N^6-SUBSTITUTED ADENOSINES. CYTOKININ AND ANTITUMOR ACTIVITIES

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Received June 3, 2011
Accepted July 21, 2011
Published online November 20, 2011

Dedicated to Professor Antonín Holý on the occasion of his 75th birthday.

A series of N^6-adenosine derivatives were synthesized by alkylation of N^6-acetyl-2′,3′,5′-tri-*O*-acetyladenosine (**1**) with alkyl halides and alcohols. It was shown that propargyl derivative **2a** is a good substrate for copper(I) catalyzed Huisgen [3+2] cycloaddition with azides. This click-reaction can be used for preparation of the libraries of 1,2,3-triazolyl modified adenosines. Biological activities of N^6-adenosines were studied in two plant and six human cancer cell assays. The remarkable parallel between cytokinin and cytotoxic activities was found. The most cytokinin active compounds **3c–3e** at the same time appeared to be the most potent cytotoxic agents.

Keywords: Nucleosides; Adenosine derivatives; Alkylation; Huisgen [3+2] cycloaddition; Cytokinins; Anticancer activity; Click chemistry; Antitumor agents.

Cytokinins are a group of plant hormones and related synthetic bioregulators that exert multiform effects on plant growth and development. Endogenous cytokinins stimulate cell division, photomorphogenesis, chloroplast development, pigment biosynthesis, regulate shoot and root growth and overall plant architecture, and counteract leaf aging and apical dominance (for recent reviews see ref.[1]).

Naturally occurring cytokinins are relatively simple N^6-substituted adenine derivatives. Common natural isoprenoid cytokinins are N^6-iso-

doi:10.1135/cccc2011114

pentenyladenine, zeatin and dihydrozeatin. In zeatin, one methyl group of isopentenyl residue is hydroxylated thus giving rise for two isomeric *trans*- and *cis*-zeatin. Dihydrozeatin has saturated isoprenoid side chain. Aromatic cytokinins such as N^6-benzyladenine (BA) and its *m*- and *o*-hydroxylated derivatives, topolins, were found in some plant species. Kinetin (N^6-furfuryladenine) can be also regarded as aromatic cytokinin. It was initially isolated from autoclaved herring sperm DNA and has been proposed to be a product of decomposition of DNA [2]. Later on the problem of its occurrence was thoroughly reinvestigated. Kinetin has been found in plant cell extracts and as a naturally occurring component of DNA [3]. Natural cytokinin bases are often modified (glucosylated, ribosylated, etc.) at different positions of the purine heterocycle or terminal hydroxyl group of the side chain[1a].

The first step of isoprenoid cytokinin biosynthesis is adenosine phosphate-isopentenyl transferase (EC 2.5.1.27) catalyzed N^6-prenylation of adenosine 5′- (mono-, di-, or tri-) phosphates with 3,3-dimethylallyldiphosphate. Nucleotides are readily dephosphorylated to corresponding nucleoside derivatives. It is believed that two enzymes, namely adenosine nucleosidase (EC 3.2.2.7) and purine nucleoside phosphorylase (EC 2.4.2.1), are involved in interconversion of cytokinin nucleoside to cytokinin base[1]. According to these transformations N^6-substituted adenines and adenosines might have similar cytokinin activities.

In recent years, cytokinins have been used as constituents of cosmetic creams to improve skin structure and reduce the signs of aging. Some cytokinins and their analogs have been shown to have antiproliferative effect on animal tumor cells, with several of them being used already in medicinal practice. Several N^6-substituted adenosines such as N^6-methyladenosine, N^6-isopentenyladenosine and some others were isolated from tRNA [4]. N^6-Isopentenyladenosine and its analogs have been recently found to possess profound anticancer activity[5].

These important biological properties of cytokinins have stimulated the search for convenient methods of synthesis of these compounds and their analogs. For the most synthetic strategies 9-N position of purine moiety should be protected. Naturally occurring nucleosides are convenient synthetic precursors for cytokinins and their analogs as soon as 9-N position is already protected. The following general approaches for preparation of N^6-alkylated adenosines could be found in the literature: (i) 1-N-Alkylation of adenosine with subsequent Dimroth rearrangement in the basic media[6], (ii) one-pot aminations of inosine[7], (iii) nucleophilic substitution of halogen in 6-position with amine[8], (iv) reduction of corresponding acyl

derivatives with $LiAH_4$[9], (v) selective N^6-alkylation of *N*^{6}-acyladenosine derivatives either with alkyl halides under phase-transfer catalysis conditions or with alcohols utilizing Mitsunobu protocol[10].

In spite of a number of available methods for the preparation of N^6-substituted adenosines there is still a room for the development of simple and reliable methods for the synthesis of this group of important natural compounds. All methods have their limitations. Methods (i) and (ii) do not require a protection of hydroxyl groups of the sugar moiety. But this synthetic advantage sometimes turns to be a disadvantage for product isolation. It is well-known that free nucleosides have spare solubility in common solvents, which limits the usage of chromatography for their purification. The same is true for methods (iii) and (iv). Besides, the preparation of starting compounds requires intermediate protection of hydroxyl groups.

Base-promoted alkylation of adenosine derivatives has been less thoroughly studied. Till now only one example (v) can be found in the literature utilizing a series of *N*^{6}-acyl-2′,3′,5′-tri-*O*-TBDMS adenosine species which were alkylated with activated alkyl halides under phase-transfer catalysis conditions. The N^6/N^1-selectivity depends strikingly on the nature of *N*^{6}-acyl group, and with the acetyl group only N^6-alkylation is observed[10a]. The disadvantage of the protocol is the necessity of TBDMS protection of ribose hydroxyl groups. This high molecular weight protective group interferes with the atom economy strategy.

In some cases the starting amines and bromides which are needed for the synthesis of N^6-substituted derivatives are not commercially available so it will be of interest to develop the synthetic scheme utilizing alcohols. In our research we have been focused on developing of a cheap and simple substrate for regioselective N^6-alkylation of adenosine not only with bromides but also with alcohols under Mitsunobu reaction conditions[11].

In our previous publication[12], we reported on the preparation of *N*^{6}-acetyl-2′,3′,5′-tri-*O*-acetyladenosine (**1**) which is a versatile starting compound for regioselective N^6-alkylation under base-promoted and Mitsunobu conditions. In the present work we give additional evidence of synthetic importance of this substrate. It was used for the synthesis of intermediate propargyl derivative **2a** which was successively used in Huisgen [3+2] cycloaddition with azides to give a series of yet unknown triazolyl analogs of aromatic cytokinins. Cytokinin and anticancer activity of N^6-substituted adenosines were studied.

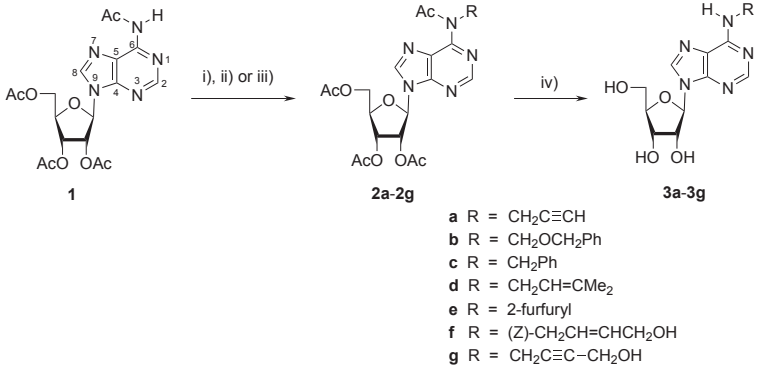
Chemistry

Adenine molecule has five nitrogen atoms which can be considered as possible sites for electrophilic attack. Position of its alkylation at different conditions has been unambiguously assigned. Strikingly no products of alkylation of exocyclic NH_2 group were detected at any conditions. In base free conditions at elevated temperature adenine is alkylated at position 3 [13], and the resulting product is successively alkylated at position 7 [14]. When strong bases are used, the site of alkylation of adenine changes from 3 to 9 [15]. Adenosine, naturally protected at position 9 with ribose moiety, in base free conditions is alkylated selectively at position N1 [16]. To achieve selective N^6-alkylation NH_2-group of adenosine should be acylated[10].

