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Parallel Solution-Phase Synthesis of an Adenosine Antibiotic Analog Library

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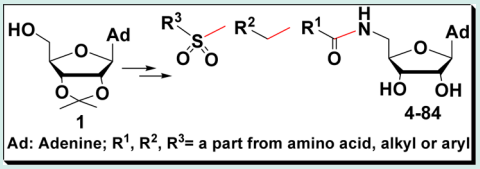
Supporting Information

ABSTRACT: A library of eighty one adenosine antibiotic analogs was prepared under the Pilot Scale Library Program of the NIH Roadmap initiative from 5′-amino-5′-deoxy-2′,3′-*O*-isopropylidene-adenosine **3**. Diverse aldehyde, sulfonyl chloride and carboxylic acid reactant sets were condensed to **3**, in solution-phase fashion, leading after acid-mediated hydrolysis to the targeted compounds in good yields and high purity. No marked antituberculosis or anticancer activity was noted on preliminary cellular testing, but these nucleoside analogs should be useful candidates for other types of biological activity.



KEYWORDS: *5′-amino, carboxamide and sulfonyl-adenosine antibiotic analogs*

INTRODUCTION

Historically, nucleoside chemistry has primarily focused on low throughput production and screening of analogs for anticancer, antifungal, and antiviral antimetabolite activities.[1−8] Beyond the traditional nucleosides with an available 5′-hydroxyl that can enter nucleoside metabolic pathways, there are numerous examples of relatively simple to complex nucleoside antibiotics that exhibit diverse alternative mechanisms of action.[9,10] Natural nucleoside antibiotics, for example, demonstrate potent activities, such as protein synthesis inhibition, glycosyltransferase inhibition, and methyltransferase inhibition, among others.[9−13] More recently, there has been a trend to develop approaches for the synthesis and screening of this class of compounds, which do not exhibit typical antimetabolite activities based on nucleoside phosphorylation and incorporation into nucleoside metabolic pathways.[14−18] In many respects, the nucleoside scaffolds offer an opportunity for numerous diverse and directionally oriented substitutions to probe for activity against diverse protein binding sites. Furthermore, many of these basic scaffolds are not well represented in commercial chemical space, including the Molecular Library Small Molecule Repository (MLSMR) [see PubChem Substance at http://www.ncbi.nlm.nih.gov/pcsubstance]. Hence, we have designed and prepared nucleoside antibiotic-like small molecule libraries under the Pilot Scale Library Program of the NIH Roadmap Initiative to probe specific or general biological activities. We report herein the initial phase of this project which is the synthesis of a library of eighty one 5′-amido-, amino-, and sulfonylamido-adenosine analogs derived from 5′-amino-5′-deoxy-2′,3′-*O*-isopropylidene-adenosine **3** (Scheme 1) using parallel solution phase chemistry.

Both **3** or its analog 5′-amino-5′-deoxy-adenosine are suitable precursors for the synthesis of a variety of biologically active nucleoside analogs[19−36] dating back to the first report describing the synthesis of 5′-amino-5′-deoxy-5′-*N*-aminoacyl peptide derivatives of guanosine, and adenosine and their effects on cell-free protein synthesis.[37] Hence, the 5′-amino group of the nucleoside ribose moiety was chosen as a site of diversification through robust peptidyl chemistry in order to prepare the target library of 5′-amido derivatives **4**−**34** (Scheme 1). The resulting nucleoside peptide library was expected to show reasonable stability to dissolution, storage and screening as evidenced for similar aminoacyl functions found in various nucleoside antibiotics, such as puromycin, gougerotin, amicetin, and blasticidin S, all known inhibitors of protein synthesis. Thus, **3** was synthesized in two steps (Scheme 1) starting from 2′,3′-*O*-isopropylidene adenosine **1**, which was reacted with triphenylphosphine, 1,3-dihydro-1,3-dioxo-2*H*-isoindole and diisopropyl azodicarboxylate to give 5′-deoxy-5′-(1,3-dihydro-l,3-dioxo-2*H*-isoindol-2-y1)-2′,3′-*O*-isopropylidene adenosine **2**. Hydrazinolysis of **2** with hydrazine monohydrate in ethanol gave **3** in 78% yield according to the previously reported method.[19]

