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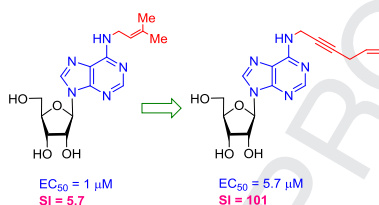
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## Graphical Abstract

**Chemical modification of the plant isoprenoid cytokinin *N*<sup>6</sup>-isopentenyladenosine yields a selective inhibitor of human enterovirus 71 replication**

pp. 1–8

Vitali I. Tararov, Aloys Tijssma, Svetlana V. Kolyachkina, Vladimir E. Oslovsky, Johan Neyts, Mikhail S. Drenichev, Pieter Leyssen, Sergey N. Mikhailov\*



## Highlights

- Ten new *N*<sup>6</sup>-substituted adenosines have been synthesized.
- *N*<sup>6</sup>-Isopentenyladenosine exerts antiviral effect on the replication of EV71.
- The structure–activity relationship for *N*<sup>6</sup>-substituted adenosines has been studied.
- The allylation of *N*<sup>6</sup>-propargyladenosine in the presence of Cu(I) was developed.
- *N*<sup>6</sup>-(5-Hexene-2-yn-1-yl)adenosine exhibits the highest selectivity index.



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## Original article

Chemical modification of the plant isoprenoid cytokinin  $N^6$ -isopentenyladenosine yields a selective inhibitor of human enterovirus 71 replicationVitali I. Tararov<sup>a</sup>, Aloys Tijssma<sup>b</sup>, Svetlana V. Kolyachkina<sup>a</sup>, Vladimir E. Oslovsky<sup>a</sup>,  
Johan Neyts<sup>b</sup>, Mikhail S. Drenichev<sup>a</sup>, Pieter Leyssen<sup>b</sup>, Sergey N. Mikhailov<sup>a,\*</sup><sup>a</sup> Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Str. 32, Moscow 119991, Russian Federation<sup>b</sup> KU Leuven – University of Leuven, Department of Microbiology and Immunology, Laboratory for Virology and Chemotherapy, Rega Institute for Medical Research, Minderbroedersstraat 10, 3000 Leuven, Belgium

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## ABSTRACT

In this study, we demonstrate that  $N^6$ -isopentenyladenosine, which essentially is a plant cytokinin-like compound, exerts a potent and selective antiviral effect on the replication of human enterovirus 71 with an  $EC_{50}$  of  $1.0 \pm 0.2 \mu\text{M}$  and a selectivity index (SI) of 5.7. The synthesis of analogs with modification of the  $N^6$ -position did not result in a lower  $EC_{50}$  value. However, in particular with the synthesis of  $N^6$ -(5-hexene-2-yn-1-yl)adenosine ( $EC_{50} = 4.3 \pm 1.5 \mu\text{M}$ ), the selectivity index was significantly increased: because of a reduction in the adverse effect of this compound on the host cells, an  $SI > 101$  could be calculated. With this study, we for the first time provide proof that a compound class that is based on the plant cytokinin skeleton offers an interesting starting point for the development of novel antivirals against mammalian viruses, in the present context in particular against enterovirus 71.

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## 1. Introduction

Common natural isoprenoid cytokinins are  $N^6$ -isopentenyladenine, *trans*- and *cis*-zeatin, and dihydrozeatin. The first step in isoprenoid cytokinin biosynthesis is carried out by the adenosine phosphate isopentenyltransferase (EC 2.5.1.27), which catalyzes the  $N^6$ -prenylation of adenosine 5'-(mono-, di-, or tri-) phosphates with 3,3-dimethylallyldiphosphate [1]. The resulting nucleotides are readily dephosphorylated to their corresponding nucleoside derivatives. These in turn serve as substrate for two other enzymes, namely adenosine nucleosidase (EC 3.2.2.7) and purine nucleoside phosphorylase (EC 2.4.2.1), which convert the cytokinin nucleosides into their cytokinin bases. Accordingly, to these transformations  $N^6$ -substituted adenines and adenosines might have similar cytokinin activities [1].

Several  $N^6$ -substituted adenosines, such as  $N^6$ -isopentenyladenosine,  $N^6$ -methyladenosine, and some others have been detected in, amongst others, tRNA [2]. Cytokinin ribosides were found to inhibit the growth of mammalian neoplastic cells *in vitro* [3,4], and, in limited clinical trials in the 1970's,  $N^6$ -isopentenyladenosine showed some antitumor chemotherapeutic effect [5]. These findings initiated an interest to synthesize novel  $N^6$ -adenosine analogs in an attempt to identify compounds with enhance antitumor activity. However, biological evaluation of a wide variety of such derivatives did not yield compounds with activity higher than that of  $N^6$ -isopentenyladenosine itself [6].

Nonetheless, encouraged by the fact that  $N^6$ -substituted adenosines have been shown to elicit biological activity in mammalian assay systems for malaria [7], African sleeping sickness [8], toxoplasmosis [9], and some others, we decided to explore the potential of  $N^6$ -isopentenyladenosine and derivatives as inhibitors of virus replication.

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## 2. Results and discussion

### 2.1. Chemistry

Recently, we developed a new and versatile method for the preparation of  $N^6$ -adenosine derivatives by regioselective  $N^6$ -alkylation of  $N^6$ -acetyl-2',3',5'-tri-*O*-acetyladenosine (**1**) [10,11]. One of the most common approaches for the preparation of  $N^6$ -alkylated adenosines is the reaction of alkylamines with 6-chloropurine riboside [12,13]. The advantage of our method is the possibility of usage both alkyl halides and alcohols for  $N^6$ -modification. This is important especially in the case when an amine is hardly available. Compounds **2a–c** were synthesized by alkylation of tetraacetate **1** with appropriate alkyl halides (Scheme 1, method A) and they were deprotected with ammonia in methanol according to well-established procedures reported in the literature.

In other cases, tetraacetate **1** was transformed into nucleosides **2d–k** using the Mitsunobu protocol (Scheme 1, method B). Although the Mitsunobu coupling promoted with  $\text{Ph}_3\text{P}$  and DEAD (diethyl azodicarboxylate) is a very popular mild chemical transformation that occurs under essentially neutral conditions, it produces at least equivalent amounts of  $\text{Ph}_3\text{PO}$  and  $(\text{NHCOOEt})_2$ . It is almost impossible to isolate pure substances **2** without repeated column chromatography on silica gel which resulted in low yields of **2**. The partially purified acetates **2** were subjected to deacetylation with the following column chromatography to give pure nucleosides **3** with reasonable overall yields.

Deacetylation with 7 M  $\text{NH}_3$  in methanol at room temperature proceeded completely during 48 h. However, the rate of deprotection of *N*-acetyl and *O*-acetyl groups in nucleosides **2** is quite different: the first one is much more stable and, as a result, an additional amount of acetamide was formed under deblocking conditions. Acetamide caused some difficulties in the purification of the final products using column chromatography on silica gel, because it was often eluted together with nucleosides **3**.

We have also tried to use 0.1 M  $\text{MeONa}$  in  $\text{MeOH}$  at room temperature for removal of acetyl groups. After 30 min, we were able to

isolate  $N^6$ -acetyl derivatives of **3** in good yields and their structure was confirmed by NMR and MS methods. Prolonged treatment results in substantial decomposition of the desired products.

Finally, we found that the use of 4 M  $\text{PrNH}_2$  in  $\text{MeOH}$  [14] at room temperature for one day is the method of choice for the preparation of nucleosides **3**. The formed *N*-propylacetamide has higher mobility on silica gel than the desired nucleosides **3**.

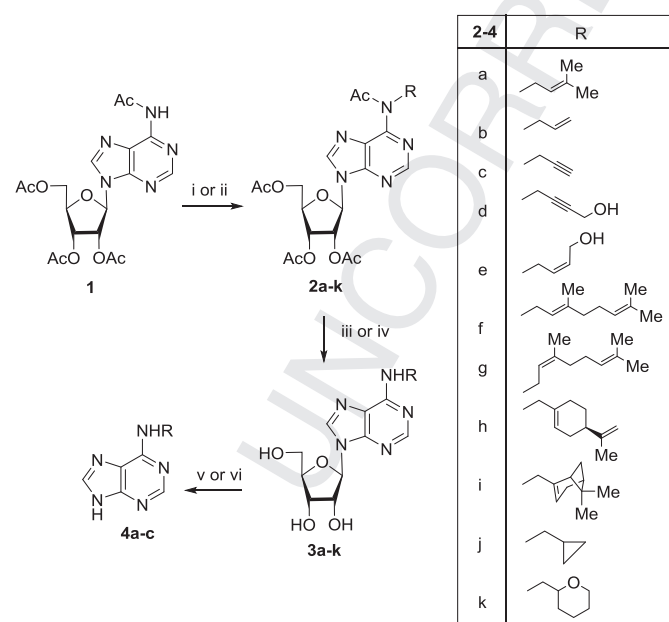
$N^6$ -Substituted adenine derivatives **4b–c** were obtained by acid hydrolysis of the nucleosides **3b–c**. The treatment of corresponding nucleosides with 0.5 M  $\text{HCl}$  at 100 °C leads to cleavage of the glycoside bond and removal of *N*-acetyl group. Unfortunately, this method was not applicable to isoprenoid derivatives due to the side reactions [15,16]. Isopentenyladenine (**4a**) was obtained via the enzymatic phosphorolysis of **3a** in the presence of recombinant purine nucleoside phosphorylase in  $\text{KH}_2\text{PO}_4$  buffer (pH 7.5) [10].

The structure of all synthesized compounds was confirmed by NMR and MS methods.

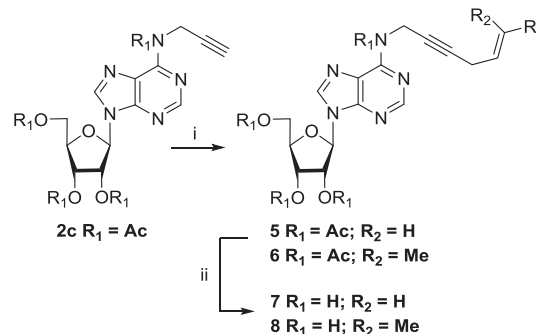
Among the synthesized derivatives,  $N^6$ -acetyl- $N^6$ -propargyl-2',3',5'-tri-*O*-acetyladenosine (**2c**) may be used for introduction of new structural motifs in the adenine moiety according to Scheme 2.

No literature examples of allylation of acetylenic nucleic base derivatives were found, though successful examples carried out on simple alkynes are available [17–19]. We found that reaction of allyl bromide and 3,3-dimethylallyl bromide with compound **2c** can be carried out in the presence of  $\text{CuCl}$  and a base at ambient temperature affording **5** and **6** in 70–80% yield. It is noteworthy that no reaction occurred with methyl iodide or benzyl bromide. This can be a piece of evidence that the mechanism of acetylenic allylation, catalyzed with  $\text{Cu(I)}$ , is more complicated than it was suggested [17], since it is limited only to allylic halides. Obviously, the participation of  $\eta^3$ -allylic complex should also be taken into consideration. The participation of binuclear copper species is also highly probable in a way similar to  $\text{Cu(I)}$  catalyzed  $[3 + 2]$  cycloaddition of azides to acetylenes [20].

