefinic proton [δ 5.80 (dq, J=8.5 and 1.5 Hz)] resonated in $CDCl_3$ at higher field than the corresponding proton [δ 6.53 (dq, J=8.5 and 1.5 Hz)][5a,b] of the (*E*)-isomer. Although the enantiomeric purity of the above sample of **9** seemed high, we were unable to determine it by means of proton nuclear magnetic resonance (^1H-NMR) spectroscopy using the chiral shift reagent tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]-europium(III) [Eu(hfc)$_3$], a method employed successfully in the case of the corresponding (*E*)-isomer,[5b] because of nonseparable broad signals of enantiomeric protons, if present.

On reduction with DIBAH in CH_2Cl_2–hexane at −78 °C for 45 min, the (*Z*)-ester **9** gave the (−)-allylic alcohol **10** in 92% yield. The carbamate **10** was then 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 (**11**) in 88% yield (from **10**).

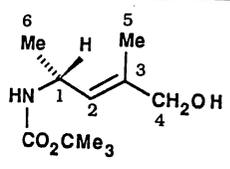
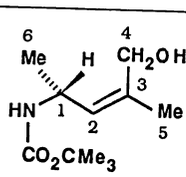
Finally, purinylation of **11** with 6-chloropurine in boiling 1-butanol containing Et_3N for 3.5 h produced (1'*R*)-1'-methyl-*cis*-zeatin (**2b**) in 75% yield. A similar condensation of **11** with 6-chloro-9-β-D-ribofuranosylpurine[14] gave the target riboside **4b** in 96% yield. The high enantiomeric purity of **2b** and high diastereomeric purity of **4b** were suggested by comparison of their chiroptical properties with those reported[5a,b] for **1b** and **3b**. The *Z* stereochemistry of the

N^6-substituents in **2b** and **4b** was confirmed by comparison of their ^{13}C-NMR spectra with those of the corresponding (*E*)-isomers (**1b** and **3b**). It may be seen from Table I that the C(4′) carbon of **2b** resonated in CD_3OD at higher field than did the corresponding carbon of **1b** by 5.8 ppm because of the γ-effect.[15] This was also the case for the C(4″) carbons of the *Z–E* isomeric nucleoside pair **4b** and **3b**. Similar upfield shifts (7.5—7.6 ppm) of the C(5′) and C(5″) signals of **1b** and **3b** in CD_3OD, relative to the corresponding signals of **2b** and **4b**, also supported the correctness of the *cis* relationship between the C(5′) and C(1′) atoms in **1b** and that between the C(5″) and C(1″) atoms in **3b**. As shown in Table II, the *Z* stereochemistry of the precursor **10** and the *E* stereochemistry of the previously synthesized isomer **12**[5a,b] were likewise characterized by upfield shifts of the C(4) signal of **10** by 6.2 ppm and of the C(5) signal of **12** by 8.4 ppm, relative to the corresponding signals of the isomeric counterpart. These upfield shifts are again interpretable in terms of the γ-effect.[15]

With the completion of the above syntheses and characterization of (1′*R*)-1′-methyl-*cis*-zeatin (**2b**) and its 9-β-D-ribofuranoside (**4b**), 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 III that in the tobacco callus bioassay the maximal yield of the callus was obtained at 4 μM **2b** and at 1 μM *cis*-zeatin (**2a**). As had seemed probable,[2b,d] the nucleoside **4b** was less active than the aglycone **2b**: it was only active at 100 μM or higher concentration. Thus, the cytokinin activity follows the order: *cis*-zeatin (**2a**) > **2b** > **4b**. The same activity order was also found in the lettuce seed germination bioassay, as shown in Table IV. These results indicate that the introduction of a methyl group into *cis*-zeatin (**2a**) at the 1′-position with the *R* configuration lowers its cytokinin activity by a factor of 4. This presents a contrast to the previous finding[5b] that the corresponding structural modification in *trans*-zeatin (**1a**) does not alter its cytokinin activity (optimum at 0.04 μM concentration in the tobacco callus bioassay). It is known that the *cis* forms (**2a** and **4a** in the zeatin series are less active than the corresponding *trans* forms (**1a** and **3a**) in the standard tobacco callus bioassay for cytokinin activity.[2b,d,16] Interestingly, such a *cis–trans* activity relationship also holds for the (1′*R*)-1′-methylzeatin series, since the maximal yield of the tobacco callus has been obtained at 0.04 μM **1b**,[5b] 4 μM **2b**, 0.4 μM **3b**,[5b] and 100 (or >100) μM **4b**.

