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THE SYNTHESIS OF CYTOKININ PHOSPHATES

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Abstract: The bifunctional phosphorylating reagent N-morpholino O,O-bis[(6-trifluoromethyl)benzotriazolyl]phosphate was used for the preparation of ribosyl zeatin 5'-phosphate **1b** and N^6-(Δ^2-Isopentenyl)adenosine 5'-phosphate **2b**, whereas the monofuntional phosphitylating reagent 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one was used for the preparation of ribosyl zeatin allylic phosphate **1d** and ribosyl zeatin diphosphate **1c**.

INTRODUCTION

The cytokinins, a group of naturally-occurring compounds which are involved in a variety of aspects of plant development, are established to be N^6-substituted derivatives of adenine, which occur either as a free-base, ribonucleoside or ribonucleotide. One of the first naturally-occurring adenosine derivatives with plant cell division promoting activity was isolated from extracts of sweet corn kernels (zeamays)[1] and was identified as 6-[4-hydroxy-3-methyl-E-but-2-enylamino]-9-(β-D-ribofuranosyl) purine **1a**, usually named ribosyl zeatin. The second adenosine derivative being isolated from extracts of lactobacillus acidophilus, was N^6-(Δ^2-Isopentenyl)adenosine i.e. 6-(3-methyl-2-butenylamino)-9-(β-D-ribofuranosyl)purine **2a**.

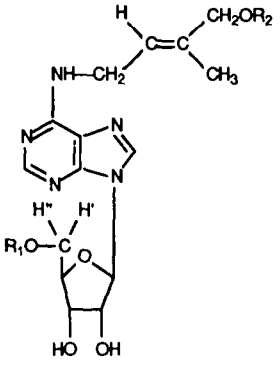
Many workers in this field suggested the presence of phosphates of cytokinin ribonucleosides; up to now only two of these derivatives have been isolated[3,4]. These compounds were assigned the structures of ribosyl zeatin 5'-phosphate **1b** and N^6-(Δ^2-Isopentenyl)-adenosine 5'-phosphate **2b**; these assignments are based on UV spectral data, enzymatic characterisation as well as independent synthesis. A number of chemical procedures has been reported for the synthesis of these ribonucleotides **1b**[6] and **2b**[4,5].

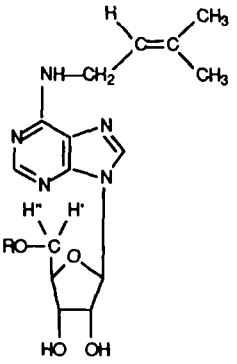
The best approach described for the phosphorylation of the alcoholic group involved the use of the powerful phosphorylating agent pyrophosphoryl chloride[5,6]. The method is simple but requires a difficult purification procedure and gives relatively low yields (≈40%).

Attempts were made to synthesize ribosyl zeatin phosphate **1b**, ribosyl zeatin diphosphate **1c** and ribosyl zeatin allylic phosphate **1d** by using either pyrophosphoryl chloride, or a mixture of 2-cyanoethylphosphate and dicyclohexylcarbodiimide in pyridine. Both attempts failed[7].

We report in detail a new and improved synthesis of **1b** and **2b**, using the phosphotriester approach, and the synthesis of **1c** and **1d**, using the phosphitetriester approach.

This synthetic study was part of a program of cooperation between our laboratory and the center of Agricultural and Biological Research at Wageningen, directed to study the mechanism and the physiological effects of cytokinins in plants.





1 a) $R_1 = R_2 = H$

1 b) $R_1 = P(O)(OH)-OH$, $R_2 = H$

1 c) $R_1 = R_2 = P(O)(OH)-OH$

1 d) $R_1 = H$, $R_2 = P(O)(OH)-OH$

2 a) $R = H$

2 b) $R = P(O)(OH)-OH$

Results and discussion

The strategy we have adopted for the synthesis of the ribonucleoside phosphates **1b**, **1c** , **1d** and **2b** consisted of the following steps.

i The preparation of properly protected ribonucleoside derivatives **6**, **8**, **9** and **10**, (see scheme 1); **ii** effective phosphorylation of the free hydroxyl groups in these ribonucleoside derivatives (see scheme 2 and 3); **iii** complete removal of all protective groups.

In our strategy dealing with the preparation of properly protected ribonucleoside derivatives we used the base-labile acetyl group for the protection of the allylic hydroxy function, the acid-labile methoxymethylidene group for the protection of the 2'-OH and the 3'-OH function and the acid-base-labile *tert*-butyldimethylsilyl (TBDMS) group for the protection of the 5'-OH function. The TBDMS group was selectively removed in the presence of the other protective groups (methoxymethylidene and acetyl groups) by fluoride ions.

6-Chloro-9-β-ribofuranosyl purine **3**, which was prepared by a procedure described before[8], was converted into 6-chloro-9-(2',3'-O-methoxymethylidene-β-D-ribofuranosyl) purine **4** (yield 70%) by treatment with trimethyl orthoformate in the presence of the monohydrate of toluene-p-sulphonic acid. The TBDMS group was introduced at the 5'-hydroxy function of **4** by treatment

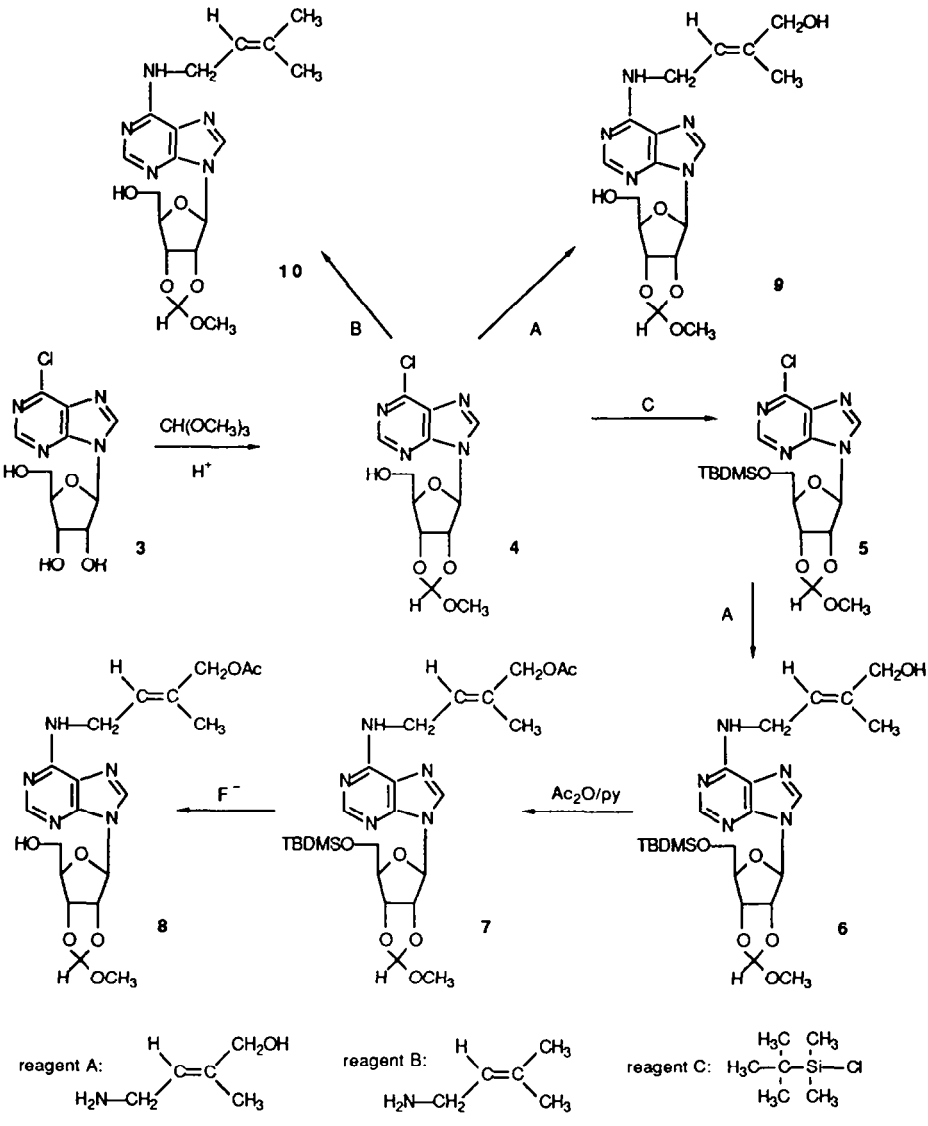
with *tert*-butyldimethylsilyl chloride in pyridine, which afforded 6-chloro-9-(2',3'-O-methoxy methylidene-5'-O-*tert*-butyldimethylsilyl-β-D-ribofuranosyl) purine **5** (yield 80%). 6-[4-Hydroxy-3-methyl-E-but-2-enylamino]-9-(2',3'-O-methoxymethylidene-5'-O-*tert*-butyldimethylsilyl-β-D-ribofuranosyl) purine **6** and 6-[hydroxy-3-methyl-E-but-2-enylamino]-9-(2',3'-O-methoxy-methylidene-β-D-ribofuranosyl) purine **9** were produced in yields of 77% and 80% respectively by coupling of 4-hydroxy-3-methyl-E-but-2-enylamine[10] with **5** and **4** respectively in refluxing n-butanol in the presence of triethylamine. Coupling of **4** with 3-methylbut-2-enylamine[11] under the same conditions as described above gave 6-[3-methylbut-2-enylamino]-9-(2',3'-O-methoxymethylidene-β-D-ribofuranosyl) purine **10** (yield 79%).

