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Solution-Phase Parallel Synthesis of Acyclic Nucleoside Libraries of Purine, Pyrimidine, and Triazole Acetamides

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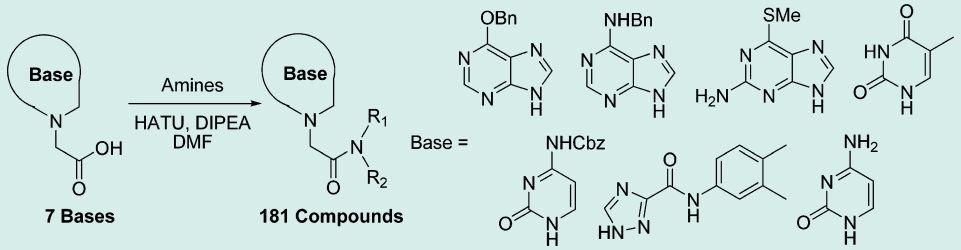
Supporting Information



ABSTRACT: Molecular diversity plays a pivotal role in modern drug discovery against phenotypic or enzyme-based targets using high throughput screening technology. Under the auspices of the Pilot Scale Library Program of the NIH Roadmap Initiative, we produced and report herein a diverse library of 181 purine, pyrimidine, and 1,2,4-triazole-*N*-acetamide analogues which were prepared in a parallel high throughput solution-phase reaction format. A set of assorted amines were reacted with several nucleic acid *N*-acetic acids utilizing HATU as the coupling reagent to produce diverse acyclic nucleoside *N*-acetamide analogues. These reactions were performed using 24 well reaction blocks and an automatic reagent-dispensing platform under inert atmosphere. The targeted compounds were purified on an automated purification system using solid sample loading prepacked cartridges and prepacked silica gel columns. All compounds were characterized by NMR and HRMS, and were analyzed for purity by HPLC before submission to the Molecular Libraries Small Molecule Repository (MLSMR) at NIH. Initial screening through the Molecular Libraries Probe Production Centers Network (MLPCN) program, indicates that several analogues showed diverse and interesting biological activities.

KEYWORDS: *solution-phase, parallel synthesis, nucleoside libraries, acyclic nucleosides, high-throughput screening, drug discovery*

INTRODUCTION

The introduction of rapid DNA sequencing and the genomic sciences, combinatorial chemistry, and cell- and protein-based assays, under automated high throughput screening (HTS) has led to a new paradigm in drug discovery.[1] The ultimate goal is to translate the massive output from these higher throughput technologies faster than historical approaches and to more rapidly establish the roles of new biological targets in human disease. The use of novel libraries of chemical probes in the MLSMR is allowing a long-term vision to be realized through new government initiatives for developing highly selective drug-like candidates that will swiftly translate to benefits in public health. In this approach, small molecule chemical probes are used to study specific enzymatic processes, protein−protein interactions, or even whole-cell metabolic pathways in a high throughput chemical biology approach.[2] As such, high quality, diverse and unique small molecules are constantly in demand in order to probe a wider range of new biological space. In response, over the last several decades, special attention has been directed at providing diverse and well-characterized sets of small molecule probes for numerous HTS screens against an ever-growing platform of new biological targets in both phenotypic and enzyme-based assays. For example, a major goal in the field of diversity-oriented synthesis has been the

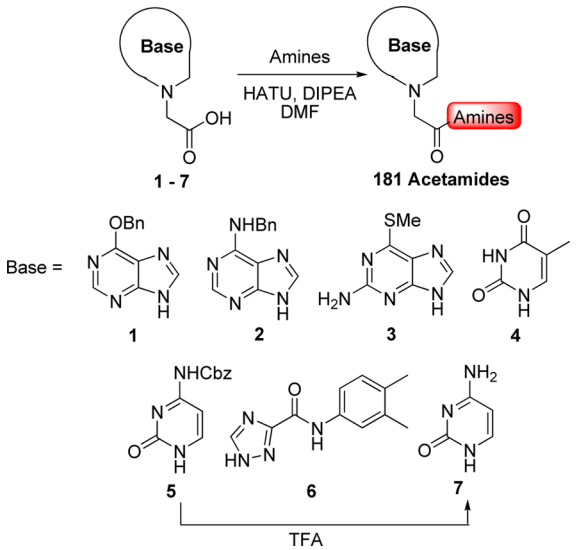


Figure 1. General representation of library structures.

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Table 1. Predicted Values of Physicochemical Properties of Library Members

entry	parameter	range	av
1	molecular weight (MW)	239−504	371.5
2	LogP	−1.88−4.29	1.21
3	LogD at pH 7.40	−3.13−4.28	0.58
4	number of H-bond donor	0−4	2.0
5	number of H-bond acceptors	3−10	6.5
6	total polar surface area (TPSA) (Å^2)	66.90−194.76	130.83
7	polar solvent accessible surface area (polar SASA) (Å^2)	76.38−298.86	187.62

efficient production of small-molecule libraries in biologically relevant chemical space.[3] Subsequently, screening across a wide range of newly developed biological HTS assays provides direct biological validation of the functional capabilities of these libraries. Toward this end, diverse varieties of natural product-based or inspired libraries have been synthesized, and early results are promising.[4]

