Tetrahedron Vol. 46, No. 6, pp. 2179-2186, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00
Pergamon Press plc

THE SYNTHESIS OF PHOSPHOR DERIVATIVES OF RIBOSYL ZEATIN

Belal Shadid and Henk C. van der Plas*

Laboratory of Organic Chemistry, Agricultural University Wageningen,
Dreijenplein 8, 6703 HB Wageningen, The Netherlands

(*Received in UK* 15 *November* 1989)

Abstract: Powdered sulfur was used for the conversion of the protected ribosyl zeatin phosphonate derivatives **2** and **3** into the corresponding thiophosphate **8** and **9**, whereas 2,2'-dipyridyldisulfide in the presence of methanol was used for the conversion of **2** and **3** into the corresponding methyl phosphates **6** and **7**. After cleavage of the protecting groups of **2**, **3**, **6**, **7**, **8** and **9** under mild acidic condition the phosphor derivatives of ribosyl zeatin **1b-1g** are obtained.

INTRODUCTION

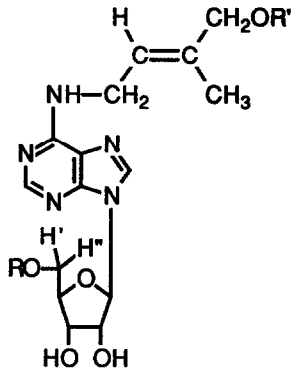
Cytokinins are a class of hormones that were first isolated from plant tissues. They regulate cell division and growth in plants and participate in the differentiation process through their interaction with other plant hormones[1]. Certain cytokinins have also been shown to affect the behaviour of mammalian cells. For example, they have been reported to regulate the growth of mammalian cells[2,3,4], to inhibit platelet aggregration[5] and to have immunosuppressive activity[6]. Cytokinins have also been employed as potential anticancer agents in clinical trials[7,8]. Naturally occurring cytokinins are N^6-substituted adenine derivatives and several highly active species have been isolated at the purine, ribonucleoside and ribonucleotide levels[9-12]. One of these adenine derivatives with plant cell division promoting activity was isolated[13] from Zeamays and was identified as 6-(4-hydroxy-3-methyl-E-but-2-enylamino)-9-(β-D-ribofuranosyl)purine i.e. **1a**, usually named ribosyl zeatin.

In an earlier work we reported on the synthesis of ribosyl zeatin phosphates[14]. Now we wish to describe the synthesis of some phosphor derivatives of ribosyl zeatin i.e. **1b-1g**. This synthetic study is part of a program of cooperation between our laboratory and the Center of Agriculture and Biological Research at Wageningen, directed to study the mechanism and physiological effects of cytokinins in plants.

RESULTS AND DISCUSSION

Recently we described a new and efficient method for the synthesis of the phosphate, methyl phosphate and thiophosphate monoester of the allylic hydroxy group in 6-(4-hydroxy-3-methyl-

E-but-2-enylamino)purine[15]. This method is based on the phosphitylation of the allylic hydroxy group with salicyl chlorophosphite yielding the allylic phosphonate monoester, and subsequent oxidation or sulphurization.