The allylic hydroxy function on **6** was acylated by treatment of **6** with acetic anhydride in pyridine in the presence of 4-dimethylaminopyridine (DAP)[12] to afford the fully protected compound **7** (yield 94%). Selective removal of the TBDMS group in **7** was performed by treatment with tetra-n-butylammonium fluoride (TBAF)[13] in dioxane to give 6-[4-acetoxy-3-methyl-E-but-2-enylamino]-9-(2',3'-O-methoxymethylidene-β-D-ribofuranosyl) purine **8** (yield 82%).

The above described procedures for the synthesis of properly protected ribonucleosides **6**, **8**, **9** and **10** proved to be relatively fast and gave these partially protected ribonucleosides in good yields. All these reaction steps of above described methodology could be performed on a relatively large scale.

The next step in our strategy deals with the effective phosphorylation of the free hydroxy groups on the properly protected ribonucleosides **6**, **8**, **9** and **10**. So far, the bifunctional phosphorylating reagent N-morpholino O,O-bis[(6-trifluoromethyl)benzotriazolyl] phosphate (reagent D) (see scheme 2) was found to be suitable for the preparation of 5'-monophosphates of nucleosides[14]. When we treated a solution of the nucleosides **10** or **8** in pyridine (see scheme 2) with reagent D in the presence of N-methylimidazole we succesfully obtained the phosphate triesters **11** and **12** respectively. These fully protected intermediates were not isolated but were hydrolyzed immediately to remove all protective groups. Treatment of intermediate **11** with pyridine/water removed the protective group 6-trifluoromethylbenzotriazolyl and subsequent treatment with acid at pH=2 removed the morpholino as well as the methoxymethylidene group providing **2b** (yield 73% based on **10**). In case of **12** after treatment with pyridine/water the acetyl protective group was removed by treatment with aqueous ammonia and the morpholino as well as the methoxymethylidene function by acid treatment at pH=2 to yield **1b** (yield 68% based on **8**). By ^{1}H NMR, ^{13}C NMR and ^{31}P NMR data (see experimental part) these structures **1b** and **2b** were unequivocally established. The Rf and UV data were identical as described in the literature[4,5,6].

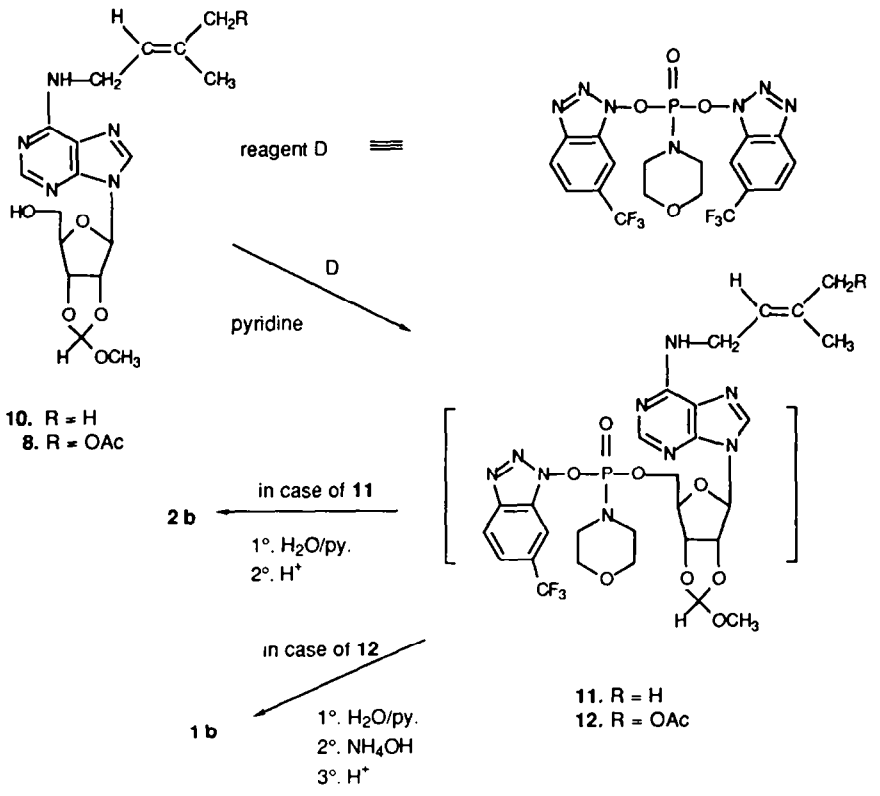
The introduction of the phosphate group at the allylic hydroxy group[7,15], leading to the compounds **1c** and **1d** by using the bifunctional phosphorylating reagent D was found to be unsuitable[16]. A new and efficient method for the phosphorylation of an allylic hydroxy group via a phosphite intermediate has been found[16]. This method is based on the initial phosphitylation of the allylic hydroxy group with salicyl chlorophosphite, followed by oxidation of the allylic phosphonate monoester to the corresponding phosphate monoester.



Scheme 1

Application of this phosphite triester-approach was already demonstrated in the preparation of the phosphate monoester of N-(4-hydroxy-3-methyl-E-but-2-enyl) phthalimide[16] and further in the preparation of the phosphate monoester of 6-(4-hydroxy-3-methyl-3-E-but-2-enylamino) purine[10].

