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Synthesis of Optically Active 6-Alkynyl- and 6-Alkylpurines as Cytokinin Analogs and Inhibitors of 15-Lipoxygenase; Studies of Intramolecular Cyclization of 6-(Hydroxyalkyn-1-yl)purines

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Optically active 6-alkynyl- and 6-alkylpurines have been synthesized. These compounds can be regarded as analogs of cytokinin plant growth hormones. The analogs were examined as growth stimulators and as inhibitors of 15-lipoxygenase (15-LO). The (*S*)-enantiomer of 6-(5-hydroxy-4-methylpent-1-yl)purine exhibited profound growth stimulation activity, especially at low concentrations. 6-(Hydroxyalkyn-1-yl)purines were found to cyclize by nucleophilic *exo* attack from the hydroxy group at the triple bond. (*Z*)-Isomers were formed under acidic conditions. Plant growth stimulators and 15-LO inhibitors were identified among the cyclization products.

Introduction

We have been studying 6-alkynyl-, 6-alkenyl-, 6-cyclopropyl-, and 6-alkylpurines as analogs of cytokinins (CK) like *trans*-zeatin (*t*-Z) and 6-benzylaminopurine (BAP) (Figure 1).[1] CKs are plant growth hormones which promote cell division and cell growth, and they are involved in the retardation of senescence. The cytokinins found in nature are 6-(alkylamino)purines, and BAP and *t*-Z are among the most potent known naturally occurring CKs. Metabolism of zeatin involves cleavage of the side chain by the enzyme system cytokinin oxidase/dehydrogenase (CKX);[2] adenine, showing no phytohormone properties, is formed irreversibly. BAP is also metabolised to adenine, but knowledge about the enzyme system is limited. 6-Substituted purines lacking the exocyclic amino functionality in the 6-position are not expected to be substrates for CKX and similar enzymes. A prolonged cytokinin effect is therefore expected. During our previous studies we found that that racemic 6-(5-hydroxy-4-methylpentyl)purine exhibited growth stimulating effect comparable to BAP and *t*-Z[1a] in the radish cotyledon assay.[3] This was somewhat surprising since it has generally been believed that an unsaturation in the side chain of CKs, like the phenyl group in BAP or alkenyl group in *t*-Z, is required for high activity.[4] We are now reporting the synthesis and plant growth stimulating effect for both enantiomers of 6-(5-hydroxy-4-methylpentyl)purine as well as synthetic intermediates. Furthermore, we have also observed that certain 6-substituted purines, originally designed as CK analogs, are inhibitors of 15-lipoxygenase (15-LO),[5] 15-LO has been implicated in oxidation of low-density lipoproteins (LDL), a process believed to be important for the development of atherosclerosis,[6] as well as for instance in prostate cancer[7] and spontaneous abortions,[8] and several compounds described herein have also been screened for activity against 15-LO.

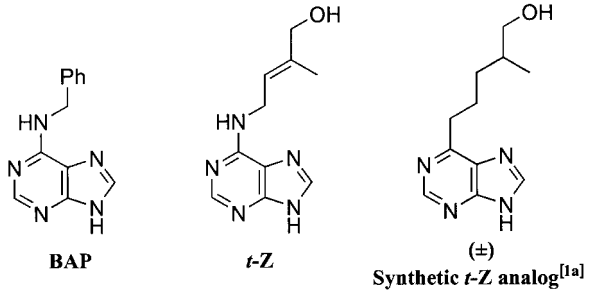

Figure 1. Structures of naturally occurring cytokinins and analogs.

Results and Discussion

Synthesis

A variety of carbon substituents can easily be introduced in the purine 6-position by palladium-catalyzed coupling reactions,[9] and for the synthesis of the enantiomerically pure targets **6** (Scheme 1), we chose Sonogashira coupling between terminal alkyne **2** and THP-protected 6-chloropu-

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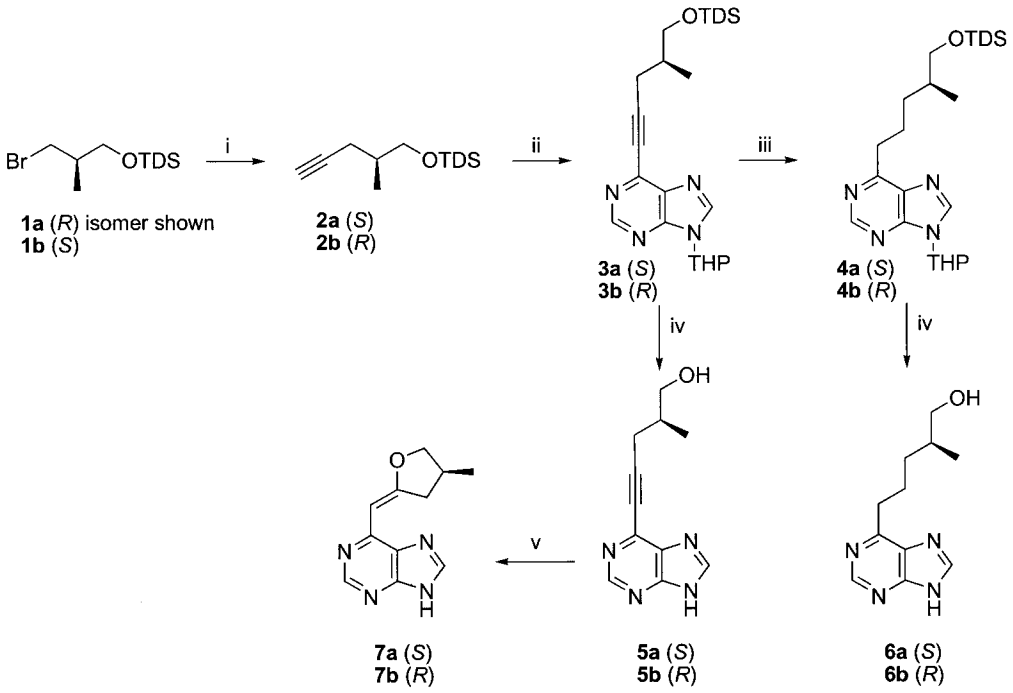


Scheme 1. *Reagents and reaction conditions:* i. LiC≡CH·EDA, DMPU, THF; ii. 6-Cl-9-THP-purine, $(Ph_3P)_2PdCl_2$, CuI, $(iPr)_2NEt$, DMF, 80 °C; iii. H_2, Pd/C, EtOAc, MeOH; iv. HCl(aq), EtOH; v. DMF, 80 °C.