1a. R' = R = H

1b. R' = $P(=O)(H)(OH)$, R = H

1c. R' = R = $P(=O)(H)(OH)$

1d. R' = $P(=O)(OH)(OCH_3)$, R = H

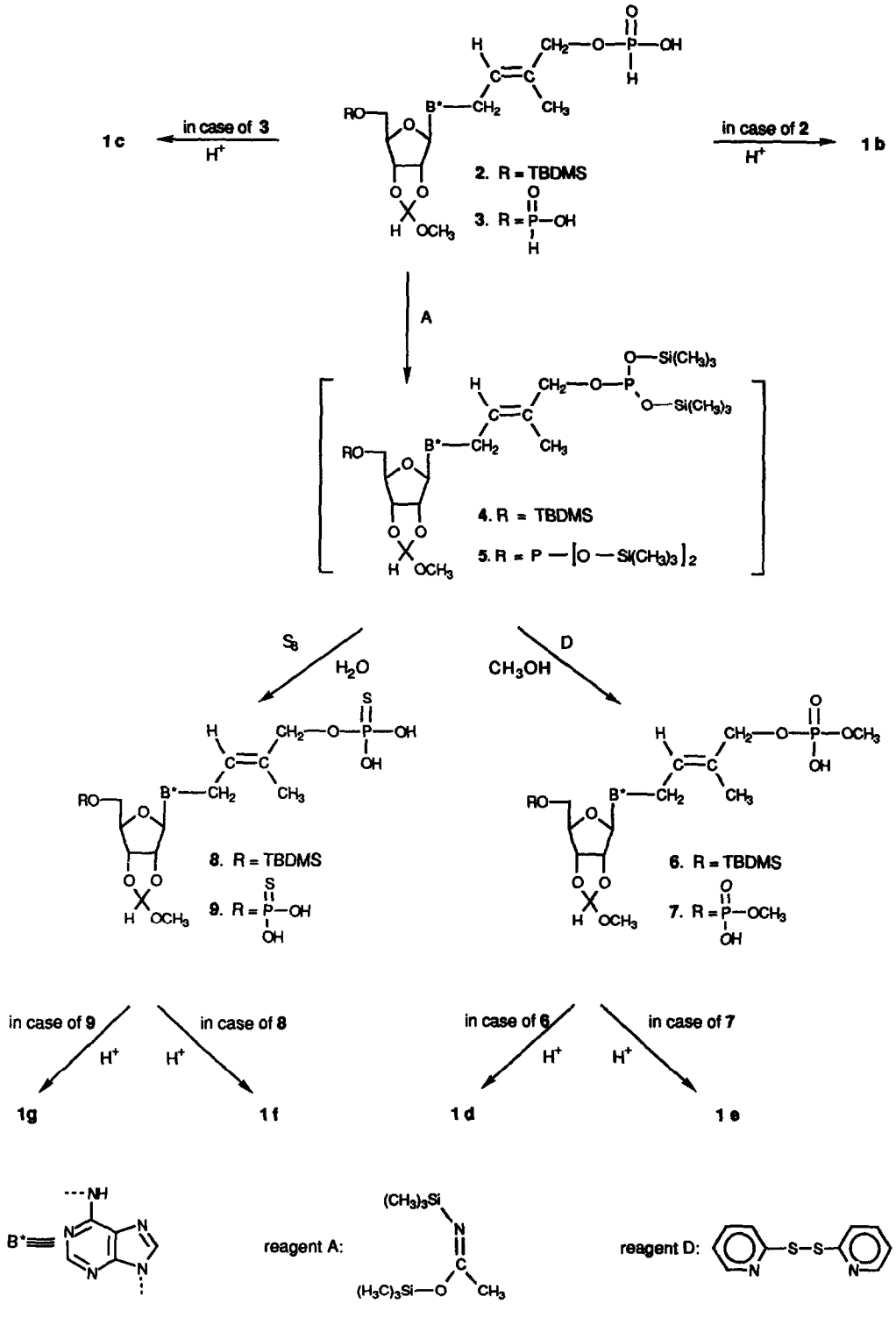
1e. R' = R = $P(=O)(OH)(OCH_3)$

1f. R' = $P(=S)(OH)_2$, R = H

1g. R' = R = $P(=S)(OH)_2$

We wanted to examine whether this methodology is also suitable for the synthesis of some phosphor derivatives of ribosyl zeatin i.e. **1b-1g.**

According to a slight modification of the procedure of Hata et al.[16] ribosyl zeatin allylic phosphonate **2** and ribosyl zeatin bis(phosphonate) **3** (both compounds **2** and **3** being prepared by a procedure described before[14]) were treated with N,O-bis(trimethylsilyl)acetamide (reagent A) in the presence of N,N-diisopropylethylamine for 15 min. to give the intermediary bis(trimethylsilyl)phosphites **4** and **5**, respectively. Without any further purification both **4** and **5** were treated with 2,2'-dipyridyldisulfide (reagent D) and the reaction solution thus obtained was treated with dry methanol affording **6** and **7**, respectively. The structures of **6** and **7** were ascertained by ^{1}H-NMR and ^{31}P-NMR spectroscopy. Treatment of **4** and **5** with powdered sulfur for 15 h and subsequent treatment with water afforded the sulfur compounds **8** and **9**, respectively. The structure of these compounds was ascertained by ^{1}H-NMR and ^{31}P-NMR spectroscopy.


