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Modular Assembly of Purine-like Bisphosphonates as Inhibitors of HIV-1 Reverse Transcriptase

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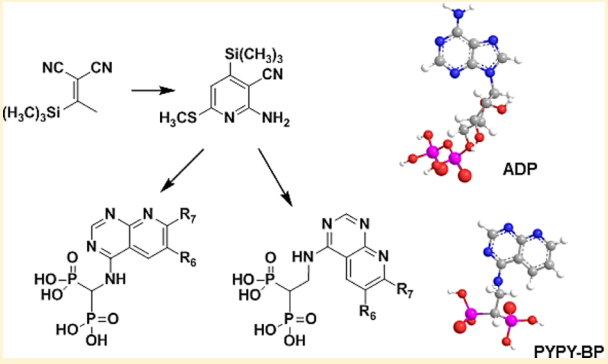
Supporting Information

ABSTRACT: Bisphosphonates can mimic the pyrophosphate leaving group of the nucleotidyl transfer reaction and effectively inhibit RNA/DNA polymerases. In a search of HIV-1 reverse transcriptase (RT) inhibitors, a new chemotype of nonhydrolyzable purine diphosphate mimic was synthesized. A modular synthetic protocol was developed, utilizing 2-amino-6-(methylthio)-4-(trimethylsilyl)nicotinonitrile as the key synthon in the preparation of highly substituted 2-aminonicotinonitriles. These building blocks were subsequently elaborated to the pyrido[2,3-*d*]pyrimidine bisphosphonates (PYPY-BPs). Biochemical screening identified analogs of PYPY-BPs that inhibit HIV-1 RT-catalyzed DNA synthesis.



INTRODUCTION

Virally encoded nucleic acid processing enzymes are essential for viral replication and represent a large family of validated therapeutic targets. Important examples include the hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase and both the reverse transcriptase (RT) and integrase (IN) of the human immunodeficiency virus type 1 (HIV-1). Nucleoside analogs were the first antiviral agents developed to bind at the polymerase active site, thus blocking DNA or RNA chain elongation. Examples include the purine bioisosteres MK-0608 (**1c**) and abacavir (**2**), which inhibit HCV NS5B[1] and HIV-1 RT,[2] respectively (Chart 1). Abacavir (**2**) is one of seven currently approved nucleoside drugs (NRTIs) for the treatment of AIDS.[2] These compounds are metabolized to their corresponding 5′-triphosphates, thus mimicking the natural substrates (e.g., **1a** is converted to **1b**). Additionally, allosteric inhibitors targeting virally encoded polymerases and integrases are also clinically validated. However, identification and development of bona fide active site inhibitors for these targets, with good biopharmaceutical properties, is a significant challenge. This is mainly due to the highly charged nature of the protein surface inside the active sites of these enzymes. Despite the uniqueness of the catalytic function of each nucleic acid processing enzyme, all of the active site cavities are characterized by having a cluster of highly conserved aspartic acid residues that bind their substrates via metal-mediated interactions.

Inorganic pyrophosphate (PPi, **3a**) is the phosphorolysis byproduct formed during each catalytic cycle of nucleotide triphosphate incorporation into a growing oligonucleotide biopolymer (Figure 1). Numerous biochemical and crystallographic studies have provided evidence that polymerases undergo a large conformational change (from an "open" to a "closed" conformation) upon binding of a nucleotide triphosphate (NTP, e.g., **1b**) in the active site, resulting in the formation of a stable complex with the primer and template (P/T) nucleic acid strands. Interestingly, studies have also shown that the same conformational change can be induced upon binding of either the NTP substrate or the PPi byproduct to the active site; it is presumed that stabilization of this complex by a small molecule can be an effective, novel mechanism of inhibiting HIV-1 RT. Several crystal structures of polymerase/P/T/PPi complexes for both DNA and RNA polymerases have been reported.[3−6]

A number of PPi bioisosteres have been previously investigated, including phosphonoformates and α,γ-diketo acids (e.g., **3b** and **4**, respectively), and shown to inhibit virally encoded nucleic acid processing enzymes (e.g., HCV-NS5B,[7] HIV-1 RT-dependent RNase H and HIV-1 IN[8]). Although the main pharmacophores of compounds such as **3b** and **4** are clearly not desirable to have in a therapeutic agent, replacement

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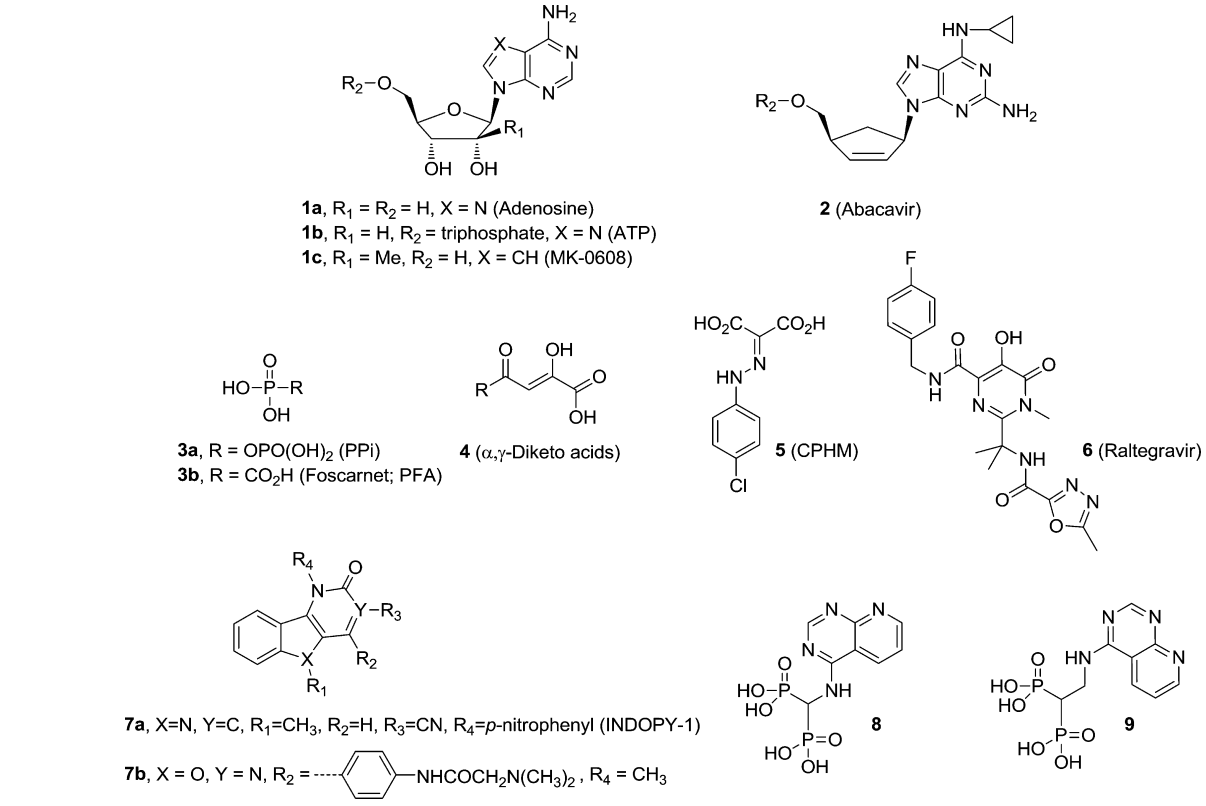
Chart 1



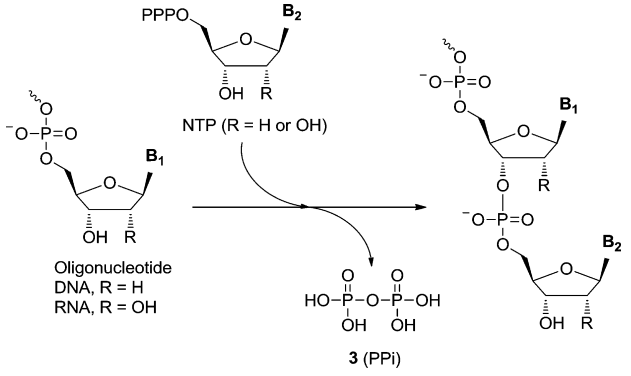


Figure 1. Oligonucleotide polymerization reaction.

of these motifs with other bioisosteres of PPi has provided drugs with significant clinical value, such as the HIV-1 IN inhibitor raltegravir (**6**).[9] The 5-hydroxy-6-oxo-1,6-dihydropyrimidine-4-carboxamide core of **6** (a structural mimic of an α,γ-diketo acid) binds to aspartate-rich active site of the HIV-1 IN via bifurcated Mg^{2+}-mediated interactions. Raltegravir represents the first clinically validated, *true* active site inhibitor of a nucleic acid processing enzyme.

Foscarnet (PFA, **3b**) is the simplest bioisostere of PPi that has been extensively investigated. It exhibits broad spectrum antiviral activity against *Herpesviridae* and *Retroviridae* and has been used for the treatment of herpes simplex (SV-1 and HSV-2) and human cytomegalovirus (HCVM)[10] infections in second line drug regimens. Stable complexes of HIV-1 RT-P/T-**3b** have been observed.[11] Recently, it was shown that **3b** can trap the HIV-1 RT/DNA complex at the pretranslocational state, in which the ultimate nucleoside monophosphate of the primer occupies the substrate binding site.[12] Similar results were previously reported for the mesoxalic acid derivative **5**, which presumably can also bind Mg^{2+} via the dicarboxylate anions.[13] These compounds inhibit HIV-1 via a mechanism that is uniquely different from that of the NRTIs and NNRTIs and have been labeled as nucleotide competitive RT inhibitors (NcRTIs). Non-pyrophosphate mimics, such as the pyridoindolone analog **7a** (INDOPY-1)[14] and benzofuropyrimidinone analog **7b**[15] have also been described as NcRTIs. Interestingly, these compounds appear to form RT-DNA/RNA ternary complexes that "freeze" the post-translocation state in a similar manner to the natural nucleotide substrate.

Our group has had a long-standing interest in designing inhibitors for enzymes that typically bind phosphate, diphosphate (pyrophosphate) or triphosphate substrates using divalent metal ions as cofactors. We previously designed bisphosphonate (BP) inhibitors of the human farnesyl pyrophosphate synthases (hFPPS), the gate-keeper enzyme of isoprenoid biosynthesis in mammalian cells.[16] Currently, bisphosphonates are clinically validated drugs for the treatment of skeletal diseases targeting the human farnesyl pyrophosphate synthase.[17] These drugs have high affinity for bone, are potent inhibitors of osteoclastic activity, and are widely used for the treatment of bone-related diseases.[18]

BPs have also been described to bind to HIV-1 RT and block the excision of 3′-azido-3′-deoxythymidine (AZT) from the 3′-end of an oligonucleotide.[19,20] However, the BPs described so far exhibited little or no activity at inhibiting RT-catalyzed RNA-templated DNA synthesis.[20] Recently, BPs were also reported to be weak inhibitors of DNA 3′-processing and strand transfer reactions catalyzed by HIV-1 IN; however, the potency of these compounds was found to be even weaker in the presence of the biologically relevant cofactor (Mg^{2+}) than other divalent metals.[21] BPs have also been reported as very

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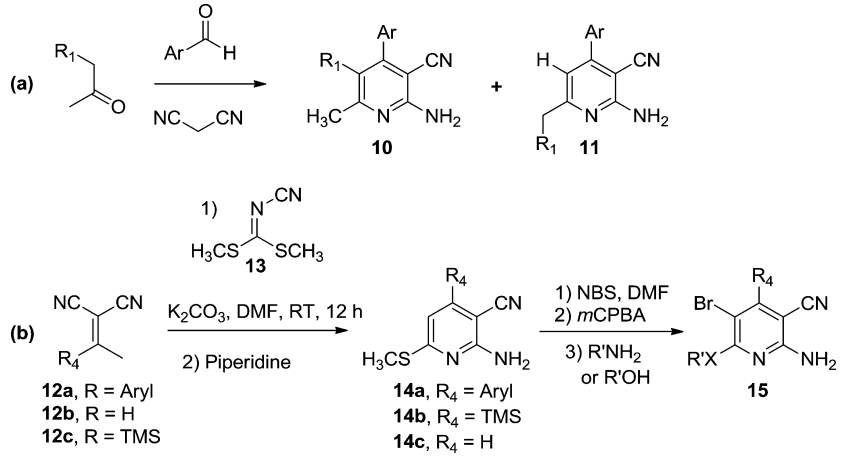
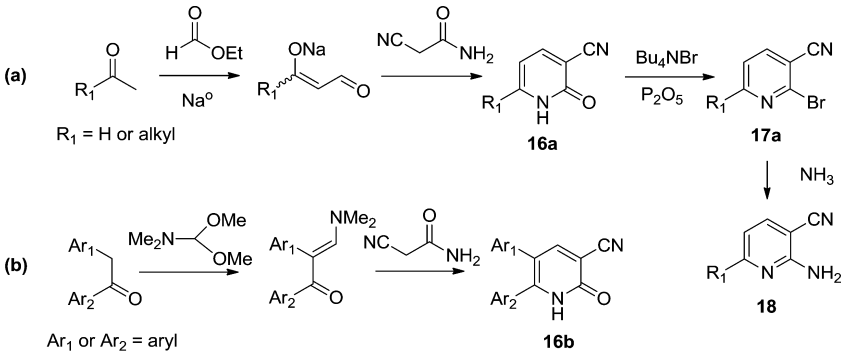
Scheme 1. Synthesis of Substituted 2-Aminonicotinonitriles: (a) Classical Hantzsch Synthesis and (b) Modified Teague Synthesis[35]



Scheme 2. Two Common Approaches for the Synthesis of 2-Aminonicotinonitriles That Are Not Substituted at C-4



weak inhibitors of HCV NS5B (IC_{50} values of >100 μM).[22] In this study, we report the synthesis of pyrido[2,3-*d*]pyrimidine bisphosphonates (PYPY-BPs) with general structures **8** and **9** as potential bioisosteres of purine-based nucleotide diphosphates (NDPs). A modular synthetic protocol was developed, utilizing 2-amino-6-(methylthio)-4-(trimethylsilyl)-nicotinonitrile (**14b**) as the key synthon to prepare highly substituted pyrido[2,3-*d*]pyrimidine bisphosphates (PYPY-BPs). In vitro biochemical screening led to the identification of several "hits" that block HIV-1 RT-catalyzed DNA synthesis at low micromolar concentrations, with IC_{50} values of 1−5 μM. A preliminary structure−activity relationship (SAR) was established that suggests a promising path forward for the optimization of this chemotype into novel antiviral agents.

CHEMISTRY

Substituted 2-aminonicotinonitriles (e.g., **10**, **11**; Scheme 1) are common synthetic precursors to the pyrido[2,3-*d*]pyrimidine scaffold, which is a privileged bioisostere of the purine nucleobase core. In recent years, pyrido[2,3-*d*]pyrimidin-4-amine-based molecules have received significant attention in drug discovery. Preclinical/clinical exploratory therapeutics of this class include antiviral agents targeting the HCV NS5A,[23] agents for the treatment of cardiovascular diseases,[24] acetylcholinesterase inhibitors,[25] and anticancer agents.[26] The 2-aminonicotinonitrile precursor to these compounds is also an important structural motif of pharmaceutical interest; examples include $P2Y_{12}$ inhibitors as antithrombotic agents,[27] antitumor agents,[28] A_{2A} adenosine receptor antagonists,[29] potential prion disease therapeutics,[30] and IKK-β kinase inhibitors.[31]

In the past, a number of useful methodologies were reported for the construction of substituted 2-aminonicotinonitriles and their conversion to pyridopyrimidines. Most of these approaches employed the classical three-component Hantzsch-type condensation/dehydrogenation reaction of ketones with aryl aldehydes, malononitrile, and ammonia (or ammonium acetate) under thermal or microwave conditions (Scheme 1a).[32] This methodology often suffers from poor yields, particularly in the case of nonsymmetrical aliphatic ketones, which leads to mixtures of regioisomers (e.g., **10** and **11**; Scheme 1). Several modifications have been reported,[33] including the ytterrbium perfluorooctanoate [$Yb(PFO)_3$] catalyzed one-pot synthesis, which provides higher yields under more favorable reaction conditions.[34] In 2008, Teague reported a modification that proved to be particularly relevant to our work.[35] This method involves condensation of 2-(1-arylethylidene)propanedinitriles (**12a**) with dimethyl *N*-cyanodithioiminocarbonate (**13**) under basic conditions to give the 2-amino-6-(methylthio)-4-arylnicotinonitriles (**14a**, Scheme 1b). This intermediate can be selectively brominated at C-5, even in the presence of an electron-rich aryl substituent at C-4, and the C-6 thiomethyl group can be oxidized to sulfoxide, before displacement with various nucleophiles to give highly substituted pyridines.[35] However, this methodology is limited by the requirement of an arylethylidene precursor (**12a**) in order for the reaction to proceed efficiently (Scheme 1b).