The Pilot Scale Library (PSL) Program was implemented to specifically produce libraries based on biologically relevant scaffolds with greater chemical diversity than provided by commercial chemical library space. Furthermore, successful approaches used to generate these libraries should be robust, leading to easily purified samples in high enough yields to allow deposition of sufficient material to supply the NIH Roadmap screening centers. Ideally, a funded grant would produce small diverse sets (∼15−30 members) from highly biologically relevant scaffolds. Sample acceptance into the MLSMR requires compounds with >90% purity by HPLC and fully characterized by NMR and MS spectral techniques. These small diverse sets would possess, in theory, broader biological relevance, and would more likely lead to hits against the numerous new targets deriving from genomics and disease pathway and metabolism analyses. For the purpose of chemical library generation, special emphasis has been given to chemical reactions in parallel and combinatorial formats using solution-phase and solid supported approaches on these diverse chemical scaffolds.[5] Solid-phase synthesis is used extensively as it has provided ease of purification. On the other hand, certain benefits naturally accrue from solution-phase approaches including good yields of higher quantities of targets based on reaction homogeneity. Both of these techniques are crucial to produce the required numbers of diverse new small molecules at reasonable cost.

Natural nucleic acids and synthetic analogues are components of numerous clinically used drugs, particularly antiviral, and anticancer agents.[6] Typical nucleoside drugs function as antimetabolites, and act through direct incorporation into nucleoside metabolic pathways interfering in downstream enzyme-mediated reactions or the general processes of RNA or DNA structure and function. Drugs based on these natural scaffolds show diverse activities including anticancer, antiparasitic, antifungal and antiviral action. For example, a number of nucleoside-based antimetabolites are used for cancer treatment such as, cytarabine (cytosine arabinoside, Ara-C), an antileukemic agent used for the treatment of acute myeloid leukemia and non-Hodgkin's lymphoma; Purinethol (6-mercaptopurine, 6-MP) used for the acute lymphoblastic leukemia, 5-fluorouracil (Efudex; 5-FU, a thymidylate synthase inhibitor used for the treatment of colon, rectal, stomach, skin, breast, and pancreatic cancer; antileukemic agents Vidaza (5-azacytidine) and Dacogene (2′-deoxy-5-azacytidine, decitabine) currently the most successful agents approved for therapy of myelodysplastic syndrome. Acyclovir [Zovirax, 9-(2-hydroxyethoxymethyl)guanine] is an acyclic guanine analogue that selectively misincorporates into DNA in infected cells via the herpesvirus thymidine kinase and is active against HSV-1 and HSV-2 infections.[7] Acyclovir, similar to the many other antiherpetic agents (penciclovir, ganciclovir, and their pro-drug forms), belongs to the group of acyclic nucleoside analogues, compounds having the sugar furanose ring substituted in a nucleoside with a polyhydroxylic

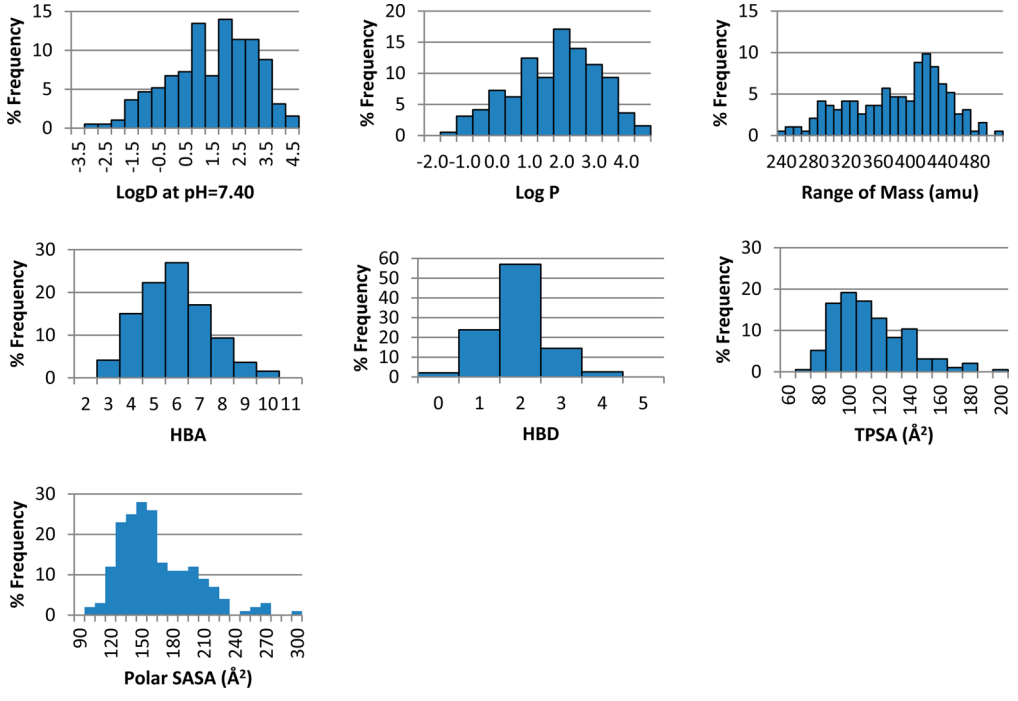

Figure 2. Graphical representations of physicochemical properties distribution of library members.