1c
in case of 3
H+
2. R = TBDMS
3. R = P(O)(OH)H
in case of 2
H+
1b
A
4. R = TBDMS
5. R = P[O—Si(CH3)3]2
S8
H2O
D
CH3OH
8. R = TBDMS
9. R = P(S)(OH)OH
6. R = TBDMS
7. R = P(O)(OCH3)OH
in case of 9
in case of 8
in case of 6
in case of 7
1g
1f
1d
1e
reagent A:
reagent D:

The cleavage of the acid-labile groups tert-butyldimethylsilyl (TBDMS) and methoxymethylidene in the derivatives **2, 3, 6, 7, 8** and **9** was effected by acid treatment at pH=2 for 3 h. The ribosyl zeatin allylic phosphonate **1b**, ribosyl zeatin bis(phosphonate) **1c**, ribosyl zeatin allylic thiophosphate **1f**, ribosyl zeatin bis(thiophosphate) **1g**, ribosyl zeatin allylic methyl phosphate **1d** and ribosyl zeatin bis(methyl phosphate) **1e** were obtained in good yields. Over-all yields in the preparation of the compounds **1b-1g** fluctuated between 70% and 90% (calculated from **2** and **3**).

The structure of **1b-1g** was proven by ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR data. The ^{1}H-NMR and ^{13}C-NMR data of the ribosyl zeatin derivatives **1b-1g** bear a strong resemblance to those of ribosyl zeatin[14]. However, there are some exceptions: i. The ^{1}H-absorption of H_5' and H_5'' appears in ribosyl zeatin **1a** as two pairs of double doublets at 3.75-3.88 ppm., due to the fact that H_5' and H_5'' have slightly different chemical shifts and $J(H_5'\text{-}H_5'') \neq J(H_5'\text{-}H_4') \neq J(H_5''\text{-}H_4')$. In **1c**, **1e** and **1g** the absorption of H_5' and H_5'' appears as 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 ^{1}H-absorption of $C{=}C\text{-}CH_2\text{-}O$ in ribosyl zeatin **1a** is found as a singlet at 3.96 ppm., but in **1b-1g** this absorption appears as a doublet at about 4.20 ppm. The doublets are caused by the $^{3}J_{H\text{-}P}$ coupling. iii. The ^{13}C-resonance of $CH_3\text{-}\underline{C}{=}C$ in ribosyl zeatin **1a** is a singlet (at about 138 ppm.), but in **1b-1g** it is a doublet. Similarly, the ^{13}C-signals of C_4' appear in ribosyl zeatin **1a** as a singlet at about 85 ppm., but in **1c**, **1e** and **1g** they appear as doublets, due to coupling of ^{13}C with ^{31}P($^{3}J_{C\text{-}P}$). Proton decoupled ^{31}P-NMR spectroscopy revealed the presence of the expected number of resonance absorptions, which further proves the structural identity and purity of the compounds.

In conclusion, the methodology described in this paper presents an elegant way for the synthesis of phosphate derivatives of ribosyl zeatin. The yields are satisfactory, the reaction proceeds smoothly under mild conditions.

EXPERIMENTAL

General procedures.

N,N-diisopropylethylamine and acetonitrile were dried by refluxing with CaH_2 for 16 h and then distiled. Methanol was dried by refluxing with magnesium methoxide and distiled before use. All liquids were stored under nitrogen. N,O-bis(trimethylsilyl)acetamide and 2,2'-dipyridyldisulfide were purchased from Janssen Chimica (Belgium). Triethylammonium bicarbonate buffer was prepared by passing a stream of CO_2 gas through a cooled (ice-water bath) 2 M solution of triethylamine in deionized water until the solution became neutral. Scheicher and Schüll DC Fertigfolien were used for TLC. The following solvent system was used: system A (isopropyl alcohol /concentrated ammomium hydroxide /water, 7:1:2, v/v). Short column chromatography was performed on Sephadex LH 20 suspended in CH_2Cl_2/CH_3OH (2:1, v/v), unless otherwise mentioned. DEAE-Sephadex A 25 was purchased from Pharmacia (Uppsala, Sweden). Cation-exchange resin (Na^+-form): a solution of NaOH (2 M; 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. ^{1}H-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.

6-(4-O-Phosphonate-3-methyl-E-but-2-enylamino)-9-(2',3'-O-methoxymethylidene-5'-O-tert-butyldimethylsilyl-β-D-ribofuranosyl) purine **2** and 6-(4-O-phosphonate-3-methyl-E-but-2-enylamino)-9-(2',3'-O-methoxymethylidene-5'-O-phosphonate-β-D-ribofuranosyl)purine **3** were prepared as described previously[14].