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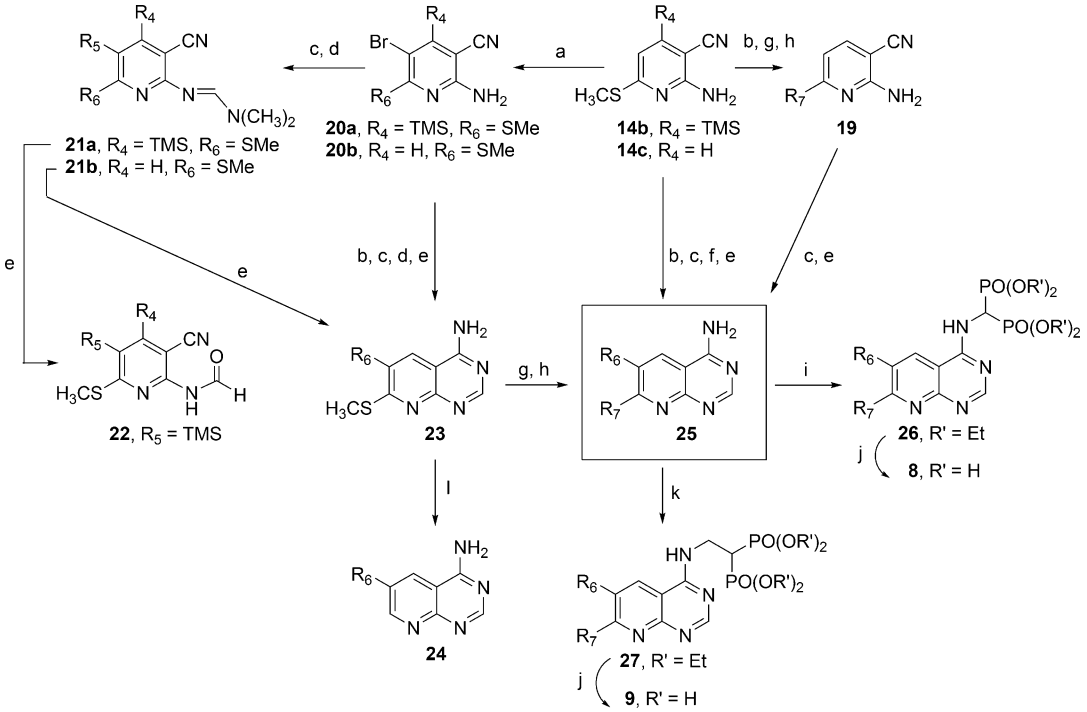
Scheme 3. Modular Synthesis of C-6/C-7 Substituted Pyrido[2,3-*d*]pyrimidin-4-amines[a]



[a]Conditions: (a) NBS in acetonitrile, 4 h, rt, 78%; (b) TBAF, THF, rt, 12 h, 90−98%; (c) *N*,*N*-dimethylformamide dimethylacetal DMF−DMA, DMF, 4−12 h, rt, 70−95%; (d) Pd-catalyzed Suzuki cross coupling, 40−90% yield; (e) NH_4OAc, AcOH, 100 °C, 1−2 h, 50−98%; (f) ArZnI·LiCl, $Pd(OAc)_2$, S-Phos, THF, 25−50 °C, 15 h, 40−45%; (g) *m*CPBA, DCM, 40 min, rt, 80%; (h) NaOR′ or HNR′R″, EtOH, 10−15 h, 80 °C, 70−80%; (i) diethyl phosphite, triethyl orthoformate, toluene or DMF, 150 °C (microwave), 1.5 h; (j) (i) TMSBr, 2−3 days, rt; (ii) MeOH; (k) tetraethylethane 1,1-diylbis(phosphonate), THF, rt 16 h; (l) Et_3SiH, Pd/C, 0 °C to rt, ∼35%.

Our goal was to explore pyrido[2,3-*d*]pyrimidine bisphosphonates (PYPY-BPs) as potential bioisosteres of purine-like NDPs. On the basis of structural and conformational considerations, we anticipated that a large aromatic substituent at C-5 of the pyridopyrimidine scaffold (originating from the C-4 substituent of the 2-aminonicotinonitrile precursor) was likely to hinder proper binding of the bisphosphonate moiety in the active site of the enzyme. For this purpose, we required the synthesis of 2-aminonicotinonitriles that were unsubstituted at C-4 and could be easily modified at C-5 and C-6 in a modular synthesis, library mode. Preparation of 2-aminonicotinonitriles from a 2-oxo-1,2-dihydropyridine-3-carbonitrile precursor (e.g., Scheme 2; **16a**[23a] or **16b**[36]) has been previously reported. Conversion of the pyridone to the corresponding C-2 halo derivative (e.g., **17a**) provides access to the desired 2-aminonicotinonitrile (e.g., **18**). Although this approach allows the preparation of the desired compounds, the synthetic protocol is linear, making the preparation of libraries of structurally diverse pyridopyrimidines challenging.

Initially, we attempted to optimize the reaction shown in Scheme 1b.[35] We explored the removal of the aromatic substituent from this ylidene (i.e., **12b**), which had a dramatic effect on the reactivity of the olefin, flipping this reagent from a Michael donor into a Michael acceptor even superior to compound **13** (Scheme 1b). For example, we prepared ylidene **12b** (via condensation of malononitrile with acetaldehyde in the presence of a catalytic amount of diethylamine) and found that this ylidene was prone to self-condensation in the presence of base. In an attempt to enhance the electrophilicity of the iminithiocarbonate **13**, we also added various alkyl iodides or TMS triflate to the reaction mixture; however, only polymerization of **12b** was observed. We then modified the ylidene with a TMS substituent (i.e., ylidene **12c**; Scheme 1b), as a masking moiety at C-4 of the desired 2-aminonicotinonitrile intermediate (**14b**). Knoevenagel condensation of acetyltrimethylsilane and malononitrile provided the stable ylidene building block **12c**, as we previously reported.[37] One-pot, two-step condensation of **12c** with the iminothiocarbonate **13** under basic conditions led to good overall conversion of the starting material to a mixture of 2-aminonicotinonitriles **14b** and the desilylated product **14c** in 2:1 ratio (Scheme 1b). Separation of **14b** from **14c** can be easily achieved by chromatography, although for our purposes this step was not required and the mixture was treated with TBAF to give exclusively intermediate **14c** in good yield (70% isolated yield). Bromination of **14b** or **14c** with NBS proceeded selectively at C-5, giving **20a** or **20b**, respectively, in good to excellent yields (70−80%). Pd-catalyzed cross coupling of **20a** or **20b** with various boronic acids or boronate esters under typical Suzuki conditions proceeded in good yield (average yields of 45−70%), although a small amount of the corresponding desilylated product was usually formed when using **20a** (∼10%). These observations suggest that replacing the TMS group of ylidene **12c** with a more stable silyl group may further improve this synthetic protocol (such optimization was unnecessary for our objectives).

Intermediates **20a** and **20b** were converted to their corresponding *N*,*N*-dimethylformamidine derivatives **21a** and **21b**, respectively, prior to cyclization. Surprisingly, whereas cyclization of **21b** with ammonium acetate in acetic acid gave the expected pyrido[2,3-*d*]pyrimidin-4-amine product **23**,

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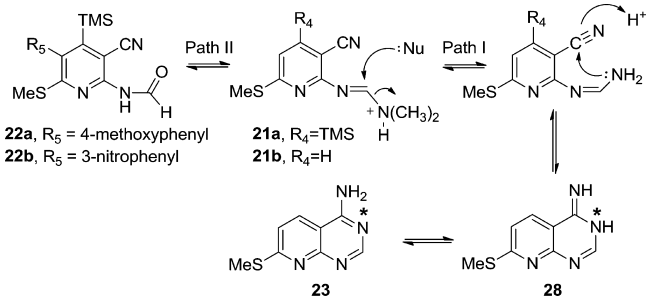
cyclization of **21a** under the same conditions produced mainly the formamide intermediate **22** (60−70% isolated yield). It appears that the steric bulk of the TMS group at C-4 disfavors formation the exocyclic imine intermediate **28** (Scheme 4, path I), leading to formation of **22** via nucleophilic attack by water present in AcOH (Scheme 4, path II). Cyclization of **21b** in the presence of $^{15}NH_4Cl$ in AcOH provided the expected ^{15}N-labeled product **23** (i.e., specifically labeled at N-3), whereas cyclization of **21a** under the same conditions did not lead to incorporation of the ^{15}N-label; the structures of compounds **22a** and **22b** were confirmed by 2D NMR, IR, and HRMS. The nitro intermediate **21s** (R_4 = TMS, R_5 = 3-nitrophenyl, R_6 = SMe) leading to **22b** was only prepared to test any electronic contributions in the formation of **22** vs the cyclized pyridopyrimidine **23** and was not pursued further to a final inhibitor.

Scheme 4. Cyclization Mechanism[a]

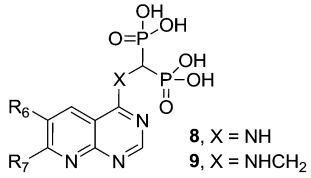


[a] The asterisk (∗) indicates incorporation of ^{15}N from $^{15}NH_4Cl$.

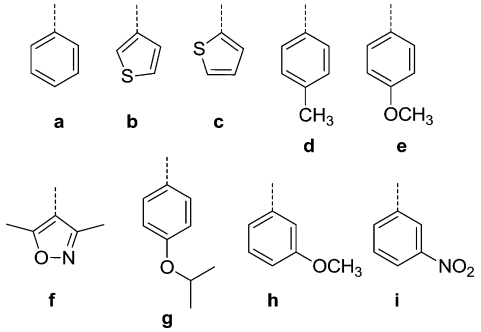
Pyridopyrimidine analogs **25**, with an aromatic moiety at C-7, were prepared via Pd-catalyzed Negishi cross-coupling reactions of **14c** with an organozinc reagent, using the protocol previously described by Knochel and co-workers (Scheme 3).[38] Alternatively, the thiomethyl group of **14c** was first oxidized with *m*-chloroperoxybenzoic acid (often generating a mixture of the sulfoxide and sulfone) which upon addition of a nucleophile, such as alkylamines or alkoxides, generated analog of intermediate **19** with R_7 as -OR or -NHR in modest to good yield (∼50−80%). Furthermore, Pd-catalyzed reductive scission of the thiomethyl ether moiety could be achieved in the presence of Et_3SiH to give the C-6 monosubstituted derivatives of general structure **24** in modest yield (Scheme 3; although this is a very modular approach for library synthesis, higher yield can be obtained directly from the 6-bromopyrido[2,3-*d*]pyrimidin-4-amine).[39]

Reaction of the 4-aminopyridopyrimidine intermediate **23**, **24**, or **25** with either diethyl phosphite and triethyl orthoformate or tetraethyl ethane-1,1-diylbis(phosphonate) led to the formation of the tetraethyl bisphosphonate esters **26** and **27**, respectively (Scheme 3). The ethyl esters were treated with TMSBr followed by methanolysis to obtain the final phosphonic acids **8** and **9** in good yield (isolated yields of ∼50% for the two steps). It is noteworthy that the overall synthetic protocol outlined in Scheme 3 is fairly robust and amenable to library synthesis. For example, intermediate **20b** could be converted directly to the bisphosphonate tetraester **26** or **27** before replacing the C-6 bromo moiety via a Pd-catalyzed cross-coupling reaction. To validate the chemistry and the amenability of this protocol for parallel synthesis, a minilibrary of compounds was synthesized; representative compounds are shown in Table 1.

Table 1. Compound Library



8a, $R_6 = R_7 = H$	**9a**, $R_6 = R_7 = H$
8b, $R_6 = H$, $R_7 = SCH_3$	**9b**, $R_6 =$ **a**, $R_7 = H$
8c, $R_6 = H$, $R_7 =$ **c**	**9c**, $R_6 =$ **b**, $R_7 = H$
8d, $R_6 = H$, $R_7 = SO_2CH_3$	**9d**, $R_6 =$ **d**, $R_7 = H$
8e, $R_6 = H$, $R_7 = OCH_3$	**9e**, $R_6 =$ **e**, $R_7 = H$
8f, $R_6 = Br$, $R_7 = H$	**9f**, $R_6 =$ **h**, $R_7 = H$
8g, $R_6 =$ **c**, $R_7 = H$	**9g**, $R_6 = Br$, $R_7 = SCH_3$
8h, $R_6 =$ **f**, $R_7 = H$	**9h**, $R_6 =$ **a**, $R_7 = SCH_3$
8i, $R_6 =$ **g**, $R_7 = H$	**9i**, $R_6 =$ **h**, $R_7 = SCH_3$
8j, $R_6 =$ **a**, $R_7 = H$	**9j**, $R_6 =$ **g**, $R_7 = SCH_3$
8k, $R_6 =$ **a**, $R_7 = SCH_3$	**9k**, $R_6 =$ **b**, $R_7 = SCH_3$
8l, $R_6 =$ **b**, $R_7 = SCH_3$	
8m, $R_6 =$ **g**, $R_7 = SCH_3$	
8n, $R_6 = Br$, $R_7 = SCH_3$	



RESULTS AND DISCUSSION

Preliminary biochemical profiling of our pyridopyrimidine bisphosphonates (PYPY-BPs) focused on the identification of low micromolar "hits" that could inhibit HIV-1 RT-catalyzed DNA synthesis. It is noteworthy that at very high concentrations, bisphosphonates may appear to be inhibiting the enzyme (i.e., giving a false positive result) because of their ability to chelate the divalent metal ion required for catalytic activity (e.g., the Mg^{2+} cofactor of HIV-1 RT). Mindful of this property, we initiated in vitro screening of our PYPY-BP analogs (Table 1) at a fixed concentration of 10 μM inhibitor and 6 mM $MgCl_2$ using a modified scintillation proximity assay (SPA) and purified HIV-1 RT.[40] Significant inhibition of primer extension (>50% inhibition) was observed with several analogs. A full dose−response inhibition curve (IC_{50}) was subsequently determined for the most promising of these compounds (Table 2).