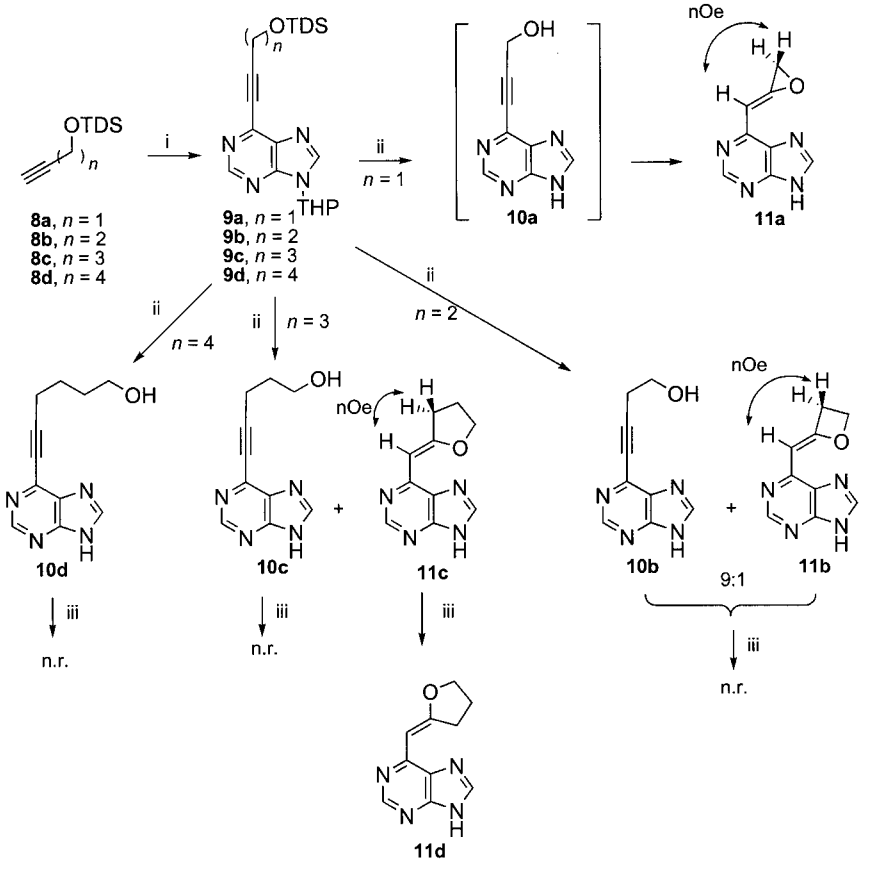
rine as the key-step. Our starting point was commercially available (*R*)- or (*S*)-3-bromo-2-methyl-1-propanol, which were *O*-protected and reacted with lithiumacetylenide–ethylenediamine complex to give the enantiomeric alkynes **2a** and **2b**. The 6-alkynylpurines **3**, formed after Sonogashira coupling, were reduced by catalytic hydrogenation and the target alkylpurines **6** were isolated after simultaneous removal of both protecting groups under acidic conditions.

Also the alkynes **3** could be deprotected to give compounds **5** when treated with dilute hydrochloric acid. We found, however, that when the alkynols **5** were heated, a ring closing reaction took place. When compounds **5** were heated at 80 °C in DMF for 28 h, starting materials were consumed and the tetrahydrofuranylmethylidenepurines **7** were isolated in moderate yields. No correlations were seen in the NOESY spectra of **7** between the CH= proton in the side chain and any of the protons on the tetrahydrofuranyl ring. Hence we propose (*E*)-configuration for compounds **7** and that cyclization had taken place by a *syn*-addition to the triple bond. We have previously reported that 6-vinylpurines readily undergo nucleophilic attack,[10] and also proposed that the high cytotoxicity found for many 6-alkenyl- or 6-alkynylpurines may be partly explained by nucleophilic attack on the electron deficient double- or triple bond.[11]

To the best of our knowledge, the cyclizations of the alkynols **5** to compounds **7** are the first examples of a synthetically useful intramolecular nucleophilic addition to an alkenyl or alkynylpurine, and there are no similar cyclization reactions on other hydroxyalkynyl heterocycles desribed in the literature. We investigated the scope and limitation of the reaction further with respect to the ring sizes that may be formed. The alkynylpurines **9** were synthesized by Sonogashira coupling and deprotected under standard acidic conditions (Scheme 2). The allene oxide **11a** was obtained directly after deprotection of compound **9a** by a "disfavored" 3-*exo-dig* cyclization,[12] and the elusive **10a** could not be isolated. NOESY spectroscopy showed that the (*Z*)-isomer was formed and that cyclization under acidic conditions resulted in an intramolecular *anti*-addition of the hydroxy group. The other extreme was compound **9d**, which was deprotected cleanly to the hexynol **10d**. Neither did the latter cyclize, even not after prolonged heating in DMF, nor did addition of acid promote cyclization.