To achieve our targeted 5′-amido compounds **4**−**34** (Figure 1), we explored several methods for peptide coupling of **3** to diverse and readily available carboxylic acid-substituted or *N*-*tert*-butyloxycarbonyl (Boc) amino acid derivatives using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and *N*,*N*′-dicyclohexylcarbodiimide (DCC). HATU [(2-(7-Aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] has been reported to rapidly provide the peptide linkage in high yield and with little or no racemization. In our hands, the best results were obtained with HATU (1 equiv) and *N*,*N*-diisoproplyethylamine (DIEA, 1.5 equiv) in acetonitrile for 30−180 min. The utility of HATU allowed the facile preparation of the title compounds with a variety substituents and ready adaptation to a parallel format using a Radleys 12-place carousel reaction station on a 0.5 mmol scale. Subsequent acid-mediated deprotection of the acetonide

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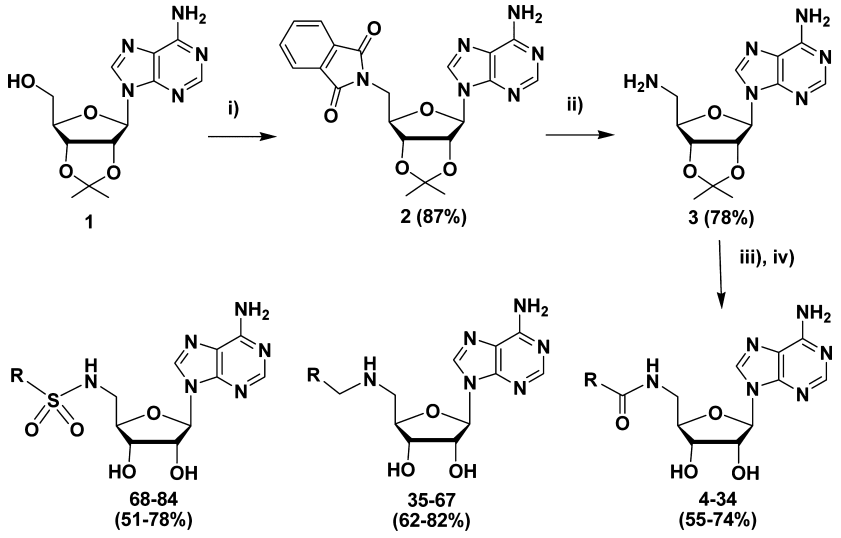
Scheme 1[a]



[a]Reagents and conditions: (i) phthalamide, PPh_3, DIAD; (ii) H_2NNH_2, EtOH; (iii) R = a part from N-Boc amino acid, HATU, DIEA, CH_3CN; R = a part from an aldehyde, MeOH, molecular sieves, 0−40 °C, $NaBH_4$; R = a part from sulfonyl chloride, DMF, $CsCO_3$; (iv) 50% formic acid, 70 °C.