For the purpose of selective preparation of N^6-adenosine derivatives we developed a method of synthesis of N^6-acetyl-2′,3′,5′-tri-*O*-acetyladenosine (**1**)[12]. Tetraacetate **1** can be alkylated at ambient temperature under traditional base assisted or Mitsunobu conditions (Scheme 1). To promote reaction of **1** with alkyl halides 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; method *A*) or K_2CO_3 (method *B*) were used as bases. DBU is a sterically hindered strong base with pK_a 11.6 [17] soluble in most organic solvents that allows to conduct alkylation reactions homogeneously. This base is widely used for the N^9-alkylation of adenine instead of the adenine sodium salt[14]. The examples of both methods are given for the synthesis of propargyl derivative **2a** with high yields 89% (method *A*) and 85% (method *B*). Applying this approach, new adenosine derivative **2b** was synthesized with the yield 70%. Interestingly, the stability of *N*,*O*-acetal system was rather high to allow deacetylation in methanolic ammonia solution.

Tetraacetate **1** is also a useful substrate for Mitsunobu alkylation[12]. The Mitsunobu reaction has become a very popular mild chemical transformation, occurring under essentially neutral conditions. In this reaction alcohol is activated and then coupled to nucleophile[11]. We found that tetraacetate **1** can be alkylated under Mitsunobu conditions with retention of regioselectivity at N^6-position. Thus reacting **1** with propargyl alcohol under activation with Ph_3P and diethyl diazadicarboxylate (DEAD) gives only one product (TLC) with ^1H NMR spectra identical to the above prepared compound **2a**. Two rounds of the Mitsunobu reaction required to consume all starting compound **1**. Scrupulosity and patience was necessary to isolate pure **2a** from Ph_3PO and $(NHCOOEt)_2$ with the yield 74% applying column chromatography (3 times with different eluents). Though poor atom economy is the main drawback of Mitsunobu reaction, nevertheless, when alkyl halides are not available Mitsunobu protocol utilizing parent al-

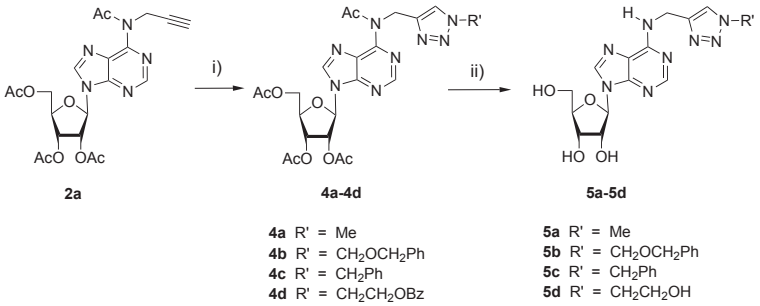
cohols has no alternatives. Its priority is evident in case of *cis*-2-butene-1,4-diol which can be used directly for preparation of **2f** without protection of one hydroxyl group. Though the intermediate compound **2f** was not possible to isolate in pure form, ammonolysis of partially purified substance gave **3f** in 52% yield based on starting compound **1**.



SCHEME 1

Synthesis of N^6-alkyladenosines. Reagents and conditions: i) Method *A*: RX, DBU, MeCN, 20 °C, 16 h; ii) Method *B*: RX, K_2CO_3, DMF, 20 °C, 16 h; iii) Method *C*: ROH, Ph_3P, DEAD, THF, 20 °C, 20 h; iv) 7 M NH_3 in MeOH, 48 h, 20 °C

The propargyl derivative **2a** was used for the synthesis of triazolyl adenosines **5a–5d** (Scheme 2). Thus **2a** was reacted with a series of azides in Huisgen [3+2] cycloaddition reaction catalyzed with CuCl in MeCN to give intermediate **4a–4d**. Corresponding azides were prepared *in situ* starting with appropriate alkylhalides. The click reaction is carried out at ambient temperature to afford **4a–4d** in good to high yields. It is noteworthy to



SCHEME 2

Synthesis of N^6-triazolylmethyl adenosine analogs. Reagents and conditions: i) alkylazide, CuCl, MeCN, 20 h, 20 °C; ii) 7 M NH_3 in MeOH, 48 h, 20 °C

mention that under these conditions the reaction of **2a** with NaN_3 gave very polar product(s) of unknown structure. Nucleosides **5a–5d** were prepared by standard ammonolysis of **4a–4d**.

Biological Results

Cytokinin Activity

The biological activity of synthesized compounds was tested using two plant assay systems. One system relies on seedlings of transgenic P_{ARR5}:*GUS* Arabidopsis expressing reporter gene *GUS* under control of the cytokinin-dependent promoter of the *ARR5* gene. The transcription of transgenic construct was shown to be sensitive and specific toward cytokinins[18]. Another assay system is based on Amaranthus seedlings which quickly respond to cytokinin by accumulation of the red pigment amaranthin[19]. In both systems N^6-benzyladenine (BA) was used as a reference cytokinin; all compounds were tested at the optimal concentration 5 μM. The results are presented in Table I and given in percents of activity of BA.

TABLE I
The cytokinin activity of N^6-adenosine derivatives **3**, **5** and **6** in per cent of BA activity

Compound	GUS test in % to BA	Amaranthin test in % to BA
3a	21.6 ± 0.04	22.1 ± 1.8
3b	37.2 ± 4.2	57.2 ± 5.2
3c	91.6 ± 24.3	98.9 ± 12.3
3d	159.6 ± 20.1	94.4 ± 1.8
3e	89.2 ± 8.5	65.6 ± 3.5
3f	59.7 ± 17.5	37.9 ± 3.5
3g	41.8 ± 1.4	50.5 ± 3.5
5a	9.2 ± 1.6	24.9 ± 6.0
5b	13.5 ± 3.5	32.8 ± 0.4
5c	10.5 ± 6.7	17.4 ± 1.1
5d	9.5 ± 0.04	27.7 ± 5.2
6	78.7 ± 19.6	76.8 ± 8.8
Without treatment	22.8 ± 9.9	26.4 ± 3.5

Results obtained with two assay systems were in good accordance. Synthesized compounds **3c**, **3d** and **3e** have shown high activity close to level of BA (Table I). Compound **3f** harboring *cis*-hydroxylated olefinic side chain as well as compound **3g** with triple bond in the aliphatic chain displayed much less activity. Compound **3a** with shortened side chain had no activity at all (control level). The same applies to a group of related compounds **5a–5d** harboring complex side chain with the 1,2,3-triazolyl ring. All these compounds were lacking cytokinin activity. O^6-Benzylinosine (**6**) with N^6 nitrogen replaced with oxygen in the BA and compound **3b** with extended distance between phenyl group and N^6-position showed some activity.

Thus, assay data fully confirmed the biological activity of ribosides **3c**, **3d** and **3f** derived from the known cytokinins.

Anticancer Activity

N^6-Isopentyladenosine derivatives are known to inhibit DNA synthesis, to cause cell-cycle arrest and to induce apoptosis. To continue these studies, concerning the relation between structure and biological activities we have examined the cytotoxic and apoptosis inducing activities of eight N^6-isopentyladenosine derivatives modified in N^6-position. The cytotoxic effect of N^6-isopentyladenosine derivatives was assessed by MTT-test in T-cell leukemia, melanoma, lung, ovarian and breast cancer cells (Table II). We have

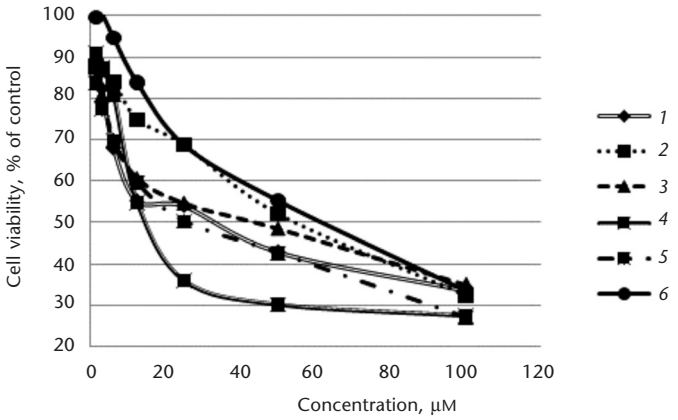


FIG. 1
The cytotoxic effect of **3e** in Jurkat cells (*1*), melanoma Mel-Kor cells (*2*), ovarian cancer cells SKOV-3 (*3*), breast cancer cells MCF-7 (*4*), lung cancer cells A549 (*5*), endothelial cell SVEC-4-10 (*6*)

also examined the ability of these compounds to influence the growth of mouse endothelial cells line SVEC-4-10. Compounds **3c–3e** at the concentration 100 μM dramatically reduced the viability of all tested cells. The data obtained indicate that the most cytotoxic effect was observed in cells of epithelial origin (lung, ovarian and breast carcinoma) (Fig. 1).