The reactivities of the butyne **9b** and pentyne **9c** were found to be more complex. Acidic deprotection of **9b** resulted in a virtually inseparable mixture of the alcohol **10b** and ca. 10% of the oxetane **11b**. NOESY spectroscopy of the cyclization product **11b** again indicated that the (*Z*)-isomer was formed under acidic conditions. No further reactions could be observed when the mixture of compounds **10b** and **11b** was heated at 80 °C in DMF.

After treatment of the pentyne **9c** with dilute HCl, two products **10c** and **11c** were formed. They were separated and isolated in 30% and 47% yields, respectively. Again, NOESY spectroscopy was employed to determine the stereochemistry of the cyclized product **11c**. In contrast to what we earlier observed for the tetrahydrofuran derivatives **5** (Scheme 1), no cyclization was observed when **10c** was heated in DMF, but a net isomerization of compound **11c** to the corresponding (*E*)-isomer **11d** took place under these set of reaction conditions.



Scheme 2. *Reagents and reaction conditions:* i. 6-Cl-9-THP-purine, $(Ph_3P)_2PdCl_2$, CuI, $(i\text{Pr})_2$NEt, DMF, 65 °C; ii. HCl(aq), EtOH; iii. DMF, 80 °C.

Cytokinin Activity

The cytokinin activities of compounds **5**–**7**, **11a**, and **11d** as well as the naturally occurring cytokinin BAP, were determined by using the radish cotyledon assay.[3] The results are presented in Figure 2 and Table 1. Several compounds exhibited significant growth stimulating ability. The effect was especially profound at low concentrations. A 1 μM solution of compound **6a** was twice as active as BAP. Also the racemate of **6a** has previously been found to stimulate radish cotyledon growth.[1a] We have now shown that (*S*)-en-

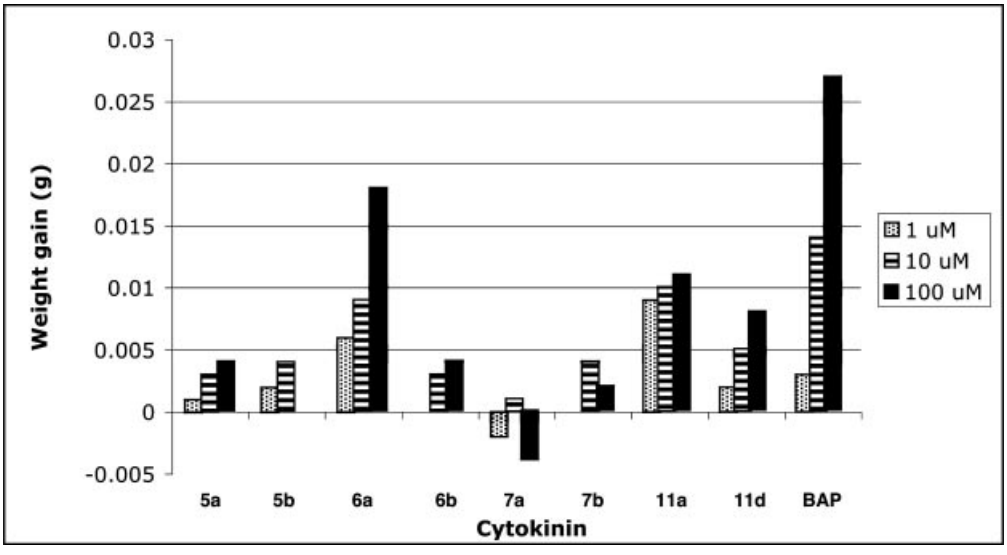


Figure 2. Weight gain (g) of cotyledons grown in the presence of cytokinin (1, 10 or 100 μM) compared to cotyledons grown without any purine added.

antiomer **6a** is substantially more active than the (*R*)-enantiomer **6b**. Lower activity for the alkynes **5** compared to the alkylpurines **6** is in accordance with our previous findings.[1] The oxirane **11a** was three times more active than 1 μM BAP. In the concentration range examined, the growth stimulation effect for compound **11a** was much less concentration dependant than for instance the effects found for BAP and compound **6a**. The allene oxide **11a** is structurally significantly different from any other known CK analogs. Moderate to low growth increase were found for the tetrahydrofuranylmethylenepurines **7** and **11d**. Compound **7a** was actually the only purine examined causing growth retardation of radish cotyledons.

Table 1. Cytokinin activity of synthetic purines compared to 1, 10 and 100 μM BAP.

Compound[a]	% Weight increase relative to BAP		
	1 μM[b]	10 μM[b]	100 μM[b]
BAP	100[c]	100[c]	100[c]
5a	33	21	15
5b	66	29	0
6a	200	64	67
6b	0	21	15
7a	–[d]	7	–[d]
7b	0	29	7
11a	300	71	40
11d	67	35	30

[a] The detailed structures of compds. **5**–**7** and **11** are found in Scheme 1. [b] % Weight gain obtained for radish cotyledon grown with 1, 10 or 100 μM purine compared to the weight gain obtained with the same concentrations of BAP. [c] Weight gain with BAP at the given concentration compared to no additive defined as 100%. [d] Retardation compared to BAP was observed.