Synthesis of 6-(4-O-methylphosphate-3-methyl-E-but-2-enylamino)-9-(β-D-ribofuranosyl)purine **1d** (ribosyl zeatin allylic methylphosphate)

The Na^+-salt of **2** (0.25 g, 0.42 mmol) was repeatedly coevaporated with acetonitrile (4x10 ml). The residue was diluted with acetonitrile (5 ml) and this mixture was treated with N,N-diisopropylethylamine (0.15 ml, 0.84 mmol) and N,O-bis(trimethylsilyl)acetamide (0.21 ml, 0.84 mmol). After 15 min. the solution was reacted with 2,2'-dipyridyldisulfide (108 mg, 0.5 mmol) for 1 h at 20°C. TLC analysis (system A) indicated the complete absence of **2**. After addition of an excess of methanol (3 ml) the solution was left for 15 h at 20°C to give **6**, Rf= 0.52 (system A). The reaction solution was concentrated to an oil and dissolved in water (20 ml). The pH of the resulting solution was adjusted to 2 by the addition of HCl (0.1 N). After 3 h at 20°C, TLC analysis (system A) showed that the cleavage of TBDMS as well as methoxymethylidene was complete. The reaction solution was washed with diethylether (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 A 25 (HCO_3^--form) suspended in triethylammonium bicarbonate buffer (0.05 M). The column was eluted with a linear gradient of triethylammonium buffer (0.05→0.50 M) for 15 h with a flow rate of 35 ml/h and fractions of 10 ml were collected. The UV-positive eluates containing **1d**, Rf=0.37 (system A) were pooled and concentrated to a small volume, coevaporated with water (4x50 ml) and lyophilized from H_2O. The residue was dissolved in water (1 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 144 mg (74% based on **2**).

Compound **1d**; ^{1}H-NMR (D_2O): δ 8.20, s, 1H, H-8; 8.10, s, 1H, H-2; 6.01, d, J=6.0 Hz, 1H, H_1'; 5.62, t, J=6.2 Hz, 1H, CH=C; dd, J(H_3'-H_2')=3.4 Hz, J(H_3'-H_4')=5.4 Hz, 1H, H_3'; 2.28, m, 1H, H_4'; 4.19, d, $^3J_{H\text{-}P}$=6.5 Hz, 2H, C=C-CH_2-O; 3.90, brs, 2H, CH_2-N; 3.86-3.50, 2xdd, J(H_5'-H_5")=12.8 Hz, J(H_5'5"-H_4')=2.6 Hz and 3.4 Hz, 2H, H_5' and H_5"; 3.46, d, $^3J_{H\text{-}P}$=10.8 Hz, 3H, P-OCH_3; 1.70, s, 3H, CH_3; ^{13}C-NMR (D_2O): δ 154.6, 152.8, 150.0, 148.1 and 119.6, 5xs (purine); 136.2, d, $^3J_{C\text{-}P}$=6.3, CH_3-C=C; 122.1, s, CH=C; 88.5, s, C_1'; 85.8, s, C_4'; 74.1, s, C_3'; 70.8, s, C_2'; 70.7, s, C=C-CH_2-O; 61.9, s, C_5'; 53.7, s, P-OCH_3; 39.2, s, CH_2-N; 14.0, s, CH_3; ^{31}P-NMR (D_2O): δ_P=2.17 ppm.

Compound **6** was identified after purification by using a column of DEAE-Sephadex A25 (HCO_3^-); the purified compound was converted into the sodium salt by passing it over Dowex 50 Wx-8 (Na^+-form).

Compound **6**; ^{1}H-NMR (D_2O): δ 8.05, s, 1H, H-8; 8.00, s, 1H, H-2; 6.10, d, J=4.1 Hz, 1H, H_1'; 6.00, s, 1H, CH-OCH_3; 5.58, t, J=6.2 Hz, 1H, CH=C; 5.5, m, 1H, H_2'; 4.80, m, 1H, H_3'; 4.60, m, 1H, H_4'; 4.10, d, $^3J_{H\text{-}P}$=7.8 Hz, 2H, C=C-CH_2-O; 4.00, m, 2H, CH_2-N; 3.60, m, 2H, H_5' and H_5"; 3.50, d, $^3J_{H\text{-}P}$=10.7 Hz, 3H, P-OCH_3; 3.15, s, 3H, CH-OCH_3; 1.70, s, 3H, CH_3; 0.88, s, 9H, (TBDMS); 0.00, s, 6H, (TBDMS); ^{31}P-NMR (D_2O): δ_P=2.19 ppm.

