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Covalent Analogues of DNA Base-Pairs and Triplets. Part 2:† Synthesis and Cytostatic Activity of Bis(purin-6-yl)acetylenes, -diacetylenes and Related Compounds

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Abstract—The title bis(purin-6-yl)acetylenes, -diacetylenes, -ethylenes and -ethanes were prepared as covalent base-pair analogues starting from 6-ethynylpurines and 6-iodopurines by cross-coupling and homo-coupling reactions and hydrogenations. The bis(purin-6-yl)acetylenes and -diacetylenes exhibited significant cytostatic activity in vitro (IC_{50} = 0.4–1.0 μmol/l).

The effect of many clinically used antitumor agents is based on DNA cross-linking[2] or on intercalation[3] to DNA. Numerous models and analogues of Watson–Crick base pairs consisting of annelated[4] or cross-linked[5] purine and pyrimidine heterocycles or even more simple aromatic rings[6,7] have been prepared. Recently, also the first covalently linked analogues of Hoogsteen triplets were prepared[1] in our laboratory. Such base-pairs/triplets 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 to duplex, they form permanent cross-links.

A number of diverse purine–purine conjugates containing linkage (9–9, 8–8, 9–8, 9–7, 9–6 and 6–6) of various lengths, including double- and triple-linked purinophanes, have been prepared[8] in order to study the π–π stacking of purine bases. The purine–purine dimers with a 6,6′-pyridine-2,6-bis(carboxamido) linker have been recently prepared[9] to study its H-bonding properties as potential artificial receptors. Methylene N^6,$N^{6'}$-linked-adenine-adenine dimers are formed by the reaction of carcinogenic formaldehyde with DNA, which was proved by independent synthesis[10] of the products. A variety of other N^6,$N^{6'}$-linked-adenine-adenine dimers, trimers and tetramers with the linkers of various lengths were prepared[11] and exhibited diverse types of biological activity (inhibition of adenosine kinase, ribosomal peptidyltransferase, etc.).

Purines bearing carbon substituents in positions 2 or 6 possess a broad spectrum of biological activity. Thus, 6-methylpurine is highly cytotoxic,[12] while 2-alkynyladenosines are an important class of adenosine receptors agonists.[13] Recently, a cytokinin activity of 6-(arylalkynyl)-, 6-(arylalkenyl)- and 6-(arylalkyl)purines,[14] a cytostatic activity of 6-(trifluoromethyl)purine riboside[15] and of 6-arylpurine ribonucleosides,[16] a corticotropin-releasing hormone antagonist activity of some 2,8,9-trisubstituted-6-arylpurines[17] and an antimycobacterial activity of 9-benzyl-6-arylpurines[18] were also reported.

A combination of the unique structural features of the above mentioned classes of compounds led us to the design of a new group of base-pair analogues **A**–**E** (Chart 1) based on covalent purine–purine conjugates linked through positions 6 and 6′ by carbon linkages. The linkers differ from rigid linear acetylene-linkage, bend *E*- or *Z*-vinylene-linkers to conformationally flexible saturated ethylene-spacer. Such carbon linkers connected to carbon atoms of the heterocycles are expected to be stable towards enzymatic degradation. Apparently, such 'extended' analogues are larger than the parent Watson–Crick base-pairs but on the other hand they could be capable of intercalation within the DNA

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replication fork and thus inhibit its synthesis 'de novo' and cell division. This is a preliminary communication reporting their synthesis and cytostatic activity.

Chemistry

The synthesis of the target compounds was based on standard acetylene chemistry (Scheme 1) using the 9-benzyl-6-ethynylpurine[19] (**1a**) as a key starting compound. Attempted Sonogashira reaction of this compound with 9-benzyl-6-iodopurine (**2a**) in presence of CuI, $Pd(PPh_3)_4$ and Et_3N in DMF did not give the expected bis(purin-6-yl)acetylene **5a** but its partly reduced *E*-ethylene derivative **3a** in 27% yield.[20] The formation of this product could be explained by a reductive addition[21] of the iodopurine **2a** on the acetylene **1a**. Catalytic hydrogenation of the ethylene derivative **3a** on Pd/C gave the fully saturated ethane derivative **4a** in good yield of 80%.