Inhibition of 15-Lipoxygenase

6-Alkynyl- and 6-alkenylpurines may have a profound inhibitory effect on 15-lipoxygenase (15-LO).[5] The inhibitory activities of compounds **5**–**7** against 15-LO from soybeans are shown in Table 2. The low activity of the alkylpurines **6** was not unexpected. We have previously found that an alkenyl or alkynyl functionality in the purine 6-position is required for activity against 15-LO.[5] The methylidenetetrahydrofurans **7** were the most active compounds examined and there were only small differences between the enantiomers. Alkynes **5** were somewhat less active than other alkynes studied before.[5] There were no correlations between cytokinin activity and inhibition of 15-LO for compounds **5**–**7**, supporting out hypothesis that there is no relationship between these two different biological activities.[5]

Table 2. 15-LO inhibitory activity of 6-substituted purines **5**–**7**.[a]

Compound	IC_{50} [μM][b]
5a	153 ± 9
5b	> 167
6a	> 167
6b	> 167
7a	93 ± 7
7b	80 ± 5

[a] The detailed structures of compds. **5**–**7** are found in Scheme 1. [b] IC_{50} for quercetin was 48 ± 2 μM.

Conclusions

Both enantiomers of the cytokinin analog 6-(5-hydroxy-4-methylpentyl)purine have been synthesized and it is shown that the plant growth stimulating effect is mainly associated with the (*S*)-enantiomer. The side chain was introduced in the purine 6-position by Sonogashira coupling followed by catalytic hydrogenation of the alkyne. It was found that 6-(hydroxyalkyn-1-yl)purines may cyclize by nucleophilic *exo-dig* attack from the hydroxy group at the triple bond. 3- and five-membered rings were easily formed and (*Z*)-isomers were formed under acidic conditions. Isomerization of tetrahydrofurans from this ring closing reaction, was observed under thermal conditions. High cytokinin activity, especially at low concentrations, was found for the purinyl allene oxide formed and some tetrahydrofuran derivatives exhibited significant inhibitory activity against 15-lipoxygenase.

Experimental Section

The ^1H NMR spectra were acquired on a Bruker Avance DRX 500 spectrometer, a Bruker Avance DPX 300 spectrometer or a Bruker Avance DPX 200 spectrometer at 500, 300, or 200 MHz respectively. The ^1H decoupled ^{13}C NMR spectra were recorded at 125, 75, or 50 MHz using the above-mentioned spectrometers. All NMR spectra were recorded at 20 °C. MS spectra under electron impact conditions were recorded with a VG Prospec instrument at 70 eV ionizing voltage, and are presented as *m*/*z* (% rel. int.). CH_4 was employed as the ionization gas for chemical ionization (CI). Electrospray MS spectra were recorded with a Bruker Apex 47e FT-ICR mass spectrometer. Elemental analyses were performed by Ilse Beetz Mikroanalytisches Laboratorium, Kronach, Germany. Melting points are uncorrected. THF was distilled from Na/benzophenone, and DMF, DMPU and dichloromethane from CaH_2. The following compounds were prepared by literature methods: 6-chloro-9-tetrahydropyranyl-9*H*-purine,[13] **1a**,[14] **8a**,[15] **8b**,[16] **8c**,[17] **8d**.[18] Cytokinin activity,[1,3] and inhibition of 15-LO[5] were determined as described before.

[(2*S*)-3-Bromo-2-methylpropoxy](1,1-dimethylethyl)dimethylsilane (1b): The compound was prepared as described for the synthesis of the enantiomer **1a**,[14] and the data were in good accordance with those reported before.[19] $[\alpha]_D$ = +11.5 (*c* = 2.0, $CHCl_3$) (ref.[14] $[\alpha]_D$ = +11.1; *c* = 1.42, CH_2Cl_2).

(*S*)-(1,1-Dimethylethyl)dimethyl[(2-methyl-4-pentynyl)oxy]silane (2a): Lithium acetylide ethylene diamine complex (620 mg, ca 6.7 mmol, ca 90% pure) was dissolved in dry THF (30 mL) at 0 °C under argon atmosphere. Compound **1a** (1.50 g, 5.61 mmol) in DMPU (15 mL) was added dropwise. The cooling bath was removed and the resulting mixture was stirred at ambient temperature for 17 h. Ice/water (25 mL) was added and the mixture was extracted with Et_2O (3 × 100 mL). The combined extracts were washed with water (100 mL), dried ($MgSO_4$) and the solvents evaporated. The product was purified by flash chromatography on silica gel eluting with acetone/hexane (1:199); yield 96%, oil. $[\alpha]_D$ =

–11.5 (c = 2.5, $CHCl_3$) (ref.[20] $[\alpha]_D$ = –15.5; c = 1.1, $CHCl_3$). The spectroscopic data were in good accordance with those reported before.

(*R*)-(1,1-Dimethylethyl)dimethyl[(2-methyl-4-pentynyl)oxy]silane (2b): The title compound was prepared from compound **1b** as described for the enantiomer **2a** above; yield > 99%, oil. $[\alpha]_D$ = +11.4 (c = 2.1, $CHCl_3$).

