Purines. LXII. Both Enantiomers of N^6-(1,3-Dimetyl-2-butenyl)adenine and Their 9-β-D-Ribofuranosides : Synthesis and Cytokinin Activity

Purines. LXII.[1] Both Enantiomers of N^6-(1,3-Dimethyl-2-butenyl)adenine and Their 9-β-D-Ribofuranosides: Synthesis and Cytokinin Activity

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Both enantiomers [(1′*R*)-6 and (1′*S*)-6] of N^6-(1,3-dimethyl-2-butenyl)adenine and their 9-β-D-ribofuranosides [(1″*R*)-16 and (1″*S*)-16] have been synthesized for the first time from both enantiomers of alanine (15) in nine steps. These aglycones and nucleosides, together with N^6-(3-methyl-2-butenyl)adenine (5) and its 9-β-D-ribofuranoside (18) as well as 9-β-D-ribofuranosyl-*cis*-zeatin (20) and 9-(2-deoxy-β-D-ribofuranosyl)-*cis*-zeatin (19), were tested for cytokinin activity in the tobacco callus bioassay. The order of their activity was 5 > (1′*R*)-6 > (1″*R*)-16 ≈ 18 > (1′*S*)-6 > (1″*S*)-16 > 20 > 19. The bioassay results are compared with those obtained previously for the derivatives modified analogously in the N^6-substituent in the *cis*- and *trans*-zeatin series.

Keywords N^6-isopentenyladenine 1′-methyl; chiral synthesis; cytokinin activity; N^6-isopentenyladenosine 1″-methyl; *cis*-zeatin 9-(2-deoxyribofuranosyl)

Cytokinins constitute a class 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] Besides a large number of synthetic cytokinins, whose activity varies from highly active to almost inactive, more than 30 naturally occurring cytokinins have so far been isolated from plants and microorganisms, and their chemical structures established.[2c,3-7] Interestingly, all these natural cytokinins are N^6-substituted adenines with or without substituent(s) on the purine nucleus,[8] and they may be structurally classified into two groups according to their N^6-substituents: (1) the zeatin [N^6-(4-hydroxy-3-methyl-2-butenyl)adenine] family (*e.g.*, type **1** or **3**) and (2) the IPA [N^6-(Δ^2-isopentenyl)adenine; N^6-(3-methyl-2-butenyl)adenine; N^6-(γ,γ-dimethylallyl)adenine] family (*e.g.*, type **5**).[9]

The zeatin family includes (1′*R*)-1′-methyl-*trans*-zeatin [(1′*R*)-**2**][4,5] and its 9-riboside [(1″*R*)-1″-methyl-*trans*-zeatin 9-β-D-ribofuranoside] [(1″*R*)-**7**],[3,5] both isolated from the culture filtrate of the gall-forming phytopathogenic bacterium *Pseudomonas syringae* pv *savastanoi*,[3,4] and 2-hydroxy-1′-methyl-*trans*-zeatin (**9**)[7,10] from methanolic extracts of a marine green alga (code No. NIO-143)[7a] or from AcOEt extracts of the culture broth of the fungus *Alternaria brassicae*.[11] These latest members are unique in that their N^6-substituents possess an asymmetric center adjacent to the N^6 atom, and their natural occurrence suggests that analogously methylated derivatives in the IPA family may also exist in nature. If synthetic reference samples were available, the search for such 1′-methyl or 1″-methyl analogues as natural products would be greatly facilitated. For this reason, together with our continuing interest in the preparation and structure–activity relationships of cytokinins,[5,7b,c,12] we have investigated the synthesis and cytokinin activity of both enantiomers of N^6-(1,3-dimethyl-2-butenyl)adenine (1′-methyl-IPA) (**6**) and of their 9-β-D-ribofuranosides [(1″*R*)-**16** and (1″*S*)-**16**] in the present study.

The synthesis of (1′*R*)-**6** and (1″*R*)-**16** started with the bromination of the allylic alcohol (*R*)-**12**, prepared from D-alanine [(*R*)-**15**] through (*R*)-**10** and (*R*)-**11** according to the previously reported procedures.[5b,7c,12i] Treatment of (*R*)-**12** with *N*-bromosuccinimide (NBS) in benzene in the presence of triphenylphosphine at room temperature for 50 min, an application of the known bromination method,[13] afforded the allylic bromide (*R*)-**13** in 83% yield. For conversion of (*R*)-**13** into the γ,γ-dimethylallylamine derivative [(*R*)-**14**], experiments using triethylsilane [Et_3SiH/2,2′-azobisisobutyronitrile (AIBN)/*tert*-dodecanethiol, boiling hexane under argon, 5 h][14] and tributyltin hydride (Bu_3SnH/AIBN, boiling benzene, 4.5 h)[15] as the reducing agents were tried, but without satisfactory results. However, Super-Hydride reduction [$LiBEt_3H$/tetrahydrofuran (THF), 25 °C, 30 min][16] of (*R*)-**13** was found to proceed smoothly, giving (*R*)-**14** in 80% yield. The carbamate (*R*)-**14** was then hydrolyzed with hydrochloric acid in 50% aqueous EtOH at room temperature for 7 h, and the basic product was isolated in the form of the oxalate (*R*)-**17** in 59% yield. Purinylation of (*R*)-**17** with 6-chloropurine in boiling 1-butanol containing Et_3N for 3 h afforded (*R*)-1′-methyl-IPA [(1′*R*)-**6**] in 92% yield. A similar condensation of (*R*)-**17** with 6-chloro-9-β-D-ribofuranosylpurine[17] gave the target riboside (1″*R*)-**16** in 98% yield.

