COVALENT ANALOGUES OF DNA BASE-PAIRS AND TRIPLETS VII.+ SYNTHESIS AND CYTOSTATIC ACTIVITY OF BIS(PURIN-6-YL)ACETYLENE AND -DIACETYLENE NUCLEOSIDES

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Dedicated to Professor Miloslav Černý on the occasion of his 75th birthday.

The title bis(purin-6-yl)acetylene and -diacetylene nucleoside derivatives were prepared as covalent base-pair analogues starting from acyl-protected 6-ethynylpurine and 6-iodopurine nucleosides by the Sonogashira cross-coupling or oxidative alkyne-dimerization reactions followed by deprotection. The key starting acyl-protected 6-ethynylpurine nucleosides were prepared by a sequence of cross-coupling reactions of protected 6-halopurine nucleosides with (trimethylsilyl)acetylene followed by a modified desilylation with TBAF in presence of acetic acid. Surprisingly, the acyl-protected nucleosides exhibited significant cytostatic activity higher than the fully deprotected title compounds.

Keywords: Purines; Nucleobases; Nucleosides; Base-pairs; Alkynes; Cross-coupling reactions; Protecting groups; Desilylation; Oxidative dimerization; Cytostatics.

The effect of many clinically used antitumor agents is based on DNA cross-linking[1] or on intercalation[2] into DNA. Numerous models and analogues of Watson–Crick base pairs consisting of annelated[3] or cross-linked[4] purine and pyrimidine heterocycles or even more simple aromatic rings[5,6] have been prepared. Such base-pairs analogues may interact with DNA (e.g. by intercalation); if incorporated into single-stranded DNA, they are complementary to abasic site of a damaged DNA strand; or, alternatively, if incorporated into duplex, they form permanent cross-links.

+ Part VI, see lit.[7]

Recently, we have designed a new group of covalent base-pair or triplet analogues (Chart 1) based on conjugates of two or three purine and/or pyrimidine bases connected with diverse carbon linkages[7]. Such carbon linkers connected to carbon atoms of the heterocycles were expected to be stable towards enzymatic degradation. Transition metal-catalyzed cross-coupling reactions or cyclomerizations were the key synthetic methodology[8] for the construction of the C-C bonds in carbon-linkages. Tris(purin-6-yl)- and tris(pyrimidin-5-yl)benzenes were prepared[9,10] as triplet analogues by cyclotrimerization of 6-ethynylpurines or 6-ethynylpyrimidines. Bis(purin-6-yl)benzenes as well as (purin-6-yl)(pyrimidin-5-yl)benzenes were prepared by double cross-coupling of phenylenebis(stannanes)[11]. Purine dimers linked through positions 6 and 6′ with acetylene, diacetylene, vinylene and ethylene linkers were prepared[12,13] by Sonogashira cross-coupling reactions of 6-ethynylpurines with 6-halopurines or 5-iodopyrimidines or by oxidative dimerizations of ethynylpurines. Similar acetylene couplings were independently used by Matsuda[14] and Marsh[15] and alternative Heck couplings by Sessler[16] for the preparation of other types of nucleobase dimers or covalent dinucleotides that were used for self-assembly or artificial receptor studies.

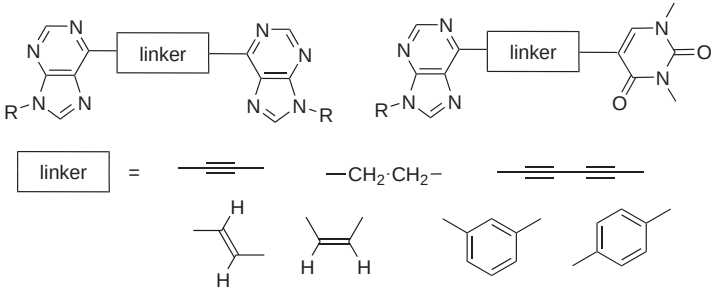


CHART 1

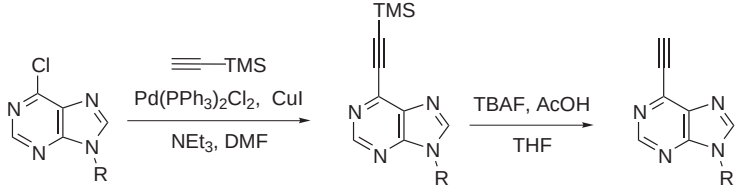
Cytostatic activity screening of the covalent base-pair analogues (Chart 1) revealed a significant antiproliferative effect of some bis(purin-6-yl)-acetylenes and diacetylenes[12], while the partly and fully saturated derivatives, as well as the phenylene-linked analogues were entirely inactive. The activity of these compounds was somewhat surprising since these base-pair analogues were just model compounds bearing simple alkyl substituents in position 9 of purine rings. Apparently, major drawback of these model compounds was their extremely low solubility in water. In order to improve the water solubility and bioavailability, as well as for potential incorporation into nucleic acids, the logical continuation of this project is to prepare

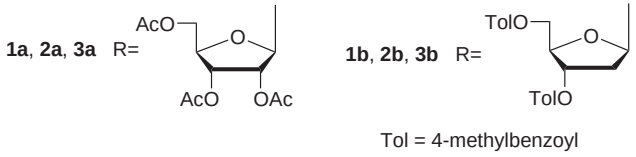
nucleoside derivatives of these compounds. This paper reports on the preparation of nucleosides derivatives of the most active bis(purin-6-yl)-acetylenes and -diacetylenes.

RESULTS AND DISCUSSION

Chemistry

The first task was to prepare suitably protected 6-ethynylpurine nucleosides as key building blocks for the acetylenic couplings and dimerizations. From our previous experiences[17] with purine nucleosides bearing C-substituents in position 6 we knew that the best protecting groups for the glycon part are acyl groups easily cleavable under mild basic conditions (usually catalytic amount of NaOMe in methanol). On the other hand, acidolabile groups are not suitable due to high acidolability of nucleosidic bonds in these compounds. Introduction of the acetylene groups was performed by the standard Sonogashira reactions of acyl-protected 6-chloropurine nucleosides **1a** and **1b** with (trimethylsilyl)acetylene in the presence of $[PdCl_2(PPh_3)_2]$, CuI and Et_3N in DMF to give the 6-[(trimethylsilyl)ethynyl]-purine nucleosides **2a** and **2b** in good yields (Scheme 1). Attempted protodesilylations of **2a** under standard conditions ($TBAF{\cdot}3H_2O$/THF, NH_3/MeOH, K_2CO_3/MeOH) led to concomitant partial de-*O*-acetylation and resulted in complex inseparable mixtures of partly and fully deprotected products. To overcome the problem of basicity/nucleophilicity of the reagent, the deprotection of TMS group was conducted with $TBAF{\cdot}3H_2O$ in

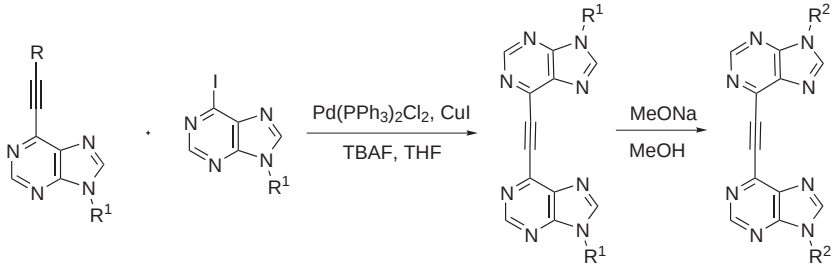




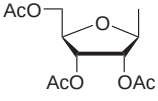
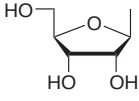
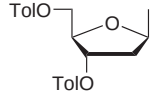
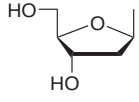
SCHEME 1

the presence of acetic acid (a similar procedure has been used[18] for protodesilylation of *S*-acylbenzenethiols) providing desired per-*O*-acetylated 6-ethynylpurine riboside **1a** in excellent yield of 98%. Analogously, the corresponding per-*O*-(4-methylbenzoyl)-6-ethynylpurine 2-deoxyriboside **3b** was prepared in the same way from **2b** in 97% yield. No transesterification with acetic acid has been observed under these conditions.