In order to examine this potential route we treated **6** and **9** in dioxane with the monofunctional reagent 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (reagent K)[17] (see scheme 3) in the presence of N,N-diisopropylethylamine for 5 min. It gave the phosphite triesters **13** and **14** respectively, which were hydrolyzed into **15** and **16** respectively. By ^{1}H NMR, ^{13}C NMR and



Scheme 2

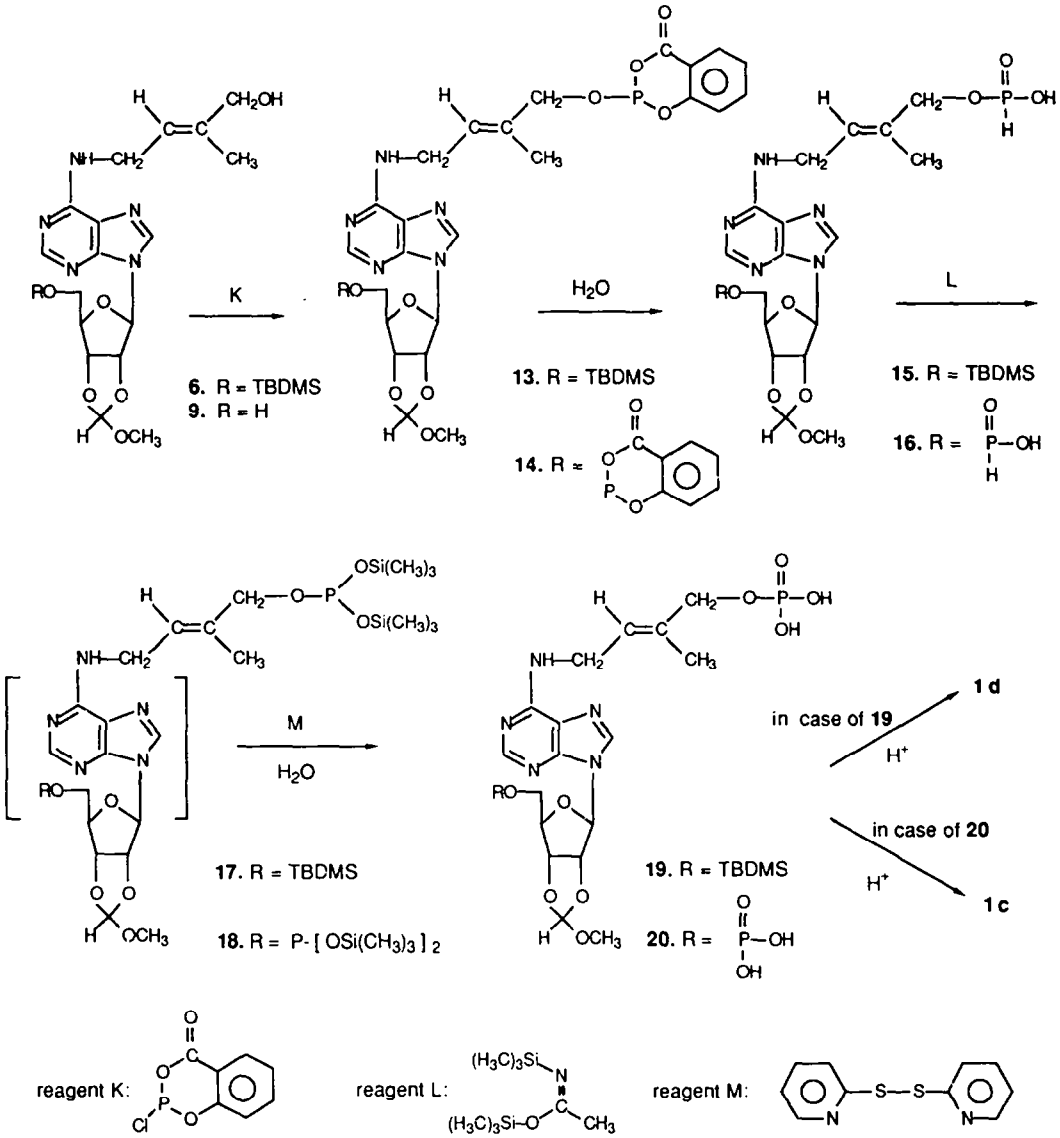
^{31}P NMR data (see experimental part) these structures were proven.

The oxidation of **15** and **16** was performed according to a slight modification the procedure of Hata et al[18]. A solution of **15** and **16** in acetonitrile when treated with N,O-bis(trimethylsilyl) acetamide (reagent L) in the presence of N,N-diisopropylethylamine for 15 min. gave the intermediates bis (trimethylsilyl phosphite) **17** and **18** respectively. Without further purification **17** and **18** were treated with 2,2'-dipyridyldisulfide (reagent M) for 1h and the reaction mixtures thus obtained were subsequently treated with water affording **19** and **20** respectively. The identity of **19** and **20** was ascertained by ^{1}H NMR and ^{31}P NMR spectroscopy.

Cleavage of the acid-labile group TBDMS and methoxymethylidene group was effected by acid treatment at pH=2 for 3h to give after workup **1d** and **1c** respectively (overall yield of **1d** 76% based on **6**, yield **1c** 70% based on **9**). The identity of these phosphate derivatives **1d** and **1c** was ascertained by ^{1}H NMR, ^{13}C NMR and ^{31}P NMR.

The ^{1}H NMR and ^{13}C NMR data of the ribosyl zeatin derivatives **1b**, **1c** and **1d** are almost identical in comparison with those of ribosyl zeatin **1a**[19]; however we mention some exceptions:

i) The ^{1}H-absorptions of H_5' and H_5'' appear in ribosyl zeatin **1a** as two pairs of double doublets



Scheme 3

at 3.75 - 3.88 ppm., due to the fact that H_5' and H_5'' have slightly different chemical shifts and $J(H_5' - H_5'') \neq J(H_5'-H_4') \neq J(H_5''-H_4')$. In **1b** and **1c**, however, the absorption of H_5' and H_5'' takes the form of an undefined multiplet at about 4.10 ppm. No conclusion can be drawn whether ^{31}P coupling occurs with H_5' and H_5''. ii) The ^1H-absorption of C=C-CH_2-O which in ribosyl zeatin **1a** is found as singlet at 3.96 ppm., appears in **1c** and **1d** as a doublet at about 4.20 ppm. The doublets are caused by the coupling of ^1H with ^{31}P ($^3J_{P-H}$). iii) The ^{13}C-signal of $CH_3\underline{C}$=C features in ribosyl zeatin **1a** as a singlet at about 138 ppm., but in **1c** and **1d** as a doublet.

Similarly the ^{13}C-signals of C_4' appear in ribosyl zeatin **1a** as a singlet at about 85 ppm., but becomes in **1b** and **1c** a doublet. The doublets are caused by the coupling of ^{13}C with ^{31}P ($^3J_{C-P}$). When we compared the ^1H NMR and ^{13}C NMR data of N^6-(Δ^2-Isopentenyl)adenine **2a**[20] with the data of its phosphate **2b**, the same observations were made as decribed above concerning the ^1H-absorption of H_5' and H_5'' and the ^{13}C-absorption of C_4'.

Proton decoupled ^{31}P NMR spectroscopy revealed the presence of the expected number of resonance absorptions, which further proved the structural identity and purity of the compounds. From these results it can be unequivocally concluded that the methods described above are simple and reliable procedures for the preparation of cytokinin phosphates. These compounds are produced in satisfactory yields without the formation of any side-products.

EXPERIMENTAL

General procedures.

