Bioorganic & Medicinal Chemistry 20 (2012) 1008–1019

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, SAR, and preliminary mechanistic evaluation of novel antiproliferative N^6,5′-bis-ureido- and 5′-carbamoyl-N^6-ureidoadenosine derivatives

Jadd R. Shelton [a], Christopher E. Cutler [a], Marcelio Oliveira [a], Jan Balzarini [b], Matt A. Peterson [a,*]

[a] Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602-5700, USA
[b] Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 14 October 2011
Revised 19 November 2011
Accepted 19 November 2011
Available online 2 December 2011

Keywords:
Anticancer nucleosides
N^6,5′-Bis-ureidoadenosines
Antiproliferative activity
BMPR1b

ABSTRACT

We have developed efficient methods for the preparation of N^6,5′-bis-ureidoadenosine derivatives and their 5′-carbamoyl-N^6-ureido congeners. Treatment of 5′-azido-5′-deoxy-N^6-(*N*-alkyl or -arylurea)adenosine derivatives (**6a**–**d**) with H_2/Pd–C or Ph_3P/H_2O, followed by *N*-methyl-*p*-nitrophenylcarbamate gave N^6,5′-bis-ureido products **7a**–**d** in 49–78% yield. Analogous derivatives in the 5′-carbamoyl-N^6-ureido series were prepared by treatment of 2′,3′-bis-*O*-TBS-adenosine (**11**) with *N*-methyl-*p*-nitrophenylcarbamate followed by acylation with appropriate isocyanates which gave **13a**–**d** in 45–69% yield. A more versatile route for obtaining potentially vast libraries of compounds from both series was achieved by treatment of 5′-*N*-methylureido- or 5′-*N*-methylcarbamoyladenosine derivatives with ethylchlorformate to give N^6-ethoxycarbonyl derivatives (**9** and **14**) in 55–63% yields, respectively. Simple heating of **9** or **14** in the presence of primary alkyl- or arylamines gave the corresponding N^6,5′-bis-ureido- or 5′-carbamoyl-N^6-ureidoadenosine derivatives in good yields (33–72% and 39–83%; **10a**–**e** and **15a**–**e**, respectively). Significant antiproliferative activities ($IC_{50} \approx 4$–10 μg/mL) were observed for a majority of the N^6,5′-bis-ureido derivatives, whereas the 5′-carbamoyl-N^6-ureido derivatives were generally less active (IC_{50} >100 μg/mL). A 2′,3′-*O*-desilylated derivative (5′-amino-5′-deoxy-5′-*N*-methylureido-N^6-(*N*-phenylcarbamoyl)adenosine, **16**) was shown to inhibit binding of 16 of 441 protein kinases to immobilized ATP-binding site ligands by 30–40% in a competitive binding assay at 10 μM. Compound **16** was also shown to bind to bone morphogenetic protein receptor 1b (BMPR1b) with a Kd = 11.5 ± 0.7 μM.

1. Introduction

Over the past several decades, nucleoside derivatives have attracted significant interest as templates for the development of anticancer and/or antiviral therapeutics.[1,2] Hundreds of such derivatives have been made, and numerous analogs have proven useful for the treatment of cancer or viral infection. Recently, a new class of nucleosides, bis-ureidoadenosine derivatives, typified by structures **1**–**3** (Fig. 1), were found to possess broad-spectrum antiproliferative activities against human cancer cells in vitro.[3] The most potent compound (**3**) was also found to exhibit selective toxicities toward human colon cancer and renal cancer cells in vitro.[4] A preliminary structure–activity relationship study (SAR) had revealed that the 2′-*O*-TBS group (TBS = *tert*-butyldimethylsilyl) was necessary for optimal antiproliferative activity, and neither N^6- nor 5′-ureido substituents were sufficient to achieve significant (IC_{50} <10 μM) antiproliferative effects in the absence of each other.[3b] Analysis of the antiproliferative effects of compound **1** using the COMPARE algorithm[5] available from the NCI Developmental Therapeutics Program,[6] pointed to a correlation between protein kinase expression and antiproliferative activity.[3c]

A competitive binding inhibition assay[7] revealed that binding of 11 of 353 protein kinases to ATP-binding site ligands was inhibited by 30–50% by compound **1** at 10 μM concentration.[3c] These data suggested that protein kinases may be molecular targets for compounds of this class, and prompted our investigation of a series of derivatives that might reasonably be expected to bind with enhanced affinity to protein kinase targets in general. The currently accepted pharmacophore binding model[8] for the ATP-binding site of protein kinases invokes a phosphate binding region near the 5′-position and a hydrophobic binding pocket in close proximity to N^6. In addition, a sugar binding pocket also exists in close proximity to the 2′,3′-hydroxyls of the ribose ring. With this model as a guide, we set out to perform an SAR to refine our understanding of the structural requirements needed for optimal antiproliferative activity. Herein we report the synthesis and biological evaluation of a series of derivatives of compound **3** with various substituents at the N^6 and/or 5′-positions. These substituents were designed to interrogate the effect of substitution at these positions as a prelude

* Corresponding author. Tel.: +1 801 422 6843; fax: +1 801 422 0153.
E-mail address: matt_peterson@byu.edu (M.A. Peterson).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.11.043

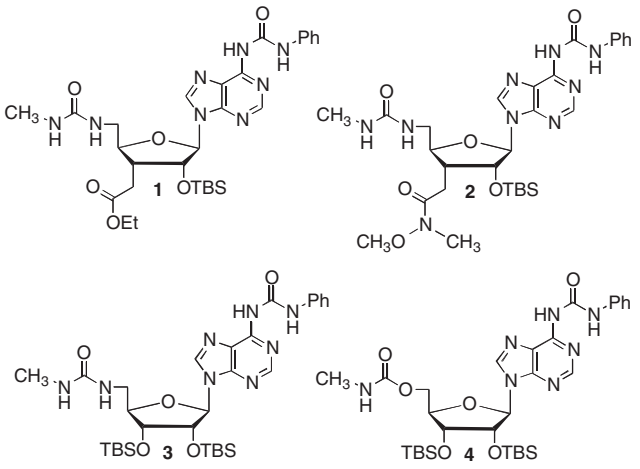


Figure 1.

to more focused SARs that could be conducted once the biomolecular receptor(s) had been unequivocally identified. A derivative of the most potent compound (**3**) lacking 2′,3′-*O*-TBS groups was also evaluated in order to determine possible reasons this substitution is necessary for optimal antiproliferative activity.

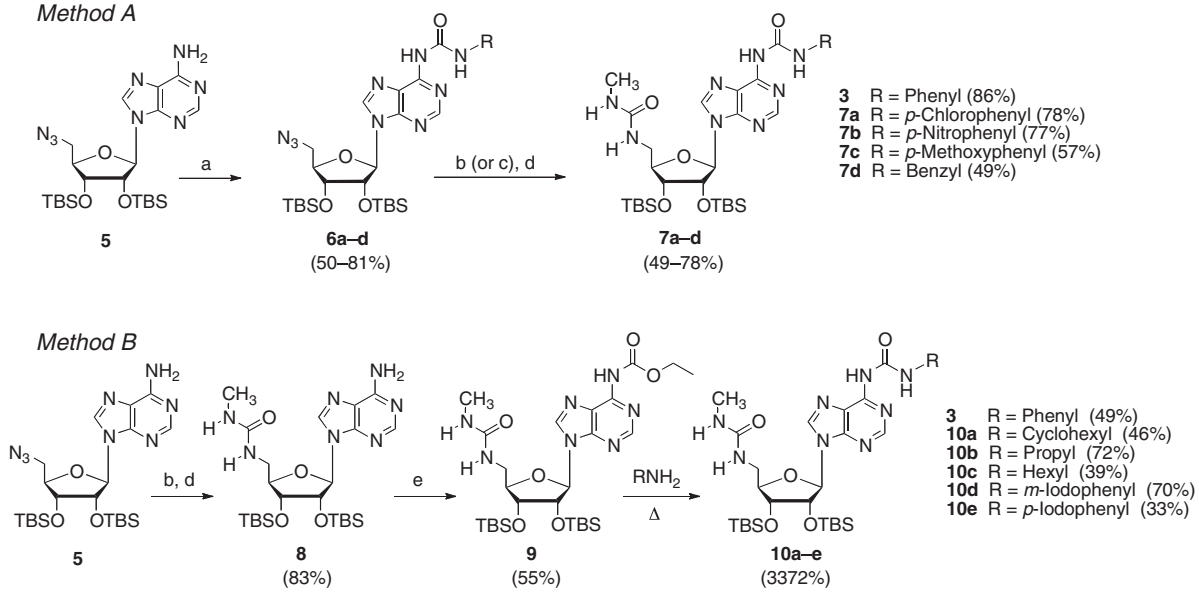
2. Results

2.1. Chemistry

In order to test the effect of varying substitution at N^6, two synthetic approaches were examined (Scheme 1). It was reasoned that varying the π-electron density in the N^6-phenyl ring might lead to tighter binding in the (assumed) hydrophobic pocket of the biomolecular receptor. Increasing or decreasing the inherent π-electron density of the phenyl ring could enhance binding in the hydrophobic pocket by fine-tuning electronic interactions between the ligand and amino acid residues in the binding pocket of the receptor, as has been observed in numerous related instances.[9]

It has been well established that face-to-face and edge-to-face bonding interactions can occur between aromatic side chains in receptors and aromatic moieties found in receptor ligands.[9a] In addition, aliphatic C–H/π-electron interactions between the receptor and ligand can occur and can be exploited to enhance molecular recognition.[10] As has been demonstrated in previously studied systems, electron-donating substituents increase the π-electron density and enhance the Lewis-basicity of phenyl rings, whereas electron-withdrawing substituents decrease π-electron density leading to increased Lewis-acidity of the phenyl ring.[9a] Fine-tuning the π-electron density of aromatic moieties is a strategy that has been applied successfully to enhance binding between ligands and hydrophobic receptors. Accordingly, we reasoned that derivatives that varied in the π-electron density of the phenyl ring (e.g., **7a**–**d**) might reasonably be expected to bind with different affinities to the hydrophobic pocket of the assumed primary biomolecular receptor(s). Thus, compounds **6a**–**d** were treated by *Method A* to prepare derivatives **7a**–**d** which vary by virtue of their possession of either electron withdrawing (**7a**,**b**) or electron donating (**7c**) substituents, or by loss of conjugation with the π-system of the phenyl ring and the urea moiety (**7d**). Yields ranged from 49–78% via this method, in general agreement with the yield obtained for the parent compound (**3**). Lower yields were observed for compounds **7a**–**d** than for **3**, owing to either the required use of Staudinger reduction conditions for compounds **7a** and **7b** (to avoid reduction of the C–Cl, or NO_2 moieties, respectively), and/or owing to the challenges associated with chromatographic separation of the diphenylurea byproducts that invariably accompanied formation of **7a**–**d**. In order to avoid these complications and to provide access to a potentially unlimited, easily accessible, compound pool, *Method B* was also investigated (Scheme 1). In this method, the azido group is reduced and the 5′-*N*-methylurea group is introduced before N^6 is acylated. Treatment of compound **8** with ethylchloroformate gave compound **9** which could potentially be converted to a broad array of N^6-aryl- or alkylurea derivatives by simple heating in the presence of a virtually unlimited number of primary aryl- or alkylamines, respectively.

In this study, relatively hindered (e.g., cyclohexyl, **10a**), unhindered (e.g., propyl, hexyl, **10b** and **10c**) primary alkyl- and arylamines (e.g., *m*-iodoaniline, *p*-iodoaniline, **10d** and **10e**) gave products in moderate to good yields, thus providing proof of concept that *Method B* could potentially be exploited for producing large libraries of derivatives **10**. There are relatively few commercially available isocyanates when compared to commercially available primary amines. Thus, *Method B* (in contrast to *Method A*



Scheme 1. Reagents: (a) R–N=C=O; (b) H_2, Pd–C; (c) Ph_3P, H_2O; (d) *p*-NO_2-$C_6H_4O_2CNHCH_3$; (e) ethylchloroformate.

which is limited due to the relatively small number of commercially available isocyanates) opens up a viable path that can be exploited for straightforward preparation of large arrays of N^6-arylurea or N^6-alkylurea derivatives from the large number of commercially available primary amines, as exemplified by the synthesis of compounds **10a–e**.

In previously reported synthetic approaches to compounds **1–3**, it had become apparent that the overall efficiency of the synthesis could be enhanced if the 5′-*N*-methylurea moiety were replaced with the isosteric *N*-methylcarbamoyl group. Replacement of the 5′-urea moiety with a 5′-carbamate would reduce the total number of steps by three (from adenosine), and also eliminate the synthetically challenging introduction of the 5′-azido group from a 5′-activated precursor (such as a 5′-chloro-, or 5′-*O*-tosyladenosine derivative).[11] For compounds **1** and **2**, the latter transformation (i.e., introduction of the 5′-azido group from a 5′-activated adenosine derivative without competing cyclonucleoside formation)[11] required fairly labor-intensive conditions, and unusual attention to detail was required to obtain optimum yields.[3c] Assuming that the isosteric 5′-*N*-methylcarbamoyl derivatives retained biological activity, the proposed NH to O substitution would be a favorable modification. Toward this end, compounds **4** (Fig. 1) and **13a–d** and **15a–e** were prepared and evaluated (Scheme 2).

Compounds **13a–d** and **15a–e** were designed to probe SAR features analogous to those of **7a–d** and **10a–e**, respectively. The syntheses proceeded smoothly and provided desired products in good to moderate overall yields from compound **11**, which was readily obtained in excellent yield in only two steps from adenosine.[12]

Table 1
Inhibitory effects of the test compounds on the proliferation of murine leukemia cells (L1210), murine mammary carcinoma cells (FM3A), human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa).

