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Synthesis and Biological Evaluation of Various Derivatives of a Broad-Spectrum Anticancer Nucleoside

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A dissertation submitted to the faculty of
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Doctor of Philosophy

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ABSTRACT

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Broad-Spectrum Anticancer Nucleoside

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Recently the Peterson lab discovered a promising anticancer adenosine derivative– 2′,3′-bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-[*N*-(methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine. This compound showed selective toxicity against human colon cancer cells in vitro with LC_{50}'s = 6–10 μM.

It was hypothesized that the lead compound exerted its cytotoxic effects by interacting with a protein kinase. A systematic Structure Activity Relationship (SAR) was undertaken in an attempt to increase the kinase-binding affinity of the lead compound. Many regions of the lead compound were examined: the N^6-phenyl urea moiety, the 5′-*N*-methyl urea group, the 2′,3′-bis-*O*-TBS groups, the nucleobase, and the ribose sugar. Results of these studies produced some promising new derivatives. In particular, one analogue exhibited potent cancer cell growth inhibition with an average GI_{50} of 0.58 μM (NCI-60). In addition, another compound showed selective toxicity for the non-small cell adenocarcinoma cell line NCI-H522 with an LC_{50} of 10 nM.

Efficient methods for the preparation of a wide variety of N^6-aryl and -alkyl substituted derivatives were developed. One versatile route involved the installation of an N^6-ethoxy carbonyl and subsequent displacement with an alkly- or arylamine. Synthetic routes for the preparation of of a variety of 2′,3′-bis-*O*-acylated analogues were also developed.

Nucleoside mono-, di-, and triphosphate bioisosteres in which the phosphoester or phosphoanhydride have been replaced by an unnatural functional group have been extensively investigated. A simple and efficient method was developed for the preparation of carbamoyl analogues of nucleoside mono-, di-, and triphosphate surrogates. This method uses a modified version of the Kočovský reaction to install mono-, di-, and triphosphate mimics in good to excellent yields (ave = 75%).

Keywords: Anticancer Nucleosides, N^6,5′-Bis-ureidoadensoines, Antiproliferative Activity, BMPR1b, Nucleotide Surrogates

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Chapter 1: Nucleoside Derivatives–a Fruitful Field for Drug Discovery

1.1. Nucleoside Derivatives as Medicinal Agents

Nucleoside derivatives (NDs) have proven to be a fruitful field for drug discovery.[1] These derivatives include analogues of the naturally occurring DNA and RNA bases, pyrimidines cytidine, thymidine, and uracil as well as purines adenine and guanine. They also include derivatives of the accompanying nucleosides and nucleotides of these bases. Figure 1 shows the five naturally occurring nucleosides.[2]

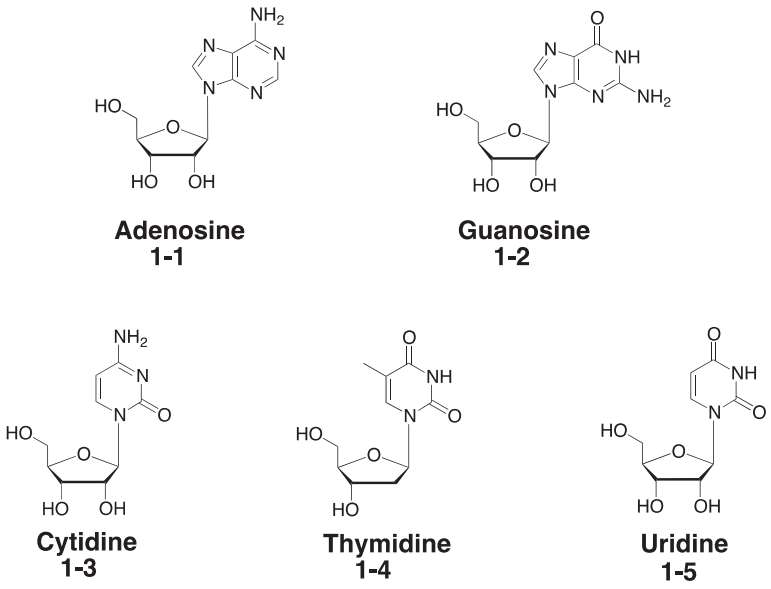

Figure 1. Naturally occurring nucleosides.

Due to the ubiquitous nature of NDs in biological settings, modifications in their structures can potentially lead to powerful biological effects. Much research has been conducted in this field producing many medicinally important compounds. NDs have proven particularly effective

as anticancer agents.[3-6] These derivatives have also been successful in producing antiviral therapeutics.[7-14] The Food and Drug Administration (FDA) has approved 37 NDs for clinical use, 14 of which are used for cancer. The structures of the 14 FDA-approved anticancer NDs are shown in Figure 2.

Mercaptopurine (**1-6)** is most commonly used to treat acute lymphatic leukemia, however, it is also used to treat various autoimmune disorders.[15, 16] Upon entrance into the cell, mercaptopurine is converted into its nucleoside and triphosphate nucleotide forms. The triphosphate nucleotide can inhibit RNA synthesis and the deoxy form can be incorporated into DNA, leading to cytotoxicity.[16, 17] Thioguanine (**1-7)** is also used in the treatment of leukemia.[16] It follows the same basic biological mechanism as mercaptopurine, but is more specific in its mode of action.[16-18]

Fluorouracil (**1-8)** is used to treat head and neck cancer, colorectal cancer, and breast cancer.[19] It enters cells either by uracil transporters or by passive diffusion. Ribosylation and phosphorylation subsequently occur.[19-21] The triphosphate nucleotide is incorporated into RNA and inhibits rRNA maturation leading to cell death.[22] The deoxy monophosphate nucleotide inhibits thymidylate synthase causing a decrease in the pools of deoxythymidine monophosphate and an increase in the pools of deoxyuridine monophosphate (dUMP).[19-21] This increase in dUMP causes an increase of deoxyuridine triphosphate (dUTP) concentration, which leads to the misincorporation of dUTP into DNA.[19] Floxuridine (**1-12)** and capecitabine (**1-14)** are FDA-approved prodrugs of fluorouracil. They were designed to have better selectivity and bioavailability than fluorouracil.[23, 24]

Azacitidine (**1-9)** is used to treat myelodysplastic syndrome (preleukemia).[25] This disease occurs when bone marrow makes misshapen or ineffective blood cells.[25, 26] Azacitidine is

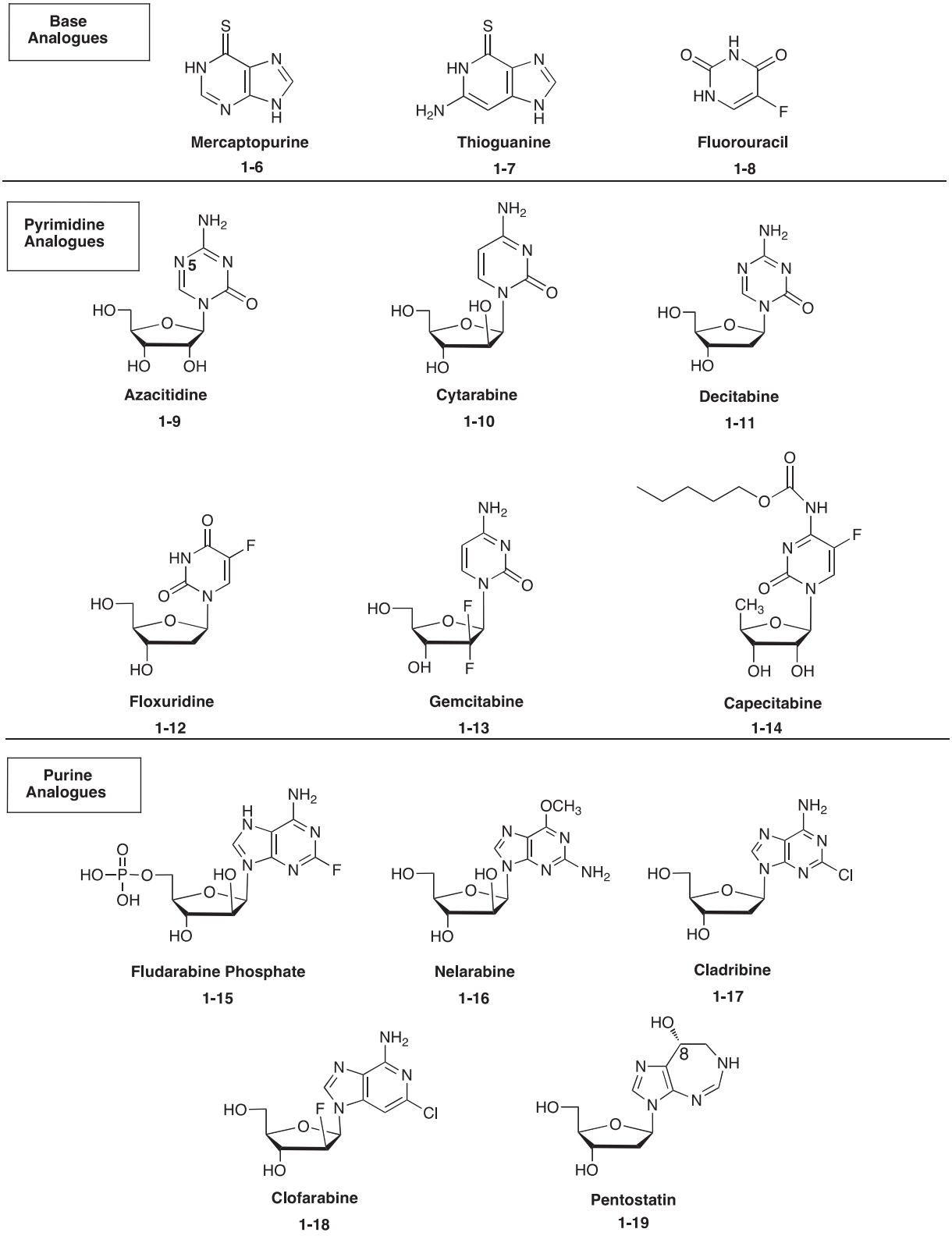

Figure 2. FDA-approved ND cancer drugs.

actively transported into cells by uridine/cytidine facilitated transport systems.[27] Following conversion to the triphosphate, it is predominantly incoporated into RNA.[27-29] This drug is structurally very similar to cytidine with the only variation being a nitrogen at the 5 position in the base. This nitrogen destabilizes the base resulting in a half-life of about four hours.[27] This decomposition destabilizes the RNA strand and disrupts RNA processing.[29]

Decitabine (**1-11)** is approved for the treatment of myelodysplastic syndrome and is also in clinical trials for the treatment of various other cancer types.[25] It is structurally very similar to azacitidine, except that it lack a 2′-OH. It also follows a similar biological mode of action, differing from azacitidine in that it can only be incorporated into DNA.

Fludarabine phosphate (**1-15)** is a commonly used drug for chronic lymphoid leukemia and low grade B- and T-cell non-Hodgkin's lymphoma.[30] The triphosphate form of the drug can be incorporated into RNA. The 2′-deoxy triphosphorylated form inhibits DNA polymerase, DNA primase, DNA ligase, and/or is incorporated into DNA.[31, 32] This eventually causes DNA strand breaks leading to apoptosis.

Nelarabine **(1-16)** was approved in 2005 by the FDA and is used to treat T-cell lymphoblastic lymphoma and T-cell acute lymphoblastic leukemia.[33] It mimics the effects of purine nucleoside phosphorylase inhibition. Purine nucleoside phosphorylase deficiencies lead to accumulation of deoxyguanosine in the plasma which leads to deoxyguanosine triphosphate mediated T-cell lymphopenia.[34, 35] The triphosphate nucleotide form of the drug is incorporated into DNA causing chain termination and inhibition of DNA synthesis, resulting in apoptotic cell death.[35, 36]

Cladribine (**1-17)** was developed by BYU professors Roland K. Robins and Morris J. Robins.[37] It was approved by the FDA in 1993 and is very effective against hairy cell leukemia

with a nearly 85% response rate. It is also used to treat acute myelogenous leukemia, chronic myelogenous leukemia, chronic lymphatic leukemia, non-Hodgkin's lymphoma, and cutaneous T-cell lymphoma.[38-41] Phosphorylated cladribine causes accumulation of DNA strand breaks and can be incorporated into growing DNA strands impairing DNA synthesis.[31, 42, 43]

Clofarabine (**1-18)** is used in the treatment of pediatric relapsed or refractory acute lymphoblastic leukemia and is structurally similar to cladribine and fludarabine.[44] The 2′-fluoro substituent renders **1-18** more hydrolytically stable at the anomeric position than cladribine and fludarabine.[45, 46] The di- and tri-phosphate forms of clofarabine inhibit ribonucleotide reductase and DNA polymerase, respectively.[44, 45, 47, 48] The triphosphate form is also incorporated into DNA causing chain termination, possibly due to the electron withdrawing nature of the fluorine atom affecting the reactivity of the 3′-OH and/or the structure of the DNA helix.[45]

Pentostatin (**1-19)** is used to treat hairy cell leukemia.[30, 49] It was originally isolated from *Streptomyces antibioticus* and found to inhibit adenosine deaminase.[50, 51] The drug acts as a transition state analogue of adenosine deaminase. The tetrahedral carbon at position 8 mimics the proposed tetrahedral carbon in the deamination of adenosine to inosine. Adenosine deaminase deficiencies cause lymphopenia, which is why pentostatin is effective against lymphoid malignancies with high adenosine deaminase activities.[49, 50, 52] Inhibition of adenosine deaminase eventually leads to DNA single-strand breaks, hindered repair mechanisms, and inhibition of transcription, all of which lead to cell death.[53]

1.2. Anticancer Nucleoside Derivative General Modes of Biological Action

Nucleoside derivatives generally act as antimetabolites to produce their biological effects.[4] Antimetabolites are compounds that inhibit the ability of a cell to use normal metabolites.

Because cancer cells generally divide faster than non-cancerous cells, they tend to take up more metabolites than normal cells. As a consequence antimetabolites are also taken up to a greater extent, thus providing the neoplastic cell with greater quantities of the anticancer NDs.

Once inside the cell, ND antimetabolites have varied biological mechanisms of action. However, broadly speaking, they exert their cytotoxic activity by one of two general mechanisms. They can also act by both mechanisms, though they predominantly exert their effects by one or the other (Figure 3).[54] The first mechanism of action (Mechanism 1) begins when the ND enters the cell and is triphosphorylated. A cellular polymerase subsequently uses the ND as a substrate and incorporates it into a growing strand of DNA or RNA. This destabilizes the strands and/or causes strand breaks leading to cell cycle arrest and apoptosis.

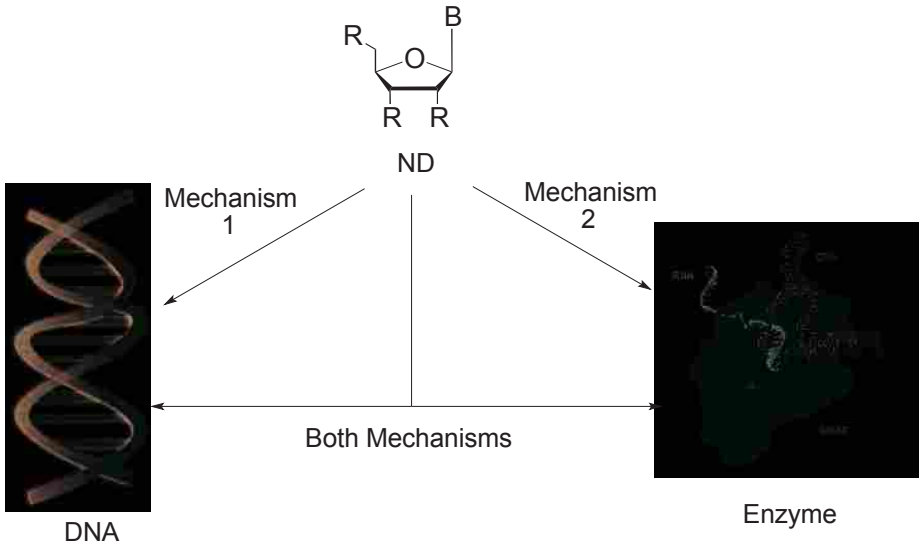

Figure 3. General mechanisms of anticancer NDs.

The second general mechanism of action (Mechanism 2) includes the ND acting as a competitive inhibitor of enzymes essential for metabolism, usually those involved in nucleoside/nucleotide synthesis. Inhibition of these enzymes disrupts intracellular

nucleoside/nucleotide pool concentrations leading to mutations in the newly synthesized DNA. Like mechanism 1, this also causes cell cycle arrest and apoptosis.

It is interesting to note that of the 14 FDA-approved anticancer drugs, base and pyrimidine analogues act mainly by mechanism 1 whereas the purine analogues primarily exert their effects via mechanism 2.[15-53]

1.3. Adenosine Derivative Research

Research focused on the development of adenosine NDs has lead to modifications in both the nucleobase and ribose sugar. Much of this research has been directed toward development of selective agonists and/or antagonists for cell surface adenosine receptors.[55] Adenosine receptors are involved in a diverse array of important physiological processes such as cell growth and differentiation, immunosupression, platelet aggregation, regulation of myocardial oxygen and coronary blood flow, and apoptosis.[56-59]

1.3.1. Discovery of Promising Anticancer Bis-Ureidoadenosine Derivatives

Research in the laboratory of Dr. Matt A. Peterson (BYU) has recently lead to the discovery of some promising anticancer adenosine derivatives (Figure 4).[60, 61] When screened against the NCI-60,[62] compounds **1-20**–**1-22** showed average growth inhibition values (GI_{50}) = 7.57 μM, 17.2 μM, and 3.13 μM, respectively.

A preliminary Structure Activity Relationship (SAR) study of compounds **1-20** and **1-21** showed that the 2′-*O*-TBS group is necessary for anticancer activity. The 5′-*N*-methyl urea and N^6-*N*-phenyl urea substitutions were also necessary. Left in doubt from these initial studies was the relative importance of the 3′-substitution in **1-20** and **1-21**. Hence compound **1-22**, which

was easier to prepare than **1-20** or **1-21**, was tested. We were pleased to find that not only was the 3′-substitution found in **1-20** and **1-21** not necessary for anticancer activity, but the 3′-*O*-*tert*-butyldimethylsilyl group (TBS) found in **1-22** gave rise to an increase in cancer cell growth inhibition as well as selective toxicity against human colon cancer cells in vitro. These data coupled with the relative synthetic ease of derivative **1-22** versus compounds **1-20** and **1-21**, made analogue **1-22** the lead compound for further studies.

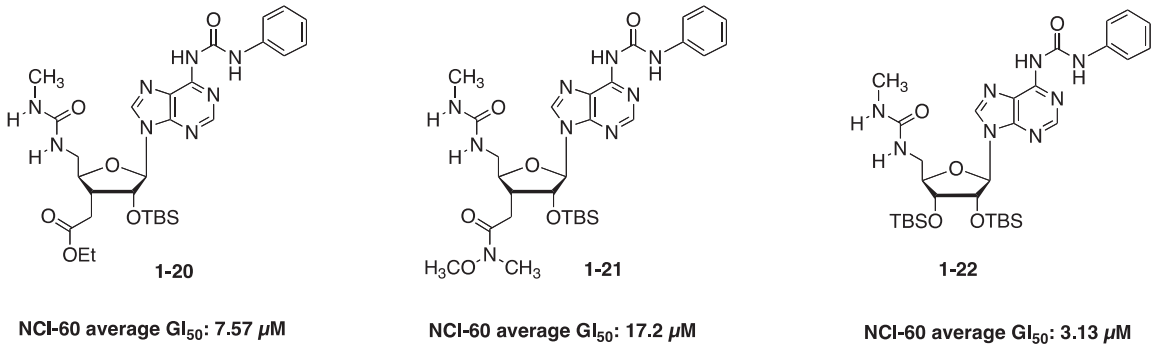

Figure 4. Promising anticancer bis-ureidoadenosine derivatives.

A COMPARE[63] analysis of the GI_{50} data for compound **1-20** suggested that protein kinases may be molecular targets for this derivative. Derivative **1-20** was screened against a panel of 353 protein kinases to determine the binding affinities of **1-20** for these kinases. Of the 353 screened kinases, **1-20** showed selective binding inhibition of 11 (each of which has been implicated a variety of cancers) with the highest inhibition of binding observed for BMPR1b (or Alk 6).[64] It was also assumed that protein kinases could be targets for the lead compound **1-22**.

1.3.2. Putative Mechanism of Action of Lead Adenosine Derivative 1-22.

Preliminarily, these data point to a putative biological mechanism of action for compound

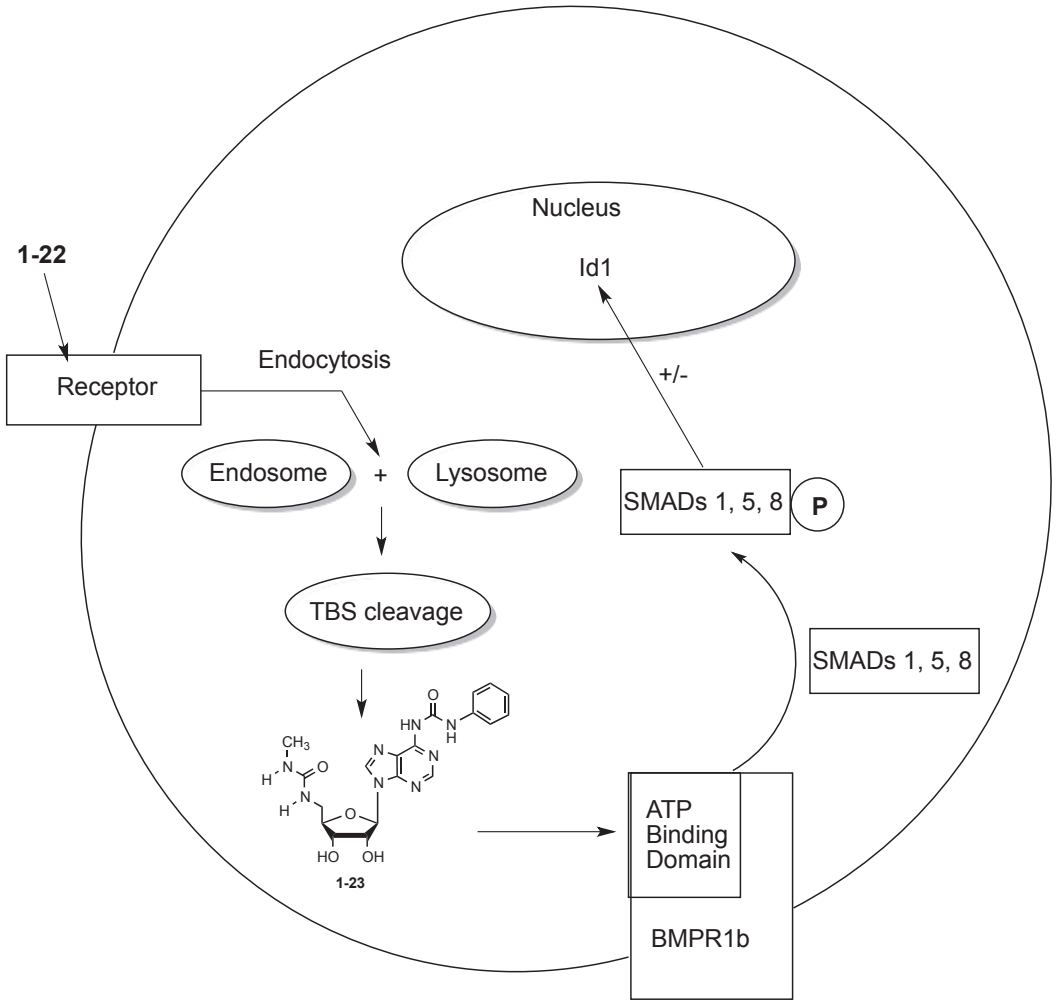

Figure 5. Putative biological mechanism of action of lead compound **1-22**.

1-22 (Figure 5). The mechanism begins with lead compound **1-22** entering the cell via an ND receptor such as one of the purine P2 or P1 adensoine receptors (e.g., A_{2A}). Upon binding to the ND receptor, the compound enters the cell via endocytosis in clathrin-coated pits. This leads to receptor and agonist localization in early endosomes.[65] The resultant endosomes join with lysosomes. The acidic environment of the lysosomes (pH ~4.5) cleaves the 2′,3′-TBS groups to give compound **1-23**. Compound **1-23** then enters the cytosol and subsequently binds and inhibits BMPR1b, a transmembrane receptor whose ATP binding site lies within the

cytoplasm.[61, 66] BMPR1b is part of a signaling cascade which activates SMADs 1, 5, and 8 by phosphorylation. These SMADs regulate the expression of Id-1.[67-70] Overexpression of Id-1 has been reported in a number of cancers including lung,[71] breast,[72] colon,[73] ovarian,[74] pancreas,[75] prostate,[76] and renal cancers.[77] Downregulation, inhibition, and/or inactivation of Id-1 have been shown to induce apoptosis in several of these cancers.[78-82]

1.4. Systematic SAR of Lead Compound 1-22

In order to increase anticancer potency as well as probe the mechanism of action of lead compound **1-22**, we undertook a systematic SAR targeting a generally-accepted model for an ATP kinase binding site.[83] In this model, the ATP-binding site has a phosphate binding region, a hydrophobic pocket, and a region for sugar interaction (Figure 6). Our SAR focused on structural modifications of the 5′-position (phosphate binding pocket), the 2′,3′-positions (sugar region), and the N^6-region (hydrophobic binding pocket) and will be discussed in subsequent chapters.

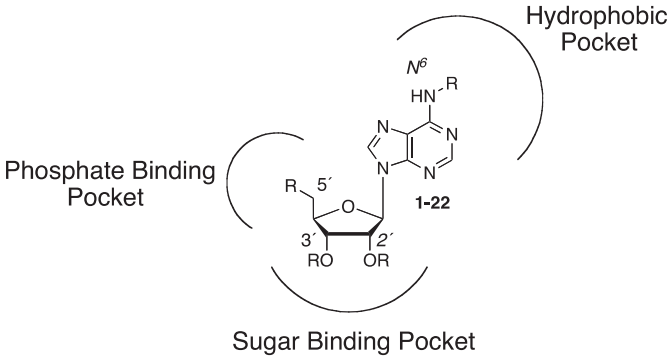

Figure 6. Putative ATP kinase binding pocket with generic adenosine derivative.

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Chapter 2: Synthesis, SAR, and Preliminary Mechanistic Evalutaion of the N^6-Position of a Promising Anticancer Bis-Ureido Compound and its 5′-Carbamate Derivatives

2.1. Introduction

A preliminary SAR of lead compound **2-1** was devised drawing on previous SARs of earlier lead compounds[1] as well as our working biological model (see chapter 1). The SAR explored the N^6-position of the lead compound. This position possibly interacts with the hydrophobic pocket of the ATP binding site of a protein kinase.[2] Compounds in the study varied in steric and electronic nature at the N^6-position. In an attempt to decrease synthetic steps, carbamate derivatives were also prepared. A derivative of **2-1** lacking the 2′,3′-*O*-TBS groups (**2-3**) was evaluated to probe the role of these substituents (Figure 1).

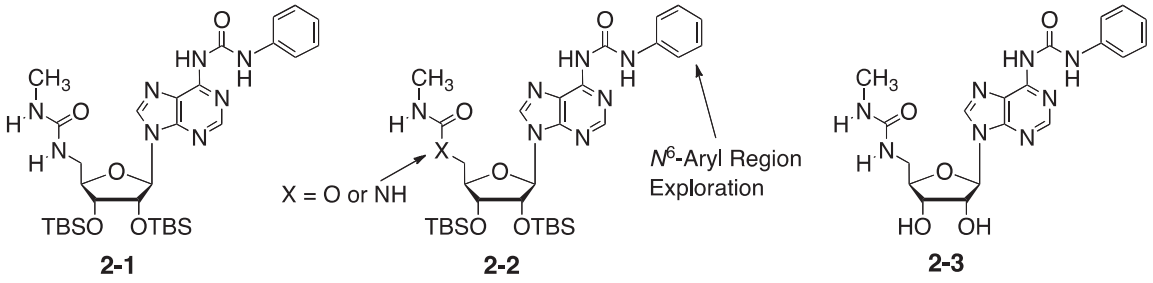


Figure 1. Preliminary SAR structures.

2.1. Chemistry

Two synthetic approaches were explored to test the effect of varying N^6- substitution. It was hypothesized that altering the π-electron density in the N^6-phenyl ring could lead to tighter binding in the hydrophobic pocket. At the time this research was conducted, the X-ray crystal structure of BMPR1b had not been published. We were thus forced to devise an SAR study

without the guidance of X-ray structural information. We began by testing the very reasonable hypothesis that variation in the nature of the N^6 substituent might lead to enhanced binding with hydrophobic amino acid residues in the ATP binding site of BMPR1b. We thus set out to systematically probe the effects of increasing or decreasing the π-electron density of the N^6-phenyl urea. Based on earlier precedent, such a systematic empirical approach could lead to improved binding in the hydrophobic pocket by fine-tuning the electronic interactions between amino acid residues in the binding pocket of the receptor and the ligand, a phenomenon that has been observed in many related instances.[3, 4]

It has been well established that edge-to-face and face-to-face non-covalent bonding interactions can occur between aromatic moieties found in receptor ligands and aromatic side chains in receptors.[3] It has also been reported that aliphatic C-H/π-electron interactions between the ligand and receptor can occur and be exploited to increase molecular recognition (see Figure 2).[5-8] Electron-withdrawing substituents decrease π-electron density leading to increased Lewis-acidity of phenyl rings, whereas electron-donating substituents increase π-electron density and increase the Lewis-basicity of the phenyl ring.[3]

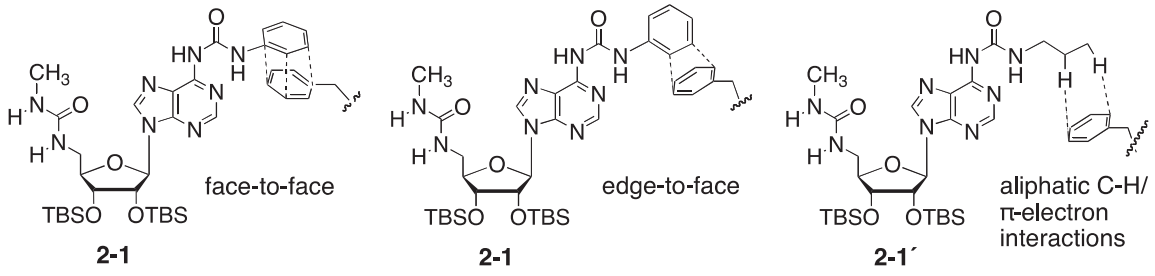
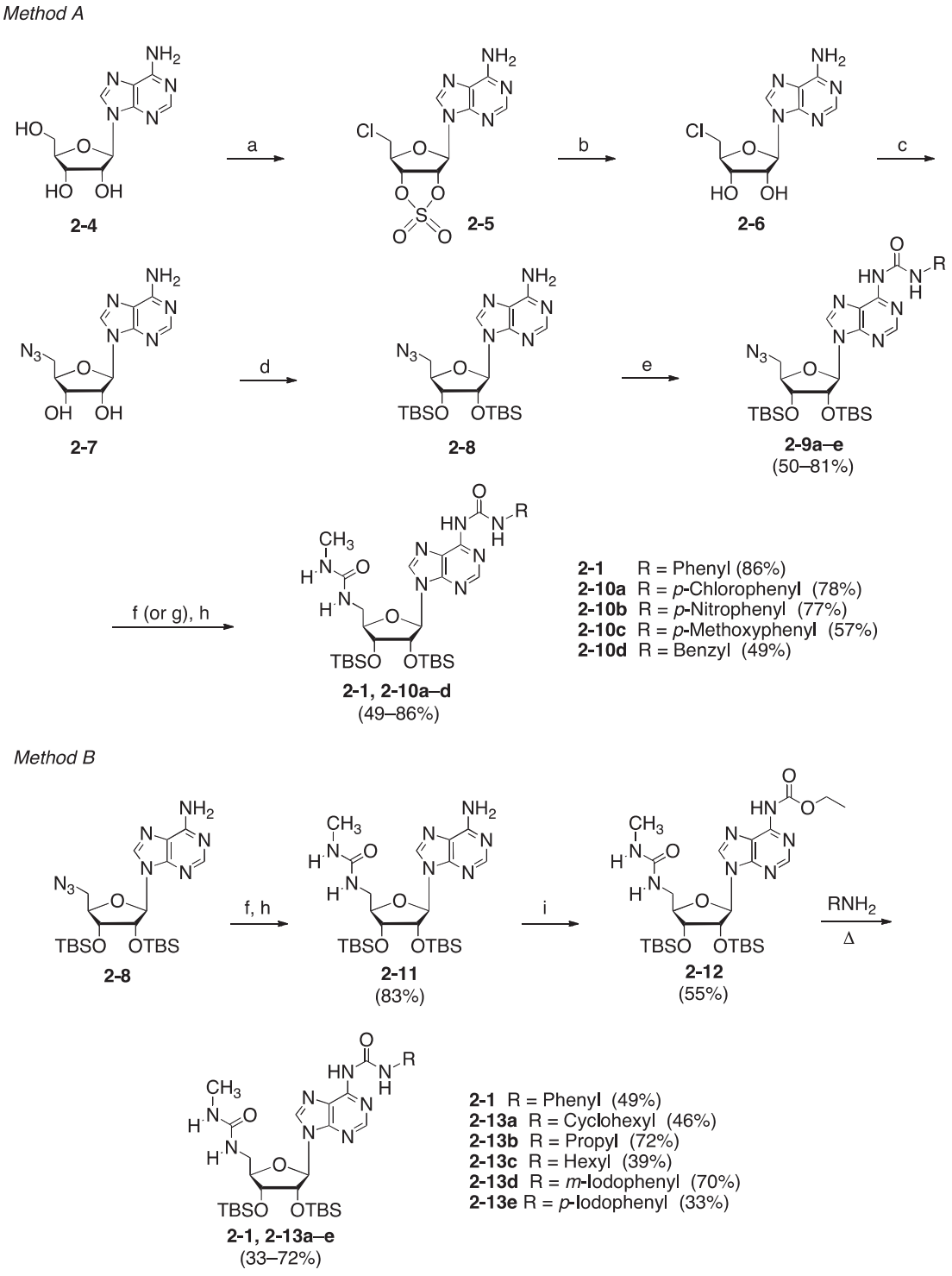


Figure 2. Non-covalent intermolecular interactions.
Possible non-covalent intermolecular interactions between lead **2-1** and **2-1´** and aromatic amino acid residues of an enzyme binding pocket.

It was reasoned that analogues of **2-1** which varied in the π-electron density of the phenyl ring (e.g., **2-10a-d**) might bind with different affinities to the hydrophobic pocket of the putative kinase receptor (Scheme 1). Therefore, compounds **2-9a-e** were prepared by *Method A*. The synthesis of **2-8** was reported previously.[1] These derivatives vary in the electronic nature of their respective N^6-aromatic rings by possessing either electron donating (**2-10c**) or withdrawing (**2-10a,b**) substituents or by loss of conjugation with the π-system of the phenyl ring with the urea moiety (**2-10d**). This method gave the desired products in yields of 49-78%. Compounds **2-10a-d** had lower yields than **2-1** due to either the difficulty in the chromatographic separation of the unavoidable biphenylurea byproducts (final synthetic step) and/or the required use of the Staudinger reduction for derivatives **2-10a,b**. The Staudinger reduction was used in order to avoid reduction of the C-Cl and C-NO_2 substituents, respectively. In order to obviate these difficulties as well as greatly increase synthetic access to a vast compound pool, *Method B* was developed (Scheme 1). This method installs the 5′-*N*-methylurea (**2-11**) followed by introduction of the N^6-ethoxycarbonyl (**2-12**). The ethoxycarbonyl has the potential of being converted to a wide variety of N^6-alky or aryl derivatives by simple heating in the presence of a wide variety of primary alky- or arylamines, respectively.

Unhindered (e.g., propyl, **2-13b**; hexyl, **2-13c**) and relatively hindered (e.g., cyclohexyl, **2-13a**) primary alkyl- and arylamines (e.g., *m*-iodoaniline, **2-13d**; *p*-iodoaniline, **2-13e**) were produced in moderate to good yields. This supports the hypothesis that *Method B* could be used to produce a large library of N^6-derivatives, because they can be prepared from a vast array of commercially available primary amines. This stands in contrast to the relatively few commercially available isocyanates needed for *Method A*.

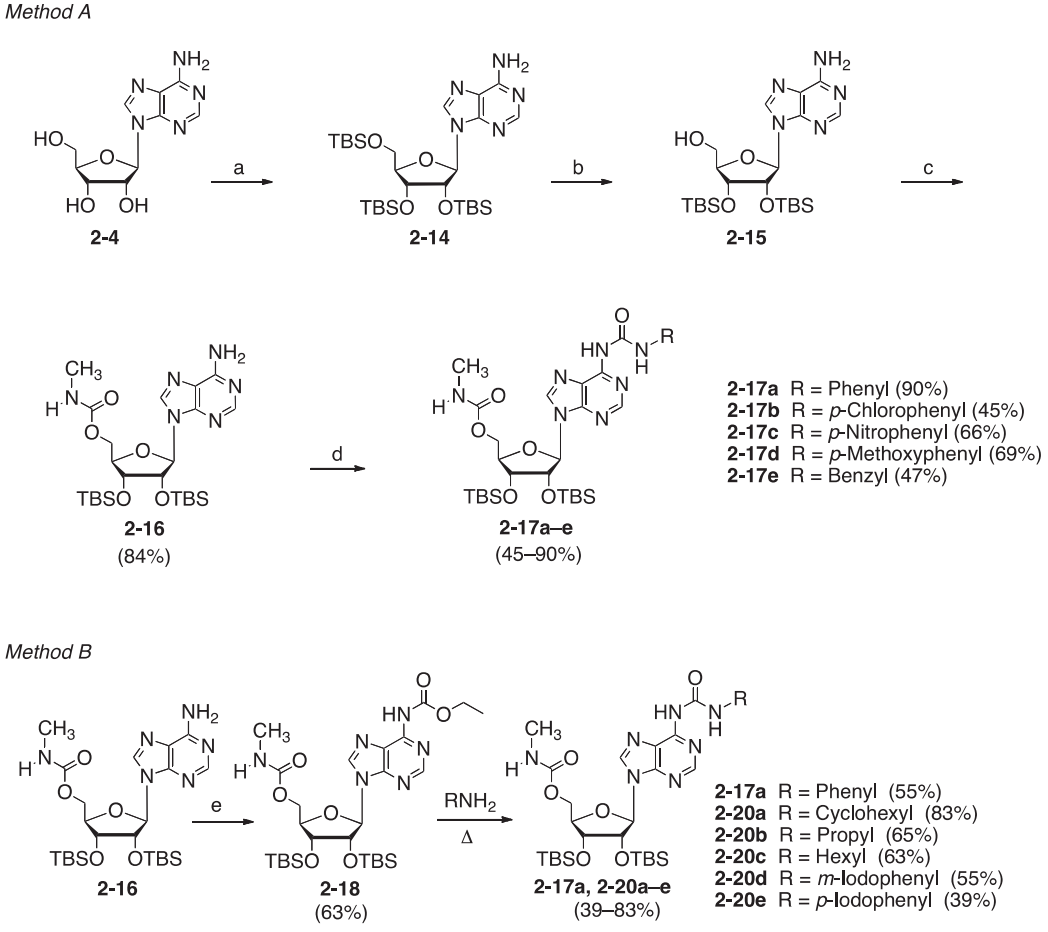
Replacement of the 5′-*N*-methylurea group of **2-1** with the isosteric 5′-*N*-



Scheme 1. 5′-Urea Methods A and B synthesis.
Reagents: (a) $SOCl_2$; (b) MeOH; (c) NaN_3, Δ; (d) TBSCl, Imid; (e) R–N=C=O; (f) H_2, Pd-C; (g) Ph_3P, H_2O; (h) *p*-NO_2-$C_6H_4OC(O)NHCH_3$; (i) ethylchloroformate. Compounds synthesized by Method A were made by Marcelio Oliveira.[9]

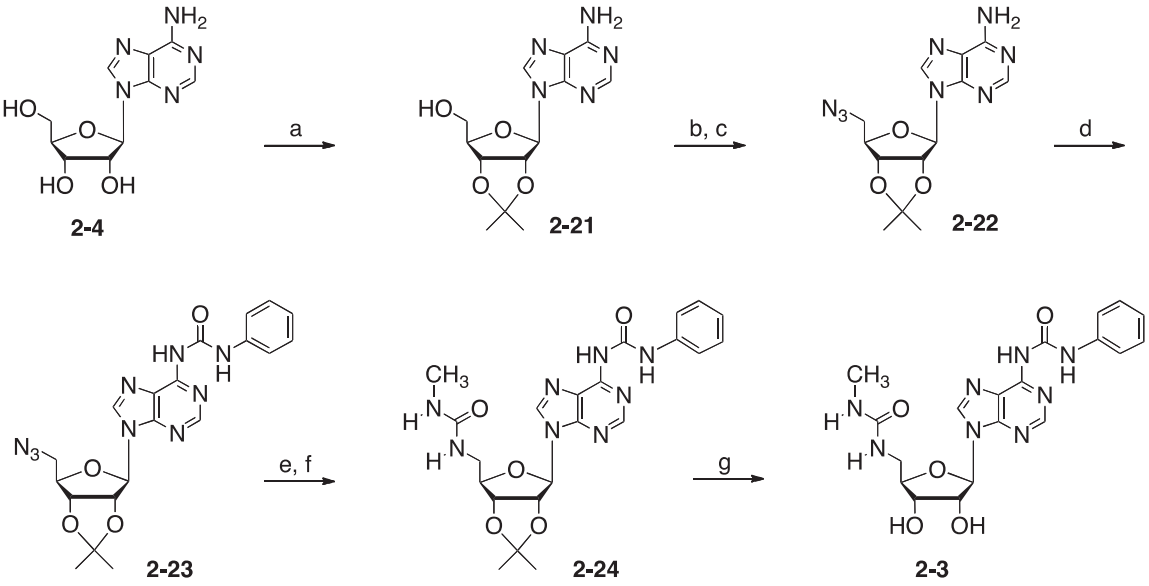
methylcarbamoyl moiety would decrease the number of synthetic steps required by three (from adenosine) and thereby greatly increase the overall efficiency of the preparation. This would be advantageous provided the carbamate derivatives retain biological activity.

Carbamate derivatives **2-17a-e** and **2-20a-e** were synthesized to probe the same SAR features as ureas **2-1, 2-11a-d**, and **2-13a-e** (Scheme 2). The syntheses proceeded freely and gave the desired products in moderate to good yields from bis-*O*-TBS derivative **2-15**, which is readily prepared from adenosine in excellent yield.[10]



Scheme 2. 5′-Carbamate Methods A and B synthesis.
Reagents: (a) TBSCl, Imid; (b) TFA, H_2O; (c) *p*-NO_2-$C_6H_4OC(O)NHCH_3$; (d) R–N=C=O; (e) ethylchloroformate.

Our proposed biological model suggests that compound **2-1** may act as a prodrug of the desilylated derivative **2-3** (Scheme 3). This compound was essentially inactive against the NCI-60, showing the importance of the 2′,3′-TBS groups for in vitro activity.[2] However, upon entrance into the cell, the TBS groups could be cleaved leaving compound **2-3** to possibly bind protein kinases. In order to determine whether **2-3** does in fact bind to protein kinases, desilylated **2-3** was prepared by a previously reported method.[2]



Scheme 3. Synthesis of **2-3**.
Reagents: (a) $HClO_4$, acetone; (b) $SOCl_2$; (c) NaN_3, Δ; (d) PhN=C=O; (e) H_2, Pd-C; (f) *p*-NO_2-$C_6H_4OC(O)NHCH_3$; (g) TFA.

2.2. Biology

2.2.1. Antiproliferative Activity

Compounds **2-1, 2-10a-d, 2-13a-e, 2-17a-e,** and **2-20a-e** were tested for their antiproliferative activity using murine leukemia L1210, murine mammary carcinoma FM3A,

human lymphoblastic leukemia CEM, and human cervix carcinoma HeLa (Table 1). The data show that derivatives **2-10a-d** and **2-13a-e** had no significantly improved biological activity relative to lead compound **2-1**, although compounds **2-10a, 2-10c, 2-10d, 2-13a,** and **2-13b** showed comparable activity. Generally inferior activities were exhibited by the carbamate analogues **2-17a-e** and **20a-e**, with the exception of **2-17c**, which showed significantly higher antiproliferative activity than the other carbamates.

Table 1. Inhibitory effects of test compounds.
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). IC_{50} (μg/ml): 50% Inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

Compound	L1210	FM3A	CEM	HeLa
2-1	3.8 ± 0.3	5.9 ± 1.1	8.3 ± 2.9	3.2 ± 0.2
2-17a	160 ± 56	> 200	> 200	>200
2-10a	5.6 ± 1.1	33 ± 2	14 ± 8	3.4 ± 0.0
2-10b	>200	>200	>200	>200
2-10c	3.8 ± 0.0	5.8 ± 0.4	5.2 ± 1.3	2.6 ± 0.8
2-10d	4.6 ± 0.9	9.2 ± 0.2	8.3 ± 0.8	3.8 ± 2.1
2-13a	20 ± 13	8.4 ± 0.8	5.2 ± 0.3	11 ± 3
2-13b	3.9 ± 0.3	4.7 ± 0.5	4.4 ± 1.1	3.3 ± 0.7
2-13c	74 ± 8	ND	68 ± 9	74 ± 9
2-13d	38 ± 17	ND	182 ± 8	21 ± 1
2-13e	43 ± 15	ND	68 ± 34	18 ± 2
2-17b	>200	>200	>200	>200
2-17c	7.7 ± 1.6	24 ± 5	15 ± 1	1.5 ± 1.0
2-17d	>200	>200	>200	>200
2-17e	101 ± 5	>200	>200	23 ± 4
2-20a	74 ± 0	>200	175 ± 8	69 ± 51

2-20b	18 ± 11	23 ± 4	59 ± 7	55 ± 32
2-20c	>200	>200	175 ± 8	88 ± 56
2-20d	>200	>200	>200	>200
2-20e	>200	>200	>200	143 ± 80

2.2.2. Protein Kinase Binding Activity

As mentioned previously, compound **2-3** was prepared to test if lead compound **2-1** possibly exhibits protein kinase inhibition after intracellular desilylation. Compound **2-3** was subjected to the competitive binding inhibition assay of Fabian et al.[11] In this assay, desilylated derivative **2-3** inhibited binding of 16 of 441 protein kinases to ATP-binding site ligands by 30-45% (Figure 3). A multi-dose binding assay showed that compound **2-3** binds to BMPR1b with a K_d = 11.5 ± 0.7 μM whereas lead **2-1** did not bind to BMPR1b at concentrations as high as 30 μM (Figure 4).

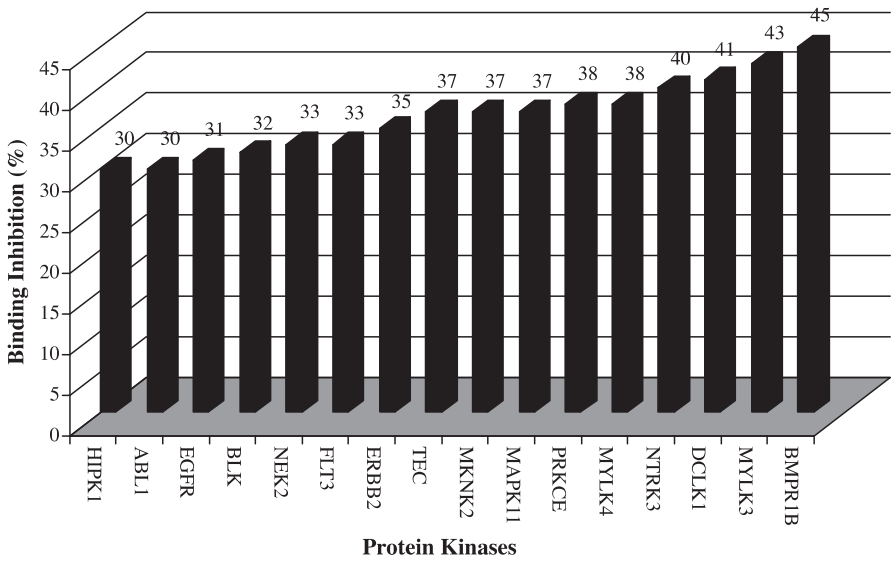
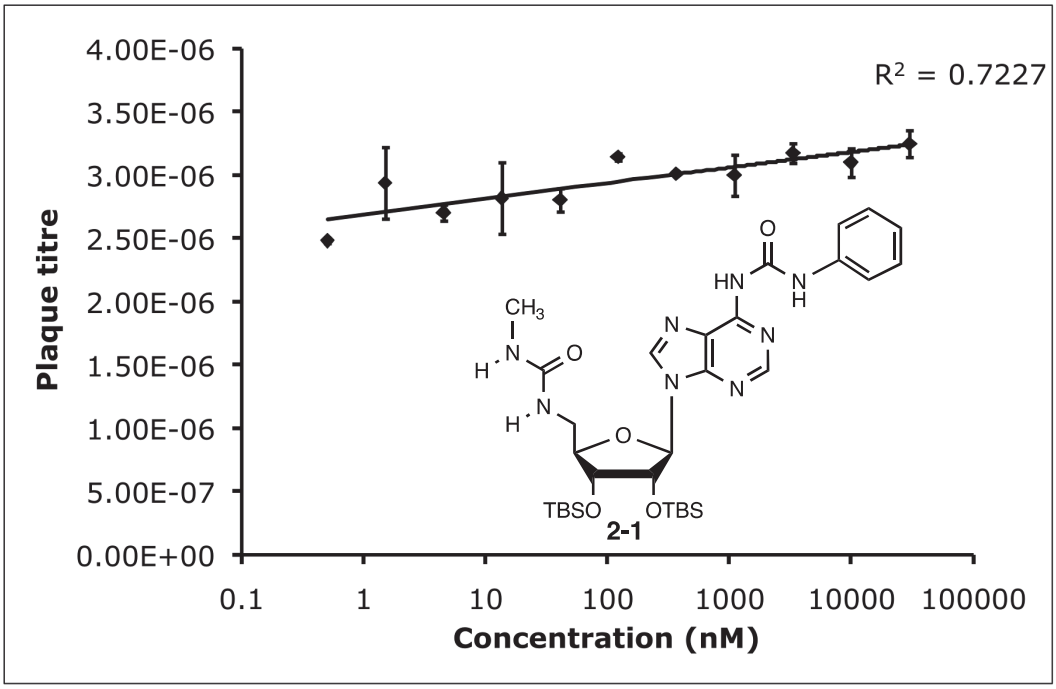


Figure 3. Inhibition assay of **2-3**.
Inhibition of binding protein kinases to immobilized ATP-binding site ligands by compound **2-3** (10 μM).



