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

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(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; ¹³C-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])

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 LiBH₄ reduction of 7 and Me₂SO oxidation of the resulting N-protected amino alcohol using SO₃–pyridine complex in the presence of Et₃N.[5a,b]) In the present study, however, reduction of 7 with diisobutylaluminum hydride (DIBAH) in CH₂Cl₂–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 CH₂Cl₂ 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]) [(CF₃CH₂O)₂P(O)CH(Me)CO₂Me, KN(SiMe₃)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
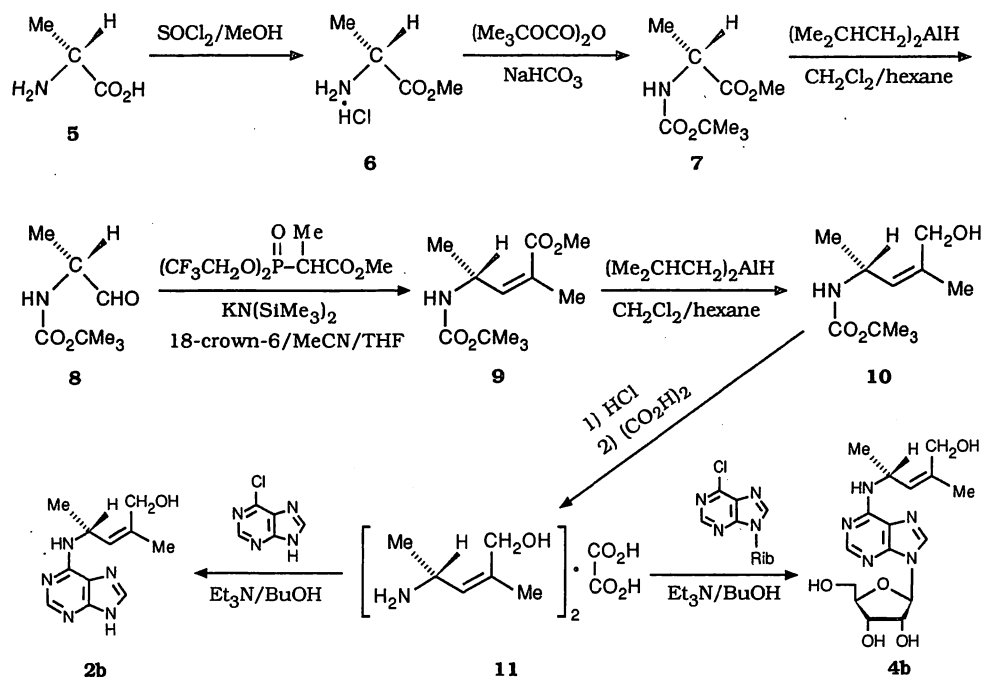