The reaction of **2c** with allylbromide and 3,3-dimethylallylbromide is to be carried out at ambient conditions but in anaerobic ( $\text{N}_2$ ) atmosphere to observe a sufficient increase in yields. We were unable to monitor both reactions by TLC on silica gel plates because in the wide range of the used solvents, the starting compound **2c** and the products **5** or **6** have quite the same mobility. To ensure the complete consumption of **2c** and for simplification of chromatographic isolation of **5** and **6**, the reaction mixtures, after stirring for 16 h in  $\text{N}_2$  atmosphere, were additionally stirred for 6–8 h on air. This causes the oxidation of the unreacted initial compound **2c** with the formation of more polar products, thus simplifying the isolation procedure. The oxidation of **2c** in solution in the presence of  $\text{Cu(I)}$  was confirmed by independent experiment in which **2c** and  $\text{CuCl}$  were stirred in  $\text{MeCN}$  in the open flask and complete conversion of **2c** to polar compounds was



**Scheme 1.** Synthesis of adenosine derivatives with unsaturated  $N^6$ -substituents. Reagents and conditions: (i) **Method A**:  $\text{RX}$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{DMF}$ , room temperature, 16 h; (ii) **Method B**:  $\text{ROH}$ ,  $\text{Ph}_3\text{P}$ ,  $\text{DEAD}$ ,  $\text{THF}$ , room temperature, 20 h; (iii) 7 M  $\text{NH}_3$  in  $\text{MeOH}$ , room temperature, 48 h; (iv) 4 M  $\text{PrNH}_2$  in  $\text{MeOH}$ , room temperature, 24 h; (v) 0.5 M  $\text{HCl}$ , 100 °C, 8 h; (vi) enzymatic phosphorolysis (*E. coli* **PNP**,  $\text{KH}_2\text{PO}_4$ , pH 7.5).



**Scheme 2.** Allylation of acetylenic adenosine derivative **2c**. Reagents and conditions: (i)  $(\text{R}_2)_2\text{C}=\text{CHCH}_2\text{Br}$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{CuCl}$ ,  $\text{BuNH}_4\text{Br}$ ,  $\text{DMF}$ , room temperature, 16 h; (ii) 7 M  $\text{NH}_3$  in  $\text{MeOH}$ , room temperature, 48 h.



observed within several hours. After the removal of acetyl groups by ammonolysis of peracetylated compounds **5** and **6**, free nucleosides **7** and **8** can be isolated in good yields.

The structure of the side chain of the compounds was unambiguously assigned by  $^1\text{H}$  NMR spectral data (the numeration of side chain carbon atoms is depicted in Fig. 1). Long-range spin–spin coupling constants were observed. In compounds **5** and **6**, the  $1\text{-CH}_2$  groups appears as triplets with  $^3J = 2.2$  Hz. In the free nucleosides **7** and **8**, significant broadening of the  $1\text{-CH}_2$  signals is observed. Such effect is general for free  $\text{N}^6$ -substituted nucleosides. A possible explanation can be found in the literature and is ascribed to ‘a chemical exchange process connected to the restricted conformational change’ [21,22]. As a result, spin–spin coupling between  $1\text{-CH}_2$  and  $4\text{-CH}_2$  is not detectable. Nevertheless, the sets of constants for other hydrogen atoms remain the same as for acetylated nucleosides **5** and **6** (Supporting information). Thus, no principle structural changes of  $\text{N}^6$ -residues occurred during deprotection. The chemical purity of **7** and **8** was established to be higher than 99% on the basis of LC–MS analysis.

## 2.2. Biological activity

In a first round, the compounds were evaluated for their potential as inhibitors of virus replication in cell-based assays for hepatitis C virus (HCV, replicon-based assay), chikungunya virus (CHIKV, virus-cell-based assay) and enterovirus 71 (EV71, virus-cell-based assay). No antiviral activity was observed in the assays for HCV and CHIKV. In contrast, a clear cell protective effect was apparent in the EV71 assay for compounds **3a**, **3b**, **3e**, **3j**, **3k**, and **7**, for which an  $\text{EC}_{50}$  value could be derived from the dose–response curves (Table 1). This value represents the concentration of compound that induces a cell protective effect of 50% and is indicative of the antiviral potency of the compound. In parallel, also uninfected cells were treated with the compounds to quantify the adverse effect of treatment on host cell metabolism. From these dose–response curves,  $\text{CC}_{50}$  values equaling the concentration of compound that reduces overall host cell metabolism by 50%, are calculated. From both values, the selectivity index ( $\text{SI} = \text{CC}_{50}/\text{EC}_{50}$ ) can be derived, a value that is indicative for the therapeutic window of a particular compound in this assay (the larger the SI, the larger the therapeutic window). However, a cell protective effect that is obtained in a virus-cell-based assay does not necessarily prove that a compound selectively inhibits virus replication. Therefore, all assay wells with a cytoprotective effect were inspected by microscope for minor signs of virus-induced cell death, or for treatment-induced host cell or monolayer abnormalities. Only compounds for which the cells at least at one concentration of compound resemble the untreated, uninfected control cells are considered as selective inhibitors of EV71 virus replication. Compound **3a** proved to be the most potent compound of this series ( $\text{EC}_{50}$  of  $1.0 \pm 0.2 \mu\text{M}$ ) while compound **7** was the most selective compound ( $\text{SI} > 101$ ). Both matched the selection criteria for a selective inhibitor of EV71 replication as outlined above, and were selected for further evaluation. The  $\text{N}^6$ -substituted adenines **4a–c** do not elicit an antiviral effect in this assay, which indicates that the ribofuranose residue is most likely essential for antiviral activity.

In a second round, the antiviral effect of compound **3a** and **7** was evaluated in virus-cell-based assays for a panel of other

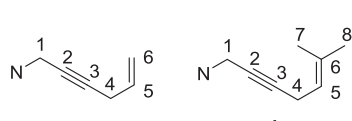


Fig. 1. Numeration of carbon atoms in  $\text{N}^6$ -substituents of compounds **5** and **6**.

Table 1  
Antiviral effect on the replication of EV71 strain BrCr in RD cells.

Compound name	$\text{CC}_{50} \pm \text{SD}^{a,b}$	$\text{EC}_{50} \pm \text{SD}^{a,b}$	SI
<b>3a</b> $\text{N}^6$ -Isopentenyladenosine	$5.7 \pm 0.3$	$1.0 \pm 0.2$	5.7
<b>4a</b> $\text{N}^6$ -Isopentenyladenine	ND	>492	ND
<b>3b</b> $\text{N}^6$ -Allyllyladenosine	$78 \pm 20$	$7.1 \pm 1.6$	11
<b>4b</b> $\text{N}^6$ -Allyllyladenine	ND	>571	ND
<b>3c</b> $\text{N}^6$ -Propargyladenosine	$166 \pm 15$	>166	ND
<b>4c</b> $\text{N}^6$ -Propargyladenine	ND	>577	ND
<b>3d</b> $\text{N}^6$ -(4-Hydroxy-2-butenyl) adenosine	ND	>298	ND
<b>3e</b> $\text{N}^6$ -(Z)-(4-Hydroxy-2-butenyl) adenosine	$122 \pm 25$	$4.7 \pm 0.7$	26
<b>3f</b> $\text{N}^6$ -Geranyladenosine	$174 \pm 10$	>124	NA
<b>3g</b> $\text{N}^6$ -Neryllyladenosine	>372	>124	NA
<b>3h</b> $\text{N}^6$ -(S)-(-)-Perillyllyladenosine	$307 \pm 7$	>125	NA
<b>3i</b> $\text{N}^6$ -(1R)-(-)-Myrtenyladenosine	>374	>125	NA
<b>3j</b> $\text{N}^6$ -Cyclopropylmethyladenosine	$22 \pm 3$	$4.6 \pm 0.8$	5
<b>3k</b> $\text{N}^6$ -(Tetrahydropyran-2-methyl)adenosine	>274	$33 \pm 5$	>8
<b>7</b> $\text{N}^6$ -(5-hexene-2-yne-1-yl)adenosine	>434	$4.3 \pm 1.5$	>101
<b>8</b> $\text{N}^6$ -(2-Methylhept-2-en-4-yne-1-yl)adenosine	>402	>335	NA

ND = Not Determined; NA = Not Active within tested range.

<sup>c</sup> Selectivity Index (SI);  $\text{SI} = \text{CC}_{50}/\text{EC}_{50}$ .

<sup>a</sup> All values are in  $\mu\text{M}$  and are based on at least three independent dose–response curves.

<sup>b</sup> On rhabdomyosarcoma (RD) cells.

enteroviruses. Interestingly, except for a very faint cell protective effect in the assay for echovirus 11 (ECHO11,  $\text{EC}_{50} = 230 \pm 45 \mu\text{M}$ ), no antiviral activity was observed against any of the other viruses. This indicates that the antiviral activity of this compound class is most likely very selective for enterovirus 71 (Table 2).

To confirm that the compounds are selective inhibitors for EV71, their antiviral effect was evaluated in virus-cell-based assays for a panel of clinical isolates of EV71 that belong to different EV71 genogroups (Table 3).

Compound **3a** proved to be equipotent against all viruses of this panel, while for **7**, the activity ranged from  $0.9 \pm 1.8 \mu\text{M}$  for EV71 strain H08300 461 #812 (genogroup C2) to  $17 \pm 4 \mu\text{M}$  for EV71 strain TW/70902/08 (genogroup B5). Also the other virus isolate that belongs to genogroup B5 appeared to be less sensitive to the antiviral effect of this compound. Because all EV71 assays have been conducted using RD (rhabdomyosarcoma) cells, these results may indicate that the compound most likely has a mechanism of action that also involves a specific viral protein rather than only a cellular factor.

## 3. Conclusions

In the present study, we reported on the synthesis of  $\text{N}^6$ -isopentenyladenosine analogs and demonstrated that these cytokinin-like compounds have potential as inhibitors of the replication of EV71 in a mammalian bio-assay. To obtain a deeper

Table 2  
Antiviral effect of **3a** and **7** against a selection of enteroviruses.

Virus	Type	Strain	$\text{EC}_{50} \pm \text{SD}^a$	
			<b>3a</b>	<b>7</b>
EV	71	BrCr	$1.0 \pm 0.2$	$4.3 \pm 1.5$
PV	1	Sabin	>298	>362
	2		>298	>290
	3		>298	>290
CV	B3	Nancy	>298	>290
ECHO	11	Gregory	>224	$230 \pm 45$
hRV	2	/	>298	>290
	14		>298	>290

EV = enterovirus, PV = poliovirus, CV = Coxsackievirus, ECHO = echovirus, hRV = human rhinovirus.

<sup>a</sup> All values are in  $\mu\text{M}$  and are based on at least three independent dose–response curves.

**Table 3**  
Antiviral effect of **3a** and **7** against a selection of EV71 clinical isolates.

Genogroup	Strain	Genbank	EC <sub>50</sub> ± SD <sup>a</sup>	
			<b>3a</b>	<b>7</b>
B2	11316	AB575927	2.0 ± 0.1	8.0 ± 2.0
B5	TW/96016/08	GQ231942	2.3 ± 0.6	12 ± 3
	TW/70902/08	GQ231936	2.5 ± 0.5	17 ± 4
C2	H08300 461/812	–	1.2 ± 0.2	0.9 ± 1.8
C4	TW/1956/05	GQ231926	1.5 ± 0.2	3.9 ± 1.1
	TW/2429/04	GQ231927	1.4 ± 0.5	4.5 ± 1.3

<sup>a</sup> All values are in  $\mu\text{M}$  and are based on at least three independent dose–response curves.

insight into the structure–activity relationship of these compounds against EV71, additional sets should be prepared, which hopefully will allow the identification of a compound with a large window of selectivity. The availability of such a compound will be essential to initiate studies into the precise molecular mechanism of action by which enterovirus 71 replication is inhibited.