For the preparation of bis(purin-6-yl)acetylene dinucleosides the Sonogashira reaction between 6-ethynylpurine **3** and 6-iodopurine nucleosides **4** was the method of choice (Scheme 2). As it was previously demonstrated[13] in 9-alkyl-6-ethynylpurines, standard conditions ([$Pd(PPh_3)_4$], CuI, Et_3N in DMF) do not give the expected bis(purin-6-yl)ethynes but (*E*)-bis-(purin-6-yl)ethenes as a result of reductive addition. For the preparation of the desired bis(purin-6-yl)ethynes, alternative protocol based on the reaction in the presence of TBAF as base in THF was found to be suitable and was also used here. Thus the treatement of equimolar amounts of protected 6-ethynylpurine **3a** and 6-iodopurine ribonucleosides **4a** in the presence of TBAF (2 equivalents) as base, CuI (20%) and [$PdCl_2(PPh_3)_2$] (10%) in THF at



2, 3 **4** **5** **7**

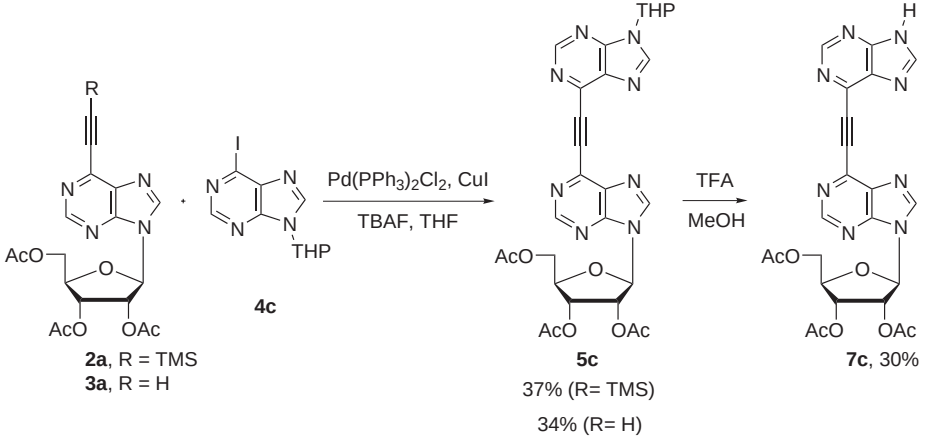
	R^1	Yield of **5**		R^2	Yield of **7**
a		52% (R= H) 42% (R= TMS)	**a**		37%
b		39% (R= H) 42% (R= TMS)	**b**		42%

SCHEME 2

room temperature afforded the desired per-*O*-acetylated acetylenic dinucleoside **5a** in an acceptable yield of 52% (Scheme 2). It should be noted that this reaction failed when 6-chloro derivative **1a** was used instead of 6-iodopurine **4a**, as well as in the absence of CuI.

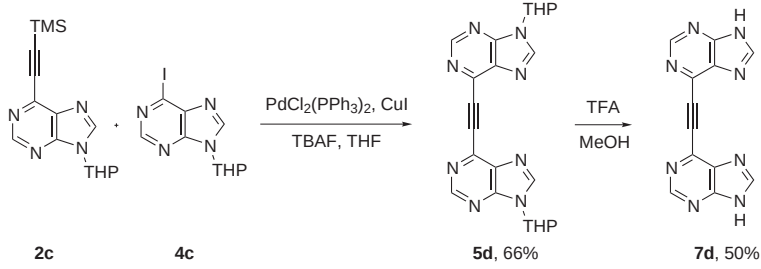
The Sonogashira reaction generally works[19] also directly with (TMS-ethynyl)aromatics instead of terminal acetylenes. The cross-coupling of 6-(TMS-ethynyl)purine derivatives with concomitant desilylation was exemplified by the reaction of TMS-alkyne **2a** with iodopurine riboside **4a** under the same conditions as for terminal acetylenes (TBAF/THF) it afforded the desired compound **5a** in 42% yield. Similarly, protected 2-deoxyribonucleoside dimer **5b** was prepared in 42% yield when 6-iodopurine 2-deoxyriboside **4b** was reacted with 6-(TMS-ethynyl)purine 2-deoxyriboside **2b** and in 39% yield from 6-ethynylpurine **3b**. Though the yields were somewhat lower (presumably due to partial deacylation), this direct coupling saves one deprotection step and thus it is synthetically useful.

An advantage of this Sonogashira coupling approach is the possibility of synthesis of unsymmetrically disubstituted acetylenes. This is important for the synthesis of bis(purin-6-yl)acetylene mononucleosides or orthogonally protected dinucleosides for incorporation into oligonucleotides or duplexes. It was exemplified by the reactions of THP-protected 6-iodopurine **4c** with 6-ethynylpurine **3a** or 6-(TMS-ethynyl)purine ribonucleosides **2a** under above mentioned conditions, which provided unsymmetrical acetylenic mononucleoside **5c** in 34 and 37% yield, respectively (Scheme 3).



SCHEME 3

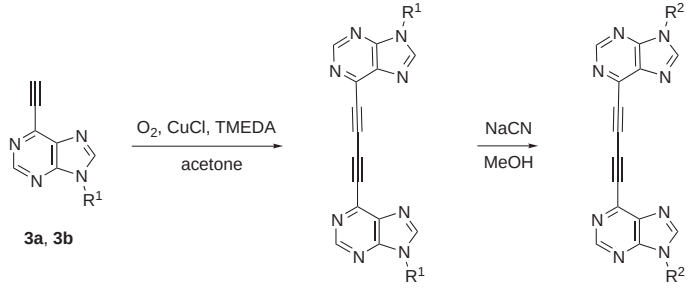
Analogously, we have also prepared symmetrical bis-THP protected bis-(purin-6-yl)acetylene **5d** as potential precursor of corresponding free base by the reaction of 9-THP-6-(TMS-ethynyl)purine **2c** and iodide **4c** in 66% yield (Scheme 4).