In conclusion, (1′*R*)-1′-methyl-*cis*-zeatin (**2b**) and its 9-β-D-ribofuranoside (**4b**), the (*Z*)-isomers of the natural cytokinins **1b** and **3b**, have now become available by synthesis in 7 steps starting from D-alanine (**5**) and proceeding through the key intermediate **8**. These syntheses demonstrate the usefulness of optically active *N*-protected α-amino aldehydes (type **8**) in stereocontrolled organic synthesis.[17] In the two bioassay systems for cytokinin activity, the *cis* base **2b** and its 9-riboside **4b** have been found to be less active than the corresponding *trans* isomers **1b** and **3b**, respectively. With synthetic samples of **2b** and **4b** now available, the search for these substances in plants and microorganisms will be facilitated.

TABLE II. ^{13}C Shieldings of Both Geometrical Isomers in an Allylic Amino Alcohol System

Carbon[b]	Chemical shift[a] in $CDCl_3$	
	 **12**	 **10**
C(1)	44.6	44.0
C(2)	127.3	129.6
C(3)	136.3	137.0
C(4)	67.7	61.5
C(5)	13.9[c]	22.3[c]
C(6)	21.8	21.2[c]
$\underline{C}O_2CMe_3$	155.2	155.6
$CO_2\underline{C}Me_3$	79.3	79.8
$CO_2C\underline{Me}_3$	28.5	28.4

a) In ppm downfield from internal Me_4Si. *b*) The carbon indicated by underscoring in the partial structure is that to which the signal has been assigned. *c*) This assignment is based on a selective proton decoupling experiment.

Experimental

General Notes All melting points were taken on a Yamato MP-1 capillary melting point apparatus and are corrected. Thin-layer chromatography (TLC) was done on Merck Kieselgel 60 F_{254} plates (0.25-mm thickness), and spots were detected by means of ultraviolet (UV) absorbance (at 254 nm) and/or by spraying with the standard $KMnO_4$ or I_2–KI reagent. Flash chromatography[18] was carried out by using Kieselgel 60 (E. Merck, No. 9385) silica gel. High-performance liquid chromatography (HPLC) was performed on a Waters ALC/GPC 204 liquid chromatograph, and peaks were located by using a UV absorbance detector operated at 254 nm. Spectra reported herein were recorded on a Hitachi 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 infrared (IR) spectrophotometer, a Hitachi M-80 mass spectrometer, a JASCO J-500C spectropolarimeter, or a JEOL JNM-FX-100 nuclear magnetic resonance (NMR) spectrometer (^{1}H 100 MHz and ^{13}C 25.0 MHz), 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 = mul-

TABLE III. Cytokinin Activity of *cis*-Zeatin Analogues Tested by the Tobacco Callus Bioassay

Compound	Average fresh weight of tobacco callus (mg)										
	Concentration of test compound (μM)										
	0	0.001	0.01	0.04	0.1	0.4	1	4	10	40	100
2b	16	—	19	26	32	153	835	1869	1252	567	—
4b	28	—	—	—	29	37	84	186	219	657	1067
cis-Zeatin (**2a**)	24	37	153	714	1160	1387	1503	—	1205	—	—

TABLE IV. Cytokinin Activity of *cis*-Zeatin Analogues Tested by Lettuce Seed Germination

Compound	Lettuce seed germination (%)							
	Concentration of test compound (μM)							
	0	*0.1*	0.4	1	4	10	40	100
2b	5.8	—	—	8.6	12.1	22.2	37.3	70.0
4b	5.8	—	—	7.4	7.3	9.3	12.8	21.8
cis-Zeatin (**2a**)	5.8	6.8	16.9	14.3	22.3	25.7	68.6	72.6

tiplet, s = singlet.