TABLE II
The cytotoxic effect of N^6-isopentyladenosine derivatives in cancer cells

Compound	IC_{50}, μM					
	SVEC-4-10	Jurkat	Mel Kor	SKOV-3	MCF-7	A549
3a	85.8 ± 3.1	92.6 ± 27.9	73.3 ± 1.19	75.8 ± 12.1	47.8 ± 11.8	18.5 ± 7.7
3c	49.7 ± 14.3	30.7 ± 6.2	96.1 ± 39.1	22.4 ± 10.9	15.1 ± 2.9	30.4 ± 15.4
3d	44.8 ± 8.1	39.1 ± 8.1	52.5 ± 11.8	28.2 ± 5.6	13.8 ± 3.2	13.6 ± 5.2
3e	48.0 ± 11.1	41.3 ± 26.8	57.4 ± 3.13	28.4 ± 6.2	19.5 ± 2.6	10.5 ± 3.7
3f	45.8 ± 4.0	128.3 ± 85.4	69.1 ± 9.8	77.9 ± 10.5	45.1 ± 9.3	65.7 ± 22.2
3g	139.2 ± 59.6	>500	87.0 ± 15.1	86.2 ± 1.76	383.4 ± 88.0	112.8 ± 31.3
5a	136.8 ± 51.1	383.3 ± 72.3	118.1 ± 35.7	168.0 ± 22.4	483.2 ± 149.4	255.5 ± 125.2
5d	140.5 ± 36.7	159.4 ± 66.5	213.6 ± 100.7	198.5 ± 48.5	236.3 ± 15.1	294.0 ± 93.2
Gremcitabine	nd	nd	nd	21.0 ± 7.3	15.7 ± 4.2	7.5 ± 3.8

[a] nd – Not determined.

TABLE III
The apoptosis induction by N^6-adenosine derivatives (100 and 25 μM) in Jurkat cells

Compound	% of apoptotic cells	
	100 μM	25 μM
3a	24.94 ± 1	21.9 ± 0.4
3c	72.4 ± 1	43.4 ± 4.8
3d	69.9 ± 0.8	45.9 ± 2.3
3e	61.6 ± 4.7	40.6 ± 1.2
3f	21.7 ± 2.7	22.1 ± 0.6
3g	9.8 ± 1.2	8.5 ± 0.6
5a	8.4 ± 1.6	8.3 ± 0.9
5d	8.2 ± 1.7	9.3 ± 1.6

Gemcitabine (2′,2′-difluoro-2′-deoxycytidine) was used as a control anticancer agent. In our experiments IC_{50} of Gemcitabine was 15.7 ± 4.2 for MCF-7 cells, 21.0 ± 7.3 for SKOV-3 cells and 7.5 ± 3.8 for A549.

We have also studied the ability of these compounds to induce apoptosis in Jurkat cells (Table III). Among eight tested compounds, only **3c**, **3d** and **3e** dose-dependently induced apoptosis in cancer cells. Low concentration (12.5 μM) of these compounds did not induce apoptosis in Jurkat cells. However, when cells were incubated with high concentrations (above 100 μM) for 48 h about 70% apoptotic cells were estimated (Fig. 2). The appearance of apoptotic bodies and chromatin condensation in MCF-7 cells was confirmed by immunocytochemistry staining with Hoechst 33258 (not shown).

Our data indicate that the cytotoxic effect was optimal for compounds **3c**, **3d** and **3e**. However, this activity was highest in cancer cells of epithelial origin. The similar cytotoxic effect of **3d** was observed in T24 bladder cancer cells and other epithelial origin cells[8e]. Interestingly, compounds **3c**, **3d** and **3e** also displays cytotoxic effect on endothelic SVEC-4-10 cells thus, giving for the first time a piece of evidence that they may be involved in tumor angiogenesis.

Recently it was shown that N^6-isopentyladenosine derivatives activate caspase-3 and -7 at the concentration of 100 μM in A549 cells[20]. Therefore, we have also studied the ability of these compounds to induce apoptosis. Flow cytometry analysis showed high level of apoptotic cells. This was confirmed by appearance of apoptotic bodies and chromatin condensation on

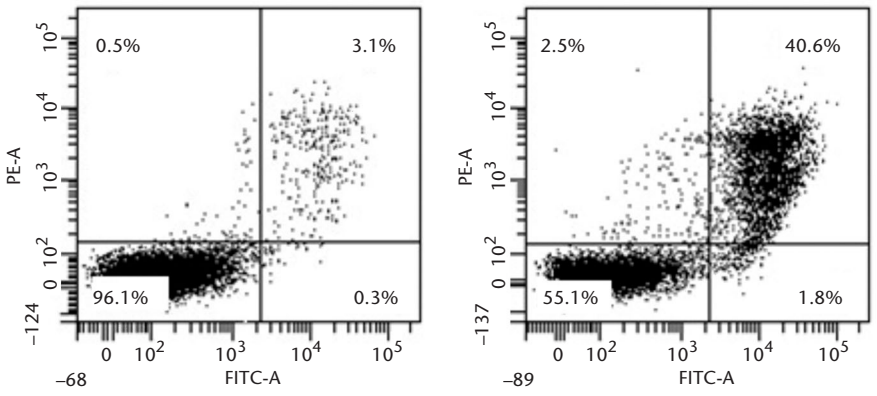


FIG. 2
The apoptosis induction in Jurkat cells by treatment with 25 μM of **3e** (right) for 48 h versus control (left) as observed with fluorescently labeled Annexin V-FITC (FITC-A) and propidium iodide (PI-A)

slides stained by Hoechst 33258. We conclude that **3c**, **3d** and **3e** induced cell death mainly by apoptosis.

However, despite the fact that the cytotoxic activity of **3c**, **3d** and **3e** was the highest among the tested derivatives, it was weaker than that of Gemcitabine (nucleoside analog), the widely used anticancer drug for treatment of patients with epithelial cancer[21].

The better understanding of mechanisms of N^6-isopentyladenosine derivatives action will help to develop a new potential class of anticancer drugs.

In conclusion, we have shown N^6-acetyl-2′,3′,5′-tri-*O*-acetyladenosine (**1**) to be a versatile starting compound for the synthesis of N^6-substituted adenosines. This compound can be alkylated either by alkyl halides in base promoted conditions or by alcohols in Mitsunobu reactions. N^6-Propargyl derivative **2a** is a useful starting compound for modification of side chain in Huisgen [3+2] cycloaddition. Cytokinin and anticancer activity of a series of compounds has been tested. In all tests the highest activity revealed N^6-adenosine derivatives modified with benzyl (**3c**), isopentenyl (**3d**) and furfuryl (**3e**) groups. Thus, the remarkable parallel between cytokinin and cytotoxic activities was found.

EXPERIMENTAL

Biological Materials and Methods

Cytokinin activity assays were performed using model plant systems with Amaranthus (*Amaranthus caudatus* L.)[19] and transgenic P_{ARR5}*:GUS* Arabidopsis (*Arabidopsis thaliana* L.) seedlings[18]. Both assay systems are specifically sensitive to cytokinins and respond to hormone application in few hours, making possible quantitative determinations. For a positive control, BA was applied.