2c **4c** **5d**, 66% **7d**, 50%

SCHEME 4

For the preparation of bis(purin-6-yl)diacetylene dinucleoside dimers, oxidative dimerizations of protected 6-ethynylpurine nucleosides **3a** and **3b** were performed (Scheme 5). Thus the addition of 6-ethynyl riboside **3a** to a stirred solution of a catalytic amount of CuCl and TMEDA in acetone in air provided protected diyne dinucleoside dimer **6a** in 68% yield. Corresponding deoxyribonucleoside dimer **6b** was prepared similarly by oxidative homocoupling of **3b** in 82% yield.



3a, **3b** **6a, 6b** **8a**, **8b**

	R^1	Yield of **6**	R^2	Yield of **8**
a	AcO, AcO, OAc	68%	HO, HO, OH	48%
b	TolO, TolO	82%	HO, HO	decomposition

SCHEME 5

As for the deprotection step, all prepared acetylenic compounds were found sensitive to basic conditions used for the cleavage of ester protecting groups (MeONa/MeOH, NEt_3/MeOH, NH_3/EtOH, NaCN/MeOH) and we have observed the formation of insoluble red colored tarry (polymeric) deposits under such conditions. The deprotections should be performed under strictly controlled conditions and the course of the reaction should be carefully monitored and the reaction quenched as soon as the deprotection is completed. Protected ethyne dimers **5a** and **5b** provided free nucleosides **7a** and **7b** on treatment with a catalytic amount of NaOMe in methanol in moderate yields of 37–42% after column chromatography (Scheme 2). Bis-(purin-6-yl)ethyne **7d** was prepared by the action of trifluoroacetic acid on THP-protected derivative **5d** (Scheme 4) and the same acid treatment was also used for partial deprotection of the mixed acetylene mononucleoside **5c** to give **7c** (Scheme 3). The deprotection of diacetylenic dimers **6a** and **6b** was even more problematic than with the acetylene dinucleosides due to more pronounced sensitivity of the diacetylene moiety to basic conditions. The cleavage of the acyl groups was carried out under milder conditions making use of NaCN [20] in MeOH. In the case of the acetyl-protected dinucleoside **6a**, the desired product **8a** was obtained in 42% yield after column chromatography, while in the case of the toluoyl-protected deoxyribonucleoside **6b** the cleavage of the acyl groups was much slower than side reactions of the diacetylene and therefore the free diacetylene deoxyribonucleoside **8b** could not be obtained (Scheme 5).

Biological Activity

The title covalent dinucleosides **5a**, **5b**, **6a**, **6b**, **7a**, **7b** and **8a**, as well as mononucleoside **5c** and base-pair **5d** were tested for their cytostatic activity – inhibition of cell growth of the following cell cultures: mouse leukemia L1210 cells (ATCC CCL 219), human promyelocytic leukemia HL60 cells (ATCC CCL 240), human cervix carcinoma HeLaS3 cells (ATCC CCL 2.2) and human T lymphoblastoid CCRF-CEM cell line (ATCC CCL 119). For experimental details of the cytostatic activity screening see[17a]. Most of the compounds exhibited a cytostatic effect in micromolar range, similarly to the parent alkyl substituted model compounds[12]. Also analogously to the previous results, cytostatic potency of these compounds towards different cell lines decreased in the order CCRF-CEM > HL60 > L1210 > HeLaS3. In general, the more hydrophobic acyl-protected derivatives were surprisingly more active than the hydrophilic free nucleosides. It may be, however, due to their better transport through the cell membrane. The activity of this

class of compounds may be in relation to the recently reported cytostatic activity of simple (arylalkynyl)purines[21].

Conclusions

In conclusion, the bis(purin-6-yl)acetylene and -diacetylene dinucleosides could be prepared in moderate yields by the Sonogashira cross-coupling reactions of acyl-protected 6-ethynyl- or 6-[(trimethylsilyl)ethynyl]purine nucleosides with 6-iodopurine nucleosides or by oxidative dimerization of the former ones. The starting protected 6-ethynylpurine nucleosides prepared by a modified procedure may find applications in some other reactions (cycloadditions, heterocyclizations, etc.). Cleavage of the acyl-protective groups is problematic due to side reactions of the acetylene or diacetylene moiety under basic conditions. Due to the high sensitivity of these systems, incorporations into oligonucleotides does not seem to be realistic. Nevertheless, these compounds, in particular the protected lipophilic ones, display interesting cytostatic activity and therefore further research in this field is desirable.

TABLE I
Cytostatic activity of the title covalent dinucleosides or base-pairs

Compound	IC_{50}, μmol l^{-1} [a]			
	CCRF-CEM	HL60	L1210	HeLa S3
5a	10.9 (±0.90)	–	NA	NA
5b	3.5 (±0.20)	NA	NA	NA
5c	1.6 (±0.16)	12.8 (±1.0)	22.7 (±1.6)	NA
5d	0.42 (±0.028)	2.0 (±0.12)	6.3 (±0.54)	4.6 (±0.30)
6a	1.8 (±0.17)	7.2 (±0.63)	21 (±2.2)	NA
6b	NA	NA	NA	NA
7a	3.0 (±0.21)	3.3 (±0.27)	NA	NA
7b	NA	NA	NA	NA
7c	NA	NA	NA	NA
7d	NA	11.5 (±0.98)	NA	NA
8a	NA	NA	NA	NA

[a] NA, not active (inhibition of cell growth at c = 10 μmol l^{-1} was lower than 20%).

EXPERIMENTAL

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 60 °C/2 kPa. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured at 25 °C on an Autopol IV (Rudolph Research Analytical) polarimeter, $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (^{1}H at 400 MHz, ^{13}C at 100.6 MHz) and on a Bruker Avance (^{1}H at 500 MHz, ^{13}C at 125.8 MHz). Chemical shifts (in ppm, δ-scale) were referenced to TMS as internal standard. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix) or EI ionization. IR spectra were recorded on a Nicolet 750 FT-IR and wavenumbers are given in cm^{-1}. DMF was distilled from P_2O_5, degassed in vacuo and stored over molecular sieves under argon. THF was refluxed with Na and benzophenone under argon and freshly distilled prior to use. Starting 6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine[22], 6-chloro-9-[2-deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]purine[23] and 9-(tetrahydropyran-2-yl)-6-[(trimethylsilyl)ethynyl]purine[10] were prepared by known procedures. Cytostatic activity tests were performed as described in[17a].