Acetonitrile, N,N-diisopropylethylamine, dioxane, N-methylimidazole, pyridine, triethylamine and trimethyl orthoformate were dried by refluxing with CaH_2 for 16h and then distilled. Dioxane was redistilled from $LiAlH_4$ (5 g/l). All liquids were stored under nitrogen. n-Butanol (analyzed grade Baker) was used without further purification. N,O-bis(trimethylsilyl)acetamide and 2,2'-dipyridyldisulfide were purchased from Janssen Chimica (Belgium). 6-Chloro-9-β-D-ribofuranosyl purine[8], 4-hydroxy-3-methyl-E-but-2-enylamine[10], 3-methyl-2-butenylamine[11], tetra-n-butylammonium fluoride (TBAF)[13], 2-chloro-4H-1,3,2-benzodioxaphoshorin-4-one[17] and N-morpholino O,O-bis[(6-trifluoromethyl)benzotriazolyl)] phosphate[14] were prepared as described previously.
Triethylammonium bicarbonate buffer was prepared by passing a stream of CO_2 gas through a cooled (ice-water bath) 2M solution of triethylamine in deionized water until solution became neutral. Scheicher and Schüll DC Fertigfolien F 1500 LS 254 were used for TLC, unless otherwise mentioned. The following solvent systems were used. System A (chloroform/methanol, 96:4, v/v), system B (chloroform/methanol 92:8, v/v), system C (chloroform/methanol, 90:10, v/v), system D (chloroform/methanol, 85:15, v/v), system E (isopropyl alcohol/concentrated ammonium hydroxide/water, 7:1:2, v/v) and system F (n-butanol/acetic acid/water, 12:3:5, v/v, Whatman No. 1 paper was used for TLC). Compound were visualized by UV-light or by spraying with the appropriate reagents. Thus compounds containing ribofuranosyl moieties were visualized by spraying with sulfuric acid/methanol (20:80, v/v). The phosphor containing compounds were visualized by spraying with Zinzadze's reagent. Short column chromatography was performed on silicagel 60 (230-400 mesh ASTM) suspended in chloroform. DEAE-Sephadex A 25 was purched from Pharmacia (Uppsala, Sweden). Cation-exchange resin (Na^+-form): a solution of NaOH (2M; 100 ml) was passed over a column packed with cation-exchange resin (Dowex 50 Wx 8, 100-200 mesh; Fluka H^+-form, 1.5x5 cm) followed by washing of the column with sterile water until pH=7. Sterile water and glass were used during the whole deblocking and purification processes.
^1H NMR spectra were measured at 300 MHz using a Bruker CXP 300 spectrometer; ^{13}C NMR spectra were measured at 75.460 MHz using a Bruker CXP 300 spectrometer; Proton noise decoupling was used. ^{31}P NMR spectra were measured at 121.470 MHz using a Bruker CXP 300 spectrometer, chemical shifts are in ppm. relative to 85% H_3PO_4 as external standard. Mass spectral data were obtained from an AEI MS 902 spectrometer equipped with a VG ZAB console.

Synthesis of 6-chloro-9-(2',3'-O-methoxymethylidene-β-D-ribofuranosyl) purine **4**
A mixture of 6-chloro-9-β-D-ribofuranosyl purine **3** (13.48 g, 47 mmol), toluene-p-sulphonic acid, monohydrate (0.83 g, 4.28 mmol) and dry trimethyl orthoformate (58 ml) was stirred for 16h at 20°C. TLC (system B) indicated complete disappearance of the starting material. The mixture was taken up in chloroform (250 ml) and washed with an aqueous solution of sodium bicarbonate (1M, 2x100 ml) and water (2x100 ml). The organic layer was dried on $MgSO_4$, concentrated to an oil, which was chromatographed on a column of silicagel. Elution with

$CHCl_3/CH_3OH$ (100:0→96:4, v/v) gave after evaporation pure **4** as oil. Yield 10.83 g (70%), Rf=0.60 (system B).
Compound **4**, ^1H NMR ($CDCl_3$): δ 8.72, s, 1H, H-8; 8.41, s, 1H, H-2; 6.34, d, J=4.3 Hz, 1H, H_1'; 5.94, s, 1H, CH-OCH_3; 5.28, m, 1H, H_2'; 5.14, m, 1H, H_3', 4.53, m, 1H, H_4'; 3.82-3.74, m, 2H, H_5' and H_5''; 3.30., s, 3H, OCH_3; ^{13}CNMR ($CDCl_3$): δ 151.71, 151.1, 150.4, 144.5 and 117.5, 5xs (purine); 119.14, s, CH-OCH_3; 92.1, s, C_1'; 87.0, s, C_4'; 84.2, s, C_3'; 80.9, s, C_2'; 62.4, s, C_5'; 52.7, s, OCH_3; HRMS, found m/z 328.0571 $C_{12}H_{13}N_4O_5{}^{35}Cl$ requires 328.0574.

Synthesis of 6-chloro-9-(2',3'-O-methoxymethylidene-5'-O-*tert*-butyldimethylsilyl-β-D-ribofuranosyl) purine **5**
To a solution of **4** (4.65 g, 14.25 mmol) in pyridine (20 ml) was added *tert*-butyldimethylsilyl chloride (2.40 g, 16 mmol) and the reaction mixture was left for 16h at 20°C. TLC (system A) indicated complete conversion of the starting compound. After the addition of ice (20 g) the reaction mixture was concentrated to an oil which was dissolved in $CHCl_3$ (200 ml) and washed with an aqueous solution of sodium bicarbonate (1M, 2x100 ml) and water (2x100 ml). The organic layer was dried on $MgSO_4$, concentrated to an oil which was chromatographed on a column of silicagel. Elution with $CHCl_3/CH_3OH$ (100:0→98:2, v/v) gave after evaporation pure **5** as oil. Yield 5.02 g (80%), Rf=0.621 (system A).
Compound **5**, ^1H NMR ($CDCl_3$): δ 8.73, s, 1H, H-8; 8.36, s, 1H, H-2; 6.37, d, J=3Hz, 1H, H_1'; 5.95, s, 1H, CH-OCH_3; 5.30 m, 1H, H_2'; 5.01, m, 1H, H_3'; 4.97, m, 1H, H_4'; 3.85-3.74, m, 2H, H_5' and H_5''; 3.30, s, 3H, OCH_3; 0.80, s, 9H (TBDMS); 0.00, s, 6H (TBDMS); ^{13}C NMR ($CDCl_3$): δ 152.1, 151.8, 150.4, 143.7 and 118.2, 5xs (purine); 119.3, s, CH-OCH_3; 91.8, s, C_1'; 87.7, s, C_4'; 84.9, s, C_3'; 81.4, s, C_2'; 63.4, s, C_5', 52.8, s, CH-OCH_3; 25.9, 18.2 and -5.5, 3xs (TBDMS); FD-MS m/z: 442(M^+) $C_{18}H_{27}N_4O_5Si^{35}Cl$; HRMS $(M\text{-}CH3)^+$, found m/z 427.1209 $C_{17}H_{24}N_4O_5Si^{35}Cl$ requires 427.1204.

Synthesis of 6-[4-hydroxy-3-methyl-E-but-2-enylamino]-9-(2',3'-O-methoxymethylidene-5'-O-*tert*-butyldimethylsilyl-β-D-ribofuranosyl) purine **6**
A solution of **5** (4.50 g, 10.16 mmol), aminoalcohol (reagent A, 1.50 g, 14.85 mmol), triethylamine (3 ml) in n-butanol (150 ml) was boiled under reflux for 3h. TLC analysis (system A) indicated that the conversion of the starting material was complete. The reaction solution was concentrated under reduced pressure to an oil, which was chromatographed on a column of silicagel. Elution with $CHCl_3/CH_3OH$ (100:0→97:3, v/v) gave after evaporation pure **6** as an oil. Yield 4.00 g (77%), Rf=0.40 (system A).
Compound **6**, ^1H NMR ($CDCl_3$): δ 8.40, s, 1H, H-8; 7.93, s, 1H, H-2; 6.32, t, J=5.35 Hz, 1H, NH-CH_2; 6.25, d, J=2.8 Hz, H_1'; 6.12, s, 1H, CH-OCH_3; 5.65, t, J=6.8 Hz, 1H, CH=C; 5.38, m, 1H, H_2'; 5.26, m, 1H, H_3'; 4.52 m, 1H, H_4'; 4.36, br s, 2H, CH_2-N; 4.04, s, 2H, C=C-CH_2-O; 3.76-3.70, m, 2H, H_5' and H_5''; 3.43, s, 3H, OCH_3; 1.73, s, 3H, CH_3; 0.93, s, 9H, (TBDMS); 0.00, s, 6H, (TBDMS); ^{13}C NMR ($CDCl_3$): δ 154.3, 153.2, 150.0, 142.0 and 119.8, 5xs (purine); 138.8, s, CH_3-C=C; 119.9, s, CH=C; 117.7, s, CH-OCH_3; 91.2, s, C_1'; 87.3, s, C_4', 84.4, s, C_3'; 81.4, s, C_2'; 66.3, s, C=C-CH_2-O; 63.0, s, C_5'; 51.6, s, CH-OCH_3; 38.2, s, CH_2-N; 13.7, s, CH_3; 25.6, 18.2 and -5.6, 3xs (TBDMS); HRMS, found m/z 507.2505 $C_{23}H_{37}N_5O_6Si$ requires 507.2513.