6-[(4*S*)-5-{[(1,1-Dimethylethyl)dimethylsilyl]oxy}-4-methylpent-1-ynyl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (3a): Ethyldiisopropylamine (1.10 mL, 6.3 mmol) followed by a solution of compound **2a** (580 mg, 2.73 mmol) in dry DMF (15 mL) were added dropwise to a stirred mixture of 6-chloro-9-tetrahydropyranyl-9*H*-purine (500 mg, 2.10 mmol), $(Ph_3P)_2PdCl_2$ (147 mg, 0.21 mmol) and CuI (80 mg, 0.42 mmol) in DMF at ambient temperature. The resulting mixture was heated at 80 °C for 23 h, evaporated and the product was isolated by flash chromatography in silica gel eluting with EtOAc/$CHCl_3$ (1:4); yield 55%, oil. $[\alpha]_D$ = –9.65 (c = 2.6, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.87 (s, 1 H, 2-H), 8.27 (s, 1 H, 8-H), 5.76 (m, 1 H, 2-H in THP), 4.16 (m, 1 H, 6_A-H in THP), 3.75 (m, 1 H, 6_B-H in THP), 3.55 (m, 2 H, CH_2OSi), 2.74 (dd J = 17.3 and 5.4 Hz, 1 H, H_A in C≡CCH_2), 2.46 (dd, J = 17.3 and 7.7 Hz, 1 H, H_B in C≡CCH_2), 2.0–1.6 (m, 7 H), 1.07 (d, J = 6.8 Hz, 3 H, CH_3), 0.86 (s, 9 H, But), 0.03 [s, 6 H, $Si(CH_3)_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 152.4 (C-2), 150.4 (C-4), 142.7 (C-8), 142.3 (C-6), 134.3 (C-5), 100.6 (C≡), 81.9 (C-2 in THP), 77.2 (C≡), 68.7 (C-6 in THP), 66.8 (CH_2O), 35.2 (CH), 31.6 (CH_2 in THP), 25.8 (CH_3, butyl), 24.7 (CH_2 in THP), 23.6 (C≡CCH_2), 22.6 (CH_2 in THP), 18.1 (C, butyl), 16.1 (CH_3), –5.5 [$Si(CH_3)_2$] ppm. HRMS (ESI): calcd. for $C_{22}H_{34}N_4O_2Si$ + H 415.2529, found 415.2532. $C_{22}H_{34}N_4O_2Si$ (414.62): calcd. C 63.73, H 8.27, N 13.51; found: C 64.03, H, 8.12, N 13.15.

6-[(4*R*)-5-{[(1,1-Dimethylethyl)dimethylsilyl]oxy}-4-methylpent-1-ynyl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (3b): The title compound was prepared from compound **2b** as described for the enantiomer **3a** above; yield 56%, oil. $[\alpha]_D$ = +9.60 (c = 2.7, $CHCl_3$). $C_{22}H_{34}N_4O_2Si$ (414.62): calcd. C 63.73, H 8.27, N 13.51; found: C 63.47, H, 8.25, N 13.10.

6-[(4*S*)-5-{[(1,1-Dimethylethyl)dimethylsilyl]oxy}-4-methylpent-1-yl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (4a): A mixture of compound **3a** (179 mg, 0.43 mmol) and Pd/C (360 mg, 0.33 mmol) in EtOAc (20 mL) and methanol (20 mL) was stirred under H_2-pressure (1 atm.) at ambient temperature for 3 h. The mixture was filtered through a Celite pad and the solvents evaporated. The product was isolated by flash chromatography on silica gel eluting with EtOAc/$CHCl_3$ (1:4); yield 68%, oil. $[\alpha]_D$ = –0.89 (c = 2.8, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.86 (s, 1 H, 2-H), 8.21 (H-8), 5.77 (m, 1 H, 2-H in THP), 4.12 (m, 1 H, 6_A-H in THP), 3.76 (m, 1 H, 6_B-H in THP), 3.41 (dd, J = 9.7 and 5.8 Hz, 1 H, H_A in CH_2OSi), 3.32 (dd, J = 9.7 and 6.5 Hz, 1 H, H_B in CH_2OSi), 3.16 (m, 2 H, C≡CCH_2), 2.1–1.6 (m, 5×CH_2), 0.85 (m, 12 H, CH_3 and But), 0.01 [s, 6 H, $Si(CH_3)_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 163.5 (C-6), 152.9 (C-2), 150.4 (C-4), 141.8 (C-8), 133.1 (C-5), 82.3 (C-2 in THP), 69.2 (C-6 in THP), 68.7 (CH_2O), 36.1 (CH), 33.9 (CH_2), 33.5 (CH_2 in THP), 32.2 (CH_2 in THP), 26.5 (CH_2), 26.3 (CH_3, butyl), 25.3 (CH_2), 23.2 (CH_2 in THP), 18.7 (C, butyl), 17.0 (CH_3), –5.0 [$Si(CH_3)_2$] ppm. MS (CI): m/z (%) = 419 (40) [M + 1], 335 (66), 217 (100). HRMS (ESI): calcd. for $C_{22}H_{38}N_4O_2Si$ + H 419.2836, found 419.2844. $C_{22}H_{38}N_4O_2Si$ (418.28): calcd. C 63.12, H 9.15; found: C 62.83, H, 8.98.

6-[(4*R*)-5-{[(1,1-Dimethylethyl)dimethylsilyl]oxy}-4-methylpent-1-yl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (4b): The title compound was prepared from compound **3b** as described for the enantiomer **4a** above; yield >99%, oil. $[\alpha]_D$ = +1.57 (c = 2.5, MeOH). $C_{22}H_{38}N_4O_2Si$ (418.28): calcd. C 63.12, H 9.15, N 13.38; found: C 62.80, H, 9.32, N 13.39.

6-[(4*S*)-5-Hydroxy-4-methylpent-1-ynyl]-1*H*-purine (5a): Hydrochloric acid (12 mL, 1 M sol.) was added to a solution of compounds **3a** (200 mg, 0.99 mmol) in ethanol (20 mL) and the resulting mixture was stirred at ambient temperature for 6 h, neutralized by the addition of $NaHCO_3$ and the solvents evaporated. The product was isolated by flash chromatography in silica gel eluting with EtOAc/EtOH (1:1); yield 70%, yellow crystals, m.p. > 300 °C. $[\alpha]_D$ = +12.3 (c = 1.0, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 8.65 (s, 1 H, 2-H), 8.38 (s, 1 H, 8-H), 5.76 (br. s, 1 H, NH or OH), 4.63 (dd, J = 15.0 and 6.0 Hz, 1 H, H_A in CH_2O), 4.12 (dd, J = 15.0 and 6.4 Hz, 1 H, H_B in CH_2O), 3.05 (m, 1 H, H_A in C≡CCH_2), 2.57 (m, 2 H, CH and H_B in C≡CCH_2), 1.12 (d, J = 6.6 Hz, 3 H, CH_3) ppm. MS (EI): m/z (%) = 216 (29) [M^+], 201 (100). HRMS (EI): calcd. for $C_{11}H_{12}N_4O$ 216.1011, found 216.1009.