Cell lines. T-cell leukemia Jurkat, lung cancer A549, breast cancer MCF-7, ovarian cancer SKOV-3 cell lines were obtained from ATCC and maintained in RPMI-1640 media (ICN Pharmaceuticals Inc, USA) supplemented with 10% fetal calf serum (ICN Pharmaceuticals Inc, USA), 2 mM glutamine (ICN Pharmaceuticals Inc, USA) and 100 U/ml penicillin/streptomycin (ICN Pharmaceuticals Inc, USA). Melanoma cell line Mel Kor was isolated from surgical species of patients with disseminated melanoma and cultured in RPMI-1640 complete medium[22]. Mouse endothelial cell line SVEC-4-10 was kind gift of Dr Grigorian[23]. The SVEC-4-10 cells were cultured in DMEM (ICN Pharmaceuticals Inc, USA) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin/streptomycin. All experiments were performed with 75–80% confluent culture.

All tested compounds were dissolved in DMSO (Sigma–Aldrich, USA) and used at final concentration from 1.6 to 100 μM. Clinical grade Gemcitabine (Gemzar, Eli Lilly, Sesto Fiorentino, FI, Italy) were used as a control.

MTT assay. The cytotoxic potency of compounds was determined in a formazan conversion assay (MTT-test). Cells (5×10^3 in 190 μl of culture medium) were plated into a 96-well plate (Costar–Corning, USA) and treated with 0.1% DMSO (vehicle control) or with increas-

ing concentrations of tested compounds for 48 h. After the completion of drug exposure, 50 μg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma–Aldrich, USA) were added into each well for additional 2 h. Formazan was dissolved in DMSO, and the absorbance at λ = 540 nm was measured. Cell viability at a given drug concentration was calculated as the percentage of absorbance in wells with drug-treated cells to that of vehicle control cells (100%). The IC_{50} (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell viability by 50%.

Flow cytometry analysis. For quantitative estimation of the number of apoptotic cells, the Annexin V-FITC staining was performed. 6×10^4 cells/ml were incubated with different concentrations of tested compounds for 48 h. ApoTarget Annexin V-FITC Apoptosis kit (Invitrogen, USA) was used for identification of apoptotic cells. The cells were analyzed by flow cytometry FACSCanto II (Becton Dickinson, USA) using FACSDiva software package.

Statistical analysis. All data on biological activity are averages of three independent experiments made in triplicates. Error bars shown in the figures represent SEM. Statistically comparisons were carried out using two-tailed Student's *t* test for comparison of means. *P* values less than 0.05 were accepted as statistically significant.

Chemical Synthesis

The solvents and materials of reagent grade were used without additional purification. Column chromatography was performed on silica gel (Kieselgel 60 Merck, 0.063–0.200 mm). The solvent system used was a gradient of EtOH in CH_2Cl_2 increasing in polarity from CH_2Cl_2 to CH_2Cl_2–EtOH 9:1. TLC was performed on Alugram SIL G/UV254 (Macherey–Nagel) with UV visualization. Melting points were determined on a Electrothermal apparatus and are uncorrected. ^1H and ^{13}C (with complete proton decoupling) NMR spectra were recorded on Bruker AMX 400 NMR instrument. Chemical shifts (δ-scale) in ppm were measured relative to the residual solvent signals as internal standards ($CDCl_3$, ^1H: 7.26 ppm, ^{13}C: 77.1 ppm; DMSO-d_6, ^1H: 2.50 ppm, ^{13}C: 39.5 ppm). Spin–spin coupling constants (*J*) are given in Hz. Double resonance technique was applied for assigning the resonances. UV spectra were recorded on a Cary300UV/VIS spectrophotometer (Varian). LC-MS analysis was performed on Surveyor MSQ instrument (Thermo Finnigan, USA), operating in APCI (atmospheric pressure chemical ionization) mode with detection of positive and negative ions, and equipped with Onyx Monollithic C18 25 × 4.6 mm Part No. CHO-7645 column. The eluent was 0.1% HCOOH in water with a gradient of solution in MeCN. Chromatographic peaks were detected simultaneously with ELSD (evaporative light scattering detector), PAD (photodiode array detector), and TIC (total ion current) detector. In all cases, only one peak was revealed and the chromatographic purity of compounds was more than 99%. N^6-Acetyl-2′,3′,5′-tri-*O*-acetyladenosine (**1**), N^6-benzyladenosine (**3c**), N^6-isopentenyladenosine (**3d**), N^6-furfuryladenosine (**3e**) and N^6-(4-hydroxy-2-butynyl)adenosine (**3g**) were prepared according to ref.[12].

N^6-Acetyl-N^6-(2-propynyl)-2′,3′,5′-tri-*O*-acetyladenosine (2a)

Method A. To a stirred solution of **1** (500 mg, 1.15 mmol) and DBU (0.34 ml, 2.3 mmol) in 5 ml of MeCN in one portion $BrCH_2C{\equiv}CH$ (0.18 ml of 80% solution in toluene, 1.7 mmol) was added at room temperature. The reaction was monitored by TLC. After 20 h, the reaction mixture was diluted with AcOEt and the solution was washed successively with 20 ml of 0.1 M HCl and brine (3 × 20 ml). The organic layer was dried over Na_2SO_4 and evaporated.

Column chromatography of the residue eluting with a gradient of EtOH in CH_2Cl_2 (1:100→1:25) gave 484 mg (89%, foam) of **2a**.

Method B. To a stirred mixture of **1** (500 mg, 1.15 mmol), K_2CO_3 (0.32 g, 2.3 mmol) in 5 ml of DMF in one portion $BrCH_2C{\equiv}CH$ (0.15 ml of 80% solution in toluene, 1.4 mmol) was added at room temperature. The reaction was monitored by TLC. The mixture was stirred at ambient temperature for 20 h and then it was diluted with AcOEt. The mixture was washed with brine (3 × 20 ml). The organic extract was dried over Na_2SO_4 and evaporated. The residue was applied to column chromatography on silica gel to give 463 mg (85%, foam) of **2a**.

Method C. A mixture of **1** (500 mg, 1.15 mmol), Ph_3P (453 mg, 1.72 mmol) and propargyl alcohol (0.1 ml, 1.72 mmol) in 5 ml of THF was stirred at room temperature until homogeneuos solution was formed. DEAD (0.27 ml, 1.72 mmol) was added in one portion. The reaction was monitored by TLC. After 20 h, the second addition of reagents (Ph_3P, propargyl alcohol and DEAD) in indicated quantities was made to achieve complete conversion of **1**. After 4–5 h, the reaction mixture was evaporated and the residue was applied to column chromatography. Three column chromatography procedures were performed to isolate pure compound: 1) elution with AcOEt, 2) elution with a gradient $CHCl_3$–EtOH 100:1→30:1, and 3) elution with a gradient CH_2Cl_2–AcOEt 4:1→CH_2Cl_2–AcOEt 2:1. The yield of **2a** was 405 mg (74%, foam). R_F 0.42 ($CHCl_3$–EtOH 25:1). ^{1}H NMR ($CDCl_3$): 2.10 (s, 3 H, AcO), 2.12 (t, 1 H, J = 2.5, HC≡C), 2.13 (s, 3 H, AcO), 2.16 (s, 3 H, AcO), 2.39 (s, 3 H, AcN), 4.40 (dd, 1 H, $J_{5'b,5'a}$ = –12.9, $J_{5'b,4'}$ = 5.2, H-5′b), 4.47 (dd, 1 H, $J_{5'a,5'b}$ = –12.9, $J_{5'a,4'}$ = 3.1, H-5′a), 4.48 (ddd, 1 H, $J_{4',5'b}$ = 5.2, $J_{4',5'a}$ = 3.1, $J_{4',3'}$ = 4.8, H-4′), 5.10 (d, 2 H, J = 2.5, CH_2N), 5.68 (dd, 1 H, $J_{3',4'}$ = 4.8, $J_{3',2'}$ = 5.6, H-3′), 5.97 (dd, 1 H, $J_{2',3'}$ = 5.6, $J_{2',1'}$ = 5.0, H-2′), 6.25 (d, 1 H, $J_{1',2'}$ = 5.0, H-1′), 8.22 (s, 1 H, H-2), 8.82 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 20.44 (AcO), 20.54 (AcO), 20.78 (AcO), 24.46 (AcN), 36.46 (N^6-CH_2), 63.09 (C-5′), 70.54 (C-3′), 71.64 (H-C≡), 73.19 (C-2′), 77.42 (C≡), 80.45 (C-4′), 86.86 (C-1′), 126.97 (C-5), 142.27 (C-8), 152.27 (C-2), 152.60 (C-4), 152.69 (C-6), 169.41 (CO), 169.59 (CO), 170.30 (CO), 170.95 (CO). MS (APCI): m/z [M + H$^+$] calculated for $C_{21}H_{24}N_5O_8$: 474.16, found 474.27.