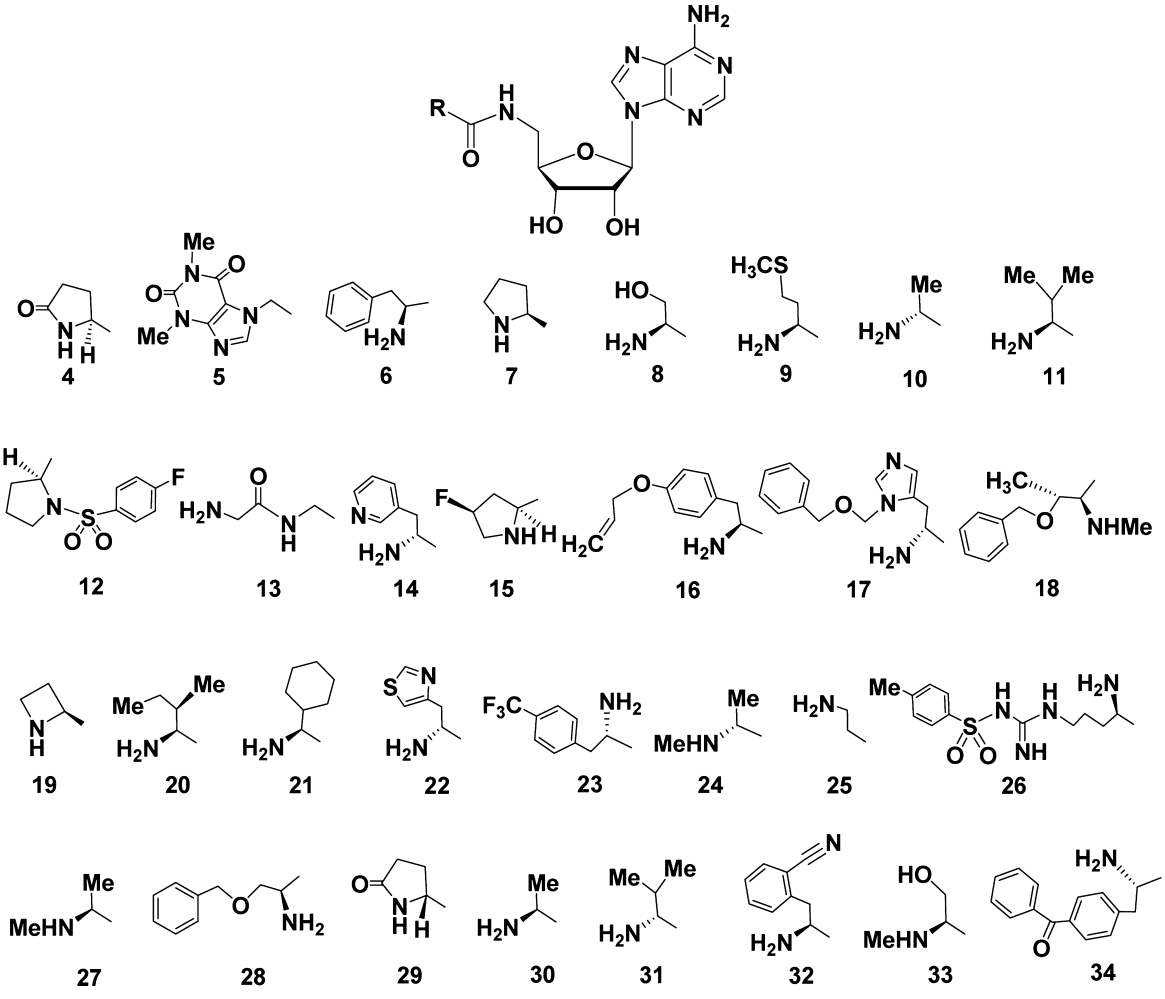


Figure 1. Structures of compounds **4**−**34**.

as well as the Boc protecting group using 50% formic acid, again in a parallel format, furnished the desired compounds **4**−**34** in quantitative yields and high purity.

Next, we prepared a diverse small library of 5′-*N*-alkyl-5′-deoxy-adenosine derivatives **35**−**67** (Figure 2) based upon the important biological activity and stability exhibited by