A parallel sequence of reactions starting from (*S*)-**12** provided (*S*)-**13** (73% yield), (*S*)-**14** (80%), (*S*)-**17** (56%), (*S*)-1′-methyl-IPA [(1′*S*)-**6**] (86%), and the nucleoside (1″*S*)-**16** (74%). The correctness of the structures of these compounds was confirmed by spectral comparison with the corresponding compounds in the (*R*)-series described above.

The above four-step route from (*R*)- or (*S*)-**10** to (*R*)- or (*S*)-**14** would be shortened by application of a Wittig-type isopropenylation to (*R*)- or (*S*)-**10**. In a pilot experiment, (±)-**10**[5c] was allowed to react with isopropyltriphenyl-

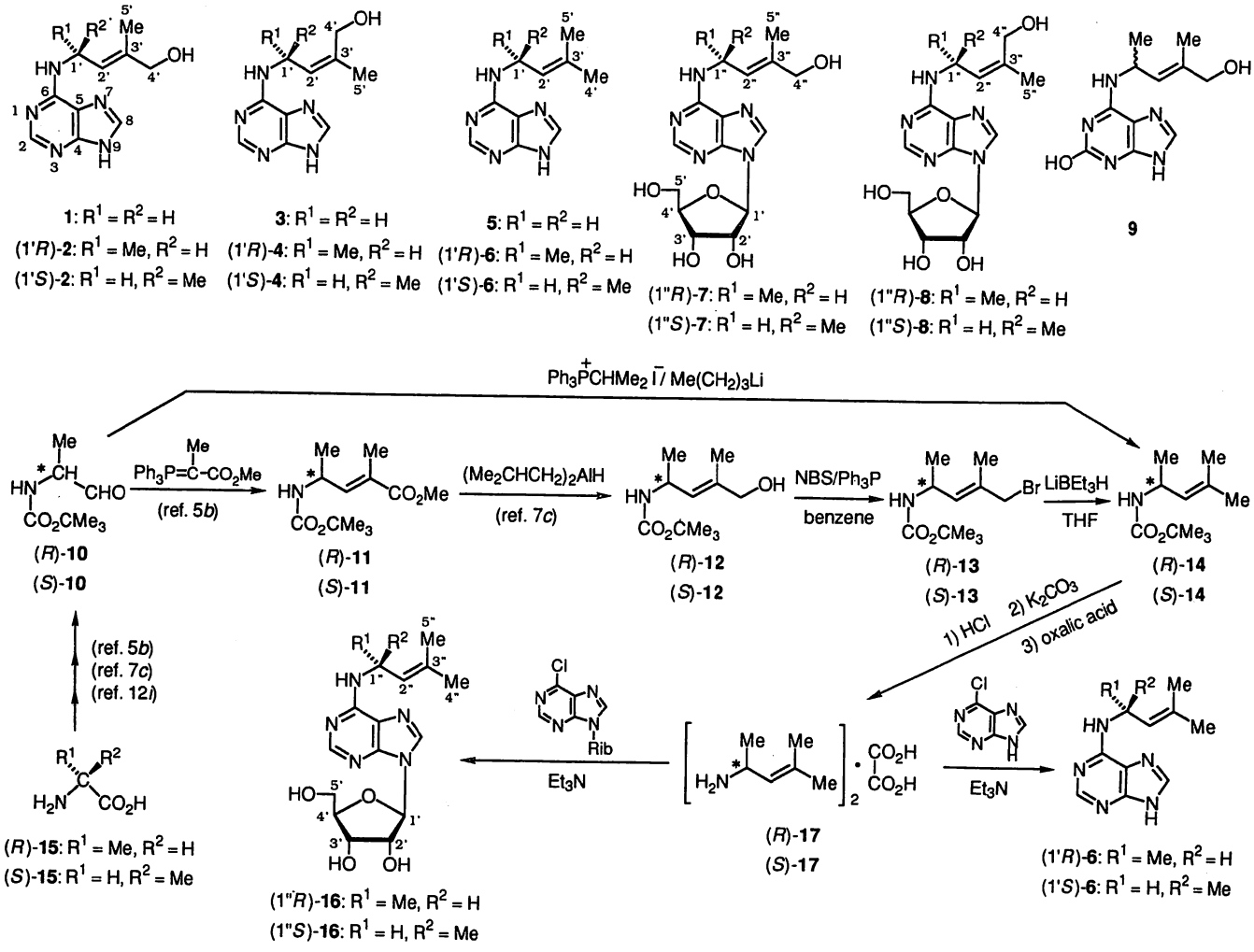
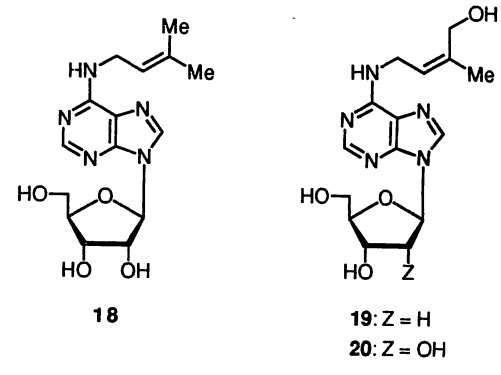