Initial evidence of a structure−activity relationship (SAR) indicated that while neither parent compounds PYPY-BPs **8a** and **9a** nor their corresponding C-7 thiomethyl ethers (e.g., **8b**) exhibited any significant activity in inhibiting HIV-1 RT, some of the C-6 substituted analogs inhibited RT with an IC_{50} value in the 10−25 μM range. Further increase in potency was observed with analogs having the thiomethyl ether moiety at C-7. For example, the potency (IC_{50}) of analogs **8i** and **9f** was

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Table 2. Activity Data of Key Compounds

compd	IC_{50} (μM)[a]	compd	IC_{50} (μM)[a]
3b	2.5 ± 1.3	**9a**	>50
5	1.5 ± 0.9	**9c**	9.0 ± 1.4
8a	>50	**9f**	20.0 ± 4.8
8b	>50	**9h**[b]	1.1 ± 0.3
8c	10.4 ± 1.4	**9i**[b]	2.4 ± 1.5
8i	10.0 ± 1.5	**9j**	7.1 ± 0.8
8m	1.8 ± 0.8[c]	**9k**	8.3 ± 1.3

[a]Inhibition of DNA primer extension catalyzed by HIV-1 RT; average IC_{50} value of three determinations. [b]Compounds also inhibit hFPPS (75–95% inhibition at 10 μM) but not hGGPPS. [c]Less than 10% inhibition was observed in the hFPPS and hGGPPS inhibition assay at 50 μM.

approximately 10-fold lower than that of the corresponding derivatives **8m** and **9i**, respectively (Table 2). The same trend in SAR was observed when analog **9b** (data not shown) was compared to **9h** (Table 2). Interestingly, the longer linker between the pyridopyrimidine core and the bisphosphonate moiety (i.e., analogs **8** vs **9**) was well tolerated for our SAR purposes. In line with our objective to identify selective inhibitors for HIV-1 RT, the most potent HIV-1 RT inhibitors identified (Table 1; IC_{50} of 1–5 μM) were also tested in our in vitro hFPPS and hGGPPS inhibition assays.[16c] Interestingly, analogs **9h** and **9i** were found to also exhibit considerable potency in inhibiting hFPPS but not hGGPPS. In contrast, inhibitor **8m** was selective in inhibiting HIV-1 RT and showed no activity in our hFPPS and hGGPPS inhibition assays even at a concentration of 50 μM.

The active site of the RT enzyme is the target of the current nucleoside drugs (NRTIs), although these compounds are substrate mimics that lead to chain termination and not true inhibitors of the RT enzyme. Preliminary evaluation of our most potent compounds in HIV mutants that are typically associated with resistance to the current nucleoside drugs, such as K65R (e.g., tenofovir) and M184V (e.g., lamivudine and emtricitabine),[45] as well as the foscarnet resistant E89K mutant,[12] was carried out. The K65R mutant appears to confer low level resistance to all compounds tested (Table 3), whereas the M184V mutant leads to a small decrease (<3-fold) in sensitivity to analog **9i**; these results are summarized in Table 3.

CONCLUSIONS

HIV/AIDS remains a major global public health problem but is manageable with highly active antiretroviral therapy (HAART), which provides a combination of drugs with different mechanisms of action that target various virally encoded enzymes. The current clinically validated RT inhibitors include nucleosides/nucleotides (NRTIs) and allosteric inhibitors (NNRTIs). The triphosphates of NRTIs bind to the active site of RT and mimic the natural dNTP substrates, leading to incorporation into the viral DNA and chain termination. Resistance to an NRTI drug can emerge due to mutation(s) in the active site of RT that introduce electronic or steric effects, thus hindering the binding of the drug. Alternatively, mutations can create a new binding site for cellular ATP, which promotes the excision of the incorporated nucleotide monophosphate drug. For example, resistance to AZT or d4T is mediated through this type of mechanism, where ATP acts like a PPi donor and removes the incorporated AZT monophosphate. This reaction can be seen as the reverse of the nucleotide incorporation (Figure 1); however, the affinity of PPi for the RT complex is presumed to be inefficient, thus disfavoring the reversibility of this reaction.

Recently, a novel class of RT inhibitors, referred to as nucleotide-competing RT inhibitors (NcRTIs), was identified.[14,15,41] Biochemical studies suggest that most of these compounds compete with the nucleotide substrate for binding to the RT active site, blocking translocation of HIV-1 RT at the post-translocation DNA/RT complex. Included in this mechanism-based category is the pyrophosphate bioisostere **3b**, which traps the RT/DNA pretranslocation complex.[12] In an effort to identify better "druglike" molecules for further optimization into novel antiviral agents with NcRTI-like mechanism of action, we explored the ability of pyridopyrimidine-based bisphosphonates (PYPY-BPs) to inhibit the HIV-1 RT-catalyzed DNA polymerization. We developed a modular protocol that is amenable to high throughput parallel synthesis of compound libraries. In the course of our methodology development, we prepared a minilibrary of analogs and identified several hits of low micromolar inhibitors, such as compound **8m** (IC_{50} ≈ 1.8 μM), which exhibits equivalent potency to foscarnet (**3b**, IC_{50} ≈ 2.5 μM) in inhibiting HIV-1 RT-catalyzed DNA synthesis. Preliminary SAR and biochemical evidence also suggests that the structural/molecular recognition can be fine-tuned to provide molecules that are selective in inhibiting HIV-1 RT versus other biological targets that are more commonly associated with bisphosphonate inhibitors, such as the human FPPS.

EXPERIMENTAL SECTION

General Procedures for Characterization of Compounds. All compounds were purified by normal phase flash column chromatography on silica gel using a CombiFlash instrument and a solvent gradient from 5% EtOAc in hexanes to 100% EtOAc and then to 20% MeOH in EtOAc, unless otherwise indicated. The homogeneity of all final compounds was confirmed to be ≥95% by reverse-phase HPLC. Only phosphonate esters with homogeneity of ≥90% were processed further to the final phosphonic acid inhibitors. HPLC analysis was

Table 3. Activity Data of Key Compounds in HIV-1 Wild-Type vs HIV Mutants

	foscarnet		8m		9h		9i	
	IC_{50}[a] (μM)	fold change	IC_{50}[a] (μM)	fold change	IC_{50}[a] (μM)	fold change	IC_{50}[a] (μM)	fold change
RT WT	1.2	1.0	0.8	1.0	2.0	1.0	1.5	1.0
RT K65R	2.8	2.4	1.8	2.1	5.2	3.4	3.3	2.1
RT E89K	5.2	4.5	0.8	1.0	1.0	0.6	2.1	1.3
RT M184V	0.8	0.7	1.4	1.6	0.6	0.4	4.2	2.7

[a]Inhibition of DNA primer extension catalyzed by HIV-1 RT; average IC_{50} values from an assay run in triplicates. All compounds were run in parallel with the wild-type RT and then three mutants (the IC_{50} values for WT are the average from this one assay run in triplicate and within the variability of the data shown in Table 2).

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performed using a Waters ALLIANCE instrument (e2695 with 2489 UV detector and 3100 mass spectrometer). Final compounds were fully characterized by ^{1}H, ^{13}C, and ^{31}P NMR and HRMS. Chemical shifts (δ) are reported in ppm relative to the internal deuterated solvent (^{1}H, ^{13}C) or external H_3PO_4 (δ 0.00 ^{31}P), unless indicated otherwise. The NMR spectra of all final bisphosphonate inhibitors were acquired in D_2O with 0.15% ND_4OD. In many cases, the Cα to the bisphosphonate of intermediates **26** and final inhibitors **8** was broad and overlapped with the solvent peak, as confirmed by the HSQC data; the HSQC data of key compounds are provided. The high resolution mass spectra of final products were recorded using electrospray ionization ($ESI^{\pm}$) and Fourier transform ion cyclotron resonance mass analyzer (FTMS).

The method for homogeneity analysis using a Waters Atlantis T3 C18 5 μm column) involved the following: solvent A, H_2O, 0.1% formic acid; solvent B, CH_3CN, 0.1% formic acid; mobile phase, linear gradient from 95% A and 5% B to 0% A and 100% B in 13 min.

Library Synthesis. General Synthetic Protocols. Suzuki cross-coupling reactions and deprotection of the phosphonate esters to the phosphonic acids were carried out using the general protocols previously reported.[16a] Reductive scission of the thioether was performed using the protocol of Graham and co-workers.[39] Selective bromination at C-5 of the 2-aminonicotinonitrile scaffolds was carried out using 1.1 equiv of *N*-bromosuccinimide in acetonitrile for 4 h in the dark at room temperature.[42] The 2-amino moieties of intermediates **20** were converted to the corresponding *N*,*N*-dimethylformamidine derivatives **21** with *N*,*N*-dimethylformamide dimethyl acetal (~10 equiv) in DMF at room temperature using a slightly modified literature procedure.[43,44]

General Protocol for the Cyclization of Intermediate 21 to the 4-Aminopyridopyrimidine Core (23 or 25). In a pressure vessel equipped with a magnetic stir bar, a compound of general structure **21** was added, with ammonium acetate (1.1 equiv) and acetic acid. The mixture was heated at 100 °C for 1−2 h. The resulting mixture was cooled and concentrated under vacuum. The residual acetic acid was coevaporated with toluene, and the residue was suspended in 30% EtOAc in hexanes. The precipitate was filtered, washed several times with 30% EtOAc in hexanes, and dried under vacuum. The crude product **23** or **25** (usually >95% homogeneity) were used in the subsequent step without further purification.

2-Amino-6-(methylthio)-4-(trimethylsilyl)nicotinonitrile (14b). The synthesis of 2-(1-(trimethylsilyl)ethylidene)malononitrile (**12c**) was previously reported.[37] In a 100 mL round-bottom flask, **12c** (2.00 g, 12.17 mmol), K_2CO_3 (1.94 g, 14.0 mmol), and dimethyl *N*-cyanodithioiminocarbonate (3.08 g, 21.1 mmol) were suspended in DMF (30 mL) and stirred for 12 h at room temperature. Piperidine (1.9 mL, 19.5 mmol) was then added, and the mixture was stirred at 60 °C for 12 h. The resulting mixture was diluted with EtOAc and water. The organic layer was collected and washed with water (3×), then brine (once), and finally dried with anhydrous $MgSO_4$. The solvent was removed under vacuum to obtain a black residue, which was purified by normal phase column chromatography on silica gel (solvent gradient from 0% to 20% EtOAc in hexanes with 0.1% Et_3N). The product was obtained as yellow oil (1.39 g, 48%). The desilylated product (**14c**) was also isolated in small amount (~10% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.63 (s, 1H), 5.12 (br_s, 2H), 2.49 (s, 3H), 0.36 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 163.9, 159.3, 154.3, 118.6, 116.2, 89.0, 13.0, −1.90. HRMS $[ESI^+]$ calculated for $C_{10}H_{16}N_3SSi$ *m*/*z*, 238.082 87; found 238.082 99 $[M + H^+]^+$.

2-Amino-6-(methylthio)nicotinonitrile (14c). The product was obtained as a light yellow powder (in >85% after the mixture of **14b** and **14c** was treated with TBAF). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (d, *J* = 8.2 Hz, 1H), 6.58 (d, *J* = 8.2 Hz, 1H), 5.14 (br_s, 2H), 2.50 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.7, 158.8, 139.8, 117.3, 111.0, 85.2, 13.2 MS $[ESI^+]$ *m*/*z*: 166.23 $[M + H^+]^+$.

2-Amino-6-methoxynicotinonitrile (19, R_4 = R_5 = H, R_6 = OMe). The product was isolated as white solid (38.7 mg, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50 (d, *J* = 8.5 Hz, 1H), 6.11 (d, *J* = 8.5 Hz, 1H), 5.13 (br_s, 2H), 3.86 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2, 159.7, 142.8, 117.7, 100.9, 81.7, 53.8. MS $[ESI^+]$ *m*/*z*: 150.15 $[M + H^+]^+$.

2-Amino-5-bromo-6-(methylthio)-4-(trimethylsilyl)-nicotinonitrile (20a). Compound **14b** was isolated as yellow-orange solid (935 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.16 (br_s, 2H), 2.44 (s, 3H), 0.54 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 164.8, 158.3, 152.6, 118.2, 113.4, 91.1, 15.3, 1.3. HRMS $[ESI^+]$ calculated for $C_{10}H_{15}BrN_3SSi$ *m*/*z*, 315.993 38; found 315.992 58 $[M + H^+]^+$.

2-Amino-5-bromo-6-(methylthio)nicotinonitrile (20b). Tetra-*n*-butylammonium fluoride (3.13 mL, 3.13 mmol) was added to **20a** (900 mg, 2.85 mmol) in THF (15.0 mL). The mixture was stirred for 12 h at room temperature. The solvent was removed under vacuum, and the residue was redissolved in EtOAc, washed with water and brine. The combined organic layers were dried over anhydrous $MgSO_4$ and then concentrated under vacuum. The crude product was purified by chromatography on silica gel (2−30% EtOAc in hexanes with 0.1% Et_3N) to give the final product as a light yellow powder (694 mg, quantitative). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.59 (s, 1H), 5.15 (br_s, 2H), 2.48 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 164.9, 157.3, 141.7, 116.0, 104.9, 86.5, 14.5. MS $[ESI^+]$ *m*/*z*: 243.95 $[M + H^+]^+$.

2-Amino-5-bromonicotinonitrile (20c, R_4 = R_6 = H). Compound **20c** was isolated as an off- white solid (438 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (d, *J* = 2.4 Hz, 1H), 7.78 (d, *J* = 2.4 Hz, 1H), 5.23 (br_s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 157.7, 153.9, 142.8, 115.2, 106.8, 92.8. MS $[ESI^+]$ *m*/*z*: 197.96 $[M + H^+]$.$^+$

(*E*)-*N*′-(3-cCyano-6-(methylthio)-4-(trimethylsilyl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21a, R_4 = TMS R_5 = H, R_6 = SMe). Product was obtained as a yellow solid (552 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H), 6.83 (s, 1H), 3.20 (s, 3H), 3.15 (s, 3H), 2.54 (s, 3H), 0.39 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 162.7, 162.1, 155.7, 154.9, 119.3, 119.1, 101.1, 41.0, 35.0, 13.1, −1.93. MS $[ESI^+]$ *m*/*z*: 293.12 $[M + H^+]^+$.

(*E*)-*N*′-(3-Cyano-6-(methylthio)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21b, R_4 = R_5 = H, R_6 = SMe). The product was obtained as a yellow orange oil that solidifies upon standing at room temperature (298.1 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H), 7.54 (d, *J* = 8.1 Hz, 1H), 6.74 (d, *J* = 8.2 Hz, 1H), 3.18 (s, 3H), 3.15 (s, 3H), 2.54 (s, 3H). MS $[ESI^+]$ *m*/*z*: 221.078 $[M + H^+]^+$.

(*E*)-*N*′-(5-Bromo-3-cyano-6-(methylthio)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21c, R_4 = H, R_5 = Br, R_6 = SMe). The product was obtained as a yellow-orange solid (246 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H), 7.70 (s, 1H), 3.19 (s, 3H), 3.16 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 162.7, 161.0, 155.8, 142.7, 116.9, 108.7, 97.7, 41.2, 35.1, 14.5. MS $[ESI^+]$ *m*/*z*: 298.99 $[M + H^+]^+$.

(*E*)-*N*′-(3-Cyanopyridin-2-yl)-*N*,*N*-dimethylformimidamide (21d, R_4 = R_5 = R_6 = H). The product was isolated as a colorless oil (115 mg, 79%). ^{1}H NMR (400 MHz, acetone-d_6): δ 8.67 (s, 1H), 8.37 (dd, *J* = 4.8, 2.0 Hz, 1H), 7.92 (dd, *J* = 7.6, 2.0 Hz, 1H), 6.97 (dd, *J* = 7.7, 4.8 Hz, 1H), 3.23 (s, 3H), 3.14 (s, 3H). MS $[ESI^+]$ *m*/*z*: 175.09 $[M + H^+]^+$.

(*E*)-*N*′-(5-Bromo-3-cyanopyridin-2-yl)-*N*,*N*-dimethylformimidamide (21e, R_4 = R_6 = H, R_5 = Br). The product was isolated as a white solid (289.7 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (s, 1H), 8.35 (d, *J* = 2.5 Hz, 1H), 7.86 (d, *J* = 2.6 Hz, 1H), 3.18 (s, 3H), 3.16 (s, 3H). MS $[ESI^+]$ *m*/*z*: 253.00 $[M + H^+]^+$.

(*E*)-*N*′-(3-Cyano-5-(4-methoxyphenyl)-6-(methylthio)-4-(trimethylsilyl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21f, R_4 = TMS R_5 = *p*-Methoxyphenyl, R_6 = SMe). The product was isolated as a yellow solid (43.5 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H), 7.09 (d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 3.85 (s, 3H), 3.21 (s, 3H), 3.15 (s, 3H), 2.39 (s, 3H), 0.06 (s, 9H). MS $[ESI^+]$ *m*/*z*: 399.16 $[M + H^+]^+$.