Synthesis of 6-(4-O-methylphoshate-3-methyl-E-but-2-enylamino)-9-(5'-O-methylphosphate-β-D-ribofuranosyl) purine **1e** [ribosyl zeatin bis(methylphosphate)]

Since compound **1e** was synthesized according to a similar procedure as described before in the synthesis of **1d**, we refrain from an extensive description and give here only some notes. The conversion of the Na^+-salt of bis(phosphonate) derivative **3** (0.25 g, 0.44 mmol) into the bis(methylphosphate) derivative **7** was realized by using N,O-bis(trimethylsilyl)acetamide (0.44 ml, 1.76 mmol), N,N-diisopropylethylamine (0.31 ml, 1.76 mmol), 2,2'-dipyridyldisulfide (228 mg, 1.1 mmol) and methanol (4 ml). The bis(methylphosphate) **7**, Rf=0.37 (system A) was acidified to pH=2. After 3 h **1e** was obtained, Rf=0.31 (system A) which was purified on a column of DEAE-Sephadex A 25 (HCO_3^--form). The column was eluted with a linear gradient of triethylammonium buffer (0.05→0.5 M) and was converted into its sodium salt. Compound **1e** was obtained as a white solid. Yield=192 mg (75% based on **3**).

Compound **1e**; ^{1}H-NMR (D_2O): δ 8.36, s, 1H, H-8; 8.14, s, 1H, H; 6.02, d, J=5.6 Hz, 1H, H_1'; 5.62, t, J=6.5 Hz, 1H, CH=C; 4.30, t, J=5.9 Hz, 1H, H_2'; 4.28, m, 1H, H_3; 4.26, m, 1H, H_4'; 4.23, d, $^3J_{H\text{-}P}$=6.6 Hz, 2H, C=C-CH_2-O; 4.21, brd, J=6.5, 2H, CH_2-N; 4.12, m, 2H, H_5' and H_5"; 3.56, d, $^3J_{H\text{-}P}$=12.6 Hz, 3H, P-OCH_3; 3.52, d, $^3J_{H\text{-}P}$=12.6 Hz, 3H, P-OCH_3; 1.70, s, 3H; ^{13}C-NMR (D_2O): δ 151.8, 153.4, 148.4, 139.5, 119.4, 5xs (purine), 136.2, d, $^3J_{C\text{-}P}$=5.60 Hz, CH_3-C=C; 123.1, s, CH=C, 87.6, s, C_1', 83.9, d, $^3J_{C\text{-}P}$=7.4 Hz, C_4'; 74.3, s, C_3', 70.8, s, C_2', 70.5, s, C=C-CH_2-O; 65.1, s, C_5', 53.2, s, 2x(P-OCH_3); 38.7, s, CH_2-N; 14.0, s, CH_3; ^{31}P-NMR (D_2O): δ_P=1.90 ppm and 2.05 ppm,

Compound **7** was identified after purification by using a column of DEAE-Sephadex A 25 (HCO_3^-); the purified compound was converted into sodium salt by passing it over Dowex 50 Wx-8 (Na^+-form).

Compound **7**; ^{1}H-NMR (D_2O): δ 8.22, s, 1H, H-8; 8.15, s, 1H, H-2; 6.30, d, J=2.6 Hz, 1H, H_1', 6.10, s, 1H, CH-OCH_3; 5.28, t, J=5.7 Hz, 1H, CH=C; 4.79, m, 1H, H_2'; 4.74, m, 1H, H_3'; 4.70, m, 1H, H_4'; 4.26, d, J=7.0 Hz, 2H, C=C-CH_2-O; 4.00, brd, J=4.2 Hz, 2H, CH_2-N; 3.90, m, 2H, H_5' and H_5"; 3.40, d, $^3J_{H\text{-}P}$=10.0 Hz, 3H, P-OCH_3; 3.36, d, $^3J_{H\text{-}P}$=10.0 Hz, 3H, P-OCH_3; 3.30, s, 3H, CH-OCH_3; 1.76, s, 3H, CH_3; ^{31}P-NMR (D_2O): δ_P=3.80 ppm and 4.21 ppm.