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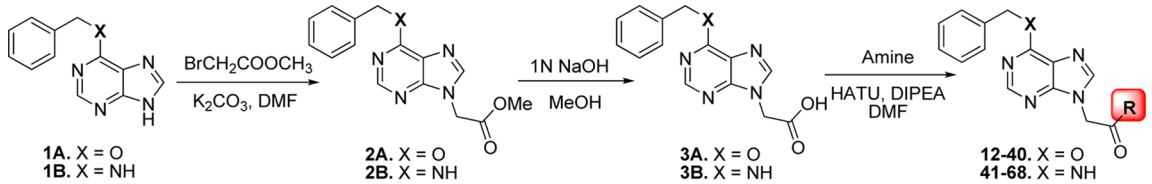
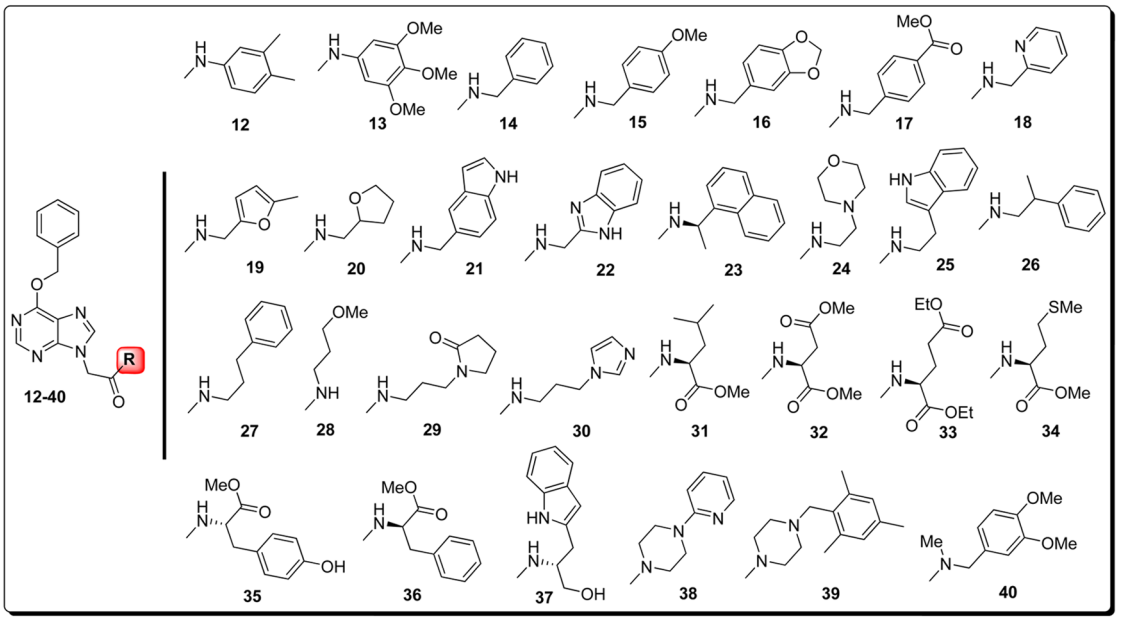
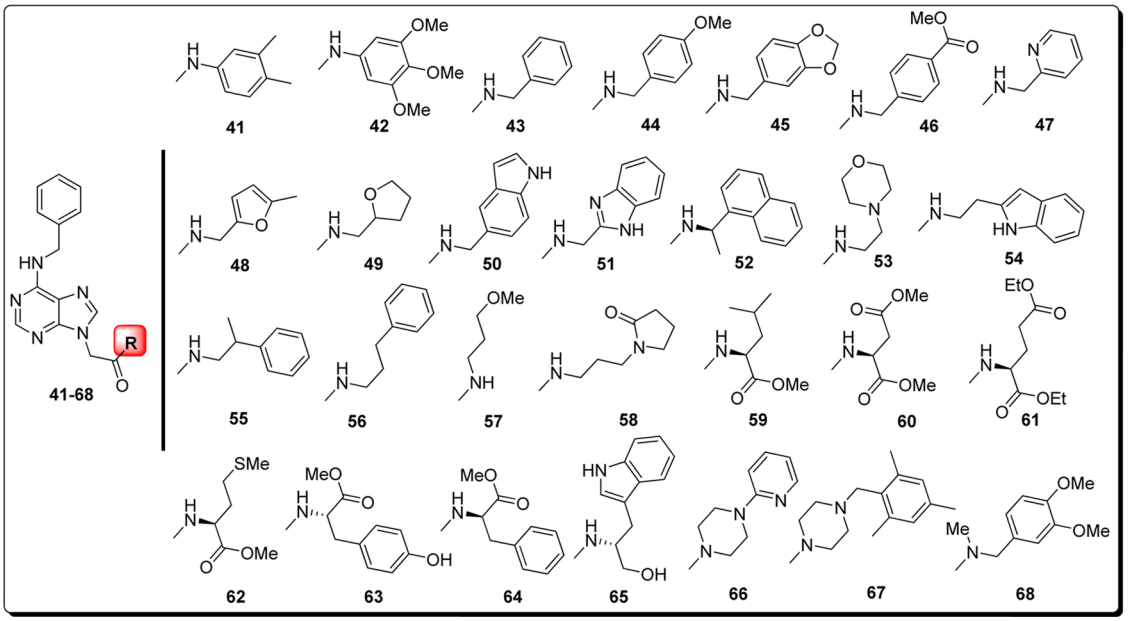
Scheme 1. Synthesis of 6-Benzylhypoxanthine-9-acetamides and 6-Benzyladenine-9-acetamides







carbon chain.[8] These compounds represent the so-called second generation of nucleoside antimetabolites where the structural resemblance to a natural metabolite is only present in the nucleic acid. Typically, acyclic nucleosides are less toxic because of preferential metabolism by pathogens such as viruses (e.g., acyclovir and gancyclovir). Our initial interest based on PSL requirements were to use these scaffolds to produce libraries that could not enter standard eukaryotic metabolism but would appear as routine and diverse three-dimensional pharmacophores filling alternative 3D chemical space. Pursuant to these interests, our nucleoside-like libraries did not contain a 5′-hydroxyl function that might be phosphorylated allowing further progression into crucial nucleoside metabolic pathways.[9] Rather, they were designed around the class of nucleoside antibiotics that have interesting and alternative modes of action.