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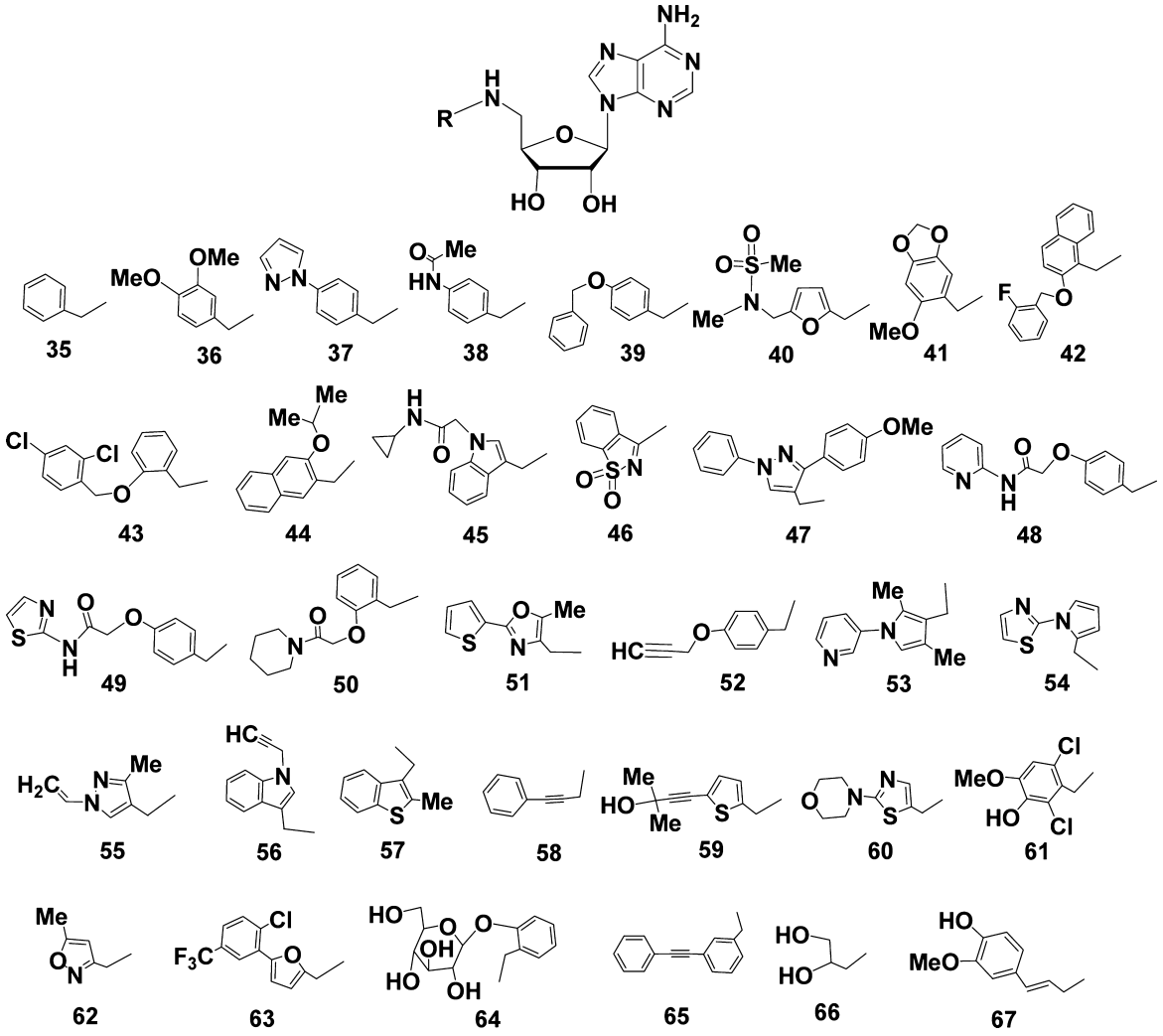

Figure 2. Structures of compounds **35**−**67**.

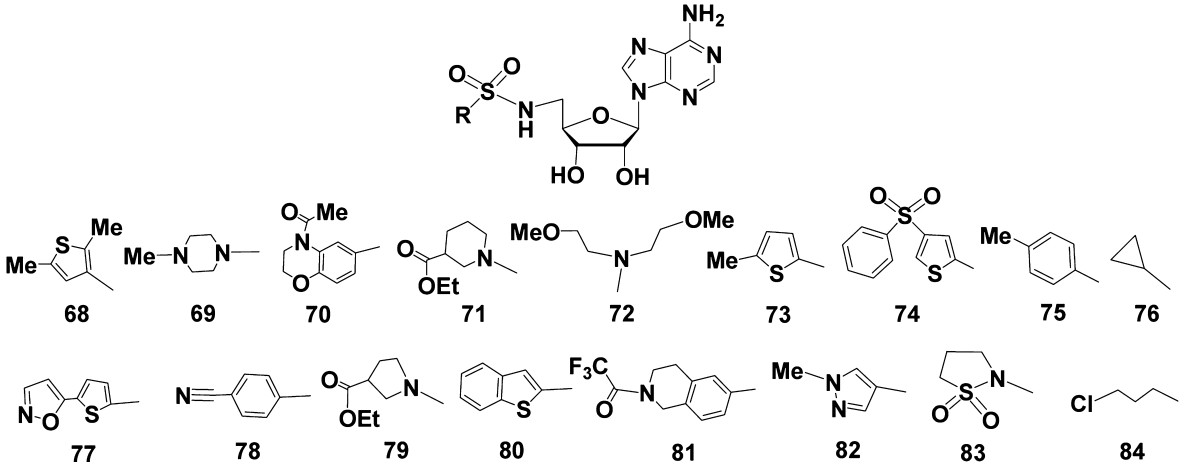

Figure 3. Structures of compounds **68**−**84**.

known natural analogues. Reductive amination is an efficient method that is readily adaptable to parallel format and, hence, we adapted this reaction to couple compound **3** with thirty three commercially available aldehydes. Successful couplings were achieved in methanol in the presence of molecular sieves to efficiently drive intermediate imine formation. It must be noted that the use of molecular sieves was crucial in terms of yield improvement and reaction time. The reaction was also readily adapted to a parallel format on a Radleys 12-place carousel reaction station at room temperature, although occasionally reactions were facilitated with less soluble aldehydes by warming for the first ten minutes at 40 °C.