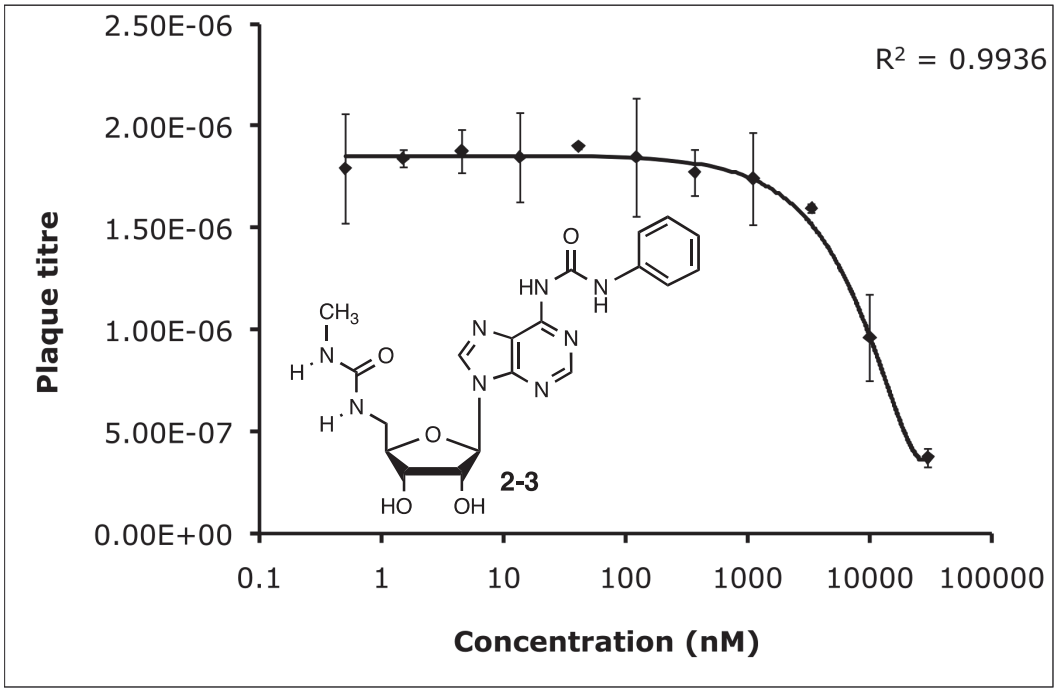


Figure 4. Equilibrium competitive binding assay of **2-1** and **2-3**.
Effect of **2-1** and **2-3** on equilibrium competition binding of BMPR1b to immobilized ATP-binding site ligand.

2.3. Conformational Analysis

Conformations of nucleosides play a key role in their biological activities.[12-15] A possible explanation for the difference in the biological activity of the urea derivatives (**2-1**, **2-10a-d**, **2-13a-e**) vs the carbamate analogues (**2-17a-e**, **2-20a-e**) could be the difference in the *syn*/*anti* conformational preference for the compounds.[16] Nuclear Magnetic Resonance (NMR) coupling constant data ($J_{1',2'}/J_{3',4'}$), and 1D-NOESY experiments show that the urea derivatives exhibit conformational preferences for the *syn* glycosyl conformer, whereas the carbamate compounds show less preference for the *syn* conformer and exhibit more rotational freedom around the glycosidic bond (Figure 5 and Tables 2 and 3).

The conformational equilibrium between *syn* and *anti* conformers (K_{eq}) may be calculated using equation 1. In this relatioship mole fractions of the S or *syn* conformer (X_S) and the mole fraction of the N or *anti* conformer (X_N) are calculated from the observed $J_{1',2'}$ and $J_{3',4'}$ values, respectively. The C2′-*endo* (S) conformations correlates primarily with a *syn* glycosyl conformation (χ = 10-30°), and the C3′-*endo* (N) correlates primarily with the *anti* glycosyl conformation (χ = 200-210°; Figure 5). Equilibrium constant calculations from equation 1 compare very well to those obtained by a full pseudorotational analysis.[17, 18]

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

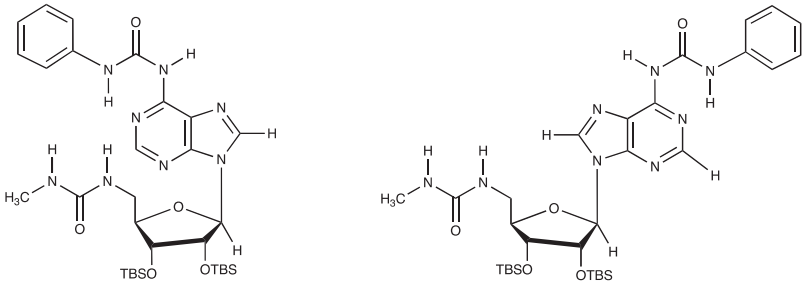


Figure 5. C2′-*endo* (S) conformer (syn); C3′-*endo* (N) conformer (anti).

Table 2. ^{1}H NMR and K_{eq} data.
^{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
2-1	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
2-10a	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
2-10b[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
2-10c	6.11 (7.5 Hz)	4.53	4.43 (1.8 Hz)	4.53	3.98, 1.14	6.27	4.96	8.94	8.65	9.52	11.9	4.1	80	20
2-10d	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
2-13a	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
2-13b	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
2-13c	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
2-13d	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
2-13e	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
2-17a	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
2-17b	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
2-17c[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
2-17d	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
2-17e	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
2-20a	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
2-20b	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
2-20c	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
2-20d	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

2-20e	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. $J_{3',4'}$ and were determined using HOMO-2DJ spectra.
[c] For hydrogen numbering, see Figure 5.
[d] Spectra for **2-10b** and **2-17c** were determined in acetone-d_6.

Intramolecular hydrogen bonding between the N3 of the adenine heterocycle and 5′ H-bond donors has been reported for several adenosine analogues.[19] In such compounds (e.g., NECA), a *syn* glycosyl conformation was observed in the solid state, and the *syn* glycosyl conformer was also predominant in solution. The 1D-NOESY data from the urea analogues are consistent with a strong preference for the *syn* conformer (Figure 6). This is consistent with intramolecular hydrogen bonding between the N3 of the adenine base and the 5′-NH of the urea moiety (see strong NOE enhancement of H-1′ and H-8 when either is irradiated, Table 3). Absence of a strong NOE when either H-1′, H-2, or H-8 are irradiated in the 5′-carbamate derivatives supports the hypothesis that these analogues are more conformationally labile. This is consistent with the fact that an intramolecular H-bonding between the 5′-O and the N3 is not

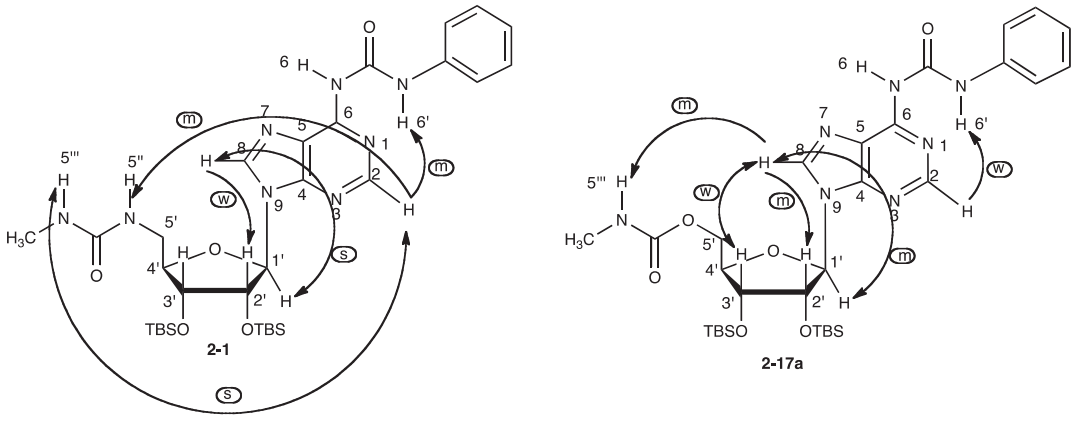


Figure 6. NOESY correlation data.
NOESY correlation data for compounds **2-1** and **2-17a**, (s = strong, m = medium, w = weak).

Table 3. 1D-NOESY data.
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]
2-1	H-8: 4.02	H-6′: 0.40	H-1′: 3.15	**2-17a**	H-8: 0.25	H-6′: 0.41	H-1′: 0.94
	H-2′: 1.03	H-5″: 0.19			H-2′: 0.22		H-5‴: 0.54
	H-3′: 0.21	H-5‴: 0.82			H-3′: 0.25		H-2′: 0.33
	H-4′: 0.71	NCH_3: 0.29			H-4′: 0.25		H-5′: 0.45
							H-5′: 0.15
2-10a	H-8: 4.64	H-6′: 0.73	H-1′: 6.43	**2-17b**	H-8: 1.81	H-6′: 0.25	H-1′: 2.00
	H-2′: 1.31	H-5″: 0.86			H-2′: 1.77		H-5‴: 0.77
	H-3′: 0.39	H-5‴: 1.63			H-3′: 0.50		H-2′: 0.48
	H-4′: 1.00	NCH_3: 0.58			H-4′: 0.50		H-5′: 0.56
							H-5′: 0.30
2-10b	H-8: 2.04	H-6′: 0.25	H-1′: 1.18	**2-17c**	H-8: 1.53	H-6′: 0.39	H-1′: 1.83
	H-2′: 0.76	H-5″: 0.16			H-2′: 0.89		H-5‴: 0.24
	H-3′: 0.17	H-5‴: 0.17			H-3′: 0.25		H-2′: 1.25
	H-4′: 0.55	NCH_3: 0.10			H-4′: 0.56		H-5′: 0.40
							H-5′: 0.47
2-10c	H-8: 2.93	H-6′: 0.00	H-1′: 3.23	**2-17d**	H-8: 1.10	H-6′: 0.33	H-1′: 0.42
	H-2′: 1.21	H-5″: 0.17			H-2′: 1.10		H-5‴: 0.76
	H-3′: 0.00	H-5‴: 0.45			H-3′: 0.23		
	H-4′: 0.84	NCH_3: 0.20			H-4′: 0.42		
2-10d	H-8: 4.02	H-6′: 0.00	H-1′: 3.52	**2-17e**	H-8: 0.96	H-6′: 0.37	H-1′: 2.02
	H-2′: 1.03	H-5″: 0.64			H-2′: 1.21		H-5‴: 0.96
	H-3′: 0.21	H-5‴: 0.33			H-3′: 0.23		H-2′: 0.56
	H-4′: 0.71	NCH_3: 0.49			H-4′: 0.41		H-3′: 0.66
2-13a	H-8: 1.90	H-6′: 0.00	H-1′: 3.46	**2-20a**	H-8: 1.50	H-6′: 0.64	H-1′: 2.49
	H-2′: 0.76	H-5″: 1.31			H-2′: 1.77		H-2′: 0.83
	H-3′: 0.30	H-5‴: 0.57			H-3′: 0.43		H-3′: 0.96
	H-4′: 0.45	NCH_3: 0.45			H-4′: 0.61		
2-13b	H-8: 2.79	H-6′: 0.62	H-1′: 5.73	**2-20b**	H-8: 1.45	H-6′: 0.56	H-1′: 2.23

	H-2′: 1.13	H-5″: 1.12			H-2′: 1.64		H-5‴: 1.15
	H-3′: 0.57	H-5‴: 0.68			H-3′: 0.32		H-2′: 1.10
	H-4′: 0.95	NCH_3: 0.54			H-4′: 0.79		H-3′: 0.88
							H-5′: 0.43
2-13c	H-8: 2.19	H-6′: 0.00	H-1′: 6.15	**2-20c**	H-8: 1.34	H-6′: 0.00	H-1′: 2.23
	H-2′: 0.97	H-5″: 0.60			H-2′: 1.47		H-5‴: 1.15
	H-3′: 0.51	H-5‴: 1.15			H-3′: 0.00		
	H-4′: 0.75	NCH_3: 0.65			H-4′: 0.60		
2-13d	H-8: 3.29	H-6′: 0.63	H-1′: 6.77	**2-20d**	H-8: 1.41	H-6′: 0.60	H-1′: 2.23
	H-2′: 1.21	H-5″: 1.12			H-2′: 1.41		H-5‴: 1.15
	H-3′: 0.39	H-5‴: 0.60			H-3′: 0.37		
	H-4′: 0.91	NCH_3: 0.71			H-4′: 0.61		
2-13e	H-8: 4.87	H-6′: 0.69	H-1′: 9.35	**2-20e**	H-8: 1.28	H-6′: 0.24	H-1′: 2.07
	H-2′: 1.45	H-5″: 1.98			H-2′: 1.12		H-5‴: 0.84
	H-3′: 0.54	H-5‴: 0.40			H-3′: 0.38		H-2′: 0.61
	H-4′: 1.21	NCH_3: 0.75			H-4′: 0.36		H-3′: 0.32

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

possible. When H-8 is irradiated in the 5′-ureas, NOE enhancement is observed only for the H-1′. When H-8 is irradiated in the 5′-carbamates, H-1′, H-5′′′, H-2′, H-3′, and H-5′ are enhanced, though 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′-urea analogues gives enhancement in the H-6′, H-5′′, H-5′′′, and the *N*-methyl of the 5′-urea moiety. These data support the conclusion that the 5′-urea derivatives are much more conformationally rigid, with H-2 being in close proximity to the 5′-urea and H-8 being proximal to H-1′. This is consistent with the *syn* glycosyl conformational preference indicated by the coupling constant data. The coupling constant and 1D-NOESY data corroboratively suggest that conformational

differences between the 5′-urea derivatives and the 5′-carbamate compounds exist and may play a role in the observed difference in biological activity.

2.4. Discussion

It is widely accepted that most biologically active nucleoside derivatives are phosphorylated before they exert their biological effect.[20-23] This usually requires that the nucleoside phosphate have either a free 5′-OH or a prodrug feature that is readily hydrolyzed to generate either a free 5′-OH or a 5′-monophosphate in vivo.[22, 23] Increasing lipophilicity of nucleoside analogues is a common strategy for increasing cell membrane permeability. One way this is accomplished is by protecting hydroxyls as acetyl, benzoyl, or isobutyryl esters, which are cleaved when the compounds enter the cell.[20, 21] Lipophilic silyl protecting groups have also been reported to be necessary for the biological activity of some nucleoside analogues. For example, TBS protecting groups at the 2′ and 5′ positions were found to be required for optimal activity of the *tert*-butyldimethyl-silyl-spiroaminooxathioledioxide-thymine (TSAO-T) class of HIV-1 reverse transcriptase (RT) inhibitors.[24] A 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 crucial role in determining the dimensions of the binding pocket as well as defining molecular recognition between the pocket and the TSAO-T ligand.[25]

The observation that the 5′-carbamates are generally much less active in vitro, although they possess the 2′,3′-bis-*O*-TBS groups, implies that the TBS groups are necessary, but not sufficient, for antiproliferative activity. The results of the protein kinase binding scan (Figure 3) suggest that the primary role of the TBS groups could be to enhance membrane permeability. Hydrolysis of the TBS groups of lead compound **2-1** within the intracellular space may give rise

to **2-3**, which clearly has greater binding affinity for BMPR1b (Figure 4). As mentioned in chapter 1, BMPR1b is part of a signaling cascade that regulates expression of Id-1, and its aberrant expression has been reported in over 20 cancers.[26-37] These data support the conclusion that protein kinases could be targets for the 5′-urea derivatives and provide preliminary evidence that the role of the TBS group may be to increase membrane permeability. In this context, lead compound **2-1** may be viewed as a prodrug of desilylated **2-3**.

The SAR designed to probe the effects of varying the 5′ and N^6 substituents showed that a 5′-urea is required for optimal antiproliferative activity. Conformational analysis revealed that the 5′-carbamate analogues are substantially more conformationally labile than the 5′-ureas. This greater degree of conformal freedom could contribute to the loss of biological activity relative to the more conformationally rigid 5′-ureas. Conformationally constrained nucleosides have been shown to have greater activities than less-rigid derivatives in a number of cases.[12-15] Our results are in agreement with these observations. Compounds **2-10b** and **2-17c** are exceptions to the general trend, suggesting that their primary targets could differ from other members of their respective series.

Varying the N^6 –position of the 5′-urea compounds produced modest to quite significant effects. Regrettably, no improvement in anticancer activity over lead **2-1** was made. Carbamate derivatives were significantly inferior to the 5′-urea analogues.

Both *Method A* and *B* (Schemes 1 and 2) were successful in preparing 5′-carbamoyl-N^6-ureidoadensosine derivatives, with *Method B* being the more synthetically versatile route.

2.5. Experimentals

2.5.1. Biology

2.5.1.1. 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 (HeLa) were evaluated as follows: an appropriate number of cells suspended in growth medium were allowed to proliferate in 200-lL-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%.

2.5.1.2. 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 anEscherichia 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.

2.5.2. Chemistry

2.5.2.1. 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 13C NMR spectra were determined using internal references at δ 7.27 ($CDCl_3$), 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. All compounds tested were

>98% pure (as determined by HPLC; 5–10% IPA/CH_2Cl_2).

2.5.2.2. General Procedure A (Acylation with Isocyanates)

A solution of the adenosine derivative (**2-8** or **2-16**) 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.

2.5.2.3. General Procedure B (Hydrogenation)

A suspension of the 5′-azido-5′-deoxyadenosine derivative **2-8** or any analogue of **2-9a-e** 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.

2.5.2.4. General Procedure C (Acylation with *N*-methyl-*p*-nitrophenylcarbamate)

A solution of the 5′-amino-5′-deoxyadenosine product derived from reduction of **2-8**, or compounds **2-9a-e**, 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.

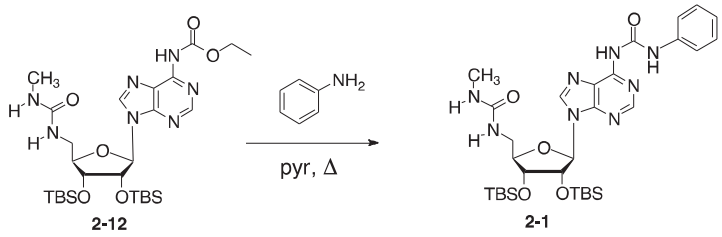
2.5.2.5. General Procedure D (Acylation with Ethylchloroformate)

A solution of adenosine derivatives **2-11** or **2-16**, 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 **2-12** or **2-18**, respectively. Volatiles were evaporated under reduced pressure, and the product was purified by flash chromatography.

2.5.2.6. General Procedure F (N^6-Urea Formation)

A solution of adenosine derivatives **2-12** or **2-18**, 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.

2.5.2.7. Compound Characterization Data

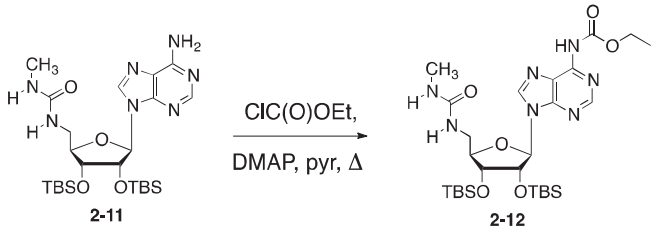


2-12 2-1

2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-[(*N*-methylcarbamoyl) amino]-N^6-(*N*-phenylcarbamoyl)]adenosine (2-1).

Treatment of **2-12** (70 mg, 0.11 mmol), aniline (17 mg, 0.18 mmol), and pyridine (1.0 mL) by general procedure E (chromatography EtOAc), gave **2-1** (36 mg, 0.054 mmol, 49%). ^{1}H 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*

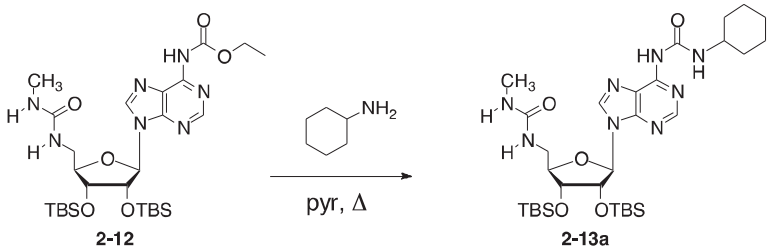
= 7.5 Hz), 7.39 (t, *J* = 8.3 Hz, 2H), 7.18 (t, *J* = 7.3 Hz, 1H), 6.51 (d, *J* = 6.0 Hz, 1 H), 6.01 (d, *J* = 7.7 Hz, 1H), 4.74–4.73 (m, 1H), 4.64 (dd, *J* = 7.5, 4.5 Hz, 1 H), 4.36 (d, *J* = 4.5 Hz, 1H), 4.18 (t, *J* = 2.5 Hz, 1H), 3.99 (ddd, *J* = 14.5, 9.0, 2.5 Hz, 1H), 3.19 (dt, *J* = 14.5, 3.1 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.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-deoxy-*N*^{6}-(ethoxycarbonyl)-5′-[(*N*-methylcarbamoyl)amino]adenosine (2-12).

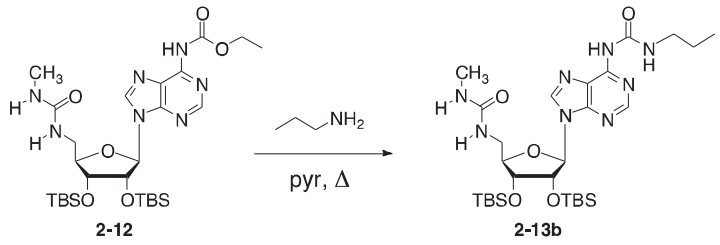
Treatment of **2-11** (80 mg, 0.145 mmol), ethylchloroformate (62 mg, 0.57 mmol), DMAP (27 mg, 0.22 mmol), and pyridine (1 mL), by general procedure D [1 h; additional ethylchloroformate (30 mg)], 1 h; chromatography 30→50% acetone/hexanes] gave **2-12** (50 mg, 0.080 mmol, 55%). ^{1}HNMR ($CDCl_3$, 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 (MH+ [$C_{27}H_{50}N_7O_6Si_2$]) = 624.3358.



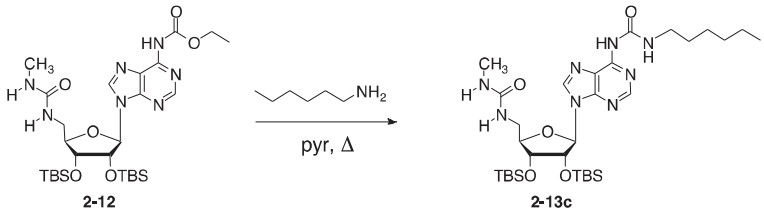
2′,3′-Bis-*O-tert*-butyldimethylsilyl-N^6-(*N*-cyclohexyl carbamoyl)-5′-deoxy-5′-[(*N*-methylcarbamoyl) amino]adenosine (2-13a).

Treatment of **2-12** (38 mg, 0.060 mmol) and cyclohexylamine (10 mg, 0.10 mmol) in pyridine (1 mL) by general procedure E [chromatography 40→60% acetone/CH_2Cl_2] gave **2-13a** (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 (MH+ [$C_{31}H_{57}N_8O_5Si_2$]) = 677.3949.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-deoxy-5′-[(*N*-methylcarbamoyl) amino]-N^6-(*N*-propylcarbamoyl)adenosine (2-13b).

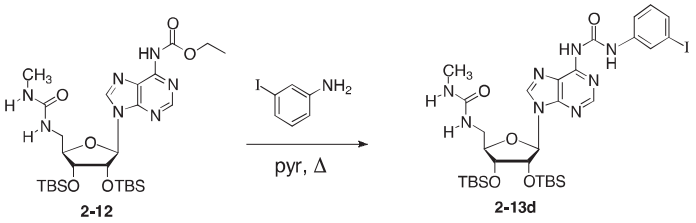
Treatment of **2-12** (38 mg, 0.061 mmol) and propylamine (11 mg, 0.19 mmol) in pyridine (1 mL) by general procedure E [chromatography 40→60% Acetone/CH_2Cl_2] gave **2-13b** (28 mg, 0.044 mmol, 72%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.10 (t, *J* = 5.0 Hz, 1H), 9.47 (s, 1H), 9.12 (s, 1H), 8.58 (s, 1H), 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 (MH+ [$C_{28}H_{53}N_8O_5Si_2Na$]) = 637.3677.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-deoxy-N^6-(*N*-hexylcarbamoyl)-5′-[(*N*-methylcarbamoyl)amino]adenosine (2-13c).

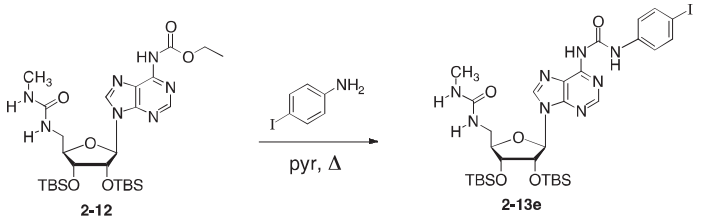
Treatment of **2-12** (19 mg, 0.031 mmol) and hexylamine (12 mg 0.12 mmol) in pyridine (1 mL) by general procedure E [chromatography 2% MeOH/CH_2Cl_2] gave **2-13c** (8 mg, 0.012 mmol, 39%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.00 (br s, 1H), 9.32 (br s, 1H), 9.02 (br s, 1H), 8.57 (s, 1H), 6.47 (d, *J* = 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 (MH+ [$C_{31}H_{59}N_8O_5Si_2$]) = 679.4204.



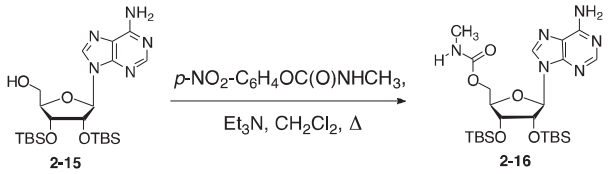
2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-*N*^{6}-[*N*-(*m*-iodophenyl) carbamoyl]-5′-[(*N*-methylcarbamoyl)amino]adenosine (2-13d).

Treatment of **2-12** (38 mg, 0.061 mmol) and *m*-iodoaniline (36 mg, 0.16 mmol) in pyridine (1 mL) by general procedure E [chromatography 2→4% MeOH/CH_2Cl_2 then 15→45% Acetone/CH_2Cl_2] gave **2-13d** (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); 13C 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 (MH+ [$C_{31}H_{50}IN_8O_5Si_2$]) = 797.2445.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-deoxy-N^6-[*N*-(*p*-iodophenyl)carbamoyl]-5′-[(*N*-methylcarbamoyl)amino]adenosine (2-13e).

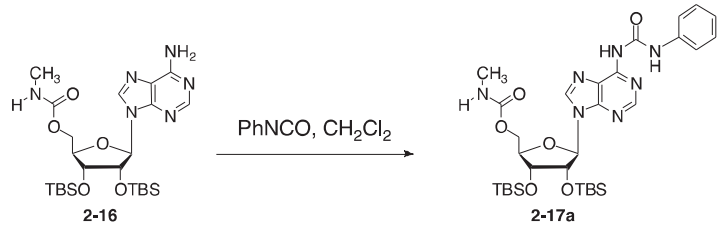
A solution of **2-12** (38 mg, 0.061 mmol) and *p*-iodoaniline (39 mg, 0.18 mmol) in pyridine (1 mL) by general procedure E [chromatography 40→50% Acetone/hexanes then EtOAc] gave **2-13e** (16 mg, 0.020 mmol, 34%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.95 (s, 1H), 8.93 (br s, 1H), 8.67 (s, 1H), 8.49 (s, 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 (MH+ [$C_{31}H_{50}IN_8O_5Si_2$]) = 797.2474.



2′,3′- Bis-*O-tert*-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)adenosine(2-16).

A solution of **2-15** (618 mg, 1.25 mmol), *p*-NO_2-$C_6H_4OCONHCH_3$ (377 mg, 1.92 mmol), and Et_3N (2.1 mL) in CH_2Cl_2 (9.5 mL) was stirred protected from moisture at 50 °C overnight.

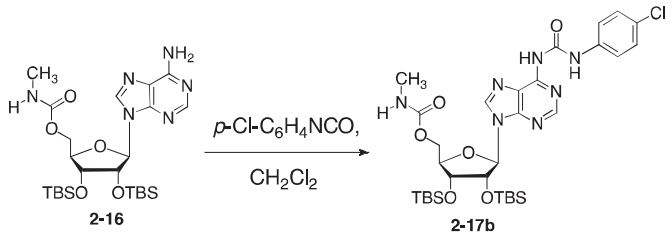
Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with EtOAc to give compound **2-16** (580mg, 1.05 mmol, 84%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.35 (s, 1H), 7.98 (s, 1H), 5.89 (d, *J* = 4.5 Hz, 1H), 5.51 (bs, 2H), 4.93 (t, *J* = 4.0 Hz, 1H), 4.72 (bs, 1H), 4.50 (dd, *J* = 4.3, 11.8 Hz, 1H), 4.33 (dd, *J* = 4.7, 11.8 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) δ 156.7, 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* 553.2985 (MH+ [$C_{24}H_{45}N_6O_5Si_2$])= 553.2990.



2′,3′- Bis-*O-tert*-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)-N^6-(*N*-phenylcarbamoyl)adenosine (2-17a).

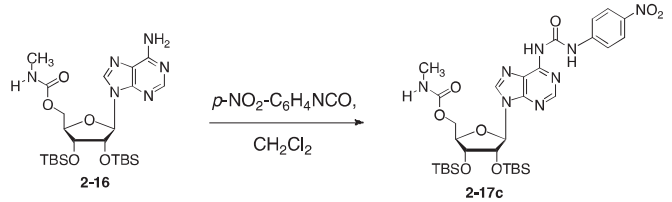
Treatment of **2-16** (100 mg, 0.181 mmol), phenylisocyanate (33 mg, 0.28 mmol), and CH_2Cl_2 (2.2 mL) by general procedure A [chromatography 30→50% EtOAc/hexanes] gave compound **2.17a** (109 mg, 0.162 mmol, 90%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.25 (s, 1H), 9.86 (bs, 1H), 8.80 (s, 1H), 8.66 (s, 1H), 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* = 3.8, 12.8 Hz, 1H), 4.34 (t, *J* = 3.89 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, 77.4, 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* 672.3356 (MH+ [$C_{31}H_{50}N_7O_6Si_2$])= 672.3353.



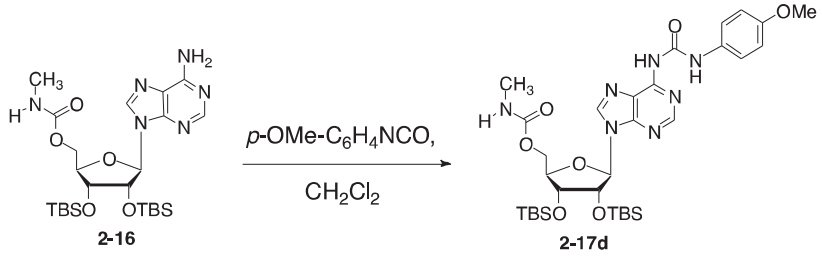
2′,3′-Bis-*O-tert*-butyldimethylsilyl-N^6-[*N*-(*p*-chlorophenyl)carbamoyl]-5′-(*N*-methylcarbamoyl)adenosine (2-17b).

Treatment of **2-16** (100 mg, 0.181 mmol), *p*-chlorophenylisocyanate (43 mg, 0.28 mmol), and CH_2Cl_2 (2.2 mL) by general procedure A [chromatography 30→50% EtOAc/hexanes] gave **2-17b** (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 (MH+ [$C_{31}H_{49}ClN_7O_6Si_2$]) = 706.2999.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)-N^6-[*N*-(*p*-nitrophenyl)carbamoyl]adenosine (2-17c).

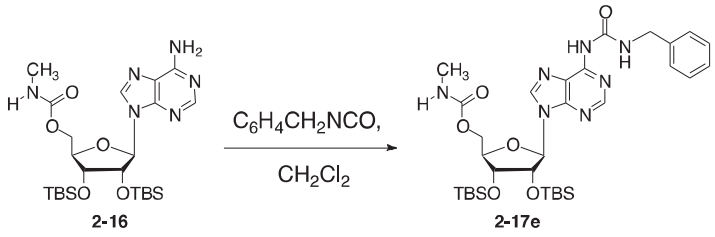
Treatment of **2-16** (100 mg, 0.181 mmol), *p*-nitrophenylisocyanate (45 mg, 0.27 mmol), and CH_2Cl_2 (2.2 mL) by general procedure A [chromatography 30% EtOAc/hexanes→100% EtOAc] gave **2-17c** (88 mg, 0.12 mmol, 66%). ^{1}H NMR (Acetone-d_6, 500 MHz) δ 12.84 (s, 1H), 9.79 (br s, 1H), 8.83 (s, 1H), 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 (MH+ [$C_{31}H_{49}N_8O_8Si_2$]) = 717.3212.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-N^6-[*N*-(p-methoxyphenyl) carbamoyl]-5′-(*N*-methylcarbamoyl)adenosine (2-17d).

Treatment of **2-16** (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 **2-17d** (88 mg, 0.125 mmol, 69%). ^{1}H NMR ($CDCl_3$, 500

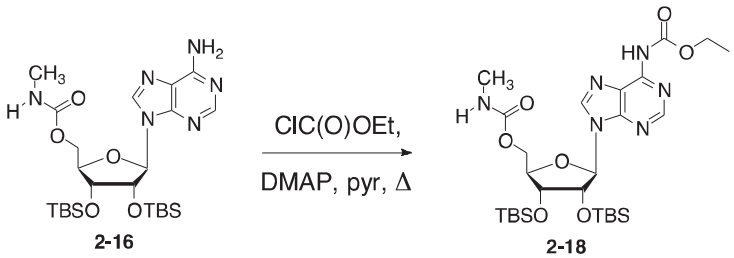
MHz) δ 12.11 (s, 1H), 10.33 (br s, 1H), 8.99 (s, 1H), 8.65 (s, 1H), 7.47 (d, *J* = 9.5 Hz, 2H), 6.91 (d, *J* = 9.0 Hz, 2H), 6.27 (d, *J* = 5.5 Hz, 1H), 6.25 (br s, 1H), 4.57–4.55 (m, 2H), 4.35 (dd, *J* =3.8, 2.8 Hz, 1H), 4.28 (dd, *J* = 5.0, 2.5 Hz, 1H), 4.21 (dd, *J* = 12.8, 2.3 Hz, 1H), 3.83 (s, 3H), 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 (MH+ [$C_{32}H_{52}N_7O_7Si_2$]) = 702.3450.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)-N^6-(*N*-benzylcarbamoyl)adenosine (2-17e).

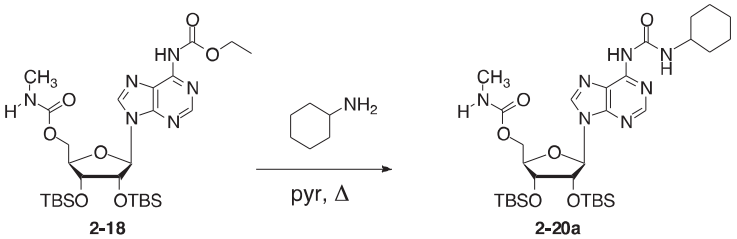
Treatment of **2-16** (100 mg, 0.181 mmol), benzylisocyanate (46 mg, 0.35 mmol), and CH_2Cl_2 (2.2 mL) by general procedure A [chromatography 30% EtOAc/hexanes→100% EtOAc] gave **2-17e** (58 mg, 0.085 mmol, 47%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.47 (t, *J* = 5.5 Hz, 1H), 10.10 (br s, 1H), 8.94 (s, 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 (MH+ [$C_{32}H_{52}N_7O_6Si_2$]) = 686.3518.



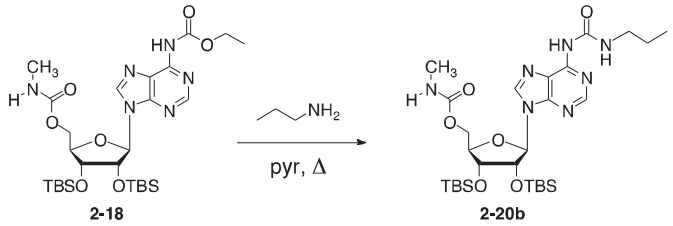
2′,3′-Bis-*O-tert*-butyldimethylsilyl-N^6-ethoxycarbonyl-5′-(*N*-methylcarbamoyl)adenosine (2-18).

Treatment of **2-16** (150 mg, 0.271 mmol), ethylchloroformate (38 mg, 0.35 mmol), DMAP (56 mg, 0.46 mmol), and pyridine (1 mL) by general procedure D [15 h; additional ethylchloroformate (48 mg); chromatography 75% EtOAc/hexanes] gave **2-18b** (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 (MH+ [$C_{27}H_{49}N_6O_7Si_2$]) = 625.3201.



2´,3´-Bis-*O-tert*-butyldimethylsilyl-N^6-[*N*-cyclohexylcarbamoyl]-5´-(*N*-methylcarbamoyl)adenosine (2-20a).

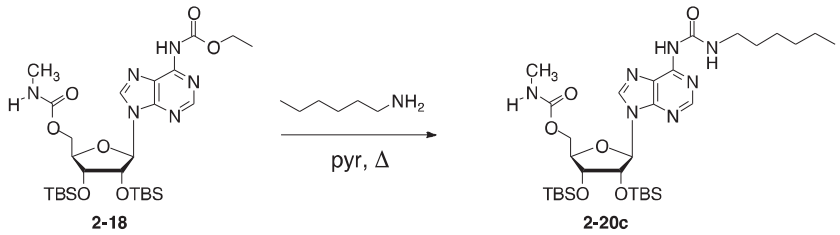
Treatment of **2-18** (50 mg, 0.08 mmol) and cyclohexylamine (12 mg, 0.12 mmol) in pyridine (1 mL) by general procedure E [chromatography 2→4% MeOH/CH_2Cl_2] gave **2-20a** (50 mg, 0.074 mmol, 93%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.00 (d, *J* = 7.5 Hz, 1H), 9.78 (br s, 1H), 8.89 (s, 1H), 8.53 (s, 1H), 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 (MH+ [$C_{31}H_{56}N_7O_6Si_2$]) = 678.3839.



2´,3´-Bis-*O-tert*-butyldimethylsilyl-5´-(*N*-methylcarbamoyl)-N^6-[*N*-propylcarbamoyl]adenosine (2-20b).

Treatment of **2-18** (75 mg, 0.12 mmol) and propylamine (11 mg, 0.19 mmol) in pyridine (1 mL) by general procedure E [chromatography 2→4% MeOH/CH_2Cl_2] gave **2-20b** (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* =

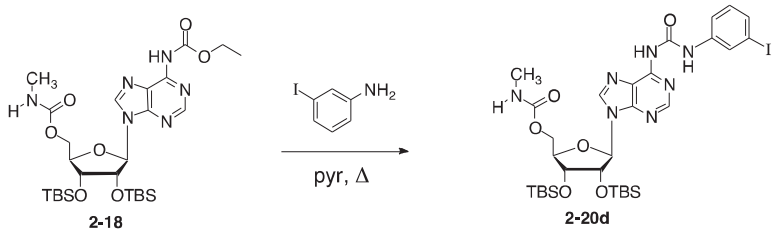
13.0, 2.5 Hz, 1H), 3.45–3.32 (m, 2H), 2.83 (d, J = 4.5 Hz, 3H), 1.70 (sext, J = 7.2 Hz, 2H), 1.04 (t, J = 7.3 Hz, 3H), 0.95 (s, 9H), 0.78 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H), –0.04 (s, 3H), –0.28 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.2, 155.5, 151.1, 150.9, 150.8, 143.4, 120.5, 87.6, 84.7, 77.3, 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 (MH+ [$C_{28}H_{52}N_7O_6Si_2$]) = 638.3518.



2-18 **2-20c**

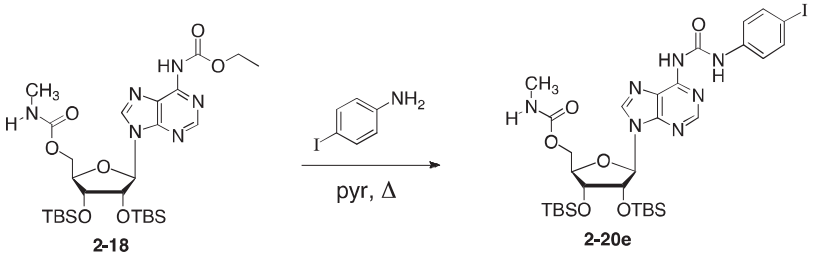
2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-N^6-[*N*-hexylcarbamoyl]-5′-(*N*-methylcarbamoyl)adenosine (2-20c).

Treatment of **2-18** (75 mg, 0.12 mmol) and hexylamine (14 mg 0.14 mmol) in pyridine (1 mL) by general procedure E [chromatography 2→4% MeOH/ CH_2Cl_2] gave **2-20c** (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 (MH+ [$C_{31}H_{58}N_7O_6Si_2$]) = 680.4004.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)-N^6-[*N*-(*m*-iodophenyl)carbamoyl]adenosine (2-20d).

Treatment of **2-18** (75 mg, 0.120 mmol) and *m*-iodoaniline (36 mg, 0.17 mmol) in pyridine (1 mL) by general procedure E [chromatography 2→4% MeOH/CH_2Cl_2] gave **2-20d** (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 (MH+ [$C_{31}H_{49}IN_7O_6Si_2$]) = 798.2328.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-N^6-[*N*-(*p*-iodophenyl)carbamoyl]-5′-(*N*-methylcarbamoyl)adenosine (2-20e).

Treatment of **2-18** (75 mg, 0.12 mmol) and p-iodoaniline (40 mg, 0.18 mmol) in pyridine (1 mL) by general procedure E [chromatography 15→25% Acetone/CH_2Cl_2] gave **2-20e** (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 (MH+ [$C_{31}H_{49}IN_7O_6Si_2$]) = 798.2328.

2.6. References

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Chapter 3: Synthesis and SAR of 2′,3′-Bis-*O*-Substituted Ureidoadenosine Derivatives: Implications for Prodrug Delivery and Mechanism of Action

3.1. Introduction

The preliminary SAR performed to optimize the anticancer activity of lead compound **3-1** indicated that varying the electronic nature or structure of the N^6-aryl or N^6-alkyl substituent was not likely to lead to better biological activity, because none of the N^6-aryl or N^6-alky derivatives showed better anticancer activity than **3-1**. The SAR also showed that the biological activity of the 5′-carbamate derivatives were substantially inferior to the 5′-urea analogues (see Chapter 2).[1] We next turned our attention to investigate the role of the 2′,3′-*O* substitution of lead compound **3-1**. Our data showed that the 2′,3′-*O*-TBS groups were necessary but no sufficient for biological activity. Our hypothesized biological mechanism of action invokes compound **3-1** as a prodrug of its desilylated derivative **3-1′** (see Chapter 1). Derivatives were designed to test if other prodrug forms of **3-1′** would be equally or possibly more biologically active than the TBS-protected compound. These derivatives included various silyl substitutions at the 2′,3′-OH position as well as some 2′,3′-*O*-esters (Figure 1).

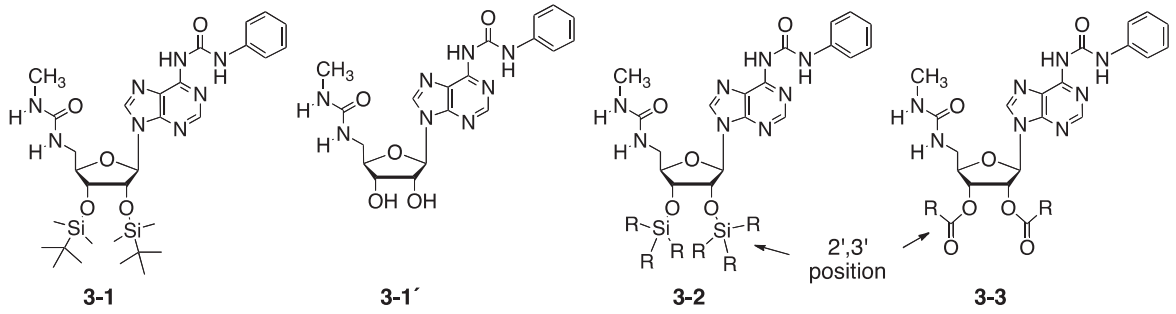
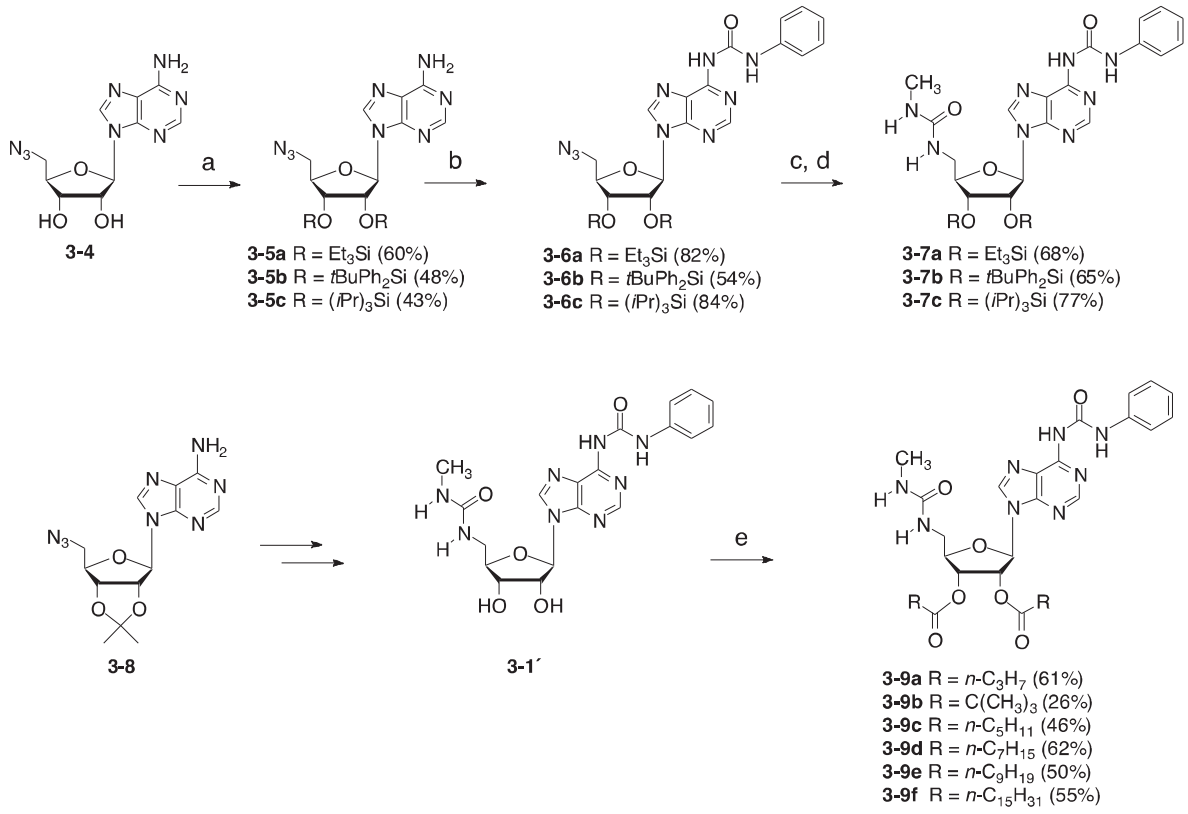


Figure 1. General SAR.
General SAR of the 2′,3′- position of lead **3-1**.

3.2. Chemistry

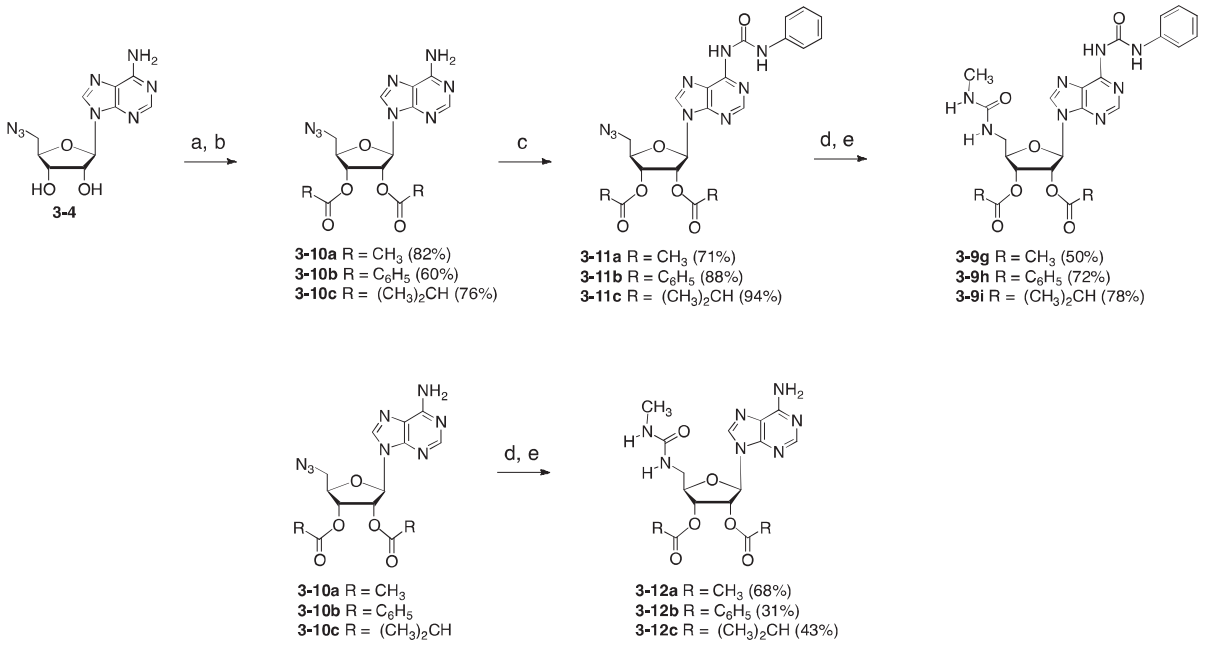
The synthesis of the 2′,3′-*O*-silyl substituted derivatives (**3-7a**–**c**) as well as 2′,3′-*O*-acylated analogues (**3-9a**–**f**) is shown in Scheme 1. It begins with silylation of azide **3-4** and furnishes compounds **3-5a**–**c** in moderate to good yields. This is followed by acylation of the N^6-position by treatment with phenyl isocyanate. A one-pot, two-step reaction sequence involving reduction of the 5′-azido group of compounds **3-6a–c** followed by acylation with *p*-nitrophenylcarbamate[2] produces compounds **3-7a–c** in 65-77% yield.