Chart 1

phosphonium iodide[18] (3 molar equiv.) in THF in the presence of butyllithium (3 molar equiv.) at room temperature for 3 h, producing (±)-**14** in 36% yield. Replacement of the phosphonium idodide by the corresponding bromide[19] in this olefination did not improve the yield of (±)-**14**, and the use of an equimolar amount of ylid or THF–hexamethylphosphoramide (HMPA) as the solvent reduced the yield. Previously, (±)-**10** had been prepared from (±)-(*N*-*tert*-butoxycarbonyl)alanine methyl ester by $LiBH_4$ reduction to the corresponding alaninol, followed by Me_2SO oxidation using SO_3–pyridine complex in the presence of Et_3N.[5c] In the present work, this two-step sequence was replaced by diisobutylaluminum hydride reduction (CH_2Cl_2–hexane, −78 °C, 75 min) to furnish (±)-**10** in one step in 76% yield, paralleling the previously reported results in both the (*R*)-[12i] and (*S*)-series.[7c] In the hope of finding a short-cut to the chiral carbamate **14**, (*S*)-**10**[5b,7c] was treated with 1.5 molar amounts of isopropyltriphenylphosphonium iodide[18] and butyllithium in THF at −78 °C for 4 h and then at 0 °C for 2 h. However, the yield and optical purity of the product [(*S*)-**14**] were so low that this approach was abandoned.

With the completion of the above syntheses of both enantiomers [(1'*R*)-**6** and (1'*S*)-**6**] of 1'-methyl-IPA and of their ribosides [(1"*R*)-**16** and (1"*S*)-**16**], it was possible



to test these products for cytokinin activity in the tobacco callus bioassay. For comparison, the cytokinin activities of the corresponding 1'- and 1"-unsubstituted derivatives (**5** and **18**) as well as 9-(2-deoxy-β-D-ribofuranosyl)-*cis*-zeatin (**19**)[12k] and 9-β-D-ribofuranosyl-*cis*-zeatin (**20**) were also assayed. Table I shows the results, together with those[5b,7c,12i] reported previously for the corresponding analogues in the *trans*-zeatin and the *cis*-zeatin series. The maximal yield of the callus was obtained at 0.04—0.1 μM **5**; 0.4—1 μM (1'*R*)-**6**; 1 μM (1"*R*)-**16**; 1 μM **18**; 1—4 μM (1'*S*)-**6**; 4 μM (1"*S*)-**16**; 4—10 μM **20**; 40 μM **19**. As in the cases of the *trans*- and *cis*-zeatin series,[5b,7c,12i] the *R*

TABLE I. Cytokinin Activity of N^6-(3-Methyl-2-butenyl)adenine Analogues and Zeatin Analogues in the Tobacco Callus Bioassay

Compound	Average fresh weight of tobacco callus (mg)											Opt. concn.[a] (μM)
	Concentration of test compound (μM)											
	0	0.001	0.01	0.04	0.1	0.4	1	4	10	40	100	
1[b]	17	153	1053	1770	1449	1444	—	—	—	—	—	0.04
(1′*R*)-**2**[b]	17	120	1065	1490	1140	622	557	—	—	—	—	0.04
(1′*S*)-**2**[b]	23	—	62	430	922	1025	1512	1006	—	—	—	1
3[c]	24	37	153	714	1160	1387	1503	—	1205	—	—	1
(1′*R*)-**4**[c]	16	—	19	26	32	153	835	1869	1252	567	—	4
(1′*S*)-**4**[d]	24	—	—	—	22	21	23	94	875	1438	161	40
5	30	89	641	1511	1110	797	623	—	—	—	—	0.04—0.1
(1′*R*)-**6**	26	—	116	281	562	1284	1395	562	475	—	—	0.4—1
(1′*S*)-**6**	21	—	24	24	23	354	1480	1331	850	—	—	1—4
(1″*R*)-**7**[b]	30	—	165	436	1419	1665	1576	722	481	—	—	0.4
(1″*S*)-**7**[b]	26	—	105	270	628	1301	1426	829	791	—	—	0.4—1
(1″*R*)-**8**[c]	28	—	—	—	29	37	84	186	219	657	1067	100[e]
(1″*S*)-**8**[d]	26	—	—	—	30	23	27	21	16	18	17	—
(1″*R*)-**16**	29	—	59	118	231	1059	1551	824	463	—	—	1
(1″*S*)-**16**	20	—	21	21	27	104	1081	1446	649	—	—	4
18	29	29	238	503	815	1238	1299	1102	638	—	—	1
19	20	—	—	—	24	52	189	674	1152	1778	1309	40
20	22	—	—	84	139	449	728	1124	1368	632	—	4—10

a) Optimum concentration. *b*) Taken from ref. 5*b*. *c*) Taken from ref. 12*i*. *d*) Taken from ref. 7*c*. *e*) Or > 100 μM.