9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-6-[(trimethylsilyl)ethynyl]purine (**2a**)

Triethylamine (1 ml) and DMF (4 ml) were added to an argon purged mixture of 6-chloropurine **1a** (413 mg, 1 mmol), CuI (10 mg, 0.05 mmol), $[PdCl_2(PPh_3)_2]$ (14 mg, 0.02 mmol), (trimethylsilyl)acetylene (0.24 ml, 1.7 mmol) and the mixture was stirred at 60 °C for 6 h. Volatiles were evaporated under reduced pressure and the residue was chromatographed on silica (hexane/AcOEt 2:1 then 1:1) affording product **2a** as yellowish oil, which after co-evaporation with diethyl ether forms foam (413 mg, 87%). $[\alpha]_D$ –34.9 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): 0.34 (s, 9 H, CH_3-TMS); 2.08, 2.12, 2.16 (3 × s, 3 × 3 H, CH_3CO); 4.43 (m, 3 H, H-5′, H-4′); 5.67 (ddd, 1 H, *J*(H-3′,H-5′) = 0.4, *J*(H-3′,H-4′) = 4.5, *J*(H-3′,H-2′) = 5.6, H-3′); 5.96 (t, 1 H, *J*(H-2′,H-1′) = 5.4, *J*(H-2′,H-3′) = 5.6, H-2′); 6.24 (d, 1 H, *J*(H-1′,H-2′) = 5.4, H-1′); 8.28 (s, 1 H, H-8); 8.94 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, $CDCl_3$): –0.47 ($(CH_3)_3Si$); 20.31, 20.49 and 20.71 (3 × CH_3); 62.94 (CH_2-5′); 70.56 (CH-3′); 73.01 (CH-2′); 80.48 (CH-4′); 86.44 (CH-1′); 98.14 (-C≡C-TMS); 106.18 (TMS-C≡C-); 134.89 (C-5); 141.78 (C-6); 143.68 (C-8); 151.25 (C-4); 152.75 (C-2); 169.26, 169.50 and 170.22 (C=O). FAB MS, *m/z* (rel.%): 475 (62) [M + H], 243 (33), 217 (100). IR (CCl_4): 1757, 1580, 1251, 1216, 856, 848. For $C_{21}H_{26}N_4O_7Si$ (474.2) calculated: 53.15% C, 5.52% H, 11.81% N; found: 52.88% C, 5.49% H, 11.63% N.

9-[2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-6-[(trimethylsilyl)ethynyl]purine (**2b**)

This compound was prepared from 6-chloropurine **1b** according to the procedure for the preparation of compound **2a** in 83% yield after column chromatography on silica (hexane/AcOEt 2:1). Yellowish foam after co-evaporation with diethyl ether. $[\alpha]_D$ –61.0 (*c* 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): 0.35 (s, 9 H, CH_3-TMS); 2.41 and 2.45 (2 × s, 2 × 3 H, CH_3-Tol); 2.87 (ddd, 1 H, J_{gem} = 14.2, $J_{2'b1'}$ = 5.8, $J_{2'b3'}$ = 2.2, H-2′b); 3.18 (ddd, 1 H, J_{gem} = 14.2, $J_{2'a1'}$ = 8.3, $J_{2'a3'}$ = 6.3, H-2′a); 4.63–4.68 (m, 2 H, H-5′b and H-4′); 4.78 (dd, 1 H, J_{gem} = 13.3, $J_{5'a4'}$ = 5.1, H-5′a); 5.84 (dt, 1 H, $J_{3'2'a}$ = 6.3, $J_{3'4'}$ = 2.2, $J_{3'2'b}$ = 2.2, H-3′); 6.58 (dd, 1 H, $J_{1'2'a}$ = 8.3, $J_{1'2'b}$ = 5.8, H-1′); 7.22 and 7.29 (2 × m, 2 × 2 H, H-*m*-Tol); 7.87 and 7.97 (2 × m,

2 × 2 H, H-*o*-Tol); 8.29 (s, 1 H, H-8); 8.85 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, $CDCl_3$): –0.43 (CH_3-TMS); 21.69 and 21.75 (CH_3-Tol); 37.83 (CH_2-2′); 63.82 (CH_2-5′); 75.03 (CH-3′); 83.23 (CH-4′); 85.01 (CH-1′); 98.28 (-C≡C-TMS); 105.75 (-C≡C-TMS); 126.32 and 126.54 (C-*i*-Tol); 129.29 (CH-*m*-Tol); 129.58 and 129.80 (CH-*o*-Tol); 134.87 (C-5); 141.50 (C-6); 143.62 (CH-8); 144.21 and 144.58 (C-*p*-Tol); 151.17 (C-4); 152.51 (CH-2); 165.92 and 166.10 (CO). IR (CCl_4): 1727, 1578, 1266, 1251, 1100, 856, 847. HR MS (FAB), calculated for $C_{31}H_{33}N_4O_5Si$ [M + H]: 569.2220; found: 569.2245.

9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-6-ethynylpurine (**3a**)

A 1 M solution of TBAF·3H_2O in THF (1 ml, 1 mmol) was dropwise added to a stirred mixture of 6-[(trimethylsilyl)ethynyl]purine nucleoside **2a** (475 mg, 1 mmol), acetic acid (69 µl, 1.2 mmol) in THF (5 ml) at –10 °C. TLC indicated the disappearance of starting TMS derivative **2a** immediately after the addition of TBAF solution. The mixture was diluted with AcOEt (15 ml) and washed with saturated aqueous ammonium chloride solution (3 × 15 ml). The organic phase was dried over $MgSO_4$ and evaporated in vacuo. The residue was passed through a short column of silica (hexane/AcOEt 1:1 then 1:2) affording product **3a** (394 mg, 98%) as yellowish foam after co-evaporation with diethyl ether. $[\alpha]_D$ –29.2 (*c* 0.2, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 2.09, 2.12 and 2.16 (3 × s, 3 × 3 H, CH_3CO); 3.75 (s, 1 H, HC≡C-); 4.39 (dd, 1 H, *J*(H-5′b,H-4′) = 4.0, J_{gem} = 11.9, H-5′b); 4.46 (dd, 1 H, *J*(H5′a,H4′) = 3.3, J_{gem} = 11.9, H-5′a); 4.48 (dt, 1 H, *J*(H-4′,H-5′a) = 3.3, *J*(H-4′,H-5′b) = 4.0, *J*(H-4′,H-3′) = 4.3, H-4′); 5.66 (dd, 1 H, *J*(H-3′,H-4′) = 4.3, *J*(H-3′,H-2′) = 5.6, H-3′); 5.97 (t, 1 H, *J*(H-2′,H-3′) = 5.6, *J*(H-2′,H-1′) = 5.6, H-2′); 6.25 (d, 1 H, *J*(H-1′,H-2′) = 5.6, H-1′); 8.31 (s, 1 H, H-8); 8.97 (s, 1 H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 20.29, 20.45 and 20.66 (3 × CH_3); 62.88 (CH_2-5′); 70.51 (CH-3′); 73.08 (CH-2′); 77.74 (-C≡CH); 80.50 (CH-4′); 86.52 (HC≡C-); 86.59 (CH-1′); 135.52 (C-5); 141.23 (C-6); 143.92 (C-8); 151.22 (C-4); 152.78 (C-2); 169.24, 169.46 and 170.16 (C=O). FAB MS, *m/z* (rel.%): 403 (55) [M + H], 278 (34), 259 (50), 243 (55), 231 (61), 145 (50), 109 (100). IR (KBr): 2113, 1749, 1581, 1232, 1095, 1049. HR MS (FAB), calculated for $C_{18}H_{19}N_4O_7$ [M + H]: 403.1254; found: 403.1261.