Scheme 1. Synthesis of **3-7a–c** and **3-9a–f**.
Reagents: (a) R_3SiCl, imid, DMF; (b) PhN=C=O; (c) H_2, Pd-C; (d) *p*-NO_2-$C_6H_4OC(O)NHCH_3$; (e) $(RC=O)_2O$. Silyl derivatives **3-7a**–**c** were synthesized by undergraduate student Christopher Cutler.

The syntheses of 2′,3′-*O*-acylated derivatives **3-9a-f** were completed in one step from 2′,3′-unprotected compound **3-1′** in good yields, except for **3-9b** which was obtained with a maximum yield of only 26%, presumably due to the steric bulk of the pivaloyl esters.

A different synthetic route to 2′,3′-*O*-acylated derivatives (**3-9g–i** and **3-12a–c**) is shown in Scheme 2. Compounds **3-9g–i** were obtained in good yields following a five-step protocol similar to the one employed to make **3-7a–c**. Derivatives **3-12a–c** were obtained in good yields following the one-pot procedure consisting of 5′-azido reduction and subsequent acylation forming the 5′-*N*-methyl urea.



Scheme 2. Synthesis of **3-9g–i** and **3-12a–c**.
Reagents: (a) $(RC{=}O)_2O$; (b) MeOH, Δ; (c) PhN=C=O; (d) H_2, Pd-C; (e) *p*-NO_2-$C_6H_4OC(O)NHCH_3$.

3.3. Biology

3.3.1. Antiproliferative Activity

Compounds **3-1, 3-7a–c**, **3-9a–i**, and **3-12a–c**, were tested for their antiproliferative activity using murine leukemia L1210, murine mammary carcinoma FM3A, human lymphoblastic leukemia CEM, and human cervix carcinoma HeLa (Table 1).

Table 1. Inhibitory effects of test compounds.
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). IC_{50} (μg/ml): 50% Inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

Compound	L1210	FM3A	CEM	HeLa
3-1	3.8 ± 0.3	5.9 ± 1.1	8.3 ± 2.9	3.2 ± 0.2
3-7a	3.8 ± 0.1	3.0 ± 0.3	4.2 ± 0.2	3.7 ± 0.4
3-7b	>200	>200	>200	104 ± 71
3-7c	>200	>200	142 ± 81	>200
3-9a	20 ± 2	18 ± 1	29	58 ± 25
3-9b	9.7 ± 3.5	15 ± 1	20	17 ± 1
3-9c	9.5 ± 0.3	20 ± 1	10 ± 2	15 ± 5
3-9d	11 ± 0	32 ± 1	12 ± 4	16 ± 9
3-9e	>100	140 ± 16	>100	>100
3-9f	>100	>200	>100	>100
3-9g	97 ± 17	150 ± 39	107 ± 8	>200
3-9h	154 ± 30	61 ± 2	>200	>200
3-9i	29 ± 4	44 ± 4	28 ± 0	73 ± 13
3-12a	112 ± 31	>200	>200	>200
3-12b	16 ± 1	36 ± 3	19 ± 8	40 ± 7
3.12c	87 ± 1	107 ± 13	88 ± 33	99 ± 14

Interestingly, the IC_{50} values for 2′,3′-bis-*O*-triethylsilyl derivative **3-7a** were very similar to those for lead compound **3-1**. Conversely, the IC_{50} values for the 2′,3′-bis-*O*-*tert*-butyldiphenylsilyl derivative **3-7b** and/or 2′,3′-bis-*O*-triisopropylsilyl analogue **3-7c** were significantly inferior to **3-1**. Acyl derivatives **3-9a-i** were generally much less active than **3-1**, especially the *O*-benzoyl, *O*-decanoyl, and *O*-hexadecanoyl derivatives (**3-9h**, **3-9e**, and **3-9f**, respectively). However, the *O*-pivaloyl, *O*-hexanoyl, and *O*-octanoyl derivatives (**3-9b**, **3-9c**, **3-9d**, respectively) showed nearly equipotent antiproliferative activities, but IC_{50} values for these compounds were from three to five times higher than those for lead **3-1**. Compounds **3-12a**–**c** (each of which lacks the N^6-phenylurea) showed generally lower activity than their corresponding N^6-substituted derivatives (**3-9g**–**i**).

3.3.2. Protein Kinase Binding Activity

As previously mentioned, compound **3-1′** binds BMPR1b with a $K_d = 11.5 \pm 0.7$ μM whereas compound **3-1** does not bind the same kinase at concentrations as high as 30 μM (Chapter 2). The negative impact of 2′,3′-*O*-substitutions on binding was also shown for several other derivatives (**3-7a**–**c** and **3-9g**–**i**). None of these compounds showed appreciable binding to BMPR1b in a competitive binding experiment at 10 μM concentration (Figure 2).

3.3.3. Docking Studies

Increasing membrane permeability of nucleosides by increasing the lipophilicity by protecting hydroxyls as acetyl, isobutyrl, or benzoyl esters is a commonly used strategy. These

esters are cleaved once the compound has crossed the cell membrane.[3, 4] In addition, TBS-protection has been shown to enhance the activities of a number of antiproliferative compounds. The activities of several of these compounds have been positively correlated with the increased lipophilicity of the active analogue (see chapter 2).[5-7] The lipophilic 2′,3′-bis-*O*- TBS groups may enhance membrane permeability of lead **3-1** and serve as a prodrug form of the active analogue **3-1′**.

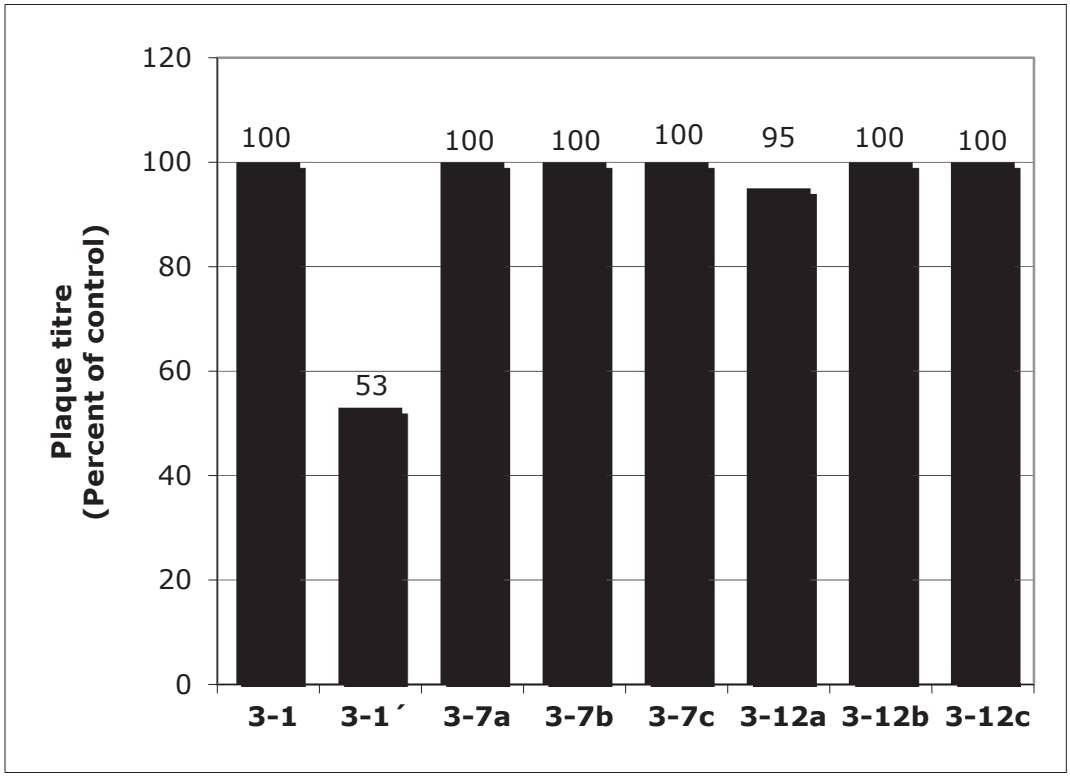


Figure 2. Competitive binding inhibition assays.
Effects of compounds on equilibrium competition binding of BMPR1b to immobilized ATP-binding site ligand. Compounds **3-1**, **3-1′**, **3-7a–c**, and **3-9g–i** at 10 μM (data expressed as percent of control).

Docking studies performed utilizing the Surflex docking program (Sybyl X 1.3) support such an interpretation. The Surflex docking program has been validated as a robust molecular docking method. In terms of docking accuracy, it performs as well as other commonly used methods. In terms of screening utility, its performance has been shown to be superior to other methods for which comparative data are available.[8, 9]

The studies docked compounds **3-1** and **3-1´** with the enzyme BMPR1b. BMPR1b is a member of the TGFβ super family of protein kinases. This enzyme (also know as Alk6) has 68% sequence homology with Alk5 (unpublished results). Assignments for the catalytic triad, gatekeeper, G-loop, and hinge region are consistent with published assignments (Figure 3).[10, 11]

The highest ranked pose for derivative **3-1´** is positioned within the ATP binding cleft of BMPR1b (pbd 3mdy) with the 5´-urea undergoing hydrogen bonding interactions with the highly conserved catalytic triad (Lys 231, Glu 244, Asp 350; yellow residues; Figure 3). The N^6-phenyl urea moiety is oriented toward the solvent accessible surface. This is consistent with the relative lack of sensitivity of the antiproliferative activity of derivatives of **3-1** to the nature of the substitution pattern in the N^6–urea moiety (see Chapter 2).

Conversely, the top ranked pose for analogue **3-1** had an almost opposite orientation to lead **3-1´**, with the N^6-phenyl urea moiety experiencing nonpolar binding interactions with the "gatekeeper" amino acid residue (Leu 277; blue residue; Figure 3) near the terminal of the catalytic cleft adjacent to the catalytic triad. In this orientation, the very hydrophobic 2´,3´-bis-*O*-TBS groups are exposed to the solvent accessible surface. If this pose were biologically relevant, then a change in the substitution of N^6-urea position would be expected to have a much greater effect than the negligible effect that was observed in vitro (Chapter 2). In addition, the

hydrophobic effect exerted by the nonpolar TBS groups extending into the aqueous environment would produce an unfavorable entropic term in the overall binding free energy.

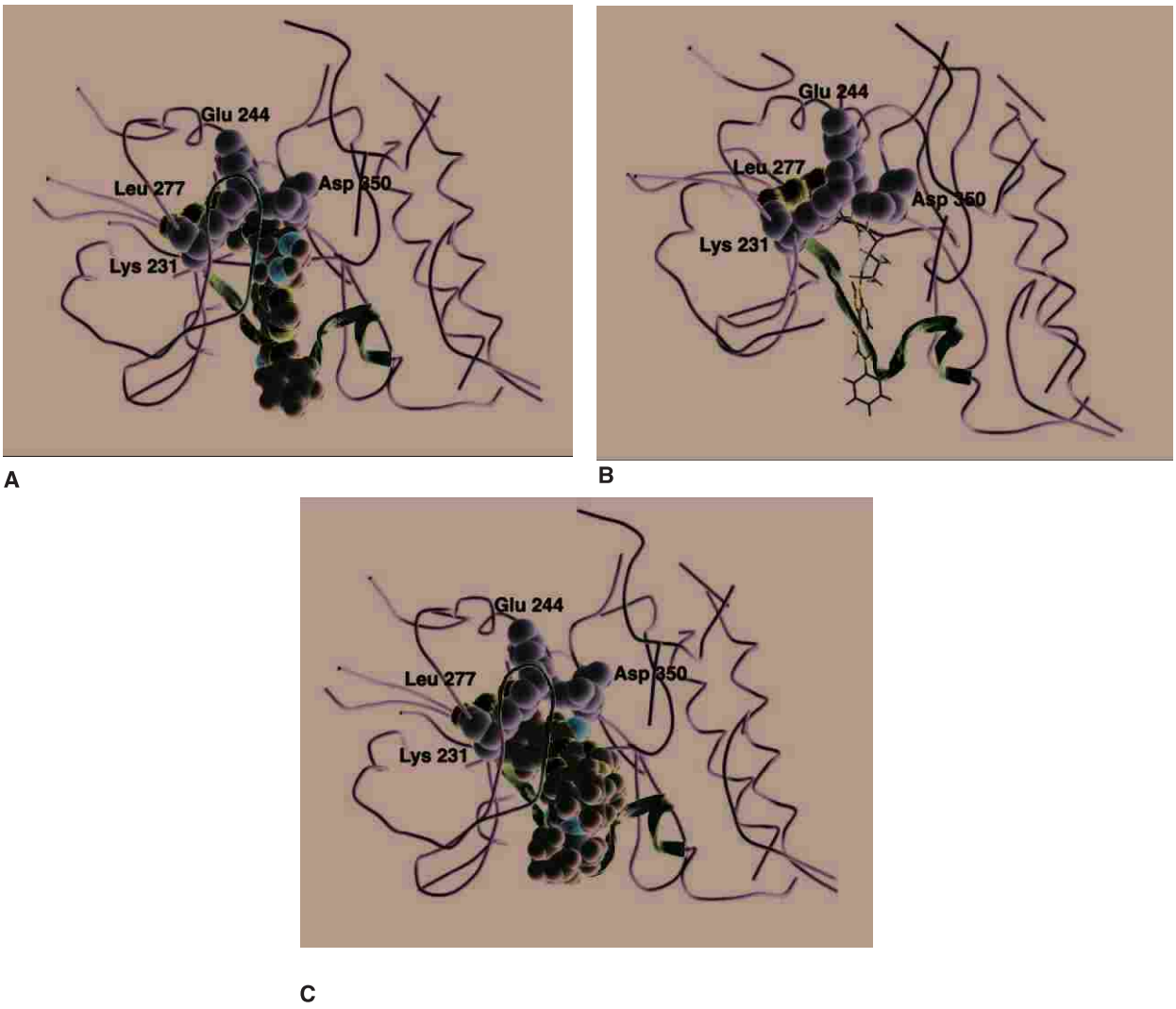


Figure 3. Docking results.
Docking results for **3-1** and **3-1´** docked into the active site of BMPR1b (pdb 3mdy). Yellow residues: catalytic triad (K231, E244, D350); blue residue: gatekeeper (L277); magenta tube: G-loop or activation loop (I210, G211, K212, G213, R214, Y215, G216); magenta ribbon: hinge region (I278, T279, D280, Y281, H282, E283, N284, G285, S286).[18] (A) Space-filling model of highest ranked pose of compound **3-1´**. (B) Tube model of highest ranked pose of compound **3-1´**. (C) Space-filling model of highest ranked pose of compound **3-1**. (Docking was performed by Dr. Matt A. Peterson).

3.4. Discussion

Efficient methods for the preparation of a variety of 2′,3′-*O*-substituted analogues of lead compound **3-1** were developed. Bis-*O*-protection of 5′-azido-5′-deoxyadenosine with either silyl or acyl protecting groups, followed by sequential acylation of the N^6- and 5′-amino groups (with phenylisocyanate or *N*-methyl-*p*-nitrophenylcarbamate, respectively) gave 2′,3′-*O*-substituted derivatives of lead compound **3-1** in good to excellent yields. This sequence, however, gave poor yields for derivatives with larger 2′,3′-*O*-acyl substituents (**3-9a**–**f**). The alternative route involving one step from desilylated derivative **3-1′** proceeded with much better overall yields.

Increased antiproliferative activity was not shown after screening compounds **3-7a**–**c**, **3-9a**–**i**, or **3-12a**–**c** against a panel of human and murine cancer cell lines. Several 2′,3′-*O*-substituted analogues showed a lack in binding affinity for BMPR1b at concentrations near the K_d for desilylated derivative **3-1′**. These results suggest the possibility that the role of the TBS groups in lead compound **3-1** is to facilitate membrane permeability. TBS group cleavage within the cytoplasm may give rise to the activated derivative **3-1′** which could target BMPR1b as its predominant biomolecular target. BMPR1b is a transmembrane receptor with serine/threonine protein kinase activity. The ATP-binding domain is within the cytoplasm and phosphorylates downstream targets (SMADs 1, 5, and 8), which regulate expression of inhibitor of differentiation gene 1 (Id1). Overexpression of Id1 has been reported in many cancers (Chapter 1). Inhibition of the BMPR1b-signaling cascade by desilylated analogue **3-1′** could account for the broad-spectrum activity of lead **3-1**.

The docking studies performed using the Surflex docking program (Sybyl X 1.3) are supportive of this putative biological model.

3.5. Experimental

3.5.1. Biology

3.5.1.1. 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 (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%.

3.5.1.2. Protein Kinase Binding 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 *E. 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 minutes 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 1x binding buffer (20% SeaBlock, 0.17x 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 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 0.5 μM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR. Binding constants (Kds) 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.

3.5.1.3. Ligand Docking

The docking study was performed using Surflex Dock in Sybyl X version 1.3 by Tripos, Inc. The structures of compounds **3-1** and **3-1′** were sketched using the Sybyl package and minimized using the conjugate gradient method until the gradient was 0.001 kcal/mol with the Tripos force field. The structure for BMPR1b (pdb 3mdy) was downloaded from the Protein Data Bank. One entire set of Chains A and B (and associated co-crystallized ligand, 4-[6-(4-piperazin-1-ylphenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl]quinoline) from the homo-dimeric crystal structure were deleted, and the remaining protein/ligand complex was prepared following the standard protocol outlined in the Surflex Dock documentation. Briefly, waters of crystallization were removed, hydrogen atoms were added, protein and ligand atom types were fixed, and ASN/GLN sidechains were oriented to maximize hydrogen bonding. A staged “hydrogen-only”

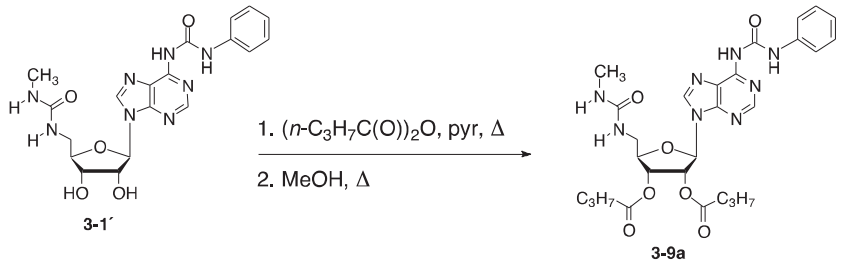
minimization of the protein and co-crystallized ligand was performed using the AMBER7 FF99 forcefield. The protomol was defined using the co-crystallized ligand with Threshold = 0.5, Bloat = 0. The structure parameters of the protein were set as rigid, while the ligand parameters were set as flexible. Docking parameters allowed for pre- and post-Dock minimization of the ligand, and molecule fragmentation was set to 20 conformations per fragment. The best 20 poses for each ligand were ranked according to the Surflex scoring function and the highest ranked poses for **3-1** and **3-1´** were selected.

3.5.2. Chemistry

3.5.2.1. General Experimental

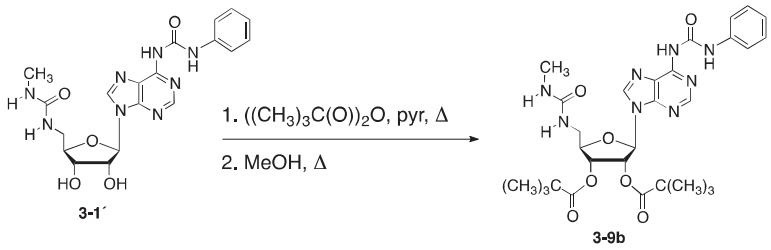
Moisture sensitive reactions were performed in flame-dried flasks under an inert atmosphere (N_2 or Ar) at ambient temperature unless otherwise indicated. Solvents (CH_2Cl_2, pyridine, EtOAc, DMF, Et_3N) were dried by passing through columns of activated alumina under Ar. TLC was performed using Merck Kieselgel 60 F254 sheets, and flash chromatography was performed with silica gel (230–400 mesh) and reagent grade solvents. ^{1}H NMR and ^{13}C NMR spectra were determined using internal references at δ 7.27 ($CDCl_3$), and δ 77.2 ($CDCl_3$), respectively. High resolution mass spectra were obtained using fast atom bombardment electrospray (ES) ionization techniques. Commercially available reagents were used as supplied.

3.5.2.2. Compound Characterization Data



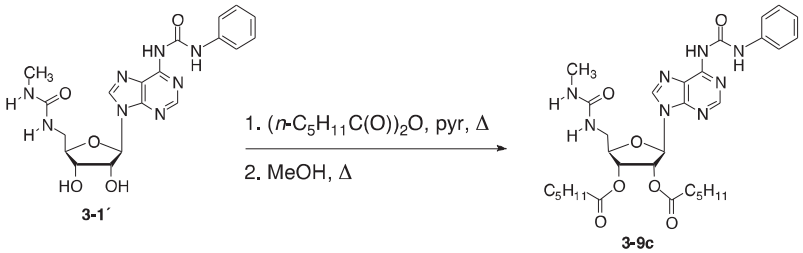
2´,3´-Bis-*O*-butanoyl-5´-deoxy-5´-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (3-9a).

A solution of **3-1´** (50 mg, 0.11 mmol) and butanoic anhydride (54 mg, 0.34 mmol), in pyridine (1.5 mL) was stirred at 70 °C overnight. Volatiles were removed under reduced pressure, and the crude mixture was added directly to a flash chromatography column and eluted with 3→7% MeOH/CH_2Cl_2 to give **3-9a** (40 mg, 0.069 mmol, 63%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.06 (s, 1H), 9.78 (s, 1H), 8.77 (s, 1H), 8.64 (s, 1H), 7.56 (dd, *J* = 8.4, 0.9 Hz, 2H), 7.39 (t, *J* = 8.0 Hz, 2H), 7.18 (t, *J* = 7.4 Hz, 1H), 6.19 (d, *J* = 6.3 Hz, 1H), 5.91 (t, *J* = 5.9 Hz, 2H), 5.60 (dd, *J* = 5.4, 3.6 Hz, 1H), 4.97 (dd, *J* = 9.0, 4.3 Hz, 1H), 4.38 (dd, *J* = 6.6, 3.3 Hz, 1H), 3.91 (ddd, *J* = 14.7, 7.2, 4.1 Hz, 1H), 3.38 (dt, *J* = 15.3, 3.6 Hz, 1H), 2.63 (d, *J* = 4.8 Hz, 3H), 2.38 (t, *J* = 7.7 Hz, 2H), 2.25 (t, *J* = 7.4 Hz, 2H), 1.70 (sext, *J* = 7.5, 2H), 1.55 (sext, *J* = 7.4, 2H), 1.00 (t, *J* = 7.5, 3H), 0.86 (t, *J* = 7.4, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.4, 172.2, 159.33, 159.30, 152.7, 151.0, 150.7, 150.6, 143.7, 137.5, 129.4, 124.9, 121.5, 121.3, 86.8, 83.6, 73.0, 71.6, 41.4, 36.0, 35.7, 27.1, 18.6, 18.2, 13.9, 13.7; MS (ES) *m/z* 582.2511 (M+ [$C_{27}H_{34}N_8O_7$]) = 582.2550.



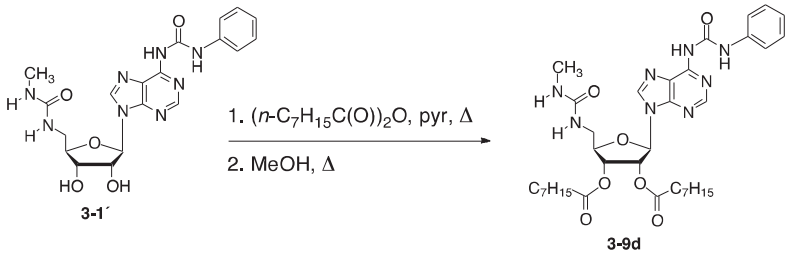
5´-Deoxy-5´-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)-2´,3´-bis-*O*-pivaloyladenosine (3-9b).

A solution of **3-1´** (28 mg, 0.063 mmol) and pivaloyl chloride (25 mg, 0.21 mmol), in pyridine (0.7 mL) was stirred at 70 °C overnight. Volatiles were removed under reduced pressure, and the crude mixture was added directly to a flash chromatography column and eluted with 3→7% MeOH/CH_2Cl_2 to give **3-9b** (10 mg, 0.016 mmol, 26%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.12 (s, 1H), 9.84 (s, 1H), 8.83 (s, 1H), 8.66 (s, 1H), 7.56 (d, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 8.0 Hz, 2H), 7.19 (t, *J* = 7.5 Hz, 1H), 6.19 (d, *J* = 6.6 Hz, 1H), 6.60–6.00 (m, 1H), 5.88 (dd, *J* = 12.0, 6.3 Hz, 1H), 5.57 (dd, *J* = 5.4, 3.3 Hz, 1H), 4.93 (dd, *J* = 8.7, 6.5 Hz, 1H), 4.35 (dd, *J* = 6.5, 3.3 Hz, 1H), 3.95 (ddd, *J* = 14.7, 7.4, 3.7 Hz, 1H), 3.38 (ddd, *J* = 14.8, 7.4, 3.7 Hz, 1H), 2.58 (d, *J* = 4.8 Hz, 3H), 1.29 (s, 9H), 1.12 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.2, 177.1, 159.3, 152.9, 151.0, 150.9, 150.6, 143.6, 137.4, 129.4, 125.1, 121.6, 121.5, 121.3, 86.7, 84.2, 73.3, 72.2, 41.5, 39.1, 39.0, 29.9, 27.4, 27.2, 27.1, 26.7; MS (ES) *m/z* 610.2863 (M+ [$C_{29}H_{38}N_8O_7$]) = 610.2871.



5′-Deoxy-2,′3′-bis-*O*-hexanoyl-5′-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (3-9c).

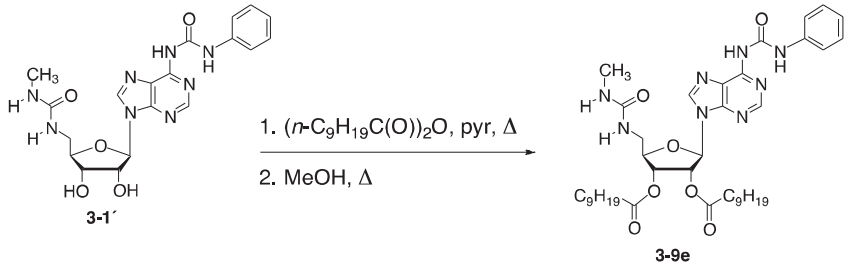
A solution of **3-1′** (40 mg, 0.09 mmol) and hexanoic anhydride (54 mg, 0.25 mmol), in pyridine (3 mL) was stirred at 70 °C overnight. Volatiles were removed under reduced pressure, and the crude mixture was added directly to a flash chromatography column and eluted with 4→7% MeOH/CH_2Cl_2 to give **3-9c** (26 mg, 0.041 mmol, 46%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.06 (s, 1H), 8.79 (s, 1H), 8.62 (s, 1H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.37 (t, *J* = 8.0 Hz, 2H), 7.17 (t, *J* = 7.3 Hz, 1H), 6.18 (d, *J* = 6.0 Hz, 1H), 5.93–5.89 (m, 2H), 5.59 (dd, *J* = 5.3, 3.8 Hz, 1H), 5.09 ("d", *J* = 4.0 Hz, 1H), 4.35 ("d", *J* = 3.0 Hz, 1H), 3.87 (ddd, *J* = 14.5, 7.3, 4.3 Hz, 1H), 3.38 (dt, *J* = 15.3, 3.8 Hz, 1H), 2.62 (d, *J* = 4.4 Hz, 3H), 2.41–2.37 (m, 2H), 2.25 (t, *J* = 7.5 Hz, 2H), 1.69–163 (m, 2H), 1.54–1.48 (m, 2H), 1.36–1.33 (m, 4H), 1.26–1.17 (m, 5H), 0.92 (t, *J* = 6.8, 3H), 0.83 (t, *J* = 7.0, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.6, 172.3, 159.4, 152.8, 151.0, 150.7, 150.5, 143.8, 137.6, 129.4, 124.9, 121.4, 121.3, 86.8, 83.4, 73.1, 71.6, 41.4, 34.1, 33.8, 31.5, 31.3, 27.1, 24.7, 24.5, 22.5, 22.4, 14.1, 14.0; MS (ES) *m/z* 638.3171 (M+ [$C_{31}H_{42}N_8O_7$]) = 638.3176.



5′-Deoxy-5′-[(*N*-methylcarbamoyl)amino]-2,′3′-bis-*O*-octanoyl-N^6-(*N*-phenylcarbamoyl)adenosine (3-9d).

A solution of **3-1′** (48 mg, 0.11 mmol) and octanoic anhydride (88 mg, 0.33 mmol) in pyridine (1.5 mL) was stirred at 70 °C overnight. Volatiles were removed under reduced

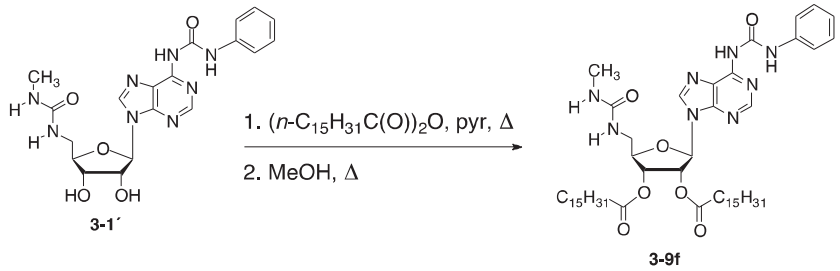
pressure, and the crude mixture was added directly to a flash chromatography column and eluted with 2→6% MeOH/CH_2Cl_2 to give **3-9d** (41 mg, 0.059 mmol, 53%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.05 (s, 1H), 9.89 (s, 1H), 8.77 (s, 1H), 8.61 (s, 1H), 7.55 (d, *J* = 7.5 Hz, 2H), 7.37 (t, *J* = 7.8 Hz, 2H), 7.16 (t, *J* = 7.9 Hz, 1H), 6.19 (d, *J* = 6.0 Hz, 1H), 5.96-5.88 (m, 2H), 5.59 (dd, *J* = 5.4, 3.9 Hz, 1H), 5.17 (bs, 1H), 4.35 (dd, *J* = 6.9, 3.6 Hz, 1H), 3.86 (ddd, *J* = 14.7, 6.9, 4.5 Hz, 1H), 3.39 (dt, *J* = 15.0, 3.8 Hz, 1H), 2.62 (d, *J* = 4.5 Hz, 3H), 2.41–2.33 (m, 2H), 2.25 (t, *J* = 7.5 Hz, 2H), 1.70–1.63 (m, 2H), 1.55–1.48 (m, 2H), 1.32–1.20 (m, 16H), 0.92–0.82 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.6, 172.3, 159.5, 152.8, 151.0, 150.7, 150.5, 143.8, 137.6, 129.4, 124.8, 121.4, 121.2, 86.8, 83.4, 73.1, 71.5, 41.4, 34.2, 33.9, 31.9, 31.8, 29.3, 29.2, 29.1, 29.0, 27.1, 25.1, 24.8, 22.8, 22.7, 14.3, 14.2; MS (ES) *m/z* 694.3801 (M+ [$C_{35}H_{50}N_8O_7$]) = 694.3802.



2´,3´-Bis-*O*-decanoyl-5´-deoxy-5´-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (3-9e).

A solution of **3-1´** (60 mg, 0.14 mmol) and decanoic anhydride (133 mg, 0.41 mmol) in pyridine (1.5 mL) was stirred at 70 °C overnight. Volatiles were removed under reduced pressure, and the crude mixture was added directly to a flash chromatography column and eluted with 2→7% MeOH/CH_2Cl_2 to give **3-9e** (53 mg, 0.07 mmol, 50%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.07 (s, 1H), 9.92 (s, 1H), 8.80 (s, 1H), 8.60 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.37 (t, *J* = 8.0 Hz, 2H), 7.16 (t, *J* = 7.4 Hz, 1H), 6.17 (d, *J* = 6.0 Hz, 1H), 5.95–5.88 (m, 2H), 5.59 (dd, *J* =

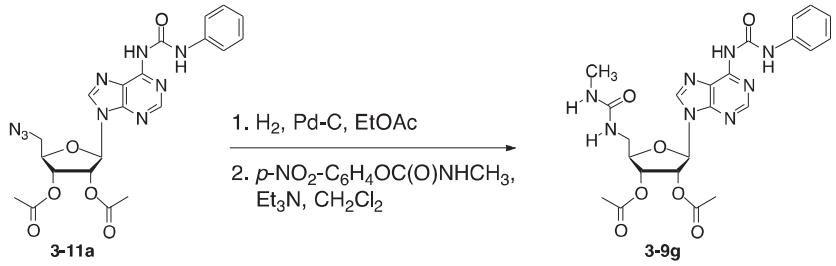
5.4, 3.9 Hz, 1H), 5.16 (“d”, *J* = 4.2 Hz, 1H), 4.34 (dd, *J* = 6.9, 3.6 Hz, 1H), 3.84 (ddd, *J* = 14.6, 6.8, 4.7 Hz, 1H), 3.40–3.36 (m, 1H), 2.62 (d, *J* = 4.8 Hz, 1H), 2.44–2.30 (m, 2H), 2.24 (t, *J* = 7.5 Hz, 2H), 1.65–1.60 (m, 2H), 1.58–1.48 (m, 2 H), 1.31–1.20 (m, 26H), 0.89 (t, *J* = 6.6, 3H), 0.85 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.5, 172.3, 159.4, 152.8, 151.0, 150.7, 150.5, 143.9, 137.6, 129.4, 124.8, 121.3, 121.2, 86.9, 83.3, 73.2, 71.5, 41.4, 34.2, 33.9, 32.1, 32.0, 29.6, 29.55, 29.51, 29.49, 29.42, 29.38, 29.37, 29.2, 27.1, 25.1, 24.8, 22.9, 22.8, 14.29, 14.25; MS (ES) *m/z* 750.4450 (M+ [$C_{39}H_{58}N_8O_7$]) = 750.4428.



5´-Deoxy-5´-[(*N*-methylcarbamoyl)amino]-2,´3´-bis-*O*-palmitoyl-N^6-(*N*-phenylcarbamoyl)adenosine (3-9f).

A solution of **3-1´** (98 mg, 0.22 mmol) and palmitic anhydride (329 mg, 0.67 mmol) in pyridine (3 mL) was stirred at 70 °C overnight. In order to ensure reaction completion, an additional aliquot of palmitic anhydride (46 mg, 0.09 mmol) was added. Volatiles were removed under reduced pressure, and the crude mixture was added directly to a flash chromatography column and eluted with 2→6% MeOH/CH_2Cl_2 to give **3-9f** (107 mg, 0.12 mmol, 55%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.00 (s, 1H), 9.58 (s, 1H), 8.69 (s, 1H), 8.65 (s, 1H), 7.58 (d, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.8 Hz, 2H), 7.18 (t, *J* = 7.4 Hz, 1H), 6.19 (d, *J* = 6.3 Hz, 1H), 5.92 (t, *J* = 5.9 Hz, 2H), 5.59 (dd, *J* = 5.4, 3.6 Hz, 1H), 4.92 (dd, *J* = 6.8, 2.4 Hz, 1H), 4.39 (dd, *J* = 6.5, 3.2 Hz, 1H), 3.93 (ddd, *J* = 14.7, 7.7, 3.8 Hz, 1H), 3.41 (dt, *J* = 14.7, 3.8 Hz,

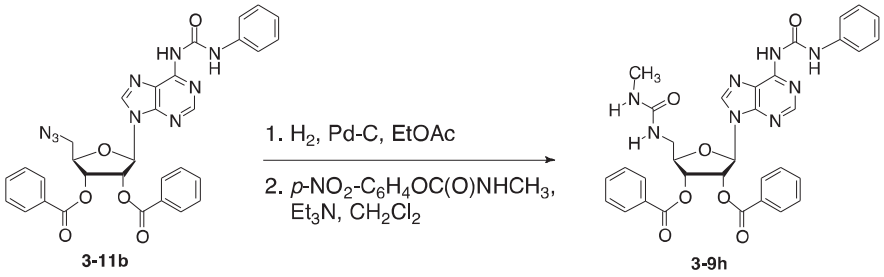
1H), 2.65 (d, J = 4.8 Hz, 3H), 2.42–2.33 (m, 2H), 2.26 (t, J = 7.7 Hz, 2H), 1.68–1.62 (m, 2H), 1.55–1.50 (m, 2H), 1.27–1.22 (m, 48 H), 0.90–0.86 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.6, 172.3, 159.4, 152.6, 151.1, 150.7, 150.6, 143.4, 137.6, 129.4, 124.9, 121.5, 121.4, 86.9, 83.6, 77.4, 73.0, 71.6, 41.5, 34.2, 33.9, 32.1, 29.94, 29.92, 29.89, 29.84, 29.80, 29.7, 29.6, 29.5, 29.45, 29.42, 29.34, 29.25, 27.2, 25.1, 25.0, 24.8, 22.9, 14.3; MS (ES) *m/z* 918.6352 (M+ [$C_{51}H_{82}N_8O_7$]) = 918.6306.



2′,3′-Bis-O-acetyl-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (3-9g).

A solution of **3-11a** (40 mg, 0.081 mmol) and 10% Pd–C (40 mg) in EtOAc (7.5 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dissolved in CH_2Cl_2 (1 mL) and crude *N*-methyl-*p*-nitrophenylcarbamate (31 mg, 0.16 mmol) and Et_3N (15 μL) were then added. The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a flash chromatography column and eluted with 5→7% MeOH/CH_2Cl_2 to give **3-9g** (21 mg, 0.040 mmol, 49%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.06 (s, 1H), 9.85 (s, 1H), 8.82 (s, 1H), 8.62 (s, 1H), 7.56 (d, J = 7.5 Hz, 2H), 7.38 (t, J = 8.0 Hz, 2H), 7.17 (t, J = 7.5 Hz, 1H), 6.22 (d, J = 6.0 Hz, 1H), 5.92–5.88 (m, 2H), 5.59 (dd, J = 5.0, 4.0 Hz, 1H), 5.03 (d, J = 4.0

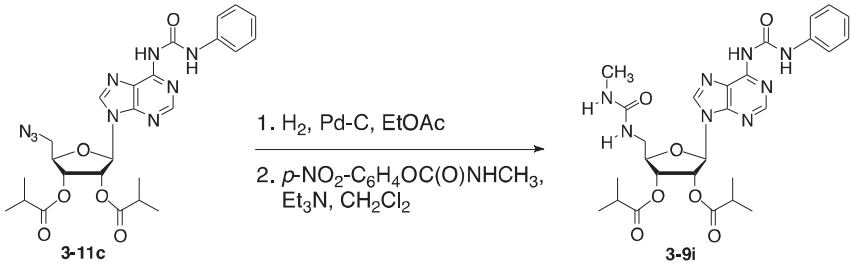
Hz, 1H), 4.38 (d, *J* = 3.5 Hz, 1H), 3.89 (ddd, *J* = 14.8, 7.3, 3.8 Hz, 1H), 3.39 (dt, *J* = 14.5, 3.3 Hz, 1H), 2.61 (d, *J* = 4.5 Hz, 3H), 2.15 (s, 3H) 2.03 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.8, 169.5, 159.4, 152.8, 151.0, 150.8, 150.6, 143.7, 137.5, 129.4, 124.9, 121.5, 121.3, 86.6, 83.3, 73.3, 71.7, 41.4, 27.1, 20.8, 20.6; MS (ES) *m/z* 527.2010 (MH+ [$C_{23}H_{27}N_8O_7$]) = 527.2003.



5′-Deoxy-2′,3′-bis-*O*-benzoyl-5′-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (3-9h).

A solution of **3-11b** (124 mg, 0.20 mmol) and 10% Pd–C (62 mg) in EtOAc (9 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dissolved in CH_2Cl_2 (2 mL) and *N*-methyl-*p*-nitrophenylcarbamate (58 mg, 0.30 mmol) and Et_3N (400 μL) were then added. The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a flash chromatography column and eluted with 5% MeOH/CH_2Cl_2 to give **3-9h** (94 mg, 0.14 mmol, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.86 (s, 1H), 8.99 (bs, 1H), 8.69 (s, 1H), 8.53 (s, 1H), 8.05 (dd, *J* = 8.5, 1.5 Hz, 2H), 7.84 (dd, *J* = 8.5, 1.0 Hz, 2H), 7.63–7.60 (m, 3H), 7.53 (t, *J* = 7.5 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 2H), 7.40 (t, *J* = 7.8 Hz, 2H), 7.33 (t, *J* = 7.8 Hz, 2H), 7.17 (t, *J* = 7.3 Hz, 1H), 6.40 (d, *J* = 6.0 Hz, 1H), 6.30 (t, *J* = 6.0 Hz, 1H), 6.25–6.03 (m, 1H), 5.99 (dd, *J* = 5.5, 3.5 Hz, 1H), 4.74–4.70 (m, 1H), 4.68 (dd, *J* = 6.5, 3.5 Hz, 1H), 4.06

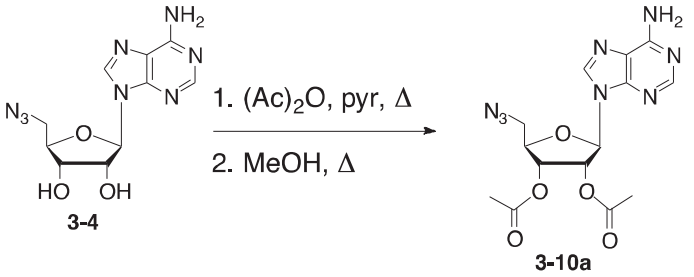
(ddd, J = 14.8, 7.8, 3.8 Hz, 1H), 3.57 (dt, J = 14.5, 3.8 Hz, 1H) 2.74 (d, J = 4.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.5, 165.2, 159.6, 152.7, 151.0, 150.8, 150.6, 150.39, 150.37, 144.4, 144.1, 137.6, 133.9, 133.8, 133.6, 130.04, 130.00, 129.94, 129.86, 129.4, 129.2, 128.8, 128.7, 128.5, 128.4, 124.7, 124.5, 121.3, 121.2, 121.0, 87.5, 87.3, 83.3, 83.2, 74.2, 74.0, 72.5, 72.3, 41.7, 41.5, 41.3, 27.2, 26.9; MS (ES) *m/z* 651.2376 (MH+ [$C_{33}H_{31}N_8O_7$]) = 651.2316.



5′-Deoxy-2′,3′-bis-*O*-isobutyryl-5′-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (3-9i).

A solution of **3-11c** (83 mg, 0.15 mmol) and 10% Pd–C (83 mg) in EtOAc (7.5 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dissolved in CH_2Cl_2 (2 mL) and crude *N*-methyl-*p*-nitrophenylcarbamate (67 mg, 0.34 mmol) and Et_3N (15 μL) were then added. The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a flash chromatography column and eluted with 5→7% MeOH/CH_2Cl_2 to give **3-9i** (68 mg, 0.12 mmol, 80%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.02 (s, 1H), 9.59 (bs, 1H), 8.73 (s, 1H), 8.66 (s, 1H), 7.57 (d, J = 7.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.18 (t, J = 7.5 Hz, 1H), 6.20 (d, J = 6.5 Hz, 1H), 5.90 (t, J = 6.0 Hz, 2H), 5.60 (dd, J = 5.3, 3.3 Hz, 1H), 4.82 (bs, 1H), 4.39 (d, J = 3.5 Hz, 1H), 3.93 (ddd, J = 14.5, 7.5, 3.5 Hz, 1H), 3.42 (dd, J = 14.5, 3.0 Hz, 1H),

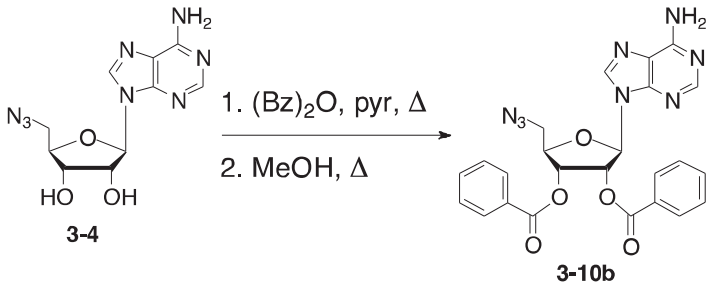
2.65 (sept, *J* = 7.0 Hz, 1H), 2.63 (d, *J* = 4.5 Hz, 3H), 2.51 (sept, *J* = 7.0 Hz, 1H), 1.24 (d, *J* = 7.0 Hz, 6H), 1.10 (d, *J* = 7.0 Hz, 3H), 1.08 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.8, 175.6, 159.2, 152.6, 151.1, 150.8, 150.6, 143.5, 137.6, 129.4, 124.9, 121.5, 110.2, 86.9, 83.8, 73.1, 71.8, 41.6, 34.1, 33.9, 29.9, 27.2, 19.2, 19.1, 18.91, 18.88; MS (ES) *m/z* 583.2663 (MH+ [$C_{27}H_{35}N_8O_7$]) = 583.2629.



3-4 3-10a

2′,3′-Bis-*O*-acetyl-5′-azido-5′-deoxyadenosine (3-10a).

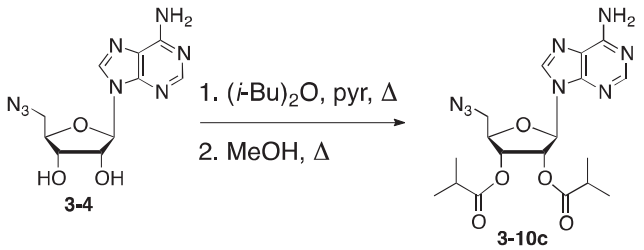
A solution of **3-4** (50 mg, 0.17 mmol), acetic anhydride (70 mg, 0.69 mmol), and pyridine (1 mL) was stirred at 70 °C overnight. Volatiles were removed under reduced pressure, and the crude extraction mixture was added directly to a flash chromatography column and eluted with 3% MeOH/ CH_2Cl_2 to give **3-10a** (52 mg, 0.14 mmol, 82%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.36 (s, 1H), 8.05 (s, 1H), 6.20 (bs, 2H), 6.21 (d, *J* = 5.5 Hz, 3H), 5.94 (t, *J* = 5.8 Hz, 1H), 5.62 (dd, *J* = 5.8, 4.3 Hz, 1H), 4.36 (dd, *J* = 8.0, 4.0 Hz, 1H), 3.78 (dd, *J* = 13.5 Hz, 5.0 Hz, 1H), 3.76 (dd, *J* = 13.3, 3.8 Hz, 1H), 2.15 (s, 3H), 2.07 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.9, 169.5, 156.0, 153.5, 150.0, 139.9, 120.2, 86.1, 81.6, 73.2, 71.5, 52.1, 20.7, 20.5; MS (ES) *m/z* 376.1274 (M+ [$C_{14}H_{16}N_8O_5$]) = 376.1244.



3-4 3-10b

5′-Azido-5′-deoxy-2′,3′-bis-*O*-benzoyladenosine (3-10b).

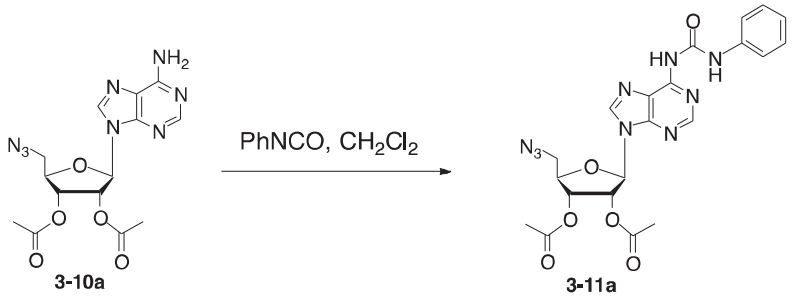
A solution of **3-4** (75 mg, 0.25 mmol), benzoic anhydride (313 mg, 1.38 mmol), and pyridine (2 mL) was stirred at 70 °C overnight. Pyridine was removed under reduced pressure and the crude residue was transferred to a pressure flask in MeOH (4 mL) and stirred at 115 °C (4 hr). Volatiles were removed under reduced pressure, and the crude mixture was added directly to a flash chromatography column and eluted with 70% EtOAc/hexanes → 100% EtOAc to give **3-10b** (75 mg, 0.15 mmol, 60%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.42 (s, 1H), 8.14 (s, 1H), 7.98 (d, *J* = 7.0 Hz, 2H), 7.94 (d, *J* = 7.5 Hz, 2H), 7.59 (t, *J* = 7.5 Hz, 1H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.37 (t, *J* = 7.8 Hz, 2H), 6.47 (d, *J* = 5.5 Hz, 1H), 6.27 (t, *J* = 6.1 Hz, 1H), 6.03 (dd, *J* = 5.5, 4.5 Hz, 1H), 5.80 (bs, 2H), 4.61 (dd, *J* = 7.8, 4.3 Hz, 1H), 3.93 (dd, *J* = 13.3, 5.0 Hz, 1H), 3.85 (dd, *J* = 13.3, 3.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.5, 165.3, 155.8, 153.7, 150.2, 139.3, 134.0, 133.9, 130.1, 130.0, 128.9, 128.8, 128.7, 120.4, 86.6, 82.1, 74.1, 72.2, 52.3; MS (ES) *m/z* 580.1560 (M+ [$C_{24}H_{20}N_8O_5$]) = 580.1557.



3-4 3-10c

5′-Azido-5′-deoxy-2′,3′-bis-*O*-isobutyryladenosine (3-10c).

A solution of **3-4** (100 mg, 0.34 mmol), isobutyric anhydride (216 mg, 1.4 mmol), and Pyridine (2 mL) was stirred at 70 °C overnight. Pyridine was removed under reduced pressure and the crude residue was transferred to a pressure flask in MeOH (4 mL) and stirred at 115 °C (2.5 hr). Volatiles were removed under reduced pressure, and the crude mixture was added directly to a flash chromatography column and eluted with 2%→5% MeOH/ CH_2Cl_2 to give **3-10c** (113 mg, 0.26 mmol, 76%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.39 (s, 1H), 8.04 (s, 1H), 6.19 (d, *J* = 6.0 Hz, 1H), 5.91 (t, *J* = 5.8 Hz, 1H), 5.64 (dd, *J* = 5.5, 4.5 Hz, 1H), 5.6 (bs, 2H), 4.33 (dd, *J* = 8.0, 4.0 Hz, 1H), 3.79 (dd, *J* = 13.2, 4.8 Hz, 1H), 3.72 (dd, *J* = 13.0, 3.5 Hz, 1H), 2.63 (sept, *J* = 7.0 Hz, 1H), 2.56 (sept, *J* = 7.0 Hz, 1H), 1.23 (d, *J* = 7.0 Hz, 3H), 1.22 (d, *J* = 7.0 Hz, 3H), 1.15 (d, *J* = 7.0 Hz, 3H), 1.12 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.8, 175.4, 155.9, 153.3, 153.2, 149.8, 138.9, 138.8, 119.9, 86.2, 86.0, 81.8, 81.6, 73.1, 72.9, 71.2, 71.1, 52.2, 52.0, 51.8, 33.9, 33.8, 33.6, 33.5, 19.1, 18.9, 18.87, 18.69, 18.54, 18.4; MS (ES) *m/z* 433.1944 (MH+ [$C_{18}H_{25}N_8O_5$]) = 433.1942.