Synthesis of 6-[4-hydroxy-3-methyl-E-but-2-enylamino]-9-(2',3'-O-methoxymethylidene-β-D-ribofuranosyl) purine **9**
A solution of **4** (3.00 g, 9.13 mmol) aminoalcohol (reagent A, 1.00 g, 10 mmol), triethylamine (3 ml) in n-butanol (150 ml) was boiled under reflux for 3h. TLC analysis (system B) indicated that the conversion of the starting material was complete. The reaction solution was concentrated under reduced pressure to an oil, which was chromatographed on a column of silicagel. Elution with $CHCl_3/CH_3OH$ (100:0→95:5, v/v) gave after evaporation pure **9** as an oil. Yield 2.90 (80%), Rf=0.44 (system B).
Compound **9**, ^1H NMR ($CDCl_3$): δ 8.26, 1H, s, H-8; 7.79, s, 1H, H-2; 6.44, t, J=4.97, 1H, NH-CH_2; 6.16, d, J=3.37 Hz, 1H, H_1', 5.93, s, 1H, CH-OCH_3; 5.62, t, J=7.21 Hz, 1H, CH=C; 5.33, m, 1H, H_2'; 5.16, m, 1H, H_3', 4.50, m, 1H, H_4'; 4.20, br s, 2H, CH_2-N; 4.01, s, 2H, C=C-CH_2-O, 3.98-3.93, m, 2H, H_5' and H_5''; 3.66, s, 3H, CH-OCH_3; 1.72, s, 3H, CH_3; ^{13}C NMR ($CDCl_3$): δ 154.7, 152.8, 147.2, 139.1 and 119.4, 5xs (purine); 139.0, s, CH_3-C=C; 120.6, s, CH=C; 117.5, s, CH-OCH_3; 92.7, s, C_1'; 87.4, s, C_4'; 85.9, s, C_3', 81.1, s, C_2'; 67.1, s, C=C-CH_2-O; 62.8, s, C_5'; 52.8, s, CH-OCH_3'; 38.2, s, N-CH_2; 13.8, s, CH_3; HRMS, found m/z 393.1649 requires 393.1648.

Synthesis of 6-[4-acetoxy-3-methyl-E-but-2-enylamino]-9-(2',3'-O-methoxymethylidene-5'-O-*tert*-butyldimethylsilyl-β-D-ribofuranosyl purine **7**
To a solution of **6** (2.00 g, 3.94 mmol) in pyridine (10 ml) was added acetic anhydride (3 ml) and 4-dimethylaminopyridine (150 mg). The reaction solution was stirred for 40h. TLC analysis

(system A) indicated that the conversion of the starting material was complete. After the addition of ice (50 g) the reaction mixture was taken up in $CHCl_3$ (150 ml) and washed with an aqueous solution of sodium bicarbonate (1M, 2x50 ml) and water (2x50 ml). The organic layer was dried on $MgSO_4$, concentrated to an oil, coevaporated with toluene (2x100 ml), the oil residue was chromatographed on a column of silicagel. Elution with $CHCl_3/CH_3OH$ (100:0→98:2, v/v) gave after evaporation pure **7** as an oil. Yield 2.03 g (94%), Rf=0.70 (system A).
Compound **7**, ^1H NMR ($CDCl_3$): δ 8.36, s, 1H, H-8; 7.92, s, 1H, H-2; 6.24, t, J=6.0 Hz, 1H, NH-CH_2; 6.11, d, J=2.6 Hz, 1H, H_1'; 6.10, s, 1H, CH-OCH_3; 5.65, t, J=6.8 Hz, 1H, CH=C; 5.36, m, 1H, H_2', 5.10, m, 1H, H_3'; 4.40, s, 2H, C=C-CH_2-O; 4.34, m, 1H, H_4'; 4.28, br s, 2H, CH_2-N; 3.75-3.70, m, 2H, H_5' and H_5''; 3.32, s, 3H, CH-OCH_3; 2.05, s, 3H, CH_3-C=O; 1.74, s, 3H, CH_3; 0.88, s, 9H (TBDMS), 0.00, s, 6H, TBDMS; ^{13}C NMR ($CDCl_3$): δ 170.0, s, CH_3-C=O; 154.6, 153,3, 150.4, 138.7 and 119.2, 5xs (purine); 134.2, s, CH_3-C=C; 121.0, s, CH=C; 118.0, s, CH-OCH_3; 90.4, s, C_1'; 86.5, s, C_4'; 83.8, s, C_3', 81.0, C_2'; 68.8, s, C=C-CH_2-O; 63.2, s, C_5'; 51.7, s, CH-OCH_3; 38.3, s, CH_2-N; 20.8, s, CH_3-C=0, 14.1 s, CH_3=C; 25.8, 18.2 and -5.5, 3xs (TBDMS); FD-MS m/z: 549 (M^+) $C_{25}H_{39}N_5O_7Si$; HRMS (M-CH_3CO_2)$^+$, found m/z 490.2483 $C_{23}H_{36}N_5O_5Si$ requires 490.2486.

Synthesis of 6-[4-acetoxy-3-methyl-E-but-2-enylamino]-9-(2',3'-O-methoxymethylidene-β-D-ribofuranosyl) purine **8**
A solution of tetra-n-butylammonium fluoride in dioxane (0.5M, 17 ml) was added to **7** (2.03 g, 3.7 mmol), which was coevaporated with dioxane and dissolved in dioxane (10 ml). The reaction solution was left for 30 min. at 20°C. TLC analysis (system C) indicated complete removal of the silyl group, water was added (2 ml) and the reaction mixture was concentrated to an oil, which was dissolved in $CHCl_3$ (200 ml) and washed with an aqueous solution of sodium bicarbonate (1M, 2x100 ml) and water (2x100 ml). The organic layer was dried on $MgSO_4$, concentrated to an oil, which was chromatographed on a column of silicagel. Elution with $CHCl_3/CH_3OH$ (100:0→96:4, v/v) gave after evaporation pure **8** as an oil. Yield 1.30 g (82%), Rf=0.56 (system C).
Compound **8**, ^1H NMR ($CDCl_3$): δ 8.00, s, 1H, H-8; 7.83, s, 1H, H-2; 6.60, t, J=5.5 Hz, 1H, NH-CH_2; 6.03, d, J=3.4 Hz, H_1'; 6.43, t, J=6.9 Hz, CH=C; 5.10, m, 1H, H_2'; 4.90, m, 1H, H_3'; 4.27, s, 2H, C=C-CH_2-O; 4.00, br s, 2H, CH_2-N; 3.66, m, 1H, H_4'; 3.35-3.0, m, 2H, H_5' and H_5''; 1.85, s, 3H, CH_3-C=C; 1.57, s, 3H, CH_3-C=O; ^{13}C NMR ($CDCl_3$): δ 171.0, s, CH_3-C=O; 154.5, 152.6, 141.0, 139.1 and 119.3, 5xs (purine); 133.9, s, CH_3-C=C; 117.5, s, CH-OCH_3; 92.4, s, C_1'; 85.8, s, C_4'; 83.3, s, C_3'; 80.8, s, C_2'; 72.1, s, C=C-CH_2-O; 62.6, s, CH_3-C=O; 13.2, s, CH_3; HRMS, found m/z 435.1742 $C_{19}H_{25}N_5O_7$ requires 435.1754.