Synthesis of 6-(4-O-thiophosphate-3-methyl-E-but-2-enylamino)-9-(β-D-ribofuranosyl) purine **1f** (ribosyl zeatin allylic thiophosphate)

The Na^+-salt of **2** (0.25 g, 0.42 mmol) was repeatedly coevaporated with acetonitrile (4x10 ml). The residue was diluted with acetonitrile (5 ml) and this mixture was treated with N,N-diisopropylethylamine (0.15 ml, 0.84 mmol) and N,O-bis(trimethylsilyl)acetamide (0.21 ml, 0.84 mmol). After 15 min. the solution was treated with powdered sulfur (1 g) for 16 hrs. TLC analysis (system A) revealed that 16 h is required to complete the conversion of phosphonate **2** into thiophosphate **8**, Rf=0.38 (system A). After this time water (1 ml) was added and the excess of sulfur was removed by filtration. The resulting solution was concentrated to a small volume and triturated with diethyl ether. The oil formed was dissolved in water (20 ml), the pH of this solution was adjusted to 2 by the addition of HCl (0.1 N). After 3 h at 20°C TLC analysis (system A) showed the complete cleavage of the TBDMS as well as the methoxymethylidene group. 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 Sephadex LH 20. The column was eluted with CH_2Cl_2/CH_3OH (2:1, v/v). The fractions containing purine product **1f**, Rf=0.28 (system A) were pooled, concentrated to an oil 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 **1f** as a white solid. Yield 167 mg (80% based on **2**).

Compound **1f**; ^{1}H-NMR (D_2O): δ 8.12, s, 1H, H-8, 8.00, s, 1H, H-2; 6.02, d, J=6.21 Hz, 1H, H_1'; 5.67, t=6.5 Hz, 1H, CH=C; 4.60, brs, 1H, H_2'; 4.14, dd, J(H_3'-H_2')=4.3 Hz, J(H_3'-H_4')=3.4, 1H, H_3'; 4.27, m,

1H, H_4'; 4.17, d, $^3J_{H\text{-}P}$=5.2 Hz, 2H, C=C-CH_2-O; 4.25 brs, 2H, CH_2-N; 3.75-3.90, 2xdd, J(H_5'-H_5'')=12.8 Hz, J($H_5'$$_5''$-$H_4'$)=2.2 Hz and 3.4 Hz, 2H, H_5' and H_5''; 1.72, s, 3H, CH_3; ^{13}C-NMR (D_2O): δ 156.9, 153.1, 148.0, 140.0, 119.5, 5xs (purine); 138.2, d, $^3J_{C\text{-}P}$=7.4 Hz, CH_3-C=C; 121.5, s, CH=C; 89.1, s, C_1'; 86.2, s, C_4'; 74.6, s, C_3'; 71.2, s, C_2'; 67.9, s, C=C-CH_2-O; 62.6, s, C_5'; 39.4, s, CH_2-N; 14.0, s, CH_3; ^{31}P-NMR (D_2O): δ_P=46.00 ppm;

Compound **8** was identified after purification by using a column of Sephadex LH 20 suspended in CH_2Cl_2/CH_3OH (2:1, v/v); the purified compound was converted into sodium salt by passing it over Dowex 50 Wx-8 (Na^+-form).

Compound **8**; ^{1}H-NMR (D_2O): δ 8.25, s, 1H, H-8; 8.15, s, 1H, H-2, 6.20, d, J=3 Hz, 1H, H_1'; 6.05, s, 1H, CH-OCH_3; 5.60, t, J=6.5 Hz, 1H, CH=C; 4.80, m, 1H, H_2'; 4.72, m, 1H, H_3'; 4.20, m, 5H, CH_2-N, H_4' and C=C-CH_2-O; 3.80, m, 2H, H_5' and H_5''; 3.32, s, 3H, CH-OCH_3; 1.74, s, 3H, CH_3; 0.85, s, 9H, (TBDMS); 0.00, s, 6H, (TBDMS); ^{31}P-NMR (D_2O): δ_P=43.01 ppm.

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

Compound **1g** was synthesized according to the same procedure as described above in the synthesis of ribosyl zeatin allylic thiophosphate **1f**; therefore only some notes are given. The sulfurization of the Na^+-salt of bis(phosphonate) derivative **3** (0.25 g, 0.44 mmol) was performed by using N,N-diisopropylethylamine (0.31 ml, 1.76 mmol), N,O-bis(trimethylsilyl)acetamide (0.31 ml, 1.76 mmol) and powdered sulfur (1g). The bis(thiophosphate) **9**, R=0.33 (system A) was acidified to pH=2. After 3 h **1g** was obtained, Rf=0.25 (system A), which was purified by using a column of Sephadex LH 20 eluting with CH_2Cl_2/CH_3OH (2:1, v/v). The purified compound was converted into its sodium salt. **1g** was obtained as white solid. Yield 227 mg (82% based on **3**).