We were interested in acyclic nucleoside structures since many of these acylic nucleoside scaffolds with an amide bond[10] were not well represented in commercial chemical space, including the MLSMR (http://www.ncbi.nlm.nih.gov/pcsubstance) (Figure 1). Furthermore, the chemistry was well established in the preparation

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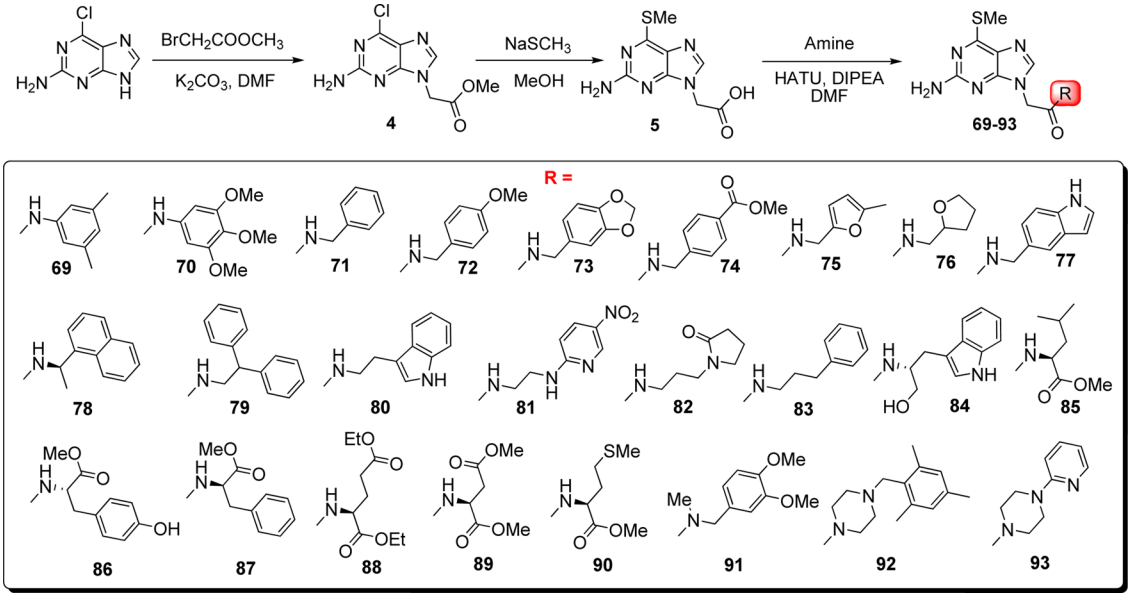
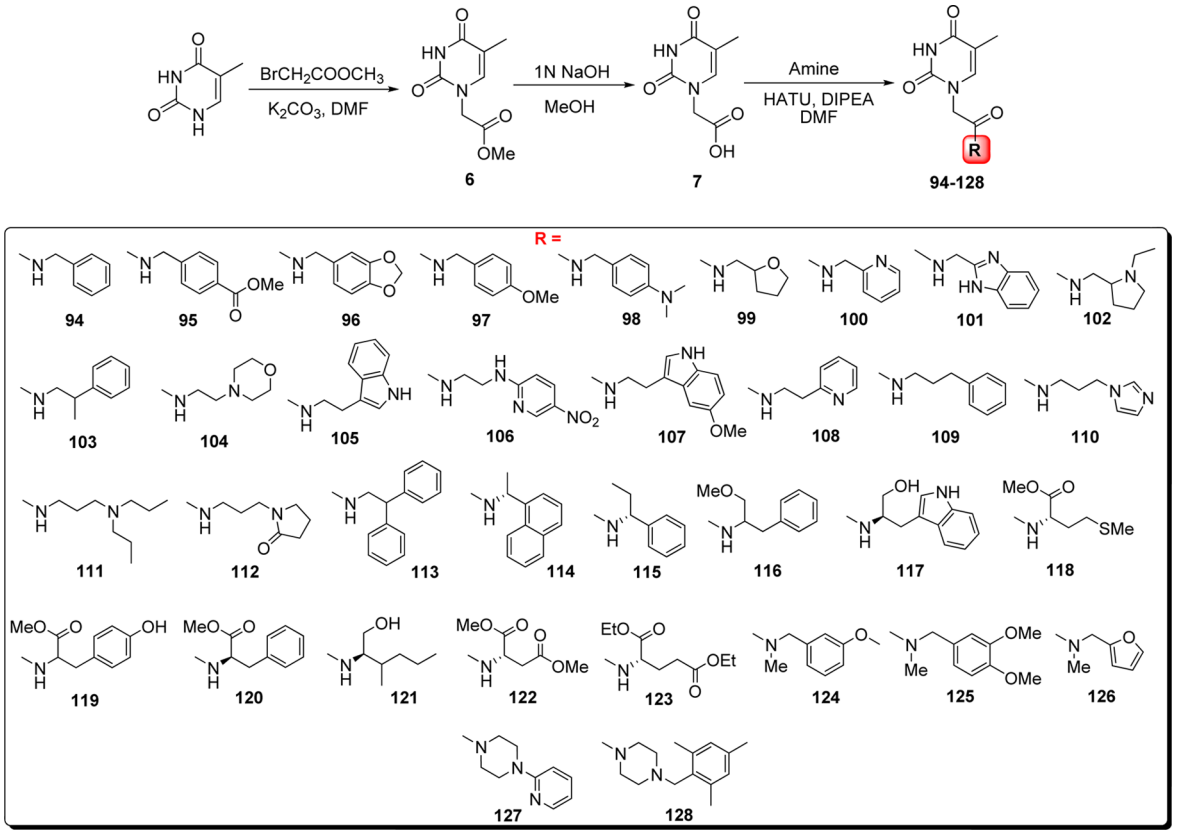
Scheme 2. Synthesis of 6-Methylthioguanine-9-acetamides and Structures of Analogues 69−93