N^6-Acetyl-N^6-benzyloxymethyl-2′,3′,5′-tri-*O*-acetyladenosine (**2b**)

The compound was prepared from **1** (500 mg, 1.15 mmol) by method *A*. The yield of **2b** was 445 mg (70%, foam). R_F 0.75 (CH_2Cl_2–EtOH 25:1). ^{1}H NMR ($CDCl_3$): 2.10 (s, 3 H, AcO), 2.13 (s, 3 H, AcO), 2.16 (s, 3 H, AcO), 2.33 (s, 3 H, AcN), 4.40 (dd, 1 H, $J_{5'b,5'a}$ = –12.5, $J_{5'b,4'}$ = 4.9, H-5′b), 4.46 (dd, 1 H, $J_{5'a,5'b}$ = –12.5, $J_{5'a,4'}$ = 3.3, H-5′a), 4.48 (ddd, 1 H, $J_{4',5'b}$ = 4.9, $J_{4',5'a}$ = 3.3, $J_{4',3'}$ = 4.6, H-4′), 4.66 (s, 2 H, OCH_2Ph), 5.68 (dd, 1 H, $J_{3',4'}$ = 4.6, $J_{3',2'}$ = 5.5, H-3′), 5.77 (s, 2 H, OCH_2N), 5.98 (dd, 1 H, $J_{2',3'}$ = 5.5, $J_{2',1'}$ = 5.3, H-2′), 6.25 (d, 1 H, $J_{1',2'}$ = 5.3, H-1′), 7.15–7.30 (m, 5 H, H-Ph), 8.20 (s, 1 H, H-2), 8.84 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 20.42 (AcO), 20.51 (AcO), 20.82 (AcO), 24.81 (AcN), 63.119 (C-5′), 70.85 (C-3′), 71.26 (CH_2-Ph), 73.19 (C-2′), 78.64 (NCH_2O), 80.68 (C-4′), 86.87 (C-1′), 127.55 (C-5), 128.30 (CH-Ph), 128.61 (CH-Ph), 129.81 (C-Ph), 142.37 (C-8), 152.41 (C-2), 153.06 (C-4), 153.26 (C-6), 169.40 (CO), 169.50 (CO), 170.31 (CO), 171.64 (CO). MS (APCI): m/z [M + H$^+$] calculated for $C_{26}H_{30}N_5O_9$: 556.20, found 474.32.

N^6-(*Z*)-(4-Hydroxy-2-butenyl)adenosine (**3f**)

Corresponding tetraacetate **2f** was prepared by method *C* starting with **1** (500 mg, 1.15 mmol). After column chromatography on silica gel (CH_2Cl_2–EtOH 100:1→20:1), partially purified product was dissolved in 7 M ammonia solution in MeOH (4 ml, 28 mmol) and the solution

was left for 48 h at ambient temperature to remove acetyl groups. Et_2O was added to the resulted slurry and the solid was filtered, washed with Et_2O and dried to give **3f** (162 mg, 42%). R_F 0.43 (CH_2Cl_2–EtOH 9:1). ^{1}H NMR (DMSO-d_6): 3.55 (ddd, 1 H, $J_{5'b,5'a}$ = –12.0, $J_{5'b,4'}$ = 3.6, $J_{5'b,OH}$ = 7.2, H-5'b), 3.67 (ddd, 1 H, $J_{5'a,5'b}$ = –12.1, $J_{5'a,4'}$ = 3.8, $J_{5'a,OH}$ = 4.6, H-5'a), 3.96 (ddd, 1 H, $J_{4',5'b}$ = 3.6, $J_{4',5'a}$ = 3.8, $J_{4',3'}$ = 2.8, H-4'), 4.07–4.23 (m, 5 H, overlapping OCH_2C=, NCH_2C=, H-3'), 4.60 (ddd, 1 H, $J_{2',3'}$ = 4.9, $J_{2',1'}$ = 6.0, $J_{2',OH}$ = 6.2, H-2'), 4.71 (t, J = 5.3, ω-OH, exchangeable with D_2O), 5.15 (d, 1 H, $J_{OH,3'}$ = 4.6, 3'-OH, exchangeable with D_2O), 5.37 (dd, 1 H, $J_{OH,5'b}$ = 7.2, $J_{OH,5'a}$ = 4.6, 5'-OH, exchangeable with D_2O), 5.41 (d, 1 H, $J_{OH,2'}$ = 6.2, 2'-OH, exchangeable with D_2O), 5.88 (d, 1 H, $J_{1',2'}$ = 6.0, H-1'), 7.91 (br s, 1 H, NH, exchangeable with D_2O), 8.20 (s, 1 H, H-2), 8.34 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6): 37.85 (CH_2N), 57.68 (CH_2OH), 62.26 (C-5'), 71.23 (C-3'), 74.27 (C-2'), 86.62 (C-4'), 88.82 (C-1'), 120.19 (C-5), 127.96 (CH=), 132.53 (CH=), 140.73 (C-8), 148.69 (C-4), 153.17 (C-2), 155.04 (C-6).

Preparation of Azides

MeN_3 was prepared by reaction of stoichiometric quantities of MeI and NaN_3 (5 mmol) in DMSO (5 ml) at ambient temperature overnight. This 1 M solution was used in the next step.

$PhOCH_2OCH_2N_3$, BnN_3 and $BzCH_2N_3$ were prepared by reaction of $PhOCH_2OCH_2Cl$, BnBr and $BzOCH_2CH_2Br$ with the 2-fold excess of NaN_3 in DMSO at ambient temperature overnight. The azides were isolated by usual water–AcOEt work-up procedure. Their purity was confirmed by ^{1}H NMR.

Alkene-Azide Cycloaddition. General Method

A mixture of **2a** (500 mg, 1.1 mmol), azide (1.65 mmol) and CuCl (23 mg, 0.22 mmol) in MeCN (10 ml) was stirred at ambient temperature overnight. The reaction was monitored by TLC. The mixture was diluted with AcOEt and washed successively with 0.1 M disodium EDTA aqueous solution and brine. The organic phase was dried over Na_2SO_4 and evaporated. The residue was applied to column chromatography on silica gel (CH_2Cl_2–EtOH 100:1→20:1).

N^6-Acetyl-N^6-[(1-methyl-1,2,3-triazol-4-yl)methyl]-2',3',5'-tri-O-acetyladenosine (**4a**). Yield 361 mg (64%, foam). R_F 0.33 (CH_2Cl_2–EtOH 25:1). ^{1}H NMR ($CDCl_3$): 2.10 (s, 3 H, AcO), 2.14 (s, 3 H, AcO), 2.15 (s, 3 H, AcO), 2.29 (s, 3 H, AcN), 4.03 (s, 3 H, Me), 4.40 (dd, 1 H, $J_{5'b,5'a}$ = –12.5, $J_{5'b,4'}$ = 5.0, H-5'b), 4.46 (dd, 1 H, $J_{5'a,5'b}$ = –12.5, $J_{5'a,4'}$ = 3.1, H-5'a), 4.48 (ddd, 1 H, $J_{4',5'b}$ = 5.0, $J_{4',5'a}$ = 3.1, $J_{4',3'}$ = 4.3, H-4'), 5.43 (s, 2 H, CH_2), 5.68 (dd, 1 H, $J_{3',4'}$ = 4.3, $J_{3',2'}$ = 5.3, H-3'), 5.99 (dd, 1 H, $J_{2',3'}$ = 5.3, $J_{2',1'}$ = 5.3, H-2'), 6.26 (d, 1 H, $J_{1',2'}$ = 5.3, H-1'), 7.70 (s, 1 H, 5-H_t), 8.40 (s, 1 H, H-2), 8.81 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 20.47 (AcO), 20.55 (AcO), 20.86 (AcO), 24.35 (AcN), 36.84 (MeN), 43.16 (N^6-CH_2), 63.08 (C-5'), 70.67 (C-3'), 73.01 (C-2'), 80.65 (C-4'), 86.87 (C-1'), 125.06 (CH_t), 126.93 (C-5), 143.48 (C-8), 144.42 (C_t), 152.46 (C-2), 152.84 (C-4), 153.15 (C-6), 169.40 (CO), 169.57 (CO), 170.36 (CO), 171.83 (CO). MS (APCI): *m/z* [M + H^+] calculated for $C_{22}H_{27}N_8O_8$: 531.20, found 531.30.