9-[2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-6-ethynylpurine (**3b**)

This compound was prepared from 6-[(2-trimethylsilyl)ethynyl]purine nucleoside **2b** according to the procedure for the preparation of compound **3a** in 97% yield. Yellowish foam after co-evaporation with diethyl ether. $[\alpha]_D$ –66.9 (*c* 0.2, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 2.41 and 2.45 (2 × s, 2 × 3 H, CH_3-Tol); 2.88 (ddd, 1 H, J_{gem} = 14.2, $J_{2'b1'}$ = 5.8, $J_{2'b3'}$ = 2.2, H-2′b); 3.18 (ddd, 1 H, J_{gem} = 14.2, $J_{2'a1'}$ = 8.2, $J_{2'a3'}$ = 6.3, H-2′a); 3.72 (s, 1 H, HC≡C); 4.64–4.69 (m, 2 H, H-5′b and H-4′); 4.80 (dd, 1 H, J_{gem} = 13.3, $J_{5'a4'}$ = 5.1, H-5′a); 5.84 (dt, 1 H, $J_{3'2'a}$ = 6.3, $J_{3'4'}$ = 2.5, $J_{3'2'b}$ = 2.2, H-3′); 6.60 (dd, 1 H, $J_{1'2'a}$ = 8.2, $J_{1'2'b}$ = 5.8, H-1′); 7.22 and 7.29 (2 × m, 2 × 2 H, H-*m*-Tol); 7.88 and 7.98 (2 × m, 2 × 2 H, H-*o*-Tol); 8.30 (s, 1 H, H-8); 8.88 (s, 1 H, H-2). ^{13}C NMR (125.8 MHz, $CDCl_3$): 21.68 and 21.74 (CH_3-Tol); 37.91 (CH_2-2′); 63.80 (CH_2-5′); 75.02 (CH-3′); 77.79 (-C≡CH); 83.31 (CH-4′); 85.04 (CH-1′); 86.28 (-C≡CH); 126.30 and 126.51 (C-*i*-Tol); 129.30 (CH-*m*-Tol); 129.56 and 129.81 (CH-*o*-Tol); 135.50 (C-5); 140.92 (C-6); 143.85 (CH-8); 144.26 and 144.61 (C-*p*-Tol); 151.10 (C-4); 152.55 (CH-2); 165.92 and 166.10 (CO). FAB MS, *m/z* (rel.%): 497 (100) [M + H], 433 (40), 303 (42), 289 (77), 263 (44). IR ($CHCl_3$): 2120, 1721, 1583, 1268, 1179, 1102. HR MS (FAB), calculated for $C_{28}H_{25}N_4O_5$ [M + H]: 497.1825; found: 497.1842.

General Procedure for the Preparation of Protected Bis(purin-6-yl)ethynes **5**

A 1 M solution of TBAF·3H_2O in THF (2 ml, 2 mmol) was dropwise added to an argon purged stirred mixture of protected 6-ethynylpurine **3** (1 mmol) or 6-(2-TMS-ethynyl)purine **2** (1 mmol), protected 6-iodopurine **4** (1 mmol), CuI (38 mg, 0.2 mmol), [$PdCl_2(PPh_3)_2$] (70 mg, 0.1 mmol) in THF (6 ml). The mixture was stirred at ambient temperature for 4 h. The solvent was removed in vacuo and the residue was chromatographed on a silica column (AcOEt/hexane 1:1 to 1:0 for **5a**, **5c**, **5d** or AcOEt/hexane 1:1 to 2:1 for compound **5b**).

*Bis[9-(2,3,5-tri-O-acetyl-*β*-D-ribofuranosyl)purin-6-yl]ethyne* (**5a**). Yield 52% from **3a** and **4a** or 42% from **2a** and **4a**. Brownish solid. M.p. 92–93 °C. $[\alpha]_D$ –60.9 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): 2.10, 2.14 and 2.17 (3 × s, 3 × 6 H, CH_3CO); 4.38–4.52 (m, 6 H, H-5′ and H-4′); 5.67 (dd, 2 H, $J_{3'2'}$ = 5.5, $J_{3'4'}$ = 4.3, H-3′); 5.95 (t, 2 H, $J_{2'3'}$ = 5.5, $J_{2'1'}$ = 5.5, H-2′); 6.28 (d, 2 H, $J_{1'2'}$ = 5.5, H-1′); 8.42 (bs, 2 H, H-8); 9.06 (s, 2 H, H-2). ^{13}C NMR (100.6 MHz, $CDCl_3$): 20.34, 20.52 and 20.78 (CH_3); 62.94 (CH_2-5′); 70.58 (CH-3′); 73.01 (CH-2′); 80.61 (CH-4′); 86.54 (CH-1′); 90.95 (C-alkyne); 135.53 (C-5); 140.54 (C-6); 144.65 (CH-8); 151.40 (C-4); 152.94 (CH-2); 169.28, 169.52 and 170.26 (CO). FAB MS, *m/z* (rel.%): 801 (35) [M + Na], 779 (100) [M + H], 521 (86), 325 (66). IR ($CHCl_3$): 1751, 1584, 1228. HR MS (FAB), calculated for $C_{34}H_{35}N_8O_{14}$ [M + H]: 779.2273; found: 779.2288.

*Bis{9-[2-deoxy-3,5-bis-O-(4-methylbenzoyl)-*β*-D-erythro-pentofuranosyl]purin-6-yl}ethyne* (**5b**). Yield 42% from **3b** and **4b** or 39% from **2b** and **4b**. White solid. M.p. 159–160 °C. $[\alpha]_D$ –109.4 (*c* 0.3, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 2.40 and 2.44 (2 × s, 2 × 6 H, CH_3-Tol); 2.90 (ddd, 2 H, J_{gem} = 14.2, $J_{2'b1'}$ = 5.9, $J_{2'b3'}$ = 2.2, H-2′b); 3.20 (ddd, 2 H, J_{gem} = 14.2, $J_{2'a1'}$ = 8.3, $J_{2'a3'}$ = 6.4, H-2′a); 4.65–4.70 (m, 4 H, H-5′b and H-4′); 4.79 (dd, 2 H, J_{gem} = 13.3, $J_{5'a4'}$ = 5.2, H-5′a); 5.85 (ddt, 2 H, $J_{3'2'a}$ = 6.4, $J_{3'4'}$ = 2.5, $J_{3'2'b}$ = 2.2, $J_{3'5'b}$ = 0.5, H-3′); 6.61 (dd, 2 H, $J_{1'2'a}$ = 8.3, $J_{1'2'b}$ = 5.9, H-1′); 7.22 and 7.29 (2 × m, 2 × 4 H, H-*m*-Tol); 7.88 and 7.98 (2 × m, 2 × 4 H, H-*o*-Tol); 8.35 (s, 2 H, H-8); 8.95 (s, 2 H, H-2). ^{13}C NMR (125.8 MHz, $CDCl_3$): 21.64 and 21.70 (CH_3-Tol); 37.89 (CH_2-2′); 63.82 (CH_2-5′); 75.07 (CH-3′); 83.33 (CH-4′); 85.13 (CH-1′); 90.74 (C-alkyne); 126.41 and 126.58 (C-*i*-Tol); 129.29 and 129.30 (CH-*m*-Tol); 129.58 and 129.81 (CH-*o*-Tol); 135.66 (C-5); 140.43 (C-6); 144.21 (CH-8); 144.23 and 144.54 (C-*p*-Tol); 151.37 (C-4); 152.59 (CH-2); 165.90 and 166.11 (CO). FAB MS, *m/z* (rel.%): 989 (78) [M + Na], 967 (100) [M + H], 615 (66). IR ($CHCl_3$): 1721, 1612, 1585, 1269, 1179, 1102. For $C_{54}H_{46}N_8O_{10}$ (966.3) calculated: 67.07% C, 4.79% H, 11.59% N; found: 66.95% C, 4.75% H, 11.43% N.