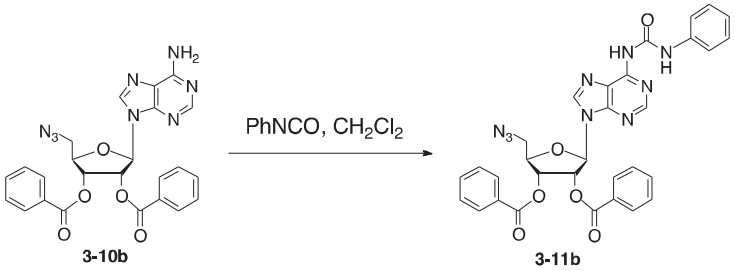


3-10a 3-11a

2′,3′-Bis-*O*-acetyl-5′-azido-5′-deoxy-N^6-(*N*-phenylcarbamoyl)adenosine (3-11a).

A solution of **3-10a** (45 mg, 0.12 mmol) and phenylisocyanate (21 mg, 0.18 mmol) in CH_2Cl_2 (2 mL) was stirred at ambient temperature (6 d). Volatiles were removed under reduced

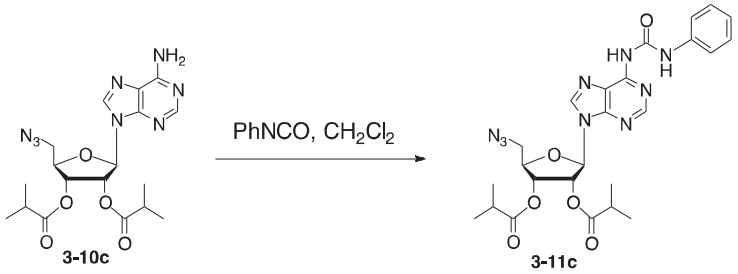
pressure, and the crude reaction mixture was added directly to a flash chromatography column and eluted with 50% EtOAc/hexanes →100% EtOAc to give **3-11a** (42 mg, 0.085 mmol, 71%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.85 (s, 1H), 9.15 (s, 1H), 8.66 (s, 1H), 8.57 (s, 1H), 7.66 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.8 Hz, 2H), 7.13 (t, *J* = 7.3 Hz, 1H), 6.26 (d, *J* = 6.0 Hz, 1H), 6.05 (t, *J* = 5.5 Hz, 1H), 5.66 (dd, *J* = 5.0, 4.5 Hz, 1H), 4.38 (dd, *J* = 8.3, 4.3 Hz, 1H), 3.79 (dd, *J* = 13.0 Hz, 5.0 Hz, 1H), 3.73 (dd, *J* = 13.0, 3.5 Hz, 1H), 2.17 (s, 3H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.8, 169.5, 151.7, 151.4, 150.6, 150.5, 142.7, 138.2, 129.2,124.1, 121.2, 120.7, 86.6, 81.8, 73.1, 71.4, 52.0, 20.7, 20.6; MS (ES) *m/z* 495.1629 (M+ [$C_{21}H_{21}N_9O_6$]) = 495.1615.



5′-Azido-5′-deoxy-2′,3′-bis-*O*-benzoyl- N^6-(*N*-phenylcarbamoyl)adenosine (3-11b).

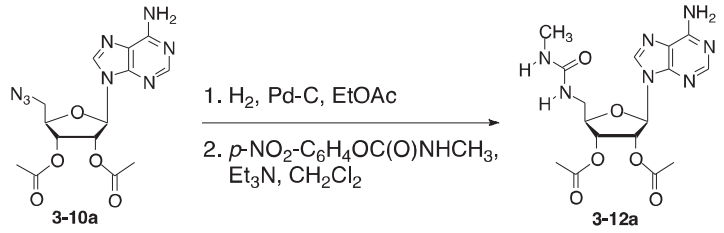
A solution of **3-10b** (120 mg, 0.24 mmol) and phenylisocyanate (43 mg, 0.36 mmol) in CH_2Cl_2 (3 mL) was stirred at ambient temperature (3 d). Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a flash chromatography column and eluted with 30→70% EtOAc/hexanes to **3-11b** (132 mg, 0.21 mmol, 88%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.77 (s, 1H), 8.70 (s, 1H), 8.59 (bs, 1H), 8.50 (s, 1H), 8.00 (d, *J* = 7.0 Hz, 2H), 7.94 (d, *J* = 7.5 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.56 (t, *J* = 7.3 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 2H), 7.39 (dd, *J* = 7.8, 2.3 Hz, 2H), 7.37 (dd, *J* = 7.8, 2.3 Hz, 2H), 7.14 (t, *J* = 7.3 Hz, 1H), 6.50 (d, *J* = 5.5 Hz, 1H), 6.36 (t, *J* = 5.5 Hz, 1H), 6.04 (dd, *J* = 5.8, 4.8

Hz, 1H), 4.64 (dd, *J* = 8.0, 4.5 Hz, 1H), 3.94 (dd, *J* = 13.3, 4.5 Hz, 1H), 3.87 (dd, *J* = 13.3, 3.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.5, 165.2, 151.7, 151.4, 150.7, 150.6, 142.8, 138.2, 133.99, 133.97, 130.04, 129.98, 129.2, 128.9, 128.8, 128.7, 128.5, 124.1, 121.3, 120.6, 87.2, 82.2, 74.0, 72.1, 52.2 MS (ES) *m/z* 619.1936 (M+ [$C_{31}H_{25}N_9O_6$]) = 619.1928.



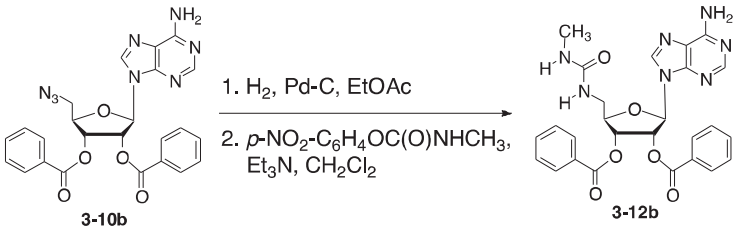
5′-Azido-5′-deoxy-2′,3′-bis-*O*-isobutyryl- N^6-(*N*-phenylcarbamoyl)adenosine (3-11c).

A solution of **3-10c** (71 mg, 0.16 mmol), phenyl isocyanate (29 mg, 0.24 mmol), and CH_2Cl_2 (3.5 mL) was stirred at ambient temperature overnight. Volatiles were removed under reduced pressure, and the crude mixture was added directly to a flash chromatography column and eluted with 30% EtOAc/hexanes →100% EtOAc to give **3-11c** (85 mg, 0.15 mmol, 94%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.88 (s, 1H), 9.27 (s, 1H), 8.65 (s, 1H), 8.60 (s, 1H), 7.66 (d, *J* = 8.0 Hz, 2H), 7.37 (t, *J* = 7.8 Hz, 2H), 7.12 (t, *J* = 7.5 Hz, 1H), 6.25 (d, *J* = 5.5 Hz, 1H), 6.03 (t, *J* = 5.8 Hz, 1H), 5.70 (t, *J* = 5.0 Hz, 1H), 4.36 (dd, *J* = 8.5, 4.0 Hz, 1H), 3.80 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.71 (dd, *J* = 13.0, 3.5 Hz, 1H), 2.64 (sept, *J* = 7.0 Hz, 1H), 2.57 (sept, *J* = 7.0 Hz, 1H), 1.23 (d, *J* = 7.0 Hz, 3H), 1.22 (d, *J* = 7.5 Hz, 3H), 1.15 (d, *J* = 7.0 Hz, 3H), 1.12 (d, *J* = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.9, 175.5, 151.8, 151.3, 150.6, 150.5, 142.8, 138.2, 129.2, 124.1, 121.2, 120.6, 87.0, 82.1, 73.1, 71.3, 52.1, 34.0, 33.8, 19.0, 19.0, 18.995, 18.987, 18.8; MS (ES) *m/z* 551.2259 (M+ [$C_{25}H_{29}N_9O_6$]) = 551.2241.



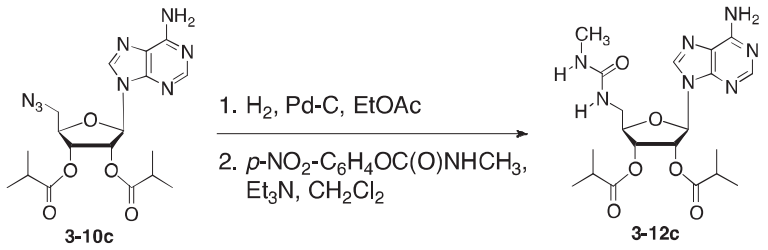
2′,3′-Bis-*O*-acetyl-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino]adenosine (3-12a).

A solution of **3-10a** (52 mg, 0.14 mmol) and 10% Pd–C (52 mg) in EtOAc (7.5 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dissolved in CH_2Cl_2 (1 mL) and *N*-methyl-*p*-nitrophenylcarbamate (37 mg, 0.19 mmol) and Et_3N (15 μL) were then added. The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a flash chromatography column and eluted with 6→10% MeOH/CH_2Cl_2 to give **3-12a** (38 mg, 0.093 mmol, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.26 (s, 1H), 7.88 (s, 1H), 6.75 (bs, 1H), 6.22 (s, 2H), 6.06 (t, *J* = 6.0 Hz, 1H), 6.01 (d, *J* = 6.5 Hz, 1H), 5.54 (dd, *J* = 5.0, 3.0 Hz, 1H), 5.03 (d, *J* = 4.5 Hz, 1H), 4.39 (d, *J* = 3.5 Hz, 1H), 3.86 (ddd, *J* = 14.3, 7.5, 4.5 Hz, 1H), 3.48 (dt, *J* = 14.5, 3.3 Hz, 1H), 2.76 (d, *J* = 5.0 Hz, 3H), 2.13 (s, 3H), 2.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.8, 169.5, 159.6, 156.1, 153.0, 149.3, 140.5, 120.8, 87.3, 83.2, 72.3, 71.9, 41.7, 27.3, 20.8, 20.6; MS (ES) *m/z* 408.1664 (MH+ [$C_{16}H_{22}N_7O_6$]) = 408.1632.



5′-Deoxy-2′,3′-bis-*O*-benzoyl-5′-[(*N*-methylcarbamoyl)amino]adenosine (3-12b).

A solution of **3-10b** (15 mg, 0.030 mmol) and 10% Pd–C (15 mg) in EtOAc (1.5 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dissolved in CH_2Cl_2 (1 mL), and crude *N*-methyl-*p*-nitrophenylcarbamate (10 mg, 0.051 mmol) and Et_3N (10 μL) were then added. The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a flash chromatography column and eluted with 8→10% MeOH/CH_2Cl_2 to give **3-12b** (5 mg, 0.0094 mmol, 31%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.42 (s, 1H), 8.02 (dd, *J* = 8.0, 1.0 Hz, 2H), 7.92 (bs, 1H), 7.84 (dd, *J* = 8.5, 1.5 Hz, 1.7H), 7.73 (dd, *J* = 5.8, 3.3 Hz, 0.3H), 7.60 (t, *J* = 7.3 Hz, 1H), 7.55–7.51 (m, 1.4H), 7.44 (t, *J* = 7.8 Hz, 1.7H), 7.32 (t, *J* = 7.8 Hz, 2H), 6.85 (d, *J* = 6.5 Hz, 1H), 6.39 (t, *J* = 6.0 Hz, 1H), 6.21 (d, *J* = 6.5 Hz, 1H), 5.94 (dd, *J* = 5.5, 3.0 Hz, 1H), 5.74 (bs, 2H), 4.67 (dd, *J* = 6.0, 3.0 Hz, 1H), 4.64 (d, *J* = 4.5 Hz, 1H), 4.12 (ddd, *J* = 14.5, 8.8, 3.1 Hz, 1H) 3.67–3.65 (m, 1H), 3.54 (dt, *J* = 14.3, 2.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.4, 165.1, 159.5, 156.2, 153.1, 149.5, 133.9, 133.8, 132.5, 131.2, 130.0, 129.9, 129.1, 128.8, 128.7, 128.6, 121.4, 88.4, 83.8, 73.0, 72.8, 41.9, 19.9, 27.6; MS (ES) *m/z* 532.1949 (MH+ [$C_{26}H_{26}N_7O_6$]) = 532.1945.



5′-Deoxy-2′,3′-bis-*O*-isobutyryl-5′-[(*N*-methylcarbamoyl)amino]adenosine (3-12c).

A solution of **3-10c** (100 mg, 0.23 mmol) and 10% Pd–C (100 mg) in EtOAc (8 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dried under vacuum pump for two days and then dissolved in CH_2Cl_2 (2 mL) and *N*-methyl-*p*-nitrophenylcarbamate (100 mg) and Et_3N (25 μL) were then added. The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a flash chromatography column and eluted with 4→10% MeOH/CH_2Cl_2 to give **3-12c** (46 mg, 0.099 mmol, 43%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.31 (s, 1H), 7.85 (s, 1H), 6.78 (bs, 1H), 6.05 (t, *J* = 6.0 Hz, 1H), 5.96 (d, *J* = 7.0 Hz, 1H), 5.93 (bs, 2H), 5.55 (dd, *J* = 5.8, 2.8 Hz, 1H), 4.76 (d, *J* = 4.5 Hz, 1H), 4.39 (d, *J* = 3.0 Hz, 1H), 3.94 (ddd, J = 14.3, 8.3, 4.3 Hz, 1H), 3.47 (dt, *J* = 14.5, 3.0 Hz, 1H), 2.80 (t, *J* = 4.5 Hz, 3H), 2.63 (sept, *J* = 7.0 Hz, 1H), 2.49 (sept, *J* = 7.0 Hz, 1H), 1.22 (d, *J* = 6.5 Hz, 6H), 1.09 (d, *J* = 7.0 Hz, 3H), 1.07 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.7, 175.5, 159.5, 156.1, 153.0, 149.4, 140.5, 121.1, 87.9, 83.7, 72.3, 71.8, 41.8, 34.0, 33.8, 27.4, 19.2, 19.1, 18.9; MS (ES) *m/z* 464.2290 (MH+ [$C_{20}H_{30}N_7O_6$]) = 464.2258.

3.6. References

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Chapter 4: Efficient Synthesis of 5′-*O*-Carbamoyl and 5′-*O*-Polycarbamoyl Nucleosides: Nucleotide Surrogates with an Uncharged Phosphoester Replacement

4.1. Introduction

The carbamoyl group (H_2NCO-) is highly prevalent in nature. Many natural products possess this moiety.[1-11] It also is found in a number of pharmaceuatically important compounds.[12-14] In addition, the *N*-substituted carbamoyl moiety is found in a number of experimental, or currently licensed, drugs.[15, 16] It also occurs in a variety of organic synthesis contexts. Its most common occurrence is possibly the well-known nitrogen protecting groups such as Cbz, Fmoc, Cbz, etc.[17]

Nucleoside phosphate derivatives have been extensively investigated.[18-23] These compounds are nucleoside mono-, di-, and triphosphates in which the phosphoester or phosphoanhydride has been replaced with an unnatural functional group. The motivation for this research has been the need for compounds with greater hydrolytic stability, membrane permeability, and/or bioavailability than naturally occurring nucleotides.[24-26] Modified nucleosides have been studied for their use as antiviral therapeutics, agonists/antagonists for P2 purinoceptors (Chapter 1), and probes for DNA polymerases[27-29] or other enzyme substrate interactions.[30-34] Modifications to the naturally occurring phosphoester, diester, and triester have been probed. The majority of these modifications were made to the oxygen in the P-O-P bridge between the αβ (X) or βγ (Y) phosphates. In these alterations, the oxygen has been replaced with an unnatural atom or bridging group (Figure 1).

The bridging groups which have been examined include: $–CH_2–$, $–CCl_2–$, $–CBr_2–$, $–CF_2–$, –CHF–, –CHCl–, –CHBr–, $–CH_2CH_2–$, $–CHSO_3–$, $–CHPO_3–$, $–CFPO_3–$, $CH_2–$, NH–, and –

CHCOOH–.[27-34] An array of nonbridging modifications have also been studied. These include $R^3 = BH_3$, SH, and Se; and R^4 = Me, Ph, NH_2, NHMe, NHPh, F, and N_3.[24-26]

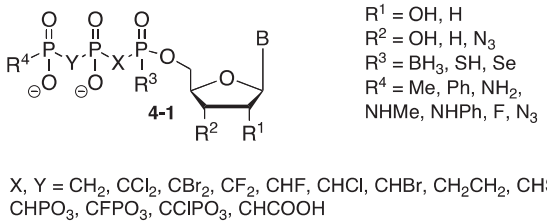


Figure 1. Modified nucleotides.

A thorough review of the literature (Reaxsys, CAS) showed that there have been few instances where the 5′-*O*-carbamoyl nucleoside monophosphate surrogates have been reported previously.[35-37] Only two examples of a 5′-*N*-carbamoyl derivative of 5′-amino-5′-deoxyuridine were reported.[36, 38] Polycarbamoyl nucleotide analogs typified by compounds **4-2c**–**4-6c** and **4-2d**–**4-6d** and their related 5′-amino-5′-deoxy analogues have not been previously investigated, to the best of our knowledge (Schemes 1 and 2). Due to the possibility that these novel nucleotide surrogates might have unusual biological properties, we set out to develop an efficient method for their synthesis. These novel derivatives may also be used to further explore the 5′-position of our lead compounds (Chapters 1–3).

4.2. Chemistry

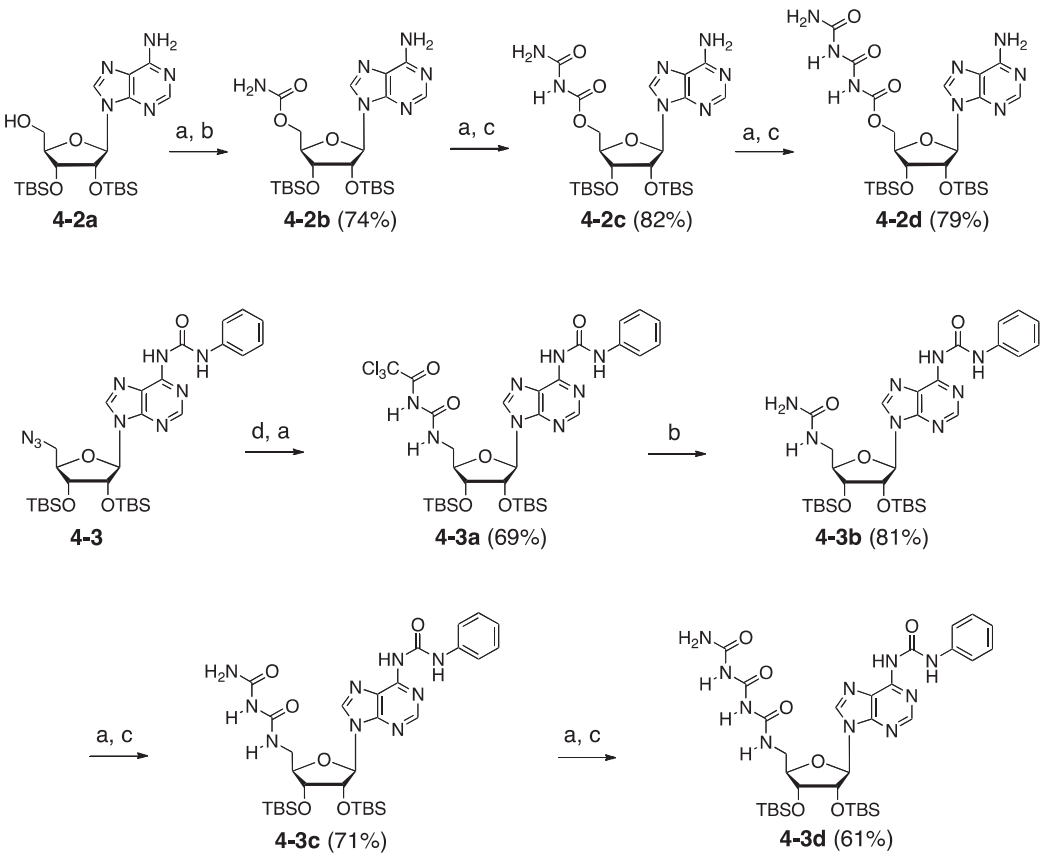
Consultation of the reported methods for installing primary carbamoyl groups lead us to the Kočovský[39] method which has been used extensively for installing carbamoyl moieties on a variety of substrates.[40] We hypothesized that this method, or a modification of thereof, could be applied in an iterative fashion allowing for the formation of polycarbamoyl nucleoside

derivatives. We also believed that this process would prove successful for the preparation of *N*-carbamoyl analogues. To the best of our knowledge, this method has never been applied previously to the synthesis of 5′-*O*- or 5′-*N*-polycarbamoyl nucleoside derivatives.

Our exploration of this chemistry began with 2′,3′-bis-*O*-*tert*-butyldimethylsilyl protected adenosine derivatives **4-2a** and **4-3** (Scheme 1). Compound **4-2a** was treated with trichloroacetylisocyanate ($Cl_3CCON=C=O$) in dry CH_2Cl_2 to give the corresponding *N*-trichloroacetylcarbamoyl analogue in excellent yield (TLC). This intermediate was analyzed by MS to verify correct mass, and then immediately carried on to the next step. The CH_2Cl_2 was removed and the crude mixture was dissolved in anhydrous MeOH and then charged with K_2CO_3. This gave the primary 5′-*O*-carbamoyl derivative **4-2b** in good yield (74%).

When compound **4-2b** was treated with $Cl_3CCON=C=O$, we were surprised to learn that the *N*-trichloroacetyl moiety was readily cleaved to polycarbamoyl **4-2c** by simply subjecting the initial *N*-trichloroacetylcarbamoyl products to standard Flash column chromatography using $MeOH/CH_2Cl_2$ mixtures as eluting solvents. This fortuitous discovery gave the product directly after column chromatography and in good yield (82%) with no need to use the extra base treatment step. This same procedure, treatment with $Cl_3CCON=C=O$ followed by Flash chromatography in $MeOH/CH_2Cl_2$, was successful in converting **4-2c** to **4-2d** (79%).

The procedure was extended to compound **4-3** in an effort to synthesize 5′-*N*-polycarbamoyl (or polyureido) derivatives (e.g., compounds **4-3b**–**d**). Derivative **4-3** was chosen because it is a key intermediate for our lead compound 2′,3′-bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)] adenosine (see Chapters 1-3). Direct conversion of **4-3** to 5′-urea **4-3b** (combining azide reduction, acylation of the resulting 5′-amino product to give the 5′-*N*-trichloroacetyl moiety,

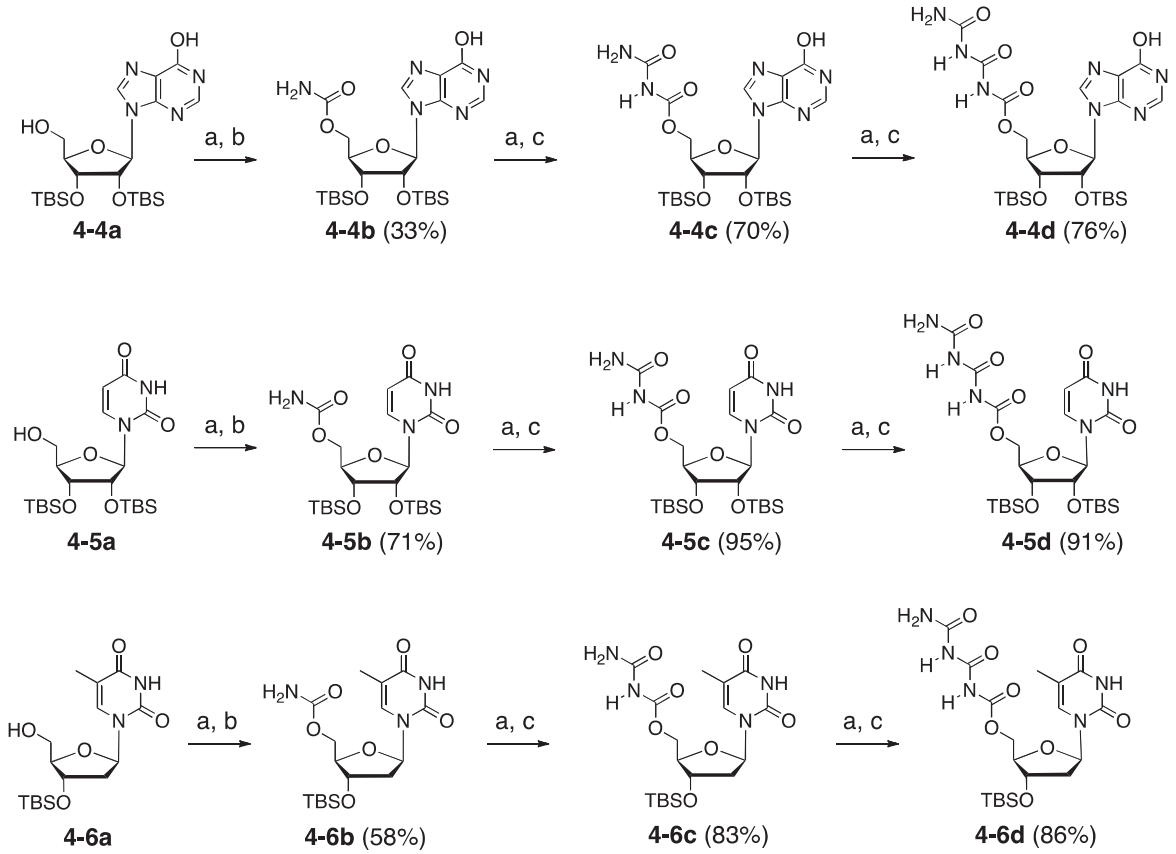


Scheme 1. Synthesis of **4-2b–d** and **4-3a–d**.
Reagents: (a) $Cl_3CCON{=}C{=}O$, CH_2Cl_2; (b) K_2CO_3, MeOH; (c) SiOH, CH_2Cl_2/MeOH; (d) H_2, Pd-C, EtOAc.

and cleavage of that moiety with K_2CO_3/MeOH) consistently produced low yields. It was decided to chromatograph intermediate **4-3a** and then treat with K_2CO_3/MeOH. This procedure gave **4-3a** in 69% and urea **4-3b** in 81% yields. Gratifyingly, treatment of **4-3b** with the isocyanate followed by Flash chromtography afforded 5′-polyureido derivatives **4-3c** and **4-3d** in good yields (71% and 61%, respectively).

In order to examine the scope and versatility of this method, it was extended to another 2′,3′-*O*-bis-TBS protected purine (**4-4a**) and two TBS-protected pyrimidines (**4-5a** and **4-6a**; Scheme 2). Treatment of compounds **4-4a**, **4-5a**, and **4-6a** following the same procedure used to form **4-2b** (treatment with $Cl_3CCON{=}C{=}O$ followed by K_2CO_3/MeOH) furnished mono 5′-*O*-

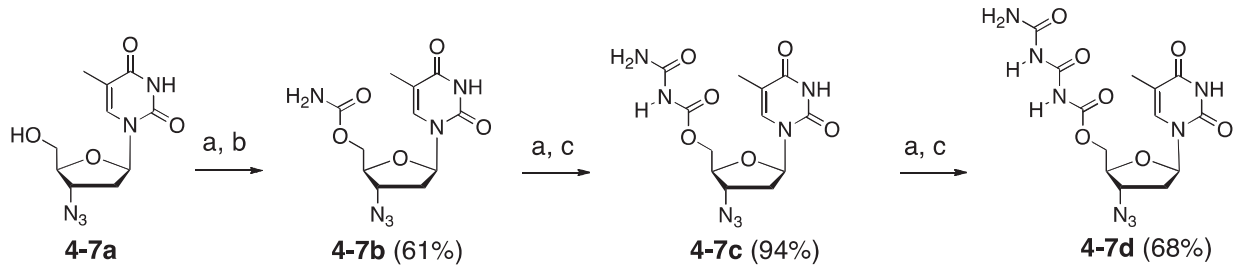
carbamoyl products **4-4b**, **4-5b**, **4-6b** in moderate to good yields (33–71%). Repeated attempts to improve the yield for inosine derivative **4-4b** were unsuccessful. The simple procedure that was successfully applied to the preparation of 5′-*N(O)*-polycarbamoyl derivatives **4-2c,d**, **4-3c,d** (Scheme 1) was applied to 5′-*O*-polycarbamoyl compounds **4-4c,d**, **4-5c,d**, and **4-6c,d** and gave these products in good to excellent yields (70–95%).



Scheme 2. Synthesis of **4-4b–d**, **4-5b–d**, and **4-6b–d**.
Reagents: (a) $Cl_3CCON{=}C{=}O$, CH_2Cl_2; (b) K_2CO_3, MeOH; (c) SiOH, CH_2Cl_2/MeOH.

To further extend the scope of this method, we prepared 5′-*O*-carbamoyl derivatives of the blockbuster HIV drug Zidovudine (also known as AZT). The analogues consisted of AZT mono-, di-, and triphosphates (**4-7b**–**c**), and their synthesis is shown in Scheme 3. Yields for

these reactions were good to excellent (61-94%), illustrating that this method is applicable to biologically relevant compounds currently used in the clinic.



Scheme 3. Synthesis of **4-7b–d**.
Reagents: (a) $Cl_3CCON{=}C{=}O$, CH_2Cl_2; (b) K_2CO_3, MeOH; (c) SiOH, CH_2Cl_2/MeOH.

4.3. Biology

A majority of the 2′,3′-bis-*O*-TBS protected mono-, di-, and triphosphate derivatives (**4-2b**,**c** – **4-6b**,**c**) are currently being screened for anticancer and antiviral activity. AZT analogues **4-7b**–**d** are also being screened for antiviral activity. Results of these studies will be reported in due course.

4.4. Discussion

We have developed an efficient method for 5′-*O*-carbamoyl and 5′-*N*-carbamoyl analogues of nucleoside mono-, di-, and triphosphates. These uncharged nucleotide surrogates may possess unusual properties relative to their parent nucleotides. A significant number of medicinally useful 2′,3′-dideoxy (and/or otherwise modified) nucleosides exist, a majority of which must be phosphorylated up to at least the monophosphate in order to exert their effects in vivo.[41-53] The

uncharged *O*-carbamoyl or *N*-carbamoyl phosphoester replacements reported here may lead to enhanced hydrolytic stability, permeability, and/or bioavailability of such medicinally important nucleoside derivatives.

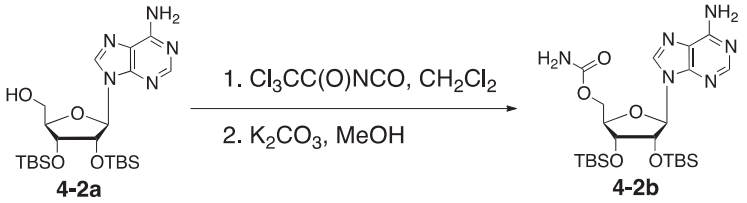
4.5. Experimentals

4.5.1. Chemistry

4.5.1.1. General Experimental

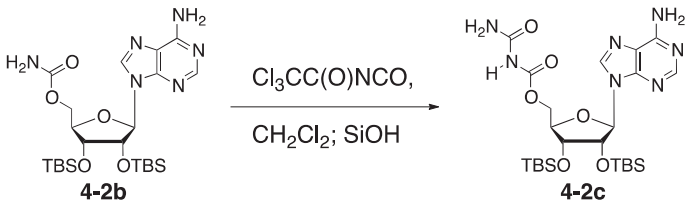
Moisture sensitive reactions were performed in flame-dried flasks under an inert atmosphere (N_2 or Ar) at ambient temperature unless otherwise indicated. Solvents (CH_2Cl_2, pyridine, EtOAc, DMF, Et_3N) were dried by passing through columns of activated alumina under Ar. TLC was performed using Merck Kieselgel 60 F254 sheets, and flash chromatography was performed with silica gel (230–400 mesh) and reagent grade solvents. ^{1}H NMR and ^{13}C NMR spectra were determined using internal references at δ 7.27 ($CDCl_3$), and δ 77.2 ($CDCl_3$), respectively. High resolution mass spectra were obtained using fast atom bombardment electrospray (ES) ionization techniques. Commercially available reagents were used as supplied.

4.5.1.2. Compound Characterization Data



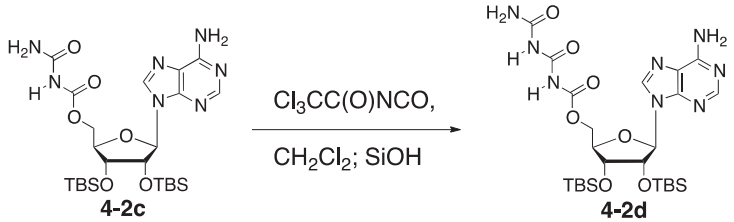
5′-*O*-Aminocarbonyl-2′,3′-bis-*O*-*tert*-butyldimethylsilyladenosine (4-2b).

To a solution of **4-2a** (66 mg, 0.13 mmol) and CH_2Cl_2 (1.4 mL) was added drop-wise trichloroacetyl isocynate (38 mg, 0.020 mmol), and the reaction was stirred at room temperature (30 min). Volatiles were removed under reduced pressure, and the flask was then purged with argon. The crude mixture was dissolved in MeOH (2.8 mL), charged with potassium carbonate (101 mg, 0.73 mmol), and stirred at room temperature (2 h). The reaction was diluted in CH_2Cl_2 (2.8 mL) and filtered via a silica gel/Frit-filter plug under vacuum. Most of the volatiles were removed under reduced pressure making sure not to remove all the solvent. The crude mixture was added directly to a flash chromatography column and eluted with 3% MeOH/CH_2Cl_2 to give **4-2b** (53 mg, 0.098 mmol, 74%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.35 (s, 1H), 7.99 (s, 1H), 5.89 (d, J = 4.2 Hz, 1H), 5.64 (bs, 2H), 4.95 (t, J = 4.2 Hz, 1H), 4.77 (bs, 3H), 4.52 (dd, J = 11.6, 3.5 Hz, 1H), 4.36 (dd, J = 10.8, 4.8 Hz, 1H), 4.33–4.27 (m, 2H), 0.94 (s, 9H), 0.84 (s, 9H), 0.11 (s, 6H), 0.02 (s, 3H), –0.13 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.3, 155.5, 153.1, 149.8, 140.4, 120.8, 90.1, 82.7, 74.6, 72.1, 64.1, 26.0, 25.9, 18.3, 18.1, –4.19, –4.50, –4.70, –4.33; MS (ES) *m/z* 538.2756 (M+ [$C_{23}H_{42}N_6O_5Si_2$]) = 538.2755.



5′-*O*-[(Aminocarbonyl)aminocarbonyl]-2′,3′-bis-*O*-*tert*-butyldimethylsilyladenosine (4-2c).

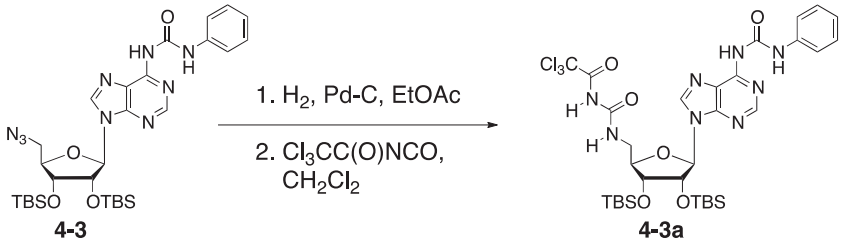
To a solution of **4-2b** (68 mg, 0.13 mmol) and CH_2Cl_2 (2.1 mL) was added drop-wise trichloroacetyl isocyanate (47 mg, 0.25 mmol), and the reaction was stirred at room temperature (1 h). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 2→6% MeOH/CH_2Cl_2 to give **4-2c** (60 mg, 0.10 mmol, 82%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.1 (s, 1H), 9.75 (s, 1H), 8.89 (s, 1H), 8.57 (s, 1H), 6.20 (d, J = 5.0 Hz, 1H), 5.55 (bs, 2H), 4.59–4.54 (m, 2H), 4.35 (t, J = 3.5 Hz, 1H), 4.29–4.25 (m, 2H), 0.95 (s, 9H), 0.80 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H), –0.02 (s, 3H), –0.22 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.0, 156.3, 151.18, 151.16, 150.4, 143.5, 120.8, 88.0, 84.0, 77.4, 72.5, 63.6, 29.9, 26.0, 25.9, –4.3, –4.6, –4.7, –5.0; MS (ES) *m/z* 581.2811 (M+ [$C_{24}H43N_7O_6Si_2$]) = 581.2813.



5′-*O*-[((Aminocarbonyl)aminocarbonyl)aminocarbonyl]-2′,3′-bis-*O*-*tert*-butyldimethylsilyladenosine (4-2d).

To a solution of **4-2c** (60 mg, 0.10 mmol) and CH_2Cl_2 (2.0 mL) was added drop-wise trichloroacetyl isocyanate (60 mg, 0.32 mmol), and the reaction was stirred at room temperature

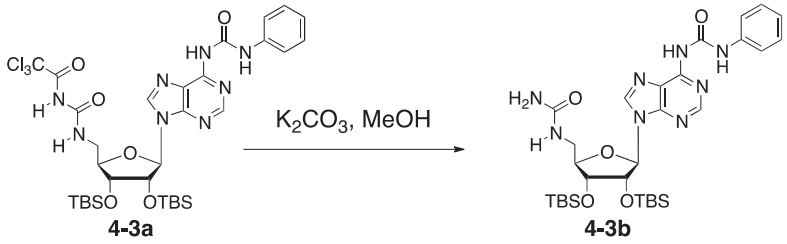
(30 min). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 2→5% MeOH/CH_2Cl_2 to give **4-2d** (51 mg, 0.82 mmol, 79%). ^{1}H NMR (DMSO–d_6, 300 MHz) δ 10.2 (s, 1H), 9.54 (s, 1H), 8.80 (bs, 1H), 8.70 (s, 1H), 7.35 (bs, 1H), 7.21 (bs, 2H), 6.04 (d, J = 6.3 Hz, 1H), 4.85 (dd, J = 6.3, 4.5 Hz, 1H), 4.46–4.34 (m, 3H), 4.19–4.14 (m, 1H), 0.92 (S, 9H0, 0.71 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H), –0.06 (s, 3H), –0.36 (s, 3H); ^{13}C NMR (DMSO–d_6, 75 MHz) δ 154.3, 154.0, 153.5, 151.2, 150.5, 150.4, 142.4, 120.2, 86.8, 82.8, 74.2, 72.0, 64.0, 25.7, 25.5, 17.7, 17.46, 17.45, –4.75, –4.82, –5.0, –5.5; MS (ES) *m/z* 624.2876 (M+ [$C_{25}H_{44}N_8O_7Si_2$]) = 624.2871.



2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-*N*^{6}-(*N*-phenylcarbamoyl)-5′-[((trichloroacetyl)aminocarbonyl)amino]adenosine (4-3a).

A solution of **4-3** (40 mg, 0.063 mmol) and 10% Pd–C (40 mg) in EtOAc (40 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dried under vacuum pump for two days and then dissolved in CH_2Cl_2 (0.5 mL) and trichloroacetyl isocyanate (15 mg, 0.08 mmol) was then added. The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a flash chromatography column and eluted with 1→4% MeOH/CH_2Cl_2 to give **4-3a** (34 mg, 0.042 mmol, 67%, over two steps). ^{1}H NMR ($CDCl_3$, 500

MHz) δ 11.80 (s, 1H), 8.74 (bs, 1H), 8.67 (s, 1H), 8.59 (bs, 1H), 8.25 (bs, 1H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.38 (t, *J* = 7.8 Hz, 2H), 7.14 (t, *J* = 7.3 Hz, 1H), 5.93 (d, *J* = 5.5 Hz, 1H), 5.01 (t, *J* = 4.8 Hz, 1H), 4.39 (t, *J* = 3.3 Hz, 1H), 4.29 (d, *J* = 3.0 Hz, 1H), 3.82 (t, *J* = 5.5 Hz, 2H), 0.97 (s, 9H), 0.80 (s, 9H), 0.16 (s, 3H), 0.15 (s, 3H), – 0.05 (s, 3H), –0.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.7, 151.8, 151.5, 151.2, 150.5, 150.4, 143.4, 138.2, 129.2, 124.2, 121.9, 120.6, 91.0, 84.1, 74.4, 73.3, 42.3, 26.0, 25.9, 18.3, 18.1, – 4.2, – 4.5, – 4.5, – 5.0; MS (ES) *m/z* 800.2220 (M+ [$C_{32}H_{47}Cl_3N_8O_6Si_2$]) = 800.2223.

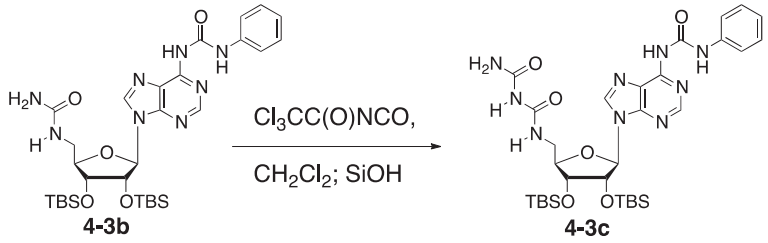


4-3a **4-3b**

5′-[(Aminocarbonyl)amino]-2′,3′-bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-N^6-(*N*-phenylcarbamoyl)adenosine (4-3b).

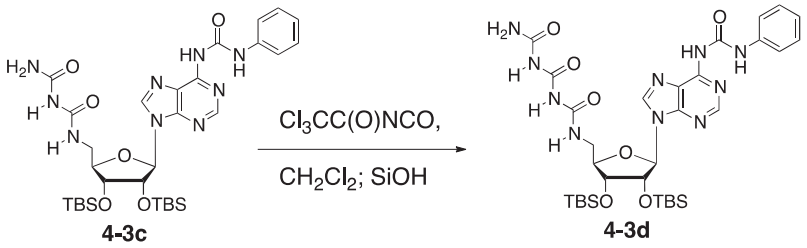
A solution of **4-3a** (30 mg, 0.037 mmol), potassium carbonate (28 mg, 0.20 mmol), and MeOH (3.7 mL) was stirred at room temperature (75 min). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 3→6% MeOH/CH_2Cl_2 to give **4-3b** (20 mg, 0.030 mmol, 81%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.9 (s, 1H), 9.26 (s, 1H), 8.65 (s, 1H), 8.62 (s, 1H), 7.57 (d, J = 7.5 Hz, 2H), 7.39 (t, J = 7.2 Hz, 2H), 7.18 (t, J = 6.9 Hz, 1H), 6.01 (d, J = 7.5 Hz, 1H), 4.72 (dd, J = 7.7, 4.7 Hz, 1H), 4.65 (bs, 2H), 4.32 (d, J = 4.5 Hz, 1H), 4.20 ("t", J approx. 4.5 Hz, 1H), 3.92 (ddd, J = 14.7, 8.4, 3.3 Hz, 1H), 3.26 (dt, J = 14.5, 3.4 Hz, 1H), 0.96 (s, 9H), 0.70 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H), –0.10 (s, 3H), –0.49 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.0, 152.4, 150.61, 150.59,

144.3, 137.6, 129.4, 124.9, 121.8, 121.4, 89.2, 87.8, 77.4, 75.2, 73.7, 41.7, 26.1, 25.8, 18.3, 18.0, –4.33, –4.39, –4.5, –5.4; MS (ES) *m/z* 656.3284 (M+ [$C_{30}H_{48}N_8O_5Si_2$]) = 656.3286.



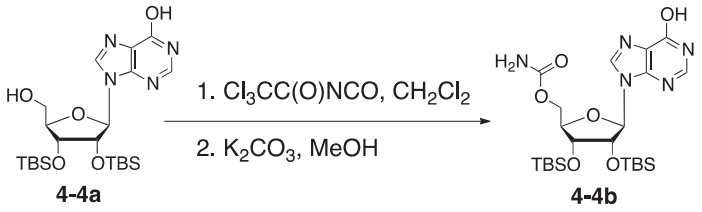
5′-[((Aminocarbonyl)aminocarbonyl)amino]-2′,3′-bis-*O-tert*-butyldimethylsilyl-5′-deoxy-N^6-(*N*-phenylcarbamoyl)adenosine (4-3c).

To a solution of **4-3b** (66 mg, 0.10 mmol) and CH_2Cl_2 (1.5 mL) was added drop-wise trichloroacetyl isocyanate (38 mg, 0.20 mmol), and the reaction was stirred at room temperature (4 h). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 2→5% MeOH/CH_2Cl_2 to give **4-3c** (50 mg, 0.071 mmol, 71%). ^{1}H NMR (acetone-d_6, 500 MHz) δ 12.1 (d, J = 5.0 Hz, 1H), 9.11 (bs, 1H), 8.80 (s, 1H), 8.76 (s, 1H), 8.38 (bs, 1H), 8.10 (bs, 1H), 7.75 (d, J = 7.5 Hz, 2H), 7.37 (t, J = 7.8 Hz, 2H), 7.10 (t, J = 7.5 Hz, 1H), 6.19 (d, J = 7.0 Hz, 1H), 5.05 (dd, J = 7.0 Hz, 1H), 4.54 (dd, J = 4.0, 1.0 Hz, 1H), 4.23 ("t", J = 5.3 Hz, 1H), 3.82–3.77 (m, 1H), 3.68 (dt, J = 14.0, 6.0 Hz, 1H), 2.84 (s, 1H), 2.81 (s, 1H), 0.91 (s, 9H), 0.75 (s, 9H), 0.20 (s, 3H), 0.18 (s, 3H), –0.03 (s, 3H), –0.35 (s, 3H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 157.14, 157.10, 157.07, 156.73, 156.66, 152.6, 152.5, 151.7, 151.6, 144.8, 144.4, 140.2, 130.2, 124.7, 122.0, 121.1, 88.7, 87.1, 76.6, 74.8, 56.4, 42.5, 42.4, 26.8, 26.6, 19.2, 18.9, –3.8, –3.9, –4.7; MS (ES) *m/z* 699.3348 (M+ [$C_{31}H_{49}N_9O_6Si_2$]) = 699.3344.



5′-[(((Aminocarbonyl)aminocarbonyl)aminocarbonyl)amino]-2′,3′-bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-N^6-(*N*-phenylcarbamoyl)adenosine (4-3d).

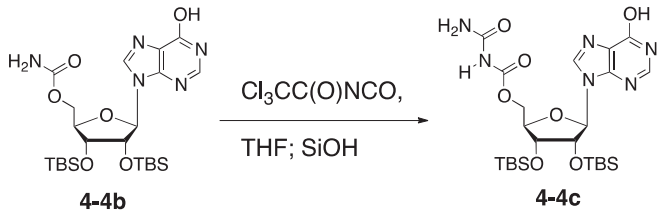
To a solution of **4-3c** (14 mg, 0.020 mmol) and CH_2Cl_2 (0.3 mL) was added drop-wise trichloroacetyl isocyanate (11 mg, 0.058 mmol), and the reaction was stirred at room temperature (1 h). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 2→6% MeOH/CH_2Cl_2 to give **4-3d** (9 mg, 0.012 mmol, 61%). ^{1}H NMR (DMSO–d_6, 500 MHz) δ 11.8 (s, 1H), 10.2 (s, 1H), 10.0 (bs, 1H), 9.55 (bs, 1H), 8.78 (s, 1H), 8.71 (s, 1H), 8.00 (bs, 1H), 7.63 (d, J = 8.0 Hz, 2H), 7.36 (t, J = 7.8 Hz, 2H), 7.27 (bs, 1H), 7.10 (t, J = 7.5 Hz, 1H), 6.75 (bs, 1H), 6.01 (d, J = 7.5 Hz, 1H), 6.01 (d, J = 7.5 Hz, 1H), 5.00 (dd, J = 7.3, 4.3 Hz, 1H), 4.39 (d, J = 4.0 Hz, 1H), 4.06 (t, J = 6.5 Hz, 1H), 3.70 (dt, J = 14.0, 6.8 Hz, 1H), 3.50 (dt, J = 14.0, 6.5 Hz, 1H), 0.92 (s, 9H), 0.68 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H), –0.10 (s, 3H), – 0.47 (s, 3H); ^{13}C NMR (DMSO–d_6, 125 MHz) δ 154.3, 153.2, 152.6, 150.9, 150.8, 150.6, 150.1, 143.3, 138.4, 129.0, 123.3, 120.8, 119.4, 87.1, 84.7, 73.6, 73.0, 41.2, 25.7, 25.4, 17.8, 17.4, –4.7, –4.8, –5.0, –5.8; MS (ES) *m/z* 742.3404 (M+ [$C_{32}H_{50}N_{10}O_7Si_2$]) = 742.3402.



4-4a **4-4b**

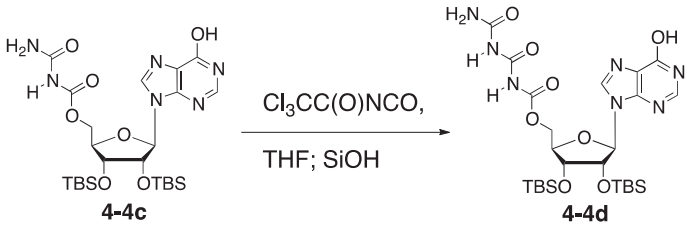
5′-*O*-Aminocarbonyl-2′,3′-bis-*O*-*tert*-butyldimethylsilylinosine (4-4b).

To a solution of **4-4a** (69 mg, 0.14 mmol) and CH_2Cl_2 (1.4 mL) was added drop-wise trichloroacetyl isocyanate (36 mg, 0.19 mmol), and the reaction was stirred at room temperature (30 min). Volatiles were removed under reduced pressure, and the flask was then purged with argon. The crude mixture was dissolved in MeOH (2.8 mL), charged with potassium carbonate (100 mg, 0.72 mmol), and stirred at room temperature (90 min). The reaction was diluted in CH_2Cl_2 (2.8 mL) and filtered via a silica gel/Frit-filter plug under vacuum. Most of the volatiles were removed under reduced pressure making sure not to remove all the solvent. The crude mixture was added directly to a flash chromatography column and eluted with 5→8% MeOH/CH_2Cl_2 to give **4-4b** (25 mg, 0.046 mmol, 33%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.23 (s, 1H), 8.16 (s, 1H), 5.94 (d, J = 4.0 Hz, 1H), 5.24 (bs, 2H), 4.69 (bs, 1H), 4.47 (d, J = 11.0 Hz, 1H), 4.37 (dd, J = 12.0, 3.5 Hz, 1H), 4.31–4.27 (m, 2H), 0.93 (s, 9H), 0.86 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H), 0.03 (s, 3H), –0.08 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.0, 156.7, 148.6, 145.2, 139.5, 125.7, 89.8, 82.8, 75.7, 71.8, 63.5, 26.0, 25.9, 18.3, 18.1, –4.2, –4.5, –4.7, –4.8; MS (ES) *m/z* 539.2600 (M+ [$C_{23}H_{41}N_5O_6Si_2$]) = 539.2595.