N^6-Acetyl-N^6-[(1-benzyloxymethyl-1,2,3-triazol-4-yl)methyl]-2',3',5'-tri-O-acetyladenosine (**4b**). Yield 624 mg (92%, foam). R_F 0.38 (CH_2Cl_2–EtOH 25:1). ^{1}H NMR ($CDCl_3$): 2.10 (s, 3 H, AcO), 2.12 (s, 3 H, AcO), 2.15 (s, 3 H, AcO), 2.32 (s, 3 H, AcN), 4.39 (dd, 1 H, $J_{5'b,5'a}$ = –12.4, $J_{5'b,4'}$ = 4.8, H-5'b), 4.45 (dd, 1 H, $J_{5'a,5'b}$ = –12.4, $J_{5'a,4'}$ = 3.3, H-5'a), 4.48 (ddd, 1 H, $J_{4',5'b}$ = 4.8, $J_{4',5'a}$ = 3.3, $J_{4',3'}$ = 4.5, H-4'), 4.48 (s, 2 H, CH_2), 5.59 (s, 2 H, CH_2), 5.63 (s, 2 H, CH_2), 5.67 (dd, 1 H, $J_{3',4'}$ = 4.5, $J_{3',2'}$ = 5.6, H-3'), 5.96 (dd, 1 H, $J_{2',3'}$ = 5.6, $J_{2',1'}$ = 5.3, H-2'), 6.24 (d,

1 H, $J_{1',2'}$ = 5.3, H-1′), 7.25–7.36 (m, 5 H, Ph), 7.84 (s, 1 H, 5-H$_t$), 8.20 (s, 1 H, H-2), 8.79 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 20.45 (AcO), 20.54 (AcO), 20.79 (AcO), 24.21 (AcN), 42.95 (N^6-CH_2), 63.09 (C-5′), 70.65 (C-3′), 71.16 (CH_2-Ph), 73.09 (C-2′), 77.64 (NCH_2O), 80.58 (C-4′), 86.84 (C-1′), 123.62 (CH_t), 127.54 (C-5), 128.28 (CH-Ph), 128.60 (CH-Ph), 129.79 (C-Ph), 142.35 (C-8), 144.93 (C_t), 152.39 (C-2), 152.96 (C-4), 153.16 (C-6), 169.38 (CO), 169.56 (CO), 170.29 (CO), 171.62 (CO). MS (APCI): *m/z* [M + H$^+$] calculated for $C_{29}H_{33}N_8O_9$: 637.24, found 637.40.

N^6-Acetyl-N^6-[(1-benzyl-1,2,3-triazol-4-yl)methyl]-2′,3′,5′-tri-O-acetyladenosine (**4c**). Yield 513 mg (78%, foam). R_F 0.42 (CH_2Cl_2–EtOH 25:1). ^{1}H NMR ($CDCl_3$): 2.11 (s, 3 H, AcO), 2.13 (s, 3 H, AcO), 2.16 (s, 3 H, AcO), 2.28 (s, 3 H, AcN), 4.40 (dd, 1 H, $J_{5'b,5'a}$ = –12.5, $J_{5'b,4'}$ = 4.8, H-5′b), 4.45 (dd, 1 H, $J_{5'a,5'b}$ = –12.5, $J_{5'a,4'}$ = 3.1, H-5′a), 4.47 (ddd, 1 H, $J_{4',5'b}$ = 4.8, $J_{4',5'a}$ = 3.1, $J_{4',3'}$ = 4.7, H-4′), 5.43 (s, 2 H, CH_2), 5.53 (s, 2 H, CH_2), 5.67 (dd, 1 H, $J_{3',4'}$ = 4.7, $J_{3',2'}$ = 5.4, H-3′), 5.96 (dd, 1 H, $J_{2',3'}$ = 5.4, $J_{2',1'}$ = 5.4, H-2′), 6.25 (d, 1 H, $J_{1',2'}$ = 5.4, H-1′), 7.17–7.23 (m, 2 H, Ph), 7.31–7.36 (m, 3 H, Ph), 7.62 (s, 1 H, 5-H$_t$), 8.20 (s, 1 H, H-2), 8.77 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 20.46 (AcO), 20.46 (AcO), 20.73 (AcO), 24.07 (AcN), 42.93 (N^6-CH_2), 54.12 (NCH_2Ph), 63.04 (C-5′), 70.59 (C-3′), 73.01 (C-2′), 80.50 (C-4′), 86.64 (C-1′), 123.74 (CH_t), 127.77 (C-5), 127.99 (CH-Ph), 128.59 (CH-Ph), 128.98 (CH-Ph), 134.61 (C-Ph), 142.35 (C-8), 145.50 (C_t), 152.30 (C-2), 152.35 (C-4), 153.09 (C-6), 169.33 (CO), 169.51 (CO), 170.23 (CO), 171.50 (CO). MS (APCI): *m/z* [M + H$^+$] calculated for $C_{28}H_{31}N_8O_8$: 607.23, found 607.38.

N^6-Acetyl-N^6-{[1-(2-benzoyloxyethyl)-1,2,3-triazol-4-yl]methyl}-2′,3′,5′-tri-O-acetyladenosine (**4d**). Yield 652 mg (93%, foam). R_F 0.27 (CH_2Cl_2–EtOH 25:1). ^{1}H NMR ($CDCl_3$): 2.10 (s, 3 H, AcO), 2.12 (s, 3 H, AcO), 2.15 (s, 3 H, AcO), 2.28 (s, 3 H, AcN), 4.39 (dd, 1 H, $J_{5'b,5'a}$ = –12.3, $J_{5'b,4'}$ = 4.8, H-5′b), 4.44 (dd, 1 H, $J_{5'a,5'b}$ = –12.3, $J_{5'a,4'}$ = 3.3, H-5′a), 4.46 (ddd, 1 H, $J_{4',5'b}$ = 4.8, $J_{4',5'a}$ = 3.3, $J_{4',3'}$ = 4.5, H-4′), 4.66 (m, 4 H, CH_2CH_2), 5.55 (s, 2 H, CH_2), 5.66 (dd, 1 H, $J_{3',4'}$ = 4.5, $J_{3',2'}$ = 5.6, H-3′), 5.95 (dd, 1 H, $J_{2',3'}$ = 5.6, $J_{2',1'}$ = 5.3, H-2′), 6.23 (d, 1 H, $J_{1',2'}$ = 5.3, H-1′), 7.42–7.49 (m, 2 H, Bz), 7.50–7.60 (m, 1 H, Bz), 7.82 (s, 1 H, 5-H$_t$), 7.95–8.01 (m, 2 H, Bz), 8.19 (s, 1 H, H-2), 8.73 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 20.46 (AcO), 20.56 (AcO), 20.79 (AcO), 24.17 (AcN), 43.06 (N^6-CH_2), 49.20 (CH_2), 62.85 (CH_2), 63.11 (C-5′), 70.67 (C-3′), 73.09 (C-2′), 80.59 (C-4′), 86.67 (C-1′), 124.18 (CH_t), 127.49 (C-5), 128.62 (CH-Ph), 129.37 (C-Ph), 129.81 (CH-Ph), 133.47 (CH-Ph), 142.37 (C-8), 144.91 (C_t), 152.36 (C-2), 152.86 (C-4), 153.14 (C-6), 166.02 (PhCO), 169.39 (CO), 169.58 (CO), 170.31 (CO), 171.62 (CO). MS (APCI): *m/z* [M + H$^+$] calculated for $C_{30}H_{33}N_8O_{10}$: 665.23, found 665.39.