## 4. Experimental section

### 4.1. General

The solvents and materials were reagent grade and were used without additional purification. Column chromatography was performed on silica gel (Kieselgel 60 Merck, 0.063–0.200 mm). TLC was performed on Alugram SIL G/UV254 (Macherey–Nagel) with UV visualization. Melting points were determined on a Electro-thermal apparatus and are uncorrected.  $^1\text{H}$  and  $^{13}\text{C}$  (with complete proton decoupling) NMR spectra were recorded on Bruker AMX 400 NMR instrument at 300 K.  $^1\text{H}$  NMR-spectra were recorded at 400 MHz and  $^{13}\text{C}$  NMR-spectra at 100 MHz. Chemical shifts in ppm were measured relative to the residual solvent signals as internal standards ( $\text{CDCl}_3$ ,  $^1\text{H}$ : 7.26 ppm,  $^{13}\text{C}$ : 77.1 ppm;  $\text{DMSO}-d_6$ ,  $^1\text{H}$ : 2.50 ppm,  $^{13}\text{C}$ : 39.5 ppm) [23]. Spin–spin coupling constants ( $J$ ) are given in Hz. Double resonance technique was applied for assigning the resonances. High resolution mass spectra (HRMS) were measured on a Bruker micrOTOF II instrument using electrospray ionization (ESI) [24]. The measurements were done in a positive ion mode (interface capillary voltage – 4500 V) or in a negative ion mode (3200 V); mass range from  $m/z$  50 to  $m/z$  3000 Da; external or internal calibration was done with Electrospray Calibrant Solution (Fluka). A syringe injection was used for solutions in acetonitrile, methanol, or water (flow rate 3  $\mu\text{L}/\text{min}$ ). Nitrogen was applied as a dry gas; interface temperature was set at 180 °C. LC–MS analysis was performed on a 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 Monolithic C18 25 × 4.6 mm Part No CHO-7645 column. The eluent was 0.1%  $\text{HCOOH}$  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%.

### 4.2. Typical procedure for alkylation of tetraacetate **1** in DMF in the presence of $\text{K}_2\text{CO}_3$ (method A)

To a stirred mixture of tetraacetate **1** (500 mg, 1.15 mmol),  $\text{K}_2\text{CO}_3$  (0.32 g, 2.3 mmol) in 5 ml of DMF in one portion alkyl bromide (1.4 mmol) was added at room temperature. The reaction was monitored by TLC. After stirring for 20 h at ambient temperature,

the conversion was completed. The reaction mixture was diluted with  $\text{AcOEt}$  and the solution was washed with brine (3 × 20 ml). The extract was dried over  $\text{Na}_2\text{SO}_4$  and evaporated. The residue was applied to column chromatography on silica gel.

#### 4.2.1. $N^6$ -Acetyl- $N^6$ -isopentenyl-2',3',5'-tri- $O$ -acetyladenosine (**2a**)

A commercial isopentenyl bromide was used for the reaction. The yield of **2a** was 410 mg (67%) as a foam;  $R_f$  0.41 ( $\text{CH}_2\text{Cl}_2$ – $\text{EtOH}$ , 25:1).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.61 (s, 6H, Me), 2.10 (s, 3H, AcO), 2.13 (s, 3H, AcO), 2.16 (s, 3H, AcO), 2.28 (s, 3H, AcN), 4.39 (dd, 1H,  $J_{5'b,5'a}$  = 12.9,  $J_{5'b,4'}$  = 5.2, H-5'b), 4.45–4.49 (m, 2H, H-5'a + H-4'), 4.84 (d, 2H,  $J$  = 6.7,  $\text{NCH}_2$ ), 5.22–5.26 (m, 1H,  $\text{CH}_2\text{CH}=\text{C}$ ), 5.69 (dd, 1H,  $J_{3',4'}$  = 4.8,  $J_{3',2'}$  = 5.6, H-3'), 5.96 (dd, 1H,  $J_{2',3'}$  = 5.6,  $J_{2',1'}$  = 5.0, H-2'), 6.23 (d, 1H,  $J_{1',2'}$  = 5.0, H-1'), 8.19 (s, 1H, H-2), 8.79 (s, 1H, H-8).

#### 4.2.2. $N^6$ -Acetyl- $N^6$ -allyl-2',3',5'-tri- $O$ -acetyladenosine (**2b**)

A commercial allyl bromide was used for the reaction. The yield of **2b** (420 mg, 77%) as a foam;  $R_f$  0.58 ( $\text{CH}_2\text{Cl}_2$ – $\text{EtOH}$ , 25:1).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 2.10 (s, 3H, AcO), 2.12 (s, 3H, AcO), 2.15 (s, 3H, AcO), 2.32 (s, 3H, AcN), 4.39 (dd, 1H,  $J_{5'b,5'a}$  = 12.8,  $J_{5'b,4'}$  = 5.2, H-5'b), 4.46 (dd, 1H,  $J_{5'a,5'b}$  = 12.8,  $J_{5'a,4'}$  = 3.0, H-5'a), 4.47 (ddd, 1H,  $J_{4',5'b}$  = 5.2,  $J_{5'a,4'}$  = 3.0,  $J_{4',3'}$  = 4.9, H-4'), 4.88 (d, 1H,  $^3J$  = 5.1,  $\text{NCH}_2$ ), 5.04 (dd, 1H,  $^2J$  = 1.0,  $^3J$  = 10.3,  $\text{C}=\text{CH}$ -cis), 5.17 (dd,  $^2J$  = 1.0,  $^3J$  = 17.2,  $\text{C}=\text{CH}$ -trans), 5.69 (dd, 1H,  $J_{3',4'}$  = 4.9,  $J_{3',2'}$  = 5.4, H-3'), 5.89 (ddd,  $^3J$  = 5.1, 10.3 and 17.2,  $\text{CH}_2\text{CH}=\text{C}$ ), 5.96 (dd, 1H,  $J_{2',3'}$  = 5.4,  $J_{2',1'}$  = 5.0, H-2'), 6.23 (d, 1H,  $J_{1',2'}$  = 5.0, H-1'), 8.19 (s, 1H, H-2), 8.78 (s, 1H, H-8).

#### 4.2.3. $N^6$ -Acetyl- $N^6$ -propargyl-2',3',5'-tri- $O$ -acetyladenosine (**2c**)

A commercial solution of propargyl bromide in toluene (80% wt) was used for the reaction. The yield of **2c** was 463 mg (85%) as a foam;  $R_f$  0.42 ( $\text{CH}_2\text{Cl}_2$ – $\text{EtOH}$ , 25:1).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 2.10 (s, 3H, AcO), 2.12 (t, 1H,  $J$  = 2.5,  $\text{HC}\equiv\text{C}$ ), 2.13 (s, 3H, AcO), 2.16 (s, 3H, AcO), 2.39 (s, 3H, AcN), 4.40 (dd, 1H,  $J_{5'b,5'a}$  = 12.9,  $J_{5'b,4'}$  = 5.2, H-5'b), 4.47 (dd, 1H,  $J_{5'a,5'b}$  = 12.9,  $J_{5'a,4'}$  = 3.1, H-5'a), 4.48 (ddd, 1H,  $J_{4',5'b}$  = 5.2,  $J_{4',5'a}$  = 3.1,  $J_{4',3'}$  = 4.8, H-4'), 5.10 (d, 2H,  $J$  = 2.5,  $\text{CH}_2\text{N}$ ), 5.68 (dd, 1H,  $J_{3',4'}$  = 4.8,  $J_{3',2'}$  = 5.6, H-3'), 5.97 (dd, 1H,  $J_{2',3'}$  = 5.6,  $J_{2',1'}$  = 5.0, H-2'), 6.25 (d, 1H,  $J_{1',2'}$  = 5.0, H-1'), 8.22 (s, 1H, H-2), 8.82 (s, 1H, H-8).

### 4.3. $N^6$ -Isopentenyladenosine (**3a**)

The tetraacetate **2a** (500 mg, 0.99 mmol) was dissolved in 7 M  $\text{NH}_3$  in MeOH (3 ml, 21 mmol) and the solution was left for 48 h at ambient temperature. The final reaction mixture was evaporated in vacuum and the residue was crystallized from water. The product was dried in vacuum over  $\text{P}_2\text{O}_5$ .

The yield of **3a** was 124 mg, (33%) as a white powder;  $R_f$  0.45 ( $\text{CH}_2\text{Cl}_2$ – $\text{EtOH}$ , 4:1). mp 130–132 °C ( $\text{H}_2\text{O}$ ); Lit.: Mp 134–136 °C [14].  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  = 1.66 (s, 3H, Me), 1.69 (s, 3H, Me), 3.55 (ddd, 1H,  $J_{5'b,5'a}$  = 12.1,  $J_{5'b,4'}$  = 3.0,  $J_{5'b,\text{OH}}$  = 7.9, H-5'b), 3.67 (ddd, 1H,  $J_{5'a,5'b}$  = 12.1,  $J_{5'a,4'}$  = 3.6,  $J_{5'a,\text{OH}}$  = 4.6, H-5'a), 3.96 (ddd, 1H,  $J_{4',5'b}$  = 3.0,  $J_{4',5'a}$  = 3.6,  $J_{4',3'}$  = 2.8, H-4'), 4.14 (ddd, 1H,  $J_{3',4'}$  = 2.8,  $J_{3',2'}$  = 5.3,  $J_{3',\text{OH}}$  = 4.8, H-3'), 4.08 (br s, 2H,  $\text{NCH}_2\text{C}$ ), 4.60 (ddd, 1H,  $J_{2',3'}$  = 5.3,  $J_{2',1'}$  = 6.0,  $J_{2',\text{OH}}$  = 6.2, H-2'), 5.16 (d, 1H,  $J_{\text{OH},3'}$  = 4.8, 3'-OH, exchangeable with  $\text{D}_2\text{O}$ ), 5.30 (dd, 1H,  $J_{\text{OH},5'b}$  = 7.9,  $J_{\text{OH},5'a}$  = 4.6, 5'-OH, exchangeable with  $\text{D}_2\text{O}$ ), 5.37–5.47 (m, 2H,  $J_{\text{OH},2'}$  = 6.2,  $\text{CCH}=\text{C}$ , 2'-OH, exchangeable with  $\text{D}_2\text{O}$ ), 5.87 (d, 1H,  $J_{1',2'}$  = 6.0, H-1'), 7.82 (br s, 1H, NH, exchangeable with  $\text{D}_2\text{O}$ ), 8.19 (br s, 1H, H-2), 8.32 (s, 1H, H-8).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  = 18.48 (Me), 26.05 (Me), 38.57 ( $\text{CH}_2\text{N}$ ), 62.24 (C-5'), 71.22 (C-3'), 74.24 (C-2'), 86.59 (C-4'), 88.82 (C-1'), 120.13 (C-5), 121.76 ( $\text{CH}=\text{C}$ ), 135.44 ( $=\text{CMe}_2$ ), 140.52 (C-8), 148.77 (C-4), 153.17 (C-2), 154.97 (C-6). MS (APCI):  $m/z$  [ $\text{M}+\text{H}^+$ ] calculated [ $\text{C}_{15}\text{H}_{22}\text{N}_5\text{O}_4$ ] 336.17, found

336.23;  $m/z$  [ $M-H^- + HCOOH$ ] calculated [ $C_{16}H_{22}N_5O_6$ ] 380.16, found 380.20.

#### 4.4. $N^6$ -Allyl adenosine (**3b**)

Tetraacetate **2b** (400 mg, 0.841 mmol) was dissolved in 7 M  $NH_3$  in MeOH (2.4 ml, 16.8 mmol) and the solution was left for 48 h at ambient temperature. The precipitate formed was filtered off and washed with ether (10 ml). The product was dried in vacuum over  $P_2O_5$ . The yield of **3b** was 182 mg (70%) as a white powder;  $R_f$  0.25 ( $CH_2Cl_2$ –EtOH, 9:1). mp 165–167 °C (MeOH); Lit.: Mp 167 °C [25].  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 3.56 (m, 1H, H-5'b), 3.66 (m 1H, H-5'a), 3.97 (m 1H,  $J$  = 2.8, H-4'), 4.15 (br s  $CH_2N$ , H-3'), 4.62 (m, 1H, OH), 5.05 (d,  $J$  = 10.3, C=CH-cis), 5.13 (m, 1H, OH), 5.15 (d,  $J$  = 17.0, C=CH-trans), 5.34 (m, 1H, H-2'), 5.40 (m, 1H, OH), 5.94–6.13 (m, 2H, overlapping signals of  $CH_2CH=C$  and H-1'), 7.98 (br s, 1H, NH), 8.20 (s, 1H, H-2), 8.34 (s, 1H, H-8).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  = 33.56 ( $NCH_2$ ), 61.66 (C-5'), 70.64 (C-3'), 73.49 (C-2'), 85.89 (C-4'), 87.95 (C-1'), 115.02 (CH=), 118.75 (C-5), 135.60 (=CH<sub>2</sub>), 139.78 (C8), 152.30 (C-2), 154.49 (C-6). HRMS:  $m/z$  [ $M+H$ ]<sup>+</sup> calculated [ $C_{13}H_{18}N_5O_4$ ] 308.1353, found 308.1357;  $m/z$  [ $M+Na$ ]<sup>+</sup> calculated [ $C_{13}H_{17}N_5O_4Na$ ] 330.1173, found 330.1176.