5′-*O*-[(Aminocarbonyl)aminocarbonyl]-2′,3′-bis-*O*-*tert*-butyldimethylsilylinosine (4-4c).

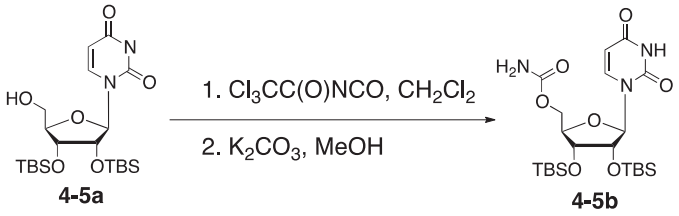
To a solution of **4-4b** (24 mg, 0.044 mmol) and THF (1.0 mL) was added drop-wise trichloroacetyl isocyanate (17 mg, 0.090 mmol), and the reaction was stirred at room temperature (2 h). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 3→9% MeOH/CH_2Cl_2 to give **4-4c** (18 mg, 0.031 mmol, 69%). ^{1}H NMR (DMSO–d_6, 500 MHz) δ 10.2 (s, 1H), 8.40 (s, 1H), 8.10 (s, 1H), 7.21 (s, 2H), 5.92 (d, J = 7.0 Hz, 1H), 4.70 (dd, J = 3.9, 2.7 Hz, 1H), 4.36–4.33 (, 3H), 4.17–4.13 (m, 1H), 0.91 (s, 9H), 0.72 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H), –0.06, –0.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.6, 154.3, 153.5, 148.5, 146.1, 139.0, 124.3, 86.3, 83.1, 74.6, 72.3, 72.0, 69.81, 69.78, 64.1, 60.2, 25.7, 25.4, 17.7, 17.5, –4.77, –4.83, –4.9, –5.6; MS (ES) *m/z* 582.2659 (M+ [$C_{24}H_{42}N_6O_7Si_2$]) = 582.2654.



5′-*O*-[((Aminocarbonyl)aminocarbonyl)aminocarbonyl]-2′,3′-bis-*O*-*tert*-butyldimethylsilylinosine (4-4d).

To a solution of **4-4c** (11 mg, 0.019 mmol) and THF (10.7 mL) was added drop-wise trichloroacetyl isocyanate (10 mg, 0.053 mmol), and the reaction was stirred at room temperature

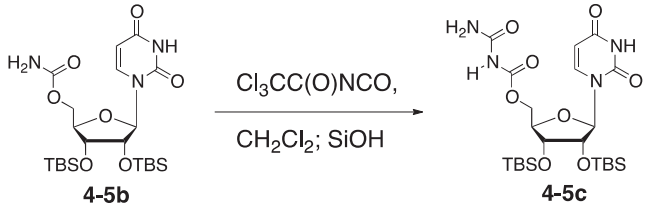
(2 h). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 4→8% MeOH/CH_2Cl_2 to give **4-4d** (9 mg, 0.014 mmol, 76%). ^{1}H NMR (DMSO–d_6, 500 MHz) δ 12.4 (s, 1H), 11.2 (s, 1.54H), 10.9 (s, 0.46H), 10.2 (s, 0.36H), 9.43 (s, 0.56H), 8.39 (s, 0.45H), 8.37 (s, 0.55H), 8.07 (s, 1H), 7.38 (s, 0.53H), 7.19 (s, 1.24 H), 5.91 (d, J = 4.2 Hz, 1H), 4.75 (dd, J = 6.5, 4.5 Hz, 0.5H), 4.68 (dd, J = 6.5, 4.5 Hz, 0.5H), 4.43 (dd, J = 12.3, 4.3 Hz, 0.5H), 4.40 (dd, J = 12.0, 5.5 Hz, 0.5 H), 4.35–4.32 (m, 2H), 4.16–4.11 (m, 1H), 0.9 (s, 9H), 0.70 (s, 9H), 0.114, 0.108, 0.094, 0.090, –0.02, –0.07, –0.34, –0.36 (8 X s, 12H); ^{13}C NMR (DMSO–d_6, 125 MHz) δ 156.5, 154.3, 153.5, 153.3, 152.9, 151.0, 149.9, 148.44, 148.39, 146.04, 145.99, 139.10, 138.98, 124.40, 124.26, 86.5, 86.3, 83.0, 82.9, 74.6, 74.5, 72.1, 72.0, 65.1, 64.0, 25.7, 25.4, 17.68, 17.67, 17.43, –4.77, –4.79, –4.85, –4.94, –4.95, –5.96, –5.64; MS (ES) *m/z* 625.2715 (M+ [$C_{25}H_{43}N_7O_8Si_2$]) = 625.2712.



5′-*O*-Aminocarbonyl-2′,3′-bis-*O*-*tert*-butyldimethylsilyluridine (4-5b).

To a solution of **4-5a** (69 mg, 0.14 mmol) and CH_2Cl_2 (1.4 mL) was added drop-wise trichloroacetyl isocyanate (50 mg, 0.27 mmol), and the reaction was stirred at room temperature (30 min). Volatiles were removed under reduced pressure, and the flask was then purged with argon. The crude mixture was dissolved in MeOH (2.8 mL), charged with potassium carbonate (100 mg, 0.72 mmol), and stirred at room temperature (90 min). The reaction was diluted in CH_2Cl_2 (2.8 mL) and filtered via a silica gel/Frit-filter plug under vacuum. Most of the volatiles

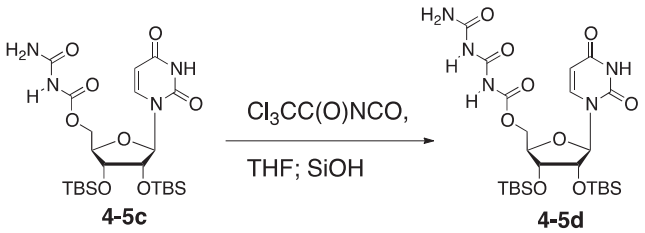
were removed under reduced pressure making sure not to remove all the solvent. The crude mixture was added directly to a flash chromatography column and eluted with 2→5% MeOH/CH_2Cl_2 to give **4-5b** (51 mg, 0.099 mmol, 71%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.68 (s, 1H), 7.61 (d, J = 8.4 Hz, 1H), 5.77 (d, J = 8.1 Hz, 1H), 5.68 (d, J = 3.0 Hz, 1H), 4.81 (bs, 2H), 4.43 (ddd, J = 13.8, 4.8, 2.4 Hz, 1H), 4.32 (d, J = 4.5 Hz, 1H), 4.29–4.24 (m, 2H), 4.01 (dd, J = 5.9, 4.4 Hz, 1H), 0.94 (s, 9H), 0.93 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H), 0.10 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.1, 156.1, 150.2, 140.3, 102.2, 91.2, 81.8, 75.3, 71.2, 63.8, 26.00, 25.97, 18.25, 18.21, –4.1, –4.3, –4.6, –4.8; MS (ES) *m/z* 515.2481 (M+ [$C_{22}H_{41}N_3O_7Si_2$]) = 515.2483.



5′-*O*-[(Aminocarbonyl)aminocarbonyl]-2′,3′-bis-*O*-*tert*-butyldimethylsilyluridine (4-5c).

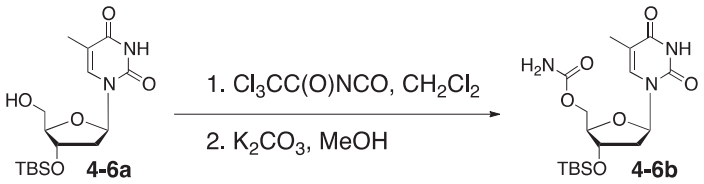
To a solution of **4-5b** (50 mg, 0.097 mmol) and CH_2Cl_2 (2.0 mL) was added drop-wise trichloroacetyl isocyanate (28 mg, 0.15 mmol), and the reaction was stirred at room temperature (18 h). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 3→6% MeOH/CH_2Cl_2 to give **4-5c** (52 mg, 0.093 mmol, 96%). %). ^{1}H NMR (DMSO–d_6, 300 MHz) δ 11.4 (d, J = 1.5Hz, 1H), 10.2 (s, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.20 (s, 2H), 5.84 (d, J = 6.0 Hz, 1H), 5.68 (dd, J = 8.1, 1.8 Hz, 1H), 4.44–4.24 (m, 3H), 4.13–4.04 (m, 2H), 0.88 (s, 9H), 0.83 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H), 0.04 (s, 3H), –0.02 (s, 3H); ^{13}C NMR (DMSO–d_6, 75 MHz) δ 162.9, 154.2, 153.5, 150.7, 140.7,

102.3, 87.0, 82.2, 73.4, 71.4, 64.0, 25.7, 25.6, 17.7, 17.6, –4.7, –4.8, –5.0; MS (ES) *m/z* 558.2544 (M+ [$C_{23}H_{42}N_4O_8Si_2$]) = 558.2541.



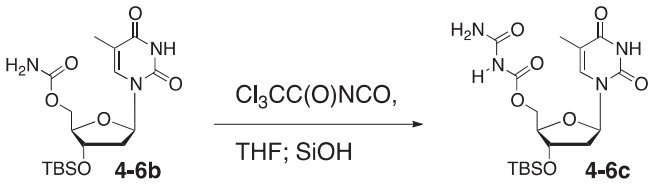
5′-*O*-[((Aminocarbonyl)aminocarbonyl)aminocarbonyl]-2′,3′-bis-*O*-*tert*-butyldimethylsilyluridine (4-5d).

To a solution of **4-5c** (69 mg, 0.12 mmol) and THF (2.5 mL) was added drop-wise trichloroacetyl isocyanate (48 mg, 0.25 mmol), and the reaction was stirred at room temperature (4 h). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 4→6% MeOH/CH_2Cl_2 to give **4-5d** (66 mg, 0.11 mmol, 89%). ^{1}H NMR (DMSO–d_6, 300 MHz) δ 11.4 (d, J = 3.0 Hz, 1H), 10.9 (s, 1H), 9.44 (s, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.39 (bs, 1H), 7.15 (bs, 1H), 5.83 (d, J = 6.0 Ha, 1H), 5.69 (dd, J = 8.1, 2.1 Hz, 1H), 4.39–4.32 (m, 3H), 4.15–4.07 (m, 2H), 0.88 (s, 9H), 0.09 (s, 3H),).08 (s, 3H), 0.04 (s, 3H), –0.02 (s, 3H); ^{13}C NMR (DMSO–d_6, 75 MHz) δ 162.9, 153.5, 152.7, 150.9, 150.7, 140.7, 102.3, 87.3, 82.0, 73.4, 71.4, 65.0, 25.7, 25.6, 17.7, 17.6, –4.7, –4.8, –5.0; MS (ES) *m/z* 601.2597 (M+ [$C_{24}H_{43}N_5O_9Si_2$]) = 601.2599.



5′-*O*-Aminocarbonyl-3′-*O*-*tert*-butyldimethylsilylthymidine (4-6b).

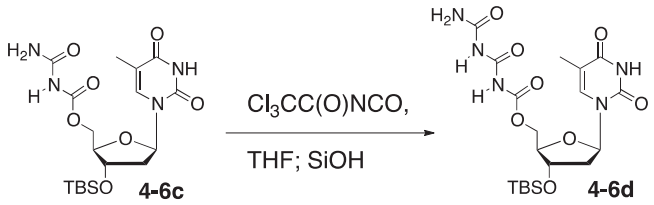
To a solution of **4-6a** (69 mg, 0.19 mmol) and CH_2Cl_2 (1.4 mL) was added drop-wise trichloroacetyl isocyanate (73 mg, 0.39 mmol), and the reaction was stirred at room temperature (30 min). Volatiles were removed under reduced pressure, and the flask was then purged with argon. The crude mixture was dissolved in MeOH (2.8 mL), charged with potassium carbonate (100 mg, 0.72 mmol), and stirred at room temperature (90 min). The reaction was diluted in CH_2Cl_2 (2.8 mL) and filtered via a silica gel/Frit-filter plug under vacuum. Most of the volatiles were removed under reduced pressure making sure not to remove all the solvent. The crude mixture was added directly to a flash chromatography column and eluted with 3→8% MeOH/CH_2Cl_2 to give **4-6b** (45 mg, 0.11 mmol, 58%). ^{1}H NMR (DMSO–d_6, 300 MHz) δ 11.3 (s, 1H), 7.44 (d, 0.9Hz, 1H), 6.71 (bs, 2H), 6.18 (dd, J = 8.1, 6.0 Hz, 1H), 4.38 ("t", J = 2.9 Hz, 1H), 4.15 (dd, J = 15.6, 12.0 Hz, 1H), 3.99 (dd, J = 11.9, 5.3 Hz, 1H), 3.91–3.88 (m, 1H), 2.29 (ddd, J = 13.4, 7.9, 5.8 Hz, 1H), 2.04 (ddd, J = 13.5, 6.0, 2.7 Hz, 1H), 1.79 (s, 3H), 0.87 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (DMSO–d_6, 75 MHz) δ 163.7, 156.4, 150.5, 135.9, 109.8, 84.5, 83.8, 72.5, 63.3, 25.7, 17.7, 12.2, –4.86, –4.92; MS (ES) *m/z* 399.1823 (M+ [$C_{17}H_{29}N_3O_6Si$]) = 399.1826.



5′-*O*-[(Aminocarbonyl)aminocarbonyl]-3′-*O*-*tert*-butyldimethylsilylthymidine (4-6c).

To a solution of **4-6b** (50 mg, 0.13 mmol) and THF (1.3 mL) was added drop-wise trichloroacetyl isocyanate (48 mg, 0.25 mmol), and the reaction was stirred at room temperature (2 h). Volatiles were removed under reduced pressure. The crude mixture was added directly to

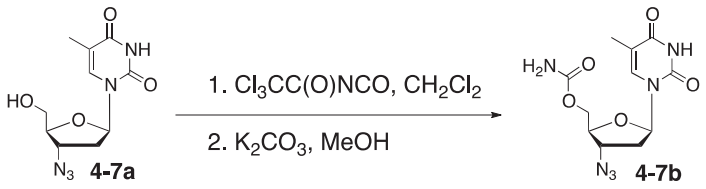
a flash chromatography column and eluted with 3→6% MeOH/CH_2Cl_2 to give **4-6c** (46 mg, 0.10 mmol, 83%). ^{1}H NMR (DMSO–d_6, 300 MHz) δ 11.2 (s, 1H), 10.1 (s, 1H), 7.40 (d, J = 1.2 Hz, 1H), 7.19 (bs, 2H), 6.19 (dd, J = 8.1, 6.3 Hz, 1H), 4.44 ('t", J = 3.0 Hz, 1H), 4.32 (dd, J = 12.2, 3.2 Hz, 1H), 4.15 (dd, J = 12.0, 4.5 Hz, 1H), 3.94 (dd, J = 6.9, 3.3 Hz, 1H), 2.41 (ddd, J = 14.1, 8.0, 6.4 Hz, 1H), 2.03 (ddd, J = 13.2, 6.0, 2.7 Hz, 1H) 1.77 (s, 3H), 0.87 (s, 9H), 0.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.7, 154.2, 153.5, 150.5, 136.0, 109.8, 83.8, 83.7, 72.2, 64.5, 25.7, 17.7, 12.2, –4.88, –4.93; MS (ES) *m/z* 442.1885 (M+ [$C_{18}H_{30}N_4O_7Si$]) = 442.1884.



5′-*O*-[((Aminocarbonyl)aminocarbonyl)aminocarbonyl]-3′-*O*-*tert*-butyldimethylsilylthymidine (4-6d).

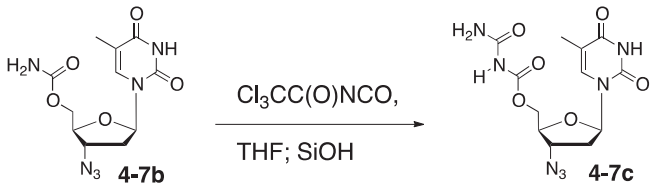
To a solution of **4-6c** (35 mg, 0.079 mmol) and THF (1.0 mL) was added drop-wise trichloroacetyl isocyanate (54 mg, 0.29 mmol), and the reaction was stirred at room temperature (90 min). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 3→7% MeOH/CH_2Cl_2 to give **4-6d** (33 mg, 0.79 mmol, 86%). ^{1}H NMR (DMSO–d_6, 300 MHz) δ 11.3 (s, 1H), 10.9 (s, 1H), 9.45 (s, 1H), 7.43 (d, J = 1.2 Hz, 1H), 7.38 (bs, 1H), 7.25 (bs, 1H), 6.19 (dd, J = 7.8, 6.3 Hz, 1H), 4.44 ("t", J = 3.2 Hz, 1H), 4.36 (dd, J = 11.9, 3.5 Hz, 1H), 3.99–3.94 (m, 1h), 2.48 (ddd, J = 13.8, 7.5, 6.3 Hz, 1H), 2.05 (ddd, J = 13.5, 6.3, 3.0 Hz, 1H), 1.77 (d, J = 0.5 Hz, 3H), 0.87 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (DMSO–d_6, 75 MHz) δ 163.7, 153.2, 153.0, 151.1, 150.5, 136.0, 109.8, 83.8,

83.6, 72.1, 65.4, 25.65, 17.6, 12.1, –4.87, –4.96; MS (ES) *m/z* 485.1938 (M+ [$C_{19}H_{31}N_5O_8Si$]) = 485.1942.



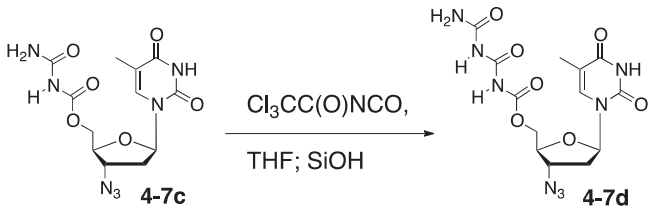
5′-*O*-Aminocarbonyl-3′-azidothymidine (4-7b).

To a solution of **4-7a** (75 mg, 0.28 mmol) and CH_2Cl_2 (2.7 mL) was added drop-wise trichloroacetyl isocyanate (105 mg, 0.56 mmol), and the reaction was stirred at room temperature (30 min). Volatiles were removed under reduced pressure, and the flask was then purged with argon. The crude mixture was dissolved in MeOH (5.4 mL), charged with potassium carbonate (155 mg, 1.1 mmol), and stirred at room temperature (60 min). The reaction was diluted in CH_2Cl_2 (5.4 mL) and filtered via a silica gel/Frit-filter plug under vacuum. Most of the volatiles were removed under reduced pressure making sure not to remove all the solvent. The crude mixture was added directly to a flash chromatography column and eluted with 2→3% MeOH/CH_2Cl_2 to give **4-7b** (53 mg, 0.17 mmol, 61%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.37 (bs, 1H), 7.24 (d, J = 1.2 Hz, 1H), 6.12 (t, J = 6.5 Hz, 1H), 4.83 (bs, 2H), 4.44 (dd, J = 12.0, 4.8 Hz, 1H), 4.36 (dd, J = 12.0, 4.8 Hz, 1H), 4.26 (ddd, J = 7.8, 7.5, 5.3 Hz, 1H), 4.10 (dd, J = 9.6, 4.5 Hz, 1H), 2.51 (ddd, J = 14.0, 6.7, 5.3 Hz, 1H), 2.39 (ddd, J = 14.0, 7.7, 6.2 Hz, 1H), 1.97 (d, J = 1.2 Hz, 3H); ^{13}C NMR (DMSO–d_6, 75 MHz) δ 163.7, 156.3, 150.4, 135.9, 109.9, 83.5, 81.2, 63.5, 60.8, 35.6, 12.2; MS (ES) *m/z* 310.1028 (M+ [$C_{11}H_{14}N_6O_5$]) = 310.1026.



5′-*O*-[(Aminocarbonyl)aminocarbonyl]-3′-azidothymidine (4-7c).

To a solution of **4-7b** (40 mg, 0.13 mmol) and THF (1.3 mL) was added drop-wise trichloroacetyl isocyanate (49 mg, 0.26 mmol), and the reaction was stirred at room temperature (30 min). Volatiles were removed under reduced pressure. The crude mixture was added directly to a flash chromatography column and eluted with 3→7% MeOH/CH_2Cl_2 to give **4-7c** (43 mg, 0.12 mmol, 94%). ^{1}H NMR (DMSO–d_6, 500 MHz) δ 11.4 (s, 1H), 10.1 (s, 1H), 7.42 (d, J = 1.0 Hz, 1H), 7.20 (bs, 2H), 6.13 (t, J = 6.3 Hz, 1H), 4.50 (dt, J = 7.3, 3.8 Hz, 1H), 4.34 (dd, J = 12.0, 2.5 Hz, 1H), 4.23 (dd, J = 12.0, 4.5 Hz, 1H), 4.06 (dd, J = 7.0, 4.0 Hz, 1H), 2.59 (dt, J = 14.0, 7.2 Hz, 1H), 2.27 (ddd, J = 13.9, 6.4, 3.9 Hz, 1H), 1.77 (d, J = 0.5 Hz, 3H); ^{13}C NMR (DMSO–d_6, 125 MHz) δ 163.6, 154.1, 153.4, 150.4, 135.8, 109.8, 83.5, 80.7, 64.9, 60.7, 35.3, 12.1; MS (ES) *m/z* 353.1081 (M+ [$C_{12}H_{15}N_7O_6$]) = 353.1084.



5′-*O*-[((Aminocarbonyl)aminocarbonyl)aminocarbonyl]-3′-azidothymidine (4-7d).

To a solution of **4-7c** (37 mg, 0.10 mmol) and THF (1.1 mL) was added drop-wise trichloroacetyl isocyanate (59 mg, 0.31 mmol), and the reaction was stirred at room temperature (90 min). Volatiles were removed under reduced pressure. The crude mixture was added

directly to a flash chromatography column and eluted with 3→7% MeOH/CH_2Cl_2 to give **4-7d** (27 mg, 0.068 mmol, 68%). ^{1}H NMR (DMSO–d_6, 300 MHz) δ 11.4 (s, 1H), 10.9 (bs, 1H), 9.44 (s, 1H), 7.44 (d, J = 0.9 Hz, 1H), 7.36 (bs, 2H), 7.27 (bs, 1H), 6.13 (t, J = 6.9 Hz, 1H), 4.50 (dt, J = 7.7, 4.0 Hz, 1H), 4.39 (dd, J = 12.0, 3.3 Hz, 1H), 4.32 (dd, J = 12.0, 4.8 Hz, 1H), 2.58–2.49 (m, 1H, overlaps with solvent), 2.30 (ddd, J = 14.0, 6.5, 4.4 Hz, 1H), 1.77 (s, 3H); ^{13}C NMR (DMSO–d_6, 75 MHz) δ 163.7, 153.2, 153.1, 151.1, 150.5, 136.0, 109.8, 83.6, 80.6, 65.5, 60.5, 35.4, 12.1; MS (ES) *m/z* 396.1146 (MH^+ [$C_{13}H_{16}N_8O_7$]) = 396.1142.

4.6. References

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Chapter 5: Synthesis, SAR, and Preliminary Biological Evaluation of Some 5′-Analogues

5.1. Introduction

Exploration of the 2′,3′- positions as well as the N^6-position of lead compound **5-1** produced better understanding of the requirements for antiproliferative activity: (1) the 5′-ureas are more conformationally rigid than the 5′-carbamates and have substantially superior anticancer activity, (2) variations can be made at the N^6-position (most of which exert little or no effect on the overall activity, thus indicating a significant degree of tolerance at this position),[1] (3) the 2′,3′-TES derivative (**3-7a**) is approximately as active as the 2′,3′-TBS derivative (**5-1**), (4) the 2′,3′-OHs can be protected as esters and retain biological activity (although inferior to that of 2′,3′-TBS protected derivatives), and (5) the desilylated (2′,3′-OH) analogue of **5-1** binds to some ATP-binding kinases which have been implicated in cancer (see Chapters 1–3). Armed with the synthetic capacity to better explore the 5′-position (Chapter 4), we turned our attention to the design of alkyl, aryl, and mono-, di-, and triphosphate bioisostere derivatives of compound **5-1**. The alkyl- and aryl- carbamoyl derivatives were developed to gain a rough understanding of what could be sterically tolerated at the 5′-position. The mono-, di-, and triphosphate bioisostere analogues were designed to more fully exploit interactions with the catalytic triad[2, 3] and gatekeeper residues of the active site of BMPR1b in hopes to enhance binding (Chapter 3).

5.2. Chemistry

The synthesis of 5′-alkyl and aryl urea and carbamate derivatives is shown in Scheme 1. Reduction of azide **5-2** followed by treatment with excess phenyl isocyanate furnished 5′-*N*-

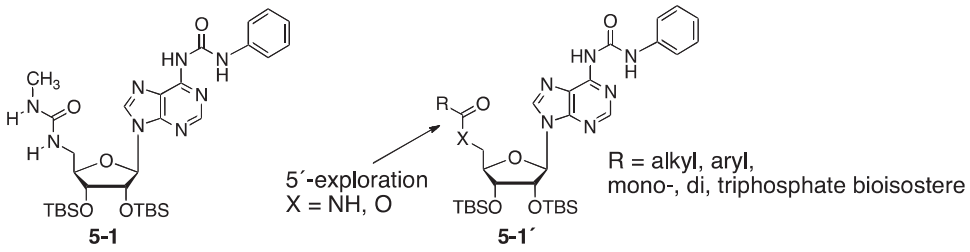
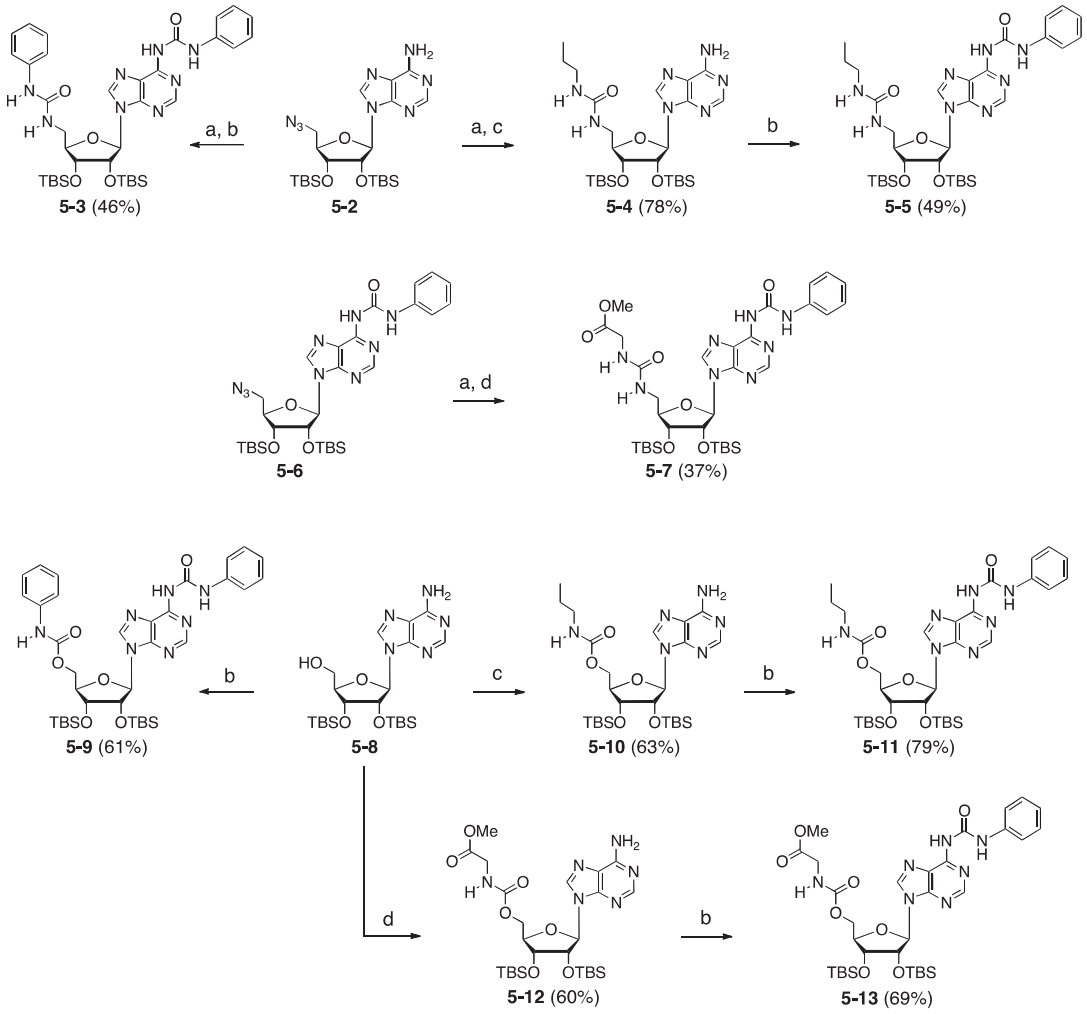


Figure 1. Exploration of the 5´-position of lead compound **5-1**.

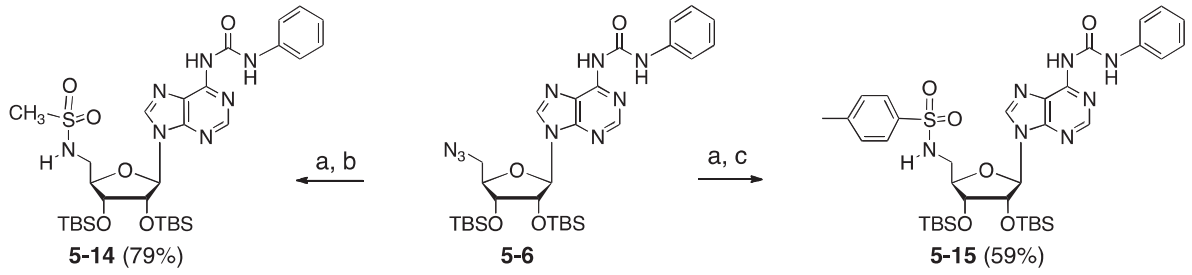


Scheme 1. Synthesis of 5´-urea and 5´-carbamate derivatives.
Reagents: (a) H_2, Pd-C; (b) PhN=C=O; (c) *p*-NO_2-$C_6H_4OC(O)NHCH_2CH_2CH_3$; (d) *p*-$NO_2$-$C_6H_4OC(O)NHCH_2C(O)OCH_3$. See Chapter 2 for preparation of starting materials **5-2**, **5-6**, and **5-8**.

phenyl urea **5-3** in 46% yield. Reduction under the same conditions (H_2, Pd-C, EtOAc) of azides **5-2** and **5-6** followed by treatment with a *p*-nitrophenyl acylating agent[4] gave derivatives **5-4** (78%) and **5-7** (37%), respectively. The modest yield of analogue **5-7** is presumably due to various side reactions of the reactive amine of **5-7** with the *N*-glycine methyl ester *p*-nitrophenylcarbamate substrate. Derivative **5-4** was acylated with phenyl isocyanate producing compound **5-5** (49%).

The carbamate derivatives (**5-9**–**5-13**) were synthesized in a similar fashion as the urea analogues. Diphenyl derivative **5-9** was made in 61% yield; analogues **5-10** and **5-12** were acylated at the 5′-position in good yield (63% and 60%, respectively). Acylation at the N^6-position of **5-10** and **5-12** gave their respective targets **5-11** (79%) and **5-13** (69%).

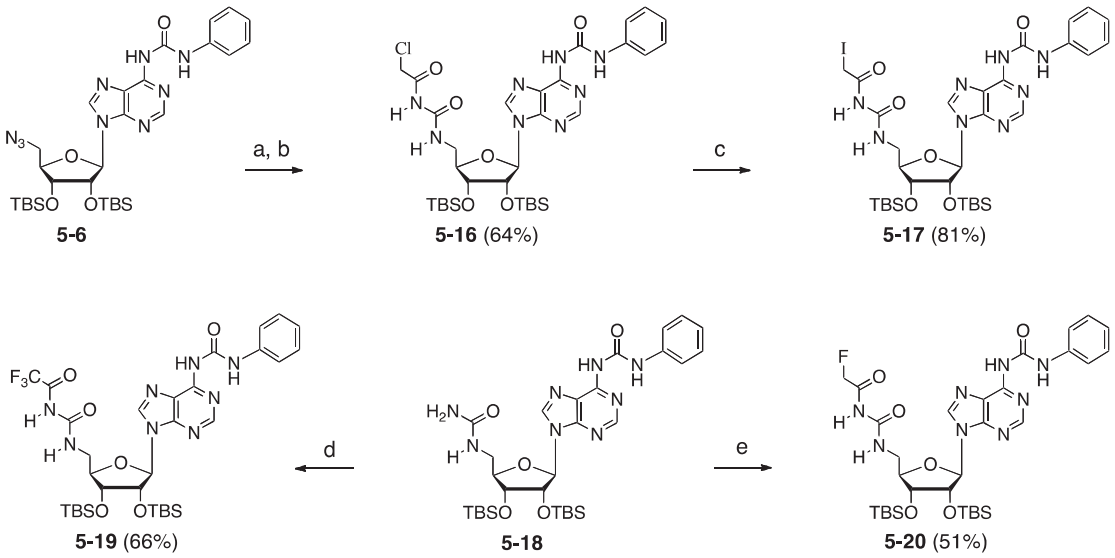
The sulfone moiety has been used as a bioisostere in medicinal chemistry.[5] Sulfonamides **5-14** and **5-15** were designed to be bioisosteres of a monophosphate of lead **5-1** (Scheme 2). The synthesis of both sulfonamides was straightforward: reduction of the 5′-azide followed by treatment with their respective sulfonly chlorides.



Scheme 2. Synthesis of sulfonamides.
Reagents: (a) H_2, Pd-C; (b) CH_3SO_2Cl; (c) $CH_3C_6H_4SO_2Cl$.

Halogenated diphosphate surrogates of lead **5-1** were conceived as possible probes for the catalytic triad region of the active site of BMPR 1b (Scheme 3). Chlorine and iodine atoms can

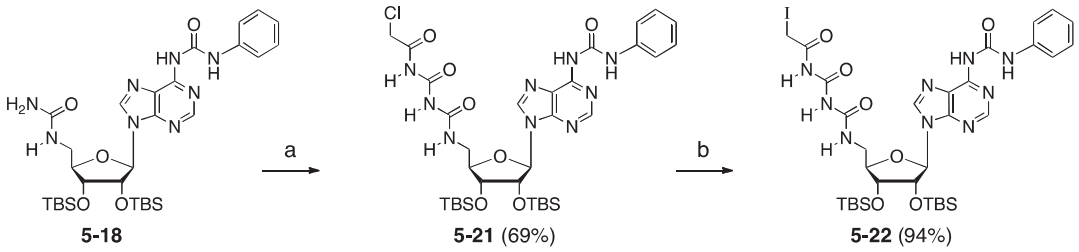
lead to increased binding affinity between the substrate and its enzyme binding pocket by halogen bonds and/or multipolar interactions.[6] In addition, fluorines have a prominant role in the field of medicinal chemistry. Incorporation of this atom (particularly the CF_3 moiety) into small molecule drugs often increases efficacy by enhancing cellular membrane permeability, promoting electrostatic interactions with targets, and decreasing oxidative metabolism of the drug.[7-10]



Scheme 3. Synthesis of halogenated diphosphate bioisosteres.
Reagents: (a) H_2, Pd-C; (b) $ClCH_2C(O)N{=}C{=}O$; (c) NaI, Acetone; (d) $(F_3CC(O))_2O$; (e) $FCH_2C(O)Cl$.
See Chapter 4 for preparation of staring material **5-18**.

Reduction of azide **5-6** followed by treatment with chloroacetyl isocyanate afforded **5-16**. The Finkelstein[11-12] reaction subsequently produced iodide **5-17** in a very good yield (81%). Trifluoro derivative **5-19** and fluoro derivative **5-20** were each prepared in one step from starting material **5-18** by treatment with trifluoroacetic anhydride and fluoroacetate, respectively. A procedure analogous to that reported by Wang[13] was used to form trifluoro compound **5-19**.

Halogenated triphosphate analogues were prepared (Scheme 4) for the same basic reasons as the halogenated diphosphate surrogates. Chloro **5-21** was prepared by treating starting material **5-18** with chloroacetyl isocyanate (69% yield). The Finkelstein reaction gave derivative **5-22** in an excellent yield of 94%.



Scheme 4. Synthesis of halogenated triphosphate bioisosteres.
Reagents: (a) $ClCH_2C(O)N=C=O$; (b) NaI, Acetone.

5.3. Biology

5.3.1. Antiproliferative Assays

Compounds **5-3**, **5-5**, **5-7**, **5-9**, **5-11**, **5-13**, **5-14**–**5-18**, **5-21**, and **5-22** were tested for their antiproliferative activity using murine leukemia L1210, human lymphoblastic leukemia CEM, and human cervix carcinoma HeLa (Table 1). Compounds **5-23** and **5-24** (Figure 2; see Chapter 4 for preparation) were tested and the results of these assays are also shown Table 1.

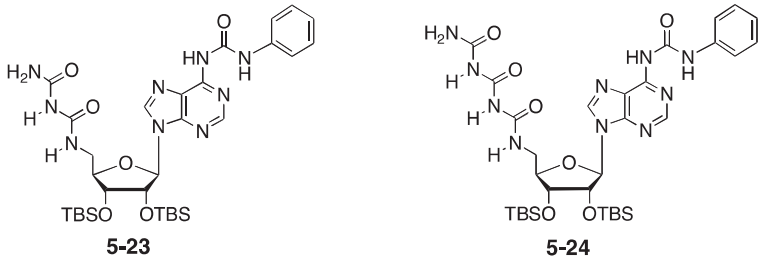


Figure 2. Di- and triphosphate bioisostere derivatives.

Table 1. Inhibitory effects of test compounds.
Inhibitory effects of the test compounds on the proliferation of murine leukemia cells (L1210), human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa). IC_{50} (μg/ml): 50% Inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

Compound	L1210	CEM	HeLa
5-1	3.8 ± 0.3	8.3 ± 2.9	3.2 ± 0.2
5-3	127 ± 61	>200	25 ± 7
5-5	56 ± 30	>200	72 ± 54
5-7	4.1 ± 0.4	11 ± 6	3.0 ± 0.4
5-9	>200	>200	>200
5-11	106 ± 15	>200	>200
5-13	19 ± 3	125 ± 37	158 ± 60
5-14	10 ± 1	77 ± 32	39 ± 1
5-15	>100	>100	>100
5-16	0.82 ± 0.48	0.46 ± 0.10	1.6 ± 0.0
5-17	6.8 ± 0.1	0.28 ± 0.07	10 ± 1
5-18	6.7 ± 0.5	7.7 ± 0.7	7.8 ± 1.2
5-21	1.9 ± 0.2	1.8 ± 0.2	8.9 ± 1.7
5-22	7.9 ± 0.6	1.3 ± 0.6	8.4 ± 1.1
5-23	5.9 ± 0.5	6.9 ± 0.1	7.5 ± 0.4
5-24	7.6 ± 0.4	8.3 ± 1.2	5.1 ± 3.9

The data show that 5′-*N*-phenyl urea **5-3** and 5′-*N*-propyl urea **5-5** are substantially inferior in activity to lead compound **5-1**. However, it is interesting to note that 5′-*N*-glycine methyl ester urea **5-7** was similar in activity to the lead. Carbamates **5-9**, **5-11**, and **5-13** were significantly inferior in anticancer activity, as had been observed with other members in the 5′-carbamate series. The sulfonamide mono-phosphate mimics **5-14** and **5-15** also showed weak biological activity, with **5-15** having essentially no activity at concentrations <100 μM. Chloro derivative **5-16** was the most active derivative tested, exhibiting IC_{50} values in the sub-μg/mL

regime. Derivatives **5-17**, **5-21**, and **5-22** exhibited significant anticancer activity only slightly higher than the lead. Mono-, di-, and triphosphate surrogates (**5-18**, **5-23**, and **5-24**, respectively) also showed significant antiproliferative activity, although IC_{50} values for these compounds were approximately two times higher than for compound **3-1**.

Compounds **5-16**, **5-21**, and **5-22** as well as fluoro derivatives **5-19** and **5-20** were submitted to the National Cancer Institute Developmental Therapeutics Program. Results from the multi-dose growth inhibition assays are shown in Tables 2–4 for compounds **5-16**, **5-21**, and **5-22**, respectively. Results from the single-dose growth inhibition assays of derivatives **5-19** and **5-20** are shown in their respective Tables 5 and 6.

Derivative **5-16** had an overall GI_{50} of 0.58 μM and LC_{50}'s in the range of 1-10 μM for many of the cell lines: six non-small cell lung, five colon, three CNS, eight melanoma, three ovarian, six renal, one prostate, and five breast cancers. The LC_{50}'s reached sub-μM concentrations for one cell line of non-small cell lung, melanoma, ovarian, and renal cancers.

Derivative **5-21** had an overall GI_{50} of 3.2 μM and LC_{50}'s in the range of 3-4 μM for cell lines of leukemia (2), non-small cell lung (1), ovarian (1), and renal cancers (1). The LC_{50}'s reached sub-μM concentrations for a non-small cell lung and melanoma cancer cell line.

Compound **5-22** had an overall GI_{50} of 0.95 μM and LC_{50}'s in the range of 3-4 μM for cancer cell lines of leukemia (1), non-small cell lung (2), colon (1), CNS (3), melanoma (1), ovarian (1), renal cancers (1), prostate (1), and breast (2). The LC_{50}'s reached concentrations of sub-μM for two leukemia, one ovarian, and two renal cancer cell lines. Impressively, derivative **5-22** inhibited a non-small cell lung adenocarcenoma cell line at a concentration of 10 nM.

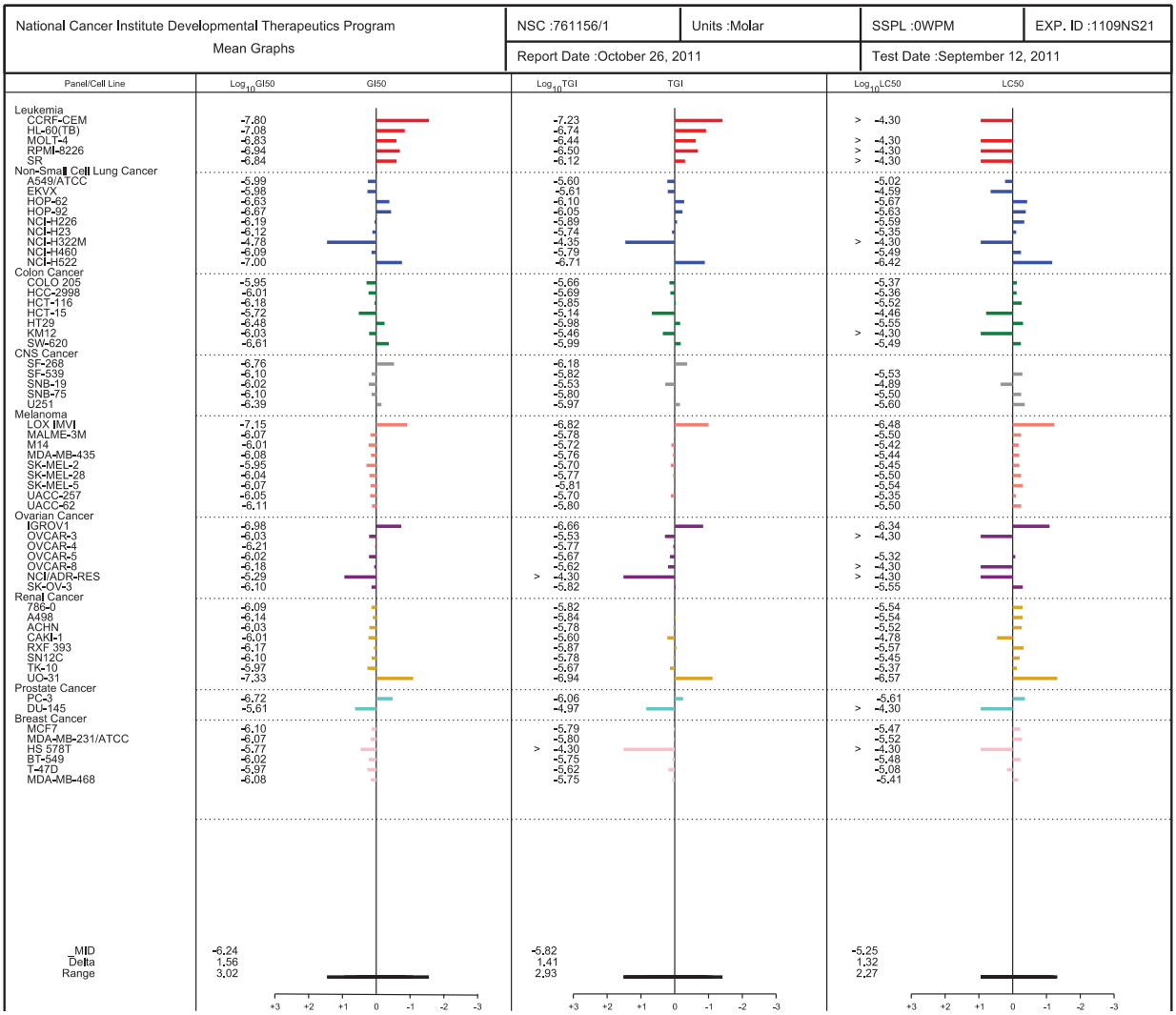
The single-dose data for trifluoro derivative **5-19** (Table 5) show some interesting trends. For example, at 10 μM this compound inhibits all of the leukemia cell lines, whereas it only

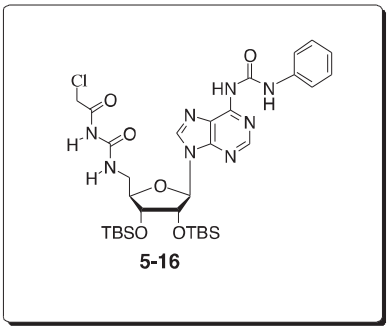
significantly inhibits one cell line each for non-small cell lung, CNS, melanoma, ovarian, and renal cancers. It is also noteworthy that both prostate cancer cell lines are significantly inhibited.

Single-dose data for fluoro **5-20** derivative (Table 6) also shows growth inhibition (at 10 μM) of all of the leukemia cell lines, but to a lesser extent than compound **5-19**. Derivative **5-20** modestly inhibits four of the six colon cancer cell lines. Interestingly, compounds **5-20** and **5-19** inhibit only one cell line of CNS (SR-268) and ovarian (OVCAR-3) cancers, with trifluoro derivative **5-19** being somewhat more potent than monofluoro derivative **5-20**.

Both compounds **5-19** and **5-20** have been selected for NIH multidose testing. Results of these assays will be reported in due course.

Table 2. Multi-dose growth inhibition for **5-16**.
GI_{50} determined from dose-response curve. LC_{50} = concentration required to reduce total cell count by 50%. Calculated as $[(T_i - T_z)/T_z)] \times 100 = -50$, where T_z = absorbance at t = 0; T_i = absorbance at t = 48h.





5-16

Table 2 (continued).