Compound	IC_{50}[a] (μg/ml)			
	L1210	FM3A	CEM	HeLa
3	3.8 ± 0.3	5.9 ± 1.1	8.3 ± 2.9	3.2 ± 0.2
4	160 ± 56	> 200	> 200	⩾200
7a	5.6 ± 1.1	33 ± 2	14 ± 8	3.4 ± 0.0
7b	⩾200	>200	>200	>200
7c	3.8 ± 0.0	5.8 ± 0.4	5.2 ± 1.3	2.6 ± 0.8
7d	4.6 ± 0.9	9.2 ± 0.2	8.3 ± 0.8	3.8 ± 2.1
10a	20 ± 13	8.4 ± 0.8	5.2 ± 0.3	11 ± 3
10b	3.9 ± 0.3	4.7 ± 0.5	4.4 ± 1.1	3.3 ± 0.7
10c	74 ± 8	ND	68 ± 9	74 ± 9
10d	38 ± 17	ND	182 ± 8	21 ± 1
10e	43 ± 15	ND	68 ± 34	18 ± 2
13a	>200	>200	>200	>200
13b	7.7 ± 1.6	24 ± 5	15 ± 1	1.5 ± 1.0
13c	>200	>200	>200	⩾200
13d	101 ± 5	>200	>200	23 ± 4
15a	74 ± 0	>200	175 ± 8	69 ± 51
15b	18 ± 11	23 ± 4	59 ± 7	55 ± 32
15c	>200	>200	175 ± 8	88 ± 56
15d	>200	>200	>200	>200
15e	>200	>200	⩾200	143 ± 80

[a] 50% Inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

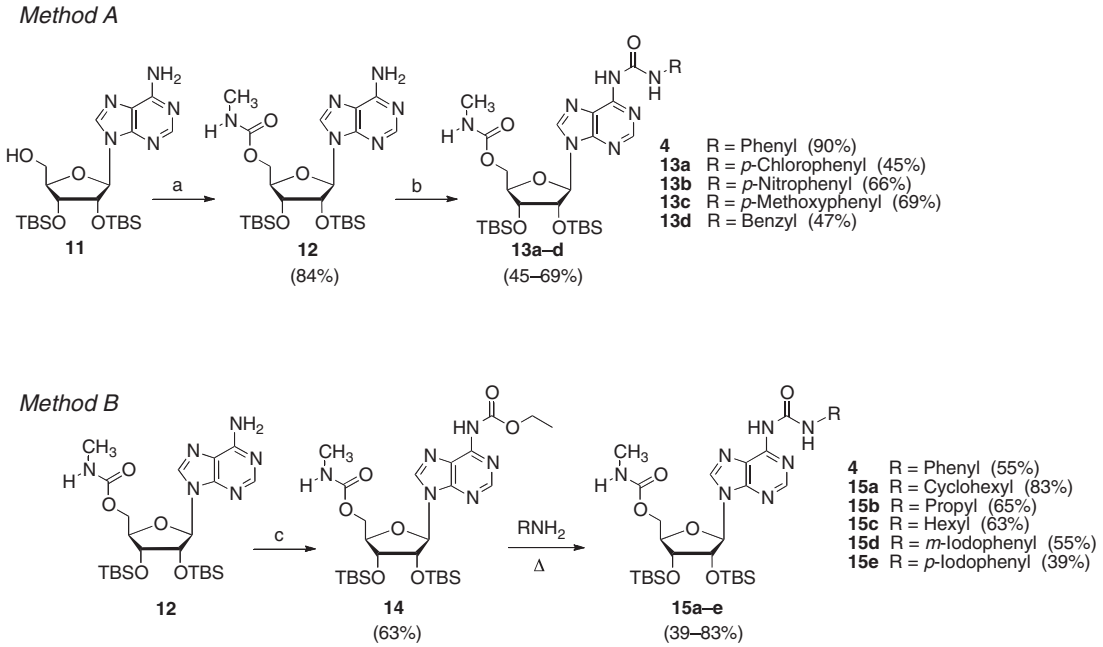
2.2. Biology

2.2.1. Antiproliferative activity

Compounds **3**, **4**, **7a–d**, **10a–e**, **13a–d**, and **15a–e** were examined for their antiproliferative activity using the following cell lines: murine leukemia L1210, murine mammary carcinoma FM3A, human lymphoblastic leukemia CEM, and human cervix carcinoma HeLa. The results of these assays are presented in Table 1. As these data illustrate, none of the derivatives **7a–d** or **10a–e** showed significantly improved activities relative to lead compound **3**, although comparable activities were exhibited by **7a**, **7c**, **7d**, **10a** and **10b**. *N*-Methylcarbamoyl analogs **4**, **13a–d** and **15a–e**, exhibited generally inferior activities, with the exception of **13b** which exhibited cytostatic activities that were markedly higher than observed for the parent compound **4**.

2.2.2. Protein kinase binding activity

In order to determine whether compound **3** exerts its antiproliferative effect via inhibition of protein kinases after intracellular hydrolysis of the TBS groups, the desilylated derivative **16** was prepared and subjected to the competitive binding inhibition assay of Fabian et al.[7] In this assay, compound **16** was shown to inhibit



Scheme 2. Reagents: (a) p-NO_2-$C_6H_4O_2CNHCH_3$; (b) R–N=C=O; (c) ethylchloroformate.

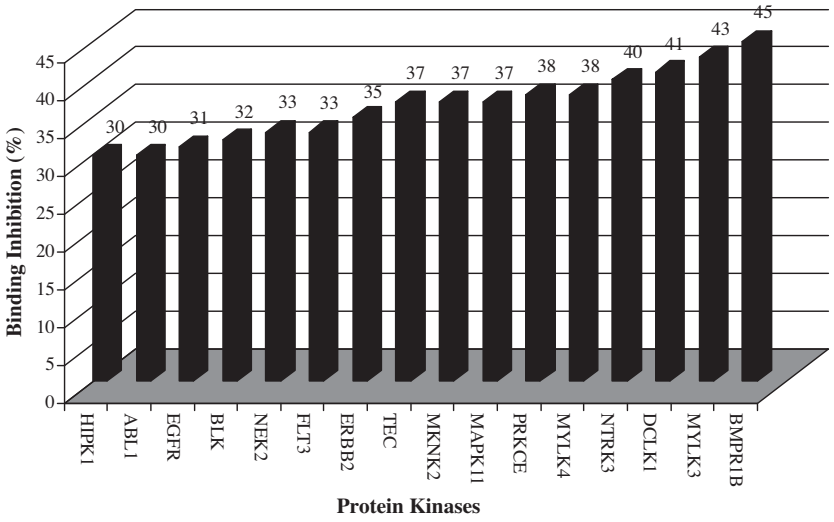


Figure 2a. Inhibition of binding of protein kinases to immobilized ATP-binding site ligands by compound **16** (10 μM).

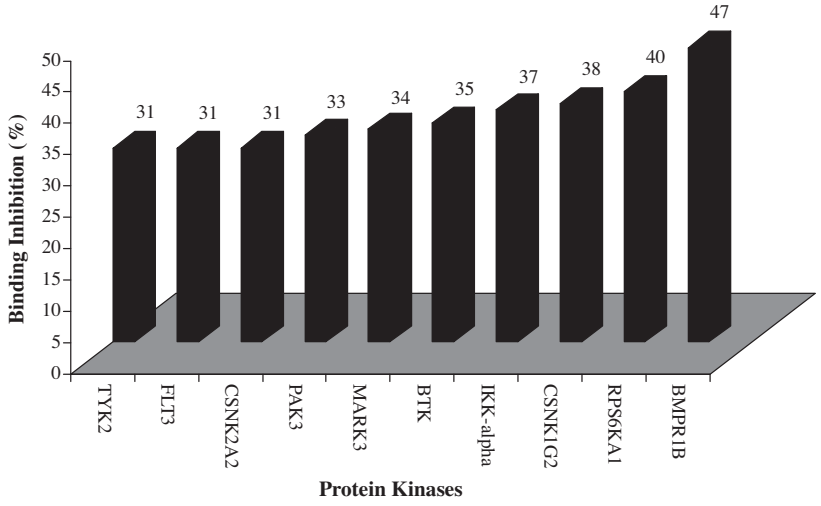


Figure 2b. Inhibition of binding of protein kinases to immobilized ATP-binding site ligands by compound **1** (10 μM).

binding of 16 of 441 protein kinases to ATP-binding site ligands by 30–45% (Fig. 2a). The binding profile for **16** varied significantly from that of **1** (Fig. 2b),[3b] but both **16** and **1** inhibited binding to bone morphogenetic protein receptor 1b (BMPR1b) to a similar degree (45% and 47%, respectively). In a multi-dose binding assay, compound **16** was found to bind to BMPR1b with a K_d = 11.5 ± 0.7 μM, whereas compound **3** did not bind to BMPR1b at concentrations as high as 30 μM (Fig. 3).

2.3. Conformational analysis

It has been well established that conformations of nucleosides play a key role in determining their biological activities.[13] In a recent communication, we had reported that one possible factor that could contribute to the difference in antiproliferative activities observed for **3** and **4** might be the difference in the *syn*/*anti* conformational preference for the two compounds (Fig. 4).[4] We now report that 5′-ureido derivatives **3**, **7a–d**, and **10a–e** each exhibit similar conformational preferences for the *syn* glycosyl conformer, while the conformational preference for the 5′-carbamate derivatives **4**, **13a–d**, and **15a–e** is much less pronounced. Such a conclusion is supported by $J_{1',2'}/J_{3',4'}$ coupling constant data, as well as results from 1D-NOESY experiments (Tables 2 and 3).

The equilibrium constant (K_{eq}) relating the mole fraction of S conformer (X_S) to the mole fraction of N conformer (X_N) was calculated from the observed $J_{1',2'}$ and $J_{3',4'}$ according to Eq. 1, and is a measure of the conformational equilibrium between the *syn* and *anti* conformers. (The C2′-*endo* (S) conformation correlates with a predominant *syn* glycosyl conformation (χ = 10–30°), whereas the C3′-*endo* (N) conformation correlates with the *anti* glycosyl conformation (χ = 200–210°).[14] Equilibrium constants calculated with Eq. 1 compare very favorably to those obtained by a full pseudorotational analysis.[14,15]

$$(K_{eq}) = X_S/X_N = J_{1',2'}/J_{3',4'} \quad (1)$$

Intramolecular hydrogen bonding between 5′ H-bond donors and N3 of the adenine heterocycle has been reported for several adenosine derivatives.[16] In such compounds (e.g., NECA), a *syn* glycosyl conformation was observed in the solid state, and the *syn* glycosyl conformer was preferred in solution. The 1D-NOESY data we obtained for the 5′-ureido derivatives are consistent with a strong preference for the *syn* conformer, which would be consistent with

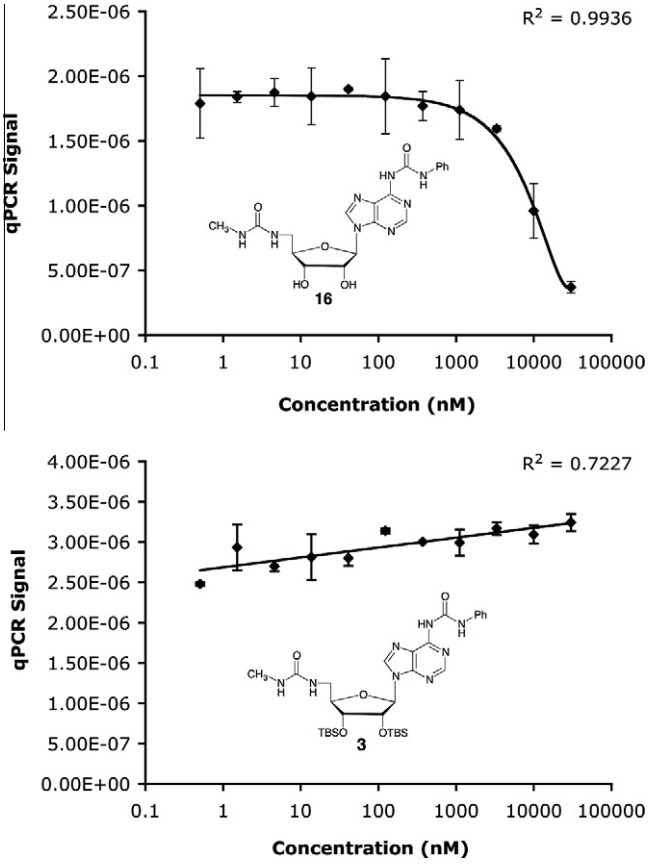


Figure 3. Effect of **3** and **16** on equilibrium competition binding of BMPR1b to immobilized ATP-binding site ligand.[7]

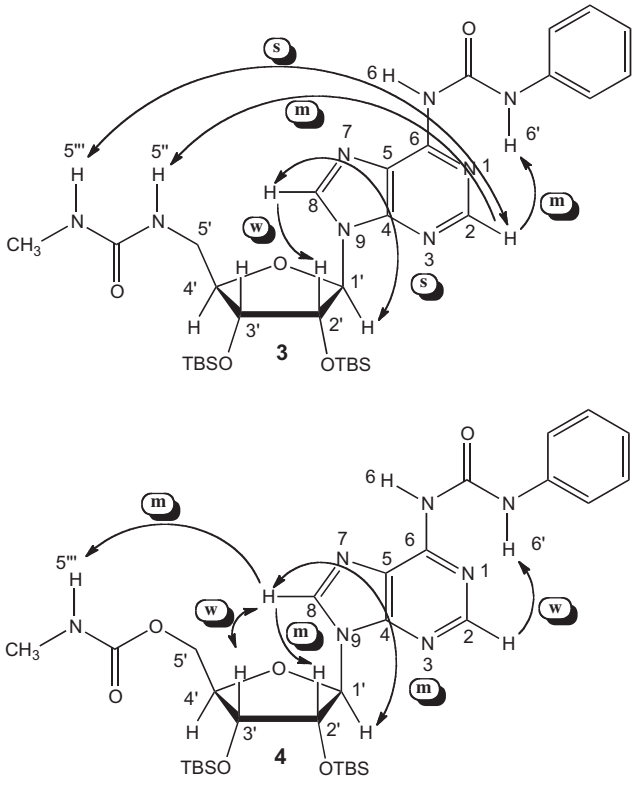


Figure 4. NOESY correlation data for compounds **3** and **4**: (s = strong, m = medium, w = weak).

intramolecular hydrogen bonding between the 5′-NH of the urea moiety and the N3 of the adenine heterocycle (see strong NOE enhancement of H-1′ and H-8 when either one is irradiated, Table 3).