#### 4.5. $N^6$ -Propargyl adenosine (**3c**)

The tetraacetate **2c** (500 mg, 1.06 mmol) was dissolved in 7 M  $NH_3$  in MeOH (3 ml, 21 mmol) and the solution was left for 48 h at ambient temperature. The final reaction mixture was evaporated in vacuum and the residue was crystallized from EtOH–H<sub>2</sub>O. The product was dried in vacuum over  $P_2O_5$ .

The yield of **3c** was 145 mg (41%) as a white powder;  $R_f$  0.33 ( $CH_2Cl_2$ –EtOH, 9:1). mp 171–174 °C (EtOH–H<sub>2</sub>O); Lit.: Mp 169 °C [23].  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 3.01 (t, 1H,  $J$  = 2.3, HC≡C), 3.56 (ddd, 1H,  $J_{5'b,5'a}$  = 12.0,  $J_{5'b,4'}$  = 3.7,  $J_{5'b,OH}$  = 7.2, H-5'b), 3.68 (ddd, 1H,  $J_{5'a,5'b}$  = 12.0,  $J_{5'a,4'}$  = 3.9,  $J_{5'a,OH}$  = 4.3, H-5'a), 3.97 (ddd, 1H,  $J_{4',5'b}$  = 3.7,  $J_{4',5'a}$  = 3.9,  $J_{4',3'}$  = 2.8, H-4'), 4.16 (ddd, 1H,  $J_{3',4'}$  = 2.8,  $J_{3',2'}$  = 5.0,  $J_{3',OH}$  = 4.7, H-3'), 4.29 (br s, 2H,  $NCH_2Ph$ ), 4.60 (ddd, 1H,  $J_{2',3'}$  = 5.0,  $J_{2',1'}$  = 5.9,  $J_{2',OH}$  = 6.2, H-2'), 5.14 (d, 1H,  $J_{OH,3'}$  = 4.7, 3'-OH, exchangeable with D<sub>2</sub>O), 5.28 (dd, 1H,  $J_{OH,5'b}$  = 7.2,  $J_{OH,5'a}$  = 4.3, 5'-OH, exchangeable with D<sub>2</sub>O), 5.41 (d, 1H,  $J_{OH,2'}$  = 6.2, 2'-OH, exchangeable with D<sub>2</sub>O), 5.90 (d, 1H,  $J_{1',2'}$  = 5.9, H-1'), 8.18 (br s, 1H, NH, exchangeable with D<sub>2</sub>O), 8.28 (br s, 1H, H-2), 8.39 (s, 1H, H-8).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  = 29.38 ( $CH_2N$ ), 61.69 (C-5'), 70.67 (C-3'), 72.47 (HC≡), 73.66 (C-2'), 81.88 (–C≡), 85.94 (C-4'), 88.03 (C-1'), 119.93 (C-5), 140.25 (C-8), 148.88 (C-4), 152.32 (C-2), 154.07 (C-6). MS (APCI):  $m/z$  [ $M+H$ ]<sup>+</sup> calculated [ $C_{13}H_{16}N_5O_4$ ] 306.12, found 306.20;  $m/z$  [ $M-H^- + HCOOH$ ] calculated [ $C_{14}H_{16}N_5O_6$ ] 350.11, found 350.14.

#### 4.6. Typical procedure for preparation of nucleosides **3d–k** by Mitsunobu reaction of tetraacetate **1** with alcohols (method B)

A mixture of **1** (435 mg, 1 mmol),  $Ph_3P$  (393 mg, 1.5 mmol) and corresponding alcohol (1.5 mmol) in 5 ml of THF was stirred at room temperature until a homogeneous solution was formed. After DEAD (0.24 ml, 1.5 mmol) was added in one portion, the stirring was continued at ambient temperature. The reaction was monitored by TLC (silica gel,  $CH_2Cl_2$ –EtOH = 97:3). If the conversion of **1** was not completed after 20 h, the additional portions of reagents ( $Ph_3P$ , alcohol and DEAD) in above indicated quantities were added to achieve full conversion of **1**. After 4–5 h, the reaction mixture was evaporated and the residue was applied to column chromatography (silica gel,  $CH_2Cl_2$ –EtOH = 97:3). Partially purified compound was dissolved in 4 M  $PrNH_2$  in MeOH solution (50 mmol) and was left for 24 h, after which the mixture was evaporated and

the residue was applied to column chromatography. The product was eluted with  $CH_2Cl_2$ –EtOH (9:1 v/v) mixture.

#### 4.6.1. $N^6$ -(4-Hydroxy-2-butenyl) adenosine (**3d**)

The yield of **3d** was 203 mg (61%, white powder);  $R_f$  0.13 ( $CH_2Cl_2$ –EtOH, 9:1). mp 160–162 °C (EtOH–H<sub>2</sub>O).  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 3.56 (ddd, 1H,  $J_{5'b,5'a}$  = 11.9,  $J_{5'b,4'}$  = 4.1,  $J_{5'b,OH}$  = 7.0, H-5'b), 3.68 (ddd, 1H,  $J_{5'a,5'b}$  = 12.0,  $J_{5'a,4'}$  = 3.6,  $J_{5'a,OH}$  = 4.6, H-5'a), 3.97 (ddd, 1H,  $J_{4',5'b}$  = 4.1,  $J_{4',5'a}$  = 3.6,  $J_{4',3'}$  = 2.8, H-4'), 4.02 (dt, 2H,  $^5J$  = 1.7,  $^3J_{CH-OH}$  = 5.8,  $OCH_2C\equiv$ ), 4.15 (ddd, 1H,  $J_{3',4'}$  = 2.8,  $J_{3',2'}$  = 5.1,  $J_{3',OH}$  = 4.8, H-3'), 4.32 (br s, 2H,  $NCH_2C\equiv$ , H-3'), 4.60 (ddd, 1H,  $J_{2',3'}$  = 5.1,  $J_{2',1'}$  = 6.0,  $J_{2',OH}$  = 6.2, H-2'), 5.10 (t,  $^3J_{Hq-CH}$  = 5.8,  $\omega$ -OH, exchangeable with D<sub>2</sub>O), 5.17 (d, 1H,  $J_{OH,3'}$  = 4.8, 3'-OH, exchangeable with D<sub>2</sub>O), 5.34 (dd, 1H,  $J_{OH,5'b}$  = 7.0,  $J_{OH,5'a}$  = 4.6, 5'-OH, exchangeable with D<sub>2</sub>O), 5.43 (d, 1H,  $J_{OH,2'}$  = 6.2, 2'-OH, exchangeable with D<sub>2</sub>O), 5.90 (d, 1H,  $J_{1',2'}$  = 6.0, H-1'), 8.14 (br s, 1H, NH, exchangeable with D<sub>2</sub>O), 8.27 (s, 1H, H-2), 8.38 (s, 1H, H-8).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  = 29.48 ( $CH_2N$ ), 49.12 ( $CH_2OH$ ), 61.70 (C-5'), 70.67 (C-3'), 73.66 (C-2'), 81.41 (C≡), 81.73 (C≡), 85.96 (C-4'), 88.04 (C-1'), 119.94 (C-5), 140.21 (C-8), 148.83 (C-4), 152.35 (C-2), 154.06 (C-6). MS (APCI):  $m/z$  [ $M+H$ ]<sup>+</sup> calculated [ $C_{14}H_{18}N_5O_5$ ] 336.13, found 336.18;  $m/z$  [ $M-H^- + HCOOH$ ] calculated [ $C_{15}H_{18}N_5O_7$ ] 380.12, found 380.14.

#### 4.6.2. $N^6$ -(Z)-(4-Hydroxy-2-butenyl) adenosine (**3e**)

The yield of **3e** was 141 mg (42%, white powder);  $R_f$  0.43 ( $CH_2Cl_2$ –EtOH, 9:1). mp 159–160 °C (EtOH–H<sub>2</sub>O).  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 3.55 (ddd, 1H,  $J_{5'b,5'a}$  = 12.0,  $J_{5'b,4'}$  = 3.6,  $J_{5'b,OH}$  = 7.2, H-5'b), 3.67 (ddd, 1H,  $J_{5'a,5'b}$  = 12.1,  $J_{5'a,4'}$  = 3.8,  $J_{5'a,OH}$  = 4.6, H-5'a), 3.96 (ddd, 1H,  $J_{4',5'b}$  = 3.6,  $J_{4',5'a}$  = 3.8,  $J_{4',3'}$  = 2.8, H-4'), 4.07–4.23 (m, 5H, overlapping  $OCH_2C\equiv$ ,  $NCH_2C\equiv$ , H-3'), 4.60 (ddd, 1H,  $J_{2',3'}$  = 4.9,  $J_{2',1'}$  = 6.0,  $J_{2',OH}$  = 6.2, H-2'), 4.71 (t,  $J$  = 5.3,  $\omega$ -OH, exchangeable with D<sub>2</sub>O), 5.15 (d, 1H,  $J_{OH,3'}$  = 4.6, 3'-OH, exchangeable with D<sub>2</sub>O), 5.37 (dd, 1H,  $J_{OH,5'b}$  = 7.2,  $J_{OH,5'a}$  = 4.6, 5'-OH, exchangeable with D<sub>2</sub>O), 5.41 (d, 1H,  $J_{OH,2'}$  = 6.2, 2'-OH, exchangeable with D<sub>2</sub>O), 5.88 (d, 1H,  $J_{1',2'}$  = 6.0, H-1'), 7.91 (br s, 1H, NH, exchangeable with D<sub>2</sub>O), 8.20 (s, 1H, H-2), 8.34 (s, 1H, H-8).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  = 37.86 ( $CH_2N$ ), 57.70 ( $CH_2OH$ ), 62.28 (C-5'), 71.26 (C-3'), 74.29 (C-2'), 86.64 (C-4'), 88.85 (C-1'), 119.83 (C-5), 127.99 (CH=), 132.56 (CH=), 140.75 (C-8), 148.68 (C-4), 153.19 (C-2), 155.07 (C-6). MS (APCI):  $m/z$  [ $M+H$ ]<sup>+</sup> calculated [ $C_{14}H_{20}N_5O_5$ ] 338.13, found 338.20;  $m/z$  [ $M-H^- + HCOOH$ ] calculated [ $C_{15}H_{20}N_5O_7$ ] 382.12, found 382.15.