(*R*)-(1-Methyl-2-oxoethyl)carbamic Acid *tert*-Butyl Ester (8) A stirred solution of **7**[5a,b,9] (1.23 g, 6 mmol) in dry CH_2Cl_2 (30 ml) was cooled to −78 °C in an atmosphere of argon, and a 1.0 M solution (12 ml, 12 mmol) of diisobutylaluminum hydride in hexane was added dropwise over 20 min. After the mixture had been stirred at −78 °C for 1 h, the reaction was quenched by adding MeOH (0.5 ml). The resulting mixture, after addition of 10% aqueous Rochelle salt (35 ml), was stirred at room temperature for 1 h. The aqueous layer was separated from the organic layer and extracted with CH_2Cl_2 (3 × 20 ml). The CH_2Cl_2 extracts and the above organic layer were combined, washed with saturated aqueous NaCl (70 ml), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave a colorless solid. Recrystallization of the solid from hexane afforded **8** (694 mg, 66%) as colorless prisms, mp 86—87 °C; $[\alpha]_D^{22}$ +35.2° (*c* = 1.00, MeOH). This sample was identical (by comparison of the IR and ^{1}H-NMR spectra) with authentic **8** [mp 90—91 °C; $[\alpha]_D^{18}$ +35.2° (*c* = 1.00, MeOH)].[5a,b]

[*R*-(*Z*)]-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoic Acid Methyl Ester (9) A solution of recrystallized 18-crown-6–MeCN complex[19] (6.93 g, 20 mmol) and methyl 2-[bis(2,2,2-trifluoroethoxy)phosphinyl]propionate $[(CF_3CH_2O)_2P(O)CH(Me)CO_2Me]$[13] (1.33 g, 4 mmol) in dry tetrahydrofuran (THF) (60 ml) was stirred at −78 °C under an atmosphere of N_2, and a 0.5 M solution (8 ml, 4 mmol) of potassium bis(trimethylsilyl)amide $[KN(SiMe_3)_2]$ in toluene (Aldrich Chemical Co.) was added dropwise over 10 min. The mixture was stirred at −78 °C for 25 min, and then a solution of **8** (695 mg, 4 mmol) in dry THF (12 ml) was added dropwise over 5 min. After the resulting mixture had been stirred at the same temperature for 30 min, the reaction was quenched by adding saturated aqueous NH_4Cl (30 ml). The reaction mixture was then stirred at room temperature, and H_2O (13 ml) was added. The aqueous layer was separated from the organic layer and extracted with ether (2 × 40 ml). The ethereal extracts and the above organic layer were combined, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave an orange oil (2.54 g). The oil was purified by means of flash chromatography[18] [hexane–AcOEt (3 : 1, v/v)] to give a colorless solid (662 mg, 68%), mp 52—53 °C, which was a 99 : 1 mixture of **9** and the (*E*)-isomer[5a,b] as estimated by means of HPLC [Hibar column LiChrosorb Si 60 (5 μm) (Cica–Merck, No. 50388), hexane–AcOEt (7 : 1, v/v), 220 p.s.i., 0.7 ml/min].[20] Recrystallization of the crude solid from hexane afforded a pure sample of **9** (593 mg, 61%) as colorless prisms, mp 54—54.5 °C; $[\alpha]_D^{22}$ −71.7° (*c* = 1.03, MeOH); IR ν_{max}^{Nujol} cm^{-1}: 3330 (NH), 1715 (α,β-unsaturated ester CO), 1682 (carbamate CO), 1645 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, d, *J* = 7 Hz, CHMe), 1.42 (9H, s, CMe_3), 1.90 (3H, d, *J* = 1.5 Hz, CH=CMe), 3.75 (3H, s, CO_2Me), 4.5 (br, NH), 4.94 (1H, dq, *J* = 8.5, 7 Hz, CHMe), 5.80 (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, 59.11; H, 8.81; N, 5.59.