(*E*)-*N*′-(3-Cyano-5-(3-methoxyphenyl)-6-(methylthio)-pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21g, R_4 = H, R_5 = *m*-Methoxyphenyl, R_6 = SMe). The product was isolated as a yellow solid (46.6 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 1H), 7.48 (s, 1H), 7.38−7.31 (m, 1H), 7.00−6.90 (m, 3H), 3.84 (s, 3H), 3.21 (s, 3H), 3.18 (s, 3H), 2.49 (s, 3H). ^{13}C NMR (126 MHz, DMSO-

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d_6): δ 161.3, 161.3, 159.6, 156.6, 141.0, 138.2, 129.8, 128.5, 121.7, 118.2, 114.9, 114.0, 95.8, 55.5, 41.1, 34.9, 13.7. MS [ESI$^+$] m/z: 327.22 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-6-(methylthio)-5-(thiophen-3-yl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21h, R_4 = H, R_5 = 3-Thiophenyl, R_6 = SMe). The product was isolated as a yellow solid (54.1 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.72 (s, 1H), 7.54 (s, 1H), 7.40 (ddd, J = 7.9, 3.9, 2.2 Hz, 2H), 7.22 (dd, J = 4.9, 1.4 Hz, 1H), 3.21 (s, 3H), 3.17 (s, 3H), 2.51 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 161.6, 161.0, 155.7, 140.5, 136.8, 128.3, 125.7, 124.3, 124.2, 118.1, 96.5, 41.1, 35.0, 13.7. MS [ESI$^+$] m/z: 303.16 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-6-(methylthio)-5-phenylpyridin-2-yl)-*N*,*N*-dimethylformimidamide (21i, R_4 = H, R_5= Phenyl, R_6 = SMe). The product was isolated as a light yellow solid (24.3 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 1H), 7.47 (s, 1H), 7.46−7.36 (m, 5H), 3.21 (s, 3H), 3.18 (s, 3H), 2.49 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 161.6, 161.2, 155.7, 140.6, 136.8, 129.3, 129.2, 128.5, 128.2, 118.2, 96.5, 41.1, 35.0, 13.7. MS [ESI$^+$] m/z: 297.21 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-5-phenylpyridin-2-yl)-*N*,*N*-dimethylformimidamide (21j, R_4 = R_6 = H, R_5 = Phenyl). The product was isolated as a yellow solid (185.3 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (s, 1H), 8.58 (d, J = 2.6 Hz, 1H), 8.00 (d, J = 2.6 Hz, 1H), 7.54−7.49 (m, 2H), 7.49−7.43 (m, 2H), 7.41−7.31 (m, 1H), 3.21 (s, 3H), 3.17 (s, 3H). MS [ESI$^+$] m/z: 251.18 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-5-(4-isopropoxyphenyl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21k, R_4 = R_6 = H, R_5 = 4-Isopropoxyphenyl). The product was isolated as a yellow solid (252.7 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H), 8.54 (d, J = 2.6, 1H), 7.94 (d, J = 2.6, 1H), 7.42 (d, J = 8.6 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 4.59 (hept, J = 6.0 Hz, 1H), 3.20 (s, 3H), 3.16 (s, 3H), 1.37 (s, 3H), 1.35 (s, 3H). MS [ESI$^+$] m/z: 309.26 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-5-(thiophen-2-yl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21l, R_4 = R_6 = H, R_5 = 2-Thiophenyl). The product was isolated as a yellow solid (51.7 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.62 (s, 1H), 8.59 (d, J = 2.6 Hz, 1H), 7.97 (d, J = 2.6 Hz, 1H), 7.31 (dd, J = 5.1, 1.1 Hz, 1H), 7.25−7.23 (m, 1H), 7.10 (dd, J = 5.1, 3.6 Hz, 1H), 3.20 (s, 3H), 3.17 (s, 3H). MS [ESI$^+$] m/z: 257.08 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-5-(thiophen-3-yl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21m, R_4 = R_6 = H, R_5 = 3-Thiophenyl). The product was isolated as a brown solid (100.5 mg, 66%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.63 (s, 1H), 8.59 (d, J = 2.5 Hz, 1H), 7.98 (d, J = 2.5 Hz, 1H), 7.45−7.41 (m, 2H), 7.31 (dd, J = 4.8, 1.6 Hz, 1H), 3.20 (s, 3H), 3.17 (s, 3H). MS [ESI$^+$] m/z: 257.08 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-5-(3,5-dimethylisoxazol-4-yl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21n, R_4 = R_6 = H, R_5 = 3,5-Dimethylisoxazol-4-yl). The product was obtained as a yellow solid (74.5 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64 (s, 1H), 8.24 (d, J = 2.4 Hz, 1H), 7.67 (d, J = 2.5 Hz, 1H), 3.22 (s, 3H), 3.18 (s, 3H), 2.41 (s, 3H), 2.26 (s, 3H). MS [ESI$^+$] m/z: 270.13 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-5-(4-methoxyphenyl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21o, R_4 = R_6 = H, R_5 = 4-Methoxyphenyl). The product was isolated as a light brown solid (110.2 mg, 66%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.63 (s, 1H), 8.53 (d, J = 2.6 Hz, 1H), 7.95 (d, J = 2.5 Hz, 1H), 7.48−7.40 (m, 2H), 7.02−6.95 (m, 2H), 3.85 (s, 3H), 3.20 (s, 3H), 3.17 (s, 3H). MS [ESI$^+$] m/z: 281.23 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-5-(*p*-tolyl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21p, R_4 = R_6 = H, R_5 = *p*-Tolyl). The product was isolated as a yellow solid (45.6 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.62 (s, 1H), 8.56 (d, J = 2.6 Hz, 1H), 7.97 (d, J = 2.6 Hz, 1H), 7.42−7.39 (m, 2H), 7.28−7.26 (m, 2H), 3.20 (s, 3H), 3.16 (s, 3H), 2.40 (s, 3H). MS [ESI$^+$] m/z: 265.14 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-6-(thiophen-2-yl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21q, R_4 = R_5 = H, R_6 = 2-Thiophenyl). **21p** was prepared following the protocol described by Knochel and co-workers.[38] To a dry argon-flushed 50 mL round-bottom flask equipped with a septum and a magnetic stirrer bar were added intermediate **21b** (100.0 mg, 0.454 mmol), $Pd(OAc)_2$ (2.5 mol %), and S-Phos (5.0 mol %) dissolved in THF (1.0 mL). After 10 min of stirring, the organozinc reagent was added dropwise and the reaction mixture was stirred at room temperature for 1 h and then at 50 °C for 16 h. The resulting reaction mixture was cooled, quenched with saturated solution of aqueous NH_4Cl, and extracted with EtOAc (3 × 15 mL). The crude product was purified by chromatography on silica gel (1−25% EtOAc in hexanes with 0.1% triethylamine). The product was isolated as a yellow solid (49.6 mg, 43%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.71 (s, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.62 (dd, J = 3.7, 1.1 Hz, 1H), 7.44 (dd, J = 5.0, 1.1 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.12 (dd, J = 5.0, 3.7 Hz, 1H), 3.20 (s, 3H), 3.19 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 162.9, 156.1, 153.9, 144.3, 142.2, 129.0, 128.2, 126.2, 118.0, 111.5, 99.9, 41.1, 35.0. MS [ESI$^+$] m/z: 257.078 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-6-(methylthio)-5-(3-nitrophenyl)-4-(trimethylsilyl)pyridin-2-yl)-*N*,*N*-dimethylformimidamide (21r, R_4 = TMS, R_5 = 3-Nitrophenyl, R_6 = SMe). Product was obtained as a yellow solid (75.5 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 (s, 1H), 8.29 (ddd, J = 8.1, 2.3, 1.3 Hz, 1H), 8.11−8.09 (t, J = 1.9 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.54 (dt, J = 7.6, 1.4 Hz, 1H), 3.23 (s, 3H), 3.18 (s, 3H), 2.42 (s, 3H), 0.05 (s, 9H). MS [ESI$^+$] m/z: 414.13 [M + H$^+$]$^+$.

(*E*)-*N*′-(3-Cyano-6-methoxypyridin-2-yl)-*N*,*N*-dimethylformimidamide (21s, R_4 = R_5 = H, R_6 = OMe). The product was isolated as a white solid (104 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (s, 1H), 7.60 (d, J = 8.4 Hz, 1H), 6.27 (d, J = 8.4 Hz, 1H), 3.89 (s, 3H), 3.15 (s, 3H), 3.13 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.0, 163.1, 155.9, 143.3, 118.6, 104.0, 93.4, 53.5, 41.0, 35.0. MS [ESI$^+$] m/z: 205.13 [M + H$^+$]$^+$.

***N*-(3-Cyano-5-(4-methoxyphenyl)-6-(methylthio)-4-(trimethylsilyl)pyridin-2-yl)formamide (22a, R_4 = TMS, R_5 = 4-Methoxyphenyl, R_6 = SMe).** The product was isolated as a light yellow solid (22.3 mg, 60%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.64 (d, J = 9.9 Hz, 1H), 8.18 (d, J = 9.8 Hz, 1H), 7.11−7.03 (m, 2H), 6.99−6.94 (m, 2H), 3.87 (s, 3H), 2.38 (s, 3H), 0.08 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 161.4, 160.2, 152.8, 151.5, 136.8, 131.8, 129.2, 117.1, 114.1, 93.4, 55.3, 14.6, 0.3. HRMS [ESI$^+$] calculated for $C_{18}H_{22}N_3O_2SSi$ m/z, 372.119 65; found 372.118 50 [M + H$^+$]$^+$.

***N*-(3-Cyano-6-(methylthio)-5-(3-nitrophenyl)-4-(trimethylsilyl)pyridin-2-yl)formamide (22b, R_4 = TMS, R_5 = 3-Nitrophenyl, R_6 = SMe).** The product was isolated as a yellow solid (32.5 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.65 (d, J = 9.8 Hz, 1H), 8.34 (ddd, J = 8.3, 2.3, 1.0 Hz, 1H), 8.21 (d, J = 9.7 Hz, 1H), 8.09 (t, J = 1.9 Hz, 1H), 7.66 (t, J = 7.9 Hz, 1H), 7.57−7.51 (m, 1H), 2.42 (s, 3H), 0.08 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.2, 161.2, 153.2, 152.2, 148.3, 138.8, 136.9, 134.1, 129.9, 125.9, 124.1, 116.7, 93.8, 14.6, 0.4. HRMS [ESI$^-$] calculated for $C_{17}H_{17}N_4O_3SSi$ m/z, 385.079 61; found 385.079 83 [M − H$^+$]$^-$.

7-(Methylthio)pyrido[2,3-*d*]pyrimidin-4-amine (23a, R_5 = R_6 = H, R_7 = SMe). Product was isolated as yellow solid (26 mg, 100%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.45 (s, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 2.65 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.0, 163.3, 159.3, 159.2, 132.9, 120.1, 105.9, 12.9. MS [ESI$^+$] m/z: 193.05 [M + H$^+$]$^+$.

6-Bromo-7-(methylthio)pyrido[2,3-*d*]pyrimidin-4-amine (23b, R_6 = Br). The product was isolated as a yellow solid (167.1 mg, 88%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.82 (s, 1H), 8.46 (s, 1H), 8.03 (br_s, 2H), 2.57 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 165.9, 162.7, 159.6, 157.6, 135.6, 114.6, 107.0, 14.7. MS [ESI$^+$] m/z: 270.96 [M + H$^+$]$^+$.

7-(Methylthio)-6-phenylpyrido[2,3-*d*]pyrimidin-4-amine (23c, R_6 = Phenyl). Product was isolated as light yellow powder (42.5 mg, 94%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.49 (s, 1H), 8.22 (s, 1H), 7.53−7.43 (m, 5H), 2.62 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD): δ 168.1, 163.6, 157.2, 155.9, 136.7, 135.5, 131.2, 129.1, 128.4, 128.2, 105.0, 12.7. HRMS [ESI$^\pm$] calculated for $C_{14}H_{13}N_4S$ m/z, 269.085 54; found 269.084 45 [M + H$^+$]$^+$.

6-(3-Methoxyphenyl)-7-(methylthio)pyrido[2,3-*d*]pyrimidin-4-amine (23d, R_6 = 3-Methoxyphenyl). Product was isolated as yellow powder (30.4 mg, 86%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.45 (s, 1H), 8.36 (s, 1H), 7.91 (br_s, 2H), 7.41 (t, J = 7.7 Hz, 1H), 7.08−7.00 (m, 3H), 3.79 (s, 3H), 2.51 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 165.4, 163.5, 159.6, 159.2, 158.1, 138.5, 133.8, 132.5,

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130.1, 122.0, 115.4, 114.5, 105.0, 55.7, 13.8. MS [ESI$^+$] m/z: 299.20 [M + H$^+$]$^+$.

7-(Methylthio)-6-(thiophen-3-yl)pyrido[2,3-*d*]pyrimidin-4-amine (23e, R_6 = 3-Thiophenyl). Product was isolated as a yellow powder (37.4 mg, 94%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.44 (s, 2H), 7.92 (br_s, 2H), 7.79 (dd, J = 2.9, 1.3 Hz, 1H), 7.70 (dd, J = 5.0, 3.0 Hz, 1H), 7.37 (dd, J = 5.0, 1.3 Hz, 1H), 2.54 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 165.5, 163.4, 159.2, 157.9, 137.2, 132.5, 129.2, 129.0, 127.0, 126.1, 105.8, 13.8. MS [ESI$^+$] m/z: 275.15 [M + H$^+$]$^+$.

6-(4-Isopropoxyphenyl)-7-(methylthio)pyrido[2,3-*d*]-pyrimidin-4-amine (23f, R_6 = 4-Isopropoxyphenyl). The product was isolated as a yellow solid (104.2 mg, 57%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.45 (s, 1H), 8.33 (s, 1H), 7.88 (br_s, 2H), 7.41 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.7 Hz, 2H), 4.70 (hept, J = 6.0 Hz, 1H), 2.53 (s, 3H), 1.31 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 165.4, 163.0, 158.6, 157.6, 157.5, 133.4, 131.8, 130.7, 128.7, 115.4, 105.4, 69.2, 21.8, 13.4. MS [ESI$^+$] m/z: 327.12 [M + H$^+$]$^+$.

Pyrido[2,3-*d*]pyrimidin-4-amine (25a, R_5 = R_6 = R_7 = H). Product was isolated as white solids (63.0 mg, 75%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.98 (dd, J = 4.4, 1.9 Hz, 1H), 8.66 (dd, J = 8.2, 1.9 Hz, 1H), 8.52 (s, 1H), 8.09 (br_s, 2H), 7.52 (dd, J = 8.2, 4.4 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.6, 159.1, 159.0, 156.4, 133.7, 121.6, 109.5.

6-Bromopyrido[2,3-*d*]pyrimidin-4-amine (25b, R_5 = R_7 = H, R_6 = Br). Product was isolated as light yellow powder (210.7 mg, 95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.03 (d, J = 2.5 Hz, 1H), 8.97 (d, J = 2.5 Hz, 1H), 8.53 (s, 1H), 8.19 (br_s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 162.9, 159.5, 157.5, 157.0, 135.6, 115.7, 110.6. MS [ESI$^+$] m/z: 224.97 [M + H$^+$]$^+$.

6-Phenylpyrido[2,3-*d*]pyrimidin-4-amine (25c, R_5 = R_7 = H, R_6 = Phenyl). Product was isolated as light yellow powder (160.0 mg, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.33 (d, J = 2.5 Hz, 1H), 8.99 (d, J = 2.5 Hz, 1H), 8.51 (s, 1H), 7.89−7.83 (m, 2H), 7.58−7.51 (m, 2H), 7.48−7.41 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.8, 159.0, 158.3, 154.8, 136.7, 133.1, 130.7, 129.7, 128.8, 127.4, 109.2. MS [ESI$^+$] m/z: 223.09 [M + H$^+$]$^+$.