#### 4.6.3. $N^6$ -Geranyl adenosine (**3f**)

The yield of **3f** was 155 mg (39%) as a white powder;  $R_f$  0.13 ( $CH_2Cl_2$ –EtOH, 97:3). mp 137–140 °C (with decomposition).  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 1.54 (s, 3H,  $CH_3$ ), 1.59 (s, 3H,  $CH_3$ ), 1.70 (s, 3H,  $CH_3$ ), 1.92–1.99 (m, 2H,  $CH_2CH_2CH=$ ), 2.00–2.08 (m, 2H,  $CH_2CH_2CH$ ), 3.55 (ddd, 1H,  $J_{5'b,4'}$  = 3.6,  $J_{5'b,5'a}$  = –12.0,  $J_{OH,5'b}$  = 7.3, H5'b), 3.68 (ddd, 1H,  $J_{5'a,5'b}$  = 12.0,  $J_{5'a,4'}$  = 3.6,  $J_{OH,5'a}$  = 4.6, H5'a), 3.96 (q, 1H,  $J_{4',3'}$  = 3.6,  $J_{4',5'a}$  = 3.6,  $J_{4',5'b}$  = 3.6 Hz, H4'), 4.09 (br s, 2H,  $NHCH_2$ ), 4.15 (ddd, 1H,  $J_{3',2'}$  = 5.2,  $J_{3',4'}$  = 3.6,  $J_{OH,3'}$  = 4.6, H3'), 4.60 (ddd, 1H,  $J_{2',1'}$  = 6.1,  $J_{2',3'}$  = 5.2,  $J_{OH,2'}$  = 6.2, H2'), 5.06 (tt, 1H,  $J_{CH-CH_2}$  = 7.0,  $J_{CH-Me}$  = 1.4 (trans)  $CHCMe_2$ ), 5.13 (d, 1H,  $J_{OH,3'}$  = 4.6, 3'-OH, exchangeable with D<sub>2</sub>O), 5.31 (br t,  $J_{CH-CH_2}$  = 6.5 Hz,  $NHCH_2CH$ ), 5.36 (dd, 1H,  $J_{5'a,5'b}$  = 12.0,  $J_{OH,5'b}$  = 7.2 Hz,  $J_{OH,5'a}$  = 4.6 Hz, 5'-OH, exchangeable with D<sub>2</sub>O), 5.39 (d, 1H,  $J_{OH,2'}$  = 6.2 Hz, 2'-OH, exchangeable with D<sub>2</sub>O), 5.88 (d, 1H,  $J_{1',2'}$  = 6.1 Hz, H1'), 7.84 (br s, 1H, NH), 8.19 (s, 1H, H8), 8.32 (s, 1H, H2).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  = 16.12, 17.48, 25.38, 25.94, (CH<sub>2</sub>, CH<sub>2</sub>, CH<sub>3</sub>, CH<sub>3</sub>), 37.68 ( $NHCH_2$ ), 61.63 (C5'), 70.65 (C3'), 73.48 (C2'), 85.84 (C4'), 87.94 (C1'), 119.75 (C5), 121.73 (CH=), 123.91 (CH=), 130.75 (=C(Me)CH<sub>2</sub>), 136.64 (=CMe<sub>2</sub>), 139.59 (C8), 148.30 (C4), 152.27 (C2), 154.35 (C6). HRMS:  $m/z$  [ $M+H$ ]<sup>+</sup> calculated



[C<sub>20</sub>H<sub>30</sub>N<sub>5</sub>O<sub>4</sub>] 404.2292, found 404.2284; *m/z* [M+Na]<sup>+</sup> calculated [C<sub>20</sub>H<sub>29</sub>N<sub>5</sub>O<sub>4</sub>Na] 426.2112, found 426.2102.

#### 4.6.4. N<sup>6</sup>-Neryladenosine (**3g**)

The yield of **3g** was 192 mg (48%) as a white powder; *R<sub>f</sub>* 0.15 (CH<sub>2</sub>Cl<sub>2</sub>–EtOH, 97:3). mp 129–130 °C (with decomposition). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ = 1.58 (s, 3H, CH<sub>3</sub>), 1.64 (s, 3H, CH<sub>3</sub>), 1.67 (s, 3H, CH<sub>3</sub>), 2.03–2.16 (m, 4H, =C(Me)CH<sub>2</sub>CH<sub>2</sub>, CH<sub>2</sub>CH<sub>2</sub>CH=), 3.55 (ddd, 1H, *J*<sub>5'b,5'a</sub> = 12.2, *J*<sub>5'b,4'</sub> = 3.5, *J*<sub>5'b,OH</sub> = 6.5, H5'b), 3.67 (ddd, 1H, *J*<sub>5'a,5'b</sub> = 12.2, *J*<sub>5'a,4'</sub> = 3.5, *J*<sub>5'a,OH</sub> = 3.8, H5'a), 3.96 (q, 1H, *J*<sub>4',3'</sub> = 3.5, *J*<sub>4',5'a</sub> = 3.5, *J*<sub>4',5'b</sub> = 3.5, H4'), 4.09 (br s, 2H, NHCH<sub>2</sub>), 4.15 (ddd, 1H, *J*<sub>OH,3'</sub> = 4.6, *J*<sub>3',2'</sub> = 4.5, *J*<sub>3',4'</sub> = 3.5, H3'), 4.60 (ddd, 1H, *J*<sub>2',1'</sub> = 6.1, *J*<sub>2',3'</sub> = 4.5, *J*<sub>OH,2'</sub> = 6.3, H2'), 5.10–5.16 (m, 2H, overlapping 3'OH, CHCMe<sub>2</sub>), 5.31 (br t, *J*<sub>CH–CH<sub>2</sub></sub> = 6.6, NHCH<sub>2</sub>CH), 5.35 (dd, 1H, *J*<sub>OH,5'b</sub> = 6.5, *J*<sub>OH,5'a</sub> = 3.8, 5'OH, exchangeable with D<sub>2</sub>O), 5.38 (d, 1H, *J*<sub>OH,2'</sub> = 6.3 Hz, 2'OH, exchangeable with D<sub>2</sub>O), 5.88 (d, 1H, *J*<sub>1',2'</sub> = 6.1, H1'), 7.80 (br s, 1H, NH), 8.18 (s, 1H, H8), 8.32 (s, 1H, H2). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ = 17.52, 23.09, 25.48, 26.08, 31.67, (CH<sub>2</sub>, CH<sub>2</sub>, CH<sub>3</sub>, CH<sub>3</sub>, CH<sub>3</sub>), 37.36 (NHCH<sub>2</sub>), 61.67 (C5'), 70.64 (C3'), 73.50 (C2'), 85.88 (C4'), 87.95 (C1'), 119.80 (C5), 122.68 (CH=), 124.05 (CH=), 131.07 (=C(Me)CH<sub>2</sub>), 136.85 (=CMe<sub>2</sub>), 139.64 (C8), 148.30 (C4), 152.29 (C2), 154.39 (C6). HRMS: *m/z* [M+H]<sup>+</sup> calculated [C<sub>20</sub>H<sub>30</sub>N<sub>5</sub>O<sub>4</sub>] 404.2292, found 404.2286; *m/z* [M+Na]<sup>+</sup> calculated [C<sub>20</sub>H<sub>29</sub>N<sub>5</sub>O<sub>4</sub>Na] 426.2112, found 426.2105.

#### 4.6.5. N<sup>6</sup>-[(S)-(-)-Perillyl]-adenosine (**3h**)

The yield of **3h** was 241 mg (60%) as a white powder; *R<sub>f</sub>* 0.11 (CH<sub>2</sub>Cl<sub>2</sub>–EtOH, 97:3). mp 158–160 °C (with decomposition). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ = 1.32–1.45 (m, 1H, H-perillyl), 1.68 (s, 3H, CH<sub>3</sub>-perillyl), 1.71–1.91 (m, 2H, CH<sub>2</sub>-perillyl), 1.98–2.13 (m, 4H, 2 × CH<sub>2</sub>-perillyl), 3.55 (dd, 1H, *J*<sub>5'b,5'a</sub> = 12.1, *J*<sub>5'b,4'</sub> = 3.2, H5'b), 3.68 (dd, 1H, *J*<sub>5'a,5'b</sub> = 12.1, *J*<sub>5'a,4'</sub> = 3.4, H5'a), 3.96 (ddd, 1H, *J*<sub>4',3'</sub> = 3.3, *J*<sub>4',5'a</sub> = 3.4, *J*<sub>4',5'b</sub> = 3.2, H4'), 4.03 (br.s, 2H, NHCH<sub>2</sub>), 4.12–4.18 (m, 1H, H3'), 4.62 (dd, 1H, *J*<sub>2',1'</sub> = 6.1, *J*<sub>2',3'</sub> = 5.2, H2'), 4.68 (s, 2H, C(CH<sub>3</sub>)=CH<sub>2</sub>–perillyl), 5.13 (br s, 1H, 3'OH, exchangeable with D<sub>2</sub>O), 5.43–5.30 (m, 2H, overlapping 5'OH, =CHCH<sub>2</sub>–perillyl), 5.53 (br s, 1H, 2'OH, exchangeable with D<sub>2</sub>O), 5.88 (d, 1H, *J*<sub>1',2'</sub> = 6.1, H1'), 7.89 (br s, 1H, NH), 8.19 (s, 1H, H8), 8.34 (s, 1H, H2). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ = 20.54, 26.57, 27.06, 29.81, (CH<sub>2</sub>, CH<sub>2</sub>, CH<sub>2</sub>, CH<sub>3</sub>), 40.52 (CHC(Me)=), 44.53 (NHCH<sub>2</sub>), 61.65 (C5'), 70.63 (C3'), 73.42 (C2'), 85.88 (C4'), 87.94 (C1'), 108.77 (CH(Me)CCH<sub>2</sub>), 119.68 (C5), 120.23 (=CH), 134.77 (C(Me)=), 139.67 (C8), 148.32 (C4), 149.25 (NHCH<sub>2</sub>C=), 152.28 (C2), 154.68 (C6). HRMS: *m/z* [M+H]<sup>+</sup> calculated [C<sub>20</sub>H<sub>28</sub>N<sub>5</sub>O<sub>4</sub>] 402.2136, found 402.2135; *m/z* [M+Na]<sup>+</sup> calculated [C<sub>20</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>Na] 424.1955, found 424.1948.

#### 4.6.6. N<sup>6</sup>-[(1R)-(-)-Myrtenyl]-adenosine (**3i**)

The yield of **3i** was 130 mg (33%) as white foam; *R<sub>f</sub>* 0.11 (CH<sub>2</sub>Cl<sub>2</sub>–EtOH, 97:3). mp 168–169 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ = 0.76 (s, 3H, CH<sub>3</sub>-myr), 1.10 (d, 1H, *J* = 8.4, H-myrtanyl), 1.22 (s, 3H, CH<sub>3</sub>-myrtanyl), 2.26–2.00 (m, 4H, myrtanyl), 2.34 (dt, 1H, *J*<sub>CHaHb</sub> = 8.4, *J*<sub>CHa–CH1</sub> = *J*<sub>CHa–CH2</sub> = 5.7, CH<sub>3</sub>Hb-myrtanyl), 3.55 (ddd, 1H, *J*<sub>5'b,5'a</sub> = 12.1, *J*<sub>5'b,4'</sub> = 3.7 Hz, *J*<sub>OH,5'b</sub> = 7.3, H5'b), 3.67 (ddd, 1H, *J*<sub>5'a,5'b</sub> = 12.1, *J*<sub>OH,5'a</sub> = 4.3, *J*<sub>5'a,4'</sub> = 3.7, H5'a), 3.96 (td, 1H, *J*<sub>4',3'</sub> = 3.4, *J*<sub>4',5'a</sub> = 3.7, *J*<sub>4',5'b</sub> = 3.7, H4'), 4.07 (br.s, 2H, NHCH<sub>2</sub>), 4.14 (ddd, 1H, *J*<sub>3',2'</sub> = 4.7, *J*<sub>3',4'</sub> = 3.7, *J*<sub>OH,3'</sub> = 4.6, H3'), 4.62 (ddd, 1H, *J*<sub>2',1'</sub> = 6.3 Hz, *J*<sub>2',3'</sub> = 4.7 Hz, *J*<sub>OH,2'</sub> = 6.2 Hz, H2'), 5.13 (d, 1H, *J*<sub>OH,3'</sub> = 4.6 Hz, 3'OH, exchangeable with D<sub>2</sub>O), 5.32–5.38 (m, 2H, overlapping =CH–myrtanyl, 5'OH), 5.39 (d, 1H, *J*<sub>OH,2'</sub> = 6.2 Hz, 2'OH), 5.88 (d, 1H, *J*<sub>1',2'</sub> = 6.3 Hz, H1'), 7.83 (br s, 1H, NH), 8.17 (s, 1H, H8), 8.32 (s, 1H, H2). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ = 20.89, 26.04, 30.61, 31.02, 37.61, 43.29, (myrtanyl), 43.79 (NHCH<sub>2</sub>), 61.65 (C5'), 70.63 (C3'), 73.40 (C2'), 85.87 (C4'), 87.88 (C1'), 116.41 (=CHCH<sub>2</sub>–), 119.55 (C5), 139.61 (C8), 145.30 (NHCH<sub>2</sub>C=), 148.50 (C4), 152.20 (C2), 154.65 (C6). HRMS: *m/z* [M+H]<sup>+</sup> calculated [C<sub>20</sub>H<sub>28</sub>N<sub>5</sub>O<sub>4</sub>] 402.2136, found

402.2133; *m/z* [M+Na]<sup>+</sup> calculated [C<sub>20</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub>Na] 424.1955, found 424.1946.