configuration at the 1′- or 1″-position in the IPA family seems to be more important than the *S* configuration in determining cytokinin activity. Although in the *trans*-zeatin series the 1′-methyl derivative (1′*R*)-**2** is as active as the 1′-unsubstituted cytokinin (**1**), the introduction of a methyl group into IPA (**5**) at the 1′-position with the *R* configuration lowers the cytokinin activity by a factor of 10. This parallels the structure–activity relationship observed in the *cis*-zeatin series[12i] (Table I). As expected,[2b,d,5b,7c,12i] the nucleosides (1″*R*)-**16** and (1″*S*)-**16** were less active than the corresponding aglycones (1′*R*)-**6** and (1′*S*)-**6**, respectively. However, this difference in cytokinin activity between the nucleoside and the aglycone is only slight, resembling that[5b] between (1″*S*)-**7** and (1′*S*)-**2**. It is interesting to note that 9-(2-deoxy-β-D-ribofuranosyl)-*cis*-zeatin (**19**) induced the maximal yield of the callus at 40 μM concentration; it is less active than 9-β-D-ribofuranosyl-*cis*-zeatin (**20**) by a factor of 4—10. The deoxyriboside **19** has been reported to lack cytokinin activity at 4.5 μM concentration in the cucumber cotyledon bioassay.[12k] Thus, the cytokinin activity of the compounds listed in Table I follows the order: *trans*-zeatin (**1**) ≈ (1′*R*)-1′-methyl-*trans*-zeatin [(1′*R*)-**2**] > IPA (**5**) > (1″*R*)-1″-methyl-*trans*-zeatin 9-riboside [(1″*R*)-**7**] > (1′*R*)-1′-methyl-IPA [(1′*R*)-**6**] ≈ (1″*S*)-1″-methyl-*trans*-zeatin 9-riboside [(1″*S*)-**7**] > (1′*S*)-1′-methyl-*trans*-zeatin [(1′*S*)-**2**] ≈ *cis*-zeatin (**3**) ≈ (1″*R*)-1″-methyl-IPA 9-riboside [(1″*R*)-**16**] ≈ IPA 9-riboside (**18**) > (1′*S*)-1′-methyl-IPA [(1′*S*)-**6**] > (1′*R*)-1′-methyl-*cis*-zeatin [(1′*R*)-**4**] ≈ (1″*S*)-1″-methyl-IPA 9-riboside [(1″*S*)-**16**] > *cis*-zeatin 9-riboside (**20**) > (1′*S*)-1′-methyl-*cis*-zeatin [(1′*S*)-**4**] ≈ 9-(2-deoxy-β-D-ribofuranosyl)-*cis*-zeatin (**19**) > (1″*R*)-1″-methyl-*cis*-zeatin 9-riboside [(1″*R*)-**8**] ⋙ (1″*S*)-1″-methyl-*cis*-zeatin 9-riboside [(1″*S*)-**8**] (inactive).

In conclusion, both enantiomers of N^6-(1,3-dimethyl-2-butenyl)adenine (1′-methyl-IPA) (**6**) and their 9-ribosides [(1″*R*)-**16** and (1″*S*)-**16**] have now become available by synthesis in nine steps starting from both enantiomers of alanine (**15**) and proceeding through the intermediates shown in Chart 1. The knowledge obtained with the synthetic cytokinin samples should aid the search for these substances in plants and microorganisms. It is also hoped that the structure–activity relationships found in the present and previous studies for the natural and unnatural cytokinins with an asymmetric center in the N^6-substituent will be useful for developing a stereochemical model[20] that explains the difference in cytokinin activity between *cis*- and *trans*-zeatins.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. See ref. 12*k* for details of chromatographies, instrumentation, and measurements. In addition, the ^{1}H-NMR spectra of the nucleosides (1″*R*)-**16** and (1″*S*)-**16** were recorded on a JEOL JNM-GSX-500 (^{1}H 500 MHz) instrument. Elemental analyses were performed mainly by Mr. Y. Itatani and his associates at Kanazawa University and partly by the staff of the Microanalytical Laboratory of Hokuriku University. The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, m = multiplet, s = singlet.

Materials The known compounds tested for cytokinin activity in the tobacco callus bioassay were taken from stocks of commercial origin or which had been prepared in our laboratories according to published procedures: N^6-(3-methyl-2-butenyl)adenine (IPA) (**5**) (purchased from Sigma Chemical Co.); N^6-(3-methyl-2-butenyl)adenosine (**18**) (Sigma Chemical Co.); 9-(2-deoxy-β-D-ribofuranosyl)-*cis*-zeatin (**19**)[12k]; 9-β-D-ribofuranosyl-*cis*-zeatin (**20**) (Sigma Chemical Co.). Other compounds were synthesized as described below.