Synthesis of 6-[3-methyl-2-butenylamino]-9-(2',3'-O-methoxymethylidene-β-D-ribofuranosyl) purine **10**
A solution of **4** (2.00 g, 6.00 mmol), aminoalcohol (reagent B, 1.02 g, 12 mmol), triethylamine (1.8 ml) in n-butanol (150 ml) was boiled under reflux for 3h. TLC analysis (system B) indicated that the conversion of starting material was complete. The reaction solution was concentrated under reduced pressure to an oil which was chromatographed on a column of silicagel. Elution with $CHCl_3/CH_3OH$ (100:0→96:4, v/v) gave after evaporation pure **10** as an oil. Yield 1.80 g (79%), Rf=0.60 (system B).
Compound **10**, ^1H NMR ($CDCl_3$): δ 8.26, s, 1H, H-8; 7.75, s, 1H, H-2; 6.14, m, 2H, NH-CH_2 and H_1'; 5.91, s, 1H, CH-OCH_3; 5.29, t, J=6.88 Hz, 1H, CH=C; 5.19 m, 1H, H_2'; 4.50, m, 1H, H_3'; 4.14, br s, 2H, CH_2-N; 3.90, m, 1H, H_4'; 3.75-3.70, m, 2H, H_5' and H_5''; 3.41, s, 3H, CH-OCH_3; 1.69, s, 3H, CH_3-C=C; 1.67, s, 3H, CH_3-C=C; ^{13}C NMR ($CDCl_3$); δ 154.6, s, 152.6, 147.1, 138.8 and 119.1, 5xs (purine); 136.2, s, $(CH_3)_2$-C=C; 120.6, CH=C; 119.9, CH-OCH_3; 92.54, s, C_1'; 87.3, s, C_4'; 83.74, s, C_3'; 81.05, s, C_2'; 62.6, s, C_5'; 52.3, s, CH-OCH_3, 25.3 and 17.5, 2xs (CH_3)$_2$-C=C; HRMS, found m/z 377.1696 $C_{17}H_{23}N_5O_5$ requires 377.1699.

Synthesis of 6-[4-hydroxy-3-methyl-E-but-2-enylamino]-9-(5'-O-phosphate-β-D-ribofuranosyl) purine **1b** (ribosyl zeatin 5'-phosphate)
A solution of phosphorylating agent N-morpholino O,O-bis[(6-trifluoromethyl)benzotriazolyl] phosphate in dioxane (reagent D, 0.2M, 4.5 ml, 0.90 mmol) was added to **6** (0.22 g, 0.5 mmol) which was coevaporated with pyridine (3x5 ml) and dissolved in pyridine (5 ml). N-methylimidazole (0.07 ml, 0,93 mmol) was then added. After 1h TLC analysis (system B) indicated complete conversion of the purine compound **8** into intermediate **12**, Rf=0.77, water was then added to the reaction mixture (3 ml). After 1h TLC (system B) showed the formation of a new product with zero mobility. The reaction mixture was evaporated and the residue was diluted with 25% aqueous ammonia (10 ml) to remove the acetyl group and the mixture was left for 20h at 20°C. The reaction mixture was concentrated to a small volume. The residue was

diluted with water (15 ml) and the pH was adjusted to 2.0 by the addition of HCl (0.1N) to remove methoxymethylidene and morpholino groups. After 15h at 20°C the reaction mixture was extracted with diethyl ether (2x100 ml). Then the pH of the aqueous layer was adjusted to 8.0 with aqueous ammonia 25%, concentrated to a small volume (1 ml) and was applied to a column of DEAE-Sephadex A25 (HCO_3^--form) suspended in triethylammonium bicarbonate buffer (0.05M). The column was eluted with a linear gradient of triethylammonium bicarbonate buffer (0.05→0.7M) for 16h with a flow rate of 35 ml/h. Fractions of 10 ml were collected and all UV-positive eluates, containing purine product **1b**, [Rf=0 (system D), Rf=0.34 (system E)], were pooled, concentrated to a small volume and coevaporated with water (4x50 ml), and lyophilized from H_2O. The residue was dissolved in water (2 ml) and applied to a column of Dowex 50 Wx-8 cation-exchange resin (Na-form, 1.5x5 cm).
The column was eluted with water and all UV-positive eluates were collected, concentrated to a small volume and lyophilized from D_2O to give **1b** as a white solid. Yield 160 mg (68% based on **6**), [Rf=0.34 (system E), Rf=0.20 (system F)]. Product identity was confirmed by UV and thin-layer chromatography, which was identical as described in the literature[7], and further with ^{31}P NMR, ^{13}C NMR and ^1H NMR.
Compound **1b**, ^1H NMR (D_2O): δ 8.39, s, 1H, H-8; 8.20, s, 1H, H-2; 6.00, d, J=5.5 Hz, H_1'; 5.59, t, J=6.6 Hz, 1H, CH=C; 4.73, t, J=5.5 Hz, H_2'; 4.48, dd, J(H_3'-H_4')=4.1 Hz, J(H_3'-H_2')=4.8 Hz, 1H; H_3'; 4.38 , m, 1H, H_4'; 4.19, brd, J=4.8 Hz, 2H, CH_2-N; 4.10, m, 2H, H_5' and H_5''; 4.00, s, 2H, C=C-CH_2-O; 1.74, s, 3H, CH_3; ^{13}C NMR (D_2O): δ 155.3, 153.4, 150.1, 140.1 and 119.8, 5xs (purine); 139.7, s, CH_3-C=C; 88.3, s, C_1'; 85.7, d, $^3J_{C-P}$=3.7 Hz, C_4'; 77.3, s, C_3'; 73.0, s, C_2'; 71.0, s, C=C-CH_2-O; 68.0, s, C_5'; 40.2, s, CH_2-N, 14.3, s, CH_3; ^{31}P NMR (D_2O): δ_P=1.10 ppm.

Synthesis of 6-[3-methyl-2-but-enylamino]-9-(5'-O-phosphate-β-D-ribofuranosyl) purine **2b** [N^6-(Δ^2-Isopentenyl)adenosine 5'-phosphate]
A solution of phosphorylating agent N-morpholino O,O-bis[(6-trifluoromethyl)benzotriazolyl phosphate (reagent D) in dioxane (0.2M, 4.77 ml), 0.95 mmol) was added to purine compound **10** (0.20 g, 0.53 mmol), which was coevaporated with pyridine (2x10 ml) and dissolved in pyridine (5 ml), N-methylimidazole (0.08 ml, 1 mmol) was then added. After 1h TLC analysis (system B) indicated complete conversion of purine compound **10** into intermediate **11**, Rf=0.82. Water was then added to the reaction mixture (3 ml). After 1h TLC analysis (system B) showed the formation of new product with zeromobility. The reaction mixture was evaporated and the residue was diluted with water (15 ml) and the pH was adjusted to 2.0 by the addition of HCl (0.1N) to remove methoxymethylidene as well as the morpholino groups. After 15h at 20°C the reaction mixture was extracted with diethyl ether (2x100 ml). The pH of the aqueous layer was adjusted to pH=8.0 with aqueous ammonia 25%. The reaction solution was concentrated to a small volume (1 ml) and was applied to a column of DEAE-Sephadex A25 (HCO_3^--form) suspended in triethylammonium bicarbonate buffer (0.05M) to give after work up and purification as described in the synthesis of **1b** the sodium salt of **2b** as a white solid. Yield 177 mg (73% based on **10**), Rf=0.35 (system E), product identity was confirmed by UV and thin-layer chromatography which was identical as described in the literature[4,5] and further with ^{31}P NMR, ^{13}C NMR and ^1H NMR.
Compound **2b**, ^1H NMR (D_2O): δ 8.42, s, 1H, H-8; 8.19, s, 1H, H-2; 6.08, d, J=6 Hz, 1H, H_1'; 5.36, t, J=5.9 Hz, 1H, CH=C; 4.70, t, J=5.8 Hz, 1H, H_2'; 4.45, dd, J(H_3'-H_4')=3.9 Hz, J(H_3'-H_2')=4.9 Hz, 1H, H_3'; 4.32, m, 1H, H_4'; 4.00, br d, J=6.3 Hz, 2H, CH_2-N; 4.08, m, 2H, H_5' and H_5''; 1.70, s, 3H, CH_3; ^{13}C NMR (D_2O): δ 154.3, 152.9, 148.1, 139.2, 118.8, 5xs (purine); 137.9, s, $(CH_3)_2$-C=C; 87.0, s, C_1'; 84.2, d, $^3J_{C-P}$=8.2 Hz, C_4'; 74.5, s, C_3'; 70.6, s, C_2'; 64.2, s, C_5', 38.9, s, CH_2-N; 24.8 and 17.3, 2xs, $(CH_3)_2$-C=C; ^{31}P NMR (D_2O): δ_P=1.3 ppm.