#### 4.6.7. N<sup>6</sup>-(Cyclopropylmethyl)adenosine (**3j**)

The yield of **3j** was 110 mg (36%) as a white powder; *R<sub>f</sub>* = 0.08 (CH<sub>2</sub>Cl<sub>2</sub>–EtOH, 97:3). mp 176–177 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ = 0.27 (td, 2H, *J*<sub>CH<sub>2</sub>CH<sub>2</sub></sub> = 5.8 Hz, *J*<sub>CH<sub>2</sub>CH</sub> = 4.2 Hz, CH<sub>2</sub>-cyclopropyl), 0.41 (td, 2H, *J*<sub>CH<sub>2</sub>CH<sub>2</sub></sub> = 6.0 Hz, *J*<sub>CH<sub>2</sub>CH</sub> = 4.2 Hz, CH<sub>2</sub>-cyclopropyl), 1.21–1.08 (m, 1H, NHCH<sub>2</sub>CH), 3.17 (d, 1H, *J*<sub>OH,2'</sub> = 5.5 Hz, 2'OH), 3.37 (br s, 1H, 3'OH, exchangeable with D<sub>2</sub>O), 3.55 (ddd, 1H, *J*<sub>5'b,4'</sub> = 3.5 Hz, *J*<sub>5'b,5'a</sub> = 12.0 Hz, *J*<sub>OH,5'b</sub> = 6.8 Hz, H5'b), 3.68 (ddd, 1H, *J*<sub>5'a,4'</sub> = 4.0 Hz, *J*<sub>5'a,5'b</sub> = 12.0 Hz, *J*<sub>OH,5'a</sub> = 4.9 Hz, H5'a), 3.95 (ddd, 1H, *J*<sub>4',3'</sub> = 7.2 Hz, *J*<sub>4',5'a</sub> = 4.0 Hz, *J*<sub>4',5'b</sub> = 3.5 Hz, H4'), 4.15 (dd, *J*<sub>3',2'</sub> = 4.7 Hz, *J*<sub>3',4'</sub> = 7.4 Hz, H3'), 4.51 (dd, 1H, *J*<sub>OH,5'a</sub> = 4.9 Hz, *J*<sub>OH,5'b</sub> = 6.8 Hz, 5'OH), 4.61 (ddd, 1H, *J*<sub>2',1'</sub> = 6.0 Hz, *J*<sub>2',3'</sub> = 4.7 Hz, *J*<sub>OH,2'</sub> = 5.5 Hz, H2'), 5.48–5.34 (m, 2H, NHCH<sub>2</sub>), 5.90 (d, 1H, *J*<sub>1',2'</sub> = 6.0 Hz, H1'), 7.88 (br s, 1H, NH), 8.18 (s, 1H, H8), 8.33 (s, 1H, H2). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ = 3.21 (CH<sub>2</sub>, CH<sub>2</sub>), 11.13 (CH<sub>2</sub>CH), 43.97 (NHCH<sub>2</sub>), 61.66 (C5'), 70.63 (C3'), 73.46 (C2'), 85.88 (C4'), 87.96 (C1'), 119.68 (C5), 139.63 (C8), 148.39 (C4), 152.29 (C2), 154.64 (C6). HRMS: *m/z* [M+H]<sup>+</sup> calculated [C<sub>14</sub>H<sub>20</sub>N<sub>5</sub>O<sub>4</sub>] 322.1509, found 322.1510; *m/z* [M+Na]<sup>+</sup> calculated [C<sub>14</sub>H<sub>19</sub>N<sub>5</sub>O<sub>4</sub>Na] 344.1329, found 344.1326.

#### 4.6.8. N<sup>6</sup>-(Tetrahydropyran-2-methyl)-adenosine (**3k**)

The yield of **3e** was 187 mg (51%) as a white powder; *R<sub>f</sub>* = 0.02 (CH<sub>2</sub>Cl<sub>2</sub>–EtOH, 97:3). mp 145–147 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ = 1.85–1.13 (m, 6H, tetrahydropyran), 3.37–3.25 (m, 1H, tetrahydropyran, overlapping with D<sub>2</sub>O), 3.50–3.40 (m, 1H, tetrahydropyran), 3.51 (br s, 2H, NHCH<sub>2</sub>), 3.57 (ddd, 1H, *J*<sub>5'b,4'</sub> = 3.6 Hz, *J*<sub>5'b,5'a</sub> = –12.1 Hz, *J*<sub>OH,5'b</sub> = 6.9 Hz, H5'b), 3.68 (ddd, 1H, *J*<sub>5'a,4'</sub> = 3.8 Hz, *J*<sub>5'a,5'b</sub> = –12.1 Hz, *J*<sub>OH,5'a</sub> = 4.4 Hz, H5'a), 3.90–3.83 (m, 1H, tetrahydropyran), 3.96 (ddd, 1H, *J*<sub>4',3'</sub> = 3.4 Hz, *J*<sub>4',5'a</sub> = 3.4 Hz, *J*<sub>4',5'b</sub> = 3.4 Hz, H4'), 4.15 (ddd, 1H, *J*<sub>3',2'</sub> = 4.7 Hz, *J*<sub>3',4'</sub> = 3.2 Hz, *J*<sub>OH,3'</sub> = 4.6 Hz, H3'), 4.61 (dd, 1H, *J*<sub>2',1'</sub> = 6.0 Hz, *J*<sub>2',3'</sub> = 4.7 Hz, *J*<sub>OH,2'</sub> = 6.2 Hz, H2'), 5.13 (d, 1H, *J*<sub>OH,3'</sub> = 4.6 Hz, 3'OH), 5.34 (dd, 1H, *J*<sub>OH,5'b</sub> = 6.9 Hz, *J*<sub>OH,5'a</sub> = 4.4 Hz, 5'OH), 5.39 (d, 1H, *J*<sub>OH,2'</sub> = 6.2 Hz, 2'OH), 5.88 (d, 1H, *J*<sub>1',2'</sub> = 6.1 Hz, H1'), 7.59 (br s, 1H, NH), 8.20 (s, 1H, H8), 8.34 (s, 1H, H2). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ = 29.07, 25.65, 22.59 (tetrahydropyran), 44.67 (NHCH<sub>2</sub>), 61.64 (C5'), 67.26 (tetrahydropyran), 70.61 (C3'), 73.47 (C2'), 75.48 (NHCH<sub>2</sub>CHO tetrahydropyran), 85.87 (C4'), 87.94 (C1'), 119.67 (C5), 139.76 (C8), 148.32 (C4), 152.25 (C2), 154.64 (C6). HRMS: *m/z* [M+H]<sup>+</sup> calculated [C<sub>16</sub>H<sub>24</sub>N<sub>5</sub>O<sub>5</sub>] 366.1772, found 366.1768; *m/z* [M+Na]<sup>+</sup> calculated [C<sub>16</sub>H<sub>23</sub>N<sub>5</sub>O<sub>5</sub>Na] 388.1591, found 388.1586.

#### 4.7. N<sup>6</sup>-Isopentenyladenine (**4a**)

Compound **4a** was obtained by the enzymatic phosphorolysis of **3a** in the presence of *E. coli* Recombinant Purine Nucleoside Phosphorylase (PNP) in KH<sub>2</sub>PO<sub>4</sub> buffer (pH 7.5) [10].

The yield of **4a** was 15 mg (50%) as a white powder; *R<sub>f</sub>* 0.43 (CH<sub>2</sub>Cl<sub>2</sub>–EtOH, 9:1). mp 218–220 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ = 1.67 (s, 3H, Me), 1.70 (s, 3H, Me), 4.08 (br s, 1H, NCH<sub>2</sub>), 5.31 (s, 1H, HCJC), 7.55 (br s, 1H, NH), 8.04 (s, 1H, H-2), 8.16 (s, 1H, H-8), 12.84 (br s, 1H, NH).

#### 4.8. Procedure for preparation of nucleobases **4b–c** by acid hydrolysis of nucleosides **3b–c**

The solution of corresponding nucleoside **3b–c** (0.2 mmol) in 0.5 M HCl (2.5 ml, 1.26 mmol) was stirred at 100 °C for 8 h. The reaction was monitored by TLC (acetonitrile–25% ammonia, 9:1). When the traces of initial nucleoside **3** had disappeared, the reaction was cooled down and neutralized to pH 7 with concentrated

NH<sub>3</sub>. The resulting precipitate was filtered, washed with cold water (2 × 5 ml), acetone (2 × 5 ml) and dried in vacuum over P<sub>2</sub>O<sub>5</sub>.

#### 4.8.1. N<sup>6</sup>-Allyladenine (**4b**)

The yield of **4b** was 26 mg (70%) as a white powder. *R*<sub>f</sub> 0.45 (acetonitrile–ammonia, 9:1). mp 223–224 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ = 4.16 (br s, 2H, NCH<sub>2</sub>), 5.06 (d, 1H, <sup>3</sup>*J* = 9.5 Hz, C=CH-*cis*), 5.17 (d, 1H, <sup>3</sup>*J* = 17 Hz, C=CH-*trans*), 5.93–6.00 (m, 1H, –CH=CH<sub>2</sub>), 7.71 (br s, 1H, H-6), 8.07 (s, 1H, H-2), 8.17 (s, 1H, H-8), 12.87 (br s, 1H, 9-NH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ = 41.94 (CH<sub>2</sub>), 114.96 (=CH), 135.91 (=CH<sub>2</sub>), 138.73 (C-8), 149.83 (C-4), 152.30 (C-2), 154.26 (C-6).

#### 4.8.2. N<sup>6</sup>-Propargyladenine (**4c**)

The yield of **4c** was 35 mg (94%) as a white powder; *R*<sub>f</sub> 0.67 (acetonitrile–25% ammonia, 9:1). mp 255 °C (with decomposition). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ = 3.01 (s, 1H, ≡CH), 4.30 (s, 2H, CH<sub>2</sub>), 7.92 (br s, 1H, H-9), 8.12 (s, 1H, H-2), 8.24 (s, 1H, H-8), 12.96 (br s, 1H, NH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ = 29.26 (CH<sub>2</sub>N), 72.38 (HC≡), 82.01 (–C≡), 117.95 (C-5), 139.51 (C-8), 147.58 (C-4), 152.16 (C-2), 153.23 (C-6).