Absence of a strong NOE when either H-1′, H-2, or H-8 are irradiated in the 5′-carbamates supports the conclusion that these compounds are much less conformationally rigid, consistent with the fact that an intramolecular H-bond cannot exist between the 5′-O and N3. Interestingly, when H-8 is irradiated in the 5′-ureido derivatives, NOE enhancement is observed for only H-1′. When H-8 is irradiated in the 5′-carbamates, H-1′, H-5‴, H-2′, H-3′, and H-5′ are enhanced, although to a much weaker extent. When H-2 is irradiated in the 5′-carbamates, enhancement is observed for only H-6′. In contrast, irradiation of H-2 in the 5′-ureido compounds gives enhancement in H-6′, H-5″, H-5‴, and the NCH_3 of the 5′-urea moiety. These data support the conclusion that the 5′-ureas are much more conformationally constrained, with H-2 being in close proximity to the 5′-urea and H-8 being in close proximity to H-1′, consistent with the *syn* glycosyl conformational preference indicated by the coupling constant data (Table 2). Taken together, the coupling constant and 1D-NOESY data suggest that conformational differences between the 5′-ureido and 5′-carbamate derivatives may play a role in the observed difference in antiproliferative activities.

3. Discussion

It is generally accepted that a majority of biologically active nucleoside analogs are phosphorylated before they exert their effect biologically.[17] This typically requires that the nucleoside possess either a free 5′-OH or a prodrug feature that is easily hydrolyzed to generate either a free 5′-OH or a 5′-monophosphate in vivo.[17c,d] A common strategy for enhancing membrane permeability has been to increase lipophilicity of the nucleoside derivative by protecting hydroxyls as benzoyl, acetyl, or isobutyryl esters that are cleaved once the compound has crossed the cell membrane.[17a,b] Lipophilic silyl protecting groups have also been reported to be required for the biological activity of nucleoside analogs. For example, TBS groups at the 2′ and 5′ positions were found to be required for optimal activity of the *tert*-butyldimethylsilyl-spiroaminooxathioledioxide-thymine (TSAO-T) class of HIV-1 reverse transcriptase (RT) inhibitors.[18] A recent crystal structure of the TSAO–T/RT complex shows that the TBS groups occupy hydrophobic pockets in the non-nucleoside RT inhibitor binding pocket, and play a critical role in defining the dimensions of the binding pocket as well as determining molecular recognition between the pocket and the TSAO–T ligand.[19]

Since our initial discovery that the N^6,5′-bis-ureidoadenosines require a TBS group for antiproliferative activity, we have been intrigued by the question of what role the TBS group might play in this class of compounds. The observation that the 5′-carbamates are in general substantially less active in the cytostatic assays in spite of the fact that they possess the 2′,3′-bis-*O*-TBS substitution, implies that the TBS groups are necessary, but not sufficient, for antiproliferative activity. The results presented in Figure 3 suggest that the primary role of the TBS groups may be to enhance membrane permeability, assuming that BMPR1b is the primary target. Hydrolysis of the TBS group of compound **3** within the intracellular milieu could give rise to **16**, which clearly has greater affinity for BMPR1b (Fig. 3). BMPR1b is a transmembrane receptor whose ATP-binding site domain lies within the cytoplasm. It is part of a signaling cascade that regulates expression of Id-1.[20] Aberrant expression of Id-1 has been reported in over 20 cancers,[21] and inhibition, inactivation, or downregulation of Id-1 have been shown to induce apoptosis in breast, ovarian, and prostate cancers.[22] In

Table 2
^{1}H NMR and K_{eq} data for 5′-ureido and 5′-carbamoyl derivatives[a,b,c]

Compound	H-1′ ($J_{1',2'}$)[b]	H-2′	H-3′ ($J_{3',4'}$)[b]	H-4′	H-5′	H-5″	H-5‴	H-8	H-2	H-6	H-6′	K_{eq}	%S	%N
3	6.17 (7.7 Hz)	4.62	4.34 (2.1 Hz)	4.62	3.97, 3.18	6.49	4.75	8.60	8.65	9.10	11.9	3.7	79	21
7a	6.14 (7.6 Hz)	4.66	4.34 (2.0 Hz)	4.66	3.99, 3.22	6.55	4.64	8.49	8.67	8.92	11.9	4.1	80	20
7b[d]	6.14 (7.5 Hz)	5.01	4.55 (1.7 Hz)	4.16	3.63, 3.59	6.28	5.61	8.84	8.83	9.50	12.7	4.4	82	20
7c	6.11 (7.5 Hz)	4.53	4.43 (1.8 Hz)	4.53	3.98, l.14	6.27	4.96	8.94	8.65	9.52	11.9	4.1	80	20
7d	6.15 (7.5 Hz)	4.41	4.48 (1.2 Hz)	4.41	3.93, 2.92	6.19	5.46	9.10	8.56	9.61	10.6	6.4	86	14
10a	6.07 (8.0 Hz)	4.54	4.44 (1.4 Hz)	4.54	4.05, 3.12	6.57	5.41	8.84	8.56	9.03	9.86	5.6	85	15
10b	6.09 (7.5 Hz)	4.52	4.44 (1.3 Hz)	4.52	4.04, 3.12	6.52	5.45	8.89	8.57	9.15	9.93	5.8	85	15
10c	6.10 (7.5 Hz)	4.52	4.46 (1.2 Hz)	4.52	4.04, 3.12	6.51	5.48	8.92	8.56	9.19	9.92	6.3	86	14
10d	6.01 (8.0 Hz)	4.63	4.38 (1.7 Hz)	4.63	4.01, 3.23	6.51	4.71	8.61	8.68	9.08	12.0	4.8	83	17
10e	5.92 (7.5 Hz)	4.72	4.31 (1.6 Hz)	4.72	4.01, 3.23	6.65	4.55	8.34	8.67	8.65	11.8	4.7	82	18
4	6.20 (5.4 Hz)	4.54	4.32 (3.1 Hz)	4.54	4.48, 4.23	…	5.90	8.84	8.64	10.0	12.2	1.7	63	37
13a	6.19 (5.5 Hz)	4.62	4.31(2.8 Hz)	4.62	4.45, 4.28	…	5.63	8.72	8.63	9.77	12.2	1.8	64	36
13b[d]	6.20 (5.5 Hz)	4.98	4.55 (3.0 Hz)	4.30	4.43, 4.42	…	6.37	8.68	8.77	9.44	12.8	1.8	64	36
13c	6.22 (5.5 Hz)	4.58	4.34 (2.9 Hz)	4.58	4.53, 4.03	…	6.10	8.89	8.63	10.1	12.1	1.9	66	34
13d	6.17 (5.5 Hz)	4.51	4.35 (3.1 Hz)	4.51	4.56, 4.21	…	6.40	8.84	8.50	9.82	10.4	1.8	64	36
15a	6.16 (5.0 Hz)	4.55	4.37 (3.2 Hz)	4.55	4.55, 4.26	…	6.38	8.74	8.53	9.41	9.87	1.7	63	37
15b	6.18 (5.5 Hz)	4.53	4.37 (3.1 Hz)	4.53	4.57, 4.24	…	6.52	8.81	8.53	9.63	9.93	1.8	64	36
15c	6.16 (5.0 Hz)	4.55	4.37 (3.0 Hz)	4.55	4.25,4.57	…	6.43	8.77	8.53	9.52	9.88	1.7	63	37
15d	6.19 (5.5 Hz)	4.62	4.34 (3.0 Hz)	4.62	4.54, 4.28	…	5.75	8.78	8.66	9.88	12.3	1.8	64	36
15e	6.18 (5.0 Hz)	4.64	4.32 (2.9 Hz)	4.64	4.46, 4.30	…	5.64	8.73	8.65	9.78	12.2	1.7	63	37

[a] Spectra were obtained in $CDCl_3$ at 500 MHz. Chemical shifts were assigned using 2D COSY spectra and are reported in ppm relative to TMS.
[b] Coupling constants are in parentheses. $J_{3',4'}$ were determined using HOMO-2DJ spectra.
[c] For hydrogen numbering, see Figure 4.
[d] Spectra for **7b** and **13b** were determined in acetone-d_6.

addition, a majority of the protein kinases inhibited by **16** (Fig. 2a) have been implicated in cancer. These data support the conclusion that protein kinases may be key targets for the N^6,5′-bis-ureidoadenosine derivatives discussed here, and provide preliminary evidence that the role of the TBS group may be to enhance membrane permeability. In this context, compound **3** would be viewed as a prodrug form of the active derivative, compound **16**.

The SAR designed to probe the effects of varying N^6 and 5′ substituents revealed that a 5′-urea is necessary for optimal antiproliferative activity. Conformational analysis showed that the 5′-carbamate derivatives are substantially less conformationally rigid, and this greater degree of conformational freedom may contribute to the observed loss of activity relative to the more conformationally constrained 5′-ureas. Conformationally restricted nucleosides have been demonstrated to have greater activities than less-restricted analogs in a number of instances,[13] and our results are in harmony with these observations. Compounds **7b** and **13b** are exceptions to this general trend, suggesting that primary targets for compounds **7b** and **13b** may differ from other members of their respective series.

The effect of varying the π-electron density of the phenyl rings of the N^6-ureido moiety ranged from somewhat modest to quite significant, with the most notable effect on antiproliferative activity being exhibited by nitro derivative **7b**. Compounds **7a**, **7c**, **7d**, and **10a**–**b** had comparable activities to compound **3**, in contrast to compounds **10c**–**e** which were approximately 10–20 times less potent.

4. Conclusion

We have developed methods for efficient preparation of N^6,5′-bis-ureido and 5′-carbamoyl-N^6-ureidoadenosine derivatives. Representative compounds from each class were prepared via the routes depicted (Schemes 1 and 2). Treatment of compounds **5** or **12** with isocyanates (*Method A*) gave N^6-ureido products **3**/**7a**–**d** and **4**/**13a**–**d** in good yields (49–86% and 45–90%, respectively). However, preparation of these compounds via this route was complicated by a number of factors, including labor-intensive chromatographic removal of diphenylurea byproducts (which occurred due to hydrolysis of excess isocyanate used to ensure complete conversion of starting material), and/or the required use of Staudinger reduction conditions when working with H_2/palladium-sensitive moieties such as C–Cl or NO_2. In addition, the number of derivatives that could be prepared by *Method A* is limited due to the somewhat restricted number of isocyanates that are commercially available. A synthetically much more versatile route was found in *Method B*. Treatment of compounds **8** or **12** with ethylchloroformate gave N^6-ethoxycarbonyl derivatives **9** and **14** in good yields (55 and 63%, respectively). Compounds **9** and **14** could be converted to N^6-ureido products (**10a**–**e** and **15a**–**e**) by simple heating in the presence of a primary amine. The relatively large selection of commercially available primary alkyl- and arylamines, together with the relative ease of preparation of such precursors, make *Method B* a versatile route providing access to a potentially vast library of compounds varying at the N^6-position.

Evaluation of compounds **4**, **7a**–**d**, **10a**–**e**, **13a**–**d**, and **15a**–**e**, against a number of cancer cell lines did not reveal any marked improvement in antiproliferative activity relative to compound **3**. The 5′-carbamoyl derivatives **4**, **13a**–**d**, and **15a**–**e** were, generally speaking, significantly less potent than the corresponding bis-ureido derivatives **3**, **7a**–**d**, and **10a**–**e**. The difference in antiproliferative activity may be at least partially attributed to a difference in conformational flexibility between the 5′-ureido and 5′-carbamoyl series.

A 2′,3′-*O*-desilylated derivative (**16**) was shown to bind to BMPR1b with a $K_d = 11.5 \pm 0.7$ μM. Compound **16** also inhibited binding of several other cancer-related protein kinases to ATP-binding site ligands in a single-dose competitive binding assay, suggesting that protein kinases may be targets for this class of compounds after intracellular hydrolysis of the 2′,3′-*O*-TBS ethers.

5. Experimental

5.1. Biology

5.1.1. Antiproliferative assays

The cytostatic effects of the test compounds on murine leukemia cells (L1210), murine mammary carcinoma cells (FM3A), human T-lymphocyte cells (CEM) and human cervix carcinoma cells

Table 3
1D-NOESY data for 5′-ureido and 5′-carbamoyl derivatives[a,b].