Preparation of Nucleosides. General Method of Ammonolysis

Corresponding compound was dissolved in 7 M ammonia solution in MeOH (5 mmol per acetyl group) and left at ambient temperature for 48 h. The resulting slurry was diluted with Et_2O. The insoluble product was filtered, washed with Et_2O and dried.

N^6-(Benzyloxymethyl)adenosine (**3b**). Yield 117 mg (56%, solid) from **2a** (300 mg, 0.54 mmol). M.p. 138–140 °C. R_F 0.57 (CH_2Cl_2–EtOH 4:1). ^{1}H NMR (DMSO-d_6): 3.57 (ddd, 1 H, $J_{5'b,5'a}$ = –11.9, $J_{5'b,4'}$ = 4.0, $J_{5'b,OH}$ = 7.2, H-5′b), 3.69 (ddd, 1 H, $J_{5'a,5'b}$ = –11.9, $J_{5'a,4'}$ = 4.0, $J_{5'a,OH}$ = 4.6, H-5′a), 3.97 (ddd, 1 H, $J_{4',5'b}$ = 4.0, $J_{4',5'a}$ = 4.0, $J_{4',3'}$ = 3.2, H-4′), 4.16 (ddd, 1 H, $J_{3',4'}$ = 3.2, $J_{3',2'}$ = 4.8, $J_{3',OH}$ = 4.9, H-3′), 4.58 (s, 2 H, OCH_2Ph), 4.61 (ddd, 1 H, $J_{2',3'}$ = 4.9, $J_{2',1'}$ = 6.0, $J_{2',OH}$ = 6.2, H-2′), 5.13 (br s, 2 H, NCH_2O), 5.14 (d, 1 H, $J_{OH,3'}$ = 4.8, 3′-OH, exchangeable with D_2O), 5.26 (dd, 1 H, $J_{OH,5'b}$ = 7.2, $J_{OH,5'a}$ = 4.6, 5′-OH, exchangeable with D_2O), 5.42 (d, 1 H, $J_{OH,2'}$ = 6.2, 2′-OH, exchangeable with D_2O), 5.93 (d, 1 H, $J_{1',2'}$ = 6.0, H-1′),

7.22–7.36 (m, 5 H, H-Ph), 8.31 (br s, 1 H, H-2), 8.44 (s, 1 H, H-8), 8.70 (br s, 1 H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 61.52 (C-5′), 68.88 ($PhCH_2$), 70.50 (C-3′), 70.50 (OCH_2N), 73.52 (C-2′), 85.77 (C-4′), 87.81 (C-1′), 119.80 (C-5), 127.17 (Ph), 127.39 (Ph), 128.06 (Ph), 138.54 (Ph), 140.43 (C-8), 149.32 (C-4), 152.10 (C-2), 154.41 (C-6). UV (H_2O), λ_{max} in nm (ε): pH 1, 260 (13 700), 266 (14 900); pH 7, 260 (16 700), 265 (17 500); pH 13, 260 (14 800), 265 (15 800). MS (APCI): *m/z* [M + H^+] calculated for $C_{18}H_{22}N_5O_5$: 388.16, found 388.14; *m/z* [M – H^+ + HCOOH] calculated for $C_{19}H_{22}N_5O_7$: 432.15, found 432.06.

N^6-[(1-Methyl-1,2,3-triazol-4-yl)methyl]adenosine (**5a**). Yield 123 mg (60%, solid) from **4a** (300 mg, 0.57 mmol). M.p. 76–78 °C. R_F 0.41 (CH_2Cl_2–EtOH 4:1). ^1H NMR (DMSO-d_6+D_2O): 3.55 (dd, 1 H, $J_{5'b,5'a}$ = –12.2, $J_{5'b,4'}$ = 3.6, H-5′b), 3.65 (dd, 1 H, $J_{5'a,5'b}$ = –12.2, $J_{5'a,4'}$ = 2.4, H-5′a), 3.94 (s, 3 H, Me), 3.97 (ddd, 1 H, $J_{4',5'b}$ = 3.6, $J_{4',5'a}$ = 2.4, $J_{4',3'}$ = 3.1, H-4′), 4.14 (dd, 1 H, $J_{3',4'}$ = 3.1, $J_{3',2'}$ = 4.9, H-3′), 4.58 (dd, 1 H, $J_{2',3'}$ = 4.9, $J_{2',1'}$ = 6.2, H-2′), 4.73 (br s, 2 H, CH_2), 5.87 (d, 1 H, $J_{1',2'}$ = 6.2, H-1′), 7.83 (s, 1 H, 5-H_t), 8.19 (br s, 1 H, NH), 8.22 (s, 1 H, H-2), 8.33 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6): 35.40 (N^6-CH_2), 36.04 (Me), 61.61 (C-5′), 70.59 (C-3′), 73.48 (C-2′), 85.85 (C-4′), 87.91 (C-1′), 119.80 (C-5), 123.59 (CH_t), 139.90 (C-8), 145.52 (C_t), 148.60 (C-4), 152.24 (C-2), 154.30 (C-6). UV (H_2O), λ_{max} in nm (ε): pH 1, 260 (23 400), 264 (24 400); pH 7, 260 (24 300), 266 (25 900); pH 13, 260 (14 800), 265 (15 800). MS (APCI): *m/z* [M + H^+] calculated for $C_{14}H_{19}N_8O_4$: 363.15, found 363.07; *m/z* [M – H^+ + HCOOH] calculated for $C_{15}H_{29}N_8O_6$: 407.14, found 407.08.

N^6-[(1-Benzyloxymethyl-1,2,3-triazol-4-yl)methyl]adenosine (**5b**). Yield 163 mg (74%, solid) from **4b** (300 mg, 0.54 mmol). M.p. 87–89 °C. R_F 0.38 (CH_2Cl_2–EtOH 4:1). ^1H NMR (DMSO-d_6+D_2O): 3.57 (dd, 1 H, $J_{5'b,5'a}$ = –12.1, $J_{5'b,4'}$ = 3.7, H-5′b), 3.68 (dd, 1 H, $J_{5'a,5'b}$ = –12.1, $J_{5'a,\ 4'}$ = 3.4, H-5′a), 3.97 (ddd, 1 H, $J_{4',5'b}$ = 3.7, $J_{4',5'a}$ = 3.4, $J_{4',3'}$ = 2.6, H-4′), 4.16 (dd, 1 H, $J_{3',4'}$ = 2.6, $J_{3',2'}$ = 5.2, H-3′), 4.52 (s, 2 H, CH_2), 4.62 (dd, 1 H, $J_{2',3'}$ = 5.2, $J_{2',1'}$ = 6.0, H-2′), 4.78 (br s, 2 H, CH_2), 5.74 (s, 2 H, CH_2), 5.90 (d, 1 H, $J_{1',2'}$ = 6.0, H-1′), 7.20–7.35 (m, 5 H, Ph), 8.08 (s, 1 H, 5-H_t), 8.25 (s, 1 H, H-2), 8.32 (br s, 1 H, NH), 8.38 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6): 35.37 (N^6-CH_2), 61.61 (C-5′), 70.42 (CH_2Ph), 70.58 (C-3′), 73.48 (C-2′), 77.31 (CH_2O), 85.84 (C-4′), 87.94 (C-1′), 119.86 (C-5), 123.33 (CH_t), 127.64 (CHPh), 127.72 (CHPh), 128.24 (CHPh), 136.78 (CPh), 139.92 (C-8), 145.94 (C_t), 148.52 (C-4), 152.22 (C-2), 154.29 (C-6). UV (H_2O), λ_{max} in nm (ε): pH 1, 260 (16 600), 264 (17 600); pH 7, 260 (17 900), 266 (18 500); pH 13, 260 (16 600), 266 (18 000). MS (APCI): *m/z* [M + H^+] calculated for $C_{21}H_{25}N_8O_5$: 469.19, found 469.12; *m/z* [M – H^+ + HCOOH] calculated for $C_{22}H_{25}N_8O_7$: 513.18, found 513.01.