#### 4.9. N<sup>6</sup>-Acetyl-N<sup>6</sup>-(hex-5-en-2-ynyl)-2',3',5'-tri-O-acetyladenine (**5**)

The reaction was carried out in N<sub>2</sub> atmosphere using standard Schlenk technique. A mixture of **2c** (500 mg, 1.06 mmol), CuCl (105 mg, 1.06 mmol), K<sub>2</sub>CO<sub>3</sub> (292 mg, 2.12 mmol), Bu<sub>4</sub>NBr (34 mg, 0.106 mmol), CH<sub>2</sub>[CHCH<sub>2</sub>Br (0.1 ml, 1.16 mmol) and DMF (5 ml) was stirred at room temperature in N<sub>2</sub> atmosphere for 16 h. Subsequently, the flask was opened and stirring was continued for an additional 8 h. The mixture was diluted with AcOEt (150 ml) and washed successively with water (30 ml) and 0.1 M water solution of disodium EDTA (3 × 30 ml). The organic layer was separated, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The residue was applied to column chromatography on silica gel.

The yield of **5** was 477 mg (88%) as a foam; *R*<sub>f</sub> 0.47 (CH<sub>2</sub>Cl<sub>2</sub>–EtOH, 25:1). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 2.10 (s, 3H, AcO), 2.13 (s, 3H, AcO), 2.16 (s, 3H, AcO), 2.37 (s, 3H, AcN), 2.80–2.86 (m, <sup>3</sup>*J* = 5.1, <sup>4</sup>*J* = 2 and 1.6, <sup>5</sup>*J* = 2.2, 2H, ≡CCH<sub>2</sub>C=), 4.40 (dd, 1H, *J*<sub>5'b,5'a</sub> = 12.9, *J*<sub>5'b,4'</sub> = 5.3, H-5'b), 4.48 (dd, 1H, *J*<sub>5'a,5'b</sub> = 12.9, *J*<sub>5'a,4'</sub> = 3.1, H-5'a), 4.48 (ddd, 1H, *J*<sub>4',5'b</sub> = 5.3, *J*<sub>5'a,4'</sub> = 3.1, *J*<sub>4',3'</sub> = 4.7, H-4'), 4.97 (ddt, 1H, <sup>2</sup>*J* = 1.7, <sup>3</sup>*J* = 10, <sup>4</sup>*J* = 1.6, C=CH-*trans*), 5.06 (ddt, 1H, <sup>2</sup>*J* = 1.7, <sup>3</sup>*J* = 17.1, <sup>4</sup>*J* = 2, C=CH-*cis*), 5.10 (t, 2H, <sup>5</sup>*J* = 2.2, NCH<sub>2</sub>), 5.65 (ddt, 1H, <sup>3</sup>*J* = 5.1, 10 and 17.1, CH<sub>2</sub>CH=C), 5.69 (dd, 1H, *J*<sub>3',4'</sub> = 4.7, *J*<sub>3',2'</sub> = 5.6, H-3'), 5.97 (dd, 1H, *J*<sub>2',3'</sub> = 5.6, *J*<sub>2',1'</sub> = 5.0, H-2'), 6.25 (d, 1H, *J*<sub>1',2'</sub> = 5.0, H-1'), 8.20 (s, 1H, H-2), 8.81 (s, 1H, H-8). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 20.50 (AcO), 20.62 (AcO), 20.85 (AcO), 23.08 (NAC), 23.08 (CH<sub>2</sub>), 37.06 (NCH<sub>2</sub>), 63.11 (C-5'), 70.65 (C-3'), 70.77 (≡C–), 73.28 (C-2'), 77.79 (–C≡), 80.54 (C-4'), 86.90 (C-1'), 116.02 (–C=), 132.32 (=CH<sub>2</sub>), 138.50 (C-4), 142.19 (C-8), 152.34 (C-2), 152.72 (C-6), 169.47 (CO), 169.67 (CO), 170.39 (CO), 170.45 (CO). MS (APCI): *m/z* [M+H]<sup>+</sup> calculated [C<sub>24</sub>H<sub>28</sub>N<sub>5</sub>O] 514.19, found 514.75.

#### 4.10. N<sup>6</sup>-Acetyl-N<sup>6</sup>-(6-Methylhept-5-en-2-ynyl)-2',3',5'-tri-O-acetyladenine (**6**)

The reaction was carried out in N<sub>2</sub> atmosphere using standard Schlenk technique. To a stirred mixture of **2c** (500 mg, 1.06 mmol), CuCl (105 mg, 1.06 mmol), K<sub>2</sub>CO<sub>3</sub> (292 mg, 2.12 mmol) and Bu<sub>4</sub>NBr (34 mg 0.106 mmol) in DMF (5 ml), 80% solution of isopentenyl bromide in toluene (0.274 ml, 2.33 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 48 h, after which it was diluted with AcOEt (150 ml) and washed successively with water (30 ml) and 0.1 M water solution of

disodium EDTA (3 × 30 ml). The extract was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The residue was applied to column chromatography on silica gel.

The yield of **6** was 417 mg (72%) as a foam; *R*<sub>f</sub> 0.5 (CH<sub>2</sub>Cl<sub>2</sub>–EtOH, 25:1). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 1.49 (s, 3H, Me), 1.62 (d, 3H, <sup>4</sup>*J* = 1, Me), 2.10 (s, 3H, AcO), 2.13 (s, 3H, AcO), 2.16 (s, 3H, AcO), 2.36 (s, 3H, AcN), 2.74 (m, 2H, <sup>3</sup>*J* = 6.9, <sup>5</sup>*J* = 2.2, ≡CCH<sub>2</sub>C=), 4.39 (dd, 1H, *J*<sub>5'b,5'a</sub> = 12.9, *J*<sub>5'b,4'</sub> = 5.4, H-5'b), 4.47 (dd, 1H, *J*<sub>5'a,5'b</sub> = 12.9, *J*<sub>5'a,4'</sub> = 3, H-5'a), 4.48 (ddd, 1H, *J*<sub>4',5'b</sub> = 5.4, *J*<sub>5'a,4'</sub> = 3, *J*<sub>4',3'</sub> = 4.7, H-4'), 5.00 (m, 1H, <sup>3</sup>*J* = 10, <sup>4</sup>*J* = 1, CH<sub>2</sub>CH=C), 5.05 (t, 2H, <sup>5</sup>*J* = 2.2, NCH<sub>2</sub>), 5.69 (dd, 1H, *J*<sub>3',4'</sub> = 4.7, *J*<sub>3',2'</sub> = 5.6, H-3'), 5.97 (dd, 1H, *J*<sub>2',3'</sub> = 5.6, *J*<sub>2',1'</sub> = 5.1, H-2'), 6.25 (d, 1H, *J*<sub>1',2'</sub> = 5.1, H-1'), 8.20 (s, 1H, H-2), 8.81 (s, 1H, H-8). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 17.86 (Me), 20.33 (AcO), 20.44 (AcO), 20.66 (AcO), 24.21 (NAC), 25.54 (Me), 45.56 (NCH<sub>2</sub>), 45.56 (CH<sub>2</sub>), 62.95 (C5'), 70.63 (≡C–), 70.67 (–C≡), 70.46 (C-3'), 73.12 (C-2'), 80.30 (C-4'), 86.81 (C-1'), 120.10 (–CH=), 127.55 (C-5), 135.75 (CMe<sub>2</sub>), 142.15 (C-8), 152.19 (C-2), 152.48 (C-4), 153.83 (C-6), 169.34 (CO), 169.52 (CO), 170.21 (CO), 171.15 (CO). MS (APCI): *m/z* [M+H]<sup>+</sup> calculated [C<sub>26</sub>H<sub>31</sub>N<sub>5</sub>O<sub>8</sub>] 542.22, found 542.22.

#### 4.11. N<sup>6</sup>-(Hex-5-en-2-ynyl)adenosine (**7**)

Compound **5** (1.2 g, 2.34 mmol) was dissolved in 7 M NH<sub>3</sub> in MeOH (6.7 ml, 46.8 mmol) and the solution was left for 48 h at room temperature. The reaction mixture was evaporated and the residue was treated with 5 ml of cold MeOH. The solid was filtered, washed with MeOH (5 ml) and dried in vacuum over P<sub>2</sub>O<sub>5</sub>.

The yield of **7** was 609 mg (75%) as a white powder; *R*<sub>f</sub> 0.28 (CH<sub>2</sub>Cl<sub>2</sub>–EtOH, 9:1). mp 136–138 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub> + D<sub>2</sub>O): δ = 2.95 (m, 2H, <sup>3</sup>*J* = 5, <sup>4</sup>*J* = 1.4 and 1.7, –CH<sub>2</sub>CH=), 3.56 (dd, 1H, *J*<sub>5'a,5'b</sub> = 12.5, *J*<sub>5'a,4'</sub> = 3.3, H-5'a), 3.67 (dd, 1H, *J*<sub>5'b,5'a</sub> = 12.5, *J*<sub>5'b,4'</sub> = 3.4, H-5'b), 3.97 (ddd, 1H, *J*<sub>4',5'b</sub> = 3.4, *J*<sub>4',5'a</sub> = 3.3, *J*<sub>4',3'</sub> = 2.9, H-4'), 4.15 (dd, 1H, *J*<sub>3',4'</sub> = 2.9, *J*<sub>3',2'</sub> = 5.3, H-3'), 4.31 (broad s, 2H, NCH<sub>2</sub>), 4.61 (dd, 1H, *J*<sub>2',3'</sub> = 5.3, *J*<sub>2',1'</sub> = 6.0, H-2'), 5.05 (ddt, 1H, <sup>2</sup>*J* = 1.7, <sup>3</sup>*J* = 10, <sup>4</sup>*J* = 1.7, –C=CH-*trans*), 5.26 (ddt, 1H, <sup>2</sup>*J* = 1.7, <sup>3</sup>*J* = 17.3, <sup>4</sup>*J* = 1.4, –C=CH-*cis*), 5.15 (d, 1H, 3'OH), 5.32 (dd, 1H, 5'OH), 5.42 (d, 1H, 2'OH), 5.77 (ddt, 1H, <sup>3</sup>*J* = 5, 10 and 17.3, CH<sub>2</sub>CH=C), 5.90 (d, 1H, *J*<sub>1',2'</sub> = 6.0, H-1'), 8.18 (br s, 1H, NH), 8.27 (br s, 1H, H-2), 8.39 (s, 1H, H-8). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 22.26 (CH<sub>2</sub>), 29.44 (NCH<sub>2</sub>), 61.58 (C-5'), 70.55 (C-3'), 73.50 (C-2'), 78.12 (≡C–), 80.34 (–C≡), 85.81 (C-4'), 87.88 (C-1'), 115.79 (–CH=), 119.80 (C-5), 132.95 (=CH<sub>2</sub>), 140.02 (C-8), 148.72 (C-4), 152.19 (C-2), 153.97 (C-6). MS (APCI): *m/z* [M+H]<sup>+</sup> calculated [C<sub>16</sub>H<sub>20</sub>N<sub>5</sub>O<sub>4</sub>] 346.14, found 346.25.

#### 4.12. N<sup>6</sup>-(6-Methylhept-5-en-2-ynyl)adenosine (**8**)

Compound **6** (1 g, 1.9 mmol) was dissolved in 7 M NH<sub>3</sub> in MeOH (5.6 ml, 40.7 mmol) and the solution was left for 48 h at room temperature. The reaction mixture was evaporated and the residue was treated with 5 ml of cold MeOH. The solid was filtered, washed with MeOH (5 ml) and dried in vacuum over P<sub>2</sub>O<sub>5</sub>.