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 761156 / 1	Experiment ID : 1109NS21	Test Type : 08	Units : Molar
Report Date : October 26, 2011	Test Date : September 12, 2011	QNS :	MC :
COMI : JRS-100 (109621)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0WPM	

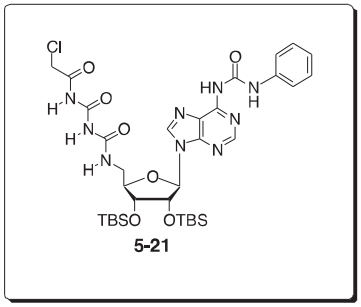
			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.3	-7.3	-6.3	-5.3	-4.3	-8.3	-7.3	-6.3	-5.3	-4.3	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.380	1.489	1.462	0.417	0.211	0.234	0.274	98	3	-44	-38	-28	1.60E-8	5.87E-8	> 5.00E-5
HL-60(TB)	0.695	2.176	2.299	1.928	0.235	0.275	0.353	108	83	-66	-61	-49	8.35E-8	1.80E-7	.
MOLT-4	0.518	1.898	1.889	2.022	0.430	0.467	0.476	99	109	-17	-10	-8	1.47E-7	3.66E-7	> 5.00E-5
RPMI-8226	0.673	1.732	1.723	1.652	0.514	0.370	0.421	99	92	-24	-45	-37	1.16E-7	3.13E-7	> 5.00E-5
SR	0.449	2.108	1.844	1.919	0.533	0.347	0.315	84	89	5	-23	-30	1.45E-7	7.58E-7	> 5.00E-5
Non-Small Cell Lung Cancer															
A549/ATCC	0.410	1.609	1.587	1.543	1.484	0.256	0.076	98	94	90	-38	-82	1.02E-6	2.53E-6	9.54E-6
EKVX	0.836	1.915	1.806	1.798	1.849	0.487	0.390	90	89	94	-42	-53	1.05E-6	2.46E-6	2.55E-5
HOP-62	0.407	1.013	0.990	1.033	0.555	0.025	0.015	96	103	24	-94	-96	2.37E-7	8.03E-7	2.12E-6
HOP-92	0.903	1.521	1.471	1.411	1.094	0.091	0.164	92	82	31	-90	-82	2.11E-7	9.00E-7	2.33E-6
NCI-H226	0.668	1.554	1.514	1.478	1.281	0.013	0.011	95	91	69	-98	-98	6.51E-7	1.30E-6	2.58E-6
NCI-H23	0.445	1.405	1.355	1.326	1.149	0.194	0.194	95	92	73	-57	-57	7.56E-7	1.84E-6	4.45E-6
NCI-H322M	0.717	1.651	1.594	1.634	1.686	1.757	0.674	94	98	104	111	-6	1.66E-5	4.44E-5	> 5.00E-5
NCI-H460	0.172	1.847	1.869	1.892	1.587	0.031	0.033	101	103	84	-82	-81	8.05E-7	1.61E-6	3.20E-6
NCI-H522	0.798	1.842	1.868	1.863	0.243	0.199	0.225	102	102	-70	-75	-72	1.00E-7	1.97E-7	3.85E-7
Colon Cancer															
COLO 205	0.315	1.180	1.266	1.273	1.277	0.119	0.025	110	111	111	-62	-92	1.13E-6	2.19E-6	4.25E-6
HCC-2998	0.424	1.639	1.592	1.517	1.584	0.170	0.109	96	90	95	-60	-74	9.81E-7	2.06E-6	4.32E-6
HCT-116	0.194	1.478	1.435	1.452	1.065	0.034	0.047	97	98	68	-82	-76	6.57E-7	1.41E-6	3.04E-6
HCT-15	0.395	1.927	1.971	1.995	1.968	0.578	0.151	103	104	103	12	-62	1.90E-6	7.26E-6	3.46E-5
HT29	0.216	1.306	1.384	1.342	0.633	0.044	0.058	107	103	38	-80	-73	3.30E-7	1.05E-6	2.80E-6
KM12	0.322	2.492	2.549	2.536	1.938	0.276	0.257	103	102	74	-14	-20	9.43E-7	3.45E-6	> 5.00E-5
SW-620	0.196	1.662	1.596	1.557	0.648	0.061	0.079	96	93	31	-69	-60	2.46E-7	1.02E-6	3.23E-6
CNS Cancer															
SF-268	0.280	1.487	1.497	1.490	0.363	0.137	0.165	101	100	7	-51	-41	1.73E-7	6.57E-7	.
SF-539	0.713	2.042	1.949	2.089	1.849	0.065	0.054	93	104	85	-91	-92	7.94E-7	1.53E-6	2.93E-6
SNB-19	0.476	1.631	1.534	1.506	1.394	0.361	0.064	92	89	79	-24	-87	9.63E-7	2.92E-6	1.30E-5
SNB-75	0.863	1.581	1.509	1.465	1.465	0.149	0.283	90	84	84	-83	-67	7.99E-7	1.59E-6	3.18E-6
U251	0.365	1.502	1.504	1.437	0.884	0.031	0.083	100	94	46	-92	-77	4.07E-7	1.08E-6	2.49E-6
Melanoma															
LOX IMVI	0.247	1.551	1.557	1.192	0.057	0.044	0.095	100	72	-77	-82	-62	7.07E-8	1.53E-7	3.30E-7
MALME-3M	0.602	1.369	1.333	1.373	1.302	0.093	0.151	95	100	91	-85	-75	8.58E-7	1.65E-6	3.18E-6
M14	0.317	1.061	1.074	1.102	1.059	0.093	0.110	102	106	100	-71	-65	9.78E-7	1.92E-6	3.77E-6
MDA-MB-435	0.551	2.358	2.295	2.223	2.082	0.151	0.163	97	93	85	-73	-70	8.31E-7	1.73E-6	3.59E-6
SK-MEL-2	0.821	1.475	1.542	1.578	1.614	0.171	0.092	110	116	121	-79	-89	1.13E-6	2.01E-6	3.58E-6
SK-MEL-28	0.533	1.369	1.340	1.320	1.359	0.072	0.059	97	94	99	-86	-89	9.17E-7	1.71E-6	3.18E-6
SK-MEL-5	0.497	2.319	2.316	2.230	2.204	0.021	0.015	100	95	94	-96	-97	8.50E-7	1.56E-6	2.86E-6
UACC-257	0.874	1.637	1.565	1.539	1.521	0.382	0.149	91	87	85	-56	-83	8.82E-7	2.00E-6	4.51E-6
UACC-62	0.563	1.935	1.872	1.751	1.677	0.101	0.074	95	87	81	-82	-87	7.76E-7	1.57E-6	3.18E-6
Ovarian Cancer															
IGROV1	0.570	1.842	1.918	1.851	0.253	0.235	0.250	106	101	-56	-59	-56	1.06E-7	2.20E-7	4.60E-7
OVCAR-3	0.235	1.824	1.915	1.902	1.463	0.183	0.174	106	105	77	-22	-26	9.39E-7	2.98E-6	> 5.00E-5
OVCAR-4	0.580	1.174	1.162	1.125	0.941	0.266	0.379	98	92	61	-54	-35	6.21E-7	1.69E-6	.
OVCAR-5	0.521	1.460	1.408	1.415	1.365	0.245	0.126	95	95	90	-53	-76	9.51E-7	2.13E-6	4.76E-6
OVCAR-8	0.498	1.768	1.725	1.689	1.273	0.359	0.332	97	94	61	-28	-33	6.65E-7	2.42E-6	> 5.00E-5
NCI/ADR-RES	0.500	1.546	1.541	1.527	1.465	1.028	0.529	100	98	92	50	3	5.11E-6	> 5.00E-5	> 5.00E-5
SK-OV-3	0.563	1.226	1.263	1.264	1.139	0.031	-0.002	106	106	87	-95	-100	7.99E-7	1.51E-6	2.84E-6
Renal Cancer															
786-0	0.661	2.199	2.189	2.227	2.015	0.039	0.143	99	102	88	-94	-78	8.08E-7	1.52E-6	2.86E-6
A498	0.701	1.863	1.554	1.653	1.604	0.066	0.053	73	82	78	-91	-93	7.30E-7	1.45E-6	2.87E-6
ACHN	0.346	1.429	1.485	1.507	1.451	0.027	0.031	105	107	102	-92	-91	9.27E-7	1.68E-6	3.03E-6
CAKI-1	0.638	2.251	2.145	2.096	2.030	0.407	0.237	93	90	86	-36	-63	9.88E-7	2.53E-6	1.64E-5
RXF 393	0.511	1.116	1.106	1.085	0.949	0.025	0.049	98	95	72	-95	-90	6.80E-7	1.35E-6	2.69E-6
SN12C	0.473	1.829	1.739	1.696	1.571	0.127	0.087	93	90	81	-73	-82	7.94E-7	1.67E-6	3.53E-6
TK-10	0.909	1.672	1.647	1.661	1.705	0.345	0.008	97	99	104	-62	-99	1.06E-6	2.12E-6	4.23E-6
UO-31	0.370	1.286	1.216	0.817	0.054	0.027	0.039	92	49	-86	-93	-90	4.69E-8	1.15E-7	2.72E-7
Prostate Cancer															
PC-3	0.364	1.009	0.962	0.901	0.532	0.059	0.092	93	83	26	-84	-75	1.91E-7	8.63E-7	2.46E-6
DU-145	0.173	1.293	1.378	1.416	1.397	0.439	0.089	108	111	109	24	-49	2.46E-6	1.06E-5	> 5.00E-5
Breast Cancer															
MCF7	0.280	1.442	1.420	1.378	1.226	0.065	0.035	98	94	81	-77	-88	7.90E-7	1.63E-6	3.39E-6
MDA-MB-231/ATCC	0.638	1.712	1.697	1.681	1.616	0.068	0.086	99	97	91	-89	-87	8.44E-7	1.60E-6	3.02E-6
HS 578T	0.449	1.769	1.730	1.693	1.607	0.667	0.669	97	94	88	17	17	1.69E-6	> 5.00E-5	> 5.00E-5
BT-549	0.868	1.773	1.843	1.847	1.796	0.155	0.118	108	108	102	-82	-86	9.62E-7	1.79E-6	3.35E-6
T-47D	0.549	1.246	1.267	1.266	1.227	0.295	0.206	103	103	97	-46	-62	1.07E-6	2.38E-6	8.41E-6
MDA-MB-468	0.579	1.178	1.149	1.123	1.083	0.190	0.137	95	91	84	-67	-76	8.40E-7	1.80E-6	3.85E-6

Table 3. Multi-dose growth inhibition for **5-21**.
GI_{50} determined from dose-response curve. LC_{50} = concentration required to reduce total cell count by 50%. Calculated as $[(T_i - T_z)/T_z)] \times 100 = -50$, where T_z = absorbance at t = 0; T_i = absorbance at t = 48h.

National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC :762610/1 | Units :Molar | SSPL :0WPM | EXP. ID :1201NS87
Report Date :March 02, 2012 | Test Date :January 09, 2012

Panel/Cell Line	Log_{10}GI50	GI50	Log_{10}TGI	TGI	Log_{10}LC50	LC50
Leukemia						
CCRF-CEM	-6.84		-6.35		-4.72	
HL-60(TB)	-5.87		-5.52		-4.30	
K-562	-5.93		-5.53		> -4.30	
MOLT-4	-5.99		-5.65		-5.32	
RPMI-8226	-5.86		-5.40		> -4.30	
SR	-6.68		-6.11		-5.47	
Non-Small Cell Lung Cancer						
A549/ATCC	-5.46		-4.89		-4.42	
EKVX	-5.81		-5.27		-4.62	
HOP-62	-5.88		-5.53		-4.96	
HOP-92	-6.07		-5.70		-5.33	
NCI-H226	-5.04		-4.78		-4.52	
NCI-H23	-5.88		-5.24		-4.65	
NCI-H460	-5.73		-4.95		> -4.30	
NCI-H522	-7.02		-6.70		-6.39	
Colon Cancer						
COLO 205	-5.11		-4.81		-4.51	
HCC-2998	-5.10		-4.77		-4.44	
HCT-116	-5.75		-5.12		-4.70	
HCT-15	-5.04		-4.68		-4.33	
HT29	-5.70		-5.17		-4.51	
KM12	-5.64		-5.03		-4.51	
SW-620	-5.68		-5.03		-4.45	
CNS Cancer						
SF-268	-5.22		-4.77		-4.32	
SF-295	-5.65		-5.01		-4.43	
SF-539	-5.06		-4.80		-4.54	
SNB-19	-4.77		> -4.30		> -4.30	
SNB-75	-5.19		-4.81		-4.44	
U251	-5.50		-4.96		-4.55	
Melanoma						
LOX IMVI	-6.99		-6.65		-6.30	
MALME-3M	-5.92		-5.51		-4.57	
M14	-5.26		-4.91		-4.56	
MDA-MB-435	-5.05		-4.69		-4.32	
SK-MEL-2	-4.99		-4.70		-4.41	
SK-MEL-28	-4.99		-4.67		-4.34	
SK-MEL-5	-5.06		-4.81		-4.55	
UACC-257	-5.27		-4.85		-4.43	
UACC-62	-5.09		-4.76		-4.43	
Ovarian Cancer						
IGROV1	-6.28		-5.79		-5.31	
OVCAR-3	-5.54		-5.02		-4.55	
OVCAR-4	-5.18		-4.75		-4.33	
OVCAR-5	-5.15		-4.84		-4.52	
OVCAR-8	-5.76		-4.96		-4.37	
NCI/ADR-RES	-5.11		-4.75		-4.39	
SK-OV-3	-5.01		-4.73		-4.45	
Renal Cancer						
786-0	-5.92		-5.33		-4.66	
A498	-5.15		-4.85		-4.54	
ACHN	-5.38		-4.94		-4.55	
CAKI-1	-5.70		-5.08		-4.51	
RXF 393	-5.62		-5.11		-4.67	
SN12C	-5.16		-4.84		-4.51	
UO-31	-6.02		-5.72		-5.41	
Prostate Cancer						
PC-3	-6.04		-5.52		-4.84	
DU-145	-5.04		-4.72		-4.40	
Breast Cancer						
MCF7	-5.09		-4.70		-4.31	
MDA-MB-231/ATCC	-5.75		-5.16		-4.63	
HS 578T	-5.26		-4.69		> -4.30	
BT-549	-5.19		-4.88		-4.58	
T-47D	-5.01		-4.71		-4.42	
MDA-MB-468	-5.53		-5.01		-4.58	
MID	-5.55		-5.11		-4.63	
Delta	1.47		1.59		1.76	
Range	2.25		2.4		2.09	



5-21

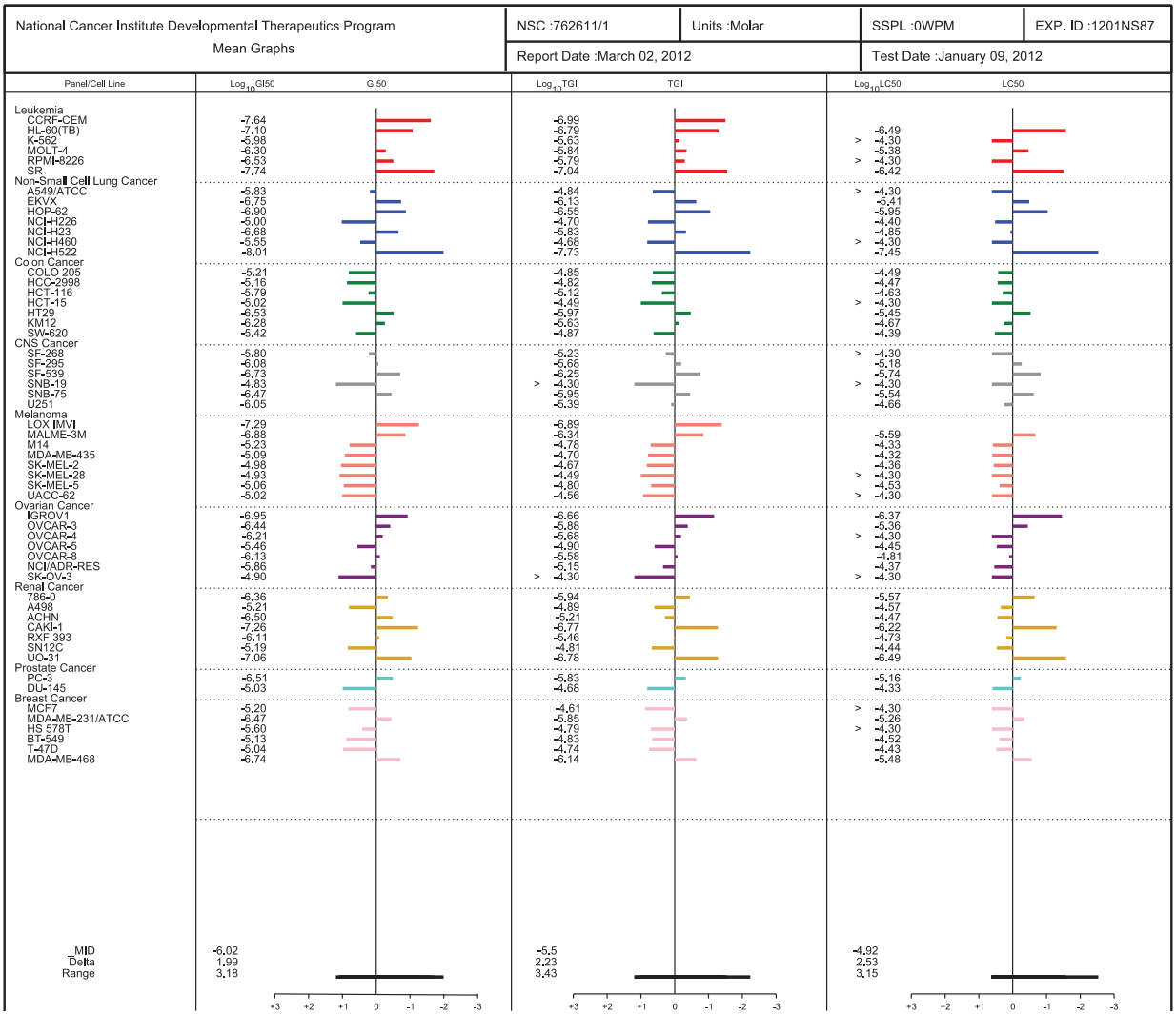
Table 3 (continued).

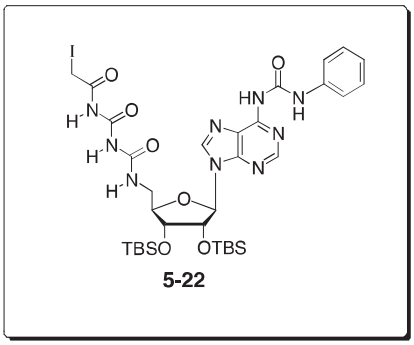
National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 762610 / 1	Experiment ID : 1201NS87	Test Type : 08	Units : Molar
Report Date : March 02, 2012	Test Date : January 09, 2012	QNS :	MC :
COMI : JRS-147 (111689)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0WPM	

		Log10 Concentration												
	Time	Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.3	-7.3	-6.3	-5.3	-4.3	-8.3	-7.3	-6.3	-5.3	-4.3	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.635	2.066	2.017	2.014	0.606	0.305	0.374	97	96	-5	-52	-41	1.44E-7	4.50E-7	.
HL-60(TB)	0.805	1.978	1.913	1.718	2.115	0.555	0.293	94	78	112	-31	-64	1.35E-6	3.03E-6	1.90E-5
K-562	0.229	1.302	1.344	1.284	1.267	0.162	0.129	104	98	97	-29	-44	1.17E-6	2.93E-6	> 5.00E-5
MOLT-4	0.657	1.806	1.540	1.927	1.776	0.308	0.294	77	111	97	-53	-55	1.03E-6	2.22E-6	4.77E-6
RPMI-8226	0.941	1.975	1.999	1.989	1.963	0.842	0.543	102	101	99	-11	-42	1.40E-6	4.00E-6	> 5.00E-5
SR	0.489	1.375	1.356	1.453	0.619	0.179	0.239	98	109	15	-63	-51	2.11E-7	7.70E-7	3.36E-6
Non-Small Cell Lung Cancer															
A549/ATCC	0.405	1.630	1.605	1.548	1.416	0.943	0.150	98	93	82	44	-63	3.48E-6	1.29E-5	3.78E-5
EKVX	0.850	1.568	1.586	1.549	1.539	0.868	0.217	103	97	96	3	-75	1.55E-6	5.39E-6	2.40E-5
HOP-62	0.354	0.749	0.764	0.747	0.783	0.243	0.050	104	99	108	-31	-86	1.31E-6	2.98E-6	1.09E-5
HOP-92	1.038	1.262	1.236	1.208	1.219	0.474	0.193	88	76	80	-54	-81	8.42E-7	1.98E-6	4.64E-6
NCI-H226	0.751	1.473	1.457	1.482	1.524	1.464	0.066	98	101	107	99	-91	9.03E-6	1.65E-5	3.03E-5
NCI-H23	0.522	1.332	1.321	1.267	1.194	0.568	0.104	99	92	83	6	-80	1.33E-6	5.81E-6	2.22E-5
NCI-H460	0.267	2.302	2.311	2.242	2.259	0.556	0.198	100	97	98	14	-26	1.87E-6	1.13E-5	> 5.00E-5
NCI-H522	0.612	1.397	1.404	1.358	0.220	0.189	0.247	101	95	-64	-69	-60	9.59E-8	1.98E-7	4.08E-7
Colon Cancer															
COLO 205	0.252	0.828	0.843	0.876	0.875	0.720	0.037	103	108	108	81	-86	7.70E-6	1.54E-5	3.06E-5
HCC-2998	0.479	1.705	1.740	1.655	1.702	1.467	0.137	103	96	100	81	-72	7.95E-6	1.69E-5	3.61E-5
HCT-116	0.139	1.067	0.919	1.047	0.931	0.335	0.003	84	98	85	21	-98	1.77E-6	7.52E-6	1.98E-5
HCT-15	0.305	1.498	1.469	1.495	1.519	1.349	0.141	98	100	102	88	-54	9.21E-6	2.08E-5	4.69E-5
HT29	0.206	1.030	1.069	1.053	1.107	0.289	0.070	105	103	109	10	-66	1.98E-6	6.78E-6	3.06E-5
KM12	0.484	2.118	2.237	2.149	2.058	0.918	0.143	107	102	96	27	-70	2.31E-6	9.39E-6	3.08E-5
SW-620	0.300	1.912	1.844	1.781	1.801	0.687	0.111	96	92	93	24	-63	2.10E-6	9.43E-6	3.53E-5
CNS Cancer															
SF-268	0.484	1.398	1.425	1.432	1.432	1.025	0.233	103	104	104	59	-52	6.04E-6	1.70E-5	4.80E-5
SF-295	0.730	1.867	1.879	1.792	1.812	1.022	0.281	101	93	95	26	-62	2.23E-6	9.85E-6	3.69E-5
SF-539	0.706	1.853	1.850	1.803	1.862	1.800	0.025	100	96	101	95	-97	8.62E-6	1.57E-5	2.86E-5
SNB-19	0.636	1.850	1.775	1.662	1.690	1.731	0.806	94	85	87	90	14	1.69E-5	> 5.00E-5	> 5.00E-5
SNB-75	0.649	1.362	1.310	1.284	1.247	1.115	0.204	93	89	84	65	-69	6.51E-6	1.54E-5	3.63E-5
U251	0.322	1.372	1.419	1.325	1.248	0.748	0.065	105	95	88	41	-80	3.17E-6	1.09E-5	2.82E-5
Melanoma															
LOX IMVI	0.185	1.244	1.198	1.192	0.092	0.036	0.053	96	95	-50	-81	-71	1.02E-7	2.25E-7	4.98E-7
MALME-3M	0.719	1.507	1.503	1.476	1.491	0.530	0.296	100	96	98	-26	-59	1.22E-6	3.07E-6	2.67E-5
M14	0.399	1.196	1.065	1.170	1.079	0.846	0.048	84	97	85	56	-88	5.51E-6	1.22E-5	2.72E-5
MDA-MB-435	0.601	2.209	2.191	2.163	2.120	1.945	0.285	99	97	94	84	-53	8.82E-6	2.05E-5	4.78E-5
SK-MEL-2	0.846	1.650	1.705	1.722	1.746	1.692	0.257	107	109	112	105	-70	1.03E-5	2.00E-5	3.86E-5
SK-MEL-28	0.403	1.152	1.144	1.150	1.094	1.137	0.177	99	100	92	98	-56	1.02E-5	2.16E-5	4.56E-5
SK-MEL-5	0.543	2.413	2.453	2.488	2.492	2.353	0.003	102	104	104	97	-100	8.66E-6	1.56E-5	2.80E-5
UACC-257	0.620	1.323	1.267	1.242	1.168	1.000	0.215	92	88	78	54	-65	5.40E-6	1.42E-5	3.71E-5
UACC-62	0.696	2.071	2.051	2.007	1.910	1.828	0.214	99	95	88	82	-69	8.17E-6	1.75E-5	3.73E-5
Ovarian Cancer															
IGROV1	0.759	2.033	2.111	2.103	1.430	0.375	0.243	106	105	53	-51	-68	5.30E-7	1.62E-6	4.93E-6
OVCAR-3	0.529	1.553	1.640	1.591	1.653	0.844	0.123	109	104	110	31	-77	2.86E-6	9.66E-6	2.82E-5
OVCAR-4	0.415	1.107	1.086	1.088	1.088	0.860	0.196	97	97	97	64	-53	6.62E-6	1.77E-5	4.72E-5
OVCAR-5	0.556	1.445	1.456	1.468	1.455	1.209	0.086	101	103	101	73	-85	7.03E-6	1.46E-5	3.02E-5
OVCAR-8	0.406	1.446	1.409	1.303	1.187	0.706	0.179	96	86	75	29	-56	1.74E-6	1.09E-5	4.26E-5
NCI/ADR-RES	0.483	1.415	1.416	1.378	1.386	1.205	0.180	100	96	97	77	-63	7.84E-6	1.78E-5	4.06E-5
SK-OV-3	0.583	1.263	1.287	1.305	1.309	1.279	0.133	104	106	107	102	-77	9.79E-6	1.86E-5	3.53E-5
Renal Cancer															
786-0	0.610	1.944	1.701	2.016	1.712	0.598	0.139	82	105	83	-2	-77	1.21E-6	4.73E-6	2.17E-5
A498	1.242	1.919	1.693	1.673	1.762	1.743	0.131	67	64	77	74	-89	7.01E-6	1.42E-5	2.87E-5
ACHN	0.348	1.511	1.540	1.617	1.575	0.875	0.067	102	109	105	45	-81	4.17E-6	1.14E-5	2.85E-5
CAKI-1	0.699	1.956	1.950	1.921	1.920	0.939	0.225	100	97	97	19	-68	2.01E-6	8.29E-6	3.11E-5
RXF 393	0.547	0.940	0.950	0.958	0.989	0.631	0.044	102	105	113	21	-92	2.42E-6	7.70E-6	2.13E-5
SN12C	0.518	2.109	2.003	1.967	2.052	1.655	0.089	93	91	96	71	-83	6.89E-6	1.45E-5	3.06E-5
UO-31	0.774	1.624	1.528	1.576	1.589	0.248	0.271	89	94	96	-68	-65	9.52E-7	1.92E-6	3.88E-6
Prostate Cancer															
PC-3	0.465	1.161	1.106	1.074	0.991	0.366	0.076	92	87	76	-21	-84	9.17E-7	3.01E-6	1.44E-5
DU-145	0.557	1.815	1.954	1.923	1.936	1.706	0.195	111	109	110	91	-65	9.19E-6	1.92E-5	4.01E-5
Breast Cancer															
MCF7	0.510	2.158	2.076	2.034	2.093	1.768	0.251	95	92	96	76	-51	8.05E-6	1.99E-5	4.93E-5
MDA-MB-231/ATCC	0.491	1.200	1.156	1.146	1.166	0.589	0.094	94	92	95	14	-81	1.80E-6	6.99E-6	2.36E-5
HS 578T	0.988	1.762	1.696	1.653	1.617	1.402	0.657	91	86	81	54	-34	5.49E-6	2.06E-5	> 5.00E-5
BT-549	0.724	1.199	1.079	1.139	1.146	1.052	0.025	75	87	89	69	-97	6.51E-6	1.31E-5	2.62E-5
T-47D	0.541	1.229	1.250	1.258	1.243	1.223	0.165	103	104	102	99	-70	9.78E-6	1.94E-5	3.83E-5
MDA-MB-468	0.622	0.934	0.934	0.933	0.949	0.728	0.113	100	99	105	34	-82	2.95E-6	9.79E-6	2.65E-5

Table 4. Multi-dose growth inhibition for **5-22**.
GI_{50} determined from dose-response curve. LC_{50} = concentration required to reduce total cell count by 50%. Calculated as $[(T_i - T_z)/T_z)] \times 100 = -50$, where T_z = absorbance at t = 0; T_i = absorbance at t = 48h.





5-22

Table 4 (continued).

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : 762611 / 1	Experiment ID : 1201NS87	Test Type : 08	Units : Molar
Report Date : March 02, 2012	Test Date : January 09, 2012	QNS :	MC :
COMI : JRS-150 (111691)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0WPM	

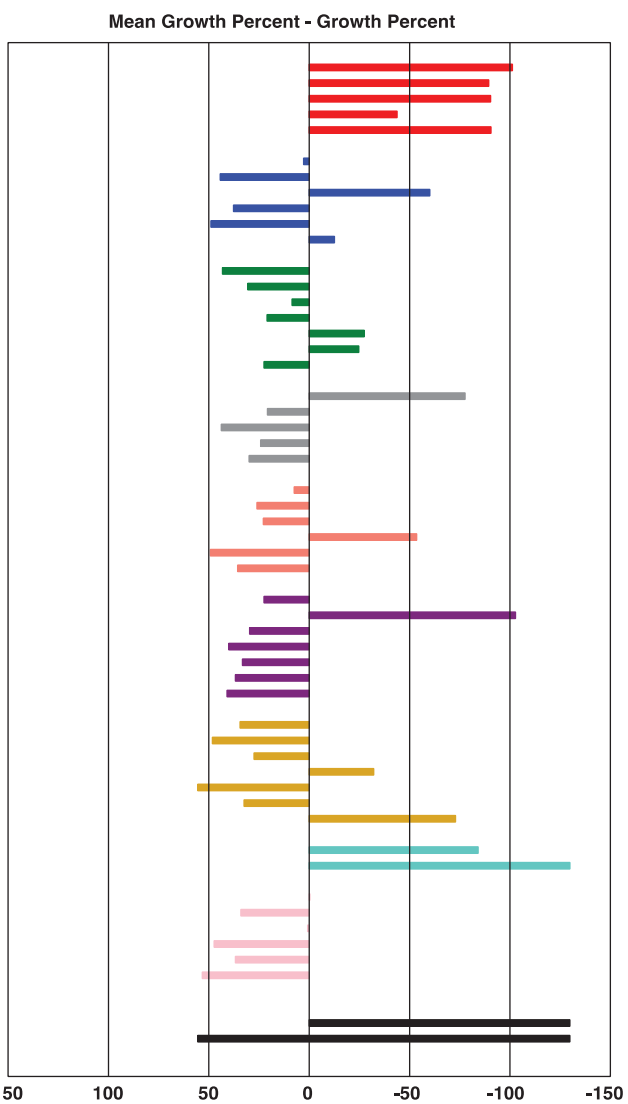
			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.3	-7.3	-6.3	-5.3	-4.3	-8.3	-7.3	-6.3	-5.3	-4.3	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.635	2.057	1.979	1.027	0.242	0.271	0.379	94	28	-62	-57	-40	2.31E-8	1.02E-7	.
HL-60(TB)	0.805	1.917	1.891	1.733	0.153	0.185	0.268	98	83	-81	-77	-67	7.98E-8	1.61E-7	3.24E-7
K-562	0.229	1.253	1.369	1.311	1.200	0.125	0.132	111	106	95	-46	-42	1.04E-6	2.37E-6	> 5.00E-5
MOLT-4	0.657	1.765	1.811	1.777	1.210	0.269	0.324	104	101	50	-59	-51	4.97E-7	1.43E-6	4.13E-6
RPMI-8226	0.941	1.975	2.015	1.979	1.307	0.623	0.511	104	100	35	-34	-46	2.98E-7	1.62E-6	> 5.00E-5
SR	0.489	1.420	1.305	0.680	0.198	0.176	0.242	88	21	-60	-64	-51	1.82E-8	9.02E-8	3.80E-7
Non-Small Cell Lung Cancer															
A549/ATCC	0.405	1.568	1.636	1.314	1.198	0.746	0.264	106	78	68	29	-35	1.47E-6	1.43E-5	> 5.00E-5
EKVX	0.850	1.587	1.571	1.558	0.938	0.362	0.214	98	96	12	-57	-75	1.76E-7	7.42E-7	3.90E-6
HOP-62	0.354	0.753	0.741	0.780	0.232	0.074	0.120	97	107	-34	-79	-66	1.26E-7	2.85E-7	1.11E-6
NCI-H226	0.751	1.513	1.517	1.507	1.577	1.510	0.255	101	99	108	100	-66	9.96E-6	2.00E-5	4.00E-5
NCI-H23	0.522	1.391	1.359	1.266	0.767	0.358	0.143	96	86	28	-31	-73	2.08E-7	1.48E-6	1.41E-5
NCI-H460	0.267	2.320	2.353	2.291	2.313	0.959	0.212	102	99	100	34	-21	2.83E-6	2.09E-5	> 5.00E-5
NCI-H522	0.612	1.345	1.354	0.145	0.139	0.134	0.174	101	-76	-77	-78	-72	9.71E-9	1.86E-8	3.55E-8
Colon Cancer															
COLO 205	0.252	0.873	0.883	0.903	0.833	0.643	0.061	102	105	94	63	-76	6.20E-6	1.42E-5	3.26E-5
HCC-2998	0.479	1.530	1.482	1.512	1.502	1.228	0.118	95	98	97	71	-75	6.98E-6	1.53E-5	3.36E-5
HCT-116	0.139	1.114	1.021	1.093	0.938	0.324	0.024	90	98	82	19	-83	1.61E-6	7.67E-6	2.37E-5
HCT-15	0.305	1.539	1.535	1.520	1.527	1.242	0.251	100	98	99	76	-18	9.45E-6	3.22E-5	> 5.00E-5
HT29	0.206	1.002	1.023	1.069	0.463	0.074	0.051	103	108	32	-64	-75	2.93E-7	1.08E-6	3.57E-6
KM12	0.484	2.155	2.180	2.257	1.351	0.363	0.172	101	106	52	-25	-65	5.29E-7	2.36E-6	2.14E-5
SW-620	0.300	1.892	1.844	1.822	1.648	1.019	0.123	97	96	85	45	-59	3.77E-6	1.36E-5	4.10E-5
CNS Cancer															
SF-268	0.484	1.374	1.388	1.444	1.335	0.520	0.246	102	108	96	4	-49	1.57E-6	5.94E-6	> 5.00E-5
SF-295	0.730	1.914	1.836	1.816	1.644	0.382	0.241	93	92	77	-48	-67	8.25E-7	2.07E-6	6.55E-6
SF-539	0.706	1.827	1.833	1.933	0.759	0.048	0.082	100	109	5	-93	-88	1.85E-7	5.59E-7	1.81E-6
SNB-19	0.636	1.760	1.728	1.664	1.662	1.678	0.665	97	91	91	93	3	1.49E-5	> 5.00E-5	> 5.00E-5
SNB-75	0.649	1.426	1.358	1.310	0.980	0.137	0.176	91	85	43	-79	-73	3.35E-7	1.12E-6	2.89E-6
U251	0.322	1.179	1.097	1.044	0.917	0.301	0.082	90	84	69	-7	-75	9.00E-7	4.10E-6	2.17E-5
Melanoma															
LOX IMVI	0.185	1.264	1.256	0.745	0.050	0.054	0.105	99	52	-73	-71	-44	5.18E-8	1.30E-7	.
MALME-3M	0.719	1.429	1.432	1.348	0.693	0.225	0.315	100	89	-4	-69	-56	1.31E-7	4.57E-7	2.58E-6
M14	0.399	1.285	1.151	1.255	1.148	0.912	0.187	85	97	85	58	-53	5.89E-6	1.66E-5	4.69E-5
MDA-MB-435	0.601	2.180	2.148	2.149	2.074	1.817	0.290	98	98	93	77	-52	8.10E-6	1.98E-5	4.84E-5
SK-MEL-2	0.846	1.580	1.633	1.676	1.727	1.596	0.349	107	113	120	102	-59	1.05E-5	2.16E-5	4.41E-5
SK-MEL-28	0.403	1.182	1.157	1.188	1.115	1.113	0.320	97	101	91	91	-21	1.17E-5	3.26E-5	> 5.00E-5
SK-MEL-5	0.543	2.494	2.517	2.525	2.522	2.429	0.030	101	102	101	97	-94	8.77E-6	1.60E-5	2.93E-5
UACC-62	0.696	2.067	2.002	1.979	1.924	1.802	0.503	95	94	90	81	-28	9.59E-6	2.77E-5	> 5.00E-5
Ovarian Cancer															
IGROV1	0.759	1.888	1.956	2.007	0.286	0.141	0.139	106	111	-62	-81	-82	1.12E-7	2.18E-7	4.24E-7
OVCAR-3	0.529	1.529	1.630	1.636	0.933	0.233	0.160	110	111	40	-56	-70	3.65E-7	1.31E-6	4.34E-6
OVCAR-4	0.415	1.130	1.111	1.115	0.831	0.268	0.210	97	98	58	-35	-50	6.11E-7	2.09E-6	> 5.00E-5
OVCAR-5	0.556	1.489	1.483	1.530	1.280	0.972	0.189	99	104	78	45	-66	3.43E-6	1.27E-5	3.58E-5
OVCAR-8	0.406	1.173	1.159	0.983	0.911	0.305	0.097	98	75	66	-25	-76	7.47E-7	2.66E-6	1.55E-5
NCI/ADR-RES	0.483	1.487	1.481	1.426	1.298	0.583	0.219	99	94	81	10	-55	1.37E-6	7.13E-6	4.24E-5
SK-OV-3	0.583	1.271	1.286	1.303	1.280	1.126	0.627	102	105	101	79	6	1.25E-5	> 5.00E-5	> 5.00E-5
Renal Cancer															
786-0	0.610	2.018	1.774	1.928	1.273	0.092	0.217	83	94	47	-85	-65	4.33E-7	1.14E-6	2.72E-6
A498	1.242	1.971	1.751	1.835	1.833	1.706	0.105	70	81	81	64	-92	6.12E-6	1.29E-5	2.70E-5
ACHN	0.348	1.571	1.606	1.635	0.787	0.425	0.135	103	105	36	6	-61	3.13E-7	6.19E-6	3.41E-5
CAKI-1	0.699	1.907	1.773	1.359	0.360	0.239	0.189	89	55	-49	-66	-73	5.54E-8	1.69E-7	6.05E-7
RXF 393	0.547	1.028	1.059	1.055	0.859	0.480	0.121	107	106	65	-12	-78	7.78E-7	3.46E-6	1.88E-5
SN12C	0.518	2.056	1.979	2.001	1.974	1.526	0.160	95	96	95	66	-69	6.52E-6	1.53E-5	3.61E-5
UO-31	0.774	1.590	1.470	1.518	0.131	0.147	0.324	85	91	-83	-81	-58	8.61E-8	1.67E-7	3.23E-7
Prostate Cancer															
PC-3	0.465	1.176	1.137	1.060	0.756	0.255	0.095	94	84	41	-45	-80	3.07E-7	1.49E-6	6.90E-6
DU-145	0.557	1.782	1.884	1.891	1.891	1.636	0.260	108	109	109	88	-53	9.29E-6	2.10E-5	4.73E-5
Breast Cancer															
MCF7	0.510	2.095	1.957	1.990	2.016	1.439	0.374	91	93	95	59	-27	6.31E-6	2.43E-5	> 5.00E-5
MDA-MB-231/ATCC	0.491	1.201	1.172	1.203	0.773	0.250	0.128	96	100	40	-49	-74	3.38E-7	1.40E-6	5.44E-6
HS 578T	0.988	1.779	1.756	1.694	1.658	1.264	0.664	97	89	85	35	-33	2.49E-6	1.64E-5	> 5.00E-5
BT-549	0.724	1.267	1.160	1.244	1.198	1.144	0.101	80	96	87	77	-86	7.35E-6	1.49E-5	3.01E-5
T-47D	0.541	1.217	1.232	1.241	1.245	1.170	0.152	102	104	104	93	-72	9.12E-6	1.83E-5	3.68E-5
MDA-MB-468	0.622	0.989	0.988	0.985	0.668	0.229	0.171	100	99	12	-63	-73	1.83E-7	7.29E-7	3.34E-6

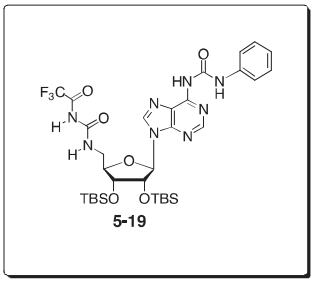
Table 5. Single-dose growth inhibition for **5-19**.
Percent calculated as: $[(T_i - T_z)/C - T_z)] \times 100$ for $T_i \geq T_z$; $[(T_i - T_z)/T_z)] \times 100$ for $T_i < T_z$; where T_z = absorbance at t = 0; T_i = absorbance at t = 48 h (10 μM test compound); C = absorbance of control at t = 48 h.

Developmental Therapeutics Program
One Dose Mean Graph

NSC: 764269 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 26, 2012
Experiment ID: 1203OS40		Report Date: Apr 25, 2012

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	-52.58
K-562	-40.75
MOLT-4	-41.72
RPMI-8226	4.89
SR	-41.89
Non-Small Cell Lung Cancer	
A549/ATCC	51.44
HOP-62	93.03
HOP-92	-11.41
NCI-H23	86.36
NCI-H322M	97.63
NCI-H460	36.12
Colon Cancer	
COLO 205	91.91
HCC-2998	79.30
HCT-116	57.22
HCT-15	69.68
HT29	21.28
KM12	23.96
SW-620	71.22
CNS Cancer	
SF-268	-28.97
SF-295	69.47
SF-539	92.46
SNB-75	72.84
U251	78.64
Melanoma	
LOX IMVI	56.17
MALME-3M	74.75
M14	71.55
MDA-MB-435	-4.83
SK-MEL-28	97.94
UACC-62	84.33
Ovarian Cancer	
IGROV1	71.24
OVCAR-3	-54.08
OVCAR-4	78.35
OVCAR-5	88.80
OVCAR-8	81.96
NCI/ADR-RES	85.46
SK-OV-3	89.66
Renal Cancer	
786-0	83.14
A498	96.91
ACHN	76.20
CAKI-1	16.56
RXF 393	104.24
SN12C	81.12
UO-31	-24.22
Prostate Cancer	
PC-3	-35.46
DU-145	-81.16
Breast Cancer	
MCF7	48.34
MDA-MB-231/ATCC	82.71
HS 578T	49.48
BT-549	96.03
T-47D	85.48
MDA-MB-468	101.94
Mean	48.68
Delta	129.84
Range	185.40





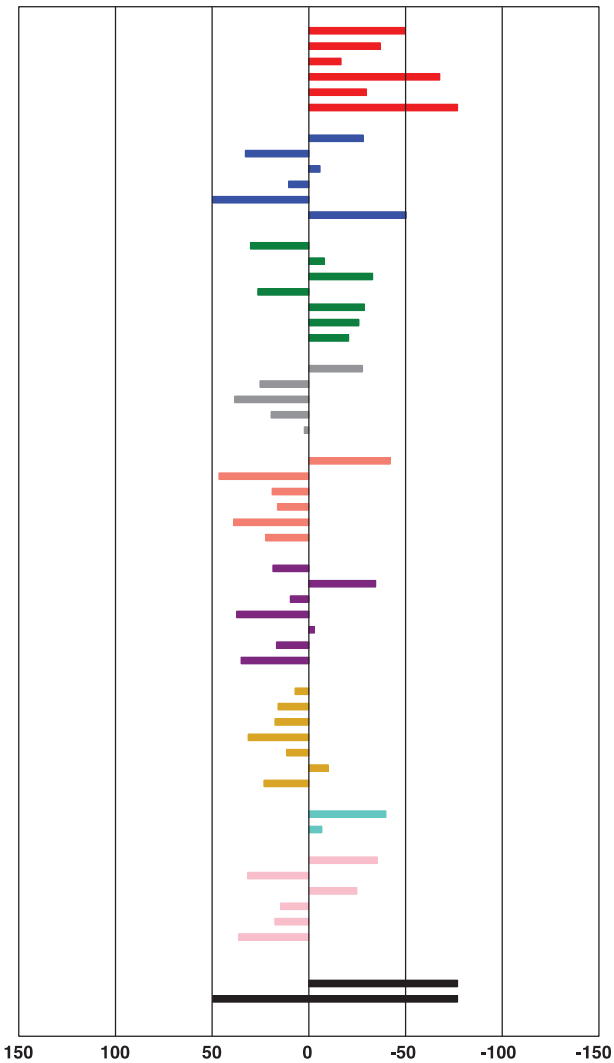
5-19

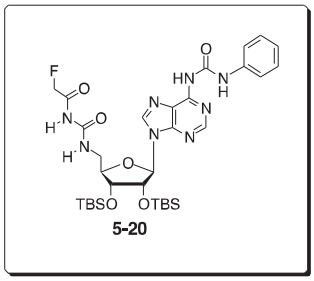
Table 6. Single-dose growth inhibition for **5-20**.
Percent calculated as: $[(T_i - T_z)/C - T_z)] \times 100$ for $T_i \geq T_z$; $[(T_i - T_z)/T_z)] \times 100$ for $T_i < T_z$; where T_z = absorbance at t = 0; T_i = absorbance at t = 48 h (10 μM test compound); C = absorbance of control at t = 48 h.

Developmental Therapeutics Program
One Dose Mean Graph

NSC: 764270 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 26, 2012
Experiment ID: 1203OS40		Report Date: Apr 25, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	4.80
HL-60(TB)	17.32
K-562	37.65
MOLT-4	-13.36
RPMI-8226	24.56
SR	-22.57
Non-Small Cell Lung Cancer	
A549/ATCC	26.19
HOP-62	87.09
HOP-92	48.51
NCI-H23	64.71
NCI-H322M	103.97
NCI-H460	4.01
Colon Cancer	
COLO 205	84.45
HCC-2998	46.27
HCT-116	21.35
HCT-15	80.65
HT29	25.61
KM12	28.48
SW-620	33.77
CNS Cancer	
SF-268	26.61
SF-295	79.54
SF-539	92.56
SNB-75	73.79
U251	56.62
Melanoma	
LOX IMVI	12.23
MALME-3M	100.71
M14	73.23
MDA-MB-435	70.48
SK-MEL-28	93.18
UACC-62	76.64
Ovarian Cancer	
IGROV1	72.87
OVCAR-3	19.78
OVCAR-4	63.82
OVCAR-5	91.53
OVCAR-8	51.42
NCI/ADR-RES	70.97
SK-OV-3	89.30
Renal Cancer	
786-0	61.38
A498	70.17
ACHN	71.79
CAKI-1	85.61
RXF 393	65.78
SN12C	44.28
UO-31	77.51
Prostate Cancer	
PC-3	14.46
DU-145	47.62
Breast Cancer	
MCF7	18.99
MDA-MB-231/ATCC	85.92
HS 578T	29.48
BT-549	68.86
T-47D	71.88
MDA-MB-468	90.52
Mean	54.29
Delta	76.86
Range	126.54





5-20

5.4. Discussion

The same synthetic route used to install the 5′-*N*-methyl urea moiety of lead compound **5-1** can be extended to other 5′-*N*-aryl (**5-3**) and 5′-*N*-alkyl derivatives (e.g., **5-4** and **5-12**). It has also proven successful for 5′-carbamates (**5-9**, **5-10**, and **5-12**). We have also developed efficient methods for preparing 5′-sulfonamide analogues (**5-14** and **5-15**). The chemistry developed in Chapter 4 was successfully used to prepare halogenated di- and triphosphate surrogates (**5-16**, **5-17**, **5-19**, **5-20**, **5-21**, and **5-22**) in good yields.

The biological results for 5′-*N*-phenyl urea **5-3** and sulfonamide **5-15** show that aryl groups are not conducive to achieving potent antiproliferative activity. Extending an alkyl substituent into the supposed phosphate-binding region of the ATP-binding pocket of BMPR1b gave mixed results. The 5′-*N*-Propyl derivative **5-5** showed inferior activity to that of the lead, but 5′-*N*-glycine methyl ester analogue **5-7** exhibited nearly equal antiproliferative activity to **5-1**. This latter result may be due to the fact that **5-7** possesses more hydrogen bond acceptor groups than **5-5** and could potentially interact with hydrogen bond donor residues within the active site, in keeping with our hypothesis.

The best antiproliferative results came from the halogenated di- and triphosphate bioisoster derivatives **5-16**, **5-17**, **5-19**, **5-20**, **5-21**, and **5-22**. The superior activities of both the chloro derivative **5-16** compared to its closely related (but halogenated) diphosphate congener **5-23** and the iodo derivative **5-22** compared to its closely related (but halogenated) triphosphate congener **5-24** suggest that the halogen atoms in **5-16** and **5-22** play a key role in determining biological activity. The promising single-dose data of fluoro derivatives **5-19** and **5-20** add further support to this assumption. This could be explained by increased halogen, multipolar, and hydrogen (for

the fluoro derivatives) intermolecular interactions[6] between the 5′- substituent and the catalytic triad of the BMPR1b binding pocket.

Triphosphate surrogates **5-21** and **5-22** exhibit selective cytotoxicity of the non-small cell lung adenocarcenoma cell line NCI-H522. This suggests that both compounds follow the same biological pathway to effect their antiproliferative activity. It is also interesting to note that iodo derivative **5-22** is significantly more cytotoxic than chloro derivative **5-21** (10 nM versus 95 nM). An SN_2 reaction between the amino acid residues of the enzyme binding pocket and the carbonyl α halogens of **5-21** and **5-22** offer a possible explanation for this observation.

5.5. Experimentals

5.5.1. Biology

5.5.1.1. Antiproliferative Assays

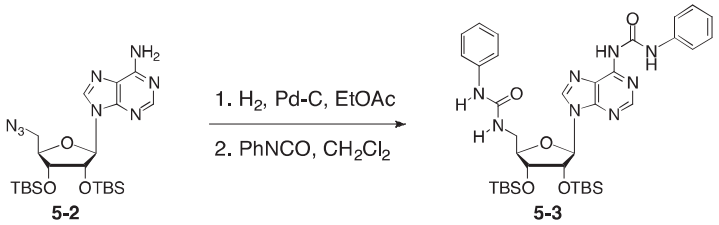
The cytostatic effects of the test compounds on murine leukemia cells (L1210), human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa) were evaluated as follows: an appropriate number of cells suspended in growth medium were allowed to proliferate in 200-lL-wells of 96-well-microtiter plates in the presence of variable amounts of test compounds at 37 °C in a humidified CO_2-controlled atmosphere. After 48 h (L1210), 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%.

5.5.2. Chemistry

5.5.2.1. 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. 1H NMR and 13C NMR spectra were determined using internal references at δ 7.27 ($CDCl_3$), 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. All compounds tested were >95% pure (except for compound **5-5** which was 94% pure) –as determined by HPLC; 5–10% IPA/CH_2Cl_2.

5.5.2.2. Compound Characterization Data

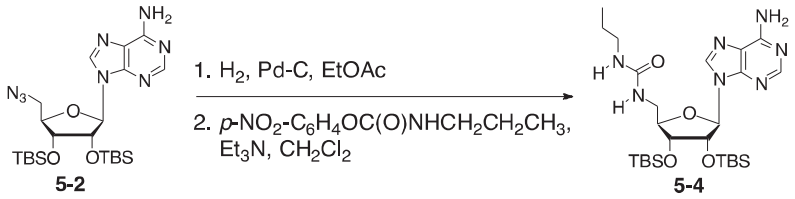


5-2 **5-3**

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

A solution of **5-2** (75 mg, 0.11 mmol) and 10% Pd–C (40 mg) in EtOAc (5 mL) was stirred for two days under an atmosphere of H_2 (balloon pressures). The catalyst was removed

via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dried under vacuum pump for two days and then dissolved in CH_2Cl_2 (3 mL) and charged with phenyl isocyanate (70 mg, 0.59 mmol). The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a Flash chromatography column and eluted with 40→60% EtOAc/hexanes to give **5-3** (44 mg, 0.060 mmol, 53%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.30 (s, 1H), 9.80 (s, 1H), 9.01 (s, 1H), 8.64 (s, 1H), 7.55 (d, *J* = 7.0 Hz, 1H), 7.49 (bs, 1H), 7.34 (t, *J* = 8.0 Hz, 2H), 7.26 (t, *J* = 4.0 Hz, 2H), 7.22 (t, *J* = 7.8 Hz, 2H), 7.16 (t, *J* = 7.5 Hz, 1H), 6.97 (t, *J* = 7.3 Hz, 1H), 4.60 (dd, *J* = 4.5, 7.5 Hz, 1H), 4.51 (d, *J* = 4.5 Hz, 1H), 4.21 (bs, 1H), 4.01 (ddd, *J* = 2.8, 7.3, 14.5 Hz, 1H), 3.30 (dt, *J* = 4.0, 14.5 Hz, 1H), 1.00 (s, 9H), 0.68 (s, 9H), 0.19 (s, 3H), 0.17 (s, 3H), –0.16 (s, 3H), –0.50 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155.9, 153.3, 151.2, 150.7, 150.5, 144.3, 139.4, 137.1, 129.4, 128.9, 125.5, 122.7, 122.2, 121.5, 119.3, 88.1, 88.0, 76.2, 73.9, 41.5, 26.1, 25.7, 18.3, 17.9, –4.3, –4.6, –5.5; MS (ES) *m/z* 755.3491 (MH+ $[C_{36}H_{52}N_8O_5Si_2]$)= 755.3457.

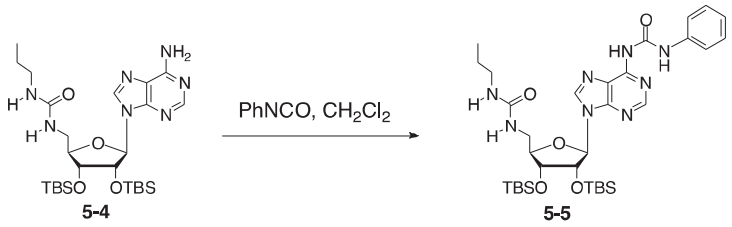


5-2 5-4

2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-[(*N*-propylcarbamoyl)amino]adenosine (5-4).