Scheme 3. Synthesis of Thymine-3-acetamides and Structures of Analogues 94−128



of the peptide nucleic acids, or PNAs, that have been studied as alternative DNA scaffolds, and, considering the ease of this chemistry, it is notable that there are very few examples of using the starting reagents for the PNAs to generate chemical diversity.[11] Hence, we have designed and prepared acyclic nucleoside antibiotic[12] 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 program, the high throughput automated parallel preparation of a small library of 181 compounds comprising 6-benzylhypoxanthine, 6-benzyladenine, 6-thiomethylguanine, thymine, Cbz-cytosine, cytosine, and 3-(3,4-dimethylphenylcarbamoyl)-1*H*-1,2,4-triazole acetamides (Figure 1 and Schemes 1−6). A set of diverse amines were chosen after a computational diversity analysis using Pipeline Pilot program with the proprietary functional class fingerprints.[13] The predicted ranges of physicochemical properties such as LogD at pH 7.0, LogP, range of masses in amu,

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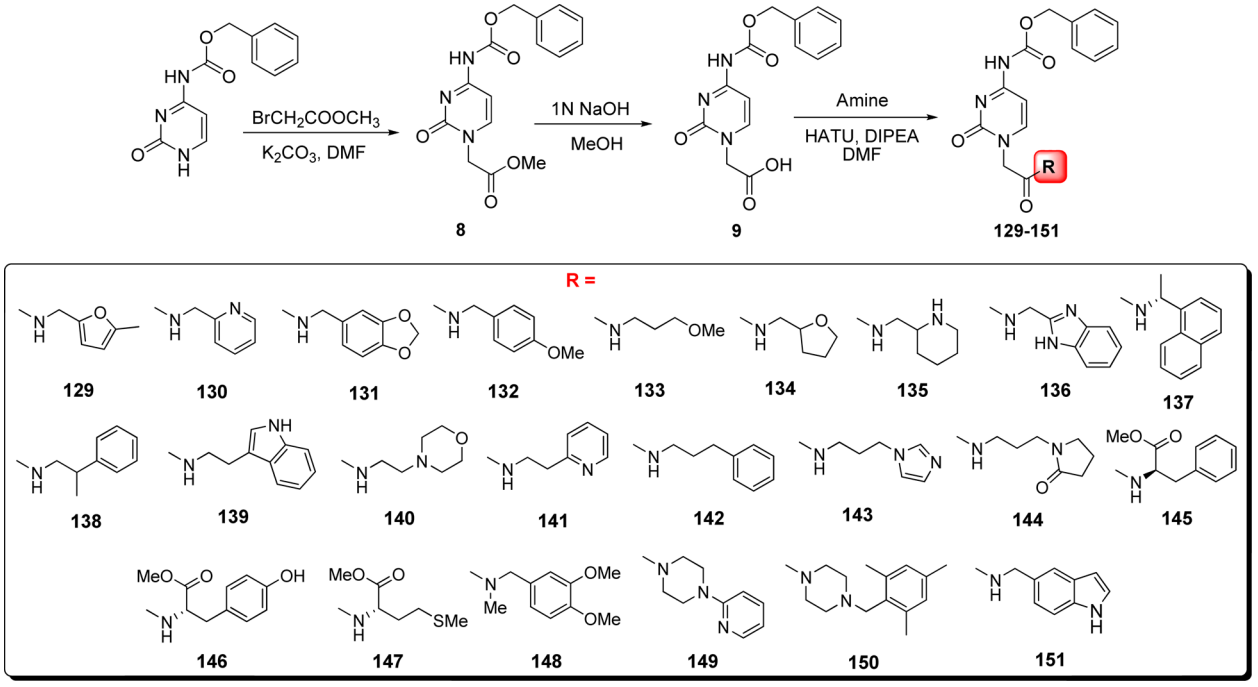
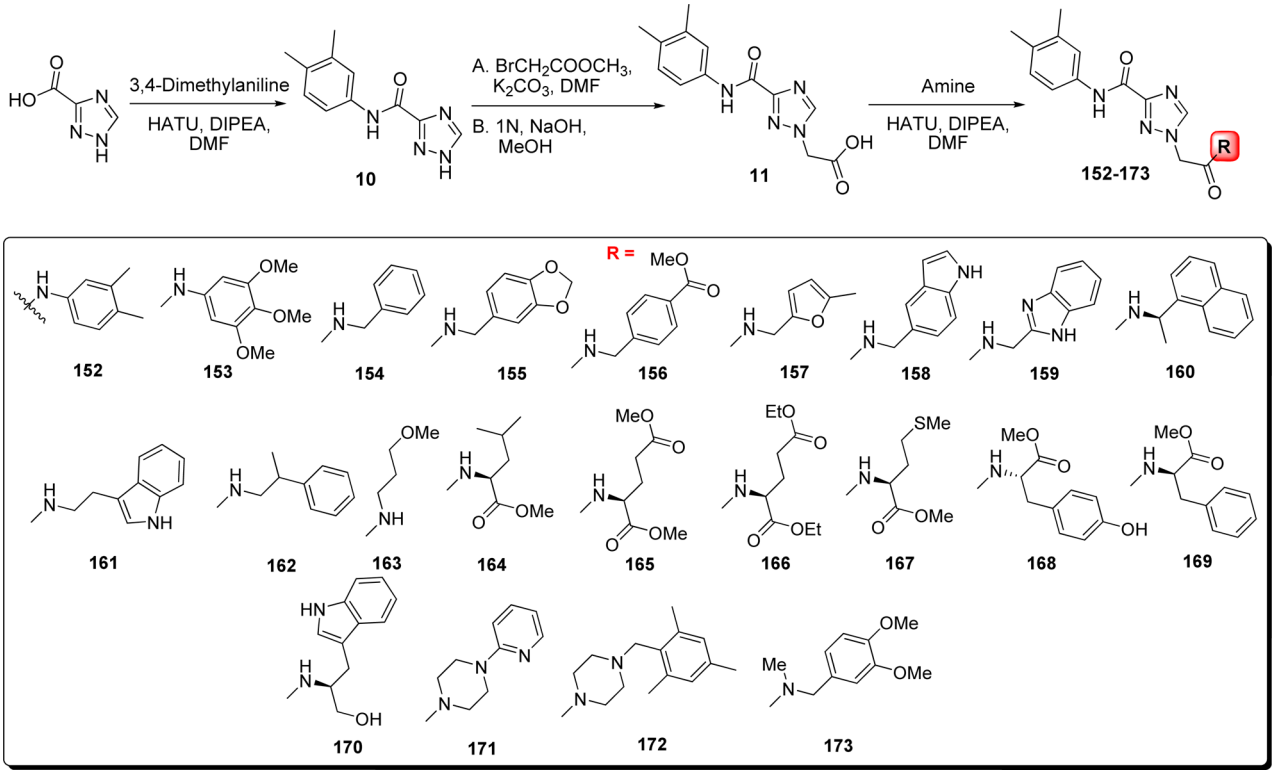
Scheme 4. Synthesis of Benzaloxycytosine-3-acetamides and Structures of Analogues 129−151