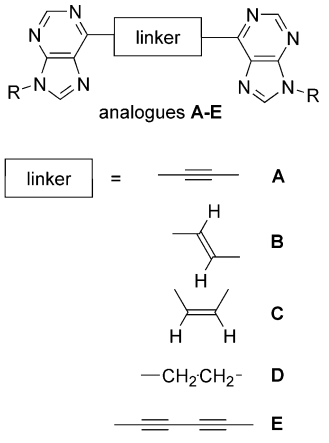
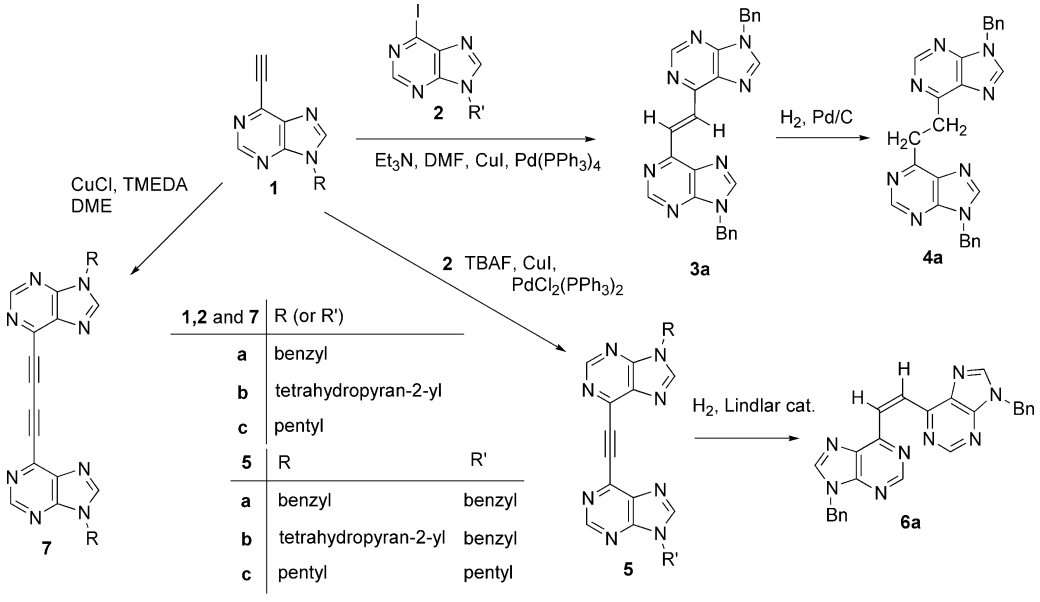


Chart 1.

For the synthesis of the acetylene derivative **5a**, an alternative method based on recently published procedure[22] has been used. The reaction of the 6-iodopurine **2a** with the terminal acetylene **1a** was made in presence of tetrabutylammonium fluoride (TBAF) as base, catalytic amount of CuI and $PdCl_2(PPh_3)_2$ in THF at room temperature to give[23] the desired acetylene **5a** in good yield of 57%. This approach has also been used for the synthesis of other related symmetrically and asymmetrically disubstituted acetylenes **5b** and **5c** differing by the substituent in the positions 9 and 9′ starting from the appropriate iodopurines **2a** and **2c** and ethynylpurines **1b** and **1c**. Catalytic hydrogenation of the acetylene **5a** on Lindlar catalyst afforded the complementary *Z*-ethylene derivative **6a** in a low yield of 11% accompanied by the fully saturated compound **4a** (19%). Oxidative homo-coupling[24] of the terminal acetylenes **1a**–**1c** in presence of CuCl and TMEDA[25] afforded[26] the 1,4-bis(purin-6-yl)diacetylenes **7a**–**7c** in good yields of 50–60%.

Cytostatic activity evaluation

The target base-pair analogues **3**–**7** were tested[27] on their in vitro inhibition of the cell growth in the following cell cultures: mouse leukemia L1210 cells (ATCC CCL 219); murine L929 cells (ATCC CCL 1); human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2); and human T lymphoblastoid CCRF-CEM cell line (ATCC CCL 119). The results (Table 1) show that while the bis(purinyl)acetylenes **5a**–**5c** and -diacetylenes **7a**–**7c** exhibited a significant cytostatic effect in these assays ($IC_{50} = 0.3$–15 μM), the partly and fully saturated derivatives bearing vinylene and ethylene linkers, compounds **3a**, **4a** and **6a**, were entirely inactive. The nature of substituents in the positions 9 of the purine rings has only little effect on the activity (derivatives **a**–**c** in each series of active compounds). Among the assays tested, the T-lymphoblastoid CCRF-CEM and leukemia L1210 cells were the most sensitive to the action of these compounds.



Scheme 1.