A solution of **5-2** (100 mg, 0.19 mmol) and 10% Pd–C (50 mg) in EtOAc (7.5 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude

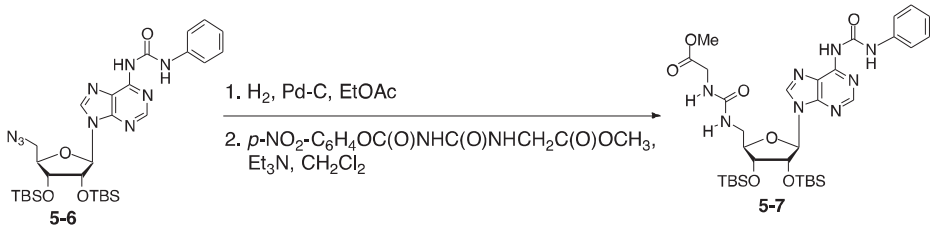
material was dried under vacuum pump for one day and then dissolved in CH_2Cl_2 (2 mL) and charged with *p*–nitrophenyl–*N*–propylcarbamate (58 mg, 0.26 mmol) and Et_3N (400 mL). The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a Flash chromatography column and eluted with 5% MeOH/ CH_2Cl_2 to give **5-4** (87 mg, 0.15 mmol, 78%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.35 (s, 1H), 7.87 (s, 1H), 7.50 (d, *J* =9.8 Hz, 1H), 5.79 (bs, 2H), 5.76 (d, *J* = 8.0 Hz, 1H), 4.85 (d, *J* = 4.8, 8.3, Hz, 1H), 4.59 (t, *J* = 5.5 Hz, 1H), 4.21 (d, *J* = 5.0 Hz, 1H), 4.19 (bs, 1H), 4.05 (dd, *J* = 8.8, 13.8 Hz, 1H), 3.30 (ddd, *J* = 6.3, 14.3, 20 Hz, 1H), 3.18–3.10 (m, 2H), 1.53 (sext, *J* = 7.3 Hz, 2H), 0.95 (s, 9H), 0.94 (t, *J* = 7.5 Hz, 3H), 0.71 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H), –0.14 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.8, 156.3, 152.3, 149.2, 142.1, 121.8, 90.6, 88.4, 73.8, 73.6, 42.4, 41.6, 26.0, 25.8, 23.9, 18.2, 18.0, 11.6, –4.3, –4.4, –4.5, –5.6; MS (FAB) *m/z* 580.3453 (MH+ [$C_{26}H_{49}N_7O_4Si_2$])= 580.3453.



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

A solution of **5-4** (59, 0.10 mmol) and phenyl isocyanate (15 mg, 0.13 mmol) in CH_2Cl_2 (1.5 mL) was stirred at ambient temperature (5 d). In order for the reaction to reach completion an additional aliquot of **5-4** (22 mg, 0.185) was added after seven hours. Volatiles were removed under reduced pressure and the crude reaction mixture was added directly to the Flash chromatography column and eluted with pure EtOAc to give **5-5** (35 mg, 0.51 mmol, 49%). ^{1}H

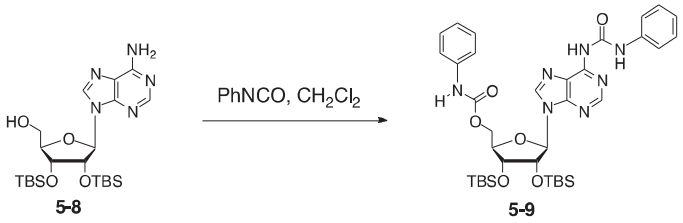
NMR ($CDCl_3$, 500 MHz) δ 12.17 (s, 1H), 9.56 (s, 1H), 8.94 (s, 1H), 8.66 (s, 1H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.39 (t, *J* = 7.8 Hz, 2H), 7.18 (t, *J* = 7.5 Hz, 1H), 6.32 (bs, 1H), 6.12 (d, *J* = 7.0 Hz, 1H), 5.06 (bs, 1H), 4.55 (dd, *J* = 4.5, 7.5 Hz, 1H), 4.44 (d, *J* = 4.5 Hz, 1H), 4.18 (s, 1H), 3.98 (dd, *J* = 3.3, 8.5 Hz, 1H), 3.18 ("d", *J* = 14.8 Hz, 1H) 3.10–3.02 (m, 2H), 1.40 (sext, *J* = 7.4 Hz, 2H), 0.97 (s, 9H), 0.84 (t, *J* = 7.3 Hz, 3H), 0.70 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H), –0.09 (s, 3H), –0.48 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.7, 152.9, 151.1, 150.6, 150.5, 144.3, 137.4, 129.4, 125.1, 121.7, 121.4, 88.4, 88.2, 76.1, 73.8, 42.2, 41.7, 26.1, 25.8, 23.8, 18.2, 17.9, 11.6, –4.3, –4.5, –5.4; MS (FAB) *m/z* 699.3834 (MH+ [$C_{33}H_{54}N_8O_5Si_2$])= 699.3855.



2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-[((*N*-methoxycarbonyl)methyl)carbamoyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (5-7).

A solution of **5-6** (50.5 mg, 0.082 mmol) and 10% Pd–C (50 mg) in EtOAc (3 mL) was stirred for one day under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dried under vacuum pump for two days and then dissolved in CH_2Cl_2 (2 mL) and combined with *p*-nitrophenyl-*N*-glycine methylester carbamate (50 mg, 0.20 mmol). The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a Flash chromatography column and eluted with 80→100% EtOAc/hexanes to give **5-7** (18 mg, 0.025 mmol, 30%, over two steps).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.86 (s, 1H), 8.92 (bs, 1H), 8.78 (s, 1H), 8.50 (s, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.39 (t, *J* = 8.0 Hz, 2H), 7.17 (t, *J* = 7.5 Hz, 1H), 5.97 (d, *J* = 8.0 Hz, 1H), 5.36 (bs, 1H), 4.76 (dd, *J* = 5.0, 7.5 Hz, 1H), 4.30 (d, *J* = 5.0 Hz, 1H), 4.21 (bs, 1H), 3.99 (dd, *J* = 5.0, 11.0 Hz, 1H), 3.99 (t, *J* = 6.0 Hz, 2H), 3.75 (s, 3H), 3.22 (dt, *J* = 2.8, 14.5 Hz, 1H), 0.96 (s, 9H), 0.71 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H), –0.13 (s, 3H), –0.51 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.6, 158.2, 151.9, 150.8, 150.8, 150.5, 144.3, 137.8, 129.3, 124.7, 122.1, 121.2, 89.6, 88.1, 74.8, 73.8, 52.4, 42.3, 41.7, 26.1, 25.8, 18.2, 18.0, –4.3, –4.3, –4.6, –5.5; MS (ES) *m/z* 729.3574(MH+ [$C_{33}H_{53}N_8O_7Si_2$) = 729.3570.

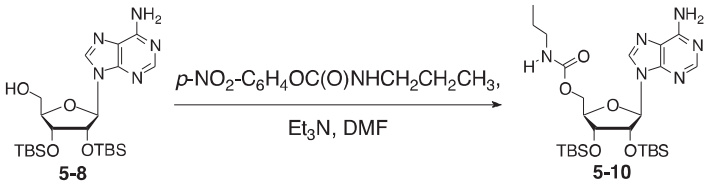


5-8 **5-9**

2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-(*N*-phenylcarbamoyl)-N^6-(*N*-phenylcarbamoyl)adenosine (5-9).

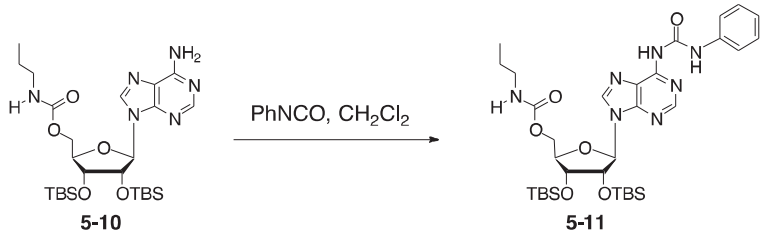
A solution of **5-8** (100, 0.20 mmol) and phenyl isocyanate (70 mg, 0.59 mmol) in CH_2Cl_2 (4.4 mL) was stirred at ambient temperature (4 d). Volatiles were removed under reduced pressure and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 25→40% EtOAc/hexanes to give **5-9** (90 mg, 0.12 mmol, 61%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.00 (bs, 1H), 8.65 (bs, 1H), 8.64 (s, 1H), 7.73 (bs, 1H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 2H), 7.26 (t, *J* = 8.0 Hz, 2H), 7.08 (t, *J* = 7.5 Hz, 1H), 7.04 (t, *J* = 7.3 Hz, 1H), 6.13 (d, *J* = 3.5 Hz, 1H), 4.72 (bs, 1H), 4.60 (dd, *J* = 3.5, 12.5 Hz, 1H), 4.48 ("d", *J* = 12.5 Hz, 1H), 4.44 (t, *J* = 4.3 Hz, 1H), 4.34 (dd, *J* = 3.3, 12.0 Hz, 1H), 0.95 (s, 9H), 0.80 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H), –0.07 (s, 3H), –0.26 (s, 3H); ^{13}C NMR

($CDCl_3$, 125 MHz) δ 153.6, 153.3, 151.2, 150.9, 150.4, 143.5, 138.2, 137.5, 129.1, 128.9, 124.9, 123.5, 122.0, 120.8, 119.2, 88.0, 84.4, 72.7, 63.2, 29.9, 26.0, 25.8, 18.2, 18.0, –4.26, –4.60, –4.72, –5.17; MS (FAB) *m/z* 734.3492 (MH+ [$C_{36}H_{52}N_7O_6Si_2$])= 734.3512.



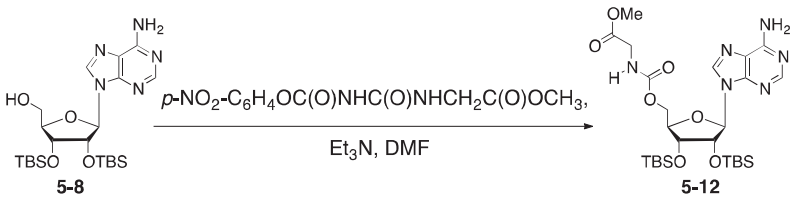
2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-(*N*-propylcarbamoyl)adenosine (5-10).

A solution **5-8** (100, 0.20 mmol), *p*-nitrophenyl-*N*-propyl carbamate (100 mg, 0.45 mmol), and Et_3N (400 μl) in DMF (2 mL) was stirred at 70 °C overnight. Volatiles were removed under reduced pressure and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 2→4% MeOH/CH_2Cl_2 to give compound **5-10** (74 mg, 0.13mmol, 63%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.35 (s, 1H), 7.98 (s, 1H), 5.89 (d, *J* = 4.5 Hz, 1H), 5.67 (bs, 1H), 5.65 (s, 1H), 4.95 (t, *J* = 4.3 Hz, 1H), 4.81 (bs, 1H), 4.48 (dd, *J* =4.0, 12.0 Hz, 1H), 4.35–4.32 (m 2H), 4.28 (t, *J* = 4.5 Hz, 1H), 3.19–3.11 (m, 2H), 1.56-1.49 (m, 2H), 0.93 (s, 9H), 0.92 (t, *J* = 7.5 Hz, 3H), 0.83 (s, 9H), 0.01 (s, 6H), 0.004 (s, 3H), –0.16 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.1, 155.8, 153.1, 149.8, 140.3, 120.8, 90.0, 82.9, 74.6, 72.2, 63.8, 43.0, 42.6, 26.0, 25.9, 23.6, 23.3, 18.2, 18.1, 11.5, 11.4, –4.23, –4.52, –4.73, –4.76; MS (ES) *m/z* 581.3298 (MH+ [$C_{26}H_{48}N_6O_5Si_2$])= 581.3303.



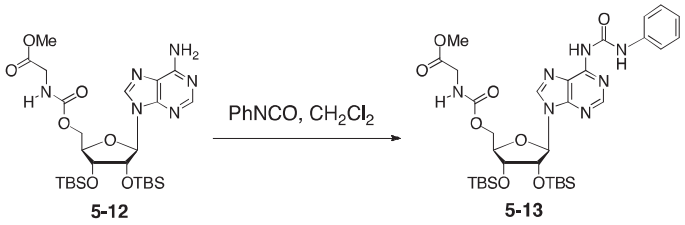
2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-N^6-(*N*-phenylcarbamoyl)-5′-(*N*-propylcarbamoyl)adenosine (5-11).

A solution of **5-10** (103, 0.18 mmol), phenyl isocyanate (33 mg, 0.28 mmol) in CH_2Cl_2 (2.2 mL) was stirred at ambient temperature (8 d). Volatiles were removed under reduced pressure and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 30→50% EtOAc/hexanes to give compound **5-11** (98 mg, 0.140 mmol, 79%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.16 (s, 1H), 9.63 (s, 1H), 8.76 (s, 1H), 8.66 (s, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.37 (t, *J* = 8.0 Hz, 2H), 7.15 (t, *J* = 7.8 Hz, 1H), 6.18 (d, *J* = 5.0 Hz, 1H), 5.72 (bs, 1H), 4.67 (t, *J* = 4.5 Hz, 1H), 4.43 (dd, *J* = 3.3, 12.3 Hz, 1H), 4.38 (t, *J* = 3.0 Hz, 1H), 4.36 (d, *J* = 3.0 Hz, 1H), 4.30 (3.3, 6.8 Hz, 1H), 2.95 (p, *J* = 6.9 Hz, 1H) 2.84 (p, *J* = 6.9 Hz, 1H), 1.35 (dq, *J* = 3.1, 7.3, H, 2H), 0.95 (s, 9H), 0.84 (s, 9H), 0.78 (t, *J* = 7.5 Hz, 3H), 0.13 (s, 3H), 0.12 (s, 3H), 0.02 (s, 3H), –0.18 (s, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 156.4, 153.0, 151.2, 150.8, 143.9, 137.9, 129.2, 124.4, 121.1, 120.8, 88.3, 84.0, 72.6, 63.4, 42.8, 29.9, 26.0, 25.9, 23.2, 18.2, 18.1, 11.3, –4.3, –4.6, –5.0; MS (FAB) *m/z* 700.3685 (MH+ [$C_{33}H_{53}N_7O_6Si_2$])= 700.3669.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-[((*N*-methoxycarbonyl)methyl)carbamoyl]adenosine (5-12).

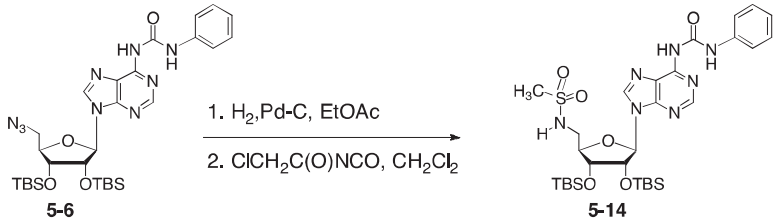
A solution of **5-8** (98 mg, 0.20 mmol), *p*-nitrophenyl-*N*-glycine methylestercarbamate (100 mg, 0.39 mmol), Et_3N (300 μL), and DMF (2 mL) was stirred 80 °C overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 50→90% EtOAc/hexanes to give **5-12** (72 mg, 0.12 mmol, 60%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.36 (s, 1H), 7.98 (s, 1H), 5.88 (d, *J* = 4.5 Hz, 1H), 5.62 (bs, 2H), 5.36 (t, *J* = 5.0 Hz, 1H), 4.99 (d, *J* = 4.5 Hz, 1H), 4.54 (dd, *J* = 4.0, 11.5 Hz, 1H), 4.36 (t, *J* = 5.0 Hz, 1H), 4.34 ("t", *J* = 2.5 Hz, 1H), 4.29, dd, *J* = 4.3, 8.8 Hz, 1H), 3.99 (d, *J* = 6.0 Hz, 2H), 3.77 (s, 3H), 1.79 (bs, 1H), 0.94 (s, 9H), 0.84 (s, 9H), 0.11 (s, 6H), 0.01 (s, 3H), –0.16 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.4, 156.1, 155.7, 153.2, 149.9, 140.5, 120.9, 90.1, 82.8, 74.5, 72.3, 64.4, 52.6, 42.9, 26.0, 25.9, 18.3, 18.1, –4.2, –4.5, –4.7, –4.7; MS (ES) *m/z* 611.3040 (MH+ [$C_{26}H_{47}N_6O_7Si_2$])= 611.3039



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-[((*N*-methoxycarbonyl)methyl)carbamoyl]-N^6-(*N*-phenylcarbamoyl)adenosine (5-13).

A solution of **5-12** (40 mg, 0.065 mmol) and phenyl isocyanate (12 mg, 0.10 mmol) in CH_2Cl_2 (1.5 mL) was stirred at ambient temperature (4d). Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography

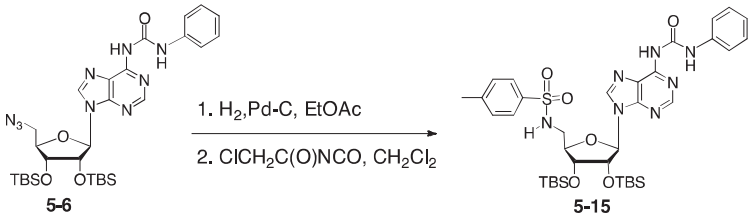
column and eluted with 30→50% EtOAc/hexanes to give **5-13** (33 mg, 0.045, 69%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.29 (s, 1H), 10.06 (bs, 1H), 8.92 (s, 1H), 8.96 (d, *J* = 8.0 Hz, 2H), 7.56 (t, *J* = 8.0 Hz, 2H), 7.14 (t, *J* = 7.3 Hz, 1H), 6.43 (bs, 1H), 6.25 (d, *J* = 5.0 Hz, 1H), 4.70 (t, *J* = 4.8 Hz, 1H), 4.46 (dd, *J* = 2.3, 12.3 Hz, 1H), 4.37 ("t", *J* = 3.8 Hz, 2H), 4.34–4.31 (m, 1H), 3.62 (s, 3H), 3.58 (dd, *J* = 6.5, 18.0 Hz, 1H), 3.32 (dd, *J* = 5.0, 18.0 Hz, 1H), 1.04 (s, 9H), 0.96 (s, 9H), 0.14 (s, 6H), 0.04 (s, 3H), –0.17 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.3, 156.5, 153.2, 151.2, 150.8, 150.4, 143.9, 137.9, 129.4, 124.5, 121.1, 120.8, 88.4, 83.9, 72.7, 64.1, 52.2, 42.3, 29.9, 26.0, 25.9, 18.3, 18.1, –4.2, –4.6, –4.6, –5.0; MS (ES) *m/z* 730.3403 (MH+ $[C_{34}H_{52}N_7O_8Si_2]$)= 730.4310.



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

A solution of **5-6** (35 mg, 0.057 mmol) and 10% Pd–C (35 mg) in EtOAc (3.5 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dried under vacuum pump for two days and then dissolved in CH_2Cl_2 (1 mL) and methylsulfonyl chloride (10 mg, 0.09 mmol), and Et_3N (15 μL) were then added. The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a Flash chromatography column and eluted with

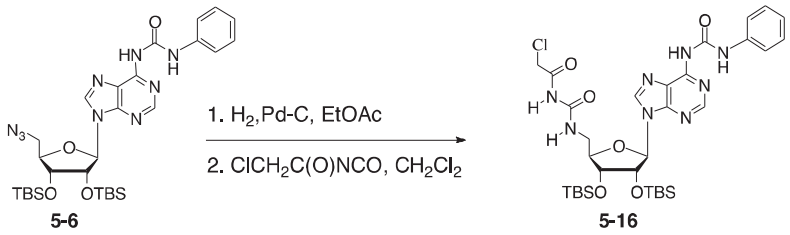
30→90% EtOAc/hexanes to give **5-14** (31 mg, 0.045 mmol, 79%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.77 (s, 1H), 8.80 (s, 1H), 8.71 (s, 1H), 8.63 (d, *J* = 9.5 Hz, 1H), 8.26 (s, 1H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.15 (t, *J* = 7.0 Hz, 1H), 5.87 (d, *J* = 7.5 Hz, 1H), 5.03 (dd, *J* = 5.0, 7.0 Hz, 1H), 4.36 (d, *J* = 4.5 Hz, 1H), 4.31 (s, 1H), 3.51 (t, *J* = 11.3 Hz, 1H), 3.40 (d, *J* =13.0 Hz, 1H), 3.01 (s, 3H), 0.97 (s, 9H), 0.72 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H), – 0.11 (s, 3H), –0.59 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 151.3, 150.9, 150.8, 149.6, 144.0, 138.0, 129.3, 124.3, 122.1, 120.6, 90.7, 87.0, 73.8, 73.6, 45.0, 40.4, 26.0, 25.8, 18.2, 17.9, –4.3, –4.3, –4.5, –5.7; MS (ES) *m/z* 691.3019 (M+ [$C_{30}H_{49}N_7O_6SSi_2$])= 691.3004.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-deoxy-N^6-(*N*-phenylcarbamoyl)-5′-[(*N-p*-toluenesulfonyl)amino]adenosine (5-15).

A solution of **5-6** (42 mg, 0.067 mmol) and 10% Pd–C (45 mg) in EtOAc (4 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dried under vacuum pump for two days and then dissolved in CH_2Cl_2 (1 mL) and charged with 4-methyltoluene chloride (15 mg, 0.079 mmol), and Et_3N (15 mL). The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a Flash chromatography column and eluted with 30→70% EtOAc/hexanes to give **5-15** (31 mg, 0.040 mmol, 62%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.76 (s, 1H), 8.89 (d, *J* = 10.2 Hz, 1H), 8.84 (s, 1H), 8.70 (bs, 1H), 8.19 (s, 1H), 7.78 (d, *J* =

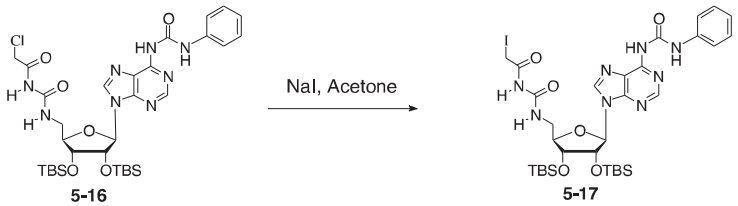
8.1 Hz, 2H), 7.67 (d, *J* = 7.8 Hz, 2H), 7.40 (t, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 7.8 Hz, 2H), 7.15 (t, *J* = 7.5 Hz, 1H), 5.81 (d, *J* = 8.1 Hz, 1H), 5.05 (dd, *J* = 5.0, 8.0 Hz, 1H), 4.24 (bs, 1H), 4.20 (d, *J* = 5.1 Hz, 1H), 3.36 (dt, *J* = 1.8, 13.4 Hz, 1H), 3.15 (dd, *J* = 1.8, 13.2 Hz, 1H), 2.42 (s, 3H), 0.94 (s, 9H), 0.74 (s, 9H), 0.12 (s, 3H), 0.09 (s, 3H), – 0.11 (s, 3H), –0.61 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.2, 151.0, 149.6, 143.9, 143.5, 138.0, 137.4 130.0, 129.3, 127.0, 124.3, 122.2, 120.6, 90.9, 87.0, 73.9, 73.5, 45.0, 26.0, 25.8, 21.7, 18.2, 17.9, –4.3, –4.4, –4.4, –5.7; MS (ES) *m/z* 767.3310 (M+ [$C_{36}H_{53}N_7O_6SSi_2$])= 767.3317.



2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-5′-[((chloroacetyl)aminocarbonyl)amino]-5′-deoxy-N^6-(*N*-phenylcarbamoyl)adenosine (5-16).

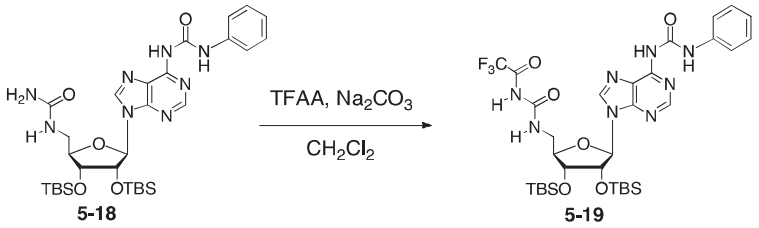
A solution of **5-6** (34 mg, 0.053 mmol) and 10% Pd–C (34 mg) in EtOAc (3.4 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dried under vacuum pump for two days and then dissolved in CH_2Cl_2 (1 mL) and charged with chloroacetyl isocyanate (7 mg, 0.059 mmol). The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a Flash chromatography column and eluted with 50→90% EtOAc/hexanes to give **5-16** (25 mg, 0.034 mmol, 64%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.82 (s, 1H), 8.95 (bs, 1H), 8.65 (s, 1H), 8.61 (bs, 1H), 8.48 (bs, 1H), 8.28 (s, 1H), 7.65 (d, *J* = 8.0 Hz,

2H), 7.38 (t, *J* = 8.0 Hz, 2H), 7.13 (t, *J* = 7.5 Hz, 1H), 5.95 (d, *J* = 5.5 Hz, 1H), 4.96 (t, *J* = 4.8 Hz, 1H), 4.31 (t, *J* = 3.5 Hz, 1H), 4.27 ("t", *J* = 8.0 Hz, 2H), 4.10 (s, 2H), 3.84–3.72 (m, 2H), 0.96 (s, 9H), 0.80 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H), – 0.06 (s, 3H), –0.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ, 167.3, 152.5, 151.3, 151.2, 150.4,150.3, 142.9, 138.2, 129.3, 124.2, 121.7, 121.7, 120.5, 89.8, 84.4, 77.4, 74.5, 73.4, 42.5, 41.9, 26.0, 25.9, 18.3, 18.1, – 4.2, – 4.5, – 4.5, – 5.0; MS (ES) *m/z* 733.3098 (M+ [$C_{32}H_{50}ClN_8O_6Si_2$])= 733.3080.



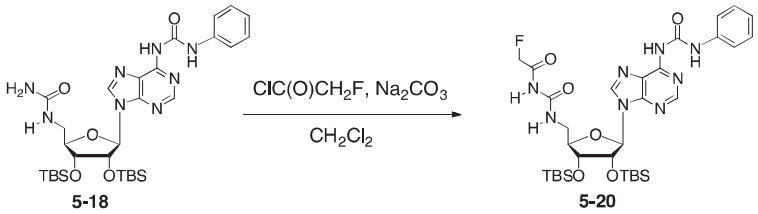
2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-deoxy-5′-[((iodoacetyl)aminocarbonyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (5-17).

A solution of **5-16** (5.5 mg, 0.0075 mmol), NaI (2 mg), and acetone (0.2 mL) was stirred at 55 °C for 30 minutes. Volatiles were removed under reduced pressure and the crude reaction mixture was added to a glass pipette with silica gel and eluted with 1→2 % MeOH/ CH_2Cl_2 to give **5-17** (5 mg, 0.0061 mmol, 81%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.75 (s, 1H), 9.09 (s, 1H), 8.51 (s, 1H), 8.67 (s, 1H), 8.17 (bs, 1H), 8.15 (s, 1H), 7.66 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 8.0 Hz, 2H), 7.14 (t, *J* = 7.3 Hz, 1H), 5.93 (d, *J* = 5.5 Hz, 1H), 4.96 (t, *J* = 5.0 Hz, 1H), 4.30 (t, *J* = 3.5 Hz, 1H), 4.26 (bs, 1H), 3.80–3.71 (m, 4H), 0.96 (s, 9H), 0.79 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H), –0.04 (s, 3H), –0.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.4, 165.2, 154.0, 151.4, 151.3, 150.4, 150.3, 142.8, 138.2, 129.3, 124.2, 121.7, 120.6, 89.7, 84.4, 74.5, 73.4, 41.9, 26.0, 25.9, 18.3, 18.1, –2.5, –4.2, –4.4, –4.5, –5.0; MS (ES) *m/z* 825.2428 (M+ [$C_{32}H_{50}IN_8O_6Si_2$])= 825.2437.



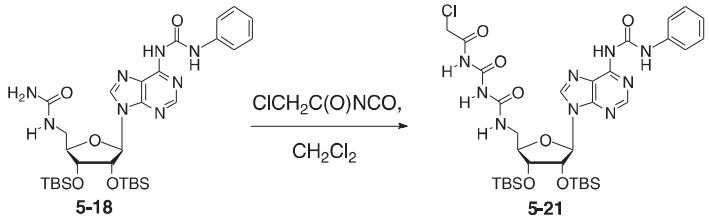
2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-deoxy-5′-[((trifluoroacetyl)aminocarbonyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (5-19).

To a solution of **5-18** (20 mg, 0.030 mmol) and sodium carbonate (10 mg, 0.094 mmol) in CH_2Cl_2 (0.6 mL) was added trifluoroacetic anhydride (11mg, 0.05 mmol) stirred at ambient temperature (4 h). The reaction was filtered with celite and a frit filter. Volatiles were removed under reduced pressure and the resulting residue was purified by silica gel chromatography (2→6% MeOH/CH_2Cl_2) to give **5-19** (15 mg, 0.020 mmol, 66%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.78 (s, 1H), 9.70 (bs, 1H), 8.64 (s, 1H), 8.51 (bs, 1H), 8.32 (t, *J* = 6.0 Hz, 1H), 8.18 (s, 1H), 7.65 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.9 Hz, 2H), 7.13 (t, *J* = 7.7 Hz, 1H), 5.9 (d, *J* = 5.7 Hz, 1H), 4.98 (dd, *J* = 4.3, 6.0 Hz, 1H), 4.32–4.25 (m, 2H), 3.83–3.77 (m, 2H), 0.95 (s, 9H), 0.78 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H), –0.08 (s, 3H), –0.34 (s, 3H) ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.8, 157.3, 152.2, 151.5, 151.2, 150.5, 150.3, 143.3, 138.2, 129.2, 124.2, 121.9, 120.6, 117.1, 113.3, 90.1, 84.0, 74.4, 73.4, 42.4, 29.9, 25.8, 18.1, –4.30, –4.54, –4.64, –5.07; MS (ES) *m/z* 752.3102 (M+ [$C_{32}H_{47}F_3N_8O_6Si_2$]) = 752.3109.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-deoxy-5′-[((fluoroacetyl)aminocarbonyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (5-20).

To a solution of **5-18** (36 mg, 0.055 mmol) and sodium carbonate (15 mg, 0.14 mmol) in CH_2Cl_2 (1.0 mL) was added fluoroacetyl chloride (18 mg, 0.019 mmol) stirred at ambient temperature for 3 hr. The reaction was filtered with celite and a frit filter. Volatiles were removed under reduced pressure and the resulting residue was purified by silica gel chromatography (2→4% MeOH/CH_2Cl_2) to give **5-20** (20 mg, 0.028 mmol, 51%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.80 (s, 1H), 8.65 (bs, 1H), 8.53 (bs, 1H), 8.44 (bs, 1H), 8.36 (bs, 1H), 8.27 (s, 1H), 7.65 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.13 (t, *J* = 7.4 Hz, 1H), 5.9 (d, *J* = 6.0 Hz, 1H), 4.97 (dd, *J* = 4.2, 5.7 Hz, 1H), 4.92 (s, 1H), 4.76 (s, 1H), 4.31–4.25 (m, 2H), 3.83–3.75 (m, 2H), 0.96 (s, 9H), 0.79 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H), –0.06 (s, 3H), –0.32 (s, 3H) ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.9, 168.6, 152.3, 151.5, 151.2, 150.4, 150.4, 143.2, 138.2, 129.2, 124.2, 121.7, 120.5, 89.7, 84.3, 80.8, 78.3, 77.4, 74.6, 73.5, 41.9, 29.9, 26.0, 25.8, 18.3, 18.0, –4.27, –4.50, –4.55, –5.02; MS (ES) *m/z* 716.3307 (M+ [$C_{32}H_{49}FN_8O_6Si_2$]) = 716.3298.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-[(((chloroacetyl)aminocarbonyl)aminocarbonyl)amino]-5′-deoxy-N^6-(*N*-phenylcarbamoyl)adenosine (5-21).

A solution of **5-18** (50 mg, 0.076 mmol) and chloroacetyl isocyanate (18 mg, 0.15 mmol) in CH_2Cl_2 (1.0 mL) was stirred at ambient temperature overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 2→4% MeOH/CH_2Cl_2 to give **5-21** (41 mg, 0.053 mmol, 69%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.97 (s, 1H), 10.78 (bs, 1H), 10.18 (bs, 1H), 8.93 (s, 1H), 8.59 (s, 1H), 8.28 (bs, 1H), 7.97 (s, 1H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.20 (t, *J* = 7.8 Hz, 2H), 7.03 (t, *J* = 7.3 Hz, 1H), 5.90 (d, *J* = 8.5 Hz, 1H), 4.86 (d, *J* = 4.0 Hz, 1H), 4.83 (bs, 1H), 4.29 (d, *J* = 4.0 Hz, 1H), 4.19 (dd, *J* = 4.3, 8.8 Hz, 1H), 4.05 (bs, 1H), 3.72 (d, *J* = 14.5 Hz, 1H), 0.98 (s, 9H), 0.79 (s, 9H), 0.20 (s, 3H), 0.16 (s, 3H), – 0.05 (s, 3H), –0.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ, 168.4, 152.7, 152.5, 150.8, 150.4, 149.9, 143.4, 137.6, 129.0, 124.2, 121.7, 120.4, 90.6, 83.4, 75.0, 71.9, 56.2, 42.8, 40.5, 29.9, 26.1, 25.8, 18.3, 18.0, – 4.0, – 4.5, – 4.5, – 4.6; MS (ES) *m/z* 776.3051 (M+ [$C_{33}H_{50}ClN_9O_7Si_2$])= 776.3060.

5-21 **5-22**

2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-[(((iodoacetyl)aminocarbonyl)aminocarbonyl)amino]-N^6-(*N*-phenylcarbamoyl)adenosine (5-22).

A solution of **5-21** (22 mg, 0.028 mmol), NaI (7.5 mg, 0.05 mmol), and acetone (0.8 mL) was stirred at 55 °C for 30 minutes. Volatiles were removed under reduced pressure and the

crude reaction mixture was added to a Flash chromatography column and eluted with 1, 3 % MeOH/ CH_2Cl_2 to give **5-22** (23 mg, 0.027 mmol, 94%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.14 (s, 1H), 11.74 (bs, 1H), 10.27 (bs, 1H), 9.19 (s, 1H), 8.60 (s, 1H), 8.33 (d, *J* = 7.2 Hz, 1H), 7.98 (Bs, 1H), 7.43 (d, *J* = 7.8 Hz, 2H), 7.13 (t, *J* = 7.8 Hz, 2H), 6.99 (t, *J* = 7.2 Hz, 1H), 5.93 (d, *J* = 3.3 Hz, 1H), 5.10 (bs, 1H), 4.78 (t, *J* = 4.2 Hz, 1H), 4.30–4.29 (m, 1H), 4.22 (dd, *J* = 8.7, 14.7 Hz, 1H), 3.93 (s, 2H), 3.73 (d, *J* = 15.3 Hz, 1H), 0.98 (s, 9H), 0.70 (s, 9H), 0.22 (s, 3H), 0.16 (s, 3H), – 0.05 (s, 3H), –0.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.8, 153.4, 153.2, 152.8, 150.7, 150.4, 149.7, 143.6, 137.4, 128.9, 124.2, 121.7, 120.6, 90.9, 83.0, 77.4, 75.3, 71.2, 39.9, 26.1, 25.8, 18.3, 18.0, – 2.8, – 3.9, – 4.3, – 4.5, – 4.7; MS (ES) *m/z* 867.2410 (M+ $[C_{33}H_{50}IN_9O_7Si_2]$)= 867.2416.

5.6. References

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Chapter 6: Additional Study of the Structural Features Required for Anticancer Activity

6.1. Introduction

Inferences for the required structural features for anticancer activity of lead **6-1** were made from SARs of previous compounds.[1] Such assumptions included the requirement of the 2′-*O*-TBS group, the 5′-*N*-methyl carbamoyl moiety, and the N^6-phenyl urea group (see Chapter 1). Further work indicated that 5′-methyl ureas were substantially more biologically active than their respective 5′-methyl carbamate analogues (Chapter 2).[2] The decision was made to further probe various regions of lead compound **6-1** to verify the essential requirements for anticancer activity. The regions to be studied included revisiting of the N^6-aryl substitution, the 5′-carbamoyl moiety, and the 2′,3′-*O*-substitutions. In addition, preliminary exploration into the impact of the nucleobase and ribose sugar were undertaken (Figure 1). Discovery of which regions, if any, are not vital for anticancer activity would simplify the synthesis as well as produce a compound which better follows Lipinski's Rule of Five.[3, 4]

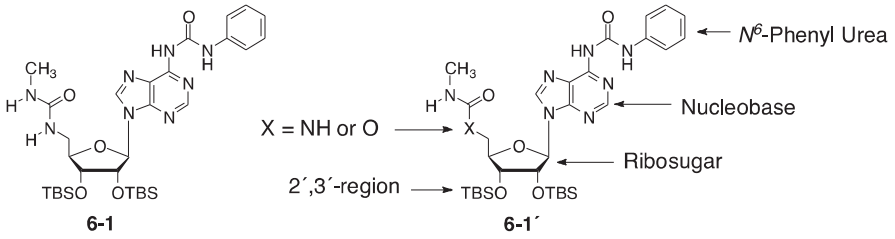


Figure 1. Additional SAR for lead compound **6-1**.

The Lapinski Rule of Five (commonly referred to as Lipinski's Rule) is the result of the analysis of many bioactive medicinal compounds. It gives four general guidelines which if

followed will produce a small molecule therapeutic agent that has better absorption and permeation properties, and, therefore, a compound more likely to be active in humans. The four general guidelines are: (1) no more than 5 hydrogen-bond donors, (2) the sum of nitrogens and oxygens (H-bond acceptors) should not be over 10, (3) a molecular weight of < 500, and (4) CLogP at or below 5. The number 5 in the rule is not for the number of requirements, but for the parameter values, which are each multiples of five. Compound **6-1** breaks Lipinski's Rule in a number of ways (mw > 500, CLogP > 5, sum of H-bond acceptors >10), thus refinement of the structure through paring off those functionalities that are not absolutely essential for antiproliferative activity was deemed an important step toward making compound **6-1** more druglike and hence more "druggable".

6.2. Chemistry

The SAR began with an evalution of several compounds that had been prepared as synthetic intermediates along the route to compounds discussed in Chapter 5. These intermediates lacked the N^6-phenyl urea moiety and included 5′-ureas as well as 5′-carbamates (Figure 2).

Preliminary biological data for the compounds in Figure 2 surprisingly showed that the 5′-carbamates were comparable in activity to the 5′-ureas. Since the 5′-*N*-methyl carbamate moiety is considerably easier to install than the 5′-*N*-methyl urea, and since the derivatives that lacked N^6-substitutuents (Figure 2) were surprisingly active, additional derivatives were prepared which lacked N^6-substitution and possessed the 5′-*N*-methyl carbamate (Scheme 1). Compounds **6-9** and **6-10** were designed to probe the effect of 2′,3′-*O*-substitution in derivatives which lacked an

N^6-phenyl urea and compounds (**6-12** and **6-14**) were designed to probe the relative importance of the 2′-*O*-TBS versus the 3′-*O*-TBS groups (Scheme 1). Derivative **6-9** was prepared by

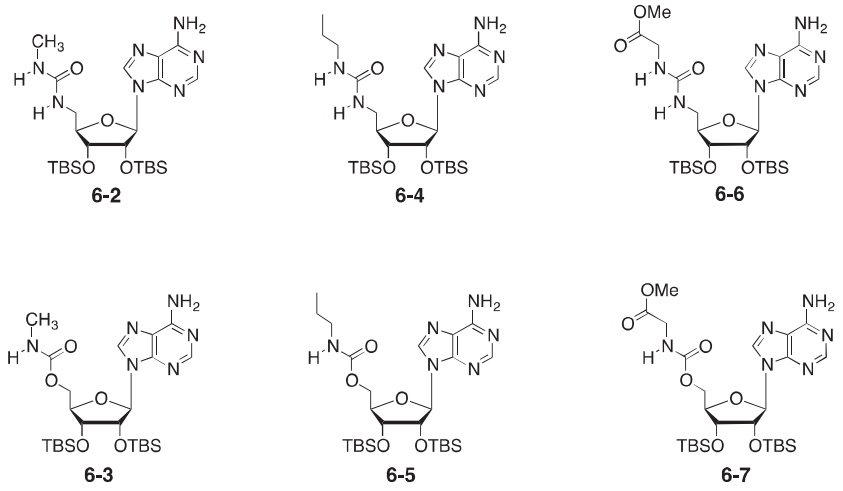
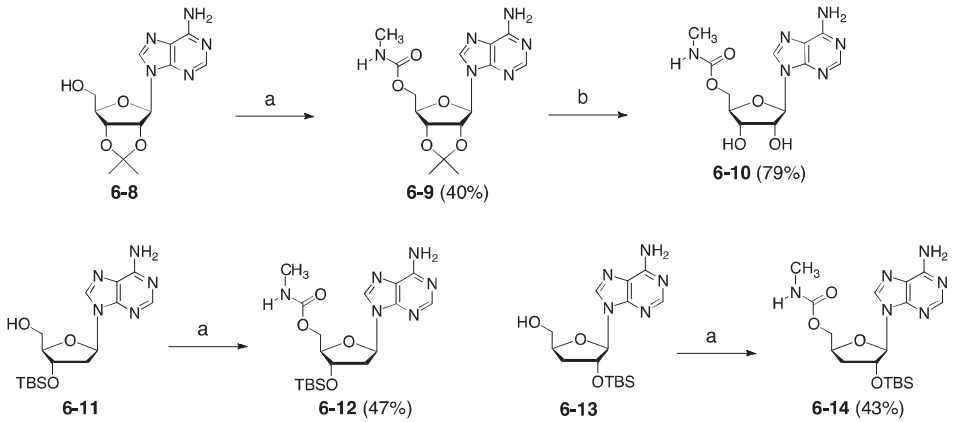


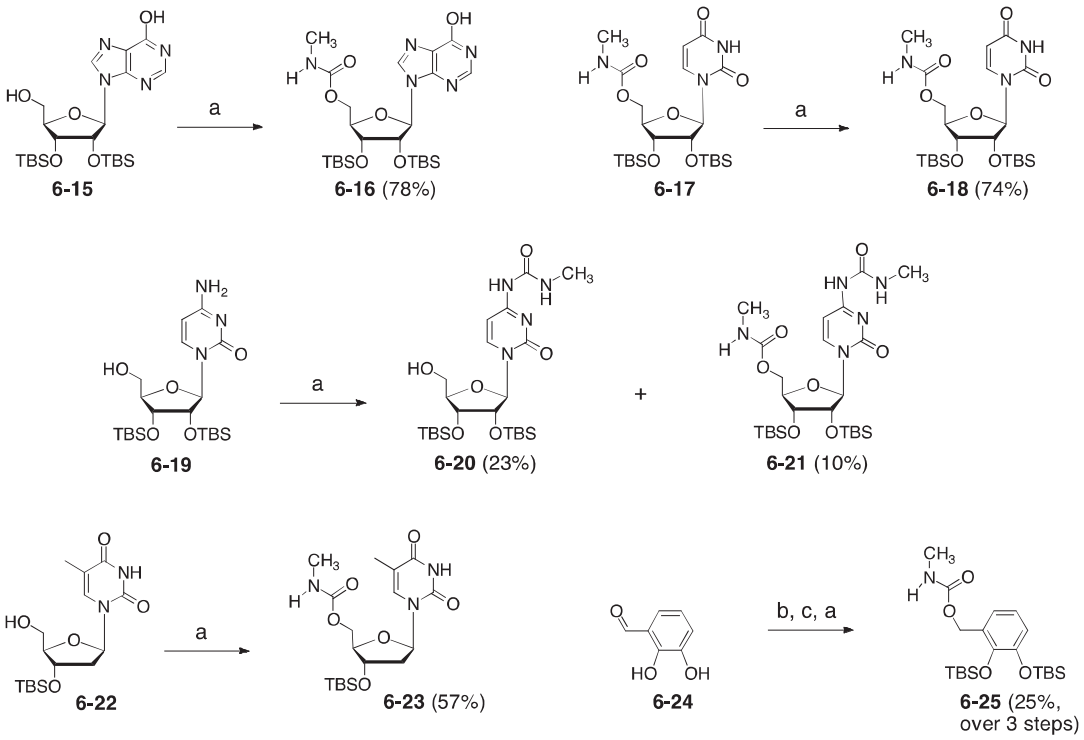
Figure 2. Derivatives **6-2**–**6-7**.
Derivatives lacking substituents at N^6 (See Chapter 5 for compound preparation).



Scheme 1. Synthesis of derivatives varying at the 2′,3′ position.
Reagents: (a) *p*-NO_2-$C_6H_4OC(O)NHCH_3$, DMF, Δ; (b) TFA, H_2O, CH_2Cl_2. See Chapter 2 for preparation of **6-8**.

treating **6-8** with *p*-nitrophenyl-*N*-methyl carbamate.[5] Removal of the isopropylidine protecting group gave **6-10** in good yield (79%). Compounds **6-12** and **6-14** were prepared in a similar fashion as **6-9**. Lower yields for these compounds are due to the extreme difficulty in chromatographic separation of **6-12** and **6-14** from their respective starting materials.

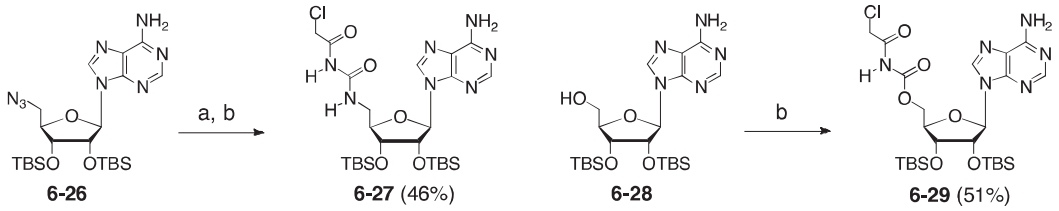
In order to probe the importance of the adenine base, inosine (**6-16**), uridine (**6-18**), cytidine (**6-20**, **6-21**), and thymidine (**6-23**) analogues were prepared (Scheme 2). Compounds **6-16**, **6-18**, and **6-23** were obtained in good yields (57–78%) after treatment of their respective TBS-protected starting material with *p*-nitrophenyl-*N*-methyl carbamate. Synthesis of cytidine derivatives gave a mixture of products **6-20** and **6-21**, in about a 2:1 ratio. The low yields are a



Scheme 2. Synthesis of nucleobase and ribose sugar analogues.
Reagents: (a) *p*-NO_2-$C_6H_4OC(O)NHCH_3$, DMF, Δ; (b) TBSCl, Imid, DMF; (c) $NaBH_4$, MeOH.

direct result of the extreme difficulty in chromatographic separation of the two products since they have nearly identical R_f values. Selective acylation of the 5′-OH is not possible due to the relatively higher reactivity of the N^4 of cytidine. The enhanced reactivity of the N^4- position of cytidine relative to the 5′-OH has also been observed in a synthesis of Sapacitabine.[6, 7] This is in keeping with the general well-documented reactivity of amines vs alcohols, but in contrast to the order of reactivity of the 5′-OH group versus the N^6-position of adenosine. Derivative **6-25** was synthesized in order to explore the effect of replacing the ribose sugar with a phenyl ring.

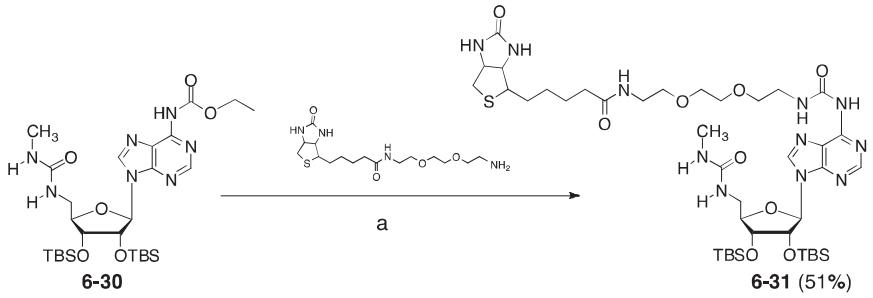
Derivatives of a potent anticancer compound discussed in Chapter 5 derivative (**5-16**) were prepared (Scheme 3). Analogue **6-27** is identical to **5-16** except that it lacks the N^6-phenyl urea moiety. Derivative **6-29** is the carbamate analogue, which is prepared from adenosine in only three synthetic steps. Preparation of the compounds was routine.



Scheme 3. Synthesis of **6-27** and **6-29**.
Reagents: (a) H_2, Pd-C, EtOAc; (b) $ClCH_2C(O)N=C=O$, CH_2Cl_2.

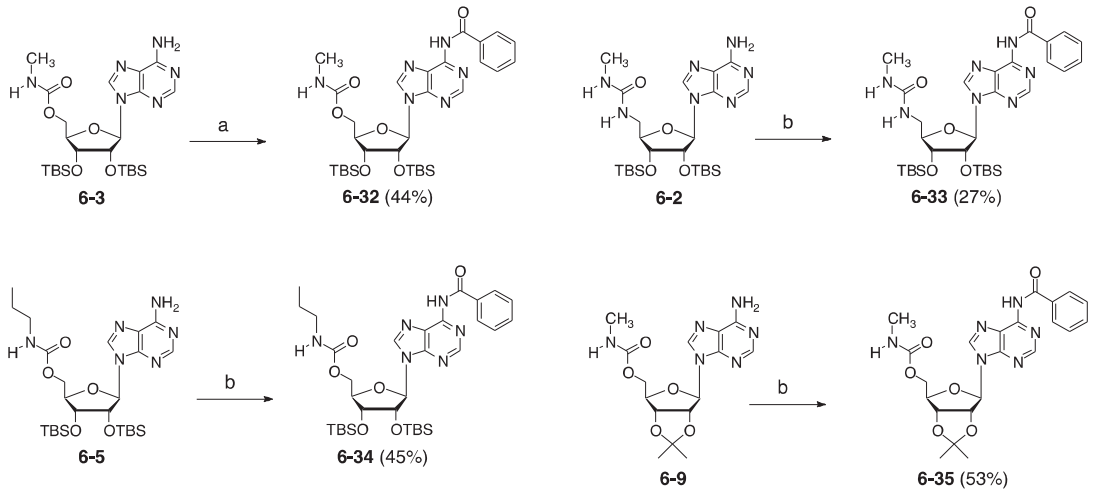
Biotin-Avidin complexes are widely used in conjuction with confocal microscopy to track the movement of molecules in biological settings.[8] Derivative **6-31** was prepared to test if a biotin-linked analogue of lead **6-1** would retain anticancer activity (Scheme 5). If it did, then it could be conjugated with Avidin *in vitro* and visualized with confocal microscopy, hopefully

shedding light on the mechanism of action of **6-1** and other members of this class of compounds. Biotinylated **6-31** was prepared in one step from starting material **6-30** in 51% yield.