[*R*-(*E*)]-(4-Bromo-1,3-dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(*R*)-13] A stirred solution of [*R*-(*E*)]-(4-hydroxy-1,3-dimethyl-2-butenyl)carbamic acid *tert*-butyl ester [(*R*)-**12**][5b,7c] (2.51 g, 11.7 mmol) and triphenylphosphine (6.14 g, 23.4 mmol) in benzene (120 ml) was cooled to 5 °C in an ice bath, and NBS (4.16 g, 23.4 mmol) was added. After the mixture had been stirred at room temperature for 50 min, the reaction was quenched by adding 10% aqueous $Na_2S_2O_3$ (120 ml). The aqueous layer was separated from the benzene layer and extracted with ether (3 × 50 ml). The ethereal extracts and the above benzene layer were

combined, washed successively with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was triturated with ether (50 ml), the insoluble solid that resulted was removed by filtration, and the filtrate was concentrated *in vacuo* to leave a brown semisolid (6.93 g). Purification of the semisolid by flash chromatography[21] [silica gel, CH_2Cl_2–hexane (5:1, v/v)] afforded (*R*)-**13** (2.70 g, 83%) as slightly yellowish needles, mp 38.5—39.5 °C; $[\alpha]_D^{23}$ +13.6° (*c*=1.00, MeOH); $[\alpha]_{365}^{23}$ +50.1° (*c*=1.00, MeOH); MS *m/z*: 280, 278 [(M+1)$^+$]; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH), 1706 (carbamate CO); ^{1}H-NMR ($CDCl_3$) δ: 1.18 [3H, d, *J*=6.5 Hz, C(1)-Me], 1.44 (9H, s, CMe_3), 1.84 [3H, d, *J*=1.5 Hz, C(3)-Me], 3.92 [2H, d, *J*=1 Hz, C(3)-CH_2Br], 4.2—4.5 [2H, m, C(1)-H and NH], 5.43 [1H, m, C(2)-H].

[*S*-(*E*)]-(4-Bromo-1,3-dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(*S*)-13] Bromination of (*S*)-**12**[5b,7c] (950 mg, 4.41 mmol) with NBS (1.58 g, 8.88 mmol) in benzene (50 ml) in the presence of triphenylphosphine (2.34 g, 8.92 mmol) at room temperature for 2 h and work-up of the reaction mixture were effected in a manner similar to that described above for (*R*)-**13**, giving (*S*)-**13** (900 mg, 73%) as slightly yellowish needles, mp 35.5—37.5 °C; $[\alpha]_D^{26}$ −12.2° (*c*=0.49, MeOH); $[\alpha]_{365}^{24.5}$ −50.0° (*c*=0.49, MeOH). The IR and ^{1}H-NMR spectra and TLC mobility of this sample were identical with those of (*R*)-**13**.

(*R*)-(1,3-Dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(*R*)-14] A solution of (*R*)-**13** (495 mg, 1.78 mmol) in dry THF (20 ml) was stirred at room temperature in an atmosphere of argon, and a 1 M solution (3.6 ml, 3.6 mmol) of $LiBEt_3H$ in THF was added dropwise over 5 min. After the mixture had been stirred at room temperature for 30 min, the reaction was quenched by adding saturated aqueous NH_4Cl (20 ml). The aqueous layer was separated from the organic layer and extracted with ether (3 × 30 ml). The ethereal extracts and the above organic layer were combined, washed successively with saturated aqueous NH_4Cl and saturated aqueous NaCl, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to leave a colorless semisolid (560 mg). Purification of the semisolid by flash chromatography[21] [silica gel, hexane–AcOEt (10:1, v/v)] yielded (*R*)-**14** (285 mg, 80%) as colorless needles, mp 40—41 °C; $[\alpha]_{365}^{23}$ −13.1° (*c*=1.00, MeOH); MS *m/z*: 199 (M$^+$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460 (NH), 1706 (carbamate CO); ^{1}H-NMR ($CDCl_3$) δ: 1.15 [3H, d, *J*=6.5 Hz, C(1)-Me], 1.43 (9H, s, CMe_3), 1.69 [6H, d, *J*=1 Hz, C(3)-Me's], 4.2—4.5 [2H, m, C(1)-H and NH], 5.00 [1H, m, C(2)-H].

(*S*)-(1,3-Dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(*S*)-14] i) By Super-Hydride Reduction of (*S*)-**13**: Reduction of (*S*)-**13** (2.50 g, 8.99 mmol) with $LiBEt_3H$ (18 mmol) in THF at room temperature for 40 min and work-up of the reaction mixture were conducted as described above for (*R*)-**14**, furnishing (*S*)-**14** (1.44 g, 80%) as colorless needles, mp 40—43.5 °C; $[\alpha]_{365}^{26}$ +11.8° (*c*=1.00, MeOH). The IR and ^{1}H-NMR spectra and TLC mobility of this sample were identical with those of (*R*)-**14**.