Scheme 5. Synthesis of 2-(3-(3,4-Dimethylphenylcarbamoyl)-1*H*-1,2,4-triazol-1-yl)acetamides and Structures of Analogues 152−173



hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), total polar surface area (TPSA in Å^2), and total polar solvent accessible surface area (SASA) (Polar SASA in Å^2) of library members are represented in Table 1 and graphical representations of physicochemical properties distribution of library members are shown in Figure 2. The predicted values of physicochemical properties for these library members are, for the most part, well within accepted parameters for small molecule libraries.

RESULTS AND DISCUSSION

Synthesis of the required starting acetic acid analogues of nucleobases (6-benzylhypoxanthine, 6-benzyladenine, 6-(methylthio)guanine, thymine, Cbz-cytosine and cytosine)

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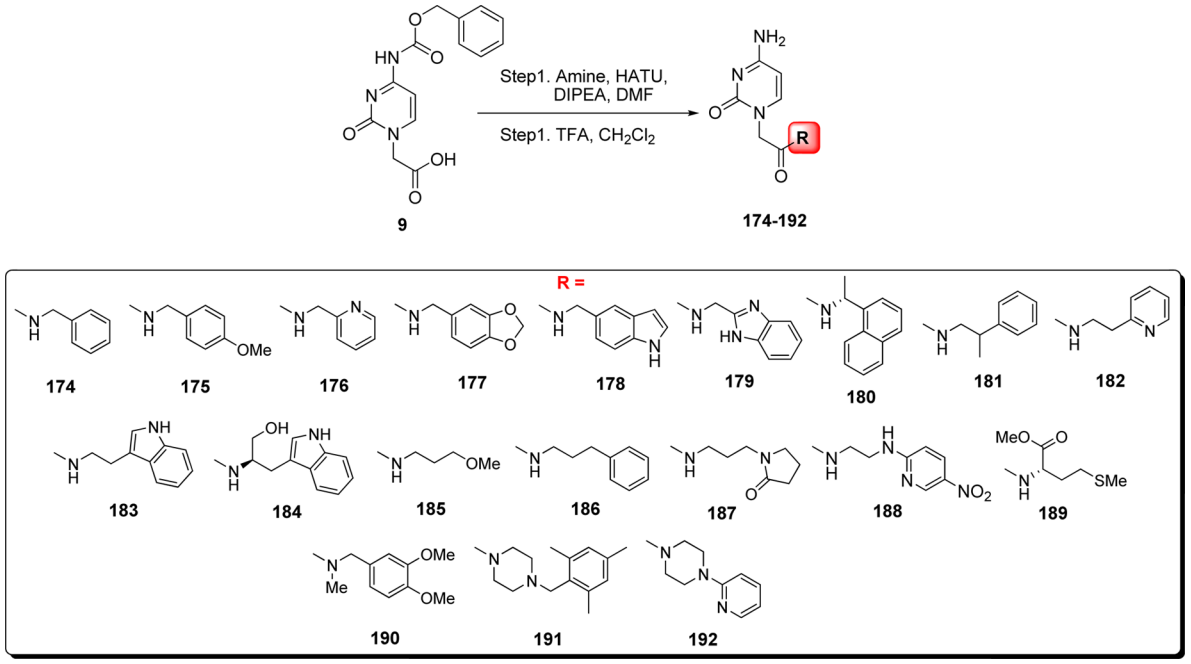
Scheme 6. Synthesis of Cytosine-3-acetamides and Structures of Analogues 174-192