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The resulting aldimines were carefully treated in situ with solid sodium borohydride for one-half hour and the reaction was then preadsorbed and dried on silica gel without further workup followed by flash chromatography purification. Similarly, the hydrolysis of the isopropylidene blocking groups of the resulting intermediates using 50% formic acid was successful leading to the desired targets **35**−**67** in quantitative yields.

The synthesis of sulfonylamide adenosine analogs **68**−**84** (Figure 3) was achieved by reacting intermediate **3** with seventeen commercially available sulfonyl chlorides. The reactions were carried out in *N*,*N*-dimethylformamide (DMF) at room temperature using cesium carbonate as base followed by hydrolysis of the isopropylidene blocking groups as described above.

BIOLOGICAL EVALUATION

The described analogs were tested in vitro for their effectiveness against *Mycobacterium tuberculosis* (*Mtb*).[38] None of the compounds showed appreciable activity at compound concentrations lower than 100 μM. Only compound **42** exhibited slight inhibition with an IC_{50} of 43 μM. Also, they were screened in vitro against three human tumor cell lines (HT29 colon, PC3 prostate, and MDA-MB-231 breast). Only compounds **39**, **42**, and **43** showed toxicity at less than 50 μM in these cells as shown in Table 1 (details of these biological studies are provided in Supporting Information).

Table 1. Effect of Prepared Analogs on Cell Growth

	cancer screen		
compound	HT29 (CC_{50})[a]	PC3 (CC_{50})[a]	MDA (CC_{50})[a]
4−**38**, **40**−**41**, and **44**−**84**	>50	>50	>50
39	**14**	**45**	**31**
42	**9**	**24**	>50
43	**12**	>50	>50

[a]CC_{50} = Concentrations of compound required for 50% growth inhibition of cancer cells.

All the prepared analogs have been submitted (20 mg) in the Molecular Libraries Small Molecule Repository (MLSMR) to be screened against a wide range of biological assays (see www.ncbi.nlm.nih.gov/pcsubstance search term Robert Reynolds). Certain analogs (Figure 4) exhibited interesting activities in these primary screens. For example, adenosine peptides **9** and **21** were found to be malaria HSP40-mediated yeast toxicity inhibitors; **16** was identified as an inhibitor of G-Protein coupled receptor kinase-2 (GRK2) (protein target = beta-adrenergic receptor kinase 1); **39** was identified as an activator of methionine sulfoxide reductase A (MsrA); compound **48** was found to be inhibitor of prion protein 5′ UTR mRNA; **58** and **63** were identified as inhibitors of the histone demethylase GASC-1 (gene amplified in squamous cell carcinoma-1) and vitamin D receptor (VDR) respectively; sulfonylamide adenosine compound **71** inhibited the interaction of LANA 1−23 peptide with purified nucleosomes (containing H2A/H2B histone dimers) while the derivative **74** was found to inhibit scavenger receptor class B using DiI-HDL.

CONCLUSION

In conclusion, a small library of eighty one adenosine antibiotic-like analogs derived from 5′-amino-5′deoxy-2′,3′-*O*-isopropylideneadenosine was prepared in good yields and high purity using a parallel solution phase format. No marked anti-tuberculosis nor anticancer activity was witnessed and all the prepared analogs have been submitted for screening in the Molecular Libraries Probe Production Centers Network (MLPCN), preliminary screening via the MLPCN has