N^6-[(1-Benzyl-1,2,3-triazol-4-yl)methyl]adenosine (**5c**). Yield 189 mg (84%, solid) from **4c** (300 mg, 0.49 mmol). M.p. 92–94 °C. R_F 0.67 (CH_2Cl_2–EtOH 4:1). ^1H NMR (DMSO-d_6+D_2O): 3.57 (dd, 1 H, $J_{5'b,5'a}$ = –12.3, $J_{5'b,4'}$ = 3.6, H-5′b), 3.66 (dd, 1 H, $J_{5'a,5'b}$ = –12.3, $J_{5'a,\ 4'}$ = 3.1, H-5′a), 3.97 (ddd, 1 H, $J_{4',5'b}$ = 3.6, $J_{4',5'a}$ = 3.1, $J_{4',3'}$ = 3.3, H-4′), 4.14 (dd, 1 H, $J_{3',4'}$ = 3.3, $J_{3',2'}$ = 4.7, H-3′), 4.58 (dd, 1 H, $J_{2',3'}$ = 4.7, $J_{2',1'}$ = 6.2, H-2′), 4.75 (br s, 2 H, CH_2), 5.49 (s, 2 H, CH_2), 5.87 (d, 1 H, $J_{1',2'}$ = 6.2, H-1′), 7.20–7.38 (m, 5 H, Ph), 7.94 (s, 1 H, 5-H_t), 8.19 (br s, 1 H, NH), 8.21 (s, 1 H, H-2), 8.32 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6): 35.49 (N^6-CH_2), 52.61 (CH_2Ph), 61.60 (C-5′), 70.58 (C-3′), 73.47 (C-2′), 85.84 (C-4′), 87.92 (C-1′), 119.69 (C-5), 122.88 (CH_t), 127.81 (CHPh), 127.96 (CHPh), 128.60 (CHPh), 136.10 (CPh), 139.87 (C-8), 145.70 (C_t), 148.52 (C-4), 152.21 (C-2), 154.28 (C-6). UV (H_2O), λ_{max} in nm (ε): pH 1, 260 (10 800), 264 (11 100); pH 7, 260 (11 900), 266 (12 500); pH 13, 260 (10 400), 267 (11 100). MS (APCI): *m/z* [M + H^+] calculated for $C_{20}H_{23}N_8O_4$: 439.18, found 439.14; *m/z* [M – H^+ + HCOOH] calculated for $C_{21}H_{23}N_8O_6$: 483.17, found 483.01.

N^6-{[1-(2-Hydroxyethyl)-1,2,3-triazol-4-yl]methyl}adenosine (**5d**). Yield 189 mg (46%, solid) from **4d** (300 mg, 0.45 mmol). M.p. 97–99 °C. R_F 0.12 (CH_2Cl_2–EtOH 4:1). ^{1}H NMR (DMSO-d_6+D_2O): 3.56 (dd, 1 H, $J_{5'b,5'a}$ = –12.2, $J_{5'b,4'}$ = 3.3, H-5'b), 3.66 (dd, 1 H, $J_{5'a,5'b}$ = –12.2, $J_{5'a,4'}$ = 3.5, H-5'a), 3.73 (t, 3 H, J = 5.3, CH_2), 3.97 (ddd, 1 H, $J_{4',5'b}$ = 3.3, $J_{4',5'a}$ = 3.5, $J_{4',3'}$ = 3.1, H-4'), 4.14 (dd, 1 H, $J_{3',4'}$ = 3.1, $J_{3',2'}$ = 5.1, H-3'), 4.32 (t, 3 H, J = 5.3, CH_2), 4.58 (dd, 1 H, $J_{2',3'}$ = 5.1, $J_{2',1'}$ = 6.2, H-2'), 4.76 (br s, 2 H, CH_2), 5.87 (d, 1 H, $J_{1',2'}$ = 6.2, H-1'), 7.85 (s, 1 H, 5-H$_t$), 8.19 (br s, 1 H, NH), 8.23 (s, 1 H, H-2), 8.33 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6): 35.41 (N^6-CH_2), 51.99 (CH_2), 59.83 (CH_2), 61.62 (C-5'), 70.59 (C-3'), 73.46 (C-2'), 85.85 (C-4'), 87.92 (C-1'), 119.78 (C-5), 123.12 (CH$_t$), 139.88 (C-8), 145.12 (C$_t$), 148.48 (C-4), 152.25 (C-2), 154.29 (C-6). UV (H_2O), λ_{max} in nm (ε): pH 1, 260 (19 900), 264 (20 800); pH 7, 260 (21 000), 266 (22 800); pH 13, 260 (19 900), 266 (21 900). MS (APCI): *m/z* [M + H$^+$] calculated for $C_{15}H_{21}N_8O_5$: 393.16, found 392.52; *m/z* [M – H$^+$ + HCOOH] calculated for $C_{16}H_{21}N_8O_7$: 437.15, found 437.05.

O^6-Benzylinosine (**6**)

To benzylalcohol (5 ml) was carefully added with stirring 60% NaH in paraffin (360 mg, 8.9 mmol) and the mixture was stirred at room temperature for additional 1 h. To the resulted mixture was added 6-chloro-9-(2',3',5'-tri-*O*-acetyribozyl)purine[24] (370 mg, 0.89 mmol). The mixture was stirred at room temperature overnight. After neutralization with AcOH (0.6 ml) the viscous solution was directly applied for column chromatography. Elution with a gradient of EtOH in CH_2Cl_2 (1:100→1:9) gave 260 mg (81%, solid) of **6**. M.p. 153–155 °C. R_F 0.63 (CH_2Cl_2–EtOH 9:1). ^{1}H NMR (DMSO-d_6): 3.57 (ddd, 1 H, $J_{5'b,5'a}$ = –12.2, $J_{5'b,4'}$ = 3.8, $J_{5'b,OH}$ = 6.0, H-5'b), 3.68 (ddd, 1 H, $J_{5'a,5'b}$ = –12.2, $J_{5'a,4'}$ = 4.2, $J_{5'a,OH}$ = 5.3, H-5'a), 3.97 (ddd, 1 H, $J_{4',5'b}$ = 3.8, $J_{4',5'a}$ = 4.2, $J_{4',3'}$ = 3.4, H-4'), 4.17 (ddd, 1 H, $J_{3',4'}$ = 3.4, $J_{3',2'}$ = 5.1, $J_{3',OH}$ = 4.9, H-3'), 4.59 (ddd, 1 H, $J_{2',3'}$ = 5.1, $J_{2',1'}$ = 5.7, $J_{2',OH}$ = 6.0, H-2'), 5.14 (dd, 1 H, $J_{OH,5'b}$ = 6.0, $J_{OH,5'a}$ = 5.3, 5'-OH, exchangeable with D_2O), 5.21 (d, 1 H, $J_{OH,3'}$ = 4.9, 3'-OH, exchangeable with D_2O), 5.49 (d, 1 H, $J_{OH,2'}$ = 6.0, 2'-OH, exchangeable with D_2O), 5.63 (d, $J_{a,b}$ = 12.7, C**H**$_b$H$_a$Ph), 5.66 (d, $J_{a,b}$ = 12.7, CH$_b$**H**$_a$Ph), 5.99 (d, 1 H, $J_{1',2'}$ = 5.7, H-1'), 7.32–7.44 (m, 3 H, H-Ph), 7.48–7.53 (m, 2 H, H-Ph), 8.56 (s, 1 H, H-2), 8.61 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6): 61.27 (C-5'), 67.79 (PhCH$_2$), 70.30 (C-3'), 73.76 (C-2'), 85.69 (C-4'), 87.81 (C-1'), 121.13 (C-5), 128.11 (Ph), 128.25 (Ph), 128.43 (Ph), 136.23 (Ph), 142.49 (C-8), 151.52 (C-2), 151.97 (C-4), 159.80 (C-6). UV (H_2O), λ_{max} in nm (ε): pH 1, 251 (11 600); pH 7, 252 (13 300); pH 13, 251 (12 500). MS (APCI): *m/z* [M + H$^+$] calculated for $C_{17}H_{19}N_4O_5$: 359.14, found 359.21.

This work was supported by the Molecular and Cellular Biology Program of the Russian Academy of Sciences, the Russian Foundation for Basic Research and the Russian Federal Program (contract No. 16.512.11.2239).

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