ii) By Wittig Reaction of (*S*)-**10**: A stirred suspension of isopropyltriphenylphosphonium iodide[18] (648 mg, 1.50 mmol) in dry THF (15 ml) was cooled to −78 °C in an atmosphere of N_2, and a 1.18 M solution (1.27 ml, 1.50 mmol) of butyllithium in hexane was added dropwise over 10 min. After the mixture had been stirred at −78 °C for 2 h, (*S*)-(1-methyl-2-oxoethyl)carbamic acid *tert*-butyl ester [(*S*)-**10**][5b,7c] (173 mg, 1 mmol) was added, and the resulting mixture was stirred first at −78 °C for 4 h and then at 0 °C for 2 h. The reaction mixture was filtered in order to remove the insoluble material, which was washed with ether. The filtrate and washings were combined and concentrated *in vacuo* to leave a yellow, viscous oil. The oil was then partitioned between $CHCl_3$ and H_2O (the pH of the aqueous layer was adjusted to 6—7 by addition of 10% aqueous HCl). The $CHCl_3$ extracts were washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave a yellow oil (295 mg). Purification of this oil by flash chromatography[21] [silica gel, hexane–AcOEt (8:1, v/v)] provided (*S*)-**14** (38 mg, 19%) as a colorless solid, mp 37.5—40 °C; $[\alpha]_{365}^{22}$ +8.3° (*c*=0.313, MeOH). The IR and ^{1}H-NMR spectra and TLC mobility of this sample were identical with those of the product obtained by method (i).

(±)-(1-Methyl-2-oxoethyl)carbamic Acid *tert*-Butyl Ester [(±)-10] A stirred solution of (±)-*N*-[(1,1-dimethylethoxy)carbonyl]alanine methyl ester[5c] (2.03 g, 10.0 mmol) in dry CH_2Cl_2 (50 ml) was cooled to −78 °C in an atmosphere of argon, and a 1.0 M solution (20 ml, 20 mmol) of diisobutylaluminum hydride in hexane was added dropwise over 20 min. After the mixture had been stirred at −78 °C for 75 min, the reaction was quenched by adding 2 N aqueous HCl (10 ml). The resulting mixture was brought to pH 4—5 by addition of saturated aqueous $NaHCO_3$, turning into a gel. The gel was then filtered with the aid of Celite 535 (Nacalai Tesque, Inc.) to obtain a clear two-layer filtrate. The aqueous layer was separated from the organic layer and extracted with $CHCl_3$. The $CHCl_3$ extracts and the above organic layer were combined, washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave a colorless solid. Recrystallization of the solid from hexane yielded (±)-**10** (1.32 g, 76%) as colorless plates, mp 77.5—79 °C (lit.[5c] mp 83.5—84.5 °C). This sample was identical (by comparison of the IR spectrum) with authentic (±)-**10**.[5c]

(±)-(1,3-Dimethyl-2-butenyl)carbamic Acid *tert*-Butyl Ester [(±)-14] A stirred suspension of isopropyltriphenylphosphonium iodide[18] (1.30 g, 3.01 mmol) in dry THF (13 ml) was cooled to 0 °C in an atmosphere of N_2, and a 1.38 M solution (2.2 ml, 3.05 mmol) of butyllithium in hexane was added dropwise over 5 min. After the mixture had been stirred at 0 °C for 1.5 h, a solution of (±)-**10** (173 mg, 1 mmol) in dry THF (2 ml) was added dropwise over 5 min, and the resulting mixture was stirred at room temperature for 3 h. The reaction was then quenched by adding saturated aqueous NH_4Cl (10 ml). The aqueous mixture was filtered in order to remove the insoluble material, which was washed with ether. The filtrate and washings were combined and concentrated *in vacuo*, and the residue was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 extracts were washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave a yellowish jelly (210 mg). The jelly was purified by means of flash chromatography[21] [silica gel, CH_2Cl_2–hexane (5:1, v/v)] to afford (±)-**14** (72 mg, 36%) as a colorless oil. The IR and ^{1}H-NMR spectra of this sample were superimposable on those of (*R*)- or (*S*)-**14**.

Replacement of isopropyltriphenylphosphonium iodide by the corresponding bromide salt[19] in the above reaction was found to be possible, but the yield of (±)-**14** was only 31%. Replacement of the solvent THF by THF–HMPA (12:3 or 13:8, v/v) was also ineffective.