Compound **1g**; ^{1}H-NMR (D_2O): δ 8.52, s, 1H, H-8; 8.13, s, 1H, H-2; 6.00, d, J=5.1 Hz, 1H, H_1'; 5.60, t, J=6.2 Hz, 1H, CH=C; 4.54, dd, J(H_3'-H_4')= 5.3 Hz, J(H_3'-H_2')=3.4 Hz, 1H, H_3'; 4.40, m, 1H, H_4'; 4.30, d, $^3J_{H\text{-}P}$=6.5 Hz, 2H, C=C-CH_2-O; 4.10, m, 4H, CH_2-N, H_5' and H_5''; 1.77, s, 3H, CH_3; ^{13}C-NMR (D_2O): δ 155.3, 153.8, 149.1, 140.5 and 119.8, 5xs (purine); 137.8, d, J=7.3 Hz, CH_3-C=C; 122.4, s, CH=C; 88.1, s, C_1'; 85.2, d, J=9.3 Hz; C_4'; 75.4, s, C_3'; 71.6, s, C=C-CH_2-O; 70.4, s, C_2'; 64.9, s, C_5'; 39.5, s, CH_2-N; 14.0, s, CH_3; ^{31}P-NMR (D_2O): δ_P=49.52 ppm and 48.40 ppm;

Compound **9** was identified after purification by using a column of Sephadex LH 20 suspended in CH_2Cl_2/CH_3OH (2:1, v/v); the purified compound was converted into the sodium salt by passing it over Dowex 50 Wx-8 (Na^+-form).

Compound **9**; ^{1}H-NMR (D_2O): δ 8.44, s, 1H, H-8; 8.13, s, 1H, H-2; 6.30, d, J=3.4 Hz, 1H, H_1'; 6.20, s, 1H, CH-OCH_3; 5.61, t, J=5.6 Hz, 1H, CH=C; 5.43, m, 1H, H_2'; 3.39, m, 1H, H_3'; 4.67, m, 1H, H_4'; 4.21, d, J=6.4 Hz, 2H, C=C-CH_2-O; 4.15, brd, 2H, CH_2-N; 4.07-3.98, m, 2H, H_5' and H_5''; 3.35, s, 3H, CH-OCH_3; 1.75, s, 3H, CH_3; ^{31}P-NMR (D_2O): δ_P=59.40 ppm.

Synthesis of 6-(4-O-phosphonate-3-methyl-E-but-2-enylamino)-9-(β–D-ribofuranosyl) purine **1b** ribosyl zeatin allylic phosphonate) and of 6-(4-O-phosphonate-3-methyl-E-but-2-enylamino)-9-(5'-O-phosphonate-β-D-ribofuranosyl) purine **1c** [ribosyl zeatin bis(phosphonate)]

The Na^+-salt of **2** (0.25 g, 0.42 mmol) and **3** (0.25 g, 0.44 mmol) respectively were dissolved in water (20 ml), the pH was adjusted to 2 with HCl (0.1 N) and this solution was kept for 3 h at 20°C. TLC analysis (system A) showed that the cleavage of the TBDMS as well as methoxymethylidene group was then complete. The solution was washed with diethyl ether (2x100 ml), neutralized with aqueous ammonia (25%), concentrated to a small volume and then 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. The amount of **1b** obtained was 166 mg (90% based on **2**) and the yield of **1c** was 200 mg (86% based on **3**) respectively.