Compound	(H-1′)[b]	(H-2)[b]	(H-8)[b]	Compound	(H-1′)[b]	(H-2)[b]	(H-8)[b]
3	H-8: 4.02 H-2′: 1.03 H-3′: 0.21 H-4′: 0.71	H-6′: 0.40 H-5″: 0.19 H-5‴: 0.82 NCH_3: 0.29	H-1′: 3.15	**4**	H-8: 0.25 H-2′: 0.22 H-3′: 0.25 H-4′: 0.25	H-6′: 0.41	H-1′: 0.94 H-5‴: 0.54 H-2′: 0.33 H-5′: 0.45 H-5′: 0.15
7a	H-8: 4.64 H-2′: 1.31 H-3′: 0.39 H-4′: 1.00	H-6′: 0.73 H-5″: 0.86 H-5‴: 1.63 NCH_3: 0.58	H-1′: 6.43	**13a**	H-8: 1.81 H-2′: 1.77 H-3′: 0.50 H-4′: 0.50	H-6′: 0.25	H-1′: 2.00 H-5‴: 0.77 H-2′: 0.48 H-5′: 0.56 H-5′: 0.30
7b	H-8: 2.04 H-2′: 0.76 H-3′: 0.17 H-4′: 0.55	H-6′: 0.25 H-5″: 0.16 H-5‴: 0.17 NCH_3: 0.10	H-1′: 1.18	**13b**	H-8: 1.53 H-2′: 0.89 H-3′: 0.25 H-4′: 0.56	H-6′: 0.39	H-1′: 1.83 H-5‴: 0.24 H-2′: 1.25 H-5′: 0.40 H-5′: 0.47
7c	H-8: 2.93 H-2′: 1.21 H-3′: 0.00 H-4′: 0.84	H-6′: 0.00 H-5″: 0.17 H-5‴: 0.45 NCH_3: 0.20	H-1′: 3.23	**13c**	H-8: 1.10 H-2′: 1.10 H-3′: 0.23 H-4′: 0.42	H-6′: 0.33	H-1′: 0.42 H-5‴: 0.76
7d	H-8: 4.02 H-2′: 1.03 H-3′: 0.21 H-4′: 0.71	H-6′: 0.00 H-5″: 0.64 H-5‴: 0.33 NCH_3: 0.49	H-1′: 3.52	**13d**	H-8: 0.96 H-2′: 1.21 H-3′: 0.23 H-4′: 0.41	H-6′: 0.37	H-1′: 2.02 H-5‴: 0.96 H-2′: 0.56 H-3′: 0.66
10a	H-8: 1.90 H-2′: 0.76 H-3′: 0.30 H-4′: 0.45	H-6′: 0.00 H-5″: 1.31 H-5‴: 0.57 NCH_3: 0.45	H-1′: 3.46	**15a**	H-8: 1.50 H-2′: 1.77 H-3′: 0.43 H-4′: 0.61	H-6′: 0.64	H-1′: 2.49 H-2′: 0.83 H-3′: 0.96
10b	H-8: 2.79 H-2′: 1.13 H-3′: 0.57 H-4′: 0.95	H-6′: 0.62 H-5″: 1.12 H-5‴: 0.68 NCH_3: 0.54	H-1′: 5.73	**15b**	H-8: 1.45 H-2′: 1.64 H-3′: 0.32 H-4′: 0.79	H-6′: 0.56	H-1′: 2.23 H-5‴: 1.15 H-2′: 1.10 H-3′: 0.88 H-5′: 0.43
10c	H-8: 2.19 H-2′: 0.97 H-3′: 0.51 H-4′: 0.75	H-6′: 0.00 H-5″: 0.60 H-5‴: 1.15 NCH_3: 0.65	H-1′: 6.15	**15c**	H-8: 1.34 H-2′: 1.47 H-3′: 0.00 H-4′: 0.60	H-6′: 0.00	H-1′: 2.23 H-5‴: 1.15
10d	H-8: 3.29 H-2′: 1.21 H-3′: 0.39 H-4′: 0.91	H-6′: 0.63 H-5″: 1.12 H-5‴: 0.60 NCH_3: 0.71	H-1′: 6.77	**15d**	H-8: 1.41 H-2′: 1.41 H-3′: 0.37 H-4′: 0.61	H-6′: 0.60	H-1′: 2.23 H-5‴: 1.15
10e	H-8: 4.87 H-2′: 1.45 H-3′: 0.54 H-4′: 1.21	H-6′: 0.69 H-5″: 1.98 H-5‴: 0.40 NCH_3: 0.75	H-1′: 9.35	**15e**	H-8: 1.28 H-2′: 1.12 H-3′: 0.38 H-4′: 0.36	H-6′: 0.24	H-1′: 2.07 H-5‴: 0.84 H-2′: 0.61 H-3′: 0.32

[a] Data given as % enhancement when proton is irradiated.
[b] Proton irradiated is in parentheses.

(HeLa) were evaluated as follows: an appropriate number of cells suspended in growth medium were allowed to proliferate in 200-μL-wells of 96-well-microtiter plates in the presence of variable amounts of test compounds at 37 °C in a humidified CO_2-controlled atmosphere. After 48 h (L1210, FM3A), 72 h (CEM) or 96 h (HeLa), the number of cells was counted in a Coulter counter. The IC_{50} value was defined as the compound concentration required to inhibit cell proliferation by 50%.

5.1.2. Protein kinase assays

The competitive binding assays were performed by DiscoveRx, Inc. according to the following general protocol. Kinase-tagged T7 phage strains were prepared in an *Escherichia coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage and incubated with shaking at 32 °C until lysis. The lysates were centrifuged and filtered to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 min at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1× binding buffer (20% SeaBlock, 0.17× PBS, 0.05% Tween 20, 6 mM DTT). All reactions were performed in polystyrene 96-well plates in a final volume of 0.135 mL. The assay plates were incubated at room temperature with shaking for 1 h and the affinity beads were washed with wash buffer (1× PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1× PBS, 0.05% Tween 20, 0.5 μM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 min. The kinase concentration in the eluates was measured by qPCR. Binding constants (K_ds) were calculated with a standard dose–response curve using the Hill equation. The Hill Slope was set to −1, and curves were fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm.

5.2. Chemistry

5.2.1. General experimental

Moisture sensitive reactions were performed in flame-dried flasks under an inert atmosphere (N_2 or Ar) at ambient

temperature unless otherwise indicated. Solvents (CH_2Cl_2, pyridine, EtOAc, DMF, Et_3N) were dried by passing through columns of activated alumina under Ar. TLC was performed using Merck Kieselgel 60 F254 sheets, and flash chromatography was performed with silica gel (230–400 mesh) and reagent grade solvents. 'Solvent A' for chromatography consisted of the separated organic phase of EtOAc/*i*-PrOH/H_2O (4:1:2). ^1H NMR and ^{13}C NMR spectra were determined using internal references at δ 7.24 ($CDCl_3$), and δ 77.23 ($CDCl_3$), respectively. High resolution mass spectra were obtained using fast atom bombardment (FAB, NaOAc/thioglycerol or thioglycerol matrix) or electrospray (ES) ionization techniques. Commercially available reagents were used as supplied. Compounds **3**–**5**,[3a,c] **11**,[12] and **16**[3c] were prepared as previously reported. All compounds tested were >98% pure (as determined by HPLC; 5–10% IPA/CH_2Cl_2).

5.2.2. General procedure A (acylation with isocyanates)

A solution of the adenosine derivative (**5** or **12**) and R–N=C=O (1.2 equiv.) in CH_2Cl_2 was stirred protected from moisture at ambient temperature until TLC showed complete consumption of starting material (5–7d). The crude reaction mixture was added to a flash chromatography column and chromatographed directly.

5.2.3. General procedure B (hydrogenation)

A suspension of the 5′-azido-5′-deoxyadenosine derivative **6** and Pd–C (10%) in EtOAc was stirred for 12–15 h at ambient temperature under H_2 (balloon pressure). The catalyst was removed by filtering through celite. Volatiles were evaporated under reduced pressure and the crude product was used without further purification.

5.2.4. General procedure C (Staudinger reduction)

A solution of the 5′-azido-5′-deoxyadenosine derivative **6** and Ph_3P (1.5 equiv.) in THF–H_2O (25:1) was heated at 85 °C for 1.5 h. Volatiles were evaporated under reduced pressure, and the product was purified by flash chromatography.

5.2.5. General procedure D (acylation with *N*-methyl-*p*-nitrophenylcarbamate)

A solution of the 5′-amino-5′-deoxyadenosine product derived from reduction of **6**, or compound **11**, *N*-methyl-*p*-nitrophenylcarbamate, and Et_3N (or Na_2CO_3), in CH_2Cl_2 (or EtOAc) was stirred at ambient temperature until TLC indicated complete conversion to product (4–6 h). Volatiles were evaporated under reduced pressure, and the product was isolated by flash chromatography.

5.2.6. General procedure E (acylation with ethylchloroformate)

A solution of adenosine derivatives **8** or **12**, 4-(dimethylamino)pyridine, and ethylchloroformate, in pyridine was stirred at ambient temperature. Additional aliquots of ethylchloroformate were added in order to achieve complete conversion to products **9** or **14**, respectively. Volatiles were evaporated under reduced pressure, and the product was purified by flash chromatography.

5.2.7. General procedure F (N^6-Urea formation)

A solution of adenosine derivatives **9** or **14**, and various primary alkyl or arylamines, in pyridine was heated at 80 °C and the reaction was followed by TLC. Volatiles were evaporated under reduced pressure, and the product was purified by flash chromatography.

5.2.7.1. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (3).

Method A. Treatment of **5** (125 mg, 0.240 mmol), phenylisocyanate (0.29 mmol), and CH_2Cl_2 (2.9 mL) by general procedure A [chromatography 20→30% EtOAc/hexanes] gave 5′-azido-2′,3′-bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-N^6-(*N*-phenylcarbamoyl)adenosine (130 mg, 0.203 mmol, 85%). This material (123 mg, 0.192 mmol) was subjected to general procedure B [10% Pd–C (50 mg), EtOAc (10 mL)] to give a crude product that was treated with *N*-methyl-*p*-nitrophenylcarbamate, Et_3N (60 μL), and CH_2Cl_2 (4 mL), by general procedure D [chromatography 75% EtOAc/hexanes→EtOAc] to give **3** (111 mg, 0.165 mmol, 86%). *Method B.* Treatment of **9** (70 mg, 0.11 mmol), aniline (17 mg, 0.18 mmol), and pyridine (1.0 mL) by general procedure F (chromatography EtOAc), gave **3** (36 mg, 0.054 mmol, 49%). ^1H NMR ($CDCl_3$, 500 MHz) δ 11.92 (br s, 1H), 9.03 (br s, 1H), 8.67 (s, 1H), 8.61 (s, 1H), 7.57 (d, J = 7.5 Hz), 7.39 (t, J = 8.3 Hz, 2H), 7.18 (t, J = 7.3 Hz, 1H), 6.51 (d, J = 6.0 Hz, 1 H), 6.01 (d, J = 7.7 Hz, 1H), 4.74–4.73 (m, 1H), 4.64 (dd, J = 7.5, 4.5 Hz, 1 H), 4.36 (d, J = 4.5 Hz, 1H), 4.18 (t, J = 2.5 Hz, 1H), 3.99 (ddd, J = 14.5, 9.0, 2.5 Hz, 1H), 3.19 (dt, J = 14.5, 3.1 Hz, 1H), 2.72 (d, J = 4.5 Hz, 3H), 0.95 (s, 9H), 0.70 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H), −0.13 (s, 3H), −0.49 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.1, 152.9, 151.0, 150.4, 150.3, 144.1, 137.1, 129.2, 125.0, 121.8, 121.2, 88.0, 87.8, 75.9, 73.5, 41.6, 26.8, 25.9, 25.6, 18.0, 17.7, −4.53, −4.79, −5.65; MS (FAB) m/z 671.3525 (MH^+ [$C_{31}H_{51}N_8O_5Si_2$]) = 671.3516.

5.2.7.2. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)-N^6-(*N*-phenylcarbamoyl)adenosine (4).

Method A

Treatment of **12** (100 mg, 0.181 mmol), phenylisocyanate (33 mg, 0.28 mmol), and CH_2Cl_2 (2.2 mL), by general procedure A [chromatography 30→50% EtOAc/hexanes] gave **4** (109 mg, 0.162 mmol, 90%). *Method B.* Treatment of **14** (50 mg, 0.09 mmol), aniline (11 mg, 0.12 mmol), and pyridine (1 mL) by general procedure F (chromatography 50% EtOAc/hexanes), gave **4** (33 mg, 0.049 mmol, 55%). ^1H NMR ($CDCl_3$, 500 MHz) δ 12.25 (s, 1H), 9.86 (br s, 1H), 8.80 (s, 1H), 8.66 (s, 1H), 7.60 (d, J =7.5 Hz, 2H), 7.38 (t, J = 7.8 Hz, 2H), 7.16 (t, J = 7.3 Hz, 1H), 6.21 (d, J = 5.0 Hz, 1H), 5.82 (d, J = 4.0 Hz, 1H), 4.64 (t, J = 4.8 Hz, 1H), 4.50 (dd, J = 12.8, 3.8 Hz, 1H), 4.34 (t, J = 3.8 Hz, 1H), 4.31–4.29 (m, 2H), 2.47 (d, J = 5.0 Hz, 3H), 0.95 (s, 9H), 0.82 (s, 9H), 0.12 (s, 6H), 0.00 (s, 3H), −0.20 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.0, 153.4, 151.4, 150.8, 150.4, 143.9, 137.8, 129.3, 124.7, 121.6, 120.6, 87.8, 84.5, 72.4, 72.9, 63.6, 29.9, 27.1, 26.0, 25.9, 18.2, 18.0, 4.29, −4.61, −4.72, −5.15; MS (FAB) m/z 672.3353 (MH^+ [$C_{31}H_{50}N_7O_6Si_2$]) = 672.3356.

5.2.7.3. 5′-Azido-2′,3′-bis-*O*-tert-butyldimethylsilyl-5′-deoxy-N^6-[*N*-(4-chlorophenyl)-carbamoyl]adenosine (6a).