Scheme 4. Synthesis of biotinylated derivative **6-31**.
Reagents: (a) Δ; Amine-PEG2-Biotin was purchased from Thermo Scientific.

Derivative **6-32** initially showed some promising antiviral activity (Scheme 5). Analogues **6-33**–**6-35** were designed to probe various aspects of its SAR. Installation of the N^6-benzoyl groups of **6-33**–**6-35** was attempted following the same procedure[9] we had applied for preparation of **6-32** (BzCl then NH_4OH, H_2O, pyridine). For these derivatives, that procedure turned out to be problematic, and it was decided to form the other N^6- benzoyl derivatives by treatment with benzoic anhydride with mild heat. This simpler reaction gave slightly higher yields for **6-34** (45%) and **6-35** (53%). Preparation of 5′-*N*-methyl urea **6-33** gave consistently low yields using either method. Perhaps this is due to the higher conformational rigidity of 5′-urea starting material **6-2** versus the 5′- carbamate starting material **6-3** (Chapter 2).[2]



Scheme 5. Synthesis of **6-32**–**6-35**.
Reagents: (a) BzCl, CH_2Cl_2, then NH_4OH, H_2O, pyridine (2:1:4); (b) $(Bz)_2O$, Δ.

6.3. Biology

6.3.1. Antiproliferative Assays

Compounds **6-2**–**6-7**, **6-9**, **6-10**, **6-12**, **6-14**, **6-16**, **6-18**, **6-20**, **6-21**, **6-23**, **6-25**, **6-27**, **6-29**, **6-31**–**6-35** were tested for their antiproliferative activity using murine leukemia L1210, human lymphoblastic leukemia CEM, and human cervix carcinoma HeLa (Table 1).

The data show that 5′-urea derivatives **6-2** and **6-4** were equal and even slightly better in activity than lead **6-1**. The 5′-urea derivative **6-6** had significant antiproliferative activity, although about 2–3 times higher than the lead. Interestingly, 5′-carbamate analogues **6-3** and **6-5** both showed slightly increased anticancer activity over **6-1**, and **6-7** showed significant activity as well. The essentially complete lack of antiproliferative activity of compounds **6-9** and **6-10** underscore the necessity of the 2′,3′-*O*-TBS groups. The weak anticancer activity of **6-12** and **6-14** indicate that biological activity is better achieved with TBS groups at both the 2′ and 3′ positions. Inosine **6-16** and uridine **6-18** derivatives showed strong anticancer activity, though

roughly two times higher than **6-1**. Cytidine analogues **6-20** and **6-21** as well as thymidine derivative **6-23** showed relatively weak antiproliferative activity. The phenyl analogue **6-25** did have antiproliferative activity, though significantly inferior to that of lead **6-1**. Derivatives **6-27** and **6-29** both showed potent antiproliferative activity, slightly superior to **6-1**. Biotinylated **6-31** showed significant biological activity, suggesting that it could be used for confocal microscopy imaging. The antiviral activity of **6-32** could not be repeated, and the compound showed weak anticancer activity. Analogues **6-33** and **6-34**, however, showed anticancer activity almost equal to lead **6-1**. The lack of activity of derivative **6-35** once again demonstrated the need of TBS groups at the 2′,3′ position.

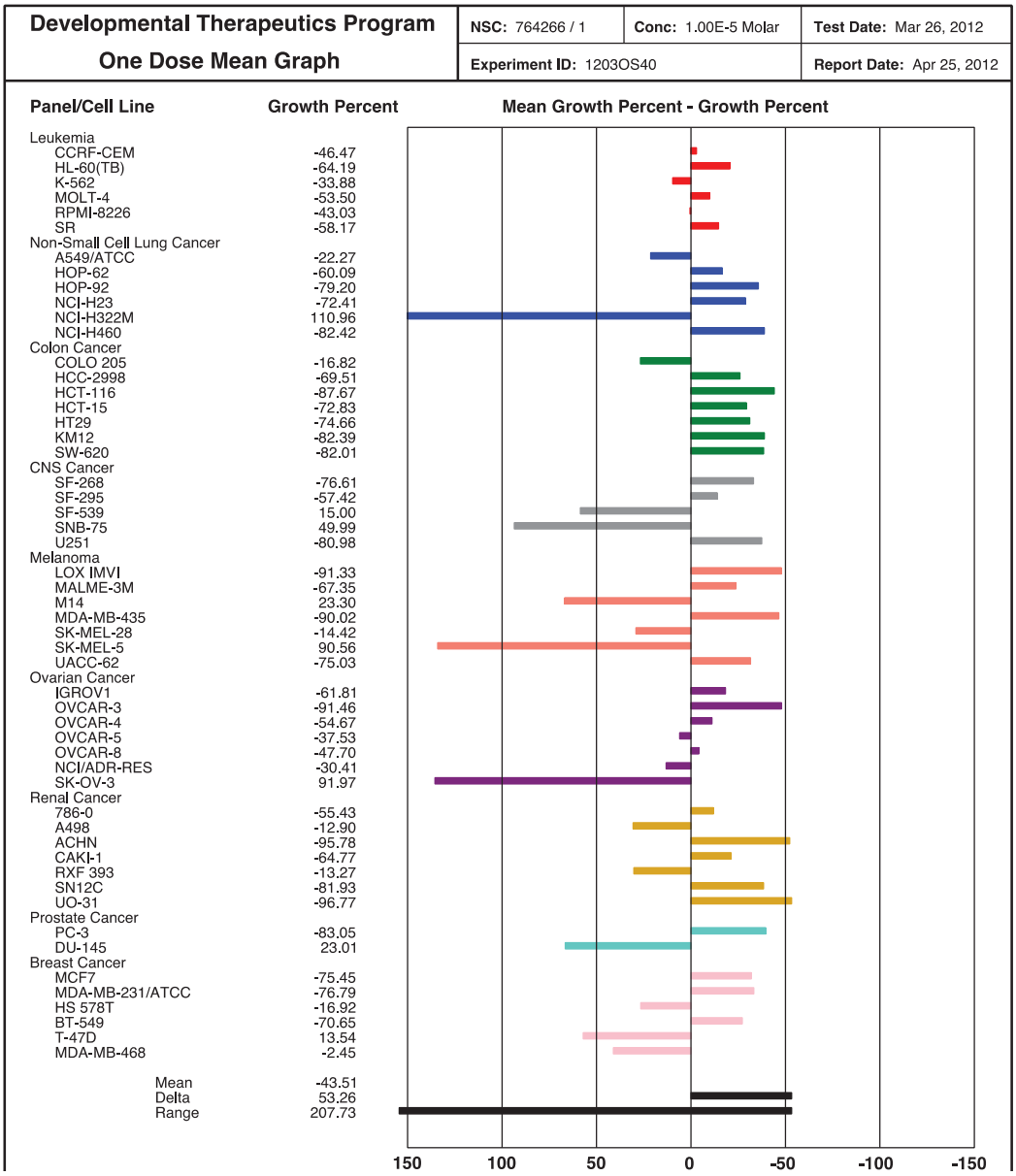
Table 1. Inhibitory effects of test compounds.
Inhibitory effects of the test compounds on the proliferation of murine leukemia cells (L1210), human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa). IC_{50} (μg/ml): 50% Inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

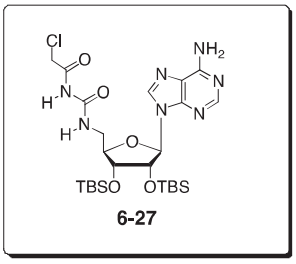
Compound	L1210	CEM	HeLa
6-1	3.8 ± 0.3	8.3 ± 2.9	3.2 ± 0.2
6-2	3.8 ± 0.1	4.2 ± 0.8	3.4 ± 0.2
6-3	2.0 ± 1.9	6.9 ± 1.9	3.1 ± 0.2
6-4	3.8 ± 0.3	3.6 ± 0.2	3.5 ± 0.1
6-5	0.12 ± 0.03	5.3 ± 0.4	2.6 ± 0.9
6-6	8.4 ± 4.6	6.6 ± 0.6	7.6 ± 3.1
6-7	0.83 ± 0.71	11 ± 3	5.4 ± 3.1
6-9	100	100	100
6-10	99 ± 2	100	100
6-12	41 ± 2	47 ± 1	54 ± 0
6-14	59 ± 3	89 ± 15	54 ± 1
6-16	7.8 ± 0.4	9.6 ± 0.8	6.6 ± 0.6

6-18	7.5 ± 0.7	8.3 ± 1.1	7.3 ± 1.3
6-20	1.3 ± 0.6	68 ± 21	26 ±12
6-21	19 ± 3	31 ± 7	14 ± 2
6-23	44 ± 4	46 ± 7	70 ± 21
6-25	51 ± 3	>100	39 ± 6
6-27	0.75 ± 0.16	0.74 ± 0.10	3.1 ± 0.7
6-29	2.2 ± 1.9	0.86 ± 0.25	2.9 ± 0.8
6-31	8.8 ± 0.0	9.2 ± 1.3	8.3 ± 0.8
6-32	47 ± 9	35 ± 24	32 ± 33
6-33	3.7 ± 0.1	3.5 ± 0.0	3.3 ± 0.1
6-34	0.63 ± 0.09	≥0.8	5.0 ± 1.0
6-35	100	100	100

Derivatives **6-27** and **6-29** were submitted to the National Cancer Institute Developmental Therapeutics Program. Compound **6-27** had modest to significant antiproliferative activity against many of the NCI-60 cell lines (Table 2). It showed strong activity against six of the seven colon cancer cell lines. Derivative **6-27** inhibited four non-small cell lung, four melanoma, and five renal cancer cells lines with growth percent values lower than the mean growth percent (Table 2). Compound **6-29** showed potent antiproliferative activity against all leukemia cell lines (Table 3). It also exhibited potent inhibition of three melanona cell lines and one non-small cell lung, ovarian, renal, and prostate cancer cell lines. Both compounds have been accepted for multi dose testing. The results will be forth coming.

Table 2. Single-dose growth inhibition for **6-27**.
Percent calculated as: $[(T_i - T_z)/C - T_z)] \times 100$ for $T_i \geq T_z$; $[(T_i - T_z)/T_z)] \times 100$ for $T_i < T_z$; where T_z = absorbance at t = 0; T_i = absorbance at t = 48 h (10 μM test compound); C = absorbance of control at t = 48 h.





6-27

Table 3. Single-dose growth inhibition for **6-29**.
Percent calculated as: $[(T_i - T_z)/C - T_z)] \times 100$ for $T_i \geq T_z$; $[(T_i - T_z)/T_z)] \times 100$ for $T_i < T_z$; where T_z = absorbance at t = 0; T_i = absorbance at t = 48 h (10 μM test compound); C = absorbance of control at t = 48 h.

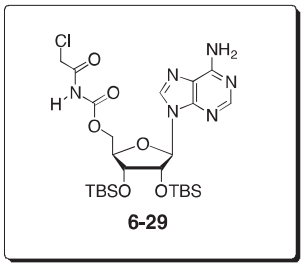
Developmental Therapeutics Program
One Dose Mean Graph

NSC: 764265 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 26, 2012
Experiment ID: 1203OS40		Report Date: Apr 25, 2012

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	-48.61
HL-60(TB)	-48.30
K-562	-37.59
MOLT-4	-54.69
RPMI-8226	-29.54
SR	-57.53
Non-Small Cell Lung Cancer	
A549/ATCC	62.55
HOP-62	81.93
HOP-92	12.72
NCI-H23	-45.00
NCI-H322M	113.01
NCI-H460	64.58
Colon Cancer	
COLO 205	27.45
HCC-2998	55.98
HCT-116	11.21
HCT-15	11.82
HT29	32.29
KM12	19.87
SW-620	-21.84
CNS Cancer	
SF-268	-19.61
SF-295	45.70
SF-539	89.78
SNB-75	101.42
U251	53.23
Melanoma	
LOX IMVI	-94.04
MALME-3M	-57.02
M14	18.41
MDA-MB-435	-28.23
SK-MEL-28	71.80
SK-MEL-5	61.39
UACC-62	75.16
Ovarian Cancer	
IGROV1	-68.27
OVCAR-3	0.63
OVCAR-4	36.94
OVCAR-5	77.45
OVCAR-8	-7.49
NCI/ADR-RES	46.64
SK-OV-3	87.77
Renal Cancer	
786-0	4.11
A498	105.37
ACHN	61.42
CAKI-1	19.31
RXF 393	63.99
SN12C	-47.55
UO-31	19.99
Prostate Cancer	
PC-3	-59.90
DU-145	46.23
Breast Cancer	
MCF7	10.57
MDA-MB-231/ATCC	35.65
HS 578T	74.46
BT-549	-17.86
T-47D	76.34
MDA-MB-468	65.01
Mean	20.74
Delta	114.78
Range	207.05

Mean Growth Percent - Growth Percent

150 100 50 0 -50 -100 -150



6-29

6.4. Discussion

The same synthetic routes followed in order to produce 2′,3′-bis-*O*-TBS-5′-*N*-methyl carbamoyl adenosine analogues can be extend to other nucleosides such as inosine, uridine cytidine, and thymidine (compounds **6-16**, **6-18**, **6-20**, **6-21**, and **6-23**, respectively). A similar synthetic route produced phenyl analogue **6-25**. Biotinylated derivatives of lead compounds (e.g., **6-31**) can be prepared using the same chemistry discussed in Chapter 2, in which a N^6-ethoxy carbonyl is displaced by a primary alkyl amine.

The biological data from this section reaffirm the importance of the 2′,3′-bis-*O*-TBS groups as well as the 5′-*N*-alkyl carbamoyl moiety for biological activity. However, the N^6-phenyl urea is not vital for activity and analogues without it are just as active, if not slightly more active, than lead **6-1** (e.g., **6-2**–**6-5**, **6-27**, and **6-29**). Not only is this moiety not needed for activity, but the N^6-region can also accommodate large substituents like biotin without losing anticancer activity (**6-31**). It is hoped that derivative **6-31** can be used for confocal microscopy studies which will shed light on the mechanism of action of our compounds. The data also showed the very interesting result that without the N^6-phenyl urea, 5′-carbamate analogues are just as active as 5′-urea analogues. This greatly increases the synthetic ease of derivative preparation as the 5′-carbamates generally require three less steps than their 5′-urea congeners. The nucleobase can also be exchanged without loss of antiproliferative activity, although adenosine still gives superior results.

Results from these studies have produced compounds that are synthetically easier to prepare than lead compound **6-1** and as good or slightly better in biological activity. These compounds also have a lower molecular weight, less hydrogen bond acceptors and donors, and, therefore, better follow Lipinski's Rule of Five.

6.5. Experimental

6.5.1. Biology

6.5.1.1. Antiproliferative Assays

The cytostatic effects of the test compounds on murine leukemia cells (L1210), human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa) were evaluated as follows: an appropriate number of cells suspended in growth medium were allowed to proliferate in 200-lL-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), 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%.

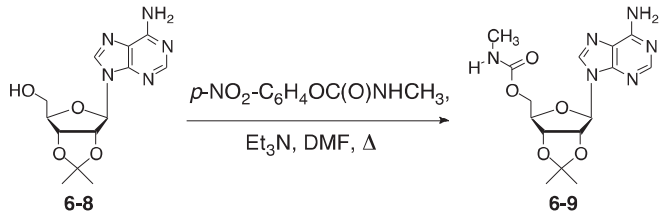
6.5.2. Chemistry

6.5.2.1. 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. 1H NMR and 13C NMR spectra were determined using internal references at δ 7.27 ($CDCl_3$), 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. All compounds tested were >95% pure

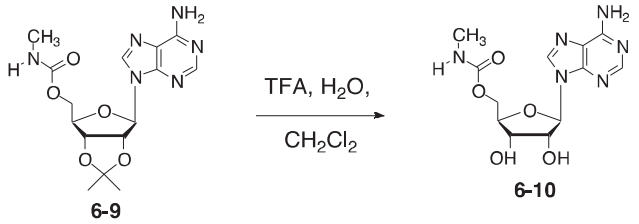
(as determined by HPLC; 5–10% IPA/CH_2Cl_2).

6.5.2.2. Compound Characterization Data



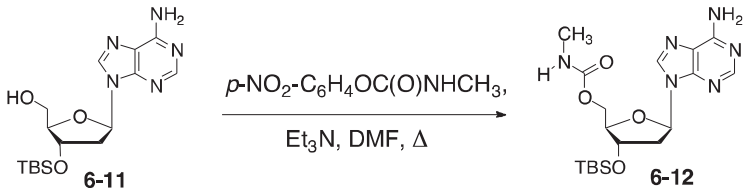
2′,3′-*O*-Isopropylidene-5′-(*N*-methylcarbomyl)adenosine (6-9).

A solution of **6-8** (200 mg, 0.65 mmol), *p*-nitrophenyl-*N*-methylcarbamate (200 mg, 1.02 mmol), Et_3N (400 μL) in DMF (3 mL) was stirred 60 °C overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 2→5% MeOH/CH_2Cl_2 to give **6-9** (95 mg, 0.26 mmol, 40%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.34 (s, 1H), 7.91 (s, 1H), 6.11 (d, *J* = 1.5 Hz, 1H), 5.96 (bs, 2H), 5.48 (dd, *J* = 2.8, 6.5 Hz, 1H), 5.04 ("t", *J* = 2.8 Hz, 1H), 4.82 (bs, 1H), 4.60 (bs, 1H), 4.47 (bs, 1H), 4.35 (dd, *J* = 4.0, 11.5 Hz, 1H), 4.23 (dd, *J* = 6.0, 11.5 Hz, 1H), 2.77 (d, *J* = 3.0 Hz, 3H), 1.62 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.3, 156.6, 155.8, 153.3, 149.5, 139.9, 120.4, 114.7, 91.2, 85.5, 84.3, 81.8, 64.6, 47.3, 27.5, 25.5; MS (ES) *m/z* 364.1484 (M+ [$C_{15}H_{20}N_6O_5$])= 364.1495.



5′-(*N*-Methylcarbomyl)adenosine (6-10).

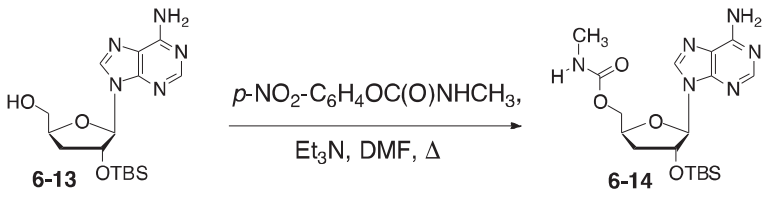
A solution of **6-9** (50 mg, 0.14 mmol), TFA (600 μL), H_2O (150 μL) in CH_2Cl_2 (3 mL) was stirred at ambient temperature for 4 hours. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 8→10% MeOH/CH_2Cl_2 to give **6-10** (35 mg, 0.11 mmol, 79%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.67 (s, 1H), 8.50 (s, 1H), 7.63 (s, 2H), 7.50 (s, 1H), 6.25 (d, J = 4.5 Hz, 1H), 5.86 (bs, 1H), 5.70 (bs, 1H), 5.00 (bs, 1H), 4.60 (d, J = 6.0 Hz, 1H), 4.50 (bs, 1H), 4.45 (s, 1H), 4.41 (bs, 1H), 2.77 (d, J = 3.0 Hz, 3H); ^{13}C NMR (DMF-d^7, 125 MHz) δ 151.6, 151.2, 147.8, 134.5, 114.1, 82.0, 77.5, 68.1, 65.7, 59.2, 25.8, 22.0; MS (ES) *m/z* 324.1189 (M+ [$C_{12}H_{16}N_6O_5$])= 324.1182.



3′-*O*-*tert*-Butyldimethylsilyl-2′-deoxy-5′-(*N*-methylcarbamoyl)adenosine (6-12).

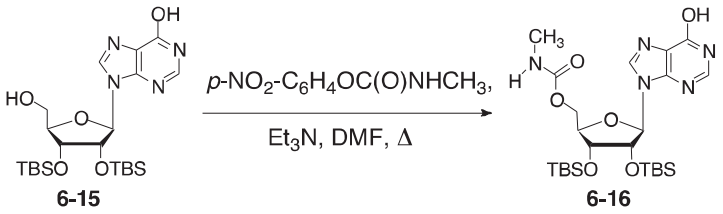
A solution of **6-11** (31 mg, 0.085 mmol), *p*-nitrophenyl-*N*-methylcarbamate (31 mg, 0.16 mmol), Et_3N (40 μL) in DMF (1 mL) was stirred at 60 °C overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash

chromatography column and eluted with 3→6% MeOH/CH_2Cl_2 to give **6-12** (17 mg, 0.040 mmol, 47%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.34 (s, 1H), 7.98 (s, 1H), 6.38 (t, *J* = 6.3 Hz, 1H), 5.93 (bs, 2H), 4.88 (bs, 1H), 4.60 (dd, *J* = 4.5, 12.9 Hz, 1H), 4.36 (dd, *J* = 6.5, 11.7 Hz, 1H) 4.24 (dd, *J* = 2.1, 11.7 Hz, 1H), 4.14 (t, *J* = 4.2 Hz, 1H), 2.84 (dd, *J* = 6.2, 13.1 Hz, 1H), 2.78 (d, *J* = 4.5 Hz, 3H), 2.44 (ddd, *J* = 4.8, 6.3, 7.2 Hz, 1H), 0.91 (s, 9H), 0.11 (s, 6H) ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.8, 155.7, 153.2, 149.7, 139.4, 120.4, 85.6, 84.9, 72.3, 64.2, 40.6, 27.8, 25.9, 18.2, –4.5, –4.7; MS (ES) *m/z* 422.2099 (M+ [$C_{18}H_{30}N_6O_4Si$])= 422.2098.



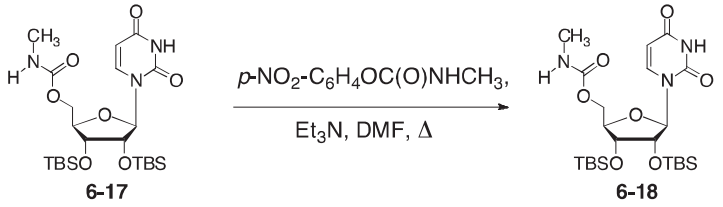
2′-*O*-*tert*-Butyldimethylsilyl-3′-deoxy-5′-(*N*-methylcarbamoyl)adenosine (6-14).

A solution of **6-13** (30 mg, 0.082 mmol), *p*-nitrophenyl-*N*-methylcarbamate (31 mg, 0.16 mmol), Et_3N (40 μL) in DMF (1 mL) was stirred at 60 °C overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 3→6% MeOH/CH_2Cl_2 to give **6-14** (15 mg, 0.035 mmol, 43%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.34 (s, 1H), 8.08 (s, 1H), 5.93 (s, 1H), 5.59 (bs, 2H), 4.89 (d, *J* = 4.5 Hz, 1H), 4.78 (bs, 1H), 4.72 (dd, *J* = 3.2, 5.3 Hz, 1H), 4.46 (dd, *J* = 2.6, 7.5 Hz, 1H) 4.36 (dd, *J* = 5.0, 12.3 Hz, 1H), 2.81 (d, *J* = 4.8 Hz, 3H), 2.16–2.06 (m, 1H), 1.96 (dd, *J* = 7.8, 13.8 Hz, 1H) 0.91 (s, 9H), 0.11 (s, 6H) ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.8, 155.4, 153.1, 139.2, 128.0, 114.1, 93.0, 79.5, 72.3, 65.3, 34.8, 27.9, 25.9, 18.1, –4.5, –4.8, –11.1; MS (ES) *m/z* 422.2104 (M+ [$C_{18}H_{30}N_6O_4Si$])= 422.2098.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)inosine (6-16).

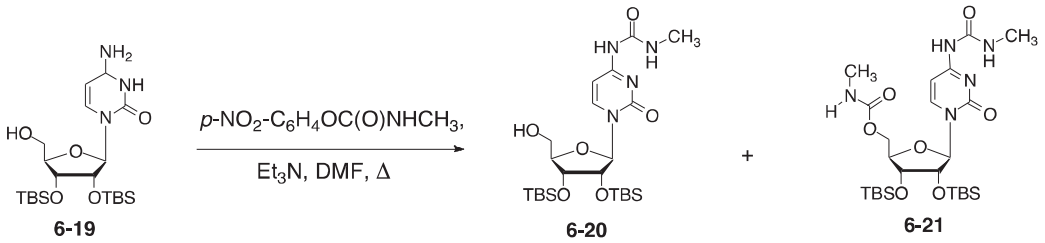
A solution of **6-15** (26 mg, 0.052 mmol), *p*-nitrophenyl-*N*-methylcarbamate (26 mg, 0.13 mmol), Et_3N (30 μL) in DMF (1 mL) was stirred at 60 °C overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 2% MeOH/CH_2Cl_2 to give **6-16** (22 mg, 0.041 mmol, 78%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.99 (bs, 1H), 8.25 (s, 2H), 5.97 (d, *J* = 3.5 Hz, 1H), 5.37 (bs, 1H), 4.58 (t, *J* = 3.5 Hz, 1H), 4.46 (dd, *J* = 3.0, 12.0.0 Hz, 1H), 4.40 (dd, *J* = 3.0, 12.0 Hz, 1H), 2.85 (d, *J* = 5.0 Hz, 3H), 0.92 (s, 9H), 0.86 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H), 0.03 (s, 3H), –0.07 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.1, 156.7, 148.7, 145.3, 139.3, 125.4, 89.5, 82.9, 76.2, 71.6, 63.2, 28.0, 26.0, 25.9, 18.2, 18.1, –4.2, –4.5, –4.7, –4.8; MS (ES) *m/z* 553.2755 (M+ [$C_{24}H_{43}N_5O_6Si_2$])= 553.2752.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)uridine (6-18).

A solution of **6-17** (29 mg, 0.061 mmol), *p*-nitrophenyl-*N*-methylcarbamate (30 mg, 0.15 mmol), Et_3N (10 μL) in DMF (1 mL) was stirred at 60 °C overnight. Volatiles were

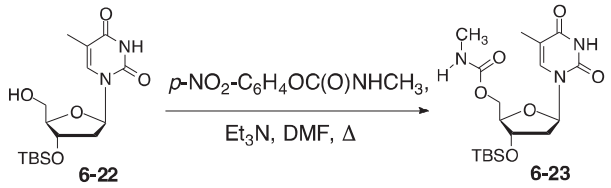
removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 2→5% MeOH/CH_2Cl_2 to give **6-18** (24 mg, 0.045, 74%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.03 (bs, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 5.73 (d, *J* = 8.0 Hz, 1H), 5.66 (d, *J* = 2.5 Hz, 1H), 4.71 (d, *J* = 4.0 Hz, 1H), 4.40 (dd, *J* = 2.0, 12.5 Hz, 1H), 4.28 (dd, *J* = 4.3, 12.0 Hz, 1H), 4.23 (bs, 1H), 4.21 (t, *J* = 3.0 Hz, 1H), 3.98 (t, *J* = 4.8 Hz, 1H), 2.84 (d, *J* = 5.0 Hz, 3H), 0.91 (s, 18H), 0.12 (s, 3H), 0.10 (s, 3H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ163.4, 156.5, 150.3, 140.3, 102.1, 91.1, 82.0, 75.4, 71.2, 71.0, 63.4, 28.0, 26.0, 26.0, 18.2, –4.1, –4.3, –4.7, –4.9; MS (ES) *m/z* 529.2637 (M+ [$C_{23}H_{43}N_3O_7Si_2$])= 529.2640.



2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-N^4-(*N*-methylcarbomyl)cytidine (6-20); **2′,3′-Bis-*O*-*tert*-butyldimethylsilyl-N^4-(*N*-methylcarbomyl)-5′-(*N*-methylcarbamoyl)cytidine (6-21).**

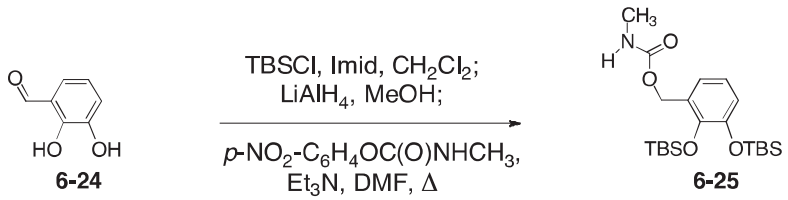
A solution of **6-19** (31 mg, 0.066 mmol), *p*-nitrophenyl-*N*-methylcarbamate (31 mg, 0.16 mmol), Et_3N (40 μL) in DMF (1 mL) was stirred at 60 °C overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 2→3% MeOH/CH_2Cl_2 to give the products **6-20** (8 mg, 0.015 mmol, 23%) and **6-21** (4 mg, 0.0068 mmol, 10%). **6-20**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.69 (bs, 1H), 9.01 (bs, 1H), 8.02 (d, *J* = 7.5 Hz, 1H), 7.71 (bs, 1H), 5.45 (d, *J* = 3.5 Hz, 1H), 4.62 (t, *J* = 3.5 Hz, 1H), 4.19–4-16 (m, 2H), 4.03 (d, *J* = 12.0 Hz, 1H), 3.75 (dd, *J* = 7.0, 11.5 Hz, 1H), 3.24 (bs, 1H), 2.84 (bs, 3H), 0.91 (s, 9H), 0.90 (s, 9H), 0.09 (s, 9H), 0.01 (s, 3H); ^{13}C NMR

($CDCl_3$, 125 MHz) δ 165.3, 157.1, 154.8, 145.7, 130.2, 110.2, 97.6, 95.7, 85.7, 73.8, 71.1, 61.4, 29.9, 26.6, 26.1, 18.3, 18.2, –4.2, –4.4, –4.6, –4.7; MS (ES) *m/z* 528.2793 (M^+ [$C_{23}H_{44}N_4O_6Si_2$])= 528.2799. **6-21**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.81 (bs, 1H), 9.21 (bs, 1H), 8.08 (d, *J* = 7.0 Hz, 1H), 7.67 (bs, 1H), 5.63 (s, 1H), 4.63 (s, 1H), 4.56 (d, *J* = 7.0 Hz, 1H), 4.40–4-28 (m, 2H), 4.21–4.16 (m, 1H), 3.89 (bs, 1H), 2.87 (d, *J* = 5.0 Hz, 3H), 2.84 (bs, 3H), 0.91 (s, 9H), 0.90 (s, 9H), 0.09 (s, 9H), 0.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.3, 157.1, 156.0, 154.8, 145.7, 130.2, 110.2, 97.6, 95.7, 85.7, 73.8, 71.1, 61.4, 29.9, 26.6, 26.5, 26.1, 18.3, 18.2, –4.2, –4.4, –4.6, –4.7; MS (ES) *m/z* 585.3017 (M+ [$C_{25}H_{47}N_5O_7Si_2$])= 585.3014.



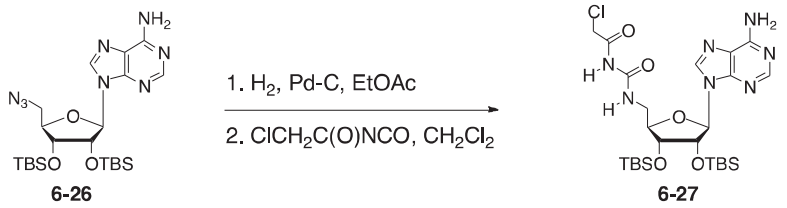
3′-*O-tert*-Butyldimethylsilyl-5′-(*N*-methylcarbamoyl)thymidine (6-23).

A solution of **6-22** (60 mg, 0.17 mmol), *p*-nitrophenyl-*N*-methylcarbamate (80 mg, 0.41 mmol), Et_3N (50 μL) in DMF (1 mL) was stirred at 60 °C overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 2% MeOH/CH_2Cl_2 to give **6-23** (40 mg, 0.097 mmol, 57%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.52 (s, 1H), 6.24 (t, *J* = 6.5 Hz, 1H), 4.70 (bs, 1H), 4.35 (d, *J* = 4.0 Hz, 1H), 4.32 (dd, *J* = 3.3, 8.8 Hz, 1H), 4.21 (dd, *J* = 5.5, 11.8 Hz, 1H), 4.06 (d, *J* = 4.0 Hz, 1H), 2.84 (d, *J* = 5.0 Hz, 3H), 2.32 (ddd, *J* = 3.0, 5.5, 13.0 Hz, 1H), 2.04 (p, *J* = 6.8 Hz, 1H), 1.94 (s, 3H), 0.90 (s, 9H), 0.09 (s, 6H) ^{13}C NMR ($CDCl_3$, 125 MHz) δ 163.7, 156.7, 150.2, 135.5, 111.2, 85.7, 85.4, 72.3, 64.3, 41.1, 25.9, 18.2, 12.8, –4.5, –4.7; MS (ES) *m/z* 413.1990 (M+ [$C_{18}H_{31}N_3O_6Si$])= 413.1982.



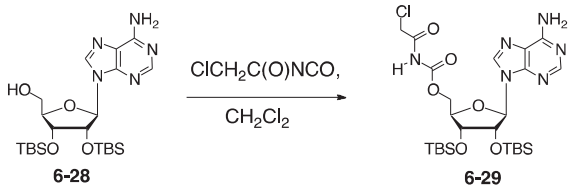
***N*-Methyl-[(2,3-bis-*O*-*tert*-butyldimethylsilyloxy)phenyl]methylcarbamate (6-25).**

A solution of **6-24** (6.5 mg, 0.047 mmol), TBSCl (18 mg, 0.12 mmol), and imidazole (26 mg, 0.38 mmol) in CH_2Cl_2 (0.5 mL) was stirred at ambient temperature (2 h). Reaction was filtered through a silica gel plug (5% EtOAc/hexanes). Volatiles were removed under reduced pressure, and the crude reaction mixture was dried overnight. The crude material was then charged with sodium borohydride (0.75 mg, 0.020 mmol) and dissolved in MeOH (0.5 mL) and stirred at room temperature for 1.5 hours. The reaction was quenched with sodium bicarbonate (0.01 mL). Volatiles were removed under reduced pressure after liquid-liquid extraction with CH_2Cl_2 and water. The crude material was dried over vacuum overnight. The crude material was then treated with *p*-nitrophenoly-*N*-methyl carbamate (7 mg, 0.036), Et_3N (5 μL) in DMF (0.5 mL) and stirred at 60 °C overnight. Volatiles were removed under reduced pressure and the crude reaction material was added directly to a Flash chromatography column and eluted with 1→5% EtOAc/hexanes to give **6-25** (5 mg, 0.012 mmol, 25%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.12–7.95 (m, 2H), 6.08 (dd, J = 5.3, 11.3 Hz, 1H), 4.91 (d, J = 8.5 Hz, 1H), 4.61 (s, 2H), 2.88 (d, J = 8.5 Hz, 3H), 0.99 (s, 9H), 0.94 (s, 9H), 0.21 (s, 6H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 154.4, 147.8, 138.8, 126.1, 120.0, 118.8, 60.3, 31.2, 28.0, 26.1, 25.8, 18.6, 18.3, –4.25, –5.12.



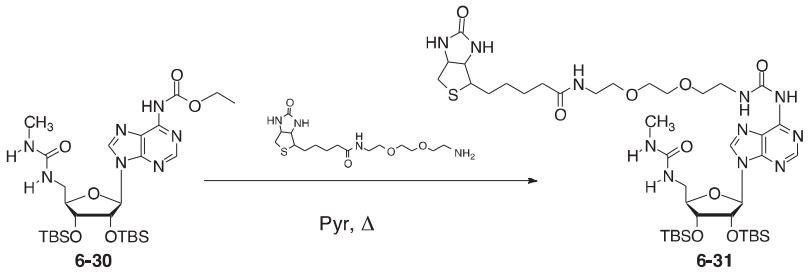
2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-[((chloroacetyl)aminocarbonyl)amino]-5′-deoxy-adenosine (6-27).

A solution of **6-26** (45 mg, 0.091 mmol) and 10% Pd–C (34 mg) in EtOAc (4.0 mL) was stirred overnight under an atmosphere of H_2 (balloon pressures). The catalyst was removed via filtration (celite) and volatiles were removed under reduced pressure. The resulting crude material was dried under vacuum pump for two days and then dissolved in CH_2Cl_2 (1 mL) and charged with chloroacetyl isocyanate (22 mg, 0.18 mmol). The mixture was stirred at ambient temperature until TLC showed complete consumption of starting material (1 d). The crude mixture was added directly to a Flash chromatography column and eluted with 2→4% MeOH/CH_2Cl_2 to give **6-27** (26 mg, 0.042 mmol, 46%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.47 (bs, 1H), 8.36 (s, 1H), 7.94 (s, 1H), 5.84 (d, *J* = 5.5 Hz, 1H), 5.82 (bs, 2H), 5.02 (bs, 1H), 4.32 (bs, 1H), 4.23 (bs, 1H), 4.08 (bs, 2H), 3.76 (d, *J* = 6.0 Hz, 2H), 0.95 (s, 9H), 0.79 (s, 9H), 0.14 (s, 3H), 0.14 (s, 3H), – 0.07 (s, 3H), –0.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ, 167.3, 155.8, 153.2, 152.8, 149.8, 140.9, 121.0, 89.9, 84.1, 74.2, 73.5, 42.6, 41.9, 26.0, 25.9, 18.3, 18.1, – 4.2, – 4.5, – 4.5, – 5.0; MS (ES) *m/z* 614.2630 (M+ [$C_{25}H_{44}ClN_7O_5Si_2$])= 614.2648.



2′,3′-Bis-*O-tert*-butyldimethylsilyl-5′-[(chloroacetyl)aminocarbonyl]adenosine (6-29).

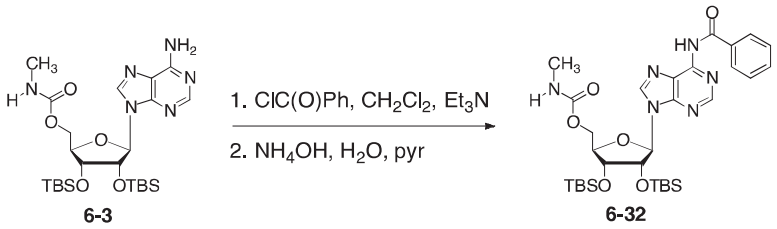
A solution of **6-28** (39 mg, 0.079 mmol) and chloroacetyl isocyanate (30 mg, 0.025 mmol) in CH_2Cl_2 (1 mL) was stirred at ambient temperature (2 h). The crude mixture was added directly to a Flash chromatography column and eluted with 2→4% MeOH/CH_2Cl_2 to give **6-29** (25 mg, 0.041 mmol, 51%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.76 (bs, 1H), 8.28 (bs, 1H), 8.07 (s, 1H), 6.25 (s, 1H), 5.78 (d, *J* = 5.5 Hz, 1H), 5.28 (t, *J* = 4.1 Hz, 1H), 4.73 (t, *J* = 5.0 Hz, 1H), 4.65 (bs, 2H), 4.56 (d, *J* = 8.7 Hz, 1H), 4.42–4.32 (m, 2H), 3.76 (d, *J* = 6.0 Hz, 2H), 0.96 (s, 9H), 0.88 (s, 9H), 0.18 (s, 3H), 0.17 (s, 3H), 0.00 (s, 3H), –0.18 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ, 169.7, 155.8, 153.3, 152.5, 149.2, 142.1, 119.9, 91.6, 82.0, 73.8, 72.6, 66.6, 44.6, 26.1, 25.9, 18.3, 18.1, – 4.0, – 4.4, – 4.5, – 4.7; MS (ES) *m/z* 614.2480 (M+ [$C_{25}H_{43}ClN_6O_6Si_2$]) = 614.2471.



***N*^{6}-[((2-(*N*-Biotinyl)amino)eth-1-yl)-2-hydroxyeth-1-yl)-2-hydroxyeth-1-yl)]carbamoyl-2′,3′-bis-*O-tert*-butyldimethylsilyl-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino]adenosine (6-31).**

A solution of starting material (40 mg, 0.064 mmol), and biotin (36 mg, 0.096 mmol) in pyridine (1 mL) was stirred at 80 °C overnight. Volatiles were removed under reduced pressure,

and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 7→9% MeOH/CH_2Cl_2 to give **6-31** (31 mg, 0.033 mmol, 51%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.13 (s, 1H), 9.44 (s, 1H), 8.82 (s, 1H), 6.82 (t, *J* = 5.2 Hz, 1H), 6.66 (bs, 1H), 6.42 (q, *J* = 3.9 Hz, 1H), 6.06 (d, *J* = 7.8 Hz, 1H), 5.65 (d, *J* = 4.5 Hz, 1H), 5.51 (bs, 1H), 4.59 (dd, *J* = 4.6, 7.5 Hz, 1H), 4.41 (d, *J* = 4.8 Hz, 1H), 4.38 (bs, 1H), 4.15 (bs, 2H), 3.96 (ddd, *J* = 2.6, 7.8, 14.5 Hz, 1H), 3.72–3.61 (m, 8H), 3.57 (t, *J* = 5.0 Hz, 2H), 3.39–3.35 (m, 2H), 3.12 (dt, *J* = 3.8, 15.0 Hz, 3H), 3.01 (6.3 Hz, 1H), 2.85 (d, *J* = 4.8 Hz, 1H), 2.79 (d, *J* = 4.5 Hz, 3H), 2.69 (s, 1H), 2.65 (s, 1H), 2.22 (bs, 1H), 2.14–2.03 (m, 2H), 1.60–1.46 (m, 2H), 1.28 (p, *J* = 7.4 Hz, 2H), 0.96 (s, 9H), 0.69 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H), –0.12 (s, 3H), –0.47 (s, 3H) ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.4, 164.0, 159.4, 155.2, 150.9, 150.8, 150.5, 143.6, 121.0, 88.0, 87.7, 75.8, 73.5, 70.4, 70.2, 70.0, 69.7, 61.8, 60.1, 55.5, 41.5, 40.3, 39.2, 35.8, 28.0, 27.9, 27.0, 25.9, 25.6, 18.1, 17.8, –4.5, –4.5, –4.7, –5.6; MS (ES) *m/z* 951.4780 (M+ [$C_{41}H_{73}N_{11}O_9SSi_2$])= 951.4852.

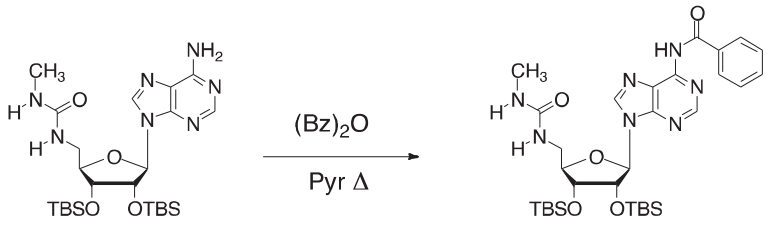


6-3 **6-32**

N^6-(*N*-Benzoyl)-2′,3′-bis-*O*-*tert*-butyldimethylsilyl-5′-(*N*-methylcarbamoyl)adenosine (6-32).

A solution of **6-3** (100 mg, 0.18 mmol), benzoyl chloride (70 mg, 0.50 mmol), Et_3N (100 µl) CH_2Cl_2 (2.8 mL) was stirred at ambient temperature overnight. Excess benzoyl chloride was added to quickly get to the bis-acylated intermediate. The intermediate was directly treated with NH_4OH, H_20, Pyr (100 µl, 25 µl, 50 µl) for one hour. A second volume of NH_4OH, H_20, Pyr was then added, and the reaction was quenched with sodium bicarbonate (200 µl) after 30

minutes. Volatiles were removed under reduced pressure and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 30→75% EtOAc/hexanes to give **6-32** (53 mg, 0.081 mmol, 44%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.98 (bs, 1H), 8.79 (s, 1H), 8.25 (s, 1H), 8.03 (d, *J* = 7.5 Hz, 2H) 7.63 (t, *J* = 7.0 Hz, 1H), 7.54 (t, *J* = 7.5 Hz, 1H), 5.99 (d, *J* = 4.0 Hz, 1H), 4.89 (t, *J* = 3.5 Hz, 1H), 4.77 (d, *J* = 4.0 Hz, 1H), 4.50 (dd, J= 3.0, 11.5 Hz, 1H), 4.36-4.29 (m, 3H), 2.83 (d, *J* = 5.0 Hz, 3H), 0.93 (s, 9H), 0.86 (s, 9H), 0.09 (s, 3H), 0.09 (s, 3H), 0.04 (s, 3H), -0.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.3, 165.1, 156.6, 152.5, 151.7, 149.7, 142.5, 133.7, 132.9, 129.0, 128.1, 124.1, 89.9, 85.4, 82.7, 75.0, 71.7, 63.7, 63.4, 27.8, 25.9, 25.8, 25.4, 18.2, 18.1, -4.23, -4.55, -4.68, -4.85; MS (FAB) *m/z* 679.3045 (MNa+ $[C_{31}H_{48}N_6O_6Si_2]$)= 679.3066.

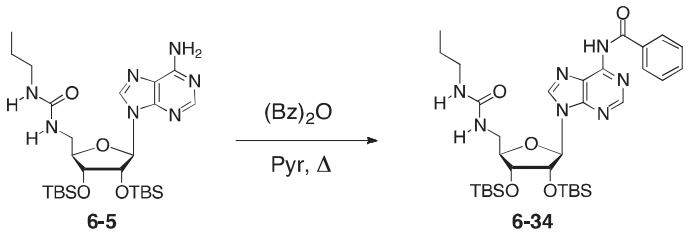


6-2 **6-33**

N^6-(*N*-Benzoyl)-2′,3′-bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-[(*N*-methylcarbamoyl)amino]adenosine (6-33).

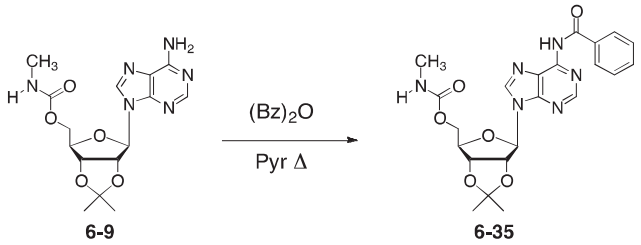
A solution of **6-2** (50 mg, 0.91 mmol) and benzoic anhydride (43 mg, 0.19 mmol) in pyridine (2 mL) was stirred at 50 °C overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 5% MeOH/CH_2Cl_2 to give **6-33** (16 mg, 0.024 mmol, 27%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.13 (s, 1H), 8.84 (s, 1H), 8.06 (s, 1H), 8.04 (d, *J* = 7.0 Hz, 2H), 7.64 (t, *J* = 7.3 Hz, 1H), 7.55 (t, *J* = 7.3 Hz, 2H), 7.03 (d, *J* = 8.0 Hz, 1H), 5.84 (d, *J* = 8.0 Hz, 1H), 4.83(dd, *J* = 4.3, 7.8 Hz, 1H),

4.47 (dd, *J* = 4.5, 9.5 Hz, 1H), 4.28 (d, *J* = 4.5 Hz, 1H), 4.22 (t, *J* = 2.5 Hz, 1H), 4.02 (ddd, *J* = 2.8, 9.0, 14.3 Hz, 1H), 3.25 (dt, *J* = 2.8, 14.5 Hz, 1H), 2.84 (d, *J* = 4.5 Hz, 3H), 0.96 (s, 9H), 0.71 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H), –0.14 (s, 3H), –0.57 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.6, 159.2, 153.2, 152.3, 151.0, 150.7, 144.0, 133.7, 133.2, 129.2, 128.0, 124.7, 90.6, 88.3, 74.0, 73.6, 41.8, 27.5, 26.0, 25.9, 25.8, 18.2, 18.0, –4.3, –4.4, –4.6, –5.5; MS (ES) *m/z* 656.3417 (MH+ [$C_{31}H_{50}N_7O_5Si_2$) = 656.3406.



N^6-(*N*-Benzoyl)-2′,3′-bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-(*N*-propylcarbamoyl)adenosine (6-34).

A solution of **6-5** (75 mg, 0.13 mmol) and benzoic anhydride (46 mg, 0.20 mmol) in pyridine (1 mL) was stirred at 50 °C overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 1% MeOH/CH_2Cl_2 to give **6-34** (40 mg, 0.058 mmol, 45%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.13 (s, 1H), 8.78 (s, 1H), 8.25 (s, 1H), 8.02 (d, *J* = 7.5 Hz, 2H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 2H), 5.98 (d, *J* = 4.0 Hz, 1H), 4.87 (bs, 2H), 4.47 (dd, *J* = 2.3, 11.8 Hz, 1H), 4.35 (dd, *J* = 4.0, 12.3 Hz, 1H), 4.31 (t, *J* = 3.3 Hz, 1H), 3.20–3.10 (m, 2H), 1.53 (sext, *J* = 7.8 Hz, 2H), 0.92 (s, 9H), 0.90 (t, *J* = 7.5 Hz, 3H), 0.84 (s, 9H), 0.09 (s, 3H), 0.02 (s, 3H), –0.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.8, 156.1, 152.7, 151.7, 149.8, 142.5, 133.8, 133.0, 129.1, 128.1, 124.1, 90.0, 82.9, 75.0, 72.0, 63.4, 43.1, 26.0, 25.9, 23.4, 18.2, 18.1, 11.4, –4.2, –4.5, –4.7, –4.8; MS (ES) *m/z* 685.3582 (MH+ [$C_{33}H_{53}N_6O_6Si_2$) = 685.3560.



N^6-(*N*-Benzoyl)-2′,3′-*O*-isopropylidine-5′-(*N*-methylcarbamoyl)adenosine (6-35).

A solution of **6-9** (50 mg, 0.14 mmol) and benzoic anhydride (46 mg, 2.0 mmol) in pyridine (1 mL) was stirred 50 °C overnight. Volatiles were removed under reduced pressure, and the crude reaction mixture was added directly to a Flash chromatography column and eluted with 2→3% MeOH/CH_2Cl_2 to give **6-35** (34 mg, 0.073 mmol, 53%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.09 (s, 1H), 8.81 (s, 1H), 8.17 (bs, 1H), 8.03 (d, *J* = 7.5 Hz, 2H), 7.62 (t, *J* = 7.3 Hz, 1H), 7.53 (t, *J* = 7.8 Hz, 2H), 6.21 (s, 1H), 5.48 (d, *J* = 5.0 Hz, 1H), 5.03 (d, *J* = 3.0 Hz, 1H), 4.72 (bs, 1H), 4.54 (d, *J* = 3.0 Hz, 1H), 4.35–4.27 (m, 2H), 2.75 (d, *J* = 4.5 Hz, 2H), 1.64 (s, 3H), 1.42 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.9, 156.4, 153.0, 151.6, 149.9, 142.9, 133.7, 133.1, 129.1, 128.6, 128.1, 124.0, 114.9, 114.1, 91.6, 85.8, 84.5, 81.7, 64.5, 27.9, 27.4, 25.6; MS (ES) *m/z* 685.3582 (MH+ [$C_{33}H_{53}N_6O_6Si_2$) = 685.3560.

6.6. References

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