1a,b



2a,b



3a,b



4a,b

a: R = H    b: R = Me
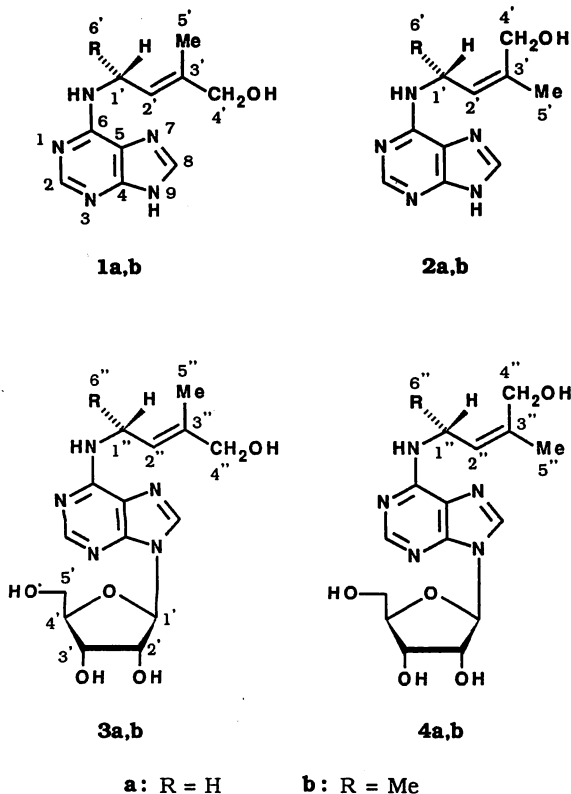
Chart 1

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

| Carbon | Chemical shift[a] | | | Carbon | Chemical shift[a] | | |
|---|---|---|---|---|---|---|---|
| | 1b[b] | 2b | | | 3b[b] | 4b | |
| | in CD$_3$OD | in CD$_3$OD | in (CD$_3$)$_2$SO | | in CD$_3$OD | in CD$_3$OD | in (CD$_3$)$_2$SO |
| 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$_4$Si. b) Data taken from ref. 5d. 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 [$\delta$ 5.80 (dq, $J$ = 8.5 and 1.5 Hz)] resonated in CDCl$_3$ at higher field than the corresponding proton [$\delta$ 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 ($^1$H-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$_2$Cl$_2$–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$_3$N for 3.5 h produced (1'$R$)-1'-methyl-$cis$-zeatin (2b) in 75% yield. A similar condensation of 11 with 6-chloro-9-$\beta$-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$_3$OD 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$_3$OD, 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 structura· modification in *trans*-zeatin (**1a**) does not alter its cytokini activity (optimum at 0.04 μM concentration in the tobacc callus bioassay). It is known that the *cis* forms (**2a** and **4a** in the zeatin series are less active than the correspondin *trans* forms (**1a** and **3a**) in the standard tobacco callu 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 tobacc callus has been obtained at 0.04 μM **1b**,[5b] 4 μM **2b**, 0.4 μ **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.

### 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$_4$Si 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 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] |
| <u>C</u>O$_2$CMe$_3$ | 155.2 | 155.6 |
| CO$_2$<u>C</u>Me$_3$ | 79.3 | 79.8 |
| CO$_2$C<u>Me</u>$_3$ | 28.5 | 28.4 |

*a*) In ppm downfield from internal Me$_4$Si. *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.

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 ($\mu$M) | | | | | | | |
| | 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 $^1H$-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 $\nu_{max}^{Nujol}$ cm$^{-1}$: 3330 (NH), 1715 (α,β-unsaturated ester CO), 1682 (carbamate CO), 1645 (C=C); $^1H$-NMR (CDCl$_3$) $\delta$: 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$_2$Me), 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 $\nu_{max}^{Nujol}$ cm$^{-1}$: 3370, 3250 (NH and OH), 1688 (carbamate CO); $^1H$-NMR (CDCl$_3$) $\delta$: 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$_2$OH), 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$_3$N. 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 $\nu_{max}^{Nujol}$ cm$^{-1}$: 3460 (OH), 1580 (COO$^-$ and NH$_3^+$); $^1H$-NMR [(CD$_3$)$_2$SO] $\delta$: 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$_2$OH), 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-(9H-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$_3$N (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) [θ]$^{18}$ (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); $^1H$-NMR (CD$_3$OD) $\delta$: 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$_2$OH), 5.1—5.4 (2H, m, CHMe and CH=CMe), 8.06 and 8.20 (1H each, s, purine protons); $^1H$-NMR [(CD$_3$)$_2$SO] $\delta$: 1.25 (3H, d, J = 6.5 Hz, CHMe), 1.69 (3H, s, CH=CMe), 4.06 (2H, br s, CH$_2$OH), 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$_3$N (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) [θ]$^{18}$ (nm): −29500 (275) (neg. max.), +54300 (218) (pos. max.); MS m/z: 365 (M$^+$); UV $\lambda_{max}^{95\% EtOH}$ 270

nm ($\varepsilon$ 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$_3$OD) $\delta$: 1.36 (3H, d, $J=6.5$Hz, CH<u>Me</u>), 1.79 (3H, s, CH=C<u>Me</u>), 3.71 and 3.90 [1H each, dd, $J=13$, 2.5Hz, 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, $J=5$, 2.5Hz, C(3')-H], 4.73 [1H, dd, $J=6.5$, 5Hz, C(2')-H], 5.30 [2H, brs, 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$_3$)$_2$SO] $\delta$: 1.25 (3H, d, $J=6.5$Hz, CH<u>Me</u>), 1.69 (3H, s, CH=C<u>Me</u>), 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$_5$O$_5$: 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.

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