Synthesis of 6-[4-O-phosphate-3-methyl-E-but-2-enylamino]-9-β-D-ribfuranosyl) purine **1d** (ribosyl zeatin allylic phosphate)
To a solution of **6** (0.20 g, 0.40 mmol) and N,N-diisopropylethylamine (0.10 ml, 0.56 mmol) in dioxane was added 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (reagent K, 114 mg, 0.56 mmol). After reaction for 5 min. at 20°C, it was found by TLC analysis (system C) that complete conversion of starting compound into a product with zeromobility has taken place. Water was added (1 ml) and the solution was left for 10 min. at 20°C to give **15** [Rf=0 (system D), Rf=0.67 (system E)]. The reaction solution was concentrated to a small volume and triturated with diethyl ether, the precipitated oil was coevaporated with acetonitrile (4x10 ml) and dissolved in acetonitrile (10 ml). The solution was treated with N,N-diisopropylethylamine (0.15 ml), 0.80 mmol) and N,O-bis(trimethylsilyl) acetamide (0.2 ml, 0.8 mmol). After 15 min. the reaction solution was further treated with 2,2'-dipyridyldisulfide (160 mg, 0.48 mmol) after 1h at 20°C TLC analysis (system E) indicated the complete conversion of **15**.

Then water was added (3 ml) and the reaction solution left for 2h at 20°C to give **19** with Rf=0.40 (system E). The reaction solution was concentrated to an oil and dissolved in water (20 ml). The pH of the resulting solution was adjusted to pH=2 by the addition of HCl (0.1N). After 3h at 20°C TLC analysis (system E) showed that the cleavage of (TBDMS) as well as methoxymethylidene was complete. The reaction solution was washed with diethyl ether (2x100 ml). The aqueous layer was neutralized to pH=8.0 with aqueous ammonia 25%, concentrated to an oil and applied to a column of DEAE-Sephadex A25 (HCO_3^--form) suspended in triethylammonium bicarbonate buffer (0.05M). The column was eluted with a linear gradient of triethylammonium buffer (0.05→0.70M) for 15h with a flow rate of 35 ml/h and fractions of 8 ml were collected. All UV-positive eluates containing **1d**, [Rf=0 (system D), Rf=0.30 (system E)] were pooled and concentrated to a small volume, coevaporated with water (4x50 ml) and lyophilized from H_2O. The residue was dissolved in water (2 ml) and applied to a column of Dowex 50 Wx-8 cation-exchange resin (Na^+-form, 1.5x5 cm). The column was eluted with water and all UV-positive eluates were collected, concentrated to a small volume and lyophilized from D_2O to give **1d** as a white solid. Yield 145 mg (76% based on **6**), Rf=0.30 (system E).
Compound **1d**, ^1H NMR (D_2O): δ 8.19, s, 1H, H-8; 8.12, s, 1H, H-2; 6.00, d, J=6.3 HZ, 1H, H_1'; 5.65, t, J=6.2 HZ, 1H, CH=C; 4.72, t, J=6.2 Hz, 1H, H_2'; 4.38, dd, J(H_3'-H_2')=5.2 Hz, J(H_3'-H_4')=3.3 Hz, 1H, H_3'; 4.24, m, 1H, H_4'; 4.16, d, $^3J_{H-P}$=5.4 Hz, 2H, C=C-$\underline{CH_2}$-O; 4.10, br s, 2H, CH_2-N; 3.88-3.75, 2xdd, J(H_5'-H_5'')=12.8 Hz, J(H_5'5''-H_4')=2.5 Hz and 3.4 Hz, 2H, H_5' and H_5''; 1.73, s, 3H, CH_3; ^{13}C NMR (D_2O): δ 154.7, 153.1, 148.2, 140.3 and 119.9, 5xs (purine); 138.2, d, $^3J_{C-P}$=7.4 Hz, CH_3-$\underline{C}$=C; 121.0, s, $\underline{CH}$=C, 88.5, s, C_1'; 85.8, s, C_4'; 74.0, s, C_3'; 70.7, s, C_2', 68.9, s, C=C-$\underline{CH_2}$-O, 61.8, s, C_5'; 38.8, s, CH_2N; 13.4, s, CH_3; ^{31}P NMR (D_2O): δp=2.12 ppm.; compounds **15** and **19** were identified after purification by using a column of DEAE-Sephadex A 25 (HCO_3^--form); the purified compounds were converted into sodium salts by passing it over Dowex 50 Wx-8 (Na^+-form).
Compound **15**, ^{1}H-NMR (D_2O): δ=8.15, s, 1H, H-8; 8.10, s, 1H, H-2; 6.95, d, $^1J_{P-H}$=632 Hz, 1H, P-H; 6.28, d, J=2.2 Hz, 1H, H_1'; 6.17, s, 1H, $\underline{CH}$-OCH_3; 5.60, t, J=6.5 Hz, 1H, CH=C; 5.40, m, 1H, H_2'; 5.17, m, 1H, H_3', 4.45, m, 1H, H_4'; 4.35, d, $^3J_{H-P}$=8 Hz, 2H, C=C-$\underline{CH_2}$-O; 4.17, br d, J=6.5 Hz, 2H, CH_2-N, 3.88-3.72, m, 2H, H_5' and H_5'', 3.42, s, 3H, CH-$\underline{OCH_3}$; 1.72, s, 3H, CH_3; 0.80, s, 9H, TBDMS; 0.00, s, 6H, TBDMS; ^{13}C NMR (D_2O): δ 155.5, 153.9, 147.2, 141.2 and 119.7, 5xs (purine); 137.1, d, $^3J_{C-P}$=5.4 Hz; CH_3-$\underline{C}$=C; 123.1, s, $\underline{CH}$-OCH_3; 120.1, s, $\underline{CH}$=C; 91.4, s, C_1'; 86.6, s, C_4'; 83.0, s, C_3'; 81.5, s, C_2', 69.8, s, C=C-$\underline{CH_2}$-O; 62,8, s, C_5'; 51.7, s, CH-$\underline{OCH_3}$; 39.7, s, CH_2-N; 26.4, 18.8 and -5.0, 3xs (TBDMS); 14.4, s, CH_3; ^{31}P NMR (D_2O): δp=6.77 ppm., $^1J_{P-H}$=633 Hz.
Compound **19**, ^1H NMR (D_2O): δ 8.5, s, 1H, H-8; 8.2, s, 1H, H-2; 6.22, d, J=4 Hz, 1H, H_1'; 6.15, s, 1H, $\underline{CH}$-OCH_3; 5.56, t, J=6.4 Hz; 1H, CH=C; 5.23, m, 1H, H_2'; 4.50, m, 1H, H_3'; 4.28, m, 5H, CH_2-N, H_4' and C=C-$\underline{CH_2}$-O; 3.82-3.75, m, 2H, H_5' and H_5''; 3.75, s, 3H, CH-$\underline{OCH_3}$; 1.74, s, 3H, CH_3; 0.88, s, 9H, TBDMS; 0.00, s, 6H, TBDMS;, ^{31}P NMR (D_2O): δp=2.20 ppm.