(*R*)-1,3-Dimethyl-2-buten-1-amine Ethanedioate (2:1) (Salt) [(*R*)-17] A solution of (*R*)-**14** (900 mg, 4.52 mmol) in 50% (v/v) aqueous EtOH (6 ml) was stirred at room temperature, and 20% aqueous HCl (5 ml) was added dropwise over 5 min. The resulting mixture was stirred at room temperature for 7 h, made alkaline and saturated with K_2CO_3 by adding anhydrous K_2CO_3 under ice-cooling, and extracted with ether. The ethereal extracts were dried over anhydrous K_2CO_3 and concentrated at atmospheric pressure to leave (*R*)-1,3-dimethyl-2-buten-1-amine as a slightly yellowish oil. The oil was dissolved in 99% (v/v) aqueous EtOH (3 ml), and the ethanolic solution was exactly neutralized by addition of a solution of oxalic acid (203.5 mg, 2.26 mmol) in 99% (v/v) aqueous EtOH (5 ml) and, if necessary, with Et_3N. The mixture was cooled in an ice bath, and the precipitate that resulted was filtered off, washed with a little 99% (v/v) aqueous EtOH, and dried to give (*R*)-**17** (381 mg, 59%) as a colorless solid, mp 219—221 °C (dec.). Recrystallization of the solid from 92% (v/v) aqueous EtOH yielded an analytical sample of (*R*)-**17** as colorless needles, mp 220—221 °C (dec.); $[\alpha]_D^{22}$ −9.7° (*c*=0.123, MeOH); $[\alpha]_{365}^{22}$ −33.6° (*c*=0.123, MeOH); IR ν_{max}^{Nujol} 1580 cm^{-1} (COO^- and NH_3^+); ^{1}H-NMR (Me_2SO-d_6) δ: 1.14 [3H, d, *J*=6.5 Hz, C(1)-Me], 1.65 and 1.69 [6H, d each, *J*=1 Hz, C(3)-Me's], 3.85 [1H, m, C(1)-H], 5.05 [1H, m, C(2)-H]. *Anal.* Calcd for $C_{14}H_{28}N_2O_4$: C, 58.31; H, 9.79; N, 9.71. Found: C, 58.22; H, 10.14; N, 9.61.

(*S*)-1,3-Dimethyl-2-buten-1-amine Ethanedioate (2:1) (Salt) [(*S*)-17] Hydrolysis of (*S*)-**14** (1.495 g, 7.50 mmol) in 50% (v/v) aqueous EtOH (10 ml) containing 20% aqueous HCl (10 ml) at room temperature for 3 h and work-up of the reaction mixture were carried out in a manner similar to that described above for (*R*)-**17**, giving (*S*)-**17** (608 mg, 56%) as a colorless solid, mp 205—208 °C (dec.). Recrystallization of the solid from 92% (v/v) aqueous EtOH yielded an analytical sample as colorless needles, mp 219—221 °C (dec.); $[\alpha]_D^{23}$ +7.1° (*c*=0.069, MeOH); $[\alpha]_{365}^{23}$ +35.7° (*c*=0.069, MeOH). *Anal.* Calcd for $C_{14}H_{28}N_2O_4$: C, 58.31; H, 9.79; N, 9.71. Found: C, 58.15; H, 9.51; N, 9.81. The IR and ^{1}H-NMR spectra of this sample were superimposable on those of (*R*)-**17**.

(*R*)-*N*^{6}-(1,3-Dimethyl-2-butenyl)adenine [(1′*R*)-6] A stirred mixture of (*R*)-**17** (86.5 mg, 0.3 mmol), 6-chloropurine (77.3 mg, 0.5 mmol), and Et_3N (0.5 ml) in 1-butanol (5 ml) was heated under reflux for 3 h. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ extracts were dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to leave a yellow solid. Purification of the solid by flash chromatography[21] [silica gel, $CHCl_3$–MeOH (15:1, v/v)] gave (1′*R*)-**6** (100 mg, 92%) as a yellowish solid,

mp 195—197 °C. Recrystallization from MeCN furnished an analytical sample as colorless minute needles, mp 195—197.5 °C; $[\alpha]_D^{20}$ −88.6° ($c=0.128$, MeOH); CD ($c=7.26\times10^{-5}$ M, MeOH) $[\theta]^{25}$ (nm): −16400 (273) (neg. max.), −5920 (252) (pos. max.), −6610 (245) (neg. max.), +58900 (215) (pos. max.); MS *m/z*: 217 (M^+); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 271 nm (ε 18800); $\lambda_{max}^{H_2O}$ (pH 1) 275 (17000); $\lambda_{max}^{H_2O}$ (pH 7) 270 (18600); $\lambda_{max}^{H_2O}$ (pH 13) 275 (18300); ^{1}H-NMR ($CDCl_3$) δ: 1.38 [3H, d, $J=6.5$ Hz, C(1′)-Me], 1.73 and 1.76 [3H each, s, C(3′)-Me's], 5.22 [2H, m, C(1′)-H and C(2′)-H], 5.93 (1H, br, NH), 7.96 and 8.44 (1H each, s, purine protons), 13.7 (1H, br, NH). *Anal.* Calcd for $C_{11}H_{15}N_5$: C, 60.81; H, 6.96; N, 32.23. Found: C, 60.78; H, 7.03; N, 32.11.

(*S*)-*N*^{6}-(1,3-Dimethyl-2-butenyl)adenine [(1′*S*)-6] Condensation of (*S*)-**17** with 6-chloropurine and work-up of the reaction mixture were conducted as described above for (1′*R*)-**6**, affording (1′*S*)-**6** in 86% yield as a colorless solid, mp 188—190 °C. Recrystallization from MeCN provided an analytical sample as colorless minute needles, mp 196—198 °C; $[\alpha]_D^{27}$ +94.0° ($c=0.152$, MeOH); CD ($c=6.92\times10^{-5}$ M, MeOH) $[\theta]^{25}$ (nm): +16800 (274) (pos. max.), +6360 (252) (neg. max.), +7080 (246) (pos. max.), −55900 (216) (neg. max.); MS *m/z*: 217 (M^+). *Anal.* Calcd for $C_{11}H_{15}N_5$: C, 60.81; H, 6.96; N, 32.23. Found: C, 60.52; H, 7.08; N, 32.16. The UV, IR, and ^{1}H-NMR spectra of this sample were superimposable on those of (1′*R*)-**6**.