*1-[9-(Tetrahydropyran-2-yl)purin-6-yl]-2-[(2,3,5-tri-O-acetyl-*β*-D-ribofuranosyl)purin-6-yl]ethyne* (**5c**). Yield 34% from **3a** and **4c** or 37% from **2a** and **4c**. Beige solid. M.p. 109–110 °C. $[\alpha]_D$ –35.6 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): 1.65–1.88 and 2.04–2.30 (m, 6 H, CH_2-THP); 2.10, 2.14 and 2.17 (3 × s, 3 × 3 H, CH_3CO); 3.81 (dt, 1 H, *J* = 11.5 and 2.6, bCH_2-O-THP); 4.20 (ddt, 1 H, *J* = 11.5, 4.0 and 2.3, aCH_2-O-THP); 4.37–4.52 (m, 3 H, H-5′ and H-4′); 5.67 (dd, 1 H, $J_{3'2'}$ = 5.5, $J_{3'4'}$ = 4.3, H-3′); 5.84 (dd, 1 H, *J* = 10.3 and 2.7, CH-O-THP); 5.99 (t, 1 H, $J_{2'3'}$ = 5.5, $J_{2'1'}$ = 5.4, H-2′); 6.28 (d, 1 H, $J_{1'2'}$ = 5.4, H-1′); 8.41 and 8.45 (2 × bs, 2 × 1 H, H-8); 9.04 and 9.06 (2 × s, 2 × 1 H, H-2). ^{13}C NMR (100.6 MHz, $CDCl_3$): 20.35, 20.53 and 20.78 (CH_3); 22.65, 24.78 and 31.80 (CH_2-THP); 62.95 (CH_2-5′); 68.87 (CH_2-O-THP); 70.59 (CH-3′); 73.02 (CH-2′); 80.61 (CH-4′); 82.26 (CH-O-THP); 86.50 (CH-1′); 90.48 and 91.33 (C-alkyne); 135.08 and 135.51 (C-5); 139.97 and 140.74 (C-6); 144.53 and 144.95 (CH-8); 151.09 and 151.38 (C-4); 152.65 and 152.94 (CH-2); 169.28, 169.53 and 170.27 (CO). FAB MS, *m/z* (rel.%): 605 (21) [M + H], 521 (100) [M + H – THP].

IR ($CHCl_3$): 1751, 1585, 1229. HR MS (FAB), calculated for $C_{28}H_{29}N_8O_8$ [M + H]: 605.2108; found: 605.2118.

Bis[9-(tetrahydropyran-2-yl)purin-6-yl]ethyne (**5d**). Yield 66% from **2c** and **4c**. Beige solid. M.p. > 315 °C (dec). ^{1}H NMR (400 MHz, $CDCl_3$): 1.65–1.89 and 2.03–2.23 (m, 12 H, CH_2-THP); 3.81 (dt, 2 H, *J* = 11.6 and 2.6, bCH_2-O-THP); 4.20 (ddt, 2 H, *J* = 11.6, 4.2 and 1.8, aCH_2-O-THP); 5.83 (dd, 2 H, *J* = 10.3 and 2.7, CH-O-THP); 8.40 (s, 2 H, H-8); 9.03 (s, 2 H, H-2). ^{13}C NMR (100.6 MHz, $CDCl_3$): 22.64, 24.77 and 31.77 (CH_2-THP); 68.83 (CH_2-O-THP); 82.17 (CH-O-THP); 90.73 (C-alkyne); 135.11 (C-5); 140.16 (C-6); 143.87 (CH-8); 151.08 (C-4); 152.56 (CH-2). FAB MS, *m/z* (rel.%): 431 (55) [M + H], 371 (24), 347 (100) [M + H – THP], 309 (80). IR ($CHCl_3$): 1585, 1495, 1447, 1334, 1322, 1087, 1046, 990. HR MS (FAB), calculated for $C_{22}H_{23}N_8O_2$ [M + H]: 431.1944; found: 431.1946.

1,4-Bis[9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]butadiyne (**6a**)

The 6-ethynylpurine **3a** (403 mg, 1 mmol) dissolved in acetone (8 ml) was dropwise added to a stirred solution of CuCl (20 mg, 0.20 mmol) and TMEDA (38 µl, 0.25 mmol) in acetone (2 ml). The mixture was stirred in air at room temperature for 1 h. Solvent was evaporated in vacuo and the residue dissolved in AcOEt (20 ml). The organic phase was washed with saturated aqueous ammonium chloride solution (2 × 20 ml), saturated aqueous Na_2EDTA solution (20 ml) and brine (20 ml), dried over anhydrous $MgSO_4$, evaporated in vacuo, and the residue purified by column chromatography on silica (AcOEt) affording dimer **6a** as yellowish foam after co-evaporation with diethyl ether (273 mg, 68%). M.p. 85–86 °C. $[\alpha]_D$ –77.2 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): 2.10, 2.14 and 2.17 (3 × s, 3 × 3 H, CH_3CO); 4.40 (dd, 1 H, *J*(H-5′b,H-4′) = 4.3, J_{gem} = 12.1, H-5′b); 4.46 (dd, 1 H, *J*(H-5′a,H-4′) = 3.2, J_{gem} = 12.1, H-5′a); 4.49 (dt, 1 H, *J*(H-4′,H-5′a) = 3.2, *J*(H-4′,H-5′b) = 4.3, *J*(H-4′,H-3′) = 4.4, H-4′); 5.66 (dd, 1 H, *J*(H-3′,H-4′) = 4.4, *J*(H-3′,H-2′) = 5.5, H-3′); 5.97 (t, 1 H, *J*(H-2′,H-1′) = 5.3, *J*(H-2′,H-3′) = 5.5, H-2′); 6.27 (d, 1 H, *J*(H-1′,H-2′) = 5.3, H-1′); 8.35 (s, 1 H, H-8); 9.00 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, $CDCl_3$): 20.33, 20.48 and 20.72 (CH_3); 62.88 (CH_2-5′); 70.48 (CH-3′); 73.03 (CH-2′); 78.44 (Pur-C≡C-); 80.52 (CH-4′); 80.87 (-C≡C-Pur); 86.54 (CH-1′); 136.18 (C-5); 140.08 (C-6); 144.36 (C-8); 151.41 (C-4); 152.85 (C-2); 169.27, 169.50 and 170.22 (CO). FAB MS, *m/z* (rel.%): 803 (100) [M + H], 663 (16), 545 (28). IR ($CHCl_3$): 2157, 1752, 1577, 1227. HR MS (FAB), calculated for $C_{36}H_{35}N_8O_{14}$ [M + H]: 803.2273; found: 803.2249.