Compound 1c; ^{1}H-NMR(D_2O): δ 8.20, s, 1H, H-8; 8.08, s, 1H, H-2 6.68, d, $^1J_{H\text{-}P}$=639.2, 1H, P-H; 6.66, d, $^1J_{H\text{-}P}$=639.2 Hz, 1H, P-H; 6.00, d, J=5.4 Hz, 1H, H_1'; 5.60, t, J=6.8 H_2, 1H, CH=C; 4.66, t, J = 5.4, 1H, H_2'; 4.39, dd, J(H_3'-H_4') = 4.4 Hz, J(H_3'-H_2') = 5.0 Hz, 1H, H_3'; 4.29, m, 1H, H_4'; 4.23, d, $^3J_{H\text{-}P}$ = 8.8 Hz, 2H, C=C-CH_2-O; 4.07, m, 4H, CH_2-N, H_5' and H_5"; 1.71, s, 3H, CH_3; ^{13}C-NMR (D_2O): δ 155.7, 154.1, 150.3, 141.0, 119.7, 5xs (purine); 136.8, d, $^3J_{C\text{-}P}$ = 7.4 Hz, CH_3-C=C; 123.3, s, CH=C; 88.1, s, C_1'; 84.7, d, $^3J_{C\text{-}P}$ = 5.4 Hz, C_4'; 77.7, s, C_3'; 71.4, s, C_2'; 69.8, s, C=C-CH_2-O; 64.0, s, C_5'; 39.2, s, CH_2-N; 14.0, s, CH_3; ^{31}P-NMR (D_2O): δ_P=7.03 ppm, $^1J_{H\text{-}P}$=634.8 Hz and δ_P=6.65 ppm, $^1J_{H\text{-}P}$=632.3 Hz.
Compound 1b; ^{1}H-NMR(D_2O): δ 8.40, s, 1H, H-8; 7.73, s, 1H, H-2; 6.74, d, $^1J_{H\text{-}P}$=636.7 Hz, 1H, P-H; 6.05, d, J=6.3 Hz, 1H, H_1'; 5.60, t, J=6.5 Hz, 1H, CH=C; 4.52, dd, J=5.4 Hz and 3.4 Hz; 1H, H_3'; 4.30, m, 1H, H_4'; 4.28, d, J=5.2 Hz, 2H, C=C-CH_2-O; 4.25, brd, 2H, CH_2-N; 3.90-3.75, 2xdd, J(H_5'5")=12.8 Hz, J(H_5'5'-H_4')=2.2 Hz and 3.4 Hz, 2H, H_5' and H_5"; 1.72, s, 3H, CH_3; ^{13}C-NMR (D_2O): δ 155.5, 153.7, 148.6, 140.2, 119.6, 5xs (purine); 135.9, d, $^3J_{C\text{-}P}$=6.0 Hz, CH_3-C=C; 122.9, s, CH=C; 88.5, s, C_1'; 86.0, s, C_4'; 73.9, s, C_3'; 70.9, s, C_2'; 69.0, s, C=C-CH_2-O; 61.7, s, C_5'; 38.7, s, CH_2-N; 14.4, s, CH_3; ^{31}P-NMR (D_2O): δ_P=6.51 ppm, $^1J_{H\text{-}P}$=634.7 Hz.

ACKNOWLEDGEMENT

We wish to thank Mr. A. van Veldhuizen for recording the ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectra.

REFERENCES

1. Skoog. F.; Armstrong. A. *Ann. Rev. Plant Physiol.* **1970**, *21*, 359.
2. Hampton. A.; Biesele. J.J.; Moore. A.E.; Brown. G.B. *J. Am. Chem. Soc.* **1956**, *78*, 5695.
3. Fleysher. M.H. *J. Med. Chem.* **1972**, *15*, 187.
4. Suk. D.; Simpson. G.L.; Mihich. E. *Cancer Res.* **1970**, *30*, 1429.
5. Kikugawa. K.; Iizuka. K.; Ichino. M. *J. Med. Chem.* **1973**, *16*, 358.
6. Hacker. B.; Feldbush. T.L. *Biochem. Pharmacol.* **1969**, *18*, 847.
7. Jones. R.; Grace. J.T.; Mittelman. A.; Woodruff. M.W. *Proc. Am. Assoc. Cancer Res.* **1968**, *9*, 35.
8. Mittelman. A.; Evans. J.T.; Cheda. G.B. *Ann. N.Y. Acac. Sci.* **1975**, *255*, 255.
9. Letham. D.S. *Bio-Science* **1969**, *19*, 309.
10. Helgeson. J.P. *Science* **1968**, *161*, 974.
11. Zachu. H.G. *Angew. Chem.*, Int. Ed. Engl. **1969**, *8*, 711.
12. Letham. D.S. *Phytochemistry* **1973**, *12*, 2445.
13. Letham. D.S. *Life Sci.* **1966**, *5*, 551.
14. Shadid. B.; van der Plas. H.C.; *Tetrahedron* **1989**, *45*, accepted for publication.
15. Shadid. B.; van der Plas. H.C.; Vonk. C.R.; Davelaar. E.; Ribot. S.A. *Tetrahedron* **1989**, *45*, 3889.
16. Hata. T.; Skin. M. *Tetrahedron Letters* **1975**, 3943.