Treatment of **5** (60 mg, 0.12 mmol), 4-chlorophenylisocyanate (0.14 mmol), and CH_2Cl_2 (1.8 mL) by general procedure A [chromatography 10% EtOAc/CH_2Cl_2] gave **6a** (40 mg, 0.06 mmol, 50%). ^1H NMR ($CDCl_3$, 300 MHz) δ 11.91 (s, 1H), 8.81 (s, 1H), 8.63 (s, 1H), 8.43 (s, 1H), 7.61 (d, J = 8.9 Hz, 2H), 7.32 (d, J = 8.9 Hz, 2H), 6.00 (d, J = 3.3 Hz, 1H), 4.85 (t, J = 4.2 Hz, 1H), 4.32 (t, J = 4.5 Hz, 1H), 4.23 (dd, J = 9.0, 4.8 Hz, 1H), 3.75 (dd, J = 13.1, 4.1 Hz, 1H), 3.69 (dd, J = 13.2, 4.8 Hz, 1H), 0.94 (s, 9H), 0.84 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H), 0.00 (s, 3H), −0.15 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.6, 150.9, 150.2, 143.0, 136.9, 129.2, 129.0, 121.7, 89.9, 83.1, 75.0, 72.5, 51.8, 26.00, 25.89, 18.26, 18.12, −4.17, −4.47, −4.63, −4.67; MS (ES) m/z ($[M+H]^+$ 674.2819 [$C_{29}H_{45}ClN_9O_4Si_2$] = 674.2816).

5.2.7.4. 5′-Azido-2′,3′-bis-*O*-tert-butyldimethylsilyl-5′-deoxy-N^6-[*N*-(4-nitrophenyl)carbamoyl]adenosine (6b).

Treatment of **5** (100 mg, 0.192 mmol), 4-nitrophenylisocyanate (0.24 mmol), and CH_2Cl_2 (2.4 mL), by general procedure A [chromatography 10% EtOAc/CH_2Cl_2] gave **6b** (97 mg, 0.141 mmol, 73%). ^1H NMR ($CDCl_3$, 500 MHz) δ 12.39 (s, 1H), 8.69 (s, 1H), 8.64 (s, 1H), 8.38 (s, 1H), 8.27 (d, J = 9.3 Hz, 2H), 7.85 (d, J = 9.3 Hz, 2H), 6.02 (d, J = 4.5 Hz, 1H), 4.86 (t, J = 4.3 Hz, 1H), 4.33 (t, J = 4.3 Hz, 1H), 4.23 (dd, J = 8.7, 4.2 Hz, 1H), 3.77 (dd, J = 13.5, 4.0 Hz, 1H), 3.72 (dd,

J = 13.5, 4.8 Hz, 1H), 0.95 (s, 9H), 0.85 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H), 0.02 (s, 3H), −0.14 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 151.2, 150.9, 150.7, 149.9, 144.4, 143.6, 143.0, 125.3, 121.6, 119.7, 90.0, 83.3, 75.1, 72.5, 51.8, 26.0, 25.9, 18.3, 18.1, −4.14, −4.42, −4.58, −4.67; MS 685.3057 (ES) m/z ([M+H]$^+$ [$C_{29}H_{45}N_{10}O_6Si_2$] = 685.3062).

5.2.7.5. 5′-Azido-2′,3′-bis-*O*-tert-butyldimethylsilyl-5′-deoxy-*N*^{6}-[*N*-(4-methoxyphenyl)-carbamoyl]adenosine (6c). Treatment of **5** (100 mg, 0.192 mmol), 4-methoxyphenylisocyanate (0.24 mmol), and CH_2Cl_2 (2.4 mL), by general procedure A [chromatography 50% EtOAc/Hexanes] gave **6c** (104 mg, 0.155 mmol, 81%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.60 (s, 1H), 8.61 (s, 1H), 8.53 (s, 1H), 8.36 (s, 1H), 7.54 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 5.98 (d, J = 4.5 Hz, 1H), 4.86 (t, J = 4.0 Hz, 1H), 4.32 (t, J = 4.5 Hz, 1H), 4.23 (dd, J = 9.5, 5.0 Hz, 1H), 3.82 (s, 3H), 3.72 (dd, J = 13.0, 4.0 Hz, 1H), 3.70 (dd, J = 13.0, 5.0 Hz, 1H), 0.94 (s, 9H), 0.84 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H), 0.00 (s, 3H), −0.16 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.3, 151.5, 150.8, 150.2, 150.1, 142.5, 131.0, 122.2, 121.2, 114.2, 89.7, 82.9, 74.7, 72.3, 55.5, 51.6, 25.8, 25.7, 18.0, 17.9, −4.4, −4.7, −4.85, −4.90; MS (ES) m/z ([M+H]$^+$ 670.3335 [$C_{30}H_{48}N_9O_5Si_2$] = 670.3317).

5.2.7.6. 5′-Azido-2′,3′-bis-*O*-tert-butyldimethylsilyl-5′-deoxy-*N*^{6}-(*N*-benzylcarbamoyl)adenosine (6d). Treatment of **5** (70 mg, 0.13 mmol), benzylisocyanate (0.16 mmol), and CH_2Cl_2 (1.8 mL) by general procedure A [chromatography 10% EtOAc/CH_2Cl_2] gave **6d** (65 mg, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.97 (br s, 1H), 8.78 (br s, 1H), 8.48 (s, 1H), 8.42 (s, 1H), 7.42–6.95 (m, 5H), 5.98 (d, J = 3.6 Hz, 1H), 4.83 (t, J = 4.2 Hz, 1H), 4.66 (d, J = 5.4 Hz, 2H), 4.34 (dd, J = 10.5, 5.1 Hz, 1H), 4.32 (t, J = 4.4 Hz, 1H), 4.22 (dd, J = 9.3, 4.5 Hz, 1H), 3.73 (dd, J = 13.8, 4.5 Hz, 1H), 3.66 (dd, J = 13.7, 5.0 Hz, 1H), 0.93 (s, 9H), 0.84 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H), 0.00 (s, 3H), −0.15 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.2, 151.0, 150.5, 142.4, 138.8, 128.6, 127.4, 127.3, 121.2, 89.7, 82.8, 74.8, 72.3, 51.6, 44.0, 29.8, 25.81, 25.70, 18.04, 17.90, −4.38, −4.70, −4.85; MS 669.3609 (ES) m/z ([M+H]$^+$ [$C_{31}H_{51}N_9O_4Si_2$] = 669.3603).

5.2.7.7. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-*N*^{6}-[*N*-(4-chlorophenyl) carbamoyl]-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino] adenosine (7a). Treatment of **6a** (60 mg, 0.09 mmol), triphenylphosphine (35 mg, 0.13 mmol), THF–H_2O (25:1, 0.6 mL) by general procedure C [chromatography; solvent A] gave a crude product that was treated with *N*-methyl-*p*-nitrophenylcarbamate (26 mg, 0.13 mmol), Et_3N (0.36 mmol), and CH_2Cl_2 (2.3 mL), by general procedure D [chromatography 30% acetone/hexanes→5% MeOH/CH_2Cl_2] to give **7a** (50 mg, 0.07 mmol, 78%). ^{1}H NMR (Acetone-d_6, 300 MHz) δ 12.30 (s, 1H), 9.86 (s, 1H), 9.03 (s, 1H), 8.77 (s, 1H), 7.79 (d, J = 9.0 Hz, 2H), 7.37 (d, J = 8.7 Hz, 2H), 6.34 (t, J = 6.0 Hz, 1H), 6.15 (d, J = 7.5 Hz, 1H), 5.74 (d, J = 5.1 Hz, 1H), 4.96 (dd, J = 7.1, 4.4 Hz, 1H), 4.54 (d, J = 4.2 Hz, 1H), 4.15 (t, J = 5.3 Hz, 1H), 3.70–3.60 (m, 2H), 2.72 (d, J = 4.5 Hz, 3H), 0.98 (s, 9H), 0.73 (s, 9H), 0.19 (s, 3H), 0.16 (s, 3H), −0.05 (s, 3H), −0.41 (s, 3H); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 159.1, 151.5, 151.4, 151.0, 150.9, 150.2, 144.1, 137.6, 128.8, 128.5, 127.8, 121.3, 120.9, 87.9, 87.1, 75.2, 73.5, 41.9, 26.4, 26.3, 25.5, 25.3, 17.8, 17.5, −5.13, −5.19, −5.32, −6.16; MS 705.3126 (ES) m/z ([M+H]$^+$ [$C_{31}H_{50}ClN_8O_5Si_2$] = 705.3131).

5.2.7.8. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino]-*N*^{6}-[*N*-(4-nitro-phenyl)carbamoyl] adenosine (7b). Treatment of **6b** (60 mg, 0.09 mmol), triphenylphosphine (29 mg, 0.11 mmol), and THF–H_2O (25:1, 0.5 mL) by general procedure C [chromatography; solvent A] gave a crude product that was treated with with *N*-methyl-*p*-nitrophenylcarbamate (22 mg, 0.11 mmol), Et_3N (0.29 mmol), and CH_2Cl_2 (1.9 mL), by general procedure D [chromatography 30% acetone/hexanes→3% MeOH/CH_2Cl_2] to give **7b** (47 mg, 0.07 mmol, 78%). ^{1}H NMR (Acetone-d_6, 300 MHz) δ 12.77 (s, 1H), 9.60 (s, 1H), 8.94 (s, 1H), 8.82 (s, 1H), 8.26 (d, J = 9.3 Hz, 2H), 8.02 (d, J = 9.3 Hz, 2H), 6.33–6.30 (m, 1H), 6.14 (d, J = 7.6 Hz, 1H), 5.70 (d, J = 4.5 Hz, 1H), 5.00 (dd, J = 4.5, 2.7 Hz, 1H), 4.55 (d, J = 4.5 Hz, 1H), 4.15 (t, J = 5.4 Hz, 1H), 3.64 (d, J = 5.1 Hz, 2H), 2.74 (d, J = 4.2 Hz, 3H), 0.98 (s, 9H), 0.72 (s, 9H), 0.19 (s, 3H), 0.17 (s, 3H), −0.04 (s, 3H), −0.14 (s, 3H); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 159.9, 152.2, 152.0, 151.8, 150.9, 145.9, 145.0, 143.9, 125.8, 122.0, 120.2, 120.1, 88.9, 88.1, 75.9, 74.4, 42.8, 42.7, 27.3, 27.1, 26.4, 26.1, 18.7, 18.4, −4.2, −4.3, −4.4, −5.3; MS 716.3366 (ES) m/z ([M+H]$^+$ [$C_{31}H_{50}N_9O_7Si_2$] = 716.3353).

5.2.7.9. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-deoxy-*N*^{6}-[*N*-(4-methoxyphenyl)carbamoyl]-5′-[(*N*-methylcarbamoyl) amino]adenosine (7c). Treatment of **6c** (100 mg, 0.15 mmol), 10% Pd–C (50 mg), and EtOAc (10 mL) by general procedure B gave a crude product that was treated with *N*-methyl-*p*-nitrophenylcarbamate, Et_3N (60 μL), and CH_2Cl_2 (4 mL), by general procedure D [chromatography 5% MeOH/CH_2Cl_2] to give **7c** (60 mg, 0.086 mmol 57%). ^{1}H NMR (Acetone-d_6, 300 MHz) δ 11.98 (s, 1H), 9.55 (br s, 1H), 9.00 (s, 1H), 8.90 (s, 1H), 7.67 (d, J = 9.0 Hz, 2H), 6.98 (d, J = 9.0 Hz, 2H), 6.40–6.32 (m, 1H), 6.19 (d, J = 7.5 Hz, 1H), 5.71 (d, J = 4.8 Hz, 1H), 4.97 (dd, J = 7.4, 4.4 Hz, 1H), 4.59 (d, J = 4.5 Hz, 1H), 4.19 (t, J = 4.7 Hz, 1H), 3.83 (s, 3H), 3.77–3.67 (m, 1H), 3.62–3.54 (m, 1H), 2.72 (d, J = 4.2 Hz, 3H), 0.98 (s, 9H), 0.73 (s, 9H), 0.19 (s, 3H), 0.17 (s, 3H), 0.00 (s, 3H), −0.42 (s, 3H); ^{13}C NMR (Acetone-d_6, 75 MHz) δ 158.9, 156.3, 151.6, 151.4, 151.0, 150.9, 150.4, 143.9, 131.5, 131.4, 121.7, 121.6, 114.0, 87.9, 87.3, 75.2, 73.6, 54.8, 41.8, 25.5, 25.2, 17.8, 17.5, −5.14, −5.19, −5.33, −6.22; MS 701.3611 (ES) m/z ([M+H]$^+$ [$C_{32}H_{53}N_8O_6Si_2$] = 701.3621).

5.2.7.10. *N*^{6}-[*N*-Benzylcarbamoyl]-2′,3′-bis-*O*-tert-butyldimethylsilyl-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino] adenosine (7d). Treatment of **6d** (126 mg, 0.192 mmol), triphenylphosphine (76 mg, 0.29 mmol), and THF–H_2O (25:1, 1.2 mL) by general procedure C [chromatography; solvent A] gave a crude product that was treated with with *N*-methyl-*p*-nitrophenylcarbamate (57 mg, 0.29 mmol), Na_2CO_3 (53 mg, 0.5 mmol), and EtOAc (8.0 mL), by general procedure D [chromatography 30% acetone/hexanes→3% MeOH/CH_2Cl_2] to give **7d** (65 mg, 0.095 mmol, 49%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.43 (br s, 1H), 9.33 (br s, 1H), 8.91 (br s, 1H), 8.55 (s, 1H), 7.39–7.36 (m, 3H), 7.32–7.29 (m, 2H), 6.34 (br s, 1H), 6.10 (d, J = 8.0 Hz, 1H), 5.27 (br s, 1H), 4.65 (d, J = 6.0 Hz, 2H), 4.49 (dd, J = 7.8, 4.8 Hz, 1H), 4.45 (d, J = 5.0 Hz, 1H), 4.13 (t, J = 5.4 Hz, 1H), 3.97 (ddd, J = 14.8, 8.0, 1.5 Hz, 1H), 2.97 (dt, J = 11.5, 3.0 Hz, 1H), 2.71 (d, J = 5.0 Hz, 3H), 0.96 (s, 9H), 0.69 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H), −0.12 (s, 3H), −0.49 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.5, 156.2, 151.7, 150.9, 150.5, 144.1, 138.2, 128.9, 127.6, 126.9, 120.8, 88.6, 86.6, 77.3, 73.9, 44.3, 41.5, 29.9, 26.9, 26.1, 25.7, 18.2, 17.9, −4.3, −4.6, −5.5; MS 685.3666 (ES) m/z ([M+H]$^+$ [$C_{32}H_{53}N_8O_5Si_2$] = 685.3672).