and 1,2,4-triazole base **A(i–vii)** in sufficient quantities was the first task to achieve the synthesis of the desired libraries **B(i–vii)** represented in Figure 1. Purine bases **A(i** and **ii)**, 2-(6-(benzyloxy)-9*H*-purin-9-yl)acetic acid (**3A**),[14] and 2-(6-(benzylamino)-9*H*-purin-9-yl)acetic acid (**3B**),[15] were synthesized as represented in Scheme 1 by reported methods. In short, alkylation with the 2-bromoacetate of commercially available 6-benzylhypoxanthine and 6-benzyladenine in the presence of K_2CO_3 produced methyl esters **2A** and **2B** in excellent yields. Further, saponification of **2A** and **2B** with 1N NaOH in methanol gave the desired acetic acid analogues **3A** and **3B** respectively. Synthesis of purine base **A(iii)**, 2-(2-amino-6-(methylthio)-9*H*-purin-9-yl)acetic acid (**5**), was accomplished as shown in Scheme 2. Starting from commercially available 6-chloroguanine which was *N*-alkylated with methyl 2-bromoacetate in the presence of K_2CO_3 in DMF for 24 h provided **4** in good yield after column purification. Reaction of precursor compound **4** with $NaSCH_3$ in MeOH not only installed the thiomethyl group on **4**, but saponified the methyl ester to provide final product 6-(methylthio)guanine acetic acid (**5**). Pyrimidine bases **A(iv** and **v)**, (thymin-1-yl)acetic acid (**7**),[16] and Cbz-cytosine acetic acid (**9**)[17] were synthesized from thymine and Cbz-cytosine respectively as reported, and the chemistry is shown in Schemes 3 and 4 respectively. Base **A(vi)**, 2-(3-(3,4-dimethylphenylcarbamoyl)-1*H*-1,2,4-triazol-1-yl)-acetic acid (**11**), was synthesized starting from commercially available 1,2,4-triazole-3-carboxylic acid as shown in Scheme 5. In the first step, 1,2,4-triazole-3-carboxylic acid was reacted overnight with 3,4-diemethylaniline with coupling reagent HATU [(2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] and base *N*,*N*-diisoproplyethyl amine (DIPEA) in DMF at room temperature to give amide **10** in 75% yield. Next, **10** was suspended in DMF and reacted overnight at room temperature with methyl bromoacetate and K_2CO_3. After standard work-up and purification by column chromatography, the methyl ester of the target acetic acid **11** was obtained as a white powder in 70% yield. Finally, saponification of the methyl ester with 1 N NaOH in MeOH at room temperature produced the desired acetic acid analogue **11** in excellent yield after purification.

Once all the starting materials were in hand, we employed several peptide coupling reagents and various solution-phase conditions in several model amide forming reactions. A number of different nucleobase carboxylic acids and various amines were reacted using alternative coupling reagents such as HBTU [2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate], DCC [*N*,*N*′-dicyclohexylcarbodiimide] or HATU to achieve our targeted acyclic amide-linked compounds **B(i–viii)** given in Figure 1. These reagents are reported to rapidly provide the peptide linkage in high yield with little or no racemization. In our hands, the best results in terms of yield and reaction purity were obtained with HATU (1.0 equiv) and DIEA (1.5 equiv) in CH_3CN for 180 min at room temperature. The reaction conditions were easily translated to a 24-well reaction block at a 100 mg scale to ensure production of sufficient pure product as per requirement of the PSL program. These reactions were performed once and the yields are not further optimized. In certain cases, efficient coupling did not result based on TLC, and these reactions were discarded and are not reported. After removal of the reaction solvent, DMF, under centrifugal evaporation using a 24-vessel rack, automated purification produced the expected pure targets. The structures of these analogues are shown in Schemes 1-5. However, synthesis of a 19-member cytosine library as shown in Scheme 6 was carried out in two steps starting from Cbz-cytosine acetic acid **9** in one pot. In step 1, the standard amide coupling was achieved and solvent was evaporated from the reaction block under centrifugal evaporation. In step 2, the Cbz-protecting group was removed by reduction. To each vessel in the block was added 20 mg of Pd/C and 2 equiv of ammonium formate followed by 5 mL of MeOH. The reaction block was shaken overnight at room temperature. The reaction mixtures were aspirated via syringe, and were further filtered through micrometer filter adapters onto RediSep

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Table 2. Representative Active Analogues in 12 Bioassays