6-[(4*R*)-5-Hydroxy-4-methylpent-1-ynyl]-1*H*-purine (5b): The title compound was prepared from compound **3b** as described for the enantiomer **5a** above; yield 89%, yellow crystals, m.p. > 300 °C. $[\alpha]_D$ = –12.4 (c = 1.0, MeOH).

6-[(4*S*)-5-Hydroxy-4-methylpent-1-yl]-1*H*-purine (6a): The title compound was prepared from compound **4a** as described for the compound **5a** above; yield 99%, oil. $[\alpha]_D$ = –7.28 (c = 1.4, MeOH). HRMS (EI): calcd. for $C_{11}H_{16}N_4O$ 220.1324, found 220.1322. The spectroscopic data were in good agreement with those found for the racemate before.[1a]

6-[(4*R*)-5-Hydroxy-4-methylpent-1-yl]-1*H*-purine (6b): The title compound was prepared from compound **4b** as described for the compound **5a** above; yield 99%, oil. $[\alpha]_D$ = +7.28 (c = 0.92, MeOH). HRMS (CI, CH_4): calcd. for $C_{11}H_{16}N_4O$ + H 221.1402, found 221.1405.

(*E*)-6-[(4*S*)-4-Methyltetrahydrofuran-2-ylmethylene]-1*H*-purine (7a): Compound **5a** (300 mg, 1.39 mmol) was heated at 80 °C in DMF for 28 h. After evaporation, the product was isolated by flash chromatography on silica gel eluting with EtOAc/EtOH (9:1); yield 31%, yellow crystals, m.p. 186–188 °C. $[\alpha]_D$ = –59.0 (c = 1.0, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 8.63 (s, 1 H, 2-H), 8.22 (s, 1 H, 8-H), 6.31 (s, 1 H, CH=), 4.27 (dd, J = 8.5 and 6.7 Hz, 1 H, H_A in CH_2O), 3.76 (dd, J = 8.5 and 6.6 Hz, 1 H, H_B in CH_2O), 3.48 (m, 1 H, H_A in CH_2), 2.96 (dd, J = 6.9 and 1.8 Hz, H_B in CH_2), 2.51 (m, 1 H, CH), 1.07 (d, J = 7.0 Hz, 3 H, CH_3) ppm. MS (EI): m/z (%) = 216 (33) [M^+], 201 (100). HRMS (EI): calcd. for $C_{11}H_{12}N_4O$ 216.1011, found 216.1006.

(*E*)-6-[(4*R*)-4-Methyltetrahydrofuran-2-ylmethylene]-1*H*-purine (7b): The title compound was prepared from compound **5b** as described for the enantiomer **7a** above; yield 41%. $[\alpha]_D$ = +59.7 (c = 1.0, MeOH). HRMS (EI): calcd. for $C_{11}H_{12}N_4O$ 216.1011, found 216.1016.

General Procedure for the Synthesis of Compounds 9: Compounds **9** were formed by Sonogashira coupling between 6-chloro-9-tetrahydropyranyl-9*H*-purine and the appropriate alkyne **8** as described above for the synthesis of compound **3a**, except for the reaction temperature: 65 °C instead of 80 °C.

6-[3-{[(1,1-Dimethylethyl)dimethylsilyl]oxy}-1-propyn-1-yl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (9a): EtOAc/hexane (3:1) was used as eluent for flash chromatography; yield 67%, oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.90 (s, 1 H, 2-H), 8.30 (s, 1 H, 8-H), 5.77 (m, 1 H, 2-H in THP), 4.67 (s, 2 H, CH_2), 4.17 (m, 1 H, THP),

3.84 (m, 1 H, THP), 2.07 (m, 3 H, THP), 1.73 (m, 3 H, THP), 0.91 (s, 9 H, But), 0.16 [s, 6 H, $Si(CH_3)_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 153.0 (C-2), 151.1 (C-4), 143.6 (C-8), 141.9 (C-6), 134.8 (C-5), 98.2 (≡C), 82.5 (C-2 in THP), 79.9 (≡C), 69.3 (C-6 in THP), 52.7 (CH_2), 32.2 (CH_2 in THP), 26.2 (CH_3, butyl), 25.2 (CH_2 in THP), 23.1 (CH_2 in THP), 18.7 (C, butyl), –4.7 [Si-$(CH_3)_2$] ppm. MS (CI): *m/z* (%) = 373 (9) [M + 1], 317 (16), 289 (92), 231 (100). HRMS (ESI): calcd. for $C_{19}H_{28}N_4O_2Si$ + H 373.2047, found 373.2054. $C_{19}H_{28}N_4O_2Si$ (372.20): calcd. C 61.26, H 7.58, N 15.04; found: C 60.90, H, 7.29, N 14.74.