[*R*-(*Z*)]-(4-Hydroxy-1,3-dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester (10) A stirred solution of **9** (584 mg, 2.4 mmol) in dry CH_2Cl_2 (5 ml) was cooled to −78 °C in an atmosphere of N_2, and a 1.0 M solution (7.2 ml, 7.2 mmol) of diisobutylaluminum hydride in hexane was added dropwise over 10 min. After the mixture had been stirred at −78 °C for 45 min, the reaction was quenched by adding a 5 M solution (4.5 ml) of AcOH in CH_2Cl_2 at −78 °C. The reaction mixture was then stirred at room temperature, and 10% aqueous tartaric acid (11 ml) and H_2O (8 ml) were added in that order. The aqueous layer was separated from the organic layer and extracted with CH_2Cl_2 (3 × 7 ml). The CH_2Cl_2 extracts and the above organic layer were combined, washed successively with saturated aqueous $NaHCO_3$ (30 ml) and saturated aqueous NaCl (30 ml), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave a colorless oil. Purification of the oil by means of flash chromatography[18] [hexane–AcOEt (3 : 1, v/v)] gave **10** (477 mg, 92%) as a colorless solid, mp 66.5—67.5 °C. Recrystallization from hexane yielded an analytical sample as colorless scales, mp 67.5 °C; $[\alpha]_D^{24}$ −3.3° (*c* = 1.00, MeOH); IR ν_{max}^{Nujol} cm^{-1}: 3370, 3250 (NH and OH), 1688 (carbamate CO); ^{1}H-NMR ($CDCl_3$) δ: 1.16 (3H, d, *J* = 6.5 Hz, CHMe), 1.42 (9H, s, CMe_3), 1.80 (3H, d, *J* = 1.5 Hz, CH=CMe), 3.65 (1H, br, NH or OH), 3.70 and 4.46 (2H, AB type d's, *J* = 11.5 Hz, CH_2OH), 4.3—4.7 (2H, m, CHMe and OH or NH), 5.03 (1H, dull d, *J* = 9.5 Hz, CH=CMe); ^{13}C-NMR (Table II). *Anal.* Calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.24; H, 10.10; N, 6.52.

[*R*-(*Z*)]-4-Amino-2-methyl-2-penten-1-ol Ethanedioate (2 : 1) (Salt) (11) A mixture of **10** (581 mg, 2.7 mmol) and 10% aqueous HCl (5.4 ml) was shaken at room temperature for 1 h, giving a slightly orange solution. The solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (27 ml), and the column was eluted with H_2O. The eluate (100 ml) was concentrated to dryness *in vacuo* to leave a yellow oil (330 mg), which was dissolved in EtOH (2.7 ml). The resulting ethanolic solution was exactly neutralized by addition of a solution of oxalic acid (122 mg, 1.35 mmol) in EtOH (1.4 ml) and, if necessary, with Et_3N. The precipitate that resulted was filtered off, washed with EtOH (3.5 ml), and dried to give a first crop (370 mg, 86%) of **11** as a colorless powder, mp 198.5—199.5 °C (dec.). The filtrate and washings were combined and concentrated *in vacuo*, and the residue was recrystallized from 99% (v/v) aqueous EtOH (1 ml), yielding a second crop (12 mg). The total yield of **11** was 382 mg (88%). Recrystallization of crude **11** from 97% (v/v) aqueous EtOH produced an analytical sample as colorless filaments, mp 199—199.5 °C (dec.); $[\alpha]_D^{23}$ −10.1° (*c* = 0.458, MeOH); IR ν_{max}^{Nujol} cm^{-1}: 3460 (OH), 1580 (COO^- and NH_3^+); ^{1}H-NMR [$(CD_3)_2SO$] δ: 1.11 (3H, d, *J* = 6.5 Hz, CHMe), 1.69 (3H, d, *J* = 1.5 Hz, CH=CMe), 3.88 and 3.99 (2H, AB type d's, *J* = 12.5 Hz, CH_2OH), 3.8—4.15 (1H, m, CHMe), 4.94 (4H, br s, OH and NH_3^+), 5.11 (1H, dq, *J* = 9, 1.5 Hz, CH=CMe). *Anal.* Calcd for $C_{14}H_{28}N_2O_6$: C, 52.48; H, 8.81; N, 8.74. Found: C, 52.26; H, 8.87; N, 8.74.