6-(4-Isopropoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-amine (25d, R_5 = R_7 = H, R_6 = 4-Isopropoxyphenyl). Product was isolated as light yellow powder (160 mg, 97%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.31 (d, J = 2.5 Hz, 1H), 8.93 (d, J = 2.5 Hz, 1H), 8.50 (s, 1H), 8.12 (br_s, 2H), 7.80 (d, J = 8.8 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 4.71 (hept, J = 6.0 Hz, 1H), 1.31 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 163.7, 158.6, 158.2, 157.8, 154.5, 132.9, 129.6, 128.7, 128.6, 116.7, 109.3, 69.8, 22.2. MS [ESI$^+$] m/z: 281.24 [M + H$^+$]$^+$.

Synthesis of 6-(Thiophen-2-yl)pyrido[2,3-*d*]pyrimidin-4-amine (25e, R_5 = R_7 = H, R_6 = 2-Thiophenyl). The product was isolated as a yellow solid (80.3 mg; 80%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.35 (d, J = 2.5 Hz, 1H), 8.93 (d, J = 2.5 Hz, 1H), 8.54 (s, 1H),, 8.30 (br_s, 2H), 7.76 (dd, J = 3.6, 1.1 Hz, 1H), 7.72 (dd, J = 5.1, 1.1 Hz, 1H), 7.25 (dd, J = 5.1, 3.6 Hz, 1H). MS [ESI$^+$] m/z: 229.05 [M + H$^+$]$^+$.

6-(3,5-Dimethylisoxazol-4-yl)pyrido[2,3-*d*]pyrimidin-4-amine (25f, R_5= R_7 = H, R_6= 3,5-Dimethylisoxazol-4-yl). The product was isolated as a yellow solid (79.5 mg, 69%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.02 (d, J = 2.4 Hz, 1H), 8.65 (d, J = 2.4 Hz, 1H), 8.56 (s, 1H), 8.19 (s, 2H), 2.32 (s, 3H), 1.91 (s, 3H). MS [ESI$^+$] m/z: 242.10 [M + H$^+$]$^+$.

6-(*p*-Tolyl)pyrido[2,3-*d*]pyrimidin-4-amine (25g, R_5 = R_7 = H, R_6 = *p*-Tolyl). The product was isolated as a light orange solid (29.4 mg, 63%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.33 (d, J = 2.5 Hz, 1H), 8.97 (d, J = 2.5 Hz, 1H), 8.52 (s, 1H), 8.17 (br_s, 2H), 7.78 (d, J = 8.1 Hz, 2H), 7.37 (d, J = 7.9 Hz, 2H), 2.38 (s, 3H), MS [ESI$^+$] m/z: 237.11 [M + H$^+$]$^+$.

6-(4-Methoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-amine (25h, R_5 = R_7 = H, R_6 = 4-Methoxyphenyl). The product was isolated as a yellow solid (85.6 mg, 95%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.32 (d, J = 2.5 Hz, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.51 (s, 1H), 8.13 (br_s, 2H), 7.83 (d, J = 8.8 Hz, 2H), 7.12 (d, J = 8.8 Hz, 2H), 3.84 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.7, 160.0, 158.7, 157.9, 154.5, 132.9, 129.6, 129.0, 128.6, 115.1, 109.3, 55.8. MS [ESI$^+$] m/z: 253.19 [M + H$^+$]$^+$.

6-(Thiophen-3-yl)pyrido[2,3-*d*]pyrimidin-4-amine (25i, R_5 = R_7 = H, R_6 = 3-Thiophenyl). The product was isolated as a light brown solid (85.4 mg, quantitative). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.43 (d, J = 2.4 Hz, 1H), 9.00 (d, J = 2.5 Hz, 1H), 8.51 (s, 1H), 8.15 (dd, J = 2.9, 1.4 Hz, 1H), 8.10 (br_s, 2H), 7.78 (dd, J = 5.0, 2.9 Hz, 1H), 7.75 (dd, J = 5.0, 1.4 Hz, 1H). MS [ESI$^+$] m/z: 229.08 [M + H$^+$]$^+$.

7-(Thiophen-2-yl)pyrido[2,3-*d*]pyrimidin-4-amine (25j, R_5 = R_6 = H, R_7 = 2-Thiophenyl). The product was isolated as a light yellow solid (28.5 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.65 (d, J = 8.6 Hz, 1H), 8.47 (s, 1H), 8.08 (d, J = 8.6 Hz, 1H), 8.05 (dd, J = 3.7, 1.0 Hz, 1H), 8.01 (br_s, 2H), 7.79 (dd, J = 5.0, 0.9 Hz, 1H), 7.23 (dd, J = 5.0, 3.8 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d6): δ 163.8, 160.2, 159.6, 158.0, 145.0, 135.3, 132.1, 129.7, 129.4, 118.0, 108.6. MS [ESI$^+$] m/z: 229.047 [M + H$^+$]$^+$.

6-(3-Methoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-amine (25k, R_5 = R_6 = H, R_7 = 3-Methoxyphenyl). The product was isolated as a yellow orange solid (28.0 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.37 (d, J = 2.4 Hz, 1H), 9.00 (d, J = 2.5 Hz, 1H), 8.54 (s, 1H), 8.16 (br_s, 2H), 7.51−7.42 (m, 3H), 7.04 (dt, J = 7.0, 2.4 Hz, 1H), 3.88 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.4, 160.0, 158.5, 157.7, 154.4, 137.6, 132.5, 130.4 (2 × C overlapping), 119.2, 113.9, 112.5, 108.7, 55.3. MS [ESI$^+$] m/z: 253.23 [M + H$^+$]$^+$.

7-Methoxypyrido[2,3-*d*]pyrimidin-4-amine (25l, R_5 = R_6 = H, R_7 = OMe). The product was obtained as a white solid (69.4 mg, 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.48 (d, J = 8.9 Hz, 1H), 8.40 (s, 1H), 7.79 (br_s, 2H), 6.94 (d, J = 8.9 Hz, 1H), 3.94 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.7, 162.9, 159.3, 159.2, 136.2, 111.7, 104.5, 54.0. MS [ESI$^+$] m/z: 177.07 [M + H$^+$]$^+$.

General Protocols for the Synthesis of Bisphosphonate Tetraethyl Esters 26. The pyrido[2,3-*d*]pyrimidin-4-amine intermediate (i.e., **23** or **25**; 1 equiv), diethyl phosphite (6 equiv), and triethyl orthoformate (1.6 equiv) were dissolved in dry DMF or toluene. The mixture was heated in a microwave at 150 °C for 1.5 h. The resulting mixture was cooled to room temperature and concentrated under vacuum. The product was purified by column chromatography on silica gel. Any product isolated with <90% homogeneity was further purified by reversed-phase preparative HPLC using a Waters Atlantis T3 C18 5 μm column: solvent A, H_2O, 0.1% formic acid; solvent B, CH_3CN, 0.1% formic acid; mobile phase, gradient from 95% A and 5% B to 5% A and 95% B in 17 min acquisition time; flow rate, 1 mL/min.

Tetraethyl ((Pyrido[2,3-*d*]pyrimidin-4-ylamino)methylene)-bis(phosphonate) (26a, R_6 = R_7 = H). The product was isolated as a light yellow solid (11.4 mg, 38%). ^{1}H NMR (500 MHz, CD_3OD): δ 9.06 (d, J = 3.0 Hz, 1H), 8.87 (d, J = 7.4 Hz, 1H), 8.76 (s, 1H), 7.65 (dd, J = 8.2, 4.4 Hz, 1H), 6.08 (t, J = 23.5 Hz, 1H), 4.29−4.14 (m, 8H), 1.30 (t, J = 7.1 Hz, 6H), 1.25 (t, J = 7.1 Hz, 6H). ^{31}P NMR (81 MHz, CD_3OD): δ 16.59. ^{13}C NMR (126 MHz, CD_3OD): δ 160.6, 157.9, 157.5, 156.2, 132.7, 122.2, 110.1, 63.8 (t, J = 3.1 Hz), 15.3−15.1 (m), C-α to the phosphonate overlaps with the solvent peak. MS [ESI$^+$] m/z: 433.13 [M + H$^+$]$^+$.

Tetraethyl (7-(Methylthio)pyrido[2,3-*d*]pyrimidin-4-ylamino)methylenediphosphonate (26b, R_6 = H, R_7 = SMe). The product was isolated as a yellow solid (37.8 mg, 30%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.74 (s, 1H), 8.11 (d, J = 8.7 Hz, 1H), 7.27 (d, J = 8.7 Hz, 1H), 6.72 (d, J = 9.7 Hz, 1H), 5.86 (td, J = 22.0, 9.7 Hz, 1H), 3.89−4.33 (m, 8H), 2.71 (s, 3H), 1.29 (t, J = 7.1 Hz, 6H), 1.20 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.3, 159.1, 159.0, 158.2, 133.0, 113.0, 104.8, 63.4−63.9 (m), 54.4, 44.8 (t, J = 147.2 Hz), 16.3−16.4 (m). ^{31}P NMR (81 MHz, $CDCl_3$): δ 17.46. MS [ESI$^+$] m/z: 479.18 [M + H$^+$]$^+$.

Tetraethyl (((7-(Thiophen-2-yl)pyrido[2,3-*d*]pyrimidin-4-yl)-amino)methylene)bis(phosphonate) (26c, R_6 = H, R_7 = 2-Thiophenyl). The product was isolated as a yellow solid (8.0 mg, 9%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.78 (d, J = 8.7 Hz, 1H), 8.70 (s, 1H), 8.09 (d, J = 8.7 Hz, 1H), 8.00 (dd, J = 3.7, 0.7 Hz, 1H), 7.70 (dd, J = 5.0, 0.8 Hz, 1H), 7.22 (dd, J = 5.0, 3.8 Hz, 1H), 6.07 (t, J = 23.5 Hz, 1H), 4.29−4.12 (m, 8H), 1.31 (t, J = 7.1 Hz, 6H), 1.26 (t, J =

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7.1 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD): δ 160.2 (t, J = 4.3 Hz), 158.5, 158.0, 157.7, 143.4, 132.9, 130.8, 128.4, 128.2, 113.0, 108.3, 63.9−63.7 (m), 16.2−14.7 (m); C-α to the bisphosphonate overlaps with the solvent peak, as determined by HSQC. ^{31}P NMR (81 MHz, CD_3OD): δ 16.67. MS [ESI$^+$] m/z: 515.12 [M + H$^+$]$^+$.

Tetraethyl (((7-(Methylsulfonyl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (26d, R_6 = H, R_7 = SO_2Me). Product was isolated as a yellow solid (20 mg, 54%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.01 (d, J = 8.5 Hz, 1H), 8.89 (s, 1H), 8.09 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 9.6 Hz, 1H), 5.93 (td, J = 22.2, 9.6 Hz, 1H), 4.39−4.00 (m, 8H), 3.48 (s, 3H), 1.35 (t, J = 7.1 Hz, 6H), 1.21 (t, J = 7.1 Hz, 6H). ^{31}P NMR (81 MHz, $CDCl_3$): δ 16.85. ^{13}C NMR (75 MHz,$CDCl_3$): δ 163.3, 161.4, 159.6, 157.7, 136.4, 116.3, 112.0, 63.9 (t, J = 3.1 Hz), 45.4 (t, J = 147.5 Hz), 38.9, 16.4 (t, J = 2.5 Hz). MS [ESI$^+$] m/z: 511.32 [M + H$^+$]$^+$.

Tetraethyl (7-Methoxypyrido[2,3-*d*]pyrimidin-4-ylamino)-methylenediphosphonate (26e, R_6 = H, R_7 = OMe). The product was isolated as a white solid (25 mg, 16%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.71 (s, 1H), 8.24 (d, J = 9.0 Hz, 1H), 6.89 (d, J = 9.0 Hz, 1H), 6.63 (d, J = 9.7 Hz, 1H), 5.87 (td, J = 22.1, 9.7 Hz, 1H), 4.11−4.31 (m, 8H), 4.10 (s, 3H), 1.28 (t, J = 7.1 Hz, 6H), 1.19 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.3, 159.1, 158.2, 148.11, 133, 113.0, 104.8, 63.6−63.8 (m), 54.4, 44.8 (t, J = 147.2 Hz), 16.3−16.4 (m). ^{31}P NMR (81 MHz, $CDCl_3$): δ 16.49. MS [ESI$^+$] m/z: 463.3 [M + H$^+$]$^+$.

Tetraethyl (((6-Bromopyrido[2,3-*d*]pyrimidin-4-yl)amino)-methylene)bis(phosphonate) (26f, R_6 = Br, R_7 = H). The product was isolated as a yellow solid (169.2 mg, 50%). ^{1}H NMR (500 MHz, CD_3OD): δ 9.16 (s, 1H), 9.11 (s, 1H), 8.78 (s, 1H), 6.04 (t, J = 23.4 Hz, 1H), 4.29−4.14 (m, 8H), 1.30 (t, J = 7.1 Hz, 6H), 1.27 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD): δ 159.9, 157.7, 157.3, 156.4, 134.6, 117.0, 111.0, 63.85 (t, J = 3.2 Hz), 15.3 (t, J = 2.8 Hz) and 15.2 (t, J = 3.0 Hz) (P-O-$CH_2\underline{C}H_3$, phosphonate groups are non-equivalent); C-α to the bisphosphonate overlaps with the solvent peak. ^{31}P NMR (81 MHz, CD_3OD): δ 16.42. MS [ESI$^+$] m/z: 511.04 [M + H$^+$]$^+$.

Tetraethyl (((6-(Thiophen-2-yl)pyrido[2,3-*d*]pyrimidin-4-yl)-amino)methylene)bis(phosphonate) (26g, R_6 = 2-Thiophenyl, R_7 = H). The product was isolated as a yellow solid (24.3 mg, 18%). ^{1}H NMR (500 MHz, CD_3OD): δ 9.37 (s, 1H), 9.10 (d, J = 2.3 Hz, 1H), 8.72 (s, 1H), 7.70 (dd, J = 3.7, 1.1 Hz, 1H), 7.59 (dd, J = 5.1, 1.1 Hz, 1H), 7.21 (dd, J = 5.1, 3.7 Hz, 1H), 6.11 (t, J = 23.6 Hz, 1H), 4.29−4.16 (m, 8H), 1.31 (t, J = 7.1 Hz, 6H), 1.27 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD): δ 160.6 (t, J = 4.1 Hz), 156.9, 156.7, 153.6, 138.8, 129.3, 128.4, 127.5, 127.0, 125.5, 110.1, 63.8 (t, J = 3.3 Hz), 45.2 (t, J = 151.0 Hz), 15.3 (t, J = 2.8 Hz) and 15.2 (t, J = 3.0 Hz) (P-O-$CH_2\underline{C}H_3$, phosphonate groups are nonequivalent). ^{31}P NMR (81 MHz, CD_3OD): δ 16.7. MS [ESI$^+$] m/z: 515.12 [M + H$^+$]$^+$.

Tetraethyl (((6-(3,5-Dimethylisoxazol-4-yl)pyrido[2,3-*d*]-pyrimidin-4-yl)amino)methylene)bis(phosphonate) (26h, R_6 = 3,5-Dimethylisoxazol-4-yl, R_7 = H). The product was isolated as a yellow solid (22.0 mg, 17%). ^{1}H NMR (400 MHz, CD_3OD): δ 9.07 (s, 1H), 8.87 (s, 1H), 8.80 (s, 1H), 6.11 (t, J = 23.5 Hz, 1H), 4.29−4.16 (m, 8H), 2.50 (s, 3H), 2.34 (s, 3H), 1.31 (t, J = 7.1 Hz, 6H), 1.26 (t, J = 7.1 Hz, 6H). ^{31}P NMR (81 MHz, CD_3OD): δ 16.60. MS [ESI$^+$] m/z: 528.17 [M + H$^+$]$^+$.