5.2.7.11. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino]adenosine (8). Treatment of **5** (100 mg, 0.192 mmol), 10% Pd–C (50 mg), and EtOAc (10 mL) by general procedure B gave a crude product that was treated with *N*-methyl-*p*-nitrophenylcarbamate (57 mg, 0.29 mmol), Et_3N (60 μL), and CH_2Cl_2 (4 mL), by general procedure D [chromatography 5% MeOH/CH_2Cl_2] to give **8** (87 mg, 0.16 mmol, 83%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.35 (s, 1H), 7.84 (s, 1H), 7.42 (d, J = 9.5 Hz, 1H), 5.75 (d, J = 4.5 Hz, 1H), 5.65 (br s, 2H), 4.84 (dd, J = 8.3, 4.8 Hz, 1H), 4.50–4.42 (m, 1H), 4.23 (d, J = 5 Hz, 1H), 4.18 (s, 1H), 4.05 (dd, J = 14.3, 10.3 Hz, 1H), 3.17 (d, J = 14.5 Hz, 1H), 2.84 (d,

J = 4.5 Hz, 3H), 0.95 (s, 9H), 0.71 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H), −0.15 (s, 3H), −0.52 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.4, 156.4, 152.4, 149.3, 142.1, 121.9, 90.6, 88.3, 73.82, 73.80, 41.8, 27.4, 26.10, 25.8, 18.2, 18.0, −4.24, −4.35, −4.59, −5.58; MS 552.3153 (ES) *m*/*z* ([M+H]$^+$ [$C_{24}H_{46}N_7O_4Si_2$] = 552.3150).

5.2.7.12. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-deoxy-*N*^{6}-(ethoxycarbonyl)-5′-[(*N*-methylcarbamoyl)amino]-adenosine (9). Treatment of **8** (80 mg, 0.145 mmol), ethylchloroformate (62 mg, 0.57 mmol), DMAP (27 mg, 0.22 mmol), and pyridine (1 mL), by general procedure E [1 h; additional ethylchloroformate (30 mg), 1 h; chromatography 30→50% acetone/hexanes] gave **9** (50 mg, 0.080 mmol, 55%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.76 (s, 1H), 8.42 (br s, 1H), 8.03 (s, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 5.81 (d, *J* = 8.5 Hz, 1H), 4.78 (dd, *J* = 8.0, 5.0 Hz, 1H), 4.49–4.46 (m, 1H), 4.35 (q, *J* = 7.2 Hz, 2H), 4.24 (d, *J* = 4.5 Hz, 1H), 4.19 (t, *J* = 2.8 Hz, 1H), 4.01 (ddd, *J* = 15.0, 9.5, 2.5 Hz, 1H), 3.22 (dt, *J* = 14.5, 2.8 Hz, 1H), 2.81 (d, *J* = 5.0 Hz, 3H), 1.37 (t, *J* = 7.3 Hz, 3H), 0.94 (s, 9H), 0.68 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H), −0.17 (s, 3H), −0.61 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.2, 152.3, 150.9, 150.6, 150.4, 143.8, 123.8, 90.5, 88.4, 73.9, 73.6, 62.6, 41.8, 29.9, 29.5, 27.4, 26.0, 25.7, 18.2, 17.9, 14.6, −4.3, −4.4, −4.6, −5.6; MS (FAB) *m*/*z* 624.3356 ([M+H]$^+$ [$C_{27}H_{50}N_7O_6Si_2$]) = 624.3358.

5.2.7.13. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-*N*^{6}-(*N*-cyclohexyl carbamoyl)-5′-deoxy-5′-[(*N*-methylcarbamoyl) amino]adenosine (10a). Treatment of **9** (38 mg, 0.060 mmol) and cyclohexylamine (10 mg, 0.10 mmol) in pyridine (1 mL) by general procedure F [chromatography 40→60% acetone/CH_2Cl_2] gave **10a** (19 mg, 0.028 mmol, 46%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.00 (d, *J* = 7.0 Hz, 1H), 9.29 (br s, 1H), 9.04 (br s, 1H), 8.57 (s, 1H), 6.48–6.43 (m, 1H), 6.14 (d, *J* = 7.0 Hz, 1H), 5.63 (br s, 1H), 4.51–4.49 (m, 2H), 4.16 (s, 1H), 4.10 (dd, *J* = 14.3, 8.8 Hz, 1H), 3.82–3.74 (m, 1H), 3.11, (dt, *J* = 14.8, 3.4 Hz, 1H), 2.84 (d, *J* = 4.0 Hz, 3H), 2.04–1.96 (m, 2H), 1.82–1.74 (m, 2H), 1.68–1.62 (m, 2H), 1.48–1.42 (m, 4H), 0.97 (s, 9H), 0.70 (s, 9H), 0.17 (s, 3H), 0.15 (s, 3H), −0.11 (s, 3H), −0.45 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.5, 154.5, 151.1, 150.8, 143.8, 121.1, 88.6, 87.3, 77.4, 76.6, 73.7, 49.6, 41.6, 33.4, 33.3, 29.9, 27.1, 26.1, 25.8, 24.8, 18.3, 17.9, −4.29, −4.60, −5.45; MS (FAB) *m*/*z* 677.3985 ([M+H]$^+$ [$C_{31}H_{57}N_8O_5Si_2$]) = 677.3949.

5.2.7.14. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino]-*N*^{6}-(*N*-propylcarbamoyl)adenosine (10b). Treatment of **9** (38 mg, 0.061 mmol) and propylamine (11 mg, 0.19 mmol) in pyridine (1 mL) by general procedure F [chromatography 40→60% Acetone/CH_2Cl_2] gave **10b** (28 mg, 0.044 mmol, 72%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.10 (t, *J* = 5.0 Hz, 1H), 9.47 (s, 1H), 9.12 (s, 1H), 8.58 (s, 1H), 6.40 (q, *J* = 4.0 Hz, 1H), 6.17 (d, *J* = 7.5 Hz, 1H), 5.71, (br s, 1H), 4.50 (dd, *J* = 13.3, 4.8 Hz, 1H), 4.48 (dd, *J* = 8.0, 5.0 Hz, 1H), 4.15 (s, 1H), 4.07 (dd, *J* = 14.3, 8.3 Hz, 1H), 3.38 (q, *J* = 6.5 Hz, 2H), 3.11 (dt, *J* = 3.5, 15.0 Hz, 3H), 2.83 (d, *J* = 4.5 Hz, 3H), 1.70 (sext, *J* = 7.2 Hz, 2H), 1.04 (t, *J* = 7.5 Hz, 3H), 0.97 (s, 9H), 0.69 (s, 9H), 0.17 (s, 3H), 0.15 (s, 3H), 0.10 (s, 3H), −0.45 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.6, 155.6, 151.3, 150.9, 150.7, 143.9, 121.4, 88.6, 87.1, 73.8, 42.4, 41.6, 29.9, 29.5, 27.1, 26.1, 25.8, 23.3, 18.3, 17.9, 11.8, 0.21, −4.10, −4.58, −5.47; MS (FAB) *m*/*z* 637.3678 ([M+H]$^+$ [$C_{28}H_{53}N_8O_5Si_2Na$]) = 637.3677.

5.2.7.15. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-deoxy-*N*^{6}-(*N*-hexylcarbamoyl)-5′-[(*N*-methylcarbamoyl)amino]adenosine (10c). Treatment of **9** (19 mg, 0.031 mmol) and hexylamine (12 mg 0.12 mmol) in pyridine (1 mL) by general procedure F [chromatography 2% MeOH/CH_2Cl_2] gave **10c** (8 mg, 0.012 mmol, 39%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.00 (br s, 1H), 9.32 (br s, 1H), 9.02 (br s, 1H), 8.57 (s, 1H), 6.47 (d, *J* = 4.0 Hz,1H), 6.13 (d, *J* = 7.5 Hz, 1H), 5.60 (br s, 1H), 4.52–4.48 (m, 2H), 4.16 (s, 1H), 4.05 (ddd, *J* = 14.3, 8.8, 1.3 Hz, 1H), 3.40 (q, *J* = 6.5 Hz, 2H), 3.12 (dt, *J* = 14.8, 3.6 Hz, 1H), 2.84 (d, *J* = 4.5 Hz, 3H), 1.66 (pent, *J* = 7.3 Hz, 2H), 1.46–1.42 (m, 2H), 1.38–1.32 (m, 2H), 0.98 (s, 9H), 0.91 (t, *J* = 6.5 Hz, 3H), 0.70 (s, 9H), 0.17 (s, 3H), 0.15 (s, 3H), −0.10 (s, 3H), −0.46 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.5, 155.3, 151.1, 150.8, 143.8, 121.2, 88.6, 87.6, 76.5, 73.8, 41.7, 40.7, 31.7, 29.9, 27.1, 27.0, 26.1, 26.0, 25.8, 22.8, 18.3, 18.0, 14.2, −4.26, −4.30, −4.56, −5.46; MS (FAB) *m*/*z* 679.4142 ([M+H]$^+$ [$C_{31}H_{59}N_8O_5Si_2$]) = 679.4204.

5.2.7.16. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-deoxy-*N*^{6}-[*N*-(m-iodophenyl)carbamoyl]-5′-[(*N*-methylcarbamoyl)amino] adenosine (10d). Treatment of **9** (38 mg, 0.061 mmol) and *m*-iodoaniline (36 mg, 0.16 mmol) in pyridine (1 mL) by general procedure F [chromatography 2→4% MeOH/CH_2Cl_2 then 15→45% Acetone/CH_2Cl_2] gave **10d** (34 mg, 0.043 mmol, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.13 (s, 1H), 9.26 (br s, 1H), 8.72 (s, 1H), 8.69 (s, 1H), 8.01 (t, *J* = 1.8 Hz, 1H), 7.55 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.11 (t, *J* = 7.8 Hz, 1H), 6.49–6.42 (m, 1H), 6.06 (d, *J* = 8.0 Hz, 1H), 4.81–4.76 (m, 1H), 4.61 (dd, *J* = 8.0, 4.5 Hz, 1H), 4.41 (d, *J* = 4.5 Hz, 1H), 4.20 (t, *J* = 2.5 Hz, 1H), 4.10 (ddd, *J* = 15.0, 8.5, 2.5 Hz, 1H), 3.24 (dt, *J* = 14.8, 3.3 Hz, 1H), 2.75 (d, *J* = 4.5 Hz, 3H), 0.97 (s, 9H), 0.71 (s, 9H), 0.16 (s, 3H), 0.15 (s, 3H), −0.11 (s, 3H), −0.48(s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.2, 152.3, 150.9, 150.6, 150.4, 144.3, 138.9, 133.8, 130.8, 129.9, 121.8, 120.4, 94.5, 88.8, 88.3, 75.7, 73.7, 41.9, 29.8, 27.3, 26.1, 25.8, 18.3, 18.0, −4.26, −4.32, −4.55, −5.46; MS (FAB) *m*/*z* 797.2482 (([M+H]$^+$ [$C_{31}H_{50}IN_8O_5Si_2$]) = 797.2445.

5.2.7.17. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-deoxy-*N*^{6}-[*N*-(*p*-iodophenyl)carbamoyl]-5′-[(*N*-methylcarbamoyl)amino] adenosine (10e). A solution of **9** (38 mg, 0.061 mmol) and *p*-iodoaniline (39 mg, 0.18 mmol) in pyridine (1 mL) [chromatography 40→50% Acetone/hexanes then EtOAc] gave **10e** (16 mg, 0.020 mmol, 34%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.95 (s, 1H), 8.93 (br s, 1H), 8.67 (s, 1H), 8.49 (s, 1H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 9.0 Hz, 2H), 6.57 (d, *J* = 6.0 Hz, 1H), 5.97 (d, *J* = 8.0 Hz, 1H), 4.70 (dd, *J* = 8.0, 4.5 Hz, 1H), 4.66–4.64 (m, 1H), 4.34 (d, *J* = 4.5 Hz, 1H), 4.22–4.19 (m, 1H), 4.00 (ddd, *J* = 15.0, 9.0, 2.5 Hz, 1H), 3.24 (dt, *J* = 15.0, 3.0 Hz, 1H), 2.78 (d, *J* = 5.0 Hz, 3H), 0.96 (s, 9H), 0.71 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H), −0.12 (s, 3H), −0.50 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.2, 151.7, 150.5, 144.3, 138.3, 137.6, 122.8, 89.5, 88.2, 87.9, 75.0, 73.6, 41.9, 29.9, 27.6, 26.1, 25.8, 18.3, 18.0, −4.28, −4.33, −4.55, −5.48; MS (FAB) *m*/*z* 797.2482 ([M+H]$^+$ [$C_{31}H_{50}IN_8O_5Si_2$]) = 797.2474.