(*R*)-*N*-(1,3-Dimethyl-2-butenyl)adenosine [(1″*R*)-16] A stirred mixture of (*R*)-**17** (86.5 mg, 0.3 mmol), 6-chloro-9-β-D-ribofuranosylpurine[17] (143.3 mg, 0.5 mmol), and Et_3N (0.5 ml) in 1-butanol (5 ml) was heated under reflux for 7 h. The reaction mixture was concentrated *in vacuo* to leave a slightly yellowish oil, which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ extracts were washed with saturated aqueous NaCl, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to leave a yellow oil. Purification of the oil by flash chromatography[21] [silica gel, $CHCl_3$–MeOH (8 : 1, v/v)] gave (1″*R*)-**16** (172 mg, 98%) as a faintly yellowish glass; $[\alpha]_D^{27}$ −94.9° ($c=0.474$, MeOH); CD ($c=6.66\times10^{-5}$ M, MeOH) $[\theta]^{25}$ (nm): −19200 (276) (neg. max.), −3750 (255) (pos. max.), −4650 (244) (neg. max.), +53900 (217) (pos. max.); MS *m/z*: 349 (M^+); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 270 nm (ε 17600); $\lambda_{max}^{H_2O}$ (pH 1) 266 (18100); $\lambda_{max}^{H_2O}$ (pH 7) 270 (18200); $\lambda_{max}^{H_2O}$ (pH 13) 270 (18300); ^{1}H-NMR ($CDCl_3$) δ: 1.31 [3H, d, $J=7$ Hz, C(1″)-Me], 1.73 and 1.75 [3H each, s, C(3″)-Me's], 3.33, 4.55, 5.87, and 6.6 (1H each, br, three OH's and NH), 3.72 (1H, d, $J=13$ Hz) and 3.93 (1H, dd, $J=13$, 1.5 Hz) [C(5′)-H's], 4.32 [1H, s, C(4′)-H)], 4.44 [1H, d, $J=5.5$ Hz, C(3′)-H], 4.99 [1H, dd, $J=7$, 5.5 Hz, C(2′)-H], 5.09 [1H, br, C(1″)-H], 5.18 [1H, d, $J=8.5$ Hz, C(2″)-H], 5.77 [1H, d, $J=7$ Hz, C(1′)-H], 7.71 and 8.22 (1H each, s, purine protons).

(*S*)-*N*-(1,3-Dimethyl-2-butenyl)adenosine [(1″*S*)-16] A stirred mixture of (*S*)-**17** (86.5 mg, 0.3 mmol), 6-chloro-9-β-D-ribofuranosylpurine[17] (143.3 mg, 0.5 mmol), and Et_3N (0.5 ml) in 1-butanol (5 ml) was heated under reflux for 5 h. The reaction mixture was worked up as described above for (1″*R*)-**16**, yielding (1″*S*)-**16** (130 mg, 74%) as a faintly yellowish glass, $[\alpha]_D^{27}$ −8.4° ($c=0.500$, MeOH); $[\alpha]_{365}^{27}$ +77.1° ($c=0.500$, MeOH); CD ($c=6.41\times10^{-5}$ M, MeOH) $[\theta]^{25}$ (nm): +12800 (277) (pos. max.), +3120 (257) (neg. max.), +5310 (242) (pos. max.), −45300 (217) (neg. max.); MS *m/z*: 349 (M^+); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 270 nm (ε 17500); $\lambda_{max}^{H_2O}$ (pH 1) 266 (18200); $\lambda_{max}^{H_2O}$ (pH 7) 270 (18100); $\lambda_{max}^{H_2O}$ (pH 13) 270 (18200); ^{1}H-NMR ($CDCl_3$) δ: 1.31 [3H, d, $J=6.5$ Hz, C(1″)-Me], 1.68 and 1.70 [3H each, s, C(3″)-Me's], 3.69 and 3.89 [1H each, br d, $J=12.5$ Hz, C(5′)-H's], 4.14, 5.5, 6.01, and 6.9 (1H each, br, three OH's and NH), 4.28 [1H, s, C(4′)-H], 4.42 [1H, d, $J=5$ Hz, C(3′)-H], 4.98 [1H, dd, $J=7$, 5 Hz, C(2′)-H], 5.05 [1H, br, C(1″)-H], 5.12 [1H, d, $J=8$ Hz, C(2″)-H], 5.77 [1H, d, $J=7$ Hz, C(1′)-H], 7.74 and 8.15 (1H each, s, purine protons).

Bioassay Procedure The cytokinin activities of **5**, (1′*R*)-**6**, (1′*S*)-**6**, (1″*R*)-**16**, (1″*S*)-**16**, **18**, **19**, and **20** were tested in the tobacco callus bioassay in a manner similar to that[5b] described previously. The results are shown in Table I.

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