[*R*-(*Z*)]-2-Methyl-4-(9*H*-purin-6-ylamino)-2-penten-1-ol [(1′*R*)-1′-Methyl-*cis*-zeatin] (2b) A stirred solution of **11** (88.1 mg, 0.275 mmol) and 6-chloropurine (77.7 mg, 0.503 mmol) in 1-butanol (5 ml) containing Et_3N (0.5 ml) was heated under reflux for 3.5 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^-) (5 ml), and the column was eluted with H_2O (100 ml). The eluate was concentrated *in vacuo*, and the residual jelly was purified by flash chromatography[18] [$CHCl_3$–MeOH (8 : 1, v/v)] to give **2b** (88 mg, 75%) as a colorless solid. Recrystallizations of the solid from AcOEt and then from MeCN yielded an analytical sample as colorless, minute prisms, mp 182—183.5 °C; $[\alpha]_D^{22}$ −128° (*c* = 0.117, EtOH); CD (*c* = 5.93 × 10^{-5} M, MeOH) $[\theta]^{18}$ (nm): −26100 (272) (neg. max.), +57700 (215) (pos. max.); MS *m/z*: 233 (M^+); UV $\lambda_{max}^{95\%\ EtOH}$ 270 nm (ε 18600); $\lambda_{max}^{H_2O}$ (pH 1) 275 (16500); $\lambda_{max}^{H_2O}$ (pH 7) 270 (17900); $\lambda_{max}^{H_2O}$ (pH 10) 272 (17000); $\lambda_{max}^{H_2O}$ (pH 13) 275 (17300); ^{1}H-NMR (CD_3OD) δ: 1.37 (3H, d, *J* = 6.5 Hz, CHMe), 1.80 (3H, d, *J* = 1 Hz, CH=CMe), 4.13 and 4.33 (2H, AB type d's, *J* = 12.5 Hz, CH_2OH), 5.1—5.4 (2H, m, CHMe and CH=CMe), 8.06 and 8.20 (1H each, s, purine protons); ^{1}H-NMR [$(CD_3)_2SO$] δ: 1.25 (3H, d, *J* = 6.5 Hz, CHMe), 1.69 (3H, s, CH=CMe), 4.06 (2H, br s, CH_2OH), 5.32 (2H, br s, CHMe and CH=CMe), 8.10 and 8.16 (1H each, s, purine protons); ^{13}C-NMR (Table I). *Anal.* Calcd for $C_{11}H_{15}N_5O$: C, 56.63; H, 6.48; N, 30.03. Found: C, 56.86; H, 6.52; N, 30.10.