Tetraethyl (((6-(4-Isopropoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (26i, R_6 = 4-Isopropoxyphenyl, R_7 = H). The product was isolated as a yellow oil (13.3 mg, 22%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.34 (d, J = 2.4 Hz, 1H), 8.83 (s, 1H), 8.45 (br_s, -NH), 7.69−7.57 (m, 2H), 7.04−6.99 (m, 2H), 5.91 (td, J = 21.9, 9.7 Hz, 1H), 4.62 (hept, J = 6.1 Hz, 1H), 4.37−4.04 (m, 8H), 1.38 (s, 3H), 1.37 (s, 3H), 1.31 (t, J = 7.1 Hz, 6H), 1.22 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.6, 158.6, 157.5, 157.3, 155.6, 134.7, 128.5, 128.4, 126.9, 116.5, 109.5, 70.0, 64.0 (t, J = 3.2 Hz) and 63.7 (t, J = 3.1 Hz) (P-O-$\underline{C}H_2CH_3$, phosphonate groups are nonequivalent), 45.1 (t, J = 147.1 Hz), 22.0, 16.4−16.3 (m). ^{31}P NMR (162 MHz, $CDCl_3$): δ 16.42. MS [ESI$^+$] m/z: 567.36 [M + H$^+$]$^+$.

Tetraethyl (((6-Phenylpyrido[2,3-*d*]pyrimidin-4-yl)amino)-methylene)bis(phosphonate) (26j, R_6 = Phenyl, R_7 = H). The product was isolated as a yellow solid (12.2 mg, 11%). ^{1}H NMR (500 MHz, CD_3OD): δ 9.36 (s, 1H), 9.14 (d, J = 2.3 Hz, 1H), 8.76 (s, 1H), 7.89−7.80 (m, 2H), 7.60−7.53 (m, 2H), 7.50−7.45 (m, 1H), 6.12 (t, J = 23.5 Hz, 1H), 4.30−4.15 (m, 8H), 1.31 (t, J = 7.1 Hz, 6H), 1.26 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD): δ 160.7, 157.2, 157.0, 155.0, 136.2, 135.3, 129.6, 129.0, 128.5, 127.0, 110.0, 63.8 (t, J = 3.3 Hz), 15.3 (t, J = 2.8 Hz) and 15.2 (t, J = 3.0 Hz) (P-O-$CH_2\underline{C}H_3$, phosphonate groups are nonequivalent); C-α to the bisphosphonate overlaps with the solvent peak. ^{31}P NMR (81 MHz, CD_3OD): δ 16.68. MS [ESI$^+$] m/z: 509.164 [M + H$^+$]$^+$.

Tetraethyl (((7-(Methylthio)-6-phenylpyrido[2,3-*d*]-pyrimidin-4-yl)amino)methylene)bis(phosphonate) (26k, R_6 = Phenyl, R_7 = SMe). The product was isolated as a light yellow solid (18.5 mg, 45%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (d, J = 9.3 Hz, -NH), 8.88 (s, 1H), 8.64 (s, 1H), 7.58−7.45 (m, 5H), 5.88 (td, J = 23.5, 9.0 Hz, 1H), 4.13−3.95 (m, 8H), 2.53 (s, 3H), 1.17 (t, J = 7.0 Hz, 6H), 1.08 (t, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 166.1, 160.5, 158.1, 157.9, 137.0, 134.6, 132.3, 129.8, 129.1, 128.9, 106.8, 63.4 (t, J = 3.0 Hz) and 63.2 (t, J = 3.0 Hz) (P-O-$\underline{C}H_2CH_3$, phosphonate groups are nonequivalent), 46.7−44.2 (m), 16.7−16.5 (m), 13.8. ^{31}P NMR (203 MHz, CD_3OD): δ 16.74. MS [ESI$^+$] m/z: 555.35 [M + H$^+$]$^+$.

Tetraethyl (((7-(Methylthio)-6-(thiophen-3-yl)pyrido[2,3-*d*]-pyrimidin-4-yl)amino)methylene)bis(phosphonate) (26l, R_6 = 3-Thiophenyl, R_7 = SMe). Product was isolated as a yellow solid (35.0 mg, 34%). ^{1}H NMR (500 MHz, CD_3OD): δ 8.68 (s, 1H), 8.53 (s, 1H), 7.74−7.70 (m, 1H), 7.57 (ddd, J = 5.0, 3.0, 0.9 Hz, 1H), 7.41−7.37 (m, 1H), 6.06 (t, J = 23.5 Hz, 1H), 4.27−4.14 (m, 8H), 2.65 (s, 3H), 1.30 (t, J = 7.1 Hz, 6H) and1.25 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD): δ 168.2, 160.40 (t, J = 4.1 Hz), 157.2, 156.7, 136.6, 130.8, 130.0, 128.1, 125.7, 125.3, 106.1, 63.8−63.7 (m), 44.9 (t, J = 149.8 Hz), 15.3 (t, J = 2.8 Hz) and 15.2 (t, J = 3.1 Hz) (P-O-$CH_2\underline{C}H_3$, phosphonate groups are nonequivalent), 12.7. ^{31}P NMR (81 MHz, CD_3OD): δ 16.74. MS [ESI$^+$] m/z: 561.108 [M + H$^+$]$^+$.

Tetraethyl (((6-(4-Isopropoxyphenyl)-7-(methylthio)pyrido-[2,3-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (26m, R_6 = 4-Isopropoxyphenyl, R_7 = SMe). The product was isolated as a yellow solid (12.6 mg, 22%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.68 (s, 1H), 8.41 (s, 1H), 7.44 (d, J = 8.6 Hz, 2H), 7.02 (d, J = 8.7 Hz, 2H), 6.06 (t, J = 23.5 Hz, 1H), 4.69 (hept, J = 6.1 Hz, 1H), 4.25−4.14 (m, 8H), 2.63 (s, 3H), 1.37 (s, 3H), 1.36 (s, 3H), 1.30 (t, J = 7.1 Hz, 6H), 1.25 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD): δ 168.6, 160.4, 158.5, 157.0, 156.7, 136.7, 130.4, 129.8, 128.6, 115.3, 106.2, 69.6, 63.9−63.7 (m), 20.9, 15.3 (t, J = 2.9 Hz) and 15.2 (t, J = 3.0 Hz) (P-O-$CH_2\underline{C}H_3$, phosphonate groups are nonequivalent), 12.7; C-α to the bisphosphonate overlaps with the solvent peak. ^{31}P NMR (162 MHz, CD_3OD): δ 16.78. MS [ESI$^+$] m/z: 613.34 [M + H$^+$]$^+$.

Tetraethyl (((6-Bromo-7-(methylthio)pyrido[2,3-*d*]-pyrimidin-4-yl)amino)methylene)bis(phosphonate) (26n, R_6 = Br, R_7 = SMe). The product was isolated as a yellow solid (55.6 mg, 30%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.40 (s, 1H), 8.65 (s, 1H), 5.82 (t, J = 23.5 Hz, 1H), 4.12−3.97 (m, 8H), 2.59 (s, 3H), 1.17 (t, J = 7.0 Hz, 6H), 1.09 (t, J = 7.0 Hz, 6H). MS [ESI$^+$] m/z: 557.03 [M + H$^+$]$^+$.

General Protocols for the Synthesis of Bisphosphonate Tetraethyl Esters 27. The pyrido[2,3-*d*]pyrimidin-4-amine intermediate (i.e., **23** or **25**; 1 equiv) and tetraethylethene 1,1-diylbisphosphonate (1.1−2.0 equiv), which was previously prepared according to literature procedures,[43,44] were dissolved in anhydrous THF. The mixture was stirred at room temperature for 16 h and then concentrated under vacuum. The product was purified by column chromatography on silica gel. Any product isolated with <90% homogeneity was further purified by reversed-phase preparative HPLC using a Waters Atlantis T3 C18 5 μm column as described for the bisphosphonate tetraesters **26**.

Tetraethyl (2-(Pyrido[2,3-*d*]pyrimidin-4-ylamino)ethane-1,1-diyl)bis(phosphonate) (27a, R_6 = R_7 = H). Product was isolated as a pale yellow solid (28.6 mg, 47%). ^{1}H NMR (500 MHz, CD_3OD): δ 9.01 (d, J = 2.8 Hz, 1H), 8.70 (s, 1H), 8.58 (dd, J = 8.3, 1.7 Hz, 1H), 7.59 (dd, J = 8.3, 4.4 Hz, 1H), 4.31−4.05 (m, 10H), 3.62−3.49 (m,

1H),, 1.30−1.27 (m, 12H). ^{13}C NMR (126 MHz, CD_3OD): δ 160.8, 158.0, 157.4, 155.7, 132.4, 121.8, 110.1, 63.5−62.5 (m), 37.9 (t, J = 3.6 Hz), 34.5 (t, J = 132.7 Hz), 15.6−14.6 (m). ^{31}P NMR (81 MHz, CD_3OD): δ 21.28. MS [ESI$^+$] m/z: 447.148 [M + H$^+$]$^+$.

Tetraethyl (2-((6-Phenylpyrido[2,3-*d*]pyrimidin-4-yl)amino)-ethane-1,1-diyl)bis(phosphonate) (27b, R_6 = Phenyl, R_7 = H). The product was isolated as a light yellow oil (16.2 mg, 23%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.31 (d, J = 2.3 Hz, 1H), 8.84 (s, 1H), 8.33 (d, J = 2.2 Hz, 1H), 7.80 (t, J = 4.8 Hz, -NH), 7.67 (d, J = 7.3 Hz, 2H), 7.52 (t, J = 7.6 Hz, 2H), 7.45 (t, J = 7.4 Hz, 1H), 4.45−4.02 (m, 10H), 2.87 (tt, J = 22.9, 6.2 Hz, 1H), 1.38 (t, J = 7.1 Hz, 6H), 1.32 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 160.4, 158.2, 157.4, 155.3, 136.7, 134.7, 129.4, 128.5, 128.1, 127.4, 109.6, 63.4−63.0 (m), 37.8 (t, J = 4.4 Hz), 36.4 (t, J = 131.9 Hz), 16.4−16.3 (m). ^{31}P NMR (162 MHz, $CDCl_3$): δ 21.11. MS [ESI$^+$] m/z: 523.21 [M + H$^+$]$^+$.

Tetraethyl (2-((6-(Thiophen-3-yl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)bis(phosphonate) (27c, R_6 = 3-Thiophenyl, R_7 = H). The product was isolated as a yellow oil (16.5 mg, 47%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.30 (d, J = 2.4 Hz, 1H), 8.77 (s, 1H), 8.33 (d, J = 2.4 Hz, 1H), 7.86 (t, J = 4.7 Hz, -NH), 7.63 (dd, J = 2.5, 1.8 Hz, 1H), 7.49−7.42 (m, 2H), 4.29−4.13 (m, 10H), 2.89 (tt, J = 22.9, 6.3 Hz, 1H), 1.37 (t, J = 7.1 Hz, 6H), 1.30 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 160.3, 158.2, 157.5, 154.6, 137.7, 129.4, 127.4, 126.9, 125.9, 122.2, 109.7, 63.4−62.9 (m), 37.7 (t, J = 4.4 Hz), 36.3 (t, J = 132 Hz), 16.5−16.3 (m). ^{31}P NMR (203 MHz, $CDCl_3$): δ 21.15. MS [ESI$^+$] m/z: 529.17 [M + H$^+$]$^+$.

Tetraethyl (2-((6-(*p*-Tolyl)pyrido[2,3-*d*]pyrimidin-4-yl)-amino)ethane-1,1-diyl)bis(phosphonate) (27d, R_6 = *p*-Tolyl, R_7 = H). The product was isolated as a yellow solid (21.2 mg, 78%). ^{1}H NMR (500 MHz, CD_3OD): δ 9.30 (s, 1H), 8.78 (s, 1H), 8.72 (s, 1H), 7.68 (d, J = 8.1 Hz, 2H), 7.36 (d, J = 7.9 Hz, 2H), 4.18 (m, 10H), 3.57 (tt, J = 23.1, 7.1 Hz, 1H), 2.42 (s, 3H), 1.33−1.25 (m, 12H). ^{13}C NMR (126 MHz, CD_3OD): δ 160.9, 157.8, 156.3, 154.2, 138.7, 134.8, 133.3, 129.7, 129.0, 126.7, 105.0, 63.3−62.8 (m), 37.9 (br), 34.5 (t, J = 132.7 Hz), 19.8, 15.3−15.2 (m). ^{31}P NMR (81 MHz, CD_3OD): δ 21.29. MS [ESI$^+$] m/z: 537.195 [M + H$^+$]$^+$.

Tetraethyl (2-((6-(4-Methoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)bis(phosphonate) (27e, R_6 = 4-Methoxyphenyl, R_7 = H). The product was isolated as a light yellow oil (15.0 mg, 46%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.25 (d, J = 2.4 Hz, 1H), 8.78 (s, 1H), 8.24 (d, J = 2.4 Hz, 1H), 7.75 (t, J = 4.9 Hz, -NH), 7.58−7.53 (m, 2H), 7.03−6.95 (m, 2H), 4.30−4.14 (m, 10H), 3.86 (s, 3H), 2.87 (tt, J = 22.9, 6.3 Hz, 1H), 1.37 (t, J = 7.1 Hz, 6H), 1.30 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 160.3, 160.0, 158.1, 157.4, 155.0, 134.2, 129.1, 128.4, 127.1, 114.8, 109.6, 63.4−63.0 (m), 55.4, 37.7 (t, J = 4.1 Hz), 36.4 (t, J = 131.8 Hz), 16.4−16.3 (m). ^{31}P NMR (162 MHz, $CDCl_3$) 21.16. MS [ESI$^+$] m/z: 553.36 [M + H$^+$]$^+$.

Tetraethyl (2-((6-(3-Methoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)bis(phosphonate) (27f, R_6 = 3-Methoxyphenyl, R_7 = H). The product was isolated as a yellow oil (30.0 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.27 (d, J = 2.4 Hz, 1H), 8.81 (s, 1H), 8.31 (d, J = 2.4 Hz, 1H), 7.81 (t, J = 4.8 Hz, -NH), 7.40 (t, J = 8.0 Hz, 1H), 7.20 (ddd, J = 7.6, 1.6, 0.9 Hz, 1H), 7.16−7.11 (m, 1H), 6.95 (ddd, J = 8.3, 2.5, 0.7 Hz, 1H), 4.30−4.14 (m, 10H), 2.89 (tt, J = 22.9, 6.3 Hz, 1H), 1.37 (t, J = 7.1 Hz, 6H), 1.31 (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 160.4, 160.2, 158.4, 157.8, 155.2, 138.1, 134.4, 130.4, 128.2, 119.7, 113.7, 113.2, 109.6, 63.4−63.0 (m), 55.4, 37.8 (t, J = 4.3 Hz), 36.4 (t, J = 131.8 Hz), 16.4−16.3 (m). ^{31}P NMR (162 MHz, $CDCl_3$): 21.15. MS [ESI$^+$] m/z: 553.35 [M + H$^+$]$^+$.

Tetraethyl (2-((6-Bromo-7-(methylthio)pyrido[2,3-*d*]-pyrimidin-4-yl)amino)ethane-1,1-diyl)bis(phosphonate) (27g, R_6 = Br, R_7 = SMe). Product was isolated as a yellow oil (107.4 mg, 44%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.72 (s, 1H), 8.14 (s, 1H), 7.65 (br_s, -NH), 4.31−4.12 (m, 10H), 2.86 (tt, J = 22.9, 6.2 Hz, 1H), 1.38 (t, J = 7.1 Hz, 6H), 1.32 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.6, 159.4, 158.8, 156.9, 132.4, 116.0, 107.1, 63.4−63.0 (m), 37.7 (t, J = 4.4 Hz), 36.3 (t, J = 131.9 Hz), 16.5−16.3 (m), 15.1. ^{31}P NMR (203 MHz, $CDCl_3$): δ 21.13. MS [ESI$^+$] m/z: 571.23 [M + H$^+$]$^+$.