PubChem Bioassay ID	assay title	active analogues (activity conc.)[a]
686979	qHTS for Inhibitors of human tyrosyl-DNA phosphodiesterase 1 (TDP1): qHTS in cells in presence of CPT.	**39** (8.2 μM), **43** (4.1 μM), **44** (10.3 μM), **45** (6.5 μM), **48** (18.4 μM), **52** (18.4 μM), **67** (5.8 μM), **69** (13.0 μM), **72** (16.4 μM), **74** (23.1 μM), **79** (14.6 μM), **91** (14.6 μM), **92** (1.4 μM), **150** (5.2 μM), **173** (18.4 μM)
686978	qHTS for Inhibitors of human tyrosyl-DNA phosphodiesterase 1 (TDP1): qHTS in cells in absence of CPT.	**14** (16.4 μM), **21** (16.4 μM), **39** (2.6 μM), **43** (2.9 μM), **44** (5.8 μM), **45** (13.0 μM), **48** (8.2 μM), **67** (1.8 μM), **69** (14.6 μM), **72** (8.2 μM), **79** (20.6 μM), **83** (14.6 μM), **91** (20.6 μM), **92** (0.8 μM)
651820	qHTS Assay for Inhibitors of Hepatitis C Virus (HCV)	**29** (0.6 μM), **39** (0.6 μM), **69** (3.5 μM), **72** (8.9 μM), **91** (4.5 μM), **92** (1.4 μM), **108** (2.8 μM), 109 (2.8 μM), **118** (0.5 μM), **139** (2.8 μM), **150** (4.5 μM)
602438	qHTS identification of modulators of interaction between CendR and NRP-1 using Fluorescence Polarization assay.	**132**, **139**, **140**, **143**, **175**, **177**, **178**, **188**
652048	qHTS of D3 Dopamine Receptor Agonist: qHTS	**55**, **58**, **67**, **77**, **185**, **188**
652051	qHTS of D3 Dopamine Receptor Potentiators: qHTS	**55**, **58**, **67**, **77**, **185**, **186**
652115	MLPCN SirT-5 Measured in Biochemical System	**45**, **73**, **76**, **94**, **110**
651640	DENV2 CPE-Based HTS Measured in Cell-Based and Microorganism Combination System Using Plate Reader	**33**, **49**, **138**, **144**, **149**
651610	HIV entry: Env-mediated Cell Fusion Measured in Cell-Based System Using Plate Reader	**67**, **150**, **169**
720504	qHTS for Inhibitors of PLK1-PDB (polo-like kinase 1 - polo-box domain)	**14** (23.8 μM), **22** (21.2 μM), **108** (21.2 μM), **125** (26.7 μM)
493131	Activator for delta FosB/delta FosB homodimer Measured in Biochemical System Using Plate Reader	**23**, **52**, **73**, **178**
504467	qHTS screen for small molecules that inhibit ELG1-dependent DNA repair in human embryonic kidney (HEK293T) cells expressing luciferase-tagged ELG1	**67** (1.0 μM), **69** (6.5 μM), 77 (23.1 μM), **92** (2.3 μM)
504444	Nrf2 qHTS screen for inhibitors	**28** (3.6 μM), **67** (3.6 μM), **74** (23.1 μM)

[a]Concentration at which compound exhibits half-maximal efficacy, AC_{50}. Extrapolated AC_{50}'s also include the highest efficacy observed and the concentration of compound at which it was observed.

solid sample loading prepacked cartridges (2.5 g of silica) followed by drying under vacuum before purification by column chromatography on automated medium pressure liquid chromatographic (MPLC) system. Each purified product was checked for purity by HPLC and characterized by NMR and mass analysis. General experiment procedures, detailed analytical data and complete list of structures along with their PubChem ID's as hyperlinks to all library members are provided in Supporting Information.

BIOLOGICAL EVALUATIONS

The prepared analogues were submitted (20 mg) to the MLSMR, the Roadmap compound repository, for further quality assessment and distribution to the various NIH screening centers as probes against a wide range of biological assays. Each submitted compound has a unique PubChem ID number (CID), and these numbers are provided herein as a hyperlink with structure in the Supporting Information section to allow the reader ready access to all PubChem information. To date, the reported libraries have been screened against 301 protein targets from 530 bioassays. Approximately 88 compounds out of 185 analogues and precursors reported in this article have shown activity in one or more assays. Several library members were active in 147 bioassays (details of the assays with assay ID's of hits are summarized in Supporting Information as a table). Representative 12 bioassays data with active library members are shown in Table 2.

All library members were also tested for inhibition of *Mycobacterium tuberculosis* (Mtb H37Rv) growth in vitro. The libraries were evaluated in a dose response (DR) format against Mtb and in a cell cytotoxicity assay using a mammalian cell line, VERO cells, as previously described.[18] Certain analogues showed Mtb growth inhibition (IC_{90} <50 μM) and these are **85** (2.0 μM), **186** (21 μM), **31** (26 μM), **39** (27 μM), **172** (29 μM), and **26** (43 μM). Also, these compounds were tested via in house HTS screening in vitro against three human tumor cell lines (HT29 colon, PC3 prostate, and MDA-MB-231 breast) as described.[9a] Only eight compounds showed cytotoxicity at less than 50 μM in one or more of these cells and data are provided in Table 3.

Table 3. Effect of Analogues on Cancer Cell Growth

	cancer cell line screen		
analogues	HT29 (CC_{50})[a]	PC3 (CC_{50})[a]	MDA-MB-231 (CC_{50})[a]
14	30	>50	42
23	6	>50	43
39	3	22	25
43	5	>50	26
48	2	>50	17
57	7	>50	>50
67	2	32	45
172	15	16	17

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

CONCLUSIONS

Herein, we report the synthesis and preliminary biological evaluation of a diverse library of 181 purine, pyrimidine, and 1,2,4-triazole acetamide analogues which were prepared in a high throughput solution-phase parallel reaction format under the Pilot Scale Library Program of the NIH Roadmap initiative. These reactions were performed in 24-well reaction blocks with automatic reagent dispensing under inert atmosphere. All compounds were characterized by NMR and HRMS, and were checked for purity by HPLC before submission to The Molecular Libraries Small Molecule Repository (MLSMR) at NIH.

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Preliminary screening was performed through the Molecular Libraries Probe Production Centers Network (MLPCN) program and further screening continues through this program (for updates see http://www.ncbi.nlm.nih.gov/pcsubstance and input search term Robert Reynolds).

■ ASSOCIATED CONTENT

Supporting Information

Additional materials as described in the text and detailed analytical data for all new analogues. 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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