5.2.7.18. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-(*N*-methylcarbamoyl) adenosine (12). Treatment of **11** (618, 1.25 mmol), *N*-methyl-*p*-nitrophenylcarbamate (377 mg, 1.92 mmol), Et_3N (2.1 mL), and CH_2Cl_2 (9.5 mL) by general procedure D [60 °C overnight; chromatography EtOAc] gave **12** (580 mg, 1.05 mmol, 84%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.35 (s, 1H), 7.98 (s, 1H), 5.89 (d, *J* = 4.5 Hz, 1H), 5.51 (br s, 2H), 4.93 (t, *J* = 4.0 Hz, 1H), 4.72 (br s, 1H), 4.50 (dd, *J* = 11.8, 4.3 Hz, 1H), 4.33 (dd, *J* = 11.8, 4.7 Hz, 1H), 4.32 (t, *J* = 4.5 Hz, 1H), 4.29 (t, *J* = 4.5 Hz, 1H), 2.82 (d, *J* = 5 Hz, 3H), 0.93 (s, 9H), 0.84 (s, 9H), 0.09 (s, 6H), 0.01 (s, 3H), −0.14 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ156.7, 155.9, 153.1, 149.7, 140.1, 120.6, 89.9, 82.7, 74.7, 72.0, 63.8, 29.8, 27.8, 25.9, 25.8, 18.2, 18.0, −4.25, −4.57, −4.75, −4.80; MS (FAB) *m*/*z* 553.2999 ([M+H]$^+$ [$C_{24}H_{45}N_6O_5Si_2$]) = 553.2985.

5.2.7.19. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-*N*^{6}-[*N*-(*p*-chlorophenyl)carbamoyl]-5′-(*N*-methylcarbamoyl) adenosine (13a).

Treatment of **12** (100 mg, 0.181 mmol), *p*-chlorophenylisocyanate (43 mg, 0.28 mmol), and CH_2Cl_2 (2.2 mL) by general proce-

dure A [chromatography 30→50% EtOAc/hexanes] gave **13a** (57 mg, 0.081 mmol, 45%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.15 (s, 1H), 9.45 (br s, 1H), 8.65 (s, 1H), 8.63 (s, 1H), 7.56 (d, J =8.5 Hz, 2H), 7.33 (dt, J = 9.7, 2.5 Hz, 2H), 6.14 (d, J = 3.0 Hz, 1H), 5.48 (d, J = 5.5 Hz, 1H), 4.68 (t, J = 4.5 Hz, 1H), 4.45 (dd, J = 12.8, 3.3 Hz, 1H), 4.35 (dd, J = 12.5, 3.0 Hz, 1H), 4.33–4.28 (m, 2H), 2.60 (d, J = 5.0 Hz, 3H), 0.94 (s, 9H), 0.82 (s, 9H), 0.11 (s, 6H), 0.00 (s, 3H), −0.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 156.9, 153.2, 151.3, 150.8, 150.2, 143.9, 136.5, 129.6, 129.3, 122.5, 120.7, 88.1, 84.4, 77.7, 77.5, 72.8, 63.7, 29.9, 27.4, 26.0, 25.9, 18.2, 18.0, −3.42, −3.77, −3.82, −4.24; MS (FAB) m/z 706.2971 ($[M+H]^+$ $[C_{31}H_{49}ClN_7O_6Si_2]$) = 706.2999.

5.2.7.20. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)-N^6-[*N*-(*p*-nitrophenyl)carbamoyl]adenosine (13b).

Treatment of **12** (100 mg, 0.181 mmol), *p*-nitrophenylisocyanate (45 mg, 0.27 mmol), and CH_2Cl_2 (2.2 mL) by general procedure A [chromatography 30% EtOAc/hexanes→100% EtOAc] gave **13b** (88 mg, 0.12 mmol, 66%). ^{1}H NMR (Acetone-d_6, 500 MHz) δ 12.84 (s, 1H), 9.79 (br s, 1H), 8.83 (s, 1H), 8.75 (s, 1H), 8.22 (dt, J = 9.5, 2.7 Hz, 2H), 7.99 (dt, J = 9.5, 2.3 Hz, 2H), 6.41 (q, J = 4.5 Hz, 1H), 6.19 (d, J = 5.5 Hz, 1H), 4.93 (t, J = 5.0 Hz, 1H), 4.53 (t, J =4.0 Hz, 1H), 4.47 (dd, J = 12.3, 4.8 Hz, 1H), 4.44 (dd, J = 11.8, 4.8 Hz, 1H), 4.31 (dd, J = 8.3, 4.3 Hz, 1H), 2.72 (d, J = 4.5 Hz, 3H), 0.98 (s, 9H), 0.81 (s, 9H), 0.18 (s, 3H), 0.16 (s, 3H), 0.03 (s, 3H), −0.20 (s, 3H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 157.5, 152.3, 152.1, 151.6, 150.7, 145.8, 144.2, 143.9, 125.7, 121.7, 120.3, 120.2, 118.9 (minor), 89.1, 84.8, 76.6, 73.6, 64.4, 27.8, 26.4, 26.2, 18.7, 18.5, −4.2, −4.4, −4.5, −4.9; MS (FAB) m/z 717.3168 ($[M+H]^+$ $[C_{31}H_{49}N_8O_8Si_2]$) = 717.3212.

5.2.7.21. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-N^6-[*N*-(*p*-methoxyphenyl)carbamoyl]-5′-(*N*-methylcarbamoyl)adenosine (13C).

Treatment of **12** (100 mg, 0.181 mmol), *p*-methoxyphenylisocyanate (53 mg, 0.36 mmol), and CH_2Cl_2 (2.2 mL) by general procedure A [chromatography 30% EtOAc/hexanes→100% EtOAc] gave **13C** (88 mg, 0.125 mmol, 69%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.11 (s, 1H), 10.33 (br s, 1H), 8.99 (s, 1H), 8.65 (s, 1H), 7.47 (d, J =9.5 Hz, 2H), 6.91 (d, J = 9.0 Hz, 2H), 6.27 (d, J = 5.5 Hz, 1H), 6.25 (br s, 1H), 4.57–4.55 (m, 2H), 4.35 (dd, J =3.8, 2.8 Hz, 1H), 4.28 (dd, J = 5.0, 2.5 Hz, 1H), 4.21 (dd, J = 12.8, 2.3 Hz, 1H), 3.83 (s, 3H), 2.38 (d, J = 4.0 Hz, 1H), 0.95 (s, 9H), 0.80 (s, 9H), 0.12 (s, 6H), −0.01 (s, 3H), −0.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.0, 156.9, 153.5, 151.3, 150.9, 150.5, 143.8, 130.7, 123.5, 120.7, 114.5, 87.8, 84.6, 72.9, 63.6, 55.8, 27.3, 26.1, 25.9, 18.3, 18.1, −4.3, −4.6, −4.7, −5.1 ; MS (FAB) m/z 702.3461 ($[M+H]^+$ $[C_{32}H_{52}N_7O_7Si_2]$) = 702.3450.

5.2.7.22. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-(*N*-methylcarbamoyl) -N^6-(*N*-benzylcarbamoyl)adenosine (13d).

Treatment of **12** (100 mg, 0.181 mmol), benzylisocyanate (46 mg, 0.35 mmol), and CH_2Cl_2 (2.2 mL) by general procedure A [chromatography 30% EtOAc/hexanes→100% EtOAc] gave **13d** (58 mg, 0.085 mmol, 47%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.47 (t, J = 5.5 Hz, 1H), 10.10 (br s, 1H), 8.94 (s, 1H), 8.52 (s, 1H), 7.40–7.30 (m, 5H), 6.63 (br s, 1H), 6.21 (d, J = 5.5 Hz, 1H), 4.69 (dd, J = 15.5, 6.0 Hz, 1H), 4.64 (dd, J = 15.8, 5.8 Hz, 1H), 4.60 (dd, J = 12.8, 2.3 Hz, 1H), 4.49 (t, J = 4.8 Hz, 1H), 4.38 (t, J = 3.5 Hz, 1H), 4.25 (dd, J = 5.3, 2.3 Hz, 1H), 4.20 (dd, J = 13.0, 2.5 Hz, 1H), 2.69 (d, J = 4.5, 3H), 0.94 (s, 9H), 0.78 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H), −0.06 (s, 3H), −0.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.9, 155.5, 150.9, 150.8, 150.4, 143.2, 138.4, 128.7, 127.5, 127.4, 127.0, 120.4, 87.6, 84.4, 76.9, 72.5, 63.1, 43.9, 27.5, 25.8, 25.6, 18.0, 17.8, −4.5, −4.85, −4.89, −5.35; MS (FAB) m/z 686.3569 ($[M+H]^+$ $[C_{32}H_{52}N_7O_6Si_2]$) = 686.3518.

5.2.7.23. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-N^6-ethoxycarbonyl-5′-(*N*-methylcarbamoyl)adenosine (14).

Treatment of **12** (150 mg, 0.271 mmol), ethylchloroformate (38 mg, 0.35 mmol), DMAP (56 mg, 0. 46 mmol), and pyridine (1 mL) by general procedure E [15 h; additional ethylchloroformate (48 mg); chromatography 75% EtOAc/hexanes] gave **14** (107 mg, 0.171 mmol, 63%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.75 (s, 1H), 8.13 (br s, 1H), 8.12 (s, 1H), 5.95 (d, J = 4.5 Hz, 1H), 4.90 (t, J = 4.3 Hz, 1H), 4.72 (d, J = 2.0 Hz, 1H), 4.50 (dd, J = 11.5, 3.5 Hz, 1H), 4.37–4.32 (m, 3H), 4.31–4.27 (m, 2H), 2.83 (d, J = 5.0 Hz, 3H), 1.37 (t, J = 7.0 Hz, 3H), 0.93 (s, 9H), 0.88 (s, 9H), 0.09 (s, 6H), 0.00 (s, 3H), −0.16 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.6, 152.9, 151.4 (minor), 151.0, 149.8, 142.2, 123.1, 89.9, 82.9, 74.9, 72.0, 63.6, 62.4, 29.0, 27.9, 26.0, 25.9, 18.2, 18.1, 14.6, −4.2, −4.5, −4.72, −4.76; MS (FAB) m/z 625.3182 ($[M+H]^+$ $[C_{27}H_{49}N_6O_7Si_2]$) = 625.3201.

5.2.7.24. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-N^6-[*N*-cyclohexylcarbamoyl]-5′-(*N*-methylcarbamoyl)adenosine (15a).

Treatment of **14** (50 mg, 0.08 mmol) and cyclohexylamine (12 mg, 0.12 mmol) in pyridine (1 mL) by general procedure F [chromatography 2→4% MeOH/CH_2Cl_2] gave **15a** (50 mg, 0.074 mmol, 93%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.00 (d, J = 7.5 Hz, 1H), 9.78 (br s, 1H), 8.89 (s, 1H), 8.53 (s, 1H), 6.76 (br s, 1H), 6.21 (d, J = 5.0 Hz, 1H), 4.60 (dd, J = 12.5, 1.5 Hz, 1H), 4.49 (t, J = 4.8 Hz, 1H), 4.40 (t, J = 3.8 Hz, 1H), 4.26–4.21 (m, 1H), 3.84–3.76 (m, 1H), 2.84 (d, J = 4.0 Hz, 3H), 2.06–1.98 (m, 2H), 1.81–1.76 (m, 2H), 1.69–1.62 (m, 2H), 1.48–1.38 (m, 4H), 0.95 (s, 9H), 0.79 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H), −0.04 (s, 3H), −0.26 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.2, 154.4, 151.0, 150.9, 150.8, 143.1, 120.6, 87.8, 84.5, 77.1, 72.7, 63.3, 49.1, 33.4, 33.3, 29.9, 27.8, 26.1, 25.9, 24.9, 18.3, 18.0, −4.3, −4.6, −4.7, −5.1; MS (FAB) m/z 678.3825 ($[M+H]^+$ $[C_{31}H_{56}N_7O_6Si_2]$) = 678.3839.

5.2.7.25. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)-N^6-[*N*-propylcarbamoyl]adenosine (15b).

Treatment of **14** (75 mg, 0.12 mmol) and propylamine (11 mg, 0.19 mmol) in pyridine (1 mL) by general procedure F [chromatography 2→4% MeOH/CH_2Cl_2] gave **15b** (50 mg, 0.078 mmol, 65%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.08 (br s, 1H), 10.08–10.06 (m, 1H), 8.99 (s, 1H), 8.54 (s, 1H), 6.99–6.94 (m, 1H), 6.24 (d, J = 5.5 Hz, 1H), 4.64 (dd, J = 13.0, 2.0 Hz, 1H), 4.46 (t, J = 4.8 Hz, 1H), 4.40 (t, J = 3.8 Hz, 1H), 4.25 (d, J = 2.5 Hz, 1H), 4.19 (dd, J = 13.0, 2.5 Hz, 1H), 3.45–3.32 (m, 2H), 2.83 (d, J = 4.5 Hz, 3H), 1.70 (sext, J = 7.2 Hz, 2H), 1.04 (t, J = 7.3 Hz, 3H), 0.95 (s, 9H), 0.78 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H), −0.04 (s, 3H), −0.28 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.2, 155.5, 151.1, 150.9, 150.8, 143.4, 120.5, 87.6, 84.7, 77.3, 72.7, 63.3, 42.2, 29.5, 27.8, 26.0, 25.8, 23.2, 18.2, 18.0, 11.8, −4.26, −4.63, −4.70, −5.20; MS (FAB) m/z 638.3512 ($[M+H]^+$ $[C_{28}H_{52}N_7O_6Si_2]$) = 638.3518.

5.2.7.26. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-N^6-[*N*-hexylcarbamoyl]-5′-(*N*-methylcarbamoyl)adenosine (15c).