[*R*-(*Z*)]-*N*-(4-Hydroxy-1,3-dimethyl-2-butenyl)adenosine [(1″*R*)-1″-Methyl-*cis*-zeatin 9-β-D-Ribofuranoside] (4b) A stirred solution of **11** (106 mg, 0.33 mmol) and 6-chloro-β-D-ribofuranosylpurine[14] (172 mg, 0.6 mmol) in 1-butanol (6 ml) containing Et_3N (0.6 ml) was heated under reflux for 3.5 h. The reaction mixture was concentrated to dryness *in vacuo*, and the residual solid was recrystallized from H_2O (5 ml), giving a first crop (176 mg, 80%) of **4b**, mp 192—197 °C. The mother liquor from the above recrystallization was passed through a column of Amberlite IRA-402 (HCO_3^-) (6 ml), and the column was eluted with H_2O (60 ml). The eluate was concentrated *in vacuo*, and the residue was recrystallized from H_2O (3 ml) to furnish a second crop (16 mg, 7%) of **4b**, mp 182—184 °C. A further crop (18 mg) was obtained by concentration of the mother liquor of this recrystallization under reduced pressure and subsequent purification of the residue by flash chromatography[18] [$CHCl_3$–MeOH (8 : 1, v/v)]. The total yield of **4b** was 210 mg (96%). Further recrystallization of crude **4b** from H_2O provided an analytical sample as colorless filaments, mp 206.5—207.5 °C; $[\alpha]_D^{23}$ −131° (*c* = 0.156, MeOH); $[\alpha]_{365}^{23}$ −676° (*c* = 0.156, MeOH); CD (*c* = 3.46 × 10^{-5} M, MeOH) $[\theta]^{18}$ (nm): −29500 (275) (neg. max.), +54300 (218) (pos. max.); MS *m/z*: 365 (M^+); UV $\lambda_{max}^{95\%\ EtOH}$ 270

nm (ε 19600); $\lambda_{max}^{H_2O}$ (pH 1) 266 (20100); $\lambda_{max}^{H_2O}$ (pH 7) 269 (20200); $\lambda_{max}^{H_2O}$ (pH 13) 269 (20400); ^{1}H-NMR (CD_3OD) δ: 1.36 (3H, d, J=6.5 Hz, CHMe), 1.79 (3H, s, CH=CMe), 3.71 and 3.90 [1H each, dd, J=13, 2.5 Hz, C(5')-H's],[21] 4.15 and 4.29 [2H, AB type d's, J=12.5 Hz, C(4'')-H's], 4.16 [1H, ddd, J=2.5 Hz each, C(4')-H], 4.31 [1H, dd, J=5, 2.5 Hz, C(3')-H], 4.73 [1H, dd, J=6.5, 5 Hz, C(2')-H], 5.30 [2H, br s, C(1'')-H and C(2'')-H], 5.94 [1H, d, J=6.5 Hz, C(1')-H], 8.19 and 8.24 (1H each, s, purine protons); ^{1}H-NMR [$(CD_3)_2SO$] δ: 1.25 (3H, d, J=6.5 Hz, CHMe), 1.69 (3H, s, CH=CMe), 3.5—3.75 [2H, m, C(5')-H's],[21] 3.85—4.25 [4H, m, C(3')-H, C(4')-H, C(4'')-H's], 4.5—4.8 [2H, m, C(2')-H and OH], 5.1—5.5 [5H, m, C(1'')-H, C(2'')-H, and three OH's], 5.89 [1H, d, J=6 Hz, C(1')-H], 7.77 (1H, d, J=8 Hz, NH), 8.19 and 8.36 (1H each, s, purine protons); ^{13}C-NMR (Table I). *Anal.* Calcd for $C_{16}H_{23}N_5O_5$: C, 52.60; H, 6.34; N, 19.17. Found: C, 52.53; H, 6.49; N, 19.02.

Bioassay Procedure The cytokinin activities of **2b**, **4b**, and *cis*-zeatin (**2a**) were tested in the tobacco callus and the lettuce seed germination bioassays in a manner similar to that described recently[5b] for the *trans* isomers **1b**, **3b**, and **1a** and related compounds. The results are given in Tables III and IV.

Acknowledgment We are pleased to acknowledge the support of this work by a grant from the Japan Research Foundation for Optically Active Compounds.

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