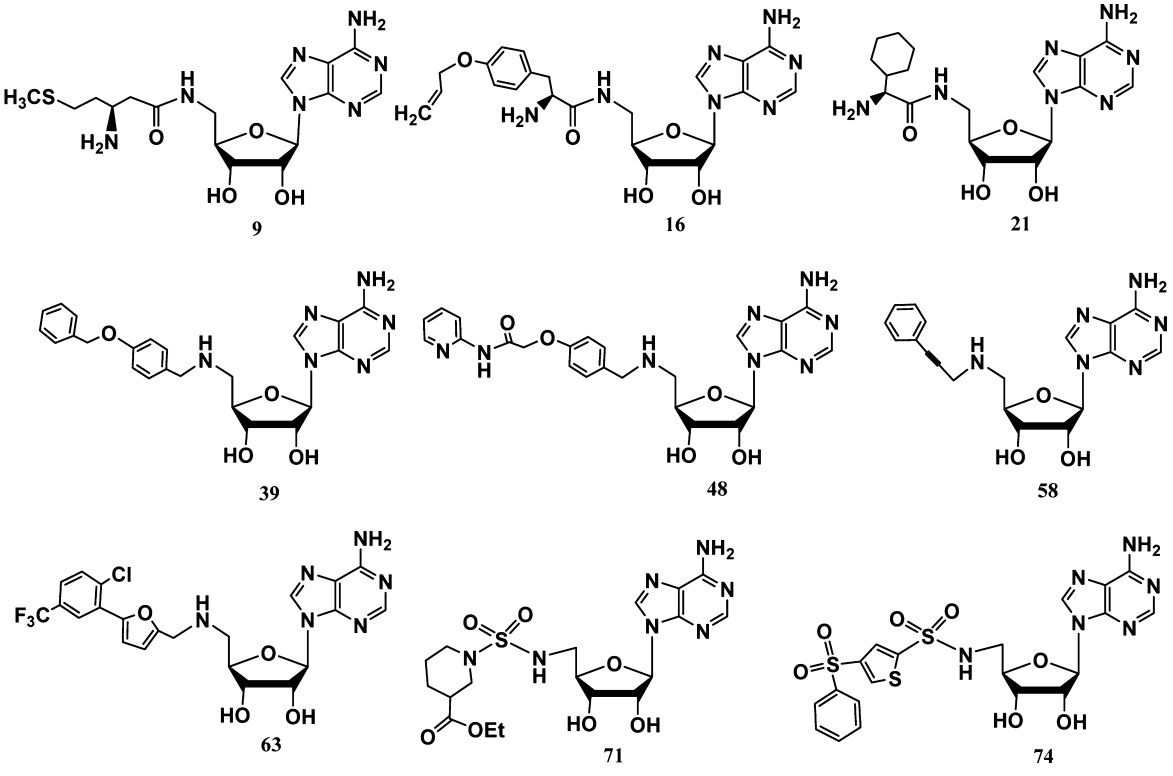

Figure 4. Some active adenosine analogues.

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indicated a variety of interesting activities although full evaluation of the libraries is still under way (see http://www.ncbi.nlm.nih.gov/pcsubstance search term Robert Reynolds).

■ ASSOCIATED CONTENT

Supporting Information

Additional material as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Robins, R. K.; Revankar, G. R. Purine analogs and related nucleosides and nucleotides as antitumor agents. *Med. Res. Rev.* **1985**, *5*, 273−296.

(2) Plunkett, W.; Saunders, P. P. Metabolism and action of purine nucleoside analogs. *Pharmacol. Ther.* **1991**, *49*, 239−268.

(3) Huryn, D. M.; Okabe, M. AIDS-driven nucleoside chemistry. *Chem. Rev.* **1992**, *92*, 1745−1768.

(4) Kolesar, J. M.; Morris, A. K.; Kuhn, J. G. Purine nucleoside analogues: fludarabine, pentostatin, and cladribine: Part 2: Pentostatin. *J. Oncol. Pharm. Pract.* **1996**, *2*, 211−224.

(5) Mansour, T. S.; Storer, R. Antiviral nucleosides. *Curr. Pharm. Des.* **1997**, *3*, 227−264.

(6) Tan, X.; Chu, C. K.; Boudinot, F. D. Development and optimization of anti-HIV nucleoside analogs and prodrugs: A review of their cellular pharmacology, structure−activity relationships and pharmacokinetics. *Adv. Drug Delivery Rev.* **1999**, *39*, 117−151.

(7) Cheson, B. D.; Keating, M. J.; Plunkett, W. *Nucleoside Analogues in Cancer Therapy*; Marcel Dekker: New York, 1997; Vol. *12*.

(8) Shuter, J. Antifungal and antiviral agents: A review. *Cancer Invest.* **1999**, *17*, 145−152.

(9) Isono, K. Current progress on nucleoside antibiotics. *Pharmacol. Ther.* **1991**, *52*, 269−286.

(10) Isono, K. Nucleoside antibiotics: Structure, biological activity and biosynthesis. *J. Antibiot.* **1988**, *41*, 1711−1739.

(11) Knapp, S. Synthesis of complex nucleoside antibiotics. *Chem. Rev.* **1995**, *95*, 1859−1876.

(12) Rosemeyer, H. The chemodiversity of purine as a constituent of natural products. *Chem. Biodiversity* **2004**, *1*, 361−401.