Treatment of **14** (75 mg, 0.12 mmol) and hexylamine (14 mg 0.14 mmol) in pyridine (1 mL) by general procedure F [chromatography 2→4% MeOH/CH_2Cl_2] gave **15c** (51 mg, 0.075 mmol, 63%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.02 (t, J = 5.5 Hz, 1H), 9.98 (br s, 1H), 8.95 (s, 1H), 8.54 (s, 1H), 6.88 (br s, 1H), 6.23 (d, J = 6.0 Hz, 1H), 4.63 (dd, J = 12.5, 2.0 Hz, 1H), 4.48 (t, J = 5.3 Hz, 1H), 4.40 (t, J = 3.8 Hz, 1H), 4.25 (dd, J = 4.5, 2.5 Hz, 1H), 4.20 (dd, J = 12.5, 2.5 Hz, 1H), 3.42–3.38 (m, 2H), 2.83 (d, J = 5.0 Hz, 3H), 1.67 (sext, J = 7.5 Hz, 2H), 1.46–1.42 (m, 2H), 1.38–1.32 (m, 4H), 0.95 (s, 9H), 0.91 (t, J = 6.8 Hz, 3H), 0.78 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H), −0.04 (s, 3H), −0.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.2, 155.4, 151.1, 151.0, 150.8, 143.3, 120.6, 87.7, 84.7, 72.7, 63.3, 40.5, 31.7, 29.9, 27.8, 27.0, 26.1, 25.8, 22.8, 18.3, 18.0, 14.2, −4.25, −4.62, −4.68, −5.17; MS (FAB) m/z 680.3915 ($[M+H]^+$ $[C_{31}H_{58}N_7O_6Si_2]$) = 680.4004.

5.2.7.27. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)-N^6-[*N*-(*m*-iodophenyl)carbamoyl]-adenosine (15d).

Treatment of **14** (75 mg, 0.120 mmol) and *m*-iodoaniline (36 mg, 0.17 mmol) in pyridine (1 mL) by general procedure F [chromatography 2→4% MeOH/CH_2Cl_2] gave **15d** (53 mg, 0.066 mmol, 55%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.35 (s, 1H), 10.22 (s, 1H), 8.90 (s, 1H), 8.68 (s, 1H), 8.02 (s, 1H), 7.56 (d, J = 10.5 Hz, 1H), 7.50 (d, J = 7.0 Hz, 1H), 7.10 (t, J = 8.3 Hz, 1H), 6.24 (d, J = 5.5 Hz, 1H), 5.97-5.92 (m, 1H), 4.59 (dd, J = 10.5, 5.0 Hz, 1H), 4.58 (dd, J = 8.5, 3.0 Hz, 1H), 4.36 (t, J = 3.5 Hz, 1H), 4.30–4.25 (m, 2H), 2.52 (d, J = 5.0 Hz, 3H), 0.96 (s, 9H), 0.80 (s, 9H), 0.13 (s, 6H), 0.00 (s, 3H), −0.24 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.0, 152.9, 151.4, 150.9, 150.2, 143.7, 139.2, 133.5, 130.8, 130.0, 120.9, 120.5, 94.5, 88.3, 84.7, 72.9, 63.8, 30.0, 27.6, 26.1, 25.9, 18.3, 18.1, −3.72, −4.02, −4.05, −4.56; MS (FAB) m/z 798.2324 ($[M+H]^+$ $[C_{31}H_{49}IN_7O_6Si_2]$) = 798.2328.

5.2.7.28. 2′,3′-Bis-*O*-tert-butyldimethylsilyl-N^6-[*N*-(*p*-iodophenyl)carbamoyl]-5′-(*N*-methylcarbamoyl)adenosine (15e).

Treatment of **14** (75 mg, 0.12 mmol) and *p*-iodoaniline (40 mg, 0.18 mmol) in pyridine (1 mL) by general procedure F [chromatography 15→25% Acetone/CH_2Cl_2] gave **15e** (37 mg, 0.046 mmol, 39%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.35 (s, 1H), 10.22 (s, 1H), 8.90 (s, 1H), 8.68 (s, 1H), 7.67 (d, J = 8.7 Hz, 1H), 7.38 (d, J = 8.7 Hz, 1H), 6.23 (d, J = 5.4 Hz, 1H), 5.97–5.92 (m, 1H), 4.59 (dd, J = 10.5, 5.0 Hz, 1H), 4.58 (dd, J = 8.5, 3.0 Hz, 1H), 4.36 (t, J = 3.5 Hz, 1H), 4.30–4.25 (m, 2H), 2.52 (d, J = 5.0 Hz, 3H), 0.96 (s, 9H), 0.80 (s, 9H), 0.13 (s, 6H), 0.00 (s, 3H), −0.24 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.7, 153.0, 151.3, 150.9, 150.2, 143.4, 138.3, 137.8, 123.1, 120.1, 88.3, 87.7, 84.3, 72.8, 63.8, 30.0, 27.5, 26.1, 25.9, 18.3, 18.1, −3.74, −4.10, −4.53; MS (FAB) m/z 798.2322 ($[M+H]^+$ $[C_{31}H_{49}IN_7O_6Si_2]$) = 798.2328.

Acknowledgments

The authors thank Mrs. Lizette van Berckelaer, Mrs. Leen Ingels, Mrs. Lies Van den Heurck, Mr. Steven Carmans, Mrs. Anita Camps, Mrs. Leentje Persoons and Mrs. Frieda De Meyer for excellent technical assistance. Financial support was provided by the K.U. Leuven (GOA 10/15) and BYU development funds.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.11.043.

References and notes

1. For discussions highlighting the role of nucleoside derivatives in anticancer therapeutics see: (a) Secrist, J. A., III *Nucleic Acids Symp. Ser.* **2005**, *49*, 15; (b) Galmarini, C. M.; Mackey, J. R.; Dumontet, C. *Lancet Oncol.* **2002**, *3*, 415; (c) Galmarini, C. M.; Popowycz, F.; Joseph, B. *Curr. Med. Chem.* **2008**, *15*, 1072; (d) Jordheim, L. P.; Galmarini, C. M.; Dumonte, C. *Rec. Pat. Antican. Drug Disc.* **2006**, *1*, 163.
2. For discussions highlighting the role of nucleoside derivatives in antiviral therapeutics see: (a) Mansour, T. S.; Storer, R. *Curr. Pharm. Des.* **1997**, *3*, 227; (b) Herdewijn, P. *Drug Discov. Today* **1997**, *2*, 235; (c) Furman, P. A.; Lam, A. M.; Murakami, E. *Future Med. Chem.* **2009**, *1*, 1429; (d) Cihlar, T.; Ray, A. S. *Antiviral Res.* **2010**, *85*, 39; (e) Mathe, C.; Gosselin, G. *Antiviral Res.* **2006**, *71*, 276; (f) Meng, W. D.; Qing, F. L. *Curr. Top. Med. Chem.* **2006**, *6*, 1499; (g) Ichikawa, E.; Kato, K. *Curr. Med. Chem.* **2001**, *8*, 385; (h) Freeman, S.; Gardiner, J. M. *Mol. Biotech.* **1996**, *5*, 125; (i) Huryn, D. M.; Okabe, M. *Chem. Rev.* **1992**, *92*, 1745.
3. (a) Peterson, M. A.; Oliveira, M.; Christiansen, M. A.; Cutler, C. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6775; (b) Peterson, M. A.; Oliveira, M.; Christiansen, M. A. *Nucleosides Nucleotides Nucleic Acids* **2009**, *28*, 394; (c) Peterson, M. A.; Ke, P.; Shi, H.; Jones, C.; McDougal, B. R.; Robinson, W. E. *Nucleosides Nucleotides Nucleic Acids* **2007**, *26*, 499.
4. Shelton, J. R.; Burt, S. R.; Peterson, M. A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1484.
5. Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1088.
6. <http://dtp.nci.nih.gov/compare/>
7. Fabian, M. A.; Biggs, W. H., III; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lélias, J.-M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J. *Nature Biotechnol.* **2005**, *23*, 329.
8. Fabbro, D.; Ruetz, S.; Buchdunger, E.; Cowan-Jacob, S. W.; Fendrich, G.; Liebetanz, J.; Mestan, J.; O'Reilly, T.; Traxler, P.; Chaudhuri, B.; Fretz, H.; Zimmermann, J.; Meyer, T.; Caravatti, G.; Furet, P.; Manley, P. W. *Pharm. Ther.* **2002**, *93*, 79.
9. (a) Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210; (b) Bissantz, C.; Kuhn, B.; Stahl, M. *J. Med. Chem.* **2010**, *53*, 5061.
10. For examples of the C–H/π-interaction in biological systems see: (a) Obst, U.; Banner, D. W.; Weber, L.; Diederich, F. *Chem. Biol.* **1997**, *4*, 287; (b) Nishio, M.; Hirota, M.; Umezawa, Y. *The CH/π Interaction*; Wiley: New York, 1998; (c) Suezawa, H.; Hashimoto, T.; Tsuchinaga, K.; Yoshida, T.; Yuzuri, T.; Sakakibara, K.; Hirota, M.; Nishio, M. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1243; (d) Vyas, N. K.; Vyas, M. N.; Quiocho, F. A. *Science* **1988**, *242*, 1290.
11. For a discussion on the complications associated with cyclonucleoside formation from 5′-activated adenosine derivatives, see Ref.[3c] and references cited therein.
12. Zhu, X.-F.; Williams, H. J.; Scott, I. A. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2305.
13. For excellent discussions of the impact of nucleoside conformation on biological activities, see: (a) Mathé, C.; Périgard, C. *Eur. J. Org. Chem.* **2008**, 1489; (b) Ford, H., Jr.; Dai, F.; Mu, L.; Siddiqui, M. A.; Niklaus, M. C.; Anderson, L.; Marquez, V. E.; Barchi, J. J., Jr. *Biochemistry* **2000**, *39*, 2581; (c) Mu, L.; Sarafianos, S. G.; Nicklaus, M. C.; Russ, P.; Siddiqui, M. A.; Ford, H., Jr.; Mitsuya, H.; Le, R.; Kodama, E.; Meier, C.; Knispel, T.; Anderson, L.; Barchi, J. J., Jr.; Marquez, V. E. *Biochemistry* **2000**, *39*, 11205; (d) Eoff, R. L.; McGrath, C. E.; Maddukuri, L.; Salamanca-Pinzon, S. G.; Marquez, V. E.; Marnett, L. J.; Guengerich, F. P.; Egli, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 7481.
14. Davies, D. B. *Nucl. Magn. Reson. Spec.* **1978**, *12*, 135.
15. Davies, D. B.; Danyluk, S. S. *Biochemistry* **1974**, *13*, 4417.
16. (a) de Zwart, M.; Kourounakis, A.; Kooijman, H.; Spek, A. L.; Link, R.; von Frijtag Drabbe K ünzel, J. K.; IJzerman, A. P. *J. Med. Chem.* **1999**, *42*, 1384; (b) Homma, H.; Watanabe, Y.; Abiru, T.; Murayama, T.; Nomura, Y.; Matsuda, A. *J. Med. Chem.* **1992**, *35*, 2881; (c) Moss, W. J.; Hamilton, H. W.; Ortwine, D. F.; Taylor, M. D.; McPhail, A. T. *Nucleosides Nucleotides* **1989**, *8*, 449.
17. (a) Li, F.; Maag, H.; Alfredson, T. *J. Pharm. Sci.* **2008**, *97*, 1109; (b) Mackman, R. L.; Cihlar, T. *Ann. Rep. Med. Chem.* **2004**, 305; (c) Krise, J. P.; Stella, V. J. *Adv. Drug Deliv.* **1996**, *19*, 287; (d) Meier, C. *Synlett* **1998**, 233.
18. Camarasa, M. –J.; San-Felix, A.; Velázquez, S.; Pérez-Pérez, M. –J.; Gago, F.; Balzarini, J. *Curr. Top. Med. Chem.* **2004**, *4*, 945.
19. Das, K.; Bauman, J. D.; Rim, A. S.; Dharia, C.; Clark, A. D., Jr.; Camarasa, M. –J.; Balzarini, J.; Arnold, E. *J. Med. Chem.* **2011**, *54*, 2727.
20. (a) Ruzinova, M. B.; Benezra, R. *Trends Cell Biol.* **2003**, *13*, 410; (b) Ying, Q. L.; Nichols, J.; Chambers, I.; Smith, A. *Cell* **2003**, *115*, 281; (c) Korchynskyi, O.; ten Dijke, P. *J. Biol. Chem.* **2002**, *277*, 4883; (d) Lopez-Rovira, T.; Chalaux, E.; Massague, J.; Rosa, J. L.; Ventura, F. *J. Biol. Chem.* **2002**, *277*, 3176.
21. (a) Ling, M. –T.; Wang, X.; Zhang, X.; Wong, Y. –C. *Differentiation* **2006**, *74*, 481; (b) Gautschi, O.; Tepper, C. G.; Purnell, P. R.; Izumiya, Y.; Evans, C. P.; Green, T. P.; Desprez, P. Y.; Lara, P. N.; Gandara, D. R.; Mack, P. C.; Kung, H. –J. *Cancer Res.* **2008**, *68*, 2250.
22. (a) Wong, Y.-C.; Wang, X.; Ling, M.-T. *Apoptosis* **2004**, *9*, 279; (b) Ling, M.-T.; Kwok, W. K.; Fung, M. K.; Wang, X. H.; Wong, Y. C. *Carcinogenesis* **2006**, *27*, 205; (c) Ling, Y. X.; Tao, J.; Fang, S. F.; Hui, Z.; Fang, Q. R. *Eur. J. Cancer Prev.* **2011**, *20*, 9; (d) Mern, D. S.; Hoppe-Seyler, K.; Hoppe-Seyler, F.; Hasskarl, J.; Burwinkel, B. *Breast Cancer Res.* **2010**, *124*, 623; (e) Mern, D. S.; Hasskarl, J.; Burwinkel, B. *Br. J. Cancer* **2010**, *103*, 1237.