Table 1. Cytostatic activity data for compounds **3**–**7**

Compd	IC_{50} (μM)[a]			
	L1210	L929	HeLa S3	CCRF-CEM
FUDR[b]	<0.02 (±0.002)	>25	>25	0.5 (±0.04)
3a	na[c]	na	na	na
4a	na	na	na	na
5a	1.8 (±0.17)	5.3 (±0.6)	na	0.9 (±0.08)
5b	0.9 (±0.08)	1.9 (±0.2)	6.0 (±0.6)	0.32 (±0.07)
5c	1.5 (±0.12)	3.0 (±0.3)	15.0 (±1.8)	0.36 (±0.03)
6a	na	na	na	na
7a	0.37 (±0.03)	6.3 (±0.5)	na	0.43 (±0.03)
7b	0.7 (±0.05)	1.0 (±0.12)	13.3 (±1.3)	0.58 (±0.04)
7c	0.5 (±0.07)	1.3 (±0.11)	15.0 (±1.1)	0.37 (±0.03)

[a]Values are means of four experiments, standard deviation is given in parentheses.
[b]1-(β-D-2-deoxy-*erythro*-pentofuranosyl)-5-fluorouracil.
[c]na, not active (inhibition of cell growth at 10 μM was lower than 20%).

In conclusion, the substituted bis(purin-6-yl)acetylenes and -diacetylenes represent a novel class of anti-neoplastic compounds. They are characterized by a rigid linear (acetylene or diacetylene) linker between the two purine rings. It is not yet clear whether the role of this spacer is just in being a linear linker of certain steric parameters or whether the alkyne forms covalent or non-covalent adducts with the target cell system (DNA or enzyme). Though we suspect that the intercalation or other interaction with DNA might be the mode of action of this class of compounds, these problems remain the subjects for further investigation.

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20. Its *E*-configuration has been assigned based on NOE of its cycloadduct with cyclopentadiene. The details are beyond the scope of this communication and will appear in a forthcoming full-paper.
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23. **Typical procedure**: A degassed 0.165 M solution of TBAF trihydrate in THF (24 mL, 4 mmol) was added dropwise to an argon purged flask containing **1a** (407 mg, 1.67 mmol), **2a** (560 mg, 1.67 mmol), CuI (60 mg, 0.3 mmol), $PdCl_2(PPh_3)_2$ (100 mg, 0.14 mmol) at ambient temperature and the mixture was stirred for 4 h. The solvent was evaporated and the residue was chromatographed on silica gel (200 g, ethyl acetate–light petroleum 1:1) to give the 1,2-bis(9-benzylpurin-6-yl)acetylene (**5a**) (420 mg, 57%), mp 254–257 °C (CH_2Cl_2/heptane), FAB MS, *m*/*z* (rel.%): 443 (5) [M + H], 91 (100). IR ($CHCl_3$): ν = 1583, 1448, 1403, 1330 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): 5.49 (s, 4H, CH_2Ph); 7.30–7.40 (m, 10H, H–arom); 8.15 (s, 2H, H-8); 9.07 (s, 2H, H-2). ^{13}C NMR (100.6 MHz, $CDCl_3$): 47.52 (CH_2Ph); 90.89 (C≡); 127.89, 128.80 and 129.28 (CH–arom.); 134.83 and 135.09 (C-5 and C–*i*-arom); 140.40 (C-6); 145.83 (CH-8); 152.04 (C-4); 152.84 (C-2). EI HR MS, found: 442.1691; $C_{26}H_{18}N_8$ [M] requires: 442.1654.
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26. **Typical procedure**: A solution of CuCl (20 mg, 0.2 mmol), TMEDA (37 μL, 0.25 mmol) in DME (2 mL) was stirred at ambient temperature while a solution of **1a** (244 mg, 1 mmol) in DME (8 mL) was added dropwise. The stirring of the mixture in air atmosphere was continued for 4 h and was allowed to stand overnight. Then the solvent was evaporated and the residue was chromatographed on silica gel (100 g, ethyl acetate–light petroleum 1:1) to give the 1,4-bis(9-benzylpurin-6-yl)butadiyne (**7a**) (137 mg, 59%); mp 215 °C dec. (CH_2Cl_2/heptane); FAB MS, *m*/*z* (rel.%): 467 (8) [M + H], 91 (100). IR ($CHCl_3$): ν = 2157, 1575, 1497, 1491, 1457, 1435 1404, 1330 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): 5.46 (s, 4H, CH_2Ph); 7.26–7.38 (m, 10H, H-arom); 8.12 (s, 2H, H-8); 9.01 (s, 1H, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): 47.55 (CH_2Ph); 78.59 and 80.68 (C≡C); 127.96, 128.83 and 129.27 (CH–arom.); 134.62 and 135.52 (C–arom and C-5); 139.72 (C-6); 145.80 (CH-8); 151.98 (C-4); 152.81 (CH-2). FAB HR MS, found: 467.1700; $C_{28}H_{19}N_8$ [M + H] requires: 467.1732. Anal. calcd for $C_{28}H_{18}N_8$ (466.5): C, 72.10; H, 3.89; N, 24.02; found: C, 71.96; H, 3.82; N, 23.81.

27. For experimental details of the assays see ref 16a.