1,4-Bis{9-[2-deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-purin-6-yl}butadiyne (**6b**)

The 6-ethynylpurine **3b** (497 mg, 1 mmol) dissolved in acetone (8 ml) was dropwise added to a stirred solution of CuCl (20 mg, 0.20 mmol) and TMEDA (38 µl, 0.25 mmol) in acetone (2 ml). The mixture was stirred in air at room temperature for 1 h after which the product precipitated as a gel. Saturated aqueous ammonium chloride solution (25 ml) and saturated aqueous Na_2EDTA (25 ml) were added and the resulting mixture was thoroughly shaken. Crude solid product was collected by suction on a Büchner funnel and washed repeatedly with saturated aqueous ammonium chloride solution (3 × 10 ml) and water (3 × 10 ml). The wet solid was dissolved in $CHCl_3$ (50 ml) and the solution was dried over anhydrous $MgSO_4$. After evaporation of $CHCl_3$ the product was passed through a short column of silica (AcOEt) affording product **6b** as brownish solid (408 mg, 82%). M.p. 114–115 °C. $[\alpha]_D$ –132.7 (*c* 0.3,

$CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): 2.42 and 2.45 (2 × s, 2 × 6 H, CH_3-Tol); 2.90 (ddd, 2 H, J_{gem} = 14.3, $J_{2'b1'}$ = 5.9, $J_{2'b3'}$ = 2.3, H-2′b); 3.18 (ddd, 2 H, J_{gem} = 14.3, $J_{2'a1'}$ = 8.1, $J_{2'a3'}$ = 6.3, H-2′a); 4.64–4.71 (m, 4 H, H-5′b and H-4′); 4.80 (dd, 2 H, J_{gem} = 13.2, $J_{5'a4'}$ = 4.9, H-5′a); 5.85 (dt, 2 H, $J_{3'2'a}$ = 6.3, $J_{3'4'}$ = 2.7, $J_{3'2'b}$ = 2.3, H-3′); 6.60 (dd, 2 H, $J_{1'2'a}$ = 8.1, $J_{1'2'b}$ = 5.9, H-1′); 7.22 and 7.29 (2 × m, 2 × 4 H, H-*m*-Tol); 7.92 and 7.98 (2 × m, 2 × 4 H, H-*o*-Tol); 8.34 (s, 2 H, H-8); 8.90 (s, 2 H, H-2). ^{13}C NMR (100.7 MHz, $CDCl_3$): 21.70 and 21.73 (CH_3-Tol); 37.91 (CH_2-2′); 63.76 (CH_2-5′); 75.02 (CH-3′); 78.48 and 80.71 (C-alkyne); 83.39 (CH-4′); 85.15 (CH-1′); 126.30 and 126.46 (C-*i*-Tol); 129.30 (CH-*m*-Tol); 129.55 and 129.80 (CH-*o*-Tol); 136.19 (C-5); 139.81 (C-6); 144.27 (CH-8); 144.31 and 144.60 (C-*p*-Tol); 151.28 (C-4); 152.60 (CH-2); 165.92 and 166.10 (CO). ESI MS, *m/z*: 991 [M + H], 1013 [M + Na]. IR ($CHCl_3$): 2156, 1721, 1612, 1576, 1268, 1179, 1121, 1102.

Bis[9-(β-D-ribofuranosyl)purin-6-yl]ethyne (**7a**)

Compound **5a** (160 mg, 0.21 mmol) in methanol (2 ml) was treated with 1 M methanolic sodium methoxide (40 µl, 0.040 mmol) at room temperature for 1 h. The mixture was evaporated with silica gel and chromatographed on a silica column (AcOEt/MeOH 10:1) affording product **7a** as yellow solid (40 mg, 37%). M.p. 155–156 °C. $[\alpha]_D$ –72.2 (*c* 0.2, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6): 3.61 (ddd, 2 H, J_{gem} = 11.7, $J_{5'bOH}$ = 5.5, $J_{5'b4'}$ = 4.0, H-5′b); 3.72 (dd, 2 H, J_{gem} = 11.7, $J_{5'aOH}$ = 5.5, $J_{5'a4'}$ = 4.1, H-5′a); 4.01 (q, 2 H, $J_{4'5'a}$ = 4.1, $J_{4'3'}$ = 4.0, $J_{4'5'b}$ = 4.0, H-4′); 4.22 (q, 2 H, $J_{3'OH}$ = 5.2, $J_{3'2'}$ = 5.1, $J_{3'4'}$ = 4.0, H-3′); 4.64 (q, 2 H, $J_{2'OH}$ = 5.8, $J_{2'1'}$ = 5.3, $J_{2'3'}$ = 5.1, H-2′); 5.13 (t, 2 H, $J_{OH5'}$ = 5.5); 5.28 (d, 2 H, $J_{OH3'}$ = 5.2); 5.61 (d, 2 H, $J_{OH2'}$ = 5.8); 6.09 (d, 2 H, $J_{1'2'}$ = 5.3, H-1′); 9.03 (s, 2 H, H-8); 9.07 (s, 2 H, H-2). ^{13}C NMR (100.6 MHz, DMSO-d_6): 61.26 (CH_2-5′); 70.31 (CH-3′); 74.07 (CH-2′); 85.91 (CH-4′); 88.11 (CH-1′); 90.24 (C-alkyne); 135.30 (C-5); 138.56 (C-6); 146.88 (CH-8); 152.02 (C-4); 152.51 (CH-2). FAB MS, *m/z* (rel.%): 527 (91) [M + H], 465 (100), 417 (73). IR (KBr): 1590, 1332, 1211, 1099, 1061, 1032. HR MS (FAB), calculated for $C_{22}H_{23}N_8O_8$ [M + H]: 527.1639; found: 527.1650.

Bis[9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purin-6-yl]ethyne (**7b**)

Compound **5b** (245 mg, 0.25 mmol) in methanol (2 ml) and THF (8 ml) was treated with 1 M methanolic sodium methoxide (40 µl, 0.040 mmol) at room temperature for 5 h. The mixture was evaporated with silica gel and chromatographed on silica (AcOEt/MeOH 10:1) affording product **7b** as pink solid (53 mg, 42%). M.p. 153–154 °C. $[\alpha]_D$ –43.2 (*c* 0.2, DMSO). ^{1}H NMR (400 MHz, CD_3OD): 2.54 (ddd, 1 H, J_{gem} = 10.1, $J_{2'b1'}$ = 6.4, $J_{2'b3'}$ = 3.8, H-2′b); 2.90 (dt, 1 H, J_{gem} = 10.1, $J_{2'a1'}$ = 6.8, $J_{2'a3'}$ = 5.8, H-2′a); 3.77 (dd, 1 H, J_{gem} = 12.1, $J_{5'b4'}$ = 4.1, H-5′a); 3.85 (dd, 1 H, J_{gem} = 12.1, $J_{5'a4'}$ = 3.5, H-5′a); 4.07 (q, 1 H, $J_{4'5'b}$ = 4.1, $J_{4'5'a}$ = 3.5, $J_{4'3'}$ = 3.4, H-4′); 4.63 (dt, 1 H, $J_{3'2'a}$ = 5.8, $J_{3'2'b}$ = 3.8, $J_{3'4'}$ = 3.4, H-3′); 6.62 (t, 1 H, $J_{1'2'a}$ = 6.8, $J_{1'2'b}$ = 6.4, H-1′); 8.87 (s, 1 H, H-8); 8.99 (s, 1 H, H-2). ^{13}C NMR (100.6 MHz, CD_3OD): 41.40 (CH_2-2′); 63.12 (CH_2-5′); 72.52 (CH-3′); 86.59 (CH-1′); 89.69 (CH-4′); 91.17 (C≡C); 136.48 (C-5); 140.33 (C-6); 147.96 (CH-8); 152.94 (C-4); 153.35 (CH-2). FAB MS, *m/z* (rel.%): 495 (25) [M + H], 443 (28), 413 (41), 355 (38), 309 (32), 278 (64), 263 (100), 231 (86). IR (KBr): 1584, 1444, 1402, 1328, 1212, 1090, 1062. HR MS (FAB), calculated for $C_{22}H_{23}N_8O_6$ [M + H]: 495.1741; found: 495.1735.