(13) Lagoja, I. M. Pyrimidines as constituent of natural biologically active compounds. *Chem. Biodiversity* **2005**, *2*, 1−50.

(14) Herforth, C.; Wiesner, J.; Franke, S.; Golisade, A.; Jomaa, A.; Link, A. Antimalarial activity of N6-substituted adenosine derivatives. *J. Comb. Chem.* **2002**, *4*, 302−314.

(15) Winans, K. A.; Bertozzi, C. R. An inhibitor of the human UDP-GlcNAc 4-epimerase identified from a uridine-based library: A strategy to inhibit O-linked glycosylation. *Chem. Biol.* **2002**, *9*, 113−129.

(16) Epple, R.; Kudirka, R.; Greenberg, W. A. Solid-phase synthesis of nucleoside analogues. *J. Comb. Chem.* **2003**, *5*, 292−310.

(17) Valade, A.; Urban, D.; Beau, J.-M. Target-assisted Selection of galactosyltransferase binders from dynamic combinatorial libraries. An unexpected solution with restricted amounts of the enzyme. *ChemBioChem.* **2006**, *7*, 1023−1027.

(18) Townsend, A. P.; Roth, S.; Williams, H. E. L.; Stylianou, E.; Thomas, N. R. New *S*-adenosyl-L-methionine analogues: Synthesis and reactivity studies. *Org. Lett.* **2009**, *11*, 2976−2979.

(19) Kolb, M.; Danzin, C.; Barth, J.; Claverie, N. Synthesis and biochemical and properties of chemically stable product analogues of the reaction catalyzed by S-adenosyl l-methionine decarboxylase. *J. Med. Chem.* **1982**, *25*, 550−556.

(20) Kolb, M.; Barth, J. Synthesis of 5′-[(3-aminooxypropyl)amino]-5′-deoxyadenosine. *Lieb. Ann. Chem.* **1985**, *5*, 1036−1040.

(21) Lever, O. W., Jr.; Vestal, B. R. Bridged isocytosine-adenosine compounds: synthesis and antibacterial evaluation. *J. Heterocycl. Chem.* **1986**, *23*, 901−903.

(22) Minnick, A. A.; Kenyon, G. L. General synthetic approach to stable nitrogen analogs of *S*-adenosylmethionine. *J. Org. Chem.* **1988**, *53*, 4952−61.

(23) Kvasyuk, E. I.; Kulak, T.; Mikhailopulo, I. A.; Charubala, R.; Pfleiderer, W. Nucleotides. Part XLV. Synthesis of new (2′−5′) adenylate trimers, containing 5′-amino-5′-deoxyadenosine residues at the 5′-end of the oligoadenylate chain, and of its analogs, carrying a 9-[(2-hydroxyethoxy)methyl]adenine residue at the 2′-terminus. *Helv. Chim. Acta* **1995**, *78*, 1777−84.

(24) Gareava, L. D.; Goryunova, O. V.; Yartseva, I. V.; Mashalova, N. A.; Mel'nik, S. Ya. Synthesis of modified purine ribonucleosides from 3′(5′)-*O*-succinyladenosine and 5′-amino-5′-deoxyadenosine. *Bioorg. Khim.* **1995**, *21*, 717−723.

(25) Ceulemans, G.; Vandendriessche, F.; Rozenski, J.; Herdewijn, P. Synthesis of an uncharged cAMP analog. *Nucleosides Nucleotides* **1995**, *14*, 117−127.

(26) Comstock, L. R.; Rajski, S. R. Expeditious synthesis of aziridine-based cofactor mimics. *Tetrahedron* **2002**, *58*, 6019−6026.

(27) Ciuffreda, P.; Loseto, A.; Santaniello, E. Deamination of 5′-substituted-2′,3′-isopropylidene adenosine derivatives catalyzed by adenosine deaminase (ADA, EC 3.5.4.4) and complementary enzymatic biotransformations catalyzed by adenylate deaminase (AMPDA, EC 3.5.4.6): a viable route for the preparation of 5′-substituted inosine derivatives. *Tetrahedron* **2002**, *58*, 5767−5771.