6-[4-{[(1,1-Dimethylethyl)dimethylsilyl]oxy}-1-butyn-1-yl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (9b): EtOAc/$CHCl_3$ (1:1) was used as eluent for flash chromatography; yield 77%, oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.86 (s, 1 H, 2-H), 8.27 (s, 1 H, 8-H), 5.76 (m, 1 H, 2-H in THP), 4.15 (m, 1 H, 6_A-H in THP), 3.89 (t, *J* = 7.5 Hz, 2 H, CH_2O), 3.74 (m, 1 H, 6_B-H in THP), 2.79 (t, *J* = 7.5 Hz, 2 H, CH_2), 2.05 (m, 3 H, THP), 1.71 (m, 3 H, THP), 0.87 (s, 9 H, But), 0.05 [s, 6 H, $Si(CH_3)_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 152.4 (C-2), 150.5 (C-4), 142.8 (C-8), 141.9 (C-6), 134.2 (C-5), 97.6 (≡C), 81.9 (C-2 in THP), 77.2 (≡C), 68.7 (C-6 in THP), 61.2 (CH_2O), 32.6 (CH_2), 25.8 (CH_3, butyl), 24.7 (CH_2), 24.1 (CH_2), 22.6 (CH_2), 18.2 (C, butyl), –5.4 [$Si(CH_3)_2$] ppm. MS (CI): *m/z* (%) = 387 (37) [M + 1], 303 (44), 245 (100), 215 (39). HRMS (ESI): calcd. for $C_{20}H_{30}N_4O_2Si$ + Na 409.2025, found 409.2030. $C_{20}H_{30}N_4O_2Si$ (386.56): calcd. C 62.14, H 7.82; found: C 62.10, H, 7.65.

6-[5-{[(1,1-Dimethylethyl)dimethylsilyl]oxy}-1-pentyn-1-yl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (9c): EtOAc/$CHCl_3$ (1:1) was used as eluent for flash chromatography; yield 87%, oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.82 (s, 1 H, 2-H), 8.26 (s, 1 H, 8-H), 5.70 (m, 1 H, 2-H in THP), 4.09 (m, 1 H, 6_A-H in THP), 3.70 (m, 2 H, 6_B-H in THP and CH_2O), 2.59 (t, *J* = 7.5 Hz, 2 H, CH_2), 2.00 (m, 3 H, THP), 1.82 (m, 2 H, CH_2), 1.65 (m, 3 H, THP), 0.79 (s, 9 H, But), 0.05 [s, 6 H, $Si(CH_3)_2$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.5 (C-2), 150.4 (C-4), 142.8 (C-8), 142.2 (C-6), 134.2 (C-5), 101.5 (≡C), 81.9 (C-2 in THP), 76.0 (≡C), 68.7 (C-6 in THP), 61.5 (CH_2O), 31.6 (CH_2), 31.1 (CH_2), 25.8 (CH_3, butyl), 24.7 (CH_2), 22.5 (CH_2), 18.1 (C, butyl), 16.4 (CH_2), –5.4 [$Si(CH_3)_2$] ppm. MS (CI): *m/z* (%) = 259 (100). HRMS (ESI): calcd. for $C_{21}H_{32}N_4O_2Si$ + H 401.2367, found 401.2358. $C_{21}H_{32}N_4O_2Si$ (400.23): calcd. C 62.96, H 8.05; found: C 62.72, H 7.91.

6-[6-{[(1,1-Dimethylethyl)dimethylsilyl]oxy}-1-hexyn-1-yl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (9d): EtOAc/$CHCl_3$ (1:1) was used as eluent for flash chromatography; yield 84%, oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.84 (s, 1 H, 2-H), 8.29 (s, 1 H, 8-H), 5.73 (m, 1 H, 2-H in THP), 4.12 (m, 1 H, 6_A-H in THP), 3.72 (m, H, 6_B-H in THP), 3.58 (t, *J* = 6.0 Hz, 2 H, CH_2O), 2.55 (m, 2 H, CH_2), 2.03 (m, 3 H, THP), 1.8–1.6 (m, 7 H, THP and 2×CH_2), 0.82 (s, 9 H, But), 0.15 [s, 6 H, $Si(CH_3)_2$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.5 (C-2), 150.4 (C-4), 149.2 (C-8), 143.3 (C-6), 134.2 (C-5), 101.8 (≡C), 82.0 (C-2 in THP), 76.1 (≡C), 68.8 (C-6 in THP), 62.4 (CH_2O), 31.9 (CH_2), 31.7 (CH_2), 25.9 (CH_3, butyl), 24.7 (CH_2), 24.5 (CH_2), 22.6 (CH_2), 19.7 (CH_2), 18.2 (C, butyl), –5.4 [$Si(CH_3)_2$] ppm. MS (CI): *m/z* (%) = 273 (100). HRMS (ESI): calcd. for $C_{22}H_{34}N_4O_2Si$ + H 415.2523, found 415.2515. $C_{22}H_{34}N_4O_2Si$ (414.62): calcd. C 63.73, H 8.27; found: C 64.12, H, 8.10.

6-(4-Hydroxy-1-butyn-1-yl)-1*H*-purine (10b) and (*Z*)-6-[Oxetan-2-ylmethylene]-1*H*-purine (11b): 6-[4-{[(1,1-Dimethylethyl)dimethylsilyl]oxy}-1-butyn-1-yl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (**9b**) (581 mg 1.5 mmol) was dissolved in MeOH (30 mL), and HCl (15 mL, 1 M) was slowly added The reaction mixture was stirred at ambient temperature for 6 h, and quenched by addition of solid $NaHCO_3$ to neutral pH. The product was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (85:15); yield 164 mg (58%) as a 9:1 mixture of compounds **10b** and **11b**. MS (EI): *m/z* (%) = 188 (3) [M^+], 158 (100).

6-(4-Hydroxy-1-butyn-1-yl)-1*H*-purine (10b): ^1H NMR (300 MHz, CD_3OD): δ = 8.82 (s, 1 H, 2-H), 8.56 (s, 1 H, 8-H), 3.84 (t, *J* = 6.4 Hz, 2 H, CH_2), 2.87–2.79 (m, 2 H, CH_2) ppm.