Synthesis of 6-[4-O-phosphate-3-methyl-E-but-2-enylamino]-9-(5'-O-phosphate-β-D-ribofuranosyl) purine **1c** (ribosyl zeatin diphosphate)

Ribosyl zeatin diphosphate **1c** was synthesized in a similar procedure as described in the synthesis of ribosyl zeatin allylic phosphate **1d**, thus diphosphonate derivative **16** was obtained from ribofuranosyl derivative **9** (0.20 g, 0.5 mmol), 1,3,2-benzodioxaphosphorin-4-one (reagent K, 0.28 g, 1.4 mmol) and N,N-diisopropylethylamine (0.26 ml, 1.4 mmol). The oxidation of diphosphonate derivative **16**, Rf=0.56 (system E) to diphosphate derivative **20** was realized by using N,O-bis(trimethylsilyl) acetamide (0.5 ml, 2 mmol), N,N-diisopropylethylamine (0.35 ml, 2 mmol) and 2,2'-dipyridylsulfide (260 mg, 1.2 mmol).
The diphosphate derivative **20**, Rf=0.35 (system E) was acidified to pH=2 for 3h to give **1c**, Rf=0.28 (system E), the latter was purified on a column of DEAE-Sephadex A25 (HCO_3^--form) eluted with a linear gradient of triethylammonium buffer (0.05→1M) and was converted into its sodium salt, **1c** was obtained as white solid after lyophylization. Yield 210 mg, (70% based on **9**), Rf=0.28 (system E).
Compound **1c**, ^1H NMR (D_2O): δ 8.40, s, 1H, H-8; 8.14, s, 1H, H-2; 6.05, d, J=5.8 Hz, 1H, H_1'; 5.65, t, J=6.6 Hz, 1H, CH=C; 4.71, t, J=5.5 Hz, 1H, H_2'; 4.45, dd, J(H_3'-H_4')=3.7 Hz, J(H_3'-H_2')=5.2 Hz, 1H, H_3'; 4.22, m, 1H, H_4'; 4.22, d, $^3J_{H-P}$=6.3 Hz, 2H, C=C-$\underline{CH_2}$-O; 4.15, br d, J=5.8 Hz, 2H, CH_2-N, 4.02, m, 2H, H_5' and H_5''; 1.74, s, 3H, CH_3; ^{13}C NMR (D_2O): δ 154.7, 153.2, 148.5, 139.7 and 119.2, 5xs (purine); 137.1, d, $^3J_{C-P}$=7.8 Hz, CH_3-$\underline{C}$=C; 122.1, s, $\underline{CH}$=C; 87.42, s, C_1'; 84.5, d, $^3J_{C-P}$=5.6 Hz, H_4'; 74.6, s, C_3', 70.7, s, C_2', 69.8, s, C=C-$\underline{CH_2}$-O, 64.28, s, C_5'; 38.8, s, CH_2-N; 13.4, s, CH_3; ^{31}P NMR (D_2O): δp=2.55 ppm. and 1.90 ppm.; compounds **16** and **20** were identified after purification by using a column of DEAE-Sephadex A 25 (HCO_3^--form); the purified compounds were converted into sodium salt by passing it over Dowex 50 Wx-8 (Na^+-form).
Compound **16**, ^1H NMR (D_2O): δ 8.11, s, 1H, H-8; 8.00, s, 1H, H-2; 6.15, d, J=2.1 Hz, 1H, H_1'; 6.04, s, 1H, $\underline{CH}$-OCH_3; 6.70, d, $^1J_{H-P}$=635 Hz, 1H, P-H; 5.60, t, J=6.5 Hz, 1H, CH=C; 6.55, d, $^1J_{H-P}$=639 Hz,

1H, P-H; 5.30, m, 1H, H_2', 5.07, m, 1H, H_3'; 4.56, m, 1H, H_4'; 4.23, d, $^3J_{H-P}$=8.6 HZ, 2H, C=C-$\underline{CH_2}$-O; 4.00, m, 4H, CH_2-N, H_5' and H_5''; 3.43, s, 3H, CH-$\underline{OCH_3}$; 1.71, s, 3H, CH_3; ^{13}C NMR (D_2O): δ 154.3, 153.0, 147.8, 135.9 and 118.9, 5xs (purine); 139.5, d, $^3J_{C-P}$=8.3 Hz, CH_3-$\underline{C}$=C; 122.9, s, CH=C; 117.9, s, CH-OCH_3; 90.1, s, C_1'; 85.5, d, $^3J_{C-P}$=7.4 Hz, C_4'; 83.2, s, C_3', 80.5, s, C_2', 68.8, s, C=C-$\underline{CH_2}$-O; 63.4, s, C_5'; 52.7, s, CH-$\underline{OCH_3}$; 38.6, s, CH_2-N, 13.4, s, CH_3; ^{31}P NMR (D_2O): δp=6.73 ppm. ($^1J_{P-H}$=634.7 Hz) and δp=6.67 ppm., ($^1J_{P-H}$=631 Hz).

Compound **20**, ^{1}H NMR (D_2O): δ 8.38, s, 1H, H-8; 8.15, s, 1H, H-2, 6.20, d, J=2.5 Hz, H_1', 6.06, s, 1H, $\underline{CH}$-OCH_3; 5.50, t, J=6.4 Hz, 1H, CH=C; 5.41, m, 1H, H_2'; 5.14 m, 1H, H_3', 4.30 m, 1H, H_4'; 4.13, br d, J=5.4 Hz, s, 4H, CH_2-N and C=C-$\underline{CH_2}$-O; 3.91, m, 2H, $H_5'$and H_5''; 3.40, s, 3H, CH-$\underline{OCH_3}$; 1.71, d, 3H, CH_3; ^{31}P NMR (D_2O): δ_P=4.30 ppm. and 4.22 ppm.

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19. Compound **1a**, ^{1}H NMR (D_2O): δ 8.16, s, 1H, H-8; 8.09, s, 1H, H-2; 5.96, d, J=5.9 Hz, 1H, H_1'; 5.54, t, J=6.35 Hz, 1H, CH=C; 4.68, t, J=5.9 Hz, 1H, H_2'; 4.36, dd, J(H_3'-H_2')= 4.9 Hz, J(H_3'-H_4')=3.4 Hz, 1H, H_3'; 4.23, m, 1H, H_4', 4.15, br s, 2H, CH_2-N; 3.96, s, 2H, C=C-CH_2-O; 3.88-3.75; 2xdd, J(H_5'-H_5'')=12.8 Hz, J($H_5'5''$-H_4')=2.5 Hz and 3.41 Hz; 2H, H_5' and H_5''; 1.69, s, 3H, CH_3; ^{13}C NMR (D_2O): δ 153.8, 152.8, 147.2, 139.7 and 119.0, 5xs (purine); 138.5, s, CH_3-$\underline{C}$=C; 120.4, s, $\underline{CH}$=C, 88.2, s, C_1', 85.8, s, C_4'; 74.1, s, C_3'; 73.6, s, C_2'; 70.7, s, C=C-$\underline{CH_2}$-O; 66.5, s, C_5'; 38.6, s, CH_2-N; 13.6, s, CH_3.
20. Compound **2a**, ^{1}H NMR (D_2O): δ 8.19, s, 1H, H-8; 8.03, s, 1H, H-2; 5.90, d, J=6.12 Hz, 1H, H_1'; 5.26, t, J=6.6 Hz, 1H, CH=C; 4.67, t, J=5.9 Hz, 1H, H_2'; 4.50, dd, J(H_3'-H_2')=5.2 Hz, J(H_3'-H_4')=3 Hz, 1H, H_3'; 4.31, m, 1H, H_4'; 4.20, br d, J=3 Hz, 2H, CH_2-N; 3.86-3.73, 2xdd, J(H_5'-H_5'')=12.2 Hz, J($H_5'5''$-H_4')=2.5 Hz and 3.4 Hz, 2H, H_5' and H_5''; 1.71, s, 3H, CH_3-C=C; 1.65, s, 3H, CH_3-C=C; ^{13}C NMR (D_2O): δ 153.9, 152.2, 147.1, 140.3 and 119.0, 5xs (purine); 137.6, s, CH_3-$\underline{C}$=C; 88.4, s, C_4', 85.5, s, C_4'; 73.9, s, C_3', 70.4, s, C_2', 61.2, s, C_5', 38.5, s, CH_2-N, 24.5 and 16.9, 2xs, $\underline{(CH_3)_3}$-C=C.