Tetraethyl (2-((7-(Methylthio)-6-phenylpyrido[2,3-*d*]-pyrimidin-4-yl)amino)ethane-1,1-diyl)bis(phosphonate) (27h, R_6 = Phenyl, R_7 = SMe). Product was isolated as a yellow oil (35.4 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.74 (s, 1H), 7.70 (s, 1H), 7.48−7.40 (m, 5H), 7.36 (br_s, -NH), 4.26−4.11 (m, 10H), 2.81 (tt, J = 22.9, 6.2 Hz, 1H), 2.65 (s, 3H), 1.35 (t, J = 7.1 Hz, 6H), 1.30 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.4, 160.3, 158.5, 157.5, 136.8, 135.2, 129.4, 128.8, 128.7, 128.6, 106.0, 63.3−62.9 (m), 37.6 (t, J = 4.4 Hz), 36.5 (t, J = 131.9 Hz), 16.5−16.2 (m), 14.1. ^{31}P NMR (203 MHz, $CDCl_3$): δ 21.20. MS [ESI$^+$] m/z: 569.27 [M + H$^+$]$^+$.

Synthesis of Tetraethyl (2-((6-(3-Methoxyphenyl)-7-(methylthio)pyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)bis(phosphonate) (27i, R_6 = 3-Methoxyphenyl, R_7 = SMe). The product was isolated as a yellow solid (20.0 mg, 66%). ^{1}H NMR (500 MHz, CD_3OD): δ 8.61 (s, 1H), 8.15 (s, 1H), 7.42−7.35 (m, 1H), 7.06−7.00 (m, 3H), 4.20−4.13 (m, 8H), 4.12−4.05 (m, 2H), 3.84 (s, 3H), 3.51 (tt, J = 23.1, 7.1 Hz, 1H), 2.61 (s, 3H), 1.30−1.26 (m, 12H). ^{13}C NMR (126 MHz, CD_3OD): δ 167.2, 160.8, 159.8, 157.8, 156.6, 138.0, 135.1, 130.1, 129.3, 121.3, 114.7, 113.8, 105.9, 63.2−62.8 (m), 54.4, 37.7 (t, J = 3.6 Hz), 34.4, 15.3−15.1 (m). ^{31}P NMR (81 MHz, CD_3OD): δ 21.37. MS [ESI$^+$] m/z: 599.178 [M + H$^+$]$^+$.

Synthesis of Tetraethyl (2-((6-(4-Isopropoxyphenyl)-7-(methylthio)pyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)bis(phosphonate) (27j, R_6 = 4-Isopropoxyphenyl, R_7 = SMe). The product was isolated as a light yellow oil (36.2 mg, 66%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.73 (s, 1H), 7.66 (s, 1H), 7.36−7.31 (m, 2H), 7.30 (t, J = 5.4 Hz, -NH), 6.97−6.90 (m, 2H), 4.65−4.57 (m, 1H), 4.26−4.12 (m, 10H), 2.81 (tt, J = 22.9, 6.2 Hz, 1H), 1.39 (s, 3H), 1.37 (s, 3H), 1.35 (t, J = 7.1 Hz, 6H), 1.30 (t, J = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.7, 160.2, 158.3, 158.3, 157.4, 135.0, 130.6, 128.7, 128.6, 115.7, 106.1, 69.9, 63.3−62.9 (m), 37.6 (t, J = 4.6 Hz), 36.5 (t, J = 131.9 Hz), 22.1, 16.5−16.3 (m), 14.2. ^{31}P NMR (203 MHz, $CDCl_3$): δ 21.22. MS [ESI$^+$] m/z: 627.38 [M + H$^+$]$^+$.

Tetraethyl (2-((7-(Methylthio)-6-(thiophen-3-yl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)bis(phosphonate) (27k, R_6 = 3-Thiophenyl, R_7 = SMe). The product was obtained as a yellow oil (16.4 mg, 52%). ^{1}H NMR (500 MHz, CD_3OD): δ 8.61 (s, 1H), 8.24 (s, 1H), 7.68 (dd, J = 2.9, 1.3 Hz, 1H), 7.57 (dd, J = 5.0, 3.0 Hz, 1H), 7.36 (dd, J = 5.0, 1.3 Hz, 1H), 4.25−4.09 (m, 10H), 3.51 (tt, J = 23.2, 7.2 Hz, 1H), 2.64 (s, 3H), 1.31−1.26 (m, 12H). ^{31}P NMR (81 MHz, CD_3OD): δ 21.30. MS [ESI$^+$] m/z: 575.12 [M + H$^+$]$^+$.

Characterization Data of Final Inhibitors. ((Pyrido[2,3-*d*]pyrimidin-4-ylamino)methylene)diphosphonic Acid (8a). Product was isolated as a light yellow solid (3.7 mg, 50%). ^{1}H NMR (500 MHz, D_2O): δ 8.84 (dd, J = 4.6, 1.8 Hz, 1H), 8.68 (dd, J = 8.3, 1.8 Hz, 1H), 8.47 (s, 1H), 7.55 (dd, J = 8.3, 4.6 Hz, 1H), 4.68 (t, J = 18.5 Hz, 1H). ^{13}C NMR (126 MHz, D_2O): δ 159.9, 158.7, 156.5, 154.8, 134.1, 121.8, 111.2, 52.50 (t, J = 124.7 Hz). ^{31}P NMR (81 MHz, D_2O): δ 12.67. HRMS [ESI$^-$] calculated for $C_8H_9N_4O_6P_2$ m/z, 319.000 28; found 319.001 39 [M − H$^+$]$^-$.

7-(Methylthio)pyrido[2,3-*d*]pyrimidin-4-ylamino)methylene-diphosphonic Acid (8b). Product was isolated as a yellow solid (21.9 mg, 96%). ^{1}H NMR (500 MHz, D_2O): δ 8.31 (s, 1H), 8.29 (d, J = 8.7 Hz, 1H), 7.30 (d, J = 8.7 Hz, 1H), 4.53 (t, J = 18.8 Hz, 1H), 2.53 (s, 3H). ^{13}C NMR (125 MHz, D_2O): δ 168.1, 159.8, 158.6, 157.3, 132.6, 119.7, 107.3, 39.7 (t, J = 142.2 Hz), 12.7. ^{31}P NMR (162 MHz, D_2O): δ 12.74. HRMS [ESI$^-$] calculated for $C_9H_{11}O_6N_4P_2S$ m/z, 364.988 00; found 364.98814 [M − H$^+$]$^-$.

(((7-(Thiophen-2-yl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)-methylene)diphosphonic Acid (8c). The product was isolated as a yellow solid (4.5 mg, 82%). ^{1}H NMR (400 MHz, D_2O: δ 8.66 (d, J = 8.7 Hz, 1H), 8.58 (s, 1H), 8.00 (d, J = 8.7 Hz, 1H), 7.93 (d, J = 3.3 Hz, 1H), 7.72 (d, J = 4.8 Hz, 1H), 7.29−7.21 (m, 1H), 5.08 (t, J = 19.3 Hz, 1H). ^{13}C NMR (126 MHz, D_2O): δ 160.1 (t, J = 4.0 Hz), 158.6, 157.5, 157.1, 142.3, 134.3, 130.9, 128.9, 128.8, 118.3, 109.0, 50.0 (t, J = 144.1 Hz). ^{31}P NMR (81 MHz, D_2O): δ 12.21. HRMS [ESI$^-$] calculated for $C_{12}H_{11}N_4O_6P_2S$ m/z, 400.988 00; found 400.987 98 [M − H$^+$]$^-$.

(((7-(Methylsulfonyl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)-methylene)diphosphonic Acid (8d). Product was isolated as a light

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yellow solid (16.3 mg, quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 8.91 (d, J = 8.4 Hz, 1H), 8.49 (s, 1H), 8.04 (d, J = 8.4 Hz, 1H), 4.59 (t, J = 19.9 Hz, 1H), 3.30 (s, 3H). ^{13}C NMR (126 MHz, D_2O): δ 163.3, 155.9, 154.7, 153.6, 132.0, 107.8, 101.8, 50.4, 48.0 (t, J = 125.3 Hz). ^{31}P NMR (162 MHz, D_2O): δ 12.25. HRMS [ESI$^-$] calculated for $C_9H_{11}N_4O_8P_2S$ m/z, 396.977 83; found 396.977 73 [M − H$^+$]$^-$.

(7-Methoxypyrido[2,3-*d*]pyrimidin-4-ylamino)methylenediphosphonic Acid (8e). Product was isolated as a white solid (31.5 mg, 75%). ^{1}H NMR (200 MHz, D_2O): δ 8.35 (d, J = 9.0 Hz, 1H), 8.28 (s, 1H), 6.86 (d, J = 9.0 Hz, 1H), 3.90 (s, 3H). ^{13}C NMR (126 MHz, D_2O): δ 167.0, 159.6, 158.4, 157.3, 135.7, 111.5, 105.5, 54.1 (Cα and -OCH_3, t, J = 124.8 Hz). ^{31}P NMR (81 MHz, D_2O): δ 12.86. HRMS [ESI$^-$] calculated for $C_9H_{11}O_7N_4P_2$ m/z, 349.010 84; found 349.010 71 [M − H$^+$]$^-$.

(((6-bromopyrido[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic Acid (8f). The product was isolated as a light yellow solid (7.10 mg, 74%). ^{1}H NMR (500 MHz, D_2O): δ 8.98 (d, J = 2.3 Hz, 1H), 8.94 (d, J = 2.4 Hz, 1H), 8.54 (s, 1H);α C-H to the bisphosphonate overlaps with the solvent peak. ^{13}C NMR (126 MHz, D_2O): δ 159.6, 158.6, 156.2, 155.3, 136.0, 116.1, 111.8, 52.4−49.7 (m). ^{31}P NMR (81 MHz, D_2O): δ 12.61. HRMS [ESI$^-$] calculated for $C_8H_8BrN_4O_6P_2$ m/z, 396.910 79; found 396.91105 [M − H$^+$]$^-$.

(((6-(Thiophen-2-yl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic Acid (8g). Product was isolated as a yellow solid (12.1 mg, 77%). ^{1}H NMR (500 MHz, D_2O): δ 9.12 (d, J = 2.3 Hz, 1H), 8.84 (d, J = 2.3 Hz, 1H), 8.47 (s, 1H), 7.54 (d, J = 3.6 Hz, 1H), 7.48 (d, J = 5.1 Hz, 1H), 7.12 (dd, J = 5.0, 3.7 Hz, 1H), 5.05 (t, J = 19.6 Hz, 1H). ^{13}C NMR (126 MHz, D_2O): δ 160.5, 157.2, 154.5, 152.8, 138.5, 128.7, 128.7, 127.4 (2 × C overlapping), 125.5, 110.4, 50.2 (t, J = 126.0 Hz). ^{31}P NMR (203 MHz, D_2O): δ 12.22. HRMS [ESI$^-$] calculated for $C_{12}H_{11}N_4O_6P_2S$ m/z, 400.988 00; found 400.987 99 [M − H$^+$]$^-$.

(((6-(3,5-Dimethylisoxazol-4-yl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic Acid (8h). The product was isolated as a yellow solid (7.2 mg, 46%). ^{1}H NMR (400 MHz, D_2O): δ 8.83 (d, J = 2.2 Hz, 1H), 8.70 (d, J = 2.2 Hz, 1H), 8.55 (s, 1H), 2.43 (s, 3H), 2.28 (s, 3H); α-CH to the bisphosphonate overlaps with the solvent peak. ^{13}C NMR (126 MHz, D_2O): δ 168.1, 160.4, 160.1, 158.8, 156.1, 155.6, 134.3, 124.2, 112.9, 110.8, 50.9 (t, J = 121.1 Hz), 10.6, 9.6. ^{31}P NMR (81 MHz, D_2O): δ 12.65. HRMS [ESI$^-$] calculated for $C_{13}H_{14}N_5O_7P_2$ m/z, 414.037 39; found 414.037 31 [M − H$^+$]$^-$.

(((6-(4-Isopropoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic Acid (8i). Product was isolated as a yellow solid (9.2 mg, 95%). ^{1}H NMR (500 MHz, D_2O): δ 8.89 (d, J = 2.2 Hz, 1H), 8.62 (d, J = 2.3 Hz, 1H), 8.34 (s, 1H), 7.45 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.9 Hz, 2H), 4.97 (t, J = 19.3 Hz, 1H), 4.52 (hept, J = 6.1 Hz, 1H), 1.18 (s, 3H), 1.17 (s, 3H). ^{13}C NMR (126 MHz, D_2O): δ 160.5, 157.2, 156.5, 153.7, 153.2, 134.1, 129.8, 128.2 (two overlapping carbons), 116.6, 110.1, 71.3, 50.2 (t, J = 124.6 Hz), 21.0. ^{31}P NMR (162 MHz, D_2O): δ 12.22. HRMS [ESI$^-$] calculated for $C_{17}H_{19}N_4O_7P_2$ m/z, 453.073 45; found 453.073 64 [M − H$^+$]$^-$.

(((6-Phenylpyrido[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic Acid (8j). The product was isolated as a light yellow solid (3.59 mg, 66%). ^{1}H NMR (500 MHz, D_2O): δ 9.22 (d, J = 1.6 Hz, 1H), 9.04 (d, J = 1.5 Hz, 1H), 8.59 (s, 1H), 7.85 (d, J = 7.4 Hz, 2H), 7.60 (t, J = 7.6 Hz, 2H), 7.51 (t, J = 7.4 Hz, 1H), 5.09−4.89 (m, 1H). ^{31}P NMR (203 MHz, D_2O): δ 12.80. HRMS [ESI$^-$] calculated for $C_{14}H_{13}N_4O_6P_2$ m/z, 395.031 58; found 395.031 82 [M − H$^+$]$^-$.

(((7-(Methylthio)-6-phenylpyrido[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic Acid (8k). The product was isolated as light yellow solid (3.9 mg, 81%). ^{1}H NMR (500 MHz, D_2O): δ 8.34 (s, 1H), 8.21 (s, 1H), 7.47−7.33 (m, 5H), 2.44 (s, 3H); α-CH to the bisphosphonate overlaps with the solvent peak. ^{13}C NMR (126 MHz, D_2O): δ 166.3, 160.2, 158.2, 156.5, 136.8, 134.6, 131.9, 129.4, 128.7, 128.6, 106.9, 51.8−49.9 (m), 13.2. ^{31}P NMR (81 MHz, D_2O): δ 12.77. HRMS [ESI$^-$] calculated for $C_{15}H_{15}N_4O_6P_2S$ m/z, 441.019 30; found 441.020 82 [M − H$^+$]$^-$.

(((7-(Methylthio)-6-(thiophen-3-yl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic Acid (8l). The product was isolated as a light yellow solid (17.9 mg, 93%). ^{1}H NMR (400 MHz, D_2O): δ 8.33 (s, 1H), 8.29 (s, 1H), 7.58 (dd, J = 2.9, 1.2 Hz, 1H), 7.44 (dd, J = 5.0, 3.0 Hz, 1H), 7.29 (dd, J = 5.0, 1.2 Hz, 1H), 2.46 (s, 3H); α-CH to the bisphosphonate overlaps with the solvent peak. ^{13}C NMR (126 MHz, D_2O): δ 166.6, 160.4, 158.2, 156.4, 136.7, 131.8, 129.9, 128.7, 126.5, 126.0, 106.9, 50.8 (t, J = 122.1 Hz), 13.4. ^{31}P NMR (81 MHz, D_2O): δ 12.84. HRMS [ESI$^-$] calculated for $C_{13}H_{13}N_4O_6P_2S_2$ m/z, 446.975 72; found 446.975 66 [M − H$^+$]$^-$.

(((6-(4-Isopropoxyphenyl)-7-(methylthio)pyrido[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic Acid (8m). The product was isolated as a yellow solid (6.8 mg, 70%). ^{1}H NMR (500 MHz, D_2O): δ 8.50 (s, 1H), 8.32 (s, 1H), 7.52 (d, J = 8.7 Hz, 2H), 7.12 (d, J = 8.7 Hz, 2H), 2.58 (s, 3H), 1.37 (s, 3H), 1.35 (s, 3H); α-CH to the bisphosphonates and i-Pr-CH overlaps with the solvent peak as confirmed by HSQC. HSQC (^{1}H−^{13}C): δ 4.79 (^{1}H) correlates to δ 50.0 (^{13}Cα), and δ 4.69 correlates to ^{13}C δ 71.1. ^{13}C NMR (101 MHz, D_2O): δ 166.8, 160.4, 158.1, 157.2, 156.4, 134.4, 131.7, 131.0, 129.8, 116.3, 106.9, 71.7, 21.1, 13.3; Cα to the bisphosphonate was observed by HSQC. ^{31}P NMR (162 MHz, D_2O): δ 13.04. HRMS [ESI$^-$] calculated for $C_{18}H_{21}N_4O_7P_2S$ m/z, 499.061 17; found 499.061 37 [M − H$^+$]$^-$.