(*Z*)-6-[Oxetan-2-ylmethylene]-1*H*-purine (11b): ^1H NMR (300 MHz, CD_3OD): δ = 8.89 (s, 1 H, 2-H), 8.56 (s, 1 H, 8-H), 7.12 (s, 1 H, CH=), 3.90 (t, *J* = 6.0 Hz, 2 H, CH_2), 2.87–2.79 (m, 2 H, CH_2) ppm.

6-(5-Hydroxy-1-pentyn-1-yl)-1*H*-purine (10c) and (*Z*)-6-[tetrahydrofuran-2-ylmethylene]-1*H*-purine (11c): 6-[5-{[(1,1-Dimethylethyl)dimethylsilyl]oxy}-1-pentyn-1-yl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (**9c**) (876 mg, 2.19 mmol) was dissolved in EtOH (44 mL), and HCl (22 mL, 1 M) was slowly added. The reaction mixture was stirred at ambient temperature for 6 h, and quenched by addition of solid $NaHCO_3$ to neutral pH. The products were separated by flash chromatography on silica gel eluting with EtOAc/EtOH (1:1); yield 134 mg (30%) of **10c** and 208 mg (47%) of **11c**.

6-(5-Hydroxy-1-pentyn-1-yl)-1*H*-purine (10c): M.p. > 300 °C, pale greenish crystalline solid. ^1H NMR (500 MHz, CD_3OD): δ = 8.82 (s, 1 H, 2-H), 8.51 (s, 1 H, 8-H), 3.74 (t, *J* = 6.1 Hz, 2 H, CH_2O), 2.72 (t, *J* = 7.1 Hz, 2 H, CH_2), 1.94–1.89 (m, 2 H, CH_2) ppm. MS (EI): *m/z* (%) = 202 (100) [M^+], 171 (26). HRMS (EI): calcd. for $C_{10}H_{10}N_4O$ 202.0855, found 202.0852.

(*Z*)-6-[Tetrahydrofuran-2-ylmethylene]-1*H*-purine (11c): M.p. 131–133 °C, yellow crystalline solid. ^1H NMR (500 MHz, CD_3OD): δ = 8.69 (s, 1 H, 2-H), 8.44 (s, 1 H, 8-H), 5.83 (s, 1 H, CH=), 4.61 (t, *J* = 6.8 Hz, 2 H, CH_2O), 2.99 (t, *J* = 7.6 Hz, 2 H, CH_2), 2.22–2.16 (m, 2 H, CH_2) ppm. MS (EI): *m/z* (%) = 202 (67) [M^+], 171 (100). HRMS (EI): calcd. for $C_{10}H_{10}N_4O$ 202.0855, found 202.0845.

6-(6-Hydroxy-1-hexyn-1-yl)-1*H*-purine (10d): Hydrochloric acid (14 mL, 1 M sol.) was added to a solution of compounds **9d** (500 mg, 1.20 mmol) in ethanol (24 mL) and the resulting mixture was stirred at ambient temperature for 6 h, neutralized by the addition of $NaHCO_3$ and the solvents evaporated. The product was isolated by flash chromatography on silica gel eluting with EtOAc/EtOH (1:1); yield 60%, off-white crystals, m.p. 142–143 °C. ^1H NMR (300 MHz, CD_3OD): δ = 8.91 (s, 1 H, 2-H), 8.58 (s, 1 H, 8-H), 3.66 (m, 2 H, CH_2O), 2.70 (m, 2 H, CH_2), 1.9–1.7 (m, 4 H, 2×CH_2) ppm. MS (EI): *m/z* (%) = 216 (54) [M^+], 171 (100). HRMS (EI): calcd. For $C_{11}H_{12}N_4O$ 216.1011, found 216.1009.

(*Z*)-6-Oxiranylmethylene-1*H*-purine (11a): Hydrochloric acid (15 mL, 1 M sol.) was added to a solution of compounds **9a** (270 mg, 0.73 mmol) in ethanol (30 mL) and the resulting mixture was stirred at ambient temperature for 6 h, neutralized by the addition of $NaHCO_3$ and the solvents evaporated. The product was isolated by flash chromatography on silica gel eluting with EtOAc/EtOH (9:1); yield 40%, off-white crystals, m.p. > 300 °C. ^1H NMR (300 MHz, [D_6]DMSO): δ = 8.89 (s, 1 H, 2-H), 8.50 (s, 1 H, 8-H), 7.40 (s, 1 H, CH=), 4.34 (s, 2 H, CH_2) ppm. MS (CI): *m/z* (%) = 175 (100) [M + 1]. HRMS (ESI): calcd. for $C_8H_6N_4O$ + H 175.0619, found 175.0620.

(*E*)-6-[Tetrahydrofuran-2-ylmethylene]-1*H*-purine (11d): (*Z*)-6-[Tetrahydrofuran-2-ylmethylene]-1*H*-purine (**11c**) (91 mg, 0.45 mmol) was dissolved in DMF (10 mL) and stirred at 80 °C for

25 h. The product was isolated by flash chromatography on silica gel eluting with EtOAc/EtOH (1:1); yield 27 mg (30%). off-white crystals m.p. 184–187 °C. ^{1}H NMR (500 MHz, CD_3OD): δ = 8.69 (s, 1 H, 2-H), 8.29 (s, 1 H, 8-H), 6.42 (br. s, 1 H, CH=), 4.28 (t, J = 7.0 Hz, 2 H, CH_2O), 3.38 (dt, J = 7.7 and 1.5 Hz, 2 H, CH_2), 2.20–2.14 (m, 2 H, CH_2) ppm. MS (EI): m/z (%) = 202 (100) [M^+], 161 (35). HRMS (EI): calcd. for $C_{10}H_{10}N_4O$ 202.0855, found 202.08523, calcd. for $C_{10}H_{10}N_4O$ 202.0855.

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