1-(Purin-6-yl)-2-[9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]ethyne (**7c**)

To a solution of **5c** (205 mg, 0.34 mmol) in MeOH (3 ml), trifluoroacetic acid (130 μl, 1.69 mmol) was added. The mixture was stirred at ambient temperature for 1 h, solid $NaHCO_3$ (145 mg) was added and the mixture was stirred for 5 min. The solid was filtered off, washed with methanol and the filtrate was evaporated. The column chromatography of the residue on silica (AcOEt/MeOH) afforded product **7c** as amorphous yellow solid (53 mg, 30%). ^{1}H NMR (500 MHz, CD_3OD): 2.07, 2.09 and 2.16 (3 × s, 3 × 3 H, CH_3CO); 4.42 (dd, 1 H, J_{gem} = 12.1, $J_{5'b4'}$ = 4.8, H-5′b); 4.48 (dd, 1 H, J_{gem} = 12.1, $J_{5'a4'}$ = 3.5, H-5′a); 4.51 (td, 1 H, $J_{4'3'}$ = 4.9, $J_{4'5'b}$ = 4.8, $J_{4'5'a}$ = 3.5, H-4′); 5.77 (t, 1 H, $J_{3'2'}$ = 5.8, $J_{3'4'}$ = 4.9, H-3′); 6.12 (dd, 1 H, $J_{2'3'}$ = 5.8, $J_{2'1'}$ = 4.9, H-2′); 6.40 (d, 1 H, $J_{1'2'}$ = 4.9, H-1′); 8.73 (brs, 1 H, H-8-PurH); 8.82 (s, 1 H, H-8-PurRf); 8.99 (brs, 1 H, H-2-PurH); 9.04 (s, 1 H, H-2-PurRf). ^{13}C NMR (125.8 MHz, CD_3OD): 20.28, 20.44 and 20.66 (CH_3); 64.08 (CH_2-5′); 71.86 (CH-3′); 74.29 (CH-2′); 81.84 (CH-4′); 88.70 (CH-1′); 90.66 and 91.16 (C-alkyne); 136.24 (C-5-PurRf); 140.40 (C-6-PurRf); 148.35 (CH-8-PurRf); 149.41 (CH-8-PurH); 152.89 (C-4-PurRf); 153.62 (CH-2-PurH); 153.88 (CH-2-PurRf); 171.24, 171.40 and 172.22 (CO). Note: Quaternary carbons of free purine moiety are not observable due to tautomerism. CH-8-PurH visible in C,H-HSQC spectrum. IR (KBr): 1745, 1684, 1595, 1211. FAB MS, *m/z* (rel.%): 521 (25) [M + H], 433 (27), 391 (36), 373 (55), 355 (100). HR MS (FAB), calculated for $C_{23}H_{21}N_8O_7$ [M + H]: 521.1533; found: 521.1549.

Bis(purin-6-yl)ethyne (**7d**)

To a solution of **5d** (100 mg, 0.33 mmol) in $CHCl_3$ (2 ml), MeOH (0.25 ml) and TFA (0.25 ml) were added. The mixture was stirred at ambient temperature for 1 h. Precipitated solid was collected by suction and washed with chloroform (5 × 2 ml) affording product **7d** as beige solid (44 mg, 50%). M.p. > 300 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6): 8.77 (s, 2 H, H-8); 9.01 (s, 2 H, H-2). We were not able to record ^{13}C NMR spectrum of compound **7d** due to tautomerism, which results in missing quaternary carbons in ^{13}C NMR spectrum. It could not be observed even using C,H-HMBC experiment due to poor solubility of **7d** in common NMR solvents. EI MS, *m/z* (rel.%): 262 (32) [M^+], 129 (100). IR (KBr): 3196, 3086, 3048, 1599, 1397, 1319. HR MS (EI), calculated for $C_{12}H_6N_8$ [M^+]: 262.0715; found: 262.0706.

1,4-Bis[9-(β-D-ribofuranosyl)purin-6-yl]butadiyne (**8a**)

Dimer **6a** (250 mg, 0.31 mmol) was treated with NaCN (15 mg, 0.31 mmol) in dry MeOH (10 ml) at room temperature for 25 min. The mixture was evaporated with silica gel and chromatographed on silica (AcOEt/MeOH 10:1) affording product **8a** as redish solid (82 mg, 48%). M.p. > 300 °C (dec). $[\alpha]_D$ –51.3 (*c* 0.2, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6): 3.60 (ddd, 2 H, J_{gem} = 12.0, $J_{5'bOH}$ = 5.5, $J_{5'b4'}$ = 4.0, H-5′b); 3.71 (ddd, 2 H, J_{gem} = 12.0, $J_{5'aOH}$ = 5.5, $J_{5'a4'}$ = 5.0, H-5′a); 4.00 (q, 2 H, $J_{4'5'a}$ = 5.0, $J_{4'3'}$ = 4.3, $J_{4'5'b}$ = 4.0, H-4′); 4.21 (q, 2 H, $J_{3'OH}$ = 5.1, $J_{3'2'}$ = 4.6, $J_{3'4'}$ = 4.3, H-3′); 4.62 (q, 2 H, $J_{2'OH}$ = 5.8, $J_{2'1'}$ = 5.3, $J_{2'3'}$ = 4.6, H-2′); 5.16 (t, 2 H, $J_{OH5'}$ = 5.5, 5′-OH); 5.28 (d, 2 H, $J_{OH3'}$ = 5.1, 3′-OH); 5.61 (d, 2 H, $J_{OH2'}$ = 5.8, 2′-OH); 6.08 (d, 2 H, $J_{1'2'}$ = 5.3, H-1′); 9.04 (s, 4 H, H-8 and H-2). ^{13}C NMR (100.6 MHz, DMSO-d_6): 61.21 (CH_2-5′); 70.28 (CH-3′); 74.11 (CH-2′); 78.51 and 79.20 (C-alkyne); 85.82 (CH-4′); 88.15 (CH-1′); 136.19 (C-5); 137.58 (C-6); 147.21 (CH-8); 151.95 (C-4); 152.53 (CH-2). FAB MS, *m/z* (rel.%): 573 (73) [M + Na], 551 (100) [M + H], 482 (42), 460 (64),

419 (67). IR (KBr): 2154, 1578, 1333, 1057. HR MS (FAB), calculated for $C_{24}H_{23}N_8O_8$ [M + H]: 551.1639; found: 551.1660.

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