(2-(Pyrido[2,3-*d*]pyrimidin-4-ylamino)ethane-1,1-diyl)diphosphonic Acid (9a). The product was isolated as light yellow solid (6.8 mg, 33%). ^{1}H NMR (500 MHz, D_2O): δ 8.87 (dd, J = 4.6, 1.8 Hz, 1H), 8.49 (s, 1H), 8.44 (dd, J = 8.3, 1.8 Hz, 1H), 7.58 (dd, J = 8.3, 4.6 Hz, 1H), 3.94−3.87 (m, 2H), 2.26−2.14 (m, 1H). ^{13}C NMR (126 MHz, D_2O): δ 160.0, 158.2, 156.2, 155.2, 133.8, 122.3, 110.7, 39.9 (br; Cβ), 38.8 (t, J = 112.7 Hz, Cα). ^{31}P NMR (81 MHz, D_2O): δ 16.72. HRMS [ESI$^-$] calculated for $C_9H_{11}N_4O_6P_2$ m/z, 333.015 93; found 333.016 52 [M − H$^+$]$^-$.

(2-((6-Phenylpyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)diphosphonic Acid (9b). The product was isolated as a yellow solid (9.7 mg, 82%). ^{1}H NMR (500 MHz, D_2O): δ 8.99 (d, J = 1.9 Hz, 1H), 8.42 (d, J = 1.8 Hz, 1H), 8.40 (s, 1H), 7.55 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.3 Hz, 1H), 3.99 (td, J = 14.1, 7.2 Hz, 2H), 2.50 (tt, J = 20.7, 7.0 Hz, 1H). ^{13}C NMR (101 MHz, D_2O): δ 160.0, 157.3, 154.7, 153.8, 135.0, 134.0, 130.1, 129.2, 128.6, 126.5, 110.1, 39.3 (br, Cβ), 39.5−37.3 (m, Cα). ^{31}P NMR (162 MHz, D_2O): δ 17.02. HRMS [ESI$^-$] calculated for $C_{15}H_{15}N_4O_6P_2$ m/z, 409.047 23; found 409.047 14 [M − H$^+$]$^-$.

(2-((6-(Thiophen-3-yl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)diphosphonic Acid (9c). The product was isolated as a yellow solid (10.5 mg, 84%). ^{1}H NMR (500 MHz, D_2O): δ 8.86 (d, J = 2.0 Hz, 1H), 8.27 (s, 1H), 8.23 (d, J = 1.9 Hz, 1H), 7.66 (d, J = 1.5 Hz, 1H), 7.47 (dd, J = 5.0, 2.9 Hz, 1H), 7.34 (d, J = 4.8 Hz, 1H), 3.96 (td, J = 14.2, 7.1 Hz, 2H), 2.54 (tt, J = 20.9, 7.1 Hz, 1H). ^{13}C NMR (126 MHz, D_2O): δ 157.5, 154.4, 151.4, 150.8, 133.5, 126.9, 126.0, 125.5, 122.8, 120.6, 107.6, 37.1 (br, Cβ), 36.1 (t, J = 114.9 Hz, Cα). ^{31}P NMR (203 MHz, D_2O): δ 16.98. HRMS [ESI$^-$] calculated for $C_{13}H_{13}N_4O_6P_2S$ m/z, 415.003 65; found 415.004 00 [M − H$^+$]$^-$.

(2-((6-(*p*-Tolyl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)diphosphonic Acid (9d). The product was isolated as a yellow solid (14.1 mg, 92%). ^{1}H NMR (500 MHz, D_2O): δ 9.06 (s, 1H), 8.49 (s, 1H), 8.45 (s, 1H), 7.49 (d, J = 7.8 Hz, 2H), 7.23 (d, J = 7.8 Hz, 2H), 4.05 (td, J = 14.4, 6.5 Hz, 2H), 2.62−2.47 (m, 1H), 2.32 (s, 3H). ^{13}C NMR (126 MHz, D_2O): δ 160.1, 157.3, 155.0, 153.7, 138.9, 134.0, 132.4, 129.9, 129.8, 126.5, 110.2, 39.5−39.2 (m, two overlapping carbons, Cα and Cβ), 20.3. ^{31}P NMR (203 MHz, D_2O): δ 17.07 and 17.04 (the two phosphonates are nonequivalent). HRMS [ESI$^-$] calculated for $C_{16}H_{17}N_4O_6P_2$ m/z, 423.062 88; found 423.062 90 [M − H$^+$]$^-$.

(2-((6-(4-Methoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)diphosphonic Acid (9e). The product was isolated as a yellow solid (10.0 mg, 86%). ^{1}H NMR (500 MHz, D_2O): δ 8.85 (d, J = 2.2 Hz, 1H), 8.35 (s, 1H), 8.11 (d, J = 2.3 Hz, 1H), 7.28 (d, J = 8.7 Hz, 2H), 6.72 (d, J = 8.7 Hz, 2H), 4.00 (td, J = 14.1, 7.2 Hz, 2H), 3.76 (s, 3H), 2.54 (tt, J = 20.9, 7.2 Hz, 1H). ^{13}C NMR (126 MHz, D_2O): δ 159.8, 158.7, 156.6, 154.0, 153.2, 133.2, 128.9, 127.4, 127.4, 114.1, 109.9, 55.1, 39.3 (br, Cβ), 38.4 (t, J = 111.5 Hz, Cα). ^{31}P NMR (203 MHz, D_2O): δ 17.05. HRMS [ESI$^-$] calculated for $C_{16}H_{17}N_4O_7P_2$ m/z, 439.057 80; found: 439.058 09 [M − H$^+$]$^-$.

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(2-((6-(3-Methoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-yl)-amino)ethane-1,1-diyl)diphosphonic Acid (9f). The product was isolated as a yellow solid (6.0 mg, 80%). ^{1}H NMR (500 MHz, D_2O): δ 8.96 (d, J = 1.8 Hz, 1H), 8.44 (s, 1H), 8.39 (d, J = 1.4 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.16 (d, J = 7.6 Hz, 1H), 6.96 (s, 1H), 6.82 (dd, J = 8.2, 1.9 Hz, 1H), 4.02 (td, J = 14.0, 7.3 Hz, 2H), 3.82 (s, 3H), 2.54 (tt, J = 20.8, 7.2 Hz, 1H). ^{13}C NMR (101 MHz, D_2O): δ 160.1, 159.1, 157.3, 154.8, 153.8, 136.6, 133.6, 130.5, 130.3, 119.4, 113.7, 111.8, 110.0, 55.3, 39.3 (br, Cβ), 38.40 (t, J = 113.9 Hz, Cα). ^{31}P NMR (203 MHz, D_2O): δ 17.02. HRMS [ESI$^-$] calculated for $C_{16}H_{17}N_4O_7P_2$ m/z, 439.057 80; found: 439.057 76 [M − H$^+$]$^-$.

(2-((6-Bromo-7-(methylthio)pyrido[2,3-*d*]pyrimidin-4-yl)-amino)ethane-1,1-diyl)diphosphonic Acid (9g). The product was isolated as a white solid (5.7 mg, 62%). ^{1}H NMR (400 MHz, D_2O): δ 8.49 (s, 1H), 8.31 (s, 1H), 4.08−3.90 (m, 2H), 2.59 (s, 3H), 2.56−2.36 (m, 1H). ^{13}C NMR (126 MHz, D_2O): δ 167.4, 159.2, 157.4, 154.7, 133.9, 115.8, 107.0, 39.3 (br, Cβ), 14.2; C-α to the bisphosphonate was observed by HSQC. HSQC (^{1}H−^{13}C): ^{1}H at δ 2.46 correlates to ^{13}Cα at δ 38.5. ^{31}P NMR (162 MHz, D_2O): δ 16.90. HRMS [ESI$^-$] calculated for $C_{10}H_{12}BrN_4O_6P_2S$ m/z, 456.914 16; found 456.914 45 [M − H$^+$]$^-$.

(2-((7-(Methylthio)-6-phenylpyrido[2,3-*d*]pyrimidin-4-yl)-amino)ethane-1,1-diyl)diphosphonic Acid (9h). The product was isolated as a yellow solid (4.7 mg, 65%). ^{1}H NMR (500 MHz, D_2O): δ 8.52 (s, 1H), 8.08 (s, 1H), 7.60−7.52 (m, 5H), 3.98−3.91 (m, 2H), 2.60 (s, 3H), 2.35−2.24 (m, 1H). ^{13}C NMR (126 MHz, D_2O): δ 167.8, 160.2, 156.0, 153.5, 136.1, 135.5, 130.9, 129.3, 129.0, 128.7, 106.0, 39.3 (Cβ), 13.4; C-α to the bisphosphonates was observed by HSQC. HSQC (^{1}H−^{13}C): ^{1}H δ 2.46 correlates to ^{13}C δ 38.5. ^{31}P NMR (203 MHz, D_2O): δ 16.84. HRMS [ESI$^-$] calculated for $C_{16}H_{17}N_4O_6P_2S$ m/z, 455.034 95; found: 455.0349 8 [M − H$^+$]$^-$.

(2-((6-(3-Methoxyphenyl)-7-(methylthio)pyrido[2,3-*d*]-pyrimidin-4-yl)amino)ethane-1,1-diyl)diphosphonic Acid (9i). The product was isolated as an off-white solid (7.9 mg, 90%). ^{1}H NMR (400 MHz, D_2O): δ 8.49 (s, 1H), 8.05 (s, 1H), 7.46 (t, J = 8.1 Hz, 1H), 7.17−7.05 (m, 3H), 3.92 (br, 2H), 3.85 (s, 3H), 2.56 (s, 3H), 2.27 (br, 1H). ^{13}C NMR (126 MHz, D_2O): δ 166.7, 160.2, 158.8, 158.0, 156.4, 138.1, 134.7, 131.4, 130.1, 122.3, 115.0, 114.7, 106.6, 55.5, 39.5−39.3 (Cβ), 39.4−38.0 (m, Cα), 13.3. ^{31}P NMR (203 MHz, D_2O): δ 16.49. HRMS [ESI$^-$] calculated for $C_{17}H_{19}N_4O_7P_2S$ m/z, 485.045 52; found 485.045 96 [M − H$^+$]$^-$.

(2-((6-(4-Isopropoxyphenyl)-7-(methylthio)pyrido[2,3-*d*]-pyrimidin-4-yl)amino)ethane-1,1-diyl)diphosphonic Acid (9j). Product was isolated as a yellow solid (4.04 mg, 37%). ^{1}H NMR (500 MHz, D_2O): δ 8.49 (s, 1H), 8.03 (s, 1H), 7.52 (d, J = 7.8 Hz, 2H), 7.12 (d, J = 8.1 Hz, 2H), 3.97−3.90 (m, 2H), 2.58 (s, 3H), 2.33−2.25 (m, 1H),, 1.37 (s, 3H), 1.36 (s, 3H); i-Pr-CH overlaps with the solvent peak; confirmed by HSQC. HSQC (^{1}H−^{13}C): δ ^{1}H δ 4.68 correlates to ^{13}C δ 71.7. ^{13}C NMR (126 MHz, D_2O): δ 167.1, 160.2, 157.8, 157.3, 156.2, 134.7, 131.2, 131.0, 129.6, 116.3, 106.6, 71.7, 39.3 (Cβ), 38.7 (Cα), 21.1, 13.4. ^{31}P NMR (203 MHz, D_2O): δ 16.94. HRMS [ESI$^-$] calculated for $C_{19}H_{23}N_4O_7P_2S$ m/z, 513.076 82; found 513.076 68 [M − H$^+$]$^-$.

(2-((7-(Methylthio)-6-(thiophen-3-yl)pyrido[2,3-*d*]pyrimidin-4-yl)amino)ethane-1,1-diyl)diphosphonic Acid (9k). The product was isolated as a light yellow solid (4.9 mg, 41%). ^{1}H NMR (500 MHz, D_2O): δ 8.48 (s, 1H), 8.12 (s, 1H), 7.76−7.72 (m, 1H), 7.58 (dd, J = 4.9, 3.0 Hz, 1H), 7.43 (dd, J = 5.0, 1.1 Hz, 1H), 3.92 (br, 2H), 2.60 (s, 3H), 2.26 (br, 1H). ^{13}C NMR (126 MHz, D_2O): δ 166.7, 160.0, 157.8, 156.0, 136.4, 131.2, 130.0, 128.5, 126.4, 126.0, 106.5, 39.4(br, Cβ), 36.9 (Cα), 13.2. ^{31}P NMR (81 MHz, D_2O): δ 17.01. HRMS [ESI$^-$] calculated for $C_{14}H_{15}N_4O_6P_2S_2$ m/z: 460.991 37; found: 460.991 84 [M − H$^+$]$^-$.

Primer Extension Polymerase Scintillation Proximity Assay (SPA).[40] The following primer sequences were used in the HIV RT primer extension scintillation proximity assay (SPA) (Bosworth and Towers, 1989). Biotinylated PPT80 (5′-ATC TTG TCT TCG TTG GGA GTG AAT TAG CCC TTC CAG TCC CCC CTT TTC TTT TAA AAA GTG GCT AAG CTC TAC AGC TGC CC-3′) was used as a template. The underlined nucleotides are the portion of the template that annealed to the primer. Primer DNA PPT (5′-TTA AAA GAA AAG GGG GG-3′) was used in the assay.

A 10-step 3-fold dilution series of the compound to be tested was prepared in either reaction buffer (50 mM Tris, pH 7.8, 50 mM NaCl, 6 mM $MgCl_2$) or DMSO, depending on compound solubility, to obtain a final concentration in the experiment of 100 μM to 10 nM. The concentration of DMSO in the final reaction did not exceed 5%.

50 nM of DNA PPT/biotinylated PPT80 primer-template hybrid was preincubated for 5 min at 37 °C in a buffer containing 50 mM Tris, pH 7.8, 50 mM NaCl, 6 mM $MgCl_2$, 4 μM dCTP, 4 μM dGTP, 4 μM dTTP, and 0.5 μM [8-3H (N)]-dATP in the presence of inhibitor to be tested. Nucleotide incorporation was initiated by the addition of the HIV RT polymerase, and reaction was allowed to proceed for 5 min. The reaction was quenched by the addition of one reaction volume of 0.5 M EDTA containing 1 mg/mL streptavidin coated PVT SPA beads. The concentration of HIV RT used in the reaction was empirically determined to produce 1000 cpm in an uninhibited positive control.

The SPA beads were then allowed to settle for approximately 8 h upon which the samples were counted using a liquid scintillation counter. The liquid scintillation count represents the total amount of incorporation of [8-3H (N)]-dATP and is related to the amount of polymerase activity. Liquid scintillation counts were converted to relative activity by dividing the activity of an inhibited experiment count by an average of at least three uninhibited positive control counts. These data were then plotted using GraphPad Prism (version 5.0). The IC_{50} values were calculated by fitting at least eight data points to a sigmoidal dose−response (variable slope) equation using GraphPad Prism. Significant figure for all experiments were determined on the basis of at least two independent replicates.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra and homogeneity data (HPLC chromatograms) for key inhibitors **8m**, **9h**, **9i**, and **9j**. 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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■ ABBREVIATIONS USED

HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase; PYPY-BP, pyrido[2,3-*d*]pyrimidine bisphosphonate; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NcRTI, nucleotide competitive reverse transcriptase inhibitor; PPi, inorganic pyrophosphate; NTP, nucleotide triphosphate; NDP, nucleotide diphosphate; PFA, foscarnet

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