The yield of **8** was 510 mg (76%) as a white powder; *R*<sub>f</sub> 0.67 (CH<sub>2</sub>Cl<sub>2</sub>–EtOH, 4:1). mp 128–130 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub> + D<sub>2</sub>O): δ = 1.55 (s, 3H, Me), 1.64 (d, 3H, <sup>4</sup>*J* = 1, Me), 2.85 (d, 2H, <sup>3</sup>*J* = 6.8, C–CH<sub>2</sub>–CH=), 3.55 (dd, 1H, *J*<sub>5'a,5'b</sub> = 12.2, *J*<sub>5'a,4'</sub> = 3.5, H-5'a), 3.65 (dd, 1H, *J*<sub>5'b,5'a</sub> = 12.2, *J*<sub>5'b,4'</sub> = 3.4, H-5'b), 3.98 (ddd, 1H, *J*<sub>4',5'b</sub> = 3.4, *J*<sub>4',5'a</sub> = 3.5, *J*<sub>4',3'</sub> = 2.8, H-4'), 4.14 (dd, 1H, *J*<sub>3',4'</sub> = 2.8, *J*<sub>3',2'</sub> = 4.9, H-3'), 4.25 (br s, 2H, NCH<sub>2</sub>), 4.56 (dd, 1H, *J*<sub>2',3'</sub> = 4.9, *J*<sub>2',1'</sub> = 6.2, H-2'), 5.09 (m, 1H, <sup>3</sup>*J* = 6.8, <sup>4</sup>*J* = 1, CH<sub>2</sub>CH=C), 5.86 (d, 1H, *J*<sub>1',2'</sub> = 6.2, H-1'), 8.23 (br s, 1H, H-2), 8.31 (c, 1H, H-8). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ = 17.16 (Me), 25.09 (Me), 29.07 (CH<sub>2</sub>), 29.38 (NCH<sub>2</sub>), 61.64 (C5'), 70.54 (C-3'), 70.61 (≡C–), 73.42 (–C≡), 73.51 (C-2'), 85.84 (C-4'), 87.89 (C-1'), 105.06 (–CH=), 119.09 (C-5), 139.85 (CMe<sub>2</sub>), 143.20 (C-8), 149.05 (C-4), 152.33 (C-2), 156.13 (C-6). MS (APCI): *m/z* [M+H]<sup>+</sup> calculated [C<sub>18</sub>H<sub>24</sub>N<sub>5</sub>O<sub>4</sub>] 374.41, found 374.30.

## 5. Antiviral assays

RD (rhabdomyosarcoma) cells were obtained from the European Collection of Cell Cultures and were maintained in minimal essential medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% sodium bicarbonate (Gibco) and 1% L-glutamine (Gibco). Cells were grown at 37 °C and 5% CO<sub>2</sub>. EV71 strains that originated from Taiwan were a generous gift from Dr. Shih-Cheng Chang (Chang Gung University, Taoyuan, Taiwan). Strain H08300 461 #812 was acquired from National Collection of Pathogenic Viruses (NCPV). Strain 11316 was a kind gift from the National Institute for Public Health and the Environment (RIVM, The Netherlands). EV71 strain BrCr was kindly provided by F. van Kuppeveld (UMCU, The Netherlands). Poliovirus 3 was a gift from J. Martin (NIBSC, Hertfordshire, UK). All other viruses belonged to the virus collection of the Rega Institute for Medical Research.

The antiviral assay for HCV was essentially performed as described by Manfroni et al. [26] while that for chikungunya virus was performed as described by Gigante et al. [27]. The antiviral assay for EV71 was performed as described by Tijsma et al. [28]. Briefly, RD cells, grown to confluence in 96-well microtiter plates were infected with ~100 CCID<sub>50</sub> of EV71 and were treated with a dilution series of the different compounds. Cultures were incubated for three days at 37 °C (5% CO<sub>2</sub>) after which residual cell viability was quantified using an MTS readout method according to the manufacturers' instructions (Promega). A similar assay setup was used for the other viruses, although with different cell lines (BGM cells for the polioviruses as well as echovirus 11, Vero cells for Coxsackievirus B3, and HeLa cells for the human rhinoviruses).

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2014.11.048>. These data include MOL files and InChIKeys of the most important compounds described in this article.

## References

- [1] T. Kamada-Nobusada, H. Sakakibara, Molecular basis for cytokinin biosynthesis, *Phytochemistry* 70 (2009) 444–449.
- [2] J. Rozanski, P.F. Crain, J.A. McCloskey, The RNA modification database: 1999 update, *Nucleic Acids Res.* 72 (1999) 196–197.
- [3] F. Colombo, F.S. Falvella, L. De Cecco, M. Tortoreto, G. Pratesi, P. Ciuffreda, R. Ottria, E. Santaniello, L. Cicatiello, A. Weisz, T.A. Dragani, Pharmacogenomics and analogues of the antitumour agent N<sup>6</sup>-isopentenyladenosine, *Int. J. Cancer* 124 (2009) 2179–2185.
- [4] J. Voller, M. Zatloukal, R. Lenobel, K. Dolezal, T. Běreš, V. Kryštof, L. Spíchal, P. Niemann, P. Dzubák, M. Hajdúch, M. Strnad, Anticancer activity of natural cytokinins: a structure–activity relationship study, *Phytochemistry* 71 (2010) 1350–1359.
- [5] A. Mittelman, J.T. Evans, G.B. Chheda, Cytokinins as chemotherapeutic agents, *Ann. N. Y. Acad. Sci.* 255 (1975) 225–234.

- [6] R. Ottria, S. Casati, A. Manzocchi, E. Baldoli, M. Mariotti, J.A.M. Maier, P. Ciuffreda, Synthesis and evaluation of in vitro anticancer activity of some novel isopentenyladenosine derivatives, *Bioorg. Med. Chem.* 18 (2010) 4249–4254.
- [7] C. Herforth, J. Wiesner, P. Heidler, S. Sanderbrand, S. Van Calenbergh, H. Jomaa, A. Link, Antimalarial activity of N<sup>6</sup>-substituted adenosine derivatives. Part 3, *Bioorg. Med. Chem.* 12 (2004) 755–762.
- [8] J.C. Bressi, C.L.M.J. Verlinde, A.M. Aronov, M.L. Shaw, S.S. Shin, L.N. Nguyen, S. Suresh, F.S. Buckner, W.C.V. Van Voorhis, I.D. Kuntz, W.G.J. Hol, M.H. Gelb, Adenosine analogues as selective inhibitors of glyceraldehyde-3-phosphate dehydrogenase of trypanosomatidae via structure-based drug design, *J. Med. Chem.* 44 (2001) 2080–2093.
- [9] Y. Ah Kim, A. Sharon, C.K. Chu, R.H. Rais, O.N. Al Safarjalani, F.N.M. Naguib, M.H. El Kouni, Synthesis, biological evaluation and molecular modeling studies of N<sup>6</sup>-benzyladenosine analogues as potential anti-toxoplasma agents, *Biochem. Pharmacol.* 73 (2007) 1558–1572.
- [10] V.I. Tararov, S.V. Kolyachkina, C.S. Alexeev, S.N. Mikhailov, N<sup>6</sup>-Acetyl-2',3',5'-tri-O-acetyladenosine – a convenient, missed out substrate for regioselective N<sup>6</sup>-alkylations, *Synthesis* (2011) 2483–2489.
- [11] S.V. Kolyachkina, V.I. Tararov, C.S. Alexeev, D.M. Krivosheev, G.A. Romanov, E.V. Stepanova, E.S. Solomko, A.N. Inshakov, S.N. Mikhailov, N<sup>6</sup>-substituted adenosines. Cytokinin and antitumor activities, *Collect. Czech. Chem. Commun.* 76 (2011) 1361–1378.
- [12] M.H. Fleysher, A. Bloch, M.T. Hakala, C.A. Nichol, Synthesis and biological activity of some new N<sup>6</sup>-substituted purine nucleosides, *J. Med. Chem.* 12 (1969) 1056–1061.
- [13] R. Ottria, S. Casati, E. Baldoli, J.A.M. Maier, P. Ciuffreda, N<sup>6</sup>-alkyladenosines: synthesis and evaluation of in vitro anticancer activity, *Bioorg. Med. Chem.* 18 (2010) 8396–8402.
- [14] M.S. Drenichev, I.V. Kulikova, G.V. Bobkov, V.I. Tararov, S.N. Mikhailov, A new protocol for selective cleavage of acyl protective groups in 2'-O-modified 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)ribonucleosides, *Synthesis* (2010) 3827–3834.
- [15] M.J. Robins, R.H. Hall, R. Thedford, N<sup>6</sup>-(Δ<sup>2</sup>-isopentenyl) adenosine. A component of the transfer ribonucleic acid of yeast and of mammalian tissue, methods of isolation, and characterization, *Biochemistry* 6 (1967) 1837–1848.
- [16] D.M.G. Martin, C.B. Reese, Some aspects of the chemistry of N(1)- and N(6)-dimethylallyl derivatives of adenosine and adenine, *J. Chem. Soc. C* (1968) 1731–1738.
- [17] V.V. Grushin, H. Alper, Copper(I) and phase transfer catalyzed allylation of alkynes, *J. Org. Chem.* 57 (1992) 2188–2192.
- [18] T. Jeffery, Copper (I) and phase transfer catalyzed allylic substitution by terminal alkynes, *Tetrahedron Lett.* 30 (1989) 2225–2228.
- [19] L.W. Bieber, M.F. Silva, Copper catalyzed regioselective coupling of allylic halides and alkynes promoted by weak inorganic bases, *Tetrahedron Lett.* 48 (2007) 7088–7090.
- [20] B.T. Worrell, J.A. Malik, V.V. Fokin, Direct evidence of a dinuclear copper intermediate in Cu(I)-catalyzed azide-alkyne cycloadditions, *Science* 340 (2013) 457–460.
- [21] S. Casati, A. Manzocchi, R. Ottria, P. Ciuffreda, <sup>1</sup>H, <sup>13</sup>C and <sup>15</sup>N NMR assignments for N<sup>6</sup>-isopentenyladenosine/inosine analogues, *Magn. Reson. Chem.* 48 (2010) 745–748.
- [22] J. Vicha, M. Malon, P. Veselá, O. Humpa, M. Strnad, R. Marek, <sup>1</sup>H-, <sup>13</sup>C-, and <sup>15</sup>N-NMR chemical shifts for selected glucosides and ribosides of aromatic cytokinins, *Magn. Reson. Chem.* 48 (2010) 318–322.
- [23] H.E. Gottlieb, V. Kotlyar, A. Nudelman, NMR chemical shifts of common laboratory solvents as trace impurities, *J. Org. Chem.* 62 (1977) 7512–7515.
- [24] P.A. Belyakov, V.I. Kadentsev, A.O. Chizhov, N.G. Kolotyrykina, A.S. Shashkov, V.P. Ananikov, Mechanistic insight into organic and catalytic reactions by joint studies using mass spectrometry and NMR spectroscopy, *Mendeleev Commun.* 20 (2010) 125–131.
- [25] M.H. Fleysher, R.G. Bernacki, G.A. Bullard, Some short-chain N<sup>6</sup>-substituted adenosine analogues with antitumor properties, *J. Med. Chem.* 23 (1980) 1448–1452.
- [26] G. Manfroni, D. Manvar, M.L. Barreca, N. Kaushik-Basu, P. Leyssen, J. Paeshuyse, R. Cannalire, N. Iraci, A. Basu, M. Chudae, C. Zamperini, E. Dreassi, S. Sabatini, O. Tabarrini, J. Neyts, V. Cecchetti, New pyrazolobenzothiazine derivatives as hepatitis C virus NS5B polymerase palm site I inhibitors, *J. Med. Chem.* 57 (2014) 3247–3262.
- [27] A. Gigante, M.D. Canela, L. Delang, E.M. Priego, M.J. Camarasa, G. Quera, J. Neyts, P. Leyssen, M.J. Pérez-Pérez, Identification of [1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-ones as novel inhibitors of Chikungunya virus replication, *J. Med. Chem.* 57 (2014) 4000–4008.
- [28] A. Tijsma, D. Franco, S. Tucker, R. Hilgenfeld, M. Froeyen, P. Leyssen, J. Neyts, The capsid binder vavendavir and novel protease inhibitor SG85 inhibit enterovirus 71 replication, *Antimicrob. Agents Chemother.* 58 (2014) 6990–6992.