(28) Manfredini, S.; Solaroli, N.; Angusti, A.; Nalin, F.; Durini, E.; Vertuani, S.; Pricl, S.; Ferrone, M.; Spadari, S.; Focher, F.; Verri, A.; De Clercq, E.; Balzarini, J. Design and synthesis of phosphonoacetic acid (PPA) ester and amide bioisosters of ribofuranosylnucleoside diphosphates as potential ribonucleotide reductase inhibitors and evaluation of their enzyme inhibitory, cytostatic, and antiviral activity. *Antiviral Chem. Chemother.* **2003**, *4*, 183−194.

(29) Jagtap, P. G.; Southan, G. J.; Baloglu, E.; Ram, S.; Mabley, J. G.; Marton, A.; Salzman, A.; Szabo, C. The discovery and synthesis of novel adenosine substituted 2,3-dihydro-1*H*-isoindol-1-ones: potent inhibitors of poly(ADP-ribose) polymerase-1 (PARP-1). *Bioorg. Med. Chem. Lett.* **2004**, *14*, 81−85.

(30) Enkvis, E.; Lavogina, D.; Raidaru, G.; Vaasa, A.; Viil, I.; Lust, M.; Viht, K.; Uri, A. Conjugation of adenosine and hexa-(D-arginine) leads to a nanomolar bisubstrate-analog inhibitor of basophilic protein kinases. *J. Med. Chem.* **2006**, *49*, 7150−7159.

(31) Bisseret, P.; Thielges, S.; Bourg, S.; Miethke, M.; Marahiel, M. A.; Eustache, J. Synthesis of a 2-indolylphosphonamide derivative with inhibitory activity against yersiniabactin biosynthesis. *Tetrahedron Lett.* **2007**, *48*, 6080−6083.

(32) Dowden, J.; Hong, W.; Parry, R. V.; Pike, R. A.; Ward, S. G. Toward the development of potent and selective bisubstrate inhibitors of protein arginine methyltransferases. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2103−2105.

(33) Mori, S.; Iwase, K.; Iwanami, N.; Tanaka, Y.; Kagechika, H.; Hirano, T. Development of novel bisubstrate-type inhibitors of histone methyltransferase SET7/9. *Bioorg. Med. Chem.* **2010**, *18*, 8158−8166.

(34) Dowden, J.; Pike, R. A.; Parry, R. V.; Hong, W.; Muhsen, U. A.; Ward, S. G. Small molecule inhibitors that discriminate between

protein arginine *N*-methyltransferases PRMT1 and CARM1. *Org. Biom. Chem.* **2011**, *9*, 7814−7821. Hong, W.; Dowden, J. Facile synthesis of *N*-6 adenosine modified analogue toward *S*-adenosyl methionine derived probe for protein arginine methyltransferases. *Chin. Chem. Lett.* **2011**, *22*, 1439−1442.

(35) Yao, Y.; Chen, P.; Diao, J.; Cheng, G.; Deng, L.; Anglin, J. L.; Prasad, B. V. V; Song, Y. Selective inhibitors of histone methyltransferase DOT1L: Design, synthesis, and crystallographic studies. *J. Am. Chem. Soc.* **2011**, *133*, 16746−16749.

(36) Felczak, K.; Chen, L.; Wilson, D.; Williams, J.; Vince, R.; Petrelli, R.; Jayaram, H. N.; Kusumanchi, P.; Kumar, M.; Pankiewicz, K. W. Cofactor-type inhibitors of inosine monophosphate dehydrogenase via modular approach: targeting the pyrophosphate binding sub-domain. *Bioorg. Med. Chem.* **2011**, *19*, 1594−1605.

(37) Robins, M. J.; Simon, L. N.; Stout, M. G.; Ivanovics, G. A.; Schweizer, M. P.; Rousseau, R. J.; Robins, R. K. Nucleoside peptides. I. Synthesis of 5′-deoxy-5′-amino-5′-*N*-aminoacyl peptide derivatives of guanosine, adenosine, and 2′-deoxyadenosine and their effect of cell-free protein synthesis. *J. Am. Chem. Soc.* **1971**, *98*, 1474−1480.

(38) Ananthan, S.; Faaleolea, E. R.; Goldman, R. C.; Hobrath, J. V.; Kwong, C. D.; Laughon, B. E.; Maddry, J. A.; Mehta, A.; Rasmussen, L.; Reynolds, R. C.; Secrist, J. A., 3rd; Shindo, N.; Showe, D. N.; Sosa, M. I.; Suling, W. J.; White, E. L. High-throughput screening for inhibitors of Mycobacterium tuberculosis H37Rv. *Tuberculosis* **2009**, *89*, 334−353.